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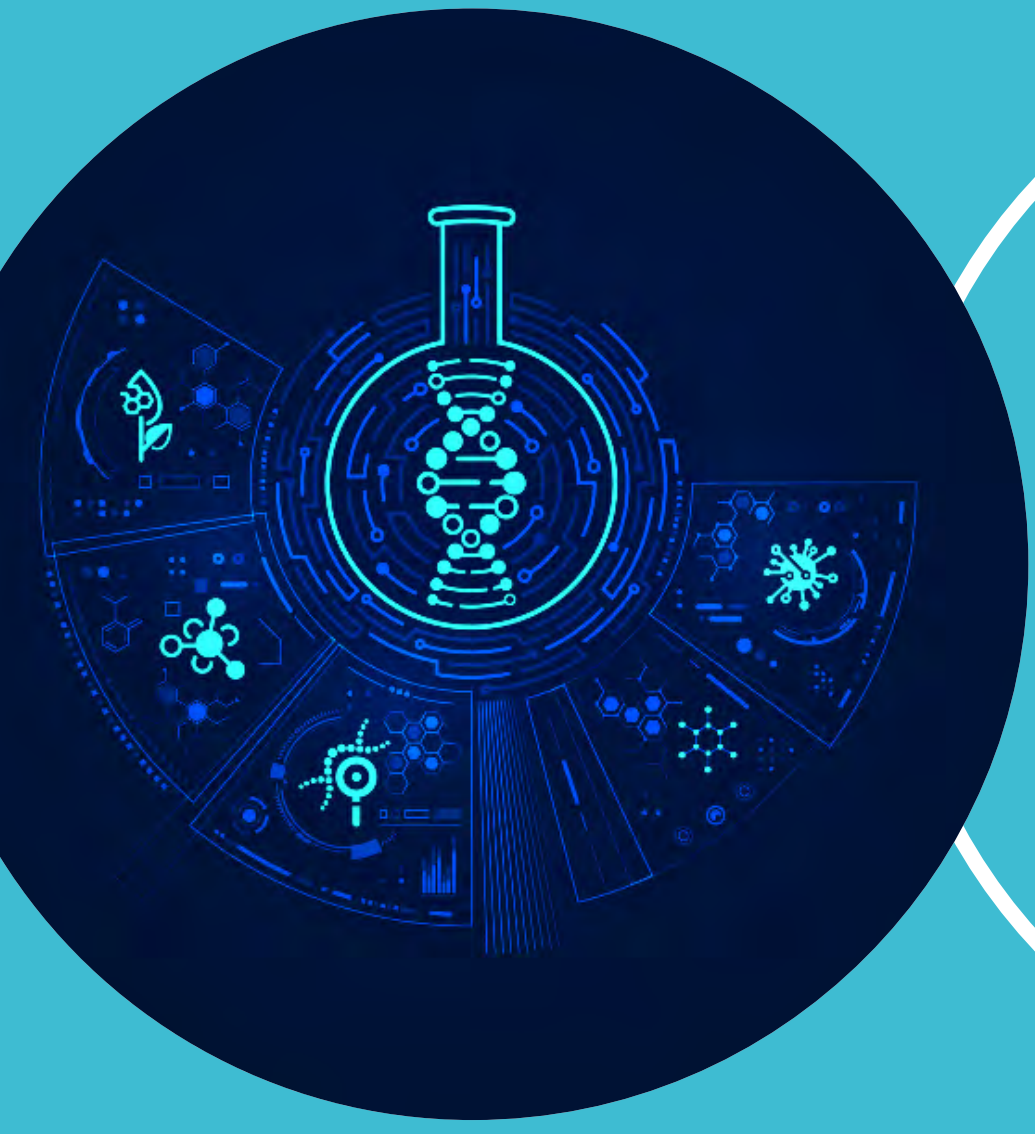
Synthetic Biology for Future Health

13–15 March 2023

Wellcome Genome Campus, UK



Abstract book

Synthetic Biology for Future Health

Wellcome Genome Campus Conference Centre,
Hinxton, Cambridge, UK
13–15 March 2023

Scientific Programme Committee:

Yasunori Aizawa
Tokyo Institute of Technology, Japan

Sonja Billerbeck
University of Groningen, The Netherlands

Tom Ellis
Imperial College London, UK

Katie Galloway
MIT, USA

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Dear Colleague,

I would like to offer you a warm welcome to the Synthetic Biology for Future Health 2023 conference, which is being delivered as a hybrid conference, with delegates from around the world joining us on campus or online. I hope you will find the talks interesting and stimulating, and find opportunities for networking with colleagues and potential collaborators during the breaks and at the poster sessions, or by participating in the online discussions though our virtual event portal. We hope you make connections that lead to new and exciting collaborations throughout your time here with us.

This event is organised by Wellcome Connecting Science (WCS), with core funding from the Wellcome Trust. WCS funds, develops and delivers training and conferences that span basic research, cutting-edge biomedicine, and the application of genomics in healthcare. Our programme includes a range of conferences and laboratory, computational, and discussion based courses at the campus or in lower and middle income countries, providing hands-on training in the latest biomedical techniques for research scientists and healthcare professionals. We have also developed a series of MOOCs, accessible to learners for free. Our recently introduced podcast Your Digital Mentor, explores topics around mentoring and career development, tackling the challenges through real stories and honest discussions from expert guests across the world. To find out more about our programme, please visit: **https://coursesandconferences.wellcomeconnectingscience.org/**

We have a strong commitment to equality, diversity and inclusion across the programme. To promote a culture of inclusion and equal representation at our events, we ensure that 50% of our programme committees, session chairs and invited speakers are women. We also work with our programme committees to invite speakers from a range of countries. To read more about our policies, please visit: **https://coursesandconferences.wellcomeconnectingscience.org/about-us/policies/**

The events team will be onsite and online to help this event run smoothly, so drop by the registration desk email us at **conferences@wellcomeconnectingscience.org**, or contact us through the event portal if you have any queries.

Finally, I hope you enjoy the conference.

Best wishes,

Dr Treasa Creavin
Head of Conferences and Online Training
treasa.creavin@wellcomeconnectingscience.org

Hybrid Conference Programme

Start (GMT)	Finish (GMT)	Presenter details
Monday 13 March 2023		
12:00	**13:00**	**Registration, lunch and networking**
12:45		Briefing for Keynote, Session 1 speakers, chair, moderator, all microphone runners & committee - Auditorium
13:00	**13:10**	**Welcome**
		Scientific Programme Committee:
		Yasunori Aizawa, Tokyo Institute of Technology, Japan
		Sonja Billerbeck, University of Groningen, Netherlands
		Tom Ellis, Imperial College London, UK
		Katie Galloway, Massachusetts Institute of Technology, USA
13:10	**14:10**	**Keynote 1**
		Chair: Tom Ellis, Imperial College London, UK
		Moderator: Sonja Billerbeck, University of Groningen, Netherlands
13:10	14:10	**Engineering Next-Generation T Cells for Cancer Immunotherapy**
		Yvonne Chen, University of California, Los Angeles, USA
14:10	14:20	**Comfort break**
14:20	**15:50**	**Session 1: Cells Engineered as Therapies**
		Chair: Tom Ellis, Imperial College London, UK
		Moderator: Jonas Koeppel, Wellcome Sanger Institute, UK
14:20	14:50	**Targeted engineering of human gut microbiome**
		Lei Dai, SIAT, Chinese Academy of Sciences, China
14:50	15:20	**Synthetic biology in biomedicine: towards the design of improved T cell therapies**
		Velia Siciliano, Instituto Italiano de Tecnologia, Italy
15:20	15:35	**A rapid and efficient platform for targeted large gene insertions in human pluripotent stem cells**
		Albert Blanch Asensio, Leiden University Medical Center, Netherlands
15:35	15:50	**A pipeline for the evolution and delivery of therapeutic cargo by Salmonella**
		Marc Biarnes Carrera, Prokarium Ltd, UK
15:50	16:35	**Refreshment break**

16:20		**Briefing for Session 2 speakers, keynote 2, chair & moderator - Auditorium**
16:35	**18:05**	**Session 2: SocioTechnological readiness and entrepreneurship**
		Chair: Sonja Billerbeck, University of Groningen, Netherlands
		Moderator: Alison Heggie, Imperial College London, UK
16:35	17:05	**Synthetic Biology from Molecular to Social Scales**
		Megan Palmer, Stanford University, USA
17:05	17:35	**Standardisation in Synthetic Biology: squaring the circle?**
		Manuel Porcar, Universidad de Valencia, Spain
17:35	17:50	**Open source toolkits to enable synthetic biology for future health in the global South**
		Jennifer Molloy, University of Cambridge, UK
17:50	18:05	**Indirect human engineering as a societal concern for synthetic genomics**
		Erika Szymanski, Colorado State University, USA
18:05	18:15	**Comfort break**
18:15	**19:15**	**Keynote 2**
		Chair: Yasunori Aizawa, Tokyo Institute of Technology, Japan
		Moderator: Katie Galloway, Massachusetts Institute of Technology, USA
18:20	19:15	**Reprogramming the Genetic Code**
		Jason Chin, University of Cambridge , UK
19:15		Dinner
19:15	23:00	Bar open (card payments only)

Tuesday 14 March 2023

09:15		Briefing for Session 3 speakers, chair & moderator - Auditorium
09:30	**11:00**	**Session 3: Engineering Gene Regulation**
		Chair: Katie Galloway, Massachusetts Institute of Technology, USA
		Moderator: Jacob Hepkema, Wellcome Sanger Institute, UK
09:30	10:00	**Host-aware construct design in mammalian cells.**
		Francesca Ceroni, Imperial College London, UK
10:00	10:30	**Teaching cells to perform new therapeutic functions**
		Mo Khalil, Boston University, USA - VIRTUAL
10:30	10:45	**Programming nuclear phase separation states and gene expression in human cells using precision targeting of synthetic intrinsically disordered regions**
		Isaac Hilton, Rice University, USA
10:45	11:00	**Towards precision targeting of non-coding RNAs using oligonucleotide catalysts**
		Maria Donde, University of Cambridge, UK
11:00	11:30	Refreshment break
11:15		Briefing for chair - Auditorium
11:30	**12:15**	**Poster Pitches**
		Chair: Sonja Billerbeck, University of Groningen, Netherlands
12:15	14:15	Lunch with posters Odd numbers 12:30 - 13:15 Even numbers 13:15-14:00
14:00		Briefing for Session 4 speakers, chair & moderator - Auditorium
14:15	**15:45**	**Session 4: Infectious Disease and Biosensing**
		Chair: Yasunori Aizawa, Tokyo Institute of Technology, Japan
		Moderator: Juliane Weller, Wellcome Sanger Institute, UK
14:15	14:45	**Engineering sustainable resources for CRISPR-based diagnostics**
		Chayasith Uttamapinant, VISTEC, Thailand
14:45	15:15	**Targeting fungal diseases using the secreted yeast toxicome**
		Sonja Billerbeck, University of Groningen, Netherlands
15:15	15:30	**Resolving spatial variation in engineered biosensor response within the mammalian gut**
		David Riglar, Imperial College London, UK
15:30	15:45	**Humanized CB1R and CB2R yeast biosensors enable facile screening of cannabinoid compounds**
		Colleen Mulvihill, University of Texas at Austin, USA
15:45	16:30	Refreshment break

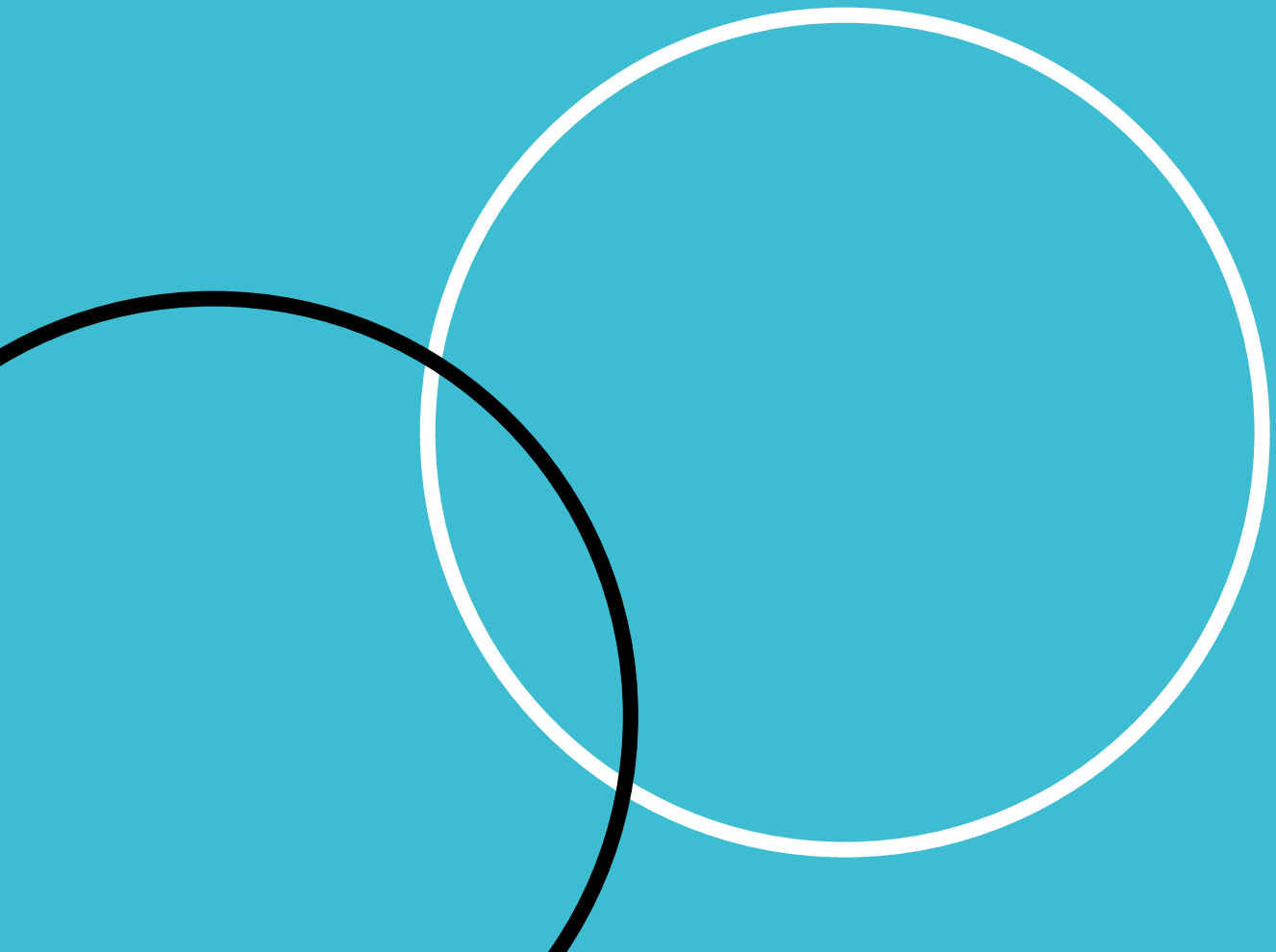
16:15		Briefing for Session 5 speakers, chair & moderator - Auditorium
16:30	**18:00**	**Session 5: Designing Synthetic Biomolecules**
		Chair: Katie Galloway, Massachusetts Institute of Technology, USA
		Moderator: Clare Robinson, Imperial College London, UK
16:30	17:00	**Build to understand -- the Secret Life of Feedback Circuits**
		Hana El-Samad, University of California, San Francisco/Altos Labs, USA
17:00	17:30	**Engineered mammalian synthetic biology towards therapy**
		Roman Jerala, Kemijski Institute, Slovenia
17:30	17:45	**Finding all our switches: biophysics at scale to understand, predict and engineer allostery**
		Ben Lehner, Wellcome Sanger Institute, UK
17:45	18:00	**Multi-input drug-controlled switches of mammalian gene expression based on engineered nuclear hormone receptors**
		Simon Kretschmer, University of California, San Francisco, USA
18:00	19:00	Reception/networking
19:00		Dinner
19:00	23:00	Bar open (card payments only)

Wednesday 15 March 2023

09:15		Briefing for Session 6 speakers, chair & moderator - Auditorium
09:30	**11:15**	**Session 6: Synthetic Genomes, Cells and Multicellularity**
		Chair: Sonja Billerbeck, University of Groningen, Netherlands
		Moderator: Juliane Weller, Wellcome Sanger Institute, UK
09:30	10:00	**Sub-megabase and biallelic genome writing technology creates multi-functional human iPS cells**
		Yasunori Aizawa, Tokyo Institute of Technology, Japan
10:00	10:30	**Limb regeneration rveals novel programming of development signaling modalities**
		Elly Tanaka, Research Institute of Molecular Pathology, Austria
10:30	10:45	**Drag-and-drop genome insertion of large sequences without double-strand DNA cleavage using CRISPR-directed integrases**
		Matthew Yarnall, MIT, USA
10:45	11:00	**Temperature-responsive synthetic cells for advanced drug delivery**
		Carolina Monck, Imperial College London, UK
11:00	11:15	**Randomizing gene regulatory regions using prime editing**
		Jonas Koeppel, Wellcome Sanger Institute, UK
11:15	11:45	Refreshment break
11:30		Briefing for panel members, chair, moderator & committee - Auditorium
11:45	**12:45**	**Panel Session: Translating tools to therapies**
		Chair: Adrian Woolfson, Replay Holding, LLC, USA
		Moderator: Yasunori Aizawa, Tokyo Institute of Technology, Japan
		Harold P. de Vladar, Ribbon Biolabs, Austria
		Jennifer Molloy, University of Cambridge, UK
		Anne-Marie Mooney, New England Biolabs UK Ltd, UK
		Serge Pampfer, EureKARE SAS, France - VIRTUAL
12:45	**13:00**	**Closing remarks and prize presentation**
		Scientific Programme Committee:
		Yasunori Aizawa, Tokyo Institute of Technology, Japan
		Sonja Billerbeck, University of Groningen, Netherlands
		Tom Ellis, Imperial College London, UK
		Katie Galloway, Massachusetts Institute of Technology, USA
13:00	13:50	Lunch and departures
13:50		**Coach departures for Stansted and Heathrow airports**
14:00		**Coach departures for Cambridge train station and city centre**

Spoken Presentation Abstracts

These abstracts should not be cited in bibliographies. Materials contained herein should be treated as personal communication and should be cited as such only with consent of the author.



Engineering Next-Generation T Cells for Cancer Immunotherapy

Yvonne Chen, Sarah Larson, Brenda Ji, Caitlin Harris, Mobina Khericha Gandhi, Andrew Hou, ZeNan Chang, Ryan Shih, Benjamin Uy, Meng-Yin Lin, Eunwoo Nam, Eugenia Zah

University of California Los Angeles, USA

The adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has demonstrated clinical efficacy in the treatment of advanced cancers, with anti-CD19 CAR-T cells achieving up to 90% complete remission among patients with relapsed B-cell malignancies. However, challenges such as antigen escape and immunosuppression limit the long-term efficacy of adoptive T-cell therapy. Here, I will discuss the development of and clinical data on next-generation T cells that can target multiple cancer antigens and resist antigen escape. I will also present recent work on tuning CAR signaling activities via rational protein design to achieve greater in vivo anti-tumor efficacy. This presentation will highlight the potential of synthetic biology in generating novel mammalian cell systems with multifunctional outputs for therapeutic applications.

Notes:

Targeted engineering of human gut microbiome

Lei Dai

SIAT, Chinese Academy of Sciences, China

In this talk, I will present our recent efforts to engineer human gut microbiome at the genetic level and the ecological level. In the first study, we developed a versatile, highly efficient CRISPR/Cas-based genome editing tool that allows markerless gene deletion and insertion in human gut Bacteroides species. We demonstrated that CRISPR/FnCas12a can be broadly applied to engineer multiple human gut Bacteroides and Parabacteroides species. CRISPR/Cas-based genome editing tools for Bacteroides will greatly facilitate mechanistic studies of the gut commensal and the development of engineered live biotherapeutics. In the second study, we developed a Gut Phage Isolate Collection (GPIC) of 209 phages isolated for 42 commensal human gut bacterial species. Infection assays revealed that GPIC phages have high host specificity and Bacteroides and Parabacteroides strains isolated from the human gut have substantial variations in phage susceptibility. We demonstrated that a cocktail of 8 GPIC phages successfully knocked down the abundance of B. fragilis in complex communities. Our study greatly expands the diversity of cultured human gut phages and provides a valuable resource for human microbiome engineering.

Notes:

Synthetic biology in biomedicine: towards the design of improved T cell therapies

Velia Siciliano

Istituto Italiano di Tecnologia, Italy

Mammalian synthetic networks build on the conjugation of efforts to design circuits that are activated when specific molecules are sensed within the cells or surrounding environment. These smart interfaces process endogenous information and implement robust responses enabling high specificity and self-containment of desired output activation. Sensing cellular state may be particularly relevant when engineering T cell-based therapies for the treatment of solid tumors. By designing combined transcriptional and post-transcriptional sensors of T cell exhaustion we will try to enhance the long-term efficacy of CAR-T cells.

Notes:

A rapid and efficient platform for targeted large gene insertions in human pluripotent stem cells

Albert Blanch-Asensio1,3, Catarina Grandela1, Babet van der Vaart1,2,3, Mariana Vinagre1,3, Eline Groen1, Niels Geijsen1,2,3, Christine Mummery1,3, Richard Davis1,3

1 Department of Anatomy and Embryology, Leiden University Medical Center, 2300RC Leiden, The Netherlands

2 LUMC hiPSC Hotel, Department of Anatomy and Embryology, Leiden University Medical Center, 2300RC Leiden, The Netherlands

3 The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden University Medical Center, 2300RC Leiden, the Netherlands

Inserting large DNA payloads (>10 kb) into specific genomic sites or replacing genomic segments in mammalian cells, such as human induced pluripotent stem cells (hiPSCs), still remains a challenging task. Applications ranging from synthetic biology to personalized medicine would greatly benefit from tools that facilitate this process. Here we have merged the strengths of different classes of site-specific recombinases and combined these with CRISPR-Cas9-mediated homologous recombination to develop a platform termed STRAIGHT-IN (Serine and Tyrosine Recombinase Assisted Integration of Genes for High-Throughput INvestigation) for stringent site-specific integration of large DNA payloads or replacement of genomic fragments at least 50 kb in size in hiPSCs. Only one copy of the DNA payload is integrated with full control of where it is targeted in the genome with no cargo size limitation detected to date (>170 kb). The procedure also permits the excision of nearly all the auxiliary DNA sequences which can lead to post-integrative silencing, simply leaving in the genome the desired payload flanked by two minimal scars (<300 bp). We have demonstrated its applicability by establishing a multi-parameter reporter hiPSC line to assess the excitation-contraction coupling cascade in derivative cardiomyocytes, and simultaneously generating >10 hiPSC lines containing disease variants present in patients with arrhythmogenic phenotypes. In addition, we recently developed an upgraded version of the platform (version 2) that allows genetically-modified hiPSC lines to be generated with 100% efficiency and within a two or three-week time period, making the procedure very rapid. Acceptor hiPSC lines with the new landing pad inserted into either the AAVS1 or CLYBL safe harbour loci have been generated and validated, and we have created several constitutive and doxycycline-inducible reporter lines to compare expression levels from both genomic regions in a number of differentiated cell types.

Notes:

A pipeline for the evolution and delivery of therapeutic cargo by Salmonella

Marc Biarnes Carrera, Georgia Petsiou, Laura Feather, Christopher J Waite, Nicolas Glanville, Paola Salerno, Marko Storch, Livija Deban

1. Prokarium Ltd, London, United Kingdom

2. Centre for Synthetic Biology and Innovation, Imperial College London, London, United Kingdom

Prokarium is pioneering the field of microbial immunotherapy. Our pipeline is designed to unlock the next level of immuno-oncology by building on the most recent advances in cancer immunology. Prokarium is focused on transforming the treatment paradigm in solid tumours by orchestrating immune-driven, long-lasting antitumor effects.

In this adaption of our platform technology, we leverage proprietary genetic engineering interventions to build on the natural ability of our bacterial strains to seek out and colonise solid tumours. We have developed attenuated Salmonella Typhi strains capable of targeting tumours without causing pathology in normal tissues. These strains can be used to deliver specific immunostimulatory cargo aimed at activating the patient's immune system to destroy tumours. However, the expression of complex therapeutic molecules in bacteria typically results in the formation of inactive inclusion bodies, with consequent little or negligible amount of active protein production.

Here, we present a biological engineering pipeline aimed at optimising the production and secretion of complex therapeutic cargo from our strain. The pipeline leverages the high throughput capabilities of the London Biofoundry to screen hundreds of sequence variants with few amino acid substitutions for each candidate which maintain the same bioactivity as the wild-type cargo but have enhanced solubility when expressed in the bacterial chassis. The final evolved candidates are then integrated into specific landing pad positions on the bacterial chromosome to generate stable cargo-secreting strains. As a proof-of-concept, we apply the pipeline to the evolution of various human cytokines and chemokines whose expression in bacteria typically results in the formation of inactive inclusion bodies, identifying at least one soluble and active variant per candidate molecule.

Notes:

Synthetic Biology from Molecular to Social Scales

Megan J. Palmer

Stanford University, USA

Scientists and engineers have increasingly complex responsibilities in designing technologies that shape – and are shaped by - global societies. This is especially true for designers of biological systems, in which new knowledge and innovation offers not only new ways to feed, fuel, heal, and make, but also uncovers new complex vulnerabilities and understandings of the living world and our roles within it. How might we prepare science and engineers to evaluate the impact of their decisions on society, empower them to explore their concerns, and enable them to work with others to alter the course of development?

I will share several studies to understand what motivates and enables scientists and engineers to consider how to advance their work to serve diverse—and sometimes conflicting—public interests and values. These studies leverage several testbeds in the field of synthetic biology, including an international student competition. I will also share theoretical work on how novel technologies can drive reconfigurations in our approaches to governance through changes in both policies and technologies. Lastly, I will describe a recent empirical study to understand how different organizations supporting research in the life sciences evaluate security concerns in practice. Throughout, I will describe the benefits of bringing together multiple disciplinary perspectives in learning how we can design biology from the molecular to the social scale, and back again.

Notes:

Standardisation in Synthetic Biology: squaring the circle?

Manuel Porcar Miralles

Universidad de Valencia, Spain

Standards are central in engineering and they can even be seen as one of the pillars of our modern, technological civilisation. Synthetic Biology has the ambitious goal of engineering life, and thus standardisation of biological systems is one of the major goals of this discipline. Living cells and biological "circuits" display similarities but also differences with machines and this poses important challenges on the current standardisation efforts. Standardisation in Synthetic Biology includes both success and failure stories and the talk will describe both, in the light of the EU-funded project BIOROBOOST.

Notes:

Open source toolkits to enable synthetic biology for future health in the global South

Jenny Molloy, Chiara Gandini, Felipe Buson [1], Fernan Federici, Tamara Matute, Isaac Núñez [2], Anibal Arce [3], Isaac Larkin [4], Scott Pownall [5]

[1] University of Cambridge, [2] Pontificia Universidad Católica de Chile, [3] Northwestern University, [4] Free Genes Project, [5] Open Science Network Society

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. DNA parts were distributed via Addgene, Free Genes and iGEM under an Open Material Transfer Agreement (which allows commercial use) and are now in >500 labs in >40 countries, including universities, research institutions and small biotech companies.

We will present the development and impact of the Research in Diagnostics Collection as a case study. This is an open source DNA toolkit containing a set of 16 ready-to-use expression vectors for E. coli for the most common enzymes used in research on diagnostics (e.g. RT-PCR, LAMP, RPA), plus a set of DNA parts ready to be assembled using MoClo standards with a tailored syntax. These include parts tailored for low-resource and sustainable protocols, such as light- and heat-inducible promoters and affinity tags for immobilisation on cellulose and silica.

We will introduce the Reagent Collaboration Network (Reclone) community that has grown around the toolkit and the training, research and entrepreneurship that it has enabled across several continents. Our work demonstrates the potential for distributed manufacturing of enzymes and other reagents to catalyse synthetic biology for global health. We will discuss future plans to create research networks and foster entrepreneurial activities that will increase the autonomy and agency of synthetic biologists to tackle health challenges, wherever they are based in the world.

Notes:

Indirect human engineering as a societal concern for synthetic genomics

Erika Szymanski, Maya Hey

Colorado State University

The prospect of human genome synthesis is often central to conversations about the ethical, legal, and social dimensions of synthetic genomics. We argue that this is the wrong locus of concern because, among other things, it suggests an unjustifiably clear distinction between human and non-human research. From a survey of genome-scale synthetic biology across the United States, we see an increasingly diverse range of plants, animals, and microbes on and beyond the canonical model organism list being justified as the right tool for a similarly diverse range of jobs. Unsurprising, these rationales often address the same central exigency: equipping future humans to be more fit and resilient against the uncertain challenges of the climate crisis and the possibility of needing to adapt to new environments. However, they can also be understood in a different way, as indirect human engineering-engineering future human capabilities without directly altering human DNA and thereby engaging the ethical concerns that doing so would entail.

Indirect human engineering does not raise all of the same ethical concerns presented by synthesizing a human genome-notably, religious prohibitions related to the specific sanctity of human identity in its relation to a creator-but it does invoke many of them. For example, privileged parents might be able to increase their offspring's resistance to disease through human genome editing, or through designer symbiotic gut microbes, or through precision pharmaceuticals, to similar effect and with similar concerns about access and inequality. While these kinds of problems are often discussed through bioethical frameworks about consent, harms, slippery slopes, embryo use, etc., we suggest an alternative approach through posthumanist theory whereby humans are understood as fuzzy-edged creatures that become (particular kinds of) human with other creatures and their environments. No human is human alone; rather, human wellbeing and identity is always co-constructed through more-than-human relationships.

Because the conditions through which we become human are distributed beyond the boundaries of an individual body or genome, concerns about engineering humans cannot be avoided by not engineering human genomes. Through a posthumanist lens, we conclude that conversations about the social and ethical dimensions of synthetic genomics need to decenter humans as discrete individuals and recenter on humans as interconnected multispecies accomplishments. Doing so should enable more open and nuanced consideration of how equity, diversity, and justice are enacted through the future landscapes that synthetic genomics may help construct, not just through future human genomes.

Notes:

Reprogramming the Genetic Code

Jason W. Chin, Ph.D.

Joint Head & Programme Leader, Division of Protein & Nucleic Acid Chemistry, Head, Centre for Chemical & Synthetic Biology, MRC Laboratory of Molecular Biology, Cambridge, UK

Professor of Chemistry and Chemical Biology, Department of Chemistry, Cambridge University, UK

Associate Faculty Synthetic Genomics, Wellcome Sanger Institute, Cambridge, UK

Fellow in Natural Sciences, Trinity College, Cambridge, UK

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 rewritten the genetic code.

Notes:

Host-aware construct design in mammalian cells

Francesca Ceroni

1. Department of Chemical Engineering, Imperial College London, South Kensington Campus, London (UK)

2. Imperial College Centre for Synthetic Biology, South Kensington Campus, London (UK)

Resource competition arises from the simultaneous use of cellular gene expression resources by different expression cassettes. This often results in undesired cross-interactions in engineered cells and can cause many of the unpredictable behaviours observed in synthetic biology. In bacteria, the phenomenon has been extensively characterised, and many tools, both experimental and computational, have been developed to achieve more robust construct engineering. Evidence has confirmed that the same problem also affects mammalian cells. Precisely, recent work has shown that gene expression resources are also limiting in mammalian cells as in microbes and that unobvious coupling of genetic circuits can arise as a consequence of synthetic cassettes drawing from the same pool of resources.

In order to take resource competition into account at the design stage, we developed a framework for the quantification of the load imposed by different genetic designs on the cellular resources of two commonly adopted cell lines, HEK293T and CHOK1.

In our workflow, a transient capacity monitor, namely a constitutively expressed mKATE, is adopted as proxy of the amount of cellular resources recruited by a competing test plasmid, expressing eGFP.

We designed a modular test plasmid architecture where promoter, kozak and polyA components can easily be exchanged. Using this, we tested the impact that eight different promoters (pJB42CAT5, PGKp, hACTBp, SV40p, UBp, CMVp, EF1ap, TETp), three Kozak sequences with different translation efficiency, and six polyA sequences (SV40pA, SV40pA_rv-, PGKpA, BGHpA, RBpA, HGHpA) have on cellular resources. From our results, promoters tend to impact resources the most compared to Kozak sequences, while polyAs also play a role in competition which is dependent on the cell line considered.

We then characterised widely adopted parts for multi-cistronic expression, such as IRES and 2A peptides, and verified that these parts can be used to achieve minimised resource footprint.

We applied our findings to the optimisation of genetic circuit performance (i.e. the TET-ON system), and we demonstrated that our framework remains relevant when applied to other proteins of interest.

Overall, we here provide direct evidence that different synthetic designs can be characterised based on the resource load they impose on mammalian cells and demonstrate that our framework can be used for the optimisation of co-expression systems, whether targeted towards fundamental and/or applied biological research.

This work provides the opportunity for the mammalian synthetic biology community to take the host response into account and achieve robust and controllable gene expression over-time.

Notes:

Teaching Cells to Perform New Therapeutic Functions

Mo Khalil

Boston University, USA

Cells use “circuits” of interacting molecules to monitor their environment and generate appropriate responses. Synthetic circuits designed to manipulate this process and program new cellular capabilities present unique opportunities for next-generation therapeutics. In this talk, I will discuss our development of synthetic biology platforms designed to teach cells (1) to perform customizable therapeutic tasks and (2) to autonomously discover new therapeutic molecules. The first study focuses on addressing a key limitation in applying synthetic circuits to enhance cell-based therapy (such as CAR-T therapy): the lack of platforms for developing gene circuits in primary human cells that can drive robust functional changes in vivo and have compositions suitable for clinical use. To fill this gap, we developed a new gene circuit engineering platform based on synthetic zinc finger regulators that are compact and based largely on human-derived proteins (synZiFTRs). We demonstrate the platform by programing new capabilities for user-defined control over therapeutic immune cell function, including circuits that can instruct primary T cells to sequentially activate multiple cellular programs to enhance anti-tumor activity in vivo. The second study focuses on cell signaling systems and in particular on GPCRs, which are important targets for pharmacological intervention and drug discovery. I will describe ongoing efforts to program cells so they can autonomously generate and discover precision modulators of GPCR signaling.

Notes:

Programming nuclear phase separation states and gene expression in human cells using precision targeting of synthetic intrinsically disordered regions

Isaac B. Hilton, Jing Li, Shizhe Liu, Sunghwan Kim, Jacob Goell, Barun Mahata, Mario Escobar, Rosa Selenia Guerra-Resendez, Yuhao Zhou, Bo Yu, Gang Greg Wang, Douglas H. Phanstiel

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Rice University Department of BioSciences, Houston, TX, United States

Rice University Systems, Synthetic, and Physical Biology Graduate Program, Houston, TX, United States

School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Recent evidence has demonstrated that biomolecular condensates are linked to diverse and dynamic cellular processes in eukaryotic cellular nuclei, including gene expression and oncogenesis. However, the ability to interrogate and unravel the complex relationships between these phase separated portions of cell nuclei, gene expression, and oncogenesis has been largely restricted to global chemical perturbation or overexpression studies. Although these strategies can be powerful, they remain limited in their capacity to elucidate, and ideally synthetically modulate, the underlying mechanisms that control specific loci. To overcome these limitations, we have isolated key segments of intrinsically disordered regions (IDRs) from human proteins known to be involved in oncogenesis and also associated with phase separation, and then fused these IDRs to deactivated CRISPR/Cas9 (dCas9) proteins. We show that different dCas9-IDR fusion compositions (and non-dCas9-fused IDRs) display differing abilities for nuclear import/export and differing propensities to form bona fide phase separated foci in human cell nuclei. We also demonstrate that by synthetically manipulating IDR composition, we can effectively tune the levels of phase separation in human nuclei, and further, that these levels of phase separation are directly proportional to the synthetic activation of precisely targeted genes. Interestingly, we also find that conventional dCas9-based transcriptional activators fail to form robust biomolecular condensates, suggesting that dCas9-IDR fusions likely function through distinct mechanisms. Using mass spectrometry and microscopy, we also demonstrate that these IDRs can co-localize and interact with important epigenetic enzymes and other cellular cofactors, delineating potential interventional nodes for new therapies. Moreover, by reconstructing known oncogenic fusions between IDRs and other human protein partners, we show that phase separation and genomic binding (using microscopy and ChIP-seq, respectively) are altered in discrete ways. Finally, we combine the application of these different fusion proteins in primary human hematopoietic stem cells (HSCs) with total RNA-seq, microscopy, and quantitative measurements of oncogenic phenotype to further explain how IDR-containing proteins drive hematopoietic cancers. Altogether our studies provide new insights into nuclear phase separation and how this phenomenon drives human gene regulation. Importantly, our work also arms synthetic biologists and other biomedical researchers with new tools and abilities to synthetically perturb, control, and study an important and multifaceted cellular process at the forefront of eukaryotic cell biology and biomedicine.

Notes:

Towards precision targeting of non-coding RNAs using oligonucleotide catalysts

Maria Donde, Adam M. Rochussen, Saksham Kapoor & Alexander I. Taylor

Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), Jeffrey Cheah Biomedical Centre, University of Cambridge, Cambridge, UK

In principle, RNA-cleaving oligonucleotide catalysts (ribozymes and DNAzymes) can be programmed to modulate the expression of virtually any gene of interest. Their high specificity and activity independent from cellular machinery has long held promise for reagents with reduced off-target effects compared with other approaches. However, clinical applications have been hampered by the limitations of DNA & RNA.

By evolving fully-artificial catalysts composed of xeno-nucleic acids (XNA), it has become possible to engineer XNAzyme catalysts with advantageous properties, for example, improved biostability and activity under physiological conditions. As a proof of concept, we have developed a series of catalysts composed of 2'-fluoroarabino nucleic acid (FANA) that selectively cleave disease-associated RNA, including individual members of the classic microRNA-17-92 cluster, suggesting a route to precise modulation of RNA regulatory networks.

Notes:

Engineering sustainable resources for CRISPR-based diagnostics

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I will discuss our development of a robust multiplexed CRISPR-based detection using orthogonal CRISPR-associated enzymes to simultaneously diagnose SARS-CoV-2 infection and pinpoint the causative SARS-CoV-2 variant of concern (VOC)—including recent globally dominant VOCs Delta (B.1.617.2) and Omicron (B.1.1.529), as well as our implementation, with approval of the FDA of Thailand, of the CRISPR-based test technology. The platform has features for point-of-care (POC) use: premixed, freeze-dried reagents for easy use and storage; convenient direct-to-eye or smartphone-based readouts; and a one-pot variant of the multiplexed detection. To reduce reliance on proprietary reagents and enable sustainable use of the technology in low- and middle-income countries, we locally produce and formulate our own recombinase polymerase amplification reaction and demonstrate its equivalent efficiency to commercial counterparts. Our tool—CRISPR-based detection for simultaneous COVID-19 diagnosis and variant surveillance which can be locally manufactured—may enable sustainable use of CRISPR diagnostics technologies for infectious diseases in POC settings.

Notes:

Targeting fungal diseases using the secreted yeast toxicome

Sonja Billerbeck

University of Groningen, Netherlands

Fungal pathogens are an emerging threat to human health and food security. Very few fungicides are available and resistance to these is rising. It is a long-standing challenge to develop new antifungals. As eukaryotic pathogens, fungi offer very few selective drug targets and we urgently need new strategies for antifungal development. Ascomycete yeasts – such as environmental isolates of Saccharomyces cerevisiae and related species – have evolved a large set of small protein toxins, so-called yeast killer toxins (or mycocins), to compete against fungi in the environment. Previous research revealed that these toxins exhibit diverse modes of action, thus, indicating that the yeast toxicome might constitute a rich source of functionally diverse but yet-untapped antifungals.

In my talk I will exemplify my group's research on the molecular functioning of these yeast-derived toxins and their modularity and engineerability towards applications in human health, food and biotech.

Notes:

Resolving spatial variation in engineered biosensor response within the mammalian gut

David T Riglar, David Carreno Yugueros, Clare M Robinson

Department of Infectious Disease, Imperial College London

Engineered biosensors have emerged as powerful tools for deployment in the mammalian gut for a range of health-related synthetic biology applications, including non-invasive memory recorders, 'sense-and-respond' biotherapeutics and as prototype disease diagnostics. However, little is known about the spatial variation of biosensing within the gut despite its likely importance, especially in a range of diseases that are known to present with spatially variable symptoms, such as inflammatory bowel disease and colorectal cancer. To address this, we have developed a set of complementary tools for spatial dissection of engineered whole-cell bacterial biosensor function within the gut of animal models. We demonstrate our ability to image biosensor strains in situ at single bacterial resolution across large 3D regions of the gut. Using a biosensor that responds to sialic acid, a key inflammatory biomarker, we visualise spatial variations in sialic acid availability within the microbiome caused due to changes in bacterial sialidase enzyme activity. Our findings have implications for our understanding of the host-microbiome interface during inflammatory disease and treatments targeting these interactions. More broadly, our approach to biosensor analysis creates powerful new opportunities to develop more robust engineered biosensors and to extract more extensive biologically information from them.

Notes:

Humanized CB1R and CB2R yeast biosensors enable facile screening of cannabinoid compounds

Colleen Mulvihill, Josh Lutgens1, Jimmy D. Gollihar2, Petra Bachanová1, Edward M. Marcotte1, Andrew D. Ellington1, Elizabeth C. Gardner1,3

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Yeast expression of human G Protein Coupled Receptors (GPCRs) can be used as a biosensor platform for the detection of pharmaceuticals. The Cannabinoid receptors type 1 and 2 (CB1/2R) are of particular interest, given the cornucopia of natural and synthetic cannabinoids being explored as therapeutics. We show for the first time that engineering the N-terminus of CB1R allows for efficient signal transduction in yeast, and that engineering the sterol composition of the yeast membrane optimizes CB2R performance. Using the dual cannabinoid biosensors, large libraries of synthetic cannabinoids and terpenes could be quickly screened to elucidate known and novel structure-activity relationships, including compounds and trends that more selectively target each of the two receptors. The biosensor strains offer a ready platform for evaluating the activity of new synthetic cannabinoids, monitoring drugs of abuse, and developing molecules that target the therapeutically important CB2R receptor while minimizing psychoactive effects.

Notes:

Build to understand -- the Secret Life of Feedback Circuits

Hana El-Samad

UCSF/Altos Labs, USA

In this talk, I take the premise that feedback control plays a universal role in the existence and persistence of life. I discuss our efforts in pushing forward technologies for understanding the quantitative properties of bio-feedback loops. I hope to engage the audience in a discussion of broad and universal roles feedback loops may play, and how to approach systematically understanding their role in health and disease and for therapeutic applications.

Notes:

Protein engineering-based regulation of mammalian cells

Roman Jerala

Department of synthetic biology and immunology, National institute of chemistry, Ljubljana, Slovenia

Proteins perform most functions in cells, such as catalysis, localization, formation of scaffolds, recognition of other proteins and nucleic acids. Recent advances in protein design offer exciting potentials not just to understand function of proteins but also to introduce new types of regulation of biological processes. Direct regulation by proteins is able to speed up the cellular response and introduce clinically relevant tools. Several strategies will be presented that have been implemented to therapeutically relevant mammalian cell systems and demonstrated in animal models. Coiled-coil modules can be used for diverse functions, from augmenting chemical regulation, targeting localization, construction of fast responsive systems and regulation of protein function. A strategy for engineering inducible split protein regulators based on human proteins (INSPIRE) uses regulation by physiological ligand or clinically approved drug. The INSPIRE platform can be used for the dynamic, orthogonal, and multiplex control of gene expression in mammalian cells and its' sensing and feedback regulation by cortisol has been demonstrated in vivo. A broadly applicable strategy called INSRTR (inserted peptide structure regulator) is based on regulation of the activity of selected proteins by peptides that form a rigid coiled-coil dimer which can allosterically disrupt the function of diverse proteins, including enzymes, signaling mediators, nucleic acid binders/transcriptional regulators, fluorescent proteins, and antibodies implemented as a sensing domain of anticancer chimeric antigen receptors on T cells. This platform enables the construction of ON/OFF protein switches, their regulation by small molecules, and logic functions with a rapid response in mammalian cells. Development of new protein design tools have potentials to expand this field much further.

Notes:

Finding all our switches: biophysics at scale to understand, predict and engineer allostery

Ben Lehner, Chenchun Weng, Andre J. Faure

Wellcome Sanger Institute, UK

Centre for Genomic Regulation (CRG), Barcelona

Thousands of proteins have now been genetically-validated as therapeutic targets in hundreds of human diseases. However, very few have actually been successfully targeted and many are considered 'undruggable'. This is particularly true for proteins that function via protein-protein interactions: direct inhibition of binding interfaces is difficult, requiring the identification of allosteric sites. However, most proteins have no known allosteric sites and a comprehensive allosteric map does not exist for any protein. Here we address this shortcoming by charting multiple global atlases of inhibitory allosteric communication in KRAS, a protein mutated in 1 in 10 human cancers. We quantified the impact of >26,000 mutations on the folding of KRAS and its binding to six interaction partners. Genetic interactions in double mutants allowed us to perform biophysical measurements at scale, inferring >22,000 causal free energy changes, a similar number of measurements as the total made for proteins to date. These energy landscapes quantify how mutations tune the binding specificity of a signalling protein and map the inhibitory allosteric sites for an important therapeutic target. Allosteric propagation is particularly effective across the central beta sheet of KRAS and multiple surface pockets are genetically-validated as allosterically active, including a distal pocket in the C-terminal lobe of the protein. Allosteric mutations typically inhibit binding to all tested effectors but they can also change the binding specificity, revealing the regulatory, evolutionary and therapeutic potential to tune pathway activation. Using the approach described here it should be possible to rapidly and comprehensively identify allosteric target sites in many important proteins.

Notes:

Multi-input drug-controlled switches of mammalian gene expression based on engineered nuclear hormone receptors

Simon Kretschmer, Nicholas Perry, Yang Zhang, Tanja Kortemme

Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA 94158, USA

In synthetic biology, precise control over gene expression in engineered cells is critical for many applications, including biomedical scenarios in which therapeutic responses could be controlled by FDA-approved small-molecule drugs. One way to achieve advanced control is via the processing of multiple inputs simultaneously that can act in a synergistic or antagonistic manner. To realize such multi-input-responsive protein switches, the nuclear hormone receptor (NHR) superfamily provides a promising scaffold framework for engineering. Besides containing domains for input-specific small-molecule binding and gene-specific DNA binding and transcriptional activation, NHRs can form heterodimers of ligand binding domains that each respond to various FDA-approved small-molecule drugs. Here, we show that reporter gene expression controlled by engineered NHR dimers can be finely adjusted by the synergistic or antagonistic action of up to three simultaneously administered drug inputs. In the case of drug synergism, the highest expression levels are accessible exclusively in the presence of drugs targeting both halves of a NHR heterodimer, allowing for increased control compared to a single-input scenario. Such high-level control validates NHRs as a versatile, engineerable platform for programming multi-drug-controlled responses and motivates further engineering of output behaviors using experimental and computational approaches.

Notes:

Sub-megabase and biallelic genome writing technology creates multi-functional human iPS cells

Yasunori Aizawa

Tokyo Institute of Technology, Japan

Our group recently developed a genome engineering platform for cells having long, diploid and repeat-rich genomes such as human cells, called Universal Knock-in System or UKiS (Nature Communications 13, 4219, 2022). UKiS allows for precise substitution of sub-megabase-scale regions into synthetic genomic sequences of our design in biallelic and scarless manners in cancer cells and human induced pluripotent stem cells (iPSCs). Both alleles of your interest in autosomes can be efficiently modified, without leaving any tags or markers disturbing downstream analyses or purposes in the genomes. UKiS has potential to expand our capability of multi-functionalization of human cell for various industrial uses including pharmaceutical and therapeutic ones. In this presentation, I will introduce our latest applications of UKiS and the other engineering tools that we have established for investigating functional meanings of the noncoding genomic regions as well as for creating human cells for drug screening, disease study cells and therapeutic modality development.

Notes:

Limb regeneration reveals novel programming of developmental signaling modalities

Elly M. Tanaka

Research Institute of Molecular Pathology, Vienna Austria

The axolotl undertakes the remarkable feat of regenerating the missing part of an amputated limb. This process involves the dedifferentiation of fibroblasts into a limb bud progenitor that re-expressed the developmental signaling molecules required to organize the complex growth and patterning of a limb. Posterior fibroblasts re-activate the program for expression of the zone of polarizing activity molecule sonic hedgehog. Here we define the molecular competency zone, and how the stability competency can be modulated in the context of regeneration.

Notes:

Drag-and-drop genome insertion of large sequences without double-strand DNA cleavage using CRISPR-directed integrases

Matthew T.N. Yarnall, Eleonora I. Ioannidi, Cian Schmitt-Ulms, Rohan N. Krajeski, Justin Lim, Lukas Villiger, Wenyuan Zhou, Kaiyi Jiang, Sofya K. Garushyants, Nathaniel Roberts, Liyang Zhang, Christopher A. Vakulskas, John A. Walker II, Anastasia P. Kadina, Adrianna E. Zepeda, Kevin Holden, Hong Ma, Jun Xie, Guangping Gao, Lander Foquet, Greg Bial, Sara K. Donnelly, Yoshinari Miyata, Daniel R. Radiloff, Jordana M. Henderson, Andrew Ujita, Omar O. Abudayyeh & Jonathan S. Gootenberg

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Programmable genome integration of large, diverse DNA cargo without DNA repair of exposed DNA double-strand breaks remains an unsolved challenge in genome editing. We present programmable addition via site-specific targeting elements (PASTE), which uses a CRISPR-Cas9 nickase fused to both a reverse transcriptase and serine integrase for targeted genomic recruitment and integration of desired payloads. We demonstrate integration of sequences as large as ~36 kilobases at multiple genomic loci across three human cell lines, primary T cells and non-dividing primary human hepatocytes. To augment PASTE, we discovered 25,614 serine integrases and cognate attachment sites from metagenomes and engineered orthologs with higher activity and shorter recognition sequences for efficient programmable integration. PASTE has editing efficiencies similar to or exceeding those of homology-directed repair and non-homologous end joining-based methods, with activity in non-dividing cells and in vivo with fewer detectable off-target events. PASTE expands the capabilities of genome editing by allowing large, multiplexed gene insertion without reliance on DNA repair pathways.

Notes:

Temperature-responsive synthetic cells for advanced drug delivery

Carolina Monck (1,2), Yuval Elani (1,2,3), Francesca Ceroni (1,2)

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Synthetic cells, which aim to replicate the architectures and behaviours of living systems, have traditionally been used as tools to further our understanding of cell biology. Recently, interest has shifted to the applications that such systems could have beyond pure research purposes. One such example is the adoption of synthetic cells as therapies where in situ drug synthesis and release are triggered by external stimuli, supporting advanced drug delivery with decreased off-target effects. To this aim, synthetic cells can be engineered to incorporate cell-free protein synthesis buffers that support genetic control and interaction with the external environment via synthetic constructs. By engineering switches - either through genetic regulation or molecular chemistry - synthetic cells can act as programmable therapeutics with tuneable cargo release.

Here we present a synthetic cell platform capable of switchable protein expression dependent on local temperature. We show temperature-dependent in situ protein synthesis in Giant Unilamellar Vesicles using RNA thermometers, which confer translational control over gene expression. We also demonstrate that the membrane pore alpha hemolysin can self-insert into GUVs, thereby making an effective and autonomous release mechanism for cargo. The system functions as a proof of concept for advanced drug delivery vehicles capable of triggered, local release, conferring a much-reduced likelihood of off-target effects. Beyond therapeutic applications, this same technology of synthetic cells capable of sense and response can be applied further afield to the circular bioeconomy, industrial chemical synthesis, biosensing and bioremediation.

Notes:

Randomizing gene regulatory regions using prime editing

Jonas Koeppel, Mélanie Gouley, Valentin Reberning, Leopold Parts

JK, MG, VR, LP: Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK

MG: Ecole Supérieure de Biotechnologie de Strasbourg, University of Strasbourg, France

VR: FH Campus Wien University of Applied Sciences, Vienna, Austria

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Enhancer clusters recruit transcription factors and activate genes from a distance of one million base pairs and more. However, how individual enhancers within a cluster interact and how their spacing and relative orientations drive gene expression is not well understood. To answer these questions, we aim to create thousands of variants of these enhancer cluster regions in their endogenous context and measure the resulting gene expression levels.

To achieve this, we used CRISPR prime editing to tile an enhancer cluster in the OTX2 homeobox gene with symmetrical loxP sites. We integrated many copies of doxycycline-inducible prime editors in the genome of the haploid HAP1 cell lines using Piggybac transposase and further increased prime editing efficiency by knocking out DNA mismatch repair. This strategy resulted in up to 80% insertion rates of loxPsym sequences after a single transfection.

Induction of Cre recombinase resulted in stochastic inversions and deletions within the enhancer cluster. We use Oxford nanopore long-read sequencing with Cas9-based enrichment to sequence the new architectures of the enhancer cluster and discover architectures with differential OTX2 expression. Our findings demonstrate the feasibility of highly efficient, multiplexed prime insertions, and will shed light on the grammar of gene expression for regulatory elements.

Poster Presentation Abstracts

Proliferation and migratory activity suppression in IL-21-silenced colorectal cancer cells

Eshtiyag Abdalkareem, Lim Boon Huat, Khoo Boon Yin

Eshtiyag Abdalkareem, Tropical Medicine Research Institute (TMRI), Khartoum, Sudan.

Lim Boon Huat, School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

Khoo Boon Yin, Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Pulau Pinang, Malaysia.

The interleukin-21 (IL-21) protein was found to be expressed at an elevated level in clinical samples of colorectal cancer patients without or with a schistosoma mansoni infection that was collected from Sudan. The IL-21 gene in HT29 and HCT116 cells were then correlated to cell proliferation and cell migration, as well as the cellular mechanisms associated with gene expressions. Our results demonstrated that silencing the IL-21 gene in HT29 and HCT116 cells increased the cytotoxic level and decreased the proliferation genes mRNA expression in both colorectal cancer cells. Moreover, specific gene silencing reduced the migration of cancer cells compared to non-silenced cancer cells.

Our results indicate the importance of silencing the IL-21 gene, which pave the way for the discovery of important factors to be used for the design of drugs or cost-effective supplements to effectively treat patients having infectious disease and colorectal cancer simultaneously in the future.

Modulating co-translational protein folding by rational design and ribosome engineering

Minkoo Ahn, Tomasz Wlodarski, Alkistis Mitropoulou, Sammy Chan, Haneessh Sidhu, Elena Plessa, Thomas Becker, Christopher Waudby, Roland Beckmann, Anais Cassaignau, Lisa Cabrita, John Christodoulou

University College London, UK

Co-translational folding is a fundamental process for the efficient biosynthesis of nascent polypeptides that emerge through the ribosome exit tunnel. To understand how this process is modulated by the shape and surface of the narrow tunnel, we have rationally engineered three exit tunnel protein loops (uL22, uL23 and uL24) of the 70S ribosome by CRISPR/Cas9 gene editing, and studied the co-translational folding of an immunoglobulin-like filamin domain (FLN5) within a tandem repeat protein. Our precise thermodynamics measurements employing 19F/15N/methyl-TROSY NMR spectroscopy together with cryo-EM and molecular dynamics simulations reveal at atomic resolution the manner in which the variations in the lengths of the loops present across species exert their distinct effects on the free energy of FLN5 folding. Using a range of extensions and truncations of these loops, we find that a concerted interplay of the uL23 and uL24 loops is sufficient to alter co-translational folding energetics, which we highlight by the opposite folding outcomes resulting from their short extensions. These subtle modulations occur through a combination of the steric effects relating to the shape of the tunnel, the dynamic interactions between the ribosome surface and the unfolded nascent chain, and its altered exit pathway within the vestibule. These results illustrate the important role of the exit tunnel structure in co-translational folding, and provide useful principles for how to remodel it to elicit a desired folding outcome.

Future Organisms: synthetic genomics and responsible innovation in the UK, the USA and Japan

Jane Calvert, Robert Smith, Koichi Mikami, Erika Szymanski

University of Edinburgh, Keio University, Colorado State University

Synthetic biologists are starting to work at new scales, building whole genomes and megabase constructs and introducing new degrees of biological complexity. These developments offer plenty of creative opportunities. They drive leaders in the field to ask questions like, 'what should be designed?' and 'which tools do we need?' However it is also important to ask, 'who gets to design?' and 'how will we relate to the new organisms that are being created?' Future Organisms is the first dedicated social scientific project on synthetic genomics, funded by UKRI and the NSF. Assembling researchers from the UK, the USA and Japan, our aims are twofold: to carry out a social scientific investigation into synthetic genomics and to develop new approaches to Responsible Research and Innovation (RRI) through this investigation. We are studying national and international policy and funding strategies for synthetic genomics and the expectations embedded within them. We are also exploring the ways in which synthetic genomics alters human relationships with other organisms by positioning humans as designers of other species. One of our key areas of investigation is large-scale genome synthesis in mammalian synthetic biology - both the challenges it presents and the opportunities it gives rise to. In this poster we will present our findings to date.

A microfluidic platform to prototype continously-transcribed RNA-based systems

Kate Collins, Dr. Claire Stanley, Prof. Guy-Bart Stan, Dr. Thomas Ouldridge

Imperial College London, UK

RNA strands can be rationally engineered to perform versatile functions such as biosensing, catalysis, and logic computation. RNA-based circuits are a promising, but relatively underexplored technology for synthetic biology. This poster will describe a microfluidic platform developed to study continuously transcribed RNA-based systems. The device contains microchambers in which DNA templates have been immobilised so that transcription reagents and waste products can be continuously added and removed from the system. As part of this work, a new method to pattern and immobilise DNA inside microfluidic devices will be described. Continuously transcribed RNA reaction networks can be monitored by fluorescence microscopy using sequence-specific fluorescent probes. Finally, potential applications of this platform will be discussed, with a focus on its role as a test bed for an RNA-based computational network toolbox for synthetic biology.

Comparing the performance of Base Editing and Prime Editing to HDR for saturation variant effect measurement

Alistair Dunham, Elin Madli Peets, Gareth Girling, Leopold Parts

Wellcome Sanger Institute, UK

Understanding the consequences of mutations can be greatly beneficial for understanding and improving human health. It allows natural variation to be understood and treated effectively and allows the development of new variant genes and regulatory sequences for synthetic applications. This has led to a range of experimental methods aiming to measure the fitness effect of variants, both in coding and non-coding regions. One of the most common methods for saturation genome editing (SGE) in human cells is utilising homology directly repair after CRISPR-Cas9 cuts to insert a library of variants, which are then tested by selection or transcriptomics. Base Editing (BE) and Prime Editing (PE) technologies can also be used to introduce a library of variants to test, with several potential advantages. BE experiments are potentially cheaper and can therefore be scaled up to larger libraries with the drawback of less precise editing. PE potentially offers a balance between cost and precision. Both can also easily target many regions of the genome in one experiment.

In this work we apply saturation BE and PE libraries to multiple genes (BE: BAP1, BRCA1 & DDX3X; PE: DDX3X) with existing HDR based SGE results to compare performance. We also screen a PE library targeting all ClinVar variants in 43 essential genes and 30 non-essential controls, alongside a range of exon level controls, to test the ability of PE experiments to assess many loci simultaneously. This design utilises a self-targeting construct so that the inserted guide sequence contains a copy of the genomic target, allowing guide sequencing to measure each guides editing efficiency, which we benchmark using genomic and scDNA sequencing. We have optimised the screening process and recently completed the second round of screens with pending analysis pending.

Discovery of a Polyethylene Terephthalate (PET) Hydrolase from the Human Saliva Metagenome and its use to Bio-functionalise PET surface by Genetic Code Expansion

*Bhumrapee Eiamthong, Piyachat Meesawat (a), Thanakrit Wongsatit (a), Jariya Jitdee (b), Worawan Bhanthumnavin (b), and Chayasith Uttamapinant (a),**

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Plastics such as polyethylene terephthalate (PET) accumulated in and polluted the environment after their use, partly due to limited availabilities of approaches to recycle them especially in resource-limited settings. Biodegradation of PET by hydrolase enzymes could be an alternative approach to recycling of PET and be combined with upcycling processes to convert PET monomers into high-value compounds. The discovery of PET hydrolases, IsPETase from environmental isolation and LCC from the microbiome of compost, has led to the development of useful engineered PET hydrolases including FAST-PETase and LCCICCG, enabling sustainable recycling of PET plastics. The human microbiome could be a potential discovery source of PET hydrolases because of the widespread use of PET in food and beverage packaging.

In this study, we discovered MG8, a PET hydrolase from the human saliva metagenome, using bioinformatics. Without any enzyme engineering, MG8 exhibits active PET degradation over several natural and engineered PET hydrolases under different temperature and salinity conditions. We also incorporated a non-canonical amino acid (2,3-diaminopropionic acid, DAP) within the active site of MG8, repurposing MG8 into a covalent binder to a PET surface. We used a split green fluorescent protein system linked with MG8(DAP) to demonstrate the binding of MG8(DAP) to PET and other polyester plastics.

The discovery of the first PET hydrolase from the human metagenome has revealed the potential of metagenomic data as a source for industrially useful enzymes. It also suggests that human microbiomes may have evolved in response to abundant use of plastics in humans. By using a synthetic biology tool such as genetic code expansion, MG8 can be repurposed as a tool for plastic functionalisation, expanding the utility of PET.

Transcriptional dysregulation in pulmonary fibrosis: A Tail of Two Mures

Thea Fennell, Ieva Berzanskyte, Rihab Gam, Minkyung Sung, Julian Gough

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Background: Idiopathic pulmonary fibrosis is a terminal inflammatory lung disease that results in permanent scarring (fibrogenesis). Bleomycin is a drug used to induce fibrosis in mouse models, typically C57Bl/6 reference mice. However, meta-analyses show inter-strain heterogeneity in fibrotic phenotype, e.g. resistance in BALB/c. Transcriptomic profiling has previously been applied to characterise murine lung fibrosis, but the dataset presented here extends it across different strains. This cross-strain comparison helps to qualify inferences from murine models and suggests genetic risk factors that could inform clinical research.

Methods: Transcriptomic datasets were generated from both C57Bl/6 and BALB/c mice. Test mice were administered bleomycin (BLM) - with tissue samples sequenced from healthy (control), fibrotic, and contralateral lungs at 7-14 days. Differentially expressed genes (DEGs) were identified by comparison between sample types within each strain, followed by network analysis of strain-specific DEGs. In addition to the transcriptomic data, strain-specific variants were identified in fibrosis-related genes. Experiments were also conducted, quantifying TGF□-induced stress fibre formation in lung cells obtained from the two strains.

Results: DEGs calculated between treated/untreated lung samples were enriched for general fibrosis-related processes across strains. Some fibrogenic processes, however, were specifically enriched in C57Bl/6 DEGs. Moreover, amongst these DEGs, the anti-fibrotic gene Ctsk was found to be upregulated in response to fibrosis in BALB/c specifically. The genetic analysis reveals a non-synonymous variant (P170L) located in the FRA1 transcription factor of BALB/c, which regulates a number of fibrosis-related genes observed to respond strain-specifically to BLM treatment. Further, human FRA1 possesses a comparable SNP (P170T) and has been linked to lung disease in GWAS studies. in vitro TGF□-induced fibrosis in lung cells was also found by experiment to be consistent with known in vivo BLM-model strain phenotypes, thus confirming that BALB/c resistance is not model-specific.

Conclusions: Transcriptional differences in response to lung fibrosis in mice have been established for C57Bl/6 and BALB/c strains, mirroring the known difference in phenotypic response whereby BALB/c is more resistant to fibrogenesis. Analysis of gene set expression - and corresponding subnetwork states - implicates the transcription-level regulation of multiple factors in fibrosis risk. Investigation of these regulatory nodes as potential therapeutic targets is thus recommended, including transcription factors (FRA1) and other regulators (e.g. Nos2, Il6). The results complement the established view of IPF: a genetically complex disease, caused by dysregulated response.

Development of an Autotransporter-based heterologous protein delivery system in Salmonella

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Bacteria are attractive hosts for heterologous protein secretion due to their ease of genetic manipulation, rapid growth, and low production cost. These advantages can be applied towards the development of bacteria-based therapeutics and are further strengthened through the ability of some intracellular bacterial pathogens to colonize tissues and stimulate an immune response. A prime example of this is in vaccine development with bacteria-based vaccines, such as Recombinantly Attenuated Salmonella Vaccines (RASVs), which can elicit a strong mucosal and humoral immunogenic response.

The modular structure of the Type V Autotransporter (AT) secretion system provides a convenient platform for protein secretion, as the native passenger can be replaced with heterologous peptides. We have re-engineered an AT system originally developed by Sevastsyanovich et al. (2012) for the efficient, regulated secretion of heterologous peptides in mono- or multiplexed-antigens in Salmonella. In initial versions, we made and evaluated peptide secretion with truncated versions of the Pet AT. Through this, we developed a more efficient secretion system, as shown by the secretion of SARS-CoV-2 immunogenic linear epitope sequences. Using in vitro secretion assays, Western blotting and immunofluorescence microscopy, we detected in vitro secretion of heterologous peptides via engineered ATs in Salmonella. We use a dual-inducible Salmonella promoter system to detect peptide secretion from extracellular and intracellular bacteria.

Our findings validate the use of an optimised AT-based system that facilitates in vitro and in cellulo peptide secretion from Salmonella. Due to the modular design, the peptides secreted by the system can easily be replaced through cloning. Therefore, this work contributes a platform for bacteria-based peptide delivery with an application starting in, but not limited to, vaccine development.

Development and local production of a multiplexed CRISPR-based diagnostic platform for SARS-CoV-2 variant surveillance

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The COVID-19 pandemic has emphasized the importance of improving health security and pandemic resilience. Gold-standard RT-PCR is commonly used for viral diagnosis in viral nucleic acid detection. However, the test requires expensive machinery and trained personnel to administer and analyze. Accurate molecular testing can be difficult to access in low- and middle-income communities, particularly in locations remote from clinical laboratories, putting residents at an increased risk of viral transmission. Point-of-care (POC) nucleic acid tests are under development and offer the opportunity to lower the barrier to broader use.

We previously developed a molecular tool for SARS-CoV-2 RNA detection by coupling recombinase polymerase amplification (RPA) with CRISPR-Cas13 RNA detection technology, and demonstrated its comparable sensitivity to the gold-standard RT-PCR. We further developed a multiplexed gene detection platform based on CRISPR and applied it to simultaneously diagnose SARS-CoV-2 infection and pinpoint the variant of concern (VOC)-such as Delta (B.1.617.2) and Omicron (B.1.1.529)-causing the infection. The features of the platform, such as freeze-dried reagents for simple handling and storage, direct-to-eye or smartphone-based readouts, and a one-pot multiplexed detection method, facilitate it suited for POC applications.

To assure sustainable production and usage of this diagnostic technology in Thailand, we locally produced biochemical components of CRISPR diagnostics, in particular RPA components, and created our own recipe of RPA that has equivalent sensitivity to commercial counterparts. Local formulation and manufacturing represent a shift toward long-term plans to provide affordable access to critical diagnostics reagents and improve diagnostic capacity in low- and middle-income countries. Our CRISPR-based detection for concurrent COVID-19 diagnosis and variant surveillance may enable the long-term application of CRISPR diagnostics for COVID-19 and other epidemic infections in POC settings.

Building Bionic Cells for Advanced Therapies

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Cells are nature's greatest source of inspiration and resources for the design of programmable systems with compartmentalized tools and information that, autonomously or by external control, perform tasks with a degree of precision relevant for physiological environments. In order to harness cellular components and fast-track the development of advanced therapeutics, one can take a top-down approach (i.e., de-constructing and rewiring pre-existing cells) as well as build cellular components from the bottom-up. We propose a strategy to leverage the sophistication of living (E. coli) cells and the robustness of synthetic organelle-like compartments (i.e., artificial liposomes) to build a bionic cell with enhanced capabilities. While the stability and viability of the system and the intrinsic functionalities of the living cell (e.g., motility, gene expression) are retained, we imparted extra functionality to the system via actuation of the artificial compartment. Furthermore, engineering effort is in the process to coat the bacterial cells with different populations of artificial compartments, interface DNA nanostructures and to develop a microfluidic platform to study short-range navigation and targeting of chemical species. We anticipate that this study could yield a simple and pragmatic approach to engineer bionic cellular systems for advanced therapies.

Non-allosteric mechanism enables rapid evolution of novel biosensors.

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Transcription factors (TFs) regulate transcription initiation rate of their target promoter through allosteric mechanisms. While they confer high signal-to-noise ratio, stringency, and robustness of their regulation, they significantly complicate the re-designing of TFs: to acquire a new response to the novel target molecules, they require not only the fabrication of selective binding pocket to the targets, but also rebuilding the information transmitting pathway harnessing ligand binding to TF-DNA binding. In this context, ligand-induced folding (LIF) is an attractive mode of action for engineering TFs (Kohn 2005, Feng 2015). Because this mode detects the ligand binding via stabilization of TF, not by its conformational change, engineering effort for creating artificial TF could be much simpler. Indeed, we found that random mutagenesis frequently converts transcription factors into ligand-induced folders, thereby resulting in excellent switchers with high stringency (Kimura 2020). In this report, we describe several unique properties that are characteristic to TFs functioning through LIF mechanism.

AraC is one of the symbolic allosteric molecular switches and regulates arabinose promoter (pBAD) both in negative and positive manner in response to L-arabinose. By the removal of the negative regulating site (O2), AraC behaves as a super-activator (always-ON phenotype) to the resulting promoter. We found that whole-gene random mutagenesis turned as much as 20% of the AraC variants into decent switchers of the O2-deficient pBAD. These switching-variants were confirmed to be the LIF-type (non-allosteric) biosensors, due to the positive (agonistic) response to D-fucose, the known antagonist of AraC. Most of these LIF-type AraC variants retained switching function to allosteric (pBAD) promoters as well, and the allosteric and non-allosteric promoters were found to be fully compatible in a single cell, enabling to be integrated into various unique and complex circuit behaviors that are otherwise difficult to achieve.

Another prominent feature of LIF-type biosensors is their surprising evolvability of their ligand response. We created 32 different AraC variants with/without five mutations previously shown to turn AraC into salicylic acid responder (Frei 2018). While the allosteric pBAD promoter required at least four mutations in AraC, the majority of the 32 variants behaved as salicylic acid responders on non-allosteric (O2-deficient) pBAD promoter. Being liberated from the requirement for conformational changes upon ligand binding, LIF-type biosensor is not only easy-to-emerge, but also become highly accessible to the new functions. Thus, LIF may be a significant evolutionary accelerator of novel regulatory/ sensory functions both in the natural and biotechnological contexts.

Spliceosome-mediated RNA trans-splicing for the repair of genetic mutations associated with CTNNB1 syndrome

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Spliceosome-mediated RNA trans-splicing (SMaRT) is an artificial exon replacement strategy that allows the replacement of exons on target pre-mRNA using a rationally designed exogenous pre-RNA trans-splicing molecule (PTM) and cell's own splicing machinery. In principle, it holds great potential for gene therapy as it could maintain the endogenous control of mRNA expression. We aimed to apply trans-splicing for CTNNB1 syndrome. CTNNB1 syndrome is a rare neurodevelopmental disorder, which is caused by de novo occurrence of loss-of-function mutations within one allele of the CTNNB1 gene. It manifests itself in an array of motor, cognitive and behavioral impairments that are directly linked to the insufficient production of β-catenin protein, encoded by CTNNB1 gene. As there is currently no treatment available for this syndrome, the aim of this study was to exploit the potential of SMaRT strategy for the replacement of exons bearing CTNNB1 syndrome-associated mutations. In this study, trans-splicing efficiency for the repair of CTNNB1 syndrome-associated mutations was cytometrically determined on the split yellow fluorescent protein (YFP) reporter in HEK293-T cells. Split YFP reporter assay successfully revealed best intron in CTNNB1 gene and PTM candidate for the efficient trans-splicing in HEK293-T cells. In order to further improve its efficiency, trans-splicing was tested in combination with short anti-sense RNAs (asRNAs) that were designed to mask various cis-splicing elements. The combination of best PTM candidate and asRNAs was tested on a split YFP reporter system and increased trans-splicing efficiency by 2-fold. In summary, our study showed that SMaRT strategy tested on reporter system in HEK293-T cells provides a good starting point for further testing of trans-splicing strategy in iPS cells, obtained from patients with a diagnosed pathogenic CTNNB1 mutation.

Establishing a Clostridium butyricum Strain for Heterologous Gene Expression

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Clostridioides difficile is a spore forming, anaerobic Gram-positive bacterium. If ingested, spores can cause C. difficile associated disease (CDAD), with symptoms ranging from diarrhoea to pseudomembranous colitis. Germination of spores in the gut is both induced and inhibited by primary and secondary hepatic bile acids, respectively, and the biotransformation between the two structures by 7α-dehydroxylation is therefore key in the onset of CDAD. Colonic bacteria capable of 7α-dehydroxylation are limited and are mainly from the Clostridia family. These species, particularly Clostridium scindens, have been implicated in bile acid-mediated colonisation resistance against C. difficile (Buffie et al., 2016). Unfortunately, research into C. scindens has been hindered by its genetic intractability. To overcome this, Clostridium butyricum, a gut commensal, was chosen as a chassis strain to study the 7α-dehydroxylation pathway, encoded by bile acid inducible (bai) genes.

An inducible CRISPR-Cas9 system RiboCas, developed by Cañadas et al (2019), was first optimised for work in C. butyricum. It was then used to develop a strain primed for gene insertion by Allelic-Coupled Exchange (ACE) (Ng et al., 2013). Three metabolically important genes were chosen to generate auxotrophic mutants, and to allow subsequent selection for insertion of bai genes into the genome by concomitant gene repair and restoration to prototrophy.

Truncation of all three chosen genes was achieved using RiboCas, and the expected auxotrophic phenotypes demonstrated on appropriate minimal media. Successful repair of all three genes was also achieved with ACE, and the expected return to prototrophy was observed in all cases. This demonstrates the suitability of all three loci for subsequent cargo insertion. Following individual assessment, a triple knockout strain of C. butyricum was generated, providing multiple sites for gene insertion. A reporter assay was then carried out to assess promoters successfully exemplified in other Clostridial genetic tools, to select the most appropriate for heterologous gene expression in the C. butyricum genome.

This work has demonstrated the successful use of CRISPR-Cas9 genome editing to generate auxotrophic gene truncations in C. butyricum, and used this to develop a triple-knockout strain that offers a vehicle for heterologous expression of bai genes. Once optimised, this strain could then also be engineered for stable expression of other genes, for example those involved in the generation of biotherapeutics, and would allow targeting to the gut.

Taking long-term policy visions seriously: the challenge for synthetic genomics in Japan

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Synthetic genomics is a field of synthetic biology characterized by its ambition to construct long-chain DNA for genome-scale (re)design. Its emergence was marked by the creation of M. mycoides JCVI-syn 1.0, and in 2016 an international consortium Genome Project-write was launched to further advance the field with the prospect of synthesizing the entire human genome. This move stimulated the research in some countries, and Japan made its commitment by starting a local research program focusing on synthetic genomics in 2018. While the international consortium makes the promise of improving our health, however, this Japanese program has little emphasis on human health. We argue that this divergence reveals the influence of the local vision of bioeconomy in the country, and that its nature can have significant implications for translating research into clinical applications.

The Japanese vision of bioeconomy was shaped in the context that the government strengthened its policy control over scientific research in the country to recover from the serious economic downturn started in the early 1990s. It was built upon the strong belief that Japan has an advantage over other countries in microbiology due to its relevance to traditional agricultural and food industries. Therefore, the effort was made to find microorganisms with desirable genetic functions and use their genetic systems to create efficient bioreactors by introducing the system to a host microorganism that could be managed at the industrial scale. Influenced by this vision, synthetic genomics in Japan is framed as development of methods for producing and then managing long-chain DNA and is aligned closely with studies on delivery of synthesized DNA into an artificial cell, which is considered as a promising novel host for any genetic systems today.

The problem with this local framing of synthetic genomics is not so much about its focus, and the effort may well provide valuable knowledge and/or techniques for clinical applications. However, because the local research program is designed without a commitment to a specific use context, such findings likely need further adjustment(s) when it comes to their applications. The adjustment(s) for clinical applications can be particularly complex or even impossible because of tight constraints on what is considered 'do-able' with respect to the bodily as well as medical environments in which they are to be used. Our analysis therefore suggests that synthetic genomics in Japan can potentially face significant challenge in delivering human health more than similar efforts in other countries.

Forest Restoration Paradigms: Emerging Opportunities for Synthetic Biology in Forestry

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Globally, there are an estimated 2 billion ha of damaged forests, and the effects of climate change indicate an even higher need for forest restoration. Revegetation, ecological restoration, functional restoration, and forest landscape restoration are the four paradigms for forest restoration that are recognised and explored. A degraded beginning point and an idealised natural forest's ending point are used to study restoration. Current restoration paradigms are significantly challenged by factors such as global change, climate variability, biotechnology, and synthetic biology, underlining the significance of having well-defined objectives centered on healthy ecosystems. The microbiological sciences and human health have received the majority of the attention to date from the rapidly expanding field of synthetic biology. The zeitgeist suggests that synthetic biology will also soon have a significant impact on forestry. Agriculture, fisheries, and forestry are the world's three core sectors; overcoming these issues will be made easier by the sector's long history of early adoption of revolutionary innovation, such as the genetic innovations that underlie synthetic biology. However, given that the business is dominated by higher plants and mammals, where big and frequently polyploid genomes and a lack of sufficient tools challenge the ability to deliver results in the short term, the use of synthetic biology within forestry may be hindered. However, the rapidly expanding area of synthetic biology, advances in genome design and synthesis, and increasingly effective molecular tools like CRISPR/Cas9 may offer prospects that go beyond the creation of novel breeds. In particular, the development of synthetic biosensors, synthetic speciation, microbial metabolic engineering, mammalian multiplexing CRISPR, innovative anti-microbials, and initiatives like Yeast 2.0 all have the potential to significantly alter forestry in the short, medium, and long terms. Particularly, synthetic biology promises to aid primary industries by boosting production and sustainability, supporting the sector's profitability in the face of global difficulties towards conservation of forests.

Keywords: Conservation, Synthetic biology, forestry, microbial.

Enzymes for Innovation: Expanding the Molecular Toolbox

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New England Biolabs sells hundreds of reagents for molecular biology, yet enzymes in the NEB catalogue represent only the tip of the iceberg in terms of the diversity and range of enzyme activities characterised at NEB. We realise that in molecular biology, development of new analysis tools often lead to new discoveries. To expand the molecular toolbox for key customers, the "Enzymes for Innovation" initiative aims to identify and release a number of enzymes having unique activities for analysing DNA, RNA, proteins and glycans that will enable development of new workflows and applications. I will discuss several examples of Enzymes for Innovation discovered from the basic research programs at NEB. We expect that these enzymes will enable key customers to generate new ideas and accelerate workflow development.

Identification of Opioid Receptor Agonists Using Self-Produced Peptide Libraries in Yeast

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G protein-coupled receptors (GPCRs) are membrane proteins that encompass a large percentage of drug targets. Signaling networks initiated from GPCR activation are complex and often drug and context specific, making drug discovery screens challenging. The "null" background of the yeast Saccharomyces cerevisiae provides a simplified platform to assay human receptor and ligand interactions. In addition to simplified signaling, the genetic tractability and cellular physiology of yeast enable tethering of peptides to the inside of the yeast cell wall in an orientation able to activate heterologous GPCRs. This strategy allows the compartmentalized screening of hundreds of thousands of peptides for their ability to agonize receptors. We demonstrate the use of this strategy for the first time as a screening tool for GPCRs. In starting with a non-specific endogenous opioid peptide, we created a library of peptide mutants and screened for those that activated each of the opioid receptors, finding general amino acid trends that inform our understanding of ligand-receptor dynamics, and peptide leads that are unique for receptor type.

Designed allosteric protein logic

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Regulation of protein activity is one of the key processes in living organisms and is achieved through diverse mechanism such as transcriptional regulation, posttranslational modifications, multimolecular complex formation and allostery. We developed a generally applicable regulation of proteins called inserted peptide structure regulator (INSRTR). A coiled coil-forming peptide was inserted into a loop of a target protein. By strategically selecting specific loops for insertion, the structure of the protein was only partially afflicted and the protein retained its function. Upon addition of a complementary coiled-coil-forming peptide to the inserted peptide (termed regulatory peptide), the inserted peptide adopted a rigid helical conformation, expanding the distance between the inserted termini in the loop. This in turn disturbed the structure at the site crucial for protein function without inducing a large conformational change in the protein.

This design principle enabled construction of ON/OFF protein switches, their regulation by small molecule and logic functions in mammalian cells. We tested this strategy on ten different proteins, namely enzymes, fluorescent protein, signaling mediators, DNA binders/ transcriptional regulators and an antibody domain in a chimeric antigen receptor on T cells.

We successfully implemented INSRTR into ten proteins with different folds and functions, demonstrating the robustness of this platform. Several positions of insertion for each protein were tested. We were able to prepare at least one variant with strong inhibition for each protein. Stronger affinity between the regulatory peptides correlated with the more potent response. Coupling of INSRTR to chemically-regulated proteases facilitates regulation of diverse proteins by small molecules, for many of the tested proteins which has not been reported before.

INSRTR platform represents a widely applicable principle of regulation for selected proteins and their coupling to chemical signals, which could be relevant for therapeutic applications.

A design and discovery pipeline for synthetic memory bacterial biosensors for the mammalian gut

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Whole-cell bacterial biosensors encoding synthetic memory recording circuits have recently emerged as exciting tools for non-invasive surveillance of the mammalian gut and other similarly inaccessible environments. However, despite the large number of sensing mechanisms used by microbes in nature, our current catalogue physiologically relevant biosensors that have been validated for use in the gut remains limited. To address this, we have developed a high-throughput, memory-based screening pipeline capable of simultaneously testing hundreds of biosensors in vitro or in vivo within the mammalian gut. Advancing on our previous work in this area, we now present the development of a computational pipeline to extract compatible sensing components from genomic databases. We also demonstrate considerable gains in robustness, cost and flexibility that have been made through cloning and analysis of uniquely DNA-barcoded biosensor strains. Through in vitro and in vivo screening of biosensor libraries we have identified a set of new engineered biosensors that respond to a range of key signals of interest in the lab and the clinic. These new biosensors can not only open multiple avenues for scientific and clinical investigation of new molecules in the gut, but also further our understanding of the bacteria from which the sensing components originate. With flexibility and broad applicability, this platform has the potential to greatly accelerate the development and discovery of new sensing components for use in engineered whole cell biosensors and various other related applications.

Module design for synthetic circuits

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Synthetic biology involves rationally engineering biological processes with the objective of obtaining behaviors in organisms that are not realized in nature. A desired behavior can potentially be realized by various synthetic circuit candidates. The question that subsequently rises is, which candidate synthetic circuit, should one build?

Each candidate synthetic circuit can be designed from parts, such as promoters, terminators etc. However, determining the combination of parts that lead to predictable and robust behavior can increase significantly the experimental effort required, let alone automate the Design-Build-Test-Learn cycle in synthetic biology. The challenges mainly rise from uncertainty (eg due to intrinsic biological variability, load on cell's resources, etc) and context dependency of a part's behavior, both of which are inherent in biology.

To minimize the experimental effort, 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. The method we introduce depends on maximizing the expected information gain for the candidate circuits assuming that a module or a set of modules were to be experimentally characterized. In contrast to previous synthetic circuit design methods, we explicitly consider all the candidate synthetic circuits accompanied by their parameter uncertainty. Due to the nature of the modules, we can also consider what has been previously built in the lab. We demonstrate our method's applicability with an in-silico study based on a real-world biological example.

CSIR Synthetic Biology and Precision Medicine Centre Bio-foundry Program : Development for first of its kind Biofoundry lab in Africa

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Background: Global bio-foundry Alliance (GBA) has been established between countries including the UK, US, Japan, Singapore, China, Australia, Denmark, and Canada through 16 research institutions. Global bio-foundry Alliance plays the key role in the synthetic biology drive towards a new global bioeconomy that is accelerated by advanced technology innovation. Establishment of Biofoundry program in South Africa and in Africa will plan key scientific and the strategic role in promoting synthetic biology and precision medicine program in Africa. This would further enable bioeconomy and industrial development towards SME program. At our CSIR Synthetic Biology and Precision medicine Centre, we are currently establishing biofoundry lab that will implement various synthetic biology and precision medicine projects in South Africa.

Methods: We are currently establishing two research components in the CSIR Synthetic Biology and Precision Medicine Centre Bio-foundry program which includes industrial synthetic biology and functional precision medicine program. We implement Biofoundry biodesign and biological engineering Design-Build-Test-Learn (DBTL) cycle into our industrial synthetic biology and functional precision medicine program. In our Industrial synthetic biology program, we are working on a) ValitaCHO: Development of superior CHO cell line system for hyper-burst protein expression system using directed evolution and synthetic biology approaches; b) Lactochassis: Designer microbes for industrial synthetic biology platform applications; In our Cancer Precision Medicine program: we are working on drug repurposing based drug sensitivity screening platform for B-cell malignancies and ovarian cancer treatment for South African patient cohort.

Results : We are currently at the Design phase of the Design-Build-Test-Learn (DBTL) cycle in our industrial synthetic biology and functional precision medicine program. We have so far have progressed in generation of the preliminary data on ValitaCHO cell-line chemstress fingerprinting profiling. We are currently designing the directed evolution approach for generation superior CHO cell line. In the Lactochassis project, we are currently designing the computational biology based genome mapping for Lactochassis. In our precision medicine platform, we are currently progressing in design and build phase of platform where we have currently procured 770 cancer drugs for drug repurposing platform which can be applied for blood and ovarian cancer cohort.

Conclusion: Using Bio-design DBTL cycle, we aim to implement our industrial synthetic biology and cancer precision medicine platform at CSIR Synthetic Biology and Precision Medicine Centre. These platforms will enable establishment of one of the first Biofoundry labs in Africa.

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Expanding the genetic code to produce semi-synthetic prions

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Prions are transmissible proteinaceous agents that cause invariably fatal neurodegenerative diseases and propagate through a poorly defined mechanism involving the templated misfolding of the native cellular prion protein (PrPC) into proteinase K (PK)-resistant infectious aggregates (PrPSc). Existing methods for direct fluorescent tagging of PrPSc are sup-optimal, as methods using fluorescent fusion proteins cause steric hindrance of templated misfolding of PrPC, and methods that require antibodies naturally preclude the use of live-cell imaging - a vital prerequisite for interrogating transient interactions.

Genetic code expansion exploits bio-orthogonal translational components, sourced from evolutionarily distant archaea, to site-specifically incorporate unnatural amino acids (UAA) into proteins by stop codon suppression. By using genetic code expansion to incorporate a UAA compatible with tetrazine-based click chemistry into PrPC in a mouse neuroblastoma cell line, we have produced fluorescent taggable PrPC with only a single amino acid modification. This modified PrPC retains its tag upon pathogenic misfolding into bona fide infectious semi-synthetic prions, allowing visualisation of both conformers. Crucially, this system is conducted in live cells and therefore will enable live-cell imaging of dynamic prion infection routes for the first time.

TF Decoys as Tools to Control Mammalian Cells

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Transcription Factors (TF) are key cellular regulators that bind TFBSs (Transcription Factor Binding Sites) and recruit transcription machinery to specific loci within a vast DNA genome. Therefore, due to their potential applications in cell engineering, intracellular TF control is highly desirable. Traditionally this is done by engineering protein abundance, post-translational modifications, and subcellular localisation. However, these techniques often rely on laborious or metabolically expensive proteins. A less established route in which the levels of TFs can be controlled is by altering the number of their TFBSs in cells via addition of DNA Sponges, repetitive DNA constructs rich in TFBSs that compete for TF binding with functional sites. Development of such DNA sponges could provide a tool for changing expression profiles within cells simply through DNA addition. However, for wide use the tool must be easy to assemble and shown to work in mammalian cells reliably.

This project addresses the significant hurdle of repeat assembly for DNA sponges by designing a cyclical Golden Gate method for their construction. We also go on to show that DNA sponges in mammalian cells can effectively repress the actions of orthogonal and native TFs as planned. Tests with sponges targeting orthogonal TFs such as TetR and dCas9 show that the presence of sponge constructs can reduce activity at active sites by up to 70%. Furthermore, repression can be controlled by sponge repeat length, the amount of construct given to cells and the level of the TF in the cell. Sponges have also been designed and assembled to perturb the human TF NRF2, a regulator of cellular response to oxidative stress. These sponges should lead to cell death likely due to reduced activation at NRF2 genomic targets known as AREs.

In conclusion, TF sponges can be used to engineer intracellular TF levels and have effects on gene circuits and cell fate. Applying this tool to a variety of native TFs may further exemplify their broad utility and potential.

Virtual Poster Presentation Abstracts

A time-resolved proteome allocation model for evaluating heterologous systems

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The correct management of the burden caused by heterologous expression in engineered bacteria is key for the success of synthetic biology projects. It is therefore desirable to develop theoretical tools that relate easily observable macroscopic culture parameters to the molecular aspects of circuitry design. To this aim, the bacterial cell can be modelled as the sum of different sets of proteins represented as fractions of the total proteome. All the proteins within a fraction are considered to have roughly a single activity and genetic regulation. Previously, other authors have considered three native fractions defined as nutrient fixators, biomass producers, and structural proteins, and used this approach to explain how the maximum growth rate of Escherichia coli responds to changes in the culture media. Here, we expand the model by deriving an equation for the evolution of a protein fraction in time, which depends on a regulatory function specifically defined for said fraction. Quantifying the GFP expression under the control of IPTG-inducible T7RNAP, we observed a linear correlation between production rate and growth rate through the whole exponential phase of three E. coli strains. From these results we propose a simple hyperbolic regulatory mode for the heterologous protein fraction. The resulting model represents a bacterial culture for heterologous protein production, which allowed us to test alternative explanations for the significant differences in yield observed in K12 versus B strains. The model allowed us to evaluate the kinetics of induction by IPTG, ionic mercury and cumaric acid, producing a quantitative assessment of the system's affinity for these inducers and how they are internalized into the cell. Finally, with this model we can study with macroscopic parameters the protein fraction's regulatory behaviour, which allowed to detect different degrees of crosstalk between native regulations and the three analysed heterologous circuits.

Characterizing the importance of thermosensitive ion channel TRPV3 for regulation of cellular pH and Ca2+ and bacterial clearance in murine macrophage functions

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Macrophages are cells that provide a defensive mechanism to body. It endorses homeostatic maintenance against pathogens, including different bacteria. Importantly, phagocytosis plays a crucial role in this process. A critical event in macrophages is when cells infected with bacteria require an acidic environment and Ca2+ intake during phagocytosis. However, most studies of macrophage are developed to stimulate pH and Ca2+-influx regulation in macrophage cells. In this process, non-selective cation channel proteins, thermosensitive ion channels, cytokinin production, and ATP-signaling pathways play crucial roles in intracellular calcium balance and pH regulation. Therefore, bacterial infection affects cells' intracellular Ca2+ and pH-level. TRP (Transient Receptor Potential channels) protein is a non-selective cation channel essential for several physiological functions. These channels are known to be activated by various pharmacological compounds, endogenous compounds, and different physical stimuli such as temperature, mechanical stimuli, cold, pain, etc. Thermosensitive TRP channels are potential candidates that can play a crucial role in regulation of macrophage functions. In this study, we used a series of novel and unique sensors that helps us monitor intracellular Ca2+ and pH separately and simultaneously. Using this approach, our study aims to characterize the importance of thermosensitive TRP channels in the regulation of cellular as well as lysosomal pH and Ca2+ levels and bacterial clearance.

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