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SANDOZ SUSTAINABILITY
SCIENCE FLASH

Science Flash

SAGROPIA – Sustainable agriculture through novel pesticides using an integrated approach

T. Bath

RTDS Group, Vienna, Austria

The EU-funded SAGROPIA project, in line with the European Union's Farm to Fork strategy, is dedicated to promoting safe, sustainable, and secure food production by reducing the use of harmful chemical pesticides in agriculture. Conventional crop protection practices, particularly in the cultivation of staple crops like potato and sugar beet, rely heavily on chemical pesticides, which pose significant risks to human health and the environment. Reducing the use of these substances is a top priority for the European Commission, especially regarding 'candidates for substitution' as outlined in Regulation (EC) No 1107/2009.

SAGROPIA focuses on developing and demonstrating biocontrol-based solutions that can effectively replace chemical pesticides, while incorporating Integrated Pest Management (IPM) strategies that are both affordable and practical for farmers. The project will introduce 13 biological and low-risk pesticides from three industry partners, with the goal of replacing several hazardous active substances such as the nematicide oxamyl and the fungicides difenoconazole, fluopicolide, and metalaxyl. In addition, SAGROPIA aims to reduce the overall use of copper-based pesticides and insecticides, including pirimicarb, lambda-cyhalothrin, and esfenvalerate, by at least 50%.

Potato and sugar beet crops are highly susceptible to diseases such as early and late blight, as well as pests like nematodes and the Colorado beetle. SAGROPIA's approach combines biocontrol solutions that have already demonstrated efficacy in specialty crops and are close to market readiness. These solutions are further refined, with scaled-up production and research into their modes of action, ensuring they meet the practical needs of European farmers.

The project emphasizes the development of holistic IPM strategies rather than simply substituting chemical pesticides. These IPM strategies will integrate the new biocontrol products into broader farm management practices, promoting a comprehensive approach to pest and disease management. Real-life trials will be conducted across five potato and sugar beet-growing regions in Europe, with the active participation of local farmers to ensure practicality and adoption.

Sustainability is at the core of SAGROPIA's work. The project not only aims to reduce harmful pesticides but also to evaluate the environmental, economic, and social impacts of its solutions. Detailed sustainability assessments will measure improved performance in terms of natural resource use, environmental impact, and economic feasibility, preparing the ground for broader adoption across Europe.

SAGROPIA seeks to ensure that biological alternatives to chemical pesticides are both accessible and economically viable for European farmers, contributing to a more sustainable and resilient agricultural system. The project's outcomes will provide valuable tools and data to help farmers transition to eco-friendly practices without sacrificing crop yield or quality.

Find more information, resources, and publications on the project's home page: https://sagropia.eu

Renewable Energy-Driven Lignin Valorization: Scalable Biocatalysis for a Sustainable Future

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Unlocking the true potential of lignin for industrial applications has long been a formidable challenge—until now. Our research focuses on the development of sustainable and efficient tools for the valorization of lignin-derived aromatic compounds (LDACs) such as vanillate, alkylguaiacols, and alkoxybenzoic acids. Through an innovative enzymatic cascade, we aim to convert lignin waste into valuable chemical intermediates. These products include protocatechuic acid and alkylcatechols, which serve as essential building blocks for the synthesis of polymers, biofuels, and pharmaceuticals.
The enzymatic process is catalyzed by three distinct oxidoreductase systems: PbdA/HaPuX/HaPuR, AgcA/AgcB (Class I and Class V P450, respectively), and VanA/VanB (a two-component Rieske oxygenase). These systems were used individually, each coupled with a hydrogen-driven cofactor regeneration system powered by the soluble hydrogenase (SH) from Cupriavidus necator. This approach allows for continuous regeneration of NAD+, enabling sustained catalytic activity without the need for large quantities of reduced cofactors, achieving a total turnover number (TTN) of over 160 for NAD+. This system enabled efficient catalysis at a fraction of the cost without compromising performance.
A critical challenge we addressed was optimizing the interplay between the O_2-dependent oxidoreductases and the H_2-dependent hydrogenase to ensure safe and efficient performance. Comprehensive studies on gas composition enabled us to fine-tune both systems to operate synergistically without compromising efficiency or safety. Notably, we maintained H2 concentrations below 4%, well within safe limits for flammability and explosion risk. This balance not only maximized atom efficiency but also ensured a secure framework for the biocatalytic process.

After successfully optimizing the batch reactions, we scaled the system to a pre-industrial prototype, utilizing an electro-driven flow chemistry setup. This system, equipped with in situ gas production via a proton exchange membrane electrolyzer, continuously supplies H_2 and O_2 to the reaction, making the entire process powered exclusively by renewable energy. The combined benefits of high atom efficiency, safe gas management, and renewable energy sourcing make this system not only highly efficient but also truly sustainable.

To enhance reactor stability and performance, we employed enzyme immobilization through adsorption and entrapment. This setup achieved over 99% yield in 5 hours, demonstrating high catalytic efficiency. To optimize the reaction environment, we kept one section of the reactor open with heavy stirring, allowing natural evaporation of formaldehyde and preventing its accumulation. The reaction was monitored online via TLC, and the flow setup enabled efficient downstream processing, yielding 82.3% recovery of the product as pure powder, confirmed by NMR.
This innovative system represents a major leap forward in the sustainable valorization of lignin-derived compounds. By combining hydrogen-driven cofactor regeneration, precise gas composition management, and electrodriven flow chemistry for upscaling, we offer a clean, scalable, and cost-effective approach to biocatalysis. This breakthrough paves the way for the future of sustainable biotechnology, contributing to a greener and more economically viable industry.

Production of the highly active thermophile PETases PHL7 and PHL7mut3 using *E. coli*

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The growing crisis of polyethylene terephthalate (PET) waste demands innovative recycling strategies. Enzymatic degradation of PET into its monomers, followed by re-polymerization, represents a promising circular economy approach. However, a significant challenge lies in the large-scale production of highly active PET-degrading enzymes without the requirement of costly downstream processing. In this study, we present an optimized process for the extracellular production of the thermophilic and highly active PETases, PHL7 and PHL7mut3, eliminating the need for expensive purification steps. By employing the growth-decoupled enGenes eX-press V2 E. coli strain and systematically varying pH, induction strength, and feed rate through a factorial-based optimization approach, we identified optimal production conditions for PHL7, resulting in a 40% increase in fermentation supernatant activity. Further improvement of the expression construct led to a 4-fold increase in activity. Applying these optimizations to the more active, temperature-stable variant PHL7mut3, we achieved complete degradation of PET film within 16 hours at 70°C, using only 0.32 mg of enzyme per gram of PET. These advancements support scalable enzymatic PET recycling, contributing to solving the global plastic waste crisis.

New possibilities for lowering serum free cultivation media costs

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Cultivated meat may be a more ethical, environmentally friendly, antibiotic-free meat alternative of the future. As of now, one of the main limiting factors for bringing cultivated meat to the market is the high cost of the cell culture media and their great dependency on serum albumins, production of which is predicted to become a major bottleneck of this industry. Here, we identified several food grade, low-price medium stabilizers, exhibiting comparable or even superior stabilization of the B8 medium in short-term cultivations, as compared to recombinant human serum albumin (HSA). We show transferability of our approach on porcine, chicken satellite cells, and CHO cell line, though significant cell-line specific differences in response to stabilizers were observed. Thus, we provide an alternative to HSA, enabling up to an overall 73% reduction of medium price.

Accelerating DBTL cycles to Develop Sustain-able Protein-Based Biomaterials through robust data models and lab-in-the-loop AI tools

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Cambrium is a biotechnology company at the forefront of the design, development, and manufacturing of novel protein building blocks for innovative materials and products. Our platform harnesses AI-based protein design and precision fermentation to facilitate efficient design-build-test-learn (DBTL) cycles, enabling the fast development of proteins with specific material properties.
We use proprietary AI-based protein design tools to design novel proteins tailored for material use cases. This computational design phase allows us to explore vast sequence landscapes and identify high-performing candidates at a low cost, in silico, before testing them in living organisms. This approach ensures that only the most promising designs are brought to the laboratory for a cost-effective evaluation.
We use Pichia pastoris as our workhorse organism for protein expression. The size of any design library and the throughput capacity of our lab have a direct impact on our ability to identify proteins with desired properties. To address this, we have established an automated, pipette-free robotic platform for protein screening. This system streamlines the construction of Pichia strains through the targeted chromosomal integration of payload DNA and enables simultaneous screening of multiple strain libraries.
Our high-throughput screening platform evaluates library candidates based on protein production, employing plate-based quantification methods that facilitate accurate comparisons. High-scoring candidates are then validated in controlled fermentations. To de-risk the scale up from plate to production scale, our screening platform includes fermentations at different scales to assess production robustness and ensure reliable results.
Finally, the learning component of our DBTL platform is enabled by a comprehensive data infrastructure, encompassing an electronic lab notebook in combination with a cloud-based data “lakehouse” architecture. Core to this infrastructure are data models for end-to-end tracking of experimental data and metadata, with automated processing to yield AI-ready data on the fly. Data generated throughout all phases of the cycle is used to train and refine ML models. These models intervene at each step of the design, from DNA to controlled fermentations. This infrastructure allows continuous improvement of the entire platform.
One notable outcome of our approach is NovaColl™, a 100% skin-identical vegan collagen. Developed with our R&D platform, NovaColl™ exemplifies the potential of AI-driven protein design. By efficiently scanning the combinatorial space of collagen molecules for specific characteristics, and screening candidates using our Pichia-based platform, we identified and scaled a lead candidate with unique structural and skin-active properties.

Dynamic Control Flux Balance Analysis Accurately Maps the Design Space for 2,3-Butanediol Production

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2,3-Butanediol is a valuable raw material for many industries. Compared to its classical production from petroleum, novel fermentation-based manufacturing is an ecologically superior alternative. To be also economically feasible, the production bioprocesses need to be well optimized. However, biotechnological process optimization is often hindered by incomplete solution space characterization, leading to suboptimal conditions.
To address this, we improved a dynamic control algorithm that uses flux balance analysis for process optimization: dcFBA. To enhance the algorithm’s efficiency we reduce the number of required Karush-Kuhn-Tucker condition constraints and improve the implementation of moving finite elements. We find that our improvements achieve a speed-up of at least a factor of four compared to a previous method, with even greater gains when process length was included in the objective function.
We applied this optimization approach to the production of 2,3-butanediol through fermentation in E. coli. Using two-stage fed-batch process simulations, we identified proportionality and trade-off regions within the solution space. This allowed us to design close-to-optimal production processes that maximized both titer and productivity, achieving a experimentally validated titer of 43.6±9.9 g/L and a productivity of 1.93±0.08 g/(Lh). Further optimization of a continuous two-reactor process may increase productivity more than threefold with minimal impact on titer and yield.
The dcFBA algorithm presented here demonstrates the potential for significant improvements in biotechnological production processes, for example, for the production of 2,3-butanediol. We are confident that our method offers a path towards economically feasible and ecologically superior alternatives to traditional petroleum-based production of many chemicals.

A novel colorimetric enzyme-coupled colony filter assay for high-throughput screening of PHB-Depolymerases.

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PHB-depolymerases (PHBDPs) are a group of hydrolytic enzymes that release 3-hydroxybutyric acid from the polyester poly-3-hydroxybutyrate (PHB). This biodegradable polymer is utilized by some bacteria as a carbon and energy storage pool. Due to its environmentally friendly nature and thermoplastic properties, it is suitable for many things such as packaging but also applications in the biomedical and pharmaceutical field. Isolated PHBDPs are useful for the controlled degradation of PHB-based structures, where they can be surface bound to the hydrophobic material, resulting in composites with interesting decomposing and release properties. Therefore, PHBDPs with specific, optimized attributes are highly desirable.
In this project, a novel high-throughput screening method was developed to identify PHBDP variants with altered enzyme properties. The screening process involves transferring bacterial colonies onto PHB-coated filter papers, followed by exposure to elevated temperatures. Colonies exhibiting altered PHBDP activity were identified by the increased release of 3-hydroxybutyric acid in their vicinity, which is visualized via the chromogenic reaction of a coupled enzyme cascade. The detection enzymes used are a 3-hydroxybutyric acid dehydrogenase (HBDH) together with a diaphorase. They convert the substrate iodonitrotetrazolium chloride (INT) into INT-formazan, resulting in a visible red spot surrounding colonies with enhanced PHBDP activity. The intensity of the red spots allowed rapid identification and selection of PHBDP mutants with superior properties.
The presented method shows significant potential for the efficient identification of PHBDPs with various improved functionality by application of selected selection pressures, enabling the development of enzymes tailored for specific industrial and environmental applications.

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Development and testing of polymer-encapsulated, amine-functionalized iron-based contrast materials in animal model

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Background
Magnetic nanoparticles (NPs), particularly superparamagnetic variants such as magnetite, maghemite, and various ferrite NPs, emerge as promising alternatives to traditional Magnetic Resonance Imaging (MRI) contrast agents (CAs). Their heightened specificity and biocompatibility make them attractive candidates [1, 2]. The escalating demand for stable and precisely tuned magnetic NPs in biomedical applications highlights their significance. However, the preparation of these NPs remains a persistent challenge.

Methods
Two different solvothermal methods (12 h reflux and a 4 min microwave, MW) were used to synthesize amine-functionalized ferrite, superparamagnetic NPs, doped with Zn2+ and Cu2+ ions. To overcome stability problems in the colloidal phase, the ferrite NPs were embedded in polyvinylpyrrolidone and could be easily redispersed in water.
The morphological characterization of the NPs was executed by High Resolution Transmission Electron Microscopy, Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS). For detecting the supramolecular interactions and crystalline structure, Fourier Transform Infrared Spectroscopy and X-ray Powder Diffraction was utilized. The in vitro and in vivo MRI measurements were performed with a PET/MR system (Mediso, Budapest, Hungary).

Results
In case of the Zn-doped NPs, the conventional solvothermal synthesis (ZnFe2O4-NH2 Refl.) resulted in a more stable system as compared to the microwave-assisted synthesis (ZnFe2O4-NH2 MW). These results were supported by DLS and AFM measurements, as well as in vitro MRI measurements, where inhomogeneities in the signal were detected.
The CuFe2O4-NH2 MW samples however showed increased colloidal stability as well as homogenous MRI signal in vitro and in vivo. After injection, consistent with other SPION NPs, both samples exhibit a concentrated presence in the hepatic region of the animals, with comparable biodistribution and pharmacokinetics suspected.

Conclusion
Our investigation shows that the ferrite NPs are a feasible candidate for a new generational, multimodal MRI CA. Their chemical properties, owning an -NH2 group holds great options for surface modifications with chelators for isotopes or fluorescent pigments for multimodal molecular imaging purposes. It must be highlighted that the preparation method as well as the nature of the applied precursors play a crucial role in the synthesis of a stable system.

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Cutting-Edge Fermentation Methods for CO_2 Utilization Using the Bioplastic-Producing Bacterium *Cupriavidus necator*

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Gas fermentation is a revolutionary technology for microbial CO2 assimilation and reduction. Thereby, the carbon is fed back into the carbon cycle and greenhouse gas emissions are mitigated. The cultivation of hydrogen-oxidizing Cupriavidus necator on CO2 for the production of bioplastics (poly-(R)-3-hydroxybutyrate (PHB)) has become a central focus of both research and industry. Current bottlenecks in cultivating this organism relate to the design of bioreactors that can be used for explosive gas mixtures of the needed substrate gases (O2, H2, CO2) and the precise control of dissolved oxygen concentrations due to biological limitations. Here we report on the bioreactor design as well as parameter optimizations for high cell density production of C. necator H16. We show how safe lab-scale fermentations can be established in order to cultivate hydrogen-oxidizing bacteria without gas limitations. By implementing continuous chemolitotrophic fermentation set ups, bacteria were grown under reproducible conditions with biomass yields of up to 15 g/L (Lambauer and Kratzer, 2022). In addition, a precise automation and control model was applied to finely adjust gas concentrations for optimal cell growth and PHB production. We showed, that low concentrations of 0.75 mg/L dissolved oxygen (dO2) significantly enhance biomass and PHB production under chemolitotrophic conditions (Lambauer et al. 2023). Further optimisation of the media composition led to a final biomass concentration of 53 g/L dry cell weight with a PHB content of 75 % with CO2 as sole carbon source. It is to highlight, that all fermentations were carried out without pH control or additional feeding. With this multi-faceted approach, we hope to improve the efficiency and sustainability of gas fermentation processes and gain new insights into critical media components under chemolitotrophic conditions.

Electro-Driven Biocatalysis for Sustainable Chemical Synthesis in a Future Bioeconomy

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A bioeconomy, essential for carbon neutrality, depends on rethinking industrial biotechnology processes. Traditional reliance on glucose from starch and sucrose is increasingly challenged by land use and food industry concerns. Bioelectrocatalysis offers an environmentally friendly alternative, enabling precise chemical transformations with minimal waste, crucial for both bulk and fine chemical production.
Our research explores the integration of biocatalysis with electrolysis and covers enzymatic and whole-cell systems using electrical energy to create new possibilities for chemical synthesis.
We developed a system of immobilised biocatalysts including O2-tolerant hydrogenases [1, 2] in a continuous flow setup using molecular hydrogen from water electrolysis for electro-synthesis of chemicals [3, 4]. This approach allowed the reliable synthesis of chiral cyclohexanedione derivatives using flavin and enoate reductase, as well as N-heterocyclic derivatives utilizing NADH, putrescine oxidase and imine reductase [3, 4]. We have integrated enzymatic systems into Cupriavidus necator for CO2 sequestration and efficient H2 oxidation, using a developed electro-fermentation reactor for safe chemical synthesis [5]. Our work gave valuable insights into interplay between biocatalysts and electrolysers, improving yield and efficiency and demonstrated scalable biotechnological advances.
Conclusion:
The integration of water electrolysers with H2-driven enzymatic systems, together with advances in lithoautotrophic biorefineries represents a significant step forward in biocatalysis. This approach has potential for scale-up and is in line with the goals of a sustainable bioeconomy, paving the way for greener and more efficient chemical production.

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Textile waste upcycling from a biotechnological perspective

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Textile waste poses a serious challenge nowadays as lifespan of clothing is decreasing together with increasing consumption. Textiles often consist of blends comprising natural fibers such as cotton, viscose or wool as well as synthetic fibers such as polyester or polyamide. Synthetic fibers are not biodegradable and therefore pollute the environment if they end up in landfills which is the currently most applied route for textile waste. For the purpose of recycling, these fiber blends need to be separated which can be achieved by biotechnological methods. Cellulosic (cotton, viscose) or protein (wool) fibers can enzymatically be hydrolyzed from blends thereby recovering pure synthetic fibers (polyester or polyamide) for re-application in textile production. At the same time, released hydrolysis monomers represent a starting point for valorization approaches which was accomplished through cultivation of different microorganisms. Glucose from cellulose hydrolysis was successfully converted into lactic acid (LA), polyhydroxybutyrate (PHB), or bacterial cellulose (BC) by Weizmannia coagulans, Cupriavidus necator, and Komagataeibacter sucrofermentans, respectively. Amino acids from wool hydrolysis could be used as nitrogen source by Chlorella vulgaris and Rhodotorula mucolaginosa to obtain valuable bioproducts including pigments (chlorophyll, carotenoids) and lipids. The obtained biopolymers (PHB, BC and PLA with LA as building block) could be re-introduced in the textile industry together with extracted pigments as natural dyes. Therefore, biotechnological tools enable not only separation of blended fabrics but also valorization of recovered fractions for a sustainable and circular bioeconomy.

EcoFusion: Pioneering Light, Air, and Nature for Microplastic Degradation and Recycling

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Microplastics are tiny bits of plastic that end up in the environment. Right now, we often dispose of plastic in ways that harm the environment. In this project we investigated a new method to recycle microplastics by creating a special enzyme that uses light, and air to break down plastics in water.
Plastic pollution is a substantial global issue, necessitating environmentally friendly alternatives to conventional disposal practices such as burial, which prove detrimental to the ecosystem. Particularly resilient plastics like polyethylene and polypropylene present challenges in degradation. The primary aim of this project is to discover an improved method for upcycling these plastics into valuable materials, aligning with the concept of a sustainable and closed-loop society.
We use light, air, a strong oxidant-producing protein, and a hydrophobic anchor to attack, degrade, and valorize microplastic in water at ambient temperatures. We apply light-controlled reactive oxygen species (ROS) producing enzymes (LOV-FPs: light oxygen voltage- fluorescent proteins) attached to a hydrophobic tail (hydrophobins) that acts as plastic-recognition element (an anchor) for the oxidative degradation of polyolefins (low-density PE, PP). The desired degradation products are organic acids, which are key platform chemicals for the chemical industry.

CSBJ

Science Flash

Structure-function relationships in NDP-sugar active SDR enzymes: Fingerprints for functional annotation and enzyme engineering

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Nucleotide Sugar active Short-chain Dehydrogenase/Reductase enzymes (NS-SDR) are involved in the biosynthesis of rare sugars and glycosides. Some NS-SDRs have been extensively studied as they are linked to metabolic disorders or virulence factor biosynthesis, others are lesser studied. Here, we combined knowledge gathered from earlier studies (typically focused only on one activity) with an in-depth analysis and overview of the different NS-SDR families (169,076 sequences). Through a structure-based multiple sequence alignment of NS-SDRs retrieved from databases, we could identify clear patterns in conservation and correlation. Supported by this analysis, we suggest extending the “hexagonal box model” of UDP-galactose 4-epimerase to an “heptagonal box model” for all NS-SDRs.[3] This specificity model consists of seven regions surrounding the NDP-sugar substrate that serve as fingerprint for each specificity. These fingerprints will be beneficial for functional annotation of NS-SDR enzymes, discovery of novel specificities and form a basis for enzyme engineering efforts. As such, we have already discovered one new specificity (i.e., promiscuous GDP-sugar 4-epimerase[2]) and found multiple new CDP-tyvelose 2-epimerase-like enzymes capable of converting UDP-glucose into UDP-mannose [3]. In addition, we have successfully created improved variants via enzyme engineering based on this in silico analysis and novel specificity model.

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Development of orthogonal pairs able to equip enzymes with non-canonical amino acids containing silicon and tertiary amines

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Protein engineering has emerged as a proficient technology to improve the catalytic performance of enzymes. It can increase promiscuous activities or even to generate biocatalysts catalyzing “new-to-nature” reactions. However, the chemistry proteins can incorporate is limited by the fixed 20 canonical amino acids prescribed by the standard genetic code. The expansion of the genetic code with non-canonical amino acids (ncAAs) carrying new side chain chemistries is a promising approach to expand the side chain chemistry of proteins and, subsequently, the chemical reactions enzymes can perform. In this project, we aim to equip enzymes with Si-groups and tertiary amines (tA) for improved bioorthogonal labeling or even “new-to-nature” biocatalysis. We will introduce the new chemistries by using non-canonical amino acids (ncAAs) as building blocks for enzyme engineering. To achieve this, we will take a computational approach which will be validated in the laboratory. We will generate orthogonal aminoacyl-tRNA synthetase/tRNACUA pairs for Si- and tA ncAAs to allow their incorporation in response to an in-frame amber stop codon. The genetic encoding of Si-ncAAs will facilitate metal-free site-selective hydrosilylation as an alternative approach to, e.g. copper(I)-catalyzed azide-alkyne “click chemistry”. tA-ncAAs will serve to replace catalytic proline in the active site of an enzyme.

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

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The development of alternative chemical production is essential for achieving a circular bioeconomy. The EU-funded project "ConCO2rde" harnesses the chemolithoautotrophic metabolism of the knallgas bacterium Cupriavidus necator to develop biorefineries that convert CO2 into high-value compounds, such as nitrogen-heterocycles, using CO2 as sole carbon source, O2 as the electron acceptor, and H2 as the electron donor. Although this system presents a carbon-neutral production method by valorizing CO2, improving product yields and creating robust platform strains for diverse biotransformations are still needed.
In this study, our aim is to expand the array of products obtained from C. necator by establishing an enzymatic cascade to produce nitrogen heterocycles. The cascade includes an engineered O2-dependent putrescine oxidase (PuOx), an NADH-dependent imine reductase (IRED), and the O2-tolerant NAD+-reducing soluble hydrogenase (SH) of C. necator, used for cofactor regeneration [1, 2, 3]. For this purpose, we constructed different C. necator strains and evaluated their protein production. Gel immunoblotting analysis and activity assays of the soluble extract of C. necator cultivated under heterotrophic and autotrophic conditions showed that IRED was heterologously produced and was active. Further investigation will focus on the production and activity of PuOx, and cultivation strategies will be optimized to achieve higher production titers of nitrogen heterocycles. Integrating the enzymatic cascade with the autotrophic metabolism of C. necator offers a promising approach for a sustainable chemical production process and contributes to advancing CO2-based bioeconomy.

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Biocatalytic Cascade for the Selective Synthesis of Asymmetric Pyrazines

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Pyrazines and, more precisely, alkyl pyrazines, are key additives in food industry which mimic the organoleptic properties of ingredients like coffee or cocoa, such as roasty, nutty or earthy smells. Despite the interest that both the scientific and industrial communities have shown in recent decades regarding these products and their derivatives, a viable solution for the selective synthesis of pyrazines in a reproducible manner within the confines of current food industry regulations has yet to be found. Consequently, the industry is compeled to continue obtaining this valuable additives as complex mixtures with low yields through extraction from molasses. In this work, we propose a simple and selective concurrent cascade procedure for the synthesis of these target molecules through a dimerisation followed by incorporation of electrophiles of different natures, under completely environmentally friendly conditions, establishing the foundations for a future efficient industrial process for their mass production.

Introducing a heterologous ribulose monophosphate pathway to *Komagataella phaffii* for increasing energy efficiency on methanol

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Metabolic engineering of microorganisms presents promising opportunities for tailoring established cell factories to support sustainable biotechnological production processes. Recently, there has been growing interest in utilizing one-carbon (C1) substrates for the biotechnological production. Methanol, for example, can be directly derived from greenhouse gases such as methane and carbon dioxide—both potent contributors to global warming. Employing native or synthetic methylotrophic cell factories holds the potential to establish a circular bioeconomy.

Komagataella phaffii (Pichia pastoris) is a prominent industrial production host, primarily recognized for heterologous protein production and, more recently, for non-protein products. This aerobic methylotrophic yeast relies on its native xylulose monophosphate (XuMP) cycle for methanol utilization. However, by looking into nature, more energy-efficient methanol utilization pathways, such as the ribulose monophosphate (RuMP) cycle observed in certain bacteria, can be identified.

In this study, our objective was to metabolically engineer K. phaffii towards a more energy-efficient methanol assimilation by incorporating the RuMP cycle from Bacillus methanolicus. We were able to re-engineer the yeast with a peroxisomal heterologous RuMP cycle. The resulting strain, designated RuMPi, demonstrated the ability to grow on methanol as the sole carbon and energy source. However, the strain exhibited an inferior biomass yield on methanol compared to the wild type, accompanied by a very long doubling time of tD = 4 days. Through 52 generations of Adaptive Laboratory Evolution (ALE), the strain significantly improved its doubling time to tD = 17 hours. Ongoing optimization efforts aim to further enhance the strain's performance, eventually offering the possibility of incorporating RuMPi in methanol-based bioeconomies.

Machine Learning for Advanced Growth Media Optimization with a Fully Automated Microbioreactor

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Bioprocesses play a pivotal role in the efficient manufacturing of specialized products such as antibiotics and enzymes. Selecting appropriate microbial strains and optimizing culture conditions are vital to ensuring the economic feasibility of these processes. One major hurdle is developing a fermentation medium that meets the specific nutritional demands of the strain, a task that is often labor-intensive and time-consuming. Currently, Design of Experiments (DoE) is the standard approach used for growth media development. Although DoE helps to reduce the number of experiments, this approach has its limitations, particularly when addressing nonlinear systems. In addition, two-stage DoE processes (screening and optimization) may discard factors too early, deeming them irrelevant before they can prove significant in later optimization stages. In this context, Machine Learning (ML) algorithms for optimization offer a potentially more efficient alternative.

We introduce a fully automated microbioreactor system that autonomously mixes nutrients from various stock solutions and tests their efficacy through iterative cycles, removing the need for manual adjustments. The system integrates a BioLector microbioreactor, which facilitates the parallel cultivation of 48 distinct cultures in a microtiter plate while simultaneously tracking key bioprocess parameters such as biomass, dissolved oxygen (DO), and pH levels in real time. The OT-2 liquid handling system automates tasks such as medium preparation, plate sterilization, and inoculation.

During the initial experiment, 48 different media compositions are determined using a DoE-based approach. After the first cultivation cycle, the system automatically cleans and sterilizes the microtiter plate, preparing it for the next round of medium development. From the second cycle onward, the medium compositions are generated by an ML algorithm using Bayesian Optimization. Fresh pre-cultures are automatically introduced into each of the 48 growth media, and this process is repeated until an optimal medium formulation is identified.

The preliminary data sets are promising. The chosen model organism was MICP-relevant bacterium Sporosarcina pasteurii. The ML-optimized medium showed a 34% improvement in the maximum backscatter value compared to the medium optimized through DoE. The ML algorithm is set to be further diversified and applied to additional strains to validate its broader applicability.

Intensified biomanufacturing with *E. coli:* Establishing of greener processes with continuous cultivation strategies

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The development of low environmental footprint manufacturing processes is a challenge in the biopharmaceutical industry. There is an emerging importance of sustainability aspects of the biopharmaceutical production and the analysis of potential risks related to the release of active pharmaceutical ingredients (APIs) to the environment [1]. The ENVIROMED project [2] focuses on the holistic assessment of (bio)pharmaceutical production, usage and final disposal. In this study, the authors are demonstrating the potential of intensified bioprocessing to mitigate the high demands of purified water, chemicals and energy throughout the upstream process.
A gate-to-gate approach was chosen to compare conventional fed-batch processing with intensified operations. “Green metrics” with respect to chemical usage and energy demands reflect the environmental footprint of the upstream process [3]. In the first step of the sustainability analysis, the system boundary of the upstream process was defined by the following steps: (I) equipment cleaning and sterilization, (II) pre-culture cultivation, (III) fermentation in the bioreactor and (IV) harvest of the cell broth. The use-case is the lab-scale production of a recombinant fragment antigen binding (Fab) using the Escherichia coli strain W3110.
The investigated intensified processes (repetitive fed-batch, chemostat, cascaded processing) were significantly decreasing energy and water/chemical demands (up to 30 %) compared to the conventional fed-batch. This is due to long equipment downtimes, repeated cleaning efforts, non-productive (non-induced) phases and high energy demands in conventional biomanufacturing. Additionally, keeping up the cell-specific productivity by tailoring process parameters was a crucial step towards the establishment of an intensified process.
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Brilliantly lazy: How *C. necator* only shines at avoiding work

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The current state of affairs, with ongoing climate crisis and depletion of fossil resources, calls for innovative industry to fulfill market demands while minimizing the impact on our planet. Biotechnology offers an attractive solution for classic petrochemistry, as microorganisms are equipped with countless pathways for the creation of value-added products from simple carbon sources such as glucose, cellulose or even CO_2. Additionally, microorganisms’ pathways can be expanded upon in with synthetic biology, unlocking near-infinite end-products.
Such genetically enhanced microorganisms are a vault of untapped potential; however, engineering efforts are more laborious than expected due to the complex and dynamic nature of microbial metabolism and regulation. It is for this reason unsurprising that when challenged with upscaling, characterized by stressful conditions and longer cultivations, the performance of the process is lower than anticipated. Furthermore, when faced higher number of generations, the productivity of these strains depreciates, reducing economic profit. This phenomenon is ubiquitous and is termed strain degeneration.
Here we describe the cunning strategies through which bacteria evade exploitation by studying engineered Cupriavidus necator for heterologous isopropanol production. Employing enhanced Green Fluorescent Protein (eGFP) as a biosensor, we have managed to follow strain degradation in the form of plasmid expression loss in continuous cultures. We have established that the expression of the isopropanol operon results in a drastic loss of plasmid expression in an increasing part of the population, which is not caused by a growth impairment. Through a multidisciplinary approach involving traditional counting methods and molecular techniques such as qPCR and sequencing we have attempted to ascertain the various mechanisms at play in this loss of expression, and through diverse strategies we have ventured to tackle the matter of strain degeneration.

Increasing electroporation efficiency in the lithoautotrophic bacterium *Cupriavidus necator* H16: A roadmap for non-model bacteria domestication

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Climate change is an urgent and collective challenge, and a portfolio of solutions is needed to reduce CO2 emissions or to increase carbon capture and utilization from the atmosphere. Nature has been evolving CO2 utilization pathways for billions of years and offers a promising repository of novel metabolisms and enzymes capable of CO2 fixation. However, non-model bacteria are recalcitrant to genetic engineering, and the application of modern genetic tools is cumbersome. One of the main barriers is the low transformation efficiency, as most tools and technologies require the delivery of DNA molecules to tune and modify the host metabolism. This transformation barrier is a common feature of all wild-type bacteria, which employ a variety of defense systems to avoid phages, plasmids, and other mobile genetic elements in their native ecological niche. To transform them with recombinant DNA, this arsenal has to be predicted, characterized and circumvented.

In our study, we performed an in-depth analysis of Cupriavidus necator H16 using bioinformatic tools to study its restriction enzymes and defense systems. By using tailored plasmids, we confirmed the functional role of three systems encoded in the genome, and through a combination of plasmid mutation and demethylation, we transformed large plasmids with higher efficiency. We also succeeded in transforming suicide plasmids via electroporation, deleting the native defense systems and creating a domesticated strain.

These findings will benefit both the C. necator H16 community and researchers working with other non-model bacteria by providing a roadmap that can be followed to increase transformation efficiency.

Moving towards the utilisation of CO_2-rich industrial off-gas streams for isopropanol formation by *Cupriavidus necator*

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Valorising CO2-rich industrial off-gas streams by microbial fermentation is appealing as it reduces CO2-emissions while harnessing an underexploited carbon source for the production of value-added compounds. The Knallgas bacterium Cupriavidus necator is a promising chassis strain, having been successfully engineered to produce various compounds. This study focuses on isopropanol formation as a model system.
An improved autotrophic shake flask system was developed to characterise several isopropanol-producing strains and compare with their heterotrophic performance. While growth and product formation rates were slower under autotrophic conditions, the product yield was comparable to heterotrophy. Scaling up to a 7.5 L bioreactor yielded promising final isopropanol concentrations of 12 g L-1 with a maximum productivity of 0.4 g L-1 h-1. This process was extensively characterized, including gas uptake rates during both the growth and product formation phases.
Biogas was evaluated as a potential gas source aiming to upgrade it by reducing the CO2 content. Cultivations in shake flasks were conducted where biogas composition supplemented with H2 and O2 was simulated using lab-grade gases. It was demonstrated that initial methane concentrations of up to 24 % did not negatively impact growth or isopropanol production. Cultivations using real industrial biogas are ongoing.
This work demonstrates the feasibility of CO2 fixation for isopropanol production at concentrations exceeding the g/L level, while providing essential data for feasibility studies and identifying process bottlenecks to guide further process intensification.

Enzymatic C=C bond cleavage: characterization and engineering of a new dioxygenase

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Aromatic DiOxygenases (ADOs), are enzymes belonging to the family of carotenoid cleaving enzymes (CCOs or CCDs). They are non-heme-iron-dependent enzymes characterised by the chelation of a Fe2+ active centre by 4 histidine residues. They activate molecular oxygen and produce two species of carbonyl compounds from a substrate with a C=C double bond.
Based on TtADO from Thermothelomyces thermophila[ ] we identified and expressed 13 novel ADOs. We call them 'ozonylases', because these enzymes may be a substitution for ozonolysis – a rather dangerous process on scale due to the formation of explosive intermediates. Among the tested enzymes, MapADO from the marine fungus Moesziomyces aphidis was found to be the most effective enzyme not only converting isoeugenol to vanillin, but also accepting 4-vinylguaiacol, hydroxyanethol, corniferyl alcohol and resveratrol as substrates. Kinetic studies revealed MapADO possessing one of the highest vmax, km and kcat for isoeugenol, with 230 µM/min, 120 µM and 240 s-1, respectively. Rationally-designed mutants of this enzyme further increased activity almost 3 times in the case of resveratrol. Mutagenesis also revealed key insights in substrate binding of MapADO. Using a 200 mL preparative scale with whole cells, we achieved quantitative conversion of 50 mM isoeugenol to the corresponding aldehyde overnight. We isolated 1.3 grams of vanillin. We are now exploring random mutagenesis with high mutation rates in combination with screening by rapid-fire MS-MS.

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Optimising the cultivation of *Crocosphaera chwakensis* for efficient production of Cyanoflan

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The skin is the largest human organ and requires adequate care. Currently there is a growing trend to avoid synthetic components in skin care products due to public awareness of their long-term risks to the environment and human health. Therefore, novel natural polymers are being explored to be used in cosmetics and personal care products as active and/or non-active ingredients. Cyanobacteria are prolific sources of added value biocompounds, such as extracellular polymeric substances (EPS) [1]. The marine cyanobacterium Crocosphaera chwakensis CCY0110 was chosen due to its ability of constantly release a complex and versatile carbohydrate polymer, named Cyanoflan[2]. Starting with the aim of implementing a constant, timely and cost-effective industrial production of Cyanoflan, the optimization of the culture conditions and the isolation procedure was envisaged. Firstly, the most important culture parameters to be tested were selected: light intensity, temperature, and agitation. Then, the growth was evaluated using standard parameters, optical density, and chlorophyll a content, throughout the cultivation period of one month. In addition, the content in total carbohydrates (intracellular and extracellular) and in carbohydrates released to the culture medium (RPS) were assessed. As expected, preliminary results suggest that all the parameters tested affect the growth of C. chwakensis and consequently Cyanoflan production, with light intensity being the most important. The light intensity inside the reactor is modelled mechanistically and coupled to a CFD simulation to transiently track the intensity cells are exposed to. The obtained parameter setpoints were integrated in modelling software to develop a digital twin of the bioreactor and associated processes aiming to improve our understanding of cyanobacterial cultivation in terms of efficiency and precision. The result of this study will pave the way for optimising the industrial-scale production of Cyanoflan, with the ultimate goal of commercialising it as a natural ingredient for cosmetics and personal care.

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Biosynthesis of zinc oxide nanoparticles from *Weissella cibaria* UPM22MT06 supernatant: High antibacterial efficacy and zero cytotoxicity

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Introduction
Advancing nanotechnology with eco-friendly nanoparticle synthesis is pivotal for expanding nanomedicine applications. The emergence of multidrug-resistant (MDR) bacteria, notably methicillin-resistant Staphylococcus aureus (MRSA), poses a significant challenge in clinical environments. This study explores the biosynthesis of zinc oxide nanoparticles (ZnO NPs) using the bacterial strain Weissella cibaria UPM22MT06, isolated from a wastewater treatment plant, as a potential eco-friendly approach to combat MRSA infections.

Methods
Weissella cibaria UPM22MT06 was cultured, and the supernatant utilized for the biosynthesis of ZnO NPs. Characterization of the nanoparticles was conducted using UV-visible spectroscopy (UV), high-resolution transmission electron microscopy (HR-TEM), dynamic light scattering (DLS), and Fourier-transform infrared spectroscopy (FTIR) to confirm their size and stability. The antibacterial efficacy of the synthesized ZnO NPs was evaluated using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods against MRSA. Additionally, cytotoxicity was assessed on the HS-27 fibroblast cell line using the MTT–(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays.

Results
Characterization revealed that the ZnO NPs exhibited a spherical morphology with sizes ranging from 2.3 to 7.5 nm. The antibacterial assays demonstrated significant antibacterial activity, with MIC values as low as 0.625 mg/mL and MBC values of 1.25 mg/mL against MRSA. Furthermore, the cytotoxicity results indicated that the ZnO NPs were non-toxic to human fibroblast cells, suggesting their safety for potential biomedical applications.

Conclusion/Impact
This study highlights the potential of Weissella cibaria UPM22MT06 for the eco-friendly synthesis of ZnO NPs, which exhibit promising antibacterial properties against MRSA while maintaining low cytotoxicity. The findings contribute to the development of green nanotechnology and offer a novel approach to addressing the growing challenge of antibiotic resistance in clinical settings.

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Posters

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2	**A. S. HILTS**	Towards holistic metabolic engineering of Methanothermobacter marburgensis using a combination of *in silico* methods
3	**A. ARASTEH KANI**	Shedding Light on Non-Canonical Amino Acid Incorporation: The Bright (and Not-So-Bright) Side of Using Dual Fluorescence Proteins
4	**B. SEMLER**	Sustainable Degradation: Novel Enzymes for Advanced Biopolymer Degradation
5	**C. KÖPPL**	Modifications of the 5' region of the CASPONTM tag's mRNA further enhance soluble recombinant protein production in *Escherichia coli*
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POSTER CATEGORIE

Biotech in General

Novel methanol-free Pichia pastoris platform for protein production

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Yeasts are established eukaryotic host systems for heterologous protein production. Their major advantages are growth to high cell densities, inexpensive media, easy genetic manipulations, and their capability of posttranslational modifications.
We use the methylotrophic yeast Pichia pastoris (Komagataella phaffii) for production of proteins and enzymes. It is known for high cell densities, high expression levels, protein secretion and good scalability. Pichia pastoris is free of endotoxins and generally recognized as safe (GRAS). Most frequently the strong methanol inducible AOX1 promoter is used for protein expression. However, the flammability and toxicity of methanol causes challenges in scale-up as well as safety concerns in food and related applications. This creates a strong demand for alternative methanol free strategies with competitive productivity.
We can build on more than 15 years of experience in toolbox development using Pichia pastoris to overcome hurdles in recombinant protein production. Building on license-free tools and own developments we have established our own toolbox for methanol-induced and methanol-free production. For applications in food and feed, we solely build on methanol-free and antibiotic-free processes.
Here, we show how we apply our expression platform for development of scalable methanol-free processes for protein production.

Towards holistic metabolic engineering of Methanothermobacter marburgensis using a combination of *in silico* methods

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The last decade has witnessed the ushering in of powerful computational methods to better understand how cells orchestrate their metabolisms. In particular, as computers have become more powerful, it has become increasingly easy to build models that are capable of approximating full metabolic networks in various organisms. However, there remains significant under-explored biological space with the potential to lead to industrial innovations. The archaea are a group of poorly characterized organisms comprising one of the three domains of life, capable of unique metabolisms and growth under extreme conditions. The lack of complete databases of genomic and proteomic information for these fascinating organisms has historically posed significant challenges, but the community is now reaching a stage where it is becoming possible to holistically study these organisms. Here, we present an update to a previously published genome-scale metabolic model (GEM) of Methanothermobacter marburgensis. We have worked to update missing metabolic steps and to incorporate information about the regulatory processes this species is capable of at the transcription level. We illustrate that these tools have the potential to lead to highly specialized cell factories and provide an overview of how we are developing these models to lead to the production of industrially relevant compounds.

Shedding Light on Non-Canonical Amino Acid Incorporation: The Bright (and Not-So-Bright) Side of Using Dual Fluorescence Proteins

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Establishing a reliable dual-fluorescence protein as a reporter for non-canonical amino acid (ncAA) incorporation can significantly enhance studies involving ncAA incorporation. One promising candidate is RGFP, a fusion protein comprising monomeric red (mCherry) and green fluorescent proteins (mEGFP), with the coding sequences of mCherry and mEGFP separated by an in-frame amber stop codon. When the amber codon remains unsuppressed, translation halts, resulting in red fluorescence. However, upon successful incorporation of an ncAA into the amber stop codon, both red and green fluorescence are observed.

While theoretically sound, the practical implementation of this dual-fluorescence protein system has presented several challenges. These include issues with the expression of both the mCherry and mEGFP components, as well as difficulties in suppressing the amber stop codon. In this presentation, we will shed light on both the bright and not-so-ideal aspects of using dual fluorescence proteins, examining the challenges faced and explaining the changes made to overcome them.

Sustainable Degradation: Novel Enzymes for Advanced Biopolymer Degradation

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This research investigates the mechanisms behind the degradation of biopolymers based on furandicarboxylic acid (FDCA), concentrating on polymers synthesized from FDCA with diols of various chain lengths. The study focuses on the characterization and optimization of novel enzymes derived from the Acidimicrobiales bacterium, as well as commercial enzymes like cutinases from Thermobifida cellulosityca (Thc_Cut1) and Humicola insolens (HiC).

Tailored novel enzymes designed for FDCA-based polymers exhibit superior performance, underscoring the importance of enzyme substrate specificity in enhancing bioplastic degradation efficiency. By optimizing enzyme expression and characterization methods, this research enhances our understanding of catalytic activity and effectiveness in breaking down biopolymers. Mechanistic studies on enzymatic hydrolysis will employ established analytical techniques such as GPC coupled with MALS to detect molecular weight changes and identify oligomers. Gravimetric analysis and SEM will track polymer decomposition, while HPLC will identify and quantify soluble hydrolysis products. NMR spectrometry will ensure the purity of monomers for polymer resynthesis.

Furthermore, this research underscores the broader implications of bioplastic hydrolysis for sustainable resource management. Exploration of alternative feedstocks and fine-tuning enzymatic pathways advocate for a holistic approach to reducing dependency on fossil resources in polymer production.
In summary, this study enhances our understanding of enzymatic bioplastic degradation and provides practical insights for the development of efficient and sustainable recycling strategies. It underscores the crucial role of enzymes in facilitating the transition towards a circular economy, where bioplastics significantly contribute to mitigating environmental impact and promoting resource efficiency.

Modifications of the 5’ region of the CASPONTM tag’s mRNA further enhance soluble recombinant protein production in *Escherichia coli*

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Escherichia coli is one of the most commonly used host organisms for the production of biopharmaceuticals, as it allows for cost-efficient and fast recombinant protein expression. However, challenging proteins are often produced with low titres or as inclusion bodies, and the manufacturing process needs to be developed individually for each protein. Recently, we developed the CASPONTM technology, a generic fusion tag-based platform process for high-titer soluble expression including a standardized downstream processing and highly specific enzymatic cleavage of the fusion tag. To assess potential strategies for further improvement of the N-terminally fused CASPONTM tag, we modified the 5’UTR and 5’ region of the tag-coding mRNA to optimize the ribosome-mRNA interactions. We found that by modifying the 5’UTR sequence of a pET30acer plasmid-based system, expression of the fusion protein CASPONTM-tumour necrosis factor α was altered in laboratory-scale carbon-limited fed-batch cultivations, but no significant increase in expression titre was achieved. However, translation efficiency was drastically altered by the new 5’UTR sequences. Surprisingly, a construct with comparatively low transcriptional efficiency, which lacked an expression enhancer sequence and carried the most favourable ΔGtotal tested, led to the highest recombinant protein formation alongside the reference pET30a construct. Furthermore, we found, that by introducing synonymous mutations within the nucleotide sequence of the T7AC element of the CASPONTM tag, utilizing a combination of rare and non-rare codons, the free folding energy of the nucleotides at the 5’ end (-4 to +37) of the transcript encoding the CASPONTM tag increased by 6 kcal/mol. This new T7ACrare variant led to improved recombinant protein titres by 1.3-fold up to 5.3-fold, shown with three industry-relevant proteins in lab-scale carbon limited fed-batch fermentations. This study, which demonstrates the influence of changes in ribosome-mRNA interactions on protein expression under industrially relevant production conditions, opens the door to the applicability of the new T7ACrare tag in biopharmaceutical industry using the previously developed CASPONTM platform process.

Exploring energy metabolism on C1 carbon sources in the yeast *Komagataella phaffii*

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The methlyorophic yeast Komagataella phaffii (Pichia pastoris) is one of the main expression systems for the production of heterologous proteins (1) and has been an attractive model organism for studies on peroxisome formation (2) and growth on C1 carbon substrates like methanol (3). With growing knowledge about the methylotrophy of K. phaffii (4), the maintenance of energy and redox state during growth on C1 substrates still isn’t fully understood. Considering the attention and potential of C1 carbon sources for bioprocesses (5), unraveling the energy metabolism of K. phaffii during methylotrophy can provide valuable insights for the future biotechnological applications.
In this project, the contribution of the tricarboxylic acid (TCA) cycle to energy formation as a central metabolic pathway bearing potential to produce carboxylic acids in addition to the cytosolic NADH supply is used as pivot point. Contrary to in silico predictions and observations in other yeasts like Saccharomyces cerevsisae (6), interruption of the TCA cycle proves notoriously difficult to introduce and leads to severe growth defect during methylotrophic growth, indicating a main role of the cycle for cellular viability. To understand the role of TCA and source of reducing power on methanol, we focus on redoxactive TCA-cycle enzymes employing NAD+/NADH as cofactor as well as external alternative dehydrogenases. Fluorescent labeling of peroxisome formation under (partial) TCA-disruption can potentially frame these results into earlier studies on peroxisome maintenance (7) and help understanding the role of the TCA cycle for the broader metabolic organization of methylotrophic growth in K. phaffii.

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Development of an SpsA-based chromatography matrix as capture step for secretory immunoglobulin A

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We envision a novel affinity-based chromatography matrix for secretory immunoglobulin A (sIgA) based on the Spore coat polysaccharide biosynthesis protein (SpsA) from Streptococcus pneumoniae. SpsA binds to the secretory component of sIgA and was immobilized on a chromatographic matrix. sIgA is composed of two monomeric IgA molecules, which initially form dimeric IgA (dIgA) through aid of the joining chain. The secretory component is a residual polypeptide of the polymeric immunoglobulin receptor and covalently bound to the Fc-regions of the dimeric IgA.
Commercially available resins utilize expensive nanobody-based ligands and exhibit small pores, which limit mass transfer. The high molecular weight of sIgA requires a large-pored backbone to ensure sufficient diffusional mass transfer which in turn facilitates economic viability. To maximize the potential of the SpsA matrix, a robust immobilization procedure was developed. We used large-pored epoxy-activated resins, which allow for coupling reactions carried out close to physiological pH, while minimizing the usage of harmful chemicals. Directed immobilization allows for a consistent attachment point, ensuring the uniform orientation of the ligand on the backbone. To achieve this, SpsA was expressed with a single cysteine residue, which can be coupled with the epoxide group to form a thioether bond. Placement of the cysteine was varied by rational design of the ligand molecule and their impact on resin performance was evaluated. Moreover, ligand densities and immobilization conditions were optimized to maximize the dynamic binding capacity. Lastly it was compared to commercially available resins in terms of volumetric productivity and mass transfer characteristics.

Ethanol driven protein expression in *K. phaffii*

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The methylotrophic yeast K. phaffii has become a widely used host for the production of recombinant proteins. K. phaffii is able to grow at fast rates to high cell densities while being a GRAS organism that is relatively easy to genetically manipulate. Wildtype K. phaffii can grow on a variety of carbon sources, ranging from hexose and pentose sugars to (sugar)-alcohols such as sorbitol, glycerol, methanol and ethanol as well as acids like acetate, lactate and oleic acid. Most often, the strong and tightly regulated methanol-induced alcohol oxidase promoter (pAOX1) is used to drive protein expression. Metabolization of C1-substrates such as methanol triggers an upregulation of heme-biosynthesis as heme is required for the activation of catalase – an enzyme present in the peroxisomes for detoxification of nascent H¬2O2 produced by oxidation of methanol. Although highly successful, the usage of methanol – a toxic and flammable alcohol - for induction is perceived problematic on industrial scales for operational and product safety concerns. A variety of methanol-free expression systems have been developed already, including constitutive promoters such as GAP, TEF, AOD or ILV5 which have can vary from stronger (pGAP or pTEF) to weaker expression (pAOD or pILV5) compared to pAOX1. K. phaffii can utilize ethanol as a carbon source and it can produce ethanol during fermentation. Ethanol metabolism through the EUT (ethanol utilization pathway) in K. phaffii has been studied to some extent albeit not as extensively as the prevalently exploited methanol metabolism. Several genes and enzymes have been identified which play a crucial role in the ethanol metabolism. ADHs are involved in the oxidation of ethanol to acetaldehyde which is followed by aldehyde dehydrogenases (ALDs) mediated oxidation of acetaldehyde to acetate. Ethanol was also used to drive protein expression under an engineered variant of the pADH2 promoter. This project aims to generate a K. phaffii strain which can produce proteins upon ethanol induction by exploring different ethanol responsive promoters. The system should allow regulation of expression to give the possibility to produce toxic proteins that could not be produced with a constitutive promoter.

Renewable Energy-Driven Lignin Valorization: Scalable Biocatalysis for a Sustainable Future

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Unlocking the true potential of lignin for industrial applications has long been a formidable challenge—until now. Our research focuses on the development of sustainable and efficient tools for the valorization of lignin-derived aromatic compounds (LDACs) such as vanillate, alkylguaiacols, and alkoxybenzoic acids. Through an innovative enzymatic cascade, we aim to convert lignin waste into valuable chemical intermediates. These products include protocatechuic acid and alkylcatechols, which serve as essential building blocks for the synthesis of polymers, biofuels, and pharmaceuticals.
The enzymatic process is catalyzed by three distinct oxidoreductase systems: PbdA/HaPuX/HaPuR, AgcA/AgcB (Class I and Class V P450, respectively), and VanA/VanB (a two-component Rieske oxygenase). These systems were used individually, each coupled with a hydrogen-driven cofactor regeneration system powered by the soluble hydrogenase (SH) from Cupriavidus necator. This approach allows for continuous regeneration of NAD+, enabling sustained catalytic activity without the need for large quantities of reduced cofactors, achieving a total turnover number (TTN) of over 160 for NAD+. This system enabled efficient catalysis at a fraction of the cost without compromising performance.
A critical challenge we addressed was optimizing the interplay between the O_2-dependent oxidoreductases and the H_2-dependent hydrogenase to ensure safe and efficient performance. Comprehensive studies on gas composition enabled us to fine-tune both systems to operate synergistically without compromising efficiency or safety. Notably, we maintained H2 concentrations below 4%, well within safe limits for flammability and explosion risk. This balance not only maximized atom efficiency but also ensured a secure framework for the biocatalytic process.
After successfully optimizing the batch reactions, we scaled the system to a pre-industrial prototype, utilizing an electro-driven flow chemistry setup. This system, equipped with in situ gas production via a proton exchange membrane electrolyzer, continuously supplies H_2 and O_2 to the reaction, making the entire process powered exclusively by renewable energy. The combined benefits of high atom efficiency, safe gas management, and renewable energy sourcing make this system not only highly efficient but also truly sustainable.
To enhance reactor stability and performance, we employed enzyme immobilization through adsorption and entrapment. This setup achieved over 99% yield in 5 hours, demonstrating high catalytic efficiency. To optimize the reaction environment, we kept one section of the reactor open with heavy stirring, allowing natural evaporation of formaldehyde and preventing its accumulation. The reaction was monitored online via TLC, and the flow setup enabled efficient downstream processing, yielding 82.3% recovery of the product as pure powder, confirmed by NMR.
This innovative system represents a major leap forward in the sustainable valorization of lignin-derived compounds. By combining hydrogen-driven cofactor regeneration, precise gas composition management, and electrodriven flow chemistry for upscaling, we offer a clean, scalable, and cost-effective approach to biocatalysis. This breakthrough paves the way for the future of sustainable biotechnology, contributing to a greener and more economically viable industry.

Ploidy and chromosome configuration in human gut-associated *Methanobrevibacter smithii*

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Like most bacteria, archaea have small circular genomes, but in contrast to bacteria, which all have a single replication origin (oriC) per chromosome, archaea have a variable number of origins (between one to more than 10), depending on the species. The oriC is usually composed of two elements: an AT-rich DNA unwinding element and a gene encoding for the initiator protein “Orc1/Cdc6.” In bacteria and archaea with a single oriC, the two replication forks usually meet on the opposite side of the origin on the chromosome—in the so-called termination region (ter). Bacterial chromosomes can assume two major configurations: 1) In oriC-ter (longitudinal) the origin is located at one cell pole and the terminus is at the other pole, and 2) in left-oriC-right (transverse), the pattern is rotated by 90° compared to the oriC-ter configuration, so that both oriC and ter are centrally positioned. Currently, we do not know how archaeal chromosomes are oriented. For the model Crenarchaeon Sulfolobus, this is because these archaea have small round cells that lack recognizable poles. In contrast, a great deal of Euryarchaeota in fact have defined poles, but an additional issue is that many species, including haloarchaea and methanogens, carry several copies of their chromosome per cell (polyploid), making it difficult to decipher the orientation of individual chromosomes.
Here, we studied the ploidy and chromosome configuration of the human gut-associated archaeon Methanobrevibacter smithii. We used qPCR to determine the ploidy of M. smithii, and unlike the current opinion, which is that all Euryarcheota are diploid or polyploid, we show that M. smithii is monoploid. Additionally, we fluorescently labeled the oriC and ter regions on the M. smithii chromosome using DNA FISH. We imaged hundreds of cells using epifluorescence microscopy and performed a quantitative image analysis to reconstruct the oriC and ter localization pattern during the M. smithii cell cycle. For both oriC and ter, one fluorescent focus was observed in non-constricting cells in the proximity of the cell pole, with the oriC being closer to the pole than the ter. Once the cells started to constrict a second focus of oriC or ter, respectively appeared and was segregated along the long axis. These results confirm that M. smithii is monoploid and suggest that the chromosome configuration is longitudinal.
Our cell biological study paves the way for a greater understanding of the biology of methanogens. Research on chromosome biology of methanogens has the potential to reveal genetic adaptations which allow them to survive and thrive under extreme conditions and adapt to various ecosystems. This in turn can provide insights into microbial resilience and lead the way to applying a cell biology approach to expand biotechnology applications of methanogens.

Oxidative biotransformation of HMF catalyzed by UPOs

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2,5-Furandicarboxylic acid (FDCA) is a versatile chemical that finds application in the polymer industry. This building block attracts great interest since it can be accessed via renewable starting materials, i.e. via the catalytic oxidation of biomass-derived 5-hydroxymethylfurfural (HMF). The inclusion of FDCA in the list of the top 12 biobased chemicals by the U.S. Department of Energy (DOE) back in 2004 promoted a wave of research resulting in a wide toolbox of catalytic oxidation methods towards FDCA. The main advantages of biocatalysts in this regard are their selectivity and mild reaction conditions, which are fundamental for a sustainable process. One challenge of enzymatic processes is
to obtain product titers to competitive levels. In this respect, unspecific peroxygenases (UPOs) have shown great potential as oxidation catalysts. Since until now only AaeUPO has been used in cascades with oxidases for the oxidation of HMF in this work we screened 23 different UPOs and found that HspUPO and UPOx8 are the first UPOs capable of performing three consecutive oxidation steps from HMF to FDCA. We also observed that the chemoselectivity in the first oxidation step can be increased by using cosolvents, although accompanied by lower conversions. The combination of HspUPO with HMFO and variants thereof in a self-sufficient cascade led to increased efficiency of the system.
Our results highlight the potential of UPOs for the synthesis of FDCA and contribute to the development of sustainable and efficient methods for the production of value-added chemicals from biomass-derived feedstocks.

pH-Dependent Promiscuous Activity of Cyanobacterial PBP-A Against *E. coli*: Experimental and Computational Insights

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Penicillin-binding proteins (PBPs) play a fundamental role in peptidoglycan (PG) biosynthesis, remodeling, and recycling in bacteria.1 PBP-A from Thermosynechococcus elongatus belongs to a cyanobacterial family closely related to class A β-lactamases. With the goal of converting PBP-A into a β-lactamase through directed evolution, we introduced it into Escherichia coli and observed growth defects linked to its enzymatic activity.2 In vitro and in vivo characterization revealed that PBP-A acts as a DD-carboxypeptidase and DD-endopeptidase with a strong preference for amidated muropeptides. Interestingly, its promiscuous activity on non-amidated PG is exacerbated under acidic conditions, leading to damage of the E. coli cell envelope. Microsecond-long molecular dynamics simulations showed a key interaction between Glu96 at the active site and the amidated substrate, which is reduced or not available in non-amidated substrates, particularly at acidic pH. This study highlights how environmental factors such as pH can influence enzyme promiscuity, leading to harmful effects and limiting the enzyme's evolutionary potential. These findings suggest that directed evolution of PBP-A should be performed under controlled conditions, such as neutral pH, to mitigate adverse effects.

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CO_2 as feedstock in microbial cultivations: gas fermentation to match microbial requirements and technical feasibilities

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Utilizing carbon dioxide (CO_2) as a feedstock in microbial cultivation presents a highly promising and sustainable approach to biomanufacturing, offering dual benefits: the reduction of greenhouse gas emissions and the production of valuable bio-based compounds. This approach leverages on the capabilities of hydrogen-oxidizing bacteria (HOB), such as Cupriavidus necator, which employs the Calvin-Benson-Bassham (CBB) cycle to fix CO_2 into organic molecules (1). In C.necator, H_2 serves as an electron donor, undergoing oxidation to provide both the energy (via ATP production) and reducing power (via NADH) needed for CO_2 fixation. Concurrently, O_2 acts as the terminal electron acceptor, ensuring the completion of the electron transport chain, thereby maintaining efficient energy flow (2). This integration of H_2 oxidation and O_2 reduction drives the conversion of CO_2 into high-value products such as polyhydroxybutyrate (PHB), a biodegradable bioplastic, or isopropanol, which is a bulk chemical (1,4). Despite its significant potential, integrating O_2 and H_2 in microbial cultivation poses technical challenges, particularly the risk of creating explosive gas mixtures (3). In principle, two process options are feasible: the utilization of gas compositions that are outside the explosion limits and the use of explosion-proof gas fermentation equipment. In both cases, careful management of gas flow rates, pressure, and reactor design is required to mitigate these risks. Here, successful cultivations of C. necator under non-explosive conditions resulted in a cell dry weight of 42.52 g/L with up to 70% PHB accumulation. Cultivation parameters have been optimized under stringent safety measures. Furthermore, the use of explosion-proof fermentation set-ups is summarized and discussed. Our results provide useful insights for the implementation of CO_2-based bioproduction in industry, regardless of whether non-explosive experimental setups or explosion-proof fermentation plants are used.

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Exploring the impact of mRNA decay and protein translation on recombinant protein secretion

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K. phaffii is a widely used organisms for commercial, heterologous protein production, however, as a budding yeast, specific productivities are tightly coupled to biomass formation. We have previously identified the translation machinery as one major rate limiting factor at slower growth rates. Overexpression of specific translation initiation factors increased the productivity of several recombinant secreted proteins up to 3-fold in industrially relevant fed-batch cultivations. In addition to this, and enhanced global cellular translation, a strong impact on mRNA levels was observed, indicating that different cellular levels of regulation are tightly interconnected. Additional RNA-Seq analysis showed that particularly mRNA stability was affected in the overexpression strains.

Therefore, as an alternative measure to increase global protein production within the cells, proteins involved in mRNA decay were deleted. Strikingly, this global intervention also led to increased global translation activity, as measured by OPP-labelling assays. Furthermore, the production of secreted heterologous proteins in fed-batch cultivations was increased by up to 4-fold.

Overall, it appears that mRNA levels can be stabilized by either hampering decay or protection by bound translation factors. This in turn has a global impact on general translation activity and with this, production of native and heterologous proteins. Additionally, our data shows the great flexibility of yeast for adaption to global intracellular changes and K. phaffii's not fully exploited potential for protein production.

Structure-function relationships in NDP-sugar active SDR enzymes: Fingerprints for functional annotation and enzyme engineering

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Nucleotide Sugar active Short-chain Dehydrogenase/Reductase enzymes (NS-SDR) are involved in the biosynthesis of rare sugars and glycosides. Some NS-SDRs have been extensively studied as they are linked to metabolic disorders or virulence factor biosynthesis, others are lesser studied. Here, we combined knowledge gathered from earlier studies (typically focused only on one activity) with an in-depth analysis and overview of the different NS-SDR families (169,076 sequences). Through a structure-based multiple sequence alignment of NS-SDRs retrieved from databases, we could identify clear patterns in conservation and correlation. Supported by this analysis, we suggest extending the “hexagonal box model” of UDP-galactose 4-epimerase to an “heptagonal box model” for all NS-SDRs.[3] This specificity model consists of seven regions surrounding the NDP-sugar substrate that serve as fingerprint for each specificity. These fingerprints will be beneficial for functional annotation of NS-SDR enzymes, discovery of novel specificities and form a basis for enzyme engineering efforts. As such, we have already discovered one new specificity (i.e., promiscuous GDP-sugar 4-epimerase[2]) and found multiple new CDP-tyvelose 2-epimerase-like enzymes capable of converting UDP-glucose into UDP-mannose [3]. In addition, we have successfully created improved variants via enzyme engineering based on this in silico analysis and novel specificity model.

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Exploring Antimicrobial Peptides from the Marine Environment: A Sustainable Alternative to Antibiotics

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Background: The increasing crisis of antibiotic resistance, driven by the overuse and misuse of antibiotics, necessitates the search for novel antimicrobial agents. This project explores the potential of bacteriocins derived from marine environments as efficient and eco-friendly alternatives to traditional antibiotics.
Objectives:1. Identify and isolate bacteriocin genes from marine samples.2. Express and characterize selected bacteriocins.3. Test the antimicrobial activity of bacteriocins against various human pathogens.4. Model and gain structural insights into the most potent bacteriocins.5. Evaluate the risk of off-target activity.
Methods:Marine samples, including seawater and sediment, were collected according to the research vessel’s cruise schedule. Despite challenges such as atmospheric conditions and technical failures, over 250 samples were obtained from the Marmara sea. Computational studies identified and isolated bacteriocin genes, followed by their expression and characterization. The antimicrobial activity of bacteriocins was tested against various microorganisms, and structural modeling was performed to understand their mechanisms. Results: Our initial findings indicate that 5 out of 20 selected candidates exhibit distinctive potential antimicrobial peptide (AMP) activity against Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis. Conclusion: Marine-derived bacteriocins offer a promising alternative to traditional antibiotics, with the potential to combat the increasing problem of antibiotic resistance. Further research is necessary to fully comprehend their environmental impact and optimize their application in clinical settings.

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Exploring the genetics governing quantitative phenotypes in the yeast *Komagataella phaffii*

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Komagataella phaffii (formerly known as Pichia pastoris) is a methylotrophic yeast widely used in industry for recombinant protein production. In particular, derivatives of the isolate CBS2612 have been used for manufacturing a wide variety of products and have been extensively studied. While much effort has been put into the thorough investigation of these industrially-relevant strains, the majority of natural isolates within this species remains unexplored. As a result, also the use of quantitative genetic tools exploiting species richness to find superior allelic variants has lagged behind in the yeast K. phaffii. Yet, as demonstrated in other yeast species, particularly isolates of Saccharomyces spp., exploiting intra-species variation can benefit strain development for industrially-relevant phenotypic traits enormously.
Using quantitative trait loci (QTL) mapping, we aimed at resolving the recombinant protein production potential, as well as temperature tolerance between a cross of a natural isolate and CBS7435, the strain background commonly used in industry. Both founder strains show highly contrasting phenotypes with regard to product yields in small-scale cultivations and also growth at non-optimum temperatures of 39°C. We generated F14 recombinant inbred lines, of which single hybrids were screened and ranked for their ability to secrete a fluorescence protein and for their growth performance at high (39°C) and at low temperatures (12°C). Hybrids exhibiting extreme values of respective phenotypes were pooled, whole-genome sequenced, and subsequently, bulk segregant analysis (BSA) was applied for the first time in this yeast.
And indeed, allele swaps confirmed TSR4, a ribosomal chaperone as causative locus for high-temperature growth, together with two other candidate regions. Additionally, biologically meaningful candidates regarding low temperature growth, as well as recombinant protein production were obtained and are currently being investigated further. This study shows for the first time that bulk segregant analysis is a powerful tool for the investigation of quantitative phenotypes in the yeast K. phaffii.

Extracellular recombinant peptide production in *Escherichia coli*

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Peptides (proteins <100 amino acids) have emerged throughout the last decades and have proven as valuable alternatives to traditional biopharmaceuticals (Henninot et al., 2018). Recombinant peptide expression in Escherichia coli represents a possibility to counteract the downsides of state-of-the-art chemical synthesis (Chan and White, 2000). For efficient production of biopharmaceuticals, release of the recombinant protein/peptide into the cultivation medium is beneficial. We therefore adjusted the outer membrane (OM) permeability of the host organism in-process to facilitate peptide release into the cultivation medium and simplify subsequent downstream processing. Fusion protein technologies were applied to facilitate soluble expression (Köppl et al., 2022; Lingg et al., 2021), detection, or subsequent affinity purification and tag removal.
By combining aforementioned technologies, we were able to express different peptides up to 5 g/L (roughly 120 mg per g biomass) during fed batch cultivations. In-process manipulation of the outer membrane resulted in distinctly increased permeability and subsequent peptide release into the medium. This was shown through various analytical assays (flow cytometry, SDS-PAGE, western blot, etc.).
We propose the usage of fusion tags and OM-adapted host strains for recombinant peptide expression. Downstream processing could be greatly simplified by this approach. This would offer advantages like easier processing of the cell suspension due to lower DNA content, easier separation of recombinant peptides, and lower levels of endotoxins.

Targeting Pests: A Quest for Safe and Selective inhibitors for Juvenile Hormone's Epoxide hydrolase

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Maintaining biodiversity requires pollinating insects. Worldwide reports of pollinator losses in re-cent years have major ecological and economic repercussions. Deterioration and loss of habitat are two factors that have a negative impact on the population of these insects. The use of plant protection products by humans, whose primary objective is to lower the pesticides among crops is however, a reason that is becoming more and more prevalent.
We think it's possible to maintain pollinators while also managing insect numbers. It is already known, that juvenile hormone epoxide hydrolase, and enzyme which is crucial for insects' meta-morphosis, can be used as a target for insect's control. However, so far developed pesticides were unspecific. In our research we are combining computational and experimental methods used for drug design, to propose highly selective compounds, for selective pests control.
We aim to explore a unique approach which is based on intramolecular voids analysis and molecu-lar dynamics simulations (MD) in co-solvents which will guide pharmacophore design. Identified differences and similarities between different insect JHEH will help in the design of new pharma-cophores and subsequently species-specific inhibitors.
Ultimately, our goal is to conduct an extensive study of selected recombinant proteins from ten organisms to best replicate the taxonomic cross-section of species and experimentally confirm the results of the computational analysis carried out.

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Boosting transformation efficiency of *Pichia pastoris*

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The methylotrophic yeast Komagataella phaffii (K. phaffii), commonly known also as Pichia pastoris (P. pastoris), is one of the most widely used hosts for recombinant protein production. During the past decades P. pastoris became increasingly popular, not only for fundamental research, but also for industrial applications in the biopharmaceutical, feed and food sectors. As P. pastoris became more and more widely applied, it became necessary to have a wide selection of genetic tools for protein production. Furthermore, cell engineering of P. pastoris to improve productivity gained increasing importance.
Nowadays cell engineering to obtain improved production strains does not only require expression of single genes, but also entire metabolic pathways or even libraries might be overexpressed. For all these purposes, an efficient transformation method yielding high numbers of transformants is a prerequisite. This work focuses on the development of a reliable, highly efficient, and fast protocol for preparing electro-competent P. pastoris cells. In literature, several protocols are available for introducing DNA into P. pastoris. Two of the most commonly used protocols for generating electro-competent P. pastoris were comparatively evaluated for their efficiency, and further adapted based on the results. So far, a 4-fold increase in the transformation efficiency over the published protocols could be achieved.

Diapers Recycler: development of decentralized complete recycling system for diaper waste.

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The “Diapers Recycler” process aims to recover valuable waste materials such as plastics and superabsorbent polymers from used diapers to reduce the need to produce new plastics and to substantially reduce the CO2 emissions for their production by applying enzymatic and mechanical waste treatment instead of usage of high amounts of fresh water or dangerous chemicals.
The waste from absorbent hygiene products nowadays represents about the 12% of the total municipal solid waste in Europe. In recent years, baby diapers waste alone has received a lot of attention due to the high volume produced and the difficult recycling processes. In Europe, the annual market demand of only disposable baby diapers exceeds 20 billion units, in fact, 40.000 diapers are used every minute, dramatically increasing the amount of dry weight waste (1.3 t/min) (Mendoza et al., 2019). So far, disposable diapers are mostly destined for landfill or incineration after their use. Lately, some processes have been developed to mechanically recycle the different diapers components. However, this methodology has some caveats regarding the separation of all the components, thus not allowing to retrieve highly pure materials. Furthermore, biological treatments by aerobic and anaerobic digestion were applied to obtain a reduced volume and a low moisture content. Nonetheless, the compost derived from the process is of low quality and difficult to commercialize (Kim et al., 2017). “Conventional” diapers recycling processes applying conditions of 250°C and 40 bar, have a high energy consumption (Odegard et al., 2018). Enzymatic approaches ensure specificity without damaging the valuable molecules. Therefore, the enzymatic treatment can be considered as a powerful strategy for a complete economic circle solution (Vecchiato et al., 2018). Using enzyme for the recycling of a material has the advantages over chemical recycling that no toxic or hazardous chemicals need to be used and that the process is carried out at a maximum temperature of 60°C. Unlike thermal recycling, no CO2 is produced. Moreover, the enzymatic recycling process is a measure to slow down the effects of climate change.
Due to the knowledge acquired with plastics and cellulose degradation, a new approach will be developed to recycle diapers locally at facilities like hospitals, nursing homes, etc. developing a clear improvement over disposal.

High-Throughput Screening of Mutations Facilitating Non-Canonical Amino Acid Incorporation via Dual Fluorescence Reporters and FACS

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The screening of gene libraries using flow cytometry and fluorescence-activated cell sorting (FACS) is a powerful method to identify and isolate cells expressing a desired trait. In this study, we developed a high-throughput screening strategy to identify mutations in aminoacyl-tRNA synthetases (aaRS) that promote the efficient incorporation of non-canonical amino acids (ncAAs). A dual fluorescence reporter system consisting of a fusion of red (mScarlet3) and green fluorescent proteins (mEGFP) was employed to monitor gene expression and ncAA incorporation. The upstream mScarlet3 coding sequence was separated from the downstream mEGFP by an in-frame amber codon at which the ncAA was incorporated. Only successful readthrough of the amber stop codon with the ncAA produced the full-length red/green fluorescence reporter. The aaRS library was designed to express various amino acid binding pocket variants to facilitate the activation and charging of ncAAs onto an amber suppressor tRNACUA. Next to the aaRS library and the amber suppressor tRNACUA, the library plasmid encoded also the dual fluorescence reporter. E. coli cells were transformed with the plasmid library allowing each cell to express a unique mutant aaRS/tRNACUA pair alongside the dual fluorescent reporter. To establish a screening procedure for mutant aaRS/tRNACUA pairs, the cells were exposed to the lysine derivatives Nε-carbobenzyloxy-L-lysine (ZK) and Nε-epsilon-((2-azidoethoxy)carbonyl)-L-lysine (AzK). For both ncAAs, orthogonal translation systems had been devised before, hence they represent perfect test compounds to set up the screening procedure. Flow cytometry was used to identify specific expression patterns based on red and green fluorescence, and FACS was employed to sort cells with the desired fluorescence characteristics. In the future, this approach may allow for the precise, high-throughput screening of mutations that enhance ncAA incorporation, with significant implications for synthetic biology, protein engineering, and the expansion of the genetic code.

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.
The potential impact of this research is significant. 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.

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.

The Gas and Pressure Controller (GPC): a device for automated closed batch cultivations and assessment of cultivation parameters of gas-converting microbial cell factories

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Gas fermentation is a key technology for microbiological production of gaseous, liquid and solid products. To advance the utilization of microbial cell factories in gas fermentation processes, their physiological and biotechnological characteristics must be understood, especially before bioprocess development shall be initiated. Here, we report the construction and operation of a novel device, the Gas and Pressure Controller (GPC), which is specifically suited to automatically control the headspace gas pressure of closed batch gas fermentations. The GPC facilitates automated gassing, sparging, monitoring and regulation of the headspace volume of cultivation bottles operated in closed batch cultivation mode in real-time. It offers a high level of flexibility for a wide range of applications including the assessment of physiological and biotechnological information that involves a pressure deviation (increase or decrease) during closed batch cultivation, the identification of putative liquid limitations and fully automated biomass production.
As proof of concept of the GPC, the physiological and biotechnological characteristics of four autotrophic, hydrogenotrophic methanogenic archaea (methanogens) were examined. This allowed the quantification of physiological limits of these methanogens through the elimination of gas limitation during growth and methane formation, thereby facilitating near optimal cultivation conditions in closed batch cultivation mode. We determined unprecedented high maximum specific methane productivity (qCH4) values for: Methanothermobacter marburgensis of 169.59 ± 12.52 mmol g-1 h-1, Methanotorris igneus of 420.21 ± 89.46 mmol g-1 h-1, Methanocaldococcus jannaschii of 364.52 ± 25.50 mmol g-1 h-1 and Methanocaldococcus villosus of 356.38 ± 20.79 mmol g-1 h-1. The qCH4 of M. marburgensis that has been obtained in this study is more than 10-fold higher compared to conventional closed batch cultivation set-ups and as high as the highest reported qCH4 value of M. marburgensis from fed-batch gas fermentation in stirred tank bioreactors.

Exploiting auto-induction by carbon source limitation to improve space-time-yields of recombinant protein production in *E. coli*

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Speed of bioprocessing is a main cost driver for pharmaceutical manufacturing. Applying promoter screening and translational fine-tuning to primary carbon source-limiting conditions, we have developed an E. coli promoter panel to achieve superior space-time-yields without addition of inducers. Using the promotor panel, we can achieve different auto-induction profiles during fermentations, allowing us to select the optimal production profile for a given protein and develop faster and cheaper manufacturing processes.

Co-expression of prokaryotic disulfide bond isomerase C in *E. coli* Origami B alters expression yield of recombinant PHB-Depolymerase

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3-Hydroxybutyric acid (3HB) is a ketone body that serves as an alternative carbon source for microorganisms, particularly under conditions where glucose is scarce. In PHB-accumulating bacteria, 3HB is generated through hydrolysis of polyhydroxybutyrate (PHB) by PHB-depolymerases, allowing these microorganisms to survive in nutrient-limited environments. In contrast, in humans, 3HB is produced in the liver through the conversion of fatty acids, especially during prolonged exercise, or starvation. Beyond its role as an energy carrier, emerging evidence suggests that 3HB also functions as a signaling molecule that influences gene expression and neuronal function. This signaling capacity of 3HB may be significant in various medical contexts, including the management of inflammation, cancer, and neurological diseases. Of the two existing enantiomers, R-3HB is rapidly catabolized and utilized by the human body, whereas S-3HB appears only transiently as an intermediate. Consequently, enantiopure R-3HB is preferred for both medical and industrial applications. To address the challenge of racemic mixtures produced during the chemical synthesis of 3HB, enzyme-catalyzed reactions are employed to selectively generate the desired enantiomer. In this project, we focused on optimizing the expression of a hydrolase to efficiently produce enantiopure R-3HB.
In a previous study a PHB-Depolymerase (PHBDP) was isolated from Acidovorax sp and recombinantly expressed in E. coli. However, early expression studies showed that the native signal sequence of PHBDP led to the protein becoming membrane-bound, placing a significant burden on the host cells and resulting in low yields of active enzyme. To avoid this shortcoming, a truncated PHBDP gene version without its native signal sequence, was expressed in E. coli Origami B cells preventing the transport of the protein from the cytoplasm to the periplasm. Additionally, intracellular co-expression of DsbC, a disulfide bond isomerase that assists in the correct folding of proteins within the cytoplasm, further improved the active expression of PHBDP. For this improvement DsbC was also expressed without a transport signal to maintain cytoplasmic localization. This refined expression system significantly boosts (66 fold) the intracellular production of active PHBDP.
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Innovative tools for recombinant protein production by *Myceliophthora thermophila*

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The filamentous fungus Myceliophthora thermophila ATCC42464 (DSM 1799), also known as Thermothelomyces thermophilus, is a promising fungal host for recombinant protein production. The special properties of this fungus, like protein secretion to very high titres, as well as, its capability to produce biologically active complex proteins of eukaryotic origin, makes it an interesting microbial host for biotechnological applications.
For the development and assessment of new expression tools and strains, it is essential to have reliable and easily detectable reporter proteins. In this context, we demonstrated the application of an unspecific peroxygenase (UPO, EC1.11.2.1) for evaluating various constitutive promoters derived from the M. thermophila genome. The fungal origin makes UPOs a promising reporter for this host. In addition, this is the first report about producing an enzyme of this class recombinantly by M. thermophila and, employing this reporter protein, the toolbox of regulatory elements for this fungal host was expanded by a new promoter engineering approach. After transformation of M. thermophila with expression cassettes with the HspUPO gene of Hypoxylon sp. EC38, linked to engineered promoter variants, expression analyses were performed by small scale cultivations and determination of the activity of secreted enzyme using a colorimetric high-throughput assay with ABTS (2,2-azina-bis-(3-ethylbenzothiozoline-6-sulfonic acid)) as a substrate. The screening identified inventive promoter variants that resulted in increased peroxidase activity.

Synthetic proteins in *Pichia pastoris*

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The genetic code is a universal set of rules for the translation of genetic information into proteins using 20 canonical amino acids (cAAs) as building blocks. The chemical diversity of cAAs is limited. Therefore, it is of great interest to introduce non-canonical amino acids (ncAAs) with unique biochemical handles into target proteins.
The eukaryotic expression host Komagataella phaffii (Pichia pastoris) is particularly attractive for production of complex proteins because it can introduce posttranslational modifications and efficiently secretes protein products into the medium. These assets combined with fast growth to high cell densities in inexpensive media make it an attractive complementary expression host to Chinese hamster ovary cells and Escherichia coli.
NcAAs can be introduced at in-frame stop codons during ribosomal protein synthesis. An orthogonal aminoacyl-tRNA synthetase recognizes the amino acid analog and charges it onto a cognate suppressor tRNA, which inserts it in response to the stop codon. Functional high-level expression of the orthogonal aminoacyl-tRNA synthetase and its cognate suppressor tRNA are key factors for success. In addition, the ncAA of choice must be sufficiently transported into the cells from the media to be available for incorporation. The feasibility of the approach has been successfully demonstrated in bacterial and mammalian expression systems. However, examples of yeast genetic code expansion remain scarce [1-3]. Our aim is to develop platform technology for the efficient incorporation of ncAAs with a reactive side chain into proteins produced by Pichia pastoris. To achieve this, we are systematically evaluating and optimizing the orthogonal translation system.

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POSTER CATEGORIE

Artificial Intelligence & Biotechnology

Investigating the Inflamed Subtype of Small Cell Lung Cancer

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Small cell lung cancer (SCLC) remains one of the most lethal forms of lung cancer, marked by rapid progression and limited therapeutic options. However, recent advances have identified molecular subtypes within SCLC, including the SCLC-inflamed (SCLC-I) subtype, which is distinguished by immune-related gene expression signatures and has shown to have benefit to immunotherapy treatments. Despite this, the mechanisms that drive the inflamed phenotype and the tumor-immune microenvironment remain underexplored, making the investigation of SCLC-I a critical priority for improving patient outcomes.

In this study, we reanalysed single-cell RNA sequencing (scRNA-seq) to characterize the SCLC-I subtype from patient-derived circulating tumor cell-derived xenograft (CDX) models. By investigating the transcriptional landscape at single-cell resolution, we aimed to gain insights into the tumor heterogeneity and immune microenvironment that define SCLC-I tumors. The CDX models offer a unique platform to recapitulate the complexity of patient tumors, allowing us to dissect the cellular composition and identify key immune and non-immune players driving the inflamed phenotype. Our scRNA-seq analysis revealed distinct cellular subpopulations within SCLC-I tumors and identified upregulated pathways. In contrast to non-inflamed SCLC subtypes, SCLC-I tumors exhibit a transcriptional signature suggestive of a more immunogenic profile, potentially explaining their better response to immunotherapies and favorable prognosis.

The urgency of further exploration into SCLC-I stems from its clinical implications. By understanding the cellular and molecular features that distinguish SCLC-I from other subtypes, we can better stratify patients for targeted therapies, offering a path toward more personalized and effective treatment strategies. Our findings underscore the importance of continued research into stratifying SCLC patients, which could pave the way for novel therapeutic approaches aimed at harnessing the immune system in patients.

In conclusion, our study provides crucial insights into the cellular architecture of the SCLC-inflamed subtype using CDX models, highlighting the potential of scRNA-seq to unravel the complexity of this unique tumor phenotype. Given the better prognosis associated with SCLC-I, its thorough characterization is of importance, with the potential to guide the development of more effective therapeutic interventions for SCLC patients

Accelerating DBTL cycles to Develop Sustainable Protein-Based Biomaterials through robust data models and lab-in-the-loop AI tools

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Cambrium is a biotechnology company at the forefront of the design, development, and manufacturing of novel protein building blocks for innovative materials and products. Our platform harnesses AI-based protein design and precision fermentation to facilitate efficient design-build-test-learn (DBTL) cycles, enabling the fast development of proteins with specific material properties.
We use proprietary AI-based protein design tools to design novel proteins tailored for material use cases. This computational design phase allows us to explore vast sequence landscapes and identify high-performing candidates at a low cost, in silico, before testing them in living organisms. This approach ensures that only the most promising designs are brought to the laboratory for a cost-effective evaluation.
We use Pichia pastoris as our workhorse organism for protein expression. The size of any design library and the throughput capacity of our lab have a direct impact on our ability to identify proteins with desired properties. To address this, we have established an automated, pipette-free robotic platform for protein screening. This system streamlines the construction of Pichia strains through the targeted chromosomal integration of payload DNA and enables simultaneous screening of multiple strain libraries.
Our high-throughput screening platform evaluates library candidates based on protein production, employing plate-based quantification methods that facilitate accurate comparisons. High-scoring candidates are then validated in controlled fermentations. To de-risk the scale up from plate to production scale, our screening platform includes fermentations at different scales to assess production robustness and ensure reliable results.
Finally, the learning component of our DBTL platform is enabled by a comprehensive data infrastructure, encompassing an electronic lab notebook in combination with a cloud-based data “lakehouse” architecture. Core to this infrastructure are data models for end-to-end tracking of experimental data and metadata, with automated processing to yield AI-ready data on the fly. Data generated throughout all phases of the cycle is used to train and refine ML models. These models intervene at each step of the design, from DNA to controlled fermentations. This infrastructure allows continuous improvement of the entire platform.
One notable outcome of our approach is NovaColl™, a 100% skin-identical vegan collagen. Developed with our R&D platform, NovaColl™ exemplifies the potential of AI-driven protein design. By efficiently scanning the combinatorial space of collagen molecules for specific characteristics, and screening candidates using our Pichia-based platform, we identified and scaled a lead candidate with unique structural and skin-active properties.

Machine Learning for Advanced Growth Media Optimization with a Fully Automated Micro-bioreactor

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Bioprocesses play a pivotal role in the efficient manufacturing of specialized products such as antibiotics and enzymes. Selecting appropriate microbial strains and optimizing culture conditions are vital to ensuring the economic feasibility of these processes. One major hurdle is developing a fermentation medium that meets the specific nutritional demands of the strain, a task that is often labor-intensive and time-consuming. Currently, Design of Experiments (DoE) is the standard approach used for growth media development. Although DoE helps to reduce the number of experiments, this approach has its limitations, particularly when addressing nonlinear systems. In addition, two-stage DoE processes (screening and optimization) may discard factors too early, deeming them irrelevant before they can prove significant in later optimization stages. In this context, Machine Learning (ML) algorithms for optimization offer a potentially more efficient alternative.

We introduce a fully automated microbioreactor system that autonomously mixes nutrients from various stock solutions and tests their efficacy through iterative cycles, removing the need for manual adjustments. The system integrates a BioLector microbioreactor, which facilitates the parallel cultivation of 48 distinct cultures in a microtiter plate while simultaneously tracking key bioprocess parameters such as biomass, dissolved oxygen (DO), and pH levels in real time. The OT-2 liquid handling system automates tasks such as medium preparation, plate sterilization, and inoculation.

During the initial experiment, 48 different media compositions are determined using a DoE-based approach. After the first cultivation cycle, the system automatically cleans and sterilizes the microtiter plate, preparing it for the next round of medium development. From the second cycle onward, the medium compositions are generated by an ML algorithm using Bayesian Optimization. Fresh pre-cultures are automatically introduced into each of the 48 growth media, and this process is repeated until an optimal medium formulation is identified.

The preliminary data sets are promising. The chosen model organism was MICP-relevant bacterium Sporosarcina pasteurii. The ML-optimized medium showed a 34% improvement in the maximum backscatter value compared to the medium optimized through DoE. The ML algorithm is set to be further diversified and applied to additional strains to validate its broader applicability.

Machine Learning-Driven Gene Signatures Distinguish Molecular Subtypes of Lung Carcinoids

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Introduction:
Lung carcinoids (LCs) are rare, heterogeneous neuroendocrine tumors classified into molecular subtypes: Carcinoid-A1, A2, B, and Supra-Carcinoid. Identifying gene signatures that distinguish these subtypes is essential for understanding tumor biology and improving patient outcomes. We developed a web application, SurvSig, designed to cluster cancer cohorts and analyze gene expression data. The app integrates machine learning to identify subtype-specific gene signatures, perform single-sample gene set enrichment analysis (ssGSEA), and calculate survival associations. This study highlights the application of an artificial neural network (ANN)-based approach to generate gene lists, providing insights into LC molecular subtypes and their biological significance.

Methods:
Using SurvSig, we applied ANN-based gene list generation to classify LC subtypes based on uploaded or system-generated gene sets. Gene expression data were subsetted by these gene lists, followed by clustering to identify molecular subtypes. ssGSEA was used to evaluate pathway enrichment, and survival analysis was calculated based on gene expression clusters. The app also supports individual gene analyses and allows for survival comparison between gene expressions. The identified gene signatures were validated on an independent cohort to ensure reproducibility of the results.

Results:
The ANN-based approach identified gene signatures that effectively distinguished LC molecular subtypes (A1, A2, B, and Supra-Carcinoid). Clustering revealed highly distinct gene expression patterns across subtypes, corresponding with known molecular markers and biological pathways. Validation on an independent cohort demonstrated the same strong clustering patterns and alignment with existing classifications. The generated clusters were robust and consistently separated the molecular subtypes, highlighting the potential of machine learning to refine the classification of lung carcinoids and enhance the understanding of their underlying biology.

Optimization of strains for antibiotic production aimed by the CavitOmiX technology

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Fermentative processes leverage the full cellular machinery of microbial hosts, such as fungi and bacteria, to produce a wide range of valuable compounds, including antibiotics. These biological systems are inherently complex and efficient, capable of performing intricate biochemical transformations that are often challenging to replicate through chemical synthesis. Enzymes in Penicillium can convert readily available and inexpensive precursors into more valuable intermediates. To make strategic genetic modifications to the strain that enhance these transformations, the responsible genes must be identified. To achieve this, in addition to sequencebased analyses, structural models for the entire Penicillium proteome were predicted. Both homology modeling and AI modeling software was applied, resulting in a total of over 30000 protein models. Using Innophore's CavitOmiX technology, the active site cavities of Penicillium enzymes were analyzed. Thereby physico-chemical properties of the cavities were calculated and compared to known cavities that bind the substrate or perform a reaction similar to the desired transformation. Together with other tools, a comprehensive bioinformatic analysis of the Penicillium proteome was performed, and a ranked list of candidate proteins and genes was generated, of which the most promising will be tested by the industrial partner.

Approaches to target double strand break repair in colorectal cancer based on gene expression

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Colorectal adenocarcinoma (CRC) is the third most common and the 2nd most deadly cancer type worldwide. The genomic background of CRC is described in detail by The Cancer Genome Atlas (TCGA) COAD, the main source of data in this project. To investigate the potential of therapeutic strategies, such as those focusing on the DNA repair profile, one needs to consider both the sequence and the expression levels on a genome-wide scale. DNA damage response (DDR) is an important target exploited by many cancer therapeutics but the efficacy of the drug often depends on the repair profile and therefore these strategies require appropriate molecular selection of the patients. DDR deficiency can be exploited in many ways, yet targeted therapeutics like Poly (ADP-ribose) Polymerase inhibitors (PARPi) are not currently approved for the treatment of CRC. In this project we explore the transcriptomic signatures and significantly discriminating features to support external groupings based on clinical data in the transcriptomic context and select key players for laboratory validation focusing on double strand break repair as the main avenue of intervention.

From Variability to Consistency: A Novel Manufacturing System for Standardized Extracellular Vesicles from telomerized human Mesenchymal Stromal Cells

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Extracellular vesicles (EV) from different cell sources including Mesenchymal Stromal Cells (MSC) offer great promises for many therapeutic applications in Regenerative Medicine and beyond. The vesicular fraction of the secretome has been shown to exert similar biological functionalities as the cells themselves. Consequently, MSC-derived EV are being investigated in multiple pre-clinical and clinical trials. However, manufacturing of EV in larger quantities and with consistent quality attributes has proven difficult. Therefore, Phoenestra has recently developed a scalable and tunable manufacturing setup based on a patented perfusion bioreactor assembly using stable, telomerized MSC (MSC/TERT). This novel setup allows for continuous harvesting of conditioned media – currently tested for up to 43 days – followed by standardized isolation steps which ensure the molecular integrity of the EV fraction. With this we have successfully manufactured EV preparations from different MSC/TERT cell lines derived from various tissues and in a highly productive and reproducible manner. The isolated EV preparations display relevant biological functionalities as tested with developed cell-based bioassays. On top of these bioassays, we perform an extensive characterization of our EV preparations based on RNA profiling and marker protein analyses. All of these analyses are used to better understand the impact of cultivation mode, media composition and downstream processing on the molecular composition and – ultimately - biological function of EVs. Our recent optimizations led to increased oxygen demand during cell manufacturing resulting in a 2-3-fold increased EV productivity, therefore our 250 ml bioreactor scale enables the supply of a clinical study.

POSTER CATEGORIE

Sustainable Bioproduction

New possibilities for lowering serum free cultivation media costs

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Cultivated meat may be a more ethical, environmentally friendly, antibiotic-free meat alternative of the future. As of now, one of the main limiting factors for bringing cultivated meat to the market is the high cost of the cell culture media and their great dependency on serum albumins, production of which is predicted to become a major bottleneck of this industry. Here, we identified several food grade, low-price medium stabilizers, exhibiting comparable or even superior stabilization of the B8 medium in short-term cultivations, as compared to recombinant human serum albumin (HSA). We show transferability of our approach on porcine, chicken satellite cells, and CHO cell line, though significant cell-line specific differences in response to stabilizers were observed. Thus, we provide an alternative to HSA, enabling up to an overall 73% reduction of medium price.

Optimising the cultivation of *Crocosphaera chwakensis* for efficient production of Cyanoflan

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The skin is the largest human organ and requires adequate care. Currently there is a growing trend to avoid synthetic components in skin care products due to public awareness of their long-term risks to the environment and human health. Therefore, novel natural polymers are being explored to be used in cosmetics and personal care products as active and/or non-active ingredients. Cyanobacteria are prolific sources of added value biocompounds, such as extracellular polymeric substances (EPS) [1]. The marine cyanobacterium Crocosphaera chwakensis CCY0110 was chosen due to its ability of constantly release a complex and versatile carbohydrate polymer, named Cyanoflan[2]. Starting with the aim of implementing a constant, timely and cost-effective industrial production of Cyanoflan, the optimization of the culture conditions and the isolation procedure was envisaged. Firstly, the most important culture parameters to be tested were selected: light intensity, temperature, and agitation. Then, the growth was evaluated using standard parameters, optical density, and chlorophyll a content, throughout the cultivation period of one month. In addition, the content in total carbohydrates (intracellular and extracellular) and in carbohydrates released to the culture medium (RPS) were assessed. As expected, preliminary results suggest that all the parameters tested affect the growth of C. chwakensis and consequently Cyanoflan production, with light intensity being the most important. The light intensity inside the reactor is modelled mechanistically and coupled to a CFD simulation to transiently track the intensity cells are exposed to. The obtained parameter setpoints were integrated in modelling software to develop a digital twin of the bioreactor and associated processes aiming to improve our understanding of cyanobacterial cultivation in terms of efficiency and precision. The result of this study will pave the way for optimising the industrial-scale production of Cyanoflan, with the ultimate goal of commercialising it as a natural ingredient for cosmetics and personal care.

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Pathway design for mixotrophic production of chemicals from CO_2 and methanol in yeasts

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Autotrophy and mixotrophy in industrial strains have the potential to mitigate climate change, making them highly relevant for sustainable biochemical production. Previous studies have engineered functional autotrophy in yeasts by integrating the Calvin-Benson-Bassham (CBB) cycle in Komagataella phaffii (Pichia pastoris) (Gassler et al. 2020) and similarly in Escherichia coli (Gleizer et al. 2019), enabling growth on CO2 as the sole carbon source. The autotrophic K. phaffii strain uses methanol for energy.
This project aims to design alternative pathways for the assimilation of both CO2 and methanol to convert CO2 into value-added compounds. Using methanol in both assimilatory and dissimilatory pathways reduces the overall methanol demand of the process. The blueprints for such a mixotrophic pathway include the bacterial serine or serine-threonine cycles. Implementing such a complex pathway in the metabolism of K. phaffii requires substantial rerouting of central metabolic fluxes to maintain growth. Using synthetic biology methods such as Golden Gate Assembly and CRISPR-Cas9, we aim to design thermodynamically feasible and energetically favorable pathways for producing organic acids from a mixed feed of methanol and CO2. This involves activating and modifying the serine cycle by introducing bacterial genes or activating native yeast pathways. The functionality of these novel metabolic modules are assessed through 13C metabolite tracing experiments

NOVABIOMA Biomanufacturing FlexCo – A startup company exploiting Plant Molecular Farming for the sustainable production of complex recombinant proteins

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NOVABIOMA Biomanufacturing FlexCo, an Austrian spin-off from BOKU University and the Medical University of Graz, is at the forefront of sustainable biologics production targeting infectious and non-communicable diseases. Utilizing Plant Molecular Farming (PMF), our platform leverages Nicotiana benthamiana as a renewable, safe, and cost-effective bioreactor for the production of complex recombinant glycoproteins, including monoclonal antibodies, vaccine subunits, and enzymes. Unlike traditional microbial or mammalian cell-based systems, plant-based expression offers unique advantages in producing structurally complex glycoproteins such as secretory IgAs, benefiting from the robust biosynthetic machinery inherent to plants.
A critical aspect of recombinant glycoprotein quality—glycosylation—affects stability, immunogenicity, biological activity, and pharmacokinetics. To address this, NOVABIOMA Biomanufacturing Flex Co employs advanced glycoengineering techniques pioneered by BOKU University, enabling the in-planta synthesis of glycoproteins with mammalian-like glycans that closely replicate natural human glycosylation patterns. Our transient expression platform facilitates rapid product development within weeks, scalable by simply increasing the number of plants, thereby offering great flexibility for high-demand scenarios, including emergency responses.
The primary objective of NOVABIOMA Biomanufacturing Flex Co is to exploit the scalability and adaptability of PMF to produce antiviral compounds, establishing a resilient platform for next-generation therapeutics. By optimizing glycoengineering processes, we enhance the functionality and stability of plant-derived antiviral proteins, presenting a promising and sustainable solution to emerging viral threats. This approach not only accelerates the timeline from development to deployment but also ensures cost-effective and environmentally friendly biomanufacturing, positioning NOVABIOMA Biomanufacturing Flex Co as a key player in the future of therapeutic protein production.

Biocatalytic Cascade for the Selective Synthesis of Asymmetric Pyrazines

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Pyrazines and, more precisely, alkyl pyrazines, are key additives in food industry which mimic the organoleptic properties of ingredients like coffee or cocoa, such as roasty, nutty or earthy smells. Despite the interest that both the scientific and industrial communities have shown in recent decades regarding these products and their derivatives, a viable solution for the selective synthesis of pyrazines in a reproducible manner within the confines of current food industry regulations has yet to be found. Consequently, the industry is compeled to continue obtaining this valuable additives as complex mixtures with low yields through extraction from molasses. In this work, we propose a simple and selective concurrent cascade procedure for the synthesis of these target molecules through a dimerisation followed by incorporation of electrophiles of different natures, under completely environmentally friendly conditions, establishing the foundations for a future efficient industrial process for their mass production.

Synthesis of 10-hydroxystearic acid from Waste Cooking Oils by an engineered *Yarrowia lipolytica*

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The development of second generation biorefineries is of great importance for the sustainable production of valuable compounds. Hydroxy fatty acids (HFAs) are a class of compounds that can be used for the production of various industrial products, such as polymers, detergents and flavourings.
Metabolic engineering of microorganisms has great potential for the sustainable and cost-effective production of chemical products and novel compounds. The unconventional yeast Yarrowia lipolytica is a suitable host for biotechnological applications due to its unique characteristics. It is an oleaginous yeast with GRAS (generally recognised as safe) status, it has a high tolerance to a wide range of pH and salinity conditions, it is able to grow on a wide range of substrates, including renewable carbon sources, and several tools for genetic manipulation facilitate its metabolic engineering.
In this work, we have engineered a Y. lipolytica strain for the one-pot biocatalytic conversion of waste cooking oils to HFAs with overexpression of the endogenous lipase Lip2, recombinant expression of the Elizabethkingia meningoseptica oleate hydratase and reduced activity of β-oxidation. This approach streamlines the synthesis process and limits the need for purification steps.

Supplementation strategies for the re-use of spent fermentation media from a β-glucosidase expression system in *Komagataella phaffii*

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In this study the re-usability of yeast fermentation media from a β-glucosidase expression system is examined. β-glucosidase is a biotechnologically relevant enzyme in processes ranging from producing bioethanol from lignocellulose to improving wine and fruit juice aromas. Following a 144-hour culture with a β-glucosidase overexpressing clone (IRA1), the secreted enzyme is purified via ion exchange chromatography (IEX). Our previous investigations have determined the consumption of elements and amino acids by K. phaffii during fermentation using ICP-MS and LC-MS/MS methods. These measurements have shown a near total depletion of amino acids with 17 of 20 being significantly reduced, with increases in levels of phosphorus, sulphur, and potassium. Conversely, levels of magnesium, boron, and calcium are reduced to almost zero. Here, β-glucosidase expression media is analysed in the same way to reveal the compositional profile following primary K. phaffii culture and subsequent IEX purification of the secreted protein. An optimal nutrient supplementation strategy to restore vital elements and amino acids in the spent media post-purification is studied. The output of these sensitive measurements will determine the optimal supplementation strategy in an industrially relevant context so that spent media may be recycled effectively. A potential benefit of this approach is instillation of a circular bioeconomy approach in an industrially relevant process.

Production of the highly active thermophile PETases PHL7 and PHL7mut3 using *E. coli*

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The growing crisis of polyethylene terephthalate (PET) waste demands innovative recycling strategies. Enzymatic degradation of PET into its monomers, followed by re-polymerization, represents a promising circular economy approach. However, a significant challenge lies in the large-scale production of highly active PET-degrading enzymes without the requirement of costly downstream processing. In this study, we present an optimized process for the extracellular production of the thermophilic and highly active PETases, PHL7 and PHL7mut3, eliminating the need for expensive purification steps. By employing the growth-decoupled enGenes eX-press V2 E. coli strain and systematically varying pH, induction strength, and feed rate through a factorial-based optimization approach, we identified optimal production conditions for PHL7, resulting in a 40% increase in fermentation supernatant activity. Further improvement of the expression construct led to a 4-fold increase in activity. Applying these optimizations to the more active, temperature-stable variant PHL7mut3, we achieved complete degradation of PET film within 16 hours at 70°C, using only 0.32 mg of enzyme per gram of PET. These advancements support scalable enzymatic PET recycling, contributing to solving the global plastic waste crisis.

Cultured Metabolites - a plant cell culture approach for the scalable production of complex metabolites

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Plant alkaloids, a diverse group of secondary metabolites found in various plant parts, exhibit substantial therapeutic properties, including anti-inflammatory, antitumor, antimicrobial, and analgesic effects. Traditionally, these valuable compounds are obtained through the cultivation and harvest of specific crops, followed by extraction and purification processes. However, this approach faces several challenges, such as environmental impact, resource-intensive farming, and environmentally harmful purification practices. Given that some alkaloids are classified by the WHO as essential medicines, it is crucial to develop a reliable and sustainable production process.
Chemical synthesis represents a potential method for producing small molecules. However, the complex chemical structure of alkaloids makes it challenging to achieve high yields in synthesis. Utilizing microbial systems for the bio-based production of alkaloids has also been the focus of research. These systems involve engineered microorganisms to express enzymes involved in the biosynthetic pathways of alkaloids. Despite significant advancements in metabolic engineering, economic feasibility remains a challenge due to low titres of the target compounds. This may be a consequence of the intricate nature of the biosynthetic pathway. Given the involvement of numerous enzymes in the process, the recreation and optimization of the pathway in a heterologous system represents a substantial challenge.
Utilizing the native host of the target alkaloids provides an advantage over recombinant systems, as the biosynthetic pathway does not require engineering and introduction into a heterologous system. To overcome the challenges presented by traditional crop farming, plant suspension culture can be used as an alternative. Unlike traditional farming, plant suspension cultures offer a highly controlled environment concerning internal and external factors, ensuring consistent alkaloid production. Additionally, plant suspension cultures can be scaled up in bioreactors, enabling the production of large quantities of alkaloids without the need for extensive agricultural land.
Ultimately, plant suspension culture stands out as a promising solution for the sustainable and scalable production of alkaloids. This approach not only mitigates the environmental challenges of traditional methods but also ensures a steady and high-quality supply of these vital compounds. As the field of biotechnology continues to evolve, plant suspension cultures will play a crucial role in the development of efficient pharmaceutical production processes.

Optimizing sustainable itaconic acid production in *Komagataella phaffii* at low pH

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Itaconic acid is a value-added organic acid with multiple applications within many different industries. Today, itaconic acid is primarily produced industrially through microbial fermentation of sugars or starch, which rely on agricultural cultivation and might compete with food production. As part of the VIVALDI project, we strive for a sustainable production using the C1 carbon source methanol with engineered Komagataella phaffii.
Insertion of the Aspergillus terreus derived heterologous pathway for itaconic acid production enabled K. phaffii to produce itaconic acid from methanol, and further strain optimization focusing on multicopy integration of the heterologous genes doubled the production capacity in fed-batch bioreactor cultivations of K. phaffii. Furthermore, several optimizations strategies were employed for upstream process intensifications, at first neglecting impacts on downstream processing. Noteworthy, an efficient upstream process itself does not necessarily equal to a sustainable process, especially when the fermentation broth composition presents economically and environmentally costly challenges for downstream processing. Ideally, isolation and purification of the itaconic acid is aided by a low pH, high product titers and a media composition reduced in salts without byproducts or excess media components. The effect of a low pH on itaconic acid production and cellular metabolism and viability was investigated.
Interestingly, at low pH methanol consumption increased together with the itaconic acid production, however, only until the viability was decreased due to the combined effect of a low pH and a high acid concentration. To challenge this issue, we focused on additional strain optimization through adaptive laboratory evolution (ALE) and identification of stress-related genes during K. phaffii cultivation at low pH. Our results generate valuable knowledge for sustainable process design and highlight the importance of an aligned approach for both upstream and downstream processing.

Enzymatic C=C bond cleavage: characterization and engineering of a new dioxygenase

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Aromatic DiOxygenases (ADOs), are enzymes belonging to the family of carotenoid cleaving enzymes (CCOs or CCDs). They are non-heme-iron-dependent enzymes characterised by the chelation of a Fe2+ active centre by 4 histidine residues. They activate molecular oxygen and produce two species of carbonyl compounds from a substrate with a C=C double bond.
Based on TtADO from Thermothelomyces thermophila[ ] we identified and expressed 13 novel ADOs. We call them 'ozonylases', because these enzymes may be a substitution for ozonolysis – a rather dangerous process on scale due to the formation of explosive intermediates. Among the tested enzymes, MapADO from the marine fungus Moesziomyces aphidis was found to be the most effective enzyme not only converting isoeugenol to vanillin, but also accepting 4-vinylguaiacol, hydroxyanethol, corniferyl alcohol and resveratrol as substrates. Kinetic studies revealed MapADO possessing one of the highest vmax, km and kcat for isoeugenol, with 230 µM/min, 120 µM and 240 s-1, respectively. Rationally-designed mutants of this enzyme further increased activity almost 3 times in the case of resveratrol. Mutagenesis also revealed key insights in substrate binding of MapADO. Using a 200 mL preparative scale with whole cells, we achieved quantitative conversion of 50 mM isoeugenol to the corresponding aldehyde overnight. We isolated 1.3 grams of vanillin. We are now exploring random mutagenesis with high mutation rates in combination with screening by rapid-fire MS-MS.

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Dynamic Control Flux Balance Analysis Accurately Maps the Design Space for 2,3-Butanediol Production

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2,3-Butanediol is a valuable raw material for many industries. Compared to its classical production from petroleum, novel fermentation-based manufacturing is an ecologically superior alternative. To be also economically feasible, the production bioprocesses need to be well optimized. However, biotechnological process optimization is often hindered by incomplete solution space characterization, leading to suboptimal conditions.
To address this, we improved a dynamic control algorithm that uses flux balance analysis for process optimization: dcFBA. To enhance the algorithm's efficiency we reduce the number of required Karush-Kuhn-Tucker condition constraints and improve the implementation of moving finite elements. We find that our improvements achieve a speed-up of at least a factor of four compared to a previous method, with even greater gains when process length was included in the objective function.
We applied this optimization approach to the production of 2,3-butanediol through fermentation in E. coli. Using two-stage fed-batch process simulations, we identified proportionality and trade-off regions within the solution space. This allowed us to design close-to-optimal production processes that maximized both titer and productivity, achieving a experimentally validated titer of 43.6±9.9 g/L and a productivity of 1.93±0.08 g/(Lh). Further optimization of a continuous two-reactor process may increase productivity more than threefold with minimal impact on titer and yield.
The dcFBA algorithm presented here demonstrates the potential for significant improvements in biotechnological production processes, for example, for the production of 2,3-butanediol. We are confident that our method offers a path towards economically feasible and ecologically superior alternatives to traditional petroleum-based production of many chemicals.

How engineering lactic acid production revealed the hidden phosphoglycolate salvage pathway in the synthetic autotrophic *K. phaffii*.

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Atmospheric CO2 levels are rising each year, primarily due to the extensive use of fossil fuels, which is a leading contributor to global warming. To address this issue, reducing CO2 emissions is crucial, and one promising approach is to utilize CO2 as a carbon source. Recent advancements have allowed for the conversion of the methylotrophic yeast Komagataella phaffii into a synthetic autotroph through the integration of the Calvin-Benson-Bassham cycle, enabling this strain to utilize CO2 as its sole carbon source. We have already demonstrated its ability to produce valuable building block chemicals such as lactic acid and itaconic acid from CO2.
During the engineering of the lactic acid-producing strain, specifically through the deletion of the CYB2 gene, we observed that the cells began secreting glycolate alongside lactic acid. This glycolate production is likely linked to the side reaction of the RuBisCO enzyme, in which oxygen is mistakenly fixed instead of CO2, thereby reducing the overall yield of carbon fixation. The byproduct of this reaction is 2-phosphoglycolate, a compound toxic to the cells, has to be recycled through energy-intensive pathways. In the synthetic autotroph K. phaffii, the mechanisms for these recycling pathways had yet to be elucidated.
Our investigations revealed that CYB2 is an essential gene in this recycling process; its disruption completely blocked the pathway for 2-phosphoglycolate salvage. By utilizing 13C tracer experiments with fully labeled glycolate as the carbon source, we were able to further delineate the route of phosphoglycolate recycling in K. phaffii. The results indicated that a significant portion of phosphoglycolate is indeed recycled and is distributed into the central carbon metabolism.
These findings provide an excellent foundation for further enhancement of growth in K. phaffii. Future efforts could focus on integrating more efficient salvage pathways or optimizing culture conditions to minimize the oxygenation rate of RuBisCO, ultimately leading to improved CO2 utilization and production efficiency.

Introducing a heterologous ribulose monophosphate pathway to *Komagataella phaffii* for increasing energy efficiency on methanol

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Metabolic engineering of microorganisms presents promising opportunities for tailoring established cell factories to support sustainable biotechnological production processes. Recently, there has been growing interest in utilizing one-carbon (C1) substrates for the biotechnological production. Methanol, for example, can be directly derived from greenhouse gases such as methane and carbon dioxide—both potent contributors to global warming. Employing native or synthetic methylotrophic cell factories holds the potential to establish a circular bioeconomy.

Komagataella phaffii (Pichia pastoris) is a prominent industrial production host, primarily recognized for heterologous protein production and, more recently, for non-protein products. This aerobic methylotrophic yeast relies on its native xylulose monophosphate (XuMP) cycle for methanol utilization. However, by looking into nature, more energy-efficient methanol utilization pathways, such as the ribulose monophosphate (RuMP) cycle observed in certain bacteria, can be identified.

In this study, our objective was to metabolically engineer K. phaffii towards a more energy-efficient methanol assimilation by incorporating the RuMP cycle from Bacillus methanolicus. We were able to re-engineer the yeast with a peroxisomal heterologous RuMP cycle. The resulting strain, designated RuMPi, demonstrated the ability to grow on methanol as the sole carbon and energy source. However, the strain exhibited an inferior biomass yield on methanol compared to the wild type, accompanied by a very long doubling time of tD = 4 days. Through 52 generations of Adaptive Laboratory Evolution (ALE), the strain significantly improved its doubling time to tD = 17 hours. Ongoing optimization efforts aim to further enhance the strain's performance, eventually offering the possibility of incorporating RuMPi in methanol-based bioeconomies.

From sewage sludge ash to fertilizer

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Sewage sludge from municipal wastewater treatment plants that is currently used as fertilizer often contains higher amounts of pollutants like per- and polyfluoroalkyl substances (PFAS), heavy metals, toxic substances and potential pathogens. Therefore, sewage sludge originating from larger wastewater treatment plants (>20,000 EW60) must be incinerated and phosphorus has to be recovered from the sewage sludge ash (SSA) according to a new Austrian legislation which will be in force from January 2033 on. In this project we will investigate the recovery of phosphorus through solubilization by acid producing bacteria for bioleaching of a carbonatized SSA. Bioleaching, the solubilization of compounds by microbial action, is often applied to solid materials like ores or solid waste streams like waste electronics and ashes. After the solubilization of phosphors, phosphorus will be obtained in form of a bioavailable phosphate-rich fertilizer with minimal toxic metal content. The phosphate fertilizer produced will then be tested on model plants, investigating plant and root growth and the dynamics of the soil microbiome. This investigation will provide a more economical and environmentally friendly alternative to comparable processes and the environmental impact will be investigated in a life cycle assessment (LCA).

Textile waste upcycling from a biotechnological perspective

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Textile waste poses a serious challenge nowadays as lifespan of clothing is decreasing together with increasing consumption. Textiles often consist of blends comprising natural fibers such as cotton, viscose or wool as well as synthetic fibers such as polyester or polyamide. Synthetic fibers are not biodegradable and therefore pollute the environment if they end up in landfills which is the currently most applied route for textile waste. For the purpose of recycling, these fiber blends need to be separated which can be achieved by biotechnological methods. Cellulosic (cotton, viscose) or protein (wool) fibers can enzymatically be hydrolyzed from blends thereby recovering pure synthetic fibers (polyester or polyamide) for re-application in textile production. At the same time, released hydrolysis monomers represent a starting point for valorization approaches which was accomplished through cultivation of different microorganisms. Glucose from cellulose hydrolysis was successfully converted into lactic acid (LA), polyhydroxybutyrate (PHB), or bacterial cellulose (BC) by Weizmannia coagulans, Cupriavidus necator, and Komagataeibacter sucrofermentans, respectively. Amino acids from wool hydrolysis could be used as nitrogen source by Chlorella vulgaris and Rhodotorula mucolaginosa to obtain valuable bioproducts including pigments (chlorophyll, carotenoids) and lipids. The obtained biopolymers (PHB, BC and PLA with LA as building block) could be re-introduced in the textile industry together with extracted pigments as natural dyes. Therefore, biotechnological tools enable not only separation of blended fabrics but also valorization of recovered fractions for a sustainable and circular bioeconomy.

Cutting-Edge Fermentation Methods for CO_2 Utilization Using the Bioplastic-Producing Bacterium *Cupriavidus necator*

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Gas fermentation is a revolutionary technology for microbial CO2 assimilation and reduction. Thereby, the carbon is fed back into the carbon cycle and greenhouse gas emissions are mitigated. The cultivation of hydrogen-oxidizing Cupriavidus necator on CO2 for the production of bioplastics (poly-(R)-3-hydroxybutyrate (PHB)) has become a central focus of both research and industry. Current bottlenecks in cultivating this organism relate to the design of bioreactors that can be used for explosive gas mixtures of the needed substrate gases (O2, H2, CO2) and the precise control of dissolved oxygen concentrations due to biological limitations. Here we report on the bioreactor design as well as parameter optimizations for high cell density production of C. necator H16. We show how safe lab-scale fermentations can be established in order to cultivate hydrogen-oxidizing bacteria without gas limitations. By implementing continuous chemolitotrophic fermentation set ups, bacteria were grown under reproducible conditions with biomass yields of up to 15 g/L (Lambauer and Kratzer, 2022). In addition, a precise automation and control model was applied to finely adjust gas concentrations for optimal cell growth and PHB production. We showed, that low concentrations of 0.75 mg/L dissolved oxygen (dO2) significantly enhance biomass and PHB production under chemolitotrophic conditions (Lambauer et al. 2023). Further optimisation of the media composition led to a final biomass concentration of 53 g/L dry cell weight with a PHB content of 75 % with CO2 as sole carbon source. It is to highlight, that all fermentations were carried out without pH control or additional feeding. With this multi-faceted approach, we hope to improve the efficiency and sustainability of gas fermentation processes and gain new insights into critical media components under chemolitotrophic conditions.

Enzymatic cascade to natural ligustrazine

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Ligustrazine (2,3,5,6-tetramethylpyrazine; TMP), a Maillard reaction product that contributes to nutty, roasted, and toasted tonalities of heat-processed food, is widely used in the food industry as a flavor ingredient (1,2). Additionally, TMP is a natural product found in Ligusticum wallichii, a herb used in traditional Chinese medicine. It contributes to cardio-and cerebrovascular health, has anti-cancer properties, and serves as a precursor for other pharmacologically active compounds.[3] The demand for natural TMP exceeds its supply from raw materials, favoring biotechnological methods.[2] Several chemoenzymatic methods rely on enhanced microbial production of acetoin followed by spontaneous condensation with ammonia, often at high temperatures (1,2,4). We set out to develop an enzymatic cascade towards TMP where all the steps are performed under mild conditions (Figure 1). A carboligase (CL) / transaminase (TA) sequence (5,6) was applied for the first time in synthesis of a short-chain vicinal amino alcohol. For the 3rd step, we tested the ability of a panel of alcohol dehydrogenases (ADHs) to catalyze the challenging oxidation of a vicinal amino alcohol to an aminoketone, which spontaneously forms TMP. 46% of TMP was produced from racemic amino alcohol standard, whereas a sequential one-pot cascade yielded up to 18% TMP. Further optimization will ensure enantiocompatibility of applied enzymes and complete consumption of acetaldehyde, which otherwise forms side-products.
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Moving towards the utilisation of CO_2-rich industrial off-gas streams for isopropanol formation by *Cupriavidus necator*

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Valorising CO2-rich industrial off-gas streams by microbial fermentation is appealing as it reduces CO2-emissions while harnessing an underexploited carbon source for the production of value-added compounds. The Knallgas bacterium Cupriavidus necator is a promising chassis strain, having been successfully engineered to produce various compounds. This study focuses on isopropanol formation as a model system.
An improved autotrophic shake flask system was developed to characterise several isopropanol-producing strains and compare with their heterotrophic performance. While growth and product formation rates were slower under autotrophic conditions, the product yield was comparable to heterotrophy. Scaling up to a 7.5 L bioreactor yielded promising final isopropanol concentrations of 12 g L-1 with a maximum productivity of 0.4 g L-1 h-1. This process was extensively characterized, including gas uptake rates during both the growth and product formation phases.
Biogas was evaluated as a potential gas source aiming to upgrade it by reducing the CO2 content. Cultivations in shake flasks were conducted where biogas composition supplemented with H2 and O2 was simulated using lab-grade gases. It was demonstrated that initial methane concentrations of up to 24 % did not negatively impact growth or isopropanol production. Cultivations using real industrial biogas are ongoing.
This work demonstrates the feasibility of CO2 fixation for isopropanol production at concentrations exceeding the g/L level, while providing essential data for feasibility studies and identifying process bottlenecks to guide further process intensification.

Intensified biomanufacturing with *E. coli*: Establishing of greener processes with continuous cultivation strategies

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The development of low environmental footprint manufacturing processes is a challenge in the biopharmaceutical industry. There is an emerging importance of sustainability aspects of the biopharmaceutical production and the analysis of potential risks related to the release of active pharmaceutical ingredients (APIs) to the environment [1]. The ENVIROMED project [2] focuses on the holistic assessment of (bio)pharmaceutical production, usage and final disposal. In this study, the authors are demonstrating the potential of intensified bioprocessing to mitigate the high demands of purified water, chemicals and energy throughout the upstream process.
A gate-to-gate approach was chosen to compare conventional fed-batch processing with intensified operations. “Green metrics” with respect to chemical usage and energy demands reflect the environmental footprint of the upstream process [3]. In the first step of the sustainability analysis, the system boundary of the upstream process was defined by the following steps: (I) equipment cleaning and sterilization, (II) pre-culture cultivation, (III) fermentation in the bioreactor and (IV) harvest of the cell broth. The use-case is the lab-scale production of a recombinant fragment antigen binding (Fab) using the Escherichia coli strain W3110.
The investigated intensified processes (repetitive fed-batch, chemostat, cascaded processing) were significantly decreasing energy and water/chemical demands (up to 30 %) compared to the conventional fed-batch. This is due to long equipment downtimes, repeated cleaning efforts, non-productive (non-induced) phases and high energy demands in conventional biomanufacturing. Additionally, keeping up the cell-specific productivity by tailoring process parameters was a crucial step towards the establishment of an intensified process.
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Ethanol direct measurement and soft sensing via off-gas measurement in shake flasks

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Respiration activity measurement in shake flask has been proven a useful tool for multiple applications during the last decades, such as media development, process development, scale up and many more. Oxygen transfer rate (OTR) and Carbon dioxide transfer rate (CTR) quantitatively analyze the physiological state of microbial cultures. The RQ (CTR/OTR) can tell about the type of substrate consumed or product formed. In this study, a Kuhner TOM device for off-gas analysis in shake flasks (Transferrate Online Monitoring) was equipped with an additional VOC sensor to allow for direct ethanol concentration measurement in the flask headspace. In addition, a soft sensor approach was applied considering stoichiometrical balances, background knowledge and boundary conditions to estimate the ethanol concentration throughout the cultivation. Both methods showed good agreement with offline measured ethanol concentrations. The results indicate that online ethanol measurement in shake flasks is well possible. Phases of ethanol formation and consumption become obvious without laborious sampling. This can be used especially in process-and media development for yeast cultivations where ethanol is often seen as undesired overflow metabolite. Application examples are shown for Batch and Fed-batch cultivations with S. cerevisiae.

POSTER CATEGORIE

Biopharma & Health

Engineering of *Komagataella phaffii* expression strains with focus on requirements for biopharmaceutical production

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Microorganisms, such as bacteria and yeast, are widely used for the production of chemicals and biomlolecules in biotechnology. Komagataella phaffii (syn. Pichia pastoris) is a particularly attractive expression host for the pharmaceutical industry due to its GRAS status, high secretion capacity, ability of protein folding and post-translational modifications. However, in the context of large-scale commercial production of biopharmaceuticals, the selection of K. phaffii expression strains and clones necessitates careful consideration of specific criteria. In addition to high productivity, which facilitates attractive cost of goods (CoGs), thereby ensuring the affordability of medicinal products for patients, the chosen expression strains must be suitable for the stringent maintenance of elevated standards in product quality. Moreover, expression strains must align with process-driven objectives, emphasizing facile and robust processability within ecologically and environmentally sustainable frameworks as well as imperative considerations of ensuring operator and facility safety. The intricate process of designing and selecting production strains that meet these diverse requirements poses a challenge for strain development, demanding the application of advanced methods in molecular strain engineering and generation as well as efficient workflows to screen and select for strain showing the required profile.
Our presentation will focus on K. phaffii strain engineering and screening strategies employed to generate strains suitable for “Chemistry, Manufacturing, and Control” (CMC) development. Within that we will scrutinize the comparison between conventional random plasmid integration for production strain engineering and targeted integration methodologies utilizing CRISPR techniques and show how strain engineering can benefit from targeted integration approaches.

Purification and characterization of recombinant neuraminidase for non-seasonal vaccine against Influenza virus

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Influenza viruses pose a significant public health threat, causing seasonal epidemics and occasional pandemics with substantial morbidity and mortality worldwide. The development of effective vaccines remains crucial for mitigating the impact of influenza virus infections. The influenza virus possesses two glycoproteins on its surface: hemagglutinin (HA), which is immunodominant, and neuraminidase (NA), which is immunosubdominant. Traditional influenza vaccines primarily target the viral surface glycoprotein HA to induce protective immunity. However, the high rate of antigenic drift limits the durability and efficacy of current vaccines. NA's higher antigenic stability may provide a longer-lasting immune response in vaccines. Recombinant NA is an attractive candidate for as broadly protective influenza virus vaccine, necessitating robust purification and characterization methods. Here we present a scalable approach for purifying recombinant NA, expressed in Sf9 insect cells utilizing the baculovirus expression system. A lean purification process, based on chromatography and tangential flow filtration, achieve high yield and purity while maintaining the target’s structural integrity and biological activity. Characterization methods, including SDS-PAGE, Western blot, size exclusion chromatography and mass spectrometry, confirm the product's structural and functional properties. Scalability from laboratory to manufacturing scale ensures reproducibility, thus accelerating the development of recombinant NA-based vaccines for improved influenza control.

Optimization of ultrashort DNA fragment visualization using sybr gold

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The most common practice for visualization of DNA fragment size is gel electrophoresis, mostly using agarose or polyacrylamide gels in combination with ethidium bromide as nucleic acid staining agent. While these gels can be bought or self-made in various concentrations and buffer conditions, working with a broad distribution of fragment sizes is still a challenge. Very long sequences tend to underlie a diffusion bias or stay trapped in the gel's pocket. Very short sequences, especially in single digit base pair fragments, are likely to diffuse so quickly that the bands are lost, while larger sequences have barely left the pockets yet. While larger sequences can often be amplified by PCR to increase concentrations, this is not feasible for oligonucleotides in a 6 to 10 bp range. This is especially true if those small oligonucleotides are products of an endonuclease digestion, resulting in random short sequences. Our aim was to develop a method which would allow us to separate the smallest of DNA fragments to check the sizes of all digested products. Since the smallest commercially available DNA ladder only works down to 10bp at the lowest band, custom oligos were ordered, and arranged to a ladder for short fragments from 50bp to 6bp. Five factors were considered for optimization: Gel type, running buffer, nucleic acid staining agent, running time and voltage. First, using self-cast agarose gels in combination with ethidium bromide did not allow to detect the custom-made ladder. Switching to pre-cast polyacrylamide gels already improved the visualization, but only after replacing ethidium bromide with SYBR Gold the custom ladder was visible even in low concentrations. For polyacrylamide gels, 2 different concentrations of pre-cast gels were tested. A 4-20% gradient gel in TBE, as well as a 15% gel in Urea/TBE. The 15% PA Urea/TBE gel shows the most promise regarding separation of the lowest two bands at 6 and 8 bp respectively, but this has yet to be achieved. Switching the running buffer from TAE to TBE additionally showed an improvement in resolution. The developed method allowed the visualization of DNA fragments resulting from a nuclease digestion (using M-SAN, ArcticZymes) *of HEK293 cell culture supernatant, without the need for a concentration step.*

Tirzepatide, a GIP/GLP1-receptor co-agonist preserves cardiac function and improves survival in mice with angiotensin II-induced heart failure

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Introduction
Incretin analogs, such as GLP1-receptor agonists, are widely used for the treatment of obesity and type 2 diabetes mellitus. Beneficial effects on major adverse cardiac events have been shown in recent clinical trials. Some preclinical and clinical (FIGHT, LIVE) studies demonstrated the effectiveness of Liraglutide (Lira) in heart failure. However, dual GIP/GLP1-receptor agonists, such as Tirzepatide (TZP) are under investigation to treat type 2 diabetes mellitus and obesity, and data regarding their use in heart failure with reduced ejection fraction (HFrEF) is lacking.

Aim
We performed a comparative study in a mouse model of HFrEF induced by continuous angiotensin II (AngII) infusion to investigate the effects of Lira and TZP on mortality and cardiovascular structural and functional changes.
Methods
AngII-induction (1.5 mg/kg/day) was carried out with osmotic minipumps implantation subcutaneously (s.c.) to 5-month-old male BALB/c mice or SHAM surgery was performed. Following this, the animals received vehicle treatment (Veh), TZP (10 nmol/day s.c.), or Lira (300 µg/day i.p.) treatment for 14 days in the following groups: SHAM/Veh (n=7), AngII/Veh (n=15), SHAM/Lira (n=7), AngII/Lira (n=15), SHAM/TZP (n=8), AngII/TZP (n=15).
Echocardiography, electrocardiography, immunohistochemistry, and qRT-PCR were used to characterize the structural, functional, and molecular features of the heart and the aorta.

Results
Mortality was significantly higher in AngII/Veh animals compared to control, while TZP treatment significantly increased the chance of surviving in the AngII group. Compared to the control TZP and Lira treatment as well as AngII-induction significantly decreased the body weight, however, the combination of treatments and AngII-induction resulted in the most severe weight loss. Treatment with both compounds preserved cardiac systolic and diastolic function compared with AngII/Veh treated animals, as shown by normal ejection fraction and E/e'. Both Lira and TZP decreased the AngII-induced elevation of cardiac fibrosis and hypertrophy markers, including Ctgf, Col3a1, and NPPA, while TZP also reduced the elevated NPPB level. On histological characterization of the abdominal aorta TZP and Lira treatment caused a significant increase in the thickness of the aortic wall in AngII groups compared to their control groups.
Conclusion
Tirzepatide reduced mortality significantly in our model. Both compounds preserved normal cardiac function and decreased markers of hypertrophy and fibrosis. Moreover, TZP and Lira increase the thickness of the aortic wall, thus may reducing the incidence of aorta aneurysms. These results suggest that Tirzepatide may have potentially beneficial effects in the treatment of HFrEF.

Understanding the effect of biostimulants on the microbial communities associated with apple

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Conventional agricultural practices rely on synthetic pesticides and fertilizers to meet the global demand for food production. However, the large-scale application of pesticides damages agricultural and natural ecosystems and directly reduces overall microbial abundance and diversity. Microbial diversity is, however, imperative for plant growth, development and productivity. Integrated management practices that incorporate biopesticides, biostimulants, and biofertilizers present promising alternatives to conventional agricultural methods. Consequently, it is essential to evaluate the impacts of different management practices on both the plant and soil microbiomes. Two commercially available microbial products (produced by Valibiotics AG) were applied as leaf treatments, either with or without a reduction in pesticides, to apple trees in conventionally managed orchards in Styria. Standard pesticide treatment served as control. High-throughput sequencing targeting the V4 hypervariable region of the 16S rRNA gene and the internal transcribed spacer (ITS1), along with quantitative real-time PCR, were applied to analyse the effect of the microbial products on the apple leaf, fruit, and seed microbiome. Microbial abundance was significantly lower in conventionally managed apple leaves and fruits treated with microbial products without pesticide reduction compared to the conventional control. The fungal community structure, as well as fungal species richness and evenness, were significantly affected by the microbial products. Although there was no significant impact on the bacterial community structure, bacterial diversity was higher in apple leaves and fruits treated with the microbial products without pesticide reduction. Additionally, bacterial and fungal genera with significantly higher or lower abundance were identified in apple leaves and fruits treated with the microbial products compared to the conventional control. Overall, the results demonstrate that the application of microbial products influences the microbial communities associated with apple leaves, fruits, and seeds. This study provides a new perspective on the impact of integrated farming by considering not only plant health and productivity but also the entire plant holobiont.

Thymic Activity Modulates Immune Checkpoint Inhibitor-Induced Cardiotoxicity: Implications for Therapeutic Strategies

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Immune checkpoint inhibitors (ICI) are monoclonal antibodies that enhance the anti-tumor activity of the immune system. However, this therapy could lead to immune overactivation, resulting in severe side effects associated with high mortality rates.
Previously, our group demonstrated that ICI increases the expression of inflammatory genes in the thymus, and furthermore in clinical studies, ICI therapy for thymoma treatment caused severe immune related adverse events, including fatal cardiotoxicity. Based on these findings, we hypothesized that thymic activity could play an important role in ICI-induced autoimmune side effects.
The main aim of our study was to investigate the correlation between thymic activity and the pathophysiological processes of immune-mediated cardiotoxic and systemic side effects during ICI therapy using a preclinical mouse model. Since the thymus gradually atrophies and thymic activity decreases with age, we examined the side effects of ICIs in two differently aged groups, plus artificially degraded the thymus with pharmacological treatment in young animals.
Based on our results, thymic immune response plays a central role in ICI-induced cardiotoxicity in mice. In aged animals undergoing ICI treatment and combined immunotherapy with parallel pharmacologically induced involution of the thymus significantly reduced side effects, indicating that the immune response following ICI treatment varies depending on thymic activity.
Thus, modulation of thymus activity may serve as a therapeutic target in reducing immune-mediated side effects and monitoring the morphology and activity may be an important factor in predicting the severity of side effects and identifying high-risk patients.

Melanoma subtype-dependent cardiotoxicity to immune checkpoint inhibitor therapy

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Confidental

Development, *In Vitro* Characterization & *In Vivo* Testing of multimodal Prussian Blue nanoparticles in an animal model

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Aim
The most commonly used Gd (III) materials lack the in vivo stability, causing toxic side-effects for the patiens; materials other than Gd (III) available lack the required in vivo contrast for the Magneti Resonance Imaging (MRI).
A possible candidate for high performance MRI contrast materials could be the FDA authorized Prussian blue (PB), which is used to treat heavy metal poisoning since 2003. Its uniquie structure allows the control of size, shape and biocompatibility, nevertheless, non-functionalized PBNPs show less significant T1 and T2 signal changes in vitro; their measured longitudinal and transversal relaxation times did not suggest their in vivo use. Therefore, functionalizing PBNPs, to achieve multimodal contrast would highly increase their impact for preclinical applications, thus in the routine procedures MRI is oftentimes coupled with other modalities providing greater functional contrast.

Material & Methods
Native PBNPs were synthesized according to as described by Shokouhimehr [1], with modifications. For citrate coated PBNPs, the process as described by Shokouhimehr [1] was applied. A two-step PBNP preparation was made.
The fluorescent labelling of PBNPs was conducted using Eosine Y, Rhodamine B and Methylene Blue (MB) that adsorbed onto the particle surface. For near infrared (NIR) fluorescent labelling, IR820 was used.
For particle characterization, Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) was used.
The in vitro and in vivo imaging was performed using a nanoScan® PET/MR system with a 1T permanent magnetic field, and a Fluorescence-labeled Organism Bioimaging Instrument (FOBI) for the fluorescent measurements.

Results
Polyethylene glycol (PEG) stabilization was required for the fluorescent labelling of the PBNPs. Only MB conjugated PBNPs demonstrated promising in vitro stability and fluorescence. In vivo, the particles accumulated in the liver, spleen and gastrointestinal tract.
For the NIR labelling, IR820 was successfully adsorbed onto the surface, facilitated by the porous nanoparticle surface. The particles showed similar characteristics in vivo, as compared to the MB labelled PBNPs. The in vivo MRI measurements confirmed the presence of the particles in the thoracic region and the liver.
CONCLUSION: This study demonstrates the synthesis and modification of biocompatible, stealth, fluorescent, and MRI-contrast-capable MB- and IR820-labeled PBNPs. The nano-system, including IR-820-conjugated PBNPs, exhibits enhanced in vitro and in vivo T1-weighted MR contrast. The study aims for clinical translation, utilizing QbD during synthesis and validating with nude mice, offering a finely tuned platform for clinical applications with potential for further development and implementation.

The Importance of Endonuclease Treatment Placement in Downstream Processing of Virus-like Particles

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Host cell DNA is a major impurity in the purification of bio-nanoparticles (BNPs) such as viruses, viral vectors, and virus-like particles (VLPs), so it would be essential to remove those. Regulatory bodies such as the FDA and EMA require that residual host cell DNA in injectable vaccines must not exceed 10 ng per dose. Isolating BNP products from host cell DNA is particularly challenging when it is present in the form of chromatin. Chromatin is composed of DNA wrapped around histone proteins, creating structures that are similar in size and surface charge to BNPs. Endonuclease treatment is widely used to reduce host cell DNA content. However, tightly packed DNA in the form of chromatin is not accessible for digestion using conventional endonucleases. Previously chromatin has been overlooked due to the absence of a clear distinction between free DNA and DNA in chromatin form, as well as the lack of suitable analytical methods for detecting and quantifying chromatin. It has been demonstrated that salt-active nucleases, combined with increased ionic strength, are significantly more effective at removing DNA in the form of chromatin than traditional endonucleases. Endonuclease treatment can be applied at various stages of the process. In this study, the placement of endonuclease treatment within the downstream process train was evaluated. The downstream process, following harvest, included flowthrough and affinity chromatography. HIV-1 Gag-based VLPs produced in HEK-293 cells were used. Two different endonucleases were assessed for the treatment step: Benzonase used as a benchmark, and M-SAN, a salt-active endonuclease. The results demonstrated that both Benzonase and M-SAN effectively digested free DNA, while M-SAN was also able to digest DNA in chromatin form. Various scenarios were tested, each differing in the point of endonuclease treatment. The outcomes from each scenario were analyzed to determine the most effective placement for reducing DNA content in the final product, improving the efficiency of downstream unit operations, and minimizing process costs.
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Infrastructure for Bionanoparticle Processes

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Bionanoparticles, i.e. viruses, virus-like particles (VLP), or extra cellular vesicles, are the next generation of biopharmaceuticals used for vaccines, gene therapy, immunotherapy and tissue regeneration. A bottleneck in rapid development of these advanced medicines is the lack of understanding the process mechanisms and scale-up of manufacturing processes. Moreover, there is a lack of advanced analytical tools which allow for the rapid and accurate quantification and characterization of bionanoparticles, especially in complex mixtures such as cell culture supernatant, in which different particle populations with similar characteristics may be present. Therefore, a full set of instruments is needed to develop integrated bioprocessing and analytical methods for generation of process understanding and characterization.
At acib, a fully equipped 400 m² biosafety level 2 laboratory is available to all partners in collaborative projects. Equipment of Upstream and downstream process development and analytical instruments for in-depth product characterization are available. Moreover, acib experts are excited to share their know-how and experience working with these new modalities.

Exploring Cell State Transitions in Small Cell Lung Cancer

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Small Cell Lung Cancer (SCLC) accounts for approximately 15% of all lung cancer cases in Hungary and is particularly notorious for its aggressive clinical course, characterized by rapid tumor growth, early metastasis, and poor prognosis largely due to the development of resistance to chemotherapy. A significant contributor to this resistance is the tumor's remarkable phenotypic plasticity, where neuroendocrine (NE) cells can transition to non-neuroendocrine (non-NE) states. This dynamic switch is often regulated by pathways such as NOTCH signaling and is a key driver of SCLC progression, therapeutic resistance, and recurrence. However, despite the well-documented importance of this plasticity, the molecular and phenotypic differences between adherent and suspension SCLC cell populations and their association with NE or non-NE states remain poorly understood. Investigating these distinctions in detail could uncover critical insights into the mechanisms of SCLC adaptability, offering potential strategies for overcoming therapeutic resistance and improving patient outcomes.
Our study aims to investigate the distinctions between adherent and suspension cell populations in two SCLC cell lines (NCI-H446 and DMS-273), specifically focusing on identifying which populations exhibit NE or non-NE traits. We seek to characterize the underlying genetic and transcriptional features that contribute to their phenotypic states.

The NEUROD1+ SCLC cell lines (such as NCI-H446 and DMS-273) are cultured under two distinct conditions: as adherent and suspension populations and as a mixed population. These two growth modes are expected to represent differing phenotypic states, with adherent cells potentially exhibiting non-neuroendocrine (non-NE) characteristics, while suspension cells are likely to retain more traditional neuroendocrine (NE) traits. We will use RNA sequencing (RNA-seq) to perform a detailed comparison of the transcriptional profiles between adherent and suspension cells. This will allow us to identify key differentially expressed genes. Key findings from the RNA-seq analysis will be validated using additional molecular techniques, including fluorescent Western blotting to quantify protein expression and qPCR to confirm the transcriptional changes. We hypothesize that adherent cells, which may resemble non-NE populations, will show upregulation of genes linked to mesenchymal or epithelial traits, while suspension cells will express higher levels of NE markers. Through this comprehensive analysis, we aim to identify critical biomarkers and transcription factors involved in the NE/non-NE plasticity, providing new insights into the mechanisms behind SCLC's aggressive behavior and resistance to therapies.

Expanding bioprocessing workflows using off the shelf ready-to-use systems: from bench to clinic

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Precision controlled biomanufacturing, cell processing tools & particles separation technologies, are essential to the production of Advanced Therapy Medicinal Products (ATMP), synthetically cultured biologics and laboratory-grown meats.

With the search for efficient, consistent, high performance & scalable biomanufacturing solutions continuing to intensify, adaptable downstream cells processing platforms have come to be of a central importance. Developing & scaling production of novel biologic therapeutics is hugely challenging & associated manufacturing costs & risk.

Manufacturing personalized (cell based) therapies is a costly and a complicated process. The goal is this study was to reduce this cost while covering the entire process using off-the-shelf components and hardware integration. A new semi-automated biomanufacturing platform for ATMP has been developed.

In this presentation we will discuss the benefits of embedding ready to use technologies into any workflow in the facility. The described workflow allows acceleration of biological drug development while lowering the associated economic costs. It meets on-demand/batch, continuous manufacturing and global decentralized production of biologics.

We highlight some in-depth technical information and practical advice to identify, develop and optimize separation process workflows for cells based microcarrier systems. This presentation will compare cells processed in a novel carefully designed single-use assembly to accommodate cryopreservation, cell growth & separation & filling, to those manufactured in a traditional way throughout the industry.

This work also confirms the use of the ready-to-use systems as educational tool as the workflow could be easily implemented in a classroom for training purpose and combined with other gene editing techniques, cells handing protocols, synthetic biology tutorials and integrated with conventional lab hardware.

The presentation will be illustrated with a set of carefully selected case studies. Data obtained from controlled rate freezing profiles and standard methods used among cryo-bags will be presented. Insights into a novel process and designed containers for therapeutic cells will be offered. We will explore how the strategy & performance may change depending on the scale up and the storage methodology. We believe this transformative strategy provides a more robust but adaptable system that preserves biological activity and product quality.

Novel analytical HPLC method for characterization and quantification of VLPs

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The development of analytical methods for the quantification and characterization of enveloped VLPs remains a major challenge due to their overlap in size, buoyant density and similarity in membrane composition to other bionanoparticles, such as host cell vesicles and chromatin. Especially in complex mixtures (e.g. cell culture supernatants), the presence of other bionanoparticles leads to inaccurate quantification of VLPs. The aim of the present work was to develop a method for accurate quantification of VLPs even when other bionanoparticles are present in the mixture. Heparin affinity chromatography has been successfully used to separate different bionanoparticle population in preparative scale. In this work we developed an analytical HPLC method using preparative grade Capto™ Heparin affinity resin and included several in-line detectors such as UV, MALS, DLS and RI. Consistent recoveries of 100% for pure VLPs samples and 88% for clarified cell culture supernatant were achieved through extensive flow rate screening, column hardware and filter evaluation and the addition of additives to the mobile phase. The developed method allows rapid and accurate quantification and characterization of VLPs, even in complex mixtures, improving downstream process development speed. In addition, the use of HPLC is non-destructive which enables the collection and further analysis of highly pure bionanoparticles.

Development of an immunological antibody approach for the treatment of Huntington's disease

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The Huntingtin (HTT) protein plays a key role in many cellular functions, and its mutated form (mtHTT) is the primary driver of the symptoms observed in the neurodegenerative disorder Huntington's disease (HD). HD is an autosomal dominant condition caused by the expansion of a CAG trinucleotide, which results in an extended polyglutamine (polyQ) stretch with the number of repeats exceeding 35 and leading to abnormal conformational changes in the protein and aggregation. Although HD predominantly affects the central nervous system, it is now understood HD to be a systemic disease (Jaramillo L.C. et al. 2022).
Additionally, studies have shown that mtHTT is present not only intracellularly but also in the extracellular environment, where it can be taken up by other cells in a prion-like manner, thereby spreading the pathological process to other tissues (Donnelly K.M. et al. 2022). For these reasons, an immunological approach can represent a promising therapeutic strategy for treating HD patients to slow down the disease progress.
The aim of this project is to develop and characterize a humanized and/or a fully human IgG antibody, based on a previously established murine antibody C6-17 (Bartl et al. 2020) targeting the caspase-6 cleavage site of HTT. These humanized anti-HTT antibodies are being characterized for their binding affinity to the epitope and their ability to mediate phagocytosis in monocytes in vitro. In particular, a phagocytosis assay was developed to evaluate immune cells activation against mtHTT.
In this poster, we present preliminary characterization of the first developed human antibody clones and their activity in the newly optimized phagocytosis assay.

Purification of measles virus by combination of salt-active nuclease treatment and heparin-affinity chromatography

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Host cell DNA is a critical impurity in downstream processing of enveloped viruses. For vaccine applications, host cell DNA content should be below 10 ng per dose in the final product. In addition to DNA in its naked form, host cell DNA is present in virus preparations in form of chromatin resulting in high DNA content in the final product. Chromatin consists of complex and large structures which include DNA and highly positively charged histones. Similar charge properties of chromatin and viruses result in cumbersome chromatographic separation. Moreover, chromatin is often similar in size to viruses, further complicating their separation. We evaluated the performance of salt-active nuclease, M-SAN, and a traditional endonuclease, Benzonase®, in the downstream processing of recombinant measles virus. Endonuclease treatment was performed after clarification and followed by a purification step using affinity chromatography with Capto™ Heparin resin. TCID50 was performed to determine the infectivity of the viruses. DNA and histones presence (in process and purified samples) were determined using PicoGreen™ assay and Western blot analysis using detecting anti-histone antibodies. The salt-active nuclease was more efficient in the removal of chromatin and consequently in the removal of host cell DNA. The combination of salt-active nuclease treatment and heparin affinity chromatography resulted in high purity and yield of measles virus. Moreover, the developed is scalable and suitable for integration in measles virus manufacturing

Identifying molecular markers for the early diagnosis of non-small cell lung cancer

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Nowadays, a painful tumour biopsy is required for the accurate diagnosis of non-small cell lung cancer, which involves several risk-factors. The most up-to-date method is "body fluid biopsy", which can detect the presence of a tumour by examining molecular tumour markers derived from blood sampling with lower risk rate. However, this test has not been used in the diagnosis of non-small cell lung cancer yet. This requires further identification of molecular tumour markers associated with the tumour stages, which allow determining an accurate diagnosis. By analysing the results of RNA-sequencing data derived from tumorous specimens, we can highlight specific network systems linked to gene expression changes that can greatly assist in the identification of tumour markers with new diagnostic value. The main aim of our study is to identify specific gene expression patterns that can be used in the early diagnosis of non-small cell lung cancer. Therefore, RNA-sequencing data from non-small cell lung tumours will be analysed through multiple statistical and screening steps. In addition to histological classification and oncological stages, we have to consider several aspects that are closely related to the development of non-small cell lung cancer. This will be followed an extensive bioinformatics study, using algorithms that allow machine learning. Our bioinformatics analysis could help in the diagnosis of other tumours as well as in the development of artificial intelligence being able to learn sequentially.

Challenge of future antibiotic resistance: the increased bacterial virulence provoked by SPR206 promotes human cell profile alterations

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Susceptible species do not always overtake resistant species due to the presence of a fitness cost. The commonly held belief that the development of antibiotic resistance would eventually lead to this outcome is not always the case. Our hypothesis is that the pattern of certain bacterial pathogens rapidly developing resistance while others remain susceptible for prolonged periods reflects species-specific differences in the impact of resistance on bacterial virulence. The exact relationship between antibiotic resistance and virulence is still unclear, in vivo assessments have shown that several antibiotic-resistant strains have altered virulence. Importantly, the molecular mechanisms underlying resistance are specific to each bacterial species. Limited overlap exists in resistance mutations and mobile resistance genes across bacterial species. Alternative resistance mechanisms may not be phenotypically equivalent, as they may incur a fitness cost in a species-specific manner. Our research has definitively shown that certain combinations of bacterial species and antibiotics have significant negative trade-offs between resistance and virulence. The cytotoxicity of bacteria on human cells has a considerable effect on the change in virulence. Our findings suggest a correlation between antibiotic resistance and cytotoxicity in human cells, which requires further investigation into the underlying mechanisms.

Inflammaging: Impact of Inflammation on Cardiopulmonary Decline in Old Mice

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Introduction
Population aging is a global phenomenon with a noticeable growth every year. The prevalence of age-related cardiovascular diseases is highly associated with advancing age. Chronic low-grade inflammation, termed inflammaging, emerged as a risk factor for multiple diseases in elderly individuals. At such pro-inflammatory status, the level of multiprotein complexes (inflammasomes) is altered, and that correlates with health and disease during aging.
Aims: Given the increase in life expectancy, further understanding of the inflammatory background in aging is crucial. We aim to assess if inhibiting a wide array of inflammasomes can ameliorate cardiovascular dysfunction in the older population.

Methods
We treated 90-week-old C57Bl/6J mice with Canakinumab, Probenecid or vehicle for six months. At the beginning of the study and before the termination, we performed echocardiography. After the treatment period, we terminated all the mice and collected their organs (heart, lung, kidney, liver, spleen, pancreas, adrenal gland and brain). One part of the collected sample was placed in Formalin for histological examination. The other part was frozen in liquid Nitrogen and then stored at -80 C for further experiments (ELISA, Western Blot).

Result
Our results did not show a significant difference in the Echocardiography parameters between groups, apart from mild improvement in the ejection fraction in the canakinumab-treated mice and an increase in the cardiac output in the Probenecid-treated group. Histological analysis of liver samples showed no significant difference in the percentage of the fibrotic area between the treated and control groups.

Conclusion
Our current study investigated the potential role of selective and non-selective inflammasome inhibition in improving the cardiovascular system in the aged population. While our results showed only an improvement in the ejection fraction in the canakinumab-treated group and a mild increase in the cardiac output in the probenecid-treated group, we aim to further investigate the role of inflammasomes (e.g., NLRC4, AIM2, NLRP1 and NLRP3) and downstream mediators in developing low-grade inflammation in the aged heart.

POSTER CATEGORIE

Out of the Box

A pipeline for the identification of disease-specific genetic biomarkers using next-generation sequencing data of cell-free DNAs in human plasma.

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Cell-free DNA in the bloodstream is the result of the shedding of DNA fragments by various tissues. The isolation of these molecules can be achieved through a routine blood draw, which is a cost-effective, rapid and non-invasive procedure. While their presence can be detected in individuals with no apparent health issues, there is substantial evidence to suggest a link between their presence and a range of clinical conditions. The combination of a large number of samples and high-throughput sequencing technologies has made it possible to generate substantial amounts of complex genomic data. This has led to a great interest in cell-free DNA in clinical settings, given its potential role as a biomarker. However, this potential can only be exploited if supported by a computational platform that simplifies the execution of the analysis and makes it reproducible and shareable across different platforms. To address the complexity of this analysis, we propose a computational pipeline that automatically chains together multiple modules, each one executed by an independent open-source software tool. The pipeline starts with the raw data pre-processing, which is an important step to remove residual adapters and filter out low-quality data. The second module is the composition analysis, whereby the average sample composition is calculated and expressed in terms of specific target regions of human DNA. A classifier algorithm is then utilised to evaluate the performance of these target regions in discriminating between the healthy and case cohorts. Its output is statistically validated by a MANOVA test. The final module runs through the original set of sequencing data to determine which sequences best match the composition derived from the pool of target regions as calculated in the previous module. The final result is a list of nucleotide sequences that have been identified as the subset of best-performing indicators of a clinical condition based on the above analysis. Thanks to the containerization technology and workflows managers, the pipeline can be shared and executed with the same functionality across different platforms, and its installation process is automated.

POSTER CATEGORIE

EU-Project

Development of orthogonal pairs able to equip enzymes with non-canonical amino acids containing silicon and tertiary amines

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Protein engineering has emerged as a proficient technology to improve the catalytic performance of enzymes. It can increase promiscuous activities or even to generate biocatalysts catalyzing “new-to-nature” reactions. However, the chemistry proteins can incorporate is limited by the fixed 20 canonical amino acids prescribed by the standard genetic code. The expansion of the genetic code with non-canonical amino acids (ncAAs) carrying new side chain chemistries is a promising approach to expand the side chain chemistry of proteins and, subsequently, the chemical reactions enzymes can perform. In this project, we aim to equip enzymes with Si-groups and tertiary amines (tA) for improved bioorthogonal labeling or even “new-to-nature” biocatalysis. We will introduce the new chemistries by using non-canonical amino acids (ncAAs) as building blocks for enzyme engineering. To achieve this, we will take a computational approach which will be validated in the laboratory. We will generate orthogonal aminoacyl-tRNA synthetase/tRNACUA pairs for Si- and tA ncAAs to allow their incorporation in response to an in-frame amber stop codon. The genetic encoding of Si-ncAAs will facilitate metal-free site-selective hydrosilylation as an alternative approach to, e.g. copper(I)-catalyzed azide-alkyne “click chemistry”. tA-ncAAs will serve to replace catalytic proline in the active site of an enzyme.

SAGROPIA – Sustainable agriculture through novel pesticides using an integrated approach

T. Bath

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The EU-funded SAGROPIA project, in line with the European Union’s Farm to Fork strategy, is dedicated to promoting safe, sustainable, and secure food production by reducing the use of harmful chemical pesticides in agriculture. Conventional crop protection practices, particularly in the cultivation of staple crops like potato and sugar beet, rely heavily on chemical pesticides, which pose significant risks to human health and the environment. Reducing the use of these substances is a top priority for the European Commission, especially regarding ‘candidates for substitution’ as outlined in Regulation (EC) No 1107/2009.

SAGROPIA focuses on developing and demonstrating biocontrol-based solutions that can effectively replace chemical pesticides, while incorporating Integrated Pest Management (IPM) strategies that are both affordable and practical for farmers. The project will introduce 13 biological and low-risk pesticides from three industry partners, with the goal of replacing several hazardous active substances such as the nematicide oxamyl and the fungicides difenoconazole, fluopicolide, and metalaxyl. In addition, SAGROPIA aims to reduce the overall use of copper-based pesticides and insecticides, including pirimicarb, lambda-cyhalothrin, and esfenvalerate, by at least 50%.

Potato and sugar beet crops are highly susceptible to diseases such as early and late blight, as well as pests like nematodes and the Colorado beetle. SAGROPIA’s approach combines biocontrol solutions that have already demonstrated efficacy in specialty crops and are close to market readiness. These solutions are further refined, with scaled-up production and research into their modes of action, ensuring they meet the practical needs of European farmers.

The project emphasizes the development of holistic IPM strategies rather than simply substituting chemical pesticides. These IPM strategies will integrate the new biocontrol products into broader farm management practices, promoting a comprehensive approach to pest and disease management. Real-life trials will be conducted across five potato and sugar beet-growing regions in Europe, with the active participation of local farmers to ensure practicality and adoption.

Sustainability is at the core of SAGROPIA's work. The project not only aims to reduce harmful pesticides but also to evaluate the environmental, economic, and social impacts of its solutions. Detailed sustainability assessments will measure improved performance in terms of natural resource use, environmental impact, and economic feasibility, preparing the ground for broader adoption across Europe.

SAGROPIA seeks to ensure that biological alternatives to chemical pesticides are both accessible and economically viable for European farmers, contributing to a more sustainable and resilient agricultural system. The project’s outcomes will provide valuable tools and data to help farmers transition to eco-friendly practices without sacrificing crop yield or quality.

Find more information, resources, and publications on the project's home page: https://sagropia.eu

MIBIREM- Innovative technological toolbox for soil and groundwater bioremediation

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MIBIREM is a collaborative European project involving 11 partners from 6 EU countries, funded by the European Union's Horizon Europe Programme. It unites advanced microbiome research centres and leading companies to tackle the cleanup of contaminated sites in Europe through microbiome-based soil bioremediation.
Hundreds of thousands of contaminated sites across Europe pose significant risks to both human health and the environment, requiring innovative remediation technologies. Conventional methods are often costly and inefficient. Biotechnological remediation, which uses living organisms such as microbes and bacteria to remove contaminants, offers a promising alternative.

MIBIREM is developing a unique toolbox designed for efficient microbiome-based environmental bioremediation. The project focuses on molecular methods for monitoring, isolating, cultivating, and depositing whole microbiomes. It includes techniques for enhancing specific microbiome functions, such as improving evolution and enrichment. The performance of these microbiomes is tested in real-world conditions, with the aim of long-term scalability for broader environmental applications.

The process begins with sampling from contaminated sites, followed by microbiome analysis to identify the bacteria present and evaluate their effectiveness in degrading contaminants. Based on these findings, the most effective microbiomes are selected and tested at actual contaminated locations.

Data gathered is then fed into a prediction tool. The tools and technologies developed will be packaged into the MIBIREM toolbox, which can be used by others to implement microbiome-based bioremediation for a variety of contaminants.
MIBIREM aims to make bioremediation accessible, economical, and eco-friendly.
Bioremediation costs 20-50% less than traditional methods. It uses minimal water, produces no waste and reduces CO2 emissions by 70-90%.

So far the partners have published the MIBIREM Handbook for sampling, a publication by TAUW in the Journal Bodem and a software MiBiPreT– a Python package for prediction and analysis in Microbiome based Remediation developed as part of the MIBIREM toolbox by the University of Utrecht.

- Link to the relevant handbook
- Link to the relevant publications

Development and testing of polymer-encapsulated, amine-functionalized iron-based contrast materials in animal model

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Background
Magnetic nanoparticles (NPs), particularly superparamagnetic variants such as magnetite, maghemite, and various ferrite NPs, emerge as promising alternatives to traditional Magnetic Resonance Imaging (MRI) contrast agents (CAs). Their heightened specificity and biocompatibility make them attractive candidates [1, 2]. The escalating demand for stable and precisely tuned magnetic NPs in biomedical applications highlights their significance. However, the preparation of these NPs remains a persistent challenge.

Methods
Two different solvothermal methods (12 h reflux and a 4 min microwave, MW) were used to synthesize amine-functionalized ferrite, superparamagnetic NPs, doped with Zn2+ and Cu2+ ions. To overcome stability problems in the colloidal phase, the ferrite NPs were embedded in polyvinylpyrrolidone and could be easily redispersed in water.
The morphological characterization of the NPs was executed by High Resolution Transmission Electron Microscopy, Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS). For detecting the supramolecular interactions and crystalline structure, Fourier Transform Infrared Spectroscopy and X-ray Powder Diffraction was utilized. The in vitro and in vivo MRI measurements were performed with a PET/MR system (Mediso, Budapest, Hungary).

Results
In case of the Zn-doped NPs, the conventional solvothermal synthesis (ZnFe2O4-NH2 Refl.) resulted in a more stable system as compared to the microwave-assisted synthesis (ZnFe2O4-NH2 MW). These results were supported by DLS and AFM measurements, as well as in vitro MRI measurements, where inhomogeneities in the signal were detected.
The CuFe2O4-NH2 MW samples however showed increased colloidal stability as well as homogenous MRI signal in vitro and in vivo. After injection, consistent with other SPION NPs, both samples exhibit a concentrated presence in the hepatic region of the animals, with comparable biodistribution and pharmacokinetics suspected.

Conclusion
Our investigation shows that the ferrite NPs are a feasible candidate for a new generational, multimodal MRI CA. Their chemical properties, owning an -NH2 group holds great options for surface modifications with chelators for isotopes or fluorescent pigments for multimodal molecular imaging purposes. It must be highlighted that the preparation method as well as the nature of the applied precursors play a crucial role in the synthesis of a stable system.

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Chemolithoautotrophic Fermentations with *Cupriavidus necator* for the Production of Isotopically Labelled Biomolecules

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Introduction
The conversion of renewable resources into valuable products remains a significant challenge for modern society. In this project, we harness the CO_2-fixation capability of the hydrogen-oxidising bacterium Cupriavidus necator to convert relatively inexpensive stable-isotope-labelled (SI-labelled) reagents into biologically relevant molecules. SI-labelled compounds are essential for biological applications in mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy but are typically very costly to synthesise using conventional methods. Our approach integrates biotechnology, chemistry, and enzymatic synthesis to enable the affordable production of target SI-labelled biomolecules from biomass containing valuable ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), amino acids, and proteins. Specifically, we focus on the isotopic labelling of two high-demand RNA modifications: pseudouridine and N^1-methylpseudouridine, aiming to facilitate prospective Bio-NMR studies in the rapidly advancing field of RNA technology.

Method
We employed the hydrogen-oxidising bacterium C. necator to produce SI-labelled biomass chemolithoautotrophically from reagents such as $^{13}CO_2$, $^2H_2$, ^2H_2O, and $^{15}NH_4Cl$. From this biomass, uniformly $^2H/^{13}C/^{15}N$-labelled unmodified nucleotides were isolated through hydrolysis, chromatographic separation, and precipitation. These nucleotides then served as starting materials for the chemo-enzymatic synthesis of pseudouridine- and N^1-methylpseudouridine-5'-triphosphates (ΨTPs and m^1ΨTPs). Here, our primary objective was to introduce suitable ^1H-^{13}C and ^1H-^{15}N isolated spin topologies into these molecules for application in Bio-NMR spectroscopy.

Results
Based on the desired isotopic labelling patterns, we synthesised ΨTPs and m^1ΨTPs in 3–5 and 4–7 steps, respectively. During this process, we also developed an innovative and sustainable enzymatic method to efficiently convert Ψ- and m^1Ψ-5'-monophosphates into their corresponding 5'-triphosphates. The overall yields, relative to the most expensive building block (ribose-5-monophosphate), were 59% and 33%, respectively. All products exhibited isotopic enrichment levels exceeding 96% and were successfully incorporated into model 45-mer RNA fragments using T7 RNA polymerase. These RNA fragments were first synthesised with standard ribonucleoside 5'-triphosphates and, subsequently, also by replacing uridine 5'-triphosphate (UTP) with either ΨTP or m^1ΨTP. Notably, the RNA synthesis kinetics were comparable across UTP, ΨTP, and m^1ΨTP.

Conclusions
In summary, we successfully developed a method to generate a library of SI-labelled ΨTPs and m^1ΨTPs and incorporated them into RNA fragments. This efficient, adaptable, and commercially viable method holds promise for future developments in the field of RNA technology. Future work will focus on producing analogous nucleoside phosphoramidites for the chemical synthesis of site-specifically labelled modified RNAs.

CO_2 Fixation for Sustainable L-Methionine Production – The Role of Acyltransferases in the Biosynthetic Pathway

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L-methionine plays an important role in several physiological processes and is of great relevance in medical and pharmaceutical applications. As the conventional chemical synthesis of L-methionine relies on harsh chemicals such as methyl mercaptan, acrolein, and hydrogen cyanide[1], it is necessary to improve the sustainability of the L-methionine production. Therefore, we aim to establish an autotrophic bioprocess using the chemolithoautotrophic bacterium Cupriavidus necator as a chassis strain. Given its versatile metabolism and capacity for high cell density growth with minimal inclusion body formation, this bacterium shows great potential for producing specific compounds from CO_2, O_2, and H_2[2].
The regulation of the intracellular amino acid concentration in bacterial cells is complex and depends on multiple factors. High intracellular concentrations lead to the activation of negative feedback mechanisms which consequently result in a decreased biosynthesis of the respective amino acid. Thus, to reach the highest possible yield of L-methionine, multiple key enzymes of the biosynthetic pathway have to be targeted by strain and metabolic engineering.
While the amino acid biosynthesis and the enzymes involved have been studied in some bacteria[3, 4], several enzymes involved in the amino acid biosynthesis in C. necator have not been studied in detail. In the biosynthetic pathway of L-methionine, homoserine is acylated to O-succinyl- or O-acetyl-L-homoserine; this step is catalyzed by the enzyme MetXS in C. necator. In this study, we confirm the exact function of MetXS and report its biochemical properties.
In E. coli, the formation of O-succinyl-L-homoserine is catalyzed by the homoserine O-succinyltransferase MetA. We herein report the characterization of MetXS and the characterization and engineering of various MetA variants with the aim of increasing the transferase activity of the enzyme. The MetA variants will be heterologously expressed in C. necator to investigate the different strains for their ability to produce increased titers of L-methionine.

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Carboxylic Acid Reductase: Towards Understanding of Bottlenecks

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Carboxylic acid reductase enzymes (CARs) are well known for the reduction of a wide range of carboxylic acids to the respective aldehydes. CARs are comprised of three domains: an adenylation domain, a phosphopantetheinyl-binding domain and a reductase domain. Acid reduction is a multi-step cascade starting with carboxylate activation, which is followed by the formation of an enzyme-tethered thioester and finally, this thioester is reduced. The enzyme consumes one equivalent of ATP for acid activation. The acyl group is transferred from the emerging AMP-anhydride to the phosphopantetheine arm as a thioester. Next, the enzyme tethered substrate is transferred to the reductase domain and reduced at the expense of one equivalent of NAD(P)H.
One of our long-term goals is to gain deep insight into the structure-function-relationship of CARs and to apply them for synthesis. In cell free systems, the reaction scheme needs to be extended by further enzymes or other catalytic steps that allow for recycling of the two costly co-factors. Although much has been accomplished already [1-3], the overall reaction works best at substrate concentrations not higher than 12 mM. While the reaction velocity is high at the beginning, it typically stalls after 8-10 h. There is clearly a need to understand in detail what is happening on the molecular level. Only with this knowledge, educated protein- and reaction engineering will become possible.
Herein we present first results towards a systematic investigation of everybody's darling CAR, which is the CAR from Mycobacterium marinum. First, we explored various tagging variations to understand which affinity tag would either C- or N-terminally give highest possible expression levels and purities of MmCAR. We chose the most promising candidate to generate single- and di-domain variants, which will enable us to study each reaction step separately.

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

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The development of alternative chemical production is essential for achieving a circular bioeconomy. The EU-funded project "ConCO2rde" harnesses the chemolithoautotrophic metabolism of the knallgas bacterium Cupriavidus necator to develop biorefineries that convert CO2 into high-value compounds, such as nitrogen-heterocycles, using CO2 as sole carbon source, O2 as the electron acceptor, and H2 as the electron donor. Although this system presents a carbon-neutral production method by valorizing CO2, improving product yields and creating robust platform strains for diverse biotransformations are still needed.
In this study, our aim is to expand the array of products obtained from C. necator by establishing an enzymatic cascade to produce nitrogen heterocycles. The cascade includes an engineered O2-dependent putrescine oxidase (PuOx), an NADH-dependent imine reductase (IRED), and the O2-tolerant NAD+-reducing soluble hydrogenase (SH) of C. necator, used for cofactor regeneration [1, 2, 3]. For this purpose, we constructed different C. necator strains and evaluated their protein production. Gel immunoblotting analysis and activity assays of the soluble extract of C. necator cultivated under heterotrophic and autotrophic conditions showed that IRED was heterologously produced and was active. Further investigation will focus on the production and activity of PuOx, and cultivation strategies will be optimized to achieve higher production titers of nitrogen heterocycles. Integrating the enzymatic cascade with the autotrophic metabolism of C. necator offers a promising approach for a sustainable chemical production process and contributes to advancing CO2-based bioeconomy.

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Increasing electroporation efficiency in the lithoautotrophic bacterium *Cupriavidus necator* H16: A roadmap for non-model bacteria domestication

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Climate change is an urgent and collective challenge, and a portfolio of solutions is needed to reduce CO2 emissions or to increase carbon capture and utilization from the atmosphere. Nature has been evolving CO2 utilization pathways for billions of years and offers a promising repository of novel metabolisms and enzymes capable of CO2 fixation. However, non-model bacteria are recalcitrant to genetic engineering, and the application of modern genetic tools is cumbersome. One of the main barriers is the low transformation efficiency, as most tools and technologies require the delivery of DNA molecules to tune and modify the host metabolism. This transformation barrier is a common feature of all wild-type bacteria, which employ a variety of defense systems to avoid phages, plasmids, and other mobile genetic elements in their native ecological niche. To transform them with recombinant DNA, this arsenal has to be predicted, characterized and circumvented.

In our study, we performed an in-depth analysis of Cupriavidus necator H16 using bioinformatic tools to study its restriction enzymes and defense systems. By using tailored plasmids, we confirmed the functional role of three systems encoded in the genome, and through a combination of plasmid mutation and demethylation, we transformed large plasmids with higher efficiency. We also succeeded in transforming suicide plasmids via electroporation, deleting the native defense systems and creating a domesticated strain.

These findings will benefit both the C. necator H16 community and researchers working with other non-model bacteria by providing a roadmap that can be followed to increase transformation efficiency.

Brilliantly lazy: How *C. necator* only shines at avoiding work

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The current state of affairs, with ongoing climate crisis and depletion of fossil resources, calls for innovative industry to fulfill market demands while minimizing the impact on our planet. Biotechnology offers an attractive solution for classic petrochemistry, as microorganisms are equipped with countless pathways for the creation of value-added products from simple carbon sources such as glucose, cellulose or even CO2. Additionally, microorganisms' pathways can be expanded upon in with synthetic biology, unlocking near-infinite end-products.
Such genetically enhanced microorganisms are a vault of untapped potential; however, engineering efforts are more laborious than expected due to the complex and dynamic nature of microbial metabolism and regulation. It is for this reason unsurprising that when challenged with upscaling, characterized by stressful conditions and longer cultivations, the performance of the process is lower than anticipated. Furthermore, when faced higher number of generations, the productivity of these strains depreciates, reducing economic profit. This phenomenon is ubiquitous and is termed strain degeneration.
Here we describe the cunning strategies through which bacteria evade exploitation by studying engineered Cupriavidus necator for heterologous isopropanol production. Employing enhanced Green Fluorescent Protein (eGFP) as a biosensor, we have managed to follow strain degradation in the form of plasmid expression loss in continuous cultures. We have established that the expression of the isopropanol operon results in a drastic loss of plasmid expression in an increasing part of the population, which is not caused by a growth impairment. Through a multidisciplinary approach involving traditional counting methods and molecular techniques such as qPCR and sequencing we have attempted to ascertain the various mechanisms at play in this loss of expression, and through diverse strategies we have ventured to tackle the matter of strain degeneration.

Production of indigo dye through gas fermentation

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Climatic events are becoming increasingly frequent and harsh worldwide, causing irreparable environmental, economic, and societal damage. The scientific community has recognised climate change as responsible for these events, for which in turn the linear economic model of “make-use-dispose” must be blamed. Hence, a transition to a circular and bio-based economic model is required more than ever to prevent the reaching of the non-returning point. Among the industries with the biggest environmental impact, the textile industry will surely face continuous economic growth in future due to the demands of an increasing world population. Unfortunately, this industry still employs non-biodegradable materials and polluting traditional chemical synthesis methods. On the other hand, it is also one of the industries for which biotechnology may play a strategic role in speeding up the transition to more sustainable production methods, thanks to the technological readiness level of biotechnological processes used for the production of sustainable and biodegradable textile materials and dyes. In this context, one of the most employed dyes in the Denim fashion industry is indigo, whose large-scale production still employs heavily polluting and health-harmful chemicals. Bio-production of indigo was first demonstrated in Escherichia coli in the 90s, and since then different microbial hosts and enzymes have been tested to improve titers and yields. However, no large-scale process for indigo bioproduction is available to date. Here, we propose the chemolitoautotroph Cupriavidus necator H16 as a cell factory for a proof-of-concept production of the indigo dye and its derivatives through gas fermentation. The dye production was achieved by expressing a synthetic operon containing a naphthalene dioxygenase (NDO), belonging to the Rieske oxygenases family. This also proved that C. necator is a good chassis for the expression of this enzymatic class, for which the Rieske community has been struggling to find an ideal expression host. The use of metabolic engineering strategies for increasing tryptophan and indigo production is planned, as well as different protein engineering approaches for improving the specificity of the NDO enzyme towards the synthesis of more valuable indigo derivatives.


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