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Solvent-free nanoparticles synthesis for encapsulation of water-soluble compounds

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22 November 2026



# esib / 2022

**EUROPEAN SUMMIT OF INDUSTRIAL BIOTECHNOLOGY**

GRAZ, AUSTRIA, NOVEMBER 14th – 16th

TOGETHER WITH






# welcome

EUROPEAN SUMMIT OF INDUSTRIAL BIOTECHNOLOGY
GRAZ, AUSTRIA, NOVEMBER 14th – 16th

TOGETHER WITH

IFPB

PICHIA 2022

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# European Summit of Industrial Biotechnology

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14th – 16th November 2022, Graz, Austria

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TWIST BIOSCIENCE

APP NOTE

**Rapid, Whole-cell Engineering of Plant Alkaloid Biosynthesis in Yeast Using Twist Gene Fragments**

Learn how Prashanth Srinivasan, PhD and his colleagues from Stanford University sped up almost 200 times the engineering of complex biological pathways in yeast by synthesizing all heterologous enzymes as Twist Gene Fragments.

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# Organizing Committee

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# Conference
## Venue

The European Summit on Industrial Biotechnology (esib) is going to take place in Graz/ Austria. The second largest city of Austria is a fascinating, beautiful and nice place. With more than 900 years of history, visitors get in touch with ancient knights, a vibrating cultural scenery that ranges from classics to modern approaches, tasty food and an inspiring nightlife. Additionally, nine universities and with colleges Graz is an internationally acknowledged location for science and research.
The conference takes place at the Messe Congress Graz, which is centrally located within walking distance from the picturesque old town:

**Messe Congress Graz**
**Messeplatz 1**
**A-8010 Graz**

## Public Transport

You can conveniently reach the conference venue by public transport (tramway). The following stops are in close walking distance to the Messe Congress Graz:

**Jakominigürtel/Messe,**
**Stadthalle, Fröhlichgasse/Messe,**
**Münzgrabenstraße/Messe.**

You can take the following tramway lines: **4, 5, 6.**

## Instructions for Poster Presenters

The poster boards will be available in the c onference venue. They will be labelled with consecutive numbers. Your poster is assigned a number, which can be found in the program. Please mount your poster at the beginning of the conference on **Nov. 14th, 2022.** Please be available during the poster sessions until the end of the conference on **Nov. 16th, 2022.**






## Welcome Drink

The IFPB Welcome Drink will take place on Sunday, November 13th, 2022 from 19:00 - 21:00. We warmly welcome you in Graz with some drinks and little snacks. Possibility for „light registration“.

VENUE

**Hotel Weitzer**
Grieskai 12-16
8020 Graz

Internet access
Name: **esib**
Password: **RCTPichia**

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# Gala Academia Dinner
## 14th November

The Gala Dinner takes place on **November 14th, 2022 at 20:30; doors open 20:00.** Meet, talk, eat, drink and enjoy an evening by interesting people! High above the old city center of Graz you will have the chance for networking with visionaries and doers, with decision makers and sceptics. Be part of it!

VENUE

**Schlossbergrestaurant**
Am Schlossberg 7
8010 Graz



### How to get there:

At **19:45 and 19:55** the **tramway "Sonderfahrt"** will wait for you just in front of the Messe Congress Graz at the stop "Stadthalle" – therefore please be on time. It will bring you to the stop "Schlossbergbahn" where you change to the "Schlossbergbahn", a funicular that will bring you directly to the top of the Schlossberg. Take a left and you will see the Schlossbergrestaurant in front of you. If you prefer to go by yourself please make sure your arrive of Schloßbergbahn between 20:00 and 20:30. The password for free transfer is **esib 2022.** Maps of Graz will be offered at the registration desk.





# Chilling Life Science
## 15th November

The Chilling Life Science takes place on **November 15th, 2022 at 21:00, Doors open 20:00.** Let the day fade away in the heart of the Schlossberg – at the Dom im Berg - with delicious fingerfood, cocktails and good company. Enjoy yourselves; it's time to chill!

VENUE

**Dom im Berg**
Schlossbergsteig
8010 Graz

### How to get there:

At **20:15 and 20:30** the **tramway "Sonderfahrt"** will wait for you just in front of the Messe Congress Graz at the stop "Stadthalle" – therefore please be on time. It will bring you to the stop "Schlossbergplatz / Murinsel". Walk via the Schlossbergplatz in the direction Schlossberg and take the entrance on the right into the Schlossberg, from there it is approx. 3-4 minutes walk uphill to the Dom im Berg. Alternatively take the left entrance, here a lift will bring you to the Dom im Berg. If you prefer to go by yourself please come to the tramway stop "Schlossbergplatz / Murinsel", and for entering Dom im Berg see description above. Maps of Graz will be offered at the registration desk.



# Biotech Breakfast
## 16th November

The Biotech Breakfast takes place on **November 16th, 2022 8:30 – 10:00** at the Messe Congress Graz. Let the day begin! Breakfast is the most important meal of the day so let's get together and start the last day of our summit with a delicious, typical Styrian breakfast to delight body & soul.

## PICHIA Dinner on November 17th, 2022

Networking event with dinner at the Gansrieglhof, a typical local Buschenschank, in Weiz. (Complementary for participants of the PICHIA 2022 PLUS ONE DAY).
Transfer **with buses at 18:15** from Messe Graz. Transfer back at **22:15 and 23:00** to Messe Graz

VENUE

**Gansrieglhof**
https://gansrieglhof.at





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# Timetable **Day 1**
# Monday, 14th November

| | HOUR | SESSION | LOCATION |
|---|---|---|---|
| | **8:15 – 11:45** | Site Visit to ZETA GmbH | |
| | **10:15 – 11:45** | European funding opportunities in Horizon Europe: What's in it for you? | **Galerie** |
| | **10:15 – 11:45** | synBiocarb glycobiology workshop | **Saal 3** |
| lfpb | **10:15 – 11:45** | Bio-based polymers | **Saal 1** |
| | **11:45 – 12:30** | **REGISTRATION RECEPTION & LUNCH POWERED BY LACTOSAN** | |
| | **12:30 – 14:30** | Welcome address & Trends in biopharma – Medicines of the future | **Saal 1** |
| lfpb | **12:30 – 14:30** | Biorecycling & sustainable processes | **Saal 10** |
| | **14:30 – 15:00** | **COFFEE BREAK & POSTER SESSION POWERED BY GIVAUDAN** | |
| | **15:00 – 16:30** | Trends in bioeconomy – Proteins of the future | **Saal 1** |
| lfpb | **15:00 – 16:30** | Enzymatic decomposition of plastics | **Saal 10** |
| | **16:30 – 17:00** | **COFFEE BREAK POWERED BY GIVAUDAN** | |
| | **17:00 – 18:30** | Trends in policy | **Saal 1** |
| lfpb | **17:00 – 19:00** | The plastics biorefinery | **Saal 10** |
| | **18:30 – 19:30** | Panel discussion | **Saal 1** |
| | **20:30** | **GALA ACADEMIA DINNER** | **SCHLOSSBERG** |




# Timetable **Day 2**
# Tuesday, 15th November

| | HOUR | SESSION | LOCATION |
|---|---|---|---|
| | **9:00 – 11:00** | Vaccines for emerging viral diseases | **Saal 11** |
| | **9:00 – 11:00** | $CO_2$, the future feedstock for biobased processes - A researcher´s perspective | **Galerie** |
| | **9:00 – 11:00** | Meat of the future | **Saal 1** |
| | **9:00 – 11:00** | Biofunctionalisation | **Saal 10** |
| | **11:00 – 11:15** | **COFFEE BREAK** | |
| | **11:15 – 12:00** | Science Flash | **Saal 1** |
| | **12:00 – 13:30** | **LUNCH BREAK & POSTER SESSION POWERED BY VALIDOGEN** | |
| | **13:30 - 15:30** | Sustainability in the value chain | **Galerie** |
| | **13:30 - 15:30** | Next generation downstream processing | **Saal 1** |
| | **13:30 - 15:30** | Nano/Bio-materials and applications | **Saal 10** |
| | **13:30 – 14:00** | PICHIA opening | **Saal 12** |
| | **14:00 – 15:30** | (Bio-) materials and chemicals, biopolymers made by *Pichia* | **Saal 12** |
| | **15:30 – 16:00** | **COFFEE BREAK POWERED BY RCT** | |



# Timetable **Day 2**
# Tuesday, 15th November

| | HOUR | SESSION | LOCATION |
|---|---|---|---|
| | **16:00 – 18:00** | Technical implementation of cultivated meat production | **Saal 1** |
| | **16:00 – 18:00** | Designing the future of Science & Art | **Saal 5** |
| | **16:00 – 18:00** | Bio-based polymers | **Saal 10** |
| | **16:00 – 18:00** | Food and feed products | **Saal 12** |
| | **18:00 – 18:15** | **COFFEE BREAK** | |
| | **18:15 – 19:45** | Poster session: esib, PICHIA, IFPB | **Foyer** |
| | **18:15 – 19:45** | Science Meets Economy B2B Matchmaking Event | **Galerie** |
| | **21:00** | **CHILLING LIFE SCIENCE POWERED BY BASF** | **DOM IM BERG** |

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# Timetable **Day 3**
# Wednesday, 16th November

| | HOUR | SESSION | LOCATION |
|---|---|---|---|
| | 08:30 – 10:00 | BIOTECH BREAKFAST & POSTER SESSION POWERED BY RIEGER | FOYER |
| | 10:00 – 12:00 | Process intensification in biopharmaceutical manufacturing | Galerie |
| | 10:00 – 12:00 | Building a new future: $CO_2$-based bioprocesses - A researcher´s perspective | Saal 10 |
| | 10:00 – 12:00 | Digitalization of enzyme engineering | Saal 1 |
| | 10:00 – 12:00 | PICHIA methods and technologies | Saal 12 |
| | 12:00 – 12:15 | COFFEE BREAK | |
| | 12:15 – 13:00 | Science Flash | Saal 1 |
| | 13:00 – 14:00 | LUNCH BREAK POWERED BY CODEXIS | |
| | 14:00 – 15:30 | Closing lecture "Bridging Tomorrow" | Saal 1 |
| | 15:30 | Closing remarks esib | Saal 1 |
| | 14:00 – 15:30 | New (Pharmaceutical) products made by *Pichia* I | Saal 12 |
| | 15:30 – 16:00 | COFFEE BREAK | |
| | 16:00 – 18:00 | New (Pharmaceutical) products made by *Pichia* II | Saal 12 |
| | 19:00 | PICHIA DINNER POWERED BY VALIDOGEN | GANSRIEGLHOF |



esib /

# **PICHIA plus one day**
# Thursday, 17th November

| | HOUR | SESSION | LOCATION |
|---|---|---|---|
| | 09:00 – 10:00 | Secretion | Hotel Weitzer |
| | 10:00 – 10:30 | COFFEE BREAK POWERD BY MODERN MEADOW | |
| | 10:30 – 12:00 | New aspects of the Pichia genome | Hotel Weitzer |
| | 12:30 – 13:30 | LUNCH BREAK POWERD BY RCT | |
| | 13:30 – 15:00 | Synthetic biology & Metablic Engineering | Hotel Weitzer |
| | 15:00 – 15:30 | COFFEE BREAK POWERED BY MODERN MEADOW | |
| | 15:30 – 17:00 | Bioprocess engineering | Hotel Weitzer |

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# Monday,
14th November

## 8:15 – 11:45
## Site visit to ZETA GmbH

**Lecture:** From a laboratory scale process to an industrial production facility – how early stage engineering can secure profitability of business. In the lecture you will learn about the common pitfalls in scale up and industrialization of biotech processes and how they can be overcome.

**After the lecture:** Workshop tour through ZETA facilities in Lieboch.

**Please note:** Participation only with pre-registration. A photo of the neg. test with the date is fine.

## 10:15 – 11:45
## European funding opportunities in Horizon Europe: What's in it for you?

Horizon Europe is the largest funding programme for research and innovation in the world. With a budget of € 95.5 billion until 2027, it tackles climate change, helps to achieve the UN's Sustainable Development Goals and boosts the EU's competitiveness and growth. The programme facilitates collaboration among researchers, innovators and industries to develop, support and implement EU policies while tackling global challenges. It supports creating and better dispersing of excellent knowledge and technologies by helping individual researchers and top class innovators to develop and deploy their ideas and support breakthrough innovations to create new markets. Interested? Get an overview of the diverse funding opportunities in Horizon Europe!

**Speakers:**
Simone Jährig (Austrian Research Promotion Agency, FFG)
Angelo Nuzzo (Austrian Research Promotion Agency, FFG)



esib /

# Monday,
14th November

## 10:15 – 11:45
## synBiocarb glycobiology workshop

The synBIOcarb European Training Network brings together a diverse team of chemists, structural biologists, biophysicists, cell biologists and protein engineers from across Europe who are pioneering the development of Synthetic Glycobiology, and four SMEs that are leading industrial innovation in glycoscience and protein engineering.

In this session, three ESRs, an SME and an academic beneficiary will present their achievements in synBIOcarb and how they benefit from being a part of this European Training Network.

**Special Session:**
Chair: Birgit Wiltschi (BOKU/acib)

**Speakers:**
Jürgen Mairhofer (enGenes Biotech GmbH)
Natalia Danielewicz (enGenes Biotech GmbH)
Francesca Rosato (University of Freiburg)
Paras Kundalia (Slovak Academy of Sciences)

# Monday,
14th November

## 12:30 – 14:30
## esib Welcome Address

**Speakers:**
Mathias Drexler (CEO acib)
Bernd Nidetzky (acib; Graz University of Technology)

## Trends in biopharma – Medicines of the future

The pandemic has impressively demonstrated that we urgently need efficient measures to be prepared for health crises of this kind as quickly as possible. What were the most important lessons learned by those who fought on the front lines? And what are the future scenarios in vaccine and drug development? Take part in discussing the most promising trends in biopharma.

**Chair:**
Bernd Nidetzky (acib; Graz University of Technology)

**Speakers:**
Florian Krammer (Icahn School of Medicine at Mount Sinai)
Philippe-Alexandre Gilbert (Bill & Melinda Gates Foundation)
Raffael Nachbagauer (Moderna)

## 15:00 – 16:30
## Trends in bioeconomy – Proteins of the future

Meat from the lab and food ingredients produced by microorganisms? Improved material properties through proteins? Society is undergoing a paradigm shift toward a more sustainable future. Our plenary speakers share with us their experiences in their fields - get inspired to new ideas for a circular bioeconomy!

**Chair:**
Bernd Nidetzky (acib; Graz University of Technology)

**Speakers:**
Mark Post replaced by Laura Jackisch (Mosa Meat)
David Kaplan (Kaplan Labs, Tufts University)



esib /

# Monday,
14th November

## 17:00 – 18:30
## Trends in policy

The European Green deal and the trend toward recycling waste streams and use of new resources also brings significant challenges in the approval of new products. How can this step be met as efficiently as possible? How can decision-makers be involved at an early stage? We take a look at new opportunities in industrial biotechnology and look forward to the perspectives of experts from industry & policy.

**Chair:**
Bernd Nidetzky (acib; Graz University of Technology)

**Speakers:**
Adrian Leip (European Commission)
Elke Duwenig (BASF)

## 18:30 – 19:30
## Podium Discussion (all speakers)

# Tuesday, 15th November

## 9:00 – 11:00
## Vaccines for emerging viral diseases

The challenge for emergent viral diseases especially in times of a pandemic is the fast reaction from initial detection of the disease to a protective vaccine. Next to the development of the expression system and production hosts, also the design and scale-up of the production process for a vaccine plays an important role in the development chain. How can we streamline and accelerate the development and analytics of production processes for vaccines and other bionanoparticle products (such as vectors for gene or cancer therapy) in the view of the current issues of supply chain shortages?

**Chair:**
Narges Lali (acib)

**Speakers:**
Andreas Wagner (Polymun Scientific)
Alexei Voloshin (3M)
Matthew Watson (Merck)
Reingard Grabherr (BOKU)

## 9:00 – 11:00
## Meat of the future

Current animal based agriculture for the production of meat products contributes to Global Warming, bears inherent inefficiencies (less calories per hectare of land than plant based food), and violates animal welfare. Biotechnology may become the key to a benign production of food that satisfies human craving for the "meaty taste". Let's compare the different approaches to substitute animal grown meat and receive some guidance for biotechnology research how to engage.

**Chair:** Kai Baldenius (Baldenius Biotech Consulting)

**Speakers:**
Seren Kell (Good Food Institute)
Lieven Thorrez (KU Leuven)
Marco van den Berg (DSM)
Martin Krenn (Buehler)
Andrew Stout (Kaplan Lab, Tufts University)



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# Tuesday, 15th November

## 9:00 – 11:00
## $CO_2$, the future feedstock for biobased processes

We live in a carbon world – life would not be possible without. But carbon has become also one of the biggest problems, we are facing today: climate crisis. Between 2011 and 2020, the yearly $CO_2$ emissions caused by human activity exceeded 38 gigatons, while $CO_2$ fixation by nature is almost 22 gigatons. To improve the balance of carbon cycle and meet the goals of the European Green Deal, net $CO_2$ emissions should be set to zero or even negative by removing $CO_2$ from the atmosphere. Using $CO_2$ as a new generation feedstock is part of the solution to address the climate crisis while transforming the EU into a sustainable resource-efficient, and competitive economy. Let's bring researchers, industrial stakeholders, and policy makers together to discuss $CO_2$ -based bioeconomies!

**Chair:**
Özge Ata (acib)
Simone Bachleitner (BOKU)

**Speakers:**
Thomas Jakl (Federal Ministry Republic of Austria, Climate Action, Environment, Energy, Mobility, Innovation and Technology)
Albert Guisasola (Universitat Autonoma de Barcelona)
Helmuth Schwab (econutri)
Gregor Tegl replaced by Simon Rittmann (Arkeon)

## 11:15 – 12:00
## Science flash

You have an idea you want to place on the market? You have an outstanding new finding you want to present? be precise and be to the point in a powerful speed presentation.

**Chair:**
Astrid Preisz

**Speakers:**
See page 38

# Tuesday, 15th November

## 13:30 – 15:30
## Sustainability in the value chain

Sustainability assessment in all three dimensions - economic, environmental and social - will form the future basis for decisions taken by consumers, funding authorities and investors. What is state of the art from an academic view point, how is the topic handled in best practice in large industries and SME? And what are the latest developments in EU taxonomy approaches for sustainable investment? Take the chance to get insights from top experts in the field. A special focus will be given to social life cycle assessment.

**Chair:**
Barbara Petschacher (acib, Graz University of Technology)

**Speakers:**
Mark Goedkoop (Pré Sustainibility)
Alexandra Florea (DSM)
Brigitte Bichler (OMV)
Jürgen Kuballa (GALAB Laboratories GmbH)

## 13:30 – 15:30
## Next generation downstream processing

The pressure caused by time to market has increased in the biopharmaceutical development. The need for platform processes became very urgent and new strategies of downstream processing are of utmost importance for purification of new modalities or to intensify legacy processes. There is no platform purification process for non-mab proteins available. How can we solve these challenges? And how can we ensure a carbon neutral biomanufacturing? We discuss solutions such as the development of new ligands for affinity chromatography, new ways for enzymatic processing of fusion proteins and radical change of chromatography materials. More efficient downstream processing will help to establish carbon neutral biomanufacturing.

**Chair:**
Nico Lingg (BOKU)

**Speakers:**
Alexei Voloshin (3M)
David O'Connell (University College Dublin)
Rainer Schneider (University of Innsbruck)
Matthias Berkemeyer (Boehringer Ingelheim RCV)



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# Tuesday, 15th November

## 16:00 – 18:00
## Technical implementation of cultivated meat production

Cultivated Meat has great potential for an environmentally neutral solution for the production of meat. In the last decade Its development has been truly remarkable, but it still needs to overcome multiple technical hurdles before becoming a reality. What are these hurdles and how can industrial biotechnology help?

**Chair:**
Aleksanda Fuchs (acib)

**Speakers:**
Andrew Stout (Kaplan Lab, Tufts University)
Peter Stogios (University of Toronto)
Florian Krainer (ZETA/Eridia)
Christoph Reisinger (acib)
Che Connon (University of Newcastle)

## 16:00 – 18:00
## Designing the future of Science & Art

Integrating science into art? Let's talk about it and get inspired by presentations about the successful collaborations between Science & Art "Fermenting Futures" and "ZELLE | ParZELLE".

**Interactive discussion (world cafè)** about potential scientific topics that would be suitable for future "Science & Art" projects. There are no limits to creativity: Photography, pottery, sculpture, painting, theatre, literature, music etc. – all disciplines of art could be interesting to illustrate the scientific topics around industrial biotechnology and life sciences.

**Chair:**
Diethard Mattanovich (BOKU)

**Speakers/Artists:**
Anna Dumitriu and Alex May (Artists, "Fermenting Futures")
Sanela Pansinger and Petra Kohlenprath (Architects and urban researchers, "ZELLE | ParZELLE")

# Tuesday, 15th November

## 18:15 – 19:45
## Science Meets Economy B2B Matchmaking Event

Are you looking for new collaborative partnerships? The matchmaking event organised by the SFG and the Enterprise Europe Network is a unique opportunity to generate new business contacts and to develop new ideas. Select interesting prospective partners and pre-book 15-minute meetings with them in advance of the event. The meetings are arranged in advance and participation is requested in the course of the registration process.

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# Wednesday, 16th November

## 10:00 – 12:00
## Process intensification in biopharmaceutical manufacturing

In this session the trends and challenges of process intensification and continuous manufacturing of biopharmaceuticals will be discussed. One focus will be the process integration of upstream and downstream processing and how processes are optimized in small scale. The other focus will be on the impact of process intensification on economic and environmental sustainability and on carbon footprint. Case studies will be presented.

**Chair:**
Alois Jungbauer (BOKU)

**Speakers:**
Florian Mayer (BOKU)
Bernhard Sissolak (Bilfinger)
Bernt Nilsson (Lund University)
Alessandro Cataldo (Takeda)
Johannes Felix Buyel (RWTH Aachen & BOKU)

# Wednesday, 16th November

## 10:00 – 12:00
## Building a new future: $CO_2$-based bioprocesses – A researcher's perspective

The increasing release of $CO_2$ has already had already observable effects on our climate. More than ever, innovative ideas are needed to reintegrate the potent greenhouse gas $CO_2$ in-to a circular bioeconomy and to ensure sustainable energy and material sources for the next generations. There is hope, however, that we can harness the power of biology to create opportunities from our very big pollution problem. To achieve net zero, we need a circular carbon economy based on $CO_2$, that, with the help of biology, enables the direct conversion into chemicals that are used to manufacture the materials of our daily life. But how can we harness biology's synthetic ability to turn atmospheric $CO_2$ into these products? And how can we bring this to industrial scale? Join in and get a clearer picture of the key researcher activities to create value from $CO_2$.

**Chairs:**
Anita Emmerstorfer-Augustin (acib, Graz University of Technology)
Sandy Schmidt (Groningen Research Institute of Pharmacy)

**Speakers:**
Heleen de Wever (VITO)
Dirk Holtmann (TU Mittelhessen)
Stephane Guillouet (INSA Toulouse)
Falk Harnisch (Helmholtz Centre for Environmental Research - UFZ)

## 12:15 – 13:00
## Science flash

You have an idea you want to place on the market? You have an outstanding new finding you want to present? be precise and be to the point in a powerful speed presentation.

**Chair:**
Olivia Laggner (acib)

**Speakers:**
See page 39



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# Wednesday, 16th November

## 10:00 – 12:00
## Digitalization of enzyme engineering

While the impact of computational methods on enzyme engineering is still significant, the potential of recent developments such as the de novo prediction of protein structures and machine-learning guided protein engineering represents a paradigm change, improving dramatically the possibilities to optimize existing biocatalysts and to create entirely new reactivities. Let's discuss different approaches and their impact on the way how we investigate and engineer biological systems for biotechnology and biocatalysis. A main focus will lie on novel protein engineering platforms that will ultimately become an integral part of academic and industrial biocatalysis in the following years.

**Chair:**
Robert Kourist (Graz University of Technology)

**Speakers:**
Stefan Lutz (Codexis)
Elina Siirola (Novartis Pharma)
Christian Gruber (Innophore
Gustav Oberdorfer (Graz University of Technology)

## 14:00 – 15:30
## Closing lecture "Bridging tomorrow"

Join in and have a look at the upcoming trends in biotechnology for the next decades. What are the visions of industry and which disruptive technologies might influence or support the missions of tomorrow? Let's shed a light on innovative views from representatives in the field of industrial biotechnology.

**Chair:**
Bernd Nidetzky (acib; Graz University of Technology)

**Speakers:**
Sang Yup Lee (Korea Advanced Institute of Science and Technology – KAIST)
Andrea Camattari (Ginko Bioworks)

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# Science Flash,
## Tue 15th November

11:15 – 12:00

| ID | NAME | TITLE |
|---|---|---|
| 01 | Alzbeta Cardova | Stable FGF2 (FGF2-STAB) as an innovative component of cultured meat media |
| 02 | Gernot Beihammer | Strategies to enhance the N-glycosylation efficiency of plant produced pharmaceuticals |
| 03 | Carlotta Mattioda | Solvent-free nanoparticles synthesis for encapsulation of water-soluble compounds |
| 04 | Touraj Eslami | Online control and optimization for protein chromatography by MPC for enhancing process economics |
| 05 | Filipa Daniela Gomes Gonçalves | Response to perspiration from functional textiles incorporating protein/fragrances complex |
| 06 | Luigi Marra | Assessment of biocatalytic $CO_2$ capture and utilization through a carbonic anhydrase - decarboxylase cascade |
| 07 | Ana Tinoco | Fusion proteins with chromogenic and keratin binding modules |
| 08 | Andrea Nigl | A new biosynthetic route from isoprenol to tulipalin A – Steps towards fermentative production of the polymer precursor |
| 09 | Vanja Kokol | Ecotoxicity and cytotoxicity of antibacterial active nanocellulose |
| 10 | Christian Witz | Efficient modeling of bioreactors – from lab scale to industrial scale |
| 11 | Sabine Schelch | Whole-cell synthesis for 3'-sialyllactose optimized through a high-flux enzymatic cascade transformation |
| 12 | Mikael Muegge | Sustainable fertilisers boosting the bio-economy |



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# Science Flash,
## Wed 16th November

12:15 – 13:00

| ID | NAME | TITLE |
|---|---|---|
| 13 | Wiebke Düttmann | Modern data management in medicine: Tbase |
| 14 | Arianna Palma | Super-fast oxidases and more: novel yeast enzymes and mechanisms of oxidative protein folding |
| 15 | Daniel Incze | Mycotoxin inactivating enzymes as feed additives |
| 16 | Petra Heidinger | Wastewater based surveillance and analysis of SARS-CoV-2 in Styria |
| 17 | David Wollborn | Predicting high recombinant protein producer strains of Pichia pastoris MutS using the oxygen transfer rate as an indicator of metabolic burden |
| 18 | Núria Bernat Camps | Methanol-free expression system PDH: a growth-decoupled alternative to classical P. pastoris promoters for recombinant protein production |
| 19 | David Schönauer | EnzyMAP AI – De-risking enzyme evolution with AI-augmented sequence-function data for the identification of the key point mutants across the entire enzyme sequence |
| 20 | Michael Baumschabl | Lactic acid from $CO_2$ using the synthetic autotroph Komagataella phaffii |
| 21 | Hannah Pia Franziska Meier | From $CO_2$ to L-methionine – engineering the pacemaker enzyme homoserine O-succinyltransferase |
| 22 | Benjamin Kogelmann | Nicotiana benthamiana as expression platform for proteins with targeted glycosylation |
| 23 | Franz Herzog | Characterizing the human immune response by proteomics aids the development of targeted biotherapeutics |
| 24 | Giovanni Davide Barone | Towards the rate limit of heterologous biotechnological reactions in recombinant cyanobacteria |

# Science Flash **01**

## Stable FGF2 (FGF2-STAB) as an innovative component of cultured meat media

**Alzbeta Cardova**[1]

[1]Enantis s.r.o., Brno, Czech Republic

Enantis, a protein engineering company, has developed a novel version of fibroblast growth factor 2 (FGF2-STAB®) with improved thermostability, longevity and a fully retained biological activity that assures appropriate cell proliferation. Thanks to a smart substitution of a couple of amino acids in the FGF2 predicted by our in-silico protein design platform, we have managed to improve half-life of FGF2 in cell cultured media more than 50 times (from 9 hours to more than 30 days at 37°C). The use of our patented FGF2-STAB® in cultured meat space is game-changing as FGF2 is one of the critical and most expensive components of the cultivation media making up to 60% of the total media cost. Replacement of standard FGF2 with our unique FGF2-STAB® allows the cellular agriculture scientists to use lower concentration of the growth factor in the media and permits less frequent media changes while the biological activity of the molecule is fully retained. This represents a significant benefit for the cultured meat and culture media companies as the media price is the main limiting factor of commercial production of cultured meat, milk and fat. We are on the mission to bring the media cost down with our innovative molecule to enable the final product to reach the market earlier, while assuring an improved and reliable function of FGF2 in the media.



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# Science Flash **02**

## Strategies to enhance the N-glycosylation efficiency of plant produced pharmaceuticals

**Gernot Beihammer**[1,2], Julia König-Beihammer[1,2], Benjamin Kogelmann[1,2], Herta Steinkellner[1], Richard Strasser[1]

[1]BOKU Wien, Vienna, Austria, [2]acib GmbH (Austrian Center of Industrial Biotechnology), Graz, Austria

Plant expression systems offer great potential for recombinant protein production due to their low cost, high scalability, and safety. One of the major challenges of using Nicotiana benthamiana as a production platform is the frequently observed underglycosylation of certain N-glycosylation sites. This underglycosylation can lead to aberrant protein folding, alter the functionality of glycoproteins and induces undesired product heterogeneity. In higher eukaryotes, N-glycosylation is mediated by the multimeric oligosaccharyltransferase (OST) complex. The OST catalyses the transfer of a preassembled oligosaccharide precursor to newly formed proteins. While homologues of the OST subunits are evolutionarily conserved and can be found in the genomes of yeast, mammals and plants, there are differences in the subunit composition and individual sequence domains that may lead to the reduced N-glycosylation efficiency.

In this project, we aim to enhance the N-glycosylation site occupancy of N. benthamiana produced glycoproteins by transient overexpression of selected catalytic OST subunits from different species. Overexpression of individual catalytic OST subunits led to aberrant subcellular localization in N. benthamiana leaves and caused an ER phenotype without enhancing the N-glycosylation site occupancy of transiently expressed human IgGs. Via co-expression of further non-catalytic OST subunits, we were able to enhance the stability and improve the subcellular localization of the ectopically expressed catalytic OST subunits. These optimized combinations for OST overexpression are currently examined for their effect on the N-glycosylation site occupancy of recombinant IgGs and may provide a strategy to overcome a current limitation for recombinant glycoprotein expression in plants.

# Science Flash **03**

## Solvent-free nanoparticles synthesis for encapsulation of water-soluble compounds

**Carlotta Mattioda**[1], Clara Mattu[1], Gianluca Ciardelli[1]

[1]Politecnico Di Torino, Torino, Italy

The increasing concerns on green manufacturing practices with low environmental impact have put pressure on the pharmaceutical industry. Of particular concern is the large number of organic solvents used in a wide range of pharmaceutical products, posing a significant risk on human and environmental health. Recently, nanoparticles(NPs) emerged as advantageous drug-delivery systems with the potential to maximize drug efficacy and minimize side effects.
Unfortunately, NPs synthesis processes are still environmentally unsustainable, due to the large amount of organic solvent involved.
This contribution describes the synthesis and characterization of NPs encapsulating proteins or nucleic acids for metastatic melanoma treatment using alternative synthesis methods that replace organic solvents with water-based solutions.
Two different green synthesis techniques have been investigated; (i)Ionic gelation was used to prepare chitosan(CS) NPs, exploiting the electrostatic interaction between the CS amino groups and tripolyphosphate(TPP), (ii)self-assembly technique to prepare siRNA/phosphate-poly(allylamine-hydrochloride)(PAH) NPs, exploiting the interactions between primary amines in the polymer and siRNAs to form stable complexes.
CS and PAH NPs with the appropriate hydrodynamic diameters (~200 nm), polydispersity index, and Z potential for high cell internalization and tissue extravasation were obtained. Preliminary in vitro tests demonstrated that particles are well tolerated by human fibroblast which has shown high viability even when treated with the highest NPs concentration (viability ~85% at 48h from the treatment).
Additional tests are currently ongoing to demonstrate the efficacy of the drug-loaded system on human fibroblasts.
Carlotta Mattioda acknowledges PON "Ricerca e Innovazione" 2014-2020 Azione IV.R "dottorati su tematiche green" for co-financing her Ph.D scholarship.



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# Science Flash **04**

## Online control and optimization for protein chromatography by MPC for enhancing process economics

**Touraj Eslami**[1,2], Nico Lingg[2,3], Alois Jungbauer[3]

[1]evon GmbH, St. Ruprecht an der Raab, Austria,
[2]University of Natural Resources and Life Sciences (Boku)
[3]Austrian Centre of Industrial Biotechnology, Vienna, Austria

The aging of chromatography columns is not taken into account while developing and executing operations. Process chromatography is now carried out with little automation or control; instead, a predefined protocol is often employed. Even essential choices, like pooling, are made after offline analysis or in accordance with predefined conditions [1]. A safety margin must be included to prevent losses during the loading and elution phases adaptively. Accordingly, we have designed an online automation framework to react to the decline in resin capacity over cycles caused by fouling or ligand degradation. Such a control strategy can automate and optimize bioprocesses to account for uncertain feed concentration or flowrate mismatches across unit operations. As a result, the system is able to maintain resin utilization at high productivity and reduces buffer consumption, similar to a countercurrent loading strategy but with lower hardware complexity [2,3]. In order to optimize the resin utilization and productivity, a velocity gradient is applied using a model predictive controller and an extended Kalman filter. The capacity degradation is constantly considered through breakthrough data each cycle, which is used to adapt the loading volume further. This is accomplished by the implemented soft sensor inside the control loop, which measures the actual loading concentration and current capacity [2]. Accordingly, this system enables automation of a chromatographic unit operation and ensures stable, long term operation.

**References DOI:**
1. https://doi.org/10.1016/j.seppur.2021.119985
2. https://doi.org/10.1002/cite.202255366
3. https://doi.org/10.1016/j.chroma.2022.463420

# Science Flash **05**

## Response to perspiration from functional textiles incorporating protein/fragrances complex

**Filipa Daniela Gomes Gonçalves**[1,2], Artur Ribeiro[2], Carla Silva[2], Artur Cavaco-Paulo[1,2]

[1]SOLFARCOS, Avenida Imaculada Conceição, nº 589, 4700-034, Braga , Portugal, [2]Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal

The unpleasant odors of perspiration are a response to daily activity and physical exercise. The presence of these odors in clothes cause social unrest and embarrassment. To tackle it, functional textiles incorporating fragrances could be an effective clothing deodorizing product. This work presents two strategies for the release of fragrances from functionalized cotton with carbohydrate-binding module (CBM)-based complexes: OBP::GQ20::CBM/β-citronellol (approach 1) and CBM::GQ20::SP-DS3-liposome/β-citronellol (approach 2). CBM from Cellulomonas fimi was fused with the odorant-binding protein (OBP::GQ20::CBM), and with an anchor peptide with affinity to the liposome membrane (CBM::GQ20::SP-DS3). In the approach 1, OBP fusion protein served as a fragrance container, whereas in approach 2, the fragrance was loaded into liposomes with a higher cargo capacity. The two strategies showed a differentiated β-citronellol release profile triggered by an acidic sweat solution. OBP::GQ20::CBM complex revealed a fast release (31.9% of the initial amount, after 1.5 h of exposure with acidic sweat solution), while the CBM::GQ20::SP-DS3-liposome complex demonstrated a slower and controlled release (5.9% after 1.5 h of exposure with acidic sweat solution). Both strategies revealed high potential for textile functionalization aimed at the controlled release of fragrances. The OBP::GQ20::CBM/β-citronellol complex is ideal for applications requiring fast release of a high amount of fragrance, whereas the CBM::GQ20::SP-DS3-liposome/β- citronellol complex is more suitable for prolonged and controlled release of a lower amount of β-citronellol.



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# Science Flash **06**

## Assessment of biocatalytic CO2 capture and utilization through a carbonic anhydrase - decarboxylase cascade

**Luigi Marra**[1,2], Hanna Knuutila[3], Antonio Marzocchella[1], Piero Salatino[1]

[1]Dip. Ingegneria Chimica dei Materiali e della Produzione Industriale - Università degli studi di Napoli Federico II, Napoli, Italy, [2]Ist. Scienze e Tecnologie per l'Energia e la Mobilità Sostenibili - Consiglio Nazionale delle Ricerche, Napoli, Italy, [3]Dep Chemical Engineering - Norwegian University of Science and Technology, Trondheim, Norway

Increasing the impact of carbon capture and utilization (CCU) processes requires novel technologies and synergy between industrial sectors. To this aim, biocatalytic pathways worth to be investigating to design feasible CCU processes based on diversified sources of energy and co-substrates. Carbonic anhydrase (CA) has been used in post-combustion $CO_2$ capture by gas-liquid absorption. The CA-based technology is relatively mature and can fit specific design criteria of full-size reactors by tuning the enzyme immobilization methods. The direct use of bicarbonate as the main product of CA-based $CO_2$ capture offers further opportunities still marginally explored. Among these, the production of phenolic carboxylic acids (PCA) catalysed by cofactor-free decarboxylases (DC) at large excess of bicarbonate has been proposed. The present study addressed the feasibility of a cascade process where CA-based $CO_2$ capture provides the bicarbonate-rich solvent to DC catalysed production of PCA. The $CO_2$ capture unit model was based on the literature data on $CO_2$ enzymatic reactive absorption in $K_2CO_3$ solutions. Lignin-derived phenols from the fractionation of a pyrolytic bio-oil were assumed as substrates of DC. The carboxylation reactor model assumed integrated PCA removal steps and an immobilized DC. Efficient immobilization techniques (covalent attachment and cross-linked enzyme aggregates) were considered in order to model two alternative continuous reactor configurations (packed bed and series of stirred tanks). Two process layouts were simulated depending on substrate availability. The sensitivity study showed the impact of phenols solubility and immobilized enzymes' efficiency on reactors size and suggested guidelines for future experimental research.

# Science Flash **07**

## Fusion proteins with chromogenic and keratin binding modules

**Ana Tinoco**[1,2], Egipto Antunes[2], Madalena Martins[2], Filipa Gonçalves[1,2], Andreia C. Gomes[3], Carla Silva[2], Artur Cavaco-Paulo[1,2], Artur Ribeiro[2]

[1]Solfarcos, Braga, Portugal, [2]CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal, [3]Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus of Gualtar, 4710-057, Portugal

Hair coloring using chemical dyes is a popular hair routine that significantly damage the hair keratin fiber. A fusion protein composed by a chromogenic blue ultramarine protein (UM) bound to a keratin-based peptide (KP) was explored, for the first time, as a hair coloring agent. The KP-UM fusion protein explores UM chromogenic nature together with the KP affinity towards hair. The KP-UM application on overbleached asian hair was able to color the hair tresses, being the color dependent on the formulation polarity. For the more polar formulations, it was obtained a purple hair color while for the formulations with higher percentage of ethanol a redder color was observed. Using confocal microscopy, it was verified that the KP-UM protein bounded to the hair cuticle and even penetrated throughout the hair fiber, reaching even the medulla. All the tested formulations recovered the mechanical properties of overbleached asian hair and the KP-UM protein proved to be safe when tested in human keratinocytes. Although based on a chromogenic non-fluorescent protein, the KP-UM presented a photoswitch phenomenon, changing from chromogenic to fluorescent depending on the wavelength selected for the protein excitation. It was then demonstrated the potential of KP-UM protein to be incorporated in new ecofriendly cosmetic formulations for hair coloration, decreasing the use of traditional dyes and reducing its environmental impact.



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# Science Flash **08**

## A new biosynthetic route from isoprenol to tulipalin A – Steps towards fermentative production of the polymer precursor

**Andrea Nigl**[1,2], Kamela Myrtollari[2,3], Marina Marić[2,4], Andreas Taden[3], Robert Kourist[2]

[1]acib GmbH - Austrian Centre of Industrial Biotechnology, Graz, Austria, [2]Institute of Molecular Biotechnology, Graz University of Technology, Graz, Austria, [3]Henkel AG & Co. KGaA, Adhesive Research, Düsseldorf, Germany, [4]Department of Biochemical Engineering, University of Zagreb, Zagreb, Croatia

Tulipalin A is a secondary defense metabolite found in plants among the Alstroemeriaceae and Liliaceae families. Besides its antimicrobial and insecticidal properties, it has been gaining increasing attention due to its applicability as a building block for various bio-based polymers. Desirable characteristics of tulipalin A for thermoplastic applications, such as high glass transition temperature and high refractive index, have led to intensive studies on its chemical synthesis. However, many of those synthesis routes are limited by low yields and costly starting materials.[1] A biotechnological approach via a microbial chassis represents a promising alternative. To enable fermentative production of the monomer from renewable resources, a suitable pathway has to be integrated and interconnected with the microbial host's metabolism.

In our study, we aim to identify and establish an artificial downstream pathway deviating from the hemiterpenoid metabolism and thereby paving the way towards a biotechnological production of tulipalin A. The synthetic route starting from isoprenol encompasses acetylation and selective oxyfunctionalization. The resulting product can then be converted to tulipalin A via acid catalysis. Here we demonstrate the exploitation of substrate promiscuity of an alkane monooxygenase to catalyze the key hydroxylation step in this artificial pathway.[2]

**References:**

[1] S. Agarwal, Q. Jin, S. Maji, in Biobased Monomers, Polymers, and Materials (Eds.: P.B. Smith, R.A. Gross), American Chemical Society, Washington, DC, 2012, pp. 197–212.

[2] A. Taden, H. Beck, K. Myrtollari, A. Nigl, R. Kourist, PROCESS FOR THE SYNTHESIS OF α-METHYLENE-γ-BUTYROLACTONE, n.d., EP 22156336.4.

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# Science Flash **09**

## Ecotoxicity and cytotoxicity of antibacterial active nanocellulose

**Vanja KOKOL**[1*], Sara NOVAK[2], Veno KONONENKO[2], Vera VIVOD[1], Damjana DROBNE[2]
[1]Faculty of Mechanical Engineering, University of Maribor, Slovenia
[2]Biotechnical Faculty, University of Ljubljana, Slovenia

Research focusing on naturally-occurring biocides with a low-risk of toxicity for humans and the environment is becoming increasingly urgent. The use of nanocellulose (NC) in this context is also increasing, due to its low cost and unique properties, which are potentially useful in many applications, such as wound dressings, composites, packaging materials, filters/adsorbents, textiles and paints. However, NC needs to be surface modified to gain an antimicrobial potential. Chemical modifications with non-leaching properties and prolonged biocidal activity are of the highest interest (Norrahim et al 2021, Materials Advances 2/11 p.3538-3551).

We have (PCT/EP2022/061122) oxidized the wood-derived cellulose nanocrystals (CNC) and cellulose nanofibrils (CNFs) via periodate oxidation (CNC/CNF-ox) to yield aldehyde groups at the corresponding C2 and C3 positions of glucopyranose units and further conjugated them with amino-bearing hydrophobic molecule (HMDA) via the Schiff-base reaction (CNC/CNF-ox-HMDA) (confirmed by FTIR spectroscopy, potentiometric titration, and zeta-potential analysis), thus to promote interactions with a negatively charged and hydrophobic bacterial cell membrane, compromising its integrity, and resulting in a bactericidal effect (Tavakolian et al. 2020, Nano-Micro Letters 12/1 p.1-23). The aquatic in vivo (to Daphnia magna, ISO 16197:2014) and human in vitro (to A549 human lung cells) toxicities, and degradation profiles in composting soil (ISO 11721-1: 2001) of native and both modified forms of CNF/CNC were also assessed in order to define their safety profile.

The CNF/CNC-ox-HMDA exhibited higher antibacterial activity than CNF/CNC-ox and higher against Gram-positive S. aureus than Gram-negative E. coli, yielding >90% of bacteria reduction after 24h of exposure at minimum (≤0.2 wt%), but potentially moderate (aquatic) and low (human) toxic (≥50 mg/L) concentrations. Hydrodynamically smaller (< 1 μm) CNC-ox-HMDA decorated with aldehyde, anionic, amino and hydrophobic surface groups was found to be the most effective, with up to log 9 of bacteria reduction at >0.4 wt.% and a bactericidal effect with bacteria catching ability (SEM imaging). While the antimicrobial less-active CNF/CNC-ox were fully biodegradable in composting soil within 24 weeks, this process was inhibited considerably for CNF/CNC-ox-HMDA, indicating their different stability and disposal after use (e.g. composting vs. recycling). Such a materials can, however, serve as an alternative to nano-biocides (such as metal nanoparticles), which are limited by their toxicity for humans and the environment. ***The research was funded by Slovenia Research Agency (Projects No. J2-3053, J2-1719, and Research Program No. P2-0424).***



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# Science Flash **10**

## Efficient modeling of bioreactors – from lab scale to industrial scale

**Christian Witz**[1], Philipp Eibl[1]

[1]Simvantage Gmbh, Graz, Austria

Transferring process understanding gained on the lab-scale to the pilot-scale and large-scale fermenters is a non-trivial task that is often based on empirical knowledge from costly experiments and empirical correlations. Ensuring optimal environmental conditions for microorganisms in large-scale production fermenters in the biopharmaceutical industry is of vital importance for safety, stability, and optimized productivity.

In this work, we present the code SimVantage (simvantage.com), which is a powerful GPU-based modeling environment for bioreactors, that utilizes the Lattice Boltzmann Method (LBM) for transient simulations of the liquid phase. It is based on an in-silico scale-independent simulation method to predict key parameters based on first principles, even suitable for large-scale fermenters.

The implementation is optimized for state-of-the-art Graphics Processing Units (GPUs) and includes the motion, breakup, and coalescence of the dispersed gas phase via the Euler-Lagrange method, transport phenomena between phases and throughout the fermentation broth, as well as the movement and distribution of microorganisms. The inclusion of the microorganism movement allows for a detailed analysis of the inhomogeneities within the fermentation broth by tracking the individual organism's lifelines and the environmental conditions of the whole population over time.

After an overview of the algorithms of the simulation code, a use-case is shown on how to transfer process conditions from the lab scale to the production scale based on the similarity of the environmental conditions of the organism population. Possible applications include reducing experimental load during scale-up, as well as the construction of representative scale-down models of large-scale fermenters.

# Science Flash **11**

## Whole-cell synthesis for 3’-sialyllactose optimized through a high-flux enzymatic cascade transformation

**Sabine Schelch**[1,2], Jasmin Zuson[1,2], Jürgen Kuballa[3], Bernd Nidetzky[1,2]

[1]acib GmbH, Graz, Austria, [2]Institute of Biotechnology and Biochemical Engineering, NAWI Graz, Graz University of Technology, Graz, Austria, [3]GALAB Laboratories GmbH, Hamburg, Germany

Enzyme cascades that enable efficient transformation of an expedient substrate into a valuable product are regarded as highly promising bio-production systems. However, despite the growing interest, there have been only limited efforts by systematic engineering approaches to optimize the flux through the cascade for enhanced process efficiency. In this study, we applied the idea of flux optimization in a multistep-enzymatic cascade for the development of a whole-cell catalyst producing 3'-sialyllactose (3SL). 3SL is a major human milk oligosaccharide[1] and its bioproduction has attracted attention considerably. The E.coli catalyst considered here co-expressed CMP-sialic acid synthetase (CSS) and sialyltransferase (ST) to enable synthesis of 3SL from N-acetylneuraminic acid, CTP and lactose. The strain development involved the design of different plasmid vectors for tunable enzyme co-expression to give CSS and ST activities in ratios suitable for efficient conversion of substrates into 3SL, predicted by a previously developed model[2].
The resulting biocatalysts co-expressing both enzymes showed a high flux through the cascade for 3SL synthesis, observable by low intermediate accumulation (10-15% of total mass balance). The flux through the cascade could be further optimized by adaptation of reaction conditions to up- or down-regulate the activity of the individual enzyme. This control of the activity ratio allowed to maintain a high flux in the cell catalyst in reactions with higher substrate concentrations, where a 3SL product titer of 44 g/L (70% yield based on 100 mM substrate) could be reached.

[1] L. Bode (2012), Glycobiology, 22(9), 1147-1162.
[2] S. Schelch (2021), Biotech. Bioeng., 118(11), 4290-4302.





# Science Flash **12**

## Sustainable fertilisers boosting the bio-economy

**Mikael Muegge**[1]
[1] RTDS Association, Vienna, Austria

SUSFERT develops sustainable, multifunctional fertilisers for phosphorus and iron supply. It combines bio-based and biodegradable coatings for controlled release, probiotics to increase nutrient availability, the renewable phosphorous source struvite, soil improvers, as well as “nutrigels".
The SUSFERT results lead to a number of key impacts including a decrease of dependency on mined phosphorus by 40%, the strengthening of the circular economy by valorising waste and by-products as well as a reduction of soil contamination by reducing fertiliser application frequency. In addition, SUSFERT demonstrates soil improvements and water holding capacity a well as controlled nutrient release through its novel “nutrigels”. It is ultimately demonstrating these innovative green technologies together in large-scale field trials and prepares data for registration dossiers for each product to speed up market entry.

www.susfert.eu

# Science Flash 13

## Modern data management in medicine: TBase

**W. Duettmann**[1, 2], V. Graf[1], M. Pfefferkorn[1], T. Gielsdorf[1], J. Bakker[1], D. Schmidt[1], K. Budde[1], B. Zukunft[1]

[1]Department of Nephrology and Medical Intensive Care, Charité - Universitätsmedizin Berlin, Berlin, Germany; [2]Berlin Institute of Health, Berlin, Germany

**Introduction:**
Clinical information system (CIS) are crucial for medical personnel, but had been developed without user input and build up data silos. The implementations of features including such as easy data extractions for data quality controls, science and case teaching or telemedicine are modern approaches for medical care. UI and UX has the potential to optimize care.

**Methods:**
The electronic patient record (EPR) TBase has been developing at Charité since 1980 for routine outpatient care. TBase still evolves and bases completely on the input of users including a section for data extraction and a telemedicine module. Basic design requests are

- One button = one feature
- "Slim design", no overload
- Quick adaption and implementation of feature requests
- Use of standardized tool (HL7 FHIR, PROMIS, Snomed CT)
- Standardization of data

The financing is still troublesome. TBase passed the privacy impact assessment and the application for Medical Product is in process.

**Results:**
More than 4500 long-term data profiles of patients is available and used for quality controls, science and case teaching. First big data analysis and prediction models were performed via certain access for (external) scientists. The telemedicine unit cares for more than 480 patients and is accepted well.

**Conclusion:**
TBase is a new approach to easy clinical day-life and improve patient care. To date, the software finds high acceptance is developed further. Implementation in other outpatient care centers and locations is ongoing.



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# Science Flash 14

## Super-fast oxidases and more: novel yeast enzymes and mechanisms of oxidative protein folding

**Arianna Palma**[1,2], Lloyd Ruddock[3], Brigitte Gasser[1,2]

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Oxidative folding in the endoplasmic reticulum (ER) has been long researched in model organisms such as yeast and mammalian cells. Yet, investigation on the kinetic and mechanistic aspects is still scarce. The present work dives into the biochemistry of disulfide bond formation in the methylotrophic yeast Pichia pastoris (syn Komagataella spp), an established host for recombinant protein secretion. Although being a budding yeast, P. pastoris is phylogenetically distant to the well-studied model Saccharomyces cerevisiae. In this study, we show that the assumption of similar organisms developing similar pathways is inaccurate. We describe uncharacterized and rare record-breaking oxidases. We illustrate human-like covalent complexation between the ER-oxidoreductase and disulfide isomerase. Finally, we propose novel molecular switches to ease the unfolded protein response.

# Science Flash **15**

## Mycotoxin inactivating enzymes as feed additives

**Daniel Incze**[1,2], Sofia Bata[1], Laszlo Poppe[2]

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Fumonisins are one of the most prevalent mycotoxins produced by certain Fusarium species, that contaminate crops globally, predominantly corn. The most important and prominent substance of the family is Fumonisin $B_1$ ($FB_1$). $FB_1$ inhibits ceramide synthase, an important enzyme of the sphingolipid biosynthesis in mammals. The altered sphingolipid metabolism can lead to different serious adverse health effects in humans and animals, therefore crops contaminated with high levels of $FB_1$ should be removed from the food or feed chain. Physical or chemical decontamination of crops are often not effective enough, however, enzymatic detoxification can be a solution to the problem. Fumonisin esterases cleave the two tricarballylic acid group of $FB_1$, leading to partially and fully hydrolysed $FB_1$ ($pHFB_1$ and $HFB_1$, respectively). In vitro studies suggested that these metabolites are significantly less toxic than $FB_1$. Fumonisin esterases are excellent candidates of food or feed additives against $FB_1$ contamination, however, kinetics and mechanism of these enzymes remain to be well characterized. The aim of the study was to understand the detailed enzymatic mechanism of two of these biocatalysts, and to fine tune an already existing product. For that reason, the enzymes were expressed in Pichia pastoris expression system, and kinetic constants were evaluated, with both $FB_1$ and $pHFB_1$ as substrate. It was found that fumonisin esterases selectively produce only one of the two possible $pHFB_1$, and that the two enzymes significantly differ in their affinity towards $pHFB_1$. We proposed a reaction mechanism based on our results, although further structure analyses are necessary.





# Science Flash **16**

## Wastewater based surveillance and analysis of SARS-CoV-2 in Styria

Thorsten Bachler[1], David Gradischnig[1], Daniel Schwendenwein[1], **Petra Heidinger**[1]

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As part of the national scale project termed “Schulstandmonitoring” of the Federal Ministry of Education, Science and Research, we analyzed samples from 24 wastewater (WW) treatment plants in Styria for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The whole project was carried out by multiple collaborators from different institutions around Austria and in total 122 WW treatment plants were sampled twice a week starting from 1st, September 2021 until 31st, August 2022. The coordination of the project was carried out by the University of Innsbruck under Heribert Insam. The aim was to establish the methodology for concentrating, isolating and quantifying the virus in our lab and the sample logistics to ensure an uninterrupted refrigeration chain and timely processing. The virus fragment concentration was then determined with RT-qPCR. In addition, total nitrogen along other population size markers were measured to normalize virus concentration for varying influx and differing population sizes in the catchment of the WW treatment plants. The relative virus load is then calculated in gene copies times million per capita per day and represents an apt parameter to map the course of the pandemic.

# Science Flash **17**

## Predicting high recombinant protein producer strains of *Pichia pastoris* MutS using the oxygen transfer rate as an indicator of metabolic burden

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The methylotrophic yeast Pichia pastoris (Komagataella phaffii) is a widely used host for recombinant protein production. In this study, a clonal library of P. pastoris MutS strains (S indicates slow methanol utilization) was screened for high green fluorescent protein (GFP) production. The expression cassette was under the control of the methanol inducible AOX promoter. The growth behavior was online-monitored in 48-well and 96-well microtiter plates by measuring the oxygen transfer rate (OTR). By comparing the different GFP producing strains, a correlation was established between the slope of the cumulative oxygen transfer during the methanol metabolization phase and the strain's production performance. The correlation corresponds to metabolic burden during methanol induction. The findings were validated using a pre-selected strain library (7 strains) of high, medium, and low GFP producers. For those strains, the gene copy number was determined via Whole Genome Sequencing. The results were consistent with the described OTR correlation. Additionally, a larger clone library (45 strains) was tested to validate the applicability of the proposed method. The results from this study suggest that the cumulative oxygen transfer can be used as a screening criterion for protein production performance that allows for a simple primary screening process, facilitating the preselection of high producing strains.



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# Science Flash **18**

## Methanol-free expression system PDH: a growth-decoupled alternative to classical *P. pastoris* promoters for recombinant protein production

**Núria Bernat Camps**[1,2], Miguel Angel Nieto-Taype[1], Jasmin E Fischer[3], Katharina Ebner[3], Francisco Valero[1,2], Anton Glieder[3], Xavier Garcia-Ortega[1,2]

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Finding novel and different-regulated expression systems for recombinant protein production (RPP) with Pichia pastoris is currently of great interest. In this work, a methanol-free expression system based on the P. pastoris heat-shock gene hsp12 promoter (PDH) was studied using the lipase B from C. antarctica (CalB) as model protein. This stress-related promoter is strongly activated under carbon-starving conditions, presenting hence a growth-decoupled regulation.

PDH regulating CalB expression exceeded the benchmark methanol-free expression system PGAP, when using a slow-release feeding technology in shake-flask cultivations. Moreover, osmotic shock conditions were applied in shake-flask to increase cell stress and further activate PDH regulation, resulting in slight increases in CalB production levels.

Furthermore, carbon-limiting fed-batches and three optimization rounds were carried out for PDH under different feeding and osmolarity strategies. In all of them, expression by the PDH outperformed the CalB titer achieved by PGAP, whose increases ranged from 1.5 to 7.8-fold. The best fed-batch approach consisted in a first biomass production phase to high cell densities, followed by an induction phase at a very low constant feeding rate. After 24h of induction, the cultivation presented a 3.4-fold increase and rather the same specific productivity than PGAP fed-batches at low and high μ, respectively.

To summarize, PDH is a regulated, methanol independent and growth-decoupled promoter that exhibited a higher efficiency than the commonly used PGAP benchmark. Thus, this novel expression system emerges as a potential alternative for P. pastoris RPP bioprocess, especially for complex and toxic recombinant proteins, which need regulated transcript levels.

# Science Flash **19**

## EnzyMAP AI – De-risking enzyme evolution with AI-augmented sequence-function data for the identification of the key point mutants across the entire enzyme sequence

**David Schönauer**[1], Eduardo Oliveira[1]

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Directed enzyme evolution has evolved towards increasing reliance on in silico designs and lessened dependence on expensive experimental screens. Now we are witnessing the emergence of in silico machine learning strategies with the promise to propel the field of enzyme evolution further, eventually reaching the goal of accessible de novo enzyme design.

Aminoverse seeks to fulfill that promise with its proprietary "EnzyMAP-AI". As a first step on this journey, EnzyMAP-AI predicts the performance of all point mutations across the entire enzyme sequence with >90% accuracy to cherry-pick for recombinatorial experiments thus generating enzymes with superior performance.

We collected and generated datasets with >150,000 mutants spanning diverse proteins and implemented a modern deep learning architecture that uses a wet-lab data input of 3-5 representative amino acids, combined with evolutionary and structural information, to predict the fitness of all 20 amino acid mutations in all positions of any given protein. This process reduces screening efforts and costs by 85% while providing complete structure-function knowledge of mutations in the target protein, proven by an internal oxidoreductase enzyme case study.

Compared to state-of-the-art deep learning models, EnzyMAP-AI achieves the same performance on the same datasets while requiring 4-times less data rendering it the fastest and most accurate approach to obtain holistic fitness-function data of the entire enzyme sequence that is universally applicable to any enzyme property and chained to real experimental data – presenting an ideal starting point to efficiently traverse the fitness landscape in downstream rational or ML-guided evolution through combinatorial libraries.





# Science Flash **20**

## Lactic acid from $CO_2$ using the synthetic autotroph *Komagataella phaffii*

**Michael Baumschabl**[1,2], Bernd M. Mititc[1,4], Christina Troyer[4], Özge Ata[1,2], Thomas Gassler[2,3], Diethard Mattanovich[1,2]

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The methylotrophic yeast Komagataella phaffii was recently converted to a synthetic autotroph by the integration of the Calvin Benson Bassham cycle. The key step here is the fixation of one molecule of $CO_2$ by the enzyme RuBisCO. We could already prove that this strain is capable of growing with $CO_2$ as a carbon source. The next step was to test the ability of this strain to produce organic acids.
We chose lactic acid as a product of choice. It is a hydroxycarboxylic acid used in the food, pharmaceutical, and chemical industry. Furthermore, it is the precursor of the biodegradable polymer poly-lactic acid (PLA). Lactic acid can be produced in Komagataella phaffii by the integration of a lactate dehydrogenase gene.
In this work, we assessed the lactic acid production in the autotrophic strain using $CO_2$ as a carbon source. This strain was able to produce up to 150 mg/L in approximately 200 hours of cultivation time. Titers were further improved up to 300 mg/L by deleting the CYB2, a gene involved in lactic acid consumption. Interestingly this knockout also led to the production of glycolate as a by-product. By strain engineering, we were able to omit the production of glycolate and remained at the same level of lactic acid production. Additionally, we compared the lactic acid consumption kinetics of the CYB2 knock-out strain compared to its parental strain. Finally, the incorporation of $CO_2$ into our product was proven by a C-labeling experiment.

# Science Flash **21**

## From $CO_2$ to L-methionine – engineering the pacemaker enzyme homoserine O-succinyltransferase

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L-methionine plays an important role in various physiological processes and is of importance for medicinal and pharmaceutical applications, but its conventional chemical synthesis relies on harsh chemicals such as methyl mercaptan, acrolein and hydrogen cyanide. In order to improve the sustainability of L-methionine production, we aim to develop an autotrophic bioprocess starting from $CO_2$, $O_2$ and $H_2$ with the chemolithoautotrophic bacterium Cupriavidus necator. Due to its versatile metabolism and ability to grow to a high cell density with minimal inclusion body formation, it is a promising organism in autotrophic biotechnology.
The regulation of the intracellular amino acid concentration in bacterial cells is complex and depends on multiple factors. To reach the highest possible yield of L-methionine, key enzymes of the biosynthetic pathway have to be targeted. A pacemaker enzyme of the L-methionine biosynthesis is the homoserine O-succinyltransferase MetA, catalyzing the formation of O-succinyl-L-homoserine.
We herein report the characterization and engineering of various MetA variants with the aim to increase the transferase activity of the enzyme. The MetA variants have been heterologously expressed in C. necator and the different strains were investigated for their ability to produce increased titres of L-methionine.



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# Science Flash **22**

## *Nicotiana benthamiana* as expression platform for proteins with targeted glycosylation

**Benjamin Kogelmann**[1,2], Stanislav Melnik[1,2], Michaela Bogner[2], Eva Stöger[2], Clemens Grünwald-Gruber[3], Herta Steinkellner[2]

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The demand of biopharmaceuticals with designed post translational modifications, like glycosylation, is constantly increasing. However, appropriate industry suited expression platforms are rare. Plants are increasingly being recognized as suitable expression system for proteins with complex glycosylation. The development of transient expression vectors, that allow harvesting of recombinant products 4-6 days post DNA construct delivery, has placed Nicotiana benthamiana (Nb), a tobacco related plant species, into focus. A series of studies demonstrate that Nb plants are well suited for glycan engineering towards human type structures [1].
Here we investigated a Nb-based glycosylation mutant that lacks plant specific xylose and fucose (FX-KO line, synthesizing so called GnGn structures), for their ability to engineer complex human type structures, including sialylation [2]. Various reporter proteins (e.g. different antibody isotypes) were transiently co-expressed along with foreign glycosylation enzymes. It is shown that FX-KO lines synthesize in a reliable manner targeted glycosylation, including sialylation. Such lines/approach may be used as a robust industrial platform for the generation of glyco-designed recombinant proteins. Designed proteins provide enormous economic benefits, as improper glycosylation generates products with reduced activities or serum half-life, as demonstrated for e.g. various antibodies, enzymes or hormones (e.g. EPO).

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# Science Flash **23**

## Characterizing the human immune response by proteomics aids the development of targeted biotherapeutics

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Mass spectrometry is a key technology for studying the proteins and their interactions that mediate different steps of the human immune response. Recent technological advancements enable the identification of the specific immunogenic epitopes generated and presented during various types of infections and diseases. The Institute Krems Bioanalytics (IKB) at the IMC University of Applied Sciences Krems offers a wide range of tailored analytical solutions for specific drug or vaccine development programs with a focus on the immunogenicity of biotherapeutics. Establishing an Orbitrap Eclipse Tribrid system, an exceptionally versatile and powerful mass spectrometer, at the IKB provides the basis for the long-term development of a technological platform that transfers cutting-edge research into diagnostic tools.

Our proteomics platform will allow a detailed characterization of immune responses at the molecular level like determining protein cleavage products of a pathogen, a tumor cell or a biotherapeutic that trigger an immune response. This will not only enable the identification of essential antigens for novel immunotherapies, but also significantly improve the classification of cancers by quantitative biomarker signatures.

A central goal will be the development of mass spectrometric technologies for the de novo sequencing of antibodies and for the analysis of the epitopes and affinities of antigen-antibody interactions. In contrast to established standard methods for recombinant antibodies in the pharmaceutical industry, these mass spectrometric approaches should enable the analysis of endogenous antibodies from blood samples. Characterizing the patient-specific antibody spectrum will provide the molecular basis for the development of tailored therapies up to personalized medicine.



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# Science Flash **24**

## Towards the rate limit of heterologous biotechnological reactions in recombinant cyanobacteria

**Giovanni Davide Barone**[1,2,3], Michal Hubacek[4], Lenny Malihan-Yap[1], Hanna C. Grimm[1], Lauri Nikkanen[4], Catarina C. Pacheco[2], Paula Tamagnini[2,3], Yagut Allahverdiyeva[4], Robert Kourist[1]

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Cyanobacteria have emerged as highly efficient organisms for the production of chemicals. Cyanobacterial photobiotransformations utilize photosynthetic electrons to form reducing equivalents such as NADPH to fuel biocatalytic reactions. These photobiotransformations are a measure to which extent photosynthetic electrons can be deviated towards heterologous biotechnological processes, such as the production of biofuels. Expressing oxidoreductases as YqjM from Bacillus subtilis in Synechocystis sp. PCC 6803, a high specific activity was obtained in the reduction of maleimides. We investigated the possibility to accelerate the NADPH-consuming redox reactions adding carbohydrates as exogenous carbon sources like D-Glucose (D-Glu) under light and darkness.

A 1.7-fold increase of activity (150 µmol min-1 gDCW-1) was observed upon addition of D-Glu at an OD750=2.5 (DCW=0.6 g L-1) in the biotransformation of 2-methylmaleimide. The stimulating effect of D-Glu was also observed at higher cell densities in light and dark conditions as well as in the reduction of other substrates. No increase in both effective photosynthetic yields of Photosystem II and Photosystem I was found upon D-Glu addition. We observed higher NADPH fluorescence when D-Glu was supplemented, suggesting increased glycolytic activity. Moreover, the system was scaled-up in an internally-illuminated Bubble Column Reactor exhibiting a 2.4-fold increase of specific activity under light-limited conditions.

The results show that under photoautotrophic conditions at a specific activity of 90 µmol min-1 gDCW-1, YqjM in Synechocystis is not NADPH saturated. This indicates that an increase of the rates of heterologous electron for catalysis and biofuel production will require funnelling further reducing power from the photosynthetic chain.

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# Poster No. 01

## $CO_2$-fixation by hydrogen oxidizing bacteria: simple lab-scale cultivation of *Cupriavidus necator*

**Vera Lambauer**[1], Regina Kratzer[2]

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Use of the greenhouse gas CO2 as a starting material for the production of chemicals and materials closes the carbon cycle. CO2, however, is a stable molecule and high energy is required to convert CO2 into more useful compounds. Aerobic, hydrogen oxidizing bacteria (Knallgas bacteria) are capable of efficient, non-phototrophic CO2 assimilation. They use H2 as electron donor and O2 as electron acceptor. The most prominent hydrogen-oxidizing bacteria, Cupriavidus necator, can accumulate polyhadroxyalkanoate as carbon storage to levels of up to 90% of the cell's dry weight. The metabolism is tractable by genetic engineering and alternative products become available. However, the development of new processes based on hydrogen-oxidizing bacteria is made difficult by the complexity of Knallgas fermentations. A gas mixture of H2, O2, and CO2 is the essential substrate for lithotrophic growth of hydrogen-oxidizing bacteria. H2 and O2 form explosive gas mixtures and both gases are badly soluble in the aqueous media. Here, we report on a reproducible, safe and simple solution to produce Cupriavidus necator on gram-scale. The presence of explosive gas mixtures demanded strict requirements regarding safety for reactor and process design i.e. construction measures in the lab and the building, specialized equipment and strict safety precautions. The Knallgas fermentations were performed in an explosion-proof bioreactor situated in a strong, ventilated hood. Cells grew under O2 control and H2 and CO2 excess. Dissolved O2 was measured on-line and was kept under a threshold value. Final biomass concentrations of 11.6 ± 1.0 gCDW/L were obtained.





# Poster No. 02

## Immobilized hexosaminidases in chemo-enzymatic preparation of α-anomers of synthetic enzyme substrates

**Helena Hronská**[1], Ema Ondrejková[1], Mária Bláhová[1], Vladimír tefuca[1], Michal Rosenberg[1]

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Synthetic enzyme substrates are powerful tools for diagnostic and research laboratories all over the world. They are widely used for early detection and identification of microorganisms, detection and quantification of enzymes as well as for studying cellular metabolism. Since glycosidases are widespread in all living organisms, they are also responsible for a large number of human diseases. Their substrates, glycosides, have an irreplaceable position among diagnostic agents. The main advantages of synthetic analogues of natural substrates are a) higher sensitivity, b)variability of the detection signal, c) rapid detection, and d) application mode (e.g. multi-test systems or culture media).
β-N-acetylhexosaminidases (EC 3.2.1.52, GH 20) are exo-glycosidases with a broad substrate specificity. Depending on the source, they can hydrolyse not only β-N-acetylgluco/galactoaminides but also β-glycosides[1].
We present here simple and convenient procedure for preparation of α-anomer of nitrophenyl galactoside derivatives using free and immobilized fungal β-N-acetylhexosaminidases. In our reaction system, α/β-substituted GalNAc derivatives were chemically synthetized and the β-substituted GalNAc derivatives were enzymatically hydrolyzed thus allowing for the easy isolation of α-anomers GalNAc derivatives. Immobilization of β-N-acetylhexosaminidase in lens-shaped polyvinyl alcohol hydrogel capsules provided a stable biocatalyst for more than 18 month long-time experiments and allowed its successful reuse in hydrolytic reactions [2].

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# Poster No. 03

## A systematic screening of Lactate oxidase on solid carriers

**Margherita Bruni**[1]

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Real-time blood sample analyses are important for health monitoring of patients in intensive cares. Lactate especially can be used to check the variations of oxygen in the body. Currently, the market offers devices able to measure lactate concentration off-line. A real-time lactate detector would improve health care quality.
To make it possible, an immobilized enzyme is needed.
Lactate oxidase from Aerococcus viridans was chosen for the purpose. It is a homotetramer of 146 kDa containing 4 FMN molecules and able to convert lactate to pyruvate using oxygen.
Our aim is to create an immobilized enzyme derivative with high activity and efficiency and to characterize it from a biochemical and a biophysical point of view. In literature, attempts of lactate oxidase immobilization can be found, but none of them seems to be suitable for the application. Since now, a systematic screening of solid carriers has never been carried out. We selected commercially available solid carriers made of hydrophobic and hydrophilic materials in combination with difference functional groups, i.e. epoxy, amine and azlactone, which can target lysine residues on the carrier surface. Additionally, immobilization by adsorption interaction was performed.
Our work shows great differences among the derivatives, but none of them can preserve more than 30 % of the free enzyme activity. Low efficiencies can be attributed to unpredicted specific orientation, rigidification, conformational changes or mass transfer limitation.
Adsorption and covalent strategies show comparable results, and stability studies are in progress.



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# Poster No. 04

## Bacteriophage derived expression enhancing tag yields powerful platform process for the production of recombinant fusion proteins

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Expression of complex recombinant proteins in E. coli is a major challenge in industrial biotechnology as the protein of interest is often produced with very low titres or misfolded as inclusion body. One possibility to circumvent these problems is the use of fusion partners. Unfortunately, fusion tags can vary greatly in their effectiveness, which is often dependent on the protein of interest. We found that a solubility tag originating from the gene 10 of the T7 bacteriophage can greatly increase recombinant protein titres of multiple relevant biopharmaceutical proteins. Due to its small size of only 22 amino acids, this fusion tag keeps the exerted additional stress on the cellular transcription machinery on a minimal level. The effectiveness of the fusion tag has been evaluated in carbon limited laboratory scale fed-batch fermentations. There, specific recombinant protein titres could be increased by a factor of greater than two compared to expression of the native protein. Since the fermentations were performed under identical conditions, this effect can be attributed solely to the fusion tag which was fused to the N-terminus of the proteins of interest. Furthermore, this fusion tag can be coupled with a pentapeptide cleavage site, resulting in highly efficient and specific cleavage by a human caspase-2 variant. The cleavage is largely independent of the N-terminal amino acid of the protein of interest, which makes this system universally applicable.

# Poster No. 05

## Sequential activation of multiple gene copies facilitates adaptation of cho cells to increased productivity

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A quick increase in productivity due to random gene amplification prevents high producers from adapting their transcriptome during cell line development. Hence, we present a molecular toolbox that enables a stepwise increase of gene copy numbers. Stable CHO cell lines containing four gene copies encoding GFP or BFP each fused to a fragment crystallizable region (Fc) were generated. Three of these genes are initially not expressed due to genetic repressor elements ("Rep"). CRISPR/Cas9-mediated deletion of the individual Reps enables specific activation of the corresponding gene. By successive activation and sorting, various CHO production clones and pools were obtained. Was used as control the same plasmid with all four gene copies active from the beginning. Our findings demonstrate that this molecular toolbox is suited to increase production loads in a stepwise manner. Intriguingly, we found that productivities only increase marginally from three to four active genes although a clear increase of intracellular fluorescence could be observed. This finding implies that a bottleneck in secretion occurs with increased productivity. Nevertheless, cell lines generated with successively activated gene copies achieved higher productivities compared to cell lines that were obtained by integration of four actively expressed gene copies. This suggests that stepwise activation of production load on the cells helps CHO cells to adapt to increasing production challenges. In summary, our study showcases how this toolbox can be employed to study the individual contribution of gene copy numbers and to eventually generate CHO production cell lines in a controlled and rational manner.



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# Poster No. 06

## Amino acid metabolism of Chinese Hamster Ovary cells: Comparison of growth and production phases in a fed-batch

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Metabolic pathways in Chinese Hamster Ovary (CHO) cells are sub optimally regulated regarding nutrient uptake rates, which, along with the excessive supply of amino acids (AA) in media, leads to the formation of by-products. Some of these by-products, such as ammonia, are toxic to cells, and their accumulation in the media negatively affects cell growth, productivity, and product quality. Here we use carbon-13 metabolic flux analysis (13C-MFA) in order to analyze AA metabolisms and to identify reformulated media compositions that reduce the formation of toxic by-products. Parental and mAb producer CHO-K1 cell lines were grown in a fed-batch culture where exponential and stationary phases were studied in detail. Comparing extracellular exchange rates for both cell lines, the producer generally showed moderately higher exchange rates for high-flux AAs and metabolites, with no considerable differences for low-flux AAs, in both phases. Comparing phases for both cell lines, major differences were observed between the exponential and stationary phases. Specifically, exchange rates significantly dropped for most measured metabolites and AAs, except for alanine and lactate, which switched to consumption, while rates remained constant for ammonia and some low-flux AAs. We observed higher glutamate consumption for the non-producer in the exponential phase which didn't coincide with higher glutamine consumption. We hypothesize that this results from the non-producer having lower flux from glutamate to alpha-ketoglutarate, which would cause glutamate accumulation. Alternatively, the producer utilizes glutamine in another pathway that doesn't produce glutamate. Currently, we are in the process of verifying our hypothesis with additional 13C experiments.

# Poster No. 07

## Media development for bovine muscle cells - a natural muscle growth cocktail

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As of now, one of the main limiting factors for bringing cultivated meat to the market is the high cost of cell culture medium. We are optimizing the medium, to lower its costs, and to raise its efficiency. For that, we are trying out growth factors and signaling molecules associated with muscle injury, and/or sport, and Hippo pathway inhibitors, all known to induce robust muscle growth in human or mice.





# Poster No. 08

## New enrichment method for proteins containing heparin binding domain

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Chitinase 3-like protein 1 (CHI3L1) is a non-enzymatic member of the glycoside hydrolase family 18 that belongs to a group of sport-induced myokines. Like many other cytokines, CHI3L1 is inherently unstable due to the presence of destabilising heparin binding domain, which is stabilised in vivo by its binding to heparin residues on the cell surface, but can also bind other polysaccharides, e.g., chitin and hyaluronic acid. rbCHI3L1 serves as an example that secretion of proteins with a heparin binding domain could be hampered by their binding to chitin in the cell wall of the yeast host. This, on the other hand, provides a convenient way to enrich such a protein in the yeast cell wall, which is also stabilised by binding to chitin and, subsequently, to wash it out in a small volume of buffer.

# Poster No. 09

## Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions

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Analysis and elucidation of protein mass transfer mechanisms is beneficial for the understanding of the fundamentals of chromatography-based separation processes. In this work we examine the intraparticle diffusion processes of human fibroblast factor 2 (hFGF2) in a crude and purified feed solution. Capto S, which is a grafted agarose-based cation exchanger is used as the stationary phase. In general, the properties of the grafted media enable an enhanced hFGF2 mass transfer facilitated by interactions of the protein with surface extenders of the media. Even faster overall effective diffusion rates compared to the free diffusivity of hFGF2 are observed. Macroscopic protein adsorption methods such shallow bed and batch uptake reveal a 40-fold decrease of the overall effective pore diffusivity Ḋe for hFGF2 in crude solution. The difference in diffusion processes is mainly caused by a decreased binding capacity as well as an increased dynamic viscosity induced by higher dsDNA and membrane lipid concentrations. Emulating the dynamic viscosity of the crude solution by adding specific amounts of glycerol to purified hFGF2 confirms the impact on the internal protein mass transfer. Moreover, confocal light scanning microscopy Investigation substantiate the slower intraparticle mass transfer rate of hFGF2 in crude solution compared to purified hFGF2.



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# Poster No. 10

## Conversion of $CO_2$ into organic acids by synthetic autotrophic yeast

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One of the biggest challenges humankind is facing these days is the climate crisis.One clear cause is the increasing atmospheric CO2 level due to human activity.Anthropogenic CO2 emission is more than can be captured by plants and microorganisms which consequently causes an imbalance in the carbon cycle.Towards a more sustainable future,we need to restore this balance.
To address this problem,we aim to enable microbial assimilation of CO2 as a carbon sink by converting it into value-added, bio-based polymers.Previously,Komagataella phaffii was converted from a methylotroph into an autotroph that can grow solely on CO2 for biomass formation while using methanol to harvest energy.In this work,we further engineer this CO2 fixing K. phaffii and use it as a platform to produce value-added organic molecules.Using synthetic biology tools and CRISPR-Cas9,we generated an autotrophic K. phaffii strain that can produce itaconic acid by fixing CO2. Balancing the synthetic itaconic acid metabolism,identifying and engineering targets in the central carbon pathway and optimizing the process parameters resulted in a final titer of 2 g/L itaconic acid in flasks. 13C-labelling experiments confirmed the incorporation of the captured CO2 into itaconic acid.We also investigated the itaconic acid production performance of the CO2-fixing K. phaffii strain in lab-scale bioreactors.To demonstrate broader applicability,the same metabolic engineering strategy was also applied to the production of lactic acid. In the light of our results,we show that the synthetic autotrophic yeast K. phaffii can be a platform for the production of value-added chemicals by the microbial conversion of CO2 for a sustainable bioprocess.

# Poster No. 11

## Protein production from carbon dioxide

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Due to the massive release of carbon dioxide into the atmosphere new technologies are required in order to provide solutions for the recycling of it into bound carbon products. One interesting product is protein for food and feed purposes. Here not only the carbon dioxide problem is an issue but also the exhausted resources of cultivable land and fish, which presently are the main basis for protein supply.
An interesting alternative for providing feed and food protein is production of single cell protein by microbes. Chemolithoautotrophic bacteria are able to use carbon dioxide as their sole carbon source. They gain the needed energy for carbon fixation by oxidation of hydrogen. This project is based on use of the bacterium Cupriavidus necator which is excellently characterized and which has already been used in large scale bioprocesses based on organic carbon sources. For autotrophic growth on carbon dioxide no large-scale processes have been developed so far, only data on cultivation in small scale lab bioreactors have been reported. A very important issue for autotrophic cultivation is realization of a high gas to liquid transfer rate, especially as hydrogen has a very low solubility in aqueous solutions. Econutri has therefore developed a specific reactor design for high gas transfer rates based on a special mixing system and application of elevated operation pressure. A bioreactor at pilot scale of 300L is already constructed and is used to produce single cell protein under lithoautotrophic growth conditions.





# Poster No. 12

## Enzymatic polycondensations in novel bio-based solvents for greener synthesis

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Polyesters are useful materials with a wide range of applications, but there is increasing concern over their sustainability. These polymers can be synthesised enzymatically, which is a sustainable alternative to traditional chemical catalysis. Despite being considered green catalysts, enzymatic transformations are still performed in organic solvents with serious environmental issues such as diphenyl ether (DPE)1. As DPE is a petroleum-based solvent that is toxic to aquatic life and classed as highly hazardous, new solvents need to be investigated to replace it. Cyrene is a bio-based dipolar aprotic solvent that has been used as an NMP substitute2, and has recently seen success as a solvent in polyester synthesis3. In this work, Cyrene and several derivatives (dioxolane Cygnet 0.0 and dioxepane Cygnet) were tested in the polycondensation of adipic acid and either 1,4-butanediol or 1,8-octanediol, using the biocatalyst Candida antarctica lipase B (CaLB). To make the entire process more sustainable, it was a secondary aim to optimise the reaction and substitute the methanol and chloroform utilized in the workup procedure with greener alternatives. This was successful; chloroform and methanol were effectively substituted for Cyrene and water, respectively. Dioxolane Cygnet was found to be the preferred solvent, giving polymers with a Mn of >22 KDa. Finally, these solvents were used to polymerize several other bio-based monomers, showing their versatility in the production of bio-based polymers.

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# Poster No. 13

## Characterisation of engineered *Cupriavidus necator* strains for isopropanol production from $CO_2$ in shake flask cultivation

**Isabell Weickardt**[1], Eric Lombard[1], Lars Blank[2], Stéphane Guillouet[1]

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Isopropanol is a platform chemical easily converted to acrylic glass or polypropylene and is used as a solvent and disinfectant. With 2.4 million tons of isopropanol produced worldwide each year from fossil resources, resulting in toxic waste and greenhouse gas emissions, alternative production processes are needed.
One possibility is the utilization of the knallgas bacterium Cupriavidus necator, known as a model organism for PHB production. It can grow on carbon dioxide which may stem from industrial gas effluents or waste gasification.
The engineering of C. necator for isopropanol production has been published. However, comparing different genetic modification approaches under small-scale autotrophic conditions is complicated by safety concerns when working with mixtures of explosive gas, and the low transfer rate of gaseous substrates.
Here, a simple and straightforward screening method on shake flask scale under aerobic, autotrophic conditions was developed. Analysis of growth, product formation and gas consumption allowed the comparison of previously published strains designed for isopropanol production in heterotrophy. The data so far suggest different product formation kinetics under autotrophic conditions compared to the available data for heterotrophic cultivation.
This work highlights the utility of shake flasks for rapid strain screening under autotrophic conditions and provides insight into isopropanol formation by C. necator from carbon dioxide, offering a solid basis for bioreactor-scale process transfer.



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# Poster No. 14

## SLAM-seq reveals early transcriptomic adaptation mechanisms upon glutamine deprivation in chinese hamster ovary cells

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During biopharmaceutical production processes, mammalian cell cultures frequently experience environmental perturbations. To compensate, cells typically preserve metabolic resources by decreasing growth and/or productivity, therefore impacting the bioprocess and/or bioproduct quality. Thus, knowing the genetic factors that govern cellular stress response(s) can facilitate targeted genetic engineering to obtain production cell lines that demonstrate a higher stress tolerance. Here, we simulated nutrient deprivation stress by transferring Chinese Hamster Ovary (CHO) cells into a glutamine-free medium and investigated transcriptional dynamics using thiol(SH)-linked alkylation for the metabolic sequencing of RNA (SLAM-seq) along with standard RNA-seq of stressed and unstressed cells. In SLAM-seq, cells were labelled with 4-Thiouridine promptly after perturbation, followed by a step of alkylation and sequencing. The method allows the differentiation between actively transcribed, nascent mRNA and total mRNA in the sample, adding an additional, time-resolved layer to classic RNA-sequencing. Early transcriptomic changes in the first hours after glutamine deprivation are reflected in the enrichment of Gene Ontology terms involved in Organic Substance Metabolic Process and Cellular Response to Stress. The cells tackle amino acid limitation by Atf4 overexpression, leading to subsequent activation of its targets, namely Asns, Slc7a11 and Trib3. As the differential expression in Atf4 was active for less than 24h, it would likely have been missed in standard experimental designs from RNA-seq. Our results describe the successful establishment of SLAM-seq in CHO cells and therefore facilitate its future use in other scenarios where dynamic transcriptome profiling in CHO cells is essential.

# Poster No. 15

## Fatty-acids esters production in the Knallgas bacterium *Cupriavidus necator*

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The main goal of the EU-funded H2020 project ConCO2rde (Grant No: 955740) is to develop high-performance autotrophic cell factories and optimise gas fermentation processes to produce bio-based chemicals and pharmaceuticals. In this frame, we focus on the metabolic engineering of the Knallgas bacterium Cupriavidus necator for efficient gas feedstock conversion. We aim to develop highly efficient, autotrophy-driven OH-fatty acid esters (hydroxyalkanoyloxy-alkanoic acids: HAAs) producing strains.

A computational approach was used to study the metabolism of C. necator. A Genome-Scale model of the organism was employed to perform different simulations to find possible metabolic engineering strategies for improved production of fatty acids, precursors of HAAs, in heterotrophic, mixotrophic and autotrophic growth conditions. The Phenotype Microarray technology based on Biolog 96-well plates was exploited to assess the ability of C. necator to utilise different carbon sources. The data obtained from this experiment were used to refine the model further.

An ester-forming enzyme, encoded by the gene rhlA from Pseudomonas aeruginosa, was expressed both in wild-type and in a C. necator strain with a deletion in the polyhydroxybutyrate (PHB) synthesis pathway to channel the excess acetyl-CoA towards the production of the targeted fatty acid esters. Two strategies were used to express the gene of interest: a genomic integration by homologous recombination guided by the Tn7 transposon and a multi-copy plasmid vector bearing the gene.

Additionally, the ability of the wild-type strain and PHB synthesis deletion strain to metabolise HAAs as a carbon source will be tested in a minimal medium.



# Poster No. 16

## Metabolic engineering of *Komagataella phaffii* for succinic acid production

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Succinic acid (SA), an intermediate of TCA cycle, is considered as one of the most important platform chemicals with a wide range of applications, including pharmaceuticals, food and beverages, cosmetics and feedstock for industrial chemicals. Especially, bio-based SA production is in demand as it offers an ecological sustainable alternative to the traditional petrochemical production process. Although bacteria naturally produce high SA titers, they are considered as sub-optimal producing hosts as they are sensitive to low pH, cost-expensive in downstream processing and prone to bacteriophage infections. Therefore, yeasts and fungi are increasingly engineered for SA production. In this study we report, that deletion of the SDH2, encoding for a subunit of the succinate dehydrogenase complex leads to SA accumulation when glucose and glycerol is used as a carbon source in Komagataella phaffii. Titers of 1.2 and 0.6 g/L were achieved in shake flask cultivations using glucose and glycerol respectively. While further genetic engineering of ICL1 and MLS1 did not improve SA production, the heterologous expression of the dicarboxylic transporter DCT-02 from Aspergillus niger helped to boost succinic acid levels up to 3 and 5 g/L on glucose and glycerol, respectively. Here, different promotor strengths were tested to find best conditions for succinate export.

# Poster No. 17

## Generation and evaluation of *Aspergillus*-specific DNA aptamers to improve diagnosis of aspergillosis

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The rapid technological, climatic and demographic changes characterizing the last few decades are exerting drastic effects on our planet. In this scenario, the occurrence of infectious diseases, including fungal infections, appears to increase steadily. Aspergillosis, caused by Aspergillus fungal species, poses a particular threat as it is estimated to affect millions of people every year. The high mortality rate of this infection is often associated with delayed diagnosis.
This project aims at the development and evaluation of DNA aptamers recognizing Aspergillus cells which can be implemented into a novel diagnostic tool for the detection of aspergillosis.
In order to select DNA aptamers which specifically recognize conidia and hyphae of Aspergillus species causing aspergillosis, a SELEX approach previously developed for bacteria [1] will be adapted for fungal cells (e.g. A. niger). The establishment of this SELEX pipeline includes setting up a robust PCR procedure to amplify ssDNA aptamers bound to fungal cells and an evaluation method to characterize the newly selected aptamers.
In order to establish a method to evaluate the aptamer binding capabilities, fluorescently labelled versions of previously developed aptamers [2] were used. Upon incubation with A. niger cells, the binding of the aptamers to the target cells was evaluated by means of fluorescence measurements and epifluorescence microscopy. The aptamer evaluation method established here will be applied to new aptamers obtained by the SELEX procedure.

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# Poster No. 18

## Unraveling the selectivity of borneol dehydrogenases based on ancestral reconstruction

**Jasmin Zuson**[1], Andrea Magdalena Chánique[1,3], Katarína Kavčiaková[1,2], Bernhard Loll[4], Daniel Kracher[1], Robert Kourist[1]

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The high selectivity of enzymes enables the design of promising biocatalyst-based synthesis routes for enantiopure compounds instead of relying on chemical synthesis or extraction processes. Currently bicyclical monoterpenols borneol and camphor are produced either as racemate through chemical synthesis from α-pinene in waste streams of turpentine production or extracted from plants in enantiopure form [1, 4]. The capacity of plants to synthesize enantiopure (+)-camphor has been attributed to the selective oxidation of (+)-borneol by borneol dehydrogenases (BDHs), first discovered in leaf homogenate of Salvia officinalis [2, 3].

Selective BDHs could be utilized to synthesize optical pure camphor, borneol or isoborneol from industrial waste streams containing α-pinene [4]. Previously, the first enantiospecific BDHs from Salvia rosmarinus have been recombinantly produced, characterized and crystallized (SrBDH1; PDB ID: 6ZYZ)[2]. The close relationship of these enzymes to unspecific BDHs suggests that BDHs from plants share a common non-selective ancestor [2]. Through ancestral reconstruction, characterization, modeling and rational design, the development and evolutionary history of enantioselectivity in SrBDH2 should be retraced. In this work, novel insight into the enantioselectivity of BDHs is gained by reconstruction of a non-selective ancestor to the (+)-borneol specific SrBDH2 and by tracing the emergence of selectivity via a natural evolutionary path.

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[4] Drienovská I., et al. (2020). Phytochemistry, Volume 172, 112227, ISSN 0031-9422.

# Poster No. 19

## Biocatalyst activity and stability in the presence of organic co-solvents

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After establishing a biotransformation on laboratory scale the addition of organic co-solvent during process intensification might be needed. Poor substrate solubility or needs to simplify downstream processing might require the solvent as additive. Consequently, the biocatalyst is exposed to solvents which might reduce its activity or even lead to a complete inactivation.
We aim to decipher the solvent resistance of different biocatalysts towards different solvents. To achieve this, we combine experimental evaluation of stability with computational methods. To have a good dataset for the computational analysis we have identified a representative set of ene-reductases for which solvent stability was assessed. We have measured the thermal stability in the presence of co-solvents and the influence of the co-solvents on the initial activity.
The addition of the tested organic solvents led to a decreased melting. The degree of destabilization seems to be dependent on the solvent type and concentration while the activity under the same conditions behaves different. In some cases the addition of the co-solvent led to an increase in the initial activity while it led to a decrease for other ene-reductases or in combination with other co-solvents. Thus, a thermal destabilization does not necessarily go together with a reduction of the initial activity.

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# Poster No. 20

## Lignocellulosic biomasses as bioresources in central europe

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In a bid to replace fossil resources by renewable raw materials, the utilization of lignocellulosic biomasses as starting materials for bioconversions to value added products was investigated. Flexibility in raw materials is a major characteristic of a phase III biorefinery where a range of different raw materials and processes are utilized. Wheat straw, miscanthus, beech, pine and spruce which were sourced from Europe were used as starting materials. The goal was to have an optimized biomass-to-sugar process with integrated unit operations. Pretreatment (steam explosion), enzymatic hydrolysis as well as filtration are the unit operations that were involved. Moreover, the conditions for steam explosion were optimized for each biomass (temperature, pH). The enzymatic hydrolysis was carried out using the enzyme cocktail from Novozymes (Cellic3®Tec3 HS). In addition, the released sugars and byproducts were analyzed using high performance liquid chromatography (HPLC). With a vacuum filtration set-up, the filterability of the biomasses was studied and the mass balances calculated. Furthermore, the effect of pretreatment conditions on filterability and sugar release was investigated. The efficiency of the whole process was determined by the degree of saccharification and the filterability.

# Poster No. 21

## Optimizing carbon catabolism for improved organic acid production in *Aspergillus niger*

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Aspergillus niger is the leading workhorse for the production of citric acid, an organic acid, that is extensively used in food and pharmaceutical processes. However, the carbon yield of this acetyl-CoA derived product is capped to 67%. The decarboxylation of pyruvate, the precursor of acetyl-CoA, loses a carbon equivalent in form of a $CO_2$ molecule. In addition, another enzymatic step in the oxidative pentose phosphate pathway requires the emission of $CO_2$ when the metabolite ribulose-5-phosphate is formed.
We aim to design a carbon conserving pathway and increase acetyl-CoA alternatively to achieve a more carbon efficient and sustainable production of the target metabolite citric acid. Therefore, the enzymatic step that connects glycolysis and the pentose phosphate pathway is engineered and downregulated to avoid excessive $CO_2$ emissions. On the other hand, putative phosphoketolases that convert sugars to acetyl phosphate, the precursor of acetyl-CoA, are screened for their enzymatic activity on diverse sugar substrates.
The hypothesis to be tested is that by combining the downregulation of the pentose phosphate pathway as well as the overexpression of an acetyl phosphate generating phosphoketolase leads to a reduction of $CO_2$ loss and hence, a more carbon efficient biocatalyis of citric acid in A. niger.



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# Poster No. 22

## Determination of enantioselectivity in plant-derived borneol dehydrogenases

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Borneol and camphor are monoterpenes used in cosmetics, fragrances, or medicine. Their pure enantiomers are primarily obtained from plants through an elaborate extraction process [1], whereas racemic mixtures are by-products of pine-tree processing [2]. Utilizing enzymes to produce pure enantiomers from waste streams would pose economical and environmental benefits. Borneol dehydrogenases (BDHs) typically show high enantioselectivity towards (+)-borneol, making them suitable for the task. However, some of the BDHs are non-enantioselective [3].
There are several approaches to determine the enantioselectivity of enzymes. A widely used method is the kinetic resolution of racemates, but since some BDHs show substrate inhibition, this strategy is not applicable. Another approach is determining kinetic constants for pure enantiomers to calculate an enantiomeric ratio. The equation utilizes KM and kcat [4]. Here the quantification is troublesome since BDHs tend to have high KM values and show low Ki and substrate solubility limits the experiments. Therefore this work looks into alternative ways to assess enantioselectivity. The determination is based on the Quick-E method by Kazlauskas [5] in comparison to the reaction rates of enantiopure substrates to characterize a set of BDHs derived from Salvia species.

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# Poster No. 23

## Biotechnological recycling of textile waste blends by simultaneous saccharification and fermentation

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There is urgent need to develop recycling concepts for textile waste since only less than 1 % is recycled into new clothes whereas 87 % is still landfilled or incinerated. Textile waste usually consists of blended fibers comprising natural fibers with synthetic ones which makes recycling challenging. Biotechnological approaches enable the specific separation of fiber blends by the application of enzymes. Cellulases depolymerize natural cellulosic fibers such as cotton or viscose to glucose leaving synthetic fibers such as polyester for direct reutilization in textile production. Recovered glucose can be used as a substrate in microbial fermentation processes to further valorize this building block. To reduce the processing steps and additionally save costs, resources and energy, the concept of Simultaneous Saccharification and Fermentation (SSF) is introduced into the biotechnological recycling process of blended textile waste. Therefore, the bacterial organism Weizmannia coagulans, that grows under the enzymatic hydrolysis process conditions, was integrated in the fiber blend separation reaction. The released glucose can directly be used by the bacterium which produces lactic acid (LA). This fermentation product can further serve as a building block for production of the biopolymer polylactic acid (PLA) and is recovered together with pure PET from the same process thereby increasing the circular application of valuable raw materials.



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# Poster No. 24

## Towards electro-driven N-heterocycle synthesis by recombinant *Rhodopseudomonas palustris* TIE-1

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In order to avert the most severe consequences of climate change, our society needs to develop more solutions to achieve reduction in greenhouse gas emissions. Changing conventional industrial processes into more sustainable alternatives will contribute significantly to achieve a more sustainable society. The bacterium Rhodopseudomonas palustris TIE-1 can offer a sustainable alternative towards fine chemical synthesis. R. palustris is able to grow photoautotrophically on $CO_2$ when it harnesses the reducing power of $H_2$ or electrons from a cathode.[1,2] Combining this with N-heterocycle synthesis, which are important precursors for pharmaceuticals, offers a sustainable approach for a sustainable fine chemical production.[3] In this study, an imine reductase (IRED) that catalyzes the synthesis of N-heterocycles from dicarbonyls and diamines[4] was heterologously produced in R. palustris via an expression plasmid. The production levels and activity of IRED in recombinant R. palustris are examined. However, integration of IRED genes in the genome will be the ultimate goal to improve production stability and eliminate the need for antibiotic addition to the culture medium. This study provides a first step towards a more sustainable N-heterocycle synthesis while also paving the way to a potential platform for more sustainable fine chemical production.

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# Poster No. 25

## Enzymatic dynamic kinetic resolution and ex-cell anodic oxidation for Levetiracetam synthesis

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Levetiracetam is an active pharmaceutical ingredient widely used as a medication for epilepsy. Synthetic routes to this chiral amino amide are low yielding and wasteful. We designed a short and highly efficient route to levetiracetam. Herein we describe the enantioselective preparation of the levetiracetam precursor molecule 2-(pyrrolidine-1-yl)butaneamide. The cobalt-dependent thermotolerant nitrile hydratase from Comamonas testosteroni (CtNHase) was engineered by directed evolution. We tailored a high throughput screening method that allowed to distinguish clones with improved (S)-selectivity or activity on colony level and screened more than 50,000 clones. Key positions were identified and combined, delivering a set of highly S-selective CtNHase variants. Racemic nitrile was applied in a fed-batch reaction to a final concentration of 167 mM and converted to the desired (S)-amide in a dynamic kinetic resolution (Fig. 1). The amide was obtained in 73% conversion and 95.2% enantiomeric excess. For the final oxidation to levetiracetam, a ligand-free ruthenium-catalysed method at a low catalyst loading was used. The oxidant was electrochemically generated in 86% yield [1].
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# Poster No. 26

## Extending the toolbox for biocatalytic aldehyde synthesis

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Aldehydes are reactive compounds and can undergo chemical transformations to numerous other functional groups. The aldehyde is an invaluable chemical handle to make all sorts of products. Aldehydes find application in the flavor and fragrance sector, because they are often volatile with characteristic olfactory properties. Nature is equipped with a portfolio of proteins with enzymatic activity to transform various precursor molecules to the respective aldehydes. Herein we focus on alkene cleavage by aromatic dioxygenase (ADO) and lipoxygenases (LOX)/hydroperoxide lyases (HPL) as well as carboxylic acid reductase (CAR).
CARs are capable to generate a broad variety of aldehydes from corresponding carboxylic acids.[1] Herein, we present novel CARs which were identified by database mining. We focused on cell free enzymatic synthesis [2] and present first results for the secretive production of CARs in P. pastoris. We used the same yeast to access volatile short-chain aldehydes through an enzymatic cascade (LOX/HPL) from unsaturated fatty acids [3]. Moreover, we study the enzymatic equivalent of ozonolysis to give carbonyl compounds like vanillin from precursors with C=C double bonds like isoeugenol. A new ADO from a marine fungus was produced in E. coli and characterized.

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# Poster No. 27

## Flavour of cheese, wine and durian: synthesis of odorous α-hydroxy pentanones with carboligases

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3-Hydroxy-2-pentanone (flavour: herbaceous, truffle; odour: caramel-sweet, buttery) and its isomer, 2-hydroxy-3-pentanone (flavour: truffle, peanut; odour: buttery, hay-like), are volatile acyloins that have been identified as flavour components of cheese, wine, durian, honey, butter, soy sauce, sherry and other foods. Acyloins are typical products of the enzymatic acyloin-type condensation reaction catalysed by thiamine diphosphate (ThDP)-dependent carboligases. The reaction usually includes a decarboxylation of an α-keto acid and its subsequent ligation to an aldehyde, leading to acyloins. By choosing complementary substrates the reaction can be directed towards one or the other isomer: pyruvate and propanal condense to 3-hydroxypentane-2-one, whereas 2-oxobutyric acid and acetaldehyde give the 2-hydroxypentan-3-one. Herein, we tested a panel of carboligases for the desired reactions reaching up to 90% product formation.

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# Poster No. 28

## *Pichia pastoris* as a producer of carboxylic acid reductases

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Carboxylic acid reductases (CARs) catalyze the reduction of carboxylic acids to aldehydes. CARs have been studied quite well in the bacterial host Escherichia coli, but little is known about the potential of the yeast Komagataella phaffi (P. pastoris) as a producer of CARs. Hence, the CAR and its activator protein (phosphopantetheinyl transferase, PPTase) from the original host Mycobacterium marinum were co-expressed, using a bidirectional promoters. Screening of several promoter variants and hundreds of integration variants revealed a MmCAR_PGAP_PTEF_MmPPTase strain as the most promising strain for intracellular production of the active MmCAR [1] and the use of the living-cell yeast biocatalyst for biotransformations. P. pastoris achieved 1.4 times higher space-time yields as E. coli for the preparation of piperonal.

P. pastoris has the ability for extracellular secretion of proteins, however, CARs are 130 kDa proteins and are hence a rather difficult target. Herein, we describe results of both, intra- and extracellular production of MmCAR [2].

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# Poster No. 29

## Accelerating the optimization of biocatalytic cascades

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The transfer of biocatalytic cascades from a laboratory scale to its industrial applications requires fine tuning of the reaction parameters. However, the high number of variables, concentrations of the different components and non-linear and unpredictable interactions leave the scientist with a difficult problem to solve. The most followed approach that optimizes singularly each enzymatic step does not consider these complex interactions present when all biocatalysts share the same reaction vessel. Therefore, with the implementation of more and more biocatalysis by the industry, new strategies towards a fast, efficient and generalizable optimization of enzymatic cascades are needed.
We took inspiration from previous active learning workflows developed by Faulon and Erb [1] which demonstrated to be highly efficient for solving complex optimization problems, for instance the improvement of the CETCH cycle. We applied it on a model cascade previously developed in our laboratory, seeing good improvement of the process [2].
We introduced some modifications, in the active machine learning workflow, necessary for an application on biocatalytic cascades to maximize the product formation and minimize the biocatalysts loading. We expanded the use of this tool for a machine learning driven biocatalyst selection, namely by providing different biocatalysts options, the most suitable combination is chosen. Moreover, we are currently implementing different sampling methodologies and multiple target variables on the algorithm that may allow a faster and more aimed improvement rate.

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# Poster No. 30

## Towards a comprehensive CRISPR-Cas9 deletion screen strategy using a paired guide RNA approach

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CRISPR-Cas9 is a powerful and versatile genome editing tool that provides a potent strategy to gather in-depth genetic information and engineer cell lines with specific genotypic and phenotypic requirements. In our study, we exploit the efficiency and flexibility of the CRISPR system by employing a paired guide RNA (pgRNA) strategy to ensure a complete functional knockout of selected genes of interest within the Chinese Hamster Ovary (CHO) genome.

To test the functionality of our approach in a pooled format, we simulated a small-scale deletion, where selected pgRNA sequences covering a broad range of deletion sizes, spanning from 12 to 106 kbps, were co-transfected with a Cas9-encoding plasmid into a CHO K1 cell line to review individual deletions. In all cases, deletion PCR conducted on genomic DNA clearly showed that deletions were introduced at the Cas9 targeted sites. Furthermore, mRNA expression surveyed by qRT-PCR also shows a decrease in gene expression, even at the pool level.

In conclusion, we successfully showed that the different design decisions employed for the pgRNA expression cassette resulted in precise and expected deletions. Overall, this study sets an essential baseline required for setting up functional genome-wide deletion screens and could be extended for similar applications within the larger scope of industrial biotechnology.

# Poster No. 31

## A rational approach to pooled CRISPR screens for the identification of essential genomic targets in CHO cells

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Pooled CRISPR screens are typically conducted using viral transduction to deliver a library of guide RNAs into each cell. On the contrary, site-specific recombinase-mediated cassette exchange (RMCE), offers a more precise system and is regarded as the most suitable virus-free alternative to obtain a cell pool, where one unique gRNA is stably integrated per cell. In large-scale screen, this advantage leads to a homogeneous genetic background, which reduces clonal variability compared to random viral integration.

Here, we exploited the potential of CRISPR-Cas9 in combination with an RMCE platform for the optimisation of a CRISPR-mediated screening approach to perform full deletions of genes in CHO cells. An in-silico pipeline for the computational design of paired guide RNAs (pgRNAs) was established to generate a library targeting a set of essential and non-essential genes. By using this approach, we were able to remove most of the bias that occurs when single gRNAs are used, allowing for the complete deletion up to 150 kb. Upon transfection of the pgRNA library, RMCE was performed to generate a cell pool with stable integration of one pgRNA per cell. Positive cell population underwent a phenotypic screen. By comparing pgRNA abundance at the end of the screen, data led to the identification of depleted candidates that will be subsequently validated.

In conclusion, a small-scale CRISPR-Cas9 deletion screen based on a viral-free delivery system was established. The genomic deletion of candidate genes from a CHO-specific library led to the identification of potential essential genomic targets for future biotechnological applications.



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# Poster No. 32

## Comprehensive meta-analysis of the cho coding transcriptome

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Chinese hamster ovary (CHO) cells are amongst the most important cell factories in biotechnology. Capable of incorporating complex post-translational modifications, they are invaluable in the production of biopharmaceuticals. They are steadily developed to improve yield and product quality through cellular engineering and by optimizing bioprocesses. High-throughput omics technologies have drastically influenced these endeavours by enabling a comprehensive molecular insight at multiple levels, leading to more rational and informed engineering decisions. Oftentimes, such datasets are created to elucidate a specific biological question and the potential to put individual datasets into greater context is left unused. With numerous RNA-sequencing datasets accumulated, we aim to seize the opportunity to conduct a large-scale meta-analysis of the CHO transcriptome to study gene expression across various cell lines and cell culture conditions. Starting from raw reads in FASTQ format, we consistently process all datasets through a reproducible workflow incorporating state-of-the-art bioinformatic tools. RNA-seq reads are mapped to the most recent reference genome assembly of the Chinese Hamster. Our approach furthermore involves novel batch adjustment techniques and sophisticated normalization methods in order to address the challenges arising from inhomogeneous data. Custom-developed R scripts assess gene expression and correlation network across several biological conditions of various cell lines. Our meta-analysis of RNA-seq data aims to elucidate the complex circuitry of the CHO transcriptome and is able to reveal transcriptomic programmes that cohere with industrially relevant phenotypes and/or differences that are specific to culture conditions or recombinant cell lines.

# Poster No. 33

## On the way: Establishing CRISPR/Cas as genome editing tool in *Cupriavidus necator* H16

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Due to the increasing public awareness of climate change , the gram-negative bacterium Cupriavidus necator is one of the rising stars in modern biotechnology. Its versatile metabolism allows to utilize CO2 fixation in the presence of H2 for the production of proteins for food and feed. Yet, the broad application of C. necator is currently limited by a small molecular biology toolkit, and, consequently, a lack of a more detailed genetic understanding. Currently available genome-editing tools rely on homologous recombination, which is notoriously inefficient in bacteria, and laborious intron-based methods. Therefore, genome editing by CRISPR/Cas would be a great alternative. A first attempt to establish CRISPR/Cas in C. necator has been published before, but the reported method never found broad application. By constructing a new, more efficient and flexible vector system we are aiming to establish CRISPR/Cas as first choice for genome editing in C. necator. Here we show that Cas9 can be efficiently expressed from a small, vector that can easily be electroporated. First testings in an eGFP expressing model strain showed a significant decrease in viability, indicating functional expression of Cas9 and the protospacer targeting the GFP locus in C. necator. Further work, e.g. to support the repair of the DNA double strand break to allow for DNA insertions is in progress.



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# Poster No. 34

## Engineering biomolecular strategies to optimise mRNA-based protein expression in CHO cells

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For the biopharmaceutical industry, CHO cells serve as the workhorse to produce recombinant proteins. It has been demonstrated that mRNA-based transfection provides a rapid and efficient way of elucidating bottlenecks within the post-translational machinery of CHO cells, emerging as a valuable alternative to common protein expression strategies (Coats et al., 2020). Compared to traditional plasmid transfection, this technique achieves high specific productivities up to 12 hours while maintaining low cellular toxicity. Due to their short circulation, resident time and low stability, transfected mRNAs have significant limitations. To overcome these limitations, structural modifications have been adopted, including elongation of poly(A) tails, modification of 5′ caps, and engineering of UTRs and nucleotides (Wadhwa et al., 2020).

This study aimed to test these modifications and to assess their effects on mRNA stability, expression time, and translation efficiency using eGFP as a reporter protein. This includes modifications of the cap and modified nucleotides, such as pseudouridine (Ψ) and 5-methylcytosine (5mC). In our optimised protocol, the mRNA was produced by in vitro transcription, followed by post-transcriptional capping and tailing. To measure protein expression levels over time, electroporation was used to deliver mRNA to our target cells. Furthermore, to quantify and compare mRNA expression under the different conditions, RNA isolation followed by RT-qPCR was performed.

Our optimisations aim to build a comprehensive working system that can be used as a valuable and time-conserved strategy. This improved method would serve as a platform for other industrially relevant proteins by significantly decreasing experimental timelines, compared to traditional methods.

# Poster No. 35

## Optimisation of a Lentiviral delivery strategy to successfully conduct genome-wide CRISPR-Cas9 screens in suspension-adapted CHO cell lines.

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Genome editing using the CRISPR-Cas9 toolbox has proved to be an efficient application that has garnered substantial interest due to its enormous potential in the context of functional genome-wide screens for developing new cell line engineering strategies. The main strength of CRISPR-Cas9 is the programmability of the system through the delivery of engineered guide RNAs (gRNAs), which can be designed to target and edit specific genomic loci.

Lentiviral vectors are the system of choice for delivering libraries containing multiple gRNAs in CRISPR-Cas9 screens. Although lentiviruses allow effective and stable integration of the cassette-of-interest into the host genome, there are critical experimental factors that can affect the outcome, including their production process as well as the susceptibility of target cells to infection.

Here, in our work, we established an optimised protocol to enhance the transduction efficiency to a level where CRISPR-Cas9 genome-wide screen in suspension-adapted CHO cells becomes feasible by using 3rd generation lentiviral vectors. Two different transduction protocols were tested, involving spinoculation and static transduction. This protocol enabled enhanced efficiencies through the optimisation of various experimental conditions involving the use of ideal culture vessels, the addition of a histone deacetylase inhibitor, an advantageous system for viral particles concentration and serum-containing medium replacement without the need for ultracentrifugation steps.

These improvisations led to an overall increase in transduction efficiency of up to ~ 20 %.

In conclusion, we achieved optimal transduction efficiencies and hence, provide a feasible, reproducible, and cost-effective system, specifically tailored for genome-wide screens in otherwise difficult-to-transduce suspension cells.





# Poster No. 36

## Exploiting substrate promiscuity of transmembrane alkane monooxygenase AlkB and homologs for key hydroxylation step in tulipalin A biosynthesis

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In our study, we aim to establish and integrate an artificial downstream pathway towards tulipalin A by deviation from the hemiterpenoid metabolism to enable its fermentative production. Tulipalin A can serve as a monomeric building block for the production of enhanced bio-based polymers. The focus of this work lies on the site-specific hydroxylation of isoprenyl acetate at the terminal $C_4$-atom and thereby leaving the adjacent methylene group intact – the crucial step in the synthetic pathway towards tulipalin A. The alkane monooxygenase AlkB from Pseudomonas putida GPo1 was found to be a suitable enzyme for that goal - it selectively hydroxylates isoprenyl acetate at the $C_4$-position.[1]

AlkB is a transmembrane non-heme di-iron monooxygenase that catalyzes the ω-hydroxylation of a broad range of substrates, including medium-chain alkanes ($C_5$ to $C_{12}$) to produce 1-alkanols. It is also known for its over-oxidation ability to aldehyde and carboxylic acid.[2] In this work, we demonstrate the optimization of the aforementioned hydroxylation step by investigating several AlkB homologs and their performance in whole-cell biotransformations and thereby showing how searching for homologs can increase the overall output.

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# Poster No. 37

## Engineering cyanobacterial chassis for improved electron supply toward a heterologous ene-reductase

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The oxygenic photosynthesis-driven cofactor recycling hosted in cyanobacteria offers a sustainable alternative for industrially relevant asymmetric catalysis, as it greatly increases the atom economy. This approach allows for the substitution of sacrificial organic co-substrates using water as the electron donor. The heterologous ene-reductase YqjM from Bacillus subtilis was previously expressed in unicellular cyanobacterium Synechocystis sp. PCC 6803 and used in light-driven biotransformations [1]. In order to further improve this light-driven whole-cell biotransformation, we focused on rational engineering of the electron transfer pathways associated with photosynthetic electron flow. For this purpose, yqjM was introduced into four different Synechocystis chassis lacking genes encoding proteins of the electron transfer pathways. These chassis include the previously constructed Synechocystis ΔhoxYH [2], and the newly generated Synechocystis Δflv3, ΔndhD2 and the double mutant ΔhoxYHΔflv3. The robustness of the chassis was evaluated following growth and oxygen evolution rates, and the functionality of YqjM was tested in whole-cell light-driven biotransformations by determining the conversion of 2-methylmaleimide (2-MM) into 2-methylsuccinimide (2-MS). The results showed no significant differences in growth and oxygen evolution rates, suggesting the robustness of the chassis is maintained when expressing YqjM. The whole-cell biotransformations results showed an improvement in the specific activity of YqjM in the mutant lacking an active bidirectional hydrogenase. In conclusion, this work demonstrates rational engineering of cyanobacterial chassis is a valid strategy to improve the productivity of a heterologous ene-reductase.

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# Poster No. 38

## Using *Pichia pastoris* as production host for animal myoglobin and hemoglobin

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In the recent years, the increasing awareness of humans about the negative influence of the meat industry on our ecosystem and on the climate, placed the recombinant animal proteins in the focus of the food biotechnology research. Thereby, animal hemoproteins are crucially important for a meaty taste and flavour. They increase the nutritional value concerning iron content and improve the biomimicry of alternative meat products. Within this project, Pichia pastoris strains expressing bovine (Bos taurus) and chicken (Gallus gallus) myoglobin and hemoglobin were generated and studied. Both animal hemoproteins expressed in yeast were further purified and characterized.

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# Poster No. 39

## Process intensification of virus-like particles in latent virus-free insect cell line

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At the University of Natural Resources and Life Sciences, we are working with a latent virus-free insect cell line called Tnms42. Previous studies have shown that this cell line can produce virus-like particles* (VLPs) with the baculovirus expression system and that it performs as well as the latent virus infected HighFiveTM cell line. Intensification of VLP production through transient infection could be achieved through continuous processing, but this faces numerous challenges: presence of defective interfering particles hinders continuous infection, virus replication is faster than cell growth, cell retention membranes and hollow fibers retain VLPs. Alternatively, VLP process intensification in small reactor vessels is possible with high cell densities. We were able to achieve a high cell density with the Tnms42 insect cell line in both shaking flasks with pseudo perfusion and in the bioreactor utilizing an alternating tangential flow filtration. With daily medium exchange the exponential growth phase could be prolonged and the total cell concentration increased by a factor of two in the shake flask and by factor four in the bioreactor (~40 $10^6$ cells/mL) in comparison to reference batch processes. Various factors, such as cell concentration at infection, multiplicity of infection and cell state at infection will be further optimized in the intensified high cell density process to reach higher volumetric VLP titers, which in turn increases the productivity of the entire process.

*Virus-like particles are - as the name suggests - similar to viruses and trigger an immune response in mammals and are therefore of interest for vaccines.



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# Poster No. 40

## Soft sensor for determining apparent oxygen consumption rate and viable cell count

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The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 2002) envisions process design by process understanding but applicable tools for industrial upstream processes are still missing. In recent years, soft-sensors are proposed as promising tools for implementation of the Quality by Design (QbD) approach. Especially the correlation between viable cell count and oxygen consumption was investigated but problems remained: Either processes had to be modified for the conventional use of $CO_2$/base pH control e.g. in off-gas analysis to exclude $CO_2$ or complex kLa models had to be set up for specific processes. We developed a non-invasive soft-sensor for cell counting based on oxygen consumption to close the gap between QbD process development and simplified online cell counting. We directly measured the apparent oxygen consumption of Chinese hamster ovary (CHO) cells by automatic and periodic interruptions of gas supply in DASGIP® bioreactors. With off-line cell counting we were able to correlate the two parameters and develop a novel soft-sensor. We also investigated the potential influence of discontinuous gas supply and could confirm no impact on cell growth behavior.

# Poster No. 41

## Diagnosis of human sepsis using circulating nucleic acids (CNA) as biomarkers

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Human sepsis is a life-threatening disease caused by several types of infectious pathogens. The diagnosis of sepsis relies thus far either on slow methods such as culture-based assays or fast but inaccurate measurements. In total 24 molecular markers were identified by high throughput sequencing, based on circular nucleic DNA (CNA) of the human genome, which in combination can be used to identify human sepsis via qRTPCR. It is possible to detect the presence of sepsis two to three days prior patients develop the first clinical signs and meet the sepsis-3 criteria with an accuracy of 87% inside of the same patient cohort for which the markers were developed and up to 81 % in blind studies of patient cohorts which were not included in the marker development. This detection method is a promising approach for an early diagnosis of human sepsis even before clinical symptoms occur.



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# Poster No. 42

## Wastewater based surveillance and analysis of SARS-CoV-2 in Styria

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As part of the national scale project termed “Schulstandmonitoring” of the Federal Ministry of Education, Science and Research, we analyzed samples from 24 wastewater (WW) treatment plants in Styria for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The whole project was carried out by multiple collaborators from different institutions around Austria and in total 122 WW treatment plants were sampled twice a week starting from 1st, September 2021 until 31st, August 2022. The coordination of the project was carried out by the University of Innsbruck under Heribert Insam. The aim was to establish the methodology for concentrating, isolating and quantifying the virus in our lab and the sample logistics to ensure an uninterrupted refrigeration chain and timely processing. The virus fragment concentration was then determined with RT-qPCR. In addition, total nitrogen along other population size markers were measured to normalize virus concentration for varying influx and differing population sizes in the catchment of the WW treatment plants. The relative virus load is then calculated in gene copies times million per capita per day and represents an apt parameter to map the course of the pandemic1,2.

# Poster No. 43

## Morphine production in genetically engineered poppy cell culture

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Morphine is classified as an essential medicine by the World Health Organization (WHO) due to its prominent role in pain management. In contrast, roughly half of the human population (an estimated 3.6 billion people) has insufficient to nonexistent access to this pain management.
The current bottleneck is that opium poppy (Papaver somniferum) is the sole source of Morphine and industrial poppy farming is affected by numerous environmental factors including weather, climate change, pests, and diseases. Establishing means of morphine production that are independent of these factors will therefore substantially improve control over product quantity and quality. Ultimately, this can result in improved global supply chain stability and reduced production costs rendering morphine available to patients in low- and middle-income countries around the globe.
Here, we will develop an innovative plant cell culture-based morphine production technology. We base our approach on optimizing cell culture conditions, ectopic expression of enzymes and elucidating the transcription factors regulating the Morphine biosynthesis pathway. Trough optimization of the growth conditions and altering the regulation we will switch on Morphine production in engineered opium poppy cells.





# Poster No. 44

## Protein-Probe – a versatile high throughput luminescent tool for label-free investigation and quantification of viruses and proteins/ enzymes

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Rapid methods for investigation of protein/enzymes and virus/vesicles properties and quantity are of high interest in modern biochemical industry. No compromise in protein properties, low aggregation and high stability, is desirable to promote stable and nontoxic protein end-products. Enzymes are widely used in industry to catalyze reactions enabling higher efficiency and reduced time of processing. Current protease activity measurements rely unfortunately to artificial detection systems. As recently proven by vaccine industry, viral based vehicles play a significant role in vaccine development, and thus rapid quantification tools are needed. Current methodologies do not support rapid measurement of production line outcome and end-products with simple, high sensitivity, and substrate-independent enzyme activity methods.

We have developed a versatile tool, Protein-Probe, which is an ultra-sensitive label-free external lanthanide chelate probe utilizing highly sensitive time-resolved luminescence detection to monitor changes in target protein structure, interactions, and activity as well as viral/vesicle count based on increased TRL-signal at room or increased temperatures. We have already shown that Protein-Probe can measure protein stability and binding properties in high throughput compatible format at nanomolar level, at least 100-fold improved sensitivity compared to existing technologies. Current protease activity detection systems are based on artificial biomolecular substrates while Protein-Probe technique provides an approach to measure enzyme activity with natural enzyme substrates at nanomolar level. Also, Protein-Probe technology provides antibody aggregation detection below 0.1%, and can be used to study and quantify cells, viruses, and extracellular vesicles. The label-free Protein-Probe technique is 384-well plate compatible, thus increasing throughput and reducing cost.

# Poster No. 45

## Immunogenicity testing is key to assessing drug safety and efficacy

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An increasing number of protein therapeutics have become available since the introduction of recombinant technologies, providing new treatment options for a wide range of diseases. Over the years, it has become apparent that many of these products bear the risk of inducing unwanted immune responses which can be associated with severe clinical consequences such as cross-reactions to the endogenous counterpart, a diminished drug efficacy, an altered pharmacokinetic profile of the drug, hypersensitivity reactions against the drug (I-IV) and/or to the release of pro-inflammatory cytokines or chemokines. Often, these events are infrequent and only become recognized at a late stage in clinical development or after marketing authorization.

Therefore, the assessment of the immunogenicity of therapeutic proteins and peptides is a key and mandatory element in biological drug development. As the immunogenicity is determined by a multiplicity of different factors, it is essential to develop a specific bioanalytical assay strategy for each protein therapeutic. The Institute Krems Bioanalytics develops tailored bioanalytical solutions to understand the interplay of the adaptive and innate immune responses in the context of immune-mediated adverse effects of biological drugs. We have long-standing expertise in the development and validation of customized and GxP compliant analytical assays for the assessment of immunogenicity and the application of theses assays in preclinical studies and clinical trials. Moreover, we specialize in cutting-edge mass spectrometry technologies like plasma proteomics, immunopeptidomics, mapping of antibody epitopes and antibody sequencing to allow a detailed characterization of immune responses at the molecular level.



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# Poster No. 46

## Gamification as a powerful tool to interest children for biotechnology

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In order to secure a qualified workforce in biotechnology, it is important to interest children in the life sciences as early as primary school age. Gameification can be used as a tool for this purpose. To reinforce the first lessons on biochemistry and provide information on enzymes, acib has developed the "Enzyme Quartet". This card game, designed for 3 players aged 5 and up, is intended to make children curious to learn more about biotechnology. 20 enzymes are introduced to the players in an easy-to-understand way. Four parameters for enzymes were chosen, but the numbers on the cards were set to be calculated by the children. In a first step, a card game was developed, the transfer of which to a digital online game is in preparation.

# Poster No. 47

## The virtual biotech accelerator as a digitaltool in business development

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Business development is the creation of long-term value for an organization from customers, markets and relationships. For the Austrian Centre of Industrial Biotechnology (acib), business development is the combination of identification of new business areas, project ideas and industrial partners. It is supported by the acib open innovation strategy. Mayor tools are project development, networking, the acib system of technical offers and close collaboration with acib scientific communication. acib developed a "Virtuell Biotech Accelerator" to enhance open innovation and to support business development.





# Poster No. 48

## Recover of highly pure monomer through enzymatic hydrolysis of polyesters

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Polyester recycling has been taking place in the last years mainly through mechanical/chemical methods. Classic approaches entail several challenges, from contaminants separation to poor quality of recycled products. Moreover, the process could result in pollutants as side products [1].
Enzymatic hydrolysis is therefore extremely promising in the green processing of plastic waste. Apart from being an eco-friendly method, biocatalysts allow to obtain high-quality monomers. These could re-enter production cycle with same grade of virgin material.
This work focuses on different polymers: Poly(ethylene terephthalate) (PET), Poly(butylene-adipate-co-terephthalate (PBAT) and blended biopolymers such as PBAT/Starch and PBAT/Polylactic acid (PLA). A commercial cutinase (Humicola insolens cutinase) is chosen for hydrolysis of above-mentioned polyesters. Released soluble monomers are quantitatively monitored through High-Performance Liquid-Chromatography.
Being Terephthalic acid (TPA) the common component of PBAT and PET, it was separated from both hydrolysates through acidification and centrifugation. Solid was then dried and characterized via FT-Infrared and NMR analysis. Especially TPA obtained from PET proved to be 95% pure, remarkably close to the purity of commercial counterpart.
In addition to TPA, Ethylene Glycol (from PET) and Adipic acid/Butanediol (from PBAT) were investigated in terms of isolation from residual liquid fractions. Low presence of contaminants is the minimal requirement for their application in resynthesis as well as for microbial fermentation [2].
Besides many advantages in terms of quality and straightforward handling, the applied methods demonstrate to be economically competitive. This represents the key factor for real industry recycling process, to overcome the current preferred virgin plastic productions from fossils [3].

# Poster No. 49

## Bio-exploration towards novel plastic-degrading microorganisms: metagenomic study from rumen for synthetic polyesters depolymerization

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In 2019 alone, 29.5 million tons of plastic waste was collected in the EU, of which only 35% was recycled. PET, the most abundant polyester, accounts for 8.4% of demand in the European plastics marke. It is clear that all aspects of the plastics lifecycle must be made more sustainable. New plastics from bio-based sources must be investigated, the procedures for polymer synthesis and processing must be 'greened' and the vast excess of waste plastic currently polluting the environment must be degraded. Microorganisms are an untapped resource in the search for novel enzymes for plastic degradation. This study investigates the hydrolytic activity of cattle rumen on synthetic polyesters. Initial screening on model substrates demonstrated the hydrolytic activity on p-NP-esters, and the subsequent attempt to hydrolyse poly(ethylene terephthalate) (PET) and poly(butylene adipate-co-terephthalate) (PBAT) was successful. HPLC analysis of hydrolysed polymer film showed showed 0.5 mM and 0.15 mM terephthalic acid was released from PBAT and PET respectively. Poly(ethylene furanoate) (PEF) was also successfully hydrolysed. Shotgun metagenomic analysis of extracted rumen DNA gave more than two million reads that could be taxonomically classified. The taxonomic proportion showed 98% of Bacteria, 1% of Eukaryota, around 0.9% of Archaea, and <0.1% viral origin in the rumen microbiome. Members of the Pseudomonas genus in particular are the most prolific within the rumen microbiome, suggesting they may play a major role in polyester hydrolysis.



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# Poster No. 50

## Hydrogen-powered production of nitrogen heterocycles in *Cupriavidus necator*

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The metabolic versatility of Cupriavidus necator makes it a suitable microbial platform for biotechnological industrial applications. Under chemolithoautotrophic conditions, C. necator grows with CO2 as a carbon source, H2 as an electron donor and O2 as an electron acceptor. The objective of this project is to create an efficient biorefinery using C. necator that transforms CO2 into fine chemicals like nitrogen (N)-heterocycles. This will be done by developing an enzymatic cascade that includes an NADH dependent imine reductase (IRED), an O2 dependent putrescine oxidase (PuOx) and an O2 tolerant NAD+ reducing hydrogenase for cofactor regeneration (SH) in C necator for N-heterocycle production (1,2,3). The first enzyme in the cascade was heterogously produced in C. necator and was found to be active as detected by immunoblots and evaluated by activity assay. Several C. necator strains were developed for the optimal production of IRED. These strains had different plasmids and genomic background, including co-expression with the chaperonin GroESL (4). This same protein production strategy will be done for PuOx to ultimately create a functional enzymatic cascade for N-heterocycle synthesis. Combination of biocatalytic cascades with the autotrophic metabolism of C. necator is an attractive alternative to develop sustainable industrial processes.

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# Poster No. 51

## Hydrogen-driven cofactor regeneration for sustainable whole-cell biotransformations in *Cupriavidus necator*

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Oxidoreductases comprise a large number of industrially relevant enzymes catalyzing the transfer of electrons from an electron donor to an electron acceptor molecule. Enzymatic redox reactions in the reductive direction often require the presence of reduced redox cofactors, commonly nicotinamide adenine dinucleotide (NADH) or its phosphorylated form (NADPH). Since the addition of such reducing equivalents in stochiometric amounts is economically prohibitive, the implementation of efficient cofactor regeneration systems is pivotal to achieve large-scale biocatalytic applications [1]. To this end, the most widespread cofactor recycling strategies rely on the partial oxidation of auxiliary organic substrates resulting in poor atom efficiencies, unwanted by-products and increased downstream processing costs [2]. Contrarily, the use of hydrogen as a reducing agent represents a clean, cheap and atom-efficient alternative. The vast potential of H2 as a substrate for growth and cofactor regeneration has been exploited by several chemolithoautotrophic organisms through the use of hydrogenases. Cupriavidus necator is one of the most extensively studied Knallgas bacteria capable of oxidizing molecular hydrogen for the provision of energy and electrons for intracellular redox reactions, thanks to its oxygen-tolerant hydrogenases (Figure 1). In this context, coupling recombinantly expressed oxidoreductases to the endogenous hydrogenases of C. necator for cofactor recycling, constitutes an efficient strategy to circumvent the limitations of employing organic cosubstrates as sacrificial electron donors [3].





# Poster No. 52

## Increasing the electroporation efficiency in the chemolithoautotrophic bacterium *Cupriavidus necator*

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Climate change is an increasingly concerning phenomenon, and novel solutions to reduce the release of greenhouse gases are necessary. Industrial biotechnology can play an important role by using engineered bacteria for the production of chemicals from carbon dioxide. In particular, Cupriavidus necator is a lithoautotrophic bacteria able to grow on $CO_2$ and $H_2$, making it a potential player in the increasingly important hydrogen-based economy. However, as many other non-model organisms, synthetic biology tools for C. necator are still underdeveloped and limit its broad applicability. In particular, low transformation efficiency of current plasmids and reliance on conjugation strongly restrains experiment throughput. The lack of standardized tools also decreases sharing of knowledge between labs, slowing down the development of the field. The goal of this project is to increase the transformation efficiency of C. necator, and to create a broad set of tools and protocols for its easy genetic engineering. To this end, several electroporation protocols were analyzed and compared in order to increase efficiency. This is an essential step in order to employ high-throughput techniques, such as combinatorial construction of metabolic pathways. The role of plasmid size was also investigated by using plasmids of increasing length. Other factors that may influence transformation efficiency were also investigated, and a strong influence of the native restriction/methylation system was confirmed. These findings will be applied to create novel genetic tools in order to efficiently modify C. necator, paving the way to a broad applicability of this chassis strain in autotrophic biotechnology.

# Poster No. 53

## Traceability of products and guide for batch definition in integrated continuous biomanufacturing

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Moving from batch processing to continuous processing is trending as a major improvement in biomanufacturing. The recent guidelines asked for tracing the material through the process in continuous mode, and it's suggested to use residence time distribution (RTD) for this purpose. In batch processing also the batch number is used to trace the material, however finding a new batch definition is a big challenge in continuous processing.

We established a simulation of a process train to compare the conventional batch definition based on an arbitrary time or volume to a new batch definition method based on the greatest common divisor (GCD) of the time period of the individual unit operations time period. We successfully showed that, by using the new batch definition based on GCD, we will have a constant periodic profile of the product. Therefore, we can define batches in a continuous process, which will lead to higher control and traceability over the process. In comparison to collecting the outlet products over arbitrary times or volumes, collecting the product based on a section defined by the GCD method meets the criteria for characterizing the residence-time distribution of the process, as advised by regulatory authorities. This method applies to a continuous process or a hybrid process in which there are only a few continuous unit operations along with batch process operations.

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# Poster No. 54

## Characterization of hollow fiber membrane modules for hydrogen transfer in bioreactors

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Greenhouse gases such as $CO_2$ can be directly used as feedstocks in microbial gaseous fermentations. One of the most attractive and well-studied microorganisms able to grow on $CO_2$ is Cupriavidus necator, which also needs $H_2$ as a source of reducing power and $O_2$ as a final electron acceptor. However, the gas mixture of $H_2$ and $O_2$ can be explosive, and supplementing hydrogen to the cultivation broth through a membrane is a promising way to keep the gaseous $H_2$ concentration below the explosive limit.

Two hollow fiber membrane modules were evaluated to compare their respective permeance to hydrogen. One contained dense polydimethylsiloxane fibers while the other contained composite fibers with a much thinner coating of polydimethylsiloxane. Both of them were first tested without liquid in the module, to only evaluate the gas transfer performances of the membranes. Then they were tested with water recirculation, under various combinations of three crucial parameters: liquid flow, gas flow and trans-membrane pressure.

As expected, the gas permeance of the coated membrane was found to be higher than that of the dense one, due to the thinner polymeric layer. When liquid recirculation was added, though, water resistance appeared to have a greater impact on the composite than on the dense membrane, since the latter had a higher permeance in all tested conditions. Additional studies are ongoing to evaluate both modules under fermentation conditions, i.e. to determine which is the most effective in providing sufficient $H_2$-supply during microbial gas fermentation.

# Poster No. 55

## Engineering alpha-ketoglutarate-dependent halogenases towards a green and sustainable synthesis of functionalized heterocycles

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Piperazines are N-heterocyclic compounds that are renowned for their multiple bioactive roles [1]. To aid in the development of its manufacturing process, we aim to engineer alphaketoglutarate dependent halogenases to optimise the production of functionalized piperazine rings [2]. We have identified through gene cluster analysis three enzymes, (BesD [3], MstM and Psm3), capable of potentially producing these halogenated heterocyclic rings. Through the use of a mixture of site directed and random mutagenesis, our group is focusing on engineer BesD to expand its substrate scope, making it able to halogenate both cadaverine and putrescine, in addition to its natural substrate, L-lysine. MstM and PsM3, already capable
of halogenating cyclic compounds, are being engineered to optimise the direct halogenation of piperazine derivatives. These engineered enzymes will be incorporated as components of enzymatic cascades in the chemolithoautotrophic organism, Cupriavidus necator, This will be a step toward making the synthesis of piperazines and its derivatives more sustainable, green and efficient in terms of economy, energy, and waste generated.

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# Poster No. 56

## Polyhydroxyalkanoate production with *Cupriavidus necator* from lignocellulosic feedstocks

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Increasing environmental pollution of petroleum-based plastics is impelling advances in developing so-called bio-polymers being biodegradable under natural conditions. Polyhydroxyalkanoates or PHAs are a group of bio-polymers with promising plastic-like material properties which are produced by microorganisms as storage compounds. Cupriavidus necator, formerly known as Ralstonia eutropha, is the most commonly used PHA producer accumulating intracellular PHA in large amounts. Lowering the production costs of PHAs is one of the central goals of this project. As the sole carbon source an extract of pre-treated lignocellulosic materials, containing primarily glucose and xylose, is used. In order to valorise all available carbon sources in the extract, growth on glucose, fructose and xylose is tested and optimized. Growing C. necator, which is able to utilize glucose by adaption, on highly concentrated minimal media with glucose added as a carbon source is reaching cell densities higher than 200 OD600 units. By introducing heterologous genes of the xylose degradation pathway, C. necator can use xylose as a carbon source. Possible inhibitors of C. necator such as vanillin, which are present in the lignocellulosic extract, are identified and used for adapting the bacterium. Given all these circumstances, C. necator is well suited for producing biodegradable bio-plastics from naturally occurring lignocellulosic feedstocks.

# Poster No. 57

## Sustainable alternative to conventional plastic: Production of (poly)-lactic acid from wheat straw hydrolysate

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Polylactic acid (PLA), a bio-based plastic is an environmentally friendly alternative to petroleum-based plastic due to its biodegradability and its wide range of applications. Lactic acid (LA), the building block for polylactic acid can be produced via microbial fermentation. But high substrate costs pose the greatest challenge to LA bioproduction. Therefore, the use of inexpensive biomasses such as lignocellulosic feedstock is a great way to lower LA production costs.

The growth behaviour of lactic acid bacteria (LAB) on different carbon sources was examined with regard to LA production and sugar consumption, as well as the influence of inhibitors in lignocellulosic material. Studying LAB growth behavior on various carbon sources revealed that LA production and sugar consumption can differ depending on the carbon source. Among all LAB strains tested, only the obligate heterofermentative L. brevis was capable of co-metabolizing glucose and xylose with high xylose consumption. When grown on wheat straw hydrolysate, growth and glucose consumption decreased for all strains. The results indicate that inhibitors present in the wheat straw hydrolysate have a slightly negative impact on sugar metabolism. LA production, however, was not affected by the lignocellulosic hydrolysate. Overall, the best LA producers on wheat straw hydrolysate were L. plantarum NCIMB 8826 followed by L. plantarum WCFS1 and L. plantarum DSM 20174.

Therefore, LAB are excellent candidates for producing LA from sugar-rich biomass because of their high LA production rate and their robustness towards lignocellulosic by-products.





# Poster No. 58

## Next Generation of Microfluidics for safe and sustainable diagnostic devices

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In the past years, biological assays shifted more and more towards miniaturization, increasing lab work efficiency and enabling high-throughput screening. Microfluidics show intrinsic ability to manipulate very small volumes of fluids in a variety of integrated ways including sample processing, accurate control of fluids and fast delivery of results [1]. The translation of lab scale devices to industry requires a large number of integrated microfluidic devices, being relevant for the high-volume manufacturing methods for upscaling of such. Roll-to-roll (R2R) imprinting enables parallel and high-throughput generation of micro or even nanostructures in various designs due to a production performed on flexible polymer foils and the possibility of post-processing steps such as bio-functionalization, chip lamination and others [2]. The Next Generation Microfluidics Open Innovation Test Bed (OITB) aims to offer customers a single-entry point to a wide range of existing cutting-edge technologies to accelerate scientific breakthroughs towards a working prototype and beyond. In this work, we present a preliminary assessment of the safety and sustainability issues associated with next-generation microfluidic devices for diagnostic applications.

Five demo cases are being used for the safety and sustainability assessment, ranging from biosensor development, molecular diagnostics and smart-phone-supported home diagnostics to pharmaceutical testing and bioprocess monitoring sensors. All of them will optimize their biochemical and molecular assays for the selected analysis prior to their upscaling. For upscaling, the R2R manufacturing process is used for rapid prototyping, enabling reliable and reproducible mass production.

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# Poster No. 59

## Milliscale devices to accelerate process development for protein precipitation and filtration

**Maria del Carme Pons Royo**[1,2], Alois Jungbauer[1,2], Peter Satzer[1,2]

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Protein precipitation may be an alternative to conventional affinity capture of antibodies. Typically, protein precipitation is performed by direct addition of the precipitating agent in a single dose. However, such an approach does not consider co-precipitation of impurities and neglects the dynamics of precipitate formation and inclusion of unwanted impurities. To overcome such drawbacks, a gradual dosage of polyethylene glycol was proposed which impacted the final purity and yield. In addition, precipitates exhibited different characteristics depending on the dosage time, resulting in different filterability in tangential flow filtration and depth filtration. To switch from batch to continuous PEG addition, precipitation conditions need to be adjusted to improve the product quality attributes and inform about the design of further purification steps. To obtain all the required data for scaling up the precipitation process, devices for continuous protein precipitation to screen the necessary parameters for process setup in as small a form as possible were developed. Such millidevices device has a volume of 1 – 5ml in the form of a tubular reactor manufactured in Polymethylmethacrylate by laser cutting. The devices have multiple injection points for controlled and precise addition of precipitating agents without valves. The designed millidevices were used to study and determine the most appropriate precipitation conditions such as the dosage time of the precipitating agent and the resulting filterability of the precipitates in tangential flow filtration and depth filtration, as well as, the apparent solubility curves of antibodies and impurities. However, the main unsolved impediment to consider protein precipitation as an option at a large scale was the elevated buffer consumption. Therefore, a powder feeding device to directly add PEG6000 in solid form combined with millidevices was used for continuous protein precipitation. Such an alternative process was evaluated concerning economics and environmental footprint and compared to batch process and current state-of-art processes for the capture step. Total costs of goods were significantly reduced and the environmental footprint was at least half of the reference processes. The findings presented will help to expedite the development of fully end-to-end platforms for continuous manufacturing of biopharmaceuticals. The economic and environmental assessment will help to guide the developers to more efficient and environmentally friendly processes.



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# Poster No. 60

## Characterization of novel NADH: FMN oxidoreductases from *Herbaspirillum seropedicae*

**Karishma Shah**[1], Robert Kourist[2], Andre Pick[3]

[1]acib Gmbh, Graz, Austria, [2]Graz University of Technology, Graz, Austria, [3]CASCAT GmbH, Straubing, Germany

Nicotinamide adenine dinucleotide (NAD+) and NADH its reduced form are important cofactors in redox reactions. Many industrially interesting oxidoreductases are dependent on nicotinamide cofactors to accomplish different transformations(1,2). These rely on the stoichiometric utilization of NAD+ cofactor; therefore, an integrated effective recycling system is essential to keep the process running at a low cost. NADH: FMN oxidoreductases and NADH oxidases are majorly employed for regeneration application(3,4). Enzyme-mediated cofactor recycling method, which uses molecular oxygen as an oxidizing agent, is one of the most evolvable and efficient ways to recycle NAD cofactor. Even though NADH: FMN oxidoreductases can reduce oxygen, their physiological roles may differ or are usually unknown. The current research focuses on the biochemical characterisation of NADH: FMN oxidoreductases from Herbaspirillum seropedicae in order to better understand their physiological role and evaluate their potential stability.

**1.** Hall M. Applications of Oxidoreductases in Synthesis: A Roadmap to Access Value-Added Products. Biocatal Pract. 2021;181–223.

**2.** Mordhorst S, Andexer JN. Round, round we go-strategies for enzymatic cofactor regeneration. Nat Prod Rep. 2020;37(10):1316–33.

**3.** Nowak C, Beer B, Pick A, Roth T, Lommes P, Sieber V. A water-forming NADH oxidase from Lactobacillus pentosus suitable for the regeneration of synthetic biomimetic cofactors. Front Microbiol. 2015;6(SEP):1–9.

**4.** Li FL, Su W Bin, Tao QL, Zhang LY, Zhang YW. Expression, biochemical characterization, and mutation of a water forming NADH: FMN oxidoreductase from Lactobacillus rhamnosus. Enzyme Microb Technol [Internet]. 2020;134(October 2019):109464. Available from: https://doi.org/10.1016/j.enzmictec.2019.109464

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# Poster No. 61

## Studies on mechanical properties of crosslinked bio-polyester/elastomer – blends and their biodegradability

**Carina Frank**[1,2], Franz Stelzer[1,2], Anita Emmerstorfer-Augustin[1,3]

[1]acib Gmbh, Graz, Austria, [2]Institute for Chemistry and Technology of Materials, Graz, Austria, [3]Institute of Molecular Biotechnology, Graz, Austria

While plastics have become highly valued for their long-lasting functional use, their very slow degradation rate has challenged society with the accumulation of plastic waste leading to big environmental problems. Therefore, an increased interest regarding sustainable products can be observed. PHB and PHBV are promising bio-based and biodegradable thermoplastics. However, PHB has a very poor thermal stability, which limits the processability and recyclability. Motivated by these limitations, this study focused on the development of a bio-polymer compound suitable for toys and aquatic materials. Strategies to improve the processability were investigated. Hence, natural rubber was used as an impact modifyer and crosslinking was carried out. Mechanical and thermal properties of the blends were analysed and compared with the neat material.
Furthermore, different extracellular PHB(V)-degrading depolymerases were selected, expressed, and characterized to monitor the degradation of the blends. Results showed that each of the heterologous depolymerase was able to degrade crosslinked PHB/PHBV/NR blends.



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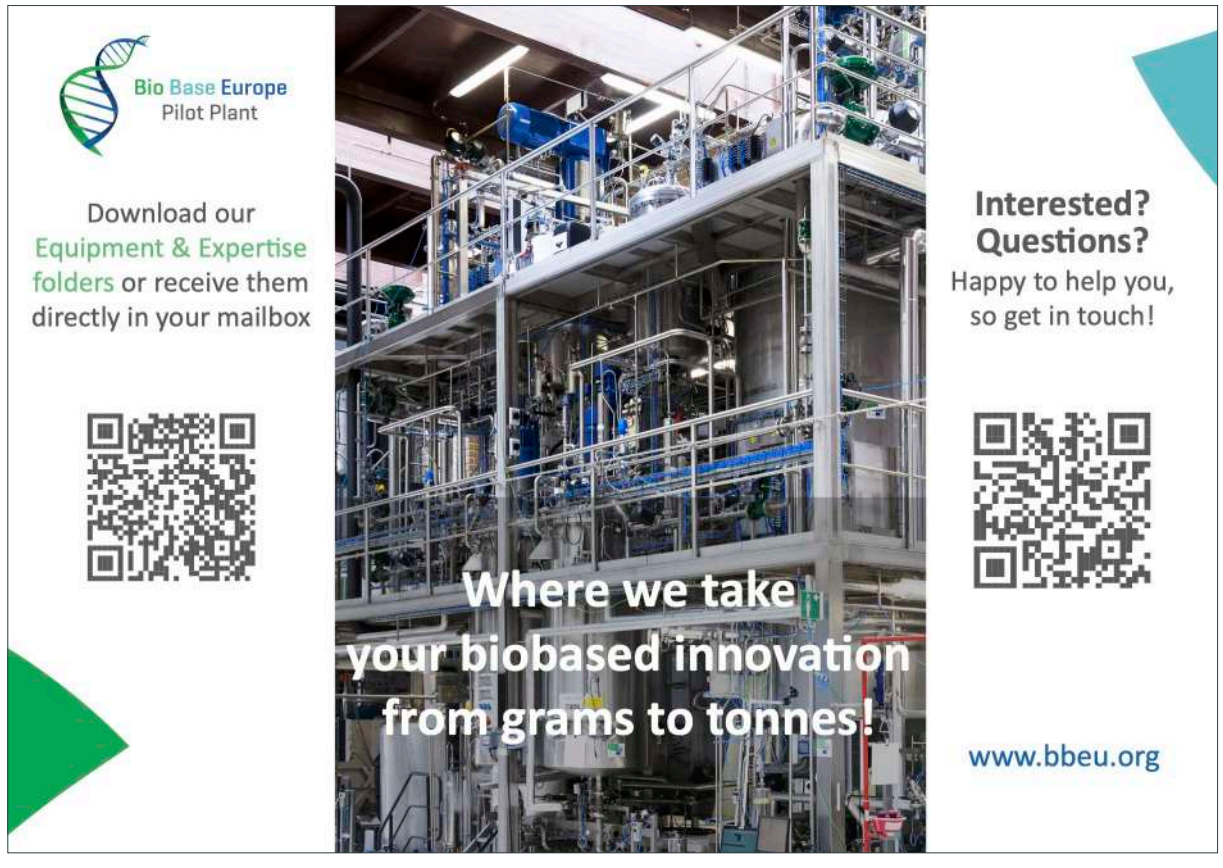
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# Monday, 14th November

**10:15 – 10:25**
## IFPB Opening
**Speaker:** Georg Guebitz

## Biobased polymers and fibres, 10:25 – 11:45

To tackle the world's plastic problem, bio-based polymers are the future. Many microbes are capable of producing biopolymers. How can we use such microorganisms as cell factories for production? Are polyhydroxyalkanoates (naturally occurring, biodegradable polyesters) the future of biopolymers? And where can we find aminopolysaccharides and aminopolysaccharide-glucan complexes?

**Chair:** Georg Guebitz (BOKU)

**10:25 – 10:55**
### Microbial Cell factories for the production of novel bacterial polymers
**Speaker:** M. Auxiliadora Prieto (Spanish National Research Council)

**10:55 – 11:15**
### Polyhydroxyalkanoates, a dream come true?
**Speaker:** Iolanda Corrado (University of Napoli "Federico II")

**11:15 – 11:35**
### Novel fungal source of aminopolysaccharides and aminopolysaccharide-glucans complexes
**Speaker:** Simona Dzurendova (Norwegian University Of Life Sciences)

# Monday,
14th November

## Biorecycling & Sustainable Processes, 12:30 – 14:30

Once plastics are no longer used, it is difficult to recycle them. Some recycling strategies will be presented in this session: depolymerization of PET by a thermotolerant enzyme scaffold or the use of the metagenomic polyester hydrolase PHL7, engineered metabolism in Pseudomonas taiwanensis, and enzymes for energy conservation in refining or recycling cellulose fibers. But how are these recycling strategies compatible with industry requirements for hyper-stable protein design? And how can we effectively use resources for functional and smart textiles?

**Chair:** Vincent Nierstrasz

### 12:30 – 13:00
### Sourcing thermotolerant enzyme scaffolds for depolymerisation of PET

**Speaker:** John Mcgeehan (World Plastics Summit)

### 13:00 – 13:20
### Engineering metabolism of plastic monomer 1,4-butanediol in an aromatics producing *Pseudomonas taiwanensis*

**Speaker:** Leonie Op De Hipt (Forschungszentrum Jülich GmbH)

### 13:20 – 13:40
### Low Carbon Footprint Recycling of Post-Consumer PET with the Metagenomic Polyester Hydrolase PHL7

**Speaker:** Paula Blázquez-Sánchez (University of Leipzig)



Monday,
14th November

### 13:40 – 13:55
### Meeting the industry's needs: hyperstable protein design

**Speaker:** Ezequiel Juritz (Enantis)

### 13:55 – 14:10
### Mechanistic study of enzymes used for energy saving in refining or recycling of cellulose fibers

**Speaker:** Martin Nagl (BOKU Wien)

### 14:10 – 14:30
### Resource effective textile processes for functional and smart textiles

**Speaker:** Vincent Nierstrasz (University of Borås)

# Monday,
14th November

## Enzymatic decomposition of plastics, 15:00 – 16:30

Worldwide, the plastic production and its use are accelerating and they are becoming one of the most discussed issues in the media and in different research sectors. In 2017, more than 27.1 million tonnes of plastic waste were produced in the EU with only 31.1 % being recycled, the residual 41.6 % were incinerated and 27.3 % landfilled. Conventional recycling still have some caveats regarding the complete separation of all the components, e.g. for multilayer and plastic mixes, thus not allowing to retrieve highly pure materials. Microbial and/or highly specific enzymatic processes are promising strategies in order to step-wise recover monomers from blended and multi-layer materials and/or for their biodegradation.

**Chair:**
Cristiano Varrone

### 15:00 – 15:15
**Enzymatic treatment of non-recycled plastic fractions**

**Speaker:** Juan Antonio Tamayo Ramos (ITENE; ENZYCLE Coordinator)

### 15:15 – 15:30
**Development of an enzymatic degradation process for thermoset material recycling**

**Speaker:** Julio Vidal (AITIIP/BIZENTE)

### 15:30 – 15:45
**Introduction of sustainable circular economy into fast fashion: Biotechnological Approaches for textile waste recycling**

**Speaker:** Felice Quartinello (acib, BOKU)



# Monday,
14th November

### 15:45 – 16:00
**Biotech synergies to solve plastic recycling and contamination challenges**

**Speaker:** Maria J. Lopez (RECOVER coordinator)

### 16:00 – 16:20
**The BioICEP project: Combination of physico-chemical pretreatments with biological depolymerization to recycle mixed plastic waste**

**Speaker:** Pablo Ferrero (AIMPLAS)



# Monday,
14th November

## The plastics biorefinery, 17:00 – 18:30

Decomposition of plastics will lead to valuable building blocks which can e.g. be used for re-synthesis of polymers or as substrates for bioproduction. In the latter case, recent advances involve the engineering of microorganisms to use pure or mixed monomers as substrates to produce biopolymers or platform molecules. Such plastics biorefineries will ultimately help to close the plastics-cycle.

**Chair:** Manfred Zinn

### 17:00 – 17:20
### Contribution of fermentation technology to a more sustainable plastic sector – the plastic biorefinery

**Speaker:** Cristiano Varrone (UPLIFT)

### 17:20 – 17:40
### Applying ligninases to resolve end-of-life issues of thermoset composite plastics

**Speaker:** Beatrice Mongili (Biosphere, BIZENTE)

### 17:40 – 18:00
### Development of enzymatic depolymerization-based processes to recycle plastic waste streams in ENZYCLE

**Speaker:** Tom Ewing (Wageningen University and Research, ENZYCLE)

### 18:00 – 18:20
### Potential application of electrospinning/electrospray in development of chitin and chitosan-based product

**Speaker:** Bahareh Azimi (Pisa University, RECOVER)



# Tuesday,
15th November

## Biofunctionalisation, 09:00 – 11:00

This session will focus on the modification of materials by microorganisms and their enzymes: e.g., innovative surface modification by fungal hydrophobins, inkjet printing of enzymes onto synthetic fabrics, and the use of laccase to crosslink proteins. How can crystallin fusion proteins improve the thermal properties of hair? Can we catalytically activate functional textiles to remove pharmaceutical residues from wastewater? And which microbiome and metabolome is already present on clothing textiles?

**Chair:** Gianluca Ciardelli

### 9:00 – 9:30
### The microbiome and metabolome present on clothing textiles

**Speaker:** Chris Callewaert (Ghent University)

### 9:30 – 9:50
### Innovative surface modification by fungal hydrophobins and their chimeric variants

**Speaker:** Alessandra Piscitelli (University of Naples Federico II)

### 9:50 – 10:05
### Inkjet printing of enzymes on synthetic fabrics

**Speaker:** Tuser Biswas (University of Borås)

### 10:05 – 10:20
### Insights on the hair structure under different drying conditions

**Speaker:** Madalena Martins (CEB - University of Minho)

# Tuesday,
15th November

**10:20 – 10:35**
**Removal of pharmaceutical residue from wastewater using catalytically active functional textiles**

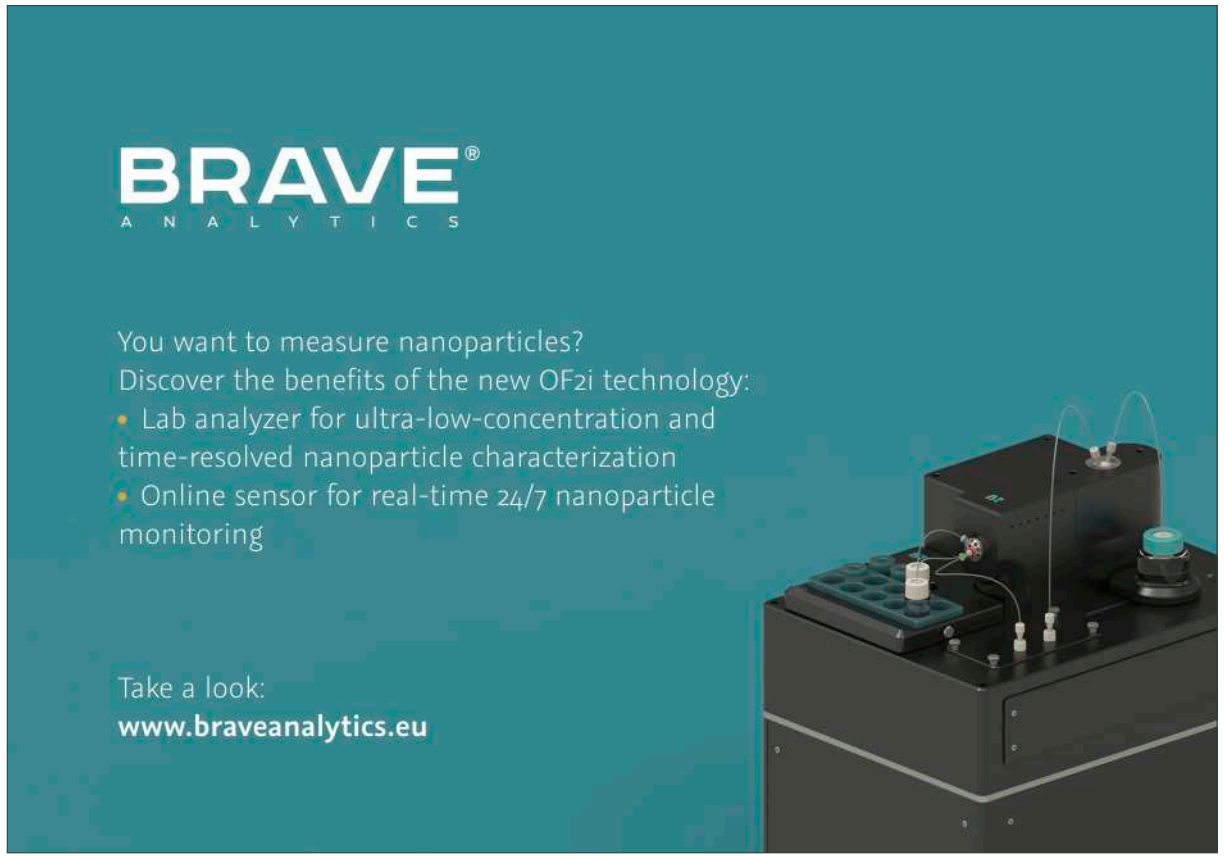
**Speaker:** Mohammad Neaz (Morshed University of Borås )

**10:35 – 10:50**
**Crosslinking of proteins with laccases: implications for fibre modification**

**Speaker:** Carla Pereira Marinho Silva (Universidade Do Minho)







# Tuesday,
15th November

## Nano/Bio-materials and Applications, 13:30 – 15:30

The areas of application for nanomaterials and biomaterials are diverse: In medicine, nanomaterials and biomaterials are used, for example, in tissue engineering and organ modeling or for patient-specific drug-releasing patches and the treatment of chronic wounds. Another area is the antibacterial activity of nanomaterials and biomaterials. Upscaling and preparation of market applications can be facilitated by the Open Innovation Test Bed on Bio-based Nanomaterials, presented as the BIOMAC project.

**Chair:** Gianluca Ciardelli

### 13:30 – 14:00
### Collagen-derived biomaterials in tissue engineering and organ modelling

**Speaker:** Gianluca Ciardelli (Politecnico Di Torino)

### 14:00 – 14:20
### Antimicrobial and antifouling nano-enabled hydrogel coating built on urinary catheters using a bottom up enzymatic approach

**Speaker:** Antonio J Puertas Segura (Universitat Politècnica De Catalunya)

### 14:20 – 14:40
### The BIOMAC Project – Access to a European Open Innovation Test Bed on Bio-based Nanomaterials

**Speaker:** Annika Frank (IBB Netzwerk GmbH)



Tuesday,
15th November

### 14:40 – 15:00
### Dual-function nanoparticles enzymatically conjugated with a custom-made polyurethane hydrogel for chronic wound treatment

**Speaker:** Giulia Crivello (Politecnico Di Torino)

### 15:00 – 15:15
### Development of a recombinant silk-elastin/essential oil biocomposite with antibacterial activity

**Speaker:** André Da Costa (SOLFARCOS)

### 15:15 – 15:30
### Green approaches to develop patient-specific drug-releasing patches for chronic wound treatment

**Speaker:** Rossella Laurano (Politecnico Di Torino)

# Tuesday,
15th November

## Bio-based Polymers, 16:00 – 18:00

Which building blocks are used for bio-based polymers? And how are they produced? The use and production of glycopolymers, polyhydroxyalkanaotes, exopolysaccharides from Rhodotorula yeasts, and Cyanoflan (a marine cyanobacterial polymer) are introduced. In addition, examples such as value-added produucts of lignin by enzymatic processes and the use of recombinant protein polymers for antimicrobial materials will be presented.

**Chair:** Artur Cavaco-Paulo

### 16:00 – 16:20
### Building blocks of polyhydroxyalkanaotes

**Speaker:** Manfred Zinn (University of Applied Sciences Western Switzerland)

### 16:20 – 16:35
### Production of high-value exopolysaccharides by *Rhodotorula* yeasts using sustainable feedstock

**Speaker:** Dana Byrtusova (Norwegian University of Life Sciences)

### 16:35 – 16:50
### Creation of antimicrobial materials using recombinant protein polymers

**Speaker:** André Da Costa (SOLFARCOS)



# Tuesday,
15th November

### 16:50 – 17:05
### Enzyme based processes for value-added products from lignins

**Speaker:** Renate Weiss (BOKU)

### 17:05 – 17:20
### Cyanoflan: a marine cyanobacterial polymer for skin well-being

**Speaker:** Rita Mota (acib GmbH)

### 17:20 – 17:35
### Sucrose bioconversion in high-added value alpha-glucans using engineered bacterial enzymess

**Speaker:** Claire Moulis (Toulouse Biotechnology Institute)

### 17:50 – 18:00
### Closing remarks

**Speaker:** Georg Guebitz

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## Microbial cell factories for the production of novel bacterial polymers

**Auxiliadora Prieto**[1]

[1]Spanish National Research Council-CSIC, Madrid, Spain

Nature offers a plethora of bacterial polymers with an infinite panel of different properties linked to their chemical structures. Their physical and chemical properties can be custom-designed for each specific application through the use of microbial biotechnology tools combined with material science. Cases such as bacterial cellulose (BC) and polyhydroxyalkanoates (PHAs) have drawn much attention due to their biodegradability, renewability and biocompatibility. BC, an extracellular biopolymer produced by bacteria of the genus Komagataeibacter spp., has been widely used in view of its excellent mechanical performance with a high capacity of retaining water in its nanofibrillar structure. PHA are intracellular biopolymers synthetized by bacteria of many genera, highlighted by their hydrophobic character and excellent barrier properties. In this work, we design cell factories for the production of novel PHAs via smart metabolic engineering and synthetic biology, to obtain next-generation-advanced materials with smart functional properties. Examples are PHAs carrying not natural groups in the side chain like halogens, biohybrid materials made of BC and PHAs, in which a polymeric component is brought to life with living organisms, and materials functionalized with anchored peptides or enzymes, with antimicrobial activity with applications as bioplastics in fields such as biomedicine and packaging.



IFPB Abstracts

## Polyhydroxyalkanoates, a dream come true?

**Iolanda Corrado**[1]**,** Giovanni Sannia[1], Cinzia Pezzella[1]

[1]Department of Chemical Sciences, University of Naples “Federico II”, Complesso Universitario di Monte Sant’Angelo, Napoli, Italy

Polyhydroxyalkanoates (PHA) are thermoplastic polyesters of 3-hydroalkanoic acids, produced as intracellular carbon and energy reserve trough microbial fermentation of different C-sources. Completely biodegradable in both soil and marine environments, PHAs are receiving an increasing interest as valid alternative to the conventional plastics. However, their commercial exploitation is strongly limited by the overall production costs. This work focuses on the exploitation of Cynara cardunculus L. as renewable feedstock for the production of two classes of PHAs. The attention has been focused on cardoon roots that are rich in inulin, a polysaccharide that can be converted into fermentable sugars by inulinases, and on cardoon seeds oil. The inulin hydrolysis by PlaI inulinases mixture was optimized by statistical approach and integrated for Polyhydroxybutyrate (PHB) production by Cupriavidus necator in both separated hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes. A maximum of 3.2 g L-1 of PHB accumulation, corresponding to 82% polymer content, was achieved in the SSF approach, and further implemented in a 7L bioreactor operated in a batch or pulsed-batch mode. On the other hand, cardoon seed oil was used in a bioprocess for mcl-PHA production by Pseudomonas resinovorans. A pulsed batch mode bioprocess was set up obtaining up to 2.3 g L-1 of mcl-PHA at 48 h. Physical blends, in which the PHB polymer matrix was added with mcl-PHA, were prepared evidencing a modulating effect of additive on PHB structural and thermal properties.

## Novel fungal source of aminopolysaccharides and aminopolysaccharide-glucans complexes

**Simona Dzurendova**[1], Boris Zimmermann[1], Dana Byrtusova[1], Ondrej Slany[2], Achim Kohler[1], Mounashree J. Urs[3], Stefan Cord-Landwehr[3], Bruno Moerschbacher[3], Milan Certik[2], Volha Shapaval[1]

[1]Faculty of Science and Technology, Norwegian University of Life Sciences, ÅS, Norway, [2]Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia, [3]Institute of Plant Biology and Biotechnology, University of Münster, Münster, Germany

Mucoromycota fungi are powerful cell factories producing a wide range of intra- and extracellular metabolites. The cell wall of Mucoromycota fungi is rich in aminopolysaccharides, such as chitin and chitosan and aminopolysaccharide-glucans complexes.

Aminopolysaccharides, chitin and chitosan, have unique properties and biological activity and are considered as one of the most versatile and promising biopolymers for the applications with stringent requirements. Global demand for these biopolymers is exceeding the current production based on seafood rest materials, mushrooms, and fungal biomass residues of acetic acid production. Mucoromycota fungi represent an important alternative source of chitin and chitosan. In addition, Mucoromycota may be the only source of natural non-chemically derived chitosans, since all industrially produced chitosans are chemically derived from chitin. Aminopolysaccharides in Mucoromycota cell wall are present in the form of complexes with glucans, which, as it was reported, may have high bioactivity in its natural state and do not need to be functionalised.

The total content of aminopolysaccharides-glucan complexes can reach up to 30-40% of the cell dry weight, and the production is highly dependent on the strain and growth conditions, such as the growth substrate composition, aeration or pH. Recently, we have identified the most powerful Mucoromycota aminopolysaccharides producers and develop fermentation process with integrated sensor-based monitoring for the production of Mucoromycota aminopoly-saccharides and aminopolysaccharide-glucans complexes using sustainable rest materials-based feedstock.



IFPB Abstracts

## Sourcing thermotolerant enzyme scaffolds for depolymerisation of PET

**John Mcgeehan**

[1]World Plastics Summit, , Monaco

The discovery of PET hydrolysis by a cutinase enzyme ~16 years ago, combined with the global pollution crisis caused by plastics waste in the environment has catalyzed a massive international community effort to discover, engineer, evolve, and implement a biological solution for polyester recycling. Prominent works from many groups in this field have demonstrated that enzymatic PET deconstruction is likely optimal near the glass transition temperature of the polymer (~70°C).
Large strides have been made towards developing effective PET hydrolases using protein engineering tools, especially in terms of the ability to deconstruct amorphous PET substrates at large scale and to high conversion extents. However, if enzymes are to offer a viable solution to polyester recycling, there is an urgent need to be able to deconstruct crystalline substrates as well, found in single-use beverage bottles and textiles. Indeed, we estimated that substantial savings from an economics, energy, and greenhouse gas emissions perspective could be avoided by reducing or removing mechanical pretreatment to make crystalline PET into an amorphous substrate for enzymatic hydrolysis.
With that motivation, we were interested to expand the suite of known thermotolerant enzymes and provide new scaffolds for the research and industrial communities. In this seminar I will present new data from the University of Portsmouth's Centre for Enzyme Innovation, NREL and the BOTTLE consortium. I will also share details of my new position, taking up the lead of the World Plastics Summit in Monaco, which I hope will open new opportunities for collaborative research and technology innovation.

## Engineering metabolism of plastic monomer 1,4-butanediol in an aromatics producing *Pseudomonas taiwanensis*

**Leonie Op De Hipt**[1], Yannic Ackermann[1], Benedikt Wynands[1], Tino Polen[1], Nick Wierckx[1]

[1]Forschungszentrum Jülich GmbH, Jülich, Germany

The plastic crisis is one of the biggest challenges of our time and drastic measures are needed, especially for plastics end-of-life. Microbiological upcycling represents a promising strategy for mixed waste streams. We are engineering metabolism of plastic monomers in an aromatic-producing Pseudomonas taiwanensis strain with the aim of upcycling plastic hydrolysates to valuable aromatic compounds. One important plastic monomer is 1,4-butanediol. Growth on 1,4-butanediol was achieved via laboratory evolution. Subsequent genome sequencing and reverse engineering revealed three genomic mutations. A mutation upstream of PVLB_10545 encoding a putative ethanol dehydrogenase was essential for growth on 1,4-butanediol. This dehydrogenase likely oxidizes 1,4-butanediol to 4-hydroxybutyrate. In P. putida this initial oxidation is performed by PedE (1), but no homologue of pedE is present in P. taiwanensis. The other two mutations enhanced growth on 1,4-butanediol. One resulted in an amino acid substitution in a LysR family transcriptional regulator (PVLB_12690), the homologue of which was also mutated in P. putida evolved on 1,4-butanediol (1). The second mutation led to an amino acid substitution in a sigma-54 dependent transcriptional regulator (PVLB_13305). The gene encoding this regulator is located upstream two genes encoding transport proteins putatively responsible for 1,4-butanediol uptake. In all, there are striking differences between 1,4-butanediol metabolism in P. putida and P. taiwanensis related to their varying dehydrogenase palette. Engineering the metabolism of this and other plastic monomers by P. taiwanensis now enables the conversion of plastic hydrolysates to aromatic platform chemicals.

(1) Li et al., 2020. Front. Microbiol. 11:382



IFPB Abstracts

## Low Carbon Footprint Recycling of Post-Consumer PET with the Metagenomic Polyester Hydrolase PHL7

**Paula Blázquez-Sánchez**, Konstantin Richter[2], Ziyue Zhao[1], Felipe Engelberger[3], Christian Wiebeler[1], Georg Künze[3], Ronny Frank[4], Jörg Matysik[1], Wolfgang Zimmermann[1], Norbert Sträter[2], Christian Sonnendecker[1]

[1]Institute of Analytical Chemistry, University of Leipzig, Leipzig, Germany, [2]Institute of Bioanalytical Chemistry, Centre for Biotechnology and Biomedicine, University of Leipzig, Leipzig, Germany, [3]Institute for Drug Discovery, Medical School, University of Leipzig, Leipzig, Germany, [4]Centre for Biotechnology and Biomedicine Molecular Biological-Biochemical Processing Technology, University of Leipzig, , Germany

Polyethylene terephthalate (PET) has become one of the most produced plastics world-wide. Despite several recycling options, the majority of the waste stream finally ends up in landfills or is incinerated. Depolymerization and recovery of the monomeric units terephthalic acid and ethylene glycol represents an alternative pathway to close the recycling loop for PET. Such kind of tertiary recycling strategy can contribute to reduce the consumption of fossil resources, the release of CO2 emissions and environmental pollution. Enzymatic recycling has emerged as a new technology to depolymerize amorphous PET (e.g. thermoform packaging) under mild conditions without the need of any pretreatment. Polyester hydrolase PHL7, isolated from a compost metagenome, can completely degrade PET thermoforms at 70°C in aqueous solution within 24 hours, using 0.6 mg of enzyme per gram of PET. For a PET film, hydrolysis rates of 91 mg TA h-1 mgenzyme-1 have been observed, making PHL7 the most active PET-degrading enzyme yet discovered.
We present a cocrystal structure of this enzyme with its hydrolysis product, terephthalic acid (TA), and elucidate the binding mode of this ligand as well as the influence of individual mutations on the hydrolytic activity and thermal stability. We also shed light on the role of leucine at position 210 for the extraordinarily high PET hydrolytic activity of PHL7.
As a proof of concept, TA recovered from the enzymatic hydrolysate was used to synthesize virgin PET, demonstrating that it is possible to close the consumption-recycling loop with a low-carbon footprint process.

## Meeting the industry's needs: hyperstable protein design

**Ezequiel Juritz**[1]

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Proteins have evolved through millions of years to meet the biochemical needs of living organisms. At Enantis, we are working on designing proteins that meet the needs of a more sustainable and cost-effective industry since 2006.
From health care to food ingredients, every space in the global industry demands not only extremely efficient processes, but also more sustainable standards – and optimized enzymes represent fundamental piece of this solution.
Our approach stems from a strong partnership between academia and industry. We gathered a multidisciplinary team focused on decoding protein structure stability patterns to design and implement protein engineering software focused on future industry needs.
The result: a set of nine proprietary software tools that are commercially available, with applications ranging from thermostability improvement to binding pocket analysis. The best validation of our approach is our own portfolio of upgraded proteins: our protein engineering know-how coupled with proprietary state-of-the-art software tools enabled us to improve the stability of our internal targets, leading to the design of five hyperstable proteins and three patent applications filed.
At Enantis we offer commercial licenses for protein engineering software that has been extensively validated in a broad spectrum of industries, as well as efficient and cost-effective protein engineering services tailored for any of your needs.



IFPB Abstracts

## Mechanistic study of enzymes used for energy saving in refining or recycling of cellulose fibers

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The refining of cellulose fibers is an essential process during paper production, aiming at improved paper properties such as increased tensile strength and smoothness, however, it is energy demanding. Pre-treatment with enzymes (e.g. endoglucanases) lowers required refiner revolutions and thus saves energy. The use of commercial enzyme formulations is convenient, but the high number of available products complicates prediction of their behaviour, as each formulation acts differently, especially among pulp types. Due to the versatile composition of the included enzymes, a lot of knowledge about the enzymes and their effects on pulp and paper products is still needed. Similarly, produced products need to be recycled in an environmentally friendly way using the most efficient enzymes. Therefore, comparative studies of the effects of commercial enzyme formulations were conducted. Moreover, endoglucanases were purified from enzyme formulations and tested in laboratory refining trials along with a commercial endoglucanase formulation to allow a mechanistic study. Extensive basic characterisation of the enzymes involved assessment of their hydrolytic activity on model substrates like derivatized cellopentaose (CellG5), carboxymethylcellulose or filter paper as well as activity on different pulps (softwood or hardwood). Further studies using sophisticated techniques like CLSM (confocal laser scanning microscopy) in combination with fluorescently labelled carbohydrate binding modules, HPLC and NMR indicated the major role of endoglucanases in refining and recycling with concomitant need of β-glucosidases to avoid product inhibition. Combination of enzyme assays, analytical methods and imaging technologies enable prediction of the most efficient enzymes based on activity of the model substrate CellG5.

# **IFPB** Abstracts

## Resource effective textile processes for functional and smart textiles.

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Research at the research group Textile Material Technology at the University of Borås focuses on the development of advanced functional and smart materials using novel, resource-effective processes to produce such materials in an effective and efficient way. Examples of such technologies in the TMT group are:

- Digital printing:
- Inkjet for functional and smart textiles
- 3D printing
- Valvejet (Chromojet)
- Supercritical $CO_2$ (liquid $CO_2$)
- Spray technology
- UV curing
- Plasma
- Catalysis and Biocatalysis

It is a very multidisciplinary domain were e.g. interface and surface science, (bio)catalysis, chemistry, biotechnology, digital technologies (inkjet, 2D and 3D printing), 3D body scanning, coating, printing, dyeing, and nanotechnology meet.





## Introduction of sustainable circular economy into fast fashion: biotechnological approaches for textile waste recycling

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Overproduction and overconsumption of textile is the result of the so-called "fast fashion" trend, where various retailers design new clothing models monthly, triggering the concept into customers to throw barely used clothes. Therefore, the life cycle of textiles became shorter and shorter, incrementing the generation of textile waste. Europeans discard 11 kilograms of textiles per person and year. 87 % of the textile waste is still landfilled or incinerated [1, 2]. Textiles usually consist of blended fibers which comprise natural and synthetic fibers, for example cellulose (cotton, viscose) together with PET or polyamide. Biotechnological approaches enable the specific separation of fibers by the application of enzymes. The cellulosic fibers are hydrolyzed by cellulases leaving pure synthetic fibers textile-to-textile recycling [3]. The recovered building block glucose from the depolymerization of cellulose can be used as a substrate in microbial fermentation processes for valorization of each component. To further improve biotechnological recycling approaches, the concept of simultaneous saccharification and fermentation (SSF) is introduced in this study. The intermediate steps of recovery and separation of the glucose solution are not required in the SSF process, since the bacterium Weizmannia coagulans, grows at the same enzymatic optimal conditions. The glucose obtained from cellulose hydrolysis is used as carbon source by the microorganism and in parallel produces lactic acid ,a potential building block for synthesis of the bio-based polylactic acid (PLA) [4]. In this concept two valuable products, pure PET and lactic acid are recovered, valorizing each maximizing and supporting the development towards a circular economy.

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IFPB Abstracts

## Biotech synergies to solve plastic recycling and contamination challenges: RECOVER project

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The increasing plastic production, the single-use materials, and the insufficient capacity of the waste management systems for the elimination or recycling have driven global plastic pollution. The complex nature of plastic waste in terms of the diversity of polymers and the presence of multilayers of different materials, as well as food scraps and other remainings, cause technical and profitability issues, preventing further expansion of recycling, which only account for around 30% in the EU. The destination of these heterogeneous waste streams is usually their disposal in landfills or incineration, with the consequent loss of resources and aggravation of environmental pollution. The BBI JU H2020-funded RECOVER project addresses these challenges by exploring new routes for the biotechnological recycling of plastics and their removal from soil and compost. The project focuses on plastic waste from food packaging and agriculture and involves the synergistic action of microorganisms, new enzymes, earthworms and insects. These combined biocatalytic systems are expected to maximize transformation performance, enable the treatment of mixed plastic waste and turn petroleum-based plastics into biodegradable plastics in a single step. This work presents the most significant advances at the lab scale on the biotransformation of plastics of fossil origin using microbial consortia capable of degrading a wide range of plastic polymers and increasing the plastic conversion efficiency of insects and earthworms by incorporating microorganisms. The optimized processes will be later scaled using real plastic waste under composting or insect culture for urban solid waste plastics and agricultural plastics or be applied directly to the soil in the case of bioremediation of contamination. In addition, a new management model will be proposed, and biofertilizers and bioplastics will be obtained for agriculture and food packaging, helping to close the loop in both productive sectors.

## Combination of physico-chemical pretreatments with biological depolymerization to recycle mixed plastic waste. BioICEP

**Pablo Ferrero**[1], Rafael Jimenez-Lorenzo[1], Olivia Attallah[2], Margaret Brennan Fournet[2], Jasmina Nikodinovic-Runic[3]

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Approximately, 367 million tonnes of polymers were produced worldwide in 2021. One of the main properties of most of these polymers are their stability and durability which can be an advantage for its use. However, this property becomes in a problem at the end of their life causing environmental problems such as the plastic pollution of different environmental compartments. BioICEP project aims to solve this problem by developing sustainable biorecycling process for plastics combining physico-chemical pretreatments with biological depolymerization.
Pretreatments such as chemical oxidation by reactive extrusion, supercritical CO2, microwave thermal depolymerization, ultrasonication and UV irradiation has been explored for different polymers. The results have shown that reactive extrusion modified the surface of recalcitrant and non-biodegradable plastics such as LDPE making it more amenable for biodegradation.
Different strains have been isolated with high metabolic capacity to degrade polymers such as PET, polyurethane, PLA, etc. and enzymes involved in the degradation of polymers have been identified and characterized.
The microbial and enzymatic degradation of plastics has been carried out obtaining as product monomers that are recovered. The main monomers recovered until now has been terephthalic acid, ethylene glycol and styrene from the pretreatment and biodegradation of PET and polystyrene, respectively.
These monomers have been used as the substrate of different fermentation to obtain bioproducts with added value, so the plastic waste is converted finally into high market demand biodegradable products such as PHB, nanocellulose and biosurfactants.

In conclusion, BioICEP has developed a combined process to enhance the plastics biorecyling.



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## Contribution of fermentation technology to a more sustainable plastic sector – the plastic biorefinery

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Recycling facilities are currently struggling when dealing with challenging mixed plastic waste, multi-layers, blends, and additives. Consequently, plastics are too often incinerated, landfilled, or spilled into the environment. The development of new biotechnological recycling technologies could contribute to alleviating this problem, by converting persistent plastic waste into more easily recyclable and/or degradable polymers.
UPLIFT is a H2020 4-year collaborative project, involving 15 partners that will work together to develop a more sustainable plastic packaging value chain in the Food and Drink sector, by applying novel biochemical upcycling technology routes and eco-design strategies. Overall aim is to produce, at a pilot scale, new eco-polymers and plastic materials that are easier to recycle.
From Biorefineries to renewable building blocks for plastics: the main idea is to integrate the fossil-based (depolymerized) plastic monomers with bio-based (fermented) building blocks, in order to obtain more carbon-neutral polymers. Eco-design of renewable and easy-recyclable eco-polymers will pave the way to a more sustainable plastic system, making post-consumer plastic waste an available feedstock for the circular economy, also thanks to a biorefinery approach. By keeping the plastics in the loop and integrating with bio-based building blocks (instead of using virgin fossil-based monomers), UPLIFT will contribute to increasing the sustainability, as well as the current recycling rates and the percentage of bioplastics used. At the same time, the project will reduce plastic waste generation and greenhouse gas emissions associated with plastic production. Overall, UPLIFT will support the transition to more efficient and circular plastic sector, integrating the current mechanical and chemical recycling. A case study with the techno-economic analysis of a plastic biorefinery will be presented, to show the viability of this approach.

## Applying ligninases to resolve end-of-life issues of thermoset composite plastics

**Mongili Beatrice**[1]

[1]Biosphere

One of the major threats to our ecosystem is the significant increase in the accumulation of plastic waste. Among plastic materials, thermoset composite materials belong to those synthetic polymers that are hard to recycle due to their structural and chemical features. These are polymers that, after a process of curing, develop a dense crosslink matrix that is hard to break. Thus, thermosets are frequently stored in landfills or incinerated, once their end-of-life is reached.
BIZENTE, a BBI-funded project under the support of EU Horizon 2020 programme, is paving the way to develop an innovative recycling process for thermoset composites based on the application of ligninolytic enzymes in combination with targeted mechanical and chemical pre-treatment.
With this approach, BIZENTE forecasts to develop a new value chain for these recalcitrant substrates. Particularly addressed to epoxy-based, vinyl- and polyester-based thermoset composite resins. In this frame, the process of enzymes production and resin biodegradation will be presented.





## Recycling processes for multilayer packaging materials based on selective enzymatic depolymerisation

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Multilayer plastics consisting of more than one type of polymer are widely applied as e.g. food packaging. Use of multilayer plastics is advantageous due to their good barrier properties or to enable efficient sealing of the packaging. However, multilayer plastics provide a major challenge from the recycling perspective, as it is extremely difficult to separate the various layers of the material to obtain pure polymer streams that can be recycled by conventional mechanical recycling. Therefore, multilayer packaging is currently essentially non-recyclable. In the BBI-JU-financed ENZYCLE project, we aim to enable recycling of multilayer packaging by harnessing the ability of enzymes to selectively depolymerise a single polymer present in a multilayer material. In particular, we are targeting multilayer packaging composed of a PET and a polyolefin layer. The PET layer is enzymatically depolymerised to yield products that can be applied in the production of new plastics. Meanwhile, a residual polyolefin layer is obtained, which may be recycled using conventional recycling technologies. This talk will discuss the latest status of these developments.

## Potential application of electrospinning/ electrospray in development of chitin and chitosan-based products

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In recent years, the development of natural biopolymer-based products suitable for different applications has received considerable attention. The key specifications of these products are biocompatibility and biodegradation, both in the environment and in the human body. The constituent polymers and their decomposition products are thus expected to be safe and without side effects. Chitin, is typically a by-product of fishery and plant biomass, but can be extracted even from insect's exoskeleton, chitin can be reused and converted to novel high-value materials for different applications, which are bio- and eco-compatible. In the running project RECOVER "Development of innovative biotic symbiosis for plastic biodegradation and synthesis to solve their end of life challenges in the agriculture and food industries", the management of hardly recyclable plastic waste generated by light weight single use packaging and by agricultural films, is improved by applying biotechnological approaches, combining microorganisms, new enzymes, earthworms, and insects to work collaboratively, to degrade plastics and in a circular economy approach chitin is extracted from larvae and insect exoskeleton, transforming this biomass into an added-value products. From chitin, chitin nanofibrils (CNs) can be introduce, these are an interesting bio-based nanomaterial with high anti-bacterial activity. We report the investigation of the possible advantageous use of CNs in combination with bacterial cellulose (BC) for food packaging applications, closing the loop of food packaging waste valorisation. In the present study BC was used to produce electrospun fibre meshes, to be surface-modified via electrospray of CNs to reach a uniform distribution. BC nanofibers with desirable size and morphology were successfully prepared and functionalized with CN using electrospinning and tested in vitro with human keratinocytes (HaCaT cells). Application of CNs improved the indirect antimicrobial activity of the electrospun fibre meshes by upregulating human defensin 2 expressions. This natural and eco-sustainable mesh is promising in skin contact and food packaging applications. Chitosan, a partially or completely N-deacetylated derivative of chitin is known for its biocompatibility, non-toxicity, antifungal activity, water-binding capacity, bioactivity, and antimicrobial properties which make it a particularly attractive substitute for synthetic polymers in different application fields. Electrospinning of poly(hydroxybutyrate-co-hydroxyvalerate)/chitosan blend was also investigated since both polymers are biobased and have great potential in the development of bio-based products for different applications. The produced fibre meshes were characterized and in vitro tested with different cell types, including HaCaT cells, showing good cytocompatibility.

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## The microbiome and metabolome present on clothing textiles

**Chris Callewaert[1]**

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Clothing textiles protect the human body against external factors. These textiles are not sterile and can harbor high bacterial counts as sweat and bacteria are transmitted from the skin. We investigate the impact of clothing textiles and laundering processes on the skin microbiome and metabolome. We found that worn T-shirts develop a differential microbiome as compared to the skin microbiome and that a specific malodour can be formed after incubation at room temperature for 24h. Each clothing textile has a selective enrichment / inhibition of microbiota. Laundering of the clothes did not result in a reduction of microbial counts; on the contrary, it resulted in a microbial exchange and selection on washed clothing fabrics. High levels of living bacteria were retrieved after the laundry process, when washing at 30°C and using an enzyme-based detergent.
Antibacterial (silver) T-shirts are a popular solution to prevent malodour. However, we found that significantly more bacteria were present on skin after wearing nanosilver clothes, as compared to the same clothes without silver incorporated. We also found higher levels of mono unsaturated fatty acids on the skin when wearing silver-incorporated textiles. Lastly, we explore new ways to solve smelly and permastink clothes, by pre-emptively incorporating non-odorous and benign bacteria in the textiles. This is called "probiotic textiles" and is regarded as a sustainable and alternative way to combat textile malodour.

## Innovative surface modification by fungal hydrophobins and their chimeric variants

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The biological interfacing of materials covers both the search for novel materials endowed with good biocompatibility, cost, and for effective immobilization procedure ensuring stable and spatial anchoring of biomolecules with a precise orientation. In this context, self-assembling amyloid proteins provide new and tangible opportunities as platforms to develop biohybrid functional materials. Indeed, adhesion to solid interfaces of amyloid protein layers can efficiently play the role of connecting bridges between organic and inorganic surfaces. Among self-assembling proteins, fungal Class I hydrophobins are characterized by high propensity to form amyloid fibrils and to efficiently and easily adhere to several conventional and nanostructured surfaces by direct deposition.
Our research group has exploited the layers of Class I hydrophobins in various application fields, both as an attractive platform to immobilize proteins and/or peptides on different surfaces, and as an antibiofilm coating to inhibit bacterial biofilm formation.
This adhesive ability has been further exploited by genetic fusion, designing and producing chimeric proteins built by fusing the self-assembling adhesive moiety of the fungal hydrophobin to various biotechnologically relevant proteins. Following this approach, different proteins and enzymes have been functionally combined to a class I hydrophobin and have been stably anchored on classical surfaces and 2D nanomaterials with a favourable orientation, thus forming a homogeneous biological layer on biodevices.
Our results establish the effectiveness and versatility of both fungal hydrophobins and their chimeric variants in surface functionalization.

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## Inkjet printing of enzymes on synthetic fabrics

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Enzymes can be immobilized on textiles to impart anti-microbial properties in a more environment-friendly manner compared to conventional biocide-based solutions. Such application requires ensuring precise, flexible and contamination-free immobilization methods that can be offered by digital printing compared to coating or screen-printing techniques. Drop-on-demand inkjet printing is a resource-efficient technology that can ensure these requirements. The use of polyester and polyamide based fabrics is rising for applications ranging from apparel and home furnishing to hygiene and medical textiles. These fibres offer superior chemical, physical, and mechanical properties due to their inert nature but challenge the printing process due to hydrophobicity and lack of functional groups. Lysozyme and tyrosinase are two enzymes showing great potential for grafting on synthetic fabrics paving the way to use them for inkjet printing as well.

Challenges for inkjet printing of enzymes on synthetic fabric surfaces come in multiple forms i.e. ink recipe formation, printer mechanics and fabric surface characteristics. The ink must maintain a suitable viscosity and surface tension for effective drop ejection and a feasible ionic nature for enzyme activity. Then, the enzyme must be able to sustain the temperature and shear stress generated inside an inkjet printhead. Finally, influential fabric characteristics include surface structure, pore size distribution, evaporation rate and binding mechanism. By considering these parameters, lysozyme and tyrosinase were successfully printed on variously modified synthetic fabrics using a combination of sustainable technologies.

## Insights on the hair keratin structure under different drying conditions

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We found that different drying conditions greatly influence the wearing fatigue of human hair. The removal of excess water with a microfiber towel (80% polyester/ 20% nylon) seems to retain more integrity on human hair when compared with drying by a blow-dry and a cotton towel. The effects of humidity and temperature were evaluated. It was found that hair dried with a microfiber towel showed strong intensity bands at 1664 cm-1 due to alpha-helix structure, while no intensity was exhibited in the spectrum of the hair treated with a blow drier and a cotton towel. A good agreement with experimental results was obtained since fatigue recovery of the hair sample dried with a microfiber towel showed double wear resistance when compared with the cotton towel and blow-drier process. These findings were supported by DSC which aided in the elucidation of the secondary structure of alpha-keratin in the stabilization of hair. Exposed to high temperatures, DSC profiles provided information about the movement of water molecules from hair fiber being the enthalpy for microfiber towel with the highest value. Contributions from damage, friction, and heat were correlated with the molecular structure of hair integrity, and the microfiber towel preserves the alpha-keratin structure.





## Removal of pharmaceutical residue from wastewater using catalytically active functional textiles

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The increase in human life expectancy coupled with the rise in population has boosted the use of pharmaceuticals. These biologically active compounds do not fully metabolize by the human body and are excreted out into the wastewater which is often resistant to conventional wastewater treatment processes. Herein, this study presents the prospects of catalytically active textiles-based heterogeneous bio-electro-Fenton reactors for the effective removal of pharmaceutical residue from wastewater. The reactor consists of a bio-anode prepared by immobilized redox enzyme on synthetic nonwoven textiles and a cathode by zerovalent iron nanoparticles immobilized functional textiles. The resultant reactor has been extensively explored for the removal of pharmaceuticals from simulated wastewater. The results of this current study will be of great importance as it is expected to deliver the much-needed upgrade in the conventional wastewater treatment system to remove persistent organic pollutants with a bio-based, sustainable, and textiles-based alternative.

**Keywords:** Biocatalysis; Enzymes immobilization; Functional textiles; Wastewater treatment.

## Crosslinking of proteins with laccases: implications for fibre modification

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In this work, we describe how proteins can be crosslinked with phenolic compounds using laccases. We have used BSA as a model protein and tyrosine as a crosslinking agent. This concept was further applied to the modification of wool and hair fibers. The shape modification was evaluated by quantifying the number of curly twists on the grafted samples. The thermal and mechanical properties of the grafted wool yarns were evaluated by TGA, DSC, and breaking strength determination. The concentration of free thiols and weight gain were assessed aiming to infer the role of the cysteine pre-treatment on the final tyrosine grafting and shape modification. The laccase-assisted grafting of tyrosine onto keratin fibers have influenced the thermal and mechanical properties however without compromising their structural integrity for the final application purposes.





## Collagen-derived biomaterials in tissue engineering and organ modelling

**Gianluca Ciardelli[1]**, Monica Boffito[1], Chiara Tonda-Turo[1], Valeria Chiono[1]

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Tissue Engineering (TE) reconstructs damaged tissues/organs exploiting biocompatible and biodegradable scaffolds, taking often advantage of natural materials' bioactive/biomimetic cues together with advanced 3D processing techniques. In this scenario, collagen-derived materials (e.g. gelatin) have played a pivotal role. This paper will review past and recent outcomes of my research in the field, focusing on how collagen-based materials have been transferred from TE to the realization of 3D tissue/organ models for disease monitoring and drug design by sustainable and ethical approaches. First research has shown the potential of enzymatic or genipin crosslinking to develop collagen-derived biomaterials, mainly exploited as fillers for hollow guides in peripheral nerve regeneration. Gelatin was also imprinted onto polymeric scaffolds by the SOFT-MI technique. A procedure for preparing bone-derived biomimetic soluble collagen was also developed "in-house". More recently, collagen coatings with custom polyurethanes were developed to model healthy and osteoporotic bone. Collagen is, moreover, a key component to design 3D skin equivalents for assessing biological safety and biocompatibility. Gelatin was exploited as bioink for 3D-bioprinting. Indeed, gelatin/nano-hydroxyapatite bioinks have permitted the realization of bone tissue models, while cellularized gelatin bioinks were used to fill synthetic scaffolds mimicking cardiac tissue. Electrospun gelatin membranes were also exploited to design alveoli and pancreatic adenomere replicas in disease modelling.

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## Antimicrobial and antifouling nano-enabled hydrogel coating built on urinary catheters using a bottom up enzymatic approach.

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Catheter-related urinary tract infections represent 40 % of all nosocomial infections and are emerged concern for the public health due to the increased mortality and morbidity worldwide. The drug-resistant bacteria and the biofilm formation on the surface of indwelling devices overcome the antimicrobial agents causing device dysfunction and severe chronic infections, associated with longer period of hospitalization and huge financial burden. Herein, antimicrobial and antifouling nano-enabled hydrogel coating was constructed on urinary catheters in an enzymatically triggered bottom-up approach. Foremost, silicone catheters were plasma-activated and preaminated with (3-Aminopropyl)triethoxysilane, allowing the laccase-catalysed grafting of the silver/phenolated lignin nanoparticles (AgPL NPs) with high antibacterial activity towards medically relevant Gram-positive and Gram-negative urinary pathogens. Subsequently, the tethered phenolic residues from the AgPL NPs were activated by laccases to phenoxy radicals, triggering an enzymatically initiated radical polymerization of anti-fouling carboxybetaine methacrylate monomers on the catheters' surface in a "grafting from" process. The developed hybrid nano-hybridized hydrogel-coating led to increased hydrophilic features of the silicone material preventing the undesirable protein attachment on the catheter's surface. The hybrid hydrogel coating demonstrated outstanding antibacterial activities reducing the Pseudomonas aeruginosa and Staphylococcus aureus viability by 4 and 3 Logs, respectively, compared to the stand-alone hydrogel coatings. Moreover, the hybrid coating prevented the formation of drug-resistant biofilms on the surface of catheters by 60 % for S. aureus and P. aeruginosa without affecting human cell viability after exposure to the nanocoatings for 7 days, showing to be promising strategy for prevention of urinary tract infections.



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## The BIOMAC Project – Access to a European Open Innovation Test Bed on Bio-based Nanomaterials

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To address global climate goals while reducing consumption and dependency on fossil resources, the European Sustainable BIObased nanoMAterials Community (BIOMAC) was established within the European Commission's Horizon2020 framework. BIOMAC's 29 core partners cooperate closely in an open innovation test bed (OITB) for nano-enabled, biobased materials derived from renewable resources. Here, cellulose nanocrystals and nanofibrilated cellulose as well as nanolignin and bionanochar find their application as additives in biobased polymers such as polylactides, polyurethanes and succinate based polyesters. 5 Internal test cases evaluate the new materials in agricultural, automotive, packaging, construction and functional textiles applications.

**The BIOMAC ecosystem is composed of:**

- 17 pilot lines covering the value chain from feedstock to product;
- three transversal service hubs as well as an exploitation and dissemination management for customer support;
- a single-entry point (SEP), represented by the IBB Netzwerk GmbH, which acts as contact point for clients and has administrative responsibilities as well as representative rights.

In December 2022, a BIOMAC open call will be launched. We are looking for partners with bionanomaterial applications who wish to take their laboratory test cases from lignocellulosic feedstock to high value, nano-enabled industrial prototypes. In return for their invaluable feedback, access will be granted free of charge to this innovative one-stop-shop set up with access to the entire bionanomaterial production and service chain.

## Dual-function nanoparticles enzymatically conjugated with a custom-made polyurethane hydrogel for chronic wound treatment

**Giulia Crivello**[1], Giuliana Orlandini[1], Angela Gala Morena Gatius[2], Clara Mattu[1], Monica Boffito[1], Tzanko Tzanov[2], Gianluca Ciardelli[1]

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Hydrogels are attractive drug delivery systems with the potential to protect their cargo and control its release. In particular, hydrogels based on synthetic polymers are gaining increasing interest by virtue of their controllable chemistry, ease of modification, and reproducibility. Moreover, the presence of specific side chains and pending functional groups in the polymer structure allows for the conjugation of drugs and other compounds resulting in improved control over drug release. Enzymes that catalyse reactions in a very specific way could also be used to control the conjugation of compounds to the polymeric chains to improve reproducibility and biocompatibility of the conjugation process.
This contribution describes an innovative system for drug delivery comprising a bioartificial supramolecular hydrogel based on a customised polyurethane and α-cyclodextrins, and nanoparticles, for application in the treatment of chronic wounds. The system has the potential to reduce inflammation and eradicate infection by virtue of dual-function nanoparticles which incorporate cobalt as antimicrobial agent, and phenolated lignin as antioxidant. The nanoparticles are enzymatically conjugated to the hydrogel by means of the amine side groups exposed along the backbone of the ad-hoc synthesised polyurethane. The oxidase enzyme laccase is exploited to oxidize the phenol groups of lignin, to allow their interaction with the amines on the hydrogel. The effects of nanoparticles conjugation to the hydrogel are studied through gelification tests, stability tests, and rheology. Moreover, the release of nanoparticles from the hydrogel and their effects on patients' wound fluids and against relevant bacterial strains are analysed in vitro.



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## Development of a recombinant silk-elastin/ essential oil biocomposite with antibacterial activity

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The development of sustainable functional materials is attracting increased attention due to current environmental pressing issues and the pursuit for new "green" alternatives, prompting the search for new material designs with enhanced functionality. In this work, we describe the formulation of "green" bioactive composites comprising a genetically engineered silk-elastin-like protein (SELP) and essential oil from Mentha piperita (MPEO) to obtain a material displaying antibacterial activity. SELPs are genetically engineered protein polymers that combine in the same molecule semicrystalline silk- and elastomeric elastin-blocks to provide tensile strength, ductility and water solubility. The essential oil from Mentha piperita (peppermint) is widely used in the pharmaceutical and cosmetic industries, showing antimicrobial properties against bacteria and classified as GRAS for both people and environment. MPEO was used for the formulation of aqueous emulsions with Tween 80 as surfactant and SELP as structural matrix for film formation. For composite formulation, several parameters were investigated. SELP/MPEO biocomposite films were obtained by solvent casting using water as a solvent, and evaluated for the antimicrobial performance against Gram-positive and Gram-negative bacteria. The SELP/MPEO films demonstrated to inhibit the growth of most of the bacterial strains and showed to retain the antibacterial activity over short-term storage for 7 days at 4 °C. These results point to the potential of using natural active fillers and recombinant protein polymers for the development of new sustainable active materials.

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## Green approaches to develop patient-specific drug-releasing patches for chronic wound treatment

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The design of patient-specific wound patches is gaining increasing interest due to the lack of wound dressings able to effectively treat chronic ulcers supporting tissue regeneration. The main factors limiting their effectiveness lie in the uncontrolled drug release mechanism and in the availability of standard formats requiring adaptation to the irregular morphology of the wound cavity. To overcome these criticisms, we engineered multi-stimuli-responsive polymers, starting from customized poly(ether urethane)s (PEUs), to obtain biomaterial-inks able to work as smart drug carriers and be processed in the form of 3D patches perfectly replicating the wound bed. This purpose was achieved by exploiting two green functionalization procedures, i.e., the water-based carbodiimide chemistry and the solvent-free plasma treatment to graft photo-sensitive (i.e., thiol and acrylate moieties) and carboxylic acid groups, respectively. PEU-based hydrogels showed responsiveness to temperature, alkaline-pH and Vis-light, which were exploited to encapsulate the payload and tune system viscosity, release their content via a pH-controlled mechanism and reinforce the network upon light exposure, respectively. Results evidenced hydrogel capability to modulate payload (e.g., Ibuprofen) release in response to wound clinical needs (e.g., alkalinity of infected wound exudate) and to be processed through solvent-free bioprinting techniques obtaining 3D constructs with patient-personalized morphology. Lastly, PEU-based biomaterial-inks showed excellent cytocompatibility according to the ISO 10993:5 regulation. Hence, the versatility of PEU chemistry and the exploitation of green functionalization and fabrication approaches resulted in the development of smart wound dressings with promising features to overcome current drawbacks.

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## Building blocks of polyhydroxyalkanaotes

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The material properties of the biopolymer polyhydroxyalkanoate (PHA) are determined by the monomeric unit composition consisting to a large percentage of enantiomerically pure [R]-3-hydroxyalkanoates. To date, more than 150 different monomers have been reported in literature that are synthesized by microorganisms, where they serve as a carbon and energy storage compound. The chemical synthesis of block copolymers revealed novel material properties by the linkage of two different polymer types.
Recently, it has been found that the polymerizing enzyme PhaC plays a crucial role in polymerizing other than 3-hydroxyalkanoates but also in sequence regulating activity. A recombinant Escherichia coli JM109 strain with the a chimeric class I PHA synthase comprising PhaCs from Aeromonas caviae and Ralstonia eutropha (formally Cupriavidus necator) has such a unique sequence-regulating capacity and spontaneously synthesizes block copolymers from a mixture of substrates. It has been found that PhaCAR provides a versatile biosynthetic system for random and block copolyesters comprising 2-, 3-, 4-, 5-, and 6-hydroxyalkanoates.

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# **IFPB** Abstracts

## Production of high-value exopolysaccharides by *Rhodotorula* yeasts using sustainable feedstock

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Nowadays, we are witnessing fast climate change all around the world. It is resulting in increased demand for bio-based products which can replace chemical and synthetic compounds. Extracellular polysaccharides are biopolymers secreted by microbes into the culture media, outside of the cell. Many of them are extensively researched for their biocompatibility, antimicrobial, antitumor, thickening properties, etc. They can be applied in food, pharmaceutical, fabric industry, or for personal care formulations. Despite their beneficial properties only a few of them have found commercial application, mostly from bacterial origin.

Rhodotorula yeasts are promising organisms for the production of high-value multifunctional biomass from food and agriculture supply chain by-products since they can utilize a wide range of growth substrates. Due to the versatile metabolic system, different Rhodotorula yeasts can co-produce a wide range of chemicals in addition to lipids: β-glucans, carotenoids, and exopolysaccharides. In this study, we present a development of the fermentation process for valorizing sugar-rich waste materials and protein by-products from the fish industry into multifunctional biomass with enhanced exopolysaccharides biosynthesis. For our purposes, twenty carotenogenic yeasts of different genera were selected for high-throughput screening in Duetz-MTPS and investigated for extracellular biopolymers production. Among the Rhodotorula yeast, several of them can overproduce exopolysaccharides when using lignocellulose feedstock compared to the synthetic media. Monosaccharide composition showed the presence of galactose, glucuronic acid, and mannose.

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## Creation of antimicrobial materials using recombinant protein polymers

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The increasing healthcare-associated infections and antibiotic-resistant microorganisms demand new antimicrobial compounds and antimicrobial materials with improved efficiency and reduced propensity to elicit resistance. By combining the antimicrobial activity of naturally occurring antimicrobial peptides (AMPs) with recombinant protein polymers, such as elastin-like recombinamers (ELRs), it is possible to create novel materials that can be explored for the development of advanced antimicrobial medical devices. In the present work, through the use of recombinant DNA technology, we have functionalized the ELR A200 with different AMP sequences to obtain biopolymers with antimicrobial activity. The antimicrobial-functionalized biopolymers were biologically produced in Escherichia coli and purified via a simplified non-chromatographic method comprising heating/cooling cycles, resorting to the thermoresponsive behaviour of A200. These were further processed into different materials namely, particles, fibres and free-standing films. The produced materials demonstrated to be highly effective against an assortment of different microorganisms including Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi in both in vitro and ex vivo assays. Further, the antimicrobial performance showed to be time-dependent and mediated by direct contact, suggesting a mechanism of irreversible cell damage and disruption. Moreover, the antimicrobial materials demonstrated to be non-cytotoxic to both normal human skin fibroblasts and human keratinocytes. This strategy can be applied to other peptides and proteins to create functionalized materials suitable for several applications. This work was supported by the strategic programme UID/BIA/04050/2020, funded by Portuguese national funds through FCT IP. RM acknowledges FCT IP for funding (CEECIND/00526/2018)





## Enzyme based processes for value-added products from lignins

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The ever-rising demand for energy and materials make it necessary to find new ways and strategies to solve this problem. There is a common consent in science, politics, and the general public that the implementation of circular economy systems is one feasible strategy to tackle the current situation. Reorientation from classic, often fossil-based, processes and technologies toward renewable-resources-based ones is necessary. Lignin is the second most abundant natural material on earth. It is generated as a waste product from paper and ethanol production. The worldwide production of lignin is approximately 100 million tonnes.The complexity and richness of its functional groups makes it attractive for converting into value-added products. Lignin has been predominantly burnt as fuel for heat and power. Enzyme (i.e. laccase) catalyzed polymerization has shown a great potential to convert lignins into value added products such as coatings or adhesives. Aside from polymerization and depolymerization of lignin, the radical reaction mechanism of laccase with lignin allows for grafting of foreign molecules onto lignocellulosic biomass or technical lignins. With this approach, the properties of lignin can be tailored into a desired direction by choosing the respective type of technical lignin, laccase, solvent and functional molecule. The increase of lignin hydrophobicity can be used as a way to improve the performance of possible lignin-based materials and covalent coupling with hydrophobic fluorophenols has been demonstrated. The polymerized version of the lignosulfonates can also replace fossil-based products especially the currently used coating materials such as those based on, polyethylene, polysulfone and so on.

## Cyanoflan: a marine cyanobacterial polymer for skin well-being

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With a more environmentally conscious society, the demand for natural alternatives to replace harmful chemical/synthetic origin compounds is rapidly increasing worldwide. In particular, the cosmetics and personal care industry has been actively seeking for natural ingredients that can contribute to the quality, performance, value, and lifespan of formulations, while promoting a sustainable and eco-friendly economy. In this context, cyanobacteria-derived products are still an unexploited resource, offering an opportunity to address key challenges in the industry. This project aims to develop innovative and added-value products from cyanobacteria, starting by Cyanoflan: a unique extracellular polysaccharidic polymer naturally produced by a marine unicellular cyanobacterium [1]. Cyanoflan is a complex and versatile macromolecule that can be applied in cosmetic and personal care formulations as a rheology modifier, showing a viscosity about 1.5x higher than xanthan gum [2]. Furthermore, in vitro and in vivo results demonstrated Cyanoflan biocompatibility with human cells and bioactivity, since it has antioxidant and anti-inflammatory properties, providing protection to the skin and promoting its regeneration [3]. In addition, the biomass surplus can be used to generate value based on a circular economy model, having the potential to be commercialized as a dietary supplement, for example. The incorporation of natural, renewable and sustainable raw materials such as Cyanoflan into commodities and premium products from the cosmetics and personal care industry will allow reaching the expanding market of environmentally conscious consumers.

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## Sucrose bioconversion in high-addes value alpha-glucans using engineered bacterial enzymes

**Claire Moulis**[1], Magali Remaud-Simeon[1], Gianluca Cioci[1], Etienne Severac[1]

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Alpha-transglucosylases produced by lactic acid bacteria are promising tools for the synthesis of novel carbohydrate structures, as they catalyze the production of high molar mass alpha-glucans from sucrose, a simple and low-cost agroresource. The best-known polysaccharide of this family is dextran, an homopolymer of glucosyl units mainly linked by alpha-1,6 osidic linkages that found various industrial applications, e.g. in food, health, and as biomaterials. However, different alpha-glucans varying in term of osidic linkage content and physico-chemical properties can be produced following the enzyme specificity used, that could open the route for new applications.

These sucrose-active enzymes are classified in the family 70 of Glycoside-Hydrolases, which comprises today around 800 sequences for only about sixty enzymes biochemically characterized, that remains very low. However, with the progress in bioinformatics, structure-function studies, screening technologies and enzyme engineering, we recently discovered new enzymes that catalyze the formation of a broad variety of new gluco-oligosaccharides and polymers.

The presentation will first focus on the structure-function relationship studies of these intriguing enzymes, distinguishable by their linkage specificity or ability to control the size of the produced polysaccharides. The resolution of some 3D structures allowed us to decipher structural features playing a key role in polymer elongation, enzyme processivity, and/or linkage specificity. These findings open promising strategies for GH70 enzyme engineering aiming at customize the alpha-glucan architectures on purpose, and some examples will be given during the presentation.

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# Poster No. 62

## Elucidating Enzymes Selectivity and Thermostability in Short-Esters and Polyesters Synthesis

**Filippo Fabbri**[1], Federico Adolfo Bertolini[1], Georg M. Guebitz[1,2], Alessandro Pellis[3]

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Various bio-based aliphatic building blocks, such as adipic acid, itaconic acid, sebacic acid and 1,4-butandiol were investigated for the biocatalyzed synthesis of polyesters [1]. Different hydrolases, namely Candida antarctica lipase B (CaLB), Humicola insolens cutinase (HiC), and the in-house produced Thermobifida cellulosilytica cutinase 1 (Thc_Cut1) [2,3] were successfully adsorbed onto polypropylene beads [4] and evaluated using a full-factorial design of experiments (DoE) for the synthesis of short-esters. The activity of the biocatalysts in transesterification was screened in a broad range of temperatures (50-90 °C) considering different carbon chain lengths for alcohols and acids (C4, C8 and C12). The DoE allowed to obtain a 4D contour response, which indicated CaLB, HiC and Thc_Cut1 temperature optima at 85 °C, 70 °C, and 50 °C, respectively. Furthermore, CaLB and HiC exhibited their selectivity towards long-chain alcohols and acids as substrate in contrast to Thc_Cut1, which was more active on short-chain monomers. Finally, the obtained model was verified by biocatalyzed synthesis of polyesters through polycondensation reactions, using the previously investigated immobilized hydrolytic enzymes. CaLB and HiC showed the best synthetic activity when used with dimethyl sebacate (DMSe) while a decrease in polymers Mw was observed with dimethyl adipate (DMA) and dimethyl itaconate (DMI). On the other hand, Thc_Cut1 led to polyesters with lower molecular masses, despite the high conversion rate achieved with DMA. These results fully confirmed our model for all of the three enzymes.

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# Poster No. 63

## Metabolic Engineering of *Pseudomonas taiwanensis* for high-yield production of 4-coumarate and derived aromatics

**Benedikt Wynands**[1], Franziska Kofler[1], Nadine da Silva[1], Nick Wierckx[1]

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Aromatics are versatile building blocks for many important products including plastics. Their vast majority is derived from fossil resources and a bio-based production is required to for the transition towards a sustainable economy. Previously, we applied Pseudomonas taiwanensis as microbial host for whole-cell biosynthesis of a range of aromatics from renewable substrates. Here, we focused on the production of 4-coumarate and derived aromatics using a P. taiwanensis strain with an increased metabolic flux to tyrosine. Multiple ammonia-lyases were evaluated for specific deamination of tyrosine. All tested tyrosine ammonia-lyases (TALs) were highly specific for tyrosine, but 4-coumarate production was limited by the enzymes' activity, indicated by residual tyrosine. When a phenylalanine ammonia-lyase (PAL) with PAL/TAL activity was used, no tyrosine was detected. Instead, trans-cinnamate accumulated as a major byproduct (2.8 mM) even surpassing the titer of 4-coumarate (1.0 mM). To make use of the higher activity of the PAL, but limit trans-cinnamate accumulation, a point mutation was introduced in the prephenate dehydratase. This reduced the flux to phenylalanine, limiting byproduct formation on the level of substrate availability. Thereby, 2.6 mM 4-coumarate were produced from glucose with a yield of ~20 % (Cmol/Cmol). Only ~0.1 mM trans-cinnamate was produced by this strain.
Subsequently, the 4-coumarate producer was used as a basis for the biosynthesis of 4-coumarate-derived aromatics (e.g., 4-hydroxybenzoate) through the expression of additional heterologous enzymes.



# Poster No. 64

## Supercritical $CO_2$ a Sustainable Support for Enzymatic Processing of Plastic Waste

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Plastic is a ubiquitous material with extensive applications. Due to its versatile properties, plastic materials are constantly in demand and the share market of plastic-based materials are expected to increase further. However, due to its non-biodegradable and minimum viable recycling procedure, plastic waste causes a massive problem.
Enzymatic degradation is a sustainable promising solution for handling plastic waste. Nevertheless, this technology is still facing hurdles such as enzyme thermo-stability and plastic crystallinity. To address these barriers, drying and encapsulation of various plastic degrading enzymes as well as plastic materials pre-treatment utilising unique properties of supercritical CO2 were investigated. The reasoning behind using supercritical CO2 is due to its natural sourcing, inert properties, and low critical point, which allows mild processing condition.
In this study, two types of PET degrading enzymes (Lipase-Novozym® 51032 and Leaf-Branched Compost Cutinase) were dried and immobilized via encapsulation in different matrices. Their performance was subsequently determined in comparison with the original liquid enzymes. It was observed that supercritical CO2 drying conditions, and the nature of the immobilised support greatly influences the stability of the enzymes. This confers great economic advantages in handling and transporting enzymes for processing plastic waste.
Supercritical CO2 pre-treatment of multi-layered plastic waste employs the ability of creating instant thermal and pressure shocks. As a result, the plastic materials were visibly shrunk, deformed, and foamed, which potentially increase the surface area for enzymatic attack shortening the degradation time.

Keywords: Plastic recycling, enzymatic degradation, enzyme drying and immobilisation, supercritical CO2, plastic foaming.

# Poster No. 65

## Contribution to the circular economy of textiles: Microbial conversion of textile monomers into biopolymers

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Global production of textile fibers amounted to 120 million tons in 2019, with polyester-based fibers accounting for more than half of the production capacity. In the context of end-of-life management of plastics, the increasing quantities of petroleum-based textiles such as PET and PU in textile fiber production, but also the amount of accumulating waste textiles, pose a major health threat to the environment and society. Contaminated textile waste streams that cannot be recycled with common recycling techniques, are a major issue. To address this problem, alternative and innovative recycling routes are indispensable.

As an alternative recycling route biological up-cycling is emerging. Textile waste is served as feedstock for bacterial fermentation, producing biological compounds such as PHA that contribute to the circular and sustainable economy of plastics.

In our project "EnzyDegTex", PET and PU monomers ethylene glycol, terephthalate, adipic acid and 1,2-butandiol will be used as feedstock for a mixed-culture of Pseudomonads. Genetic engineering should enable growth on textile monomers or improve the metabolization to higher levels. Corresponding toxicity tests should provide information on the tolerance towards additives or other chemical compounds that are commonly used in textile processing. In addition, targeted extraction or adaptive laboratory evolution could avoid or reduce inhibitory effects of certain chemicals.

As a so-called "bioplastic", PHA has the potential to replace a part of the petroleum-based plastics. Since many Pseudomonads can produce PHA, genetic engineering methods and process-optimization are to be used in this project to improve the production.



# Poster No. 66

## Biocatalytic synthesis of amides with lipases in organic solvent

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Amide formation is one of the most important reactions in industrial pharmaceutical synthesis. Although the synthetic approaches appear to be simple, they suffer in fact from many drawbacks. The synthesis requires toxic or hazardous reagents to activate the ester or acid component and have a poor atom economy. So far, many strategies to obtain amides have been published, but several problems have not been solved, yet. Challenges are for example the direct transformation of carboxylic acids or esters with amines. Known chemical strategies require the hydrolysis of the ester, activation of the carboxylic acid via SOCl2 leading to a reactive acyl chloride which reacts with the amine.

For these challenges, biocatalysis may offer a solution, especially because biocatalysis is known for using harmless reagents and working under mild conditions.

Our aim is to identify a biocatalytic access to amide formation, especially by the help of hydrolases starting from bulky acyl donors. Screening several hydrolases, we decided to work with the lipase from Sphingomonas sp. HXN-200 (SpL). SpL shows high activity in aminolysis towards various acid and esters with amines in organic solvents. New substrates have been tested and functional studies on this protein shall be conducted to understand its reaction behavior.

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# Poster No. 67

## Enzymatic degradation of mycotoxins in animal nutrition

**Markus Aleschko**[1], Sebastian Fruhauf[1], Michaela Thamhesl[1], Manuela Killinger[1], Andreas Höbartner-Gußl[1], Patricia Fajtl[1], Doris Hartinger[1], Patricia Menczik[1], Heidi E. Schwartz-Zimmermann[2], Kristina Djinovic-Carugo[3], Gudrun Vogtentanz[1], Gerd Schatzmayr[1], Wulf-Dieter Moll[1]

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Fungal mycotoxins represent a genuine risk to livestock production. Two mycotoxins frequently contaminating crops worldwide are fumonisins (FUM) and zearalenone (ZEN). Our goal is to develop enzyme-based feed additives to detoxify FUM, ZEN and other mycotoxins in the gastrointestinal tract of animals.

FUM inhibit ceramide synthase, a key enzyme in sphingolipid metabolism. The resulting imbalance of sphingolipids and sphingoid bases upon FUM exposure is linked to esophageal cancer and neural tube defect in humans, and mycotoxicoses in domestic animals like porcine pulmonary edema and equine leucoencephalomalacia.

The resemblance between zearalenone and the primary female hormone, estrogen, leads to interference with the endocrine system especially of swine, causing hyperestrogenism and impaired fertility.

We isolated fumonisin esterase FumD (FUMzyme®) from Sphingopyxis sp. MTA144. It hydrolyzes two tricarballylic acid (TCA) side chains from the FUM molecule. We proved efficacy of FumD to degrade FUM in vivo by following the restoration of sphingolipid metabolism.

Zearalenone lactonase ZenA (ZENzyme®) was isolated from Rhodococcus erythropolis strain PFA D8-1. Subsequently, we identified several homologs and confirmed their activity in vivo by quantifying ZEN and the non-estrogenic hydrolysis product HZEN in urine and feces.

By combining directed evolution and rational design, we increased thermostability and catalytic activity of FumD and ZenA to withstand feed processing and to efficiently degrade mycotoxins in the challenging gastrointestinal environment.

FUMzyme® is the first and only EU- and FDA-authorized enzymatic feed additive for mycotoxin degradation and part of the Mycofix® product line. Recently, ZENzyme® was launched as another solution for mycotoxin risk management.



# Poster No. 68

## Oxidative Alkene cleavage with novel metal-dependent carotenoid cleaving oxygenases as an alternative to ozonolysis

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Ozonolysis is one of the best-known chemical methods for the cleavage of C=C bonds and can be used for the synthesis of aldehydes and ketones, however, ozonolysis is scarcely applied on large scale due to the highly explosive nature of reaction intermediates. Therefore, we searched for an enzymatic alternative based on the Aromatic DiOxygenase (ADO) from Thermothelomyces thermophila.[1] Sequence similarity search led to several hypothetical carotenoid cleavage oxygenases (CCO) with promising properties. All of them are putative non-heme iron-dependent alkene cleaving enzymes and typically for CCOs the metal ion is chelated between 4 histidine residues.

The new enzymes found in the search were used in a one-pot whole cell reaction system to convert isoeugenol to vanillin. From all the candidates tested TsADO from Talaromyces stipitatus and MapADO from the marine fungus Moesziomyces aphidis showed significant conversion to vanillin. For example, using MapADO as ‘ozonylase’ for this reaction, more than 75 times more product was achieved compared to the already described ADO.[1]

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# Poster No. 69

## Acetylated cellulose nanomaterial as a reinforcing agent in chitosan-based foils for flexible packaging

**Ana Oberlintner[1],** Bla Likozar[1], Uro Novak[1]

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Biopolymers are showing a great potential to replace as an alternative to conventional bioplastic materials, benefiting primarily the packaging industry, as completely biodegradable option. Chitosan, obtained from chitin, the second most abundant polymer on Earth, is among the most promising ones as it possess the ability to form foils suitable for flexible packaging materials. The properties of the biopolymer-based foils important for such applications, for example tensile strength and water vapor permeability, can be further improved by incorporation of cellulose nanocrystals (CNCs) isolated from cellulose, that is available worldwide and is thus cheap and biodegradable. However, as cellulose's surface is rich in hydroxyl groups, it is highly hydrophilic in its nature and does not mix well into chitosan matrix. To overcome this limitation, cellulose can be chemically modified, reducing abundancy of surface hydroxyl groups.

With this in mind, in the present research CNCs were acetylated with acetic anhydride in pyridine medium to three different degrees of substitution, which was confirmed by ATR-FTIR and incorporated into chitosan foils in amount of 3 wt. % with regards to chitosan. Moisture content, mechanical properties and water vapor permeability of the foils were evaluated after conditioning at 50 % RH for 48 hours and compared to biopolymer foils without CNCs and biopolymer foils with incorporated pristine CNCs. It was observed, that acetylation of CNCs leads to better incorporation into chitosan matrix and improves tensile strength as well as water vapor barrier, pushing bio-based foils closer to the market need for biodegradable for flexible packaging.



# Poster No. 70

## Combining enzymatic hydrolysis and microbial fermentation for valorisation of organic municipal solid waste

**Annika Putz[1],** Chiara Falcone[1], Sabine Frühauf[1], Markus Neureiter[1]

[1]University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology, IFA-Tulln, Institute of Environmental Biotechnology, Tulln an der Donau, Austria

Organic waste represents an accessible, renewable and valuable resource. Different waste fractions and valorisation routes are available; however, efficient implementation requires suitable pre-treatment processes. Within the EU-project CAFIPLA (Horizon 2020, BBI) different types of organic wastes are pre-treated and the resulting liquid and solid fractions serve as input for the carboxylate- and fibre-platforms to generate products like chemicals, bioplastics, and fibres. Here, organic municipal solid waste (OMSW) is pre-treated by combining mechanical and enzymatic processes. Enzymatic hydrolysis is applied to increase the amount of soluble organic substances in the liquid fraction. The present approach combines enzymatic hydrolysis as a mild pre-treatment for OMSW with lactic acid production by Bacillus coagulans in a simultaneous saccharification and fermentation process (SSF). The strain's ability to convert different sugars released by the enzymatic activity to lactic acid is evaluated. In addition, factors such as time of inoculation, inoculum strength or substrate addition during SSF will be tested and optimised. Combining enzymatic hydrolysis and microbial fermentation within one pre-treatment process improves the valorisation of organic municipal solid waste and should direct product formation towards lactic acid. As a result, an enhanced pre-treatment efficiency and increased product yields are expected.

**Acknowledgements:**
This project has received funding from the Bio-based Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation programme under grant agreement No 887115. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio-based Industries Consortium.

# Poster No. 71

## Plant based biopolymer hydrophobic/ oleophobic coatings for natural cellulosics textile fibers

**Petra Jerič**[1], Bla Likozar[1], Uro Novak[1]

[1]National Institut Of Chemistry, Ljubljana , Slovenia

The use of synthetic chemicals in the textile industry and their influence on the environment and health is becoming an ever bigger issue. New research has shown that chemicals from clothes can penetrate into our body where they can disturb the balance of hormones and can even lead to cancer. Main chemical sources in textile production are the coloring phase and textile coatings where hazardous synthetic chemicals (eg. polyflouro alkyl subtances –PFAS) are used to increase the characteristics of textile material. Finally the synthetic fibers, can restrict release of toxins from our skin through restricting water perspiration.

In this study a development of new hydrophobic and breathable biopolymer coating for natural fibers was executed. Natural pigments were added directly into the coating. Different versions of coatings were prepared using three types of polysaccharides: corn starch, agar and tapioca starch. Homogenized mixture was heated and evenly distributed on organic bamboo fabric and left to dry at room temperature. Final treatment with the top layer of coconut oil and waxes (candelilla, jojoba and almond) was applied to the biomaterial coating increasing its hydrophobic/oleophobic properties. Mechanical properties with the help of a tensile tester and contact and slide angles under which the water and short-chain alkane testing fluid fall on the textile with coatings were performed by employing of a tensiometer. The obtained results confirmed the material hydrophobicity and oleophobicity, while keeping the advantages of natural fibers like breathability, natural antibacterial activity and good water (sweat) absorption from the inside of fabric.



# Poster No. 73

## Towards a sustainable hygiene industry: Development of a circular economy of bio-based superabsorbent polymers

**Frederik Völker**[1]

[1]RWTH Aachen University, Aachen, Germany

Today's hygiene industry faces a global challenge of outermost importance. With increasing world population, urbanization and simplified access to fundamental everyday use objects, the demand for single-use hygiene products rises as well. However, end-of-life treatment options are rarely obtainable for such disposable articles. Apart from partial chemical and mechanical recycling, which are itself hampered by troublesome product component separation as well as poor quality of segregated product streams, hardly any biotechnological treatment options exceeding lab-scale applications are available.

Moreover, the main functional component of these products, the superabsorbent polymer network, is chiefly synthesized from fossil feedstocks and only few bio-degradable alternatives exist. Thus, single-use products such as diapers are mainly incinerated, contributing to climate change as well as to the loss of reusable resources.

The presented project aims to develop a circular economy of a bio-based, bio-degradable superabsorbent polymer based on the poly(amino acid) Poly-γ-glutamic acid (γ-PGA) produced by metabolically engineered Bacillus subtilis. As a first step, we aimed to increase product titers and process yield by streamlining the microbial γ-PGA synthesis pathway by multiple gene deletions associated with the organisms' overflow metabolism. A decline in culture pH was observed during the cultivation of the newly generated strain, indicating a metabolic imbalance. We show that the genetic modifications led to a steep increase in extracellular pyruvate levels, evoked by the altered carbon flux of the mutant strain. Based on this, we propose a new approach to re-distribute glycolytic flux towards the TCA cycle by heterologous gene expression.

# Poster No. **74**

## Cyanophycin modifications toward applications in tissue scaffolding

**Natalia Kwiatos**[1], Deniz Atila, Vignesh Kumaravel, Alexander Steinbüchel

[1]ICRI-BioM, Lodz University of Technology, Lodz, Poland

Cyanophycin is an amino acid polymer, also referred to as cyanophycin granule polypeptide or multi-L-arginyl-poly-L-aspartate. It is naturally synthesized in cyanobacteria and some heterotrophic bacteria, and it serves as a temporary storage compound primarily for nitrogen, but also for energy and carbon(Frommeyer et al., 2016). It is composed of aspartic acid in the backbone and arginine residues as the side chains. Cyanophycin is a biobased polymer with a great potential for applications in many areas including medicine and food technology. Despite the fact that features of cyanophycin do rarely allow this polymer to be used as a sole material, its biodegradability and environmentally friendly production encourages studies on widening its applicability (Kwiatos & Steinbüchel, 2021). According to previous studies, cyanophycin is a biocompatible material, thus it has potential to be used for tissue scaffolding(Tseng et al., 2016).
Herein, several methods for cyanophycin cross-linking, one enzymatic cross-linking method and three chemical approaches using a) glutaraldehyde, b) methacrylic anhydride c) genipin as cross-linking agents are presented. Moreover, the success of the cross-linking processes, the properties of the cross-linked products, and potential applicability of cyanophycin in tissue scaffolding were investigated.

Frommeyer, M., Wiefel, L. & Steinbüchel, A. (2016). Crit Rev Biotechnol. 36, 153–164.
Kwiatos, N. & Steinbüchel, A. (2021). Front Bioeng Biotechnol. 9, 984.
Tseng, W. C., Fang, T. Y. & Chen, S. Y. (2016). Biochem Eng J. 105, 97–106.



# Poster No. **75**

## Enzymatic synthesis of polygallate in fed-batch conditions and its characterisation

Marina Ti ma[1], Darijo ibalić[2], Ana Bucić-Kojić[3], Jelena Vukov-Parlov[4], Tatjana Tomić[5], **Mirela Planinić**[6]

[1]University Of Osijek, Faculty Of Food Technology Osijek, Osijek, Croatia, [2]University Of Osijek, Faculty Of Food Technology Osijek, Osijek, Croatia, [3]University Of Osijek, Faculty Of Food Technology Osijek, Osijek, , [4]Central Testing Laboratory, Research & Development, INA-Industrija nafte d.d, Zagreb, Croatia, [5]Central Testing Laboratory, Research & Development, INA-Industrija nafte d.d, Zagreb, Croatia, [6]University Of Osijek, Faculty Of Food Technology Osijek, Osijek, Croatia

The enzymatic oxidation of gallic acid catalysed by laccase from Trametes versicolor to produce polygallate was performed in 100 cm3 magnetically stirred glass reactors in 12-cycle repetitive batch conditions at following process conditions: T = 27 °C, pH 5, n = 220 rpm, c0, gallic acid = 0.085 mmol dm-3, ɣ0, laccase= 0.099 mg cm-3. Gallic acid was added every 30 min in amount to obtain initial concentration approximately the same as it was in the beginning of the first cycle. The product was precipitated in methanol and dried to obtain the polymer as black powder which was then characterized by FTIR and NMR spectroscopies.
The FTIR spectrum was acquired by means of attenuated total reflectance (ATR) technique on a Shimadzu Tracer 100 spectrometer. The ATR spectrum was recorded in the single reflection configuration, over the 4000 – 400 cm-1 spectral range with the resolution of 4 cm-1. The total scans were 128. The NMR experiments were performed at 298 K and chemical shifts were reported relative to tetramethylsilane (TMS) internal standard. One-dimensional 1H NMR spectra were recorded on a Bruker Avance Neo 300 NMR spectrometer in DMSO d6 and D2O using a C/H BB 5 mm probe with 1000 scans and 10 s recycle delay.
The synthesised product, polygalate, may have various applications, in the cosmetics industry as a component of hair dyes, in the textile industry for dyeing textiles, in 3D printing for medical applications, etc.

# PICHIA 2022

## Scientific Committee:

**Brigitte Gasser,** (BOKU), Austria
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**Knut Madden,** BioGrammatics, Inc., United States
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# Tuesday, 15th November

## TOPIC 1, 13:30 – 15:30: (Bio-) materials and chemicals, biopolymers made by *Pichia*

The desire to uncouple the need of animal farming and production of highly demanded animal derived materials and products, as well as to implement environmentally friendly bioprocesses with reduced $CO_2$ footprint and independence from fossil resources drive new developments in industrial biotechnology and enable new products and industrial design. How can Pichia pastoris contribute to these needs? How can we benefit towards a change to a $CO_2$ neutral bioeconomy?

**13:30 – 13:50**
### Welcome to the PICHIA 2022

**Speakers:**
Anton Glieder (Graz University of Technology, bisy)
Tom Chappell (BioGrammatics, Carlsbad)

**13:50 – 14:20**
### Production of protein-based polymers in *Pichia pastoris*

**Speaker:** Marc Werten (Wageningen Food & Biobased Research)

**14:20 – 15:00**
### Production and characterization of a recombinant protein in *Pichia pastoris*

**Speaker:** Lixin Dai (Modern Meadow)

**15:00 – 15:30**
### Conversion of $CO_2$ into organic acids by synthetic autotrophic yeast

**Speaker:** Özge Ata (BOKU)



PICHIA 2022

# Tuesday, 15th November

## TOPIC 2, 16:00 – 18:00: Food and feed products

Leghemoglobin from Impossible Food demonstrated the demand for novel food ingredients and the technical, legal and economic feasibility of new commercial processes and products for fermentation enabled food production employing Pichia pastoris. What's coming next?

**16:00 – 16:20**
### Shaping the future of milk with *Pichia*

**Speaker:** Laura Navone (Eden Brew)

**16:20 – 16:50**
### Large-scale manufacturing of egg proteins by precision fermentation

**Speaker:** Ranjan Patnaik (EVERY)

**16:50 – 17:20**
### Making biology easier to engineer: unlocking the potential of accelerated DBTL cycle

**Speaker:** Andrea Camattari (Ginkgo Bioworks)

**17:20 – 17:40**
### Lactoferrin- A New Category of Recombinant Food Proteins

**Speaker:** Amanda Fischer (TurtleTree)

**17:40 – 18:00**
### Scaling Up Synthetic Biology Industrial Products in a Disrupted Global Supply Chain Environment

**Speaker:** Alex Berlin (Solar Biotech)

# Wednesday, 16th November

## TOPIC 3, 10:00 – 12:00: *Pichia* methods and technologies

To unlock the full potential of Pichia pastoris as host for biotechnological applications a continuous evolution of the tools and methods for genetical manipulation and cultivations process is required. The four presentations of this session promise an update on the latest developments and will provide a glimpse on what we can expect in future.

**10:00 – 10:40**
### Utilising *Pichia pastoris* cell-free protein synthesis for the production of vaccine targets

**Speaker:** Rochelle Aw, (Northwestern University, Imperial College London)

**10:40 – 11:05**
### CRISPR/MAD7 technologies for *P. pastoris* genome and transcriptome engineering

**Speaker:** Florian Weiss & Andrea Hönikl (Graz University of Technology)

**11:05 – 11:30**
### Tangential-flow filtration as an alternative to centrifugation for biomass clarification in *Pichia pastoris* cultures

**Speaker:** Juan Moreno-Cid (Bionet Engineering)

**11:30 – 12:00**
### Dynanamic feeding for *Pichia pastoris*

**Speaker:** Julian Kopp (TU Wien)



PICHIA 2022

# Wednesday, 16th November

## TOPIC 4, 14:00 – 18:00: New (pharmaceutical) products made by *Pichia*

Most pharmaceutical antibodies are produced by CHO cell lines. Are we going to change this with new antibody formats and -products. Can we make use of the specific technological advantages of Pichia? How can we provide better and more economic and dynamic solutions in an ongoing SARS-CoV-2 crisis? Can we still improve the secretion efficiency for pharmaceutical proteins? How about new opportunities by providing pure and medically active food components to support human health and well-being?

**14:00 – 14:45**
### Neo-glyco-engineered VHH antibodies from *Pichia* for chemo-orthogonal coupling

**Speakers:** Nico Callewaert (Ghent University)

**14:45 – 15:30**
### Process development and cGMP manufacturing of an effective recombinant COVID-19 vaccine

**Speaker:** Mehmet Inan (Akdeniz University)

**16:00 – 16:25**
### Industrial bioprocesses where switching to the use of *Pichia pastoris* led to improved performance

**Speaker:** Alison Arnold (Ingenza)

**16:25 – 17:10**
### QTL analysis of protein secretion in *K. phaffi*

**Speaker:** Kenneth Wolfe (Conway Institute)

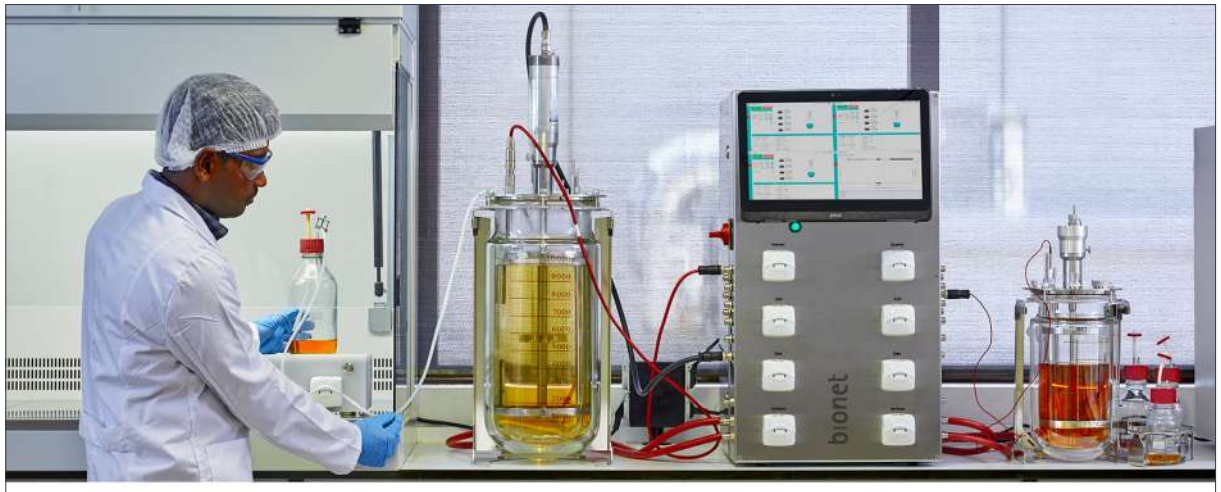
# Wednesday, 16th November

**17:10 – 17:35**
**_Pichia_ expression platform design for biopharmaceutical protein production**

**Speaker:** Daniel Degreif (Sanofi-Aventis Deutschland GmbH)

**17:35 – 18:00**
**High-productivity methanol-free protein production processes with *P. pastoris***

**Speaker:** Aid Atlic (VALIDOGEN GmbH)






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# Thursday, 17th November

## TOPIC 5, 09:00 – 10:00: Secretion

A reason why Pichia pastoris is often preferred over other microbial host in biotechnological applications is the yeasts' ability to secret high titers of recombinant protein with high purity to the extra cellular environment. Although mostly very similar technologies are evaluated for protein secretion by P. pastoris, the speakers in this session will offer alternatives and demonstrate that optimizing the secretion process is critical for maximizing the productivity of P. pastoris.

**9:00 – 9:30**
**Going beyond the limit: impact of increasing global translation activity on the productivity of recombinant secreted proteins in *Pichia pastoris***

**Speakers:** Jennifer Staudacher (BOKU)

**9:30 – 10:00**
**Driving proteins through the co-translational translocation pathway for improved protein secretion in *P. pastoris***

**Speaker:** Pau Ferrer (Universitat Autònoma de Barcelona)

# Thursday, 17th November

## TOPIC 6, 10:30 – 12:00: New aspects of the *Pichia* Genome

New products made by Pichia pastoris received FDA approval and GRAS status in the past years. Legal approval, as well as modern research relies on detailed information about the genome of P. pastoris and possible side products, originating from the host. Genome sequence data with high coverage and new genome annotations became available in the past years and enable -omics based research and reliable characterizations of host- and expression strains and also of products and their final formulations. Expression bottle necks can be identified, and the benefits of diverse host strain lines might contribute to a next generation of host – and production strains. But do we already know everything and how similar or diverse are the different common strains and how big is the impact of applied engineering technologies on the host genome?

### 10:30 – 11:00
***Pichia* production host engineering by systemic host changes**

**Speaker:** Christoph Kiziak (Lonza)

### 11:00 – 11:20
***Komagataella phaffii:* QTL mapping for strain development**

**Speaker:** Marina Jecmenica (BOKU)

### 11:20 – 11:40
**Whole genome sequencing analysis of effects of CRISPR/Cas9 in *Komagataella phaffii*: A buddi(ng yeast) in distress**

**Speaker:** Veronika Schusterbauer (bisy)

### 11:40 – 12:00
**Metabolomics and metabolic engineering reveals a hidden methanol, formate and $CO_2$ assimilation pathway in *Komagataella phaffii* – the oxygen-tolerant reductive glycine pathway**

**Speaker:** Bernd Mitic (BOKU)



PICHIA 2022
PLUS ONE DAY

# Thursday, 17th November

## TOPIC 7, 13:30 – 15:30: Synthetic Biology & Metabolic enineering

Going beyond protein secretion and manufacturing of intracellular proteins by Pichia pastoris we just start to discover the benefits of this expression platform for small molecule manufacturing and new opportunities for protein production by engineered metabolic pathways. Do we have the right tools for metabolic engineering and synthetic biology for small molecule manufacturing. What do we know about existing metabolic pathways and the hidden potential of P. pastoris? Will we be able to provide economic new biomanufacturing routes for natural small molecules to generate more safe and reliable alternatives to limited supply by extraction from plants or animal sources or can we even provide a more diverse and more useful diversity of potential APIs by engineered Pichia strains?

### 13:30 – 14:00
***Pichia pastoris* for sustainable manufacturing of alkaloid drugs**

**Speakers:** Gita Naseri (Humboldt-Universität zu Berlin)

### 14:00 – 14:30
**Engineering Adh2 for improved methanol utilization in *Komagataella phaffii***

**Speaker:** Charles Moritz (BOKU)

### 14:30 – 15:00
**Auxin-induced degradation of target proteins in *K. phaffi***

**Speaker:** Anita Emmerstorfer-Augustin (Graz University of Technology)

# Thursday, 17th November

## TOPIC 8, 15:30 – 17:15: Bioprocess engineering

New strains, proteins and chemicals, made by Pichia pastoris open new opportunities for medical treatments, industrial manufacturing, nutrition and even (biomedical-) material sciences. But what else is needed to realize early-stage ideas and dreams and to translate product inventions into innovations in our daily life? Bioprocess technologies and Down-stream Processing are often areas of industrial and secret know how. How can we benefit from the scientific knowledge in the Pichia community to change our dreams to reality in near future?

**15:30 – 16:00**
### Is medium heterogeneity really a problem in large scale bioreactor?
**Speaker:** Patrick Fickers (University of Liège)

**16:00 – 16:20**
### Applying Dynamic Time Warping to Machine Learning based Automation of *Pichia pastoris* Fermentations
**Speaker:** Stefan Hauer (ZHAW, Wädenswil, TU Wien)

**16:20 – 16:40**
### Methanol-free promoter PDH: a growth-decoupled system for recombinant protein production in *Pichia pastoris*
**Speaker:** Núria Bernat-Camps (Universitat Autònoma de Barcelona)

**16:40 – 17:00**
### AI Models applied in RQ control to optimize recombinant protein production in *Pichia pastoris*
**Speaker:** Arnau Gasset (Universitat Autònoma de Barcelona)

**17:00 – 17:15**
### Closing words
**Speaker:** Anton Glieder (Graz University of Technology, bisy)





# PICHIA Abstracts

## Production of protein-based polymers in *Pichia pastoris*

**Marc Werten**[1]

[1] Wageningen Food & Biobased Research, The Netherlands

So-called protein-based polymers are increasingly being used in the fabrication of (biomedical) performance materials. Natural examples of such polymers are collagen, silk, and elastin. These self-assembling and structure-forming proteins feature repetitive amino acid sequences that can be conveniently mimicked and expanded upon through recombinant DNA technology. Different polymer sequences can be combined into multifunctional block copolymers. Although protein-based polymers are usually produced in Escherichia coli, our group has predominantly used the methylotrophic yeast Pichia pastoris in view of its capacity for efficient secretory production. In this talk I will present a brief overview of our more than two decades of research in this area. Properties and biosynthesis of various block copolymers will be summarized. Challenges that may be faced when using P. pastoris for the production of these polymers will be discussed, such as proteolytic degradation and intracellular self-assembly.

# PICHIA Abstracts

## Production and characterization of a recombinant protein in *Pichia pastoris*

**Lixin Dai**[1]

[1]Modern Meadow Inc., USA

Collagen and its derivative proteins have been widely used as a natural ingredient for many applications. Most commercially available collagens are animal-derived collagen type I and other forms of collagen, such as type III collagen, are far less prevalent in animals, making extraction and purification extremely difficult and expensive. Here, we report the production of a 50 kDa protein produced in yeast that is 100% identical to the human collagen type III alpha chain. We report the industrialization of both the fermentation and purification processes to produce a final recombinant protein product. This final protein product was shown to be safe for general applications to human skin and compatible with common formulation protocols, including ethanol-based formulations. This recombinant protein was also shown to uniquely stimulate collagen type III production and secretion by primary human dermal fibroblasts.



PICHIA 2022

PICHIA Abstracts

## Conversion of $CO_2$ into organic acids by synthetic autotrophic yeast

**Özge Ata**[1,2], Michael Baumschabl[1,2], Lisa Lutz[1,2], Diethard Mattanovich[1,2]

[1] Austrian Centre of Industrial Biotechnology (acib), Vienna, Austria
[2] Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

One of the biggest challenges humankind is facing these days is the climate crisis. One clear cause is the increasing atmospheric $CO_2$ level due to human activity. Anthropogenic CO2 emission is more thancan be captured by plants and microorganisms which consequently causes an imbalance in the carbon cycle. Towards a more sustainable future, we need to restore this balance.
To address this problem, we aim to enable microbial assimilation of $CO_2$ as a carbon sink by converting it into value-added, bio-based polymers. Previously, Komagataella phaffii (Pichia pastoris) was converted from a methylotroph into an autotroph that can grow solely on $CO_2$ for biomass formation while using methanol to harvest energy (Gassler et al. 2020). In this work, we further engineer this $CO_2$ fixing K. phaffii and use it as a platform to produce value-added organic molecules. Using synthetic biology tools and CRISPR-Cas9, we generated an autotrophic K. phaffii strain that can produce itaconic acid by fixing $CO_2$. Balancing the synthetic itaconic acid metabolism, identifying and engineering targets in the central carbon pathway and optimizing the process parameters resulted in a final titer of 2 g/L itaconic acid in shake flasks. $^{13}$C-labelling experiments confirmed the incorporation of the captured $CO_2$ into itaconic acid. We also investigated the itaconic acid production performance of the $CO_2$-fixing K. phaffii strain in lab-scale bioreactors. To demonstrate broader applicability, the same metabolic engineering strategy was also applied to the production of lactic acid. In the light of our results, we show that the synthetic autotrophic yeast K. phaffii can be a platform for the production of value-added chemicals by the microbial conversion of $CO_2$ for a sustainable bioprocess.

Gassler, T., Sauer, M., Gasser, B., Egermeier, M., Troyer, C., Causon, T., Hann, S., Mattanovich, D., & Steiger, M. G. (2020). The industrial yeast Pichia pastoris is converted from a heterotroph into an autotroph capable of growth on $CO_2$. Nature biotechnology, 38(2), 210–216. https://doi.org/10.1038/s41587-019-0363-0

# PICHIA Abstracts

## Shaping the future of milk with *Pichia*

**Laura Navone**[1]

[1] Eden Brew, Australia

Laura is the R&D Director of Eden Brew, and animal-free dairy company. We make milk proteins with precision fermentation and reconstruct milk using sustainable bioprocesses. This talk will present Eden Brew and provide an overview of our mission and science platform.




PICHIA 2022

# PICHIA Abstracts

## Large Scale Manufacturing of Egg Proteins using Precision Fermentation

**Ranjan Patnaik**

EVERY Co., California, USA

Sourcing of animal proteins through the age old practice of factory farming of animals has a significant negative impact on the environment and is unsustainable. In recent years, precision fermentation (https://gfi.org/fermentation/) has emerged as an alternative manufacturing platform that enables production of nature equivalent animal proteins without the use of animals but rather by harnessing the power of yeast, fungi and other microbial species to make these animal proteins. The EVERY Co. (https://theeverycompany.com/do) has developed a proprietary technology platform that integrates microbial strain engineering efforts with rapid isolation and prototyping of functional animal proteins into a variety of end use applications such as in baking goods and ready to drink (RTD) beverages, to name a few. This platform has been successfully leveraged to produce multiple egg-white proteins at very large scale and at very high productivities without compromising the desired functionality of the proteins. In this presentation, I will share some of the key technical & non-technical learnings and insights from our journey towards commercializing multiple egg-white proteins made by precision fermentation at industrial scale.

# PICHIA Abstracts

## Making biology easier to engineer: unlocking the potential of accelerated DBTL cycle

**Andrea Camattari**[1]

[1]Ginkgo Bioworks, Boston, USA

The Design-Build-Test-Learn (DBTL) cycle is a key concept of synthetic biology, borrowed from traditional engineering disciplines and representing an increasingly adopted metabolic engineering framework for a more systematic and efficient approach to strain development than historical efforts in a variety of industrial domains.

At Ginkgo Bioworks, DBTL cycles are considered a key element to fulfill our mission to make biology easier to engineer. In this context, the pivotal concept of Cell Development Kits (CDKs) has been introduced: by analogy with Software Development Kits (SDKs), allowing software houses to streamline their platform development, we aim to greatly accelerate strain development by ultimately generating a data-driven ecosystem for constantly understanding and improving performance.

Another key development we are implementing at Ginkgo Bioworks is directly related to the predictability of resource (and time) allocation for our platform, as a direct consequence of CDK workflows. A fundamental issue for innovation in biotechnology is related to the difficulty to scale operations maintaining an ever-improving virtuous cycle: applying the CDK concept, it is possible to apply and benefit from concepts of lean industry 4.0, since process modularity allows to finally consider and assimilate a biotech process to large-scale manufacturing.





# PICHIA Abstracts

## Lactoferrin- A New Category of Recombinant Food Proteins

**Amanda Fischer**[1]

[1]Turtletree, Woodland, United States

TurtleTree is a biotech company committed to creating a new generation of sustainable foods based on milk, a unique and rich source of nutritional and functional ingredients.

TurtleTree is interested in milk because its composition has been precisely tuned through evolution to be a complete source of nutrition as well as a source of bioactive proteins critical for gut health and immune system development. Each mammalian species produces milk with proteins that vary in structure and function. In addition, the overall composition of milk changes throughout the period of lactation to meet the nutritional needs of the offspring. These qualities present an untapped potential for human nutrition.

Through the use of precision fermentation as a sustainable platform for production of these ingredients, we are able to generate much larger volumes as well as novel sources of these molecules than would otherwise be possible to purify from cow's milk. Our first bioactive milk protein product will be recombinant lactoferrin, a unique milk protein critical for gut and immune health.

Precision fermentation has been utilized for over 50 years for pharmaceutical and industrial enzyme production. These two industries have shaped the existing regulatory standards and technology landscape but have vastly differing requirements for purity and safety. Since 2018, four precision fermentation-based food protein products have been approved by the FDA. New regulatory standards and technologies are needed for food-grade products. TurtleTree aims to help shape this new path for production of safe, healthy and economical products by bringing lactoferrin to the market.

# PICHIA Abstracts

## Utilising *Pichia pastoris* cell-free protein synthesis for the production of vaccine targets

**Rochelle Aw**[1,2], Alex Spice[2], Farzana Alam[2], Michael Jewett[1], Karen Polizzi[2]

[1]Northwestern University, Evanston, United States, [2]Imperial College London, London, United Kingdom

Vaccines have been identified as one of the most important public health inventions. Between 2010 and 2015 vaccines were predicted to have been responsible for preventing at least 10 million deaths. However, the World Health Organisation (WHO) have recently reported that on their Global Vaccine Action Plan that 19.4 million people did not receive lifesaving vaccinations in 2018. Part of the problem with this is that vaccines are costly and rapid response to outbreaks, such as Ebola, Zika and SARS-CoV-2, are challenging. It is imperative that research into rapid scale-up, low cost production, and improved supply chain distribution expand into potentially unexpected research areas in order to solve this global challenge.

Pichia pastoris is an attractive host for vaccine manufacturing due to its high volumetric productivity and capabilities of performing post-transformational vector amplification. Due to the affordable culturing conditions of P. pastoris this makes this platform a strong competitor compared to the more traditionally used mammalian cell culture. We have produced a range of vaccine targets from SARS-CoV-2 receptor binding domain (RBD), Human papilloma virus (HPV) virus-like particles (VLP) and a Rabies glycoprotein-G (RABV-G) trimeric protein using our yeast platform. In addition to utilising traditional in vivo methods for protein production we have developed a P. pastoris cell-free protein synthesis (CFPS) platform to evaluate protein production on a small scale. Our platform is capable of producing protein subunits and virus-like particles that can be used to evaluate production in hours as opposed to days.



PICHIA 2022

PICHIA Abstracts

## Tangential-Flow Filtration as an alternative to centrifugation for biomass clarification in *Pichia pastoris* cultures

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In bioprocesses, the stages after biomass formation and growth, known as upstream (USP), follow the stages of dewatering, isolation, recovery, and concentration of the product of interest, known as downstream (DSP). These stages can represent between 50-70% of the total cost of the process, and many of them entail losses during the process. In cultures with the yeast Pichia pastoris, it is necessary to apply a biomass clarification stage to separate it from the product of interest, generally secreted into the medium. The most widely used technology for clarification is centrifugation. Tangential flow filtration (TFF) is an alternative to centrifugation. With this technology, it is possible to apply biomass clarification to a wide range of microorganisms using membranes with a pore size of the order of 0.1-0.45 microns. In this work, the application of TFF has been studied to apply a biomass clarification of a P. pastoris culture alternative to centrifugation. For this purpose, the Bionet TFF M1 system has been used using two different types of membranes (ceramic and hollow fiber) and at different temperatures at scalable conditions and comparing the results to a scalable condition for the equivalent centrifugation.

# PICHIA Abstracts

## CRISPR/MAD7 technologies for *Pichia pastoris* genome and transcriptome engineering

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated (Cas) systems are powerful and programmable tools for genome editing based on RNA-guided DNA cleavage. Inscripta (CO, USA) introduced MAD7 nuclease, an engineered Class 2 Type V Cas enzyme (Cas12a) from Eubacterium rectale favoring YTTN PAM sites, which extends the portfolio of the originally published and patented toolbox. We tested and evaluated different combinations of MAD7 codon optimizations, nuclear localization signals and scaffold RNA sequences in terms of gene disruption efficiencies in Komagataella phaffii (Pichia pastoris) and established an alternative to widely used Cas9 and Cpf1 nucleases. To expand the CRISPR/MAD7 toolbox, we introduced three mutations (D877A, E962A and D1213A) to the MAD7 genes to obtain a nuclease deficient MAD7 (dMAD7) allowing it to be used as road blocker for transcriptional regulation. The data obtained by targeting the promoters of reporter proteins eGFP, HRP, GOX or HNL with different gRNAs and gRNA combinations suggests that the CRISPR/dMAD7 system is indeed suitable for transcription regulation as lower reporter levels or activities were obtained.



PICHIA 2022

# PICHIA Abstracts

## The effect of oxidative stress tolerance on the production of heterologous protein in *Pichia pastoris*

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Metabolism of methanol in methylotrophic yeast Pichia pastoris increases the amount of ROS (reactive oxygen species). During adaptation to the methanol, transcription factor PpYap1 activates the expression of the glutathione redox system via upregulation of glutathione reductase 1 (Glr1). Also, PpPMP20 (peroxisomal glutathione peroxidase) is the most important antioxidant enzyme in the glutathione redox system as a central compartment of the ROS reduction machinery in peroxisomes. The gene co-expression network-based analysis showed that the expression of the Yap1 and PMP20 have a 0.90 and 0.98 percent positive correlation with the AOX1 gene in P. pastoris, respectively. Each Yap1 and PMP20 gene under the control of AOX1 and GAP promoters was transferred into a heterologous glucose oxidase (GOX) producing Pichia pastoris. The expression and secretion of GOX, cell viability, and intracellular ROS in overexpressed strains were investigated in the culture medium containing 1 to 8% Methanol as an inducer. The gene co-expression network-based analysis showed that the expression of the Yap1 and PMP20 have a 0.90 and 0.98 percent positive correlation with the AOX1 gene, respectively. The overexpression of Yap1 under the control of both AOX1 and GAP promoters showed a similar 50% increment in secretory production of heterologous GOX in the 4% methanol medium. Inductive and continuous overexpression of pmp20 showed a 60% and 80% increase in the secretion of GOX into the culture media containing 4 percent of methanol, respectively. Also, all overexpressions significantly elevated the viability of yeast cells in all media and drastically decreased the intracellular ROS.

# PICHIA Abstracts

## Neo-glyco-engineered VHH antibodies from Pichia for chemo-orthogonal coupling.

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The VHH domain of camelid heavy-chain only antibodies is the most versatile, stable and clinically validated single-domain affinity module. It was discovered at VIB more than 30 years ago and its engineering has been at the foundation of a wide range of biotech companies and products, including several approved clinical drugs. Our laboratory is further diversifying the utility of VHH domains for versatile use in novel biopharmaceutical drug concepts. For many of these, convenient site-selective coupling of function-modulating molecules to the VHH module is required. Whereas selective reactions to the (modified) protein backbone can be used, this is often not fully chemo-orthogonal or interfering with favorable manufacturability characteristics of the VHH.

Hence, we have set out to structure-inspired scanning of the VHH surface for sites that could accommodate the incorporation of an N-glycosylation site with as minimal as possible sequence alterations, while not interfering with antigen binding. The analysis of N-glycan site occupancy of a large number of such candidate mutant VHHs revealed 4 sites that were virtually stoichiometrically substituted with a glycan upon expression in Pichia. In a first application, we demonstrated efficient lung macrophage phagosome uptake both in vitro and in vivo (upon inhaled administration) of high-mannose glycan-modified Pichia-produced VHHs.

Production of such VHHs in a GlycoDelete Pichia strain further engineered for efficient beta-1,4-galactosylation of the single-GlcNAc product of GlycoDelete engineering, afforded LacNAc-substituted VHHs. Using Pichia-produced fungal galactose oxidase to functionalize the C6 of the Galactose moiety, with concomitant click-chemistry using catalyzed oxime ligation allowed for the stoechiometric formation of VHH-conjugates with both small molecules and polymers. This versatile, cost-effective and highly scalable methodology for antibody-conjugate formation on VHHs opens up a large range of future applications in drug development.As a baseline chassis for all Pichia engineering work in our lab, including the work described here, we make use of our OPENPichia strain background that is parental to historically used commercial strains of Pichia, is entirely free to operate incl. for commercial manufacturing purposes, is distributable from our strain collection, and which we continue to further characterize and optimize.



PICHIA 2022

# PICHIA Abstracts

## Process development and cGMP manufacturing of an effective recombinant COVID-19 vaccine candidate

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Vaccine development based on economically accessible technological platforms is essential to achieve herd immunity to end the COVID-19 pandemic. Pichia pastoris is one of these platforms. This talk describes the development of a vaccine candidate based on the SARS-CoV-2 receptor binding domain (RBD) protein in Pichia pastoris. A two-step fed-batch fermentation and three-step purification process were developed at a 5-L scale. The final product was formulated with alum and CpG. The current Good Manufacturing Practice (cGMP) compliant production of Covid-19 was achieved at the 10-L scale. The results indicate a consistent and robust process. Potency assays demonstrated that SARS-Cov-2 RBD was immuno-protective against live challenge with SARS-Cov-2 and elicited neutralizing antibodies in the vaccinated mice.

# PICHIA Abstracts

## Industrial bioprocesses where switching to the use of *Pichia pastoris* led to improved performance.

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At Ingenza we engineer biological systems to make everything from therapeutics to enzymes and consumer products, addressing challenges in human health and the global environment. In order to achieve these goals, we require a toolbox of different microorganisms. Increasingly Pichia pastoris has become the preferred choice of host microorganism in recombinant protein production offering many advantages over other microorganisms. In this presentation we will discuss a number of different real examples of where switching from the more typically used host microorganisms to Pichia pastoris has led to the successful development of industrial bioprocesses which otherwise would not have been possible.



PICHIA 2022

# PICHIA Abstracts

## Identification of genetic variants in *Komagataella phaffii* that contribute to increased yields of secreted heterologous proteins

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K. phaffii is widely used as a host for the efficient production of heterologous proteins, but all the strains that are commonly used in industry are derived from a single source, the Phillips Petroleum strain called CBS7435 or NRRL Y-11430. We hypothesized that other isolates of K. phaffii might contain genetic variants that affect heterologous protein secretion. To identify such variants, we made genetic crosses between a CBS7435 strain and strains derived from two other isolates, Pp2 (NRRL Y-17741, from an oak tree) and Pp4 (NRRL YB-378, from an elm tree). We identified two quantitative trait loci (QTLs) affecting secretion of a test protein, β-glucosidase (BGL), and mapped them with single-nucleotide resolution. A major QTL was mapped to the gene HOC1, which codes for an enzyme that mannosylates cell wall proteins. We found that HOC1 is defective in CBS7435-background strains due to a frameshift mutation, whereas HOC1 is intact in the Pp2 and Pp4 genetic backgrounds. The HOC1 mutation in CBS7435 makes its cell wall thinner, which probably increases its permeability to heterologous proteins. By comparing CBS7435 to its natural isolate progenitor NRRL Y-7556/CBS2612, we infer that the HOC1 mutation originated in the 1970s during selection by Phillips Petroleum for K. phaffii strains showing increased methanol utilization. A second QTL mapped to IRA1, where the allele in strain Pp2 has an amino acid substitution that increases BGL secretion but reduces cell viability. However, this IRA1 substitution did not increase secretion of two other heterologous proteins when tested.

# PICHIA Abstracts

## *Pichia* expression platform design for biopharmaceutical protein production

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Pichia pastoris (syn. Komagataella phaffii) is one of the cornerstones of Sanofi's microbial expression platform. The use of the methanol-inducible expression system for production was the golden standard for years, delivering high titers and unsurpassed quality. However, for large scale manufacturing, the use of methanol as toxic substance increases the complexity of HSE & safety requirements. Hence, Sanofi's microbial expression platform is looking for an alternative system that is capable of matching the performance of methanol-based processes but without the drawbacks associated with the use of methanol. Having a safe- and eco-friendly process design in mind, Sanofi is working on establishing a methanol-free expression platform. Recent developments to optimize the methanol-free process have emphasized the potential of this new platform to achieve commercially competitive productivity and product quality. Further progress in the field of strain-engineering and process design will aid to advance the Pichia-based production platform further.



PICHIA 2022

PICHIA Abstracts

## High-productivity methanol-free protein production processes with *Pichia pastoris*

**Aid Atlic**[1]

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The most widely used approach for recombinant protein production using Pichia pastoris as host is to express the gene of interest under control of AOX1 promoter applying methanol induction. However, many companies prefer not to use methanol due to safety concerns (e.g. for medical, food and feed products). To address this issue, VALIDOGEN has developed a set of AOX1 promoter variants that enable methanol-free protein production at multi-gram-per-liter titers. Methanol-free fermentation processes offer several advantages over those with methanol induction, such as low safety issues due to omittance of hazardous compounds, more economical production plant requirements and lower oxygen demand. Using components of VALIDOGEN's UNLOCK PICHIA toolbox and process development methods, we established strategies for maximization of space-time yield as well as total product titer. Examples and case studies will be presented that show the applications of high-productivity methanol-free protein production processes with Pichia pastoris at various scales.

# PICHIA Abstracts

## Going beyond the limit: impact of increasing global translation activity on the productivity of recombinant secreted proteins in *Pichia pastoris.*

**Jennifer Staudacher**[1,2,3],

Corinna Rebnegger[1,2,3], Diethard Mattanovich[1,3], Brigitte Gasser[1,2,3]

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Yeasts, especially Pichia pastoris (syn. Komagataella phaffii), are widely used organisms for commercial, heterologous protein production. P. pastoris is known for its high secretory efficiency and biomass yield, however specific productivities are low and tightly coupled to biomass formation. This highly impacts production processes, which are commonly not run at the maximum growth rate, thereby resulting in suboptimal productivities. To tackle this issue, we evaluated transcriptomics datasets of P. pastoris. These showed a clear downregulation of protein translation related genes with decreasing growth rates, thus revealing the yeast translation machinery as our cellular engineering target.
By overexpression of selected differentially expressed translation factors, we identified translation initiation as the main rate-limiting step. Specifically, factors associated with the closed-loop conformation, a structure that increases stability and rates of translation initiation before start codon scanning is initiated, showed the strongest effects. Overexpression of these factors alone or in combination increased titers of different heterologous proteins by up to 3-fold in fed-batch processes. Global translational activity, as measured by OPP-labelling assays, correlated nicely to the enhanced secreted recombinant protein levels. Furthermore, selected transcript levels and total protein content were higher in the engineered cells. Translation factor overexpression therefore has a global effect on the cell. Concludingly, our work displays not only the interconnection of different protein synthesis steps but also the capacity P. pastoris has for protein production, and indicates that this host organism is not at its limit yet.



PICHIA 2022

# PICHIA Abstracts

## Driving proteins through the co-translational translocation pathway for improved protein secretion in *P. pastoris*

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Protein secretion has been long recognized as a limiting process in which many roadblocks have been identified. Consequently, a wide range of engineering strategies has been investigated. However, these often have resulted in limited improvements and/or protein-specific effects.
The most commonly used secretion signal in P. pastoris is the N-terminal portion of pre-pro-α-factor from S. cerevisiae. However, this secretion signal promotes posttranslational translocation into the endoplasmic reticulum (ER), so proteins that can fold in the cytosol may be inefficiently translocated and thus poorly secreted [1]. Efficient co-translational translocation into the endoplasmic reticulum (ER) was achieved via secretion signal engineering. In particular, we engineered a hybrid secretion signal consisting of the S. cerevisiae Ost1 signal sequence, which promotes co-translational translocation into the ER, followed by the α-factor pro region. This chimeric secretion signal was fused to three diverse proteins, improving secretion in all cases, suggesting that such strategy should be effective for improving the secretion of a broad range of recombinant proteins, particularly for proteins that can fold in the cytosol and for oligomeric proteins. Importantly, the strains carrying the improved secretion signal showed improved performance at bioreactor scale (e.g. higher maximum specific growth rates) than their counterparts carrying the conventional secretion signal. These results were further corroborated in fed-batch cultivations, where the final product concentration and volumetric productivities were also shown to be improved.

[1] Barrero JJ, Casler JC, Valero F, Ferrer P, Glick BS. Microb Cell Fact. 2018 17(1):161.

# PICHIA Abstracts

## *Pichia* production host engineering by systemic host changes

**Christoph Kiziak**[1], Núria Adelantado[1], Jennifer Staudacher[2], Corinna Rebnegger[2], B rigitte Gasser[2,3], and Diethard Mattanovich[2,3]

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**Key Words:** Pichia, productivity, fermentation, protein secretion

Protein production has a continuous need for the improvement of production systems regarding product titers and quality, but also processability, robustness and reliability. The improvement process can follow two major principles: Incremental optimization of existing systems until reaching an intrinsic limitation of the production system or process, or Systemic changes to overcome limitations of existing systems or enable new applications or processes.

Here, we show three examples for Pichia pastoris (syn. Komagataella phaffii) wherein systemic changes of theproduction platform led to extensive changes of protein production and host performance. The consequences for the development of production process will also be highlighted.
New promoter system to dramatically reduce fermentation time and complexity to enable yeast production processes in "bacterial" fermentation times of 2-3 days with high space time yields.
Transcription factor knock-out impacting various cellular processes including transcriptional activity, mating, respiration, cellular membranes, cell cycle genes, etc. Systemic increase of mRNA levels leading to global effects on the cell, such as increased transcript levelsand total protein content in the engineered cells.

Such approaches of changing basic parameters of a production system to create "novel" Pichia production hosts instead of incrementally increasing an existing system have the potential to overcome intrinsic limitations of the system. However, the far-reaching changes of the production host will have consequences not only for strain generation and screening, but also for the whole production process. In addition to the potential higher risk of such approaches, the time&cost-benefit ratio of such developments needs to be carefully considered.



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PICHIA Abstracts

## *Komagataella phaffii*: QTL mapping for strain development

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Komagataella phaffii is among the most widely used cell factories for recombinant protein production. The majority of research on improving its industrially relevant traits is based on one species (K.phaffii), specifically on the strain background of CBS7435. The genus of Komagataella., however, consists of seven different species with various natural isolates from different natural habitats. Consequently, strains show considerable inter- and intraspecies phenotypic variation including traits of industrial relevance.
Two such traits are the subject of this study, namely temperature tolerance and recombinant protein production. Both phenotypes are regulated in a complex manner, making it difficult to pinpoint the exact genetic determinants contributing to observed differences in these phenotypes.
The expression of both phenotypes was investigated among a set of natural isolates belonging to all seven currently known Komagataella species. Initial crosses were performed with K.phaffii strains only upon identification of four parent strains showing sufficiently large genetic variation, and high variation in production potential and growth at non-optimum temperatures of 12 and 39°C; we then designed a Quantitative Trait Loci mapping experiment for bulk segregant analysis. Recombinant inbred lines were established and resulted in 600 randomly selected F14 hybrids exhibiting a wide range of protein secretion yields and temperature tolerance. For both traits, hybrids were ranked according to their phenotypes to generate high, low and random pools for whole genome re-sequencing. Ongoing bioinformatical analysis yielded first QTL regions, which are currently being examined in detail to get a higher resolution of the beneficial genetic determinants responsible for both phenotypes.

# PICHIA Abstracts

## Whole genome sequencing analysis of effects of CRISPR/Cas9 in *Komagataella phaffii*: A buddi(ng yeast) in distress.

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CRISPR/Cas is an effective tool for genome engineering. Especially in the industrially important non-conventional yeast Komagataella phaffii, CRISPR/Cas represents a simple and efficient alternative to methods relying mainly on homologous recombination. To characterize on- and off-target mutations caused by CRISPR/Cas9 followed by non-homologous end joining (NHEJ) repair, we chose a diverse set of CRIPSR/Cas targets and conducted whole genome sequencing on 146 CRISPR/Cas9 engineered single colonies. We compared the outcomes of single target CRISPR transformations to multiplexing experiments with two targets. Furthermore, we examined the extent of possible large deletions by targeting a large genomic region, which is likely to be non-essential. The analysis of on-target mutations showed an unexpectedly high number of large deletions and chromosomal rearrangements at the CRISPR target loci. We also observed an increase of on-target structural variants in multiplexing experiments as compared to single target experiments. The multiplexing of two targets within a putatively non-essential region led to a truncation of chromosome 3 at the target locus in multiple cases, causing the deletion of 20 genes and several ribosomal DNA repeats. The identified de novo off-target mutations were rare and randomly distributed, with no apparent connection to unspecific CRISPR/Cas off-target binding sites.



PICHIA 2022

PICHIA Abstracts

## Metabolomics and metabolic engineering reveals a hidden methanol, formate and $CO_2$ assimilation pathway in *Komagataella phaffii* – the oxygen-tolerant reductive glycine pathway

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Komagataella phaffii (Pichia pastoris) has a well-known and in detail described main methanol assimiliation pathway, the xylulose 5-phosphate pathway. By applying C-methanol and C-formate labeling as well as reverse labeling combined with advanced GC-HRMS and LC-IM-QTOFMS metabolomics methodologies, we revealed an alternative methanol and the native formate assimilation pathway, which co-assimilates $CO_2$ . Generally, co-assimilation of $CO_2$ is desirable as it renders production processes more sustainable. By knocking out DAS1 and DAS2 with CRISPR/Cas9, the xylulose 5-phosphate pathway was blocked. Isotopologue distribution analysis of intracellular metabolites in combination with C-methanol labeling, C-formate labeling or reverse labeling was applied to investigate pathway activities. To evaluate the labeling degree in the folate pathway, the development of a fit-for-purpose folate extraction method was necessary, and selective analysis was subsequently performed with LC-IM-QTOFMS. The isotopologue distribution analysis results revealed that the oxygen-tolerant reductive glycine pathway is active: methanol is dissimilated to formate, which is then fixated by the tetrahydrofolate cycle to form methylenetetrahydrofolate. Glycine is provided by de-novo synthesis via reaction of methylenetetrahydrofolate with $CO_2$. A methyl group transfer from methylenetetrahydrofolate to glycine leads to serine, which is then degraded to pyruvate and used as a precursor for all biomass formation. All enzymes catalyzing these reactions are encoded in K. phaffii. Further targeted knockouts and overexpressions of this pathway confirmed that alternative methanol, formate and $CO_2$ assimilation in this yeast indeed occurs via the oxygen-tolerant reductive glycine pathway. This pathway activity was previously only known as a synthetic route in E. coli.

# PICHIA Abstracts

## *Pichia pastoris* for sustainable manufacturing of alkaloid drugs

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Naturally occurring alkaloids have substantially contributed to the pharmaceutical industry for more than a century, but the low abundance of alkaloids makes their extraction inefficient. The use of natural alkaloids is expected to increase with the expanding and aging global population, thus causing ecosystem stress. The yeast Pichia pastoris offers promising alternatives to sources from nature for producing value-added alkaloids. I will mainly focus on describing a synthetic biology platform for sustainable biomanufacturing alkaloids in yeast, involving other disciplines such as bioprocess engineering and protein engineering. Such a platform not only allows for cost-effective production of pharmaceutical drugs but has potential to also addresses the challenges of food supply sustainability.



PICHIA 2022

PICHIA Abstracts

## Engineering Adh2 for improved methanol utilization in *Komagataella phaffii*

**Moritz C**[1,2], Hermann E[2], Ata Ö[1,2], Peterbauer C[2], Mattanovich D[1,2]

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Komagataella phaffii (Pichia pastoris) is a yeast species of high biotechnological interest in part due to its ability to utilize methanol to generate both biomass (assimilation) and energy (dissimilation). It was recently demonstrated that in the absence of the methanol oxidizing enzymes Aox1 and Aox2 K. phaffii can utilize methanol through the native alcohol dehydrogenase 2 (Adh2) enzyme, significantly increasing in the amount of cellular energy (NADH) harvested from each molecule of methanol. While superior in terms of efficiency, the methylotrophic growth of this strain remains limited by the low activity of Adh2 towards methanol. We have therefore aimed to use enzyme engineering to produce variants of Adh2 with improved in vivo activity towards methanol using both targeted and random mutagenesis approaches. For the targeted approach, molecular dynamics simulations of K. phaffii Adh2 with the substrates and products of the reaction were used to select eight different residues for site saturation mutagenesis (SSM). A novel in vivo biosensor coupled with flow cytometry was then used to assess the performance of the enzyme variants compared to the wild type form. We have additionally developed a workflow employing a high throughput biosensor screening strain and fluorescence activated cell sorting (FACS) that enables the screening of larger libraries of enzyme variants generated through error-prone PCR. Here we present the workflows and results from the two Adh2 engineering approaches, which may in the future be applied to generate more carbon-efficient K. phaffii strains for the production of industrial chemicals from methanol.

# PICHIA Abstracts

## Auxin-induced degradation of target proteins in *K. phaffii*

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The auxin-inducible degron (AID) system is a useful technique to rapidly deplete any protein of interest 'on-demand'. The system uses the plant hormone auxin, it's in vivo binding target, IAA17 (the AID-tag), and its adaptor for E3 ubiquitin ligase, TIR1. To generate AID-based conditional knockout strains, a target gene fused to the AID-tag sequence must be integrated into the endogenous target gene locus by homologous recombination. Ectopically expressed TIR1 protein allows cells to rapidly degrade AID-fused target proteins upon the addition of auxin. In order to establish the AID-system in K. phaffii, we constructed robust integration plasmids expressing TIR1 from promoters of different strength that can easily be transformed and efficiently be integrated into any K. phaffii strain expressing a target gene fused to an AID-tag. First, we tested auxin-induced degradation of the glycerol kinase Gut1. Moderate expression of TIR1 resulted in complete degradation of the target protein within several minutes. Second, we show that the absence of all three Wsc type sensors is detrimental for cell growth, which indicates that these are the dominant cell wall sensors in this yeast. Third, down-regulation of Erg1, an essential enzyme of the ergosterol biosynthetic pathway, resulted in quick and efficient accumulation of squalene, a pharmaceutically relevant reagent. We conclude that AID is an extremely powerful tool that, for the first time, enables the analysis of gene essentiality and function in K. phaffii.



PICHIA 2022

# PICHIA Abstracts

## Is medium heterogeneity really a problem in large scale bioreactor?

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Medium heterogeneities are inherent in industrial scale bioreactor due to their inefficient mixing abilities, especially when operated in fed-batch. Consequently, cells face periodically harsh culture conditions that may impact their metabolism. Cell response to transient perturbations (TPs), namely high methanol concentration combined with hypoxia, have been investigated using a P. pastoris strain bearing a pAOX1-EGFP construct in a two compartment STR-STR scale down systems. TPs were design based on hydraulic circulation time found at 10 and 100 m3 scale; giving a residence time for cells under TPs of 60 and 180 seconds. Although, TPs negatively affect cell growth; methanol consumption rate, oxygen uptake rate and EGFP expression level increased unexpectedly as mixing efficiency decrease. In scale down systems, part of the cells population grew as pseudofilament with increased EGFP fluorescence level. Similarly, FLO (FLO5, FLO11) and ERG (ERG11, ERG25) genes involved in flocculation and ergosterol metabolism, respectively, were also upregulated. When the secretory lipase CalB was considered instead of intracellular EGFP, a similar trend was found at gene expression level. By contrast, severe TPs were found to decrease the extracellular CalB titer, most probably due to a stronger UPR. Indeed, UPR genetic markers, namely HAC1, KAR2, ERO1 and PDI, were significantly increased at higher medium heterogeneity. Nevertheless, using a methanol/sorbitol co-feeding strategy, the UPR activity could be lowered with beneficial effect on the CalB extracellular titre.

# PICHIA Abstracts

## Applying Dynamic Time Warping to Machine Learning based Automation of *Pichia pastoris* Fermentations

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For the analysis and classification of time series, there is a single algorithm that consistently outperforms all other methods: Dynamic time warping (DTW), often in combination with k-nearest neighbors (kNN). While kNN-DTW has increasingly gained attention in biotechnology, it is still only rarely applied, despite its promising potential.
In our presentation we showcase two different usecases for application of this powerful algorithm in high-density fed-batch fermentations of the industrial expression platform Pichia pastoris.
In the first section of the presentation, we demonstrate a simple example of how knn-DTW can be applied to detect process events. We focus on end-of-batch detection using only standard in-line sensors as inputs for processes that vary in scale, control parameters, and organisms. We demonstrate methods to create robustness against sensor biases and sudden faults and how relevant hyperparameters influence performance.
In the second section of the presentation, we apply DTW to trend and pattern mining of flow cytometry data to control population age via tailored feed rates. Changes in morphology and genealogical age of Pichia pastoris are monitored using at-line flow cytometry with bud scar staining.





# PICHIA Abstracts

## A dynamic feeding strategy for recombinant *Pichia pastoris* strains

**Julian Kopp**[1], Mihail Besleaga[1] and Oliver Spadiut[1]

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Since the publication of the "Pichia Fermentation Process Guidelines" by the Invitrogen Corporation in 2002, many fermentation strategies for P. pastoris have been developed. However, diverse strain specific parameters need to be determined in order to set up a proper feeding regime for fed-batch cultivations. Here, we present a fast screening method to extract essential strain characteristic parameters, which are required to set up a dynamic feeding strategy for P. pastoris strains, based on the specific substrate uptake rate ($q_s$).

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# **PICHIA** Abstracts

## AI Models applied in RQ control to optimize recombinant protein production in *Pichia pastoris*

**Arnau Gasset**[1], Albert Sales[1], Joeri Van Wijngaarden[2], Xavier Garcia-Ortega[1], José Luis Montesinos-Seguí[1], Toni Manzano[2], Francisco Valero[1]

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Oxygen limitation has been shown to improve recombinant protein production (RPP) in Pichia pastoris, under the regulation of the GAP promoter. This environmental stress (hypoxia) can be monitored by the respiratory quotient (RQ). A step further is to design and to implement a control algorithm using RQ as a controlled variable to maintain the desired hypoxic conditions in the culture acting on agitation rate.

This strategy has been studied at three different stages: first, a manual-heuristic control system was tested, obtaining 2-fold higher productivities with respect to oxygen non-limiting conditions with a P. pastoris strain producing heterologous Candida rugosa lipase 1 (Crl1). Then, an automatic control algorithm has been implemented, considerably reducing labour and time costs while maintaining the 2-fold increase in productivity and also increasing precision and stability. Finally, as a more attractive and scalable strategy from an industrial perspective, an RQ-control algorithm based on artificial intelligence (AI) has been designed, implemented and tested. Continuous data feeding is conducted from the fermenter to an on-line server, where an AI model predicts on-line the proper agitation to maintain a constant RQ throughout the fermentation process.

As conclusion, it can be stated that an innovative physiological control has been implemented based on an AI approach allowing to achieve results comparable to those obtained with classical methods, increasing 2-fold yields and productivities compared to standard oxygen conditions (normoxia). This opens a window for this data-driven technologies to be implemented in bioprocesses based on microbial fermentation including both pharma and biotech industrial sectors.

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# Poster No. 75

## Enzymatic synthesis of polygallate in fed-batch conditions and its characterisation

Marina Ti ma[1], Darijo ibalić[2], Ana Bucić-Kojić[3], Jelena Vukov-Parlov[4], Tatjana Tomić[5], **Mirela Planinić[6]**

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The enzymatic oxidation of gallic acid catalysed by laccase from Trametes versicolor to produce polygallate was performed in 100 cm3 magnetically stirred glass reactors in 12-cycle repetitive batch conditions at following process conditions: T = 27 °C, pH 5, n = 220 rpm, c0, gallic acid = 0.085 mmol dm-3, ɣ0, laccase= 0.099 mg cm-3. Gallic acid was added every 30 min in amount to obtain initial concentration approximately the same as it was in the beginning of the first cycle. The product was precipitated in methanol and dried to obtain the polymer as black powder which was then characterized by FTIR and NMR spectroscopies.

The FTIR spectrum was acquired by means of attenuated total reflectance (ATR) technique on a Shimadzu Tracer 100 spectrometer. The ATR spectrum was recorded in the single reflection configuration, over the 4000 – 400 cm-1 spectral range with the resolution of 4 cm-1. The total scans were 128. The NMR experiments were performed at 298 K and chemical shifts were reported relative to tetramethylsilane (TMS) internal standard. One-dimensional 1H NMR spectra were recorded on a Bruker Avance Neo 300 NMR spectrometer in DMSO d6 and D2O using a C/H BB 5 mm probe with 1000 scans and 10 s recycle delay.

The synthesised product, polygalate, may have various applications, in the cosmetics industry as a component of hair dyes, in the textile industry for dyeing textiles, in 3D printing for medical applications, etc.





# Poster No. 76

## Upstream process development for a VHH-Fc antibody in the *Pichia* expression system in the scope of pandemic readiness

**Semiramis Yılmaz,** Chiara Lonigro[1], Erhan Çıtak[1], Delphine Devriese[2], Loes van Schie[1], Pieter Vanhaverbeke[1], Katrien Claes[1], Nico Callewaert[1]

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The development of both time- and cost-efficient upstream production processes is becoming more crucial for all biomanufacturers, as wholeheartedly experienced in the COVID19 pandemic. Pichia expression system is biotech's most favored yeast expression system in these respects, although it brings significant engineering challenges for at-scale manufacturing originating from the methanol utilization metabolism (high aeration and cooling requirements, safe handling requirement of methanol). Here we aimed to develop a platform for a VHH-Fc molecule (an anti-Covid antibody), only pursuing methanol-free options of Pichia technology. We have mainly focused on strain development and process optimization. For establishing an optimized baseline VHH-Fc expressing strain, several Pichia strains were generated considering the promoters, the secretion leader sequence, gene copy number, and overexpression of certain secretory system-enhancing factors. The generated strains were tested on small scale (24-well plate). The promising ones were then characterized in Fed-Batch operation mode on a 3L bioreactor scale by performing a kinetic analysis for investigation of the changes in the state variables (specific rates and yield coefficients). As a result, we obtained very similar performance parameters at each run that was controlled with a different specific growth rate, suggesting a robust process. The verification of the process which assures the product titer and folding properties of the molecule was done further on 6L and 10L scales consecutively.

# Poster No. 77

## Use of the OPENPichia toolbox for rapid prototyping of affinity-enhanced heavy chain-only antibody variants against SARS-CoV-2

Chiara Lonigro, **Loes van Schie**, Wim Nerinckx, Kenny Roose, Bert Schepens, Sandrine Vanmarcke, Annelies Van Hecke, Gitte Michielsen, VIB-CMB COVID-19 Rapid Response Team, Xavier Saelens and Nico Callewaert

VIB-UGent Center for Medical Biotechnology, VIB, Technologiepark-Zwijnaarde 75, 9052 Ghent, Belgium. Department of Biochemistry and Microbiology, Ghent University, Technologiepark-Zwijnaarde 75, 9052 Ghent, Belgium

Antibody-based therapeutics could be part of the first line of defense against infectious disease outbreaks, providing passive immunization to the general population, and immune-compromised people in particular, until vaccine-induced immunity is reached. To generate and screen antibody variant libraries against new pathogens – such as SARS-CoV-2 – in the early phase of a pandemic, rapid recombinant protein production is key. However, expression in the mammalian expression hosts typically used for monoclonal antibodies is tedious and expensive and the use of an alternative host system such as Pichia pastoris, capable of processing complex eukaryotic proteins, could be advantageous. Guided by molecular modeling, we designed a set of anti-SARS-CoV-2 heavy chain-only antibodies with point mutations that could potentially increase antigen affinity. These antibody variants were then generated rapidly using a Golden Gate-based modular cloning toolkit and constitutive expression in OPENPichia. Crude Pichia supernatant could be used for prototyping the antibodies by biolayer interferometry and in vitro characterization of binding to SARS-CoV-2 spike protein on the surface of mammalian cells. This allowed us to prioritize antibody variants with enhanced affinity in our intended bivalent Fc fusion drug context for further characterization and development, within a week upon the arrival of synthetic DNA fragments. The antibody variant selected for further drug development based on early prototyping in Pichia eventually led to XVR011, a stable, anti–COVID-19 biologic produced in mammalian cells that were evaluated in the clinic. To increase the accessibility of such therapeutics, future strain engineering and process optimization steps could potentially move Pichia beyond prototyping and turn it into a cost-effective host for large-scale heavy-chain antibody production.





# Poster No. 78

## Systems metabolic engineering of *Pichia pastoris* for 3-Hydroxypropionic acid production from glycerol

Albert Fina[1], Stephanie Heux[2], Joan Albiol[1], **Pau Ferrer**[1]

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3-hydroxypropionic acid (3-HP), a three carbon organic acid, has been identified as one of the top platform chemicals from biomass by the US Energy Department and, consequently, development of cell factories for efficient 3-HP production has gained remarkable attention in recent years. So far, production of 3-HP for revalorization of multiple carbon sources has been reported using a variety of microorganisms, including bacteria and yeast. Pichia pastoris (syn. Komagataella phaffii) is able to grow on crude glycerol, which is an abundant, inexpensive and renewable feedstock produced as a main by-product of the conventional biodiesel production process. Moreover, glycerol is very attractive for production of organic acids such as 3-HP due to its higher degree of reduction compared to glucose.
In this study, P. pastoris was metabolically engineered to produce 3-HP via the malonyl-CoA pathway, using glycerol as sole carbon source. With the goal to increase further 3-HP, we explored multiple metabolic engineering strategies and studied their effects at the metabolic flux level using a high-throughput 13C-based metabolic flux analysis platform. Fed-batch cultures of the final engineered strains with a pre-programmed exponential feeding strategy produced up to 37 g/L of 3-HP with the yield and productivity of 0.19 Cmol/Cmol glycerol and 0.71 g/L/h, respectively [1]. To the best of our knowledge, this is the highest 3-HP productivity achieved in yeast.

[1] Fina et al. (2022). Front. Bioeng. Biotechnol. 10:942304. doi: 10.3389/fbioe.2022.942304.

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# Poster No. 79

## Metabolic Engineering of *Pichia pastoris* for renewable methanol bioconversion into 3-Hydroxypropionic acid (3-HP), a platform chemical for acrylic acid and bioplastics production

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Methanol is increasingly gaining attraction as renewable carbon source to produce specialty and commodity chemicals [1], as it can be generated from sustainable raw materials. In this context, aerobic methylotrophs such as Pichia pastoris are potentially attractive cell factories to produce a wide range of products from this highly reduced substrate.

3-Hydroxypropionic acid (3-HP) was identified as one of the top future value platform chemicals from biomass [2], with a huge market potential as building block. 3-HP can be naturally produced by several bacteria through different biosynthetic routes. Recently, we have successfully expressed the malonyl-CoA pathway in P. pastoris to produce 3-HP from glycerol [3]. After further metabolic engineering, the best strain produced up to 37.05 g L-1 of 3-HP in fed-batch cultures [4].

In this study, the alternative β-alanine metabolic pathway has been established in P. pastoris for the first time with the expression of three different heterologous genes: PAND from Tribolium castaneum, BAPAT from Bacillus cereus and YDFG from Escherichia coli K-12 [5]. Initially, the expression of these genes was placed under the control of the constitutive GAP promoter. An additional series of strains was engineered carrying the same set of genes under the control of different methanol inducible promoters.

A first series of small-scale screening experiments were performed with 24 deep-well plates using 1.5% (v/v) methanol as C-source. The best strain produced 1.32 g L-1 of 3-HP, with a final product yield of 0.118 Cmol Cmol-1. To further characterize these strains, bioreactor-scale cultures are being performed.





# Poster No. 80

## Pathway design for mixotrophic production of biochemicals from $CO_2$ and methanol in yeasts

**Golnaz Memari**[1,2], Özge Ata[1,2], Diethard Mattanovich[1,2]
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Autotrophy and mixotrophy in industrial strains have the potential to contribute to the mitigation of climate change,and they are therefore of a great interest towards a more sustainable production of biochemicals.

In previous studies, functional autotrophy was engineered in yeasts by integrating the Calvin-Benson-Bessham (CBB) cycle in Pichia pastoris (Komagataella phaffii), and similarly in Escherichia coli , allowing growth on CO2 as the sole carbon source. The autotrophic P. pastoris strain uses methanol for energy supply. In this project, we aim to design alternative pathways for assimilation of both CO2 and methanol for the conversion of CO2 into value-added compounds. Using methanol in both assimilatory and dissimilatory pathways lowers the overall methanol demand. A blueprint for such a mixotrophic pathway is the
bacterial serine cycle and modifications thereof.

By using methods for synthetic biology such as Golden Gate Assembly and CRISPR-Cas9, we are aiming to design thermodynamically feasible and energetically favorable pathways for the production of organic acids from a mixed feed of methanol and CO2. For this purpose, additional enzymatic loops are added to the serine cycle by either introducing bacterial genes or activating native yeast pathways. Functionality of the novel cyclic pathways is assessed by the capacity to produce the target molecules and intermediates from methanol and CO2.

# Poster No. 81

## On the functional expression of fungal LPMOs in *K. phaffii*

**Lukas Rieder**[1,2], Katharina Ebner[3], Anton Glieder[1], Morten Sørlie[2]

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The Lytic Polysaccharide MonoOxygenases are mono-copper enzymes that catalyze the oxidative cleavage of glycosidic bonds. Thus, LPMOs are crucial for the efficient degradation of recalcitrant sugar polymers making them indispensable for natural and industrial saccharification processes. Despite major breakthroughs regarding the catalytic mechanism, research aiming to discover the full potential for biotechnological applications of these enzymes is still at the beginning.

To obtain large quantities of secreted recombinant LPMO of fungal origin, we decided to use Komagataella phaffii, as production host. For the methanol free production of LsAA9A and NcAA9C, the genes were cloned into the commercially available pBSY3Z and the newly assembled pBSYPGCW14Z that use different promoters for the regulation of LPMO transcription. A second challenge connected to the recombinant production of LPMOs is the processing of the signal peptide as the mature LPMO requires a histidine at the N-terminal position. As it was shown previously that, the α-mating sequence is not suitable for the expression of LPMOs, we tested the OST1 and the pre-OST1-pro-α-factor for the functional secretion of NcAA9C.

To validate the established 3-step LPMO production protocol, the homogeneity of the produced enzymes was verified using SDS-PAGE and quantified using the Bradford assay. The functionality of the LPMOs made with the different plasmids and SP was assessed from the oxidization of PASC using classical monooxygenase reaction conditions with subsequent product analysis via HPAEC-PAD chromatography. In addition to assessing the activity of the NcAA9C SP variants on PASC, the processing of the N-terminus was investigated using MALDI-ToF MS.





# Poster No. 82

## Effect on methanol feeding strategies on itaconic acid production in *Komagataella phaffii*

**Manja Mølgaard Severinsen**[1], Simone Bachleitner[1], Özge Ata[1,2], Lisa Lutz[2], Diethard Mattanovich[1]

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The overall purpose of this project is to generate Komagataella phaffii (Pichia pastoris) strains capable of energetically favorable production of organic acids in fed-batch cultivations. Itaconic acid is a value-added organic acid which can be synthesized from cis-aconitate, an intermediate of the tricarboxylic acid cycle. MeOH-induced expression of the heterologous gene cadA enabled K. phaffii to produce itaconic acid, and additional expression of the mitochondrial (mttA)- and cytoplasmic membrane transporters (mfsA) increased the itaconic acid yield significantly.

Itaconic acid production procedure has two phases: in the first phase biomass is produced utilizing glycerol, while in the second phase methanol is used as an inducer for itaconic acid production and serves at the same time as a carbon source. However, K. phaffii is also sensitive to methanol concentrations. Thus, different feeding strategies and process optimizations are in focus of this work. In our first strategy the glycerol batch phase was followed by a glucose/MeOH Co-Feed to slowly adapt the cells to methanol, and is finally replaced by a linear MeOH feeding rate, controlling the growth rate at 0.05 h-1. In our second strategy, biomass production was decoupled from itaconic acid production by nitrogen-limitation, and the glycerol batch was followed by a MeOH-pulse and a limited MeOH-feed. Both strategies are evaluated here, to optimize itaconic acid production in K. phaffii.

# Poster No. 83

## Effect of specific growth rate on recombinant protein production by *Pichia pastoris* under hypoxic and normoxic conditions

**Albert Sales[1],** Arnau Gasset[1], Francisco Valero[1], Xavier Garcia-Ortega[1], Jose-Luís Montesinos-Seguí[1]

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It has been described that the implementation of controlled hypoxic conditions increases the performance of PGAP-regulated Pichia pastoris bioprocesses for recombinant protein production (RPP). Nevertheless, further research focused on studying the interaction of oxygen-limiting conditions with other key parameters are essential to optimize the performance of these bioprocesses. One of the parameters is the specific growth rate (μ), which influences the physiologic state of the cell factory.

The present study compares the production of the heterologous Candida rugose lipase 1 (Crl1) at different μ (0.030, 0.065, 0.10 and 0.12 h-1), both in normoxic and hypoxic conditions in carbon-limiting fed-batch cultures. According to previous results, the respiratory quotient (RQ) was kept between 1.3 and 1.4 for hypoxic fermentations. To achieve these conditions an innovative automatic control based on the stirring rate as manipulated variable was designed and implemented.

Results confirm the positive effect of the oxygen limitation, leading a shift from respiratory to respiro-fermentative metabolism and increasing the productivity of the bioprocess up to 2-fold for the specific growth rates tested. Production parameters were significantly higher for cultures grown under hypoxic conditions. When comparing cultures at different μ, it was observed the same production kinetics pattern indistinctly for either normoxic or hypoxic cultures. The specific product generation rate (qp) increases linearly when increasing the specific growth rate (μ). These results support the idea that a rational study of the production process allows the design and implementation of innovative operation strategies to enhance RPP improving the efficiency of these bioprocesses.





# Poster No. 84

## Producing Nanobody® molecules modified with free Cysteines in *Pichia pastoris*

**Peter Schotte[1]**

[1]sanofi, Zwijnaarde, Belgium

Modifying and producing Nanobody® molecules with extra cysteines often result in a drop in titer and a heterogenic product due to cysteine reactive adducts from cellular or media components. This heterogeneity complicates and reduces the efficiency of the thiol coupling chemistry to toxins, radioactive tracers and with other cysteine modified proteins. Addition of cysteine, glutathione or cysteamine to the cultivation medium reduces the heterogeneity of the Nanobody-cysteine product and shifts the balance from the dimer to a monomeric Nanobody population. In some cases, we also observed a significant increase in titer of the Nanobody® molecules when cysteamine and glutathione capping agents were added to the culture medium.

# Poster No. 85

## Moving toward methanol-free protein expression

**Pauline Dechaene**[1]
[1]Sanofi, Zwijnaarde, Belgium

The current NANOBODY® platform uses the yeast Pichia pastoris (syn. Komagataella phaffii) as expression host. This yeast is known for its methanol-inducible expression system, allowing it to become one of the preferred hosts for microbial biotechnology in general and for NANOBODY® production in particular. Despite this system being the cornerstone of the NANOBODY® platform for years, the use of methanol can present challenges within a large scale production setting. Hence there is a need for alternative "methanol-free" systems. In order for the NANOBODY® platform to evolve to a methanol-free platform, a feasibility study was performed within the Sanofi Early CMC department aiming to generate a new platform strain and -process, capable of producing multi-gram quantities of NANOBODY® molecules.





# Poster No. 86

## Producing real dairy sustainably through precision fermentation with yeast

**Albert Fina**[1], Sergi Monforte[1], Esther Paulo[1], Zoltan Toth-Czifra[1], Gabriel Mora[1]

[1]Real Deal Milk SL, Barcelona, Spain

Cows are inefficient at producing milk. 144 L of water are needed to produce 1 L of milk. Moreover, the dairy industry takes responsibility for 4% of the greenhouse gas emissions worldwide. Low milk prices have forced farmers to intensify classical milk production, undermining cows' welfare. Thus, sustainable alternatives to classical milk production are needed to feed the growing world population.

In Real Deal Milk we are developing yeast strains that produce milk proteins. The main two groups of milk proteins include whey proteins and caseins. Caseins interact with each other making a supramolecular structure called micelle. Getting micelles is crucial towards using such proteins to make cheese. Micelles exist in suspension. When treated with rennet or acid, interactions within the micelle-forming proteins change and trigger curdling, the starting point of the cheese-making process. Therefore, the milk obtained from microbial fermentation can be used to produce the same cheese variants we are already familiar with. Such trait represents a competitive advantage compared to plant-based milk substitutes, as yeast-based dairy allows to preserve the food culture associated with dairy products from every region. Production of vegan products which are almost identical to traditional animal-based products would make the transition towards an animal-free diet easier for the largest market of potential customers, the omnivores.
Our research has achieved the generation of yeast strains producing caseins that are able to form micelles. The current research is focused on increasing the product yield and fine-tuning micelle properties using both strain and bioprocess engineering strategies.

# Poster No. 87

## Enhancing expression of a Fumonisin B1 degrading carboxylesterase (FumD) in *Pichia pastoris* by chaperone co-expression

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Fumonisin B1 (FB1) is a mycotoxin frequently present in grain, grain derived products and animal feed. It shows cancerogenous and teratogenous effects on mammals and its degradation is of high interest. In the bacterium Sphingopyxis sp. MT144 the consecutive action of two enzymes FumD and FumI, a carboxylesterase and an aminotransferase, respectively, was shown to degrade FB1.
Since recombinant expression of FumD in E. coli was only partly successful - formation of inclusion bodies and poor solubility of recombinant protein - Komagataella phaffii (P. pastoris) was evaluated as alternative expression host early on. (Heinl et al., 2010) Although this endeavor was more successful publications still indicate significant bottlenecks in enzyme production, which can partially be overcome by overexpression of helper proteins. (Ruth et al., 2014)
We aimed to improve secreted FumD production with K. phaffii as host, focusing on balanced co-expression of helper factors. Briefly, since the FumD has three predicted disulfide bridges we evaluated simultaneous overexpression of the FumD with the chaperone protein disulfide isomerase (PDI) to potentially enhance correct protein folding and consequently titer of active enzyme. To fine-tune expression profiles of both enzymes and therefore allow the system to unfold its whole potential, FumD and the PDI were co-expressed using various differently regulated synthetic bidirectional promoters (BDPs). Evaluating the actions of constitutive, de-repressible and methanol inducible promoters in six different combinations, we were able to increase the titer of secreted FumD 2 to 2.5-fold in comparison to the respective monodirectional control strains.





# Poster No. 88

## Biochemical characterization of the β-glucosidase Glu1B from Coptotermes formosanus produced in *Pichia pastoris* and identification of residues responsible for the high specific activity toward cellobiose and resistance to glucose

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β-glucosidases are enzymes that hydrolyze β-1,4-glycosidic bonds from non-reducing terminal residues in β-D-glucosides, with the release of glucose. β-glucosidases currently used for the saccharification of lignocellulosic biomass have low efficiency in hydrolyzing cellobiose and are inhibited by glucose, contrary to what would be desirable. We engineered Pichia pastoris strains to produce the β-glucosidase Glu1B from the termite Coptotermes formosanus, and biochemically characterized the recombinant enzyme. After 36 h of methanol induction in shake flasks, the P. pastoris KM71BGlu strain produced and secreted 4.1 U/mL (approx. 26 mg/L) of N-glycosylated β-glucosidase Glu1B. The recombinant product had an optimum pH of 5.0, optimum temperature of 50°C, residual activity at 40°C higher than 80%, specific activity toward cellobiose of 431-597 U/mg protein, and a Ki for glucose of 166 mM. The protein structure was stabilized by Mn 2+ and glycerol. The high specific activity of the recombinant β-glucosidase Glu1B was correlated with the presence of specific residues in the glycone (Gln455) and aglycone (Thr193 and Hys252) binding sites, along with linker residues (Leu192, Ile251, and Phe333) between residues of these two sites. Moreover, the resistance to inhibition by glucose was correlated with the presence of specific gatekeeper residues in the active site (Met204, Gln360, Ala368, Ser369, Ser370, Leu450, and Arg451). Based on its biochemical properties and the possibility of its production in the P. pastoris expression system, the β-glucosidase produced and described in this work could be suitable as a supplement in the enzymatic hydrolysis of cellulose for saccharification of lignocellulosic biomass.

# Poster No. **89**

## Sequence engineering of an Aspergillus niger tannase to produce in *Pichia pastoris* a single-chain enzyme with high specific activity

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Tannin acyl hydrolases or tannases (E.C.3.1.1.20) are enzymes that hydrolyze the ester bond of tannins to produce gallic acid and glucose. We engineered the Aspergillus niger GH1 tannase sequence and Pichia pastoris strains to produce and secrete the enzyme as a single-chain protein. The recombinant tannase was N-glycosylated, had a molecular mass after N-deglycosylation of 65.4 kDa, and showed activity over broad pH and temperature ranges, with optimum pH and temperature of 5.0 and 20°C. Furthermore, the single-chain tannase had an 11-fold increased specific activity in comparison to the double-chain A. niger GH1 tannase, which was also produced in P. pastoris. Structural analysis suggested that the high specific activity may be due to the presence of a flexible loop in the lid domain, which can control and drive the substrate to the active site. In contrast, the low specific activity of the double-chain tannase may be due to the presence of a disordered and flexible loop that could hinder the substrate's access to the binding site. Based on its biochemical properties, high specific activity, and the possibility of its production in P. pastoris, the tannase described could be used in food and beverage processing at low and medium temperatures.



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# Poster No. **90**

## *Komagataella phaffii:* QTL mapping for strain development

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Komagataella phaffii is among the most widely used cell factories for recombinant protein production. The majority of research on improving its industrially relevant traits is based on one species (K.phaffii), specifically on the strain background of CBS7435. The genus of Komagataella., however, consists of seven different species with various natural isolates from different natural habitats. Consequently, strains show considerable inter- and intraspecies phenotypic variation including traits of industrial relevance.

Two such traits are the subject of this study, namely temperature tolerance and recombinant protein production. Both phenotypes are regulated in a complex manner, making it difficult to pinpoint the exact genetic determinants contributing to observed differences in these phenotypes.

The expression of both phenotypes was investigated among a set of natural isolates belonging to all seven currently known Komagataella species. Initial crosses were performed with K.phaffii strains only upon identification of four parent strains showing sufficiently large genetic variation, and high variation in production potential and growth at non-optimum temperatures of 12 and 39°C; we then designed a Quantitative Trait Loci mapping experiment for bulk segregant analysis. Recombinant inbred lines were established and resulted in 600 randomly selected F14 hybrids exhibiting a wide range of protein secretion yields and temperature tolerance. For both traits, hybrids were ranked according to their phenotypes to generate high, low and random pools for whole genome re-sequencing. Ongoing bioinformatical analysis yielded first QTL regions, which are currently being examined in detail to get a higher resolution of the beneficial genetic determinants responsible for both phenotypes.

# Poster No. 91

## Improving the Expression of Unspecific peroxygenases (UPOs) Using Signal Peptide Domain Engineering

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Unspecific peroxygenases (UPOs) are enzymes with great potential for industrial biocatalysis as they are able to perform highly efficient selective oxidations using only hydrogen peroxide as a co-substrate. Recombinant expression of UPOs has, however, proved to be challenging, with only modest expression levels reported for secreted expression from yeasts such as S. cerevisiae and K. phaffi, although directed evolution of the enzyme from Agrocybe aegerita (AaeUPO) has produced an enzyme variant PaDa-I with increased expression levels and activity in these yeasts.(1)
In this study we aimed to develop a universal protocol for the functional expression and secretion of target enzymes of interest in K. phaffi, using truncated constructs of AaeUPO with its native signal peptide and the mutant signal peptide from the PaDa-I variant as a model. The signal peptide was subjected to directed evolution and the platform was developed by taking advantage of S. cerevisiae genetic machinery to reconstitute plasmids in vivo,(2) allowing the development of mutant libraries which will be easily screened by using Gaussia luciferase as a marker.
High-level expression cassettes identified in S. cerevisiae will be applied to the full-length enzyme, then transferred to K. phaffi for fermentation.

**1.** P. Molina-Espeja, E. Garcia-Ruiz, D. Gonzalez-Perez, R. Ullrich, M. Hofrichter and M. Alcalde, Appl. Environ. Microbiol., 2014, 80, 3496–3507.

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# Poster No. 92

## Protein production dynamics and physiological adaptation of recombinant *Pichia pastoris* at near-zero growth rates

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Recently, we implemented retentostat cultivation for the yeast Pichia pastoris (syn. Komagataella spp) (Rebnegger et al., 2016), a popular host for recombinant protein production. Retentostat cultivation is a form of continuous cultivation where the biomass is fully retained within the bioreactor and the supply of limiting substrate per biomass becomes increasingly restricted. This naturally results in a steady decrease in the specific growth rate, theoretically culminating in a scenario where nutrients are fully spent on cellular maintenance and growth ceases.
To investigate if recombinant protein production continues at extremely slow growth, P. pastoris secreting a camelid single-domain antibody fragment (vHH) was cultivated in aerobic glucose-limited retentostats, reaching biomass doubling times of nearly two months. Production levels of vHH were monitored by quantitative microfluidic-capillary-electrophoresis as well as SDS-PAGE. Additionally, quantitative physiological data including cellular macromolecular composition as well as an in-depth analysis of global transcriptional changes was obtained and will be discussed in the context of recombinant protein production and cellular adaptation to near-zero growth conditions.

Rebnegger C., et al. 2016 Appl Environ Microbiol 82(15):4570-4583

# Poster No. 93

## Beyond engineering - What do we know about *Pichia pastoris*?

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Many advantages have contributed to the fact that Pichia pastoris is nowadays a widespread host for recombinant protein production and frequently used for large scale production of different proteins. Due to novel trends in the last few years, Pichia pastoris has become more and more relevant for food production.
The more Pichia pastoris is used in large scale production, becoming involved in everyday life (e.g., food production), the more we want to know about its behavior and viability. So far, basic scientific research in the field of Pichia pastoris has mainly been focused on understanding and optimizing genetic engineering and protein production of the methylotrophic yeast.
Although Saccharomyces cerevisiae is only a "distant relative" of P. pastoris, they are often compared to each other without considering basic differences, such as carbon source utilization, growth, temperature sensitivity etc. In fact, Pichia pastoris is generally described as a "robust" microorganism, less sensitive to temperature fluctuations and other environmental parameters than S. cerevisiae.
However, there are still open questions: e.g., within which temperature or pH range can P. pastoris survive in the lab or in the environment?





# Poster No. 94

## Implementation of CRISPR-dMAD7 in *Komagataella phaffii*

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The CRISPR-Cas9 system became the standard tool for genome editing as it can be used for the deletion and insertion of genes in almost any organism. Key to the tool is a guide RNA (gRNA), that targets a specific locus when complexed with the Cas9 nuclease which in return introduces a double strand break in the genomic DNA. A tool evolved from the classic CRISPR-Cas9 system is the nuclease deficient CRISPR-dCas9 system in which the nuclease is inactive and therefore not able to cut DNA. Still the nuclease can bind to DNA (e. g. in a promoter region) to form a road blocker for the RNA polymerase which provides a unique opportunity for the regulation of gene expression.

Previously the CRISPR-dCas9 was shown to be functional for transcription regulation in K. phaffii. To have an alternative to the Cas9-based system we aimed to adapt the CRISPR-dCas system based on the MAD7 nuclease described by Inscripta. To engineer the required nuclease deficient MAD7, three mutations (D877A, E962A and D1213A) were introduced into the coding sequence. To find optimal conditions for the newly implemented tool, we tested different gRNAs, gRNA-combinations, target promoters, MAD7-codon optimizations and integration loci for their impact on eGFP, HRP, GOX or HNL expression. Our results suggest that CRISPR-dMAD7 is indeed suitable for transcription regulation as lower expression levels/ activities for the reporter proteins were obtained making it an attractive tool for the usage in K. phaffii.

# Poster No. **95**

## Genome sequence analysis of a transformed *Pichia pastoris* KM71 strain

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The Pichia pastoris KM71 strain (his4 arg4 aox1Δ::SARG4) is a histidine auxotrophic strain suitable for generating transformants with methanol utilization slow (Muts) phenotype after transformation with a linearized expression vector harboring a functional copy of the histidinol dehydrogenase (HIS4) gene. This strain comes from the GS115 strain by a partial deletion of the AOX1 gene and a replacement with the Saccharomyces cerevisiae ARG4 gene. We sequenced the genome of a transformed P. pastoris KM71 strain using the Ilumina MiSeq system, assembled de novo the four chromosomes and mitochondrial DNA using the new high-throughput AGATAGenomics system, and the obtained sequences were compared with the reported genome of the GS115 strain. The results obtained suggest that five tandem copies of the expression cassette were integrated into a single location in the yeast genome. Furthermore, multiple point mutations were observed with respect to the described sequences for the GS115 strain. These differences may alter the physiological performance of the KM71 strain compared to the GS115 strain, in addition to the Muts phenotype.





# Poster No. **96**

## Unspecific Peroxygenases: Screening for Stereoselectivity

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Unspecific peroxygenases (UPOs) have shown high potential for the stereoselective oxygenation of nonactivated carbon atoms.[1,2] Current screening strategies for UPOs rely mostly on spectrophotometric assays, are substrate specific and do not assay the potential stereoselectivity of the enzyme.[3] The determination of the enantiomeric excess (e.e.) often presents a bottleneck when screening large (bio)catalyst
libraries for stereoselectivity.[4]
Here we report a fluorometric one-pot, one-step coupled enzyme assay for the high-throughput screening of UPO hydroxylation stereoselectivity and activity. The reaction of interest is the UPO catalyzed hydroxylation of ethylbenzene. In a cascade fashion, the enantiomers of 1-phenylethanol formed in the UPO reaction are further oxidized by two stereocomplementary alcohol dehydrogenases (LkADH and ADH-A) with concomitant reduction of the nicotinamide cofactor. The difference in the increase of NAD(P)H fluorescence in the alcohol dehydrogenase (ADH) catalyzed reporter reactions correlates directly with the stereoselectivity of the UPO of interest.

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# Poster No. 97

## Enhancing economic factors of protein purification process to the edges via model predictive controller

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The current view in preparative and industrial chromatography is that dynamic binding capacity governs the process economy. Increased dynamic binding capacity and column utilization is obtained at the expense of productivity. The dynamic binding capacity of a chromatography column increases with residence time until it reaches a plateau, whereas productivity has an optimum. Therefore, the loading step of a chromatographic process is a balancing act between productivity and column utilization. An online optimization approach for capture chromatography is presented. A residence time gradient during the loading step can be employed to improve the traditional trade-off between productivity and resin utilization. The extended Kalman filter as a soft sensor the nonlinear model predictive controller are used to perform online optimization using the pore diffusion model as a simple mechanistic model. When a soft sensor for the product is placed before and after the column a nonlinear model predictive controller can forecast the optimal condition to maximize productivity and resin utilization. The controller can also account for varying feed concentrations. The robustness of the controller has been examined under the extreme change of the feed concentration in a range of 50%. The online optimization was demonstrated with two model systems: monoclonal antibody purification by protein A affinity chromatography and lysozyme by a cation exchanger. Using this optimization strategy with a controller saves buffer up to 43% and increases the productivity together with resin utilization in a similar range as a multi-column continuous counter current loading process.



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# Poster No. 98

## More enzymes more opportunities for oxyfunctionalization: Sixty novel unspecific peroxygenases for biocatalysis

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Unspecific peroxygenases (UPOs, EC 1.11.2.1) are heme thiolate enzymes divided into two classes (long and short UPOs) which combine the advantages of peroxidases (one electron transfer) and peroxygenases (two electron transfers). They were found in Ascomycota and Basidiomycota [1] and catalyze oxyfunctionalization reactions using hydrogen peroxide as a co-substrate to activate C-H bonds [2]. UPOs are naturally secreted and stable enzymes and therefore they are of great interest for industrial applications, including e.g. the production of agrochemicals, insecticides, dye precursors or pharmaceuticals [3]. However, until recently, only a few UPOs have been recombinantly expressed and characterized. Therefore, our goal was to expand the repertoire of novel UPOs and to test those for potential interesting activities [1]. In this study, we used computational methods (e.g. sequence alignment and homology modelling) to identify novel sequences which then were codon-optimized and cloned via the BioXPTM 3200 system [4] into Pichia pastoris (Komagataella phaffii) expression vectors. In three consecutive steps, either the native secretion signal (nss), or the truncated alpha-mating factor secretion signal (Dα) [5], were combined with the pDC or the pDF: 1st step (pDC + Dα); 2nd step (pDF + Dα); 3rd step (pDF + nss). We successfully produced more than sixty UPOs of different origin. Functionality of the novel UPOs was determined by colorimetric high-throughput assays using typical UPO substrates to determine peroxidase (ABTS, 2,6-DMP) and peroxygenase (naphthalene) activity, respectively. The new UPO panel expands the repertoire of oxidative multi-tool enzymes.

# Poster No. **99**

## Predicting high recombinant protein producer strains of *Pichia pastoris* MutS using the oxygen transfer rate as an indicator of metabolic burden

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The methylotrophic yeast Pichia pastoris (Komagataella phaffii) is a widely used host for recombinant protein production. In this study, a clonal library of P. pastoris MutS strains (S indicates slow methanol utilization) was screened for high green fluorescent protein (GFP) production. The expression cassette was under the control of the methanol inducible AOX promoter. The growth behavior was online-monitored in 48-well and 96-well microtiter plates by measuring the oxygen transfer rate (OTR). By comparing the different GFP producing strains, a correlation was established between the slope of the cumulative oxygen transfer during the methanol metabolization phase and the strain's production performance. The correlation corresponds to metabolic burden during methanol induction. The findings were validated using a pre-selected strain library (7 strains) of high, medium, and low GFP producers. For those strains, the gene copy number was determined via Whole Genome Sequencing. The results were consistent with the described OTR correlation. Additionally, a larger clone library (45 strains) was tested to validate the applicability of the proposed method. The results from this study suggest that the cumulative oxygen transfer can be used as a screening criterion for protein production performance that allows for a simple primary screening process, facilitating the preselection of high producing strains.



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# Poster No. **100**

## Increasing the carbon efficiency of citric acid production

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Citric acid is one of the most important organic acids produced by fermentation with the filamentous fungus Aspergillus niger using glucose as substrate. Citric acid is produced in the TCA cycle from oxaloacetate and acetyl-CoA. In glycolysis, 1 mol glucose is converted into 2 mol of pyruvate. 1 mol of pyruvate is carboxylated to oxaloacetate, the other is decarboxylated to acetyl-CoA, resulting in the net reaction:

$$Glucose + 3\ NAD+ + H_2O \rightleftharpoons Citrate + 3\ NADH$$

While this pathway leads to a theoretically balanced carbon yield it is not redox balanced and re-oxidizing the NADH leads to high oxygen consumption and heat release. Mixed-substrate conversion allows to incorporate CO2, a cheap carbon source, into products with higher oxidation states than the co-substrate. This is a promising strategy to fix CO2 in an industrial process and increase the total carbon yield of the process without requiring oxygen as an electron acceptor, hence reducing the need for extensive cooling.

This research aims to increase the carbon efficiency of citric acid production by developing a synthetic pathway that avoids decarboxylation, hence leading to a net CO2 assimilation during the mixed-substrate production of citric acid. The pathway, expressing the respective genes under control of methanol regulated promoters, is being incorporated into the yeast Komagataella phaffii to create an orthogonal test system. Preliminary results of this research have shown that the citric acid transporter genes were successfully expressed in K. phaffii. After evaluation, the best pathway variants will be transferred into A. niger.

# Poster No. **101**

## Development of a SNARE-based cell fusion system in *Pichia pastoris*

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SNAREs are the main parts of the fusion machinery in synaptic vesicles in mammalian cells. The main fusion proteins involved are VAMP (or synaptobrevin), also known as the v-SNARE, and the complex made up of SNAP-25 and syntaxin as the t-SNARE. It was previously shown that flipped SNAREs can also be used to fuse two mammalian cells. In this study, we modified this system with the aim of generating cell fusion between two Pichia pastoris yeast cells. The mutated SNAREs proteins, as reported by (Hu et al. 2003), were used and were codon-optimized for expression in P. pastoris. The outward orientation was achieved using the alpha-factor, which was fused to the N-terminus of each SNARE mediating protein. A strain expressing flipped VAMP2 carries a cytosolic DsRed and the second strain expressing flipped syntaxin1, flipped SNAP-25, and a secreted complex of VAMP2 was created, expressing a nuclear GFP.

Yeast spheroplasts were generated after harvesting in the exponential growth phase and a Zymolase treatment. Spheroplasts of strains with compatible SNARE systems were incubated together, and the fusion events were visualized by fluorescence microscopy. After 3 hours, a fusion efficiency of about 16% was obtained. The fused cells were recovered on plates containing both dominant selection markers. In conclusion, we showed that P. pastoris spheroplasts can be fused using a SNARE mediating procedure. This system can be further developed as a tool for cross-species hybridization (e.g., Pichia spp. & Saccharomyces spp.) which enables the implementation of different pathways in a yeast host cell.





# Poster No. **102**

## Protease production in Pichia: inhibitor co-expression prevents autocatalytic proteolysis

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Chronic wounds are difficult to treat because of dead tissue and debris, part of which is fibrin. The removal of debris is the first step of chronic wound treatment. A non-invasive and effective way is maggot therapy, in which live fly larvae are applied that lyse and consume dead tissue. However, most patients find this treatment revolting.

This study describes the identification and production of the major fibrin-degrading protease from medicinal maggots and the production thereof in Pichia pastoris.

A cDNA library from total RNA of medicinal maggots was constructed and transferred into Escherichia coli for gene expression. Clones showing proteolytic activity on casein were further analysed for fibrinolytic activity. Positive clones producing fibrin-degrading proteases were subsequently sequenced. One single gene sequence was identified encoding for a novel trypsin-like serine protease (Aurase). Subsequently, the gene was cloned into P. pastoris expression vectors for enzyme production. Aurase was secreted with its native pro-peptide that usually keeps the protease inactive. During the fermentation process, single protease molecules became active by cleavage of the pro-peptide, which resulted in an autolytic chain reaction that activated other molecules and ultimately lead to the loss of most of the Aurase. Such an unstable process would not have been suitable for the production of an enzyme in medical applications. In order to prevent self-activation of the secreted protease, the proteinaceous protease inhibitor aprotinin was co-secreted in the production strain. This successfully prevented premature activation of the protease and greatly increased the production yield.

# Poster No. **103**

## Unfolded protein response biosensors for recombinant protein expression

**Helena Godon**[1,2], Martina Puricelli[3,4], Sonakshi De[1,2], Núria Adelantado[3], Christoph Kiziak[3], Brigitte Gasser[1,2], Diethard Mattanovich[1,2]

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The yeast Pichia pastoris is regarded as an attractive workhorse for the synthesis of valuable products. One of its key features as an industrial protein production host, is its ability to secrete high amounts of recombinant proteins. However, the secretion efficiency also depends on the protein target. Therefore, strain engineering and improvement is paramount to generating high producing, industrially applicable strains. The unfolded protein response (UPR) significantly impacts the recombinant protein production and secretion in eukaryotic cell factories, including P. pastoris. This cellular stress response is triggered when unfolded proteins accumulate inside the endoplasmic reticulum (ER) lumen, exceeding the protein folding capacity of the ER. The UPR modulates the transcription of UPR-responsive genes to help restore the ER homeostasis. The transcription of one of these UPR-responsive genes, the KAR2 gene has been used as an indicator to evaluate the manifestation and strength of the UPR in yeast cells. Here, the putative promoter sequence of KAR2 is coupled with a fluorescent marker gene (eGFP) to generate a UPR biosensor strain that can be used as chassis for recombinant protein expression. The UPR biosensor provides a signal for folding stress and thus for target protein secretion, which can be used for clone selection during screening, scaling up to bioreactor and during fermentation development by better understanding the cell folding stress during recombinant protein production. The UPR biosensor will give elements to guide rational engineering strategies towards optimal heterologous protein production.



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# Poster No. **104**

## Whole Genome Sequencing and RNA-seq for *K. phaffii* strains downregulated with dMAD7

**Carla Magdalena Aguilar Gomez**[1], Julian Krappinger[1,3], Andrea Hönikl[2], Julia Feichtinger[3], Anton Glieder[1,2]

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The dMAD7 system was used to downregulate the expression of eGFP under a PDF promoter in two K. phaffii strains. Through the downregulation experiment two different phenotypes exhibiting low and high reduction of expression, AH_6 and AH_11 respectively, were obtained; episomal and genomic integration were suspected. To elucidate why the same gRNA and plasmid produced different phenotypes, we investigated Whole Genome Sequencing (WGS) data of 3 randomly selected samples from each phenotype and strand-specific paired-end RNA-seq (ssRNA-seq) data from each population. The integration loci of the eGFP plasmid and dMAD7 plasmid were identified and inspected using the WGS data. No dMAD7 plasmid was found in AH_6, but a marker exchange happened. In AH_11, the dMAD7 plasmid was integrated in the promoter region in the gene HAP3-1. Differential gene expression analysis (DGEA) of the ssRNA-seq data revealed the downregulation of the HAP3-1 in AH_11 and also a low amount of reads mapping to the dMAD7 sequence in AH_6. More analysis is undergoing to corroborate the hypothesis of episomal and genomic integration.

# Poster No. 105

## Towards the production of recombinant animal myoglobin: Engineering of heme biosynthesis pathway of *Pichia pastoris*.

**Mihaela Neofitova**[1,2,3], Viktorija Vidimce-Risteski[1], Karin Reicher[1], Harald Pichler[2,1]

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The hemoproteins myoglobin and hemoglobin are responsible for the meaty flavour and characteristic colour of cooked meat. Therefore, their addition to plant-based meat alternatives is considered beneficial. Heme plays an essential role in the function of these proteins. We can take advantage of the naturally occurring heme biosynthesis pathway of P. pastoris to recombinantly produce both hemoglobin and myoglobin in high quantities. The targeted knock-out of four genes in P. pastoris, which are thought to be essential in the heme downregulation and degradation, should allow for higher concentrations of heme and, as a result, also more active hemoproteins. To prove this hypothesis, two model strains were used, the first secreting horseradish peroxidase and the second expressing chicken myoglobin intracellularly.





# Poster No. 106

## Carbon source-based selection of ARS plasmids in a triosephosphate isomerase-deficient *Pichia pastoris* strain

**Florian Weiss**[1], Kjeld Olesen[2], Sanne Jensen[2], Anton Glieder[1]

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Selection markers are used for the detection of successful transformation events of host cells in genetic modification procedures. Genomic integration of recombinant DNA, typically the method of choice with Pichia pastoris, leads to stable genotypes. Non-integrative, episomal ARS plasmids on the other hand require constant selection pressure for propagation and maintenance. For this, antibiotic resistance genes are convenient dominant selection markers in small scale experiments but become economically unfeasible for large scale cultivations. To avoid antibiotics, prototrophic markers that complement deficiencies of auxotrophic host strains can be used. However, the prototrophic selection effect is easily diminished due to cross-feeding resulting in marker-less subpopulations.

Alternatively, carbon source utilization markers like GUT1 in P. pastoris or the triosephosphate isomerase gene (TPI1) in Saccharomyces cerevisiae can be used. TPI catalyzes the interconversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate in the glycolytic pathway which enables carbon source-based selection in complex media in an TPI1 null mutant host strain. Thus, the TPI1 marker is very attractive for processes that demand permanent selection pressure.

In our endeavor to expand the portfolio of antibiotic free marker systems for the use in P. pastoris we turned our attention to the TPI marker system. The required TPI1-deficient P. pastoris platform strain was generated by the in-house-developed CRISPR-MAD7 system. For the plasmid restoring the TPI activity the gene was amplified from the genome. The emerging marker system allows transformant selection with glycerol as sole carbon source in P. pastoris and is suitable for cultivation processes employing ARS plasmids.

# Poster No. 107

## Matrix attached region sequence converts an expression vector into an episomal plasmid for *K. phaffii*

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Matrix attached regions (MARs) are defined as DNA sequences which bind specifically to nucleoskeleton (or the nuclear matrix) in vitro. There are several studies showing that the presence of MARs in expression construct improves the transgene expression. However, there are no reports about the influence of MARs on recombinant protein production in K.phaffii. Therefore, we aimed to investigate how MARs affects the gene expression in K. phaffii.

Our study revealed that a human MAR sequence incorporated into an enhanced green fluorescent protein (eGFP) expression construct converts the construct into an episomal plasmid. These findings are supported by experimental data demonstrating the differences in eGFP fluorescence level between two groups of transformants. The first group included clones transformed with the linear eGFP expression construct and this group had higher eGFP expression. The second one consisted of the clones transformed with the circular eGFP expression plasmid harbouring the MAR sequence and the low eGFP expression level was observed in this group. In order to find an explanation for that, several samples from analysed cell cultures were plated on YPD and YPD-Zeocin plates. As a result, the comparable number of colonies on both plates were noticed for clones from the first group. In contrast, the second group had few times less colonies on YPD-Zeo plates than on YPD plated. It leads to the conclusion that plasmid harbouring MARs is not integrated into genome and, as in case with other episomal plasmids, K. phaffii loses it in the absences of the selection pressure.





# Poster No. 108

## Functional characterization of the *Pichia pastoris* 1033 gene promoter and transcriptional terminator

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A strong promoter is not always the most favorable choice for heterologous protein production, especially if the correct folding of the protein and/or post-translational processing is the limiting step. Furthermore, weak promoters are useful for expressing genes that produce toxic proteins to the host cells and also in synthetic biology applications. Therefore, in this work, we functionally characterized the promoter ($P_{1033}$) and transcriptional terminator ($T_{1033}$) of a constitutive gene (i.e., the 1033 gene) with a weak non-methanol-dependent transcriptional activity. We constructed two Pichia pastoris strains with two combinations of the regulatory DNA elements (i.e., $P_{1033}$-TAOX1 and $P_{1033}$-$T_{1033}$ pairs) in the expression cassette. We selected single-copy clones of each construct and evaluated the impact of the regulatory element combinations on the transcript levels of the heterologous gene in cells grown in glucose or glycerol as a carbon source, and on the extracellular product/biomass yields (Yp/x). The results indicate that the $P_{1033}$ has a 2-3% transcriptional activity of the GAP promoter activity and it is modulated by cell growth and the carbon source. Moreover, the TAOX1 and $T_{1033}$ terminators did not contribute to a differentiated transcriptional activity of the reporter gene in glycerol, while in glucose TAOX1 increases the transcriptional activity of the heterologous gene with respect to $T_{1033}$. Although the highest heterologous gene transcription levels were attained in glucose-grown $P_{1033}$-TAOX1 cells, the highest Yp/x values were obtained in the glycerol cultures for the two strains. The findings indicate that low heterologous gene transcript levels along with glycerol cultures increase protein secretion.

# Poster No. **109**

## A novel *Pichia pastoris* promoter active at a low specific growth rate

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Pichia pastoris can be grown to high biomass concentration in a bioreactor, which is an advantage since generally protein production is linked to cell growth and the extracellular protein titers are directly proportional to the biomass concentration. Nevertheless, the biomass generated in large quantities becomes a by-product that is difficult to give a profitable outlet. Pichia pastoris cells can remain viable at a very low specific growth rate (μ). Therefore, using an expression cassette with an active promoter under a low μ value would produce the recombinant protein with only a small increase in biomass, which in turn would increase the product/biomass yield (Yp/x). Mining homemade RNA-seq data from P. pastoris KM71 strains grown in glycerol, we located an endogenous promoter with these properties. We engineered a P. pastoris strain to drive the expression of the reporter gene with this promoter. With this strain, two bioreactor cultures were completed in two phases: a glycerol batch phase followed by a glycerol-fed batch phase. The glycerol-fed batch phase was carried out with an exponential feeding rate of glycerol to generate a constant μ of 0.02 or 0.08 h-1. The transcript levels of the heterologous gene for cells from the two cultures, evaluated by RT-qPCR, did not present significant differences. Nevertheless, they were higher than the expression levels of the endogenous gene of the promoter used in the expression cassette. Moreover, the culture at 0.02 h-1 showed higher extracellular Yp/x and product/substrate yield (Yp/s) than that of the culture at 0.08 h-1.





# Poster No. **110**

## Boosting transformation efficiency of *Pichia pastoris*

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The methylotrophic yeast Komagataella phaffii (K. phaffii), commonly known also as Pichia pastoris (P. pastoris), is one of the most widely used hosts for recombinant protein production. During the past decades P. pastoris became increasingly popular, not only for fundamental research, but also for industrial applications in the biopharmaceutical, feed and food sectors. As P. pastoris became more and more widely applied, it became necessary to have a wide selection of genetic tools for protein production. Furthermore, cell engineering of P. pastoris to improve productivity gained increasing importance. Nowadays cell engineering to obtain improved production strains does not only require expression of single genes, but also entire metabolic pathways or even libraries might be overexpressed. For all these purposes, an efficient transformation method yielding high numbers of transformants is a prerequisite. This work focuses on the development of a reliable, highly efficient, and fast protocol for preparing electro-competent P. pastoris cells. In literature, several protocols are available for introducing DNA into P. pastoris. Two of the most commonly used protocols for generating electro-competent P. pastoris were comparatively evaluated for their efficiency, and further adapted based on the results. So far, a 4-fold increase in the transformation efficiency over the published protocols could be achieved.

# Poster No. 111

## Protein production of recombinant mutated hyaluronidase from wasp venom (*Vespa tropica*) using yeast expression system.

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Recombinant mutated hyaluronidase (rmVesT2a) protein is the protein that can bind hyaluronic acid without hydrolysable activity. The aim of this study is the production of rmVesT2a protein from wasp venom (Vespa tropica) using a yeast expression system. For this study, the hyaluronidase gene (VesT2a gene; wild type) is cloned from wasp venom. After that, VesT2a gene is mutated at the catalytic site (D107N and E109Q) using the site-directed mutagenesis technique. Next, mVesT2a gene is optimized for codon optimization for Pichia pastoris. Then, this mVesT2a gene is assembled into recipient BB1 and dBB3 using the GoldenPiCS with different promoters which are PAOX1 and PGAP. At the present, we can produce the recombinant protein that MW is approximately 30 kDa by P. pastoris. However, these rmVesT2a proteins must have to be confirmed by some analytical technique such as LC/MS-MS.





# Poster No. 112

## Impact of oxygen availability on organelle-specific redox potentials and stress in recombinant protein producing *Pichia pastoris*

**Aliki Kostopoulou**[1,2], Corinna Rebnegger[2], Brigitte Gasser[1,2], Diethard Mattanovich[1,2]

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The methylotrophic yeast Pichia pastoris is one of the most effective and established expression hosts for heterologous protein production. The redox balance of its secretory pathway, which is multi-organelle dependent, is of high importance for recombinant protein production. Redox imbalance and oxidative stress are two main factors that can influence protein production and secretion, especially the redox potential of the ER where protein folding and disulfide bond formation occur. Glutathione is the main redox buffer of the cell and its redox conditions can be determined by the status of glutathione redox couple (GSH-GSSG). In vivo measurements of the glutathione redox potential in different subcellular compartments can be achieved by genetically encoded redox sensitive fluorescent probes (roGFPs).1,2 The aim of this study is to investigate the impact of oxygen availability on the redox potentials of different organelles in glucose-limited chemostat cultures of non-producing and producing Pichia pastoris strains. It was found that the switch from normoxic to hypoxic conditions affected the redox potentials of all investigated organelles, the specific oxygen uptake rate as well as the accumulation of reactive oxygen species (ROS). Also, as reported previously, hypoxic conditions led to increased recombinant protein secretion.3 Consequently, a better understanding of oxidative stress and redox homeostasis of the P. pastoris strains can lead to improved production of therapeutic disulfide-bonded protein in industrial setups.

# Poster No. 113

## Bidirectional promoters - future of recombinant protein production

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The majority of recombinant proteins is produced using monodirectional promoter systems (MDPs). MDPs are well-established, available for a variety of inducers, characterized for tunability but, unfortunately, lack in flexibility and versatility. Bidirectional promoter systems (BDPs) are able to boost recombinant protein production via multi-gene expression. Thereby, the implementation of BDPs would allow co-expression of chaperones increasing the amount of correctly folded protein. In this study, we compared MDPs and BDPs, trying to express an unspecific peroxygenase in the yeast Komagataella phaffii BSYBG11. To assist the folding of our target enzyme, we introduced the protein disulfide isomerase in the BDP construct. Both genes were under control of a derepressed promotor, with additional promotor boost for the Heme containing protein. We characterized BDPs and MDPs in fed-batch cultivations under (i) derepressed feeding and (ii) methanol-glycerol mixed feed induction. Results show that higher enzyme productivity can be achieved (i) when applying BDPs instead of MDPs and (ii) that derepressed feeding outperforms methanol addition for the expression of unspecific peroxygenase. We present that BDPs are a promising solution for recombinant enzyme expression.





# Poster No. 114

## Terminators for tuning gene expression in the methylotrophic yeast *Ogataea polymorpha*

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The methylotrophic yeast Ogataea polymorpha (Pichia angusta) is an upcoming host for bio-manufacturing due to its unique physiological properties, including its thermotolerance and broad substrate spectrum, and particularly its ability to utilize methanol as the sole carbon and energy source. However, metabolic engineering tools for O. polymorpha are still rare. In our study, we characterized the influence of terminators on gene expression throughout batch cultivations with glucose, glycerol, and methanol as carbon sources as well as mixes of these carbon sources. Aside from homologous terminators, we also tested heterologous terminators from Pichia pastoris and Saccharomyces cerevisiae. For this characterization, a short half-life GFP variant was chosen, which allows a precise temporal resolution of gene expression. By varying the terminators alone, a 6-fold difference in gene expression was achieved with the homologous MOX terminator boosting gene expression on all carbon sources by around 50% compared to the second strongest AOX1 terminator from Pichia pastoris. It was shown that this exceptional increase in gene expression is achieved through a stabilized mRNA, which results in an increased transcript level in the cells. We further found that different pairing of promoters and terminators or the expression of a different gene (β-galactosidase gene) did not influence the performance of the terminators. Consequently, this study shows how terminators can be used as independent elements to tune gene expression and can be applied as genetic tools also among different yeast species.

# Poster No. 115

## Are yeast promoters active in *E. coli* under cloning conditions?

**Sandra Moser**[1], Patricia Menczik[1], Lukas Sturmberger[1]

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The construction of P. pastoris expression cassettes very often relies on an intermediate cloning step in E. coli. Prerequisite for this process is the inertness of the yeast promoter in the bacterial cell and therefore its lack of protein expression from the designated plasmid to be constructed. Challenges in cloning error-free plasmids containing certain enzyme sequences prompted us to investigate this assumption. In doing so, we analyzed 23 different yeast promoters for their transcriptional response in E. coli DH10B cells under cloning conditions. For the different promoters tested in this experiment, we observed a broad spectrum of transcriptional activities when measuring OD600-normalized fluorescence protein levels, ranging from no expression to levels rivaling strong constitutive E. coli promoters. This finding has important implications for the generation of yeast expression constructs which might have negative effects on E. coli cell viability.





# Poster No. 116

## Promoter discovery and engineering for *Komagataella phaffii*

**Katharina Ebner**[1], Corina Dörner[1], Stefan Ertl[1], Jasmin Fischer[1], Sandra Schein[1], Christian Schmid[1], Anton Glieder[1]

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In the last decades Komagataella phaffii (K. phaffii), formally known as Pichia pastoris, a methylotrophic crabtree negative yeast, rose to be one of the major workhorses for recombinant protein production. (Bill et al., 2014) However, most of the promoter sequences available for K. phaffii are derived from its endogenous methanol utilization (MUT) pathway, making methanol the major inducer of expression. (Ahmad et al., 2014) Since methanol is toxic and flammable, high concentrations are not desired in large-scale applications. Therefore, expanding the toolbox of promoter sequences for K. phaffii not dependent on methanol induction is one of the major challenges in this field.

Based on transcriptome sequencing data of a wild type K. phaffii strain we were able to identify methanol independent endogenous regulatory sequences potentially suited for recombinant protein expression. Following the experimental determination of the fully functional promoter length, the activity was characterized using different cultivation modes (feed and starvation) and carbon sources (glucose, glycerol, methanol and xylitol) using intracellularly expressed eGFP as reporter. Additionally, the applicability of the promoter sequences was investigated with selected industrially and pharmaceutically relevant proteins - CalB, HSA and hGH - showcasing the suitability for production of a wide range of different proteins. Furthermore, for one promoter sequence variants were created to potentially elucidate the promoter specific control mechanisms.

# Poster No. 117

## Synthetic proteins in *Pichia pastoris*

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The genetic code is universal set of rules for translation of the genome encoded information into proteins, using 20 canonical amino acids as building components. Since their functional diversity is limited, it is of great interest to introduce non-canonical amino acids with unique biochemical handles into target proteins. Expanding the genetic code of organisms is thus becoming an indispensable tool for protein site-specific labelling, e.g. for production of next-generation therapeutics such as antibody-drug conjugates.

Komagataella phaffii (Pichia pastoris) is a widely used expression host capable of proper protein folding, posttranslational modifications, and secretion of complex proteins. These assets in combination with fast growth to high cell densities in inexpensive media make it an attractive complementary expression host to, for instance, Chinese hamster ovary (CHO) cells and Escherichia coli.

Non-canonical amino acids can be introduced at in-frame amber codons during ribosomal protein synthesis. This requires that their orthogonal aminoacyl-tRNA synthetase and its cognate amber suppressor tRNA are encoded in the host and optimally expressed. Additionally, the amino acid of choice must be sufficiently transported into the cells from the media to be available for incorporation. The feasibility of the approach has been successfully demonstrated in bacterial and mammalian expression systems; however, examples of yeast genetic code expansion remain scarce.

Our aim is to develop a platform technology for the efficient incorporation of non-canonical amino acids with a reactive side chain into target proteins in Pichia pastoris. To achieve this, we intend to systematically evaluate and optimize the orthogonal translation system.





# Poster No. 118

## Flo8 – a versatile regulator for improving recombinant protein production in *Pichia pastoris*

**Corinna Rebnegger**[1,2,3], Viktoria Kowarz[3], Mirelle Flores[2,3], Christoph Kiziak[4], Diethard Mattanovich[1,3], Brigitte Gasser[1,2,3]

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Flo8 is a main transcriptional regulator of pseudohyphal growth in yeast. Recently, it was shown that disruption of FLO8 in the popular recombinant protein production host Pichia pastoris (syn Komagataella spp) abolishes pseudohyphal growth and significantly reduces cell-to-surface adherence, making the mutant an interesting base strain for research and industry [1,2]. However, knowledge on the physiological impact of the mutation remains scarce and comprehensive studies employing FLO8-deficient strains for recombinant protein production are lacking. Here, we re-analysed published RNAseq data of the P. pastoris wildtype and Δflo8 mutant cultivated in glucose-limited chemostats at a fast and slow growth rate set-point [2], revealing that Flo8 affects the expected flocculation targets, but also mating, respiration, cell cycle genes as well as catabolite repression and that its actions are specific to the respective growth condition. Furthermore, we tested the Δflo8 mutant in combination with the strong glucose-regulated (methanol-independent) GTH1 promoter (PG1) [3] and its engineered derivative PG1-3 [4] for recombinant protein production in small scale screenings and bioreactor cultivations. It was demonstrated that PG1 and PG1-3 expression strength was significantly elevated in the Δflo8 mutant, resulting in substantially enhanced recombinant protein titers.

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# Poster No. **119**

## Recombinant production of plant enzymes in *Pichia pastoris*

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Plant secondary metabolites are often being masked or inactivated in plants by the glycosylation of active molecules. However, these molecules are usually highly value added and are worth to be produced by biocatalysis process. Plants it selves also contains hydrolases which cleaves the sugar part of these molecules and may be therefore applied for the biocatalytic production of value-added compounds. These enzymes in plants usually contains native signal sequences, which transports the nascent protein through the endoplasmic reticulum membrane in plants. Identification of such sequences are key for an effective overproduction of recombinant enzymes also in Pichia pastoris. The deletion of the native signal sequence may result to as high as 40-fold activity increase compared to the expression of native, non-treated hydrolases. The further effective upscale of recombinant enzymes production in bioreactors results in further almost 100-fold activity increase compared to flask cultivations. These results could be applied to the expression of other plant enzymes.

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# Poster No. **120**

## AI Models applied in RQ control to optimize recombinant protein production in *Pichia pastoris*

**Xavier Garcia-Ortega**[1], Arnau Gasset[1], Albert Sales[1], Joeri Van Wijngaarden[2], José Luis Montesinos[1], Toni Manzano[2] and Francisco Valero[1],

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**Session:** Model driven strain & fermentation process development
The yeast Pichia pastoris is currently considered the second preferred microbial host for recombinant protein production (RPP), specially since numerous products biomanufactured with this cell factory are FDA approved for therapeutics and food applications. Oxygen limitation has been shown to improve significantly RPP productivities and yields in bioprocesses regulated by GAP promoter. This environmental stress (hypoxia) can be monitored by the respiratory quotient (RQ), being then a transferable parameters between different fermentation systems. A step further is to design and to implement a control algorithm using RQ as a controlled variable to maintain the desired hypoxic conditions in the culture acting on agitation rate.
This strategy has been studied at three different stages: first, a manual-heuristic control system was tested, obtaining 2-fold higher productivities with respect to oxygen non- limiting conditions with a Pichia strain producing Candida rugosa lipase 1 (Crl1) as a model protein. Then, an automatic control algorithm has been implemented, considerably reducing labour and time costs while maintaining the 2-fold increase in productivity and also increasing precision and stability. Finally, as a more attractive and scalable strategy from an industrial perspective, an RQ-control algorithm based on artificial intelligence (AI) has been designed, implemented and tested. Continuous data feeding is conducted from the fermenter to an on-line server, where an AI model predicts on-line the proper agitation to maintain a constant RQ throughout the fermentation process.
As conclusion, it can be stated that an innovative physiological control has been implemented based on an AI approach allowing to achieve results comparable to those obtained with classical methods, increasing 2-fold yields and productivities compared to standard oxygen conditions (normoxia). This opens a window for this data-driven technologies to be implemented in bioprocesses based on microbial fermentation including both pharma and biotech industrial sectors.

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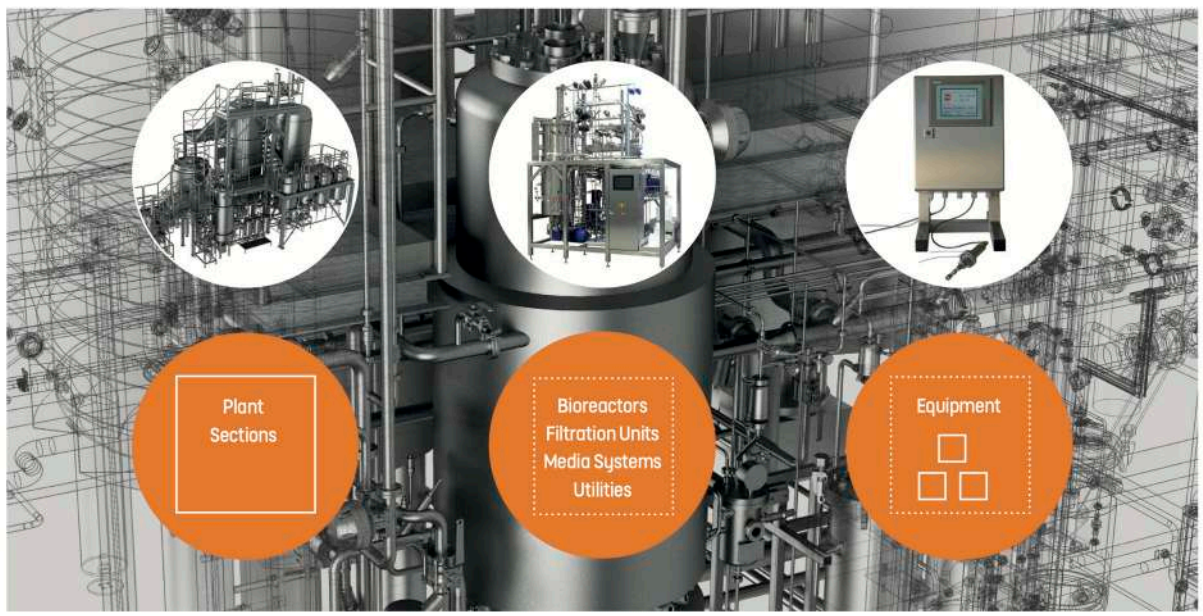
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