



Industrial  
Biotechnology  
Forum 2018

# PROGRAM

**March**  
**13 / 14**  
**2018**

— Venue —  
**TUM Garching**



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## Patronage of the IBF 2018



*The Industrial Biotechnology Forum 2018 is under the patronage of the Bavarian State Minister of Economic Affairs and Media, Energy and Technology, Ms. Ilse Aigner, and the State Secretary in the Bavarian Ministry of Economic Affairs and Media, Energy and Technology, Mr. Franz Josef Pschierer.*

We are particularly delighted to be patrons of the Industrial Biotechnology Forum this year, as this conference is now established as a biennial international conference series especially dedicated to all aspects of Industrial Biotechnology. Seeing the success of the event in 2016, it is certain to say that the IBF conferences are supposed to become the leading meet-up event in this sector.

As the program indicates, the IBF 2018 virtually builds the foundation for a long-lasting and successful meeting series by combining excellence, internationality and representation of academia and industry in a very unique approach. The close connection of scientific achievements and economic commercialization reflects the essence of the Bavarian policy for transferring scientific highlights to the market. Only with a tight link between science and economy, a sustainable and value adding network of all stakeholders can develop, thus generating a new, a bio-based economy.

The concept of the IBF is the result of an alliance between two "Bavarian stars": the elite and entrepreneurial Technical University of Munich and the extremely spry and thriving Industrielle Biotechnologie Bayern Netzwerk GmbH (abbr. IBB Netzwerk GmbH). We support the IBB Netzwerk since 2008 to foster technology transfer and to promote Industrial Biotechnology and a sustainable economy. Thus, we are very pleased to see how well the Industrial Biotechnology is meanwhile established in Bavaria. Approximately 340 companies with roughly 40,000 employees work in this sector in Bavaria creating new solutions for food, cosmetics or plastics – to name only a few fields of application.

We wish the organizers as well as all participants a very interesting, successful and inspiring IBF 2018!

**Ilse Aigner**

*Bavarian State Minister  
of Economic Affairs and Media,  
Energy and Technology;  
Deputy Minister-President of Bavaria*

**Franz Josef Pschierer**

*State Secretary in the Bavarian Ministry  
of Economic Affairs and Media,  
Energy and Technology*



*Prof. Dr. Zorbas /  
Industrielle Biotechnologie  
Bayern Netzwerk GmbH –  
organizer*



*Prof. Dr. Liebl und Prof. Dr. Sieber /  
Technical University of Munich –  
scientific responsibility*



## Welcome to the IBF 2018!

The Industrial Biotechnology Forum (IBF) is our new biennial international scientific conference series highlighting new developments, recent scientific results and future trends in Industrial Biotechnology. Organized by the IBB Netzwerk GmbH (the network organization of Bavaria for the advancement of Industrial Biotechnology and sustainable economy) and under the scientific responsibility of the Technical University of Munich, the IBF intends to open the doors to scientists from academy and industry worldwide for an intense interdisciplinary exchange on this field.

The launching event in 2016 was a great success having attracted more than 170 international participants. With the IBF 2018, we want to continue this success story. Besides high-impact research presentations from academy and industry, the IBF 2018 will be accompanied by an enlarged industrial and poster exhibition.

Particular highlights are the patronage of the Bavarian State Minister of Economic Affairs and Media, Energy and Technology, Ms. Ilse Aigner, and of the State Secretary in the Bavarian Ministry of Economic Affairs and Media, Energy and Technology, Mr. Franz Josef Pschierer. The Ministry also invites to the Conference Dinner on the evening of the first conference day.

The IBF 2018 highlights the newest developments within the scientific core competences of Industrial Biotechnology: on the one hand, Enzyme Catalysis and Metabolic Engineering, i.e. technologies delivering new and efficient biocatalysis pathways; and on the other hand, Bioprocess Engineering and Upscaling as well as Bioseparation Engineering to enable use of new biocatalysts in an efficient way on an industrial scale. In addition, two Special Sessions about topical subjects are scheduled, one on Synthetic Biotechnology and another on Bioinformatic Trends in Biotechnology.

The invited speakers are international experts not only from Academia but also from globally acting enterprises as well as from medium size companies. Thus, the intersectoral character and the economic relevance of Industrial Biotechnology is demonstrated. Chosen speakers from submitted abstracts complement every session of the conference. In the breaks, there is plenty of time to visit the industrial and the poster exhibition and to reflect and exchange ideas with the experts and with each other.

We invite you cordially to submit your recent results for oral or poster presentation and to participate at this international and interdisciplinary event about Industrial Biotechnology science.

**Prof. Dr.  
Haralabos Zorbas**

*Managing Director  
Industrielle Biotechnologie  
Bayern Netzwerk GmbH*

**Prof. Dr.  
Wolfgang Liebl**

*Chair of Microbiology  
Technical University of Munich*

**Prof. Dr.  
Volker Sieber**

*Rector TUM Campus Straubing for  
Biotechnology and Sustainability;  
Chair of Chemistry of Biogenic Resources,  
Technical University of Munich*

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**ThermoFisher**  
S C I E N T I F I C

SPONSORS



PARTNERS



## Scientific Committee

### Prof. Dr. Sonja Berensmeier

Bioseparation Engineering,  
Technical University of Munich, Garching

### Prof. Dr. Thomas Brück

Werner Siemens-Chair of Synthetic Biotechnology,  
Technical University of Munich, Garching

### Prof. Dr.-Ing. Andreas Kremling

Systems Biotechnology,  
Technical University of Munich, Garching

### Prof. Dr. Wolfgang Liebl

Microbiology,  
Technical University of Munich – School of  
Life Sciences, Freising-Weihenstephan

### Prof. Dr.-Ing. Mirjana Minceva

Biothermodynamics,  
Technical University of Munich, Freising

### Prof. Dr. Volker Sieber

Chemistry of Biogenic Resources,  
Technical University of Munich Campus Straubing  
for Biotechnology and Sustainability, Straubing

### Dr. Axel Trefzer

Synthetic Biology R&D,  
Thermo Fisher Scientific GENEART GmbH,  
Regensburg

## Organizer and financier



Netzwerk GmbH

IBB Netzwerk GmbH is a network and service organization in the field of Industrial Biotechnology and sustainable economy. Its goal is to catalyze the implementation

of valuable scientific knowledge into innovative, marketable products and processes.

IBB Netzwerk GmbH manages inter alia the cooperation networks "MoDiPro", "UseCO<sub>2</sub>", "Waste2Value" and "BioPlastik" and supports the partners in the development of R&D projects.

IBB Netzwerk GmbH is the organizer and financier of the Industrial Biotechnology Forum, which now takes place for the second time.

### CONTACT

#### Industrielle Biotechnologie Bayern Netzwerk GmbH

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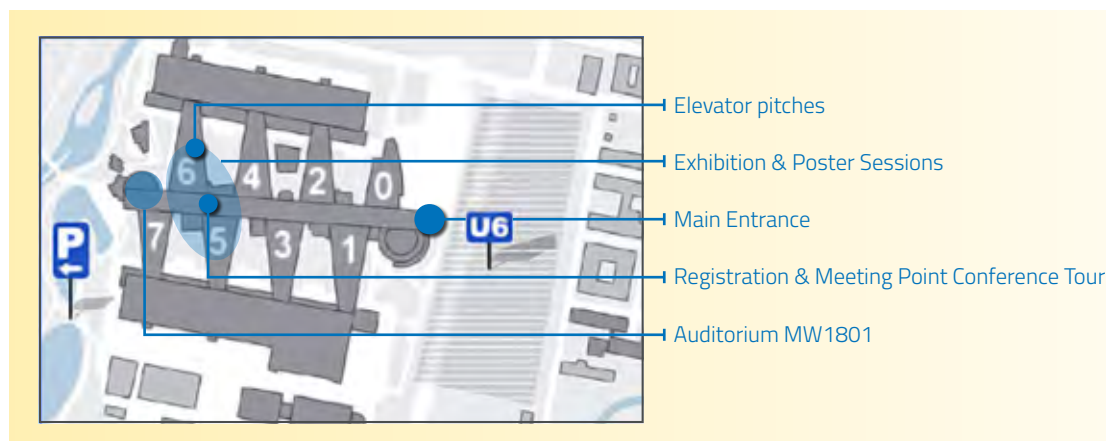
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# Where, when & what?

## Industrial Biotechnology Forum 2018

March 13-14 (Tuesday-Wednesday)



### Conference Venue

TUM Department of Mechanical Engineering  
Boltzmannstraße 15  
85748 Garching b. München (Germany)

The TUM Department of Mechanical Engineering is part of the Garching Research Center, subway station "Garching-Forschungszentrum" (U6)

### Registration and information desk

The registration and information desk is located in the rear part of the TUM Department of Mechanical Engineering, ground floor, and is open for participant registration on Tuesday, March 13th, from 9:00 a.m. and on Wednesday, March 14th, from 8:00 a.m.

### Conference sessions

The welcome addresses and all scientific presentations will take place in lecture hall MW 1801 in the rear part of the TUM Department of Mechanical Engineering, 1<sup>st</sup> floor.

### Exhibition, posters & elevator pitches

The exhibition with industry booths and scientific posters will be accessible during the entire IBF 2018. Elevator pitches and poster presentations take place on Tuesday, March 13th, from 5:30 p.m. in the exhibition area. Scientific posters can be placed directly after registration on Tuesday, March 13th, from 9:00 until 10:30 a.m.

### Conference Dinner

The Bavarian Ministry of Economic Affairs and Media, Energy and Technology invites to the conference dinner at the Ludwig-Erhard hall (BayStMWi, Prinzregentenstraße 28, 80538 München). A supervised cloakroom will be provided. You need to be registered for the dinner beforehand. Meeting point for the bus transfer is on Tuesday, March 13th, at 6:50 p.m. next to the registration desk. Your conference badge acts as identification, please keep it visible with you.

### Conference Tour

For registered participants, conference tours to the TUM Pilot Plant for Industrial Biotechnology in Garching are taking place on Wednesday, March 14th, from 4:30 p.m. until approx. 6:00 p.m. The tours will be organized in two groups. The participants will meet at 4:20 p.m. (group 1) and 4:50 p.m. (group 2) respectively. Meeting point is next to the registration desk. You will find all necessary information on your 'Info card' (see next paragraph).

### Info card

During registration, you receive an 'Info card' together with your conference badge. On this card, information is provided regarding your participation at the conference dinner and conference tour, and, if applicable, your poster number and the number of your exhibition booth. Please check the provided information.

# 1<sup>st</sup> Conference day

## Tuesday, March 13, 2018

07:00	Registration for exhibitors
09:00	Registration for conference participants
09:00	Exhibition opening
11:00	Conference opening and welcome address
	<ul style="list-style-type: none"> <li>Prof. Dr. Haralabos Zorbas, Managing Director Industrielle Biotechnologie Bayern Netzwerk GmbH</li> <li>Prof. Dr. Wolfgang Liebl, Chair of Microbiology, Technical University of Munich</li> <li>Prof. Dr. Volker Sieber, Rector TUM Campus Straubing for Biotechnology and Sustainability; Chair of Chemistry of Biogenic Resources, Technical University of Munich</li> </ul>
11:30	1 <sup>st</sup> Session: Enzyme Catalysis – Chair: Prof. Dr. Volker Sieber
	<p><b>Talk 1.1</b> Prof. Dipl.-Ing. Dr. techn. <b>Bernd Nidetzky</b>, Institute of Biotechnology and Biochemical Engineering, Technische Universität Graz; CSO of The Austrian Centre of Industrial Biotechnology (acib) Austria – <i>“Enzymes and sugars: biotransformations of recalcitrant substrates, small and large”</i></p> <p><b>Talk 1.2</b> Dr. <b>Kai-Uwe Baldenius</b>, Head of Biocatalysis Research, BASF SE, Germany – <i>“Biocatalysis: We create chemistry – with a little help from enzymes!”</i></p> <p><b>Talk 1.3</b> Dr. <b>Katrin Schullehner</b>, Phytowelt GreenTechnologies GmbH, Germany – <i>“Improving Lignan Biosynthesis in E. coli by Engineering of Plant P450 Oxidoreductases”</i></p> <p><b>Talk 1.4</b> <b>Agathe Bronikowski</b>, Institute of Biochemistry II, Heinrich-Heine University Düsseldorf, Germany – <i>“A new manganese peroxidase: High yield production and mutagenesis towards versatile peroxidase”</i></p>
13:00	Luncheon
14:00	2 <sup>nd</sup> Session: Metabolic Engineering – Chair: Prof. Dr. Wolfgang Liebl
	<p><b>Talk 2.1</b> Prof. Dr. <b>Philippe Soucaille</b>, Université de Toulouse, Institut National des Sciences Appliquées (INSA), Toulouse; CSO of METabolic Explorer, Bipole Clermont-Limagne, Saint Beauzire, France – <i>“The Weizmann Process Revisited for the Continuous Production of n-Butanol”</i></p> <p><b>Talk 2.2</b> Dr. <b>Jürgen Eck</b>, CEO B.R.A.I.N. AG, Germany – <i>“Engineering Biology: From strain improvement to Designer Bugs”</i></p> <p><b>Talk 2.3</b> Dr. <b>Hannes Link</b>, Dynamic Control of Metabolic Networks, Max Planck Institute for terrestrial Microbiology, Germany – <i>“Engineering metabolic regulation: Why so many feedbacks?”</i></p> <p><b>Talk 2.4</b> Dr. <b>Armin Ehrenreich</b>, Department of Microbiology, Technical University of Munich, Germany – <i>“Construction of Tailored Acetic Acid Bacteria Strains for Oxidative Fermentations”</i></p>
15:30	Coffee break
16:00	Special Session: Synthetic Biotechnology – Chair: Dr. Axel Trefzer
	<p><b>Talk 3.1</b> Prof. Dr. <b>Sven Panke</b>, Bioprocess Laboratory, Department of Biosystems Science and Engineering, ETH Zürich, Switzerland – <i>“Design and evolution of novel chemical functions”</i></p> <p><b>Talk 3.2</b> Dr. <b>Jonathan Chesnut</b>, Senior Director R&amp;D Synthetic Biology, Thermo Fisher Scientific, USA – <i>“Something old, something new: Improving genome editing efficiency over CRISPR with a new generation of TALE nucleases”</i></p> <p><b>Talk 3.3</b> Dr.-Ing. <b>Jochen Schmid</b>, Chair of Chemistry of Biogenic Resources, Technical University of Munich Campus Straubing for Biotechnology and Sustainability, Germany – <i>“Paenibacillus polymyxa as novel chassis organism for heterologous”</i></p> <p><b>Talk 3.4</b> <b>Sabine G. Wagner</b>, Systems Biotechnology, Technical University of Munich, Germany – <i>“Intracellular dynamics during heterologous protein expression”</i></p>
17:30	Poster session
17:30	Exhibition with “elevator pitches”
19:00	Bus departure to the Bavarian Ministry of Economic Affairs and Media, Energy and Technology
20:00	The Bavarian Ministry of Economic Affairs and Media, Energy and Technology invites to the Ludwig-Erhard hall, Munich
	Speech of State Secretary Mr. Franz Josef Pschierer
20:15	Conference Dinner
22:30	Bus departure back to Garching



# 2<sup>nd</sup> Conference day

## Wednesday, March 14, 2018

08:00	Further registration for conference participants
08:00	Exhibition opening
09:00	<b>4<sup>th</sup> Session: Bioprocess Engineering / Upscaling – Chair: Prof. Dr.-Ing. Andreas Kremling</b>
	<b>Talk 4.1</b> Prof. Dr.-Ing. <b>Irina Smirnova</b> , Institute of Thermal Separation Processes, Hamburg University of Technology, Germany – <i>“Integration of enzymes in reactive separation processes: extraction and distillation”</i>
	<b>Talk 4.2</b> Dr. <b>Gernot Jäger</b> , Bioprocesses, Covestro Deutschland AG, Germany and Dr. <b>Wolf Klöckner</b> , Engineering & Technology, Bayer AG, Germany – <i>“Biobased Aniline: A sustainable new route to a strategic raw material”</i>
	<b>Talk 4.3</b> Dr. <b>Maria Alexandri</b> , Department of Bioengineering, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Germany – <i>“Robust lactic acid production from defatted rice bran using a thermophilic Bacillus coagulans strain”</i>
	<b>Talk 4.4</b> Dr.-Ing. <b>Thomas Hahn</b> , Molecular Biotechnology, Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany – <i>“Xylonic acid from hemicellulose hydrolysates”</i>
10:30	Coffee break
11:00	<b>Special Session: Bioinformatic Trends in Biotechnology – Chair/Moderation: Prof. Dr. Thomas Brück</b>
	<b>Talk 5.1</b> Prof. Dr. <b>Walter Thiel</b> , Theoretical Chemistry, Director at Max Planck Institute of Coal Research at Mülheim, Germany – <i>“Theoretical Studies of Enzymatic Reactions”</i>
	<b>Talk 5.2</b> Prof. Dr. <b>Victor Guallar</b> , Founder and Head of the Advisory Board at Nostrum Biodiscovery, ICREA Professor at Barcelona Supercomputing Center, Spain – <i>“Got Enzymes? A la C arte Design through Molecular Modelling”</i>
	<b>Talk 5.3</b> Dr. <b>Hubert Bernauer</b> , ATG:biosynthetics GmbH, Germany – <i>“Expression relevant molecular parameters for genetically re-coding synthetic genes aiming on functionally refactoring biochemical gene clusters”</i>
	<b>Talk 5.4</b> <b>Manuela Gottardi</b> , Institute for Molecular Bioscience AK Boles, Goethe University Frankfurt, Germany – <i>“Metabolic engineering of Saccharomyces cerevisiae for the production of aromatic compounds of industrial relevance”</i>
12:30	Poster awards
12:45	Luncheon and Exhibition
14:15	<b>6<sup>th</sup> Session: Bioseparation Engineering – Chairs: Prof. Dr. Sonja Berensmeier, Prof. Dr.-Ing. Mirjana Minceva</b>
	<b>Talk 6.1</b> Dr. <b>Simone Dimartino</b> , Institute for Bioengineering, School of Engineering, University of Edingburgh, UK – <i>“3D printed chromatography columns: changing the face of downstream processing”</i>
	<b>Talk 6.2</b> Dr. <b>Fabrice Gentile</b> , Biotech & Biopharma Business Manager at Ypso-Facto, France – <i>“Using modeling for a rational design of continuous chromatographic Bioprocesses”</i>
	<b>Talk 6.3</b> Dr. <b>Cristina Peixoto</b> , Animal Cell Technology Unit, Instituto de Biologia Experimental e Tecnológica (iBET), Portugal – <i>“Improving Downstream Processing of Influenza VLPs using a Click Chemistry strategy”</i>
	<b>Talk 6.4</b> Prof. Dr. <b>Ludo Diels</b> , Flemish Institute for Technological Research – VITO, Separation & Conversion Technology, Belgium – <i>“Membrane separation technology as a valuable and efficient tool in the value chain of wood towards chemicals and materials”</i>
15:45	Closing remarks
16:30	Optional visit to the TUM Pilot Plant for Industrial Biotechnology
17:00-18:00	End of Conference (depending on tour participation)

Talk  
1.1



## Prof. Dipl.-Ing. Dr. techn. Bernd Nidetzky

Institute of Biotechnology and Biochemical Engineering,  
University of Graz, Austria

[bernd.nidetzky@acib.at](mailto:bernd.nidetzky@acib.at)

[www.tugraz.at/institute/biote/home/](http://www.tugraz.at/institute/biote/home/)

### MAIN RESEARCH FIELD

- Applied enzymology and enzyme technology
- Metabolic and reaction engineering for biocatalysis (fine chemical synthesis) and renewable resource utilization
- Glycobiotechnology
- Heterogeneous enzyme catalysis, interactions of enzymes with solid surfaces

### EDUCATION AND EMPLOYMENT

Study of Technical Chemistry Graz University of Technology; doctoral degree in biotechnology, Graz University of Technology

- |           |  |
|-----------|--|
| 1992–1999 | PostDoc and Research assistant (biochemical engineering), Institute of Food Technology, University of Natural Resources and Life Sciences, Vienna, Austria |
| 1999–2002 | Associate professor, Institute of Food Technology, University of Natural Resources and Life Sciences, Vienna, Austria                                      |
| 2002      | Full professor of biotechnology, Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Austria                            |
| 2004      | Head of Institute of Biotechnology and Biochemical Engineering   |
| 2011      | Vice Dean of Faculty of Technical Chemistry, Process Engineering, and Biotechnology  |
| 2012      | Head of Field of Expertise in Human- and Biotechnology at Graz University of Technology  |
| 2014      | Scientific director (CSO) of the Austrian Centre of Industrial Biotechnology   |

# Dr. Kai-Uwe Baldenius

Head of Biocatalysis Research, BASF SE, Germany

[kai.baldenius@basf.com](mailto:kai.baldenius@basf.com)  
[www.basf.com](http://www.basf.com)



Talk  
1.2

## MAIN RESEARCH FIELD

- Enzymes as robust, industrially suitable biocatalysts (e.g. lipases, amidases, nitrilases, hydratases, cyclases, alcohol dehydrogenases, transaminases, transglutaminase; phosphatases)
- Enzyme catalysis for carbohydrate synthesis and transformation
- Process development for large scale biotransformations

## ACADEMIC CURRICULUM VITAE

Kai Baldenius is a chemist by formation. After receiving a PhD from Hamburg University, he spent a post-doc research year at The Scripps Research Institute before he joined BASF in 1993.

In BASF Kai started his career in the central research doing process development work for vitamins and other fine chemicals. He took over responsibilities in production, marketing and sales, before he returned to research.

Since 2009, Kai led the BASF biocatalysis research group. In March 2018, he has been appointed Principal Scientist for Biocatalysis at BASF. In addition, he currently serves as visiting professor at the University of Manchester.



## Prof. Dr. Philippe Soucaille

Université de Toulouse, METabolic EXplorer, France

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[www.insa-toulouse.fr](http://www.insa-toulouse.fr)

### MAIN RESEARCH FIELD

The work carried out within the PEEP team (for Pathways Evolution and Engineering in Prokaryotes), is dedicated to the exhaustive analysis of bacterial metabolism through a Systems Biology approach in order to improve its knowledge and identify new regulatory mechanisms. Based on the expertise acquired on Genome Scale Model and Metabolic Pathway Engineering, the concept of microbial cell factory is developed: new metabolic pathways are created and the metabolism is rationally modified and reoriented to maximize the substrate / product conversion efficiency for the production of biological synthons for chemistry and / or energy. The team brings together a dozen people including five permanent researchers.

### ACADEMIC CURRICULUM VITAE

Philippe Soucaille serves as the Chief Scientific Officer at Metabolic Explorer and is a Professor of Microbial Physiology at the National Institute for Applied Sciences (University of Toulouse, France). He is an expert on the physiology and molecular genetics of bacteria. He has published more than 80 papers in this field and is inventor or co-inventor on more than 60 patents. He and his group at INSA pioneered methods for the in vivo evolution of enzymes and metabolic pathways and successfully applied them to the production of several bulk chemicals. Previously, he served as senior scientist and project leader at Genencor International Inc, where he managed the 1, 3-propanediol project, an industrial collaborative project with DuPont de Nemours involving more than 40 scientists. This was a major breakthrough in the development of chemicals from clean, renewable raw materials. He holds a BS degree in Microbiology from the University of Toulouse and received MA and PhD degrees in Biochemical Engineering from INSA.

# Dr. Jürgen Eck

CEO B.R.A.I.N. AG, Germany

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[www.brain-biotech.de](http://www.brain-biotech.de)



Talk  
2.2

## MAIN RESEARCH FIELD

BRAIN is a pacemaker of the bioeconomy and a high-tech pioneer of sustainable applied biotechnology. BRAIN stands for the biologisation of both industry and the consumer world. The Group develops and markets product and process innovations based on species diversity and its own BioArchive. Its work focuses on bioactive natural compounds, nature-based enzymes and customised high-performance microorganisms for sustainable applications in the consumer goods and chemicals industries.

## ACADEMIC CURRICULUM VITAE

Dr Jürgen Eck received his doctorate title at TU Darmstadt for research in biochemistry and molecular genetics. Together with Dr Holger Zinke, member of the German Bioeconomy Council, he became co-founder of BRAIN in 1993. From the start Dr Eck was responsible for developing BRAIN's technology platforms and its global academic network. In 2000 he was appointed to the Management Board as CTO and in 2015 he took over the CEO position. Dr Eck led BRAIN to a successful IPO in February 2016.



## Prof. Dr. Sven Panke

Bioprocess Laboratory, Department of Biosystems Science and Engineering, ETH Zürich, Switzerland

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[www.bsse.ethz.ch/bpl](http://www.bsse.ethz.ch/bpl)

### MAIN RESEARCH FIELD

The research of the BioprocessLaboratory revolves around the design of novel bioprocesses for the pharmaceutical and chemical industry. We address fundamental issues in biocatalyst discovery, biocatalyst engineering and process engineering. This set of activities reflects the interdisciplinary set of competences, from engineering to molecular biology, that is required to design successful processes. This includes, in the framework of our activities in synthetic biology, our work towards the transfer of more and more engineering concepts into the world of bio"engineering", with the ultimate goal of converting biotechnology from a discovery science into a true engineering discipline.

### PROFESSIONAL EXPERIENCE

- |             |  |
|-------------|--|
| 2017        | Full Professor, Department of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland   |
| 2009        | Associate Professor, Department of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland  |
| 2007 – 2008 | Associate Professor, Bioprocess Laboratory, Institute of Process Engineering, Department of Mechanical and Process Engineering (D-MAVT), ETH Zurich, Switzerland |
| 2006        | Appointment as Full Professor at TU Berlin; Germany - declined   |
| 2004        | Appointment as Full Professor at TU Hamburg, Germany - declined  |
| 2001 – 2007 | Assistant Professor, Bioprocess Laboratory, Institute of Process Engineering, Department of Mechanical and Process Engineering (D-MAVT), ETH Zurich, Switzerland |
| 1999 – 2001 | Biocatalysis research scientist, DSM Pharma Products, Geleen, The Netherlands  |
| 1999        | Ph.D., ETH Zurich, Institute of Biotechnology (Prof. B. Witholt)   |
| 1995 – 1999 | Graduate research assistant, Institute of Biotechnology (Prof. B. Witholt), ETH Zurich, Switzerland  |
| 1995        | Diploma, Biochemical Engineering, Technical University of Braunschweig, Germany  |
| 1993 – 1994 | Predoctoral fellow, Centro de Investigaciones Biologicas (CIB-CSIC, Prof. V. de Lorenzo), Madrid, Spain  |
| 1991 – 1993 | Research assistant, Dptmt of Microbiology, German National Research Centre for Biotechnology (GBF, Prof. K. N. Timmis), Braunschweig, Germany                    |

### AWARDS

2004 DSM Research Award

2000 ETH Silver medal

# Dr. Jonathan Chesnut

Senior Director R&D Synthetic Biology, Thermo Fisher Scientific, USA

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Talk  
3.2

## MAIN RESEARCH FIELD

Jon Chesnut received a PhD in Cell and Molecular Biology from UC Davis (1994), and did postdoctoral studies at the University of Colorado in recombinant antibody technology development (1994–95). Prior to graduate school, he was employed at the Salk Institute (1983–84) and Hybritech Inc. in La Jolla, CA (1984–88). After his post doctoral research at Colorado he joined Invitrogen as a research scientist (1995). Since then he has led the development of various prokaryotic and mammalian cell

cloning and expression systems. He has led groups focused on development of TOPO and Gateway cloning as well as the human embryonic cell engineering technologies. He now leads the Synthetic Biology Research and Development Team in the Life Sciences Solutions Group of Thermo Fisher Scientific in Carlsbad, CA. His group is focused on developing tools for the entire Synthetic Biology and Cell Engineering workflow, specifically Bioinformatics, Genome Editing, and Cellular Analytics.

## ACADEMIC CURRICULUM VITAE

### Education and Training

- 1994          PhD. Cell and Molecular Biology, University of California, Davis
- 1984          B.A Biology, University of California, San Diego

### Employment History

- 3/14          Present Sr. Director, Synthetic Biology R&D, Thermo Fisher Scientific
- 4/13 – 3/14   Sr. Director, Synthetic Biology R&D, Life Technologies
- 5/11 – 4/13   Research Fellow, Synthetic Biology R&D, Life Technologies
- 2009 – 2011   Research Fellow, Global Science and Innovation Office, Life Technologies
- 2006 – 2009   Director, Primary and Stem Cell Systems R&D, Invitrogen Corporation (Platform development for mammalian cell engineering focusing on human embryonic stem cells)
- 2001 – 2006   R&D Manager/ Principle Scientist, Group Leader, Invitrogen Corporation (Product development for cloning technologies/Gateway cloning group lead)
- 1998 – 2001   Senior Scientist, Group Leader, Invitrogen Corporation (Product development for cloning technologies/TOPO cloning group lead)
- 1995 – 1998   Scientist, Group Leader, Invitrogen Corporation (Product development for mammalian expression and protein detection)
- 1994 – 1995   University of Colorado Health Sciences Center, Denver, Colorado (Development of recombinant antibody technologies)
- 1988 – 1994   U. C. Davis Section of Cell and Molecular Biology, Davis, California (PhD candidate/ research associate focused on class II transcriptional regulation/Thesis "The Role of RNA Polymerase II Phosphorylation in the Early stages of Transcription")
- 1985 – 1988   Hybritech Inc., La Jolla, California (Development of human serum diagnostic assays)
- 1983 – 1984   The Salk Institute, La Jolla, California



## Prof. Dr.-Ing. Irina Smirnova

Institute of Thermal Separation Processes, Hamburg University of Technology, Germany

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[www.tuhh.de/v8](http://www.tuhh.de/v8)

### MAIN RESEARCH FIELD

Research topics of prof. Smirnova include experimental and theoretical works concerning high-pressure engineering, supercritical fluids, accompanied by the thermodynamics of biological relevant systems and downstream processes. Her group investigates innovative separation processes, mainly extraction under different conditions. High pressure extraction processes with supercritical fluids are applied for the production of nano- and mesoporous materials (aerogels) in lab and pilot scale, including sol-gel process, coating and impregnation with active ingredients, providing the aerogels with exceptional properties for a variety of applications.

Another area of research is the study of a selective fractionation of lignocellulosic biomass by liquid hot water hydrolysis (LHW)/extraction, enzymatic biomass degradation and supercritical CO<sub>2</sub> extraction. Surfactant-based extraction is applied for the in-situ separation of valuable compounds from microalgae cultures. Scale up of this process process is proposed based on the lab and pilot experiments in cooperation with industrial partners. The concept of the in-situ separations is further extended to the enzymatic catalyzed reactive distillation. For further design and optimization of separation processes molecular methods like COSMO-RS and MD simulations are applied and extended.

### ACADEMIC CURRICULUM VITAE

Prof. Smirnova got her PhD in Chemical Engineering from TU Berlin and has continued her academic carrier with habilitaion at the University of Erlanegen. In 2008 she became a chair of the Institute of Thermal Separation Process at Hamburg University of Technology.

Prof. Smirnova supervises currently 18 PhD students and 3 PostDoc and is involved in numerous scientific projects on the national (BMBF, BMWI, AIF,DFG) and international (EU) level. She is a Member of Process Net Working Parties "Thermodynamics", "Adsorption", "Fluidverfahrenstechnik" and Vice Chair of Working Party "High Pressure Technology".

Further she is a member of the editorial board "Journal of supercritical fluids" and "Annual Review in Chemical and Biomolecular Engineering". Since 2014 she is a scientific head of the "Graduate Academy for Technology and Innovation" of TUHH and in the time period from 01.02.2015- 01.02.2017 was the Dean of Chemical Engineering Department TU Hamburg-Harburg. Overall she has published over 90 papers and 6 patents.



# Dr. Gernot Jäger

Bioprocesses, Covestro Deutschland AG, Germany

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Talk  
4.2

## MAIN RESEARCH FIELD

Gernot Jäger is responsible for biocatalytic and bio-based processes at Covestro. He joined Covestro (formerly Bayer MaterialScience) in 2012 and has held various positions in innovation management, process research, project portfolio management and catalysis.

He has also played an active role on a range of committees, including GDCh (sustainable chemistry, board), CLIB2021 (biotechnology cluster, board) and the German chemicals association VCI (covering topics regarding renewable resources).

His research areas have been industrial biotechnology, pharmaceutical biotechnology, process development/conceptual design, and bioanalytics.

Dr. Gernot Jäger published more than 25 publications, book chapters and patents in the field of biotechnology.

## ACADEMIC CURRICULUM VITAE

Dr. Gernot Jäger majored in biotechnology at RWTH Aachen University. He earned his PhD (summa cum laude) in bioprocess engineering at RWTH Aachen University in 2012.



## Dr. Wolf Klöckner

Engineering & Technology, Bayer AG, Germany

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### MAIN RESEARCH FIELD

Expert in the field of fermentation and purification at Bayer since more than 4 years.  
Experience in the field of industrial biotechnology:

- Development of new biotech-processes
- Design, optimization and scale-up of bioreactors
- Purification and downstream processing of fermentation products

Experience in the field of cell culture technology:

- Upstream process development for pharmaceutical production processes
- Design and optimization of cell culture bioreactors

More than 20 publications, book chapters and patents in the field of biochemical engineering.

### EDUCATION

- 2008 – 2014 Ph.D. in Biochemical Engineering (Dr.-Ing), RWTH Aachen University  
Thesis: Characterization and Application of Orbitally Shaken Disposable Bioreactors for Plant Cell Suspension Cultures
- 2007 – 2008 Diploma thesis at Bayer Technology Services, Group Enzyme & Fermentation Technology
- 2001 – 2008 Dipl.-Ing. in Biochemical Engineering, RWTH Aachen University

# Prof. Dr. Walter Thiel

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Talk  
5.1

## MAIN RESEARCH FIELD

Our central field of research is Theoretical and Computational Chemistry, in particular Quantum Chemistry. We focus on theoretical developments that extend the scope of computational methodology, especially for large molecules, and we apply theoretical methods to study specific chemical problems, mostly in close cooperation with experimental partners. The activities of the group cover a broad methodological spectrum:

- ab initio methods
- density functional theory
- semiempirical methods
- combined quantum mechanical/molecular mechanical methods

Recent applications from these areas address the rovibrational spectra of small molecules, catalytic reactions of transition metal compounds, excited-state dynamics, and enzymatic reactions. They thus range from accurate calculations on small molecules to the approximate modeling of very complex systems with thousands of atoms.

## ACADEMIC CURRICULUM VITAE

- since 2017    Emeritus, Max-Planck-Institut für Kohlenforschung
- since 2001    Honorary Professor, Universität Düsseldorf
- since 1999    Director, Max-Planck-Institut für Kohlenforschung
- 1992 – 1999    Full Professor, Universität Zürich
- 1987          Visiting Professor, University of California at Berkeley
- 1983 – 1992    Associate Professor, Universität Wuppertal
- 1981          Habilitation, Universität Marburg
- 1973 – 1975    Postdoctoral fellow, University of Texas at Austin (M.J.S. Dewar)
- 1971 – 1973    Doctoral studies, Universität Marburg (A. Schweig)
- 1966 – 1971    Chemistry studies, Universität Marburg
- 1949          Born in Treysa/Germany



## Prof. Dr. Victor Guallar

Founder and Head of the Advisory Board at Nostrum Biodiscovery,  
ICREA Professor at Barcelona Supercomputing Center, Spain

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### MAIN RESEARCH FIELD

Our lab focuses on the theoretical modelling of biochemical and biophysical processes at a molecular, atomic, and electronic level of detail. We place emphasis both on development of methods and on their application to specific systems. Particular areas of interest include: protein engineering, drug design and software development, where we aim at real applications and in their overlap with industrial developments.

#### Main objectives include:

- Developing our in-house Monte Carlo (PELE) code for better sampling of protein/DNA-ligand interactions
- Develop new approaches for mapping in silico protein engineering focusing on: protein mutation, substrate migration and chemical process.
- Improving the user experience in interactive molecular modelling by combining biophysics/ biochemistry software with state-of-the-art graphics, High Performance Computing and Artificial Intelligence.
- Application studies of our (and others') modelling techniques for addressing specific biomedicine and biotechnology problems, aiming to establish collaboration with top experimental labs and industries.

### ACADEMIC CURRICULUM VITAE

Currently an ICREA Professor, Dr. Guallar performed his PhD between the University Autonomous of Barcelona (Barcelona, Spain) and UC Berkeley (Berkeley, USA). After three years as a postdoctoral researcher at Columbia University (New York, USA), in 2003 he was appointed assistant professor at Washington University School of Medicine (St Louis, USA), before moving his group to the Barcelona Supercomputing Center in 2006. In the last 10 years, his laboratory (EAPM) has grown considerably, keeping a productive international character, and developing important contributions in computational biophysics, such as the protein-ligand modeling software PELE, and biochemistry, including computational algorithms to study long-range electron transfer processes and enzyme engineering. As a BSC researcher, Prof. Guallar has secured ~4 million euros in external funding in the last 6 year, including the award of a prestigious Advanced ERC grant.

# Dr. Simone Dimartino

Institute for Bioengineering, School of Engineering,  
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Talk  
6.1

## MAIN RESEARCH FIELD

Dr. Dimartino interests lie in the application of additive manufacturing (3D printing) technology into chemical and biochemical engineering. Together with Prof. Fee (University of Canterbury), he was the first in the world to conceive and propose the concept of 3D printing of highly ordered porous structures. This idea was demonstrated in 2014, with the first 3D printing of a porous beds with applications in chromatography (J. Chromatography A, 1333, 18-24). Yet, this same concept idea can be employed in any operation based on fluid-solid contacting, such as catalysis, drug delivery, absorption, adsorption, filtration, etc.

The possibility to 3D print and control the structure of the porous bed at the microscale, as opposite to random porous media, opens up the opportunity to unravel fundamental questions on fluid-flow, mass transport mechanisms and kinetic phenomena. In turn, their understanding helps direct efforts in applied research, e.g. to determine the appropriate morphology of the porous bed as well as apposite operating conditions for certain fluid-solid contacting operations.

Dr. Dimartino's research group is currently focusing on the development of new 3D printable materials and geometries for applications in bioseparations (chromatography), biocatalysis (immobilized cell and enzyme bioreactors) and removal of CO<sub>2</sub> from polluting streams.

## ACADEMIC CURRICULUM VITAE

Dr. Dimartino is a Lecturer at the Institute for Bioengineering at the University of Edinburgh, UK. He received his PhD from the University of Bologna (2009) followed by a PostDoc at the University of Canterbury (New Zealand).

He has been always working on the development of new stationary phases for chromatography, spanning from membranes to monoliths to fibre-based media. He now employs 3D printing methods for the fabrication of chromatography media with perfectly ordered internal morphology. The novelty and quality of this research has been recognised with a number of international awards, including 3 best poster awards in 3 continuous years at the PREP conference series, and the Csaba Horvath Young Scientist Award at HPLC 2016.

Dr. Dimartino is co-author of over 30 papers in international journals. He also is in the Editorial Board of Scientific Reports, open access journal from the publishers of Nature.



## Dr. Fabrice Gentile

Biotech & Biopharma Business Manager at Ypso-Facto, France

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### MAIN RESEARCH FIELD

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- Ypso-Proxima® for multi-operation processes, including production cost evaluation.

### ACADEMIC CURRICULUM VITAE

Fabrice Gentile has worked with a wide variety of organizations in the fields of cell culture and fermentation to produce MAbs and recombinant proteins for clinical lots production. He has also been involved in biofuel process development. During his career, he applied a theoretical approach to solve practical cases in R&D departments as well as production units, and has been involved in many process transfer from lab to production. Fabrice has a PhD in Biochemical Engineering from the Institut Polytechnique de Lorraine (Nancy, France). He has now joined Ypso-Facto for bioprocess optimization activities.

## Call for papers



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The workshop is organized within the cooperation network "Models for diagnostic and process optimization (MoDiPro)". The workshop is held in German. Further information and registration at <http://bit.ly/MoDiPro-Workshop>.

#### Date/time

April 26th, 2018, 9:30 a.m. until 5:00 p.m., afterwards get-together

#### Venue

Innovations- und Gründerzentrum für Biotechnologie  
"Ellipse 1" im Haus 7, 3. OG  
Am Klopferspitz 19  
D-82152 Martinsried

#### Organizer

Industrielle Biotechnologie Bayern Netzwerk GmbH as management organization of the cooperation network MoDiPro

# TUM Research Center for Industrial Biotechnology



Researchers in the TUM Pilot Plant for Industrial Biotechnology

The TUM Research Center for Industrial Biotechnology is an interdisciplinary association of 9 TUM professors and their research groups of 3 faculties of the Technical University of Munich (Department of Mechanical Engineering, Department of Chemistry and TUM School of Life Sciences Weihenstephan) with the objectives

- to establish Industrial Biotechnology (“White Biotechnology”) as a strongly interdisciplinary research and educational program at TUM,
- to constitute interdisciplinary research networks across multiple faculties regarding prospective topics in Industrial Biotechnology,
- to promote transfer of technologies by an early integration of companies, in particular small and middle-sized companies, in research projects and
- to organize the scientific education within the Master’s Program Industrial Biotechnology of the Munich School of Engineering (MSE).

The main technical facility of this interfaculty research center is the **TUM Pilot Plant for Industrial Biotechnology** located in Garching, which

- enables the production of sufficiently large amounts of new biocatalysts, functional proteins, and biomaterials for TUM scientists,
- offers the possibility to evaluate novel biotechnological production processes on a technically relevant scale,
- promotes transfer of technologies via the demonstration of technical processes, and
- contributes to a practically oriented education of graduate students in the field of Industrial Biotechnology.

The TUM Pilot Plant for Industrial Biotechnology enables to perform bioprocesses up to the m<sup>3</sup>-scale and to obtain highly purified biotechnological products up to the kg-scale. The pilot plant facilities can be used by scientists of TUM and by qualified scientists of other collaborating research institutions or private enterprises.



The industrial demand for outstanding individuals who can work across a number of disciplines and have a broad and applicable expert knowledge of biosciences and process engineering is continually rising. TUM is meeting this increased demand by offering a **Master's program in Industrial Biotechnology (IBT)** at the Munich School of Engineering (MSE). In the first two semesters, graduates with very good bachelor's degrees in biosciences or engineering are taught the complementary interdisciplinary fundamentals, based on a specially tailored curriculum. The program continues with scientific training in the following four key areas: enzyme engineering, metabolic engineering, bioprocess engineering and bioseparation engineering. The intensive study program is complemented by a mentoring program. The design of the IBT study program is unique in Germany and one of the first of its kind in Europe. Successful completion of the Industrial Biotechnology master's program offers most successful graduates a seamless transition to a doctorate, especially in the research groups associated in the TUM Research Center for Industrial Biotechnology.



Prof. Dirk Weuster-Botz, Director TUM Research Center for Industrial Biotechnology

#### CONTACT AND INFORMATION

##### Prof. Dr.-Ing. Dirk Weuster-Botz

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## Talks from applicants

Talk  
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### Improving Lignan Biosynthesis in *E. coli* by Engineering of Plant P450 Oxidoreductases

**Speaker: Dr. Katrin Schullehner**

**Katrin Schullehner, Peter Welters, Guido Jach**

*Phytowelt GreenTechnologies GmbH, Germany*

**Abstract:** Lignans comprise a large group of structurally diverse natural polyphenols found in plants. Lately a rise of interest has been displayed since lignans play important roles as lead compounds in drug discovery but also as supplements in health improving diets. To the detriment of a stable and cost-efficient supply lignan content in plants is very low and prone to seasonal variation, therefore new approaches for an efficient and sustainable lignan production are highly desirable. Aim of the BMBF funded project LignaSyn is to develop alternative biotechnological approaches for production of lignans from renewable resources like sugar or molasses by implementing multiple enzyme cascades into microbial strains.

Not only during functionalisation of lignans but also for biosynthesis of the monolignolic precursors hydroxylation reactions catalysed by cytochrome P450 monooxygenases (CYPs) are important steps

towards the final high value products, e.g. sesamin, matairesinol or podophyllotoxin. Heterologous expression of plant derived CYPs in *E. coli* is not trivial and usually a bottleneck in fermentative processes. CYPs are membrane bound and form a complex with cytochrome P450 oxidoreductases which act as electron donor and are essential for enzyme activity. These cytochrome P450 oxidoreductases (CPRs) are a feasible target for enzyme modification. Specific point mutations but also swapping of whole domains improved CPR activity. Based on these data a library of hybrid CPR was created and applied to different cytochrome P450 monooxygenase classes thus proving that (a) the most suitable CPR needs to be identified for each specific CYP and (b) that by selecting the most efficient CPR as electron donor for a given CYP, its enzyme activity and therefore strain productivity can be improved by a multiple.

Talk  
1.4

### A new manganese peroxidase: high yield production and mutagenesis towards versatile peroxidase

**Speaker: Agathe Bronikowski**

**Agathe Bronikowski, Katja Koschorreck, Vlada B. Urlacher**

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**Abstract:** Manganese peroxidases, lignin peroxidases, and versatile peroxidases secreted by white rot fungi are supposed to play an essential role in lignin degradation in nature. Thus, these ligninolytic enzymes have attracted significant attention of biochemists, biotechnologists and bioengineers as potential biocatalysts for lignin valorization. Among these three types, manganese peroxidases, though being more abundant in nature, have a narrower substrate spectrum and are not able to directly oxidize high-redox potential substrates as compared to lignin and versatile peroxidases.

Herein we demonstrate how properties of a new manganese peroxidase from the basidiomycete *Moniliophthora roreri*, designated as MrMnP1, were shifted towards those of a versatile peroxidase. The *mrmnp1* gene was cloned and highly expressed in *Pichia pastoris*, yielding 132 mg per L medium of active enzyme. Biochemical characterization has demonstrated that MrMnP1 is a typical short manganese peroxidase, which accepts and oxidizes

Mn<sup>2+</sup> and some low redox potential substrates like ABTS and 2,6-DMP in the presence of H<sub>2</sub>O<sub>2</sub>. A catalytic tryptophan found in lignin and versatile peroxidases but absent in manganese peroxidases was introduced in MrMnP1 (mutation A172W) in order to extend its substrate scope towards high-redox potential substrates like lignin or lignin model compounds and textile dyes. The mutant A172W gained activity towards the lignin model substrates guaiacylglycerol- $\beta$ -guaiacyl ether, veratryl alcohol and the high-redox potential dye Reactive Black 5. Furthermore, five amino acids in close proximity to the catalytic tryptophan were replaced towards those present in a versatile peroxidase, and their influence on biochemical characteristics like pH stability, pH optimum or substrate oxidation was evaluated. Most MrMnP1 double mutants demonstrated not only increased activity and stability as compared to the single mutant A172W, but also the substrate spectrum was broadened.

## Talk 2.3 Engineering metabolic regulation: Why so many feedbacks?

Speaker: Dr. Hannes Link

Timur Sander, Niklas Farke, Christoph Diehl, Timo Glatter, Hannes Link

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**Abstract:** Dysregulating metabolic feedback is a common approach to create industrial production strains. However, how feedback-dysregulation influences physiology of the host has not been characterized. Especially primary metabolism is feedback regulated at many layers and novel 'omics' approaches emerged to study their function *in vivo*<sup>1,2,3</sup>. Here, such approaches demonstrate that feedback-dysregulation has a strong effect on stability and robustness of *Escherichia coli* metabolism. By combining CRISPR-based genetic perturbations, metabolomics and proteomics we first explored the function of negative feedback in amino acid biosynthesis of *E. coli*. These data show that allosteric and transcriptional feedbacks are simultaneously active, and that the interplay between both regulatory layers accomplishes a deliberate overabundance of biosynthetic enzymes. As an approach to integrate these data systematically we used ensemble modeling, suggesting that overabundant enzymes prevent bottlenecks in metabolic pathways. To validate this prediction we used CRISPR

interference for perturbations of enzyme expression, and indeed feedback-dysregulated strains were extremely fragile against modulating enzyme levels. These results provide a mechanistic understanding why feedback dysregulation can result in instable production strains, and pave the way for novel alternative metabolic engineering strategies.

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## Talk 2.4 Construction of Tailored Acetic Acid Bacteria Strains for Oxidative Fermentations

Speaker: Dr. Armin Ehrenreich

A. Ehrenreich, S. Gruber, M. Bimmer, W. Liebl

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**Abstract:** Acetic acid bacteria have flavin and pyrroloquinoline quinone (PQQ) dependent membrane-bound dehydrogenases that oxidize in a stereo- and regio-specific manner various polyols, sugars and sugar derivatives under preservation of their carbon skeleton. Those enzymes have an active site facing to the periplasm and feed their electrons in an electron transport chain with oxygen as terminal acceptor. This permits rapid oxidation of large amounts of substrates with a high yield and simultaneously avoids a problem common to other whole-cell biocatalysts in biotechnological processes, i.e. the mass transfer limitation of substrate transport into the cytoplasm. Typical acetic acid bacteria contain 5 to 15 such dehydrogenases with narrow to very broad substrate spectra. This physiology enables the construction of strains that can be exploited in biotechnology, expressing single membrane-bound dehydrogenases that allow rapid and specific oxidation of substrates and avoids competing and follow-up oxidations by multiple dehydrogenases in those tailored strains for oxidative fermentations.

We have developed and applied a comprehensive toolbox of methods to construct strains to be used as oxidative catalysts that are devoid of inapt dehydrogenases and express selected biotechnologically relevant membrane-bound dehydrogenases, and to characterize substrates and products of these enzymes. Characterization of membrane-bound dehydrogenases towards their substrates and products is key for the forward-looking strategy to construct strains with an oxidative metabolism specifically engineered for new biotechnological processes.

Interestingly, we found that homologous membrane-bound dehydrogenases with only minor sequence differences from different strains of acetic acid bacteria showed vast differences in their substrate spectra. Various mutagenesis strategies further increase the variability of those enzymes.



## Talk 3.3 *Paenibacillus polymyxa* as novel chassis organism for heterologous exopolysaccharide production

Speaker: Dr.-Ing. Jochen Schmid

Jochen Schmid, Christoph Schilling, Marius Rütering and Volker Sieber

Chair of Chemistry of Biogenic Resources, Technical University of Munich, Campus of Biotechnology and Sustainability, Schulgasse 16, 094315 Straubing, Germany

**Abstract:** *Paenibacillus polymyxa* produces a highly viscous hetero exopolysaccharide of unique rheological properties with high specific production rates [1]. On sucrose, *Paenibacillus polymyxa* can produce the fructose-based levan in high amounts, thus representing a highly versatile exopolysaccharide producing organism [2]. The genome of *P. polymyxa* DSM 365 shows a great variety of genes involved in sugar nucleotide synthesis, which serve as precursors for the exopolysaccharide biosynthesis. Just recently, we developed a tailored CRISPR-Cas9 system for *P. polymyxa* DSM 365 showing a high efficiency for this Gram-positive microorganism [3]. By that approach we were able to delete large genomic regions (>18kb) as well as single genes or perform integration of foreign genes into the genome and to unravel the biosynthesis pathway of the highly viscous heteropolysaccharide. Taking all that together, *P. polymyxa* can be turned into a chassis organism for the expression of

heterologous exopolysaccharide encoding gene clusters, by deleting all, or only selected native genes involved in exopolysaccharide production. Additionally, negative aspect such as spore formation are deleted and the genome will be reduced to a minimum for having a powerful production organism.

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## Talk 3.4 Intracellular dynamics during heterologous protein expression

Speaker: Sabine G. Wagner

Sabine G. Wagner, K. Pflüger-Grau, and Andreas Kremling

Systems Biotechnology, Technical University of Munich, Garching / Munich

**Abstract:** Genetically engineered organisms are frequently applied in biotechnological processes. Compared to the wild type strains these production hosts have to fulfil additional tasks. Further resources are needed to supply the increased demand for replication, transcription, and translation. These resources are then missing for cellular maintenance and growth. For that reason, heterologous production can lead to a metabolic burden and, associated therewith, an underperforming biotechnological process.

The aim of this work is to uncover such bottlenecks and quantitatively describe the distribution of the resources between the intrinsic processes, which are needed for maintenance, and the extra load, that is introduced by the heterologous gene. To this end, we analysed at different energetic states the expression of the model protein mCherry as proxy of any heterologous load. In order to shed light on the intracellular metabolic dynamics, the behaviour of stressed *E. coli* cells was analysed by quantification of the extracellular metabolites that accumulated along growth. Several fermentative by-products were detected, which indicate a change in the metabolic behaviour during heterologous expression. The cellular demand of energy rises with increasing growth rate and

production. At a certain point of time, cells can no longer cover their requirements via aerobic pathways and need to switch to anaerobic pathways even without oxygen limitation. As consequence, the substrate taken up cannot be metabolized in an optimal way.

Furthermore, the expression-process itself was analysed. Therefore, we generated the reporter-plasmid pTRA, which enables *on-line* visualization of transcriptional and translational processes. We performed a case study in which we monitored the specific mRNA and protein production. Though the system still has some shortcomings, it is a promising tool that might contribute to understand the delicate equilibrium between transcription, translation and degradation during protein production. This systematic approach to understand the cellular processes and limitations during foreign protein production will lay the basis for the mathematical description of the relation of load to burden by integrating flux and resource distributions. Having a functional model in hands should allow the identification of bottlenecks during heterologous protein production with the aim to improve the overall process.

Talk  
4.3Robust lactic acid production from defatted rice bran using a thermophilic *Bacillus coagulans* strain**Speaker: Dr. Maria Alexandri****Maria Alexandri, A.K. Neu, R. Schneider, J. Venus**

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**Abstract:** The development of robust bioprocesses utilizing renewable resources as feedstock for the production of platform chemicals, like lactic acid, is crucial for the development of novel biorefineries. Lactic acid consists of one of the top C3 platform chemicals, with various applications not only in the food sector but also in the pharmaceutical, chemical and cosmetic industries. One of the most important applications of lactic acid involves the production of polylactic acid (PLA), a biodegradable polymer. Even though the market for PLA is increasing, the major bottleneck is its production cost. The utilization of renewable resources, suitable as both carbon and nitrogen sources together with the adequate downstream process would decrease the overall cost. Defatted rice bran (DRB) is an industrial by-product stream, produced in vast amounts in China. Currently, it is either given as animal feed or incinerated in the landfills, causing serious environmental issues. Since it is rich in starch and proteins, it can be considered a potential candidate for the biotechnological production of lactic acid.

The aim of this study is the identification of a suitable thermophilic strain for lactic acid production using DRB hydrolysate as a sole nutrient source. At the beginning of the study, five different batches of rice bran were characterized. Then, both starch and proteins were hydrolysed in order to produce a fermentation substrate, rich in glucose and free-amino-nitrogen (FAN). Forty-four thermophilic strains, isolated from various sources, were tested for their ability to grow on the DRB hydrolysates. Lab-scale fermentations were subsequently carried out using eleven strains that showed the highest growth in the initial test. The performance of a *Bacillus coagulans* isolate was the most promising in terms of yield, productivity and L-lactic acid purity. Process stability was then evaluated in continuous fermentation mode using cell recycling. Current results indicate that high lactic acid productivities can be achieved from the complete exploitation of DRB.

Talk  
4.4

## Xylonic acid from hemicellulose hydrolysates

**Speaker: Dr.-Ing. Thomas Hahn****Thomas Hahn<sup>1</sup>, Nadine Gammel<sup>1</sup>, Max Hesse<sup>1</sup>, Katja Patzsch<sup>2</sup>, Sandra Torkler<sup>2</sup>, Susanne Zibek<sup>1</sup>**<sup>1</sup> Fraunhofer IGB, Molecular Biotechnology, Nobelstraße 12, 70569 Stuttgart, Germany<sup>2</sup> Fraunhofer CBP, Biotechnological processes, Am Haupttor (Gate 12, Building 1251), 06237 Leuna, Germany

**Abstract:** Due its chelating power, gluconic acid is an important component of food products, solvents, adhesives and paints<sup>1</sup>. The production is based on the chemical oxidation or the whole-cell conversion of glucose<sup>2</sup> and is commonly obtained from agricultural products originally designated to produce food products. A compound not based on agricultural products in competition to food production is thus highly demanded whereas drop-in substitution of gluconic acid should not affect product quality. A compound meeting the two requirements is xylonic acid. It can be obtained by conversion of xylose which from hardwood lignocellulose. The aim of our project is thus the biotechnological conversion of hemicellulose-derived xylose to xylonic acid.

**Selection of a suitable microorganism**

The economic viability of an enzymatic conversion process is limited due to the cofactor recycling that should be implemented in the process for enzymatic oxidation. Thus, a whole-cell transformation was focused. *Gluconobacter sp.* was the microorganism of choice since it has large oxidative potential, is not capable to use xylose as substrate.

**Process development for xylonic acid production**

Cultivation investigations and xylose conversion experiments in shaking flasks revealed optimal growth characteristics and requirements for increased xylonic acid production. In order to raise oxygen-supply and to control pH without excessive calcium carbonate addition growth and production phase were transferred to the bioreactor. Different feeding strategies of xylose were performed concerning the bioreactor production: 1. A pulsed- or continuous fed-batch. Applying an appropriate strategy, the final xylonic acid concentration could be increased exceeding 300 g/L.

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Talk  
5.3

## Expression relevant molecular parameters for genetically re-coding synthetic genes aiming on functionally refactoring biochemical gene clusters

**Speaker: Dr. Hubert Bernauer****Gregor Zipf, Josef Maier, Hubert Bernauer**

ATG:biosynthetics GmbH, Germany

**Abstract:** For >10 years now ATG:biosynthetics developed a multi-parameter *in parallel* gene sequence re-coding platform technology (EvoMAG<sup>IS-v2.0</sup> (*in silico* evolution mediates to artificial genomes) for re-factoring synthetic biochemical genes and clusters (BGC) thereof. Multi-parametric sequence *in silico* optimization calculations of regulator regions and coding sequences of pathway genes are performed *in parallel*.

Comparative evolutionary analyses of existing ortholog natural operon structures in different organisms are valuable informational sources for the redesign of e.g. artificial biochemical gene clusters with intended function. Detailed comparative analyses of related gen-, transcript- and proteomes allow for the extraction of host-specific natural design rules, which are applied during sequence optimization of artificial genes. Performing thorough molecular analyses of evolutionary genomic data in terms of ortho-, para- and xenologs of the target host genomes, the regulatory regions and motives as well as the specific codon use can be extremely valuable for identifying relevant formal-functional expression parameters on several levels for refactoring e.g. BGCs.

During the 17 years of its existence ATG continuously improved bioinformatics tools suitable by applying iterative modes of design of experiments (DoE). The setups of sequence data are describing relevant functional sequence parameters *in detail*. Statistical methods for correlating expression level data with sequence features are enabling iterative experimental design strategies for use of active learning heuristics. This is a prerequisite for speeding up overall pathway optimization processes.

Constructive molecular biology applications for to express natural compound producing artificial genes – *in vivo* and *in vitro* – need basic molecular design concepts and strategies, synthetic bioinformatics services, suitable multi-gene expression vector systems and functionally predictive sequence modulations. The ATG: **FlexTEC** – vector systems due to its systematic standardized modularity are the ideal basis for realizing DNA-constructions where systematic assembly and specific de-assembly as well of exchange of genetic elements and parts plays a predominant role and is advantageous for reaching the desired objectives.

Talk  
5.4Metabolic engineering of *Saccharomyces cerevisiae* for the production of aromatic compounds of industrial relevance**Speaker: Manuela Gottardi****Manuela Gottardi<sup>1</sup>, Mislav Oreb<sup>1</sup>, Jan Dines Knudsen<sup>2</sup>, Wanda Dischert<sup>3</sup>, Paola Branduardi<sup>4</sup> and Eckhard Boles<sup>1</sup>**<sup>1</sup>Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany.<sup>2</sup>Terranol A/S, c/o Section for Sustainable Biotechnology, Aalborg University, Copenhagen A.C. Meyers Vænge 15, DK-2450 Copenhagen, SV, Denmark<sup>3</sup>Metabolic Explorer, Biopôle Clermont Limagne, 63360, Saint-Beauzire, France.<sup>4</sup>Department of Biotechnology and Biosciences, University of Milano – Bicocca, P.zza della Scienza 4, 20126, Milano, Italy.

Contact: gottardi.bio@gmail.com

**Abstract:** Aromatic chemicals comprise a wide variety of industrially relevant compounds, which find applications in several fields, ranging from the plastic to the medical, food, aroma and fragrance fields. Generally, aromatic compounds are chemically synthesized from petroleum-derived sources, especially bulk chemicals, or extracted from plants. Nevertheless, both approaches present drawbacks, like dependence on fossil resources and the high environmental footprint, or, when using plants, challenging extraction methods and low extraction yields.

Therefore, white biotechnology is posing its attention to the metabolic engineering of microorganisms, among them the yeast *Saccharomyces cerevisiae*, in order to produce aromatics from renewable sources and waste materials. We will present a study

on the production of *trans*-cinnamic acid and its derivatives from engineered *S. cerevisiae* strains. In particular the study focuses on the biosynthesis of the aroma compounds naturally present in the cinnamon essential oil, cinnamaldehyde, cinnamyl alcohol and hydrocinnamyl alcohol. The establishment of the pathway for their production in yeast was achieved via expression of a heterologous phenylalanine ammonia lyase enzymes as well as an aryl carboxylic acid reductase. Interestingly, our results show that cinnamaldehyde is partly diverted to the synthesis of cinnamyl methyl ketone by the carbolygase activity of yeast pyruvate decarboxylase. This pathway enables the biosynthesis of an aromatic intermediate related to the chemotherapeutic drug daunomycin.

## Talk 6.3 Improving Downstream Processing of Influenza VLPs using a Click Chemistry strategy

**Speaker: Dr. Christina Peixoto**

**S.B. Carvalho<sup>1,2</sup>, J.M. Freire<sup>3</sup>, M.G. Moleirinho<sup>1,2</sup>, F. Monteiro<sup>1,2</sup>, D. Gaspar<sup>3</sup>, M.A.R.B. Castanho<sup>3</sup>, G.J.L. Bernardes<sup>3,4</sup>, C. Peixoto<sup>1,2</sup>, P.M. Alves<sup>1,2</sup>, M.J.T. Carrondo<sup>1,2,5</sup>**

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**Abstract:** Virus-like particles (VLPs) constitute a promising platform in vaccine development and targeted drug-delivery. However, most applications use simple, non-enveloped VLPs that present less technical challenges, not only to produce and purify, but also in terms of characterization, compared to enveloped VLPs.

Recent advances in upstream processing, new product quality requirements and other regulatory issues, as well as the search for more cost-effective processes, led to the need to develop more efficient downstream processes for biopharmaceuticals<sup>1</sup>. In that sense, new monitoring and product characterization methods, which can be applied at all stages of downstream processing, are needed.

Here is reported a valuable platform for the downstream processing and monitoring of the in vivo production of site-specifically functionalized enveloped Influenza VLPs. This strategy involves a two-step procedure that consists of residue-specific replacement of methionine by an analog (azidohomolanine) that enabled for post-expression functionalization with a fluorophore<sup>2</sup>.

Importantly, this platform does not impact VLP production or purification processes, and allows functionalization without deleterious effect on hemagglutinin biological function. As a proof of concept a complete downstream processing was performed, including clarification, capture and polishing steps. A flow cytometry analysis (FACS) step was added to achieve a refined discrimination and separation between VLPs and baculovirus – the major impurity of the process<sup>3</sup>. This was further confirmed using atomic force microscopy (AFM). This tool allowed accurately monitoring our product, achieving higher product recovery yields and higher impurity removal levels. The versatile system presented here is broadly applicable to the production of functionalized enveloped VLPs, for vaccine design, targeted drug delivery and molecular imaging.

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## Talk 6.4 Membrane separation technology as a valuable and efficient tool in the value chain of wood towards chemicals and materials

**Speaker: Prof. Dr. Ludo Diels**

**Ludo Diels, Kelly Servaes, Pieter Vandezande, Richard Vendamme, Karolien Vanbroekhoven, Anita Buekenhoudt**

*Flemish Institute for Technological Research – VITO, Separation & Conversion Technology, Mol, Belgium*

**Abstract:** Bio-based chemicals are expected to grow significantly and increase their share of the global chemical production in the coming years. Today nearly all aromatic chemicals and building blocks originate from fossil oil, whereas lignin – nature's second most abundant polymer after cellulose – could provide a valuable renewable aromatic resource for the chemical industry. Conversion of presently underutilized lignin by-products (e.g. from pulp and paper industries) into high performance biomaterials calls for development of new and/or optimized process technologies. In this respect, cost-effective downstream separation and purification processes are of utmost importance. Generally, membrane processes can be considered a "natural" technology for separation of complex biomass streams. Thanks to their low energy requirements, mild processing conditions, scalability, moderate cost to performance ratio and flexibility in equipment design, membranes are a highly attractive separation technology for tomorrow's lignocellulosic biorefineries.

VITO's research activities are dedicated to valorization of biomass through conversion of lignocellulose into value-added bio-aromatics, primarily for use as building blocks within the chemical industry. Depolymerization of lignin typically results in complex mixtures comprising a wide array of phenolics, bearing a variety of oxygen-based functionalities and covering a broad range of molecular weights. However, in many cases, valorization of these lignols can only be pursued from well-defined fractions. In this context, the principal focus is the development and demonstration of membrane processes for fractionation and purification of lignins and lignin degradation fragments to enable the use of these molecules in chemical and materials applications.

VITO is co-initiator of Biorizon, an industry driven Shared Research Center, focusing on technology development for the production of functionalized biobased aromatics for performance materials, chemicals & coatings supported by a Roadmap, inspired by an industry driven community. As the leading institute, VITO coordinates the development of lignin derived bioaromatics by its own technology and collaboration with many different technology providers ([www.biorizon.eu](http://www.biorizon.eu)).

Being involved in various national (e.g. ArboRef, MAIA) and international (e.g. BIO-HArT) running initiatives in which different depolymerization processes of wood/lignin are envisaged, more insight is gained in the potential of membrane processes in the value chain of wood/lignin towards bio-based aromatics. The separation efficiency of commercial polymeric and ceramic membranes as well as in-house developed functionalized ceramic membranes with carefully selected molecular weight cut-offs is evaluated and demonstrated at a larger scale aiming at a proof-of-concept of the membrane-based fractionation/purification. In this talk, the potential of membrane processes in different aspects of the wood-to-aromatics value chain will be illustrated through some recent case studies. The potential applicability of the resulting fractions of lignin derivatives in polymer applications will be discussed.

# 1<sup>st</sup> Session: Enzyme Catalysis

Poster  
1.1

## Expression of archaeal genes for improved enzyme performance

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**Abstract:** Working within “extremozyme” systems for the continued deconstruction and conversion of biomass and carbon sources into chemicals and alcohols is a promising strategy. Modern enzymatic deconstruction strategies (such as Ammonia Fiber Expansion) require different phases of biomass preparation, which are time consuming, costly, and results in high inhibitor concentrations to fermentative microbes. The use of “one-pot” enzymatic cascade strategies overcomes these demerits.

The use of dihydroxyacid dehydratases (DHADs) as biocatalysts in these one-pot enzymatic cascades for the conversion of biomass continue to develop. DHADs catalyze the dehydration of a wide range of substrates<sup>1</sup> and it has been shown that DHADs are [Fe-S] cluster dependent<sup>2</sup>. Thermal tolerance in DHADs of archaeal origins has proven successful, however activities on various substrates have been suboptimal. This is where traditional enzyme engineering techniques such as rational design and directed evolution can be

the key to success in targeting substrate specificity and activity, while maintaining the reliable thermal tolerance of these archaeal enzymes.

Additionally, engineering thermal tolerance in mesophilic enzymes would be a parallel approach. This is due to the fact that the enzymes already possess high activities on a variety of substrates. Practical application by protein engineers to create new and novel enzymes gives us an insight into the tools available for modifying existing enzymes. New methods outside of the conventional protein engineer’s toolbox have emerged specifically for these needs that are presently being exploited in this research.

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Poster  
1.2

## Improving enzymes’ tolerance towards harmful conditions

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**Abstract:** As long as the use of enzymes is restricted to mild reaction conditions, the scope of industrial bioconversions is necessarily limited<sup>1</sup>. This might be overcome by improving enzymes’ tolerance towards harmful conditions like monophasic organic solvents or high concentrations of aldehydes.

Water is a poor solvent for many applications in chemical industry. Most organic compounds of commercial interest are very sparingly soluble and are often unstable in aqueous solutions<sup>2</sup>. The use of nearly anhydrous organic solvents as reaction media for enzymatic reactions provides numerous industrially attractive advantages and will allow direct integration of enzymes into chemical processes. However, most enzymes are inactivated in organic solvents<sup>3</sup>. This is investigated by using different monooxygenases as model biocatalysts.

Enzymes can also be inactivated by aldehydes and is largely due to the formation of Schiff bases with lysine residues present on enzymatic surfaces<sup>4</sup>. Engineering lysines on the surface and adjacent residues might render an enzyme more resistant to aldehydes. However, many surface residues are critical for the functioning of the enzyme and cannot be readily changed<sup>5</sup>. This is investigated by using different aldolases as model enzymes.

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Poster  
1.3

## A new experimental approach to evaluate temperature vs. pH activity profiles for biotechnological enzymes

Petra Kornberger, Jonathan Herlet, Simon Heinze<sup>1</sup>, Wolfgang H. Schwarz, Wolfgang Liebl, Vladimir V. Zverlov

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**Abstract:** The easy assessment of an enzyme's suitability for a combination of process parameters is of crucial importance when designing biotechnological processes on either lab- or industrial scale. Temperature and pH are two of the most important process factors on enzyme activity. Conventional approaches to test the effect of those factors on enzyme activity act in the assumption of a two-dimensional correlation between temperature and activity as well as pH and activity. New approaches determine the relation in a three-dimensional matter but are statistics-based, complex to

design and their accuracy to the actual data may vary. We therefore developed a method which allows a fast and easy determination of the effect that temperature and pH have on the enzyme's activity simultaneously by using 96-well plates, multichannel pipettes and a gradient PCR cycler. With this basic technical equipment, it is possible to produce contour plots of pH, temperature and activity which are based directly on experimental data. The method was tested for several widespread glycoside hydrolase assays as well as model and complex substrates.

Poster  
1.4

## 4-Hydroxy-2-oxoglutarate as a novel donor compound for MenD-catalyzed 1,2- and 1,4-addition reactions with Michael acceptors

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**Abstract:** Thiamine diphosphate (ThDP)-dependent enzymes are well-established catalysts in the field of asymmetric synthesis<sup>1</sup>. The ThDP-dependent enzyme MenD (SEPHCHC synthase) from *E. coli* uses its physiological donor, 2-oxoglutarate, which is decarboxylated and performs a unique Stetter-like 1,4 addition to  $\alpha,\beta$ -unsaturated Michael acceptors (isochorismate, 2,3-*trans*-CHD) or acrylic acid<sup>2,3</sup>. As well, MenD is able to perform 1,2-addition reactions with a variety of aliphatic or benzylic aldehydes<sup>3,4</sup> to form stereospecific *S*-hydroxyketones. This makes MenD a promising novel biocatalyst for C-C bonding reactions. Here we report that MenD is able to utilize the alternative donor, 4-hydroxy-2-oxoglutarate (HOG) as a novel donor compound for 1,4- (see scheme) as well as for 1,2-addition reactions and leads to more functionalized products. HOG was synthesized either by a chemical reaction (product: *R,S*-HOG) or by aldolase reactions (from glyoxylate and pyruvate<sup>5</sup>).

We will present data on the acceptor substrate spectrum of MenD with HOG<sup>6</sup>.

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Poster  
1.5

## *In silico* modeling of enzymes – substrate promiscuity

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**Abstract:** Enzyme engineering based on *in silico* modeling is a valuable tool to enlarge the substrate specificity of wild type enzymes.

Here, we describe a modeling process for variegating the substrate specificity of a PHA-synthase. PHA-synthases catalyze the polymerization of 3-hydroxybutyryl-CoA (3HB-CoA) into poly(3-hydroxybutyrate) (PHB). The resulting polymer can be used for industrial applications. By modifying the substrate specificity of PHA-synthases, the monomer composition of the polymer can be adjusted.

Class I PHA-synthase from *Ralstonia eutropha* forms a dimer. Based on the crystal structure, the subunits form a substrate channel where the monomer can enter the active center within the enzyme<sup>1</sup>. We used the bioinformatic tools YASARA and PyMol for docking studies with different substrates. Several criteria were applied

to select amino acid positions that were supported to vary the substrate specificity. The most important criteria was the distance from the reactive moiety of the substrate to the catalytically active cysteine of the enzyme. Additionally, the position of the substrate within the enzyme was evaluated. Positions, in which the substrate was close to the catalytically active center and in which the resulting polymer was placed next to the product exit channel of the protein were chosen for further analysis. These docking processes pointed out some amino acid residues which might interact with the substrate binding and result in a broader substrate specificity of the enzyme.

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Poster  
1.6

## Engineering Ketoacid Decarboxylases: Building Towards Feasible Alcohol Productions in Archaea

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**Abstract:** High production cost of biofuels is a major drawback rendering its utilization to replace gasoline. Cell-free minimized cascade reactions from glucose to ethanol and isobutanol have been demonstrated before<sup>1</sup>. The cascade reactions will be adapted further in *Sulfolobus acidocaldarius*, a thermoacidophilic archaea. *S. acidocaldarius* will serve as an ideal chassis for biofuels production due to its ability to withstand high temperature and low pH environment. These two beneficial factors will facilitates one pot production of alcohol (biomass hydrolysis and alcohol fermentations) followed by direct separation of alcohols, thus lowering production cost. However, there are no keto acid decarboxylases (PDC & KDC)<sup>2,3</sup> found in archaea or thermophilic bacteria. Hence, in order to successfully introduce the cascades into *S. acidocaldarius*, these two key enzymes have to be engineered towards improved thermostability.

Several approaches have been reported for improving thermostability of enzymes. Hitherto, there are no general methods for increasing enzymes' thermostability<sup>4</sup>. Therefore, in this project several approaches will be employed, such as rational designs and directed evolution. More recent method, i.e. ancestral reconstruction will also be considered<sup>5</sup>.

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## Biocatalytic Disproportionation Reactions: Towards an Atom-Efficient Synthetic Platform

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**Abstract:** *Intermolecular* hydride transfer between two aldehyde molecules leads to the formation of equimolar amounts of the corresponding alcohol and carboxylic acid. While the chemical reaction is limited to non-enolizable aldehydes ('Cannizzaro reaction'), an asymmetric biocatalytic equivalent was successfully applied to racemic-substituted aldehydes. A single alcohol dehydrogenase (ADH) was sufficient to catalyze the disproportionation reaction using catalytic amounts of the nicotinamide cofactor as hydride shuttle.<sup>1</sup> Overall redox neutral, this biotransformation is synthetically attractive as it furnishes enantiopure molecules and does not rely on stoichiometric reagents and/or sacrificial co-substrates.

Aiming at improving the applicability of this protocol further, a statistical optimization was conducted based on Design of Experiment. While this approach led to major improvements in the outcome of the reaction in terms of yield and selectivity, two products are obtained simultaneously and further valorization requires an additional separation step. To increase the atom-

efficiency of the process further, two alternative strategies were developed: i) A convergent bi-enzymatic route relied first on the ADH-catalyzed asymmetric disproportionation of 2-arylpropanals to generate two enantioenriched product molecules – alcohol and carboxylic acid – which was followed by the enzymatic oxidation of the alcohol to the carboxylic acid in a stereoconvergent manner; ii) *Intramolecular* hydride transfer was shown possible on di-functionalized aldehydes, leading to the formation of single products, which was thermodynamically supported by intramolecular cyclization.

### Acknowledgements

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## Identification and Optimization of Exopolysaccharides (EPS) from Microalgae

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**Abstract:** Microalgae are photosynthetic microorganisms that are being researched extensively as sources for valuable products such as carbohydrate polymers with sometimes rare functional groups like methyl (CH<sub>3</sub>) or sulfate groups (SO<sub>4</sub><sup>-2</sup>)<sup>1,2</sup>. In general, two main types of polysaccharides are generated by microorganisms, intracellular polysaccharides, which serve as energy storage and the extracellular polysaccharides (EPS), exported into the surrounding. Microbial EPS, such as xanthan, gellan, scleroglucan, and levan, to name a few, are valuable biopolymers with several applications in the cosmetic, nutraceutical, and surfactant industries<sup>3,4</sup>. Although, the main sources of EPS are bacteria and some fungi, microalgae are being explored to obtain those polymers, what is also the focus of this study. Two important aspects are presented in this work. Firstly, the most promising EPS producing microalgal strain will be identified by a screening approach of different type strains and novel isolates. By the first screening rounds, it was found that a yet undescribed Rhodophyta strain (*Porphyridium sordidum*), is a highly promising EPS producer. The EPS obtained from *P. sordidum* was evaluated and was compared to the *Porphyridium purpureum* polymer. Secondly, the process conditions for EPS production have to be optimized, by identifying the most suitable conditions that could enhance the growth rate and EPS yield of *P. sordidum* and

*P. purpureum*. Thus, several conditions, such as media composition and illumination were evaluated in different cultivation vessels such as air bubbled cylinders and photobioreactors. An in-depth characterization of the polymers and optimization strategies is on the way to further enhance productivity and EPS yields of these two highly promising microalgae strains.

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## Photobiocatalytic Module for Redox Reactions

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**Abstract:** Developing sustainable and green routes for the production process of materials has been widely acknowledged as a key challenge for the 21<sup>st</sup> century.

Many redox enzymes potentially are very useful tools for establishing greener routes in organic synthesis which is due to highly selective reduction, oxidation and oxyfunctional-isation reactions. However, the point of supplying this class of enzymes with the redox equivalents (hydrides) needed for catalysis is the major challenge and bears the drawback that it naturally requires the reduced nicotinamide cofactor NAD(P)H, which is expensive and easily tends to decompose due to hydrolysis.

Based on the model of nature's photosynthesis this project aims at splitting water by visible light and transfer the electrons to a variety of enzymatic redox reactions. The usage of water as electron donor

instead of other sacrificial substrates would be a smooth way to recycle cofactors without producing waste and thus, increase the atom efficiency and the greenness of this system. Such a system can be established by using a semi-conductor as photocatalyst and in some cases – like recent research showed – a direct coupling to an oxidoreductase<sup>1,2</sup>. Since this is surely not possible for all redox enzymes a second approach aims to use cheap and more stable synthetic analogues of the natural nicotinamide cofactor.

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## 2<sup>nd</sup> Session: Metabolic Engineering

Poster  
2.1

### Manipulation of the hydrocarbon biosynthesis pathway in *Micrococcus luteus*

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**Abstract:** *Micrococcus luteus* naturally produces olefins (unsaturated aliphatic hydrocarbons) and represents a promising host to produce hydrocarbons as constituents of biofuels and lubricants. In this work, we alter the genes for key enzymes of the olefin biosynthesis pathway in *M. luteus* and demonstrate how these genes can be used to manipulate the production of specific olefins.

Because the first metabolic steps of branched chain amino acid (BCAA) degradation lead also to the formation of primer molecules for olefin biosynthesis, targeted alterations in the BCAA metabolism can be used in order to produce olefins with a desired isomer composition (iso-iso- or anteiso-anteiso-branching). By generating deletion and up-mutants of several gene candidates involved in the BCAA metabolism or its regulation, we could identify the gene cluster encoding the components of the branched chain  $\alpha$ -keto acid dehydrogenase (BCKD) complex, as well as the genes for the downstream steps in the degradation of valine, leucine and isoleucine. Overexpression of the BCKD gene cluster resulted in about threefold increased olefin production whereas deletion of the

cluster led to a drastic reduction in branched chain fatty acid content and a complete loss of olefin production. The specificities of the acyl-CoA dehydrogenases of the BCAA degradation pathways were deduced from the fatty acid and olefin profiles of the respective deletion mutant strains. We show that the isomer composition of the *M. luteus* olefins strongly depends on the availability of branched acyl-CoA primer molecules for fatty acid biosynthesis, which in turn can be manipulated by altering the individual BCAA degradation pathways. The olefin biosynthesis system of *M. luteus* seems to be relatively unspecific, allowing the incorporation of straight chain acyl-CoA primers and even of primers not found in nature, i.e. even-numbered anteiso-branched acyl-CoA.

In conclusion, our experiments allow the unambiguous assignment of specific functions to the genes for key enzymes of the branched chain amino acid metabolism of *M. luteus*. We also show how this knowledge can be used to engineer the isomeric composition and the chain lengths of the olefins produced by this organism.

Poster  
2.2

### A novel tool for metabolic pathway engineering of *Clostridium saccharobutylicum* NCP262

Ching-Ning Huang, Wolfgang Liebl and Armin Ehrenreich

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**Abstract:** *Clostridium saccharobutylicum* NCP 262 is a solventogenic bacterium, which was used in South-Africa for the industrial production of acetone, butanol and ethanol (ABE). The lack of a genetic manipulation system for *C. saccharobutylicum* currently limits the use of metabolic pathway engineering to improve the yield, titer and productivity of n-butanol production by this microorganism. In this study, we developed a marker-less deletion system for *C. saccharobutylicum* using the *codBA* genes from *Clostridium ljungdahlii* as a counter-selection marker. The *codB* gene encodes a cytosine permease which facilitates the entry of cytosine into cell, and the *codA* gene encodes a cytosine deaminase that convert 5-fluorocytosine to 5-fluorouracil which is toxic to the cell. First, we constructed a suicide vector, containing

a *catP* gene for thiamphenicol resistance, *codBA* genes as counter-selection markers and DNA segments up- and downstream of the chromosomal deletion target fused together. Then we introduced the construct into *C. saccharobutylicum* by tri-parental conjugation. The single crossover integrants were selected on 2xYTG plates supplemented with 15  $\mu$ g/ml thiamphenicol and 10  $\mu$ g/ml colistin and the double crossover mutants having a deleted gene of interest were selected on MES-MM plates with 5-fluorocytosine. Using this tool, we first constructed a markerless-restrictionless mutant, *C. saccharobutylicum* $\Delta$ *hsdR1* $\Delta$ *hsdR2* and successfully demonstrated unmethylated DNA transfer into this strain. This method was further used in a strategy to create a butyrate minus mutant of *C. saccharobutylicum* to produce n-butanol at high yield.

Poster  
2.3

## Ready-to-use (Di-)terpenes – a modular approach for their microbial production

**Katarina Kemper, Max Hirte, Monika Fuchs and Thomas Brueck**

*Werner Siemens-Chair of Synthetic Biology, Department of Chemistry, Technical University of Munich*

**Abstract:** Microorganisms such as *E. coli* are demanded hosts for heterologous production of bioactive substances with pharmaceutical, food or other applications. In the struggle for maintaining the supply of terpene-based drugs and for exploring new bioactive substances, microbial production of these compounds has become a serious and up-to-date alternative to chemical synthesis. Due to the complexity of their natural biosynthetic pathways, transfer from plants or marine organisms to a heterologous host often turns out to be challenging. For a successful establishment of a desired production system, a synergistic approach that addresses as well the optimization of

metabolic fluxes from sustainable carbon sources as the efficient scaledup production is presented here. One main focus of our work is the extraction and purification of diterpene products that allow further chemical or enzymatic conversion *in vitro*. With our modular built plasmid based production system, exchange or integration of enzymes, promoter systems or ribosomal binding sites can be realized fast and easy. Therefore, we are able to adjust the existent system to produce several different diterpenes in a time and cost effective straightforward way that currently result in yields up to 300 mg/L.

Poster  
2.4

## Model-driven Development of a Dynamic Process for Succinic Acid Production in *Escherichia coli*

**Diana Kreitmayer<sup>1</sup> and Andreas Kremling<sup>1</sup>**

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**Abstract:** Succinic acid is a widely used platform chemical in the food-, cosmetics and pharma-industries, as well as a precursor for polymers. *Escherichia coli* produces succinic acid as a natural fermentation by-product. Commercial biotechnological production of succinic acid from renewable resources, in contrast to a non-sustainable petrochemical production, has first started in 2010. Here we use succinic acid production as a case study of employing a model-driven approach to design a producer strain starting from wildtype *E. coli* MG1655.

Modeling is then further applied to optimize the production process. First, we used Flux Balance Analysis (FBA) to estimate flux distributions of wildtype and mutant strains based on exchange rates. By comparing those against a theoretical distribution corresponding with an optimal product yield we identified knockout targets. This process lead to an experimentally implemented strain with a yield of 1.36 mol succinic acid per mol glucose, 79 % of the

maximal theoretical yield. Since growth and product formation compete for the carbon source, we developed a two-phase process strategy. The first process step is an aerobic growth phase to increase the amount of biocatalyst, followed by a second, anaerobic process phase with high product yield. A simple kinetic model was used to estimate the time course of extracellular variables.

To further increase process performance, we are currently testing a genetic switch that allows us to dynamically regulate gene expression of *ptsG* depending on the process phase. Concurrently we are developing a model for the genetic switch to further optimize process control. This will allow us to model the time course of extracellular variables, as well as the switching process. This model will be validated against further experimental findings. It can then be used to optimize our process according to initial concentrations, duration and switching.

Poster  
2.5

## Access to N-methylated amino acids by fermentation

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**Abstract:** N-methylated amino acids are found in many pharmaceutically active compounds and have been shown to improve pharmacokinetic properties as constituents of peptide drugs since N-methylation of amino acids may result in conformational changes, improved proteolytic stability and higher lipophilicity of the peptide drug<sup>1</sup>. Synthesis of N-methylated amino acids by chemical and biocatalytic approaches is known, however, production by a fermentative route from sugars has not yet been developed. *Corynebacterium glutamicum* is commercially used for the fermentative production of amino acids such as L-lysine at the million-ton-scale. Metabolic engineering of *C. glutamicum* led to an expanded product range of proteinogenic amino acids like L-serine<sup>2</sup> and L-valine<sup>3</sup> – but also  $\omega$ -amino acids like  $\gamma$ -aminobutyrate<sup>4</sup> and 5-aminovalerate<sup>5</sup>.

Here, a one-step conversion of sugars and methylammonium into the N-methylated amino acid N-methyl-L-alanine was developed. A whole-cell biocatalyst was derived from a pyruvate overproducing *C. glutamicum* strain<sup>6\*</sup> by heterologous expression of the N-methyl-L-amino acid dehydrogenase gene from *Pseudomonas putida*. As

proof-of-concept, N-methyl-L-alanine titers of 31.7 g L<sup>-1</sup> with a yield of 0.71 g per g glucose were achieved in fed-batch cultivation. The *C. glutamicum* strain was engineered further to extend this green chemistry route to production of N-methyl-L-alanine from alternative feed stocks such as starch or the lignocellulosic sugars xylose and arabinose.

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Poster  
2.6

## Pathway and enzyme engineering in *Saccharomyces cerevisiae* for de novo biosynthesis of mandelic and 4-hydroxymandelic acid

Mara Reifenrath, Maren Bauer, Eckhard Boles

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**Abstract:** Mandelic acid (MA) and 4-hydroxymandelic acid (HMA) are important fine chemicals and find use in the cosmetic and pharmaceutical industry. Today they are mainly produced using chemical approaches. We recently published how expression of bacterial hydroxymandelate synthases and engineering of the amino acid pathway enabled de novo biosynthesis of both MA and HMA in *S. cerevisiae* (Metab. Eng.; DOI: 10.1016/j.ymben.2018.01.001). Engineering of the upstream shikimic acid pathway, introducing deletions in downstream pathways including the Ehrlich pathway and comparing hydroxymandelate synthases from various organisms allowed for the production of more than 1 g/L HMA, which is to our knowledge the highest titer reported for HMA production with microorganisms in shake flasks.

Aside from its native substrate 4-hydroxyphenylpyruvate, hydroxymandelate synthases also accept phenylpyruvate as a substrate and convert it to MA, however yielding much lower titers compared to the native product HMA. Nevertheless, we achieved a 3.5-fold increase in MA production by preventing the native substrate 4-hydroxyphenylpyruvate from binding to the hydroxymandelate synthase by site-specific engineering. Even more promising, deletion of *TYR1* which is responsible for conversion of prephenate to 4-hydroxyphenylpyruvate led to a more than 20-fold increase in MA production. Combined with the pathway engineering, we could achieve a final titer of 236 mg/l MA, which is a more than 200-fold increase compared to the wildtype strain expressing only hydroxymandelate synthase.

## Development of optimized *Aspergillus niger* strains for highly efficient D-galacturonic acid generation from pectin-rich, agricultural residues

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**Abstract:** In conjunction with the German national research strategy “BioEconomy 2030”, the BMBF fosters the development of novel applications for the use of renewable resources in the chemical industry. As such, pectin is one of the main components in residual agricultural products like sugar beet pulp. Due to the variety of achievable fermentation products from D-galacturonic acid (D-GalA) as the main backbone component of pectin, it is considered a highly promising second generation feedstock for biotechnological fermentations. Naturally, saprotrophic fungi play an important role in biomass degradation. Amongst these, *Aspergillus niger* is known for its strong pectinolytic capabilities making it a perfect candidate for industrial scale pectin de-polymerization. As a consequence of the complex chemical structure, de-polymerization of pectin to D-GalA is facilitated through a large set of secreted enzymes, collectively referred to as pectinases and tightly regulated on the transcriptional level. The panregulon-like regulation of a large subset of pectinase genes by two central transcription factors (TF), namely GaaR and RhaR, has recently been described in *A. niger* (Alazi et al., 2016, Gruben et al., 2014). TF engineering of GaaR and RhaR aiming at a global increase of pectinase expression is therefore applied to realize highly efficient pectin de-polymerization. Notably, GaaR-/RhaR-activities were shown to be modulated by substrate-related inducers (Alazi et al., 2017, Gruben et al., 2014), carbon catabolite repression (CCR) and nuclear exclusion in the case of GaaR (Niu et al., 2017). For that matter, constitutive activity, improved nuclear localization and reduced sensitivity of GaaR/RhaR towards CCR will improve pectinase production in *A. niger* for industrial D-GalA-supply.

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## 3<sup>rd</sup> Session: Synthetic Biotechnology

### Poster 3.1 GeneArt Strings DNA products – fast and economic solutions for synthetic biology

**Dr. Claudia Chiocchini, Nikolai Netuschil, Krishna Vattem, Michael Liss, and Axel Trefzer**

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**Abstract:** Thermo Fisher Scientific offers an extensive portfolio around de novo gene synthesis which consistently keeps up-to-date with the latest demands and applications. As the world market leader in gene synthesis we provide the complete service chain from design, optimization and de novo synthesis of genes up to protein expression thereof. Our Directed Evolution products deliver top-of-the-line quality, showcasing our technology leadership in the market.

Fast-turnaround and cost-effective GeneArt™ Strings™ DNA Fragments became an important part of our gene synthesis service and can be used not only for cloning but for a variety of other applications like in vitro and in vivo transcription and translation.

Based on the fast and reliable availability of GeneArt Strings, we developed downstream applications that will lead to expressed and purified protein using the time advantage but not at the expense of quality. Several downstream formats are feasible leading from small scale expression in eukaryotic cells down to 96well IVT expression.

By combining our longstanding expertise in the production of GeneArt Combinatorial Libraries with GeneArt Strings DNA Fragments, we are able to offer an economical directed evolution solution to price sensitive academic and industrial customers alike: GeneArt Strings DNA Libraries allow for the introduction of all IUPAC defined ambiguous nucleotides into the customer sequence while providing the advantages of GeneArt Strings DNA Fragments of short production time, low price, flexible sequence design and full gene optimization options.

## 4<sup>th</sup> Session: Bioprocess Engineering / Upscaling

Poster  
4.1

### Development of an enzyme cascade for the production of cellobiose and its implementation in industrial scale

**Thomas Häßler<sup>1</sup>, Timo Koch<sup>1,2</sup>**

<sup>1</sup> Pfeifer & Langen GmbH & Co. KG, Germany

<sup>2</sup> Savanna Ingredients GmbH, Germany

**Abstract:** Cellobiose is a naturally occurring disaccharide consisting of two  $\beta$ -1,4 linked glucose monomers. Cellobiose has several promising properties to be used as a functional carbohydrate in feed and food applications. E.g. cellobiose is considered to be a prebiotic and does not affect blood glucose or insulin levels. It supports the growth of intestinal bacteria or probiotics when given in combination.

As a reducing sugar, cellobiose has extensive potential in food applications. It is able to undergo Maillard reactions resulting in a browning effect similar to that of sucrose, which is most often desired in baked products. Furthermore, the reactive properties of cellobiose positively affect its use in encapsulations, where it prevents sensitive compounds such as fats and oils with high amounts of polyunsaturated fatty acids or flavours from oxidation. With a lower water activity and sweetness than sucrose (cellobiose = 0.2; sucrose = 1.0), other potential application as filler in pills or effervescent tablets and to improve mouth feeling or even as replacer for lactose e.g. in fermented meat products are possible.

The production of cellobiose is based on a two-step enzymatic cascade. In the first place sucrose phosphorylase (EC 2.4.1.7) catalysis the reaction of sucrose to glucose-1-phosphate (G1P) and fructose. With G1P and additional glucose cellobiose phosphorylase (EC 2.4.1.20) synthesizes cellobiose. Whereby, fructose obtains as a high value co-product. Due to the enzyme engineering of both enzymes substrate and product inhibition were minimized and activity was improved. This allowed a simplified process where the cascade reaction takes place in one reactor. The process was designed with recycling of enzymes, unused substrates, co-substrates, condensates and intermediates.

A crystallization process developed by which cellobiose was efficiently separated from a four-phase-process liquor.

The process was finally up-scaled to a production capacity of 100 t crystalline cellobiose per annum.

Poster  
4.2

### Chiral amines synthesis and *in-situ* product recovery for process intensification for application in organic fine chemicals and pharmaceuticals

**Claudia Matassa<sup>1</sup>, Matthias Höhne<sup>2</sup>, Dominic Ormerod<sup>1</sup>, Karolien Vanbroekhoven<sup>1</sup>, Yamini Satyawali<sup>1</sup>**

<sup>1</sup> VITO, Mol, Belgium

<sup>2</sup> Institute of Biochemistry, Greifswald, Germany

**Abstract:** Chiral amines integrate the backbone of several active pharmaceutical ingredients (APIs) used in modern medicine for treatment of a vast range of diseases. However, despite the demand, their synthesis remains challenging. Besides a range of chemicals and enzymatical methods, chiral amine synthesis using transaminases (EC 2.6.1.W) represents a useful alternative to access this important class of compounds. Even though transaminases exhibit excellent stereo and regioselectivity and the potential for high yield, the reaction suffers from a number of challenges including the thermodynamic equilibrium, product inhibition, low substrate solubility etc.

In this work, an attractive process strategy for "*in-situ* product recovery in transamination" is presented. It involves the use of novel "High molecular weight" amines as donor substrates in

transamination reactions. The choice of donor substrate is of high importance for amine transaminase reaction and process design. In contrast to common donor substrates such as alanine or isopropyl amine, we present a novel approach that makes it superfluous to administer amino donor molecules in excess, which, as known, leads to substantial losses of these molecules. The equilibrium shift towards the product side is greatly boosted without affecting the purity of the resulting product stream. On the contrary, our process leads to a substantial increase in product purity and yield. Results demonstrate the immense potential of these novel amine donors for developing a continuous transamination process.



## Development of Lipase Mediated Epoxidation Process for Monoterpenes in Deep Eutectic Solvents

**Sumanth Ranganathan<sup>1</sup>, Sandra Zeitlhofer<sup>2</sup>, Prof. Volker Sieber<sup>1,2</sup>**

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<sup>2</sup> Fraunhofer Institute of Interfacial Engineering and Biotechnology (IGB) Bio-, Electro and Chemo Catalysis BioCat Branch Straubing, Schulgasse 11a, Straubing, 94315, Germany

**Abstract:** Functionalised terpenes are of great value to the flavour and fragrance industries<sup>1</sup>. One such functionalisation process, viz. epoxidation, can be performed using chemical or enzymatic means. However, a critical demerit of both the processes is the use of organic solvents in excess per final product produced. This aspect is harmful for the operator as well as the environment. In order to overcome this and devise a green and non-polluting process, alternate reaction media such as deep eutectic solvents (DES) can be used. These DES are advantageous because of their low melting, non-volatile, biological origin, general degradability and harmless nature<sup>2</sup>. This work deals with the feasibility studies, development and optimisation of a lipase mediated terpene epoxidation process in DES.

First, numerous DES mixtures were screened for fluidity. The two best DESs, choline chloride : glycerol at a molar ratio of 1 : 2 and an equimolar mixture of choline chloride : sorbitol yielded a complete conversion within 8 hours. Following this, process optimisation by the design of experiments (DoE) – Taguchi approach in a crossed array technique was done. Although successful, a major issue in

both the DES media was the formation of impurities (esters). To overcome this, a novel co-substrate plus solvent DES was used. The new mixture was a 1 : 2 molar ratio of choline chloride: urea-H<sub>2</sub>O<sub>2</sub>, which was better than the other two mixtures described previously. A complete turnover was achieved within 2 hours (4x faster) for 3-carene and 3h (2.67x faster) for limonene and  $\alpha$ -pinene. Moreover, it is worth mentioning that the downstream operations for the DES based system are easier than the organic solvent process.

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## 6<sup>th</sup> Session: Bioseparation Engineering

### Poster 6.1 Effect of Carbon and Nitrogen Sources on Growth and Lipid Profile of the Oleaginous Yeast *C. oleaginosus*

**Dania Awad, Thomas Brück**

*Werner Siemens-Lehrstuhl für Synthetische Biotechnologie, Technische Universität München*

**Abstract:** Climate change and a rise in global population have driven the industry towards a search for clean and sustainable alternatives. While first-generation biodiesel relaxes the aforementioned concerns, it poses an ethical dilemma in its competition with the food industry. Biodiesel production via oleaginous microorganisms offers a sustainable energy source, requires less labor and resources, is not limited by season or climate, and most crucially does not impact food security. *Cutaneotrichosporon oleaginosus* is an oleaginous yeast capable of high lipid accumulation of up to 60% of its dry weight under specific nutrient-starvation conditions.

Additionally, *C. oleaginosus* is strongly adapted to changing nutrient supply as it utilizes a broad monosaccharide spectrum of hexoses and pentoses. Cultivation medium dictates both lipid yield and profile. Consequently, a thorough media optimization provides a better understanding of the lipogenic process and offers a preliminary insight on cost analysis prior to any industrial scale-up. In this study, we present a thorough optimization of culturing conditions for *C. oleaginosus* that covers 12 nitrogen sources and 10 carbon sources of variable concentrations extensively investigated with the aid of Surface Response Methodology (SRM).

### Poster 6.2 Deep eutectic solvents in downstream processing

**Franziska Bezold, Maria Weinberger, Mirjana Minceva**

*Biothermodynamics, TUM School of Life and Food Sciences Weihenstephan, Technical University of Munich, 85354 Freising, Germany*

**Abstract:** Deep eutectic solvents (DES) have attracted attention as new designer solvents in recent years. They are formed by hydrogen bond acceptor (HBA) and donor (HBD) molecules which, when combined, show a considerable melting point depression compared to the pure compounds. DES are formed by a large number of HBA and HBD species, such as sugars, alcohols and polyols, carboxylic acids, and quaternary ammonium salts. The properties of a DES can be tailored by selecting different HBA and HBD combinations, as well as, to a certain extent, by selection of the molar ratio of HBA and HBD. Many HBA and HBD are available in food grade quality and the DES can be considered non-toxic green solvents. Applications of DES are, for example, the use as a green extraction medium for compounds from plant material, enhancement of stability and solubility of natural compounds or drugs, liquid-liquid extraction, use as solvents in chromatography or as a reaction medium. The nearly limitless number of combinations makes DES versatile, however, the selection of an appropriate pairing is challenging and time consuming. Thermodynamic models and systematic screening methods reduce the experimental effort in the selection of a DES and are generally faster than trial and error approaches and at the same time require less material.

In this work, a strategy for solvent selection for an application of DES in centrifugal partition chromatography (CPC) is presented. CPC uses the phases of a biphasic solvent system as stationary and mobile phases in a solid-support free column. Target compounds injected into the column are separated according to the difference in their partition coefficients in the biphasic solvent system. A solvent system selection strategy was evaluated for an application to DES-based biphasic systems, and the prediction quality of a thermodynamic model was explored for a model mixture of natural compounds. The prediction of thermodynamic phase equilibria and partition coefficients was solely based on the molecular structure of molecules present in the mixture. It was found that the prediction quality was sufficient to perform solvent system screening and the selection approach was applied to a mixture of different forms of vitamin E. The mixture could be separated with the DES-based biphasic solvent system selected with the computational screening.

Poster  
6.3

## Purification of diterpenes from aqueous fermentation broth

Grozdev L., Berensmeier S.

*Bioseparation Engineering Group, Technical University of Munich, Garching, Germany*

**Abstract:** Paclitaxel and other highly functionalized diterpenes show a strong activity against certain cancer forms, but their production in semisynthetic or plant cell suspension processes are limited in titer and yield. Another approach is the biotechnological production of less functionalized bioactive derivatives in fast growing and cost effective recombinant bacteria. Due to low concentrations, toxicity and extreme hydrophobicity of these taxanes, purification remains a major challenge for the process development. The chromatographic adsorption as the first unit operation after cell removal can lead to an effective capturing step with a good selectivity and very high recovery.

In comparison to the already existing phytoextraction, the taxanes are solubilized in the aqueous phase of the supernatant. This is a big advantage since solid-liquid extraction with strong organic solvents is no longer a necessary process step.

The best suited adsorption material was tested to capture them selectively and most efficiently from the aqueous phase. Their process integrity was investigated in terms of capacity, recovery and back-pressure. Following the adsorption, elution plays a major role in terms of desorption strength and maximum solubility of the taxanes in the fluid phase. Consequently, another selective step can be implemented throughout gradient elution with a feasible organic modifier.

Moreover, solvents for elution were screened with a simulation based on COSMO-RS (COnductor like Screening MOdel for Real Solvents), where the highest solubility in pure solvents and in mixtures with water were calculated. This approach was found to be a fast and robust way to screen solvents for the experimental use.

This work is part of the SysBioTerp project, funded by the German Federal Ministry of Education and Research.

Poster  
6.4Two different approaches for integrated product removal of a hydrophobic diterpene from *E. coli* fermentation broth

L. Janoschek, F. Schmitz, S. Berensmeier

*Bioseparation Engineering Group, Technical University of Munich, Garching, Germany*

**Abstract:** Taxanes like Paclitaxel and its diterpene derivatives are natural cytostatic compounds with diverse medical applications, especially in anti-tumor treatment. High technical and monetary efforts are necessary for their production in semi-synthetic or plant-based processes. Therefore, synthesis of terpenes and taxoids in heterologous but well-known organisms like *Escherichia coli* recently become a topic of interest. However, achieving high titers of terpene substances remains a major challenge due to the low solubility of these hydrophobic compounds. Also, batch-wise downstream processing lacks efficiency as a consequence of this. Using an integrated process, where the product is continuously removed from the fermentation medium, one might overcome these obstacles. With direct adsorption as well as membrane-assisted extraction with a hollow-fiber membrane contactor, two different approaches for the capture of a diterpene were investigated and then integrated in a fed-batch fermentation process.

The early diterpene precursor taxa-4,11,-diene was produced in *E. coli* in a stirred-tank fed-batch cultivation process on minimal medium. Nevertheless, yields were still limited, as the hydrophobic terpenes show low solubility in aqueous solution, which limits diffusive secretion from the cells into the cultivation medium

and results in low extracellular product concentrations. Thus, the compounds should be continuously removed during the process in order to shift the equilibrium towards an extended production. This was addressed by the application of a basket filled with polymeric XAD-2 adsorber, which was immersed in the cultivation medium. This integrated adsorber module was able to remove the taxa-4,11-diene from the broth during the fermentation process. In an alternate process, the cell broth was pumped through a lab-scale membrane contactor with PTFE membranes. From this stream, the produced taxa-4,11-diene was continuously extracted in a circulating n-heptane phase and by this purified from the fermentation broth.

Both techniques for product stripping from cultivation medium have shown promising results for the removal of taxoids like taxa-4,11-diene. For both integrated approaches a significant increase in taxa-4,11-diene yields was achieved, which, in combination with the simultaneous purification, facilitates a more efficient process.

This work is part of the SysBioTerp Project (FKZ 031A305A), funded by the German Federal Ministry of Education and Research.

Poster  
6.5

## Separation of metal binding entities aided by post-column terbium luminescence spectroscopy

**W. Jurkowski, M. Heilmann, A.M. Becker; R. Buchholz and T. Brück**

*Werner Siemens-Lehrstuhl für Synthetische Biotechnologie, Technical University of Munich*

**Abstract:** We have developed a simple chromatographic isolation method of heavy metal binding components from any unknown biological sample employing lanthanide luminescence. It was designed to be used for identification of active components from biosorbents, but can be easily adapted for other applications. It relies on a post column addition of terbium and a subsequent analysis of fractions by luminescence excitation spectroscopy to detect interactions with the metal observed as quenching, shifts or sensitization. In biological samples binding domains of calcium, zinc or magnesium can be easily substituted by lanthanides making

terbium a good model substance for many applications. Besides a chromatography system, only a spectrofluorometer is required making it far less expensive than alternative approaches based on Inductively Coupled Plasma (ICP). Furthermore, the method is non-destructive and gives first predictions about the nature of the involved molecules by comparing their spectra with model substances. In our application, we have extracted metal binding polysaccharides from a cyanobacterium and a green algae in order to characterise them by enzymatic cleavage and NMR.

Poster  
6.6

## Crystallization of a small molecule in aqueous solvents

**L. Martin, S. Berensmeier**

*Technical University of Munich, Bioseparation Engineering Group, Garching*

**Abstract:** The biotechnological market is a fast-growing section since the availability of cost-effective and productive, but at the same time resource-friendly processes for a large product range in the medical and pharma industry are required. Here, the industrial use of halophilic bacteria as a potent production strain for biomolecules is still underestimated. The need of high salt content in the surrounding makes a process less susceptible to contaminations, but particularly forces the cell to produce unique, cell protective substances, so called compatible solutes. The characteristic of compatible solutes qualifies these molecules as potent products on the medical market and for cosmetic industries.

Accordingly, a new amino acid derivative with osmoprotective abilities is to be produced in *Halomonas elongata*. Desalting is the main challenge of the downstream process, since standard purification steps are limited, due to interfering interaction with the salt, and the small size of the target molecule normally requires energy intense separation methods like electrodialysis. Therefore, crystallization is applied for formulation as well as concentration and desalting, offering a very selective and efficient process step.

Crystallization or recrystallization is one of the major and oldest purification methods in industry. Here, temperature, pH and concentration of components mainly influence the progress of crystallization and at the same time are the parameters to be controlled in industrial processes. Therefore, solid material is dissolved in a suitable solvent and the desired product is slowly crystallized, mostly by either cooling the solvent, adding an antisolvent or concentrating the sample by evaporation.

Since, solubility is the key parameter of crystallization, the behaviour of the molecule in various solvents was investigated. With COSMO-RS, a software to calculate thermodynamic relevant parameters for solvation, a simulative approach was firstly applied to narrow the complexity of experimental procedure. Further, crystallization methods were experimentally performed in promising solvents and under several process conditions. Kinetic studies were additionally used for a better characterisation of the crystallization progress.

This work is part of the HOBBIT (FKZ 031B0363C) Project, funded by the German Federal Ministry of Education and Research.

## Liquid-liquid chromatography: a versatile separation technology with high potential for the biotech industry

**Raena Morley, Johannes Goll, Mirjana Minceva**

*Department: Biothermodynamics, Technical University of Munich*

**Abstract:** Solid support-free liquid-liquid chromatography (LLC) combines the advantages of liquid-liquid extraction (high loading capacity) and conventional chromatography (high selectivity). In LLC the stationary and mobile phases are the two immiscible phases of a biphasic liquid system. The stationary phase is held in place during operation as the result of the column geometry and the application of a centrifugal field. Separation occurs due to the differing partitioning behavior of the feed components. LLC offers several advantages over chromatographic techniques with a solid stationary phase: no irreversible adsorption, high sample loading due to availability of the entire stationary phase volume for solute partitioning, and a nearly limitless selection of tailorably solvent systems. The presence of a liquid stationary phase also allows for high operational flexibility. Either phase of the biphasic system may be employed as the stationary phase, and the roles of the phases may be switched during operation. This characteristic has led to the development of several operating modes unique to LLC.

Two such LLC-specific operating modes are sequential centrifugal partition chromatography (sCPC) for continuous binary separations

and trapping multiple dual mode (trapping MDM) for ternary separations involving the recovery of intermediately-eluting components. To facilitate the selection of the operating parameters, design strategies involving mathematical short-cut models and numerical simulations have been developed by the group.<sup>1,2</sup> It has recently been demonstrated that the sCPC short-cut design model can be used to successfully separate multi-component mixtures into two desired feed streams at high purity and yield (>99%) and for components with separation factors as low as 1.6.<sup>3</sup> Additionally, the trapping MDM process has been successfully utilized for the recovery of an intermediately-eluting compound at maximum productivity with purity and yield >95%.<sup>4</sup> These results demonstrate the potential of LLC as an alternative method for the purification of complex biological feedstocks following an initial capture step.

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## Liquid-liquid chromatography: A promising unit operation for the purification of bioactive minor hop compounds

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**Abstract:** Solid support-free liquid-liquid chromatography, also known as countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC), is a highly selective, versatile and scalable separation technique for the isolation, purification and separation of active compounds from natural resources, biotech and biosynthetic products<sup>1</sup>. The separation is achieved as a result of the different partitioning of the mixture compounds between two phases of a multi-solvent biphasic system. One of the phases is kept in place with the help of a centrifugal force field, while the second one is pumped through.

In the past few years, natural phenolic plant compounds have shown promise due to their versatile bioactive potential. As an excellent source of a wide variety of such biologically active constituents, hop (*Humulus lupulus* L.) has been attracting scientific attention. Xanthohumol (XN) and other prenylated flavonoids belong to the most studied polyphenolic hop compounds<sup>2</sup>. Recent studies strongly emphasize their potential for the treatment of different diseases. Especially, some minor XN analogues were shown to exceed the bioactivity of XN itself<sup>3, 4</sup>. Existing methods for the isolation and purification of minor hop compounds are complex and inefficient. They involve several extraction and chromatographic steps, such as flash chromatography and preparative HPLC, and they suffer from low overall yield and irreversible adsorption<sup>2</sup>. Therefore, new strategies for the isolation and production of minor components from plant extracts in high purity as well as sufficient amounts are needed.

In the present study, CCC/CPC is demonstrated to be a promising technology for isolating and purifying Xanthohumol C, a minor hop compound, in sufficient amounts for their further analysis and

subsequent bioactivity studies. Two different separation strategies for the purification of the minor hop compound Xanthohumol C were evaluated. The first method consisted of a two-step liquid-liquid chromatographic process, where a capture and enrichment step was followed by another CCC purification step. A second production strategy involved a one-step semi-synthesis starting from XN and a subsequent CCC/CPC purification. The second approach led to higher Xanthohumol C purity and productivity, while simultaneously requiring less solvent. The proposed methods thus provide a further step towards the exploitation of CCC/CPC as a highly flexible unit operation in downstream processing of active minor ingredients from crude extracts.

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Poster  
6.9

## New non-chromatographic stationary materials for antibody affinity purification

Matthias Freiherr von Roman, Priyanka Padwal, Constanze Finger, Paula Fraga-García, Sebastian P. Schwaminger, Yasmin Kaveh Baghbaderani, Sonja Berensmeier

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**Abstract:** In the 1980s, the first pharmaceutical product based on monoclonal antibodies (mAbs) was approved by the US authority FDA for use in humans. Since then, mAbs gained continuously in importance. On this account, it is not surprising that mAbs still retain the largest segment in the global biopharmaceutical market. Over all these years, the fermentation process during antibody production was optimized in terms of high product titers (over  $10 \text{ g L}^{-1}$ ). Expressed mAbs must be processed subsequently in downstream. However, the purification by affinity chromatography still represents the bottleneck of the overall mAb production due to limitations in binding capacity, bed dimensions and flowrates. In a previous publication (Freiherr von Roman and Berensmeier 2014), we outlined the impact of polymerization of the immunoglobulin G (IgG)-binding domain B of Protein A on different parameters. Increasing the number of B-domains from 2 to 9 led to an almost linear relationship between the number of human IgG molecules bound to the ligand and the B-domain count regarding in-solution-experiments. However, coupling these ligands to a stationary phase revealed the effect of steric hindrance in two ways: The molar ligand density on chromatography media reached a maximum using the B4-ligand, despite the use of the same molar concentrations of ligand during coupling reaction. Besides,

the molar binding stoichiometry of the immobilized ligand was reduced, when compared to binding in solution. These effects led to a maximum equilibrium binding capacity with the ligand B8. Continuation of B-domain polymerization resulted in decreased IgG binding capacity on account of steric effects due to the porous nature of chromatography stationary phases. Therefore, establishing non-porous materials as an alternative is a desirable aim. Immobilizing Protein A and Protein G ligands on magnetic micro- and nanoparticles could have the potential to overcome the mentioned limitations. The concentration and separation effects are thereby mediated by inducing magnetic fields. Especially the use of magnetic nanoparticles (MNPs) will take advantage of small size and thus a high specific surface. The possibility of these low-cost MNPs to be used bare or with different coatings and functionalizations, qualifies them for protein immobilization applications in different environmental conditions.

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Poster  
6.10

## Rational Design of Iron Oxide Binding Peptide Tags

Sebastian Schwaminger<sup>1</sup>, Silvia Blank-Shim<sup>1</sup>, Priya Anand<sup>2</sup>, Monika Borkowska-Panek<sup>2</sup>, Karin Fink<sup>2</sup>, Paula Fraga-García<sup>2</sup>, Wolfgang Wenzel<sup>2</sup>, Sonja Berensmeier<sup>1</sup>

<sup>1</sup> *Technical University of Munich, Bioseparation Engineering Group, Germany*

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**Abstract:** Biomolecule recognition plays an important role in nature as well as for modern industrial applications. Therefore, the development of new ways of biomolecule recognition and design of affinity binding tags is of great importance in protein science. Magnetic iron oxide nanoparticles are an interesting counterpart for peptide tags as their properties facilitate an easy handling and manipulation. Furthermore, these particles can be synthesized with a low-cost co-precipitation route.

The key to the design of high-affinity peptide tags lies in an in-depth understanding of surface-peptide recognition patterns. We combine a model and an experimental approach. The Explicit Implicit Solvation Model (EISM) which has already been used for the design of affinity binding to gold surfaces is parameterized by data from peptide arrays incubated with iron oxide nanoparticles. EISM is an example of an implicit model of the surface with a computational protocol based on the Simulation of Molecular and Nanoscale Systems (SIMONA) engine, performing Monte Carlo calculations,

supported by metadynamics, using force field methods as a system description. The EISM model has been parameterized for several surfaces, using empirical data as a basis for defining the affinity of amino acids to inorganic surfaces in certain experimental conditions.

Peptide arrays are used to develop new affinity tags for metals and metal oxides and therefore used for the parameterization of the computational model. This technique is particularly well suited, since iron oxide MNP stain distinctively, leaving dark spots when bound to peptides on a white cellulose membrane.

The goal of our investigation is to extrapolate specific peptide-surface interactions to rationally design iron oxide binding tags which can be used for various applications. Our main issue is therefore to identify different peptide sequences specifically binding bare magnetite nanoparticles in order to provide a computer-based platform of tag which can be genetically engineered and fused to proteins like existing affinity tag systems.

## Potential-Controlled Chromatographic Separation of Small Molecules

T. Turrina, P. Fraga-García, S. Berensmeier

*Technical University of Munich, Bioseparation Engineering Group*

**Abstract:** Downstream processing is a major part of biotechnology, which includes the capture and purification of small and large biomolecules. However, industrial processes, such as ion exchange chromatography, are limited in multifunctional applications and require additional regeneration steps to elute the column with high salt gradients. To overcome these limitations, our research focuses on the development of a separation process, which combines electrochemical and chromatographic effects. This innovative potential-controlled chromatography (PCC) promises to be an efficient and economical alternative to conventional concepts. Developed in the second half of the 20<sup>th</sup> century and enhanced by several groups, Brammen et al. (J. Sep. Sci., 2016) designed an optimized three electrode setup, which enables work with nanomaterials. Applying a potential to the stationary phase, counter-charged and polarized molecules of the mobile phase are adsorbed on the solid surface, which simultaneously operates as a working electrode. Subsequently, switching the potential, the electrosorbed molecules desorb without the need of any additives. Based on Brammen's successful application of multi-walled carbon nanotubes, a project was initiated to analyse PCC via a multi-

scale concept to ascertain the electrochemical and mass transfer effects occurring at the nano, micro and macro levels using both simulations and experiments. The main target is to optimize and validate the existing separation process by improving its area of application. Two different resin materials are deployed due to their (electro)chemical and economic advantages: 1) carbon nanotubes (CNT-K), which have excellent conductivity and high specific surface area (200 m<sup>2</sup>/g) yet a complex structure; and 2) glassy carbon (Sigradur G), which provides decisive advantages for the simulation due to its spherical structure and its narrow particle size distribution. In the current research, the packing quality of these resin materials in the PCC column is analysed in dependence on the mobile phase parameters, the membrane/wire effects and the decisive potential influence using small carbon acids and aromatic amino acids as analytes. Additionally, a detailed material characterization is performed to determine the physical quantities and the adsorption effects. To validate the experimental results, a random glassy carbon particle packing is simulated in OpenFOAM to analyse the packing's structure, hydrodynamics and salt concentration profiles at the macro level.



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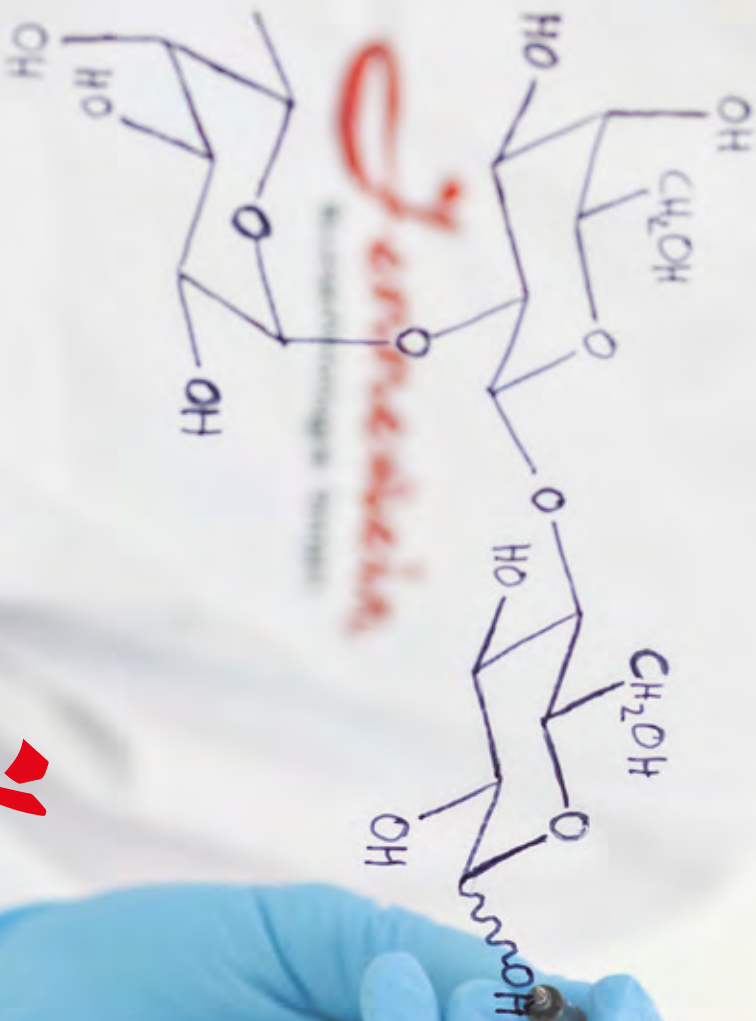
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*See you again in spring 2020!*



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