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Abstracts

Poster & Oral Presentations

125 Posters • 43 Oral Presentations

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Poster Presentations

Poster 1: Dual-Node Carbon Catabolite Repression Circuit Governs the Growth–Secretion Switch in an Industrial Non-Model Fungal Cell Factory

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Filamentous fungi are widely used as industrial cell factories for enzyme production, where productivity depends on balancing biomass accumulation with protein secretion. However, the regulatory systems governing this growth–secretion switch remain poorly understood, particularly in industrial non-model hosts. Carbon catabolite repression (CCR), mediated by the conserved C2H2 zinc-finger repressor Mig1/CreA, is a global nutrient-sensing mechanism controlling carbon utilization and metabolic allocation, yet its full regulatory architecture in production fungi remains unresolved. Using the industrially relevant non-model fungus *Talaromyces pinophilus* (formerly *Penicillium funiculosum* NCIM1228), we identify Mig3, a previously uncharacterized Eurotiales-specific C2H2 transcription factor that functions redundantly with Mig1/CreA as part of a dual-node CCR circuit. Mig3 binds canonical Mig1/CreA response elements with elevated DNA affinity, and simultaneous loss of Mig1 and Mig3 is synthetically lethal, revealing an essential shared regulatory function beyond classical carbon repression. Integrative transcriptomic, ChIP-seq, and proteomic analyses show that Mig1/CreA and Mig3 directly coordinate mitochondrial and ribosomal gene networks required for respiratory and translational capacity while repressing alternative carbon metabolism and secretion-associated pathways. Consistent with this central role in cellular resource allocation, perturbation of either regulator alters respiratory function and increases sensitivity to mitochondrial and ribosomal inhibitors. Together, these results identify a systems-level regulatory module governing the transition between growth and enzyme secretion and provide new targets for engineering improved industrial fungal cell factories.

KEYWORDS Carbon repression, synthetic lethality, Secretion, Biomass, Fungal cell factories, regulation

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Poster 2: From Suicide Enzyme to Catalytic Engine: Reprogramming THI4 for Carbon-Efficient Plants

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Reducing atmospheric CO_2 is essential for limiting global warming to 1.5 °C and achieving net-zero emissions by 2050. Beyond carbon capture technologies and reforestation, engineering plants with improved metabolic efficiency represents a promising strategy to enhance biological carbon sequestration. One overlooked inefficiency lies in thiamin (vitamin B1) biosynthesis, particularly in the enzyme THI4. In plants and fungi, the thiazole synthase THI4 functions as a suicide enzyme: it donates sulfur from an active-site cysteine to form the thiazole ring and becomes irreversibly inactivated after a single catalytic cycle. Continuous replacement of THI4 imposes a substantial energetic burden on cells and contributes to respiratory carbon loss. In contrast, certain prokaryotic THI4 enzymes lack the active-site cysteine and instead use sulfide as a sulfur donor, enabling true catalytic turnover. However, these enzymes typically originate from anaerobic organisms and function poorly under oxygen-rich conditions. To address this limitation, we are applying continuous directed

evolution using the OrthoRep system in yeast to engineer catalytic THI4 variants capable of functioning under aerobic, plant-like conditions. In parallel, we are exploring natural diversity within the plant kingdom to identify non-cysteine THI4 variants. Our genomic and biochemical analyses reveal catalytic THI4 paralogs in cereals such as barley, wheat, and oat, suggesting that plants may naturally evolve mechanisms to reduce this metabolic inefficiency. However, natural evolution operates over long timescales, and we need to act faster. Leveraging synthetic biology tools, such as laboratory evolution and genome editing, provides a powerful approach to rapidly introduce beneficial mutations and transform energetically costly enzymes into more efficient catalytic systems that ultimately improve plant productivity and carbon storage. Together, these findings highlight catalytic THI4 engineering as a promising synthetic biology strategy to improve plant metabolic efficiency and enhance carbon sequestration.

KEYWORDS Carbon sequestration, Directed Evolution, Suicide enzyme

Poster 3: Engineering Genetic Information with Modified Nucleic Acids

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Natural organisms use a four-letter genetic alphabet that generates 64 triplet codons to encode proteins composed of the 20 canonical amino acids. If an additional unnatural base pair (UBP) orthogonal to the A–T and G–C pairs is introduced, up to 152 codons can theoretically be created, enabling the incorporation of multiple distinct noncanonical amino acids (ncAAs) into proteins.

Here, we employed the dNaM–dTPT3 UBP in *E. coli* to encode ncAAs through the transcription and translation of unnatural codons. Using a systematic screening approach, we identified nine unnatural codons that efficiently support ncAA incorporation. Furthermore, we demonstrated the simultaneous decoding of three unnatural codons in a single gene, representing one of the largest expanded-codon sets reported at that time.

To improve the robustness of *E. coli* toward unnatural nucleic acids, we developed a β-lactamase–based selection system in which a UBP is placed at the essential Ser70 position, making enzyme expression strictly dependent on UBP retention and decoding. This system enabled optimization of the nucleotide transporter. We will also discuss the potential of unnatural nucleic acid chemistries beyond UBPs as synthetic genetic information elements, opening new avenues for protein engineering and nucleic acid–based therapeutic applications.

KEYWORDS modified nucleic acids, genetic code expansion

Poster 4: Machine-Learning Tools to Elucidate Species-Specific Promoter Design Rules Across the Proteobacteria

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Despite decades of research into bacterial promoters, accurately predicting promoter activity from DNA sequence remains a challenge, even in model organisms. In the absence of systematic studies, there is a general assumption that the same promoter sequence will have similar activity across closely related species. However, research has shown that when the same promoters are transferred among Proteobacteria within the same taxonomic class, their rank order of expression is often inconsistent.

Uncovering a phylogenetic correlation in promoter activity, where closely related species exhibit similar promoter activity levels, could guide the discovery of effective promoters in non-model species. Here, we employed high-throughput screening to measure expression from a promoter library in 15 Proteobacterial species in the Alpha-, Beta-, and Gammaproteobacteria to generate a comprehensive expression dataset. We first generated feature vectors that encoded biochemical, biophysical and compositional properties of the sequences. We leveraged these encodings to train machine learning classifiers for each taxonomic class and achieved an AUC score exceeding 0.75 in all three models. To uncover phylogenetic relationships, we tested the performance of models trained on data from a single taxonomic class in classifying promoter activity from species of that same class compared to species from other classes. Finally, we applied explainable AI for model interpretation to compare feature importance profiles across the three models to elucidate species-specific promoter design rules. This work will enable a more targeted approach to identifying promoters with predictable activity across species, streamlining genetic circuit design for a range of genetic engineering applications.

KEYWORDS promoter, gene expression, proteobacteria, machine learning, AI, broad host range

Poster 5: **Unlocking the full genetic potential of *Streptomyces* through synergizing systems and synthetic biology**

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Streptomyces are soil-dwelling Gram-positive bacteria that are major producers of natural products, known as secondary metabolites (SMs). These compounds exhibit diverse pharmaceutical activities, including antimicrobial, antifungal, anticancer, and immunosuppressive properties. Although *Streptomyces* genomes typically contain more than 30 SM biosynthetic gene clusters (smBGCs), most of these clusters remain unlinked to their corresponding products. Furthermore, many smBGCs are silent under standard laboratory conditions, which limits their practical utilization. To fully exploit the genomic potential of *Streptomyces*, I have applied systems and synthetic biology approaches to: (1) identify novel smBGCs; (2) elucidate regulatory mechanisms to activate silent clusters; (3) rationally enhance SM production through multi-omics–based bottleneck analysis; and (4) reprogram SM biosynthetic machinery using retrobiosynthetic strategies. Systems biology provides a global understanding of secondary metabolism and identifies key engineering targets, whereas synthetic biology and retrobiosynthesis enable the design and production of new-to-nature compounds beyond the scope of conventional metabolic engineering.

KEYWORDS Systems biology, Synthetic biology, Secondary metabolites, Retrobiosynthesis

Poster 6: **Investigating DNA as a crowding agent in Synthetic Transcription Systems**

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In vitro transcription and translation enable synthetic biologists to produce on demand RNAs and proteins, and to investigate the molecular dynamics that control their production. Therefore, understanding the factors that enhance in vitro transcription is relevant both for bioproduction and

molecular biology. Synthetic “crowding” agents can mimic the cell's packed environment and enhance the rate of in vitro transcription and translation. However, less is known on the impact biological “crowding” may have on transcription. The nucleus and nucleoid contain millions of base pairs of DNA that are not transcribed at a given time. Can the presence of noncoding DNA near transcribed regions affect RNA production? In this study, transcription of a gene encoding the enhanced Broccoli fluorescent RNA aptamer was quantified using fluorescence as a readout. We found that in a defined T7 RNA Polymerase system and PURExpress the addition of certain noncoding plasmids can enhance transcription of a gene on the target plasmid. In addition, transcription increases as the concentration of noncoding plasmids increases. Next, we investigated if this increase in transcription is due to a crowding effect. A FRET assay was performed to quantify the hybridization of ssDNA in the presence of noncoding plasmids. Plasmid DNA enhanced the hybridization of ssDNA suggesting plasmids exclude volume like macromolecular crowders. This novel understanding of DNA as a potential crowding agent can help optimize cell free systems for increased bioproduction. In addition, these insights shed light on the dynamics of transcription in nuclei and suggest evolutionary constraints to minimizing genomes.

KEYWORDS Cell Free Expression, Molecular Crowding

Poster 7: Dynamic Gene Regulation in *Bacillus subtilis*

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Bacillus subtilis is a widely used Gram-positive chassis for industrial enzyme production, metabolic engineering, and synthetic biology. Despite its importance, the relationship between core promoter architecture and operator-based regulation in this organism remains insufficiently defined, limiting predictable gene circuit design. To systematically dissect how intrinsic promoter strength interacts with operator-mediated repression, we first constructed a synthetic promoter library by varying the −35 and −10 elements to generate a graded spectrum of basal transcriptional strengths. This established a quantitative reference set of core promoter architectures in *B. subtilis*. We then integrated tet operator (tetO) sequences at defined positions within these promoters, placing them in the spacer between the −35 and −10 elements, downstream of the promoter, or at both sites. This design enabled direct assessment of how operator placement influences intrinsic promoter strength. Using the TetR–tetO system, selected for its low leakiness and graded induction behavior, we expressed TetR constitutively and quantified repression and dose-dependent activation in response to anhydrotetracycline. We evaluated whether inducible regulation preserves the strength hierarchy defined by −35 and −10 variation and how spatial operator positioning affects repression efficiency and dynamic range. Together, these results define architectural principles for constructing tunable and inducible transcriptional modules in *B. subtilis*, supporting more predictable circuit and pathway engineering in this industrially relevant host through an expanded regulatory toolkit.

KEYWORDS Promoter architecture, Tunable Transcriptional Regulation, Synthetic Biology Toolkit, *B. subtilis*

Poster 8: Development of a thermostable dextransucrase for the production of a-1,6-glucosylated Rebaudioside A derivatives at elevated temperature

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Steviol glycosides from *Stevia rebaudiana* are widely used as non-caloric sweeteners. Even so, their sensory profile and formulation performance often benefit from enzymatic tailoring. Glucosylation has been actively explored to soften undesirable taste notes. In addition, biocatalytic conversion provides a practical route to rebaudioside analogs that are rarely found in nature and can show improved physicochemical behavior. In this study, dextransucrase is used to transfer glucose from sucrose onto rebaudioside A, generating an α-1,6-glucosylated rebaudioside A derivative. This modification is commonly associated with higher aqueous solubility and improved stability across a range of pH and temperature conditions. For industrial translation, the main bottleneck is maintaining enzyme performance under heat. Operating at elevated temperature can speed up conversion, lower contamination risk, and integrate more smoothly with multi-enzyme reaction schemes. These advantages require a catalyst that remains active and stable when heated. To meet this need, we engineer dextransucrase for enhanced thermostability while preserving efficient transglucosylation. Structure-guided site selection will direct mutagenesis, and directed evolution will be applied to explore and enrich improved variants. Screening will be performed under elevated-temperature conditions to identify enzymes that sustain productive formation of the α-1,6-glucosylated rebaudioside A derivative at higher operating temperatures. The proposed workflow is expected to offer a practical enzyme-engineering approach for producing diverse glycosylated natural product derivatives and for developing durable biocatalysts suitable for industrial biotransformations.

KEYWORDS Synthetic biology, Enzyme engineering, Directed evolution, Thermostable biocatalyst, Dextransucrase

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Poster 9: Microbial Daisyworld: A Synthetic Biology Experiment Toward Testing Gaia Theory

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James Lovelock and Lynn Margulis first proposed the Gaia theory in the 1970s, positing that interactions between organisms and their environment shape Earth into a self-regulating system that maintains conditions favorable to life. Rather than being a collection of isolated components governed by chance, Earth functions as an integrated whole. A formal expression of this idea emerged with the Daisyworld model, a theoretical construct that demonstrated how homeostasis could arise as an emergent property of life-environment interactions. Despite the conceptual power of Daisyworld and similar models, a gap remains between theory and experimental validation. This study bridges that gap by using synthetic biology to construct a microbial analog of Daisyworld. We engineered a two-strain *Escherichia coli* consortium in which one strain produces acid and the other produces base, mimicking the temperature-modulating roles of black and white daisies in Lovelock's work. Their opposing effects on pH create a dynamic, self-regulating system that stabilizes environmental conditions near the optimal growth range for both strains. To test this system, we designed and

characterized the recombinant strains, calibrated continuous bioreactors, and quantified key parameters for mathematical modeling. Through controlled experiments, we aim to show that microbial feedback can stabilize environmental pH via population-level adjustments, offering empirical support for the hypothesis that simple biological loops can drive environmental homeostasis. Life-environment feedbacks are key to understanding global change.This study introduces the first experimental framework for investigating biosphere-level regulation using synthetic biology, offering new tools for ecological monitoring and potentially for ecosystem restoration.

KEYWORDS Synthetic biology, Microbial ecology, Daisyworld, Gaia theory, pH regulation, homeostasis

Poster 10: A Growth-Coupled Biosensor Platform for Engineering High Malonyl-CoA-Producing Strains

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Conventional enzyme engineering approaches largely focus on selecting and combining only the top-performing variants, while discarding variants with intermediate or low activities. Although effective for improving enzyme performance, such approaches overlook epistatic interactions among amino acid mutations, whereby mutations with minimal individual effects can produce unexpected synergistic or antagonistic outcomes when combined. In contrast to traditional enzyme engineering, which relies on phenotypic observations to guide genotype analysis, recovering overlooked information requires a deeper understanding of and explicit matching between phenotype and genotype. Here, we present a growth-coupled biosensor-based high-throughput screening platform that establishes a direct linkage between enzyme amino acid sequences and enzymatic performance. This platform enables systematic collection of comprehensive datasets spanning high-, intermediate-, and low-performing variants, which are used to train machine learning models. This data-driven strategy facilitates not only genotype-based phenotype prediction but also rational enzyme design that quantitatively accounts for epistatic interactions. As a representative application, we target acetyl-CoA carboxylase (ACC), a key enzyme controlling intracellular malonyl-CoA production. Malonyl-CoA is a central precursor for high-value natural product biosynthesis like polyketides, and ACC catalytic efficiency directly determines its cellular availability. By applying this platform to diverse ACC variants, we aim to elucidate genotype–phenotype relationships at scale and rationally design ACC enzymes that maximize malonyl-CoA production.

KEYWORDS 1. Enzyme engineering 2. Genotype–phenotype matching, 3. High-throughput screening 4. Biosensor 5. Acetyl-CoA carboxylase (ACC)

Poster 11: Sustainable Production of High-Value Chemicals from Renewable C1 Feedstocks

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Sustainable Production of High-Value Chemicals from Renewable C1 Feedstocks Abdullahi Muhammad Labbo1,2, Bashir Rumah1, Klaus Winzer1, Ying Zhang1 1Synthetic Biology Research

Centre (SBRC), University of Nottingham, UK. 2Sokoto State University, Sokoto, Nigeria The continued reliance on non-renewable carbon-based energy sources for chemical production is increasingly unsustainable due to resource limitations and the environmental impacts of greenhouse gas emissions. Consequently, there is growing interest in sustainable, environmentally friendly approaches to producing high-value bio-based chemicals. Conventional fermentation processes typically rely on sugar-based substrates, which can limit both economic and environmental sustainability. Fermentation-based biological processes utilising one-carbon (C1) feedstocks, such as methane and methanol, offer significant potential for sustainable production of chemicals, fuels, and pharmaceuticals from renewable resources. These processes also provide an opportunity to reduce greenhouse gas emissions by converting industrial waste gases from sectors such as steel manufacturing, oil refining, anaerobic digestion, and natural gas processing into valuable products In this work, we demonstrate the use of methylotrophs as a microbial platform for the production of the compatible solute ectoine from C1 feedstocks. A combination of strain optimisation and bioprocess development was employed to enhance the production of novel compatible solutes. Culture conditions were systematically optimised, and the process was successfully scaled up from small-scale cultures to laboratory bench-top bioreactors, demonstrating the feasibility of methylotroph-based ectoine production under controlled bioprocess conditions.

KEYWORDS Methylotrophs, C1 feedstocks, Compatible solutes, Sustainable biotechnology

Poster 12: Effect of cell division-related genes on cellular growth in a genomically minimal cell

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Many bacterial species control cell division and regulate cellular size; however, it remains unclear how cellular growth and fitness depend on cell division and cellular size, in part due to their complex genetic bases in natural bacteria. Engineered to have the smallest known genome among cells capable of autonomous growth and division, the genomically minimal cell JCVI-syn3B provides a simplified model system to study the genetic requirements for cell division. JCVI-syn3B retains genes in the highly conserved division and cell wall (dcw) cluster, such as the bacterial tubulin homolog ftsZ and its membrane anchor sepF. In JCVI-syn3B, transposon bombardment reveals that genes in the division and cell wall cluster are non-essential for cellular growth. The metabolic cost of expressing ftsZ and sepF has not been quantified, and it remains unclear how these genes contribute to cell division in mycoplasmas such as JCVI-syn3B, which do not have a peptidoglycan cell wall. In this work, to estimate the metabolic cost of expressing non-essential genes, we measure the doubling time of JCVI-syn3B and related strains. We observe faster growth upon deletion of ftsZ, sepF, and an adjacent gene of unknown function, which we interpret with quantitative metabolic models. We estimate cellular size distributions by super-resolution confocal microscopy and observe no significant difference in cellular size upon deletion of ftsZ, suggesting alternative mechanisms of cell division in JCVI-syn3B.This work will clarify the influence of cell division on cellular growth and size, using JCVI-syn3B to study genes and mechanisms shared in more complex cells.

KEYWORDS Genomically minimal cells, JCVI-syn3A, bacterial division, metabolic burden

Poster 13: **Identification of chromosomal integration sites in *Escherichia coli* Nissle 1917 for expression of therapeutic protein cargoes in a safe bacterial chassis**

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Escherichia coli is a common host in synthetic biology, and non-pathogenic strains can be used without further attenuation for therapeutic applications. The probiotic strain *E. coli* Nissle 1917 (EcN) is a promising live biotherapeutic candidate, but its clinical translation as a modular chassis requires safe, stable chromosomal integration sites for therapeutic payloads. This work focuses on the identification of non-essential genetic loci in EcN encoding adhesins, fimbriae, autotransporters and toxin-associated factors, and on their evaluation as potential insertion sites for expression of protein cargoes. Building on a conjugative donor–recipient system, we implemented a workflow in which suicide integration vectors carrying homologous recombination arms of the selected loci flanking a Ptac-GFP gene reporter are delivered by bacterial conjugation and resolved to leave scarless insertions without antibiotic resistance markers or vector backbone sequences. For each targeted site, we first characterized expression profiling under induced and non-induced conditions using the inducible Ptac-GFP reporter to identify loci offering favorable expression windows for future integration of therapeutic payloas. Altogether, our work outlines a conjugation-driven, markerless strategy to both attenuate EcN and open defined chromosomal integration sites, advancing this strain as a safer, genome-programmable chassis for future biomedical applications.

KEYWORDS *E. coli* Nissle, live biotherapeutics, genome engineering

Poster 14: **Fungal Biosynthesis of Antimicrobial Peptides for Enteric Methane Mitigation Anna Donnan1, Nicole M. Tosto1, Emily J. Parker2, Sarah A. Kessans1**

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New Zealand's agricultural sector contributes nearly half of national greenhouse gas emissions. Enteric fermentation from pasture-grazed livestock accounts for 82% of methane emissions—a potent climate pollutant targeted for 24–47% reduction by 2050 relative to 2017 levels. More than NZD 283 million has been invested into research related todietary methane mitigation, genetics, vaccines, manure management, and chemical inhibitors. However, New Zealand's pasture-based systems—covering 40% of land with non-native grasses such as perennial ryegrass—limit dependable inhibitor delivery, as methane suppressants must contact each mouthful of feed to be effective.

Antimicrobial peptides (AMPs) inhibit methanogenic archaea and bacteria, with results illustrating dose-dependent methane reductions in rumen in vitro studies without impairing digestion. Despite regulatory approval across multiple sectors, AMP production in native hosts remains low-yield and cost-prohibitive, driving the need for novel heterologous expression platforms.

Epichloë festucae, the natural endophyte of ryegrass that provides 75% of New Zealand's livestock feed, represents a promising candidate for an in situ methane mitigation strategy. As an Ascomycota symbiont, *E. festucae* possesses large cellular volumes, efficient secretion pathways and post-translational modification capabilities that could support in planta AMP biosynthesis. This approach has the potential to establish a pasture-based delivery system for continuous methane inhibitor production, reducing reliance on feed processing or controlled dosing.

Using synthetic biology's design–build–test–learn framework, this project aims to optimise expression architecture in *E. festucae* across transcriptional, translational, and post-translational levels. Beyond methane mitigation, engineered *E. festucae* offers a platform for in situ production of bioactives, advancing sustainable agriculture without altering pastoral systems.

KEYWORDS Antimicrobial peptides (AMPs) *Epichloë festucae* Methane emissions Enteric fermentation Heterologous expression Synthetic biology

Poster 15: **Hybrid Biocatalytic Cascades aided by Machine Learning**

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Enzymatic cascades offer an efficient route for one-pot syntheses, enabling access to unstable intermediates and overcoming thermodynamic barriers conventional chemistry struggles with. However, their broader application remains limited. Optimizing conditions for multiple enzymes in a large parameter space is experimentally taxing, and the scope of reactions is constrained by the catalytic repertoire of natural enzymes. Our work addresses these challenges through closed-loop automation and the use of designer enzymes. We employ an artificial enzyme containing para-aminophenylalanine (LmrR-pAF) integrated via amber codon suppression that catalyzes the formation of a hydrazone chromophore, producing a measurable color shift. This reaction enables continuous monitoring in a 96-well plate format. Automated liquid handling ensures reliable and high-throughput condition screening, while a Bayesian optimization algorithm proposes new conditions based on prior results, completing a fully self-optimizing experimental cycle. We validate this approach on a two-step enzymatic cascade combining 5-hydroxymethylfurfural oxidase (HMFO) with the designer enzyme LmrR-pAF. Subsequently, we expand to larger systems, including cofactor recycling, and more challenging transformations, such as optimizing conditions for decarboxylases to catalyze reverse reactions. This closed-loop framework provides a generalizable strategy to accelerate the development of hybrid enzymatic cascades.

KEYWORDS enzyme cascades, artificial enzymes, automation, machine learning

Poster 16: Multimodal Mutational Scanning to Reveal the Functional and Adaptive Landscapes of an Entire Virus

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Viruses are essentially biological information encoded in nucleic acids that can be packed in protein capsids for propagation. In most cases, viral genomes are sufficient to recruit the host's machinery and specify the additional biological functions required to complete their complex life cycle. Despite the apparent simplicity of these genetic systems, the quantitative relationships between viral genotype and phenotype remain poorly understood at the genome scale. To address this fundamental gap, we conducted a comprehensive functional analysis of the 6,032-nucleotide genome of *Junonia coenia* densovirus (JcDV), which can notably infect invasive, crop-damaging caterpillars. Using high-throughput DNA synthesis and cloning, we generated 300,000 barcoded point mutations (substitutions, insertions and deletions) that systematically span the JcDV genome. Through the development of quantitative phenotypic screens based on barcode sequencing in insect-derived cell lines and live caterpillar, we have undertaken to systematically assess the genomic variations impact on different stages of JcDV's life cycle, including: host cell targeting and entry, gene expression, replication, genome packaging and virion stability. Here we describe the mutant library construction strategy along with exemplary data on transcription, replication and viral fitness in selected genomic regions. These preliminary results pave the way for more extensive experiments. Eventually, this work will advance our fundamental understanding of how complex biological systems function, laying data informed foundations for engineering enhanced biocontrol agents with applications in sustainable agriculture, gene therapy, and synthetic biology.

KEYWORDS Deep mutational scanning, Virus, Synthetic genomics

Poster 17: Coupling biosensors to logic circuits in soil bacteria

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Synthetic biologists have focused for decades on engineering biosensors and genetic logic circuits. However, these goals have traditionally been pursued separately, even though natural cells seamlessly sense inputs and process them into outputs through integrated information flow. This ability to compute environmental inputs underpins our work. Here, we present a library of biosensors responsive to environmentally relevant signals, coupled to genetic logic gates. As chassis organisms, we use the soil bacteria *Pseudomonas putida* KT2440 and *Pseudomonas protegens* Pf-5. We developed a modular biosensing platform for environmental information processing and logic integration. Biosensors for iron, oxygen, temperature, and light were constructed in standard format. Chemical stimuli are integrated through the endogenous regulatory networks of these soil bacteria, while physical cues are sensed via engineered regulatory cascades, thereby expanding the range of environmental signals these organisms can process. To enable signal computation, each sensor was coupled to a library of Boolean NOT logic gates. We experimentally validated the system in both strains, demonstrating functional modularity and portability across *Pseudomonas* species. The logic

gates exhibited host- and input-dependent performance. While some gates showed input-specificity, others could be coupled to two different inputs. Notably, one logic gate was successfully regulated by three independent inputs, revealing a versatile "plug and play" compatibility between sensors and logic modules.

Together, this work establishes a plug-and-play framework for microbial environmental computing, opening new opportunities for the design of adaptable, context-dependent synthetic circuits.

KEYWORDS *Pseudomonas*, biosensors, genetic logic circuits, environmental inputs

Poster 18: Evolutionary engineering of a yeast coculture platform for biomanufacturing

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Synthetic microbial communities have the potential to bring novel design strategies to biotechnological processes, but these rely on our ability to control and stabilise their population composition. Metabolite cross-feeding has been studied as mechanism to achieve this, but its applicability in yeast communities is limited by their low metabolite secretion rates. A poor understanding of their export mechanisms has so far limited the success of engineering interventions to enhance cross-feeding, remaining an obstacle to the implementation of competitive bioprocesses. In our work, we use adaptive laboratory evolution (ALE) to approach this question. By selecting for community-level growth and community composition metrics, we isolated a cross-feeding coculture with significant improvements in growth performance. We studied the effect of ALE on biomanufacturing by introducing a biosynthesis pathway split across the ancestral and evolved cocultures and observed that the evolved chassis improved both titre and productivity, while ensuring the coexistence of both biosynthetic modules. Retracing the steps of ALE on transcriptomic data, we found that it had alleviated a general metabolic slowdown resulting from limited access to cross-fed metabolites, and it had dampened a nutritionally triggered replicative stress response, both undesired in a bioproduction context. Our results illustrate the relevance of ALE as a tool to mitigate the effects of engineered obligate cross-feeding in yeast, and how this is a key enabling step for their use in bioproduction setups.

KEYWORDS SynComms, bioproduction, ALE, cocultures

Poster 19: **Identification of a Key Bottleneck Enzyme for Enhanced Glycolic Acid Production Using Flux Balance Analysis**

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Microbial production of glycolic acid (GA) from renewable carbon sources represents a sustainable alternative to conventional chemical synthesis. The Dahms pathway converts xylose to GA through a relatively simple enzymatic route; however, its application is limited by low productivity and growth inhibition arising from metabolic bottlenecks. In this study, flux balance analysis was applied to systematically investigate these limitations, identifying aldehyde dehydrogenase (AldA) as a key constraint in the pathway. We further identified a novel AldA from *Buttiauxella agrestis* (BaAldA) with improved catalytic performance compared to *E. coli* AldA (EcAldA), exhibiting a 1.69-fold lower Km and a 1.49-fold higher kcat/Km. Expression of BaAldA in *E. coli* increased GA production by 1.59-fold. In fed-batch fermentation, the engineered strain produced 22.70 g/L GA with a yield of 0.497 g/g xylose, corresponding to 98.2% of the theoretical yield. These results demonstrate that BaAldA is an efficient enzyme for GA biosynthesis and highlight the effectiveness of flux analysis in identifying and relieving metabolic bottlenecks in engineered metabolic pathways.

KEYWORDS Glycolic acid, Dahms pathway, Aldehyde dehydrogenase, Metabolic flux balance analysis

Poster 20: **Toward Self-Driving Enzyme Engineering: Continuous Evolution Coupled to Growth-Based Selection**

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Continuous evolution approaches reimagine traditional enzyme engineering strategies by performing the necessary diversification, selection, and amplification steps within a single host cell. By eliminating time-consuming and laborious molecular biology steps, continuous evolution systems do not only accelerate enzyme engineering campaigns, but also provide the opportunity to study how mutations accumulate and shape enzyme function over time. Toward establishing a versatile and fully integrated continuous evolution platform for both enzyme optimization and understanding of molecular adaptation, we interfaced a recently-developed selection strategy with continuous small-scale selections in bioreactors. Specifically, we applied an iterative workflow, consisting of error-prone PCR, continuous selection, and sequence analysis, to the N-L-carbamoylase from *Sinorhizobium meliloti*, SmLcar. Throughout these cycles, increasing selection pressures enriched catalytically improved variants, allowing beneficial mutations to emerge and spread throughout the populations. To track evolutionary trajectories, we analyzed populations at multiple time-points by high-throughput sequencing (Oxford Nanopore®). The generated data provided a dynamic view of mutation frequencies, revealing which variants rose to dominance, which mutations persisted or were lost, and

how evolutionary pathways developed. By combining continuous selection, controlled environmental pressures, and real-time population analysis, this platform moves toward a more autonomous and data-driven paradigm for enzyme engineering.

KEYWORDS Continuous evolution , Mutation dynamics , Adaptive landscapes

Poster 21: Engineering Living Bacterial Therapeutics for Cancer Immunotherapy

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Solid tumors pose a major challenge to CAR-T cell therapy by restricting immune cell infiltration and creating an immunosuppressive tumor microenvironment. Here, we present a synthetic biology–based microbial immunotherapy platform built on the probiotic strain *Escherichia coli* Nissle 1917 (ECN) to enhance T cell activation and improve the tumor specificity of CAR-T cells in solid tumors. ECN was metabolically engineered to overproduce L-arginine, an immunomodulatory amino acid known to support T cell activation and modulate immunosuppressive conditions within tumors. In parallel, we designed a genetic circuit that enables ECN to secrete synthetic antigens into the tumor microenvironment, allowing localized and selective activation of CAR-T cells at the tumor site. By combining localized metabolic immunomodulation with programmable antigen secretion, this platform offers a modular strategy to reprogram the tumor microenvironment and enhance the precision of CAR-T cell responses. This work highlights the potential of engineered probiotics as living immunotherapeutic devices to overcome key limitations of CAR-T therapy in solid tumors.

KEYWORDS Synthetic biology, Metabolic engineering, Tumor therapy

Poster 22: The Synthetic D-Threose-Dependent Glycolaldehyde Assimilation Pathway for the Biosynthesis of 2,4-Dihydroxybutyric Acid in *E. coli* and *Pseudomonas putida* KT2440

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Biotechnological processes are gaining industrial relevance as both substrate and product spectra expand and production efficiencies improve. However, these processes still predominantly rely on sugars as primary carbon and energy sources, creating competition with food and feed production. To mitigate this, alternative non-food feedstocks are required. Ethylene glycol, which may be sourced from plastic waste, non-edible biomass, or CO_2 via electrocatalytic reduction, has emerged as a promising next-generation feedstock [1]. Recently, we presented a new-to-nature metabolic route for assimilating ethylene glycol (EG) via the synthetic threose-dependent glycolaldehyde assimilation pathway [2]. This linear pathway produces 2,4-dihydroxybutyric acid (DHB), from which bioplastics [3] and a methionine analog [4] can be derived. Pathway functionality was first demonstrated in *E. coli*, resulting in production of 0.8 g L^{-1} DHB at a yield of 0.15 molDHB $molEG^{-1}$. Although enzyme engineering improved kinetic parameters and specificity of two key pathway enzymes, the primary metabolic bottleneck of the initial oxidation remained. In *E. coli*, this reaction is catalyzed by a NAD^+-dependent alcohol dehydrogenases and is therefore thermodynamically unfavorable under physiological conditions. Replacing the microbial chassis with *Pseudomonas putida* KT2440 provided

access to irreversible PQQ-dependent ethylene glycol oxidation. Combined with further strain engineering and gene expression optimization, we show a substantial improvement in DHB titers and yields. [1] Wagner et al., 2023, doi: 10.1016/j.biotechadv.2023.108276. [2] Frazão et al., 2023, doi: 10.1038/s41467-023-37558-x. [3] Frazão et al., 2019, doi: 10.1038/s41598-019-48091-7. [4] Walther et al., 2017, doi: 10.1038/ncomms15828.

KEYWORDS ethylene glycol, *Pseudomonas putida* KT2440, next-generation feedstock, 2,4-dihydroxybutyric acid, metabolic engineering

Poster 23: A polymeric shielded caffeine bacterial biosensor for safe and targeted bacterial cancer therapy.

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Engineered bacteria represent a promising cancer therapeutic platform due to their intrinsic ability to colonize tumor microenvironment (TME) and deliver therapeutic agents directly in situ. However, bacterial therapies face major limitations, including systemic toxicity and insufficient control over therapeutic delivery limiting their clinical translation. To address these challenges, we have developed a bacterial therapeutic platform combining spatial and temporal control of bacterial activity. Spatial control is provided by a PEG-poly(L-lysine) block copolymer grafted to *Escherichia coli* Nissle membrane. The polymer, functionalized with a pH-sensitive moiety, is engineered to disassemble under TME acidic conditions, allowing bacterial delivery at the tumor site. We have developed a bacterial coating protocol enabling a shielding compatible with full bacteria viability and preventing 58% of specific bacterial surface antigens recognition of anti-*E.coli* antibodies in in-vitro conditions. To further enable temporal control of therapeutic delivery, we have built ligand-responsive genetic circuit relying on the CadC-pCadBA system, in which the transmembrane receptor CadC is fused to a caffeine-binding VHH. Caffeine binding induces CadC dimerization, thereby activating transcription from the pCadBA promoter. To optimize biosensor performance in-vivo, a library of 500 promoter-ribosome binding site variants controlling CadC expression was constructed and screened. This strategy enabled identification of biosensor variants with minimal basal activity in absence of inducer while maintaining strong activation upon caffeine stimulation. Together, these results establish a dual-gated bacterial therapeutic platform combining targeting spatial control through pH-responsive polymer shielding and temporal control through externally inducible genetic circuit, providing a strategy for precise and controllable bacterial cancer therapy.

KEYWORDS Bacterial cancer therapy, *Escherichia coli* Nissle 1917, PEG polymer shielding, caffeine biosensor

Poster 24: Tessellating Voronoi pattern through bacterial chemotaxis

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Voronoi diagrams partition space into regions defined by proximity to discrete seeds and emerge across scales in physics, biology, and materials science. In microbial systems, spatially separated inoculation points can generate Voronoi-like sectoring during colony expansion. Here, we develop a synthetic platform in *Escherichia coli* to program bacterial Voronoi pattern formation in soft agar. By controlling motility and chemotaxis, and by tuning environmental parameters such as agar

concentration, we quantify how cellular and physicochemical factors interact to shape macroscopic spatial organization. We demonstrate that sector boundary formation is governed by the interplay between bacterial motility, growth, and chemoattractant diffusion and consumption. When chemoattractant gradients dissipate before opposing migration fronts meet, stable cell-depleted gaps emerge, generating Voronoi tessellations. In contrast, when bacterial diffusivity approaches chemical diffusivity, fronts merge and boundaries collapse. Coupling quantitative experiments with mathematical modeling enables predictive reconstruction of patterns, such as weighted Voronoi, 3D distributions and composite Voronoi pattern using two bacterial strains with different motility rate.

KEYWORDS Voronoi, pattern formation, motility, chemotaxis

Poster 25: DUET: Dual-Chassis Strategy for Bridging Adaptive Evolution and Rational Design for Synthetic Biology

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Genome streamlining and pathway refactoring enable the construction of controllable microbial chassis for both fundamental studies and biotechnological applications. However, these strategies create a trade-off: rational design reduces genetic complexity, whereas adaptive laboratory evolution (ALE) relies on metabolic redundancy. Here, we introduce a dual-chassis framework using *Acinetobacter baylyi* ADP1 and its genome-streamlined derivative ISx to resolve this conflict. As a proof of concept, we redesigned the β-ketoadipate pathway, a central metabolic hub for aromatic catabolism. We systematically streamlined this pathway by deleting individual branches. We then created a synthetic entry point for protocatechuate (PCA) catabolism by reconstructing a minimal PCA module coupled to the catechol (CAT) pathway. ALE restored efficient PCA utilization through this engineered pathway, and reverse engineering identified the mutations responsible for this recovery. Together, these results establish a chassis-level strategy for combining rational metabolic streamlining with evolutionary recovery, providing a general framework for engineering robust and controllable microbial systems.

KEYWORDS genome streamlining, ALE, dual-chassis, *Acinetobacter baylyi* ADP1, beta-ketoadipate pathway

Poster 26: Growth-coupled selection of tyrosine phenol lyase variants via genetic code expansion

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Directed evolution is essential for unlocking the catalytic potential of enzymes in chemical synthesis. Growth-coupled selections, which link catalytic activity to cellular survival, enable the simultaneous interrogation of millions of variants and drastically accelerate enzyme engineering. However, applying such selections to synthetically useful C–C bond-forming reactions remains challenging, as their products rarely confer a direct fitness advantage. To meet this challenge, we present a growth-coupled selection strategy for tyrosine phenol lyases (TPLs), which catalyze the enantioselective synthesis of tyrosine analogues from substituted phenols. Specifically, we leverage genetic code expansion to render cells addicted to a TPL-generated tyrosine analogue. Using this platform, we identify TPL

variants with high activity toward 2-nitrophenol, an electron-poor substrate that the wild-type enzyme cannot process. Through iterative rounds of growth-based selection, we uncover multiple evolutionary solutions to convert this challenging substrate and obtain biocatalysts with synthetically relevant rates and turnover numbers. Because the selection logic is substrate-agnostic – requiring only the biosynthesis of a non-canonical amino acid – we also present proof of principle for its expansion to other C–C bond-forming reactions catalyzed by (designer) biocatalysts.

KEYWORDS directed evolution, growth-coupled selection, genetic code expansion

Poster 27: Evolving a synthetic genetic circuit in *Escherichia coli*

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Both electric and synthetic genetic circuits (SGCs) are modular networks used to design non- and living systems with desired properties. The characteristics of well-performing circuits are modularity, robustness, predictable behavior and subsequent scalability – all of which are exhibited by electric circuits upon construction. However, SGCs, when created in practice, rely on long optimization periods based on trial and error. Such difference is caused by context – non-living systems are largely universal whilst biological entities are heavily context-dependent. This project aims to provide an alternative – hypothetically, a more natural – approach to SGC design in *Escherichia coli*. The proposed approach involves 1) introducing an overparameterized SGC template in the cell, 2) training the cell to learn from the environment that mimics the desired circuit behavior, and 3) profiling cellular genome and transcriptome to characterize the evolved circuit topology, dynamics and host context. It is hypothesized that, if carefully incentivized, a cell with a template rather than a rationally engineered topology can develop network characteristics by fitting it into the existing biological context through evolutionary tuning. The outcomes are expected to lead to insights into designing an overparameterized SGC template, achieving emergent behavior and extracting network topology information from single cell-level characterization.

KEYWORDS synthetic genetic circuits, evolution, emergent behavior, network topology

Poster 28: Microbial synthesis of hydroquinone from waste feedstocks

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Hydroquinone (HQ) (1, 4-dihydroxybenzene) is a bulk chemical widely used across multiple industries, including pharmaceuticals, photographic processing, and in production of high-performance thermoplastics. This drives a global market volume of ~80 K tonnes annually with a CAGR > 5 %. HQ is currently synthesised at industrial scale via chemical synthesis from fossil-fuel derived benzene, concurrent with high greenhouse gas emissions. As such there is widespread demand for more sustainable alternatives. To this end, we have developed a novel bioprocess for HQ production from renewable or waste C-rich feedstocks via tyrosine as the key intermediate. This involved integrating Tyr overproduction through constitutive expression of feedback resistant enzymes in the Shikimate pathway, and heterologous expression of tyrosine phenol lyase (TPL) and a regiospecific P450 hydroxylase, enabling maximum HQ titres of 220 mg/L at shaker flask scale starting from sustainable feedstocks. As HQ degradation was observed as a key bottleneck,

metabolomics and transcriptomics studies were performed to investigate cellular responses to HQ accumulation and inform further genetic optimisation. Together, this work outlines a novel bio-process for sustainable HQ production from C-rich waste streams, supporting a circular economy and decreasing reliance on finite fossil fuels.

KEYWORDS Metabolic engineering, waste upcycling, circular economy

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Poster 29: A rapid expression optimisation strategy applied on a growth-coupled heterologous Entner-Doudoroff pathway in *Escherichia coli*

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When implementing a heterologous metabolic pathway, the initial focus is on establishing basic functionality, often using strong (or inducible) constitutive promoters. However, this often leads to high metabolic burdens, intermediate toxicity or other issues. These effects can result in suboptimal pathway functionality, or even complete failure of establishing the pathway. Further optimization to resolve these issues is sometimes done using labor intensive genetic library construction and screening or time-consuming adaptive laboratory evolution. As an alternative to these labor-intensive optimization strategies, an effective co-optimisation of expression of all genes in a given pathway is needed. We propose and present preliminary results of a strategy that enables such co-optimisation. We applied this co-optimisation strategy on the implementation of a heterologous four-gene Entner-Doudoroff (ED) pathway in *Escherichia coli*. Rather than relying on native regulation or constitutive overexpression, each ED gene was expressed trough orthogonal well-titratable, inducible promotors, allowing independent and systematic tuning of expression within a single strain. A broad range of transcription-strength combinations was explored by varying inducer concentrations in 96-wells plates. Our preliminary results suggest that this strategy enables rapid identification of expression conditions that support fast growth and related high flux through the heterologous pathway. In the future, we aim to further develop this workflow and to finalise the ED optimisation with an improved expression unit and multiple optimisation iterations. Afterwards, we will test its limits by expanding to larger pathways such as synthetic CO2 fixation pathways.

KEYWORDS Pathway expression optimization, Entner-Doudoroff pathway, inducible promotors

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Poster 30: A recombinase-programmable viral sensor for recording discrete molecular events and amplifying them into macroscopic light signals

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Site-specific recombinases are key components of synthetic gene circuits, enabling precise and irreversible DNA rearrangements that can encode logic and memory in living systems. However, quantifying their activity in planta in a direct, sensitive, and non-destructive manner remains a significant challenge. Here, we present a genetically encoded reporter system that functions as a whole-plant biosensor for recombinase activity. This system records discrete DNA recombination events and converts them into a macroscopic, self-amplifiable signal. The platform is based on a stably integrated, deconstructed Tobacco Mosaic Virus (TMV) replicon in *Nicotiana benthamiana*, maintained in an inactive state by an inverted segment of the viral RNA-dependent RNA polymerase

(RdRp) flanked by recombination sites. Upon recombinase-mediated rearrangement, the viral replicon is restored, triggering autonomous replication. To monitor these events, we coupled replicon activation to a fungal-derived autobioluminescence pathway, enabling substrate-free, real-time imaging in intact tissues. Each recombination event leads to localized viral amplification of the reporter signal, generating discrete luminescent foci that act as spatially resolved records of recombinase activity. By linking a single DNA-level event to a self-amplifying optical output, this system enables the direct visualization and quantification of recombination across whole tissues without destructive assays. This approach provides a practical framework for implementing and evaluating recombinase-based memory, logic, and event-recording circuits in plant synthetic biology.

KEYWORDS Plant Synthetic Biology; Site-specific recombinases; Viral replicons; Autobioluminescence

Poster 31: A Biosensor-Driven High-Throughput Screening Strategy for Efficient Aromatic Polyketide Production in *Escherichia coli*

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Polyketides are secondary metabolites of significant industrial value. Although polyketide synthases (PKSs) have been successfully expressed in *Escherichia coli*, the rapid optimization of production strains is currently hindered by the low throughput of traditional analytical techniques, such as HPLC and LC-MS. While malonyl-CoA biosensors can be employed to indirectly measure the polyketide production capacity, biosensors that can explicitely indicate the final polyketide product levels are not available. To overcome these limitations, we developed a colorimetric biosensor that enables the visual detection of C16 aromatic polyketide production through red pigmentation. The broad applicability of this system was demonstrated in both Gram-negative (*Pseudomonas putida*) and Gram-positive (*Bacillus subtilis*) species. By integrating this biosensor with a genome-wide CRISPR interference (CRISPRi) library-assisted high-throughput screening, we identified 20 knockdown target genes that increased the target polyketide production by over 50%. Subsequent combinatorial knockdown of these targets further increased the production of C16 aromatic polyketides, with triple-knockdown strains exhibiting the highest titers. These genetic modifications also proved effective in enhancing the biosynthesis of other polyketides with different carbon chain lengths. Mechanistic investigations revealed that the observed improvements were not directly correlated with increased malonyl-CoA flux or enhanced PKS stability, which necessitate further exploration. This robust and versatile platform provides a high-throughput solution for the accelerated engineering of polyketide-producing microbial cell factories. This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (No. RS-2024-00440975) (RS-2024-00398252).

KEYWORDS polyketide, high-throughput screening, CRISPR interference, biosensor

Poster 32: Bioengineering self-sustaining *E.coli* for PLA degradation and upcycling

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This project focuses on polylactic acid (PLA), an industrially compostable bioplastic widely used in the packaging and 3D printing industry, driving a rapidly growing market (3.3 USD billion by 2028, 17%

CAGR) due to increasing public awareness of plastic pollution and demand for sustainable materials. Although this material can be produced from renewable sources and/or be degraded by microorganisms under industrial composting conditions, its unmanaged post-consumer waste can lead to microplastics accumulation, changes in soil communities and generate greenhouse gasses after their biological degradation. Therefore, it is imperative to develop novel technologies to valorise its post-consumer waste and ensure long-term sustainability of this growing sector. To address this, we sought to leverage the innate biodegradability of PLA through engineering a common industrial biotechnology strain to degrade, bio assimilate and upcycle PLA waste. We developed a one-pot system for simultaneous PLA biodegradation and metabolic upcycling, using a single engineered strain of *Escherichia coli*, engineered for secretion of a PLAase and subsequent conversion to (1) protocatechuate and (2) lycopene. Intriguingly, we found that the system was self-sustaining, with cultures grown in the presence of PLA waste achieving higher biomass and product titres than those lacking PLA. This allows continuous biomass and target chemical production over time. This work demonstrates the vast untapped potential of biodegradable plastics as an immediate resource for circular bioeconomy, further incentivising their widespread use in a more sustainable plastics future.

KEYWORDS Plastic upcycling; Plastic degradation; Sustainable chemicals production

Poster 33: **Sustainable Microbial Synthesis of Levodopa from PET Plastic Waste**

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The transition to a defossilized chemical industry which uses abundant waste feedstocks is vital to mitigate climate change and build a resilient circular economy (Nature 649, 267). An emerging way to achieve this is to utilise biology, which can catalyse chemical reactions under milder conditions and produce an array of compounds that are difficult to synthesize traditionally. We propose to leverage these approaches to produce the Parkinson's drug L-DOPA (also known as Levodopa) from polyethylene terephthalate (PET) plastic waste via engineered *Escherichia coli* strain (Nature Sustainability, manuscript accepted). Co-expression of a seven gene pathway in *E. coli* BL21(DE3) enables the conversion from the PET plastic monomer TPA to L-DOPA, and we found that expressing importer tpaK from *Rhodococcus jostii* RHA1 greatly improved TPA uptake and conversion. The pathway intermediate PCA strongly inhibited the final enzymatic step to L-DOPA, which was confirmed by in vitro data and molecular docking simulations. By splitting the pathway into two strains, one that produces catechol from TPA that is followed by a strain catalysing the synthesis reaction to L-DOPA, we were able to achieve up to 4.7 g/L under mild aqueous conditions. This process was then performed at shake flask scale using post-consumer stamping foils (a novel and abundant source of PET waste), and subsequently isolated as a TFA-salt. To further improve sustainability, we found that glucose derived from bread waste was comparable to commercial preparations, and we used *Chlamydomonas reinhardtii* to capture CO2 released during catechol generation.

KEYWORDS Metabolic Engineering, Biotechnology, Health

Poster 34: PURE makes PURE: reconstitution of the PURE cell-free system from self-synthesized proteins

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Building a universal biochemical constructor—an autonomously self-replicating biochemical system—is a major challenge in bottom-up synthetic biology. The PURE (Protein Synthesis Using Recombinant Elements) cell-free system is an ideal starting point for exploring self-regeneration, and its 36 non-ribosomal proteins constitute the primary macromolecular components that must be regenerated. In this poster, we demonstrate that the PURE system can be reconstituted from proteins synthesized by PURE itself. We first show that each of the 36 non-ribosomal proteins can be individually synthesized in PURE. We then purify the PURE-synthesized proteins as pooled subsets and show that all subsets can recover the functionality of the ΔPURE reaction (a PURE reaction lacking the corresponding proteins). We then reconstitute a fully functional PURE system by combining the subsets. Finally, we show that all 36 non-ribosomal PURE proteins can be synthesized simultaneously in a single PURE reaction and, after purification, can reconstitute a functional PURE system. Together, these results establish that the non-ribosomal protein components of the PURE system can be self-regenerated, representing a critical step toward the realization of a universal biochemical constructor and, ultimately, the construction of a synthetic cell.

KEYWORDS bottom-up synthetic biology, cell-free system, PURE, protein regeneration

Poster 35: Development of a *Yarrowia lipolytica* co-culture system for ginger essential oil bioproduction

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Ginger essential oil (GEO) presents a plant-based and biodegradable alternative to synthetic biocontrol agents, with synergistic potential as an anti-phytopathogenic, weed and pest control solution. Although presenting several advantages, its widespread adoption within agriculture has been limited due to key challenges associated with its unsustainable and uneconomical extraction from plant material. Metabolic engineering offers the possibility to develop a stable, inexpensive, and environmentally-friendly bioproduction platform through microbial cell factories. Given the limitations associated with engineering a single strain, this project aimed to develop a proof-of-concept for a modular co-culture system that can produce an alternative to GEO, with major constituent compounds produced at ratios found in nature, utilising specialised strains of the oleaginous yeast *Yarrowia lipolytica*. This study successfully constructed *Y. lipolytica* strains capable of producing terpene compounds found in GEO, employing two key strategies: gene expression modulation and pathway engineering. In the latter, MVA pathway boosting both in the cytoplasm and peroxisome was explored. To achieve production of three major constituents of GEO at a tunable ratio, a co-culture strategy based on orthogonal auxotrophies was designed. Orthogonal auxotrophies in *Y. lipolytica* were characterised, as well as the impact of amino acid supplementation on growth rate. Co-cultures with desired production profiles were achieved through the fine-tuning of inoculation ratio and media composition. Successful de novo bioproduction of a range of sesquiterpenes was achieved in *Y. lipolytica*. Initial findings suggest that the co-culture design explored shows potential as a model for a bioproduction platform producing a mixture of value-added compounds.

KEYWORDS metabolic engineering, *Yarrowia lipolytica*, co-culture, biocontrol, terpenes, MVA pathway

Poster 36: Alien condensates as human-orthogonal organelles for programmable biochemistry

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Biomolecular condensates are membraneless compartments organizing cellular biochemical reactions. While they enrich functionally related proteins across virtually every biological process, their tight integration with native pathways limits their use as orthogonal tools in mammalian cells. We present a species-orthogonal strategy repurposing extremophile proteins to build human-orthogonal condensates with programmable function. We express two such proteins in human cells: antifreeze glycoprotein AFGP from Antarctic icefish and desiccation protein CAHS2 from tardigrade. Both form condensates in human cells. Quantitative proteomics on FACS-isolated alien condensates reveals they are compositionally sparse, recruiting 1-2 orders of magnitude fewer human proteins than endogenous nucleoli or nuclear speckles. AFGP condensates are driven by dispersed LXF/LIF motifs in an Ala-Ala-Thr backbone. Mutating these motifs abolishes condensation despite equivalent hydrophobicity. Remarkably, installing this grammar into human PCF11 or plant GRP IDRs "fishifies" them, redirecting partitioning specifically into AFGP condensates. CAHS2 condensates demonstrate programmable function: split-nanoluciferase fragments reconstitute only when co-concentrated inside, creating an insulated, light-emitting reaction compartment absent from human biochemistry. These alien condensates form modular synthetic organelles insulated from native pathways. Our species-orthogonal approach reveals natural condensation diversity beyond human grammars, providing composable platforms for pathway insulation and spatial control in mammalian synthetic biology.

KEYWORDS biomolecular condensates, intrinsically disordered regions (IDRs), extremophile proteins, synthetic organelles

Poster 37: Large-scale Combinatorial Engineering of a Key C1-fixation Pathway Enzyme for Superior Acetogen Cell Factories

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Sustainable biotechnologies that convert waste carbon into fuels and chemicals are critical in addressing the global climate crisis. Gas fermentation using acetogenic bacteria has emerged as a promising technology to valorise carbon-rich gaseous and solid waste streams via the Wood–Ljungdahl pathway (WLP). The WLP enables acetogen bacteria to fix CO and CO_2 into biomass and value-added products. Integrated proteome and flux analyses in the model-acetogen *Clostridium autoethanogenum* have revealed that formate tetrahydrofolate synthase (Fhs), which catalyzes the ATP-dependent conversion of formate to 10- formyl-THF, is the most abundant enzyme during autotrophy, yet its apparent in vivo catalytic rate is significantly lower than for other WLP enzymes. This disparity suggests that high enzyme abundance compensates for limited catalytic efficiency, identifying Fhs as a target for optimization to increase WLP throughput. Here, we developed a

combinatorial protein engineering strategy to improve Fhs catalytic performance. Structural analysis identified 10 candidate sites that influence active-site geometry and substrate interactions, leading to 31 rational amino acid substitutions. We used NEBuilder HiFi assembly to construct a combinatorial plasmid library comprising ~200,000 Fhs variant sequences. High- throughput electroporation of *C. autoethanogenum* was performed. Next, the pooled population will be screened under autotrophic conditions to select Fhs variants supporting enhanced gas fixation, enabled by real-time gas pressure dynamics. Lastly, cells with superior variants will be isolated, sequence-verified, and beneficial Fhs mutation(s) reverse engineered into the endogenous enzyme. Altogether, this work establishes a scalable high-diversity, growth-coupled engineering framework for optimization of C1-fixing enzymes in an obligate anaerobe.

KEYWORDS acetogen, gas fermentation, formate tetrahydrofolate synthase, protein engineering

Poster 38: Engineering bacterial multicellular structures for therapeutic applications

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Probiotic bacteria have broad therapeutic potential across mucosal and epithelial surfaces, yet their clinical translation is limited by poor persistence and unreliable colonization. Bacterial self-assembly is a key determinant of probiotic survival and adhesion with aggregate size critically influencing therapeutic success. For example, smaller assemblies could be used for gastrointestinal delivery as the size must remain below the threshold for intestinal clearance. Whereas larger assemblies could enable probiotic-based modalities such as anticavity pastes, topical treatments for vaginal infections, and wound dressings that inhibit pathogenic biofilm. Despite this importance, existing aggregation strategies offer limited control over aggregate dimensions. Here, we present a programmable platform for bacterial self-assembly based on the surface display of elastin-like polypeptides (ELPs), self-binding polymers composed of repeating pentapeptides with a single variable guest residue. By systematically varying ELP guest-residue and pentapeptide repetitions, we demonstrate a correlation between amino acid sequence to assembly size. Using these principles, we generated bacterial assemblies spanning the micron- to centimeter-scale range of living structures. Notably, we found that ELPs containing aromatic residues consistently produce the largest assemblies, despite comparable surface hydrophobicity across the sequences tested.

This work provides a generalizable strategy for engineering bacterial multicellularity with precise physical control, enabling enhanced colonization and persistence across diverse host environments. Moreover, this platform supports integration of additional genetic functionalities, including tissue-specific adhesion and controlled therapeutic delivery. This positions programmable bacterial self-assembly as a foundation for the next generation of engineered probiotics and living materials.

KEYWORDS Engineered living materials, probiotics, therapeutic delivery, self-assembly

Poster 39: From heteroscedastic data to robust parameters in plate-reader assays

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Synthetic biology relies on well-characterised biological parts that can be rationally assembled into circuits of increasing complexity. Microplate readers are widely used to quantify growth and fluorescence during part and circuit characterisation. For instance, biosensor dose-response curves generated using microplate readers are routinely fit to Hill functions using ordinary least squares (OLS) to estimate key parameters such as dynamic range and sensing threshold (IC_{50}). However, plate-reader measurements are typically heteroscedastic, with residual variance increasing with fluorescence/OD, violating the constant-variance assumption underlying OLS and many common statistical tests.

Here, we present simple methods to model measurement noise in plate-reader data, leading to improved fits and more reliable parameter confidence intervals. We are implementing these approaches in a user-friendly Python package to facilitate adoption across the synthetic biology community. In summary, our results show that inappropriate statistical analysis can introduce avoidable variability on top of biological variation, and we propose simple methods to extract more accurate and reproducible insights from the same data.

KEYWORDS synthetic biology, plate-reader assay, dose-response curve, biosensor, Hill function, parameter estimation, heteroscedasticity

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Poster 40: SynBridge: a versatile framework for bidirectional interconversion between BioBrick and MoClo assembly standards

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While traditional BioBrick standards laid the foundational groundwork for synthetic biology, current Golden Gate-based methods like Modular Cloning (MoClo) have revolutionized the field through simultaneous multi-part assembly. However, effectively integrating the vast repository of legacy BioBrick parts with contemporary MoClo systems remains a significant logistical bottleneck. To address this, we present SynBridge (Synthetic Bridge), a novel bidirectional framework designed for seamless interconversion between BioBrick and MoClo assemblies. It comprises two core operations: (i) Bri2Mo converts BioBrick devices into a MoClo-compatible allowing them to be incorporated into hierarchical assemblies. (ii) Mo2Bri executes the reverse process, conferring BioBrick compatibility onto newly MoClo-assembled devices. This interoperability workflow was experimentally validated by assembling and testing a canonical BioBrick construct (LuxR-mediated RFP expression) alongside a MoClo-assembled device (thermoregulated GFP expression). Assembly fidelity and functional expression were quantified via phenotypic population percentages and relative fluorescence

characterization. Our results demonstrate a robust system that successfully bridges generations of standards, ensuring the strategic repurposing of legacy genetic parts. Ultimately, SynBridge is a highly resource- and time-effective framework that promotes backward compatibility, maximizes part reusability and consolidates the idempotent roots of synthetic biology.

KEYWORDS Synthetic Biology, DNA standards, Modular Cloning, Biobricks, Golden Gate

Poster 41: **Enabling Synthetic Methanotrophy: Cell Factory Design for Methane Bioconversion**

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The transition towards a sustainable bioeconomy is driving demand for engineering biology solutions that leverage microbial cell factories to convert low-cost feedstocks into high-value products. IDEA Bio operates as a cell factory design studio, providing synthetic biology services to support both research and industry. IDEA Bio enables the design–build–test–learn (DBTL) cycle by integrating parallel bioreactor cultivation with advanced analytics, supported by deep cellular characterisation. This approach generates quantitative datasets that inform rational design, accelerate development timelines, and improve reproducibility at lab-scale, increasing confidence in scale-up. As a case study, we highlight the development of a synthetic methanotrophic bacterium. *Escherichia coli* was engineered to express soluble methane monooxygenase (sMMO), enabling methane-to-methanol conversion. Due to its multi-subunit nature, recombinant sMMO requires extensive optimisation for heterologous expression in *E. coli*. To address this, a platform is being developed using a gas-tight deep-well plate system for cultivation, coupled with biochemical analysis of methanol production. This platform will enable rapid screening of key expression parameters and is also applicable for assessing protein variant activity in engineering workflows. Lead conditions and protein candidates are subsequently validated in parallel bioreactors with off-gas analysis to further characterise strain performance. Although experiments are ongoing, this integrated approach, combining detailed strain characterisation, and multi-omics data comparison, is expected to systematically evaluate strains and process conditions, identify key bottlenecks in the metabolic pathway, and guide subsequent optimisation. Overall, this work demonstrates how IDEA Bio facilitates the translation from concept idea to laboratory-scale discovery, advancing towards industrially relevant bioprocesses.

KEYWORDS bacteria, C1 Bioprocess, strain engineering, fermentation

Poster 42: **Bacterial sigma factor-dependent promoters for orthogonal transcription in *Saccharomyces cerevisiae***

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The development of orthogonal genetic tools is essential for advancing synthetic biology and enabling precise control of gene expression across diverse organisms. In this study, we designed and characterised orthogonal σ70-dependent promoters for *S. cerevisiae* by leveraging the structural and functional homology between bacterial sigma factors and eukaryotic transcription factors. A rational design approach was employed to combine bacterial core promoter elements with yeast-specific promoter features, including poly(dA:dT) tracts and scanning regions, to create hybrid promoters that

function through the interaction between the bacterial σ70 and native RNAPII in *S. cerevisiae*. These promoters were further optimised through randomisation of the spacer region between the -35 and -10 boxes, resulting in four different libraries of variants with diverse expression levels and activation ratios. After screening of these libraries, 22 promoter variants were selected for further characterisation. The results revealed that the poly(dA:dT) tracts significantly impacted the orthogonality, while the scanning region had a bigger impact on the promoter strength. Furthermore, randomisation of the spacer region had an influence on both. This work demonstrates the feasibility of engineering cross-domain transcription systems and provides a foundation for developing universal genetic tools applicable to both prokaryotic and eukaryotic hosts.

KEYWORDS Promoter, sigma factor, RNAPII, orthogonal, cross-species, *E. coli*. *S. cerevisiae*

Poster 43: **Harnessing yeast isogenic heterogeneity for improved bioproduction**

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Phenotypic heterogeneity in clonal microbial populations plays a pivotal role in shaping ecological dynamics and resilience. This variability among genetically identical cells influences microbial interactions, adaptation, and survival strategies in complex environments. While heterogeneity in nature supports population resilience, it has profound implications for biotechnology and medicine, impacting bioproduction efficiency, antimicrobial resistance, and therapeutic outcomes. Our work focuses on developing integrated experimental and computational tools to study microbial heterogeneity at single-cell resolution, using integrated single-cell multi-omics. By dissecting the cellular drivers of heterogeneity and the metabolic consequences that result from differential cell adaptation, we aim to harness microbial cell factories, optimising bioproduction through precision engineering. In this study, we identify amino acid and carbon starvation as triggers of bet-hedging, where subpopulations of cells either favour growth or survival, and demonstrate their profound impact on microbial cell factories.

KEYWORDS Phenotypic heterogeneity, Yeast, Biotechnology

Poster 44: **From PET glycolysis to PHAs: a synthetic consortium approach**

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A chemo-biological strategy for plastic upcycling is presented. We couple chemical depolymerization of polyethylene terephthalate (PET) with a division-of-labor microbial consortium to convert PET-derived waste into polyhydroxyalkanoates (PHAs). PET was depolymerized via glycolysis to bis(2-hydroxyethyl) terephthalate (BHET), bypassing limitations of in vivo degradation for crystalline materials. A two-species consortium was then established using *Comamonas testosteroni* RW31, which assimilates terephthalic acid (TPA) and accumulates poly(3-hydroxybutyrate) (PHB), and *Pseudomonas putida* JM37, which consumes ethylene glycol (EG) and produces medium-chain-length PHA (mcl-PHA). Each population was engineered to secrete one of two synergistic PET-hydrolyzing enzymes -PETase and MHETase- to hydrolyze BHET to TPA and EG, creating a metabolic

co-dependency and supporting growth and polymer accumulation. This setup concurrently valorizes both monomers and enables co-production of both PHAs in one bioprocess (PHB from the TPA specialist and mcl-PHA from the EG specialist). Community dynamics were monitored during bioconversion in a selected consortium, which was able to fully convert commercial and post-consumer BHET to PHA in 24 h. This consortium was able to accumulate predominantly PHB, reaching ~25–30% of cell dry weight. Overall, this engineered consortium improves robustness through a division of labor strategy, supporting plastic circularity via synthetic biology. This chemo-biological approach is modular and adaptable to other depolymerized plastics for scalable industrial applications. This work was supported by the Spanish project MOLA (PID2024-162673NB-I00 MICIU/AEI/ 10.13039/501100011033).

KEYWORDS microbial consortia; PET upcycling; glycolysis; bioplastics; PHB; PHAs; division of labor, *Pseudomonas putida*, *Comamonas testosteroni*

Poster 45: **Engineering Turing patterns in a synthetic microbial consortium**

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Turing patterns are ubiquitous in nature and represent a core mechanism of self-organized biological structure. Yet, engineering robust synthetic Turing patterns in living systems remains a major challenge due to the narrow parameter space of classical activator–inhibitor systems. Here, we demonstrate that cross-diffusion can overcome these constraints, enabling diverse and stable spatial patterns in a synthetic community composed of two amino acid–auxotrophic *Escherichia coli* strains. Moreover, rapid diffusion of small-molecule metabolites through agar, combined with slow cellular movement, greatly expands the conditions for Turing instability. Experimentally, we observe a range of patterns, including stripes, rings, dots, and labyrinth-like patterns, and capture their dynamics with a mathematical model based on competitive Lotka–Volterra equations incorporating cross-diffusion. Together, these results bridge theoretical Turing mechanisms and experimental synthetic biology, establishing a robust, scalable platform for programmable spatial organization. Moreover, our findings suggest that cross-diffusion may also play a key role in natural pattern formation, realizing Turing's vision of pattern formation from simple physical principles.

KEYWORDS pattern formation, Turing pattern, Voronoi pattern, microbial consortium

Poster 46: **Engineering Phototrophic Biomineralisation for Carbon Sequestratio**

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Climate change and biodiversity loss pose escalating risks to human societies. Limiting global warming to 1.5–2 °C, as outlined by the Intergovernmental Panel on Climate Change, requires reaching net-zero carbon emissions by 2050 and deploying substantial carbon dioxide removal. However, existing carbon capture and storage technologies often entail high energy demand, significant economic costs, and potential environmental trade-offs. Biological carbon sequestration offers an alternative approach. In particular, phototrophic biomineralisation by cyanobacteria and algae—where CO_2 is converted into stable carbonate minerals—represents a promising strategy for long-term carbon storage. Despite its potential, the mechanisms governing microbial biomineralisation and its

scalability remain poorly understood. Here, I propose to combine synthetic biology and quantitative phenotyping to investigate and engineer carbon sequestration through biomineralisation in the well-characterised marine cyanobacterium *Synechococcus* PCC 7002. First, I will present comparative data characterising biomineralisation capacity across diverse species of algae and cyanobacteria. I will then describe early genetic engineering efforts in PCC 7002, examining how targeted modifications influence biomineralisation rates and alter calcium carbonate crystal morphology. By integrating metabolic model, genetic engineering, and materials characterisation, this project aims to elucidate the biological controls of carbonate formation and establish a foundation for scalable, low-energy, and environmentally compatible carbon sequestration technologies.

KEYWORDS biomineralisation, synthetic biology, cyanobacteria, carbon capture, sustainability, biomaterial

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Poster 47: **Fungal-bacterial synthetic consortia enhance power output and plant-beneficial metabolite production in hydroponic-associated microbial fuel cells**

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Microbial fuel cell (MFC) technology enables the simultaneous treatment of wastewater and generation of bioelectricity. In this study, we explore MFCs as functional modules to be integrated into hydroponic cultivation systems, acting as a prosthetic rhizosphere capable of enhancing plant nutrition. To this end, we compared the performance of different rationally designed consortia in MFCs fed with mock wastewater and root exudates. The selected microorganisms were the electroactive bacterium *Shewanella oneidensis*, the plant growth–promoting rhizobacterium (PGPR) *Pseudomonas putida*, and the lignocellulose-degrading dimorphic fungus *Ophiostoma piceae*. This approach aimed to promote metabolic complementarity between electroactive, plant-associated, and biomass-degrading microorganisms, enhancing substrate utilization and bioelectrochemical activity. In addition, the effect of the quorum sensing (QS) analogue molecule 1-dodecanol was assessed. Electrochemical and microbiological parameters were evaluated, including growth, feedstock assimilation, anode colonization, voltage, current and power output, as well as the production of redox-active molecules and plant growth–promoting substances (PGPS), such as flavins and siderophores. Among the configurations tested, the consortium combining *S. oneidensis*, *P. putida*, and *O. piceae* showed the highest electrical production potential and the greatest degree of organic matter removal, indicating improved wastewater treatment, with the addition of 1-dodecanol further enhancing system performance. Moreover, extracellular siderophore production was detected in MFCs containing *P. putida*, suggesting a possible contribution to plant nutrition. Overall, our results suggest that syntrophic microbial consortia can enhance electricity generation while increasing the functional value of MFCs in integrated hydroponic systems, to our knowledge representing the first incorporation of a dimorphic fungus into MFC microbial consortia.

KEYWORDS Microbial fuel cells, *Shewanella oneidensis*, *Pseudomonas putida*, *Ophiostoma piceae*, plant growth promoting, quorum sensing

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Poster 48: **From Targets to Titers: High-Throughput Biofoundry Engineering at Scale**

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Once optimization of fermentation process has maximized cellular output, further gains in product titers can come from metabolic engineering approaches. With the explosion of AI, machine learning, omics data, and genome-scale models, we can now generate thousands of potential engineering targets. The challenge is no longer what to engineer; it's how to rapidly test it all. That's where biofoundries come in. At the BRIGHT Biofoundry, we deploy high-throughput strain engineering, real-time monitoring, and phenotypic screening to rapidly cycle through Design–Build–Test–Learn (DBTL) iterations. We integrate computational design with scalable experimental platforms to extensively explore design space, including: enzyme libraries and pangenome homologue mining; AI-driven protein engineering to push pathway performance; and genome-scale CRISPRi libraries to uncover high-impact genetic targets. We pair this with rapid, decision-ready screening methods: growth-coupled selection systems, fluorescence biosensors, and streamlined chemical or enzymatic endpoint assays. The result: faster iteration, smarter designs, and higher-performing strains. Come to my poster to see how we turn massive target lists into real biological gains.

KEYWORDS Biofoundry, Strain Engineering,

Poster 49: **Re-engineering carbon fixation: Carbon monoxide dehydrogenase as a metabolic control point in *Clostridium autoethanogenum*.**

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Clostridium autoethanogenum, a model gas-fermenting acetogen, is a promising microbial chassis for converting C1 gases into fuels and chemicals. Central to autotrophic metabolism are carbon monoxide dehydrogenase (CODH) enzymes, which couple carbon fixation to energy conservation and product formation. Interestingly, the primary CODH, AcsA, in the wild-type *C. autoethanogenum* JA1-1 strain is uniquely truncated, and this truncation is lost in the superior LAbrini strain following autotrophic adaptive laboratory evolution of JA1-1. In addition, protein expression of monofunctional CODH CooS1 has remained high and differentially regulated in *C. autoethanogenum* in various autotrophic conditions. Here, we investigated the functional significance of replacing acsA stop codon with leucine (Leu_SNP) or serine (Ser_SNP), and the effect of cooS1 deletion in *C. autoethanogenum*. Autotrophic batch and chemostat characterisation revealed significantly altered growth, carbon flux, and by-product distributions in SNP strains, whereas cooS1 deletion produced minimal and condition-dependent effects. Structural modelling of the SNP-derived AcsA variants identified no major conformational changes compared to wild-type AcsA. Transcriptomics revealed extensive transcriptional changes associated with reduced robustness and altered by-product profiles in Leu_SNP strain, whereas cooS1 deletion had limited transcriptional changes. Our study broadens current understanding of CODH function in acetogen metabolism and offers engineering targets for improving acetogen cell factories.

KEYWORDS acetogen, autotrophy, Wood-Ljungdahl pathway, reverse genetic engineering, CODH/ACS, chemostat, transcriptomics

Poster 50: **Building a modular carotenoid pathway beyond plastid limits in *Phaeodactylum tricornutum***

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Diatoms are promising chassis for sustainable pigment production, but plastid-localized carotenoid pathways are tightly regulated and strong upregulation can impair photosynthesis. In *Phaeodactylum tricornutum*, the periplastidial compartment (PPC), a cytosolic remnant of secondary endosymbiosis, contains isoprenoid precursors and, together with the cytosol, may offer an extraplastidial space for metabolic engineering with reduced competition from native plastid pathways. Here, we established a modular carotenoid pathway in the PPC/cytosol of *P. tricornutum* and tested whether extraplastidial pigment accumulation can be achieved without compromising photosynthetic function. Heterologous genes were assembled on extrachromosomal episomes using standardized uLoop workflows. The pathway comprised crtE, crtB and crtI from Pantoea ananatis, expressed alone or in combinations, and construct variants were designed to tune subcellular targeting among the PPC, cytosol, and chloroplast. Expression was screened by flow cytometry, carotenoids and intermediates (including phytoene) were quantified by HPLC-DAD, and photophysiology was assessed by PAM fluorometry (Fv/Fm, NPQ). Best-performing strains were subsequently upscaled in a photobioreactor to evaluate growth under an industrially relevant setup. In the cytosol, crtB was the only single gene yielding a detectable product, leading to phytoene accumulation. Targeting crtB to the PPC also produced phytoene, but at lower levels, consistent with a more limited GGPP pool. Co-expression with crtI enabled low but detectable lycopene formation in both compartments, while strains expressing crtE–crtB–crtI accumulated higher lycopene. The PPC showed the highest apparent phytoene-to-lycopene conversion, likely due to reduced precursor competition. Importantly, engineered lines maintained sustained photosynthetic performance, suggesting minimal interference with chloroplast function.

KEYWORDS PPC, carotenoids, chloroplast, diatoms

Poster 51: **Harnessing metabolic single-cell heterogeneity to optimise lipid production in *Yarrowia lipolytica***

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With the urgent need for a clean, circular and sustainable economy, genetically engineered microbes offer a promising alternative to produce a variety of high-value chemicals from renewable resources. However, despite substantial progress, affordable bioproduction remains limited by insufficient yields. Notably, metabolic heterogeneity, a non-genetic cell-to-cell variability, leads to differences in production performance between individual cells. This results in low-producing subpopulations that significantly reduce overall production and negatively impact efficiency, robustness and reproducibility during fermentation. To address this challenge, we investigate lipid production in the oleaginous yeast *Yarrowia lipolytica*. Using high-throughput flow cytometry, Raman spectrometry,

fluorescence imaging and multi-omics analysis, we obtain a comprehensive and quantitative map of lipid production heterogeneity. This enables us to leverage beneficial single-cell variability and inform engineering strategies to significantly enhance strain performance. Ultimately, by harnessing metabolic heterogeneity, our research allows maximized yields and supports the development of more efficient and robust microbial production platforms.

KEYWORDS Metabolic heterogeneity, bioproduction, lipid, *Yarrowia lipolytica*

Poster 52: **CascadeMAP: Autonomous Microfluidic Self-driving Lab for Optimizing Enzyme Cascades**

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Enzyme cascades demand laborious optimization of multiple parameters, such as enzyme ratios, pH, buffer composition or temperature, requiring efficient data collection and learning hidden patterns in the data. Here, we introduce CascadeMAP, an autonomous microfluidic self-driving lab for optimizing enzyme cascades. CascadeMAP presents a synergistic fusion of microfluidics for rapid collection of high-fidelity data at a minimal cost for reagents and machine learning to intelligently navigate the experiments [1]. CascadeMAP was employed to optimize two model multienzyme systems: (i) a glycerol detection cascade and (ii) a metabolic pathway for degradation of toxic pollutant 1,2,3-trichloropropane [2]. Those two examples showcase both fluorescence and label-free Raman detection. By employing the Bayesian optimization [3], we demonstrate a fully autonomous convergence to optimal parameters within a single experiment consisting of 100 consecutive learning cycles conducted completely without human intervention [4]. Thanks to its versatility, we anticipate that CascadeMAP will emerge as a valuable tool in metabolic engineering for optimizing complex metabolic networks. [1] Vasina, M., et al. Biotechnol. Adv. 66 (2023) 108171. [2] Dvorak, P., et al. ChemBioChem. 15 (2014) 1891–1895. [3] Maceiczyk, R.M., deMello, A., J. Phys. Chem. C 118 (2014), 20026–20033. [4] Vasina et al., under preparation (2026). This work was supported by the EU's Horizon Europe Programme, Grant agreement No. 101136607 (CLARA Project), co-funded by the EU from the Operational Programme Jan Amos Komenský (OP JAK) (project "Center for Artificial Intelligence and Quantum Computing in System Brain Research, reg. no. CZ.02.01.01/00/23029/0008437) and by the Czech Ministry of Education (no. GX25-17329X).

KEYWORDS Enzyme Cascades, Metabolic Engineering, Machine Learning, Microfluidics, Active Learning, Biocatalysis, Closed-loop Optimizations, Bayesian Optimization

Poster 53: Coupling cyanide detoxification to synthetic one-carbon and nitrogen assimilation in *Pseudomonas putida* for production of added-value compounds

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Coupling cyanide detoxification to synthetic one-carbon and nitrogen assimilation in *Pseudomonas putida* for production of added-value compounds Cyanide is a highly toxic, industrial contaminant, one-carbon compound whose widespread use poses significant environmental and health hazards. In this study, we developed a synthetic biology platform in *Pseudomonas putida* for the bioconversion of cyanide into value-added products, leveraging the enhanced serine-threonine cycle design. By integrating cyanide detoxification via heterologous cyanide dihydratases and engineering one-carbon (C1) assimilation pathways, we achieved growth-coupled selection on cyanide as the sole nitrogen—and auxiliary carbon—source via L-serine biosynthesis. We demonstrated the revalorisation of cyanide-derived carbon via the production of L-lactate at significantly high titres. This work establishes a robust and modular framework for synthetic cyanotrophy and trash-to-treasure conversion, offering a promising strategy for sustainable bioproduction using toxic C1 compounds as feedstocks.

KEYWORDS bioproduction, bioremediation, growth-coupled selection, *Pseudomonas*

Poster 54: Untapping prophages' role in antibiotic resistance across the Pseudomonadota human pathogens

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Antibiotic resistance is one of the most pressing global health issues. Common infections are becoming hard to treat, and traditional antibiotics are no longer effective against several deadly drug-resistant bacteria. The WHO list of critical pathogens includes mostly Gram-negative bacteria, mainly from the Pseudomonadota phylum (formerly Proteobacteria). Horizontal spread of antibiotic resistance genes mainly occurs via mobile genetic elements, such as plasmids, transposons, and integrons. Bacteriophages (phages), viruses that infect bacteria, are also capable of mobilising genes across populations. However, the role of phages integrated into their host genomes (prophages) on antibiotic resistance is less clear. Here, we characterised the prophages present in 27 strains from alpha, beta, and gammaproteobacteria (Pseudomonadota phylum) along with their ARGs. Three different tools were used to predict prophage clusters and the Resistance Gene Identifier (The Comprehensive Antibiotic Resistance Database) to examine the resistomes. Each strain contained between one and sixteen putative prophages, mostly from the Siphoviridae and Myoviridae families, and eighteen not classified. Specific transposon elements are located within some prophage clusters. Interestingly, six phage-plasmids were predicted for highly perilous pathogens. All the ARGs were found within chromosomes, except for two strains, with plasmid-harboured genes. A high diversity of ARGs was detected across species, mainly linked to efflux and antibiotic target alteration, with a small set of bacteria sharing clinically relevant genes. Furthermore, dissection of co-localisation and genetic context of prophages and mobile elements provide significant insights into prophages' potential roles in transmission and dissemination of ARGs that can be harnessed as biotechnological tools.

KEYWORDS antibiotic resistance, prophages, phages

Poster 55: Phase separation to buffer growth-mediated dilution in synthetic circuits

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Fluctuations in host cell growth pose a critical challenge for maintaining reliable function in synthetic gene circuits. Growth-mediated dilution causes a global reduction in circuit component concentrations, which can significantly destabilize circuit behavior. However, effective strategies to counteract this problem remain lacking. Here, we present a phase-separation-based strategy to directly mitigate dilution effects. By fusing transcription factors (TFs) to intrinsically disordered regions (IDRs), we drive the formation of transcriptional condensates that concentrate TFs at their target promoters. These condensates buffer against prolonged rapid dilution of TF concentration and preserve bistable memory in self-activation circuits across variable growth conditions. We further show that this approach improves production efficiency in a cinnamic acid biosynthesis pathway. Together, our results establish liquid-liquid phase separation as an emerging design principle for constructing resilient synthetic circuits that maintain robust performance under dynamic growth conditions.

KEYWORDS synthetic gene circuit gene circuit stability circuit robustness synthetic condensate buffering capacity spatial control of gene expression condensate-promoter co-localization circuit memory retention

Poster 56: Modular Synthetic Cross-Kingdom Promoters Enable Coordinated Expression in *Escherichia coli* and *Saccharomyces cerevisiae*

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Synthetic biology and metabolic engineering increasingly demand predictable and interoperable gene expression across phylogenetically distant organisms, as the need for portable genetic systems and transferable metabolic pathways continues to grow. However, fundamental differences in promoter architecture and transcriptional logic across kingdoms remain a key bottleneck in developing universal expression platforms. Here, we designed a set of modular hybrid promoters that enable tunable and quantitatively consistent gene expression in both *Escherichia coli* and *Saccharomyces cerevisiae*. These promoters integrate bacterial -10/-35 motifs and Shine–Dalgarno sequences with minimal yeast TATA boxes and Kozak sequences to ensure transcriptional and translational compatibility. The promoter set supported weak, moderate, and strong expression with high relative consistency across species. Applied to the biosynthetic pathway for the valuable pigment prodeoxyviolacein, the hybrid promoters enabled coordinated production in both hosts. This work establishes a broadly compatible promoter architecture and provides a foundational toolkit for cross-kingdom, multi-host synthetic biology.

KEYWORDS Synthetic biology, hybrid promoter

Poster 57: Dual-mode CRISPRa/i for genome-scale metabolic rewiring in *Escherichia coli*

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CRISPR (clustered regularly interspaced palindromic repeats)-mediated transcriptional regulation is a powerful and programmable approach for controlling gene expression. While CRISPR-based gene repression is well established in bacteria, simultaneous activation and repression remain challenging due to the limited availability of effective bacterial activation domains. Here, we provide an efficient dual-mode CRISPR activation and interference (CRISPRa/i) system that integrates an evolved protospacer adjacent motif (PAM)-flexible dxCas9 with an engineered *Escherichia coli* cAMP receptor protein (CRP). Through systematic optimization of the CRP domains and linkers, we developed a versatile effector capable of precise gene expression control when combined with dxCas9. Our dxCas9–CRP system demonstrated robust activation of upstream regulatory regions and effective repression of coding sequences, enabling targeted and programmable gene regulation. Using dual-fluorescent reporters, we validated the ability of this system to concurrently regulate multiple genes. Furthermore, with pooled guide RNA libraries, we applied the dxCas9–CRP system to increase violacein production in *E. coli* via genome-scale activation and repression in a coordinated manner, successfully identifying key regulatory targets that significantly increase production. Overall, this dual-mode CRISPRa/i system advances the potential for bacterial metabolic pathway rewiring, providing precise and flexible control for a wide range of biotechnological applications.

KEYWORDS CRISPR activation, CRISPR interference, synthetic biology

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Poster 58: Plasmid Eco-Evolutionary Dynamics in Intracellular Domains using Open Random Generalized Lotka-Volterra Systems

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Plasmids are widespread mobile genetic elements that shape microbial evolution by mediating horizontal gene transfer and amplifying genetic variation. Within cells, plasmids coexist alongside the chromosome as intracellular populations coupled through shared molecular resources and replication control mechanisms, forming ecological assemblages whose interactions extend beyond competition and influence host–plasmid fitness and evolutionary trajectories. How these context-dependent interactions propagate across levels of biological organization remains incompletely understood. Recent experiments with plasmid synthetic cocultures show that ecological context regulates multilevel selection dynamics, highlighting the need for theoretical frameworks that generalize these findings to natural systems. We propose a plasmid-centric model treating plasmid populations as ecological communities undergoing continuous evolutionary innovation. Using an open random generalized Lotka–Volterra (orgLV) system, plasmid variants are represented as interacting populations with randomly structured ecological parameters that exhibit inheritance-induced correlations across mutations. Mutation generates a stochastic influx of novel variants, effectively opening the system in interaction space while preserving statistical similarity between parent and offspring traits. These mechanisms link community assembly and evolutionary diversification, generating ecological regimes in which plasmid–mutant diversity and coexistence are maintained by an emergent stochastic niche structure that extends beyond classical models of genetic drift. We

further show that global density regulation introduces frequency-dependent selection, modulating mutant takeover dynamics and reproducing experimentally observed scaling relationships between mutation rates and plasmid abundance. Our results suggest that intracellular plasmid populations behave as evolving ecological systems in the disordered-interaction limit, providing a quantitative framework for understanding and engineering intracellular genetic ecosystems using synthetic plasmid communities.

KEYWORDS plasmids, polyploidy, heteroplasmy, mutation rates, intracellular ecology, modelling

Poster 59: Emergent Bioengineering

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The biosphere is undergoing unprecedented transformation driven by global warming, habitat loss, and resource depletion, threatening biodiversity through widespread species extinctions and population declines. While conservation and restoration remain essential, the risk of irreversible tipping points demands new strategies. Synthetic biology offers one such approach: engineering existing ecosystems by modifying functional traits of resident communities to enhance resilience and prevent abrupt shifts. Despite and because of public concern, advances in biosafety and control have been achieved, mostly at the cellular scale. Yet after decades of bioremediation efforts, a central question emerges: not only whether interventions can be perfectly controlled, but whether they can persist and sustain ecological function. Meeting this challenge requires a paradigm shift in design philosophy, from classical to emergent engineering, embracing adaptation, feedback, and multiscale complexity as the foundation of ecosystem design.

KEYWORDS synthetic biology \sep ecosystem engineering \sep ecological resilience, complex systems, emergence, biodiversity, biocontainment

Poster 60: Discovery of a Biocatalyst from *Bacillus thuringiensis* JNU01 for Polyethylene Degradation

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Polyethylene (PE) is a persistent synthetic polymer with strong environmental resistance that degrades slowly, and the importance of developing biological treatment strategies is increasingly recognized. In this study, Bacillus species with PE-degrading ability were isolated from landfill samples, and among them, *Bacillus thuringiensis* JNU01 demonstrated the highest growth rate in PE-containing medium. Structural analysis confirmed that oxygen-containing functional groups, including hydroxyl, carboxyl, and amide groups, were introduced into the PE structure upon treatment with *B. thuringiensis* JNU01. Scanning electron microscopy (SEM) observations revealed distinct damage on the PE film surface following microbial treatment. Additionally, various alkane-derived

compounds originating from PE were detected through gas chromatography-mass spectrometry (GC-MS) analysis. Gene expression analysis results indicated that the CYP102A5.v1 mRNA level increased in the presence of PE, suggesting involvement in the degradation process. Enzyme activity analysis confirmed that CYP102A5.v1 catalyzes the hydroxylation reaction of PE, and this was reconfirmed through NADPH oxidation analysis and Fourier transform infrared spectroscopy (FT-IR) analysis. Collectively, these findings suggest that B. thuringiensis JNU01 can contribute to polyethylene degradation, and CYP102A5.v1 can act as a potential biocatalyst for PE hydroxylation reactions.

KEYWORDS Polyethylene, *Bacillus*, CYP102A5

Poster 61: Split-HPT: A flexible tool to study protein-protein interactions with expanded capabilities at a scale

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Protein-protein interactions (PPI) underlie virtually every cellular process and the function of most disease-associated proteins. Identification and characterization of these interactions is key to fully understand the complexity of biological systems and the mechanisms that dictate the beneficial and pathological relationships between them. Current methods to identify/validate PPIs are complex, labour-intensive, organism-specific and often require specialized equipment. These limitations prevent the adoption of those methods in most laboratories as a routine, easy-to-implement and affordable approach. To overcome existing limitations, we have developed a new method based on the interaction-dependant reconstitution of split hygromycin phosphotransferase (HPT), which inactivates the antibiotic hygromycin B in the growth medium conferring cell survival. Hygromycin B target, the ribosome, is conserved across all life, hence the antibiotic is active in both eukaryotic and prokaryotic cells, providing a universal survival selection. We have validated this Split-HPT system across human, bacterial and viral PPIs, diverse eukaryotic and prokaryotic hosts, and as a screening tool for small molecules that mediate PPIs. The Split-HPT system allows for rapid and straightforward multi-organism study of PPIs at high-throughput with no specialised equipment required.

Poster 62: Shikimate pathway-Dependent Catabolism: enabling near-to-maximum production yield of aromatics

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Aromatic compounds represent ~40% of all chemical building blocks, yet they remain mostly petroleum-derived. Microbial production offers a sustainable alternative but is typically limited by low yields due to carbon loss in central metabolism. In this study, we developed a novel shikimate pathway-dependent catabolism (SDC) in *Pseudomonas putida* that couples growth to the production of shikimate-derived aromatic molecules. By combining metabolic modeling, rational engineering, and adaptive laboratory evolution, we rewired central metabolism so that the shikimate pathway functions

as the primary catabolic route to the glycerol catabolic end-product pyruvate. This growth-coupled design strategy enabled aromatics production up to 89% of the pathway's maximum theoretical yield, among the highest reported for microbial systems. Current work focuses on expanding the platform to alternative carbon sources and further strain optimization to increase productivity, demonstrating the potential of the SDC as a modular chassis for efficient bio-based aromatic synthesis.

KEYWORDS Metabolic engineering, *Pseudomonas putida*, Shikimate pathway, Growth-coupled production, Adaptive laboratory evolution, Bio-based aromatics

Poster 64: A High-Resolution Mutational map of RNA Polymerase Reveals Orthogonal Control of Growth and Biosynthesis

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Regulation of growth is crucial for chassis engineering, as it determines biomass yields and impacts global metabolic flux. Bacterial RNA polymerase or RNAP plays a central role in growth control, making it a desirable engineering target. However, the limited understanding of the RNAP mutational landscape - due to previous inabilities to introduce genomic mutations at scale - has hindered its rational engineering. To overcome this, we developed and utilized a Cas9-recombineering-mediated error-prone mutagenesis or CREPE, which allows us to characterize thousands of genomic mutations in targeted regions of essential bacterial genes. Using CREPE, we targeted a specific region of the RNAP RpoB subunit previously implicated in adaptation across diverse production-relevant conditions. We characterized the effects of ~6,000 mutations in this region across multiple growth conditions and genetic backgrounds. We discovered that this highly conserved region in vital for growth rate regulation. We identified two functionally orthogonal modules within the region. Mutations in one module altered transcription elongation to improve growth across conditions, while mutations in the other altered transcription initiation to favor amino acid biosynthesis. Due to resource constraints, improved growth (which controls biomass yields) negatively affected amino acid biosynthesis (which provides precursors for several value added products). This trade-off represents a key challenge in chassis engineering. Importantly, variants combining the effects of the two modules yielded strains that successfully overcame the trade-off. By mapping a high-throughput mutational landscape of this previously unstudied RNAP region, we have transformed it into a target for the rational engineering of growth control.

KEYWORDS Deep mutational scanning, RNA polymerase, Mutational landscape, Genome engineering, Growth control

Poster 65: **Engineering good viruses for crop trait reprogramming**

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Viruses can be engineered to deliver nucleic acids, peptides and proteins for plant trait reprogramming (Pasin et al. 2024, Nat Rev Bioeng, doi:10.1038/s44222-024-00197-y). By analyzing approved uses of virus-based products for the US and EU markets, we present thought-provoking viewpoints: (i) while it is considered safe to administer live recombinant viruses for human and livestock vaccination, none is registered for plants; (ii) humans and pets can benefit of virus-based gene therapies, but crops cannot; (iii) recombinant viruses can be released into the environment for wildlife immunization, but their use in agricultural settings is not-yet authorized. By combining a cumulative knowledge in crop genomics and of the plant virosphere, we highlight the potential of virus-based technology for fast-track improvement of Solanaceae crops (Pasin et al. 2024, Hortic Res, doi:10.1093/hr/uhae205). We showcase the use of engineered RNA viruses to enable CRISPR-Cas9-based precision breeding of agronomic traits of tomato (*Solanum lycopersicum*) (Uranga et al. 2024, Hortic Res, doi:10.1093/hr/uhad279), as well as to trigger custom RNA interference and functional genomics of scarlet eggplant (*Solanum aethiopicum*), an underutilized crop mainly cultivated in Africa and Brazil (García et al. 2025, Plant Biotechnol J, doi:10.1111/pbi.70254). Overall, we present a compelling vision on the potential of virus-based innovations for on-demand reprogramming of traits of mainstream and underutilized crops, contributing to global and local food supply.

KEYWORDS Crop biodesign; Viral vector; RNA biologicals

Poster 66: **Overcoming Noise in Synthetic Genetic Oscillators Using Phase-Based Logic**

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Bacteria respond to environmental stimuli through genetically encoded regulatory networks. By constructing synthetic genetic circuits in cells, we can create biocomputers that can adapt, self-heal, and self-replicate. Using biological properties such as biosensing, molecule synthesis, and catalysis, biocomputers can offer solutions for diagnostics, biomedicine, and environmental monitoring. Their ability to operate in diverse settings makes them a sustainable alternative to silicon-based devices, reducing reliance on resource- and energy-intensive manufacturing. Most synthetic biological computation currently relies on Boolean logic, where information is encoded as binary states (0/1) based on whether signals lie below or above defined thresholds. However, existing methods for implementing Boolean logic in living systems are prone to noise due to biological variability, overlapping molecule number distributions, and molecular interactions. This limits the precision, robustness, and scalability of biocomputing. Here, we explore phase-based logic as an alternative computational approach, in which binary signals are encoded in the phase of oscillatory biomolecular signals rather than their amplitude. Using mathematical modelling and electrical engineering principles, we demonstrate in simulation that a synthetic oscillator can be induced to stabilise in two

distinct states corresponding to binary signals 0 and 1. We further present work on microfluidic devices aimed at experimentally validating these results using *E. coli* genetic oscillators coupled through quorum signalling, enabling controlled cell-cell communication and long-term dynamic measurements. Phase-based logic offers a more precise and noise-resistant framework for biological computation, enabling more reliable synthetic genetic circuits and paving the way for scalable, sustainable biocomputing systems.

KEYWORDS synthetic genetic oscillators, biocomputing, phase-based logic, microfluidics

Poster 67: **Bioluminescent sentinel plants enable autonomous diagnostics of viral infections**

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Plants engineered with synthetic genetic programs can transform how we monitor and manage the extension of crop pests and diseases. Here, we establish a bioluminescent platform in *Nicotiana benthamiana* for autonomous viral sensing based on the fungal bioluminescence pathway (FBP). We first demonstrate that recombinant viruses can deliver missing pathway components, enabling spatially resolved tracking of infection dynamics. Leveraging this starting point, we developed a dualoutput sentinel circuit that uses a protease-responsive Bioluminescence Resonance Energy Transfer (BRET) module to report infection through a virus-triggered spectral shift in luminescence. In the absence of infection, plants emit a stable yellow glow indicating system integrity. Upon infection with potyviruses, cleavage of the BRET fusion by the virus-encoded NIa-Pro protease activates a distinct colour change detectable with low-cost imaging. This modular design is compatible with other pathogens carrying specific proteases and supports future multiplexing strategies. Our results highlight the potential of synthetic sentinel gene circuits as autonomous biosensors for precision crop protection.

KEYWORDS autobioluminescence, *Nicotiana benthamiana*, sentinel plants, biosensors, viral infections, plant synthetic biology, BRET

Poster 68: **Metabolic Engineering of *Saccharomyces cerevisiae* for Enhanced Polyhydroxybutyrate Production via NADPH Regeneration and Acetyl-CoA Flux Optimization**

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Polyhydroxybutyrate (PHB) is a prominent biodegradable polyester and a sustainable alternative to petroleum-based plastics. In this study, we established a PHB biosynthetic pathway in *Saccharomyces cerevisiae* by introducing expression cassettes containing the phaA, phaB, and phaC genes from *Cupriavidus necator* H16. While the heterologous expression of phaABC alone did not result in detectable PHB, production was successfully initiated by employing an engineered *S. cerevisiae* strain equipped with a pyruvate-malate cycle designed for NADH-to-NADPH conversion. To further maximize PHB titers, the intracellular acetyl-CoA pool was bolstered by overexpressing ERG10 (acetyl-CoA acetyltransferase) and ACS1 (acetyl-CoA synthetase 1). Furthermore, fermentation conditions were optimized to minimize competitive ethanol formation by evaluating various carbon sources and concentrations. Systematic cultivation using glucose and xylose (10–40 g/L) revealed that

the highest PHB yield was achieved at a carbon concentration of 10 g/L. These findings underscore the importance of integrating cofactor engineering, enhanced precursor availability, and bioprocess optimization to develop *S. cerevisiae* as a robust microbial factory for sustainable bioplastic production.

KEYWORDS *Saccharomyces cerevisiae*, Polyhydroxybutyrate, Metabolic engineering, Cofactor engineering

Poster 69: Harnessing Synthetic Biology to Engineer Microbes for CO_2-Based Bioproduction

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Rewiring microbial metabolism to convert CO_2 into value-added chemicals represents one of the grand challenges of Synthetic Biology. While atmospheric CO_2 drives climate change, it also constitutes the most abundant and scalable carbon feedstock on Earth. Coupled with rapidly advancing technologies for CO_2 capture and reduction to one-carbon (C1) intermediates such as formate, this creates a unique opportunity to establish sustainable, electricity-driven bioproduction platforms. In this talk, I will present our work on engineering synthetic formate assimilation in *Escherichia coli*. I will discuss formate as a microbial feedstock, focusing on its transport, metabolic integration, and the physiological constraints associated with introducing synthetic C1 pathways into a heterotrophic host. Using the recently established Serine Threonine Cycle (STC) as a model system, I will demonstrate how heterotrophic metabolism can be systematically rewired for C1 assimilation through evolution-assisted engineering. I will further present insights from integrated multi-omics analyses of evolved STC strains, which revealed the molecular and regulatory adaptations enabling formatotrophic growth via this pathway. Finally, I will discuss recent efforts to accelerate microbial and enzyme evolution to overcome current performance limitations and optimize growth on C1 substrates. Together, this work highlights how synthetic biology and metabolic engineering can be combined with evolutionary strategies to develop robust microbial platforms for CO_2-based bioproduction.

KEYWORDS Synthetic Metabolism, CO2 valorization, sustainability

Poster 70: Synthetic biology approaches for modification of natural product-producing Actinobacteria

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Actinobacteria can be considered the most relevant microorganisms, when it comes to production of natural products, with applications ranging from food preservation, agriculture, personal care to medicine, including antibiotics, anticancer agents and immunosupresants. Among them, *Streptomyces* are the most commonly studied and understood species. However, the field of natural products discovery is still severely limited due to the absence of efficient and straightforward *Streptomyces* genome engineering methods. The vast majority of genetic enginnering methods, that are used for discovery of novel natural products, is slow, laborious, error-prone and time consuming. One of the established methods of discovery focuses on the expression of cloned biosynthetic gene clusters (BGCs) in heterologous conditions. However, the development of such heterologous hosts, majority of which are genome reduced, remains highly expensive practice. Here, we present novel strategies and technologies, that will simplify the development of heterologous host strains and their

application to engineer commonly used model strains, e.g. *Streptomyces albidoflavus* J1074. As a part of our approach, we work on develpment of agnostic, metabolite-independent biosensors, that can be used to link cloned BGCs to detected lead compounds. We plan to automate the generation of hosts, cloning of BGCs, their heterologous expression and subsequent detection of target compounds via use of appropriate synthetic biosensor. We believ, that our approach will improve the success rate of heterologous expression of BGCs in particular and natural products discovery efforts in general.

KEYWORDS Natural products, biosensors, automation, Actinobacteria

Poster 71: Empowering the Next Generation of SynBio: AI-Driven Bioprocess Intensification for the Biomanufacturing of Precision Fermentation Products in *Pichia pastoris*

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Driving the transition toward a sustainable circular bioeconomy, the yeast *Pichia pastoris* (reclassified as *Komagataella phaffii*) emerges as an advantageous microbial cell factory for the biomanufacturing of precision fermentation products and recombinant proteins. Capable of achieving high cell densities on low-cost, renewable substrates, it efficiently secretes high-purity targets. To maximize this potential and tackle industrial scale-up challenges, next-generation bioprocess intensification is essential. Transitioning from conventional fed-batch systems to continuous biomanufacturing provides significantly higher volumetric productivity. Chemostat operations can reduce required bioreactor volumes up to 10-fold, dramatically lowering capital expenditures and facility footprints while maintaining continuous steady-state conditions that ensure superior product quality for novel synthetic biology applications. To further elevate efficiency and overcome scale-up limitations, both fed-batch and continuous cultivations can be integrated with artificial intelligence through machine learning-based digital twins and Model Predictive Control (MPC). Scale-up remains a critical bottleneck in the industry; however, applying MPC across these cultivation methods facilitates seamless transitions to larger volumes. Digital twins leverage cloud-enabled data and soft-sensors to calculate real-time metrics, allowing young researchers to accurately predict bioprocess behavior. These models power MPC systems for self-regulated fermentation. Instead of merely maintaining baseline stability, MPC uses digital twin predictions to dynamically optimize interconnected parameters like feed flow and agitation. Ultimately, AI-driven predictive modeling and intensified biomanufacturing create a self-optimizing framework that improves productivity, reduces costs, and empowers emerging scientists to translate sustainable precision fermentation innovations into commercial viability.

KEYWORDS Precision fermentation, bioprocess intensificaction, *Pichia bioprocess*, Continuos biomanufacturing, Scale-up

Poster 72: **Understanding division of labour through an aromatic degradation pathway**

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Division of labour is a fundamental principle across biological systems, from multicellular organisms to microbial communities. Understanding how and why division of labour evolves provides insight into major evolutionary transitions and can inform the rational design of microbial consortia for biotechnology. Here, we use *Pseudomonas putida* as a model to investigate division of labour within the toluene degradation pathway, a well-characterised system for the breakdown of aromatic pollutants. This pathway comprises two functional modules: an upper operon that oxidises toluene to benzoate, a diffusible metabolic intermediate, and a lower operon that catabolises benzoate to support growth using it as a sole carbon source. Natural heterogeneity in the expression of these operons has been observed in wild-type populations, suggesting the potential for metabolic specialisation. Using synthetic biology approaches, we engineered specialist strains expressing either the upper or the lower operon, alongside a generalist strain expressing both modules. By competing and culturing these strains under varying environmental conditions, we examine how ecological context influences the relative fitness of division of labour versus metabolic generalism.

KEYWORDS Division of labour, Metabolic engineering, Microbial consortia, Aromatic degradation, *Pseudomonas putida*

Poster 73: **Expanding Strategies for Bacterial Nanocellulose Production from Organic Wastes**

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Approximately 1.3 billion tons of food waste is generated each year globally. These organic wastes are rich in sugars, making them the prime substrate of microbial cell biorefineries. Bacterial nanocellulose (BNC) is an increasingly valuable material produced by *Komagataeibacter* species. BNC is a biofilm of ultrapure, crystalline cellulose nanofibrils that are biodegradable and biocompatible with applications from textiles to medicine. The highest cost in producing BNC is the refined glucose-rich media, limiting its competitiveness with cheap, unsustainable materials. An advantageous strategy to improve BNC production is to utilize organic wastes. Host characteristics greatly impact the success of production on varying media, as well as the quality and quantity of BNC. In this study, 9 BNC-producing strains were assessed to identify high-potential candidates for optimizing yield in waste media through SynBio host generation and wild-type strain co-culture development. We identified *Komagataeibacter medellinensis* ID13488 as a uniquely applicable strain for synthetic biology tools in waste media due to its versatile growth preferences, fast growth, glucose productivity, high competency and heterologous gene expression. Additionally, novel co-culture conditions of combined *Komagataeibacter* strains were developed to improve yields on sucrose-rich waste media. Material properties were assessed to compare the quality between standard and waste media in both inocula.

KEYWORDS Biomaterials, sustainability, synthetic biology, waste valorization

Poster 74: Modular Loop Engineering Enables Reprogramming of the Temperature-Activity Profile in PET Hydrolases

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Polyethylene terephthalate (PET) pollution problem demands advanced recycling strategies, with biocatalysis emerging as a sustainable solution. However, many existing PET hydrolases exhibit modest heterologous expression in *E. coli*, and their catalytic activity is often confined to a narrow temperature range, limiting their practical versatility. In this study, we engineered PET hydrolases that can be effectively produced and function under diverse temperatures. We explored candidate PET hydrolases as potential engineering scaffolds and identified the thermostable enzyme, TfCut2, as a stable and highly expressible backbone, despite its catalytic performance being restricted to a specific temperature. To overcome these challenges, we engineered chimeric enzymes using TfCut2 as the main architecture and systematically replacing its active-site-proximal loops with homologous elements from the highly active mesophilic enzyme PsPETase. The chimeric constructs were heterologously expressed to analyze protein production, and their catalytic activity was characterized at different temperatures. All chimeric variants retained the soluble production robustness of the TfCut2 scaffold. The engineered chimeras exhibited enhanced catalytic activity across ambient and elevated temperatures compared to the wild-type chassis. Our study establishes a rational modular engineering strategy in which enhanced catalytic properties can be incorporated into a robust biocatalyst, thereby reprogramming its temperature-activity profile without compromising structural integrity. Such synthetic constructs provide versatile platforms for real-world applications, including industrial recycling processes and bioremediation, contributing to more sustainable PET waste management.

KEYWORDS PET hydrolase, chimeric enzyme, modular engineering, temperature-activity reprogramming

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Poster 75: Precision Delivery of Biotherapeutic Effectors via Responsive Engineered Probiotics

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Programmable bacteria constitute an emerging approach in synthetic biology to control bacterial gene expression and deliver therapeutic payloads directly to disease sites. In this context, the Technological Research Accelerator in Synthetic Biology (ART Synbio) has established an integrated pipeline to design, express and characterize bacterial therapeutic effectors. This pipeline extends through in vivo validation in mice to evaluate the therapeutic activity of the engineered bacteria. We focused, here, on the probiotic strain *Escherichia coli* Nissle (EcN), as a chassis for cancer therapy. Specifically, we selected nanobodies that block PD-L1 and CTLA-4 with the aim of preventing tumor immune evasion and inhibiting colorectal cancer (CRC) progression. As a proof of concept, we designed a bispecific PD-L1/CTLA-4 nanobody, performed biochemical characterization, including surface plasmon resonance (SPR) and ELISA assays, and demonstrated that it elicits an anti-tumor response in murine CRC models. To achieve controlled therapeutic delivery, we programmed EcN with inducible genetic circuits and repurposed secretion machinery to allow on-demand expression and release of the bispecific nanobody within the tumor microenvironment. Altogether, this work establishes a versatile

pipeline for engineering configurable bacteria, that could serve as low-cost diagnostic biosensors or as targeted vehicles for the precise delivery of therapeutics.

KEYWORDS Therapeutic Bacteria, Nissl, Therapeutic effectors, Cancer, Inflammation

Poster 76: **Implementation of the synthetic Arabinose 5-phosphate dependent glycolaldehyde assimilation (SAGA) pathway in *Pseudomonas putida***

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Ethylene glycol (EG) is a promising substrate for biochemical product syntheses as it can be sourced sustainably from CO2 or plastic waste. The synthetic, arabinose 5-phosphate dependent glycolaldehyde assimilation (SAGA) pathway has been recently developed to improve carbon efficiency for the conversion of EG to acetyl-CoA. This pathway performs poorly in *E. coli*, because NAD-dependent EG oxidation is thermodynamically unfavorable. Transferring the pathway to *Pseudomonas putida* KT2440 overcomes this barrier, as its native PQQ-dependent dehydrogenases oxidize EG irreversibly. In this study, the SAGA pathway was extended to enable the formation of mevalonate (MVA). Carbon tracing experiments were used to quantify the contribution of synthetic and native EG-assimilating pathways and glucose metabolism to the formation of acetyl-CoA. Deletion of the two known GA dehydrogenases resulted in detectable SAGA pathway activity. To improve GA assimilation, we identified all GA reductases in KT2440, which when deleted, eliminated GA to EG conversion. Limited regeneration of the GA-acceptor glyceraldehyde-3-phosphate (GA3P) was addressed by supplying glucose as a co-substrate. Alleviation of cofactor competition permitted simultaneous EG and glucose uptake. Together with optimizing expression of all genes encoding SAGA pathway enzymes, this resulted in improved contribution of the synthetic pathway to the total acetyl-CoA production. Additional removal of the major GA3P dehydrogenase gapA increased the SAGA pathway contribution to acetyl-CoA formation by 91 %. When glucose was provided at reduced rates using feed beads, the share of EG-derived acetyl-CoA increased to 17% out of which 10 % were channeled through the SAGA pathway.

KEYWORDS synthetic metabolic pathway, ethylene glycol assimilation, *Pseudomonas putida*, acetyl-CoA

Poster 77: **Efficient Production of Glycosylated Anthraquinones via Metabolic Engineering and a Bipartite Cross-Kingdom Coculture of *Escherichia coli* and *Saccharomyces cerevisiae***

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Complex natural products derived from plants and animals are highly valued in the cosmetic, food, and pharmaceutical industries. However, their industrial utilization is constrained by expensive extraction processes and limited natural availability. Microbial biosynthesis has emerged as a promising alternative to reduce production costs and ensure a stable supply. Nevertheless, the microbial production of complex natural products remains challenging due to lengthy biosynthetic pathways and inefficient heterologous enzyme expression in single-host systems. To address these limitations, microbial coculture distributes biosynthetic tasks across multiple hosts, providing an

effective solution by assigning pathway modules to the most suitable organisms. In this study, a bipartite cross-kingdom coculture system combining metabolically engineered *Escherichia coli* and *Saccharomyces cerevisiae* for efficient production of glycosylated anthraquinones was investigated. The biosynthetic pathway for aglycone anthraquinone formation was introduced into *E. coli*, while downstream glycosylation steps were implemented in *S. cerevisiae*. To enhance coculture stability, an essential cross-feeding strategy was employed in which each microorganism was engineered to be auxotrophic for an amino acid, rendering the partner strain as the sole source of the required nutrient. As an initial result, flask cultivation of *E. coli* strain achieved aglycone anthraquinone production at a titer of 19.79 mg/L. Overall, *E. coli–S. cerevisiae* coculture platform highlights the potential for the cost-effective and sustainable biosynthesis of complex natural products from inexpensive feedstocks. [This work was supported by the National Research Foundation of Korea grant funded by the Korea government (RS-2024-00398252) and the Global Scholarship funded by the Hyundai Motor Chung Mong-Koo Scholarship (GSB-25-01965).]

KEYWORDS *Escherichia coli*; *Saccharomyces cerevisiae*; Metabolic engineering; coculture system; glycosylated anthraquinones

Poster 78: **Workflow for optimizing bacterial biosensor performances in fecal samples: Towards the reliable monitoring of bile acids in inflammatory bowel diseases.**

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Bacterial biosensors are genetically engineered bacteria that generate measurable outputs, such as fluorescence or therapeutic effectors, in response to specific molecules. They are promising tools for targeted therapies and continuous health monitoring. Compared with conventional diagnostic methods, bacterial biosensors are inexpensive, user-friendly, and can achieve high sensitivity and specificity. However, their clinical translation remains limited by poor performance in complex biological samples, where biosensors often exhibit low signal-to-noise ratios, limited sensitivity, and reduced reproducibility. Here, we propose a workflow to optimize biosensor performance in complex clinical matrices such as fecal samples. As a proof of concept, we used a previously reported bile acid bacterial biosensor based on the VtrA/VtrC receptor from *Vibrio parahaemolyticus*. Bile acids, microbiota-derived metabolites, have recently emerged as promising non-invasive biomarkers of inflammatory bowel disease (IBD) activity. We first assessed the matrix effect of fecal samples on biosensor performance using murine fecal solutions. To improve performance in complex samples, we constructed a plasmid library containing 3,700 promoter–RBS combinations independently regulating the expression of VtrA and VtrC. Screening of this library enabled the identification of optimal biosensor expression levels ensuring low leakiness and high fold change directly in murine fecal samples. In parallel, we performed a homology search for VtrA/VtrC systems by combining amino-acid sequence analysis and structural fold comparisons. Some homologous systems exhibited distinct bile acid response profiles. This workflow represents a key milestone towards developing bacterial biosensors capable of detecting multiple bile acids in human fecal samples and ultimately improving disease monitoring in patients with IBD.

KEYWORDS Bacterial biosensor, Bile acids, Inflammatory bowel diseases

Poster 79: **Heterologous Engineering of Receptors using OrthoRep In vivo Evolution: or HEROINE**

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Graphical abstract: https://drive.google.com/file/d/1FoaRCb-F6Yvhr39YSs6yHUYRSxQCBRD7/view?usp=sharing G-protein-coupled receptors (GPCRs) represent the largest family of druggable targets, yet engineering these membrane proteins for improved function remains challenging. Cell-based biosensors coupling GPCR signalling to a measurable output offers an approach for functional optimization and structural understanding, yet existing methods require extensive library construction and screening. Here, we present a novel platform for continuous in vivo evolution of GPCRs using the OrthoRep system. Our yeast-based opioid biosensors couple human opioid receptor activation to growth via the pheromone response pathway and integrated the sequences for the mu-opioid receptor onto the OrthoRep P1 plasmid which is replicated by an error-prone DNA polymerase. This enables continuous mutagenesis during ligand-dependent selection: better signalling leads to better growth. Different receptor subtypes were passaged up to 130 generations with various ligands. Population mutation dynamics were characterized, and variants of interest were validated by flow cytometry and growth assays. We also developed an automated high-throughput evolution pipeline integrating liquid handling and real time growth monitoring. We show DAMGO selection yielded evolved variants with up to 19-fold improved sensitivity, with convergent mutations at positions W31R, S57P, and L341S emerging across independent lineages. Flow cytometry confirmed these improvements in clean sensor backgrounds. Parallel loperamide selection produced enrichment at I148, I300, and E393 aa positions, demonstrating ligand-specific adaptation. We extend this platform to delta and kappa opioid receptors, showing generalizability for biosensor optimization and receptor engineering. The mutation trajectories captured during selection provide empirical structure-function data that can inform and validate AI-based protein design tools.

KEYWORDS GPCR, directed evolution, protein engineering, high-throughput screening, biosensors

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Poster 80: **Uncovering fundamental mechanisms of cell-free protein synthesis using tailored coarse-grained models**

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Cell-Free Protein Synthesis (CFPS) has revolutionised molecular biology research and continues to be a fundamental methodology in synthetic biology today. However, many mechanistic principles remain poorly understood due to their complexity, limiting our ability to predict CFPS behaviour. In this work, we introduce a pipeline for studying mechanistic principles in CFPS that combines computational models with experimental validation in a Design-Build-Test-Learn cycle. First, we develop a small, versatile, coarse-grained mathematical ordinary differential equation (ODE) model describing the key processes in CFPS. We tailor the basic model by targeted expansion to a range of questions, predicting system behaviour under competing mechanistic hypotheses. The model predictions are then compared to our experimental data from batch reactions using *E. coli* extract. By means of mechanistic comparison and Bayesian inference, we deploy this pipeline to address existing knowledge gaps: we present results showing rate limiting steps in reaction kinetics, the role resources play in reaction termination, and important sources of batch-to-batch variability. We foresee that the results presented

here can expand our knowledge of CFPS, guide model selection, improve predictability, and motivate similar investigations in the field.

KEYWORDS Cell-free protein synthesis, Modeling, DBTL

Poster 81: Synthetic Design of *Yarrowia lipolytica* Platforms for Lignocellulosic Sugar Co-Utilization: From Division of Labor to Transporter Engineering

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Yarrowia lipolytica is an oleaginous yeast of high industrial relevance due to its capacity to accumulate large amounts of triacylglycerides under nutrient-limiting conditions. However, its inability to degrade lignocellulosic biomass (LCB), due to the lack of hydrolytic enzymes, and to co-assimilate LCB-derived sugars limits its application in sustainable bioprocesses. Expanding both the substrate range and hydrolysis capacity of *Y. lipolytica* is therefore key for the valorization of agro-industrial residues within circular bioeconomy. Here, we engineered a multifunctional *Y. lipolytica* strain (YBXT-XR-BGL3) expressing a xylose reductase pathway for pentose assimilation and secreting the fungal β-glucosidase (BGL3) and β-xylosidase (BxTw1) from *Talaromyces amestolkiae*, enabling the hydrolysis and utilization of both cello- and xylo-oligosaccharides. To alleviate metabolic burden, this strain was benchmarked against a division-of-labor strategy using a synthetic consortium composed of cellobiose- and xylooligosaccharide-specialized strains. Both approaches achieved lipid titers up to 0.67 g L^{-1}, while the consortium showed enhanced lipid accumulation driven by dynamic population shifts. Building on this platform, we address a major bottleneck in LCB valorization: the lack of glucose and xylose co-consumption due to competition at transport level. Sugar uptake was engineered by expressing native, heterologous, and rationally mutated transporters in a transporter-deficient *Y. lipolytica* chassis (Δhxt1–4), enabling simultaneous glucose and xylose consumption. Overall, this work demonstrates how modular strain design, division of labor, and transporter engineering can be combined to expand the metabolic capabilities of *Y. lipolytica* for sustainable lipid production from complex substrates. This work was funded by the Spanish project MOLA (PID2024-162673NB-I00 MICIU/AEI/ 10.13039/501100011033).

KEYWORDS *Y. lipolytica* Consortium Lignocellulosic biomass Metabolic engineering Transport engineering

Poster 82: De novo design of sequence specific DNA binding proteins

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The ability to design sequence-specific DNA-binding proteins from first principles would fundamentally expand the programmable toolkit of synthetic biology. However, the de novo creation of compact, protein-based DNA binders with entirely novel structural folds remains an unsolved

challenge. Here, we present a deep learning–driven framework for programmable DNA binder design using RFDiffusion3. Our pipeline integrates diffusion-based backbone generation, sequence design, structural validation, and iterative in silico resampling to create monomeric proteins that recognize defined double-stranded DNA sequences. Targeting biologically relevant DNA elements, computationally filtered design sets (e.g., 96-variant plates) routinely yield functional, sequence-specific binders without iterative experimental optimization. Designed proteins exhibit strong on-target selectivity with minimal off-target binding, and experimental performance correlates with in silico structural and interface confidence metrics. Importantly, our approach generates topologically novel folds not derived from known DNA-binding families, expanding the accessible structural space for programmable DNA recognition. Together, this work establishes a scalable and generalizable strategy for engineering synthetic DNA-binding proteins, providing a foundation for synthetic transcription factors, gene circuits, and next-generation genome-targeting technologies.

KEYWORDS de novo protein design, DNA binding proteins, diffusion models, structure prediction

Poster 83: The Coli Toolkit (CTK): A modular *E. coli* toolkit for genetic circuits

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Genetic circuits are a central pillar of synthetic biology, enabling engineered cells to process information, make decisions, and respond predictably to their environment. However, building and iterating genetic circuits remains challenging due to time-consuming cloning workflows, limited standardization, and the cost of assembling multi-part constructs. For this purpose, modular cloning systems have been developed, which allow for easier reuse of parts, and for the sharing of parts between scientists. Here, I present the Coli Toolkit (CTK), a Golden Gate–based cloning toolkit for the bacterium *Escherichia coli*, based on the widely adopted Yeast Toolkit (YTK). The toolkit provides users with 156 parts, ranging from promoters and RBSs to transcriptional repressors, terminators, and cloning backbones. These modular parts enable scientists to rapidly, efficiently, and predictably assemble functional plasmids. To facilitate circuit-level design, the CTK includes 20 pre-characterized NOT gates that can be readily combined to generate user-defined genetic circuits.

To support the adoption and adaptation of the CTK to the synthetic biology community, the CTK provides a computational tool to increase efficiency in de novo DNA synthesis orders. By clustering small fragments - like promoters, RBSs, and terminators – the cost of de novo synthetized DNA fragments can be reduced by more than 50%. Together, these tools shift the focus of genetic circuit engineering from the mechanics of construction to the exploration of circuit behavior and complexity. Features such as customized sequestration, degradation, and secretion expand the design space, and open the door to new modalities of genetic circuits.

KEYWORDS Genetic Circuits, Toolkit, MoClo, Golden Gate, *E. coli*

Poster 84: Biofortification of *Solanum lycopersicum* through the incorporation of the fungal bioluminescence pathway to enhance hispidin synthesis

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Plant biofortification with antioxidant compounds is a key strategy for developing functional crops. While vitamins and flavonoids are well-studied, the potential of styrylpyrones like hispidin remains unexplored. Hispidin, known for its antioxidant and therapeutic properties, is a central metabolite in the fungal bioluminescence pathway (FBP), where it is synthesized from caffeic acid. Here, we engineered transgenic *Solanum lycopersicum* var. Micro-Tom lines constitutively expressing the FBP to evaluate hispidin production and its physiological impact. The plants exhibited continuous, autonomous bioluminescence, primarily concentrated in the fruits during maturation, confirming in vivo hispidin synthesis. qPCR and LC-MS analyses verified strong expression of hispidin synthase (HispS) and stable hispidin accumulation across fruit ripening stages—immature green (IG), mature green, and breaker+7—though light emission did not directly correlate with total hispidin content. Importantly, heterologous expression of the FBP did not disrupt the plant's metabolic balance. Levels of naringenin-chalcone remained comparable to or higher than those of wild-type plants. Furthermore, DPPH assays revealed that hispidin significantly enhanced the antioxidant profile of IG fruits, though this effect was not observed in later stages. These findings demonstrate that endogenous caffeic acid in tomato is sufficient to sustain the FBP, successfully biofortifying the fruit with hispidin without detrimental metabolic effects, while providing a bioluminescent reporter system for metabolite accumulation.

KEYWORDS Biofortification, Hispidin, Fungal bioluminescence pathway, *Solanum lycopersicum*, Antioxidants

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Poster 85: Establishing a synthetic nanobody yeast-surface display library for its use in structural biology and synthetic biology.

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In the past, the production of antibodies for different purposes relied on the immunisation of animals, but much of the antibodies resulted to be unspecific and unreproducible for different applications they were meant to be used for and, since 2015, the use of animals was declared obsolete. A protocol for yeast surface display of a synthetic nanobody library was published in 2018 by the Kruse Lab, allowing the identification of high affinity nanobodies to a particular antigen. Nanobodies are a specific type of antibodies produced by camelids (llamas, camels, alpacas, etc.), that are composed solely by heavy chains instead of having two identical heavy (VH) and light chains (VL) polypeptides as the conventional ones. Such kind of antibodies bind to their target antigens through a single variable domain, termed VHH, which contains the entire antigen-binding surface. Their three-complementarity determining region (CDR) loops contain all the necessary biochemical features to achieve nano-molar binding affinity to a given antigen and have superior qualities for many applications relative to IgG's, due to their smaller size and stability. Nanobodies have been employed effectively to trap transient conformations of medically relevant proteins for structural biology, facilitate non-invasive diagnostic imaging, super resolution imaging of protein complexes, and as next generation of cancer therapies and other diseases. In this project, we aim to develop a new yeast-

surface display platform to screen nanobodies for binding to specific antigens, that can have further applications in diagnostics or therapeutics.

KEYWORDS Nanobodies, nanobody library, yeast-surface display, golden gate cloning, IGLM

Poster 86: Direct conversion of brown macroalgae into polyhydroxybutyrate using engineered *Vibrio* sp. dhg

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Polyhydroxyalkanoates (PHAs) are promising biodegradable alternatives to petroleum-based plastics; however, their commercial viability is limited by high production costs, primarily due to the expense of carbon sources. Marine biomass, particularly brown macroalgae, could be a viable alternative to enhance both cost competitiveness and environmental sustainability. Here, we engineered *Vibrio* sp. dhg, a marine bacterium with native capability to metabolize marine biomass, for the direct conversion of brown macroalgae into polyhydroxybutyrate (PHB). A recombinant *Vibrio* sp. dhg strain was engineered to produce PHB by introducing a synthetic phaCAB gene cluster derived from *Cupriavidus necator*, following promoter screening and ribosome binding sites optimization. The engineered strain achieved 55.6% PHB accumulation with glucose and successfully produced PHB using alternative carbon sources, including alginate and mannitol. Using mimetic media with different alginate-to-mannitol ratios, optimal PHB production reached a content of 65.7% and a titer of 11.14 g/L in fed-batch cultivation. Moreover, the direct conversion of Saccharina japonica hydrolysate as the sole carbon source yielded a 38.2% PHB content and a 5.46 g/L titer in fed-batch fermentation, demonstrating the feasibility of using actual marine biomass. This study highlights the potential of *Vibrio* sp. dhg as a versatile platform for converting brown macroalgae into value-added bioplastics, suggesting a potential route toward sustainable, cost-effective PHA production and the development of a marine bioeconomy.

KEYWORDS Marine biomass; Brown macroalgae; Polyhydroxyalkanoate; Polyhydroxybutyrate; Synthetic biology; Metabolic Engineering

Poster 87: Gene-Encoded Osmotic Actuation Enables Programmable Permeability Switching in Microfluidically Generated Polymeric Artificial Cells

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A practical goal in synthetic cells is to link genetic programs to controllable exchange across a compartment boundary. Most approaches tune permeability by inserting membrane channels or altering membrane composition. While powerful, these strategies can be limited by insertion feasibility and the narrow range of achievable pore sizes—often too small to pass macromolecular

cargo. Here we demonstrate an alternative route: encoding osmotic actuation inside polymersomes to stretch the membrane and expand intrinsic pores, thereby increasing permeability.

Using a PDMS microfluidic platform, we generate Pluronic L121 polymersomes encapsulating *E. coli* TXTL with dextran (70 kDa) and DNA encoding dextranase. Dextranase-mediated cleavage increases the number of osmolytes, driving an osmotic shift and swelling within polymersomes. PL121 membranes are intrinsically nanoporous and permeable to small molecules; in controls (no dextranase DNA), 10 kDa fluorescent tracers remain retained overnight.

In contrast, dextranase-programmed polymersomes reproducibly swell and shift to a higher-permeability state. Across hundreds of vesicles, a consistent subpopulation undergoes pronounced swelling and a permeability transition within hours after encapsulation. Some polymersomes expand to more than twice their initial diameter without bursting. Following swelling, we observe loss of 20 kDa tracers and release of expressed mScarlet (26.4 kDa), suggesting an increased effective transport cutoff.

Together, these results establish gene-driven osmotic actuation as a simple, microfluidics-compatible route to programmable transport in polymer-membrane synthetic cells. Ongoing work aims to synchronize and externally control actuation using inducible chemical inducers or optogenetics) and to translate permeability switching into triggered release on demand.

KEYWORDS Cell-free Expression, Microfluidics, Polymersomes, Membrane Permeability, Osmotic Actuation

Poster 88: Developing novel biosensors using AI and yeast

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Biosensors have the potential to revolutionize medical diagnostics, personal health and environmental contaminant monitoring, food safety, biodefense, and industrial processes. Biosensors are powerful tools for detecting target ligands, but their development is often hindered by the labor-intensive process of identifying specific binding pairs. Usually, biosensors require two ligand binding pairs which are built either by searching the literature for known domains or by performing protein engineering techniques. While nature has evolved many metabolites and protein binding domains, there are not enough known domains to generate biosensors for all desirable target molecules.

This project focuses on the development of novel biosensors using high-throughput screening and AI techniques. We have developed the Simultaneous Yeast Display (SYD) technique, which leverages yeast mating to identify chemically induced dimerization (CID) systems for biosensor applications. By modifying yeast cells to mate only in the presence of specific ligands, we can screen libraries of protein-ligand pairs, efficiently discovering new binding pairs. In proof-of-concept experiments, we demonstrated successful interactions using high-affinity protein pairs such as cohesin-dockerin, as well as ligand-binding systems including Pro1a-Pro1b in the presence of progesterone.

To improve the limit of detection due to background noise, we further developed rationally designed libraries, incorporating AI-generated ligand-binding domains, and pivoted toward using a Yeast Three Hybrid (Y3H) system. Specifically, we applied AI tools to generate binding pairs against resolved structures, such as maltose-binding protein and cholic acid. This approach allowed for more targeted screening and enhanced the probability of identifying effective CID systems. This system, coupled with

split-ubiquitin variations, provides a robust platform for developing biosensors tailored to different target molecules.

Poster 89: Modeling growth-associated and non-growth-associated metabolism through flux balance analysis (FBA) and chemical master equations (CME)

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With the smallest known genome, proteome, transcriptome, metabolome, etc., the genomically minimal cell JCVI-syn3B has provided a simplified model system for the most complete whole-cell models (WCMs) of any cell to date. WCMs of JCVI-syn3B have simulated thousands of coupled reactions that describe metabolism, transcription, translation, and other essential processes. A fraction of simulated cells exhibited a metabolite-depleted state interpreted as cellular death; however, current WCMs of JCVI-syn3B have not yet modeled regulatory interactions, such as cooperativity and allostery in glycolysis, that could reduce metabolite fluctuations. Here, we adapt the existing state-of-the-art whole-cell flux balance analysis (FBA) (Breuer et al., 2019) and dynamical chemical master equation (CME) (Fu et al., 2025) to include cooperative and allosteric regulation of two glycolytic enzymes, phosphofructokinase and pyruvate kinase, predicted to maintain high ATP levels in mammalian glycolysis (Choe et al., 2025). We characterize how these regulatory mechanisms buffer glycolysis against the fluctuating demand for nucleotides and charged tRNAs by stochastic processes, such as transcription and translation. Experimentally, we measure the glucose uptake rate, a key input to the FBA and CME models. Using data from both experiments and simulations, we derive a function that predicts doubling time based on the glucose concentration in the medium and we estimate growth-associated and non-growth-associated metabolic costs in JCVI-syn3B.

KEYWORDS JCVI-syn3B, genomically minimal cells, flux balance analysis, chemical master equation, growth- vs non-growth-associated metabolism.

Poster 90: Engineering Probiotic Strains for the Breakdown of Harmful Metabolites Linked to Autism Spectrum Disorders

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Autism spectrum disorders (ASD) are serious neurodevelopmental conditions affecting approximately 75 million people worldwide, with a rising prevalence and no current treatment of core symptoms. As a result, there is an urgent need for innovative therapies. Core symptoms of ASD, such as social behavior deficits and repetitive behaviors, are often accompanied by anxiety and gastrointestinal dysfunction. Disruptions in the microbiota-gut-brain axis are believed to contribute to the development of ASD, and growing evidence suggests that microbiota-based therapies could be effective. Abnormal levels of certain microbial metabolites have been observed in ASD patients, and recent research has established a causal link between elevated levels of these microbial metabolites

and the selective induction of core ASD symptoms. Particularly p-cresol has been shown to induce social deficits and stereotypies/restricted interests in rodent models. We aim to develop a probiotic strain equipped with enzymatic pathways for p-cresol degradation. Different candidates are being assessed to serve as therapeutic delivery vehicles for the p-cresol degrading enzyme (Phenol-hydroxylase) such as different lactic acid bacteria (LAB) strains like Lacticaseibacillus casei and *Lactobacillus gasseri*. They are of particular interest due to their GRAS status which makes them of great interest in vivo gut therapy, however the lack of literature on engineered LAB may pose a challenge. Thus, part of this project focuses on the development and optimization of systems enabling enzyme production in probiotic, food grade LAB which will later be tested- in a mouse model.

KEYWORDS Probiotics, Lactobacilli, Autism Spectrum Disorder, Live Bacterial Therapies

Poster 91: **The Split GFP Rheostat: an effective tool for quantifying protein expression costs**

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As synthetic genetic constructs grow in size and complexity, they impose a greater burden on host cells, risking construct loss due to negative selection. This burden arises from a competition for gene expression resources, particularly for transcription and translation. Since sequence properties of coding sequences significantly impact the expression burden, computational optimisation has been proposed as a way of identifying sequences with the least burden. To quantify the contribution of these properties, we have developed a system that enables us to measure the expression burden of any arbitrary coding sequence in a direct and sequence-specific manner. We have designed a library of >100 synthetic genetic sequences and experimentally measured their expression and cellular growth rate in *E. coli*. The results suggest that sequence properties such as length, codon optimization, as well as variation in codon optimization along the sequence length, all play a role in the cost required to express a peptide of a given amino acid composition. Incorporating this data into a mathematical model will enable the prediction of gene expression burden of any arbitrary protein coding sequence. Such a model will help design coding sequences with lower burden, thus enabling circuits with higher evolutionary robustness.

KEYWORDS *E. coli*, gene expression cost, burden, resource allocation

Poster 92: **A host-aware coarse-grained model of *Saccharomyces cerevisiae* links metabolism, gene expression and growth**

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Understanding how microbes allocate finite cellular resources between metabolism, gene expression, and growth is crucial for resolving growth strategies and predicting optimal engineering approaches. Here we present a host-aware coarse-grained model of *Saccharomyces cerevisiae*, encoded by a set of ordinary differential equations that couples a simplified biosynthetic metabolic model with transcriptional and translational resource biogenesis model through and nutrient-signalling. We calibrated our framework to transcriptomic and proteomic datasets from glucose-limited chemostats

across dilution rates, enabling concurrent estimation of promoter strengths to be estimated from mRNA fractions and translation initiation efficiencies from protein fractions. We quantiatively reproduce growth rate-dependent transcriptome and proteome allocation, capture metabolic trends (including the Crabtree effect and diauxic shift dynamics). Our model explains recent experimental results of *S. cerevisiae* resource allocation under translational inhibition, successfully reproducing the observation that growth rates decreases without a concurrent increase ribosome abundance and revealing that this is because yeast operates near to its the maximal peptide elongation and ribosome synthesis limits which prevents further ribosome supply upon inhibition (a strategy which differs markedly from *E. coli*). Our model quantitively predicts heterologous gene expression burden and associated resource drainage during RFP production. We explored gene expression and fermentation strategies to improve protein production and show that amino acid supplementation, partially relieves burden, and increases both productivity and yields from batch processes. We anticipate that this flexible, validated host-aware framework will be useful to synthetic biologists and can be readily adapted to other eukaryotes through recalibration.

KEYWORDS Synthetic biology, Coarse-grained modelling, *Saccharomyces cerevisiae*, Resource allocation

Poster 93: Engineering the skin commensal *Cutibacterium acnes* for the treatment of skin diseases

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Cutibacterium acnes is a natural skin commensal with well-established interactions with the human host. It inhabits human hair follicles, making it the most abundant bacteria in the sebaceous sites of the skin (Fourniere et al. 2020). Recent work discovered that *C. acnes* applied onto the skin are able to engraft and modulate the hosts skin microbiome composition (Paetzold et al. 2019). Additionally, the population of *C. acnes* in each hair follicle is mainly clonal (Conwill et al 2022). *C. acnes* colonization changes throughout our life, and its presence is associated to skin homeostasis by producing acidic molecules impede the growth of pathogens. By leveraging synthetic biology and microbiome engineering, we transformed *C. acnes* into a living biotherapeutic platform capable of producing and delivering targeted molecules directly to the skin. We created strains with inducible expression systems and plan to expand this toolbox to only work within the natural skin environment where *C. acnes* proliferates: the microaerophilic, acidic and lipophilic environment of the pilosebaceous units. We also plan to create sophisticated biocontainment systems that impede *C. acnes* growth outside of hair follicles, controlling its spread. Thus, making *C. acnes* a controlled live biotherapeutic for the treatment of different skin conditions such as acne, anti-aging or wound healing.

KEYWORDS *C. acnes*, skin, biotherapeutic, homeostasis, biocontainment, engineering

Poster 94: Microbial metabolic engineering for the production of immunomodulatory metabolites that synergize with checkpoint inhibitors in cancer

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In recent years, increasing attention has focused on understanding the role of microbiota in reshaping the host immune system. Pioneering studies have shown that immune checkpoint inhibitor (ICI) efficacy depends on specific gut bacteria, while metabolomic analyses of responders have identified microbial metabolites associated with improved outcomes. However, stimulating the overproduction of these metabolites by the resident microbiome remains challenging. In contrast, *E. coli* represents a tractable platform with well-characterized metabolic networks, safe probiotic strains such as Nissle and a robust genome editing toolkit. Importantly, bacteria also have a natural ability to colonize and proliferate within tumors, making them attractive therapeutic vehicles. This project proposes the development of a battery of *E. coli* strains, optimized through metabolic pathway engineering and heterologous gene expression, to overproduce metabolites that enhance ICI responses in cancer treatment. Each strain will be evaluated alone or in combination with ICIs in subcutaneous tumor models. The most effective candidates will then be combined into a single strain to maximize metabolic output and therapeutic impact. The final objective is to assess whether engineered bacteria can potentiate ICI efficacy in clinically relevant orthotopic models, including colorectal cancer (CRC) and glioblastoma. CRC is particularly relevant due to its gastrointestinal origin, where direct interaction with gut-derived metabolites is expected. In contrast, glioblastoma represents an aggressive brain tumor with limited therapeutic progress, allowing exploration of microbiome–brain communication through the gut–brain axis and its potential systemic immunomodulatory effects.

KEYWORDS Metabolic reprogramming, *E. coli*, Cancer, Immune checkpoint inhibitors

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Poster 95: Computational Protein Design for Sequence-Specific Endonucleases

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Endonucleases that create DNA strand breaks are an integral tool in modern gene-editing therapies. Existing endonuclease technologies, such as CRISPR-Cas, have various limitations, including PAM motif requirements and large size, making therapeutic delivery challenging. It is desirable to build PAM-independent, sequence-specific, and compact, deliverable endonucleases. This project aims to use computational protein design to construct such endonucleases. Specifically, de novo designed sequence-specific DNA minibinders are rigidly fused to a nuclease catalytic domain, scaffolding all essential residues for phosphodiester cleavage while retaining the specificity for the minibinder binding sites. RFdiffusion3 is used to generate protein backbones, ProteinMPNN to assign amino acid sequences, and AlphaFold3 to predict and rank protein structures yielded by these sequences. Cell-free systems are used to express the nuclease designs; cleavage is assessed by incubating designs with fluorescently-labeled DNA, running the products through gel electrophoresis to separate by size, and measuring product yield with a fluorescent gel imager. It is expected that the majority of endonuclease affinities will retain the affinity and specificity of the fused minibinder motif and a smaller fraction will exhibit cleavage activity. Cleaving designs can achieve native-like activity using directed evolution,

which mimics natural selection toward a desired goal. Compact, PAM-independent endonucleases that match state-of-the-art editing efficiency have far-reaching therapeutic potential, allowing for editing of previously inaccessible tissues and gene targets. These programmable nucleases would also enable precise DNA-level control in synthetic biology, serving as modular components for gene circuits, programmable recombination, and conditional genome editing in engineered organisms.

KEYWORDS Nuclease, Protein Design, Gene Editing

Poster 96: Topology-guided engineering of synthetic RNA thermometers through InDel assembly

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Directed evolution strategies for structured RNAs predominantly explore sequence variation through nucleotide substitutions. In contrast, methods that enable controlled and systematic exploration of RNA structural space remain limited. Because regulatory RNA function depends on architectural features such as length and structural organization, expanding access to topological design space is an important challenge. Here, we introduce an InDel assembly strategy that enables programmable variation in RNA sequence, length, and structural architecture, and apply it to engineer synthetic RNA thermometers that regulate gene expression via temperature-dependent structural transitions. Using defined structural building blocks, we constructed a library incorporating bulges, internal loops, and multi-branch junctions, enabling the controlled diversification of RNA topology. Using a GFP reporter, we identified functional RNA thermometers exhibiting robust temperature-responsive regulation in the temperature range 25-37°C. Quantitative characterization of five RNA thermometer variants at both protein and mRNA levels revealed distinct thermal-response profiles and architecture-dependent regulatory behaviors at single-cell resolution. Our results establish InDel assembly as a powerful strategy for exploring RNA topological space and for engineering structured RNA regulators with tunable functional responses.

KEYWORDS RNA thermometer, RNA engineering, Directed Evolution, Library design

Poster 97: Combinatorial engineering of RNA recognition motif proteins for efficient post-transcriptional regulation

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Post-transcriptional regulatory strategies differ substantially between eukaryotes and prokaryotes. The RNA recognition motif (RRM) is the most widespread RNA-binding domain in nature and is particularly abundant in animals. The relative scarcity of RRM-containing proteins in prokaryotes presents an opportunity to exploit RRM–RNA interactions to expand post-transcriptional regulatory capabilities in these organisms. In this context, the mammalian RNA-binding protein Musashi-1 (MSI-1), which contains two RRM domains (RRM1 and RRM2), has previously been shown to function efficiently in *Escherichia coli* as an orthogonal translation factor. Here, we engineered MSI-1 to better understand and enhance its RNA-recognition properties for improved post-transcriptional regulation. We dissected the roles of RRM1 and RRM2 and found that each domain alone can repress translation,

with RRM2 exhibiting stronger repression than RRM1. Building on these insights, we designed three novel proteins: one consisting of two tandem RRM1 domains, a second one with two RRM2 domains and another composed of two full MSI-1 units. This last construct showed markedly increased RNA affinity, highlighting the regulatory potential of tandem RRM architectures. We also established a new anti-termination system to broaden the regulatory capabilities of the engineered MSI proteins, showing that they can function effectively as activators of gene expression. Finally, we examined the ability of these MSI variants to modulate gene expression at the single-cell level, and assessed their capacity to control two independent genes. Overall, this work advances our understanding of RRM-containing proteins and expands the post-transcriptional regulation toolkit available for use in prokaryotic systems.

KEYWORDS Genetic circuits; Post-transcriptional regulation; Protein engineering; RNA recognition motif; Synthetic biology

Poster 98: Engineering *Geobacter sulfurreducens* to outperform microbial electrochemical biosensors for detecting aromatic hydrocarbon contaminants in water

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Microbial electrochemical biosensors are based on the capacity that some bacteria, so-called electroactive, exhibit for coupling their metabolism with the ability for extracellular electron transfer. Such novel generation of biosensors offers real-time monitoring of environmental events, making them attractive tools for the detection of pollutants. In this study, we modify electroactive bacteria, *Geobacter sulfurreducens*, to generate a biosensor in which the presence of a pollutant (eg, aromatic hydrocarbon) regulates the organism's electroactive response. *G. sulfurreducens* generates an electrical current by oxidizing acetate in the tricarboxylic acid (TCA) cycle. Such electroactivity is shut down in a synthase citrate-deficient strain (*G. sulfurreducens* ΔgltA). This work proposes to control TCA cycle through citrate synthase gene (gltA) expression by two independent and exogenous promoters/transcription factor pairs (XylS/Pm and CprK1-PDB3), induced by 3-Methylbenzoate and 3-chloro-4-phenylacetic acid, respectively. The expression of gltA restores the activity of the TCA cycle, thus generating an electric current. To optimize these expression systems in *G. sulfurreducens*, five different ribosome binding sites (RBSs) were tested. These RBSs were coupled to the pTrc promoter to drive green fluorescence protein expression in non-integrative plasmids. Their performance was then evaluated by measuring fluorescence levels. Based on our results, the bicistronic RBSs BCD12 and the native gltA's RBS were chosen for the expression of gltA, coupled to Pt7-LacO, XylS/Pm, and CprK1-PDB3. Each construct is delivered into *G. sulfurreducens*' genome through electroporation and mating techniques using the Tn7 transposase system.

KEYWORDS Microbial electrochemical biosensors, inducible promoters, RBSs, environmental pollution, electroactivity

Poster 99: **Engineering synthetic differentiation circuits for bacterial therapeutic combination**

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Establishing synthetic microbial consortia in competitive environments is often compromised by stochastic colonization bottlenecks, where founder effects lead to the unpredictable dominance of a single strain. Here, we overcome this challenge by engineering a differentiation abacus, a scalable, single-layer recombinase architecture that enables a single progenitor cell to differentiate into up to twelve distinct subpopulations. By arranging competitive excision sites in a linear array, we demonstrate that differentiation ratios can be programmed through rationally tuning recombination-site kinetics and inter-site spacing. This architecture allows the generation of strictly mutually exclusive phenotypes with tunable composition, scaling from simple two-state systems to complex multi-state ensembles without the need for multilayered regulation. Finally, we validate the system's utility in a mouse tumor model, showing that in situ differentiation establishes robust, homogeneous consortia that overcome the colonization variability associated with pre-assembled mixtures. This work provides a versatile and scalable framework for reliably controlling consortia composition for bioproduction, synthetic ecology, and engineered living therapies. https://www.biorxiv.org/content/10.64898/2025.12.17.694810v1

KEYWORDS Synthetic Biology; Synthetic Differentiation; Site-specific recombination; Serine Integrases; microbial consortia; Recombinase Circuits; Bacterial Cancer Therapy.

Poster 100: **Engineering Synthetic Microbiomes for Carbon Efficient Waste-To-Chemicals Conversion**

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Microbiomes offer a powerful alternative to pure cultures for biotechnology, able to convert organic waste directly into sustainable products using solid or gaseous substrates. By dividing labour across organisms, microbiomes also significantly reduce metabolic burden, boosting product titers and yields. Currently, natural microbiomes are used to convert organic waste into biogas; however, these process only ~60% of waste and lose ~40% of available carbon as CO_2. In this work, we address these technology gaps by engineering synthetic microbiomes, focusing on two key shortcomings: carbon inefficiency and susceptibility to contamination.

We have established carbon-efficient synthetic microbiomes comprising lactic acid bacterium (LAB), chain elongating bacterium (CEB) and acetogenic bacterium (ACB). LAB break down food waste into lactic acid, which is consumed by the CEB to produce medium-chain fatty acids (MCFAs). CEBs lose ~60% of available carbon as CO_2, but that carbon is consumed by ACB and redirected back as acetate, to be used by CEB for further MCFA production, greatly improving efficiency.

Grown in anaerobic batch experiments, we've demonstrated microbiome flexibility across several levels of complexity from bi-cultures to quad-cultures, with a focus on efficient use of ACB-derived

acetate as a key technical challenge. Metabolite production is analyzed via GC-MS and HPLC, enabling a robust mass balance to validate high carbon efficiency. Our next project aims to genetically engineer LAB to implement a novel toxin-antitoxin strategy using heterologous bacteriocin expression. Together, these projects help tackle two major challenges facing synthetic microbiome use in bioprocess design, significantly improving carbon efficiency.

KEYWORDS Microbiome, Synthetic Biology, Genetic Engineering

Poster 101: A Modular Assembly Platform for Engineering Microcin B17: Expanding the Functional Sequence Space of RiPPs

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Antimicrobial resistance remains a pressing global health challenge, driving the need for novel antibiotic alternatives. Ribosomally synthesised and post-translationally modified peptides (RiPPs) represent a promising but underexplored class of antimicrobials. Microcin B17 (MccB17), a well-characterised RiPP produced by *E. coli*, contains azol(in)e heterocycles formed through cyclization of cysteines and serines, and inhibit DNA gyrase in related organisms. However, MccB17 analogue development in vivo remains constrained by the unclear mutational tolerance of its biosynthetic and export machinery. To address this, we developed a modular assembly platform designed to systematically explore the mutational tolerance of MccB17. Our semi-rational approach segments the core peptide-encoding DNA into synthetic blocks that can be assembled via Indel assembly, enabling diversity at two scales: block-level mixing for sequence insertions/deletions and codon-level degeneracy for targeted amino acid changes. This design allows simultaneous exploration of MccB17 core peptide length and sequence composition. Starting from two libraries targeting heterocycle-forming motifs and inter-azole linkers, we screened a pool of 1.6×10^4 transformants, identifying 23 novel active variants. Two variants showed enhanced inhibitory activity against *E. coli* compared to the wild-type MccB17. These variants were further characterised using SDS-PAGE and mass spectrometry to confirm their expression and post-translational modifications. These results expand our understanding of MccB17's sequence flexibility and establish a platform for RiPP discovery and engineering. Overall, this modular and scalable block-assembly strategy accelerates the discovery of peptide-based antimicrobials.

KEYWORDS Antimicrobial peptide; Indel Assembly; Microcin B17; RiPP

Poster 102: Preparing Synthetic Biology applications for deliberate release: bringing a microbial-based nitrate sensor from lab bench to field

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Synthetic biology applications range from biomanufacturing to in situ bioremediation, biomining, as well as in vivo therapeutics and diagnostics for health and the environment. While some of these applications, such as biomanufacturing of chemicals, require the use of genetically modified microorganisms (GMMs) in confined or semi-confined environments, others entail the use of such microorganisms in open environments. However, few GMMs have made it to the commercial or

deployment stage. Because of the need to maintain high safety standards, the precautionary principle is applied, which entails the requirement for strict and detailed risk assessments for such applications. This process can be lengthy, requiring extensive detailed information and additional experiments. These requirements are often overlooked during the design stage, leading to the abandonment of risk assessment processes and creating the so-called "valley of death" between research and commercialization or deployment. The application of the Safe-and-Sustainable-by-Design (SSbD) framework, which considers safety and sustainability aspects, can facilitate technology transfer if applied from the design stage onwards. We analyzed the SSbD framework and applied its relevant aspects throughout the development of a novel microbial whole-cell nitrate biosensor. Embedding such considerations and translating them into laboratory practices enables a smoother risk assessment process. Key aspects include the removal of antibiotic resistance genes, testing physical and biological containment strategies, and evaluating the microbial biosensor in environments that closely resemble the envisioned end use. Our research aims to provide an initial guideline and demonstrate a practical implementation of SSbD thinking for synthetic biology applications envisioned for deliberate release.

KEYWORDS microbial whole-cell biosensor, technology transfer, Safe-and-Sustainable-by-Design

Poster 103: Barcode-resolved microfluidic screening for high-throughput biosensor evolution

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Directed evolution enables the discovery and optimization of proteins and genetic circuits without requiring detailed knowledge of sequence–function relationships. By iteratively introducing mutations and selecting improved variants, this approach has been used to develop systems with novel activities and therapeutic potential. To evaluate large mutant libraries, image-based pooled screens offer a powerful strategy: they combine single-cell resolution with high-throughput optical readouts, directly linking genotype to phenotype. My project will apply this principle to barcoded biosensor libraries, where each protein variant is associated with a unique DNA barcode, enabling scalable mapping of sequence to function. As a proof of concept, we have implemented the sequential FISH process to detect barcoded constructs encoding different fluorescent proteins. Key steps have been demonstrated, including maintenance of cell viability, fixation, and some probe-based detection of barcodes on a flow cell platform. The next stage is to refine the FISH workflow on the mother machine and apply it to a small protein library such as magnetic protein variants. An advanced automated liquid-handling system will be integrated with the microfluidic platform, increasing throughput and reproducibility and enabling the eventual application of this approach to larger protein libraries and biosensing targets such as nanobody-based sensors. This framework has the potential to uniquely identify thousands of protein variants by leveraging modular 14-bit barcodes detected across multiple hybridization rounds, opening the door to scalable, image-based directed evolution.

KEYWORDS microfluidics, synthetic biology, barcoding, FISH

Poster 104: Optimization of Plasmid Curing from Genetically Engineered *Clostridium autoethanogenum*

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Accumulation of greenhouse gases from combustion of fossil fuels drives climate change and threatens biosustainability on Earth. Microbial gas fermentation realizes the capture of CO_2 toward biomanufacturing of value-added products. Acetogens are attractive biocatalysts here, as they use CO_2 as their sole carbon source with H_2. Metabolic engineering of novel cell factories is, however, hampered by the slow and complex genetic engineering workflows. Here, we developed different approaches to optimize plasmid curing from genetically engineered strains of the model acetogen *Clostridium autoethanogenum*. Interestingly, a CRISPR/Cas9-based curing plasmid (C-plasmid) targeting the origin of replication both in the target editing plasmid and in the C-plasmid did not improve curing over a non-targeting control plasmid. Strikingly, plasmid curing by making cells electrocompetent (ECCs) and by non-transformative electroporation of ECCs or buffer-washed glycerol stocks showed 14–100% curing efficiencies across the approaches for five different genetically engineered *C. autoethanogenum* strains. The most time-efficient approach with non-transformative electroporation of buffer-washed glycerol stocks also cured an editing plasmid from *Escherichia coli*, with ~97% efficiency. This work both improves genetic engineering workflows for *C. autoethanogenum* by significantly accelerating plasmid curing and offers methods to potentially ease plasmid curing in other microbes.

KEYWORDS Plasmid curing, CRISPR/Cas9, Gas fermentation, Acetogen, *Clostridium autoethanogenum*

Poster 105: Development of Autobioluminescent Sentinel Plants for the Early Remote Detection of Viral Infections

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Continuous monitoring of plant health is critical to safeguard global crop productivity and biosecurity against devastating viral infections. Synthetic biology offers powerful tools to engineer plants not just for enhanced agricultural traits, but as living biosensors capable of generating real-time biological data. In this work, the *Nicotiana benthamiana* plant model is employed as a living platform to develop a self-sustained optical reporter system that adapts the fungal bioluminescence pathway for the continuous, non-invasive monitoring of crop infections. A virus-inducible bioluminescent genetic circuit was implemented using deconstructed geminivirus vectors, where the luminescent output is tightly coupled to the viral replication machinery. This creates an OFF → ON switching logic, enabling sentinel plants to report spatiotemporal viral dynamics prior to the appearance of macroscopic symptoms and providing a direct readout of viral activity. To expand this synthetic diagnostic toolkit, multispectral bioluminescence systems based on bacterial pathways and Bioluminescence Resonance Energy Transfer (BRET) architectures were explored, generating a versatile multicolor palette. These advanced biosensors act as optical detectors for potyvirus infections, incorporating orthogonal switches dependent on viral protease activity. Here, engineered cleavable peptide linkers enable defined spectral shifts in direct response to infection. These results highlight the power of synthetic biology to create autobioluminescent sentinel plants capable of detecting infections before any visible pathological phenotype emerges. By acting within this crucial early remote detection window, these engineered biosensors offer a robust, proactive strategy to safeguard crops worldwide.

KEYWORDS Bioluminescence, Sentinel Plants, Biosensor, Viral Detection, BRET

Poster 106: Enhancing Efficiency of Protein Expression by Utilizing mRNA Circularization via PIE System

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Circular RNA can enhance protein synthesis efficiency by evading exonuclease attack. Permuted Intron-Exon system (PIE), a method for RNA circularization, leverages Group I intron splicing mechanism to join the ends of RNA. As PIE mainly relies on intron secondary structure formation, it may be applicable in bacterial cells. Here, we present CRESEnT(Circularized RNA Expression for Stable and Enhanced Translation) system, which utilizes PIE in bacterial cell to achieve in vivo mRNA circularization. We demonstrated that mRNA using PIE could be circularized, as detected by droplet digital PCR. Moreover, circular RNA showed improved protein expression compared to linear RNA, which exon was removed to prevent circularization. To further enhance RNA circularization, we optimized the distance between coding sequence and intron, and incorporated hybridization arms to improve intron interaction. We also evaluated our system under various conditions to confirm the versatility of circularized mRNA. Finally, we applied PIE system to the biosynthesis of metabolites and demonstrated the utility of circular RNA in enhancing metabolite yields. We anticipate that our RNA circularization method can be utilized in bacterial cell to boost the gene expression and metabolite production.

KEYWORDS synthetic biology, circular RNA, PIE system

Poster 107: Living Reservoir Computing with *E. coli* for Diagnostics, Biosensing, and Real-Time Biological Computation

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We present a systems-level approach to biological sensing and computation in which *Escherichia coli* acts as a living reservoir computer, performing complex information processing through its growth dynamics. By controlling nutrient media composition, we show that these dynamics encode nonlinear transformations that outperform linear regression, support vector machines, and multilayer perceptrons across a range of regression and classification tasks. Using simulations based on genome-scale metabolic models from multiple bacterial species, we establish a strong link between phenotypic diversity and computational capacity, demonstrating that learning performance increases with the richness of metabolic phenotypes. We apply this principle to accurately classify early-stage COVID-19 plasma samples (mild versus severe cases) using only bacterial growth data, revealing strong diagnostic potential based on simple experimental devices such as plate readers. To extend this framework to temporal computational problems and real-time biodetection, we integrate these

systems into bioreactors that enable a direct interface between living cells and computers. Together, these results position biological reservoir computing as a robust, scalable, and low-cost platform for intelligent biosurveillance, diagnostics, and hybrid bio-digital computation, while providing new mechanistic insights into the computational capabilities of living systems.

KEYWORDS Biological reservoir computing; real-time biosensing; genome-scale metabolic models

Poster 108: Systems-Level Characterization of the NtrC Regulon in Methylocystis sp. MJC1 under Nitrogen Limitation

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Nitrogen limitation enables polyhydroxybutyrate (PHB) accumulation in Methylocystis sp. MJC1 up to 44.5% of cell dry weight; however, further metabolic engineering failed to substantially increase PHB levels, indicating a systemic regulatory constraint. Transcriptomic analysis under nitrogen-limited conditions revealed strong repression of carbon assimilation pathways, including methane oxidation, serine cycle and ethylmalonyl-CoA(EMC) pathway, whereas the tricarboxylic acid(TCA) cycle was markedly upregulated. Gene set enrichment analysis(GSEA) showed that deletion of the global nitrogen regulator NtrC restored carbon metabolic gene expression, implicating NtrC as a central driver of metabolic reprogramming. Integration of ChIP-exo and RNA-seq identified 38 direct NtrC regulon genes, regulated via activation, steric hindrance–mediated repression or synergistic control. Functional grouping of these targets revealed four modules: (i) nitrogen assimilation and C/N sensing, (ii) nitrogen fixation coupled to Fe–S and electron transfer systems, (iii) stress survival and proteostasis, and (iv) regulation of nitrogen-rich biosynthetic pathways. Collectively, activation of this NtrC-centered program reinforces C/N sensing, leading to global downregulation of carbon assimilation. However, the TCA cycle is selectively upregulated to meet the elevated ATP and redox demand generated by nitrogenase activation and associated electron transfer and Fe–S biogenesis during N_2 fixation, thereby imposing an energetic burden that constrains PHB accumulation under nitrogen-limited conditions.

KEYWORDS systems biology, methanotroph, transcriptomics, NtrC, metabolic rewiring

Poster 109: The pLX and SynViP synthetic biology framework for accelerated plant virus prototyping and engineering

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Viruses can be engineered to deliver nucleic acids, peptides, and proteins for reprogramming crop traits [1]. However, characterizing novel viruses and developing customizable viral vectors remain major bottlenecks. To address this, we developed pLX, a set of T-DNA vectors enabling advanced cloning methods for efficient assembly of infectious clones of both RNA and DNA viruses [2,3]. Building on this, we conceived SynViP, a synthetic genomics framework whose design was guided by local %GC compositional biases uncovered in a plant virome dataset. SynViP supports seamless

assembly of linear and circular DNA without subcloning and is insensitive to fragment ends. It enabled de novo rescue of a plant RNA virus from a digital sequence [4]. Metaviromics have resulted in an unprecedented number of novel viruses, including in crops like tomato [5], yet their impacts remain unclear. Using SynViP, we assembled a full-length clone of Plantago tobamovirus 1 (PTV1), an RNA virus newly identified by metatranscriptomics, and we are characterizing its biology. Finally, we adapted a LC-MS-based untargeted metabolite profiling workflow for fast, high-throughput evaluation of plant responses to virus infections. Together, we present the pLX and SynViP-based framework as a flexible, robust means for accelerating the prototyping and engineering of known and novel plant viruses, as well as of next-generation viral vectors for crop improvement.

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KEYWORDS Virus synthetic genomics, Plant virome, Metagenomics, Viral vector

Poster 110: High-Throughput Screening for Synthetic Biology: from gene to product

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The increasing complexity of biotechnological optimization challenges requires the construction and detailed characterization of ever-expanding protein libraries, DNA parts libraries (such as promoters and terminators), and cell libraries. To construct and analyze these libraries, the availability of a flexible, fully automated high-throughput molecular and online screening platform is essential.

At the Centre for Synthetic Biology (Ghent University), state-of-the-art infrastructure is available for the processing of (engineered) microbes and enzymes, and the identification of (new) products in a high-throughput fashion. A custom-made robotics platform equipped with a molecular biology module for high-throughput DNA assembly and cloning (i.e., liquid handler, colony picker, incubators), and a coupled screening module (i.e., plate readers; up to 32 microplates in parallel) to enable the on-line monitoring of the kinetics and dynamics of the engineered strains and/or enzymes and their production capacity. Together with a range of complementary tools like large incubators, (U)HPLC(-MS) for metabolite/product analysis, a preparative HPLC system specialized for carbohydrate purification, freeze dryer, fermenters and other laboratory instruments, the above-mentioned infrastructure and instruments are now organized in one of UGent's Core Facilities: “High-Throughput Screening for Synthetic Biology: from gene to product”. Through this Core Facility the infrastructure and instruments are made available to researchers of both academic and industrial background.

The Core Facility is a complete biofoundry including all steps in the SynBio chain, from DNA manipulations all the way to final product analysis.

KEYWORDS High-throughput, automation, synthetic biology, service, biofoundry

Poster 111: **CUTibacterium acnes Engineered for Antimicrobial peptide Momentary Production**

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Our skin is colonized by a myriad of bacteria, fungi and viruses that are collectively called the skin microbiota. The most common and consequential bacterial skin pathogen in humans is *Staphylococcus aureus*. It is an invasive pathogen responsible for varied ailments that commonly evolves resistance to antibiotics. Antimicrobial peptides (AMPs) have drawn interest from the scientific community because they are less prone to resistance development. However, they typically require complex formulations to enhance their halflife due to low stability, which decrease their efficiencies when topically applied. I propose to genetically engineer *Cutibacterium acnes*, one of the most prevalent and abundant commensal microbes of the human skin, to produce AMPs for the treatment of *S. aureus-associated* skin infections. For this, I will develop a novel expression platform based on a synthetic linear plasmid with features of bacterial telomeres that will be progressively degraded after a limited number of replication cycles. I will also screen AMPs for their potency and specificity against *S. aureus* and the best candidates will be encoded on the linear plasmid. The use of the linear plasmid as a platform of AMPs production will (i) ensure that the delivery of the AMP is limited in time to prevent the development of antibiotic resistance, and (ii) provide a stringent biocontainment of the modified bacteria, as engineered cells eventually revert to their wild-type genotype upon programmed plasmid loss. The linear plasmid with pre-programmed degradation will be a foundational tool for future engineering of commensal bacteria as live therapeutics.

KEYWORDS *Cutibacterium acnes*, *Staphylococcus aureus*, plasmids, biocontainment, antimicrobial peptides, skin microbiota engineering, translational synthetic biology, transient gene expression

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Poster 112: **Programmable Genetic Biosensors for Propionate Monitoring in Complex Biological Samples from IBD Patients**

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Inflammatory bowel diseases (IBD) are associated with dysregulated microbiota-derived metabolites, particularly short-chain fatty acids (SCFAs), which modulate epithelial barrier integrity and immune responses. Among them, propionate has emerged as a key metabolic marker linked to gut homeostasis. However, routine metabolite quantification of propionate in fecal samples remains technically demanding and costly. To address this challenge, we aim to develop engineered living cells capable of directly detecting propionate in fecal samples from IBD patients, thereby enabling low-cost, programmable metabolite monitoring through synthetic genetic circuits. We engineered a modular whole-cell biosensing circuit in *Escherichia coli* Nissle 1917. The initial design coupled the transcription factor PrpR to its cognate promoter PprpB, driving GFP as circuit output. While the sensor detected physiological concentrations of propionate, its dynamic range and signal-to-noise ratio decreased in complex matrices such as murine feces, revealing chassis circuit context interference and suboptimal regulator expression. To optimize circuit performance, we implemented quantitative expression tuning through a combinatorial promoter–RBS library controlling PrpR levels. Iterative fluorescence-activated cell sorting (FACS) applied a dual-selection strategy to suppress basal leakiness and enrich variants exhibiting inducible responses under fecal matrix conditions. Screening identified variants exhibiting distinct response profiles, ranging from low to high sensitivity to propionate. We

are integrating complementary variants into a multi-threshold sensing architecture to expand dynamic range and enable graded quantification of propionate in clinically relevant samples.

KEYWORDS Genetic circuits; Whole-cell biosensors; Propionate sensing; Synthetic biology; Inflammatory bowel disease; Complex biological matrices

Poster 113: Reprogrammable Multicellular Biosensors for Environmental Contaminant Detection Based on Wireless Biological Computation

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Synthetic biology offers essential nature-based solutions for environmental monitoring and the ecological transition. However, the development of complex biosensors is often hindered by metabolic burden in a single cell and the "wiring problem" in multicellular computation, where the need for multiple distinct signaling molecules to connect cellular modules limits system scalability. To overcome these constraints, we present a novel framework integrating reprogrammable biological circuits with a newly developed self-configuration technology that enables wireless biological computation on multicellular *Saccharomyces cerevisiae* consortia. Our architecture utilizes wireless computation, which simplifies biosensor design by removing the requirement for intercellular chemical signaling molecules. This approach significantly reduces the genetic complexity and metabolic costs associated with traditional multicellular systems. Central to this work is the implementation of self-configurable cells based on CRISPR-Cas9. This technology allows cells to undergo autonomous internal genetic reorganization through a dedicated learning stage. During this phase, cells detect specific environmental stimuli and adapt their internal logic (for instance, switching from NOT to Identity behaviors) to acquire the ability to generate a functional output in response to that input. As a proof of concept, this technology has been applied to detect environmental contaminants, specifically endocrine disruptors. Self-configuring wireless circuits offer a scalable and flexible platform capable of identifying multiple pollutants simultaneously with high specificity. By enabling cells to "learn" and adapt to specific scenarios, this technology provides a robust tool for environmental monitoring that eliminates the need for constant manual re-engineering.

KEYWORDS Biosensor, Yeast, Biocomputation, Multicellular, Biological circuits

Poster 114: Towards predictive synthetic genome design: quantitative characterization of designed synthetic promoters and Ribosome Binding Sites

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Understanding how genotype dictates phenotype is a complex task and a crucial first step towards the predictive engineering of living systems. With the rapid progress of computational biology and artificial intelligence, new tools are continuously being developed to better understand natural genomes and to design synthetic ones. The most direct link between genotype and phenotype lies in protein coding genes and the various mechanisms by which cells regulate gene expression, such as

promoters and ribosome binding sites (RBSs). These represent some of the most direct and tunable elements, as they modulate protein abundance and consequently affect the phenotype. In this work, we aim to experimentally and quantitatively characterize the Promoter and RBS Calculator tool developed by De Novo DNA in the context of synthetic genome design. We will employ different design strategies by combining promoters and RBSs of different strengths with reporter proteins and aptamers to evaluate their individual and combined impacts on protein production and growth in *E. coli*, including methods to determine absolute cellular protein abundances (proteomics). Based on this analysis we hope to enable more robust genomic designs that lead to more predictable quantitative phenotypes. This study will provide insights into the principles and constraints governing synthetic genome design, with significant implications for both industrial biotechnology and synthetic biology.

KEYWORDS Promoter; Ribosome Binding Site; Synthetic cell; Genome design

Poster 115: Integrated Tn-seq and MAGE Assisted Rapid Genome Engineering Targeting in *Escherichia coli*

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Improving microbial strains is essential for the economic feasibility of bio-based chemical production; however, the intricate nature of metabolic networks and gene interactions makes identifying effective genetic engineering targets challenging. We developed iTARGET, integrated Tn-seq and MAGE Assisted Rapid Genome Engineering Targeting workflow. An integrated approach combining in situ transposon mutagenesis, biosensor-guided selection, and multiplex automated genome engineering (MAGE) to identify novel and synergistic genetic targets that are challenging to predict through rational design. In the first phase, in situ transposon mutagenesis generated genetic diversity within a single batch culture, allowing biosensor-driven enrichment of high-producing mutants. Transposon sequencing (Tn-seq) was then performed to identify critical genomic targets. In the second phase, MAGE enabled the creation of combinatorial knockout (KO) libraries, and high-throughput screening revealed synergistic gene interactions. Applying iTARGET to naringenin (NRN) production enriched high-producing mutants, achieving a population-level titer 1.7-fold higher than that in the control. Next-generation sequencing identified nine unpredictable genetic targets, achieving a 2.3-fold titer increase with single KOs. Further combinatorial KOs revealed synergistic effects, with a double-KO mutant producing a 2.8-fold improvement. By integrating mutagenesis and selection into a single batch, iTARGET accelerates the discovery of challenging genetic targets and enables the exploration of synergistic gene interactions through high-throughput identification of combinatorial KOs, enhancing bio-based chemical production.

KEYWORDS iTARGET, Bio-based chemical production, Unpredictable synergistic genetic targets, Integrated high-throughput strain engineering

Poster 116: SCOUT: A High-Throughput Strategy for Navigating Natural Microbial Diversity to Identify Genetically Tractable Chassis Organisms

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The development of efficient microbial bioprocesses for unconventional feedstocks hinges on identifying chassis organisms that possess both robust catabolic strength and high genetic tractability. While conventional model organisms such as *Escherichia coli* offer advanced engineering toolsets, they often exhibit physiological limitations and sluggish growth on non-native substrates. To bridge this gap, we present SCOUT (Selection of bacterial Chassis Organisms Under Target conditions), a systematic framework designed to rapidly screen environmental isolates for native metabolic competence and compatibility with synthetic biology tools. By implementing SCOUT, we identified *Pseudomonas postechii* TPA1, a novel chassis characterized by an unprecedented growth rate (0.78 h-1) on terephthalic acid (TPA), significantly outperforming previously characterized TPA-utilizing strains. TPA1 demonstrated efficient co-metabolism of TPA and ethylene glycol derived from PET hydrolysate, confirming its industrial potential for plastic upcycling. Beyond native catabolism, we successfully engineered TPA1 with a heterologous indigoidine biosynthetic pathway, achieving a titer of 869 mg/L directly from 200 mM TPA. The platform's versatility was further validated through the successful isolation of a separate, high-performance chassis for styrene bioconversion. Collectively, these results establish SCOUT as a high-impact platform for expanding the repertoire of industrial microbes. By streamlining the discovery of organisms that integrate natural metabolic power with modern engineering standards, SCOUT accelerates the transition toward a circular bioeconomy and sustainable biorefinery development.

KEYWORDS PET, Terephthalate, Plastic upcycling, Biorefinery, Microbial consortium, Chassis organism, Genetically tractable

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Poster 117: Expanding Biology for Plastic Upcycling and Bioproduction with Biocompatible Photochemistry

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Synthetic biology enables the manipulation of microorganisms to produce chemicals efficiently from sustainable feedstocks, thereby facilitating the reduction of greenhouse gas emissions. To address the global challenge of plastic waste, our lab develops innovative biotechnological approaches to transform this persistent material into valuable resources. We achieve this by interfacing cellular metabolism with in situ non-enzymatic catalysis, which expands the range of target molecules and overcomes the limitations of enzymes. We have previously demonstrated the biotransformation of PET plastic-derived terephthalic acid (TPA) into higher-value products, including the nylon precursor adipic acid. Here, we apply a novel biocompatible photochemistry approach to bypass the enzymatic limitations that limit scalability of this promising process. Furthermore, we demonstrate that integrating this synthetic chemical capability into living cells enables the one-pot biosynthesis of PET precursors.

KEYWORDS Engineering Biology, Plastic Upcycling, Metabolic engineering, biocompatible chemistry

Poster 118: Non-catalytic vitamin B6 management by PLPHP1/2 proteins buffers amino acid homeostasis in *Arabidopsis thaliana*

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Metabolic plasticity is an evolutionary strategy that enables plants to grow and reproduce even when facing challenging environmental conditions. Because coenzymes act as shared resources across multiple metabolic pathways, regulating their availability provides a powerful, system-level mechanism to coordinate metabolic flux without altering individual enzyme abundance. Vitamin B6 in its form as pyridoxal 5′-phosphate (PLP) is required for ~4% of annotated enzymatic activities, disproportionately concentrated in amino acid metabolism. Besides PLP, other derivatives of vitamin B6 (vitamers) exist in cells that can serve as a source of the coenzyme. Work of the past two decades established de novo and salvage pathways in plants generating the coenzyme and maintaining balance between vitamer forms, essential for plant growth and development. We recently identified two PLP HOMEOSTASIS PROTEINS (PLPHPs) that manage vitamin B6 in plants. Our data support a model in which PLPHP1/2 act as non-catalytic buffers of the cellular vitamin B6 pool, constraining diversion into storage or degradation pathways and thereby stabilizing PLP availability for active metabolism. Although PLPHP1/2 are dispensable for development under standard growth conditions, metabolic profiling reveals pronounced shifts in amino acid steady states, particularly within the aspartate-derived network. These imbalances are strongly exacerbated under challenging environmental conditions, indicating that PLPHP-mediated vitamin B6 management is specifically required when metabolic flux must be reconfigured. Together, our results identify coenzyme buffering as a critical but underexplored regulatory layer that enables metabolic plasticity, highlighting PLPHP1/2 as a potential leverage point for engineering robust amino acid metabolism under fluctuating environmental conditions.

KEYWORDS coenzyme, vitamin B6, plants, metabolic plasticity

Poster 119: Comparative genome-scale Tx- and Tl-CRISPRi for accurate evaluation of gene functions in bacteria

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CRISPR interference (CRISPRi) has emerged as a powerful platform for functional genomics in bacteria; however, transcription-level CRISPRi (Tx-CRISPRi, dCas9-mediated) is frequently confounded by polar effects within operons. Here, we systematically compared genome-scale transcription-level and translation-level CRISPRi (Tl-CRISPRi, dCas13-mediated) for gene fitness evaluation. We first refined dCas13 guide RNA design principles for genome-scale Tl-CRISPRi, demonstrating that both spacer binding position and GC content in the stem-distal region critically influence dCas13-mediated translation knockdown efficiency. Guided by these design rules, we performed small-scale pooled Tx- and Tl-CRISPRi screens and showed that Tl-CRISPRi can successfully distinguish essential and nonessential genes by analyzing guide RNA depletion and correct false-positive essentiality calls in Tx-CRISPRi arising from transcriptional polarity. We subsequently expanded this approach to genome-scale pooled screens using approximately 16,000 dCas9 guides and 17,000 dCas13 guides. Parallel analysis of the two CRISPRi modalities enabled

improved interpretation of gene essentiality and functional relationships within polycistronic operons, including toxin–antitoxin systems. In particular, Tl-CRISPRi facilitated functional dissection of antitoxin–toxin pairs that are difficult to resolve using transcriptional repression alone. Together, our results demonstrate that genome-scale Tl-CRISPRi complements Tx-CRISPRi and enhances the resolution of gene fitness evaluation in bacteria. Harnessing dual CRISPRi modalities provides a more accurate and comprehensive framework for functional genomics in prokaryotic systems.

KEYWORDS Genome-scale CRISPRi, dCas9, dCas13, Tl-CRISPRi, functional genomics

Poster 120: Reprogramming RNA recognition specificity of a eukaryotic RNA-binding protein enables tunable translation control in bacteria

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Engineering RNA–protein interactions offers a powerful strategy to expand the post-transcriptional regulatory repertoire in synthetic biology. Here, we describe the repurposing and reprogramming of a eukaryotic RNA-binding protein containing RNA recognition motifs (RRMs) using phage display to alter its native specificity from a polyuridine (UUUUUU) motif to a redesigned UUAGGA target sequence. Directed evolution yielded a variant, A1, containing six mutations relative to the parental protein, enabling selective recognition of the new RNA motif. Assembly of up to four RNA-binding domains generated A1(4F), which exhibited enhanced affinity for its cognate target. Evaluation of different RNA designs in a cell-free system demonstrated that A1(4F) efficiently repressed translation in a motif-dependent manner in vitro. Implementation of A1(4F) in *Escherichia coli* enabled robust and tunable translation regulation. Inducible expression of A1(4F) resulted in up to 30-fold repression of sfGFP, accompanied by pronounced shifts in single-cell fluorescence distributions. Importantly, we applied this regulatory system to achieve phenotypic control by modulating the expression of an antibiotic resistance gene, thereby influencing bacterial survival. Finally, we show that the engineered eukaryotic A1 variant functions orthogonally to an MSI-based translation regulator—another eukaryote-derived RNA-binding protein—allowing parallel and independent control of gene expression through specific RNA motif recognition. Together, our results demonstrate that the high plasticity of RRM proteins allows creating modular, programmable, and orthogonal translation regulators, expanding the toolbox for multiplexed control of gene expression in synthetic biological systems.

KEYWORDS Genetic circuits; Post-transcriptional regulation; RNA recognition motif; Synthetic biology.

Poster 121: Segmental Swapping of Homologous Enzymes for Higher Cadaverine Production

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Cadaverine, which has potential applications in medicine, agriculture, and polyamide production, is biologically produced through the decarboxylation of L-lysine. Given the potential of the polyamide market, considerable efforts have been made to improve its biological production. In *Escherichia coli*, lysine decarboxylase exists in two forms, CadA and LdcC, and CadA is known to exhibit higher catalytic

activity than LdcC. However, cadaverine production remains limited because an increase in intracellular pH destabilizes the decameric structure of CadA and inhibits its activity. In this study, based on structural analysis, a chimeric CadA enzyme, CL2, was engineered by replacing its pH-sensitive region with a structurally stable counterpart derived from LdcC. The resulting BLCL2 strain expressing CL2 produced 1.12 g/L of cadaverine in flask culture, which was 1.96-fold higher than that of the BLC strain expressing wild-type CadA. Compared with wild-type CadA, CL2 showed enhanced pH stability and improved affinity for pyridoxal 5-phosphate, its cofactor.

KEYWORDS Cadaverine, Enzyme Engineering, CadA, Lysine

Poster 122: **From gene to function: a high-content screening platform for DNA-acting enzymes, demonstrated on polymerases challenged with non-canonical DNA template bases.**

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Variant-exploration campaigns for enzymes, whether framed as directed evolution, mutational scanning, or functional variant screening, are accelerating, yet many approaches prioritise large libraries paired with low-information readouts.

Here, we present a workflow that supports high-content, variant-resolved functional profiling. It integrates combinatorial library construction, clonal identification, and microplate-scale expression of mutant enzymes in a rapid, economical, gene-agnostic format. Compact, traceable mutant libraries can then be evaluated directly against diverse, enzyme-tailored substrate panels in a multiplexed format, with outcomes deconvolved by DNA barcoding and sequencing. We apply the platform to DNA polymerases expressed cell-free and challenged with non-canonical DNA template bases. Next-generation sequencing allows us to move beyond a binary “hit/no-hit” outcome, enabling variant-resolved quantification of processivity, substitution preferences, and incorporation patterns during DNA synthesis in the presence of a library of non-canonical bases. The resulting multiplexed readout captures rich phenotypes across hundreds of enzyme and template variants in a single experiment. Such polymerase behaviour on non-canonical or damaged bases is directly relevant to mutagenesis and genome instability, and impacts technologies spanning sequencing and nucleic-acid diagnostics. We complement our measurements with in silico structural analysis to map mutations onto active-site and DNA-interaction networks, rationalising incorporation profiles and guiding further exploration. Rapid library generation paired with high-content readouts facilitates iterative refocusing of follow-on designs towards informative regions of sequence space. This accelerates discovery and mechanistic ranking of DNA polymerases and, in principle, other DNA-acting enzymes, for synthetic genetics, sequencing, and nucleic-acid diagnostics at scale.

KEYWORDS Multiplexed functional profiling, High-content screening, DNA polymerases, Non-canonical DNA bases, Directed evolution, Cell-free

Poster 123: **Marker free chassis development for NextGen protein *K. phaffii* production hosts**

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Innovative methods are needed to construct novel efficient host strains without the use of antibiotic markers. Employing a shuttle plasmid based approach new strains were generated for coproduction of proteins and biochemicals and in addition the efficiency of that next generation plaform strains goes far beyond so far existing platforms. Those marker free new strains will faciltate future. additional new applcations for economic production processes for food and feed applications

KEYWORDS innovative strains, marker free, food&feed products

Poster 124: **A versatile M13 phagemid toolbox for tuneable DNA-based communication in bacterial consortia**

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Intercellular communication is key to distributed genetic circuits operating across multicellular bacterial consortia. Although many signal types have been used, including quorum-sensing molecules, secondary metabolites, pheromones, peptides, and nucleic acids, phage-packaged DNA provides a highly programmable route for transmitting genetic information between cells. Here, we present a library of several M13 phagemid variants with distinct replication origins, including those derived from the Standard European Vector Architecture (SEVA) collection, designed to tune sender-cell growth and secretion dynamics. We systematically characterise how intracellular phagemid copy number changes with cellular growth physiology and how these changes affect phage secretion rates. In co-culture, these dynamics shape resource competition and modulate communication outcomes between sender and receiver cells. Using the intercellular CRISPR interference system, i-CRISPRi, we quantify phagemid transfer frequencies and identify rapid-transfer variants for efficient communication. This phagemid toolbox expands the available design space for DNA-payload delivery and for engineering programmable intercellular communication in multicellular bacterial circuits.

KEYWORDS distributed genetic circuits, DNA-based communication, M13 phagemid, bacterial consortia, intercellular CRISPRi

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Poster 125: **Harnessing Light for Bacterial Nanocellulose Production**

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University of British Columbia

Fashion is among the highest industries in greenhouse gas emissions, resource consumption, and human and ecotoxicity. Meanwhile, demand for textiles continues to rise and the climate crisis requires immediate action. Therefore, the fashion industry is seeking more sustainable practices. An attractive alternative for cellulosic fibres and leather is bacterial nanocellulose (BNC). BNC is produced as a biofilm of pure cellulose nanofibrils. It is of particular interest for its purity, crystallinity, tensile strength, water-holding capacity, biodegradability, productivity and ability to be produced from waste. However, its commercial competitiveness remains constrained by high costs and low yields. In this study, we investigate proteorhodopsin (PR) as a new method of improving BNC yield. PR is a light-

driven proton pump from proteobacteria. PR contributes to the proton motive force (PMF), powering ATP production and other PMF-dependent processes such as substrate transport. Heterologous PR expression is an emerging strategy in metabolic engineering to address a key challenge in chemotrophic biomanufacturing: balancing carbon use between energy generation and product synthesis. Engineering PR-based phototrophy in *E. coli* has shown yield improvement and light-dependent carbon flow re-direction towards product for ATP-limited bulk chemical synthesis. The BNC production pathway is ATP intensive. Further, ATP is an inhibitor of a central branching point between BNC synthesis and energy production. Despite its potentially useful impact on ATP supply and carbon flow, PR has not been leveraged for BNC production. For the first time, we engineered PR-driven phototrophy in a high BNC-producer strain and investigated its promise for yield improvement.

KEYWORDS Biomaterials, Fashion, Metabolic Engineering, Phototrophy, Proteorhodopsin

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Poster 126: Engineering the novel extremophile alga *Chlamydomonas pacifica* for high lipid and high starch production as a path to developing sustainable and commercially relevant strains

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Microalgae represent a powerful platform for the production of sustainable commodity products, owing to their high photosynthetic efficiency, ability to grow on non-arable lands and non-potable water, and potential to generate a broad spectrum of bioproducts, such as biofuels and biomaterials. However, scaling microalgae as a bioresource has faced obstacles, notably in the cost and risk of biomass production due to vulnerabilities during large-scale cultivation. In this study, we first demonstrated the complete cycle of developing the novel extremophile species *Chlamydomonas pacifica* for commercial bioproduct production. This newly discovered microalga exhibits exceptional resilience to extreme environmental conditions, including high pH (>11.5), elevated salinity (up to 2% NaCl), and high temperatures (up to 42°C). Through mutagenesis, breeding, and selection, we further evolved *C. pacifica* to tolerate high light intensity (>2000 μE/m²/s). Genetic engineering efforts significantly enhanced lipid production by 28% and starch accumulation by 27%, without impacting growth. We scaled up cultivation of the engineered strains in pilot-scale raceway ponds (80 liters capacity) and converted the biomass into biodiesel and thermoplastic polyurethane precursors, showcasing the viability of this extremophile for sustainable bioeconomy applications.

KEYWORDS extremophile, microalgae, laboratory evolution, transcription factors, polyurethane, biodiesel

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Poster 127: Designing and Understanding (de)fluorinating Enzymes With Protein Language Models

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Protein language models (pLMs) and explainable artificial intelligence (xAI) provide powerful tools to investigate the molecular determinants of enzyme function. We apply these approaches to carbon-

fluorine (C-F) bond formation and cleavage. C-F bonds are present in approximately 20% of small-molecule drugs and in many persistent materials, including per- and polyfluoroalkyl substances (PFAS), which are associated with adverse health outcomes. While enzymatic C-F bond formation is too inefficient for industrial applications, the molecular features that drive defluorination activity remain poorly understood, hindering the design of novel dehalogenases with activity towards PFAS. Here, we use generative AI models aiming at improving the catalytic efficiencies of Fluorinases and xAI techniques to understand the molecular features that drive defluorination activity in dehalogenases. In both cases, we apply lab-in-the-loop approaches to iteratively increase enzymatic activity and refine the detected molecular signatures associated with defluorination activity. Together, this project establishes an AI-guided framework for improving enzymatic fluorination while uncovering the molecular basis of biological defluorination.

Poster 128: **A synthesis-free in vivo binary data encoding platform using programmable base editing for DNA data storage.**

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National University of Singapore

The need for sustainable storage of cold data is becoming ever larger. DNA offers an attractive alternative for long-term data storage due to its durability, density, and minimal resource footprint. Here, we present a novel DNA-based data encoding and storage method with unprecedented writing fidelity, speed and scalability. We employ a small defined set of “Alphabet” *E. coli* cells to deliver guide RNAs to a writer cell, programming base edits at defined target positions at a synthetic, non-functional “writing sequence.” Binary code data are represented as position-specific base edit states of “0” and “1”. The in vivo encoded data is then amplified, barcoded and stored as DNA amplicons which can be subsequently sequenced and decoded back to binary code. Our read filtering strategy allows us to reduce intrinsic sequencing errors by an order of magnitude, enabling accurate decoding at higher writing densities. We evaluated multiple orthogonal base editors to increase data density and improve recovery. The best-performing base editors were used to store and retrieve two of humanity’s culturally and technologically significant artifacts: i) a greeting included on NASA’s Voyager Golden Record (6,804 bits; 851 bytes) and ii) a photograph of Navajo petroglyphs from crow canyon (25,792 bits; 3,224 bytes). This synthesis-free information encoding approach uses minimal reagents and microlitre-scale volumes, and demonstrates efficient and scalable in vivo DNA data encoding for sustainable, long-term, low-cost data storage.

KEYWORDS DNA data storage, base editing, CRISPR, genetic engineering

Oral Presentations

ORAL PRESENTATION

Biotechnologies for space sustainability: a tool to close the loop in space and on Earth

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The next decades of space exploration will bring humans far beyond Earth's orbit, where resources are scarce and resupply is unviable. This demands integrated solutions for life support, resource acquisition, and recycling grounded in circular strategies that maximize resource efficiency and sustainability. Within this landscape, microbial biotechnologies in biomanufacturing, biomining, and waste recycling show great promise. Principles of biological in situ resource utilization (ISRU) have been successfully demonstrated through experiments such as BioRock and BioAsteroid, conducted on the International Space Station, which demonstrated the extraction of key metals from extraterrestrial and analog materials, highlighting the potential for scaling these systems. In parallel, solutions for biological (e.g., food, blackwater, urine) waste upcycling are progressing. However, future long-duration lunar and Martian missions are expected to generate waste streams dominated by synthetic materials (e.g., plastics, consumables, electronics), accounting for more than 70% of payload mass. Although complex, this waste is rich in carbon, hydrogen, oxygen, and metals, and thus represents a substantial but underexploited local resource for space habitats. Our research addresses this gap by focusing on the microbial-mediated degradation, recycling, and upcycling of synthetic waste for space applications. Using approaches from cell biology, bioengineering, and synthetic biology, we investigate natural biodegradation mechanisms under space-relevant conditions (microgravity and radiation), identify targets to enhance process efficiency, and engineer pathways to convert degradation products into useful feedstocks. Moreover, this work contributes to the development of integrated, sustainable systems and advances biotechnologies relevant to terrestrial circular economy efforts aligned with the United Nations Sustainable Development Goals.

KEYWORDS space sustainability, waste recyclings, plastic recycling, e-waste recycling, space biotechnology

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ORAL PRESENTATION

Natural and engineered fungal–bacterial consortia in biocatalysis for waste valorization

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Center for Biological Research (CIB-CSIC)

Polymeric waste is the most abundant waste material on the planet and represents a promising feedstock for producing valuable compounds within a circular-economy framework. This includes natural polymers from lignocellulosic biomass, such as cellulose and hemicelluloses, as well as synthetic polymers with high environmental impact, like plastics (i.e. polyethylene terephthalate (PET)). Biotechnological valorization of these residues typically involves pretreatment, enzymatic depolymerization to release monomers, and their subsequent conversion into value-added products by specialized microorganisms. When depolymerizing enzymes are expressed heterologously in these strains, a consolidated bioprocess (CBP) can be achieved, in which degradation and bioproduction

occur in a single organism. Alternatively, distributing biodegradation and biosynthesis across different members of a microbial consortium offers advantages such as broader functional capacity, task compartmentalization, and reduced metabolic burden. Establishing natural or synthetic microbial consortia requires a deep understanding of interspecies interactions. In our work, we explore both natural-inspired and engineered communities. Mimicking natural cooperation, we developed a synthetic interkingdom consortium of the fungus *Ophiostoma piceae* and the bacterium *Pseudomonas putida* to convert agro-industrial residues into value-added compounds. In parallel, we engineer designed consortia involving *Yarrowia lipolytica*, *Comamonas testosteroni*, and *P. putida* JM37, integrating complementary metabolic capabilities for advanced biocatalysis and waste valorization.

KEYWORDS Lignocellulose, Plastics, quorum sensing

ORAL PRESENTATION

Exploring and harnessing CRISPR's hidden diversity

Chase Beisel (*chase.beisel@immune.engineering*)
Botnar Institute of Immune Engineering

CRISPR is best known as a revolutionary tool for genome surgery, yet its origins lie in RNA-guided adaptive immune systems in bacteria and archaea. These CRISPR-Cas immune systems have proven incredibly diverse, with numerous RNA-guided nucleases exhibiting ranging properties ripe for technological exploitation. Here, I will describe my group's efforts to explore an overlooked branch of these nucleases, revealing new properties that lend to new and improved applications. Through this talk, I intend to convey the benefit of continuing to mine biological diversity as building blocks for further advances in synthetic biology.

KEYWORDS CRISPR

ORAL PRESENTATION

Synthetic biology approaches for host-aware cell engineering

Francesca Ceroni (*f.ceroni@imperial.ac.uk*)
Imperial College London

Our lab at Imperial College focuses on adopting synthetic biology to improve cell engineering of bacteria and mammalian cells. In my talk I will cover some of the projects we have for the design of host-aware strategies that improve control of expression and cell behaviour.

KEYWORDS cell engineering, sythetic biology

ORAL PRESENTATION

Simulating a “for-loop” in the human genome: design and evaluation of recombinase genetic programs that count to three

George Chao (*gchao@mit.edu*), Esther Mintzer, Evan Appleton, Clair S. Gutierrez, Lilia Evgeniou, Tristan Daifuku, Tim Wannier, George M. Church
Harvard Medical School

Pluripotent stem cells differentiate into downstream cell types by receiving various signals, integrating information, and executing the respective differentiation operations - a process similar to computer

algorithms. While we can now design biosensors that enable cells to respond to diverse stimuli, maintaining cellular memory that is retained through cell divisions and directs downstream behaviors remains challenging. We set out to design a method for heritable cellular memory through a genome-integrated recombinase genetic program (RGP) that leverages recombinase-mediated DNA recombination to increment a "counter locus" up to three times, with corresponding changes to gene expression. We first curated and screened a library of known recombinases under a unified assay and found that many had high activity in human cells. Using top performers, we built several designs to test key RGP design constraints and integrated them into the AAVS1 safe harbor locus of K562 cells. Evaluating RGPs using imaging and flow cytometry, we found that the design utilizing tyrosine recombinases for successive excisions (TRex) led to the highest program efficiency. We then demonstrate potential applications for TRex, including tracing cells across divisions and enabling cells to remember encounters with malignant cells. The TRex framework provides researchers with powerful capabilities for long-term cellular memory and conditional expression, with diverse potential applications, and represents a step towards a generalized programming language for biology.

KEYWORDS synthetic biology, genetic circuit, recombinase, genome editing, nanopore sequencing

ORAL PRESENTATION

From Bioprospecting to Biomining - Harnessing Biology for Sustainable Rare Earth Extraction

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Modern technologies along with the future of renewable energy require rare earth elements (REEs). As global demand grows, the need for cleaner, more resilient supply chains has become urgent. Unfortunately, current REE extraction, refinement, and separation pipelines rely on chemically intensive processes that require large quantities of high-grade input material and generate substantial toxic waste while meanwhile requiring large energy inputs. The recent discovery of microorganisms that naturally utilize rare earth elements has revealed a promising biological alternative. Building on this foundation, we aimed to harness and enhance biological systems to transform REE biomining. As part of this effort, we have identified a large library of lanthanide binding proteins that are capable of single-stage elemental purification of individual rare earths from a mixed feedstock. Concurrently we are refining and engineering novel microbial strains that can leach, solubilize, and beneficiate REEs from a wide range of complex, low-grade, or unconventional feedstocks such as fly ash. Together, these efforts could revolutionize REE mining by establishing a scalable, environmentally sustainable bioprocess.

KEYWORDS Rare Earth Elements, Biomining, Lanthanides, Environmental Isolates

ORAL PRESENTATION

Developing *Clostridium novyi*-NT as an Intravenous, Metastatic Pancreatic Cancer Therapeutic

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Pancreatic cancer has a dismal five-year survival rate of 13%, with most tumors metastasizing prior to clinical detection. Current therapeutics are unable to deliver drugs to avascular areas. The oncolytic bacteria, *Clostridium novyi*-NT (CnNT), are attracted to hypoxic/necrotic niches in solid tumor cores. While clinical trials of intratumoral injections are promising, many tumors are inaccessible. Intravenous (IV) injections of CnNT encountered rapid clearance without sepsis by the macrophage phagocytosis system. In the parallel field of nanoparticles drug delivery, tumor accumulation was improved by integrating the RGD motif (Arg-Gly-Asp) with affinity for the αvβ3 integrin overexpressed on tumor cells and tumor-associated epithelium.

A genetic editing system was developed to stably and constitutively modify the CnNT surface with a proof-of-concept modification expressing the RGD motif in the spore surface. Spores with enhanced affinity to a surface coated with αvβ3 integrin were generated. RGD-CnNT biodistribution was analyzed in an immunocompetent, syngeneic pancreatic cancer mouse model using histological analysis, tissue homogenization with differential PCR, and immunoblot cytokine analyses. RGD-CnNT exhibited two-fold increased circulation time with a 30% increase in pancreas and tumor localization. Elevated inflammation was tumor specific in CnNT-treated cohorts, with no adverse immune stimulation observed. RGD-CnNT was present in the central necrotic tumor area a mere twenty-four hours post IV administration. Customization of CnNT using synthetic biology methodology is a viable and potent avenue to develop novel cancer therapeutics capable of targeting a broad spectrum of solid tumors, including those with limited treatment availability and thus the worst prognoses.

KEYWORDS *Clostridium novyi*-NT, Cancer, Pancreatic Cancer, Pharmacy, Genetic Engineering, CRISPR/Cas

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ORAL PRESENTATION

Engineering microbial consortia for mixed plastic upcycling

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The chemical complexity and compositional variability of post-consumer mixed plastic waste, however, pose substantial challenges to unlocking its untapped potential though upcycling. In this study, we bridged the gap by demonstrating a hybrid, mixed plastic upcycling process that integrates transition-metal-free chemical catalysis with an engineered microbial community to convert post-consumer mixed plastic waste into valuable chemicals. In the initial chemical catalytic step, a quintuplet plastic mixture, comprising low-density polyethylene (LDPE), polypropylene (PP), high-density polyethylene (HDPE), poly(ethylene terephthalate) (PET), and polystyrene (PS), was depolymerized via oxidative degradation using either nitric acid or p-toluenesulfonic acid as catalysts. The resulting effluent containing diverse oxygenates was subsequently utilized as feedstocks for bioconversion. To combat the substrate complexity of the effluent streams resulting from mixed plastic

deconstruction, we designed and constructed an engineered microbial consortium for the bio-catalytic step by leveraging the principle of DOL. This community can completely and efficiently metabolize diverse oxygenates, regardless of the compositional variability of the effluents from post-consumer mixed plastic waste. Based on this, we conceptualized a one-pot process to funnel diverse oxygenated compounds from mixed plastic, oxidative depolymerization into target products, selecting lycopene and lipids as key chemicals. The consortium with the initial cell density ratio of 9/1 produced approximately 1248 mg/L of lycopene and 0.37 g/L of total lipids when cultivated in minimal medium supplemented with diluted post-consumer mixed plastic deconstruction products. Overall, this research emphasizes the significance of microbial consortium-based biotechnologies as viable alternatives to petroleum-based refineries for producing valuable chemicals from mixed plastic waste streams.

KEYWORDS Mixed plastic; Microbial Consortium; Plastic bio-upcycling; Synthetic biology

ORAL PRESENTATION

Boosting microbial carbon efficiency through synthetic chemolithoheterotrophy

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Chemoheterotrophs regenerate reducing power such as NAD(P)H by oxidizing carbon in central metabolism, leading to significant CO_2 emission that limits carbon yields. We implemented synthetic chemolithoheterotrophy in *Pseudomonas putida*, using O_2-tolerant, NAD^+-reducing hydrogenases to draw electrons from H_2. This approach increased biomass yield by ~21% and reduced CO_2 emissions by up to 37% in bioreactors, demonstrating redox-enabled carbon conservation under aerobic conditions. Constraint-based models predict potential yield gains of up to ~60% and flux redistribution toward anaplerotic carboxylation, facilitating CO_2 co-assimilation and, in specific scenarios, net CO_2 uptake. To enforce this phenotype, we created an NAD(P)H-auxotroph by deleting key $NAD(P)^+$-dependent decarboxylases, thereby rendering growth dependent on external electrons. Adaptive laboratory evolution with supplemented electron sources led to biomass yields close to predicted optima. Proteomic analysis revealed a downshift in oxidative decarboxylation and upregulation of anaplerotic pathways, confirming CO_2 recycling and redox reconfiguration. Constraint-based simulations further evaluated the potential impact on bioproduction, predicting substantial gains when carbon oxidation is replaced by inorganic electron supply. Overall, these findings establish hydrogen-powered redox control and growth-coupled evolution as effective strategies for enhancing carbon assimilation and surpassing traditional carbon yield limits in aerobic bioprocesses.

KEYWORDS Pseudomonads; redox metabolism; inorganic electron donor; mixotrophy

ORAL PRESENTATION

Mechanistic Analysis of Programmed Iteration by Module 5 of the Nocardiosis-Associated Polyketide (NOCAP) Synthase

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University of Colorado Boulder

Assembly-line polyketide synthases (PKSs) possess multimodular architectures in which each module harbors the requisite protein domains to catalyze a single round of polyketide chain elongation and post-elongation modifications. Exceptions to this paradigm are modules that catalyze multiple elongation cycles, a phenomenon referred to as "programmed iteration". The molecular mechanism that allows PKS modules to iterate remains poorly understood. For example, Module 5 of the nocardiosis-associated polyketide (NOCAP) synthase catalyzes three elongation cycles during the biosynthesis of its undecaketide product, although in the absence of downstream modules, it has been shown to catalyze five elongation cycles. To understand the context-dependent control of its iterative capacity, we combined in vitro analysis of purified Module 5 of the NOCAP synthase with in vivo studies in *E. coli*. Our findings reveal that, while the ability to iterate is an inherent property of Module 5, protein-protein interactions with its downstream module (Module 6) are key determinants of the number of elongation cycles catalyzed by Module 5 within the context of the complete assembly line. We also show that the intrinsic ability of Module 5 to iterate can be strongly influenced by the identity of its substrate. Our findings highlight the potential of Module 5 of the NOCAP synthase to reveal fundamentally new insights into the mechanistic differences between iterative and assembly-line PKSs.

KEYWORDS Polyketide Synthases, Natural Products, Mechanistic Enzymology

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ORAL PRESENTATION

Bridging continuous and discrete evolution of large metabolic pathways

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Directed evolution methods face a trade-off between the precise control of classical discrete approaches and the throughput of modern continuous systems. We have recently established a robust phage-based system for near-continuous evolution of large gene clusters while maintaining discrete checkpoints. Lytic Selection and Evolution (LySE) selectively replicates and mutagenizes the target gene cluster through alternating cycles of lysis and transduction in *Escherichia coli*. We have engineered hypermutagenic T7 DNA polymerases (T7 DNAP) fused to dual adenine-cytosine deaminases to install all possible transition mutations at similar frequencies. By relieving pressure from maintaining genome fidelity, we achieve 3.82 × 10-5 substitutions per base – 160,000 times higher than the genomic mutation rate of *E. coli*. LySE-mediated transduction enables selection for desired metabolic functions by coupling gene cluster performance to host fitness, while eliminating genomic off-target mutations by refreshing the host in each cycle. Using LySE, we have evolved a 25-fold increase in tigecycline resistance in 5 cycles, and a 50.9% increase in end-point biomass of a bacterial strain that utilizes the PET monomer, ethylene glycol, as its sole carbon source.

KEYWORDS Directed evolution, metabolic engineering, metabolic pathways, DNA replication, DNA editing, genome engineering, bacteriophages

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ORAL PRESENTATION

Towards “Autonomous” Minimal Cells as Next-Gen Therapeutics

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Institute for Bioengineering of Catalonia (IBEC)

Recent advances in the engineering of cell-free systems with integrated biomimetic functions have enabled the development of sophisticated cell mimics for therapeutic applications, including tissue angiogenesis and vasodilation. To date, however, the majority of such cell-free systems are programmed to release therapeutics that are synthesized in situ, by using provided precursors. Recently I took inspiration from the way nature operates tightly integrated metabolic and genetic networks for engineering a cell-free system that is capable to self-sustain autonomously using CO_2, by simultaneously deploying 53 enzymes recruited from across all domains of life. Building on this transformative achievement, and its demonstrated feasibility, I now seek to explore its potential for the bottom-up engineering of advanced therapeutic platforms.

KEYWORDS Synthetic Metabolism, Cell-Free systems, Nanomedicine

ORAL PRESENTATION

Scalable In Vitro Models of Early-Life Gut Microbiomes for Discovery and Rapid Testing of Microbiome Modulation Strategies

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Early-life gut microbiomes shape immune development and risk of allergic disease, yet mechanistic study is limited by the complexity, individuality, and poor manipulability of native communities. Here, we developed a scalable in vitro system, infant gut communoids, that are stable, preserve donor-specific structure, and enable high-throughput functional interrogation of stool-derived microbial communities. Fecal microbiota from one-year-old infants ($n = 27$), stratified by subsequent asthma or eczema development, were serially passaged under two medium conditions. Communoids stabilized rapidly, could be cryopreserved, and reproducibly revived. We retained all samples from two passage time points ($n = 108$ communoids) for genome-resolved metagenomics and biochemical assays. Across the communoids over 80% of donor stool species were recovered. Across media conditions and passages, communoid composition clustered strongly with their source infant, demonstrating robust retention of community identity despite environmental perturbation. While disease status explained a small fraction of community compositional variance, communoids revealed functional immune phenotypes not apparent from composition alone. Supernatants from communoids derived from infants who did not develop disease contained higher lipopolysaccharide levels and elicited higher NF-κB activation in a human macrophage model. Furthermore, we observed transcriptional response programs in RAW 264.7 macrophages treated with communoid supernatant that differed significantly based on disease status. These results indicate that stable, donor-specific functional states can emerge from model microbial communities maintained entirely in vitro. Together, these results establish infant gut communoids as a high-throughput, tractable model system capturing donor-specific

microbiome features, enabling discovery and testing of microbiome-based interventions for human health.

KEYWORDS Microbiome, Model Systems, Inflammatory Disease, Metagenomics, Multi-omics

ORAL PRESENTATION

Next-generation synthetic biology: an application-driven perspective

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Imperial College London

Advances in synthetic biology—from directed evolution to AI-driven design—are accelerating the translation of research into real-world applications, including advanced therapies, novel materials, and engineered enzymes. In this talk, we will highlight our recent research in the context of these emerging methodologies.

KEYWORDS protein design, gene therapies, enzyme engineering, materials

ORAL PRESENTATION

Autonomous learning gene circuits in *E. coli* via DNA-encoded weight updates and plasmid memory

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i2sysbio

Synthetic gene circuits already implement logic, sensing, and even neural-network–like architectures, but they typically execute fixed programs because their parameters do not update from experience. To address this gap, we engineer an autonomously learning gene circuit in *Escherichia coli* that performs local weight updates encoded in DNA—training the computation in its biological substrate rather than in an external optimizer. This physical neural network uses a DNA memory implemented by persistent copy-number tuning in duplicate-origin plasmids: the circuit stores each synaptic weight as a population-level plasmid ratio and converts task performance into durable weight changes through antibiotic-mediated population modulation. With negative feedback alone, bacterial agents implement a reinforcement-learning loop that improves performance on simplified decision trees for tic-tac-toe and other 3×3 board games. Because the update rule acts locally and depends only on circuit activity and outcome, the approach remains compatible with standard synthetic biology modules, including non-linear interactions. We further confirm that combinatorial promoters obey the same rule and support non-linear elements, including a rationally designed XOR gate. These results suggest a route towards programmable adaptive behaviour in biological computing systems.

KEYWORDS artificial intelligence, gene circuits, engineered *E. coli*

ORAL PRESENTATION

Yeast-based biosensors for accelerated screening campaigns

Emil Jensen (*emdaje@dtu.dk*)
Technical University of Denmark

I will present my team's recent successes towards using GPCR-based yeast biosensors for accelerated testing of cell factory designs and bioactive molecules, including combined in silico and experimental

screening of compound libraries containing several hundreds of pharmaceutically relevant molecules and identification of unknown drug off-targets.

KEYWORDS Yeast Synthetic Biology & G Protein-Coupled Receptors

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ORAL PRESENTATION

On Cybergenetics: From Genetic Control Circuits to Living Therapeutics

Mustafa Khammash (*mustafa.khammash@bsse.ethz.ch*)
ETH Zurich

Living cells achieve robustness through feedback, yet most engineered systems remain open-loop. I will show how control-theoretic principles can be systematically implemented in biological systems to achieve robust adaptation and precise dynamic regulation. I will highlight recent advances in cybergenetics and discuss their implications for programmable, closed-loop cell-based therapies.

KEYWORDS Cybergenetics; biological control

ORAL PRESENTATION

Sourdough starter-inspired living materials grown from probiotic consortia with engineered division-of-labor

Yong Lai[1] (*yonglai@ust.hk*), Tzu-Chieh Tang[2]
[1] *Department of Chemical and Biological Engineering, Hong Kong University of Science and Technology*
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Synthetic genetic circuits, engineered networks of genetic components that perform user-defined logical functions in living cells, enable precise control over cellular behavior. As circuits become more complex to support sophisticated functions, implementing them within a single cell population becomes difficult. Tasks can instead be distributed among different populations in engineered consortia. However, selecting appropriate consortia and harnessing the natural traits of each species for such a division of labor remain unsolved challenges. Here, we report the creation of SINERGY, an engineered synthetic sourdough starter in which functional modules are distributed between the two constituent species. These modules, consisting of sensing, cross-kingdom communication, and response, enable in vitro biosensing and in vivo drug delivery and modulate the gut ecosystem in an animal disease model. SINERGY not only preserves the long-standing safety and health-promoting properties of the natural components but also endows symbiotic microbes with programmable functionalities, facilitating broad applications in biomedicine.

KEYWORDS Division of labor, engineered living materials, synthetic sourdough starter, probiotic consortia, GPCR biosensors, cross-kingdom communication, in situ drug delivery, inflammatory bowel disease

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ORAL PRESENTATION

Cell-Free Nucleic Acid Diagnostics for Rapid Pathogen Detection and Single-Nucleotide Variant Identification

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[1] *Department of Chemical Engineering, POSTECH, Republic of Korea*

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Cell-free systems provide a powerful platform that harnesses the biochemical machinery of living cells while retaining the controllability of in vitro systems. This direct access to the core biological machinery offers an ideal foundation for developing novel molecular diagnostics. Here, we present two cell-free nucleic acid diagnostic technologies for rapid pathogen detection and variant identification. The first is a highly sensitive, one-pot fluorescence assay capable of detecting pathogenic RNA within 30 minutes. The system employs a sustained isothermal reaction cascade in which SplintR ligase joins DNA probes upon target recognition, after which T7 RNA polymerase transcribes an RNA aptamer that binds a fluorogenic dye to generate a signal. When evaluated against 40 nasopharyngeal SARS-CoV-2 samples, the assay achieved excellent clinical performance, with 95% positive predictive value and 100% negative predictive value. Building on this foundation, the second technology enables single-nucleotide polymorphism (SNP) discrimination—critical for variant identification and treatment selection. This approach couples target-dependent probe ligation at the SNP site with cell-free transcription of a reporter sequence, completing discrimination within 20 minutes. Results are visualized on lateral flow assay (LFA) strips, making the platform suitable for point-of-care deployment. Key advantages include straightforward probe design rules that eliminate time-consuming empirical optimization and robust mutation discrimination even in the presence of peripheral sequence variations. Both technologies were validated using clinical samples, demonstrating their potential for rapid, specific detection of viral and bacterial pathogens and their variants under field-deployable conditions.

KEYWORDS Cell-free system, Nucleic acid diagnostics, Single-nucleotide polymorphism (SNP), Point-of-care diagnostics

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ORAL PRESENTATION

Domesticating environmental bacteria as emerging chassis for biorefineries

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[1] *Department of Biological Sciences and Bioengineering, Inha University*

[2] *Institute of AI-driven Industrial Biotechnology, Inha University*

The efficient conversion of non-conventional feedstocks is central to realizing sustainable and carbon-efficient biorefineries. Natural microbial diversity represents an untapped reservoir of metabolic and physiological potential that can be harnessed toward this goal. This presentation introduces efforts to domesticate environmental isolates as emerging microbial chassis through the integration of systems and synthetic biology approaches. By combining large-scale genomic and transcriptomic analyses with rational strain engineering, we explore how naturally robust bacteria can be transformed into versatile platforms for bioprocessing of marine biomass, plastic-derived monomers, and lignin-based aromatics. Together, these efforts underscore the value of natural microbial diversity not only for expanding the feedstock spectrum but also for accelerating the transition toward scalable, carbon-efficient biorefineries.

KEYWORDS Biorefineries, host engineering, plastic upcycling

ORAL PRESENTATION

Fitness-profiling-guided Genome Editing Accelerates Evolution of *Pseudomonas putida* for Improved Coumarate Conversion

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[1] *Department of Biological Sciences and Bioengineering, Inha University*
[2] *Institute of AI-driven Industrial Biotechnology, Inha University*

Pseudomonas putida KT2440 is a premier chassis for biomass valorization, yet the efficient utilization of lignin-derived aromatics like p-coumarate remains a primary industrial bottleneck. In this study, we employed a systematic approach combining Random Barcode Transposon Sequencing (RB-TnSeq) and CRISPR/dCas9-mediated base editing to reconfigure the metabolic landscape of *P. putida* for enhanced aromatic catabolism. Using RB-TnSeq, we identified key genetic targets that significantly influence fitness during growth on p-coumarate. To modulate these targets, we implemented an inducible cytidine base editing (CBE) system to introduce premature stop codons, facilitating rapid and multiplexed gene attenuation. The resulting engineered strains demonstrated substantially increased growth kinetics on p-coumarate as the sole carbon source, significantly outperforming the wild-type and previous benchmarks. Whole-genome sequencing of base-edited isolates revealed critical recurrent mutations in key regulatory nodes, highlighting specific pathways essential for optimized aromatic metabolism. To demonstrate industrial utility, these optimized strains were further engineered for medium-chain-length polyhydroxyalkanoate (MCL-PHA) production. Fed-batch fermentation confirmed superior bioproduction titers and yields compared to the parental strain. Our findings provide a robust strategy for genome-scale metabolic rewiring, establishing a high-performance *P. putida* platform for converting lignin-derived aromatics into value-added bioproducts.

KEYWORDS *Pseudomonas*, coumaric acid, base editing

ORAL PRESENTATION

Synthetic genome reorganization reveals phenotypic robustness in bacteria

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Genome organization is thought to constrain bacterial physiology. Here, we establish a high-throughput system for generating extensive genome architectural diversity and apply it to probe physiology under altered genome organization. We engineered the fast-growing bacterium *Vibrio natriegens* to undergo stochastic duplications, translocations, inversions, and deletions across chromosome-scale regions, generating populations with a wide range of genome architectures. We find that multiple distinct genome architectures sustain stable physiology and, in some cases, enhanced growth, providing a striking example of phenotypic robustness. These results indicate that

bacteria can tolerate substantial chromosomal restructuring more readily than previously appreciated and further suggest that genome reconfiguration may contribute to phenogenetic drift, enabling evolutionary exploration while preserving phenotype. Our work provides a framework for advancing the understanding and engineering of bacterial genomes.

KEYWORDS Genome engineering, synthetic biology, genome reorganization, bacterial physiology

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ORAL PRESENTATION

Design meets evolution: Theory and practice

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The prevailing view of biological evolution is not unlike bricolage/pastiche/tinkering—in sharp contrast with the rational engineering that is the trademark of contemporary Synthetic Biology. Yet, different paths often lead to solutions that coincide or converge whether they emerge from naturally-occurring evolution or rationally designed. Such a conjunction—often presented as a mere anecdote—in fact reveals the ability of biological systems to physically explore solution spaces and gravitate towards information-rich attractors, which can be found through different routes. This scenario evokes one of physical heterotic computing, a non-conventional type of data processing in which the solution to a problem is not delivered through numerical calculations but through its embodiment in a material object. Once left to undergo a physical process the object manages a large number of parameters for reaching a multi objective optimum. The course of information is thus a physical flow and the outcome is a physical currency. The consequences of this notion for bioengineering are remarkable, as it enables solutions to multi-objective optimization challenges not yet amenable to all-rational approaches. The ensuing technical question is how to bring about hyper-diversification not only of genomic sequences but also environmental and context-dependent parameters for securing the desired performance of a given synthetic device. This issue will be illustrated with a number of practical cases picked from adaptation scenarios of environmental bacteria where stress-induced genetic variability was key to find ideal outcomes to otherwise intractable design hitches of biotechnological interest.

KEYWORDS Design; Evolution; Computing; Information; 2,4 Dinitrotoluene; *Pseudomonas putida*

ORAL PRESENTATION

Expanding the Genetic Lexicon with Xenonucleic Acids

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The 4-letter genetic alphabet of natural DNA (A, T, G, C) are often regarded as a “perfect” solution for storing and transmitting biological information. Yet this alphabet only represents a possible minimal set, not the upper limit of what is possible. In this talk, I will describe how our group is engineering genetic systems with unnatural base pairing xenonucleic acids (ubp XNAs) to expand the information and functional capabilities of biological systems. We combine mechanistic studies of replication, transcription, and translation with the development of next-generation tools for sequencing, and quantitative measurement to establish a modern framework for studying, and scaling, expanded genetic alphabets.

KEYWORDS Nucleic Acids, Expanded Genetic Alphabets, Xenobiology

ORAL PRESENTATION

Programming the cellular behavior of the human microbiome

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University Pompeu Fabra, Dept. of Medicine and Life Sciences (MELIS)

Synthetic gene circuits are invaluable tools for programming the behavior of microbes, a requirement for using them to tackle health and environmental challenges. A major frontier in the field is the development of autonomous, programmable systems that operate within diverse bacterial cells—including those found in the human microbiome—without the need for continuous external intervention. Here, we present our efforts to develop molecular tools and circuits that enable the precise programming of the human microbiome – including gut, lung and skin microbiome. By engineering both model and non-model species, we illustrate the common tradeoff between relevant chassis selection and engineering capacity of that particular chassis. Indeed, our work includes examples of both complex circuit behaviours (implemented in model bacteria) and niche-specific non-model species engineering (albeit with lower complexity of the programmed actions). Even more, we also exemplify how the knowledge acquired through both approaches enables the advancement towards the engineering of complex behaviours in relevant non-model bacterial chassis.

KEYWORDS synthetic gene circuits, microbiome engineering, non-model organism

ORAL PRESENTATION

Systems biology approaches to engineer robust bioproduction microbial hosts and processes

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Microbial strains provide versatile chassis for valorizing abundant and alternative carbon sources into useful products. However, engineering bacterial strains for biomanufacturing involves challenges beyond pathway construction. Productive strains must use multiple carbon sources, tolerate process stresses, sustain high flux to product, and perform reliably at scale. In this talk, I will present systems and synthetic biology approaches used to engineer a range of robust bacterial production hosts for biofuels and bioproducts. I will highlight the development and use of genetically encoded biosensors to directly link intracellular state to measurable outputs. These sensors enable high-throughput screening and rapid iteration through automated design–build–test–learn workflows. I will also discuss how large experimental datasets generated from these platforms are analyzed using artificial intelligence and machine learning approaches to guide strain design, identify key engineering targets, and optimize cultivation parameters. Together, these methods enable faster, data-driven development of bacterial strains and processes with improved and scalable production phenotypes.

KEYWORDS systems biology, KT2440, synthetic biology, biosensor, isoprenol, SAF, bioproducts

ORAL PRESENTATION

Rewriting skin microbes to treat skin chronic inflammation

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The skin is the most accessible organ in the human body. Yet, conventional topical delivery methods lack specificity and dose control due to limitations in ingredient stability or absorption. As an alternative, bacteria represent a promising dynamic delivery system for the treatment of disease. While gut bacteria have been extensively engineered to address metabolic disorders or limit cancer progression, the skin remains largely underexplored. Here, we explore the use of *Cutibacterium acnes*, the most abundant skin commensal, as therapeutic delivery platform to treat skin inflammatory diseases like atopic dermatitis. We have pioneered the first set of tools for this bacteria, including streamlined genome integration to ensure cargo stability. With these tools, we have engineered therapeutic secretion of AI-design immune modulators and boosted *C. acnes* metabolites beneficial for atopic dermatitis amelioration. These strategies open the door to new therapeutic delivery modalities in the skin.

KEYWORDS *Cutibacterium acnes*, skin, microbiome, microbiome engineering

ORAL PRESENTATION

High-yield biosynthesis of synthetic proteins in next-generation genome-recoded organisms

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While expanding the chemical repertoire of proteins with non-standard amino acids (nsAAs) promises breakthroughs in biotechnology, medicine, and sustainability, significant challenges remain. Host constraints and inefficiencies in orthogonal translation systems currently limit efficient bioproduction. Here, we present a genomically recoded *Escherichia coli* strain that enables high-yield production of synthetic proteins with site-specific incorporation of two distinct nsAAs at up to six sites. Building on our recently published Ochre strain, which relies exclusively on UAA as the stop codon, we rationally engineered release factors 1 and 2 to facilitate efficient reassignment of UAG and UGA codons by orthogonal translation systems. Additionally, we genomically integrated T7 RNA polymerase to enhance transcriptional output, enabling high expression of synthetic proteins. Combining these optimizations with two orthogonal aminoacyl-tRNA synthetase/tRNA pairs, we demonstrate the unprecedented high production of a synthetic protein, ELP-3x(TGA-TAG)-GFP, containing two nsAAs at three distinct sites each in. We demonstrate 15 combinations of dual nsAA incorporation with high yields and achieve ~180 mg/L in shaking flask cultures with Nε-Boc-L-lysine and para-acetyl-L-phenylalanine, a significant advancement toward industrial scalability. In summary, our work establishes an efficient microbial chassis for the biosynthesis of chemically enhanced proteins, demonstrating potential for scalability for biotechnological applications.

KEYWORDS Genetic code expansion, non-standard amino acids, orthogonal translation systems, translation

ORAL PRESENTATION

Growth-coupling strategies and evolutionary approaches to establish microbial pigment production from one-carbon feedstocks

Prof. Pablo Ivan Nikel (*pabnik@dtu.dk*)
Technical University of Denmark

Formate is an attractive one-carbon (C1) feedstock for biomanufacturing due to its low cost, high solubility, and compatibility with electricity-driven CO_2 reduction. However, natural assimilation of formate is limited to a few bacterial species. I will discuss the engineering of the soil bacterium *Pseudomonas putida* to assimilate formate as its sole carbon and energy source via the linear reductive glycine pathway. Through a combination of rational design and adaptive laboratory evolution (ALE), initial strains were optimized for mixotrophic growth, leading to mutations in promoter regions of synthetic genes and the native genome that facilitated metabolic integration. Strict formatotrophy was established by introducing a formate dehydrogenase gene and applying growth-coupled selection. Building on this platform, microbial growth was linked to the biosynthesis of complex metabolites. As an example, a formatotrophic *P. putida* strain was engineered to couple xanthommatin production—a color-shifting animal pigment—with growth by creating a 5,10-methylenetetrahydrofolate auxotrophy dependent on endogenous formate levels. This modular system was further refined through ALE, enabling efficient, gram-scale pigment biosynthesis. These findings establish *P. putida* as the first strictly formatotrophic member of its genus and highlight its potential as a robust host for scalable, growth-coupled production of high-value compounds from C1 substrates.

KEYWORDS Metabolic engineering, *Pseudomonas putida*, Growth-coupling, C1 feedstock

ORAL PRESENTATION

Feedstock-efficient conversion through hydrogen and formate-driven metabolism in *Escherichia coli*

Justin Panich[1] (*justinpanich@lbl.gov*), Robert L. Bertrand[2], Aidan E. Cowan[2], Jacob B. Roberts[2], Lesley J. Rodriguez[3], Juliana Artier[2], Emili Toppari[4], Edward EK Baidoo[3], Yan Chen[3], Christopher J Petzold[5], Graham A Hudson[2], Patrick M Shih[2], Steven W Singer[5], Jay D. Keasling[2]

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Product yields for biomanufacturing processes are often constrained by the tight coupling of cellular energy generation and carbon metabolism in sugar-based fermentation systems. To overcome this limitation, we engineered *Escherichia coli* to utilize hydrogen gas (H_2) and formate ($HCOO^-$) as alternative sources of energy and reducing equivalents, thereby decoupling energy generation from carbon metabolism. This approach enabled precise suppression of decarboxylative oxidation during acetate growth, with 86.6 ± 1.6 % of electrons from hydrogen gas (via soluble hydrogenase from *Cupriavidus necator* H16) and 98.4 ± 3.6 % of electrons from formate (via formate dehydrogenase from *Pseudomonas* sp. 101) offsetting acetate oxidation. Hydrogen gas supplementation led to a titratable and stoichiometric reduction in CO_2 evolution in acetate-fed cultures. Metabolomic analysis

suggests that this metabolic decoupling redirects carbon flux through the glyoxylate shunt, partially bypassing two decarboxylative steps in the TCA cycle. We demonstrated the utility of this strategy by applying it to mevalonate biosynthesis, where formate supplementation during glucose fermentation increased titers by 57.6 % in our best-performing strain. Flux balance analysis further estimated that 99.0 ± 2.8 % of electrons from formate were used to enhance mevalonate production. These findings highlight a broadly applicable strategy for enhancing biomanufacturing efficiency by leveraging external reducing power to optimize feedstock and energy use.

KEYWORDS biomanufacturing, hydrogen, formate dehydrogenase, CO2, metabolic engineering

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ORAL PRESENTATION

ChIP-mini: a low-input ChIP-exo protocol for elucidating DNA-binding protein dynamics in bacteria

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[2] *Advanced Materials and Chemic al Engineering, University of Scie nce and Technology (UST), Rep ublic of Korea*

Genome-wide identification of binding profiles for DNA-binding proteins from the limited number of bacteria in various studies is crucial for understanding transcriptional regulation and cellular processes but remains challenging, as the current ChIP-exo is designed for high-input bacterial cells (>1010). Here, we developed an optimized ChIP-mini method, a low-input ChIP-exo utilizing a 5,000-fold reduced number of initial bacterial cells and an analysis pipeline (DiffExo), to identify genome-wide binding dynamics of DNA-binding proteins in samples with limited bacterial cell numbers. Collectively, this work establishes a broadly adaptable platform for elucidating DNA-binding protein dynamics in diverse intracellular pathogens during infection, with potential applications extending to biofoundry automation through high-throughput experiments at the 96-well plate scale.

KEYWORDS Chromatin Immunoprecipitation, ChIP-exo, Intracellular pathogens, Biofoundry, Automation

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ORAL PRESENTATION

Engineering synthetic acetogenesis and novel terpenoid biosynthesis

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University of California, Los Angeles

Nature possesses a broad spectrum of catalytic functions that are spread throughout different organisms. To fully harness nature's full catalytic abilities for solving global energy and carbon challenges, we must search for metabolic pathways in the pan-genome-scale metabolic networks. Here we present two novel pathways that we discovered: synthetic acetogenesis and terpenoid diacid biosynthesis. Our synthetic acetogenesis includes gluconeogenesis (GNG) and non-oxidative glycolysis (NOG) that together produce acetate from CO2. The reductive acetyl-CoA pathway (also known as the Wood-Ljungdahl pathway) is a natural acetogenesis pathway that chemolithoautotrophs use to convert inorganic carbon to biomass and bioproducts. Our GNG-NOG pathway carries out acetogenesis by integrating the assimilation of one-carbon units through pyruvate formate lyase, carbon reduction through gluconeogenesis, nonoxidative cleavage through the pentose phosphate pathway and phosphoketolase. Using enzymes derived from *Escherichia coli*, *Bacillus subtilis*, and Bifidobacterium adolescentis, synthetic acetogenesis enables cells to proliferate and produce acetate using one-carbon units. The ensuing acetate may serve as a direct substrate to lipogenesis and natural

product synthesis via acetyl-CoA. To add to our repertoire of value-added products, we searched for new metabolites emerging in engineered *Yarrowia lipolytica* that has enhanced flux through the mevalonate pathway. We identified a novel class of bifunctionalized terpenoids and mapped their biosynthetic pathways. Our discovery provides novel carbon upcycling routes and a glimpse into novel engineering strategies utilizing uncharted metabolism.

KEYWORDS Metabolic engineering, C1 metabolism, metabolomics, terpenoid, isoprenoid

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ORAL PRESENTATION

Ian Paulsen, ARC Centre of Excellence in Synthetic Biology

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ARC Centre of Excellence in Synthetic Biology, Macquarie University, Sydney, Australia

Cyanobacteria are responsible for about 25% of all photosynthesis and form the foundation of marine and terrestrial food chains globally. Our research investigates the uptake and metabolism of alternative (organic) nutrients by these ancient microbes. In particular, the impact of nutrient utilisation on the geographic distribution of marine cyanobacteria. Our research also demonstrated that marine cyanobacteria are mixotrophic, not obligate photoautotrophs - they can metabolise organic nutrients and fix atmospheric carbon concurrently. Within the Australian Research Council Centre of Excellence in Synthetic Biology, we expanded our research to explore how cyanobacterial carbon fixation can be combined with the bioproduction of high-value compounds. In collaboration with Prof. Esteban Marcellin's group at the University of Queensland and Bondi Bio Pty Ltd, the team has engineered squalene-producing (emollient terpenoid) cyanobacteria with the highest reported productivity under optimised growth conditions. Physiological and multi-omic characterisation also revealed the impact of bioproduction on the strain's photosynthetic capability. More recently, we have been investigating a fast-growing strain of cyanobacteria, *Synechococcus* sp. PCC11901, which exhibits unprecedented biomass accumulation (OD750 >100). Our team has developed a highly efficient transformation method for this strain, which eases genetic engineering for bioproduction, and has also conducted in-depth metabolic characterisation. Overall, our work not only sheds light on how cyanobacterial carbon capture supports marine food chains worldwide but also harnesses cyanobacterial photosynthesis to revolutionise synthetic biology and biomanufacturing.

KEYWORDS Cyanobacteria, Synthetic Biology

ORAL PRESENTATION

Protein engineering and design to develop efficient protease inhibitors

Maryam Sarmazdeh (*maryamr@unr.edu*)

Chemical and Materials Engineering, University of Nevada, Reno

Proteins are versatile biological building blocks whose evolved structures and functions can be reprogrammed through biomolecular engineering to create new molecular activities. My research group focuses on protein engineering and design to develop innovative tools and therapeutics for drug delivery, biosensing, and sustainability. We employ a combination of rational design and combinatorial protein engineering approaches to create new bioengineering platforms and designer protein scaffolds. In this talk, I will highlight our recent efforts to engineer protease inhibitor biomolecules as potential therapeutics. Metalloproteinases are key regulators in a range of diseases, making them attractive therapeutic targets; however, broad-spectrum small-molecule

metalloproteinase inhibitors have largely failed in clinical trials due to poor selectivity and off-target effects. In contrast, protein-based scaffolds offer enhanced specificity and tunability, enabling more precise inhibition. Our research integrates structural biology, advanced directed protein evolution, and yeast surface display with high-throughput library screening to develop novel protein-based metalloproteinase inhibitors with enhanced affinity and selectivity. The therapeutic potential of TIMP and engineered TIMP variants has been evaluated using a simplified vitro blood–brain barrier model and glioblastoma multiforme cells. Together, these studies reveal the structural determinants underlying improved binding affinity and selectivity, deepen our understanding of protein–protein interactions, and enable targeted delivery, dynamic modulation of complex biological systems, and real-time bioimaging—opening new avenues for therapeutic intervention and mechanistic discovery.

KEYWORDS Protein engineering and design, Directed evolution, Protease inhibitors

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ORAL PRESENTATION

Towards solar-powered growth of autotrophic *Escherichia coli* using photoelectrochemistry

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[2] *University of Cambridge*

Carbon-neutral biomanufacturing requires microbial platforms that valorize CO_2, but the fully integrated coupling of renewable energy sources with microbial CO_2 fixation remains a major challenge. Here, we present the design of an integrated biohybrid system that autonomously converts solar energy and CO_2 into microbial biomass. We achieved this by systematically integrating a semi-artificial leaf that converts CO_2into formate, which is then consumed by an engineered, autotrophic *Escherichia coli* (*E. coli*). First, we employed adaptive laboratory evolution to overcome slow autotrophic microbial growth rates, resulting in a 5-fold decrease in the time required for growth on formate following dilution. Second, we established electrode-microbe compatibility, showing that the adapted strain can grow directly using formate electrochemically generated from CO_2 by a bio-cathode featuring co-immobilized formate dehydrogenase and carbonic anhydrase. Third, we replaced the electrical energy source with sunlight, developing a biophotoelectrochemical device to support *E. coli* growth. Finally, we propose a fully integrated design of an autonomous 'semi-artificial leaf' device that couples solar-driven CO_2-to-formate conversion and O_2 evolution within situmicrobial growth in a single reactor, thereby replicating natural photosynthesis using a semi-biological *E. coli* platform.

KEYWORDS Autotrophic *Escherichia coli*, Solar-powered biomanufacturing, Formate bioeconomy, Carbon-neutral biorefineries, Semi-artificial photosynthesis.

ORAL PRESENTATION

Cyborg cells for programmable medicine

Cheemeng Tan (*cmtan@ucdavis.edu*)
University of California Davis

Imagine a world where engineered entities outperform their natural counterparts, offering superior functionality and efficacy in treating challenging chronic diseases. My lab is pursuing this vision by merging engineering approaches and biology to create nano- and micro-scale biohybrid systems. I will discuss our pioneering work on “Cyborg Cells”, a new class of cells that incorporate synthetic materials into their cytosol. Cyborg Cells do not divide but preserve desired therapeutic functions, including

cellular metabolism, protein synthesis, and drug secretion. They also acquire new abilities to resist stressors that otherwise kill natural cells. The Cyborg Cells are applied to treat various diseases, including microbial infection, cancers, aging, and cell/tissue damage. Our work uses a unique and holistic approach that integrates synthetic and systems biology. Furthermore, our work establishes a new paradigm in cellular bioengineering by creating non-living synthetic devices that mimic the complexity of life, and may soon redefine the limits of what engineered living systems can achieve.

KEYWORDS Synthetic biology, mammalian cells, bacteria, cell therapy

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ORAL PRESENTATION

Designing Burden-Aware Control for Host- and Resource-Robust Gene Circuits

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Arizona State University

Synthetic gene circuits operate within living cells that impose fundamental constraints through limited resources and growth coupling. While a wide range of control strategies have been proposed to improve robustness, these approaches often incur substantial expression burden and architectural complexity, making them difficult to scale to multi-module systems. As circuit complexity increases, the cost of control itself can become a dominant failure mode. Here, I argue that effective gene circuit control must be explicitly burden-aware and host-aware, and I present our efforts to design control strategies that mitigate resource competition and growth-mediated effects with minimal overhead. In particular, I highlight a phase separation–based strategy that spatially organizes gene regulatory components, reducing effective dilution and buffering circuits from host-induced perturbations without increasing global expression load. Together, these results illustrate how accounting for the burden cost of control through engineered intracellular organization enables more robust and context-resilient synthetic gene circuits.

KEYWORDS Synthetic gene circuits; burden-aware control; host–circuit interactions; resource competition; growth coupling; RNA-based regulation; feedback and feedforward control; phase separation; intracellular organization; robustness and scalability

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ORAL PRESENTATION

Predictive biotechnology – A case study on 4-phenol oxidase

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Enzymes are Nature´s catalysts and have a great impact on (organic)synthesis Knowledge about enzymes and their functionality is steadily increasing. Frequently novel enzymes and reactions are described. Herein, we present a streamlined approach of enzyme mining in order to rationally select enzymes with proposed functionalities from the ever-increasing amount of available sequence data. We want to predict enzyme functionality and applicability based on the amino acid sequence: work related to predictive biotechnology. In a case study on 4-phenol oxidases eight enzymes were selected from about 300 sequences on basis of the properties of first shell residues of the catalytic center. A broad sequence space was covered on base of a first bioinformatic screening approach including known enzymes. To handle numerous sequences and easily rationalize or even predict sequence-function relations we created a computational tool, called amino acid cluster analysis (A2CA;

https://doi.org/10.57760/sciencedb.09549). It allows to correlate phylogenetic information with the data from a respective multiple sequence alignment as well as to link any other data such as activity, binding, structural data. Thereafter, selected candidates were produced and fully characterized. Correlations between important residues identified and enzyme activity yielded robust sequence-function relations, which were exploited by site-saturation mutagenesis. The application of a novel oxidase screening assay resulted in 16 active enzyme variants which were up to 90-times more active than respective wildtype enzymes. The results were supported by kinetic experiments and structural models. The newly introduced amino acids confirmed the correlation studies which overall highlights the successful logic of the presented approach.

KEYWORDS oxidases, functional annotation, biocatalysis

ORAL PRESENTATION

Empowering Synthetic Biology through Next-Generation Genome Engineering Technologies

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Enabling technologies for precise and efficient genetic manipulation are essential to realize the vast potential of synthetic biology. At MySynBio Lab, our efforts are dedicated to building next-generation tools specifically designed for industrially relevant microbes. To tackle the limitations of CRISPR-Cas-based genome editing, such as large protein size and off-target effects, we developed STAGE (*Streptomyces*-compatible TnpB-Assisted Genome Editing), a compact, high-fidelity, non-CRISPR-Cas genome engineering toolkit for *Streptomyces*. STAGE enables flexible gene deletion, large-fragment DNA excision, and multiplexed base editing with no detectable off-target activity. For multi-layered gene regulation, we systematically engineered a Cas13d-based RNA-targeting system named SONAR (Synthetic Orthogonal Nucleic acid Activation and Repression) that separates RNA cleavage from translational activation and repression, enabling multi-layered and reversible gene regulation. This allows us to achieve precise translational activation, gene-specific translation repression, and targeted transcript degradation within a single, highly modular platform. In addition, we developed a blue light–inducible sigma factor assembly system, which enables robust and reversible gene activation (>500-fold induction) without the need for chemical inducers, offering a new level of control and biosafety for engineered microbes. Taken together, these enabling technologies significantly expand the synthetic biology toolkit and pave the way for more programmable, reliable, and application-oriented microbial engineering.

KEYWORDS Genome editing; CRISPR-Cas13; Translation regulation; Transcription regulation; Optogenetics

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ORAL PRESENTATION

Programmable RNA trans-splicing for scaling up cellular logic computation

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Synthetic genetic circuits program the cellular input–output relationships to execute customized functions. However, efforts to scale up these circuits have been hampered by the limited number of reliable regulatory mechanisms with high programmability, performance, predictability and

orthogonality. Here we report a class of split-intron-enabled trans-splicing riboregulators (SENTRs) based on de novo designed external guide sequences. SENTR libraries provide low leakage expression, wide dynamic range, high predictability with machine learning and low crosstalk at multiple component levels. SENTRs can sense RNA targets, process signals by logic computation and transduce them into various outputs, either mRNAs or noncoding RNAs. We subsequently demonstrate that digital logic operation with up to six inputs can be implemented using multiple orthogonal SENTRs to regulate a single gene simultaneously and coupling SENTRs with split intein-mediated protein trans-splicing. SENTR represents a powerful and versatile regulatory tool at the post-transcriptional level in *Escherichia coli*, with broad biotechnological applications.

KEYWORDS genetic circuit design, split intron, RNA trans-splicing, cellular logic computation

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ORAL PRESENTATION

Programming Materials through Synthetic Biology -

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Programming Materials through Synthetic Biology - Wilfried Weber Synthetic biology enables the creation of materials with advanced properties and functions fostering advances in therapeutics, biosensing, manufacturing, and construction. In this keynote, I will highlight three directions illustrating how biological principles can be used to program materials. First, I will present information-processing biohybrid materials. By integrating light- and chemically responsive biological switches into polymers and connecting modules through diffusible signals, we assembly biomolecular circuits directly in materials. These systems perform operations such as signal amplification, light-pulse counting, and binary encoding and decoding. Second, I will discuss programming the material properties of transcription factors within mammalian cells. Liquid-like condensates co-localize with target promoters and enhance gene expression up to four-fold compared to non-condensed factors, while gel-like assemblies silence promoters. We demonstrate this tunability using synthetic and natural transcription factors acting on transgenic and endogenous genes. Third, I will describe our approach to engineer microbially augmented wood composites. To navigate the vast genetic, compositional, and processing design space, we built a robotic platform for automated material fabrication and mechanical characterization. A transformer model trained on this library enables inverse design of formulations with defined properties. These materials can be further programmed for coloration, porosity, or damage reporting, and can be scaled via mold-based casting or additive manufacturing of free-form furniture components. Together, these efforts show how synthetic biology is transforming materials into systems that sense, compute, adapt, and communicate, enabling new classes of functional and sustainable material technologies.

KEYWORDS Adaptive Materials, Engineered Living Materials; Optogenetics

ORAL PRESENTATION

Metabolic engineering of an *E. coli* probiotic for in situ production of serotonin in the gut

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The gut microbiome is a critical regulator of human health, maintaining homeostasis and immunity through the production of bioactive metabolites. Tryptophan derivatives, specifically serotonin, serve as essential mediators of the gut–brain axis, influencing gastrointestinal function, neurological health, as well as gut motility. Given the link between metabolite dysregulation and neuropsychiatric disorders, this study describes the engineering of an *Escherichia coli* probiotic strain, *E. coli* Nissle 1917, for the stable, in situ biosynthesis of these compounds. By integrating heterologous pathways and cofactor regeneration modules, and optimizing enzyme efficiency, we developed a genetically stable probiotic platform. These engineered strains offer a promising living therapeutic strategy for treating gut as well as mental health conditions through the gut–brain axis.

KEYWORDS metabolic engineering, probiotic, gut-brain axis, serotonin

ORAL PRESENTATION

Engineered commensal biosensors reveal spatial metabolic dynamics of sialic acid in the inflamed gut

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Host mucin-derived sialic acids are key drivers of microbial colonisation, growth, and pathogenicity in the mammalian gut. However, their study is complicated by pronounced spatiotemporal dynamics: many gut metabolites, including sialic acids, are rapidly consumed by the microbiota, transformed, or absorbed by the host, meaning that conventional measurements of faeces or bulk digesta often fail to reflect their true local availability. In contrast, engineered whole-cell bacterial biosensors provide an in situ read-out of metabolite exposure at the precise time and location of use, before these molecules are depleted. Here, we demonstrate regional increases in sialic acid bioavailability in the inflamed mouse gut using an engineered *Escherichia coli* biosensor that reports sialic acid exposure via a NanR-regulated transcriptional circuit. The biosensor robustly colonises the mouse gut and remains functional for at least six weeks. Using organ-scale mesoscopic imaging at single-bacterium resolution, we observe strong correlations between disease status and biosensor activity across two models of intestinal inflammation. Longitudinal profiling along the gut reveals regional disparities between maximal sialic acid sensing and peak host inflammatory responses in a murine colitis model. Together, these findings illuminate the complex spatial dynamics underlying shared host-microbiome metabolism and demonstrate the broader potential of engineered bacterial biosensors to monitor the in situ bioavailability of rapidly turned-over metabolites within the gut.

KEYWORDS biosensor inflammation gut-microbiome commensal