We Are Continuous Ready
Scalable, integrated platform technologies for continuous biomanufacturing

Watch our video and learn more:
www.pall.com/continuous

Continuously Improving Bioprocesses
### Scientific Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michael Bergmann</td>
<td>Medical University of Vienna</td>
</tr>
<tr>
<td>Kalina Duszka</td>
<td>University of Vienna</td>
</tr>
<tr>
<td>Anton Glieder</td>
<td>TU Graz</td>
</tr>
<tr>
<td>Sigismund Huck</td>
<td>Medical University of Vienna</td>
</tr>
<tr>
<td>Annemarie Käsbohrer</td>
<td>Vetmeduni Vienna</td>
</tr>
<tr>
<td>Günther Koraimann</td>
<td>University of Graz</td>
</tr>
<tr>
<td>Alexander Lichius</td>
<td>University of Innsbruck</td>
</tr>
<tr>
<td>Dorothea Orth-Höller</td>
<td>Medical University of Innsbruck</td>
</tr>
<tr>
<td>Andreas Posch</td>
<td>Ares Genetics GmbH</td>
</tr>
<tr>
<td>Matthias Steiger</td>
<td>ACIB</td>
</tr>
<tr>
<td>Eva Untersmayr-Elsenhuber</td>
<td>Medical University of Vienna</td>
</tr>
<tr>
<td>Viktoria Weber</td>
<td>Danube University Krems</td>
</tr>
<tr>
<td>Konstantin Bergmeister</td>
<td>Medical University of Vienna / BG-Klinik</td>
</tr>
<tr>
<td>Klaus Graumann</td>
<td>Novartis / Sandoz</td>
</tr>
<tr>
<td>Beatrice Jahn-Schmid</td>
<td>Medical University of Vienna</td>
</tr>
<tr>
<td>Georg Klima</td>
<td>Boehringer Ingelheim</td>
</tr>
<tr>
<td>Kaisa Koskinen</td>
<td>Medical University of Graz</td>
</tr>
<tr>
<td>Alexandra Lusser</td>
<td>Medical University of Innsbruck</td>
</tr>
<tr>
<td>Winfried Neuhaus</td>
<td>AIT</td>
</tr>
<tr>
<td>Brigitte Pertschy</td>
<td>University of Graz</td>
</tr>
<tr>
<td>Claudia Preininger</td>
<td>AIT</td>
</tr>
<tr>
<td>Sandra Siegert</td>
<td>IST Austria</td>
</tr>
<tr>
<td>Joseph Strauss</td>
<td>BOKU</td>
</tr>
<tr>
<td>Andreas Villunger</td>
<td>Medical University of Innsbruck</td>
</tr>
<tr>
<td>David Berry</td>
<td>University of Vienna</td>
</tr>
<tr>
<td>Mohamed Elgendy</td>
<td>University of Vienna</td>
</tr>
<tr>
<td>Michael Jantsch</td>
<td>Medical University of Vienna</td>
</tr>
<tr>
<td>Bon-Kyoung Koo</td>
<td>IMBA</td>
</tr>
<tr>
<td>Margit Laimer</td>
<td>BOKU</td>
</tr>
<tr>
<td>Astrid Mach-Aigner</td>
<td>TU Wien</td>
</tr>
<tr>
<td>Christa Nöhammer</td>
<td>AIT</td>
</tr>
<tr>
<td>Bruno Podesser</td>
<td>Medical University of Vienna</td>
</tr>
<tr>
<td>Monika Schmoll</td>
<td>AIT</td>
</tr>
<tr>
<td>Ines Swoboda</td>
<td>FH Campus Vienna</td>
</tr>
<tr>
<td>Martin Wagner</td>
<td>Vetmeduni Vienna</td>
</tr>
</tbody>
</table>

### Organizing Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michael Sauer (Chair)</td>
<td>BOKU</td>
</tr>
<tr>
<td>Angela Sessitsch</td>
<td>AIT</td>
</tr>
<tr>
<td>Alexandra Khassidov</td>
<td>ÖGMBT</td>
</tr>
<tr>
<td>Sergey Zotchev</td>
<td>University of Vienna</td>
</tr>
</tbody>
</table>
# Table of Contents

- Table of Contents ......................................................................................................................... 2
- General Information ....................................................................................................................... 4
- Venue floor plans ............................................................................................................................. 6
- Overview ........................................................................................................................................ 9
- Welcome Address .......................................................................................................................... 13
- Satellite Events .............................................................................................................................. 15
- Invited Speakers ............................................................................................................................ 19
- Detailed Program ............................................................................................................................ 31
  - Biophysics Austria Annual Meeting - Monday, September 17th, 2018........................................ 31
  - Monday, September 17th, 2018 .................................................................................................. 33
  - Tuesday, September 18th, 2018 ................................................................................................. 35
  - Wednesday, September 19th, 2018 ............................................................................................ 39
  - Thursday, September 20th, 2018 ............................................................................................... 42
- Poster Table - Biophysics ................................................................................................................. 45
- Poster Table - Poster Session 1 ....................................................................................................... 46
- Poster Table - Poster Session 2 ....................................................................................................... 47
- Poster Table - Poster Session 3 ....................................................................................................... 48
- Monday 17th September 2018 ......................................................................................................... 49
  - Biophysics Austria Annual Meeting ......................................................................................... 50
  - Opening & Opening Plenary .................................................................................................... 59
  - Plant biotechnology ................................................................................................................. 60
  - Neuroimmunology .................................................................................................................... 64
  - From RNAomics to function .................................................................................................. 68
  - Life Science Awards & Anniversary Ceremony ....................................................................... 72
  - Poster Session Biophysics ....................................................................................................... 76
- Tuesday 18th September 2018 ......................................................................................................... 80
  - Plenary 1 .................................................................................................................................... 81
  - Plenary 2 .................................................................................................................................... 82
  - Poster Flash 1 ............................................................................................................................. 83
  - Optimization of microbial workhorses for biotechnology ....................................................... 87
  - Next-generation pathogen & antibiotic resistance diagnostics ............................................. 91
  - Biological barriers in health and disease ................................................................................ 95
  - Poster Session 1: Plant biotechnology ................................................................................. 99
  - Poster Session 1: Optimization of microbial workhorses for biotechnology ...................... 104
  - Poster Session 1: Neuroimmunology ..................................................................................... 112
  - Poster Session 1: Next-generation pathogen & antibiotic resistance diagnostics .............. 114
  - Poster Session 1: New trends in allergy diagnosis and therapy ........................................... 116
  - Poster Session 1: From RNAomics to function .................................................................. 121
  - Poster Session 1: Biological barriers in health and disease ................................................. 126
  - Poster Session 1: Extracellular vesicles in coagulation and inflammation ....................... 129
  - Molecular microbiology ........................................................................................................ 132
  - New trends in allergy diagnosis and therapy ....................................................................... 136
  - Extracellular vesicles in coagulation and inflammation ...................................................... 142
  - DK BioToP ............................................................................................................................. 146
  - DK ICA ................................................................................................................................. 149
- Wednesday 19th September 2018 ............................................................................................... 152
  - Plenary 3 .................................................................................................................................. 153
  - Plenary 4 .................................................................................................................................. 154
  - Poster Flash 2 .......................................................................................................................... 155
  - Microbial chemical production .............................................................................................. 159
  - Microbiomes: interplay of microbes, their hosts and environments .................................. 163
  - Pluripotent stem cells and neural differentiation ................................................................. 167
  - Poster Session 2: Microbial chemical production ............................................................... 171
  - Poster Session 2: Biopharmaceutical technologies ............................................................... 175
Table of Contents

Poster Session 2: Microbiomes: interplay of microbes, their hosts and environments ........................................ 181
Poster Session 2: Pluripotent stem cells and neural differentiation ................................................................. 186
Poster Session 2: Cancer metabolism, autophagy and cell death ................................................................. 188
Poster Session 2: Biomaterials in surgery ........................................................................................................ 195
Poster Session 2: Varia .................................................................................................................................. 197
Biopharmaceutical technologies ...................................................................................................................... 201
Cancer metabolism, autophagy and cell death ................................................................................................. 205
Biomaterials in surgery .................................................................................................................................. 209
Thursday 20th September 2018 ....................................................................................................................... 213
Plenary 5 ......................................................................................................................................................... 214
Plenary 6 ......................................................................................................................................................... 215
Poster Flash 3 .................................................................................................................................................. 216
Synthetic biology ............................................................................................................................................. 220
Antimicrobial drugs: drug screening and prudent use ................................................................................... 223
Translational oncology I ............................................................................................................................... 227
Poster Session 3: Molecular microbiology ................................................................................................... 231
Poster Session 3: Synthetic biology ............................................................................................................... 241
Poster Session 3: Translational oncology ...................................................................................................... 243
Poster Session 3: Antimicrobial drugs: drug screening and prudent use ..................................................... 256
Molecular microbiology II ............................................................................................................................. 259
Antimicrobial resistance: transfer of bugs and genes in diverse ecosystems ............................................ 262
Translational oncology II ............................................................................................................................... 266
Author Index .................................................................................................................................................... 270
Participant Index ............................................................................................................................................. 281
Acknowledgements ........................................................................................................................................ 297
General Information

Taxi:
+ 43 (0)1 31 300
+ 43(0)1 40 100

Venue address:
Campus of the University of Vienna
Spitalgasse 2
1090 Vienna, Austria

The full Abstractbook is available for download as PDF at the ÖGMBT Annual meeting website on the “Program” page and after the meeting in the “Jahretagung Archiv” section.

WiFi is available at the venue, please get your network login code at the registration desk.

Biophysics Day Tickets are only valid on Monday September 17th where the Biophysics Satellites Meeting takes place.

Coffee points, lunch bags, dinner:
Coffee, beverages, dinner and lunch bags will be provided in the lecture hall C2 foyer and Aula. Please note the different lunch bag points (C2 foyer: meat, gluten free, Aula: vegetarian, vegan). You will find coupons for the lunch bags with your food preference you declared within your registration in your badge.

Talks:
Please give your presentation (in PowerPoint or PDF format) to the technician in the break before the talk via USB-stick. Best Student Talk Award: The three winners will be determined by an online participant vote during Award Ceremony II on Thursday. To access the voting system, use the URL or scan the QR code on the right.

Poster Flash:
Additional to poster presentations selected participants will give a Poster Flash (2 minutes, 2 PDF pages). The presentations should have been submitted online before conference beginning. Poster Flash 1 takes place on September 18th, 10:20-10:35, Poster Flash 2 on September 19th, 10:20-10:35 and Poster Flash 3 on September 20th, 10:20-10:35.

Posters:
Poster presentations will be split in three poster sessions, poster session 1 (September 18th, 13:30-15:30), poster session 2 (September 19th, 13:30-15:30) and poster session 3 (September 20th, 13:30-15:30)

- Posters from poster session Biophysics Austria must be on display until the Lunch break on 17th and removed latest until 9:00 next day the 18th.
- Posters from poster session 1 must be on display Thursday until the Lunch break on 18th and removed latest in the Coffee Break on the next day (19th, 10:30-11:00).
- Posters from poster session 2 must be on display until the Lunch break on 19th and removed latest in the Coffee Break on the next day (20th, 10:30-11:00).
- Posters from poster session 3 must be on display until the Lunch break on 20th and removed in the last Coffee Break on this day (20th, 17:00-17:30).

Best poster awards: The nine awards will be nominated by an evaluation committee and awarded at the Award Ceremony II on Thursday. Information on the assigned poster numbers and the location can be found on pages 44-47. Posters can be stored at the registration. Please contact the helpers if you want to store your poster.
ÖGMBT General Assembly:
Be an active part of ÖGMBT and shape the future with us! We cordially invite all ÖGMBT members to participate in the ÖGMBT General Assembly session on September 18th from 13:00 – 14:00.

Networking events:
We invite all participants and exhibitors to our networking events:
17th September: 10 years ÖGMBT Party starting 18:00

We reserve the right to use for documentation and promotion purposes any photographs/video staken at the event without the express written permission as already agreed by participants during the registration process.

The language of the meeting is English.

Please note that smoking inside the congress building is strictly forbidden!
Venue floor plans

LECTURE HALL C1

10th OGBMT Annual Meeting
TEN YEARS LIFE, SCIENCE & MOLECULES
Sep. 17-20 2018
Campus of the University of Vienna
Venue floor plans

AULA

IP1 Life & Health Science Cluster Tirol
IP2 Life Science Karriere Services
M11 New England Biolabs
M12 Eurofins Genomics
M13 Berthold Technologies
M14 VWR International
M15 Metrohm Inula
M16 Advanced Analytical Technologies
M17 Charles River
S6 Biomedica Medizinprodukte
S7 Stemcell Technologies
S8 Roche Diagnostics
S9 KML Vision
S10 Thermo Fisher Scientific
S11 Open Science

10th GMBT Annual Meeting

TEN YEARS LIFE, SCIENCE & MOLECULES
Sep. 17-20 2018
Campus of the University of Vienna
# Overview

**Monday 17\textsuperscript{th} September 2018**

<table>
<thead>
<tr>
<th>Satellites</th>
<th>Biophysics Austria Annual Meeting</th>
<th>YLSA Career Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>LH B</em> 09:50 – 19:00</td>
<td><em>LH C2</em> 10:00 – 13:00</td>
</tr>
</tbody>
</table>

| Invited Speaker | Christine Ziegler, Gehard Schütz & Georg Pabst |

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00 - 13:00</td>
<td>Lunch / Break / Science Art Exhibition</td>
</tr>
<tr>
<td>13:00 - 14:00</td>
<td>Opening &amp; Opening Plenary: Joshua Rosenthal <em>LH C1</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parallel Tracks</th>
<th>Track 1</th>
<th>Track 2</th>
<th>Track 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:15 - 15:45</td>
<td>Plant biotechnology <em>LH A</em></td>
<td>Neuro-immunology <em>LH C2</em></td>
<td>From RNAomics to function <em>LH C1</em></td>
</tr>
</tbody>
</table>

| Invited Speaker | Stefan Jansson | Marco Prinz | Christian R. Eckmann |

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:45 - 16:15</td>
<td>Break / Science Art Exhibition</td>
</tr>
<tr>
<td>16:15 - 17:25</td>
<td>Life Science Awards Austria 2018 Ceremony <em>LH C1</em></td>
</tr>
<tr>
<td>17:25 - 18:00</td>
<td>Anniversary Ceremony <em>LH C1</em></td>
</tr>
<tr>
<td>18:00 - 21:00</td>
<td>10 years ÖGMBT party</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9:00-9:40</td>
<td>Plenary 1</td>
</tr>
<tr>
<td>9:40-10:20</td>
<td>Plenary 2</td>
</tr>
<tr>
<td>10:20-10:35</td>
<td>Poster Flash 1</td>
</tr>
<tr>
<td>10:35-11:00</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>11:00-12:30</td>
<td>Parallel Tracks</td>
</tr>
<tr>
<td></td>
<td>Invited Speaker</td>
</tr>
<tr>
<td>12:30-13:30</td>
<td>Lunch</td>
</tr>
<tr>
<td>13:00-14:00</td>
<td>ÖGMBT General Assembly</td>
</tr>
<tr>
<td>13:30-15:30</td>
<td>Postersession 1</td>
</tr>
<tr>
<td>15:30-17:00</td>
<td>Parallel Tracks</td>
</tr>
<tr>
<td></td>
<td>Invited Speaker</td>
</tr>
<tr>
<td>17:00-17:30</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>17:30-18:20</td>
<td>DK BioToP</td>
</tr>
<tr>
<td>18:20-21:00</td>
<td>Networking</td>
</tr>
</tbody>
</table>
### Overview

**Wednesday 19th September 2018**

<table>
<thead>
<tr>
<th>Time</th>
<th>Track 1</th>
<th>Track 2</th>
<th>Track 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-9:40</td>
<td>Plenary 3</td>
<td>Paul Cos</td>
<td>LH C1</td>
</tr>
<tr>
<td>9:40-10:20</td>
<td>Plenary 4</td>
<td>Marina Šantić</td>
<td>LH C1</td>
</tr>
<tr>
<td>10:20-10:35</td>
<td></td>
<td>Poster Flash 2</td>
<td>LH C1</td>
</tr>
<tr>
<td>10:35-11:00</td>
<td></td>
<td>Coffee Break</td>
<td></td>
</tr>
<tr>
<td>11:00-12:30</td>
<td>Parallel Tracks</td>
<td><strong>Microbial chemical production</strong> LH A</td>
<td><strong>Microbiomes: interplay of microbes, their hosts and environments</strong> LH C1</td>
</tr>
<tr>
<td></td>
<td>Invited Speaker</td>
<td>Irina Borodina</td>
<td>Buck Hanson</td>
</tr>
<tr>
<td>12:30-13:30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30-15:30</td>
<td></td>
<td>Postersession 2</td>
<td><strong>Aula &amp; LH C1 foyer</strong></td>
</tr>
<tr>
<td>15:30-17:30</td>
<td>Parallel Tracks</td>
<td><strong>Biopharmaceutical technologies</strong> LH A</td>
<td><strong>Cancer metabolism, autophagy and cell death</strong> LH C1</td>
</tr>
<tr>
<td></td>
<td>Invited Speaker</td>
<td>Christoph Pistek</td>
<td>Wolfram Weckwerth &amp; Peter Vandenabeele</td>
</tr>
<tr>
<td>Time</td>
<td>Activity</td>
<td>Track 1</td>
<td>Track 2</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9:00-9:40</td>
<td>Plenary 5</td>
<td>Arnold J. M. Driessen</td>
<td>LH C1</td>
</tr>
<tr>
<td>9:40-10:20</td>
<td>Plenary 6</td>
<td>Henning Walczak</td>
<td>LH C1</td>
</tr>
<tr>
<td>10:20-10:35</td>
<td>Poster Flash 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:35-11:00</td>
<td>Coffee Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00-12:30</td>
<td>Parallel Tracks</td>
<td>Synthetic biology</td>
<td>Antimicrobial drugs: drug screening and prudent use</td>
</tr>
<tr>
<td></td>
<td>Invited Speaker</td>
<td>LH A</td>
<td>LH C2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roman Jeralal</td>
<td>Marc Stadler</td>
</tr>
<tr>
<td>12:30-13:30</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30-15:30</td>
<td>Postersession 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aula &amp; LH C1 foyer</td>
<td></td>
</tr>
<tr>
<td>15:30-17:00</td>
<td>Parallel Tracks</td>
<td>Molecular microbiology II</td>
<td>Antimicrobial resistance: transfer of bugs and genes in diverse ecosystems</td>
</tr>
<tr>
<td></td>
<td>Invited Speaker</td>
<td>LH A</td>
<td>LH C2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alexander Harms</td>
<td>Annemarie Kaesbohrer</td>
</tr>
<tr>
<td>17:00-17:30</td>
<td>Coffee Break / Exhibitor Quiz I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30-18:00</td>
<td>Award Ceremony II / Exhibitor Quiz II / Closing remarks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Welcome Address

10 years ÖGMBT - 10 years of life, science and molecules

It’s time to celebrate! We welcome you in Vienna to the ÖGMBT Annual Meeting, celebrating the 10th anniversary of the foundation of our lively society. This meeting is the largest Life Science meeting in Austria bringing together 500 participants from academia and industry – from Austria and abroad. We are particularly happy to welcome many participating PhD students and early stage researchers showcasing their work. The meeting provides a prime opportunity for you to start building your professional networks and the ÖGMBT YLSA (Young Life Scientists Austria) organized a fantastic session with career talks.

We are very grateful to all session chairs, who come from different institutions spread all over Austria, who helped bringing together an exciting programme covering three streams:

Biotechnology and molecular biology
Cancer and human disease
Medical microbiology and immunology

We are also happy to welcome all participants of the Biophysics Austria Annual Meeting which is held as satellite meeting on September 17th.

The meeting provides ample time for science, but we hope that you also appreciate the occasions for networking and connecting. The winners of the popular Life Science Research & PhD Awards Austria 2018 will be honoured and the ÖGMBT welcomes all participants and guests to the 10 years ÖGMBT party with many surprises on the first day of the conference. Our corporate sponsors cordially invite all attendees to enjoy wine and cheese (prime biotech products, of course) on the second evening of the conference during our longstanding networking event “wine & science”.

We are happy that you celebrate with us,

Michael Sauer, Sergey Zotchev, Angela Sessitsch and Alexandra Khassidov
Culture of Tomorrow

The new CellXpert® C170i CO₂ Incubator
Are you looking for a 170 L class CO₂ incubator that provides flexibility for the future, makes monitoring and documentation easy, and provides optimized growth conditions, even for your sensitive cells? An incubator that also saves money and is produced to the highest standards of quality?

> Stay flexible for the future
> Safety for your cells
> Save money

www.eppendorf.com/CellXpert

Your local distributor: www.eppendorf.at
Eppendorf Austria GmbH · Ignaz Köck Straße 10 · 1210 Wien · Austria
Tel. +43 1 890 13 64-0 · Fax: +43 1 890 13 64-20 · www.eppendorf.at

Eppendorf®, the Eppendorf Brand Design, CellXpert®, and VisionBox® are registered trademarks of Eppendorf AG, Germany. All rights reserved, including graphics and images. Copyright © 2018 by Eppendorf AG.

Order no.: XM02111026/EN/20180716/AG/573131
Satellite Events

**What do you want to do after your graduation? No idea? YLSA has some for you!**

**SATELLITE WORKSHOP Program**

10:00 – 10:30  ISG Personalmanagement International: Which skills do you need for your first job? What are typical entry positions?
10:30 – 10:45 All about YLSA, Introduction to our activities
10:45 – 13:00 Career Talks and Speakers Corner
Claudia Schandl (Patent Attorney Trainee @ REDL Patent Attorneys)
Daniel Kiesenhofer (Project Manager @ Ecoduna)
Udochuku Richson (Consulting @ FFG)
Silvia Herold (Editor @ Springer Nature)
Michael Durchschlag (Corporate Microbiologist & Qualified Person @ Iason)

Registration in advance is needed: Email: ylsa.ost@gmx.at

**When: September 17th, 10:00 – 13:00**
**Where: Lecture hall C2 and Career Corner tents**

---

**Communicating Science with Creativity**

**SCIENCE ART EXHIBITION AND FASHION SHOW by Beata Edyta Mierzwa (Beata Science Art)**

Creativity is an integral part of both science and art, and combining these two seemingly different disciplines creates unique ways to communicate science. This satellite event will focus on how researchers can use art to add some creativity to the conventional forms of scientific communication, and to convey complex biological concepts in an intuitive way.

We will highlight creativity in science with a space for science-inspired art, featuring an art exhibition and fashion show with works by Beata Science Art, who combines her passions for science and art to communicate science. We highly encourage participants to bring their own pieces of art of any kind - drawings, paintings, sculptures, jewelry, etc - for a unique opportunity to display their works, and to celebrate the beauty of science with fellow scientists and artists!

**When: September 17th, 2018**
12:00 – 13:00 Open to conference attendees
15:45 – 16:15 Open to conference attendees
17:45 – 21:00 Open to conference attendees & guests

**Where: Lecture Hall Center + Aula**

**Own pieces of art of any kind:**
Poster walls and pins will be available to attach your works of arts. Bar tables can also be used for the presentation of your art objects.
Annual Biophysics Austria Meeting

SATELLITE MEETING

Biophysics is the bridge between biology and physics. To foster interactions with biology, it has thus become tradition to liaise with the ÖGMBT and hold the Annual Biophysics Austria Meeting as satellite of the Annual ÖGMBT meeting. The meeting highlights recent advances in cell biophysics, membranes, channels & transporters, nanoscale biophysics, proteins, nucleic acids, systems biophysics, theoretical biophysics, biophysical techniques, and new emerging areas.

Further details are announced within the main scientific program.

When: September 17th, 10:00-19:00
Where: Lecture hall A

organized by Biophysics Austria

SMARTer solutions for single-cell transcriptomics and genomics

SATELLITE WORKSHOP

Since the emergence of next-generation sequencing (NGS), the importance and demand for single-cell analysis have risen rapidly. As a result, single-cell RNA- and DNA-seq has been gaining prominence not only in basic research fields, but also in clinical fields.

By leveraging on its patented SMART technology, Takara Bio has always been at the forefront of single-cell NGS research by providing the capability to obtain full-length mRNA sequence information.

Attend our seminar to learn more about SMARTer ICELL8 cx: The Open Platform for high-throughput single-cell NGS, SMARTer PicoPlex & SMART-seq – latest versions and see how they can improve your single-cell research.

Speaker: Dr. François-Xavier Sicot - Senior Product Manager, Takara Bio Europe

When: September 18th, 17:30-18:20
Where: Lecture hall C2
A convincing application is the ticket to the interview - Improve your CV

CV Check by Gisela Zechner

“HR professionals spend only few minutes to read your application and to decide either to invite you for an interview or not. Few minutes and rows to catch the attention and to convince with your personality”

Is there a job, you want to apply for right now? Are you generally interested in optimizing your CV and cover letter? Then take the chance for a quick check of your individual resume and cover letter at the life-science info point.

Do you have some specific questions concerning the interview? Let’s talk about and analyse it to strengthen your interview skills.

Registration in advance is favoured: E: office@life-science.eu subject: CV check ÖGMBT
Free of charge for the visitors of the conference.

When: September 17th - 20th (duration about 15 – 20 minutes/CV)
Where: Exhibition area, Aula
Breaking performance barriers in single-cell NGS library preparation

Discover our industry-leading technologies for single-cell RNA and DNA sequencing and speed your journey to the most elusive answers:

- **SMART-Seq® v4 Kits**: identify the highest number of transcripts (full-length or 3’end only) from single cells with high reproducibility.
- **SMARTer® Human scTCR a/b Profiling Kit**: obtain full-length, paired TCR a/b mRNA sequence information from single T cells.
- **PicoPLEX® DNA-Seq Kit**: detect aneuploidy and copy-number variation (CNV) with accurate and highly reproducible single-cell whole genome amplification.
- **ICELL8™ Single-Cell System**: high-throughput single-cell automation platform for scalable and advanced single-cell applications.

Learn more by visiting www.takarabio.com

MEET US AT OUR BOOTH!

Learn more about our NGS solutions at our seminar:

**“SMARTer solutions for single-cell transcriptomics and genomics”**

Presented by Dr. François-Xavier Sicot
Senior Product Manager, Takara Bio Europe

Room: HS C2  
Tuesday 18th September 2018
17:30 - 18:20

Takara

Clontech Takara cellartis
www.takarabio.com
Invited Speakers

Cosmas Arnold, Research Institute of Molecular Pathology (IMP), AT

Dr. Cosmas Arnold is a senior postdoctoral researcher and research associate in the laboratory of Prof. Dr. Alexander Stark at the Research Institute of Molecular Pathology (IMP, part of the Vienna BioCenter (VBC)) in Vienna, Austria. His research focus is regulatory and functional genomics and the development of advanced, next-generation sequencing-based methods. During his PhD (obtained from the University of Vienna), he developed the first genome-wide enhancer activity assay, STARR-seq (Arnold et al., Science 2013), which was awarded by the 2013 VBC PhD award. During his early postdoctoral work, he co-discovered the existence of different transcriptional programs that are defined by the compatibility between different classes of enhancers and promoters (Zabidi & Arnold et al., Nature 2015). His continued interest in developing novel methods to study transcriptional regulation resulted in the developed of STAP-seq, a method to study promoters and determine the responsiveness of genomic DNA sequences to enhancers, i.e. their transcriptional output levels or promoter strengths (Arnold & Zabidi et al., Nature Biotechnology 2017). This work was awarded with the Life Science Research Awards Austria 2018.

Irina Borodina, Technical University of Denmark, DK

Irina Borodina is Senior Scientist and Group Leader at the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark. Her group focuses on advancing the methodologies for metabolic engineering of yeast cell factories for sustainable production of bulk and high-value chemicals. Irina Borodina received her Chemical Engineering degree from Kaunas University of Technology in Lithuania in 2001 and her Ph.D. degree in Biotechnology from Technical University of Denmark in 2007. She has authored and co-authored 37 peer-reviewed articles, which have been cited over 1,600 times, and she is co-inventor of 9 patent applications. She received Jay Bailey Young Investigator Award in Metabolic Engineering in 2016. Irina is co-founder and CEO of a biotech start-up company BioPhero ApS.

Alain Brisson, UMR-CBMN, FR

Alain Brisson studied physical chemistry, biochemistry and neurobiology and obtained a thesis diploma from Paris-7 University and a PhD dedicated to the structural analysis of membrane proteins by electron crystallography from the University of Grenoble (1975-80). A.R. Brisson held successively academic positions as scientist at the Grenoble Nuclear Energy Center (80-87), visiting scientist at Stanford University (82-84), research director at INSERM in Strasbourg (87-94), Professor of Chemistry at the University of Groningen (94-01), group leader at the European Institute in Chemistry and Biology in Bordeaux (2001-11) and Professor of Biochemistry at the University of Bordeaux (2001-2017). He is currently Emeritus Professor at the University of Bordeaux in CNRS unit CBMN. He was president of the French Society for Microscopies from 2004 to 2006 and was nominated senior member of the Institut Universitaire de France from 2011 to 2016.

The central focus of his research has been membrane biology, with the objective of elucidating biological processes occurring at cell membranes and the relationship between structure and function of protein-membrane complexes, mainly by imaging methods (cryo-electron microscopy, atomic force microscopy) and physico-chemical methods (Q-CMD, flow cytometry). Since a decade, his research focuses on the field of
extracellular vesicles and exosomes, with the objectives to determine their structure, phenotype and concentration in health and disease situations, to identify disease-specific EV signatures and develop standardized methods of EV quantification and purification. His group has major expertise in cryo-electron microscopy, immuno-gold labeling, flow cytometry and protein-conjugated nanoparticle synthesis.

**Emilio Casanova, Medical University of Vienna, AT**

Emilio Casanova is a professor at the Medical University of Vienna since 2014. After completing his PhD in molecular biology at the University of Leon, Spain in 1997 he performed two post-doctoral trainings in Heidelberg and Basel where he became an expert in transgenic mouse models. In 2006 he joined the Ludwig Boltzmann Institute for Cancer Research in Vienna as group leader focusing on the use of transgenic mouse models to study tumorigenesis. His actual research interests are 1) generating genetically modified mouse models to understand KRAS driven lung adenocarcinoma and 2) developing of euchromatin-containing expression vectors to produce recombinant proteins on mammalian cells. He is an editor of a book in Methods of Molecular Biology about “Mouse Models of Cancer”.

**Thibaud Coradin, Sorbonne Université - CNRS, FR**

Thibaud Coradin obtained his PhD in Materials Science at Université Paris 11 in 1997. He was a post-doc in the Royal Institution of Great Britain (London) and Temporary Assistant Lecturer in Ecole Nationale Supérieure de Chimie Paris. He was appointed as a CNRS research fellow in 1999, in the Laboratoire de Chimie de la Matière Condensée de Paris where he developed biomimetic approaches to cell encapsulation in inorganic and then in collagen-based materials. He was promoted Research Director in 2007 and funded the “Materials and Biology” team in 2009, gathering chemists, biologists and physicists to study cell-materials interactions and their application in biofunctional materials design. He published 180 papers, co-edited 2 books related to bio-nanoscience, deposited 3 patent applications and gave 30 invited talks in international conferences.

**Paul Cos, University of Antwerp, BE**

Paul Cos was born in Turnhout (Belgium) in 1970. In 2001, he became a Doctor in Pharmaceutical Sciences at the University of Antwerp, Belgium. After a post-doc fellowship at the Research Fund (FWO) - Flanders, he was appointed associate professor in 2009 and professor in 2014 at the University of Antwerp. He is one of the directors of LMPH, which is a research unit at the University of Antwerp with more than 30 staff members (website: https://www.uantwerpen.be/en/rg/lmph/).

His main research interests are macrophage-pathogen interactions, with a focus on bacterial biofilms and oxidative stress. Paul Cos has an extensive expertise in the management of multi-partner research projects, including FWO, IWT-SBO (SBO-Resist), GOA-BOF and EU-FP7 projects (ITN Print-AID and ITN DED3). He is a board member of the Belgian Society of Microbiology (BSM) and BSM delegate for the Federation of European Microbiological Societies (FEMS).
Richard Daneman, UCSD, US

Richard Daneman received his Bachelor of Science from McGill University, in Montreal Canada majoring in biochemistry. He then received his Ph.D in developmental biology from Stanford University where he studied the molecular mechanisms that regulate blood-brain barrier formation in the laboratory of Dr. Ben Barres. Dr. Daneman then started his own lab as a Sandler Fellow at UCSF, before moving to a position as Assistant Professor in the departments of Pharmacology and Neuroscience at the University of California, San Diego. Dr. Daneman focuses his studies on understanding the molecular mechanisms that regulate blood-brain barrier (BBB) function during health and disease. He has won a number of awards including the Rita Allen Foundation Milton E. Cassel Scholar Award, the Klingenstein-Simons Fellowship Award in Neuroscience, the ASPET Neuropharmacology Early Career Award and the American Association of Anatomists Young Investigator Award. His lab uses a combination of cellular, molecular and genetic approaches to understand the mechanisms of BBB formation and function, addressing important questions such as: What are the molecules in CNS vascular cells that form the BBB? What are the signaling mechanisms that regulate the formation of the BBB during development, and dynamic function throughout life? What are the molecular mechanisms that lead to BBB disruption during neurological disease? The overall goal of his work is to elucidate these mechanisms, such that we will be able to develop therapeutics to modulate the barrier to treat neurological diseases.

Arnold Driessen, University of Groningen, NL

Arnold Driessen is Full Professor in Molecular Microbiology at the University of Groningen (NL) and member of the Kluyver Center of Genomics of Industrial Fermentation. He obtained his degree in biology at the University of Groningen in 1983. In 1987, he received his PhD with cum laude. In 1990, after postdoctoral research at UCLA, USA, he returned to the Netherlands and since then leads a research group that amongst others studies antibiotic and natural production formation in filamentous fungi. Driessen is flagship leader in BE-Basic, an international public-private partnership in the field of sustainable chemistry and ecology, funded by the Dutch government. Driessen is member of the Royal Dutch Academy of Arts and Sciences.

Christian R. Eckmann, Martin Luther University Halle-Wittenberg, DE

Christian Eckmann obtained a diploma in Molecular Genetics at the University of Vienna in 1995. Supported by a DOC stipend from the Austrian Academy of Sciences, he elucidated in his PhD thesis the molecular basis of adenosine-deaminase-mediated mRNA editing and graduated from the same University in 1998. As a postdoc, he joined the lab of Judith Kimble, a Howard Hughes Medical Investigator (HHMI) at the University of Wisconsin-Madison, to follow his interests in developmental RNA regulation in germ cells and to be trained in the genetic animal model system, C. elegans. After co-discovering the unique family of regulated cytoplasmic polyA polymerases, he joined the newly established MPI of Molecular Cell Biology and Genetics (Dresden) in 2004. Initially as a junior and later as a senior independent research group leader, he establish a broad research program on post-transcriptional RNA regulatory networks, developmental mRNP dynamics, and germ cell-specific RNA granule biology. In 2014, he got accepted into the Heisenberg Program of the German Research Council (DFG) and moved to the Martin Luther University of Halle-Wittenberg, where he became professor for Developmental Genetics in 2016. His long-standing research interests unite the two worlds of RNA and Germ Cell Biology. While specifically working on the molecular functions of RNA-modifying enzymes and RNA-binding proteins, he recently developed also a strong interest in the post-translational control mechanism of RNA regulators during germline specification and formation. His research philosophy is motivated by a strong commitment to interdisciplinary approaches, for which he also received funding from the Human Frontiers Science Project, as a Long-term Stipend holder and Program Grant awardee.
Alexander Harms, University of Copenhagen, DK

Dr. Alexander Harms is a microbiologist studying the molecular mechanisms that underly the failure of antibiotics to kill some bacterial cells despite their genetic susceptibility to the treatment. He received his PhD degree at the University of Basel (Switzerland) for work on evolutionary links between bacterial stress response loci known as toxin-antitoxin (TA) modules and host-targeted effector proteins of bacterial pathogens. Subsequently, he joined Prof. Kenn Gerdes’ group at the University of Copenhagen (Denmark) that was renowned for studying the role of TA modules in the formation of bacterial persisters. These dormant cells are hallmarkmed by their tolerance to antibiotic treatment and considered to be main culprits behind the recalcitrance of chronic or relapsing bacterial infections. Dr. Harms uncovered that the common view on *Escherichia coli* persister cell formation as being a phenomenon driven by TA module activation is wrong and he unraveled how a number of biological and technical artefacts have promoted this misconception in previous studies. Consequently, his current work is taking a new view on the molecular basis of bacterial antibiotic tolerance.

Stefan Jansson, Umeå university, SE

Dr. Stefan Jansson is Professor and Head of the Department of Plant Physiology at Umeå University, Sweden, a part of Umeå Plant Science Centre. He got his PhD in plant cell- and molecular biology at Umeå University in 1992 and has in his career focused first and foremost on basic research of photosynthesis light harvesting, but since 2001 also on genetic, genomics and natural variation in aspen, in particular in relation to phenology. He was also leading the project to sequence the first conifer genome (Norway spruce). In the last years, he has been much involved in the debate on GMOS, in particular in relation to novel plant breeding techniques like CRISPR. He is a member of Royal Swedish Academy of Sciences and the Royal Swedish Academy of Engineering Sciences and president of Scandinavian Plant Physiology Society (SPPS).

Roman Jerala, National institute of chemistry, SI

Roman Jerala is head of the Department of synthetic biology and immunology at the National institute of chemistry in Ljubljana, Slovenia. He received his PhD at the University of Ljubljana and postdoctoral training at the University of Virginia. Since 1996 he has been at the National institute of chemistry, initially working on the NMR of proteins and later switching towards signaling in innate immunity and synthetic biology, which are currently two most important research topics in his group. Research in the field of innate immunity has been primarily dedicated to the investigation of the molecular mechanisms of signaling of Toll-like receptors using combination of molecular modeling, molecular biology and immunology with implications and applications for vaccines and cancer. The main areas of synthetic biology in his group include i) design of bionanostructures, ii) engineering of mammalian cell signaling, iii) information processing by mammalian cells and iv) scaffolding of the biosynthetic pathways. His group demonstrated single-layer logic NOR gates based on TALE-KRAB repressors that allowed construction of logic gates in mammalian cells and orthogonal bistable genetic switches and designed cell logic was also used for the design of anti-inflammatory therapeutic cellular device. One of his most original achievement was the invention of a new principle for the construction of protein folds, called “coiled-coil protein origami” (CCPO), based on the concatenated modular building blocks, which is also the topic of his recent ERC Advanced Grant project MaCChines. He is member of the editorial board of ACS Synthetic Biology, Journal of Biological Chemistry and Innate immunity. He has been elected as an EMBO member and member of the Academia Europaea and has been known in the synthetic biology community as a mentor of successful iGEM student research projects.
Somanath Kallolimath, Universität für Bodenkultur, AT

Dr. Somanath Kallolimath is a postdoctoral researcher in the Department of Applied Genetics and Cell biology at University of Natural Resources and Life Sciences, Vienna (BOKU). After completion of his M.Sc Engineering in Medical and Pharmaceutical Biotechnology from IMC University of Applied Sciences, Krems, Austria. He joined Biomolecular Technology of Proteins (BioToP), an International Ph.D. program to pursue his research career at BOKU, Vienna.

During his Ph.D. research, “Production of recombinant protein with polysialylated N-glycans in Nicotiana benthamiana” he has developed a plant-based expression platform for the production of polysialylated N-glycans. His work is published in the renowned scientific journal PNAS, titled "Engineering of complex protein sialylation in plants". He holds a patent for his work on Production of polysialylated polypeptides in plants. He has presented his research work in several national and international conferences. His current research interests are focused on 1) Production of recombinant proteins in Nicotiana benthamiana 2) In planta glycoengineering 3) Synthesis of polysialylated recombinant glycoproteins in plants.

Annemarie Käsbohrer, University of Veterinary Medicine, Vienna, AT

Annemarie Käsbohrer is veterinarian with specialisation in microbiology and epidemiology. Since 2006, she is Head of Unit for Epidemiology, Zoonoses and Antimicrobial resistance at the Federal Institute for Risk Assessment. In addition, since April 2016 she is professor and head of institute of Veterinary Public Health at the Veterinary University in Vienna. She has very much expertise in the areas monitoring and surveillance of zoonotic pathogens in the food chain; collection and analysis of data related to risk factors for zoonotic agents and antimicrobial resistance and the impact of risk factors on the spread of these; she was involved in the development and validation of epidemiological models and exposure assessment of zoonotic pathogens and antimicrobial resistance.

Christopher Landowski, VTT Technical Research Centre of Finland Ltd., FI

Christopher Landowski is the Research Team Leader of the Protein Production Team at VTT Technical Research Institute of Finland. His research expertise covers metabolism of anticancer prodrugs, membrane transport proteins for nutrients and peptidomimetics, and recombinant protein production in industrial microbes. He earned his Ph.D. in Pharmaceutical Sciences from the University of Michigan (USA) in 2005. He has done postdoctoral work in Harvard Medical School (USA) and University of Bern Medical School (CH). At VTT his research work is aimed at understanding secretion related processes in filamentous fungi and applying this knowledge to create improved commercial production strains. He is currently Principal Investigator on an Academy of Finland funded research project to study sugar sensing and enzyme secretion in filamentous fungi (2016-2020).
Alexander Loy, University of Vienna, AT

Alexander Loy is Professor for Microbial Communities at the Department of Microbiology and Ecosystem Science (University of Vienna, Austria), managing director of the Austrian Microbiome Initiative (AMICI), and faculty member of the Austrian Polar Research Institute (APRI). Research of the Loy group focuses on ecology and evolution of sulfur microorganisms, the function of the complex symbiotic microbiota of animals and humans in health and disease, and the development of molecular and isotope-labeling methods for studying uncultivated microorganisms in their natural environment.

Alexander Loy received his PhD in Microbiology in 2003 at the Technical University of Munich in Germany. In the same year, he was awarded a Marie Curie postdoctoral fellowship to join the newly founded Department of Microbial Ecology at the University of Vienna in Austria, where he established his own research group in 2006 based on third-party grants and was Assistant Professor (2009-2013) and Associate Professor (2013-2017). He obtained his Habilitation (venia docendi) in Microbiology and received the Young Scientist Award of the City of Vienna in 2012. He has published 74 peer-reviewed papers with a total impact factor of 506 and more than 7,900 citations (Google Scholar H-index of 41).

Matthias Muhar, Research Institute for Molecular Pathology, AT

Matthias Muhar completed his master’s degree in molecular biology at the University of Vienna in 2014 prior to joining the PhD program of the Vienna BioCenter (VBC). As a doctoral fellow of the Austrian Academy of Sciences he performs his graduate research under the supervision of Johannes Zuber at the Research Institute of Molecular Pathology (IMP), Vienna. His doctoral work aims at deciphering mechanisms of gene regulation in cancer, with a particular focus on leukemia. To this end, he employs advanced deep sequencing methods to probe cellular responses to established and emerging cancer therapeutics as well as chemical-genetic manipulation of essential transcriptional regulators. By these means he addressed the impact of BET bromodomain inhibitors (BETi) on leukemic gene expression, contributions of their molecular target BRD4 as well as regulatory functions of the transcription factor and common human oncogene, MYC.

Roman Necina, Shire, AT

Roman is heading the Process Science and Technical Services team at Shire. In his current role he is responsible for all process, assay and formulation development activities across all modalities, medical device development, QbD and technical support for GMP products across the internal and external manufacturing network. Roman has 25 years experience in development and launch of biopharmaceuticals and has served as VP Biopharmaceutical Production & Process Science for Boehringer Ingelheim, SVP Quality & Regulatory Compliance and as VP Technical Operations for Intercell. Roman graduated at the University for Agriculture in Vienna, Austria and holds a Ph.D. in biotechnology.
Liam O'Mahony, University College Cork, IE

Prof. Liam O’Mahony received his BSc in Microbiology from University College Cork, Ireland in 1994 and his PhD in Immunology was awarded in 1998 by Trinity College Dublin, Ireland. Thereafter, Dr. O’Mahony performed post-doctoral research at the Department of Microbiology, University College Cork, Department of Medicine, University College Cork and the Digestive Diseases Division, UCLA. Dr. O’Mahony was a Principal Investigator at the Alimentary Pharmabiotic Centre, University College Cork, from 2003 to 2008. From 2009 to 2018, he was head of Molecular Immunology at the Swiss Institute of Allergy and Asthma Research, University of Zürich, Switzerland. He is currently the Prof. of Immunology at the Departments of Medicine and Microbiology, APC Microbiome Ireland, National University of Ireland, Cork, Ireland. His research interests are focused on the molecular basis for microbe and metabolite modulation of mucosal inflammatory responses.

Stefan Rose-John, University of Kiel, DE

Stefan Rose-John studied at the University of Heidelberg Biology with the subsidiary subjects Chemistry and Physics. After receiving his doctorate, Stefan Rose-John worked as a postdoc at the Michigan State University in the USA. Thereafter, Stefan Rose-John became a junior group leader at the German Cancer Research Center in Heidelberg, Germany. After moving to the RWTH Aachen, Stefan Rose-John was habilitated in Biochemistry. He became Associate Professor of Pathophysiology at the University of Mainz. In 2000, he moved to the University of Kiel where he became full Professor and Director of the Institute of Biochemistry.

Since many years, his laboratory is focused on understanding the molecular biology of cytokines. A major aspect of his work has started with the discovery of a naturally occurring proteolytic cleavage of the Interleukin-6 receptor, which leads to the generation of a soluble Interleukin-6 receptor. He has discovered that the complex of soluble Interleukin-6-receptor and Interleukin-6 stimulates cells, which express the signal transducing receptor subunit gp130 but not the ligand binding subunit Interleukin-6 receptor. In the absence of the sIL-6R such cells do not respond to IL-6. He has called this process ‘trans-signaling’ and he has shown in the past years that ‘trans-signaling’ has a prominent role in inflammation, neuronal survival, hematopoiesis, and tumor defense. He is currently developing the concept that IL-6 ‘trans-signaling’ is an emergency reaction of the human immune system and that disruption of ‘trans-signaling’ can be used therapeutically for the treatment of chronic inflammatory diseases and cancer. Indeed, he generated a specific IL-6 ‘trans-signaling’ antagonist, which – in animal models – has proven effective in blocking chronic inflammatory diseases such as Crohn’s diseases, Rheumatoid Arthritis and inflammatory colon cancer, is now being tested in Phase II clinic trials in patients with inflammatory bowel disease.

Joshua Rosenthal, Marine Biological Laboratory/ Univ of Chicago, US

Joshua Rosenthal is a Senior Scientist at the Marine Biological Laboratory in Woods Hole, Massachusetts. He received his Ph.D. in Biology from Stanford University and completed his postdoctoral training in biophysics and physiology at UCLA. Before coming to the Marine Biological Laboratory, he rose from Assistant to Full Professor at the University of Puerto Rico’s Medical Sciences Campus. Dr. Rosenthal’s research focuses on the process of RNA editing from a variety of angles. His group has shown that mRNA recoding is unusually frequent in cephalopods. They are interested in what it’s being used for and how the underlying machinery for RNA editing differs in this taxon. Other projects aim to use RNA editing as a vehicle for therapeutics.
John W.A. Rossen, University of Groningen, University Medical Center Groningen, NL

Prof. dr. John W. A. Rossen has an almost 30-year history in molecular virology/microbiology and more than 144 peer reviewed publications (Google Scholar H-index 33; Scopus H-index 27). He is tenure track Professor at the University of Groningen and head of the molecular unit which has recently implemented the use of next generation sequencing for routine clinical microbiology and infection prevention. The method is used to determine the genetic relationship between pathogens (used to guide infection prevention measures) and for the molecular detection and further characterization of (emerging) pathogens. This includes analyses for revealing (new) antibiotic resistance mechanisms and for determining the virulence of pathogens resulting in improved risk assessment and infection prevention. In addition, based on comparing whole genomes of bacteria, tailor-made diagnostic tests are developed used for specific detection of outbreak and or virulent bacterial strains. Nowadays his research is focused on implementing metagenomics into clinical microbiology. In his research group “Personalised Microbiology” several PhD students, Post-Docs and technicians work together to investigate not only patient samples but also samples taken from animals, food and water - thereby realizing the one health principle in microbiology. Currently, Prof. dr. Rossen is involved in the supervision of 9 PhD students. He is secretary of the ESCMID study group for genomic and molecular diagnostics (ESGMD) and treasurer of the Dutch Society of Medical Microbiology.


Ana Ruiz-Saenz, UCSF, US

Dr. Ana Ruiz-Saenz is a senior scientist at the University of California San Francisco (UCSF). She obtained a degree in Biochemistry and a Ph.D in Molecular Biology from the University Autonoma of Madrid. In 2013, she was awarded with a Ramon Areces postdoctoral fellowship and she joined the laboratory of Dr. Moasser at UCSF where she has focused her research on the progression and resistance to therapies of HER2-amplified breast cancers. In an effort to explore novel therapeutically approaches, she found a novel mechanism to target the currently undruggable HER3 and explored the potential of HER2 to overcome the requirement of HER3 in HER2-amplified cancers. In collaboration with scientists at the University of California San Diego and The Netherlands Cancer Institute (NKI) she is also studying the role of Src in therapeutic resistance of BRAF(V600E) colorectal tumors. She is actively involved in Science Communication initiatives, mentoring and has participated in numerous international conferences.

Marina Santic, Medical faculty, University of Rijeka, HR

Dr. M. Santic is full professor at the Department of Microbiology, Medical Faculty, University of Rijeka. She is running several projects related to the research of intracellular pathogens and Host pathogen interaction with special focus on F. tularensis and L. pneumophila. M. Santic (maiden name Radulic) has published more than 40 papers in high impact journal including “Science” and “Journal of Experimental Medicine” in addition to the book chapters. She frequently reviews proposals for national and international programs, institutes and papers. M. Santic has been awarded Best Scientist in Croatia, 2007, Best Scientist at the University of Rijeka two years in a row, 2005 and 2006 and Best Scientist of Primorska-goranska County 2010.
Invited Speakers

Kerstin Schipper, Heinrich Heine University Düsseldorf, DE

Kerstin Schipper obtained her Ph.D. in the field of plant pathology in 2009 from Philipps University Marburg. She conducted her doctoral project under the supervision of Prof. Regine Kahmann at the Max Planck Institute for Terrestrial Microbiology. In her work she investigated the interaction of the corn smut fungus *Ustilago maydis* with its host plant maize on the level of secreted fungal effectors. Since then, protein secretion is in the center of her research interest. After a short postdoctoral time at the MPI in Marburg, she moved on to the Heinrich Heine University Düsseldorf where she started her own group in 2011. Sticking with the model fungus *Ustilago maydis*, she now dedicates her work to more applied aspects. One major focus is the dissection of a novel unconventional secretion pathway and its potential applications for heterologous protein production.

Marc Stadler, Helmholtz-Zentrum für Infektionsforschung (HZI), DE

Marc Stadler studied biology in Kaiserslautern and received his PhD in 1993 on the subject of new antibiotics and nematicides from predacious fungi. His PhD project was supported by a grant form the German National Scholarship Foundation. After a DFG-funded post-doctoral stay at University of Lund/Sweden in natural product chemistry, he joined the pharmaceutical industry in 1995 and worked in the natural products department of Bayer Healthcare (Pharma Division). Together with other Bayer researchers, he later co-founded the company InterMed Discovery GmbH in 2006 as an MBO, where he worked as Department Head until April 2012. During his industrial career (17 years in total) he was responsible for the fungal and microbial culture collections, the fermentation and biotechnological process development, as well as a natural product chemical laboratory. Concurrently, he was teaching at University of Bayreuth, where he completed his habilitation in 2009 and received the venia legendi in Mycology. He is a globally recognized expert in industrial microbiology and mycology, as well as fungal biodiversity research. He also acts on the Editorial Boards of leading mycological journals such as *Studies in Mycology* and *Fungal Diversity*, was appointed in 2016 as Editor-in-Chief of *Mycological Progress*, and is Acting Vice President of the International Mycological Association (IMA). He took over his current position at HZI in 2012 and is teaching biology and biotechnology at Technical University of Braunschweig. He has coauthored over 250 original papers, reviews and patents.

Dora Clara Tărlungeanu, IST Austria, AT

Dora Clara Tărlungeanu completed a BSc and an MSc in Pharmacy at the University of Medicine and Pharmacy “Iuliu Hatieganu” in Cluj-Napoca (Romania) before joining IST Austria in 2013. Her main research interests include understanding the role of genetics in the onset of complex diseases, such as autism spectrum disorder, and contributing to the development of individualized therapies for such conditions. She worked on the research project “The Branched Chain Amino Acids in Autism Spectrum Disorders” at IST Austria, and has published the results in the prestigious and leading biology journal *Cell*. During her PhD studies, Dora presented her research results at several international conferences and earned an award for the best poster at the BioParadigms conference in Lausanne, Switzerland in 2017. Besides her scientific research interests, she was a leading member of the organizing committees for IST Austria’s Young Scientist Symposium 2016 and the Science Industry Day 2017. She is a very motivated and dedicated scientist who is genuinely interested in tackling medical challenges and in using her skills to develop innovative medical treatments as part of her future career.
Henning Walczak, UCL Cancer Institute, GB

Professor Henning Walczak is Head of the Department of Cancer Biology at the Cancer Institute of University College London (UCL), Chair of the Centre for Cell Death, Cancer and Inflammation (CCCI) and Scientific Director of the Cancer Research UK - UCL Centre. Following his PhD in 1995 at the German Cancer Research Centre (DKFZ, Heidelberg, Germany), he performed postdoctoral research at Immunex Corporation in Seattle (WA, USA). After returning to Europe in 1998, he became group leader at the DKFZ in 2000 following receipt of a BioFuture Prize awarded by the German Ministry for Science and Education. In October 2007 he was appointed as Chair of Tumour Immunology at Imperial College London, and joined UCL in January 2013 to assume his current position. In 2012 Professor Walczak was awarded an ERC Advanced Grant and received a Wellcome Trust Senior Investigator Award. His research on cancer has been funded through Programme grants from Cancer Research UK since 2008. Professor Walczak’s research focuses on cell death and ubiquitin in inflammation, cancer and auto-immunity. He is particularly interested in unravelling the mechanisms how different death receptor-ligand systems such as the TNF and TRAIL systems are regulated and how they impact cancer cell survival and cancer-related inflammation. His research aims at developing novel cancer therapies on the basis of specifically inducing cancer cell death and by therapeutically directing the type of death induced in cancer cells to convert cancer-related inflammation from being immuno-regulatory to enabling the immune system to recognise and kill cancer cells.

Wolfram Weckwerth, university of Vienna, AT

Wolfram Weckwerth integrates system-theoretical ideas with genome-scale molecular analysis using genomics, transcriptomics, proteomics, phosphoproteomics and metabolomics to understand and predict the genotype - phenotype-relationship. He investigates plant, microbial, animal and human systems. Working in the field of metabolomics since 2000, Wolfram Weckwerth has established metabolomics, proteomics and phosphoproteomics platforms in Germany and Austria. In 2008 he moved as a full professor to the University of Vienna and founded the Department of Molecular Systems Biology (MOSYS). Since 2013 he is Head of the newly founded Department of Ecogenomics and Systems Biology comprising a full PANOMICS platform. Since 2015 Wolfram Weckwerth is founding chair of the Vienna Metabolomics Center (VIME) (https://vime.at/). VIME has a focus on biomedical research questions and algorithms for data processing and integration, biochemical interpretation, structural elucidation of unknown metabolites as well as metabolomics/life sciences databases. Wolfram Weckwerth published more than 180 publications on systems biology applications, multiomics analysis and data-driven inverse modelling, edited several books and is special chief editor of Frontiers Metabolomics.

Therese Wohlschlager, University of Salzburg, AT

Therese Wohlschlager is a postdoctoral researcher in the Christian Doppler Laboratory (CDL) for Biosimilar Characterization that was established at the University of Salzburg in 2013. During her studies of biotechnology at BOKU Vienna, Therese performed her diploma thesis at the Institute for Glycomics, Griffith University (Australia) where she gained experience in fungal glycosylation and glycan analysis. Therese then received a PhD from ETH Zurich (Switzerland) where she studied lectin-glycan interactions in fungal defense. Within the CDL for Biosimilar Characterization Therese engages in the development of analytical tools for the characterization of glycosylated biopharmaceuticals employing high-performance liquid chromatography (HPLC) and mass spectrometry (MS). Her research focuses on intact protein and protein subunit characterization in order to study glycosylation and other post-translational modifications in their molecular context. The translational aspect of Therese’s research is reflected by a long-standing collaboration of the CDL for Biosimilar Characterization with Novartis (Kundl, Austria) and Thermo Fisher Scientific (Waltham, MA, USA).
Peter Zilla, Univ. of Cape Town, ZA

After graduating as “Doctor of Medicine” at the University of Vienna in 1980 he obtained a DrMed. degree from the University of Zurich, a “Habilitation” from the University of Vienna and a PhD degree from the University of Cape Town. He is a registered general-, vascular- and cardiothoracic surgeon.

After he spent his initial three post-graduate years in basic science he commenced his surgical career with his residency at the University Hospital Vienna from 1983 to 1989. His subsequent surgical positions were as Senior Resident at the Department of Cardiovascular Surgery, University Hospital Zurich (1989-1990) followed by staff surgeon positions in Austria and Cape Town where he has been Head of the Chris Barnard Department of Cardiothoracic Surgery for the past two decades.

Pioneering tissue engineering since 1983, his group developed a method of culturing the patient’s own endothelial cells on prosthetic surfaces. Addressing the need for ‘home grown’ solutions for patients in developing countries who have no access to cardiac surgery, Professor Zilla founded a University of Cape Town Start-Up Company in 2008.

He is author of >200 peer reviewed full papers and patents (104 times first author or corresponding author) with almost 10,000 citations and an H-index of 45. Apart from >40 filed or issued US/PCT patents he is the editor of 5 books and has authored numerous book chapters. He obtained international academic and industry grants of >14 million Euros and secured 20mio Euros for his University start-up company since 2008. For his research he has received several prestigious awards such as the Alexis Carrel and the Alain Carpentier Award. He was the organizer of 6 major international conferences in 4 different countries; is a member and executive council member of 10 international societies; was president of ISACB from 1994-98 and is on the editorial board of major international journals and was Associate Editor of ‘Biomaterials’ (IF 8).
Rent Scientific Equipment
Benefit from Scientific Services

Equipment

- Use state-of-the-art devices
- Pay-by-use
- Save investments & maintenance costs
- Stay financially flexible

Services

- Benefit from simple advice only or full service solutions
- Enjoy attention to your needs
- Profit from world renown expertise
- Save time & money

Interested? Questions? Contact Us!

http://eq-vibt.boku.ac.at/

Partner to Academia & Industry
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:50 - 10:00</td>
<td>Opening</td>
<td>Welcome by Thomas Stockner</td>
<td>LH B</td>
</tr>
<tr>
<td>10:00 - 12:00</td>
<td>Session 1</td>
<td>Structure and Simulations</td>
<td>Chair: Thomas Stockner</td>
</tr>
<tr>
<td>10:00 - 10:45</td>
<td>Keynote</td>
<td>Christine Ziegler (Universität Regensburg, DE)</td>
<td>How lipids regulate membrane transport proteins</td>
</tr>
<tr>
<td>10:45 - 11:00</td>
<td></td>
<td>Denis Knyazev (Johannes Kepler University, AT)</td>
<td>Partition coefficient of arginine between translocon interior and lipid phase</td>
</tr>
<tr>
<td>11:00 - 11:15</td>
<td></td>
<td>Jan Walther Perthold (University of Natural Resources and Life Sciences, Vienna, AT)</td>
<td>Automated Free Energy Calculation for Drug Design: Accelerated Enveloping Distribution Sampling</td>
</tr>
<tr>
<td>11:15 - 11:30</td>
<td></td>
<td>Dániel Szöllösi (Medical University of Vienna, AT)</td>
<td>ABCB1 nucleotide binding domain dimerization cycle</td>
</tr>
<tr>
<td>11:30 - 11:45</td>
<td></td>
<td>Jozef Hritz (University of Natural Resources and Life Sciences, AT)</td>
<td>Protein homodimerization from perspective of structural biology and biophysics. Case study of 14-3-3ζ protein.</td>
</tr>
<tr>
<td>11:45 - 12:00</td>
<td></td>
<td>Andreas Horner (Johannes Kepler University, AT)</td>
<td>Single-file transport of water through membrane channels</td>
</tr>
<tr>
<td>12:00 - 13:00</td>
<td>Lunch / Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00 - 14:00</td>
<td></td>
<td>Biophysics Austria Generalversammlung</td>
<td>LH B</td>
</tr>
<tr>
<td>14:00 - 15:45</td>
<td>Session 2</td>
<td>Biophysical Methods</td>
<td>Chair: Birgit Plochberger</td>
</tr>
<tr>
<td>14:00 - 14:30</td>
<td>Keynote</td>
<td>Gerhard Schütz (TU Wien, AT)</td>
<td>Superresolution Microscopy Images: What they tell us about protein clusters – and what they don’t</td>
</tr>
<tr>
<td>14:30 - 14:45</td>
<td></td>
<td>Magdalena Schneider (TU Wien, AT)</td>
<td>Overcoming blinking artifacts in nanocluster detection with two-color STORM</td>
</tr>
<tr>
<td>14:45 - 15:00</td>
<td></td>
<td>Enrico F. Semeraro (University of Graz, AT)</td>
<td>Time-Resolved X-ray Studies of Antimicrobial Peptide Activity in Live E. coli on the Nano- to Micrometer Scales</td>
</tr>
<tr>
<td>15:00 - 15:15</td>
<td></td>
<td>Jose L. Toca-Herrera (BOKU, AT)</td>
<td>Atomic Force Microscopy as imaging and mechanical device</td>
</tr>
<tr>
<td>15:15 - 15:30</td>
<td></td>
<td>Hannah Seferovic (Johannes Kepler University Linz, AT)</td>
<td>The all-electric AFM allows imaging of biological samples in opaque liquids</td>
</tr>
<tr>
<td>15:30 - 15:45</td>
<td></td>
<td>Ingo Ohlenschläger (Nikon Cee GmbH, AT)</td>
<td>Nikon's all-new inverted microscope platform for advanced imaging. See more than before!</td>
</tr>
<tr>
<td>15:45 - 16:15</td>
<td></td>
<td>Coffee Break</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session 3</td>
<td>Membranes and Signalling</td>
<td>Chair: Elena Pohl</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>--------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>16:15 - 16:45</td>
<td>Keynote</td>
<td>Georg Pabst (University of Graz, AT)</td>
<td>Structure and Interleaflet Coupling in Asymmetric Lipid Membranes</td>
</tr>
<tr>
<td>16:45 - 17:00</td>
<td>Lisa Marx (University of Graz, AT)</td>
<td>X-Ray Studies of Antimicrobial Peptide Activity in <em>E. coli</em> Inner and Outer Membrane Mimics</td>
<td></td>
</tr>
<tr>
<td>17:00 - 17:15</td>
<td>Olga Jovanovic (Veterinärmedizinische Universität Wien, 1210 Wien, AT)</td>
<td>Uncoupling effect of 2,4 dinitrophenol strongly depends on the membrane lipid composition</td>
<td></td>
</tr>
<tr>
<td>17:15 - 17:30</td>
<td>Jürgen Kreiter (University of Veterinary Medicine, AT)</td>
<td>Electrophysiological characterization of adenine nucleotide translocase (ANT) – mediated proton leak in mitochondria</td>
<td></td>
</tr>
<tr>
<td>17:30 - 17:45</td>
<td>Rainer Schindl (Medical University of Graz, AT)</td>
<td>Calcium and calmodulin regulation of autophagy transcription factors</td>
<td></td>
</tr>
<tr>
<td>18:00 - 19:00</td>
<td>Poster</td>
<td>Poster Session Biophysics</td>
<td>Aula</td>
</tr>
</tbody>
</table>
### Monday, September 17th, 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Location</th>
</tr>
</thead>
</table>
| 10:00 - 13:00 | **YLSA Career Session**  
*Moderation: Judith Frei & Elisabeth Fitz*                                                    | LH C2    |
| 10:00 - 10:30 | **ISG Personalmanagement International:**  
Which skills do you need for your first job? What are typical entry positions?   |          |
| 10:30 – 10:45 | **All about YLSA, Introduction to our activities**                                                 |          |
| 10:45 – 13:00 | **Career Talks and Speakers Corner**  
Claudia Schandl (Patent Attorney Trainee @ REDL Patent Attorneys)  
Daniel Kiesenhofe (Project Manager @ Ecoduna)  
Udochuku Richson (Consulting @ FFG)  
Silvia Herold (Editor @ Springer Nature)  
Alfons Felice (CTO @ Directsens)                                                    |          |
| 12:00 - 13:00 | **Lunch / Break /Science Art Exhibition**                                                          |          |
| 13:00 - 13:20 | **Opening Ceremony**  
*Michael Sauer, chair organizer*                                                                | LH C1    |
| 13:20 - 14:00 | **Opening Plenary**  
Joshua Rosenthal (Marine Biological Laboratory/ Univ of Chicago, US)  
High-level RNA editing in the behaviorally complex cephalopods.  
*Chair: Michael F. Jantsch*                                                           | LH C1    |
| 14:15 - 15:45 | **Track 1 Plant biotechnology**  
*Chair: Margit Laimer*                                                                             | LH A     |
| 14:15 - 14:45 | **Keynote**  
Stefan Jansson (Umeå university, SE)  
Genome editing using CRISPR/Cas9 in plants and the EU GMO legislation |          |
| 14:45 - 15:00 | Markus Freudhofmaier (University of Natural Resources and Life Sciences, Vienna, AT)  
Genetic improvement of the biofuel crop *Jatropha curcas* (L) by CRISPR/Cas9 mediated genome editing |          |
| 15:00 - 15:15 | Jennifer Schwestka (University of Natural Resources and Life Sciences, AT)  
Utilizing *in planta* zein protein bodies for oral vaccine applications |          |
| 15:15 - 15:30 | Souleymane Bado (University of Natural Resources and Life Sciences, AT)  
Mutation induction in * Coffea spp* to counteract the impact of a changing climate |          |
| 15:30 - 15:45 | Hansjörg Stampfli (AIT - Austrian Institute of Technology, AT)  
The GSK3 kinase ASKα contributes to early immune signaling and acclimation to environmental stress. |          |
| 14:15 - 15:45 | **Track 2 Neuroimmunology**  
*Chair: Sandra Siegert*                                                                               | LH C2    |
| 14:15 - 14:45 | **Keynote**  
Marco Prinz (University of Freiburg, DE)  
From yolk sac to neurodegeneration: the multiple facets of microglia |          |
| 14:45 - 15:00 | Alessandro Venturino (Institute of Science and Technology IST Austria, AT)  
Direct effects of general anesthesia on microglia |          |
| 15:00 - 15:15 | Carmen Hagen (Medizinische Universität Innsbruck, AT)  
On the day-to-day functional relations between interleukin-6 and mood, irritation and mental activity in a breast cancer survivor Running title: Dynamics between interleukin-6 and emotions |          |
| 15:15 - 15:30 | Maike Werning (Medical University of Vienna, MFPL, AT)  
A study on PanK2 enzyme pathology in erythrocytes from PKAN patients |          |
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
</table>
| 15:30 - 15:45 | Andrea Vogel (Institute for Vascular Biology and Thrombosis Research, AT)  
The Role of PI3K/PTEN in Microglia Functions during Homeostasis and Neuroinflammation |
| 14:15 - 14:54 | From RNAomics to function  
Track 3  
Chairs: Alexandra Lusser & Michael F. Jantsch  
LH C1 |
| 14:15 - 14:45 | Christian R. Eckmann (Martin Luther University Halle-Wittenberg, DE)  
C. elegans germ cell development: a GoLDmine to unearth post-transcriptional RNA control mechanisms |
| 14:45 - 15:00 | Maria Kalyna (University of Natural Resources and Life Sciences - BOKU, AT)  
Exitrons, alternatively spliced internal regions of protein-coding exons: from their discovery to pan-cancer profiling |
| 15:00 - 15:15 | Andrea Tanzer (University of Vienna, AT)  
From RNA structure to function - The RNA structureome |
| 15:15 - 15:30 | Clemens Heissenberger (University of Natural Resources and Life Sciences, AT)  
NSUN5 methylates 28S rRNA and modulates cell proliferation in humans and mice |
| 15:30 - 15:45 | Brigitte Pertschy (University of Graz, AT)  
Guardians of the ribosomal proteins - In search of dedicated chaperones and importins |
| 15:45 - 16:15 | Lunch / Break / Science Art Exhibition |
| 16:15 - 17:25 | Awards  
Life Science Awards Austria 2018 Ceremony  
Moderation: Lukas Huber  
LH C1 |
| 16:15 - 17:25 | Life Science PhD Award Austria 2018 – Basic Science  
Dora Clara Tarlungeanu (IST Austria, AT)  
The branched chain amino acids in autism spectrum disorders. |
| 16:15 - 17:25 | Life Science PhD Award Austria 2018 – Applied research  
Somanath Kalloolimath (Universität für Bodenkultur, AT)  
Production of recombinant protein with polysialylated N-glycans in Nicotiana benthamiana |
| 17:25 - 18:00 | Anniversary Ceremony  
Moderation: Angela Sessitsch  
LH C1 |
| 18:00 - 21:00 | 10 years ÖGMBT party |
**Tuesday, September 18th, 2018**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Title</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00 - 09:40</td>
<td>Plenary Lecture</td>
<td>Kerstin Schipper (Heinrich Heine University Düsseldorf, DE)</td>
<td>Unconventional secretion in the emerging fungal model <em>Ustilago maydis</em>: From discovery to application.</td>
<td>LH C1</td>
</tr>
<tr>
<td>09:40 - 10:20</td>
<td>Plenary Lecture</td>
<td>Richard Daneman (UCSD, US)</td>
<td>Regulation of the blood-brain barrier in health and disease</td>
<td>LH C1</td>
</tr>
<tr>
<td>10:20 - 10:35</td>
<td>Flash Talks</td>
<td>Katharina Novak (TU Wien, AT)</td>
<td>Overexpression of an acetylation-insensitive acetyl-CoA synthetase in <em>E. coli W</em> and its effect on glucose and acetate co-utilization in batch and continuous cultures</td>
<td>LH C1</td>
</tr>
<tr>
<td>10:22 - 10:24</td>
<td></td>
<td>Markus Schosserer (University of Natural Resources and Life Sciences, Vienna, AT)</td>
<td>Ribosomal RNA methylation by rram-1 modulates development and healthy lifespan</td>
<td></td>
</tr>
<tr>
<td>10:24 - 10:26</td>
<td></td>
<td>Felix Locker (University of Veterinary Medicine, AT)</td>
<td>The influence of ketogenic diet on psoriasiform-like skin inflammation</td>
<td></td>
</tr>
<tr>
<td>10:26 - 10:28</td>
<td></td>
<td>Razieh Karimi Aghcheh (Georg August University Göttingen, DE)</td>
<td>Insight to the mechanism of action of fungi fitness regulator</td>
<td></td>
</tr>
<tr>
<td>10:28 - 10:30</td>
<td></td>
<td>Norhan Mahfouz (Ares Genetics GmbH, AT)</td>
<td>Antimicrobial Resistance Markers in Molecular Diagnostics: Good Enough for the Clinic?</td>
<td></td>
</tr>
<tr>
<td>10:30 - 10:32</td>
<td></td>
<td>Florian Ehrlich (medical university of vienna, AT)</td>
<td>Molecular adaptations of epidermal barrier keratins in association with evolutionary land-to-water transitions of mammals</td>
<td></td>
</tr>
<tr>
<td>10:35 - 11:00</td>
<td></td>
<td>Coffee Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00 - 12:30</td>
<td>Track 1</td>
<td>Optimization of microbial workhorses for biotechnology</td>
<td><strong>Chairs: Monika Schmoll &amp; Anton Glieder</strong></td>
<td>LH C1</td>
</tr>
<tr>
<td>11:00 - 12:30</td>
<td>Keynote</td>
<td>Christopher Landowski (VTT Technical Research Centre of Finland Ltd., FI)</td>
<td>Tailoring <em>Trichoderma reesei</em> for optimal production of heterologous proteins</td>
<td>LH C1</td>
</tr>
<tr>
<td>11:30 - 11:45</td>
<td></td>
<td>David J. Wurm (TU Wien, AT)</td>
<td>The time has come: Introducing QbD into inclusion body refolding processes</td>
<td></td>
</tr>
<tr>
<td>11:45 - 12:00</td>
<td></td>
<td>Jürgen Zanghellini (University of Natural Resources and Life Sciences, Vienna, Austria, AT)</td>
<td>Designing production envelopes and yield spaces of cell factories</td>
<td></td>
</tr>
<tr>
<td>12:00 - 12:15</td>
<td></td>
<td>Jonas Ramoni (Ares Genetics GmbH, AT)</td>
<td>Optimization of the industrial workhorse <em>Trichoderma reesei</em> towards improved xylanase production</td>
<td></td>
</tr>
<tr>
<td>12:15 - 12:30</td>
<td></td>
<td>Sabrina Beier (Austrian Institute of Technology, AT)</td>
<td>Phosphodiesterases impact sexual development and the block of cellulase gene expression in light in <em>Trichoderma reesei</em></td>
<td></td>
</tr>
</tbody>
</table>
Detailed Program

11:00 - 12:30 Track 2  
**Next-generation pathogen & antibiotic resistance diagnostics**  
*Chairs: Andreas Posch & Dorothea Orth-Höller*  
**LH A**

11:00 - 11:30 **Keynote**  
**John W.A. Rossen** (University of Groningen, University Medical Center Groningen, NL)  
Next-generation sequencing for next generation clinical microbiology and infection prevention.

11:30 - 11:45 **Noa Wolff** (AIT Austrian Institute of Technology, AT)  
Culture-independent identification of pathogens and antibiotic resistance genes via a ligation-based microarray chip

11:45 - 12:00 **Matthias Pilecky** (Donau Universität Krems, AT)  
Influence of Antibiotic Pretreatment on Molecular Diagnostics in a Staphylococcus aureus Blood Stream Infection Model

12:00 - 12:15 **Stephan Beisken** (Ares Genetics GmbH, AT)  
Evaluation of clinical isolate *de novo* sequencing for pathogen & antibiotic resistance diagnostics using BGISEQ-500

12:15 - 12:30 **Stefan Howorka** (University College London, GB)  
Hand-held DNA-sequencing and biosensing with nanopores

11:00 - 12:30 Track 3  
**Biological barriers in health and disease**  
*Chairs: Eva Untersmayr-Elsenhuber & Winfried Neuhaus*  
**LH C2**

11:00 - 11:30 **Keynote**  
**Liam O'Mahony** (University College Cork, IE)  
Microbial modulation of mucosal immunity

11:30 - 11:45 **Barbara Bachmann** (Ludwig Boltzmann Institute for Experimental and Clinical Traumatology & Vienna University of Technology, AT)  
Effects of growth factor gradients and indirect flow on vasculature-on-chip

11:45 - 12:00 **Grace Lin** (AIT-Austrian Institute of Technology GmbH, AT)  
The role of oral mucosa epithelia for biomarker diagnostics in saliva

12:00 - 12:15 **Anna Gerhartl** (AIT Austrian Institute of Technology, AT)  
Effects of cerebral ischemia on the integrity of the human blood brain barrier: A comparative study with *in vitro* and *in vivo* data

12:15 - 12:30 **Julia Lachner** (Medical University of Vienna, AT)  
Characterization of the epidermal differentiation complex (EDC) gene EDDM: Adaptation of epidermal differentiation facilitated the evolution of feathers

12:30 - 13:30 Lunch

13:30 - 15:30 Poster Session 1

15:30 - 17:00 Track 1  
**Molecular microbiology I**  
*Chairs: Günther Koraimann & Brigitte Pertschy*  
**LH C1**

15:30 - 16:00 **Keynote**  
**Thibaud Coradin** (Sorbonne Université - CNRS, FR)  
A materials chemistry perspective on cell encapsulation

16:00 - 16:15 **Valeria Ellena** (University of natural resources and life sciences, AT)  
Towards a sexual cycle in *Aspergillus niger*

16:15 - 16:30 **Alex Lichius** (University of Innsbruck, AT)  
CRIB reporter technology to study cellular recovery mechanisms from host-induced hyphal depolarisation in mycoparasitic *Trichoderma* species.
### Detailed Program

#### Track 2

16:30 - 16:45
**Hoda Bazafkan (University of Innsbruck, AT)**
Tracing the TOR kinase pathway and its role in *Trichoderma atroviride* mycoparasitism

16:45 - 17:00
**Sabrina Jenull (Medical University of Vienna, AT)**
Out of control - how the loss of the Hir1 histone chaperone affects virulence of the human fungal pathogen *Candida albicans*

**New trends in allergy diagnosis and therapy**
*Chairs: Ines Swoboda & Beatrice Jahn-Schmid*

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:30</td>
<td><strong>Christian Lupinek (Medical University of Vienna, AT)</strong></td>
<td>New Trends in Allergy Diagnosis and Therapy</td>
</tr>
<tr>
<td>15:30</td>
<td><strong>Tanja Kalic (Medical University of Vienna, AT)</strong></td>
<td>Individuals allergic to bony fish tolerate cartilaginous fish due to the low allergenicity of their parvalbumins</td>
</tr>
<tr>
<td>15:50</td>
<td><strong>Sandra Pfeiffer (FH Campus Wien, AT)</strong></td>
<td>Ulo c 1 - a novel Alt a 1 cross-reactive allergen from the fungus <em>Ulocladium chartarum</em></td>
</tr>
<tr>
<td>16:05</td>
<td><strong>Maria R. Strobl (Medical University of Vienna, AT)</strong></td>
<td>Characterization of the affinity of Mal d 1-specific antibodies induced by sublingual immunotherapy with recombinant Bet v 1 or Mal d 1</td>
</tr>
<tr>
<td>16:35</td>
<td><strong>Jasmine Karacs (Medical University of Vienna, AT)</strong></td>
<td>Alum and monophosphoryl-lipid A as trigger for extracellular trap release from human neutrophils <em>in vitro</em></td>
</tr>
<tr>
<td>16:50</td>
<td><strong>Maximilian Kmen (FH Campus Wien, AT)</strong></td>
<td>Myosin light chain 2 - a novel fish allergen</td>
</tr>
<tr>
<td>17:00</td>
<td><strong>Angelika Tschepp (Medical University of Vienna, AT)</strong></td>
<td>Contribution of conformational and linear IgE epitopes to Ara h 2-specific IgE-binding – <em>in vitro</em> and <em>in vivo</em> studies</td>
</tr>
</tbody>
</table>

#### Track 3

15:30 - 16:00
**Alain Brisson (UMR-CBMN, FR)**
Imaging Extracellular Vesicles, Exosomes and Microparticles in Plasma and Conditioned Media

**Extracellular vesicles in coagulation and inflammation - Supported by ASEV**
*Chair: Viktoria Weber*

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:30</td>
<td><strong>Carla Tripisciano (Danube University Krems, AT)</strong></td>
<td>Extracellular vesicles from different settings support thrombin generation via different pathways</td>
</tr>
<tr>
<td>16:00</td>
<td><strong>Johannes Schmid (Med. Univ. Vienna, AT)</strong></td>
<td>Complex links between inflammation, cancer and thrombosis</td>
</tr>
<tr>
<td>16:15</td>
<td><strong>Birgit Fendl (Danube University Krems, AT)</strong></td>
<td>Differential interaction of platelet-derived extracellular vesicles with leukocyte subsets in human whole blood</td>
</tr>
<tr>
<td>16:45</td>
<td><strong>Andrea De Luna (Danube University of Vienna, AT)</strong></td>
<td>Different production technology of blood derived products influences origin of extracellular vesicles</td>
</tr>
</tbody>
</table>

17:00 - 17:30 **Coffee Break**
<table>
<thead>
<tr>
<th>Time</th>
<th>Room</th>
<th>Speaker and Affiliation</th>
<th>Title</th>
<th>Presenter and Affiliation</th>
</tr>
</thead>
</table>
| 17:30 - 18:20 | DK   | **DK BioTop**  
*Chair: Jürgen Zanghellini* | Linda Schwaigerlehner (University of Natural Resources and Life Sciences, AT)  
Are somatic mutations of mAbs responsible for low expression and thermal stability? | Dominik Jeschek (University of natural resources and life sciences, AT)  
Phospholipid vesicles to determine the transport functionality of mitochondrial carrier proteins |
| 17:30 - 17:45 | LH C1 | **Chair: Jutta Horejs-hoeck** | Helen Strandt (University of Salzburg, AT)  
The Activation State of Langerhans Cells Determines the Fate of Cytotoxic T Cells | Tamara Scheidt (University of Salzburg, AT)  
Phosphorylation: an Important Signal in Biological Chemistry |
| 17:45 - 18:00 | LH C1 | **Chair: Jutta Horejs-hoeck** | Bianca Chichirau (University of Salzburg, AT)  
*Helicobacter pylori*-controlled c-Abl localization promotes cell migration and limits apoptosis | **Chair: Jutta Horejs-hoeck**  
**DK ICA**  
**Chair: Jutta Horejs-hoeck** |
<p>| 18:00 - 18:15 | LH A  | <strong>Chair: Jutta Horejs-hoeck</strong> | Specialized ribosomes in cellular senescence |
| 18:20 - 21:00 | <em>Wine &amp; Science</em> | | |</p>
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00 - 09:40</td>
<td>Plenary Lecture</td>
<td>Paul Cos (University of Antwerp, BE)</td>
<td>LH C1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Can we develop a ROS-based antibacterial therapy?</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chair: Michael Sauer</td>
<td></td>
</tr>
<tr>
<td>09:40 - 10:20</td>
<td>Plenary Lecture</td>
<td>Marina Santic (Medical faculty, University of Rijeka, HR)</td>
<td>LH C1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Emerging zoonosis: one strategy, multiple host</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chair: Michael Bergmann</td>
<td></td>
</tr>
<tr>
<td>10:20 - 10:35</td>
<td>Flash Talks</td>
<td>Poster Flash 2</td>
<td>LH C1</td>
</tr>
<tr>
<td>10:20 - 10:22</td>
<td></td>
<td>Hyelin Na (Institute of Molecular Biotechnology, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Establishment of a human endometrial cancer organoid library</td>
<td></td>
</tr>
<tr>
<td>10:22 - 10:24</td>
<td></td>
<td>Katja Knapp (Innsbruck Medical University, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dissecting the role of the PIDDosome in hepatocellular carcinoma</td>
<td></td>
</tr>
<tr>
<td>10:26 - 10:28</td>
<td></td>
<td>Pia Hager (Medical University of Vienna, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Establishment of an in vitro phosphate buffer induced calcification model</td>
<td></td>
</tr>
<tr>
<td>10:28 - 10:30</td>
<td></td>
<td>Alice Rassinger (BOKU, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Identification of a citrate exporter protein CexA for citric acid production in <em>Aspergillus niger</em></td>
<td></td>
</tr>
<tr>
<td>10:30 - 10:32</td>
<td></td>
<td>Stefanie Duller (Medical University of Graz, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interplay of hospital microbiome and resistome – connecting pathogenic infection risk, healthy microbes and environmental biodiversity in and functional hospital setting</td>
<td></td>
</tr>
<tr>
<td>10:35 - 11:00</td>
<td>Coffee Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00 - 12:30</td>
<td>Track 1</td>
<td>Microbial chemical production</td>
<td>LH A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chairs: Matthias Steiger &amp; Alex Lichius</td>
<td></td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td>Keynote</td>
<td>Irina Borodina (Technical University of Denmark, DK)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Engineering yeasts for production of high-value metabolites</td>
<td></td>
</tr>
<tr>
<td>11:30 - 11:45</td>
<td></td>
<td>Stefan Pflügl (TU Wien, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microbial production of 2,3-butanediol: a comparison between <em>Escherichia coli</em> and <em>Vibrio natriegens</em></td>
<td></td>
</tr>
<tr>
<td>11:45 - 12:00</td>
<td></td>
<td>Florian Csarman (University of Natural Resources and Life Sciences, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>How oxidoreductases support biomass hydrolysis</td>
<td></td>
</tr>
<tr>
<td>12:00 - 12:15</td>
<td></td>
<td>Jaime Felipe Guerrero Garzón (University of Vienna, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bioactive <em>Amycolatopsis</em> sp. from the Mongolian steppe: identification of secondary metabolites and genome mining yielding novel lasso peptides</td>
<td></td>
</tr>
<tr>
<td>12:15 - 12:30</td>
<td></td>
<td>Hannes Russmayer (CD Laboratory for Biotechnology of Glycerol, BOKU Wien, AT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entirely oil palm based 1,3-propanediol production with <em>Lactobacillus diolivorans</em></td>
<td></td>
</tr>
<tr>
<td>11:00 - 12:30</td>
<td>Track 2</td>
<td>Microbiomes: interplay of microbes, their hosts and environments</td>
<td>LH C1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chairs: Kaisa Koskinen &amp; David Berry</td>
<td></td>
</tr>
</tbody>
</table>
### Detailed Program

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:00 - 11:30</td>
<td><strong>Keynote</strong>&lt;br&gt;Buck Hanson (University of Vienna, AT)&lt;br&gt;New human microbiome functions: How a vegetarian diet supports an exclusive physiological niche for gut commensals and contributes to hydrogen sulfide production</td>
</tr>
<tr>
<td>11:30 - 11:45</td>
<td><strong>Filomena Nogueira</strong> (CCRI, AT)&lt;br&gt;Bacteria and fungi: (un)healthy relationship</td>
</tr>
<tr>
<td>11:45 - 12:00</td>
<td><strong>Benjamin Zwirzitz</strong> (FFoQSI GmbH, AT)&lt;br&gt;Microbiota of the gut-lymph node axis: depletion of mucosa-associated segmented filamentous bacteria and enrichment of <em>Methanobrevibacter</em> by Colistin sulfate and Linco-Spectin in pigs</td>
</tr>
<tr>
<td>12:00 - 12:15</td>
<td><strong>Manuela-Raluca Pausan</strong> (Medical University of Graz, AT)&lt;br&gt;The unexplored Human Archaeome</td>
</tr>
<tr>
<td>12:15 - 12:30</td>
<td><strong>Markus Eder</strong> (University of Graz, AT)&lt;br&gt;Surface layer proteins of lactobacilli – Unraveling their structure will help us understand their probiotic effects</td>
</tr>
<tr>
<td>11:00 - 12:30</td>
<td><strong>Track 3</strong>&lt;br&gt;<strong>Pluripotent stem cells and neural differentiation – supported by</strong>&lt;br&gt;<em>Chairs: Sigismund Huck &amp; Bon-Kyoung Koo</em></td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td><strong>Keynote</strong>&lt;br&gt;Joshua Bagley (IMBA, AT)&lt;br&gt;Brain organoid fusion models human interneuron migration</td>
</tr>
<tr>
<td>11:30 - 11:45</td>
<td>Erik J. Vrij (IMBA, AT)&lt;br&gt;Recapitulating early embryonic development of the mouse using stem cells</td>
</tr>
<tr>
<td>11:45 - 12:00</td>
<td><strong>Sebastien Couillard-Despres</strong> (Paracelsus Medical University, AT)&lt;br&gt;Maturation and network integration of non-proliferative neuronal precursors in the adult murine piriform cortex</td>
</tr>
<tr>
<td>12:00 - 12:15</td>
<td><strong>Hannes Steinkellner</strong> (Medical University of Vienna, AT)&lt;br&gt;Generation of functionally active neurons using direct conversion from MeCP2 deficient male fibroblasts</td>
</tr>
<tr>
<td>12:15 - 12:30</td>
<td><strong>Iliaidy G. Soztekin</strong> (Medical University of Vienna, TR)&lt;br&gt;Generation of induced pluripotent stem cell derived cardiomyocytes from long QT-syndrome mouse model</td>
</tr>
<tr>
<td>12:30 - 13:30</td>
<td><strong>Lunch</strong></td>
</tr>
<tr>
<td>13:30 - 15:30</td>
<td><strong>Poster Session 2</strong></td>
</tr>
<tr>
<td>15:30 - 17:00</td>
<td><strong>Track 1</strong>&lt;br&gt;<strong>Biopharmaceutical technologies</strong>&lt;br&gt;<em>Chairs: Georg Klima &amp; Klaus Graumann</em></td>
</tr>
<tr>
<td>15:30 - 16:00</td>
<td><strong>Keynote</strong>&lt;br&gt;Christoph Pistek (Shire, AT)&lt;br&gt;Smart biopharmaceutical manufacturing enables global access of meaningful therapies</td>
</tr>
<tr>
<td>16:00 - 16:15</td>
<td><strong>Christoph Eilenberger</strong> (Technical University Vienna, AT)&lt;br&gt;3D Cellular Spheroid Age as critical Parameter for Drug Toxicity Screenings</td>
</tr>
<tr>
<td>16:15 - 16:30</td>
<td><strong>Benjamin Bayer</strong> (University of Natural Resources and Life Sciences, AT)&lt;br&gt;Design of experiments in the PAT/QbD concept - a comparative study of various design spaces for <em>Escherichia coli</em> fed-batch processes</td>
</tr>
<tr>
<td>16:30 - 16:45</td>
<td><strong>Matthias Berkemeyer</strong> (Boehringer Ingelheim RCV, AT)&lt;br&gt;Integrated process development for microbial expressed proteins</td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>16:45 - 17:00</td>
<td>Sergey Gusenkov (Novartis, AT) Understanding the structure-function relationship between disulfide bridging and potency in etanercept</td>
</tr>
<tr>
<td>15:30 - 17:30</td>
<td>Track 2 Cancer metabolism, autophagy and cell death Chairs: Mohamed Elgendy &amp; Andreas Villunger</td>
</tr>
<tr>
<td>15:30 - 16:00</td>
<td>Keynote Wolfram Weckwerth (university of Vienna, AT) Multomics and data-driven inverse modelling in molecular medicine</td>
</tr>
<tr>
<td>16:00 - 16:15</td>
<td>Sepideh Aminzadeh Gohari (Paracelsus Medical University, AT) Ketogenic diet as an adjuvant therapy for solid tumors</td>
</tr>
<tr>
<td>16:15 - 16:30</td>
<td>Sebastian Hofer (University of Graz, AT) Effects of dietary polyamine supplementation on mitochondria and the aging brain.</td>
</tr>
<tr>
<td>16:30 - 16:45</td>
<td>Thomas W. Grunt (Medical University of Vienna &amp; Ludwig Boltzmann Cluster Oncology, AT) Key cell regulation systems: cell signaling, lipid metabolism and epigenetics - how do they cooperate in cancer?</td>
</tr>
<tr>
<td>16:45 - 17:00</td>
<td>Valentina Sladky (Medical University of Innsbruck, AT) The PIDDosome in liver development, regeneration and tumorigenesis</td>
</tr>
<tr>
<td>17:00 - 17:30</td>
<td>Keynote Peter Vandenabeele (UGent-VIB, BE) Potency of different cell death modalities to induce an anti-tumor response</td>
</tr>
<tr>
<td>15:30 - 17:00</td>
<td>Track 3 Biomaterials in surgery Chairs: Konstantin D Bergmeister &amp; Bruno K. Podesser</td>
</tr>
<tr>
<td>15:30 - 16:00</td>
<td>Keynote Peter Zilla (Univ. of Cape Town, ZA) Tissue engineering of heart valves: a critical appraisal</td>
</tr>
<tr>
<td>16:00 - 16:15</td>
<td>Mario Rothbauer (Vienna University of Technology, AT) A nanobiotechnology-advanced lab-on-a-chip for blood cell immobilization and phenotyping</td>
</tr>
<tr>
<td>16:15 - 16:30</td>
<td>Magdalena Eilenberg (Medical University of Vienna, AT) Nanofibrous, bioabsorbable polycarbonate urethane for small diameter vessel replacement</td>
</tr>
<tr>
<td>16:30 - 16:45</td>
<td>Bernhard Priv. Doz. Dr. Winkler (KH Hietzing, AT) TRITON artificial tissue graft for small diameter application in cardiovascular procedures- preliminary results</td>
</tr>
<tr>
<td>16:45 - 17:00</td>
<td>Martin Aman (Medical University Vienna, AT) Innovationen in der Mensch-Maschine Schnittstelle zur Steuerung und Feedback bionischer Prothesen</td>
</tr>
</tbody>
</table>
### Thursday, September 20th, 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Topic</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00 - 09:40</td>
<td>Plenary Lecture</td>
<td>Arnold Driessen (University of Groningen, NL)</td>
<td>Engineering of the filamentous fungus <em>Penicillium chrysogenum</em> as cell factory for natural products</td>
<td>LH C1</td>
</tr>
<tr>
<td>09:40 - 10:20</td>
<td>Plenary Lecture</td>
<td>Henning Walczak (UCL Cancer Institute, GB)</td>
<td>To TRAIL or not to TRAIL in cancer therapy</td>
<td>LH C1</td>
</tr>
<tr>
<td>10:20 - 10:35</td>
<td>Flash Talks</td>
<td>Poster Flash 3</td>
<td></td>
<td>LH C1</td>
</tr>
<tr>
<td>10:20 - 10:22</td>
<td>Roman Labuda</td>
<td>Roman Labuda (University of Veterinary Medicine, Vienna (VetMed), AT)</td>
<td>Discovery of novel bioactive compounds through fugal and bacterial co-culture.</td>
<td></td>
</tr>
<tr>
<td>10:22 - 10:24</td>
<td>Johannes Pitsch</td>
<td>Johannes Pitsch (Johannes Kepler Universität Linz, AT)</td>
<td>Identification of fungi occurring in bread fermentation baskets</td>
<td></td>
</tr>
<tr>
<td>10:24 - 10:26</td>
<td>Michela Luciano</td>
<td>Michela Luciano (University of Salzburg, AT)</td>
<td>Role of the NLRP3/IL-1β axis in Acute Myeloid Leukemia (AML)</td>
<td></td>
</tr>
<tr>
<td>10:26 - 10:28</td>
<td>Victoria Stary</td>
<td>Victoria Stary (Medical University Vienna, AT)</td>
<td>Tumor-associated macrophages of rectal cancer polarize to the proinflammatory M1 phenotype after irradiation in patients and co-cultures</td>
<td></td>
</tr>
<tr>
<td>10:28 - 10:30</td>
<td>Bianca Chichirau</td>
<td>Bianca Chichirau (University of Salzburg, AT)</td>
<td>Identification of the functional role of the bacterial effector and oncoprotein CagA expressed by <em>Helicobacter pylori</em> in immune cells</td>
<td></td>
</tr>
<tr>
<td>10:30 - 10:32</td>
<td>Nisit Watthanasakphuban</td>
<td>Nisit Watthanasakphuban (BOKU, AT)</td>
<td>Functional verification of menaquinone biosynthesis genes of <em>Lactobacillus plantarum</em> WCFS1 and <em>Lactobacillus buchneri</em> DSM20057 by complementation of the respective defective mutants of <em>Lactococcus lactis</em> subsp. <em>cremoris</em> NZ9000</td>
<td></td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td>Track 1: Synthetic biology</td>
<td>Roman Jerala (National institute of chemistry, SI)</td>
<td>Designable modules in synthetic biology for the design of gene circuits to protein origami nanostructures</td>
<td>LH A</td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td>Keynote</td>
<td>Thomas Gassler (University of Natural Resources and Life Sciences, Vienna, AT)</td>
<td>Switching the metabolism of <em>Pichia pastoris</em> to efficiently assimilate CO₂</td>
<td></td>
</tr>
<tr>
<td>11:30 - 12:00</td>
<td></td>
<td>Christian Derntl (TU Wien, AT)</td>
<td>Synthetic transcription factors and expression system in <em>Trichoderma reesei</em></td>
<td></td>
</tr>
<tr>
<td>12:00 - 12:15</td>
<td></td>
<td>Sarah Noel Galleguillos (University of Natural Resources and Life Sciences, AT)</td>
<td>Efficient calculation of microbial production envelopes</td>
<td></td>
</tr>
<tr>
<td>12:15 - 12:30</td>
<td></td>
<td>Sergey Zotchev (University of Vienna, AT)</td>
<td>Synthetic biology and metabolic engineering toward the discovery of novel bioactive secondary metabolites from actinomycete bacteria</td>
<td></td>
</tr>
<tr>
<td>11:00 - 12:30</td>
<td>Track 2: Antimicrobial drugs: drug screening and prudent use</td>
<td>Sergey Zotchev (University of Vienna, AT)</td>
<td>Synthetic biology and metabolic engineering toward the discovery of novel bioactive secondary metabolites from actinomycete bacteria</td>
<td>LH C2</td>
</tr>
</tbody>
</table>
### Detailed Program

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:00 - 11:30</td>
<td><strong>Keynote</strong> &lt;br&gt; <strong>Marc Stadler</strong> <em>(Helmholtz-Zentrum für Infektionsforschung (HIZ), DE)</em> &lt;br&gt; Discovery and preclinical development of novel anti-infectives from fungal and microbial sources</td>
</tr>
<tr>
<td>11:30 - 11:45</td>
<td><strong>Bor Kavcic</strong> <em>(IST Austria, AT)</em> &lt;br&gt; Diverse effects of translation bottlenecks on antibiotic action underlie drug interactions</td>
</tr>
<tr>
<td>11:45 - 12:00</td>
<td><strong>Reinhard Beyer</strong> <em>(University of Natural Resources and Life Sciences, AT)</em> &lt;br&gt; Antifungal susceptibility of Candida blood stream isolates collected during a 10 year period from Austria between 2007 and 2016</td>
</tr>
<tr>
<td>12:00 - 12:15</td>
<td><strong>Gerald Geroldinger</strong> <em>(Univ. of Veterinary Medicine, AT)</em> &lt;br&gt; Anthracene endoperoxides: impact on <em>Leishmania</em></td>
</tr>
<tr>
<td>12:15 - 12:30</td>
<td><strong>Günther Koraimann</strong> <em>(University of Graz, AT)</em> &lt;br&gt; A non-canonical RNA polymerase drives <em>tra</em>-operon expression of F-like plasmids.</td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td><strong>Track 3</strong> &lt;br&gt; <strong>Translational oncology I</strong> &lt;br&gt; <em>Chairs: Christa Noehammer &amp; Michael Bergmann</em></td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td><strong>Keynote</strong> &lt;br&gt; <strong>Stefan Rose-John</strong> <em>(University of Kiel, DE)</em> &lt;br&gt; The role of IL-6 and ADAM17 in inflammation and cancer</td>
</tr>
<tr>
<td>11:30 - 11:45</td>
<td><strong>Rudolf Oehler</strong> <em>(Medical University of Vienna, AT)</em> &lt;br&gt; Immunological differences between colorectal cancer and normal mucosa uncover a prognostically relevant immune cell profile</td>
</tr>
<tr>
<td>11:45 - 12:00</td>
<td><strong>Daniel Drev</strong> <em>(Medical University Vienna, AT)</em> &lt;br&gt; Influence of fibroblast derived SPARC on colorectal cancer migration and invasiveness</td>
</tr>
<tr>
<td>12:00 - 12:15</td>
<td><strong>Irene Maier</strong> <em>(University of California, Los Angeles, US)</em> &lt;br&gt; Anti-inflammatory microbiota restriction augments skeletal bone structure</td>
</tr>
<tr>
<td>12:15 - 12:30</td>
<td><strong>David Pereyra</strong> <em>(Medical University of Vienna, AT)</em> &lt;br&gt; Oncological outcome after liver resection for malignant entities can be modulated via selective serotonin reuptake inhibition</td>
</tr>
<tr>
<td>12:30 - 13:30</td>
<td><strong>Lunch / Lunch Workshop</strong></td>
</tr>
<tr>
<td>13:30 - 15:30</td>
<td><strong>Poster Session 3</strong></td>
</tr>
<tr>
<td>15:30 - 17:00</td>
<td><strong>Track 1</strong> &lt;br&gt; <strong>Molecular microbiology II</strong> &lt;br&gt; <em>Chairs: Günther Koraimann &amp; Brigitte Pertschy</em></td>
</tr>
<tr>
<td>15:30 - 16:00</td>
<td><strong>Keynote</strong> &lt;br&gt; <strong>Alexander Harms</strong> <em>(University of Copenhagen, DK)</em> &lt;br&gt; One step back, two steps forward: Toxin-antitoxin modules and persister formation of <em>Escherichia coli</em></td>
</tr>
<tr>
<td>16:00 - 16:15</td>
<td><strong>Nela Nikolic</strong> <em>(IST Austria, AT)</em> &lt;br&gt; Autoregulation of <em>mazEF</em> expression underlies growth heterogeneity in bacterial populations</td>
</tr>
<tr>
<td>16:15 - 16:30</td>
<td><strong>Tobias Bergmiller</strong> <em>(IST Austria, AT)</em> &lt;br&gt; Biased partitioning of the multidrug efflux pump AcrAB-ToIC underlies long-lived phenotypic heterogeneity</td>
</tr>
<tr>
<td>16:30 - 16:45</td>
<td><strong>Petra Pusic</strong> <em>(University of Vienna, AT)</em> &lt;br&gt; Metabolic regulation of antibiotic resistance in <em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>
### Detailed Program

**16:45 - 17:00**

Silvia Schönthaler (AIT Austrian Institute of Technology GmbH, AT)

Low-cost DNA amplification-independent microarray characterisation of pathogens

---

**15:30 - 17:00 Track 2**

**Antimicrobial resistance: transfer of bugs and genes in diverse ecosystems**

*Chairs: Martin Wagner & Annemarie Käsbohrer*

LH C2

**15:30 - 16:00**

Keynote

Annemarie Käsbohrer (University of Veterinary Medicine, Vienna, AT)

Antimicrobial resistance: Transfer of bugs and genes in diverse ecosystems

---

**16:00 - 16:15**

Mahdi Ghanbari (Biomin Research center, AT)

In-feed antibiotic effects on the swine intestinal microbiome and resistome, as revealed by shotgun metagenomics

**16:15 - 16:30**

Anna Andersson (IST Austria, AT)

Biased partitioning of the multidrug efflux pump AcrAB-TolC underlies long-lived phenotypic heterogeneity.

**16:30 - 16:45**

Christoph Schüller (University of Natural Resources and Life Sciences, Vienna, AT)

The open research platform "BiMM - Bioactive Microbial Metabolites" - a high-throughput biotic and chemical interaction approach to discover novel bioactive compounds

**16:45 - 17:00**

Andrea Schabauer (University of Veterinary Medicine Vienna, AT)

Udder health of Austrian dairy cows: Mastitis, pathogens, clinical signs, and antimicrobial resistance

---

**15:30 - 17:00 Track 3**

**Translational oncology II**

*Chairs: Lukas Kenner & Philipp Staber*

LH C1

**15:30 - 15:45**

Keynote

Ana Ruiz-Saenz (UCSF, US)

HER2 amplification in tumors activates PI3K/Akt signaling independent of HER3

---

**15:45 - 16:00**

Wolfgang Gruber (University of Salzburg, AT)

DYRK1B as therapeutic target in Hedgehog/GLI-dependent cancer cells

Karin Schelch (Medical University of Vienna, AT)

YB-1 – master regulator in mesothelioma malignancy and potential therapeutic target

**16:00 - 16:15**

Andrija Matak (Medical University Graz, AT)

Stochastic phenotype switching leads to intratumor heterogeneity in human liver cancer

**16:15 - 16:30**

Belinda Schmalzbauer (Medical University of Vienna, AT)

Investigation of the methyltransferase KMT2C in *in vitro* systems of prostate cancer

---

**16:45 - 17:00**

Keynote

Emilio Casanova (Medical University of Vienna, AT)

KRAS mutated lung adenocarcinoma depends on EGFR/ERBB signaling

---

**17:00 - 17:30**

Coffee Break

---

**17:30 - 18:00**

Award Ceremony II / Exhibitor Quiz II /Closing remarks

*Moderation: Michael Sauer & Angela Sessitsch*

LH C1
Poster Table

Poster Table - Biophysics

Posters from poster session Biophysics Austria must be on display until the Lunch break on 17th and removed latest until 9:00 next day the 18th.

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Poster Session Biophysics</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSBA-01</td>
<td>Thomas Stockner</td>
<td>Aula</td>
</tr>
<tr>
<td>PSBA-02</td>
<td>Christof Hannessschläger</td>
<td>Aula</td>
</tr>
<tr>
<td>PSBA-03</td>
<td>Sudarat Tharad</td>
<td>Aula</td>
</tr>
<tr>
<td>PSBA-04</td>
<td>Begüm Dikecoglu</td>
<td>Aula</td>
</tr>
<tr>
<td>PSBA-05</td>
<td>Sanja Curcic</td>
<td>Aula</td>
</tr>
<tr>
<td>PSBA-06</td>
<td>Sarah Stainer</td>
<td>Aula</td>
</tr>
</tbody>
</table>

Advanced Analytical Technologies Inc. (AATI) develops, manufactures and markets high-throughput, fully-automated nucleic acid and genetic analysis systems. The company’s product portfolio includes instruments for the parallel analysis of DNA, RNA, genomic DNA, double-stranded DNA, gene editing (CRISPR/Cas9) using capillary electrophoresis (CE) with fluorescence detection. The company’s flagship product - the Fragment Analyzer - is recognized as the best-in-class, multi-channel, automated fluorescence-based CE detection system for the simultaneous analysis of the quantity and quality of nucleic acids.


The Austrian Society for Tissue Engineering and Regenerative Medicine (ASTERM) aims at linking groups and individuals in the field of basic and application oriented research on human cells and tissues, thereby strengthening the interdisciplinary exchange on current research approaches and methods. For this purpose, e.g. events for different target groups in the field of cell-based therapies are organized. An essential aspect of our goals is the promotion of young scientists.
# Poster Table - Poster Session 1

Posters from poster session 1 must be on display on Thursday until the Lunch break on 18th and removed latest in the Coffee Break on the next day (19th, 10:30-11:00).

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Plant biotechnology</th>
<th>Location</th>
<th>Poster #</th>
<th>New trends in allergy diagnosis and therapy</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1:PB-01</td>
<td>Kathrin Göritzer</td>
<td>C1 Foyer</td>
<td>PS1:ADT-01</td>
<td>Cancelled</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:PB-02</td>
<td>Martin Maier</td>
<td>C1 Foyer</td>
<td>PS1:ADT-02</td>
<td>Marcel Tisch</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:PB-03</td>
<td>Pascal Mülner</td>
<td>C1 Foyer</td>
<td>PS1:ADT-03</td>
<td>Athina Trakaki</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:PB-04</td>
<td>Margit Laimer</td>
<td>C1 Foyer</td>
<td>PS1:ADT-04</td>
<td>João Grilo</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:PB-05</td>
<td>Muhammad Ahmad</td>
<td>C1 Foyer</td>
<td>PS1:ADT-05</td>
<td>Piotr Humeniuk</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:PB-06</td>
<td>Silvia Madritsch</td>
<td>C1 Foyer</td>
<td>PS1:ADT-06</td>
<td>Manuel Reithofer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:PB-07</td>
<td>Öykü Üzülmez</td>
<td>C1 Foyer</td>
<td>PS1:ADT-07</td>
<td>Patricia Roman Carrasco</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMB-01</td>
<td>Michael Egermeier</td>
<td>C1 Foyer</td>
<td>PS1:ADT-08</td>
<td>Flora Stübl</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMB-02</td>
<td>Antonia Volpini de Maestri</td>
<td>C1 Foyer</td>
<td>PS1:ADT-09</td>
<td>Stefan Kabasser</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMB-03</td>
<td>Artur Schuller</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-04</td>
<td>Johannes Bitter</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-05</td>
<td>Markus Gorfer</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-06</td>
<td>Mai-Lan Pham</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-07</td>
<td>Wolfgang Hinterdolber</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-08</td>
<td>Kay D. Novak</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-09</td>
<td>Gerald Klanert</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-10</td>
<td>Lukas Johannes Pfeifenberger</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMB-11</td>
<td>Martin Altvater</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF1-01</td>
<td>Katharina Novak</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF1-04</td>
<td>Razieh Karimi Aghcheh</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:NIB-01</td>
<td>Andrea Rampacher</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:NIB-02</td>
<td>Manuela Paunovic</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:NPA-01</td>
<td>Clemens Kittinger</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF1-05</td>
<td>Norhan Mahfouz</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Optimization of microbial workhorses for biotechnology</th>
<th>Location</th>
<th>Poster #</th>
<th>From RNAomics to function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1:OMP-01</td>
<td>Michael Egermeier</td>
<td>C1 Foyer</td>
<td>PS1:RNA-01</td>
<td>Florian Ebner</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMP-02</td>
<td>Antonia Volpini de Maestri</td>
<td>C1 Foyer</td>
<td>PS1:RNA-02</td>
<td>Mamta Jain</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMP-03</td>
<td>Artur Schuller</td>
<td>C1 Foyer</td>
<td>PS1:RNA-03</td>
<td>Konstantin Licht</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMP-04</td>
<td>Johannes Bitter</td>
<td>C1 Foyer</td>
<td>PS1:RNA-04</td>
<td>Michael F. Jantsch</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMP-05</td>
<td>Markus Gorfer</td>
<td>C1 Foyer</td>
<td>PS1:RNA-05</td>
<td>Utkarsh Kapoor</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMP-06</td>
<td>Mai-Lan Pham</td>
<td>C1 Foyer</td>
<td>PS1:RNA-06</td>
<td>Sabrina Summer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMP-07</td>
<td>Wolfgang Hinterdolber</td>
<td>C1 Foyer</td>
<td>PF1-02</td>
<td>Markus Schosserer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:OMP-08</td>
<td>Kay D. Novak</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMP-09</td>
<td>Gerald Klanert</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMP-10</td>
<td>Lukas Johannes Pfeifenberger</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:OMP-11</td>
<td>Martin Altvater</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF1-01</td>
<td>Katharina Novak</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF1-04</td>
<td>Razieh Karimi Aghcheh</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:NIB-01</td>
<td>Andrea Rampacher</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS1:NIB-02</td>
<td>Manuela Paunovic</td>
<td>C1 Foyer</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Neuroimmunology</th>
<th>Location</th>
<th>Poster #</th>
<th>Biological barriers in health and disease</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1:NI-01</td>
<td>Andrea Rampacher</td>
<td>C1 Foyer</td>
<td>PS1:BB-01</td>
<td>Winfried Neuhaus</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:NI-02</td>
<td>Manuela Paunovic</td>
<td>C1 Foyer</td>
<td>PS1:BB-02</td>
<td>P. Luza Szabo</td>
<td>Aula</td>
</tr>
<tr>
<td>PS1:NPA-01</td>
<td>Clemens Kittinger</td>
<td>C1 Foyer</td>
<td>PS1:EV-01</td>
<td>Ulrike Kegler</td>
<td>Aula</td>
</tr>
<tr>
<td>PF1-05</td>
<td>Norhan Mahfouz</td>
<td>C1 Foyer</td>
<td>PS1:EV-02</td>
<td>Anja Buhmann</td>
<td>Aula</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Next-generation pathogen &amp; antibiotic resistance diagnostics</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1:NPA-01</td>
<td>Clemens Kittinger</td>
<td>C1 Foyer</td>
</tr>
<tr>
<td>PF1-05</td>
<td>Norhan Mahfouz</td>
<td>C1 Foyer</td>
</tr>
</tbody>
</table>
### Poster Table - Poster Session 2

Posters from poster session 2 must be on display until the Lunch break on 19th and removed latest in the Coffee Break on the next day (20th, 10:30-11:00).

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Microbial chemical production</th>
<th>Location</th>
<th>Poster #</th>
<th>Microbiomes: interplay of microbes, their hosts and environments</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS2:MCP-01</td>
<td>Damiano Totaro</td>
<td>C1 Foyer</td>
<td>PS2:MB-01</td>
<td>Günther Koraimann</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:MCP-02</td>
<td>Claudia Juno</td>
<td>C1 Foyer</td>
<td>PS2:MB-02</td>
<td>Christina Kumpitsch</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:MCP-03</td>
<td>Harald Pichler</td>
<td>C1 Foyer</td>
<td>PS2:MB-03</td>
<td>Guofen Li</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:MCP-04</td>
<td>Lena Wohlschlager</td>
<td>C1 Foyer</td>
<td>PS2:MB-04</td>
<td>Claudia Preininger</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:MCP-05</td>
<td>Robin Hoheneder</td>
<td>C1 Foyer</td>
<td>PS2:MB-05</td>
<td>Cintia Csorba</td>
<td>Aula</td>
</tr>
<tr>
<td>PF2-05</td>
<td>Alice Rassinger</td>
<td>C1 Foyer</td>
<td>PS2:MB-06</td>
<td>Johannes - Paul Fladerer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:MCP-01</td>
<td>Microbial chemical production</td>
<td>Location</td>
<td>PS2:MB-01</td>
<td>Microbiomes: interplay of microbes, their hosts and environments</td>
<td>Location</td>
</tr>
<tr>
<td>PS2:BT-01</td>
<td>Hannelore Breitenbach-Koller</td>
<td>C1 Foyer</td>
<td>PS2:MB-07</td>
<td>Ursula Sauer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:BT-02</td>
<td>Klaus Kraitsy</td>
<td>C1 Foyer</td>
<td>PF2-06</td>
<td>Stefanie Duller</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:BT-03</td>
<td>Klaus Stolze</td>
<td>C1 Foyer</td>
<td>PF2-01</td>
<td>Hyelin Na</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:BT-04</td>
<td>Jens Kastenhofer</td>
<td>C1 Foyer</td>
<td>Poster #</td>
<td>Pluripotent stem cells and neural differentiation</td>
<td>Location</td>
</tr>
<tr>
<td>PS2:BT-05</td>
<td>Gabriela Eder</td>
<td>C1 Foyer</td>
<td>PS2:SC-01</td>
<td>Ji-Hyun Lee</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:BT-06</td>
<td>Alexander Pekarsky</td>
<td>C1 Foyer</td>
<td>PS2:BT-01</td>
<td>Hyelin Na</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:BT-07</td>
<td>Bernhard Sissolak</td>
<td>C1 Foyer</td>
<td>Poster #</td>
<td>Cancer metabolism, autophagy and cell death</td>
<td>Location</td>
</tr>
<tr>
<td>PS2:BT-08</td>
<td>Špela Knez</td>
<td>C1 Foyer</td>
<td>PS2:CM-01</td>
<td>Lisa Milchram</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:BT-09</td>
<td>Alexandra Pum</td>
<td>C1 Foyer</td>
<td>PS2:BT-07</td>
<td>Julie Krainer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:BT-10</td>
<td>Isabella Thiel</td>
<td>C1 Foyer</td>
<td>Poster #</td>
<td>Cancer metabolism, autophagy and cell death</td>
<td>Location</td>
</tr>
<tr>
<td>PF2-03</td>
<td>Sergey Gusenkov</td>
<td>C1 Foyer</td>
<td>PS2:CM-02</td>
<td>Carina Hasenöhrl</td>
<td>Aula</td>
</tr>
<tr>
<td>Poster #</td>
<td>Biopharmaceutical technologies</td>
<td>Location</td>
<td>PS2:CM-03</td>
<td>Andreas Weber</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:V-01</td>
<td>Helmhuth Haslacher</td>
<td>C1 Foyer</td>
<td>PS2:CM-04</td>
<td>Leopold Eckhart</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:V-02</td>
<td>Philipp Zeiger</td>
<td>C1 Foyer</td>
<td>PS2:CM-05</td>
<td>Magdalena Grill</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:V-03</td>
<td>Michael Karbiener</td>
<td>C1 Foyer</td>
<td>PS2:CM-06</td>
<td>Magdalena Grill</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:V-04</td>
<td>Tanja Grossmann</td>
<td>C1 Foyer</td>
<td>PS2:CM-07</td>
<td>Margit Cichna-Markl</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:V-05</td>
<td>Andrijana Kirsch</td>
<td>C1 Foyer</td>
<td>Poster #</td>
<td>Biomaterials in surgery</td>
<td>Location</td>
</tr>
<tr>
<td>PS2:V-06</td>
<td>Alice Rassinger</td>
<td>C1 Foyer</td>
<td>PS2:CM-08</td>
<td>Jürgen Thanner</td>
<td>Aula</td>
</tr>
<tr>
<td>PF2-03</td>
<td>Sergey Gusenkov</td>
<td>C1 Foyer</td>
<td>PS2:CM-09</td>
<td>Dominik E. Indra</td>
<td>Aula</td>
</tr>
<tr>
<td>Poster #</td>
<td>Varia</td>
<td>Location</td>
<td>PS2:CM-10</td>
<td>Jasmin Huber</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:CM-02</td>
<td>Carina Hasenöhrl</td>
<td>Aula</td>
<td>PF2-02</td>
<td>Katja Knapp</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:CM-03</td>
<td>Andreas Weber</td>
<td>Aula</td>
<td>Poster #</td>
<td>Biomaterials in surgery</td>
<td>Location</td>
</tr>
<tr>
<td>PS2:CM-04</td>
<td>Leopold Eckhart</td>
<td>Aula</td>
<td>PS2:CM-05</td>
<td>Magdalena Grill</td>
<td>Aula</td>
</tr>
<tr>
<td>PS2:CM-05</td>
<td>Magdalena Grill</td>
<td>Aula</td>
<td>Poster #</td>
<td>Biomaterials in surgery</td>
<td>Location</td>
</tr>
</tbody>
</table>
**Poster Table - Poster Session 3**

Posters from poster session 3 must be on display until the Lunch break on 20\textsuperscript{th} and removed in the last Coffee Break on this day (20\textsuperscript{th}, 17:00-17:30)

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Molecular microbiology</th>
<th>Location</th>
<th>Poster #</th>
<th>Translational oncology</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS3:MM-01</td>
<td>Vera Pils</td>
<td>C1 Foyer</td>
<td>PS3:TO-01</td>
<td>Lisa Gabler</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-02</td>
<td>Gernot Zarfel</td>
<td>C1 Foyer</td>
<td>PS3:TO-02</td>
<td>Manuela Hofner</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-03</td>
<td>Elisabeth Damisch</td>
<td>C1 Foyer</td>
<td>PS3:TO-03</td>
<td>Thomas Dillinger</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-04</td>
<td>S. Andreas Angermayr</td>
<td>C1 Foyer</td>
<td>PS3:TO-04</td>
<td>Reinhold Hofbauer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-05</td>
<td>Krisztian Twaruschek</td>
<td>C1 Foyer</td>
<td>PS3:TO-05</td>
<td>Dafina Ilijazi</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-06</td>
<td>Diane Barbay</td>
<td>C1 Foyer</td>
<td>PS3:TO-06</td>
<td>Bianca Dietrich</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-07</td>
<td>Sebastian Diechler</td>
<td>C1 Foyer</td>
<td>PS3:TO-07</td>
<td>Ingrid Walter</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-08</td>
<td>Cancelled</td>
<td>C1 Foyer</td>
<td>PS3:TO-08</td>
<td>Franziska Vogl</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-09</td>
<td>Maja Plesko</td>
<td>C1 Foyer</td>
<td>PS3:TO-09</td>
<td>Zsuzsanna Valko</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-10</td>
<td>Georg Sandner</td>
<td>C1 Foyer</td>
<td>PS3:TO-10</td>
<td>Katja Zappe</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-11</td>
<td>Arthur Sedivy</td>
<td>C1 Foyer</td>
<td>PS3:TO-11</td>
<td>Stefanie Brezina</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-12</td>
<td>Kalina Duszka</td>
<td>C1 Foyer</td>
<td>PS3:TO-12</td>
<td>Monika Sachet</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-13</td>
<td>Sabine Bernegger</td>
<td>C1 Foyer</td>
<td>PS3:TO-13</td>
<td>Walter Pulverer</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-14</td>
<td>Dubraska C. Moreno Ruiz</td>
<td>C1 Foyer</td>
<td>PS3:TO-14</td>
<td>Jürgen Lebhard</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:MM-15</td>
<td>Jennifer Staudacher</td>
<td>C1 Foyer</td>
<td>PS3:TO-15</td>
<td>Nevena Jankovic</td>
<td>Aula</td>
</tr>
<tr>
<td>PF3-02</td>
<td>Johannes Pitsch</td>
<td>C1 Foyer</td>
<td>PS3:TO-16</td>
<td>Melanie Kienzl</td>
<td>Aula</td>
</tr>
<tr>
<td>PF3-05</td>
<td>Bianca Chichirau</td>
<td>C1 Foyer</td>
<td>PS3:TO-17</td>
<td>Raheleh Sheibani-Tezerji</td>
<td>Aula</td>
</tr>
<tr>
<td>PF3-06</td>
<td>Nisit Watthanasakphuban</td>
<td>C1 Foyer</td>
<td>PS3:TO-18</td>
<td>Loan Tran</td>
<td>Aula</td>
</tr>
<tr>
<td>Poster #</td>
<td>Synthetic biology</td>
<td>Location</td>
<td>Poster #</td>
<td>Antimicrobial drugs: drug screening and prudent use</td>
<td>Location</td>
</tr>
<tr>
<td>PS3:SB-01</td>
<td>Michael Tscherner</td>
<td>C1 Foyer</td>
<td>PS3:TO-20</td>
<td>Georg Csukovich</td>
<td>Aula</td>
</tr>
<tr>
<td>Poster #</td>
<td>Antimicrobial drugs: drug screening and prudent use</td>
<td>Location</td>
<td>Poster #</td>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>PS3:AD-01</td>
<td>Judith Quirgst</td>
<td>C1 Foyer</td>
<td>PS3:TO-20</td>
<td>Georg Csukovich</td>
<td>Aula</td>
</tr>
<tr>
<td>PS3:AD-02</td>
<td>Andrea Schabauer</td>
<td>C1 Foyer</td>
<td>PF3-03</td>
<td>Michela Luciano</td>
<td>Aula</td>
</tr>
<tr>
<td>PF3-01</td>
<td>Roman Labuda</td>
<td>C1 Foyer</td>
<td>PF3-04</td>
<td>Victoria Stary</td>
<td>Aula</td>
</tr>
</tbody>
</table>
Monday 17th

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Monday 17th September 2018
Monday 17th: Biophysics Austria Annual meeting

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Biophysics Austria Annual Meeting

Chair: Thomas Stockner
BPA-01  How lipids regulate membrane transport proteins
Caroline Overa, Gregor M Madej, Lifei Fu, Izabela Waclawska, Veronika Heinz, Christine Ziegler
Biophysik, Universität Regensburg, Germany

Regulated solute transport across the cellular membrane is a major strategy to counteract various stress types in cells and its molecular understanding is one of the cutting-edge questions in biological science.

Our structural, functional and biophysical work on the betaine transporter BetP, a member of the BCCT family, has provided unique insights into the process of stress regulated transport. BetP responds to osmotically driven changes in the membrane and in cytoplasmic K⁺-concentration. We elucidated the complex regulatory interaction mechanism of BetP during stress response between terminal domains and anionic lipids by X-ray crystallography, single particle cryo-electron microscopy, HDX and FTIR. Recently, we have solved a single particle cryo-EM structure of a homologous choline BCCT symporter to 4.4 Å, which provides new insights into the evolution of regulatory lipid interaction sites in secondary transporters.

BPA-02  Partition coefficient of arginine between translocon interior and lipid phase
Denis Knyazev, Mirjam Zimmermann, Roland Kuttner, Peter Pohl
Institute of Biophysics, Johannes Kepler University, Austria

Whether the peptide segment is transmembrane depends on the hydrophobicity of individual amino acids. In the cytoplasmic membrane of bacteria as well as in the endoplasmic reticulum membrane of eukaryotes the incorporation of transmembrane proteins is facilitated by translocation protein complex. It is long debated if the translocon merely catalyzes the insertion into lipid membrane or rather influences the partition. Partition coefficients of individual amino acids between apolar and polar phase coincide to the large extent in both physical-chemical and biological scales. As the former was derived from the distribution of peptides between apolar solvent and water phase, and the latter from the insertion probabilities in vivo, the similar partition for individual amino acids supports the view that the translocon is a passive facilitator. On the other hand, there are indications that in vivo insertion is a non-equilibrium process. Indeed, otherwise we would have topology homologs for most of proteins, whereas such examples seem to be rather an exception from the rule. Thus, biological system where partition could be directly observed online. Here we report partition coefficient of arginine obtained in vitro from partitioning of the peptide segment between the SecYEG interior and lipid phase and measured with single channel electrophysiology. For that we use purified and reconstituted into planar lipid bilayer SecYEG as in (Knyazev et al. 2014) to form translocation intermediate with proOmpA-based construct, where we introduced a transmembrane segment with variable position and number of arginines.

Knyazev, Denis G.; Winter, Lukas; Bauer, Benedikt W.; Siligan, Christine; Pohl, Peter (2014): Ion conductivity of the bacterial translocation channel SecYEG engaged in translocation. In: The Journal of biological chemistry 289 (35), S. 24611–24616. DOI: 10.1074/jbc.M114.588491.

BPA-03  Automated Free Energy Calculation for Drug Design: Accelerated Enveloping Distribution Sampling
Jan Walther Perthold, Chris Oostenbrink
Institute for Molecular Modeling and Simulation (MMS), University of Natural Resources and Life Sciences, Vienna, Austria

Enveloping distribution sampling (EDS) is an efficient approach to calculate multiple free-energy differences from a single molecular dynamics (MD) simulation. However, the construction of an appropriate reference-state Hamiltonian that samples all states efficiently is not straightforward. We propose a novel approach for the construction of the EDS reference-state Hamiltonian, related to a previously described procedure to smoothen energy landscapes. In contrast to previously suggested EDS approaches, our reference-state Hamiltonian preserves local energy minima of the combined end-states. Moreover, we propose an intuitive, robust and efficient parameter optimization scheme to tune EDS Hamiltonian parameters. We demonstrate the proposed method with established and novel test systems and conclude that our approach allows for the automated calculation of multiple free-energy differences from a single simulation. Accelerated EDS promises to be a robust and user-friendly method to compute free-energy differences based on solid statistical mechanics.
BPA-04  ABCB1 nucleotide binding domain dimerization cycle
Dániel Szőllősi, Thomas Stockner
Institute of Pharmacology, Medical University of Vienna, Austria

P-glycoprotein (a.k.a. MDR1 or ABCB1) is expressed in cellular barriers and causes resistance against chemotherapy if expressed in cancer cells. The pseudo-symmetric ABCB1 consists of two transmembrane domains (TMD) that bind and transport the substrate, and of two nucleotide binding domains (NBDs) that energize the transport by ATP binding and hydrolysis. It is accepted that dimerization is triggered by ATP binding, which is the first power stroke leading to a close NBD-NBD association. This dimerization is assumed to cause conformational changes and induce the switching to the outward facing conformation.

To study the energetics of NBD dimer formation and the influence of nucleotides, we performed biased and unbiased molecular dynamics simulations of isolated NBDs. Potential of mean force curves show that the apo configuration favors the dimeric conformation, but a deep energy minimum (~ -42 kJ/mol) is reached in the presence of ATP. Conserved motif interaction network analyses revealed that ATP stabilizes the NBD dimer by forming strong attractive interaction with both domains. These forces are multilayered and consist of electrostatic, hydrophobic and water-mediated interactions between the nucleotide and the NBDs. The NBD dimer is opened by the formation of ADP3- and HPO42- , which drastically changes interactions within the nucleotide binding site (NBS) and the free energy hypersurface. ATP hydrolysis does therefore, serve as the energy input and facilitates both the closure and following hydrolysis the opening of the NBD dimer allowing the ABCB1 transport cycle.

This work was supported by the Austrian Science Fund (FWF), Special Research Program SFB 3524.

BPA-05  Protein homodimerization from perspective of structural biology and biophysics. Case study of 14-3-3ζ protein.
Jozef Hritz1, Zuzana Trošanová2, Petr Louša2, Zuzana Jandová3, Jan Tungli2, Tomáš Brom2, Gabriel Žoldák4, Chris Oostenbrink3
1 Institute of Molecular Modeling and Simulation, University of Natural Resources and Life Sciences, Austria
2 Structural Biology, CEITEC-MU, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
3 Institute of Molecular Modeling and Simulation, University of Natural Resources and Life Sciences, Vienna A-1190, Austria
4 Center for Interdisciplinary Biosciences, Technology and Innovation Park P.J. Šafárik University, Jesenná 5, 041 54 Košice, Slovakia

Large number of proteins are forming homodimers. Here, we are presenting experimental and computational tools for the characterization of stable homodimers in terms of biophysical and structural properties of the dimer dissociation. First, fluorescence assays analyzed by ad-hoc mathematical models as presented here allow efficient and reliable determination of thermodynamic and kinetic parameters of dimer-monomer equilibria. To quantify microscopic dynamics between monomers and homodimers, we have designed sensitive and efficient fluorescent assays based on the Förster resonance energy transfer (FRET) and self-quenching (SQ) phenomena. The applicability of these approaches is shown here for the determination of dissociation constant (Kd) and dissociation and association rate constants (koff and kon, respectively) of 14-3-3ζ dimer-monomer equilibria. The most important biophysical factors altering this equilibrium are presented here.

Second, two mutations were introduced into the 14-3-3ζ dimer interface and the corresponding change in the binding affinity was predicted by alchemical free energy perturbation method. The obtained results are compared with the experimental values.

This work was supported by the research grant from the Czech Science Foundation (grant no. GA. 15-34684L) and the Austrian Science Fund (grant number 1999-N28)

References:
**BPA-06  Single-file transport of water through membrane channels**

**Andreas Horner**, Christine Siligan, Johannes Preiner, Peter Pohl

1 University of Applied Sciences Upper Austria, Franz-Fritsch-Straße 11, 4600 Wels, Austria
2 Institute of Biophysics, Johannes Kepler University, Austria

Macroscopic laws of hydrodynamics do not apply to narrow biological channels. Since the discovery of water channels more than a quarter of a century ago, the physical laws of water flux through sub-nanometer wide channels remained elusive: Published single channel permeability values ($p_f$) vary by three orders of magnitude, even though (i) length variations of these channels are limited by membrane thickness and (ii) their pore diameter is fixed to the dimensions of one water molecule. To obtain water flow determinants, we quantified $p_f$ of a diverse set of water facilitators, including cation selective peptides (gramicidin), potassium channels (KcsA) and aquaporins (AQP1, AQP4, AQP5, AQZ, GlpF). Therefore, we measured (i) the amount of fluorescently labeled and reconstituted proteins in the vesicular membrane by both fluorescence correlation spectroscopy (FCS) and high-speed atomic force microscopy, and (ii) the intensity of scattered light as a means to assess vesicular water efflux via our new adaptation of the Rayleigh-Gans-Debye equation (1). Using these methods we made the following observations: 1. The number of hydrogen bonds a water molecule may form inside narrow channels serves as the major determinant of $p_f$ (1). 2. Positively charged residues near the channel mouth may increase $p_f$ two or three times, conceivably by lowering the dehydration penalty at the channel mouth (2). 3. Transition state theory provides a useful link between $p_f$ and the activation energy for single file transport (3).

(1) A Horner, F Zocher, J Preiner, N Ollinger, C Siligan, SA Akimov, P Pohl. The mobility of single-file water molecules is governed by the number of H-bonds they may form with channel-lining residues. Science Advances, 1, 2, e1400083, 2015
(3) A. Horner, P. Pohl. Comment on “Enhanced water permeability and tunable ion selectivity in subnanometer carbon nanotube porins”. Science, 359, eaap9173, 2018

---

**BPA-07  Superresolution Microscopy Images: What they tell us about protein clusters – and what they don’t**

**Benedikt Rossboth**, Andreas Arnold, Haisen Ta, Rene Platzer, Florian Kellner, Johannes B. Huppa, Mario Brameshuber, Florian Baumgart, Gerhard J. Schütz

1 Max Planck Institute for Biophysical Chemistry, Göttinger, Germany
2 Medical University of Vienna, Austria
3 Institute of Applied Physics, TU Wien, Austria

The organization and dynamics of proteins and lipids in the plasma membrane, and their role in membrane functionality, have been subject of a long-lasting debate. Developments in superresolution microscopy have facilitated for the first time the direct imaging of cellular structures at length scales far below the optical diffraction limit. Indeed, when applied to the plasma membrane the presence of a variety of protein nanoclusters was revealed, which lead to speculations whether nanoclustering was a general feature of plasma membrane proteins. Particularly in T lymphocytes, clustering of signaling proteins has been proposed to represent a fundamental mechanism for cell activation. Recently, however, doubts were raised whether imaging artifacts inherent to PALM/STORM might have influenced or even caused the observation of some of those protein clusters. To approach these concerns, we developed a method to robustly discriminate clustered from random distributions of molecules detected with single molecule localization microscopy-based techniques like PALM and STORM (1). In my talk I will present the application of superresolution techniques to different proteins expressed at the T cell plasma membrane. Particularly, I will show that the T cell receptor complex is randomly distributed at the plasma membrane of non-activated T cells.

BPA-08  Overcoming blinking artifacts in nanocluster detection with two-color STORM

Magdalena Schneider, Andreas M. Arnold, Florian Baumgart, Gerhard J. Schütz
Institute of Applied Physics, TU Wien, Austria

Observations using single molecule localization microscopy have led to the belief that the majority of tested membrane proteins are organized in clusters at sizes below the diffraction limit. These nanoclusters are thought to play an important role in cellular signaling. However, concerns about the existence of nanoclusters have been fueled by the notion that virtually all fluorescent probes show complex blinking behavior including long-lived dark states. This results in localization clusters due to the repeated observation of single molecules. Existing post-processing approaches commonly struggle to reliably distinguish real molecular clustering from such blinking artifacts.

Here, we present a novel analytical method using information from two-color STORM experiments for overcoming the erroneous detection of clustering due to fluorophore blinking. Targeting the same protein species with differently labeled antibodies allows for the calculation of distance distributions from both color channels. Molecular clusters exhibit a characteristic bias towards shorter distances. Applying toroidal shifts to the data breaks correlations between the two color channels, thus providing realizations of the null hypothesis of independence (randomly distributed molecules). This allows for statistical significance tests without the necessity of additional calibration. We evaluate the limits of the method with Monte Carlo simulations and experiments on clustered and randomly distributed membrane proteins.

BPA-09  Time-Resolved X-ray Studies of Antimicrobial Peptide Activity in Live E. coli on the Nano- to Micrometer Scales

Enrico F. Semeraro1, Lisa Marx1, Theyencheri Narayanan2, Karl Lohner1, Georg Pabst1
1 Institute of Molecular Biosciences (Biophysics), University of Graz, Austria
2 ESRF-The European Synchrotron, 38043 Grenoble, France

Novel antibiotics based on antimicrobial peptides (AMPs) are promising candidates for defending the spread of diseases caused by multi-resistant pathogenic bacteria. Some AMPs primarily interacts with the cell wall, causing bacterial death by physically disrupting the barrier function of the cell envelope. Notwithstanding the number of works that explore the relationship between AMP activity and membrane architecture [1], the dynamics and full mechanism that lead to cell death are currently not clear. Here, we investigated the effect of different membrane-active peptides on live E. coli by means of high-resolution (Ultra) Small-angle X-ray Scattering (USAXS/SAXS) measurements performed on the ID02 beamline (ESRF, France).

Time-resolved USAXS/SAXS technique allowed to probe these systems in the appropriate length and time scales. Thanks to a very wide scattering vector (q) range, a multi-scale model provided valuable information about structural changes in both submicron and nanoscales (cell-body and cell-envelope, respectively) [2]. Results showed that key events occur on a few second, which is a time scale that was not previously accessible, and on a wide length scale, including electron-density variations in both cell-body and cell-envelope. In addition, the peptide-induced alterations of the bacterial structure exhibited well-defined time-evolutions which depended on the specific AMP. As an example, the mode of action of O-LF11-215 (derivative of the human lactoferricin) consisted of two distinct time regimes. Within the first 40 seconds, the structural parameters showed large-scale alterations (submicron range), possibly related to cell shrinking and loss of cytoplasmic content. At a later stage instead, results were addressed to a structural disintegration of the cell-envelope on the nanometer scale.

References
BPA-10 Atomic Force Microscopy as imaging and mechanical device

Maria Sumarokova, Jagoba Iturri, Andreas Weber, Sudarat Tharad, Alberto Moreno-Cencerrado, Jose L. Toca-Herrera

Nanobiotechnology /Biophysics, BOKU, Austria

The versatility of the atomic force microscopy (AFM) for investigating questions related to biophysical and biomaterials science is presented, since AFM can be used as imaging and mechanical machine. In particular, we will present results concerning the characterization of (macro) molecules at different interfaces (e.g. crystallization processes, lipid-protein interactions), molecular/colloidal forces, and biomaterials visco-elasticity. An outlook of the different possibilities to combine the scanning probe microscopy with other microscopy techniques will be briefly discussed.

BPA-11 The all-electric AFM allows imaging of biological samples in opaque liquids

Hannah Seferovic¹, Michael Leitner¹, Sarah Stainer¹, Clemens Kemptner¹, Christian Schwalb², Pinar Frank², Stefan Hummel², Andreas Ebner¹

¹ Institute for Biophysics, Johannes Kepler University Linz, Austria
² GETec Microscopy GmbH, 1220 Vienna, Austria

In our study we optimized an all-electric atomic force microscope (AFM) to investigate biological samples in opaque liquids. The all-electric AFM is working with self-sensing cantilevers using piezoresistors integrated in a Wheatstone bridge configuration, providing high stability during imaging [1]. This overcomes the usage of optical beam deflection readout of commercial AFM systems, which detect the cantilever deflection by laser light, reflected from the coated backside of the cantilever [2]. As no optical readout is needed, the all-electric AFM offers the opportunity to investigate light-sensitive samples as well as samples in opaque environments. The self-sensing cantilevers are easy to handle and user-friendly as no laser alignment is needed. Due to its compact design, the all-electric AFM can be easily combined with other microscopy or spectroscopy techniques or can be integrated in larger cantilever arrays. In addition, imaging of samples, which are difficult to access due to their geometry, is possible [1]. Thus, with the all-electric AFM we investigated samples in biological relevant liquids and tested the performance. We showed that imaging of samples in non-transparent liquids is possible, where today’s commercial AFM systems with an optical readout are limited.

As biological systems, erythrocyte ghost cells have been imaged with the all-electric AFM in dry state as well as in deionized water and physiological buffer solutions. In addition, we were able to perform measurements in opaque liquids such as milk, cell-culture medium, serum and diluted blood. The recorded images have been compared and analysed regarding their topographical structure. For erythrocytes this study was a proof of concept for a new sample preparation.

BPA-12  Nikon's all-new inverted microscope platform for advanced imaging. See more than before!

Ingo Ohlenschläger
Nikon Cee GmbH, Austria

Large-format camera sensors and improvements in data processing capabilities of PCs have facilitated and evolved research trends towards complex microscope applications including an increasing demand for faster and also more reliable data acquisition. Nikon’s all-new inverted microscope platform, the ECLIPSE Ti2, is ready to meet the needs of today’s researchers. With its unparalleled ultra-wide 25mm field of view (FOV) which is up to twice as wide as competitive systems, it enables researchers to truly maximize the utility of large-format detectors. As an added benefit, intensities across this large FOV are also more even resulting in more reliable data in less time. A newly designed and exceptionally stable drift-free platform as well as the latest generation of the industry leading Perfect Focus System (PFS) help to even meet the most challenging microscope application in terms of stability and focus maintenance, while on the other hand unique hardware-triggering capabilities enhance even the most challenging, high-speed imaging applications. An accessible back aperture with photo access and intelligent built in sensors detecting the status of different components on the microscope support the validation of optical technologies and sense whether the microscope is correctly set up for a particular imaging technique. This leads to an easy enhancement of image quality but also to a reduction of potential user errors. Finally, compatible with many existing Nikon and third-party systems, the Ti2 offers significantly more upgrade options than any other microscope allowing seamless integration of future upgrades without requiring system wide modifications.

BPA-13  Structure and Interleaflet Coupling in Asymmetric Lipid Membranes

Barbara Eicher1, Drew Marquardt2, Fred Heberle3, John Katsaras3, Georg Pabst1

1 University of Graz, Austria
2 Department of Chemistry and Biochemistry, University of Windsor, Windsor, Ontario, Canada
3 Shull Wollan Center, Oak Ridge National Laboratory, Oak Ridge Tennessee, USA; The Bredesen Center for Interdisciplinary Research and Graduate Education, University of Tennessee, Knoxville, Tennessee, USA; Biology and Soft Matter Division, Oak Ridge

Biological membranes are asymmetric including the distribution of membrane lipids. Of recent, asymmetric large unilamellar lipid vesicles (aLUVs) have emerged as a new platform for studying fundamental membrane biophysics pertaining to membrane with the advantage to be amenable for broad variety of experimental techniques. In our recent studies we have focused primarily on neutron and X-ray scattering techniques, solution NMR or differential scanning calorimetry to interrogate leaflet specific structural properties of aLUVs on the sub-nanometer scale [2-4]. In particular, this allowed us to address transbilayer coupling and passive lipid flip/flop. Lipid flip/flop, for example was found to be slow in the fluid phase and practically absent in the gel phase. However, membrane defects associated e.g. with the lipid’s melting transition or solid supported bilayers may cause an increase of lipid translocation [3]. Transleaflet coupling was observed only for coexisting gel and fluid phases, but not for all-fluid bilayers. In the case of DPPC/POPC aLUVs, for example, DPPC-enriched gel domains in the outer leaflet were significantly disordered by a coexisting fluid inner leaflet enriched in POPC [1]. For POPE/POPC aLUVs in turn, we found transbilayer coupling when POPE was enriched on the inner leaflet, but not for the reversed system [4]. Hence, transbilayer coupling depends strongly on lipid composition and in the case of DPPC/POPC most likely on partial hydrocarbon chain interdigitation, whereas intrinsic lipid curvature apparently dominates the coupling of leaflets in POPE/POPC aLUVs.

Monday 17th: Biophysics Austria Annual meeting

**BPA-14  X-Ray Studies of Antimicrobial Peptide Activity in *E. coli* Inner and Outer Membrane Mimics**

**Lisa Marx**, Enrico Semeraro, Karl Lohner, Georg Pabst

Institute of Molecular Biosciences/Biophysics Division, University of Graz, Austria

Infectious diseases caused by multi-resistant pathogenic bacteria are rapidly gaining grounds world-wide. One highly promising strategy to combat infectious diseases is based on antimicrobial peptides (AMPs), effector molecules of innate immunity. We performed time-resolved X-ray scattering experiments on live *Escherichia coli* (*E. coli*) under the attack of AMPs. A preliminary global analysis in terms of a multi core-shell model [1] is highly encouraging, however, we need to further substantiate the obtained results at different levels of structural hierarchy. In order to get access to distinct structural information at the different length scales, the *E. coli* scattering intensity is dissected into different components, starting with lipid-only mimics of the inner (IM) and outer (OM) membrane as a bottom up approach.

As a mimic of the inner bacterial membrane we investigated large unilamellar lipid vesicles (LUVs) containing a mixture of phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) to study the specific interactions of AMPs with IM lipids. Further, asymmetric LUVs (aLUVs) were investigated as a mimic of the OM. These vesicles contain a mixture of PE and PG on the inner leaflet of the lipid bilayer and lipopolysaccharides (LPS) on the outer leaflet, using a protocol recently developed by the Pabst group [2]. This enables us to learn about contributions of the IM and OM to the more complex systems of live *E. coli*, as well as specific interactions of the peptides with this lipid matrix. This will be further exploited to ultimately correlate time dependent changes on diverse levels of structural hierarchy in bacteria due to activity of AMPs.

**BPA-15  Uncoupling effect of 2,4 dinitrophenol strongly depends on the membrane lipid composition**

**Olga Jovanovic**¹, Lars Gille¹, Mario Vazdar², Elena E. Pohl¹

¹ Veterinärmedizinische Universität Wien, 1210 Wien, Austria
² Division of Organic Chemistry and Biochemistry, Rudjer Boskovic Institute, Zagreb, Croatia

2,4 dinitrophenol (DNP) is an artificial uncoupler of oxidative phosphorylation in mitochondria and was often used in therapy against obesity in the mid-1930s, which has resulted in a number of negative consequences. Recent studies suggest that depending on the applied concentration, DNP shows either beneficial or toxic effects, suggesting that different molecular mechanisms underlay organism intoxication or the amelioration of several diseases such as diabetes, hepatic steatosis, neuronal dysfunction and degeneration.

In this work we investigated the role of membrane lipid composition on uncoupling effect of DNP combining the measurements of total membrane conductance, order parameter and Z-potential with MD simulations [1, 2]. Our experiments revealed that DNP (i) strongly increases the total membrane conductance and decreases order parameter in membranes formed from 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cardiolipin (CL) compared to membranes composed from 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), DOPC and CL, (ii) leads to a similar increase in the negative Z-potential in both lipid systems. MD simulations show that DNP anions are localised 5 times closer to nitrogen atoms in DOPEs than to those in DOPCs.

We propose that “trapping function” of the PE is based on ability of amine group to form hydrogen bond with DNP anion. We hypothesize that the abundance of lipids with hydrogen bond donor feature in the membrane may play a regulatory role towards the DNP action.

**References**


BPA-16  Electrophysiological characterization of adenine nucleotide translocase (ANT) – mediated proton leak in mitochondria

Jürgen Kreiter, Anne Rupprecht, Elena E. Pohl

Physiology and Biophysics, University of Veterinary Medicine, Austria

The adenine nucleotide translocase (ANT) is the most abundant protein in the inner mitochondrial membrane and exchanges cytosolic ADP with mitochondrial ATP. Apart from its main function, evidence has emerged that ANT is involved in mediating leakage of protons, which is sensitive to free fatty acid (FFA) content and ANT specific inhibitor carboxyatractysolide (CATR)\(^1\). In a model system with artificial bilayer membranes reconstituted with recombinant mANT1\(^2\), we measure membrane conductance in the presence of FFA arachidonic acid, PNs, and ANT specific inhibitor CATR and bongkrekic acid (BKA). We show that ANT mediated proton leak is activated by arachidonic acid and inhibited by all PNs except GMP, CATR and BKA. Our results contribute to understand the mechanism of proton transport mediated by ANT.

\(^{1}\) Brand et al. (2005), Biochem. J., Volume 392, 353–362
\(^{2}\) Beck et al. (2006), BBA-Bioenergetics, Volume 1757, 474–479

BPA-17  Calcium and calmodulin regulation of autophagy transcription factors

Romana Schober Schober\(^1\), Vivian Pogenberg\(^2\), Irene Frischauf\(^1\), Victoria Lunz\(^1\), Matthias Wilmanns\(^2\), Rainer Schindl\(^3\)

\(^1\) JKU Linz, Austria
\(^2\) EMBL Hamburg, Germany
\(^3\) Gottfried Schatz Forschungszentrum, Medical University of Graz, Austria

TFEB and its family members MITF, TFEC and TFE3 are master regulators of autophagy and liposome biogenesis. Upon cell starvation TFEB, but also the mitophagy transcription factor MITF translocate from the cytosol into the nucleus to trigger gene regulation. Interestingly, starvation induces binding of MITF and Calmodulin in the nucleus, in a calcium dependent manner. The binding of Calmodulin to MITF could be efficiently blocked by point mutations of a putative CaM binding site. Moreover, these MITF mutants fail to translocate to the nucleus upon starvation. These results suggest that calcium and Calmodulin play an essential role to regulate the activation of transcription of autophagy and mitophagy transcription factor family.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Opening & Opening Plenary

Opening by Michael Sauer

Chair: Michael F. Jantsch

OP-01 High-level RNA editing in the behaviorally complex cephalopods.

Joshua Rosenthal
Eugene Bell Center, Marine Biological Laboratory/ Univ of Chicago, United States of America

The coleoid cephalopods are the most behaviorally complex invertebrates, exhibiting comparable sophistication to higher vertebrates. They have achieved this through a different evolutionary path because they split from the vertebrate lineage close to 800 million years ago. Have vertebrates and cephalopods evolved common or unique mechanisms for neural complexity? RNA editing by adenosine deamination is a mechanism which all multicellular metazoans use to change genetic information. Catalyzed by the ADAR (Adenosine Deaminases the Act on RNA) family of enzymes, select adenosines in RNA are converted to inosine, a biological mimic for guanosine. When this process occurs within messenger RNAs, it can recode genetic information, creating multiple protein products from a single gene (recoding). Researchers have long appreciated RNA editing’s potential to create genetic diversity. Transcriptome-wide screens for recoding events, however, have demonstrated that editing is infrequently used for this purpose in most organisms. In mammals, flies and nematodes, only a tiny fraction of brain messages harbor a recoding site. Recent work by our team has shown that coleoid cephalopods are a clear exception. The majority of transcripts in their brain transcriptome have at least one recoding event, and many have far more. These sites are enriched in transcripts that encode proteins that are specific for neural function, like ion channels and those that regulate the synaptic vesicle cycle. These editing events are dynamic, being regulated by diverse factors like the physical environment, time of day and age. Furthermore, mRNA editing efficiency is even regulated within squid neurons. The mechanistic underpinnings of high-level recoding in coleoids is not understood, however there are some notable differences in the process compared to other taxa. For example, ADARs are not restricted to the nucleus and editing activity is clearly present in other regions of the neuron. This raises the possibility that cephalopods can regulate protein function regionally within cells.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Plant biotechnology

Chair: Margit Laimer
PB-01  Genome editing using CRISPR/Cas9 in plants and the EU GMO legislation
Stefan Jansson
UPSC, Dept of Plant Physiology, Umeå university, Sweden

Genome editing using CRISPR/Cas9 is a tool with huge potential for basic plant science, but also for plant breeding but the latter potential can only realized if the plants generated would not fall under the definition of a genetically modified organism (GMO), as such are very hard or impossible to use in agriculture. The legal details concerning GMOs differ between counties, and the differences have been further pinpointed when the first examples of plants produced by CRISPR/Cas9 genome editing has been evaluated by competent authorities. In the European union (EU) the resistance towards GMOs has been massive, and the scepticism has also extended to against genome edited plants, but the EU definition of GMOs could not readily be applied to genome edited plants so it is not clear if they are included or not and this have left researchers, breeder and the public in a legal limbo. The scientific community have tried to get clarity, but so far with meagre success. In order to get a concrete case to test, we used CRISPR/Cas9 generated Arabidopsis plants lacking a photosynthetic protein, PsbS, and submitted a question whether they would fall under the GMO legislation. In November 2015, The Swedish Board of Agriculture announced their opinion that plants that have been modified using CRISPR-Cas9 where DNA where only a piece of a genes has deleted and but no novel DNA added, not fall within the scope of the GMO legislation. This opened up the possibility to, in Sweden, grow such plants under the same conditions as “normal crops”, i.e. without the supervision of any authority. The implications of this decision, and the follow-up activities, including the cultivation and cooking of Brassica plants in the summer of 2016, will be presented. The meal got significant attention and has so far been reported in ca 300 media in ca 40 countries. In this talk, the focus will be put on the consequences of the decision and the meal, in particular intriguing issues around the fact that there will now be plants grown in Sweden (and other parts of the world) which are not covered by the GMO legislation, while the legal status of the very same plants in other EU countries is still unclear. This challenges core values of EU like free movement of goods, and pinpoints the inability of EU to adjust its legislation around GMOs to the development in science and technology. The consequences of the ruling of the European Court of Justice on July 25 wil also be discussed.

PB-02  Genetic improvement of the biofuel crop Jatropha curcas (L) by CRISPR/Cas9 mediated genome editing
Markus Freudhofmaier, Fatemeh Maghuly, Margit Laimer
Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

The undomesticated plant Jatropha curcas belongs to the Euphorbiaceae family and is widely distributed in the tropics and subtropics. The seed oil of J. curcas has gained increasing interest for the production of biodiesel, since the uncertainty of non-renewable fuel resources and the effort to environmental protection. However, the genetic improvement of J. curcas is still necessary to gain better biodiesel quality and to remove toxic compounds. Furthermore the reduction of allergenic proteins would reduce the risk for workers during harvest and seed processing. The current work aims at exploring the still untapped potential of J. curcas by CRISPR/Cas9 mediated gene editing. Six genes related to the fatty acid biosynthesis, allergen content and toxin level were selected and analyzed. For each gene a separate knock-out vector containing the Cas9 gene, up to six gRNA expression cassettes and the plant selectable marker nptII were constructed and validated by sequencing. The knock-out vectors were introduced separately to J. curcas leaf discs via Agrobacterium-mediated transformation. Transformed cells were selected on medium containing kanamycin and whole plants will be regenerated from mutant lines by somatic embryogenesis. Induced INDEL mutations will be detected by sequencing and the successful knock-out of targeted genes validated by phenomic and genomic analyses.
PB-03 Utilizing in planta zein protein bodies for oral vaccine applications

Jennifer Schwestka¹, Marc Tschofen¹, Stefan Vogt², Johannes Grillari², Eva Stoger¹

¹ Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Austria
² Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

Storage organelles found in plants such as protein bodies have been successfully used as tools for accumulation and encapsulation of recombinant proteins. Fusion of gamma-zein, a major storage protein of maize, to a protein of interest leads to the incorporation of the chimeric protein into the stable and protected environment inside newly induced protein bodies (PBs). Since zein PBs have been shown to be recalcitrant against digestion by various proteases, found in the gastric milieu [1] and elicit a stronger immune response compared to soluble antigen [2], they hold promise for the bioencapsulation of oral vaccines.

The Peyer’s patch is located beneath the follicle-associated epithelium of the small intestine and plays a crucial role in the induction of mucosal immune response, when it is reached by an antigen. Therefore, we are interested in the uptake of zein-encapsulated vaccines in the intestine. To study this, we fused gamma-zein to green fluorescent protein (GFP) and recombinantly expressed them in leaves of Nicotiana benthamiana.

For isolation and purification, we established a workflow that utilizes two sequential steps of tangential flow filtration, leading to isolated PBs of 1 µm average size.

We studied their uptake in vitro in different human cells including colon epithelial cells, as well as macrophage-like cells by using flow cytometry and confocal microscopy. Zein PBs displayed an increased cellular uptake rate compared to 1 µm polystyrene beads.

Due to their hydrophobic nature, zein PBs tend to aggregate at high concentrations, which exacerbates quantification and characterization. Since it is necessary for future applications to avoid aggregation, we set out to measure zeta-potential in different buffers to obtain a stable dispersion. Furthermore, we characterized PBs in terms of particle count, size distribution and protein content to enable future oral applications.


PB-04 Mutation induction in Coffea spp to counteract the impact of a changing climate

Souleymane Bado, Fatemeh Maghuly, Margit Laimer

Plant Biotechnology Unit (PBU), Dept. Biotechnology, University of Natural Resources and Life Sciences, Austria

Coffee is on the most valuable commodity tree crops worldwide. Nevertheless, the natural diversity in Coffea ssp. is in shortage of pre-breeding genotypes or cultivars with tolerance/resistance to the most devasting diseases and pests, e.g. coffee leaf rust and coffee berry borer, whose impact is being amplified by changing climatic conditions. Mutation breeding through the use of physical and chemical mutagens combined with tissue culture techniques has proven to be effective for broadening genetic variability in many species, subsequently the production of pre-breeding materials and development of improved cultivars. Since coffee offers a wide range of explants and the susceptibility to physical mutagens depends to type of explants, the first step in any mutation breeding approach involves the determination of the explants sensitivity to the mutagens to be applied. Therefore, various explants (seeds, in vitro cuttings, leaves, callus, embryogenic callus, globular and torpedo stage somatic embryos) were exposed to gamma irradiation to establish the optimal mutation induction dose. The evaluation criteria were: shoot and root length, leaf area, survival rate, growth rate. In agreement with results obtained in other plant species, coffee seeds showed the highest tolerance to gamma irradiation (105 - 150Gy) compared to vegetative explants, in which cellular explants (shoot cutting and calli) also exhibited more resistance (40 - 60Gy) than shoot cuttings (10 - 30Gy). This pioneering work provides information on the most suitable explants and data on optimal dose treatments for mutation induction in tissue cultures of coffee, to be exploited for coffee improvement.
PB-05 The GSK3 kinase ASKα contributes to early immune signaling and acclimation to environmental stress.

Hansjörg Stampfl1, Silvia Dal Santo2, Peter Stasnik1, Karoline Steinberger1, Claudia Jonak1

1 Center for Health & Bioresources, AIT - Austrian Institute of Technology, Austria
2 Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Dr. Bohr-Gasse 3, 1030 Vienna, Austria

Unfavorable environmental conditions and pathogen infections limit plant growth and development and thus reduce agronomic yield. Plants have evolved complex cellular and physiological mechanisms to prevent damage and ensure growth under stress conditions. These responses are controlled by different stress-type specific but also common and interacting signaling pathways, which may inhibit each other, explaining the trade-off between biotic and abiotic stress responses. In our work, we identified the Arabidopsis GSK3 kinase ASKα as a positive regulator of both abiotic and biotic stress responses. Plants deficient in ASKα are more sensitive to high soil salinity and are more susceptible to Pseudomonas infection, whereas plants with increased ASKα activity are more resistant to salt stress and pathogen infection. Salt stress and pathogen infection enhance ASKα activity, which in turn phosphorylates and thereby increases cytosolic glucose 6-phosphate dehydrogenase (G6PD) activity. G6PD is the key enzyme of the oxidative pentose phosphate pathway providing NADPH. Remarkably, while under salt stress conditions, enhanced G6PD activity contributes to the removal of excess levels of ROS via the ascorbate/glutathione cycle and thus stress tolerance, upon pathogen infection G6PD activity is necessary for ROS production by NADPH oxidases and a successful defense response. Overall, our data provide evidence that ASKα and G6PD constitute a signaling module that links protein phosphorylation cascades to metabolic adjustment under both abiotic and biotic stress conditions.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna,
September 17-20, 2018, Vienna, Austria

Neuroimmunology

Chair: Sandra Siegert
NI-01  From yolk sac to neurodegeneration: the multiple facets of microglia

Marco Prinz

University of Freiburg, Germany

The diseased brain hosts a heterogeneous population of myeloid cells, including parenchymal microglia, perivascular cells, meningeal macrophages and blood-borne monocytes. To date, the different types of brain myeloid cells have been discriminated solely on the basis of their localization, morphology and surface epitope expression. However, recent data suggest that resident microglia may be functionally distinct from bone marrow- or blood-derived phagocytes, which invade the CNS under pathological conditions. During the last few years, research on brain myeloid cells has been markedly changed by the advent of new tools in imaging, genetics and immunology. These methodologies have yielded unexpected results, which challenge the traditional view of brain macrophages. On the basis of these new studies brain myeloid subtypes can be differentiated with regard to their origin, function and fate in the brain (1,2).

References:

NI-02  Direct effects of general anesthesia on microglia

Alessandro Venturino, Rouven Schulz, Gloria Colombo, Sandra Siegert

Institute of Science and Technology IST Austria, Austria

General anesthesia is commonly used during surgical procedures to induce an unconscious state, muscle relaxation and absence of pain sensation. Two commonly used anesthetics in animal research are isoflurane, a GABAa receptor agonist, and KXA, which consists of the NMDA receptor blocker ketamine, the paralyzer xylazine, and the sedative acepromazine. Anesthetic drugs either increase neuronal inhibition or block excitation to depress nerve-cell function, however their effect on glial cells is not well understood.

Here, we focus on microglia, the brain macrophages, which express a repertoire of neurotransmitter and ATP receptors, and are able to respond to abnormal neuronal firing rates. We induced general anesthesia with either isoflurane or KXA in adult C57BL/6J animals and analyzed microglia morphology in different brain regions after 4 and 48 hours. We found that KXA induced morphological changes in microglia after 4 hours across all brain regions, which were recovered after 48 hours. This effect was not observed with isoflurane at the selected time points. Next, we performed in vivo 2-photon imaging of Cx3CR1-GFP+/- animals before, during and after anesthesia induced by KXA. We found that microglia indeed change their morphology in response to KXA administration within 4 hours.

To reveal whether KXA directly affects microglia, we prepared primary microglia cell culture and imaged their response upon KXA application. We observed that microglia rapidly reduced their movement and remained immobile. This effect was only observed with ketamine and not with xylazine or acepromazine. Next, we investigated the changes on the proteomic level. We found that several lysosomal associated proteins are downregulated, whereas cytoskeleton remodeling was increased. Surprisingly, the endosomal/lysosomal trafficking molecule Cd68 was upregulated. When we investigated changes of Cd68 expression level in the brain, we verified the Cd68 increase, however in a region-specific manner. We rescued this effect when we applied an antagonist of ketamine.

In summary, we show that microglia respond to KXA induced general anesthesia. This has important implications for general anesthesia in clinical research.
NI-03  On the day-to-day functional relations between interleukin-6 and mood, irritation and mental activity in a breast cancer survivor Running title: Dynamics between interleukin-6 and emotions

Christian Schubert¹, Carmen Hagen⁰

¹ Clinical Department of Medical Psychology, Innsbruck Medical University, Innsbruck, Austria
² Medizinische Universität Innsbruck, Austria

Interleukin-6 (IL-6) is a pleiotropic cytokine with pro- and anti-inflammatory properties orchestrating a brougth spectrum of immunological and non-immunological (1,2). In patients with breast cancer, high levels of circulating IL-6 are associated with tumor progression, recurrence and shorter survival time (3). Besides its direct effect on cancer progression, IL-6 is also related to behavioral side effects of breast cancer such as fatigue, depression and cognitive disturbances (4). As evidence linking IL-6 to poor outcome and low quality of life in breast cancer patients grows, it becomes increasingly important to understand how psychosocial factors of daily life impact synthesis and function of this versatile cytokine. Stress theories consider emotions the final link in the chain from environmental triggers to biological response, what makes them a suitable target in psychoneuroimmunology (PNI) (5). However, studies on emotions and IL-6 in breast cancer patients are rare and data is equivocal. This data inconsistency could be due to methodological limitations: Most studies in PNI measure variables only at a few time points and pool data across individuals thereby ignoring dynamic aspects of the emotion-immune interplay. This ‘integrative single-case study’ investigated the bidirectional cause-effect-relations between various emotional states (i.e., mood, irritation, mental activity) and urinary IL-6 levels in a 49-year old female breast cancer survivor under conditions of ’life as it is lived’. During a period of 28 days the patient collected her entire urine in 12 h intervals for IL-6 measurement and completed every morning and evening a list of adjectives regarding mood, irritation and mental activity. ARIMA modeling revealed a 4-day cycle in the IL-6 time series. Furthermore, cross-correlational analyses after controlling for serial dependencies (significance level: p<0.05) showed that decreases in mood and mental activity, as well as increases in irritation were followed by increases in urinary IL-6 levels with temporal delays between 12 and 36 h. In the opposite direction of effect, increases in urinary IL-6 levels were followed by increases in mood and mental activity, as well as decreases in irritation with temporal delays between 48 and 72 h. These cross-correlational results are strong indicators of real-life negative feedback loops and demonstrate that IL-6 could be involved in health as well as in sickness behavior.

NI-04  A study on PanK2 enzyme pathology in erythrocytes from PKAN patients

Maike Werning⁰, Ernst Müllner⁵, David Baron⁵, Thomas Klopstock⁵, Ulrich Salzer⁵

¹ Department of Anaesthesia, Intensive Care Medicine and Pain Medicine, Medical University of Vienna
² Friedrich Baur Institute, Neurological Clinic, Ludwig Maximilian University, Munich
³ Department of medical Biochemistry, MFPL, Medical University of Vienna, MFPL, Austria

Neuroacanthocytosis (NA) syndromes comprise a group of very rare neurodegenerative disorders characterized by neurodegeneration within the basal ganglia and the occurrence of misshaped erythrocytes called acanthocytes. One of these disorders is pantothenate kinase-associated neurodegeneration (PKAN), which is caused by mutated pantothenate kinase 2 (PANK2). PanK catalyzes the first and rate-limiting step in coenzyme A (CoA) biosynthesis. By studying the molecular alterations of mutated Pantothenate kinase 2 (PanK2) in PKAN erythrocytes, we aim to increase our understanding of the molecular mechanisms underlying neurodegeneration in NA. We confirmed the presence of PanK2 in normal erythrocytes by quantitative Western blot analyses and established a quantitative pantothenate kinase activity assay. Erythrocytes of 15 PKAN patients with different mutations were analyzed and compared to wild-type and healthy carriers. 8 patients exhibited no, 5 low (≤ 30% relative to control) and 2 considerable (41% and 57%) PanK2 activity. Western blot analysis revealed low (<10%) PanK2 expression in 9 patients, intermediate in 4 patients (<25%) and considerable PanK2 expression (45% and 74%) in 2 patients. By combining these data we are able to identify PanK2 mutations that rather affect the activity and others that affect the stability of the PanK2 protein. Together with the clinical data, this research will provide valuable insight in the molecular cause of PKAN neuropathology. Moreover, this study highlights that erythrocytes are a valuable source for studying enzyme pathology which could be interesting also in the context of other neurodegenerative diseases.
The Role of PI3K/PTEN in Microglia Functions during Homeostasis and Neuroinflammation

Andrea Vogel, Melanie Hofmann, Julia S. Brunner, Martina Kerndl, Hannes Datler, Omar Sharif, Gernot Schabbauer

Medical University Vienna, Institute for Vascular Biology and Thrombosis Research, Austria

Microglia, the resident tissue macrophages of the immune privileged central nervous system (CNS) are crucially involved in brain development, pathogen recognition and maintaining tissue homeostasis by scavenging dying cells. As central immune effector cells in the brain, microglia activation is an extensively-described feature in many CNS diseases such as multiple sclerosis. However, their exact role and impact on disease pathogenesis remains controversial and ill-defined. The Phosphatidylinositol 3-kinase (PI3K) is a central cellular hub and myeloid PTEN deficiency strikingly skews the inflammatory phenotype of macrophages. We therefore hypothesized that PI3K/PTEN influences microglia functions during homeostasis and inflammation. To determine a potential role of PI3K/PTEN in microglia we analyzed total neural cell count and microglia frequency in mice overexpressing PTEN (PTEN^{Tr^+}) or in myeloid specific PTEN knock out mice (PTEN^{fl/fl}LysM^{Cre^+}). We further investigate frequency and activation state of microglia and other myeloid cell populations in autoimmune-driven CNS inflammation. Therefore experimental autoimmune encephalomyelitis (EAE) was induced and brain leucocytes were analyzed by flow cytometry. We observed that PI3K/PTEN expression notably affected microglia numbers in brains of healthy, adult mice. In contrast myeloid PTEN deficiency, resulting in sustained PI3K activity led to higher cell counts of microglia. By using tdTomato reporter mice we could confirm that LysMcre recombinase targets approximately 70% of the microglia pool. In a murine model of multiple sclerosis, microglia change their activation state and upregulate important activation marker such as MHCII. Beside activated and resting microglia, EAE bearing mice further exhibit a population of infiltrating monocytes. Myelin scavenging, representing a crucial process in the resolution of autoimmune-driven CNS inflammation, was reduced upon PI3K inhibition in vitro. Overall, our data provide new insights in how PI3K activity shapes microglia phenotypes in homeostasis and neuroinflammation.
Monday 17th: From RNAomics to function

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

*From RNAomics to function*

Chairs: Alexandra Lusser & Michael F. Jantsch
RNA-01  C. elegans germ cell development: a GoLDmine to unearth post-transcriptional RNA control mechanisms

Christian Eckmann
Developmental Genetics, Martin Luther University Halle-Wittenberg, Germany

Tissue and organ formation requires the generation of complex protein expression patterns. Regulated protein synthesis, in the form of cytoplasmic post-transcriptional mRNA regulation, is a powerful mechanism to shape protein gradients across cells and tissues, in time and space. While translational control is a ubiquitous phenomenon in physiological gene expression control, gene-specific translational control is predominantly emanent during metazoan germ cell development, early embryogenesis and a key process in memory formation.

Research in my lab aims to uncover the molecular mechanisms of post-transcriptional mRNA regulation and elucidates how cytoplasmic mRNA-binding and mRNA-modifying proteins build RNA regulatory networks and how they dynamically organized themselves in tissue-specific RNA granules. We study these gene expression mechanisms and subcellular liquid-liquid-phase transitions during germline specification and formation, using Caenorhabditis elegans as our main animal model. My talk will focus on cytoplasmic poly(A) polymerase complexes, a unique class of conserved mRNA regulators. I will discuss their mechanism of action and their roles in animal development.

RNA-02  Exitrons, alternatively spliced internal regions of protein-coding exons: from their discovery to pan-cancer profiling

Peter Venhuizen¹, Ido Tamir², Yamile Marquez³, Andreas Sommer², Maria Kalyna¹

¹ Department of Applied Genetics and Cell Biology (DAGZ), University of Natural Resources and Life Sciences - BOKU, Austria
² Vienna Biocenter Core Facilities GmbH (VBCF), Austria
³ Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Spain

Previously, we have identified unusual alternative splicing events that allow intra-exonic protein-coding sequences to be differentially spliced (Marquez et al., 2012, 2015). As these events involve introns with both exonic and intronic features, we named them exitrons (exonic introns). Exitrons utilize all canonical core splicing signals, but these are usually weak. Since they are internal parts of the annotated protein-coding exons, they do not contain stop codons. At least 3.7% of human protein-coding genes contain exitrons as evidenced by analyses of seven tissue and HER-2/ERBB2 positive breast cancer transcriptomes. More than half of the exitrons have sizes of multiples of three nucleotides. Splicing of these exitrons results in internally deleted protein isoforms often with changes in protein domains, disordered regions and various post-translational modification sites, thus broadly impacting protein function. Exitrons are present in genes that are frequently mutated in cancers and that are commonly used as cancer markers and potential targets for anticancer therapeutics. Splicing of ~9% of exitrons is misregulated in Her-2/ERBB2 positive breast cancer affecting genes with known functions in cancer. We will present and discuss analyses of other cancer types to identify further instances of misregulated exitron splicing events with potential roles in cancer.

RNA-03 From RNA structure to function - The RNA structureome

Andrea Tanzer, Bernhard Thiel, Veerendra Gadekar, Roman Ochsenreiter, Ivo Hofacker

Department of Theoretical Chemistry, University of Vienna, Austria

RNA secondary structures have proven essential for understanding the regulatory functions performed by noncoding RNAs like microRNAs and RNA elements such as riboswitches and attenuators. This success is in part due to the availability of efficient computational methods for predicting RNA secondary structures, for instance the ViennaRNA package.

In a recent genome wide screen we identified over 600,000 conserved RNA structure elements in the mouse genome. We explicitly include repeat-masked regions to explore the potential of transposable elements and low complexity regions to give rise to regulatory RNA elements. By comparing our results to gene annotations, repeat classes, transcriptomics data and RNA editing sites we propose functional classes of these RNA elements.

3'-UTRs of protein coding genes and small non-coding RNAs are enriched for structures, while coding sequences are depleted. Repeat associated RNA elements make up about 95% of the homologous loci identified and are, as expected, predominantly found in intronic and intergenic regions. Nevertheless, we report structures enriched in specific genome elements, such as 3'-UTRs and IncRNAs.

We built a publicly available track hub to visualize our results via the UCSC genome browser and thereby make the underlying data easily accessible to the research community. With this presentation we wish to encourage researchers from other disciplines to use this resource for further functional analysis of the RNA elements predicted in our study.

RNA-04 NSUN5 methylates 28S rRNA and modulates cell proliferation in humans and mice

Clemens Heissenberger1, Fabian Nagelreiter1, Elena Stelzer1, Teresa Krammer1, Yulia Gonskikh2, Norbert Polacek2, Martin Koš3, Johannes Grillari1, Markus Schosserer1

1 Department of Biotechnology, University of Natural Resources and Life Sciences, Austria
2 University of Bern
3 Heidelberg University

Eukaryotic ribosomes are complex molecular machines composed of proteins and four different ribosomal RNAs, which are extensively modified. Up to now, three general types of ribosomal RNA modifications are known: 2'-O-methylations, pseudouridinylations and base methylations. The importance of such modifications for organismal fitness was evidenced by a recent report of our group, showing that lack of a single C5 methylation on rRNA, which is introduced by NSUN5, increased the lifespan and stress resistance of yeast, worms and flies.

Here, we provide a basic molecular characterization of human and mouse NSUN5. For this purpose, we deleted NSUN5 in HeLa cells and mice by CRISPR/Cas9, identified the corresponding methylation site on ribosomal RNA and investigated its effects on global protein synthesis and translational fidelity. Furthermore, we identified the catalytic center of NSUN5, as well as its protein domains responsible for nucleolar localisation. Interestingly, lack of NSUN5 decreases cell size and proliferation, both in human and murine cells.

We anticipate that this work will help to elucidate how subtle alterations of the translational machinery, such as lack of a single base modification in close proximity to the decoding centre, impact on cellular growth and ribosome function.
RNA-05  Guardians of the ribosomal proteins - in search of dedicated chaperones and importins
Ingrid Rössler, Julia Unterluggauer, Jutta Hafner, Brigitte Pertschy
Institute for Molecular Biosciences, University of Graz, Austria

The ribosome is an outstanding molecular machine that synthesizes all cellular proteins and is assembled as well as matured in a complex process involving more than 200 ribosome biogenesis factors. In the course of this process ribosomal proteins have to be transported into the nucleus to be incorporated into pre-ribosomal particles. Thereby, chaperones and importins protect the ribosomal proteins from aggregation and guard them from the cytoplasm through the nuclear pore complex into the nucleus.

Up to the present only a few specific chaperones for ribosomal proteins have been identified and characterized. In this study, we performed a screen to discover so far unknown specific chaperones, importins and other non-ribosomal interaction partners of ribosomal proteins. All ribosomal proteins of the 40S subunit were purified via "tandem affinity purification" and potential interaction partners were identified by semi-quantitative mass spectrometry. This analysis revealed that ribosomal proteins are bound to different importins, suggesting they utilize different import pathways. Moreover, we identified several so far unknown specific partners of ribosomal proteins. Our data suggest that these bind to their ribosomal protein clients and increase their solubility, thereby promoting the efficient assembly of new ribosomes.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Life Science Awards & Anniversary Ceremony

Moderation: Lukas Huber

Awards sponsored by

[Brands and logos]
AW-01  The branched chain amino acids in autism spectrum disorders.

Dora Clara Tarlungeanu
Neuroscience, IST Austria, Austria

Autism spectrum disorders (ASD) are a group of genetic disorders often overlapping with other neurological conditions. Despite the remarkable number of scientific breakthroughs of the last 100 years, the treatment of neurodevelopmental disorders (e.g. autism spectrum disorder, intellectual disability) remains a great challenge. Recent advancements in genomics, like whole-exome or whole-genome sequencing, have enabled scientists to identify numerous mutations underlying neurodevelopmental disorders. Given the few hundred risk genes that were discovered, the etiological variability and the heterogeneous phenotypic outcomes, the need for genotype- along with phenotype-based diagnosis of individual patients becomes a requisite.

Driven by this rationale, in a previous study we described mutations, identified via whole-exome sequencing, in the gene BCKDK – encoding for a key regulator of branched chain amino acid (BCAA) catabolism - as a cause of ASD. Following up on the role of BCAAs, in the study described here we show that the solute carrier transporter 7a5 (SLC7A5), a large neutral amino acid transporter localized mainly at the blood brain barrier (BBB), has an essential role in maintaining normal levels of brain BCAAs. In mice, deletion of Slc7a5 from the endothelial cells of the BBB leads to atypical brain amino acid profile, abnormal mRNA translation and severe neurological abnormalities. Interestingly, we demonstrate that BCAA intracerebroventricular administration ameliorates abnormal behaviors in adult mutant mice. Furthermore, whole-exome sequencing of patients diagnosed with neurological disorders helped us identify several patients with autistic traits, microcephaly and motor delay carrying deleterious homozygous mutations in the SLC7A5 gene.

In conclusion, our data elucidate a neurological syndrome defined by SLC7A5 mutations, support an essential role for the BCAAs in human brain function and might have a crucial impact on the development of novel individualized therapeutic strategies for ASD.

AW-02  Production of recombinant protein with polysialylated N-glycans in Nicotiana benthamiana

Somanath Kallolimath
Department für Angewandte Genetik und Zellbiologie, Universität für Bodenkultur, Austria

Polysialic acid (polySia) is a unique post-translational modification composed of sialic acids in α 2,8 linkage, specifically found on Neural Cell Adhesion Molecules (NCAM) and certain neuro-invasive bacteria. PolySia enhances the bioavailability of therapeutic proteins, promotes repair of damaged neural cells and is involved in anti-inflammatory activities [1]. However, the enormous structural complexity together with technological hurdles in producing recombinant proteins with polySia render their functional investigation challenging.

Plants display a remarkable tolerance towards manipulation of their intrinsic glycan biosynthetic pathways. Gene knock-in and knock-down/out strategies are intensively applied to reconstruct human type glycosylation. We have generated plants to produce recombinant glycoproteins with terminal sialic acid (ΔXTFTSia) [2]. Here we set out to engineer polysialylation into the commonly used plant expression host Nicotiana benthamiana. By expression of two human polysialyltransferases (ST8Sial I or ST8SialIV) in the ΔXTFTSia platform, we observed autopolySialylation of the recombinant enzymes. Importantly, by co-expression of Ig5FN1, a well-known naturally polysialylated domain of NCAM, we showed synthesis of polysialylated structures on the recombinant Ig5FN1. HPLC analysis of the plant-produced Ig5FN1 shows that it carries complex N-glycans with a chain length of up to 40 sialic acid residues. Moreover, plant-produced polySia is functionally active, as determined by the inhibition of microglia activation assay [3].

To evaluate the impact of NCAM domains on polysialylation, we designed erythropoietin (EPO) fusions with one of the two NCAM domains (EPOig5, EPOFN1) and EPOlc. The results indicate that, at least in plants, the mode of action of human polysialyltransferases does not involve docking to the FN1 domain. At this point, it is clear that polysialylation is possible for plant-produced EPO.

In conclusion, the plant-based polySia platform will allow both the investigation of the functional role of polySia and the development of polySia-dependent therapeutics with enhanced or novel functions.

References
AW-03  Genome-wide assessment of sequence-intrinsic enhancer responsiveness at single-base-pair resolution

Cosmas Arnold
Research Institute of Molecular Pathology (IMP), Austria

Differential gene expression is the driving force that governs the coordinated differentiation of cells and thus the development and metabolism of multi-cellular organisms. Differential gene expression has commonly been attributed to dynamic enhancer activities that are sequence-intrinsic, allowing them to be studied outside of their native environment e.g. in massively parallel reporter assays. In contrast, the contribution of core promoters to the regulation of gene expression remained largely unexplored. Although a key function of core promoters is to convert enhancer activities into gene transcription, how they respond to activating enhancer input, i.e. their enhancer responsiveness, has not been systematically assessed on a genome-wide level.

To study core promoter function and enhancer responsiveness, we developed a novel plasmid-based method, Self-Transcribing Active Core Promoter-sequencing (STAP-seq). We assessed the sequence-intrinsic responsiveness for millions of candidate genomic sequences from the Drosophila melanogaster genome. In brief, we cloned candidate fragments at the position of the core promoter (also called minimal promoter) in transcriptional reporter constructs with or without a strong enhancer, transfected the resulting library into cells, and quantified the transcripts that initiated from each candidate for each setup by deep sequencing. In the presence of a single strong enhancer, the enhancer responsiveness of different sequences differs by up to 1,000-fold, and different levels of responsiveness are associated with genes of different functions.

Importantly, we observe that core promoters can respond differentially to enhancers associated with developmental (i.e. cell-type specific) or housekeeping gene transcription, a finding indicative of transcriptional programs being separated at the level of enhancer – core-promoter specificity. We also identify sequence features that predict the responsiveness of different core promoters to different enhancers and show that the respective genes differ in function, thus offering an explanation how they are employed for the regulation of gene expression.

AW-04  SLAM-seq defines direct gene-regulatory functions of the BRD4-MYC axis

Matthias Muhar
Research Institute for Molecular Pathology, Austria

Misregulated gene expression underlies diverse human diseases including cancer, for which key transcriptional regulators such as BRD4 and MYC have emerged as promising therapeutic targets. To understand transcriptional and cellular functions of these and other factors, it is crucial to know which genes they directly control. Experimentally, this is achieved by perturbing a regulator and measuring subsequent changes in gene expression. However, conventional genetic tools combined with RNA sequencing cannot distinguish directly regulated mRNAs from secondary effects due to slow and diverse kinetics in mRNA- and protein turnover. To accurately measure immediate responses to acute cellular perturbations, we adopted SLAM-seq, a novel method for mapping and quantifying newly synthesized transcripts within the total mRNA pool.

SLAM-seq relies on the alkylation of metabolically labeled RNA, which can be detected by deep sequencing. We first applied SLAM-seq to study inhibitors of intracellular signaling, CDK9 and BET bromodomain proteins, capturing specific and global responses in transcription at time scales precluding the onset of secondary effects. Profiling responses to combinatorial drug treatment further revealed the transcriptional basis of known drug synergies.

We next generalized this approach for the study of transcriptional regulators such as BRD4, using a chemical-genetic strategy. Proteins tagged with a short recognition peptide (AID) can be licensed for degradation by an exogenous adapter protein, Tir1 upon addition of its inducer auxin. Auxin treatment of genetically engineered cancer cells triggered degradation of AID-tagged BRD4 within 30 minutes. Subsequent SLAM-seq profiling revealed a broad role of BRD4 in mRNA production, which we could further attribute to its function in release of paused RNA polymerase 2. The transcription factor MYC, together with a small number of additional BRD4-targets showed pronounced hypersensitivity to BET bromodomain inhibition, which could account for its therapeutic effects. To assess the consequences of targeting MYC for cancer therapy, we performed chemical-genetic degradation of MYC followed by SLAM-seq, which revealed a selective role in the regulating cellular metabolism across different cancer types. In summary, our study defines gene-regulatory functions of BRD4 and MYC in cancer, and establishes a simple, fast and scalable approach to map direct transcriptional targets of any gene or pathway.
Native mass spectrometry unravels highly complex glycosylation patterns in biopharmaceuticals

Therese Wohlschlager
Department of Biosciences, University of Salzburg, Austria

The commercial use of biopharmaceuticals, i.e. therapeutic proteins, has revolutionized modern medicine. Manufacture of biopharmaceuticals is performed in bacterial or eukaryotic expression systems, requiring extensive purification of the target product. In order to ensure highest-level safety and efficacy of the drug compounds, rigorous control of a large set of chemical, physical, and biological properties is obligatory. Characterization of glycosylation is conventionally performed at the released glycan or glycopeptide level using generic high-performance liquid chromatography (HPLC) and high-resolution mass spectrometry (MS) methods. Intact protein characterization, on the other hand, represents a powerful alternative approach in that it may resolve co-existing glycoforms and reveal highly distinct glycosylation patterns.

We implemented native MS for the characterization of intact Etanercept (Enbrel™), a highly N- and O-glycosylated recombinant Fc-fusion protein applied in the therapy of arthritic diseases. Taking advantage of the higher spatial resolution at lower charge states detected under native conditions, more than 80 different isoforms of the 130 kDa protein were distinguishable in the highly complex mass spectra. Assignment of specific glycoforms was achieved upon enzymatic digestion of the molecule using a set of glycosidases and proteases. Information gained at lower structural levels (i.e. glycopeptides, protein subunits) was successfully integrated to facilitate glycoform annotation at higher structural levels (i.e. whole protein upon partial deglycosylation) through the application of advanced computational tools. Finally, we demonstrate native MS as a rapid fingerprinting tool for the assessment of batch-to-batch variability at the intact protein level.

Comprehensive information on glycoform heterogeneity, fast analysis with minimal sample preparation and product-characteristic fingerprints render our method highly attractive for quality control as well as for comparability studies. Assessment of biosimilarity and relative quantification of glycoforms will be further explored in the future.
Monday 17th: Poster Session Biophysics

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session Biophysics
PSBA-01  **ABC transporters with a degenerate NBS stay active by avoiding nucleotide occlusion**

Yaprak Dönmez-Cakil, Katalin Goda, Daniel Szöllősi, Gábor Szalóki, Zahida Parveen, Dóra Türk, Gergely Szakács, Peter Chiba, Thomas Stockner

1 Pharmacology, Medical University of Vienna, Austria
2 Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Nagyerdői krt. 98, 4032 Debrecen, Hungary
3 Institute of Medical Chemistry, Center for Pathobiology and Genetics, Medical University of Vienna, Waehringerstrasse, 10, 1090 Vienna, Austria
4 Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar Tudósok körúja 2, H-1117 Budapest, Hungary
5 Institute of Cancer Research, Medical University of Vienna, Borschkegasse 8A, 1090 Vienna, Austria

A significant number of ABC exporters carry a degenerate nucleotide binding site (NBS), which shows a strongly reduced ATPase activity. The hallmark of the degenerate NBS, which is typically NBS1, is the substitution of the catalytic glutamate in the Walker B motif. The multidrug transporter ABCB1 (P-glycoprotein) and the bile salt export pump ABCB11 (BSEP) are both full-length members of the ABCB subfamily and share 49% sequence identity, but importantly, ABCB1 has two canonical NBSs, while in ABCB11 one is degenerate. A comparison of their NBD interfaces reveals that the entire interface differs in only four residues, which are all located in NBS1. The divergent residues are the catalytic glutamate of the Walker B motif of NBD1, the Q-loop residue S474 and the residues G1178 and Q1180 in the signature sequence of ABCB1. The respective residues in ABCB11 are M584, E502, R1221, and E1223. When introducing the analogous catalytic glutamate mutation (E556M) into ABCB1, the transporter becomes transport incompetent and conformationally restrained. However, when including the additional three mutations, the transporter regains the ability to hydrolyse ATP and undergo conformational changes also in the TMDs, which are reminiscent of wild type ABCB1. Simulations showed that the single mutant is conformationally locked and occludes ATP similar to WT ABCB1. The prevention of hydrolysis by the E556M mutation therefore leads to an arrest of the transport cycle. In contrast, the quadruple mutant changes the mode of ATP binding by altering the geometry of the NBD dimer and weakening the interactions between ATP and the NBS. Thus, the non-canonical NBS1, as present in ABCB11, has the ability to escape the conformationally locked state of the single catalytic glutamate mutant (E556M) by avoiding ATP occlusion.

Acknowledgements
Supported by the Austrian Science Fund (SFB 35).

PSBA-02  **Passive membrane permeability of ascorbic acid**

Christof Hannesschläger, Peter Pohl

Institut f. Biophysik Molecular and Membrane Biophysics, Johannes Kepler University Linz, Austria

Vitamin C (VC) - a collective term for the different oxidation and protonation forms of ascorbic acid (AscH) - is an essential micronutrient that serves as (i) a potent antioxidant and (ii) a cofactor of a manifold of enzymatic processes. Its role in health is related to redox balance maintenance, which is altered in diseases such as obesity, cancer, neurodegenerative diseases, hypertension and autoimmune diseases. Despite its importance, VC uptake has been poorly investigated. Available literature values for the passive membrane permeability \( P \) of lipid bilayers for AscH scatter by about 10 orders of magnitude. Here, we show by voltage clamp that \( P \) of AscH’s anionic form is negligible. To cross the membrane, the deprotonated AscH picks up a proton in the membrane vicinity and releases it on the other side of the membrane. This leads to a near-membrane pH drop that was visualized by scanning pH microelectrodes. The AscH concentration dependent pH profiles indicated \( P = 1.1+/-0.1 \times 10^{-8} \text{ cm/s} \). Thus, AscH’s \( P \) is comparable to that of sorbitol and much lower than that of other weak acids like acetic acid or salicylic acid.
**PSBA-03**  
**Effect of cholesterol on lipid binding behavior of Bacillus thuringiensis cytolytic protein Cyt2Aa2**  

Sudarat Tharad{1}, Öykü Üzülmez{1}, Boonhiang Promdonkoy{2}, Jose L. Toca-Herrera{1}  

{1} Nanobiotechnology, University of Natural Resources and Life Sciences (BOKU), Austria  
{2} Biosources research laboratory, National center for Genetic Engineering and Biotechnology, National Sciences and Technology Development Agency, 113 Phahonyothin Road, Khlong Nung, Khlong Luang, Pathumthani 12120, Thailand  

*Bacillus thuringiensis* (Bt) is a well-known bacterium in agricultural field because of its pesticidal property. Cytolytic toxin (Cyt toxin) is a member of delta-endotoxin family. Its cytolytic activity exerts against insect and mammalian cells. The cytolytic mechanism has been studied for a decade but it is still under discussion. Two models are proposed: pore forming and detergent-like action models. In this work, the weight ratio between POPC and cholesterol (Chol) was varied to form lipid membrane models of the insect cells (5:0 and 5:0.2 ratios) and mammalian cells (5:1, 5:2, and 5:3 ratios). Cyt2Aa2 from Bt subsp. * darmstadiensis* was used throughout in this experiment. The lipid binding behavior of Cyt2Aa2 on the membrane models was elucidated by combining Quartz Crystal Microbalance with Dissipation (QCM-D) and Atomic Force Microscopy (AFM). QCM-D results revealed that cholesterol enhanced the kinetics of lipid-Cyt2Aa2 binding. Interestingly, at lipid/Chol bilayer of 5:3 ratio, Cyt2Aa2 changed its binding behavior after the frequency and dissipation values reach to -30 Hz and 2.3x10^{-6} (saturated value of the other lipid mixtures), respectively. The changes of behavior led to a significant increasing of frequency (-207 Hz) and dissipation (43x10^{-6}) values but the system did not reach equilibrium. In addition, AFM measurements provided additional information of Cyt2Aa2/lipid structure. After 2 hours of incubation, a strip pattern was observed for Cyt2Aa2/lipid layer at the lipid/Chol of 5:0 and 5:0.2 ratios. On one hand, no patterned structure was found for the Cyt2Aa2/lipid/Chol layer of 5:1 and 5:2 ratios. Specifically, the reorganization of the layer and the observed ring shape structures were detected for the lipid/Chol of 5:3 ratio. The subsequent structures were detected after Cyt2Aa2 introduction; (i) strip pattern with hole, (ii) disappearance of hole, and (iii) ring shapes. A strong repulsion between the AFM tip and the Cyt2Aa2/lipid also supports the idea of Cyt2Aa2 binding onto the membrane model of 5:3 ratio was different from the other ratios. Our findings suggest that although cholesterol is not necessary for Cyt2Aa2 binding onto lipid bilayers, it increases the binding rate and changes the binding behavior, maybe because cholesterol reduces a lipid mobility and/or changes lipid acyl chain packing. Finally, our results suggest that the disruption mechanism of Cyt2Aa2 either on insect cells or on mammalian cells might be different.

---

**PSBA-04**  
**Human Monoclonal Antibody Binding to Poly N-Acetylglucosamine with Single Molecule Force Spectroscopy**  

Begüm Dikecoglu{1}, Gerald B. Pier{2}, Peter Hinterdorfer{1}  

{1} Applied Experimental Biophysics, Institute of Biophysics, Austria  
{2} Division of Infectious Diseases, Department of Medicine, Brigham and Women’s Hospital/Harvard Medical School, Boston, Massachusetts  

Poly-N-acetylglucosamine (PNAG) has been identified as surface polysaccharide antigen expressed by many microbial pathogens during human and animal infection. The group of Gerald Pier developed a human monoclonal antibody (Mab) - F598- that bound to PNAG and evidenced its biomedical relevance.[1,2] Here, nanomechanical molecular properties of MAb F598 that bound to PNAG antigen was studied using single molecule force spectroscopy. For this, PNAG antigen was tethered to AFM tips via a flexible polyethylene glycol (PEG) linker and MAB F598 was tethered to a silicon nitride surface using the same chemistry, or vice versa. Antibody-antigen binding events were studied in force-distance cycles and revealed force profiles reflecting multiple parallel and sequential antibody-antigen bond breakages.

References  
PSBA-05  Novel small molecule photoswitches for precise control of native TRPC channels
Oleksandra Tiapko1, Sanja Curcic1, Annarita Graziani1, Niroj Shrestha1, Toma Glasnov2, Gema Guedes de la Cruz2, Rainer Schindl1
1 Gottfried Schatz Research Center (for Cell Signaling, Metabolism and Aging), Chair of Biophysics, Medical University of Graz, Austria
2 Institute of Chemistry, University of Graz, Graz, Austria

Introduction: Lipid-gated TRPC3 channels are highly expressed in neuronal- and cardiovascular tissues. Precise pharmacological control of their activity in native cells can provide important insight into organ function/dysfunction and is expected to serve as a basis of novel therapies. Photopharmacology opens an avenue towards exceptionally high spatial and temporal accuracy in intervention. We have recently developed a novel optical probe (OPtoDArG1) suitable to govern recombinant TRPC channels and set out to generate further small molecule photoswitches that allow for selective control of native TRPC channels.

Methods: We synthetized a series of compounds with potential activity at the TRPC3-channel complex and integrated an azabenzene moiety into these structures to enable photoisomerization by light. Compounds were screened for TRPC modulation during light induced cis-trans isomerization at the level of ionic currents, cellular Ca2+ as well as functional parameters.

Results: Using the azobenzene photoswitch moiety, we generated a light-sensitive GSK1702934A2 derivative (OptoBI-1) that allows for efficient, optical control of TRPC3 channel activity and associated cellular Ca2+ signalling. Importantly, OptoBI-1 clearly outperforms photolipids in terms of efficient and precise manipulation of TRPC-mediated Ca2+ signaling. OptoBI-1 was found to enable not only control of recombinant TRPC channels but also high-precision temporal control of TRPC3-linked cell functions in native cells such neurons, vascular endothelial- and mast cells.

Conclusions: We introduce a novel photopharmacological strategy for control of native TRPC conductances.


PSBA-06  Localization and quantification of clinically relevant epitopes on ultra-flat red blood cell ghosts
Sarah Stainer, Andreas Ebner
Institute of Biophysics, Johannes Kepler University Linz, Austria

Atomic force microscopy (AFM) is a powerful tool when it comes to analyzing biological samples in their physiological environment. A sharp tip is scanned over a surface. By using an optical deflection read out system, images with nanometer resolution can be obtained. Due to its ability to measure in liquid environment and by minimizing the applied force on the substrate, biological samples can be analyzed. However, the ability of AFM goes further than its imaging capability. If a ligand is attached to the AFM tip, receptor-ligand interactions can be detected and analyzed. Furthermore, an application has been developed that combines the imaging mode and the recognition detection ability of the device. This technique is called “Simultaneous Topography and Recognition Imaging” (TREC), where not only topographical information is recorded, but also an image is created simultaneously that unravels the location of receptor-ligand interactions.

We used TREC imaging to investigate the epitope location of Rhesus Factor D on red blood cells. A special preparation protocol was developed to produce flat erythrocytes by flushing out the cells interior, creating a perfect ultra-flat substrate for TREC studies. Whole Erythrocytes were imaged to deduce the RhD antigen quantity and distribution patterns over the cell. We developed an effective approach to elucidate erythrocyte antigen location and quantity, which is promising to further map distributions of other clinically relevant binding epitopes like weak-D and partial-D RhD antigens.
Tuesday 18th

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Tuesday 18th September 2018
P1-01 Unconventional secretion in the emerging fungal model *Ustilago maydis*: From discovery to application.

*Kerstin Schipper*, Marius Terfrüchte, Michèle Reindl, Jörn Aschenbroich, Kai Hußnätter

Institute for Microbiology, Heinrich Heine University Düsseldorf, Germany

The corn smut fungus *Ustilago maydis* is currently gaining increasing momentum as a valuable system for both basic and applied biology. Its biotechnological potential includes the production of enzymes for novel applications or biomass degradation and the production of valuable secondary metabolites. In addition, the fungus contains not only the classical eukaryotic secretion system but also an additional pathway for unconventional secretion which can be exploited for the production of specific recombinant proteins. Unconventional secretion has first been observed for the chitinase Cts1 during basic research. This enzyme does not harbor a classical N-terminal secretion signal and thus, circumvents the Endoplasmic Reticulum passed during conventional secretion. Instead, it is translocated to the fragmentation zone during cytokinesis and released by dividing yeast cells. Interestingly, Cts1 can deal as a carrier to export heterologous proteins of interest, thereby evading N-glycosylation. Hence, we aim to exploit Cts1-mediated unconventional secretion to establish a novel protein production platform. Pharmaceutical proteins are one promising target of this secretory pathway, because inappropriate N-glycosylation may lead to allergic reactions in humans. The system is currently evaluated and optimized on different levels including extracellular protease activity, culturing conditions and downstream processing to achieve competitive protein yields in the future.
Vascular endothelial cells in the central nervous system (CNS) form a barrier that restricts the movement of molecules and ions between the blood and the brain. This blood-brain barrier (BBB) is crucial to ensure proper neuronal function and protect the CNS from injury and disease. Although the properties of the BBB are manifested in the endothelial cells, transplantation studies have demonstrated that the BBB is not intrinsic to the endothelial cells, but is induced by interactions with the neural cells. Here we use a genomic, genetic and molecular approach to elucidate the cellular and molecular mechanisms that regulate the formation and function of the BBB. We have identified a critical role for pericytes in regulating the permeability of CNS vessels by inhibiting the properties that make endothelial cells leaky. In particular, pericytes limit the rate of transcytosis through endothelial cells as well as the expression of leukocyte adhesion molecules in CNS endothelial cells, which limits CNS immune infiltration. Furthermore, we have developed methods to highly purify and gene profile endothelial cells from different tissues, and by comparing the transcriptional profile of brain endothelial cells with those purified from the liver and lung, we have generated a comprehensive resource of transcripts that are specific to the BBB forming endothelial cells of the brain. We have further examined the profile of CNS endothelial cells following injury and disease and have identified molecular mechanisms by which pericytes control BBB formation, which are then disrupted during neurological disease leading to BBB dysfunction.
Tuesday 18th: Poster Flash 1

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Flash 1

Chair: Monika Schmoll & Winfried Neuhaus
**PF1-01 Overexpression of an acetylation-insensitive acetyl-CoA synthetase in E. coli W and its effect on glucose and acetate co-utilization in batch and continuous cultures**

Katharina Novak, Lukas Flöckner, Anna Maria Erian, Philipp Freitag, Christoph Herwig, Stefan Pflügl

Institute of Chemical, Environmental and Biological Engineering, TU Wien, Austria

*Escherichia coli* W is used for the production of several metabolites and recombinant proteins, especially due to its high stress tolerance as well as low acetate excretion. Most studies were performed using glucose as carbon source and simultaneous co-utilization of glucose and other substrates such as acetate is promising, but still demanding. Acetate assimilation and dissimilation is regulated by the activity of several enzymes. One of the key enzymes, the high affinity acetyl-CoA-synthetase, is on one hand regulated on a transcriptional level by carbon catabolite repression, and is on the other hand inactivated by post-translational acetylation. To improve acetate uptake in the presence of glucose and thus enable efficient co-utilization, *E. coli* W was genetically engineered to overexpress an acetylation insensitive acetyl-CoA-synthetase. This strain was characterized in batch and continuous cultures using glucose, acetate and a mixture of both. In batch cultures with glucose and acetate, acetate uptake during the exponential growth phase was 2.7-fold higher in the engineered strain overexpressing an acetylation insensitive acetyl-CoA synthetase, thus indicating more efficient co-utilization of glucose and acetate. The engineered strain showed decreased batch durations when acetate was used as the sole source of carbon, possibly by circumventing the toxicity of acetate more efficiently. In accelerostat cultivations, *E. coli* W was shown to be a naturally efficient co-utilizer of glucose and acetate over a broad range of dilution rates (0.20 – 0.70 h⁻¹). The overexpression of acetylation insensitive acetyl-CoA-synthetase resulted in acetate accumulation and cell wash-out at lower dilution rates compared to the control strain. Gene expression analysis revealed in imbalance in the ratio of *acs* and *pta-ackA*. Higher expression levels of the ATP-consuming *acs* could cause increased energy dissipation and hence explain the early wash-out. Additionally, the membrane proteins *yjcH* and *actP*, genes co-transcribed with acetyl-CoA synthetase showed significant down-regulation at higher dilution rates. In this study, *E. coli* W was shown to be able to efficiently co-utilize glucose and acetate, revealing different behaviors in batch and continuous processes.

**PF1-02 Ribosomal RNA methylation by rram-1 modulates development and healthy lifespan**

Clemens Heissenberger¹, Teresa Krammer¹, Fabian Nagelreiter¹, Jarod A. Rollins², Santina Snow², Aric Rogers², Johannes Grillari¹, Markus Schossberger¹

1 Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
2 Mount Desert Island Biological Laboratory, Bar Harbor, Maine, USA

The ribosome has been seen for decades as a static machine that translates mRNAs into proteins. However, over the last few years it became clear that it rather represents a highly dynamic structure that responds to various stimuli by adapting its structure and, as a consequence, its function. Such structurally distinct ribosomes are postulated to be “specialized ribosomes” comprising peculiar functional properties and are thus considered to be engaged in translating specific subsets of cellular messages (Filipovska and Rackham, 2013; Xue and Barna, 2012). Although ribosomal RNA is extensively modified by methylations and pseudouridyations (Rozenzki et al., 1999), the functional roles of such modifications in regulating translation and physiology are not yet fully understood.

*m³A674* methylation of 26S rRNA is introduced by rram-1 in *Caenorhabditis elegans* (Yokoyama et al., 2018), is preferentially associated with fully assembled 80S ribosomes and modulates translation of a specific set of mRNAs. Interestingly, both rram-1 expression and methylation at A674 are influenced by age and feeding protocol. Furthermore, knockout of rram-1 delays development, extends lifespan and improves locomotion at advanced age. Thus, methylation of ribosomal RNA represents an important regulator of organismal aging and our work will contribute to a better understanding of the underlying molecular mechanisms.
PF1-03  The influence of ketogenic diet on psoriasiform-like skin inflammation

Felix Locker¹, Julia Stockinger², Sepideh Aminzadeh-Gohari², Daniela Weber³, Philippe Sanio², Andreas Koller², René Günther Feichtinger², Barbara Kofler², Roland Lang²

1 Department of Biomedical Sciences, University of Veterinary Medicine, Austria
2 Department of Pediatrics, Research Program for Receptor Biochemistry and Tumor Metabolism, Paracelsus Medical University, Salzburg, Austria.

Psoriasis is an inflammatory skin disease characterized by increased neo-vascularization, keratinocyte hyperproliferation, a pro-inflammatory cytokine milieu and immune cell infiltration and is associated with metabolic syndrome, obesity and diabetes. Several reports indicate that dietary intervention with ketogenic diet (KD), a high in fat and low in carbohydrate diet, and omega-3 fatty acids (ω-3 FA) can reduce inflammation and angiogenesis. Therefore, the aim of the present study was to elucidate the impact of a 4:1 (ratio of fat to carbohydrate + protein) long chain triglyceride based KD (LCT-KD) ± ω-3 FA and LCT-KD with 30% medium chain triglycerides (MCT) (LCT/MCT-KD) ± ω-3 FA, to prevent, delay or reduce severity of Imiquimod (IMQ)-induced psoriasis-like skin inflammation in mice compared to standard diet (SD) ± ω-3 FA.

Six weeks old male mice (C57BL/6N) were adapted to KD only diets over a 10 day period. Thereafter, mice were fed KDs 7 days prior to daily topical application of IMQ on depilated back skin for 3 or 6 consecutive days. Disease severity, changes in vascularization, neutrophil infiltration and myeloperoxidase (MPO)-activity as well as mRNA levels of key cytokines were assessed.

In comparison with SD, LCT/MCT-KD ± ω-3 FA significantly increased disease severity and showed increased neutrophil influx and MPO activity in the skin. Furthermore, IL-1β and IL-17A mRNA were significantly increased in LCT-KD and LCT/MCT-KD supplemented with ω-3 FA. However, LCT-KD + ω-3 FA showed no increased skin inflammation, neutrophil activity and abundances compared to SD. Mice fed with SD + ω-3 FA showed a markedly reduced skin inflammation and reduced neo-vascularization upon IMQ treatment as compared to the SD group. Our results demonstrate that addition of MCTs to KD worsens psoriasiform-like skin inflammation in mice indicating that the dietary FA composition is crucial in mediating pro- or anti-inflammatory effects.

The study was supported by the Paracelsus Medical University research fund (PMU-FFF E-16/24/125-LAL).

PF1-04  Insight to the mechanism of action of fungi fitness regulator

Razieh Karimi Aghcheh

Dept. of Molecular Microbiology and Genetics, Georg August University Göttingen, Germany

Understanding of the coordination of metabolic and morphological functions of fungi is required for a biotechnological control of the formation of desired bioactive products. The fungal putative methyltransferase LaeA/LAE1 homologs their gene products act at the interphase between secondary metabolism, cellulase production and development. Lack of the corresponding genes results in significant physiological changes including loss of secondary metabolite and lignocellulose degrading enzymes production. The molecular mechanism of LaeA/LAE1 function which may link epigenetic to transcriptional control is unknown. Only an automethylation function of Aspergillus nidulans LaeA was found at methionine-207 which is close to the AdoMet binding site. The biological significance of LaeA automethylation is yet unknown because mutant proteins revealed that the methylation site Met207 is not required for secondary metabolites (SMs) biosynthesis. Aspergilli and Trichoderma represent different biotechnologically significant species with significant differences in the LaeA/LAE1 and their target proteins. The conserved Met207 in LaeA/LAE1 of several filamentous fungi is replaced by a lysine residue at the respective position in T. reesei LAE1; T. reesei LAE1 is unable to complement a laeA-null mutant of A. nidulans and this protein and A. nidulans LaeA differ also from each other by a hypervariable N-terminal and C-terminal region. Here, we present our most recent data about the potential interaction partners of LaeA/LAE1 and the importance of N-and C-terminal ends of this protein in its interaction. Furthermore, the role of Met207 for the action of T. reesei LAE1 will be discussed.
**PF1-05 Antimicrobial Resistance Markers in Molecular Diagnostics: Good Enough for the Clinic?**  
**Norhan Mahfouz, Inês Ferreira, Stephan Beisken, Andreas Posch**  
Ares Genetics GmbH, Austria

Antimicrobial resistance (AMR) is a global health threat with a projected annual casualty rate of > 10 Mio by 2050. In molecular diagnostics, AMR is routinely assessed by PCR, a molecular biology method to detect the presence or absence of known AMR markers. One drawback of PCR is the limited number of markers that can be detected in parallel, especially for multi-drug resistant bacteria.

Driven by declining costs of Next Generation Sequencing (NGS), NGS is increasingly replacing PCR for pathogen characterization and outbreak monitoring but is not yet used for AMR testing in clinical routine. In contrast to PCR, NGS enables genome-wide detection of AMR markers and can provide a high resolution assessment of AMR.

Public resources like The Comprehensive Antibiotic Resistance Database, ARG-ANNOT or ResFinder have emerged as repositories for AMR determinants and many software tools have been developed for NGS-based, i.e. genotype-based, AMR detection based on these databases.

However, *in silico* genotype-based methods may suffer from high false positive and / or false negative rates. In order to achieve accurate AMR diagnostic tests based on bacterial genotyping, the gap between AMR genotype detection and AMR phenotype prediction needs to be closed. This is especially challenging as the detection of a single AMR determinant may not always translate to phenotypic resistance with a clinically significant MIC change.

To address this problem, we evaluate the diagnostic performance of publicly available AMR determinants in testing for resistance against different antibiotics on a dataset of bacterial isolates exhibiting genotypic and phenotypic diversity. We use our proprietary Ares Database comprising a representative set of thousands of thoroughly profiled clinical isolates from different bacterial species that were collected globally over the last 30 years.

The results show the advantages and limitations of current AMR resources for clinical practice and diagnostic use. There is significant room for improvement in diagnostic performance that, *inter alia*, can be achieved by introducing novel combinations of AMR determinants as well as newly discovered resistance markers to existing combinations of AMR determinants from public resources. The results highlight the instrumental role of a curated AMR database combining whole-genome pathogen information with antibiotic resistance profiles for the translation of NGS-based testing from research to clinical practice.

---

**PF1-06 Molecular adaptations of epidermal barrier keratins in association with evolutionary land-to-water transitions of mammals**  
**Florian Ehrlich, Heinz Fischer, Bettina Strasser, Erwin Tschachler, Leopold Eckhart**  
dep. of dermatology, medical university of vienna, Austria

Skin appendages such as hair and nails depend on keratins that build the cytoskeleton of cornifying cells. Other keratins are expressed at the epidermal barrier to the environment. Fifty four different keratins play specialized roles in human epithelia and appendages and similar numbers are present in other terrestrial mammals. Here, we performed a comparative genomics study to determine the conservation and loss of suprabasal epidermal keratin genes in different phylogenetic lineages of mammals. We found a striking decrease in the number of keratins in cetaceans (dolphins and whales) which corresponds to the decrease in the complexity of their skin. Keratins specifically expressed in hair follicles and nails were lost while a minimal set of hair keratin-like proteins were conserved, perhaps to facilitate the formation of hard papillae on the tongue. However, also the diversity of keratins in the epidermis is reduced in cetaceans. In contrast to the basal epidermal keratins K5 and K14, which are highly conserved among mammals, the suprabasal epidermal keratins K1, K2 and K10 have been inactivated in cetaceans. Transcriptomics of dolphin skin showed high expression levels of keratins K6 and K17, which are markers of stress and disease-associated epidermal thickening in humans, and, remarkably, the thickness of the epidermis has dramatically increased during the evolution of cetaceans. These data suggest that epidermal gene expression has been remodelled in cetaceans, and skin appendage- and skin barrier-specific cytoskeletal proteins of terrestrial mammals are dispensable in a fully aquatic environment.
Tuesday 18th: Optimization of microbial workhorses for biotechnology

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Optimization of microbial workhorses for biotechnology

Chairs: Monika Schmoll & Anton Glieder
OMB-01  Tailoring *Trichoderma reesei* for optimal production of heterologous proteins

Christopher Landowski

Industrial Biotechnology, VTT Technical Research Centre of Finland Ltd., Finland

*Trichoderma reesei* is a filamentous fungus that is used world-wide as a host for industrial enzyme production. It is a low cost production system that secretes its native enzymes at levels exceeding 100 g/L of culture medium. *T. reesei* is capable of high levels of protein production, but is also an active secretor of proteases.

Host proteases are often a major barrier limiting production of heterologous proteins. We have adapted the fungus to become more suitable for therapeutic protein production by reducing secreted protease activity and altering glycosylation pathways needed for adding mammalian glycoforms. After deleting or silencing the most critical protease genes, the secreted protease activity was reduced over 30-fold. Expression levels and product quality of several therapeutic proteins improved dramatically after multiple protease deletions and optimization of culture conditions. Monoclonal antibodies could be produced up to 7.6 g/L, Fab antibody fragments up to 8.2 g/L, interferon alpha-2b at 7.9 g/L, and insulin-like growth factor fusion protein at 8 g/L. Human glycoforms such as G0 and FG0 were produced on monoclonal antibodies.

The strongest promoters used in this system typically require inducing molecules, which create limitations and generate extra costs in the process. To simplify and improve the production process we have employed a novel orthogonal expression system based upon a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The great benefit is that the new expression system allows for high level, constitutive expression in diverse growth conditions and growth stages. Multiple sTF-binding sites can be designed to enable selection of a wide range of target gene expression levels, from very low to extremely high.

We have taken advantage of this unique expression system to improve native and heterologous protein production in the industrial microbe *T. reesei*. We have been able to produce high levels of secreted proteins without the use of inducing molecules. For instance, with *T. reesei* we could produce the *Candida antarctica* lipase B and bovine beta-lactoglobulin at high levels and high purity when grown in glucose medium. The new universal expression system allows for simpler media and bioprocesses to be used for industrial protein production.

OMB-02  The time has come: Introducing QbD into inclusion body refolding processes

David Wurm¹, Britta Eggenreich¹, Christoph Herwig¹, Gerald Berghammer², Oliver Spadiut¹

¹ Institut für Verfahrenstechnik, Umwelttechnik und Technische Biowissenschaften, TU Wien, Austria
² Bilfinger Industrietechnik Salzburg GmbH

The prominent recombinant host organism *E. coli* tends to produce insoluble protein aggregates of the recombinant product, so-called inclusion bodies (IBs). In such case a harvesting, solubilization and refolding step is necessary to obtain correctly folded and active product. To date, IB processing, especially the process step refolding, is still based on empiricism rather than sound process knowledge contradicting current quality by design (QbD) guidelines. Here we demonstrate the benefit of a novel refolding vessel, which allows the control of different process parameters, for refolding of model proteins as well as industrially relevant proteins such as a single chain variable fragment and a therapeutic enzyme.

Within this work we able to develop a platform tool for controlled refolding of proteins from *E. coli* inclusion bodies. By our approach we were able to increase the refolding yield by reducing formation of agglomerates and precipitates. We showed that the protein concentration during refolding could be significantly increased using a fed batch approach and the correct refolding conditions. This saves costs by reducing the vessel and we were able to increased the space time yield more than 10 fold.

We believe that this novel strategy which complies with the QbD guidelines as requested by the FDA will soon be generally implied in production processes as this approach sheds light onto the black box “refolding” and allows more flexibility and more efficient production processes.
OMB-03  Designing production envelopes and yield spaces of cell factories
Steffen Klamt\textsuperscript{1}, Stefan Müller\textsuperscript{2}, Georg Regensburger\textsuperscript{3}, Jürgen Zanghellini\textsuperscript{4}

\begin{itemize}
\item \textsuperscript{1} Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
\item \textsuperscript{2} Faculty of Mathematics, University of Vienna, Austria
\item \textsuperscript{3} Institute for Algebra, Johannes Kepler University Linz, Austria
\item \textsuperscript{4} Austrian Center of Industrial Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria, Austria
\end{itemize}

Production rates and yields are key parameters of biochemical transformation processes. While optimal rates and phase spaces are readily studied with flux-balance analysis (FBA) approaches, optimal yields and yield spaces are rarely systematically analyzed. Often elementary flux modes (EFMs) are used to characterize yields and yield-optimal pathways. However, EFMs characterize the unbounded flux cone and are incompatible with non-zero flux bounds and allocation constraints often used in FBA. By resorting to the concept of elementary flux vectors (EFVs), it is possible to generalize the idea of unique metabolic pathways to also account for inhomogeneous linear constraints. We show that any rate-optimal FBA solution sits in an optimal polyhedron spanned by (certain) EFVs. This holds true not only for rate-optimal but for yield-optimal solutions too, which cannot be found by standard FBA approaches. Next, we demonstrate that (optimal) yield spaces can be readily calculated even in genome-scale metabolic models by linear-fractional programing without explicitly enumerating EFVs. Although phase spaces and yield spaces often are of similar shapes (and therefore sometimes confused), they carry very different information. In a realistic analysis based on \textit{E. coli}, we show how these complementary pieces of information can be used to understand and optimally shape the metabolic capabilities of cell factories with any desired yield and/or rate requirements. We conclude that EFVs provide an unifying framework for the theoretical description and analysis of any constraint-based model under arbitrary linear constraints. More specifically, EFVs close the gap between biased FBA approaches and unbiased EFM approaches and allow one to fully characterize and shape metabolic phase spaces and yield spaces. This reinforces the fundamental importance of EFVs (or EFMs) as the “coordinates of metabolism”. However, an explicit enumeration of EFVs is not required as phase spaces and yield spaces can be efficiently computed even in genome-scale metabolic networks.

OMB-04  Optimization of the industrial workhorse \textit{Trichoderma reesei} towards improved xylanase production
Jonas Ramoni\textsuperscript{1}, Martina Marchett-Deschmann\textsuperscript{2}, Verena Seidl-Seiboth\textsuperscript{3}, Bernhard Seiboth\textsuperscript{4}

\begin{itemize}
\item \textsuperscript{1} Ares Genetics GmbH, Austria
\item \textsuperscript{2} Institute of Chemical Technologies and Analytics, TU Wien, 1060 Vienna, Austria
\item \textsuperscript{3} Institute of Science and Technology Austria, Am Campus 1, 3400 Klosterneuburg, Austria
\item \textsuperscript{4} Molecular Biotechnology, Research Division Biochemical Technology, Institute of Chemical Engineering, TU Wien, Gumpendorferstraße 1a, 1060 Vienna, Austria.
\end{itemize}

\textit{Trichoderma reesei} is a paradigm for the regulation and industrial production of plant cell wall-degrading enzymes. Among these, five xylanases, including the glycoside hydrolase (GH) family 11 XYN1 and XYN2, the GH10 XYN3, and the GH30 XYN4 and XYN6, were described. By genome mining and transcriptome analysis, a further putative xylanase, encoded by \textit{xyn5}, was identified. Analysis of \textit{xyn5} from the genome-sequenced reference strains \textit{T. reesei} QM6a and RUTC-30 shows that it encodes a non-functional, truncated form of XYN5. Employing multi-strain sequencing a non-truncated putatively functional orthologue of \textit{xyn5} was identified in a \textit{Trichoderma spp.} wild-type isolate. \textit{In-silico} analysis and 3D modeling of this gene revealed that the encoded XYN5 has significant structural similarities to xylanases of the GH11 family, including a GH-typical substrate binding groove and a carbonylato pair in the active site. The putatively functional \textit{xyn5} gene was recombinantly overexpressed in a hemicellulose negative QM6a derivative and the corresponding protein was purified to apparent homogeneity. The pH and temperature optima and the kinetic parameters of the purified XYN5 were determined to be pH 4, 50 °C, and \(V_{\text{max}} = 2646 \text{ nkat/mg} \) with a \(K_m \) of 9.68 mg/ml. Furthermore, the functional \textit{xyn5} allele was used to replace the mutated version in \textit{T. reesei} which led to a significant improvement of the overall xylanolytic activity of the fungus. These findings are of particular importance as GH11 xylanases are of high biotechnological relevance, and \textit{T. reesei} is one of the main industrial workhorses to produce such lignocellulose-degrading enzymes.
OMB-05  Phosphodiesterases impact sexual development and the block of cellulase gene expression in light in *Trichoderma reesei*

Sabrina Beier\(^1\), Eva Stappler\(^1\), Wolfgang Hinterdobler\(^1\), Doris Tisch\(^2\), Lukas Feiler\(^1\), Jianping Sun\(^3\), Louise N. Glass\(^3\), Monika Schmoll\(^1\)

\(^1\) Austrian Institute of Technology, Austria
\(^2\) Research Area Gene Technology and Applied Biochemistry, Vienna University of Technology, Getreidemarkt 9, 1060 Wien, Austria
\(^3\) Plant and Microbial Biology Department, University of California, Berkeley, CA 94720, USA

Plant cell wall degradation is of major importance for sustainable production of materials and fuel in the future. The biotechnological workhorse *Trichoderma reesei* produces the required enzymes as well as heterologous proteins for industrial applications.

We showed previously, that cellulase gene expression is regulated by light in *T. reesei* and that the photoreceptor ENV1 is involved in this process. Thereby, light tolerance with respect to cellulase production is increased in early high producer mutants such as QM9414. However, in the wild-type QM6a, cellulase levels drop dramatically in light. ENV1 is essential for elevated cAMP levels during growth in light and exerts its function at least in part via the cAMP pathway. It was assumed that ENV1 acts by dampening the function of phosphodiesterases. We tested this hypothesis by investigating mutants in the phosphodiesterase genes *pde1* and *pde2* in *Neurospora crassa*, which showed only minor regulatory effects with respect to cellulase formation or gene regulation upon growth on cellulose.

In contrast, in *Trichoderma reesei* we found that indeed PDE2 dampens transcript levels of the major cellulase gene *cbh1* in light on cellulose and this effect is mediated by ENV1. Accordingly, the light dependent growth defect of mutants lacking ENV1 prevails in double mutants with phosphodiesterases. Additionally our first data also suggest an effect on secondary metabolism.

In summary, we found an important contribution of a phosphodiesterase to cellulase gene expression as well as rewiring of the associated light dependent signaling pathway between *T. reesei* and *N. crassa*.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Next-generation pathogen & antibiotic resistance diagnostics

Chairs: Andreas Posch & Dorothea Orth-Höller
NPA-01  Next-generation sequencing for next generation clinical microbiology and infection prevention.
John W. Rossen¹, Monika Chlebowicz¹, Natacha Couto¹, Silvia García-Cobos¹, Anna M. Kooistra-Smid², Alexander W. Friedrich¹

¹ Department of Medical Microbiology and Infection Prevention, University of Groningen, University Medical Center Groningen, Netherlands
² Certe, Department of Medical Microbiology, Groningen, The Netherlands

Current molecular diagnostics of human pathogens provide limited information that is often not sufficient for outbreak and transmission investigation. Next generation sequencing (NGS) determines the DNA sequence of a complete bacterial genome in a single sequence run, and from these data, information on resistance and virulence, as well as information for typing is obtained, useful for outbreak investigation. The obtained genome data can be further used for the development of an outbreak-specific screening test. Like every new technology adopted in microbiology, the integration of NGS into clinical and routine workflows must be carefully managed. As the microbiology laboratories have to adhere to various national and international regulations and criteria for their accreditation, quality control issues for using WGS in microbiology, including the importance of proficiency testing, are presented. In addition, applications of NGS in the clinical setting are discussed, such as outbreak management, molecular case finding, characterization and surveillance of pathogens, rapid identification of bacteria using the 16S-23S rRNA region, taxonomy, and metagenomics approaches on clinical samples. Finally, we share our vision on the use of NGS in personalised microbiology in the near future, pointing out specific requirements.

NPA-02  Culture-independent identification of pathogens and antibiotic resistance genes via a ligation-based microarray chip
Noa Wolff, Michaela Hendling, Silvia Schönthaler, Ivan Barišić

Health & Bioresources, AIT Austrian Institute of Technology, Austria

Careless use of antibiotics is discussed as a major reason for the appearance of antibiotic resistance [1]. Therefore, it is of crucial importance to be able to identify pathogens and their putative antibiotic resistance genes, resulting in a proper disease treatment. Today's gold standard, however, are culture-based identification methods, which are time-consuming, error-prone and inapplicable in terms of certain pathogen strains.

A DNA-based method, utilizing a ligase-dependent labelling method, was described by Barišić and co-workers, able to reliably identify pathogens [2, 3]. We have further enhanced this method towards a multiplex detection that enables high-throughput screening, thereby not only identifying pathogens, but also resistance genes and virulence factors.

For this purpose, 810 genes were chosen that encode either clinically relevant antibiotic resistance genes or virulence factors. In addition, phylogenetic marker sequences were added to clearly identify the clinically most important pathogens. The required DNA probe sequences were designed using the oligonucleotide design tool Oli2go [4] and immobilized on the microarray chip surface.

After pre-testing with synthetic DNA, PCR-amplified cell lysates of clinical samples were analysed by means of this microarray technique, which is capable of pathogen and resistance gene identification within 5 hours. In a next step, it needs to be further refined towards the application of unamplified cell lysates.

References
NPA-03 Influence of Antibiotic Pretreatment on Molecular Diagnostics in a Staphylococcus aureus Blood Stream Infection Model

Matthias Plecky1, Anita Schildberger1, Ludwig Knabi2, Dorothea Orth-Höller2, Viktoria Weber3

1 Department for Biomedical Technology, Donau Universität Krems, Austria 
2 Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Schöpfstraße 43, A-6020 Innsbruck, Austria 
3 Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis, Department for Health Sciences and Biomedicine, Danube University Krems, Dr.-Karl-Dorrek-Strasse 30, 3500 Krems, Austria

Background: Rapid pathogen identification is a prerequisite for efficient treatment of septic patients. qPCR methods offer some advantages, as compared to classical blood culture, as they are much faster and do not require selective culture steps. However, the isolation of pathogen DNA from samples is a critical step, especially when using pathogen enrichment, and little is known about possible influence factors, such as bilirubin, lipids, heparin, or antibiotics on the preanalytical process. We therefore investigated the effect of commonly used antibiotics on the recovery of S. aureus from whole blood by a commercial system for pathogen diagnostics (GINA pathogen DNA Enrichment, CubeDX, St. Valentin, Austria).

Methods: Whole blood anticoagulated with heparin, freshly drawn from healthy donors, was inoculated with three culture type strains of S. aureus (1000 CFU/mL). Samples were incubated for 4 h at 37°C to allow the pathogen to adapt to and grow in the whole blood matrix, after which they were aliquoted and treated with piperacillin, ciprofloxacin, vancomycin, clindamycin, or 0.9 % NaCl as a control at 37°C with agitation for further 1.5 h. Each sample was plated on LB-agar 3-fold to determine colony forming units (CFU). DNA was isolated using the GINA pathogen extraction kit and qPCR was performed using S.aureus specific primers.

Results & Discussion: While CFU/mL decreased in blood culture in the presence of antibiotics, Ct values remained stable, providing evidence that pathogen inactivation by antibiotic treatment did not result in loss of pathogen DNA during enrichment. In conclusion, our results indicate that pathogen enrichment by the GINA kit is not prone to the influence of antibiotics pre-treatment in case of S. aureus blood stream infections. It is likely that our findings also apply to other pathogen enrichment systems based on selective lysis and centrifugation.

NPA-04 Evaluation of clinical isolate de novo sequencing for pathogen & antibiotic resistance diagnostics using BGISEQ-500

Marko Fritz1, Jing Zou2, Stephan Beisken1, Valentina Galata3, Jingjing Wang2, Yong Chen2, Jonas Ramoni1, Norhan Mahfouz2, Yongping Li4, Achim Plum5, Andreas Keller3, Hui Jiang2, Hongdong Tan2, Andreas E. Posch1

1 Ares Genetics GmbH, Austria 
2 MGI, BGI-Shenzhen, Shenzhen 518083, China 
3 Chair for Clinical Bioinformatics, Saarbrücken, 66123, Germany 
4 BGI Genomics, BGI-Shenzhen, Shenzhen, 518083 China 
5 Curetis GmbH, Holzgerlingen, 71088, Germany

Antimicrobial resistance (AMR) is a global health threat with a projected annual casualty rate of more than 10 Mio by 2050. In molecular diagnostics, AMR is routinely assessed by PCR, a molecular biology method to detect the presence or absence of genetic AMR markers. In contrast to PCR, whole genome sequencing (WGS) allows to read a pathogen’s entire genome for subsequent interpretation via a genetic antibiotics resistance database to comprehensively identify all AMR markers within a single test.

Performant next generation sequencing (NGS) platforms are a key prerequisite for high-quality WGS and subsequent AMR marker detection. While several bioinformatics challenges need to be solved to fully unlock the potential of NGS in infectious disease testing, here we evaluate the applicability of NGS platforms for AMR diagnostics from clinical isolates. We present a one-on-one comparison of BGI (BGISEQ-500) and Illumina (HiSeq 2000/2500) sequencing technology based on a comprehensive set of multi-drug resistant pathogens including Klebsiella pneumoniae and Escherichia coli that are deeply sequenced using 100 base paired-end technology on both platforms. Isolates are selected from the ARES Database (ARESdb), which combines genetic AMR markers with resistance phenotypes for thousands of thoroughly profiled pathogens that have been collected globally over the last 30 years.

In this study, both NGS platforms are systematically evaluated on the raw read, assembly and annotation level and, finally, tested for AMR marker recovery based on ARESdb as well as public resources including the Comprehensive Antibiotic Resistance Database [1]. Resistance phenotypes as determined via broth microdilution included in ARESdb are used as ground truth to assess clinical utility of both sequencing technologies and resolve platform specific discrepancies in AMR marker detection.

The results provide first insights into the utility of the combinatorial probe-anchor synthesis (cPAS) based BGI sequencing platform for pathogen and antibiotic resistance diagnostics. For the selected set of clinical isolates, BGISEQ-500 performs comparatively to Illumina sequencing technology, increasing the options of available NGS platforms for the translation of NGS-based infectious disease testing from research to clinical practice.

NPA-05  Hand-held DNA-sequencing and biosensing with nanopores

Stefan Howorka

Department of Chemistry, University College London, United Kingdom

Portable DNA sequencing and biosensing can advance research, bedside-diagnostics, and homeland security. I describe how label-free sensing is achieved with atom-scale designed membrane nanopores. In this strategy, nanopores act as electronic sensors that detect when individual molecules pass the pores' nanoscale hole. The temporary blockages cause changes in ionic pore current. The approach has helped pioneer portable DNA sequencing with protein pores\(^{(1)}\) to discriminate individual bases. More recently, synthetic pores have been built by folding DNA strands into defined channels\(^{(2)}\). The DNA nanopores are relevant as they overcome the narrow size range of protein pores and thereby accommodate folded protein analytes. The DNA nanostructures are also easier to rationally design than proteins\(^{(3)}\) and thereby enable new applications, also in synthetic biology\(^{(4)}\).

(1) *Nature* 2014 516 250;
(2) *Nat. Nanotechnol.* 2016 11 152;
(3) *Nat. Nanotechnol.* 2017 12 619;
(4) *Science* 2016 352 890; *Nat. Chem.* 2017 9 611;
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Biological barriers in health and disease

Chairs: Eva Untersmayr-Elsenhuber & Winfried Neuhaus
BB-01  Microbial modulation of mucosal immunity
Liam O’Mahony
University College Cork, Ireland

The mucosal immune system is intimately connected with the vast diversity of microbes present within the gut and on mucosal surfaces. The discovery of novel molecular mechanisms, which mediate host-microbe-nutrient communication, have highlighted the important roles played by microbes and dietary factors in influencing mucosal immune responses. The balance between immune tolerance and inflammation is regulated in part by the crosstalk between innate and adaptive immune cells and the microbiota. For example, certain Bifidobacteria strains have been shown to induce Tregs in humans, while bacterial metabolites, such as short chain fatty acids, drive immunoregulatory responses by binding to g-protein coupled receptors. Importantly, histamine secreting microbes are increased in the gut microbiota of asthma patients and their levels correlate with disease severity. Many human studies now clearly provide strong associations between the composition and metabolic activity of the bacterial microbiota and the development of allergic disease and asthma. The importance of the microbiota to treatment success has been clearly illustrated in cancer patients undergoing immunotherapy (PD-1 blockade) – treatment responders have a different microbiota to non-responders. However, significant gaps in our knowledge on the natural induction of tolerance by microbes still exist, especially early in life. The continuing development of microbial-based immunoregulatory protocols that fully replicate natural tolerance processes are critical for both the prevention and treatment of allergic disorders. Significant research is still required to fully appreciate and understand the complexities of tolerance development to the microbiota and its associated importance for tolerance induction to potential allergens. The potential detrimental effects of antibiotic use on resident microbial communities is another important topic for future research and microbiome-replacement and recovery protocols may be required in future clinical practice following antibiotic therapy.

BB-02  Effects of growth factor gradients and indirect flow on vasculature-on-chip
Barbara Bachmann1, Sarah Spitz2, Mario Rothbauer3, Christian Jordan3, Michaela Purscher4, Severin Mühleder5, Eleni Priglinger4, Heinz Redl5, Wolfgang Holnthoner5, Peter Ertl2

1 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology & Vienna University of Technology, Austria
2 Institute of Applied Synthetic Chemistry, Vienna University of Technology, Austria
3 Institute of Chemical Engineering, Vienna University of Technology, Austria
4 Department of Biochemical Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria
5 AUVA Research Centre, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Austria

Recreating vasculature for tissue engineering in vitro remains vital as non-perfusable tissue constructs are restricted in nutrient supply and waste removal. Microfluidic devices have emerged as an integral tool to mimic both physiologic and pathologic cellular microenvironments. While currently employed vasculature-on-chip models are routinely used to investigate the effect of nutrient gradients [1] or interstitial flow [2] on endothelial cell sprouting, limited research elucidates the influence of these factors on physiologic cocultures of endothelial cells and supporting mural cells. Here, we show that not only is microfluidics ideally suited to create and monitor spatiotemporal gradients in three-dimensional hydrogel cell cultures but also that growth factor supply and elution as well as type of flow regime greatly affect vascular network formation in a co-culture model of human adipose-derived stem/stromal cells and human umbilical vein endothelial cells. In a static model without flow, vascular networks showed a dependence on nutrient diffusion, as network parameters peaked at distance 2 millimeters from the nutrient supply and subsequently declined. In contrast, application of indirect flow enhanced endothelial cell sprouting due to increased delivery of nutrients into the construct, but failed to initiate the formation of mature vascular networks. By comparing vessel parameters, we found that dynamic cultivation increased sprouting activity but failed to produce vascular networks with similar vessel area coverage and average vessel length as static cultures. Assisted by finite volume CFD simulations of distribution of differently sized molecules, we found that while indirect interstitial flow in the medium channel enhances HUVEC sprouting activity, it attenuates vascular network formation due to increased growth factors elution.

References

Acknowledgements
This work was supported from the European Regional Development Fund in frame of the project Kompetenzzentrum MechanoBiologie (ATCZ133) in the Interreg V-A Austria - Czech Republic programme, the European Union’s Horizon 2020 research and innovation programme (685817) and the City of Vienna Tissue Engineering International Project (MA 23, #14-06).
Saliva harbors a quantity of proteins, exosomes, inorganic molecules and cell debris among others, whereby the composition varies depending on the body health status. This offers huge opportunities to use saliva for non-invasive biomarker analysis, especially for critical patient types such as elderly and children. Until now, the proteome and transcriptome of the saliva have been intensively analyzed and biomarkers for Sjögren’s Syndrome, oral cancer, HIV, autism and even epithelial ovarian carcinoma have been defined.

For the evaluation of potential biomarkers in saliva, it is necessary to understand the origin of those molecules and how their appearance in saliva is linked to the investigated diseases. For this purpose, it is essential to clarify the transport of these substances from blood into saliva across the blood-saliva barrier. The blood-saliva barrier is defined as the sum of epithelial layers of the oral cavity and salivary glands.

Recently, an oral mucosa epithelium model using the buccal carcinoma human cell line TR146 was developed at AIT using a transwell model setup. It is assumed that most of the molecules present in saliva cross the blood-saliva barrier using the paracellular pathway. Therefore, a focus during model establishment was set on barrier characterization. The barrier integrity was monitored using TEER (transepithelial electrical resistance) and the permeability of paracellular marker. Further characterization was performed using real-time PCR, high-throughput PCR, HE as well as immunofluorescence stainings. At first the model was used to investigate the transport of tryptophan, a biomarker for Alzheimer and depression, whereby our obtained results suggested an active transport from blood into the direction of the saliva compartment. Additionally, the functionality of various ABC-transporters was proven, which could be linked to a reduced availability to orally administered drugs. Current studies in our group deal with the investigation of the transport of markers for inflammation and cardiovascular diseases from serum into saliva.

In conclusion, we have optimized a model of the oral mucosa using specific media supplements and airlift cultivation. This model has proven its value for human biomarker research. Future efforts focus on the establishment of human cell models of the salivary gland epithelium in order to study the contribution of this barrier to the transport process of biomarkers across the blood-saliva barrier.
**BB-04 Effects of cerebral ischemia on the integrity of the human blood brain barrier: A comparative study with in vitro and in vivo data**

Anna Gerhartl1, Nadja Pracser3, Alexandra Vladetic1, Sabrina Oerter2, Malgorzata Burek3, Carola Y. Förster3, Michael Bohnert2, Antje Appelt-Menzel4, Marco Metzger4, Winfried Neuhaus1

1 Molecular Diagnostics, AIT Austrian Institute of Technology, Austria  
2 Institute of Forensic Medicine, University of Würzburg, Würzburg, Germany  
3 Department of Anesthesiology and Critical Care, University of Würzburg, Würzburg, Germany  
4 Department of Tissue Engineering and Regenerative Medicine, University Hospital Würzburg, Germany

The blood-brain barrier (BBB) is damaged during ischemic insults such as stroke or traumatic brain injury. This contributes to vasogenic edema and subsequent impairment and loss of neurons. As the exact mechanisms of BBB disruption during cerebral ischemia is still unknown we focused on a comprehensive analysis of several tight junction proteins including JAM 1-3, ZO1-3, occludin, tricellulin, CLDN1-25 as well as a series of ABC- and SLC-transporters in vitro as well as in vivo.

For this purpose a high-troughput qPCR chip (“Barrier-Chip”) using the Fluidigm® platform was developed, validated and applied. In order to assess the effects on the human BBB in vitro, human immortalized hCMEC/D3 cells were cultivated in transwell models as either mono- or co-culture with the rat glioma cell line C6 or a mixture of human primary astrocytes and human primary pericytes. To mimic cerebral ischemia, cells were subjected to oxygen glucose deprivation (OGD) with oxygen levels of 0.1% or 1% for 5 hours. The obtained results showed that OGD treatment did not affect the viability of hCMEC/D3 cells, but significantly increased the expression of hypoxia markers such as VEGFa and SLC2A1 revealing cellular responses to applied hypoxic conditions. The co-cultivation with astrocytes and pericytes or rat glioma cells significantly enhanced barrier breakdown shown by TEER (transendothelial electrical resistance) and fluorescein data. Using the newly developed “Barrier-Chip” revealed that the expression of several tight junction and transporter proteins was significantly altered indicating a complex, underlying regulation network. These alterations in mRNA expression levels were then compared to biopsy data obtained from capillaries isolated from ipsilateral and contralateral brain samples of patients suffering from traumatic brain injury or as controls from myocardial infarct or sudden cardiac death.

In summary, the presence and regulation of several tight junction proteins at the human BBB, especially claudins, were altered indicating a complex, underlying regulation network. These alterations in mRNA expression levels were then compared to biopsy data obtained from capillaries isolated from ipsilateral and contralateral brain samples of patients suffering from traumatic brain injury or as controls from myocardial infarct or sudden cardiac death.

Acknowledgements: This work was supported by Stiftung SET zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen, project 060 to W.N. and M.M..

**BB-05 Characterization of the epidermal differentiation complex (EDC) gene EDDM: Adaptation of epidermal differentiation facilitated the evolution of feathers**

Julia Lachner1, Veronika Mlitz2, Karin Brigit Holthaus1, Supawadee Sukseeree1, Marcela Hermann2, Erwin Tschachler3, Lorenzo Alibardi3, Leopold Eckhart1

1 Department of Dermatology, Medical University of Vienna, Austria  
2 Department of Medical Biochemistry, Medical University of Vienna, Vienna, Austria  
3 Dipartimento di Scienze Biologiche, Geologiche ed Ambientali (BiGeA), University of Bologna, Bologna, Italy

The epidermal differentiation complex (EDC) is a cluster of genes that are specifically expressed during the late stages of epidermal keratinocyte differentiation. Comparative genomics has suggested an origin of the EDC in terrestrial vertebrates and its diversification in amniotes. The most complex epidermal appendages of vertebrates are the feathers, which consist of interconnected dead keratinocytes that are filled with heavily cross-linked proteins. Although the molecular architecture determines essential functions of feathers, only few feather proteins have been characterized with regard to their evolution and expression pattern. Here, we identify Epidermal Differentiation protein containing DPCC Motifs (EDDM) as a cysteine-rich protein that has co-evolved with feathers. The EDDM gene is located within the avian EDC and shares the exon-intron organization with EDC genes of other amniotes, including humans, indicating an origin by gene duplication and subsequent sequence modification. The EDDM protein contains multiple sequence repeats and has an unusually high cysteine content of 23%. We raised an antibody against an EDDM-specific epitope and performed immunohistochemical investigations. EDDM was specifically expressed in barbs and barbules of feathers. By contrast, the feather pulp and sheath were immunonegative for EDDM. The results of this study add EDDM to the growing list of feather proteins, also including feather beta-keratins (corneous beta-proteins), EDCRP, and EDMTFH, that have evolved within the EDC.
Tuesday 18th: Poster Session 1: Plant biotechnology

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

*Poster Session 1: Plant biotechnology*
**PS1:PB-01  Glyco-engineering in *Nicotiana benthamiana* for investigation of structure-function relationships of different human IgA glycoforms**

Kathrin Göritzer¹, Daniel Maresch², Friedrich Altmann², Jan Novák¹, Christian Obinger², Richard Strasser¹

¹ Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences Vienna, Austria
² Department of Chemistry, University of Natural Resources and Life Sciences Vienna

Immunoglobulin A (IgA) is increasingly gaining attention as a biopharmaceutical for the treatment of infectious diseases and cancer. However, the full potential of recombinant IgAs as therapeutic antibodies is not explored yet, also owing to the fact that structure-function relationships of these extensively glycosylated proteins are not well understood. To study the influence of glycosylation on the biophysical and immunological properties, homogenously glycosylated IgA is required. In that terms plants such as *Nicotiana benthamiana* have emerged as promising expression systems for the production of proteins with tailored human-like glycans. Here, monomeric IgA1 and IgA2 variants of the HER2-binding clinical antibody Trastuzumab were transiently expressed in *Nicotiana benthamiana* ΔXF plants, in which enzymes responsible for generating non-human N-glycan structures are eliminated. By co-infiltration of the respective mammalian glycosyltransferases, IgAs carrying homogenous oligomannosidic, paucimannosidic, complex terminally galactosylated or sialylated N-glycans were generated. To validate the capacity of the plant-based system to produce functional IgA, the constructs were additionally expressed in human embryonic kidney (HEK293) cells. Plant- and HEK-produced IgA variants were subjected to detailed characterization to assess site-specific glycosylation, overall structure, stability as well as binding to antigen and common receptors like FcαRI. While glycosylation had no effect on antigen-binding, it was shown that increased complexity of glycosylation was not only beneficial for conformational and thermal stability but also for binding to FcαRI. These findings indicate important roles of the IgA N-glycans for its structure and function and provide important input for future drug development.


**PS1:PB-02  Advancement of 3-MCPD and glycidol ester analysis in edible oils by GC-MS**

Martin Maier, Bettina Schwarzinger, Julian Weghuber

CoE Lebensmitteltechnologie, FH Oberösterreich, Austria

The analysis of foodstuff for various foodborne contaminants became increasingly important. In the last few years, also refined edible oils occurred as source for carcinogenic food contaminants, namely 3-monochloro-propanediol (3-MCPD) and glycidol that are mainly present as fatty acid esters. Although there are three official methods from American Oil Chemist’s Society, International Organization for Standardization and Deutsche Gesellschaft für Fettwissenschaft for the analysis of 3-MCPD and glycidol esters, there are several negative aspects in existing methods. These methods are based on the indirect determination of 3-MCPD and glycidol, where the glycidol content is calculated from the difference in 3-MCPD content of two reaction batches. Besides, the analytes have to be derivatized directly before GC/MS analysis, as the derivatives are very instable. In this context, existing methods are very elaborate. In order to reduce analysis time and effort for sample preparation, an advancement of existing methods is desired for simultaneous determination of 3-MCPD and glycidol esters in edible oils. Efforts resulted in a single reaction batch analysis method, whereat glycidol is converted to monobromo-propanediol. Both process contaminants are further derivatized by phenylboronic acid and analyzed by GC/MS. Additionally, experiments for the stabilization of the derivatives were carried out and resulted in the stabilization of the derivatized analytes for at least 5 hours in a solvent mixture of iso-octane and pyridine. Although our experiments to achieve an advancement of the existing methods were successful, the method remains prone to errors as it includes saponification, a washing step, an extraction step and two reaction steps. Also the conversion of 3-MCPD to glycidol during saponification contributes to the absolute need for the usage of d3-3-MCPD as internal standard. In any case, the method has to be carried out very conscientiously to achieve reliable results.
PS1:PB-03  Assessment of the microbiota of imperishable survival structures to identify volatile-producing biocontrol agents

Pascal Mülner, Philipp Wagner, Alessandro Bergna, Dženana Sarajlić, Barbara Gstöttenmayr, Kristin Dietel, Tomislav Cernava, Gabriele Berg

1 ABiTEP GmbH, Germany
2 Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria
3 ACIB GmbH, Petersgasse 14, 8010 Graz, Austria

Fungal pathogens such as *Sclerotinia sclerotiorum* (Lib.) de Bary and *Rhizoctonia solani* (Kühn) are the cause of extensive crop damage across the globe. Sclerotia, consisting of an outer layer with high concentration of melanin and medullary hyphae with extended vacuoles, are formed by both fungi to persist severe environmental conditions. Sclerotia-associated microbial communities were explored by cultivation-dependent and –independent methods to identify potential novel biocontrol agents and to obtain deeper insights into the microbiome. Data from a 16S rRNA amplicon study showed that the sclerotiome of *Rhizoctonia solani* is highly similar to the microbiota of surrounding soil, while microbial communities of the unaffected potato peel display significant differences. Distinctive bacterial lineages were associated with healthy and sclerotia-affected potato peel. *Flavobacteriaceae* and *Caulobacteraceae* were primarily found in unaffected areas, while *Phyllobacteriaceae* and *Bradyrhizobiaceae* were associated with the presence of sclerotia of *Rhizoctonia solani*. In addition to various *Bacillus* species, isolates assigned to the *Enterobacter*, *Pseudomonas* and *Buttiauxella* genus exhibited promising antagonistic activity towards fungal pathogens. Bioactive substances like acetoin, dimethyl disulfide and diverse alkylated pyrazines were detected in the volatilome of antagonists belonging to species of *Bacillus* and *Pseudomonas*. Previous studies demonstrated the pronounced antimicrobial effects of these diazine derivatives. These microbial volatile organic compounds are able to alter the morphology of sclerotia and increase the layer of non-viable hyphae. Moreover, distinct combinations of isolated antagonists led to a significant increase in the pathogen inhibition. The results exhibit that fungal survival structures are associated with a specific microbiome, which is also a reservoir for new biocontrol agents.

PS1:PB-04  Determination of genetic variability and selection of interesting genotypes of undomesticated accessions of *Cornus mas*.

Fatemeh Maghuly, Sabine Mansky, Souleymane Bado, Margit Laimer

PBU, DBT, BOKU, Austria

Consumer interest in health foods has increased market demand for high quality fruits such as the Cornelian cherry. Seed propagation and long term human selection have given rise to a great diversity of trees, so this makes them ideal candidates as parents for genetic improvement. A thorough evaluation of the genetic resources of the native accessions is essential for selecting genotypes for future breeding programs especially those with traits associated with hardiness and disease resistance.

To make efficient use of the genetic variability available in the region, new methods for the identification of suitable varieties of *C. mas* need to be established for practical use in the Austrian cornelian cherry breeding.

An *in vivo* collection of reference plants was assembled in the glasshouse of the PBU, BOKU to provide comparative and starting material for the experiments. In addition, 400 of the selected genotypes in the project region (Pielach- Traisen- and Gölsen-valley) were sampled in the field, labeled and selected a) for tissue culture establishment and b) for further analyses. A method for the initiation of tissue culture starting from glasshouse model plants was established and optimized for the subsequent establishment of field derived material. From vegetative buds of six reference plants from the glasshouse, from six seed sources (Austria, Iran) and twenty provenances from the project region (from over 100 year old outdoor plants) actively growing shoot cultures could be established.

Of the 400 selected individuals from the project region and some known varieties as reference plants, DNA samples were extracted and subjected to genetic analyzes using ISSR markers. With regard to health-related ingredients, methods for the enrichment of proteins from Dirndl fruits and pollen have been developed. First evidence of the presence of PR proteins could be confirmed.
PS1:PB-05  How do microorganisms modulate alkannin and shikonin production in Boraginaceae plants? An insight from comparative transcriptomics.

Muhammad Ahmad¹, Angela Sessitsch¹, Philipp Rödel², Eva Maria Sehr¹

¹ Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, Austria
² Institut für Pflanzenkultur e.K., Solkau, Schnega, Germany

Alkannin, shikonin (A/S) and their derivatives, known as red naphthoquinones, are the commercially interesting class of plant secondary metabolites which are produced in the roots of different species of the Boraginaceae family. Specifically, red color extracts from the roots of Alkanna, Echium and Lithospermum were traditionally used as dyes and in herbal preparations in both Europe and Asia for several centuries. Currently, they have applications in cosmetic, food and pharmaceutical industries based on their broad-spectrum biological activities such as wound healing, antibacterial, anticancer, and antioxidant.

However, biosynthesis of A/S in Boraginaceae is shaped by specific cultivation regimes which often lead to low yield of these compounds and thus limits their commercial utilization. Recent studies suggest that plants are colonized by a variety of microorganisms which during their interaction with plants can enhance the biosynthesis of secondary metabolites. Such a system can be exploited for improving A/S production and to meet increasing demand from industry. However, little is known regarding the changes in Boraginaceae species transcriptome in response to treatment with microorganisms and its relationship with modulation of A/S contents. In this project, using RNA-sequencing, we aim to explore the effect of selected microorganisms on the transcriptome of Lithospermum erythrorhizon Siebold & Zucc. 1846 and to reveal differential expressed genes involved in pathways leading to the enhanced production of A/S.

Currently, we are establishing a system to assess the interaction between the plants and microorganisms. In a test approach, clonal plant individuals will be inoculated at various stages of plant growth with four chosen bacterial strains known for secondary metabolites induction. In a first step, the microbial effect on A/S production will be assessed using qPCR. In future, we will use this system for investigating transcriptional modulation in response to inoculation with microorganisms isolated from Boraginaceae species themselves.

Acknowledgements: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 721635.

PS1:PB-06  A cross-species transcriptomics approach to identify genes involved in drought stress in European oak species

Silvia Madritsch³, Elisabeth Wischnitzki³, Peter Kortrade¹, Agnes Burg⁰, Silvia Fluch⁰, Wolfgang Brüggemann¹, Eva M. Sehr⁰

¹ Department of Ecology, Evolution and Diversity, Goethe University Frankfurt, Max-von-Laue-Str. 13, Biologicum, 60438 Frankfurt, Germany
² Bioresources, AIT Austrian Institute of Technology, Austria

The impact of climate change that comes with a dramatic increase of long periods of extreme summer drought associated with heat is a fundamental challenge for European forests. As a result, forests dominated by relatively drought-sensitive coniferous and broadleaf species but also mixed oak forests of Central European and Mediterranean countries are expected to shift their distribution patterns towards north-east, which may lead to a dramatic loss in value of European forest land. Thus, unravelling key processes that underlie drought stress (DS) tolerance is not only of great scientific but also of utmost economic and ecologic importance to survive future heat and drought wave scenarios.

In the current study we applied cross-species comparative transcriptomics to reveal DS-related molecular patterns of the less tolerant deciduous pedunculate oak (Q. robur) via the deciduous but quite tolerant pubescent oak (Q. pubescens) to the very tolerant evergreen holm oak (Q. ilex subsp. ilex). RNA of leaves of individuals under DS and of a control group (CO) was extracted, sequenced and de novo assembled for each species. We found 415, 79, and 222 differentially expressed genes in Q. robur, Q. pubescens, and Q. ilex, respectively. Gene ontology (GO) enrichment analysis showed important differences in biological processes going on in each species. Orthologous gene family analysis gave further insight of similarities and differences of drought regulated genes across the analysed species. We found 165 orthologous groups that may play an important role in drought stress adaption.

This study unveils new regulatory candidate genes in the context of DS response in European oaks and provides a first set of DS associated genetic markers, that have the potential to accelerate breeding programs or assisted migration projects to maintain forest structure and the economical and ecological value of forests in the face of climate change.
**PS1:PB-07  Expression of Bet v 1 in *Nicotiana benthamiana* plants based on in-planta assembly of deconstructed TMV vector modules**

Öykü Üzülmez, Vanessa Mayr, Angelika Tschepp, Chiara Palladino, Heimo Breiteneder
Department of Pathophysiology & Allergy Research, Medical University of Vienna, Austria

**Background**
IgE-mediated allergies affect 25-30% of the world’s population. Recombinant allergens offer certain advantages in diagnosis and therapy. Correct post-translational modifications (PTMs) might be crucial for the allergen’s full IgE-binding capacity. However, protein expression systems such as yeasts and bacteria have limitations for PTMs. Therefore, plant-based expression systems may improve the production of plant-derived allergens.

**Methods**
*Agrobacterium tumefaciens* was transformed with separate tobacco mosaic virus (TMV)-based vector modules that were used for the infiltration of *N. benthamiana* plants. The TMV-based genes were delivered in two plasmids, as a 5’ and a 3’ module, each incorporated into the T-DNA of separate *A. tumefaciens* transformants. The 5’ TMV module encodes proteins that are necessary for translation. The 3’ TMV module harbours major birch pollen allergen Bet v 1. The 5’ and 3’ modules were brought together in-planta via the phiC31 integrase which is encoded within a co-delivered 3rd TMV-based vector. The plants were infiltrated by applying vacuum while submerging the leaves into a suspension of transformed agrobacteria. Transcription of the Bet v 1 mRNA was achieved by a viral vector encoded RNA-dependent RNA polymerase from a subgenomic promoter.

**Results**
The expression level of purified Bet v 1 was at 12 mg/kg leaf fresh weight 16 days post-infiltration. The rBet v 1 was able to be detected by immunoblotting using both monoclonal anti-Bet v 1 antibody Bip 1 as well as the IgE from allergic donors’ sera. The *N. benthamiana*-produced Bet v 1 harbours modifications such as phosphorylation and glycosylation.

**Conclusions**
We established the usefulness of the deconstructed TMV-based vector system by expressing the major birch pollen allergen in *N. benthamiana*. This system offers an advantage for its exchangeable 3’ module for producing an allergen of interest. Future plans include the expression of more complex plant-derived allergens to achieve site-specific PTMs and testing their effects on the IgE-binding. Supported by the Austrian Science Fund doctoral program W1248-B30.
Poster Session 1: Optimization of microbial workhorses for biotechnology
PS1:OMB-01  **Yarrowia lipolytica: A metabolic multi-talent for the bio-refinery of glycerol**  
Michael Egermeier$^{1,2}$, Hans Marx$^{1,2}$, Hannes Russmayer, Hans Marx$^{1,2}$, Michael Sauer$^{1,2,3}$

1 Christian Doppler Laboratory for Biotechnology of Glycerol, Vienna, Austria  
2 University of Natural Resources and Life Sciences (BOKU), Austria  
3 Austrian Centre for Industrial Biotechnology (ACIB GmbH), Vienna, Austria

The biodiesel-byproduct glycerol represents a cheap and abounding substrate for large scale microbial conversion processes to enhance the economic viability of biodiesel production. The oleaginous yeast *Yarrowia lipolytica* readily consumes glycerol for biomass formation and produces value-added metabolites and microbial lipids. This non-conventional yeast is known to be very sensitive to changes in the pH which can lead to a complete alteration of the product pattern in a strain. *lipolytica* thrives in many different habitats ranging from cheese and meat to soil, to unlikely places such as fuel tanks. The source of isolation has great impact on the metabolic characteristics of this microorganism and is the first important point to consider for the choice of a novel host for industrial applications and the basis for strain engineering and evolution. While some strains convert the excess glycerol to polyols (mainly mannitol and erythritol), other strains of the same species shift their metabolism from polyol production towards the secretion of citric acid.

In a comprehensive screening experiment, we investigated the ability of 20 strains of *Y. lipolytica*, including the primary lab strains as well as 12 completely new isolates from dairy products, to convert glycerol into citric acid and polyols in a bioreactor under different pH conditions. We could show, that strains from a common origin behave comparably, even though completely unlike their relatives from diverging habitats$^1$. 

Based on this observation we selected two strains (DSM-3286 and DSM-21175) showing diverse metabolite patterns to investigate the transcriptional regulation of this phenotype using RNA-Seq and to identify possible engineering targets. The specific glycerol uptake rate drops dramatically after nitrogen depletion, thus in the production phase. Regulatory changes were observed in transport mechanisms, glycerol utilization and major metabolic pathways.

The combined knowledge from generic strain screenings and intrinsic omics-analysis will promote the development of *Y. lipolytica* towards a custom-tailored microbial cell-factory.

PS1:OMB-02  **Combinatorial optimization strategies to increase Human P450 catalytic Activity in whole cell Biotransformations**  
Astrid Weninger$^0$, Anna Alkofer$^0$, Christian Schmid$^0$, Johannes Bitter$^0$, Antonia Volpini de Maestri$^1$, Monika Pranjić$^0$, Margit Winkler$^0$, Anton Glieder$^0$

1 TU Graz IMBT  
2 IMBT, TU Graz, Austria

Human cytochrome P450 biotransformations are of great potential for drug metabolite synthesis. However, recombinant production yields and product titers are low, which has been the most limiting factor for their application in large scale processes. In this study, we combined protein engineering with expression-, strain-, and process-optimization to obtain high level human cytochrome P450 producing *P. pastoris* strains for whole-cell biocatalysis.

The research for this work has received funding from the European Union (EU) project ROBOX (grant agreement n° 635734) under EU’s Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1
PS1:OMB-03  Genome integrated expression systems - versatile tools to answer fundamentals in recombinant protein production with E. coli.

Artur Schuller\textsuperscript{0}, Monika Cserjan\textsuperscript{0}, Johanna Jarmer\textsuperscript{1}, Christopher Tauer\textsuperscript{0}, Gerald Striedner\textsuperscript{0}

\textsuperscript{1} Doktor-Boehringer-Gasse 5-11, 1120 Wien
\textsuperscript{2} Department of Biotechnology, CB Laboratory for production of next-level biopharmaceuticals in E. coli, University of Natural Resources and Life Sciences Vienna, Austria

For the production of biopharmaceuticals in E. coli, genome integrated T7-based expression systems offer significant advantages in terms of product yield and quality. Compared to plasmid-based systems there is no plasmid mediated metabolic load and no variation in gene dosage during production. Consequently, such single copy systems also allow for in-vivo investigations of fundamentals in recombinant protein production. However, to answer special research questions like the characterization of the long-term response of cells to recombinant gene expression, the strength of the T7 system is still too high. Apart from that, the T7 RNA polymerase (RNAP) is prone to mutations under production conditions. Therefore alternative promoter systems have to be evaluated.

Aim of this work was to investigate the suitability of two constitutive phage-derived promoters that were recognized by the $\sigma^{32}$ E. coli RNAP. For expression rate control we introduced lacO binding sites to the promoter sequences. Five promoter/operator combinations with the model proteins GFP and an antibody Fab' fragment were investigated regarding expression strength, tunability, basal expression and cell growth in plasmid-based and plasmid-free BL21 expression systems. The resulting set of 20 production clones was cultivated and compared under fed-batch like conditions in BioLector micro-titer fermentations and for detailed characterization lab-scale fed-batch cultivations were conducted.

Independent from the promoter/operator combination all plasmid-based systems showed expression rates similar to the T7 based reference system with increasing copy numbers after induction. Furthermore, we observed considerable basal expression under C-limited conditions in the feed phase. In contrast, for all genome integrated systems we saw significant impact of the promoter/operator combination on expression level and systems leakiness. These results clearly confirm the necessity of single copy genome integrated systems for detailed characterization of host cell response to recombinant gene expression. In future we will use these genome integrated systems to investigate the cell response, the system stability and the mutation characteristics of E. coli under long-term production conditions in chemostat cultivations.

PS1:OMB-04  Combinatorial optimization strategies to increase human P450 catalytic activity in whole cell biotransformations

Astrid Weninger, Anna Alkofer, Christian Schmid, Johannes Bitter, Antonia Volpini de Maestri, Monika Pranjic, Margit Winkler, Anton Glieder

IMBT, TU Graz, Austria

Human cytochrome P450 biotransformations are of great potential for drug metabolite synthesis. However, recombinant production yields and product titers are low, which has been the most limiting factor for their application in large scale processes. In this study, we combined protein engineering with expression-, strain-, and process-optimization to obtain high level human cytochrome P450 producing P. pastoris strains for whole-cell biocatalysis.

The research for this work has received funding from the European Union (EU) project ROBOX (grant agreement n° 635734) under EU’s Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1.
PS1:OMB-05  Characterization of *Trichoderma reesei* nature isolates and their improvement for biological biomass pretreatment and cellulase production by sexual crossing

Samira Basyouni-Khamis¹, Guofen Li⁰, Wolfgang Hinterdobler⁰, Markus Gorfer⁰, Monika Schmoll⁰

¹ University of Natural Resources and Life Sciences Vienna; Institute of Agricultural Engineering
² Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, Austria

The development and implementation of alternative energy generation such as biogas and biofuel is a major goal to reduce the demand of crude oil for energy production. *Trichoderma reesei* represents the model organism for investigation of plant cell wall degrading enzymes and their regulation mechanisms.

With locally isolated *T. reesei* strains, we aim to improve the biomass pretreatment and conversion of cellulosic plant materials to chemical building blocks and subsequent production of biogas, bioethanol or platform chemicals by crossing. The isolated strains showed different levels of cellulase production and were able to undergo mating with sexually competent *T. reesei* strains of opposite mating type (CBS999.97). In addition, the isolated strains show different responses to injury in terms of sporulation. Besides phenotypic analysis, UP-PCR analysis confirmed uniqueness of the strains isolated and excluded contamination with lab strains.

We successfully implemented a screening program to select *T. reesei* strains with specific attributes by sexual crossing and media based selection. This way we specifically select progeny with a high ability to secrete cellulases but with a low consumption of glucose and preferred utilization of hemicellulose for growth.

For screening of the several hundred progeny per crossing cycle, we apply a high-throughput-method for enzymatic analysis. Our first results indicate that our selection process yields strains with improved cellulolytic and xylanolytic efficiency compared to their parental strains and even exceeding those of the hypersecreting industrial strain RUT-C30. Our specifically developed biotest system allows us to assess inhibitory secondary metabolites produced by the selected strains in order to prevent problems in subsequent fermentations.

With further strain improvement we aim to get *T. reesei* strains with high levels of cellulytic enzymes but reduced uptake of fermentable sugars. Along with a favourable combination with white rot fungi for lignin degradation these local *Trichoderma* strains will contribute to optimized biomass pretreatment of cellulosic plant material such as maize straw.

PS1:OMB-06  Protein display on the cell surface of *Lactobacillus plantarum* for the development of whole-cell biocatalysts

Mai-Lan Pham, Anh- Minh Tran, Dietmar Haltrich, Thu- Ha Nguyen

Food Biotechnology Lab, Department of Food Science and Technology, BOKU- University of Natural Resources and Life Sciences, Vienna, Austria

*Lactobacillus plantarum* is considered as a potential cell factory because of its GRAS (generally recognized as safe) status and long history of use in food applications. It has the potential for the *in situ* delivery of proteins to a host and also for the production of food-related enzymes. By displaying different enzymes on the surface of *L. plantarum* cells these could be used as whole-cell biocatalysts for the production of oligosaccharides. In this present study, we aimed to display a chitosanase from *Bacillus subtilis* on the cell surface of *Lactobacillus plantarum* WCFS1. The target protein chitosanase was fused with three different truncated forms of a cell wall anchor and cloned into lactobacillal food-grade expression vectors. The resulting fusion proteins were then expressed in *L. plantarum* WCFS1. The surface localization of secreted protein was confirmed by flow cytometry analysis and immunofluorescence microscopy. This study proved that heterologous proteins can be anchored efficiently onto the cell surface of *L. plantarum* and *L. plantarum* chitosanase-displaying cells should be of interest for the production of potentially ‘prebiotic’ oligosaccharides.
PS1:OMB-07 Functions of G-protein coupled receptors in secondary metabolite production in *Trichoderma reesei*

Wolfgang Hinterdobl⁰, Jana M. Krautloher⁰, Stefan Böhmdorfer¹, Monika Schmoll⁰

¹ University of Natural Resources and Life Sciences Vienna, Division of Renewable Resources, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria
² Center for Health & Bioresources, AIT Austrian Institute Of Technology, Austria

Mimicking the complex natural habitat of microorganisms and their interactions with others under laboratory conditions still is one of the major limiting factors in studying the underlying molecular mechanisms which often stay silent under axenic conditions.

The filamentous ascomycete *Trichoderma reesei* (syn. *Hypocreas jecorina*) represents a workhorse for biotechnological applications, and decades of strain improvement resulted in detailed knowledge of nutrient requirements, regulation mechanisms and regulators of enzyme production and secondary metabolism. The discovery of sexual development in *T. reesei* permits an in-depth investigation of intra-species chemical communication inevitable for successful mating. We recently showed that fungi recognize potential mating partners. Beside the upregulation of pheromone precursor genes and pheromone receptors, the pattern of secreted compounds changes in the presence of different partners. Hence, *T. reesei* applies a language of pheromones and secondary metabolites to communicate with mating partners. Our studies revealed a time-dependent shift in secondary metabolite production upon perception of a mating partner and we could show an influence on the growth rate in the receiving fungus. The perception of signals released from nearby fungi – and other microorganisms – is at least in part mediated via activation of G-protein coupled receptors (GPCRs) inducing a cascade of molecular adjustments to its surrounding habitat within the fungal mycelium. Among others, the glucose sensors CSG1 and CSG2 are involved in the chemical response to environmental conditions, which is in agreement with transcriptome data indicating that CSG1 influences secondary metabolism. The activation of gene clusters responsible for secondary metabolite production leads to a specific response in metabolite production and secretion.

We investigated the dependency of the reaction to a mating partner on GPCR-mediated signals by comparing the secondary metabolite patterns of 30 receptor-deficient *T. reesei* strains under sexual and asexual conditions.

PS1:OMB-08 Optimization of *CYP153A6* expression by bidirectional promoter shuffling

Anna-Maria Hatzl⁰, Raphaela Wimmer⁰, Lukas Johannes Pfeifenberger⁰, Kay Domenico Novak⁰, Anton Glieder¹

¹ Petersgasse 14, 8010 Graz
² IMBT, TU Graz, Austria

The versatile vector system pBSYBiE21 was developed for the bidirectional expression of proteins in *E. coli*. “Bidirectional” in this expression strategy means that two open reading frames can be placed on one plasmid. This expression strategy has two main advantages:

Antibiotic selection markers can be spared by placing two open reading frames on one plasmid. Protein titers can be easily optimized by shuffling bidirectional promoters with different properties. Balanced protein titers of two or more enzymes involved in a biocatalytic synthesis are crucial to avoid bottlenecks and increase yields. This is for example relevant for cytochrome P450 enzymes and their corresponding reductases.

The *CYP153A6 operon* from *Mycobacterium* sp. HXN-1500 encodes for the CYP 450 enzyme CYP153A6 and its corresponding ferredoxin reductase (FdR) and ferredoxin (Fdx). The CYP153A6 is one of the few well-studied candidates of the CYP153 enzyme family and proved to be an excellent biocatalyst for preparative hydroxylation of medium chain length alkanes and cycloalkanes.

This work received funding from the EU project ROBOX (grant agreement nr. 635734) under EU’s H2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1.

Any statement made herein reflects only the author’s views. The European Union is not liable for any use that may be made of the information contained herein.
PS1:OMB-09  A cross-species whole genome siRNA screen in Chinese hamster ovary cells identifies novel engineering targets

Gerald Klanert1, Daniel J Fernandez2, Marcus Weinguny1, Peter Eisenhut1, Eugen Bühler2, Michael Melcher3, Steven A Titus2, Andreas B Diendorfer1, Elisabeth Gludovacz4, Vaibhav Jadhav1, Su Xiao5, Madhu Lal2, Joseph Shiloach5, Nicole Borth6

1 Department for Biotechnology, University of Natural Resources and Life Sciences, Austria
2 Division of Preclinical Innovation, NCATS, NIH, Rockville, MD, USA
3 Austrian Centre of Industrial Biotechnology, Graz; University of Natural Resources and Life Sciences, Vienna, Austria
4 Medical University of Vienna
5 Biotechnology Core Laboratory, NIDDK, NIH, Bethesda, MD, USA
6 Austrian Centre of Industrial Biotechnology, Graz; University of Natural Resources and Life Sciences, Vienna, Austria

High-throughput siRNA screens were only recently applied to cell factories to identify novel engineering targets which are able to boost cells towards desired phenotypes. While siRNA libraries exist for model organisms, no CHO-specific library is publicly available, hindering the application of this technique in CHO cells. The optimization of these cells is of special interest, as they are the main host for the production of therapeutic proteins. Here, we performed a cross-species approach by applying a mouse whole-genome siRNA library to CHO cells, and developed an in silico method to identify functioning siRNAs. With this method, we were able to identify several genes that, upon knockdown, enhanced the specific productivity in the primary screen. A second screen validated two of these genes, Rad21 and Chd4. The knockdown of both genes was tested in additional CHO cell lines, confirming the induced high specific productivity phenotype.

PS1:OMB-10  Optimization of CYP153A6 expression by bidirectional promoter shuffling

Anna-Maria Hatzl0, Raphaela Wimmer0, Lukas Johannes Pfeifenberger0, Kay Domenico Novak0, Anton Glieder1

1 Petersgasse 14, 8010 Graz
2 IMBT, TU Graz, Austria

The versatile vector system pBSYBiE21 was developed for the bidirectional expression of proteins in E. coli. “Bidirectional” in this expression strategy means that two open reading frames can be placed on one plasmid. This expression strategy has two main advantages. Antibiotic selection markers can be spared by placing two open reading frames on one plasmid. Protein titers can be easily optimized by shuffling bidirectional promoters with different properties. Balanced protein titers of two or more enzymes involved in a biocatalytic synthesis are crucial to avoid bottlenecks and increase yields. This is for example relevant for cytochrome P450 enzymes and their corresponding reductases. The CYP153A6 operon from Mycobacterium sp. HXN-1500 encodes for the CYP 450 enzyme CYP153A6 and its corresponding ferredoxin reductase (FdR) and ferredoxin (Fdx). The CYP153A6 is one of the few well-studied candidates of the CYP153 enzyme family and proved to be an excellent biocatalyst for preparative hydroxylations of medium chain length alkanes and cycloalkanes.

This work received funding from the EU project ROBOX (grant agreement nr. 635734) under EU’s H2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1. Any statement made herein reflects only the author’s views. The European Union is not liable for any use that may be made of the information contained herein.
PS1:OMB-11  Towards sustainable production of lactic acid
Martin Altvater, Siniša Petrik, Diethard Mattanovich, Michael Sauer
The Austrian Center of Industrial Biotechnology (ACIB), Austria

Lactic acid is an organic acid that is used in food, cosmetic and pharmaceutical industries and that serves as building block for polyactic acid (PLA), a biodegradable and biocompatible polymer. The global lactic acid market is estimated to increase from 714.2 kilo tons in 2013 to 1,960.1 kilo tons by 2020 with a revenue of USD 4.3 billion. Currently, optically pure lactic acid is mainly produced by sugar fermentation of lactic acid bacteria. However, this production process has some drawbacks. Particularly, lactic acid bacteria are sensitive to low pH. Therefore, neutralizing agents have to be added to the production medium making the separation and purification of lactic acid very costly. In the presented project, we use *S. cerevisiae* as production organism for lactic acid. Budding yeast has many advantages over *Lactobacilli* such as the simple nutritional requirements or the tolerance to low pH, making it an excellent cell factory for producing organic acids. Since *S. cerevisiae* cells do not naturally produce lactic acid, metabolic engineering is required in the first place to develop a lactic acid producing strain. Initially, the genes coding for pyruvate decarboxylases were deleted. By overexpressing a heterologous lactate dehydrogenase, pyruvate can then be converted into lactic acid. Furthermore, a combinatorial approach of modifications of gene expression levels, and evolutionary engineering of the yeast production strain will improve the final lactic acid yield.

PF1-01  Overexpression of an acetylation-insensitive acetyl-CoA synthetase in *E. coli* W and its effect on glucose and acetate co-utilization in batch and continuous cultures
Katharina Novak, Lukas Flöckner, Anna Maria Erian, Philipp Freitag, Christoph Herwig, Stefan Pflügl
Institute of Chemical, Environmental and Biological Engineering, TU Wien, Austria

*Escherichia coli* W is used for the production of several metabolites and recombinant proteins, especially due to its high stress tolerance as well as low acetate excretion. Most studies were performed using glucose as carbon source and simultaneous co-utilization of glucose and other substrates such as acetate is promising, but still demanding. Acetate assimilation and dissimilation is regulated by the activity of several enzymes. One of the key enzymes, the high affinity acetyl-CoA-synthetase, is on one hand regulated on a transcriptional level by carbon catabolite repression, and is on the other hand inactivated by post-translational acetylation. To improve acetate uptake in the presence of glucose and thus enable efficient co-utilization, *E. coli* W was genetically engineered to overexpress an acetylation insensitive acetyl-CoA synthetase. This strain was characterized in batch and continuous cultures using glucose, acetate and a mixture of both. In batch cultures with glucose and acetate, acetate uptake during the exponential growth phase was 2.7-fold higher in the engineered strain overexpressing an acetylation insensitive acetyl-CoA synthetase, thus indicating more efficient co-utilization of glucose and acetate. The engineered strain showed decreased batch durations when acetate was used as the sole source of carbon, possibly by circumventing the toxicity of acetate more efficiently. In accelerostat cultivations, *E. coli* W was shown to be a naturally efficient co-utilizer of glucose and acetate over a broad range of dilution rates (0.20 – 0.70 h⁻¹). The overexpression of acetylation insensitive acetyl-CoA-synthetase resulted in acetate accumulation and cell wash-out at lower dilution rates compared to the control strain. Gene expression analysis revealed in imbalance in the ratio of *acs* and *pta-ackA*. Higher expression levels of the ATP-consuming *acs* could cause increased energy dissipation and hence explain the early wash-out. Additionally, the membrane proteins *yjCH* and *actP*, genes co-transcribed with acetyl-CoA synthetase showed significant down-regulation at higher dilution rates. In this study, *E. coli* W was shown to be able to efficiently co-utilize glucose and acetate, revealing different behaviors in batch and continuous processes.
**PF1-04  Insight to the mechanism of action of fungi fitness regulator**

**Razieh Karimi Aghcheh**  
Dept. of Molecular Microbiology and Genetics, Georg August University Göttingen, Germany

Understanding of the coordination of metabolic and morphological functions of fungi is required for a biotechnological control of the formation of desired bioactive products. The fungal putative methyltransferase LaeA/LAE1 homologs their gene products act at the interphase between secondary metabolism, cellulase production and development. Lack of the corresponding genes results in significant physiological changes including loss of secondary metabolite and lignocellulose degrading enzymes production. The molecular mechanism of LaeA/LAE1 function which may link epigenetic to transcriptional control is unknown. Only an automethylation function of *Aspergillus nidulans* LaeA was found at methionine-207 which is close to the AdoMet binding site. The biological significance of LaeA automethylation is yet unknown because mutant proteins revealed that the methylation site Met\(^{207}\) is not required for secondary metabolites (SMs) biosynthesis. *Aspergilli* and *Trichoderma* represent different biotechnologically significant species with significant differences in the LaeA/LAE1 and their target proteins. The conserved Met\(^{207}\) in LaeA/LAE1 of several filamentous fungi is replaced by a lysine residue at the respective position in *T. reesei* LAE1; *T. reesei* LAE1 is unable to complement a laeA-null mutant of *A. nidulans* and this protein and *A. nidulans* LaeA differ also from each other by a hypervariable N-terminal and C-terminal region. Here, we present our most recent data about the potential interaction partners of LaeA/LAE1 and the importance of N-and C-terminal ends of this protein in its interaction. Furthermore, the role of Met\(^{207}\) for the action of *T. reesei* LAE1 will be discussed.
Tuesday 18th: Poster Session 1: Neuroimmunology

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 1: Neuroimmunology
PS1:NI-01  Galanin is a potent modulator of cytokine/chemokine expression of human macrophages

Andreas Koller1, Susanne M Brunner0, Rodolfo Bianchini0, Felix Locker0, Andrea Rampacher9, Sandra Schlager2, Barbara Kofler0

1 Research Program for Receptor Biochemistry and Tumor Metabolism, University Hospital for Pediatrics of the Paracelsus Medical University, Salzburg, Austria
2 University Institute for Blood Group Serology and Transfusion Medicine, Paracelsus Medical University, Salzburg, Austria
3 University Hospital for Pediatrics of the Paracelsus Medical University, Salzburg, Austria, Research Program for Receptor Biochemistry and Tumor Metabolism, Austria

The regulatory peptide galanin (GAL) is broadly distributed in the central/peripheral nervous system but also in non-neuronal tissues. GAL exerts its diverse physiological functions via three G-protein coupled receptors (GAL1-3-R). Overall, regulatory peptides are important players in the cross-communication between nervous and immune system and are in focus as therapeutics for diverse inflammatory diseases. Various studies on inflammatory animal models and immune cells revealed a pro- and anti-inflammatory capacity of GAL suggesting a complex regulation of GAL signaling at the tissue and cellular level. However, the microenvironment of tissues upon an immune challenge is dynamic and depends on the type of stimulus and duration, and might be important for the specific actions of GAL during inflammation. We aimed at elucidating the role of GAL in immunity in more detail, with a special focus on macrophages.

CD14+ monocytes were isolated from human buffy coats and differentiated for 6 days with GM-CSF (M0-GM) or M-CSF (M0-Mφ), without or with GAL co-treatment. Differentiated cells were treated with GAL alone for 20 hours or they were polarized with IFNγ+LPS, IL-4, or IL-10 to generate M1-GM-Mφ, M2a-M-Mφ, or M2c-M-Mφmacrophages, respectively. Polarization was performed with and without GAL co-treatment. Relative mRNA expression levels of inflammatory cytokine and chemokines, and the GAL system were analyzed by qPCR. Protein levels of cytokines and chemokines, and GAL in cell culture supernatants were analyzed by ELISA.

We found that macrophage subtypes exhibited varied GAL secretion and a distinct balance of GAL1-R and GAL2-R expression. GAL itself affected the cytokine/chemokine expression profile of macrophages differently, depending on differentiation and polarization and mainly modulated the expression of chemokines (CCL2, CCL3, CCL5 and CXCL8) and anti-inflammatory cytokines (TGF-β, IL-10 and IL-1Ra) especially in type 1 macrophages. Cytokine/chemokine expression of IFNγ+LPS polarized macrophages were upregulated whereas cytokine/chemokine expression levels of unpolarized macrophages were downregulated upon GAL treatment after 20 hours.

This study displays the regulation of important cytokines/chemokines of macrophages by GAL depending on specific cell activation.

Acknowledgements

This work was supported by the Austrian Research Promotion Agency [FFG, 822782/THERAEP] and Paracelsus Medical University Salzburg [PMU-FFF, R-17/01/086-KOL].

PS1:NI-02  B-cells in the multiple sclerosis brain

Manuela Paunovic, Joana Machado-Santos, Anna Tröscher, Monika Bradl, Hans Lassmann

Neuroimmunology, Center of Brain Research/ Medical University of Vienna, Austria

Multiple sclerosis (MS) is a chronic inflammatory disease in which active demyelination and neurodegeneration are associated with brain-infiltrating lymphocytes. Interestingly, CD4+ T cell-directed therapies in MS patients are largely ineffective, while B cell-directed therapies turn out to be very efficient. This observation contrasts our lack of knowledge about the phenotypes and functions of B cells in MS lesions.

To fill this gap, we used selected, well characterized archival autopsy samples of MS brains from patients with acute, relapsing-remitting, and progressive disease, and characterized the B-cell populations in these tissues using immunohistochemistry. B-cells were identified by the surface marker CD20 and plasma cells by the cytoplasmic immunoglobulin content and the expression of CD138. To further determine other distinct B-cell subtypes, like memory B-cells and plasma blasts, we performed additional stainings for CD27 and CD38. We found that CD20+ B-cells were particularly numerous in patients with acute MS, relapsing-remitting MS and in the rare classical active lesions of progressive MS. On the contrary, the numbers of plasma cells and the plasma cell / B-cell ratio were significantly higher in lesions from patients with progressive MS in comparison to those in acute MS. This suggests that infiltrating B-cells gradually transform into plasma cells with disease chronicity, which is supported by the expression of the markers for B-cell survival and plasma blast differentiation, CD27 and CD38.

In next experiments, we plan to analyze the antigen-specificity of these infiltrating B-cells to clarify their role in MS.

This work is supported by the Austrian Science Funds (FWF, project I3335-B27).
Tuesday 18th: Poster Session 1: Next-generation pathogen & antibiotic resistance diagnostics

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 1: Next-generation pathogen & antibiotic resistance diagnostics
PS1:NPA-01  Evaluation of qPCR as a rapid detection method for *Legionella pneumophila*

Daniela Toplitsch, Sabine Platzer, Rita Baumert, Gernot Zarfel, Franz Mascher, Clemens Kittinger
Inst. for Hygiene, Medical University of Graz, Austria

The number of diseases caused by waterborne pathogens is steadily rising globally. Waterborne diseases are a serious threat because of their ability to infect a high number of individuals in a short time span, such as during outbreaks of Legionellosis. This significantly highlights the need for the rapid detection and quantification of bacteria in environmental water samples, which as of yet relies on time consuming and work-intensive culture dependent methods. The aim of this study was to investigate an already established qPCR method for the detection of *Legionella pneumophila* (L. pneumophila) in environmental water samples and following comparison of the qPCR method with the standard culture methods for *Legionella* detection according to ISO 111731. Our study reached a negative predictive value (NPV) for *L. pneumophila* of 83.87 % and for *L. pneumophila* serogroup (sg) 1 the calculated NPV was 97.44 %. On the other hand, the positive predictive value (PPV) for *L. pneumophila* was 54.00 % and for *L. pneumophila* sg1 the calculated NPV was 25.00 %. Our study showed a correlation between qPCR and culture with an R² value of 0.9140 for *L. pneumophila*, whereas no correlation was observed for the detection of *L. pneumophila* sg1. Elevated detection of *L. pneumophila* by qPCR is displayed in the low PPV value as well as the mean log difference of the methods of 2.54 x 10⁻¹. qPCR values are on average 3.48 fold higher than culture values, which goes in line with findings in other current literature on this topic. Furthermore, our findings suggest that the sample type, such as drinking water, or cooling water, strongly influences cultivability and qPCR results. qPCR shows obvious benefits due to easier sample handling and the rapid generation of results, which proposes the need for an evaluation and validation of different qPCR tools in the own laboratory before implementation of the method as a stand-alone technique in to the routine water quality assessment.

PF1-05  Antimicrobial Resistance Markers in Molecular Diagnostics: Good Enough for the Clinic?

Norhan Mahfouz, Inês Ferreira, Stephan Beisken, Andreas Posch
Ares Genetics GmbH, Austria

Antimicrobial resistance (AMR) is a global health threat with a projected annual casualty rate of > 10 Mio by 2050. In molecular diagnostics, AMR is routinely assessed by PCR, a molecular biology method to detect the presence or absence of known AMR markers. One drawback of PCR is the limited number of markers that can be detected in parallel, especially for multi-drug resistant bacteria.

Driven by declining costs of Next Generation Sequencing (NGS), NGS is increasingly replacing PCR for pathogen characterization and outbreak monitoring but is not yet used for AMR testing in clinical routine. In contrast to PCR, NGS enables genome-wide detection of AMR markers and can provide a high resolution assessment of AMR. Public resources like The Comprehensive Antibiotic Resistance Database, ARG-ANNOT or ResFinder have emerged as repositories for AMR determinants and many software tools have been developed for NGS-based, i.e. genotype-based, AMR detection based on these databases.

However, in *silico* genotype-based methods may suffer from high false positive and/or false negative rates. In order to achieve accurate AMR diagnostic tests based on bacterial genotyping, the gap between AMR genotype detection and AMR phenotype prediction needs to be closed. This is especially challenging as the detection of a single AMR determinant may not always translate to phenotypic resistance with a clinically significant MIC change.

To address this problem, we evaluate the diagnostic performance of publicly available AMR determinants in testing for resistance against different antibiotics on a dataset of bacterial isolates exhibiting genotypic and phenotypic diversity. We use our proprietary Ares Database comprising a representative set of thousands of thoroughly profiled clinical isolates from different bacterial species that were collected globally over the last 30 years.

The results show the advantages and limitations of current AMR resources for clinical practice and diagnostic use. There is significant room for improvement in diagnostic performance that, *inter alia*, can be achieved by introducing novel combinations of AMR determinants as well as newly discovered resistance markers to existing combinations of AMR determinants from public resources. The results highlight the instrumental role of a curated AMR database combining whole-genome pathogen information with antibiotic resistance profiles for the translation of NGS-based testing from research to clinical practice.
Tuesday 18th: Poster Session 1: New trends in allergy diagnosis and therapy

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 1: New trends in allergy diagnosis and therapy
PS1:ADT-02  The role of glucocorticoids in B cell development and function
Marcel Tisch, Katia Schöler, Verena Labi, Jan Wiegers, Andreas Villunger
Developmental Immunology, Medical University, Innsbruck, Austria

Glucocorticoids are steroid hormones that play an important role in many physiological processes. Clinical use has shown that they are capable of suppressing inappropriate immune responses. Hence, they are widely used as treatment for autoimmune diseases, dampening of inflammation and many other therapeutic approaches. However, little is known about the physiological relevance of endogenous glucocorticoids during development and function of the immune system. B cells, as key players of the adaptive immune system, have been reported to express high levels of the glucocorticoid receptor and several reports show that glucocorticoid treatment both in vitro and in vivo impacts B cells in terms of development, differentiation and cell death. The generation and analysis of a B cell glucocorticoid receptor knock out mouse (Mb1creGRfl/fl) will help expand our understanding of how glucocorticoids influence B cell biology. Our study aims to shed light on the role of endogenous glucocorticoids in modulating B cell development, germinal center reactions and antibody production.

PS1:ADT-03  Role of high-density lipoprotein, composition and function in allergy
Athina Trakaki, Eva Sturm1, Gunter Sturm2, Akos Heinemann1, Gunther Marsche1
1 Otto Loewi Research Center, Unit of Pharmacology, Medical University of Graz, Austria
2 Department of Dermatology and Venereology, Medical University of Graz, Austria

Background: High Density Lipoprotein (HDL) exerts several beneficial properties, such as anti-oxidative and anti-inflammatory activities. Allergic rhinitis (AR) is an IgE-mediated disease, which is predominantly caused by environmental allergen exposure in genetically predisposed individuals. Recent studies suggest that HDL particles are implicated in allergic diseases.

Purpose: The aim of the study was to assess whether AR affects HDL composition and function and if HDL isolated from AR patients affects immune cell function.

Methods: 24 control subjects, 31 AR patients in the active (acute) and 23 AR patients in the inactive (non-acute) disease state, which were age matched, were determined in terms of HDL associated apolipoproteins and lipids. Anti-oxidative, anti-inflammatory and cholesterol efflux potential of HDL, as well as effect of HDL on eosinophil effector responses were evaluated.

Results: Reduced levels of apolipoprotein A-I (apoA-I), p<0.01, were observed in AR patients and reduced levels of free cholesterol, p<0.05, were observed in non-acute AR patients compared to control subjects. Increased levels of apoA-II, apoC-III and triglycerides, p<0.01, p<0.05 and p<0.001 respectively, were observed in non-acute AR compared to control subjects. The ability of HDL to modulate eosinophil effector responses was evaluated. AR-HDL (acute and non-acute) markedly inhibited agonist induced eosinophil activation, p<0.001 and p<0.001 respectively, and chemotaxis, p=0.091 and p<0.001 respectively, compared to control subjects. The anti-inflammatory potential of HDL was evaluated by its ability to inhibit LPS induced activation of the NF-κB pathway in monocytes. Decreased anti-inflammatory capacity of non-acute AR-HDL was observed, p<0.001, compared to control subjects. The anti-oxidative capacity of HDL was assessed by determining the oxidation of the fluorescent dye dihydrorhodamine. Reduced anti-oxidative capacity of AR-HDL, p<0.05 and p<0.001 respectively, was observed compared to control subjects. Cholesterol efflux potential of serum HDL (serum depleted of apoB containing particles) was evaluated in macrophages. We observed improved cholesterol efflux capacity of non-acute AR serum HDL, p<0.001, compared to control subjects.

Conclusion: AR markedly alters HDL composition and affects HDL functional properties. Understanding the features of altered HDL functionality in AR might lead to new diagnostic and therapeutic approaches.
We have recently performed a sublingual immunotherapy (SLIT) with the recombinant major birch pollen allergen rBet v 1. Twenty individuals received a daily dose of 25 µg of rBet v 1 and 20 individuals received placebo for 16 weeks. Compared to the placebo group, SLIT with rBet v 1 induced significant levels of Bet v 1-specific IgG4 antibodies. Furthermore, the post-SLIT sera prevented the IgE-mediated activation of basophils indicating the induction of Bet v 1-specific blocking antibodies. Various tree pollens contain Bet v 1-homologous major allergens such as Aln g 1 (alder), Cas s 1 (chestnut tree), Cor a 1 (hazel), Fag s 1 (European beech), Ost c 1 (hornbeam), Que a 1 (white oak), which through IgE-cross-reactivity cause allergic reactions in birch pollen-allergic patients. Here, we sought to investigate if SLIT with Bet v 1 induced antibodies can prevent IgE-mediated reactions to these homologous pollen allergens. We first tested the presence of IgG4 antibodies against the different pollen allergens by ELISA. We found that SLIT with rBet v 1 induced a significant increase of IgG4 against all Betv1 homologous pollen allergens except for Cor a 1. Currently, the capacity of the post-SLIT sera containing specific IgG4 antibodies to inhibit basophil activation by the different homologs are being performed. Primary experiments provide evidence for a cross-blocking capacity of sera collected post SLIT with rBet v 1.

**Methods:** An iNKT reporter system was engineered by introducing the human iNKT TCR into a human leukemic Jurkat T cell line carrying an NF-kB-driven fluorescent transcriptional reporter construct (Jkt-iNKT). BW-CD1d, a human CD1d transfected thymoma cell line, was generated and used as antigen presenting cells. Reporter induction (NF-kB-driven eGFP-expression) in Jkt-iNKT cells was measured by flow cytometry. The specificity and sensitivity of our system was compared to IL-2 production by murine DN32.D3 iNKT cell hybridomas, following activation by a-Galactosylceramide (a-GalCer)-loaded recombinant CD1d molecules, as well as co-culture assays utilizing BW-CD1d cell lines.

**Results:** Jurkat cells stably expressing the human iNKT TCR receptor (Jkt-iNKT) were shown to specifically react with iNKT antigens presented in the context of CD1d. The detection limit for a-GalCer was similar for Jkt-iNKT and DN32.D3 cell lines.

**Conclusions:** Our Jurkat-based iNKT cell reporter cell line is a useful tool to study the capacity of lipid antigens to activate human iNKT TCR. In addition, our reporter system is remarkably faster and more cost-effective, compared to traditional iNKT cell assays.
PS1:ADT-06  Alum-adjuvanted allergoids induce IgE-blocking IgG antibodies in allergen-specific immunotherapy

Manuel Reithofer, Simone Lisa Böll, Claudia Kitzmüller, Fritz Horak, Mahtab Sotoudeh, Barbara Bohle, Beatrice Jahn-Schmidt

Institute for Pathophysiology and Allergy Research, Medical University of Vienna, Austria

In addition to allergen-specific immunotherapy (AIT) with intact allergen extracts, chemically modified extracts with less IgE-binding activity, named allergoids, have been introduced for conventional AIT of IgE-mediated allergy already in the 1990's. They were envisaged as alternative vaccine formulation in order to reduce potential side effects and to improve the effectiveness of AIT by increasing doses and providing more favorable regimens. These allergoid vaccines are used frequently and have been reported to be clinically effective. However, data regarding the allergen-specific humoral immune response induced by these allergoids are scarce. The objective of this study was to investigate the induction of allergen-specific IgG antibodies and their potential IgE-blocking activity by allergoid AIT. Sera from allergoid-treated grass pollen-allergic individuals were compared to sera from patients that had undergone AIT with intact grass pollen allergen. Both subcutaneous vaccines contained alum as adjuvant. AIT with allergoid triggered similar levels of allergen-specific IgG antibodies as evaluated by ELISA. Yet, the capacity to inhibit IgE as determined by inhibition of facilitated allergen binding to CD23 and basophil activation tests was partly reduced in sera of patients undergoing AIT with allergoid. In conclusion, although allergoids induce high amounts of IgG, these antibodies cannot totally block the formation of IgE immune complexes or the activation of effector cells by native allergen.

Supported by Austrian Science Fund FWF DK-1248

PS1:ADT-07  Alpha-Gal bound to lipids, but not to proteins, is able to cross the intestinal epithelium explaining the delayed allergic symptoms in meat allergic patients

Patricia Román Carrasco¹, Barbara Lieder², Veronika Somoza³, Marta Ponce⁴, Zsolt Szépfalusi⁵, Wolfgang Hemmer³, Ines Swoboda³

¹ Molecular Biotechnology, FH-Campus Wien, Austria
² Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Vienna,
³ Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria
⁴ Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria
⁵ FAZ-Floridsdorf Allergy Center, Vienna, Austria

The carbohydrate galactose-α-1,3-galactose (α-Gal) is expressed on mammalian proteins and lipids and causes an unusual delayed allergic reaction. In individuals with IgE antibodies against α-Gal the onset of the allergic symptoms occurs 3 to 6 hours after ingesting mammalian meat. To better understand the reason for the delay in the onset of allergic symptoms we investigated whether protein-bound or lipid-bound α-Gal is responsible for them and analyzed the capacity of α-Gal conjugated proteins and lipids to cross the intestinal epithelium.

Extracts of proteins and lipids from cooked beef were prepared, digested in vitro and then added to Caco-2 cells grown on permeable inserts. The presence of α-Gal in the basolateral medium was investigated by immunoblotting, thin-layer chromatography (TLC) with immunostaining and ELISA and its allergenic activity was analyzed in a basophil activation test. α-Gal containing peptides were not detected in the basolateral medium, after incubating Caco-2 cells with digested beef proteins, and those peptides that could have crossed the Caco-2 monolayer did not activate basophils from an α-Gal allergic patient. Instead α-Gal was detected in the basolateral medium, when Caco-2 cells were incubated with lipids extracted from beef. Furthermore, these α-Gal lipids, were able to activate the basophils of an α-Gal allergic patient in a dose-dependent manner. We thus showed that only α-Gal carried on lipids, but not on proteins is able to cross the intestinal epithelium and trigger an allergic reaction. The slower digestion process of α-Gal conjugated lipids might explain the delay in the appearance of symptoms since it takes for dietary lipids a longer time than for proteins to reach the circulation.
**PS1:ADT-08  An In Ovo Model for Testing Insulin-mimetic Compounds**

Renate Haselgrubler, *Flora Stübl*, Verena Stadlbauer, Peter Lanzerstorfer, Julian Weghuber

FH-Wels, Austria

Type 2 diabetes mellitus (T2DM), a complex and multifactorial metabolic disease which results in elevated blood glucose levels, is caused by insulin resistance and β-cell failure. Various strategies, including the application of insulin-sensitizing drugs as well as the injection of insulin, are used to treat or at least reduce the symptoms of T2DM. In addition, the application of herbal compounds has attracted increasing attention for the therapy and prevention of T2DM. Thus, it is necessary to find efficient test systems to identify and characterize insulin-mimetic compounds. We present a modified chick embryo model (hens egg test, HET) which enables testing of synthetic compounds and herbal extracts with insulin-mimetic properties. Originally it has been developed to determine the potential irritancy of chemicals but was modified accordingly and termed Gluc-HET. Fluorescence microscopy-based primary screens, which quantifies the translocation of Glucose transporter 4 to the plasma membrane, where used to identify compounds, which lead to an increase of intracellular glucose concentrations in adipocytes. Further to this in-vitro primary screening procedure we used the Gluc-HET as an in-ovo approach for the effectiveness of blood glucose-reducing properties. The approval by an ethics committee is not needed since the use of chicken embryos during the first two-thirds of embryonic development is not considered an animal experiment. Determination of different buffer parameters, such as temperature, composition and volume as well as variations in the incubation time of the fertilized eggs led to a reliable and manageable system for the characterization and screening of antidiabetic compounds.

**PS1:ADT-09  Purification and characterization of a new allergenic walnut vicilin – “Jug r 6”**

Stefan Kabasser, Pawel Dubiela, Sabine Geiselhart, Merima Bublin, Christoph Nagl, Karin Hoffmann-Sommergruber

Inst. f. Pathophysiologie u. Allergieforschung, Medizinische Universität Wien, Austria

**Background:** Walnuts are potent inducers of food allergic symptoms, ranging from mild up to severe reactions in sensitized individuals. So far, five allergens were identified, among those Jug r 2 belonging to the vicilin family. Based on the identification of an IgE-reactive clone from a cDNA library in 1999 (Teuber et al.), the sequence fragment of Jug r 2 was determined. However, only limited data is known about the natural form of the protein. Therefore, this study aimed to purify and characterize the physicochemical and allergenic properties of Jug r 2.

**Methods:** Walnut kernels were ground and defatted twice (1:5 w/v) by n-hexane. Proteins were extracted from walnut flour by 7 volumes of buffer (20 mM Tris/HCl, 0.5 M NaCl, 5% PVPP, 10 mM DTT, pH=8) for 4 hours at 4°C. Subsequently, purification was performed starting with concanavalin A affinity followed by anion exchange and size exclusion chromatography. The purified protein was identified by MALDI-TOF mass spectrometry and LC-MS/MS. Secondary structure was assessed by CD spectroscopy and the IgE binding activity of vicilin was tested in ELISA and Western Blot using sera from 77 walnut allergic patients.

**Results:** In SDS-PAGE the purified vicilin migrates as a single band at around 50 kDa. CD spectroscopy confirmed a stable secondary structure of the protein. MALDI-TOF mass spectrometry revealed a molecular mass of 47.1 kDa, which is in disagreement with previously identified Jug r 2 (48.2 kDa) (database access. no: Q9SEW4). However, results from IgE-ELISA showed that 26% of walnut allergic patients had specific IgE against the purified 47.1 kDa protein. Therefore, the protein was included as an additional walnut allergen in the WHO/IUIS list of allergens as “Jug r 6”. Additionally, sequence analysis revealed high sequence identity with other allergenic vicilins such as from hazelnut, Cor a 11 (72%), sesame seeds, Ses i 3 (60%) and pistachio, Pis v 3 (62%). Results from inhibition ELISA showed IgE cross-reactivity between Jug r 6 and proteins from those food sources. In future, Jug r 6 could be used in diagnostic tests to verify IgE sensitization to walnut and to predict allergic cross-reactions with other tree nuts.
Tuesday 18th: Poster Session 1: From RNAomics to function

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 1: From RNAomics to function
PS1:RNA-01  Beyond RNA-editing: Non-canonical functions of ADAR1

Florian Ebner, Prajakta Bajad, Cornelia Vesely, Michael Jantsch
Center for Anatomy and Cell Biology, Medical University of Vienna, Austria

The recognition of widespread and diverse RNA modifications and their influence on genetic information has sparked epitranscriptomic research with impact on biology and medicine. The most abundant modification known is RNA-editing by adenine to inosine deamination, found in all classes of RNAs but rRNAs. Posttranscriptional A-to-I editing of RNA is an essential mechanism. For example, mutations in the editase ADAR1 (Adenosine deaminases acting on RNA) cause Aicardi–Goutières syndrome, a potentially severe inflammatory disorder. Accordingly, full knockout of the Adar1 gene or exchange of the gene with an editing-deficient Adar1 variant are both embryonically lethal in mice due to uncontrolled interferon-driven inflammation. On the molecular level, editing prevents recognition of cellular dsRNA by the cytosolic innate immune receptor MDA-5 (Melanoma Differentiation-Associated protein 5), therefore blocking downstream interferon production and hyperinflammation. Lethality of mice with editing-deficient point mutant ADAR1 is fully rescued by additional knockout of MDA-5. These double knockout animals display no interferon overproduction and are healthy and fertile (Liddicoat et al. Science 2015). On the other hand, ADAR1 knockout animals with additional ablation of MDA-5 or the downstream scaffolding protein MAVS (Mitochondrial Antiviral-Signaling Protein) display a severe developmental delay and premature lethality, albeit diminished inflammation. Intriguingly, inducible knockout of ADAR1 in adult animals shows only mild effects without lethal outcome. This divergence in the grade of rescue between full knockout and mutated, editing-deficient ADAR1 raises the possibility of non-canonical functions of ADAR1 besides RNA-editing.

Here we investigate the possibility of editing-independent functions of ADAR1 during post-natal development. We show that in utero lethality of ADAR1 knockout is attenuated by concomitant knockout of mitochondrial adaptor protein MAVS. The severe developmental phenotype is recapitulated upon combining ADAR1 knockout with concomitant MDA-5 knockout. Our data show that double-knockout animals (ADAR1 and MAVS) have abnormal proportions of B cells, neutrophils and macrophages. Macrophage numbers are diminished in vivo and in vitro, accompanied by lower phagocytic activity. Altogether, this hints at editing-independent functions of ADAR1 during post-natal development and will be further investigated in detail.

PS1:RNA-02  Functional consequences of A-I Flna RNA editing

Mamtà Jain1, Tomer Mann2, Maja Stulic3, Michael Jantsch5
1 Bar Ilan University, Israel
2 CACB, Medical University of Vienna, Austria

Adenosine deaminases acting on RNA convert adenosine to inosines in all classes of RNA. As inosines are recognized as guanosines by most cellular machineries, A to I editing can lead to a codon exchange in mRNAs. A conserved target of A to I editing that leads to recoding are the mRNAs encoding the actin crosslinking proteins Filamin A and Filamin B. Editing of either mRNA leads to the conversion of a glutamine (Q) to an arginine (R) codon. The editing induced amino acids exchange occurs in repeat 22 of either Filamin protein. This region has been identified to be a highly interactive region of Filamins. To determine the impact of this conserved and regulated amino acids exchange we have generated mice harboring an uneditable allele of Filamin A. Phenotypic analysis of these mice demonstrate cardiovascular problems with hypercontractile smooth muscle cells. Moreover, these mice are highly susceptible to develop colitis. Mice carrying a preedited allele of Filamin A have also been generated and are currently being investigated for their organismic phenotypes.
PS1:RNA-03  Splicing controls tissue-specific regulation of A-to-I editing sites in pre-mRNAs

Konstantin Licht¹, Utkarsh Kapoor¹, Fabian Amman², Ernesto Picardi³, David Martin¹, Michael Jantsch¹

¹ Center for Anatomy and Cell Biology, Medical University of Vienna, Austria
² University of Vienna, TBI, Waehringer Strasse 13, Vienna, Austria
³ University of Bari, Piazza Umberto 1, Bari, Italy

Most metazoan mRNA-processing - including mRNA-splicing and adenosine to inosine (A-to-I) editing - occurs co-transcriptionally. During A-to-I editing a genomically encoded adenosine is deaminated to inosine by adenosine deaminases acting on RNA (ADARs). As inosine has different base-pairing characteristics, A-to-I editing affects various cellular processes ranging from the recoding of transcripts to the recognition of RNAs by the innate immune system. ADAR enzymes require double-stranded RNA for binding. These editing-competent stems are frequently formed between exons and introns, suggesting that editing and splicing require co-transcriptional coordination.

Here, we use nascent RNA-seq to identify A-to-I editing events that occur co-transcriptionally. We identified almost 100,000 editing sites (~80% novel) that primarily locate to intronic regions. As A-to-I editing frequently depends on intronic sequences we reasoned that the efficiency of splicing is a major factor regulating editing. Indeed, inhibition of splicing changed the editing levels of hundreds of sites. Intronic and exonic editing levels primarily increase, suggesting that reduced splicing efficiency leads to an increased exposure of intronic and exonic sequences to ADAR enzymes. Consistently, we also observe changes in editing levels for mice where the alternative splicing factors NOVA1 or NOVA2 are depleted, suggesting that alternative splicing factors can act as editing modulators. Finally, we show that across tissues, intron retention rates correlate with editing levels. We therefore suggest that splicing efficiency contributes to tissue-specific differences in editing levels.

PS1:RNA-04  Consequences of Filamin Editing

Mamta Jain, Laura Cimatti, Maja Stulic, Michael Jantsch

Center of Anatomy and Cell Biology, Division of Cell- and Developmental Biology, Medical University of Vienna, Austria

Adenosine deaminases acting on RNA convert adenosine to inosines in all classes of RNA. As inosines are recognized as guanosines by most cellular machineries, A to I editing can lead to a codon exchange in mRNAs.

A conserved target of A to I editing that leads to recoding are the mRNAs encoding the actin crosslinking proteins Filamin A and Filamin B. Editing of either mRNA leads to the conversion of a glutamine (Q) to an arginine (R) codon. The editing induced amino acids exchange occurs in repeat 22 of either Filamin protein. This region has been identified to be a highly interactive region of Filamins. To determine the impact of this conserved and regulated amino acids exchange we have generated mice harboring an uneditable allele of Filamin A. Phenotypic analysis of these mice demonstrate cardiovascular problems with hypercontractile smooth muscle cells. Moreover, these mice are highly susceptible to develop colitis. Mice carrying a preedited allele of Filamin A have also been generated and are currently being investigated for their organismic phenotypes.
**PS1:RNA-05  Global Interplay of A-to-I RNA Editing and pre-mRNA Splicing**

_Utkarsh Kapoor¹, Fabian Amman², Konstantin Licht¹, Michael F. Jantsch¹_

¹ Department of Cell & Developmental Biology, Medical University of Vienna, Austria  
² Theoretical Biochemistry Group, University of Vienna, Währinger Strasse 17, 1090, Vienna, Austria

Adenosine deamination type editing and pre-mRNA splicing are tightly interlinked processes. Adenosine deaminases acting on RNA (ADARs) recognize double stranded structures, typically formed between adjacent complementary sequences within one RNA. Frequently, editing events that lead to the recoding of mRNAs are defined by base pairing between the exonic editing site with an adjacent intronic editing complementary sequence (ECS). Consequently, editing needs to occur before the ECS is removed by splicing, implying that the speed of splicing affects the extent of editing. Conversely, it has been shown that inhibition of editing can interfere with splicing. Lastly, nuclear and cytoplasmic editing levels can vary, indicating selective processing and/or export of mRNAs, depending on their editing status.

Using inhibitors of splicing and genetic mouse models in which either one of the two editing enzymes ADAR1 or ADAR2 are deleted, we determine the interplay of pre-mRNA splicing and RNA editing. Our studies show an unexpected high level of intronic RNA editing events, exceeding the previously known number of editing events in the mouse. We show further, that inhibition of pre-mRNA splicing increases the rate of RNA editing, primarily in introns and UTRs. Lastly, we can show that inhibition of editing by ADAR1 can strongly affect alternative splicing while ADAR2 only has a minor impact on splicing.

Currently, we are determining whether tissue specific splice rates can affect editing rates in vivo and explain the observed tissue specific variation in editing patterns despite relatively constant levels of the editing enzymes.

---

**PS1:RNA-06  The human homolog of a bacterial endonuclease is essential for mitochondrial gene expression.**

_Sabrina Summer¹, Alexandre Smirnov², Anna Smirnova², Nina Entelis², Ivan Tarassov², Walter Rossmanith¹_

¹ Center for Anatomy and Cell Biology, Medical University of Vienna, Austria  
² Université de Strasbourg, Strasbourg, France

Mammalian mitochondria contain a small genome encoding 13 polypeptides essential for oxidative phosphorylation (OXPHOS), which are translated by dedicated mitochondrial ribosomes. Additionally, the mitochondrial proteome includes approximately 1500 further proteins that are encoded in the nucleus and imported into the organelle, where they are involved in mitochondrial functions.

Many of these proteins are orthologs of bacterial proteins that have outlasted the evolutionary remodeling of the mitochondrial genome, suggesting that they have essential functions in mitochondria. One of them is the putative endoribonuclease C21ORF57/YBEY, whose bacterial counterpart is proposed to be involved in the maturation and quality control of ribosomal RNAs.

Here we show that human YBEY is involved in the post-transcriptional regulation of gene expression in mitochondria. YBEY-knockout cells exhibit impaired OXPHOS activity associated to severely decreased steady-state levels of the mitochondrial encoded OXPHOS subunits, ultimately leading to mitochondrial dysfunction. We observed a severe decrease of mitochondrial ribosomal RNA, apparently resulting in a lack of actively translating mitoribosomes, although ribosomal RNA processing was not altered. Moreover, we identified a mitochondrial RNA-binding protein as a key interactor of YBEY, potentially mediating its functions.
PF1-02 Ribosomal RNA methylation by rram-1 modulates development and healthy lifespan

Clemens Heissenberger¹, Teresa Krammer¹, Fabian Nagelreiter¹, Jarod A. Rollins², Santina Snow², Aric Rogers², Johannes Grillari¹, Markus Schosserer¹

¹ Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
² Mount Desert Island Biological Laboratory, Bar Harbor, Maine, USA

The ribosome has been seen for decades as a static machine that translates mRNAs into proteins. However, over the last few years it became clear that it rather represents a highly dynamic structure that responds to various stimuli by adapting its structure and, as a consequence, its function. Such structurally distinct ribosomes are postulated to be “specialized ribosomes” comprising peculiar functional properties and are thus considered to be engaged in translating specific subsets of cellular messages (Filipovska and Rackham, 2013; Xue and Barna, 2012). Although ribosomal RNA is extensively modified by methylations and pseudouridinylation (Rozenski et al., 1999), the functional roles of such modifications in regulating translation and physiology are not yet fully understood.

$m^3A674$ methylation of 26S rRNA is introduced by rram-1 in Caenorhabditis elegans (Yokoyama et al., 2018), is preferentially associated with fully assembled 80S ribosomes and modulates translation of a specific set of mRNAs. Interestingly, both rram-1 expression and methylation at A674 are influenced by age and feeding protocol. Furthermore, knockdown of rram-1 delays development, extends lifespan and improves locomotion at advanced age.

Thus, methylation of ribosomal RNA represents an important regulator of organismal aging and our work will contribute to a better understanding of the underlying molecular mechanisms.
Tuesday 18th: Poster Session 1: Biological barriers in health and disease

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

*Poster Session 1: Biological barriers in health and disease*
PS1:BB-01 Human induced pluripotent stem cell based in vitro models of the blood-brain barrier: the future standard for models in health and disease

Winfried Neuhaus
Center Health and Bioresources, Unit Molecular Diagnostics, AIT, Austria

There is an urgent and tremendous need for human disease models in drug development in order to improve preclinical predictability. In the case of brain disorders drugs have to cross the blood-brain barrier (BBB) to enter the central nervous system (CNS). It was estimated that more than 95% of the drugs cannot cross the BBB. In the case of biopharmaceuticals, it seems to be even more difficult for them to overcome the BBB and reach their target sites. The functionality of the BBB is altered in chronic as well as acute diseases of the central nervous system such as Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), epilepsy, pain, brain tumor, stroke, and traumatic brain injury. Currently no standard in vitro model of the human blood-brain barrier (BBB) with in-vivo like properties is established, although huge demands from basic research as well as pharmaceutical industry exist. Novel developments applying brain endothelial cells differentiated from human induced pluripotent stem cells (hiPSCs) for BBB in vitro models are very promising, because these models exhibit in vivo-like high paracellular tightness and expression of functional active transporters [1]. Since hiPSCs could be generated from several cell types, it seems that there is no limit for the establishment of personalized, human, disease BBB in vitro models. hiPSC-based BBB in vitro model differentiation protocols as well as disease models for e.g. Huntington's disease or stroke will be presented. The question will be discussed in how far current hiPSC-based models reflect the human in vivo BBB and the mechanisms during diseases, and which parameters might be considered for further improvement of these models (e.g. epigenetic, gender or environmental influences). In conclusion, hiPSCs-based BBB models possess enormous potential for human preclinical disease models especially to elucidate and reflect disease and species dependent differences.


Acknowledgement: This study was supported by the SET foundation (Stiftung zur Förderung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen) project 060 to Winfried Neuhaus and Marco Metzger.

PS1:BB-02 Characterization of cardiac and vascular function in a Duchenne Muscular Dystrophy in mice

P. Lujza Szabó1, Ouafa Hamza1, Milat Inci1, Ines Gonçalves1, Karlheinz Hilber2, Janine Ebner2, Bruno K. Podesser1, Attila Kiss1

1 Center for Biomedical Research, Medical University of Vienna, Austria
2 Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive, progressive muscle wasting disease. Besides skeletal muscle degeneration, increasingly important source of morbidity and mortality is dilated cardiomyopathy leading to heart failure, arrhythmias and vascular dysfunction. There are several animal models for this human disease are developed. The most widely used is the dystrophin deficient mdx mouse, which develops severe symptoms at late onset (9-14 months old). Therefore, we aimed to assess the development of cardiac and vascular dysfunction in young mice (3 and 6 months old) to investigate the pathophysiological processes in the early stage of the disease.

Male mdx and Bl/10 mice were used (3 and 6 months old). Echocardiography was used to assess left ventricular (LV) ejection fraction (LVEF) and hemodynamic function was monitored by an invasive method involving the determination of LV systolic pressure (LVSP) and the rate of LV pressure development (+dP/dt). Vascular reactivity was performed by wire myography. We observed the early signs of the cardiac dysfunction as a slight LV dilatation in mdx mice at age 6 months old in compared to wt littermates and 3 month old (p<0.05, respectively). Of importance, endothelial dysfunction on aorta segments were observed at age of 3 months in mdx mice in compared to wt littermates (p<0.05). Our study demonstrated the early sign of endothelial dysfunction a mouse model of DMD, which may contribute to the progression of cardiomyopathy. Thus, targeting the preservation of endothelial function in DMD might be a therapeutic approach for preserving cardiac function.
**PF1-06  Molecular adaptations of epidermal barrier keratins in association with evolutionary land-to-water transitions of mammals**

Florian Ehrlich, Heinz Fischer, Bettina Strasser, Erwin Tschachler, Leopold Eckhart  
Dep. of dermatology, medical university of vienna, Austria

Skin appendages such as hair and nails depend on keratins that build the cytoskeleton of cornifying cells. Other keratins are expressed at the epidermal barrier to the environment. Fifty four different keratins play specialized roles in human epithelia and appendages and similar numbers are present in other terrestrial mammals. Here, we performed a comparative genomics study to determine the conservation and loss of suprabasal epidermal keratin genes in different phylogenetic lineages of mammals. We found a striking decrease in the number of keratins in cetaceans (dolphins and whales) which corresponds to the decrease in the complexity of their skin. Keratins specifically expressed in hair follicles and nails were lost while a minimal set of hair keratin-like proteins were conserved, perhaps to facilitate the formation of hard papillae on the tongue. However, also the diversity of keratins in the epidermis is reduced in cetaceans. In contrast to the basal epidermal keratins K5 and K14, which are highly conserved among mammals, the suprabasal epidermal keratins K1, K2 and K10 have been inactivated in cetaceans. Transcriptomics of dolphin skin showed high expression levels of keratins K6 and K17, which are markers of stress and disease-associated epidermal thickening in humans, and, remarkably, the thickness of the epidermis has dramatically increased during the evolution of cetaceans. These data suggest that epidermal gene expression has been remodelled in cetaceans, and skin appendage- and skin barrier-specific cytoskeletal proteins of terrestrial mammals are dispensable in a fully aquatic environment.
Tuesday 18th: Poster Session 1: Extracellular vesicles in coagulation and inflammation

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

*Poster Session 1: Extracellular vesicles in coagulation and inflammation*
PS1:EV-01  Comparative studies on plasma and saliva-derived circulating biomolecules as a basis for the discovery of epigenetic markers applicable in early Type 2 diabetes diagnosis

Ulrike Kegler, Manuela Hofner, Walter Pulverer, Klemens Vierlinger, Andreas Weinhäusel, Christa Nöhammer
Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, Austria

Special focus and aim of our research activities at AIT, the Austrian Institute of Technology, is to define reliable biomarkers suitable for early and non-invasive disease diagnosis from body fluids such as serum/plasma and saliva. We concentrate our studies mostly on epigenetic mechanisms, including DNA methylation and miRNA, which play an important role in gene regulation and have been found to be altered in human diseases as well as already manifest in early state of diseases. Furthermore autoantibodies have caught our attention. Due to the evident advantages of saliva our current special interest is saliva diagnostics and to investigate saliva for its suitability for any type of circulating biomarker that can be found in this easy-to-access and completely non-invasive body fluid. Using previously for plasma/serum cancer diagnostics defined DNA-methylation – as well as autoantigen biomarkers we tested their feasibility for saliva diagnostics by comparing biomarker paired serum and saliva samples from a breast cancer patient cohort. In addition, we also compared cell-free saliva protein-, DNA methylation - and miRNA profiles of healthy individuals with those obtained from healthy serum or plasma samples applying multiplex protein-panel assays (OLINK Proseek), genome-wide DNA methylation microarray or small RNA Seq respectively. In all above mentioned studies we could successfully show the feasibility of using saliva as a diagnostic matrix since the performance in saliva was similar to serum/plasma and marker profiles showed quite some overlap. By this we demonstrate that saliva is a suitable and most interesting sample matrix for future applications in the area of medical diagnostics. We currently use our previous proof-of-concept insights as a sound basis in an ongoing research project aiming for the discovery of salivary epigenetic biomarkers to be potentially used in future early non-invasive diagnosis of type 2 diabetes.

PS1:EV-02  Comparison of exosome isolation procedures for use in saliva

Anja Buhmann, Ulrike Kegler, Manuela Hofner, Christa Noehammer
Center of Health and Bioresources, Austrian Institute of Technology, Austria

First discovered by Pan and Johnstone in 1983, exosomes have become the focus of exponentially growing interest. Exosomes represent tiny, by a membrane-protein containing lipid bilayer surrounded vesicles (30-150nm), which are constantly secreted by all healthy and abnormal cells and found in abundance in all body fluids. There is emerging evidence that exosome-mediated cell-to-cell communication is of importance in both health and disease. Together with the finding that e.g. tumor-derived exosomes contain a specific RNA and protein cargo this holds tremendous potential for exosomes as biomarkers and is anticipated to lead to the development of exosome-based minimally invasive diagnostics and next generation therapies within the next few years. Especially circulating biomarkers derived from body fluids such as blood or serum have great potential, but each sample withdrawal holds potential risks like e.g. danger of infection. Therefore biomarker analysis in saliva, which is a natural ultrafiltrate of blood, could be a potential simple and safe alternative for the future. Due to these advantages of saliva our current special interest is saliva diagnostics and to investigate saliva for its suitability for any type of circulating biomarker that can be found in this easy-to-access and completely non-invasive body fluid.

Here we report on the evaluation of different strategies for exosome isolation from human saliva including the subsequent isolation of DNA and microRNA to identify the best isolation protocol. Five different commercial kits for exosome isolation were tested and compared for their use with saliva and combined with different kits for DNA and/or microRNA isolation. Furthermore we will present our best practice isolation procedure which includes exosome digestion with DNase and RNase to get rid of nucleic acids attached to the outside of exosomes.
The influence of ketogenic diet on psoriasiform-like skin inflammation

Felix Locker¹, Julia Stockinger², Sepideh Aminzadeh-Gohari², Daniela Weber², Philippe Sanio², Andreas Koller², René Günther Feichtinger², Barbara Kofler², Roland Lang²

¹ Department of Biomedical Sciences, University of Veterinary Medicine, Austria
² Department of Pediatrics, Research Program for Receptor Biochemistry and Tumor Metabolism, Paracelsus Medical University, Salzburg, Austria.

Psoriasis is an inflammatory skin disease characterized by increased neo-vascularization, keratinocyte hyperproliferation, a pro-inflammatory cytokine milieu and immune cell infiltration and is associated with metabolic syndrome, obesity and diabetes. Several reports indicate that dietary intervention with ketogenic diet (KD), a high in fat and low in carbohydrate diet, and omega-3 fatty acids (ω-3 FA) can reduce inflammation and angiogenesis. Therefore, the aim of the present study was to elucidate the impact of a 4:1 (ratio of fat to carbohydrate + protein) long chain triglyceride based KD (LCT-KD) ± ω-3 FA and LCT-KD with 30% medium chain triglycerides (MCT) (LCT/MCT-KD) ± ω-3 FA, to prevent, delay or reduce severity of Imiquimod (IMQ)-induced psoriasiform-like skin inflammation in mice compared to standard diet (SD) ± ω-3 FA.

Six weeks old male mice (C57BL/6N) were adapted to KD only diets over a 10 day period. Thereafter, mice were fed KDs 7 days prior to daily topical application of IMQ on depilated back skin for 3 or 6 consecutive days. Disease severity, changes in vascularization, neutrophil infiltration and myeloperoxidase (MPO)-activity as well as mRNA levels of key cytokines were assessed.

In comparison with SD, LCT/MCT-KD ± ω-3 FA significantly increased disease severity and showed increased neutrophil influx and MPO activity in the skin. Furthermore, IL-1b and IL-17A mRNA were significantly increased in LCT-KD and LCT/MCT-KD supplemented with ω-3 FA. However, LCT-KD + ω-3 FA showed no increased skin inflammation, neutrophil activity and abundances compared to SD. Mice fed with SD + ω-3 FA showed a markedly reduced skin inflammation and reduced neo-vascularization upon IMQ treatment as compared to the SD group. Our results demonstrate that addition of MCTs to KD worsens psoriasiform-like skin inflammation in mice indicating that the dietary FA composition is crucial in mediating pro- or anti-inflammatory effects.

The study was supported by the Paracelsus Medical University research fund (PMU-FFF E-16/24/125-LAL).
Molecular microbiology I

Chairs: Günther Koraimann & Brigitte Pertschy
MM1-01  A materials chemistry perspective on cell encapsulation
Francisco Fernandes, Thibaud Coradin
Sorbonne Université - CNRS, France
Cell encapsulation within solid supports has several expected benefits over 2D configurations. Not only the 3D matrices can protect the cells against external physical or chemical stresses but, for many organisms, it is close to their natural environment, i.e. within tissues. However compared to molecules or even biomolecules, the requirements for preserved cell viability impose strong limitations on the acceptable chemical and physical conditions of the encapsulation process. Thus the use of ionotropic, thermotropic and, to some extent, pH-induced formation of hydrogels from proteins or polysaccharides remains the most popular strategy for cell encapsulation. Moreover it is amenable to most recent processing approaches, such as 3D printing, electrospinning and freeze-casting.
In contrast, the full polymerization of an organic matrix in the presence of cells is highly challenging as it should be performed in water and using non-toxic monomers, initiators and/or catalysts. In this context, the use of the inorganic sol-gel process allowing to form silica gels in aqueous solutions, near neutral pH and at moderate ionic strength has been shown to be particularly attractive for cell encapsulation. These gels are mechanically strong and their transparency/porosity can be tuned. Over the last 20 years, a wide range of cells have been successfully immobilized via the sol-gel process, with targeted applications as bioreactors and biosensors.
What comes out of these works is that sol-gel bioencapsulation may not better than traditional biopolymer-based processes but that it provides interesting environments to study cell-materials interactions. Meanwhile, combining the two strategies to form bio-hybrid materials is probably the most promising route for future developments of new cell encapsulation hosts.
Selected reviews & recent references

MM1-02  Towards a sexual cycle in Aspergillus niger
Valeria Ellena, Michael Sauer, Matthias Steiger
Biotechnology Department, University of natural resources and life sciences, Austria
In recent years an increasing number of filamentous fungi previously considered asexual, such as Aspergillus fumigatus, have been shown to possess a cryptic sexual cycle, triggered only under highly specific conditions. However, for around 20% of the known fungal species, including Aspergillus niger, a sexual cycle has not been found yet. A. niger is a relevant industrial microorganism, currently used as a versatile cell factory for the production of organic acids and enzymes. The discovery of a sexual cycle in A. niger would not only broaden the current biological knowledge of this organism but also allow to unravel new strategies for strain improvement.
In this study, we report some strong indications of the sexual potential of A. niger.
First, sclerotia formation was observed in the sequenced A. niger strain ATCC 1015, containing the MAT1-1 gene, when plated in combination with an opposite mating type A. niger strain. Sclerotia are considered pre-mature sexual structures which can mature to cleistothecia, the latter being an important requirement for the occurrence of a sexual cycle in Aspergilli. The observed sclerotia are often formed at the contact zones between the colonies derived from the two strains, suggesting an interaction between them. Also, the typical presence of liquid droplets on top of the formed structures can be often observed.
Second, a reduction of the normal asexual conidiation can be observed when the two opposite mating type strains are plated together on the same plate. This reduction is even more drastic when sclerotia are formed.
Transcriptional analysis of genes involved in the sexual and the asexual development is planned. The comparison of gene expression between the strains inoculated alone on plate or in combination with the opposite mating-type strain is of particular interest.
Overall, our findings strongly indicate that steps towards a complete sexual cycle of A. niger can be achieved.
MM1-03  CRIB reporter technology to study cellular recovery mechanisms from host-induced hyphal depolarisation in mycoparasitic *Trichoderma* species.

Laura Hackl, Dubraska Moreno, Linda Salzmann, Susanne Zeilinger, Alexander Lichius
Department of Microbiology, University of Innsbruck, Austria

Regulation of cell polarity is essential to life. Filamentous fungi grow by indefinite polarised tip extension providing outstanding model systems to study cell polarity. Core components of the polarised growth machinery are (1) landmark proteins that designate nascent sites of cell cortex extension, (2) small GTPases that initiate the formation of cortical activity clusters from which (3) F-actin tracks polymerise to guide (4) targeted delivery of secretory vesicles. Complex shuttling and feedback mechanisms focus GTPase activity in plasma membrane clusters to direct cortex protrusion in response to external and internal signalling cues.

The first fluorescent CRIB reporter (Cdc42/Rac1-Interactive Binding reporter) in a filamentous fungus was established in *Neurospora crassa* to functionally distinguish the small GTPases Cdc42 and Rac1 during negative and positive chemotropism (Lichius et al., 2014; doi: 10.1242/jcs.141630).

We now established CRIB reporters in the mycoparasite *Trichoderma atroviride* to monitor spatio-temporal dynamics of activated GTPases during pre-contact host sensing, and found the first evidence that hyphae of the mycoparasite undergo transient mass depolarisation and disassembly of the tip growth machinery when challenged by a host defence response. This phenomenon shows host-specificity and ranges from simple tip growth termination, over isotropic expansion and multipolarisation, to plasmolytic-like detachment of the plasma membrane from the apical cell wall. We improved the biophysical properties of the CRIB reporter along with custom-build TipTracker software to allow a more detailed and automated quantification of CRIB dynamics in live-cell imaging time courses of individual hyphae and on the level of the whole colony. We are further expanding this technology to other mycoparasitic Trichoderma species, including *T. asperellum*, *T. virens* and *T. harzianum*, in order to compare the effects of host-induced disruption of polarised tip growth in various parasite/host interactions and to elucidate how different Trichoderma species are either non-susceptible to or finally overcome host defence mechanisms, resume tip growth and eventually digest their various prey fungi. We expect that new insights into the molecular mechanisms of species-specific host defence and mycoparasitic attack strategies will allow a much more targeted application of *Trichoderma* species as biocontrol agents against plant-pathogenic fungi in agriculture.

---

MM1-04  Tracing the TOR kinase pathway and its role in *Trichoderma atroviride* mycoparasitism

Hoda Bazafkan, Martina Schenk, Rossana Segreto, Lea Atanasova, Susanne Zeilinger
Microbiology, University of Innsbruck, Austria

Mycoparasitic *Trichoderma* species are conventionally used as biocontrol agents and as a substitute to the less favoured chemical pesticides. Yet, the molecular mechanisms governing their mycoparasitic activity are not well understood. The TOR (target-of-rapamycin) pathway is an important regulator of cell growth and differentiation in response to nutrient availability. Considering the importance of nitrogen regulation of secondary metabolism and virulence in fungi, we intended to elucidate the function of the TOR pathway components in nitrogen sensing and mycoparasitism in *Trichoderma atroviride*.

To this end, we generated deletion mutants of several genes whose products are acting upstream and downstream of the TOR kinase including *tsc1*, *tsc2*, *rhe2*, *npr1* and *are1*. *tor1* - coding for the Tor kinase as the central component of the pathway - turned out to be an essential gene whose stable deletion was not possible. We hence generated conditionally silenced mutants by expressing *tor1* under the control of the tunable Tet-On expression system. Growth of the Δ*tsc1*, Δ*tsc2*, Δ*rhe2* and Δ*npr1* deletion mutants on different nitrogen sources was similar or slightly improved compared to the wild type, whereas the Δ*are1* mutant showed impaired growth on nearly all nitrogen sources. Confrontation assays on complete media with *Rhizoctonia solani* as fungal prey revealed similar mycoparasitic activities as the wild type for Δ*npr1* and Δ*rhe2*, Δ*tsc1*, Δ*tsc2* and Δ*are1*, however, could not overgrow *R. solani* and secreted metabolites of the Δ*are1* mutant further showed a heavily reduced inhibitory activity against *Botrytis cinerea*. In addition, deletion of *are1*, *tsc1* or *tsc2* resulted in a complete loss of *prb1* expression irrespective of the culture conditions, while this mycoparasitism-relevant protease-encoding gene was induced in the wild type upon confrontation with *R. solani*. Altogether our data point to an important role of the TOR pathway in the response of *T. atroviride* to nitrogen and in regulation of mycoparasitism-related functions.
Fungal pathogens pose a major threat to plants, animals, humans and hence, entire ecosystems. Thriving and surviving in harsh microenvironments mostly depends on the instant adaptation, fitness and development of virulence mechanisms. *Candida albicans* is an extraordinary example of how a human fungal pathogen senses and integrates environmental signals to mount an appropriate transcriptional response allowing rapid adaptation and survival within different host niches. Dynamic transcriptional changes are linked to chromatin organization which profoundly shapes the transcriptional landscape. One paradigm for the interconnection of chromatin regulators and swift transcriptional adaptation is the *C. albicans* yeast-to-hyphae transition, a major fungal virulence factor responsible for host invasion and tissue damage. Fungal morphogenesis is highly dynamic and occurs in response to various host signals which trigger transient transcriptional activation and repression involving fungal chromatin re-organization. The HIR histone chaperone complex facilitates histone deposition onto chromatin in a replication-independent manner and thus, co-regulates transcription. We hypothesized that defects in chromatin assembly may deregulate morphogenetic genes, thereby affecting pathogen fitness *in vivo*. Here, we show that genetic removal of the HIR complex subunit *HIR1* leads to decreased sensitivity to morphogenetic signals, resulting in impaired hyphal formation. Transcriptomic profiling revealed that *HIR1*-deficient cells show decreased transcriptional amplitudes of both gene repression and activation during hyphal initiation suggesting that Hir1 assists in fine-tuning transcriptional responses. Since fungal morphogenesis is linked to virulence, we investigated the host response upon challenge with *hir1Δ/Δ* cells and found that infection with *HIR1*-deficient fungal cells dramatically decreases host survival in a murine model of disseminated Candidiasis. Strikingly, assessment of leukocyte recruitment and fungal burdens of infected kidneys indicates that the hypervirulence of the *HIR1* mutant is caused by decreased pathogen clearance. In summary, illuminating the nature of Hir1-assisted transcriptional control has great potential to provide novel insights into fungal pathophysiology and host response mechanisms.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

New trends in allergy diagnosis and therapy

Chairs: Ines Swoboda & Beatrice Jahn-Schmid
ADT-01  New Trends in Allergy Diagnosis and Therapy

Christian Lupinek
Dept. of Pathophysiology and Allergy Research, Medical University of Vienna, Austria

Allergen-immunotherapy (AIT) is the only treatment of IgE-mediated allergies that can convey a sustained therapeutic effect so far. However, AIT is quite cumbersome a therapy as the treatment regimen provides a considerable number of vaccinations over three years on average. To improve the patient’s condition, it is key (1.) to accurately identify the culprit allergen sources as a prerequisite for selection of the appropriate vaccines and (2.) that the allergen composition of the vaccine matches the patient’s sensitisation profile. While composition of vaccines can hardly be tested in clinical centres, accuracy of allergy diagnosis was markedly improved by introducing allergen molecules for IgE-testing. With purified allergens it became possible to differentiate between genuine sensitisation and cross-reactivity to different allergen sources. In the last years, allergen microarrays emerged as useful diagnostic tools aiding allergologists in unravelling complex sensitisation patterns. In addition to allergy diagnosis, microarrays were shown to be versatile tools for the monitoring of AIT and even to predict the therapeutic outcome. This could be demonstrated, e.g., with peptide microarrays for oral immunotherapy of milk or peanut allergic subjects. The background of this is that one major mechanism of action of AIT is the induction of blocking antibodies that compete with IgE for binding to the allergen molecules. As microarrays comprise only low amounts of protein, presence of antibodies competing with IgE for allergen-binding leads to a decline of IgE-signal intensities measured.

Furthermore, in cohort studies it was shown that it is possible to predict the onset of allergy in children based on the detection of preclinical IgE-sensitisations. This finding was corroborated independently for different major allergen sources like birch pollen and house dust mite. Therefore, it is now feasible to coin precise strategies for the preventive vaccination of preschool children at high risk to develop symptoms of allergy. Recently, a novel generation of vaccines for AIT which are based on allergen-derived peptides and with a markedly improved safety profile was successfully applied in patients in a clinical trial. In combination, these novel diagnostic and therapeutic tools will be studied in future trials investigating the possibility to prevent, delay or mitigate the onset of allergies in children.
ADT-02  Individuals allergic to bony fish tolerate cartilaginous fish due to the low allergenicity of their parvalbumins

Tanja Kalic¹, Françoise Morel², Christian Radauer¹, Thimo Ruethers³, Aya Taki³, Ines Swoboda⁴, Christiane Hilger⁵, Andreas Lopata³, Martine Morisset², Karin Hoffmann-Sommergrube³, Christine Hafner⁶, Annette Kuehn⁵, Heimo Breiteneder¹

¹ Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Austria
² National Unit of Immunology and Allergology, Centre Hospitalier de Luxembourg, Luxembourg
³ Molecular Allergy Research Laboratory, Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia
⁴ Molecular Biotechnology Section, University of Applied Sciences, Vienna, Austria
⁵ Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg
⁶ Department of Dermatology, Karl Landsteiner University of Health Sciences, St. Poelten, Austria

Background and Objectives: Beta-parvalbumins from bony fish are highly potent and cross-reactive food allergens. The allergenic potency and clinical cross-reactivity of alpha-parvalbumins from cartilaginous fish has not been investigated in detail. We conducted a detailed study characterizing the immunological and functional allergenic properties of parvalbumins from both bony and cartilaginous fish in patients with fish allergy.

Methods: Purified beta-parvalbumins from cod, carp, salmon, tilapia and Asian seabass and alpha-parvalbumins from shark and ray were tested for their allergenic activity in 18 patients with clinically confirmed allergy to fish using IgE ELISA, basophil activation test and skin prick test (SPT). To explore the possible tolerance upon consumption of cartilaginous fish, 11 patients were exposed to ingestion of ray either by clinical work-up or food challenges.

Results: IgE levels for parvalbumins in sera of fish-allergic patients were significantly lower for shark and ray alpha-parvalbumins in comparison to IgE levels for any of the tested beta-parvalbumins. While 17/18 patients had IgE to cod parvalbumin (0.2-65.1kUa/L), only three patients had sIgE to ray parvalbumin (1kUa/L-46kUa/L). No significant difference in IgE levels was found among the different beta-parvalbumins. Basophil response to alpha-parvalbumins was significantly lower than the response to beta-parvalbumins, measured by the percentage of CD63⁺ basophils and the area under the dose response curve (AUC). The average percentage of activated basophils after stimulation with 100 ng parvalbumin/ml was 23-26% for beta- and only 1-2% for alpha-parvalbumins. AUC was 6- to 7-fold lower for alpha than for beta-parvalbumins. Low reactivities to fish alpha-parvalbumins were also demonstrated by SPT. Absence of clinical reactivity upon ingestion of ray was observed in 10 of 11 patients.

Conclusion: Low allergenicity of alpha-parvalbumins from cartilaginous fish was demonstrated. Furthermore, cartilaginous fish was shown to be well tolerated by patients allergic to bony fish. Inclusion of cartilaginous fish and their parvalbumins in routine diagnosis of fish allergy may prevent unnecessary food restrictions.

This research is supported by doctoral program W1248-B30 (MCCA), the COST Action FA1402 (ImpARAS), and the Ministry of Higher Education and Research, Luxembourg.
**ADT-03**  Ulo c 1 - a novel Alt a 1 cross-reactive allergen from the fungus *Ulocladium chartarum*

Sandra Pfeiffer¹, Peter Sandler¹, Katharina Nöbauer², Ebrahim Razzazi-Fazeli², Katja Sterflinger³, Ines Swoboda¹

¹ Molecular Biotechnology, FH Campus Wien, Austria
² VetCORE Facility for Research, University of Veterinary Medicine, Vienna, Austria
³ Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

Fungi are ubiquitous in our environment and even though they represent one of the most important inducers of respiratory allergy, allergies caused by molds are often underdiagnosed due to the poor quality of mold allergen extracts and the still insufficient characterization of mold allergens. Therefore, we aimed to identify and characterize allergens from *Ulocladium chartarum*, which is one of the most common mold species occurring in indoor environments in Austria and belongs to the same family as *Alternaria alternata*, the best studied mold allergen source.

To identify novel fungal allergens, IgE immunoblots with sera from mold allergic patients were carried out, followed by nano-LC-QTOF mass spectrometric analysis of IgE reactive protein bands. This led to the identification of the first allergen from *U. chartarum*. This novel allergen, designated Ulo c 1, shows high sequence homology to Alt a 1, the major *A. alternata* allergen. A full-length cDNA coding for Ulo c 1 was amplified from *U. chartarum* mRNA using the RACE-PCR approach and was cloned into the bacterial expression vector pET-17b. Recombinant Ulo c 1 was expressed in *Escherichia coli* as a soluble protein with a C-terminal hexahistidine tag and was purified by Nickel-affinity chromatography.

To characterize the IgE binding capacity of rUlo c 1, ELISA plate-bound rUlo c 1 was incubated with sera from patients sensitized to the genus *Alternaria*. These experiments showed that rUlo c 1 represents an IgE-reactive molecule with strong IgE-binding capacity. To investigate whether Ulo c 1 shares epitopes with Alt a 1, rUlo c 1 and *U. chartarum* extract were exposed to a rabbit antiserum raised against Alt a 1. The anti-Alt a 1 antiserum recognized recombinant as well as natural Ulo c 1, indicating the presence of epitopes conserved between Alt a 1 and Ulo c 1. Furthermore, IgE inhibition blots, carried out with sera from fungal sensitized patients, showed that rUlo c 1 was able to inhibit patients’ IgE binding to Alt a 1, indicating the cross-reactivity between Ulo c 1 and Alt a 1.

In summary, we identified and characterized Ulo c 1, a new allergen from *U. chartarum*, which shows cross-reactivity to Alt a 1, the major *A. alternata* allergen. Recombinant Ulo c 1 represents an IgE reactive molecule that could be applied for component-resolved diagnosis and could therefore improve diagnosis of mold allergy.

This study was funded by project 856337 of the Austrian Research Promotion Agency (FFG).

**ADT-04**  Characterization of the affinity of Mal d 1-specific antibodies induced by sublingual immunotherapy with recombinant Bet v 1 or Mal d 1

Maria R. Strobl¹, Christian Lupinek¹, Claudia Kitzmüller¹, Gabriela Sánchez Acosta², Tamar Kinaciyan², Barbara Bohle²

¹ Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Austria
² Department of Dermatology, Medical University of Vienna, Austria

High structural similarity between the major birch pollen allergen Bet v 1 and the major apple allergen Mal d 1 results in immunological cross-reactivity which causes food allergy in up to 70% of birch pollen allergic individuals upon eating fresh apple. Bet v 1-sensitized individuals with birch pollen-related apple allergy (BPRAA) were treated with sublingual immunotherapy (SLIT) using recombinant (r)Bet v 1, rMal d 1 or placebo for 16 weeks within the framework of a single center, double-blind, placebo-controlled pilot study to investigate the effect of SLIT on BPRAA. Significant clinical improvements were recorded in the rMal d 1-treated group, in contrast to patients who were administered rBet v 1 or placebo. SLIT induced allergen-specific IgG4 antibodies which may block the formation of IgE-allergen complexes. Thereby, cross-linkage of FceRI molecules on mast cells and subsequent degranulation is prevented and allergic responses are hampered. Notably, rMal d 1-specific IgG4 antibodies were found in post-SLIT sera of both verum groups, however, only those induced by SLIT with rMal d 1 showed IgE-blocking capacity. We hypothesize that rMal d 1-specific IgG4 antibodies induced by SLIT with rMal d 1 have a higher affinity to the apple allergen than rMal d 1-specific IgG4 induced by SLIT with rBet v 1. To test this hypothesis, we intend to analyze and compare the binding characteristics of rMal d 1-specific antibodies in post-SLIT sera of both patient groups by surface plasmon resonance. Subsequently, we will correlate possible differences in binding strength to rMal d 1 with clinical efficacy of SLIT. Thereby, we will gain a deeper understanding of the SLIT-induced antibody response and mechanism of tolerance induction in BPRAA.
ADT-05  Alum and monophosphoryl-lipid A as trigger for extracellular trap release from human neutrophils in vitro

Jasmine Karacs, Manuel Reithofer, Barbara Bohle, Beatrice Jahn-Schmid
Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Austria

Most vaccines used in subcutaneous allergen-specific immunotherapy (AIT) contain aluminium hydroxide (alum) as adjuvant. Another adjuvant being used in AIT is the TLR-4-ligand monophosphoryl-Lipid A (MPLA), a detoxified derivative of lipopolysaccharide (LPS). Neutrophils are the most abundant leukocytes in peripheral blood and represent the first line defence against invading microbes. In response to strong bacterial or particulate stimuli, e.g. fungal hyphae, neutrophils have the ability to release DNA together with toxic granular proteins (such as myeloperoxidase, elastase or LL-37) as so-called neutrophil extracellular traps (NETs). These NETs are able to trap and kill microbes, but as modified endogenous DNA they may also represent danger-associated molecular patterns (DAMPs) in the initiation of innate immune responses. Adjuvant-induced NETs may play an important role in the initiation of immune responses of AIT vaccines. In this study, a human in vitro model is used to investigate the role of neutrophils and their NETs in the initiation of immune responses in the presence of alum and/or MPLA. Human neutrophils were freshly isolated from peripheral blood of donors and incubated with medium, as negative control, PMA or ionomycin as positive controls or alum and MPLA, respectively. NET formation was visualized by fluorescence microscopy and quantitatively assessed by DNA release assays. Stimulation of neutrophils at optimum concentrations with alum induced strong DNA release, whereas MPLA, similar to LPS, induced much less DNA release. Confocal fluorescence microscopy confirmed these differences. Alum induced marked NET release with typical co-localization of DNA and granular proteins, while only a few neutrophils released typical NETs upon stimulation with MPLA.

So far, our data show that especially alum is a strong trigger for NET release, while MPLA only weakly induces NET formation. Further experiments will address potential synergistic effects of both adjuvants and the different pathways leading to NET induction.

ADT-06  Myosin light chain 2 - a novel fish allergen

Maximilian Kmen1, Horer Stefanie1, Julia Hable3, Patricia Román-Carrasco1, Katharina Nöbauer2, Ebrahim Razzazi-Fazeli2, Hemmer Wolfgang1, Santiago Quirce4, Ioanna Manolaraki5, Ines Swoboda2
1 Molecular Biotechnology, FH Campus Wien, Austria
2 VetCore Research Facility, University of Veterinary Medicine, Vienna, Austria
3 FAZ Floridsdorfer Allergiezentrum GmbH, Vienna, Austria
4 Department of Allergy, Hospital La Paz Health Research Institute (IdiPAZ), Madrid, Spain
5 Allergy Unit 2nd Paediatric Clinic, University of Athens, Greece

Fish is one of the 8 most common food allergen sources and affects approximately 1% of the global population. The most common clinical symptoms of fish allergy are urticaria, angioedema, asthma, rhinitis as well as nausea and vomiting. However, fish can even cause anaphylactic shock reactions. Patients suffering from fish allergies produce specific IgE antibodies against fish proteins. Most patients allergic to fish display IgE reactivity to parvalbumin, a 12 kDa calcium-binding protein, which is highly resistant to enzymatic digestion as well as heat treatment. Other fish allergens described are aldolase, enolase, and collagen. Yet, these allergens are less frequently recognized by patients’ IgE than parvalbumin.

In this study, we aimed to identify further allergens involved in fish allergy. For this, we used sera from 20 Spanish children allergic to fish and performed IgE immunoblots with a protein extract from cooked salmon. Mass spectrometry of a protein of 20 kDa, which was recognized by the majority of patients, allowed us to identify myosin light chain 2 (MLC-2) as a new fish allergen. A cDNA coding for MLC-2 was cloned from salmon mRNA by reverse transcriptase PCR and subcloned into the bacterial expression vector pET-17b. This enabled production of recombinant MLC-2 in Escherichia coli. The recombinant molecule will now be investigated regarding its biochemical and immunological features, in order to evaluate its applicability as a new tool for diagnosis of fish allergic individuals.

Acknowledgment: This study was funded by research grant P25868 of the Austrian Science Fund (FWF) and by project 856337 of the Austrian Research Promotion Agency (FFG).
ADT-07  Contribution of conformational and linear IgE epitopes to Ara h 2-specific IgE-binding – in vitro and in vivo studies

Angelika Tscheppe¹, Dieter Palmberger², Christian Radauer³, Leonie S. Van Rijt¹, Merima Bublin¹, Christine Hafner³, Wolfgang Hemmer⁵, Vanessa Mayr¹, Chiara Palladino¹, Adrian Logiantara³, Ronald Van Ree³, Reingard Grabherr², Heimo Breiteneder¹

¹ Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria
² Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
³ Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands
⁴ Department of Dermatology, University Hospital St. Pölten, Karl Landsteiner University of Health Sciences, St. Pölten, Austria
⁵ Floridsdorf Allergy Center, Vienna, Austria

Introduction: Ara h 2 is the most important peanut allergen. Derivatives with reduced IgE-binding capacities are promising candidates for allergen specific immunotherapy. Little is known about the contributions of conformational and linear epitopes to binding of patients’ IgE. Therefore, we aimed to define the patient-specific IgE epitope profiles of Ara h 2.

Methods: An Ara h 2 mutant (mt) lacking most linear epitopes and the wild-type protein (wt) were expressed in the baculovirus-insect cell system. Purified allergens and the natural protein (n) were reduced and alkylated (red/alk) to destroy the conformational epitopes. IgE-binding was tested by ELISA using sera of 55 Ara h 2 allergic children and adults. Basophile activation tests were performed using whole blood of 8 Ara h 2 sensitized patients. C3H/HeOUJ mice were orally sensitized with peanut extract and challenged intraperitoneally with the various proteins to determine their anaphylactogenic potencies.

Results: Mt, wt and nAra h 2 showed the predicted masses and structures. Complete reduction and alkylation was verified by mass spectrometry. IgE-binding was reduced in a patient specific manner by up to 50% due to the loss of conformational epitopes by reduction/alkylation (p<0.001). Likewise, IgE-binding was reduced by up to 70% when linear epitopes were mutated (p<0.001). Patients with high levels of Ara h 2 specific IgE tended to recognize primarily linear epitopes (r=0.305, p=0.025), while patients with low levels of Ara h 2 specific IgE recognized mainly conformational epitopes (r=-0.5, p=0.0001). Basophil activation tests showed the lowest activation upon stimulation with the reduced and alkylated mutant. In vivo, mice reacted with anaphylaxis upon challenge with mt, wt and n but not with the red/alk proteins. Furthermore, the challenge did not affect the levels of Ara h 2 specific IgE or IgG1 antibodies.

Conclusions: The obtained results indicate that both epitope types are important for allergen specific IgE-binding in a patient specific manner. In contrast to the published literature, full destruction of the 3D structure is required when designing safe hypoallergens.

Supported by the Austrian Science Fund doctoral program W1248-B30 and grant P 30936-B30, the Medical University of Vienna and Cost Action FA1402 (ImPARAS).
Extracellular vesicles in coagulation and inflammation

Chair: Viktoria Weber

Supported by ASEV
**EV-01** Imaging Extracellular Vesicles, Exosomes and Microparticles in Plasma and Conditioned Media

Alain Brisson  
University of Bordeaux, UMR-CBMN, France

Most cells release vesicles upon activation. These vesicles, called extracellular vesicles (EV), are limited by a protein-rich lipid membrane and contain cytoplasmic elements. Two main types of EV are distinguished, depending on their mechanism of formation: microvesicles or exosomes form at the plasma membrane, while exosomes form within multivesicular bodies (MVB) and are released after fusion of MVB with the plasma membrane. EV raise high interest, due to the diversity of their physiopathological roles, e.g. in coagulation, inflammation, cell-cell communication, cancer or cardiovascular diseases, as well as their biomedical applications, as biomarkers, therapeutic agents or drug delivery systems (1). However, despite intense research activity, our understanding on what are EV, what are their functions and mechanisms of action remains largely unknown. This is due to the difficulty of characterizing EV, which results mainly from the heterogeneity of EV suspensions and the small size of EV - most of them ranging from 50 to 500 nm. The characterization of EV suspensions requires the combination of biochemical, structural, biophysical and functional methods (2). Since the initial discovery of EV, electron microscopy (EM) has played a major contribution in EV research, providing unique information on their structure, size and phenotype. Nowadays, cryo-transmission EM is the gold standard method for imaging molecules or particles ranging in size from nm to few hundred nm in complex media, at nm resolution. This presentation will illustrate the contribution of cryo-EM combined with immuno-gold labeling to the field of EV, including the description of the main EV populations in plasma of healthy individuals, the search for EV signatures in diseases, the imaging of MVB-derived exosomes and the control quality of EV isolation procedures (3-5).

2- Coumans et al., Circulation Research 2017, 120:1632-1648.  
5- Spinelli et al., J. Extracell. Ves. 2018, 7(1).

**EV-02** Extracellular vesicles from different settings support thrombin generation via different pathways

Carla Tripisciano1, René Weiss2, Tanja Eichhorn1, Andreas Spittler2, Michael Bernhard Fischer3, Viktoria Weber1  
1 Center for Biomedical Technology, CD-Laboratory for Innovative Therapy Approaches in Sepsis, Danube University Krems, Austria  
2 Core Facility Flow Cytometry & Department of Surgery, Research Lab, Medical University of Vienna  
3 Center for Biomedical Technology, Danube University Krems

Introduction: We investigated the thrombogenicity of extracellular vesicles (EVs) from blood products from healthy donors and the contribution of different molecules. We compared platelet and red blood cell EVs to EVs from cell culture environment.

Methods: Platelet and red blood cell units were obtained from whole blood using an automated blood collection system (Version 5.0, Gambro BCT). Different vesicle fractions were enriched by sequential centrifugation (20,000 g and 100,000 g), after cell removal (2500 g). EVs from mononuclear cells were enriched with the same protocol.

Results: EV fractions were normalized to protein content, and characterized by nanoparticle tracking analysis, cryo-electron microscopy, imaging flow cytometry, and flow cytometry using lactadherin as marker for phosphatidylserine (PS), CD41 for platelets, CD235a for red blood cells, and CD45 for leukocytes. EVs supported thrombin generation mainly due to exposure of PS, as this thrombogenicity was inhibited by pre-incubation with annexin V. On the opposite, pre-treatment with anti-tissue factor (TF) mAb (HTF-1) did not affect thrombin generation induced by platelet or red blood cell EVs, while it reduced thrombin generated by LPS-stimulated mononuclear cells. Upon inhibition of factor XII (contact pathway), no thrombin was generated by blood-derived EVs, whereas EVs from LPS-stimulated mononuclear cells triggered the formation of thrombin via the extrinsic pathway. Remarkably, EVs from unstimulated mononuclear cells did not support thrombin generation by either pathway. By removing residual blood-derived contaminants from red blood cell and platelet EVs via size exclusion chromatography, no decrease in thrombin generation was observed, excluding the co-enrichment of soluble clotting factors.

Conclusion: Our data indicate the ability of EVs to support thrombin generation by exposing PS, while the ability to trigger coagulation is restricted to EVs from pathological settings with the presence of functionally active TF. As the ability to support coagulation differs in EVs from different settings, the presence of surface-bound coagulation factors in blood-derived EVs is currently under investigation.
**EV-03  Complex links between inflammation, cancer and thrombosis**

Bernhard Moser, Bernhard Hochreiter, José Basílio, Johannes A. Schmid

Inst. of Vascular Biol. and Thrombosis Research, Med. Univ. Vienna, Austria

Inflammation is the physiological response of the organism to all different kinds of stress situations, whether it is the invasion of pathogens, mechanical or physical injury or the occurrence of danger-associated molecular patterns (DAMPs). All of these stimuli activate the NF-κB signaling pathway, triggering a network of kinases, as well as transcription factors that alter the cellular program to cope with the stressor. In most cases cell survival mechanisms are induced such as anti-apoptotic proteins. However, this might have detrimental consequences in case of simultaneous oncogenic mutations, providing a survival advantage to transformed cells. Moreover, many types of cancer represent an inflammatory stimulus themselves and are interpreted by the organism as “wounds that do not heal”. Furthermore, inflammation is evolutionary tightly linked to blood coagulation, as injuries require not only efficient blood clotting but also a rapid immune defense. This is the basis for increased thrombotic risks that come along with chronic inflammatory disorders, as well as cancers. We have evidence that signaling kinases of the NF-κB pathway phosphorylate and stabilize an important oncogene and furthermore that this gene binds to the promoter region of tissue factor (coagulation factor III) thereby enhancing its expression, which may result in the release of TF-positive microvesicles from cancer cells into the circulation. This provides a molecular explanation for a functional link between inflammation, elevated oncogene levels and increased thrombotic risks, which are observed in a high percentage of cancers.

**EV-04  Differential interaction of platelet-derived extracellular vesicles with leukocyte subsets in human whole blood**

René Weiss¹, Marion Gröger², Sabine Rauscher², Birgit Fendi³, Tanja Eichhorn¹, Michael B. Fischer³, Andreas Spittler⁴, Viktoria Weber¹

¹ Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis, Danube University Krems, Austria
² Core Facility Imaging, Medical University of Vienna, Vienna, Austria
³ Center for Experimental Medicine, Danube University Krems, Krems, Austria
⁴ Core Facility Flow Cytometry & Surgical Research Laboratories, Medical University of Vienna, Vienna, Austria

**Introduction:** We have previously found that circulating extracellular vesicles (EVs) are primarily associated with granulocytes and monocytes, but scarcely with lymphocytes. In this context, we studied the association of EVs with immune cells, particularly with monocyte subsets.

**Methods:** Association of EVs with immune cells was visualized by flow cytometry, imaging flow cytometry and confocal microscopy. Monocyte subsets were identified directly in whole blood based on their CD14 and CD16 expression as classical (CM; predominantly phagocytic; CD14⁺⁺CD16⁻), intermediate (IM; phagocytic and pro-inflammatory; CD14⁺⁺CD16⁺), and non-classical monocytes (NCM; mainly pro-inflammatory; CD14⁻CD16⁺⁺) using flow cytometry. The association of monocyte subsets with platelet-derived EVs was detected using lactadherin (LA) as marker of phosphatidylserine and CD41 as platelet marker. In addition to their characterization in whole blood, we studied the association of platelet-derived EVs with monocytes isolated from PBMCs by negative depletion of non-monocytes.

**Results:** Imaging flow cytometry and confocal microscopy confirmed the preferential interaction of platelet EVs with monocytes and granulocytes. The distribution of monocyte subsets in freshly drawn whole blood was 86.1 ± 2.1% CM, 4.9 ± 1.1% IM, and 9.0 ± 2.6% NCM, respectively. Freshly isolated monocytes exhibited an almost identical distribution. Overnight resting, however, induced a significant shift towards IM (4.9 ± 1.1% vs. 59.1 ± 24.0%). On average, 5.5 ± 3.6% of all CM, 16.6 ± 6.1% of all IM, and 3.5 ± 2.1% of all NCM were CD41⁺LA⁺, indicating their association with platelet EVs (n=6). Storage of whole blood induced an increase in monocyte-EV aggregates to 66.3 ± 12.1% for CM, to 80.1 ± 8.7% for IM, and to 28.4 ± 11.1% for NCM, indicating the preferential association of EVs with CM and IM.

**Conclusion:** Monocyte subsets are differentially associated with platelet-derived EVs. The mechanisms and functional implications of this differential association remain to be elucidated.
Extracellular vesicles (EVs) have been increasingly recognized as central players in intercellular communication. Depending on their cargo, they can influence various biological functions in the recipient cells and have been implicated in the progression of various diseases. EVs are present in most physiological fluids, including plasma and synovial fluid. In this study, we investigated the presence of EVs within two autologous blood products, platelet rich plasma (PRP) and hypACT serum, in order to gain understanding of the mechanisms of action of these products. PRP, in particular, is frequently clinically applied to support the treatment of osteoarthritis, but its specific mechanism of action remains to be elucidated.

PRP, and hypACT serum were produced from whole blood derived from the same donor using standardized, but distinct isolation protocols. EDTA or citrate was used in PRP samples as anticoagulants. The abundance and average size of EVs in these blood products were determined by flow cytometry and nanoparticle tracking analysis (hypACT serum: 4923 EVs/µl ± 504, 156 nm ± 0.72; PRP (EDTA): 2554 EVs/µl ± 251, 149 nm ± 5.9; PRP (citrate): 8673 EVs/µl ± 3864, 158 nm ± 13.04). The cellular origin of the EVs was determined using flow cytometry with CD41 as platelet marker and CD235a as red blood cell marker, while lactadherin (LA) served as marker for phosphatidylserine exposing EVs.

The majority of EVs in hypACT serum originated from platelets (LA+/CD41+). Interestingly, the two anticoagulants had a strong impact on the cellular origin of EVs present in PRP. EVs from PRP samples including EDTA as an anticoagulant descended mainly from red blood cells (LA+/CD235a+) whereas EVs from citrate treated PRP samples mainly originated from platelets (LA+/CD41+). Next, we isolated EVs from the respective blood products by ultracentrifugation as well as by size exclusion chromatography and verified successful isolation by staining with EV-specific markers Annexin V and CD63. These isolated EVs will be further used to investigate their role in cell culture models of inflammation and osteoarthritis. In conclusion, our data reveal differences between PRP and hypACT serum both with respect to EV counts and cellular origin, which may have implications for the therapeutic application of these blood products. In addition, our results demonstrated that anticoagulants have a strong impact on the cellular origin of EVs and subsequently on their biological function.
Tuesday 18th: DK BioToP

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

DK BioToP

Chair: Jürgen Zanghellini
DKB-01  Are somatic mutations of mAbs responsible for low expression and thermal stability?

Linda Schwaigerlehner, Patrick Mayrhofer, Renate Kunert

Department of Biotechnology, University of Natural Resources and Life Sciences, Austria

The antibody expression potential of individual monoclonal antibodies (mAbs) can differ significantly, despite recombinant chinese hamster ovary (CHO) cell lines are developed and cultivated under comparable conditions. We conclude that these expression differences might result from the intrinsic antibody structure and its interaction with cellular compartments of the folding and secretion machinery (Mayrhofer et al., 2014; Sommeregger et al., 2016). To explore responsible factors we defined a population of four mature naturally occurring mAbs and designed a germline derived cognate mAb of each. We assigned each naturally occurring mAb to a designed mAb variant composed of the nearest related germline genes. To compare interrelated mature and germline antibody pairs we express them stably in a defined chromosomal environment in CHO cells. In order to standardize the production process, semi-continuous perfusion experiments were performed. Although the antibodies differ only in their variable sequence, different expression potentials are observed in the IgG expressing CHO clones. Intracellular and secreted product was analyzed by immunoblotting to enable tracking of heavy and light chain fragments as well as accumulation of certain antibody fragments. In addition, we performed differential scanning calorimetry (DSC) measurements of the purified IgG variants. Two germline variants showed up a significant increase in thermal stability compared to their associated mature antibody. These methods help us to investigate the phenomenon of differential antibody expression levels and their correlation to thermal stability properties.

Acknowledgments

This work was supported by the PhD program BioToP (Biomolecular Technology of Proteins) funded by FWF under Project W1224. Furthermore, it was supported by EQ-BOKU VIBT GmbH and the BOKU Core Facility for Biomolecular and Cellular Analysis.

References


DKB-02  Phospholipid vesicles to determine the transport functionality of mitochondrial carrier proteins

Dominik Jeschek1, Matthias G. Steiger2, Diethard Mattanovich2, Michael Sauer2

1 Department of Biotechnology, University of natural resources and life sciences, Austria
2 Department of Biotechnology, BOKU - University of Natural Resources and Life Sciences Vienna, Muthgasse 18, Vienna, Austria
3 CD Laboratory for Biotechnology of Glycerol, Muthgasse 18, Vienna, Austria

Mitochondrial carrier proteins play a key role in many metabolic pathways. Some of these membrane transport proteins carry di- and tricarboxylic acids across the mitochondrial membrane. The construction of efficient cell factories to produce secondary metabolites requires a deeper understanding of transport capabilities. However, dissecting these highly hydrophobic proteins requires the development of suitable expression systems and membrane-like environments to retain their functionality.

In our study, we employ synthetic phospholipid vesicles to incorporate fully functional membrane proteins for transport activity assays. In the formed proteoliposomes, membrane transporters are embedded into a phospholipid bilayer, which surrounds an aqueous inner compartment. These constructs enable the simulation of distinct cellular compartments in vitro due to possible variations in the composition of phospholipids, integral membrane proteins and enclosed inner substances. We established a cell-based and a cell-free process to construct these systems, ranging from the expression to the incorporation of membrane transporters into vesicles. Usage of proteoliposomes in combination with specific assays to analyze substrate fluxes provides details of the carrier process. The information obtained helps to develop metabolic models and metabolic engineering strategies incorporating details about transport processes, which has great potential to push the frontiers of microbial organic acid production.
Historically, the ribosome has been regarded as a static machine and all ribosomes within a cell or organism were assumed to be identical. This simplified view has been challenged in recent years, when the existence of heterogeneous ribosome populations was reported and evidence for structurally “specialized” ribosomes emerged. Heterogeneous ribosomes may differ in rRNA, ribosomal protein composition or differential modifications of ribosomal proteins or rRNA. The functional consequences of these structural specializations are still poorly understood. However, it is apparent that modulation of protein synthesis represents an important aspect of the cellular response to different environmental conditions or certain cellular states.

Down-regulation of NSUN5, a methyltransferase responsible for a site-specific methylation of ribosomal RNA, lead to an increased lifespan and stress resistance in *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*. Subsequent cell culture experiments with human cells revealed that NSUN5- knock out reduced global protein synthesis via modulations intrinsic to the ribosomes.

After this proof-of-principle that already a single nucleotide-modification of rRNA can drastically alter the cellular state and affect whole organism lifespan, we are now interested in senescence-associated differences of ribosomal structure and function.

Senescence is a cellular state in which cells enter an irreversible cell cycle arrest. It has been shown that the presence of senescent cells *in vivo* is associated with several age-related pathologies, including kidney fibrosis, atherosclerosis and many cancers, while removal of senescent cells in mice was shown to ameliorate these conditions.

Using human dermal fibroblasts (HDFs) as a model, we have established protocols to induce and confirm the senescent state of cells *in vitro*. So far, our data indicates increased global protein synthesis in senescent cells and we are currently investigating potential changes to the ribosomal structure and function, as the underlying mechanism of this effect is still unknown.
Tuesday 18th: DK ICA

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

DK ICA

Chair: Jutta Horejs-hoeck
DKI-01 The Activation State of Langerhans Cells Determines the Fate of Cytotoxic T Cells

Helen Strandt¹, Douglas Florindo Pinheiro², Daniel H. Kaplan³, Dagmar Wirth³, Iris Karina Gratz³, Peter Hammerl⁴, Josef Thalhamer⁴, Angelika Stockelinger⁰

¹ Department of Dermatology, University of Pittsburgh, Pittsburgh, PA 15261 ² Department of Immunology, University of Pittsburgh, Pittsburgh, PA 15261
³ Helmholtz Centre of Infection Research, 38102 Braunschweig, Germany
⁴ Biosciences, University of Salzburg, Austria

Langerhans cells (LCs) in the epidermis are known to act as antigen-presenting cells (APC). However, the presence of multiple dendritic cell (DC) populations in the skin has complicated the analysis of these cells functions. In order to assess LC function in vivo, we have generated transgenic mice in which antigen presentation was inducible and limited to LCs. In these mice, tamoxifen (TAM) treatment induced transgenic expression of different antigens (Ags) including non-secreting GFP-OVA in LCs but no other DC subsets. LCs presenting GFP-OVA could stimulate endogenous OVA-specific cytotoxic CD8⁺ T cell (CTLs) under steady-state conditions, inducing these CTLs to proliferate, kill target cells, and secrete IFNγ. However, these mice were resistant to cutaneous challenge with OVA during the contraction phase of the CTL response, suggesting the induction of tolerance. This tolerance was confirmed to be Ag-specific and was dependent on regulatory T cells (Tregs), as Treg depletion reversed tolerance, allowing expansion of IFNγ-secreting CTLs in OVA-challenged mice. To investigate whether the activation state of LCs affected the nature of the CTL response, LCs were stimulated in vivo with anti-CD40 and polyinosinic:polycytidylic acid (poly I:C) concurrently with TAM treatment, which induced presentation of GFP-OVA, and then challenged with cutaneous OVA as before. OVA-specific CTLs expanded by these activated LCs did not exhibit tolerance but were instead strongly activated by cutaneous OVA challenge, suggesting that a memory response had been induced. Supporting these findings, CD62L and IL-7R expression, both expressed on central memory T cells, were upregulated on CTLs primed by activated LCs but not on CTLs primed by steady-state LCs. This in vivo system allows isolated analysis of LC Ag presentation and identifies conditions under which these cells can either tolerize CTLs or promote from them a robust recall response. (This project was funded by the FWF: P25243-B22 and W01213).

DKI-02 Phosphorylation: an Important Signal in Biological Chemistry

Tamara Scheidt¹, Humberto Jorge-Gonczarowska¹, Wolfgang Gruber³, Margherita Dell’Aica³, René Zahedi³, Fritz Aberger³, Christian G. Huber⁰

¹ 2Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V., Dortmund, Germany
³ University of Salzburg, Austria

Question: Dysfunctions in phosphorylation cascades can promote the development of various diseases such as cancer. The holistic approach of functional phosphoproteomics using HPLC-MS based techniques has become an important research tool to investigate the phosphoprotein composition of tumor cells in a particular state. Within this study the comprehensive attempt should help to better understand the mechanisms of action involved in the function of the oncogenic GLI transcription factor proteins after smoothened (SMO) dependent activation and inhibition of the oncogenic hedgehog (Hh) pathway.

Methods: Human medulloblastoma cell cultures (DA0Y cells) were treated for two different time points with the Hh activator smoothened agonist (SAG) and the antagonist Vismodegib, which selectively blocks Hh signaling. Treatment with epidermal growth factor (EGF) was used as a control for increased early phosphorylation events. After cell lysis, the protein content was isolated and digested with trypsin by filter-aided sample preparation. Protein quantification was enabled by 10-plex TMT labels. Enrichment of phosphopeptides was performed with titanium dioxide beads. Hydrophilic interaction chromatography prior to reversed-phase high-performance liquid chromatography mass spectrometry was applied to characterize the phosphoprotein composition. Peptide identification was performed by tandem mass spectrometry using data-dependent acquisition.

Results: More than 10 000 phosphosites out of 8000 protein groups were identified. Several phosphosites were found to be differentially regulated in the smoothened activated cells compared to Vismodegib treatment. Diverse proteins involved in Hedgehog dependent intracellular signaling and cell cycle control varied among assorted treatments and timepoints.
DKI-03  *Helicobacter pylori*-controlled c-Abl localization promotes cell migration and limits apoptosis

Gernot Posselt¹, Maria Wiesauer¹, Bianca Chichirau¹, Daniela Engler², Linda Krisch¹, Gabriele Gadermaier³, Peter Briza³, Sabine Schneider⁴, Francesco Boccellato⁵, Thomas Meyer⁵, Cornelia Hauser-Kronberger⁶, Daniel Neureiter⁶, Anne Müller², Silja Wessler¹

¹ Biosciences, University of Salzburg, Austria
² Institute of Molecular Cancer Research, University of Zurich, Switzerland
³ Department of Biosciences, Division of Allergy and Immunology, University of Salzburg, Austria
⁴ Paul-Ehrlich-Institute, Langen, Germany
⁵ Max Planck Institute for Infection Biology, Berlin, Germany
⁶ Department of Pathology, Paracelsus Medical University Salzburg, Austria

The class-I carcinogen *Helicobacter pylori* (Hp) activates the non-receptor tyrosine kinase c-Abl to phosphorylate the oncoprotein cytotoxin-associated gene A (CagA). The role of c-Abl in CagA-dependent pathways is well established; however, the knowledge of CagA-independent c-Abl processes is scarce. Here, we investigated the activity and subcellular localization of c-Abl in vitro and in vivo and unraveled the contribution of c-Abl in CagA-dependent and -independent pathways to gastric Hp pathogenesis. We report a novel mechanism and identified strong c-Abl threonine 735 phosphorylation (pAblT735) mediated by the type-IV secretion system (T4SS) effector D-glycer-β-D-manno-heptose-1,7-bisphosphate (βHBP) and protein kinase C (PKC) as a new c-Abl kinase. pAblT735 interacted with 14-3-3 proteins, which caused cytoplasmic retention of c-Abl, where it potentiated Hp-mediated cell elongation and migration. Further, the nuclear exclusion of pAblT735 attenuated caspase-8 and caspase-9-dependent apoptosis. Importantly, in human patients suffering from Hp-mediated gastritis c-Abl expression and pAblT735 phosphorylation were drastically enhanced as compared to type C gastritis patients or healthy individuals. Pharmacological inhibition using the selective c-Abl kinase inhibitor Gleevec confirmed that c-Abl plays an important role in Hp pathogenesis in a murine in vivo model. In conclusion, Hp actively promotes cytoplasmic localization of activated c-Abl through PKC-mediated pAblT735 phosphorylation to increase Hp-mediated EMT-like processes, while preventing excessive apoptosis.
Wednesday 19th

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Wednesday 19th September 2018
Wednesday 19th: Plenary 3

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Plenary 3
Chair: Michael Sauer

P3-01 Can we develop a ROS-based antibacterial therapy?
Paul Cos
LMPH, University of Antwerp, Belgium

Bacteria can grow as single cells (planktonic mode of growth) or as a sessile community, also known as a biofilm. According to the National Institutes of Health (NIH), biofilms are involved in over 80% of all bacterial infectious diseases. The biofilm lifestyle gives many advantages to the microorganisms involved. The extracellular matrix offers protection against host defense mechanisms and can shield the sessile cells from certain antimicrobial compounds like oxidizing agents. It is therefore pivotal to study the interaction between reactive oxygen species (ROS), for example produced by macrophages and neutrophils, and sessile bacteria. This presentation focuses on the challenges and pitfalls of measuring oxidative stress in bacteria with the aim of setting standards. The most common methods used in free radical research will be discussed with a specific focus on their advantages and shortcomings. Besides the fingerprinting methods, free radicals can also be detected with electron spin resonance (ESR). ESR studies on the measurement of free radical production in macrophages and animals will be discussed.

The potential of a ROS-based antibacterial therapy will be reviewed. Antibiotic-mediated killing of bacteria can be mediated by ROS-dependent mechanisms. In a recent study the effects of different classes of antibiotics were compared against both planktonic and sessile cultures. An alternative approach to treat biofilm infections is to locally increase the oxidative stress, like e.g. with a cold plasma pen. Although neutrophils play a crucial role in our innate immune defense against pathogens, they may also contribute to tissue damage at the site of inflammation. Selective modulation of the neutrophils’ oxidative burst could offer an interesting pharmacological approach. Mycothione reductase is an enzyme that plays a pivotal role in the redox homeostasis of Mycobacterium tuberculosis. Like many Actinomycetes, M. tuberculosis produces mycothiol to protect the organism against oxidative stress and the druggability of this target will be reviewed.
Emerging zoonosis: one strategy, multiple host

Marina Santic
Microbiology and Parasitology, Medical faculty, University of Rijeka, Croatia

*Francisella tularensis* is an intracellular pathogen and is the etiological agent of tularemia in humans and animals. The bacterium is transmitted from infected animals to humans by multiple routes and can cause disease of varying severities. Due to ease of transmission, low infectious dose and severity of infection, *Francisella tularensis* has been classified as Tier 1 select agent. It is known that *Francisella* uptake and replication occurs in a variety of cell types including phagocytic and non-phagocytic cells. However it is still unknown what type of host cells provide growth niches essential for virulence of this bacterium. It is still the mystery how bacterium could adapt and to so many different reservoirs (250) and niche, existing with rather small genome. Once inside different cells type the life cycle is unique comparing to other intracellular pathogen. Within the cell, the bacterium is circulating between the intracellular compartment; the phagosome (*Francisella* containing phagosome), and the vacuole (*Francisella* containing vacuole). The incidence of tularemia in Europe and worldwide is increasing, while there is still no vaccine available to prevent this zoonotic disease. This overview will summarize the important aspects and different faces of tularemia in nature.
Wednesday 19th: Poster Flash 2

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Flash 2
PF2-01  Establishment of a human endometrial cancer organoid library

Hyelin Na
Institute of Molecular Biotechnology, Austria

Endometrial cancer is a type of cancer that forms in the lining of the uterus. Endometrial cancer is the sixth common cancer and the fourth prevalent cancer in female worldwide. Incidence of endometrial cancer have increased in many countries for decades. In more developed regions, incidence and mortality are higher that less developed regions. The signs and symptoms of endometrial cancer, such as abnormal vaginal bleeding or discharge, enable the disease to be diagnosed at an early stage. Due to early detection, most patients have low-grade and early stage diseases. The early-detected endometrial cancers can be cured by hysterectomy and surgery. However, except main surgical treatments, most patients don’t have any benefit from current targeted therapeutics. Low-grade endometrial cancers are often treated with radiotherapy, whereas some endometrial cancers with an advanced stage are treated with chemotherapy. These adjuvant therapies can help the cures, but it can be harder on some patients because it causes more side effects. In this aspect, the importance of understanding individual patient endometrial cancer might be strongly emphasized. For the study of individual patient endometrial cancer, organoid culture systems have recently emerged as a novel platform for unmet clinical needs. Organoid culture systems allow patient-derived cancer cells to be successfully cultured in the laboratories and used quickly for test against adjuvant therapy, leading to develop the individual treatment strategies for cancer. To date, several lines of patient-derived endometrial cancer organoids have been generated, but large-scale functional analysis of endometrial cancer has not been performed. In this study, we established a patient-derived endometrial cancer organoid library and refined organoid culture conditions. This endometrial cancer organoid library will provide a valuable prognostic platform for functional analysis and rapid test of individual endometrial cancer treatment.

PF2-02  Dissecting the role of the PIDDosome in hepatocellular carcinoma

Katja Knapp
Division of Developmental Immunology, Innsbruck Medical University, Austria

The PIDDosome is an activation platform for Caspase-2 additionally containing the proteins PIDD1 and RAIDD. The PIDDosome functions as a sensor for supernumerary centrosomes which can occur upon cytokinesis failure or endoreduplication. In such polypliod cells, the PIDDosome activates Caspase-2, starting a signaling cascade which triggers cell cycle arrest via cleavage of Mdm2, p53 stabilization and p21 induction. Failed cell division and centrosome amplification promote genomic instability which is considered to be a hallmark of cancer. In the liver, however, polyploidization of hepatocytes is part of normal organogenesis devoid of malignant transformation. Previously, we could show that the PIDDosome has a key role in regulation of hepatocyte polyploidization in vivo during liver development and regeneration. To investigate the consequences of PIDDosome deficiency on tumor development we used a carcinogen-driven hepatocellular carcinoma mouse model. Surprisingly, we found that PIDDosome knockout mice exhibit significantly fewer tumors than wildtype mice. As the total tumor burden was similar in all genotypes, histopathological analysis will be performed to assess potential differences in differentiation stage and proliferative index of the tumors. Analysis of the ploidy state of tumor cells will show whether this effect is directly due to PIDDosome loss or if the effect is secondary to increased hepatocyte ploidy, regardless of its cause. Moreover, samples of human HCC patients will be analyzed with regards to the ploidy state and activation of the PIDDosome pathway to reveal the relevance of the centrosome-PIDDosome axis in human tumorigenesis.
**PF2-04 Establishment of an *in vitro* phosphate buffer induced calcification model**

**Pia Hager**, Sabrina Rohringer, Helga Bergmeister, Bruno K. Podesser, Barbara Kapeller, Karl H. Schneider

1. Department of Biomedical Research, Medical University of Vienna, Austria
2. Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna

**Introduction**
Calcium mineral deposition (calcification) in tissues is a multifactorial process that frequently accompanies atherosclerosis or valvular heart disease. Patients suffering from hyperphosphatemia or chronic inflammatory diseases such as rheumatoid arthritis are facing an increased risk to develop symptoms of vascular calcification due to permanent administration of corticoid drugs. We developed a calcification model to mimic and compare several pathological risk factors *in vitro*. Cardiovascular tissue specific cells can be stimulated by elevated levels of inorganic or organic phosphates, corticoids and ascorbic acid to trigger cell calcification.

**Materials and Methods**
Endothelial cells (EC) and fibroblasts (FB) were seeded in 24 well plates and stimulated with calcification media (CM). The calcification media (CM1 and CM2) consisted of regular cultivation medium for each cell type (DMEM for FB and Medium 200 for EC) supplemented with 2 mM Phosphate (CM1) or 10 mM β-Glycerophosphate, 50 mg/ml Ascorbic acid and 10 nM Dexamethasone (CM2). Cells cultivated with the regular cultivation medium were used as control. Culture plates were incubated for 6, 24, 48, 72, and 96 hours before histological staining was performed to determine proliferation rates and calcium deposition.

**Results**
Both cell types showed a change in proliferation rates when incubated in the calcification media. CM1 induced significant increase and CM2 significantly decreased the proliferation rate of both cell types during the first 96 hours of incubation. Furthermore, CM2 was more adequate to induce calcification in both cell types compared to CM1 medium. Each experiment was performed three times for both cell types and showed little divergence within each group.

**Conclusion**
We established a simple and reproducible *in vitro* test for investigating the influence of different proteins or drugs on cell types involved in calcium deposition. Creating high technical repetitive accuracy within our model was an important factor to enable future calcification studies.

**PF2-05 Identification of a citrate exporter protein CexA for citric acid production in *Aspergillus niger***

**Alice Rassinger**, Diethard Mattanovich, Michael Sauer, Matthias Steiger

1. Department of Biotechnology, BOKU, Austria
2. Austiran Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 18, Vienna, Austria; Department of Biotechnology, BOKU - University of Natural Resources and Life Sciences Vienna, Muthgasse 18, Vienna, Austria; CD Laboratory for Biotechnology of Gl

Citric acid is a commonly used organic acid, which is applied as food additive or preservative, and is used for further production of bulk chemicals in the industry. Amongst various microbial hosts, *Aspergillus niger* remains one of the top producers of citric acid. It has been widely studied in terms of media composition, the metabolic flow through the TCA, bioprocess optimization and most notably the substrate uptake. However, the export of citric acid remains still obscure. Based on transcriptomic data and a similarity in sequence to a putative itaconic acid transport gene from *Ustilago maydis*, a candidate citrate exporter protein (CexA) was identified. A deletion of cexA leads to an abolished citric acid accumulation. A heterologous expression of CexA in baker’s yeast (*S. cerevisiae*), which naturally does not secrete citric acid into the media, leads to accumulation of citric acid. On top of that, we found that CexA is a specific transporter for citrate and does not transport structurally similar compounds like isocitrate in *S. cerevisiae*. To summarize, the identification of CexA presents a new target for further strain engineering in *A. niger* and can be used to create new citric acid producing microbes.
PF2-06  Interplay of hospital microbiome and resistome – connecting pathogenic infection risk, healthy microbes and environmental biodiversity in and functional hospital setting

Stefanie Duller
Department of Internal Medicine, Medical University of Graz, Austria

Hospital acquired infections are a serious problem worldwide. The risk of acquiring pathogenic infections is higher in hospital than in other environments, and these infections are also more often fatal. The risk is not only related to invasive procedures or inadequate hygiene; infection can also be transferred from patient to patient, via personnel, surfaces, or routinely used equipment. Resistant (pathogenic) bacteria are frequently found in hospital surfaces, despite strict disinfection procedures. Consequently, the microorganisms that inhabit hospital indoor environment can influence the patient recovery and outcome, and potential link between hospital indoor environment-associated microbial communities and hospital-acquired infections has been suggested.

In this project we explore the microbiomes and resistomes of different hospital areas and sites with different cleaning and disinfection procedures, such as operating room, intensive care unit, waiting areas and toilets, and study if purposely increased environmental biodiversity in hospital can decrease the prevalence of (opportunistic) pathogenic microorganisms and the extent of horizontal gene transfer, reflecting the probability for developing new hospital pathogens. Our focus is on the whole microbial communities, and their relation to the pathogenic, life-threatening organisms and resistance. Resolving the bacterial community structure in hospital environments will be critical for understanding the dissemination of antibiotic resistance genes and development of antibiotic resistance.

The results will help us to understand the microbial dynamics in the hospital indoor environment and to support safe recovery of the patients. In this presentation, we will summarize the latest results of this project.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

**Microbial chemical production**

Chairs: Matthias Steiger & Alex Lichius
MCP-01  Engineering yeasts for production of high-value metabolites
Irina Borodina
The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark

One of the major applications of synthetic biology is development of novel cell factories for sustainable production of bulk and specialty chemicals. The recent advances in CRISPR-based genome editing of yeast made construction of yeast cell factories cheaper and faster. These genetic tools facilitate iterative cycles of metabolic engineering, where the cellular metabolism is systematically re-wired towards higher titer, rate and yield of the target products. I will present examples of engineering yeast *Saccharomyces cerevisiae* and oleginous yeast *Yarrowia lipolytica* for production of high-value metabolites, such as aromatic nutraceuticals, carotenoid feed additives, and insect pheromones for environmentally friendly pest control.

MCP-02  Microbial production of 2,3-butanediol: a comparison between *Escherichia coli* and *Vibrio natriegens*
Anna Maria Erian¹, Martin Gibisch², Christoph Herwig³, Stefan Pfügl⁴

¹annaerian@gmail.com
²martin.gibisch@web.de
³christoph.herwig@tuwien.ac.at
⁴Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Austria

2,3-butanediol is an interesting chemical due to the broad range of potential applications. In the present study, microbial production of 2,3-butanediol was evaluated in detail in *E. coli* and *Vibrio natriegens*. The genetic construct was established with special emphasis on (i) the use of genes from three different donor organisms and (ii) the use of different promoter-gene combinations to study the effect on the overall flux through the 2,3-butanediol pathway.

Different *E. coli* strains were tested in shake flask batch cultivations. Under these conditions *E. coli* W was able to produce 17 g/l 2,3-butanediol using minimal media supplemented with 50 g/l glucose, while production in the other strains tested was dependent on yeast extract supplementation. Bioreactor cultivations using aerobic batches for biomass generation and microaerobic pulsed-feeds for product formation showed formation of up to 37 g/l 2,3-butanediol and 17 g/l acetoin with a total diol productivity of 0.76 g/l h. Compared to shake flask cultivations, significant amounts of mixed fermentation products were accumulated. Two knock-out strains were tested to reduce by-product formation and increase glucose uptake. The best strain produced 68 g/l 2,3-butanediol with a productivity of 0.93 g/l h. Additionally, a 50 % increase in product yield due to decreased by-product formation compared to wild type *E. coli* W was observed.

Compared to *E. coli*, *V. natriegens* shows high substrate uptake rates under aerobic and anaerobic conditions, a property highly desirable for microbial chemical production. Therefore we were curious if *V. natriegens* could be a suitable organism for production of 2,3-butanediol. Indeed, with the same genetic construct used for *E. coli* W 2,3-butanediol production could be demonstrated, marking the first example of metabolic engineering of *V. natriegens* for the production of a metabolite not naturally produced by the organism. The productivity was 1.5-fold higher compared to *E. coli* W in bioreactor cultivations.

Proof-of-principle to replace pure glucose with sucrose from sugar beet molasses could be shown for both *E. coli* W and *V. natriegens*.

Use of defined minimal media without expensive components like yeast extract and the use of cheap complex substrates paired with the right choice of host organism as shown in this study is likely to play an important role in the development of economical and sustainable microbial production processes for chemicals.
MCP-03  How oxidoreductases support biomass hydrolysis

Florian Csarman, Lena Wohlschlager, Daniel Kracher, Roland Ludwig

Department of Food Science and Technology, University of Natural Resources and Life Sciences, Austria

Since 2010, the contribution of oxidoreductases in biomass hydrolysis has received increased attention, after a new enzyme, lytic polysaccharide monooxygenase (LPMO) was discovered to boost the activity of commercial hydrolase cocktails. Ninety percent of lignocellulose-degrading fungi contain lpmo genes. LPMOs catalyze the initial oxidative cleavage of recalcitrant polysaccharides after activation by an electron donor, which opens new points of attack for cellobiohydrolases [1]. LPMO and auxiliary enzymes form an oxidative machinery that increases the efficiency of hydrolytic enzymes twofold. This talk will describe the nature of the oxidative enzymes, their interactions and key intermediate substrates. Understanding the source of enzymatic substrates is fundamental to exploit LPMOs for biomass processing. Using genome data and biochemical methods, we characterized and compared different extracellular electron sources for LPMO: cellobiose dehydrogenase [2], phenols procured from plant biomass or produced by fungi, and GMC oxidoreductases that regenerate LPMO-reducing diphenols. The availability of extracellular electron donors is obligatory to activate fungal oxidative attack on polysaccharides [3]. The talk will cover the discovery of LPMO, its structure, catalytic mechanism, substrate specificity and reaction products, as well as the boosting effect LPMO exerts on cellulase activity. Finally, an outlook on the potential methods to investigate enzyme interaction on biomass surfaces by fluorescence microscopy, surface plasmon resonance and scanning electrochemical microscopy will be given.


MCP-04  Bioactive Amycolatopsis sp. from the Mongolian steppe: identification of secondary metabolites and genome mining yielding novel lasso peptides

Jaime Felipe Guerrero-Garzón0, Martin Zehl1, Ernst Urban2, Yanru Cao3, Yi Jiang3, Chenglin Jiang3, Christian Rückert4, Tobias Busche4, Jörn Kalinowski4, Sergey B. Zotchev0

1 Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria
2 Department of Pharmaceutical Chemistry, University of Vienna, 1090 Vienna, Austria
3 Yunnan Institute of Microbiology, Yunnan University, 650091 Kunming, Yunnan, P. R. China
4 Center for Biotechnology (CeBiTec), Universität Bielefeld, Bielefeld, Germany
5 Pharmacognosy Department, University of Vienna, Austria

Actinomycete bacteria belonging to the rare genus Amycolatopsis are of special importance because of their capacity to produce several medically important antibiotics. In the current work, a new strain YIM10 identified as a member of the genus Amycolatopsis was isolated from a soil sample collected in the Mongolian steppe. Cultivation of YIM10 in various fermentation media yielded extracts active against Bacillus subtilis. One of the bioactive extracts prepared from the culture grown in liquid medium with soy flour and glycerol was investigated further using fractionation and high-resolution LC-MS and MS/MS. Major metabolites produced by YIM10 in these conditions, as confirmed by NMR, were found to be various tiglosides, that did not display antimicrobial activity. The compound active against B. subtilis was purified, and its structure determined by NMR as 1,2,4-trimethoxynaphthalene. Interestingly, this compound was previously isolated from a plant and a fungus, but never from a bacterium, and its antibiotic activity has not been reported.

In order to obtain a deeper insight into the biosynthetic potential of YIM10, its genome was completely sequenced. The YIM10 genome is represented by a 10.32 Mbp circular chromosome and a 39.9 kb plasmid. According to the analysis by the antiSMASH software it contains at least 40 secondary metabolite biosynthetic gene clusters (BGCs). Several BGCs of YIM10 appeared to be unique, making this strain a good candidate for genome mining in search for novel bioactive secondary metabolites.

Cloning and successful expression of a unique lasso peptide BGC from YIM10 in heterologous Streptomyces hosts was accomplished, yielding production of several novel lasso peptides.

Keywords: Amycolatopsis sp., secondary metabolites, antibiotic activity, complete genome, biosynthetic gene clusters, heterologous expression, lasso peptides.
MCP-05  Entirely oil palm based 1,3-propanediol production with *Lactobacillus diolivorans*

Hannes Russmayer¹, Javier Arteaga², Alexander Bauer², Hans Marx¹, Michael Sauer³

¹ Department of Biotechnology, CD Laboratory for Biotechnology of Glycerol, BOKU Wien, Austria
² Department of Sustainable Agricultural Systems, Institute of Agricultural Engineering, BOKU – University of Natural Resources and Life Sciences, Tulln, Austria
³ CD Laboratory for Biotechnology of Glycerol, Muthgasse 18, 1190 Vienna, Austria; Department of Biotechnology, BOKU – University of Natural Resources and Life Sciences, Vienna, Austria; ACIB – Austrian Centre of Industrial Biotechnology, Vienna, Austria

Biodiesel is produced via transesterification of long chain fatty acids derived from plant oil, such as palm oil with methanol to fatty acid methyl esters. The major by-product of the transesterification processes is crude glycerol, which amounts to 10% of the total biodiesel production. The price of crude glycerol is rather low (0.05 – 0.25$ per pound). Therefore, to optimize the income of biodiesel biorefineries a value adding process converting glycerol into higher value chemicals, such as 1,3-propanediol, is desirable.

An already established value adding process under industrial relevant conditions is the conversion of glycerol to 1,3-propanediol with *Lactobacillus diolivorans*, yielding up to 90 g/L of 1,3-propanediol. However, the process is based on the addition of sugar, as *L. diolivorans* is not able to grow on glycerol as sole carbon source. However, pure glucose – while most commonly used in the academic lab - is a rather expensive carbon source for industrial biomass formation. Lignocellulosic hydrolysates are less expensive alternatives. A potential source for such hydrolysates are empty fruit bunches (EFB). EFBs are the main waste products of oil mills and constitute the solid residue of the fresh palm fruit after oil extraction. Due to their high content of cellulose (45% w/w) and hemicellulose (18% w/w) they are a potential source of sugars. To make the sugars available for the later use as carbon source, a pre-treatment of the EFB is necessary. The method of choice in this case is steam explosion followed by an enzymatic hydrolysis.

The aim of this study was to evaluate the potential of 1,3-propanediol production by *L. diolivorans* under industrial relevant and economically feasible conditions. Therefore, the ability of 1,3-propanediol production on crude glycerol in combination with EFB hydrolysates was tested. Additionally, different condition for steam explosion of EFB were tested in order to maximize the sugar concentrations in the hydrolysates.
Wednesday 19th: Microbiomes: interplay of microbes, their hosts and environments

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Microbiomes: interplay of microbes, their hosts and environments

Chairs: Kaisa Koskinen & David Berry
MB-01  New human microbiome functions: How a vegetarian diet supports an exclusive physiological niche for gut commensals and contributes to hydrogen sulfide production
Buck Hanson
Department of Microbiology and Ecosystem Science, University of Vienna, Austria
The gut microbiota facilitates the degradation of complex dietary compounds and in the process produces many metabolites that can influence host physiology and health. However, most genetic functions encoded by the gut microbiota are unknown. This generally limits interpretations of metagenome and metatranscriptome data of the human microbiome, but also our understanding of how individual dietary compounds are metabolized by individual gut microbiota members and impact the host. In my talk, I will introduce a hitherto unknown metabolic feature of the human gut microbiota - degradation of sulfoquinovose (SQ). SQ is the polar, sulfonate-containing head group of sulfoquinovosyl diacylglycerol (SQDG), a sulfolipid of plants and other photosynthetic organisms that is among the most abundant organosulfonates in the biosphere. SQ is syntrophically degraded by human gut microorganisms with concomitant acetate and hydrogen sulfide (H₂S) production. SQ represent a new, ‘green-diet’-derived source of H₂S in the gut. H₂S is an important ‘Janus-faced’ metabolite in the gut that exerts either beneficial or detrimental activity on its host. Possible implications of the different dietary- and host-derived sources of H₂S on host health will be discussed in the context of reciprocal interplay between host diet, microbiota composition, and gut barrier function.

MB-02  Bacteria and fungi: (un)healthy relationship
Filomena Nogueira¹, Leonel Pereira², Sabrina Jenull³, Karl Kuchler³, Thomas Lion¹
¹ CCRI, Austria
² Labdia Labordiagnostik GmbH
³ Medical University of Vienna, Max F. Perutz Laboratories
The interactions between pathogens occurring during polymicrobial infections remain poorly understood. Only recently, with the expansion of microbiome research, scientists became aware of the complex relationships between microorganisms. Importantly, opportunistic bacteria and fungi that have been living as commensals may turn into pathogens. Such alterations cause an imbalance of the microbiota that must be taken into account for diagnostics and treatment of infections. Often, bacteria and fungi occupy the same niches which results in either a synergistic or antagonistic interaction. Bacteria and fungi communicate via secretion of quorum sensing molecules and production of virulence factors which may also impact the response of the immune system and induce alterations in the host. Therefore, we aim to characterize the interaction of relevant human pathogens that occupy the same niches such as Candida, Aspergillus and Klebsiella. The capacity of biofilm formation and structural integrity of bacteria and fungi alone and in co-culture were assessed by crystal violet staining, confocal microscopy and qPCR. Our data have shown a suppressive effect on fungal growth and biofilm formation by the bacteria in an in vitro co-culture. However, fungal growth could be restored in the presence of UV-, and heat-killed bacteria as well as upon antibiotic treatment. Analysis of the cell viability has also shown that the bacteria do not kill the fungus but rather exert a suppressive effect. Furthermore, analysis of the proteome and metabolome revealed proteins and small molecules as key factors mediating Candida, Aspergillus and Klebsiella interaction. Molecules identified as regulators of this interaction will provide novel insights potentially exploitable for diagnostics and treatment of polymicrobial infections.
MB-03  Microbiota of the gut-lymph node axis: depletion of mucosa-associated segmented filamentous bacteria and enrichment of Methanobrevibacter by Colistin sulfate and Linco-Spectin in pigs

Benjamin Zwirzitz¹, Beate Pinior², Barbara Metzler-Zebeli³, Monika Handler¹, Kristina Gense¹, Christian Knecht⁴, Andrea Ladinig⁴, Monika Dzieciol², Stefanie Wetzels¹, Martin Wagner¹, Stephan Schmitz-Esser⁵, Evelyne Mann¹
¹ FFGQSI GmbH, Austria
² Institute for Veterinary Public Health, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria
³ Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria
⁴ University Clinic for Swine, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria
⁵ Department of Animal Science, Iowa State University, Ames, IA 50011, USA

In-feed antibiotics alter the gut microbiome and consecutively might also affect translocation processes of microorganisms to lymphatic tissues. As a result, intestinal microbiota and the host immune system could fall into an imbalanced state, making the host susceptible to recurrent infections and dysbiosis. Hence, understanding the variant effects of antibiotics on the microbiome in specific tissues is of vital importance for animal production and health. Here, we provide the first comparative study of microbial communities in pig feces, ileum, and ileocecal lymph nodes under the influence of antibiotics using 16S rRNA gene high-throughput sequencing. Furthermore, we also investigated the microbiome of ileocecal lymph nodes by cultivation, generating 95 isolates, and by sequencing the metatranscriptome of a single lymph node sample.

The Proteobacteria-dominated lymph node microbiome represented a sub-fraction of the gut microbiome with a significant lower diversity compared with ileum and feces. In each analyzed tissue, we identified phylotypes susceptible to antibiotic treatment that hold profound impacts on the host physiological and immunological state, with the lymph node microbiome being affected by antibiotics to a lesser extent compared with feces and ileum. Pigs that received antibiotics harbored significantly reduced amounts of segmented filamentous bacteria along the ileal mucosa and increased amounts of Methanobrevibacter, a methanogenic Euryarchaeote in fecal samples compared to the control group. Thus, we believe that segmented filamentous bacteria might play an important role in antibiotic-induced dysbiosis not only in farm animals but also in humans. Additionally, antibiotic treatment of livestock might have effects on global biogeochemical cycles that have been unnoticed so far e.g. antibiotic treatment promotes Methanobrevibacter growth, which could potentially lead to higher methane emissions of livestock.

RNA-sequencing of a lymph node unveiled expressed transcripts used for bacterial metabolic core processes like amino acid and carbohydrate metabolism, therefore proving the metabolic activity of bacteria in lymph nodes. Our results indicate that pathogenic bacteria could escape antibiotic treatment, if they are translocated to lymph nodes. In general ileocecal lymph nodes harbor a more diverse and active community of microorganisms than previously assumed.

MB-04  The unexplored Human Archaeome

Manuela-Raluca Pausan, Kaisa Koskinen, Alexander Mahnert, Alexandra Perras, Christine Moissl-Eichinger
Internal Medicine, Medical University of Graz, Austria

The human microbiota is composed of trillions of microorganisms from all three domains of life (Bacteria, Eukarya and Archaea), as well as viruses. These microorganisms function like an “invisible organ” helping in energy harvest and storage, contributing to metabolic functions, protecting against pathogens, and educating the immune system. Most studies target the bacterial community in the human body, and the non-pathogenic archaeal community and its diversity remains largely hidden. Since archaea have been associated mainly with the human gut and oral microbiome, little is known about the presence and diversity of archaea in other body sites.

In this study, we aim to explore the archaeal diversity and presence in different sites of the human body (e.g. vagina, oral, skin, respiratory, urinary and gastrointestinal tract). We apply NGS approach, using both amplicon sequencing and metagenomics to discover the archaeal communities within the human body. For determining the ratio between Bacteria and Archaea qPCR will be implemented.

Preliminary results indicate that the human archaeome is more diverse than expected, being composed of archaea from several phyla including Euryarchaeota, Thaumarchaeota and Woesearchaeota. Furthermore, we show for the first time that the archaeal community within the gastrointestinal tract differs based on location, and is not entirely dominated by Methanobrevibacter. We also observed that archaea are body site specific like bacteria. The ratio between bacteria and archaea based on 16S rRNA gene qPCR results varies from 200:1 for stool sample, to almost 1:1 for nose and appendix samples. Our study allowed us to retrieve a novel picture of the human archaeome. However, the function of these archaeal communities is yet to be uncovered.
**MB-05**  Surface layer proteins of lactobacilli – Unraveling their structure will help us understand their probiotic effects

Markus Eder¹, Andela Dordić¹, Krishna Mohan Padmanabha Das², Natalia Kulminskaya¹, Elisabeth Damisch¹, Theodor Sagmeister¹, Ulla Hynönen², Airi Palva², Janet Vonck³, Nermina Malanovic¹, Monika Oberer¹, Tea Pavkov-Keller⁴

¹ Institute of Molecular Biosciences, University of Graz, Austria  
² Faculty of Veterinary Medicine, University of Helsinki, Finland  
³ Structural biology, Max-Planck-Institute of biophysics, Germany  
⁴ Institute of Molecular Biosciences, University of Graz and ACIB GmbH, Austria

Surface layers (S-layers) are 2D crystalline lattices of proteins which cover the whole surface of many archaeal and bacterial cells. Since these proteins are in close contact with the environment they fulfil many important tasks like bacterial adherence to other cells, protection against life-threatening conditions, maintenance of the cell shape and, auto-coaggregation. They play an important role in the stimulation of gut dendritic cells by interacting with specific receptors. Interaction with the cell wall occurs by binding to teichoic acids [1,2,3,4].

These proteins are not just important for our immune system. They are equally important in the role of lactobacilli in the microbiome of the gut. Lactobacillus S-Layers harbor outstanding therapeutic potential, especially for vaccines. It was shown that binding of the Lactobacillus vaccine vector, mediated by the S-layer protein, to the mucosal membrane of the host may be an advantage when a mucosal delivery route of the vaccine is considered [5].

Our goal is to characterize the surface layer proteins SlpA of *L. acidophilus* and *L. amylovorus*. Both species are of high biological and medical relevance because of their probiotic properties. We produced several soluble fragments as well as the full-length proteins assembling into 2D-crystals. We elucidated the 3D structures of all fragments by x-ray crystallography or NMR.

The obtained results allow us to learn more about the self-assembly formation, cell wall binding and surface exposed areas available for the interactions with gut cells and dendritic cells.

References

4. Smit, E. and P.H. Pouwels, One repeat of the cell wall binding domain is sufficient for anchoring the Lactobacillus acidophilus surface layer protein (2002) J Bacteriol, 184(16)  
Wednesday 19th: Pluripotent stem cells and neural differentiation

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Pluripotent stem cells and neural differentiation

Chairs: Sigismund Huck & Bon-Kyoung Koo

Supported by ANA

Austrian Neuroscience Association
**SC-01  Brain organoid fusion models human interneuron migration**

Joshua Bagley  
IMBA - Institut für Molekulare Biotechnologie GmbH, Austria

Development of the forebrain involves the migration of GABAergic interneurons over long distances from subcortical into cortical brain regions. Although defects in interneuron migration are implicated in neuropsychiatric diseases such as Epilepsy, Autism, and Schizophrenia, model systems to study this process in humans are currently lacking. To develop a suitable model system, we used a novel 3D cerebral organoid co-culture system to study human interneuron migration. We observed robust long-distance migration of interneurons from ventral into dorsal forebrain regions. These migrating interneurons can produce various interneuron subtypes, and live-imaging analysis shows features consistent with tangential interneuron migration. Moreover, reduced migration through drug-induced inhibition of chemotaxis receptors highlights the utility of this system for drug-screening. Therefore, our results demonstrate that cerebral organoid co-cultures can model complex interactions between different brain regions. Combined with reprogramming technology, this system offers a possibility to analyze complex neurodevelopmental defects using cells from neuropsychiatric disease patients, and to test potential therapeutic compounds.

**SC-02  Recapitulating early embryonic development of the mouse using stem cells**

Erik Vrij¹, Yvonne Scholte op Reimer², Roman Truckenmüller², Niels Geijsen³, Clemens van Blitterswijk², Nicolas Rivron⁴

¹ Koo’s lab, IMBA, Austria  
² Merln Institute for Regenerative Medicine  
³ Hubrecht Institute  
⁴ Merln Institute for Regenerative Medicine, Hubrecht Institute

The preimplantation blastocyst of the mouse consists of three distinct lineages: the pluripotent epiblast that develops into the fetus, and two extraembryonic lineages—trophoblast (TE) and primitive endoderm (PrE)—both critical for survival and patterning of the embryo. We have recently shown that blastoids, embryonic and trophoblast stem cell-based models of the E3.5 blastocyst, allow for the genetic and physical disentanglement of the embryonic and trophoderm niches, unlocking new avenues to investigate embryonic development (REF). Although the PrE-niche is necessary to authentically recapitulate the blastocyst-stage embryo, it is often undeveloped in blastoids. Here, we derived a method for the directed differentiation of 3D aggregates of naïve pluripotent stem cells towards PrE by employing high-content screening using soluble signaling pathway modulators. Differentiated stem cell aggregates formed an outer layer of PrE while maintaining a pluripotent epiblast niche. The PrE-niche appeared functional, demonstrated by the serum- and gel-free selforganization into structures resembling the early post-implantation embryo including a pro-amniotic cavity, extraembryonic endoderm including a basal membrane and a polarized Oct4-positive epiblast. We then translated these PrE-findings into the blastoid.
SC-03 Maturation and network integration of non-proliferative neuronal precursors in the adult murine piriform cortex

Sébastien Couillard-Després¹, Bruno Benedetti¹, Richard König², Dominik Dannehl¹, Christina Kreutzer¹, Maria Belles³, Markus Ritter⁴, Thomas M. Weiger⁵, Juan Nacher³, Maren Engelhardt⁶, Ludwig Aigner²

¹ Institute of Experimental Neuroregeneration, Paracelsus Medical University, Austria
² Inst Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria; Spinal Cord Injury and Tissue Regeneration Center Salzburg
³ Cell Biology Dept, Universitat de Valencia, Spain
⁴ Inst of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, Austria
⁵ Cell Biology and Physiology, University of Salzburg, Austria
⁶ Inst Neuroanatomy, Medical Faculty Mannheim, Heidelberg University, Germany

Tangled cells (TCs) are post-mitotic immature cortical neurons characterized by their expression of doublecortin (DCX) and polysialylated neuronal cell adhesion molecule (PSA-NCAM). TCs arise during embryonic corticogenesis and remain detectable in some cortical areas in adult mammals, e.g., the piriform cortex of mice. Previous studies have shown that the number of TCs decreases over the lifespan. Using a DCX-CreER²floxed/GFP transgenic mouse line, we followed the fate of TCs in the piriform cortex over time and observed that their number remained roughly constant and that numerous TCs developed into morphologically mature glutamatergic neurons (TBR1⁺, CaMKII⁺).

We used the AIS as a surrogate marker for functional maturation of TCs into neurons. We observed the emergence of an AIS on TCs concomitant with their maturation in “complex” neuron morphology and the increase of synaptic inputs. We thus questioned whether TCs eventually become upon maturation equivalent to other principal neurons of the piriform cortex. Patch clamp experiments and morphometric analysis of the axon initial segment (AIS) were carried out on TCs and TC-derived complex cells in acute brain slices and histological sections.

TCs were small, had no AIS, received no synaptic input and produced one action potential at best when chronically depolarized. Complex cells that recently matured were instead endowed with AIS, received sparse synaptic input, produced small inward and outward currents, and fired action potentials at low frequencies and with slow kinetics. Following several months of maturation, complex cells displayed increased amounts of synaptic input, large capacitance, large inward and outward current, and sharp action potential kinetics. However, action potentials were fired at low maximal frequencies and cells had a remarkably high rheobase, implying scarce excitability. Strikingly, while principal neurons typically received a mixed glutamatergic and GABAergic synaptic input, gabazine completely blocked postsynaptic currents in complex cells of any age, suggesting exclusive GABAergic input.

On one hand, the odd physiological features of complex cells lead to question their physiological role in the adult brain; on the other, their unique features suggest that complex cells are new coding elements in the piriform cortex rather than the simple replacement or addition of homologous coding units to the preexisting network components.

SC-04 Generation of functionally active neurons using direct conversion from MeCP2 deficient male fibroblasts.

Anna Huber¹, Daniela Ackerbauer¹, Bronwen Connor², Hannes Steinkellner¹, Franco Laccone¹

¹ Institute of Medical Genetics, Medical University of Vienna, Austria
² Department of Pharmacology and Clinical Pharmacology, Centre for Brain Research, School of Medical Science, FMHS, University of Auckland, Auckland 1023, New Zealand

Rett Syndrome (RTT) is a postnatal progressive neurodevelopmental disorder predominantly caused by a mutation in a gene coding for the methyl-Cpg-binding protein 2 (MeCP2), a transcriptional regulator possessing both activation and repression properties. MeCP2 is essential for the development of the CNS. The purpose of the study is the establishment of a human cell model for RTT via a non-integrating reprogramming strategy, to characterize it and compare it with a healthy control. Direct reprogramming provides many advantages compared to iPSC cell reprogramming as it is faster and does not rejuvenate cells, which is crucial for studying neurodevelopmental disorders.

Human fibroblasts from a RTT patient and a healthy control are transfected with two episomal plasmids coding for the reprogramming factors PAX6 and SOX2. Transfected cells are kept under specific reprogramming conditions for 6 weeks, resulting in induced neuronal progenitor cells (iNPs), which are then differentiated into neurons. Changes in cell morphology are observed during the reprogramming process via bright field microscopy. Quantitative real-time PCR of iNPs displayed higher expression of progenitor-associated genes such as NCAM1, NES and FOXG1 in addition to PAX6 and SOX2. Neurons showing typical morphology were successfully stained with TUJ1 and MAP2.

In conclusion, fibroblasts from a RTT patient and a healthy control are successfully reprogrammed and differentiated into neurons expressing neuronal lineage markers. For further characterization of the human cell model, RNA sequencing and electrophysiological analysis are ongoing. This cell line can serve as a model to understand RTT phenotypes and to investigate different strategies to overcome this incurable disease.
SC-05  Generation of induced pluripotent stem cell derived cardiomyocytes from long QT-syndrome mouse model

İlayda G. Soztekin¹, Esra Cagavi², Sevilay Sahoglu³, Tuba Akgul³, Enes Kazcı³, Gizem Ors³

¹ Neuroscience, Medical University of Vienna, Turkey
² Regenerative and Restorative Medical Research Center (REMER), Department of Medical Biology, International School of Medicine, Istanbul Medipol University, Istanbul, Turkey
³ Regenerative and Restorative Medical Research Center (REMER), Istanbul Medipol University, Istanbul, Turkey

Cardiac diseases, whether inherited or acquired, is the most common cause of mortality worldwide. Long QT syndrome (LQTS) is the most common cardiac arrhythmia that can lead to sudden cardiac death. Mutations in KCNQ1 and other cardiac ion channel genes lead to LQTS. Various transgenic mouse models of arrhythmia enable researchers to investigate the molecular mechanisms behind rhythm disorders and to develop novel therapy approaches in vivo. In our research, we have obtained two transgenic LQTS mouse models that are either a knock-out (Kcnq1⁻/⁻) or acquired a mutation (Kcnq1A340E/A340E) in Kcnq1 gene [1, 2]. We have validated disease phenotype by electrocardiography recordings of the wild type and transgenic mice. We have demonstrated that both transgenic mice showed prolongation of QT interval and QRS amplitude similar to clinical phenotype in humans. In order to create the disease phenotype in vitro, we have generated induced pluripotent stem cells (iPSC) by reprogramming of mouse fibroblasts via tet-FUW-OKSM virus [3]. Similar to ESC, the expression of stemness factors (Oct4, Sox2, nanog) at the mRNA or protein level were only observed in newly generated iPSC colonies, but not the control somatic cells analysed by qRT-PCR and immunocytochemistry. Following characterization of transgenic mouse iPSCs, we differentiated them into cardiomyocytes to explore disease phenotype in vitro. Spontaneous beating were observed under light microscope starting from 7 days after embryoid body formation of iPSC. In conclusion, due to invasive nature and limitations in human cell sources, transgenic arrhythmia mouse models and mouse iPSC-derived cells provide an invaluable opportunity to study disease mechanisms and provide a renewable isogenic or autologous cell source for in vivo transplantation studies.

Keywords: iPSCs, LQTS, KCNQ1 gene, Disease Models, Transgenic Mice

Acknowledgements: This study is supported by TUBITAK under 1003 Scientific and Technological Research Projects Funding Program by project no: 213S194.

Reference:
Wednesday 19th: Poster Session 2: Microbial chemical production

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna,
September 17-20, 2018, Vienna, Austria

Poster Session 2: Microbial chemical production
PS2:MCP-01  Novel microfluidic and analytical approaches for screening engineered strains: downscaling of *S. cerevisiae* cultivations for lactic acid production

**Damiano Totaro¹, Mario Rothbauer², Yi-Yu Chen³, Michael Sauer¹, Matthias Steiger¹, Hsiang-Yu Angie Wang³, Peter Ertl², Diethard Mattanovich¹**

¹ acib GmbH, Austria  
² Vienna University of Technology, Faculty of Technical Chemistry - Institute of Applied Synthetic Chemistry, Getreidemarkt 9, 1060 Vienna, Austria  
³ National Tsing Hua University, Department of Engineering and System Science No. 101, Section 2, Kuang-Fu Road, Hsinchu, Taiwan 30013

The development of a biotechnological process depends on the optimization of a number of parameters, such as the strain, the cultivation mode, pH, temperature, dissolved oxygen concentration, growth behaviour and others. Establishing the right protocol requires a huge amount of work and time, therefore it is not surprising that there is an increasing demand for novel systems able to perform parallel processes and high-throughput analyses.

Microfluidics - and the miniaturization of laboratory instruments in general - has become a powerful tool for biotechnology and its applications have constantly increased in the last decade. This project aims at a multi-module microfluidic platform with a cultivation system and integrated sensors for operational and product parameters monitoring able to screen multiple strains. Small-scale devices provide us with the opportunity to have a better control of the whole experimental system and to reduce cost in the early stage of industrial process development.

Up to now, the basic module of the platform has been designed and fabricated. The cultivation chamber is made of PDMS (Polydimethylsiloxane) and can be used under both batch mode and perfusion mode conditions. The first experiments have been performed with the model system *Saccharomyces cerevisiae* (both wild type and a lactic-acid producing engineered strain) which could efficiently be grown both in batch and perfusion mode. Three sensing methods have been characterized and proved to be effective at detecting dissolved oxygen, biomass and pH during experiments on chip. All these strategies are non-invasive and they allow to continuously acquire data about the process occurring inside the chamber without the need of sampling.

PS2:MCP-02  Are metabolic pathways robust with respect to biomass composition?

**Claudia Juno¹, Jürgen Zanghellini²**

¹ ACIB, Austria  
² Austrian Center of Industrial Biotechnology, Vienna, Austria; Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

Genome-scale metabolic models and flux-balance analysis (FBA) have tremendously helped in our understanding of the genotype-phenotype relationship. A key ingredient of any (genome-scale) metabolic model is the experimentally determined biomass function. About one third of all incorrect FBA predictions are caused by an inaccurately measured biomass composition. While the biomass composition strongly influences the quality of predicted flux distributions, an effect on the underlying pathway structure has not been investigated so far. Here we systematically study the effect of variations in the biomass composition on the metabolic capabilities of *Escherichia coli* in terms of elementary flux modes (EFMs). EFMs are unique, minimal steady-state flux distributions, which can be thought of as the smallest functional units representing a particular metabolic feature. Preliminary data indicate that the spectrum of available EFMs, i.e. the total set of metabolic capabilities, is little affected by variations in the biomass composition. However, the stoichiometric ratio within EFMs changes significantly. Thus, we conclude that an EFM analysis allows one to robustly characterize metabolic capabilities, but not necessarily their strength.
PS2:MCP-03  Whole-cell (+)-ambrein production in the yeast *Pichia pastoris*
Sandra Moser¹, Gernot A. Strohmeier¹, Erich Leitner³, Thomas J. Plocek², Koenraad Vanhessche², Harald Pichler³

¹ acib GmbH, Petersgasse 14, 8010 Graz
² ACS International S.A., 184 Route de St-Julien, CH-1228 Plan-les-Ouates, Switzerland
³ Molecular Biotechnology, Graz University of Technology, Austria

(-)-Ambrox constitutes one of the most sought-after fragrances in perfume industry. In nature, its precursor molecule, (+)-ambrein, is found as a major component of ambergris, an intestinal excretion of sperm whales. Upon exposure to sea and sunlight, oxidative degradation of (+)-ambrein to (-)-ambrox, and related compounds that constitute the characteristic smell of ambergris, takes place. As the supply of ambergris is naturally limited and unreliable, alternative sources for (-)-ambrox had to be found. Currently, most of the (-)-ambrox is synthesized chemically using diterpenoids such as sclareol, cis-abienol or labdanolic acid as starting materials. Yeasts are well suited to produce sesqui- and triterpenoids, like (+)-ambrein, based on their endogenous and effective farnesyl diphosphate and squalene biosynthetic routes.

Targeting a central enzyme in the sterol biosynthesis pathway, we could strongly enhance precursor supply for triterpenoid biosynthesis in *Pichia pastoris*. Heterologous expression of a triterpene cyclase cascade in *P. pastoris* and, particularly, development of suitable analytical methods provided conclusive evidence of whole-cell (+)-ambrein production. Enzyme engineering approaches markedly enhanced (+)-ambrein levels. Finally, scale-up to 5 L bioreactors confirmed that metabolically engineered *P. pastoris* represents a valuable, whole-cell system for high-level production of (+)-ambrein.

PS2:MCP-04  Recombinant expression and characterization of fungal extracellular enzymes involved in lignocellulose degradation
Lena Wohlschlager¹, Florian Csarman¹, Tobias Obermann¹, Katrin Kropatsch¹, Bernhard Seiboth², Roland Ludwig¹

¹ Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria
² Research Division Biochemical Technology, Institute of Chemical, Environmental and Bioscience Engineering, Technical University of Vienna

Wood-degrading fungi secrete a diverse arsenal of biocatalysts capable of lignocellulose breakdown that act in a concerted manner. Project OXIDISE investigates and compares two basidiomycete fungi – white-rot *Phanerochaete chrysosporium* and brown-rot *Fomitopsis pinicola* – in terms of their degradation machinery and aims to explore the distribution and interaction of an assorted set of their oxidoreductases and cellulases on synthetic and natural substrates.

An initial secretome study was performed to identify and verify relevant lignocellulose-active enzymes. Subsequently, the recombinant production of the selected enzymes is required. Cellobiose dehydrogenase from *P. chrysosporium* and laccases from *F. pinicola* were already successfully produced and characterized using *Trichoderma reesei* as expression host. Challenges included the generation of stable transformants as well as screening for expression. Cellulases such as cellobiohydrolases and endoglucanases were expressed in the yeast *Pichia pastoris*.

Methods to determine the distribution and interaction of enzymes include fluorescence microscopy, scanning electrochemical microscopy and surface plasmon resonance.
PS2:MCP-05  Microbial upgrading of a side stream from cellulose fibre production
Robin Hoheneder¹, Robert H. Bischof², Michael Sauer³

1 Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
2 Lenzing AG, Werkstraße 2, 4860 Lenzing, Austria
3 University of Natural Resources and Life Sciences, Department of Biotechnology, Muthgasse 18, 1190 Vienna, Austria

On our way to a zero-waste-economy, valorisation of side-streams from industrial processes becomes increasingly important. Biotechnological approaches are among the key-technologies for conversion of low value compounds derived from renewable resources. Naturally occurring or metabolically engineered organisms are used to produce valuable products like enzymes, chemicals or biofuels.

The aim of this study is to valorize a side-stream of the sulfite process for dissolving wood pulp. Starting point is the identification of microorganisms, capable to use several or all substrates of this side stream as carbon source. For this purpose, pathway- and enzyme-databases have been screened to search both already known and potential new pathways, catabolizing the compounds of interest. Additionally, a literature research has been performed to identify promising organisms. After this in-silico screening, 15 different species were identified for experimental analyses. These 15 organisms are tested regarding their capability to grow on the different substrates since growth is the indicator for the presence of the desired pathway. The corresponding microorganism could therefore serve as host organism for production of valuable compounds from the carbon source in question.

The final aim is the construction of a cell factory converting the entire side stream of the sulfite process into valuable products.

PF2-05  Identification of a citrate exporter protein CexA for citric acid production in Aspergillus niger
Alice Rassinger¹, Diethard Mattanovich¹, Michael Sauer², Matthias Steiger¹

1 Department of Biotechnology, BOKU, Austria
2 Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 18, Vienna, Austria; Department of Biotechnology, BOKU - University of Natural Resources and Life Sciences Vienna, Muthgasse 18, Vienna, Austria; CD Laboratory for Biotechnology of Gl

Citric acid is a commonly used organic acid, which is applied as food additive or preservative, and is used for further production of bulk chemicals in the industry. Amongst various microbial hosts, Aspergillus niger remains one of the top producers of citric acid. It has been widely studied in terms of media composition, the metabolic flow through the TCA, bioprocess optimization and most notably the substrate uptake. However, the export of citric acid remains still obscure.

Based on transcriptomic data and a similarity in sequence to a putative itaconic acid transport gene from Ustilago maydis, a candidate citrate exporter protein (CexA) was identified. A deletion of cexA leads to an abolished citric acid accumulation. A heterologous expression of CexA in baker’s yeast (S. cerevisiae), which naturally does not secrete citric acid into the media, leads to accumulation of citric acid. On top of that, we found that CexA is a specific transporter for citrate and does not transport structurally similar compounds like isocitrate in S. cerevisiae. To summarize, the identification of CexA presents a new target for further strain engineering in A. niger and can be used to create new citric acid producing microbes.
Poster Session 2: Biopharmaceutical technologies
**PS2:BT-01  Ribosomal proteins as drug targets for customized protein therapy in rare disease epidermolysis bullosa (eb).**

Andreas Friedrich\(^1\), Jakob Müller\(^1\), Jacqueline Teufl\(^1\), Philipp Radlzer\(^1\), Akim Strohmeyer\(^1\), Thomas Karl\(^1\), Claudia Mößhammer\(^1\), Norbert Müller\(^2\), Petr Rathner\(^2\), Christopher Gerner\(^3\), Helmut Hintner\(^4\), Michael Breitenbach\(^1\), Johann Bauer\(^5\), Hannelore Breitenbach-Koller\(^6\)

\(^1\) Biosciences, University of Salzburg, Austria
\(^2\) Johannes Kepler University Linz, Inst. Of Organic Chemistry, Altenberger Straße 69, 4040 Linz, Austria
\(^3\) University of Vienna, Department of Analytical Chemistry, Universitätsring 1, 1010 Vienna
\(^4\) SALK, Department of Dermatology, Paracelsus Private Medical University Salzburg, Müllner Hauptstrasse 48, 5020 Salzburg, Austria

Homohzygous recessive premature termination codon (PTC) mutation R635X in the skin anchor protein LAMb3 prevents production of full-length LAMb3 protein and consequently formation of the trimeric laminin 322 complex, linking epidermis and dermis. PTCs trigger the nonsense-mediated mRNA decay pathway and production of a truncated, potentially deleterious protein. A PTC in the LAMB3 gene results in an early lethal form of rare disease eb, Herlitz junctional eb (JEB-H). Gene therapy developed by one of us (J.W. Bauer, 1) was able to rescue a terminal ill patient with JEB-H. However, this procedure has to be adapted to every single patient and is not able to repair LAMB3 defects of epidermal linings of inner organs. Therefore, a systemic therapy, able to restore full-length production of the mutant LAMB3 allele, would be of great benefit for these patients. We study the ribosome as a target for systemic intervention in human disease (2, 3, 4, 5) and identified ribosomal protein rpL35/uL29 as specific target for therapeutic intervention and repair of the human LAMB3-PTC mRNA. Genetic depletion of rpL35/uL29 promotes readthrough repair of the LAMB3R635X mutant messenger RNA. We have identified by bioinformatics studies an orphan drug that binds to rpL35/uL29 with the aim to mimic genetic depletion of rpL35/uL29. Indeed, we did observe a dose dependent response of LAMB3PTC repair upon administration of the small molecule drug. Furthermore, rpL35/uL29 was expressed as a fusion protein with maltose binding protein (MBP) and the rpL35/uL29 protein was cleaved by the TEF protease. rpL35/uL29 was subjected to CD analysis to monitor structural integrity in solution. Studies investigating affinities of candidate small molecule to rpL35/uL29 are under way. In order to learn about possible modifications of rpL35/uL29 in patient cells, we studied a compound heterozygote cell line and a proteomic analysis is presented here. Furthermore, we performed genetic analysis to demonstrate the robustness of the rpL35/uL29 target.

Acknowledgement: We thank the Paris Lodron University Salzburg, M. Auer, F. Lottspeich, H.W. Mewes and C. Brandl for continuous support and DEBRA Austria, OeNB (Nr. 16531) and Land Salzburg (Nr. 20102-P1601041-FP01-2016) for funding.


**PS2:BT-02  Mouse behaviour pipelines for various disease models**

Klaus Kraitsy, Jernej Hren, Sylvia Badurek

Vienna Biocenter Core Facilities GmbH, Austria

In our core facility, we provide behavioral assays to answer questions on disease models in mice. Beside other subjects, we address the field Learning and Memory as well as Anxiety and Depression. Symptoms of anxiety and depression or deficits in learning and memory can occur independently. However, anxiety and depression-like symptoms can influence learning and memory function. We established pipelines to phenotype mouse lines, addressing questions on Learning and Memory as found in Alzheimer’s Disease or Dementia, congenital mental deficits as well as several cognitive disorders. In another line of research we combine the TSE Phenomaster System, which is a highly flexible metabolic cage assembly, with well-known but also cutting-edge motoric assays. Thus, we established behaviour pipelines addressing Parkinson’s Disease models, but also models for stroke or traumatic brain injuries. Using the Phenomaster System, we developed another pipeline as well, which can be used for models addressing metabolic questions like thermoregulation, respiration or calory consumption.
PS2:BT-03  HPLC/MS and EPR investigations of copper complexes with green tea lyophilisates pretreated by solid phase extraction

Klaus Stolze¹, Lars Gille², Bernard A. Goodman³

² Institute of Pharmacology and Toxicology, Dept. Biomedical Sciences, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria
³ College of Physical Science and Engineering, Guangxi University, Nanning, 530004 Guangxi, China

In this work, green tea (Camellia sinensis) leaf extracts were used as a model system for investigating metal complexing abilities of plant leaf extracts in general. The beneficial health effects of green tea are thought to arise from a combination of metal chelating, antioxidant, and free radical scavenging capabilities of their polyphenols (e.g. epigallocatechin gallate) [1] but we have shown that hot aqueous extracts of tea leaves also contain hydrophilic, low molecular weight compounds that form stable copper complexes at pH 2 [2,3]. Unfortunately HPLC investigation of plant extracts using conventional reversed phase columns (such as C18, CN or phenyl columns) gives little information about strongly hydrophilic compounds since they elute during the first few minutes without adequate resolution. Furthermore, the use of specialized columns such as HILIC or aqueous normal phase, specialized amino acid or carbohydrate columns requires some information about the anticipated compounds before they can be used successfully. Therefore, in order to get more general information about compounds present in green tea, we separated the original extract into several SPE subfractions. The first step was to get a sufficiently concentrated solution by lyophilisation of the original green tea extract (1g / 50 ml boiling water / 10min / filtered); then, 30mg lyophilized powder was dissolved in 1ml H₂O and separated using three types of SPE column, namely reversed phase (C18), strong anion exchange (SAX) and strong cation exchange (SCX). All fractions were characterized using a Waters Micromass Quattro micro LC-MS/MS and their copper complexes were investigated with a Bruker EPR spectrometer. Amino acids (mainly theanine, glutamine, glutamic acid, asparagine, and aspartic acid) and carboxylic acids (quinic acid, oxalic acid, malic acid, citric acid and succinic acid) were identified, and these formed mixed ligand Cu(II) complexes, which exhibited EPR spectra at lower pH values than complexes with the known individual ligands. Parameters for these mixed ligand complexes were obtained by computer simulation of the EPR spectra obtained both, at room temperature and at 77 K.


PS2:BT-04  A growth decoupled E. coli expression system allows high-yield recombinant production of antibody fragments

Jens Kastenhofer⁶, David Wurm⁶, Jürgen Mairhofer⁷, Gerald Striedner⁷, Oliver Spadiut⁶

¹ enGenes Biotech GmbH, Vienna, Austria
⁶ Biochemical Engineering, TU Wien, Austria

Antibody fragments produced in microbials have gained increased importance in the recent past due to their economic advantages in production and improved pharmacokinetics in treatment of various diseases. Until now, recombinant protein production in Escherichia coli has been a growth-related process. Due to the consequent high metabolic burden, recombinant proteins thus end up in insoluble inclusion bodies. Specifically for antibody fragments this poses a major drawback, since they are preferably transplanted to the periplasm for proper folding in their soluble form. Here, I present a novel non-growth associated expression system wherein recombinant antibody fragment production can be decoupled from cell growth. In this system the host RNA polymerase inhibitor Gp2 is expressed under control of the araP₅BAD promoter. Thus, host protein expression can be inhibited upon induction with L-arabinose, leading to a shift of metabolism from growth towards antibody fragment production and resulting in improved soluble product titers. I will introduce this system, give insights into its physiology, and show bioreactor data as well as productivity data and compare them to the state-of-the-art BL21(DE3) system.
Development of hydrogels derived from human placenta extracellular matrix for surface coating of small diameter vascular grafts

Gabriela Eder¹, Sabrina Rohringer¹, Roman Lieber¹, Ursula Windberger¹, Karl H. Schneider², Helga Bergmeister²

¹ Department of Biomedical Research, Medical University of Vienna, Austria
² Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna

Introduction: Efficient reendothelialization of acellular vascular grafts is crucial for graft patency since endothelial cells line the lumen of blood vessels in vivo, serve as a barrier function and inhibit thrombosis. To improve cell seeding efficiency, a hydrogel for graft coating was developed and tested to create additional anchoring points for the cells and to support cell adhesion and migration. Human placenta tissue has major advantages to be used as tissue source, due to its molecular composition and its wide availability as clinical waste product. Therefore, decellularized human placenta arteries and umbilical cords were used as basic materials to create placenta extracellular matrix hydrogels (PECM-HG).

Material and Methods: PECM-HG were produced by tissue decellularization followed by an enzymatic digestion using acidic pepsin solution. PECM-HG were biochemically analyzed for quantification of possible DNA residuals, collagen and proteoglycan. Rheological tests with different hydrogel concentrations were performed to examine the polymerizing behavior of the material. Scanning electron microscopy (SEM) was used to visualize fiber structure of PECM-HG coated plane surfaces. Cytotoxicity and migration assays were carried out using PECM-HG coated culture plates. Gelatin coatings were used as a control. After the characterization, PECM-HG coated and non-coated vascular grafts were incubated in a perfusion bioreactor system to be compared to each other.

Results: PECM-HG showed low amount of DNA residuals and preservation of extracellular matrix proteins. SEM analysis showed specific fibrous structures dependent on hydrogel concentrations used during surface coating. Cell binding and viability assays showed enhanced cell adherence and increased proliferation rates when seeded on surfaces coated with PECM-HG compared to gelatin coated or untreated surfaces.

Outlook: Hydrogels made out of placental extracellular matrix showed beneficial properties for cell reseeding. Vascular graft reseeding experiments in a bioreactor have been started and will be evaluated as a next step. An actual improvement of the vascular graft recellularization process would create a fundamental basis for future experiments in the field of tissue remodeling and regeneration using a pulsatile flow bioreactor. Furthermore, the hydrogel may serve as a material for drug delivery purposes or as a bio-ink for printing tissue constructs for in vivo applications.

A success story – Inclusion bodies are more than molecular waste

Alexander Pekarsky, Britta Eggenreich, David Johannes Wurm, Oliver Spadiut

Institute of Chemical, Environmental and Biological Engineering, Technical University Vienna, Austria

Coeliac disease (CD) is one of the most common food-related chronic disorders mediated by storage proteins of different grains that occur throughout a great range of our everyday food. Hence, an agent has to be found that combines a secure, efficient and affordable solution for patients. Although research has been eager to introduce therapeutic solutions, currently no approved therapy exists and patients are bound to maintain a lifelong and expensive diet.

We developed a novel tandem single chain Fragment variable (tscFv) acting as a neutralizing agent against prolamins. Additionally, a cost-efficient, fast and reproducible production process has been developed to obtain actively refolded and secure product from Escherichia coli-derived inclusion bodies. Inclusion bodies are high molecular aggregates of partly denatured protein that form during recombinant protein production. Although high space-time yields are possible and cost-efficient production protocols exist, inclusion body formation is often unwanted for industrially relevant proteins. This is due to protein specific conditions that have to be evaluated empirically and low refolding yields. Further this protein specific process step is still a black-box when it comes to proper process control and process optimization outside the shaking flask.

In my presentation I am going to introduce this novel tscFv against CD, present our integrated and cost-efficient strategy for the production and high-yield refolding of the tscFv, highlight the necessity of controlled refolding vessels to characterize and improve the usually uncontrolled refolding process and show how the refolding step is influenced by process perturbations during the prior production step.
PS2:BT-07  Direct measurement of monoclonal antibody charge distributions from cell culture supernatants: Unveiling of process impacts

Bernhard Sissolak¹, Nico Lingg², Wolfgang Sommeregger³, Gerald Striedner¹, Karola Vorauer-Uhl¹

¹ University of Natural Resources and Life Sciences, Vienna, Austria
² ACIB - Austrian Centre of Industrial Biotechnology GmbH, Vienna, Austria
³ Bilfinger Industrietechnik Salzburg GmbH, Salzburg, Austria

Charge heterogeneity of monoclonal antibodies (mAb) is regarded as a critical quality attribute (CQA). It is a sum factor of several post-translational modifications. Which, in turn, are of high importance regarding product quality, safety and efficacy. Monitoring and controlling charge heterogeneity would be of particular importance for manufactures and process developers. However, pre-purification could possibly falsify the results and should be avoided to gain information on the impact of your process parameters on the product quality. The work presented here, adapted a cation exchange chromatography method applied in a case study to demonstrate its applicability.

Cation exchange chromatography (CEX) is routinely used and considered as the golden standard for determining charge heterogeneity. A CEX method based on pH gradient elution, previously published by Lingg et al., 2013, was used. The case study consisted of a chinese hamster ovary (CHO) cell fed-batch process, with varying process temperature and glucose feed.

The CEX method was successfully adapted to the cell culture environment. No major impurities or matrix effects were distorting the results. This method was then applied in a case study. Whereas no impact on the overall IgG titer was evident, the impact on the micro heterogeneity was huge. The charge heterogeneity of the recombinant produced mAb greatly differed from the pharmaceutical standard. Acidic variants remained the majority. The emergence of basic species could be linked to be a function of process temperature. Interestingly, the occurrence of acidic ones were dependent on the amount of main variant and glucose concentration in the supernatant. These reaction could be described by a 2nd order model and is favored at higher temperatures. To the best of our knowledge we are the first who determined antibody charge distributions directly from supernatant with a CEX method using a pH gradient. This fingerprinting platform method can be used for any basic mAb with a pI between (8 – 10).

PS2:BT-08  Cultivation of CHO-K1 cells for biomass component quantification

Špela Knez, Diana Széliová, David Ruckerbauer, Isabella Thiel, Michael Hanscho, Jürgen Zanghellini, Nicole Borth

Biotechnology, BOKU, Austria

Chinese hamster ovary (CHO) cells are the primary host organism for the production of protein biopharmaceuticals. Significant improvements in product yield and cell growth were achieved in the past years by bioprocess and media optimization, directed evolution and targeted genetic engineering. However, a deeper understanding of the underlying processes in the cells is still limited. Recently a CHO-specific genome scale metabolic model was created in a large community effort. This model is a comprehensive resource of CHO metabolism. Using the toolsets provided by metabolic modelling, we are now starting to get valuable insights into the cells’ metabolism, their protein production capabilities and their possible limitations. One of the essential inputs for the model is biomass composition. It has been shown that using cell line and condition specific biomass together with bioprocess data is a key determinant of accurate model predictions. Currently, however, the model uses estimates and literature values, since comprehensive data about CHO cell composition, specifically of individual cell lines, or over the duration of a batch, are lacking.

Here we present quantitative data on the biomass components (total protein and amino acid composition, lipids, DNA, RNA) and dry mass for both a host and a producer cell line over the full duration of the exponential phase of the batch. Each of these cell lines was grown with and without glutamine supplementation.

We investigate the changes over the batch as well as differences between the different cell lines and conditions in order to determine which compounds need to be measured for every cell line of interest and which can be approximated by a CHO-generic value. Furthermore, we will answer which components need to be measured several times during a batch and which only once.
PS2:BT-09  Expression, purification and characterisation of recombinant eotaxin-3
Alexandra Pum1, Tanja Gerlza2, Christina Trojacher3, Andreas J. Kungl1
1 Schuberstraße 1 8010 Graz
2 Pharmaceutical Chemistry, Karl-Franzens Universität Graz, Austria

Chemotactic cytokines represent a subgroup of cytokines, which are mostly basic proteins with a molecular mass of 6 to 14 kDa. One of their main function is to bind and activate G-protein coupled receptors, which are localized on the surface of leukocytes. The resulting recruitment of leukocytes to the site of inflammation plays an important role in acute and chronic inflammatory diseases. A further important interaction constitutes the binding of chemokines to glycosaminoglycans (GAGs), such as heparan sulfate and chondroitin sulfate, which can be found on the vasculature endothelium. This binding is crucial for immobilisation, activation and presentation of chemokines. [1] This work focuses on eotaxin-3, a chemokine of the CC family, which was first discovered in 1999. Eotaxin-3 has a GAG-binding domain, as well as the ability to bind and activate the chemokine receptor CCR3, expressed on eosinophilic cell surface. [2] The underlying investigation includes protein expression and functional analysis, description and evaluation of different purification steps, as well as the characterization of the recombinant protein. The analysis provides insight into the protein’s folding state, stability, glycosaminoglycan binding affinity and its ability to elicit recruitment of eosinophilic leukocytes. For this purpose different methods, including circular dichroism spectroscopy, isothermal fluorescence titration and modified Boyden Chamber assay have been applied to characterise recombinant purified Eotaxin-3. Taking into account previous R & D work in our research group, it can be hypothesized that dominant-negative mutants of eotaxin-3, characterised by higher GAG binding affinity combined with eliminated CCR3 activating potential, could be a therapeutic route to interfere with chronic inflammatory diseases such as eosinophilic esophagitis. [1] [2] Rossi, D., & Zlotnik, A. (2000). The Biology of Chemokines and their Receptors. Annual Review of Immunology, 18(1), 217–242. https://doi.org/10.1146/annurev.immunol.18.1.217

PS2:BT-10  Determination of maintenance energy in CHO cells
Isabella Thiel3, Diana Széliová4, David Ruckerbauer1, Špela Knez5, Michael Hanscho1, Jürgen Zanghellini1, Nicole Borth6
1 University of Natural Resources and Life Sciences, Muthgasse 11, 1190 Vienna
2 AG Borth, University of Natural Resources and Life Sciences Vienna, Austria

Chinese Hamster Ovary (CHO) cells are the pharmaceutical industry’s major working horse for the production of therapeutic proteins. In order to gain knowledge of and identify potential bottlenecks in CHO’s metabolism, we are currently working on refining a recently established metabolic model of CHO. Important information for the model is the composition of the biomass, uptake and secretion rates of key metabolites and the so-called maintenance energy. Maintenance energy is the non-growth associated energy demand that is required by the cell to keep up intracellular processes, such as protein turnover, independent of growth and generation of the product of interest. Since CHO cells typically grow on nutrient-rich media and use multiple carbon sources, it is not possible to calculate the turnover of ATP. Here we attempt to define maintenance requirements in terms of minimal uptake demands. Therefore, we performed continuous fermentations and measured the uptake of glucose and amino acids, as well as the secretion of by-products at different growth rates. Extrapolating to a hypothetical growth rate of zero gives the non-growth associated uptake and secretion of glucose, amino acids, and by-products. While this approach will not give a number on the required maintenance energy in terms of ATP, we obtain a maintenance term that is composed of the metabolites necessary to keep the cell alive. Thus, we can correct the uptake and secretion rates to obtain values which are purely growth-associated. This will improve model predictions for the growth rate as well as protein production. We aim to compare host and producer cell lines with different glutamine supplementation in order to get a comprehensive overview.

PS2:BT-11  Production of Fabs in E. coli – a comparison of expression systems
Oliver Spadiut
Biochemical Engineering, TU Wien, Austria

In my talk I will compare different E. coli expression platforms for the production of Fabs and discuss their advantages and challenges to be tackled.
Poster Session 2: Microbiomes: interplay of microbes, their hosts and environments
PS2:MB-01  Analysis of microbial communities in Austrian sewage systems affected by MICC.
Sarah Pycha1, Cyrill Grengg2, Florian Mittermayr3, Sabine Kienesberger1, Martin Dietzel2, Günther Koraimann1
1 IMB, University of Graz, Austria
2 Institute of Applied Geosciences, Graz University of Technology, Rechbauerstraße 12, 8010, Graz, Austria
3 Institute of Technology and Testing of Building Materials, Graz University of Technology, Inffeldgasse 24, 8010, Graz, Austria

Microbial induced concrete corrosion (MICC) is one of the main problems affecting operational lifetime and function of subsurface infrastructure, especially sewage water systems, worldwide. Besides substantial rehabilitation and maintenance costs, health and environment related issues, due to leakage of gas or liquids, are recognized. MICC is a process that takes place at the surface of aerated parts of concrete wastewater networks and involves a succession of microbial communities, each characteristic for different stages of corrosion. Key microbes within this type of concrete corrosion are chemolithotrophic sulphur oxidizing bacteria (SOB) that gradually lower the surface pH to extreme values below 2 by production of sulphuric acid (1).

To better understand the role of bacteria and fungi during early and later stages of MICC, specimens representing standard concrete or alternatives such as geopolymers with different composition were placed in manholes strongly affected by MICC. In situ placed samples were analysed after different periods for chemical, mineralogical and micro-structural alterations, as well as for the presence of surface located biofilms by fluorescent staining and imaging. In addition, microbial communities were determined by sequencing cloned 16S rDNA (V5V6) or by bacterial 16S microbiome profiling (V3V4).

We present results that show the presence of typical SOBs during early stages (up to 6 months of specimen exposure). However, the bacterial community composition is strongly influenced by the construction material that was used. The obtained results will help to develop more durable construction materials that can be used for rehabilitation purposes or as a replacement for concrete in sewage water systems (2).


PS2:MB-02  The correspondence of the nasal microbiome and olfactory function – or: The nose-brain axis
Christina Kumpitsch1, Kaisa Koskinen1, Florian Fischmeister2, Christine Moissl-Eichinger1, Veronika Schöpf2
1 Internal Medicine, Medizinische Universität Graz, Austria
2 University of Graz, Austria

Background and question. The loss of smell is an incisive event, either caused by mechanical impact, infection or subtly during the process of ageing. Besides effects on psychological, social and behavioral performance and thus affecting the quality of life tremendously, the loss of the sense of smell induces a reorganization in the functional network structure of the human brain.

The ability to smell is mediated by olfactory sensory neurons in the ceiling of the nose, the olfactory mucosa. This area is also inhabited by numerous microorganisms. In general, the human body is associated with trillions of microorganisms (the microbiome) which appear to have tremendous effects on health, disease, behavior and other aspects of human life. The microbiome is capable to communicate with the human body cells and affects functions of human tissues and even brain. This project aims to decipher the role of the nasal microbiome in olfactory function, dysfunction and regain.

Methods: Neuroimaging biomarkers and olfactory function are correlated with microbiome measures such as diversity, abundance and functional data (metatranscriptomics).

Results: In a completed pilot study, we have shown that the microbial community composition in the olfactory mucosa mirrors the capability to smell. Volunteers with impaired smelling capacity showed an increase of specific (anaerobic) microbial groups, such as butyrate producing microorganisms.

Conclusion: Understanding the microbial community in the olfactory mucosa increases therapeutic opportunities, and possibly allows monitoring and predicting smell therapy success in future.
**PS2:MB-03  Sensing and reacting to nutrients is balanced with plant and mating partner sensing in *Trichoderma reesei* and aimed at optimal association with plants**

Guofen Li1, David Turra1, Stefanie Kindel1, Ursula Sauer2, Antonio Di Pietro1, Monika Schmoll3

1 University of Cordoba, Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceia3, 14071 Córdoba, Spain
2 Austrian Institute of Technology, Austria

The natural environment of the potent cellulase producer *Trichoderma reesei* (syn. *Hypocrea jecorina*) is a tropical forest, where it degrades cellulosic plant biomass. Thereby, we found that sensing of a precisely defined concentration of glucose by the GPCR CSG1 impacts posttranscriptional regulation of cellulase gene expression, which determines whether degradative enzymes are formed or not. On natural substrates this sensing defect causes a morphological change as well. The sensing pathway for glucose further involves subunits of the heterotrimeric G-protein pathway and components of the cAMP pathway.

In the plant pathogen *Fusarium oxysporum*, plant sensing is accomplished by pheromone receptors responding to secreted plant peroxidases. Here we investigated the relationship between plant sensing, sexual development and nutrient detection in *T. reesei*.

We found that *T. reesei* is able to sense plant root exudates and peroxidases via pheromone receptors, suggesting that this mechanism is conserved between *Trichoderma* and *Fusarium*. Interestingly, a nutrient-rich medium such as malt extract (ME) resulted in bipolar and multipolar germination without discernible chemotropism, whereas exudates from soybean roots or from a compatible mating partner in the presence of ME caused unipolar germination with a robust chemotropic response. Hence, signals related to sexual development and plant sensing override nutrient regulated germination. Accordingly, we found that the presence of soybean germenmtes promotes sexual development of *T. reesei* as do compounds releasing nitric oxide, a signaling molecule, which is produced by plants to counteract oxidative stress and involved in plant pathogen interaction.

In comparative chemotropic assays we found that in *T. reesei* the priority for chemotropic response to a plant is highest, followed by a mating partner and glucose has lowest priority. We propose that *T. reesei* is attracted to plants by a mechanism that integrates directed hyphal growth and sexual development for efficient adaptation to the host and potentially also for optimized antagonism of competitors and plant pathogens. Thereby, association with a plant has higher priority than availability of nutrients.

**PS2:MB-04  Encapsulation of Gram negative bacteria for probiotic soil amendment and foliar spray**

Andrea Alber, Natalia Hatrakova, Sara Doppler, Claudia Preininger

AIT Austrian Institute of Technology, Austria

Microbial fertilizers are promising alternatives to agrochemicals in sustainable agriculture; however the lack of effective formulations is a major limitation for their successful application in the field. Encapsulation of microbes for soil amendment or UV-protection in foliar spray represents a promising approach to stabilize especially Gram negative bacteria (e.g. *Paraburkholderia phytofirmans* PsJN, *Pseudomonas* sp.) over longer times.

Herein we will present microbial alginate beads extruded from a bacteria/alginate slurry and respective core-shell particles providing an interior liquid core of effective bacteria for plant growth promotion of maize. We have characterized both microbial alginate beads and core shell capsules in terms of surface morphology, shape, size and viability over storage time at ambient temperature and link these data to the chemical composition of the capsules, the release profile of the bacteria and the type of bacteria. Starch was added as a filler to influence the time course of bacterial release upon rehydration. Use of skimmed-milk, molasses as well as the combination thereof successfully preserved cell counts and resulted in extended storage period of dry formulations. Subjection to mild stress conditions during the growth phase of bacteria made the cells more resistant to desiccation stress: PsJN grown in Luria Bertani medium of low water activity obtained by addition of glycerol or sucrose was able to survive longer times than bacteria cultivated in basic LB medium.

Elucidating microbial responses to multiple factors including the properties of the used materials, the formulation process itself and the environmental conditions during processing and storage, allows to estimate the driving factors that impact stability (viability, shelf life, metabolic activity), and performance (plant penetration) of foliar sprays and will help other researchers to optimize their choices in this area.
PS2:MB-05  Microbial diversity of Boraginaceae species with medicinal properties  

Cintia Csorba¹, Nebojsa Rodic², Angela Sessitsch³, Günter Brader¹, Vassilios P. Papageorgiou², Andrea N. Assimopoulou²  
¹ Health and Bioresources, AIT Austrian Institute of Technology, Austria  
² Aristotle University of Thessaloniki (AUTH), School of Chemical Engineering, Thessaloniki, Greece  

There has been a growing interest for plant-derived bioactive secondary metabolites (SM) for cosmeceutical and pharmaceutical applications. Effective and profitable production systems and extraction methods require a sufficient concentration of the compound of interest in plant tissues, therefore novel approaches for increasing SM yields along with an optimized cultivation scheme are needed.  

Positive effects of endophytic microorganisms on the SM production of plants have been shown several times. Application of microbial symbionts might be a sustainable, cheap and effective way for improving SM yield.  

Alkannin and shikonin (A/S) are secondary metabolites produced in the root system by members of the Boraginaceae family. A/S possess high biological activity and beneficial medicinal properties, such as anti-inflammatory effects, accelerating wound-healing and tissue-regeneration, as well as antibacterial, antitumor and antiplatelet activity.  

We studied the microbial diversity, in focus with bacteria, in different sections of the root system in Echium vulgare L. and Alkanna tinctoria L., both biosynthesizing A/S, including the rhizosphere and bulk soil in order to have a better understanding of the dynamics of the microbiome in this plant family. In addition, secondary metabolite content analysis has been carried out. Moreover, we examined the key genera in the microbiome of Alkanna tinctoria L. in five different vegetation stages under greenhouse conditions together with SM analysis.  

According to our preliminary results we will discuss if presence or absence of specific bacterial taxa may influence the concentration of A/S in the root systems of these Boraginaceae species.  

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 721635

PS2:MB-06  Cuticular hydrocarbons in the symbiosis of leafcutter ants and antimicrobial-producing bacteria  

Johannes-Paul Fladerer, Franz Bucar  
Department of Pharmacognosy, Institute of Pharmaceutical Science, Austria  

The composition of cuticular hydrocarbons (CHC) on the ants’ surface has to be adapted to environmental conditions. In arid habitats the CHC profile has to prevent the ant from drying out while in tropic habitats a water-repellent surface can be adaptive. Additionally, ants are using CHCs in interspecific recognition as well as in intraspecific nest mate recognition. Therefore, the CHC profile can be adjusted by different glands like the metapleural gland and Dufour’s gland. In leafcutter-ants, which are living in a symbiosis with antimicrobial-producing bacteria like Pseudonocardia inhabiting the ants’ surface, the CHC profile may influence the growth of the bacteria or might be used for interspecific communication between the symbionts. As a first step to investigate the impact of CHCs in the symbiosis of leafcutter ants and antimicrobial-producing bacteria we analyzed the CHC profiles of three different species of leafcutter-ants containing two genera, Atta and Acromyrmex, by GC-EI-MS. These results were compared with CHC profiles of the thermophilic ant Messor aciculatus and the tropic ant Polyrhachis dives. Our research could show high quantitative and qualitative differences in the CHC profile of the investigated ants. The CHC profiles of all leafcutter-ants were very similar and contained more amides like tetradecanamide and hexadecanamide. In contrast to that, on Messor alcohols like pentadecanol, pentacosanol and heptacosanol could be identified while the profile of Polyrhachis contained a number of different higher substituted alkanes like 2-methylpentacosan and 8-pentylheptacosan. The similarity between the two different tropic genera of leafcutter-ants living in symbiosis with the same genus of bacteria and the difference of those ants to the also tropic Polyrhachis suggests an impact of the identified compounds on the symbiosis. The profile of Messor enables the evaluation of the environmental influence.  

Key words: hydrocarbons, GC-EI-MS, Atta, Acromyrmex
PS2:MB-07  Single cell microbiology using atomic force microscopy and scanning electron microscopy

Ursula Sauer, Sara Doppler, Stefanie Kindel, Kathrin Mokesch, Katharina Zimmermann, Claudia Preininger

Center for Health and Bioresources, AIT Austrian Institute of Technology, Austria

Thinking of microbes most of us associate high numbers of cells or colonies, and many methods to study their physico-chemical properties such as XPS, infrared spectroscopy, and contact angle measurements do not provide information on single cell level. Advanced microscopy technologies on the other hand allow studying surfaces of single cells at high resolution [Dufrêne 2014].

Profound knowledge on adhesion mechanisms and forces involved allows us to control colonization on host surfaces in order to either prevent or promote the formation of biofilms. As a result, adhesion free surfaces or surfaces where bacteria adhere and stay can be designed [Angeloni et al. 2014]. The properties of both interacting surfaces, i.e. the substrate and the microbes, play a role in the attachment process and consequently are crucial for colonization or pathogenicity. Parameters involved are cell surface charge, acid-base character and hydrophobicity which can be determined by contact angle measurements on bacterial lawns [van der Mei et al. 1998]. The ultrastructure of the cell surfaces, mechanical properties and molecular interactions can be visualized by atomic force microscopy (AFM) using topographic imaging and force spectroscopy. A prerequisite for applying AFM is the non-destructive immobilization of cells. This can be done by random dispersion on suitable substrates, mechanical trapping in porous membranes [Dague et al. 2007] or in highly ordered arrays created by chemical patterning [Cerf et al. 2008] or microstructured surfaces [Dague et al. 2011]. Force spectroscopy on the other hand requires immobilization of cells on the tip of an AFM cantilever.

We will show close-ups of microbes colonizing plants such as grapevine pathogens and plant growth promoting bacteria on corn, and discuss their adhesion behaviour in the light of the physico-chemical properties of cells and substrates.

References
Dague et al. Nanotechnology 2011, 22: 395102
Dague et al. Yeast 2007, 24: 229
Dufrêne mBio 2014, 5(4):e01363-14
van der Mei et al. Colloids and Surfaces B: Biointerfaces 1998, 11:213-221

PF2-06  Interplay of hospital microbiome and resistome – connecting pathogenic infection risk, healthy microbes and environmental biodiversity in and functional hospital setting

Stefanie Duller

Department of Internal Medicine, Medical University of Graz, Austria

Hospital acquired infections are a serious problem worldwide. The risk of acquiring pathogenic infections is higher in hospital than in other environments, and these infections are also more often fatal. The risk is not only related to invasive procedures or inadequate hygiene; infection can also be transferred from patient to patient, via personnel, surfaces, or routinely used equipment. Resistant (pathogenic) bacteria are frequently found in hospital surfaces, despite strict disinfection procedures.

Consequently, the microorganisms that inhabit hospital indoor environment can influence the patient recovery and outcome, and potential link between hospital indoor environment-associated microbial communities and hospital-acquired infections has been suggested.

In this project we explore the microbiomes and resistomes of different hospital areas and sites with different cleaning and disinfection procedures, such as operating room, intensive care unit, waiting areas and toilets, and study if purposely increased environmental biodiversity in hospital can decrease the prevalence of (opportunistisch) pathogenic microorganisms and the extent of horizontal gene transfer, reflecting the probability for developing new hospital pathogens. Our focus is on the whole microbial communities, and their relation to the pathogenic, life-threatening organisms and resistance. Resolving the bacterial community structure in hospital environments will be critical for understanding the dissemination of antibiotic resistance genes and development of antibiotic resistance.

The results will help us to understand the microbial dynamics in the hospital indoor environment and to support safe recovery of the patients. In this presentation, we will summarize the latest results of this project.
Poster Session 2: Pluripotent stem cells and neural differentiation
PS2:SC-01  Identifying isthmus stem cells and dynamics of stem cells in stomach
Juergen Fink, Seungmin Han, David J. Jörg, Sebastian R. Merker, Min Kyu Yum, Ji-Hyun Lee, Benjamin D. Simons, Bon-Kyoung Koo
1 Wellcome Trust–Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge, CB2 1QR, UK
2 The Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, CB2 1QN, UK
3 Department of Visceral, Thoracic and Vascular Surgery, University Hospital Carl Gustav Carus, Medical Faculty, Technische Universität Dresden, Fetscherstr. 74, 01307 Dresden, Germany
4 Koo lab, Institute of Molecular Biotechnology (IMBA), Austria

The corpus epithelium in stomach is composed of long single-layered glands. Each gland is histologically divided into gastric pit, isthmus, neck and base region from gastric lumen to the base of stomach epithelium. Many researchers have thought there must be stem cell populations in the isthmus region where highly proliferating cells are located, even though another stem cell population was reported at the base because of the slow dividing ratio of the stem cells at the bottom. Although several markers have been proposed for isthmus stem cells, the identity of isthmus stem cells (IsthSCs) and the interaction between different stem cell populations are still unclear. In our study, we could show that corpus glands are compartmentalized into two independent zones using unbiased genetic labelling and biophysical modelling. Actively-cycling stem cells maintained the pit-isthmus-neck region whereas slow-cycling reserve stem cells supported the base. Actively-cycling IsthSCs follow “punctuated” neutral drift dynamics because existence of long-lived parietal cell acts as a barrier of lateral expansion of stem cell derived clones. We propose Stmn1 as a new marker of IsthSC through bulk RNA-seq analysis and comparison with previous analysis. We confirmed the dynamics of IsthSC using Stmn1 lineage tracing and we are now studying about potency of IsthSCs after deletion of base stem cells.

PF2-01  Establishment of a human endometrial cancer organoid library
Hyelin Na
Institute of Molecular Biotechnology, Austria

Endometrial cancer is a type of cancer that forms in the lining of the uterus. Endometrial cancer is the sixth common cancer and the fourth prevalent cancer in female worldwide. Incidence of endometrial cancer have increased in many countries for decades. In more developed regions, incidence and mortality are higher that less developed regions. The signs and symptoms of endometrial cancer, such as abnormal vaginal bleeding or discharge, enable the disease to be diagnosed at an early stage. Due to early detection, most patients have low-grade and early stage diseases. The early-detected endometrial cancers can be cured by hysterectomy and surgery. However, except main surgical treatments, most patients don’t have any benefit from current targeted therapeutics. Low-grade endometrial cancers are often treated with radiotherapy, whereas some endometrial cancers with an advanced stage are treated with chemotherapy. These adjuvant therapies can help the cures, but it can be harder on some patients because it causes more side effects. In this aspect, the importance of understanding individual patient endometrial cancer might be strongly emphasized. For the study of individual patient endometrial cancer, organoid culture systems have recently emerged as a novel platform for unmet clinical needs. Organoid culture systems allow patient-derived cancer cells to be successfully cultured in the laboratories and used quickly for test against adjuvant therapy, leading to develop the individual treatment strategies for cancer. To date, several lines of patient-derived endometrial cancer organoids have been generated, but large-scale functional analysis of endometrial cancer has not been performed. In this study, we established a patient-derived endometrial cancer organoid library and refined organoid culture conditions. This endometrial cancer organoid library will provide a valuable prognostic platform for functional analysis and rapid test of individual endometrial cancer treatment.
Wednesday 19\textsuperscript{th}: Poster Session 2: Cancer metabolism, autophagy and cell death

10\textsuperscript{th} ÖGMBT Annual Meeting 2018 & 10\textsuperscript{th} Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 2: Cancer metabolism, autophagy and cell death
PS2:CM-01  Epigenetic markers for minimal-invasive transplant rejection monitoring
Lisa Milchram1, Walter Pulverer1, Gabriel Beikircher1, Stephan Pabinger1, Klemens Vierlinger1, Martin Andreas2, Paul Werner2, Kathrin Freystätter2, Martin Jung1, Andreas Weinhäuser1

1 Molecular Diagnostics, AIT Austrian Institute of Technology, Austria
2 Medical University of Vienna, Division of Cardiac Surgery, Währinger Gürtel 18-20, 1090 Wien

Transplant rejection events are the major risk factor of long-term survival and for quality of life of solid organ recipients. Although donor and recipient are carefully cross-matched prior transplantation, the risk of the recipient’s immune system rejecting the donor’s organ remains. The gold standard for rejection monitoring is biopsy. Transplanted patients undergo 3-4 biopsies/year in order to detect rejection events on time for adapting immunosuppressive therapy. Recent work showed that apoptotic processes in the transplant releases circulating cell-free DNA (cfDNA) into the bloodstream. Levels of graft-derived cfDNA start to elevate weeks prior to the histological manifestation of the rejection event and can therefore be used for transplant reception monitoring. Thus, we focused on the development of a minimal invasive test, which allows tissue specific enrichment of cfDNA derived from heart in an access of background DNA to monitor transplant rejection. Public methylation data (derived from heart, PBMCs and other organs, e.g. kidney) was used to identify a set of regions, which is highly methylated in heart tissue and unmethylated in PBMCs and other organs. Prior amplification the unmethylated background DNA is cleaved by methylation sensitive restriction enzymes to enable the specific enrichment of tissue-derived cfDNA.

22848 CpGs showed statistical differential methylation between all investigated tissues (p<0.001). Filtering for CpGs with a fold-change >2.5 restricted the selection down to 730 CpGs. The final selection contained 96 assays, which enables the targeted amplification of heart tissue in an access of background DNA. 57 out of the 96 assays were technically qualified (75-130% PCR efficiency, R2>0.95) and a number of at least 13 assays showed discriminatory power between the tissues. Results showed that an amount of 23 pg heart DNA spiked into 24 ng PBMC DNA can be reliably detected. Therefore the detection limit is 0.1%.

Our method enables tissue specific detection of cfDNA derived from heart with very high sensitivity and specificity, based on tissue specific DNA methylation profiles. Upcoming transplant rejection events can be detected weeks prior to their clinical manifestation and allows fine regulation of the immunosuppressive therapy at very early stages. Our tool has the potential to replace the current standard but cumbersome biopsy by a minimal-invasive method using a simple blood draw.

PS2:CM-02  Monoacylglycerol lipase (MAGL)-deficient mice exhibit alterations in the tumor microenvironment composition
Carina Hasenöhrl1, Melanie Kienzl1, Magdalena Grill1, Julia Kargl1, Rudolf Schicho3
1 Otto Loewi Research Center, Medical University of Graz, Austria
2 BioTechMed, Graz, Austria
3 Otto Loewi Research Center, Medical University of Graz, Austria

Background/Aims:
Dysregulated metabolic pathways, as observed in many cancers, lead to the biosynthesis of several pro-tumorigenic lipid-signaling molecules. Monoacylglycerol lipase (MAGL) hydrolyses monoacylglycerols to glycerol and free fatty acids and, thus, contributes to the “lipolytic” pathway of cancer cells. One of the main substrates of MAGL, 2-arachidonoyl glycerol, is a key signaling molecule of the endocannabinoid system, a network recently shown to modulate immune cell populations present in tumors. Here, we investigate whether MAGL expressed in the tumor microenvironment influences tumor growth.

Methods:
A K-ras+/−/p53−/− lung adenocarcinoma cell line (KP) was engrafted into the right flank (500.000 cells/mouse) of MAGL-deficient mice and wild-type littermates. After tumor excision, 28 distinct subpopulations of tumor-infiltrating leukocytes were analyzed by flow cytometry.

Results:
Tumors of MAGL-deficient mice (n=11) were significantly smaller than those of wild-type littermates (n=10) and showed prominent alterations of the tumor microenvironment. MAGL-deficient mice had elevated monocyte and eosinophil infiltration, as well as decreased B cell infiltration. Additionally, a shift from naïve to effector T cells was observed, and PD1 expression was strongly up-regulated, both on CD4+ and CD8+ T cells.

Conclusion:
MAGL-deficient mice show a stronger activation of the adaptive immune system resulting in an increased anti-tumor response and smaller tumor sizes.
PS2:CM-03  Studying the effect of estrogen receptor-interacting substances on the mechanical and adhesive properties of human breast cancer cells via AFM

Andreas Weber1, Jagoba Iturri1, Rafael Benitez2, Maria del Mar Vivanco3, José Luis Toca-Herrera1

1 Institute of Biophysics, DNB1, University of Natural Resources and Life Sciences Vienna (BOKU), Austria
2 Department of Mathematics for Economics and Business, Universitat de Valencia, Avda. Blasco Ibáñez, 13. 46010 Valencia, Spain
3 Cell Biology and Stem Cells Unit, CIC bioGUNE, Bilbao, Bizkaia Science and Technology Park, building 801A, Derio, Spain

The study of mechanical properties of eukaryotic cells has gained an increased interest in recent years. An important finding in this field is that different cellular states (e.g. disease, cancer, age ...) lead to significant alterations in those properties [1]. By studying cell mechanics, one can therefore derive important knowledge to further understand the complex mechanisms of e.g. cancer formation. In addition, the quantification of those changes can be used as novel diagnostics tool [2].

In this study, atomic force microscopy (AFM) in force spectroscopy mode was used to determine, at the nanoscale, the mechanical properties of MCF-7 (Michigan Cancer Foundation) human breast cancer cells. The time and concentration dependent effect on cell mechanics by different agents interacting with the estrogen receptor (tamoxifen, resveratrol, estradiol) was studied. Those studies were performed on a normal MCF-7 cell line (taken as reference) as well as on a tamoxifen resistant line [4]. Thus, mechanisms-related factors such as cell elasticity, adhesion, as well as cell rheology were obtained (and their dependence upon incubation with the aforementioned substances). Batch data processing was performed by means of self-developed R language protocols (afmToolkit) [5]. Complementarily, fluorescence microscopy experiments were performed to study cytoskeletal rearrangements. By this combined methodology new insights on drug treatment influence (or resistance) on cancer cells could be achieved.

References

PS2:CM-04  Essential roles of autophagy in Merkel cells, sweat glands and enamel epithelium

Supawadee Sukserre, Heidemarie Rossiter, Sophie Bergmann, Uwe Yacine Schwarze, Reinhard Gruber, Florian Gruber, Erwin Tschachler, Leopold Eckhart

Department of Dermatology, Medical University of Vienna, Austria

Autophagy contributes to the turnover of proteins and to the removal of damaged organelles in eukaryotic cells. The relevance of autophagy for the homeostasis of the different cell types of the skin has remained incompletely understood. Here, we deleted the essential autophagy gene Atg7 by the Cre-LoxP system in keratin K14-expressing epithelial cells and their progeny in the mouse. A transgene encoding the autophagy reporter GFP-LC3 was introduced to facilitate the assessment of autophagic activity in situ, and sequestosome 1 (Sqstm1)/p62 was immuno-localized as an endogenous substrate of autophagy. The suppression of autophagy was compatible with epithelial keratinization in the interfollicular epidermis where Sqstm1/p62 was only slightly increased in the absence of Atg7. By contrast, Sqstm1/p62 showed massive accumulation in Merkel cells and secretory cells of the sweat glands of Atg7f/f K14-Cre mice, indicating that in epithelial cells undergoing a differentiation-associated switch from K5/K14 to K8/K18 expression, autophagy is required for normal protein homeostasis. Ameloblasts which form the enamel of teeth also differentiate from K14-positive precursors and express K8/K18. In Atg7f/f K14-Cre mice, the enamel epithelium showed an aberrant distribution of GFP-LC3 and accumulation of p62. Impaired secretion of iron from Atg7f/f K14-Cre ameloblasts led to change in the color of incisors from normal yellow to white. In summary, these results suggest that K8/K18-positive cells of the skin and of the enamel-forming epithelium require the autophagy gene Atg7 for normal homeostasis and function.
PS2:CM-05  Cell-specific localization of receptors and enzymes of the Endocannabinoid system in intestinal inflammation and colon cancer

Magdalena Grill, Carina Hasenöhrl, Melanie Kienzl, Rudolf Schicho
Otto Loewi Research Center, Pharmacology Section, Medical University of Graz, Austria

Cannabis has been suggested to provide improvement in inflammation and cancer of the gastrointestinal (GI) tract. The Endocannabinoid system (ECS), which is comprised of cannabinoid (CB) receptors and enzymes involved in synthesis/ degradation of endogenous ligands, is, therefore, a potential therapeutic target. However, detailed mechanisms of beneficial effects are missing, especially due to the lack of specific antibodies for receptor detection such as CB2 and GPR55. In the present study, we used quantitative RT-PCR and in situ hybridization (ISH) RNAsecope combined with immunohistochemistry to show cell-specific distribution of the receptors CB1, CB2, GPR55 and the endocannabinoid degrading enzyme monoacylglycerol lipase (MGL) in mouse models as well as human biopsies of intestinal inflammation and colon cancer. Murine ISH probes were validated using the respective knockout mouse.

In healthy murine tissue, CB1 mRNA levels were high in a subpopulation of neurons, low levels were found in the lamina propria (LP) and the longitudinal muscular layer. CB2 mRNA was mainly detected in B220+ cells, lower gene expression was found in F4/80+ and CD3+ cells. The GPR55 gene was highest expressed in F4/80+ and CD3+ cells. Low expression of all three receptors was detected in epithelial cells. We barely found CB2 or GPR55 mRNA in neurons. MGL mRNA was highly detectable in cells of the circular and longitudinal muscular layers, some neurons, epithelial cells and cells of the LP. Total GPR55 as well as MGL gene expression was reduced in inflamed tissue. While part of this could be explained due to the destruction of epithelial cells, we asked whether immune cells were involved. Interestingly, there were no changes of GPR55 and MGL gene expression in CD3+ positive cells, whereas F4/80+ cells showed reduced expression of both genes. Reduced MGL gene expression was also found in the muscular layers of the inflamed colon. In human biopsies of healthy subjects, low levels of GPR55 mRNA were detected in epithelial cells and cells of the LP. In biopsies of ulcerative colitis (UC) patients, GPR55 mRNA was locally increased in epithelial cells. Very low CB2 receptor mRNA levels were found in healthy subjects as well as UC patients.

In summary, our study revealed novel cellular details regarding the gene expression of members of the ECS. The study confirms ISH as a valid and trustworthy method to verify target expression in specific cells of intact/ fixed tissues.

PS2:CM-06  Study of serological biomarkers in systemic auto-inflammatory diseases

Julie Krainer1, Silvia Schönthaler1, Sandra Siebenhandel1, Marco Gattorno2, Andreas Weinhäusel1
1 Center for Health & Bioresources, AIT - Austrian Institute of Technology, Austria
2 IRCSS Istituto Giannina Gaslini, UOC Pediatra II, Pediatric Rheumatology, Genova, Italy

Systemic auto-inflammatory diseases (SAID) is a group of mendelian diseases resulting from a dysregulation of the innate immune system. In contrast to autoimmune diseases, SAIDs lack high-titer autoantibodies or antigen specific T-cells and are characterized by recurrent fever attacks, unprovoked inflammation, abdominal pain, arthritis and cutaneous signs. In some patients with inherited SAIDs, molecular analysis is able to provide a definitive diagnosis whereas other patients (70-80%) with clinical picture highly consistent with auto-inflammatory disease lack a molecular validation. The main goal of this study is to evaluate serological biomarkers of patients in both active and inactive disease state using Familial Mediterranean Fever (FMF), Cryopyrin-Associated Auto-inflammatory Syndrome (CAPS), Mevalonate Kinase Deficiency (MVK), Tumor necrosis factor (TNF)-associated periodic syndrome (TRAPS) as model diseases for defined SAIDs and undefined SAID patients (uSAID).

In total 90 patient plasma samples of patients in active and inactive disease state (FMF n=7/6, CAPS n=7/6, MVK n=5/4, TRAPS n=6/4, uSAID n=17/28) are analyzed using the OLINK’s Proseek 92-plexed Inflammation panel that covers a broad variety of chemokines, interleukines and fibroblast growth factors. For 19 patients paired samples (n=38) for active and inactive disease are available (FMF n=10, CAPS n=12, MVK n=8, TRAPS n=8).

Biostatistical analysis of the 92 serological biomarkers revealed a significant (|log2FC| > 1; p < 0.05) upregulation of one interleukin in active disease during paired group comparison based on disease activity for FMF, CAPS, TRAPS and all active/inactive pairs combined analysis. Further findings in pairwise group comparisons of active disease samples show two biomarkers that are significantly upregulated disease specific (|log2FC| > 1; p < 0.05).

Based on the findings we hypothesize that measuring relevant serological biomarkers in active disease state have the potential to improve diagnosis of patients with undefined SAIDs.
Hypermethylation of CDKN2A exon 2 in breast cancer

Melanie Spitzwieser¹, Andreas Jenik⁰, Katja Zappe², Elisabeth Entfellner³, Bettina Werner⁴, Walter Pulverer⁵, Lukas Uhlik⁵, Petra Heffeter⁵, Stefan Hacker⁴, Georg Pfeiler³, Margit Cichna-Markl⁰

¹ Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna
² Molecular Diagnostics, Austrian Institute of Technology
³ Institute of Cancer Research, Department of Medicine I, Medical University of Vienna
⁴ Department of Plastic and Reconstructive Surgery, Medical University of Vienna
⁵ Department of Obstetrics and Gynecology, Division of Gynecology and Gynecological Oncology, Medical University of Vienna
⁰ Department of Analytical Chemistry, University of Vienna, Austria

Cyclin-dependent kinase inhibitor 2A (CDKN2A, p16) plays a crucial role in cell cycle regulation. Silencing of CDKN2A by promoter hypermethylation has been reported to be a frequent event in a variety of cancer types including breast cancer. Recent studies suggest that in addition to regulatory elements, the methylation status of transcribed gene regions may be associated with gene expression. Hypermethylation of CDKN2A exon 2 has previously been linked to late stage oesophageal cancer.

In the present study, we aimed to investigate if the methylation status of CDKN2A exon 2 is associated with breast cancer. We developed an assay based on methylation sensitive high resolution melting (MS-HRM) analysis, allowing to determine the average methylation status across 10 CpG sites in CDKN2A exon 2. When analysing tissue specimens from 18 breast cancer patients, tumor tissues showed significantly higher CDKN2A exon 2 methylation levels than tumor-adjacent and tumor-distant tissues. In the tissue specimens from breast cancer patients, CDKN2A exon 2 was significantly higher methylated than in breast tissues from healthy women serving as control. Surprisingly, we found exon 2 more frequently methylated than the promoter. Only in tumors of two patients, the promoter was higher methylated than in normal breast tissues from healthy women.

To investigate if CDKN2A exon 2 methylation is associated with gene expression, we determined the methylation status in a variety of breast cancer cell lines. Since we have learned that CDKN2A exon 2 may be methylated heterogeneously, we applied a novel in-house pyrosequencing assay, providing the methylation status of 11 individual CpG sites in CDKN2A exon 2. In addition, we determined the extent of gene expression at both the mRNA and protein level. Preliminary results indicate that in breast cancer, CDKN2A exon 2 methylation is associated with gene expression at both levels.

The role of HSP90 in patients with thymic epithelial tumors and benign thymic pathologies.

Jürgen Thanner¹, Ana-Iris Schiefer², Leonhard Müllauer³, Cecilia Veraar³, Sophie König³, Panja Böhm³, Philipp Hacker², Stefan Janik⁴, Walter Klepetko³, Hendrik Ankersmit⁵, Bernhard Moser¹

¹ Thoracic Surgery, Medical University Vienna, Austria
² Clinical Institute of Pathology, Medical University Vienna, Austria.
³ Division of Cardiothoracic and Vascular Anesthesia and Intensive Care, Medical University of Vienna, Vienna, Austria
⁴ Department of Otolaryngology Head and Neck Surgery, Medical University of Vienna, Vienna, Austria.
⁵ Head FFG Project “APOSEC”, FOLAB Surgery, Medical University Vienna, Vienna, Austria.

Background: Thymic Epithelial Tumors (TETs) are the most frequent anterior mediastinal tumors and have a unique association with autoimmune Myasthenia Gravis. So far, no risk factors or biomarkers for TETs were incorporated into clinical practice. Heat shock family proteins (HSP) were found in several tumor entities. They are involved in tumor cell survival and tumor growth.

Methods: Our team performed enzyme-linked immunosorbent assays in serum of 114 patients with TETs, patients with benign thymic pathologies (n=20) and volunteers (n=58) in order to detect HSP90. Data are reported as mean ± standard deviation or median (range) [ng/ml].

Results: There was a statistically significant higher expression of HSP90 in patients with thymomas 9.3 (1.1 – 36.5) and thymic carcinomas 17.3 (3.2 - 47.5) compared to healthy volunteers 6.0 (0.9 – 13.4), respectively, p<0.001. While there was no statistically significant difference between benign thymic pathologies 6.2 ± 0.9 and volunteers (p=0.935). There were statistically significant differences between WHO histopathological subtypes (A, AB, B1, B2, B3, micronodular thymoma, Thymic carcinoma and thymic neuroendocrine tumors; p=0.037). Greatest HSP90 concentrations were present in WHO type B3 thymomas 16.7 ± 2.7 and thymic carcinomas 17.3 (3.2 - 47.5), while the lowest serum concentrations were found in WHO type AB thymomas 4.9 (1.1 - 27.0). There was a significant decrease of HSP90 in serum of patients with TETs after complete tumor resection (p=0.014).

Conclusions: The expression of HSP90 in TETs and patient serum warrants future study about heat shock protein pathophysiology and tumorigenesis. The prognostic role of HSP90 will be explored.
**PS2:CM-09  Analysis of the impact of L-carnitine, as a nutrigenomic factor, on various nuclear receptor pathways**

Dominic Indra, Lars Zver, Reinhold Hofbauer  
Center of Medical Biochemistry, Division of Molecular Genetics, Max F. Perutz Laboratories, Medical University of Vienna, Austria

The main function of L-carnitine is the transport of activated long-chain fatty acids from the cytosol into the mitochondrial matrix, where these fatty acids then undergo β-oxidation. However, previous experiments showed that it is also involved in the regulation of the expression of a multitude of genes. Therefore, the aim of this thesis was to prove on DNA level as well as on protein level that L-carnitine influences the activity of certain nuclear receptor pathways and thereby amplifying and targeting the signaling effect. Furthermore, the so-called “L-carnitine effect” was investigated, where gene expression is up- as well as downregulated when L-carnitine was supplemented to cells previously cultivated under L-carnitine deficiency. Regarding the protein level, we wanted to reveal early and late effects changing the activity of nuclear receptor pathways under various L-carnitine concentrations via luciferase activity measurement. Based on the results of these experiments, distinct nuclear receptor pathways were selected and the expression of key genes of these pathways was then tested for L-carnitine susceptibility via qPCR. On DNA level, electrophoretic mobility shift assays with the respective 32p-labeled DNA-oligonucleotides representing the putative promoter binding sites of GRα, HNF4α, PPARα and RXRα/RARβ were performed to check the effect of acetyl-L-carnitine on the transcription of the CRAT gene as well as to find potential mediators of the “L-carnitine effect”. Our results suggest that L-carnitine most likely has an impact on the ER, PPAR, RAR, VDR, RXR, LXR and HNF4 signaling pathways. Remarkably, some of these effects are also time-dependent. This is consistent with the findings of the band shift experiments, where the transcription factors PPARα, HNF4α and RXRα/RARβ located in the CRAT promoter are affected by L-carnitine in their binding activity towards the putative binding sites. This opens additional new possible nutrigenomic target sites within the ER, VDR and LXR pathways. Furthermore, significant changes in the gene expression of CYP2R1, ALDH1A1, HSD11B2 and HMGCR caused by various L-carnitine supplementation conditions was observed, revealing different regulation patterns.

This research was supported by the Herzfelder’sche Familienstiftung.

**PS2:CM-10  Technical qualification of high density protein and peptide microarrays**

Jasmin Huber, Peter Hettegger, Silvia Schönthaler, Regina Soldo, Stephan Pabinger, Lisa Milchram, Andreas Weinhäusel  
Center for Health & Bioresources, Austrian Institute of Technology, Austria

High density protein and peptide microarrays enable simultaneous screening of several thousand targets from just a few microliters of plasma. Among others, routine applications in research include epitope screening, drug discovery, biomarker development and biosensor technologies. However, the reliability of these platforms has to be confirmed. We present an experimental approach capable of estimating the inter-individual discriminating power of these platforms. The approach relies on pure and cross-wise mixed IgG samples of two individuals in order to (1) test for significant DIRAGs (differentially reactive antigens) between pure samples. (2) Significant inter-individual DIRAGs (p<0.05) are then used for correlation analyses on signal intensities of cross-wise mixed samples. We tested this approach on our 16k protein microarray and our 392k peptide array. IgG isolated from serum or plasma of two individuals is mixed in descending (logarithmic) proportions. Four replicates of pure sample at 100% are subjected to a class comparison analysis (p<0.05), deducing significant inter-individual DIRAGs. Resulting DIRAGs and relative IgG amounts of both individuals are subjected to a correlation analysis (Pearson’s correlation coefficient). The correlation coefficients are visualized in a histogram.

The 16k protein microarray, covering 5449 recombinantly expressed human proteins deduced from 15,417 cDNA clones, revealed 4638 DIRAGs. 97% of these DIRAGs showed correlation coefficients (r) of >0.5 or <0.5, 72% and r > 0.812 (or < -0.812). Preliminary results from our 392k peptide array, 16mer (6aa overlap) peptides deduced from preceding 16k experiments, revealed >100,000 peptides as differentially reactive (p < 0.05) between the individuals. 80% of the significant DIRAGs showed r >0.5 (or r <0.5). 38% gave correlation coefficients >0.8 (or r <0.8). For both platforms, >50% of significant features gave correlation coefficients >0.5.

Summarized, we developed a method using differences in immunosignatures as basis for technical qualification of high density protein and peptide microarrays. We applied this method to our high density protein and peptide microarrays and could successfully confirm their reliability.
Dissecting the role of the PIDDosome in hepatocellular carcinoma
Katja Knapp
Division of Developmental Immunology, Innsbruck Medical University, Austria

The PIDDosome is an activation platform for Caspase-2 additionally containing the proteins PIDD1 and RAIDD. The PIDDosome functions as a sensor for supernumerary centrosomes which can occur upon cytokinesis failure or endoreduplication. In such polyploid cells, the PIDDosome activates Caspase-2, starting a signaling cascade which triggers cell cycle arrest via cleavage of Mdm2, p53 stabilization and p21 induction. Failed cell division and centrosome amplification promote genomic instability which is considered to be a hallmark of cancer. In the liver, however, polyploidization of hepatocytes is part of normal organogenesis devoid of malignant transformation.

Previously, we could show that the PIDDosome has a key role in regulation of hepatocyte polyploidization in vivo during liver development and regeneration. To investigate the consequences of PIDDosome deficiency on tumor development we used a carcinogen-driven hepatocellular carcinoma mouse model. Surprisingly, we found that PIDDosome knockout mice exhibit significantly fewer tumors than wildtype mice. As the total tumor burden was similar in all genotypes, histopathological analysis will be performed to assess potential differences in differentiation stage and proliferative index of the tumors. Analysis of the ploidy state of tumor cells will show whether this effect is directly due to PIDDosome loss or if the effect is secondary to increased hepatocyte ploidy, regardless of its cause. Moreover, samples of human HCC patients will be analyzed with regards to the ploidy state and activation of the PIDDosome pathway to reveal the relevance of the centrosome-PIDDosome axis in human tumorigenesis.
Wednesday 19th: Poster Session 2: Biomaterials in surgery

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 2: Biomaterials in surgery
Establishment of an in vitro phosphate buffer induced calcification model

Pia Hager¹, Sabrina Rohringer¹, Helga Bergmeister², Bruno K. Podesser¹, Barbara Kapeller¹, Karl H. Schneider²

¹ Department of Biomedical Research, Medical University of Vienna, Austria
² Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna

Introduction
Calcium mineral deposition (calcification) in tissues is a multifactorial process that frequently accompanies atherosclerosis or valvular heart disease. Patients suffering from hyperphosphatemia or chronic inflammatory diseases such as rheumatoid arthritis are facing an increased risk to develop symptoms of vascular calcification due to permanent administration of corticoid drugs. We developed a calcification model to mimic and compare several pathological risk factors in vitro. Cardiovascular tissue specific cells can be stimulated by elevated levels of inorganic or organic phosphates, corticoids and ascorbic acid to trigger cell calcification.

Materials and Methods
Endothelial cells (EC) and fibroblasts (FB) were seeded in 24 well plates and stimulated with calcification media (CM). The calcification media (CM1 and CM2) consisted of regular cultivation medium for each cell type (DMEM for FB and Medium 200 for EC) supplemented with 2 mM Phosphate (CM1) or 10 mM β-Glycerophosphate, 50 mg/ml Ascorbic acid and 10 nM Dexamethasone (CM2). Cells cultivated with the regular cultivation medium were used as control. Culture plates were incubated for 6, 24, 48, 72, and 96 hours before histological staining was performed to determine proliferation rates and calcium deposition.

Results
Both cell types showed a change in proliferation rates when incubated in the calcification media. CM1 induced significant increase and CM2 significantly decreased the proliferation rate of both cell types during the first 96 hours of incubation. Furthermore, CM2 was more adequate to induce calcification in both cell types compared to CM1 medium. Each experiment was performed three times for both cell types and showed little divergence within each group.

Conclusion
We established a simple and reproducible in vitro test for investigating the influence of different proteins or drugs on cell types involved in calcium deposition. Creating high technical repetitive accuracy within our model was an important factor to enable future calcification studies.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 2: Varia
PS2:V-01 Tackling irreproducibility of research by harmonized standardization of pre-analytical processes within the Austrian Biobank network BBMRI.at

Helmuth Haslacher¹, Michaela Bayer¹, Heidelinde Fiegl², Marlene Gerner³, Philipp Hofer³, Melanie Korb⁴, Sabrina Neururer⁵, Katharina Plattner¹, Eberhard Steiner⁶, Cornelia Stumptner⁷, Monika Wieser⁴, Andrea Wutte⁵, Kurt Zatloukal⁷, Georg Goebel⁵

¹ Biobank Graz, Medical University of Graz, Graz, Austria
² Department of Obstetrics & Gynaecology, Medical University of Innsbruck, Innsbruck, Austria
³ MedUni Wien Biobank, Department of Pathology, Medical University of Vienna, Vienna, Austria
⁴ VetCore Facility for Research, University of Veterinary Medicine Vienna, Vienna, Austria
⁵ Department of Medical Statistics, Informatics and Health Economics, Medical University of Innsbruck, Innsbruck, Austria
⁶ Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Innsbruck, Austria
⁷ Institute of Pathology, Medical University of Graz, Graz, Austria
⁸ BBMRI-ERIC, Graz, Austria
⁹ Department of Laboratory Medicine, Medical University of Vienna, Austria

Background
Irreproducible biomedical research exposes humans and animals to reducible risks and, at the same time, causes enormous waste of research funding worldwide [1, 2]. Irreproducibility is often caused by low quality biomaterial [1]. Hence, standardization of material processing procedures is highly recommended [3]. The Austrian biobank network BBMRI.at aimed to harmonize its preanalytical processes on the basis of international standards.

Methods
BBMRI.at comprises of all Austrian Medical Universities, the Veterinary University of Vienna, the Universities Klagenfurt and Vienna, and the Paracelsus Medical University Salzburg. Its workpackages on “sample management” (WPII) and “quality management” (WPIII) coordinated the harmonization of quality management on the basis of ISO 9001:2008/2015 and of pre-analytical processes on the basis of Technical Specifications (TS) on the pre-examination phase published by the CEN/TC 140. Compliance with ISO 9001 was mutually assessed in the framework of biobank-specific cross-audits. Adherence to CEN/TS was assessed with an online self-assessment tool developed by WPI together with the European Biobanking infrastructure BBMRI-ERIC [4].

Results
Five years after project start, six out of seven participating biobanks/biobank sections were included into certified ISO 9001 quality management systems. All participating universities successfully completed the evaluation of preanalytical SOPs and the first round of intra-consortial cross-audits. According to the results of the CEN/TS self-assessments, BBMRI.at biobanks currently hold about 1.35 million samples that were processed with respect to international specifications.

Conclusion
The Austrian Biobank network has developed a common approach to diminish irreproducibility of scientific results based on biological samples. Harmonizing pre-analytical sample processing between biobanks may reduce individual workload and increase comparability of samples derived from different resources.

Acknowledgements
We would like to thank all supporters of BBMRI.at for their valuable contribution. BBMRI.at is funded by the Austrian Ministry of Education, Science and Research (GZ 10.470/0016-II/3/2013).

References

PS2:V-02 Three-dimensional localization microscopy using deep learning

Alexander Jesacher¹, Philipp Zelger², Klemens Kaser³, Benedikt Rossoboth³, Lukas Velas³, Gerhard Schütz²

¹ Division for Biomedical Physics, Medical University of Innsbruck, Müllerstraße 44, 6020 Innsbruck, Austria
² Institute of Applied Physics, Vienna University of Technology, Getreidemarkt 9, 1060 Vienna, Austria
³ Biomedical Physics, Medical University of Innsbruck, Austria

To access the nanometer regime of axial localization in biological samples we use Supercritical Angle Fluorescence (SAF) microscopy [1]. This enables the accurate axial localization of light emitters in close vicinity to a coverslip. The underlying physical concept is that the near field couples into the glass and turns into waves which propagate at high angles. The power contained in the SAF light strongly depends on the emitter’s distance to the glass. The shape of the resulting point spread function depends on the SAF fraction. We apply techniques of Maximum likelihood estimation and neural networks to estimate the axial positions a fluorophores from a single image.

PS2:V-03 Cigarette smoke-induced shift of extracellular matrix synthesis by vocal fold fibroblasts – a possible mechanism in the pathogenesis of Reinke’s Edema?

Michael Karbiener1, Barbara Darnhofer2, Tanja Grossmann1, Ruth Birner-Gruenberger2, Markus Gugatschka1

1 Medical University Graz, Austria
2 Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria / Omics Center Graz, BioTechMed-Graz, Graz, Austria / Austrian Center of Industrial Biotechnology, Graz, Austria

Voice production is based on proper vibration of the vocal folds, which is in turn dependent on the physiological constitution of vocal fold (VF) epithelium and underlying connective tissue (the VF lamina propria). Reinke’s edema (RE) is a benign lesion of the VFs characterized by an edema of the most superficial layer of the VF lamina propria. The resultant VF swelling is accompanied by a significant deepening of the voice, hoarseness, and can also become obstructive in the most severe setting. Current treatments are only symptomatic (e.g. microsurgery), with the true pathophysiology of RE remaining to be elucidated.

Because RE is diagnosed almost exclusively in smokers, we aim to characterize the effects of cigarette smoke on the behavior of cells residing in the VF. In a first project, we analyzed the global effects of cigarette smoke extract (CSE) on human vocal fold fibroblasts (hVFF) by label-free quantitative proteomics. At non-toxic concentrations, CSE evoked a significant upregulation of pathways linked to metabolism of xenobiotics and reactive oxygen species (ROS), while gene ontology (GO) terms linked to DNA replication/ proliferation were significantly downregulated. Further, 3 proteins that give rise to the main fibrillar collagens of the VF lamina propria – COL1A1, COL1A2, COL3A1 – were significantly downregulated in hVFF exposed to CSE. On the contrary, protein levels of UDP-glucose 6-dehydrogenase (UDGH), which generates a molecular building block (UDP-glucuronate) for glycosaminoglycan (GAG) synthesis, was significantly induced by CSE. As hyaluronic acid (HA) is one of the most prominent GAGs in the VF mucosa, we analyzed further components in the HA biosynthesis pathway. Indeed, mRNA levels of hyaluronan synthases 2 and 3 (HAS2, HAS3) were significantly upregulated in hVFF exposed to CSE. In line with this, CSE-exposed hVFF exhibited a significantly increased biosynthesis of hyaluronic acid.

Collectively, this is the first description of a cigarette smoke-induced shift in synthesis of ECM components by hVFF. As for RE, the reduced production of fibrillar collagens and the concomitant increase in hyaluronan secretion by hVFF might be a pathogenetic driver of this disease. For instance, changed biomechanical properties of the vocal folds might increase the stress for resident capillary endothelial cells and/or impact on angiogenesis. Future studies employing hVFF from RE patients and endothelial cells will test this hypothesis.

PS2:V-04 Relationship between Pro-Fibrotic Signaling and Vitamin D3 in Human Vocal Fold Fibroblasts

Tanja Grossmann, Michael Karbiener, Markus Gugatschka

Division of Phoniatrics, Medical University Graz, Austria

Introduction: The hormonally active form of vitamin D3 (1,25-dihydroxyvitamin D3; 1,25(OH)2D3) holds therapeutic promise as a possible anti-fibrotic agent in various tissues, yet has not been tested for effects on vocal folds (VF). In the proposed project we aimed to investigate the relationship between pro-fibrotic signaling, the vitamin D receptor (VDR) and its natural ligand vitamin D3 in human VF fibroblasts (hVFF).

Material and methods: Immortalized hVFF (i-hVFF) and near primary hVFF (np-hVFF) were used as in vitro fibrogenesis models. Vitamin D3 was exogeneous administered at physiological (1 nM) and pharmacological (100 nM) concentrations, and cells were stimulated with Transforming Growth Factor beta-1 (TGF-β1). Additionally, silencing of VDR was performed using small interfering RNAs. Experiments were analyzed at protein and mRNA levels.

Results: We could identify a significant up-regulation of VDR at mRNA and protein levels in both types of used hVFFs due to the pro-fibrotic cytokine TGF-β1. Vitamin D3 administration at pharmacological concentrations further elevated the TGF-β1-induced expression of the myofibroblast marker α smooth muscle actin (α-SMA) which was found to be reduced upon siRNA-mediated knockdown of VDR also in both types of hVFFs.

Conclusion: In contrast to other studies using fibroblasts from other tissues, our finding of a synergistic interaction of TGF-β1 and vitamin D3 on hVFF transdifferentiation into pro-fibrotic myofibroblasts suggest vitamin D3 to act as positive regulator of fibrotic gene expression in hVFFs. However, the dependency of TGF-β1-induced myofibroblast differentiation on VDR could, potentially open a possibility of treatment to prevent or reverse vocal fold scars.
**PS2-V-05  Construction of a phono-mimetic bioreactor for the study of vocal fold biology**

Andrijana Kirsch¹, David Hortobagyi¹, Claus Gerstenberger¹, Theresa Stachl², Simone Neugebauer¹, Michael Karbiener¹, Markus Gugatschka¹

¹ Division of Phoniatrics, ENT University Hospital, Medizinische Universität Graz, Austria  
² Graz University of Technology

The human vocal folds are a part of the larynx that enable humans to produce speech sounds (phonation). Unlike in other tissues in the human body, vibration occurs naturally in the vocal folds at frequencies of 100-1000 Hz. The vocal folds consist of three layers: epithelium, lamina propria and muscle. The lamina propria structure and viscosity is crucial as it influences vocal fold vibration and thereby phonation. It is difficult to obtain healthy vocal fold tissue for molecular studies as injury to the vocal folds causes scarring which can have a life-long detrimental impact on voice quality. Although ex vivo cultivation of vocal fold cells is possible, the static nature of classical in vitro cell culture lacks the biophysical microenvironment of the native tissue.

We therefore developed a phono-mimetic bioreactor which mimics the vibration of vocal folds in vivo. It consists of a 6-well cell culture plate with a flexible silicone bottom on top of a loudspeaker in a custom designed housing. The vibration is generated through an audio amplifier and a mobile audio player. The audio output voltage was used as a measure of vibration intensity. Human vocal fold fibroblasts were plated onto a 6-well plate with a flexible bottom and allowed to attach for 24 hours, after which they were transferred to the bioreactor for 48h with 8h vibration (frequency range from 50-250 Hz) and 16h static to resemble the average human phonation pattern. After 48h, the cells were harvested for RNA and protein analyses and compared to static control cells. The vibration conditions altered the expression levels of proteins forming the extracellular matrix. In particular, there was an elevation in hyaluronan synthase 2 (HAS2) mRNA expression, indicating an increased production of hyaluronic acid that plays a key role in vocal fold lamina propria viscosity.

Further research should be done to test if other, easily obtainable, fibroblast types could be modulated to obtain a vocal fold fibroblast-like phenotype. This would be a step towards vocal fold tissue engineering for patients with impaired phonation due to vocal fold injury.
Biopharmaceutical technologies

Chairs: Georg Klima & Klaus Graumann
BT-01  Smart biopharmaceutical manufacturing enables global access of meaningful therapies
Roman Necina
Process Science & Technical Services, Shire, Austria

New product development is time and resource consuming and risky. Todays biopharmaceutical processes are based on platform approaches, standardized unit operations and single-use technology. While this approach is increasing process robustness and accelerating availability of clinical material patient requirements are still not met and affordability of meaningful therapies is still a hurdle for global launches. Significant simplification of biopharmaceutical manufacturing processes and increase of productivity are needed to significantly decrease cost of goods and to further accelerate product development.

BT-02  3D Cellular Spheroid Age as critical Parameter for Drug Toxicity Screenings
Christoph Eilenberger¹, Mario Rothbauer¹, Eva Ehmoser², Peter Ertl¹, Seta Küpcü²
¹ Technical University Vienna, Austria
² Institute of Synthetic Bioarchitectures, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Vienna, Muthgasse 11, 1190 Vienna, Austria

The enhanced predictive power of 3D multi-cellular spheroids in comparison to conventional monolayer cultures makes them a promising drug screening tool. However, clinical translation for pharmacology and toxicology is lagging its technological progression. Even though spheroids show a biological complexity resembling native tissue, standardization and validation of drug screening protocols are influenced by continuously changing physiological parameters during spheroid formation. Such cellular heterogeneities impede the comparability of drug efficacy studies and toxicological screenings. In this paper we demonstrated that aside from already well-established physiological parameters, spheroidal age is an additional critical parameter that impacts drug diffusivity and toxicity in 3D cell culture models. HepG2 spheroids were generated and maintained on a self-assembled ultra-low attachment nanobiointerface and characterized regarding time-dependent changes in morphology, functionality as well as anti-cancer drug resistance. We demonstrated that spheroidal aging directly influences drug response due the evolution of spheroid micro-structure and organo-typic functions, that alter inward diffusion, thus drug uptake.
Wednesday 19th: Biopharmaceutical technologies

**BT-03  Design of experiments in the PAT/QbD concept - a comparative study of various design spaces for *Escherichia coli* fed-batch processes**

**Benjamin Bayer**, Moritz von Stosch, Markus Luchner, Mark Dürkop, Gerald Striedner

1 CEAM - Newcastle University, United Kingdom
2 Department of Biotechnology, University of Natural Resources and Life Sciences, Austria

The data generation for meaningful statistical Design of Experiments (DoE) is often time-consuming. However, DoE possesses a key role in the quality-by-design (QbD) implementation for advanced process understanding and control in the context of the US Food and Drug Administration’s PAT/QbD guide (2004) to release new biotherapeutics. Besides, improved quality control also the time-to-market release of new drugs is of crucial interest. Reducing this factor while maintaining the required product quality levels is of high interest for manufacturers. Therefore, different DoE approaches were investigated and their respective explanatory power was compared to determine, if a reduced design space may lead to similar results in a shortened period of time. The comparison of the used DoEs was performed using chemometric black box modeling of the process data and on-line fluorescence signals for the off-line analytes (biomass, product titer and plasmid copy number).

For this study, three varying parameters each with three levels, namely growth rate, induction strength and cultivation temperature, were chosen to set up the design space. For this purpose, an *Escherichia coli* fed-batch cultivation in the 20 L scale expressing recombinant human Cu/Zn superoxide dismutase (SOD) was used.

One full factorial, two fractional factorial and a Box-Behnken design were performed. To investigate the significance of the chosen DoEs different regression models were used. Further, as decision criteria for the DoE of choice the respective 5-fold (5xCV), the Leave-One-Fermentation out (LoFo-CV) cross-validation as well as the test set error were used. Thereby, it has been shown that an evident difference is visible between the number of experiments needed to get meaningful results for the various black box models as well as for the chosen DoE itself. Concluding, in order to accelerate the time-to-market release of new biopharmaceuticals it should also be taken care of choosing a compatible regression model to the selected DoE.

Moreover, the application of a hybrid model as well as intensified experiments (Von Stosch & Willis, 2016) would add in to the findings presented in this study reducing time-to-market even further, while still maintaining required quality levels.

**BT-04  Integrated process development for microbial expressed proteins**

**Cornelia Walther**, Simon Haidinger, Dario Boras, Astrid Dürauer, Cécile Brocard

1 University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria
2 Process Science Austria, Boehringer Ingelheim RCV, Austria

*E. coli* is a well-established host for low cost and high yield production of non-glycosylated proteins. Process development and troubleshooting activities for upstream processes, regularly carried out in form of DoE, are typically based on evaluation of product titers only. Yet, high titer does not necessarily guarantee for successful downstream development, crucial to evaluate the overall productivity of the production process.

To lever the complexity of manufacturing development for non-platform biotherapeutics, we have developed a holistic approach based on a high throughput development (HTPD) platform. Integration of the whole process chain including fermentation, purification and analytics allows for examination of interdependencies between upstream and downstream development. In our setup automated screening and optimization unit operations can either be applied as standalone module or in combination as miniaturized process chain. Here we present two case studies showing 1) the application of our HTPD platform during optimization of a fermentation process and 2) its relevance for troubleshooting activities to study, for instance, depletion of a product-related impurity. In both cases, the influence of upstream conditions on the whole purification process could be appreciated very rapidly (within 7 days) and with low amount of material (2g biomass per upstream condition).
BT-05 Understanding the structure-function relationship between disulfide bridging and potency in etanercept

Sergey Gusenkov, Robert Mayer, Alfred Rupprechter, Michael Fuchs, Fabian Higel, Wolfgang Mueller, William Lamanna, Johann Holzmann

Physico-Chemical Characterization, Novartis, Austria

Erelzi (GP2015) – an approved biosimilar product to the reference Enbrel® – consists of 934 amino acids with total 29 disulfide bridges and comprises a homodimer of the extracellular ligand-binding portion of human TNF-alpha linked to the Fc portion of an human IgG1.

The GP2015 manufacturing process was designed to deliver a product with a bioactivity well within the overall distribution of Enbrel® batches. As all Enbrel® batches were sourced from markets and represent released products, the measured overall distribution in bioactivity defined the range of acceptable bioactivity for the biosimilar product GP2015. Notably, recent batches of Enbrel® have showed a reduced bioactivity in the lower half of the overall distribution, which required the understanding of the structure-function relationship and, therefore, triggered further investigation. Considering that wrongly bridged variants are able to rearrange under physiological redox conditions and the fact that their impact on bioactivity can be described by a linear correlation, the model for the in vivo potency computation was created, allowing fulfillment of the equivalence testing requirements. Thus, elucidation of the structure and its effect on bioactivity was crucial for the approval of GP2015 as first biosimilar to Enbrel® in the US.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Cancer metabolism, autophagy and cell death

Chairs: Mohamed Elgendy & Andreas Villunger
CM-01 Multiomics and data-driven inverse modelling in molecular medicine

Wolfram Weckwerth

Ecogenomics and Systems Biology, university of Vienna, Austria

Genome sequencing and systems biology are revolutionizing life sciences. In the last decade transcriptomics and RNAseq techniques revealed that dynamics of mRNA represent only a small part of a complex regulatory biochemical network which is yet unpredictable from genome sequences. Consequently, proteomics and metabolomics emerge as cornerstone technologies for gene function analysis and genome-scale reconstruction of dynamic metabolic networks. Here, an integrated proteomics/phosphoproteomics/metabolomics platform suited for functional genomics and systems biology is presented. This platform serves also as the basis for a recently established Vienna Metabolomics Center (VIME) (https://metabolomics.univie.ac.at/). A convenient workflow for data processing, integration and mining will be presented. This strategy is based on the data mining toolbox COVAIN (COVAriance Inverse) for data integration, multivariate statistical analysis, machine learning and genome-scale metabolic modelling. A novel algorithm for data-driven inverse calculation of biochemical regulation from high throughput metabolomics data implemented in COVAIN will be presented. We applied this algorithm to the analysis of mTOR-dependent immune system modulation. Activation of immune cells is accompanied by a metabolic reconfiguration of their cellular energy metabolism including shifts in glycolysis and mitochondrial respiration that critically regulate functional effector responses. However, while current mass spectrometry strategies identify overall or flux-dependent metabolite profiles of cells or tissues, they fail to comprehensively identify the checkpoint nodes and enzymes that are responsible for different metabolic outputs. Here, we demonstrate that a data-driven inverse modelling approach from mass spectrometry metabolomics data can be used to identify causal biochemical nodes that influence overall metabolic profiles and reactions. Using multiomics metabolomics, proteomics, phosphoproteomics, transcriptomics analysis as well as enzymatic activity measurements we identified metabolic signatures of energy signaling and macrophage differentiation. The presented concept of data-driven inverse modelling and multiomics analysis allows for the systematic integration of genome-scale metabolic reconstruction, prediction and analysis of causal biochemical regulation.

CM-02 Ketogenic diet as an adjuvant therapy for solid tumors

Sepideh Aminzadeh Gohari1, Daniela Weber1, Silvia Vidalí1, David Licha2, René Günther Feichtinger1, Peter Kölblinger3, Johannes Adalbert Mayr4, Wolfgang Sperl4, Christian Huber2, Roland Lang3, Barbara Kofler1

1 Pediatrics, Paracelsus Medical University, Austria
2 Bioanalytical Research Laboratories, University of Salzburg, Salzburg, Austria.
3 Department of Dermatology, Paracelsus Medical University, Salzburg, Austria
4 Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria

The ketogenic diet (KD) - a high fat/low carbohydrate diet targets the Warburg effect, a biochemical phenomenon in which cancer cells predominantly utilize glycolysis instead of oxidative phosphorylation (OXPHOS) for ATP production. Since some types of cancer have low OXPHOS activity and lack the ability to metabolize ketone bodies, the rationale in providing a KD as adjuvant cancer therapy is to reduce circulating glucose, and consequently insulin levels, and to induce ketosis. Thereby, cancer cells will be starved of energy while normal cells will survive. It has been shown that neuroblastoma (NB) and other tumors with high glycolytic activity can be targeted by KD. The aim of the present study was to determine the effect of KD on melanoma (MEL), breast cancer (BC) and NB with different genetic alterations. Furthermore, we aimed to gain some mechanistic insight in the action of KD therapy.

We established xenografts with NB (SH-SY5Y, SKNBE(2)); BC (MDA-MB-468); MEL (WM3311, WM47) in CD-1 nu/nu mice. The NB, BC and MEL-bearing mice were fed with standard diet (SD) and KDs. KD therapy in NB and BC was combined with a low dose of chemotherapy. To investigate whether the level of protein content in the diet can influence the anti-tumor effect of KD, a diet matching the low protein content of the KD (SDLP) and a diet containing essential amino acids only (no protein) (SDEA) were used.

The KDs significantly decelerated the growth of NB, BC and MEL and thereby increased survival in KD-treated animals compared to SD-fed animals. As expected, KDs increased the level of ketosis in all tumor-bearing mice, whereas blood glucose levels were only partially reduced. We have previously shown that KDs can be deleterious in the treatment of renal cell carcinoma by provoking a raise of interleukin-6 and C-reactive protein expression in the liver. However, this was not the case for NB, BC and MEL bearing xenografts. The reduction of dietary protein to 8% in SDLP did not affect growth of NB xenografts, whereas SDEA caused a significant growth reduction of NB xenografts. Plasma amino acid levels of certain non-essential amino acids, like glutamine and serine were elevated in the SDEA-fed mice. Metabolomic analysis revealed that KDs induced an upregulation of the tricarboxylic acid cycle and pentose phosphate pathway metabolites in the plasma of NB-bearing mice and tumor.

Our studies indicate that KDs could be considered as part of multimodal therapy of NB, BC and MEL.
CM-03  Effects of dietary polyamine supplementation on mitochondria and the aging brain.

Sebastian Hofer1, Sabrina Schröder1, Christopher Dammbrück1, Mark Marcello2, Viktoria Pogatschnigg3, Ronald Mek1, Andreas Zimmermann1, Tobias Pendl1, Muammer Ücal1, Ute Schäfer1, Stephan Sigrist4, Bence Racz2, Tobias Eisenberg2, Frank Madeo1

1 Institute of Molecular Biosciences, University of Graz, Austria
2 Department of Neurosurgery, Medical University of Graz, Graz, Austria
3 Institute of Biology/Genetics, Freie Universität Berlin, Berlin, Germany

Background: Life expectancy has been steadily increasing during the last decades, accompanied by accumulating incidence of age-related diseases. Among early hallmarks of brain aging is decreased cognitive performance, with the underlying mechanisms poorly understood. Recent studies have revealed neuroprotective, anti-aging and lifespan-extending effects of autophagy-inducing polyamines, in yeast, flies, worms and mice. Aims: Here we focused on nutritional polyamine supplementation as a protective strategy in hippocampal synaptic plasticity, learning and mitochondrial physiology during aging to pre-clinically evaluate the therapeutic potential against age-associated maladies. Methods: We used mice and flies as model organisms to study nutritional polyamine-supplementation in aging. In addition to standard methods, we also performed electron microscopy, high-resolution respirometry, behavioral tests and biochemical assays. Results: Nutritional polyamine supplementation led to elevated respiratory competence through complex I in aged Drosophila, which was dependent on functional autophagy. In accordance, we found that ATP levels in fly heads and mitochondrial abundance in presynaptic boutons were increased. Furthermore, the treatment restored mitochondrial abundance in the hippocampal CA1 region of aging mice. We speculate that these improvements are interconnected with changes in synaptic plasticity: perforated post-synaptic densities (PSD) and multi-synaptic boutons (MSB) were positively influenced upon polyamine supplementation. Furthermore, aged treated mice performed superior in a spatial and temporal learning test, indicating that the observed changes in synaptic plasticity are reflected in an improvement of cognitive capacity. Conclusions: Taken together, our data suggest that increased nutritional polyamine uptake might be therapeutically employed in treating neurological disorders with underlying mitochondrial defects.

CM-04  Key cell regulation systems: cell signaling, lipid metabolism and epigenetics - how do they cooperate in cancer?

Thomas W. Grunt

Signaling Networks Program, Division of Oncology, Department of Medicine I & Comprehensive Cancer Center, Medical University of Vienna & Ludwig Boltzmann Cluster Oncology, Austria

Development of resistance to anti-cancer drugs is caused by the high degree of regulatory plasticity in malignant cells. This plasticity is supported by the intricate architecture of networks that consist of crucial pathways, including mitogenic signaling, metabolic homeostasis, and epigenetic control of gene expression. Important functional interaction has been identified between growth signaling and lipid metabolism, growth signaling and epigenetics as well as between lipid metabolism and epigenetics. Elucidation of the molecular mechanisms of this relationship is required for understanding the promotive function of metabolism and its disorders (e.g. hyperphagia, diabetes, metabolic syndrome) for development of cancer and progression to resistant disease. Cancer-specific perturbations of signaling, metabolism and epigenetics can be cause and/or consequence of malignant transformation. Evidence indicates that these regulatory systems interact with each other to form highly flexible and robust cybernetic networks that promote malignant growth and confer treatment resistance (1). Deciphering these plexuses using holistic approaches known from systems biology can be very instructive for the future design of novel anticancer strategies. Here I will discuss novel findings elucidating the multiple molecular interdependence between cancer-specific signaling, cell metabolism and epigenetics in order to provide an insightful understanding how major cancer machineries interact with each other during cancer development and progression, and how this knowledge may be used for future co-targeting strategies. Funding from the Medical Scientific Fund of the Mayor of the City of Vienna, from the ‘Initiative Krebsforschung’ of the Medical University of Vienna, and from the Herzfelder Familienstiftung, Vienna, Austria, is highly acknowledged. (1) Grunt TW. Trends Endocrinol Metab. 2018;29:86-98.
CM-05  The PIDDosome in liver development, regeneration and tumorigenesis

Valentina Sladky¹, Katja Knapp³, Elsbeth van Liere², Simon Schultz³, Michael Trauner³, Tatjana Stojakovic⁴, Hubert Scharnagl⁴, Alain de Bruin², Luca Fava⁵, Andreas Villunger¹

¹ Developmental Immunology, Medical University of Innsbruck, Austria
² Pathobiology, Utrecht University, NL
³ Hans Popper Laboratory, Medical University Vienna
⁴ Universitätsklinikum Graz
⁵ CIBIO, Università degli Studi di Trento, I

Caspase-2 and its activation platform, a protein complex dubbed the “PIDDosome” consisting of PIDD1 and the adapter protein RAIDD, have been implicated in several physiological processes. Yet, its involvement in cellular processes such as metabolism or the DNA damage responses is still controversial. Recently, we were able to demonstrate that the PIDDosome acts as sensor for supernumerary centrosomes which results in caspase-2 activation and p53-mediated cell cycle arrest. Overamplification of centrosomes can be a result of failed cytokinesis or endoreduplication leading to polyploidization which is associated with genomic instability and tumorigenesis. However, cytokinesis failure also occurs during normal organogenesis in several mammalian tissues such as the liver. Although the physiological role of hepatocyte polyploidization is not fully understood, balanced polyploidization appears to be crucial for liver integrity. We could show that hepatocytes from PIDDosome-deficient mice displayed a significant ploidy increase compared to wild type animals. Since hepatocytes are prone to fail cytokinesis in periods of extensive liver growth, which occurs both postnatally and during tissue regeneration, we focused on proliferating hepatocytes during normal development as well as after partial hepatectomy. We found that caspase-2 is specifically upregulated in proliferating hepatocytes during both liver development and liver regeneration. Caspase-2 levels are controlled by transcription factors crucial for cell cycle regulation and hepatocyte polyploidization. When hepatocytes are proliferating, the PIDDosome acts as brake: PIDDosome-dependent caspase-2 activation restricts polyploidization via p53. Our results clearly demonstrate for the first time that caspase-2 and the PIDDosome are part of the network regulating hepatocyte ploidy and play the key role in preventing excessive polyploidization. Moreover, using a carcinogen-driven mouse model for hepatocellular carcinoma (HCC), we investigated the consequences of PIDDosome loss on tumorigenesis and found that the centrosome-PIDDosome-p53 axis significantly affects the tumor burden and HCC development.

CM-06  Potency of different cell death modalities to induce an anti-tumor response

Peter Vandenabeele

VIB-UGent Center for Inflammation Research, UGent-VIB, Belgium

Immunogenic cell death (ICD) induces an antitumor response upon vaccination. This effect has been mainly attributed to the expression at the cell surface and the release of damage associated molecular patterns (DAMPs), and the production of chemokines and cytokines. The concept of immunogenic cell death has been mainly studied in the context of mitoxantrone induced apoptosis. In order to compare how different cell death modalities are able to elicit an anti-tumor response following vaccination, we developed inducible models of apoptosis, necroptosis and ferroptosis. Apoptosis is associated with caspase activation, necroptosis with RIPK3-dependent MLKL phosphorylation and ferroptosis with lipid peroxidation at the membrane. These essentially different mechanism of execution of cell death may affect the eventual outcome of immunogenicity. The three cell death modalities were compared on their capacity to protect upon vaccination against a non-orthotopic challenge of tumor cells and to develop an antigen-specific CTL response. While apoptosis was mainly driven by a dominant antigen epitope, necroptosis engaged a broader antigen spectrum. The efficacy of the necroptotic model was confirmed in vivo by treatment of a primary tumor with mRNA coding for MLKL and protected against distal and disseminated tumor formation in syngeneic mouse melanoma and colon carcinoma models. Although ferroptosis was a very efficient way to kill tumor cells, it was not able to build up an antitumor response. The mechanisms of this inability are under further investigation.
Wednesday 19th: Biomaterials in surgery

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Biomaterials in surgery

Chairs: Konstantin D Bergmeister & Bruno K. Podesser
BS-01  Tissue engineering of heart valves: a critical appraisal

Peter Zilla

Christiaan Barnard Dept. for Cardiothoracic Surgery, Univ. of Cape Town, South Africa

Fifty years after the groups in Huston and Seattle for the first time pursued the concept of autologous tissue generation in cardiovascular prostheses and 30 years after the term ‘tissue engineering’ was coined, the translation into routine clinical practice has not emerged beyond the limited field of skin burns. This is even more disappointing in view of the fact that all three basic approaches were already well described half a century ago: the use of cultured cells; facilitated tissue regeneration and the fall-out healing of circulating cells.

It also shows that the subsequent attempt to present a chronology of earlier ‘in vitro’ (Tissue Engineering) and later ‘in vivo’ (Tissue Regeneration) approaches ignores the coexistence of these avenues from the very beginning.

The excellent long-term results of autologous endothelial lining in >400 vascular patients showed the benefit of the end-goal. Yet, the desire to prematurely simplify; the short-term involvement of individual scientists; the lack of clustered research; the convenient misinterpretation of animal models and the unawareness of previous research (eg. decellularization approaches oblivious to the acellularity of long-term implanted allografts) have stalled the break-through to widely applicable solutions.

Without coordinated long term strategies we may lose another half-century before we see tissue engineered/regenerated heart valves in routine clinical use.

BS-02  A nanobiotechnology-advanced lab-on-a-chip for blood cell immobilization and phenotyping

Mario Rothbauer¹, Martin Frauenlob², Karoline Gutkas², Michael B. Fischer³, Eva-Kathrin Ehmoser², Seta Küpcü², Peter Ertl¹

¹ Institute of applied Synthetic Chemistry, Vienna University of Technology, Austria
² University of Natural Resources and Life Sciences, Department of Nanobiotechnology, Institute for Synthetic Bioarchitectures, Muthgasse 11, 1190 Vienna, Austria
³ Department of Life Science and Biomedicine, Danube University Krems, Dr. Karl Dorrekstrasse 30, 3500 Krems, Austria

Identification, isolation, separation as well as analysis of subpopulations of cells from complex cellular samples such as blood is crucial for early detection of human diseases. For instance, iterations of cell phenotypes including surface receptor expression, cell size and frequency are of known diagnostic value. A major challenge in this field lies in reproducible immobilization of antibodies thus cell capture within biochips. In particular, poor antibody binding and site-oriented antibody binding is a well-known issue for state-of-the-art plastic surfaces commonly used for industrial mass production.

Here, we present a microfluidic chip with a selective nanobiointerface based on self-assembled S-layer proteins for on-chip cell capture and in situ cell phenotyping,
**BS-03  Nanofibrous, bioabsorbable polycarbonate urethane for small diameter vessel replacement**

Magdalena Eilenberg1, Marjan Enayati1, Daniel Ehebruster1, Christian Grasi2, Ingrid Walter3, Barbara Messner4, Stefan Baudis4, Paul Potzmann5, Christoph Kaun6, Bruno K. Podesser1, Johann Wojta5, Helga Bergmeister1

1 Center for Biomedical Research, Medical University of Vienna, Waehringer Guertel 18-20, A-1090, Vienna, Austria  
2 Centre of Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria  
3 Department of Pathobiology, Veterinary University, Vienna, Austria  
4 Surgical Research Laboratories-Cardiac Surgery, Department of Surgery, Medical University of Vienna, Vienna, Austria  
5 Institute of Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria  
6 Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria

The clinical demand for synthetic small diameter vascular substitutes for aorto-coronary bypass and peripheral vascular surgery could be met with biodegradable materials. Thermoplastic polycarbonate urethane (PCU) exhibits optimal biomechanical properties without secondary inflammation due to its non-acidic degradation by-products. The aim of this study was to evaluate PCU in a short- and long-term follow-up in a rat model.

Methods: The in-vivo performance of electrospun PCU conduits was investigated by an infrarenal abdominal aortic replacement model in Sprague-Dawley rats. Grafts were retrieved after 7 days, 1, 6 and 12 months (n= 28) and compared to non-degradable polytetrafluoroethylene (ePTFE) controls (n = 28). Biomechanical analysis, histology and immunohistochemistry were performed for further evaluation. The in-vitro inflammatory potential was assessed by a rat macrophage-culture.

Results: Overall patency was excellent in PCU- and ePTFE-grafts (96.4% vs. 92.9%, p=0.55), without significant change of inner diameter in both materials. The median wall thickness was not significantly reduced over time, due to PCU’s slow degradation characteristics. In PCU a quicker full endothelialization (p=0.05), an increased and sustained transmural cellular ingrowth (p<0.001) and a reduced inflammatory response (p=0.02) was observed in comparison to ePTFE.

Conclusion: PCU grafts acted as temporary scaffolds for the newly forming artery consisting of a fully covered endothelial layer, a structured smooth muscle cell layer and microvessels. Despite the slow degradation of PCU, an extended foreign body reaction did not occur. PCU conduits seem promising for a safe, long-term approach as small diameter vessel replacement.

**BS-04  TRITON artificial tissue graft for small diameter application in cardiovascular procedures- preliminary results**

Bernhard Winkler

Biomedical Research, KH Hietzing 1. Chir, KH Hietzing, Austria

Background An increasing number of people (up to 30% according to WHO report on cardiovascular diseases 2016) who require cardiac or vascular surgery or even a dialysis shunt cannot be provided with suitable autologous bypass material due to pre-existing diseases or already used bypassmaterial. Existing artificial vascular prostheses have serious limitations.

Materials and Methods Mechanical issues: Implantation of artificial vascular graft with supporting 4.0 and 4.5 mm mesh scaffolds with a length of 150mm and burst strength > 800 mm Hg. All grafts were autoclaved according to hospital and culture.

Implantation: In-vivo porcine models were anaesthetized and intubated. The artificial vascular grafts were implanted off-pump without the use of extracorporeal circulation to the left coronary artery (LAD- left anterior descending) with proximal anastomoses to the ascending aorta. Standard off pump dose of heparin, protamine reversal, standard sternal and wound closure.

Results Coronary angiographies postoperative: Initial postoperative angiography of the artificial graft bypass to the LAD showing patency with distal outflow and excellent results without dissection or no narrowing at the distal anastomoses. Clinical course: All porcine models recovered fully within 24h hours, taking solid food and water 24h after intervention. Despite the inert antithrombogenic scaffold material for the first series aspirin is administered in usual dosage. No bleeding or signs of infection over 4 weeks. Follow-up: Coronary angiography at 4 weeks after implantation revealed patent flow in artificial graft bypass graft without any signs of burst or dissection. The explanation with macroscopic and histological analysis was performed 4 weeks after the initial implantation. No signs of burst or dissection and no haematomas were discovered.

Conclusion The provision of artificial grafts, in particular small-diameter artificial grafts, is highly desirable, in order to provide means of an optimal therapeutic artificial vascular graft, which can be used for a cardiovascular bypass operation. It is an object of the present project to improve on the mentioned state of the art, in particular to provide safe and efficacious artificial grafts, which could be used instantly after unpacking, without the limitations of the existing artificial grafts.
**BS-05 Innovationen in der Mensch-Maschine Schnittstelle zur Steuerung und Feedback bionischer Prothesen**

**Martin Aman¹, Matthias Sporer², Christopher Festin², Martin Schmoll³, Hermann Lanmüller³, Christian Hofer⁴, Michael Russold⁴, Roman Ruff⁴, Klaus-Peter Hoffmann⁵, Oskar Aszmann²**

¹ CD Laboratory for Extremity Reconstruction, Department of Surgery, Medical University Vienna, Austria
² CD Labor für Wiederherstellung von Extremitätenfunktionen, Univ. Klinik für Chirurgie, Medizinische Universität Wien
³ Zentrum für Medizinische Physik und Biomedizinische Technik, Medizinische Universität Wien
⁴ Otto Bock Healthcare Products GmbH, Wien
⁵ Fraunhofer Institut, St Ingbert, Deutschland

Bionische Prothesen sind ein essentieller Bestandteil der Versorgung von Amputationsverletzten. Entscheidender und limitierender Faktor für eine intuitive und verlässliche Steuerung ist die Schnittstelle zwischen Mensch und Maschine - das Interface. Eine wichtige Rolle derzeitiger Entwicklungen spielt hierbei die Möglichkeit mittels neuraler Stimulation sensorisches Feedback zu erzeugen, sowie eine optimierte und intuitivere Steuerung bionischer Prothesen zu ermöglichen.

**Fragestellung:**
Ziel dieser Arbeit war es, im Ratten- und Kaninchenmodell verschiedene Nerven und Muskel Schnittstellen auf Biokompatibilität und technische Fragestellungen zu testen um ihre Anwendung in der prothetischen Versorgung zu evaluieren.

**Methodik:**
Im Rattenmodell wurden zuerst einerseits longitudinale, intraneurale Elektroden sowie Cuff-Elektroden über verschiedene Zeiträume getestet und deren Auswirkungen auf Nerven und Muskelarchitektur evaluiert. Des Weiteren wurde ein implantierbares Vollsystem, das Elektroden zur simultanen Steuerung und zum sensorischen Feedback umfasst, im Kaninchenmodell evaluiert und auf Durchführbarkeit, Biokompatibilität, Stimulierbarkeit und optimierte Datenübertragung getestet.

**Ergebnisse:**

**Schlussfolgerung:**
Zukunftweisende Forschungsfragen müssen zuerst im Tiermodell beantwortet wie die Kombination aus sensorischem Feedback und intuitiver, motorischer Steuerung in bionische Vollsysteme integriert werden kann, um so langfristig in die klinische Anwendung implementiert werden zu können. Schnittstellen mit dem peripheren Nerven besitzen den Vorteil sowohl efferent als auch afferent genutzt werden zu können. Hierbei ist jedoch immer ein Kompromiss zwischen Selektivität und Invasivität zu finden.

Sowohl die Fibrosierung des Nerven als auch die Herausforderung der Signalübertragung direkt über den Nerv mit sich verändernden Impedanzen, rücken hochauflösende Muskelableitungen zur Steuerung bionischer Prothesen in den Vordergrund.
Thursday 20th September 2018
P5-01  Engineering of the filamentous fungus *Penicillium chrysogenum* as cell factory for natural products

Arnold Driessen
Molecular Microbiology, University of Groningen, Netherlands

*Penicillium chrysogenum* (renamed *P. rubens*) is the most studied member of a family of more than 350 *Penicillium* species that constitute the genus. Since the discovery of penicillin by Alexander Fleming, this filamentous fungus is used as a commercial β-lactam antibiotic producer. For several decades, *P. chrysogenum* was subjected to a classical strain improvement (CSI) program to increase penicillin titers. This resulted in a massive increase in the penicillin production capacity, paralleled by the silencing of several other biosynthetic gene clusters (BGC), causing a reduction in the production of a broad range of BGC encoded natural products (NP). Several approaches have been used to restore the ability of the penicillin production strains to synthetize the NPs lost during the CSI. This allowed for the identification of numerous NPs and the elucidation of the respective pathways. Furthermore, a CSI-optimized strain of *P. chrysogenum* has been converted into a generic platform for NP production by removal of expressed BGC. The versatility of this platform was demonstrated with the production of NPs with potential commercial value.
P6-01  To TRAIL or not to TRAIL in cancer therapy

Henning Walczak
Molecular Biology, UCL Cancer Institute, United Kingdom

We previously showed that TRAIL can induce cell death in cancer cells in vivo, importantly without causing any untoward damage to normal cells. Whilst we are convinced that apoptosis-inducing therapies combining novel, high-activity forms of recombinant TRAIL with potent sensitisers to TRAIL-induced apoptosis will provide significant benefit for cancer patients, we recently discovered a previously unrecognised, pro-tumourigenic function of the endogenous TRAIL–TRAIL receptor system. This talk will portray our latest results on the therapeutic opportunities in cancer treatment that arise from recent gains in the understanding of the mechanisms of resistance to TRAIL-induced apoptosis and how we can overcome this as well as of the tumour biological functions of the TRAIL–TRAIL receptor system and its interplay with the tumour microenvironment.
Thursday 20th: Poster Flash 3

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Flash 3
**PF3-01 Discovery of novel bioactive compounds through fugal and bacterial co-culture.**

Roman Labuda⁰, Martin Gratzi¹, Desislava Yankova¹, Kathrin Rychli⁰, Markus Bacher², Christoph Schüller¹, Jseph Strauss¹, Martin Wagner⁰

---

¹ University of Natural Resources and Life Sciences, Vienna (BOKU) DAGZ, Department of Applied Genetics and Cell Biology
² University of Natural Resources and Life Sciences, Vienna (BOKU) DAGZ, Department of Chemistry
³ Institute of Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna (VetMed), Austria

The majority of drugs used in medicine are small molecules of natural or synthetic origin. One of the strongest drivers for the discovery of bioactive compounds for medicinal use was activity screening of compounds derived from natural sources such as plants and microbes. Taking this approach, nature’s chemical diversity has been exploited successfully over the last decades and brought up the most useful and important medical drugs, such as antibiotics (derived from bacteria and fungi), anti-cancer drugs or anti-inflammatory substances. The annotation of microbial genomes revealed that many more genes are present than metabolites known for each sequenced species. Thus generally, microbes do not activate the genes under laboratory conditions and thus underlying biosynthetic pathways potentially producing novel, so far not yet identified products remain inactive. Only under the very diverse and probably competitive conditions of growth in natural habitats these genes might be expressed and the corresponding products might be produced to serve as defense or signaling compound. The goal of the research facility, Bioactive Microbial Metabolites (BiMM, bimm-research.at) is to identify new active substances from fungi and bacteria. This platform comprises high-throughput equipment and provides know-how and scientific personnel to run high content screens for the identification and characterization of novel bioactive metabolites. To obtain bioactive metabolites a library of fungal and bacterial strains were exposed during combinatorial growth (i.e. fungi and bacteria co-culture) in different growth conditions or in the presence of compounds that interfere with epigenetic regulation of fungal gene clusters. We will report on the successful upscaling, preparation and identification of bioactive and novel substances induced by fungal and bacterial co-culture.

---

**PF3-02 Identification of fungi occurring in bread fermentation baskets**

Johannes Pitsch¹, Nicole Ollinger¹, Stefan Huemer², Julian Weghuber¹

---

¹ Biophysics, Johannes Kepler Universität Linz, Austria
² Fischer Brot

The fermentation of dough is a method used since thousands of years in order to make bread more digestible and to extend its shelf life. As a large diversity of microorganisms ubiquitary occurs in the environment, it is hardly possible to achieve a fermentation driven by a single organism and to avoid the growth of unwanted species of bacteria, yeasts and moulds in fermentation racks and chambers. Due to climatic conditions, mould appears at fermentation racks in bakeries. Producers of large scale devices and bakeries try to solve this well-known problem.

To cope with mould and inhibit its formation, cultures on fermentation racks were isolated and transferred on agar plates. Different kinds of agar had to be tested as separation of cultures proved to be a challenge. Yeast in bakery showed significantly faster growth rates compared to mould. For separation of yeast from other cultures, standard microbiological methods like dilution and dilution streaks were unsuccessful. Subsequent culture transfer between malt-extract-, Rose-Bengal-, plate-count- and Czapek-Dox-Agar was necessary to get rid of yeast and to obtain pure cultures.

Beneath standard microbiological identification methods like microscopy, q-PCR was utilized for fungal DNA identification. Via ITS- (internal transcribed spacer) and β-tubulin-primers, specific regions for fungi were amplified and sequenced afterwards. For testing purposes, SEM (scanning electron microscopy) and CT (computer tomography) methods were tested for their applicability.

These methods in combination proved to complement each other and led to identification of several fungal strains with potential health risks.

Quantification and identification methods for mycotoxins have to be established to assess the actual health risk for employees and consumers. Afterwards, evaluation and development of preventive measures will have to be carried out.
PF3-03  Role of the NLRP3/IL-1β axis in Acute Myeloid Leukemia (AML)
Michela Luciano¹, Lukas Zell², Stephanie Binder³, Jutta Horejs-Hoeck⁴
¹ Biosciences, University of Salzburg, Austria
² lukas.zell@hotmail.com
³ stephanie.binder@sbg.ac.at
⁴ jutta.horejs_hoeck@sbg.ac.at

Background: Acute myeloid leukemia (AML) is a clonal haemopoietic disorder characterized by hyperproliferation and aberrant differentiation of myeloid progenitor cells. This leads to the accumulation of non-functional cells, termed myeloblasts, in the bone marrow and in peripheral blood resulting in impaired haematopoiesis and bone marrow failure. Intensive induction chemotherapy has remained the main treatment for AML patient since decades. Yet, the wide heterogeneity driving AML clearly affects the clinical response to chemotherapy. Therefore, one of the main challenge is the identification of unifying mechanisms, involved in the disease initiation and progression, regardless of the mutational status. It has been shown that enhanced IL-1β signaling is frequently observed in hematological disease, including myeloproliferative neoplasm and chronic and acute myeloid leukemia. Recently, Carey et al. have shown that proliferation of almost all AML patient-samples depends on IL-1β signaling, irrespective of the diverse genetic and molecular abnormalities. Therefore, the aim of this study is to investigate the functional role of IL-1β and the molecular mechanisms promoting its secretion in human AML cell lines (hAML).

Results and methods: We show that IL-1β secretion, detected by ELISA, is significantly higher in hAML cells compared to healthy controls. Moreover, higher IL-1β secretion correlates with enhanced cell proliferation, analyzed by BrdU. Moreover, IL-1β treatment of primary human AML cells induces the release of various inflammatory mediators, as shown by multiplex analyses. Interestingly, blocking of IL-1β signaling by using two different αIL-1β antibodies, results in a concentration-dependent decrease of cell proliferation. Furthermore, qRT-PCR analyses have revealed that an increase of the inflammasome component NLRP3, correlates with enhanced proliferation rates of hAML cell lines. Inhibition of NLRP3 activation by the NLRP3 inhibitor (CP-456773 sodium salt or CRID3) leads to a significant decrease in cell proliferation.

Conclusions: Our study shows that the IL-1β signaling pathway could be involved in promoting the survival and the proliferation of hAML cell lines. Moreover, we have found that activation of the NLRP3 inflammasome could be a key mechanisms leading to IL-1β secretion.

PF3-04  Tumor-associated macrophages of rectal cancer polarize to the proinflammatory M1 phenotype after irradiation in patients and co-cultures

Victoria Stary¹, Daniela Unterleuthner², Brigitte Wolff³, Johanna Strobl³, Andrea Beer⁴, Helmut Dolznig⁵, Michael Bergmann⁶
¹ Department for Surgery, Medical University Vienna, Austria
² Institute of Genetics, Medical University of Vienna, Vienna, Austria
³ Department of Dermatology, Medical University of Vienna, Vienna, Austria
⁴ Clinical Institute of Pathology, Medical University of Vienna, Vienna, Austria

Tumor-associated macrophages initiate anti-tumoral (M1) or immunosuppressive (M2) responses depending on their polarization status.

To test the effects of radiotherapy, we ex vivo irradiated tissue samples of human rectal cancer and assessed the phenotype of macrophages, T cells and NK cells by flow cytometry. We evaluated their distribution after short course radiotherapy (n=45) and compared findings to non-pretreated rectal cancer (n=25) using an immunostaining approach. We further investigated the influence of cancer-associated fibroblasts and cancer cells on the polarization of macrophages after ex vivo irradiation using 3D co-culture models.

Irradiated rectal cancer samples contained less CD68⁺ macrophages (18.2±2 vs 12.6±3%CD68⁺/total leukocytes) with a viability of >92% in both groups. Stainings of markers associated with the M1- (CD64, CCR7, iNos, TNFα, HLA-DR, CD86) or M2-like (CD206, CD163, IL-10, IL-4) phenotype revealed an increase of M1/M2-ratio arguing for a shift from M2- to M1-like macrophages due to irradiation. Macrophages of ex vivo irradiated rectal cancer samples displayed increased phagocytosis. Irradiated tissue sections demonstrated diminished T cell counts (109.7±8.68 vs 45.7±17.26 CD3⁺cells No./mm²) but elevated infiltration of NK cells (50.3±15.51 vs 75.9±9.43 CD56⁺CD3 cells No./mm²). Irradiation of 3D co-culture models led to a dose dependent increase of M1/M2. Untreated macrophages in co-cultures without fibroblasts tended to be less M2 but more M1-like. Neutralizing IL-10 antibody induced M1-like macrophages. Treatment with recombinant IL-10 partly rescued the effects of irradiation.

Our findings highlight macrophages as effector cells upon irradiation by enhancing their anti-tumoral activities and diminishing their immunosuppressive behavior. This study provides a rationale for future investigation aiming for immune-modulation of macrophages to ensure optimal anti-cancer immune-activation.
**PF3-05**  Identification of the functional role of the bacterial effector and oncoprotein CagA expressed by *Helicobacter pylori* in immune cells

Bianca Chichirau  
Biosciences, University of Salzburg, Austria

CagA (cytotoxin associated gene A) is one of the most studied virulence factor of the gastric pathogenic bacterium *Helicobacter pylori* (*H. pylori*). Its expression is strongly connected to the development of gastric diseases and MALT lymphoma. CagA is injected into the cytoplasm of infected epithelial cells via a specialized type IV secretion system, where it is phosphorylated at the tyrosine residues of the EPIYA motifs by Src and c-Abl family kinases. Recent studies have shown that translocation of CagA also occurs in B cells, however the signal transduction pathways regulated by this protein are still not very well defined. Here, we generated various bacterial strains presenting different genomic mutations of *cagA* gene, which we then further used in infection experiments to investigate the role of CagA on B cells proliferation, apoptosis and differentiation. The successful translocation of CagA in immune cells has been validated in short term infections performed with the isogenic *H. pylori* mutant strains. The bacterial adherence to the gastric epithelial cells did not vary significantly amongst the strains, as observed in CFU assays and Alamar Blue experiments. B cell viability was assessed through MTT analyses which revealed that during shorter time points of infection, there is an increase of the proliferation rate in cagA positive infected immune cells as compared to the ones either infected with cagA deletion strain or remaining uninfected. However, long term infections led to a decrease in the cell survival rate of the cells infected with the cagA positive strain. mRNA expression analyses performed with both infected and uninfected samples revealed an up-regulation and down-regulation of several sets of genes involved in the apoptotic pathway, NF-κB signalling cascade or in the activation of GTPase. Further studies such as qRT-PCR, immunofluorescence assays will be conducted for identifying the impact of CagA in B cell regulation. Moreover, the possibility of using these bacterial mutant strains in mouse model infections will be investigated.

**PF3-06**  Functional verification of menaquinone biosynthesis genes of *Lactobacillus plantarum* WCFS1 and *Lactobacillus buchneri* DSM20057 by complementation of the respective defective mutants of *Lactococcus lactis* subsp. *cremoris* N29000

Nisit Watthanasakphuban  
Food Science and Biotechnology, BOKU, Austria

Lactic Acid Bacteria are non-respiring, facultatively anaerobic fermentative bacteria and are typically cultivated under (micro)anaerobic conditions. In several LAB including *Lactococcus lactis* a respiration-like behavior was observed upon addition of heme to the medium, leading to stimulated aerobic growth, reduced acidification, improved growth efficiency and stress resistance. The components of a respiratory chain: electron donor (NADH dehydrogenases), electron shuttle (LAB use menaquinones) and heme-requiring terminal cytochrome oxidase are present in a non-redundant fashion in *Lac. lactis*. Supplementation of both heme and menaquinone led to respiratory behavior in several additional species, indicating an incomplete respiratory chain lacking quinones. The genome of *L. plantarum* WCFS1 contains only *menA* and *menG*, encoding the last two (of eight) pathway enzymes for menaquinone synthesis. The genome of *L. buchneri* DSM 20057 contains *menE*, *menB*, *menA* and *menG*, the genes encoding the first four steps in the pathway are absent. Both genomes contain the genes for the *bd*-type cytochrome oxidase (*CydABCD*). We determined the functionality of the remaining menaquinone pathway genes in *L. plantarum* and *L. buchneri* by functional complementation of respective *L. lactis* deletion strains for reconstituting a complete and functional menaquinone biosynthesis pathway in *L. plantarum* and *L. buchneri*. 
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

*Synthetic biology*

Chairs: Astrid R. Mach-Aigner & Sergey Zotchev
**SB-01  Designable modules in synthetic biology for the design of gene circuits to protein origami nanostructures**

**Roman Jerala**

Synthetic biology and immunology, National institute of chemistry, Slovenia

Modularity has been extensively used in engineering for the rapid and cost effective construction of complex devices and structures. Nanoscale molecular scaffolds and machines require self-assembly in the complex environment, which demands orthogonal building elements. Nucleotide sequence provides a large and easily accessible combinatorial diversity that can be used to program biological systems. Designable DNA-binding TALE domains can be used to construct an almost limitless number of artificial transcriptional regulators which enable construction of orthogonal genetic logic NOR gates. NOR gates allow construction of any logic function, which has been demonstrated by all 16 two-input logic gates in mammalian cells and on bistable switches, which involve feedback loops and competition for binding sites. This type of designed regulation can be used to regulate therapeutic cells.

An even more fundamental challenge for synthetic biology is construction of new protein folds in contrast to modifications or combinations of the existing protein domains. A modular engineering approach based on designed orthogonal coiled-coil building elements has been used to design completely new protein folds, composed of a single polypeptide chain of concatenated coiled-coil building elements. This represents a new type of topological protein folds not found in the nature so far, where the fold is defined by the order of interacting segments defining the final topology.

**SB-02  Switching the metabolism of Pichia pastoris to efficiently assimilate CO₂**

**Thomas Gassler¹, Brigitte Gasser¹, Michael Sauer¹, Matthias Steiger², Diethard Mattanovich³**

¹ University of Natural Resources and Life Sciences, Vienna, Austria
² Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 18, Vienna, Austria

The methylotrophic yeast *P. pastoris* (syn.: *Komagataella phaffii*) assimilates methanol efficiently via the xylulose-5-phosphate pathway. We have shown recently that this pathway is entirely localized to the yeast peroxisomes and has striking similarities to the Calvin-Benson-Bassham (CBB) cycle used e.g. by plants to assimilate CO₂ (Rußmayer et al., 2015, BMC Biology), which is likewise compartmentalized in chloroplasts. Recent advances in genome editing technics now facilitate the engineering of a great variety of organisms including *P. pastoris*. We exploited this high similarity and this new set of genetic tools to integrate a synthetic version of the CBB cycle.

The novel *P. pastoris* strain created by metabolic engineering has its methanol assimilation pathway re-wired to a CO₂ fixation pathway resembling the CBB cycle. This new yeast strain has the ability to efficiently assimilate CO₂ into biomass. For energy supply, any source yielding NADH can be used due to a modular metabolic design. At present methanol oxidation is used for this purpose. With this technology, it is possible to utilize CO₂ as valuable resource for biotechnological applications and to assimilate it into different bio-based products. Currently, this CO₂-assimilating *P. pastoris* strain is the most efficient recombinant microorganism engineered for using CO₂ as carbon source and significantly outcompetes other engineered systems for CO₂-fixation like *Escherichia coli* or *Saccharomyces cerevisiae*. The advantage of the *P. pastoris* platform is the ability to accumulate biomass to very high cell densities exceeding 100 g/L. Thus, high space-time yields are in reach for a CO₂-fixation platform based upon this microbial chassis.
SB-03  Synthetic transcription factors and expression system in *Trichoderma reesei*

Christian Derntl, Thiago M. Mello de Sousa, Katharina J. Regnat, Alice Rassinger, Robert L. Mach, Astrid R. Mach-Aigner
ICEBE, TU Wien, Austria

The ascomycete *Trichoderma reesei* is widely used in the industry for the production of cellulases and hemicellulases. Additionally, it has been established as model organism. For both applications, inducible gene expression is favourable. We have developed a series of synthetic transcription factors and promoters that can be used for strong and controllable gene expression using estradiol as cheap and otherwise non-interfering inducer. Our research towards the construction of synthetic transcription factors has also lead to further insights into transcription factor architecture and their conserved motifs in filamentous fungi. In particular, we studied the work mode of Xyr1, the main activator of cellulases and xylanases, in detail and could identify an additional regulatory mechanism, i.e. posttranslational activation.

SB-04  Efficient calculation of microbial production envelopes

Sarah Noel Galleguillos, Matthias Gerstl, Jürgen Zanghellini
Department of Biotechnology, University of Natural Resources and Life Sciences, Austria

Phenotypic phase planes, sometimes called production envelopes, are an important tool in constraint-based analysis of metabolic networks. They allow one to characterize the full metabolic capabilities of an organism as function of selected reaction fluxes of interest. However, phase planes are most often evaluated for only two fluxes of interest (typically a product of interest as a function of growth), as the computational work load scales exponentially with the number of selected fluxes.

Here we present an efficient algorithm for the fast calculation of phase planes in multi dimensions in any genome-scale metabolic model. We use concepts developed in computational geometry to efficiently enumerate the vertices of the phase plane, resulting from the projection of the total metabolic capabilities onto the dimensions of interest (biomass, product and substrate fluxes). With our approach, phase plane analysis is no longer restricted to two or three reactions of interest. This is particularly important for the unbiased analysis of microbial communities. In fact, it is for the first time now becoming possible to use phase planes to characterize multiple interactions and dependencies between members of a microbial community.

SB-05  Synthetic biology and metabolic engineering toward the discovery of novel bioactive secondary metabolites from actinomycete bacteria

Sergey B. Zotchev
Department of Pharmacognosy, University of Vienna, Austria

Several decades of bioprospecting in the bacterial kingdom yielded many important anti-microbial and anti-cancer agents (e.g. tetracycline, vancomycin, nystatin, doxorubicin etc), although the rate of discovery declined steeply over the last 20-25 years. The main reasons for that were high costs of screening coupled to frequent re-discovery of already known bioactive molecules.

Certain types of bacteria, in particular those belonging to the order Actinomycetales, are prolific producers of bioactive compounds. Today, approaches based on post-genomic technologies, such as synthetic biology and metabolic engineering, open completely new possibilities for drug discovery from these bacteria. Actinomycetes’ genomes were shown to harbor dozens of orphan gene clusters that are silent under laboratory conditions, but can be activated via specific manipulation or heterologous expression. Also, natural product biosynthetic pathways can be altered in order to generate new derivatives with improved pharmacological properties.

In this presentation, examples of successful synthetic biology- and metabolic engineering-based approaches to engineering actinomycetes for drug discovery and development will be highlighted.

**Keywords**: Actinomycete bacteria, secondary metabolites, biosynthetic gene clusters, synthetic biology, metabolic engineering
Thursday 20th: Antimicrobial drugs: drug screening and prudent use

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Antimicrobial drugs: drug screening and prudent use

Chair: Joseph Strauss
AD-01  Discovery and preclinical development of novel anti-infectives from fungal and microbial sources
Marc Stadler
MWIS, Helmholtz-Zentrum für Infektionsforschung (HZI), Germany

Novel lead structures for development of antibiotics and other anti-infective agents are in great demand, owing to the threatening increase of resistance among pathogenic microbes. During the past years, we have therefore invested substantially in a revival of natural product-based discovery at the Helmholtz-Centre for Infection Research (HZI). During this work, we are focusing on rare actinobacteria, myxobacteria and filamentous fungi as sources and found various new molecules from extracts that showed selective activities in various natural product screening assays. They were isolated by preparative chromatography and their chemical structures were determined NMR spectroscopy, HR-mass spectrometry and other spectral methods. The further development of these lead compounds involves biotechnological production in up to 350 litre scale and matching downstream processing procedures, in order to provide material in multi gram quantities for pharmacological experiments and medicinal chemistry programmes. The general workflow established in our department for discovery of novel bioactive compounds will be outlined. In addition, some examples for recent highlights resulting from this approach will be presented.

AD-02  Diverse effects of translation bottlenecks on antibiotic action underlie drug interactions
Bor Kavcic¹, Gasper Tkacik¹, Tobias Bollenbach²
¹ RG Tkacik (Systems biology and biophysics), IST Austria, Austria
² University of Cologne, Cologne, Germany

Rapid growth of bacteria requires a well-orchestrated translation machinery which is modulated internally by translation factors and perturbed by certain antibiotics (translation inhibitors), which act on various steps in translation. When translation inhibitors are combined, they interact diversely, as the combined effects range from synergistic to antagonistic. Such interactions are difficult to predict since the mechanisms remain unknown. We hypothesize that the interactions between antibiotics arise from their kinetic properties together with the interplay of different stages in which ribosomes are halted. To test how halting of the ribosomes affects the efficacy of antibiotics, we constructed bacterial strains in which we control the abundance of different translation factors. Measurements of the impact of the translation bottlenecks on the action of antibiotics showed how antibiotic efficacy depends on the targeted process in the translation cycle. This approach enabled the direct prediction of the interaction type between some of the antibiotics. These results provide new insights into the mechanisms of translation inhibitors and their combinations as well as translation itself.
AD-03 Antifungal susceptibility of Candida blood stream isolates collected during a 10 year period from Austria between 2007 and 2016

Reinhard Beyer1, Kathrin Spettel1, Joseph Strauss2, Christoph Schüller2, Birgit Willinger1

1 Division of Clinical Microbiology, Department of Laboratory Medicine, Medical University of Vienna
2 Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Austria

Objectives: We assessed the current status quo of clinical Candida isolates collected from hospitals all over Austria between 2007 and 2016 in high-throughput. We determined antifungal resistance and abundance and explored subsets of each Candida spp.

Methods: More than 1300 clinical Candida isolates from blood cultures were tested against fluconazole (FLZ), voriconazole (VOR), posaconazole (POS), itraconazole (ITR), isavuconazole (ISA), anidulafungin (ANI), caspofungin (CAS) and micafungin (MCA) according to the EUCAST method of broth microdilution with additional recording of the growth curves. We performed rigid quality control via growth curve assessment and inclusion of two reference strains. Minimal inhibitory concentrations (MIC) were quantified according to EUCAST guideline E.DEF 7.3.1 and resistance was evaluated using clinical breakpoints provided by EUCAST.

Results: The species distribution was C. albicans (56%), C. glabrata (19%), C. parapsilosis (9%), C. tropicalis (5%) and C. krusei (3%). We identified a low level incidence of other Candida species and fungi. While the total number of isolates increased over time, species abundance and resistance rates remained constant. Multi-resistant isolates occur rarely and mostly in species already intrinsically resistant to one class of antifungals.

Conclusions: Over the last decade, distribution of Candida species and antifungal resistances remain largely constant in Austria and are comparable with other European countries. MIC determination in large collections can be improved by high-throughput liquid handling and by recording growth parameters.

AD-04 Anthracene endoperoxides: impact on Leishmania

Gerald Geroldinger1, Matthias Tonner1, Werner Fudickar2, Sritama De Sarkar3, Lianet Monzote4, Katrin Staniek1, Torsten Linker1, Mitali Chatterjee3, Lars Gille1

1 Deptm. Biomedical Sciences, Univ. of Veterinary Medicine, Austria
2 Chemistry Department, University of Potsdam, Golm/Potsdam, Germany
3 Department of Pharmacology, Institute of Postgraduate Medical Education & Research, Kolkata, India
4 Parasitology Department, Institute of Tropical Medicine “Pedro Kouri”, Havana, Cuba

Leishmaniasis, caused by unicellular protozoal parasites of the genus Leishmania, is transmitted upon the bite of an infected sandfly carrying its promastigote form. The amastigote form of the parasite develops in the host organism causing various clinical manifestations. These parasites show increased resistance development against established therapies. Therefore new therapy options are needed urgently. Endoperoxides that are already established in the therapy of malaria have shown antileishmanial effects in vitro and in vivo. Anthracene endoperoxides (AcEPs) that are so far used for technical application (e.g. photolithography) present a potential candidate for a new pharmacological approach in antileishmanial therapy.

The antileishmanial efficiency of AcEPs was studied using resazurin and MTT based viability assays in different Leishmania species and life stages (L. tarentolae promastigotes (LtP) and amastigotes, L. donovani promastigotes) as well as in J774 macrophages as model for the mammalian host. A xylenol orange assay elucidated the reduction of AcEPs by Fe2+ identifying it as a potential activator. The subsequent formation of radicals was observed by EPR spectroscopy using DMPD spin trap in the chemical system and in LtP. The release of singlet oxygen upon UV irradiation under biomimetic conditions and the formation of superoxide radicals in LtP were observed by EPR spectroscopy. The AcEPs influence on LtP oxygen consumption and on the mitochondrial function was examined using OxoPlates®. It was shown that the studied AcEPs observed (i) were able to kill Leishmania efficiently in vitro at IC50 values of a low micromolar range, (ii) also showed a certain host cell toxicity in J774 macrophages, (iii) are activated by iron (II), cleaving the peroxide-bridge, (iv) form oxygen- and carbon-centered radicals upon cleavage and release 1O2 upon UV irradiation, (v) generate carbon-centered radicals in Leishmania which subsequently trigger the formation of superoxide radicals and (vi) that they impair the mitochondrial functions in LtP. These findings suggest that AcEPs present a potential group of compounds for the development of new antileishmanial drugs. Nevertheless, further approaches will have to be identified to improve their pharmacodynamic and pharmacokinetic properties, including the reduction of the host cell toxicity.

Special thanks to the Austrian Science Fund (FWF) for supporting the present study under grant P 27814-B22.
AD-05 A non-canonical RNA polymerase drives tra-operon expression of F-like plasmids.

Anja Haubenhofer, Karin Bischof, Kathrin Froshauer, Günther Koraimann

University of Graz, Austria

Horizontal gene transfer by bacterial conjugation is highly relevant because it is a major cause of the development and persistence of antibiotic-resistance. Through bacterial conjugation, non-pathogenic bacteria can acquire virulence and antibiotic resistance genes, thereby transforming harmless bacterial commensals into health threatening pathogens. Conjugative plasmids with F-like conjugation modules are widely distributed in commensal and pathogenic enterobacteria like Escherichia, Shigella, Salmonella and Klebsiella species and often carry virulence and/or multiple antibiotic resistance genes (1). Bacterial cells harboring F-like conjugative plasmids do not constitutively express genes needed for the assembly of the type IV secretion system by which single-stranded DNA is delivered to recipients cells. Rather, signaling molecules and/or environmental cues activate DNA transfer gene expression in a subpopulation of potential donors (2).

Here we present data aimed to unravel the molecular nature of the transcription machinery that enables transcription of more than 30 DNA transfer genes in F-like plasmids (termed tra operon). The plasmid encoded PAS domain containing TraJ protein is required for transcription of the whole operon, however mechanistic details on how this is achieved are unknown. Based on previous experimental evidence that indicated a direct interaction of TraJ with RNA polymerase (RNAP) of Escherichia coli we carried out experiments to characterize the TraJ-RNAP complex. We show that TraJ interacts with core and σ70 containing RNA polymerase of E. coli. Furthermore, we find that a TraJ-RNAP complex purified from E. coli displays transcriptional activity.

Results of our experiments indicate that TraJ is not a standard transcription activator protein but rather directly interacts with RNAP core and σ70. This unique transcription machinery is specifically directed to the main tra operon promoter, P_Y.

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Translational oncology I

Chairs: Christa Noehammer & Michael Bergmann
TO1-01 The role of IL-6 and ADAM17 in inflammation and cancer

Stefan Rose-John
Biochemistry, University of Kiel, Germany

Cytokines receptors exist in membrane bound and soluble form. The IL-6/soluble IL-6R complex stimulates target cells not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. We have named this process 'trans-signaling'. The soluble IL-6R is generated via ectodomain shedding by the membrane bound metalloprotease ADAM17. Soluble gp130 is the natural inhibitor of IL-6/soluble IL-6R complex responses. The dimerized recombinant soluble gp130Fc fusion protein is a molecular tool to discriminate between gp130 responses via membrane bound and soluble IL-6R responses. Interestingly, depending on the animal model used, global blockade of IL-6 signaling by neutralizing monoclonal antibodies and selective blockade of IL-6 trans-signaling can lead to very different consequences. Inhibition of IL-6 trans-signaling but not global IL-6 blockade was beneficial in several inflammation and cancer models. The extent of inflammation is controlled by trans-signaling via the soluble IL-6R. Using the sgp130Fc protein or sgp130Fc transgenic mice we demonstrate in animal models of inflammatory bowel disease, peritonitis, rheumatoid arthritis, atherosclerosis, pancreatitis, lupus erythematoses, colon cancer, ovarian cancer, pancreatic cancer, nephrotic nephritis and high fat diet induced obesity that IL-6 trans-signaling via the soluble IL-6R is the crucial step in the development and the progression of the disease. Therefore, sgp130Fc is a novel therapeutic agent for the treatment of chronic inflammatory diseases and cancer and it underwent phase I clinical trials as an anti-inflammatory in 2013/2014. Phase II clinical trials in patients with autoimmune diseases such as inflammatory bowel disease have started in 2016 in Germany and in 2018 in China, Taiwan and South Korea.


TO1-02 Immunological differences between colorectal cancer and normal mucosa uncover a prognostically relevant immune cell profile

Katharina Strasser1, Hanna Birnleitner2, Andrea Beer3, Dietmar Pils2, Marlene Gerner4, Klaus Schmetterer4, Thomas Bachleitner-Hofmann2, Anton Stift3, Michael Bergmann2, Rudolf Oehler2

1 CBmed Graz and Department of Surgery, Medical University of Vienna, Vienna, Austria
2 Department of Surgery, Medical University of Vienna, Austria
3 Department of Pathology, Medical University of Vienna, Vienna, Austria
4 Department of Laboratory Medicine, Medical University of Vienna

T cells in colorectal cancer (CRC) are associated with improved survival. However, checkpoint immunotherapies antagonizing the suppression of these cells are ineffective in the great majority of patients. To better understand the immune cell regulation in CRC, we compared tumor-associated T lymphocytes and macrophages to the immune cell infiltrate of normal mucosa. Human colorectal tumor specimen and tumor-distant normal mucosa tissues of the same patients were collected and phenotypes and functionality of tissue-derived T cells and macrophages were characterized using immunohistochemistry, RNA in situ hybridization, and multiparameter flow cytometry. Prognostic effects of the observed differences between distant mucosa and tumor tissue on the overall survival were examined using gene expression data of 298 CRC patients. CRC tissues contained more CD39 and Helios-expressing regulatory T cells compared to distant mucosa. On the contrary, we observed a substantial amount of IFNy-producing cells and a lack of PD-L1 in the tumors. This was accompanied by a higher CD14+/CD206+ macrophage ratio, as well as altered CX3CR1 and CD64 expression levels on tumor macrophages. The combined gene expression data of increased FOXP3, IFNy, CD14, and decreased CD206 correlated with a lower overall survival of CRC patients. These data reveal that the CRC microenvironment promotes the coexistence of seemingly antagonistic suppressive and pro-inflammatory immune responses. This immune cell profile is associated with poor prognosis and provides the explanation why a blockade of the PD1/PD-L1 axis is ineffective in CRC. This should be taken into account when designing novel treatment strategies.
TO1-03  Influence of fibroblast derived SPARC on colorectal cancer migration and invasiveness

Daniel Drev¹, Andrea Bileck², Zeynep Erdem¹, Andrea Beer³, Gerald Timelthaler³, Anton Stift⁴, Christopher Gerner², Brigitte Marian¹

¹ Cancer Research Institute, Medical University Vienna, Austria
² Institute of Analytical Chemistry, University of Vienna, Austria
³ Clinical Institute of Pathology, Medical University of Vienna
⁴ Department of Surgery, Medical University of Vienna, Austria

Interaction of tumors with their surrounding tumor microenvironment has been identified as an important factor in development and progression of various types of cancer, including colorectal cancer (CRC). To determine the role of cancer associated fibroblasts in CRC, we analyzed human CRC tissue and paired normal mucosa with tandem mass spectrometry. Mapping of resulting protein data to a stromal contribution map allowed us to identify proteins of high abundance in CRC and of stromal origin. One of these proteins was SPARC, a non-structural glycoprotein of the ECM. Consistently, immunohistochemistry staining demonstrated upregulation of SPARC in the tumor stroma compared to normal adjacent mucosa and co-localization with the mesenchymal marker αSMA. Moreover, analysis of public available RNA expression datasets of more than five hundred CRC patients correlated reduced recurrence free survival with high SPARC mRNA levels. In vitro adhesion assays resulted in a decrease of CRC cell attachment to human colonic primary fibroblasts expressing normal amounts of SPARC compared to fibroblasts where this gene was knocked down (KD). In addition, migration of cancer cells was increased on fibroblast layers producing normal amounts of SPARC compared to SPARC KD cells. Moreover, by using 3D co-cultures, we showed a reduction of cancer cell invasion into the fibroblast matrix when SPARC levels were decreased. Taken together, our results suggest that SPARC is a pro-migratory, pro-invasive protein when expressed by colonic fibroblasts in the tumor microenvironment and targeting this interactive protein in CRC might be beneficial for patients with high SPARC expression.

TO1-04  Anti-inflammatory microbiota restriction augments skeletal bone structure

Irene Maier¹, Jared Liu¹, Paul M Ruegger², Julia Deutschmann³, Janina Patsch⁴, Thomas Helbich⁴, James Borneman⁵, Robert H Schiestl¹

¹ Department of Environmental Health Sciences, University of California, Los Angeles, United States of America
² Department of Microbiology and Plant Pathology, University of California, Riverside, Riverside, CA,
³ Department for Radiologic Technology, University of Applied Sciences Wiener Neustadt for Business and Engineering Ltd., Lower Austria, Austria,
⁴ Department of Biomedical Imaging and Image-Guided Therapy, Medical University of Vienna, Vienna, Austria,
⁵ Departments of Pathology and Environmental Health Sciences, Geffen School of Medicine and School of Public Health, University of California, Los Angeles, 650 Charles E. Young Dr. South, Los Angeles, CA-90095, USA.

Intestinal microbiota play a direct role in nutrient, fatty acid and DNA metabolism. From ancient to very recent scientific efforts, the microbiome has been considered an indirect and functional sensor for molecular pathways, which orchestrate energy balance, immune responses as well as cell regenerative processes in various tissues. We previously reported that microbiota restriction promoted higher levels of systemic radiation-induced genotoxicity, proliferative lymphocyte activation, and polarization of metabolic pathways towards apoptosis. Restricted intestinal microbiota dysbiosis based on increased abundance of Lactobacillus johnsonii (LBJ) on the other hand prohibits inflammation-associated lymphomagenesis and imparts alleviation of a glycolytic phenotype underlying the elevation of onco-protective metabolites. Bacterial indicator phylotypes, including an unclassified Gram-negative bacterium cTPY-13, Barnesiella intestinihominis, and Lactobacillus johnsonii strains were found more abundant in wildtype restricted microbiota (RM) mice and increased in prevalence after whole body irradiation in conventional microbiota (CM) mice. Muribaculum intestinale was found highest in female small intestines of RM mice, which were lacking Ureaplasma felinum. By contrast, Muribaculum intestinale was increased in male colons in the radiation-resistant CM phenotype. Endogenous LBJ strains influenced phylotype densities and reduced their abundance in irradiated CM mice compared to RM mice as assessed in intestinal mucosal cells, while the adaptive repair of chromosomal DNA lesions was induced more efficiently in CM than RM mice. The expression of interleukin-17, however, was reduced by high-linear energy transfer radiation in RM mice, particularly in females. RM female mice showed improved micro-architectural bone structure compared with CM mice at necrosis-unrelated phase after six weeks post particle radiation exposure. Genotoxic and anti-inflammatory traits in peripheral blood or tissue were associated with bacterial strains along with differential abundances of bacterial indicator phylotypes before and after radiation treatment.
**TO1-05  Oncological outcome after liver resection for malignant entities can be modulated via selective serotonin reuptake inhibition**

**David Pereyra**, Robin Padickakudy, Philipp Jonas, Eva Braunwarth, Christine Brostjan, Stefan Stättner, Thomas Grünberger, Patrick Starlinger

1 Department of Surgery, Medical University of Vienna, Austria  
2 Department of Surgery, Rudolfstiftung Hospital, Vienna  
3 Department of Visceral, Transplantation and Thoracic Surgery, Medical University of Innsbruck

**Background**

Intra-platelet serotonin has been implicated in the process of liver regeneration and in the development of disease recurrence after liver resection for malignant diseases. While the effect of serotonin on liver regeneration and tumor promotion were only observed in independent experiments, we recently demonstrated a bivalent association in patients undergoing liver resection. This raised the question whether pharmacologic modification of intra-platelet serotonin might be beneficial for this patient cohort.

**Methods**

497 patients were included out of our prospectively maintained institutional data base. Perioperative intake of selective serotonin reuptake inhibitors (SSRI) was recorded. Patients were followed up for postoperative liver dysfunction (LD), severe morbidity and disease recurrence.

**Results**

52 patients (10.5%) were treated with SSRI during the perioperative course. Patients with SSRI intake showed a significantly higher incidence of severe morbidity (16.6% vs 29.5%, p=0.031) and LD (10.4% vs 25.0%, p=0.004). On the contrary, patients with SSRI intake showed a significantly decreased incidence of disease recurrence after 6 months (23.3% vs 4.7%, p=0.005) and after 12 months (44.2% vs 24.4%, p=0.015), which could also be confirmed in the subgroup analysis of patients with colorectal cancer liver metastases (p=0.024, p=0.048, respectively).

**Conclusion**

Within this study, we present solid evidence for a central impact of serotonin modification on the surgical and oncological outcome of patients undergoing liver resection. Intriguingly, treatment with SSRI seems to exert a dual effect on patients’ outcome via disruption of both liver regeneration and tumor growth. Further, our data elucidates a potential pro-tumorigenic role of SSRIs, which clearly has to be confirmed in prospective trials.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 3: Molecular microbiology
PS3:MM-01 Oxidized phospholipid species contribute to the pro-inflammatory activity of senescent fibroblasts

Vera Pils¹, Marie Narzt², Ionela-Mariana Nagelreiter³, Michael Mildner³, Lucia Nanic¹, Ivica Rubelj¹, Lucia Terlecki-Zaniewicz¹, Christopher Kremslehner², André Branco¹, Dominik Authoried¹, Johannes Grillari¹, Florian Gruber², Ingo Lämmermann¹

¹ Biotechnology, BOKU - University of Natural Resources and Life Sciences, Austria
² Departement of Dermatology, Medical University, Division for Biology and Pathobiology of the Skin, Vienna, Austria
³ Institut Ruđer Bošković, Laboratory for Molecular and Cellular Biology, Zagreb, Croatia

Cellular senescence describes a permanent cell cycle arrest. With age, senescent cells accumulate in various tissues, which is causatively linked to a state of low-grade chronic inflammation and age-related disorders and diseases. The senescence associated secretory phenotype (SASP), a cocktail of pro-inflammatory factors, whose known members are, as of now, only limited to proteins and extracellular vesicles, majorly contributes to this phenomena. However, there is still only limited knowledge on lipid mediators in the context of senescence.

Here, we investigated whether the intracellular or secreted composition and amount of bioactive lipid mediators are altered in senescent fibroblasts. Using tandem mass spectrometry, we identified two lysophosphatidylcholines, 1-palmitoyl-sn-glycero-3-phosphorylcholine (lyso-PPC) and 1-stearoyl-sn-glycero-3-phosphorylcholine (lyso-SPC), to be significantly upregulated intracellularly with the replicative age of human dermal and foreskin fibroblasts. However, this increase was not observed after cells were immortalized by TERT transduction. Moreover, the increase of lyso-PPC and lyso-SPC was not limited to replicative senescence, but was also confirmed in various telomere independent stress-induced premature senescence models (H2O2, tBHP, UVB). The amount of lyso-PPC and -SPC secreted per cell was highly elevated in senescent cells, whereas the composition of the secreted lipidome was not altered. Hence, lyso-PPC and -SPC seem to accumulate intracellularly during senescence. The sub-cellular localization of these lipids will be further investigated to gain a greater understanding of their intracellular metabolism during senescence.

When fibroblasts were exposed to lyso-PPC or -SPC, we found that both dose-dependently induced cytokine expression. We also detected chemoattractive effects on human peripheral blood monocytes. However, lyso-PPC and lyso-SPCs also disturbed the interaction of macropages and senescent cells via the TLR2/CD36 axis. Thus, we propose that these lysophosphatidylcholines are members of the SASP contributing to the chronic pro-inflammatory environment not only directly, but also indirectly, by disturbing the immunoclearance of senescent cells. Therefore, they are likely to play a prominent role in the development and progression of age-related diseases.

PS3:MM-02 Rps10 mediated Tigecycline resistance in Escherichia coli

Gernot Zarfel¹, Daniela Toplitsch², Clemens Kittinger³, Valentina Percher³, Brigitte Pertschy⁴

¹ Diagnostic & Research Center for Molecular BioMedicine, Medical University of Graz, Austria
² Medical University of Graz Diagnostic & Research Center for Molecular BioMedicine; University of Graz, Institute of Molecular Biosciences
³ Carinthian University of Applied Sciences
⁴ University of Graz, Institute of Molecular Biosciences

Tigecycline is a last line antibiotic belonging to the tetracycline family, which are potent inhibitors of translation elongation. Tigecycline binds bacterial 30S ribosomal subunits at the A-site, where it prevents delivery of aminoacyl-tRNA.

Several mutations have been described to mediate resistance to tigecycline, including allelic versions of the rpsJ gene, coding for the ribosomal protein Rps10 (uS10). While the largest part of Rps10 is positioned on the solvent exposed side of 30S subunits, two beta strands connected by an unstructured loop dive deeply into the ribosomal RNA, reaching close to the A site on the intersubunit side of the 30S subunit. All amino acid exchanges known to cause tigecycline resistance lie in the loop region of Rps10. Notably, Tigecycline does not directly bind to the Rps10 loop, but its binding site is ~10 Å apart in helix 31 of the 16S rRNA. This raises the question how mutations at a physically distant site can cause resistance to tigecycline.

In this project, we are functionally characterizing mutants in the Rps10 loop in order to gain a better understanding of the effects of Rps10 loop mutation on the ribosome. Moreover, we are aiming to unravel the mechanism by which these effects lead to Tigecycline resistance.
**PS3:MM-03** The attachment of the Lactobacillus surface-layer array to the bacterial cell

*Elisabeth Damisch*, Markus Eder
Andela Dordić, Ulla Hynönen, Airi Palva, Janet Vonck, F. Berni, J. D. C. Codée
Monika Oberer, Tea Pavkov-Keller

1. Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland
2. Structural biology, Max-Planck-Institute of Biophysics, Frankfurt am Main, Germany
3. Institute of Molecular Biosciences, University of Graz and ACIB GmbH, Graz, Austria
4. Institute of Chemistry, Leiden University, Netherlands
5. University of Graz, Institute of Molecular Biosciences, Austria

Surface layers (S-layers) are 2D paracrystalline lattices of proteins or glycoproteins which cover the whole cell surface of many Archaea and bacteria. Since these proteins are in close contact with their habitat they fulfill many vital tasks like bacterial adherence to other cells or substrates, protection against life-threatening conditions and maintenance of the cell shape [1,2].

S-layer proteins of lactobacilli species have a highly basic pl and are between 25-71 kDa in size [2,3]. They are attached to the cell wall by interaction with lipoteichoic acids (LTA) [2]. It is reported that they are involved in auto-coaggregation and adherence and therefore are significant for the stimulation of gut dendritic cells by interacting with specific receptors [4]. Our goal is to characterize the surface layer proteins SlpA and SlpX of *Lactobacillus acidophilus*, which are both necessary to build up the protective S-layer coat. By changing the composition of the S-layer coat, the organism is able to adapt to the changing environmental conditions and threats, e.g. osmotic stress [5].

For structure-function characterization, we designed several protein fragments. Soluble fragments were purified and subjected to crystallization. Optimized crystals of the C-terminal fragments, containing the LTA-binding domain, diffracted to 1.8 and 2.2 Å. Crystal structures were solved by SeMet-SAD and the later by molecular replacement. To further characterize the binding of the S-layer to bacterial cell we performed NMR titration experiments and isothermal titration calorimetry measurements with the C-terminal fragment of the protein.

**References:**


---

**PS3:MM-04** Growth rate-dependent drug target-expression level controls the dose-sensitivity to the antibiotic trimethoprim

*S. Andreas Angermayr*, Tobias Bollenbach

Institute for Biological Physics, University of Cologne, Germany

Understanding how pathogens respond to antibiotics is relevant for establishing effective disease treatments and for fighting drug resistance evolution. The environment affects the growth of bacteria and the efficacy of antibiotics. Using a set of six different antibiotics—representing the main modes of action—we tested how growth rate influences their efficacy. For trimethoprim—an antibiotic used widely to treat urinary tract infections—we have found a reduction of susceptibility for slow growing *Escherichia coli* cultures. The folate-synthesis inhibitor trimethoprim shows a consistently observed very shallow dose-response curve. Systematically using different growth limitations—encompassing a broad range of different growth rates—we can change the steepness of the dose-response curve for trimethoprim. Combining the results, we have established a general growth-mediated negative feedback loop that controls the dose-sensitivity for trimethoprim. Next, we asked for the molecular mechanism governing susceptibility and dose-sensitivity. Lowered growth rate leads to an increased drug target-expression level which in turn lowers the dose-sensitivity to the drug. The correlation between the expression level and the reduction in susceptibility can further be rationalized by bacterial growth laws. The growth-mediated negative feedback offers an explanation for the shallow dose-response curve for trimethoprim. These results have implications for antibiotic efficacy in clearing infections and for resistance evolution. Negative growth-mediated feedback offers an opportunity for slowing the evolution of drug resistance.
Positive-negative selectable markers based on fusion genes allow efficient marker-free gene editing in *F. graminearum* as well as Cre-loxP aided recycling of transformation markers for consecutive knockouts

**PS3:MM-05**

Krisztian Twaruschek¹, Thomas Svoboda¹, Gerlinde Wiesenberger¹, Pia Spörhase¹, Marta Piatkowska², Franz Berthiller², Gerhard Adam³

¹ Applied Genetics and Cell Biology, BOKU, Austria
² BOKU, Department of Agrobiotechnology, Center for Analytical Chemistry, Konrad Lorenz Straße 20, 3430 Tulln, Austria

Marker-free genetic engineering of filamentous fungi, such as *F. graminearum*, has proven to be a laborious task in the past, mainly due to the inability to efficiently recycle previously integrated markers. To amend this shortcoming, a series of bifunctional marker genes was generated by N-terminal fusion of the Herpes simplex virus thymidine kinase (HSVtk) to the resistance markers *hph* (hygromycin B), *nptII* (G418) and *nat1* (nourseothricin). These fusion genes can be selected for using the respective antibiotics in the fungus, and also in *E. coli*, while additionally allowing forced excision of the marker via 5-fluoro-2-deoxyuridine (FdU) counterselection. Here, we demonstrate the applicability of HSVtk fusion genes by constructing a *TRI8* allele-swapped *F. graminearum* PH-1 transformant. *TRI8* encodes an esterase that uses 3,15-diacetyl-deoxynivalenol as a substrate, and cleaves off an acetyl group from either position 3 or position 15, depending on the allele. PH-1 possesses the *TRI8* allele responsible for producing 15-acetyl-deoxynivalenol (15-ADON), however, the 3-acetyl-deoxynivalenol (3-ADON) chemotype is also frequently encountered in the wild.

An isogenic PH-1-derived strain, carrying the 3-acetyl *TRI8* allele, was generated in two steps. First, *TRI8* was deleted by integration of the HSVtk-*nat1* marker, after which the marker was replaced with the 3-acetyl *TRI8* allele via homologous recombination. The resulting strain no longer possessed the nourseothricin resistance of its precursor Δ*tri8::HSVtk-nat1* strain, and was PCR-verified on both flanks for correct integration. As expected, the chemotype was switched from 15-ADON to 3-ADON production. Furthermore, we constructed a *F. graminearum* PH-1-derived mutant that carries seven consecutive gene deletions using the Cre-loxP system for marker recycling. Treatment of protoplasts with recombinant Cre recombinase removes loxP-flanked cassettes very efficiently, reducing the screening effort required to obtain marker-free deletion strains.

The Cat8 transcription factors regulate carbon source utilization in *Pichia pastoris*

**PS3:MM-06**

Diane Barbay, Diethard Mattanovich, Brigitte Gasser

Biotechnologie, ACIB, Austria

The methylotrophic yeast *Pichia pastoris* (syn *Komagataella spp*) plays a major role in the synthesis of high amounts of recombinant proteins for biopharmaceutical or industrial purposes. Its ability to use methanol as a carbon source, its non-fermentative utilization of glucose and its efficient growth on glycerol are key metabolic features that make it attractive for bioprocess development. However, many complex secretory proteins still cannot be produced at the desired high levels during industrial production processes. Concerted engineering of the cellular response by modulation of transcriptional regulatory proteins has proven to be a promising approach to increase productivity in *P. pastoris*.

The transcription factors Cat8 and Sip4 were described in different yeasts to have very similar DNA binding domains and to be necessary for derepression of a variety of genes under non-fermentative growth conditions via binding to the Carbon Source Responsive Elements (CSREs). *P. pastoris* has two putative transcription factor homologs of Cat8, termed Cat8-1 and Cat8-2. It is yet unclear in which cellular processes they are involved and if one of them is Sip4.

In order to study the roles of the Cat8 homologs in *P. pastoris*, transcription factors knock-outs were generated using CRISPR/Cas9-based homology-directed genome editing. The ability of the mutant strains to grow on different nutrient sources was tested, and transcript levels of some selected genes from the carbon metabolism were quantified to see how their regulation patterns on different carbon sources were affected in the absence of either or both Cat8 homologs.
The cytotoxin associated gene A (CagA) from the human pathogen Helicobacter pylori (H. pylori) is strongly associated with development of chronic gastritis, ulceration, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. CagA is injected into host cells via a type-4 secretion system (T4SS). In the cytosol, CagA is tyrosine-phosphorylated at specific sites harboring Glu-Pro-Ile-Tyr-Ala (EPIYA) sequence motifs, which can be distinguished in EPIYA-A, -B, -C and -D motifs. These motifs occur in different numbers and combinations in individual H. pylori isolates and are highly important for pathogenesis. CagA phosphorylation leads to activation of signaling pathways and induces cell migration, anti-apoptosis and proliferation. In infected immune cells, CagA cleavage was detected in previous studies, leading to a ~100 kDa N-terminal fragment and a ~40 kDa C-terminal fragment exhibiting unknown functions in infected cells.

In this work, the effect of EPIYA motif variations on CagA phosphorylation in B-cells was investigated in infection experiments with the B-cell line MEC1. Further, CagA cleavage and phosphorylation of the C-terminal fragment was analyzed in MEC1 cells infected with clinical isolate strains harboring different EPIYA motif combinations. In another approach, CagA phosphorylation and cleavage was investigated using isogenic mutant strains harboring several variations of EPIYA-C motifs. It was observed that EPIYA-C and EPIYA-D motifs are crucially important for CagA EPIYA-A and EPIYA-B phosphorylation, whereas CagA cleavage was phosphorylation independent. Additionally, EPIYA-A motifs enhanced phosphorylation of EPIYA-C or EPIYA-B motifs. The experiments further demonstrated that repetitions of EPIYA-C motifs increased phosphorylation of the full length CagA and the C-terminal fragment in B-cells. Among different strains, CagA cleavage was observed in B-cells leading to formation of a ~100 kDa N-terminal fragment and C-terminal fragments with different molecular weight, correlating to the number of EPIYA motifs. The data from this work indicate, that CagA cleavage is a common mechanism in infected B-cells among different H. pylori strains. Further the importance of EPIYA-C motifs for phosphorylation of CagA in B-cells was proved. Additionally, the results from this work indicate that variations of EPIYA-A motifs might increase CagA phosphorylation and therefore could contribute to the pathogenicity of these strains.
Factors such as global warming or the increasing demand for meat in developing countries located in hot climatic zones, lead to a rising heat stress of farm animals. This heat stress results in reduced performance as well as decreasing health. Furthermore, the intestinal health of weaned pigs and fast-growing broiler is very problematic in the first weeks of life. Because of the EU ban of antibiotic performance enhancers in 2006 and restrictions in South Korea (2011) and USA (2017) as well as the increasing problematic of antibiotic resistances, research for alternative substances has become more and more important. Besides pre- and probiotics, especially phytogetic substances are very promising. Phytogetic substances comprise plant-derived products like herbs, spices or essential oils. Compared to synthetic and nature identical single substances, phytogetic feature a broader effectiveness. As a matter of fact, natural additives result in a key feature of multi-species animal nutrition. The addition of feed additives in the livestock sector should not only account for economic efficiency and the quality aspect of animal products, but should also consider food safety and the compliance to environmental guidelines. The antioxidative impacts of phytogetic substances lead to an increasing oxidative stability of nutrients in animal food and feed. The antimicrobial effects result in a reduction of pathogenic and unwanted microorganisms in the intestinal tract.

Selected phytogetic substances are characterized via molecular biological methods in order to identify substances containing preventative impacts on oxidative stress, inflammatory parameters and degradation of the intestinal barrier resulting in positive effects on animal health. For this purpose, cell culture models using viability tests as well as gene expression analysis are applied. In addition, tissue samples from animal experiments are investigated using gene expression analysis by quantitative real-time PCR. Precisely, we are working on gene expression analysis of broiler tissue samples. The genes of interest including heat shock proteins, anti-oxidative protection enzymes and tight junction parameters are analyzed for possible up- or down regulation in order to quantify putative effects regarding to heat stress and intestinal health.

**PS3:MM-10**  
**Protective mechanisms of phytogetic compounds in various live-stock species**

Georg Sandner\(^1\), Verena Stadlbauer\(^1\), Andreas Müller\(^2\), Julian Weghuber\(^1\)

\(^1\) Food Technology and Nutrition, University of Applied Sciences Upper Austria, Austria  
\(^2\) Delacon Biotechnik GmbH

In recent years through science we experience what is called the replication crisis (one can even find this on wikipedia: https://en.wikipedia.org/wiki/Replication_crisis). More than 50% of published results are not reproducible, which equates billions of dollars down the drain and working hours in vain.

In a joined effort, the COST initiative ARBRE-MOBIEU (Association of Resources for Biophysical Research in Europe - Molecular Biophysics in Europe: https://arbre-mobieu.eu/) and P4EU (Protein Production and Purification Partnership in Europe: https://p4eu.org/) collected statistical data about the status of protein samples delivered for scientific characterization and subsequent interaction measurement. In addition a minimum quality control protocol was established that will tackle sample quality issue related reproducibility problems. Conclusions from those collected data show, that conducting the minimum quality check for proteins (which takes about one day) can predict the rate of success for subsequent measurements and can therefore help reducing costs and manpower spent on poor samples (soon to be published).

Another effort by ARBRE-MOBIEU is the benchmarking of biophysical equipment throughout european labs and the creation of standard operation procedures (SOP) for protein related measurements. So far benchmarks for circular dichroism (CD) spectroscopy and isothermal titration (ITC) measurements have been conducted and a benchmark related to microscale thermophoresis (MST) is developed right now. The establishment of a standard sample for protein interaction, that can be used to cross validate measurements from different biophysical equipment is planned as well.

The Vienna Biocenter Core Facilities GmbH (VBCF: https://www.vbcf.ac.at/home/), a government funded non profit research facility, provides subsidized access to equipment and know how about protein characterization and interaction measurements (as well as various other specializations). Together with the Central European Institute of Technology (CEITEC: https://www.ceitec.eu/) VBCF implements an EU funded Interreg project to facilitate partnership and strengthening the scientific impact of the Vienna (Austria) - Brno (Czech Republic) region. Throughout this Interreg project SOPs for minimal quality control are established (conforming the suggestions from ARBRE-MOBIEU and P4EU) and full services for protein characterization are developed to be available for public access in 2019.

**PS3:MM-11**  
**Tackling the reproducibility crisis by establishing standards and minimal quality criteria for recombinant protein measurements, a combined effort by ARBRE, P4EU, VBCF and CEITEC.**

Arthur Sedivy, Peggy Stolt-Bergner  
Protein Technologies, Vienna Biocenter Core Facilities GmbH, Austria
PS3:MM-12  Complementary intestinal and microbiome responses to caloric restriction

Kalina Duszka1, Sandrine Ellero-Simatos2, Ghim Siong Ow3, Marianne Defernez4, Eeswari Paramalingam5, Adrian Tett1, Shi Ying2, Jürgen König1, Arjan Narbad4, Vladimir Kuznetsov1, Hervé Guillou1, Walter Wahl1

1 Department of Nutritional Sciences, University of Vienna, Austria
2 Toxalim, Université de Toulouse, Toulouse, France
3 A*STAR Biomedical Sciences Institutes, Singapore
4 Quadram Institute Bioscience, Norwich, Norfolk, UK
5 Lee Kong Chian School of Medicine, Nanyang Technological University Singapore, Singapore

The intestine is a key organ for energy and micronutrient absorption as well as for interactions of the whole organism with microbes. It constitutes a first-line organ exposed to diet changes. Thus, its response to caloric restriction (CR) is thought to be more complex than that of any other organ. We asked whether the adaptations of host and microbiome to CR are interconnected, and what pathways are involved in the intestinal CR phenotype. We submitted mice to 25% CR and we saw that this diet induced a polarization of intestinal epithelium gene expression. These changes are characterized by an up-regulation and down-regulation of the metabolic and immune/inflammatory systems, respectively. The HNF, PPAR, STAT and IRF families of transcription factors were identified as potentially critical players in this process, particularly Ppara and Lsgf3. The impact of CR on metabolic genes was mimicked by inhibition of the mTOR pathway. Furthermore, CR triggered microbiota and metabolites composition changes in proximal as well as the distal gastrointestinal tract. Performed assignment of bacteria to metabolites suggests novel CR-dependent metabolic inputs of several bacterial strains. Faecal metabolites analysis identified changes in the concentration of multiple metabolites; notably, among others branch chain amino acids, short and medium chain fatty acids. Both small intestine content and faeces showed a decrease in choline. Further experiments using germ-free mice and CR-specific microbiota transfer proven that the gene expression polarization observed for CR mice is independent of the microbiome and its metabolites. The holistic approach that we applied allowed us to characterize various aspect of host and microbiota response to CR.

PS3:MM-13  Helicobacter pylori HtrA activity and E-Cadherin cleavage is influenced by divalent cations

Sabine Bernegger, Markus Huemer, Thomas P. Schmidt, Gernot Posselt, Silja Weßler

Biosciences, University of Salzburg, Austria

High temperature requirement A (HtrA) is a chaperone and serine protease expressed by the human pathogen and class I carcinogen Helicobacter pylori (Hp). During infection HtrA is able to actively cleave the adherens junction (AJ) protein and tumor suppressor E-Cadherin on gastric epithelial cells, which induces disruption of the integrity of the gastric epithelium and thereby enables the bacterium to enter the intercellular space. Further, E-Cadherin shedding also contributes to carcinogenesis as it interferes with signaling pathways in the host cell. The functionality of AJs is mostly established by calcium dependent, homophilic interactions of the extracellular domains of E-Cadherin between neighboring cells. Hence, the aim of this project was to investigate the effect of calcium as well as other divalent cations on E-Cadherin cleavage by HtrA and on the activity of HtrA directly. A special focus lies on identifying ions with an inhibitory effect on HtrA.

For this purpose in vitro cleavage experiments with recombinant human E-Cadherin and HtrA as well as casein zymography and in vitro degradation assays were performed in the presence or absence of different divalent cations. In this context a strong inhibitory effect of calcium, copper and zinc was observed on HtrA mediated E-Cadherin cleavage, which suggests that these ions mask putative cleavage sites upon binding to E-Cadherin. However, in casein degradation assays and zymography, we observed that calcium did not affect HtrA activity, while zinc and copper efficiently blocked HtrA mediated casein proteolysis. Further, nickel and cobalt and, to a lesser extent, also manganese and calcium ions seemed to activate the proteolytic activity of HtrA. From these data we conclude that calcium ions blocked E-cadherin cleavage through direct binding to E-cadherin, while zinc and copper exhibited direct inhibitory effects on the HtrA protease. So far it is not clear how copper and zinc ions interfere with HtrA and modulate its protease activity. Hence, ion binding assays are currently being performed with HtrA and recombinant human E-Cadherin in isothermal titration calorimetry (ITC) and microscale thermoporesis (MST) experiments to determine whether the observed inhibitory effects of zinc and copper are caused by binding directly to HtrA or indirectly by binding to the E-Cadherin molecule. This knowledge would facilitate the development of more potent and highly specific HtrA inhibitors combating Hp infections.
PS3:MM-14  Evaluation of MAPK singalling in the recovery from host-induced tip depolarization during in *T. atroviride* mycoparasitism

Dubraska Moreno-Ruiz⁰, Alexander Lichius⁰, Mark Fricker¹, Susanne Zeilinger⁰

¹ Department of plant biology, University of Oxford, England.

² Institute for Microbiology, University of Innsbruck, Austria

*Trichoderma atroviride* is a mycoparasitic fungus used as biological control agent against plant-pathogenic fungi. Because sensing and recognition of prey-derived signals are essential for the successful mycoparasitic fungus-fungus interaction, we have established microcolony confrontation assays that analyse the virulence of *T. atroviride* towards different host species including *Rhizoctonia solani*, *Fusarium oxysporum* (both are plant root pathogens), *Botrytis cinerea* (foliar plant pathogen) and *Sclerotinia sclerotiorum* (attacks all parts of the plants). Cdc42-Rac1 Interactive Binding (CRIB) reporters highlight the activity of GTPases during polarized tip growth. In this study, the first CRIB reporter was established in a mycoparasite to dissect the initiating stage of the mycoparasitic process, comprising host recognition and chemoattraction. Confrontation assays using CRIB reporter technology revealed for the first time that localised GTPase activity undergoes significant changes in the vicinity of host hyphae, indicative for extensive host defense responses that disrupt the polarized tip growth apparatus of the mycoparasite. The severity of stress-induced depolarization depends on the host-species. For instance in confrontation with *B. cinerea*, the CRIB signal completely vanishes from *T. atroviride* tip apices and becomes internalized into what appear to be degradation vacuoles, whereas in confrontation with *S. sclerotiorum* the apical CRIB signal disappears from tip apices but is not seen internally. These differences lead us to hypothesise that *T. atroviride* is challenged by different kinds of host defense responses which likely have to be overcome in different ways to finally achieve mycoparasitic overgrowth of the host mycelium. The perception of host-derived signals is integrated with changes of the polarized tip growth apparatus via GTPase and MAPK signaling pathways. Previous studies showed that gene deletion of the MAP kinases Tmk1 and Tmk3 (MAPKs of the Slt2 and Hog1 pathways, respectively) affect mycoparasitism only partially, or not at all. Interestingly, the involvement of Tmk2 (MAPK of the Fus3 pathway) has so far not been investigated. We therefore generated a *atmk2* mutant by integrating the CRIB reporter into the *tmk2* locus, and are now in the process of evaluating its virulence against various hosts, and compare the dynamics of tip depolarisation and repolarisation in these interactions.

PS3:MM-15  Impact of puromycin on different *Pichia pastoris* strains

Jennifer Staudacher, Brigitte Gasser

Department of Biotechnology, University of Natural Resources and Life Sciences, Austria

Protein synthesis is regulated at several cellular levels, starting with transcription of the gene of interest and subsequent translation of the mRNA. To better understand cellular processes of protein production, rate measurements at limiting steps are necessary. While abundant methods are available to determine transcription levels, measuring translation is still a challenge. Approaches such as feeding cells with labelled amino acids are either expensive or depend on radioactivity. Ribosome based techniques, such as ribosome-profiling, are biased for slowly translated mRNAs. In recent years, the antibiotic puromycin was used as a possible alternative. Puromycin incorporates itself into nascent polypeptide chains, leading to termination. At low concentrations, the rate of translation can be measured. Multiple methods (e.g. Nagelreiter et. al, 2018) were published for different organisms, demonstrating its possibilities. For yeasts, specifically *Saccharomyces cerevisiae*, establishing the puromycin-based method proved to be problematic since three gene knockouts and high antibiotic concentrations were needed.

Hitherto *Pichia pastoris* (syn. *Komagataella* spp), a yeast commonly used for recombinant protein production, was not tested for susceptibility to this antibiotic. Interestingly, in the *P. pastoris* genome we did not find some of the genes responsible for conferring puromycin resistance as in *S. cerevisiae*. Strains of *P. pastoris* with and without two single gene knockouts were used here to ascertain said susceptibility to puromycin. We performed experiments to determine the minimum inhibitory and fungicidal concentrations. Based on these data, we conducted spiking experiments to identify the incubation time needed to inhibit cell growth, equalling the incorporation time.

We found puromycin to inhibit growth of *P. pastoris* and therefore measurement of overall translation rates should be possible. For this a single knockout is necessary. The gene is known to influence cell wall permeability, indicating this might be the most problematic part in antibiotic uptake. This work opens the way towards establishing a valuable tool for observation and manipulation of yeast cells during protein production.

PF3-02 Identification of fungi occurring in bread fermentation baskets

Johannes Pitsch¹, Nicole Ollinger¹, Stefan Huemer², Julian Weghuber¹

¹ Biophysics, Johannes Kepler Universität Linz, Austria
² Fischer Brot

The fermentation of dough is a method used since thousands of years in order to make bread more digestible and to extend its shelf life. As a large diversity of microorganisms ubiquitously occurs in the environment, it is hardly possible to achieve a fermentation driven by a single organism and to avoid the growth of unwanted species of bacteria, yeasts and moulds in fermentation racks and chambers. Due to climatic conditions, mould appears at fermentation racks in bakeries. Producers of large scale devices and bakeries try to solve this well-known problem.

To cope with mould and inhibit its formation, cultures on fermentation racks were isolated and transferred on agar plates. Different kinds of agar had to be tested as separation of cultures proved to be a challenge. Yeast in bakery showed significantly faster growth rates compared to mould. For separation of yeast from other cultures, standard microbiological methods like dilution and dilution streaks were unsuccessful. Subsequent culture transfer between malt-extract-, Rose-Bengal-, plate-count- and Czapek-Dox-Agar was necessary to get rid of yeast and to obtain pure cultures.

Beneath standard microbiological identification methods like microscopy, q-PCR was utilized for fungal DNA identification. Via ITS- (internal transcribed spacer) and β-tubulin-primers, specific regions for fungi were amplified and sequenced afterwards. For testing purposes, SEM (scanning electron microscopy) and CT (computer tomography) methods were tested for their applicability.

These methods in combination proved to complement each other and led to identification of several fungal strains with potential health risks.

Quantification and identification methods for mycotoxins have to be established to assess the actual health risk for employees and consumers. Afterwards, evaluation and development of preventive measures will have to be carried out.

PF3-05 Identification of the functional role of the bacterial effector and oncoprotein CagA expressed by Helicobacter pylori in immune cells

Bianca Chichirau

Biosciences, University of Salzburg, Austria

CagA (cytotoxin associated gene A) is one of the most studied virulence factor of the gastric pathogenic bacterium Helicobacter pylori (H. pylori). Its expression is strongly connected to the development of gastric diseases and MALT lymphoma. CagA is injected into the cytoplasm of infected epithelial cells via a specialized type IV secretion system, where it is phosphorylated at the tyrosine residues of the EPIYA motifs by Src and c-Abl family kinases. Recent studies have shown that translocation of CagA also occurs in B cells, however the signal transduction pathways regulated by this protein are still not very well defined. Here, we generated various bacterial strains presenting different genomic mutations of cagA gene, which we then further used in infection experiments to investigate the role of CagA on B cells proliferation, apoptosis and differentiation. The successful translocation of CagA in immune cells has been validated in short term infections performed with the isogenic H. pylori mutant strains. The bacterial adherence to the gastric epithelial cells did not vary significantly amongst the strains, as observed in CFU assays and Alamar Blue experiments. B cell viability was assessed through MTT analyses which revealed that during shorter time points of infection, there is an increase of the proliferation rate in cagA positive infected immune cells as compared to the ones either infected with cagA deletion strain or remaining uninfected. However, long term infections led to a decrease in the cell survival rate of the cells infected with the cagA positive strain.

mRNA expression analyses performed with both infected and uninfected samples revealed an up-regulation and down-regulation of several sets of genes involved in the apoptotic pathway, NF-kB signalling cascade or in the activation of GTPase. Further studies such as qRT-PCR, immunofluorescence assays will be conducted for identifying the impact of CagA in B cell regulation. Moreover, the possibility of using these bacterial mutant strains in mouse model infections will be investigated.
PF3-06  Functional verification of menaquinone biosynthesis genes of *Lactobacillus plantarum* WCFS1 and *Lactobacillus buchneri* DSM20057 by complementation of the respective defective mutants of *Lactococcus lactis* subsp. *cremoris* NZ9000

Nisit Watthanasakphuban  
Food Science and Biotechnology, BOKU, Austria

Lactic Acid Bacteria are non-respiring, facultatively anaerobic fermentative bacteria and are typically cultivated under (micro)anaerobic conditions. In several LAB including *Lactococcus lactis* a respiration-like behavior was observed upon addition of heme to the medium, leading to stimulated aerobic growth, reduced acidification, improved growth efficiency and stress resistance. The components of a respiratory chain: electron donor (NADH dehydrogenases), electron shuttle (LAB use menaquinones) and heme-requiring terminal cytochrome oxidase are present in a non-redundant fashion in *Lac. lactis*. Supplementation of both heme and menaquinone led to respiratory behavior in several additional species, indicating an incomplete respiratory chain lacking quinones. The genome of *L. plantarum* WCFS1 contains only *menA* and *menG*, encoding the last two (of eight) pathway enzymes for menaquinone synthesis. The genome of *L. buchneri* DSM 20057 contains *menE, menB, menA* and *menG*, the genes encoding the first four steps in the pathway are absent. Both genomes contain the genes for the *bd*-type cytochrome oxidase (*CydABCD*). We determined the functionality of the remaining menaquinone pathway genes in *L. plantarum* and *L. buchneri* by functional complementation of respective *L. lactis* deletion strains for reconstituting a complete and functional menaquinone biosynthesis pathway in *L. plantarum* and *L. buchneri*. 
Thursday 20th: Poster Session 3: Synthetic biology

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 3: Synthetic biology
PS3:SB-01  A synthetic biology approach to combat fungal infections

Michael Tscherner¹, Tobias W Giessen¹, Laura Markey², Carol A Kumamoto², Pamela A Silver¹

¹ Department for Medical Biochemistry, Medical University of Vienna, MFPL, Austria
² Graduate Program in Molecular Microbiology, Sackler School of Graduate Biomedical Sciences and Department of Molecular Biology and Microbiology, Tufts University, Boston, Massachusetts, USA

Due to a limited set of antifungals available and problems in early diagnosis invasive fungal infections caused by Candida species are among the most common hospital-acquired infections with staggering mortality rates. Here, we describe an engineered system able to sense and respond to the fungal pathogen Candida albicans, the most common cause of candidemia. In doing so, we identified hydroxyphenylacetic acid (HPA) as a novel molecule secreted by C. albicans. Furthermore, we engineered E. coli to be able to sense HPA produced by C. albicans. Finally, we constructed a sense-and-respond system by coupling the C. albicans sensor to the production of an inhibitor of hypha formation thereby preventing fungal-induced epithelial damage. This system could be used as a basis for the development of novel prophylactic approaches to prevent fungal infections.
Thursday 20th: Poster Session 3: Translational oncology

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

*Poster Session 3: Translational oncology*
PS3:TO-01  ETS1 as key molecule in \textit{BRAF} and \textit{TERT}-promoter mutation-driven glioma
Lisa Gabler\textsuperscript{1}, Johannes Gojo\textsuperscript{2}, Daniela Löttsch\textsuperscript{3}, Dominik Kirchofer\textsuperscript{1}, Sushilla van Schoonhoven\textsuperscript{1}, Christine Pirker\textsuperscript{4}, Hannah M. Schmidt\textsuperscript{1}, Dominik Enslé\textsuperscript{1}, Mirjana Stojanovic\textsuperscript{4}, Jennifer Hsu\textsuperscript{1}, Christine Haberler\textsuperscript{5}, Irene Slavc\textsuperscript{2}, Walter Berger\textsuperscript{1}
\textsuperscript{1} Medical University of Vienna, Institute of Cancer Research, Austria
\textsuperscript{2} Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria; Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Austria
\textsuperscript{3} Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria; Department of Pediatrics and Adolescent Medicine
\textsuperscript{4} Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria
\textsuperscript{5} Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Austria; Institute of Neurology, Medical University of Vienna, Austria
Tumors of the central nervous system constitute the most lethal malignancies in pediatric patients. Interestingly, brain tumors such as pleomorphic xanthoastrocytoma or epithelioid glioblastoma often harbor genomic alterations of \textit{BRAF} (e.g. V600E) leading to hyperactivation of the MAPK pathway. Moreover, activating point mutations in the \textit{TERT} promoter have been described in certain glioma subtypes. However, the role of \textit{TERT} promoter mutations in \textit{BRAF} mutated gliomas has not yet been investigated.
Herein, we interrogated the impact of \textit{BRAF} mutations on \textit{TERT} promoter activity. We applied cell viability and clonogenicity assays and explored \textit{TERT} promoter activity by luciferase reporter assays. Gene expression and downstream signaling upon MAPK pathway inhibition or siRNA knock-down were characterized by use of q-RT-PCR and Western blots, respectively. A panel of primary glioma cell lines with differential \textit{BRAF} and \textit{TERT} promoter mutation status was treated with MAPK pathway inhibitors. Remarkably, \textit{TERT} expression as well as \textit{TERT} promoter activity was only downregulated in tumors with both \textit{BRAF} and \textit{TERT} promoter mutations. As \textit{TERT} promoter mutations are known to generate de novo ETS-binding sites we thought to investigate the role of ETS in further detail. Notably, ETS1 activation and gene expression were significantly downregulated by MAPK inhibitors in \textit{BRAF} mutated tumors. Intriguingly, knock-down experiments proved the functional role of ETS1 as bridge molecule interconnecting MAPK pathway and telomerase reactivation in double mutant tumors. Strikingly, only tumor cells harboring both mutations were sensitive towards ETS-targeting therapy.
In conclusion, our data suggest that concomitant \textit{BRAF} and \textit{TERT} promoter mutations synergistically drive tumor growth. Additionally, we present ETS1 as major player linking the MAPK pathway with telomerase reactivation, thus, ETS-inhibition might represent a promising therapeutic target in these highly aggressive gliomas.

PS3:TO-02  Sample preanalytics & reliable biomolecule isolation – key issues in cell-free saliva diagnostics
Manuela Hofner, Ulrike Kegler, Florian Sammer, Suzana Ilic, Janine Niederhöfer, Klemens Vierlinger, Isabella Stocker, Christa Nöhammer
Center for Health and Bioresources, AIT Austrian Institute of Technology GmbH, Austria
Biomarkers (BM) are measurable biochemical or molecular parameters, which can constitute an indicator for a biologically normal or pathological process. In the medical field they become more and more important, where they can serve as „predictive BM“ (risk of disease), „diagnostic BM“ (diagnosis of disease) or „prognostic BM“ (progression of disease). A particular great potential has been demonstrated for circulating biomarkers found in plasma or serum. However, each blood withdrawal holds potential risks like e.g. danger of infection and can only be performed by a specially trained person. Biomarker analysis in saliva, being an ultrafiltrate of blood, should therefore be a potential simple, safe and non-invasive alternative for the future. Along these lines our current special interest is saliva diagnostics, however, before circulating biomarker-based diagnostic tests can be developed, it is crucial to establish standardized protocols for saliva preprocessing, covering e.g. sample preparation and biomarker isolation.
Here we will report on our recent research activities related to the thorough evaluation of a range of different saliva collection approaches and downstream procedures, including isolation methods for circulating DNA, (mi)RNA and immunoglobulins – which all are challenged by the low levels of circulating salivary biomarkers as well as the special matrix composition of saliva.
PS3:TO-03  Identification of biomarkers for prostate cancer by DNA methylation analysis

Thomas Dillinger1, Elisa Redl2, Walter Pulverer3, Bettina Sprinzl4, Melanie R. Hassler5, Janine Scheibelreiter5, Hajaralsadat Shafiei Afarani5, Helmut Dolznig6, Lukas Kenner6, Andreas Weinhäusel7, Gerda Egger6

1 Ludwig Boltzmann Institute Applied Diagnostics, Austria
2 Clinical Institute of Pathology, Medical University of Vienna, 1090 Vienna, Austria
3 Health & Environment Department, Molecular Diagnostics, Austrian Institute of Technology (AIT), 1190 Vienna, Austria
4 Institute for Medical Genetics, Medical University of Vienna, 1090 Vienna, Austria
5 Clinical Institute of Pathology, Medical University of Vienna, 1090 Vienna, Austria; Ludwig Boltzmann Institute for Cancer Research, 1090 Vienna, Austria; Unit of Pathology of Laboratory Animals (UPLA), University of Veterinary Medicine Vienna, 1210
6 Ludwig Boltzmann Institute Applied Diagnostics, 1090 Vienna, Austria; Clinical Institute of Pathology, Medical University of Vienna, 1090 Vienna, Austria

Introduction: Prostate cancer is the second leading cause of cancer related mortality in men. Elevated levels of the biomarker prostate specific antigen (PSA) are detected in serum of prostate cancer patients. However, PSA levels increase not only in patients with prostate cancer but also under certain benign conditions, resulting in a high level of false positives and makes more specific biomarkers necessary. DNA-hypermethylation of CpG-islands in the promoters of tumor suppressor genes frequently occurs in tumors, resulting in epigenetic silencing. Changes in the DNA-methylation can be measured, making hypermethylated genes promising new biomarkers for prostate cancer detection.

Methods: The genome-wide DNA-methylation profiles of primary prostate cancer and adjacent normal tissue was assessed by using the Infinium HumanMethylation450 BeadChip from Illumina®. Methylation-specific qPCR (qMSP) was used to validate the hypermethylation of the top significant differentially methylated genes in an independent patient cohort. To investigate the biological function of one of the target genes, CRISPR-Cas9 was used to generate knockout cell lines as well as stable transfection of prostate cancer cell lines. Monolayer and spheroid invasion assays were performed.

Results: The DNA-methylation profiles of primary prostate cancers were compared to normal adjacent tissue. Principal component analysis of the obtained data clustered the samples into two distinct groups, based on the differentially methylated CpG sites. We identified several promoter regions of genes that were hypermethylated in prostate cancers. These were successfully validated in an independent cohort using qMSP. Among the top significant hypermethylated genes we found SERPINB1, a serine protease inhibitor shown to play a role for invasiveness in other tumor entities. We identified a negative correlation between SERPINB1 expression and the invasive potential of different prostate cancer cell lines.

Conclusion: Differential methylation between prostate cancer and normal prostate epithelium allows for the clear classification of prostate cancer and normal tissue and provides insight into biologically relevant targets for prostate cancer development. As a next step we aim to design assays for non-invasive detection of our epigenetic biomarker panel in liquid biopsies and urine samples of prostate cancer patients. Furthermore, we plan to do xenograft models with the SERPINB1 knockout cell lines.

PS3:TO-04  A Novel Gene Therapy Approach to Cancer Treatment: a Superactive Thymidine Kinase 1 (superTK1)

Kleissner Theresa, Canfora Eva Maria, Christian Scherhäufli, Sven Budik, Stefan Sack, Julia Scholz, Reinhold Hofbauer

Ctr. of Medical Biochemistry, Div. Med. Genetics, MFPL, MUW, Austria

We have developed a set of mutant human thymidine kinases 1 (TK1), called super TK1, that have at least >12-fold specific activity than the wild type enzyme. Integrated in efficient and tight controllable expression systems, the super TK1 turned out to be suitable for gene therapy of solid tumors. Established as stable transfectants in tumor cells it generates enhanced thymidine monophosphate levels, immediately leading to early S-phase cell cycle arrest (cytostatic effect) and subsequently to cell death (cytotoxic effect). The concept has already been proven in vitro with the pDNA vector pUHD10.3Hyg_superTK1 in combination with a dual Tet-silencing system in several human tumor cell lines (PC-3, MFM223, HeLa, A375, MCF7). The super TK1 was expressed in the presence of various concentration levels of deoxythymidine (dTh) and cell viability assays showed a forceful growth inhibition with doxycyclin induced expression of superTK1. Existing cell culture data with pUHDSuperTK1 stable transfectants showed significant growth reduction under doxycyclin superinduction even at minute dTh levels of <5µM dTh, being very close to serum dTh concentrations. In separate test series we optimized the inhibitory levels of AraC and 5-FU and optimized the lowest dTh concentrations in combination with AraC or 5-FU. The latter being necessary for future animal studies, proving the cytostatic/toxic gene therapy with super TK1 in a preclinical model.
PS3:TO-05  DNA methylation profile differentiates BCG responders from failures in high-risk non-muscle-invasive bladder cancer

Dafina Ilijazi1, Melanie R. Hassler1, Walter Pulverer2, Ursula Lemberger1, Iris Ertl1, David D’Andrea1, Andrea Haitel3, Andreas Weinhaeusel1, Gerda Egger3, Shahrokh F. Shariat1

1 Department of Urology, Medical University of Vienna, Austria
2 Austrian Institute of Technology, Health & Environment Department
3 Medical University of Vienna, Clinical Institute of Pathology, Vienna, Austria

Introduction & Objectives: Intravesical Bacillus Calmette-Guérin (BCG) instillation is the standard of care therapy for patients with high-risk recurrence with the aim of reducing and delaying progression of high-risk non-muscle-invasive bladder cancer (NMIBC). Predictive biomarkers based on molecular characteristics of NMIBC, such as DNA methylation, could be of value in identifying patients at a higher risk of BCG failure. Our aim was to study genome-wide DNA methylation profiles of BCG responders and failures in order to establish a DNA methylation signature predictive of BCG response and identify new therapeutic targets.

Materials & Methods: In a retrospective study, genome-wide DNA methylation profiles high-risk NMIBC tumours from 26 BCG responders and 27 BCG non-responders were obtained by using the Infinium Methylation EPIC BeadChip. Response to BCG was defined based on the guidelines of European Association of Urology. Differentially Methylated Regions (DMRs) were analysed with Ingenuity Pathway Analysis (IPA) and enriched biological themes were identified with the Database for Annotation, Visualization and Integrated Discovery (DAVID). Confirmation of DMRs was performed in the same patient cohort by bisulfite-sequencing of target regions.

Results: Differential DNA methylation was seen in 2001 CpG sites between BCG responders and non-responders (p=9.8x10^-4). BCG responders showed less DNA methylation in active genomic regions such as CpG islands and gene promoters and increased DNA methylation in repressed regions such as heterochromatin and repeats. Significant DMRs were associated with 693 genes belonging to biological processes like bacterial invasion of cells, endocytosis, chemokine signaling and focal adhesion. Selected DMRs were validated in the same patient cohort through bisulfite-sequencing identifying 22 targets with promising biomarker potential. Limitations of the study include its retrospective design and the need for an external validation.

Conclusions: DNA methylation profiling can distinguish patients responding to intravesical BCG immunotherapy from those that may require alternative therapeutic approaches. In patients not responding to BCG, failure may be associated with regulation of genes involved in bacterial invasion of cells. We are currently validating these findings in an independent cohort in order to deliver a tool for clinical decision making on the optimal therapeutic strategy for individual patient.

PS3:TO-06  E-cadherin as a molecular marker of epithelial-mesenchymal transition in breast cancer

Bianca Dietrich, Heidi Miedl, Isabella Bogad, Martin Schreiber

Department of Obstetrics and Gynecology, Medical University, Austria

The tumor suppressor protein E-cadherin is a key cell-to-cell adhesion molecule that is critical for the formation and maintenance of adherens junctions. E-cadherin is a glycoprotein that binds to E-cadherin molecules on adjacent epithelial cells in calcium-dependent homotypic interactions, to maintain the cells within epithelial cell sheets. Carcinoma cells, especially in breast tumors, often undergo an epithelial-mesenchymal transition (EMT) to enable migratory, invasive and stem cell properties. The loss of E-cadherin expression is one of the best described alterations of carcinoma cells undergoing an EMT. E-cadherin is encoded by the CDH1 gene, which is located on chromosome 16q22.1 and spans a region of 100 kb. This locus includes a 5’ high-density CpG island in intron 1 that may be important for transcriptional regulation. It is thought that a decreased expression of CDH1 caused by epigenetic silencing via DNA methylation may promote tumor formation, invasion and metastasis. Here we analyzed the expression of E-cadherin in 120 human breast tumor samples with immunohistochemistry and qRT-PCR. We found that low expression of E-cadherin is associated with a poor overall and disease-free survival, a high proliferation index, a negative progesterone receptor status and a high tumor grade. Using various DNA methylation assays (COBRA, methylation specific PCR and qPCR) we characterized in detail CDH1 promoter methylation status in the same tumors to further correlate it with E-cadherin expression, prognosis, and clinical/histopathological characteristics of human breast cancer. Moreover, we are analyzing the CDH1 locus for copy number variations, particularly homo-/heterozygote deletions, as well as other mutations which can contribute to the loss of E-cadherin. Our data will contribute to a better understanding of the impact of CDH1 promoter methylation or mutation on the biology, important clinical parameters, and outcome of human breast cancer.
PS3:TO-07  Austrian Biobanking and Biomolecular resources Research Infrastructure BBMRI.at – a partner for research using biological samples

Cornelia Stumptner¹, Johann Eder², Georg Göbel³, Melanie Goisau⁴, Christian Gülly¹, Elisabeth Haschke-Becher⁵, Helmuth Haslacher⁵, Sabrina Neururer³, Christine Ruckenbauer⁶, Susanne Öchsner⁴, Petra Story¹, Ingrid Walter⁶, Monika Wieser⁶, Kurt Zatloukal¹

¹ VetCore Facility for Research, University of Veterinary Medicine, Austria
² Alpen-Adria University of Klagenfurt, Klagenfurt, Austria
³ Medical University of Innsbruck, Innsbruck, Austria
⁴ University of Vienna, Vienna, Austria
⁵ Medical University of Vienna, Vienna, Austria
⁶ University of Veterinary Medicine, Vienna, Austria

Background
Biological samples such as tissues, blood, cells and biomolecules (like RNA or DNA) are valuable resources for biomedical research. The availability and exploration of these resources in a high and standardized quality need to be assured.

Methods
The 5 Austrian biobanks at the Medical Universities of Vienna, Graz, Innsbruck and Salzburg and the University of Veterinary Medicine collect, process, and store biological resources and related medical, health and lifestyle data and distribute them to researchers to contribute to the improvements of research and human health. They are all members of the Austrian Biobanking and BioMolecular resources Research Infrastructure BBMRI.at (www.bbmri.at), the Austrian node of the European Biobanking Infrastructure BBMRI-ERIC.

BBMRI.at helps Austrian biobanks in their efforts to support researchers by providing access to high quality biological samples, associated biomedical data, biobanking services and research cooperation.

Results
BBMRI.at’s main activities address the implementation of common sample, data and quality management in biobanks to provide access to quality-defined samples. Examples of achievements are: (1) All BBMRI.at biobanks have committed themselves to establish the recently published pre-analytical quality standards (i.e. Technical Specifications of the European Committee for Standardization (CEN/TS)). (2) BBMRI.at developed an IT-based Self-Assessment-Survey, which allows users to evaluate if their sample handling procedures meet the requirement of the standards. (3) Training courses on sample processing, quality management and biobanking are held for researchers. (4) BBMRI.at provides an online catalog of biobank sample collections available in Austria (www.bbmri.at/catalog). (5) Moreover, BBMRI.at holds public events such as discussion forums with citizens on biobanking-related topics, guided biobank tours, workshops for children and adults (Kids University, Long Night of Research).

Conclusion
Since its foundation in 2013 BBMRI.at has brought together the Austrian biobanking community and developed it further with respect to sample, quality and data management. Annually several million samples are collected which are together with already archived samples provided for academic and industrial research.

Acknowledgements
BBMRI.at is funded by the Austrian Ministry of Education, Science and Research (GZ 10.470/0016-II/3/2013).
PS3:TO-08  Biobanking as Basis for Translational Research in Medicine: Model of Biobank Graz
Franziska Vogl, Annemarie Marold, Gabriele Hartl, Petra Story, Karine Sargsyan
Biobank Graz, Medical University of Graz, Austria

Background
In the last years, sample analysis technologies have become more and more sophisticated. Concomitantly access to high-quality well-documented human biospecimens has gained importance. Nevertheless, researchers complain about an inadequate access to quality biospecimens. This problem is also reflected by a high number of irreproducible results and contributes to failure in oncology trials. The quality of published data directly correlates with the quality of the biospecimens used for a study. This is where Biobanks can support researchers with high-quality data-rich biospecimens.

Methods
Biobanks are collections of biological samples and their associated data, organized in a structured, readily analysable format. Biobank Graz is a central research facility of Medical University of Graz and one of the largest biorepositories in Europe comprising millions of well-organised human blood and tissue samples and provides logistics and infrastructure for optimal support of researchers. Currently, Biobank Graz has more than 20 millions of samples in storage (see BBMRI catalog: http://bbmri.at/catalog) that are collected from selected patients at LKH University Hospital Graz and donors who have agreed and signed an informed consent form.

Results
The samples from Biobank Graz were used for a wide range of research projects from academia as well as industry. The scope of these studies ranges from clinical studies, epidemiological studies and research in the fields of microbiology, biomedicine, pharmacy as well as biotechnology. 192 publications using samples, data or services from Biobank Graz were counted in 2017 only. The cohorts, publications and clinical studies are listed on the homepages of the Medical University of Graz, Biobank Graz and BBMRI.at.

Conclusion
Biobanks are a valuable source for high-quality data-rich biospecimens in translational research. Biobank Graz provides one of the largest collections of biospecimens in Europe and contributes to the creation of new findings and knowledge by providing samples, data and research services.

PS3:TO-09  The FAK inhibitor BI853520 exerts anti-tumor activity in malignant pleural mesothelioma
Zsuzsanna Valkó1, Viktoria Laszlo2, Ildiko Kovacs3, Judit Oszsvar2, Tamas Garay4, Mir Alireza Hoda2, Thomas Klikovits2, Walter Klepetko2, Walter Berger5, Michael Grusch5, Irene Waizenegger6, Balazs Hegeduš7, Balazs Döme2
1 Department of Tumor Biology, National Korányi Institute of Pulmonology, Budapest, Hungary
2 Division of Thoracic Surgery, Medical University of Vienna, Austria
3 Department of Tumor Biology, National Korányi Institute of Pulmonology, Hungary
4 2nd Department of Pathology, Semmelweis University, Hungary
5 Institute of Cancer Research and Comprehensive Cancer Center, Department of Medicine I, Medical University of Vienna, Austria
6 Boehringer Ingelheim Austria GmbH, Vienna, Austria
7 Department of Thoracic Surgery, Ruhrlandklinik, University Clinic Essen, Germany

Background: Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that regulates a wide variety of cellular processes including proliferation, growth factor signaling, migration and survival. Preclinical studies identified FAK as a target in malignant pleural mesothelioma (MPM), especially in tumors with loss of merlin (NF2). In the present study, we evaluated the anti-tumor potential of BI853520, a highly selective FAK inhibitor in preclinical models of MPM.

Methods: IC50 values of adherently growing MPM cell lines (n=3) were measured using Sulforhodamine B (SRB) assay. The migratory activity of 4 MPM cell lines was quantified with 2D videomicroscopy. Spheroid formation assays were performed to test the effect on 3D tumor growth. The expression of merlin protein and the effect of BI853520 on the phosphorylation of FAK and its downstream targets in 2D and 3D cell cultures were investigated by Western blot analyses. Human MPM cells were inoculated orthotopically into SCID mice and treated with 20 mg/kg BI853520 daily intraperitoneally for three weeks. Cell proliferation, apoptosis and microvessel density (MVD) were measured in the xenografts tumors using Ki67, terminal deoxynucleotidyl transferase dUTP nick-end (TUNEL) and CD31 labeling, respectively.

Results: BI853520 had a mild effect on 2D cell growth and migration, but it effectively reduced the growth of MPM spheroids, independently from merlin expression. While FAK phosphorylation was reduced upon treatment, activation of Erk, Akt or S6 remained unaffected both in adherent and spheroid cultures. In vivo, FAK inhibition significantly reduced tumor weight, MPM cell proliferation and angiogenesis.

Conclusion: BI853520 has limited growth inhibitory potential in adherent in vitro cultures but demonstrates a more potent activity both in spheroid formation and in orthotopic xenografts in vivo. Based on our findings, further studies are warranted to explore the clinical utility of BI853520 in human MPM.
PS3:TO-10  Validation of assays for the determination of MGMT promoter methylation levels in glioblastoma

Katja Zappe1, Ru Wang Qiu1, Sabine Spiegler-Kreinecker2, Margit Cichna-Markl1

1 Department of Analytical Chemistry, University of Vienna, Austria
2 Department of Neurosurgery, Neuromed Campus, Kepler University Hospital, Linz, Austria

O6-methylguanine-DNA methyltransferase (MGMT) is a crucial DNA repair protein, which removes cytotoxic alkyl adducts from the O6 position of guanine. However, for the success of chemotherapy with alkylating agents such as temozolomide (TMZ), low activity of MGMT is of advantage. For patients suffering from glioblastoma multiforme (GBM), the most prevalent type of primary malignant tumors of the central nervous system, standard therapy comprises treatment with the drug TMZ. Silencing of MGMT by promoter methylation is associated with lower MGMT activity and longer overall survival of GBM patients. The methylation status of the MGMT promoter is considered a predictive biomarker for the effect of a treatment with TMZ.

Despite intensive research, no optimal predictive detection assay exists so far, but pyrosequencing (PSQ) is considered the method of choice. However, studies on the validation of PSQ methods, especially those dealing with the influence of an amplification bias on over- or underestimation of the detected DNA methylation levels, are scarce.

In this study, we assessed the accuracy and repeatability of two PSQ assays by sequencing the same 12 CpGs of the MGMT promoter in forward and reverse direction. We analyzed bisulfite converted standards of known methylation status and DNA extracts from the T98G cell line and primary cell lines, which were established from GBM patients. GBM samples were grouped by MGMT expressing and non-expressing GBMs according to Western blot results. The repeatability of the assays turned out to be sample dependent. For determining the accuracy, accurately methylated and unmethylated standards are required. Since the commercial standards were of poor quality, we started with in-house preparation of unmethylated and completely methylated standards.

Our data support the theory of a PCR or sequencing bias caused by secondary structures of CG rich regions. With our study, we want to increase the precision and accuracy of PSQ methods, which are necessary for analyses aimed to be implemented in clinical routine applications.

PS3:TO-11  Targeted plasma metabolite profiling to identify markers for colorectal cancer detection

Stefanie Brezina3, Andreas Baierl2, Pekka Keski-Rahkonen3, Thomas Bachleitner-Hofmann4, Michael M. Bergmann5, Anton Stift4, Judith Karner-Hanusch4, Gernot Leeb5, Tanja Gumpenberger1, Nina Habermann6, Alexis B. Ulrich7, Augustin Scalbert4, Cornelia M. Ulrich4, Andrea Gsur1

1 Institute of Cancer Research, Medical University of Vienna, Austria
2 Department of Statistics and Operations Research, University of Vienna, Austria
3 Biomarkers Group, International Agency for Research on Cancer, Lyon, France
4 Department of Surgery, Medical University Vienna, Austria
5 Hospital Oberpullendorf, Burgenland, Austria
6 Genome Biology, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
7 Department of General, Visceral and Transplantation Surgery, University of Heidelberg, Germany
8 Huntsman Cancer Institute Salt Lake City, Utah, USA

BACKGROUND. Colorectal cancer is a major public health concern worldwide, remaining the third leading cause of cancer-associated mortality. Novel blood-based biomarkers for colorectal cancer detection and risk prediction could complement existing screening methods. Metabolomics is a powerful approach to unravel metabolic changes associated with carcinogenesis and is increasingly applied as the method of choice for biomarker discovery.

MATERIAL & METHODS. Targeted metabolomics analysis was performed on plasma samples from 385 newly diagnosed colorectal cancer patients, and 543 cancer-free controls, using mass spectrometry and the Biocrates AbsoluteIDQ p180 Kit, covering 188 endogenous metabolites. Samples included in this study derived from the European MetaboCCC consortium, comprising five colorectal cancer cohorts, aiming to investigate metabolic profiles across the continuum of colorectal carcinogenesis. We applied a comprehensive data analysis strategy establishing and validating several multivariate PLS-DA models using an independent discovery and validation data set.

RESULTS. Data on 146 metabolites (acylcarnitines, amino acids, biogenic amines, sphingolipids, glycerophospholipids) were applicable for further analysis in all study samples. The best performing PLS-DA model comprised 57 metabolites (4 components), obtaining an area under the curve (AUC) of 0.87 in the validation data set. Best predictive metabolites included in several prediction models included glutamic acid, histidine, tryptophan, octadecenoylcarnitine (C18:1), hydroxyphosphatidylcholines-acyl C17:0, C18:2 and C28:1, as well as phosphatidylcholine-diacyl C42:3.

CONCLUSION. Our findings suggest that blood-based metabolic markers have the potential to be utilized as non-invasive clinical biomarkers for colorectal cancer detection and may offer a promising diagnostic tool to complement existing screening strategies.

KEYWORDS. Colorectal cancer, targeted Metabolomics, Biocrates AbsoluteIDQ p180, Biomarkers
PS3:TO-12 Immunomodulatory biomarkers in neoadjuvant chemotherapy of breast cancer

Kerstin Wimmer¹, Monika Sachet², Ruth Exner³, Michael Gnant³, Martin Filipits³, Peter Dubsky³, Rudolf Oehler², Florian Fitzal¹

¹ Department of Surgery and Comprehensive Cancer Center
² Dept. of Surgery, Medical University of Vienna, Austria
³ Institute of Cancer Research, Department of Medicine I

Introduction: Neoadjuvant chemotherapy (NAC) with epirubicin/cyclophosphamid followed by docetaxel (E/C→D) is currently one standard-of-care therapy option in women with early, high-risk or locally advanced breast cancer. While some patients respond excellently to preoperative therapy, in other patients significant tumor shrinkage cannot be achieved. The success of anti-tumor therapy is strongly affected by the interplay between cancer cells and immune cells. A reliable marker predicting response to NAC would be clinically useful.

Aim: We investigated the impact of NAC on circulating immunomodulatory parameters. We also examined whether changes in these parameters correlate with the response to NAC measured by the Residual Cancer Burden (RCB) score determined after neoadjuvant treatment.

Methods: To detect drug-specific effects, two different NAC regimens in primary breast cancer patients scheduled to pre-operative therapy were compared. 39 patients with conventional anthracycline/taxane sequence (E/C→D, n=39) and 40 patients with reverse sequence (D→E/C) were included. Blood plasma samples were collected at three time points - "baseline" (before NAC), "midterm" (after the first six cycles of NAC) and "surgery" (after NAC before operation). The plasma levels of uPA, uPAR, TIM-3, MCP-1, MCP-2, OPG, IP-10, CD 27, Eotaxin, Tweak, TRAIL, PD-L2, M-CSF and VEGF-A were determined either by using ELISA or a multiplex bead array immunoassay.

Results: We will present that some of the investigated parameters are differentially regulated depending on the administered NAC scheme. This confirms that NAC affects the immune system in a drug-specific manner. Comparing these results with the RCB-score, will reveal the predictive value of this response.

PS3:TO-13 DNA methylation status of circulating tumor DNA enables therapy response monitoring and assessment of tumor burden

Walter Pulverer⁶, Jagdeep Singh Bhangu¹, Andrea Beer⁴, Martina Mittelboeck¹, Thomas Gruenberger³, Michael Bergmann⁵, Rudolf Oehler⁴, Andreas Weinhäusel⁶, Thomas Bachleitner-Hofmann⁴

¹ Medizinische Universität Wien
² Health&Bioresources, AIT Austrian Institute of Technology GmbH, Austria

Neoadjuvant chemotherapy (neoCtx) followed by hepatic resection is the treatment of choice for patients with colorectal cancer liver metastasis (CLM). However, only about 70% of patients respond to neoCtx. Treatment response is generally assessed using radiologic imaging after several cycles of chemotherapy. Earlier assessment of response would be desirable since non-responders could be switched early to an alternative and more effective chemotherapy regimen, also unnecessary toxicity of ineffective treatment can be avoided. Recent evidence suggests that circulating free methylated tumour DNA is a highly sensitive biomarker and may more accurately reflect tumour burden and treatment response than conventional markers for CRC.

The presented study focused on 2 topics:

1. identification of DNA methylation markers, which correlates with SEPT9 and with tumour burden, and
2. evaluation if the methylation markers can be used for therapy response prediction.

Thirty-four patients with CLM who received neoCtx prior to intended hepatic resection were included in this prospective non-randomized study. Peripheral blood plasma was collected at baseline and before each cycle of neoCtx. Isolated circulating tumour DNA was digested with 4 different methylation sensitive restriction enzymes and then analysed for aberrant methylation of 48 CRC-associated genes using a microfluidic qPCR device. Methylation marker levels were correlated with baseline tumour volume and treatment response and compared with the standard tumour markers CEA and CA 19-9.

The methylation markers SEPT9, DCC, BOLL and SFRP2 were present in all patients at baseline and displayed a stronger correlation with tumour volume than CEA and CA 19-9. Serial measurement of these methylation markers allowed for discrimination between operated and non-operated patients already after 1 cycle of neoCtx with high sensitivity and specificity. The early dynamic changes of SEPT9 and DCC also seemed to correlate with pathohistological response. Methylation values of eleven out of the 48 tested CRC-associated markers showed a strong correlation (>0.80) with SEPT9. Our data suggest that serial measurements of CRC-associated methylation markers could be a particularly valuable tool for early response assessment in patients receiving neoCtx for CLM. We also identified a set of eleven markers, who have the potential to strengthen the value of the CRC-marker SEPT9.
PS3:TO-14 The effects of SNP285 and SNP309 in the MDM2 promoter on breast cancer risk and the expression of MDM2 and p53.

Jürgen Lebhard, Heidi Miedl, Martin Schreiber

Department of Obstetrics and Gynecology, Medical University of Vienna, Austria

The tumor suppressor p53 is a central regulator of cell cycle arrest and apoptosis, which is one of the main obstacles cancer cells have to overcome in their development. This can be achieved either through direct mutation of the p53 gene, deregulation of p53 expression, or aberrant degradation of p53 protein. One major negative regulator of p53 is MDM2, which is in a negative feedback loop with p53. Two single nucleotide polymorphisms (SNPs) in intron 1 of MDM2 can deregulate the expression of MDM2. One of these SNPs, SNP309 (rs2279744 T>G) is associated with an increased risk of breast cancer in Asians but not Europeans, and with an earlier onset of breast cancer in patients with Li-Fraumeni syndrome (LFS). The second SNP, SNP285 (rs117039649 G>C), which only occurs in European populations, tends to be associated with a reduced breast cancer risk. It has been suggested that the effects of these promoter SNPs are a result of a change of binding affinity for transcription factors, mainly SP1.

The genotypes of SNP285 and SNP309 were determined in 406 breast cancer patients and 254 controls via TaqMan genotyping assays. We found that only a small fraction of the examined population carries SNP285 C and only in its heterozygote form (4.7% [12/254] of the healthy controls and 5.6% [23/406] of the breast cancer patients). No SNP285 C homozygote carrier was found. Interestingly, the rare G-allele of SNP309 was associated with a non-significantly increased breast cancer risk in the recessive genetic model, and a delayed onset of breast cancer. SNP285 was not associated with an altered breast cancer risk or age at onset, consistent with previous reports.

We next analysed the impact of SNP309 and SNP285 on MDM2 promoter activity and transcription factor binding by conducting Electrophoretic Mobility Shift Assays (EMSA) with biotin labelled Probes each harbouring one of the 4 possible SNP combinations (G/G; T/G; G/C; T/C) and purified Sp1 protein; Chromatin Immune Precipitation with an SP1 antibody on different breast cancer cell lines; and reporter assays by cloning MDM2 intron 1 into a luciferase reporter plasmid and generating the 4 possible SNP genotype combinations via in vitro mutagenesis. The results of these experiments will be presented.

PS3:TO-15 Characterization of hypoxia-induced changes in a novel three-dimensional canine osteosarcoma model

Nevena Jankovic1, Melanie Korb2, Veronika Strasser2, Georg Csukovich1, Ingrid Walter1

1 University of Veterinary Medicine, Vienna, Austria
2 VetCORE, University of Veterinary Medicine Vienna

Osteosarcoma is one of the most common forms of malignant bone cancer in dogs and adolescent humans. The most distinct inter-species similarity is the location of the metastases in the lungs. This study investigated the applicability of a 3D canine osteosarcoma model for hypoxia studies mimicking the environment in micro-metastases. Therefore, the regional dynamics of 3D osteosarcoma cell cultures were studied by characterizing HIF-1α (Hypoxia-Inducible Factor 1 alpha) and STIP1 (Stress Induced Phosphoprotein 1) as hypoxia markers due to their pro-angiogenic and anti-apoptotic abilities.

The 3D culture was formed by cultivating canine D17 osteosarcoma cells as spheroids which are known to develop a hypoxic core. Samples for western blot were used untreated or exposed to 100mM cobalt chloride to stabilize hypoxic conditions prior to sample preparation. The established 3D microtumor model was analyzed by focusing on protein expression (immunohistochemistry, western blot) as well as gene regulation at three different time points (day 3, 7 and 14). The gene regulation analysis was done by comparing 3D cultures with standard monolayer samples using RT-qPCR. For the detection of gene regulation in specific spheroid regions (necrotic core, proliferative outer layers), cryosections of spheroids were analyzed using laser microdissection and RT-qPCR.

The immunohistochemical detection of HIF-1α and STIP1 demonstrated an increase in the protein expression reaching its peak on day 7; this dynamic change was corroborated by western blot which showed a 1.5 fold change between day 3 and 7 for HIF-1α and a 1.7 fold change between day 7 and 14 for STIP1. RT-qPCR demonstrated an increased HIF-1α mRNA expression on day 3 and 7 (1.96 and 3.3 fold difference vs. the untreated monolayer), while STIP1 expression was elevated on day 7 and 14 (1.63 and 1.42 fold difference vs. the untreated monolayer). The laser dissected samples identified the regions accountable for the production of the two proteins. HIF-1α was mainly produced in the necrotic center of the day 7 spheroids and the proliferative and necrotic region of the day 14 spheroids. The STIP1 results identified both regions of day 7 spheroids and the necrotic center of day 14 spheroids to be involved in the increased mRNA production.

In conclusion, the increase of HIF-1α and STIP1 expression has identified the canine D17 spheroids as a suitable in vitro model.
PS3:TO-16 The role of eosinophils in murine colon cancer
Melanie Kienzl1, Carina Hasenoehrl1, Julia Karigl1, Rudolf Schicho2
1 Otto-Loewi-Research Center, Pharmacology, Medical University of Graz, Austria  
2 BioTechMed, Graz, Austria

Background
Eosinophils are cells of the innate immune system and function as key players in inflammatory diseases. Their role in the development of tumors, however, is less clear. Hence, infiltration of eosinophils into the stroma of solid tumors has been reported, e.g. in melanoma, squamous cell carcinoma and colon cancer. On the one hand, they contribute to the spread of tumors by secreting proteins known to facilitate formation of metastasis and by polarizing macrophages to the tumor promoting M2 phenotype. On the other hand, eosinophils act in a tumor-suppressive manner via recruitment of CD8+ T cells and normalization of tumor vessels. Especially in tumors of colon cancer, tissue eosinophilia accounts for a good outcome in patients.

We aimed to elucidate the role of eosinophils on tumor growth in in vitro assays and in murine tumor models of colon cancer.

Methods and Results
 Supernatants of different colorectal cancer (CRC) cell cultures were used as chemoattractants in 5 µm trans-well plates. Murine eosinophils showed an up to 3-fold increase in their migration to cell line-conditioned supernatants over unconditioned medium. Coincubation of eosinophils with colon carcinoma cells for 24 hrs led up to a 50% decrease in tumor cell viability when compared to medium alone. In order to investigate the role of eosinophils in colon cancer in vivo, we performed an inflammation-induced colon cancer model as well as a subcutaneous tumor model with CRC cell lines. A monoclonal Siglec-F antibody, that depletes eosinophils in vivo, was administered i.p. to evaluate the influence of eosinophils on tumor growth in Balb/c mice. In parallel experiments, eosinophils were injected i.v. into eosinophil deficient GATA-1 mice to compare tumor development with untreated GATA-1 mice. Preliminary data suggest that presence of eosinophils may alter tumor growth.

Conclusion
We show that eosinophils are important effector cells that mediate cytotoxicity in CRC cells and increase chemotaxis to CRC cells in conditioned supernatants. The influence of eosinophils on the tumor microenvironment will be therefore investigated in closer detail in in vivo models.

PS3:TO-17 Data mining approaches to investigate the biological roles of established biomarkers
Raheleh Sheibani-Tezerji, Thomas Dillinger, Loan Tran, Gerda Egger
Applied Diagnosis, Ludwig Boltzmann institute and General Hospital of Vienna, Austria

One of the challenges in medical science is how to translate scientific findings into better clinical results. Information and data are the main input for such a translational process. In the era of postgenomics, public databases such as the Cancer Genome Atlas, have contributed to the development of various Omics data types from 33 cancer types. Omics analysis of these available data have facilitated the discovery of effective cancer biomarkers. Aside from biomarker discovery, access to such a big database provides significant insight into understanding biological changes, modifications and functions of established biomarkers in certain patients. The purpose of this study is to provide an independent analysis of public cancer databases from primary or metastatic tumor samples to investigate the biological roles of known biomarkers and add novel perspectives to biomarker discovery and application.

Existing public cancer databases from TCGA and GEO were used for data mining and to evaluate biological response in cancer patients. We analyzed the correlation between PSMA (and CXCR4) gene expression levels with survival rate, genome-wide gene expression pattern, functional enrichment and reverse phase protein array data in two groups of patients with high and low expression of PSMA (and CXCR4). Both biomarkers are well established for PET imaging of prostate cancer and colorectal cancer with liver metastasis in advanced patients.

Our results showed unique and shared biological roles of PSMA and CXCR4 in two groups of prostate and colorectal patient samples, respectively. We illustrated the differences between the patients on different levels of data especially on gene expression and pathway analysis. The correlation between gene expression data (RNA) and protein data was very low, which could be due to the lack of metadata for protein expression and low number of patient samples available. We present here data mining of publicly available cancer data bases to develop and provide additional support for further use of known biomarkers such as PSMA and CXCR4. These findings will help us to translate our biological knowledge into medical practice for disease prevention, diagnosis or treatment. Translational biology can support personalized medicine to construct predictive models from early diagnosis to monitoring prognosis and predict response to treatments.
PS3:TO-18  Development of 3-D tumor organoids as a preclinical model for colorectal cancer

Loai Tran1, Thomas Dillinger1, Raheleh Sheibani2, Helga Schachner3, Julia Schuler1, Helmut Dolznig4, Andrea Beer2, Judith Stift2, Michael Bergmann5, Thomas L. Mindt1, Markus Zeitlinger1, Judith Simon1, Markus Mitterhauser1, Gerda Egger1

1 Molecular Pathology, Ludwig Boltzmann Institute Applied Diagnostics, Austria
2 Department of Pathology, Medical University of Vienna, Vienna, Austria
3 Oncotest, Charles River, Freiburg, Germany
4 Institute for Medical Genetics, Medical University of Vienna, Vienna, Austria
5 Department of Surgery, Medical University of Vienna, Vienna, Austria

Organoids are 3-D cell clusters that mimic the native organ microstructures and are derived from self-organizing mammalian pluripotent or adult stem cells in vitro. It has been shown previously that tumor organoids reproduced the grade and differentiation capacity of their parental tumors in vitro and in xenografts. Here, we derived organoid cultures from tumorogenic and adjacent healthy tissue obtained from the same patient diagnosed with colorectal cancer. This approach enables the evaluation of the disease state while controlling for potentially confounding factors in the healthy specific genetic background. The organoids can be cultivated in co-culture with cancer associated fibroblasts and are characterized on a histopathological and molecular level. The analysis of the genetic and epigenetic composition of the organoids allow for correlations to be made between the organoid phenotype and a specific mutational and expression profile. Further, organoid-based orthotopic mouse tumor xenograft models will be established for more complex in vivo testing and tumor characterization. For translational-based research, organoids provide the possibility of high throughput analysis of samples from individual patients bridging the gap between basic research and precision medicine. A biobank of human organoids presents a platform for biomarker testing, as well as drug or small molecule screening.

PS3:TO-19  VetBioBank-An Animal Tumor Tissue Bank

Ingrid Walter, Stefanie Burger, Melanie Korb, Stefan Kummer, Marlene Mötz, Christine Ruckenbauer, Monika Wieser

Technology platform VetCore/VetBiobank, University of Veterinary Medicine, Vienna, Austria

Tissues are a precious resource for research, as they enable the application of a wide spectrum of analytical methods. The quality of tissue samples has a major impact on the results and should therefore have highest priority. To reach excellent tissue sample quality, highly standardized procedures and protocols are a prerequisite. Recently, specifications of the European Committee for Standardization (CEN/TS) for preanalytical sample processing were published/released. The Austrian National Node of the European Biobanking and BioMolecular resources Research Infrastructure (BBMRI.ERIC) comprises all four Austrian Medical Universities and the University of Veterinary Medicine Vienna with their biobanks. All BBMRI.at biobanks have committed themselves to establish a pre-analytical sample management according to the appropriate CEN/TS standards. These standards include e.g. for the sampling of tissues (ONR CEN/TS 16826:1-2; ONR CEN/TS 16827: 1-3) documentation of ischemia times, transportation conditions, details of processing, kind of fixation, fixation time for FFPE samples, and more.

The sample strategy of the VetBioBank is to collect clinical tumor samples and “healthy” samples processing them into different sample types, such as FFPE samples, optimal cutting temperature compound-embedded samples for cryosections, pure shock frozen aliquots, and aliquots in RNA later. All frozen samples are stored in the gas phase of liquid nitrogen (-170°C) in monitored tanks, located in a camera surveilled archive. FFPE tissue blocks are also stored under monitored conditions. HE stained slides are prepared from each FFPE sample and scanned to make a digital slide available for evaluation at any time. To date approximately 3000 tumor samples (e.g. feline and canine lymphoma, sarcoma, mastocytoma and squamous cell carcinoma) and 1100 “healthy” samples, which comply to the CEN/TS regulations, are available.

Companion animals are affected by similar diseases, in particular several neoplasms, like humans. Therefore, the spontaneous arising tumor often resembles the human tumor disease and is therefore a better model, as induced tumors in laboratory rodents. It really makes sense to put the same effort in standardized quality management for human and animal samples, to enable comparative translational research.

BBMRI.at is funded by the Austrian Ministry of Education, Science and Research (GZ 10.470/0016-III/3/2013).
PS3:TO-20  Analysis of a three-dimensional canine osteosarcoma–angiogenesis in vitro model

Georg Csu Kovich, Waltraud Tschulien, Veronika Strasser, Nevena Jankovic, Ingrid Walter

University of Innsbruck, Austria

Osteosarcomas are malignant bone tumours and occur more frequently in dogs than in humans, however, both species share significant similarities and therefore the canine osteosarcoma is an accepted model for the human disease. Survival rates in both species are poor because of the high aggressiveness of the tumour and early development of metastases primarily in the patient’s lungs. The devastating spreading process could be inhibited by targeting the vascularisation of the tumour metastases.

This project was aimed to test a three-dimensional (3D) osteosarcoma cell culture system in terms of its applicability to study angiogenic processes in vitro. The applied model is based on a co-culture system of canine osteosarcoma spheroids (D17 canine osteosarcoma cells) and canine lung endothelial cells mimicking the in vitro situation in the growing metastases. We hypothesised that the tumour spheroids produce angiogenic factors due to the hypoxic conditions in the spheroid core and influence proliferation and immigration of the adjacent endothelial cells into the spheroid microtumour. Therefore, canine osteosarcoma spheroids were cultured in direct physical contact or indirect contact (separated by a transwell membrane) with the endothelial cells for 5 days. Single cultures of D17 spheroids and endothelial cells were used as controls. After incubation time, cell cultures were analysed by transmission electron microscopy (TEM), immunohistochemistry (endothelial cell markers CD31, Caveolin-1 and von Willebrand factor as well as proliferation marker Ki67) and by gelatine zymology to determine activities of matrix metalloproteinases 2 and 9.

Results showed that endothelial cells do migrate into tumour spheroids in direct co-culture mimicking the angiogenic process in vitro. Endothelial cells displayed characteristics such as caveolae and formed bilayered structures as shown by TEM. Endothelial cell proliferation was enhanced in both co-culture groups compared to control groups. Important for understanding the processes in the tumour microenvironment, gelatine zymology showed increased ratios of active matrix metalloproteinase 2 in co-cultures without direct contact in contrast to co-cultures with direct contact between tumour spheroids and endothelial cells. We conclude that this 3D co-culture system is well suitable to study angiogenic processes in microtumours.

PF3-03  Role of the NLRP3/IL-1β axis in Acute Myeloid Leukemia (AML)

Michela Luciano¹, Lukas Zell², Stephanie Binder³, Jutta Horejs-Hoeck⁴

¹ Biosciences, University of Salzburg, Austria
² lukas.zell@hotmail.com
³ stephanie.binder@sbg.ac.at
⁴ jutta.horejs_hoeck@sbg.ac.at

Background: Acute myeloid leukemia (AML) is a clonal haemopoietic disorder characterized by hyperproliferation and aberrant differentiation of myeloid progenitor cells. This leads to the accumulation of non-functional cells, termed myeloblasts, in the bone marrow and in peripheral blood resulting in impaired haematoipoiesis and bone marrow failure. Intensive induction chemotherapy has remained the main treatment for AML patient since decades. Yet, the wide heterogeneity driving AML clearly affects the clinical response to chemotherapy. Therefore, one of the main challenge is the identification of unifying mechanisms, involved in the disease initiation and progression, regardless of the mutational status. It has been shown that enhanced IL-1β signaling is frequently observed in hematological disease, including myeloproliferative neoplasm and chronic and acute myeloid leukemia. Recently, Carey et al. have shown that proliferation of almost all AML patient-samples depends on IL-1β signaling, irrespective of the diverse genetic and molecular abnormalities. Therefore, the aim of this study is to investigate the functional role of IL-1β and the molecular mechanisms promoting its secretion in human AML cell lines (hAML).

Results and methods: We show that IL-1β secretion, detected by ELISA, is significantly higher in hAML cells compared to healthy controls. Moreover, higher IL-1β secretion correlates with enhanced cell proliferation, analyzed by BrdU. Moreover, IL-1β treatment of primary human AML cells induces the release of various inflammatory mediators, as shown by multiplex analyses. Interestingly, blocking of IL-1β signaling by using two different aIL-1β antibodies, results in a concentration-dependent decrease of cell proliferation. Furthermore, qRT-PCR analyses have revealed that an increase of the inflammasome component NLRP3, correlates with enhanced proliferation rates of hAML cell lines. Inhibition of NLRP3 activation by the NLRP3 inhibitor (CP-456773 sodium salt or CRID3) leads to a significant decrease in cell proliferation.

Conclusions: Our study shows that the IL-1β signaling pathway could be involved in promoting the survival and the proliferation of hAML cell lines. Moreover, we have found that activation of the NLRP3 inflammasome could be a key mechanisms leading to IL-1β secretion.
**PF3-04  Tumor-associated macrophages of rectal cancer polarize to the proinflammatory M1 phenotype after irradiation in patients and co-cultures**

**Victoria Stary**, Daniela Unterleuthner, Brigitte Wolf, Johanna Strobl, Andrea Beer, Helmut Dolznig, Michael Bergmann

1 Department for Surgery, Medical University Vienna, Austria
2 Institute of Genetics, Medical University of Vienna, Vienna, Austria
3 Department of Dermatology, Medical University of Vienna, Vienna, Austria
4 Clinical Institute of Pathology, Medical University of Vienna, Vienna, Austria

Tumor-associated macrophages initiate anti-tumoral (M1) or immunosuppressive (M2) responses depending on their polarization status. To test the effects of radiotherapy, we ex vivo irradiated tissue samples of human rectal cancer and assessed the phenotype of macrophages, T cells and NK cells by flow cytometry. We evaluated their distribution after short course radiotherapy (n=45) and compared findings to non-pretreated rectal cancer (n=25) using an immunostaining approach. We further investigated the influence of cancer-associated fibroblasts and cancer cells on the polarization of macrophages after ex vivo irradiation using 3D co-culture models.

Irradiated rectal cancer samples contained less CD68+ macrophages (18.2±2 vs 12.6±3%CD68+/total leukocytes) with a viability of >92% in both groups. Stainings of markers associated with the M1- (CD64, CCR7, iNos, HLA-DR, CD86) or M2-like (CD206, CD163, IL-10, IL-4) phenotype revealed an increase of M1/M2-ratio arguing for a shift from M2- to M1-like macrophages due to irradiation. Macrophages of ex vivo irradiated rectal cancer samples displayed increased phagocytosis. Irradiated tissue sections demonstrated diminished T cell counts (109.7±8.68 vs 45.7±17.26 CD3+ cells No./mm²) but elevated infiltration of NK cells (50.3±15.51 vs 75.9±9.43 CD56+CD3 cells No./mm²). Irradiation of 3D co-culture models led to a dose dependent increase of M1/M2. Untreated macrophages in co-cultures without fibroblasts tended to be less M2 but more M1-like. Neutralizing IL-10 antibody induced M1-like macrophages. Treatment with recombinant IL-10 partly rescued the effects of irradiation.

Our findings highlight macrophages as effector cells upon irradiation by enhancing their anti-tumoral activities and diminishing their immunosuppressive behavior. This study provides a rationale for future investigation aiming for immune-modulation of macrophages to ensure optimal anti-cancer immune-activation.
Thursday 20th: Poster Session 3: Antimicrobial drugs: drug screening and prudent use

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Poster Session 3: Antimicrobial drugs: drug screening and prudent use
Endoperoxides (EP) may act as promising drug candidates against protozoal diseases, including malaria and leishmaniasis. Previous studies have shown that in Leishmania these drugs need intracellular activation by iron compounds to exert their pharmacological function. The EP artemisinin (Art) is preferably activated by heme compounds and that its activation could be linked to a so far not characterized heme degradation pathway in Leishmania. Therefore, the aim of the current study was to elucidate a possible link between heme oxygenase (HO) activity and activation/degradation of Art in Leishmania tarentolae promastigotes (LtP). For this purpose subcellular fractionation of LtP was performed and assays for the determination of HO activity and analysis of Art degradation by gas chromatography were established. For fractionation of LtP a sequence of differential centrifugations was performed resulting in a nuclear/cell debris fraction (NCF), a large granule fraction (LGF), a small granule fraction (SGF) and a microsomal fraction (MF). The HO activity in LtP fractions was assessed using a coupled enzymatic assay using NADPH, hemin and rat liver cytosolic supernatant (providing biliverdin reductase) and subsequent extraction and photometric analysis of the reaction product bilirubin. For analysis of Art degradation a GC method with flame ionization detection was set up. According to a GC/MS analysis peaks at 8.2 and 7.8 min were assigned to Art and a pyrolysis product of Art, respectively. The sum of the peak areas was used for quantification of Art in hexane extracts of LtP fractions. Applying these methods we obtained the following results: (i) Interaction of Art with LtP resulted in decline of Art due to degradation. (ii) HO activity is concentrated in SGF and MF fractions. (iii) Especially SGF and partially MF is highly active in converting Art to breakdown products. The coincidence of HO activity and Art degradation in SGF and MF of LtP suggests a possible role of HO activity for Art activation. Although these data are not a final proof of this relationship they pave the way to the identification of this so far unknown HO protein in LtP with no or poor homology to mammalian HO and its pharmacological importance for EP activation.

We acknowledge the financial support by FWF under grant P 27814-B22.

**PS3:AD-02  Gentisaldehyde and its Derivative 2,3-Dihydroxybenzaldehyde Show Antimicrobial Activities against Bovine Mastitis Staphylococcus aureus**

Andrea Schabauer1, Christoph Zutz1, Barbara Lung2, Martin Wagner1, Kathrin Rychli2

1 Institute of Milk Hygiene, University of Veterinary Medicine Vienna, Austria
2 Veterinary Health Service Laboratory, Ried im Innkreis

Bovine mastitis is a worldwide disease of dairy cattle associated with significant economic losses for the dairy industry. One of the most common pathogens responsible for mastitis is Staphylococcus (S.) aureus. Due to the development and spreading of antibiotic resistance, the search for novel antimicrobial substances against S. aureus is of great importance. The aim of this study was to evaluate the two dihydroxybenzaldehydes for the prevention of bovine mastitis. Therefore we determined the minimal inhibitory concentration (MICs) of gentisaldehyde (2,5-dihydroxybenzaldehyde) and 2,3-dihydroxybenzaldehyde of a diverse set of 172 bovine mastitis S. aureus isolates using an automates robot-based microdilution method. To characterize the bovine isolates we determined the genotype by spa-typing, the antimicrobial resistance to eight antibiotic classes using the disk diffusion method and the MICs of three commonly used antiseptics (benzalkonium chloride, chlorhexidine and iodine). Further, we investigated the cytotoxicity of gentisaldehyde and 2,3-dihydroxybenzaldehyde in bovine mammary epithelial MAC-T cells using the XTT assay. The S. aureus strains showed a high genetic diversity with 52 different spa-types, including five novel types. Antibiotic susceptibility testing revealed that 24% of isolates were resistant to one antimicrobial agent and 3% of isolates were multi-resistant. The occurrence of antibiotic resistance strongly correlated with the spa-type. Both dihydroxybenzaldehydes showed antimicrobial activities with a MIC50 of 500 mg/L. The MIC of gentisaldehyde significantly correlated with that of 2,3-dihydroxybenzaldehyde, whereas no correlation was observed with the MIC of the three antiseptics. Cytotoxicity testing using bovine mammary epithelial MAC-T cells showed that gentisaldehyde and 2,3-dihydroxybenzaldehyde are not toxic at MIC50 and MIC90 concentrations. In conclusion, gentisaldehyde and 2,3-dihydroxybenzaldehyde exhibited antimicrobial activities against a diverse range of bovine mastitis S. aureus strains at non-cytotoxic concentrations. Therefore, both compounds are potential candidates as antiseptics to prevent bovine mastitis and to reduce the use of antibiotics in dairy cows.
**PF3-01 Discovery of novel bioactive compounds through fungal and bacterial co-culture.**

**Roman Labuda**, Martin Gratzl, Desislava Yankova, Kathrin Rychli, Markus Bacher, Christoph Schüller, Jseph Strauss, Martin Wagner

1 University of Natural Resources and Life Sciences, Vienna (BOKU) DAGZ, Department of Applied Genetics and Cell Biology
2 University of Natural Resources and Life Sciences, Vienna (BOKU) DAGZ, Department of Chemistry
3 Institute of Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna (VetMed), Austria

The majority of drugs used in medicine are small molecules of natural or synthetic origin. One of the strongest drivers for the discovery of bioactive compounds for medicinal use was activity screening of compounds derived from natural sources such as plants and microbes. Taking this approach, nature’s chemical diversity has been exploited successfully over the last decades and brought up the most useful and important medical drugs, such as antibiotics (derived from bacteria and fungi), anti-cancer drugs or anti-inflammatory substances. The annotation of microbial genomes revealed that many more genes are present than metabolites known for each sequenced species. Thus generally, microbes do not activate the genes under laboratory conditions and thus underlying biosynthetic pathways potentially producing novel, so far not yet identified products remain inactive. Only under the very diverse and probably competitive conditions of growth in natural habitats these genes might be expressed and the corresponding products might be produced to serve as defense or signaling compound. The goal of the research facility, Bioactive Microbial Metabolites (BiMM, bimm-research.at) is to identify new active substances from fungi and bacteria. This platform comprises high-throughput equipment and provides know-how and scientific personnel to run high content screens for the identification and characterization of novel bioactive metabolites. To obtain bioactive metabolites a library of fungal and bacterial strains were exposed during combinatorial growth (i.e. fungi and bacteria co-culture) in different growth conditions or in the presence of compounds that interfere with epigenetic regulation of fungal gene clusters. We will report on the successful upscaling, preparation and identification of bioactive and novel substances induced by fungal and bacterial co-culture.
10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Molecular microbiology II

Chairs: Günther Koraimann & Brigitte Pertschy
One step back, two steps forward: Toxin-antitoxin modules and persister formation of *Escherichia coli*

**Alexander Harms,** Cinzia Fino, Michael A. Sørensen, Szabolcs Semsey, Kenn Gerdes

BASP Centre, Department of Biology, University of Copenhagen, Denmark

Bacterial persisters are a dormant subpopulation of cells that exhibit greatly elevated tolerance to stresses like antibiotic treatment and are considered to be main culprits behind the recalcitrance of chronic or relapsing infections. The antibiotic tolerance of persister cells is generally seen as a consequence of their dormant physiology in which the cellular processes commonly poisoned by bactericidal antibiotics are inactive. Various pathways of persister formation have been described that likely mirror the notorious heterogeneity of persister cells forming in bacterial cultures. Among these, our group had prominently reported a pathway of *Escherichia coli* persister formation driven by the activation of ten toxin-antitoxin (TA) modules in response to signaling triggered by stochastic peaks of the second messenger (p)ppGpp via polyphosphate and protease Lon.

After these results had attracted both acclaim and skepticism over the years, we systematically revisited the experimental basis of previous studies using a set of methodological improvements and bacterial whole-genome sequencing. Most importantly, we discovered that several of our *E. coli* mutant strains had been inadvertently infected with bacteriophage phi80, a notorious laboratory contaminant. New experiments demonstrated that previously published defects of these mutant strains in antibiotic tolerance were caused by lysogenization with phi80 and not due to inactivation of any specific genetic pathway. We therefore concluded that no evidence for a role of spontaneously activated TA modules in persister formation of unstressed *E. coli* remained. Furthermore, we found that also the cryptic prophages present in the *E. coli* K-12 genome can distort the results generated with commonly used setups of persister experiments. Our current work is focusing on the roles of (p)ppGpp and TA modules in bacterial stress responses including, but not restricted to, persister formation from a fresh perspective.

Autoregulation of *mazEF* expression underlies growth heterogeneity in bacterial populations

**Nela Nikolic**1, Tobias Bergmiller1, Alexandra Vandervelde2, Tanino Albanese3, Lendert Gelens2, Isabella Moll3

1 IST Austria, Austria
2 University of Leuven
3 University of Vienna

Toxin-antitoxin systems are modulators of the physiological response to adverse conditions, and one of the hallmarks of bacterial tolerance to antibiotics and nutrient starvation in the model bacterium *Escherichia coli* as well as in different species of pathogenic bacteria. In this study, we were particularly interested in the *mazEF* toxin-antitoxin system, and the regulation of its expression. The encoded toxin MazF is an endoribonuclease that sequence-specifically cleaves single-stranded RNA regions. Thereby its activation leads to reduction of overall translation and consequently inhibition of bacterial growth. Although autoregulation of *mazEF* expression through the MazE antitoxin-dependent transcriptional repression has been biochemically characterized, less is known about post-transcriptional autoregulation, as well as how both of these autoregulatory features affect growth of single cells during conditions that promote MazF production. Here, we demonstrate post-transcriptional autoregulation of *mazEF* expression dynamics by MazF cleaving its own transcript. Single-cell analyses of bacterial populations during ectopic MazF production indicated that two-level autoregulation of *mazEF* expression influences cell-to-cell growth rate heterogeneity. The increase in growth rate heterogeneity is governed by the MazE antitoxin, and tuned by the MazF-dependent *mazF* mRNA cleavage. Also, both autoregulatory features grant rapid exit from the stress caused by *mazF* overexpression. Time-lapse microscopy revealed that MazF-mediated cleavage of *mazF* mRNA leads to increased temporal variability in length of individual cells during ectopic *mazF* overexpression, as explained by a stochastic model indicating that *mazEF* mRNA cleavage underlies temporal fluctuations in MazF levels during stress. Taken together, the dynamics of *mazEF* expression during prolonged stressful periods fosters controlled growth heterogeneity within clonal populations, and facilitates the efficient exit from stress.
MM2-03  Biased partitioning of the multidrug efflux pump AcrAB-TolC underlies long-lived phenotypic heterogeneity

Tobias Bergmiller, Anna Andersson, Kathrin Tomasek, Enrique Balleza, Daniel Kiviet, Robert Hauschild, Gasper Tkacík, Calin Guet
IST Austria, Austria

The molecular mechanisms underlying phenotypic variation in isogenic bacterial populations remain poorly understood. We report that AcrAB-TolC, the main multidrug efflux pump of Escherichia coli, exhibits a strong partitioning bias for old cell poles by a segregation mechanism that is mediated by ternary AcrAB-TolC complex formation. Mother cells inheriting old poles are phenotypically distinct and display increased drug efflux activity relative to daughters. Consequently, we find systematic and long-lived growth differences between mother and daughter cells in the presence of subinhibitory drug concentrations. A simple model for biased partitioning predicts a population structure of long-lived and highly heterogeneous phenotypes. This straightforward mechanism of generating sustained growth rate differences at subinhibitory antibiotic concentrations has implications for understanding the emergence of multidrug resistance in bacteria.

MM2-04  Metabolic regulation of antibiotic resistance in Pseudomonas aeruginosa

Petra Pusic, Elisabeth Sonnleitner, Udo Bläsi
University of Vienna, Austria

Pseudomonas aeruginosa (Pae) is a major cause of nosocomial infections and a persistent pathogen in the lungs of cystic fibrosis (CF) patients, where it can form anaerobic biofilms. In addition to the intrinsic and acquired antibiotic resistance, the resistance against antibiotics is enhanced in Pae biofilms. Transcriptome studies revealed known and unknown non-coding RNAs that were up-regulated in anoxic biofilms grown in medium resembling the milieu of CF lungs. Among them was the regulatory RNA CrcZ, which was previously shown to bind to, and to sequester the RNA chaperone Hfq. Our previous studies uncovered CrcZ and Hfq as key players in the regulation of carbon catabolite repression and that both impact on the susceptibility of Pae to different antibiotics during anoxic growth. We show that Hfq is required for anoxic biofilm formation and that CrcZ RNA interferes with this process by competition for Hfq. Similarly, CrcZ cross-regulates susceptibility of P. aeruginosa to different antibiotics.

MM2-05  Low-cost DNA amplification-independent microarray characterisation of pathogens

Silvia Schönthaler, Noa Wolff, Michaela Hendling, Ivan Barisic
Health & Bioresources, AIT Austrian Institute of Technology GmbH, Austria

Antimicrobial resistances have become a major problem in global health care. Their appearance is promoted by careless use of antibiotics [1]. Therefore, a reliable and not least affordable identification method is essential. In our previous studies, we introduced a reliable DNA-based microarray, using a novel ligation-based DNA probe concept to provide the required specificity [2, 3]. Here, we combined this approach with a terminal deoxynucleotidyl transferase reaction to reduce the detection costs, especially regarding high-throughput analyses. For that purpose, 45 clinically most relevant antibiotic resistant genes and virulence factors [4] were detected by alternative labelling via elongating of the detection oligonucleotides with a dNTP mixture containing biotin-dUTP. In a second step, these were detected via fluorescence-labelled streptavidin. We proved the reliability of our system using synthetic DNA, then proceeding via PCR-amplified cell lysates of clinical isolates and phylogenetic markers utilizing 16S rRNA genes, and showed comparability with industrially produced labelled DNA nucleotides. The introduced method keeps the possibility to use any other detection system relying on a streptavidin conjugate, while the method itself is capable of being applied to all types of DNA-based microarray analyses.

References

10th ÖGMBT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Antimicrobial resistance: transfer of bugs and genes in diverse ecosystems

Chairs: Martin Wagner & Annemarie Käsbohrer
AR-01 Antimicrobial resistance: Transfer of bugs and genes in diverse ecosystems
Annemarie Käsbohrer
University of Veterinary Medicine, Vienna, Austria

In the last decade, multidrug resistance in Gram-negative bacteria in clinical settings increased worldwide. Horizontal gene transfer contributes largely to the complexity of the problem, among them resistance to cephalosporins, carbapenems and polymyxins. Antimicrobial resistance to cephalosporins, commonly mediated by extended-spectrum β-lactamases (ESBL), is prevalent in the community, in animals, foods, and the environment. Many factors contribute to the broad distribution, among them the usage of antimicrobials in humans and animals. Horizontal transmission of resistance genes, located on transmissible elements, probably plays a much greater role than clonal spread. Two major transmission pathways have to be considered: human-to-human transmission and the exchange of resistance genes between humans, animals, food, and their environment.

Recently, carbapenemase-producing Enterobacteriaceae (CPE) arose as a major concern in human medicine. The isolation of VIM-1-producing *Escherichia coli* and *Salmonella* spp. in 2011–12 from German swine and poultry farms raised concerns that livestock might emerge as a reservoir for CPE. In fact, spread and persistence of the β-lactamase gene (*bla*) VIM-1 in the swine population for at least 4 years was demonstrated. Detailed genomic analyses are needed to uncover potential transmission pathways of these isolates.

Polymyxin compounds (e.g., colistin) have for a long time only been used in exceptional cases in medicine because of the potential of severe side effects. Now, they see a revival as last-resort antibiotics to treat severe infections caused by multidrug-resistant, especially carbapenemase-producing Gram-negative bacteria. Until 2015, resistance against polymyxin was thought to be associated only with chromosomal mutations. In 2015, Liu et al. described a novel genetic determinant, and showed that the mcr-1 (mobilsable colistin resistance-1) harbouring plasmid was transferable. Up to now, mcr-1 prevalence in isolates from inpatients seems to be quite low but widely spread in animals and foods suggesting low transfer frequency from food-producing animals to humans. Further studies are needed to understand the different pathways and the factors having an impact on colonization and transmission. Multiple measures, on the human and veterinary side, have to complement each other to tackle this public health issue. A One Health approach needs to be rigorously established.

AR-02 In-feed antibiotic effects on the swine intestinal microbiome and resistome, as revealed by shotgun metagenomics
Mahdi GHANBARI1, Paul D. COTTER2, Fiona Crispie2, Birgit Antlinger3, Viviana Klose1
1 Biomin Research center, Austria
2 Teagasc Food Research Centre, Moorepark, Fermoy, Cork, and APC Microbiome Ireland, Ireland

Part of discovering and implementing alternatives to in-feed antibiotics is understanding the effects of antibiotics on the membership and resistome of the gut microbiota in livestock animals. Oxytetracycline is an in-feed antibiotic that is widely used in swine production to prevent enteric infections and to improve feed efficiency. In this study, we used the whole metagenome shotgun sequencing approach, to monitor the effect of antibiotic feed additive at a therapeutic level on the dynamics of gut microbiota composition and resistome in post-weaned swine over a 21 day period. Eight pigs received feed containing oxytetracycline and eight received unamended feed. After 1-week of continuous oxytetracycline administration, all pigs were switched to a maintenance diet without oxytetracycline. DNA was extracted from feces taken before, during, and following (2-week withdrawal) oxytetracycline treatment. Phylotype analysis showed the gradual development of the non-medicated swine gut microbiota over the 3-week study, and that the oxytetracyclin-treated pigs had significant differences in bacterial structure and membership relative to non-medicated pigs. Gut resistome analysis revealed the presence of 648 antibiotic resistance genes (ARGs), with piglets receiving in-feed antibiotic were not only significantly rich (p < 0.05) in ARGs but also carried the higher relative abundance of ARGs. The differential abundance analysis showed that the antibiotic administration significantly enriched the abundance of 41 ARGs (q < 0.05), mainly from the tetracycline, beta-lactams and multi drug resistance class. These findings will help to optimize therapeutic schemes of the antibiotic usage in swine production and may inform alternative strategies that preserve the performance benefits while reducing potential risks to human and animal health.
AR-03  Biased partitioning of the multidrug efflux pump AcrAB-TolC underlies long-lived phenotypic heterogeneity.

Anna M. C. Andersson, Tobias Bergmiller, Calin Guet, Gasper Tkacik
Tkacik Group, IST Austria, Austria

The molecular mechanisms underlying phenotypic variation in isogenic bacterial populations remain poorly understood. We report that AcrAB-TolC, the main multidrug efflux pump of *Escherichia coli*, exhibits a strong partitioning bias for old cell poles by a segregation mechanism that is mediated by ternary AcrAB-TolC complex formation. Mother cells inheriting old poles are phenotypically distinct and display increased drug efflux activity relative to daughters. Consequently, we find systematic and long-lived growth differences between mother and daughter cells in the presence of subinhibitory drug concentrations. A simple model for biased partitioning predicts a population structure of long-lived and highly heterogeneous phenotypes. This straightforward mechanism of generating sustained growth rate differences at subinhibitory antibiotic concentrations has implications for understanding the emergence of multidrug resistance in bacteria.

AR-04  The open research platform "BiMM - Bioactive Microbial Metabolites" - a high-throughput biotic and chemical interaction approach to discover novel bioactive compounds

Christoph Schüller¹, Roman Labuda², Kathrin Rychli², Martin Wagner², Joseph Strauss¹
¹ DAGZ, University of Natural Resources and Life Sciences, Vienna, Austria
² Institute of Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna

We discover and characterize bioactive compounds by mimicking conditions in which biotic interactions between different microbial genera occur. Directed generation of biotrophic interactions in co-culture (e.g. bacteria-fungi or fungi-fungi) or drug induced interaction is followed by bioactivity testing of produced metabolites, enzymes, effectors and other compounds. These extracts are generated in a high-throughput format using automated liquid and solid handling systems. Possible new active metabolites are followed up and characterized using activity guided fractionation and NMR and MS based identification. The high throughput infrastructure is designed as open research platform providing hardware and expertise for dedicated collaborative projects in the field of innovative bioactive metabolite and enzyme production and characterization of their properties and function.

We have screened approximately 40,000 co-cultures of different combinations of bacteria and fungi and tested these against six indicator organisms (S.aureus, P.aeruginosa, A.nidulans, S.cerevisiae, C.abicans, F.oxisporum). Among these combinations we identified several interactions producing extracts and compounds with antibacterial and antifungal activity. The hallmark of our screening effort is the induction of an antimicrobial activity in the co-culture which is absent in the axenic cultures. This parameter reduces the likelihood of rediscovery of known active substances.
Bovine mastitis is a global problem which has a negative economic impact on the dairy industry through the loss of both milk and money. Furthermore, severe clinical mastitis negatively affects animals’ welfare. Appropriate information on clinical picture, causative agents and antimicrobial susceptibility are important for treatment and management of disease. The aim of the current study was to evaluate both the influence of various mastitis background factors on the microbiological findings and the influence of species or genotypes on the presence of antimicrobial resistance. Therefore, 3020 quarter milk samples of 647 dairy cows on 166 Austrian farms were collected. Microbial species, spa-genotype (Staphylococcus (S.) aureus) and antimicrobial susceptibility were investigated. A multinomial logistic regression model was applied to investigate the effect of possible categorical influencing covariates (mastitis score, somatic cell count, body temperature, appetite, sampler, reason for sampling) on the microbiological findings (pathogen groups). Additionally, a generalized linear model (GLM) with canonical logit link and binomial response factor was used to analyse the effects of genotypes and pathogen species on the occurrence of antimicrobial resistance. Staphylococci were most common pathogens (16.5% of samples) represented by 34 spa-genotypes. Almost 50% of Enterobacteriaceae were resistant to beta-lactams and 18% were multi-resistant. The majority of streptococci were susceptible to benzylpenicillin. The mastitis score significantly influenced the occurrence of pathogen groups ($\chi^2 = 245.24, p < 0.001$). Enterobacteriaceae isolates had significantly high odds of being present in severe mastitis cases (log odds = 2.84; $p = 0.014$) compared to streptococci. By contrast, staphylococci had significantly lower odds (log odds = -2.24; $p = 0.001$ and log odds = -3.04; $p = 0.007$) of occurring in moderate and severe mastitis cases, respectively, compared to streptococci. This study revealed novel and significant associations between the detection of certain bacterial groups and mastitis severity. Additionally, differences in the occurrence of antimicrobial resistance were observed between bacterial species and S. aureus spa-genotypes. The findings of this study on causative udder pathogens like species, genotypes and the antimicrobial resistance are of great importance for mastitis management and prevention.
Thursday 20th: Translational oncology II

10th ÖGMJT Annual Meeting 2018 & 10th Life Science Meeting Vienna, September 17-20, 2018, Vienna, Austria

Translational oncology II

Chairs: Lukas Kenner & Philipp Staber
TO2-01  HER2 amplification in tumors activates PI3K/Akt signaling independent of HER3
Ana Ruiz-Saenz

Medicine Dept., UCSF, United States of America

Current evidence suggests that HER2-driven tumorigenesis requires HER3. This is likely due to the unique ability of HER3 to activate PI3K/Akt pathway signaling, which is not directly accessible to HER2. By genetic elimination of HER3 or shRNA knockdown of HER3 in HER2-amplified cancer cells, we find residual HER2-driven activation of PI3K/Akt pathway signaling that is driven by HER2 through direct and indirect mechanisms. Indirect mechanisms involved second messenger pathways including Ras or Grb2. Direct binding of HER2 to PI3K occurred through p-Tyr1139, which has a weak affinity for PI3K but becomes significant at very high expression and phosphorylation. Mutation of Y1139 impaired the tumorigenic competency of HER2. Total elimination of HER3 expression in HCC1569 HER2-amplified cancer cells significantly impaired tumorigenicity only transiently, overcome by subsequent increases in HER2 expression and phosphorylation with binding and activation of PI3K. In contrast to activation of oncogenes by mutation, activation by overexpression was quantitative in nature: weak intrinsic activities were strengthened by overexpression, with additional gains observed through further increases in expression. Collectively, these data show that progressive functional gains by HER2 can increase its repertoire of activities such as the activation of PI3K and overcome its dependeny on HER3.

TO2-02  DYRK1B as therapeutic target in Hedgehog/GLI-dependent cancer cells
Wolfgang Gruber1, Martin Hutzinger1, Dominik Patrick Elmer1, Thomas Parigger1, Christina Sternberg1, Lukasz Cegielkowski1, Mirko Zaja2, Johann Leban3, Susanne Michel2, Svetlana Hamm2, Daniel Vitt4, Fritz Aberger1

1 Department of Biosciences, University of Salzburg, Austria
2 4SC AG, Planegg-Martinsried, Germany
3 Department of Pediatrics, Medical University of Vienna, Austria
4 Immunic AG, Planegg-Martinsried, Germany

Aberrant Hedgehog (HH)/GLI signaling is causally involved in the initiation and progression of numerous cancer entities and therefore constitutes a promising target for anti-cancer therapy. Clinically advanced inhibitors of the HH pathway targeting the essential HH effector Smoothened (SMO) are promising therapeutic drugs. However, rapid development of drug resistance, SMO-independent GLI activation and severe side effects limit the application of these SMO modulators. Here we report on the identification of the Dual-Specificity-Tyrosine-Phosphorylation-Regulated Kinase 1B (DYRK1B) as a critical positive regulator of HH/GLI signaling downstream of SMO. Genetic and chemical inhibition of DYRK1B in cancer cells resulted in marked repression of HH signaling and GLI1 expression. Importantly, DYRK1B inhibition profoundly impaired GLI activity in both SMO-inhibitor sensitive and resistant settings. We further introduce a novel small molecule DYRK1B inhibitor, DRYKi, with suitable pharmacologic properties to impair SMO-dependent and SMO-independent oncogenic GLI activity. Furthermore we provide evidence that DYRK1B function is required in GLI-dependent tumor-initiating cancer stem cells in vitro and in vivo.

Our study paves the way for the use of DYRK1B inhibitors for the treatment of HH/GLI-associated cancers where SMO antagonists fail to provide therapeutic benefits due to a priori or acquired resistance. Preclinical testing of an optimized small molecule for a phase I clinical trial is ongoing (Gruber et al. Oncotarget 2016).
**TO2-03  YB-1 – master regulator in mesothelioma malignancy and potential therapeutic target**

Karin Schelch1, Thomas G. Johnson2, Kadir H. Sarun2, Annette Lasham3, Michael Grusch1, Glen Reid4

1 Institut of Cancer Research, Medical University of Vienna, Austria
2 Asbestos Diseases Research Institute, Sydney, Australia
3 Department of Molecular Medicine and Pathology, University of Auckland, New Zealand
4 School of Medicine, The University of Sydney, Australia

Malignant pleural mesothelioma (MPM) is a devastating malignancy affecting the pleural linings of the lung. It is caused by the inhalation of asbestos fibres, but the exact mechanisms are unclear. MPM is characterised by aggressive growth, local invasion, and frequent resistance of chemo- and radiotherapy. Resulting in limited therapeutic options for patients. Y-box binding protein 1 (YB-1) is a multifunctional oncoprotein, often up-regulated in cancer and associated with aggressiveness, drug resistance and poor patient outcome. The aim of this study was to characterise the biological roles of YB-1 in MPM and evaluate its potential as a novel therapeutic target.

YB-1 was highly expressed in MPM cell lines compared to normal non-malignant mesothelial cells, as determined by qPCR and immunoblot. Cells stably overexpressing YB-1 through an expression plasmid showed significantly increased migratory capacity, which was assessed by live cell videomicroscopy followed by single cell tracking. We show that YB-1 is secreted by mesothelial and MPM cells upon asbestos exposure and other stresses including hypoxia and LPS. Secreted soluble YB-1 (sYB-1) is bioactive and stimulates cell migration and expression of the EMT-related genes Snail and Twist. We then knocked down YB-1 via siRNA transfection and observed significantly reduced MPM cell growth, colony formation, migration and invasion in vitro. YB-1 knockdown also sensitised MPM cell lines to cisplatin and vinorelbine chemotherapy, two commonly prescribed drugs in the management of MPM. Finally, when MPM cells transfected with YBX1-specific siRNA were intraperitoneally injected into SCID mice, we observed significantly reduced tumour growth compared to mice injected with control siRNA-transfected cells.

Conclusion: We have shown that YB-1 is an important driver of malignant behaviour in MPM and may represent a novel therapeutic target in this disease, which urgently needs one. Our data highlight a crucial role of both intracellular YB-1 and sYB-1 in the regulation of migration and invasion, which are key characteristics of MPM. Asbestos-induced YB-1 secretion might be a factor in early MPM development. Additionally, YB-1 knockdown reduces MPM growth in vitro and in vivo and sensitised MPM cells to chemotherapy. The central role YB-1 appears to play in the biology and its apparent role in the drug resistance of MPM make it a promising new target for the development of a potential novel therapy.

**TO2-04  Stochastic phenotype switching leads to intratumor heterogeneity in human liver cancer**

Andrija Matak1, Pooja Lahiri1, Ethan Ford2, Daniela Pabst1, Karl Kashofer1, Dimitris Stellas3, Dimitris Thanos3, Kurt Zatloukal3

1 Institute of Pathology, Medical University Graz, Austria
2 University of Western Australia, 35 Stirling Highway, Crawley WA 6009 Australia
3 Biomedical Research Foundation, Academy of Athens, 4 Soranou Efesiou Street, Athens 11527, Greece

Intratumor heterogeneity is increasingly recognized as a major factor impacting diagnosis and personalized treatment of cancer. We characterized stochastic phenotype switching as a novel mechanism contributing to intratumor heterogeneity and malignant potential of liver cancer. Clonal analysis of primary tumor cell cultures of a human sarcomatoid cholangiocarcinoma identified different types of self-propagating sub-clones characterized by stable (keratin-7 positive or keratin-7 negative) phenotypes and an unstable phenotype consisting of mixtures of keratin-7 positive and negative cells, which lack stem cell features but may reversibly switch their phenotypes. Transcriptome sequencing and immunohistochemical studies with the markers Zeb1 and CD146/MCAM demonstrated that switching between phenotypes is linked to changes in gene expression related but not identical to epithelial-mesenchymal transition. Stochastic phenotype switching occurred during mitosis and did not correlate with changes in DNA methylation. Xenotransplantation assays with different cellular sub-clones demonstrated increased tumorigenicity of cells showing phenotype switching, resulting in tumors morphologically resembling invasive component of primary tumor and metastasis. Our data demonstrate that stochastic phenotype switching contributes to intratumor heterogeneity and that cells with a switching phenotype have increased malignant potential.
TO2-05  Investigation of the methyltransferase KMT2C in in vitro systems of prostate cancer

Belinda Schmalzbauer1, Tanja Limberger2, Thomas Dillinger3, Gerda Egger3, Sabine Lagger4, Lukas Kenner1

1 Clinical Institute for Pathology, Medical University of Vienna, Austria
2 (2) Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria
3 (3) Ludwig Boltzmann Institute for Applied Diagnostics, Vienna, Austria
4 (4) Unit of Laboratory Animal Pathology, University of Veterinary Medicine, Vienna, Austria

Introduction
Prostate cancer (PCa) is the most frequently diagnosed malignancy in men. Exome sequencing studies identified the histone methyltransferase KMT2C, a member of the mixed-lineage leukemia (MLL) protein family, as recurrently mutated in PCa. The SET domain harboring the catalytically active center of KMT2C, conveys H3K4me1 at enhancer regions which represents a mark of actively transcribed chromatin. In this study we aim to investigate the role of KMT2C in PCa in vitro models and its mechanistic contribution to tumorigenesis.

Material and Methods
We use established human cell lines and mouse PCa organoids lacking the tumor suppressor PTEN to generate a comprehensive set of KMT2C mutants exploiting the CRISPR/Cas9 system. To monitor the impact of catalytically inactive KMT2C or complete loss of KMT2C on cellular proliferation we use fluorescence-activated cell sorting (FACS). To elucidate phenotypic changes caused by KMT2C mutations we established monoclonal populations from those cultures. Changes in gene expression are further investigated by quantitative Real-time PCR and Western Blot analysis.

Results
Preliminary experiments show that catalytically inactivating mutations of KMT2C that do not disturb total KMT2C protein levels lead to a proliferation advantage in a human PCa cell line. In contrast, complete loss of KMT2C is lethal to those cells. To test the effect of KMT2C mutations in an additional model system we have successfully established mouse PCa organoids. Initial experiments verify that the histological morphology of this three-dimensional culture closely resembles the respective primary tumors.

Conclusion
Our findings suggest that catalytically inactive mutants of KMT2C increase cellular growth, whereas complete loss of KMT2C restricts cellular proliferation. Investigating the mechanistic role of KMT2C in PCa will help to understand its contribution to tumorigenesis in patients with catalytically inactivating KMT2C mutations.

TO2-06  KRAS mutated lung adenocarcinoma depends on EGFR/ERBB signaling

Emilio Casanova
Department of Physiology, Medical University of Vienna, Austria

Several clinical trials using first generation EGFR Tyrosine Kinase Inhibitors (TKIs, erlotinib and gefitinib) in patients suffering of lung adenocarcinoma (AC) harboring activating mutations in the KRAS gene have shown disappointing results. Therefore, it has become a dogma that KRAS driven lung AC do not respond to TKIs and targeting EGFR is not considered a therapeutic option to treat patients suffering of KRAS mutated lung AC. Challenging these observations, we demonstrate that KRAS driven lung AC depends on ERBB signaling and responds to the pan-ERBB inhibitor afatinib.

Analysis of human and murine tumor samples showed activation of the ERBB receptors and expression of their ligands in KRAS driven lung AC. Furthermore, human KRAS mutated advance tumors (stage II and more advance AC) were enriched in the ERBB gene expression signatures compared to stage I tumors. Experimentally, we found that genetic deletion of EGFR in a GEMM of KRAS driven lung AC or in human xenografted A549 cells significantly reduced tumorigenesis, irrespectively of the p53 status. Tumors lacking EGFR showed less cell proliferation and reduced activation of KRAS downstream effectors. As expected, pharmacological inhibition of EGFR using erlotinib or gefitinib failed to inhibit tumorigenesis in KRAS driven lung AC experimental models. However, afatinib (an irreversible pan-ERBB inhibitor) was effective in human/murine cell lines, xenografts, PDXs and GEMM experimental models. Detailed analysis of this observation revealed that genetic deletion or pharmacology inhibition (erlotinib/gefitinib) of the EGFR in KRAS driven lung AC results in a tumor-escape mechanism relying in the activation of other ERBB receptor family members (namely ERBB2 and ERBB3) that can be blocked with the pan-ERBB inhibitor afatinib.

In conclusion, our data shows that KRAS driven lung AC is depending on ERBB signaling. Importantly, we have unraveled a tumor-escape mechanism depending on the (re)activation of non-EGFR ERBB receptors that explains the poor results in previous clinical trials using erlotinib and gefitinib in patients suffering KRAS mutated lung AC. In agreement with this, pan-ERBB inhibition using afatinib effectively abrogates KRAS driven lung AC. Thus, afatinib or other pan-ERBB inhibitors should be a therapeutic option to treat patients suffering of KRAS driven lung AC.
**Author Index**

<table>
<thead>
<tr>
<th>Author</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aberger, Fritz</td>
<td>150, 267</td>
</tr>
<tr>
<td>Ackerbauer, Daniela</td>
<td>169</td>
</tr>
<tr>
<td>Adam, Gerhard</td>
<td>234</td>
</tr>
<tr>
<td>Ahmad, Muhammad</td>
<td>102</td>
</tr>
<tr>
<td>Aigner, Ludwig</td>
<td>169</td>
</tr>
<tr>
<td>Akgul, Tubia</td>
<td>170</td>
</tr>
<tr>
<td>Albanese, Tanino</td>
<td>260</td>
</tr>
<tr>
<td>Alber, Andrea</td>
<td>183</td>
</tr>
<tr>
<td>Aleti, Gajender</td>
<td>235</td>
</tr>
<tr>
<td>Aliabadi, Lorenzo</td>
<td>98</td>
</tr>
<tr>
<td>Alkofer, Anna</td>
<td>105, 106</td>
</tr>
<tr>
<td>Altmann, Friedrich</td>
<td>100</td>
</tr>
<tr>
<td>Alvater, Martin</td>
<td>110</td>
</tr>
<tr>
<td>Aman, Martin</td>
<td>212</td>
</tr>
<tr>
<td>Aminzadeh Gohar, Sepideh</td>
<td>206</td>
</tr>
<tr>
<td>Aminzadeh-Gohari, Sepideh</td>
<td>85, 131</td>
</tr>
<tr>
<td>Amman, Fabian</td>
<td>123, 124</td>
</tr>
<tr>
<td>Andersson, Anna</td>
<td>261</td>
</tr>
<tr>
<td>Andersson, Anna M. C.</td>
<td>264</td>
</tr>
<tr>
<td>Andreas, Martin</td>
<td>189</td>
</tr>
<tr>
<td>Angermayr, S. Andreas</td>
<td>233</td>
</tr>
<tr>
<td>Ankersmit, Hendrik</td>
<td>192</td>
</tr>
<tr>
<td>Antlinger, Birgit</td>
<td>263</td>
</tr>
<tr>
<td>Appelt-Menzel, Antje</td>
<td>98</td>
</tr>
<tr>
<td>Arnold, Andreas</td>
<td>53</td>
</tr>
<tr>
<td>Arnold, Andreas M.</td>
<td>54</td>
</tr>
<tr>
<td>Arnold, Cosmas</td>
<td>74</td>
</tr>
<tr>
<td>Arteaga, Javier</td>
<td>162</td>
</tr>
<tr>
<td>Aschenbroich, Jörg</td>
<td>81</td>
</tr>
<tr>
<td>Assimopoulos, Andreana N.</td>
<td>184</td>
</tr>
<tr>
<td>Aszmann, Oskar</td>
<td>212</td>
</tr>
<tr>
<td>Atanasova, Lea</td>
<td>134</td>
</tr>
<tr>
<td>Autheried, Dominik</td>
<td>232</td>
</tr>
<tr>
<td>Bauer, Johann</td>
<td>176</td>
</tr>
<tr>
<td>Baumert, Rita</td>
<td>115</td>
</tr>
<tr>
<td>Baumgart, Florian</td>
<td>53, 54</td>
</tr>
<tr>
<td>Bayer, Benjamin</td>
<td>203</td>
</tr>
<tr>
<td>Bayer, Michaela</td>
<td>198</td>
</tr>
<tr>
<td>Bazafkan, Hoda</td>
<td>134</td>
</tr>
<tr>
<td>Beer, Andrea</td>
<td>218, 228, 229, 250, 253, 255</td>
</tr>
<tr>
<td>Beier, Sabrina</td>
<td>90</td>
</tr>
<tr>
<td>Beikircher, Gabriel</td>
<td>189</td>
</tr>
<tr>
<td>Beisken, Stephan</td>
<td>86, 93, 115</td>
</tr>
<tr>
<td>Belles, Maria</td>
<td>169</td>
</tr>
<tr>
<td>Benedetti, Bruno</td>
<td>169</td>
</tr>
<tr>
<td>Benitez, Rafael</td>
<td>190</td>
</tr>
<tr>
<td>Berg, Gabriele</td>
<td>101</td>
</tr>
<tr>
<td>Berger, Walter</td>
<td>244, 248</td>
</tr>
<tr>
<td>Berghammer, Gerald</td>
<td>88</td>
</tr>
<tr>
<td>Bergmann, Michael</td>
<td>218, 228, 250, 253, 255</td>
</tr>
<tr>
<td>Bergmann, Michael M.</td>
<td>249</td>
</tr>
<tr>
<td>Bergmann, Sophie</td>
<td>190</td>
</tr>
<tr>
<td>Bergmeister, Helga</td>
<td>157, 178, 196, 211</td>
</tr>
<tr>
<td>Bergmüller, Tobias</td>
<td>260, 261, 264</td>
</tr>
<tr>
<td>Bergna, Alessandro</td>
<td>101</td>
</tr>
<tr>
<td>Bernegger, Sabine</td>
<td>237</td>
</tr>
<tr>
<td>Berthiller, Franz</td>
<td>234</td>
</tr>
<tr>
<td>Beyer, Reinhard</td>
<td>225</td>
</tr>
<tr>
<td>Bianchini, Rodolfo</td>
<td>125</td>
</tr>
<tr>
<td>Bierbaumer, Lisa</td>
<td>97</td>
</tr>
<tr>
<td>Billeck, Andrea</td>
<td>229</td>
</tr>
<tr>
<td>Binder, Stephanie</td>
<td>218, 254</td>
</tr>
<tr>
<td>Birner-Gruenberger, Ruth</td>
<td>199</td>
</tr>
<tr>
<td>Birnleitner, Hanna</td>
<td>228</td>
</tr>
<tr>
<td>Bischof, Karin</td>
<td>226</td>
</tr>
<tr>
<td>Bischof, Robert H.</td>
<td>174</td>
</tr>
<tr>
<td>Bitter, Johannes</td>
<td>105, 106</td>
</tr>
<tr>
<td>Bläsi, Udo</td>
<td>261</td>
</tr>
<tr>
<td>Boccellato, Francesco</td>
<td>151</td>
</tr>
<tr>
<td>Bogad, Isabella</td>
<td>246</td>
</tr>
<tr>
<td>Bohle, Barbara</td>
<td>118, 119, 139, 140</td>
</tr>
<tr>
<td>Böhm, Panja</td>
<td>192</td>
</tr>
<tr>
<td>Böhndorfer, Stefan</td>
<td>108</td>
</tr>
<tr>
<td>Bohnert, Michael</td>
<td>98</td>
</tr>
<tr>
<td>Böll, Simone Lisa</td>
<td>119</td>
</tr>
<tr>
<td>Bollenbach, Tobias</td>
<td>224, 233</td>
</tr>
<tr>
<td>Boras, Dario</td>
<td>203</td>
</tr>
<tr>
<td>Borneman, James</td>
<td>229</td>
</tr>
<tr>
<td>Borodina, Irina</td>
<td>160</td>
</tr>
<tr>
<td>Borth, Nicole</td>
<td>109, 179, 180</td>
</tr>
<tr>
<td>Brader, Günter</td>
<td>184, 235</td>
</tr>
<tr>
<td>Bradl, Monika</td>
<td>113</td>
</tr>
<tr>
<td>Brahms, Heimo</td>
<td>103, 138, 141</td>
</tr>
<tr>
<td>Brezina, Stefanie</td>
<td>249</td>
</tr>
<tr>
<td>Brisson, Alain</td>
<td>143</td>
</tr>
<tr>
<td>Briza, Peter</td>
<td>151</td>
</tr>
<tr>
<td>Brocard, Cécile</td>
<td>203</td>
</tr>
</tbody>
</table>
Author Index

Hageng, Pla................................................. 157, 196
Haidinger, Simon........................................ 203
Haitel, Andrea............................................. 246
Haltrich, Dietmar........................................ 107
Hamm, Svetlana......................................... 267
Hammerl, Peter.......................................... 150
Hamza, Ouafa............................................ 127
Han, Seungmin.......................................... 187
Handl, Monika........................................... 165
Hanneschläger, Christof................................. 77
Hanscho, Michael....................................... 179, 180
Harms, Alexander....................................... 260
Hartl, Gabriele........................................... 248
Haschke-Becher, Elisabeth............................... 247
Haselgruber, Renate.................................... 120
Hasenohrl, Carina....................................... 189, 191
Haslacher, Helmut...................................... 198, 247
Hassler, Melanie R...................................... 245, 246
Hatrakova, Natalia..................................... 183
Hatzl, Anna-Maria..................................... 108, 109
Haubenhofer, Anja..................................... 226
Hauschild, Robert...................................... 261
Hauser-Kronberger, Cornelia......................... 151
Heberle, Fred............................................ 56
Heffeter, Petra.......................................... 192
Hegedüs, Balazs........................................ 248
Heinemann, Akos....................................... 117
Heinz, Veronika.......................................... 51
Heissenberger, Clemens................................. 70, 84, 125, 148
Helbich, Thomas........................................ 229
Hemmer, Wolfgang.................................... 119, 141
Hendling, Michaela.................................... 92, 261
Herbinger, Birgit........................................ 235
Hermann, Marcela....................................... 98
Herwig, Christoph....................................... 84, 88, 110, 160
Hettegger, Peter......................................... 193
Higel, Fabian............................................ 204
Hilber, Karlheinz........................................ 127
Hilger, Christiane....................................... 138
Hinterdobler, Wolfgang................................. 90, 107, 108
Hinterdorfer, Peter..................................... 78
Hintermser, Helmut..................................... 176
Hochreiter, Bernhard................................... 144
Hoda, Mir Alineza...................................... 248
Hofacker, Ivo............................................ 70
Hofbauer, Reinhold..................................... 193, 245
Hofer, Christian......................................... 212
Hofer, Philipp........................................... 198
Hofer, Sebastian........................................ 207
Hohmann, Klaus-Peter................................. 212
Hohmann-Faunberger, Karin........................... 138
Hohmann-Faunberger, Karin........................... 118, 120
Hofmann, Melanie....................................... 67
Hofner, Manuela......................................... 130, 244
Hoheneder, Robin....................................... 174
Holthner, Wolfgang.................................... 96
Holthaus, Karin Brigit................................ 98
Holzmann, Johann....................................... 204
Horak, Fritz............................................... 119
Horvais-Hoeck, Jutta................................. 218, 254
Horner, Andreas......................................... 53
Hortobagyi, David....................................... 200
Howorka, Stefan........................................ 94
Hren, Jernej............................................... 176
Hritz, Jozef............................................... 52
Hsu, Jennifer............................................ 244
Huber, Anna.............................................. 169
Huber, Christian......................................... 206
Huber, Christian G..................................... 150
Huber, Jasmin............................................ 193
Huemer, Markus......................................... 237
Huemer, Stefan.......................................... 217, 239
Humeniuk, Piotr......................................... 118
Humel, Stefan............................................ 55
Huppa, Johannes B...................................... 53
Hußnätter, Kai........................................... 81
Hutzinger, Martin....................................... 267
Hynönen, Ulla........................................... 166, 233

I

Iljic, Suzana.............................................. 244
Ilijazi, Dafina............................................ 246
Inci, Milat................................................. 127
Indra, Dominik.......................................... 193
Itrurri, Jagob............................................. 55, 190

J

JadHAV, VaiBHav.......................................... 109
Jahn-Schmid, Beatrice................................. 119, 140
Jain, Mamta.............................................. 122, 123
Jandová, Zuzana........................................ 52
Janik, Stefan............................................. 192
Jankovic, Nevena.................................... 251
Jankovic, Nevena.................................... 254
Janssen, Stefan.......................................... 61
Jantsch, Michael........................................ 122, 123
Jantsch, Michael F..................................... 124
Jarmer, Johanna......................................... 106
Jenik, Andreas........................................... 192
Jenull, Sabrina........................................... 135, 164
Jeral, Roman............................................. 221
Jesacher, Alexander................................... 198
Jesek, Dominik........................................... 147
Jiang, Chenglin.......................................... 161
Jiang, Hui................................................. 93
Jiang, Yi.................................................... 161
Johnson, Thomas G..................................... 268
Jonak, Claudia........................................... 63
Jonas, Philipp............................................ 230
Jordan, Christian........................................ 96
Jörg, David J............................................. 187
Jorge-Gonzalezewska, Humberto............... 150
Jovanovic, Olga......................................... 57
Jung, Martin............................................. 189
Jun, Claudia............................................... 172

K

Kabasser, Stefan.......................................... 120
Kalic, Tanja................................................ 138
Kalnowski, Jörn......................................... 161
Kalolimath, Somanath................................ 73
Kaly, Maria............................................... 69
Author Index

Kapeller, Barbara .................................................. 157, 196
Kaplan, Daniel H. ........................................... 150
Kapoor, Utkarsh .................................................. 123, 124
Karacs, Jasmine .................................................. 140
Karbiener, Michael ......................................... 199, 200
Kargl, Julia ......................................................... 189, 252
Karimi Aghcheh, Razieh .................................. 85, 111
Karl, Thomas ....................................................... 176
Kanner-Hansuch, Judith .................................. 249
Käsbohmer, Annemarie .................................. 263, 265
Kaser, Klemens .................................................. 198
Kashhofer, Karl ................................................... 268
Kastenhofer, Jens ............................................. 177
Katsaras, John ...................................................... 56
Kaun, Christoph ................................................ 211
Kavnic, Bor ......................................................... 224
Kazci, Enes .......................................................... 170
Kegler, Ulrike ..................................................... 130, 244
Keller, Andreas ................................................... 93
Kellner, Florian ..................................................... 53
Kemptner, Clemens ....................................... 55
Kenner, Lukas ..................................................... 245, 269
Kerndl, Martina .................................................. 67
Keski-Rahkonen, Pekka .................................. 249
Kienesberger, Sabine .................................. 182
Kienzl, Melanie .................................................. 189, 191, 252
Kinaciyon, Tamar .................................................. 118, 139
Kindel, Stefanie .................................................. 183, 185
Kirchofer, Dominik ......................................... 244
Kirsch, Andrijana .............................................. 200
Kiss, Attila ............................................................ 127
Klittcher, Clemens .......................................... 115, 232
Kitzmüller, Claudia ............................................ 119, 139
Kiviet, Daniel ...................................................... 261
Klamt, Steffen .................................................... 89
Klanert, Gerald ..................................................... 109
Klepetko, Walter ............................................... 192, 248
Klikovits, Thomas ............................................. 248
Kloosstock, Thomas ........................................... 66
Klose, Viviana ..................................................... 263
Kmen, Maximilian ............................................. 140
Knabl, Ludwig ..................................................... 93
Knapp, Katja ......................................................... 156, 194, 208
Knecht, Christian ............................................... 165
Knez, Špela .......................................................... 179, 180
Knayzev, Denis ................................................... 51
Koch, Lisa ............................................................... 97
Kofler, Barbara .................................................. 85, 113, 131, 206
Kölblinger, Peter .............................................. 206
Koller, Andreas .................................................. 85, 113, 131
König, Jürgen ...................................................... 237
König, Richard .................................................... 169
König, Sophie ...................................................... 192
Koo, Bon-Kyoung ............................................... 187
Kooistra-Smid, Anna M ......................................... 92
Koraimann, Günther ........................................... 182, 226
Korb, Melanie ................................................... 198, 251, 253
Kortrade, Peter .................................................... 102
Kos, Martin .......................................................... 148
Koš, Martin ........................................................... 70
Koskinen, Kaisa ................................................. 165, 182
Kovacs, Ilidko ...................................................... 248
Krachter, Daniel ................................................. 161
Krainer, Julie .......................................................... 191
Krafts, Klaus ......................................................... 176
Krammer, Teresa ................................................. 70, 84, 125
Krautloher, Jana M ............................................... 108
Kreiter, Jürgen ...................................................... 58
Kremslehner, Christopher ................................... 232
Kreutzer, Christina .............................................. 169
Krisch, Linda ......................................................... 151
Kropatsch, Katrin .............................................. 173
Kuchler, Karl ....................................................... 135, 164
Kuehn, Annette .................................................. 138
Kulminskaya, Natalia .......................................... 166
Kumamoto, Carol A ........................................... 242
Kummer, Stefan ................................................. 253
Kumpitsch, Christina .......................................... 182
Kunert, Renate ..................................................... 147
Kungl, Andreas J ..................................................... 180
Küpcü, Seta ............................................................ 202, 210
Kuten, Olga ........................................................... 145
Kuttner, Roland .................................................. 51
Kuznetsov, Vladimir ............................................ 237

L

Labi, Verena ......................................................... 117
Labuda, Roman ............................................... 217, 258, 264
Laccone, Franco ................................................ 169
Lachner, Julia ....................................................... 98
Laczka, Zsombor ................................................. 145
Ladining, Andrea .................................................. 165
Lagger, Sabine ...................................................... 269
Lahirii, Pooja ......................................................... 268
Laimer, Margit ..................................................... 61, 62, 101
Lal, Madhu .......................................................... 109
Lamanna, William .............................................. 204
Lämmermann, Ingo .............................................. 232
Landowski, Christopher ....................................... 88
Lang, Roland ...................................................... 85, 131, 206
Lanmüller, Hermann ........................................... 212
Lanzerstorfer, Peter ............................................ 120
Lasham, Annette ................................................ 268
Lassmann, Hans .................................................. 113
Laszlo, Viktoria ................................................... 248
Leban, Johann ..................................................... 267
Lebhard, Jürgen .................................................... 251
Lee, Ji-Hyun ........................................................ 187
Leeb, Gernot ......................................................... 249
Leitgeb, Tamara ..................................................... 97
Leitner, Erich ....................................................... 173
Leitner, Michael ................................................... 55
Lemberger, Ursula .............................................. 246
Li, Guofen ............................................................ 107, 183
Li, Yongping ........................................................ 93
Licha, David ........................................................ 206
Lichius, Alexander ............................................ 134, 238
Licht, Konstantin ................................................. 123, 124
Lieber, Roman ...................................................... 178
Lieder, Barbara ................................................... 119
Limerberger, Tanja .............................................. 269
Lin, Grace .............................................................. 97
Lingg, Nico ........................................................... 179
Linker, Torsten ..................................................... 225
Lion, Thomas ......................................................... 164
Liu, Jareed ........................................................... 229
Locker, Felix ......................................................... 85, 113, 131
Logiantara, Adrian ............................................. 141
M

Mach, Robert L.................................................. 222
Machado-Santos, Joana ..................................... 113
Mach-Aigner, Astrid R........................................ 222
Madedo, Gregor M............................................... 165
Madedo, Frank................................................... 207
Madrid, Silvia.................................................... 102
Maghuly, Fatemeh.............................................. 61, 62, 101
Mahfouz, Norhan .............................................. 86, 93, 115
Mahnert, Alexander........................................... 165
Maier, Irene....................................................... 229
Maier, Martin.................................................... 100
Mairhofer, Jürgen............................................... 177
Malianovic, Nermina.......................................... 166
Mann, Evelyne................................................... 165
Mann, Thomas.................................................. 122
Manolaraki, Ioanna............................................. 140
Mansky, Sabine.................................................. 101
Marcello, Mark.................................................. 207
Marchetti, Deschmann, Martina............................ 89
Maresch, Daniel................................................. 100
Marian, Brigitte................................................ 229
Markey, Laura.................................................... 242
Marold, Annemarie............................................. 248
Marquardt, Drew................................................ 56
Marquez, Yasmine............................................... 69
Marsche, Gunther.............................................. 117
Martin, David.................................................... 123
Marx, Hans....................................................... 105, 162
Marx, Lisa........................................................ 54, 57
Mascher, Franz ................................................ 115
Mata, Andrija..................................................... 268
Mattanovich, Diethard...................................... 110, 147, 157, 172, 214, 221, 234
Mayer, Robert.................................................. 204
Mayr, Johannes Adalbert.................................... 206
Mayr, Vanessa.................................................. 103, 141
Mayrhefer, Patrick............................................. 147
Mekpl, Ronald................................................... 207
Melcher, Michael................................................ 109
Mellor, Thiago M............................................... 222
Merger, Sebastian R.......................................... 187
Mesner, Barbara............................................... 211
Metzger, Marco................................................ 98
Mitteilbock, Barbara......................................... 165
Meyer, Thomas.................................................. 151
Michel, Susanne................................................ 267
Miedl, Heidi ...................................................... 246, 251
Milchram, Lisa.................................................. 189, 193
Mildner, Michael .............................................. 232
Mindt, Thomas.................................................. 253
Mittelbock, Martina.......................................... 250
Mitterhauser, Markus........................................ 253
Mittermayr, Florian........................................... 182
Mitz, Veronika................................................... 98
Moissl-Eichinger, Christine................................. 165, 182
Mokesch, Kathrin.............................................. 185
Moll, Isabella..................................................... 260
Monszte, Lianet.................................................. 225
Morel, Francoise............................................... 138
Moreno, Dubraska.............................................. 134
Moreno-Cencerrado, Alberto............................... 55
Moreno-Ruiz, Dubraska...................................... 238
Morisset, Martine............................................... 138
Moser, Bernhard................................................. 144, 192
Moser, Sandra................................................... 173
Mühlhammer, Claudia........................................ 176
Mühl, Marlene................................................... 253
Mueller, Wolfgang............................................. 204
Muhar, Matthias................................................ 74
Mühlender, Severin.......................................... 196
Müller, Leonhard............................................... 192
Müller, Andreas................................................ 236
Müller, Anne..................................................... 151
Müller, Jakob..................................................... 176
Müller, Norbert.................................................. 176
Müller, Stefan................................................... 89
Müller, Ernst..................................................... 66
Müller, Pascal................................................... 101

N

Na, Hyelin........................................................ 156, 187
Nacher, Juan..................................................... 169
Nagelreiter, Fabian............................................ 70, 84, 125, 148
Nagelreiter, Ionela-Mariana................................. 232
Nagl, Christoph................................................ 120
Nanic, Lucia..................................................... 232
Narayanan, Theyencheri..................................... 54
Narbad, Arjan................................................... 237
Narzt, Marie..................................................... 232
Necina, Roman.................................................. 202
Nehrer, Stefan.................................................. 145
Neugebauer, Simone......................................... 200
Neuhaus, Winfried............................................ 97, 98, 127
Neureiter, Daniel............................................... 151
Neururer, Sabrina.............................................. 198, 247
Nguyen, Thu-Ha............................................... 107
Niederhöfer, Janine.......................................... 244
Nikolic, Nela..................................................... 260
Nöbauer, Katharina.......................................... 139, 140
Noe, Kimber, Christa......................................... 130
Noe, Elina, Filomena.......................................... 164
Nöhöhammer, Christa........................................ 130, 244
Novak, Jan....................................................... 100
Novak, Katharina............................................... 84, 110
Novak, Kay Domenico........................................ 108, 109

O

Oberer, Monika................................................. 166, 233
Oberraman, Tobias.......................................... 173
Obinger, Christian............................................ 100
Obritschauer, Walter........................................ 265
Ochsrein, Romana............................................ 70
Author Index

P

Pabinger, Stephan .................................................. 189, 193
Pabst, Daniela .......................................................... 268
Pabst, Georg ............................................................. 54, 56, 57
Padickakudy, Robin .................................................. 230
Padmanabha Das, Krishna Mohan .................................. 166
Paladino, Chiara ......................................................... 103, 141
Palmer, Dieter ........................................................... 141
Palva, Ari ................................................................. 166, 233
Papageorgiou, Vassilios P ........................................... 184
Paramalingam, Eeswari .............................................. 237
Parigger, Thomas ....................................................... 267
Parveen, Zahida .......................................................... 77
Paster, Wolfgang ......................................................... 118
Patsch, Janina ............................................................ 229
Paunovic, Manuela ..................................................... 113
Pausan, Manuela-Raluca .............................................. 165
Pavkov-Keller, Tea ...................................................... 166, 233
Pekarsky, Alexander ................................................... 178
Pendl, Tobias ................................................................ 207
Percher, Valentina ....................................................... 232
Pereira, Leonel ............................................................ 164
Pereyra, David ............................................................ 230
Perras, Alexandra ......................................................... 165
Perthold, Jan Walther .................................................. 51
Pertschy, Brigitte ........................................................ 71, 232
Petrik, Siniša ............................................................... 110
Pfeifer, Lukas Johannes ................................................ 108, 109
Pfeiffer, Sandra .......................................................... 139
Pfeiffer, Georg ........................................................... 192
Pflügl, Stefan ................................................................ 84, 110, 160
Pham, Mai-Lan ........................................................... 107
Piatkowski, Marta ......................................................... 234
Picardo, Ernesto .......................................................... 123
Pichler, Harald ............................................................ 173
Picard, Gerald B. .......................................................... 78
Pilecky, Matthias ........................................................ 93
Pilis, Dietmar .............................................................. 228
Pilis, Vera .................................................................. 232
Pinior, Beate ............................................................... 165, 265
Pinke, Christine .......................................................... 244
Pitsch, Johannes ........................................................ 217, 239
Plattner, Katharina ....................................................... 198
Platzer, Rene .............................................................. 53
Platzer, Sabine ............................................................ 115
Pleško, Maja ............................................................... 235
Ploczek, Thomas J ......................................................... 173
Plum, Achim ............................................................... 93
Podesser, Bruno K ......................................................... 127, 157, 196, 211
Pogatschnigg, Viktoria .................................................. 207
Poggenberg, Vivian ....................................................... 58
Pohl, Elena E ............................................................... 57, 58
Pohl, Peter ................................................................. 51, 53, 77
Polack, Norbert ............................................................ 70, 148
Ponce, Marta ............................................................... 119
Posch, Andreas .......................................................... 86, 115
Posch, Andreas E ........................................................ 93
Posselt, Gernot ........................................................... 151, 235, 237
Potzmann, Paul ........................................................... 211
Pracser, Nadja ............................................................. 98
Pranjić, Monika ........................................................... 105, 106
Preiner, Johannes ....................................................... 53
Preininger, Claudia ...................................................... 183, 185
Priglinger, Eleni ........................................................... 96
Prinz, Marco ............................................................... 65
Promdonk, Boonhiang .................................................. 78
Pulverer, Walter ......................................................... 130, 189, 192, 245, 246, 250
Pum, Alexandra ........................................................ 180
Purtscher, Michaela .................................................... 96
Pusic, Petra ................................................................. 261
Pycha, Sarah ............................................................... 182

Q

Qiu, Ru Wang .............................................................. 249
Quirce, Santiago ......................................................... 140
Quirgst, Judith ............................................................ 257

R

Racz, Bence ............................................................... 207
Radauer, Christian ....................................................... 138, 141
Radler, Philipp ........................................................... 176
Ramoni, Jonas ............................................................ 89, 93
Ramsauer, Andreas ..................................................... 113
Rassinger, Alice .......................................................... 157, 174, 222
Rathner, Petr .............................................................. 176
Ratz, Dieter ................................................................. 148
Rauscher, Sabine ........................................................ 144
Razzazi-Fazeli, Ebrahim ................................................ 139, 140
Rechtsheler, Justyna ..................................................... 235
Redl, Elisa ................................................................. 245
Redl, Heinz ................................................................. 96
Regensburger, Georg ................................................... 89
Regnat, Katharina J ....................................................... 222
Reid, Glen ................................................................. 268
Reindl, Michèle ........................................................... 81
Reithofer, Manuel ......................................................... 119, 140
Rhodes, Nadja ............................................................ 97
Riedelberger, Michael .................................................. 135
Ritter, Markus ............................................................. 169
Rivron, Nicolas ........................................................... 168
Rödel, Philipp .............................................................. 102
Rodic, Nebojša ............................................................ 184
Rogers, Aric ............................................................... 84, 125
Rohrer, Katharina ....................................................... 157, 178, 196
Rollins, Jarod A .......................................................... 84, 125
Román Carrasco, Patricia ............................................. 119
Román-Carrasco, Patricia ........................................... 140
Rose-John, Stefan ....................................................... 228
Rosenthal, Joshua ....................................................... 59
Rossboth, Benedikt ..................................................... 53, 198
Rossen, John W .......................................................... 92

276
<table>
<thead>
<tr>
<th>Author</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sachet, Monika</td>
<td>250</td>
</tr>
<tr>
<td>Sack, Stefan</td>
<td>245</td>
</tr>
<tr>
<td>Sagmeister, Theodor</td>
<td>166</td>
</tr>
<tr>
<td>Sahoglu, Sevilay</td>
<td>170</td>
</tr>
<tr>
<td>Salzer, Ulrich</td>
<td>66</td>
</tr>
<tr>
<td>Salzmann, Linda</td>
<td>134</td>
</tr>
<tr>
<td>Sammer, Florian</td>
<td>244</td>
</tr>
<tr>
<td>Sánchez Acosta, Gabriela</td>
<td>139</td>
</tr>
<tr>
<td>Sandler, Peter</td>
<td>139</td>
</tr>
<tr>
<td>Sandner, Georg</td>
<td>236</td>
</tr>
<tr>
<td>Sanio, Philippe</td>
<td>85, 131</td>
</tr>
<tr>
<td>Santic, Marina</td>
<td>154</td>
</tr>
<tr>
<td>Sarajlić, Dženanica</td>
<td>101</td>
</tr>
<tr>
<td>Sargsyan, Karine</td>
<td>248</td>
</tr>
<tr>
<td>Sarun, Kadir H.</td>
<td>268</td>
</tr>
<tr>
<td>Sauer, Michael</td>
<td>105, 110, 133, 147, 157, 162, 172, 174, 221</td>
</tr>
<tr>
<td>Sauer, Ursula</td>
<td>183, 185</td>
</tr>
<tr>
<td>Scalbert, Augustin</td>
<td>249</td>
</tr>
<tr>
<td>Schabauer, Andrea</td>
<td>257, 265</td>
</tr>
<tr>
<td>Schabauer, Gernot</td>
<td>67</td>
</tr>
<tr>
<td>Schachner, Helga</td>
<td>253</td>
</tr>
<tr>
<td>Schäfer, Ute</td>
<td>207</td>
</tr>
<tr>
<td>Scharnagl, Hubert</td>
<td>208</td>
</tr>
<tr>
<td>Scheibeler, Janine</td>
<td>245</td>
</tr>
<tr>
<td>Scheidt, Tamara</td>
<td>150</td>
</tr>
<tr>
<td>Schelch, Karin</td>
<td>268</td>
</tr>
<tr>
<td>Schenk, Martina</td>
<td>134</td>
</tr>
<tr>
<td>Scherhäuf, Christian</td>
<td>245</td>
</tr>
<tr>
<td>Schicho, Rudolf</td>
<td>189, 191, 252</td>
</tr>
<tr>
<td>Schiefer, Ana-Iris</td>
<td>192</td>
</tr>
<tr>
<td>Schiestl, Robert H</td>
<td>229</td>
</tr>
<tr>
<td>Schilddberger, Anita</td>
<td>93</td>
</tr>
<tr>
<td>Schindl, Rainer</td>
<td>58, 79</td>
</tr>
<tr>
<td>Schipper, Kerstin</td>
<td>81</td>
</tr>
<tr>
<td>Schlager, Sandra</td>
<td>113</td>
</tr>
<tr>
<td>Schmalzauer, Belinda</td>
<td>269</td>
</tr>
<tr>
<td>Schmetterer, Klaus</td>
<td>228</td>
</tr>
<tr>
<td>Schmid, Christian</td>
<td>105, 106</td>
</tr>
<tr>
<td>Schmid, Johannes A.</td>
<td>144</td>
</tr>
<tr>
<td>Schmidt, Hannah M.</td>
<td>244</td>
</tr>
<tr>
<td>Schmidt, Thomas P.</td>
<td>237</td>
</tr>
<tr>
<td>Schmitz-Esser, Stephan</td>
<td>165</td>
</tr>
<tr>
<td>Schmoll, Martin</td>
<td>212</td>
</tr>
<tr>
<td>Schmoll, Monika</td>
<td>90, 107, 108, 183</td>
</tr>
<tr>
<td>Schneider, Karl H.</td>
<td>157, 178, 196</td>
</tr>
<tr>
<td>Schneider, Magdalena</td>
<td>54</td>
</tr>
<tr>
<td>Schneider, Sabine</td>
<td>151</td>
</tr>
<tr>
<td>Schober, Romana Schober</td>
<td>58</td>
</tr>
<tr>
<td>Schöler, Katia</td>
<td>117</td>
</tr>
<tr>
<td>Scholte op Reimer, Yvonne</td>
<td>168</td>
</tr>
<tr>
<td>Scholz, Julia</td>
<td>245</td>
</tr>
<tr>
<td>Schöntaler, Silvia</td>
<td>92, 191, 193, 261</td>
</tr>
<tr>
<td>Schöpf, Veronica</td>
<td>182</td>
</tr>
<tr>
<td>Schosserer, Markus</td>
<td>70, 84, 125, 148</td>
</tr>
<tr>
<td>Schrahböck, Alexander</td>
<td>235</td>
</tr>
<tr>
<td>Schreiber, Martin</td>
<td>246, 251</td>
</tr>
<tr>
<td>Schröder, Sabrina</td>
<td>207</td>
</tr>
<tr>
<td>Schubert, Christian</td>
<td>66</td>
</tr>
<tr>
<td>Schüller, Julia</td>
<td>253</td>
</tr>
<tr>
<td>Schuller, Artur</td>
<td>106</td>
</tr>
<tr>
<td>Schüller, Christoph</td>
<td>217, 225, 258, 264</td>
</tr>
<tr>
<td>Schultze, Simon</td>
<td>208</td>
</tr>
<tr>
<td>Schulz, Rouven</td>
<td>65</td>
</tr>
<tr>
<td>Schütz, Gerhard</td>
<td>198</td>
</tr>
<tr>
<td>Schütz, Gerhard J.</td>
<td>53, 54</td>
</tr>
<tr>
<td>Schweigerlehner, Linda</td>
<td>147</td>
</tr>
<tr>
<td>Schwab, Christian</td>
<td>55</td>
</tr>
<tr>
<td>Schwarze, Uwe Yacine</td>
<td>190</td>
</tr>
<tr>
<td>Schwarzinger, Bettina</td>
<td>100</td>
</tr>
<tr>
<td>Schwesta, Jennifer</td>
<td>62</td>
</tr>
<tr>
<td>Sedyivy, Arthur</td>
<td>236</td>
</tr>
<tr>
<td>Seferovic, Hannah</td>
<td>55</td>
</tr>
<tr>
<td>Segreto, Rossana</td>
<td>134</td>
</tr>
<tr>
<td>Sehr, Eva M.</td>
<td>102</td>
</tr>
<tr>
<td>Sehr, Eva Maria</td>
<td>102</td>
</tr>
<tr>
<td>Seiboth, Bernhard</td>
<td>89, 173</td>
</tr>
<tr>
<td>Seidl, Veronique</td>
<td>235</td>
</tr>
<tr>
<td>Seidl-Seiboth, Verena</td>
<td>89</td>
</tr>
<tr>
<td>Semeraro, Enrico</td>
<td>57</td>
</tr>
<tr>
<td>Semeraro, Enrico F.</td>
<td>54</td>
</tr>
<tr>
<td>Semsy, Szabolcs</td>
<td>260</td>
</tr>
<tr>
<td>Sessitsch, Angela</td>
<td>102, 184</td>
</tr>
<tr>
<td>Sgouras, Dionyssios</td>
<td>235</td>
</tr>
<tr>
<td>Shafiei Afarani, Hajaralsadat</td>
<td>245</td>
</tr>
<tr>
<td>Shariat, Shahrokh F.</td>
<td>246</td>
</tr>
<tr>
<td>Sharif, Omar</td>
<td>67</td>
</tr>
<tr>
<td>Sheibi, Raheleh</td>
<td>253</td>
</tr>
<tr>
<td>Sheibi, Tezerji, Raheleh</td>
<td>252</td>
</tr>
<tr>
<td>Shiloach, Joseph</td>
<td>109</td>
</tr>
<tr>
<td>Shrestha, Niroj</td>
<td>79</td>
</tr>
<tr>
<td>Siebenhandel, Sandra</td>
<td>191</td>
</tr>
<tr>
<td>Siegert, Sandra</td>
<td>65</td>
</tr>
<tr>
<td>Sigrist, Stephan</td>
<td>207</td>
</tr>
<tr>
<td>Siligan, Christine</td>
<td>53</td>
</tr>
<tr>
<td>Silver, Pamela A</td>
<td>242</td>
</tr>
<tr>
<td>Simon, Judith</td>
<td>253</td>
</tr>
<tr>
<td>Simons, Benjamin D.</td>
<td>187</td>
</tr>
<tr>
<td>Singh Bhangu, Jagdeep</td>
<td>250</td>
</tr>
<tr>
<td>Sioung Ow, Ghim</td>
<td>237</td>
</tr>
<tr>
<td>Sisoslok, Bernhard</td>
<td>179</td>
</tr>
<tr>
<td>Sladky, Valentina</td>
<td>208</td>
</tr>
<tr>
<td>Slavc, Irene</td>
<td>244</td>
</tr>
<tr>
<td>Smirnov, Alexandre</td>
<td>124</td>
</tr>
<tr>
<td>Smirnova, Anna</td>
<td>124</td>
</tr>
<tr>
<td>Snow, Santina</td>
<td>84, 125</td>
</tr>
<tr>
<td>Soldo, Regina</td>
<td>193</td>
</tr>
<tr>
<td>Sommer, Andreas</td>
<td>69</td>
</tr>
<tr>
<td>Sommergasser, Wolfgang</td>
<td>179</td>
</tr>
</tbody>
</table>
Author Index

Somoz, Veronika .......................................................... 119
Sonnleitner, Elisabeth ............................................. 261
Sørensen, Michael A. .................................................. 260
Sotoudeh, Mahtab ......................................................... 119
Sotzekin, Ilaya G. ......................................................... 170
Spadiut, Oliver ............................................................ 88, 177, 178, 180
Sperl, Wolfgang .......................................................... 206
Spettel, Kathrin ............................................................ 225
Spieg-Kreinecker, Sabine ............................................. 249
Spittler, Andreas .......................................................... 143, 144
Spitz, Sarah ................................................................. 96
Spitzwieser, Melanie .................................................... 192
Sporer, Matthias ........................................................... 212
Spörhase, Pi ................................................................. 234
Sprinzel, Bettina ........................................................... 245
Stachi, Theresa ............................................................. 200
Stadlbauer, Verena ....................................................... 120, 236
Stadler, Marc ............................................................... 224
Stainer, Sarah ............................................................... 55, 79
Stampfl, Hansjörg ........................................................ 63
Staniek, Katrin ............................................................. 225
Stappfer, Eva ............................................................... 90
Starlinger, Patrick ........................................................ 230
Stary, Victoria ............................................................ 218, 255
Stasnik, Peter ............................................................... 63
Stättner, Stefan ........................................................... 230
Staudacher, Jennifer ..................................................... 238
Stefanie, Horer .............................................................. 140
Steiger, Matthias ......................................................... 133, 157, 172, 174, 221
Steiger, Matthias G. ...................................................... 147
Steinberger, Karoline .................................................... 63
Steinberger, Peter ......................................................... 118
Steiner, Eberhard ........................................................ 198
Steinkellner, Hannes .................................................... 169
Stellas, Dimitris .......................................................... 268
Stelzer, Elena ............................................................... 70
Sterflinger, Katja ........................................................... 139
Sternberg, Christiana ................................................... 267
Stift, Anton ................................................................. 228, 229, 249
Stift, Judith ................................................................. 253
Stock, Isabella ............................................................. 244
Stockinger, Julia ........................................................... 85, 131
Stockner, Thomas ......................................................... 52, 77
Stoeckligner, Angelika .................................................. 150
Stog, Eva ................................................................. 208
Stojakovic, Tatjana ...................................................... 208
Stojanovic, Mirjana ...................................................... 244
Stolt-Bergner, Peggy .................................................... 236
Stolze, Klaus .............................................................. 177
Story, Petra ................................................................. 247, 248
Strandt, Helen ............................................................. 150
Strasser, Bettina .......................................................... 86, 128
Strasser, Katharina ....................................................... 228
Strasser, Richard ........................................................ 100
Strasser, Veronika ......................................................... 251, 254
Strauss, Joseph ............................................................ 225, 264
Strauss, Joesph ............................................................ 217, 258
Striedner, Gerald ......................................................... 106, 177, 179, 203
Stroblo, Johanna .......................................................... 218, 255
Strobi, Maria R. ............................................................ 139
Strohmeyer, Gernot A. ................................................... 173
Strohmeyer, Akim ........................................................ 176
Stübli, Flora ............................................................... 120
Stulic, Maja ............................................................... 122, 123
Stumpfner, Cornelia ..................................................... 198, 247
Sturm, Eva ................................................................. 117
Sturm, Gunter ............................................................ 117
Sukseree, Supawadee ................................................... 98, 190
Sumarokova, Maria ..................................................... 55
Summer, Sabrina .......................................................... 124
Sun, Jianping .............................................................. 90
Swoboda, Thomas ....................................................... 234
Swoboda, Ines ........................................................... 119, 138, 139, 140
Szabó, P. Lujza ............................................................ 127
Szákacs, Gergely ........................................................ 77
Szalóki, Gábor ............................................................. 77
Széliová, Diana ............................................................ 179, 180
Szépfalusi, Zsolt ........................................................... 119
Szöllösi, Dániel ............................................................ 52
Szöllösi, Daniel ............................................................ 77
T

Ta, Haisen ................................................................. 53
Taki, Aya ................................................................. 138
Tamir, Ido ............................................................... 69
Tan, Hongdong ............................................................ 93
Tanzer, Andrea ........................................................... 70
Tarassov, Ivan ............................................................ 124
Tarlungeanu, Dora Clara ............................................. 73
Tauer, Christopher ..................................................... 106
Terfrüchte, Marius ....................................................... 81
Terlecki-Zanievicz, Lucja ........................................... 232
Tett, Adrian ............................................................... 237
Teuff, Jacqueline ........................................................ 176
Thalhammer, Josef ...................................................... 150
Thanner, Jürgen .......................................................... 192
Thanos, Dimitris ........................................................ 268
Tharad, Sudanar .......................................................... 55, 78
Theresa, Kleissner ....................................................... 245
Thiel, Bernhard ............................................................ 70
Thiel, Isabella ............................................................ 179, 180
Tiapko, Oleksandra .................................................... 79
Timelhalter, Gerald ..................................................... 229
Tisch, Doris .............................................................. 90
Tisch, Marcel ............................................................ 117
Titus, Steven A .......................................................... 109
Tkacik, Gasper ............................................................ 224, 261, 264
Toca-Herrera, Jose L .................................................... 55, 78
Toca-Herrera, José Luis ............................................... 190
Tomasek, Kathrin ........................................................ 261
Tonner, Matthias ........................................................ 225, 257
Toplitsch, Daniela ....................................................... 115, 232
Toto, Damin ............................................................... 172
Trakaki, Athina ........................................................... 117
Tran, Anh-Minh ........................................................ 107
Tran, Loan .............................................................. 252, 253
Trauner, Michael ......................................................... 208
Tripisciano, Carla ......................................................... 143
Trojacher, Christina .................................................... 180
Trostanov, Zuzana ...................................................... 52
Trösch, Anna ............................................................. 113
Truckenmüller, Roman ................................................. 168
Tschacher, Erwin ......................................................... 86, 98, 128, 190
Tscheppe, Angelika .................................................... 103, 141
Tscherner, Michael ..................................................... 135, 242
Tschoden, Marc .......................................................... 62
Tschulenk, Waltraud .................................................... 254
Tungli, Jan ............................................................... 52

278
Author Index

Türk, Dóra................................................................. 77
Turra, David......................................................... 183
Twaruschev, Krisztian............................................. 234

U

Úcal, Muammer...................................................... 207
Uhlik, Lukas.......................................................... 192
Ulrich, Alexi B...................................................... 249
Ulrich, Cornelia M.................................................. 249
Unterleuthner, Daniela......................................... 218, 255
Unterluggauer, Julia.............................................. 71
Urban, Ernst.......................................................... 161
Üzümez, Öyku......................................................... 78, 103

V

Valko, Zsuzsanna.................................................... 248
van Bitterswijik, Clemens....................................... 168
van Liere, Elsbeth.................................................. 208
Van Ree, Ronald.................................................... 141
Van Rijt, Leonie S................................................... 141
van Schoonhoven, Sushilla.................................... 244
Vandenabeele, Peter.............................................. 208
Vanderveelde, Alexandra...................................... 260
Vanhessche, Koenraad......................................... 173
Vazdar, Mario....................................................... 57
Velas, Lukas.......................................................... 198
Venhuizen, Peter................................................... 69
Venturino, Alessandro.......................................... 65
Veraar, Cecilia........................................................ 192
Vesely, Cornelia................................................... 122
Vidal, Silvia............................................................ 206
Vierlinger, Klemens................................................ 130, 189, 244
Villunger, Andreas............................................... 117, 208
Vitt, Daniel........................................................... 267
Vladetic, Alexandra.............................................. 97, 98
Vogel, Andrea........................................................ 67
Vogl, Franziska..................................................... 248
Vogt, Stefan.......................................................... 62
Volpini de Maestri, Antonia.................................... 105, 106
von Stosch, Moritz................................................... 203
Vonck, Janet.......................................................... 166, 233
Vorauer-Uhl, Karola............................................... 179
Vrij, Erik............................................................... 168
Weber, Daniela....................................................... 85, 131, 206
Weber, Viktoria....................................................... 93, 143, 144, 145
Weckwerth, Wolfram................................................ 206
Weghuber, Julian................................................... 100, 120, 217, 236, 239
Weiger, Thomas M.................................................. 169
Weinguny, Marcus.................................................... 109
Weinhaeusel, Andreas............................................. 246
Weinhäusel, Andreas............................................. 130, 189, 191, 193, 245, 250
Weiss, René........................................................... 143, 144, 145
Weninger, Astrid.................................................... 105, 106
Werner, Bettina...................................................... 192
Werner, Paul.......................................................... 189
Werning, Maike..................................................... 66
Wessler, Silja.......................................................... 151, 235
Weißler, Silja.......................................................... 237
Wetzels, Stefanie.................................................... 165
Wiegers, Jan........................................................... 117
Wiesauer, Maria..................................................... 151
Wiesenberger, Gerlinde.......................................... 234
Wieser, Monika...................................................... 198, 247, 253
Willinger, Birgit...................................................... 225
Wilhmanns, Matthias............................................... 58
Wimmer, Kerstin..................................................... 250
Wimmer, Raphaela.................................................... 108, 109
Windberger, Ursula............................................... 178
Winkler, Bernhard................................................... 211
Winkler, Margit...................................................... 105, 106
Wirth, Dagmar....................................................... 150
Wischnitzki, Elisabeth.............................................. 102
Wohlschlager, Lena............................................... 161, 173
Wohlschlager, Therese........................................... 75
Wojta, Johann....................................................... 211
Wolf, Brigitte.......................................................... 218, 255
Wolf, Noa............................................................... 92, 261
Wolfgang, Hemmer................................................ 140
Wurm, David.......................................................... 88, 177
Wurm, David Johannes............................................. 178
Wutte, Andrea........................................................ 198

X

Xiao, Su................................................................. 109

Y

Yankova, Desislava.................................................. 217, 258
Ying, Shi............................................................... 237
Yum, Min Kyu........................................................ 187

Z

Zahedi, René............................................................. 150
Zaja, Mirko............................................................. 267
Zanghellini, Jürgen.................................................. 89, 172, 179, 180, 222
Zappe, Katja.......................................................... 192, 249
Zarfel, Gernot......................................................... 115, 232
Zatoukal, Kurt....................................................... 198, 247, 268
Zehl, Martin........................................................... 161
Zeilinger, Susanne.................................................. 134, 238
Zeillinger, Markus................................................... 253
Zelger, Philipp....................................................... 198
### Author Index

<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zell, Lukas</td>
<td>218, 254</td>
</tr>
<tr>
<td>Ziegler, Christine</td>
<td>51</td>
</tr>
<tr>
<td>Zilla, Peter</td>
<td>210</td>
</tr>
<tr>
<td>Zimmermann, Andreas</td>
<td>207</td>
</tr>
<tr>
<td>Zimmermann, Katharina</td>
<td>185</td>
</tr>
<tr>
<td>Zimmermann, Mirjam</td>
<td>51</td>
</tr>
<tr>
<td>Žoldák, Gabriel</td>
<td>52</td>
</tr>
<tr>
<td>Zotchev, Sergey B.</td>
<td>161, 222</td>
</tr>
<tr>
<td>Zou, Jing</td>
<td>93</td>
</tr>
<tr>
<td>Zutz, Christoph</td>
<td>257</td>
</tr>
<tr>
<td>Zver, Lars</td>
<td>193</td>
</tr>
<tr>
<td>Zwirzitz, Benjamin</td>
<td>165</td>
</tr>
<tr>
<td>Zwolanek, Florian</td>
<td>135</td>
</tr>
</tbody>
</table>
Participant Index

Ahmad Muhammad  
Center for Health & Bioresources  
AIT Austrian Institute of Technology GmbH  
Austria  
E: muhammad.ahmad@ait.ac.at

Altvater Martin  
The Austrian Center of Industrial Biotechnology (ACIB)  
Austria  
E: martin.altvater@boku.ac.at

Aminzadeh Gohari Sepideh  
Pediatrics  
Paracelsus Medical University  
Austria  
E: s.aminzadeh-gohari@salk.at

Andersson Anna  
Tkacik Group  
IST Austria  
Austria  
E: anna.andersson.ist@gmail.com

Angermayr S. Andreas  
Institute for Biological Physics  
University of Cologne  
Germany  
E: s.a.angermayr@uni-koeln.de

Arnold Cosmas  
Research Institute of Molecular Pathology (IMP)  
Austria  
E: cosmas.arnold@imp.ac.at

Auer Julia  
Open Science-Lebenswissenschaften im Dialog  
Austria  
E: auer@openscience.or.at

Bachmann Barbara  
Ludwig Boltzmann Institute for Experimental and Clinical Traumatology & Vienna University of Technology  
Austria  
E: barbara.bachmann@tuwien.ac.at

Bado Souleymane  
Plant Biotechnology Unit (PBU), Dept. Biotechnology  
University of Natural Resources and Life Sciences  
Austria  
E: bado_souleymane@hotmail.com

Bagley Joshua  
IMBA - Institut für Molekulare Biotechnologie GmbH  
Austria  
E: joshua.bagley@imba.oeaw.ac.at

Barbay Diane  
Biotechnologie ACIB  
Austria  
E: diane.barbay@boku.ac.at

Barta Andrea  
Max F Perutz Laboratories Med. Uni Wien  
Austria  
E: andrea.barta@meduniwien.ac.at

Bauer Elisabeth  
Applied Live Science  
FH Campus Wien  
Austria  
E: elisabethbauer@live.at

Baumfried Oliver  
Applied Live Science / Molecular Biotechnology  
FH Campus Wien  
Austria  
E: oliver.baumfried@gmx.at

Bayer Benjamin  
Department of Biotechnology  
University of Natural Resources and Life Sciences  
Austria  
E: benjamin.bayer@boku.ac.at

Bazafkan Hoda  
Microbiology  
University of Innsbruck  
Austria  
E: hoda.bazafkan@uibk.ac.at

Beier Sabrina  
Austrian Institute of Technology  
Austria  
E: sabrina.beier@ait.ac.at

Beisken Stephan  
Ares Genetics GmbH  
Austria  
E: stephan.beisken@ares-genetics.com

Bergmann Andreas  
Szabo-Scandic  
Azerbaijan  
E: a.bergmann@szabo-scandic.com

Bergmann Michael  
Surgery  
Med Univ Vienna  
Austria  
E: michael.bergmann@muv.ac.at

Bergmeister Konstantin D  
Department of Hand, Plastic and Reconstructive Surgery - Burn Center - BG Trauma Center Ludwigshafen  
Germany  
E: kbergmeister@gmail.com

Bergmiller Tobias  
IST Austria  
Austria  
E: tobias.bergmiller@gmail.com

Berkemeyer Matthias  
Process Science Austria  
Boehringer Ingelheim RCV  
Austria  
E: matthias.berkemeyer@boehringer-ingelheim.com

Bermann Manfred  
BIOLAB GmbH  
Austria  
E: office@biolab.co.at
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Location</th>
<th>E-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernegger Sabine</td>
<td>Biosciences</td>
<td>University of Salzburg</td>
<td><a href="mailto:sabine.bernegger@sbg.ac.at">sabine.bernegger@sbg.ac.at</a></td>
</tr>
<tr>
<td>Berry David</td>
<td>Dept. of Microbiology and Ecosystem Science</td>
<td>University of Vienna</td>
<td><a href="mailto:berry@microbial-ecology.net">berry@microbial-ecology.net</a></td>
</tr>
<tr>
<td>Beyer Reinhard</td>
<td>Department of Applied Genetics and Cell Biology</td>
<td>University of Natural Resources and Life Sciences</td>
<td><a href="mailto:reinhard.beyer@boku.ac.at">reinhard.beyer@boku.ac.at</a></td>
</tr>
<tr>
<td>Bitter Johannes</td>
<td>IMBT</td>
<td>TU Graz</td>
<td><a href="mailto:j.bitter@student.tugraz.at">j.bitter@student.tugraz.at</a></td>
</tr>
<tr>
<td>Blaha Thomas</td>
<td>VWR International GmbH</td>
<td>Austria</td>
<td><a href="mailto:thomas.blaha@vwr.com">thomas.blaha@vwr.com</a></td>
</tr>
<tr>
<td>Boras Michaela</td>
<td>Sales</td>
<td>PALL BIOTECH</td>
<td><a href="mailto:michaela_boras@europe.pall.com">michaela_boras@europe.pall.com</a></td>
</tr>
<tr>
<td>Borodina Irina</td>
<td>The Novo Nordisk Foundation Center for Biosustainability</td>
<td>Technical University of Denmark</td>
<td><a href="mailto:irbo@biosustain.dtu.dk">irbo@biosustain.dtu.dk</a></td>
</tr>
<tr>
<td>Brandstetter Simon</td>
<td>THP Medical Products</td>
<td>Austria</td>
<td><a href="mailto:s.brandstetter@thp.at">s.brandstetter@thp.at</a></td>
</tr>
<tr>
<td>Breitenbach-Koller Hannelore</td>
<td>Biosciences</td>
<td>University of Salzburg</td>
<td><a href="mailto:hannelore.breitenbach-koller@sbg.ac.at">hannelore.breitenbach-koller@sbg.ac.at</a></td>
</tr>
<tr>
<td>Brezina Stefanie</td>
<td>Institute of Cancer Research, Medical University of Vienna</td>
<td>Austria</td>
<td><a href="mailto:stefanie.brezina@meduniwien.ac.at">stefanie.brezina@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Brisson Alain</td>
<td>University of Bordeaux</td>
<td>UMR-CBMN</td>
<td><a href="mailto:a.brisson@cbmn.u-bordeaux.fr">a.brisson@cbmn.u-bordeaux.fr</a></td>
</tr>
<tr>
<td>Buhmann Anja</td>
<td>Center of Health and Bioresources</td>
<td>Austrian Institute of Technology</td>
<td><a href="mailto:anjabuhmann01@gmail.com">anjabuhmann01@gmail.com</a></td>
</tr>
<tr>
<td>Casanova Emilio</td>
<td>Department of Physiology</td>
<td>Medical University of Vienna</td>
<td><a href="mailto:emilio.casanova@lbicr.lbg.ac.at">emilio.casanova@lbicr.lbg.ac.at</a></td>
</tr>
<tr>
<td>Chichirau Bianca</td>
<td>Biosciences</td>
<td>University of Salzburg</td>
<td><a href="mailto:bianca.chichirau@sbg.ac.at">bianca.chichirau@sbg.ac.at</a></td>
</tr>
<tr>
<td>Chichirau Bianca</td>
<td>Biology</td>
<td>University of Salzburg</td>
<td><a href="mailto:bianca-elena.chichirau@stud.sbg.ac.at">bianca-elena.chichirau@stud.sbg.ac.at</a></td>
</tr>
<tr>
<td>Cichna-Markl Margit</td>
<td>Department of Analytical Chemistry</td>
<td>University of Vienna</td>
<td><a href="mailto:margit.cichna@univie.ac.at">margit.cichna@univie.ac.at</a></td>
</tr>
<tr>
<td>Coradin Thibaud</td>
<td>Sorbonne Université - CNRS</td>
<td>France</td>
<td><a href="mailto:thibaud.coradin@sorbonne-universite.fr">thibaud.coradin@sorbonne-universite.fr</a></td>
</tr>
<tr>
<td>Cos Paul</td>
<td>LMPH</td>
<td>University of Antwerp</td>
<td><a href="mailto:paul.cos@uantwerpen.be">paul.cos@uantwerpen.be</a></td>
</tr>
<tr>
<td>Damisch Elisabeth</td>
<td>University of Graz</td>
<td>Institut of Molecular Biology</td>
<td><a href="mailto:elisabeth.damisch@edu.uni-graz.at">elisabeth.damisch@edu.uni-graz.at</a></td>
</tr>
<tr>
<td>Daneman Richard</td>
<td>Pharmacology and Neurosciences</td>
<td>UCSD</td>
<td><a href="mailto:rdaneman@ucsd.edu">rdaneman@ucsd.edu</a></td>
</tr>
<tr>
<td>Couillard-Despres Sebastien</td>
<td>Institute of Experimental</td>
<td>Paracelsus Medical University</td>
<td><a href="mailto:s.couillard-despres@pmu.ac.at">s.couillard-despres@pmu.ac.at</a></td>
</tr>
<tr>
<td>Cozzarini Helena</td>
<td>Molecular Biotechnology</td>
<td>FH Campus Wien</td>
<td><a href="mailto:helena.cozzarini@gmail.com">helena.cozzarini@gmail.com</a></td>
</tr>
<tr>
<td>Crvenjak Nadja</td>
<td>University of Vienna</td>
<td>Austria</td>
<td><a href="mailto:ncrvenjak@yahoo.com">ncrvenjak@yahoo.com</a></td>
</tr>
<tr>
<td>Csarman Florian</td>
<td>Department of Food Science and Technology</td>
<td>University of Natural Resources and Life Sciences</td>
<td><a href="mailto:florian.csarman@boku.ac.at">florian.csarman@boku.ac.at</a></td>
</tr>
<tr>
<td>Csorba Cintia</td>
<td>Health and Bioresources</td>
<td>AIT Austrian Institute of Technology</td>
<td><a href="mailto:cintia.csorba@ait.ac.at">cintia.csorba@ait.ac.at</a></td>
</tr>
<tr>
<td>Curcic Sanja</td>
<td>Gottfried Schatz Research Center (for Cell Signaling, Metabolism and Aging),</td>
<td>Chair of Biophysics</td>
<td><a href="mailto:sanja.curcic@medunigraz.at">sanja.curcic@medunigraz.at</a></td>
</tr>
<tr>
<td>Damisch Elisabeth</td>
<td>University of Graz</td>
<td>Institut of Molecular Biology</td>
<td><a href="mailto:elisabeth.damisch@edu.uni-graz.at">elisabeth.damisch@edu.uni-graz.at</a></td>
</tr>
<tr>
<td>Daneman Richard</td>
<td>Pharmacology and Neurosciences</td>
<td>UCSD</td>
<td><a href="mailto:rdaneman@ucsd.edu">rdaneman@ucsd.edu</a></td>
</tr>
</tbody>
</table>
Participant Index

Das Anand K.
Institute of Applied Physics
Vienna University of Technology (TU Wien)
Austria
E: anandkantdas@gmail.com

De Luna Andrea
Center for Regenerative Medicine
Danube University of Vienna
Austria
E: andrea.deluna-preitschopf@donau-uni.ac.at

Dern tl Christian
ICEBE
TU Wien
Austria
E: christian.derntl@tuwien.ac.at

Diechler Sebastian
Biosciences
University of Salzburg
Austria
E: Sebastian.Diechler@stud.sbg.ac.at

Dietrich Bianca
Department of Obstetrics and Gynecology
Medical University
Austria
E: d.bianca94@gmail.com

Dikecoglu Begüm
Applied Experimental Biophysics
Institute of Biophysics
Austria
E: beguem.dikecoglu@jku.at

Dillinger Thomas
Ludwig Boltzmann Institute Applied Diagnostics
Austria
E: thomas.dillinger@ibiad.lbg.ac.at

Dollack Dinah
Molecular Biotechnology
FH Campus Wien
Austria
E: dinah.dollack@stud.fh-campuswien.ac.at

Domanegg Kevin
Molecular Biotechnology
FH Campus Wien
Austria
E: kevin.domanegg@stud.fh-campuswien.ac.at

Drev Daniel
Cancer Research Institute
Medical University Vienna
Austria
E: daniel.drev@meduniwien.ac.at

Driessen Arnold
Molecular Microbiology
University of Groningen
Netherlands
E: a.j.m.driessen@rug.nl

Duller Stefanie
Department of Internal Medicine
Medical University of Graz
Austria
E: stefanie.duller@medunigraz.at

Duszk a Kalina
Department of Nutritional Sciences
University of Vienna
Austria
E: kalina.duszka@univie.ac.at

Ebner Florian
Center for Anatomy and Cell Biology
Medical University of Vienna
Austria
E: florian.ebner@meduniwien.ac.at

Eckhart Leopold
Department of Dermatology
Medical University of Vienna
Austria
E: leopold.eckhart@meduniwien.ac.at

Eckmann Christian R.
Developmental Genetics
Martin Luther University Halle-Wittenberg
Germany
E: christian.eckmann@genetik.uni-halle.de

Eder Gabriela
Department of Biomedical Research
Medical University of Vienna
Austria
E: gabriela.e94@gmx.at

Eder Markus
Institute of Molecular Biosciences
University of Graz
Austria
E: markus.eder@uni-graz.at

Egermeier Michael
University of Natural Resources and Life Sciences (BOKU)
Austria
E: michael.egermeier@boku.ac.at

Egger Dominik
Department of Biotechnology
University of Natural Resources and Life Sciences
Austria
E: dominik.egger@boku.ac.at

Ehrlich Florian
dep. of dermatology
medical university of vienna
Austria
E: florian.ehrlich@meduniwien.ac.at

Eilenberger Christoph
Technical University Vienna
Austria
E: christoph.eileenberger@tuwien.ac.at

Elgendy Mohamed
University of Vienna
Austria
E: mohamed.elgendy@univie.ac.at

Ellena Valeria
Biotechnology Department
University of natural resources and life sciences
Austria
E: valeria.ellena@boku.ac.at

Englisch Rainer
THP Medical Products
Austria
E: r.englisch@thp.at

Entler Barbara
Department Life sciences
University of Applied Sciences Krems
Austria
E: barbara.entler@fh-krems.ac.at
Ergoth Stefan
Department for Biotechnology
BOKU
Austria
E: stefan.ergoth@boku.ac.at

Fendl Birgit
Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis
Danube University Krems
Austria
E: birgit.fendl@donau-uni.ac.at

Fernandes Rosenegger Paloma
Molekulare Biotechnologie
FH Campus Wien
Austria
E: fernandes.pah@gmail.com

Fitz Elisabeth
YLSA
Austria
E: elisabeth.fitz@tuwien.ac.at

Fladerer Johannes - Paul
Department of Pharmacognosy
Institute of Pharmaceutical Science
Austria
E: johannes.fladerer@uni-graz.at

Frei Judith
Molekulare Biotechnologie, 166/5
YLSA-ÖGMBT
Austria
E: judi.frei@hotmail.com

Freudhofmaier Markus
Biotechnology
University of Natural Resources and Life Sciences, Vienna
Austria
E: markus.freudhofmaier@boku.ac.at

Friedrich Andreas
Biosciences
University of Salzburg
Austria
E: andreas.friedrich@stud.sbg.ac.at

Fuchs Jürgen
LISAvienna
Austria
E: fuchs@LISAvienna.at

Gabler Lisa
Medical University of Vienna
Institute of Cancer Research
Austria
E: lisa.gabler@meduniwien.ac.at

Galleguillos Sarah Noel
Department of Biotechnology
University of Natural Resources and Life Sciences
Austria
E: sarah.galleguillos@boku.ac.at

Gassler Thomas
University of Natural Resources and Life Sciences, Vienna
Austria
E: thomas.gassler@boku.ac.at

Gerhartl Anna
Molecular Diagnostics
AIT Austrian Institute of Technology
Austria
E: anna.gerhartl@ait.ac.at

Geroldinger Gerald
Deptm. Biomedical Sciences
Univ. of Veterinary Medicine
Austria
E: gerald.geroldinger@vetmeduni.ac.at

Ghanbari Mahdi
Biomin Research center
Austria
E: mahdi.ghanbari@biomin.net

Gille Lars
Deptm. Biomedical Sciences
Univ. of Veterinary Medicine
Austria
E: Lars.Gille@vetmeduni.ac.at

Glaser Walter
ÖGMBT
Austria
E: walter.glaser@oegmbt.at

Glössl Josef
Department für Angewandte Genetik und Zellbiologie
Universität für Bodenkultur Wien
Austria
E: josef.gloessl@boku.ac.at

Gorfer Markus
Center for Health & Bioresources
AIT Austrian Institute of Technology GmbH
Austria
E: Markus.Gorfer@ait.ac.at

Göritzer Kathrin
Applied Genetics and Cell Biology
University of Natural Resources and Life Sciences Vienna
Austria
E: kathrin.goeritzer@boku.ac.at

Gotzmann Josef
Central Facility Biooptics
Max F. Perutz Laboratories
Austria
E: josef.gotzmann@meduniwien.ac.at

Graumann Klaus
Phoenestra GmbH
Austria
E: klaus.graumann@a1.net

Grill Magdalena
Otto Loewi Research Center, Pharmacology Section
Medical University of Graz
Austria
E: ma.grill@medunigraz.at

Grilo João
Institute for Pathophysiology and Allergy Research
Medical University of Vienna
Austria
E: joao.rodriguesgrilo@meduniwien.ac.at

Grimm Matthias
Bartelt
Austria
E: matthias.grimm@bartelt.at
Groschner Klaus  
Gottfried-Schatz-Research-Center - Biophysics  
Medical University of Graz  
Austria  
E: klaus.groschner@medunigraz.at

Grossmann Tanja  
Division of Phoniatics  
Medical University Graz  
Austria  
E: tanja.grossmann@medunigraz.at

Gruber Wolfgang  
Department of Biosciences  
University of Salzburg  
Austria  
E: Wolfgang.Gruber2@sbg.ac.at

Grunt Thomas W.  
Signaling Networks Program, Division of Oncology, Department of Medicine I & Comprehensive Cancer Center  
Medical University of Vienna & Ludwig Boltzmann Cluster Oncology  
Austria  
E: thomas.grunt@meduniwien.ac.at

Gschmeidler Brigitte  
Open Science-Lebenswissenschaften im Dialog  
Austria  
E: gschmeidler@openscience.or.at

Guerrero Garzón Jaime Felipe  
Pharmacognosy Department  
University of Viena  
Austria  
E: jaime.felipe.guerrero.garzon@univie.ac.at

Gusenkov Sergey  
Physico-Chemical Characterization  
Novartis  
Austria  
E: sergey.gusenkov@novartis.com

Hagen Carmen  
Medizinische Universität Innsbruck  
Austria  
E: carmen.hagen@tsn.at

Hager Pia  
Department of Biomedical Research  
Medical University of Vienna  
Austria  
E: piahager93@gmail.com

Hannschläger Christof  
Institut f. Biophysik Molekular and Membrane Biophysics  
Johannes Kepler University Linz  
Austria  
E: Christof.Hannschlaeger@jku.at

Harms Alexander  
BASP Centre, Department of Biology  
University of Copenhagen  
Denmark  
E: alexander.harms@bio.ku.dk

Hartl Lukas  
Microsynth Austria  
Austria  
E: lukas.hartl@micsynth.at

Hartmann Michael  
BioCat GmbH  
Germany  
E: hartmann@biocat.com

Hasenöhrl Carina  
Otto Loewi Research Center  
Medical University of Graz  
Austria  
E: carina.hasenoehrl@medunigraz.at

Haslacher Helmut  
Department of Laboratory Medicine  
Medical University of Vienna  
Austria  
E: helmuth.haslacher@meduniwien.ac.at

Hawlitschek Gerhard  
Molecular Devices (Germany) GmbH  
Germany  
E: gerhard.hawlitschek@moldev.com

Healy Thomas  
Bioprocess Sales  
Eppendorf Austria GmbH  
Austria  
E: healy.t@eppendorf.at

Heidari Hamid  
Operational Marketing  
Eppendorf Austria GmbH  
Austria  
E: heidari.h@eppendorf.at

Heissenberger Clemens  
Department of Biotechnology  
University of Natural Resources and Life Sciences  
Austria  
E: clemens.heissenberger@boku.ac.at

Hinterdoblter Wolfgang  
Center for Health & Bioresources  
AIT Austrian Institute Of Technology  
Austria  
E: wolfgang.hinterdoblter@ait.ac.at

Hirschmu TATJANA  
Austria  
E: tatjana.hirschmu@gmail.com

Hofbauer Reinhold  
Ctr. of Medical Biochemistry, Div. Med. Genetics  
MFPL, MUW  
Austria  
E: reinhold.hofbauer@meduniwien.ac.at

Hofer Sebastian  
Institute of Molecular Biosciences  
University of Graz  
Austria  
E: sebastian.hofer@uni-graz.at

Hofner Manuela  
Center for Health and Bioresources  
AIT Austrian Institute of Technology GmbH  
Austria  
E: manuela.hofner@ait.ac.at

Hoheneder Robin  
Biotechnology  
University of Natural Resources and Life Sciences, Vienna  
Austria  
E: Robin.Hoheneder@boku.ac.at

Holz Patrick  
LI-COR Biosciences GmbH  
Germany  
E: patrick.holz@licor.com
Horner Andreas  
Institute of Biophysics  
Johannes Kepler University  
Austria  
E: andreas.horner@jku.at

Houska Sebastian  
Sales  
Eppendorf Austria GmbH  
Austria  
E: houska.s@eppendorf.at

Howorka Stefan  
Department of Chemistry  
University College London  
United Kingdom  
E: s.howorka@ucl.ac.uk

Hren Jernej  
pcPHENO  
Vienna Biocenter  
Austria  
E: jernej.hren@vbcf.ac.at

Hritz Jozef  
Institute of Molecular Modeling and Simulation  
University of Natural Resources and Life Sciences  
Austria  
E: jozef.hritz@ceitec.muni.cz

Huber Anna  
Institute of Medical Genetics  
Medical University of Vienna  
Austria  
E: anna.b.huber@meduniwien.ac.at

Huber Jasmin  
Center for Health & Bioresources  
Austrian Institute of Technology  
Austria  
E: jasmin.huber@ait.ac.at

Huck Sigismund  
Center for Brain Research  
Medical University Vienna  
Austria  
E: sigismund.huck@meduniwien.ac.at

Humeniuk Piotr  
Department of Pathophysiology and Allergy Research  
Medical University of Vienna  
Austria  
E: piotr.humeniuk@meduniwien.ac.at

Illjazi Dafina  
Department of Urology  
Medical University of Vienna  
Austria  
E: dafinailijazii@gmail.com

Indra Dominic E.  
Center of Medical Biochemistry, Division of Molecular Genetics  
Max F. Perutz Laboratories, Medical University of Vienna  
Austria  
E: dominic.indra@univie.ac.at

Jahn-Schmid Beatrice  
Inst. of Pathophysiology and Allergy Research  
Medical University of Vienna  
Austria  
E: beatrice.jahn-schmid@meduniwien.ac.at

Jain Mamta  
CABC  
Medical University of Vienna  
Austria  
E: mamta.jain@meduniwien.ac.at

Jankovic Nevena  
University of Veterinary Medicine, Vienna  
Austria  
E: nevena.jankovic@vetmeduni.ac.at

Jansson Stefan  
UPSC, Dept of Plant Physiology  
Umeå university  
Sweden  
E: stefan.jansson@umu.se

Jantsch Michael F.  
Center of Anatomy and Cell Biology, Division of Cell- and Developmental Biology  
Medical University of Vienna  
Austria  
E: Michael.Jantsch@meduniwien.ac.at

Jennull Sabrina  
Max F. Perutz Laboratories, Vienna Biocenter  
Medical University of Vienna  
Austria  
E: sabrina.jennull@meduniwien.ac.at

Jerala Roman  
Synthetic biology and immunology  
National institute of chemistry  
Slovenia  
E: roman.jerala@ki.si

Jeschek Dominik  
Department of Biotechnology  
University of natural resources and life sciences  
Austria  
E: dominik.jeschek@boku.ac.at

Jovanovic Olga  
Veterinärmedizinische Universität Wien, 1210 Wien  
Austria  
E: olga.jovanovic@vetmeduni.ac.at

Jungwirth Emilian  
Institut of Computational Biotechnology  
Graz University Of Technology  
Austria  
E: emilian.jungwirth@student.tugraz.at

Juno Claudia  
ACIB  
Austria  
E: claudiajuno@acib.at

Jurrar Tin  
University of Veterinary Medicine  
Vienna  
Austria  
E: jurrar.tin@gmail.com

Kabasser Stefan  
Inst. f. Pathophysiologie u. Allergieforschung  
Medizinische Universität Wien  
Austria  
E: stefan.kabasser@meduniwien.ac.at

Kabinger Florian  
molecular Biotechnology  
FH Campus Wien  
Austria  
E: florian.kabinger@stud.fh-campuswien.ac.at

Kainz Katharina  
University of Graz  
Austria  
E: katharina.kainz@uni-graz.at
Kalemasi Denis
Department of Biotechnology
University of Natural Resources and Life Sciences
Austria
E: denis.kalemasi@boku.ac.at

Kalic Tanja
Institute of Pathophysiology and Allergy Research
Medical University of Vienna
Austria
E: tanja.kalic@meduniwien.ac.at

Kallolimath Somanath
Department für Angewandte Genetik und Zellbiologie
Universität für Bodenkultur
Austria
E: somanath.kallolimath@boku.ac.at

Kalyna Maria
Department of Applied Genetics and Cell Biology (DAGZ)
University of Natural Resources and Life Sciences - BOKU
Austria
E: mariya.kalyna@boku.ac.at

Kapoor Utkarsh
Department of Cell & Developmental Biology
Medical University of Vienna
Austria
E: utkarshkapoor87@gmail.com

Karacs Jasmine
Institute of Pathophysiology and Allergy Research
Medical University of Vienna
Austria
E: jasmine.karacs@meduniwien.ac.at

Karbiener Michael
Medical University Graz
Austria
E: michael@karbiener.at

Karimi Aghcheh Razieh
Dept. of Molecular Microbiology and Genetics
Georg August University Göttingen
Germany
E: karimi.razieh@gmail.com

Karuthedom George Sobha
Center for Biomedical Technology
Danube University Krems
Austria
E: sobha.karuthedom@gmail.com

Käsbohrer Annemarie
University of Veterinary Medicine, Vienna
Austria
E: annemarie.kaesbohrer@vetmeduni.ac.at

Kastenhofer Jens
Biochemical Engineering
TU Wien
Austria
E: jens.kastenhofer@tuwien.ac.at

Kavcic Bor
RG Tkacik (Systems biology and biophysics)
IST Austria
Austria
E: bor.kavcic@ist.ac.at

Kegler Ulrike
Center for Health & Bioresources
AIT Austrian Institute of Technology GmbH
Austria
E: Ulrike.Kegler@ait.ac.at

Kepplinger Christian
SALES
PALL BIOTECH
Austria
E: christian_kepplinger@pall.com

Khassidov Alexandra
ÖGBMT
Austria
E: alexandra.khassidov@oegmbt.at

Kienzl Melanie
Otto-Loewi-Research Center, Pharmacology
Medical University of Graz
Austria
E: melanie.kienzl@medunigraz.at

Kinz Elena
Open Science-Lebenswissenschaften im Dialog
Austria
E: kinz@openscience.or.at

Kirsch Andrijana
Division of Phoniatics, ENT University Hospital
Medizinische Universität Graz
Austria
E: andrijana.kirsch@medunigraz.at

Kittinger Clemens
Inst. for Hygiene
Medical University of Graz
Austria
E: clemens.kittinger@medunigraz.at

Klanert Gerald
Department for Biotechnology
University of Natural Resources and Life Sciences
Austria
E: gerald.klanert@boku.ac.at

Klima Georg
Process Science
Boehringer-Ingelheim RCV GmbH & Co KG
Austria
E: georg.klima@boehringer-ingelheim.com

Klocker Helmut
Urology
Medical University Innsbruck
Austria
E: helmut.klocker@i-med.ac.at

Klymiuk Ingeborg
Medical University Graz
Austria
E: ingeborg.klymiuk@medunigraz.at

Kmen Maximilian
Molecular Biotechnology
FH Campus Wien
Austria
E: maximilian.kmen@gmx.at

Knapp Katja
Division of Developmental Immunology
Innsbruck Medical University
Austria
E: katja.knapp@student.i-med.ac.at

Knez Špela
Biotechnology
BOKU
Austria
E: spela.knez@gmail.com

287
Knyazev Denis  
Institute of Biophysics  
Johannes Kepler University  
Austria  
E: denis.knyazev@jku.at

Kobalter Simon  
Institute of Molecular Biotechnology  
technical University of Graz  
Austria  
E: simon.kobalter@gmx.at

Koller Manuela Sophie  
Veterinärmedizinische Universität Wien  
Austria  
E: sop.koller@gmail.com

Koo Bon-Kyoung  
IMBA  
Austria  
E: bonkyoung.koo@imba.oeaw.ac.at

Koorakula Raju  
LIVESTOCK RESISTOME  
FFoQSI GmbH  
Austria  
E: raju.koorakula@ffoqsi.at

Koraimann Günther  
University of Graz  
Austria  
E: guenther.koraimann@uni-graz.at

Korb Melanie  
Technology platform  
VetCore/VetBiobank  
University of Veterinary Medicine, Vienna  
Austria  
E: melanie.korb@vetmeduni.ac.at

Koskinen Kaisa  
Medical University of Graz  
Austria  
E: kaisa.koskinen@medunigraz.at

Krainer Julie  
Center for Health & Bioresources  
AIT - Austrian Institute of Technology  
Austria  
E: julie.krainer@ait.ac.at

Kraitsy Klaus  
Vienna Biocenter Core Facilities GmbH  
Austria  
E: klaus.kraitsy@vbc.ac.at

Kraus Thomas  
Sales  
PALL BIOTECH  
Austria  
E: thomas_kraus@europe.pall.com

Kreiter Jürgen  
Physiology and Biophysics  
University of Veterinary Medicine  
Austria  
E: Juergen.Kreiter@vetmeduni.ac.at

Kresnik Rebecca  
University of Veterinary Medicine  
Austria  
E: rebecca.kresnik@gmx.at

Kukleci Egzon  
Food Hygiene  
University of Veterinary Medicine  
Vienna  
Austria  
E: egzonn_k@msn.com

Kumpitsch Christina  
Internal Medicine  
Medizinische Universität Graz  
Austria  
E: christina.kumpitsch@medunigraz.at

Lachner Julia  
Department of Dermatology  
Medical University of Vienna  
Austria  
E: julia.lachner@meduniwien.ac.at

Laimer Margit  
PBU, DBT, BOKU  
Austria  
E: margit.laimer@boku.ac.at

Landowski Christopher  
Industrial Biotechnology  
VTT Technical Research Centre of Finland Ltd.  
Finland  
E: christopher.landowski@vtt.fi

Lebhard Jürgen  
Department of Obstetrics and Gynecology  
Medical University of Vienna  
Austria  
E: juergen.lebhard@stud.fh-campuswien.ac.at

Lee Ji-Hyun  
Koo lab  
Institute of Molecular Biotechnology (IMBA)  
Austria  
E: ji.lee@imba.oeaw.ac.at

Leitner Lucia M.  
Dept. of Medical Biochemistry  
Medical University of Vienna  
Austria  
E: lucia.leitner@univie.ac.at

Li Guofen  
Austrian Institute of Technology  
Austria  
E: guofen.li.fl@ait.ac.at

Lichius Alex  
Department of Microbiology  
University of Innsbruck  
Austria  
E: alexander.lichius@uibk.ac.at

Licht Konstantin  
Center for Anatomy and Cell Biology  
Medical University of Vienna  
Austria  
E: konstantin.licht@meduniwien.ac.at

Lin Grace  
AIT-Austrian Institute of Technology GmbH  
Austria  
E: grace.lin@ait.ac.at

Locke Felix  
Department of Biomedical Sciences  
University of Veterinary Medicine  
Austria  
E: Felix.Locker@vetmeduni.ac.at

Loy Alexander  
Department of Microbiology and Ecosystem Science  
University of Vienna  
Austria  
E: loy@microbial-ecology.net

Luciano Michela  
Biosciences  
University of Salzburg  
Austria  
E: Michela.Luciano@sbg.ac.at
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Country</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupinek Christian</td>
<td>Dept. of Pathophysiology and Allergy Research</td>
<td>Austria</td>
<td><a href="mailto:christian.lupinek@meduniwien.ac.at">christian.lupinek@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Dept. of Pathophysiology and Allergy Research</td>
<td>Medical University of Vienna</td>
<td>Austria</td>
<td><a href="mailto:christian.lupinek@meduniwien.ac.at">christian.lupinek@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Lusser Alexandra</td>
<td>Division of Molecular Biology, Biocenter</td>
<td>Austria</td>
<td><a href="mailto:alexandra.lusser@i-med.ac.at">alexandra.lusser@i-med.ac.at</a></td>
</tr>
<tr>
<td>Mach-Aigner Astrid R.</td>
<td>TU Wien</td>
<td>Austria</td>
<td><a href="mailto:astrid.mach-aigner@tuwien.ac.at">astrid.mach-aigner@tuwien.ac.at</a></td>
</tr>
<tr>
<td>Madritsch Silvia</td>
<td>Bioresources</td>
<td>Austria</td>
<td><a href="mailto:silvia.madritsch@ait.ac.at">silvia.madritsch@ait.ac.at</a></td>
</tr>
<tr>
<td>Mahfouz Norhan</td>
<td>Ares Genetics GmbH</td>
<td>Austria</td>
<td><a href="mailto:norhan.mahfouz@ares-genetics.com">norhan.mahfouz@ares-genetics.com</a></td>
</tr>
<tr>
<td>Maier Irene</td>
<td>Department of Environmental Health Sciences</td>
<td>USA</td>
<td><a href="mailto:irene.maier@meduniwien.ac.at">irene.maier@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>CoE Lebensmitteltechnologie</td>
<td>FH Oberösterreich</td>
<td>Austria</td>
<td><a href="mailto:martin.maier@fh-wels.at">martin.maier@fh-wels.at</a></td>
</tr>
<tr>
<td>Manhart Michael</td>
<td>Biozym Biotech Trading</td>
<td>Austria</td>
<td><a href="mailto:michael.manhart@biozym.com">michael.manhart@biozym.com</a></td>
</tr>
<tr>
<td>Mansfeld Agata</td>
<td>BioCat GmbH</td>
<td>Germany</td>
<td><a href="mailto:mansfeld@biocat.com">mansfeld@biocat.com</a></td>
</tr>
<tr>
<td>Marold DI Annemarie</td>
<td>Biobank Graz</td>
<td>Austria</td>
<td><a href="mailto:biobank@medunigraz.at">biobank@medunigraz.at</a></td>
</tr>
<tr>
<td>Marx Hans</td>
<td>Biotechnologie</td>
<td>Austria</td>
<td><a href="mailto:hans.marx@boku.ac.at">hans.marx@boku.ac.at</a></td>
</tr>
<tr>
<td>Marx Lisa</td>
<td>Institute of Molecular Biosciences/Biophysics Division</td>
<td>Austria</td>
<td><a href="mailto:lisa.marx@uni-graz.at">lisa.marx@uni-graz.at</a></td>
</tr>
<tr>
<td>Matak Andrija</td>
<td>Institute of Pathology</td>
<td>Austria</td>
<td><a href="mailto:andrijamatak@gmail.com">andrijamatak@gmail.com</a></td>
</tr>
<tr>
<td>Mattend Erwin</td>
<td>Austria</td>
<td>Austria</td>
<td><a href="mailto:erwin.mattes@hotmail.com">erwin.mattes@hotmail.com</a></td>
</tr>
<tr>
<td>Miedl Heidi</td>
<td>MUW</td>
<td>Austria</td>
<td><a href="mailto:heidi.miedl@meduniwien.ac.at">heidi.miedl@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Miksch Christoph</td>
<td>Eurofins Genomics</td>
<td>Austria</td>
<td><a href="mailto:christophmiksch@eurofins.com">christophmiksch@eurofins.com</a></td>
</tr>
<tr>
<td>Milchram Lisa</td>
<td>Molecular Diagnostics</td>
<td>Austria</td>
<td><a href="mailto:lisa.milchram@ait.ac.at">lisa.milchram@ait.ac.at</a></td>
</tr>
<tr>
<td>Moreno Ruiz Dubraska C.</td>
<td>Institute of Microbiology</td>
<td>Austria</td>
<td><a href="mailto:Dubraska.Moreno-Ruiz@uibk.ac.at">Dubraska.Moreno-Ruiz@uibk.ac.at</a></td>
</tr>
<tr>
<td>Muhar Matthias</td>
<td>Research Institute for Molecular Pathology</td>
<td>Austria</td>
<td><a href="mailto:matthias.muhar@imp.ac.at">matthias.muhar@imp.ac.at</a></td>
</tr>
<tr>
<td>Mülner Pascal</td>
<td>ABITEP GmbH</td>
<td>Germany</td>
<td><a href="mailto:muelner@abitep.de">muelner@abitep.de</a></td>
</tr>
<tr>
<td>Na Hyelin</td>
<td>Institute of Molecular Biotechnology</td>
<td>Austria</td>
<td><a href="mailto:hyelin.na@imba.oeaw.ac.at">hyelin.na@imba.oeaw.ac.at</a></td>
</tr>
<tr>
<td>Nagelreiter Fabian</td>
<td>Department of Biotechnology</td>
<td>Austria</td>
<td><a href="mailto:fabian.nagelreiter@boku.ac.at">fabian.nagelreiter@boku.ac.at</a></td>
</tr>
<tr>
<td>Negbert Clemens</td>
<td>Cardiac Surgery Research Laboratory</td>
<td>Austria</td>
<td><a href="mailto:clemens.nebert@meduniwien.ac.at">clemens.nebert@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Necina Roman</td>
<td>Process Science &amp; Technical Services</td>
<td>Austria</td>
<td><a href="mailto:roman.necina@shire.com">roman.necina@shire.com</a></td>
</tr>
<tr>
<td>Neuhaus Winfried</td>
<td>Center Health and Bioresources, Unit Molecular Diagnostics</td>
<td>Austria</td>
<td><a href="mailto:winfried.neuhaus@ait.ac.at">winfried.neuhaus@ait.ac.at</a></td>
</tr>
<tr>
<td>Nikolic Nela</td>
<td>IST Austria</td>
<td>Austria</td>
<td><a href="mailto:nela.nikolic@ist.ac.at">nela.nikolic@ist.ac.at</a></td>
</tr>
<tr>
<td>Neohammer Christa</td>
<td>Molecular Diagnostics</td>
<td>Austria</td>
<td><a href="mailto:christa.neohammer@ait.ac.at">christa.neohammer@ait.ac.at</a></td>
</tr>
<tr>
<td>Nogueira Filomena</td>
<td>CCRI</td>
<td>Austria</td>
<td><a href="mailto:filomena.nogueira@ccri.at">filomena.nogueira@ccri.at</a></td>
</tr>
<tr>
<td>Novak Katharina</td>
<td>Institute of Chemical, Environmental and Biological Engineering</td>
<td>Austria</td>
<td><a href="mailto:katharina.novak@tuwien.ac.at">katharina.novak@tuwien.ac.at</a></td>
</tr>
</tbody>
</table>

289
Participant Index

O'Mahony Liam
University College Cork
Ireland
E: liam.omahony@siaf.uzh.ch

Oberbauer Vera
Molecular Biotechnology
FH Campus Wien
Austria
E: vera.oberbauer@stud.fh-campuswien.ac.at

Oehler Rudolf
Department of Surgery
Medical University of Vienna
Austria
E: rudolf.oehler@meduniwien.ac.at

Ohlenschläger Ingo
Nikon Cee GmbH
Austria
E: Ingo.Ohlenschlaeger@nikon.com

Orth-Höller Dorothea
Division of Hygiene and Medical Microbiology
Medical University Innsbruck
Austria
E: dorothea.orth@i-med.ac.at

Otahal Alexander
Regenerative Medicine
Danube University Krems
Austria
E: alexander.otahal@donau-uni.ac.at

Pabst Georg
University of Graz
Austria
E: georg.pabst@uni-graz.at

Paunovic Manuela
Neuroimmunology
Center of Brain Research/ Medical University of Vienna
Austria
E: manuela.paunovic@meduniwien.ac.at

Pekarsky Alexander
Institute of Chemical, Environmental and Biological Engineering
Technical University Vienna
Austria
E: alexander.pekarsky@tuwien.ac.at

Pereyra David
Department of Surgery
Medical University of Vienna
Austria
E: david.pereyra@meduniwien.ac.at

Perthold Jan Walther
Institute for Molecular Modeling and Simulation (MMS)
University of Natural Resources and Life Sciences, Vienna
Austria
E: jan.perthold@boku.ac.at

Pertschy Brigitte
Institute for Molecular Biosciences
University of Graz
Austria
E: brigitte.pertschy@uni-graz.at

Pfeiffer Sandra
Molecular Biotechnology
FH Campus Wien
Austria
E: sandra.pfeiffer@fh-campuswien.ac.at

Pflügl Stefan
Institute of Chemical, Environmental and Bioscience Engineering
TU Wien
Austria
E: stefan.pfluegl@tuwien.ac.at

Pham Mai-Lan
Food Biotechnology Lab, Department of Food Science and Technology
BOKU- University of Natural Resources and Life Sciences, Vienna
Austria
E: mailanpham.22@gmail.com

Pichler Harald
Molecular Biotechnology
Graz University of Technology
Austria
E: harald.pichler@tugraz.at

Pilecky Matthias
Department for Biomedical Technology
Donau Universität Krems
Austria
E: matthias.pilecky@donau-uni.ac.at

Pilgram Ottwin Christian
World Courier (Austria) GmbH
Austria
E: ocpilgram@worldcourier.at

Pils Vera
Biotechnology
BOKU - University of Natural Resources and Life Sciences
Austria
E: vera.pils@boku.ac.at

Pitsch Johannes
Biophysics
Johannes Kepler Universität Linz
Austria
E: pitsch.j@gmail.com

Plesko Maja
Biotech Campus Tulln
Fachhochschule Wiener Neustadt GmbH
Austria
E: plesko@tulln.fhwn.ac.at

Plochberger Birgit
Medical Engineering
University of Applied Sciences Upper Austria
Austria
E: birgit.plochberger@fh-linz.at

Podesser Bruno K.
Zentrum für Biomed. Forschung Medizinische Universität Wien
Austria
E: bruno.podesser@meduniwien.ac.at

Pohl Elena E.
Physiology and Biophysics
University of Veterinary Medicine Vienna
Austria
E: elena.pohl@vetmeduni.ac.at

Posch Andreas
Ares Genetics GmbH
Austria
E: andreas.posch@ares-genetics.com
<table>
<thead>
<tr>
<th>Name</th>
<th>Organization</th>
<th>Country</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimesberger Oliver</td>
<td>F&amp;E Jungbunzlauer Austria AG</td>
<td>Austria</td>
<td><a href="mailto:oliver.preimesberger@jungbunzlauer.com">oliver.preimesberger@jungbunzlauer.com</a></td>
</tr>
<tr>
<td>Preininger Claudia</td>
<td>AIT Austrian Institute of Technology</td>
<td>Austria</td>
<td><a href="mailto:claudia.preininger@ait.ac.at">claudia.preininger@ait.ac.at</a></td>
</tr>
<tr>
<td>Prinz Marco</td>
<td>University of Freiburg</td>
<td>Germany</td>
<td><a href="mailto:marco.prinz@uniklinik-freiburg.de">marco.prinz@uniklinik-freiburg.de</a></td>
</tr>
<tr>
<td>Priv. Doz. Dr. Winkler Bernhard</td>
<td>Biomedical Research, KH Hietzing 1. Chir KH Hietzing</td>
<td>Austria</td>
<td><a href="mailto:bernhard.winkler@wienkav.at">bernhard.winkler@wienkav.at</a></td>
</tr>
<tr>
<td>Pulverer Walter</td>
<td>Health&amp;Bioresources AIT Austrian Institute of Technology GmbH</td>
<td>Austria</td>
<td><a href="mailto:walter.pulverer@ait.ac.at">walter.pulverer@ait.ac.at</a></td>
</tr>
<tr>
<td>Pum Alexandra</td>
<td>Pharmaceutical Chemistry Karl-Franzens Universität Graz</td>
<td>Austria</td>
<td><a href="mailto:alexandra.pum@edu.uni-graz.at">alexandra.pum@edu.uni-graz.at</a></td>
</tr>
<tr>
<td>Pusic Petra</td>
<td>University of Vienna</td>
<td>Austria</td>
<td><a href="mailto:petra.pusic@univie.ac.at">petra.pusic@univie.ac.at</a></td>
</tr>
<tr>
<td>Quirgst Judith</td>
<td>Univ. of Veterinary Medicine</td>
<td>Austria</td>
<td><a href="mailto:judith.quirgst@gmail.com">judith.quirgst@gmail.com</a></td>
</tr>
<tr>
<td>Radauer Christian</td>
<td>Department of Pathophysiology and Allergy Research Medical University of Vienna</td>
<td>Austria</td>
<td><a href="mailto:christian.radauer@meduniwien.ac.at">christian.radauer@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Radkohl Astrid</td>
<td>Institute of Molecular Biotechnology Technical University of Graz Austria</td>
<td>Austria</td>
<td><a href="mailto:astrid.radkohl@student.tugraz.at">astrid.radkohl@student.tugraz.at</a></td>
</tr>
<tr>
<td>Ramoni Jonas</td>
<td>Ares Genetics GmbH</td>
<td>Austria</td>
<td><a href="mailto:jonas.ramoni@outlook.at">jonas.ramoni@outlook.at</a></td>
</tr>
<tr>
<td>Ramsacher Andrea</td>
<td>University Hospital for Pediatrics of the Paracelsus Medical University, Salzburg, Austria</td>
<td>Research Program for Receptor Biochemistry and Tumor Metabolism</td>
<td><a href="mailto:andrea.ramsacher@gmail.com">andrea.ramsacher@gmail.com</a></td>
</tr>
<tr>
<td>Reithofer Manuel</td>
<td>Institute for Pathophysiology and Allergy Research Medical University of Vienna</td>
<td><a href="mailto:Manuel.reithofer@meduniwien.ac.at">Manuel.reithofer@meduniwien.ac.at</a></td>
<td></td>
</tr>
<tr>
<td>Rodgarkia Chantal</td>
<td>THP Medical Products</td>
<td>Austria</td>
<td><a href="mailto:c.rodgarkia@thp.at">c.rodgarkia@thp.at</a></td>
</tr>
<tr>
<td>Roman Carrasco Patricia</td>
<td>Molecular Biotechnology FH-Campus Wien</td>
<td>Austria</td>
<td><a href="mailto:patricia.roman-carrasco@fh-campuswien.ac.at">patricia.roman-carrasco@fh-campuswien.ac.at</a></td>
</tr>
<tr>
<td>Rose-John Stefan</td>
<td>Biochemistry University of Kiel</td>
<td>Germany</td>
<td><a href="mailto:rosejohn@biochem.uni-kiel.de">rosejohn@biochem.uni-kiel.de</a></td>
</tr>
<tr>
<td>Rosenthal Joshua</td>
<td>Eugene Bell Center Marine Biological Laboratory/ Univ of Chicago</td>
<td>United States of America</td>
<td><a href="mailto:jrosenthal@mbl.edu">jrosenthal@mbl.edu</a></td>
</tr>
<tr>
<td>Rossen John W.A.</td>
<td>Department of Medical Microbiology and Infection Prevention University of Groningen, Medical Center Groningen</td>
<td>Netherlands</td>
<td><a href="mailto:john.rossen@gmail.com">john.rossen@gmail.com</a></td>
</tr>
<tr>
<td>Rothbauer Mario</td>
<td>Institute of applied Synthetic Chemistry Vienna University of Technology</td>
<td>Austria</td>
<td><a href="mailto:mario.rothbauer@tuwien.ac.at">mario.rothbauer@tuwien.ac.at</a></td>
</tr>
<tr>
<td>Rotheneder Johann</td>
<td>Medical Biochemistry Medical University of Vienna</td>
<td>Austria</td>
<td><a href="mailto:johann.rotheneder@meduniwien.ac.at">johann.rotheneder@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Ruiz-Saenz Ana</td>
<td>Medicine Dept. UCSF</td>
<td>United States of America</td>
<td><a href="mailto:ana.ruiz-saenz@ucsf.edu">ana.ruiz-saenz@ucsf.edu</a></td>
</tr>
<tr>
<td>Russmayer Hannes</td>
<td>Department of Biotechnology CD Laboratory for Biotechnology of Glycerol, BOKU Wien</td>
<td>Austria</td>
<td><a href="mailto:hannes.russmayer@boku.ac.at">hannes.russmayer@boku.ac.at</a></td>
</tr>
<tr>
<td>Sachet Monika</td>
<td>Dept. of Surgery Medical University of Vienna</td>
<td>Austria</td>
<td><a href="mailto:monika.sachet@meduniwien.ac.at">monika.sachet@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Sandner Georg</td>
<td>Food Technology and Nutrition University of Applied Sciences Upper Austria</td>
<td>Austria</td>
<td><a href="mailto:georg.sandner@fh-wels.at">georg.sandner@fh-wels.at</a></td>
</tr>
<tr>
<td>Santic Marina</td>
<td>Microbiology and Parasitology Medical faculty, University of Rijeka Croatia</td>
<td>Croatia</td>
<td><a href="mailto:marina.santic@medri.uniri.hr">marina.santic@medri.uniri.hr</a></td>
</tr>
</tbody>
</table>
Sauer Michael  
Biotechnology  
BOKU  
Austria  
E: michael.sauer@boku.ac.at

Sauer Ursula  
Center for Health and Bioresources  
AIT Austrian Institute of Technology  
Austria  
E: ursula.sauer@ait.ac.at

Scharl Jessica  
Sales  
Charles River  
Germany  
E: jessica.scharl@crl.com

Scheidt Tamara  
University of Salzburg  
Austria  
E: tamara.scheidt@sbg.ac.at

Schelch Karin  
Institut of Cancer Research  
Medical University of Vienna  
Austria  
E: karin.schelch@meduniwien.ac.at

Schildberger Anita  
Danube University Krems  
Austria  
E: anita.schildberger@donau-uni.ac.at

Schindl Rainer  
Gottfried Schatz Forschungszentrum  
Medical University of Graz  
Austria  
E: Rainer.Schindl@medunigraz.at

Schipper Kerstin  
Institute for Microbiology  
Heinrich Heine University Düsseldorf  
Germany  
E: kerstin.schipper@hhu.de

Schmalzbauer Belinda  
Clinical Institute for Pathology  
Medical University of Vienna  
Austria  
E: belinda.schmalzbauer@outlook.com

Schmid Johannes  
Inst. of Vascular Biol. and Thrombosis Research  
Med. Univ. Vienna  
Austria  
E: johannes.schmid@meduniwien.ac.at

Schmidt Stefan  
VWR International GmbH  
Austria  
E: stefan.schmidt@vwr.com

Schmoll Monika  
Center for Health and Bioresources  
AIT Austrian Institute of Technology  
Austria  
E: monika.schmoll@ait.ac.at

Schmölzer Birgit  
Sales  
Greiner Bio-One  
Austria  
E: birgit.schmoelzer@gbo.com

Schnabl Siegfried  
Eurofins Genomics  
Austria  
E: siegfriedschnabl@eurofins.com

Schneider Magdalena  
Institute of Applied Physics  
TU Wien  
Austria  
E: magdalena.schneider@tuwien.ac.at

Scholze Petra  
Center for Brain Research  
Medical University of Vienna  
Austria  
E: petra.scholze@meduniwien.ac.at

Schönthaler Silvia  
Health & Bioresources  
AIT Austrian Institute of Technology GmbH  
Austria  
E: silvia.schoenthaler@ait.ac.at

Schossberger Markus  
Department of Biotechnology  
University of Natural Resources and Life Sciences, Vienna  
Austria  
E: markus.schossberger@boku.ac.at

Schreiber Martin  
Obstetrics & Gynecology  
Medical University of Vienna  
Austria  
E: martin.schreiber@meduniwien.ac.at

Schroeder Wolfgang  
Marketing  
PALL BIOTECH  
Austria  
E: wolfgang_schroeder@europe.pall.com

Schubert Maria  
molecular biotechnology  
FHCW  
Austria  
E: maria.schubert@stud.fh-campuswien.ac.at

Schuller Artur  
Department of Biotechnology  
CD Laboratory for production of next-level biopharmaceuticals in E. coli, University of Natural Resources and Life Sciences Vienna  
Austria  
E: artur.schuller@boku.ac.at

Schüller Christoph  
DAGZ  
University of Natural Resources and Life Sciences, Vienna  
Austria  
E: Christoph.schueller@boku.ac.at

Schütz Gerhard  
Institute of Applied Physics  
TU Wien  
Austria  
E: schuetz@iap.tuwien.ac.at

Schwaigerlehner Linda  
Department of Biotechnology  
University of Natural Resources and Life Sciences  
Austria  
E: linda.schwaigerlehner@boku.ac.at

Schwestka Jennifer  
Department of Applied Genetics and Cell Biology  
University of Natural Resources and Life Sciences  
Austria  
E: jennifer.schwestka@boku.ac.at
Sedivy Arthur
Protein Technologies
Vienna Biocenter Core Facilities GmbH
Austria
E: arthur.sedivy@vbcf.ac.at

Seferovic Hannah
Institute for Biophysics
Johannes Kepler University Linz
Austria
E: hannah.seferovic@gmx.at

Selimovic Semir
Bartelt
Austria
E: ses@arctiko.com

Semeraro Enrico F.
Institute of Molecular Biosciences (Biophysics)
University of Graz
Austria
E: enrico.semeraro@uni-graz.at

Sessitsch Angela
AIT Austrian Institute of Technology
Austria
E: angela.sessitsch@ait.ac.at

Sheibani-Tezerji Raheleh
Applied Diagnosis
Ludwig Boltzmann Institute and General Hospital of Vienna
Austria
E: raheleh.sheibani@lbiad.lbg.ac.at

Sicot François-Xavier
Takara Bio Europe
France
E: francois-xavier_sicot@takarabio.com

Siegert Sandra
Institute of Science and Technology
Austria
E: sandra.siegert@ist.ac.at

Simon Sylvia
Takara Bio Europe
France
E: sylvia_simon@takarabio.com

Sissolak Bernhard
University of Natural Resources and Life Sciences, Vienna
Austria
E: bernhard.sissolak@boku.ac.at

Sitte Harald
Medizinische Universität Wien
Austria
E: harald.sitte@meduniwien.ac.at

Sladky Valentina
Developmental Immunology
Medical University of Innsbruck
Austria
E: valentina.sladky@i-med.ac.at

Soztekin ilayda G.
Neuroscience
Medical University of Vienna
Turkey
E: gokce.gorgulu@gmail.com

Spadiut Oliver
Biochemical Engineering
TU Wien
Austria
E: oliver.spadiut@tuwien.ac.at

Sperl Eva
Seedfinancing
Austria Wirtschaftsservice
Austria
E: E.Sperl@aws.at

Staber Philipp
Div. of Hematology and Hemostaseology
Medical University Vienna
Austria
E: philipp.staber@meduniwien.ac.at

Stadler Marc
MWIS
Helmholtz-Zentrum für Infektionsforschung (HZI)
Germany
E: mst12@helmholtz-hzi.de

Stainer Sarah
Institute of Biophysics
Johannes Kepler University Linz
Austria
E: sarah.stainer@stud.sbg.ac.at

Stampfl Hansjörg
Center for Health & Bioresources
AIT - Austrian Institute of Technology
Austria
E: hansjoerg.stampfl@ait.ac.at

Stary Victoria
Department for Surgery
Medical University Vienna
Austria
E: victoria.stary@meduniwien.ac.at

Staudacher Jennifer
Department of Biotechnology
University of Natural Resources and Life Sciences
Austria
E: jennifer.staudacher@boku.ac.at

Steiger Matthias
Systems biology & microbial cell engineering
ACIB GmbH
Austria
E: matthias.steiger@boku.ac.at

Steinkellner Hannes
Institute of Medical Genetics
Medical University of Vienna
Austria
E: hannes.steinkellner@meduniwien.ac.at

Sternberg Christina
Department for Pathobiology
Institute of Pathology and Forensic Veterinary Medicine, Unit of Laboratory Animal Pathology
Austria
E: christina.sternberg@vetmeduni.ac.at

Stockner Thomas
Pharmacology
Medical University of Vienna
Austria
E: thomas.stockner@meduniwien.ac.at

Stolze Klaus
Univ. Veterinary Medicine Vienna
Austria
E: klaus.stolze@vetmeduni.ac.at

Strandt Helen
Biosciences
University of Salzburg
Austria
E: helen.strandt@sbg.ac.at
Participant Index

Strauss Joseph  
Appl. Genetics & Cell Biology  
BOKU University - Campus Tulln  
Austria  
E: joseph.strauss@boku.ac.at

Streck Christina  
Operational Marketing  
Eppendorf Austria GmbH  
Austria  
E: streck.c@eppendorf.at

Strobl Maria R.  
Institute of Pathophysiology and Allergy Research  
Medical University of Vienna  
Austria  
E: maria.r.strobl@meduniwien.ac.at

Stübl Flora  
FH-Wels  
Austria  
E: Flora.Stuebl@fh-wels.at

Summer Sabrina  
Center for Anatomy and Cell Biology  
Medical University of Vienna  
Austria  
E: sabrina.summer@meduniwien.ac.at

Swoboda Ines  
Section Biotechnology  
FH Campus Wien  
Austria  
E: ines.swoboda@fh-campuswien.ac.at

Szabo P. Lujza  
Center for Biomedical Research  
Medical University of Vienna  
Austria  
E: petra.szabo@meduniwien.ac.at

Szöllösi Dániel  
Institute of Pharmacology  
Medical University of Vienna  
Austria  
E: daniel.szolloesi@meduniwien.ac.at

Tadic Jelena  
University of Graz  
Austria  
E: jelena.tadic@uni-graz.at

Tanzer Andrea  
Department of Theoretical Chemistry  
University of Vienna  
Austria  
E: at@tbi.univie.ac.at

Tarlungeanu Dora Clara  
Neuroscience  
IST Austria  
Austria  
E: dora_tarlungeanu@yahoo.com

Thanner Jürgen  
Thoracic Surgery  
Medical University Vienna  
Austria  
E: juergenthanner@gmx.at

Tharad Sudarat  
Nanobiotechnology  
University of Natural Resources and Life Sciences (BOKU)  
Austria  
E: sudarat.tharad@boku.ac.at

Thiel Isabella  
AG Borth  
University of Natural Resources and Life Sciences Vienna  
Austria  
E: thiel@gmx.at

Tisch Marcel  
Developmental Immunology  
Medical University, Innsbruck  
Austria  
E: Marcel.t@online.de

Toca-Herrera Jose L.  
Nanobiotechnology /Biophysics  
BOKU  
Austria  
E: jose.toca-herrera@boku.ac.at

Toca-Herrera Jose L.  
Nanobiotechnology  
BOKU  
Austria  
E: jltocah@gmail.com

Totaro Damiano  
acib GmbH  
Austria  
E: damiano.totaro@boku.ac.at

Trakaki Athina  
Otto Loewi Research Center, Unit of Pharmacology  
Medical University of Graz  
Austria  
E: athina.trakaki@medunigraz.at

Tran Loan  
Molecular Pathology  
Ludwig Boltzmann Institute Applied Diagnostics  
Austria  
E: loan.tran@lbiad.lbg.ac.at

Tripisciano Carla  
Center for Biomedical Technology, CD-Laboratory for Innovative Therapy Approaches in Sepsis  
Danube University Krems  
Austria  
E: carla.tripisciano@donau-uni.ac.at

Tscheppe Angelika  
Department of Pathophysiology and Allergy Research  
Medical University of Vienna  
Austria  
E: angelika.tscheppe@meduniwien.ac.at

Tscherner Michael  
Department for Medical Biochemistry  
Medical University of Vienna, MFPL  
Austria  
E: michael.tscherner@univie.ac.at

Twaruschek Krisztian  
Applied Genetics and Cell Biology  
BOKU  
Austria  
E: krisztian.twaruschek@boku.ac.at

Untersmayr-Elsenhuber Eva  
Institute of Pathophysiology and Allergy Research  
Medical University of Vienna  
Austria  
E: eva.untersmayr@meduniwien.ac.at

Üzülmez Öykü  
Department of Pathophysiology & Allergy Research  
Medical University of Vienna  
Austria  
E: oeykue.uezuelmez@meduniwien.ac.at
Valko Zsuzsanna  
Department of Tumor Biology  
National Korányi Institute of Pulmonology, Budapest  
Hungary  
E: zsuzsanna.valko@meduniwien.ac.at

Vandenabeele Peter  
VIB-UGent Center for Inflammation Research  
UGent-VIB  
Belgium  
E: Peter.Vandenabeele@irc.vib-UGent.be

Venhuizen Petrus  
University of Natural Resources and Life Sciences (BOKU)  
Austria  
E: peter.venhuizen@boku.ac.at

Venturino Alessandro  
Institute of Science and Technology IST  
Austria  
E: alessandro.venturino@ist.ac.at

Villunger Andreas  
Med Uni Innsbruck  
Austria  
E: andreas.villunger@i-med.ac.at

Villunger Matthias  
Med Uni Innsbruck  
Austria  
E: matthias.villunger@student.i-med.ac.at

Vogel Andrea  
Medical University Vienna  
Institute for Vascular Biology and Thrombosis Research  
Austria  
E: andrea.vogel@meduniwien.ac.at

Vogl Franziska  
Biobank Graz  
Medical University of Graz  
Austria  
E: franziska.vogl@medunigraz.at

Volpini de Maestri Antonia  
IMBT  
TU Graz  
Austria  
E: volpinidemastri@student.tugraz.at

Vrij Erik J.  
Koo’s lab  
IMBA  
Austria  
E: erikvrij@gmail.com

Wagner Martin  
Farm Animals and Veterinary Public Health  
University for Veterinary Medicine  
Austria  
E: martin.wagner@vetmeduni.ac.at

Walczak Henning  
Molecular Biology  
UCL Cancer Institute  
United Kingdom  
E: h.walczak@ucl.ac.uk

Walter Ingrid  
VetCore Facility for Research  
University of Veterinary Medicine  
Austria  
E: Ingrid.Walter@vetmeduni.ac.at

Wanzenböck Elisa  
Institut für Lebensmittelwissenschaften  
Universität für Bodenkultur  
Austria  
E: elisa.wanzenboeck@boku.ac.at

Watthanasakphuban Nisit  
Food Science and Biotechnology  
BOKU  
Austria  
E: nisitwb@gmail.com

Weber Andreas  
Institute of Biophysics, DNBT  
University of Natural Resources and Life Sciences Vienna (BOKU)  
Austria  
E: andreas.weber@boku.ac.at

Weber Viktoria  
Health Sciences and Biomedicine  
Danube University Krems  
Austria  
E: viktoria.weber@donau-uni.ac.at

Weckwerth Wolfram  
Ecogenomics and Systems Biology  
University of Vienna  
Austria  
E: wolfram.weckwerth@univie.ac.at

Weiler Angelika  
Technopol Tulln  
ecoplus.Niederösterreichs  
Wirtschaftsagentur GmbH  
Austria  
E: technopol-tulln@ecoplus.at

Weissmann Peter  
Sales  
Eppendorf Austria GmbH  
Austria  
E: weissmann.p@eppendorf.at

Weitzer Georg  
Abteilung für Molekulare Genetik, Zentrum für Medizinische Biochemie  
MFPL, MUW  
Austria  
E: georg.weitzer@univie.ac.at

Werdan Barbara  
Sales  
PALL BIOTECH  
Austria  
E: barbara_werdan@europe.pall.com

Werning Maike  
Department of medical Biochemistry, MFPL  
Medical University of Vienna, MFPL  
Austria  
E: maike.werning@univie.ac.at

Wieser Monika  
Technology platform  
VetCore/VetBiobank  
University of Veterinary Medicine, Vienna  
Austria  
E: monika.wieser@vetmeduni.ac.at

Winter, MA Robert  
World Courier (Austria) GmbH  
Austria  
E: rwinter@worldcourier.at

Wohlschlager Lena  
Department of Food Science and Technology  
University of Natural Resources and Life Sciences, Vienna  
Austria  
E: lena.wohlschlager@boku.ac.at
Wohlschlager Therese  
Department of Biosciences  
University of Salzburg  
Austria  
E: therese.wohlschlager@sbg.ac.at

Wolf Brigitte  
Dept. of Surgery, Research Laboratories  
Medical University of Vienna  
Austria  
E: brigitte.wolf@meduniwien.ac.at

Wolf Noa  
Health & Bioresources  
AIT Austrian Institute of Technology  
Austria  
E: Noa.Wolff.fl@ait.ac.at

Wossidlo Mark  
Department of Cell- and Developmental Biology  
Medical University of Vienna  
Austria  
E: mark.wossidlo@meduniwien.ac.at

Wurm David J.  
Institut für Verfahrenstechnik, Umwelttechnik und Technische Biowissenschaften  
TU Wien  
Austria  
E: david.wurm@tuwien.ac.at

Yu Josef  
Department of Surgery  
Medical University of Vienna  
Austria  
E: josef.yu@univie.ac.at

Zanghellini Jürgen  
Austrian Center of Industrial Biotechnology  
University of Natural Resources and Life Sciences, Vienna, Austria  
Austria  
E: juergen.zanghellini@boku.ac.at

Zappe Katja  
Department of Analytical Chemistry  
University of Vienna  
Austria  
E: katja.zappe@univie.ac.at

Zarfel Gernot  
Diagnostic & Research Center for Molecular BioMedicine  
Medical University of Graz  
Austria  
E: gernot.zarfel@medunigraz.at

Zelger Philipp  
Biomedical Physics  
Medical University of Innsbruck  
Austria  
E: philipp.zelger@i-med.ac.at

Zell Lukas  
Department of Biosciences  
University of Salzburg  
Austria  
E: lukas.zell@hotmail.com

Zhigalin Andrew  
clalit  
Israel  
E: andreizh12@gmail.com

Ziegler Christine  
Biophysik  
Universität Regensburg  
Germany  
E: Christine.Ziegler@biologie.uni-regensburg.de

Zilla Peter  
Christiaan Barnard Dept. for Cardiothoracic Surgery  
Univ. of Cape Town  
South Africa  
E: peter.zilla@uct.ac.za

Zipfl Nicole  
Szabo Scandic HandelsGmbH  
Austria  
E: n.zipfl@szabo-scandic.com

Zotchev Sergey  
Department of Pharmacognosy  
University of Vienna  
Austria  
E: sergey.zotchev@univie.ac.at

Zwirzitz Benjamin  
FFoQSI GmbH  
Austria  
E: Benjamin.Zwirzitz@vetmeduni.ac.at
Acknowledgements

We would like to thank the following people for their contributions and patient support for making the meeting possible: Michael Sauer together with the organizing committee, the members of the scientific committee, the Invited Speakers, Walter Glaser, Birgit Putz, all the helpers, partners and sponsors.
The ÖGMBT gratefully acknowledges the support by the following sponsors & exhibitors:

**SILVER SPONSORS**

- TaKaRa
- PALL Biotech
- LIMA Vienna
- GE Healthcare

**AUSTRIA**

- Microsynth
- Advanced Analytical
- Axonlab
- bartelt
- berthold...

**BIOLAB**

- bioRad
- biolab
- biomediqa
- Biozynex
- charles river

**EPPENDORF**

- eppendorf
- EQ Equipment Bock
- eurofins / Genomics
- greiner bio-one

**KML VISION**

- KML Vision
- BioCat
- Polymun

**LICOR**

- LI-COR

**Life-science**

- life-science
- BioLabs GmbH
- Metrohm

**SARSTEDT**

- Sachs + Schmid
- SARSTEDT

**ÖGTERM**

- open science
- Roche
- vwr

**Medical Products**

- ThermoFisher Scientific
- THP

**World Courier**

- Ana
- Austrian Neuroscience Association
- leXogen

**Media Partners**

- Chemie Report
- Austrian Life Sciences
- Biofaction