Swiss-UK

Synthetic Biology Conference

Conference Program

Welcome note

Dear colleagues and friends,

On behalf of the organizing committee, it is our great pleasure to welcome you to the beautiful Lausanne to participate the Swiss-UK Meeting on Synthetic Biology. We have been eagerly anticipating this event supported by a grant for UK–Switzerland collaborations, and are thrilled to bring together experts from both Switzerland and the United Kingdom to discuss the latest research and advancements in this exciting field.

As co-organizers of this conference, we are honored to be part of such a distinguished group of professionals. We are confident that the next few days will be filled with engaging discussions, and potential future collaborations. This conference is a unique opportunity for us to learn from and inspire each other, as we explore the many facets of synthetic biology. It is a chance to share our research, exchange ideas, and forge new collaborations with colleagues from around the world.

Over the next few days, we will have the opportunity to hear from a diverse range of speakers, covering topics such as synthetic genomes, cell-free & mammalian synthetic biology, control and regulation of genetic networks, and applications in biomanufacturer, healthcare and industry. We have also scheduled poster sessions and networking opportunities, to encourage deeper engagement and discussion among attendees. As we explore the frontiers of this rapidly evolving field, we hope that this conference will inspire us all to think more deeply about the possibilities of synthetic biology, and to consider new and innovative approaches to the world's most pressing challenges.

We hope that you find this conference to be a valuable and enjoyable experience, and that it provides a platform for new collaborations and breakthroughs. Finally, we would like to thank all the sponsors and organization team, who make this meeting possible.

Once again, welcome to Lausanne!

Sincerely,

Yolanda & Patrick

Swiss-UK
Synthetic Biology Conference

August 29th - 30th, 2023
Lausanne, Switzerland

Sponsors

The Swiss-UK Synthetic Biology Conference 2023 is funded by the UK Research and Innovation.

UK Research and Innovation

We thank these organisations for their generous support to this conference.

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Layout: Astri Kusumawardhani

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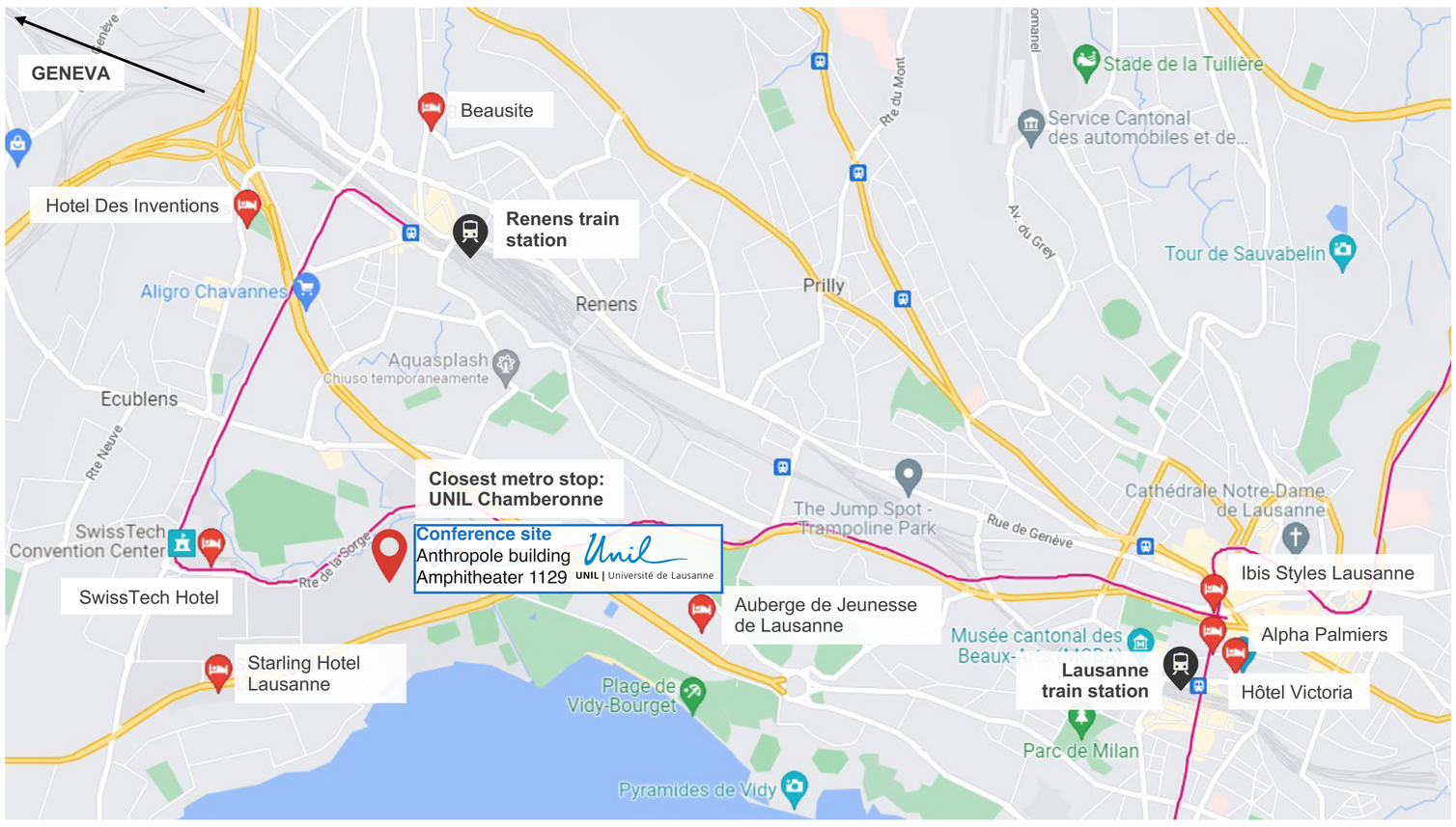
Conference information

Location

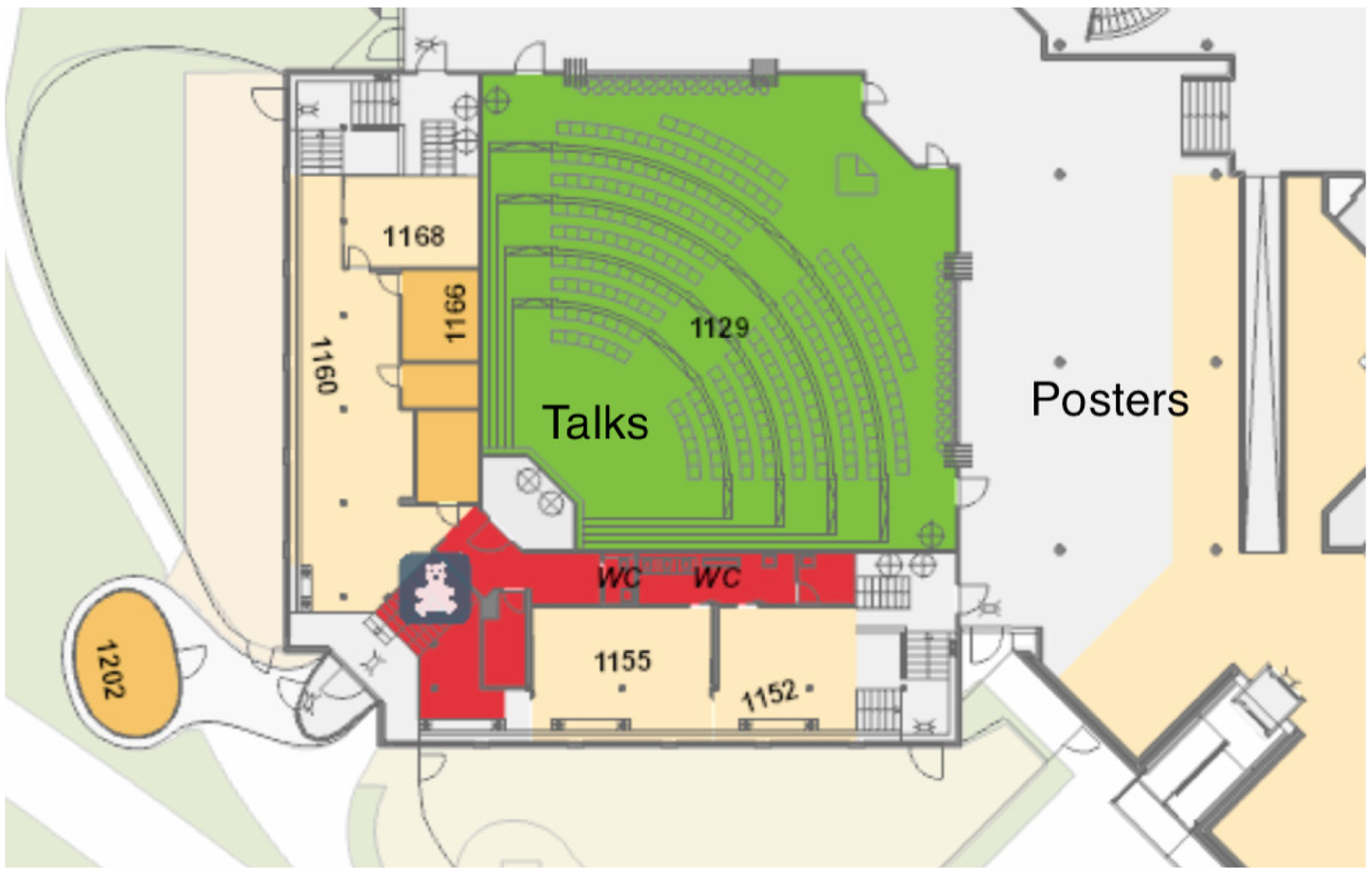
The symposium will be located at the Anthropole building (1015 Chavannes-près-Renens) of the University of Lausanne (Lausanne, Switzerland).

The map below gives an overview of the area, indicating:

- the conference location,
- nearby hotels and,
- metro stops and train stations.



Layout of the the conference site – Anthropole building, Amphitheater 1129



For Lunch – walk to Unithèque building (also called the “Banana”) from the conference location (~7 to 10 minutes walk):
For Conference dinner – walk to Geopolis building (~10 to 15 minutes walk) or one metro stop from UNIL - Chamberonne station to UNIL - Mouline station.

Getting here

From Geneva airport (Genève aéroport) to Lausanne station (Lausanne gare).
The train, metro, and bus tickets can be bought on the SBB / CFF website, app, or at the station. Price one way: 27 CHF = £24.
The UNIL – Chamberonne station on the metro M1 line is the closest metro stop to the conference centre. Its exit is just outside the building.
From Lausanne gare to UNIL-Chamberonne costs:
One way : 3.70 CHF = £3.26
Day ticket: 9.30 CHF = £8.19
You can also reach the Anthropole building from Morges gare by taking Bus 701 to Ecublens VD, Allée de Dorigny bus stop. The Anthropole building is 550 metres from this stop.

Emergency numbers

European emergency no: 112
Ambulance: 144
Police: 117

Fire service: 118
Conference organiser (Yolanda Schaerli): +41 (0) 21 692 56 02

What to see around Lausanne

On the shore of Lake Geneva, with a wonderful view of the Alps, the city of Lausanne is the perfect combination of nature and innovation. You can enjoy the panorama with a classic excursion, or by doing stand-up paddling or a boat trip on the lake. A few minutes from the city are the Lavaux terraces, terraced vineyards sloping down towards the lake, a UNESCO heritage site. Thanks to its two university centres, UNIL and EPFL, Lausanne is at the forefront of research and education. Come and visit us and don't miss the opportunity to explore this wonderful region!
More info at:
https://www.lausanne-tourisme.ch/en/
https://www.myswitzerland.com/en-ch/destinations/lake-geneva-region-vaud/

Internet connection

The wifi networks available for visitors are:

A. eduroam (https://eduroam.org/where/)
- if you are a member of eduroam partner institutions

B. public-unil - SMS Self-Registration (https://unil.ch/ci/wifi-en#visitors)
- free and instant access

C. public-unil - Login Event (https://unil.ch/ci/wifi-en#visitors)
The following access is available from August 29th to August 30th 2023, with credentials:
username: SwissUK23
password: SwissUK23

Swiss-UK
Synthetic Biology Conference

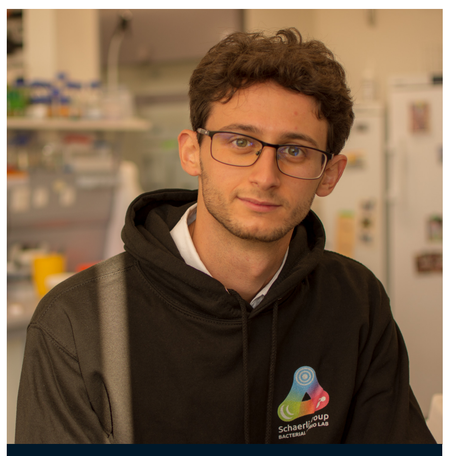
August 29th - 30th, 2023
Lausanne, Switzerland

Organisers

University of Lausanne

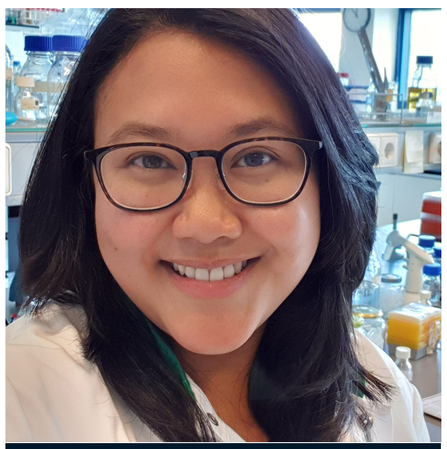
Yolanda Schaerli
Associate Professor

Estelle Pignon
PhD student



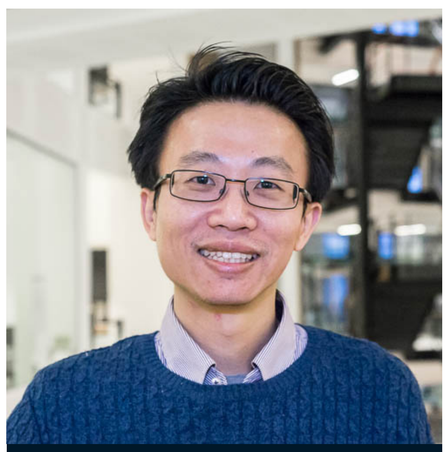
Emanuele Boni
PhD student

Içvara Barbier
Post-doc



Astri Kusumawardhani
Post-doc

University of Manchester



Patrick Cai
Professor

Stefan Hoffman
Post-doc

Mark McCullough
Post-doc

Ellie Payne
Manager

James Sanders
PhD student

Raymond Wan
Post-doc

Schedule

Tuesday, August 29th 2023		
08:30-09:00	Registration	
09:00-09:15	Opening remarks by organizers	
Session 1a	**Synthetic codes and genomes**	
09:15-09:45	Jason Chin	Reprogramming the genetic code
09:45-10:15	Patrick Cai	Design, Construction, and Functional Characterization of a tRNA Neochromosome in Yeast
10:15-10:25	Pawel M. Mordaka	Establishing design principles for codon compression in the *Chlamydomonas reinhardtii* chloroplast genome
10:25-10:55	Coffee break	
Session 1b	**Synthetic codes and genomes**	
10:55-11:25	Tom Ellis	Context and control in modular synthetic genomes
11:25-11:55	Kathrin Lang	Expanding the genetic code – new chemistries and tools for biology
11:55-12:05	Xinyu Lu	Re-writing the yeast genome using synthetic gene clustering and epigenetic regulation
12:05-13:15	Lunch	
Session 2a	**Networks, control and regulation**	
13:15-13:45	Mustafa Khammash	Opto-cybergenetics: a tango of beams and genes
13:45-14:15	Yolanda Schaerli	Engineering CRISPR-based synthetic circuits in bacteria
14:15-14:25	Cara Deal	Engineering orthogonal transcription for the development of a cross-bacterial, orthogonal and tuneable gene expression toolbox
14:25-14:35	Laura Catón	Bioengineering bacterial photonic crystal: a novel sustainable colour generation
14:35-15:35	**Poster Session: Odd numbers & Coffee break**	
Session 2b	**Networks, control and regulation**	
15:35-16:05	Lucia Marucci	Programming cells: from models to data and back
16:05-16:35	Jörg Stelling	Model-based design of synthetic circuits under uncertainty
16:35-16:45	Jean-Baptiste Lugagne	Deep neural networks for predicting single cell response landscapes and controlling gene expression
16:45-17:00	Sponsor talk (Singer Instruments)	
17:00-18:45	Beer Session (sponsored by Singer Instruments)	
19:00	Conference Dinner	

Schedule

Wednesday, August 30th 2023		
08:30-09:00	Set-up	
Session 3a	**Biomanufacturing with synthetic biology**	
09:00-09:30	Serina Robinson	Data-driven approaches for microbial enzyme and pathway discovery
09:30-10:00	Jenny Molloy	Towards distributed global manufacturing using synthetic biology
10:00-10:10	H. Adrian Bunzel	Photocatalytic enzymes by computational design and directed evolution
10:10-10:25	Sponsor talk (Thermo Fisher)	
10:25-10:55	Coffee break	
Session 3b	***In vitro* synthetic biology**	
10:55-11:25	Maartje M. C. Bastings	Multivalency as Geometric Puzzle: Engineering (super)selectivity at the biointerface with DNA
11:25-11:55	Sebastian Maerkl	On biochemical constructors and synthetic cells
11:55-12:05	Taniya Chakraborty	RNA origami-based hardware for synthetic cells
12:05-13:15	Lunch	
Session 4a	**Mammalian synthetic biology and healthcare**	
13:15-13:45	Martin Fussenegger	Toward a world of electrogenetics
13:45-14:15	Imre Berger	How synthetic biology unlocked the covid-19 response in Bristol, UK
14:15-14:25	Javier Santos-Moreno	Domestication of the human skin bacterium *Cutibacterium acnes* for medical applications
14:25-14:40	Sponsor talk (Nuclera)	
14:40-15:40	**Poster Session: Even numbers & Coffee break**	
Session 4b	**Mammalian synthetic biology and healthcare**	
15:40-16:10	Sarah Lovelock	Biocatalytic Approaches to Nucleic Acid Therapeutics Manufacturing
16:10-16:40	Karen Polizzi	Integrating living systems into biomanufacturing processes
16:40-17:15	Closing statements & awards ceremony	

Studentship awardees

We are pleased to bestow studentships to some of our outstanding PhD students to participate in the Swiss-UK Synthetic Biology Conference 2023. These studentships covers their registration fee, accommodation, and travel expenses.

The following participants are awarded the Swiss-UK Synthetic Biology Studentship:

Xinyu Lu	Imperial College London
Laura Catón	University of Cambridge
Sandie Lai	University of Warwick
Juliane Weller	Wellcome Sanger Institute
Rebecca E. Sizer	University of York
Mai P. Tran	University of Heidelberg and Max Planck Institute for Medical research
Daniela Gomes	1 CEB-Centre of Biological Engineering, Universidade do Minho
Lucas Henrion	University of Liège, Gembloux Agro Bio-Tech
Scott Stacey	Department of Engineering Science, University of Oxford
Eleftheria Kelefioti Stratidaki	Department of Biosystems Science and Engineering, ETH Zürich

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Session 1a:
Synthetic codes and genomes

Jason Chin
University of Cambridge

Reprogramming the genetic code

In terrestrial life, DNA is copied to messenger RNA, and the 64 triplet codons in messenger RNAs are decoded – in the process of translation – to synthesize proteins. Cellular protein translation provides the ultimate paradigm for the synthesis of long polymers of defined sequence and composition, but is commonly limited to polymerizing the 20 canonical amino acids. I will describe our progress towards the encoded synthesis of non-canonical biopolymers. These advances may form a basis for new classes of genetically encoded polymeric materials and medicines. To realize our goals we are re-imagining some of the most conserved features of the cell; we have created new ribosomes, new aminoacyl-tRNA synthetase/tRNA pairs, and organisms with entirely synthetic genomes in which we have re-written the genetic code.

Prof. Jason Chin is currently a Programme Leader at the Medical Research Council Laboratory of Molecular Biology (MRC-LMB), where he is also Head of the Centre for Chemical & Synthetic Biology (CCSB). He is Professor of Chemistry & Chemical Biology at the University of Cambridge, and holds a joint appointment at the University of Cambridge Department of Chemistry. He is also a fellow in Natural Sciences at Trinity College, Cambridge. He developed the first approaches to systematically expand the genetic code of eukaryotic cells and pioneered approaches, that are now widely used, for defining protein interactions by genetically encoding photocrosslinking amino acids.

Session 1a:
Synthetic codes and genomes

Patrick Cai
University of Manchester

Design, Construction, and Functional Characterization of a tRNA Neochromosome in Yeast

Here we report the design, construction and characterization of a tRNA neochromosome, a designer chromosome that functions as an additional, de novo counterpart to the native complement of *Saccharomyces cerevisiae*. Intending to address one of the central design principles of the Sc2.0 project, the ~190 kb tRNA neochromosome houses all 275 relocated nuclear tRNA genes. To maximize stability, the design incorporated orthogonal genetic elements from non-*S. cerevisiae* yeast species. Furthermore, the presence of 283 rox recombination sites enable an orthogonal SCRaMbLE system capable of adjusting tRNA abundance. Following construction, we obtained evidence of a potent selective force once the neochromosome was introduced into yeast cells, manifesting as a spontaneous doubling in cell ploidy. Furthermore, tRNA sequencing, transcriptomics, proteomics, nucleosome mapping, replication profiling, FISH and Hi-C were undertaken to investigate questions of tRNA neochromosome behavior and function. Its construction demonstrates the remarkable tractability of the yeast model and opens up new opportunities to directly test hypotheses surrounding these essential non-coding RNAs.

Prof. “Patrick” Yizhi Cai received his PhD in Genetics, Bioinformatics and Computational Biology from Virginia Tech in the USA. Prof. Cai had his postdoctoral fellowship under Jef Boeke in the Johns Hopkins University School of Medicine. From 2013 to 2017, Prof. Cai had his own research group at the University of Edinburgh with a prestigious Chancellor’s Fellowship and in 2017, Prof. Cai moved to the University of Manchester as the new chair professor in synthetic genomics. Prof. Cai’s lab focuses on Computer Assisted Design for Synthetic Biology, Neo Chromosome design and synthesis in the yeast, and DNA assembly automation.

Session 1a: Synthetic codes and genomes contributed talk

Establishing design principles for codon compression in the *Chlamydomonas reinhardtii* chloroplast genome

Pawel M. Mordaka, Kitty Clouston, Harry O. Jackson, Saul Purton, Alison G Smith

Department of Plant Sciences, University of Cambridge

A key ambition of synthetic biology is to be able to design and build entire genomes from their constituent parts. Even for bacterial genomes, with an average size of 3 Mbp and a few thousand genes, this is extremely challenging given the complex regulation and as-yet unknown features that ensure integrity of both function and maintenance of a genome. We have been focusing instead on the *Chlamydomonas reinhardtii* chloroplast genome, since its small size (~200 kbp) means it is easier to test a number of fundamental characteristics of genome structure and organisation and transformation is via homologous recombination allowing precise modifications to be made. *C. reinhardtii* can grow heterotrophically in the presence of acetate, thus allowing it to dispense entirely with photosynthesis - and hence also all photosynthetic genes in the chloroplast genome. We have established a synthetic biology workflow that allows us rapidly to delete and insert genes without leaving selection markers and are using this approach to determine what would constitute a viable minimal genome as well as to refactor gene clusters. We have also been able to recode chloroplast genes, reducing the number of codons required. This will form the basis of attempts to rewrite the universal genetic code and introduce non-canonical amino acids by reassigning the unused codons. Ultimately this will enable us to design a completely synthetic chloroplast genome. As well as addressing basic biological questions about what constitutes a functioning genome, our approach will generate novel and naive chassis as production systems for metabolites of interest and new-to-nature proteins.

Session 1b:
Synthetic codes and genomes

Tom Ellis
Imperial College London

Context and control in modular synthetic genomes

The international project to construct a synthetic version of the yeast genome (Sc2.0) has been one of the highest visibility research projects in synthetic biology in the last decade. As this draws to a close, Sc2.0 partners are now beginning to use the tools and knowledge of synthetic yeast genome assembly to ask new questions of biology and genomics, and develop new biotechnologies. As a milestone towards custom, modular genome, we are now using synthetic genome workflows with multiplex CRISPR to examine and exploit Synthetic Genome Modules (SGMs), where sets of genes that encode a common function are relocated from their native genomic loci into new synthetic defragmented or refactored gene clusters in the chromosomes. Following success using our SGM method to fine-tune pheromone sensing for biosensor cells, we are now employing it to the explore the minimal gene set for the cell cycle. This new work is revealing how changes in the genomic context of a gene can lead to changes in its expression levels and alter the regulatory control it has within a complex gene network. This highlights the importance of choosing the correct arrangements of genes when designing synthetic chromosomes, offering a new challenge for developing 'design rules'. As well as gene arrangement, gene content is also critical for synthetic genomes, and using our cell cycle SGM we are able to use combinatorial screening to determine the minimal gene content for robust function.

Tom Ellis is Professor in Synthetic Genome Engineering at Imperial College London. Tom has a degree in Molecular Biology from Oxford University and a PhD in DNA-binding Pharmacology from Cambridge University. Tom worked in a drug development company in London, then spent two years as a postdoc investigating synthetic biology at Boston University before starting his own group at Imperial College London. His research team develop synthetic biology and genome engineering tools for Baker's yeast and apply these in projects to make therapeutic molecules, biological sensors and functional living materials.

Session 1b:
Synthetic codes and genomes

Kathrin Lang
ETH Zürich

Expanding the genetic code – new chemistries and tools for biology

Nature uses a limited set of twenty amino acids to synthesize proteins. In recent years it has become possible to site-specifically incorporate designer amino acids with tailored chemical properties into proteins in living cells by reprogramming the genetic code. Together with developments in designing chemical reactions that are applicable to and selective within living systems, these strategies have begun to have a direct impact on studying biological processes.

In this talk I will present our lab's efforts to expand the genetic code and to endow proteins with novel chemical moieties within their physiological environment. By site-specifically incorporating artificial designer amino acids into proteins, we have developed tools to image and probe proteins,[1,2] to study protein-protein interactions and stabilize low-affinity protein complexes[4-6] and to re-engineer and manipulate molecular networks and biological pathways such as ubiquitylation in living cells.[7-9]

We envision that these approaches and technologies will enable the study of biological processes that are difficult or impossible to address by more classical methods.

[1] K. Lang et al.; Chem. Rev. 2014, 114, 4764.
[2] S.V. Mayer et al.; Angew. Chem. Int. Ed. 2019, 58, 15876.
[3] M. Cigler et al.; Angew. Chem. Int. Ed. 2017, 56, 15737.
[4] T.A. Nguyen et al.; Angew. Chem. Int. Ed. 2018, 57, 14350.
[5] T.A. Nguyen et al.; Angew. Chem. Int. Ed. 2022, 61, e20211108.
[6] J. Du et al., Nat. Commun. 2021, 12, 460, doi: 10.1038/s41467-020-20702-2.
[7] M. Fottner et al.; Nat. Chem. Biol. 2019, 15, 276.
[8] M. Fottner et al.; Nat. Commun. 2021, 12, 6515.
[9] M. Fottner et al.; J. Am. Chem. Soc. 2022, 144, 13118.

Kathrin Lang is Full Professor of Chemical Biology at the ETH Zürich, Switzerland. In her research she applies concepts from organic chemistry and protein engineering to develop new tools for investigating, modulating and designing novel molecular networks in living cells. Her group is especially active in enabling approaches to expand the genetic code and to endow proteins with novel chemical reactivities within their physiological environment. By equipping proteins with novel functionalities her group aims to (i) design and apply bioorthogonal reactions to image proteins and control enzyme activity in living cells, (ii) develop chemical tools to map protein-protein interactions and (iii) deploy novel chemoenzymatic approaches to study ubiquitylation/deubiquitylation pathways.

Session 1b: synthetic codes and genomes
contributed talk

Re-writing the Yeast Genome using Synthetic Gene Clustering and Epigenetic Regulation

Xinyu Lu, William Shaw, Klaudia Ciurkot, Tom Ellis

Imperial College London

Synthetic genomics is a field in synthetic biology aiming to assemble whole genomic DNAs while manipulating the genome content into a customised way. Saccharomyces cerevisiae yeast, a model organism with its whole genome sequence determined and well understood, is a good candidate for genome de novo redesign and synthesis. Here, we demonstrate the feasibility of reorganising conditionally essential pathways by synthetic gene clustering and co-regulating the clustered genes to optimise the genome function. Two synthetic chromosome clusters were generated through the genetic relocation of the genes involved in histidine and tryptophan biosynthesis. To achieve effective co- regulation of clustered genes, we engineered an epigenetic master regulation switch to reversibly control native silencing of the targeted synthetic clusters. We also applied the Synthetic Chromosome Rearrangement and Modification by LoxPsym-mediated Evolution (SCRaMbLE) system to induce dynamic changes that can optimise pathway function, while also examining the evolutionary implications of gene layout under specific conditions. These investigations will enable us to get a better understanding of the rules underlying the natural eukaryotic genome organisation and provide new principles for the artificial design, creation and evolution of synthetic modular genomes.

Session 2a:
Network, control, and regulation

Mustafa Khammash
ETH Zürich

Opto-Cybergenetics: A Tango of Beams and Genes

Cybergenetics refers to the application of control and communications concepts to regulate genetic systems. This can be achieved by using light to interface genetically engineered living cells with digital computers in order to achieve precise real-time closed-loop control. Here we present several biological and technological platforms for opto-cybergenetics and demonstrate their use in a multitude of synthetic biology applications.

Mustafa H. Khammash is Professor of Control Theory and Systems Biology at the Department of Biosystems Science and Engineering at ETH Zürich. Prof. Khammash works at the interface of control theory, systems biology, and synthetic biology. He develops computational and experimental methodologies for the analysis and design of biological networks. He has been creating control theoretic methods to reverse-engineer endogenous regulation and to engineer synthetic gene circuits for the robust and precise control of living cells, with applications to industrial and medical biotechnology.

Session 2a:
Network, control, and regulation

Yolanda Schaerli
University of Lausanne

Engineering CRISPR-based synthetic circuits in bacteria

Synthetic gene circuits allow us to govern cell behaviour in a programmable manner for user-defined tasks. Transcription factors constitute the 'classic' tool for synthetic circuit construction but some of their inherent constraints, such as insufficient modularity, orthogonality and programmability, limit progress in realizing more ambitious designs. CRISPR (clustered regularly interspaced short palindromic repeats) technology offers new and powerful possibilities for synthetic circuit design.

I will present our efforts in engineering CRISPR-based synthetic circuits in bacteria. First, I will show how we used CRISPR interference to build synthetic circuits such as an oscillator ("CRISPRlator") and incoherent feed-forward loops in *Escherichia coli*. The ease of building such circuits let us to explore the genotype-phenotype map of small network motifs and gain insights into their evolution and function. I then will present that the CRISPRlator also functions in *Streptococcus pneumoniae* and how we used it to demonstrate that variation in capsule production is beneficial for pneumococcal fitness in traits associated with pathogenesis. Finally, I will present how we can combine CRISPRi with CRISPR activation (CRISPRa) in the same circuits.

Yolanda Schaerli is an associate professor (since 2023) of synthetic biology at the Department of Fundamental Microbiology, University of Lausanne, Switzerland. Previously, she was assistant professor at the University of Lausanne (2017-2022), junior group leader at the University of Zurich, Switzerland (2014-2017), postdoc at the Centre for Genomic Regulation, Barcelona, Spain (2010-2014) and PhD student at the University of Cambridge, UK (2007-2010). Her group carries out research in bacterial synthetic biology with a focus on synthetic gene regulatory networks capable of forming spatial and temporal patterns.

Session 2a: Network, control, and regulation contributed talk

Engineering orthogonal transcription for the development of a cross-bacterial, orthogonal and tuneable gene expression toolbox

Cara Deal, Lien De Wannemaeker, Brecht De Paepe, Antoni Planas, Marjan De Mey

Centre for Synthetic Biology - UGent

The ability to engineer predictability into biology is a core aim of synthetic biology. This is especially important in the development of robust microbial cell factories that can function in harsh industrial conditions. In this context, orthogonal expression of the gene of interest can help to relieve the environmental and host-related influences that often result in bioprocess variability, thus solving one of the main bottlenecks of industrial biotechnology.

Although tools for orthogonal gene expression exist, they are currently limited in their host range and often exert significant metabolic burden on the host. This research aims to address these limitations by creating a gene expression tool that utilises the host's native transcription machinery. Building on existing research that shows that the sigma factor from one organism can be used to control orthogonal gene expression in a second host, we aim to develop and characterise a system that regulates transcription initiation in hosts across the bacterial domain.

To this end we demonstrate the use of rational DNA libraries and a highly sensitive screening method to select promoter sequences that do not interact with the host metabolism. This independence from host cellular processes was subsequently confirmed in a number of diverse bacterial organisms. For each of the selected promoters, a cognate transcription initiation factor will be engineered which activates transcription specifically and selectively at this sequence - resulting in a set of orthogonal and tuneable promoter-RNAP cognate pairs that can be utilised to build complex and orthogonal genetic circuits.

Such a genetic toolbox is a key enabling technology in industrial biotechnology, allowing predictable engineering in diverse bacterial hosts, and facilitating the transfer of these genetic circuits between hosts of industrial interest. As well as proving a functional tool, such sigma factor engineering can also provide interesting insights into the fundamental mechanisms of transcription initiation.

Session 2a: Network, control, and regulation contributed talk

Bioengineering bacterial photonic crystal: a novel sustainable colour generation

Laura Catón, Alexandre Campos, Daniel Domínguez-Pérez, Aldo Barreiro, Constantinos Patinios, Raymond Staal, Silvia Vignolini, Colin Ingham

University of Cambridge

Colour is nearly ubiquitous within the living world and may provide sustainable colourants for industry. Structural colour (SC) is a consequence of light interacting with ordered nanostructures, and it is responsible for nature's most vivid and brightest colours. Despite the plethora of work describing the frequency and diversity of SC in living organisms, little is known about the underlying genes, biochemical pathways, and evolution at the basis of SC.

To approach this problem, we developed a model organism, *Flavobacterium* IR1, which rapidly self-organises into an ordered nano-scale 2D periodic lattice crystalline colony, with an angle-dependent coloured patterning when illuminated with white light. This assembly process can potentially create engineered living materials with aesthetic and functional properties. IR1 is genetically amenable, allowing exploration of the genes that specify colony organisation and creating SC.

We first generated a transposon insertion mutant library, conducted comparative proteomic analyses on selected IR1 SC mutants and used these data to modify key genes using a CRISPR-Cas12 gene editing system. A detailed picture of the relationship between genes and biochemical pathways is being developed. For example, a key transcriptional regulator (hypA16) is responsible for SC tuning in IR1. The consequences of its KO shift the colour from green to red and cell morphology. A demonstration of genetic complementation and the implications of this KO on the proteome will be presented. Using synthetic biology, we work to understand SC and develop enhanced new bacterial-coloured optical structures as potential substitutions for unsustainable pigments.

Session 2B:
Network, control, and regulation

Lucia Marucci
University of Bristol

Programming cells: from models to data and back

The ability to program ad hoc cells and biological processes offers exciting opportunities in basic research, in the biotechnology industry and in the clinic. Computer-aided design can significantly accelerate design-build-test-learn cycles for cellular programming; however, the lack of established design tools which can cover biological functions across scales, and difficulties in engineering systems robust to changes and perturbations, still represent major roadblocks.

In this talk, I will present two complementary approaches to rationally and robustly program cellular phenotypes. I will first discuss how computer-aided cell design can be supported by whole-cell models (WCMs), which are mathematical models aimed at capturing the function of all genes and multiscale processes within a cell. The design of minimal bacterial genomes will be used as a proof-of-concept; I will also show how machine learning can support WCMs' output interpretation and solve their computational burden challenge. The second approach, named cybergenetics, leverages feedback control to engineer robust cellular phenotypes; different applications will be presented (e.g., control-based analysis of gene network dynamics, drug combination therapy design).

Our tools and results should make the design of complex cellular phenotypes and laboratory engineering a step closer.

Dr Lucia Marucci is a Fellow of the Engineering and Physical Sciences Research Council (EPSRC) and an Associate Professor in Systems and Synthetic Biology at Bristol (UK). She also co-directs the Bristol Centre for Engineering Biology (BrisEngBio) and the Bristol BioDesign Institute (BBI). Her interdisciplinary group works at the interface of systems and synthetic biology with control engineering and computer science and is focused on the development of automated strategies to understand and design complex cellular phenotypes.

Session 2B:
Network, control, and regulation

Joerg Stelling
ETH Zürich

Model-based design of synthetic circuits under uncertainty

Synthetic biology deals with substantial challenges due to uncertainty that traditional engineering disciplines do not face to the same extent. This opens questions such as: Which gene circuit architecture will let us achieve a given (complicated) design objective? Which are the most informative experiments for circuit design? And how can we deal with non-genetic cell-to-cell variability? The talk will present computational design methods based on mechanistic mathematical models that address these questions. Their common element is the use of computational / mathematical approaches that systematically represent uncertainty in circuit architectures, (unseen) experimental data, or individual cell behaviors. Example applications include the design of orthogonal signaling pathways and of informative sub-circuits to characterize experimentally for circuit selection. The examples help to argue that the computational design of synthetic circuits under uncertainty provides an engineering framework for real-world applications.

Joerg Stelling is Professor of Computational Systems Biology at the Department of Biosystems Science and Engineering at ETH Zurich. He works at the interface of systems and synthetic biology, focusing on the analysis and synthesis of biological networks using—and further developing—methods from systems theory and computer science. His main aims of current research are to understand how inter-individual variability impacts the functioning of complex networks in biological systems, and to provide concepts and methods for the rational design of biological systems despite uncertain and non-robust components and interactions.

Session 2B: Network, control, and regulation
contributed talk

Deep neural networks for predicting single cell response landscapes and controlling gene expression

Jean-Baptiste Lugagne, Heidi E. Klumpe, Caroline M. Blassick, Mary J. Dunlop

Boston University

Single-cell gene expression dynamics are increasingly recognized as pivotal in understanding biological processes. These dynamics however are inherently stochastic, making it challenging to identify links between expression and function by simply observing natural fluctuations. This stochasticity also necessitates extensive data to derive statistically robust conclusions. In response to these challenges, we introduce a novel platform for single-cell feedback control of gene expression in Escherichia coli, using automated microscopy and optogenetics. Our key innovation is to develop and train a deep neural network to predict the response of the CcaSR optogenetic system rapidly and accurately. This network is then used in a model predictive control framework, enabling us to enforce arbitrary and cell-specific gene expression dynamics on thousands of single cells in parallel and generate complex time-varying patterns. We demonstrate the framework's capabilities by controlling the expression of the *tetA* antibiotic resistance gene, thereby correlating expression to dynamic functional outcomes. We then further investigate the capabilities of our approach by computationally simulating single cell responses, allowing us to easily incorporate different sources of noise and to explore alternative genetic circuit designs. This in silico study reveals that both the size of the training set and the length of past data provided as inputs influence prediction quality. For example cascaded genetic circuits, which introduce delays, necessitated more past data. Finally, our initial attempts to predict a single trajectory for a bistable auto-activation circuit revealed that our network architecture was ill-suited for multimodal dynamics. To overcome this, we updated the network architecture to predict the full distribution of potential cell responses instead, successfully predicting bimodal expression landscapes. In conclusion, we present the first neural network-based model for predicting gene expression and use this to control gene expression experimentally. We also explore potential extensions of this work to alternative experimental setups and genetic systems.

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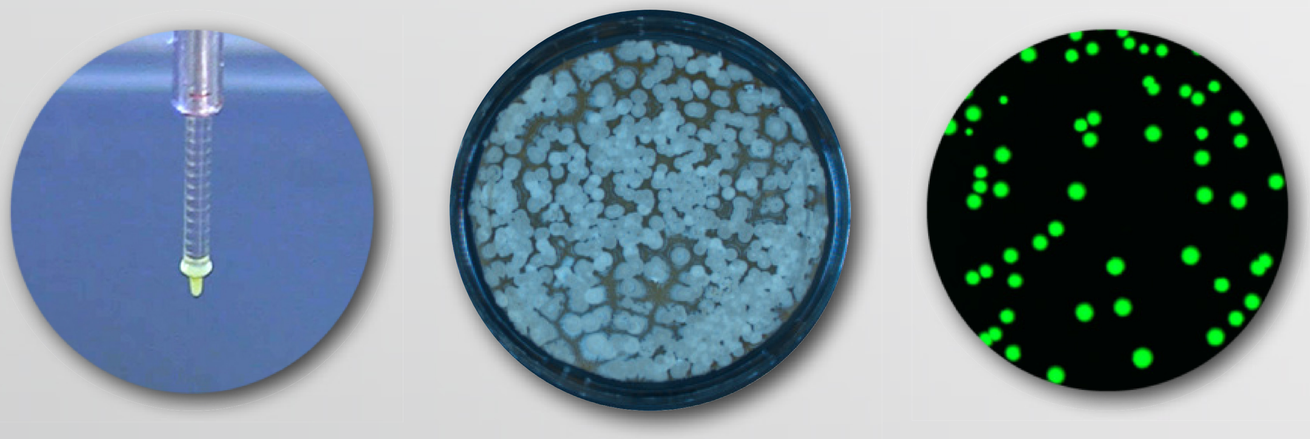

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Session 3a:
Biomanufacturing with synthetic biology

Serina Robinson
EAWAG

Data-driven approaches for microbial enzyme and pathway discovery

The rapidly evolving field of synthetic biology requires the inclusion of new enzymes in the toolbox of biological parts, e.g., for the biosynthesis and biodegradation of specialized molecules. A significant portion of microbial diversity, known as the "uncultivated majority," has remained difficult to study in the laboratory setting, making it a potential gold mine for new enzymes and functions. In this talk, I will describe how we have accessed this untapped resource through metagenomic sequencing and recombinant protein production methods. A major bottleneck in this process is the identification of promising enzyme candidates among the vast number of uncharacterized protein sequences - a task akin to searching for a needle in a haystack. Here I will cover our research to narrow the metagenomic sequence search space to mine protein-coding sequences for the biosynthesis and biodegradation of organic molecules. Specifically, I will cover how we have incorporated statistical learning, secondary structural features, and genomic context into targeted metagenomic enzyme discovery workflows. We use design-build-test-learn cycles to construct new enzyme libraries, screen their functions, and learn patterns of enzyme-substrate specificity for iterative library design. Our overall goal is to advance automated discovery methods from (meta)genomes to build and optimize metabolic pathways.

Serina Robinson is a tenure-track group leader leading the Microbial Specialized Metabolism group in the Department of Environmental Microbiology at EAWAG. Prior to starting at EAWAG in September 2021, she was a postdoctoral fellow under the guidance of Jörn Piel at ETH Zurich. She obtained her PhD in Microbiology and MSc in Bioinformatics and Computational Biology from the University of Minnesota under the supervision of Larry Wackett.

Session 3a:
Biomanufacturing with synthetic biology

Jenny Molloy
University of Cambridge

Towards distributed global manufacturing using synthetic biology

Enzymes are critical for all synthetic biology research and every molecular diagnostic test, making them vital tools for global health. However, enzymes are primarily manufactured in Europe, North America and parts of Asia and, most often, require a cold distribution chain for stability. This means that they can be expensive and unreliable to ship to other parts of the world. For example, this has been a longstanding problem for researchers building diagnostics and biosensors. Supply chains broke down further under the pressure of the COVID-19 pandemic, demonstrating the impact of reagent availability on health innovation and health security in many low and middle income countries. As a response to the need to distribute the means of enzyme production and to enable more equitable access to synthetic biology, we developed a number of open source DNA toolkits containing >200 interchangeable modules of enzymes, reporters and useful building blocks, plus protocols and expression handbooks; which are now in >500 labs in >40 countries, including universities, research institutions and small biotech companies. I will present the development and impact of these toolkits and the potential for distributed manufacturing of enzymes and other reagents to catalyse synthetic biology for global health: increasing the autonomy and agency of synthetic biologists to tackle health challenges wherever they are based in the world.

Dr Jenny Molloy is a Senior Research Associate at the University of Cambridge building technologies for an open, globally inclusive and equitable bioeconomy through the Open Bioeconomy Lab. She develops biomanufacturing tools and technologies that are sustainable by design and deployable in low-resource contexts. Her group also investigates the most effective ways that their research can make a positive impact in developing and emerging economies, particularly the effect of open licensing and collaborative development with end users. Jenny was a member of the World Economic Forum Global Future Council on Synthetic Biology, chairs an independent multi-sectoral working group on Local Production and Diagnostic and has co-founded four social enterprises and nonprofits supporting the development and deployment of open source tools for science.

Session 3a: biomanufacturing with synthetic biology contributed talk

Photocatalytic Enzymes by Computational Design and Directed Evolution

H. Adrian Bunzel, James A. Smith, Thomas A. A. Oliver, Michael R. Jones, Adrian J. Mulholland, J. L. Ross Anderson

ETH Zürich

The global energy crisis challenges us to develop more efficient strategies for the sustainable production of energy. Given the excellent efficiency of natural photosynthetic proteins, biohybrid photovoltaic devices present an attractive solution for solar energy conversion. However, their composition, stability, and complexity can limit their inclusion into photovoltaic devices. Here, we combined computational design and directed evolution to overcome these limitations and create tailor-made photoenzymes [1].

Photo-biocatalysts were designed by introducing photosensitizer binding sites into heme-containing helical bundle proteins. The designed binding sites were specific for the target photosensitizer and readily transplanted into other helical bundles. The best design was highly evolvable and reached nanomolar ligand affinity after mutagenesis and screening. The evolved enzyme generated 2.6 times higher photocurrents than the photosensitizer alone, primarily driven by increased photostability. Evolvability is a unique advantage of our protein-based approach over abiological photovoltaic and will be critical to developing efficient biohybrid systems.

Our work provides a methodological framework for creating highly efficient photoactive enzymes by design and evolution. The transferable nature of our approach will allow sustainably targeting countless other critical transformations such as nitrogen fixation, CO_2 reduction, H_2 production, and water splitting.

1. H. Adrian Bunzel, James A. Smith, Thomas A. A. Oliver, Michael R. Jones, Adrian J. Mulholland, J. L. Ross Anderson: Photovoltaic enzymes by design and evolution. bioRxiv 2022, doi:10.1101/2022.12.20.521207.

Session 3B:
In vitro synthetic biology

Maartje M. C. Bastings
EPFL

Multivalency as Geometric Puzzle: Engineering (super)selectivity at the biointerface with DNA

Understanding and manipulating precise interactions between materials and biology – the biointerface – is key to ensure optimal performance of diagnostics and therapeutics. Functional materials for biological applications, e.g. vaccines or implants, work best when their interaction with cells is precise. If not, side effects and toxicity might occur. Interactions are labeled superselective, when they happen only in a very specific (cellular) context and as such, present a strategy to enhance the therapeutic effect of bioactive materials.

Selective multivalent interactions are traditionally engineered with a focus on the balance of valency and affinity, and often a good amount of structural flexibility is present. In my laboratory, we hypothesized that rigidity at the nanoscale could be a strong determinant of super-selectivity. We combine insights from biophysics and tools from DNA nanotechnology to engineer materials with a controlled flexibility/rigidity balance which allows to present molecules and organize interactions in precise spatial patterns. I will show how structural mechanical properties on the nanoscale determine the selectivity of interactions between DNA and lipid membranes, in immune activation pathways, and how they are critical for super-selective Multivalent Pattern Recognition (MPR). Exploiting programmable flexibility within the well-defined DNA molecule, our research presents a new engineering strategy to investigate the impact of nanorigidity in functional soft matter, surface order and communication with life.

Maartje Bastings is a materials engineer who specializes in the synthesis of DNA-based supramolecular materials using multivalency as driving force in molecular design and functional performance. Her goal is to implement multivalent pattern recognition (MPR) as fundamental concept in the assembly of dynamic surfaces, control mechanical properties in soft matter, and explore the extent of conserved patterns in cellular signaling processes. Crossing supramolecular materials engineering with biophysics and cell biology creates a research space with the potential to advance the impact of DNA-nanotechnology both as functional material and as bioengineering tool.

Session 3B:
IN VITRO SYNTHETIC BIOLOGY

Sebastian Maerkl
EPFL

On biochemical constructors and synthetic cells

Cell-free synthetic biology emerged as a viable in vitro alternative for biological network engineering. Cell-free synthetic biology implements biological systems in a coupled transcription – translation reaction and therefore is a well-defined environment that is easier to control and interrogate than complex cellular systems. I will discuss several technological and methodological advances including the development of microfluidic chemostat devices, a high-throughput microfluidic device, and a method to easily produce a recombinant cell-free system. With these tools we were able to rapidly prototype genetic networks and transplant them into living hosts and engineered gene regulatory networks from the bottom-up with synthetic Zinc-finger transcriptional regulators. More recently, we were able to demonstrate that it is possible to create a partially self-regenerating cell-free system that continuously produces up to seven essential proteins and does so for an extended period of time. This work lays the foundation for the development of a universal biochemical constructor and may ultimately enable the creation of a synthetic cell.

Prof. Maerkl is Associate Professor at the Ecole Polytechnique Federale de Lausanne (EPFL). His research group works at the interface of engineering and biology and is active in the areas of systems biology, synthetic biology and molecular diagnostics. His specific biological interests lie primarily in reverse engineering gene regulatory networks, transcriptional regulation, transcription factor biophysics, cell-free synthetic biology, protein engineering, and in developing next-generation molecular diagnostic devices. To address these requirements his group is developing novel, state-of-the-art microfluidic technologies and molecular methods to address current limitations in biological engineering.

Session 3b: in vitro synthetic biology
contributed talk

RNA origami-based hardware for synthetic cells

Mai P. Tran, **Taniya Chakraborty**, Erik Poppleton, Franziska Giessler,
Luca Monari, Kerstin Göpfrich

University of Heidelberg and Max Planck Institute for Medical research

DNA nanotechnology enabled the design structural and in part, functional mimics of proteins. However, functional DNA structures often require thermal annealing and chemical modifications, which is incompatible with production in synthetic cells. In this regard, co-transcriptional folding of RNA origami could represent a new and thus far unexplored frontier for the making of custom-made molecular hardware in synthetic cells. Here, we produce RNA origami by co-transcriptonal folding from a DNA template inside of giant unilamellar lipid vesicles (GUVs) for the first time. We regulate the on-demand production with external triggers namely ions and rNTPs, transported into the GUV via ionophores or membrane pores, respectively. With the goal of making cytoskeleton-like functional components, we design the first 3D RNA nanotubes from self-assembled ssRNA tiles and successfully transcribe them inside GUVs. With sequence modifications on the DNA template, we control the length, reaching several micrometers, and the persistence length of the RNA nanotubes and achieve the formation of closed rings. Coarse-grained simulations of the ssRNA tiles and their assemblies confirm the experimental data. Showcasing the unique possibilities of RNA origami, we induce RNA cortex formation in GUVs by introducing membrane-binding aptamers, circumventing the need for chemical functionalization. Altogether, this work pioneers theuse of RNA-based hardware in synthetic cells.

Session 4a:
Mammalian synthetic biology & healthcare

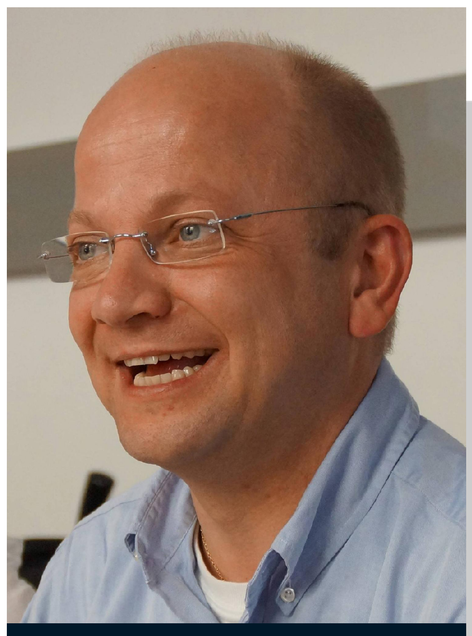
Martin Fussenegger
ETH Zürich

Toward a world of electrogenetics

With the advent of the internet of things, interconnected electronic devices are starting to dominate our daily lives and are reaching the control complexity of living systems, and yet work radically different: While human metabolism uses ion gradients across insulated membranes to simultaneously process slow analog chemical reactions and communicate information in multicellular systems via soluble or volatile molecular signals, electronic devices use multicore central processing units to control the flow of electrons through insulated metal wires with gigahertz frequency and communicate information across networks via wired or wireless connections. While analog biological systems and digital electronic devices efficiently work in their respective worlds there are no efficient interfaces between electronics and genetics. We will report our first attempts to design direct electro-genetic interfaces and our progress toward a world of ElectroGenetics and the internet of the body.

Martin Fussenegger is Professor of Biotechnology and Bioengineering at the Department of Biosystems Science and Engineering (D-BSSE) of the ETH Zurich in Basel as well as at the University of Basel. His research focuses on mammalian cell engineering, in particular on the assembly of synthetic gene circuits that process complex control and closed-loop expression logic as well as on the production of theranostic designer cell implants that interface with host metabolism to correct prominent metabolic disorders.

Session 4a:
Mammalian synthetic biology & healthcare



Imre Berger
University of Bristol

How synthetic biology unlocked the covid-19 response in Bristol, UK

A core technology in our laboratory is the MultiBac platform, which we developed originally to produce complex biologics that were hitherto unaccassible. MultiBac relies on a small non-human viral genome which we continuously optimize, for delivering customized multicomponent DNA circuitry to programme insect or mammalian cells and tissues, outfitting them with specific functionalities. During the COVID-19 pandemic, we deployed the MultiBac platform to provide SARS-CoV-2 antigens including the Spike glycoprotein to clinicians investigating this novel disease outbreak. In the process, we fortuitously discovered a druggable pocket, and a potent pan-coronavirus antiviral drug, in the SARS-CoV-2 Spike. Moreover, we used MultiBac to prepare novel nature-inspired reagents for active and passive immunization against coronavirus infectious disease.

Prof. Imre Berger was trained as a biochemist at Leibniz University and Medical School (MHH) in Hannover (Germany), MIT (Cambridge, USA), and ETH Zurich (Switzerland). He carried out his PhD work at MIT with Alexander Rich, a founding father of molecular biology. Since 2019, Prof. Berger is Founding Director of the Max Planck Bristol Centre for Minimal Biology. He has pioneered synthetic virus-derived nanosystems for DNA and protein delivery and genome engineering. The tools he developed are accelerating research and development in academia and industry world-wide. He holds international patents in protein and DNA technologies, founded four biotech companies, publishes prolifically in leading periodicals, and received numerous distinctions.

Session 4a: Mammalian synthetic biology & healthcare
contributed talk

Domestication of the human skin bacterium *Cutibacterium acnes* for medical applications

Javier Santos-Moreno, Guillermo Nevot, Nastassia Knoedlseder, Lorena Toloza, Marc Güell

University Pompeu Fabra

A prominent goal of synthetic biology is the development of living medicines based on engineered bacterial or mammalian cells. The genetic modification of the human microbiome presents fewer risks for the host than the editing of human cells, and bacterial therapeutics have been employed to treat metabolic disorders, to limit cancer progression, or to address microbiome dysbiosis [1]. In contrast to the numerous attempts to program gut bacteria for therapeutics, the engineering of the skin microbiome has remained largely unexplored despite its enormous potential.

Cutibacterium acnes, a bacterium that thrives within the sebaceous follicles, is the most abundant human skin microbe and has demonstrated promising engraftment properties when transplanted from a donor to a recipient individual [2]. Despite being an ideal chassis for the development of living skin biotherapeutics, the engineering of *C. acnes* has long been hampered by the extremely low transformation efficiency and the lack of (even basic) molecular biology tools available.

Here we reverted this situation and rendered *C. acnes* an engineerable chassis for skin disorders. First, we increased the transformability of *C. acnes* by several orders of magnitude. This paved the way for the generation of an engineering toolbox for *C. acnes* that includes modular plasmids and cloning schemes, a promoter library, a library of fluorescent reporters, and gene expression control tools such as CRISPRi and inducible transcription factors. The use of these tools enables the construction of synthetic gene circuits to control *C. acnes* behaviour in a programmable manner, and the development of strains that produce and secrete molecules with therapeutic potential.

1. McNerney, M.P., Doiron, K.E., Ng, T.L., Chang, T.Z. & Silver, P.A. Theranostic cells: emerging clinical applications of synthetic biology. Nat. Rev. Genet. 22, 730-746 (2021).

2. Paetzold, B. et al. Skin microbiome modulation induced by probiotic solutions. Microbiome 7, 95 (2019).

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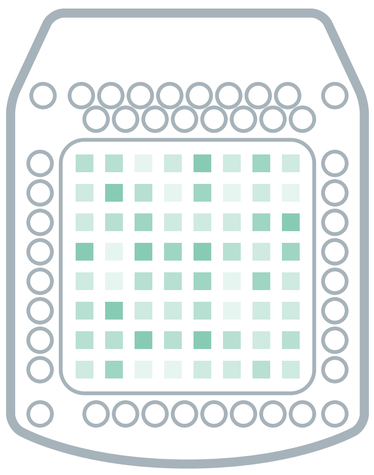

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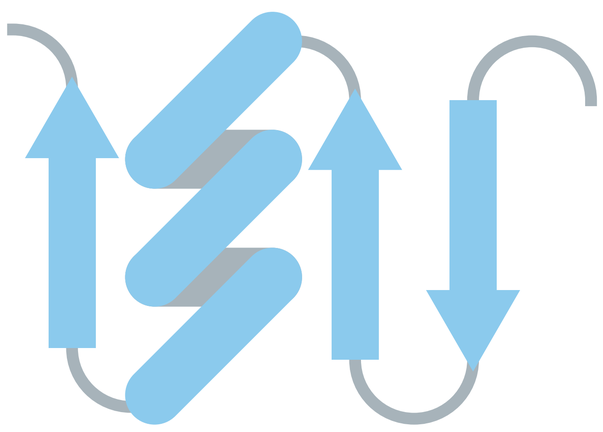

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Session 4B:
Mammalian synthetic biology & healthcare

Sarah Lovelock
University of Manchester

Biocatalytic Approaches to Nucleic Acid Therapeutics Manufacturing

Therapeutic oligonucleotides bind to mRNA to modulate the production of disease related proteins and have emerged as a new drug modality for the treatment of a range of disease areas including genetic disorders and viral infections. Until recently, approved therapeutics were limited to the treatment of rare diseases, however in 2020 Inclisiran was approved for the treatment of atherosclerotic cardiovascular disease, which affects approximately 30 million people in the USA alone. This emergence of therapeutic oligonucleotides for common diseases now creates a significant manufacturing challenge as existing methods of chemical synthesis, which rely on iterative coupling, capping, oxidation and deprotection to achieve stepwise extension of sequences immobilized on solid supports, are not suitable for large scale applications.

This talk will describe a transformative biocatalytic approach to efficiently produce oligonucleotides in a single operation, where polymerases and endonucleases work in synergy to amplify complementary sequences embedded within catalytic self-priming templates. This approach uses unprotected building blocks, aqueous conditions and can be used to produce diverse oligonucleotide sequences containing a range of pharmaceutically relevant modifications. Our methodology is showcased through the synthesis of a range of clinically relevant molecules.

Sarah received her PhD from the University of Manchester where she worked under the supervision of Prof. Nicholas Turner. Following her PhD, she moved to GSK where she worked in the Advanced Manufacturing Technologies team. She later moved back to academia and in 2020 she was awarded a UKRI Future Leader Fellowship and started her own independent research group. Sarah's interests are in the development of biocatalytic approaches for sustainable pharmaceutical manufacturing.

Session 4B:
Mammalian synthetic biology & healthcare

Karen Polizzi
Imperial College London

Integrating living systems into bio-manufacturing processes

Synthetic biology allows us to rapidly develop whole-cell biosensors (WCB) for metabolites and proteins that are critical to measure in biomanufacturing processes. However, successfully deploying these WCB for processing monitoring requires overcoming some practical challenges. Coexistence of different populations of cells and isolation of tasks between biosensing and producing cells can provide enhanced robustness and channels available resources into specialisation. In addition, the design-build-test-learn cycle in bacteria is rapid, meaning biosensors can be engineered quickly, but these cells grow quickly and can outcompete producing cells in mixed co-cultures. We have developed a materials science solution to help address this challenge, creating a system we call “living analytics in a multilayer polymer shell (LAMPS)”. LAMPS is an encapsulation method that facilitates the coculture of two cells with vastly different growth rates. Using the example ofmammalian and bacterial co-culture, we show that LAMPS enable the formation of a synthetic bacterial-mammalian cell interaction. Our work serves as a proof-of-concept for further applications in bioprocessing since LAMPS combine the simplicity and flexibility of a bacterial biosensor with a viable method to prevent runaway growth that would disturb mammalian cell physiology.

Karen Polizzi is a Professor of Biotechnology in the Department of Chemical Engineering at Imperial College London and a member of the Imperial College Centre for Synthetic Biology. Her research group applies synthetic biology to upstream bioprocess development including engineering cells for improved recombinant protein expression and the development of associated analytical technology.

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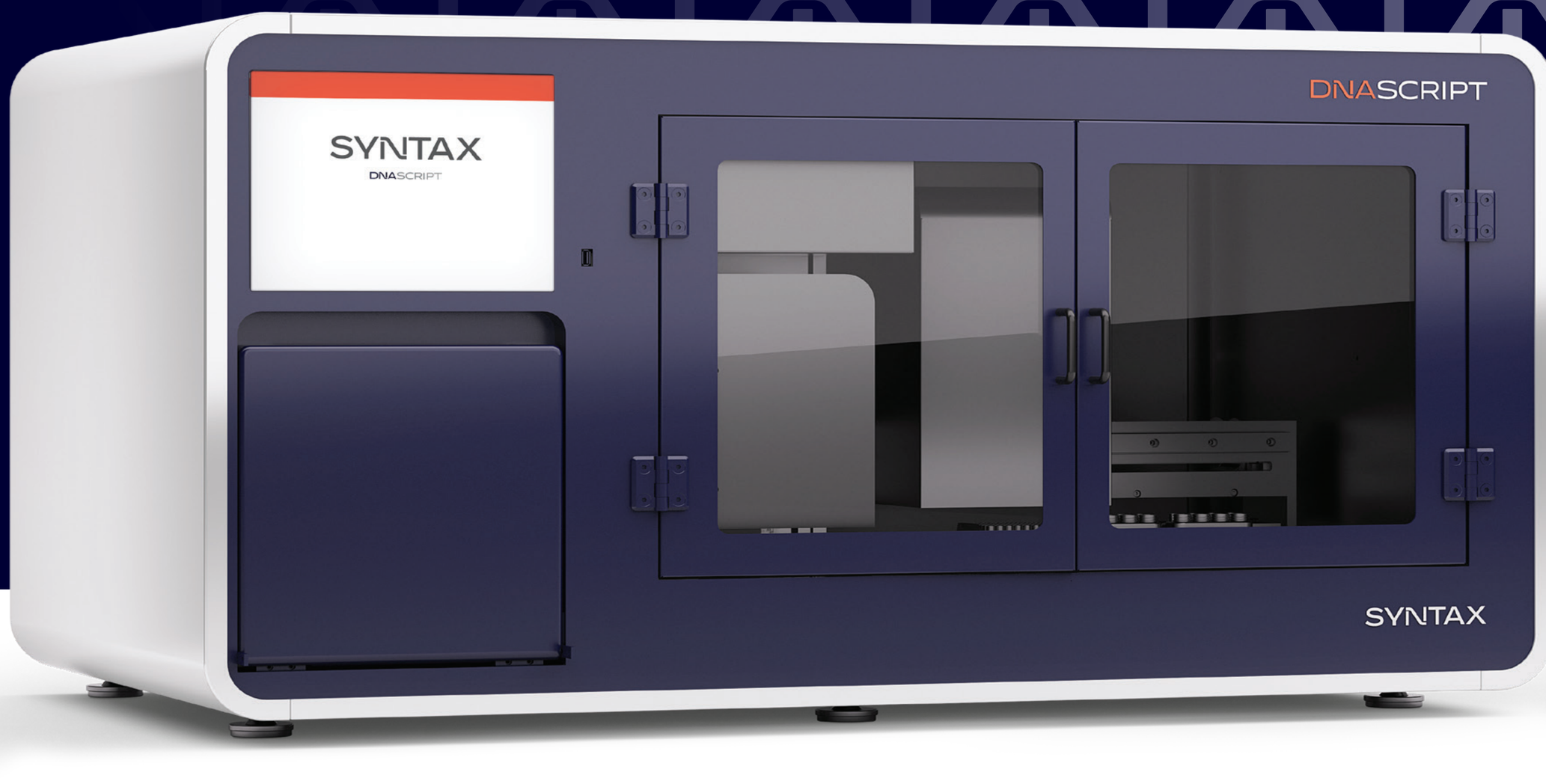

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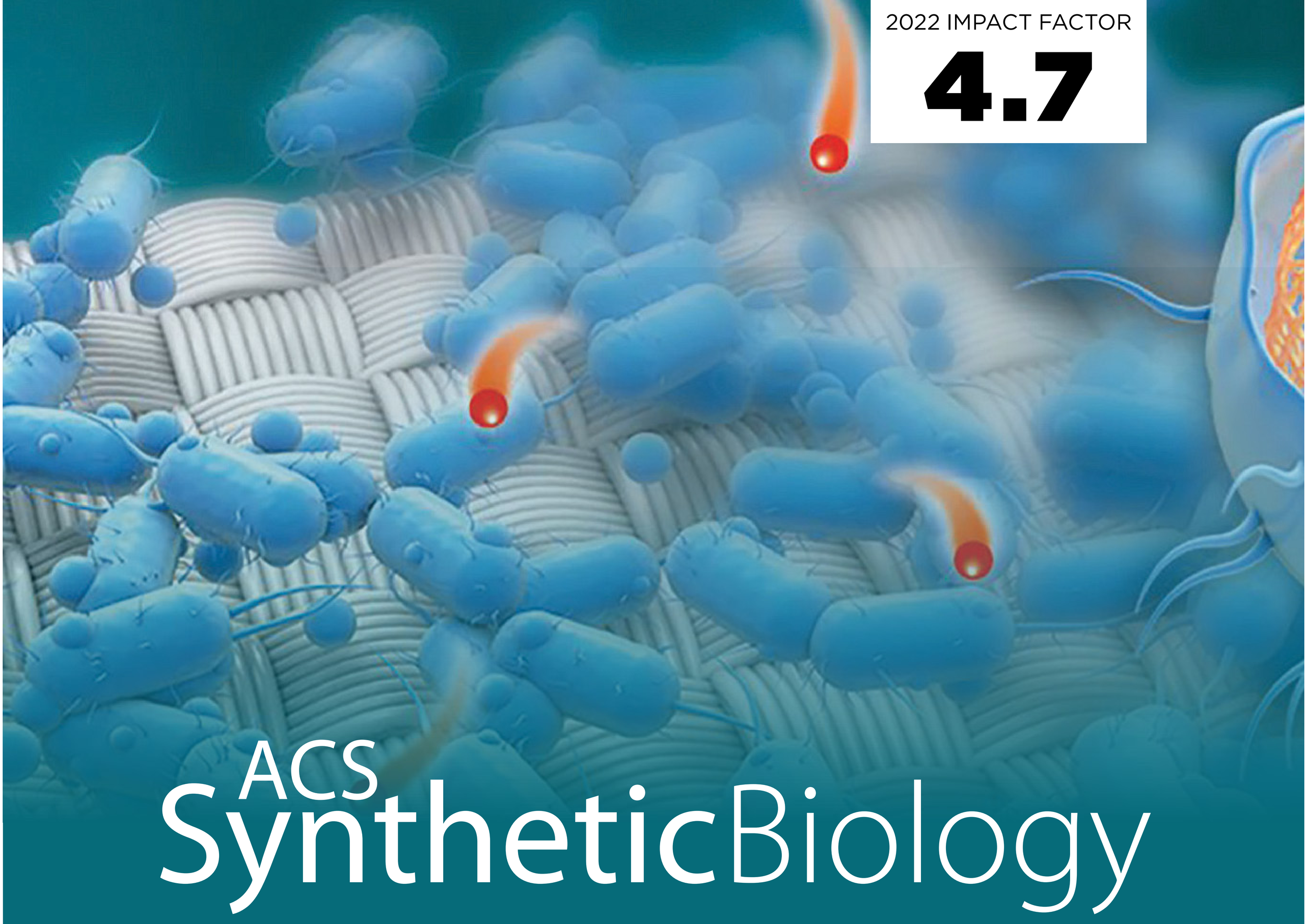

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Poster Session
Day 1 - August 29th, 2023

1
3D Laser Printing in Synthetic Cells

Tobias Abele, Tobias Messer, Lucas Diedrich, Matthias Brosz, Frauke Gräter, Camilo Aponte-Santamaría, Martin Wegener, Kerstin Göpfrich

Max Planck Institute for Medical Research, Heidelberg, Germany

Toward the ambitious goal of manufacturing synthetic cells from the bottom up, the deterministic positioning of components inside the compartment has remained elusive. Here, by using two-photon 3D laser printing, 2D and 3D hydrogel architectures are manufactured inside preformed giant unilamellar lipid vesicles (GUVs) with high precision and nearly arbitrary shape.[1] The required water-soluble photoresist is brought into the GUVs by diffusion in a single mixing step. Beyond this proof-of-principle demonstration, a transmembrane structure acting as a pore is 3D printed, thereby allowing for the transport of biological cargo, including DNA, into the synthetic compartment. We further investigated the permeation of various photoresist molecules through lipid membranes of different compositions. With experiments and molecular dynamics simulations we find a correlation between permeability and cytotoxicity.[2] With this, our work provides insights that are relevant for both, the bottom-up synthetic biology community as well as the bioprinting community.

[1] Abele *et al.*, *Adv. Mater.*, 2021 [2] Diedrich *et al.*, *Adv. Func. Mater.*, submitted

Poster Session
Day 2 - August 30th, 2023

2

Three-dimensional dynamics of genetic circuits

Lorea Alejaldre, Angeles Hueso-Gil, Jesus Miro, Huseyin Tas, Ángel Goñi-Moreno

Centro de Biotecnología y Genomica de Plantas (CBGP, UPM-INIA/CSIC)

Integration of genetic constructs into the genome improves stability and reliability compared to plasmid-based approaches. But this also formulates many new questions. What are the best coordinates for the constructs to be inserted? Should all components of the circuit be close together or far apart from each other? Indeed, location matters. It is known that some locations do not affect the cell, nor the construct, thus are widely used; other positions however, affect expression levels. This suggests that the behavior of a synthetic circuit also depends on its whereabouts within the volume of the cell. Indeed, uneven distribution of the transcriptional and translational machinery, and different chromosomal conformations modulate the function of genomic inserts. In addition, spatial distances between different elements of a genetic circuit can also have phenotypic consequences. Here we explore the three-dimensional space of *Pseudomonas putida* KT2440 using a set of expression systems, genetic inverters and toggle switches. We performed genomic integrations using the random transposase systems Tn5 and Himar1 to reduce integration bias–as well as Tn7 for consistent insertions in the same place. Individual clones were sequenced and characterized with flow cytometry. Our results show that functional differences arise depending on genomic locations and distances. Furthermore, we investigated how the induction of intracellular crowding via PHA formation modifies the 3D functioning of constructs. Our results suggest 3D features can be used to rationally modify the performance of circuits without changing their genetic sequences. Formalizing the genomic context allows researchers to select regions based on phenotypes of interest.

Poster Session
Day 1 - August 29th, 2023

3

Patterned engineered living materials based on *Escherichia coli* biofilms

Roberto Avendaño, Javier Santos-Moreno, Yolanda Schaerli

Bacterial Synthetic Biology Laboratory, Department of Fundamental microbiology.
University of Lausanne, Switzerland.

Bacterial biofilms possess properties that are highly desirable for the next generation of materials: they emerge through self-organization controlled by a genetic program, they can self-repair and adapt to environmental cues, and they are driven by sustainable energy sources and biodegradable. However, in order to realize the full potential of these materials, we need to be able to control their spatiotemporal organization. The aim of this project is to establish synthetic circuits to control the spatial expression of curli fibers in of *Escherichia coli* biofilms to produce patterned functionalized living materials. To this end, we use a synthetic toggle switch, composed of two mutually inhibitory nodes. Controlled by diffusible molecules it can produce discrete stripes of gene expression. So far, we successfully coupled the curli production with the toggle switch patterning, so that each node creates a specific type of curli fiber and we are currently functionalizing our patterned engineered living biofilm material.

4

Engineering of Synthetic Human Chromosomes

Ravishankar Babu, Patrick Cai

Manchester Institute of Biotechnology, University of Manchester

Since the beginning of late 90's considerable progress has been made to construct human artificial chromosomes (HAC's). However, construction of artificial chromosomes in mammalian cells has been challenging due to the complex structure of the chromosomes, poorly defined alpha satellite DNA comprising of tandem array of repeats around 170 bp which are chromosome specific and acquisition of host sequences due to chromosome truncation. In this study we aim to capture segments of mammalian genome using CATCH (Cas9- assisted targeting of chromosome segment) and introduce into yeast as YAC (yeast artificial chromosome). Once introduced in yeast these YAC's are further edited using CRISPR-Cas9 and integrated back into the mammalian genome. This will enable efficient engineering and construction of human synthetic chromosomes for studying chromosome functions, gene therapy and construction of synthetic metabolic pathways.

Keywords: HAC, YAC, CATCH, CRISPR-Cas9

5

Promoter engineering to create quorum sensing-based genetic circuits

Jasmine De Baets, Brecht De Paepe, Marjan De Mey

Ghent University

The microbial production of new compounds of interest has increased rapidly since the rise of synthetic biology. Both the introduction of new pathways and the tweaking of existing ones allow to regulate the flux towards the end product of interest. However, often growth and production are competing for the same resources. Additionally, the introduced heterologous genes and their corresponding protein product can lead to toxicity for the cells, thereby hampering growth. Therefore, it might be more beneficial to separate the growth and production in time.

The most well-known and straightforward manner to obtain this is by the use of inducible promoters. Unfortunately, the cost and sometimes toxicity of the inducers impede its industrial application. An elegant solution for this can be found by drawing inspiration from natural cell-cell communication systems, such as quorum sensing. In nature, these systems are used to activate certain pathways once a high cell density is obtained, thereby forming the perfect starting point for creating automated switches between growth and production. By constructing different quorum sensing-based genetic circuits in *E. coli*, we aim to expand the current toolbox of quorum sensing-based switches. To achieve these complex genetic circuits, a new quorum sensing-regulated promoter was engineered, requiring two different quorum sensing molecules for its activation.

Poster Session
Day 2 - August 30th, 2023

6

Lessons of the tRNA neochromosome and towards parallelized neochromosome constructions

Alba Ballerini, Daniel Schindler, Jef Boeke, Yizhi Cai

University of Manchester

The Sc2.0 project of the International Synthetic Yeast Consortium aims to design, construct and characterize the first de novo designed synthetic eukaryotic genome. Several chromosomes, each in a single strain, have been synthesized and characterized in recent years. The individual chromosomes are gradually consolidated into a single cell by mating-based procedures. Currently, the most synthetic strain contains 7.5 chromosomes in a single cell. The synthetic yeast project has made several design changes to the sequence, including the restructuring of its genomic architecture by moving all 275 nuclear encoded tRNAs to a de novo designed 17th chromosome, the tRNA neochromosome. Construction and characterization of the tRNA neochromosome have been completed.

Here we present the results of the first tRNA neochromosome. More importantly, we have taken the lessons learned from our first version and designed, constructed, and are in the process of characterizing two additional tRNA neochromosomes. The tRNA neochromosome 2.0 differs from the first version only in the removal of the rox sites - Dre/rox is an orthogonal recombinase system to the Cre/lox system used in synthetic yeast chromosomes to SCRaMbLE the chromosomes. The tRNA neochromosome 2.0 is an important step in the creation of the final Sc2.0 strain to remove tRNA arrays that were integrated into the synthetic chromosomes during the consolidation process. The third version does not cluster tRNAs by their chromosomal order, but by their isoacceptor. By its design, the tRNA neochromosome 3.0 should reduce the loss of tRNAs because recombination should only occur between isoacceptors, resulting in the presence of at least one isoacceptor. To enable rapid and reliable construction of neochromosomes, we developed a new hierarchical assembly workflow called HOMeRuN. This accelerated construction method opens up exciting possibilities for more efficient and streamlined assembly of synthetic chromosomes.

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Synthetic gene circuits combining CRISPRi and CRISPRa in *E. coli*: importance of equal guide RNA binding affinities to avoid context-dependent effects

Içvara Barbier, Astri Kusumawardhani, Lakshya Chauhan, Pradyumna Vinod Harlapur, Mohit Kumar Jolly, Yolanda Schaerli

University of Lausanne

Gene expression control based on CRISPR has emerged as a powerful approach for constructing synthetic gene circuits. While the use of CRISPR interference (CRISPRi) is already well-established in prokaryotic circuits, CRISPR activation (CRISPRa) is less mature and combination of the two in the same circuits is only just emerging. Here, we report that combining CRISPRi with SoxS-based CRISPRa in *Escherichia coli* can lead to context-dependent effects due to different affinities in the formation of CRISPRa and CRISPRi complexes, resulting in loss of predictable behaviour. We show that this effect can be avoided by using the same scaffold guide RNA structure for both complexes.

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Design of a modular gene delivery system for large scale DNA integration in mammalian cells

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Shuttling DNA to cells is a multipurpose requirement ranging from engineering cell lines to expressing recombination proteins. However, delivery of large scale exogenous DNA into mammalian cells for expression has been a bottleneck in scaling up from microbial synthetic biology to mammalian synthetic biology. We, therefore, present the design of a modular gene delivery system. This system includes a gene delivery vector along with other components of a recently developed genome integration tool PASTE (Programmable addition via site specific insertion elements), all of which have been modularized to facilitate easy exchange of parts. The modular version of the components of PASTE system have been constructed using EMMA (Extensive mammalian modular assembly). The latter is a modular cloning system suited for the development of diverse mammalian expression vectors. We thus combined PASTE with EMMA, creating EMMA-PASTE. EMMA PASTE and the modular vector will facilitate easier and cost effective creation of genome integration tools for biotechnological research and industrial purposes. We anticipate that our system will not only bridge the gap of DNA delivery but also aid in easy reproducibility with minimal resources across the scientific community.

9

Engineering a multi-step differentiation program in *Escherichia coli*

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Astri Kusumawardhani, Yolanda Schaerli

University of Lausanne

In nature, complex structures such as tissues, organs and entire organisms are generated from a single cell that contains all essential information for development and differentiation. Despite genetic identity, cells in an organism diverge morphologically and functionally due to various underlying mechanisms such as gene regulatory networks, cell-to-cell communication, stochasticity, mechanical forces, and lateral inhibition. Synthetic biology adopts a bottom-up approach, studying individual cell differentiation building blocks in isolation and then combining them to progressively increase system complexity.

In this study, we combine two building blocks to create a multi-step differentiation program in the model prokaryote *Escherichia coli*. First, we employ a well-characterized genetic module, the toggle switch, to break the homogeneity and stochastically differentiate into two subpopulations: senders and receivers. Next, we enable further differentiation of a subset of receivers that are in close proximity to a sender through cell-to-cell communication, implemented via the quorum sensing system LuxI-LuxR. Finally, we plan to utilize an orthogonal quorum sensing system, LasI-LasR, to engineer a third differentiation step, demonstrating the sequential differentiation of a homogeneous cell population into four subpopulations. The overarching objective is to create a toolbox for engineering robust and reliable differentiation programs in living cells.

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Photocatalytic Enzymes by Computational Design and Directed Evolution

H. Adrian Bunzel, James A. Smith, Thomas A. A. Oliver, Michael R. Jones, Adrian J. Mulholland, J. L. Ross Anderson

ETH Zurich

The global energy crisis challenges us to develop more efficient strategies for the sustainable production of energy. Given the excellent efficiency of natural photosynthetic proteins, biohybrid photovoltaic devices present an attractive solution for solar energy conversion. However, their composition, stability, and complexity can limit their inclusion into photovoltaic devices. Here, we combined computational design and directed evolution to overcome these limitations and create tailor-made photoenzymes [1].

Photo-biocatalysts were designed by introducing photosensitizer binding sites into heme-containing helical bundle proteins. The designed binding sites were specific for the target photosensitizer and readily transplanted into other helical bundles. The best design was highly evolvable and reached nanomolar ligand affinity after mutagenesis and screening. The evolved enzyme generated 2.6 times higher photocurrents than the photosensitizer alone, primarily driven by increased photostability. Evolvability is a unique advantage of our protein-based approach over abiological photovoltaic and will be critical to developing efficient biohybrid systems.

Our work provides a methodological framework for creating highly efficient photoactive enzymes by design and evolution. The transferable nature of our approach will allow sustainably targeting countless other critical transformations such as nitrogen fixation, CO_2 reduction, H_2 production, and water splitting.

1. H. Adrian Bunzel, James A. Smith, Thomas A. A. Oliver, Michael R. Jones, Adrian J. Mulholland, J. L. Ross Anderson: Photovoltaic enzymes by design and evolution. bioRxiv 2022, doi:10.1101/2022.12.20.521207.

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Epigenetic approaches to enhance productivity of biopharmaceuticals

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Monoclonal antibodies (mAbs) constitute 25% of the biopharmaceutical market. High production titres of mAbs in industry are obtained by integrating transgenes into mammalian cell genomes. However, transgene expression can be unstable over long term culture due to epigenetic silencing mechanisms, thus requiring costly and time-intensive stability studies to be carried out. Past solutions to combat silencing have included use of drugs to inhibit histone deacetylases or DNA methyltransferases. This untargeted approach has unpredictable global effects and requires subsequent removal of the drug. As an alternative strategy, I have developed a CRISPR/dCas9 system to directly target epigenetic modifiers to a transgene promoter. Using this system, I have demonstrated that transgene silencing can be achieved by targeting histone deacetylase enzyme to a CMV promoter driving an eGFP reporter gene. Conversely, I have shown that increasing histone acetylation by targeting the histone acetyltransferase p300 to a CMV promoter can significantly increase expression of a mAb transgene, as well as increasing mAb titre and specific productivity. Future work will involve testing these tools at an industrial level with the ultimate aim of making these critical medicines cheaper to produce and thus more accessible.

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Designing feedback controllers for enhanced long-term performance of synthetic gene circuits

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Synthetic gene circuits have applications across a variety of fields from the chemicals industry to biomedicine. However, these engineered systems often exert a “burden” on their host by sequestering cellular resources away from growth towards circuit function. This reduces host growth and therefore evolutionary fitness. As engineered populations grow, error-prone DNA replication introduces mutations into circuit components. Mutant non-functional strains often have higher growth rates and so out-compete their fully functioning ancestral strain. Over time, circuit function is eventually “evolved out”, often within days. This represents a fundamental roadblock to the deployment of engineered microbes where medical therapies or industrial applications require long term performance over a number of weeks.

Here we consider this problem from a systems engineering point of view, where the onset of mutations is considered an uncertainty in the system’s parameters and the resulting growth competition between mutants is an environmental disturbance. Incorporating negative feedback control can enhance a system’s robustness to both parametric uncertainty and external disturbances, making it an attractive strategy to improve the long-term performance of gene circuits. In this work, we develop a multiscale dynamic model of mutation, gene expression, cell growth and population competition to assess the performance improvements achievable by incorporating negative feedback. We demonstrate that whilst negative feedback does improve circuit longevity, the additional burden caused by controller resource consumption can reverse this benefit and in fact reduce long-term performance. We establish a series of simple design guidelines to inform the engineering of negative feedback systems with low resource consumption that can improve evolutionary longevity.

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A novel 101 kb synthetic reversed sequence reveals default chromatin states

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For many eukaryotes, there is evidence that the majority of the genome is transcribed, yet annotated transcripts account for much smaller portions. The basis for this discrepancy is unclear, and it remains uncertain whether excess transcription is functional, or a byproduct of pervasive transcription initiation. Understanding the default state of a genome would help determine whether any observed transcriptional activity is functional. We sought to address this question by introducing a novel 101 kb locus into the genomes of two eukaryotic organisms, *S. cerevisiae* and *M. musculus*, and characterizing its activity. The locus was designed by reversing, but not complementing, the sequence of the human HPRT1 locus, including ~30 kb of both upstream and downstream regulatory regions, allowing retention of sequence features like mononucleotide content and repeat frequency but ablating coding information and transcription factor binding sites. We also compared this reversed locus with a synthetic version of the normal human HPRT1 locus in both organismal contexts. Although neither the synthetic HPRT1 locus nor its reversed version evolved any yeast promoters, we observed widespread activity of both loci in yeast. In contrast, when integrated into the genome of mESCs, the synthetic HPRT1 locus showed transcriptional activity corresponding precisely to the HPRT1 coding sequence, whereas the reverse locus displayed no activity at all, but was repressed by Polycomb machinery. We tested the generalizability of these findings by integrating the reverse locus at a second genomic location, and characterized a version of the reverse locus in which all CpGs have been removed to assess their role in this context. These results show that novel genomic sequences lacking coding information are active in yeast, but repressed in mESCs, indicating a major difference in default genomic states between these two divergent eukaryotes, with implications for understanding pervasive transcription and the birth of new genes.

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Optogenetic control of a synthetic genetic clock

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Genetic clocks keep time within living organisms in order to program periodic behaviour. An intensive genetic analysis has revealed that, from bacteria and fungi to plants and animals, these clocks share similar motifs in the underlying gene regulatory networks. From a reverse perspective, synthetic biologists have tackled the problem of designing from scratch minimal gene networks that can produce periodic patterns of gene expression. The repressilator was the first experimental realisation of a synthetic genetic clock based on a three nodes network of mutually repressing transcription factors. Although the repressilator has been further engineered to show robust oscillations in single cells, phase drifts inevitably lead to damping of oscillations in the population signal.

Here we show that a population of repressilators can be entrained to an external light cue by integrating an optogenetic module in the repressilator. With both experiments and mathematical models we show that entrainment is achieved through a phase response curve that mimics the behaviour of natural circadian rhythms. Fine tuning of the dynamic range of the optogenetic component was essential to preserve limit cycle oscillations in unperturbed cells while ensuring a significant phase response to light. We study a broad range of phenomena that are typical of forced nonlinear oscillators, such as synchronisation, entrainment and detuning. Our results show that optogenetic systems can be integrated into synthetic gene networks to achieve greater control and precision.

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Engineering a symbiont as a biosensor for the honey bee gut environment

Audam Chhun, Silvia Moriano-Gutierrez, Florian Zoppi, Amélie Cabirol, Philipp Engel, Yolanda Schaerli

University of Lausanne, Switzerland

The honey bee is a powerful model system to probe host-gut microbiota interactions, and an important pollinator species for natural ecosystems and for agriculture. While bacterial biosensors can provide critical insight into the complex interplay occurring between a host and its associated microbiota, the lack of methods to non-invasively sample the gut content, and the limited genetic tools to engineer symbionts, have so far hindered their development in honey bees. Here, we built a versatile molecular toolkit to genetically modify symbionts and reported for the first time in the honey bee a technique to sample their feces. We reprogrammed the native bee gut bacterium *Snodgrassella alvi* as a biosensor for IPTG, with engineered cells that stably colonize the gut of honey bees and report exposure to the molecules in a dose-dependent manner through the expression of a fluorescent protein. We showed that fluorescence readout can be measured in the gut tissues or non-invasively in the feces. These tools and techniques will enable rapid building of engineered bacteria to answer fundamental questions in host-gut microbiota research.

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Neuromorphic computing built from synthetic gene circuits

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Biological computers based on gene circuits have emerged as a promising solution for challenges in biotechnology and medicine. However, the limitations of the digital framework, relying on logic gates, hinder its ability to capture dynamic cellular signals and adapt to cell heterogeneity and environmental changes. To address these limitations, we propose a new computing platform that integrates artificial neural network (ANN) design principles into gene circuits in living cells.

Our neuro-inspired gene circuits provide flexibility and enable soft classification, facilitating signal processing with tunable decision boundaries. We introduce a genetically regulated perceptron that combines weighted analog inputs and non-linear functions to implement ANNs in cells. Experimental demonstrations showcase the platform's potential, including programmable logic gates, circuit optimization using AI algorithms, and adaptable data converters for biosensing applications.

Through small molecule induction, we can modify the computational behavior of perceptron circuits, transitioning from OR to AND logic. Additionally, AI algorithms significantly reduce the required number of experiments for circuit optimization. Furthermore, our platform facilitates the construction of a two-bit analog-to-digital converter (ADC) capable of converting a full dynamic range to four distinct output states.

These findings establish the foundation for efficient in silico cell adaptation, enabling the execution of supervised learning algorithms using synthetic gene circuits. Future advancements built upon this platform have the potential to yield autonomous cells capable of self-learning, revolutionizing biotechnology, and medicine.

In conclusion, our study demonstrates the potential of neuro-inspired gene circuits as a computing platform within living cells, bridging the gap between digital and analog circuit design. This research paves the way for scalable, robust, and adaptive biological systems, enhancing capabilities in recognition, adaptation, and decision-making.

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Cellular Surveillance: DNA-Based Recording to Monitor and Memorize Biological Events

Gabrielle David, Raymond T. O'Keefe, Yizhi Cai

University of Manchester

Continuous monitoring of intra- and extracellular environments, ranging from open fields and rivers to within a single cell, is often limited by costly equipment, labor intensity, or invasive techniques. However, it may be possible to develop monitoring devices on the scale of single cells. Novel synthetic biology techniques now make it possible to engineer organisms with refined biorecording capabilities, using DNA as a recording medium on which a cellular "memory" of past events can be written. Through a range of mechanisms including recombinases, CRISPR nucleases, base editing, polymerases, and prime editing, intra- and extracellular events can be recorded along with temporal, spatial, and magnitude information. These devices have the potential to record information about cell lineage, cellular processes, environmental pollutants and disease biomarkers, and even apply this information to actuate downstream cellular operations. Although the realisation of such applications still faces many challenges, further innovation in the realm of synthetic biology is likely to drive DNA-based recording towards this exciting goal.

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Engineering orthogonal transcription for the development of a cross-bacterial, orthogonal and tuneable gene expression toolbox

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Centre for Synthetic Biology - UGent

The ability to engineer predictability into biology is a core aim of synthetic biology. This is especially important in the development of robust microbial cell factories that can function in harsh industrial conditions. In this context, orthogonal expression of the gene of interest can help to relieve the environmental and host-related influences that often result in bioprocess variability, thus solving one of the main bottlenecks of industrial biotechnology.

Although tools for orthogonal gene expression exist, they are currently limited in their host range and often exert significant metabolic burden on the host. This research aims to address these limitations by creating a gene expression tool that utilises the host's native transcription machinery. Building on existing research that shows that the sigma factor from one organism can be used to control orthogonal gene expression in a second host, we aim to develop and characterise a system that regulates transcription initiation in hosts across the bacterial domain.

To this end we demonstrate the use of rational DNA libraries and a highly sensitive screening method to select promoter sequences that do not interact with the host metabolism. This independence from host cellular processes was subsequently confirmed in a number of diverse bacterial organisms. For each of the selected promoters, a cognate transcription initiation factor will be engineered which activates transcription specifically and selectively at this sequence - resulting in a set of orthogonal and tuneable promoter-RNAP cognate pairs that can be utilised to build complex and orthogonal genetic circuits.

Such a genetic toolbox is a key enabling technology in industrial biotechnology, allowing predictable engineering in diverse bacterial hosts, and facilitating the transfer of these genetic circuits between hosts of industrial interest. As well as proving a functional tool, such sigma factor engineering can also provide interesting insights into the fundamental mechanisms of transcription initiation.

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Synthetic biology in Europe: current community landscape and future perspectives

Stefano Donati, Içvara Barbier, Daniela A. García-Soriano, Stefano Grasso, Paola Handal-Marquez, Koray Malci, Louis Marlow, Cauã Westmann, Adam Amara

University of Lausanne

Synthetic biology has captivated scientists' imagination. It promises answers to some of the grand challenges society is facing: worsening climate crisis, insufficient food supplies for ever growing populations, and many persisting infectious and genetic diseases. While many challenges remain unaddressed, after almost two decades since its inception a number of products created by engineered biology are starting to reach the public. European scientists and entrepreneurs have been participating in delivering on the promises of synthetic biology. Associations like the European Synthetic Biology Society (EUSyn-BioS) play a key role in disseminating advances in the field, connecting like-minded people and promoting scientific development. In this perspective article, we review the current landscape of the synthetic biology community in Europe, discussing the state of related academic research and industry. We also discuss how EUSynBioS has helped to build bridges between professionals across the continent.

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Exploiting naturally promiscuous post-translational N-acyltransferases for lipopeptide engineering

Johannes Eckert, Nina M. Bösch, Florian Hubrich, Daniel Richter,
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Peptide therapeutics are prized for their target specificity, bioavailability, and (bio)synthetic accessibility. Typical peptides comprising the 20 standard amino acid building blocks suffer from rapid proteolytic degradation, but several strategies exist to enhance their stability–such as incorporation of unnatural amino acids, conformational constraints, and conjugates. Maturation enzymes from the biosynthesis of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products offer a rich resource for peptide-modifying biocatalysts. Genome mining such pathways led to the discovery of novel enzyme functions: for example, radical-mediated peptide epimerases that irreversibly install D-amino acids, atypical arginases that hydrolyze peptidyl arginine to ornithine, and N-acyltransferases that conjugate diverse fatty acid moieties from endogenous lipid pools to the sidechain of ornithine/lysine residues. Such naturally promiscuous enzymes offer a promising mechanism for diversification of gene-encoded peptide libraries for drug development as the substrate peptide sequence is easily altered by standard codon mutagenesis. The sidechain N-acyltransferases are of particular interest since lipidation is known to fine-tune the bioactivity of peptide therapeutics–increasing serum half-life and in some cases facilitating cell permeability or improving function against membrane targets. We have bioinformatically surveyed the natural diversity of the N-acyltransferases, with biochemical characterization of prioritized lipopeptides through heterologous production to demonstrate C10-C18 fatty acylations. Mutagenesis studies have helped to elucidate the underlying principles of substrate-specificity and demonstrate tolerance to diverse peptide substrates, while maintaining fidelity of the attached fatty acid. Furthermore, natural and artificial lipopeptide pathways are being reconstructed using synthetic biology principals to allow rapid recombineering with additional post-translational enzymes in different bacterial hosts. Insights garnered from these studies will help establish branched lipopeptides as a new source of natural and engineered bioactive agents.

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Emergent digital biocomputation through spatial diffusion and engineered bacteria

Alex J.H. Fedorec, Neythen J. Treloar, Ke Yan Wen, Linda Dekker, Qing Ong, Gabija Jurkeviciute, Enbo Lyu, Jack Rutter, Luca Rosa, Alexey Zaikin, Chris P. Barnes

University College London

Building computationally capable biological systems has long been an aim of synthetic biology, with potential applications ranging from biosafety and environmental testing to health diagnostics and personalised medicine. Here we present work for the design of bio-computers which use spatial patterning to process information. Our computers are composed of bacterial colonies which, inspired by patterning in embryo development, receive information in the form of diffusible morphogen-like signals. A computation is encoded by the physical locations of the sources of morphogen and the output colonies. Inspired by a logic optimisation approach from electronic engineering, we show that we can compute, at least, all 4-input digital logic functions with a set of only four engineered bacteria. Using *Escherichia coli,* we construct 2- and 3-input digital computers, and even demonstrate proof-of-concept diagnostic devices using biosensing inputs. Our approach will open up new ways to perform biological computation, with applications in bioengineering, biomaterials and biosensing. Ultimately, these computational bacterial communities will help us explore information processing in natural biological systems.

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Developing and Optimising a Pipeline for Synthesising and Characterising Promoters

Max Freedman, Yizhi Cai

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Polymerase cycling assembly (PCA) is a polymerase chain reaction variant technique where single stranded sequences called oligonucleotides have an overlapping sequence that can bind together and build into a full sequence. The project aims to make a pipeline for synthesising and characterising multiple longer DNA sequences from oligonucleotide pools. The synthesis is done using polymerase cycling assembly, while characterising is done by fluorescent activated cell sorting and sequencing (FACS-sequencing). The synthesis is optimised throughout the project by using gradients of different variables. The pipeline can be used for developing DNA libraries which can in turn be used to synthesise parts for metabolic engineering. The result is an optimised protocol for producing DNA sequences. The results also include multiple ways the protocol can be improved. The resulting coverage of the DNA sequences indicated the protocol requires significant further optimisation. The need for further optimisation is seen in the 1st oligonucleotide pool which only has a coverage of 82 of 122 intended sequences. Further optimisation would be required for the pipeline to be practical in laboratory use.

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Heterologous pulcherrimin production in *Saccharomyces cerevisiae* confers inhibitory activity on *Botrytis* conidiation

Florian M. Freimoser, Marina Mahler, Mark McCullough, Alexander O. Brachmann, Lukas Nägeli, Maja Hilber-Bodmer, Jörn Piel, Stefan Hoffmann, Yizhi Cai

Agroscope

Pulcherrimin is an iron (III) chelate of pulcherriminic acid that plays a role in antagonistic microbial interactions, iron metabolism and stress responses. Some bacteria and yeasts produce cyclodileucine, which is oxidized by a cytochrome P450 to form pulcherriminic acid. Pulcherrimin production has been engineered and optimized in bacteria, but could not be transferred to *Saccharomyces cerevisiae* in the past. Here, we integrated multiple copies of the PUL1 and PUL2 genes from the pulcherrimin-producing yeast *Metschnikowia pulcherrima* in the *S. cerevisiae* genome to create a eukaryotic, heterologous model to study pulcherrimin metabolism and functions. Multiple integrations of PUL1 and PUL2, at the URA3 locus on chromosome V, resulted in pink and red *S. cerevisiae* colonies, which is indicative of pulcherrimin formation. The coloration correlated positively with the number of PUL1 and PUL2 genes. The presence of pulcherriminic acid and its precursors and degradation products was confirmed by mass spectrometry. In vitro competition assays with the plant pathogenic fungus *Botrytis caroliana* revealed an inhibitory activity on conidiation by the engineered, strong pulcherrimin-producing *S. cerevisiae* strain. The results presented here document that the PUL1 and PUL2 genes from *M. pulcherrima* are sufficient to transfer pulcherrimin production to *S. cerevisiae*. The need for multiple copies of both genes and the slower production, as compared to the native pulcherrimin-producer *M. pulcherrima*, highlight that *S. cerevisiae* is an inefficient producer of pulcherriminic acid. The strains generated here are thus a starting point for engineering and optimising this biosynthetic pathway in the future. The observation of reduced *Botrytis* conidiation in the presence of a pulcherrimin-producing *S. cerevisiae* strain confirms that pulcherrimin plays a role in microbial interactions. The heterologous *S. cerevisiae* model for pulcherrimin production is a novel tool to study the biosynthesis and functions of this molecule at a new level in the future.

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Using synthetic biology approaches to improve the allotopic expression of COX2 in *Saccharomyces cerevisiae*

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Mitochondria are crucial organelles in eukaryotes, particularly producing the bulk of cellular energy. Surprisingly, they still contain a small, bacterial-like genome which has been retained over two billion years of evolution. This additional genome is a puzzling heritage considering both the cost of its maintenance and the obvious risk to store DNA in a ROS-producing organelle. This resulted in a dual-genetic control of the mitochondrial function, which combined with the high mutation rate of the mitochondrial genome (mtDNA), makes mitochondrial diseases among the most frequent genetic diseases (1 in 5000 in adults). Unfortunately, no effective treatment has been discovered yet, but the gene therapy approach appears to be the most promising method to alleviate the deleterious effects of these human afflictions. However, such approach is particularly challenging as it involves the nuclear relocation and expression of a healthy copy of a mitochondrial gene (referred to as allotopic expression) in an attempt to recover the function of the endogenous and mutated mitochondrial counterpart. Even though several studies have demonstrated the potential of allotopic expression to partially recover of the mitochondrial function, improving the allotopic expression system itself has only been scarcely explored. In such a context, we aimed at harnessing the potential of synthetic biology tools to improve the allotopic expression of the COX2 gene using the budding yeast *Saccharomyces cerevisiae*. Particularly, by screening for the best expression conditions (encompassing vector copy number, promoter, MTS or amino acid substitution), we could massively increase the mitochondrial incorporation of the allotopic Cox2p and significantly increase the recovery of the mitochondrial respiration. To conclude, this study brings new insights on the actual limitations of allotopic expression and could possibly help improve gene therapy treatment to cure mtDNA diseases.

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Combinatorial approaches to achieve de novo production of prenylated flavonoids in *Escherichia coli*

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Prenylflavonoids are characterized by the presence of a lipophilic prenyl side-chain in the flavonoid skeleton exhibiting a wide range of bioactivities. They are present only in residual amounts in nature and their extraction is difficult and environmentally unfriendly. Using microorganisms as microbial cell factories is an interesting alternative to produce prenylflavonoids in an efficient and cheaper way. In this work, we designed, constructed, and validated a biosynthetic pathway to produce prenylnaringenins in *Escherichia coli* for the first time. Firstly, tyrosine ammonia-lyase, 4-coumarate-CoA ligase, chalcone synthase and chalcone isomerase were expressed to produce the intermediary naringenin. An optimized *E. coli* strain was able to produce 689.5 mg/L of naringenin. Then, a prenyltransferase (PT) was expressed to produce prenylnaringenins. Four different PTs were first tested in vitro to evaluate their ability to convert naringenin into a prenylated compound. From the tested PTs, two were derived from plants (PT from *Humulus lupulus* (HlPT) and PT from *Sophora flavescens* (SfPT)). Since plant PTs are membrane-bound enzymes, two alternative soluble PTs from fungi (CdpC3PT and AnaPT from *Neosartorya fischeri*) were also tested. All the PTs showed in vitro ability to convert naringenin into a prenylated compound. Then, these PTs were expressed in the previously constructed and optimized *E. coli* strain. In vivo production experiments were carried out to produce prenylnaringenins using only glucose as substrate and it was demonstrated that prenylnaringenins were produced but only in residual amounts. To overcome this limitation, an optimized *E. coli* strain able to produce high amounts of the extender substrates of the pathway (malonyl-CoA and dimethylallyl pyrophosphate) will be constructed sorting to synthetic biology approaches. This work represents a step- forward to achieve for the first time de novo production of prenylflavonoids in *E. coli*.

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Membrane-spanning RNA origami nanopores

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Synthetic membrane nanopores are relevant for synthetic biology and single-molecule sensing alike. DNA nanotechnology has recently provided notable examples of functional synthetic pores. However, the need for chemical functionalization limits their potential because of the high cost of large-scale production and the incompatibility with expression in cells. Here, we realize an RNA origami nanopore which self-assembles into lipid membranes by aptamer-target interactions, circumventing the need for chemical functionalization.

Our structure is composed of eight RNA duplexes which are interconnected by RNA dovetails and arranged around a central channel with a numerical diameter of 8 nm and a length of 27 nm. Each duplex displays a “loop out” which serves as binding sites for extended aptamer sequences, completing the 0.9 kilobase large RNA origami pore complex. To overcome the energy barrier for membrane insertion, we chose lipid membrane-binding aptamers, namely a biotin aptamer, which binds biotinylated DOPE lipids.

The RNA nanopore is produced by in vitro transcription from a DNA template at high yield. We confirm its correct assembly and functional membrane insertion by atomic force microscopy (AFM), and single channel ionic current recordings. The latter reveal discrete insertion steps with a conductance of 0.8 nS (in 150mM NaCl) as well as distinct voltage-dependent closure and gating patterns that resemble those observed in traditional protein pores.

Overall, our work showcases a route towards the large-scale production of nucleic acid nanopores. Genetically encoded RNA origami pores could be expressed in vitro as well as in cells as biophysical tools, components of synthetic cells or single-molecule sensors.

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Genetic Code Expansion

Aimee Haggarty, Yizhi Cai, Anthony Green

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The genetic code has been expanded by redirecting specific codons to encode amino acids beyond the 20 standard amino acids. Current enzyme design and engineering strategies rely predominantly on the twenty natural amino acids, greatly impacting the range of mechanisms that can occur within the active site of an enzyme. Using genetically encoded non-canonical amino acids (ncAAs), biocatalysts can be optimised, improving their stability and activity. Genetic code expansion is well developed in *E. coli* with over 200 ncAAs encoded to date, but is underdeveloped in yeast due to the eukaryotic background which provides additional challenges such as problematic release factor competition and poor transcription of many prokaryotic tRNAs. These limitations can be potentially over- come by the development of Sc2.0. The unique synthetic chromosomal rearrangement and modification by loxP-mediated evolution (SCRaMbLE) function built into Sc2.0 can allow insertions, inversions, deletions and translocations of the genomic background to occur, removing features that are detrimental to GCE. Future synthetic yeasts with a greater number of codons available for ncAA encoding will allow a wide-range of non-natural chemistries to be built into protein-based biomaterials, biotherapeutics and catalysts.

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Switching cost as a main driver of phenotypic heterogeneity in continuous bioprocess

Lucas Henrion, Juan Andres Martinez, Vincent Vandenbroucke, Mathéo Delvenne, Frank Delvigne, Alexander Grünberger

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Isogenic cell populations possess the ability to cope with unpredictable environmental changes by expressing a wide range of phenotypes. Although this adaptation is advantageous in natural settings, it is often undesirable in applications such as bioproduction, synthetic biology, and biomedicine, as it hinders control over the cellular population behavior. However, there is limited knowledge regarding the diversification profiles exhibited by cell populations.

In our study, we focused our analysis on various phenotypes in continuous culture, including carbon source utilization and stress response across multiple model organisms such as bacteria and yeast. Remarkably, our findings revealed a connection between diversification and the associated fitness cost of cell switching. To isolate the influence of the switching cost on population dynamics, we developed a stochastic model that successfully replicated the experimentally observed dynamics. This modeling approach led us to identify three distinct diversification regimes: constrained (at a low switching cost), dispersed (at medium and high switching costs), and bursty (for very high switching costs).

Furthermore, we utilized a cell-machine interface, the Segregostat, to demonstrate the feasibility of exerting different levels of control over these diversification regimes. This is particularly relevant in industrial settings where production load, i.e., fitness cost, exists. By employing this framework, we aim to improve the induction robustness of a system well known for its high production load, *E. coli* BL21 T7, thereby enhancing control and efficiency in bioproduction.

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Synthetic auxotrophy for a non-canonical amino acid in yeast

Stefan A. Hoffmann, David Leeming, Tarryn Miller, Bryan Leland, Yizhi Cai

University of Manchester

Synthetic biology holds immense promise to tackle key problems we are facing, for instance in resource management, environment, and health care. However, comprehensive safety measures need to be developed and put into place for the safe use of genetically engineered microorganisms, especially for open-environment applications. Previously, stringent synthetic auxotrophy for non-canonical amino acids (ncAAs) has been created in an *E. coli* strain devoid of TAG stop codons and release factor 1. The approach leverages genetic code expansion by an orthogonal translation system incorporating ncAAs at TAG stop codons. These stop codons are placed in essential genes such that in the presence of the ncAA enough functional essential protein is being made, whereas its absence results in a truncated non-functional gene product. We have now engineered synthetic auxotrophy in a widely used *S. cerevisiae* strain (BY4742).

We have developed a high-throughput pipeline to identify suitable positions for TAG codon placement in essential genes for creating biocontainment with minimal fitness perturbations. Firstly, we used a protein structure-based approach to predict sites likely to tolerate substitution with bulky residues. We analysed 401 protein structures of yeast essential proteins in silico and of each chose up to 10 positions for experimental assessment. Then, using the Inscripta Onyx platform, these ~4000 thousand intended edits were created as a pooled, barcoded library in yeast with a genomically integrated orthogonal translation system. This library was screened by growing it both with and without the ncAA and sequencing barcodes. We determined barcode frequency changes over time, looking for barcodes depleted from the population without the ncAA but maintained in its presence. The edits associated with the 48 highest scoring barcodes were then individually introduced for a focused screening. Multiple TAG edits have been combined in a single strain to achieve stringent containment while maintaining high fitness.

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Remodelling DNA filaments for bottom-up synthetic biology

Maja Illig, Kevin Jahnke, Marlene Scheffold, Lukas Weise, Jan Kierfeld,
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ZMBH Uni Heidelberg

The control of filamentous cytoskeletal systems is one of the dedicated aims of bottom- up synthetic biology to engineer self-dividing synthetic cells and equip them with mechanical cell-to-cell communication pathways. A molecular engineering approach to achieve specific functionality from the nanoscale to the microscale requires programmability in order to design self-assembly.

This work reinforces how DNA nanotechnology paves the way to create biocompatible nanostructures that can mimic cellular entities. Here, we demonstrate the remodeling of entirely synthetic filaments made from DNA nanotubes: (i) Towards bottom-up synthetic cell division, we can rationally design a ring structure made from bundled filaments. We can control the ring formation by engineering of a synthetic crosslinking peptide and we further constrict the ring diameter by external triggers (Illig & Jahnke et al. Biorxiv 2023). (ii) Towards mechanotactic synthetic cells, a transmembrane signalling pathway enables the reconfiguration of the cytoskeleton made from DNA filaments. The stimulus- induced clustering of transmembrane entities results in mechanical remodeling of the internal DNA cytoskeleton (Jahnke & Illig et al., Advanced Functional Materials 2023).

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A disordered tether to iLID improves photoswitchable protein patterning on model membranes

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Reversible protein patterning on model membranes is crucial to reproduce spatiotemporal protein dynamics in vitro. One of the most commonly used optogenetic tools is the improved light-induced dimer (iLID). However, for iLID the reversion dynamic of its blue light dependent interaction with Nano is decreased when iLID is bound to a surface compared to both interaction partners being in solution. The introduction of a disordered domain to iLID's N-terminus serves as an unstructured elongated linker between the sur- face and the protein while keeping the photosensory domain unaltered. This supposedly facilitates the protein's conformation change upon light dark transitions. The engineered version named disiLID was evaluated in comparison to iLID in solution with competitive fluorescence polarization binding assays, on supported lipid bilayers using quartz crystal microbalance with dissipation monitoring (QCM-D) and confocal fluorescence microscopy, as well as on giant unilamellar vesicles (GUVs) with confocal fluorescence microscopy. We showed that disiLID has improved recruitment of Nano under blue light and better reversibility in the dark. This enables protein patterning on membranes with higher spatiotemporal precision.

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tRNA reassignment in the chloroplast of *Chlamydomonas*

Harry Oliver Jackson, Pawel Mordaka, Alison Smith, Saul Purton

University College London

The universal genetic code forms the basis of life on Earth by providing an informational link between genes and their protein outputs. 64 triplet codons in the DNA encode for 20 amino acids (AA) and three stop commands. It is not clear whether the universal genetic code is the product of early optimisation or a ‘frozen accident’, which would potentially allow for the establishment of other theoretically viable arrangements of the code in a so-called ‘Genome Recoded Organism’ (GRO). We seek to explore rearrangement and expansion of the genetic code by exploiting the streamlined, self-contained genetic system of the chloroplast in the green alga Chlamydomonas reinhardtii, thereby reducing the technical complexities of creating GROs. A critical question at the design stage is whether anticodon changes within a chosen tRNA perturb the specificity of AA charging by its aminoacyl-tRNA synthetase. To explore this, we tested all 26 chloroplast tRNAs in vivo by introducing into the genome a second copy of a tRNA gene where the anticodon has been modified to recognise an unused stop codon (UGA). Correct charging of the tRNAs was tested using a luminescent reporter gene carrying the stop codon. The results of these trials will be presented, along with a suite of genetic engineering tools which use tRNA reassignment to create activation switches.

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Building a Platform for Human tRNA Synthetic Genomics

Joshua S. James, Wei Leong Chew, Patrick Cai

University of Manchester

With over 600 in the human genome, tRNAs have been notoriously difficult to study using traditional techniques due to their abundance and distribution, as well as their size, structure, and array of post-transcriptional modifications. As a result, many questions regarding human tRNA biology remain unanswered.

Synthetic genomics, where genomes are designed and built from the bottom up in order to answer fundamental biological questions, presents a powerful strategy to address this shortcoming. Already we have seen the Sc2.0 Synthetic Yeast Project relocate all tRNA genes to a dedicated tRNA Neochromosome, where integrated features enable high-throughput characterisation. While a synthetic human genome is still a distant milestone, we can begin to leverage advances in DNA synthesis and genome engineering to apply the principles and lessons of Sc2.0 to the human genome.

As such, we have developed a synthetic genomic complementation platform and deployed it to probe human tRNA biology at a previously unobtained resolution. Working by isoacceptor family, we assemble tRNA genes into arrays, before integrating them into the human genome and removing endogenous copies.

This facilitates an ‘investigation via synthesis’ approach, where multiple tRNA variants can be synthesised and tested, depending on the aspect of tRNA biology being addressed. Additionally, we adopt the Cre/LoxP-based SCRaMbLE system from Sc2.0, facilitating high-throughput, recombination-mediated assessment of individual tRNA gene essentiality, and minimum viable tRNA portfolio. Furthermore, an inbuilt translocation function is included, facilitating the investigation of tRNA gene interactions with genomic context and architecture.

Accompanying the platform, we have also developed several additional tools. These include a simple yet powerful mechanism to achieve iterative, site-specific integration of DNA payloads into the genome, and a multiplexed prime-editing knockout and clonal screening pipeline. Overall, our platform represents an innovative and flexible new approach for the investigation and dissection of previously intractable genomic questions.

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A bioelectronic interface that couples engineered bacterial biosensors to a custom-integrated circuit

Amritha Janardanan, Soner Sonmezoglu, Stefano Sonedda, Diana Coroiu, James Flewellen, Michel Maharbiz, Teuta Pilizota

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The bacterial flagellar motor is one of nature's rare molecular machines. Its direction of rotation is regulated by the chemotactic network, which can sense down to nanomolar concentrations of specific chemicals on the time scale of seconds. The motor can thus serve as a biosensor with unprecedented speed and sensitivity. However, at the resolution needed motor speed and rotational direction is currently detected optically, using complex equipment. A step change in harnessing the sensing potential of the motor is to detect its rotation electrically and with high throughput. Here we demonstrate such detection using a custom-designed integrated circuit with micron-sized electrodes.

Our bioelectronic interface is based on a single bacterium with a rotating motor close to a micron-sized electrode, which is then probed with a high-frequency voltage to measure the impedance change due to the rotating flagella. The impedance oscillation we obtain coincides with the speed of rotation seen optically. Several configurations of these electrodes are integrated into an integrated circuit chip roughly the size of one mm^2, with the electronics to amplify and filter the signal with minimal noise. We control the inflow and outflow to the electrode area using a microfluidics channel that can allow the delivery of the analyte. We further demonstrate the capability of the technology for chemical sensing by developing a bacterial biosensing chassis that can sense nanomolar concentrations of rhamnose and send the signal, which is the frequency of rotational direction changes, to the motor.

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Characterizing membrane-binding RNA aptamers for synthetic cell engineering

Tim Karrasch, Erik Poppleton, Alessandra Griffo, Camillo Aponte-Santamaría, Frauke Gräter, Kerstin Göpfrich

Max Planck Institute for Medical Research

RNA exists in an interesting position among biomacromolecules. A single substitution on the sugar imbues it with much wider chemical capabilities than DNA. While RNA can also carry information (mRNA), it can also exhibit regulatory activity (siRNA, riboswitches) or enzymatic activity (rRNA, ribozymes). More than 20 years ago, Michael Yarus' lab demonstrated a surprising capability of RNA: binding and disruption of liquid-ordered phospholipid membranes by choline-binding RNA aptamers [1][2]. These RNA aptamers likely function in complexes; however, the exact structure and mechanism of action of these RNA structures remains unknown.

To expand the synthetic biology toolkit, we are interested in building fully-RNA hardware for use in synthetic cells. Previously, DNA-based membrane active components were developed using chemical functionalization of strands produced using solid-phase synthesis[3]. Instead, we would like to use these lipid-binding aptamers as functional moieties, avoiding costly synthesis and allowing both in vitro and in vivo production.

To engineer with these components, their mechanism of binding and which regions of the structures are key for their activity and which are usable for incorporation into larger DNA- or RNA-based nanodevices must be understood. We present all-atom and coarse-grained MD simulations to characterize the structural landscape of these aptamers and experiments using designed DNA and RNA origami structures to probe the spatial tolerance and binding orientation of aptamer complexes. These structures were characterized with atomic force microscopy and single-molecule ionic current measurements to assess membrane binding and disruption.

References:

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Yeast + Bacteria = Engineered Living Material

Anastasiya Kishkevich, Wolfgang Ott, Will Shaw, Tom Ellis

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Budding yeast *Saccharomyces cerevisiae* are well known to co-exist with bacteria in symbiotic cultures which are used to produce fermented food and beverages. One of the examples is kombucha - fermented drink made from tea by symbiotic co-culture of bacteria from *Acetobacter spp.* and yeast. During fermentation bacteria produce layers of ultrapure cellulose at the liquid-air interface called pellicle. Inspired by this symbiotic relationship, we developed a novel engineered living material where genetically engineered yeast cells are incorporated into the pellicle. We demonstrated that this biomaterial exhibits catalytic activity when yeast are present and secrete enzymes into the pellicle. To advance physical properties of cellulose we are engineering yeast to secrete elastin-like polypeptides (ELPs) with cellulose binding modules (CBMs) which will be incorporated into growing cellulose matrix. ELPs are repetitive biopolymers which highly responsive to environmental stimuli such as changes in temperature or pH. Thus, presence of ELPs in the living material will alter mechanical properties and could make material responsive to environmental factors. We used combinatorial approach based on MoClo Yeast Toolkit to generate a library of fusion proteins with various combinations of promoters, signal sequences and cellulose binding modules. We assessed the levels of secretion via yeast surface display and flow cytometry and identified MoClo parts by sequencing. Combinations of parts with high levels of surface display were then assembled without display protein fusion. Resulted ELP-CBM fusions were successfully secreted into the media. We will next assess ELP-CBM incorporation into the pellicle and test properties of the biomaterial.

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Engineering phagemid-based intercellular communication for distributed computing in *Escherichia coli* consortium

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Building large synthetic genetic circuits or combining multiple circuits in a single bacterial cell is a major challenge as they become too complex and inflict a high metabolic burden on the host cell. Distributing the circuit's function in a multicellular consortium can address this issue. This system will have further potential applications beyond biocomputing, for example, in biomedical therapy and bioprocess technology. In this project, we aim to engineer distributed computing in a consortium of *Escherichia coli*. Our system combines 1.) cascaded CRISPR-interference (CRISPRi) gene regulation to create single and multi-input logic gates and 2.) M13 phages for establishing intercellular communication in *E. coli*. Intercellular communication is achieved by sending M13 phages carrying a single guide RNA (sgRNA) on a M13 phagemid between the donor and receiver cell populations. The donor cells will transmit this sgRNA constitutively or in the presence of chemical inducer. In combination with the catalytically dead Cas9 protein (dCas9), the transmitted sgRNA created transcriptional inhibition or activation of a reporter gene in the intended receiver cell population. Using this system, we constructed six orthogonal NOT gates. Upon receiving M13 phagemid encoding the corresponding sgRNA, the receiver cells successfully inhibit transcription 13- to 25-fold with negligible off-target interactions in more than 95% receiver cell population within 4 hours of co-incubation with the donor cells. Moreover, we have successfully layered NOT gates to build larger circuits, such as YES/buffer gates, NOR gates, OR gates and an AND gate, which also exhibit high degree of robustness and orthogonality.

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Engineering post-transcriptional gene expression circuits in *S. cerevisiae*

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The engineering of synthetic genetic circuitry has primarily relied on the use of transcriptional components such as promoters, transcription factors, and terminators. This project explores an alternative (and complementary) route based on posttranscriptional components including RNA-binding proteins, degrons, aptamers, and aptazymes, with a view to determining whether these might provide advantageous features such as relatively fast response times, reduced noise, or enhanced dynamic range. We initially focused on comparative analysis of posttranscriptional regulatory devices in yeast capable of translational switching. Degron:RNA-binding protein fusions were investigated as a potential route to achieving translational chemical-/light- induced modulation of degrons fused to the bacteriophage MS2 coat RNA-binding protein (MS2CP). By targeting the degron:repressor fusions to MS2 stem-loop (MS2SL) structures within the 5' untranslated region we were able to render target-mRNA translation responsive to the intracellular concentration of the degron:repressor fusion. However, we discovered that establishing translational regulation via a degron-repressor fusion can be an excessively time-consuming process involving adjustments of both the structure of the fusion con- struct and of the abundance of the target mRNA. In marked contrast, we find that RNA aptamers/aptazymes can be utilised in flexible ways to create switching devices as well as logic gates (NOR, NAND) that manifest a number of desirable properties, and which can be combined to beneficial effect with transcriptional components. Quantitative characterization, both at the population and single-cell levels, suggests a number of potential applications of these novel systems.

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Humanized nucleosomes reshape replication initiation and rDNA/nucleolar integrity in yeast

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Eukaryotic DNA wraps around histone octamers forming nucleosomes, which modulate genome function by defining chromatin environments with distinct accessibility. These well-conserved properties allowed “humanization” of the nucleosome core particle (NCP) in Saccharomyces cerevisiae at high fitness costs. Here we studied nucleosome- humanized yeast-genomes to understand how species-specific chromatin affects nuclear organization and function. We found a size increase in human-NCP, linked to shorter free linker DNA, supporting decreased chromatin accessibility. 3-D humanized-genome maps showed increased chromatin compaction and defective centromere clustering, correlated with high chromosomal aneuploidy rate. Site-specific chromatin alterations were associated with lack of initiation of early origins of replication and dysregulation of the ribosomal (rDNA and rRNA) metabolism. This latter led to nucleolar fragmentation and rDNA-array instability, through a non-coding RNA dependent mechanism, leading to its extraordinary, but entirely reversible, intra-chromosomal expansion. Overall, our results reveal species-specific properties of the NCP that define epigenome function across vast evolutionary distances.

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Harnessing Synthetic Gene Circuits for Customized Gene Expression Patterns

James P. B. Lloyd, Florence Ly, Patrick Gong, Jahnvi Pflueger, Tessa Swain, Christian Pflueger, Elliott Fourie, Muhammad Adil Khan, Brendan N. Kidd, Ryan Lister

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Plant carbon fixation, a vital process for capturing energy, profoundly influences various aspects of our lives, including food, clothing fibers, medicines, building materials, and even the production of human therapeutics. However, current plant biotechnology relies on a limited repertoire of genetic parts, restricting the customization of spatiotemporal and conditional gene expression patterns. Synthetic gene circuits have the potential to integrate multiple customizable input signals through a processing unit constructed from biological parts to produce a predictable and programmable output. Presented here is a suite of recombinase-based gene circuits to achieve activation of transgenes in YES, OR and AND gates, repression in NOT, NOR and NAND gates, and both activation and pression in an A NIMPLY B gate. This work demonstrates the successful manipulation of plant gene expression, both in isolated cells and stably transformed multicellular plants, by utilizing specific developmental cues to trigger activation. This highly compact programmable gene circuit platform provides new capabilities for engineering sophisticated transcriptional programs and previously unrealized traits into plants.

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Re-writing the Yeast Genome using Synthetic Gene Clustering and Epigenetic Regulation

Xinyu Lu, William Shaw, Klaudia Ciurkot, Tom Ellis

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Synthetic genomics is a field in synthetic biology aiming to assemble whole genomic DNAs while manipulating the genome content into a customised way. *Saccharomyces cerevisiae* yeast, a model organism with its whole genome sequence determined and well understood, is a good candidate for genome de novo redesign and synthesis. Here, we demonstrate the feasibility of reorganising conditionally essential pathways by synthetic gene clustering and co-regulating the clustered genes to optimise the genome function. Two synthetic chromosome clusters were generated through the genetic relocation of the genes involved in histidine and tryptophan biosynthesis. To achieve effective co- regulation of clustered genes, we engineered an epigenetic master regulation switch to reversibly control native silencing of the targeted synthetic clusters. We also applied the Synthetic Chromosome Rearrangement and Modification by LoxPsym-mediated Evolution (SCRaMbLE) system to induce dynamic changes that can optimise pathway function, while also examining the evolutionary implications of gene layout under specific conditions. These investigations will enable us to get a better understanding of the rules underlying the natural eukaryotic genome organisation and provide new principles for the artificial design, creation and evolution of synthetic modular genomes.

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Deep neural networks for predicting single cell response landscapes and controlling gene expression

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Single-cell gene expression dynamics are increasingly recognized as pivotal in understanding biological processes. These dynamics however are inherently stochastic, making it challenging to identify links between expression and function by simply observing natural fluctuations. This stochasticity also necessitates extensive data to derive statistically robust conclusions. In response to these challenges, we introduce a novel platform for single-cell feedback control of gene expression in *Escherichia coli*, using automated microscopy and optogenetics. Our key innovation is to develop and train a deep neural network to predict the response of the CcaSR optogenetic system rapidly and accurately. This network is then used in a model predictive control framework, enabling us to enforce arbitrary and cell-specific gene expression dynamics on thousands of single cells in parallel and generate complex time-varying patterns. We demonstrate the framework's capabilities by controlling the expression of the *tetA* antibiotic resistance gene, thereby correlating expression to dynamic functional outcomes. We then further investigate the capabilities of our approach by computationally simulating single cell responses, allowing us to easily incorporate different sources of noise and to explore alternative genetic circuit designs. This in silico study reveals that both the size of the training set and the length of past data provided as inputs influence prediction quality. For example cascaded genetic circuits, which introduce delays, necessitated more past data. Finally, our initial attempts to predict a single trajectory for a bistable auto-activation circuit revealed that our network architecture was ill-suited for multimodal dynamics. To overcome this, we updated the network architecture to predict the full distribution of potential cell responses instead, successfully predicting bimodal expression landscapes. In conclusion, we present the first neural network-based model for predicting gene expression and use this to control gene expression experimentally. We also explore potential extensions of this work to alternative experimental setups and genetic systems.

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Computer-Aided Manufacturing for Synthetic Genomics

Mark McCullough, Yizhi Cai

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The ability to synthesize ("write") template-independent DNA is a key enabling technology for engineering biology, expanding the possibilities of researchers to gain fundamental insights into genome structure and function with new-to-nature designs.

However, the prevailing DNA synthesis technologies for the past 40 years are derived from the same chemical approach. DNA synthesis vendors have both common and specific synthesis limitations, which – if present in a customer's order – preclude the biological realization of a design through delays, price premiums or rejection and redesign.

We describe plans and progress in developing software to process a synthetic genomic design into biological reality via an integrated software system. This entails leveraging genetic code redundancy to avoid synthesis constraints in coding regions, interaction with synthesis provider APIs to assess, score and order DNA subunits, and multi-objective optimization of workflows to meet the priorities of specific users.

Genome designs may leave vast chromosomal tracts preserved, whether due to design specifications, preservation of essential features etc. For such intervals, capturing the existing template from the wild-type organism or a prepared genomic library would be more practical than synthesis. Thus, our CAM software will compare and filter intervals based upon similarities between a genome design and its wild-type counterpart, to process specific intervals accordingly via template capture or synthesis.

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Development of high-performance GPCR juxtacrine signalling toolbox for synthetic multicellularity in yeast

Fankang Meng, William Shaw, Keith Kam, Tom Ellis

Imperial College London

Engineering multicellularity opens the door to the creation of complex synthetic tissues and organoids with customised functionality. The development of genetic tools to establish multicellularity in yeast is critical, not only for deepening our understanding of evolution, but also for advancing synthetic biology and facilitating drug discovery. While some cell-cell communication tools based on diffusible signalling molecules have been explored in yeast, there remains a paucity of modular and orthogonal cell-cell juxtacrine signalling and cell-cell specific adhesion systems.

In this study, we developed a high-performance juxtacrine signalling toolbox based on fungal mating peptide/G-protein-coupled receptor (GPCR) pairs in *Saccharomyces cerevisiae*. These orthogonal GPCR juxtacrine signalling switches were applied to induce cell-cell communication and adhesion to create complex communication programmes and patterns within cellular communities. We have also demonstrated the ability of GPCR juxtacrine signalling to act as a genetic sensor for engineering and evolution of protein- protein interactions. The development of this juxtacrine GPCR signalling toolbox here provides a stepping stone towards future efforts in engineering complex multicellularity and its applications in diverse fields.

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Establishing design principles for codon compression in the *Chlamydomonas reinhardtii* chloroplast genome

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A key ambition of synthetic biology is to be able to design and build entire genomes from their constituent parts. Even for bacterial genomes, with an average size of 3 Mbp and a few thousand genes, this is extremely challenging given the complex regulation and as-yet unknown features that ensure integrity of both function and maintenance of a genome. We have been focusing instead on the *Chlamydomonas reinhardtii* chloroplast genome, since its small size (~200 kbp) means it is easier to test a number of fundamental characteristics of genome structure and organisation and transformation is via homologous recombination allowing precise modifications to be made. *C. reinhardtii* can grow heterotrophically in the presence of acetate, thus allowing it to dispense entirely with photosynthesis - and hence also all photosynthetic genes in the chloroplast genome. We have established a synthetic biology workflow that allows us rapidly to delete and insert genes without leaving selection markers and are using this approach to determine what would constitute a viable minimal genome as well as to refactor gene clusters. We have also been able to recode chloroplast genes, reducing the number of codons required. This will form the basis of attempts to rewrite the universal genetic code and introduce non- canonical amino acids by reassigning the unused codons. Ultimately this will enable us to design a completely synthetic chloroplast genome. As well as addressing basic biological questions about what constitutes a functioning genome, our approach will generate novel and naive chassis as production systems for metabolites of interest and new-to-nature proteins.

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Applications of Autonucleolytic Chassis in Microbial and Mammalian Synthetic Biology

Darren N. Nesbeth, Geoffrey Howe, Desmond M. Schofield, Sadfer Ali, Mehtap Bal, Matt Wasmuth, Miguel-Angel Perez-Pardo, Shaukat Ali, Giulia Massaro, Ahad A. Rahim, Milena Rivera, Aminat Omotosho, Daniel Bracewell, Chris Mason, Eli Keshavarz-Moore, John Ward

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The ‘chassis’ concept in synthetic biology is a call to master implementing greater engineering control of the physical and chemical properties of cells. Controlled cellular disassembly is one avenue for chassis design, in which cells self-remove elements of their own componentry. We have designed, built, and tested autonucleolytic microbial and mammalian chassis to remove DNA from either their external environment or their own debris after mechanical disassembly.

For industrially-scaled synthetic biology processes, a major challenge is the presence of impurities such as host cell DNA, proteins, and other cell components. These impurities can compromise the quality, safety, and effectiveness of the final product, and therefore need to be eliminated to meet regulatory requirements. By removing DNA contaminants within the process stream, these autonucleolytic host cells can streamline purification steps, potentially leading to faster process times, less material usage, and overall increased efficiency.

In some bioprocesses, especially those related to the production of therapeutics, host cell DNA can be a safety concern. DNA impurities might lead to adverse reactions in patients or decrease the effectiveness of the drug. Thus, an autonucleolytic host cell can potentially degrade these impurities can increase the safety profile of the drug product.

Reducing host cell DNA to acceptable levels is a regulatory requirement for many biotherapeutics. Autonucleolytic chassis can provide an efficient, reproducible method to achieve these DNA reduction targets, facilitating easier regulatory compliance. The use of such chassis could contribute to the sustainability of synthetic biology. Fewer resources (materials, energy) may be needed for downstream processing and cleanup, and less waste might be generated, contributing to more sustainable and eco-friendly production processes.

We present applications of autonucleolytic chassis in a selection of biomanufacturing contexts: microbial production of antibody-based therapeutics and mammalian-based genetic tools for engineering therapeutic T cells, gene therapy tools and vaccines.

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Genome scrambling technologies to produce high value chemicals in the synthetic yeast Sc2.0

Eloise O'Connor, Patrick Cai, Jason Micklefield

University of Manchester

Natural products (NPs) are specialised metabolites produced by plants and microorganisms which have many valuable properties for the pharmaceutical, cosmetic, textile, and food industry. In this study, we use synthetic biology techniques to engineer the synthetic yeast Sc2.0 for the efficient production of NPs. Sc2.0 is an extensively re-engineered *Saccharomyces cerevisiae* genome with recombination sites integrated throughout the genome which enables the use of the genome rearrangement technology, Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution (SCRaMbLE). SCRaMbLE can be used to generate strains that produce NPs at higher yields and with increased tolerance to their intrinsic properties. Here we demonstrate the use of SCRaMbLE in conjunction with a one-pot combinatorial assembly technique that contains a range of promoters and terminators to create a library of different expression levels for optimising the production of the NP, indigoidine.

Indigoidine is a natural pigment that is an eco-friendly replacement for synthetic dyes in the textile industry. The pigment is produced by a non-ribosomal peptide synthetase (NRPS), a class of proteins that produce other high value NPs including vancomycin, cyclosporin and oxazinomycin. Therefore, from this study we can take the insights on the effect of the genetic elements from the combinatorial assembly and the wider genomic rearrangements from SCRaMbLE and apply them to other, more complex NRPS systems or biosynthetic pathways.

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PLASTICBUSTERS, microplastic capturing biofilms

UNIL iGEM team 2023

University of Lausanne

Microplastics are tiny plastic particles that are less than 5 mm in size. They come from a variety of sources, including plastic bags, bottles, car tires and packaging. Unfortunately, they can be found in many everyday items such as cosmetics, clothing, and even our food. Nowadays, microplastics are contaminating our oceans, rivers, and lakes, and harming aquatic life. Switzerland is the European country with the highest plastic consumption per capita, at 127 kilograms per person. Of all this plastic waste, 14’000 tons end up in soil and water.

Our team’s project aims to trap and extract microplastics found in nature. The main targets of our project are the particles that flow through various water streams into lakes. We aim to capture them with an *E. coli* biofilm to, One of the components of the biofilm’s extracellular matrix is the Curli fibers. To achieve our goal of trapping the microplastics, we will fuse peptides capable of binding specifically to various types of plastic, including PS, PP, and PE, to the Curli fibers.

Once the effectiveness of these engineered biofilms in retaining microplastics has been tested, we would like to find possible applications of our biofilm to real-life situations. Some ideas are, for example, the application of the biofilm inside drains carrying rainwater or inside sewage treatment plants.

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Design of genetic circuits to manipulate the spatial distribution of a particular protein for its enhanced accumulation in bacterial minicells

Junhyeon Park, Juhyun kim

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Microbial-based delivery systems have been developed; but, it is still challenging to use the system effectively due to its immunogenicity and toxicity to the host. To overcome these limitations, we can use bacterial minicells. They are small and anucleate cells that are produced by abnormal cell division. Deletion of genes associated with cell division allows us to create such non-living, non-division, and this results in generating constitute the same structure as the parent cell except for genomic DNA. These characteristics of minicell can reduce toxicity and increase the lifespan and are relatively safer when compared with bacterial cells when we use them as drug delivery systems. To use the system effectively, it is necessary to concentrate target proteins in minicells. To this end, we have developed genetic circuits to manipulate the spatial organization of a particular protein, and we have employed both a small DNA and a gene, Whose products migrate to cell poles. This engineering will serve as a framework to enhance the accumulation of desired proteins in minicells as bio-delivery systems.

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Amino acid uptake rate determines community structure between auxotrophic *E. coli* strains

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University of Lausanne

The microbial world is highly structured. Spatial patterns are produced as members of microbial communities grow and interact. Using a synthetic community of engineered *E. coli* strains, we aim to understand the effect of individual interactions on pattern formation. We use *E. coli* strains passively exchanging amino acids. We follow the spatial organization and abundance of each member of the community on solid media with time-lapse confocal microscopy and flow cytometry. With amino acids present, our members are competing and separate in space. However, without amino acids, the members rely on each other for growth and arrange in a mixed pattern. We observed that one member can form thicker regions isolated from its partner, i.e., the interaction range from its partner is larger. We demonstrate experimentally that the interaction range can be changed by increasing the uptake rate of the amino acids exchanged. Indeed, by over-expressing a specific proline importer, we successfully changed the spatial pattern of the community, balancing the region thickness and increasing the mixing of the community. Our methods and results lay foundations for understanding how metabolic exchanges influence microbial community structures.

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Rapidly assemble genes in your laboratory using automated enzymatic oligo synthesis

Steven D. Quistad, Anne Céline Derrien, Florence Mahé, Xavier Godron

DNA Script

Gene assembly is a central application to a wide range of scientific fields from vaccine development to synthetic biology. Most gene assembly methods utilize chemically synthesized gene fragments as a starting point which must be ordered from a commercial vendor. This legacy service is prone to long delays when sequences are complex or when oligo supply chains are disrupted, resulting in uncertain research timelines.

The SYNTAX system is an automated oligo synthesis platform powered by Enzymatic DNA Synthesis (EDS) technology that can print oligos directly in your lab within a matter of hours. This all-in-one benchtop solution enables researchers to design and assemble 1-2 kb genes in three days compared to waiting weeks using current commercial oligo suppliers.

To demonstrate the utility of the SYNTAX system in gene assembly the 1.7 kb Influenza A hemagglutinin gene was used as a model system. Three double stranded DNA (dsDNA) blocks were generated from EDS-synthesized ssDNA oligos using the PCA approach followed by error correction. The dsDNA blocks were then assembled using a commercially available kit, transformed into BL21 competent cells and colonies were selected for Sanger Sequencing confirmation.

By providing the user with complete control over their oligo synthesis pipeline the SYNTAX system can ensure consistent productivity and accelerate the iterative design-build-test cycle.

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Towards a novel *Vibrio natriegens* chassis for synthetic biology

Lea Ramming, Daniel Stukenberg, María del Carmen Sánchez Olmos, Anke Becker, **Daniel Schindler**

Max Planck Institute for Terrestrial Microbiology

The fastest growing bacterium known today, *Vibrio natriegens*, can replicate with a generation time of less than 10 minutes. It has a bipartite genome with chromosome sizes of 3.2 and 1.9 Mb. *V. natriegens* is a non-pathogenic marine bacterium with similar cultivation conditions like *E. coli*. Recent efforts established several tools and made *V. natriegens* genetically tractable. *V. natriegens* is receiving increasing attention and is becoming a chassis for basic science and biotechnology.

We recently fused the two chromosomes of *V. natriegens* and characterized the resulting strain synSC1.0 (synthetic single chromosome strain v1.0), which contains a single 5.2 Mb sized chromosome. The rationale behind this was to create a chassis strain in which the chromosome maintenance machinery of the second chromosome could serve as a platform for heterologous expression. To our surprise, this strain showed little deviation in growth rate compared to the wild-type. This suggests that the split genome is not a prerequisite for rapid growth, and that DNA replication is not an important growth rate-limiting factor.

Here we present the characterization of *V. natriegens* synSC1.0 and our efforts towards the application of expression platforms derived from the second chromosome controlled by the endogenous machinery.

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Site-Specific, Bioorthogonal Protein Labeling by Tetrazine Ligation using Endogenous β-Amino Acid Dienophiles Derived from Natural Product Biosynthesis

Daniel Richter, Edgars Lakis, Sarolt Magyari, Thomas A. Scott, Anna L. Vagstad, Jörn Piel

ETH Zurich

Recent advancements in bioorthogonal chemistry have enabled the study of proteins inside living cells. Selective modification of a specific protein in the complex mixture of proteins, macromolecules and other metabolites in living cells allows visualization and modification of proteins involved in diseases. Derivatization with fluorescent dyes enables tracking proteins of. Intracellular labeling enables investigation of proteins involved in disease and subsequent modification to cause physiological changes.

However, bioorthogonal reactions pose a few challenging requirements to be met. Reactions need to proceed selectively, fast, and in aqueous environments. Common approaches today use unnatural amino acids installed in proteins by amber codon suppression or enzymatic ligations. These approaches can suffer from low yields, metabolic changes and interferences in natural pathways which is why novel approaches are needed. Here we leveraged an enzymatic reaction naturally occurring in the biosynthesis of ribosomally synthesized and post-translationally modified peptides (RiPPs) for use in bioorthogonal labelling of proteins. By utilizing the excision of tyramine from protein backbones by a radical S -adenosyl methionine (rSAM) enzyme, we have incorporated a bioorthogonal aminopyruvate residue in ribosomally produced proteins. This building block does not naturally occur in common model organisms and reacts with commercially available tetrazine derivatives in a fast and selective manner. We have characterized and studied this reaction and shown that the reaction proceeds in aqueous media and intracellularly in *Escherichia coli* (*E. coli*). We have demonstrated the utility of this method by producing and purifying a Her2-binding Affibody, which we characterized to be fully functional after conjugation. Additionally, we have labeled the bacterial cell division protein FtsZ with a fluorescent dye to investigate the localization of the protein in *E. coli*. We anticipate that the method will be useful for future in vivo studies of cellular proteins and as a new method for the engineering of protein conjugates.

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Facilitating *E. coli* Delivery of Xenomolecules (FEDEX): Development of Novel Transport Systems for the Uptake of New-to-Nature Molecules

Emilio Rodriguez-Robles, David Müller, Tilmann Kuenzl, Tania Roberts, Philippe Marliere, Sven Panke

ETH Zurich

A long-standing goal in the fields of synthetic biology and xenobiology is to incorporate new-to-nature molecules into the biochemical processes of living organisms. New- to-nature molecules can be used in a number of ways, including expanding the cellular capabilities in terms of production of biomolecules or modifying the fundamental properties of bacteria. However, one of the major obstacles when attempting to incorporate these molecules into bacterial metabolism is ensuring their intracellular availability in the cytoplasm. To overcome this, I am working on the development of synthetic uptake systems in *Escherichia coli* following the strategy of “portage transport”. This involves chemically attaching the new-to-nature molecule (cargo) to a natural molecule (vector) that drives the uptake because the vector is specifically recognized by a bacterial transporter.

In my work, I focus on the development of two distinct uptake systems based on two different vector molecules. To ensure the complete functionality of these systems, one of the primary goals is to identify or engineer promiscuous inner membrane transporters through directed evolution. This process aims to enhance the transporters’ ability to import any cargo-vector molecule. To achieve this, I have established metabolic selection schemes for each vector molecule, wherein the growth of *E. coli* depends on the successful import of any cargo-vector construct.

This approach has enabled us to successfully import a previously impermeant non-canonical amino acid into *E. coli*. Currently, we are utilizing this method in directed evolution campaigns to improve the uptake capabilities of transporters for new-to-nature molecules of interest.

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Synthetic Mammalian-Bacterial Protein-Protein Communication

Roee Samuel, Ramez Daniel

Technion

Currently, synthetic biology most often incorporates genetic circuits that take effect within a single cell - the cell detects changes in the internal composition and generates an output. In recent years a few genetic circuits started involving communication between cells. However, these genetic circuits either involve direct communication between two cells of the same type (e.g. mammalian cells with mammalian cells, bacteria with bacteria) or indirect communication between two different types of cells (e.g. bacteria releasing immune modulators to diffuse and be picked up by immune cells, mammalian cells releasing bacterial quorum-sensing molecules to the environment to modulate bacterial growth). Here, we present a system allowing direct protein-protein communication between bacteria and mammalian cells. Unlike indirect forms of communication, direct communication in the form of protein-protein interaction requires the physical presence of both the sender and the receiver of the signal. Thus, this form of communication provides a more precise form of communication - unlike indirect communication which can result in communication with cells outside the immediate microenvironment of the cell, direct communication can only affect cells within the immediate surrounding of the cell. This flexible well-regulated platform based on efficient prokaryote-eukaryote interaction provides a new toolbox to program host cells interacting with their environments.

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Expanding the Genetic Code of Synthetic Yeast

James Sanders, Stefan Hoffmann, Anthony Green, Patrick Cai

University of Manchester

The twenty canonical amino acids that form nature's chemical toolbox establish the possibilities of protein-based chemical interactions within the cell. Expanding these fundamental building blocks to encompass non-canonical amino acids with novel side chains unlocks an extraordinary space for biological design with applications in designer enzymes, biomaterials, biotherapeutics and biocontainment.

This project leverages the synthetic yeast genome - Sc2.0 - to uncover genomic backgrounds that facilitate enhanced translation of new amino acid chemistries. Using the highly plastic genome of Sc2.0, we are able to generate massive structural variation by exploiting the globally embedded loxP sites that allow Cre recombinase to create insertions, inversions, deletions, and translocations of chromosomal segments. From this diversity, we propose to uncover fundamental knowledge on the complexity of translation as well as new insights into engineering genetically re-encoded organisms.

So far in this project, we have found a group of pyrrolysine orthogonal translation systems that are highly active in yeast. Pyrrolysine synthetase-tRNA pairings have been explored extensively in *Escherichia coli* due their flexible active site and high orthogonality to endogenous components. We have further shown that these enzymes are capable of accepting active site transplants evolved in *E. coli* while maintaining high activity in yeast - opening up a toolbox of amino acids for the organism from existing research.

Building upon this work, we are now utilising the unique functionality of Sc2.0 in conjunction with the most active pyrrolysyl system to further improve performance through changes to the genetic background evolved with the SCRaMbLE function. We envision that this will provide fresh insight into the adaptability of the genetic code that will excel genetic code expansion research in all eukaryotes.

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SCRaMbLEing synthetic yeast genomes for incipient speciation

Leandro Vieira dos Santos, Patrick Yizhi Cai

University of Manchester

The Sc2.0 project is a groundbreaking initiative in synthetic biology that aims to redesign and construct a fully synthetic version of the eukaryotic model *Saccharomyces cerevisiae*. The global Sc2.0 consortium lead the chromosome synthesis, assembly, and debugging of each 16 synthetic chromosomes, introducing different key design features into the genome structure. One main feature of the custom designed version is SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution), a powerful evolutionary tool to induce massive and stochastic rearrangements in synthetic chromosomes, creating recombinant versions in genome content and architecture. SCRaMbLE had been extensively validated as a tool for diverse biotechnological applications, creating strains more tolerant or producing higher titers of the desired product. In this project, we aim to use the programmed ability to rearrange genomes in Sc2.0 to investigate how genomic divergence leads to hybrid dysfunction and incipient speciation, while precisely mapping the genetic divergence boundary that delineates species. For this purpose, we create a synthetic platform to induce and select speciation events in a high- throughput pipeline. The HTS protocol is based on four systems: i. an HTS selection system to identify pre-zygotic reproductive isolation (RI) events in SCRaMbLED cells; ii. a flow cytometry-based system to quantify hybridization and measure incipient RI; iii. an inducible system for self-compatibility coupled with flow cytometry-based ploidy determination; iv. a haploid selection system to propagate individual matings separately for post-zygotic RI events. All four systems were successfully integrated into the synthetic genome using CRISPR/Cas, creating a platform cell to investigate inducible speciation. Whole genome sequencing and Hi-C will be used to identify genomic patterns associated with hybrid dysfunction and distinguish between different models of speciation. Based on the genomic divergence of SCRaMbLEd cells, we expect to uncover the mechanistic basis for recombination-mediated evolutionary events in hybrid yeasts at the boundary of speciation.

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Paving the way for the construction of an autotrophic *Escherichia coli* strain

Marion Schulz, Valérie Delmas, Mélodie Cadillon, Anne Berger, Ivan Dubois, Madeleine Bouzon, Volker Döring

L2BMS/CEA/DRF/Institut de Biologie François Jacob/Genoscope/UMR8030

Natural auxotrophic microorganisms convert CO_2 into biomass using energy from light or the oxidation of inorganic molecules. When expressing biosynthetic pathways for the production of commodity chemicals or fuels, they offer the possibility to industrially valorize CO_2. However, these organisms often lack genetic malleability and are limited in product range. Current knowledge of bacterial metabolism and various genome modification techniques make the implementation of an autotrophic metabolic regime in a heterotrophic background feasible. *Escherichia coli*, a facultative anaerobic heterotroph, is a versatile organism with a flexible metabolic network and proven qualities as a biotechnological platform organism, thus being a preferred target for such adaptation.

The project is based on the usage of *E. coli* strains that rely on the supply of formate for growth. NAD-dependent formate dehydrogenases (FDH) capable of the efficient conversion of CO_2 to formate are structurally complex and operate with metal centers often entailing O_2 toxicity. Recently, high in vitro reduction of CO_2 to formate upon oxidation of NADH in the presence of O_2 was reported for the Mo-dependent complex and soluble FDH from *Cupriavidus necator* (CnFDH).

In this project, we focused on the functional in vivo expression of CnFDH in a NADH-auxotrophic *E. coli* strain, lacking the lpd gene specifying lipoamide dehydrogenase. In this genetic context, NAD-dependent oxidation of formate to CO_2 can be selected, using acetate as sole carbon source. We were able to demonstrate the in vivo activity of the CnFDH-operon expressed either from a plasmid or from the operon integrated in the host chromosome. Experiments using automated continuous culture in selective conditions (GM3 technology) enabled us to select evolved descendants growing on formate as sole energy source with a generation time half as long as the original strain. This first step opens the possibility to harness the high biotechnological potential of this enzyme.

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A versatile strategy for countering mutation spread in engineered cell populations by directly sensing gene expression burden

Kirill Sechkar, Harrison Steel

University of Oxford

Mutation spread in engineered cell populations is a key challenge in synthetic biology. Competition for the host cell's gene expression resources exerted by synthetic genes on the host's native genes, known as burden, impairs cell viability. Therefore, loss of synthetic gene expression due to DNA mutation can give a cell a growth advantage, hence mutants eventually displacing engineered cells in the population. Mutation spread can be countered by coupling synthetic gene expression to survival, such as making the cell reliant on a metabolite produced by heterologous proteins or overlapping a synthetic gene's DNA sequence with that of a vitally important gene. However, due to their nature, these approaches must be tailored for each particular synthetic gene circuit to be retained.

In contrast, we propose a versatile design that prevents mutation spread regardless of the synthetic genes' identity by sensing burden directly. Leveraging the experimentally documented dependence of self-activating genes' behaviour on burden, our design disables the host cell's growth when synthetic gene expression loss brings burden below a specified threshold.

Using a coarse-grained resource-aware bacterial cell model, we study our circuit's switching behaviour, demonstrating how its burden threshold can be tuned simply by changing an inducer's concentration in the culture medium. Without re-engineering its genetic components, the same design can therefore favour the retainment of various synthetic circuits burdening the cell to different extents.

We present a simulated scenario where our design successfully reduces the cell's viability upon synthetic gene expression loss even when other approaches fail. Finally, we simulate our circuit's effect on a population level, showing how it slows mutation spread in a turbidostat.

Easily adaptable and not tied to particular genes or genetic modules, our generalised burden-sensing approach may expand the range of synthetic biology applications benefitting from increased robustness to mutations.

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Compartmentalising terpenoid biosynthesis in yeast mitochondria

Austin Semple, Kewin Gombeau, Yizhi Cai

University of Manchester

The growing threat of climate change has encouraged efforts to more sustainably pro- duce a broad range of resources used in different industries. A large class of natural plant secondary metabolites named terpenoids are among the chemicals for which improved methods of production are sought. Due to these compounds having large-scale demand in industries involved in flavour, fragrance, and therapeutic drugs, their engineered biosynthesis in microorganisms is seen as a better alternative to direct extraction from plants or synthesis by organic chemistry. As bioengineering approaches still require optimisation, many methods are being tested to improve metabolic flux to ultimately yield microbial strains which can meet industrial demand. Previously, production of the terpenoid squalene has been improved through compartmentalisation of some pathway components in mitochondria. Here, we harness mitochondrial targeting pre-sequences to localise pathway enzymes required for β-carotene and indigoidine biosynthesis, to *Saccharomyces cerevisiae* mitochondria. The compact compartmentalisation of reactants and high concentrations of reaction precursors and cofactors in this organelle make it a desirable hub for production, with the caveat of potential limitations to metabolic function. Through a growth assay and appraisal of pigment coloration, it could be concluded that targeting each of the terpenoid pathways to mitochondria did not improve overall production of the pigments. However, further analyses must be carried out to gain insights into the cause of this, as the sub-organellar localisation of such reactions has potential to impact the mitochondria-localised functions and be a burden for cells.

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Using Deep Learning to Generate Synthetic Proteins with Reduced Immunogenicity

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University of Toronto

Adenoviruses are the preferred delivery system for synthetic gene circuits as they carry a relatively high payload of 38kb. However, environmental exposure means that most people exhibit potent pre-existing immune responses to many adenovirus serotypes. Neutralising antibodies also preclude repeated administration with the same serotype. Progress toward addressing immunogenicity has been very limited using conventional methods (deploying rare serotypes, serotypes targeting other species, and capsid engineering) and a new approach is urgently needed. Deep generative modelling could potentially be used generate synthetic viral vector serotypes without epitopes for pre-existing neutralizing antibodies. However, to date, generative models for protein design have focused on small proteins with lots of training data, meaning they are often not suitable for generating proteins with the most potential for high clinical impact - including adenoviral hexons.

To address this, we developed a compact variational autoencoder, with only 12.4 million parameters, that could be efficiently trained on the limited natural sequences. In contrast to the current state-of-the-art, the model was able to generate high-quality adenovirus hexon sequences that were folded with strong confidence by Alphafold2 to produce structures essentially identical to natural hexons. Molecular dynamics simulations confirmed structure stability and protein- protein interface integrity. ProteinVAE can be used to generate a broad range of synthetic adenovirus serotype sequences without epitopes for pre-existing neutralizing antibodies. It could be used more broadly to generate different types of viral vector (e.g., AAV), and other therapeutically interesting proteins.

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Battle of the Barriers: Preventing Transgene Silencing with Insulator Elements

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Therapeutic recombinant proteins are a principle product of the biopharmaceutical industry, 60-70% of which are produced by integrating transgenes into the genomes of mammalian cells. These systems often produce highly variable and often unstable transgene expression patterns over prolonged culture. This is largely due to epigenetic mechanisms silencing the recombinant protein transgene over time. To combat this problem, vectors have been engineered to include genetic elements or barriers that protect against silencing, thereby improving recombinant protein production. Many endogenous barriers contain tRNA genes (tDNAs), examples of which have been shown to block the spread of epigenetic silencing activities.

I have assessed the ability of bioinformatically predicted tDNA barriers to reduce transgene silencing in an industrially relevant way using an established eGFP reporter assay. Here, CHO-K1 cells were transfected with constructs containing an eGFP gene flanked by a multitude of different barrier elements, and eGFP expression was monitored over 10 weeks by flow cytometry. My results demonstrate that a subset of tDNA clusters can prevent expression instability over a period of 10 weeks to a greater extent than the current industry gold standard barrier, UCOE. Further, I have shown that the UCOE and tDNA barriers can be combined to produce an optimised vector that promotes enhanced and sustained transgene expression. If these results transfer to a more industrially relevant setting, tDNA barriers could improve biomanufacturing stability significantly.

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Could Synthetic Biology Create a Second Origin of Life?

Stoyan K Smoukov

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From artificial cells to molecular motors, we have worked on minimal systems with few components where we could understand all the chemistry and physics. While far from the complexity of RNA or myosin contracting our muscles, we have been able to generate artificial morphogenesis and rechargeable swimmers from just two chemicals in addition to water. The oil we use provides compartmentalization and the surfactant is similar to half a membrane bilayer. The synthetic control is amenable to polymerization and further interpenetration for multi-functionality, [1] and novel supercapacitor architectures, [2] including moving to actuating and self-sensing materials, [3]. The process of artificial morphogenesis uses single molecules and phase transitions to shape emulsion droplets [4], and we have already grown desired polymer shapes bottom-up.[5] Thermodynamic, mathematical,[6] and molecular dynamic modelling[7] allow us to start making predictions for highly non-intuitive processes. Non-living systems we designed can harness temperature fluctuations to raise their own internal energy and show spontaneous droplet division.[8] We also grow millions of microscopic swimming robots, virtually for free, by cooling emulsion droplets that transform into elastic swimmers and a novel type of active matter.[9] In this meeting I'd like to explore with colleagues how bottom-up Synthetic Biology could help bring about a new origin of life from minimal abiotic components, its potential benefits, and any need for containment.[10]

Prof. Smoukov (>4000 citations, H-index = 35) researches phase transitions in confinement, fundamentals of minimal synthetic life, and autonomous material robotics. References:

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Investigating the role of sponge RNAs in synthetic and systems biology

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Synthetic biology holds the potential to revolutionise life science research and the bioeconomy, but challenges such as host burden, crosstalk, evolutionary instability, and a lack of robustness in designed biological systems limit its impact. One solution addressing some of these challenges involves designing negative feedback control circuits. While building such circuits using small RNAs (sRNAs) ensures they have minimal burden on the host cell.

Sponge RNAs, a class of sRNAs that regulate other sRNAs instead of mRNAs, play a key role in gene regulatory networks across various bacterial species where they regulate processes including metabolism, stress response, and global gene regulation. Despite their apparent importance, sponge RNAs' benefits to gene regulatory networks and the mechanisms by which they operate remain underexplored. As a result, sponge RNAs have to date not been used in synthetic gene circuits.

Our initial work has focused on characterising genetic parts; designing and validating novel synthetic sRNAs and cognate synthetic sponge RNAs; and mathematical modelling. We expand upon previous synthetic sRNA research, which largely uses the MicC sRNA scaffold, by developing a synthetic sRNA based on the natural ChiX sRNA scaffold. After validating our synthetic sRNA, we designed several synthetic sponge RNAs based on the natural ChiZ and ChbBC sponge RNAs and validated these by using a simple synthetic gene circuit. Additional work has focused on using mathematical modelling to guide future synthetic gene circuit design.

Future work will use our simple synthetic gene circuit to interrogate the role that sponge RNAs play in bacterial gene regulation. This work and mathematical modelling will be used to inform the design-build-test-learn process for developing novel synthetic gene circuits that use sponge RNAs. This work ultimately aims to expand the synthetic biology toolbox enabling synthetic biologists to tackle some of the outstanding challenges in the field.

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Photovoltaic enzymes by design and evolution

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Growing global energy demands and pressing needs for sustainable energy solutions call for a reassessment of the ways we generate energy. Solar energy has emerged as a promising candidate. Nevertheless, efficient conversion of solar energy into electrical energy remains limited, as commercial silicon solar cells only reach solar efficiencies of up to 19%. Here, we follow a novel alternative approach to harness solar energy by computationally designing de novo heme proteins capable of binding photoactive dye molecules, and improving their photoefficiency through directed evolution [1]. Design is aimed at establishing an electron transport chain within the protein to transfer exited electrons from the dye to heme. Subsequently, the protein facilitates the injection of the electron into the electrode of a photovoltaic cell to increase the generated photocurrents.

In this project, the heme-binding protein is used as a foundation to generate photoenzymes with enhanced activity by: (1) introducing charged tags to locate the protein to the photanode in the solar cell, (2) designing binding sites for different photosensitizer ligands to broaden the spectrum of the absorbed light, (3) including binding sites for electron donor and acceptor ligands to improve the efficiency of the electron transport chain, and (4) applying directed evolution to improve photoefficiency. This strategic approach allows us to tailor the protein's properties and create a system for efficient electron transport, while also gaining insight into the fundamental origins of photocatalysis.

The integration of synthetic biology methods established in this project will enable overcoming current limitations in solar energy conversion, and may be extended to other redox-driven challenges such as H_2 production or CO_2 fixation.

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Exploiting the Biochemical Potential of Marine Invertebrate-Associated *Acidobacteria*

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To combat pressing medical threats such as cancer or infectious diseases, the discovery of novel drug leads supplying the pharmaceutical development pipeline is crucial. A major source of such bioactive molecules has been natural products, wherein bacterial symbionts of marine invertebrates have been identified as largely untapped producers of unprecedented chemical entities. Historically, natural products were identified through cultivation of their producer. However, most bacteria, including symbionts of marine invertebrates, remain uncultivated. Advances in metagenome sequencing have helped to link metabolites isolated from hosts to the underlying biosynthetic gene cluster (BGC) in the actual symbiotic producer. For some invertebrate-derived substances with significant pharmaceutical potential, the producers and BGCs remain elusive. One approach to make these compounds accessible without relying on laborious total synthesis is to identify alternative, cultivated producers.

Here, we used a global bioinformatic strategy for trans-AT polyketide synthase analysis and prediction to identify a biosynthetically rich acidobacterial taxon with a remarkably “sponge-like” metabolic profile. To date, the widely distributed phylum *Acidobacteria* is poorly characterized, lacks identified natural products, and contains few cultivated members, among them *Sulfidibacter corallicola* and *Acanthopleuribacter pedis*, both harboring around 40 BGCs. To verify the predicted acidobacterial chemistry, we characterized from *A. pedis* new congeners of the phorboxazoles and calyculins previously only known from marine sponges. In addition, we genomically predicted the structure for another polyketide almost identical to phorboxazoles in *S. corallicola*. Preliminary LC-MS and NMR data suggest that the predicted compound is indeed produced under laboratory conditions. Cultivation of alternative hosts can thus improve the sustainable sourcing of marine natural products.

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Sc3.0: A novel approach to minimize the synthetic yeast genome

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Recent advances in synthetic genomics now allow the synthesis of organisms' entire genomes. The most extensively altered genome built to date is the ongoing Sc2.0 project, aiming to create a synthetic designer *S. cerevisiae*. One of the key features of the Sc2.0 project is the insertion of symmetrical loxP sites downstream of every non-essential gene. Upon Cre recombinase induction Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution (SCRaMbLE) takes place. SCRaMbLE shuffles the synthetic genome, evolving the synthetic yeast strains toward a desired phenotype by the creation of large genotype libraries. We have shown that SCRaMbLE can minimise the synthetic yeast genome. However, SCRaMbLE cannot delete sequences located within essential rafts, loxPSym flanked units harbouring an essential gene due to essentiality restrictions. Supplementing the Sc2.0 strain with a Neochromosome housing essential genes would increase the deletion power of SCRaMbLE, facilitating further compaction of the genome. Here, we describe the construction and characterisation of three versions of the essential Neochromosomes harbouring the essential genes of chromosome III (eNeo). The activity of the orthogonal promoters was comparable to the native expression levels of *S. cerevisiae*'s promoter counterparts. In addition, the function of the essential genes driven by orthogonal regulation proves to be functional and compensates for the absence of the native essential gene in the SynIII strain. Nevertheless, we developed a new SCRaMbLE reporter: the Elementary Random integration of the loxPSym-URA3-loxPSym cassette (ERICA), which enables random integration in the synthetic chromosome. The genome rearrangement events were deconvoluted via nanopore sequencing. Introducing the eNeo in the SynIII strain reduces the size of chromosome III effectively, not only by deleting non-essential genes but also by allowing more chromosomal sequences within the essential raft to be eliminated via SCRaMbLE. We anticipate combing SCRaMbLE with eNeo will be an invaluable approach to minimising the synthetic genome.

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Module selection for designing synthetic circuits

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Rationally engineering gene circuits with the objective of obtaining a novel behavior (ie not realized in nature) is a challenging task. Employing dynamic mechanistic mathematical modeling and utilizing existing gene circuit design methods already improves the situation by identifying synthetic gene circuits (represented as mathematical models) that yield the desired behavior equally well (in terms of criteria such as robustness, feasibility or other optimality metrics). Yet which candidate synthetic gene circuit should one build? Going a step back and designing effective and informative experiments minimizes time and cost of the Design-Build-Test-Learn cycle. We propose a model-based sequential experiment design method based on building smaller informative 'modules'. In essence, a module is a sub-circuit whose experimental characterization provides information about the feasibility of using specific parts and interconnections to construct and discriminate within the set of candidate synthetic circuits. By employing these modules as in-silico experiments, we can make predictions regarding which one is expected to yield the highest information gain for selecting the candidate synthetic circuits. We illustrate our method with two examples concerning the design of simplified protein accumulation synthetic circuits and realistic stripe forming synthetic circuits.

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RNA origami-based hardware for synthetic cells

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DNA nanotechnology enabled the design structural and in part, functional mimics of proteins. However, functional DNA structures often require thermal annealing and chemical modifications, which is incompatible with production in synthetic cells. In this regard, co-transcriptional folding of RNA origami could represent a new and thus far unexplored frontier for the making of custom-made molecular hardware in synthetic cells. Here, we produce RNA origami by co-transcriptonal folding from a DNA template inside of giant unilamellar lipid vesicles (GUVs) for the first time. We regulate the on-demand production with external triggers namely ions and rNTPs, transported into the GUV via ionophores or membrane pores, respectively. With the goal of making cytoskeleton-like functional components, we design the first 3D RNA nanotubes from self-assembled ssRNA tiles and successfully transcribe them inside GUVs. With sequence modifications on the DNA template, we control the length, reaching several micrometers, and the persistence length of the RNA nanotubes and achieve the formation of closed rings. Coarse-grained simulations of the ssRNA tiles and their assemblies confirm the experimental data. Showcasing the unique possibilities of RNA origami, we induce RNA cortex formation in GUVs by introducing membrane-binding aptamers, circumventing the need for chemical functionalization. Altogether, this work pioneers the use of RNA-based hardware in synthetic cells.

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Implementation of an orthogonal cysteine translation initiation system in *E. coli*

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The use of genetically engineered microorganisms (GEMs) is rising in various sectors such as medicine, agriculture, and the environment (Brooks and Alper, 2021). While these organisms possess significant potential in bioproduction, their deployment raises safety concerns for human and environmental health (Stirling and Silver, 2020). This risk of genetic pollution should certainly not be neglected and the demand for “safe GEMs” is a real concern. Therefore, technologies to avoid or restrict genetic communication between natural species and synthetic biodiversity, and to prevent GEMs from propagating and surviving in undesired environments, are necessary for the progress of science and industry, and must be conceptualized and deployed to anticipate this risk.

We have engineered an orthogonal, cysteine-initiated translation system by repurposing the initiator tRNA (fmet-tRNAi) to cysteine (cys-tRNAj). This alteration renders translation initiation of certain proteins absolutely dependent on the presence and functionality of the cys-tRNAj, ensuring that target proteins are expressed under strictly controlled conditions. We have demonstrated the ability of the cys-tRNAj to initiate translation from ΔMet Cys templates using several reporters. We further modified the cys-tRNAj through directed and targeted mutagenesis and evaluated the functionality and efficiency of each mutant, providing insight into the sequence-function relationships of fmet-tRNAi. The highest-performing mutants were selected for system optimization. System improvement was achieved by overexpression of cysteine-tRNA ligase and the replacement of wild-type translation components with heterologous genes, reducing cross-reactivity and increasing efficiency and specificity. The integration of non-canonical bases into the cys-tRNAj and the establishment of system inducibility are envisaged for future improvements.

Taken together, this technology opens up exciting possibilities for the precise control of gene expression in *E. coli* for bioproduction and biocontainment (when targeting an essential gene). It propels both large-scale industrial bioproduction and our understanding of translation fundamentals.

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Restructuring the chloroplast genome of the non-vascular plant, *Marchantia polymorpha*

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Over the last several decades, plants have been developed as a platform to produce useful recombinant proteins, where the plastid offers many advantages for production of foreign proteins. Plastids can promote very high levels of gene expression due to the presence of high numbers of genome copies in the cell and lack of gene silencing. To fully exploit this potential, chloroplast biologists across the world have been tapping the chloroplast genome as a medical bioreactor for therapeutics. Here, we have attempted to restructure the chloroplast genome by deleting around 10kb of its genome and developed transgenic offspring. The new line exhibits similar growth phenotype to WT under normal physiological conditions.

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DNA hydrogel-based phantom cells as novel tool for biological research

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In any biological system, cells rely on physicochemical cues from the surrounding cells and matrix to control their size, stiffness and developmental fate. Accurately deducing these cues and their influence on adjacent and global cellular behaviour, while controllably enacting locally confined changes when desired, remains unattained. Introducing cell-sized, biocompatible, multi-functional “phantom cells” with known physicochemical properties will allow us to closely study and manipulate these processes in biological systems.

Here, we introduce DNA hydrogel-based beads as synthetic phantom cells. The beads are formed in a templated manner by encapsulating double-stranded DNA Y-motif precursors with short single-stranded DNA linkers into water-in-oil droplets. Binding of the DNA linker strands to sticky-end overhangs on the Y-motifs then facilitates the gelation of the mixture in the droplets over time resulting in spherical DNA beads. Destabilization of the droplet shells releases the DNA beads into an aqueous solution.

We show that the DNA beads can be produced within the relevant size and stiffness range of living soft tissues and form rudimentary proto-tissues themselves. Further, the beads can be introduced into 2D cell layers and 3D organoids and guide their development and function. Introducing photocleavable moieties to the DNA network, the beads can be either selectively broken down [1] or attached compounds released upon light trigger. As a first application, we utilize this function to release Wnt-surrogate fusion proteins inside *O. latipes* retinal organoids following short-term UV exposure. This way, we controllably trigger the formation of retinal pigment epithelium with spatiotemporal precision. Overall, we showcase the tuneability of DNA beads as phantom cells in tissues.

[1] Walther, T., Jahnke, K., Abele, T., Göpfrich, K., Printing and Erasing of DNA- Based Photoresists Inside Synthetic Cells. Adv. Funct. Mater. 2022, 32, 2200762. https://doi.org/10.1002/adfm.202200762

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Development of Software for the Computer-aided Design of Genomes

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As synthetic biology involves larger genomes, software will continue to become a necessary part of the design process. Similar to their importance to the design of architectural blueprints, software for the computer-aided design (CAD) of genomes help users manage and modify designs.

We describe our proposal for such a program by first listing the desired features, followed by the proposed architecture that would help us achieve our goals. At its very core, our system needs to support basic edit operations such as adding and deleting of sequences. These edit operations need to be performed either at single locations or in “batch” at numerous locations simultaneously throughout a chromosome or in a region within a chromosome, based on the locations of genomic features such as genes or exons. From a computational point of view, it would be desirable if edits were performed in parallel by leveraging the computational power of the underlying computer.

In order to meet our needs, we are proposing a system that consists of a web-based graphical front-end with the majority of the computation performed by a back-end command-line program running on a Linux-based server. Preliminary experiments with an early prototype will show our expectations for such a system.

Finally, we will briefly summarise some earlier related work by others, which serve as inspiration for our system. We will present the similarities and differences between our system and their’s.

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Inserting short sequences with prime editing

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Short sequences can be precisely written into a selected genomic target using prime editing without creating double-strand breaks. This facilitates correcting pathogenic deletions, controlling gene expression, modifying proteins, and many other exciting applications. To characterize features that influence insertion efficiency, we designed a library of 3,604 sequences up to 69 nt in length and measured their insertion frequency into four genomic sites in three human cell lines, using different prime editor systems. We discover that insertion sequence length, nucleotide composition and secondary structure all affect insertion rates, and that mismatch repair proficiency is a strong determinant for the shortest insertions. We also discover that 3' flap nucleases TREX1 and TREX2 suppress the insertion of longer sequences. Combining the sequence and repair features into a machine learning model, we can predict 70% of the repeatable variation in insertion frequency for new sequences. We further investigated interactions between insertion sequences and target sites by measuring the insertion rate of 1002 sequences into 2992 synthetic target sites. The tools we provide facilitate optimal design choices for inserting short sequences into genomes.

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High-resolution adaptive landscapes to study bacterial cis-regulatory evolution

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New gene regulation is a key source of novel traits and innovations in biological evolution. The evolution of gene regulation has been extensively studied in eukaryotes but much less so in prokaryotes. Prokaryotic genes, similar to their eukaryotic counterparts, are regulated by transcription factors (TFs), which activate or repress gene expression by binding to specific sites on DNA known as transcription factor binding sites (TF- BSs). This interaction either facilitates or inhibits gene transcription by RNA polymerase. Global TFs control the expression of numerous genes, while local TFs regulate only a few. Mutations in individual TFBSs are central to regulatory evolution because they allow changes in the expression of individual genes. In this study, we employed a synthetic biology approach to investigate the adaptive landscapes of TFBSs for multiple TFs in *Escherichia coli*. We utilized massively parallel reporter assays and high-throughput DNA sequencing techniques to explore the adaptive landscapes associated with TFs responsible for regulating hundreds (CRP, Fis, and IHF), dozens (LasR), and only two genes (TetR). While each TF exhibited specific landscape topologies aligned with their biological roles, we observed common trends such as increased ruggedness (number of peaks) and epistatic interactions compared to eukaryotic TFs. Moreover, our findings revealed that local regulators possessed fewer peaks with high binding affinities than global regulators. Surprisingly, we found that these few high peaks were readily attainable through adaptive evolution simulations, with substantial contingency effects observed in the evolution of such genotypes. Finally, we highlight that the framework developed through this study not only advances our understanding of bacterial regulatory evolution but also holds promise for prototyping and engineering novel regulatory systems in synthetic biology applications.

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Construction of the artificial expression of the Type III secretion system(T3SS)

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One of the major functions of the Type III secretion system (T3SS) is to secrete proteins in the early stages of infecting host cells by Gram-negative bacteria. Owing to the effectors secreted using this system, human pathogens such as *Salmonella* and *Escherichia coli* exhibit pathogenicity. Since T3SS can transport various proteins outside of the cell, we can also exploit it as a drug delivery system for vaccines and cancer therapies. The T3SS needle, however, is metabolically expensive to synthesize the element, their regulation is strictly controlled and only emerged during the pathogenic process, and these cellular programming made us difficult to investigate the whole effectors and use the system as a protein delivery tool. To overcome the limitation, we designed a genetic circuit with essential genes in the T3SS gene cluster of *Salmonella* SPI-1. This design includes regulation of the system expression, modification of the expression level of the OrgB gene, codon optimization of the InvC gene, and labeling of the GFP gene to monitor the synthesis of the T3SS. To this end, we synthesized all the DNA fragments and assembled them with a DNA assembly approach to create the genetic circuit. This artificial expression of the T3SS enables us not only to detect unidentified secreted effectors but also to use it as a protein delivery tool.