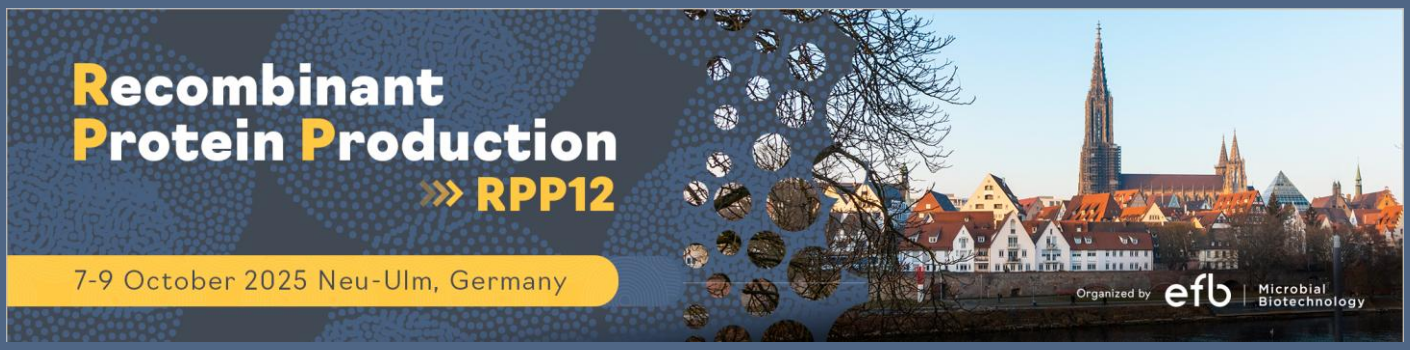


# Programme and abstracts

efb | Microbial Biotechnology

# 12th Conference
# on
# Recombinant Protein Production

European Federation of Biotechnology

Microbial Biotechnology Division

**PROGRAMME AND ABSTRACTS**

Neu-Ulm, Germany

7 - 9 October 2025

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| **Ralf Takors** | University of Stuttgart, Germany |

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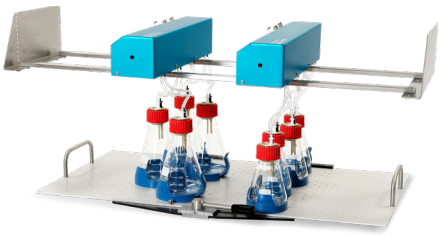

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# Scientific Programme

## Overview

| 7 October 2025 | |
|---|---|
| *10:00* | *Registration* |
| **Pre-conference workshop** | |
| 10:10 | Introduction |
| 10:10 | **F. Spreitzer** |
| 10:25 | **A. Tugrul** |
| 10:40 | **C. Meyer** |
| 10:55 | **M. Riedl** |
| 11:10 | **S. Subramaniam** |
| 11:25 | **L. Hofer** |
| 11:40 | **S. Jaya Sankar** |
| 11:55 | **F. Simon** |
| 12:10 | **S. Kutzner** |
| 12:25 | **A. Frank** |
| *12:40* | *Lunch* |
| 14:00 | **Opening** |
| 14:10 | **Keynote lecture: M. DeLisa** |
| **Session 1: Chassis engineering for recombinant production of proteins** | |
| 14:55 | **A. Robinson** |
| 15:25 | **F. Bernhard** |
| 15:45 | **Á. R. Lara** |
| 16:05 | **E. Bonnaud** |
| *16:25* | *Coffee break* |
| 16:55 | **B. Thevelein** |
| 17:15 | **B. Gasser** |
| 17:35 | **B. Wang** |
| 17:55 | **M. Scherer** |
| 18:15 | **Poster session** (odd numbers) |

| 8 October 2025 | |
|---|---|
| **Session 2: Impact of production conditions on cellular performance** | |
| 9:00 | **M. Q. Rodrigues** |
| 9:30 | **A. Ohara** |
| 9:50 | **N. Palombi** |
| 10:10 | **V. Waffenschmidt** |
| *10:30* | *Coffee break* |
| 11:00 | **J. Barczyk** |
| 11:20 | **F. Grilli** |
| 11:40 | **L. Neutsch** |
| *12:00* | *Lunch* |
| 13:00 | **Keynote lecture: T. Tuller** |
| **Session 3: Model-enabled protein, cell, and process engineering** | |
| 13:45 | **P. Neubauer** |
| 14:05 | **A. Zelezniak** |
| 14:35 | **V. Gross** |
| 14:55 | **D. Pranoto** |
| *15:15* | *Coffee break* |
| **Session 4: New horizons for biopharmaceutical proteins** | |
| 15:45 | **N. Callewaert** |
| 16:15 | **E. Garcia-Fruitós** |
| 16:35 | **B. Binay** |
| 16:55 | **R. Kumar** |
| 17:15 | **A. Semerci** |
| 17:35 | **S. Suppmann** |
| 17:55 | **Poster session** (even numbers) |

| 9 October 2025 | |
|---|---|
| 9:00 | **Poster pitches and awards** |
| **Session 5: Precision fermentation of food proteins** | |
| 9:30 | **N. Aro** |
| 10:00 | **L. Ruddock** |
| 10:20 | **F. Beck** |
| 10:40 | **A. Escrich** |
| *11:00* | *Coffee break* |
| 11:30 | **E. H. Benatto Perino** |
| 11:50 | **P. Shemesh** |
| 12:10 | **A. Pelzer** |
| *12:30* | *Lunch* |
| **Session 6: Contributions to sustainable protein biomanufacturing** | |
| 13:30 | **D. Mattanovich** |
| 14:00 | **I. Lenitz Etxaburu** |
| 14:20 | **K. Brechun** |
| 14:40 | **N. Agmon** |
| *15:00* | *Coffee break* |
| 15:30 | **J. Kopp** |
| 15:50 | **S. Sawant** |
| 16:10 | **P. Satzer** |
| *19:00* | *Conference dinner* |

## Full programme

| 7 October 2025 (Tuesday) | |
|---|---|
| *10:00 – 14:00* | *Registration* |
| **Pre-conference workshop**<br>Chair: **Ralf Takors,** University of Stuttgart, Germany | |
| 10:10 – 10:10 | Introduction and welcome address |
| 10:10 – 10:25 | Uncovering guidelines for designing well-secretable proteins<br>**Fiona Spreitzer,** BOKU, Austria |
| 10:25 – 10:40 | Inhibiting aggregation of therapeutic proteins<br>**Arda Deniz Tugrul**, NIBRT, Ireland |
| 10:40 – 10:55 | Model guided design of *E. coli* strains for the heterologous production of dopamine<br>**Cedric Meyer**, IBVT-University of Stuttgart, Germany |
| 10:55 – 11:10 | Understanding transcriptional variance in CHO cells: a quantitative meta-analysis<br>**Markus Riedl**, BOKU, Austria |
| 11:10 – 11:25 | Advancing CHO cell productivity: a combined approach of cell engineering using novel transposon vectors and proteomics<br>**Sujay Subramaniam**, University of Greifswald, Germany |
| 11:25 – 11:40 | Multi-omics characterisation reveals temporal dynamics of cellular and metabolic behaviour of a CHO-based mAb process<br>**Larissa Hofer**, BOKU, Austria |
| 11:40 – 11:55 | Scale-down bioreactor studies on heterologous protein production in stringent response modulated *E. coli* chassis<br>**Sidharth Jaya Sankar**, IBVT- University of Stuttgart, Germany |
| 11:55 – 12:10 | ECOnti - advancing integrated continuous manufacturing with microbial cells<br>**Florian Simon**, BOKU, Austria |
| 12:10 – 12:25 | CASPON®: a fusion-protein platform technology for efficient recombinant biopharmaceutical protein production<br>**Sophie Kutzner**, Boehringer Ingelheim, Austria |

| 7 October 2025 (Tuesday) | |
|---|---|
| 12:25 – 12:40 | Cationic polymers reduce (high-risk) host cell proteins in monoclonal antibody purification processes<br>**Anna Frank**, BOKU, Austria |
| *12:40 – 14:00* | *Lunch (only for pre-conference workshop participants)* |
| 14:00 – 14:10 | **Opening and welcome address** |
| 14:10 – 14:55 | **Keynote lecture:** Adventures in synthetic glycobiology: enabling customized protein glycosylation with engineered bacterial cells<br>**Matthew DeLisa,** Cornell University, USA |
| **Session 1: Chassis engineering for recombinant production of proteins**<br>Chairs: **Nicole Borth,** BOKU, Austria & **Elena Garcia-Fruitós,** IRTA, Spain | |
| 14:55 – 15:25 | Autophagy and AKT-stimulated cellular proliferation synergistically improve antibody production in CHO cells<br>**Anne Robinson,** Carnegie Mellon University, USA |
| 15:25 – 15:45 | Cryo-EM structures of human membrane protein complexes from a streamlined cell-free protein production platform<br>**Frank Bernhard,** Goethe University Frankfurt, Germany |
| 15:45 – 16:05 | Streamlined cells are superior bioproduction hosts<br>**Álvaro R. Lara,** Aarhus University, Denmark |
| 16:05 – 16:25 | *Haloalkaliphilic Archaea* as new cellular chassis for the bioproduction of recombinant proteins<br>**Emma Bonnaud,** INSA-Lyon, France |
| *16:25 – 16:55* | *Coffee break* |
| 16:55 – 17:15 | Creation of a superior 2G bioethanol yeast strain with high enzyme secretion capacity<br>**Bart Thevelein,** NovelYeast- Ghent University, Belgium |
| 17:15 – 17:35 | Flo8 – a versatile regulator for improving recombinant protein production in *Komagataella phaffii*<br>**Brigitte Gasser,** ACIB, Austria |
| 17:35 – 17:55 | Improving industrial protein secretion in *Bacillus subtilis* using RNA-seq and Ribo-seq<br>**Biwen Wang**, Hanze University of Applied Science, The Netherlands |

## 7 October 2025 (Tuesday)

| | |
|---|---|
| 17:55 – 18:15 | From recombinant expression to functional reconstitution: characterization of outer membrane porins and their mass transport<br>**Maike Scherer**, FAU Erlangen-Nürnberg, Germany |
| 18:15 – 22:00 | **Poster session** (odd numbers), exhibition & welcome buffet |

## 8 October 2025 (Wednesday)

**Session 2: Impact of production conditions on cellular performance**

Chairs: **Marco Oldiges,** FZJ, Germany & **Álvaro R. Lara,** Aarhus University, Denmark

| | |
|---|---|
| 9:00 – 9:30 | Engineering ferritin nanoparticles for next-generation vaccines: strategies for antigen display and biomanufacturing<br>**Margarida Queluz Rodrigues,** iBET, Portugal |
| 9:30 – 9:50 | The impact of methanol concentration on recombinant protein glycosylation in *Pichia pastoris* SuperMan5<br>**Andre Ohara**, Imperial College London, UK |
| 9:50 – 10:10 | The hidden cost of protein secretion: signal peptide-induced stress in *Bacillus subtilis*<br>**Noemi Palombi**, University of Greifswald, Germany |
| 10:10 – 10:30 | High-throughput investigation of recombinant bifunctional protein secretion by *Corynebacterium glutamicum*<br>**Vera Waffenschmidt**, Forschungszentrum Juelich GmbH, Germany |
| *10:30 – 11:00* | *Coffee break* |
| 11:00 – 11:20 | Mimicking large-scale mixing conditions in a laboratory-scale single multi compartment bioreactor system<br>**Jonas Barczyk**, IBVT-University of Stuttgart, Germany |
| 11:20 – 11:40 | Time-resolved absolute protein quantification to optimize recombinant protein production in *Bacillus subtilis*<br>**Francesca Grilli**, University Medical Centre Groningen, The Netherlands |
| 11:40 – 12:00 | Engineering genealogical age and cell clustering patterns for improved recombinant protein production in *Pichia pastoris* bioprocesses<br>**Lukas Neutsch**, ZHAW - Zurich University of Applied Sciences, Switzerland |
| *12:00 – 13:00* | *Lunch* |
| 13:00 – 13:45 | **Keynote lecture:** Tackling fundamental challenges in biotechnology with computational models and AI<br>**Tamir Tuller**, Tel Aviv University, Israel |

## 8 October 2025 (Wednesday)

**Session 3: Model-enabled protein, cell, and process engineering**

Chairs: **Chris Oostenbrik,** BOKU, Austria & **Verena Siewers,** Chalmers, Sweden

| | |
|---|---|
| 13:45 – 14:05 | Advancing the expression of difficult to express proteins with self-driving labs: from model-based design to deep process insight<br>**Peter Neubauer**, TU-Berlin, Germany |
| 14:05 – 14:35 | Leveraging generative AI for applications in synthetic biology<br>**Aleksej Zelezniak**, Chalmers University of Technology, Sweden |
| 14:35 – 14:55 | Optimising recombinant membrane protein production by *E. coli* cell factories using a whole cell model<br>**Viktoriia Gross**, University of Warwick, UK |
| 14:55 – 15:15 | Minimization of a *Bacillus subtilis* signal peptide by rational design<br>**Dicky Pranoto**, University Medical Centre Groningen, The Netherlands |
| *15:15 – 15:45* | *Coffee break* |

**Session 4: New horizons for biopharmaceutical proteins**

Chairs: **Peggy Stolt-Berger,** Boehringer, Germany & **Gerald Striedner,** BOKU, Austria

| | |
|---|---|
| 15:45 – 16:15 | Development of the OPENPichia open-access industrial *Pichia pastoris* protein production system and use for manufacturing of anti-infectious VHH-Fc antibodies<br>**Nico Callewaert**, VIB-Ghent University, Belgium |
| 16:15 – 16:35 | Broad-spectrum antimicrobial recombinant proteins: a potential strategy for combating infectious diseases<br>**Elena Garcia-Fruitós**, IRTA, Spain |
| 16:35 – 16:55 | High-yield production and functional characterization of a mutant human β-glucocerebrosidase enzyme in *Pichia pastoris*<br>**Barış Binay**, Gebze Technical University, Turkey |
| 16:55 – 17:15 | A novel, thermostable sarcosine oxidase from *Bacillus* megaterium for biomedical applications: Kinetic characterization and active aggregation in *E. coli*<br>**Rahul Kumar**, Indian Institute of Technology Bombay, India |

## 8 October 2025 (Wednesday)

| | |
|---|---|
| 17:15 – 17:35 | Bifunctional cancer antibody engineering for solid tumors<br>**Asli Semerci**, Bilkent University, Turkey |
| 17:35 – 17:55 | A protein production platform for diagnostics and immune therapy of emerging infections<br>**Sabine Suppmann**, Fraunhofer ITMP-IIP, Germany |
| 17:55 – 22:00 | **Poster session** (even numbers), exhibition and buffet |

| 9 October 2025 (Thursday) | |
|---|---|
| 9:00 – 9:30 | **Poster pitches and awards** |
| **Session 5: Precision fermentation of food proteins**<br>Chairs: **Klaus Liebeton,** BRAIN Biotech, Germany & **John Morrissey,** University of Cork, Ireland | |
| 9:30 – 10:00 | Development of *Trichoderma reesei* strains for high-level production of the sweet protein brazzein<br>**Nina Aro,** Technical Research Centre, Finland |
| 10:00 – 10:20 | High level production of brazzein, a natural disulfide-rich sweet protein using engineered *E.coli*<br>**Lloyd Ruddock**, University of Oulu, Finland |
| 10:20 – 10:40 | Recombinant production and characterization of selected functional proteins from chicken eggs<br>**Franziska Beck**, Technical University Munich, Germany |
| 10:40 – 11:00 | Milk caseins production through precision fermentation in *E. coli*<br>**Ainoa Escrich**, AINIA, Spain |
| *11:00 – 11:30* | *Coffee break* |
| 11:30 – 11:50 | Recombinant production of bovine caseins in *E. coli* and genome-reduced *Bacillus subtilis*: chassis optimization and production performance<br>**Elvio Henrique Benatto Perino**, University of Hohenheim, Germany |
| 11:50 – 12:10 | Optimizing *Pichia pastoris* as a fermentation platform for alternative protein production<br>**Paz Shemesh**, Technion-Israel Institute of Technology, Israel |
| 12:10 – 12:30 | Alternative proteins – chances & challenges in microbial production<br>**Alexander Pelzer**, BRAIN Biotech AG, Germany |
| *12:30 – 13:30* | *Lunch* |

| 9 October 2025 (Thursday) | |
|---|---|
| **Session 6: Contributions to sustainable protein biomanufacturing**<br>Chairs: **Pau Ferrer,** UAB, Spain & **Joachim Klein,** Lonza, Switzerland | |
| 13:30 – 14:00 | Single carbon substrates as sustainable feedstocks for protein production<br>**Diethard Mattanovich**, BOKU, Austria |
| 14:00 – 14:20 | Recombinant production and characterization of endolysins targeting *Candidatus* Liberibacter<br>**Ibai Lenitz Etxaburu**, AINIA, Spain |
| 14:20 – 14:40 | Antibiotic-free plasmid selection and maintenance via inducible complementation<br>**Katherine Brechun**, Gen-H - Genetic Engineering Heidelberg GmbH, Germany |
| 14:40 – 15:00 | Synthetic biology and bioprocess engineering for scalable, cost-effective components in cultivated meat production<br>**Neta Agmon**, Alagene, Israel |
| *15:00 – 15:30* | *Coffee break* |
| 15:30 – 15:50 | Soft-sensor–driven real-time physiological state estimation to facilitate repetitive fed-batch cultivations<br>**Julian Kopp**, TU Wien, Austria |
| 15:50 – 16:10 | Developing a recombinant barnacle cement protein as a biomimetic adhesive coating<br>**Shrutika Sawant**, University of Galway, Ireland |
| 16:10 – 16:30 | Scaling bioprocesses by AI generated 3D printed bioreactors mimicking large scale conditions<br>**Peter Satzer**, p4b GmbH, Austria |
| *19:00 – 22:30* | *Conference dinner* |

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# Oral presentations

## Pre-conference workshop

**Uncovering guidelines for designing well-secretable proteins**

Fiona Spreitzer (1); Michael Traxlmayr (2); Clemens Peterbauer (3); Brigitte Gasser (1)

(1) BOKU University, Department of Biotechnology and Food Science, Institute of Microbiology and Microbial Biotechnology, Austria

(2) BOKU University, Department of Natural Sciences and Sustainable Resources, Institute of Biochemistry, Austria

(3) BOKU University, Department of Biotechnology and Food Science, Institute of Food Technology, Austria

Utilizing the secretory apparatus of yeasts is a popular approach for recombinant protein production. However, prediction of production levels is notoriously difficult. Even small changes in the amino acid sequence, such as those introduced through mutagenesis in protein engineering, can significantly impact protein stability and production yields.

Studies based on expression data of a variety of proteins have shown correlations of expression with sequence parameters like fraction of charged amino acids or associated biosynthetic cost. Looking more specifically into sequence variation, the comparison of protein mutants revealed that secretion level correlates with thermodynamic stability. However, studies on variants were often limited by the number of experimentally tested mutants to reveal broader principles.

For our current study, we generated a model protein library with fully randomized surface patches. Using yeast surface display and cell sorting, we simultaneously screened and sorted millions of protein variants by their expression level. Based on DNA and amino acid sequences in each expression category we calculated descriptors (e.g. composition, physiochemical parameters, distances between certain amino acids) and determined their importance for explaining expression differences via machine learning. With this naïve approach we can identify and rank both expected and surprising parameters contributing to the displayed protein level. Ultimately, this study aims to provide deeper insights into surface properties dictating protein expressibility and secretability.

**Inhibiting aggregation of therapeutic proteins**

Arda Deniz Tugrul

NIBRT, Ireland

**Model guided design of *E. coli* strains for the heterologous production of dopamine**

Cedric Meyer

Institute of Biochemical Engineering, University of Stuttgart, Germany

Transcription and translation form a complex, non-linear system, where in vivo protein production rates depend on a wide range of factors, including codon usage, ribosome concentration, and substrate availability. This study leverages a detailed kinetic model of transcription and translation to predict the production of the two enzymes in the heterologous dopamine biosynthesis pathway in Escherichia coli. These predicted enzyme concentrations are then used to constrain reaction fluxes in a genome-scale metabolic model (GEM), thereby creating a link between gene expression and metabolic output. This hybrid modeling framework enables in silico optimization of heterologous pathways and serves as a predictive design tool within the Design-Build-Test-Learn (DBTL) cycle. Our approach aims to accelerate strain engineering and improve dopamine production through model-guided design.

**Understanding transcriptional variance in CHO cells: a quantitative meta-analysis**

Markus Riedl; Nicolas Marx; Nicole Borth

BOKU University, Austria

A deep understanding of Chinese Hamster Ovary (CHO) cells' molecular complexity is key to improving growth, productivity, and product quality. While omics technologies and transcriptome analyses have advanced insights into gene expression, they often miss broader transcriptomic patterns. Notably, gene expression variability across diverse CHO lines and culture conditions remains unexplored. Although CHO cells largely express the same genes, differences in expression levels can significantly affect key traits. This study examines gene expression variance across various CHO lines to identify both stable and variable transcriptome elements linked to important cellular phenotypes.

We analysed RNA-sequencing data from 17 studies spanning diverse biological conditions and experimental setups. Raw reads were processed via a scalable workflow, including normalization and data transformation. Gene expression variance was quantified, ranked, and correlated to identify global patterns of transcriptomic variability.

Our analysis showed low correlations of variance rankings across datasets, suggesting that CHO cells' genetic instability extends to the transcriptome. Housekeeping genes showed stable expression, linked to core processes like transcription and RNA biosynthesis, while highly variable genes related to signalling and extracellular matrix components. Ongoing work explores links between transcriptional variance, gene regulatory signatures, and industry-relevant cellular traits.

**Advancing CHO cell productivity: a combined approach of cell engineering using novel transposon vectors and proteomics**

Sujay Subramaniam (1); Alexander Reder (2); Ulrike Mäder (2); Mark Smales (3); Jan Maarten van Dijl (1); Uwe Völker (2)

(1) University Medical Center Groningen, the Netherlands

(2) University Medicine Greifswald, Germany

(3) University of Kent, United Kingdom

Chinese Hamster Ovary (CHO) cells are preferentially applied for manufacturing biopharmaceuticals, accounting for over 85% of the current market products. However, high production costs and inconsistent gene expression limit their application potential. Therefore, our research focuses on enhancing protein production in CHO cells by utilizing a novel transposon-based expression platform, which offers lowered toxicity to host cells and more efficient gene integration compared to currently available systems. Using a well-optimized protocol, we successfully generated first stable cell pools in a significantly shorter time than standard transfection protocols. Our present results demonstrated a 12-fold increase in monoclonal antibody (mAb) production in stable cell lines. In parallel, we conducted extensive proteomic analyses to evaluate the cellular burden associated with mAb overexpression in CHO cells. Following this approach, we uncovered differential stress responses and alterations in metabolic pathways. Altogether, our proteomic studies identified 20% more proteins than previously reported, which greatly enhances our ability to pinpoint specific production bottlenecks for further investigation. These insights will guide the refinement of our expression platform to support high protein yields while minimizing cellular stress. Our findings suggest that in-depth proteomic analyses will provide the knowledge base needed to engineer CHO cells with enhanced production capacities, reduced cellular burden and improved manufacturing efficiency for biotherapeutics.

**Multi-omics characterisation reveals temporal dynamics of cellular and metabolic behavior of a CHO-based mAb process**

Larissa Hofer (1); Dominik Hofreither (2); Thomas Rauter (3); Thomas Berger (4); Veronika Schäpertöns (4); Laura Liesinger (2); Wolfang Esser-Skala (3); Nikolaus Fortelny (4); Ruth Birner-Gruenberger (2); Christian G. Huber (4); Nicolas Marx (1); Jerneja Štor (1); Nicole Borth (1)

(1) BOKU University, Austria

(2) TU Wien, Austria

(3) Salzburg Lodron Universität, Austria

(4) Paris Lodron Universität Salzburg, Austria

Achieving consistent product quality and accurately identifying critical process parameters (CPPs) remain significant challenges in biopharmaceutical process development. A major obstacle is the limited availability of detailed characterisation data and the difficulty in comparing cell lineages across different laboratories. In this study, we present the first comprehensive, multi-level characterisation of a publicly available Chinese hamster ovary (CHO) production cell line from the National Institute of Standards and Technology (NIST), establishing a benchmark for bioprocess comparability. Controlled bioprocesses were evaluated at constant 37 °C and with a temperature downshift to 32 °C on day 6 in a 0.7 L fed-batch system. High-frequency sampling monitored cell growth, nutrient consumption, metabolic by-products, nucleotide sugar donors, and product quality. Multi-omics characterization, including transcriptomic, (phospho)proteomic, and epigenomic analyses, was performed at 17 time points, supported by the R package SplineOmics. Results reveal detailed cellular and metabolic adaptations, including stationary phase heterogeneity, stress response activation, and cellular hypertrophy after temperature shifts. CQAs showed increasing free light chain and decreasing galactosylation post-shift, more so at constant temperature. Linking metabolic and regulatory changes to CQAs supports precise bioprocess modeling. The NISTCHO cell line and this dataset provide a valuable resource for optimizing bioprocesses and enhancing comparability across labs.

**Scale-down bioreactor studies on heterologous protein production in stringent response modulated *E. coli* chassis**

Sidharth Jaya Sankar

Institute of Biochemical Engineering, University of Stuttgart, Germany

*Escherichia coli* (*E. coli*) is widely used for large-scale recombinant protein production. However, in large bioreactors, uneven mixing and limited mass transfer create fluctuating nutrient and oxygen levels, triggering stress responses in the bacteria. This stress reduces cellular performance, increases energy maintenance, and lowers protein yield, growth, and productivity, ultimately affecting product titre and process economics. One approach is to engineer robust chassis that is capable of enduring these stress conditions and prevent large scale performance loss. *E. coli* SR strain is engineered to avoid global stress regulation system called stringent response by modulating the ppGpp (guanosine pentaphosphate) to basal level, which is the key controller of this mechanism. The strain exhibits increased tolerance to industrial-scale stresses positioning it as a potential robust chassis for large scale bio-manufacturing. Investigations using scale down bioreactors (stirred tank bioreactor- plug flow bioreactor) that mimics industrial stress conditions facilitate comprehensive metabolic, transcriptional and production data. Our preliminary experimental data indicates that, *E. coli* SR sustains the fraction of super folded green fluorescence protein producing cells at a consistent level after 28-hours of large-scale stress exposure during production, comparable to results obtained 6-hours after the start of production phase. In contrast, the parental strain *E. coli* MG1655 parental strain experiences a reduction of 20% in protein producing cells under the same conditions.

**ECOnti - advancing integrated continuous manufacturing with microbial cells**

Florian Simon

enGenes Biotech GmbH, Austria

Continuous manufacturing with *Escherichia coli* (*E. coli*) is often limited by process instability and low productivity due to rapid growth and high mutation rates.

We developed a novel *E. coli* host cell line for growth-decoupled production of recombinant proteins, plasmid DNA, and small molecules. This technology, enGenes-e$^{X}$press, uses synthetic biology to enhance expression. Upon induction, co-expression of phage-derived Gp2 inhibits σ70-dependent transcription, halts cell division, and redirects metabolism toward product synthesis. This boosts productivity under industrial conditions and enables extracellular protein secretion.

In a continuous two-stage chemostat setup (enGenes-e$^{X}$cite), growth and production are spatially separated: biomass is generated in the first reactor, production induced in the second. Cell division stops, increasing stability and maintaining productivity.

This presentation highlights: i) the enGenes-e$^{X}$press technology, ii) the enGenes-e$^{X}$cite process, iii) strain development, iv) integrated continuous upstream/downstream processing, and v) automation and digitalization, including in-line analytics and hybrid modeling. Potential applications in compact manufacturing and techno-economic modeling are discussed.

**CASPON®: a fusion-protein platform technology for efficient recombinant biopharmaceutical protein production**

Sophie Kutzner; Karin Koch; Martin Wagenknecht; Vignesh Rajamanickam; Daniel Elsner; Martin Voigtmann; Wolfgang Buchinger; Stefan Krahulec; Michael Graf; Daniel Fleischanderl; Matthias Berkemeyer; Cécile Brocard

Boehringer Ingelheim RCV GmbH & Co KG, Austria

The CASPON® platform technology, a fusion-protein-based system developed for Escherichia coli, offers a powerful solution for the efficient production, purification and quantification of soluble biopharmaceuticals [1]. The system consists of an N-terminal fusion tag and an ultrafast and highly efficient circularly permuted caspase-2 protease. Both in combination enable enhanced protein solubility, protein purification and precise removal of the fusion tag to yield an authentic N-terminus of the protein of interest (PoI) [2].

The incorporation of an affinity tag facilitates cost-effective purification of the PoI and supports automated screening approaches for optimal purification conditions for downstream processing. Moreover, the platform allows for accurate, PoI-independent titer quantification using an automated immunoassay. Thus, the CASPON® platform technology supports high-throughput screening at small scale across upstream, downstream, and analytical workflows, while also enabling scalable production at industrial levels.

[1] Elsner et al.: Scar-free tag removal by CASPON® enzyme with broad physicochemical stability in biomanufacturing – A case study of five proteins. Separation And Purification Technology 2024, 130832.

[2] Lingg et al.: CASPON platform technology: Ultrafast circularly permuted caspase-2 cleaves tagged fusion proteins before all 20 natural amino acids at the N-terminus. New Biotechnology 2022, 71, 37–46.

**Cationic polymers reduce (high-risk) host cell proteins in monoclonal antibody purification processes**

Anna-Carina Frank (1); Gregor Stitz (1); Timon Kalchmayr (1); Clara Hofmann (2); Natalie Deiringer (3); Felicitas Guth (3); Christine Rösch (3); Elisabeth Grünstein (3); Rainer Hahn (1); Peter Satzer (1)

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Chinese hamster ovary (CHO) cell culture processes are continually optimized to increase mAb titers. The enhancement of mAb titers is often associated with a higher cell density and elevated concentrations of process-related impurities, including host cell DNA and host cell proteins (HCPs). Certain CHO HCPs have been identified as high-risk. They may have a negative effect on product or formulation stability, potentially trigger immunogenicity or directly impact biological functions in humans. Conventional purification processes often encounter challenges in removing these impurities due to potential interactions of the high-risk HCPs with mAbs.

It has been demonstrated that certain cationic polymers form floc complexes with negatively charged species, like mammalian cells, DNA and proteins with a low isoelectric point. These floc complexes exhibit improved removal through depth filtration in comparison to un-flocculated cell broth. The application of certain cationic polymers in flocculation has been shown to reduce impurities. The use of certain cationic polymers may result in at least a reduction of certain high-risk HCPs.

We examined the efficacy of certain cationic polymers in clarifying cell culture fluid; a significant reduction in host cell DNA (99%), total HCPs (~30%), and certain high-risk HCPs (>50%) was observed during cell removal.

Flocculation in the primary recovery of mAb processes may enhance process performance by reducing the particle burden for depth filtration and substantially decreasing overall process-related impurities and (high-risk) HCPs.

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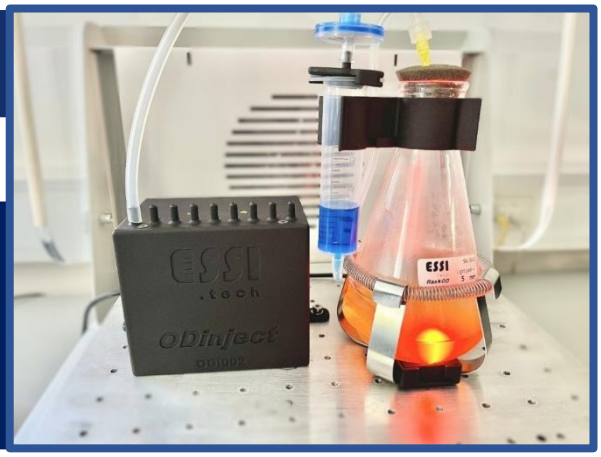


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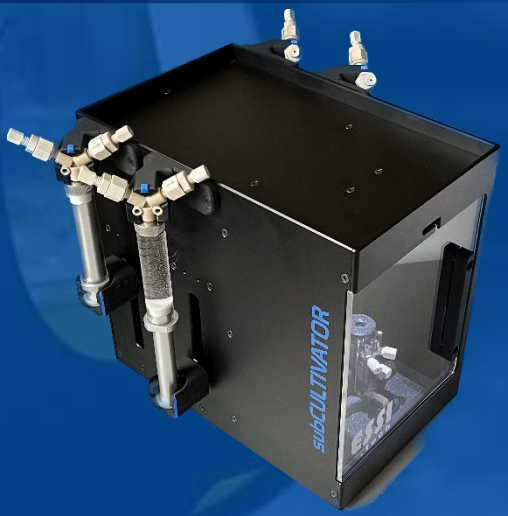


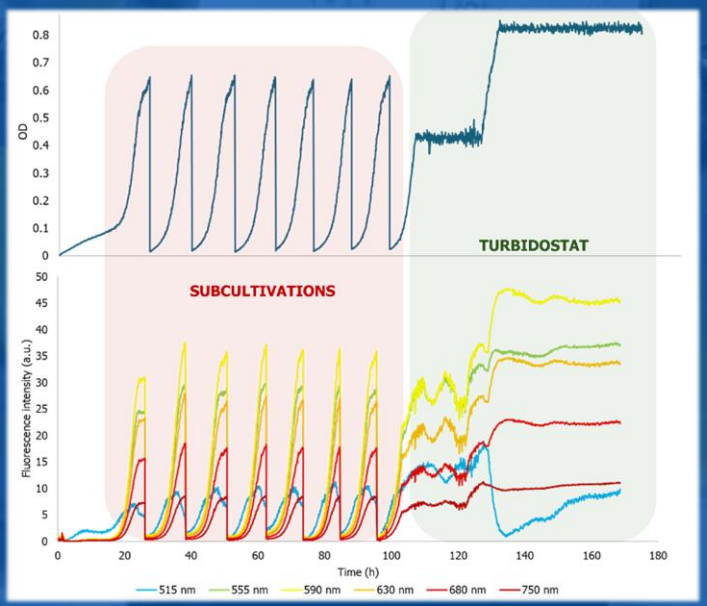


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## Keynote lecture

**Adventures in synthetic glycobiology: enabling customized protein glycosylation with engineered bacterial cells**

Matthew DeLisa

Cornell University, United States

Complex carbohydrates, or glycans, are involved in almost every human disease and biological process. Hence, the field of glycoscience has the potential to broadly impact society in areas as diverse as medicine, energy, and materials. However, the recombinant production of desirable glycomolecules, especially glycans and glycoproteins, has been hampered by the lack of easily controlled and widely accessible glycosylation systems. To address this shortcoming, a new discipline called synthetic glycobiology has emerged that enables biosynthesis of custom glycans and glycoproteins, some containing unnatural sugars, using engineered enzymes, pathways, and host organisms that catalyze prescribed glycosylation reactions. In this talk, I will discuss some of our recent progress towards the purposeful alteration and rational construction of diverse glycosylation systems in both living bacterial cells as well as cell-free reaction environments. By leveraging chemical and molecular biological approaches in conjunction with metabolic engineering and synthetic biology tools, our bacterial glycoengineering work has been instrumental in increasing knowledge about glycosylation networks and producing desired glycans and glycoconjugates.

## Session 1: Chassis engineering for recombinant production of proteins

**Autophagy and AKT-stimulated cellular proliferation synergistically improve antibody production in CHO cells**

Anne S. Robinson Robinson

Carnegie Mellon University, United States

Over the past decade, engineered producer cell lines have led to antibody yield increases over tenfold based on an improved understanding of the cellular machinery influencing cell health and protein production. In this study, we developed strategies to enhance cell viability and prolong cell culture duration, ultimately improving production lifetimes. By overexpressing the cell surface adenosine A2A receptor (A2AR), the Akt pathway was activated, resulting in improved cellular proliferation. Alternatively, by inducing autophagy through chemical supplementation or temperature down-shift, we were able to significantly enhance cellular-specific productivity. The utilization of multiple culture longevity-prolonging strategies enabled up to a three-fold increase in total antibody production as well as three-fold higher cell-specific productivity. Interestingly, the expression levels of the autophagy pathway protein Beclin-1 appeared to correlate best with the total antibody production of the cellular proteins examined.

**Cryo-EM structures of human membrane protein complexes from a streamlined cell-free protein production platform**

Frank Bernhard

Goethe University Frankfurt, Germany

G protein coupled receptors are pharmaceutical targets of prime importance and key players in most human diseases. We could reduce the overall complexity of membrane protein production by optimizing cell-free expression systems based on efficient E. coli lysates. Nascent membrane proteins are inserted already cotranslationally into preformed nanoparticle membranes. Limiting translocation machineries and critical steps such as cell culture handling, surface expression or detergent extraction are not necessary. The process ensures efficient target production and the developed fluorescence independent NSEC tool enables the rapid quality assessment of synthesized membrane proteins irrespective of type, origin or function.

We show cryo-EM structures of cell-free generated human G protein coupled receptors in complex with their cognate heterotrimeric G proteins. The histamine-2 receptor, the ß1-adrenergic receptor, and the free fatty acid receptor 2 were synthesized as full-length proteins without any truncations, deletions or fusion partners. The structural analysis revealed ligand binding pockets and identified new interaction clusters modulated by the presence of flexible loop regions that are involved in the regulation of signal transduction. For comprehensive biochemical studies, we implement the nanotransfer technique for inserting cell-free synthesized membrane proteins back into the membranes of living cells. We demonstrate the functional analysis of cell-free generated and transferred GPCRs in the plasma membranes of cultivated cell-lines.

**Streamlined cells are superior bioproduction hosts**

Alvaro R. Lara

Aarhus University, Denmark

A series of Escherichia coli streamlined strains was developed by removing the expression of genes encoding extracellular structures and unessential enzymes. The streamlined strains exhibited improved metabolic performance, including lower overflow metabolism and ATP maintenance coefficient, as well as higher growth rate, compared to their parental strain. The intracellular levels of ATP were monitored using a genetic sensor, showing the improved resource stewardship of the streamlined cells. The streamlined strains were tested as cell factories to produce plasmid DNA (pDNA) in batch cultures, exhibiting a 23 % increase in the specific pDNA production rate, compared to the parental strain. Recombinant protein expression was evaluated in microbioreactors in batch and fed-batch mode. In batch mode, recombinant protein yield from biomass was up to 82% higher in the streamlined strains than in the parental strain. Furthermore, in fed-batch mode, the recombinant protein yield was 79% greater in the streamlined cells compared to the parental strain. Our results show the benefits of reducing cellular complexity on the biomanufacturing of pDNA and recombinant proteins in culture schemes typical of industrial settings.

***Haloalkaliphilic Archaea* as new cellular chassis for the bioproduction of recombinant proteins**

Emma Bonnaud (1); Philippe OGER (2); Mathieu Orzalisi (3); Yoann Louis (2)

(1) UMR CNRS 5240-Laboratoire Microbiologie, Adaptation et Pathogénie, France

(2) MAP-UMR CNRS 5240, France

(3) SEGULA TECHNOLOGIES, France

Climate change and resource scarcity drive the development of greener industrial processes. In this context, extremozymes (extreme microbial enzymes) can replace traditional chemical synthesis steps. However, their production is limited by the lack of suitable cellular chassis. The production of a large number of extremozymes requires extremophilic chassis, which are not available. Haloalkaliphilic Archaea, which thrive in hypersaline (>35 g/L NaCl) and hyperalkaline (pH>9) environments, are promising chassis. Their extreme intracellular environment makes them ideal hosts for producing of recombinant haloalkaliphilic proteins, which often misfold or aggregate in conventional systems (i.e. Escherichia coli).

We are developing a cellular chassis based on the haloalkaliphilic archaea Natronomonas pharaonis, aiming to establish a genetic toolbox adapted to this host and eventually to other halophilic and haloalkaliphilic archaea. Several overexpression vectors were constructed, enabling controlled production of proteins of interest. One of them is based on a tryptophan-inducible promoter. A novel uracil auxotroph derivative of N. pharaonis was created to enlarge the catalogue of selection markers available in this species. Furthermore, since the standard transformation protocol for N. pharaonis is not very efficient (~8 CFU/µg DNA) and slow, to improve host manipulability and robustness, a new transformation protocol has been developed using a combination of high hydrostatic pressurization and fast depressurization.

**Creation of a superior 2G bioethanol yeast strain with high enzyme secretion capacity**

Bart Thevelein (1); Mekonnen Demeke (2); Johan Thevelein (2)

(1) UGent, NovelYeast, Belgium

(2) NovelYeast, Belgium

Introduction: The economically feasible production of lignocellulosic bioethanol is compromised by the hydrolysis of lignocellulosic biomass due to high cellulase enzyme cost. To tackle this, we are constructing Cellusec® yeast strains that can secrete high amounts of cellulase enzymes while also excelling in other critical properties such as inhibitor tolerance, xylose utilization and thermotolerance. Results: Consecutive introduction of three copies of β-glucosidase allowed the first strain to consume 0.5% w/v cellobiose in 18h which increased to 0.9% and 1.3% in 18h with two and three inserted copies, respectively. Subsequent expression of CBHI, CBHII, RfGH5-4 and overexpression of HAC1i in the same strain resulted in gradually increasing Avicel hydrolysis capacity, reaching a maximum capacity of 22% in 48h. Enzyme secretion from Cellusec® strains increased the final ethanol titer from 4.1% v/v to 5.7% v/v in fermentation of sugarcane bagasse hydrolysate. Finally, whole genome transformation (WGT) was applied to improve thermotolerance, which allowed the strain to fully ferment all sugars in sugarcane bagasse hydrolysate at 40°C. Conclusion: Rational engineering of Cellusec® successfully allowed it to ferment cellobiose at a high rate and to partially hydrolyze Avicel, which led to a significant increase in ethanol titer in lignocellulose hydrolysate fermentations. WGT was shown to be a reliable method to improve strain thermotolerance, and allowed the strain to be used in high temperature SSF (Simultaneous Saccharification and Fermentation) processes.

**Flo8 – a versatile regulator for improving recombinant protein production in *Komagataella phaffii***

Corinna Rebnegger (1); Mirelle Flores-Villegas (2); Sonakshi De (1);
Diethard Mattanovich (2); Brigitte Gasser (2)

(1) Austrian Centre of Industrial Biotechnology (acib), Austria

(2) BOKU University, Department of Biotechnology and Food Science, Institute of Microbiology and Microbial Biotechnology, Austria

Flo8 is a key transcriptional regulator of flocculation and pseudohyphal growth in yeast. Disruption of *FLO8* in the popular recombinant protein production host Komagataella phaffii (Pichia pastoris) prevents pseudohyphal growth and reduces cell-to-surface adherence, making the *flo8*Δ mutant valuable for research and industry. However, knowledge of the physiological impact of the mutation remained scarce.

Transcriptomic analysis of *FLO8*-deficient K. phaffii revealed systemic changes. In addition to flocculation genes, Flo8 affects genes involved in cell cycle, mating, respiration, and catabolite repression. One notably upregulated gene in *flo8*Δ was *GTH1*, encoding a high-affinity glucose transporter in K. phaffii. Its promoter $P_{GTH1}$ is established as a strong, glucose-regulatable alternative to methanol-induced promoters.

Thus, we tested the *flo8*Δ mutant in combination with $P_{GTH1}$ and its improved derivatives ($P_{G1X}$) for recombinant protein production in small scale screenings and bioreactor cultivations. Both $P_{GTH1}$ and $P_{G1X}$ demonstrated significantly elevated expression strength in *flo8*Δ, resulting in substantially enhanced recombinant protein titers. Consistently, secreted protein yields of several different secreted recombinant proteins were strongly enhanced (up to 8-fold higher) as well. Yields could be further increased when combining *flo8*Δ with secretion enhancing factors.

In summary, *K. phaffii flo8*Δ has many advantageous characteristics, such as reduced surface growth and increased productivity, making it an excellent chassis strain for recombinant protein production.

**Improving industrial protein secretion in *Bacillus subtilis* using RNA-seq and Ribo-seq**

Biwen Wang (1); Leendert Hamoen (2)

(1) Hanze University of Applied Science, the Netherlands

(2) University of Amsterdam, the Netherlands

Bacillus subtilis and its close relatives are widely used in the industrial-scale production of enzymes. Despite many years of research, the secretion bottlenecks and the corresponding stress regulatory mechanisms in the production organism itself are still not well understood. To address this gap in knowledge and address secretion bottlenecks, we implemented 2 unbiased deep-sequencing-based techniques: transcriptome profiling (RNA-seq) and ribosome profiling (Ribo-seq) to study potential secretion bottlenecks. Transcriptome analyses revealed that B. subtilis cells overexpressing AmyM exhibited a distinct profile compared to XynA overexpressing cells, however there were also similarities and in both cases expression of CtsR controlled genes was downregulated. Inactivation of CtsR increased XynA production by more than 25 %. Interestingly, the production of XynA peaks well before AmyM reaches its optimal secretion rate, despite both being driven by the same promoter. Analysis of transcriptome and ribosome profiling data indicated that the difference in secretion timing is not due to transcriptional or translational regulation. Our findings suggest that both secreted proteases and the intracellular protease LonA influence reduced secretion in the stationary phase. Deletion of LonA led to a 140% increase in XynA production and a 20% increase in AmyM production. The combination of RNA-seq and Ribo-seq offers detailed genome-wide data for comparing various experimental conditions, uncovering secretion bottlenecks and enabling a data-driven approach to enhance enzyme secretion.

**From recombinant expression to functional reconstitution: characterization of outer membrane porins and their mass transport**

Maike Scherer; Kathrin Castiglione

FAU Erlangen-Nürnberg, Institute of Bioprocess Engineering, Germany

Outer membrane porins (Omps) play a crucial role in the selective transport of molecules across bacterial membranes [1] and into engineered nanoreactors [2]. Comparative studies on their recombinant expression and mediated transport rate are essential for the development of a toolbox of functional porins.

The recombinant expression of Omps remains challenging due to their hydrophobicity, folding requirements, and potential cellular toxicity. Successful overexpression requires overcoming bottlenecks in membrane insertion and solubility issues, often necessitating tightly controlled expression systems and adapted purification strategies. In this work, the strain *E. coli* BL21 (DE3) omp8 was used to recombinantly express OmpF, its engineered variant of larger pore size OmpFΔ [3], and phosphoporin E (PhoE). Membrane solubilization resulted in a PhoE yield of 0.37 mg per gram cell wet mass - three times and almost seven times higher compared to OmpF and OmpFΔ, respectively.

To assess mass transport, the Omps were reconstituted into nano-scale polymer vesicles that are generated by self-assembly of amphiphilic block-copolymers while entrapping enzymes in their lumen. A luminescence assay based on the oxidation of the substrate coelenterazine by encapsulated *Gaussia* luciferase was established. Under mass transport-limited conditions, a comparison of the transport activities of OmpF, OmpFΔ and PhoE was possible showing the highest transport rates of 154 molecules per second for the large OmpFΔ porin.

[1] 10.1111/1751-7915.70122

[2] 10.1021/acscatal.7b00776

[3] JBC 271(34): 20676-20680

## Session 2: Impact of production conditions on cellular performance

**Engineering ferritin nanoparticles for next-generation vaccines: strategies for antigen display and biomanufacturing**

Margarida Queluz Rodrigues; António Roldão

iBET, Portugal

Ferritin nanoparticles offer a self-assembling, multivalent platform suitable for antigen display in vaccine development.

To expand design flexibility, we developed a site-specific antigen conjugation strategy using tyrosinase-mediated coupling and engineered cysteine reactivity. This method enabled the precise and stable display of antigens from SARS-CoV-2 and Rift Valley fever virus (RVFV), preserving epitope integrity and reactivity to neutralizing antibodies.

In parallel, we addressed the manufacturability and characterization of ferritin-based vaccines using the RVFV Gn antigen as a model. Genetic fusion constructs were expressed in baculovirus-insect cell systems, and production parameters were optimized to improve yield and particle quality. The resulting nanoparticles retained proper 24-mer assembly. Structural and immunological analyses confirmed effective antigen display and in vitro immune activation in dendritic cells.

Together, these strategies support the use of ferritin nanoparticles as a scalable platform for vaccine development against emerging infectious diseases.

**The impact of methanol concentration on recombinant protein glycosylation in *Pichia pastoris* SuperMan5**

Andre Ohara; Zhang Pengyue; Xinqi Cao; Roberto Donini; Stuart Haslam; Karen Polizzi

Imperial College London, United Kingdom

The methylotrophic yeast Pichia pastoris (also known as Komagataella phaffii) is a prominent platform for recombinant protein production, offering benefits such as thermo- and osmotolerance, high-density growth, and efficient protein secretion. Its ability to metabolize methanol, an increasingly available carbon source, enhances its cost-effectiveness and sustainability for industrial use. As a eukaryotic host, P. pastoris ensures proper protein folding and post-translational modifications (PTMs), including glycosylation, which affect stability, solubility, and bioactivity. Depending on the intended applications of the protein, glycosylation can be either beneficial or undesirable. The impact of induction conditions on glycosylation of proteins secreted by P. pastoris SuperMan5 was examined, using the DS-1 (G2P[4]) VP8* rotavirus capsid protein as a model. An ELISA-based screening system was employed for clone selection and media optimization, with results showing easy integration into automated workflows. Methanol concentration was found to impact both N- and O-linked glycosylation complexity, shaping the glycosylation profile of the target protein as well as the P. pastoris secretome. This study underscores the importance of optimizing cultivation conditions to enhance protein yield, refine glycosylation, and minimize impurities, all of which are crucial for large-scale production and efficient downstream processing. It also suggests a method for easy modulation of glycosylation depending on the target application and the desired level of glycosylation.

**The hidden cost of protein secretion: signal peptide-induced stress in *Bacillus subtilis***

Noemi Palombi (1); Johanna Wörtl (2); Francesca Grilli (2); Aysegül Öktem (2); Tjeerd van Rij (3); Jolanda Neef (2); Dörte Becher (4); Sandra Maaß (4); Jan Maarten van Dijl (2)

(1) Universität Greifswald, Germany

(2) University of Groningen, University Medical Center Groningen, the Netherlands

(3) DSM-firmenich, the Netherlands

(4) University of Greifswald, Institute of Microbiology, Germany

The secretory production of recombinant proteins greatly facilitates downstream processing. Signal peptides (SPs) are well known as core-essential drivers of protein secretion. Yet, the broader impact of SPs on host cell physiology has remained underexplored. In this study, we investigated how different SPs fused to the same heterologous protein, AmyQ, influence secretion efficiency and cellular stress responses in the popular bacterial cell factory *Bacillus subtilis*. To this end, a library of strains expressing AmyQ with diverse SPs [1] was characterized by enzyme activity assays, Western blotting, and proteome analyses.

Our findings reveal a complex relationship between secretion efficiency and AmyQ enzymatic activity, where increased secretion does not necessarily correspond to improved performance. This indicates potential compromises in protein folding or stability. Furthermore, proteome analyses show that individual SPs elicit distinct physiological adaptations, including modulation of stress-related proteins, redox balance and metabolic pathways. Notably, certain SPs that lead to modest AmyQ secretion levels induce significant intracellular responses, highlighting the importance of SPs as modulators of cellular stress, beyond their generally acknowledged role as protein sorting signals.

Altogether, our findings highlight (i) the importance of considering SP-induced secretion stress when designing protein production systems, and (ii) the need to balance secretion efficiency with “cell factory health”.

Grasso, S., et al. (2023) DOI: 10.1021/acssynbio.2c00328

**High-throughput investigation of recombinant bifunctional protein secretion by *Corynebacterium glutamicum***

Vera Waffenschmidt (1); Ines Vogt (2); Tetiana Kurkina (2); Ulrich Schwaneberg (2); Wolfgang Wiechert (1); Marco Oldiges (1)

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(2) RWTH Aachen University, Germany

Bifunctional proteins (BiFuProts) are a promising tool for a more sustainable and healthy food industry. Consisting of an adhesion-promoting peptide and a functional domain, they enable specific targeting and functionalisation of surfaces. Streamlining the secretory production of BiFuProts and gaining insights into the influencing factors on protein secretion by *Corynebacterium glutamicum* were the aims of this work.

Since predicting optimal secretion conditions for heterologous proteins is complex, affected by factors like target protein, signal peptide, and process parameters, extensive strain library screenings are necessary to establish an efficient production process. With the aid of automated routines for central cloning steps as well as for the cultivation and assaying of strains, we were able to investigate several rationally designed strain libraries for BiFuProts expressed with 24 different Sec-type secretion signal peptides in only a few weeks to months.

With these screenings, the best-performing secretion strains for the target BiFuProts were identified and cultivation conditions were optimised. The thorough screening of strain libraries for several similar target proteins also revealed a possible link between target protein sequence and signal peptide performance.

Overall, this work did not only improve the production process for proteins that will be used in sustainable food and packaging applications, but it will also accelerate future efforts to develop efficient bioprocesses for secretion of heterologous proteins.

**Mimicking large-scale mixing conditions in a laboratory-scale single multi compartment bioreactor system**

Jonas Barczyk (1); Ryan Rautenbach (2); Michael Schlüter (2); Ralf Takors (1)

(1) Institute of Biochemical Engineering, Germany

(2) Institute of Multiphase Flows, Germany

In industrial-scale cell culture processes, the formation of heterogeneous zones due to slow mixing is an important topic for bioprocess development [1,2]. However, doing research on cell culture performance in production-scale bioreactors is difficult, since the operation of bioreactors with large volumes is expensive.

This study addresses the issue of investigating heterogeneities in industrial-scale bioprocesses which influence cell culture performances and product quality. Recently, a single multi-compartment bioreactor system (SMCB) was advanced to mimic a slow-mixing and a fast-mixing zone in the same bioreactor. The scale-down reactor model shows a range of different mixing properties and is tested with an optical mixing time determination method [3]. These zones are able to mimic mixing conditions that are present in a large-scale processes of 12 500 liter [4].

In the past, the SMCB model could already link different power inputs to changes in product quality based on glycan analysis of the produced antibodies [5].

More fed-batch cultivations with mammalian cells are now investigated to determine the influence of industrial-scale mixing on cell culture performance in the 3-liter laboratory-scale system.

References:

[1] Zakrzewski et al., 2022; [2] Zhao et al., 2024; [3] Fitschen et al., 2021;

[4] Hofmann et al., 2025; [5] Gaugler et al., 2024

**Time-resolved absolute protein quantification to optimize recombinant protein production in *Bacillus subtilis***

Francesca Grilli (1); Doerte Becher (2); Jan Maarten van Dijl (1); Sandra Maass (2)

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Understanding recombinant protein production (RPP) in bacteria is crucial to enhance protein yields, and protein quantification is a very valuable tool to achieve this objective. In fact, the comprehensive absolute protein quantification of all host proteins expressed during the production process presents several advantages: it allows us to quantify the relocation of proteins in different cell compartments, calculate the secretion rate of proteins of interest and understand the cellular stress response to heterologous protein production. All this information will allow us to understand the bacterial physiology and to solve critical bottlenecks in RPP.

Therefore, a reliable method for absolute protein quantification is extremely important. Thanks to high-resolution mass spectrometry instruments, the advance of data analysis and the improvement in sample preparation, it is now possible to achieve both an accurate identification and quantification of cytosolic proteins, membrane proteins and proteins in the culture supernatant in a single experiment. The recently developed methods are robust and sensitive, as they allow us to quantify protein amounts in the order of femtomoles and molecules/cell.

Here the entire method, from the sample preparation to the data analysis, is shown exemplarily for *Bacillus subtilis* producing the α-amylase AmyQ in a time-resolved manner. The results allow us to understand how RPP affects the bacterial physiology during the growth.

**Engineering genealogical age and cell clustering patterns for improved recombinant protein production in *Pichia pastoris* bioprocesses**

Alexander Hämmerli (1); Miriam Hartmann (1); Marco Fluri (2); Irina Druzhinina (3); Lukas Neutsch (1)

(1) ZHAW - Zurich University of Applied Sciences, Switzerland

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(3) Royal Botanic Gardens Kew, United Kingdom

Pichia pastoris (also known as Komagataella phaffii) is a widely utilized host for recombinant protein production. However, compared to model organisms like S. cerevisiae, detailed single-cell level understanding of how genealogical age influences recombinant protein expression remains limited. Budding yeasts exhibit complex heterogeneous population structures due to asymmetric cell division and varying chronological and genealogical ages. Traditional models and optimization approaches often rely on averaged population variables, overlooking this critical single-cell heterogeneity.

This study pioneers a comprehensive high-throughput analysis to link cell division history and morphology to product accumulation in P. pastoris bioreactor cultivations, in close-to-industry settings. Selective bud scar staining, fluorescence microscopy, and flow cytometry – established tools for yeast population analysis – have been adapted to account for the partially clustered growth pattern of P. pastoris, harnessing improved information depth.

Our findings reveal that the genealogical age distribution of AOX-inducible P. pastoris populations can be intentionally modulated through the specific feed rate applied during the biomass expansion phase. This engineered age pattern, including characteristic cell clustering behaviors, persists significantly into the subsequent induction phase. Notably, we found that genealogical age engineering directly influences both the quantity and intracellular distribution of recombinant proteins, with huge potential for optimized bioprocess performance.

## Keynote lecture

**Tackling fundamental challenges in biotechnology with computational models and AI**

Tamir Tuller

Tel Aviv University, Israel

A large proportion of intracellular biological processes and phenomena are mediated by many subtle interactions between the genetic material and various macromolecular machines. These interactions can't be efficiently predicted and designed without the help of computational models, algorithms, and AI.

I will review the computational pipeline we are developing for tackling various fundamental challenges in biotechnology such as gene expression optimization, genetic stability, and biosafety using this approach. The presentation will include various examples from fields such as food tech, biosensors, and bio-pharmacy.

## Session 3: Model-enabled protein, cell, and process engineering

**Leveraging generative AI for applications in synthetic biology**

Aleksej Zelezniak

King's College London/Chalmers University of Technology

The central molecular dogma processes, foundational to life sciences, can be precisely controlled to catalyse advancements in biomedicine and biotechnology. Mastery of these processes can lead to transformative therapies and facilitate the transition from a fossil-centric to a bio-based society. To achieve this, our research merges computer and biological sciences by integrating generative machine learning (ML) techniques with synthetic biology. Our primary focus spans two directions: i) enhancing gene regulation for advanced protein expression systems, and ii) refining protein engineering to amplify biotechnology-relevant properties. Through applied science, we elucidate fundamental molecular mechanisms. Our development of quantitatively predictive ML models unveils the underlying mechanistic connections driving the observed molecular phenomena, e.g. such as gene expression control. In this talk, I will provide insights into the applications of generative ML within synthetic biology, share our team's recent results, and outline our forward-looking research trajectory.

**Optimising recombinant membrane protein production by *E. coli* cell factories using a whole cell model**

Viktoriia Gross (1); Alexandra Trigg (2); Douglas Browning (2); Alexander Darlington (1)

(1) University of Warwick, United Kingdom

(2) Aston University, United Kingdom

Membrane proteins are essential for cell survival and growth. Their heterologous production has a global market size of ~USD2Bn and underpins the discovery of medicines, biopesticides, antifungal and vaccine development. However, obtaining high yields of functional protein remains a significant challenge. A key cause of low productivity is the poor growth of production hosts. Heterologous production burdens cells as key cellular resources, such as amino acids and ribosomes, are diverted from host growth towards engineered processed and saturation of the cell's export machinery elicits cellular stress responses. These complex and nonlinear interactions complicate engineering efforts.

Here we present a coarse-grained mathematical model of recombinant membrane protein production by Escherichia coli cell factories. Our mechanistic model captures key aspects of cell physiology perturbed by membrane protein synthesis including cell metabolism, gene expression, protein export via the Sec system and biosynthesis of host enzymes, the host translocon and ribosomes. We fit our model to existing data sets and show that it successfully reproduces observations of the changes in resource allocation over different growth conditions, as well as the burden caused by introducing recombinant membrane protein into the system.

By coupling our new cell model with a multiscale model of fermentation and multi-optimisation routines, we are developing induction and gene expression strategies which optimise membrane protein yield. We are now testing these strategies experimentally.

**Minimization of a *Bacillus subtilis* signal peptide by rational design**

Dicky Pranoto (1); Danilo Milo (2); Lorenzo de la Parra Soto (2); Jolanda Neef (2); Girbe Buist (2); Jan Maarten van Dijl (2)

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The secretory (Sec) pathway is the major pathway of *Bacillus subtilis* for protein export from the cytoplasm to the extracellular milieu. An amino-terminal signal peptide is required to initiate protein translocation across the cytoplasmic membrane via Sec. Generally, Sec pathway signal peptides of *B. subtilis* are around 25 – 35 amino acids long and tend to be relatively hydrophobic compared to signal peptides from other organisms. In view of the importance of signal peptides for high-level secretory protein production in *B. subtilis*, understanding how signal peptide structure and length influence the efficiency of protein secretion is crucial. Accordingly, the present study was aimed at investigating the effects of signal peptide size and, in particular the subdomain sizes of a signal peptide, in relation to protein secretion efficiency. To this end, a rational engineering approach was employed for the design of shortened signal peptides that still can drive effective protein secretion. This involved *in silico* interaction studies with the signal recognition particle, which is involved in protein targeting from the ribosome to the membrane. Our results show that a *B. subtilis* signal peptide needs at least 8 – 10 hydrophobic amino acids to function properly. On this basis, we managed to construct a smaller signal peptide that is equally efficient as the original signal peptide.

**Advancing the expression of difficult to express proteins with self-driving labs: from model-based design to deep process insight**

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Bioprocess development is inherently complex and time-intensive, involving numerous interdependent decisions. The KIWI-biolab addresses this challenge by implementing a self-driving laboratory concept, integrating model-based design of experiments (mbDoEs), automated experimentation, real-time analytics, and intelligent process control. Central to this approach is the use of mathematical models that capture the dynamic behaviour of cell factories.

In this presentation, we will highlight two core capabilities of the KIWI-biolab: (i) the rapid and robust calibration of complex models via controlled, information-rich experiments, and (ii) direct opening of the KIWI-biolab for collaboration with external modelling groups for the concurrent evaluation of alternative models. These features will be illustrated with two applications: optimization of the expression of difficult-to-produce proteins at the examples of the regulatory hydrogenase from Cupriviadus necator in Escherichia coli, and the biocatalytic characterization of recombinantly produced kinases under varying conditions. Together, these examples demonstrate how self-driving labs like the KIWI-biolab can significantly accelerate and enhance the development of processes for recombinant proteins.

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## Session 4: New horizons for biopharmaceutical proteins

## Development of the OPENPichia open-access industrial *Pichia pastoris* protein production system and use for manufacturing of anti-infectious VHH-Fc antibodies

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The yeast *Komagataella phaffii* (historically referred to in biotechnology as *Pichia pastoris*) is widely used for producing recombinant proteins, with many used in the clinic, in industrial processes and in food. However, the origin and derivation of the basic strain NRRL Y-11430, from which all commercial hosts are derived, is formally undocumented, and access to its patent-associated deposit has had costs, complexities and enduring restrictions much beyond the lifetime of the original patents.

Our studies and those of others in the field suggest that NRRL Y-11430 was derived from the *K. phaffii* type strain deposited in the UC Davis Phaff Yeast Strain collection in 1954. We started from an equivalent type strain to develop an open-access *Pichia* chassis strain. The industrial NRRL Y-11430 is more transformable than the *K. phaffii* type strain, and we discovered that it has a HOC1 ORF truncation, altering cell wall mannan. Introducing this mutation in the type strain indeed confers high transformability and higher secretion for select proteins. We named this chassis strain as OPENPichia. At a not-for-profit one-time contribution to costs of development and resource maintenance&expansion at VIB, we provide the genome-sequenced type, a his4 auxotroph, and a fully synthetic modular expression vector-building toolkit under liberal distribution licenses, incl. free use for commercial manufacturing purposes, as an unencumbered OPENPichia synthetic biology resource[a]. Further strain modifications that are off-patent are continuously being implemented and added to the resource, incl. protease-knockout strains generated by IP-unencumbered genome engineering techniques.

Illustrating the industrial readiness of the system, we have used OPENPichia as the chassis for all of our work in engineering a stabilized non-N-glycosylated VHH-Fcγ antibody format, developing optimized manufacturing strains and simple repeat-fed-batch processes. In this collaboration with the Gates Foundation, we achieved space time yield of production at par with highly optimized CHO fed batch production of this antibody type, which is much more suited to yeast-based production than hIgG1. We demonstrate this technology by manufacturing of sarbecovirus-neutralizing antibodies[b]. In related work, we have engineered VHH-Fcα molecules and oligomeric VHH molecules for production in Pichia, to be formulated into dried feed/food to combat gastro-intestinal pathogens. We have demonstrated efficacy in a swine model of Enterotoxigenic *E. coli* (ETEC), of use in veterinary medicine[c], a product that has been full developed by our startup Animab with a goal for market entry in 2026. We have recenty built on this technology for development of an food-administered antibody that can prevent lethal *Clostridiodes difficile* disease in the mouse model, and anticipating utilization to combat human diarrheal pathogens, incl. in nosocomial and resource-poor settings.

[a]Van Herpe, D.,…, Claes, K. and Callewaert, N. OPENPichia: licence-free Komagataella phaffii chassis strains and toolkit for protein expression. Nature Microbiology volume 9, pages 864–876 (2024)

[b]Lonigro, C., …., Claes, K. and Callewaert, N. Yeast-based production platform for potent and stable heavy chain-only antibodies. Preprint of part of the work at bioRXiv: https://doi.org/10.1101/2024.03.04.580093

[c]Virdi, V.,…., Callewaert, N. Yeast-secreted, dried and food-admixed monomeric IgA prevents gastrointestinal infection in a piglet model. Nature Biotechnology 2019 May;37(5):527-530. doi: 10.1038/s41587-019-0070-x

**Broad-specctrum antimicrobial recombinant proteins: a potential strategy for combating infectious diseases**

Elena Garcia-Fruitós; Sergi Travé-Asensio; Eva Balan-Gutiérrez; Cristina Saubi; Jose Vicente Carratalá; Francesc Fàbregas; Anna Arís

IRTA, Spain

In recent years, bacterial infections associated to antibiotic resistant pathogens have not stopped growing and the development of therapeutic alternatives is a major challenge. Many are the alternatives under study, with a big focus on antimicrobial peptides (AMPs) including host defense peptides (HDPs) and bacteriocins. Both bacteriocins and HDPs exhibit broad-spectrum antibacterial activity, a reduced probability of resistance, and absent or limited toxicity to mammalian cells.

As an alternative to chemical synthesis, recombinant production could become a cost-effective option for the AMPs production. However, the cationic nature of these short peptides makes their production challenging. Aiming to develop a universal platform for the efficient production of broad-spectrum antimicrobials, we have used a strategy based on the design of multidomain proteins by fusing different AMPs into a larger polypeptide. Also, different bacterial expression systems including *Escherichia coli* and *Lactococcus lactis* have been compared, proving that in many cases the AMP activity is determined by the recombinant host. The antimicrobial proteins designed showed a broad antibacterial activity against relevant pathogens affecting human and animal health, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and Mycoplasma, among others. In conclusion, our results highlight the promising role of the engineered AMP produced recombinantly as assets in the therapeutical toolkit of antimicrobial resistance challenge.

**High-yield production and functional characterization of a mutant human β-glucocerebrosidase enzyme in *Pichia pastoris***

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Gaucher disease is a lysosomal storage disorder caused by a deficiency of the enzyme β-glucocerebrosidase (GBA). Current enzyme replacement therapies rely on mammalian expression systems that are often expensive and yield limited amounts of enzyme. In this study, we developed a high-yield production platform for recombinant human GBA using Pichia pastoris GlycoSwitch® expression system. Three GBA mutants (Glu235Gly, Glu235Asp, and Glu235Ser) were rationally designed to enhance enzymatic activity through site-directed mutagenesis based on structural insights into the enzyme's active site and domain interactions. Among these, the Glu235Ser mutant demonstrated the highest enzymatic activity, exhibiting a 60% increase compared to the wild-type.

The mutant strains were cultured under various pH, temperature, and methanol induction conditions to identify the optimal production parameters. Optimal enzyme expression was achieved at pH 6, 18°C, and 2% methanol, with peak activity observed at 192 hours in shake-flask cultures. Subsequently, large-scale fermentation was conducted in a 3-L stirred bioreactor, yielding an enzyme activity of 4501 U/L, approximately 1.5-fold higher than that obtained in flask-scale production.

These results underscore the potential of P. pastoris as an efficient and scalable host for the production of therapeutic GBA variants. The Glu235Ser mutant, in particular, offers promise for developing cost-effective enzyme replacement therapies for Gaucher disease.

**A novel, thermostable sarcosine oxidase from *Bacillus megaterium* for biomedical applications: Kinetic characterization and active aggregation in *E. coli***

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Enzymes with diagnostic relevance are emerging as a vital class of recombinant proteins, prompting efforts to improve their production. Sarcosine oxidase (SOX) is a clinically important flavoenzyme used in diagnostic assays for creatinine and prostate cancer biomarkers. However, SOX production is impaired by its low soluble expression and high purification costs. These limitations can be overcome by protein expression through active aggregation, enabling the production of stable and functional enzymes with minimal downstream processing. In this study, we report the expression and kinetic characterization of a novel monomeric SOX from *Bacillus megaterium* in *E. coli* and its capacity to form catalytically active aggregates. The purified enzyme showed optimal activity at 45°C and pH 8.0, with a specific activity of 1.1 µmol/min/mg ($V_{max}$ = 0.03046 mM/min, $K_m$ = 0.688 mM, $k_{cat}/K_m$ = 1.5 $mM^{-1}s^{-1}$). The thermal half-life was 7.5 h at 45°C, with 90% activity retained up to 4.5 h. Notably, fusion with aggregation-inducing tags (particularly TorA) promoted active SOX aggregation without inactivation in inclusion bodies. Active aggregation was maximum (100%) at 37°C and reduced linearly with decreasing temperature. The use of linkers, particularly SSG, further improved active aggregation by ~13-fold. This work presents, for the first time, a robust SOX variant and its active aggregation using specific tags. These functionally active aggregates, being recoverable by simple centrifugation over chromatography, suggest strong potential for integration into biosensors and diagnostic assays.

**Bifunctional cancer antibody engineering for solid tumors**

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The non-uniform nature of cancer necessitates diverse treatment approaches, and antibody engineering has introduced various promising options for developing new therapies, including different antibody structures. One of the most promising tools is bispecific antibodies (BiAbs), which can simultaneously target two different epitopes, enhancing cellular toxicity and triggering a humoral immune response.

In this study, we developed a BiAb that binds to the T-cell receptor CTLA4 to prevent immune evasion and to the cancer receptor EGFR to activate cell death pathways. Additionally, we incorporated the Fc region, which has immune activation capabilities, and we genetically enhanced the stability of the antibody subunits under various conditions.

To optimize protein production and facilitate extracellular secretion, we added genomic regulators and signal sequences to our vector structure. The produced antibodies were purified and characterized, and their binding kinetics were assessed. We tested the purified antibody molecules against different cancer cell lines and natural killer cells, comparing their efficacy to that of their parental monospecific antibodies, as well as evaluating their costimulatory effects.

The cancer cell killing activity was monitored through XCelligence, alongside morphological assessments using cellular assays and characterisation studies (like SPR). Results indicate that bispecific anti-CTLA4-EGFR antibodies show significant potential for use in clinical settings as a novel treatment strategy, demonstrating higher effectiveness than the monospecific components.

**A protein production platform for diagnostics and immune therapy of emerging infections**

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Protein production for diagnostics and therapeutic interventions of infectious diseases is facing multiple challenges such as difficult-to-express target proteins, access to patient samples, cross-reactivity and most importantly speed. At the Fraunhofer Institute for Translational Medicine and Pharmacology, Immunology, Infection and Pandemic Research, ITMP-IIP, we have established a wide portfolio for generating antigens and antibodies as well as alternative specific binders. As a case study, we will present the current progress in vaccine-derived poliovirus type 2 (cVDPV2) VP1 capsid protein expression, purification, and biophysical characterization. Recently, cVDPV2 was detected in wastewater samples from major european cities, including Munich, Barcelona, Warsaw, and London. To support surveillance and immunity monitoring, we are developing a serological assay targeting the immunodominant VP1 capsid protein of poliovirus on the Elecsys immunoassay platform (Roche Diagnostics). Following antigen validation, an assay will be applied to the analysis of blood samples from a defined study cohort. The goal is to assess antibody prevalence against cVDPV2 and evaluate the feasibility of high-throughput screening for poliovirus vaccination and exposure to cVDPV2. This approach aims to facilitate early detection of immunity gaps and silent circulation.

## Session 5: Precision fermentation of food proteins

**Development of *Trichoderma reesei* strains for high-level production of the sweet protein brazzein**

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Brazzein is a thermostable, water-soluble sweet-tasting protein originally derived from the fruit of Pentadiplandra brazzeana. It exhibits sweetness 500–2000 times greater than sucrose on a molar basis and maintains stability across a broad pH range and under thermal stress, making it an attractive candidate for use in low-calorie food formulations. To enable scalable production, we explored recombinant expression of brazzein in the filamentous fungus Trichoderma reesei, a well-established industrial host.

We systematically evaluated multiple production strategies, including the use of different T. reesei strains, cellulase and synthetic promoters, signal peptides, and carrier proteins to optimize expression. Based on small scale-cultivation results, the most promising strains were selected for bioreactor cultivations, where production titers were quantified using HPLC. Our optimized strains achieved brazzein yields of up to 10 g/L, demonstrating the feasibility of T. reesei as a robust platform for industrial-scale brazzein production.

**High level production of brazzein, a natural disulfide-rich sweet protein using engineered *E. coli***

Lloyd Ruddock

University of Oulu, Finland

The world is facing an obesity epidemic, associated with a massive rise in related healthcare issues and costs at nearly 3% of world GDP. There is an urgent need for a consumer accepted zero calorie alternative to sugar that: i) Has a clean sweet taste and no unpleasant aftertaste; ii) Has biophysical properties that make it amenable for use in the food and beverage industry; iii) Is perceived as being natural; iv) Has sustainable and economic production.

Brazzein was first described in 1994. It is a naturally occurring sweet protein found in the fruit of the Oubli shrub (Pentadiplandra Brazzeana Baillion) that has been used as a natural sweetener in West Africa for centuries. Of the naturally occurring sweet proteins Brazzein has the highest relative sweetness and the best taste profile. In most reported tests it tastes like sugar with no-side tastes. This makes it unique. Brazzein also has other important bioproperties that make it very amenable for use in the food and beverage industries: it is highly soluble, very highly thermostable and pH stable over a wide range, especially at acidic pH values. Brazzein has just entered the market in the food industry, but its large scale and economic production remains challenging due to the large number of disulfide bonds it contains.

Here we report production and purification of Brazzein on the g/L scale from a previously reported engineered *E.coli* strain at the 10L scale, along with novel cell engineering approaches which facilitate economic scaling for this and any other protein with disulfide bonds.

**Recombinant production and characterization of selected functional proteins from chicken eggs**

Franziska Beck; Philipp Noll; Marius Henkel

Technical University Munich, Germany

Egg proteins are highly valued for their nutritional and techno-functional properties, making them a valuable ingredient in foods, like baked goods and desserts. However, concerns regarding animal welfare, environmental impact, and human health risks from conventional egg production are driving the development of sustainable alternatives. While plant-based proteins have been widely studied, replicating the multifunctional properties of egg proteins in a nature-identical way remains challenging. Precision fermentation (PF) offers a promising approach for producing recombinant egg proteins with native properties. This study focuses on the microbial production of selected egg proteins with relevant functional properties, e.g., emulsion and foam formation. Therefore, bacterial systems are used as expression hosts. Protein expression is induced chemically, followed by IMAC purification for further characterization. Initial ovalbumin experiments yielded a biomass-specific production rate of $q_{max}$ = 71.5 mg/g·h, already being within the range of low-productivity industrial processes. Optimizations aim to enhance production efficiency and protein functionality by scaling from shake flasks to bioreactors and implementing a fed-batch strategy. Results will be shared in an open-access database and compared to existing literature to promote knowledge exchange. Challenges like post-translational modifications, solubility, secretion, and scale-up must be addressed so that PF can emerge as a scalable, sustainable solution to meet global demand for animal-free functional egg proteins.

**Milk caseins production through precision fermentation in *E. coli***

Ainoa Escrich

AINIA, Spain

Caseins are the major milk proteins responsible for the nutritional and functional properties of dairy products. Their recombinant production remains challenging due to their intrinsic disorder, aggregation tendencies, and complex post-translational modifications such as phosphorylation. In this work, we present a tag-free recombinant expression system in *Escherichia coli* for the production of four major bovine caseins: $\alpha_{S1}$-, $\alpha_{S2}$-, β-, and κ-casein. The proteins were successfully expressed and purified without the use of affinity tags, simplifying downstream processing and preserving native-like properties.

To replicate natural phosphorylation patterns, recombinant casein kinase was employed, enabling efficient in vitro phosphorylation. Comparative analyses with commercial caseins and kinases confirmed structural and functional similarity, including phosphorylation levels and solubility. Micellar structures are being assembled from phosphorylated recombinant caseins for use in plant-based cheese. Additionally, non-micellar recombinant caseins are under evaluation for direct incorporation into plant-based beverages, where micelle formation is not required.

This work highlights the potential of microbial precision fermentation to produce functional dairy proteins in a scalable, animal-free system. It supports the development of sustainable, high-quality food ingredients and contributes to global efforts toward more resilient and environmentally responsible food systems.

**Recombinant production of bovine caseins in *E. coli* and genome-reduced *Bacillus subtilis*: chassis optimization and production performance**

Elvio Henrique Benatto Perino

University of Hohenheim, Germany

The microbial production of milk proteins represents a sustainable alternative to animal-derived ingredients but is challenged by post-translational complexity and aggregation tendencies of caseins. We explored two complementary microbial systems for the recombinant expression of bovine caseins: genome-reduced *Bacillus subtilis* for αS1-casein and protease-deficient *E. coli* strains for β-casein. The expression of αS1-casein in *B. subtilis* IIG-Bs-20-5-1, a midi-genome strain with reduced proteolytic and metabolic side activities, enabled casein production at 1.6 mg/gCDW and titers of 56.9 mg/L in a defined fed-batch process. Notably, the casein localized in inclusion bodies, requiring pre-cleaning via urea solubilization and IMAC. Zeta potential and particle size analyses revealed pH-dependent aggregation distinct from the native reference, underlining the need for chassis-specific downstream optimization. In parallel, *E. coli* BL21-Gold (DE3) Δ*clpP*/Δ*clpQ* strain achieved soluble β-casein yields up to 5.6 mg/gCDW. A two-phase fed-batch strategy with temperature modulation improved productivity, while downstream recovery via high-pressure homogenization and IMAC yielded 5.94 g of high-purity β-casein. Together, these studies demonstrate how chassis engineering and cultivation strategy can mitigate stress responses and degradation, enabling efficient production of difficult-to-express milk proteins. Our work highlights the potential of bacterial hosts for precision fermentation in food biotechnology.

**Optimizing *Pichia pastoris* as a fermentation platform for alternative protein production**

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The increasing global demand for sustainable alternative proteins emphasizes the need for efficient microbial production platforms. *Pichia pastoris* is widely recognized for its ability to produce high cell densities and secrete recombinant proteins directly into the growth medium, simplifying downstream purification. In this study, three wild-type *P. pastoris* strains (X-33, NRRL Y-11430, and NRRL Y-7556) were screened for optimal growth and protein expression. Optimization included developing a defined growth medium, screening carbon sources, ploidy level, and fermentation conditions. High-cell-density fermentation was conducted in a 3.7 L bioreactor. Glycerol resulted in over 40% higher biomass concentrations than glucose, with Y-7556 reaching 244 g DCW/L in 48 hours- the highest biomass concentration reported for this yeast. The system was validated using mCherry and applied to produce recombinant canola napin (rNapin) and potato patatin. Expression of rNapin under the GAP promoter yielded 5.9 ± 0.1 g/L, 2.4-fold higher than AOX1, while diploid NRRL Y-11430 enhanced yield to 8.8 ± 0.1 g/L. rNapin exhibited strong solubility and emulsifying capacity under acidic conditions but lacked foaming ability. Patatin expression further confirmed the platform's robustness. This work demonstrates an efficient and scalable system for producing food-grade alternative proteins using *P. pastoris*.

**Alternative proteins – chances and challenges in microbial production**

Naumer Christian; Tiffert Yvonne; Domeyer Jan-Eike; Liebeton Klaus; Alexander Pelzer

BRAIN Biotech AG, Germany

Removing animals from the menu is recommendable for the health of both humans and the planet. Alternative proteins are what food technologists anticipate as how microorganisms shall take over a significant part of providing safe and healthy protein into the nutrition of meat and dairy loving consumers.

As many bulk plant proteins fall short of being able to replace animal proteins 1:1 in food for reasons like amino acid profile and functionality, an alternative would be for microorganisms to fill the gap. The use of microorganisms in a process called precision fermentation, which describes the recombinant production of food protein, provides animal proteins without consuming animals.

The economically feasible production of such proteins is a process that depends on various factors. From a technical point of view, the microbial production strain is one decisive factor. To reach high protein production yields, specific adaptations for each protein of interest are required. The construction of a production strain starts with selecting a capable microorganism, designing a gene expression cassette followed by expression, strain and fermentation optimization. To characterize development candidates and quantify protein production yields under relevant conditions, processes are set up in bioreactors prior to scale up.

This talk will showcase versatile microbial expression platforms, measures that have been taken to get into the area of commercially viable alternative protein production and other exciting possibilities that could arise in the field of precision fermentation.

**Single carbon substrates as sustainable feedstocks for protein production**

Diethard Mattanovich

BOKU University Vienna, Austria

Biotechnology offers ample solutions for carbon neutral production processes which have the potential to replace environmentally destructive manufacturing at large scale. However, most biotech processes depend on agricultural products as feedstocks, so scaling up is deemed to run into a limitation of available substrates, and to create a competition with food supply for human nutrition.

Single carbon feedstocks like methanol, formic acid or $CO_2$ can be produced from carbon containing process gases and even from $CO_2$ in the air, essentially basing carbon source production on $CO_2$ and electrical energy, instead on agricultural land use. Of these C1 substrates, methanol has been employed widely already for recombinant protein production by using methylotrophic yeasts (mainly *Komagataella phaffii* and *Ogataea polymorpha*). Methanol assimilation in yeasts, however, suffers from some energetic inefficiencies which reduce biomass and product yield markedly. We address these limitations by metabolic engineering, and the performance of rewired pathways will be discussed. Further, we have engineered the methanol assimilation cycle for direct $CO_2$ assimilation, creating a fully chemo-autrotrophic yeast strain that can grow and produce on $CO_2$ as its only carbon source. While formic acid has not been considered a substrate for yeasts, we have demonstrated recently that K. phaffii assimilates formic acid via the reductive glycine pathway, and we succeeded in debottlenecking the pathway by a single gene knockout to achieve growth.

**Recombinant production and characterization of endolysins targeting *Candidatus* Liberibacter**

Ibai Lenitz Etxaburu

AINIA, Spain

Endolysins are phage-derived enzymes that degrade bacterial peptidoglycans with high specificity, making them a sustainable alternative to conventional antimicrobials. Their targeted action minimizes disruption to the soil microbiome, and to date, no bacterial resistance has been reported. This project focuses on the identification, recombinant expression, and functional evaluation of endolysins targeting Candidatus Liberibacter (CL), a major citrus pathogen.

Bacteriophages will be isolated from infected plant material, and their genomes sequenced to identify endolysin-encoding genes. Selected genes will be optimized in silico and cloned into a production host for recombinant expression. The resulting proteins will be purified and assessed for antimicrobial activity and host specificity in vitro. Stabilization strategies, including microencapsulation, were explored to enhance formulation stability.

This approach integrates molecular biology, protein engineering, and formulation science to develop precise, scalable biocontrol agents. The project supports eco-friendly agriculture by providing a novel, resistance-free strategy for managing bacterial plant diseases.

**Antibiotic-free plasmid selection and maintenance via inducible complementation**

Katherine Brechun; Marion Förschle; Marlen Schmidt; Harald Kranz

Gen-H, Genetic Engineering Heidelberg GmbH, Germany

Antibiotic resistance genes used on plasmids for biomanufacturing raise safety concerns and are viewed critically by regulatory authorities. Eliminating antibiotics in biomanufacturing also has economic advantages; antibiotic supplementation is prohibitively expensive and adds production costs, including additional product purification and wastewater pre-treatment to prevent environmental contamination. Recently, we introduced a method for antibiotic-free plasmid selection and maintenance in *E. coli* that uses inducible complementation of an essential gene. An essential gene in the production strain is replaced with an inducible copy so that in the absence of the inducer, a plasmid expressing the essential gene is required for growth. The ability to induce the genomic copy of the essential gene enables antibiotic-free transformations, without needing cumbersome helper plasmids or repetitive strain modifications. Here, we show this strategy enables robust plasmid maintenance in a wide range of *E. coli* strains. We characterize growth rates and protein expression in the engineered strains, and find the modified strains performed equally well or better than their unmodified counterparts. In addition, we explore the flexibility of this strategy by using different inducible promoters and essential genes. Finally, we show antibiotic-free maintenance of two plasmids simultaneously using two essential genes. Overall, we demonstrate this strategy offers robust plasmid maintenance and high experimental flexibility, without requiring any antibiotic resistance genes.

**Synthetic biology and bioprocess engineering for scalable, cost-effective components in cultivated meat production**

Yoav Keshet, Neta Agmon

Alagene, Israel

Scaling the production of alternative proteins requires integrated bioprocess solutions to address challenges in microbial strain optimization, precision fermentation, and downstream purification.

Alagene is a biotechnology CRO specializing in synthetic biology and bioprocess design for microbial-based production systems. Our expertise spans microbial strain engineering, high-throughput screening and bioassay development, fermentation optimization, and downstream processing (DSP) development, enabling a robust, scalable, and cost-efficient biomanufacturing processes.

A practical demonstration of this expertise is Alagene's role in the Cultivated Meat Consortium, where we focused on producing recombinant growth factors, critical components of serum-free media, using microbial expression systems. The project combined iterative strain engineering, bioassay-guided selection, fermentation optimization, and DSP development to achieve over a 5-fold increase in growth factor expression and a projected 1,000-fold cost reduction, while maintaining biological activity and compliance with food-grade standards. This case study illustrates how integrated bioprocess design can overcome technical bottlenecks and significantly enhance the scalability and economic feasibility of alternative protein manufacturing.

**Soft-sensor–driven real-time physiological state estimation to facilitate repetitive fed-batch cultivations**

Julian Kopp; Rüdiger Lück; Oliver Spadiut

TU Wien/Institute of Chemical, Environmental and Bioscience Engineering, Austria

The microbial host *Escherichia coli* is widely used for commercial biotherapeutic production, yet recombinant proteins are still predominantly manufactured in fed-batch mode. Transitioning to continuous biomanufacturing with microbial systems remains uncommon, largely due to population heterogeneities causing time-dependent fluctuations in productivity. Repetitive fed-batch (RFB) processing offers a promising alternative by lowering the environmental footprint and potentially increasing the overall space–time yield. However, in RFB, the timing of intermediate harvests is critical to maintain cell viability across cycles while achieving product yields comparable to conventional fed-batch processes.

In our study, we employed a phosphate-limited auto-inducible *E. coli* W3110 strain to produce a recombinant fragment antigen-binding (Fab) domain. A mechanistic model, coupled with a Kalman filter, was developed to accurately predict the optimal harvest time – being crucial for this auto-inducible system. This approach allows for robust RFB cultivation by ensuring both high productivity and sustained cell performance across repeated cycles. I will present this novel strategy and demonstrate its superiority over state-of-the-art methods.

**Developing a recombinant barnacle cement protein as a biomimetic adhesive coating**

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INTRODUCTION: Marine organisms can adhere to a variety of surfaces in wet environments. Cell-Tak, derived from the mussel Mytilus edulis, is a commercial adhesive used in surface adherence of suspension cells but it is costly, environmentally damaging to harvest and purify, and unsuitable for in vivo applications. We propose that cp19k, a barnacle cement protein from Pollicipes pollicipes, is a promising alternative adhesive suitable for in vitro and/or in vivo use.

METHODOLOGY: cp19k was expressed in E. coli BL21 (DE3) and purified via metal affinity and ion exchange chromatography. Self-assembly of the protein into fibrils under varying pH and NaCl concentrations was analysed using Transmission Electron Microscopy (TEM). β-amyloid content of fibrils formed under optimal conditions was confirmed using a Thioflavin T assay. Adhesion experiments on hydrophilic and hydrophobic polystyrene surfaces were conducted with monomeric and fibrillar cp19k to assess their adhesion efficiencies. Cytocompatibility was evaluated using THP-1 monocytes and an MTS assay.

RESULTS: Optimised expression and purification yielded 2–2.5 mg/L of cp19k protein. TEM revealed fibrils in all conditions, with higher concentrations in low pH/high salt and high pH/low salt environments. Fibril morphology varied between conditions. Non-fibrillar cp19k adhered best in low pH/low salt, while fibrils remained adhesive when switched to high pH/high salt, mimicking natural secretion in the barnacle. THP-1 cells remained viable on cp19k coated surfaces after 72 hours.

**Scaling bioprocesses by AI generated 3D printed bioreactors mimicking large scale conditions**

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Bioprocess scale-up remains a critical bottleneck in translating laboratory fermentation to industrial production, largely due to scale-specific effects such as nutrient gradients and oxygen limitations. Conventional scale-up follows a stepwise approach, with discrepancies in yield, quality, and robustness often emerging only after substantial resource investment. We present a novel technology that integrates evolutionary AI design, CFD, and additive manufacturing to reproduce large-scale mixing dynamics in lab-scale systems. The workflow employs a generative evolutionary algorithm to design internal static structures within existing lab-scale vessels to change the mixing behavior of the laboratory system to match the production-scale conditions. The algorithm iteratively modifies reactor wall geometries, evaluates mixing characteristics automatically through CFD, and converges on designs matching the large-scale mixing curve. This method requires no extensive biological and host dependent training datasets as it mimics the physical conditions of large-scale production vessels in small benchtop bioreactors. We demonstrate modified small-scale bioreactors achieving long mixing times of up to >150 s for bioreactors for both mammalian and microbial processes. Large-scale effects were confirmed experimentally through delayed growth rates, reduced product titers, and elevated by-product formation despite identical peak cell densities. This approach enables cost-effective scale-down testing, allowing early detection of scale-up risks and robust upstream process design.

## Poster presentations

# Poster 1

**Continuous biomanufacturing for enzyme production with *Bacillus licheniformis*: the challenge of biomass segregation**

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Continuous biomanufacturing can offer superior productivity over more established bioprocess types like batch and fed batch: The process runs at optimum productivity for an extended period of time, thus improving the overall volumetric productivity. The use of equipment is thereby maximized and downtimes tremendously reduced. However, continuous biomanufacturing also comes with challenges: Production organisms are often under significant stress of high-level protein production which causes a genetic drift towards non-producing genotypes.

We applied continuous biomanufacturing to Bacillus licheniformis producing a protease. Productivity was maintained at a high level for several generations but dropped significantly with increasing cultivation time. Population analysis via Next Generation Sequencing (NGS) revealed the emergence of a subpopulation, that shared a distinct single nucleotide deletion in the promotor region of the protease gene. The segregation of biomass into these two populations correlated with the drop in productivity, as this subpopulation made up most of the cells with increasing cultivation time. We consistently observed the emergence of this subpopulation, but the speed of biomass segregation was dependent on the dilution rate and the cultivation time. Finally, we developed a mathematical model describing the biomass segregation and subsequent productivity loss to predict process conditions for maximum overall productivity, yielding superior process performance over the established fed-batch process despite the observed biomass segregation.

# Poster 2

**Peroxidase production in *Komagataella phaffii*: analysis of glycerol derepression, scale-up robustness and crude glycerol utilization**

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Polyester resins used in boat and wind turbine construction rely on cobalt-based catalysts, recently classified as carcinogenic. Heterologously produced peroxidases in *Komagataella phaffii* offer a sustainable and safer drop-in alternative. To enable industrial application, a scalable bioprocess must be developed and optimized.

At 1 L bioreactor scale, a benchmark process employing the $P_{DF}$ promoter under glycerol derepression was established. It included an initial batch phase followed by two fed-batch phases. Fourteen combinations of seven exponential and six constant feeding strategies were evaluated in biological duplicates. The highest peroxidase activity (4.4 U/mL), a 5-fold improvement over other strategies, was achieved with exponential feeding followed by constant feeding at 20 % of the maximum glycerol uptake rate.

Process robustness was tested in a scale-down plant with two stirred-tank compartments (STR-STR, volume ratio 80:20), simulating large-scale oxygen and substrate inhomogeneities. Under alternating substrate and oxygen availability in the range of 150 seconds, activity remained stable at 4.5 U/mL, confirming robustness and scale-up potential.

The utilization of crude glycerol - a biodiesel production waste stream containing 10 % sodium chloride, 4 % organic matter, and 0.5 % methanol - yielded 3.4 U/mL, a 15 % improvement over purified glycerol. Extending the peroxidase production phase further raised activity above 12 U/mL, highlighting the economic and sustainability potential of this process.

**(Re)Discovering *Pichia* – overcoming cellular limitations… and common prejudices**

Aid Atlić; Roland Weis

VALIDOGEN GmbH, Austria

Key bottlenecks in the Pichia pastoris expression system can be overcome utilizing VALIDOGEN's comprehensive UNLOCK PICHIA® toolbox, addressing challenges at every stage from translation and transcription to protein folding and secretion. In addition, contrary to the common belief that methanol is necessary for optimal performance VALIDOGEN's exclusive AOX1 promoter variants enable highly efficient methanol-free processes. Through innovative approaches production times can be significantly shortened, further enhancing process efficiency and maximizing productivity.

**A MAD7-based genome editing system for *Escherichia coli***

Mund Markus; Wadim Weber; Daniel Degreif; Christoph Schiklenk

Sanofi-Aventis Deutschland GmbH, Germany

A broad variety of biomolecules is industrially produced in bacteria and yeasts. These microbial expression hosts can be optimized through genetic engineering using CRISPR tools. Here, we designed and characterized such a modular genome editing system based on the Cas12a-like RNA-guided nuclease MAD7 in Escherichia coli. This system enables the efficient generation of single nucleotide polymorphisms (SNPs) or gene deletions and can directly be used with donor DNA from benchtop DNA assembly to increase throughput. We combined multiple edits to engineer an E. coli strain with reduced overflow metabolism and increased plasmid yield, highlighting the versatility and industrial applicability of this approach.

# Poster 5

**Recombinant expression of spider venom enzymes: pitfalls, progress and prospects**

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Spiders employ venoms to overpower prey that are primarily composed of neurotoxins, linear peptides and enzymes. The latter received little scientific attention thus far and despite their great translational potential, functional data on spider venom enzymes is scarce. Hence, a more comprehensive understanding is sought, providing insights into their biological role and facilitating the development of novel biotechnological applications. However, their chemical isolation is prevented by minuscle venom yields available from most spiders. Recombinant expression emerged as a promising tool to overcome these hurdle but little efforts were made to establish the technology for different enzyme families. Particularly few works explored the important technical aspects, including strain selection, culture conditions and product purification. Here we explore these aspects using multiple spider venom enzymes as a case study, with particular emphasis on an Astacin-like metalloprotease from *Loxosceles intermedia*. The enzymes were produced as fusion proteins utilizing diverse *E. coli* strains and pinpoint the most effective production strains and conditions. Thioredoxin A, a 6x-His-Tag and an factor Xa cleavage site allowed the efficient purification and we report in detail the purification of the mass spectrometry-confirmed metalloprotease from inclusion bodies. This exploratory study outlines the technical details and potential pitfalls encountered during the development of this production process and provides an important baseline for future attempts to express spider venom enzymes.

# Poster 6

**A recombinant production platform for M1 and other mycocins**

Tobi Schilling

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Mycocins are fungal proteinaceous killer toxins which specifically target and eliminate other fungi. Mycocins can be used as powerful and safe agents for granting food safety when applied as a natural food preservative, or in the clinical context for an alternative treatment of infections caused by multi-antibiotic-resistant fungi. M1 is a specific mycocin which is 88 amino acids in length and stabilized by 5 disulfide bonds. As opposed to most other known mycocins, M1 has shown a high potency on a wide spectrum of target hosts as well as stability function under remarkable physicochemical conditions. Due to their high potency, but concomitantly high demands for cellular resources for complex oxidative folding, mycocins are produced and secreted in only quite low titers by their natural producers.

This research project aims to build a commercially relevant yeast-based production platform for M1 and other mycocins. For that, the first step is to overcome general metabolic bottlenecks by eliminating unnecessary auxotrophies, and to increase secretion rates by targeted genomic deletions and insertions. Second, we are going to screen natural mycocin producers with a multi-omics approach to identify relevant cellular factors up-regulated during the mycocin production phase. The third step will encompass the recombinant integration of those identified cellular factors into our chassis system.

# Poster 7

**Developing of a modular workflow to engineer *Xanthobacter* SoF1 for recombinant food protein production from air and renewable energy**

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Hydrogen-oxidizing bacteria (HOB) are a diverse group of microorganisms capable of utilizing hydrogen gas ($H_2$) as an energy source while fixing carbon dioxide ($CO_2$) via the Calvin-Benson-Bassham (CBB) cycle. This unique metabolism makes HOB highly attractive for the sustainable bioproduction of food proteins from non-arable feedstocks helping alleviate the planetary crisis. Among these, Xanthobacter species, particularly Xanthobacter SoF1, has shown potential as a highly sustainable food source due to its efficient $CO_2$ fixation capabilities and nutritious biomass.

However, the absence of suitable genetic tools is currently limiting its industrial exploitation. To address this limitation, engineering workflow tailored to Xanthobacter SoF1 for recombinant protein production, with potential applicability across other Xanthobacter species. This workflow streamlines strain development and genetic manipulation, enhancing the applicability of SoF1 for sustainable bioproduction. As part of this workflow, we developed a Modular Cloning Kit (SoClo) and identified optimal growth conditions using phenotype microarrays to accelerate strain development in heterotrophic conditions. The workflow's effectiveness was validated using plate screenings, flow cytometry, and activity assays.

The successful development of this toolkit establishes a robust platform for genetic manipulation of Xanthobacter SoF1, advancing its potential for applications in sustainable bioproduction and $CO_2$ fixation.

# Poster 8

**Engineered yeast as a versatile cell factory for recombinant growth factors**

Xiang Li; Verena Siewers

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Growth factors (GFs) are signaling proteins that promote cell proliferation, wound healing, and differentiation. Over the past two decades, recombinant GFs (rGFs) have gained prominence in regenerative medicine, tissue engineering, cultivated meats, and cancer therapies. However, scalable, cost-effective production of bioactive rGFs remains a major challenge.

To address this, we are engineering yeast strains for high-yield secretion of rGFs. Starting with a strain previously evolved for enhanced protein secretion via random mutagenesis and microfluidic sorting, we used CRISPR-Cas9 to delete two vacuolar proteases, aiming to reduce degradation of recombinant proteins. We then integrated rGF genes with various signal peptides into plasmids to identify those that best promote secretion. Secreted protein levels were analyzed using Western blotting and ELISA. Our engineered strains successfully expressed and secreted multiple rGFs, including BMP-2, bFGF, EGF, and M-CSF into the culture supernatant. This work demonstrates the potential of yeast as a sustainable and versatile platform for efficient rGF production, paving the way for more accessible biopharmaceuticals, cellular agriculture, and industrial biotechnology.

# Poster 9

**_Kluyveromyces lactis_ as a host for commercial (recombinant) protein production**

Remon Boer; Ruqaya Darweesh; Diny van Helvert; Tim Weenink; Chang Liu; Vicky Sereti; Noel van Peij; Jan van Leeuwen

dsm-firmenich, the Netherlands

For more than three decades, dsm-firmenich has employed the non-conventional yeast *Kluyveromyces lactis* as a safe and robust microbial platform for the (heterologous) expression of recombinant proteins. This system underpins the commercial production of key food enzymes such as Maxilact® (β-galactosidase) and Maxiren® (chymosin), which are widely utilized in dairy processing. All of these production strains are derived from the same lineage, meaning they all originate from the same ancestor strain after classical mutagenesis and/or genetic engineering (v/d Dungen et al). Current strain engineering efforts are centered on enhancing the secretory capacity of *K. lactis* through the rational deployment of co-expression of protein-specific chaperones. By means of state-of-the-art protein modelling, protein-protein interactions between the heterologous protein expressed and (native) chaperones can be predicted in silico and proposed as relevant leads for wet-lab testing. Our latest innovation Maxiren®EVO has profited from this Design Build Test Learn-approach and expression levels could be boosted by overexpression of a *K.lactis* endogenous chaperone

Myrthe W. van den Dungen, Rémon Boer, Lonneke C. Wilms, Yulia Efimova, Hanna E. Abbas,

The safety of a *Kluyveromyces lactis* strain lineage for enzyme production,

Regulatory Toxicology and Pharmacology, Volume 126, 2021, 105027, ISSN 0273-2300,

https://doi.org/10.1016/j.yrtph.2021.105027.

# Poster 10

**The pivotal impact of dissolved oxygen on lactate accumulation in mammalian bioprocesses**

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Dissolved oxygen (DO) is a critical bioprocess parameter significantly influencing lactate metabolism in Chinese Hamster Ovary (CHO) cells. A robust switch from lactate production to consumption enhances metabolic efficiency, growth, and productivity, yet it remains not fully understood. Many early-stage projects suffer from metabolic imbalance, leading to lactate accumulation, decreased productivity, and occasional process dropouts. The desirable switch has been attributed to reaching peak cell density, glucose depletion, glutamine depletion, high extracellular lactate concentration, and changes in pH or temperature (Hartley et al., 2018).

This study aims to determine how DO levels and exposure times affect lactate metabolism and overall process performance. We identify input factor combinations and predictive readouts to foresee the fate of lactate metabolism early in the process. By analyzing stage-dependent DO effects in conjunction with other influential parameters, such as temperature, feed, and seeding density, in high-throughput ambr®250 systems, we pinpoint critical time frames and investigate the interplay between growth, metabolism, and productivity. The goal is to enhance our knowledge of CHO cell metabolic sensitivity to DO and establish development tools in line with cellular dynamics that make processes robust to lactate accumulation. To achieve this, we use a modelling approach that considers variable input factor settings at specific intervals to predict end-of-process outcomes and performance response curves for the entire bioprocess.

**The pET OPT collection of expression plasmids: Improved versions of old favourites**

Daniel Daley

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We have constructed the pETOPT collection by re-engineering the five most commonly used expression plasmids for recombinant protein production in Escherichia coli (pET28, pET22, pET15, pET30, pET32). Each pETOPT plasmid has been adapted for strains with either high or low levels of the T7 RNA polymerase by incorporating improved genetic modules for transcriptional initiation, transcriptional termination, translation Initiation and plasmid selection and maintenance. The plasmids are available with either the Tn903.1MIN(kana25) and Tn3.1MIN (amp20) antibiotic resistance markers, so that users have more flexibility. pETOPT plasmids contain the same basic architecture as original pET expression plasmids, but they support higher production titres and are maintained better over long inductions.

# Poster 12

**Design of a bacterial expression platform to produce an industrial enzyme for food applications**

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(2) CYGYC BIOCON, Spain

(3) IQS (Ramon Llull University), Spain

Enzymes play a key role in food processing by enabling selective biochemical transformations and supporting clean-label, sustainable formulations. However, production of certain enzymes is limited by low native expression levels and activation requirements. To overcome these bottlenecks, we developed a modular expression platform in *Bacillus subtilis*, a GRAS host with high secretion capacity and suitability for food applications.

Using Golden Gate method, we combinatorially assembled expression constructs from libraries of interchangeable parts, including **promoters** (constitutive, inducible, growth-phase dependent), **signal peptides** (Sec and Tat pathways), and **coding sequences** of target proteins. Constructs were then transformed into *B. subtilis* 168 and its protease-deficient derivative KO7-S.

To evaluate expression, the extracellular fraction of the culture was analysed. Initial testing with mCherry protein revealed effective secretion, with fluorescence and SDS-PAGE confirming extracellular presence. Promoter-Signal peptide combinations were then tested with the target enzyme, and extracellular activity was quantified. In strain 168, enzyme activation occurred via native extracellular proteases. Among all combinations, **Pgrac100-amyQ*** yielded the highest expression level and the construct was selected for subsequent scale-up and bioprocess optimization.

Overall, our results highlight the potential of a standardized modular expression platform for extracellular enzyme production in *B. subtilis*, offering a scalable strategy for the industrial production of low-yield proteins.

# Poster 13

**Uncovering guidelines for designing well-secretable proteins**

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Utilizing the secretory apparatus of yeasts is a popular approach for recombinant protein production. However, prediction of production levels is notoriously difficult. Even small changes in the amino acid sequence, such as those introduced through mutagenesis in protein engineering, can significantly impact protein stability and production yields.

Studies based on expression data of a variety of proteins have shown correlations of expression with sequence parameters like fraction of charged amino acids or associated biosynthetic cost. Looking more specifically into sequence variation, the comparison of protein mutants revealed that secretion level correlates with thermodynamic stability. However, studies on variants were often limited by the number of experimentally tested mutants to reveal broader principles.

For our current study, we generated a model protein library with fully randomized surface patches. Using yeast surface display and cell sorting, we simultaneously screened and sorted millions of protein variants by their expression level. Based on DNA and amino acid sequences in each expression category we calculated descriptors (e.g. composition, physiochemical parameters, distances between certain amino acids) and determined their importance for explaining expression differences via machine learning. With this naïve approach we can identify and rank both expected and surprising parameters contributing to the displayed protein level. Ultimately, this study aims to provide deeper insights into surface properties dictating protein expressibility and secretability.

**Engineering a methanol-free, inducible protein production system in *Pichia pastoris***

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myBIOS, Austria

Conventional methanol induction in *Pichia pastoris* (aka *Komagataella phaffii*) is a well-established method for driving recombinant protein production, yet it raises significant safety and toxicity concerns that limit its industrial applicability. myBIOS developed a novel methanol-free inducible system that employs a non-toxic inducer, eliminating the hazards associated with methanol. We engineered *Pichia pastoris* strains to express horseradish peroxidase (HRP) as a model protein, then conducted a comprehensive comparison between the traditional methanol-based method and the new methanol-free approach. Our evaluations focused on various key parameters, including cell growth dynamics, protein yield, metabolite production, and overall process robustness. Notably, the methanol-free system achieved protein expression levels that were competitive with, and in some cases surpassed, those obtained with methanol induction. These findings underscore the potential of adopting a safer, more scalable platform for industrial recombinant protein production, paving the way for broader applications in biotechnology.

**Fast & ferrous: optimising expression and scale-up of an oxygen-tolerant [FeFe]-hydrogenase**

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Biocatalysis offers a sustainable alternative to traditional metal-based catalysts for chemical transformations. Among biocatalysts, [NiFe]- and [FeFe]-hydrogenases are particularly promising due to their reversible hydrogen production and oxidation activity, enabling applications in chemical synthesis and biotechnology. While recombinant production of these hydrogenases typically requires multiple accessory proteins, [FeFe]-hydrogenases are generally produced more efficiently at scale due to their comparatively simpler requirements. The enzyme CbA5H, a [FeFe]-hydrogenase from *Clostridium beijerinckii*, stands out for its oxygen tolerance and catalytic robustness. Previously, we demonstrated its feasibility as an effective biocatalyst for hydrogenation processes of industrial relevance. We also achieved a >20-fold improvement in purified CbA5H yield from 10 L bioreactors using *Escherichia coli*, obtaining up to 29 mg/L of active enzyme (Cleary et al., 2024). This highlighted the strong scalability potential of CbA5H production in this host. To build on this progress, further optimisation is needed to achieve cost-effective production at larger scales (10–100 L). We are currently optimising recombinant production of CbA5H through alternative induction strategies and plasmid configurations, as well as fermentation refinement. Ultimately, this work aims to scale up CbA5H production to establish this enzyme as an industrially viable biocatalyst for sustainable chemical manufacturing and biotechnology.

S. E. Cleary, et al. (2024). *ChemCatChem*. DOI: 10.1002/cctc.202400193.

**Effect of induction temperature on endoxylanase production in a recombinant *Pichia pastoris* strain**

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*Pichia pastoris* (currently classified as *Komagataella phaffii*) is a widely used host system in biotechnology for the production of recombinant proteins, due to its ability to grow in simple media, reaching high cell densities, and metabolize methanol as sole carbon source. In this study, the KM71 strain was used to express the xylanase GtXyn10A from the fungus *Gloeophyllum trabeum*, an enzyme with potential applications in the production of xylooligosaccharides (XOS) of prebiotic interest.

The fermentation process was designed in four phases: batch phase (initial growth), growth phase (glycerol feeding), adaptation phase (glycerol–methanol feeding), and induction phase (methanol feeding). The objective was to evaluate the effect of induction temperature (20, 25, and 30 °C) on recombinant protein production and enzymatic activity. Preliminary assays were also conducted with variations in pH (5.0 and 5.5) and supplementation with PTM1 trace solution.

Results showed that, although methanol was completely consumed at 20 °C and 25 °C, xylanase expression was significantly higher at 30 °C, with a 2.3- and 2.2-fold increase compared to the other temperatures. This condition also led to methanol accumulation of up to 15 g/L.

In conclusion, induction at 30 °C enabled the highest xylanase production, while also offering operational advantages by reducing cooling requirements. This result offers a favorable balance between biological efficiency and industrial feasibility, supporting its potential for future scale-up in recombinant enzyme production.

**Model-based control of tangential flow filtration and multi column chromatography in a continuous bioprocess**

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Continuous bioprocessing represents a transformative approach in biomanufacturing, offering improved efficiency, product consistency, and cost-effectiveness. This study investigates the integration of model-based control for two critical unit operations - tangential flow filtration (TFF) and multi-column chromatography (MCC) - within a continuous microbial bioprocess. The whole process chain is orchestrated via the Qubicon software, which interfaces with all process equipment, including pumps, sensors, and balances. For TFF, our framework enables precise flux control in steady-state via a designated pump controller. Real-time anomaly detection triggers dynamic responses, such as cleaning-in-place (CIP) upon exceeding membrane pressure thresholds, while an automated bleed manages cell debris removal. This system was demonstrated stable during two weeks of autonomous operation. The permeate, containing the product of the bioprocess, flows directly into the MCC. In the MCC feed product concentration is monitored via automated HPLC. The MCC column switching times are determined and dynamically adjusted by Novasign hybrid models that integrate product concentration, cycle count, and conductivity data. Method selection is optimized via a lookup-table comparison, ensuring consistent purification performance. This model-based control approach was validated over 10 days of continuous operation. Our results highlight the robustness of automated, model-based control in continuous bioprocessing, enabling real-time decision-making, reduced downtime, and enhanced product quality.

**Recombinant production of interfering RNA in bacteria: a game changer in pest management**

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AINIA, Spain

RNA interference (RNAi) is a promising strategy for controlling agricultural pests and plant-parasitic organisms. It offers species-specific gene silencing with minimal environmental impact. In this study, we present a microbial platform for the recombinant production of double-stranded RNA (dsRNA) targeting genes essential for the survival and development of pests.

We have engineered DNA molecules that contain convergent promoters flanking pest-specific gene fragments, which enables the synthesis of complementary RNA strands. After testing its efficacy in the target organism, we designed expression vectors containing a single promoter to control the transcription of a palindromic target gene sequence divided by a non-specific sequence. This results in an RNA molecule with a hairpin-like structure (hpRNA), which enhances RNA stability. To increase hpRNA accumulation, we used RNase III-deficient bacterial strains and optimised the induction parameters. The hpRNA will be purified using a cost-effective protocol that avoids the use of organic solvents and enzymes, yielding high-purity RNA that is suitable for field application.

This study supports the development of microbial cell factories for the sustainable production of RNA-based biopesticides, contributing to the advancement of precision agriculture through environmentally friendly pest management solutions.

**Automated flow cytometry for high-throughput screening of rationally engineered strain libraries**

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Forschungszentrum Jülich GmbH, Germany

The “AutoBioTech” innovation lab is a versatile automation platform enabling the rational design and generation of engineered microbial strain libraries. Standardised modules facilitate automated plasmid assembly and transformations of prokaryotic model organisms in a reliable routine. However, the screening of generated libraries remains a bottleneck. We have addressed this by custom integration of a MACSQuant® X flow cytometer (Miltenyi Biotec) into the platform, enhancing its analytical capabilities. Flow cytometry allows monitoring of physical cell properties and fluorescence, giving insight into cell size and granularity, cell count, viability, and gene expression.

Using our standardised molecular cloning modules, we created a mini-library in *Escherichia coli*, demonstrating combinatorial variation of molecular parts, such as promotors, whereas different fluorescent protein represent the gene of interest. We screened the library at-line with our custom flow cytometry method, processing a 96 well plate in under 12 h and generating huge amount of high-resolution data that directly enter our bespoke data processing pipeline. The pipeline is based on purpose-built Python scripts that automatically sort, group, and analyse the gigabytes of data, apply machine-learning algorithms, and visualise the processed data for the researcher’s convenience and quick interpretation. Hereby, we have created a fast and reliable screening method that delivers valuable insight into morphology, viability, protein expression and aggregation patterns across the demonstrator library.

**CASPON®: A fusion-protein platform technology for efficient recombinant biopharmaceutical protein production**

Sophie Kutzner; Karin Koch; Martin Wagenknecht; Vignesh Rajamanickam; Daniel Elsner; Martin Voigtmann; Wolfgang Buchinger; Stefan Krahulec; Michael Graf; Daniel Fleischanderl; Matthias Berkemeyer; Cécile Brocard

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The CASPON® platform technology, a fusion-protein-based system developed for Escherichia coli, offers a powerful solution for the efficient production, purification and quantification of soluble biopharmaceuticals [1]. The system consists of an N-terminal fusion tag and an ultrafast and highly efficient circularly permuted caspase-2 protease. Both in combination enable enhanced protein solubility, protein purification and precise removal of the fusion tag to yield an authentic N-terminus of the protein of interest (PoI) [2].

The incorporation of an affinity tag facilitates cost-effective purification of the PoI and supports automated screening approaches for optimal purification conditions for downstream processing. Moreover, the platform allows for accurate, PoI-independent titer quantification using an automated immunoassay. Thus, the CASPON® platform technology supports high-throughput screening at small scale across upstream, downstream, and analytical workflows, while also enabling scalable production at industrial levels.

[1] Elsner et al.: Scar-free tag removal by CASPON® enzyme with broad physicochemical stability in biomanufacturing – A case study of five proteins. Separation And Purification Technology 2024, 130832.

[2] Lingg et al.: CASPON platform technology: Ultrafast circularly permuted caspase-2 cleaves tagged fusion proteins before all 20 natural amino acids at the N-terminus. New Biotechnology 2022, 71, 37–46.

**Production of enzymes of interest from a simple carbon source by a genetically modified strain of *Talaromyces versatilis***

Julien Lacombe

ADISSEO FRANCE SAS, France

Adisseo, one of the world leaders in nutritional solutions and additives for animal feed and health, has developed and is continuously optimizing the proprietary filamentous fungus *Talaromyces versatilis* to produce ROVABIO®, a range of enzymatic cocktails used as additives for feed digestibility.

This fungus is also being developed towards a recombinant proteins production platform.

A specific mutagenesis work led to the discovery of a mutation in xylose reductase gene resulting in a lowered glucose catabolite repression for the expression of glycoside hydrolases, especially hemicellulases.

This allowed us to use a set of hemicellulases promoters to overproduce proteins of interest in the presence of glucose.

More precisely, the expression system we have developed includes the combination of 3 genetic modifications: mutation of xylose reductase gene, copies addition of xlnR transcription factor, a positive regulator of (hemi)cellulolytic genes, and copies addition of the target protein-encoding gene under the control of a xlnr-regulated promoter.

This system has been successfully implemented to overproduce the arabinofuranosidase TvAbf51a with different promoters in a glucose medium.

Competing interests: Adisseo has submitted patent applications for this work (WO2023/217911 and WO2025/099395).

**PEGylation as a therapeutic protein aggregation prevention strategy**

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Protein aggregation presents a key challenge in therapeutic protein development due to its immunogenicity, toxicity, and the cost of aggregate removal. Continuous quality monitoring and optimisation are essential to ensure aggregate-free purity in drug formulations. This study investigates polyethylene glycol (PEG) modification (1.2 kDa PEG) to mitigate aggregation, focusing on monobody fragments (Mobs)—small antibody derived proteins with high-affinity binding to target antigens, including the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Despite their promise, Mobs remain underexplored in terms of structural modification and formulation design. To evaluate their stability and functional assay, both non-specific NHS-PEGylation and site-specific Maleimide-PEGylation (Mal-PEGylation) were applied and compared. A range of techniques was used, including CD, SDS-PAGE, Microfluidic CE, Qubit quantification, FITC fluorescence, and the Prometheus Panta system, which integrates nanoDSF, DLS, SLS, and Backreflection for real-time thermal and colloidal stability analysis. PEGylation preserved secondary structure and increased melting temperature. NHS-PEGylation showed greater stability but impaired RBD binding, while Mal PEGylation retained binding with moderate stabilisation. Aggregation assays confirmed NHS-PEG's superior suppression of stress-induced aggregation, at the cost of activity. This study highlights the stability–activity trade-off and shows Mal-PEGylation as a promising, function preserving protein modification tool for antiviral Mob stabilisation.

**Teaching synthetic biology for recombinant protein production: development of laboratory course for MSc students in industrial biotechnology**

Fiorella Masotti; Paola Branduardi; Vittorio Giorgio Senatore; Immacolata Serra; Valeria Mapelli

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Recombinant protein production is a crucial expertise for biotechnology students, regardless of the production scale the student and future professional biotechnologist will face. For this reason, we have developed a course for MSc students in Industrial Biotechnology blending frontal lectures and laboratory activities. Frontal lectures aim to give the theoretical understanding of synthetic biology tools; while practical sessions engage the students to generate a GRAS yeast secreting a recombinant protein.

Here, we present the vision, the structure, and the results of the course, wherein the students learn to use bioinformatics tools to design vectors and expression cassettes based on the Easy-MISE toolkit (Maestroni *et. al.*, 2023) for gene integration in *Saccharomyces cerevisiae* exploiting the CRISPR-Cas9 system. Thanks to the modularity of the toolkit, students test the effect of different promoters and of different genome integration *loci* on the expression kinetics of a secreted recombinant laccase from the white-rot fungus *Trametes polyzona* (Bucchieri *et al*., 2024). The performance of the different *S. cerevisiae* strains generated is tested in E-flasks and is scaled-up to 2 L controlled bioreactors, where the production process is followed over time monitoring the enzyme activity.

We believe that such a course is an essential experience for future industrial biotechnology professionals, providing an exhaustive overview of the entire design-build-test scheme and the basis for troubleshooting extracellular protein production processes in eukaryotic microorganisms.

**Rational *Komagataella phaffii* strain engineering for a high-secreting and stable expression platform**

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Although *Komagataella phaffii* is rare in nature, a few different natural isolates have been identified. In addition, a large number of derivatives of the NRRL Y-7556 type strain has been generated in different labs and many of those are used for different industrial applications. Several single nucleotide polymorphisms that enhance recombinant protein production have been identified by genetic comparison and quantitative trait loci analyses of different isolates and mutants of *Komagataella phaffii* and published in the past years. In addition, a possible effect by the presence of natural killer plasmids was discussed in literature. The aim of this study was to identify current limitations of the available platform strains and to engineer those strains for enhanced, robust and scalable recombinant production and high protein titers.

While neither *K. phaffii*'s natural killer plasmids nor previously published *RVB1*$^{K8E}$ or *RCR2*$^{D196E}$ mutations influenced the production of the tested reporters, the *IRA1*$^{N200D}$ mutation resulted in up to twofold increased activity levels of glucose oxidase and VHH-eGFP fusion protein in the supernatant, but did not affect eGFP or *Candida antarctica* lipase B production. This mutant, however, showcased severe flocculation and other physiological detriments.

**Multi-omics characterisation reveals temporal dynamics of cellular and metabolic behavior of a CHO-based mAb process**

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Achieving consistent product quality and accurately identifying critical process parameters (CPPs) remain significant challenges in biopharmaceutical process development. A major obstacle is the limited availability of detailed characterisation data and the difficulty in comparing cell lineages across different laboratories. In this study, we present the first comprehensive, multi-level characterisation of a publicly available Chinese hamster ovary (CHO) production cell line from the National Institute of Standards and Technology (NIST), establishing a benchmark for bioprocess comparability. Controlled bioprocesses were evaluated at constant 37 °C and with a temperature downshift to 32 °C on day 6 in a 0.7 L fed-batch system. High-frequency sampling monitored cell growth, nutrient consumption, metabolic by-products, nucleotide sugar donors, and product quality. Multi-omics characterization, including transcriptomic, (phospho)proteomic, and epigenomic analyses, was performed at 17 time points, supported by the R package SplineOmics. Results reveal detailed cellular and metabolic adaptations, including stationary phase heterogeneity, stress response activation, and cellular hypertrophy after temperature shifts. CQAs showed increasing free light chain and decreasing galactosylation post-shift, more so at constant temperature. Linking metabolic and regulatory changes to CQAs supports precise bioprocess modeling. The NISTCHO cell line and this dataset provide a valuable resource for optimizing bioprocesses and enhancing comparability across labs.

**Application of a fluorescent $H_2O_2$ biosensor to identify and mitigate intracellular redox stress**

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Yeasts are widely used organisms for commercial, heterologous protein production, especially Pichia pastoris (syn. Komagataella phaffii). This yeast is specifically known for its naturally high secretion efficiency, which is commonly engineered with countless approaches to further enhance its protein production capabilities. High production, however, comes with drawbacks for the cells, such as generation of H2O2 during oxidative protein folding. These might lead to reduced viability and cellular stress responses. Therefore, using in vivo biosensors to measure stresses becomes invaluable for choosing bioprocess and cell engineering targets, as dye-based detections are often unspecific and do not allow for real-time measurements.

In this work we tested the applicability of a genetically-encoded, fluorescent, H2O2-responsive biosensor, named HyPer7, which was recently adapted for use in P. pastoris. By cultivating cells in a microbioreactor (Biolector - M2P Labs), on-line monitoring of the biosensor oxidation/reduction status and therefore redox stress, was possible. Comparisons between wild type and recombinant protein production strains showed a clear difference in redox stress. Indeed, high producing strains also showed higher oxidation signals. Further engineering with redox stress abolishing strategies reduced the obtained oxidation signals again, pointing towards reduced cellular stress.

Therefore, our data shows the applicability of a redox biosensor to identify and mitigate intracellular redox stresses occurring during recombinant protein production.

**Secretory signals shape stability: protein degradation of a target protein in *K. phaffii***

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*Komagataella phaffii* (*K. phaffii*), a methylotrophic yeast, is widely used for the production of recombinant proteins. Its capacity to secrete proteins with the use of secretion signals into the culture medium makes it an attractive host, as it simplifies downstream processing and reduces overall production costs. Efficient secretion relies on signal peptides which are cleaved by peptidases upon translocation during the secretory pathway. Correct signal peptide processing is critical for the proper function of certain proteins, including lytic polysaccharide monooxygenases (LPMOs) and antibodies.

However, other endogenous proteases and peptidases present in *K. phaffii* can also negatively affect recombinant protein production by degrading the target protein or causing fragmentation, ultimately reducing yield. In this study, we demonstrate that signal peptides influence the susceptibility of a target protein to degradation by native *K. phaffii* proteases. Understanding the relationship provides a valuable insight into optimizing expression systems for enhanced protein stability and yield.

**Microbial chassis engineering for high-quality enzyme production**

Katharina Ebner; Veronika Schusterbauer; Moritz Bürgler; Anton Glieder

bisy GmbH, Austria

Enabling scalable, high-titer recombinant enzyme production is essential for applications in biocatalysis, pharmaceuticals, food processing, and environmental technologies. *Komagataella phaffii* (formerly *Pichia pastoris*) is a widely used yeast platform for recombinant protein production, particularly for high-yield secretory expression of industrial enzymes. In this study, we present the second-generation engineering of a *K. phaffii* chassis optimized for the high-level secretory production of unspecific peroxygenases (UPOs), a versatile class of heme-thiolate enzymes with significant biocatalytic potential. Through rational genome engineering and targeted knockout of an abundant, secreted host protein, we substantially reduced the complexity of the host secretome while maintaining high titers of the recombinant enzyme. This reduction in host cell protein contamination simplified downstream purification and improved the overall yield and purity of the target enzymes. The resulting strain provides a novel and efficient chassis for the recombinant production of UPOs and other heme proteins, offering a cleaner and more scalable expression system.

**CRISPR-based tools for rational Pichia pastoris strain engineering**

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*Pichia pastoris* (*Komagataella phaffii*) is a major eukaryotic host for recombinant protein production in academic research and the biopharmaceutical industry. Currently, the generation of *P. pastoris* production strains is based on random integration of expression cassettes for the protein of interest and – if necessary – auxiliary proteins. Multiple copies of the cassettes are often integrated, mostly as unstable direct repeats. This copy number variability as well as locus-specific effects result in considerable productivity differences between individual clones, demanding tedious and time-consuming screening efforts to identify the most productive strain.

To enable rational *P. pastoris* strain engineering, we developed CRISPR-based tools for targeted expression cassette integration. We evaluated how integration efficiency is influenced by the employed CRISPR nuclease, nuclease expression level, guide RNA expression cassette architecture, and genomic target site location. Furthermore, we established a landing pad strategy for the simultaneous expression cassette integration at multiple loci, facilitating the generation of strains with a defined cassette copy number in one transformation. We envision that our tools will streamline the precise engineering of rationally designed and genetically stable *P. pastoris* production strains. This will substantially reduce the need for extensive clone screening and accelerate the delivery of new innovative biopharmaceuticals to patients.

All authors are Sanofi employees and may hold shares and/or stock options in the company.

**Harnessing promoters from diverse yeast species to expand the synthetic biology toolbox of *Komagataella phaffii***

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Komagataella phaffii (formerly Pichia pastoris) is a well-known yeast species widely used for the production of recombinant proteins. Its advantages, such as the ability to grow to high cell densities, secretion of pure heterologous proteins, effective post-translational processing, and established genetic manipulation techniques make it an ideal host for industrial-scale bioproduction. However, to enable broader use of K. phaffii in more advanced synthetic biology applications, it is important to further expand the repertoire of available promoters.

Our research aims to address this need by looking for new functional promoters for K. phaffii in other yeast species. Several studies have already shown that even though orthologous regulatory sequences often don't share any sequence similarities, some of them can be functional across different species. Using comparative genomic techniques, we have identified a set of candidate promoter sequences from selected yeast species. These promoters were cloned upstream of a reporter gene using Golden Gate cloning, and K. phaffii strains transformed with the constructed plasmids were tested for their ability to express the reporter under a variety of different conditions.

By introducing novel promoters, we expand the synthetic biology toolbox for K. phaffii, unlocking new opportunities for more efficient and innovative applications of this already powerful production host.

**Optimizing trace elements for improved protein production in *Komagataella phaffii***

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Komagataella phaffii (formerly Pichia pastoris) is widely used as a platform organism to produce recombinant proteins. Its robustness, ability to grow to high cell densities in simple mineral media, as well as the ability to secrete heterologous proteins to the culture supernatant have made it a preferred host in industrial biotechnology. The recognition of its benefits to the industry fuelled extensive research that focused on optimizing various aspects of its use, including strain engineering, process development, and media formulation. A critical factor in successful bioprocesses is the use of a well-balanced mineral medium, including adequate trace element supplementation. The commonly used Pichia Trace Minerals solution (PTM0) provides essential metals required for cell growth and protein production.

Aiming at formulating a new, more minimal PTM0 solution for optimizing protein production, we altered the concentrations of different metal salts during batch and feed phases in fed-batch cultivations of a CBS7435 production strain background.

In this study, we demonstrate that reducing the iron concentration in the trace element solution leads to a significant increase in antibody fragment titers in the supernatant. This improvement suggests that excess iron may negatively impact protein production, possibly through cytotoxicity effects and/or metabolic imbalances stressful for the cell. Our findings underline the importance of critically assessing even well-established media components for media optimization in K. phaffii-based expression systems.

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**Comparative characterization of α-1,2-fucosyltransferases for improved *de novo* biosynthesis of 2'-fucosyllactose in *Escherichia coli***

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Human milk oligosaccharides (HMOs) are structurally diverse, unconjugated glycans naturally found in human breast milk[1]. The trisaccharide 2'-fucosyllactose (2'-FL) represents the most abundant HMO. Supplementation of infant formula with 2'-FL supports immune system development similar to breastfeeding[2]. Its addition to infant formula is approved by both the Food and Drug Administration (FDA) and European Food Safety Authority (EFSA)[3].

In the *de novo* biosynthesis of 2'-FL using *Escherichia coli*, low α-1,2-fucosyltransferase activity is hypothesized to be a key rate-limiting step[4]. The challenges here are both the poor soluble expression of the enzymes known to date and their low specific activity. Starting with the known cyanobacterial α-1,2-fucosyltransferase from *Thermosynechococcus vestitus* (WP_011056838.1), its soluble expression in *E. coli* NiCo21 (DE3) was successfully improved by optimisation using the PROSS algorithm[5]. In a next step, this enzyme was compared with other α-1,2-fucosyltransferases identified in a taxonomy-based screening in the phylum of cyanobacteria or described in the literature. All variants were assessed for their soluble expression and catalytic activity with lactose as the acceptor substrate.

The current results provide a basis for the development of improved strains for the microbial 2'-FL production.

References

1. doi: 10.1093/glycob/cws074

2. doi: 10.3945/jn.116.236919

3. doi: 10.3390/nu10091161

4. doi: 10.1002/jsfa.12224

5. doi: 10.1093/bioinformatics/btaa1071.

**RapidFire-enabled screening of *K. phaffii* expression strains**

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Komagataella phaffii is a powerful host for the heterologous production of proteins with a wide range of applications, from low-price food and feed proteins to highly specialized biocatalysts and biopharmaceuticals. In the food and feed sector, protein engineering is often limited by regulatory constraints requiring unaltered amino acid sequences. As a result, the focus shifts to specialized host strains tailored to the specific needs of each protein.

The Agilent RapidFire system enables automated sample cleanup via solid-phase extraction (SPE) followed by direct injection into an ESI mass spectrometer, with analysis cycle times of 10–15 seconds per sample. Enzyme expression can be quantified indirectly by measuring product formation from enzymatic conversions. In the absence of enzymatic activity, expression levels can instead be assessed by quantifying selected peptides that are specific to the heterologous protein.

As a proof of concept, we used a protein-specific RapidFire method for fast screening of more than 5000 K. phaffii transformants and identified a clone producing 50% more human serum albumin (HSA). Such results would have been difficult to achieve using traditional screening methods. This simple screening technology now also allows testing combinations of different hosts and different genetic expression cassettes for the identification of biocatalysts with new activities and of strains for optimized expression of food and feed proteins where no efficient enzymatic high throughput screening methods exist.

**HYDROCOW: engineering hydrogen oxidizing *Xanthobacter* Sof1 for the net-zero secretion of milk protein**

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Milk protein plays an important role in nutrition. However, conventional milk production has a significant environmental impact ranging from greenhouse gas emissions to extensive land use. To mitigate this, we aim to establish Xanthobacter Sof1 as a microbial protein secretion platform that converts CO2 into milk protein. X. SoF1 uses the energy from H2 to fixate CO2, avoiding competition with agricultural carbon feedstocks. Notably, this organism can already be cultivated at commercial scale and high density under green H2 conditions.

Currently, there are no published studies investigating protein secretion in X. SoF1, and no established strategy exists for expressing and exporting dairy proteins in this host. To fill this gap, we are developing three complementary strategies to enable heterologous protein secretion. First, we integrate supernatant proteomics with genome annotation to identify native secretion pathways. Second, we use both heterologous and native signal peptides to target proteins to the highly conserved Sec and Tat pathway. Third, we express heterologous secretion systems from other Gram negatives to facilitate protein export.

Cellulase serves as a model protein to facilitate high-throughput screening of secretion efficiency. Preliminary results show cellulase secretion mediated by heterologous signal peptides. Secreting strains are first identified under heterotrophic growth and then validated under H2 fermentation at SolarFoods.

**Turning green when things go wrong: a biosensor toolbox for monitoring recombinant protein production in *E. coli***

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Cellular stress is caused by OFF-pathway events and a key factor influencing the yield and quality of recombinant proteins. Too much stress can lead to reduced growth, protein degradation or even cell death, making it imperative to reduce or avoid stress during recombinant protein production. To address this, we developed a biosensor toolbox designed to detect OFF-pathway events during recombinant protein production in Escherichia coli. This toolbox includes biosensors that monitor protein aggregation in the cytoplasm and periplasm, as well as bottlenecks in the Sec translocon. Each biosensor expresses a fast-folding and fast-degrading variant of green fluorescent protein (GFP), allowing for a simple real-time detection by measuring whole-cell fluorescence. We validated the biosensors in this toolbox systematically using model proteins known to either aggregate or fold properly, with additional testing of Sec and periplasmic sensors using proteins with or without Sec-dependent targeting sequences. All biosensors showed specific activation in response to their intended stress condition and remained inactive otherwise which was also confirmed by SDS-PAGE and Western blot analysis. Our toolbox offers a simple and user-friendly method for identifying cellular stress during recombinant protein production. The insights this toolbox provides can be used during process optimization and research into protein folding, cellular stress and quality control, making it a valuable tool for both industrial applications and basic research.

**Empowering bioprocess development: scaling-up expression of unspecific peroxygenases with a parallel mini bioreactor system**

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Efforts to scale up recombinant protein production (RPP) are critical for advancing in bioprocess development for industrial use. In recent years, attention has been focused on the production of unspecific peroxygenases (UPOs). UPOs, as heme-thiolate enzymes, exhibit remarkable oxidative capabilities, rendering them invaluable in various industries, including pharmaceuticals, bioremediation and fine chemicals synthesis. A set of proprietary *Komagataella phaffii* UPO expression strains was validated in its RPP performance in a parallel-mini-bioreactor system and compared to shake flask cultures.

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**Towards recombinant production of the bacteriocin enterocin L50 with *Corynebacterium glutamicum***

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Due to emerging antibiotic resistant pathogens alternative antimicrobial strategies are urgently needed. Bacteriocins are antimicrobial peptides produced by bacteria that may help to address this need. Enterocin L50 (EntL50) is a leaderless two-peptide bacteriocin that is naturally produced by *Enterococcus faecium* L50 (Cintas *et al*., 1995) and shows antimicrobial activity against a wide variety of Gram-positive bacteria including human pathogens (Cintas *et al*., 1998). The natural producer *E. faecium* is classified as a human pathogen causing urinary tract infections (Treitman *et al.*, 2005) and is thus not suitable as a host for the biotechnological production of EntL50. *Corynebacterium glutamicum* is a Gram-positive soil bacterium that is widely used in biotechnology and has been previously established as host for recombinant bacteriocin productions (Goldbeck *et al.*, 2021; Desiderato *et al*., 2024). Here, we report on efforts to establish recombinant production of EntL50 using *C. glutamicum*. A first *C. glutamicum* producer of EntL50 was established by expression of the structural genes *entL50A* and *entL50B* (Cintas *et al*., 1998) for the two peptides of EntL50 using the pXMJ19 platform. Antimicrobial activity against *Lactococcus lactis* was observed in exponential phase mainly in the cytosolic fraction suggesting that EntL50 is produced but not secreted by the recombinant *C. glutamicum* producer. To achieve secretory production, further strains were generated by cloning various secretion signals upstream of the EntL50 structural genes and activity in supernatants was tested.

**Effects of pH based extraction on melanin structure and property**

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Industries like textile and paints involve use of synthetic dyes and pigments generating non-biodegradable and carcinogenic by-product wastes released into the environment. To address this, natural pigments are being explored that are cost effective and involve sustainable manufacturing. One such biopolymer, melanin, is nature's own pigment found across all kingdoms of life attributing to the diverse colour patterns in birds to UV protection in mammalian skin. Commercially, melanin is extracted from *Sepia officianalis* by sacrificing the animal leading to low yield. Microbes are hereto considered more viable option with minimal invasive environmental impact. Melanin separation from microbial culture broth involves pH-based extraction. But the effects of pH on melanin structure, composition, and properties is not well understood. In this study, tyrosinase from *Bacillus* was recombinantly expressed in *E.coli* for biocatalytic synthesis of bacterial dopa melanin. We utilized characterization techniques like FTIR, EPR, UV absorption and antioxidant assay to establish if structure and biological property of bacterial dopa melanin gets affected by pH used for extraction. FTIR suggests changes in functional group composition at different pH used for extraction. While EPR is greatly influenced by pH, showing disappearance of free radical species of melanin at high pH. This study forms the basis to understand the effects of pH on melanin synthesis and extraction that will need further analysis of process intensification and validation to meet industrial demand.

**A cyclic multiplex co-transformation approach to customize *V. natriegens* for protein production**

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*V. natriegens*' fast growth and high carbon uptake rates render this bacterium a promising host for industrial applications. It is an emerging chassis with a versatile metabolism and a broad range of sophisticated genetic engineering tools being established in recent years. Several studies utilized this host for protein production, however, some with moderate success which is attributed to an incomplete understanding of the overall protein turnover in *V. natriegens*.

Comparing different fluorophores and media compositions, we found that *V. natriegens* can efficiently degrade target proteins at distinct stages of the growth phase. This indicates that native proteases might play a crucial role in the life cycle of those proteins and shows our incomplete understanding of the overall protein turnover in *V. natriegens*. We mapped over 100 putative proteases and peptidases on the two chromosomes and designed a library of linear DNA to delete those proteases using natural transformation. In a cyclic approach of co-transforming the library of linear DNA fragments we can enrich beneficial protease deletions and receive strains with a random combination of deleted proteases.

Additional fluorescence selection markers are used to validate a positive impact on heterologous protein expression. Promising candidates are pooled by single cell screening and sorting for the upcoming cycle. Applying this approach, we aim to identify relevant proteases to eventually improve protein production and to gain a better understanding of the degradation machinery in *V. natriegens*.

**Establishing recombinant production of an antiviral Kunitz domain with *Corynebacterium glutamicum***

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Biotechnological production of therapeutic proteins and peptides often requires sophisticated processes including fermentation of recombinant expression hosts and complex downstream processing.

Kunitz domains are characterized by an a/b-fold with three disulphide bonds involving six cysteine residues. Trypstatin is a fragment encompassing the second of two Kunitz domains of the serine protease inhibitor bikunin and has recently been described as a potent inhibitor of transmembrane protease serine 2 (TMPRSS2), a protease expressed in human epithelia cells. In the lung, TMPRSS2 plays a critical role in entry of coronaviruses including SARS-CoV-2 by cleaving the spike (S) protein to facilitate fusion of the viral and host cell membranes. Thus, trypstatin is an interesting candidate for clinical application and its recombinant production may be of economic interest.

Here, we report on establishment of a first Corynebacterium glutamicum strain for production of trypstatin. C. glutamicum was was previously reported to secrete active, disulfide bond-containing proteins/peptides suggesting correct conformation. Moreover, its lack of endotoxins is an advantage for future clinical application of trypstatin. To facilitate secretory production of trypstatin a library of secretion signals was screened. Strains showing release of trypstatin into the supernatant were further characterized by fed batch fermentations. Successful production of trypstatin was verified by mass spectrometry and activity of the product was confirmed by its ability to prevent infection using in vitro models.

**A novel, high-performance pyruvate decarboxylase from *Aspergillus niger* for sustainable biomanufacturing of (R)-phenylacetylcarbinol**

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The growing emphasis on sustainable biomanufacturing necessitates the identification and engineering of novel enzymes to improve these processes. (R)-phenylacetylcarbinol (PAC) is a chiral pharmaceutical intermediate used in the synthesis of ephedrine and related drugs. (R)-PAC can be synthesized chemically from cyanohydrins or enzymatically using pyruvate decarboxylase (PDC). The enzymatic route involves pyruvate decarboxylation and its subsequent carboligation with benzaldehyde to form (R)-PAC. The enzymatic route, despite being eco-friendly, is limited by low activity and reduced benzaldehyde tolerance of PDC. Thus, the identification and engineering of novel PDCs has gained attention. We hypothesized that fungal PDCs may outperform yeast and bacterial PDCs due to the adaptability of fungi to various stress conditions. Therefore, in this study, we focussed on the expression and kinetic characterization of a novel PDC from *A. niger* (AnPDC) in *E. coli* for improved (R)-PAC production. The purified AnPDC exhibited higher carboligation activity (6.67 µmole/min/mg) compared to decarboxylation (3.05 µmole/min/mg), which is highly preferred for (R)-PAC synthesis. The observed Hill coefficient of 1.8 suggests positive cooperativity for pyruvate binding, with an apparent $K_m$ of 29.5 mM. Additionally, AnPDC's high activity over a wide range of pH (4 to 7) and temperature (25°C to 45°C) and impressive benzaldehyde tolerance (>100 mM) renders it suitable for industrial operations. Thus, this study presents a novel PDC from *A. niger* as an efficient biocatalyst for (R)-PAC synthesis.

**Transcription factors as tools to boost recombinant protein production by *Komagataella phaffii***

Tanja Schleichert

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Transcription factors (TFs) are central regulators of gene expression, offering a precise and versatile means to control cellular function in microbial systems like *Komagataella phaffii* (previously *Pichia pastoris*), a key organism for recombinant protein production and synthetic biology. TFs represent powerful tools for rewiring regulatory networks to optimize recombinant protein production and to enhance protein yield. Advances in -omics technologies and genome editing have revealed previously unknown TFs and their targets, enabling the design of synthetic TFs and regulatory circuits. Overexpression of native or engineered TFs has emerged as a key strategy to enhance target gene expression by activating or fine-tuning relevant endogenous or synthetic promoters. This approach can significantly boost expression levels while reducing or even eliminating reliance on methanol, a common inducer with safety and regulatory drawbacks. Moreover, screening of libraries overexpressing TFs enable the identification of strains with an optimal balance of TF/target gene expression ("jackpot" clones) in addition to the employment of preferred promoters for TF overexpression.

**Genome engineered variants of *Komagataella phaffii* to increase titres of secreted protein products**

David Keßler

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The non-conventional, methylotrophic yeast Komagataella phaffii is an established host for the production of recombinant protein. However, yields are still highly dependent on the individual strain as well as the protein of interest. Both, published scientific literature, as well as, in-house data strongly suggest, that protein production titres may be significantly enhanced by genome mutagenesis. Potentially interesting specific mutations have been identified in published studies, and also in inhouse studies of the past years. To test this hypothesis with recombinant strains, each producing a different secreted protein, several mutations were introduced into the genomes of K. phaffii mutS strains that secreted either Candida antarctica lipase A (CalA), Thermomyces lanuginosus lipase (LipT), Aspergillus niger glucose oxidase (GOX1) and an anti-IL 6 receptor VHH nanobody as reporter proteins. Titres of secreted proteins were compared under different production conditions and cultivation scales ranging from 96 DWP to lab scale bioreactor. Overall, the effect of the mutations on the strain productivities varied drastically among the production scales and were highly dependent on the reporter protein.

**An open-source, genome-engineered *E. coli* chassis for inducible recombinant protein overexpression**

Edjohn Aaron Macauyag (1); Jenny Molloy (2)

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Transitioning microbial strains from research to industrial use often involves high costs and complicated licensing issues. While BL21(DE3) derivatives and the pET expression system are industry standards for *E. coli* protein production, their expired patents still create licensing barriers, forcing companies to deal with intellectual property restrictions. To address this, we developed OBT7 - an IP-free *E. coli* expression strain based on K-12 MG1655, obtained from the NRRL. Using lambda red recombination, we inserted the T7 RNA polymerase gene from the Reclone DNA collections into the genome. Our results show that the engineered OBT7 strain produces 3 to 4 times higher levels of mCherry fusion proteins at 12 hours post-IPTG induction compared to the commercial BL21 strain, highlighting its potential as a cost-effective, licensing-free alternative for recombinant protein production. Variants of the strain with knockdowns and knockouts of Lon and Ompt proteases, as well as knock-ins of Sgrs, were also tested and provided new insights into the effects of timing and presence of protease expression during recombinant protein overproduction in *E. coli*.

**Leaky but efficient - comparative extracellular production of a polyethylene terephthalate degrading cutinase in *Escherichia coli* and *Corynebacterium glutamicum***

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Biocatalytic recycling of polyethylene terephthalate (PET) is a promising sustainable alternative to chemical and thermomechanical processes [1]. However, its industrial implementation depends on the cost-effective biocatalyst production. Therefore, the extracellular production of a thermostable cutinase $ICCG_{DAQI}$ was compared between *Corynebacterium glutamicum*, using a signal peptide-based secretion system [2], and *Escherichia coli* without targeted secretion. In litre-scale batch and fed-batch cultivations, *E. coli* exhibited a substantially higher extracellular cutinase release, likely due to membrane-destabilizing effects of the cutinase, as supported by membrane integrity assays [3]. Under optimised fed-batch conditions at 30 °C with lactose-based autoinduction, cultivation of *E. coli* achieved up to 660 mg $L^{-1}$ extracellular cutinase and 137 U $mL^{-1}$ esterase activity [3]. Crude *E. coli* supernatants enabled the efficient hydrolysis of PET fibres (10 g $L^{-1}$) within 18 h at 70 °C and pH 9, with terephthalic acid yields of up to 97.8%, without the need for chromatographic protein purification [3]. These results highlight the potential of extracellular cutinase release using *E. coli* as a biocatalyst production platform to reduce downstream processing costs and facilitate scalable biocatalytic plastic recycling.

[1] Kawai F. (2021). Catalysts 11:206. https://doi.org/10.3390/catal11020206

[2] Hemmerich J. *et al*. (2019). Biotechnol Bioeng 116:644–655. https://doi.org/10.1002/bit.26873

[3] Fritzsche S. *et al*. (2024). Microb Cell Fact 23:274. https://doi.org/10.1186/s12934-024-02547-2

**Amino acid pathway engineering influences heterologous protein yields in genome-reduced *Bacillus subtilis***

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*Bacillus subtilis* is a non-pathogenic Gram-positive bacterium with strong protein secretion capacity, making it a valuable expression platform for recombinant proteins. Nonetheless, producing heterologous proteins in *B. subtilis* can be challenging, with many proteins of industrial or therapeutic remaining difficult to express in conventional strains. To overcome unwanted or energy-wasting traits such as sporulation, the application of genome-reduced strains like the IIG-Bs27-39 strain has been explored. This strain lacks key genes for proteolysis, sporulation and germination, displays stable growth in M9 minimal medium, and offers an ideal chassis for metabolic rewiring. The present study is focused on the amino acid metabolism pathways, especially for arginine and glutamate metabolism, which may influence protein production. To explore this idea, we deleted the *rocDEF-rocR* and *rocG* genes to modulate arginine catabolism and glutamate biosynthesis, respectively. Results will be presented on the effects of these mutations on the secretion of various heterologous proteins in the parental *B. subtilis* 168 strain and the genome-reduced strain IIG-Bs27-39. These results show that metabolic fine-tuning in genome-reduced *B. subtilis* can further enhance its ability to produce challenging proteins at increased yields.

**Optimization of fed-batch production of recombinant BsPdaC-CD in *Escherichia coli* using a Taguchi L9 experimental design**

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The production of sequence-defined partially acetylated chitooligosaccharides (paCOS) is a promising strategy for the valorisation of chitin-rich waste into high-value bioactive compounds [1]. The catalytic domain of a deacetylase from Bacillus subtilis (BsPdaC-CD), known to act not only on N-acetylmuramic acid (MurNAc) residues of peptidoglycan but also on N-acetylglucosamine (GlcNAc) units of chitooligosaccharides, was selected as a strong candidate enzyme for paCOS production [2].

In this work, a fed-batch process to produce the recombinant protein BsPdaC-CD in Escherichia coli BL21 was developed and optimized using a Taguchi L9 ($3^4$) design of experiments [3]. Across nine experimental runs, variability in biomass concentration was observed, primarily linked to differences in biomass yield efficiency rather than substrate availability, underscoring the complex interplay between operational conditions and cellular metabolism. Analysis of volumetric BsPdaC-CD production and space-time yield (STY) highlighted significant variability, with temperature and feeding flux rate emerging as the most influential factors. Lower induction temperatures effectively reduced inclusion body formation but did not result in higher overall BsPdaC-CD production, challenging assumptions drawn from small-scale shake flask experiments.

Overall, this study demonstrates the utility of the Taguchi L9 methodology in systematically identifying critical process factors and lays the groundwork for further optimization and industrial application of recombinant protein production processes.

**Scaling of a fed-batch process for efficient heterologous production of active recombinant [NiFe]-hydrogenase**

Francisco De La Fuente; Qin Fan; Oliver Lenz; Peter Neubauer; Matthias Gimpel

TU Berlin, Germany

Hydrogenases are key enzymes for utilizing molecular hydrogen as an alternative energy source and hold significant promise as biocatalysts due to their unique redox properties. However, their industrial-scale production is limited by low yields in native hosts, high costs of homologous expression systems, and challenges related to their complex structure, maturation process, and sensitivity to gases like $O_2$ and CO. The regulatory hydrogenase (RH), one of the four $O_2$-tolerant [NiFe]-hydrogenases (MBH, SH, AH, RH), from *Cupriavidus necator* H16, has been selected as model for heterologous production in E. coli. Previous studies using the fed-batch like EnPresso B medium achieved over 100-fold higher yields of active RH in shake flask cultivations, and lactose-based autoinduction further boosted RH production. The process was successfully transferred to a glucose limited fed-batch process in a 2 L stirred tank bioreactor, which resulted in significant higher RH yields, however, with lower specific yields and reduced RH solubility.

In this study, we focused on improvement of the fed-batch production aiming at an increase in RH activity and solubility. We applied the 2mag mini-bioreactor system to scale-down the process to screen relevant cultivation parameters (e.g. inducer concentration, metal ion supplementation, etc.). Based on the results we improved the hydrogenase production resulting in higher soluble RH yields and activity. The enhanced process was scaled up again to confirm the scale-down results, showcasing the reproducibility and robustness of the developed method.

**Impact of feeding strategies on recombinant xylanase production in *Pichia pastoris***

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The effect of different feeding strategies on recombinant xylanase production in Pichia pastoris was evaluated. The fermentation process was divided into four stages: a batch phase for cell growth, a glycerol feeding phase for biomass accumulation, a transition phase with glycerol and methanol, and a final induction phase with methanol to trigger recombinant protein expression. Four feeding strategies were tested: linear glycerol with linear methanol feed (LG-LM), linear glycerol with constant methanol feed (LG-CM), exponential glycerol with linear methanol feed (EG-LM), and exponential glycerol with constant methanol feed (EG-CM).

Exponential glycerol feeding led to a biomass increase of two-fold, however this did not relate to higher protein production. This may be due to stress caused by the high cell density, a high amount of methanol was accumulated, probably affecting recombinant protein production. The highest enzyme total activity was achieved using the LG-LM strategy, where 8.6x10^6 UI were obtained in the bioreactor, a 130% increase as compared to the EG-LM strategy. After about 80h, methanol started to accumulate in the LG-LM strategy, then the methanol feeding strategy could be further optimized, to maximize protein production and decrease methanol usage.

In conclusion, modifying the feeding strategy significantly improved xylanase production, with LG-LM showing the greatest potential. Future work will include the modification of the methanol feeding rate to decrease methanol accumulation, with the goal of reducing process costs for further industrial scale-up.

**Understanding transcriptional variance in CHO cells: a quantitative meta-analysis**

Markus Riedl; Nicolas Marx; Nicole Borth

BOKU University, Austria

A deep understanding of Chinese Hamster Ovary (CHO) cells' molecular complexity is key to improving growth, productivity, and product quality. While omics technologies and transcriptome analyses have advanced insights into gene expression, they often miss broader transcriptomic patterns. Notably, gene expression variability across diverse CHO lines and culture conditions remains unexplored. Although CHO cells largely express the same genes, differences in expression levels can significantly affect key traits. This study examines gene expression variance across various CHO lines to identify both stable and variable transcriptome elements linked to important cellular phenotypes.

We analysed RNA-sequencing data from 17 studies spanning diverse biological conditions and experimental setups. Raw reads were processed via a scalable workflow, including normalization and data transformation. Gene expression variance was quantified, ranked, and correlated to identify global patterns of transcriptomic variability.

Our analysis showed low correlations of variance rankings across datasets, suggesting that CHO cells' genetic instability extends to the transcriptome. Housekeeping genes showed stable expression, linked to core processes like transcription and RNA biosynthesis, while highly variable genes related to signalling and extracellular matrix components. Ongoing work explores links between transcriptional variance, gene regulatory signatures, and industry-relevant cellular traits.

**ECOnti - advancing integrated continuous manufacturing with microbial cells**

Florian Simon

enGenes Biotech GmbH, Austria

Continuous manufacturing with *Escherichia coli* (*E. coli*) is often limited by process instability and low productivity due to rapid growth and high mutation rates.

We developed a novel *E. coli* host cell line for growth-decoupled production of recombinant proteins, plasmid DNA, and small molecules. This technology, enGenes-e$^{X}$press, uses synthetic biology to enhance expression. Upon induction, co-expression of phage-derived Gp2 inhibits σ70-dependent transcription, halts cell division, and redirects metabolism toward product synthesis. This boosts productivity under industrial conditions and enables extracellular protein secretion.

In a continuous two-stage chemostat setup (enGenes-e$^{X}$cite), growth and production are spatially separated: biomass is generated in the first reactor, production induced in the second. Cell division stops, increasing stability and maintaining productivity.

This presentation highlights: i) the enGenes-e$^{X}$press technology, ii) the enGenes-e$^{X}$cite process, iii) strain development, iv) integrated continuous upstream/downstream processing, and v) automation and digitalization, including in-line analytics and hybrid modeling. Potential applications in compact manufacturing and techno-economic modeling are discussed.

**Recombinant isobutyrate production with the acetogen *Eubacterium callanderi* 'Marburg'**

Barbara Rühle; Cristiano Da Silva Lameira; Frank Robert Bengelsdorf

University of Ulm, Germany

The resources of fossil fuels are limited. Furthermore, their usage is contributing to the generation and emission of greenhouse gases. Together with changes in ecosystems they are important drivers of climate change. One example is the synthesis of isobutyrate which is currently done via an acid-catalyzed Koch carbonylation process, which requires propylene as precursor. Alternative strategies are needed to achieve sustainable isobutyrate production without resorting to raw materials or fossil fuels.

*Clostridium luticellarii* is as *Eubacterium callanderi* an acetogen which can use C1 molecules such as CO, $CO_2$ and methanol as substrates and convert them by the Wood-Ljungdahl pathway to e.g., acetate and butyrate. Isobutyrate is catalyzed by the action of an isobutyryl-CoA-mutase (ICM) in *C. luticellarii*. The ICM is composed of a large (IcmA) and a small subunit (IcmB) which binds the cofactor vitamin B12. The respective genes were cloned under a constitutive promoter resulting in the plasmid pJIR751_$P_{fd}$_icm which was introduced in *E. callanderi*. During heterotrophic growth of *E. callanderi* [pJIR751_$P_{fd}$_icm] no production of isobutyrate was observed. This absence was due to the lack of an adenosyltransferase (ATR) which activates cob(II)alamin and a G-protein metallochaperone (Mea) which loads the active cofactor to the ICM. Respective genes were cloned to construct the plasmid pJIR751_$P_{fd}$_icm_mea_ATR and the corresponding strain *E. callanderi* [pJIR751_$P_{fd}$_icm_mea_ATR]. Heterotrophic and autotrophic growth experiments will be analyzed for growth and production kinetics.

**A unique C-terminal α-helix in *Corynebacterium glutamicum* pyruvate:quinone oxidoreductase mediates lipid interaction and native purification**

Cristiano da Silva Lameira; Bernhard J. Eikmanns; Frank R. Bengelsdorf

Institute of Molecular Biology and Biotechnology of Prokaryotes, Germany

Pyruvate:quinone oxidoreductase (PQO) catalyzes the oxidative decarboxylation of pyruvate to acetate and $CO_2$, transferring electrons to quinones. In *Corynebacterium glutamicum*, a Gram-positive, non-pathogenic actinobacterium widely applied in amino acid production, the physiological role of PQO remains unclear, but its activity varies with growth phase, growth rate and carbon source[a]. High-resolution crystal structures of native PQO were determined, which revealed a C-terminal amphipathic α-helix formed by the last 17 residues[a]. Anchored via a glycine-rich loop, this helix is positioned opposite the FAD-binding domain and appears to mediate lipid interaction. This structural feature also enables selective, reversible purification of native PQO from cell extracts via detergent phase partitioning, without the need for affinity tags. Functional analysis showed that truncation of the α-helix abolishes detergent-mediated activation, decreases turnover number, increases pyruvate affinity, and reduces detergent binding. These results indicate that the α-helix is important for both enzyme regulation and membrane interaction. In addition, it provides a practical handle for native purification, with potential relevance for the structural and functional study of other membrane-associated oxidoreductases.

[a]da Silva Lameira, C., Münßinger, S., Yang, L., Eikmanns, B.J. and Bellinzoni, M. (2024), *Corynebacterium glutamicum* pyruvate:quinone oxidoreductase: an enigmatic metabolic enzyme with unusual structural features. FEBS J, **291**: 4501-4521. https://doi.org/10.1111/febs.17232

**Recombinant collagen-derived-protein-based hydrogels for tissue engineering applications**

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Hydrogels are a commonly used material in tissue engineering with further possible use in other clinical applications. These hydrogels can be of synthetic origin, such as polyethyleneglycol, however biological molecules such as hyaluronic acid, collagen and gelatin are mostly used. The advantage of biological materials is that they provide inherent bioactivity including cell binding sites and biodegradability. In the area of protein-based hydrogels, gelatin modified with methacrylate groups (GelMA) is the most commonly used material due to its advantageous properties. However, as an animal-derived material, GelMA displays disadvantages including quality variation due to the natural sourcing of the material, the possible risk of pathogen transmission, as well as religious or ethical concerns related to its animal origin. Furthermore, the thermal gelation of GelMA frequently introduces challenges in material handling. To address this issue, we developed a thermally non-gelling recombinant hydrogel based on a collagen-derived protein expressed in *Komagataella phaffii*. The resulting material was biocompatible and displayed cellular adhesivity. The recombinant collagen was methacrylated and yielded a highly defined recombinant GelMA. In a next step, we varied the chain length of the expressed protein and investigated the resulting physical material properties. Our studies highlight how biotechnology can be used in the design of highly specific biomaterials of uniform quality, which circumvents many of the limitations associated with naturally-sourced hydrogel materials.

**Recombinant cysteine-containing proteins for thiol-ene based hydrogel applications**

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Institute of Technical Chemistry, Leibniz University Hannover, Germany

Hydrogels are widely used in tissue engineering due to their ability to mimic the extracellular matrix. In such applications, proteins with natural binding sites for cells are particularly suitable. For hydrogel formation, chemical modifications to the proteins are frequently necessary. Here, methacrylation is one of the most common types of modification and results in photopolymerizable biomaterials such as gelatin methacrylate (GelMA). However, initiation of GelMA polymerization requires high concentrations of free radicals and long UV exposure, leading to lower cell viability. An alternative crosslinking mechanism is the use of thiol-ene chemistry which utilises the reaction of a thiol and an alkene such as norbornene-functionalized gelatin (GelNB) and dithiothreitol (DTT). The curing of the GelNB hydrogels is faster while also requiring lower photoinitiator concentrations. Also, the biocompatibility of the precursor material is higher. However, the commonly used crosslinker DTT is frequently cytotoxic at the concentration required for GelNB/DTT hydrogels. For this reason, biocompatible molecules that can be used as crosslinkers are being investigated. One possibility are cysteine-containing proteins, which naturally carry thiol groups. In this case, additional biofunctionality can be engineered into the hydrogel by careful choice of the thiolated partner. In this study, a recombinant human fibrinogen gamma chain (FGG) was expressed in *Escherichia coli* in inclusion bodies. The expressed protein was then investigated for its applicability in thiol-ene hydrogels.

**Digital transformation for efficient recombinant protein production processes**

Tobias Baumgartner (1); Xavier Garcia-Ortega (2); Miguel Angel Nieto-Taype (1)

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The digital transformation of bioprocess development offers significant opportunities to improve efficiency, data quality, and decision-making. In our current initiative, we are implementing an integrated data management platform to centralize experimental data acquisition, storage, and visualization, ensuring seamless access and traceability. In parallel, advanced modeling tools are being introduced to facilitate statistical analysis and predictive simulations. Together, these efforts will streamline process optimization workflows, enhance reproducibility, and accelerate the identification of optimal cultivation strategies. This approach aims to establish a robust digital backbone for data-driven bioprocess development. First examples, experiences, and learnings from this study will be shared.

**From acetate to food: advancing innovative protein production**

Greta Gecse

Novonesis, Denmark

In 2022, a UN-led report reveales that over 250 million people faced severe hunger. Technology can play a role in creating a circular carbon economy to combat significant global challenges like malnutrition, biodiversity loss, and climate change. To address this, there is a need for an economically viable, sustainable, and environmentally friendly food production system capable of feeding a growing global population. With this goal in mind, the Bill Gates Foundation and the Novo Nordisk Foundation are funding a consortium to utilize CO2 for human food protein production

This approach aims to convert CO2 into acetate using electricity, which will serve as a fermentation substrate for food protein production. While technically challenging, it bypasses land use and offers vast potential for sustainable food and other microbial-based products. Developing strains optimized for acetate, rather than glucose, is essential for success. This project hypothesizes that microorganisms will undergo metabolic rewiring to use acetate as a carbon source. Fermentation optimization and scaling are crucial, with strains needing to match glucose-based production performance eventually. The project includes Pilot studies where strains selected from R&D are scaled. Due to the distinct carbon source, acetic acid, additional factors must be considered when designing the scale-up process and equipment.

**Mathematical modelling of TX-TL dynamics in *Pseudomonas putida* KT2440**

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Transcription-translation (TX-TL) coupling represents a potential rate-limiting factor in the biosynthesis of proteins. Precise and efficient modeling of this process is crucial for enhancing the overall yield of the target protein product. The study uses a deterministic, dynamic model founded on ordinary differential equation (ODE) designed to simulate the *in vivo* transcription and translation of individual gene sequences into their respective protein sequences in *Pseudomonas putida*. This model originates from an *in vivo* model developed by Spindler *et al* [1] to accurately represent *Escherichia coli* protein biosynthesis. Currently, the model is being translated from Matlab to the programming language Julia for application in *P. putida,* Julia's open-source advantage and computational efficiency make it the optimal choice over Matlab and Python. The TX-TL model currently under development in Julia closely approximates the results of the Matlab-based model when applying kinetic parameters identified for *E. coli*, with a low error percentage for protein concentration. Following the assessment of metabolite concentrations and distinctive physiological attributes, the model will undergo further refinement to suit the requirements of the *P. putida,* TX-TL modeling can be used for precise simulation of gene-to-protein translation in *P. putida*, crucial for optimizing protein production in synthetic biology.

1. https://pubs.acs.org/doi/10.1021/acssynbio.4c00578

**Scale-down bioreactor studies on heterologous protein production in stringent response modulated *E. coli* chassis.**

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*Escherichia coli* (*E. coli*) is widely used for large-scale recombinant protein production. However, in large bioreactors, uneven mixing and limited mass transfer create fluctuating nutrient and oxygen levels, triggering stress responses in the bacteria. This stress reduces cellular performance, increases energy maintenance, and lowers protein yield, growth, and productivity, ultimately affecting product titre and process economics. One approach is to engineer robust chassis that is capable of enduring these stress conditions and prevent large scale performance loss. *E. coli* SR strain is engineered to avoid global stress regulation system called stringent response by modulating the ppGpp (guanosine pentaphosphate) to basal level, which is the key controller of this mechanism. The strain exhibits increased tolerance to industrial-scale stresses positioning it as a potential robust chassis for large scale bio-manufacturing. Investigations using scale down bioreactors (stirred tank bioreactor- plug flow bioreactor) that mimics industrial stress conditions facilitate comprehensive metabolic, transcriptional and production data. Our preliminary experimental data indicates that, *E. coli* SR sustains the fraction of super folded green fluorescence protein producing cells at a consistent level after 28-hours of large-scale stress exposure during production, comparable to results obtained 6-hours after the start of production phase. In contrast, the parental strain *E. coli* MG1655 parental strain experiences a reduction of 20% in protein producing cells under the same conditions.

**Optimizing the recombinant lactate production with *Acetobacterium woodii***

Kira Baur; Frank Bengelsdorf

Ulm University, Germany

Microbial gas fermentation, utilizing CO2 and H2 as carbon and energy sources, is an approach to reduce climate change-forcing CO2 emissions. The acetogen A. woodii reduces CO2 via the Wood-Ljungdahl pathway (WLP) and produces natively acetate. A. woodii was genetically modified to produce lactate as second product. Therefore, a D-lactate dehydrogenase (LdhD) from L. mesenteroides was expressed recombinantly in A. woodii and reduces pyruvate to lactate. Pyruvate originates from acetyl-CoA and CO2 autotrophically by the action of a pyruvate:ferredoxin oxidoreductase. Our approach is to raise pyruvate levels by expressing a pyruvate-formate lyase (Pfl) from C. pasteurianum in A. woodii_LdhD. Mostly, the Pfl catalyzes the reaction of pyruvate and CoA to formate and acetyl-CoA. In our strain A. woodii_LdhD_Pfl, the Pfl should act in a reverse manner, converting formate and acetyl-CoA to pyruvate to branch the WLP. Since the driving force of pyruvate reduction to lactate via LdhD is high, Pfl should proceed as intended. A. woodii_LdhD and A. woodii_LdhD_Pfl were cultivated autotrophically in a bottle-scale. A. woodii_LdhD reached a max. OD600 of 1.1 ± 0.1, while A. woodii_LdhD_Pfl reached a peak OD600 of 0.9 ± 0.03. A. woodii_LdhD and A. woodii_LdhD_Pfl yielded a lactate level of 14.8 ± 3.0 mM and 19.9 ± 4.8 mM, respectively. Acetate remained the main product, with a concentration of 183 ± 35 mM produced by A. woodii_LdhD, and 173 ± 31 mM by A. woodii_LdhD_Pfl. This indicates that Pfl performed as expected, leading to an increase in lactate production using A. woodii_LdhD_Pfl.

**The missing link: connecting cultivation conditions and refolding performance via inclusion body biophysical properties**

Joan Cortada Garcia (1); Matthias Rüdt (2); Aymerick Bussien (1); Holger Morschett (1); Hauke Holm (1); Chiara Mazzucchelli (1); Karlheinz Flicker (1)

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Inclusion bodies (IBs) frequently form during the expression of heterologous proteins in *Escherichia coli* and are therefore important in pharmaceutical production. While it is accepted that cultivation conditions affect cultivation performance, the link between these conditions and refolding performance remains unexplored. This study proposes that this interplay relies, at least partially, on the inherited biophysical properties of the IBs. Using a design of experiments approach, this study systematically explored how cultivation conditions – postinduction temperature, pH, and feed rate – affect the production of IBs containing anti-desipramine single-chain variable fragment antibodies as a model therapeutic protein. Various biophysical properties of the IBs – including hydrophobicity, secondary structure, and particle size – were characterized, and their relationship to cultivation parameters and refolding performance was analyzed. Key findings revealed that higher feed rates and temperatures increased the product titer and IB size. Larger IBs facilitated refolding, while a higher content of amyloid structures, occurring locally without a strong link to the cultivation parameters, hampered protein solubilization and refolding efficiency. Higher protein content in the IBs adversely affected the refolding yield due to a hidden coupling between cultivation and refolding. This study establishes IB biophysical properties as critical factors for linking upstream and refolding process performance, offering actionable insights to enhance bioprocess robustness and efficiency.

**Driving performance and process reproducibility in recombinant protein manufacturing through optimal selection of yeast-derived bionutrients**

Alessandro Ciranna; Burcin Boran; Marie Schöpping; Abhishek Somani

Ohly GmbH, Germany

In industrial biomanufacturing the selection of optimal media components is a critical lever for enhancing recombinant protein expression, productivity, batch consistency, and overall process economics. Among these components, yeast-derived bionutrients (YDBs), such as yeast extracts and peptones, play an important role by supplying essential amino acids, peptides, vitamins, cofactors, and trace elements that support microbial growth and protein biosynthesis.

The performance of YDBs is influenced by factors such as the host organism, expression system, fermentation parameters, downstream processing requirements, and physicochemical characteristics of the target protein. Therefore, selecting the most suitable YDB, or combination thereof, is essential for maximizing yield and ensuring process robustness. In addition to performance, the inherent complexity and natural variability of YDBs demand exceptional batch-to-batch consistency to maintain reproducibility across production runs.

This work highlights case studies demonstrating how process performance indicators in recombinant protein production are strongly influenced by the choice of Ohly X-SEED® YDBs. The data emphasizes the importance of optimal nutrient selection to unlock the full potential of model microbial expression systems. Furthermore, the results presented clearly demonstrate the outstanding consistency and functional reliability of Ohly X-SEED® YDBs for recombinant protein manufacturing.

**Strategies to improve plasmid stability in *Escherichia coli* cell factories: comparing antibiotic selection systems for industrial protein production**

Mirko Frigerio Verga; Daniela Bucchieri; Valeria Mapelli; Immacolata Serra; Paola Branduardi

Università Milano-Bicocca, Italy

*Escherichia coli* cell factories for recombinant protein production typically harbour a plasmid containing the gene of interest. Maintaining these plasmids requires selective pressure, typically achieved through the use of antibiotics. The choice of antibiotic resistance plays a critical role in the overall stability and scalability of the production process, making it a key factor in determining its feasibility.

In this study, we compare two antibiotic resistance systems; one based on β-lactam antibiotics, where cell growth and protein induction must be decoupled to preserve plasmid stability and another system based on aminoglycoside antibiotics, which enables a simpler and more robust process. Our findings highlight the advantages of the aminoglycoside-based system in supporting a more stable and scalable production platform for recombinant proteins.

# Poster 64

**Synthetic biology and bioprocess engineering for scalable, cost-effective components in cultivated meat production**

Orly Savion

Alagene, Israel

Scaling the production of alternative proteins requires integrated bioprocess solutions to address challenges in microbial strain optimization, precision fermentation, and downstream purification.

Alagene is a biotechnology CRO specializing in synthetic biology and bioprocess design for microbial-based production systems. Our expertise spans microbial strain engineering, high-throughput screening and bioassay development, fermentation optimization, and downstream processing (DSP) development, enabling a robust, scalable, and cost-efficient biomanufacturing processes.

A practical demonstration of this expertise is Alagene's role in the Cultivated Meat Consortium, where we focused on producing recombinant growth factors, critical components of serum-free media, using microbial expression systems. The project combined iterative strain engineering, bioassay-guided selection, fermentation optimization, and DSP development to achieve over a 5-fold increase in growth factor expression and a projected 1,000-fold cost reduction, while maintaining biological activity and compliance with food-grade standards. This case study illustrates how integrated bioprocess design can overcome technical bottlenecks and significantly enhance the scalability and economic feasibility of alternative protein manufacturing.

**Advancing cultured meat production: cost-effective growth factor development through precision fermentation and synthetic biology**

Neta Agmon

Alagene, Israel

Alagene, as part of a pioneering consortium, has developed a serum-free growth medium to advance cultured meat production, focusing on optimizing growth factor components through advanced strain development, cell engineering, and synthetic biology. Our approach integrates precision fermentation, high-throughput screening, automation, and bio-manufacturing, allowing us to refine yeast and bacterial strains for improved growth factor production. By enhancing fermentation and downstream processing techniques, we achieved a three-figure cost reduction in growth hormones, marking a significant step toward economically viable and environmentally sustainable cultured meat.

**From weeks to days: turbocharging high-throughput antibody discovery powered by ALiCE® for HTPE**

Sunny Singh, Yannick Flaskamp, Thomas Scott, Johannes Gottschalk, Ridhima Gomkale, Harendra Mahto, Hannes Juergens, Jonathan Fauerbach

LenioBio GmbH, Germany

Accelerating recombinant protein production is essential for therapeutic antibody discovery, but traditional mammalian systems like HEK293 require 13-28 days and 15-18 steps, limiting throughput to 10 plates/month. LenioBio's ALiCE® cell-free platform, based on tobacco BY-2 lysates, transforms high-throughput protein expression (HTPE) into a 3-day, 4-step process, enabling 10 plates/week and easy automation integration.

The workflow involves inserting genes into an expression cassette using plasmid, linear DNA, dbDNA, or mRNA templates, adding to ALiCE lysate for 24-h incubation, and one-step purification with tags like C-tag, HaloTag, Strep-Tag, or Protein A. ALiCE excels at expressing challenging proteins—antimicrobial peptides, membrane receptors, growth factors, GPCRs, full IgGs, multispecifics, and virus-like particles (VLPs)—with >90% success and 200-400 µg/mL antibody yields.

In HTPE of 22 human IgGs (50 µL reactions), post-purification yields were 5-20 µg, confirmed by UV/VIS and SDS-PAGE. ALiCE-produced Adalimumab matched CHO standards in purity (native PAGE, SEC-HPLC) and binding (SPR). Multispecifics like Faricimab and VLPs were also produced, supporting non-canonical amino acids, bioconjugation, and cofactors.

ALiCE reduces timelines and costs, enhancing Design-Build-Test-Learn cycles for AI-driven engineering. This scalable platform advances protein innovation from discovery to commercialization, redefining biotherapeutics.

**Efficiency enhancement in mAb production: streamlining bioprocesses with peptides to boost CHO cell culture performance**

Sabrina Metze; Johanna Peters; Stephan Brinkmann; Anne Benedikt; Christina Jost; Tomislav Trescec

Evonik Operations GmbH, Germany

Conventional media formulations for CHO cell cultivation typically utilize dual-feed systems (main feed and alkaline feed), which pose inherent process challenges and increase risks. This study addresses the need for a more efficient and streamlined approach to cell culture.

We conducted a case study involving two CHO cell lines to evaluate the performance of the single-feed system. The methodology focused on comparing cell growth, viability, and monoclonal antibody titer against conventional dual-feed formulations.

Our findings indicate that the pH-neutral, single-feed system significantly enhances cell culture performance with reduced complexity. Notably, we observed improved cell growth and viability for a CHO DG44 cell line, with monoclonal antibody titers improvements reaching up to 67%, demonstrating substantial benefits over traditional methods.

The transition to a single-feed system not only reduces complexity in bioprocessing but also contributes to cost efficiency in biomanufacturing. This research highlights the potential of innovative media formulations to optimize CHO cell cultivation and improve overall productivity.

**SmartRoute™ to protein production: integrating early-stage strain and process development for high-titer recombinant protein production in *Bacillus subtilis***

Bor Klančnik

Acies Bio d.o.o., Slovenia

Industrial-scale recombinant protein production often suffers from a late focus on bioprocess design, leading to scale-up challenges, performance drops, and high costs. **SmartRoute™ to Protein Production** is an integrated approach bridges the gap between lab results and manufacturing realities, providing a blueprint for stress-resilient recombinant protein production. We developed a proprietary B. subtilis industrial strain platform, achieving multi-g/L titers across diverse target proteins. Strain engineering revealed that many genetic modifications are to some degree protein-specific and have limited transferability between different target proteins. In contrast, a robust, well-optimized bioprocess proved broadly applicable, enabling consistent high performance across different proteins. From proof-of-concept onwards, we eliminated complex medium components, optimized seed train architecture, fine-tuned feeding strategies, and designed media for industrial scalability and cost efficiency. This approach boosted titers from initial few g/L to over 40 g/L, demonstrating that systematic, early process development can mitigate production stress, enhance robustness, and deliver competitive manufacturing performance. Our results emphasize that coupling strain engineering with proactive bioprocess optimization is key to overcoming productivity hurdles and achieving industrially viable recombinant protein production. Well-engineered process framework can reduce risk, lower production costs, and improve performance under industrial conditions.

**Multiplex gene synthesis on bench at ultra-low cost**

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Producing a target protein at high quality and yield typically requires testing multiple DNA sequences and expression constructs under various culture conditions—a process that is labor-intensive, costly, and can take one to two months per iteration. The challenge is even greater for long or complex genes. We have developed an automated, bench-top approach for multiplex gene synthesis at a cost as low as €2 per gene. This method has no gene size limitations and can achieve turnaround times as short as three days.

**Sustained recombinant β-casein production in *E. coli* via population-balance modeling of the cascaded continuous reactor system**

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The cascaded continuous stirred-tank reactor (CCSTR) is a promising strategy for sustainable biomanufacturing of recombinant proteins. By physically separating growth and production environments, CCSTRs sustain production through continuous rejuvenation of the production stage with fresh, uninduced cells, mitigating cell aging and adaptation to metabolic burden. However, the quantitative effects of core operating conditions—such as reactor volumes, dilution rates, and biomass concentrations—on protein accumulation titers remain poorly understood. While increases in biomass-specific protein productivity rate ($q_P$) are often linked to higher substrate uptake ($q_S$) and increasing dilution rates, these factors do not necessarily translate into higher intracellular product accumulation due to competing residence-time and metabolic burden constraints.

We introduce a mechanistic population-balance model for CCSTRs that quantifies phenotypic subpopulations arising from continuous seeding with fresh cells from the growth reactor. The model is structured around the quantification of the cell aging process ($d\phi/dt$) and its influence on biomass growth $\mu(q_S, \phi)$ and $q_P$ rates. The model is trained using existing dynamic time-series data from chemostat fermentations as well as steady-state data from CCSTR campaigns. Applied to β-casein production in recombinant *E. coli* strains, the model identifies operating conditions that significantly enhance protein accumulation, enabling the rational design of experiments and advanced control strategies for the CCSTR system.

**An engineered secretion platform for peptide drugs**

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The complexity of type 2 diabetes mellitus (T2DM) requires innovative therapeutic strategies that enable localized and sustained peptide delivery. Incretin-based treatments, such as GLP-1 and Exendin-4, are potent glucose-lowering agents but are limited by rapid degradation and the need for frequent administration. In this study, we designed different E. coli strains capable of producing and secreting GLP-1 and Exendin-4 using a MicL-based outer membrane release system. Two expression strategies were implemented: a constitutive architecture using the ProD promoter and an inducible design regulated by LacI/aTc system. In both cases, incretin genes were fused to secretion signal peptides to facilitate extracellular export, while MicL was co-expressed to enhance membrane release. Constructed plasmids were transformed into different E. coli strains, and peptide production was quantified via ELISA. Purified peptides were structurally characterized, and their biological activity was evaluated using MTT assays in MIN6 cells. The effects of the constitutive and inducible systems were compared in terms of secretion efficiency and inducibility, with additional assessment of the impact of MicL-mediated release on peptide yield.

**Production of recombinant bevacizumab antigen binding fragment in *Komagataella phaffii***

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Bevacizumab is a humanized IgG monoclonal antibody that targets Vascular Endothelial Growth Factor (VEGF), blocking angiogenesis and improving survival in metastatic colorectal cancer. Antigen-binding fragments (Fabs) have a shorter half-life and lack the fragment crystallizable (Fc) domain, reducing excessive immune activation and offering potential as a cost-efficient, non-glycosylated therapeutic. *Komagataella phaffii* (*Pichia pastoris)* is an attractive host for recombinant antibody production due to its rapid growth, high protein yield, and ease of genetic manipulation.

In this study, heavy chain Fab and light chain genes of Anti-VEGF sequences were individually ligated into an expression vector under the control of the synthetic *ADH2* promoter, which was transformed into the *K. phaffii* MK115 strain. Two haploid strains expressing either the heavy and light chains were mated to create diploid *K. phaffii* cells capable of Fab production. The best diploid *K. phaffii* clone was identified and optimized in shake flasks, with pH 5 and 25 °C. Bioreactor production followed by purification with Protein G chromatography yielded 93,4 mg/L of Fab. Surface plasmon resonance confirmed VEGF binding with a Kd value of 6.59 nM. Overall, the recombinant production of Anti-VEGF Fab fragments was achieved using the diploid *K. phaffii*.

**From bond orders to β-sheets — a next-gen protein ReaxFF**

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Efficient design and optimization of proteins, whether for therapeutics, enzymes or biomanufacturing, requires an understanding of how proteins interact with peptides, ligands and their environment and how it affects their structure, stability and activity. While classical force fields like CHARMM and AMBER allow long-timescale simulations of entire proteins, they are limited by rigid atom types that restrict their transferability. Conversely, quantum methods like DFT, while able to model bond breaking and formation, are computationally limited to small fragments. To bridge this gap, we are developing a new CHONS ReaxFF parameter set for proteins and peptides, placing special emphasis on structural stability and nonbonded interactions. This approach aims to combine accuracy and application-oriented approaches, providing the foundation for a wide range of applications. At the core is a curated training set, combining dipeptide conformers, bond/angle/torsion scans, dimers capturing intrinsic protein interactions and residue–water interactions. Reference data comes from in-house high-level QM calculations and databases like the BioFragment Dataset and SPICE. The Parameter optimization uses a competitive particle swarm optimization combined with a function-partitioned strategy. Validation includes Cα RMSD drift, Ramachandran plots, and comparison to QM and crystallography. Early results demonstraced enhanced interaction-energy accuracy, robust structural stability and realistic conformational sampling, laying the groundwork for chemically accurate, scalable protein simulation.

**CRISPR/Cas9-mediated ADH2 gene deletion in *Komagataella phaffii***

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Komagataella phaffii (formerly Pichia pastoris) is a widely used host for recombinant protein production, yet many efficient strains are constrained by licensing restrictions and dependence on antibiotic markers, limiting regulatory compliance and scalability. This study focused on developing a license-free, marker-free strain using the OPENPichia platform (Claes et al., 2024). CRISPR-Cas9 genome editing was applied to delete the ADH2 gene, and Golden Gate Assembly-based homologous recombination enabled scarless genomic modifications, minimizing risks of instability (Gassler et al., 2019). Deletion of ADH2 significantly reduced ethanol utilization, as shown by growth and carbon utilization assays. Comparative analyses demonstrated that Δadh2 strains in the OPENPichia background maintained higher biomass yields and growth rates than commercialized counterparts, suggesting improved metabolic flexibility. SDS-PAGE analysis of recombinant α-amylase production confirmed that ethanol induction strategies strongly affected yields; notably, Δadh2 strains outperformed wild type, indicating that slower ethanol uptake enhances promoter induction efficiency while also providing process safety benefits. Overall, the engineered strain combines the productivity of proprietary strains with the regulatory and operational advantages of a license-free platform. These findings highlight CRISPR-Cas9 as a precise and efficient tool for robust strain construction and underscore the potential of targeted metabolic engineering to improve safety and yield in large-scale bioprocessing.

**Reducing ER's toll booths: combining co-translational translocation pathway with ERV29 overexpression for improved protein secretion in *Komagataella phaffii***

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The hybrid secretion signal pre-Ost1-pro-αMF, consisting of the Ost1 signal sequence followed by the α-factor pro region drives efficient cotranslational translocation of proteins into the endoplasmatic reticulum (ER) in yeast. It has shown increased protein secretion efficiency compared to the conventional α-Factor Pre-pro-leader (post-translational translocation) using different model proteins in *Komagataella phaffii* [1]. However, when the performance of this hybrid secretion signal was challenged in bioreactor scale fed-batch cultivations using a fluorescent model protein (E2Crimson), this protein seemed to be intracellularly accumulated in late phases of the cultivations, resulting in decreasing specific production rates over time [2]. Further transcriptional analyses suggested that this accumulation was presumably taking place at the ER level, triggering the Unfolded Protein Response (UPR) and, consequently, the ER Associated Degradation (ERAD).

To circumvent this hurdle, we hypothesised that overexpression of *ERV29* could efficiently increase the export rate of our model protein from the ER to the Golgi, thereby reducing the accumulation of protein at the ER. Erv29 is a transmembrane protein that efficiently recognizes the pro region from the secretion signal and sends the proteins to the ER- Exit Sites (ERES). Small scale cultivations experiments confirmed the positive effect of *ERV29* overexpression on E2Crimson secretion levels.

References:

[1] Barrero et al (2018). Microb Cell Fact. 17:161.

[2] Barrero et al (2021). New Biotechnol. 60:85-95.

**Development of a versatile platform for the production and purification of single-domain antibodies (VHH) in microbial hosts**

Florence Xhonneux (1); Régine Freichels (1); Quentin Nera (1); Raika Yamagiwa (1); Masahiro Aratake (2); Kojiro Ishiguro (1); Laurent Jost (1)

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Single-domain antibodies (VHH), derived from camelid heavy-chain antibodies, are increasingly recognized for their potential in drug development, diagnostics, and life science research. These antigen-binding fragments can be engineered for multivalency or multi-specificity and exhibit diverse biochemical properties.

Despite their diversity, VHH share structural and biochemical characteristics that enabled the development of a robust, flexible platform for microbial production and purification using Pichia pastoris (also known as Komagataella phaffii) and Escherichia coli.

To build this production and purification toolbox, we selected a broad panel of VHH varying in valency, hydrophobicity, and isoelectric point, ensuring a representative and challenging set for platform optimization. These VHH were expressed in both hosts under optimized fermentation and extraction conditions.

Comprehensive characterization of the VHH panel allowed us to identify the most suitable capture resins, followed by optimization of binding and elution conditions. Polishing steps were developed by screening multiple resins and parameters to ensure high purity. Initial process development was conducted at small scale and subsequently scaled up to 50 liters.

This poster presents a case study of one VHH produced in P. pastoris, demonstrating high-quality output confirmed by a full set of release quality controls.

Our newly established platform, built on deep expertise in microbial expression and protein engineering, offers a scalable, cGMP-compliant solution for rapid development and manufacturing of VHH.

**GMP VHH platform for rapid tox and clinical access**

Pascal Bolon (1); Régine Freichels (1); Quentin Nera (1); Laurent Jost (1); Thomas Windhausen (1); Raika Yamagiwa (1); Masahiro Aratake (2); Kojiro Ishiguro (1); Hiroyuki Kanamaru (1)

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At Kaneka Eurogentec, we offer an advanced GMP manufacturing platform for the rapid, high-quality production of VHH modalities using microbial systems. With over 40 years of expertise in recombinant protein expression, our platform utilizes P. pastoris (K. phaffii) and E. coli to deliver efficient, scalable production, from process development to GMP release, in under 8 months.

This platform has been successfully validated with a wide array of VHH molecules, including mono-, bi-, and trivalent formats (12–40 kDa, pI 5–9). It supports preclinical to commercial needs while minimizing production risks and ensuring flexibility across various expression systems.

Key Advantages:

- **Microbial Hosts**: Scalable expression in P. pastoris and E. coli.
- **Speed to GMP**: Delivery of GMP-grade material within 8 months.
- **Broad Compatibility**: Supports a variety of VHH structures and physicochemical profiles.
- **Cost-Efficient**: Standardized USP/DSP using economical reagents and resins.
- **Comprehensive QC**: Core quality control assays included customized tests available.
- **Scalable Production**: Smooth transition from small-scale to commercial manufacturing.
- **End-to-End Support**: From research-grade material to full GMP production.
- **Accelerated Timelines**: Quick-to-tox and clinic-ready solutions with streamlined workflows.
- **Expert Team**: Dedicated project managers and protein specialists.

Whether you're advancing a therapeutic candidate or scaling for market, we provide the reliability, flexibility, and speed to accelerate your VHH program. Contact us to explore how we can support your development goals.

**Construction and evaluation of *Pseudomonas*-expression vectors for the generation of disparity mutator strains**

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PFAS (per- and polyfluoroalkyl substances) have been widely used since the 1940s in industrial and commercial products and can now be detected in the environment worldwide, contaminate soil and water and can harm human health. Although PFAS are known to be highly resistant to biodegradation, there are some microbial species that can at least partially degrade these compounds, e.g. some *Pseudomonas* spp. These have been shown to be able to break down PFAS to some extent and to have a high tolerance to the toxic degradation product fluoride.

In our project we aim to generate *Pseudomonas* mutants with enhanced PFAS degradation abilities by creating disparity mutator strains expressing proofreading deficient variants of DnaQ and challenging these strains with PFAS. In a first step we generated *Pseudomonas*-expression vectors with inducible promoters. After introducing a tetracycline resistance cassette and a *lacI*-repressor gene into the starting vector, three different *E. coli*-derived promoters ($P_{lac}$, $P_{trp}$, $P_{trc}$) were tested for their functionality in *Pseudomonas*. The reporter protein mCherry was used to measure expression levels of the different promoters and varying inductor levels in the BioLector microfermentation system. It turned out that the $P_{lac}$ based promoters are too weak, whereas $P_{trp}$ based promoters are hard to control by varying IPTG concentrations. Best performance regarding expression strength and controllability was reached with $P_{trc}$ based promoters which will be used for the generation of the *dnaQ*$^{mut}$-mutator-plasmids.

**Non-canonical amino acid incorporation and click chemistry as a plug-and-play vaccination platform enabled by LenioBio's ALiCE® cell-free expression platform**

Maja Lieven, Jorge Armero Gimenez, Jonathan Fauerbach

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Non-canonical amino acids (ncAAs) can provide recombinant proteins with novel transformative functionalities beyond the limits of nature, such as orthogonal reaction groups. Cell-free protein synthesis (CFPS) represents the most promising methodology to introduce ncAAs into recombinant proteins. Unlike prokaryotic CFPS systems, LenioBio's ALiCE® cell-free eukaryotic platform enables production of complex proteins requiring posttranslational modifications at scale and with high yield. Here, as an example, we apply ncAA incorporation in ALiCE® to enable click chemistry bioconjugation of the receptor binding domain (RBD) of influenza hemagglutinin to pre-assembled hepatitis B core (HBc) virus-like particles (VLPs), constructing a novel plug-and-play vaccine platform.

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