Engineering Life: State of the Art and Ethical Challenges

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ABSTRACTS

Keynotes

Principles of Synthetic Genomes Modules

Tom Ellis, Imperial College Centre for Synthetic Biology & Department of Bioengineering, Imperial College, London

The international project to construct a synthetic version of the yeast genome (Sc2.0) has been one of the highest visibility research projects in synthetic biology in the last decade. As this grand project draws to a close, Sc2.0 partners are now beginning to use the tools and knowledge of synthetic yeast genome assembly to ask new questions of yeast biology and genomics, and develop new biotechnologies. As a milestone towards custom, modular genome, we are now using synthetic genome workflows and multiplex CRISPR to examine and exploit Synthetic Genome Modules (SGMs), where sets of genes that encode a common function are relocated from their native genomic loci into new synthetic defragmented or refactored gene clusters in the chromosomes. We have used our SGM method to fine-tune pheromone sensing for biosensor systems, and are now employing it to the explore the minimal gene set for the cell cycle. In new work, we have written SGMs for aromatic amino acid biosynthesis pathways and are using these as a test bed for building new tools for inducible heterochromatin-silencing and other forms of master regulation.

Toward A World of ElectroGenetics

Martin Fussenegger, Department of Biosystems Science and Engineering, ETH Zurich, Zurich

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

Rapid Discovery of High-Affinity Antibodies by Deep Screening

Philipp Holliger, MRC Laboratory of Molecular Biology, Cambridge

Engineering Life with Molecular Systems

Thomas Ward, NCCR MSE & Department of Chemistry, University of Basel, Basel

This presentation will summarize the efforts of the thirty groups involved in the National Center of Competence “Molecular Systems Engineering”. I will first outline the general concept of Engineering Life with Molecular Systems and go on to present recent applications of our approach to i) mimic life-like features in artificial systems, and ii) complement cellular systems with molecular or cellular prosthetics The presentation will end with the future perspectives that Molecular Systems Engineering offer to address unmet medical challenges.

Showcase presentations

Engineering Chemically-Controlled Cytokine-Based Immunotherapies

Lucia Bonati, School of Engineering, EPFL, Lausanne

Cytokines are key signal mediators of the immune system playing an essential role in the initiation, modulation, and resolution of immune responses. Despite their unique ability to modulate the immune system, the translation of cytokine-based therapies to the clinic has been greatly hindered by severe toxicities due to the pleiotropy and off-targeting effects of many cytokines.
Here, we present a general strategy that enables precise control over cytokine activity. We control the cytokine’s activity by selectively masking the receptor binding site with a fused chemically-responsive domain, which could be unmasked with a competing molecule (Venetoclax). To achieve this, Bcl-2 was fused to the cytokine and the BIM-BH3 interaction motif was transplanted to sites in close proximity to the cytokine’s receptor binding site. In absence of a competing molecule, Bcl-2 bound the cytokine with high affinity blocking the interaction site between the cytokine and its receptor (ON-switch). Upon addition of Venetoclax, the interaction between Bcl-2 and BIM-BH3 motif was disrupted, consequently restoring the cytokine’s activity. We have developed switchable mutants for a range of different cytokines (IL-2, IL-10, and IL-15) that are important for cancer immunotherapy. Moreover, we showed that in presence of Venetoclax, their activities can be selectively and fully restored.
Overall, this drug-responsive switch strategy may achieve spatiotemporal control of cytokine activities *in vivo* and thus improve the safety and clinical applicability of cytokine therapeutics.

Engineering Synthetic Cells from Soft Matter to Life-Like Behaviours

Claudia Contini, Department of Chemistry, Imperial College, London

Our cells are the most elaborated biological machine. They are continuously releasing and up taking billions of molecules in the environment, and they use this communication system to process information and act accordingly. However, manipulating and programming cells and understanding their intrinsic complexity is challenging as cells are often difficult to decode and manipulate. My research involves the creation of artificial cell-like systems (protocells) from

scratch by using synthetic or hybrid molecular building blocks, which are fully controllable by design and mimic the fundamental structure or mechanisms of natural cells. This multidisciplinary endeavour aims to create a broad range of products for functional applications and, at the same time, an increased understanding of biological systems and mechanisms.

Regulation of Transgene Expression by the Natural Sweetener Xylose

Silvia Galvan, Department of Biosystems Science and Engineering, ETH Zurich

Next-generation gene- and cell-therapies require precise regulation of the therapeutic output in order to achieve increased safety and efficacy, thereby facilitating successful clinical translation. For this reason, synthetic biologists are now focusing on the development of engineered gene networks that can accurately control the therapeutic protein in response to externally administered signal inputs. In this work, capitalizing on the basic components involved in the regulation of xylose in *E. coli*, we have engineered SWEET (sweetener-inducible expression of transgene), an orthogonal, highly sensitive and precise mammalian gene switch responsive to the natural sweetener xylose. SWEET functionality was confirmed in vivo as a cell- and gene-based therapy. Wild type mice implanted with encapsulated SWEET-engineered cells showed increased blood levels of a cargo protein when taking xylose-sweetened drinks. Moreover, in a proof-of-concept therapeutic application study, type-1 diabetic mice engineered with insulin-expressing SWEET showed lowered glycemia and increased insulin levels when administered this fairly diabetic-compliant sweetener. Based on the in vitro and in vivo performance of the xylose gene switch, we believe that SWEET is a versatile platform that can be implemented for the treatment of a wide range of chronic diseases and it has the potential to seamlessly integrate into patients' life-style and food habits.

Artificial Vesicular Systems with Interdependent Modules

Stephan Hirschi, Department of Biochemistry, University of Oxford

The bottom-up assembly of biological and chemical components opens exciting opportunities to engineer artificial vesicular systems for applications with previously unmet requirements. The modular combination of scaffolds and functional building blocks enables the assembly of complex molecular systems with biomimetic or new-to-nature functionalities. Inspired by the compartmentalised organisation of cells and organelles, lipid or polymer vesicles are widely used as model membrane systems. Furthermore, they form the basis for engineering artificial vesicular systems such as nanoreactors. Membrane proteins fulfil central roles in these systems, mediating aspects such as energy supply and transport of nutrients or waste products. Due to the vectorial nature of transport processes, the controlled assembly of membrane transport proteins into the vesicular membrane is key. However, many commonly employed reconstitution methods result in random membrane protein orientations. To address this issue, we have used engineered protein and scaffold modules to create preferential interactions that favour unidirectional insertion of the membrane proteins into the artificial membranes. These developed strategies will pave the way for creating increasingly complex vesicular systems with interdependent modules.

From order to chaos: The act of a last resort antibiotic

Selen Manioglu, Novartis Institutes for BioMedical Research, Basel

Antibiotic resistance is a growing threat to global health, and our antibiotic arsenal against multi-drug-resistant (MDR) bacteria has been defused immensely over the last 30 years. Polymyxins, a member of last-resort antibiotics, are *still* effective against these MDR pathogens. Although it has been in clinics since the late 1940s, the mechanistic details of polymyxin's action on the bacterial membrane were unclear. Recent findings of our study demonstrated that polymyxins interact with membrane lipids in a particular way and form ordered crystalline structures that alter the mechanical properties of the membrane considerably.

Noninvasive Assessment of Gut Function Using Transcriptional Recording Sentinel Cells

Florian Schmidt, Department of Biosystems Science and Engineering, ETH Zurich

Bacteria in the gut dynamically respond to each another, as well as to their host's diet and immune system. However, current approaches to measuring changes in gene expression in this setting have been limited and are generally invasive. We developed Record-seq a CRISPR-based recording method to capture the transcriptional changes that occur in bacteria as they pass through the intestines. Deep sequencing of these DNA recordings revealed characteristic signatures based on dietary, host, and microbiota contents at different levels of the intestine. This work provides perspectives on how diet, inflammation, and microbial interactions in the body shape the health of mammalian hosts. Bacteria in the gut dynamically respond to each another, as well as to their host's diet and immune system.

A Two-Enzyme Cascade for Precise Methylation of Small Molecules and Peptides

John Reed, Department of Chemistry, University of Basel

Late-stage methylation is a key technology in the development of pharmaceutical compounds. Methyltransferase (MT) biocatalysis provides a powerful option to insert methyl groups into complex molecules with high regio- and chemoselectivity. Large-scale application of methyltransferases is hampered by their dependence on the expensive and unstable co-substrate *S*-adenosylmethionine (SAM). A two-enzyme cascade has been developed, capable of recycling SAM to its unmethylated congener S-adenosylhomocysteine (SAH). Using simple electrophilic methyl donors (e.g. MeI or MeOTs), substrates can be methylated using a catalytic amount of SAH. This methodology can be used to introduce other groups such as fluoromethyl, enabling even greater molecular complexity.

Reprogramming the Genetic Code for Genetic Isolation

Jerome Zürcher, MRC Laboratory of Molecular Biology, Cambridge

The near-universal genetic code defines the correspondence between codons in genes and amino acids in proteins. It is widely hypothesized that refactoring the structure of the genetic code will create organisms with new properties, and may create a genetic firewall to limit the escape of genetic information from synthetic organisms. We refactored genetic code/decoder systems in a synthetic strain *E. coli.* This organism exhibits semantic- and functional- orthogonality with respect

to the code/decoder system for the canonical code. We thereby create orthogonal, and mutually orthogonal, horizontal gene transfer systems, which permit the transfer of genetic information between organisms that use the same genetic code, but restrict transfer of genetic information between cells that use different genetic codes. Moreover, we show that locking an orthogonal code into synthetic organisms completely blocks invasion by mobile genetic elements including viruses.