Imperial College
Centre for Synthetic Biology

Online Workshop on nucleic acids, synthetic biology and artificial life: Engineering and controlling out-of-equilibrium molecular systems

29-31 March 2021

UKRI Engineering and Physical Sciences Research Council

UKRI Biotechnology and Biological Sciences Research Council

Imperial College London

ROYAL ACADEMY OF ENGINEERING

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This workshop is part of the EPSRC-funded project "Genetic Engineering of Nucleic Acid Control Architectures" (GENACA), EP/P02596X/1.

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Contents

Welcome from Guy and Tom

Nucleic acid nanotechnology is an incredibly versatile platform for constructing rationally-designed molecular systems. Much of the current attention is focussed on self-assembling structures that are designed to reach a static equilibrium, or computational architectures that compute a single function by relaxing to equilibrium.

One of the signature features of living systems, however, is that they operate continuously rather than relaxing to equilibrium. The circuits built by synthetic biologists from re-purposed cellular components reflect this principle. Components are continuously produced and degraded, and the system is dynamically responsive to a changing environment. Feedback control architectures, which reject external perturbations to maintain certain properties of key molecular species, are a particularly elegant example.

Other researchers seek to mimic specific functions of living systems — such as replication, or cellular division — using de novo chemical designs. In doing so, they grapple with the challenges of designing and controlling far-from equilibrium systems with innovative chemical motifs.

We are gathering researchers with expertise across these disciplines for a workshop to discuss how nucleic acid engineering can be made more lifelike. In doing so, we hope to explore how nucleic acid nanotechnology can be applied more broadly to synthetic biology and the engineering of minimal life-like systems.

In this context, we've come up with three overarching questions that we'd like to address over the course of the workshop:

- What do we need to make DNA/RNA nanotech in living cells more predictable and usable?
- In what ways should we be looking to further emulate natural molecular systems? In what ways should we be trying to do things differently?
- What key applications/features can DNA/RNA nanotech in living cells enable?

We hope these questions will provide food for though throughout the workshop, and we have set aside a slot on the final day to discuss them explicitly.

Enjoy the workshop!

Prof. Guy-Bart Stan and Dr Thomas Ouldridge, Imperial College London

Timetable

All times BST (GMT+1, UTC+1).

Monday, 29 of March

	Welcome	
12:30–13:00	**Logging in and checking functionality**	
13:00–13:30	**Thomas Ouldridge** Imperial College London	Welcome remarks
	Synthetic cells	
13:30–14:00	**Wilhelm Huck** Radboud University	Putting Humpty Dumpty back together again – towards building a synthetic cell
14:00–14:30	**Oscar Ces** Imperial College London	Artificial Cells and Cellular Bionics
14:30–15:00	**Break and discussion**	
	Nucleic acids in synthetic biology	
15:00–15:30	**Peng Yin** Wyss Institute, Harvard University	Engineering programmable molecular devices
15:30–16:00	**Julius Lucks** Northwestern University	Programming cell-free synthetic biology diagnostics with RNA/DNA nanotechnologies
16:00–16:30	**Break and discussion**	
	Nucleic acids in synthetic biology	
16:30–17:00	**Georg Seelig** University of Washington	Designing sequences for gene expression control and DNA computing with machine learning
17:00–17:30	**Elisa Franco** University of California, Los Angeles	Dynamic self-assembly of encapsulated DNA nanotubes
17:30–18:00	**Break and discussion**	

Tuesday, 30 of March

	Molecular circuits: Principles and experiments	
13:00–13:30	**Mustafa Khammash** ETH Zurich	Synthetic Devices for Dynamic Signal Processing
13:30–14:00	**Andrew Phillips** Microsoft Research Cambridge	Programming Languages for Molecular and Genetic Circuits
14:00–14:30	**Break and discussion**	
	Nucleated self-assembly	
14:30–15:00	**Damien Woods** Hamilton Institute, Maynooth University	21 molecular algorithms using reprogrammable DNA self-assembly
15:00–15:30	**Rebecca Schulman** Johns Hopkins University	Biochemical reaction networks that regulate crystal growth and heal crystal defects
15:30–16:00	**Break and discussion**	
	Synthetic biology	
16:00–16:30	**Domitilla del Vecchio** MIT	Mitigation of context-dependence in mammalian synthetic biology
16:30–17:00	**Richard Murray** Caltech	9 years, 2 months, 16 Days to Go: Status and Progress on Implementing Synthetic Cells
17:00–17:30	**Break and discussion**	
17:30 onwards	**Social event**	

Wednesday, 31 of March

	Molecular templating	
13:00–13:30	**Andrew Turberfield** University of Oxford	Programming Synthesis
13:30–14:00	**Dieter Braun** LMU Munich	Tracking the first evolutionary steps in non-equilibrium settings
14:00–14:30	**Break and discussion**	
	Nucleic acids and enzymes	
14:30–15:00	**Yannick Rondelez** ESPCI Paris Science Lettres	Molecular networks as tools to optimize enzymes
15:00–15:30	**John Reif** Duke University	DNA Nanoscience on the Surface of Cells
15:30–16:00	**Break and discussion**	
	Overarching questions	
16:00–16:30	**Guy-Bart Stan** Imperial College London	Discussion on overarching questions
16:30–17:00	**Lee Cronin** University of Glasgow	The origin of chemical reactions
17:00–17:30	**Erik Winfree** Caltech	Thinking about molecular self-assembly that thinks
17:30–18:00	**Break and discussion**	

List of Abstracts – Talks

Monday 29th

Putting Humpty Dumpty back together again – towards building a synthetic cell

Wilhelm Huck

Radboud University

A cell is the common unit structure shared by all living organisms, but even 'simple' prokaryotic cells are extremely complex chemical reactors. One of the grand fundamental challenges of modern science is to reveal the basic operating principles of life. While we have extensive knowledge about the molecular building blocks that form the basis of modern life, we do not understand how these building blocks collectively operate to define life as we know it. Cellular life, which provides the fundament of all organisms, appears to be the result of a collection of highly controlled, energy consuming, dynamic self-assembly and self-organization processes that lead to autonomous entities that can reproduce, transfer information, interact, and evolve. Understanding the physical-chemical principles of these collective processes poses a formidable challenge, which needs to be overcome if we want to be able to understand life itself, and influence biological processes in a rational way in the future. In our research, we use microfluidic techniques to create cell-like environments that allow us to probe the impact of the physical aspects of the cell on key biochemical processes such as transcription and translation. I will also give an overview of our current attempts to reconstruct a living .

Relevant publications:

1. Transcription and Translation in Cytomimetic Protocells Perform Most Efficiently at Distinct Macromolecular Crowding Conditions, Mahesh A. Vibhute, Mark H. Schaap, Roel J.M. Maas, Frank H.T. Nelissen, Evan Spruijt, Hans A. Heus, Maike Hansen and Wilhelm T.S. Huck, ACS Synth. Biol. 2020, 9, 10, 2797–2807.
2. Macromolecularly crowded protocells from reversibly shrinking monodisperse liposomes, N.-N. Deng, M.A. Vibhute, L. Zheng, M. Yelleswarapu and W.T.S. Huck, J. Am. Chem. Soc., 2018, 140, 7399-7402.
3. The Nanotechnology of life-inspired systems, B.A. Grzybowski and W.T.S. Huck, Nature Nanotechnology 2016, 11, 585-592.
4. Macromolecular crowding creates heterogeneous environments of gene expression in picolitre droplets, M.M.K. Hansen, L.H.H. Meijer, E. Spruijt, R.J.M. Maas, M. Ventosa Rosquelles, J. Groen, H.A. Heus and W.T.S. Huck, Nature Nanotechnology 2016, 11, 191-197.
5. Monodisperse uni- and multi-compartment liposomes, N.-N. Deng, M. Yelleswarapu and W.T.S. Huck, J. Am. Chem. Soc. 2016, 138, 7584-7591.
6. Complexity of molecular crowding in cell-free enzymatic reaction networks, E. Spruijt, E. Sokolova and W.T.S. Huck, Nature Nanotechnology, 2014, 9, 406-407.

Artificial Cells and Cellular Bionics

Oscar Ces

Imperial College London

This talk will outline novel strategies for biomembrane engineering that are capable of fabricating vesicles, multisomes and artificial /cells tissues on demand. These platforms are being used to construct complex multi-compartment artificial cells where the contents and connectivity of each compartment can be controlled. These compartments are separated by biological functional membranes that can facilitate transport between the compartments themselves and between the compartments and external environment. These technologies have enabled us to engineer multi-step enzymatic signalling cascades into the cells leading to in-situ chemical synthesis and systems that are capable of sensing and responding to their environment. The talk will also highlight recent work in our group in the field of cellular bionics where we are manufacturing artificial cell-real cell hybrid systems where the combined ensemble performs user defined functions.

Engineering programmable molecular devices

Peng Yin

Wyss Institute, Harvard University

I will discuss our recent work on engineering programmable molecular devices using synthetic DNA strands and their applications in bioimaging and biosensing, including sequencing based DNA nanoscape, ultrasensitive immunoassay, and single-molecule proteomics.

Programming cell-free synthetic biology diagnostics with RNA/DNA nanotechnologies

Julius Lucks

Northwestern University

Poor water quality affects over two billion people across the globe. While we can't often see or taste water contaminants, molecular machinery can. Here I will present our latest research on developing a 'pregnancy test for water' - a cheap, fast and reliable approach that allows anyone, anywhere to detect if their water is contaminated. Our approach builds off of advances in cell-free synthetic biology – extracting the machinery of natural organisms to perform their functions but in test tube reactions instead of living cells. Using the principles of synthetic biology, we can 'rewire' natural biosensors to produce visible signals when specific contaminants are present in a water sample. The addition of synthetic RNA genetic circuits optimizes these reactions to detect contaminants with high degrees of sensitivity and specificity. We have also expanded this platform by creating an interface with toe-hold mediated strand displacement circuits, a dynamic DNA nanotechnology that enables molecular computation through programmable interactions between nucleic acid strands. This work is creating a new application area for cell free synthetic biology and nucleic acid molecular programming that promises to increase the scale at which we can monitor the health of ourselves and our environment.

Designing sequences for gene expression control and DNA computing with machine learning

Georg Seelig

University of Washington

Machine learning models that accurately predict function from sequence are becoming a powerful tool for molecular design. In this talk, I will first present our work on building models that can predict gene function (e.g. translation rate) from DNA sequence. What sets our approach apart from earlier work is that computational models are trained on very large synthetic gene libraries with millions of members. The massive size of the training data allows us to improve upon models trained exclusively on genomic data. We then use these predictors together with novel design algorithms to identify regulatory elements that result in precisely defined levels of gene expression. In the second part of the talk, I will briefly introduce a technique for executing similarity search over a DNA-based database of 1.6 million images. Queries are implemented as hybridization probes, and a key step in our approach is to learn an image-to-sequence encoding ensuring that queries preferentially bind to targets representing visually similar images.

Dynamic self-assembly of encapsulated DNA nanotubes

Elisa Franco

University of California, Los Angeles

Biological cells adapt, replicate, and self-repair in ways that are unmatched by man-made devices. These processes are enabled by the interplay of receptors, gene networks, and self-assembling cytoskeletal scaffolds. Taking inspiration from this architecture, we follow a reductionist approach to build synthetic materials by interconnecting nucleic acid components with the capacity to sense, compute, and self-assemble. Nucleic acids are versatile molecules whose interactions and kinetic behaviors can be rationally designed from their sequence content; further, they are relevant in a number of native and engineered cellular pathways, as well as in biomedical and nanotechnology applications. I will illustrate our work on self-assembling DNA scaffolds that can be programmed to respond to environmental inputs and to canonical molecular signal generators such as pulse generators and oscillators. I will discuss recent work on the encapsulation of these dynamic scaffolds inside emulsion droplets serving as a mimic of cellular compartments. I will stress how mathematical modeling and quantitative characterization can help identify design principles, guide experiments, and explain observed phenomena.

Wednesday 30th

Synthetic Devices for Dynamic Signal Processing

Mustafa Khammash

ETH Zurich

Signaling pathways govern the basic activity of living cells and facilitate their coordination. However, the mechanisms by which dynamic signals are decoded into appropriate gene expression patterns remain poorly understood. Here I will describe the development of synthetic networked pathways that recapitulate dynamic cellular information processing. By exploiting light-responsive transcriptional regulators with differing response kinetics, I will show how synthetic pathways can be engineered to perform nonlinear signal processing and decoding functions to extract information from dynamic signals. Applications of these pathways for precise multidimensional regulation of heterologous metabolic pathways and for synthetic morphogenesis will be demonstrated.

Programming Languages for Molecular and Genetic Circuits

Andrew Phillips

Microsoft Research Cambridge

Computational nucleic acid circuits show great potential for enabling a broad range of biotechnology applications, including smart probes, high-precision in vitro diagnostics and, ultimately, computational therapeutics inside living cells. This diversity of applications is supported by a range of implementation strategies, including nucleic acid strand displacement, localisation to substrates, and the use of enzymes. This talk presents a programming language that allows a broad range of computational nucleic acid circuits to be designed and analysed at the domain level. The language extends standard logic programming with an equational theory to express nucleic acid molecular motifs. We also demonstrate how similar approaches can be incorporated into a programming language for designing genetic circuits that are inserted into cells to reprogram their behaviour. The language is part of a broader system used to characterise genetic components for programming populations of cells that communicate and self-organise into spatial patterns. More generally, we anticipate that languages and software for programming molecular and genetic circuits will accelerate the development of future biotechnology applications.

21 molecular algorithms using reprogrammable DNA self-assembly

Damien Woods

Hamilton Institute, Maynooth University

In recent work [1] we designed a reprogrammable set of 355 DNA strands, or DNA tiles, capable of implementing a wide variety of algorithms. These tiles undergo algorithmic self-assembly: a form of molecular computation where molecules attach to a growing nanostructure and where each attachment executes a logical instruction, or step, of the computation.

Writing a new DNA algorithm is easy: just choose a subset of our DNA tiles. We implemented a total of 21 6-bit algorithms, including bit-copying, sorting, recognizing palindromes and multiples of 3, random walking, obtaining an unbiased choice from a biased random source, electing a leader, simulating cellular automata, generating deterministic and randomised patterns, and running a period-63 counter. The average per-tile error rate over the 21 different programs was less than 1 in 3000.

A number of technical challenges needed to be overcome to implement our large algorithmic tile set including: out-of-equilibrium growth from an input-providing seed structure, robustness to assembly errors, reconfiguration of the final DNA nanostructure for AFM imaging, and DNA sequence design of 355 DNA strands with stringent energetics requirements. Our reprogrammable architecture enabled programming while at the bench: we could come up with a new algorithm in the morning and implement it on the same day. Ideas from theoretical computer science were used to show what kinds of computations our system is capable of. The talk will showcase how the development of such multipurpose molecular machines, reprogrammable without detailed knowledge of the machine's physics, could establish a creative space where high-level molecular programmers can flourish.

Relevant publications:

[1] Diverse and robust molecular algorithms using reprogrammable DNA self-assembly. Woods*, Doty*, Myhrvold, Hui, Zhou, Yin, Winfree, Nature, 2019, 567:366-372. *Joint lead co-authors.

Biochemical reaction networks that regulate crystal growth and heal crystal defects

Rebecca Schulman

Johns Hopkins University

Living cells control self-assembly and maintain the structure of self-assembled complexes using chemical reaction networks. These networks can offset monomer depletion by synthesizing or activating monomers to stabilize growth conditions or reconfigure or repair assembled structures after they have formed. Here we show how simple chemical reaction networks can be designed to direct these processes in engineered self-assembly processes. Using DNA nanotubes as a model system, here we show that coupling a generic reversible bimolecular monomer buffering reaction to a crystallization process leads to reliable growth of large, uniformly sized crystals even when crystal growth rates change over time. We also find that maintaining the chemical potential after nanostructures have grown can prevent their degradation in the presence of nucleases. These and other simple chemical reaction schemes could be applied broadly as simple means to regulate and sustain crystal growth and assembled structures, and could facilitate the self-assembly of complex, hierarchical synthetic structures.

Mitigation of context-dependence in mammalian synthetic biology

Domitilla del Vecchio

MIT

Engineering biology has tremendous potential to impact a number of applications, from energy, to environment, to health. As the sophistication of engineered biological networks increases, the ability to predict system behavior becomes more limited. In fact, while a system's component may be well characterized in isolation, the salient properties of this component often change in rather surprising ways once it interacts with other components in the cell or when the intra-cellular environment changes. This context-dependence of biological circuits makes it difficult to perform rational design and often leads to lengthy, combinatorial, design procedures where each component is re-designed ad hoc when other parts are added to a system. In this talk, I will overview some causes of context-dependence, focusing mostly on mammalian genetic circuits, and will demonstrate how the problem of insulating a genetic module from context can be mathematically formulated as a control theoretic problem of disturbance attenuation. I will show two solutions in mammalian cells: feedforward control and feedback control. In particular, the feedback control architecture uses a covalent modification cycle as a key process to reach quasi-integral control, thus enabling attenuation of various disturbances arising from changes in intra-cellular context. These solutions support rational and modular design of sophisticated genetic circuits and can serve for engineering mammalian biological circuits that are more robust and predictable.

9 years, 2 months, 16 Days to Go: Status and Progress on Implementing Synthetic Cells

Richard Murray

Caltech

On 15 June 2010, I gave a talk at the 2nd International Workshop on Bio-Design Automation in which I declared that I wanted to see the creation of a fully programmed nanoscale machine, with all components specified and designed by engineers (no magic components). It should be a “general purpose" machine with capabilities that include sensing of environment, import/export of chemicals, communications (with other machines), locomotion, and energy harvesting/metabolism. In this talk I will give an update on various elements of creating such a machine, including our progress and frustrations with various subsystems. Depending on time, interest, and how early in the morning I have to get up, I’ll touch on metabolism, input/export machinery, computational modeling and design tools, and preliminary ideas about how to implement locomotion. This work is done jointly with the Build-A-Cell consortium in the US, which is an open collaboration supporting the science and engineering of building synthetic cells.

Wednesday 31st

Programming Synthesis

Andrew Turberfield

University of Oxford

The molecular machines of the Central Dogma - the ribosome and nucleic acid polymerases - underpin all life. Analogous machinery for programmed synthesis will play an essential role in creating future artificial life. I will discuss how far current schemes for DNA-programmed synthesis take us towards this goal, and the challenges still to be overcome. I will also explore the scientific and technological possibilities that a synthetic ribosome would create.

Tracking the first evolutionary steps in non-equilibrium settings

Dieter Braun

LMU Munich

We have driven the first steps of molecular evolution with non-equilibrium settings, often temperature gradients, but also with a recent focus on air-water interfaces. These settings accumulate molecules, select them for length, enable strand separation and allow continuous feeding through bulk solutions. Using sequencing, we see diverse pathways in sequence space that are able to create structure from randomness. These experimental findings make us better understand what are the pitfalls towards open ended evolution. In many cases still, these studies are performed with the help of a protein, but we converge towards RNA only experiment, driven by hybridization, gravity, temperature and hopefully, autocatalysis.

Molecular networks as tools to optimize enzymes

Yannick Rondelez

ESPCI Paris Science Lettres

Artificial molecular networks are rationally designed to process information at the molecular level. They can convert molecular signal, amplify them, apply specific functions (e.g. non-linear thresholding) or combine multiple signals. Although our ability to construct these networks mostly relies on nucleic acid chemistry and biochemistry, DNA-based molecular computers can be connected upstream and downstream to other chemistries or molecular processes. Additionally microfluidic partitioning allows to use a single network to perform millions or billions of parallel operations in independent compartments.

We have built PEN DNA networks to connect an enzymatic activity (upstream) and the amplification of the gene encoding that activity (downstream). Because evolution is based on a loop from phenotype to genotype and back, these networks, together with a protein expression process, allow us to program enzyme evolution, toward targets dictated by an artificial program.

Experimentally, we selected a specific endonuclease as the target activity. We built a network, which detect this activity and accordingly produces short DNA oligo for use as PCR primers. These primers can then be used to run the PCR of the enzyme's gene, a process called IPA-PCR, for Isothermal Primer amplification-PCR. Bacteria expressing the genetic library are then encapsulated in individual droplets together with the IPA-PCR components. After lysis and upon isothermal incubation followed by thermal cycles, he molecular program will ensure that, in each of 107 droplets, only gene expressing variants with the desired activity will replicate. The MP thus encodes an artificial fitness function (i.e. a function connecting specific phenotypic properties to reproductive success). Droplets are lysed afterwards to retrieve a library enriched in the best mutant genes, which can be analyzed by Next-Generation-Sequencing, or submitted to a new selection cycle. We will discuss how this approach can be used to explore enzyme's sequence spaces.

DNA Nanoscience on the Surface of Cells

John Reif

Duke University

The field of DNA nanoscience has achieved many astonishing capabilities, such as moderate molecular-scale computation and complex 3D self-assembled nanostructures. Biological environments present a very challenging, but also potentially promising domain of application of DNA nanoscience. While in vivo degradation is an obstacle for DNA nanodevices, it is not insurmountable, and the benefits of DNA computing can still be applied to in vitro assays. We describe two aspects of our current work on DNA-based cancer identification and targeted drug delivery. First, DNA hairpins implementing a

cascading chain reaction circuit bind to the membrane surfaces of particular cancer cell lines using a set of distinct aptamers, each for targeting a specific membrane protein. The selection of distinct aptamers attached one-to-one to each hairpin of the cascade implements a multi-input logical AND to identify a specific cell line matching the corresponding membrane protein profile. Thus, the circuit only generates an output if all membrane proteins, and therefore all hairpins, are present on the cell surface. Output can then be transduced into fluorescent indicators for in vitro assays, or flow downstream to bind and trigger DNA capsule drug delivery vehicles to implement in vivo therapeutics. Second, we have designed peptoid-coated, well-sealed DNA origami capsules for safe and effective drug delivery. DNA capsules loaded with therapeutic drugs can then be programmed to recognize a general signal produced by the cell identification cascade circuit, rather than tailoring its own limited mechanisms per each specific target cell. This effectively increases the fan-in logic and processing power of this DNA-based drug delivery system. A peptoid coating enhances in vivo stability and cellular uptake of the delivery vehicle that is guided to its target via signals output by the identification circuit. We envision that this system can be used to implement highly complex, multi-target, programmable therapeutics for drug-resistant diseases.

The origin of chemical reactions

Lee Cronin

University of Glasgow

What constrains how a chemical reaction emerges, and what reactions were required for the emergence of life? This is important since discussions about the emergence of life often are built around narratives regarding the prebiotic plausibility of the formation of the chemicals required to form a cell. In addition, the goal to make life in the lab is stuck around poor definitions of life, and over engineering to address these definitions. A solution would be to identify a feature exclusively associated with all life and develop a detection system for that feature that could be used for making life in the lab and the search for alien life.

Thinking about molecular self-assembly that thinks

Erik Winfree

Caltech

Thinking was once considered a unique privilege of humankind, but modern understanding has evolved to accept the depth and richness of animal cognition, and there are serious arguments about in what sense a single cell might be considered to "think". The next natural question would be to ask about a network of molecules, or even a single molecule – can it think? Is it just a matter of degree, as Darwin said, and not a matter of kind? Leaving that question aside for the philosophers, we can ask clearer questions about information processing, and attempt to characterize information processing in simple molecular systems. Remarkably, the perhaps simplest framework for molecular interactions – passive self-assembly – already exhibits rich information dynamics including the ability to simulate Turing machines, cellular automata, Boolean circuits, and neural-network-like pattern recognition – both theoretically and in experimental demonstrations using DNA nanotechnology.

List of Posters

1. Exploring and exploiting non-ideal PCR behavior towards molecular computation in the clinic

John Goertz[1]

[1] Imperial College London

The classical understanding of polymerase chain reaction (PCR) holds that every target amplicon in solution is replicated with every thermal cycle, corresponding to an amplification rate of 100%. However, we've found that amplification rate depends strongly on the length and GC content of the amplicon, ranging from less than 50% to potentially greater than 100%. These results challenge classical PCR design heuristics, but we can also take advantage of these out-of-equilibrium dynamics to enable model-driven design of novel amplification architectures such as asymmetric and competitive designs. Competitive PCR, where multiple distinct amplicon sequences compete for the same primers, is of particular interest for development of novel medical diagnostics. We present an empirical exploration of such non-idealities in PCR behavior through Gaussian process regression, a probabilistic, small-data approach to machine learning. We are using this strategy to engineer novel multiplex reactions with a tailored response to each target, creating the potential to diagnosis disease from a complex pattern of biomarkers in a single reaction.

2. Light-activated gene expression inside synthetic cells

Jefferson Smith[1]

[1] University of Oxford

Liposomes containing an in vitro transcription-translation system represent minimal synthetic cells that can perform specialised functions according to an encapsulated DNA template. Hence, regulation at the DNA-level enables control over synthetic cell activities. Protein expression inside the liposomes has previously been regulated using small-molecule sensitive transcription factors and riboswitches, however, these rely on the diffusion of small molecules across the lipid bilayer and are often leaky. Here, we utilise chemically modified, light activatable-DNA templates to provide tighter regulation over synthetic cell activities and control them in a spatiotemporal manner using patterned light. Light-activated DNA is constructed by introducing 7 amino C6 thymine bases that are conjugated to photocleavable biotinylated linkers into the T7 promoter of a linear DNA template. Monovalent streptavidin binds at each biotin and sterically hinders transcription of the downstream gene by RNA polymerase in the absence of light. However, after UV irradiation, the photocleavable linker-streptavidin complex is liberated and transcription/translation can proceed. Using this approach, we have demonstrated that protein expression inside giant unilamellar vesicles can be activated using light as an external stimulus and using we can control this spatiotemporally using pattern illumination. Using this platform, we established a means to mediate synthetic cell-bacteria cell communication through the controlled in-situ synthesis of acyl homoserine lactones involved in quorum sensing.

3. Programming Cell-Free Biosensors with DNA Strand Displacement Circuits

Kirsten Jung[1]

[1] Northwestern University

Cell-free biosensors are emerging as powerful platforms for monitoring human and environmental health. Here, we expand the capabilities of biosensors by interfacing their outputs with toehold-mediated strand displacement circuits, a dynamic DNA nanotechnology that enables molecular computation through programmable interactions between nucleic acid strands. We develop design rules for interfacing biosensors with strand displacement circuits, show that these circuits allow fine-tuning of reaction kinetics and faster response times, and demonstrate a circuit that acts like an analog-to-digital converter to create a series of binary outputs that encode the concentration range of the target molecule being detected. We believe this work establishes a pathway to create "smart" diagnostics that use molecular computations to enhance the speed, robustness, and utility of biosensors.

4. A free-energy landscape model of RNA/DNA hybrid strand displacement reaction kinetics

Francesca Smith[1]

[1] Imperial College London

Toehold-mediated strand displacement (TMSD) and variants thereof, such as toehold exchange have become nearly ubiquitous tools for engineering complex nucleic acid reaction systems. These motifs allow robust kinetic control of reaction circuits, enabling a broad range of emergent behaviour. While the kinetic and thermodynamic properties of DNA/DNA TMSD and toehold exchange have been extensively and systematically characterised, RNA/DNA hybrid strand displacement reactions remain under-explored. Importantly, although DNA is more stable and cost-effective, RNA is much more informative as a biomarker. Thus, a greater understanding of RNA/DNA strand displacement kinetics will allow broader integration of TMSD into biological research and clinical diagnostics. We present a free-energy landscape model to predict the kinetics of RNA/DNA strand displacement systems. Following experimental characterisation and parameterisation, this model has the potential to inform the rational design of RNA/DNA strand displacement systems with optimised reaction kinetics. Such applications include reversible RNA detection systems capable of capturing real-time, out-of-equilibrium target concentration dynamics.

5. A modular, dynamic, DNA-based platform for regulating cargo distribution and transport between lipid domains

Roger Rubio Sánchez [1]

[1] University of Cambridge

Biological membranes feature highly evolved proteo-lipid machinery able to co-localise in lipid rafts, nano-scaled assemblies believed to underpin signal transduction, amongst other cellular processes. Bottom-up synthetic biology aims to replicate life-like behaviours in model artificial cells, often using synthetic lipid bilayers as passive enclosures that lack the functional complexity associated to their biological analogues. DNA nanotechnology has emerged as a popular choice for biomimicry, coupling bio-inspired nano-devices with model membranes using amphiphilic oligonucleotides. In fact, amphiphilic DNA nanostructures also undergo partitioning in lipid domains, evoking the affinity of proteins for raft microenvironments. Here, we regulate the lateral distribution of DNA nanostructures in phase-separated membranes by exploiting the tendency of cholesterol and tocopherol motifs to respectively enrich liquid-ordered (Lo) and liquid-disordered (Ld) domains. By prescribing combinations of multiple anchors, changes to nanostructure topology, and size, our DNA architectures are programmed to achieve partitioning states that span the energy landscape. In addition, the functionality of our approach is showcased with a responsive biomimetic DNA device that dynamically achieves ligand-induced reconfiguration and mediates cargo transport between lipid domains. Our synergistic platform paves the way for the development of next-generation biomimetic DNA-based architectures, that can achieve sensing and communication in synthetic cellular systems.

6. A smart polymer for sequence-selective binding, pulldown, and release of DNA targets

Krishna Gupta [1]

[1] Leibniz Institute of Polymer Research Dresden

Selective isolation of DNA is crucial for applications in biology, bionanotechnology, clinical diagnostics and forensics. We herein report a smart methanol-responsive polymer (MeRPy) that can be programmed to bind and separate single- as well as double-stranded DNA targets. Captured targets are quickly isolated and released back into solution by denaturation (sequence-agnostic) or toehold-mediated strand displacement (sequence-selective). The latter mode allows 99.8% efficient removal of unwanted sequences and 79% recovery of highly pure target sequences. We applied MeRPy for the depletion of insulin, glucagon, and transthyretin cDNA from clinical next-generation sequencing (NGS) libraries. This step improved the data quality for low-abundance transcripts in expression profiles of pancreatic tissues. Its low cost, scalability, high stability and ease of use make MeRPy suitable for diverse applications in research and clinical laboratories, including enhancement of NGS libraries, extraction of DNA from biological samples, preparative-scale DNA isolations, and sorting of DNA-labeled non-nucleic acid targets.

7. Bioinspired Peptide-Nucleotide Nanofibers

Simone Hendrikse [1]

[1] University of Melbourne

In nature, fibrous assemblies continuously assemble and disassemble to fulfill a variety of functions, including providing structural support and driving cell motility. Inspired by the hierarchically complex structures they form that are necessary to perform these essential tasks, both natural and synthetic mimics have been developed. By creating synthetic mimics, control over structure, and hence, function can be achieved. Moreover, new capabilities, not achievable in nature, can be realized. For example, the Tobacco Mosaic Virus (TMV) forms helical cylindrical fibrous assemblies with very high persistence lengths. The highly infectious RNA of the virus is coated by so-called coat proteins, that protect the virus from enzymatic degradation, and keeps the virus stable for several years within infected tobacco leaf products, e.g., infected cigars. The assembly of virion TMV includes synergistic effects of both the RNA and already aggregated coat proteins (i.e., disks or small helices). In the absence of RNA, protein modules are intrinsically disordered, whereas upon RNA binding, a highly organized hybrid structure is obtained with extensive intermolecular interactions both laterally and axially, including salt-bridges. Fascinated by the hybrid self-assembled structure of RNA with coat proteins in virion TMV, we developed peptide-nucleotide hybrid polymers that also form rigid fibers. Our peptide-nucleotide fibers are formed as a consequence of lateral non-covalent interactions between the peptide sequences, while having pendant mononucleotides, altogether forming rigid rods in aqueous solutions. Despite having similar peptide sequences, installing distinct mononucleotides at the periphery of the polymers induced different structure morphology; adenine mononucleotide functionalized polymers formed micrometers long tape-like structures, whereas thymine formed short straight rods. The ability to facilitate complementary binding with its artificial complementary mimic and canonical RNA and DNA is currently being investigated. These polymers will serve as templates for the non-enzymatic synthesis of DNA and RNA.

8. Compartmentalising DNA crystals with reaction-diffusion processes for application as a membrane-less synthetic cell

Adrian Leathers [1], ***Lorenzo Di Michele***[2]

[1] University of Cambridge
[2] Imperial College London

Developing compartmentalised functional molecular structures is a crucial step toward engineering artificial cellular systems. Here, we present our findings from using reaction-diffusion processes to pattern amphiphilic DNA crystals and show an example of application as a prototype membrane-less synthetic cell. We have designed, produced and characterised micron-size functional units that are formed by self-assembling crystallisation of amphiphilic building blocks consisting of DNA structures with cholesterol tags. These DNA building blocks are able to host dynamic DNA circuits, which have been used to trigger disassembly, response cascades and interaction between different functional units [1,2,3]. In this project, we interface the crystals with a competitive DNA reaction-diffusion circuit, which is used to form up to 5 different compartments in a single DNA crystal unit. The components of the reaction-diffusion circuit competitively bind to single stranded DNA overhangs incorporated into the amphiphilic building blocks of the crystals. The pore size of the crystals allows shorter DNA strands to diffuse faster than longer strands inside of the crystal structure, thus shorter strands bind first to the overhangs. Longer DNA strands on the other hand diffuse slower through the crystal but are able to displace shorter strands from the overhangs via toehold mediated strand displacement. The presence of diffusing strands of multiple lengths creates concentric binding patterns in the DNA crystals. At any given time, the reaction-diffusion patterning can be stopped and frozen in time, by adding excess amounts of complementary DNA. Finally, we use this approach to create a core-shell patterning in a DNA crystal, where the core contains DNA template for a fluorescent RNA aptamer and the shell contains binding sites for said aptamer. A T7 RNA Polymerase is able to synthesize fluorescent RNA aptamer in the core, which then binds to the shell. Our approach can be thus used to create a highly simplified membrane-less synthetic cell.

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9. Direct Prebiotic Synthesis of DNA/ RNA Nucleosides and Nucleotides

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This presentation focusses on our recent discovery of a straightforward path to deoxyribonucleosides [1], starting from simple, prebiotically available molecules. The formation of deoxyribonucleosides is achieved under prebiotic conditions in water in high regio- and stereoselectivity, starting from all canonical purine and pyrimidine bases, by condensation with acetaldehyde and sugar-forming precursors. Furthermore, this pathway provides also interesting structures that can be considered as potential progenitors of our known DNA. A detailed stereochemical analysis proves the ingenuity of nature to minimize the number of possible stereoisomers and it gives important insights, why deoxyribose and ribose were favored over all other sugars [2]. Furthermore, a comprehensive pathway to prebiotic relevant molecules and a previously undiscovered layer is shown that exhibits evolutionary behavior already at the molecular level and catalyzes numerous processes which probably contributed to the emergence of life.

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10. CSIR Technology Platform for Synthetic Biology and Precision Medicine

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Synthetic Biology is an emerging technology that uses the principles of physics, chemistry, mathematics, and biology. Synthetic biology is the technology enabler that could be applied across all fields such as agriculture, food, health, chemical, and industrial manufacturing sector. Similarly, Optimizing Drug discovery and Translation is one of the Global Challenges for precision medicine and is the critical factor in achieving UN Sustainable Development Goals 3 Good Health and Well Being. WHO reports Cancer is the second leading cause of death globally with the estimated 9.6 million deaths in 2018 (1 in 6 death is due to cancer). In addition, 70% of mortality from cancer occur in low- and middle-income countries such as South Africa. Leukemia is one of the top ten most common cancer in South Africa. In addition, leukaemia is the most common childhood cancer (25.4% of all cancers) in South Africa, which is similar to rates in other countries. Currently, there is drive at South Africa by DSI, CSIR and MRC to establish a precision medicine program that would address the needs of South African Patient cohort. The functional precision medicine strategy is designed to directly identify tailored drug regimens that target individual patient´s cancer cells and give benefit to the same donors by supporting clinical decision-making. We aim for this project to serve as a proof of concept to showcase whether individually designed high throughput drug sensitivity screening along with microfluidics based single cell drug screening can provide patient benefit with limited material available and to build competence on existing drug sensitivity screening at CSIR using newly developed platform technologies such as microfluidics based single cell drug screening. The proposed project is therefore well aligned with the strategy of present drive of South African Precision Medicine initiative.

11. End-to-end transport of small molecules through microns-long DNA-based nanochannels

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With growing interests of using artificial nanopores and nanochannels in biosensing and single-molecular studies, DNA-based nanopores of 5-10 nm diameters show transport of small molecules and proteins across. Yet those DNA nanopores have lengths below 100 nm and the molecular transport only occurs across lipid membranes. Nanochannels of longer lengths can be used as conduits for carrying molecules on the cell-size scale or between compartments apart. Here, we design microns-long DNA nanochannels of 7-nm diameter capable of transporting of small molecules. Kinetics analysis suggests a continuum diffusion model can describe the transport phenomenon within the DNA nanochannel. The reduced transport upon bindings of DNA origami caps to the channel ends reveal the molecules mostly transport from one channel end to the other rather than leak across channel walls. The nanochannels can be further exploited to study intercellular signaling or to enable transport between synthetic cells.

12. Enzyme-free autonomous catalytic templating of DNA complexes far-from-equilibrium

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The use of templates is an established method to produce sequence-controlled assemblies, like biological polymers. Crucially, templated biological processes, like protein formation, require the eventual spontaneous detachment of the product from its template. Product detachment ensures that the polymers can perform its biological function and the reusability of the template to act as a catalyst for the polymer formation. Current artificial systems lack an enzyme-free templating mechanism capable of producing spontaneous product separation without external intervention. Given the fundamental significance of catalytic templating in contexts like biology, it is desirable to develop fully programmable synthetic systems that implement similar functionality. DNA provides a fantastic framework for the rational bottom-up design of systems of this sort, thanks to the predictability of DNA interactions. In this work, we build an only-DNA system capable of templating the catalytic production of sequence-specific dimers. The system is composed of two sets of complementary monomers, A and B, and a template, which recognises and catalyses the polymerisation of a specific monomer from each subset, e.g. monomer A1 and monomer B3. The mechanism can autonomously regenerate its template thanks to handhold-mediated strand displacement. This displacement reaction increases the polymerisation rate of specific monomers with a DNA-DNA transient interaction, the handhold. After the dimer product is formed, the transient nature of the handhold ensures the autonomous detachment of the template, so it can keep catalysing the product formation. The result is a far-from-equilibrium product distribution biased towards template-complementary products, instead of the equiprobable product distribution that would result in equilibrium. We measure and model the kinetics of the templating system, and we use this insight to catalyse, with a scarce amount of template, at least 20-fold of a specific dimer out of nine possible equally stable products.

13. In situ generation of RNA complexes for synthetic molecular strand displacement circuits in autonomous system

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Synthetic molecular circuits implementing DNA or RNA strand-displacement reactions can be used to build complex systems such as molecular computers and feedback control systems. Despite recent advances, application of nucleic acid-based circuits in vivo remains challenging due to a lack of efficient methods to produce their essential components – multi-stranded complexes known as “gates” – in situ, i.e. in living cells or other autonomous systems. Here, we propose the use of naturally occurring self-cleaving ribozymes to cut a single-stranded RNA transcript into a gate complex of shorter strands. We designed the ‘self-excising unit’ by connecting two hammerhead ribozymes that cut 5’ upstream and 3’ downstream. After successful cleavages, the self-excising unit dissociates from the RNA transcript leaving an RNA gate capable of performing strand-displacement reactions. We first confirmed the functionality of each ribozyme in the self-excising unit by running the RNA transcript in a denaturing PAGE gel. We also showed that after self-cleavages, the self-excising unit dissociates from the gate in a native condition. Finally, we demonstrated real-time and in-situ strand displacement reactions using RNA inputs and gates produced via in-vitro transcription. Our circuit could discriminate input RNAs with different toeholds and can be assembled as a two-step cascade. Our method opens a new possibility for operation RNA strand-displacement circuits via autonomous and continuous production of RNA strands in a stoichiometrically and structurally controlled way.

14. High-quality Simulation of Cotranscriptional RNA Folding with BarMap-QA

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Non-coding RNAs are major players in many cellular processes, and their function is often mediated by their structure. The structure, in turn, is dynamically changing during the course of the molecule's lifetime, especially during its transcription. These dynamics can be crucial for the behaviour of the molecule, as in the case of transcriptional riboswitches. Therefore, analyzing the folding process of an RNA is a vital step in understanding its function. The experimental probing of RNA structures, however, is laborious and time-consuming, and a precise real-time measurement of cotranscriptional folding is hardly possible at all. This makes computational folding simulations an attractive alternative for the analysis of both natural and synthetic RNA molecules. With the BarMap framework, Hofacker et al. laid the theoretical foundation for the simulation of dynamic folding scenarios using the concepts of coarse-grained RNA energy landscapes and mappings between those. Their prototypical software, however, is very general and leaves many choices and intermediate steps up to the user. Additionally, it does not provide any quality metrics, and the interpretation of the verbose output is cumbersome. This puts a high burden on utilizing their scripts to analyze the folding pathway of an actual molecule. BarMap-QA addresses these issues for the specific case of cotranscriptional folding by embedding BarMap in a semi-automatic pipeline that offers a full simulation run with a single command as well as fine-grained tuning options for experts. Additionally, three novel quality scores have been added to verify the validity of each simulation step, enabling the user to make informed trade-offs between performance and precision. The package is complemented by configurable filtering and visualization tools, which display the entire results in a single, clean plot or on the command line. As exemplary demonstration, a synthetic, cotranscriptional theophylline riboswitch is analyzed. The source code of the software is available under a free license. Additionally, a platform-independent Docker image including all dependencies is provided, which makes cotranscriptional folding analysis as easy as possible.

15. Principles and molecular mechanisms for autonomous and accurate copying of long templates

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Templated polymer copying is fundamental to the synthesis of the nucleic polymers and proteins in biology, and is a promising framework for the synthesis of synthetic, sequence- and length-controlled copolymers. In biological copying processes, such as transcription, accuracy is generated by base-pair interactions between the copy monomers and the template. Unlike the guiding interactions in self-assembly, these interactions are transient as the copy and template eventually separate. Biological systems excel at copying templates autonomously; the process is chemically driven, without systematic time-varying conditions. However, current synthetic approaches to length and sequence control in templated copying either rely on non-autonomous chemical protocols or sacrifice tight control for autonomy. Models of polymerisation that account for the separation of copy and template confirm that accurate copying is an intrinsically non-equilibrium process. DNA nanotechnology holds potential for the implementation of such processes. Recently, a novel DNA strand displacement reaction motif was used to template the completion of dimers. Despite recent advances in our understanding of sequence-control, little attention has been directed toward controlling the length distribution of copolymers in an autonomous template copying. Here, we ask what kinds of monomer-template and monomer-monomer interactions grant sequence and length control in autonomous copolymer synthesis. We simulate the stochastic dynamics of whole and partial copolymers and monomers on a single, finite-length template. We address the conflict between autonomy and length control in a simple model. We introduce mechanisms which autonomously synthesise copolymers with nearly exact lengths and sequences, that spontaneously separate from arbitrarily long templates. These mechanisms could be implemented with DNA strand displacement reactions and may be beneficial for granting control over other autonomous copolymerisation protocols. By identifying the engineering principles of control in autonomous template copying systems, we present a key step toward the creation of self-replicating synthetic copolymers.

16. Programmable Ultrasensitive Molecular Amplifier for compartmentalized single-enzyme analysis

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Enzymes are formidable catalysts that, beside their essential biological role, have profoundly transformed academic research, biotechnologies and industrial processes. In particular, DNA-related enzymes have become essential tools for molecular biology and molecular technology. These include polymerases, nucleases, ligases and many more, which together provide the basic molecular biology toolbox. The ubiquity, importance and subtlety of enzymes make accurate tools for their detection and characterization extremely important. Combining concepts from molecular programming and microfluidics technology, we propose a versatile framework for the single-molecule detection of a wide range of DNA-processing enzymes, which we demonstrate on endonucleases such as nickase, restriction enzyme, AP-endonuclease, RNAse as well as N-glycosylases, polymerase, ligase and kinase. The detection of these enzymes is based on a Programmable Ultrasensitive Molecular Amplifier (PUMA). It includes a plug-and-play sensing module, which connects the targeted activity to the generation of a short DNA trigger, and a thresholded – bistable – DNA amplification system, which produces a fluorescent readout. When isolated in picoliter-size water-in-oil droplets, single enzymes, with turnover rate too slow to be observed with traditional (linear) substrate conversion strategies, can be detected and counted. The digital approach allows accurate and quantitative detection. We demonstrated the versatility and specificity of this approach over a dozen a enzymes. In addition, the distribution of activity of an enzyme population can be estimated using a more complex circuits, where a linear amplifier is plugged downstream of the bistable sensing module, in order to recreate an analog signal correlated to the enzyme activity. Such assays represent an important step toward the characterization and in vitro evolution of enzymatic activities.

17. Responsive core-shell DNA particles destabilise lipid bilayers and trap bacteria

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Lipid bilayers play an important role in cellular biology as they act as the filter between the inner and outer environment of the cell. Their integrity, often associated with a proper functioning and vitality of the cells, can be compromised by a number of biological and synthetic agents, including antimicrobial peptides, amyloid aggregates, polymer particles and metal particles with charged coatings. Such agents, frequently considered to be toxic and highly undesirable, have a variety of beneficial applications such as the ability to control membrane leakage, which can be harnessed for biosensing and therapeutics. Here, we present a novel type of synthetic, DNA-based particles capable of disrupting lipid membranes. The particles have a core-shell structure and self-assemble from cholesterol-DNA nanostructures, named C-stars, responsible for the formation of membrane-adhesive core, and all-DNA nanoconstructs forming a protective hydrophilic corona around the core. These aggregates are stable in solution in the presence of liposomes and their size can be prescribed by changing the annealing protocol leading to self-assembly. The protective corona can be selectively displaced upon an addition of a trigger DNA oligonucleotide. The latter exposes the cholesterol-rich particle core and induces membrane disruption as caused by aggregation of the DNA-cholesterol complexes on the vesicle's surface, resulting in membrane rupture and cargo release. Furthermore, once activated, the core-shell particles assemble into a sticky DNA net capable of surrounding and immobilizing cell-like objects, as we exemplify with E. coli entrapment. This is reminiscent of the action of innate-immune cells, which can eject their genetic material to create a DNA net (Neutrophil Extracellular Trap) able to entrap pathogens. The design of the particles can be easily adapted to create antimicrobial or drug delivery systems.

18. Cation-responsive hydrogels from non-canonical amphiphilic DNA nanostructures

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Thanks to its biocompatibility, versatility and programmable interactions, DNA has gained traction as building block for a variety of 2D and 3D architectures, nanomachines and nanomaterials. Given the exploding interest in nanomedicine, there has been growing attention on the possibility of using DNA to produce stimuli-responsive carriers, particularly in the form of hydrogels [1], with applications in artificial cells, biosensing, tissue engineering and drug delivery and release platforms. However, the use of such nanomaterials in vivo is still hampered by the inability of most state-of-the-art DNA nanostructures to reversibly modify their structure in response to physiological stimuli, such as cation concentration or pH. So far, most implementations rely on conventional strand displacement reactions, which use non biologically available, single-stranded DNA fuel as input. I will present how embedding cation-responsive, DNA G-Quadruplex (G4) motifs into amphiphilic DNA nanostars [2, 3] generates nanostructures, dubbed ""Quad-Stars"", capable of assembling into responsive hydrogels via an easy, enzyme-free, one-pot reaction route. I will show that the incorporation of G4 structures is feature-enabling, allowing for structural and (to a certain degree) kinetic control over the self-assembly/disassembly by exposure to potassium (K+) ions, or lack thereof via chelation, under physiologically relevant conditions. The cargo-loading capabilities of the attached hydrophobic cholesterol moieties and the combined reversibility and responsiveness make Quad-Stars an excellent candidate for next-gen biosensors and responsive drug delivery carriers.

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19. Detailed Balanced Chemical Reaction Networks are Generalized Boltzmann Machines

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Stochastic Chemical Reaction Networks (CRNs) represent a biophysically realistic and expressive modeling framework for biological systems. Detailed Balanced CRNs (dbCRNs) are a subclass of CRNs which admit an equilibrium distribution. This poster builds off our previous work on Chemical Boltzmann Machines (Poole et al. DNA 2017) where we have shown that dbCRNs can exactly implement a stochastic neural network model called a Boltzmann Machine. This relationship illustrates that stochasticity can be advantageous because it allows a CRN to compactly represent complex distributions. We have shown that these CRNs are capable of inference, defined as computing conditional distributions, by "clamping" a subset of their species to a particular value. Finally, we have shown that the equilibrium distributions of dbCRNs can be learned by an external in silico non-CRN algorithm that tunes the energies of individual molecular species. In new work, we have shown that the above results regarding inference and learning apply to any dbCRN, not just the Chemical Boltzmann Machine constructions. In this poster, we examine physical processes that can tune species' energies and are potentially implementable in a cell-like environment. The first uses a special kind of detailed balanced chemostat to control carefully designed chemical potentials that maintain detailed balance. The second uses a simple feedback circuit implemented as a non-detailed balanced CRN. These constructions provide equilibrium and non-equilibrium physical implementations that control the inference and learning process in dbCRNs. We then analyze these processes thermodynamically to provide lower bounds on the costs of inference and learning. These results illustrate possible mechanisms whereby a biochemical system in a small volume, such as a cell, can represent and adapt to its environment.

20. Helicase expedites a catalytic DNA reaction by selectively removing the product from the catalyst

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The primer exchange reaction (PER) is a DNA-based isothermal catalytic reaction that appends a new domain with a user-specified sequence onto a single-stranded reactant. PER has shown promise, among others, as a signal amplification tool for visualization of mRNA's in cells. However, as for any catalytic reaction, its rate is limited by Sabatier's principle which requires that the binding strength between the reactant and the catalyst is strong enough for substantial binding but weak enough that the binding is reversible and the product doesn't poison the catalyst. Consequently, PER only works for primers that are approximately 10 nucleotides long at 37C. Here we show that RepX, an ATP-dependent helicase that exclusively unwinds DNA duplexes with a single stranded 3' overhang, can increase the rate of PER by up to two orders of magnitude in the strong binding regime. This extends the range of applicability of PER to longer reactants and lower temperatures, where the reaction would otherwise be prohibitively slow. We show that RepX enhances the rate by selectively removing the product - and not the reactant - from the catalyst. Our findings suggest a general strategy of "dissipative catalysis" in which catalytic reactions are enhanced at the cost of energy consumption by actively removing product from the catalyst.

21. Integral feedback in synthetic biology: Negative-equilibrium catastrophe

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A central goal of synthetic biology is the design of molecular controllers that can manipulate the dynamics of intracellular networks in a stable and accurate manner. To address the fact that detailed knowledge about intracellular networks is unavailable, integral-feedback controllers (IFCs) have been put forward for controlling molecular abundances. These controllers can maintain accuracy in spite of the uncertainties in the controlled networks. However, this desirable feature is achieved only if stability is also maintained. In this paper, we show that molecular IFCs suffer from a hazardous instability called negative-equilibrium catastrophe (NEC), whereby all nonnegative equilibria vanish under the action of the controllers, and some of the underlying molecular abundances blow up. We show that unimolecular IFCs do not exist due to a NEC. We then derive a family of bimolecular IFCs that are safeguarded against NECs when unimolecular networks are controlled. However, we show that, without a detailed knowledge of the controlled systems, NECs cannot be prevented when IFCs are applied on bimolecular networks.

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Useful Information

The workshop will be run using Microsoft Teams. Although it is possible to log directly into talks using links from our email, we recommend that you do go through the exercise of logging on to Teams properly. Doing so will allow you to access the rest of the workshop, which has been organised into “channels" (visible on the left-hand side of the Teams window). Please note that you may need to toggle between Imperial’s Teams network and that of your home institution; there is a menu to the right of the search bar that allows you to do so.

Video Talks will be delivered live on the “Links to talks" channel in teams. You should all be able to access these talks once they have been initiated by Guy or Tom. Please do mute your microphone unless you are the speaker - it would be nice, however, to see few faces over video! **Please do not record the talks from your end - we will be recording the talks of the speakers who give permission for it.**

We are not planning to have significant Q&A sessions directly after each talk; instead, there will be 30 min discussion sessions after each pair of talks. If you have questions for the speakers, please note them down and go to “Post-talk-discussion room 1/2" as appropriate. The speaker will be hosting a meeting there.

Posters should be uploaded by poster presenters by the start of the workshop. Each has its own channel, numbered as recorded in this abstract book. The discussion periods are intended to allow time to visit posters, but of course you can do it at any time. It is recommended that you contact the presenter to arrange to meet in the channel at a given time, where you can start a meeting using the “meet now" button in the top right. You can try to reach the presenter through the Teams “Chat" function; alternatively, look up their email in the list of participants/on the poster channel itself.

Social event on Tuesday evening. After the last discussion session on Tuesday, we have scheduled a free socialising session using “Wonder". You can find the link pinned in the “General" channel. Wonder is a platform that allows for dipping in and out of conversations as if you were all in a room together. It’s a bit weird to start off with, but you’ll get the hang of it!

Technical Problems. We do not have a technical support team, which is why we are trying to test as much functionality as possible ahead of time. However, if you do have a problem, please post it in the “Technical Issues" channel. We will answer as soon as we can, but we encourage other users to help out if they can.