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14. ABSTRACT

SEED 2015 was focused on advances in the science and technology emerging from the field of synthetic biology. We broadly defined this as technologies that accelerate the process of genetic engineering. It highlighted new tool development, as well as the application of these tools to diverse problems in biotechnology, including therapeutics, industrial chemicals and fuels, natural products, and agriculture. Systems span from in vitro experiments and viruses, through diverse bacteria, to eukaryotes (yeast, mammalian cells, plants).

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SEED Synthetic Biology: Engineering, Evolution & Design

2015

Synthetic Biology: Engineering, Evolution & Design

June 10-13, 2015

Boston Park Plaza, Boston, MA

SEED Synthetic Biology: Engineering, Evolution & Design

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10:15 AM-12:15 PM	**Session 1: Systems Modelling**	**Imperial Ballroom**
12:15-2:00 PM	Lunch	New Statler
2:00-4:00 PM	Registration Check In	Mezzanine Foyer
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8:00-8:30 AM	Breakfast	New Statler
8:30-9:30 AM	**Keynote: Prosthetic Gene Networks for Biomedical Applications — Martin Fussenegger, *ETH Zurich***	**Imperial Ballroom**
9:30-11:00 AM	**Session 9: Biological Parts**	**Imperial Ballroom**
11:00-11:30 AM	Coffee Break	Mezzanine Foyer
11:30 AM-1:00 PM	**Session 10: Microbial Communities and Microbiome Genomics**	**Imperial Ballroom**
1:00-1:30 PM	**ACS Synthetic Biology Young Investigator Award Lecture – Tim Lu, *MIT***	**Imperial Ballroom**

Welcome Address

Greetings!

We want to welcome you to Boston, Massachusetts for the Synthetic Biology: Engineering, Evolution and Design (SEED) Conference 2015, brought to you by the Society for Biological Engineering (SBE), an AIChE Technological Community.

SEED 2015 will focus on advances in the science and technology emerging from the field of synthetic biology. We broadly define this as technologies that accelerate the process of genetic engineering. It will highlight new tool development, as well as the application of these tools to diverse problems in biotechnology, including therapeutics, industrial chemicals and fuels, natural products, and agriculture. Systems will span from *in vitro* experiments and viruses, through diverse bacteria, to eukaryotes (yeast, mammalian cells, and plants).

We hope you're ready for a tremendous conference. On Wednesday, you will have the opportunity to hear from opening keynote Mark Ptashne, the Ludwig Chair of Molecular Biology at the Memorial Sloan–Kettering Cancer Center. Pamela Silver, Co-Chair of this conference and Professor of Systems Biology at Harvard Medical School, will be our second keynoter on Thursday. Friday will feature a keynote from Jennifer Doudna, Professor of Chemistry and of Molecular and Cell Biology at the University of California, Berkeley. The final keynote you will hear on Saturday will be from Martin Fussenegger, professor of biotechnology and bioengineering at the Department of Biosystems Science and Engineering at ETH Zurich.

Sessions throughout the week are filled with other top researchers and thought leaders in Synthetic Biology from academia and industry. A special session Friday afternoon will also provide students with the chance to give talks on their leading research in the field.

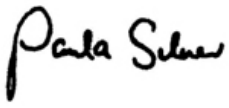
In addition to oral presentations, the conference will also feature two poster sessions. There will also be a special session on receiving NIH funding and a session for students to learn about publishing their work in the ACS Synthetic Biology journal. Additionally, breakfasts, lunches, a reception, and an off-site social mixer hosted by Ginkgo Bioworks will ensure that you have the opportunity to hear every speaker while also having time to interact with your peers.

A lot of work has gone into making this conference a success. We would not have been able to do this without the contributions of our Organizing Committee, who were instrumental in selecting our invited speakers and shaping the program. We extend additional thanks to all of our corporate sponsors, academic supporters, and media partners without whom the conference could not happen.

Finally, we would like to thank you for attending the conference. We hope these four days will be pleasant, educational, and inspiring.

Sincerely,

Dan Gibson
Conference Chair



Pam Silver
Conference Chair

Conference Chairs

- Dan Gibson, Synthetic Genomics/J. Craig Venter Institute
- Pam Silver, Harvard University

Organizing Committee

- Barry Canton, Ginkgo Bioworks
- Matthew Chang, National University of Singapore
- Michelle Chang, UC Berkeley
- Cynthia Collins, RPI
- Doug Densmore, Boston University
- Jim Haseloff, Cambridge University
- Richard Murray, California Institute of Technology
- Kristala Jones Prather, MIT
- Chris Voigt, MIT
- Peng Yin, Harvard University

Special Events

Student Information Session on Submitting Papers to ACS Synthetic Biology
Students can sit down with the Managing Editor of *ACS Synthetic Biology* during the Thursday and Friday poster sessions to learn about how to publish their research in the journal.

NIH Mentoring Session
On Friday, June 12, the NIH will be presenting a mentoring session on receiving NIH funding. It will include a 1 hour presentation during lunch, followed by a Q+A session during that evening's poster session. The NIH is a predominant funder of biomedical research. Its funding process differs significantly from other funding agencies such as DoD or DoE that may be more familiar to the synthetic biology community. This presentation will mainly focus on basic funding concepts and processes for gaining an NIH grant. Particular attention will be paid to approaches for submitting synthetic biology applications to NIH. The speakers will also discuss relevant funding opportunities and provide a general overview of the current status of NIH funding in synthetic biology. Though targeted to new investigators, the information may be valuable for a variety of career stages, including potential SBIR applicants.

Ginkgo Bioworks Off Site Party
Ginkgo Bioworks will be hosting an off-site party at their headquarters on Friday, June 12 after the poster session. Busing will be provided to and from the Boston Park Plaza, with the first bus leaving at 7:00 PM. Light food and drinks will be provided. Ginkgo's address is: 27 Drydock Avenue 8th Floor, Boston, MA, 02210.

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SPEAKERS

Speakers should plan to sit in the front of the room during their sessions. Personal laptops will not be connected to the projector for individual presentations. Keynote speakers will have 1 hour for their presentations and follow up questions. All other speakers will have 30 minutes for their talk and questions. Please help us keep to the schedule.

Presentations at the SEED 2015 Conference will be handled by our on-site presentation management system, LaunchPad. Presenters, please review the following guidelines for uploading and presenting your talk:

1. File Preparation
LaunchPad supports virtually any file type and does not place restrictions on size. For best results, bring your files to the conference in their original format (not converted) on a USB flash drive to the on-site technician located at the tech table in the back of the session room. If you plan to use multimedia such as videos, please bring the source files as well, even if you have previously embedded the files in your presentation. Please submit your presentation files to the LaunchPad technician at least an hour before your session.

2. Upload and Testing
The technician will assist you with uploading your files using an upload kiosk. In most cases this process takes just a few minutes. Here you can look through your presentation and make any last-minute edits. Please note that the podium computer uses “presenter mode” in supported applications and presenter notes ARE visible if you choose to include them.

3. Presenting
Launching your talk on LaunchPad is very straightforward. On the screen you will see a list of the presenters in your session, labeled by their number and last name (e.g. 01 Smith). Click on your name, and your presentation will launch automatically into full screen. There will be a combination wireless remote / laser pointer at the podium with which to advance your slides, or you can use the keyboard / trackpad if you prefer. When you are finished, instead of closing your presentation just press the glowing “RESET” button to the left of the laptop. This will close your presentation and return to the main LaunchPad screen for the next speaker. Similarly, if the presenter before you did not reset the podium and you do not see the list of speakers, pressing “RESET” will take you back to the main LaunchPad screen.

POSTER PRESENTERS

Poster sessions will take place on Thursday and Friday evenings. Even-numbered posters should present on Thursday, June 11. Odd-numbered posters should present on Friday, June 12. Posters presenters can set up their posters the morning of their poster session starting at 8:00 AM. Posters should be taken down immediately after each poster session. Poster numbers are listed with the abstracts in section 9 of the program book.

CONTACT INFORMATION

SBE staff will be available at registration check-in during the following times:

Wednesday, June 10, 8:00 AM – 12:15 PM, 2:00 PM – 4:00 PM
Thursday, June 11, 7:30 AM – 12:00 PM
Friday, June 12, 7:30 AM – 12:00 PM
Saturday, June 13, 7:30 AM – 9:30 AM

For questions following the conference, you may reach Derrick Wu by email at derrw@aiche.org or by phone at 646.495.1313.

Technical Program

Wednesday, June 10th	
9:00-9:15 AM	Welcome
9:15-10:15 AM	**Keynote: Regulation: Mechanisms and Logics - Mark Ptashne, Sloan Kettering Cancer Center**
10:15 AM-12:15 PM	**Session 1: Systems Modelling**
10:15-10:45 AM	Learning the Sequence Determinants of Exon Definition from Millions of Random Synthetic Sequences - Georg Seelig, University of Washington
10:45-11:15 AM	Scalable System-Wide Design of TF- and dCas9-Based Genetic Circuits - Howard Salis, Penn State
11:15-11:45 AM	Quantifying Host-Circuit Interactions with a Mechanistic Chassis Model - Andrea Weisse, University of Edinburgh
11:45 AM-12:15 PM	Biological Insights from a Computational Simulation of the M13 Bacteriophage Life Cycle - John Fisk, Colorado State University
12:15-2:00 PM	Lunch
2:00-4:00 PM	**Session 2: Biomedical Applications**
2:00-2:30 PM	Spatial and Quantitative Optimization of Engineered Multi-Element Therapeutic Proteins - Jeff Way, Wyss Institute
2:30-3:00 PM	Toward Rational Engineering of Phage to Combat Multidrug-Resistant Pathogens - Kyle Cady, SGI
3:00-3:30 PM	Engineering Smarter and Stronger T Cells for Cancer Immunotherapy - Yvonne Chen, UCLA
3:30-4:00 PM	µsynth: A Versatile Microfluidic Device for Automating the Synthetic Biology Process - Steve Shih, JBEI
4:00-4:30 PM	Coffee Break
4:30-6:30 PM	**Session 3: Biological Circuits and Context**
4:30-5:00 PM	Use of an Enzyme-Coupled Biosensor to Engineer a BIA Fermentation Pathway from Glucose in *Saccharomyces cerevisiae* - John Dueber, UC Berkeley
5:00-5:30 PM	CRISPR Genetic Circuits for Genome Control and Interfacing - Stanley Qi, Stanford University
5:30-6:00 PM	The Programmable Laboratory - Eric Klavins, University of Washington
6:00-6:30 PM	Synthetic Approaches to Studying Cis-Regulation - Sri Kosuri, UCLA
6:30-7:30 PM	Reception
Thursday, June 11th	
8:00-8:30 AM	Breakfast
8:30-9:30 AM	**Keynote: Designing Biology for a Healthy World - Pam Silver, Harvard University**
9:30-10:00 AM	Coffee Break
10:00 AM-12:00 PM	**Session 4: Metabolism, Metabolomics and Engineering Metabolism**
10:00-10:30 AM	The Development of Platform-Based Technologies for the Optimization of Sustainably Produced Chemicals - Stephanie Culler, Genomatica
10:30-11:00 AM	Metabolite Valves: Dynamic Control of Metabolic Flux for Pathway Engineering - Kris Prather, MIT
11:00-11:30 AM	Gene Circuits for Self-Tuning Metabolic Pathways - Diego Oyarzun, Imperial College London
11:30 AM-12:00 PM	Protecting Groups for Improved Control of Indigo Biosynthesis - Tammy Hsu, UC Berkeley
12:00-1:30 PM	Lunch
1:30-3:00 PM	**Session 5: Sensors**
1:30-2:00 PM	What do Soybeans Have to do with Sustainable use of our Oceans? - Virginia Ursin, Monsanto
2:00-2:30 PM	Engineering Robust Hosts to Improve Microbial Biofuel Production - Mary Dunlop, University of Vermont
2:30-3:00 PM	Targeted Integration of Genes into the Maize Genome Facilitated By Cas9-gRNA System - Huirong Gao, Pioneer
3:00-3:30 PM	Coffee Break
3:30-5:30 PM	**Session 6: Advancing Synthetic Biology Tools, Automation and Analytical Technologies**
3:30-3:45 PM	Introduction - Alicia Jackson, DARPA
3:45-4:10 PM	Broadband DNA Synthesis - Emily Leproust, Twist Bioscience
4:10-4:35 PM	Ben Gordon, MIT/Broad Foundry
4:35-5:05 PM	Synthetic Biology Open Language (SBOL): Community-Driven Standard for Communication of Synthetic Biology Designs - Jacob Beal, Raytheon
5:05-5:30 PM	Ron Weiss, MIT

Thursday, June 11th	
5:30-7:00 PM	**Poster Session A**
Friday, June 12th	
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10:00 AM-12:00 PM	**Session 7: DNA and RNA Based Synthetic Biology**
10:00-10:30 AM	Designing and Verifying Molecular Circuits Made of DNA - Erik Winfree, Caltech
10:30-11:00 AM	Powered DNA Strand Displacement Circuits for Continuous Environmental Monitoring and Memory - Rebecca Schulman, Johns Hopkins University
11:00-11:30 AM	Design of a Toolbox of RNA Thermometers - Shaunak Sen, Indian Institute of Technology
11:30 AM-12:00 PM	Dynamic RNA Nanotechnology - Niles Pierce, Caltech
12:00-1:30 PM	Lunch and NIH Mentoring Session
1:30-3:30 PM	**Session 8: Minimal Systems**
1:30-2:00 PM	Bottom Up Construction of Dynamic Biomolecular Materials - Elisa Franco, UC Riverside
2:00-2:30 PM	Minimizing a Bacterial Genome by Global Design and Synthesis - John Glass, J.Craig Venter Institute
2:30-3:00 PM	Reconstructing Anaerobic Microbiomes from the 'Bottom-up': New Techniques to Decipher Interwoven Metabolism Michelle O'Malley, UC Santa Barbara
3:00-3:30 PM	Semi-Synthetic tRNA Complement Mediates *in Vitro* Protein Synthesis - Kirill Alexandrov, The University of Queensland
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4:00-5:15 PM	**Student Session**
4:00-4:15 PM	A Modular Protein Architecture for Generalized RNA Targeting: Development and Application to RNA Monitoring, Control, and Protein Scaffolding - Daniel A. Martin-Alarcon, MIT
4:15-4:30 PM	Designing Conservation Relations in Layered Synthetic Biomolecular Networks - Thomas Prescott, Oxford
4:30-4:45 PM	Directed Evolution of Acid-Tolerant Phenotype Using an Engineered pH-Riboswitch - Hoang Long Pham, National University of Singapore
4:45-5:00 PM	Enabling Selection in Directed Evolution of Enzymes Via Cellular Engineering - Neda Hassanpour, Tufts
5:00-5:15 PM	Quantifying and Modeling Compositional Context Effects on Synthetic Biocircuits in *E. coli* - Enoch Yeung, Caltech
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7:00 PM	Off-Site Party at Ginkgo Bioworks
Saturday, June 13th	
8:00-8:30 AM	Breakfast
8:30-9:30 AM	**Keynote: Prosthetic Gene Networks for Biomedical Applications - Martin Fussenegger, ETH Zurich**
9:30-11:00 AM	**Session 9: Biological Parts**
9:30-10:00 AM	Controlling Organisms Electrically with Synthetic Biology - Caroline Ajo-Franklin, LBL
10:00-10:30 AM	Towards the High– Throughput Construction of Fluorescent Biosensors - Stacy Anne-Morgan, UC Berkeley
10:30-11:00 AM	A System for Unprecedented Ribosome Engineering in Living *E. coli* - Erik Carlson, Northwestern University
11:00-11:30 AM	Coffee Break
11:30 AM-1:00 PM	**Session 10: Microbial Communities and Microbiome Genomics**
11:30 AM-12:00 PM	Engineering Nitrogen Fixing Symbiotic Associations in Cereals - Giles Oldroyd, John Innes Institute
12:00-12:30 PM	Insights from a Global View of Secondary Metabolism: Small Molecules from the Human Microbiota - Michael Fischbach, UCSF
12:30-1:00 PM	Synthetic Biology Engineering of Biofilms As Nanomaterials Factories - Peter Nguyen, Harvard University
1:00-1:30 PM	**ACS Synthetic Biology Young Investigator Award Lecture - Tim Lu, MIT**

WEDNESDAY, JUNE 10
KEYNOTE

Regulation: Mechanisms and Logics

Mark Ptashne*
Sloan Kettering Institute, New York, NY

"…certain phenomena are "artificial" in a very specific sense: they are molded by goals or purposes (e.g. by natural selection)…Engineering, medicine…are concerned not with necessity but with the contingent…in short, with design...the requirements of design can be met fully by a modest adaptation of ordinary declarative logic." Herbert A Simon, The Sciences of the Artificial, 3rd Edition (1996)

Regulation of transcription illustrates these points. Our understanding of molecular mechanisms rationalizes the otherwise contradictory logics of control in disparate organisms. And the principles apply to a wide array of biological control processes.

SESSION 1: SYSTEMS MODELLING

Learning the Sequence Determinants of Exon Definition from Millions of Random Synthetic Sequences

Alexander B. Rosenberg[1], Rupali P. Patwardhan[2], Jay Shendure[2] and Georg Seelig*[1,3]
[1]Department of Electrical Engineering, University of Washington, Seattle, WA
[2]Department of Genome Sciences, University of Washington, Seattle, WA
[3]Department of Computer Science, University of Washington, Seattle, WA

Many of the genetic variants in coding regions of human genes cause disease through altered RNA splicing. Measuring the splicing effects of all exonic variants is infeasible, while training predictive models is challenging due to the limited number of variants with experimental data. Here we develop a novel approach that allows us to accurately predict the effects of these variants on splicing. Rather than examining splicing of genomic sequences, we measure splicing patterns of millions of randomized sequences, encompassing 100 million bases of variation. The large size of our dataset allows us to improve current models of splicing as well as gain new mechanistic insights. From these data we learn that multiple sequence motifs regulate exon definition additively rather than cooperatively. We also show that the same motifs regulate exon definition in alternative 5', 3', and cassette exon splicing. Our model of exon definition and model of the human 5' splice site greatly improve prediction of the effects of variants on both alternative 5' and cassette exon splicing. Our results suggest that large scale assays of random or synthetic sequences can also be used to improve our understanding of other complex forms of gene regulation, such as translation or transcription.

Scalable System-Wide Design of TF- and dCas9-Based Genetic Circuits

Iman Farasat[1] and Howard M. Salis*[1, 2]
[1]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA,
[2]Department of Biological Engineering, Pennsylvania State University, University Park, PA

Engineered genetic circuits can reprogram organisms with complex decision-making capabilities that are only limited by the availability of genetic regulators, the designed architecture of their interactions, and the host's capacity. As more well-characterized regulators have become available, it remains a significant challenge to design larger genetic circuits, while accounting for the physical differences in the regulators' interactions, sequence-dependent changes in expression, and non-linear host-circuit coupling. To accelerate the design of large genetic circuits with many regulators, we have developed and experimentally validated a system-wide mechanistic model of genetic circuits that predicts a circuit's input-output dynamical behavior in terms of the circuit's DNA sequence, biophysical measurements of the regulators, and the host's specifications. The model encompasses genetic circuits that use either traditional transcription factors as well as dCas9-based transcriptional regulation using guide RNAs. The model combines system-wide statistical thermodynamic models of transcription and translation that predict how changing transcription factor levels, Cas9 levels, guide RNA levels, guide RNA sequences, RBS sequences, and genome specifications alters the transcription of every promoter and the translation of every coding sequence in the circuit. The model then uses kinetics to determine circuit and host-circuit dynamics.

We experimentally validated our modeling approach by constructing and characterizing several genetic circuits. First, we developed a new approach to measure the binding free energies of a transcription factor by designing and characterizing a series of genetic circuits incorporating auxiliary binding sites. Second, we tested the model's calculations by characterizing how different circuit characteristics (plasmid copy number, translation rates, and TF binding sites) and host growth conditions controlled the circuit's input-output relationship. Third, we characterized a series of signal amplification genetic circuits using different regulators and regulator expression levels to critically test the model's ability to account for changes in regulator binding free energies and binding occupancies. Fourth, we characterized a series of dCas9-based NOT gates to test the effects of changing Cas9 levels, guide RNA levels, and the guide RNA's binding sites on circuit function. Overall, we found that the model could accurately predict circuit behaviors, while accounting for several non-intuitive and non-linear behaviors, including the effects of altering TF/guide RNA levels and adding additional TF/guide RNA binding sites.

Finally, based on these results, we propose a new dimensionless unit, the Ptashne number (Pt), that combines many circuit characteristics into a single number that accurately predicts the transcription rates of the circuit's promoters. Using the Pt number, the design space for an N-regulator circuit is transformed from a 5^N-dimensional space (5 variables per regulator) into an N-dimensional space (1 variable per regulator). We show how using the Pt number will accelerate the rational design of genetic circuits with many regulators.

Quantifying Host-Circuit Interactions with a Mechanistic Chassis Model

Andrea Weisse*
SynthSys – Synthetic & Systems Biology, University of Edinburgh, Edinburgh, United Kingdom

Cells have finite resources. Committing resources to one task thus reduces the amount of resource available to other tasks. Intracellular processes consequently do not work in isolation but continually interact with the rest of the cell.

If we aim to design complex synthetic circuits with predictable functions then we need to understand how these circuits compete for resources with their hosts. We thus need a comprehensive and quantitative understanding of these interactions. Host-circuit interactions can alter the designed function of a circuit, reduce the fitness of the host, and ultimately impose a negative selection pressure on cells with functioning synthetic circuits. Although mathematical modelling is an integral part of synthetic biology's design cycle, most models do not include explicit interactions with the host. These models cannot predict the impact of host-circuit interactions, resulting in an inefficient design process and lengthy trial-and-error iterations to appropriately tune circuit expression.

Here we consider three trade-offs that because of limitations in levels of cellular energy, free ribosomes, and proteins are faced by all living cells and construct a mathematical model that comprises these trade-offs. Our model describes the mechanisms of protein synthesis and how cells extract resources from their environment. It further couples gene expression with growth rate and growth rate with a growing population of cells. We show that the model recovers Monod's law for the growth of microbes and two other empirical relationships connecting growth rate to the mass fraction of ribosomes.

Our model can be used as a tool to quantify host-circuit interactions for the 'host-aware' design of synthetic gene circuits. It predicts the reallocation of proteome to accommodate the extra resource demand by a circuit, the resulting drop in growth rate and the quality of the circuit's functionality in response to environmental factors. The interplay between a circuit, its host, and the host's environment can be directly incorporated into the design to minimize the impact of cellular trade-offs and resource competition on the circuit function.

Biological Insights from a Computational Simulation of the M13 Bacteriophage Life Cycle

Steven Smeal, Margaret Schmitt, Ashok Prasad and John Fisk*
Chemical and Biological Engineering, Colorado State University, Fort Collins, CO

As the biotechnological applications of M13 phage particles continue to expand in scope and complexity, a quantitative, holistic understanding of the biological processes and interactions that govern the life cycle of the phage will foster the creation of new platforms with rationally designed control elements that are more amenable to engineering. The filamentous phage life cycle employs 11 phage-encoded proteins to direct a complex and coordinated program of action in the infected cell. Unique among bacteriophage, the filamentous phages act as true parasites of bacteria: infected cells continue to grow and divide as progeny phage particles are continuously produced. To be a successful parasite the phage must invade the cell and quickly establish itself in a limited manner that allows the cell to survive while at the same time avoiding the cell's attempts to dislodge it. Bacteriophage M13, the most well studied F-pilus specific filamentous phage of *E. coli*, is able to co-opt the infected cell and control the extent and timing of progeny production across many cellular generations. We have constructed a genetically-structured, experimentally-based computational simulation of the life cycle of M13 to evaluate and expand the system level understanding of this biotechnologically-relevant phage. Our deterministic chemical kinetic simulation integrates 50 years of experimental observations and explicitly includes the molecular details of phage DNA replication, mRNA transcription, protein translation and phage particle assembly, as well as the competing protein-protein and protein-nucleic acid interactions that control the timing and extent of phage production. Many aspects of M13 biology are faithfully reproduced by the simulation, including the production levels and timing of the shift between replicative form and preassembly complex DNA, quantities of phage mRNA and proteins, and the time course of assembly and release of progeny phage. Extending the simulation across multiple cell generations recapitulated the dynamic steady state behavior observed in infected cell populations and elucidated underappreciated elements of the controlling architecture of the phage life process, in particular the role of translational attenuation in resource allocation by phage protein p5. The p5 translational attenuation control mechanism was originally hypothesized to be central to the coordinated control of the levels of phage DNA in in the cell and the switch from replicative form to single-stranded DNA synthesis. Subsequent experiments called this central role into question. Our simulation

of the phage life cycle matches the behavior of experiments that indicated that translational attenuation of the DNA synthesis initiation protein p2 were unimportant, but finds a role for translational self-attenuation of p5 in controlling resource allocation. Our simulation not only provides a quantitative description of phage biology, but also highlights gaps in the present understanding of M13 biology. Understanding the subtleties of regulation will be important for maximally exploiting the phage as scaffolds for nanoscale devices from biosensors to batteries. Our simulation should find utility helping to prioritize future biochemical experiments and design new synthetic phage-like systems.

SESSION 2: BIOMEDICAL APPLICATIONS

Spatial and Quantitative Optimization of Engineered Multi-Element Therapeutic Proteins

Jeffrey C. Way*[1], Devin Burrill[1], Avi Robinson-Mosher[1], James J. Collins[1,2], and Pamela Silver[1,3]
[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA
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The current paradigm of drug development is to create single-function molecules that bind tightly to their targets and inhibit their function. In contrast, natural processes, including defense against pathogens and disease, involve many elements – multiple protein domains, weak interactions that function cooperatively, geometrically optimized elements, and so on. The spatial and quantitative aspects of such systems are tweaked in the course of evolution to create defenses that look quite different from the simple molecules designed by humans. Our goal is to create complex therapeutic proteins and cells that mimic natural systems. We have engineered proteins that deliver an activating hormone or cytokine, either erythropoietin or interferon alpha, to specific target cells. The quantitative aspect is to mutate the cytokine and reduce its activity, so that cell binding is driven by an antibody element to which the cytokine is fused. The spatial aspect is primarily to use a linker that allows simultaneous binding of the cytokine and antibody elements to their receptors, and also maximizes the rate of the cytokine binding after the antibody element has already bound. We also developed course-grained molecular dynamics software to simulate the movement and binding of such proteins, with the goal of optimizing the engineering choices before making the proteins.

Erythropoietin (Epo) is a hormone, produced by the kidneys in response to low blood oxygen, that stimulates red blood cell production. Commercial Epo (e.g. Procrit, Epogen) is used to treat anemia in kidney failure and cancer patients. In 2004, sales were about $10B, but since then the use of Epo has dropped significantly due to clinical trials showing that treated patients suffered from clotting events leading to heart attacks, strokes, and deep vein thrombosis. These effects are due to Epo action on cells other than red blood cell precursors, such as platelet precursors and blood vessel endothelial cells. To avoid these side effects, we constructed a form of Epo that is fused to an antibody and targeted to late red blood cell precursors. Upon treatment of mice, the engineered protein stimulates production of only red blood cells, while controls and commercial forms of Epo stimulate red blood cells and platelets to a similar extent.

Interferon alpha (IFNalpha) is a cytokine that represents the primary response to virus infection. IFNalpha is used in treatment of hepatitis and certain cancers, but a major side effect is 'flu-like symptoms', so that long-term high-dose treatments are burdensome. We generated a fusion protein consisting of a weakened IFNalpha fused to antibody V regions that bind to a tumor-specific surface marker. Animal testing is in progress.

These experiments illustrate how protein engineering can be added to the repertoire of synthetic-biological tools to create novel functions that go beyond what is possible by manipulating transcriptional circuits.

Toward Rational Engineering of Phage to Combat Multidrug-Resistant Pathogens

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The global threat of multidrug resistant (MDR) bacterial pathogens requires rapid development of new antimicrobials and has revitalized interest in exploiting lytic bacteriophage to treat human infections. Challenges presented by traditional phage therapy, involving cocktails of individual phages, include formulation complexity to overcome inherent narrow host-range specificity, accessibility to bacterial pathogens embedded within the exopolysaccharide matrix (biofilms), development of phage-resistance and the associated uncertainty of the regulatory approval framework, have limited successful clinical adoption. Synthetic biology driven engineering of phage, to impart key drug-like properties, provides an approach to overcome these traditional limitations. ~300 *Pseudomonas aeruginosa* (*P.a.*) MDR clinical isolates were obtained and a large collection of lytic *P.a.* phage was established. Next generation sequencing, developed bioinformatics analysis and an advanced genetic toolbox were systematically employed to quickly correlate diverse underlying genetic determinants with phenotypic properties to inform and power a design/build/test pipeline of accelerated, iterative phage engineering. Enabled by rapid construction of engineered *P.a.* phage genomes and direct transformation into *P.a.* hosts for lytic phage production, this platform has produced engineered phage chassis with designed, expanded host-range and enhanced lytic activity. Ongoing development of this platform focuses

upon overcoming host resistance mechanisms to produce engineered phage therapy product candidates with defined and enhanced properties for clinical applications.

Engineering Smarter and Stronger T Cells for Cancer Immunotherapy

Yvonne Chen*
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Adoptive T cell therapy for cancer has demonstrated exciting potential in treating relapsing cancers. In particular, T cells that express synthetic chimeric antigen receptors (CARs) specific for the B-cell marker CD19 have shown impressive results in clinical trials for various B-cell malignancies, prompting avid interest from both scientific and entrepreneurial communities in recent years. However, CD19 CAR-T cell therapy remains the only robustly effective T-cell immunotherapy to date, and several obstacles remain to be overcome before the full potential of adoptive T-cell therapy can be realized. My laboratory is pursuing several strategies for the engineering of T cells with stronger anti-tumor functions and greater robustness against evasive mechanisms employed by cancer cells. I will discuss the design, construction, and implementation of multi-input CARs to increase tumor specificity and decrease the probability of mutational escape by tumor cells. I will present the design of synthetic circuits to reroute signaling pathways triggered by tumor-secreted cytokines, thus negating the immunosuppressive effects of the tumor microenvironment. Finally, I will discuss efforts to engineer a cytotoxic protein that triggers target-cell death upon recognition of intracellular oncoproteins, thus expanding the repertoire of detectable tumor markers beyond surface-bound antigens. These strategies combine to address critical limitations facing adoptive T-cell therapy, providing potential treatment options for diseases that are otherwise incurable with current technology.

µsynth: A Versatile Microfluidic Device for Automating the Synthetic Biology Process

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The synthetic biology process of specification, design, build, and test follows an iterative process that often requires extensive manual intervention. This process is used to engineer new microbes that contain the necessary genetic circuit and metabolic pathways to produce the required outputs for a wide range of applications such as bio-based chemicals and biofuels.[1] While current available tools are useful in improving the synthetic biology process, further improvements in physical automation would help lower the barrier of entry into this field. For example, to build and test, there are currently automation tools to aid these processes[2-4] but are still relatively underserved in terms of physical automation technologies to build and to test DNA assembly.

Here, in response to this challenge, we introduce a new automated and versatile microfluidic device that can assemble DNA plasmids using three assembly methods (Golden Gate, Gibson, and TAR cloning) with on-chip transformation. Our microfluidic device takes advantage of two droplet microfluidic platforms[5]: droplet-in-flow and digital microfluidics to integrate the various molecular biology steps. The microfluidic device comprises of a digital microfluidic chip for dispensing droplets and mixing droplets on-demand. The second part of the device consists of a channel region with integrated microvalves that will be used to incubate/store droplets and to conduct on-chip transformation. To demonstrate the utility of our device, we used our device to assemble two sets of 16 plasmids: one set of plasmids for Golden Gate and Gibson assembly which contains a p15A origin of replication gene and kanamycin selection marker for bacteria and one set of plasmids for TAR cloning which contains both a 2-micron origin of replication gene with a tryptophan selection marker for yeast and a F1 origin of replication gene with an ampicillin selection marker for bacteria. Both sets of plasmids have the same DNA inserts: four promoter (Prom) variants (1, 2, 9, 11) and four bicistronic design (BCD) variants (1, 2, 20, 21) coupled with a *gfp* gene. Figure 3 shows the purification of the parts on a 1% agarose gel. Each assembled DNA plasmid is transformed into *E.coli* or *S. cerevisiae* using on-chip integrated electroporation. We also further evaluated our plasmids by Sanger sequencing. Figure 4 shows the results of our sequencing data for Golden Gate assembly (Gibson and TAR cloning not shown) using our microfluidic method. Excluding the beginning and ends of Sanger sequencing reactions, we obtained a high percentage of perfect sequence clones (95%) for the region spanning the BCD, insulator, promoter, and vector backbone.

We created the first automated microfluidic platform that integrates three DNA assembly methods with on-chip transformation and with minimal reagent use. As a proof-of-principle we showed a platform that can assemble two sets of 16 plasmid combinations, which we hope that we (or others) can expand on this microfluidic platform to assemble more combinations with integrated culture and screening.

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SESSION 3: BIOLOGICAL CIRCUITS AND CONTEXT

Use of an Enzyme-Coupled Biosensor to Engineer a BIA Fermentation Pathway from Glucose in *Saccharomyces cerevisiae*

John Dueber*
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The benzylisoquinoline alkaloids (BIAs) represent a large family of natural products rich in potential bioactivities including analgesics, antitumor candidates, antitussives, and antibiotic. There have been ample recent advances in engineering *S. cerevisiae* strains that can convert fed intermediate reticuline into multiple products of interest, including morphine, codeine, noscapine, and dihydrosanguinarine in *S. cerevisiae*. These pathways contain numerous P450 enzymes, a class of enzyme that frequently demonstrate superior expression in eukaryotic hosts. However, direct fermentation of reticuline from central metabolism in yeast requires an elusive tyrosine hydroxylase activity to produce L-DOPA. To identify an enzyme capable of catalyzing this activity, we constructed an enzyme-coupled biosensor: production of a colored, fluorescent metabolite in the presence of L-DOPA. Further, we used this screenable phenotype to isolate a mutant that preferentially performs the desired hydroxylation by lowering the undesired further oxidation of L-DOPA to the melanin biosynthetic pathway. Replacing the enzyme for biosensor metabolite production with the reticuline biosynthetic enzymes enabled the production of this major BIA branchpoint intermediate.

CRISPR Genetic Circuits for Genome Control and Interfacing

Stanley L. Qi
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Genetic reprogramming of cell fate and function requires complex control of the genome. Rationally designed synthetic circuits provide an invaluable approach to both rewire and interrogate the endogenous host network. We have developed the bacterial CRISPR immune system as a toolkit for sequence-specific transcriptional activation or repression of genes in diverse organisms including both bacterial and eukaryotic cells. We show that the basic CRISPR and CRISPRi tools can be further designed to carry out more sophisticated control to modulate multi-gene network. For example, using combinations of RNA codes, the CRISPRi tools enables switchable control of branched metabolite pathways. Here I will talk about the basic CRISPR tools for synthetic biology, the uses for constructing complex genetic circuits, and its uses for understanding biology.

The Programmable Laboratory

Eric Klavins*
University of Washington, Seattle, WA

The design-build-test cycle in synthetic biology requires build and test pipelines that are highly reproducible, maximally informative, and scalable. Traditional laboratory practices where a small team of researchers designs and performs their own experiments in a unstructured lab, in contrast, are difficult to reproduce and scale. In this talk, I will describe several recent efforts to standardize how protocols and workflows are specified using formal, executable programming languages. I will argue that treating experimental workflows as code is leading to a revolution in how experimental work is done and offers huge advantages, such as easy integration into upstream design software and easy collaboration. I will focus in particular on my lab's experience with Aquarium, our human-in-the-loop laboratory operating system that can be used to run experimental workflow programs reliably and reproducibly. I will also describe several projects in yeast synthetic biology that Aquarium has enabled.

Synthetic Approaches to Studying Cis-Regulation

Sri Kosuri*
Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA

In this talk, I will discuss our efforts to bring our DNA synthesis capacities to genomic scales in order to systematically test hypotheses of cis-regulatory control. First, we have leveraged DNA microarrays to assemble gene-sized constructs to both lower costs and increase scales of gene synthesis. Second, we developed methods to characterize large libraries of synthetic DNA in multiplex using next-generation sequencing. We synthesized ~27,000 combinations of promoter, ribosome binding site, and peptide leader sequences in an effort to look at the composability of regulatory elements governing gene expression in *E. coli*. We are able to quantify both the transcription and translation levels of each member of the library independently, leading to insights on how regulatory elements in combination affect transcription and translation rates. Finally, I will discuss our new efforts at UCLA of both increasing scale of DNA syntheses and applying these methodologies to human cell lines.

THURSDAY, JUNE 11
KEYNOTE

Designing Biology for a Healthy World

Pamela A. Silver*
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The engineering of Biology presents infinite opportunities for therapeutic design, diagnosis, prevention of disease and new sustainability strategies. Here, I will present concepts and experiments that begin to address how we approach these problems in a systematic way. By one strategy, we engineer components of the microbiome to act as both diagnostics and therapeutics. In one example, we have engineered natural gut bacteria to record the exposure of animals to antibiotics and inflammation and to count the number of cell divisions as the bacteria pass through the gut. We can engineer the same bacteria to secrete toxins that could result in localized killing of pathogens and to act in a communal manner. Taken together, these experiments have far-reaching implications for the use of biology to prevent and treat disease in the future. Towards sustainability, we seek better ways to engineer cells to harvest sunlight and/or electricity from solar power. We have engineered the interface between living organisms and chemistry and created the 'bionic leaf.' Here, we have devised a novel way to store solar energy that mimics photosynthesis at the same or better efficiency.

SESSION 4: METABOLISM, METABOLOMICS AND ENGINEERING METABOLISM

The Development of Platform-Based Technologies for the Optimization of Sustainably Produced Chemicals

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Genomatica, San Diego, CA

Genomatica has established an integrated computational/experimental metabolic engineering platform to design, create, and optimize novel high-producing organisms and bioprocesses. Here we present the use of our platform to develop *E. coli* strains for the production of the industrial chemical 1,4-butanediol (BDO) from carbohydrate feedstocks. These efforts have culminated in commercial scale production of over 5M lbs of BDO from dextrose. BDO is a chemical intermediate (>3B lbs/yr) that goes into a variety of products including automotive, electronics and apparel (such as spandex), and is currently produced commercially through energy-intensive petrochemical processes using hydrocarbon feedstocks. Therefore, this product represents an opportunity to make a significant impact on the replacement of traditional petrochemical processes with benign bioprocesses using renewable feedstocks.

We are continuing to enhance our technology platform, enabling faster development of new processes with increased chance of success. Here we describe the application and development of one such new technology, cell-free transcription-translation (TX-TL), towards improving the design-build-test-learn cycle for the construction of a high-performing microorganism capable of producing BDO and other industrial chemicals from carbohydrates. We used systems biology approaches including 13C-flux analysis, proteomics, and metabolomics to characterize the production of BDO in TX-TL as compared to traditional fermentation processes. The presentation will highlight the successful integration of the TX-TL technology into our rational and high-throughput strain engineering platform, and will show the potential advantages for using cell-free systems towards the optimization of sustainably produced chemicals.

Metabolite Valves: Dynamic Control of Metabolic Flux for Pathway Engineering

Kristala L.J. Prather*
Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

Microbial strains have been successfully engineered to produce a wide variety of chemical compounds, several of which have been commercialized. As new products are targeted for biological synthesis, yield is frequently considered a primary driver towards determining feasibility. Theoretical yields can be calculated, establishing an upper limit on the potential conversion of starting substrates to target compounds. Such yields typically ignore loss of substrate to byproducts, with the assumption that competing reactions can be eliminated, usually by deleting the genes encoding the corresponding enzymes. However, when an enzyme encodes an essential gene, especially one involved in primary metabolism, deletion is not a viable option. Reducing gene expression in a static fashion is possible, but this solution ignores the metabolic demand needed for synthesis of the enzymes required for the desired pathway.

We have developed "metabolite valves" to address this challenge. The valves are designed to allow high flux through the essential enzyme during an initial period where growth is favored. Following an external perturbation, enzyme activity is then reduced, enabling a higher precursor pool to be diverted towards the pathway of interest. We have designed valves with control at both the transcriptional and post-translational levels. In both cases, key enzymes in glucose metabolism are regulated, and two different compounds are targeted for heterologous production. We have measured increased concentrations of intracellular metabolites once the valve is closed, and have demonstrated that these increased pools lead to increased product yields. These metabolite valves should prove broadly useful for dynamic control of metabolic flux, resulting in improvements in product yields.

Gene Circuits for Self-Tuning Metabolic Pathways

Diego Oyarzún*
Biomathematical Sciences, Imperial College London, London, United Kingdom

Metabolic imbalances impair growth and limit the performance of engineered pathways. These imbalances arise from, for example, the accumulation of toxic intermediates, the depletion of metabolites for survival of the host, or the onset of native regulatory mechanisms that counteract pathway activity. Such limitations can be overcome with gene circuits that adapt pathway expression in response to the metabolic state of the host or changes in conditions of the bioreactor. When appropriately designed, gene circuits cause a pathway to self-tune its expression levels and match production goals with a reduced impact on the host. Recent implementations have showcased how gene circuits can improve pathway yield, yet so far we do not have quantitative procedures for the rational design of circuit architectures or their components, including e.g. the strength and sensitivity of promoters.

Here I will discuss our recent progress in the design of gene circuits for robust pathway engineering. We use a combination of mathematical modelling and computer simulations to learn how promoter design and circuit architecture shape pathway activity. We aim to obtain design guidelines to achieve pathway self-tuning, to control variability of fluxes across a culture, and to engineer novel phenotypes for large-scale multicellular circuitry. I will first show how promoter design affects a pathway's response to perturbations and the accumulation of toxic intermediates. I will then introduce the notion of "metabolic noise", i.e. variability caused by stochastic fluctuations in the expression of enzymes, to explain how promoter strengths can be used to control the amplification or attenuation of pathway variability across a culture. To conclude I will discuss recent results on multi-promoter architectures to implement novel metabolic responses, ultimately scaling-up the functionality of circuits that interface metabolism with the genetic machinery.

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Protecting Groups for Improved Control of Indigo Biosynthesis

Tammy M. Hsu*, Zachary N. Russ and John E. Dueber
Bioengineering, UC Berkeley, Berkeley, CA

Indigo is a widely used dye in the textile industry, but the water insolubility of the indigo molecule makes the dyeing process challenging. The current industry standard uses toxic reducing agents to reduce indigo to leucoindigo, a soluble but unstable intermediate. We are developing a more environmentally friendly, biomimetic process to produce indigo in *E. coli*. In the leaves of indigo plants such as Polygonum tinctorium, indole is oxidized to indoxyl and immediately glucosylated to make stable, soluble indican. When the leaf tissue is damaged, a β-glucosidase can hydrolyze indican back to indoxyl, which dimerizes into indigo in the presence of oxygen.

Looking to the indigo plant for inspiration, we have engineered *E. coli* to protect indoxyl in its monomeric form by glucosylation. We purified and identified the native indoxyl glucosyltransferase from *P. tinctorium*, and we have used it to protect biosynthesized indoxyl *in vivo*. The resulting indican can be exported from the cell and used for dyeing applications. The addition of β-glucosidase can "deprotect" indican, forming indigo. The strategy of biological protecting groups could also be used to make other reactive products of interest more tractable.

SESSION 5: AGRICULTURE AND WATER

What do Soybeans Have to do with Sustainable use of our Oceans?

Virginia Ursin*, Jeff Hass, Byron Froman and Henry Valentin
Monsanto, Mystic, CT

Essential fatty acids, or EFAs, are fatty acids that humans and other animals must ingest because the body requires them but cannot synthesize them. Two fatty acids are known to be essential for humans, the omega-3 fatty acid, α-linolenic acid (ALA) and an omega-6 fatty acid, linoleic acid (LA). Other fatty acids are "conditionally essential," meaning that humans can synthesize them from EFAs, but they can become limited under some conditions. These include two long-chain derivatives of ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Multiple health organizations recommend specific intake levels of ALA, EPA and DHA to promote optimal health. One such recommendation is the consumption of two fish meals per week, preferably fatty fish. This recommendation is problematic for a number of reasons, including sustainability and relative safety of the marine fish which are highest in EPA and DHA. Through the metabolic engineering of the fatty acid biosynthesis pathway in soybean seeds, we have created a land-based, sustainable source of biologically active omega-3 fatty acid, well suited for human and animal diets. The resulting product is soybean oil rich in the omega-3 fatty acid, stearidonic acid (SDA), a naturally occurring precursor to EPA and DHA. We have shown that when ingested, SDA increases beneficial omega-3 levels, specifically EPA, in humans, poultry and some fish species. Compared to EPA and DHA, SDA combines superior stability and sensory characteristics for use in a variety of applications, making this oil suitable for incorporation into a wide variety of foods. Through incorporation into commonly consumed foods, SDA Soy oil can provide health benefits of

fish consumption, while decreasing dependence on marine sources of omega-3 fatty acids.

Engineering Robust Hosts to Improve Microbial Biofuel Production

Mary Dunlop*
College of Engineering and Mathematical Sciences, The University of Vermont, Burlington, VT

A major challenge when using microorganisms to produce biofuels is that next-generation fuels are often toxic to cells. Microbes that thrive in oil rich environments (around natural oil seeps or near oil spills) have mechanisms for tolerating the toxic effects of hydrocarbons. Our goal is to identify tolerance mechanisms from these hydrocarbon-tolerant microbes and express them in biofuel production hosts to improve yields. For example, we have demonstrated that efflux pumps are effective at increasing biofuel tolerance. By isolating pump genes and expressing them heterologously in a biofuel-producing strain of *E. coli*, we have been able to increase biofuel yields. We next asked whether tolerance mechanisms could be used in combination, expressing multiple efflux pumps in the same strain. We found that combinations of pumps can increase tolerance, but the benefits are limited by toxic effects associated with pump overexpression. In addition, we are searching for novel tolerance strategies using a genomic library approach to select for mechanisms that further improve biofuel tolerance. The overall goal of this research is to enhance microbial synthesis of next-generation biofuels by developing tools for improving microbial tolerance of biofuel production conditions.

Targeted Integration of Genes into the Maize Genome Facilitated By Cas9-gRNA System

Huirong Gao*
Applied Technology System, DuPont Pioneer, Johnston, IA

Maize is one of the most important crops in the world. A growing global population requires a continuous effort to increase maize productivity and yield stability. Transgenic approach plays an important role in crop improvement. Currently, transgenes are randomly inserted into maize genome. When more than one gene are used to create a favorable trait or a group of traits, the transgenes reside in various parts of chromosomes, making the subsequent breeding costly and prolonging the time to bring products to farmers. Although molecular stacking in a transformation construct can combine multiple transgenes into one locus, this strategy is not always desirable because breeders often want to have the option to segregate out some of the transgenes to meet their trait goals. A preferred design would be placing multiple closely linked transgenes in designated chromosome regions. Previously, transgenes can be precisely inserted at a small number of locations in the genome with help of protein-based double strand break reagents like zinc-finger nucleases, homing endonucleases (meganucleases) and TALENs. Recently, the Cas9-gRNA system makes it possible to target transgenes into almost any location in the genome which would allow multiple transgenes to be precisely inserted at pre-selected, characterized, and closely linked genomic regions. Targeted integration of transgenes in maize via Cas9-gRNA will be presented.

SESSION 6: ADVANCING SYNTHETIC BIOLOGY TOOLS, AUTOMATION AND ANALYTICAL TECHNOLOGIES

Broadband DNA Synthesis

Emily Leproust*
Twist Bioscience, San Francisco, CA

Twist has developed a proprietary semiconductor-based synthetic DNA manufacturing process featuring a 10,000-well silicon platform capable of producing synthetic biology tools, such as oligonucleotides, genes, pathways, chassis and genomes. By synthesizing DNA on silicon instead of on traditional 96-well plastic plates, the Twist DNA synthesis platform overcomes the current inefficiencies of synthetic DNA production, and enables cost-effective, rapid, high-quality and high throughput synthetic gene production. The Twist Bioscience platform has the potential to greatly accelerate the development of personalized medicine, sustainable chemical production, improved agriculture production as well as new applications such as *in vivo* diagnostics, biodetection and data storage.

TBD

Ben Gordon
MIT/Broad Foundry, Cambridge, MA

Synthetic Biology Open Language (SBOL): Community-Driven Standard for Communication of Synthetic Biology Designs

Jake Beal*
BBN Technologies, Cambridge, MA

The re-use of previously validated designs is critical to the evolution of synthetic biology from a research discipline to an engineering practice. The Synthetic Biology Open Language (SBOL) is an emerging data and diagrammatic standard for visualizing and exchanging designs within the synthetic biology community. SBOL represents synthetic biology designs in a community-driven, formalized format for exchange between software tools, research groups and commercial service providers. As a community-driven standard, SBOL continues to be updated as synthetic biology evolves, with the aim of providing critical communication

and interchange capabilities for various aspects of the synthetic biology workflow. In this community update, we present an overview of the standard's current capabilities and ongoing work, including:

- A data model for representing both the structure of complex genetic designs and their operation, including support for reuse of existing modules, composition of regulatory models, and combinatorial genetic libraries.
- Unambiguous diagrams for communicating genetic designs
- Interoperability with other standards for representing structure and behavior
- Synthetic biology tools enabled by SBOL
- Software libraries to facilitate adoption of SBOL by new synthetic biology tools
- Demonstration of software that supports the standard and can support your research

Finally, we provide an updated roadmap for future development, as well a method for the broader synthetic biology community to provide input to ensure that the developing standards can best serve their needs and practices.

TBD

Ron Weiss*
MIT, Cambridge, MA

FRIDAY, JUNE 12
KEYNOTE

Re-Writing Genomes: Discoveries to Applications

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Physical Biosciences Division, Lawrence Berkeley National Lab, Berkeley, CA

Bacteria have evolved elegant systems for protecting their genomes from invasive elements using enzyme systems that detect and destroy foreign nucleic acids. I will discuss our research on CRISPR systems and related pathways in which enzymes recognize and manipulate nucleic acids in a sequence-guided fashion. Many of these enzymes have potential utility for various applications in research and the clinic, and have been harnessed based on a fundamental understanding of their biochemical and biological functions. I will describe the process of discovery and invention that enabled the CRISPR-Cas9 system to emerge as a revolutionary technology, underscoring the role of basic research as an engine of innovation and tool development.

SESSION 7: DNA AND RNA BASED SYNTHETIC BIOLOGY

Designing and Verifying Molecular Circuits Made of DNA

Erik Winfree*
Caltech, Pasadena, CA

Inspired by the information processing core of biological organisms and its ability to fabricate intricate machinery from the molecular scale up to the macroscopic scale, research in synthetic biology, molecular programming, and nucleic acid nanotechnology aims to create information-based chemical systems that carry out human-defined molecular programs that input, output, and manipulate molecules and molecular structures. For chemistry to become the next information technology substrate, we will need improved tools for designing, simulating, and analyzing complex molecular circuits and systems. Using dynamic DNA nanotechnology as a model system, I will discuss how programming languages can be devised for specifying molecular systems at a high level, how compilers can translate such specifications into concrete molecular implementations, how both high-level and low-level specifications can be simulated and verified, and how these techniques can be used to design, implement, and understand nucleic-acid circuits that exhibit specified non-equilibrium dynamics.

Powered DNA Strand Displacement Circuits for Continuous Environmental Monitoring and Memory

Dominic Scalise and Rebecca Schulman*
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In vitro biomolecular circuits seek to emulate *in vivo* chemical reaction networks that can control the concentrations of chemical outputs in response to the concentration levels of chemical inputs. While such circuits can be built using small molecules, proteins, RNA and DNA components, enzyme-free circuits based on strand displacement reactions offer important benefits in terms of scaling, robustness to physical conditions and reliable *in silico* design. Currently, DNA strand displacement circuits can perform complex computations, but they lack the capacity to respond dynamically to environmental changes because portions of the circuit can become nonfunctional after a single input/output cycle. This limitation makes it impossible to build systems that can monitor changes in the physical environment or store state information.

We are building a system of DNA-based strand displacement circuits that overcome these limitations. The circuits are based on two types of components that operate on separate time scales: fast reactions between computational components compute an answer based on the current input concentrations, while a second class of components slowly

restores the components to a steady state value. In many ways this system mirrors the process of *in vivo* computation, in which transcription and degradation processes restore concentrations to a steady state, while fast reaction produce an output state dependent on the concentration of circuit inputs.

Design of a Toolbox of RNA Thermometers

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RNA thermometers mediate responses to temperature changes in various natural circuits, and have been developed in a synthetic context as well. However, a toolbox of RNA thermometers with different sensitivities to temperature is lacking. Here, we address this issue using a combination of computational and experimental methodologies. We analysed a set of available synthetic RNA thermometers through a quantification of their activity as a function of temperatures in a cell-free expression system as well as through a computation of their melt profiles. Based on this, we computed melt profiles of a library of RNA thermometers and found that the library contained RNA thermometers with a range of sensitivities and thresholds in their response to temperature. We constructed this library and found, through preliminary measurements, a wide range of responses to temperature, which in some cases matched the computational predictions. The constructed library represents a toolbox of RNA thermometers with different sensitivities and is foundational work towards synthetic biology applications such as efficient control of large volume chemical reactors, precise spatiotemporal control of gene expression as well as tools to engineer robustness to temperature in biomolecular circuits.

Dynamic RNA Nanotechnology

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Division of Engineering & Applied Science, California Institute of Technology, Pasadena, CA; Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA

The programmable chemistry of nucleic acid base-pairing plays central roles in the biological circuitry within living organisms and provides a rich design space for the emerging discipline of dynamic nucleic acid nanotechnology. Nucleic acid molecules can be engineered to interact via prescribed hybridization cascades to execute diverse functions including catalysis, amplification, logic, and locomotion. To date, these efforts have been primarily directed at engineering DNA devices and circuits that operate *in vitro*. By contrast, synthetic RNA hybridization cascades have been relatively little-explored, yet hold great promise for engineering programmable signal transduction *in vivo*. Because biological RNAs interface with diverse endogenous pathways, small conditional RNAs (scRNAs) that interact and change conformation to transduce between detection of programmable RNA inputs and production of biologically active, programmable RNA outputs provide a conceptually appealing framework for engineering programmable conditional regulation in living organisms. This talk will describe: 1) mechanistic design elements that can be combined in diverse ways to engineer shape and sequence transduction with scRNAs, 2) a physically sound, computationally efficient sequence design algorithm for scRNA reaction pathway engineering that supports diverse user-specified sequence constraints, including biological constraints (pre-publication software available at nupack.org), 3) conceptual opportunities and practical challenges for engineering scRNA-mediated programmable conditional regulation in living cells.

SESSION 8: MINIMAL SYSTEMS

Bottom Up Construction of Dynamic Biomolecular Materials

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Cells develop, grow and reconfigure their shape via the interplay of molecular materials and circuits. Cytoskeletal scaffolds, for example, continuously adapt their shape in response to the outputs of signaling pathways and gene networks. We want to harness this general architecture and develop artificial materials with similar adaptation properties, bypassing the complexity of the cellular environment. I will describe the construction of minimal dynamic molecular scaffolds with nucleic acid nanostructures whose assembly is directed by nucleic acid signals and circuits. Nucleic acids are an ideal biomolecular material due to the programmability of their base pair interactions. I will discuss in particular the control of growth and decay in a system of DNA nanotubes, a synthetic analog to cellular microtubules.

Minimizing a Bacterial Genome by Global Design and Synthesis

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We have developed and used a whole genome design approach to create an approximately minimal bacterial genome from the 1078 kb synthetic *Mycoplasma mycoides* JCVI-syn1.0 genome we synthesized in 2010. To achieve a successful design, we made extensive use of Tn5 transposon mutagenesis and deletion analysis to identify genes that were non-essential. We developed design rules to remove the non-essential genes without disrupting the function of the remaining essential genes. To troubleshoot designs, we divided the genome into 8 approximately equal segments, each of which could be tested individually, or in various combinations with wild type *M. mycoides* syn1.0 segments. After several cycles of design and testing, we achieved a viable 574 kb genome with 38 RNA genes and 478 protein coding genes. When installed by transplantation, the resulting cell, *M. mycoides* JCVI-syn2.0, grows continuously in independent culture and has a genome smaller than that of any such cell found in nature. It is our working model of a minimal cell, the result of a compromise between small genome size and an experimentally useful replication rate. It retains almost all genes involved in synthesis and processing of macromolecules. It also contains 95 genes of undefined function, some of which may specify previously undiscovered biological functions. The 385 genes removed include 118 of unassigned function, 73 involved in mobile elements and DNA modification and restriction, 66 lipoprotein genes, as well as 73 involved in transport, catabolism, and other metabolic processes. JCVI-syn2.0 grows with a doubling time of 86 min. and its colony morphology is similar to *M. mycoides* JCVI-syn1.0. However, dramatic changes in cellular morphology are observed, including filamentous structures and giant vesicles more than 1000 times the volume of *M. mycoides* JCVI-syn1.0 cells.

The *M. mycoides* JCVI-syn2.0 system provides a platform for completely defining the gene functions required for life, developing genome design rules, and elucidating incompletely understood essential biological functions such as cell growth and division.

Reconstructing Anaerobic Microbiomes from the 'Bottom-up': New Techniques to Decipher Interwoven Metabolism

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Anaerobic microbiomes are among the most diverse microbial communities on earth, yet the mechanisms that control community structure and stability are poorly understood. For example, the rumen microbiome within large herbivores consists of four interdependent microbial populations (bacteria, protozoans, fungi, and methanogens) that work together to drive crude biomass into sugars and fermentation waste products. However, the interdependency of these microbes and their impact on microbial metabolism has been difficult to characterize since the vast majority of these microbes cannot be cultured. To characterize interwoven metabolism of anaerobes, we have focused on an interdependent sub-population within the rumen microbiome – anaerobic fungi and their associated hydrogenotrophic archaea (methanogens). In this community, gut fungi hydrolyze cellulose-rich biomass in the herbivore rumen, and a syntrophic partnership with methanogens allows fungi to thrive by siphoning hydrogen to methane. Our goal is to understand this natural syntrophy, and mimic it to engineer synthetic, stable anaerobic consortia that funnel crude biomass to sustainable chemicals.

To construct anaerobic consortia from the "bottom-up", we have overcome culture challenges by isolating anaerobic fungi with dependent methanogens from herbivore fecal materials. These environmental co-cultures establish a simplified system to model the cooperative action of the anaerobes, and our prokaryotic/eukaryotic systems are stable in culture > 60 weeks. Using RNA-Seq, we have modeled the regulatory patterns for fungal genes from the *Piromyces*, *Neocallimastix*, and *Anaeromyces* genera during hydrolysis of reed canary grass and discovered several conserved regulons of novel genes that govern function, which are catabolite repressed. Addition of methanogens (e.g. Methanocorpusculum) during biomass breakdown shows that fungal-methanogen syntrophy drastically accelerates cellulose and lignocellulose breakdown by the fungi. Furthermore, the excess sugar hydrolysates and metabolites from an *Anaeromyces*/Methanocorpusculum co-culture enable metabolic linkage of non-native facultative anaerobes to the consortium. Strains of *S. cerevisiae* and *E. coli* were engineered to produce Flavin based fluorescent protein (FbFPs) as a reporter of co-culture growth on excess sugars (5-8 g/L) left over from fungal cellulose breakdown. We will discuss stability of these synthetic co-culture platforms, and strategies we have used to optimize production of n-butanol by an engineered *E. coli* strain in co-culture with anaerobic fungi. As anaerobic fungi are not yet genetically tractable, our strategy offers a path forward to make value-added products directly from crude lignocellulose by compartmentalizing lignocellulose breakdown and production in co-cultured microbes.

Semi-Synthetic tRNA Complement Mediates *in Vitro* Protein Synthesis

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Genetic code expansion enables the site-specific incorporation of unnatural amino acids into proteins. The majority of the efforts in this direction focused on reassignment of termination or quadruplet codons. While the redundancy of genetic code provides a large number of potentially reasonable codons this approach has received much less attention due to the inevitable competition with endogenous tRNAs. Here we report an *in vitro* protein synthesis system with a controllable and simplified tRNA complement. To develop it we established a quantitative *in vitro* peptide synthesis assay that allowed us to analyse the ability of synthetic tRNAs to decode all of 61 sense codons. Systematic analysis of 48 synthetic *E.coli* tRNA species demonstrated that despite the lack of posttranscriptional modifications the majority of them were able to support protein translation with exceptions for three amino acids. For these 3 amino acids, we purified their specific native tRNAs from *E.coli* native tRNA mixture to homogeneity and demonstrated that isolated tRNAs retain their activity. Using a combination of synthetic tRNAs and purified specific native tRNAs we formulated a semi-synthetic tRNA complement coding for all 20 amino acids. We demonstrated that it was able to restore protein translation activity of tRNA-depleted *E.coli* lysate to a level comparable to that of native tRNA. Using this system we expressed three different sequences coding for superfolder *GFP*. This novel semi-synthetic translation system is powerful tool for tRNA engineering and provides a platform for reassignment of at least 9 sense codons.

STUDENT SESSION

A Modular Protein Architecture for Generalized RNA Targeting: Development and Application to RNA Monitoring, Control, and Protein Scaffolding

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A key goal of synthetic biology is to program cellular processes towards scientific and engineering goals. Recent advances in modular strategies for DNA editing have expanded our access to the eukaryotic genome, but our ability to target the transcriptome remains limited by the proteins available for RNA binding. Many RNA-binding proteins are known that bind to specific sequences, raising the question of whether a modular protein architecture could be generated with appropriately concatenated modules capable of targeting arbitrary RNA sequences. Here we present a system capable of such targeting, based on the human Pumilio homology domain (PumHD) protein. We developed a set of modular protein building blocks, one for each of the four RNA bases, which can be concatenated in chains to bind arbitrary single-stranded RNAs. We call our system Pumilio based assembly, or Pumby.

We validated the binding specificity of proteins based on Pumby modules to arbitrary RNA sequences, as well as their ability to detect particular transcripts in human cells. We further discovered that Pumby chains can report the translation state of specific open reading frames within those transcripts, enabling monitoring of protein production in live cells. We demonstrated the use of Pumby proteins to trigger RNA degradation and, conversely, to upregulate the translation of open reading frames, even those not preceded by ribosome binding sites. All these applications of Pumby proteins can be applied to unmodified native genes, without the need to introduce exogenous RNA target sequences.

Our system may open up many frontiers in the observation and control of RNA processes in living cells. We anticipate that the Pumby architecture could enable the orthogonal, simultaneous manipulation of multiple RNA targets within a cell. RNA manipulation could provide an additional layer of control over classical transcriptional genetic circuits. The availability of multiple orthogonal RNA-binding proteins may significantly augment the potential of mRNA as an intracellular scaffold for generating novel biomolecules. The Pumby architecture opens the door for many scientific inquiries and biotechnological applications of RNA in living systems.

Designing Conservation Relations in Layered Synthetic Biomolecular Networks

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In Synthetic Biology, biomolecular networks are designed and constructed to perform specified tasks. Design strategies for these networks tend to centre on tuning the parameters of mathematical models to achieve the specified behaviour, and implementing these parameters experimentally. For example, we typically manipulate the strength and rates of the interactions between transcription factors and genetic promoters to design the dynamics of genetic regulatory networks. This design strategy assumes a fixed stoichiometric structure to the network, which pre-defines its possible behaviours by constraining the space in which the concentrations can take values. These constraints are manifested as a number of fixed, linear conservation relations. Such a pre-definition may be too restrictive for the purposes of designing the specified dynamics. Our recent work has investigated the extent to which the state space of a synthetic network can also be designed and shaped by parametric tuning. We have exploited layered timescale separation to implement new, nonlinear, tuneable conservation relations, which hold for all times beyond a fast transient and remain satisfied for all possible slow-scale dynamics. This strategy can be applied to the design of genetic regulatory networks through the construction of fast protein-protein interactions. Hence we can flexibly constrain

the state space of a genetic regulatory network such that new, nonlinear, tuneable constraints can be independently imposed on the allowed trajectories of an arbitrary genetic regulatory network.

Directed Evolution of Acid-Tolerant Phenotype Using an Engineered pH-Riboswitch

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Extracellular pH constitutes an environmental signal which directs phenotype evolution and metabolism in living cells. Understanding the molecular triggers and events to which this process is regulated will enable the systematic reprogramming of biological systems with improved tolerance and production of short chain fatty acids for colonic disease prevention. To facilitate the discovery and interrogation of acid-tolerant phenotype during adaptive evolution, we designed and characterized a pH-sensing device that enables programmable gene expression control in prokaryotic hosts. Firstly, a rational learn-by-design approach was employed to enhance performance and invert behavior of a natural pH-riboswitch. The pH-sensing device was then decoupled from cellular context effects using an orthogonal phage RNAP to generate the riboswitch transcript. We also demonstrate the robustness and versatility of our system in a myriad of microbial chassis including probiotics. We envision the flexibility and simplicity of our system to facilitate the development of next generation designer probiotic and nutraceuticals.

Enabling Selection in Directed Evolution of Enzymes Via Cellular Engineering

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Directed evolution has been used extensive to engineer enzymatic activity for the synthesis of fuels, chemicals, and bioactive compounds. The process of directed evolution consists of an iterative process of creating mutant libraries and choosing desired phenotypes through screening or selection until the enzymatic activity reaches the desired goal. Screening is accomplished through linking the desired property to some visual output using colorimetric or fluorometric assays, while selection links the desired property to survival of host cell. Between the two, selection is more desirable because it offers far higher throughput capabilities with lesser effort. Furthermore, in screening, every mutant in the library needs to be examined individually for the desired property, whereas using a selection enables elimination of the majority of undesirable candidates. However, selection is harder to implement since it requires identification of a unique selection mechanism for every given directed evolution undertaking. That is, given an enzymatic reaction, the challenge is to automatically identify a consumption pathway from the desired product to a metabolite native to the host cell. In our prior work, presented at International Workshop on Bio Design Automation, June 2014, we developed an algorithm, ASF (Automated Selection Finder), for constructing a selection pathway utilizing metabolites and reactions catalogued in a database (e,g., KEGG). Here, we improve on our earlier work by providing a systemic way to engineer the host cell to maximize the yield through the selection pathway.

Given a selection pathway, we describe in this work a computational method for coupling the pathway with cell survival. Our method aims to maximize the yield of the selection pathway. To reach this goal, our method restricts carbon uptakes, making the selection pathway the only possible carbon source within the host. The added pathway thus becomes essential for producing cellular biomass. Additionally, our method identifies possible knockout targets to improve the yield through the pathway. To assess the effectiveness of our approach, we applied our method to construct selection pathways for several beneficial metabolites including xylitol, aniline, methanol, and D-ribulose-1, 5-bisphosphate. We used *Escherichia coli* as the host organism. For all compounds except aniline, high-consumption pathways were identified. Further, for xylitol and D-ribulose-1, 5-bisphosphate, our method identified high-consumption pathways that were experimentally validated and confirmed in the literature.

Quantifying and Modeling Compositional Context Effects on Synthetic Biocircuits in *E. coli*

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In the past two decades, our ability to rapidly design and implement novel synthetic biocircuits has increased dramatically. Nonetheless, our power to assemble these biocircuits into large-scale genetic programs to implement useful functions is limited by the lack of modularity, or robustness, in existing biocircuit modules. In general, there are three main sources of failure in engineering more complex biocircuits: compositional context, host context, and environmental context1. In this work we discuss how compositional context, i.e. the spatial arrangement, spacing, and orientation of biological parts, can affect gene expression in a biocircuit. We introduce a simple biocircuit comprised of two genes - each expressing an mRNA aptamer (mSpinach2 and MG aptamer3) and show that by varying the relative orientation of the two genes, we achieve significantly different gene expression. We show that by increasing the relative spacing

between genes, we can decrease the differences in gene expression, effectively insulating against compositional context effects. We then discuss how compositional context introduces intermediate supercoiling between genes and derive a novel mathematical modeling framework that recapitulates the trends seen in our experimental data. We perform time-lapse single cell microscopy experiments to show that by modulating compositional context, it is possible to affect the amplitude and duration of burstiness in gene transcription. Our experimental and modeling results corroborate recent work4, showing that burstiness is a function of supercoiling state and more importantly, that compositional context can be used to modulate the properties of pulsatile gene expression. Additionally, we demonstrate that compositional context effects can be used to enforce a more fundamental form of (positive and negative) feedback, with time-scales much faster than those achievable by protein-mediated feedback. We show that the design choice of gene orientation and spacing can be used to implement simple logic such as XOR logic and dual positive feedback. Finally, we demonstrate our findings using variants of the genetic toggle switch5, showing how compositional context design choices can be used to improve latching of the toggle switch.

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SATURDAY, JUNE 13
KEYNOTE

Prosthetic Gene Networks for Biomedical Applications

Martin Fussenegger*
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Since Paracelsus' (1493-1541) definition that the dosing makes the drug the basic treatment strategies have largely remained unchanged. We continue to use a precise prescribed dose of a small-molecule drug, a protein therapeutic or a therapeutic transgene to constitutively modulate or complement the activity of a disease-relevant target. However, this treatment concept does neither consider the metabolic dynamics nor the interdependence of the most important pathophysiologies of the 21st century such as obesity, diabetes and cardiovascular disorders. Synthetic biology-inspired prosthetic networks may act as metabolic prostheses that provide the dynamic interventions, the immediate pre-disease action and the multi-target capacity required to meet with the treatment challenges of the future. Prosthetic networks consist of synthetic sensor-effector gene circuits that (i) seamlessly operate in implanted designer cells, (ii) constantly sense, monitor and score metabolic disturbances in peripheral circulation, (iii) process OFF-level concentrations of pathologic metabolites, and (iv) coordinate an adjusted therapeutic response in an (v) automatic and self-sufficient manner. We will present our latest generation of synthetic mammalian gene circuits and provide a few examples of prosthetic networks operating in animal models of prominent human diseases to highlight the challenges and impact of synthetic biology on future biomedical applications.

SESSION 9: BIOLOGICAL PARTS

Controlling Organisms Electrically with Synthetic Biology

Caroline Ajo-Franklin*
Materials Sciences Division, Lawrence Berkeley National Lab, Berkeley, CA

Since both organisms and devices use electrons as information and energy carriers, interfacing living cells with electrodes offers the opportunity to control key biological processes electronically. I will describe our efforts to engineer bi-directional electronic communication between living cells and non-living systems by installing electron nanoconduits into cell membranes. We have recently demonstrated that by transplanting synthetic genes into the model organism *Escherichia coli* we can express these electron nanoconduits and confer upon these cells the ability to reduce metal ions, solid metal oxides, and electrodes. Additionally, current production by engineered *E. coli* causes these cells to shift their metabolism towards more oxidized products, demonstrating that this electronic interface can control intracellular state. Lastly, we have shown that these *E. coli* can accept, as well as donate, electrons from an electrode. Thus, this work provides a blueprint for electron conduits which bi-directionally move energy and information between living cells and non-living systems, and has direct applications in bioenergy, bioremediation, biosynthesis, biosensing, and biocomputing.

Towards the High – Throughput Construction of Fluorescent Biosensors

Stacy-Anne Morgan*, Dana Nadler, Kaitlyn Kortright and David Savage
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Rapid quantification of *in vivo* metabolite concentrations is a significant challenge in understanding and engineering metabolism. It has been demonstrated that single fluorescent protein biosensors can reliably act as *in vivo* sensors of metabolite concentrations with high specificity, a linear response, a wide dynamic range and on relevant spatiotempo-

ral scales. However, rational approaches to the construction of such sensors are time consuming and difficult, resulting in few reported examples. Therefore, the high-throughput construction of fluorescent protein-based biosensors would greatly enhance our abilities to meet the current and future challenges of probing and manipulating metabolism. Towards this, we report on the development of a general, transposon-mediated strategy that was applied to the construction of a sensor for trehalose, an important metabolite for which a reliable sensor is lacking. In this approach, a modified transposon was used to create a library of green fluorescent protein insertions within *T. litoralis* D-trehalose/D-maltose-binding protein (TMBP). The library was then subjected to successive rounds of fluorescence activated cell sorting (FACS) in the presence and absence of trehalose to obtain a sensor with high sensitivity and specificity. From these results, we have learned about the generality of this technique for constructing fluorescent biosensors.

A System for Unprecedented Ribosome Engineering in Living *E. coli*

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The *Escherichia coli* ribosome is a 2.4 MDa molecular machine that consists of a large subunit and a small subunit, and is the key catalyst in gene expression. It is responsible for synthesizing proteins from natural amino acids in a sequence-defined fashion with impressive speed and accuracy. Expanding the repertoire of ribosome substrates and functions would be greatly beneficial for the advancement of systems and synthetic biology. However, as with any biological system, engineering objectives are often completely opposed to the growth and reproduction objectives of the organism. This problem can be solved through the use of a specialized ribosome that translates only a specific type of engineered messenger RNAs (mRNAs) and avoids translation of native cellular mRNAs. So far, efforts to construct such orthogonal ribosomes have focused on modifying the small subunit alone because orthogonality is endowed by modifying the Shine-Dalgarno sequence of an mRNA and the corresponding complementary sequence in the 16S ribosomal RNA (rRNA) of the small subunit. Unfortunately, free exchange between the subunits meant the large subunit, which is responsible for peptide bond formation and protein excretion, could not be extensively engineered. Here we address this challenge. Specifically, we show that an engineered ribosome with tethered subunits (termed Ribo-T), which contains a single hybrid rRNA composed of small and large subunit rRNA sequences, is capable of protein synthesis *in vitro* and *in vivo*. Considering that the ribosome is one of nature's most evolved, fine-tuned and conserved structures, it is surprising that we were able to engineer a ribosome with inseparable subunits. One of the exciting implications of Ribo-T is the possibility of introducing mutations in large ribosomal subunits that would be deleterious if introduced in an untethered ribosome (e.g., dominant lethal). We show the ability to evolve Ribo-T by selecting otherwise dominantly lethal rRNA mutations in the large ribosomal subunit that facilitate translation of challenging protein sequences. We anticipate that Ribo-T will advance fundamental understanding of the ribosome, enable dual translation systems in cells, and catalyze a new paradigm for synthesis and evolution of abiological polymers.

SESSION 10: MICROBIAL COMMUNITIES AND MICROBIOME GENOMICS

Engineering Nitrogen Fixing Symbiotic Associations in Cereals

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The ability to take up mineral nutrients, particularly nitrogen and phosphorus, is generally the major limitation to plant growth. Because of this farmers apply nitrogen and phosphorus through fertiliser application to promote crop growth. Sustained yields are dependent on this fertiliser application, but it comes at a high price, both in the cost of the fertiliser and the environmental damage that results from its use. A number of plant species have evolved beneficial interactions with micro-organisms that facilitate the uptake of these nutrients. Legumes form symbiotic interactions with mycorrhizal fungi that facilitate phosphate uptake and with rhizobial bacteria that provide the plant with a source of nitrogen. The establishment of these symbioses involves a molecular communication between the plant and the symbiotic micro-organisms in the soil. Mycorrhizal fungi and rhizobial bacteria release signals that are recognised by the host plant and lead to developmental changes associated with the accommodation of the symbionts. Genetic dissection in the legume Medicago truncatula has defined the signalling pathways involved in these symbioses. A number of the genes required for the mycorrhizal interaction are also necessary for the rhizobial interaction, indicating a conserved symbiosis signalling pathway. This implies that the evolution of nodulation involved the recruitment of a signalling pathway already functioning in mycorrhizal signalling. This signalling pathway is present in most plant species, including cereals suggesting that engineering the perception of rhizobial bacteria in cereals is simplified and requires an understanding of the legume specific components that activate and are activated by the common symbiosis signalling pathway. We are in the process of engineering this

signalling pathway in cereals to promote the recognition of rhizobial bacteria as the first step in engineering biological nitrogen fixation into cereal crops.

Insights from a Global View of Secondary Metabolism: Small Molecules from the Human Microbiota

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The discovery of natural products – small molecules from microbes often used as drugs – has been an ad hoc pursuit for almost a century. The rapidly growing database of microbial genome sequences offers new opportunities to leverage genomics and bioinformatics toward discovering natural products and characterizing their roles in mediating interspecies interactions. This seminar will describe two convergent, ongoing lines of research: our use of genomics and bioinformatics to identify biosynthetic gene clusters and predict the structures of their small molecule products, and our efforts to identify and characterize small molecules produced by the human microbiota.

Synthetic Biology Engineering of Biofilms As Nanomaterials Factories

Peter Nguyen*[1], Zsofia Botyanszki[2], Tony Cho[1], Pei-Kun Tay[1], Peng Yin[3] and Neel Joshi[1]
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Programmable self-assembling living systems are the next generation of bioinspired advanced materials, and will integrate synthetic biology and materials science approaches towards reengineering abundant and robust biomaterials, such as bacterial biofilms. We have recently introduced "Biofilm-Integrated Nanofiber Display" (BIND) as a strategy for the programmable functionalization of the *E. coli* biofilm extracellular matrix by genetically fusing various peptide domains to the amyloidogenic protein CsgA, the key proteinaceous component of the biofilms. We find that these engineered CsgA fusion proteins are successfully secreted by the cellular export machinery and self-assemble into a network of extracellular amyloid nanofibers that displays the fusion peptide of interest in high density. The displayed peptide domains maintain their function and confer various non-natural functions to the biofilms as a whole. The BIND platform thus reconceptualizes the microbial communities as autonomous factories for the production of self-assembling bulk nanomaterials. Our results suggest that BIND is a novel strategy for the efficient broad functionalization of biofilms via engineered peptide or protein domains. Using this technology, large-scale structures could be constructed from on-demand nanomaterials with spatially programmable immobilized protein or peptides and controlled bulk mechanical properties.

Even-numbered posters will be presented in Poster Session A on Thursday, June 11.
Odd-numbered posters will be presented in Poster Session B on Friday, June 12.

1. Sbol Visual: Standard Schematic Diagrams for Synthetic Genetic Constructs

Jacqueline Quinn*[1], Robert Sidney Cox III*[2], Aaron Adler*[3], Jake Beal*[3], Swapnil Bhatia*[4], Yizhi Cai*[5], Joanna Chen*[6], Kevin Clancy*[7], Michal Galdzicki*[8], Nathan J Hillson*[9], Akshay Maheshwari*[10], Chris J. Myers*[11], Umesh P*[12], Matthew Pocock*[13], Cesar Rodriguez*[1], Larisa Soldatova*[14], Guy-Bart Stan*[15], Neil Swainston*[16], Anil Wipat*[14] and Herbert Sauro*[17]

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Synthetic Biology Open Language (SBOL) Visual is an effort to standardize visual representations of synthetic biological constructs. SBOL Visual 1.0 consists of a set of standardized glyphs for representing designs of synthetic genetic systems as contiguous constructs. Each symbol corresponds to a sequence element in physical DNA, including simple regulatory elements such as promoter or restriction sites. The symbols can be used as stencils to make illustrations on the computer or by hand, for rendering in a web browser, as images for software design, or as a formal symbology for aiding communication and instruction. SBOL Visual symbols are currently used by a number of commercial and academic software tools. SBOL Visual is particularly suited to represent the design of multi-component artificial genetic constructs, and we cite several examples where SBOL Visual has been employed for scientific descriptions of such constructs.

SBOL Visual is a free and open standard with resources made available for the broadest possible use. The symbols are provided for personal, academic, and commercial use in a variety of image formats, including PNG and SVG. SBOL Visual is developed by members of SBOL Developers Group as a visual, human-readable counterpart to the SBOL standard data format for representing the design of genetic constructs to ensure cross-compatibility with other biological standards. We encourage any interested practitioners to download the images, use and modify them freely, suggest changes, and join the SBOL Visual community to participate in developing the standard: http://www.sbolstandard.org/visual

2. Protease-Based Synthetic Sensing and Signal Amplification

Kirill Alexandrov*
Institute for Molecular Bioscience, Univeristy of Queensland, St Lucia, Australia

The bottom-up design of protein-based signaling networks is a key goal of synthetic biology – yet, it remains elusive due to our inability to tailor-make signal transducers and receptors that can be readily compiled into defined signaling networks. Here, we report a generic approach for the construction of protein-based molecular switches based on artificially autoinhibited proteases. Using structure-guided design and directed protein evolution, we created signal transducers based on artificially autoinhibited proteases that can be activated following site specific proteolysis, and also demonstrate the modular design of an allosterically regulated protease receptor following recombination with an “affinity clamp” peptide receptor. We also create an integrated signaling circuit based on two orthogonal autoinhibited protease units that can propagate and amplify molecular queues generated by the protease receptor. Finally, we will present a generic two component receptor architecture based on proximity-based activation of two autoinhibited proteases. Overall, the approach allows the design of protease-based signaling networks that, in principle, can be connected to any biological process. We will illustrate application of this technology to the development of point of care diagnostics as well as cellular engineering.

3. Considering Dynamic Constraints during Strain Optimization

Sara Amin*, Mona Yousofshahi and Soha Hassoun
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Microbial cells have been successfully engineered to produce a large variety of biomolecules useful as biofuels, drugs and drug-precursors, and bioplastics. One challenge in maximizing the production of a target metabolite within a microbial cell is identifying gene modifications in the form of up-regulation, down-regulation, or knockout. While many such interventions are identified in an ad hoc manner based on experimental expertise, several computational tools have been developed for strain optimization. Strain optimization is formulated as an optimization problem specified in terms of two variables: flux variables and control (decision)

variables that correspond to the presence or absence of regulation for each reaction and in each direction (up/down). Importantly, solutions to strain optimization problems must respect new bounds imposed due to regulation modification. A gene knock out modifies the upper and lower flux bounds to be zero, while up/down regulation defines new upper/lower bounds on reaction fluxes. Changes in bounds due to gene modifications impose new steady-state constraints, referred to as dynamic constraints, on the system as a whole. Another challenge in strain optimization is identifying an optimal fold change (e.g., 0.5, 2x, 5x, or 10x), instead of just identifying the fold change direction (up or down regulation). Prior computational approaches do not consider updating the steady state boundaries nor identify gene fold modification.

We propose a new strain optimization formulation that identifies fold changes required to maximize cellular yield. The fold changes are treated as a random variable with a probability distribution reflecting uncertainty in implementing engineering interventions. Each fold change can be one of three values, 2x, 5x, or 10x, compared to a 1x non-regulated fold change. Simulated Annealing (SA) is used to identify the optimal interventions and their fold changes. Flux Balance Analysis is utilized as a fitness function, evaluating the product yield using updated steady state conditions. We have applied this method to maximize the production of antibody in the Chinese Hamster Ovary (CHO) cell, and pyruvate in *Escherichia coli*. Our results show that SA is capable of identifying several intervention sets with different fold changes values that result in the same yield value of a desired product. Our results also show increased predicted maximum yield value due to utilizing dynamic constraints that update steady-state bounds.

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4. Quantitative Gene Circuits and Computationally Designed Transcriptional Sensors in Plants

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Plants are an attractive system for application of synthetic biology. Due to the long life cycle of plants, rigorous quantitative characterization of genetic components, assembly and testing of complex gene circuits, can take several months to years. For this reason, there is currently a very limited number of characterized genetic parts available for plants. We have developed methodology that allows rapid testing and quantitative characterization of a large number of synthetic genetic parts in isolated plant cells. We used this methodology to select genetic components for assembly of complex gene circuits and predict their function in transgenic plants. We have developed quantitatively tuned genetic circuits, such as positive feedback loops and toggle switches, which can be used to precisely control traits in plants with external inputs. In addition, we have developed a computationally redesigned transcriptional sensor in plants. Computationally redesigned proteins are engineered to be destabilized in the absence of the ligand and stabilized by ligand binding, leading to transcriptional activation in plants. These advances are allowing synthetic biology to take root in plants.

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5. Approaches to Improving the Potential of *Azotobacter vinelandii* as a Biofertilizer

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The industrial Haber-Bosch process responsible for a large portion of ammonia production comes at the expense of fossil fuels. *Azotobacter vinelandii* is a widely studied model diazotrophic (nitrogen fixing) bacterium, and also an obligate aerobe, differentiating it from many other diazotrophs that require environments low in oxygen for the function of the nitrogenase enzyme. This property makes *A. vinelandii* a key target strain for a range of nitrogen biosynthetic processes, including co-culture in mass algal production facilities. Our laboratory has been developing approaches and tools to screen for elevated nitrogen production through rational pathway manipulation and random insertional mutagenesis approaches. Several approaches have resulted in elevated production of specific nitrogen compounds that are sufficient to support the growth of either algae or traditional agricultural crops. Current efforts to expand this approach are studying the potential of developing new symbiotic relationships between *A. vinelandii* and target crops, while further efforts to utilize either plant exudates, residuals or cellulosic by-products would improve the application of biofertilizers further. Studies of transcriptional changes in a high nitrogen production strain are providing a glimpse of how the cell self regulates various processes that enable the flow of significant metabolic flux towards nitrogen fixation, and new tools developed recently should result in further strain enhancements that will improve the application for important nitrogen containing bioproducts. Finally, studies demonstrating the potential to apply new biofertilizer strains to both algae and traditional crops will be presented.

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6. CX1: A Web-Based Application for Augmenting Open Synthetic Biology Laboratory Automation

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Individual experimentation done at the laboratory bench is

the foundation of classical life science R&D, but this craftsman approach has substantial barriers to entry and limits information sharing, reproducibility, and scaling. Digital design, process management and automation, common in many areas of product development and manufacturing including pharmaceutical development, are now finding their way into everyday bioscience. Collectively, they have the potential to dramatically accelerate the iterative design, build, test, learn (DBTL) cycle of synthetic biology. Recently Transcriptic, Inc., has leveraged advances in robotics and software to automate the build and test components of the cycle and make them available via the cloud. However, many researchers still lack the computer programming expertise required to implement the laboratory operations to augment or replace their routine bench work. Working with Transcriptic and their Autoprotocol language, we have developed a web-based application called CX1 to provide an easy-to-use framework for designing, visualizing, controlling, and distributing JSON-based Autoprotocol constructs. The aim of CX1 is to augment openly available synthetic biology lab automation. To demonstrate the utility of the CX1 application, a process development pipeline has been implemented to direct the assembly and characterization of a synthetic PhiX174 bacteriophage from design through *Escherichia coli* plaque assay.

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7. On the Engineering of Effective Biological Computations

Jacob Beal*
Raytheon BBN Technologies, Cambridge, MA

Engineering biological cells to perform computations has a broad range of important potential applications, including precision medical therapies, biosynthesis process control, and environmental sensing. Implementing predictable and effective computation, however, has been extremely difficult to date, due to a combination of poor composability of available parts and of insufficient characterization of parts and their interactions with the complex environment in which they operate. I demonstrate how this situation can be improved by quantitative signal-to-noise analysis of the relationship between computational abstractions and the variation and uncertainty endemic in biological organisms. This analysis takes the form of a delta-SNR function for each computational device, which can be computed from measurements of a device's input/output curve and expression noise. These functions can then be combined to predict how well a circuit will implement an intended computation, as well as evaluating the general suitability of biological devices for engineering computational circuits. Applying signal-to-noise analysis to current repressor libraries shows that no library is currently sufficient for the engineering of many-layer circuits with significant SNR in their outputs. The analysis also indicates key targets to remedy this situation and vastly improve the range of computations that can be used effectively in the implementation of biological applications.

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8. Bridging the Gap: A Roadmap to Breaking the Biological Design Barrier

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This talk presents an analysis of an emerging bottleneck in organism engineering, and paths by which it may be overcome. Recent years have seen the development of a profusion of synthetic biology tools, largely falling into two categories: high-level "design" tools aimed at mapping from organism specifications to nucleic acid sequences implementing those specifications, and low-level "build and test" tools aimed at faster, cheaper, and more reliable fabrication of those sequences and assays of their behavior in engineered biological organisms. Between the two families, however, there is a major gap: we still largely lack the predictive models and component characterization data required to effectively determine which of the many possible candidate sequences considered in the design phase are the most likely to produce useful results when built and tested. As low-level tools continue to mature, the bottleneck in biological systems engineering is shifting to be dominated by design, making this gap a critical barrier to progress. Considering how to address this gap, we find that widespread adoption of readily available analytic and assay methods is likely to lead to rapid improvement in available predictive models and component characterization models, as evidenced by a number of recent results. Such an enabling development is, in turn, likely to allow high-level tools to break the design barrier and support rapid development of transformative biological applications.

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9. A Worldwide Baseline for Reproducibility in Synthetic Biology.

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Rapid improvements in our ability to genetically engineer biological organisms offer the potential for revolutionary applications for medicine, manufacturing, agriculture, and the environment A major barrier to transition from principle to practice, however, is the frequent sensitivity of biological systems to small changes in their cellular or environmental context. This makes it difficult to reproduce or build on prior results in the lab, let alone to ensure desirable behavior in a deployed application.

A first step toward addressing these issues is to quantify

the degree of variability exhibited by engineered genetic constructs across multiple laboratories. To this end, we present the results of the first large-scale interlab study of reproducibility in synthetic biology, carried out by students at 45 institutions worldwide during the 2014 International Genetically Engineered Machine (iGEM) Competition. Participants were asked to measure fluorescence from three BioBrick constructs constitutively expressing *GFP* in *E. coli*. Only around half of the teams were able to produce valid measurements for all three constructs, and nearly all measurements were in non-comparable relative units. Those teams with valid measurements, however, exhibited a high degree of consistency in observed fluorescence, with a mean error of only 1.9-fold. Teams also exhibited a high degree of variation in the details of how measurement protocols were executed.

These results are extremely promising, showing that engineered genetic constructs can exhibit a high degree of consistency in behavior even in the face of variations in context. At the same time, the limits encountered in this study highlight the need for adoption of calibrated measurements producing standardized units and for protocol automation to reduce the impact of "cultural art" in laboratory methods. Finally, this study also demonstrates how critical issues in science and reproducibility can be addressed through "citizen science" by involving undergraduates around the world.

10. Logic Gates for Engineering Programmable Interactions in Microbial Consortia

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The current state of synthetic biology relies heavily upon cultivation of single species of model microorganisms in tightly controlled laboratory environments. Such, often highly engineered, domesticated strains suffer from poor stability and resilience in natural settings due to fluctuating environmental conditions and competition from communities of indigenous microbes. To address these challenges, we have developed a generalizable framework for design and engineering of autonomous synthetic microbial consortia capable of operating safely within unpredictable and dynamic natural environments. At the center of the proposed platform lies photoautotroph-heterotroph interactive partnership. Such associations, which naturally assemble to take advantage of services carried out by individual members, are ubiquitous in nature and mediate key ecological processes such as energy capture, carbon fixation, and nutrient cycling. Genetically tractable photoautotrophic organisms, such as cyanobacteria, can sustain and drive an engineered consortium through production of O_2, organic C, and, in some cases, fixed inorganic or organic N. While metabolite exchange provides ways for functional compartmentalization, a tight control and a high level of communication and interaction between members of the consortium are required. As demonstrated in single-species models, computational logic gates provide a controllable and modular approach to programming desired cellular behavior. Applying logic gates so that output responses are sustained rather than transient to communities of bacteria, as opposed to single cells, will allow for more complex interactions and offers a novel way to precisely engineer synthetic multi-species systems. This presentation discusses opportunities for implementing logic-based controls into metabolically interdependent phototrophic consortia for improved communication, functional stability, and controllable output.

11. Engineering Emergent Properties in Synthetic Microbial Communities

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Traditionally, engineers and synthetic biologists exert control over microbial metabolism by adding or removing genes and pathways from a single host. However, new paradigms have been realized though the 'consortia' concept of engineering and compartmenting metabolic function within assigned community members. Beneficial emergent properties, such as enhanced productivity and/or stability, can be engineered into synthetic or thoughtfully constructed microbial communities. This presentation focuses on how emergent properties have been realized from community-engineering. Binary consortia were either metabolically engineered (via genome reduction) or naturally prone to cooperate in a 'producer-consumer' motif. These systems ranged from synthetic *Escherichia coli* co-cultures engineered for mutualistic exchange-detoxification of acetic acid to artificial wild-type assemblies of phototrophic cyanobacteria and heterotrophic counter parts. These distinct binary cultures demonstrated enhanced biomass productivity and resistance to metabolite feed-back inhibition. For example, the effect of oxygen availability on metabolically engineered *E. coli* systems was found to be a controlling factor for acetic acid exchange and subsequent phenotype-specific spatial patterning in biofilms and biomass productivity in chemostats. Similarly, oxygen gradients were imposed on a constructed consortium consisting of wild-type photoautotroph (*Thermosynechococcus elongatus*) and chemoheterotroph (*Meiothermus ruber*), which responded with an increased resistance to the growth inhibition effect

of high oxygen-tension. This research lends critical insight to a grand-challenge for biological engineers and synthetic biologists aiming to capitalize on knowledge gained from model systems and phenomenological observation to rationally design microbial communities for controllable outputs.

12. Complex Design, Simple Implementation: The Development of Red Light-Regulated Nuclear Protein Shuttling and Enzyme-Free DNA Assembly

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Protein shuttling in and out of the nucleus represents a key step in controlling cell fate and function. Here we demonstrate a red light-inducible and far-red light-reversible synthetic system for controlling nuclear localization of proteins in mammalian cells and zebrafish. We synthetically reconstructed and validated the red light-induced *Arabidopsis thaliana* phytochrome B nuclear import mediated by phytochrome-interacting factor 3 in a non-plant environment. Based on this principle we induced nuclear import and activity of target proteins by the spatiotemporal projection of light patterns using a smartphone display. A synthetic transcription factor was translocated into the nucleus of mammalian cells and zebrafish to drive transgene expression. We describe the first plant phytochrome-based optogenetic *in vivo* application in vertebrates and expand the toolbox of available light-regulated molecular devices. The development of such complex cellular networks requires suitable molecular cloning methods facilitating the implementation of the underlying design. Here, we describe AQUA (advanced quick assembly), a simple and versatile seamless assembly approach. It does not require any kits, enzymes or preparations of reagents and is the simplest assembly cloning protocol to date. We demonstrate the applicability and versatility of AQUA Cloning in selected proof-of-principle applications. These include (i) targeted insertion-, deletion- and site-directed point-mutagenesis, (ii) combined cloning and protein expression, (iii) combinatorial cloning, and (iv) the one step, one pot de novo assembly of multiple DNA fragments into a single circular plasmid encoding a complex light- and chemically-regulated Boolean A NIMPLY B logic operation.

13. Cell-Cell Communication in Yeast Using Auxin Biosynthesis and Auxin Responsive Crispr Transcription Factors

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A true engineering framework for synthetic multicellular systems requires a programmable means of cell-cell communication. Such a communication system would enable complex behaviors, such as pattern formation, division of labor in synthetic microbial communities, and improved modularity in synthetic circuits. However, it remains challenging to build synthetic cellular communication systems in eukaryotes due to a lack of molecular modules that are orthogonal to the host machinery, easy to reconfigure, and scalable. Here, we present a novel cell-to-cell communication system in *Saccharomyces cerevisiae* (yeast) based on CRISPR transcription factors and the plant hormone auxin that exhibits several of these features. Specifically, we engineered a sender strain of yeast that converts indole-3-acetamide (IAM) into auxin via the enzyme iaaH from *Agrobacterium tumefaciens*. To sense auxin and regulate transcription in a receiver strain, we engineered a reconfigurable library of auxin degradable CRISPR transcription factors (ADCTFs). Auxin-induced degradation is achieved through fusion of an auxin sensitive degron (from IAA co-repressors) to the CRISPR TF and co-expression with an auxin F-box protein. Mirroring the tunability of auxin perception in plants, our family of ADCTFs exhibits a broad range of auxin sensitivities. We characterized the kinetics and steady state behavior of the sender and receiver independently, and in co-cultures where both cell types were exposed to IAM. In the presence of IAM, auxin is produced by the sender cell and triggers de-activation of reporter expression in the receiver cell. The result is an orthogonal, rewireable, tunable, and arguably scalable cell-cell communication system for yeast and other eukaryotic cells.

14. Hands-Free Control of Heterologous Gene Expression in Batch Cultures

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Autonomous control of heterologous gene expression can simplify batch-culture bioproduction by obviating the need for monitoring growth condition. Existing technologies include the uses of special media, wasteful cell-cell communication or biochemical sensors designed only for specific synthetic pathways. There is a need for simple, resource-efficient and general-purpose autonomous control system responding to changing growth condition in batch cultures.

We exploited *E.coli* stationary-phase promoters as sensors for growth-phase transition in batch cultures. We used a recombinase switch to relay transcription signal between a stationary-phase promoter and an output heterologous gene. Specifically, transcription signal from the stationary-phase promoter triggers recombinase expression; recombinase enzyme then inverts a constitutive promoter that turns ON the output gene. We demonstrated that we could predict switching efficiency given measured promoter dynamic ranges and recombinase cassettes with standardized translation initiation elements. We showed that the recombinase switch amplifies signal fold change, lowers cell-to-cell signal variability and eliminates signal fluctuation.
We introduced an autonomous control system for heterologous gene expression. Our system uses stationary-phase promoters and recombinase switches to sense and relay endogenous signals during growth-phase transition. Unlike other existing technologies, our system does not require special media or resource-consuming production of cell-cell signaling molecules. Together, this work provides a simple, generic and efficient tool for batch-culture bioproduction.

15. Development and Experimental Validation of a Mechanistic Model of *in Vitro* DNA Recombination

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Synthetic Biology is a highly interdisciplinary field with the aim of establishing engineering protocols for the construction of synthetic biological circuits. One of the first synthetic biological devices to be built was the genetic toggle switch in *E. coli* [1]. Such transcriptional memory devices have paved the way for the creation of bi-stable genetic switches based on DNA recombination. These site-specific recombinases (SSRs) mediate distinct recombination events that give rise to two stable DNA states. Hence, DNA recombination has huge potential as a tool for DNA sequence assembly with numerous potential applications including biological data storage [2], DNA-based counting systems [3] and the assembly of metabolic pathways [4].

Attempts to experimentally design and build synthetic systems using recombinases have thus far been hindered by a lack of validated computational models that capture the mechanistic basis of DNA recombination. The predictive capabilities of such models could be exploited by Synthetic Biologists to reduce the number of iterative cycles required to align experimental results with design performance requirements. Here, we develop and validate the first detailed mechanistic model of DNA recombination, with a focus on how efficiently recombination can occur, and the model features required to replicate and predict experimental data.

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16. High-Throughput Picodroplet-Based Analysis of Biosynthetic Libraries

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Biosynthesis of high value chemicals by engineered microorganisms is an application of synthetic biology that offers both economic and environmental advantages. This application is increasing the need for high-throughput screening tools that can facilitate the detection of the best performance among a library of designed microbes. For this reason we are developing a high-throughput, miniaturised Mass Spectrometry (MS) tool for profiling synthetic designed libraries (1).

Combining microfluidics based picodroplet technology for cell encapsulation and sorting together with Mass Spectroscopy we aim to rapidly screen, identify and retrieve the best cell "hits" among synthetic metabolic pathway libraries. Based on this novel approach we will be able to determine which construct has the genetic combination that gives the best biosynthesis performance. To test this new tool we have designed three libraries of two synthetic metabolic pathways using molecular engineering techniques (2,3,4).

We chose two previously described synthetic pathways to produce non-natural amino acids (5,6) and focus on improving their level of expression. Various strategies have been explored such as the use of homologue genes from other organisms, varying the DNA copy number, transcription levels or translation activity. Then, using a pioneering picodroplet-based technology (7) that enables not only for the testing of up to 200,000 samples per day by MS, using miniaturised input volumes (400-700 pL) but also for retrieving identified 'hits' in a reproducible manner, we will select single cells, analyse their production of this non-natural amino acids and finally select and recover the best performing clones among the different profiles obtained for further studies.

This will enable new scientific breakthroughs, higher throughputs, lower screening costs, shorten design-build-test cycle and thus, be of interest to the current MS user base in the synthetic biology market and other sectors.

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17. Deletion of Glyoxylate Shunt Pathway Genes Results in a 3-Hydroxybutyrate Overproducing Strain of *Ralstonia eutropha*

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The glyoxylate shunt pathway was examined in the polyhydroxyalkanoate (PHA) producing bacterium, *Ralstonia eutropha*. The deletion of the aceB gene, encoding malate synthase, which catalyzes the second reaction of the glyoxylate shunt, resulted in severe growth defects in cultures containing acetate or fatty acids as the main carbon source. Further deletions of phaB genes, encoding acetoacetyl-CoA reductase enzymes, resulted in little or no growth of *R. eutropha* on acetate. Metabolomic studies revealed that the aceB deletion strain overproduced pyruvate and 3-hydroxybutyrate, both at 15X higher levels than the wild-type strain, when cultured in acetate as the main carbon source. To further enhance production of these compounds, the PHA biosynthetic pathway was disabled in the *R. eutropha* aceB mutant by an in-frame deletion of the phaC (PHA synthase) gene. The resulting strain produced even higher levels of 3-hydroxybutyrate at the onset of nitrogen limitation. This study has provided insight to the construction of a strain of *R. eutropha* that potentially produces value-added compounds when grown on carbon feedstocks containing acetate or fatty acids.

18. Rapid Microfluidic Assay for Bacterial Electrotransformation

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Electroporation results from exposure of cells to electric fields of sufficient strength to disrupt the plasma membrane. The local trans-membrane voltage (TMV) significantly increases during exposure of cells to external electric fields. When the local TMV exceeds a critical threshold, pores are created on the cell membrane, allowing transport of ions and macromolecules (e.g. DNA/RNA) across the membrane. There is vast empirical literature establishing protocols to increase the electrocompetency (capacity for DNA uptake by electroporation) of cells, a process that is currently time consuming and lacks rea-time feedback. Despite the tremendous need, there are currently no protocols for improving bacterial electrocompetency without relying upon time-consuming empirical experimental processes.

Here, we present a rapid microfluidic platform capable of determining electric field thresholds for electroporation. In our microfluidic platform, a converging microchannel generates a linear gradient of electric field. Fluorescence-encoded DNA plasmids or nucleic acid stains permeate cell membranes in regions where the electric field is sufficiently high. We correlate the fluorescent region of bacteria with the range of electric fields that results in electroporation. Analysis of the fluorescence images from a single experiment provides electroporation conditions that would otherwise require hundreds of experiments conducted in parallel.

Our microfluidic platform quantitatively characterizes the electric field required for successful electroporation of microorganisms, one of the parameters for determining electrocompetency. The assay is based on a PDMS microfluidic channel with an active electroporation region spanning 2-4 mm that enables the generation of high electric fields (5-15 kV/cm) at applied voltages of 1000-3000 V. This geometry has been optimized to yield a linear variation of electric field strength, facilitating correlation of cell location within the chip and local electric field.

Under typical operation, the inlet to the device is connected via flexible tubing and a hollow stainless steel needle to a syringe pump for controlled delivery of the cell-dye mixture to the microfluidic device. The outlet is connected via a needle and tubing to an external reservoir, enabling the sample to be collected for downstream analysis. The needles serve as the electrical contacts required to deliver the electric field from the MicroPulserTM (Bio-Rad, Hercules, CA) to the sample within the channel. The assay utilizes SYTOX® (Life Technologies, Grand Island, NY), a dye which cannot penetrate the intact cell envelope and only fluoresces once it binds to intracellular DNA; therefore, it is an effective marker for determining electroporation conditions. We have utilized numerical simulations in COMSOL® (Burlington, MA) to develop a “ruler” that allows us to determine the electric field that correlates with the onset of electroporation. The simulations allow us to quantitatively determine the electric field range in regions of dye uptake.

We envision this rapid microfluidic platform as a tool to

enable and optimize genetic transformation of intractable or previously challenging microbial chassis. This platform systematically samples a continuous spectrum of electric fields; differing from the traditional trial and error approach with discrete steps. Results of this study will broaden the scope of bacteria available for applications in synthetic biology and genetic engineering.

19. Cellulose Production in *Escherichia coli*

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Over the last 30 years, attention has been paid to bacterial cellulose (BC). BC exhibits unique physical, chemical and mechanical properties when compared to plant-based cellulose, including high purity and biocompatibility. It is currently being used in the production of a wide variety of products such as paper, tissue-engineering products, electronics, biomedical devices, textiles, and food. Although *Acetobacter xylinum* is the most efficient producer of BC, it has many weaknesses such as long doubling time compared to most other bacteria, low productivity and its susceptibility to culture conditions. These limit the cost-effective production of BC. *E.coli* is an industrially attractive microorganism because it is a well-characterized host for protein expression and it has a significantly high growth rate. Herein, *E. coli* C41 (DE3) was selected as the host organism for the expression of bacterial cellulose synthase operon (bcs) of *A.xylinum*. The expression system was created using the pET-Duet1 carrying bcs operon (bcsABCD) and pCDF carrying upstream of the bcs operon. The upstream region of the bcs operon contains two genes: cmcax (encoding endo-β-1,4-glucanase) and cppAx (encoding cellulose complementing protein). All bcs genes were successfully transferred and expressed in *E.coli* C41 (DE3). The cells were cultured in LB medium at 37°C and 180 rpm before IPTG induction. Following 0.05 mM IPTG induction, culture temperature shifted to 23°C. After 24 hour culturing, cellulose production was obtained with a yield of 0.026 g/L. Cellulose samples were analyzed by FTIR spectra and scanning electron microscopy. SEM images clearly showed the shape and size distribution of the microfibrils. The microfibrils have lengths of more than 800-1000μm and diameters of the fibres are between 10–20 μm. The expressions of bcs proteins were analysed by SDS page. The system stability was also monitored by plasmid stability test. The percentage of plasmid harboring cells was obtained 98% of the total viable cells even after 24-hr culturing. This proves that the system has been created for BC production is consistent. This study is novel in functional BC production in *E. coli* by the transfection of the cellulose production ability of *A. xylinum* and a big step to create valuable strains for the effective production of BC. BC productivity is affected by many factors such as the composition of the culture medium, environmental factors (pH, temperature, dissolved oxygen content) and the type of cultures used (culture flasks, agitated fermenters). In order to increase the yield of production in *E. coli*, culture conditions should be optimised by further studies. [1-3].

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20. Introducing Synbis – the Synthetic Biology Information System

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An increasing concern of Synthetic biology is the rational design of pathways. The goal is to build a new pathway or modify an existing one, so that it is endowed with a given dynamic and may operate under a given set of constraints. Typically, the rational design cycle involves an in-silico design and modelling phase on series of CAD software (at part level and/or circuit level [1,2]), followed by construction of the circuit and a testing phase. Depending on the results, the process may be repeated several times until the goal is met.

Such iterative approach has proven its worth and become mainstream in several fields of engineering. In the case of synthetic biology, rational design requires a large catalogue of well characterised chassis, plasmids and bioparts (fundamental parts like promoters or RBS but also common devices such as logic gates, oscillators, pulse generators …). Furthermore, such a catalogue must not only be available online, but it must also be presented in a user-friendly manner and support widely-used data standards to integrate with existing and future CAD tools [3]. Although several well-known repositories exist (the iGEM parts registry [4], JBEI [5] or the virtual parts repository [6] to name a few), we are not aware of any existing repository that meets all those requirements.

Acknowledging this crucial need, the Centre for Synthetic Biology and Innovation at Imperial College (CSynBI) has launched a multi-faceted effort to support the automated characterisation of biological parts – focusing in particular on:

1. The support for existing data standards and development of a complementary, robust data standard for the acquisition of experimental data.
2. The construction of a common IT-spine to track and store

all data as they are processed and curated.
3. A robust dissemination strategy, enabling public access to high quality biopart information.

In this paper we present the work conducted on points 2 and 3, and introduce SynBIS (the Synthetic Biology Information System).

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21. RNA Aptamer-Based Biosensors for Multiplexed *in Vitro* Diagnostics and Circuit Engineering

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The need for metabolic sensors spans both *in vitro* and *in vivo* applications, particularly in the design, evolution and optimization of pathways and genetic control systems. In addition, there is great demand for orthogonal sensors to detect and propagate signals independently while maintaining the ability to be coupled for complex diagnostics or pathway design. We are developing RNA aptamer-based sensors that process molecular inputs into programmable nucleic acid outputs, with the goal of generating a robust multiplexed small molecule sensing assay on common laboratory equipment such as a qPCR machine. These sensors are engineered using a multi-state RNA folding design strategy where transient but metastable intermediate states are engineered along the co-transcriptional folding trajectory. Binding of the small molecule input to the aptamer domain directs the folding pathway towards an active state and generates a fluorescent signal through a nucleic acid strand displacement reaction. We are currently expanding this work to include sensors for a broader panel of small molecules, while simultaneously optimizing the nucleic acid circuits for multi-signal detection *in vitro*, with the goal of generating complete biosensor devices for the eventual use in mammalian cell systems.

22. Rational Engineering of Erythropoietin for Targeted Red Blood Cell Production

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In tuning the quantitative parameters of protein-protein interactions, evolution often employs combinations of weak physical contacts to achieve specificity. This natural phenomenon can be applied to the engineering of targeted, protein-based drugs with improved cell-type specificity relative to their existing counterparts. Our approach uses mutagenesis to weaken a signaling protein's affinity for its receptor on non-target cells, combined with its fusion to a cell-specific targeting element via a flexible linker. Targeting should be driven by the targeting element, and despite the mutation, subsequent binding of the mutated protein should occur due to its high local concentration at the cell surface. We have applied this approach to the development of an erythropoietin (EPO)-based therapeutic that targets human glycophorin A (huGYPA) on red blood cell precursors (RBCs). Recombinant EPO has been used for two decades to treat forms of anemia associated with end-stage renal failure, AIDS, chemotherapy, and hemoglobinopathies. Clinical use of EPO has recently decreased due to concerns over the drug's off-target effects, including tumor recurrence and platelet formation or activation, which may lead to coronary disease or thrombosis. Using a transgenic mouse strain that expresses huGYPA, we have demonstrated that our targeted version of EPO stimulates RBC production without activating platelet formation. We also show that our compound has a similar serum half-life to Aranesp, a long-acting EPO derivative that is used clinically. In patients, targeting EPO to RBCs should allow higher doses to fully restore RBC levels without increasing the risk of cardiovascular events or cancer progression. In addition, the targeted EPO developed here should have an extended serum half-life and reduced immunogenicity relative to existing EPO drugs. More broadly, this work establishes the utility of our approach as a general platform for the rational design of targeted therapeutics.

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23. Tunable Protein Degradation for Control of Synthetic and Endogenous Bacterial Systems

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Tunable control of protein degradation in bacteria would provide a powerful research tool to develop synthetic gene circuits and probe natural cellular systems. Here we use components of the Mesoplasma florum tmRNA system to create a targeted degradation system in *E. coli* that provides control of both the initial steady-state level and the inducible degradation rate of the targeted protein. We demon-

strate the system's use in synthetic circuit development and in exogenous control of core bacterial processes including peptidoglycan biosynthesis, cell division and chemotactic motility. We transfer the system to *Lactococcus lactis* to establish its broad functionality in bacteria, and we create a 238-member library of tagged essential proteins in *E. coli* that can serve as both a research tool to study essential gene function and an applied system for antibiotic discovery. The synthetic degradation system is modular, requiring a small peptide tag and a single protease gene, does not require disruption of host systems, and can be transferred to other bacteria with minimal modification.

24. *De Novo* Designed Toehold Repressors

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RNA regulators of gene expression are becoming increasingly important components of the synthetic biology toolbox. RNA is a versatile and designable molecule, and is one of the most powerful substrates for engineering gene expression at our disposal. Moreover, the ability to propagate information directly via RNA-RNA interactions creates the basis for the bottom-up design of genetic networks to implement logic or carry out complex patterns of gene expression. An important class of such regulators is RNA-mediated repressors, which repress downstream gene expression in response to a trans-acting antisense RNA. Rational mutagenesis and sequence mining have been successful in creating libraries of orthogonal RNA repressors that function on the transcriptional and translational levels. However, the construction of large networks will require additional regulators with enhanced dynamic range and reduced crosstalk. Inspired by recent work demonstrating the successful forward design of RNA-mediated translational activators called toehold switches, we sought to use a similar approach to create de novo designed RNA translational repressors. Using the Nucleic Acid Package (NUPACK), we successfully inverted the structural logic of toehold activators to create toehold repressors capable of attenuating gene expression up to 98% (50-fold repression). In this design, the structure of the toehold repressor frees the ribosome binding site (RBS) and start codon, allowing translation to proceed in a default ON state. Binding of a designed trans-acting antisense RNA leads to structural rearrangement and formation of a hairpin, which occludes the RBS and start codon, preventing translation in the OFF state. This approach shows promise for creating a large library of independent regulators with large dynamic range.

25. A "Basic" Approach to the DNA Assembly Bottleneck: A New Standard Designed for Flexibility, Reliability and Automation

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The ability to quickly and reliably assemble DNA constructs is one of the key enabling technologies for synthetic biology. We have developed BASIC (Biopart Assembly Standard for Idempotent Cloning), a new physical standard for DNA parts which exploits the principle of orthogonal linker-based DNA assembly. The standard employs a new robust method for assembly, based on type IIs restriction cleavage and ligation of linker oligonucleotides with single stranded overhangs that determine the assembly order. BASIC achieves efficient, parallel assembly with great accuracy: small (4 parts) constructs are routinely assembled with near-100% accuracy, while larger (7 parts) assemblies achieved 90% accuracy. Additionally the linkers can be used as composable parts for RBS tuning or the creation of fusion proteins. This standard format is compatible with a wide range of DNA parts since it only requires a single forbidden restriction site and does not employ any PCR amplifications, and is also conceptually simple at both the design and experimental levels, being based on an idempotent and single-tier organisation where all parts and composite constructs are maintained in the same format. Finally BASIC has been designed with automation in mind, using a workflow that is easily performed on robotic liquid handling platforms.

26. Characterization of Crispri Dynamics Using Optogenetics and Mathematical Modeling

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CRISPR interference (CRISPRi) can be used to repress transcription from virtually any promoter in bacteria, yeast, or mammalian cells [1-3], and to construct multilayered synthetic gene circuits [4,5]. In this system, inactivated nuclease dCas9 forms a complex with a 102-nucleotide long single guide RNA (sgRNA), which is then directed to a segment of DNA complementary to a 20-nucleotide region in the sgRNA. If the DNA segment is a promoter or the coding region of a gene, dCas9 will then block transcription initiation or elongation, effectively acting as a transcriptional repressor. Though the steady state response from sgRNA to output transcription rate has begun to be studied, a thorough characterization of CRISPRi dynamics has not been performed. A quantitative understanding of CRISPRi dynamics would enable researchers to create precise, time varying perturbations in a wide range of natural gene networks, and establish design principles for engineering analog gene circuits.

Here, we study CRISPRi repression dynamics in *E. coli* using our previously developed CcaS/CcaR optogenetic system [6]. We use light-induced sgRNA expression to find that the dose-response curve follows a power-law-like scaling, and that it is very sensitive to small levels of sgRNA, in agreement with previous work [4]. Under typical operating conditions, repression is found to occur within minutes, with expression from the target decreasing within one cell cycle, whereas de-repression requires 2-3 cell cycles. Using a combination of mathematical modeling and experiments, we find that fast repression occurs because sgRNA:dCas9:DNA forms quickly, whereas slow de-repression occurs due to a need for stable sgRNA:dCas9 complexes to be diluted and new DNA to be synthesized. We then use the mathematical model to identify specific system re-designs that allows us to tune and accelerate de-repression dynamics. Finally, we use a combination of modeling and experiments with competing sgRNAs to study how competition for dCas9 (i.e. dCas9 loading) affects system performance. This work should inform future optimizations to CRISPRi system that will allow the reliable and predictable construction of large analog circuits in single cells.

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27. Transcriptional Bursting in Confined Cell-Free Reactions

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Transcriptional bursting, bursts of transcription followed by periods of inactivity, is widely observed across all life[1,2,3,4,5,6,7]. This phenomenon is important for many cell fate decisions such as proviral latency in HIV[8,9,10,11,12]. The widely studied two-state (or random telegraph) model is characterized by the transitions between ON and OFF, kON and kOFF respectively[13,14,15,16,17,18]. Many mechanisms have been proposed including: supercoiling[19], transcription factor kinetics[20], chromatin remodeling[21,22,] and transcriptional reinitiation[23,24,25]. However, these studies neglect spatial correlations caused by *in vivo* crowding (40%26) and confinement (~10 femtoliters)[27,28] that may contribute to transcriptional bursting[29,30]. We present experimental and simulation evidence of transcriptional bursting in confined cell-free protein synthesis reactions absent the proposed molecular mechanisms of bursting. And demonstrate that the magnitude and dynamics of bursting may be manipulated by control of confinement.

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28. Resource Allocation and Whole Cell Response in Heterologous Gene Expression

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The expression of exogenous proteins triggers physiological changes in the host cell, usually leading to decreased growth due to consumption of cellular resources. This loading-effect results in undesired cross-interactions and can cause many of the unpredictable behaviours characteristic of the failures common to synthetic biology. Working in standard *E. coli* strains, we show here that the 'load' of exogenous expression mainly impacts on the cell's translational resources and that this effect can be measured with a plate-based assay and predicted by a mathematical model of protein synthesis. We demonstrate that for the control of gene expression, riboswitches are less costly for the cell compared to equivalent transcription factor regulators, due to fewer resources being required for the production of RNA parts compared to proteins.

Extending from this we show preliminary RNAseq analysis of the *E. coli* transcriptome response to induced exogenous expression in the cell. Transcriptome profiling by this method provides a route to identify native genes whose expression changes in response to burden. We find that expression of many genes associated with general stress response, including ibpA, htpG, dnaK and lon, rapidly increase in the first 15 minutes of synthetic overexpression from an engineered plasmid. These native genes are thus useful biomarkers for host cell stress response to exogenous expression and their promoters can be exploited to engineer future burden-based feedback genetic devices.

29. Robust and Tunable Genetic Switch Behavior Using Antisense Transcription

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Antisense transcription has been extensively recognized as a regulatory mechanism for gene expression across all kingdoms of life. Growing evidence suggests the presence of non-coding cis antisense RNAs that regulate gene expression. Recent studies also indicate the role of transcriptional interference in regulating expression of neighboring genes due to traffic of RNA polymerases from adjacent promoter regions. Previous models investigate these mechanisms independently; however, combined effect of both mechanisms and the regulatory potential in a single model has not been developed yet. We present a mathematical modeling framework for antisense transcription that combines the effects of both transcriptional interference and cis-antisense regulation. Our model predicts a tunable switch in gene expression as a result of transcriptional interference during convergent transcription. Addition of cis antisense RNA interaction tends to further sharpen the switching response. When implementing additional positive and negative feedback loops from proteins encoded by these genes, the system response acquires a bistable behavior. Our model shows that combining these multiple-levels of regulation allows fine-tuning of system parameters to give rise to a highly tunable output, ranging from a simple-first order response to biologically complex higher-order response such as tunable bistable switch. This presents an important insight into functional role of antisense transcription and its importance towards design of synthetic biological switches.

30. C4td Cure for Type Two Diabetics

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Cure for type 2 diabetics permanently forever. It sounds too good to be true: reversing type 2 diabetes through exercise and healthy eating. No person suffering from diabetes has ever been cured of diabetes through pharmaceutical medications. It has never happened in the history of medicine. And yet type-2 diabetes can be cured at a rate of 90% - 95% with a living foods diet. Risks and challenges human trial after the C2TD injection produce

31. Feedback Control of Synthetic Genetic Circuits for the Mitigation of Resource Competition

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A recurring challenge in the design of genetic circuits is context dependence, the fact that the behavior of a functional module is influenced by the state of the wider cellular milieu with which it interacts. One key player in context dependence is the scarcity of shared cellular resources, especially those required for transcription and translation during gene expression. Because of competition for these limited resources, the behavior of modules becomes coupled in

subtle ways, preventing circuits from working as expected. This poster proposes a classical feedback control approach to mitigate the steady state effects of the competition for resources necessary for gene expression. In particular, we analyze and compare the ability of several inhibitory feedback regulation architectures to reduce the interdependence between different gene expression processes due to resource limitations.

32. Emergent Population-Level Genetic Oscillations in a Synthetic Bacterial Microconsortium

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To date, the majority of synthetic gene circuits have been constructed to operate within single, isogenic cellular populations. It has been proposed that synthetic microconsortia will provide a means of engineering novel population-level phenotypes that are difficult to obtain with single strains. While circuits that use multiple strains simultaneously to achieve population-level phenotypes have been reported, they generally use a single intercellular signaling molecule and hence the variety of their phenotypes is limited. Here, we use two genetically distinct populations of *Escherichia coli* and two different signaling mechanisms to engineer a bacterial microconsortium that exhibits robust oscillations in gene transcription. Specifically, we used two different bacterial quorum sensing systems to construct an "activator" strain and a "repressor" strain that respectively up- and down-regulate gene expression in both strains. When co-cultured in a microfluidic device, the two strains form coupled positive and negative feedback loops at the population-level. The interacting strains exhibit robust, synchronized oscillations that are absent if either strain is cultured in isolation. We used a combination of mathematical modeling and targeted genetic perturbations to better understand the roles of circuit topology and regulatory promoter strengths in generating and maintaining these oscillations. We found that the dual-feedback topology was robust to changes in promoter strengths and fluctuations in the population ratio of the two strains. These findings demonstrate that one can program population-level dynamics through the genetic engineering of multiple cooperative strains.

33. A Barcoding Strategy Enabling Higher-Throughput Library Screening By Microscopy

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Dramatic progress has been made in the design and build phases of the design-build-test cycle for engineering cells. However, the test phase usually limits throughput as many outputs of interest are not amenable to rapid analytical measurements. For example, phenotypes such as motility, morphology, and subcellular localization can be readily measured by microscopy, but analysis of these phenotypes is notoriously slow. To increase throughput, we developed microscopy-readable barcodes (MiCodes) composed of fluorescent proteins targeted to discernible organelles. In this system, a unique barcode can be genetically linked to each library member, making possible the parallel analysis of phenotypes of interest. As a first demonstration, we MiCoded a set of synthetic leucine zippers (SYNZIPs) to allow an 8x8 matrix to be tested for specific interactions in a pool. A novel microscopy-readable two-hybrid fluorescence localization assay for probing candidate interactions in the cytosol was also developed using a bait protein targeted to the peroxisome and a prey protein tagged with a fluorescent protein. This work introduces a generalizable, scalable platform for making microscopy amenable to higher-throughput library screening experiments, thereby coupling the power of imaging with the utility of combinatorial search paradigms.

34. Combinatorial Engineering of *Saccharomyces Cerevisiae* for Terminal Alkene Production

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Biological production of terminal alkenes has garnered a significant interest due to their industrial applications such as lubricants, detergents and fuels. Here, we engineered the yeast *Saccharomyces cerevisiae* to produce terminal alkenes via a one-step fatty acid decarboxylation pathway and improved the alkene production using combinatorial engineering strategies. In brief, first, we screened and characterized eight fatty acid decarboxylases (OleT) to enable and enhance alkene production in *S. cerevisiae*. We then developed a fatty acid-overproducing strain to boost the precursor availability, which could enhance the metabolic flux and resulted in a higher production titer. We then improved the enzyme cofactor accumulation through cofactor genetic engineering. We then enhanced the cell growth in rich medium and tuned the enzyme expression by optimizing the combinations of the promoters and plasmids. Finally, we further increased the alkene production by optimizing the culturing conditions in bioreactors. This study represents the first report of terminal alkene biosynthesis in the yeast *S. cerevisiae*, and we envision that the abovementioned combi-

natorial engineering approaches could provide insights into devising engineering strategies to improve the production of fatty acid-derived biochemicals in *S. cerevisiae*.

35. Crispr-Based Genome Editing Tools: New Applications and Streamlined Workflows

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CRISPR-Cas9 systems provide a platform for high efficiency genome editing that can lead to innovative applications in cell engineering. However, the delivery of Cas9 and synthesis of guide RNA (gRNA) remain two steps that limit overall efficiency and general ease of use. Here we describe novel methods for rapid synthesis of gRNA and for delivery of Cas9 protein/gRNA complexes into a variety of cells through liposome-mediated transfection or electroporation. We will present a streamlined cell engineering workflow that goes from gRNA design to analysis of edited cells in as little as three days and results in highly efficient genome editing and biallelic knockout of multiple genes in hard-to-transfect cells. The reagent preparation and delivery to cells requires no plasmid manipulation so is amenable for high throughput, multiplexed genome-wide cell engineering.

Further, we will show data using lentivirus-based CRISPR delivery for high-throughput screening of mammalian cell populations. Until recently RNAi has been the main tool for performing loss of function studies in mammalian cell populations. Using CRISPR technology, we are now able to perform complete gene knock-out studies which hold promise for clearer phenotypes and fewer false readouts as what is currently seen with the variable knock-down of expression using RNAi. We are creating gene family-specific arrayed libraries of CRISPR-lenti particles. These new tools enable high throughput, arrayed gene knockout screens using various cell types, including our proprietary CellSensor lines that give a fluorescent readout of various signaling pathway activities.

These two CRISPR-based gene-editing platforms represent the latest in the rapid evolution of editing tools for mammalian genomes by simplifying and increasing the cell engineering workflow and providing a pre-designed, ready to use platform for efficient compound screening in mammalian cell lines.

36. Combigem for Systematic and Massively Parallel Analysis of Drug-Gene Combinations for Cancer Therapeutics

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The chemosensitivity of cancer cells is under the influence of multiple cellular pathways and genetic factors. Combinatorial drug therapies can achieve higher efficacy than individual drugs since targeting multiple pathways can be synergistic. Understanding drug-gene interactions should enable us to devise optimized therapeutics for sensitizing cancer cells to drugs and reversing drug resistance. Conventional approaches for analyzing a large number of drug-gene combinations are low-throughput, and require huge effort and resources. Harnessing the power of synthetic biology and next-generation sequencing technologies, we developed CombiGEM (Combinatorial Genetics *En Masse*) as a powerful platform for high-throughput functional characterization of combinatorial genetic perturbations in human cells, which can be broadly applied in biomedical research. We applied the CombiGEM technology to achieve scalable assembly of microRNA (miRNA) overexpression constructs. We generated high-coverage combinatorial miRNA libraries and performed systematic screens to identify combinatorial miRNA effectors that sensitized drug-resistant ovarian cancer cells to chemotherapy and/or inhibited cancer cell proliferation. We identified and validated a list of miRNA combinations that act synergistically to achieve anti-cancer phenotypes when expressed in combination. This effort revealed new insights into complex miRNA interaction networks, including previously unknown interactions between miRNAs that modulate drug resistance and cell growth phenotypes.

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37. Decoupling Growth and Metabolism in *Escherichia coli*

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Microbial chemical production involves an intrinsic tradeoff between biomass formation and product formation. Theoretically, the ideal resolution of this tradeoff is production during stationary phase, in which growth is limited but carbon is nevertheless abundant. However, microbes typically respond to such conditions by shutting down central metabolism. Decoupling growth and metabolism is therefore one of the central problems in host engineering for synthetic biology. In this study, we characterize the metabolism of the model bacterium *Escherichia coli* under a variety of stationary phase conditions and show significant variation in metabolic activity that strongly depends on the limiting nutrient.

We find an unusual phenotype of high glucose uptake rates under conditions of magnesium limitation, suggesting a lack of intrinsic barriers to enhancing metabolic activity under other starvation conditions. Based on quantitative metabolomics, we suggest a role for phosphoenolpyruvate in regulating glucose uptake. We further focus on the condition of nitrogen limitation, where a key regulatory interaction is the inhibition of the glucose uptake enzyme PtsI by accumulation of alpha-ketoglutarate. We find that by manipulating the expression of PtsI, we can enhance metabolic rates under nitrogen starvation, and we investigate the effect of this enhancement on the productivity of several biofuel pathways. We will also discuss how high-throughput and single-cell based screening and selection approaches will be used to further enhance stationary phase productivity for renewable biochemical and biofuel production.

38. Engineering Bacteriophages for Specific Bacterial Targeting

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Interest in bacteriophages has seen a rebirth in recent years as the result of increasing antibiotic resistance in bacterial infections, the recognition of their important role in the human microbiome, and the development of new tools and approaches for engineering designer phages. In the past year, we demonstrated the creation of sequence-specific antimicrobials by using phages as delivery vehicles for RNA-guided nucleases. Ongoing work has focused on expanding the application of this technology to target new bacteria. As we gain experience in optimizing the system for different targets, we aim to create a platform that can be translated into a programmable system for specific removal of bacteria to alleviate infection and dysbiosis in microbial communities.

39. Synthetic Yeast Based Cell Factories for the Production of Plant Natural Colors

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The vast majority of today's consumers prefer natural over chemically synthesized pigments in their food products and the global market for natural colors is increasing and expected to reach $2.3 billion by 2019. Among the industry's preferred natural food colors are anthocyanins, which are pigments extracted from edible fruits and vegetables, such as grape, elderberry, red radish and black carrot. Being polyphenols, increasing attention has been paid to their health benefits as potent antioxidants.
Anthocyanins are a class of flavonoids that are derived from shikimic acid and acetyl-CoA. They are extensively glycosylated, acylated and/or methylated resulting in immense diversity; and up to date more than 600 compounds have been identified. These decorations with sugar, methyl, aromatic and aliphatic groups influence the molecular properties of anthocyanins, such as their solubility in water and color shade.

The complex structures of anthocyanins are difficult to mimic by chemical synthesis and their production is therefore limited to extraction from the natural resource, typically resulting in low yields, variable quality and supply, and high production costs. To serve the increasing need for plant natural food colors, it is desirable to develop cheap and reliable production methods. Our aim is therefore to transfer the production of anthocyanins to a microbial host.

The baker's yeast *Saccharomyces cerevisiae* is an intensively applied cell factory for the production of renewable fuels and chemicals. Its genetics and physiology have been extensively studied and recent publications show successful metabolic engineering of acetyl-CoA metabolism and the shikimate pathway, greatly improving yields of different classes of plant natural products. Furthermore, yeast supports heterologous production of e.g. membrane bound cytochrome p450s and glycosyltransferases and synthesis of the flavonoid naringenin, the precursor of anthocyanins, has been demonstrated previously. Therefore, we selected *S. cerevisiae* as microbial host for the production of anthocyanins.

To exploit yeast as cell factory for the biosynthesis of a wide range of natural food colors, we are applying synthetic biology tools to develop an anthocyanin production screening platform. This platform uses a combinatorial approach based on mating a library of gene candidates for decorating enzymes to strains containing the synthetic pathways for the production of defined anthocyanin scaffolds. Novel biosynthetic production systems are constructed by shuffling genes originating from different plant species, expanding the biochemical space of anthocyanins and steering required molecular properties for utilization in the food industry.

40. Crispathbrick: Modular Combinatorial Assembly of Type II-a Crispr Arrays for dCas9-Mediated Multiplex Transcriptional Repression in *E. coli*

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Programmable control over an addressable global regulator

would enable simultaneous repression of multiple genes and would have tremendous impact on the field of synthetic biology. It has recently been established that CRISPR/Cas systems can be engineered to repress gene transcription at nearly any desired location in a sequence-specific manner, but there remain only a handful of applications described to date. In this work, we report development of a cloning procedure called "CRISPathBrick," enabling rapid modular assembly of natural type II-A CRISPR arrays capable of simultaneously repressing multiple target genes in *E. coli*. Iterative incorporation of spacers into this CRISPathBrick feature facilitates the combinatorial construction of arrays, from a small number of synthetic, user-defined DNA parts, which can be utilized to generate a suite of complex phenotypes. We demonstrate assembly and functionality of these arrays in *E. coli* by repressing genomic and plasmid-based reporter genes, as well as endogenous virulence factors and biosynthetic genes, using a single plasmid in multiple divergent strains. We show that significant repression is achieved in engineering hosts like BL21, K-12, and DH5alpha, in addition to wild-type probiotic strain Nissle 1917 and virulent strain K5. The value of addressable, orthogonal master transcriptional regulators like dCas9 cannot be overstated; thus, we have developed a two-plasmid toolkit for facile construction and characterization of CRISPR arrays. The first plasmid, pCRISPathBrick, possesses all components necessary to achieve repression of both heterologous and endogenous targets in *E. coli*, guided by the genetic program encoded in its readily expandable CRISPR array. The second plasmid, pCRISPReporter, is compatible with pCRISPathBrick and contains a transcriptionally-insulated multiple cloning site, where genomic promoters regions and genes of interest can be inserted to create a translational fusion with any desired reporter. With these two plasmids, we demonstrate facile characterization of synthetic spacers, quantified in terms of repression of the target protein-reporter fusion. Finally, we show that CRISPathBrick can be used to repress endogenous metabolic targets in *E. coli*, mediating up to 2.5-fold improvement in production of the heterologous plant natural product naringenin (from 7.6 mg/L to 18.9 mg/L). We believe that the tools developed here will be incredibly valuable for scientists in many disciplines, from systems and synthetic biology to metabolic engineering and the basic sciences.

41. Isolation and Charactization of Novel Terpene Synthases from Diverse Metagenomic Libraries

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Nature produces a stunning diversity of molecular functionality, of which humanity has only exploited a small fraction for industrial and energy applications. To date, chemical space has been confined to that enabled by petroleum feedstocks or abundant natural products, in turn putting strong constraints on achievable material properties. Terpenoids are one of the most abundant and diverse classes of biomolecules produced in nature, collectively enabling a variety of therapeutic, energy, and cosmetic applications. However, this class of molecules remains recalcitrant to synthetic chemistry approaches, necessitating the use living systems. Although there has been recent outstanding progress in the high-level microbial production of several key terpenoids, much of the remaining molecular diversity is locked within production hosts which produce small product titers and are expensive to cultivate. This limitation is primarily due to a lack of diverse biological parts, as current databases of protein function are currently populated by proteins which conform to well-understood sequence-function relationships. Interestingly, despite this known diversity, recent genomics investigations have shown a vast untapped reservoir of bacterial terpene synthases residing in the genomes of uncultivable organisms living in the soil, indicating a vast array of terpenoids waiting to be discovered and offering a wider palette of biological parts for the production of these compounds in robust microbial hosts. However, in order to validate these predictions and expand our catalogs of useful biocatalysts, techniques for high-throughput enzyme discovery, characterization, and annotation are urgently needed. In this work, we exploited the toxicity of a key node in the terpenoid biosynthesis pathway in order to develop a high-throughput approach for discovery of terpene synthase activity. We first develop optimized culture conditions and screening protocols in order to maximize enrichment of terpene synthases. Then, we experimentally mine hundreds of gigabases of genetic material isolated from diverse habitats in order to discover novel biocatalysts with the ability to produce new classes of terpenoid molecules in *E. coli*. After biochemical characterization of terpenoid products generated by these novel enzymes, we then perform bioinformatics analysis to generate improved predictors of terpene synthase function and use these predictors in a focused approach to experimentally investigate terpene synthase activity in sequenced microbial genomes.

42. Heterologous Expression and Characterization of Hybrid Two-Component Systems from Gut Bacteria

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Two component systems (TCSs) are the primary signal transduction modality by which bacteria sense and respond to the environment. Classical TCSs are comprised of an inner membrane bound sensor histidine kinase (SK) and a cytoplasmic transcription factor known as a response regulator (RR). In the presence of an extracellular input signal, the SK phosphorylates the RR, which then binds to a promoter, activating transcription. Recently, a new TCS subfamily called the hybrid TCSs (HTCSs) has been identified wherein the SK and RR are fused into a single membrane-bound protein. While the functional implica-

tions of this architecture are poorly understood, HTCSs are found extensively throughout the genus *Bacteroides*, which comprise approximately 50% of the human gut microbiome. Additionally, HTCSs have been shown to sense diverse host- and diet-derived carbohydrates, which are linked to host physiology and disease. Hindering their further characterization and use for synthetic biology, no full-length HTCS has been expressed or characterized outside the native organism. Here, we demonstrate that a HTCS can be expressed and function in *E. coli*, though with considerable toxicity and a weaker transcriptional response than is observed in the native organism. Through systematic expression of truncation variants of several human gut-derived HTCSs, we have identified an N-terminal predicted signal peptide targeting the Sec translocon as the major source of toxicity. Complete removal of this domain results in a dramatic reduction in toxicity of the overexpressed protein but is accompanied by a loss of ligand sensitivity in the fructose sensor BT1754. We are currently working to optimize protein expression and enhance promoter output through protein and promoter engineering as well as identify the ligands sensed by HTCSs without known inputs. This work has implications in understanding the dynamics of the human gut microbiome, understanding the functional implications of the HTCS architecture, and engineered novel diagnostics and therapeutic agents for the human gut.

43. Visualising Individual Molecular Features with Fluorescence Digital Molecular Imaging (DMI)

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Understanding individual molecular information from a large biomolecular complex, while maintaining its native environment, represents a key challenge in biology. Recent advances in fluorescence super-resolution microscopy have shown images of sub-cellular features and synthetic nanostructures down to ~15 nm in size, but direct optical observation of individual molecular-scale (~5 nm) features within a macromolecular complex ("Digital Molecular Imaging", or DMI) has yet to be demonstrated. In this paper, we establish three technical requirements for meeting this challenge with localisation based microscopy, and demonstrate our ability to achieve DMI with DNA-PAINT (Point Accumulation for Imaging in Nanoscale Topography), a method which utilises programmable transient oligonucleotide hybridisation, on synthetic DNA nanostructures. In particular, we examined the effect of high photon count, high blinking statistics and appropriate blinking duty cycle on imaging quality, and reported fluorescence imaging of a densely packed triangular lattice pattern with 5 nm point-to-point distance. Using oligonucleotide conjugated small labelling agents, this imaging capability potentially provides a high-resolution, highly quantitative and single-molecule approach for studying structural heterogeneity, modifications and interaction networks towards a systems understanding of cellular biology with molecular-scale resolution.

Project Summary
A key challenge for super-resolution fluorescence microscopy is to directly visualise individual molecular targets in situ, which are mostly around 5 nm in size (protein subunits, membrane receptors), and are in close proximity to each other. A method that addresses these challenges must:
(1) Provide ultra-high, "molecular" resolution (~5 nm target-to-target distance)
(2) Visualise individual molecular targets (clear separation close targets)
(3) Operate from within compact native environment (dense cluster of targets)

Despite considerable recent development (PALM, STORM, STED, etc.), the limited imaging resolution of currently demonstrated methods (down to 15 nm) still failed to address this challenge and allow visualisation of closely packed and interacting molecular components.

We describe here a framework which enables stochastic localisation based super-resolution microscopy methods to achieve the above challenge, a scenario which we termed Digital Molecular Imaging (DMI), and present our result of the first super-resolution image achieving such quality on a synthetic DNA nanostructure template (5 nm point-to-point distance, each individual target is visualised, and within densely labelled grid pattern).

We propose a framework for achieving DMI of four technical requirements, and demonstrate their effects *in silico* on improving imaging quality. We show that well-controlled blinking kinetics supports high localisation precision (down to 1 nm), high image Signal-to-Noise Ratio (SNR), and low fraction of false localisations (<5%).

We show implementation of the above framework with DNA-PAINT method (Jungmann *et al*. Nature Methods, 2014), by exploiting the flexibility in blinking kinetics control offered by tuneable oligonucleotide hybridisation. We also develop a novel DNA nanostructure based drift correction algorithm that achieved <2 nm residual drift on a commercial microscope setup. We finally demonstrate the DMI imaging quality on a regular triangular pattern with 5 nm point-to-point distance constructed on a synthetic DNA nanostructure.

44. New Evidence for the Effect of Human Chromatin on Cas9-Mediated Editing

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The CRISPR/Cas9 system is a popular genome engineering tool that has the potential to be developed into a safe gene therapy, where errors in a patient's genome can be precisely corrected with no off-target effects. By simply modifying the targeting sequence of the guide RNA, one can direct Cas9 to virtually any gene to generate mutations, deletions, or small insertions. However, bioengineers have yet to fully understand how the Cas9/RNA complex, a bacterial macromolecule, interacts with the complexities of a human genome that is packaged with nuclear proteins in a structure known as chromatin. So far, previous studies have only provided direct evidence that DNA methylation does not interfere with Cas9-mediated genome editing. However, genome-wide binding studies suggest Cas9 access to target DNA may be reduced by chromatin. The impact of the multiprotein complexes that make up the bulk of chromatin remains unclear. Using an inducible, transgenic cell line, we have directly measured chromatin inhibition of Cas9 editing efficiency at a reporter transgene that is stably integrated into a chromosome. Upon addition of doxycycline, the transgenic cells express a fusion protein that induces the accumulation of histone methylation, Polycomb complexes, and nucleosome compaction at a luciferase transgene. This switchable chromatin system allows us to compare the editing efficiency of a specific luciferase-targeted Cas9/gRNA complex in the presence and absence of compacted chromatin. We observed a decrease in editing efficiency at specific sites when chromatin is induced at the Cas9/gRNA target, luciferase. These results provide the first direct evidence that closed chromatin interferes with Cas9 function at a single site in the human genome.

45. An Innovative Cloning Platform for Pathway Engineering

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To establish synthetic biology capability for transferring CAM gene modules into C3 species, we developed an innovative method for assembling DNA fragments *in vitro*. Several cloning strategies are available to support genetic engineering (DePaoli *et al*., 2014). However, the current availability of cloning methods imposes several limitations to seamless cloning of large and multi-gene constructs. Our technology, called "TNT-cloning", assembles DNA parts in a simple, fast, efficient and flexible manner. Our system combines DNA fragments to be cloned in one single universal library, leaves no undesirable sequences behind, allows "one-pot" reaction with up to 3 fragments to be joined at once and automatically maintains the open reading frame (ORF) in-frame between genes of interest. By combining all cloning elements into one single universal library, the method allows a pre-determined assembly without the need of sequence homology or linkers/adaptors, which results in a 'scar-free' product. Importantly, any fragment regardless the sequence is compatible with our system. In addition to the cloning system, a new buffer, called 'TNT-buffer', was developed to allow quick and simultaneous processing of DNA fragments, enhancing the efficiency several times compared with current commercial products. The vectors represent a binary platform, making the final gene construct reusable as well as immediately ready for plant transformation. Additionally, the set of plasmids support secondary and tertiary assembling in a loop format, with an exponential reduction of steps for assembling a large number of DNA parts. We demonstrated that the system is wholly functional by cloning, assembling and testing several fragments ranging from 30 bp to 12 kb. Because this technique is compatible with isothermal (Gibson) assembly, virtually any fragment can be used as an element in the library and circularized without the need to carry the binary-backbone, expanding the technology's use to other systems and making it of special interest for constructing new plasmids/circular molecules. This novel cloning platform will accelerate the creation of multiple gene constructs necessary for CAM engineering and will greatly support the construction and tuning of multi-combinatorial pathways in a wide range of organisms.

Note: The TNT cloning system is protected under the invention disclosure 201403357, DOE S-124,978 and is patent pending. The TNT-buffer formulation is protected under the invention disclosure 201403356, DOE S-124,977 and is patent pending.

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46. Engineering Transcriptional Regulator Effector Specificity Using Computational Design and *in Vitro* Rapid Prototyping: Developing a Vanillin Sensor

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The pursuit of circuits and metabolic pathways of increasing complexity and robustness in synthetic biology will require engineering new regulatory tools. Feedback control based on relevant molecules, including toxic intermediates and environmental signals, would enable genetic circuits to react appropriately to changing conditions. In this work, variants of qacR, a tetR family repressor, were generated by computational protein design and screened in a cell-free transcription-translation (TX-TL) system for responsiveness to a new targeted effector. The modified repressors target vanillin, a growth-inhibiting small molecule found in lignocellulosic hydrolysates and other industrial processes. Promising candidates from the in vitro screen were further characterized in vitro and in vivo in a gene circuit. The screen yielded two qacR mutants that respond to vanillin both in vitro and in vivo. We believe this process, a combination of the generation of variants

coupled with in vitro screening, can serve as a framework for designing new sensors for other target compounds.

47. Developing a DNA Polymerase-Based Biosensor for Neural Recording

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Progress in neural recording is critical to better understand the brain and to evolve treatments for brain-related illnesses. Further advances in mapping brain activity are limited by the low spatiotemporal resolution of current technologies. Our objective is to create a molecular recording device that combines the rapid writing capability of DNA polymerases with the robust information storage capability of DNA to generate dense spatiotemporal data on neural activity. Such a recording device, combined with the advent of highly parallel next generation sequencing technologies, has the potential to simultaneously monitor neurons at time scales matching mental activity[1-3].

Ca^{2+} fluctuations on the millisecond timescale are indicative of neuron firing. We aim to engineer a DNA polymerase-based biosensor that responds to high calcium by writing errors into DNA. By coupling the nucleotide misincorporation rate of a DNA polymerase with levels of Ca^{2+} in the environment we can read out the relative Ca^{2+} concentration at a particular time by sequencing the copied DNA[1-3].

As a first step toward building a Ca^{2+} biosensor, we are interested in understanding the metal cation-polymerase fidelity landscape to determine strategies for engineering DNA polymerase recorders. In order to screen metal-dependent fidelity properties of a multitude of DNA polymerases, we have developed a novel medium throughput method for rapidly identifying changes in DNA polymerase error rate across a wide spectrum of metal cation and DNA sequence context conditions. Unlike former methods for determining polymerase fidelity, which rely on bacterial transformations[4] or gel electrophoresis[5], this *in vitro* technique is scalable (100 conditions/day) and allows for standardization of fidelity changes. Additionally, since DNA polymerase properties are difficult to study in their full complexity, this screen will allow us to decouple the singular and combinatorial effects of environment and sequence context on inducing replication errors.

Using this screen, we will identify polymerase candidates, environmental conditions and DNA template sequences that facilitate recording metal cation time course information via changes in fidelity. We expect these results to catalyze synthetic biology efforts toward a high-performance metal cation sensing and storage system based on incorrect nucleotides being incorporated into DNA. This first demonstration of a divalent metal recording platform will inform future development of a Ca^{2+}-specific biosensor for neural recording applications.

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48. Control of the Asymmetric Segregation of Proteins at Division Thanks to a Self-Assembling Peptide

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Asymmetric division may give rise to daughter cells with different fates. It often results from the unequal distribution of cellular components due to either random segregation of diffusive molecules or as a result of a localizing mechanism, placing molecules in specific locations within the cell prior to division. Unequal distribution of cellular components plays an important mechanistic role in a wide range of systems such as stem cells differentiation and renewal, bacterial sporulation control, antibiotic persistence, diauxie, and aging.

So far, most of asymmetric division studies were mostly observational and at best measured the degree of asymmetry using single-cell microscopy. We go one step further and use a synthetic approach in order to actively control the distribution of specific cellular components. By fusing proteins to a ionic self-assembling peptide (ELK16) we took advantage of the nucleoid macromolecular crowding effect to localize slowly diffusing, big assemblies at *E. coli*'s pole.

We were then able to observe their asymmetric segregation during several divisions using time-lapse microscopy and microfluidic systems.We show that fused enzymatic activity is retained *in vivo*. As example, inducing asymmetry of the

aminoglycoside-resistance enzyme (APH(3')-IIIa), resulted in polar segregation where only cells containing the enzyme cluster grew and survived multiple generations in the presence of the antibiotics (e.g. Kanamycin). In this work we created and characterized a useful tool to control the segregation of proteins inside *E. coli*, enabling the study of the fitness landscape governed by asymmetric division both at the single cell and at the population level. Moreover this might be used as a tool to cluster enzymes in order to favor metabolic channeling as recently shown as well as a way to differentiate monoclonal cell population.

49. Targets of Opportunity : Hypergraph Analysis of the Retrosynthetic Design Space

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As biomanufacturing progresses towards the goal of producing a wide array of natural product compounds *in vivo*, many retrosynthetic design methods have emerged for finding and comparing pathways to a specific target compound. Little attention, however, has been given analyzing the space of all potential natural product pathways accessible through retrosynthesis. Rather, studies focus on a specific target compound, selecting heterologous genes to express in a chassis organism in order to generate a synthetic natural product pathway. This work examines the Retrosynthetic Design Space (RDS) containing all heterologous pathways which could potentially be added to a specific chassis organism. By examining the entire RDS, we directly compare the costs and benefits of pathways across different target compounds rather than only between pathways for the same compound. This analysis reveals which compounds are more easily accessible today and directs the efforts of the synthetic biology community to the most pressing challenges in biomanufacturing.

We present an analysis of the RDS for the chassis organism E. Coli constructed from publicly available databases of biochemical reactions and enzymes. The RDS is represented as a hypergraph with metabolites as nodes and biochemical transformations as hyperedges. By traversing the hypergraph we determine the set of Pareto-optimal pathways which could be potentially added to the chassis organism and which target compounds these pathways would theoretically produce. By analyzing these pathways with graph-theoretic algorithms, we are able to compare targets with inter-pathway metrics, such as centrality and clustering, as well as intra-pathways metrics such as thermodynamics and toxicity. Specifically we: (1) Rank target compounds and compound classes by difficulty using multiple scoring objectives, including, pathway length, pathway thermodynamics, enzyme availability, intermediate toxicity, and RDS centrality. (2) Identify hub compounds in the RDS which are key intermediates for multiple target pathways, including key host metabolites which serve as precursors. (3) Identify gaps in the RDS for which further investigation could unlock additional natural product targets. From this analysis we identify "targets of opportunity" which are not only easier to reach, but also serve as intermediates for producing more difficult compounds in the future.

50. Construction and Characterization of a Refactored Actinorhodin Biosynthetic Cluster

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Next-generation sequencing technologies have enabled single genomes as well as complex environmental samples (metagenomes) to be sequenced at an ever-increasing pace. Advanced bioinformatics analysis of the resulting genomics and transcriptomics data is revealing an unprecedented catalogue of proteins, and pathways that encode a myriad of novel catalytic activities and a vast diversity of novel compounds of potential value for environmental and biomedical applications.

Characterizing the products of predicted biosynthetic pathways remains challenging as (i) Clusters originate from diverse organisms many of which are difficult to culture and manipulate, (ii) Biosynthetic clusters are often under transcriptional or translational repression by unknown regulatory mechanisms, (iii) Biosynthetic clusters are often large and complex in terms of sequence composition, (iv) Molecule production may require the presence of unknown substrates or co-factors.

In order to systematically characterize the products of biosynthetic clusters we are building a scalable pipeline for pathway refactoring which includes automated design, efficient DNA construction and assembly, sequence verification, strain generation and compound detection.
As a proof-of-principle for our pipeline we designed, built and tested a fully refactored actinorhodin pathway rACT, which was synthesized from scratch using synthetic promoters and terminators, *in-silico* calculated RBSs, and optimized coding sequences. In total rACT comprised 22 Kb of synthetic sequence, which was organized as 5 contiguous operons that did not mimic the natural cluster architecture in terms of orientation, order or operon composition. The rACT DNA design was carefully screened for sequence features that might compromise synthesis efficiency and any such features present in the original design were removed

by codon juggling. The resulting 'polished' DNA design was partitioned to optimize the assembly junctions, and synthesized using standard methods. Construct assembly was performed using a two-step process involving Gibson followed by yeast Gap-repair cloning. The integrity and sequence composition of rACT was verified using Pacific Biosciences sequencing technology.

The rACT cluster was integrated into a Streptomyces coelicolor strain lacking the native actinorhodin cluster (delACT strain). The resulting rACT strain was cultured and compared to the delACT strain as well as to wildtype S. Coelicolor. Samples were collected at days 2, 4 and 6 of culture in triplicates and subjected to transcriptomics, proteomics and metabolomics analyses. Actinorhodin as well as all of its metabolic intermediates were detected by LC-MS in the rACT and wildtype strains but not in the delACT strain. Interestingly, actinorhodin production was detected earlier in the rACT strain than in the wildtype strain showing a clear de-coupling of growth and molecule production.

Our design strategy, build pipeline and full OMICS analysis will be presented in detail as we seek to further refine and scale our genomes to molecules pipeline.

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51. Modelling Population Cell Cycle Heterogeneity to Deconvolute Population-Level Measurements

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Synthetic biology uses mathematical modelling, combinedcwith *in silico* simulations to guide the design and optimization of synthetic genetic circuits (SGC). These circuits are usually modelled as a system of ordinary differential equations (ODE). For example, the represillator and the toggle switch, two of the founding SGC's, were successfully designed using this method [1][2]. The models employed in these studies did not, however, predict the level of variations in the oscillatory signals or the desynchronisation observed between even small number of generations when expressed in *Escherichia Coli* (*E.Coli*). Even if the molecular mechanisms and biochemistry of the transcription and translation mechanism controlling these SGC's are quite well understood, biological noise dictates these non-deterministic expression dynamics [3]. This noise is a combination of the probabilistic nature of the biochemical reactions, called intrinsic noise, and extrinsic noise, that refers to variations in other cellular components that may affect the transcription and translating machinery of the gene or system of interest [4]. For example, in the scope of synthetic biology, the latter noise could arise from cell variability of gene copy numbers from different plasmid copy numbers or from asymmetric cell duplication [5][6][7]. While the effects of intrinsic noise is being increasingly well formalised through stochastic modelling, extrinsic noise is more difficult to elucidate because of the vast number of potential sources and their uncertain influence on the system of interest [3].

To obtain a quantitative understanding of any biological system, intracellular concentrations over time courses are required [8]. However, most -omics technologies sample populations instead of individual cells. At the population level, the accumulation of biological noise leads isogenic cells to quickly become phenotypically heterogneic, a property that is undesirable for the accurate estimation of expression levels [9]. To reduce the influence of extrinsic noise, basic microbiological practices dictate that single celled organisms be sampled during exponential growth of culture, to assure that individuals have a similar molecular profile [7]. However, even under these stringent experimental conditions, cells are seldom homogeneous [10]. This makes the output of such measurements a statistical property of the predominant molecular state of the population, that cannot be used as a reliable estimation of discrete intracellular concentrations [11][8].

Although the origins of heterogeneity are diverse and predominantly still to be elucidated, in some cases, they depend on known biological mechanisms [10][7], such as the cell cycle [12][13]. Indeed, many genes are differentially expressed based on the growth cycle state of the cell [14]. To deconvolve the dynamics of time course data and formalise the influence of cell cycle extrinsic noises on measured sample expression, it is necessary to understand cell cycle heterogeneity in populations of cells. The term deconvolution, in this case, is used not as a strict mathematical term, but to illustrate that population measurements are the sum of individual expression. Using an individual based simulation method to represent the inherent heterogeneity of bacterial populations, combined with a realistic growth model, we simulate the cell cycle dynamics of a population of cells in conditions of restricted and exponential growth.

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52. Engineering a Synthetic Regulon in *Saccharomyces Cerevisiae* for Efficient Xylose Utilization

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Saccharomyces cerevisiae is one of the most widely used microbes for generating biofuels using lignocellulosic feedstock. Since this yeast cannot utilize xylose, the second most abundant sugar in lignocellulose, several metabolic engineering approaches have been attempted in the recent past for efficient xylose consumption. However, growth on xylose is still poor and economically unfeasible for biofuel production. We hypothesize that further enhancement in xylose utilization and biofuel production can be made by addressing the issue from a regulatory perspective rather than a metabolic perspective. Therefore, our work aims at developing a regulon (genome-wide regulatory infrastructure) to enhance the growth and biocatalytic fitness of this yeast for enhanced respiratory growth and advanced biofuel (non-ethanolic) production using xylose. To achieve this, we developed a protein sensor-actuator system that regulates the expression of aerobic xylose metabolism based on its concentration. The well-characterized galactose sensor protein, Gal3p, was engineered for increased affinity towards xylose through semi-rational protein engineering. Targets for mutagenesis were identified using *in silico* docking studies and high-throughput screens were developed based on G418 resistance and *GFP* fluorescence. In these screens, expression of kanMX and *gfp*, placed under GAL promoters, were triggered by a regulatory cascade initiated by the binding of Gal3p to xylose. Initial selection was performed in G418 selection plates and the strains that showed resistance were further quantitatively screened for increased fluorescence. The results reveal further scope for increasing xylose sensitivity in Gal3p mutant variants. Future work involves up- and down-regulating genes and pathways required for enhanced aerobic growth on xylose under the control of this synthetic regulatory system. Such a strain will act as a platform to engineer advanced biofuels using lignocellulosic biomass.

53. RNA-Guided Gene Drives Can Efficiently Bias Inheritance in Wild Yeast

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Inheritance-biasing elements known as "gene drives" may be capable of spreading genomic alterations made in laboratory organisms through wild populations. We previously considered the potential for RNA-guided gene drives based on the versatile CRISPR/Cas9 genome editing system to serve as a general method of altering populations1. Here we report molecularly contained gene drive constructs in the yeast *Saccharomyces cerevisiae* that are typically copied at rates above 99% when mated to wild yeast. We successfully targeted both non-essential and essential genes and showed that the inheritance of an unrelated "cargo" gene could be biased by an adjacent drive. Our results demonstrate that RNA-guided gene drives are capable of efficiently biasing inheritance when mated to wild-type organisms over successive generations.

54. An Ultra High Throughput Screening Platform for Discovery and Engineering of Full-Length Antibodies in Soluble and Secreted Forms

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Traditional display techniques for antibody engineering have two major limitations. First, the antigen binding affinities are normally detected with single-chain variable fragment (scFv) or fragment antigen-binding (Fab). However, the physicochemical properties and expressibilities of those fragments may not correlate with their cognate full-length formats. Second, high throughput screening or selections require high quality, soluble recombinant antigen, which can be challenging or even intractable with membrane proteins such as G-protein-coupled receptors (GPCRs) and ion-channel receptors.

To overcome these limitations, we have developed a novel high throughput screening platform for the discovery and engineering of full-length monoclonal antibodies (mAbs) in soluble, secreted forms. This state-of-the-art technology utilizes hydrogel microdroplets (GMDs) coupled with fluorescence-activated cell sorting (FACS). The GMDs provide a linkage between phenotype and genotype. Both bulk emulsion and microfluidic compartmentalization have been used to co-encapsulate antibody secreting cells (*Pichia* pastoris or *Saccharomyces cerevisiae*) and antigen bearing cells (cancer cell lines) in picoliter-volume hydrogels. Full-length antibodies secreted by recombinant yeast will bind with the cognate antigens on the surface of mammalian target cells within the same droplet, which can then be

labeled with fluorophore conjugated secondary antibodies. Fluorescently stained GMDs can be sorted by FACS at rates of 2,000 events per second or more. It has enabled us to easily screen millions of clones per day, which is impossible with the conventional microtiter well plate- based method. Furthermore, this technology is very cost-efficient. The expenses for the entire setup (except for the FACS system) were estimated to be only several thousand dollars, while each round of GMDs production costs around 50 USD.

We successfully validated our approach by the enrichment and one-step isolation of anti-EGFR IgG secreting *Pichia* pastoris from a 1:10,000 mixture of *Pichia* cells secreting negative control anti-CCR5 IgG antibodies. We also applied the GMD-FACS screening platform to distinguish cancer cells with different surface expression levels of EGFR.

In general, we have presented an innovative high throughput screening platform which is efficient, robust, and cheap for discovery and engineering of secreted, full-length mAbs. The broad utility of this methodology has been demonstrated through the screening of a spike-in library and identification of different cancer cells. In addition, this platform can also be utilized to isolate and engineer affinity proteins such as F(ab')2, nanobodies, and various non-immunoglobulin scaffold proteins. Our GMD-FACS technique can be a valuable tool to facilitate antibody discovery in both academic and industrial settings.

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55. Genomically Encoded Analog Memory with Precise *in Vivo* DNA Writing in Living Cell Populations

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Cellular memory is crucial to many natural biological processes and sophisticated synthetic biology applications. Existing cellular memories rely on epigenetic switches or recombinases, which are limited in scalability and recording capacity. In this work, we use the DNA of living cell populations as genomic "tape recorders" for the analog and distributed recording of long-term event histories. We describe a platform for generating single-stranded DNA (ssDNA) *in vivo* in response to arbitrary transcriptional signals. When coexpressed with a recombinase, these intracellularly expressed ssDNAs target specific genomic DNA addresses, resulting in precise mutations that accumulate in cell populations as a function of the magnitude and duration of the inputs. This platform could enable long-term cellular recorders for environmental and biomedical applications, biological state machines, and enhanced genome engineering strategies.

56. Metabolic Engineering of *Saccharomyces Cerevisiae* for Production of Alkanes from Free Fatty Acids

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In view of concerns regarding depletion of petroleum resources and environmental issues arising from heavy reliance on fossil fuel for energy, much attention has been paid to developing microbial biosynthesis of biofuels for generating sustainable and renewable energy sources. Alkane is an ideal biofuel candidate because it is a major component of fossil fuel and is high in energy density. Although production of alkane in bacterial hosts has been extensively explored in the past few years, research on alkane biosynthesis in yeast lags far behind. Despite *Saccharomyces cerevisiae* being a robust industrial production host, biosynthesis of alkane from fatty acyl-CoA in *S. cerevisiae* was reported only recently. However, the yield was limited partly by low production of the aldehyde intermediate. Here, we report the engineering of *S. cerevisiae* for improved production of aldehyde from free fatty acid source for alkane biosynthesis.

Oxidoreductases that convert fatty acids to aldehydes are present in plants for signaling against biotic and abiotic stresses. We functionally expressed a plant oxidoreductase heterologously in *S. cerevisiae* to produce aldehydes from free fatty acids *in vivo*. Co-expression of aldehyde decarbonylases effected production of alkanes. The heterologous alkane production pathway was further optimized by gene deletions to increase the availability of free fatty acids to serve as substrate and selecting appropriate promoters for gene expression to improve metabolic flux. The engineered *S. cerevisiae* described here is a highly promising platform for alkane production from free fatty acids and the findings in this work will facilitate future development of alkane-producing yeast hosts.

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57. Intracellular Spectral Recompositioning of Light for Improving Photosynthetic Efficiency in the Marine Diatom *Phaeodactylum tricornutum*

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Marine diatoms that usually accumulate high amounts of lipids generate as much as 40% of total organic carbon produced each year in the sea. Marine diatoms are expected to be a promising resource for future clean energy supply, as

well as for sustainable production of bioactive compounds such as high value nutraceuticals and active pharmaceutical ingredients. The light energy conversion efficiency is a decisive factor in intensive cultivation of diatoms in photobioreactors (PBRs) for determining the economical feasibility and limits of diatom-based cell factories and relevant industrial applications. Maximizing the photosynthetic efficiency may significantly improve biomass productivity and reduce the overall direct and indirect energy costs while imposing high-intensity illumination on algal cultures. We hypothesized and show here that Intracellular Spectral Recompositioning of light (or ISR) can increase the quantum yield of light if the otherwise wasted portion of blue light spectrum is shifted to green which diatoms have evolved to harvest through accessory photosynthetic pigments. We demonstrate that ISR can be employed to largely improve photosynthetic efficiency of the diatom *Phaeodactylum tricornutum* in flatpanel PBRs. We show that an addition of 2 μM of BODIPY fluorescent dye increased the photosynthetic efficiency from 4.9±1.0 % to 7.5±1.2 % in high-density culture of P. tricornutum under combined red and blue light-emitting diode illumination, on the basis of cell growth rate over a 24-hr period. To biogenically implement ISR, green fluorescent proteins (*GFP*s) as well as other FPs were elected with high quantum yield, photostability and extinction coefficient, and heterologously expressed in P.tricornutum cells. Transformants were pooled and then quantitatively screened using fluorescence-activated cell sorting (or FACS) for high expression of fluorescent proteins. Evaluation of transformant P. tricornutum cells is in progress and it is expected to optimize both intracellular and intercellular light delivery and redistribution in flat-panel PBRs through integrating selected fluorescent proteins into the existing light harvesting complexes. The carotenoid metabolism in transformants and cell tolerance to light stress will also be studied for optimizing biomass productivity as well as photosynthetic efficiency. This study may also provide a method to minimize cell damage caused by light stress of photosynthetically active radiation.

58. Synthetic Biology in a Chip: A Digital Microfluidic Platform for Cell Transformation, Culture and Expression

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Synthetic biology experiments require optimization of pathways consisting of many genes and other genetic elements. Given the large number of alternatives available for each element, optimization of a pathway can require a large number of experiments. Currently, these experiments are done manually using fairly large amounts of costly reagents per experiments making the process very expensive, extremely slow and irreproducible. We have developed a digital microfluidic platform that uses aqueous droplets suspended in an oil phase as discrete reaction chambers (or incubators) to completely automate the molecular biology steps. It integrates all critical steps of transformation and culture in one chip including plasmid addition, transformation by heat-shock, addition of selection medium, culture and phenotypic readout. The flexibility of digital microfluidics and peltier modules allows quick optimization of heat-shock parameters permitting transformation of a variety of plasmids and cell types. The chip was validated by introducing a variety of plasmids into *E. coli* including; plasmids containing genes for fluorescence proteins GFP, BFP and RFP, plasmids with selection markers for ampicillin or kanamycin, and plasmids built using golden gate assembly. Compatibility of this platform with multiple organisms was demonstrated by transforming DNA plasmids followed by culture of eukaryotic cells including; S.cerevisiae and A. niger. Employing the microfluidic device for heat-shock of GFP plasmid into *E. coli* cells gave excellent transformation efficiencies up to 4.4 × 106 ± 0.2 × 106 CFU/μg DNA, similar to benchtop results, though requiring ~100-fold less reagent. Reducing the duration of heat-shock steps afforded a throughput of 0.1 droplets/s, though with slightly compromised transformation efficiencies (9.0 × 105 ± 0.9 × 105 CFU/μg DNA). This afforded the autonomous generation and processing of up to 100 discrete heat-shocked droplets in just seventeen minutes. The microfluidic device, because of its ability to integrate and automate molecular biology steps and use ~100-fold less reagents, will be of great use to researchers for optimization of synthetic biology pathways to produce biofuels, pharmaceuticals, and other chemicals or biochemicals.

59. Design and Implementation of Reprogrammable dCas9 Transcription Factor Wires

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Presently, the size and complexity of engineered synthetic gene networks is limited by the small number well-characterized and robust genetic components. Here, we introduce and demonstrate a framework for creating orthogonal repressing transcription factor wires which exploit the programmability of the CRISPR/Cas9 system in *S. cerevisiae*. Genetic logic circuits such as NOR gates and layered cas-

cades have been implemented in bacteria and eukaryotes using CRISPRi, but are limited by non-cooperative response functions of the guide RNA (gRNA), leading to leak and signal degradation as the number of layers increase [1,2]. To achieve leak free off states, we use a eukaryotic system and a dCas9 fused with a strong chromatin remodeling repression domain (RD). In our system, a transcription factor wire consists of 1) a dCas9-RD fusion protein that when recruited to DNA represses transcription of its target and 2) a guide RNA (gRNA) which when complexed with the dCas9-RD binds a specific DNA sequence. These wires link the output of Pol II gRNA expression cassettes to their cognate gRNA responsive Pol II promoters (pGRRs). Pol II promoters are used as wire nodes, instead of noncoding RNA Pol III promoters, because of their variety and engineerability in *S. cerevisiae* [3]. To consistently express gRNA from Pol II promoters novel computational design methodologies, which consider minimum free energy and kinetic simulations of RNA structure, are used to augment and extend existing ribozyme-flanked gRNA expression methods [4]. Our synthetic pGRRs contain two discrete gRNA target sites and function as NOR gates. Therefore, systems of wires with minimal crosstalk can create any logic function. Building an orthogonal set of gRNAs and pGRRs is challenging due to the promiscuity of dCas9-gRNA complexes formed with imperfect DNA complements [5,6]. We developed a computational design strategy that generates sets of functional and orthogonal transcription factor wire libraries, enabling the construction of larger and more complex gene networks. To demonstrate the functionality of these wires, we created a library of 20 NOR gates and created a 4-component sequential repression cascade in *S. cerevisiae*. This work advances the state of the art for cellular logic and provides a framework for creating more complex circuits.

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60. Quantitative Metabolic Modeling for Biofuel Production at the Joint Bioenergy Institute

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The goal of the Joint BioEnergy Institute (JBEI) is to produce fundamental scientific discoveries to enable the development of commercially viable processes for large-scale conversion of lignocellulosic biomass into fuels.

The Quantitative Metabolic Modeling (QMM) directorate at the Fuels Synthesis Division is devoted to developing models of metabolism in order to improve biofuel production in a rationally directed fashion. We will describe how the QMM directorate fits in the general JBEI strategy and how we use experimental, computational and mathematical tools to achieve our goal. We will first show how the use of 13C labeling data allows us to effectively constrain metabolic fluxes for genome-scale models and make quantitative predictions. Secondly, we will show how we used quantitative targeted proteomic data to guide metabolic pathway engineering and increase the production titers of limonene, a biofuel molecule. Finally, we will show how new web-based tools for standardized data storage (the Experiment Data Depot, EDD) and interactive flux visualization (Multiomics Visualization Tool, MvT) produce the necessary base to make these predictions possible.

61. Adaptation of Bacteriophage on Synthetic Transcription Machinery

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With the emergence of synthetic biology, it remains unclear if synthetic parts (i.e. altered activity, substrate specificity, orthogonality, etc.) can significantly alter the evolutionary trajectory of the host organism. In this study, T7 bacteriophages lacking their wild-type RNA polymerase (T7Δ1) were passaged on an engineered T7 RNA polymerase designed to recognize an orthogonal promoter. T7 RNA polymerase plays a key step in the life cycle to the bacteriophage as it controls expression of 17 critical promoters. We hypothesize that massive changes in the regulatory architecture would emerge to better accommodate the orthogonal RNA polymerase. After 100 passages on the orthogonal polymerase, we observed multiple changes in multiple promoters and the evolved strains had a higher overall fitness than the ancestor, reaching that of wild-type T7 bacteriophage. Additionally, the evolved strains were able to accommodate insertion of the orthogonal RNA polymerase back into their genomes, whereas the ancestor T7Δ1 was not. This provides an example of how simple design strategies may frequently require further evolutionary optimization. Our

work raises the question whether synthetic biology can be used for laboratory speciation events and the development of orthogonal viruses.

62. Optogenetic Regulation of Proteolysis Using an Engineered Light-Switchable Split-Tev Protease

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Cells use proteases and proteolytic cleavage to control diverse processes which include protein activation or inactivation by removal of inhibitory or catalytic domains, mediation of protein degradation by revealing stabilizing or destabilizing residues, and initiation of protein translocation by removal of peptide signal sequences or exposure of latent ones. Due to the potential for regulating such a diverse array of cellular processes, proteases and proteolytic cleavage are excellent candidates for synthetic control.

Here, we have engineered a synthetic light-switchable protease by fusing split halves of the NIa tobacco etch virus protease (TEVp) to the light sensing proteins Phytochrome B (PhyB) and Phytochrome Interacting Factor 6 (PIF6). PhyB and PIF6 form high affinity heterodimers under red light but rapidly dissociate under far-red light, making them ideal candidates for reconstituting otherwise inactive split-TEV fragments1. By coupling the catalytic activity of TEVp to the light-dependent activity of PhyB and PIF6 we are able to rapidly, photoreversibly, and quantitatively tune TEVp activity using time-varying ratios of red and far-red light. This result suggests that split TEV fragments rapidly become functional and non-functional upon dimerization and dissociation of PhyB and PIF6. We show that this light-switchable protease can be used to cleave and modify target proteins in-vivo, and that light-gated proteolytic cleavage can be engineered to initiate target protein degradation through the conserved N-end rule pathway in the model eukaryotic organisms *Saccharomyces cerevisiae* and *Dictyostelium discoideum*.

Using a custom multichromatic LED-tissue culture plate device, we have characterized the spectral, steady-state, and dynamic performance of the photoswitchable TEVp protein degradation system. We measured action spectra (activating and deactivating) and determined peak activating and deactivating wavelengths to be 648 nm and 721 nm, respectively, which correlate closely with in-vitro measured PhyB Pr/Pfr absorbance peaks spectra (660/730 nm). The steady-state dose response to 648 nm light intensity follows a Hill-like relationship with fold-change = 4.5, n = 1.39, k = 0.02 µmol/m2*s. Step-function response dynamics, τON,1/2 = 150 min, are fast compared to gene expression dynamics (cell-cycle time scales, 12 hrs in *D. discoideum*) but slow compared to reported half-times of N-end rule substrates (< 10 min). We have constructed a mathematical model that indicates dynamic range can be increased and target protein half-life under red light decreased by optimizing expression level of system components relative to host proteolysis components and we are currently implementing these optimizations in-vivo. Finally, our plate based characterizations will enable us to couple light-switchable TEVp with other optogenetic tools2,3,4 without crosstalk for multi-channel control of protein production and degradation. This tool has many applications in science and biotechnology including improving control of dynamics and noise in eukaryotic synthetic circuits and the fabrication and modification of protein materials *in vitro*.

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63. Design of Modular Parts for Synthetic Systems Inspired By Anaerobic Fungi

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Anaerobic fungi in the hindgut of large herbivores are among the most robust organisms at degrading crude lignocellulose. They achieve this efficiency through the production of large, multi-enzyme complexes called fungal cellulosomes. The fungi also act synergistically with other microorganisms in the microbiome, such as archaea, bacteria, and protozoa. By elucidating the parts responsible for efficient biomass degradation at both the protein and cellular level, we seek to replicate this efficiency in synthetic systems.

At the protein level, fungal cellulosomes are similar to bacterial cellulosomes in that the protein-protein interactions are mediated through parts termed the dockerin and cohesin. However, many differences exist. The dockerin domains exist in tandem repeats and bear no species specificity like those in the bacterial systems. Furthermore, the exact sequence for the cohesin module has yet to be established. Through analysis of transcriptomic data for three fungal isolates, patterns governing the native placement of dockerin domains on fungal cellulases were characterized. By recombinantly grafting these dockerin domains onto similar enzymes from other organisms, the original activity of the enzymes were retained while allowing for incorporation of these exogenous enzymes into fungal cellulosomes. This was demonstrated for the TmCel5A, TmManB, TmXynA, and TmXynB from *Thermotoga maritima*. These incorporated enzymes demonstrated a greater level of synergy with

the native cellulosomes when compared to the catalytic domain without the grafted dockerins. Similarly, the newly grafted dockerin modules were replaced with carbohydrate binding modules, demonstrating that this approach can be extended to other protein binding domains. The eventual goal is to create entirely synthetic cellulosomes, which could be applied to any biocatalytic process.

At the cellular level, the anaerobic fungi have also been shown to interact closely with methane producing archaea (methanogens). The methanogens siphon hydrogen and other metabolites from the fungi, allowing the fungi to more efficiently produce energy by increasing the flux through their hydrogenosomes. This increased energy is hypothesized to increase production of cellulases, accelerating the degradation of lignocellulose in co-culture. To further investigate this mechanism, native fungal/methanogen co-cultures were isolated from herbivore fecal materials. These co-cultures were maintained together and also separated into monocultures, effectively creating parts for synthetic co-cultures. By introducing the methanogens into cultures of other well-characterized anaerobic fungi, stable synthetic co-cultures were established. With this proof of concept, other parts to the consortia can be introduced, such as non-native methanogens capable of funneling other accumulating metabolites like acetate. These stable synthetic consortia should increase the efficiency of conversion of crude biomass into sustainable chemicals.

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64. Optimizing Glycoengineered *E. coli* with Synthetic Small Regulatory RNAs

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One of the most important modifications for many proteins is N-linked glycosylation, which modulates an array of therapeutic properties from pharmacokinetic activity to immunogenicity. Large-scale production of therapeutic glycoproteins is currently accomplished using eukaryotic cell culture platforms; however, recent advances in *Escherichia coli*-based glycoprotein synthesis could enable higher yields and better control over glycoform. These *E. coli*-based platforms involve heterologous, multi-enzyme pathways for glycan synthesis that require optimization for production-scale yields. The aim of this study is to optimize *E. coli* for increased glycan production with synthetic small regulatory RNAs (sRNAs). We constructed a panel of 41 synthetic sRNAs to repress the endogenous expression of *E. coli* genes that were predicted by a genome-scale flux balance model to reduce sugar precursor levels in the N-linked heptasaccharide biosynthetic pathway from Campylobacter jejuni. We then screened our sRNA panel for improved glycan production with a fluorescence-based assay of cell-surface displayed glycans and identified *E. coli* strains with over 50% increases in fluorescence intensity. Further work is anticipated to identify even greater improvements by screening multiplexed sRNA libraries with fluorescence-activated cell sorting (FACS)-based monitoring of glycan production. Parallel work is ongoing to optimize an engineered biosynthetic pathway that produces eukaryotic core mannose3-N-acetylglucosamine2 (Man3GlcNAc2) glycans in *E. coli*. Our approach of using sRNAs for metabolic flux optimization provides an effective strategy for increasing glycan yields and opens the door for more complex regulation of *E. coli*-based glycosylation by integration within genetic circuits.

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65. MinMCell: Designing Minimal Microbial Cells

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A minimal cell can be viewed as the kernel of life. A minimal microbial cell would be a perfect framework to understand how the minimal parts (e.g., genes, pathways, networks, compartments) work together to make a living cell. The long-term goal is to try to understand which are the minimal biochemical objects required for life which will provide unexpected (and probably counterintuitive) insights into how cells function.

More pragmatically, in this research work we developed a suite of tools and algorithms, MinMCell, to infer a minimal microbial cell; to this purpose we tackled three different organisms: *E. coli* iJO1366, S. cerevisiae iTO977 and M. genitalium iPS189.

The goal of this research has been to identify a minimal set of essential genes needed to the cell to divide and grow in pure culture.

In order to discover a minimized genome the designed approach is able to maintain the essential genes and the lethal pairs of genes (genes encoding an essential function) and concurrently to erase (knock-out) the non-essential genes.

We used the public databases [1-4] and the literature [5-10] in order to classify the essential genes and the lethal genes of the three analyzed organisms. The genes to knock-out have been identified by an ad-hoc algorithm for genome-scale re-design based on Pareto optimality.

For the *E. coli* in anaerobic condition, we obtained a minimal strain with 857 knock-out genes with a biomass production of 0.554h-1. Moreover, inspecting the obtained Pareto Front it is possible to determine the strain with a

maximum value of biomass (1.0334937h-1, basically the biomass value of the wild type *E. coli* and a high number (but not the maximal one) of genes off 479; hence it is possible to re-design a bacterium to achieve the same biomass performance of the wild type *E. coli*erasing the 11,171% of the redundant and non-essential genes.

Overall, the designed method identified 35413 distinct feasible strains (the biomass values belong to the following feasible set (0.05h-1, 1.0334937h-1]) and 28 Pareto optimal strains (non-dominated strains with respect the maximization of biomass and number of knock-out genes).

Similar results have been obtained for the other two organisms. For the S. cerevisiae, in anaerobic and carbon-limited conditions, we discovered a minimal set of 424 non-essential genes that can be knocked-out in order to the cell to divide and grow (biomass value of 1.800568e-13h-1). While for the M. genitalium, in anaerobic condition, the algorithm detected 39 knout-out genes with a biomass value of 0.707h-1.

The designed method doesn't disrupt the essential protein-coding genes, lethal genes, genes that encode proteins with potential redundant essential functions and genes encoding proteins of unknowns functions. For the three organisms the disruption of some genes accelerated bacterial growth. A comparative genome analysis completes the present study in order to detect the common essential genes.

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66. An optogenetic system for nuclear translocation of viral nanoparticles and tunable gene delivery

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The use of viruses to deliver nucleic acids for various applications, ranging from fundamental biological studies to clinical translation, is an active area of research recently fueled by the European approval of an adeno-associated virus (AAV) based product for human use. AAV is a promising vector able to deliver genes, parts of genetic circuits, RNAi, and most recently CRISPR/Cas. However, *in vivo* viral gene delivery still suffers from low gene expression at the target site and leaky off-target expression.

With the hopes of improving targeting and efficiency of gene delivery, vectors have been designed to be stimulus-responsive against a variety of inputs, including pH, temperature, and enzyme concentration in the extracellular environment. Although promising, engineering vectors to be responsive to exogenous stimuli, rather than endogenous inputs, may render the delivery process more controllable both temporally as well as spatially.

Additionally, mainly extracellular steps of the gene delivery process have been manipulated so far. Once inside the cell, gene vectors are challenged by several obstacles like sequestration in endosomes, ubiquitination and clearance by autophagosomes, cytosolic trafficking, and perinuclear accumulation. This makes nuclear localization a formidable rate-limiting step and a major determinant of effective gene delivery.

To overcome this problem, we have engineered AAV controllable by externally applied light. The virus is genetically engineered to display surface-exposed Phytochrome interacting factor 6 (PIF6) moieties capable of binding to Phytochrome B (PhyB). In HeLa cells constitutively expressing PhyB tagged with a C-terminal nuclear localization sequence (NLS), we are able to exert control over virus nuclear localization and gene delivery by varying the intensity, duration, and wavelength of light. Red light activates PhyB-NLS to bind to AAV-PIF6 and promote nuclear accumulation, and far-red light deactivates PhyB. A transfer function has been established relating the ratio of red:far-red light to the overall gene delivery by the virus, and spatial control of light is being investigated to enable patterned gene expression.

Combining optogenetic tools with viral gene delivery can lead to the development of predictable and controllable delivery platforms, which will be broadly useful in gene therapy and mammalian synthetic biology research. Other light-sensitive proteins such as the Light-Oxygen-Voltage (LOV) domains are also under investigation for use in gene delivery. Future applications include organized gene expression from regulated patterns of light in 2D and in 3D biomaterials for regenerative medicine applications.

67. Piace: Parallel Integration and Chromosomal Expansion of Biofuels Pathways in *E. coli*

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Robust fermentation of biomass-derived sugars into biofuels demands the reliable microbial expression of underlying metabolic pathways. Previous studies have shown that plasmid-based expression systems, which may suffer from instability issues (despite selective pressure), can result in inconsistent and highly variable biofuel production rates and titers. An established mitigation strategy, Chemical Induced Chromosomal Expansion (CIChE; Tyo *et al*. 2009), exploits recA-mediated copy number expansion of a pathway post integration at a single chromosomal locus, and offers the gene copy numbers of plasmids while maintaining pathway stability even without the addition of antibiotics (a significant process cost-savings). Innovating beyond CIChE, we are developing PIACE (Parallel Integration and Chromosomal Expansion) to facilitate the simultaneous and independently-adjustable expansions of multiple pathways at separate chromosomal loci. PIACE offers a set of BglBrick-compatible suicide vectors, each of which site-specifically integrates into a distinct chromosomal locus via a single homologous cross-over and has high efficiency for transformation and chromosomal integration of long (> 3kb) pathways that reside in super-coiled sequence-validated plasmids. As a proof of principle for PIACE, we have integrated rfp and *gfp* at two separate chromosomal loci, expanded rfp and *gfp* independently in the presence of heat-curable plasmid pRedi2RecA, and demonstrated that both expansions are stable over 50 cellular generations in the absence of antibiotic selection pressure. We are now applying PIACE to the chromosomal stabilization and optimization of the isopentenol fuel pathway in *E. coli* DH1. PIACE affords an efficient, tunable method for improving the robustness, productivity, and titers of engineered metabolic pathways.

68. Synthetic RNA Networks for Sophisticated *in Vivo* Computation

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Synthetic gene networks have generally employed well-characterized natural parts rewired to provide new functionality; however, the limited number and crosstalk between such parts has hindered the construction of more complex networks that can operate robustly in living cells. We have recently developed a new type of synthetic biological device called the toehold switch that addresses these limitations by providing dozens of orthogonal parts each with a wide dynamic range of gene expression. These validated new parts provide us with the opportunity to construct much more complex *in vivo* synthetic networks. Here, we present a new molecular programming strategy that employs long information-processing strands of RNA to evaluate the status of networks of short de-novo-designed RNAs being transcribed within the cell. These synthetic RNA computational networks, or ribocomputers, are enabled by sets of highly orthogonal toehold switches and self-assembling RNA complexes that act as conditional activators of gene expression. Ribocomputers can compute expressions combining AND, OR, and NOT operations for universal combinatorial logic in *E. coli*, and they exploit *in silico* RNA sequence design to greatly reduce the time and effort required to construct new functional systems. These systems can efficiently evaluate multi-input AND and OR logic operations using a single computational layer and routinely provide ON/OFF ratios greater than 20. Furthermore, multiple ribocomputers can be active within a cell at the same time providing multi-output capabilities. All these features make ribocomputers an important new addition to the synthetic biology toolkit and highlight the strengths of programmable RNA-based regulation for realizing sophisticated *in vivo* logic.

69. Simplexes Characterize the Coupling in Gene Expression Due to Competition for Rnap and Ribosomes

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The limited availability of shared cellular resources couples the expressions of any two genes. This, among other factors, renders the behavior of gene circuits context-dependent. As a result, our ability of predicting the emergent behavior of a complex gene network from that of the composing modules remains limited, posing a challenge in systems and synthetic biology. To overcome this limitation, here we characterize the coupling among protein concentrations due to competition for the shared transcriptional/translational machinery in gene networks with arbitrary topology.

In particular, we show that the set of protein concentrations that are simultaneously realizable is given by the intersection of simplexes. Exploiting these simplexes, we further characterize how changing the concentration of one protein affects the concentration of other proteins, even in the absence of regulatory linkages among them. The fact that the vertices of these simplexes are straightforward to measure experimentally renders this approach easy to employ in practice.

This simplex-based method aids the rational and predictable

design of complex biocircuits as follows. First, it provides a simple tool to check whether the desired behavior of a large gene circuit is realizable. If the transcriptional/translational resources are insufficient, the simplexes aid the partitioning of circuits into smaller components (subnetworks), each realizable within a single cell. Second, we can predict how tuning various biochemical parameters affect the strength of interdependence among protein concentrations. Therefore, we can mitigate the coupling among components, facilitating the modular design of complex gene circuits. Finally, standard pathway optimization problems, such as maximizing the concentration of protein complexes or maximizing the rate at which a reaction occurs, can be formulated as convex optimization problems. These problems can be naturally interpreted in the context of economics, giving rise to concepts such as the price and utility of proteins.

70. A General Method for Sensing Small Molecules in Eukaryotes

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Biosensors for small molecules can be used in applications that range from metabolic engineering to orthogonal control of transcription. We describe a method to create biosensors starting with a computationally designed ligand-binding domain (LBD) that, in principle, can be generated for any target molecule. The LBD is fused to either a fluorescent protein or a transcriptional activator and is destabilized by mutation such that the fusion accumulates only in cells containing the target ligand. We illustrate the power of this method by developing biosensors for digoxin and progesterone. Addition of ligand to cells expressing a biosensor activates transcription in yeast, mammalian cells and plants, with a dynamic range of up to ~100-fold.

We use the biosensors to improve the biotransformation of pregnenolone to progesterone and to regulate CRISPR activity. In concert with computational LBD design approaches, this method should enable the generation of biosensors for a broad range of molecules.

71. Replicable Single-Stranded DNA Origami

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DNA has been used to create a variety of complex nanoscale shapes and devices since the birth of DNA nanotechnology in 1982. One main challenge is to create complex nanostructures from a single-strand of DNA, like proteins which typically fold from a single polymer. Here we report the design and synthesis of single-stranded DNA origami structures (ssOrigami) containing up to 4,000 nucleotides, which can fold into designed shapes. Such ssOrigami can be easily replicated by polymerases *in vitro* and *in vivo*. ssOrigami structures can also be used as a template for amplification by PCR.

In previous studies, multiple DNA strands were successfully designed to self-assemble into complex structures with or without the help of a long scaffold strand. However, biological macromolecules, such as mRNAs and proteins, typically fold from a single polymer into a well-defined structure. The ability to fold DNA nanostructures in this manner would enable robust assembly and even replication of such structures from a unimolecular template. However, folding complex nanostructures from a single molecule of single-stranded DNA (ssDNA) still remains challenging for the field of DNA nanotechnology, due to the complexity of the topology in most existing strategies and the difficulty of large-scale clonal production of suitable DNA sequences.

In our study, ssOrigami structures with different sizes ranging from 1,000 to 4,000 nucleotides (nt) were assembled. Three diamond shape ssOrigami containing 1k, 1.6k and 2.3k nt were demonstrated, and they folded with high yield. More complicated and larger ssOrigami structures were also achieved. While the folding path is less obvious than simple diamond shapes, these complex ssOrigami structures were also successfully assembled with good quality. ssOrigami of up to 4,000 nt in size were demonstrated, which are larger than 99.99% of all proteins in the human proteome and three times the size of the largest catalytic rRNA (16S rRNA). This result will greatly broaden our vision and understanding about the self-assembly capability of macro-biomolecules, especially when such a procedure can happen during a short (2 hour) annealing step.

Unlike multi-stranded DNA systems such as DNA origami and DNA bricks, which contain hundreds of distinct components and assemble with undesirable defects and heterogeneity, ssOrigami is a homogenous, 'pure' system. Additionally, ssOrigami can be easily replicated and amplified by polymerases *in vitro* and *in vivo*, which can greatly decrease the cost of DNA nanotechnology, especially when large amount of DNA nanostructures are required. Finally, ssOrigami structures can be used as a template for amplification by PCR.

72. Optogenetic Frequency Analysis of Bacterial Two-Component Systems and Synthetic Gene Circuits

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Synthetic biology is not yet a mature engineering discipline, due in part to a poor understanding of the dynamical input/output (I/O) properties of genetic components. Despite the fact that gene regulatory systems are inherently dynamic,

most attempts to characterize them have been static. We have previously engineered green/red (CcaS/R) and red/far red (Cph8/OmpR) light-switchable two component systems (TCSs) with transcriptional outputs in *E. coli*1,2. In recent work, we developed quantitatively predictive mathematical models of the I/O dynamics of these signaling pathways by measuring their gene expression outputs in response to step changes in light input3. However, this characterization relied upon intuition to design non-linear models and iterative characterization and validation experiments. In contrast, the electrical engineering community has developed standardized, automatable, and scalable system identification (SI) methods that employ frequency analysis to construct linear transfer function (TF) models. These dynamical TF models are used to systematically characterize the performance of engineered components, reliably predict the behavior of those components when they are composed with others, and thereby design new systems with high predictability.

Here, we have combined frequency analysis, optogenetics, light-switchable TCSs and synthetic gene circuits to develop a standardized I/O characterization framework for transcriptional regulatory parts. Specifically, we subject *E. coli* populations to sinusoidally oscillating light inputs spanning a wide range of frequencies, and monitor the corresponding gene expression outputs with high temporal resolution. We then fit the sinusoidal response data to a small set of TF models, selecting one with the best fidelity for each system. We have validated this approach by characterizing both light-switchable TCSs and using the resulting TF models to predict gene expression dynamics in response to non-sinusoidal, time-varying inputs, with only 5% error (RMSE) over 12-hour experiments. This result demonstrates that frequency analysis can supplant our previous intuition-guided approach. Additionally, our TF models have yielded a better understanding of the TCSs' filtering characteristics, noise, and signal transduction limits, motivating characterization of other optogenetic components. We are also extending optogenetic frequency analysis to a wide range of genetic circuit components, including the Voigt TetR inverter family4 and large sets of CRISPRi-based inverters, and using the data to create technical data sheets. Finally, we will use the resulting TF models to design larger genetic circuits with predictable dynamic properties. Our standard, scalable, and quantitatively rigorous approach will help synthetic biology transition into a mature engineering discipline.

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73. Logic Gates As sRNA Dependent Transcriptional Attenuators: A Pause for Thought

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Within the field of synthetic biology, genetic circuits have become progressively more complex, requiring more sophisticated regulation and leading to a drive to generate logic gates within biological systems that are orthogonal, scalable, respond rapidly and perform multilayer logical operations. The reprogrammable nature of RNA folding and binding combined with rapid synthesis and degradation within biological systems provides useful tools to achieve this.

It is possible to dispense with the requirement for proteins to propagate a signal through a logical operation which in the past has acted as a constraint on the size of orthogonal libraries, using instead sRNA dependent transcriptional attenuators. Here we present a new genetic learning algorithm for the de novo design and synthesis of the sequences required for sRNA dependent transcriptional attenuators. The Algorithm uses a combination of RNA folding predictions and dimer formation kinetics, aided by the use of transcriptional pausing sites to increase the time window for sRNA-Attenuator interactions.

Sequences generated by the algorithm are currently being tested and work to date will be presented.

74. Development of Modular, Self-Assembling Synthetic Protein Complexes As Screening Platforms for Enhanced Enzyme Discovery

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At the forefront of enzyme discovery, Novozymes continues to search for better ways to screen a growing diversity of glycosyl hydrolases in order to improve enzyme cocktails for second-generation biofuel production. To this end, we have successfully integrated a synthetic biology approach into our next generation of enzyme discovery platforms. Specifically, we have leveraged the available natural diversity of cellulosomal components to develop an artificial mini-cellulosome as the basis for a versatile cell-free method to screen combinations of multiple enzyme activities. In the process, we have engineered a highly thermostable scaffoldin comprised of distinct cohesin domains that stably bind cognate dockerins with high specificity for extended periods under lignocellulose hydrolysis conditions. These protein-protein interactions serve as respective binding modules for covalently-linked libraries of different enzyme classes. Our synthetic cellulosome approach has been adapted to an automated HTS system through which enzymes of synergistic quality are

more efficiently identified compared to past conventional approaches. Finally, we have successfully employed this novel screening platform to discover promising new enzyme candidates tailored for improved hydrolytic performance on AFEX-PCS, an industrially-relevant biomass substrate.

75. Seeing the Invisible, and Considering Context: Exploiting Crosstalk to Measure Genetic Construct Activity

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The ability to quantify the activity of genetic parts that do not produce spectroscopically quantifiable products would support the reusability of parts in a forward engineering context. A potential solution, described here, is a competitive assay performed in an *in vitro* transcription-translation (IVTT) system. In an *in vitro* environment, expression of orthogonal genes are coupled by competition for limited resources, which can be exploited for measuring the activity of "invisible" genetic constructs. Several new results from previously presented work are highlighted here:

1. The activity of the non-fluorescent construct of interest can be measured by exploiting resource-based coupling in a competitive assay.
2. A linear relationship between the activities of two constructs is predicted only under specific conditions.
3. The relative activities of constructs are context-dependent: assay measurements cannot yet be ported between expression systems.

The above results suggest that, while the competitive assay is feasible, appropriate conditions that define a context must be identified. Future work will focus on identifying assay conditions using a validated model-based framework, potentially leading to a procedure for characterizing the capacity of both *in vitro* and *in vivo* expression contexts.

76. Regulation of Biomass Degrading Enzymes in Anaerobic Gut Fungi and Their Application in Synthetic Co-Culture Systems

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To support renewable technologies, it is necessary to develop more efficient methods to extract sugars from crude plant biomass (lignocellulose). Plants contain cellulose that depolymerizes into fermentable sugars for microbial biofuel production. However, in crude biomass, cellulose is trapped within lignin, hemicellulose and other biopolymers that complicate its hydrolysis. To address this issue, one can turn to nature, particularly to microbes that routinely degrade plant biomass. Many large herbivores, such as cows and horses, harbor a consortium of microbes in their digestive tracts that convert recalcitrant biomass into sugars. Within this consortium, anaerobic gut fungi are the primary colonizers of plant material, and represent a rich source of biomass degrading enzymes. We have isolated several novel strains of gut fungi from herbivores at the Santa Barbara Zoo to characterize their ability to release sugars from crude biomass. We have used transcriptomic analysis to identify specific enzymes required for the breakdown of plant material including cellullases (GH5, GH9, GH48), hemicellulases (GH10, GH11, GH43), and accessory enzymes (Polysaccharide deacetylases). Through examining the regulatory pattern of these enzymes during growth on a variety of carbon sources, those that are most important for degradation of crude plant material can be identified. We have also used transcriptomics to identify sensor proteins and transcription factors that may be responsible for the global regulation of these enzymes. Through examination of putative regulatory proteins and the environmental stimuli that trigger their response, we can develop methods to control gut fungal metabolism and the production of biomass degrading enzymes.

While tools to engineer gut fungi directly are severely underdeveloped, another way to incorporate them into industrial processes is to create co-culture systems. Natively, gut fungi maintain a syntrophic relationship with archaeal methanogens by which the fungi produce CO_2 and H_2 that the methanogens convert into methane. This relationship results in enhanced biomass breakdown by the fungus. In a synthetic system gut fungi are used for their degrading power to release sugars from biomass (~5 g/L released from cellulosic substrates). This excess sugar is then used to fuel production of a value-added chemical in a model microbe, such as *S. cerevisiae* or *E. coli*. We have used the production of Flavin-based fluorescent proteins (FbFPs) to quantify growth in both systems and production of n-butanol in *E. coli* to assess the ability to produce a fuel molecule in this system. If methanogens are incorporated into this synthetic system, it is expected that the amount of sugar available for value-added chemical production will increase. By coupling the lignocellulose-degrading capabilities of the gut fungi with the production capacity of model microbes, many different products may be generated directly from biomass.

77. Engineering Caspases with Altered Specificities

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Caspases are proteases that play important regulatory roles in apoptotic cell death, neurodegeneration and inflammation. They are amongst the most specific of all proteases, and are therefore excellent targets for engineering new specificity. Unfortunately, because they are heterotetramers, they are not amenable to traditional engineering approaches like phage display and are difficult to tackle by rational design due to their flexible active sites. Such characteristics prompted our lab to develop a *GFP*-based reporter of caspase activity that has enabled us to perform a directed evolution screen and evolve caspases with new functions.

In our first application of this technology, we engineered caspase-7, which recognizes and cleaves the amino acid sequence DEVD, to recognize and cleave the new sequence VEID, which is recognized by caspase-6 but not caspase-7. Simple substitution of the homologous residues in caspase-6 dramatically diminished activity, but did not alter the specificity, indicating that a more holistic evolutionary procedure would be needed. The caspase-7 variants with the most strongly altered specificity displayed kinetic values similar to wild-type caspase-6 and mimicked caspase-6 cleavage patterns with protein substrates. Using N-terminomics we assessed the substrate specificity profile for the most successful engineered caspase. We found that had completely altered the specificity using our directed evolution approach. The resulting WebLogos for the engineered caspase were completely superimposable with caspase-6 and unlike the parent scaffold, caspase-7. Our seven crystal structures of evolved caspases bound to either DEVD or VEID substrates allowed us to observe how the new residues in the active site contributed to the change in specificity. In particular, F282 plays a critical role by arching inward to create a small pocket around the newly introduced isoleucine at the P2 peptide position. This unexpected finding underscores why rational design is insufficient for altering substrate recognition.

78. The Joint Genome Institute's Synthetic Biology Internal Review Process

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Serious concerns have been raised over the possible intentional use of synthetic biology approaches to engineer pathogenic organisms as well as the possible accidental environmental release of genetically engineered organisms. Scientists pursuing synthetic biology research must diligently consider issues such as these. As such, the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) has developed a Synthetic Biology Internal Review process to assess, beyond technical and scientific merit, certain broader aspects (e.g. environmental, biocontainment, biosafety, or biosecurity) of the research proposals associated with the JGI's DNA synthesis program.

79. Controlling Multiple Gene Expression in *Saccharomyces cerevisiae* by Optogenetic Switches

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Our group is interested in the regulated production of small to large cohorts of proteins and peptides and the establishment of a toolkit for the modular assembly of circular expression yeast artificial chromosomes (xYAC) in *S. cerevisiae*. To simplify induction of protein production we will provide a reliable and easy to use induction system: Light-induced TALE-transcription factors based on two different light-sensing systems from *Arabidopsis thaliana*. While the CRY2/CIB1 system is blue light-sensitive and has no need for an exogenous cofactor, the red light-sensing PhyB/PIF3 interaction depends on a chromophore, which is not available in yeast. To avoid the exogenous supply of the chromophore, yeast will be engineered to express the necessary cofactor biosynthesis genes along with the red light-sensing proteins. In order to engineer two autonomous and non-interfering light-dependent gene switches, we will combine the red and the blue light-sensing systems on one single xYAC. With this approach it will be possible to easily regulate gene expression of a large number of proteins in a reversible, specific, dose-dependent and orthogonal manner totally independent of each other just by applying light of different wavelength. Results on the optimization of chromophore biosynthesis, evaluation of the individual light-induction systems, and the parallel use of red and blue light-controlled gene expression within our xYAC platform will be presented.

80. Engineering *Clostridium thermocellum* for High Ethanol Yield

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Clostridium thermocellum, a gram-positive, thermophilic anaerobic bacterium, is a candidate organism for consolidated bioprocessing, due to its ability to solubilize cellulosic biomass and ferment said biomass into organic acids and ethanol. There have been several attempts to date to engineer *C. thermocellum* to produce ethanol at high yield; however, the maximum reported yield is 60% of theoretical.

Our group has previously engineered another thermophilic anaerobe, *Thermoanaerobacter saccharolyticum*, to produce ethanol at >90% theoretical yield. We have characterized and identified the genes that play a major role in pro-

ducing the high ethanol yield, and now seek to transfer the key genes of this pathway into *C. thermocellum* to improve ethanol production.

In order to do this, we have improved our plasmid-based expression system to use in our organism. We have used this new plasmid to express adhE, a gene that encodes activities thought to play a role in the last two steps of ethanol production.

81. Construction of Gamma-Aminobutyric Acid Producing Recombinant *Escherichia coli* by Introduction of Synthetic Scaffolds

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Escherichia coli were engineered for the direct production of gamma-aminobutyric acid from glucose by introduction of synthetic protein scaffold. In this study, three enzymes consisting GABA pathway (isocitrate dehydrogenase, glutamate synthase and glutamate decarboxylase) were connected via synthetic protein scaffold. By introduction of scaffold, 0.92 g/L of GABA was produced from 10 g/L of glucose while no GABA was produced in wild type *E. coli*. The optimum pH and temperature for GABA production were 4.5 and 30oC, respectively. When competing metabolic network was inactivated by knockout mutation, maximum GABA concentration of 1.3 g/L was obtained from 10 g/L glucose. The recombinant *E.coli* strain which produces GABA directly from glucose was successfully constructed by introduction of protein scaffold. This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant number: PJ011116) by RDA, and Basic Science Research Program by the Ministry of Education (NRF-2014R1A1A2054726).

82. Construction of Novel Malate Sensing Recombinant *Escherichia coli* by Engineering of Bacterial Two-Component System

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In an attempt to develop a high throughput screening system for screening microorganisms which produce high amounts of malate, a MalKZ chimeric HK-based biosensor was constructed. Considering sequence the similarity among *Escherichia coli* MalK with *Bacillus subtilis* MalK and *E. coli* DcuS, the putative sensor domain of MalK was fused with the catalytic domain of EnvZ. The chimeric MalK/EnvZ (MalKZ) TCS induced the ompC promoter through the cognate response regulator, OmpR, in response to extracellular malate. Real-time quantitative PCR and *GFP* fluorescence studies showed increased ompC gene expression and *GFP* fluorescence as malate concentration increased. By using this strategy, various chimeric TCS based bacteria biosensors can be constructed, which may be used for the development of biochemical-producing recombinant microorganisms. This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant number: PJ011116) by RDA, and Basic Science Research Program by the Ministry of Education (NRF-2014R1A1A2054726).

83. Improvement of DNA Synthesis

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In the few last years the price for DNA synthesis has decreased rapidly, opening up new markets for commercial gene synthesis suppliers. However, the current technology is reaching a limit with respect to cost reduction, constrained by synthesis scale and error rate.

In our efforts to keep offering our customers top quality synthetic DNA products, we continuously improve our oligonucleotide and gene synthesis workflows. We further develop parts of the process regarding cost reduction, time reduction, higher stability and higher efficiency. This allows us to target the market demand of lower costs for synthetic genes.

We will show data illustrating our efforts to reduce error rate both by improved oligo synthesis as well as error correction of assembled genes. In combination these developments continue to drive overall improvements in DNA synthesis.

84. Engineering Light- and Pharmacologically Triggered Extracellular Biological Switches

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Synthetic molecular switches are the key components to obtain control of mammalian cells. While the majority of the developed switches focuses on intracellular processes we now transfer concepts of synthetic biology out of the cell. In this context, we (i) engineered pharmacologically switchable viral vectors enabling precise control of the transfer of genetic information into cells and (ii) a synthetic extracellular matrix the mechanical properties of which can be modulated by light.

For the chemical switch controlling viral infectivity we used adeno-associated viral particles (AAV) due to their high relevance in gene-therapeutic approaches. We engineered the system in such a way that infection of the target cells occurs only in the presence of the rapamycin structural analog AP21967.

The development of the light-tunable extracellular matrix was realized by combining light-gated molecular switches from the field of optogenetics with cell-compatible polymers from material sciences. To this aim, we covalently coupled the cyanobacterial phytochrome Cph1 to an eight-arm polyethylene glycol forming a biohybrid hydrogel. Illumination with red light triggers dimerization of Cph1, thereby increasing the number of crosslinks within the hydrogel and thus enhancing its stiffness. Vice versa, far-red light illumination induces Cph1 monomerization and decreases hydrogel stiffness. Due to the fast switching properties of Cph1, the stiffness can be modulated reversibly within seconds. By incorporation of RGD cell adhesion motifs our hydrogel serves as suitable matrix for primary cells as well as cell lines. Since variations in matrix stiffness can modulate cellular signaling pathways and even direct cell differentiation, we propose that our engineered light-tunable extracellular matrix will be a unique tool for studying matrix-cell interactions.

85. Resource Limitations Lead to Hidden Interactions in Gene Networks

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Protein production in gene networks relies on the availability of resources necessary for transcription and translation, which are found in cells in limited amount. As various genes in a gene network compete for a common pool of resources, including RNA polymerase and ribosomes, a hidden layer of interactions among genetic circuits arises. In this work, we develop a general modeling framework using deterministic reaction rate equations to account for these interactions. We verified the model with a two-stage activation cascade and a one-stage repression circuit in experiments with *E coli*. Resource limitations introduce (1) a feed-forward loop in the interaction graph of an activation cascade, which shows counterintuitive negative or biphasic steady-state responses, and (2) a positive auto-regulation in a repression circuit, which leads to bistability confirmed by hysteresis behavior.

86. Expanding Synthetic Biology's Toolkit By *in Vitro* Evolution: Characterization of a Fast and Uniquely Unnatural Hammerhead Ribozyme

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Ribozymes, and more specifically hammerhead ribozymes, have been successfully used in synthetic biology approaches to artificially control gene expression via trans- or cis-acting mechanisms. A typical Hammerhead ribozyme consists of a highly conserved catalytic core of around 15 nucleotides flanked by three based-paired stems and two loops. This structural arrangement includes specific non- Watson-Crick interactions that prime the ribozyme for activity. Furthermore, kissing-loop interaction of loop II with loop I, which often occurs in naturally occurring ribozymes, mediates enhanced catalytic activity in natural instances of these ribozymes. In this study, we describe characterization of an atypical hammerhead ribozyme obtained through *in vitro* evolution that features an unusually short stem-loop II structure and a high catalytic activity. The minimal stem-loop II structure and the retaining of full functionality of this structure motivated us to characterize this molecule further. Extensive mutational analyses and biochemical assays evaluating the effects of point mutations on catalysis were done for this purpose. Our analyses showed sensitivity of catalytic rates to specific sequences of stem-loop II and stem I structure. We carried out de novo *in silico* simulations to obtain plausible 3D structural models that can explain our observed biochemical experiments, providing a deeper insight on the structural arrangement as well as a plausible three-dimensional folding pattern that would account for the high catalytic activity, or lack of, in certain mutants. Molecular dynamics simulations to further refine a plausible structure for this ribozyme are ongoing. This study has further been complemented by multiple genome-wide scans for naturally occurring RNA molecules sharing this specific RNA motif. These scans have yielded no significant hits in 108 eukaryotic and prokaryotic genomes searched so far, suggesting a lack of natural occurrences of this ribozyme. This apparent under-representation in extant genomes, suggest that this specific hammerhead ribozyme might have been under negative selective pressures throughout evolution. These characteristics underline a positive potential in using this specific ribozyme motif in synthetic biology approaches. The ease of activating/deactivating this ribozyme through its peripheral structures sets forth a solid, promising ground, to generation of an effective tool for efficient modulation of gene expression.

87. Directed Evolution of CRISPR Endoribonucleases Using Phage-Assisted Continuous Evolution (PACE)

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A panel of sequence-specific RNA-cleaving enzymes would enhance our ability to manipulate RNA structure and func-

tion and aid in the analysis of coding and non-coding RNAs found in human and other genomes. We have developed a selection system that enables the phage-assisted continuous evolution (PACE) of RNA-cleaving enzymes. Using PACE, we have evolved variants of the CRISPR endoribonuclease Csy4 that demonstrate altered substrate specificity.

89. Multiplexed Screens of Olfactory Receptor Binding

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Animal olfaction is extremely sensitive and precise mechanism for chemical detection with the ability to distinguish very dilute compounds from complex mixtures. This sense is mediated by molecules binding to one or more olfactory receptors (ORs), which are a subtype of G protein-coupled receptors (GPCRs) that mediate signal transduction by stimulating cyclic AMP (cAMP) production. Current methods for monitoring OR activation are either qualitative or rely on luminescent reporters not practical for screening thousands of receptors against thousands of ligands. Advancements in next generation sequencing and genome editing techniques allow us to develop a novel platform to measure multiplexed responses from a library of engineered human cells each clonally expressing unique ORs on the genome using landing pad integration. Receptor activation is linked to a cAMP responsive reporter expressing a barcode that identifies each OR and measure quantitatively using RNAseq. Apart from chemical sensing, the system enables us to pursue a variety of applications such as: deciphering functional differences in ORs among populations, screening drugs that interact with G protein coupled receptors, and engineering receptors with novel specificities.

90. Epathoptimize: A Combinatorial Approach for Transcriptional Balancing of Metabolic Pathways

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Recent advancements in synthetic biology give scientists the ability to balance a variety of factors involved in gene regulation to improve product titers, yields, and productivities. Using a library of isopropyl ăD-1-thiogalactopyranoside (IPTG)-inducible mutant T7 promoters of varied strength, a combinatorial method was developed for transcriptional balancing of the violacein pathway. Violacein is a potent bioactive compound that has been shown to have various antitumor, antifungal, and anticancer properties. The violacein pathway is also an excellent model for exploratory metabolic engineering efforts into pathway regulation and control due to many colorful intermediate and side products allowing for easy analysis and strain comparison. Upon screening approximately 4% of the initial library, several high-titer mutants were discovered that resulted in up to a 63-fold improvement over the control strain. With further fermentation optimization, titers were improved to 1829±46 mg/L, a 2.6-fold improvement in titer and a 30-fold improvement in productivity over previous literature reports. Future applications of this technology towards improving the microbial production of biofuels, pharmaceuticals, and commodity chemicals could expedite the transition from the laboratory to the marketplace.

91. From *Bacillus subtilis* Gene Expression Decomposition to Synthetic Biology

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We aimed at decomposing gene expression into distinct and well characterized genetic elements (gene location, TATA box, transcription start, translation initiation region, etc.) that could be rationally assembled to control gene expression across growth conditions. To address this issue, we revisited the contribution of replication, transcription and translation to global mechanisms allowing bacteria to modulate abundance of single proteins with respect to the growth rate. Growth rate has long been regarded as a global variable modulating macromolecular cellular contents but the growth-rate-dependent variation of the transcription machinery abundance was recently shown to clearly influence gene expression.

Regarding translation, we demonstrated that translation efficiency strongly depends on growth rate and that cells can differentially translate individual transcripts as function of the growth rate without dedicated regulators. The mechanism underpinning this translational regulation relies on the selective titration of the free (untranslating) ribosome fraction by the transcript-specific translation initiation regions. We identified this mechanism by developing a knowledge-based, nonlinear, mathematical model of translation, followed by successive cycles of predictions and experimental validations using a combination of specific

and global quantitative approaches. Similar modi operandi were adopted to characterize the intrinsic properties of replication and transcription. Integrating knowledge from these three fundamental cellular processes allowed us to acquire genome-wide the quantitative parameters that characterize the native *B. subtilis* genetic sequences. We then rationally designed a library of synthetic constructs combining a selection of different genetic elements giving rise to any desired gene expression profile across growth conditions.

The intrinsic properties of these hard-wired regulatory layers of gene expression open novel avenues for synthetic biology, particularly in the potential it offers to bypass the need for multiple specific regulators to modulate complex synthetic circuits.

92. Engineering of a High Lipid Producing *Yarrowia lipolytica* Strain

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Microbial lipid production has received interest both for the synthesis of specialty lipids and for the production of fuels and chemicals from various feedstocks. Microbial lipids are produced by many oleaginous organisms including the well characterized yeast *Yarrowia lipolytica*. Lipid yield in oleaginous organisms can be increased through the up-regulation and/or down-regulation or deletion of genes involved in the lipid pathway. In this study, we describe a strain-engineering strategy applying combinatorial screening of overexpression and deletion genetic targets that led to the construction of a high lipid-producing yeast biocatalyst. The resulting strain NS432 that combines three genetic modifications selected for their effect on lipid production reached 76% fatty acid content and 0.21 g lipid per g glucose yield in batch glucose fermentation. In fed-batch glucose fermentation NS432 produced 92 g/L lipid at a productivity of 0.68 g.L-1.hr-1, which is 3.7 and 3.2-fold higher in titer and productivity, respectively, over previously reported *Y. lipolytica* engineered biocatalysts.

93. A Synthetic Developmental Circuit to Investigate the Effects of Adhesion

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The design and engineering of multicellular systems is an important step for the future of synthetic biology. A crucial hurdle to the engineering of such systems is the understanding of the self-organisation that shapes the organism, often involving a multitude of intercellular interactions. In order to begin to simplify and disentangle such interactions, we express a primitive synthetic developmental circuit in *E. coli* colonies to investigate cell-cell adhesion. Adhesion is an important developmental mechanism, and here we isolate and observe its effects in a well-defined context. We first characterize a method to segregate bacterial colonies into spatially distinct domains of gene expression. This is achieved by transforming the cells with two similar inducible copy number plasmids in the high copy number regime. The inducer is then removed so plasmid copy number falls whereby plasmid incompatibility ensures that each cell is eventually left with only one of the original two plasmids. The segregating plasmids also contain an inducible ag43 protein that causes adhesion between cells. By observing and measuring boundaries between domains with differing adhesive properties, we demonstrate the effects of adhesion on the boundary formation process. CellModeller software is used to model the cells and their physical interactions, providing insight into the mechanistic details.

94. Cell-Free Metabolic Engineering for Butanol Production

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Cell-free biological systems have emerged as one approach to decouple the cell's physiological and evolutionary objectives from the engineer's process objectives to enable high yielding biomolecular transformations. In this work, we used a crude cell lysate approach to reconstitute the 17-step Clostridium 1-butanol biosynthetic pathway in extracts of *Escherichia coli*. We prove the ability to mix 5 crude lysates each with selectively over-expressed enzymes to activate the entire 1-butanol production pathway *in vitro* with high productivities and yields. We additionally demonstrate the ability to quickly optimize these cell-free systems by modulating the physicochemical environment to improve yields and productivities. Furthermore, we establish for the first time the ability to combine cell-free protein synthesis of enzymes with cell-free metabolic engineering to activate the metabolic pathway towards 1-butanol. This work provides a proof-of-concept that not only can we perform chemical synthesis by utilizing cellular metabolism in crude lysates but also that we can construct biosynthetic pathways by using crude lysates for both catalyst synthesis and utilization in one pot. This opens the door for novel biosynthetic pathway prospecting and rapid prototyping of metabolism by cell-free synthetic biology.

95. 2-Quinoxalinecarboxylic Acid Production in *Escherichia coli*

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Quinoxaline, also known as benzopyrazine, is an important pharmaceutical and industrial compound. Despite their simple chemical structure, quinoxaline and quinoxaline derivatives display a myriad of useful therapeutic properties including antibacterial, antiviral, antiparasitic, and antitumor activities. They also possess unique electronic and luminescent properties and are used in the production of organic semiconductors, light emitting diodes, and solar panels. The most common method for large scale production of quinoxaline compounds is condensing benzene-1,2-diamine with 1,2-diketones, by heating for several hours in organic solvent. It is therefore desirable to develop a biological method for quinoxaline preparation that does not require use of solvents and does not produce toxic waste products. We are engineering *E. coli* to produce 2-quinoxalinecarboxylic acid. In particular, we are using the native Sec pathway of *E. coli* to export the biosynthesized 2-quinoxalinecarboxylic acid.

96. Self-Assembly through Toehold-Mediated Four-Way Branch Migration

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Self-assembly is a fundamental mechanism in the formation of complex and functional biological systems, and the ability of information-carrying DNA species to confer specific two- and three-dimensional shapes is a testament to the programmability and versatility of nucleic acids. This precise control of structure at the nanoscale could have profound implications for DNA probing and small molecule drug delivery. Here, we explore the possibility of utilizing toehold-mediated four-way branch migration to sequentially assemble complex structures, ranging from simple k-arm junctions to more intricate closed shapes.

97. Engineered Antibiotic Marker-Free Probiotic Dual Expression Chassis

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Probiotics present an attractive strategy for long-term delivery of therapeutic molecules *in vivo* as they may colonise as part of the human microbiota. Synthetic biology genetic circuits that facilitate the expression of these therapeutic molecules are usually stabilized in cells on plasmid by antibiotic resistance genes as selection markers. However, antibiotic resistance genes are not suitable for *in vivo* application as constant antibiotic selection pressure is required for plasmid retention. Moreover, the presence of antibiotic resistance genes introduces the risk of potential horizontal transfer of resistance to the native microbiome. In this study, we engineered an antibiotic marker-free dual expression system in a probiotic strain, Escsherichia coli Nissle. The essential genes involved in alanine and aspartate metabolisms were deleted from the bacterial genome to create a double auxotrophic *E. coli* strain. The auxotrophic phenotypes could be rescued by the balanced co-expression of the deleted genes on two separate plasmids to achieve plasmid selection and retention. To further enhance the dual plasmid system, a regulatory switch was implemented on the two co-expressed plasmids to ensure their stability. Our study demonstrates that the co-expressed plasmids could be maintained in the probiotic expression chassis without antibiotic selection. This dual expression system selected for auxotrophic complementation can be used for gastrointestinal delivery of therapeutic molecules *in vivo* with a high degree of biosafety.

98. Engineering *E. coli* Nissle to Degrade Toxic Compounds in Mouse Models of Metabolic Disease

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The complex communities of microbes that live within the human gastrointestinal (GI) tract greatly impact human health through metabolic processes, their interactions with each other, and their interactions with the immune system. When functioning in concert with their hosts, the gut microbiota can supply nutrients, degrade toxic compounds, and prevent overstimulation of the immune system. The gut microbiota, as a whole, is so active that its combined metabolic output can be thought of as an additional organ. By manipulating these processes through genetic engineering, we can generate microbes that supply missing metabolites and degrade specific toxic compounds in order to relieve inborn metabolic disorders and supplement organ deficiencies.

Urea cycle disorders (UCD) are a collection of genetic conditions with an estimated incidence of 1:8,500 births, in which the patient is unable to convert nitrogen to urea, resulting in the toxic accumulation of ammonia systemically. This hyperammonemic state can lead to CNS impairment, affecting neurological function, impacting normal development and in some cases, can be fatal. Importantly, as much as 70% of excess ammonia in hyperammonemic patients accumulates in the GI tract, supporting the use of a modified gut microorganism-based therapeutic. We have engineered Nissle, a probiotic strain of *E. coli*, to reduce the concentration of blood ammonia in those suffering from hyperammonemia caused by UCD. Nissle has been modified to respond to the gut environment and activate a carefully optimized ammonia consumption pathway. Through *in vivo* studies, we have shown that our engineered Nissle strain can sequester the ammonia produced via digestion, thus

preventing diffusion of ammonia into the blood. Lowering blood ammonia levels also decreased mouse mortality compared to controls. Our work demonstrates that engineered microbes can be introduced into the gut microbiota, sense their local environment in that compartment, and affect an intended response in order to treat metabolic disorders. This prototype provides a meaningful example of how the tools developed in synthetic biology can be translated into the clinic.

99. *In Vivo* Characterization of Stress-Responsive Promoters in the Normal and Inflamed Murine Gastrointestinal Tract

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Research in synthetic biology has led to the generation of various biological systems using specific promoter sequences for the activation of gene expression in response to particular environmental or chemical stimuli. Those systems will likely be key in the development of synthetic therapeutic organisms requiring a precise spatiotemporal control of their activation in patients suffering from the targeted medical syndromes. Thus far, however, all of the studies describing those biological systems have been performed *in vitro*, and additional information on their behavior in animal disease models is needed to confirm their utility for potential therapeutic applications. Using the well-known probiotic *E. coli* Nissle strain carrying promoter-fluorescent protein reporter constructs, we conducted the first comprehensive *in vivo* study of low-oxygen, oxidative stress and nitrosative stress-responsive promoters in the gastrointestinal tract of healthy mice and murine IBD (Inflammatory Bowel Disease) models. Our data are providing valuable insights into the kinetics, fine localization, and level of those promoters' activities along the mouse GI tract and how they differ between a normal and inflamed gut. In parallel, our studies are providing important information regarding the environmental stresses synthetic therapeutic organisms would face in the context of IBD, a knowledge that will play a critical role in engineering more efficient microbial therapeutic agents.

100. The Effect of Cellular Noise on MSC Cell Fate Decisions

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Variation in gene expression level, termed as cellular noise, is a ubiquitous characteristic among all cell populations. Despite this inherent stochasticity, cells such as mesenchymal stem cells (MSCs) still have the ability to undergo important cell fate decisions. To investigate the role of noise in cell fate decisions, we are examining the differentiation efficiency of MSCs in the presence of various amounts of exogenous noise. To accomplish this, we are building genetic circuits to control fluctuations of noise in MSCs and assessing the differentiation outcome based on this noise. We have found that cellular noise is an important mechanistic factor that influences a MSC differentiation potential. Further, these results suggest that cellular noise is a tool that can be used to control cell differentiation.

101. Engineering Artificial Mechanosensitive Cells By Combining Cell-Free Expression and Ultrathin Double Emulsion Template

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Assembling biological parts into a biologically functional system using a bottom-up *in vitro* reconstitution approach offers the possibility of designing artificial cells with the ability of sensing and responding to external stimuli. Artificial cells are defined as the encapsulation of biologically active material in a biological or synthetic membrane. Here, we describe a robust and general method to produce artificial cells for the purpose of mimicking one or more behaviors of a cell. A microfluidic double emulsion system is used to encapsulate a mammalian cell-free expression system that is able to express membrane proteins into the bilayer or soluble proteins inside the vesicles. The development of a robust platform that allows the assembly of artificial cells is valuable in understanding subcellular functions and emergent behaviors in a more cell-like environment as well as for creating novel signaling pathways to achieve specific cellular behaviors. As a test bed, we are engineering mechanosensitive vesicles to respond to the external physical environment. The platform development and inclusion of additional information processing components could potentially open up new applications in biosensing and medicine.

102. Large-Scale Determination of Causative Mutations for Enhanced Chemical Tolerance in Evolved Strains of *Escherichia coli*

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Overcoming product toxicity remains one of the largest barriers toward achieving economically viable cell factory processes. Even relatively non-toxic products impose signif-

icant stresses at the economically-relevant titers ultimately required for commodity chemical production, which are often in excess of 100 g/L. We have utilized a robotic platform to rapidly evolve multiple parallel populations of *E. coli* K-12 MG1655 for enhanced growth in the presence of toxic concentrations of 11 chemicals representing diverse functional classes that have significant interest as polymer precursors, biofuels, and bulk chemical intermediates. These include dicarboxylic acids, diamines, monocarboxylic acids, diols, and aromatic acids. Whole genome resequencing of over 200 strains isolated from nearly 90 independently evolved populations has enabled the determination of the genetic basis of tolerance to each chemical. The mutations in evolved strains were biased toward loss-of-function mutations, which were reconstructed in combinations and shown to be major drivers of tolerance in many cases. Additional combinations of probable gain-of-function mutations were reconstructed using oligonucleotide-mediated recombineering and a conjugation-based genome shuffling approach. Based on successful reconstructions of tolerance phenotypes, novel mechanisms of tolerance have been inferred, and numerous new gene targets conferring increased tolerance have been discovered. Cross-compound screening of all evolved isolates has also revealed specific sets of mutations that confer tolerance toward multiple classes of compounds. Ongoing work includes continuing to explore the mechanistic basis for tolerance and testing tolerant mutants for endogenous production capability.

103. Reconstructing a Plant Hemicellulose Biosynthetic Pathway in the Heterologous Fungal Host *Pichia pastoris*

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The plant cell wall is made of a variety of polysaccharides including the hemicellulose xyloglucan, which is composed of a backbone of β-1,4-linked glucan that is highly substituted with xylose as well as other monosaccharides. Xyloglucan has a wide range of applications including thickening, gelling, and stabilizing agents in the food industry, during weaving of textiles, and as adhesive and binding agents in the pharmaceutical industry.

In the last decade, the enzymes involved in the biosynthesis of the xyloglucan backbone and substitutions were identified. To provide a testing platform for candidate genes involved in xyloglucan biosynthesis and to investigate the activities of these genes independently from endogenous plant systems, we attempt to reconstruct the plant xyloglucan biosynthetic pathway in the heterologous fungal host *Pichia pastoris.* Our efforts resulted in the production of a glucan backbone in the yeast *Pichia* pastoris. Subsequent addition of a cytosolic UDP-glucose dehydrogenase as well as a UDP-glucuronic acid decarboxylase made the synthesis of the nucleotide sugar UDP-xylose possible, which is required as a donor substrate for backbone xylosylation. Finally, the addition of a UDP-xylose transporter to transfer UDP-xylose into the lumen of the golgi to the previously mentioned gene resulted in the production of a xylosylated glucan as determined by HPAEC-PAD, Q-TOF and GC-MS analysis.

104. Metabolic Engineering for the Production of Medium-Chain Fatty Acids and Derivatives in Baker's Yeast

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Medium-chain fatty acids (MCFAs, with carbon chain length of 6 to12) and their derivatives are valuable compounds which can be used as food additives, anti-pathogenic agents, nutraceuticals, and drop-in fuels. Thus, they are very attractive target compounds for microbial bioproduction. In this study, we aimed to metabolically engineer baker's yeast to produce MCFAs and their derivatives. We enhanced the metabolic flux towards MCFA production by using combinatorial metabolic engineering approaches, including competitive pathway engineering, heterologous pathway reconstitution, and cofactor supply engineering. Blocking of competitive pathways to MCFA biosynthesis by multi-gene deletion led to significant accumulation of C8-C12 fatty acids as well as long-chain fatty acids. Further enhancement in MCFA production was achieved by introduction of a mammalian MCFA biosynthesis pathway into the MCFA-accumulating mutant. Subsequently, we demonstrated the conversion of MCFAs into valuable compounds by reconstituting and functionally characterizing a dietary lipid biosynthesis pathway in the MCFA-producing strain. Our findings could shed new light on metabolic engineering for developing microorganisms for the production of valuable fatty acids and derivatives. Additionally, the obtained MCFA-producing yeast strains could be applied to produce other value-added compounds.

105. Engineering Acyl Carrier Protein to Enhance Production of Shortened Fatty Acids

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We engineered the specificity of the acyl-carrier protein (ACP), an essential enzyme of fatty acid metabolism, to alter the *E. coli* lipid pool and enhance production of medium-chain fatty acids as biofuels precursors. A homology model of the *S. elongatus* ACP was constructed showing a hydrophobic pocket harboring the growing acyl chain. Amino acids within the pocket were mutated to increase steric hindrance to the acyl-chain, and gas chromatography-mass spectrometry analysis performed on cellular extracts identified mutants that, when over-expressed in *E. coli*, increased the proportion of shorter-chain lipids; I75W and I75Y showed the strongest effects. We further demonstrated increased production of lauric acid in *E. coli* also expressing the C12-specific acyl-ACP thioesterase from *Cuphea palustris*.

106. Engineering Auto-Regulatory Genetic Circuits That Enable Modular and Programmable Functionalities for Biotechnological Applications

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Microbes can be reprogrammed to demonstrate biotechnologically important functionalities such as biochemicals production. However, reprogramming microbes to perform complex and coordinated tasks often requires the use of additional exogenous chemicals such inducers, which can increase production costs, introduce host toxicity and incompatibilities with industrial scale-up. To overcome this challenge, we developed auto-regulatory genetic circuits by introducing novel sensors and feedback loops into microbial systems. Not only our engineered systems are independent of costly inducers, they are capable of regulating protein expression based on desired features such as substrate concentration and cell-density. One example is the use of our genetic circuit in auto-regulatory lignocellulosic bioconversion. In this system, the genetic circuit was engineered to regulate enzyme expression based on lignocellulose substrate cues and cell-density. In another example, we used our cell density-dependent circuit to facilitate product extraction in *Escherichia coli*. Macromolecular product extraction in *E. coli* often requires chemical, enzymatic, and/or mechanical lysis, thus increasing production costs. To aid in the extraction of biochemical products and simplify the product harvest process, we developed a genetic circuit that enabled cell density-dependent auto-regulatory lysis based on a transducer-switch scheme. As a proof of concept, we demonstrated the applicability of the lysis circuit to biotechnological applications by employing plasmid DNA extraction as a test bed. We envision that our engineered auto-regulatory circuits that enable programmable functionalities can be readily applied to a range of biotechnological applications.

107. Use of Pacific Biosciences Single Molecule Sequencing to Assay DNA Ligase Fidelity

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DNA ligases, key enzymes in molecular biology and synthetic biology, catalyze the joining of single strand breaks in duplex DNA (nicks) as well as the joining of multiple dsDNA fragments. Ligation fidelity has important consequences for the yield, accurate assembly, and maximum number of fragments that can be assembled concurrently in synthetic biology applications such as Golden Gate cloning. In this study, we have applied Pacific Biosciences single-molecule sequencing technology to develop a new method to characterize the fidelity of ligation when joining DNA fragments with short (three or four base) 5'-overhangs. Pools of substrates containing all possible overhang sequences were ligated with T4 DNA ligase, and the ligation products were read directly by sequencing individual molecules. This work has allowed us to determine the overall mismatch ligation frequency, the mismatch frequency for each individual subsequence, and to characterize the particular mismatches that have a high propensity for ligation. This methodology is easily adaptable to other dsDNA substrate structures, and provides a method to screen for ligases or buffer conditions that provide higher fidelity ligation reaction outcomes. The information generated by this study will allow the optimization of DNA fragment assembly protocols, both by the development of high fidelity ligases and by the avoidance of non-complementary overhang pairs prone to misligation.

108. Modular Design of Transcriptional Regulatory Parts by Insulating Operators from Their Cognate Promoters

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Predictably assembling genetic circuits requires a diversity of compositional DNA parts encoding defined functions. However, when assembling the parts, their defined functions are fragile, and usually interfered by other parts and their host cells. Several strategies have been developed to insulate some specific parts from their genetic context (e.g. RBS). Here, we describe a modular architecture to insulate the operators from their cognate promoters (recognized by T7 RNAP or sigma ECF11) in *E. coli*. In the modular architecture, the regulatory functions of different operators were described by a novel mathematical framework; meanwhile promoter's activities were independent of the operators. More than 1000 transcriptional regulatory parts could be combinatorially constructed from a promoter and operator library, and their regulatory functions are easily predicted by

a simple model without any fitting parameter. The versatile modular parts library is powerful toolbox to facilitate the construction of sophisticated genetic circuits.

109. Massively Parallel Combinatorial Genetics to Probe and Treat Human Diseases

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Combinatorial gene sets are important in coordinately regulating complex biological phenotypes and human diseases. For example, multiple biological factors are needed to differentiate stem cells into distinct cell types and to reset somatic cells into induced pluripotent stem cells. Targeting multiple pathways via combinatorial drug therapies can yield enhanced efficacy over standard monotherapies. Moreover, even though genome-wide association studies have identified multiple individual loci involved in multifactorial phenotypes, such loci can only account for a minor fraction of disease heritability on their own. Genetic interactions may be significant in explaining this missing heritability but existing strategies for systematically mapping the functions of high-order gene combinations are limited in the order of genetic complexity that can be studied and the throughput and scale that can be achieved.

To overcome these bottlenecks and enable massively parallel characterization of genetic combinations, we created a broadly applicable technology for the rapid and scalable assembly of high-order barcoded combinatorial genetic libraries that can be readily quantified in pooled screens with next-generation sequencing. We have applied this platform, Combinatorial Genetics *En Masse* (CombiGEM), to bacterial and human systems to discover novel combinatorial gene sets that underlie important diseases.

For example, new therapies are urgently needed to drug-resistant bacterial infections, which constitute a significant growing threat to global health. Thus, we sought to identify novel combinatorial genetic perturbations that were effective against drug-resistant bacteria. Using CombiGEM, we built ~34,000 overexpression constructs containing nearly all pairwise combinations of *Escherichia coli* transcription factors (TFs). By leveraging high-throughput pooled screens together with Illumina sequencing, we discovered diverse genetic combinations that modulated antibiotic-resistance phenotypes in carbapenem-resistant Enterobacteriaceae (CRE). Specifically, we found multiple TF pairs that enhanced antibiotic killing by up to 1,000,000-fold. By delivering these specific genetic combinations into target bacteria via phagemids and co-treating with antibiotics, we significantly increased the killing of highly drug-resistant *E. coli* harboring New Delhi metallo-betalactamase-1 (NDM-1). Furthermore, we assembled libraries of three-wise TF combinations with over four million unique members and validated that these could be quantified via high-throughput sequencing, thus demonstrating the scalability of CombiGEM.

Furthermore, we have established CombiGEM for high-throughput combinatorial screening in human cells. For example, we constructed high-coverage barcoded libraries containing 1,521 two-wise and 51,770 three-wise combinations of 39 human microRNA (miRNA) precursors. We carried out systematic screens with these libraries to discover combinatorial miRNA effectors that sensitized drug-resistant ovarian cancer cells to chemotherapy and/or inhibited cancer cell proliferation. Highly ranked hits from these pooled screens were validated for their ability to suppress drug resistance and block cancer cell growth. Thus, this effort uncovered novel insights into miRNA networks underlying drug-resistance and cell-proliferation phenotypes in cancer. Furthermore, this work identified new miRNA combinations that may be useful as effective anti-cancer therapeutics.

In summary, our work establishes a powerful technology for massively parallel combinatorial genetics using the tools of synthetic biology. We envision that CombiGEM will be useful for the high-throughput profiling of multifactorial genetic combinations that regulate a myriad of phenotypes relevant to biomedical, biotechnology, and basic science applications.

110. Evaluation of TALE- and Crispr/Cas9-Based Synthetic Transcription Factors for Heterologous Gene Expression in *Saccharomyces cerevisiae*

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Metabolic engineering in yeast, with the goal to introduce new biochemical pathways, calls for highly specific and orthogonal regulation of heterologous gene expression. Endogenous transcription factors and promoters are not suitable for this purpose, as they would strongly affect gene expression of the host. Synthetic transcription factors (synTFs) with programmable DNA-binding specificity provide one way to overcome this challenge and to ultimately construct complete gene regulatory networks. We aim to use TALE- and CRISPR/Cas9-based transcription factors in combination with synthetic promoters to control expression of a multitude of genes on circular expression yeast artificial chromosomes, which we call xYACs. Currently, we are characterizing different synTFs using a combination of flow cytometry based reporter gene assays, growth assays and host gene expression analysis. Our approach will finally yield a library of synTFs and corresponding synthetic promoters covering a wide range of expression strength and enable us to specifically modify expression levels of individual genes. The use of synTFs and their careful characterization will reduce negative effects on the host´s gene

expression and overall growth fitness. In combination with a method allowing the modular assembly of xYACs and a blue and red light-sensitive induction system, our toolbox will allow easy and fast construction of new pathways in yeast.

111. Enabling Rapid Protein and Circuit Engineering with High-Throughput Microfluidic Devices

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We developed several methods and microfluidic approaches to enable the rapid engineering and quantitative characterization of proteins and genetic circuits *in vitro*. Protein engineering remains challenging, primarily due to the cumbersome experimental processes currently required for expressing, purifying and quantitatively characterizing proteins. We developed a novel solid-phase gene synthesis approach allowing the combinatoric assembly of protein variants. We directly coupled our gene synthesis method with high-throughput on-chip protein expression and characterization, allowing us to drastically reduce the time required for a complete design-test cycle. We applied our novel protein-engineering platform to the generation of hundreds of Zn-finger transcription factors and the precise quantitation of the binding specificity of each variant.

In order to streamline genetic network engineering we have recently demonstrated that genetic networks can be implemented and characterized *in vitro* using a microfluidic chemostat. This chemostat approach allows complex networks such as oscillators to be run *in vitro*. We have previously shown that several biologically relevant regulatory mechanisms can be implemented on this *in vitro* platform and we built and characterized the first *in vitro* genetic oscillator. Over the last year we focused on testing whether we can functionally implement genetic networks that are known to work *in vivo* in order to assess whether genetic networks can be designed or modified *in vitro* and consequently transferred *in vivo*. We have successfully implemented the Elowitz repressilator *in vitro* and performed a comprehensive analysis of the system that would be difficult to achieve *in vivo*. We additionally designed and implemented novel 3 node oscillators, and the first 5 node oscillators *in vitro* and we will present data on how these systems behave *in vivo*.

112. Produce Steviol Glycosides in Engineered Yeast

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Steviol glycosides are natural products isolated from *Stevia rebaudiana* leaves, and are widely used as high intensity, low-calorie sweeteners. Naturally occurring steviol glycosides have the same base structure (steviol) and differ in the content of carbohydrate residues (e.g. glucose, rhamnose, and xylose residues) at the C13 and C19 positions of the diterpene steviol. *Stevia* extracts are commercially available, which typically contain stevioside (ST) and rebaudioside A (Reb A) as the primary compounds. Although these two compounds are much sweeter than sucrose on per unit base, they tend to have strong off-flavor taste, limiting their applications in foods and beverages. Other steviol glycosides, which demonstrate many desirable taste properties, typically are present in *Stevia* extracts at much lower concentrations.

The majority of the steviol glycosides are formed by several glycosylation reactions of steviol, which typically are catalyzed by the UDP-glycosyltransferases (UGTs) using uridine 5'-diphosphoglucose (UDP-glucose) as a donor of the sugar moiety. In plants, UGTs are a very divergent group of enzymes that transfer a glucose residue from UDP-glucose to steviol. In order to identify the key UGTs in the biosynthesis pathway of steviol glycosides, bioinformatics analysis was performed and selected UGT candidate genes were enzymatically characterized. This work identified several UGTs that demonstrated 1, 3-13-O-glucose, 1, 3-19-O-glucose, 1, 2-13-O-glucose, 1, 2-19-O-glucose and/or 1, 6-13-O-glucose glycosylation activity to produce 11 steviol glycosides including 6 known steviol glycosides (rebaudioside G, rebaudioside KA, rebaudioside A, rebaudioside E, rebaudioside D and rebaudioside M) and 5 novel steviol glycosides. Furthermore, we can produce these 11 steviol glycosides in large scale by using engineered UGT proteins and recombinant yeast cells. This recombinant yeast system has produced yields as high as 93% while providing a novel approach to successfully produce these desirable minor steviol glycosides at an industrially relevant scale.

113. Engineered Probiotics That Detect and Kill the Human Pathogen *Vibrio cholerae*

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New synthetic biology techniques have enabled us to engineer the human microbiota for therapeutic purposes. In this project, we have engineered the probiotic lactic acid bacteria *Lactococcus lactis* to detect *Vibrio cholerae*, the causative agent of the infectious disease cholera. We used the CqsS sensor domain from the *V. cholerae* CAI-1 quorum-sensing system to create a hybrid two component system in *L. lactis* that can directly detect the *V. cholerae* CAI-1 signal. Incorporation of a TetR-based signal inverter into the detection circuit allowed us to couple CAI-1 recognition to activation of the output module, where a combination of several antimicrobial peptides will be used to specifically kill *V. cholerae*. We have tested the circuit *in vitro* and in

co-culture conditions and will carry out *in vivo* tests in a *V. cholerae* mouse infection model in the near future. This novel design uses the pathogen's own sensing system to detect and kill it.

114. Genome Engineering of Genomically Recoded *Escherichia coli* Enhances Site-Specific Incorporation of Non-Standard Amino Acids in Cell-Free Protein Synthesis

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Site-specific incorporation of nonstandard amino acids (NSAAs) into proteins and biopolymers by amber suppression makes possible new chemical properties, new structures, and new functions. However, competition between release-factor 1 (RF1) and orthogonal tRNAs have limited the technology. Here, we describe the development of a high yielding cell-free protein synthesis (CFPS) platform from crude extracts of genomically recoded *Escherichia coli* lacking release factor 1. Because this recoded strain has not been previously optimized for CFPS, we exploited multiplex automated genome engineering (MAGE) to design and construct synthetic genomes that, upon cell lysis, lead to improved extract performance. To stabilize template DNA, messenger RNA, amino acids, and protein products in CFPS, we targeted the deletion of 15 potential negative effectors. More than 50 strains were generated and tested in CFPS, allowing us to catalogue the systems impact by making numerous gene deletions both individually and in combinations. The protein synthesis activities of our most productive cell extracts were more than four-fold greater as compared to the extract from the parent strain, achieving more than 1.6 mg/ml of superfolder green fluorescent protein (sf*GFP*). We also show high efficiencies for producing modified sf*GFP* containing p-acetylphenylalanine at single and multiple positions. Our work has implications for using whole genome editing for CFPS strain development, expanding the chemistry of biological systems, and cell-free synthetic biology.

115. Development and Characterization of a Nucleoside Kinase That Accepts Unnatural Deoxyribonucleoside P, a Part of an Expanded DNA

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One of the more ambitious long-term goals of synthetic biology seeks to construct living cells that accept and use biopolymers different from DNA, RNA, and proteins. For example, the Romesberg group at The Scripps Research Institute reported last year that a strain of *Escherichia coli* could maintain an unnatural nucleotide pair whose structure greatly deviates from the standard Watson-Crick geometry. Since the Scripps group could not obtain kinases to convert fed nucleosides intracellularly to the triphosphates needed for DNA replication, they instead altered the *E. coli* to have transport systems that transported their nucleoside triphosphate analogs from the growth medium into the cell directly.

Our approach is different. We seek to construct a host cell that creates the triphosphates of components of an unnatural genetic system inside of the cell. This would allow the relatively inexpensive (and much more stable) nucleosides to be added to the growth medium, where we have now shown that natural nucleoside transporters bring them inside of the cells. This strategy requires (a) that the structure of the added nucleotides not differ too greatly from the structures of the standard nucleotides, so that (b) we can engineer standard nucleoside and nucleotide kinases to accept them in a synthetic metabolic pathway that makes the triphosphates intracellularly.

Over the past five years, we have developed a set of alternative nucleotides that create a 12 letter artificially expanded genetic information system (AEGIS). AEGIS pairs are joined by alternative patterns of hydrogen bond donor and acceptor groups. In my work, I have examined one of these AEGIS components, 2-amino-8-(1'-β-D-2'-deoxyribofuranosyl)-imidazo[1,2-a]-1,3,5-triazin-4(8H)-one, trivially called P. DNA polymerases, RNA polymerases, and reverse transcriptases have all been developed to copy and transcribe DNA and RNA containing P and its pair, 6-amino-5-nitro-3-(1'-β-D-2'-deoxyribofuranosyl)-2(1H)-pyridone (trivially called Z).

A nucleoside kinase from *Drosophila melanogaster* (DmdNK) was engineered to accept unnatural nucleoside dP to produce unnatural nucleoside monophosphates dPMP. This is the first phosphorylation step to synthesize the corresponding nucleoside triphosphate dPTP, a substrate for DNA polymerases.

In this study, the activity of DmdNK Q81E mutant was measured in a three-step coupled-enzyme assay [1] and its kinetic parameters were calculated. These results showed that the Q81E mutation allowed the engineered kinase to accept dP as a substrate. The Michaelis constant (K_M), the turnover rate (k_{cat}), and specificity constant (k_{cat}/K_M) for dP were similar to those for standard nucleosides dA, dT, dC.

To formally complete the construction of an artificial metabolism *in vitro*, I am also developing nucleoside mono-

phosphate kinases (NMPK) to phosphorylate dPMP to give dPDP. I will describe work adding these to nucleoside diphosphate kinase (NDPK), which I have already shown phosphorylate dPDP to give dPTP, the third phosphorylation step to synthesize triphosphate substrates for DNA polymerases. Once transferred into living cells, these enzymes should allow the formation of dPTP *in vivo*, and represent an example of a standard problem-solution in synthetic biology.

Reference

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116. Real-Time Optogenetic Control of Intracellular Protein Concentration in Microbial Cell Cultures

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Studies of signaling and transcriptional networks are limited by the tools currently available to systematically perturb and interrogate such networks. Standard genetic tools such as deletion, overexpression, and mutation are effective for identifying network components but less suitable for understanding kinetics and identifying feedback interactions. Here we describe the development of a system for real-time light-inducible control of gene expression in *S. cerevisiae*. Controlling transcription enables fine-tuned temporal modulation of protein levels useful for interrogating genetic networks.

Light-induction is achieved through fusion of the *Arabidopsis thaliana* proteins cryptochrome 2 (CRY2) and its interaction partner (CIB1) to appropriate DNA-binding and activation domains (1). CRY2 and CIB1 naturally dimerize on blue-light exposure, bringing the DNA-binding domain and the activation domain together to drive expression of the desired gene. We characterize the kinetics, off-target effects, and dose-response of this induction system and develop improved fusion proteins to allow for specific and controllable induction.

To implement real-time control we developed an integrated robotics system. Cells from a steady-state chemostat culture are continuously imaged for fluorescence. Information from this imaging is used to adjust the inducing light levels to vary the concentration of the fluorescently-tagged protein. By controlling the light-intensity, pulse frequency, and pulse duration we are able to maintain a steady-state protein concentration of our choosing. By modulating the inducing light we can produce dynamic protein level perturbations such as pulses and oscillations. This tool allows us to flexibly perturb protein levels to interrogate the kinetics and structure of genetic networks.

[1] Kennedy, MJ *et al* 2010 Nature Methods 7: 973-975

117. Serine Integrase Recombinational Assembly (SIRA) for Rapid Optimization of Metabolic Pathways

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A major target in Synthetic Biology is to build microbial cell factories for greener and more economical production of chemicals of therapeutic and industrial importance. Building these cell factories requires genetic engineering of metabolic pathways to produce high yields of compounds for large-scale production. However, engineered metabolic pathways require considerable optimization to limit interference with existing metabolic networks, diversion of cellular resources, and accumulation of toxic intermediates which compromise strain performance. DNA assembly is one of the biggest rate-limiting steps in metabolic pathway optimization. Serine Integrase Recombinational Assembly (SIRA) is a site-specific recombination-based DNA assembly method that uses a one-pot reaction for rapid multi-part assembly1. With SIRA technology, genes and regulatory sequences in the form of linear DNA parts integrate into a landing site on target DNA. The assembly process can easily construct gene assemblies using rational or combinatorial approaches, include multiple gene variants at all positions, and facilitate varied gene expression levels by incorporating degenerate ribosome binding sites. Using the carotenoid and violacein biosynthetic pathways as model systems, we have shown assembly and optimization of two fully functional pathways in just two days. Once DNA parts are assembled, SIRA allows targeted addition, deletion, and replacement of genes and DNA elements. This permits much easier optimization of complex genetic systems than was previously possible. This method is currently being applied to enhancing production of therapeutically and industrially important isoprenoids in *E. coli*.

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118. Precision Expression Control in *Bacteroides thetaiotaomicron*, a Human Commensal Bacterium

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The human microbiota plays an integral role in diverse human physiological processes including metabolism, development and immunity. Manipulation of the native microflora

using genetically engineered microbes is a promising avenue for cellular therapies and diagnostics. However, dominant members of the microbiota are difficult to robustly and reliably engineer. Here, we describe tools for engineering *Bacteroides thetaiotaomicron*, a relatively abundant species of bacteria that resides in the human gastrointestinal tract. We identify a library of constitutive promoters and ribosome binding sites that span a 10,000-fold range in expression. This library is used to construct a recombinase-based memory gate that stores permanent memory on the *B. thetaiotomicron* chromosome. Additionally, we describe a series of fully orthogonal, sugar-inducible promoters able to conditionally elicit up to a 100-fold difference in gene expression. Finally, we demonstrate programmable knockdown of gene expression using CRISPRi targeted to both synthetic constructs and endogenous genes implicated in antimicrobial peptide resistance and carbohydrate metabolism. Together, these biological parts serve as a fundamental resource for the study and development of engineered commensal organisms that can positively impact human health.

119. Towards Construction of Programmable Cells to Prevent Infectious Diseases

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Signal sensing is critical in bacteria that live in fluctuating environments. However, even a group of isogenic bacteria, subject to identical culture conditions, often exhibit heterogeneity in phenotypes. This multi-stability can be explained by stochastic transitioning among multiple stable states of gene expression which are often the results of positive feedback loops in gene regulatory networks. Although such a "bet-hedging" strategy can increase bacterial fitness in unpredictable environments, such stochastic processes can prevent synthetic biologists from predictably engineering microbial cells that need to face uncontrolled environments. Thus, to fully exploit microbes in synthetic biology applications, complex gene regulatory systems need to be better understood. To this end, we propose to construct "robust" genetic sensors and circuits that endow bacteria with the ability to sense environmental signals (e.g., temperature, light, pH, and oxygen level), perform computational operations, and implement actuations (i.e., generation of toxins to kill parasite eggs).

By building sensors and circuits from the bottom-up, we have gained design principles for construction of programmable cells. Specifically, we have built robust logic gates that contain modular sensors for temperature or oxygen levels that can be more meaningful signals in the environment than chemical inducers that are used in the lab. Furthermore, we have constructed bistable switches that can generate noise-tolerant responses to fluctuating environmental inputs. These switches are based on the sequestering interaction between a transcription factor (an activator) and an anti-activator along with multiple positive feedback loops. Importantly, these switches are functional over a wide range of parameters and endow the bacteria with a long-lasting memory (~weeks). We are assembling these modular sensors, logic gates, and memory devices to construct robust programmable probiotic strains that can reproduce in the intestine where parasite eggs are produced, and come out of the body with the parasite eggs and kill them. We will present progress towards development of such engineered microbes, which can be programmed to kill parasite eggs only when user-defined conditions are met.

120. The JBEI Experiment Data Depot

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Synthetic Biology research draws on several disciplines and ties together diverse data sets. Collaboration efforts between researchers is often hampered by inaccessible or difficult-to-access data. Results are scattered across instruments, document folders, and email accounts. Discovering whom to ask for a copy of the needed data can be a frustrating and time-consuming process. Deciphering data in varied and exotic spreadsheet layouts and file formats can also add delays.

The Experiment Data Depot (EDD) is a database and associated set of software tools meant to ease the management and organization of "actionable" experimental data sets -- meaning data that can be fed directly into computational models and other analysis tools. The aim of the EDD is explicitly not to be a generic sample-tracking or laboratory information management system (LIMS). Rather, it is designed to be a repository of data in a Design-Build-Test-Learn workflow, focused on capturing information resulting from primarily the Test phase. Data is organized into Studies, with read/write access controls for supervisors, colleagues, and collaborators. Each Study collects metadata on the experiment setup and measurement protocols used, and links to strain records in the JBEI Inventory of Composable Elements (ICE). Data import from lab instruments - such as mass spectrometers and gene sequencers - is facilitated with custom extensions. The ability to inspect data and metadata is integrated into the user interface,

along with exports to SBML, Excel, and CSV files for use in other software packages.

121. Programmable Control of Acetate Metabolism *Escherichia coli*

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Acetate production in *Escherichia coli* fermentations reduces the total yield and quality of recombinant protein products. Acetate production occurs due to metabolic overflow in the presence of excess glucose, is increased when oxygen levels are low, and is facilitated by the phosphate acetyltransferase (pta) gene. We have engineered a genetic program that measures levels of glucose, oxygen, and acetate in the cell, combines the information into two Boolean AND gates, and produces an output that reduces acetate accumulation in batch cultures of *E. coli*. The output is composed of three modules, each regulating the expression of pta at different levels of the central dogma. A CRISPRi guide RNA module reduces the transcription of the gene, an interfering RNA that reduces translation, and a LON protease that degrades the enzyme. Together, these outputs enable real-time control of pta expression in response to conditions that lead to acetate formation in *E. coli*.

122. Modified Transposons for Enhancing Protein Function

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Multi-domain proteins are a common and essential component of the cellular proteome and contribute complex functions that single domains cannot achieve. The field of protein engineering uses sophisticated techniques to modify proteins for a given purpose, but generally focuses on single domains without consideration of the protein's overall topology. In this work, we set out to develop tools that enable the high-throughput sampling of different domain connections. A novel method of random transposon-mediated domain insertion was devised which efficiently created large plasmid libraries of domain fusions. As a proof of concept, we made fluorescent biosensors for small molecules by inserting circularly permuted *GFP* into ligand-binding proteins. We also demonstrated the generality of this method by constructing a pool of functional dCas9 variants with insertions of a PDZ protein-protein interaction domain. These successful protein-engineering efforts illustrate the advanced functions possible with multi-domain constructs and how this transposon-based insertion method can facilitate the creation and study of this new class of synthetic proteins.

123. Mining for Novel Regulatory Noncoding RNA in Bacteria

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Produced during futile attempts by the RNA polymerases to clear the promoter region and transition to transcriptional elongation phase, the synthesis of "abortive" RNAs have been known for decades in all kingdoms of life. However, until the recent identification of abortive RNA in the bacteriophage T7 with transcriptional antitermination function, they had been considered only as "junk" RNAs. Functional abortive RNAs have not, however, been identified to exist endogenously in more complex biological systems such as bacteria. In this work, we describe an *in silico* predictive approach to identify possible new regulatory noncoding RNAs in bacteria and evaluate whether these abortive RNAs might be able to serve biological functions in *E. coli*. Abortive RNAs generated from 3,780 transcriptional units (obtained from RegulonDB) were used as query sequences within their respective transcription units to search for possible binding sites. Sites that fell within known regulatory features, based upon data obtained from RegulonDB and WebGeSTerDB, were then ranked based upon the standard free energy of annealing (reported by UNAfold) of the transcript to the binding site. Matches with a sufficiently high standard free energy of annealing were then explored further as likely sites for abortive RNA-mediated regulation. We will showcase one such result – the uncovering of a potentially novel abortive RNA-mediated regulation of an alcohol dehydrogenase in *E. coli*. We also discuss the potential application of using such functional abortive RNAs in a design-based approach to regulating gene expression in synthetic systems. Future experimental validation of this and other loci may reveal the pervasive physiological role of this new class of noncoding RNAs in *E. coli*.

124. Geneart® Strings™ DNA Libraries – the Fast and Economic Way of DNA Library Production

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Thermo Fisher Scientific offers an extensive portfolio around de novo gene synthesis which consistently keeps up-to-date with the latest demands and applications. Particularly, our Directed Evolution products deliver top-of-the-line quality, showcasing our technology leadership in the market. Current processes for DNA library production in which diversity is introduced into a sequence verified template are time and labor intensive and thus make degenerated libraries products at the upper end of the customer value continuum. Here we report the development of GeneArt®

Strings™ DNA Libraries, a low cost library product that will allow our customers to obtain libraries much faster and at significantly reduced cost.

By combining our longstanding expertise in the production of GeneArt® Combinatorial Libraries with our very successful low cost gene synthesis product GeneArt® Strings™ DNA Fragments, we are able to offer an economical directed evolution solution to price sensitive academic and industrial customers alike: GeneArt® Strings™ DNA Libraries allow for the introduction of all IUPAC defined ambiguous nucleotides into the customer sequence while providing the advantages of GeneArt® Strings™ DNA Fragments of short production time, low price, flexible sequence design and full gene optimization options.

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125. Structure and Assembly of the Cyanobacterial Carboxysome Matrix

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Intracellular microcompartments are potentially useful tools for metabolic regulation and optimization. The cyanobacterial β-carboxysome is a natural microcompartment used to concentrate bicarbonate and carbon dioxide, while excluding oxygen, in the vicinity of the carbon fixing enzymes ribulose-1,5-bisphosphate carboxylase (RuBisCO) and carbonic anhydrase (CA). Our lab has previously shown that in *Synechococcus elongatus* β-carboxysomes assemble in an inside-to-outside manner, with RuBisCo aggregations budding off existing carboxysomes prior to encapsulation by an outer protein shell. Based on previous observations and known protein structures, it is hypothesized that this initial protein aggregation (termed the carboxysome matrix) has an ordered lattice-like structure and is a key aspect of β-carboxysome assembly, guiding both the final size and number of carboxysomes within the cell. Here we describe our current efforts to elucidate the spatial and temporal pattern of internal carboxysome matrix assembly using super resolution microscopy (SIM) and *in vitro* techniques. To this end we have established a β-cyanobacterial line expressing a fluorescently tagged copy of the inner matrix structural protein CcmM for *in vivo* imaging alongside fluorescently tagged RuBisCo and carboxysome shell proteins. We have also purified the short isoform of CcmM (M35), RuBisCo small and large subunits, and shell components for characterization with electron microscopy. We hope that by developing an improved picture of carboxysome self-assembly using these techniques, we will provide a useful foundation for producing synthetic protein-based microcompartments and engineering improved photosynthetic efficiency in plants and cyanobacteria.

126. A Rule-Based Library for the Verification and Modification of Synthetic DNA Sequences

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Many synthetic biology research groups design complex DNA sequences *in silico* according to biological discoveries and rules, but order the physical sequence from DNA synthesis vendors. Before the physical synthesis process starts, at latest, the designed sequences must be verified against rules of DNA synthesis methods and technologies. If a sequence violates a rule, then corrective actions are performed manually that depend on the type of rule and the designers' knowledge. Moreover, manual sequence modifications are hard to reenact, error-prone, time-consuming, and circumvent fully automated workflows.

Various algorithms have been developed to verify sequences against rules *in silico*. Biological advances have shown that rules can be mainly categorized into two types: repeats and GC content. Both types can occur in the entire sequence (i.e. globally) or in specific regions (i.e. locally). Direct repeats denote k-mers (e.g. polymers, dimers, trimers) and inverted repeats indicate potential undesired secondary structures (e.g. hairpins). Here, we demonstrate the Sequence Polishing Library (SPL) and its integration into an automated workflow at the DOE Joint Genome Institute. Currently, the SPL imports sequence information from GenBank, FASTA, FASTQ, or SBOL file formats. Besides finding only exact matches of sequence repeats, the SPL can find repeats to a configurable degree of similarity. Therefore, SPL programmatically integrates the BBMap bioinformatics library (http://sourceforge.net/projects/bbmap).

For the specification of rules, we develop a yet simple but expressive language, which also supports the specification of corrective actions. The SPL differentiates between human, recommended, and automated corrective actions. Per default, human corrective actions must be performed since which action to perform depends on where and what type of violation occurs. In order to modify sequences in an automated fashion, information is needed about the sequences' structure. For example, if a coding sequence contains repeats, then organism-specific codon tables can be consulted to modify the DNA sequence. If a promoter violates a rule, then the SPL can recommend alternative promoters to the designer. Such structural information can be specified, for example, using the GenBank or SBOL format.

The SPL can be invoked programmatically via an Application Programming Interface (API) and utilized manually via a web-based User Interface (UI). Both interfaces provide expressive feedback about rule violations and corrective actions. Therefore, we believe that synthetic biology researchers as well as DNA synthesis vendors can benefit from utilizing the SPL, contributing to the automation of internal and cross-organizational workflows.

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127. A 3-D Printed Hardware Platform for Quantitatively Characterizing the Performance Features of Diverse Optogenetic Transcriptional Regulatory Systems in *E. coli* and *S. cerevisae*

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There has recently been rapid development of optogenetic tools for optically regulating gene expression in microbes. These tools exhibit diverse spectral responses, light sensitivities, photoconversion and dark reversion rates, and chromophore requirements, which significantly impact their suitability for different experiments. Literature data on these performance characteristics is limited as different studies have performed varied measurements, resulting in only partial assessments. Furthermore, data are of variable quality and are reported in different arbitrary units. Altogether, this makes direct comparisons of the performance of these tools very difficult.

Here, we address this problem by developing a framework to quantitatively assess these and other performance metrics using a custom hardware device termed the LED Plate Apparatus (LPA). The LPA is a 3D-printed device incorporating a programmable array of through-hole LEDs which holds a 24-well microplate, providing an isolated light environment for each well. The two LEDs per well are reconfigurable, enabling them to be easily swapped for LEDs of any desired wavelength. The LPA can be programmed with complex light sequences using the Light Program Interface (LPI), a custom webtool that produces files which are loaded onto the LPA using a SD card. We are using the LPA and LPI to perform characterization experiments on many previously published but incompletely-characterized light-inducible systems, such as pDusk and BphS/MrkH in *E. coli* and PhyB/PIF6 and Cry/CIB in S. cerevisae. Our data include the action spectra (both activating and deactivating), steady-state light dose response, and step-function response dynamics. Using these characterization datasets, we are producing datasheets on the performance metrics of the current generation of *E. coli* and *S. cerevisae* optogenetic tools.

Furthermore, we are using these data to produce predictive mathematical models of the light-to-gene-expression signaling of each system that will enable a) easy selection of appropriate fluorescent protein reporters to combine each tool with and b) the design of multi-LED light input signals which can generate desired gene expression signals *in vivo*, even when programming multiple systems simultaneously. Our approach should be readily compatible with optogenetic proteolysis systems and mammalian cells. This work dramatically lowers the "barrier to entry" for scientists and engineers wishing to perform optogenetic experiments in their lab by opening up access to an easy-to-use standardized suite of hardware, software, and wetware tools.

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128. Automated Statistical Design of Experiments for Metabolic Engineering

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The growing field of metabolic engineering for natural product production typically involves transplant of a heterologous pathway comprising multiple genes into a host organism. A common challenge in metabolic engineering is balancing enzyme expression levels in this pathway to maximize product production. Finding this optimal balance requires designing, building and testing many strains with different combinations of genetic components (genes, promoters, ribosome binding sites, etc). Since pathways can contain non-linear interactions, the number of potential strains grows exponentially with the length of the pathway. Software tools make it easy to design constructs to cover the entire space of potential strains, however, the cost of building and testing constructs means that only a small fraction of the design space can be experimentally validated, even in a high-throughput environment. Thus effective Design of Experiments (DoE) is key to pathway optimization given limited experimental resources.

We approach this DoE challenge as a combinatorial optimization problem. The goal is to find a high target-producing strain out all potential strains in the pathway design space, while minimizing the total number of constructs which must be actually built and tested. The field of combinatorial optimization offers many algorithms for approaching such a problem. We propose an Estimation of Distribution Optimization (EDO) framework with an iterated regression model of the target metabolic pathway using proteomics and metabolomics data. This approach leverages multiple rounds of experiments to narrow in on the goal strain. In each round we first build a statistical model from the previous round's omics data to predict target product production for any potential strain in the design space. We then sample from this model to generate the DoE for the next round. After each round, the model accuracy increases and is able to more

effectively direct the next round of experiments.

Previous approaches to guide metabolic engineering show that statistical models are able to capture non-obvious interactions between pathway components, but fail to realize the potential of machine learning as a tool for automated DoE. Previous methods, which employ PCA and linear regression, are limited in their ability to capture arbitrary complex interactions in high-dimensional data sets and require significant manual interpretation. Machine learning algorithms allow us to train arbitrarily complex regression models and the EDO framework provides automated, statistically sound, DoE.

We test the effectiveness of this approach on several existing metabolic engineering data sets. Our toolkit predicts high-yield strains for different target natural products on distinct pathways and suggests additional experiments which are likely to yield even higher production levels. These predictions are computed without the need for manual interpretation, however, the toolkit also provides visualizations of high-dimensional omics data for qualitative representations of the pathway model.

129. Paper-Based Synthetic Gene Networks

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Synthetic gene networks are incredibly powerful tools, with wide-ranging uses in reprogramming and rewiring organisms. To date, there has not been a way to harness the vast potential of these networks beyond the constraints of a laboratory or *in vivo* environment. Here, we present an *in vitro* paper-based platform for synthetic biology that promises to provide both a new venue for synthetic biologists to operate, and a much-needed medium for the safe deployment of engineered gene circuits out of the lab. It enables the simple, sterile and abiotic distribution of synthetic biology-based technologies for the clinic, global health and industry. Based on commercially available cell-free transcription and translation systems, bacterial and mammalian components can be freeze-dried onto paper and other porous substrates to create poised synthetic gene networks that are stable for long-term storage at room temperature and are activated by rehydration. The resulting engineered materials have the transcription and translation properties of a cell and can host genetically-encoded tools. We demonstrate this technology with small molecule and RNA actuation of genetic switches, the characterization of novel gene circuits, and the construction of paper-based sensors for glucose and mRNAs, including antibiotic resistance genes and strain-specific Ebola virus sensors. Moreover, the rational design and rapid prototyping elements of the system provide a powerful combination for accelerating research. For greater practical use, gene circuits were enhanced with colorimetric outputs for detection by the naked eye, as well as with the fabrication of a low cost, electronic optical interface for quantification and possible automation of reactions. These low cost, paper-based synthetic gene networks have the potential to bring bio-based sensors, counters, timers and simple logic to portable devices.

130. Metabolic Engineering of *Ralstonia eutropha* for Biosynthesis of Natural and Unnatural Polyhydroxyalkanoates from Renewable Resources

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Polyhydroxyalkanoates (PHAs) have been considered as promising environmentally friendly alternatives of petroleum-based polymers due to their material properties that are similar to those of chemically synthesized plastics. Since the cost effective production of PHAs is one of the most important factors for the commercialization of PHAs, much effort has been devoted to the development of microorganisms able to efficiently utilize cheap carbon sources such as sucrose and glycerol. For example, sucrose is one of the most abundant and least expensive carbon sources extracted from sugarcane and sugarbeet. Thus, development of microorganisms capable of utilizing cheap carbon sources can provide cost-competitiveness of fermentation-driven products.

Here, we report recombinant *Ralstonia eutropha* strains able to produce natural and unnatural PHAs from various renewable resources derived from biomass as a carbon source and detailed results will be presented in this presentation.

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131. Predictable Design in Biological Engineering: Debugging of Synthetic Circuits by *in Vivo* and *in silico* Approaches

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The bottom-up design process of biological systems is one of the hallmarks of synthetic biology. This approach is adopted in all the fields of engineering and, similarly, it will significantly boost the potential of living systems engineering. However, our ability to predictably engineer genetic systems from sets of quantitatively characterized parts is still limited due to unpredicted interactions among circuit components. The evaluation of the bottom-up approach in a wide number of model systems can elucidate the actual predictability boundaries where the designed systems can behave as intended. The subsequent study of non-functional or unpredictable systems offers the opportunity to determine specific features, parts and conditions that significantly affect predictability. In this work, a set of increasingly complex model systems was considered. All of them were bottom-up designed from sets of pre-characterized parts, but the quantitative characterization of the final systems resulted to be unpredictable, thus requiring a debugging process, herein reported, based on both experimental procedures and mathematical modelling.
The model systems included: 1) a library of novel synthetic repressible devices, 2) transcriptional regulators cascades, 3) a feedback-controlled circuit, 4) enzyme production systems, and 5) circuits controlled by small RNAs. Mathematical models describing the quantitative behaviour of the systems were developed during the design step. Such models were used to investigate the unpredictable experimental output of the systems, measured via population-based, single-cell fluorescence measurements, or enzyme assays. Since the bottom-up design relies on parameter estimates, obtained during biological parts characterization, sensitivity analysis was coupled with Monte Carlo simulations to study the propagation of the uncertainty of parameter values towards the final output of the system. The contribution of biological noise in interconnected circuits was also studied *in silico*, via stochastic models, and *in vivo*, via single-cell measurements, to evaluate its importance in output predictability. The re-characterization of parts in a multi-faceted fashion was finally carried out to deepen the knowledge of parts functioning and identify possible conditions where specific modules do not have predictable behaviour. Crosstalk among parts like transcription factor-promoter pairs, identified via ad-hoc experiments, have also been crucial to elucidate previously unpredicted and non-modelled molecular interactions.

Taken together, the studied systems spanned a wide range of design architectures, parts and strains, and their debugging process enabled to decouple the contributions of context-dependent variability of biological parts, cell-to-cell variability, parameter estimation uncertainty, circuit mutations due to genetic instability, crosstalk, and metabolic burden.

132. The Directed Evolution of Halogenases for Late-Stage Functionalization of Bioactive Molecules

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Halogenation is a ubiquitous and vital process in the development of pharmaceuticals; an estimated quarter of all pharmaceuticals and agrochemicals are halogenated, and halogenation plays an integral role in the synthesis of many more. Despite this, the prevalent methods of halogenation suffer from a lack of site-selectivity and harsh reaction conditions, making the production of halogenated drug candidates difficult and environmentally unfriendly. To this end I have been working on the development of enzyme catalysts which perform site-selective halogenations of drug candidates in environmentally sustainable conditions. My preliminary work focused on improving the expression of natural halogenating enzymes in *E. coli*, particularly one called RebH, and exploring the scope and selectivity of wild-type RebH on a range of substrates on the preparative scale. My work now is focused on the directed evolution of RebH to further enhance its activity and expand its substrate scope with two primary goals: first, the halogenation of biologically active substrates, with a focus on bacterial biofilm inhibitors, in order to alter their biological properties; and second, the enantioselective halogenation of prochiral symmetrical compounds. Through the introduction of mutations in the genetic code for RebH, a variety of slightly altered RebH mutants are obtained and screened for changes in activity. Improved variants are characterized and further altered, thus recapitulating the process of evolution at a much accelerated pace in our laboratory. This research will both elucidate the role various mutations have in altering the activity of RebH and produce RebH variants useful for the development of novel drug candidates.

133. Metabolic Pathfinding Using Atom Tracking and Thermodynamics

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Annotated repositories of large-scale, molecular datasets provide a wealth of information to researchers designing novel biosynthetic pathways. However, utilizing information from these sources requires solutions to big data challenges: development of computational algorithms to produce pathfinding solutions from voluminous and complex data, and improvement of rankings to more easily ascertain the most biologically feasible pathways from high-throughput pathfinding solution sets.

Our work focuses on developing graph-based search methods for metabolic pathways on the atomic level. By tracking individual carbon atoms from the starting compounds to final target compounds using data from the KEGG RPAIR database, our algorithms can find non-standard pathways composed of enzymatic reactions that span multiple species and conserve varying proportions of atoms from the initial compound. Earlier work has examined algorithms that produced linear pathways as well as certain topologies of branched pathways.

To improve the biological relevance of pathways found by our algorithms, we are investigating the use of Gibbs free energy calculations for sorting and filtering out thermodynamically unfavorable pathways with the greatest aggregate free energy of formation given known cellular conditions including approximate values for pH and substrate concentrations. Our computational work will be complemented by experimental validation to test our predictions of feasible synthetic pathways.

134. How to Best Control Gene Expression in Cell Populations in Real-Time

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Automatic control is an engineering approach to regulate the behaviour of a system in an automated and precise manner. Controllers are used in everyday appliances from thermostats to microwaves, as well as, in complex and large systems such as airplanes and power-plants. The design principle is based on negative feedback, where the quantity to be measured (e.g. the temperature) is measured and compared to the desired value to yield a "control error". The controller changes some physical process in the system (e.g. switches the boiler on or off) to reduce the control error to zero. Thanks to advances in molecular biology and biotechnology, control engineering methods have been recently proposed to regulate gene expression in living cells using a variety on computational and experimental approaches. Due to its very recent application to biological systems, it is not yet clear what control strategy works best. Here we provide a computational and experimental comparison of the two main control strategies proposed in literature: proportional-integral (PI) control and model predictive control (MPC). We then propose and test an innovative control strategy, Zero Average Dynamics (ZAD) control, which has been extensively used in electrical power converters but never in biological systems. We used a microfluidics-based control strategy we recently presented [Menolascina F. *et al*, PLOS Comp. Biolo. 2014; Fiore G. *et al*, Chaos, 2013; Menolascina F. *et al*, Automatica, 2011] to control in real-time the expression of a fluorescent reporter protein from the inducible *GAL1* promoter in yeast *Saccharomyces cerevisiae*. In this set-up the computer can monitor cell fluorescence via a time-lapse fluorescent microscope and either feed cells with galactose, thus inducing expression from the *GAL1* promoter, or glucose to repress gene expression. The control tasks we investigated are: (i) "set-point" control in which the protein has to be maintained at 50% of its maximum value in galactose, for 1500 min and (ii) "tracking-control" in which the protein has to maintain a value of 75% for 500 min, then 50% for another 500 min and 25% for the remaining 500 min. Experiments confirm the numerical simulation, proving that MPC and ZAD strategies can achieve successfully the regulation of gene expression in living cells for both set-point and tracking control, whereas the PI strategy has a worse performance, at least for the "tracking" control task. The ZAD strategy however is much less computationally expensive than the MPC and does not require a detailed mathematical model of the system to be controlled. In conclusion our work shows that it is now possible to achieve precise regulation of gene expression in eukaryotic cells thus making possible to investigate cellular behaviour in completely new ways.

135. Codon Compression Algorithms for Saturation Mutagenesis

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Saturation mutagenesis is employed in protein engineering and genome-editing efforts to generate libraries that span amino acid design space. Traditionally, this is accomplished by using degenerate/compressed codons such as NNK (N = A/C/G/T, K = G/T), which covers all amino acids and one stop codon. These solutions suffer from two types of redundancy: (a) different codons for the same amino acid lead to bias, and (b) wild type amino acid is included within the library. These redundancies increase library size and downstream screening efforts. Here, we present a dynamic approach to compress codons for any desired list of amino acids, taking into account codon usage. This results in a unique codon collection for every amino acid to be mutated, with the desired redundancy level. Finally, we demonstrate that this approach can be used to design precise oligo libraries amendable to recombineering and CRISPR-based genome editing to obtain a diverse population with high efficiency.

136. ICE: A Distributed and Interconnected Biological Part Registry

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Advances in synthetic biology research and genetic design automation, along with the availability of rapid and reliable DNA sequencing has resulted in a steady increase in the number of complex engineered biological devices. Maintaining information about the constituent parts of these devices, along with the ability to share them with other members in the synthetic biology community as well-characterized components with the goal of reusability is a challenge that is not fully addressed by any of the existing registry software platforms.

We have developed an open-source web-based platform for synthetic biology information management known as the Inventory of Composable Elements (ICE). It includes a distributed software platform to enable efficient sharing of biological elements across labs in the synthetic biology research community. Referred to as "Web of Registries", this feature aims to increase the efficiency of bioengineering by enabling scientists to publish and share datasets across multiple ICE installations in potentially different geographic locations. Thus, users from one ICE instance (e.g. the JBEI public repository) can query and access parts, sequences, strains and Arabidopsis seeds of interest stored in other connected ICE instances (e.g. the Synberc repository). ICE also integrates browser-based tools such as VectorEditor and PigeonCAD for sequence annotation, manipulation, analysis and visualization. Support for data formats such as GenBank, FASTA and the Synthetic Biology Open Language (SBOL), ensures compatibility with existing and emerging tools for synthetic biologists.

137. Genome Engineering to Support Rational Design for Metabolic Engineering in Bacillus Subtilis

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Genome engineering is a rapidly growing field of microbial research but still remains limiting when developing *Bacillus subtilis*-based cell factories. *B. subtilis* is the model organism for the Gram-positive bacteria, is GRAS (Generally Regarded As Safe) and is heavily used in industry. We successfully designed and developed *B. subtilis* chassis strains lacking functions such as sporulation, antibiotic resistance, mobile DNA elements, prophages, isozymes while retaining deletion making functions. This latter work represents a proof-of-concept for strain development for biotechnologies. To develop adapted chassis strains it is critical to be able to modify the genome "*à la carte*". We are currently developing a set of novel recombineering methodologies based on single strand annealing proteins (SSAP) in *B. subtilis*. Such recombinases mediating oligonucleotide-targeted mutagenesis will serve to introduce point mutations in multiple loci, enabling simultaneous modification of several chromosomal targets. We are also developing a CRISPR-Cas system for *B. subtilis* using the inactivated Cas9 as an interference system for gene expression modulation. Altogether this will result in an integrated toolbox for synthetic biology and metabolic engineering. These new methods for rapid introduction of multiple point mutations combined with the interference system should lead to the development of robust and high-performing bacterial cell factories.

138. New Promoters to Improve Heterologous Protein Production in *Penicillium chrysogenum*

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Filamentous fungi such as *Aspergillus* and *Penicillium* are used as hosts for expression and secretion of heterologous proteins and secondary metabolites. Studies have shown the promiscuous nature of fungi in recognizing transcriptional and translational elements present in genes from other fungal species. However, only few promoters have been characterized so far in *Penicillium chrysogenum*. To address this need, a set of heterologous and homologous promoters were tested in a reporter system. We combined available synthetic biology tools such as BioBricks and *in vivo* homologous recombination in *Saccharomyces cerevisiae* in order to engineer twelve *Penicillium* and *Aspergillus* promoter pathways driving the expression of *GFP* while constitutive expression of DsRed was used as an internal strandard. These pathways were tested using the BioLector technology in fermentations with a *P. chrysogenum* strain and classified in strength and expression profile. This catalog of promoters will be used in future synthetic biology projects to tune the expression of target genes.

139. Organised Natural Structures Using Synthetic Biology

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Engineering living organisms that can be utilized in a variety of applications (e.g. valuable compound biosynthesis, applications in materials) is now the main focus of the novel field of Synthetic Biology. The idea is to take advantage of S.cerevisiae's unique growth and adhesion characteristics to create cell patterns such as fractal formations that are interesting and useful. Specifically, we are implementing novel gene networks that control S.cerevisiae's budding behavior to induce pseudohyphal growth in both haploid and

diploid strains. Through the characterization and genetic control of the key natural regulators that control pseudohyphal growth we aim to reprogram cells to grow in controllable branched fractal patterns. For a true fractal pattern to occur, cells are needed to differentiate as the colonies grow and therefore novel regulatory mechanisms to control the timing and/or location where genes are switched on and off are being implemented. By controlling cell patterns available surface area is also controlled affecting the availability of growth media, oxygen and other factors to the organism. Ultimately, unique applications in fields such as biomaterials and metabolic engineering are enabled.

140. Domesticating the Genome of a Naturally Transformable Bacterium

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As the field of synthetic biology moves towards more ambitious genome engineering efforts, the need for chassis organisms better suited for genome modification is becoming more apparent. The bacterium *Acinetobacter baylyi* ADP1 could provide an ideal next-generation synthetic biology chassis due to its natural transformability. We conducted a 1000-generation adaptive evolution experiment in ADP1 to study the stability of this strain. We observed the gradual loss of transformability over time and, furthermore, observed the emergence of a high-aggregation phenotype. The genomes of evolved clones were sequenced to determine the genetic basis of these phenotypic changes. We found that the activity of ADP1's sole IS-element family IS1236 was the main driver behind these changes and also observed the loss of a 49-kb prophage region that had been previously used as a landing site for integrating heterologous genes into the chromosome. We deleted all copies of IS1236 from the ADP1 genome to create an IS-less strain (ADP1-ISx) with increased genetic stability. This strain showed a much greater decrease in the frequency of gene-inactivating mutations than expected from the rates of spontaneous IS1236 insertions. Unexpectedly, ISx also has improved transformability and a reduced rate of autolysis. In sum, our work demonstrates the utility of 'domesticating' the genomes of microbial strains by removing selfish DNA elements and has generated a new bacterial chassis (ADP1-ISx) with improved transformability and genomic stability for future genome engineering efforts.

141. Synthetic Nucleic Acid Nanostructures Regulate Native Triplex and CRISPR/Cas Genomic Recombination, Restriction, and Repair Potentials

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Synthetic oligo- and peptide- nucleic acid nanostructures have been more predictably designed and externally delivered into the intracellular milieu. These deployed technologies interact with, and influence, the cytoplasmic and nuclear molecular machinery in order to regulate potentials involved in genomic targeting and editing. It is demonstrated that synthetic nucleic acid nanostructures composed of various nucleobase and backbone modifications can regulate the genomic recombination rate, the sequence-specific restriction of a locus, and the endogenous repair pathways. The formation of a triplex nanostructure, by exogenously introduced PNA molecules with the duplex chromosomal and episomal DNA, is shown to elevate the cell's targeted recombination potential. Alternatively, the formation of a RNA-guided CRISPR/Cas nanostructure, again by exogenously introduced RNA molecules with Cas nucleases, is shown to initiate the cell's targeted double-strand restriction potential. Recombinagenic donor DNA molecules co-opt these elevated recombination or initiated restriction potentials to form competing nanostructures that act as homology-dependent templates, sans edits to be introduced, thus potentiating repair. Safety and efficacy of these nanostructures is achieved by leveraging the performance profile of the cell's own endogenous recombination, restriction, and repair machineries in concert with these sequence-specific and localizing-in-tandem molecules. Progenitor cells drugged with designed molecules, and primed with chemical cell modulators, safely and effectively redesign the genome, which are then propagated to cellular progeny. These molecular technologies are developed to remediate the underlying genomic causes of monogenic human diseases, engineer living genetic codes, improve crop characteristics, and defend against outbreaks, and thus has well-positioned technology profiles in the synthetic biology of healthcare, biotechnology, agrotechnology, and national security.

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142. Sentinels of Disease: Engineering Bacteria to Sense and Remember *Salmonella* Infection in the Mammalian Gut

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Enteric bacterial infections are a leading cause of global morbidity and mortality. Non-typhoidal *Salmonella* (NTS) species alone infect around 100 million people, and kill over 100 thousand of these, annually. Lack of accessibility to adequate medical care during severe infections is one of the major causes of death. The development of new,

cheap and easy-to-administer diagnostics and therapeutics is therefore an important step towards better monitoring and treating this disease. We recently developed proof-of-concept engineered bacteria that sense the mammalian gut environment and remember its state for at least a week to be monitored by fecal sampling. Using 'Gut-on-a-Chip' microfluidic organ models and mouse models of *Salmonella* infection we have further developed this work to produce bacterial reporters that respond to markers of *Salmonella* infection *in vitro* and *in vivo*. This approach could provide a cheap and easy means to monitor infection and provides an important basis for the ongoing development of inducible therapeutics and other non-invasive diagnostic approaches using engineered bacteria.

143. Spatial and Kinetic Modeling for Targeted Fusion Proteins

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Interferon alpha is a cytokine that has shown efficacy in treating cancer, but its use is limited due to severe side effects. We wish to target the anti-proliferative effects of interferon to cancer cells by following the chimeric activator approach. This consists of making a fusion protein between interferon alpha and an antibody fragment that binds to EGFRvIII, an epitope specific for glioblastoma cells, and also mutating residues in the receptor binding sites of interferon to weaken its affinity. The cell specificity of interferon's activity is then driven primarily by the antibody fragment binding to EGFRvIII. We have built a model to predict the activity of a given antibody fragment-interferon fusion based on the properties of the peptide linker between the two domains and on the strength of the mutation to interferon's binding site. A constrained Brownian dynamics component of the model predicts the effect of the linker properties on cooperative binding kinetics. Those results are used in an ordinary differential equation model of binding state transitions to make quantitative predictions of experimental results. Our antibody fragment-interferon fusion construct achieves a 100-fold increase in specificity on target cells, as measured by shift in EC50 in a proliferation assay, and is well-predicted by our model.

144. High-Throughput Screening of Membrane Transport Proteins in Synthetic Lipid Vesicles

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Membrane transport proteins mediate the exchange of ions, small molecules, or macromolecules across the cell membrane and play critical roles in many biological systems. Engineered transporters have myriad applications that include biosensing, metabolic engineering, and experimental tools for probing neural function. Nevertheless, transporter engineering has been limited by the lack of robust and quantitative high-throughput assays suitable for screening large transporter libraries. Cell-based assays are difficult due to the toxicity of heterologously expressed membrane proteins, the confounding effects of endogenous transporters, and the inability to precisely control the intracellular environment. We present a new method for high-throughput transporter screening in synthetic lipid vesicles. Microfluidic flow focusing produces double emulsion templates that, through a novel dewetting mechanism, are converted into monodisperse giant unilamellar vesicles. The vesicles can be loaded with cell-free expression systems to express the desired transporters in the membrane, where they function. This allows the transporter variants to be assayed and sorted at high throughput using commercial fluorescence activated cell sorters. Synthetic lipid vesicles enable high-throughput screening of membrane transport proteins, and represent a robust platform for the directed evolution of transporters with new biochemical properties.

145. Development and Characterization of the Peroxisome for Metabolic Engineering

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Engineered metabolic pathways often suffer from undesirable interactions with the production host's native cellular processes. Evolution has solved the problem of metabolic crosstalk by segregating distinct cellular functions into membrane-bound organelles. By improving the modularity and efficiency of protein import into the yeast peroxisome, determining the natural chemical permeability of the peroxisomal membrane, and characterizing both the rate and capacity of protein import, we have transformed the peroxisome into a useful option for compartmentalization and sequestration of proteins and pathways. With this new, characterized system, we demonstrate successful compartmentalization of a model pathway and inducible, rapid sequestration of essential proteins. These advances will serve as the basis for turning the peroxisome into a well-characterized synthetic organelle for use in limiting crosstalk in pathways and protein sequestration.

146. Initial Steps Towards the Establishment of a Multigene Engineering Platform in *Drosophila melanogaster*

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The application of synthetic biology principles for the analysis of biology and disease in multicellular organisms is a recently growing field. Currently there is no standard DNA assembly methodology to facilitate multigene engineering in *Drosophila melanogaster*. To overcome the existing limitations on the design and construction of new genetic combinations in this model organism and enable the exchange of genetic building blocks, we propose the establishment of a platform founded on the synergistic combination of two transgenesis technologies: GoldenBraid2.0, a synthetic assembly platform that facilitates multigene engineering via Type IIs restriction enzyme assembly, and P[acman], the high-capacity site-specific transgenesis platform developed for *Drosophila melanogaster*. Apart from providing an optimized cloning strategy that generates fully exchangeable genetic elements for multigene engineering, the platform will include the most advanced tools for genetic engineering in *Drosophila*. Our broad technological framework aims to establish the foundations of Synthetic Biology in the fly field and can be easily extrapolated towards other model organisms.

Here we present an initial group of elements characterized in *Drosophila* Schneider 2 (S2) cells. These elements include a strategically chosen group of functionally-tested, premade genetic parts to build frequently-used modules such as constitutive, tissue-specific and inducible expression cassettes as well as fluorescent protein and other reporter units common to *Drosophila* research. These elements are components of an ever-growing open collection of DNA parts that will be available to any interested researcher. This platform, in combination with other shared resources in the fly community (e.g., Bloomington *Drosophila* Stock Center, BACPAC Resources, *Drosophila* Genomics Resource Center, and Addgene), will facilitate the expansion of complex synthetic biology advances in *Drosophila melanogaster*.

147. Biomolecular Implementation of a Quasi Sliding Mode Controller Using an Ultrasensitive Cell Signalling Pathway

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A fundamental aim of synthetic biology is to achieve the capability to design and implement robust embedded biomolecular feedback control circuits. An approach to realize this objective is facilitated by using abstract "chemical reaction networks" (CRNs). This approach can impart a good degree of predictive functionality to the wet-lab circuits since these networks can model a large class of chemical and biological processes, and can be well analysed using advanced mathematical techniques. The existing work within this framework concerns the design and implementation of linear time-invariant systems only: an example of such systems is the well-known "proportional+integrator" (PI) controller derived recently by Yordanov *et al*. In practice, however, the PI controllers must be combined with a nonlinearity to overcome the wind-up effect associated with the integrator action. Here, we extend this approach to allow the implementation of nonlinear controllers using the sliding mode control theory whose strong performance and robustness characteristics have been widely recognised for a number of decades in the more traditional control engineering application domains. We show how a signalling cycle with ultrasensitive response dynamics can provide a biomolecular implementation of a nonlinear quasi sliding mode controller. We implement our controller on a prototype of a biological pathway, specifically a first-order low pass filter and demonstrate that our nonlinear controller outperforms a PI controller by facilitating faster response dynamics without introducing overshoots in the transient response.

148. Synthetic DNA Delay Circuits for Temporal Coordination

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Biological systems use chemical circuits to coordinate elaborate sequences of events, from the timing of cell divisions inside the embryo, to the sustained rhythms of circadian clocks. Similar control over the timing of events in synthetic systems remains challenging, in part because of the complexity of designing de novo gene regulatory networks. Here we demonstrate a simple molecular circuit, consisting of only three species of synthetic DNA complexes in buffer, which automatically releases a target sequence of DNA into solution after a tunable delay period. The delay time can be specified on the order of hours to days, and multiple orthogonal delay circuits can be combined to coordinate the release of different sequences at independent times. This bare-bones circuit facilitates precise timing of target events inside of *in vitro* DNA networks without external stimulation.

149. Engineering Bacterial Finite State Machines

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Cells use genetic circuits to make complex decisions such as differentiation, sporulation, or the prioritized utilization of sugars to optimize energy payoffs. The ability to engineer cellular decision making would have widespread applications in basic research and engineering. Finite state

machines (FSMs) may serve as a useful tool for engineering complex cellular decisions1. FSMs are a framework whereby decisions can be explicitly specified as a collection of states and a mapping of the transitions among states. In computer engineering, FSMs are implemented using layered networks of digital logic gates (AND, OR, NOT). Digital-like logic gates can be implemented inside of cells using repressors (or activators) to modulate transcription from promoters. Thus, decision-making circuits can be implemented inside of cells as layered networks of promoters and repressors.

The implementation of complex decision-making circuits inside of cells requires many orthogonal promoter-repressor modules. We use the CRISPRi transcriptional repression system2 as a scalable platform for generating a large number of mutually orthogonal repressors in *E. coli*. CRISPRi allows for programmed repression of target promoters via the expression of a protein (dCas9) and a small guide RNA (sgRNA) with ~20 base pairs of homology to the target promoter DNA sequence. To ensure that CRISPRi repressors uniquely target only their intended cognate promoters, we developed a standardized procedure whereby an sgRNA target site (operator) is randomly generated in the spacer region of an otherwise invariant promoter. We demonstrate that this approach allows for the construction of families of promoters that can be strongly repressed by partner sgRNAs with complementary sequences, while retaining very similar overall transcription rates. We have used this approach to construct 9 highly orthogonal promoters and cognate sgRNAs. We achieve fold repression values ranging from ~25x to several hundred or more, and present a set of 7 promoter-repressor units with minimum orthogonal range of 50x (as defined by Nielsen *et al*.3). We also extended the above design to incorporate a second input sgRNA, resulting in NOR logic.

Working towards more complex logic circuits, we next incorporated one NOT gate and three NOR gates to construct a 2-to-1 multiplexer circuit (MUX) in *E. coli*. The MUX is a signal selection device commonly used in electrical engineering which selects one of several possible input signals and forwards it as the output signal. In a biological context, the MUX allows us to assess the activities of two different promoters using a single reporter gene (e.g. *GFP*) based on the presence of an input selector signal such as a chemical inducer.

In addition to the combinatorial circuits (i.e. circuits whose outputs depend only on the current inputs) we have constructed to date, we also plan to use the CRISPRi system to construct sequential digital logic circuits with feedback and memory (e.g. toggle switches and flip-flops). Together, these technologies will be leveraged to implement finite state machines inside of bacteria.

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150. Creating a Riboswitch-Based Whole-Cell Biosensor for BPA

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Detection of small organic molecules using riboswitches in whole-cell biosensors could allow for screening of environmental pollutants, such as bisphenol A (BPA), in an effective and economical manner. Riboswitches are RNA sequences typically embedded within the 5' untranslated region of mRNA that are capable of regulating gene expression and, when paired with a reporter gene, can signal the presence of small organic molecules. In order to create a riboswitch in *Escherichia coli* that could bind BPA and, upon binding, translationally upregulate the expression of *gfp*, we investigated (1) the best method of engineering a novel BPA-activated riboswitch via dual genetic selection and (2) the effectiveness of using such a riboswitch to detect BPA.

Dual genetic selection was performed using a *tetA-gfp* reporter downstream of a mutagenized *thiM* riboswitch. Two *E. coli* libraries – Library 1 (3×10^4 members) and Library 2 (4×10^6 members) – were subjected to positive selection followed by negative selection. Library 1 was only subjected to one round of selection, whereas Library 2 was subjected to several rounds of selection under increasingly stringent selection conditions. These libraries were then screened for BPA-activated riboswitches via a *GFP* assay. Pre-selection sequencing of random members revealed that Library 1 and Library 2 were both highly diverse; no conserved sequences were identified within each library. Initial analysis of post-selection hits suggests that multiple rounds of selection may be necessary. While more investigations must be conducted to verify these preliminary results and to determine the effectiveness of using a riboswitch to detect BPA, this technology serves as a promising method for detecting pollutants in the environment.

151. Viral Based Design: Remodeling the Hippo Pathway from Tumor Suppression to Oncogenesis

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During the process of evolution viruses have learned to tailor cellular functions to their own needs. Due to the frugal nature of viral design, viral regulatory proteins are small, multipurpose, and efficient. For this reason, viruses curate

a treasure-trove of molecular information for the synthetic biologist, providing a glimpse into a natural process of "reverse-engineering" of cellular functions. The polyomavirus middle T antigen (PyMT) induces oncogenic transformation by hijacking cellular signaling effectors. Activation of the tyrosine kinase, c-Src, is a key step, imperative for viral oncogenesis. However, the underlying mechanism of Src activation by PyMT remains elusive. We addressed this fundamental question by examining the virus-host interaction between PyMT and the Hippo tumor suppressor pathway. The Hippo pathway is a major regulator of cellular proliferation. PyMT physically interacts with the Hippo pathway effector Taz (WWTR1). In proliferating cells Taz is nuclear and coactivates the pro-oncogenic TEAD transcription factors. Taz phosphorylation by Hippo pathway core kinase, Lats, leads to Taz nuclear exclusion and a consequent downregulation of TEAD coactivation. We found that Taz was required for transformation by PyMT in a counter-intuitive manner. Despite the cells undergoing oncogenic proliferation, Taz was exclusively cytoplasmic in the presence of PyMT, an observation expected to have rendered Taz deprived of its reported pro-proliferative function. Indeed, PyMT markedly inhibited TEAD coactivation by Taz, an event associated with tumor suppression. We resolved the molecular basis of the enigmatic Taz cytoplasmic retention by PyMT by demonstrating that PyMT activated the Lats tumor suppressor kinase leading to Taz nuclear exclusion. Next, we searched a role for cytosolic Taz in PyMT oncogenesis and identified Shp2, a phospho-tyrosine phosphatase, as an important player. Taz regulates Shp2 subcellular localization via physical interaction. PyMT induced cytosolic localization of Taz and Shp2. We demonstrate how PyMT designs a new network in oncogenesis where, counter- intuitively, the tumor suppressor Hippo pathway plays a critical role. We propose that understanding viruses is of great value in designing sophisticated synthetic modules.

152. Facilitating Unconventional Yeast Engineering for Biorenewables Production

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Many of the 1800 known yeast species have highly unusual metabolic, biosynthetic, physiological and fermentative capacities that are not possessed by model yeasts such as *Saccharomyces cerevisiae*. To date, strides have primarily made on transferring genetic elements attributed to the desired traits from native sources to model hosts. However, as the outcomes of long-term natural evolution in particular environments, many high-performing functions are granted by a network of genes through a complex hierarchy of regulations, many of which have not been clearly elucidated. When the predicted relevant genes were incorporated into model hosts, many functions were impaired due to the alteration of physiological backgrounds, general missing of desired regulations, lack of mechanisms for cofactor recycling, or simply expression imbalance. On the other hand, nowadays, we are no longer retarded by genome sequencing, and if we could establish generic design rules, hence shortening tool development cycle for unconventional microbes, we can directly leverage, engineer and continuously improve the treasures granted by nature.

Here we report our recent success in establishing the first stable expression platform for engineering Scheffersomyces stipitis, one of the species with the highest native capability for xylose fermentation. The current expression plasmid is extremely unstable, being lost within 2 days of cultivation. With the perfect integration of *in silico* prediction and high throughput screening, we were able to isolate all eight centromere sequences from S. stipitis genome in a monthly manner. The identified centromeres significantly enhanced the stability of the episomal vector, resulting in 3-fold increase in lactic acid production. Isolating a functional centromere in an efficient manner by far is still a missing piece from literature. Immunoprecipitation with specific kinetochore protein antibodies is the predominant method, but suffers from the extensive experiment procedures and the prerequisite of antibody that needs to be developed for each species. We are currently investigating the generalizability of our hybrid approach in rapid centromere identification in a broad spectrum of unconventional yeasts. In contrast to the previous developments in this field that were fairly scattered and short of systematicness, we are ambitious to elucidate design principles and establish platform technologies to enable rapid functional modifications of a series of high-potential yeast species.

153. Modular Engineering of Bacterial Two-Component Systems to Encode Novel Signaling Properties

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Humans are populated by trillions of bacteria that sense and respond to host-, microbe-, and diet- derived molecules. A major goal of synthetic biology is to engineer microbes with custom sense-respond behaviors for therapeutic and diagnostic applications. Two-component systems (TCSs) are the primary means by which bacteria sense the environment. In a canonical TCS, a histidine kinase phosphorylates its cognate response regulator, eliciting a transcriptional response. However, natural TCSs contain specific steady-state and dynamical input/output (I/O) properties that have evolved to permit the organism to best adapt to different environmental signals. To leverage and multiplex the tens of thousands of TCSs known from sequence for useful applications, a generalizable strategy for designing desired TCS signaling properties is needed.

Here, we exploit the modular protein domain architecture of TCSs to program diverse signaling properties. Using our previous *E. coli* light-switchable TCSs, we identify domains and conserved mutations to create new signaling modules that can increase or decrease pathway activity, and show that this approach is generalizable across different TCSs. Furthermore, using these elements we engineer simple feedback and feedforward loops to encode ultrasensitivity, acceleration, pulsing, delay, and memory. We are applying this framework to engineer probiotic bacteria that can sense and remember exposure to diagnostic profiles of disease-associated human gut metabolites and respond with therapeutic molecules.

154. Mammalian Genetic Circuit Construction Utilizing the Q System

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It is highly desirable to have additional transcriptional regulatory tools that can be applied independently of existing systems in mammalian cells. The Q system is a binary expression system that was originally discovered in *Drosophila*. The regulatory parts have been moved upstream of the fluorescent molecule, *GFP*, and has been demonstrated to work in *Drosophila*, C. elegans, and zebrafish. Here the regulatory parts are introduced as a new component for future genetic circuits in mammalian cells. The regulatory component of the Q system contains two genes of interest QF and QS along with the necessary binding sites (QUAS). QF acts as a transactivator for genes downstream of its binding site, while QS inhibits the binding of QF and prevents transcription. The predictable binary manner of the system makes the system a prime candidate for inclusion into the synthetic biology repertoire. To demonstrate the Q system's candidacy it was introduced to mammalian cells to determine functionality. Plasmids containing QF and binding sites upstream of a reporter gene were found to function as predicted. However, the repression predicted for QS was absent and thus deemed unfit for inclusion in genetic circuits. Additionally, QF and QUAS were coupled with existing components to predictably control gene expression through genetic circuits such as Boolean logic gates and an oscillatory system. The Q system demonstrates its candidacy for inclusion into the synthetic biology repertoire to advance the construction of genetic circuits in a controlled and predictable manner.

155. Incorporating Antimicrobial Peptides As Therapeutic Modules in Engineered Probiotics

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156. Enabling Multiplex Genome Engineering in Yeast By RNAi and Crispr

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Successful metabolic engineering practice often requires simultaneous manipulation of many genes. Whereas recombination-based genetic engineering (recombineering) is able to create combinatorial genetic diversity on a genome scale, such method is mainly limited to bacterial cells. Here we report a strategy that can generate multiplex modifications in the *Saccharomyces cerevisiae* genome, by combining the advances in full-length cDNA library construction, RNA-interference technology and CRISPR-Cas system. Briefly, upon introduction of specific double-stranded breaks (DSBs) in the repetitive sequences by CRISPR-Cas nucleases, both the overexpression and knockdown cassettes of every yeast gene can be integrated at high efficiency into the genomic loci of repetitive sequences. This process may be iteratively performed to accumulate dozens of genetic modifications in a single cell of an evolving yeast population. We fully automated this process through an integrated robotic platform, enabling generation of vast genetic diversity from which new or improved properties may emerge. We envision this new tool can greatly accelerate genome engineering in *S. cerevisiae* for basic and applied biological research and medicine.

157. The Joint Genome Institute's Synthetic Biology Biosecurity DNA Screening Process

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Concerns have been raised that individuals with malicious intent could exploit DNA synthesis technology in order to obtain genetic elements from organisms and toxins that would otherwise be difficult to obtain. In response to these concerns, the U.S. Department of Health and Human Services issued the Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA. The voluntary Guidance outlines the U.S. government's recommendations for screening double-stranded DNA to ensure that existing regulations [1] are followed, encourage best practices in addressing biosecurity concerns, and reduce the risk that individuals with ill intent may exploit the application of nucleic acid synthesis technology to obtain genetic material derived from or encoding Select Agents or Toxins, or agents on the EAR's Commerce Control List (CCL).

In accordance with the Guidance, the U.S. Department

of Energy Joint Genome Institute has developed a DNA screening pipeline to screen all sequences that are synthesized through its synthetic biology program. The screening method detects "sequences of concern" of at least 200 nucleotides in length on either DNA strand, and the resultant polypeptides from translations using the three alternative reading frames on each DNA strand (or six-frame translation). Sequences are aligned to the sequences in GenBank's nucleotide and protein databases rather than a curated database, to ensure that it automatically adapts as new sequences are added to GenBank. A "Best Match" approach is used to determine whether a query sequence is unique to Select Agents or Toxins, or CCL-listed agents, toxins or genetic elements, and to minimize false positives from closely related organisms or highly conserved "house-keeping genes" which do not pose a biosecurity threat.

[1] Select Agent Regulations (SAR) and, for international orders, the Export Administration Regulations (EAR)

158. Effects of Cell-to-Cell Variability in MarA Expression on *Escherichia coli* Antibiotic Resistance

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Traditionally, bacterial populations are viewed as individual cells that share both genotypic and phenotypic identity. Consequently, when individual cells within a population demonstrate elevated antibiotic resistance levels this was assumed to be the result of genetic mutation. However, recent studies have successfully demonstrated that there exist disparities between genotypic identity and phenotypic identity. For instance, studies have shown that, as a mechanism to evade the lethal effects of the bactericidal drugs, bacteria cells are capable of switching between a regular drug susceptible state and a drug tolerant dormant state (persister) by stochastic phenotypic variation. In addition, recent work by our group has suggested that transient antibiotic resistance can stem from stochastic pulsing in genes related to antibiotic resistance. In particular, we are focusing on a transcription factor, MarA from *Escherichia coli*. MarA is known to play an essential role for the regulation of more than 40 downstream antibiotic resistance genes, such as the AcrAB-TolC efflux pump genes and micF gene. In our study, we aim to demonstrate that MarA expression is heterogeneous for each individual cells within an isogenic bacterial population. We hypothesize that differences in MarA expression may render individual bacteria with different antibiotic resistant levels. To achieve this goal, we are exposing wild-type *E. coli* cells to various antibiotics. Plasmids that carry a fluorescence protein gene controlled by a marA promoter are used as reporters for MarA expression. In an effort to attain a precisely controlled external environment, we are using a microfluidic chip to control addition of antibiotics and have coupled this with time-lapse microscopy. Our preliminary data indicate that there are differences in expression of fluorescence proteins between individual cells within a microcolony, which reflects noisy MarA expression. In addition, when antibiotics are added, cells demonstrate different antibiotic resistance levels, which correlated with the noisy MarA expression. Hence, the isogenic bacteria population could use this mechanism as a "bet-hedging" strategy, which could eventually lead to a new bacteria population with pre-adapted antibiotic resistance ability.

159. Thermal Effects on Cell Death Induced By High-Intensity, Ultrashort Electric Pulses

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Nanosecond and high intensity electric pulse induced irreversible electroporation has been shown to eliminate melanoma tumor cells with needle electrodes, while thermal effects are typically not considered. Our results of self-consistent analyses of cells show that thermal effects due to high-intensity, ultrashort pulses could facilitate poration over small sections of the membrane. Short-term temperature increases of several degrees Celsius above the physiological temperature could allow a considerable reduction in the pulse duration and amplitude for pore formation. Thermally launched bioresponses are similar in nature to those triggered electrically. Molecular Dynamics simulations indicate an increased probability for pore formation in the plasma membrane with lower pulse amplitude or smaller pulse durations at elevated temperatures. Synergistic effects enhance the benefits at a lower energy cost and better utilize bioelectric effects for therapeutic applications. Therapeutic applications include the treatment of tumors in internal organs by adding thermal effects as a synergistic mechanism. The treatments use antennas to reach internal organs and require the application of even shorter subnanosecond pulses, in order to obtain a reasonably small focal volume.

160. Reengineering for Robust Photorespiratory Bypass to Improve Photosynthesis and Crop Production

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Over the past 50 years improvements in grain crop yield potential has maintained food supplies as demand has increased. As climate changes globally and human population increases traditional methods of crop improvement have become less effective in adapting and improve agricultural production on ever limiting land and water availability. Improving photosynthetic efficiency has been a long standing goal toward increasing output and crop yield that

has to date only played a minor role in crop improvements. At 25°C and current CO_2 levels roughly 25% of Rubisco activity is the fixation of oxygen instead of carbon dioxide resulting in the conversion of RuBP to one molecule of phosphoglycerate and one molecule of glycolate. C3 plants recover the carbon in glycolate through the C2 photorespiratory pathway. The C2 pathway uses energy in the form of ATP and reducing equivalents and loses fixed CO_2 resulting in a reduction in photosynthetic efficiency by 30%. Synthetic biology has provided new opportunities in altering photorespiratory metabolism to improve photosynthetic efficiency. Indeed metabolic bypasses to photorespiration have been generated and have demonstrated improvements in growth. Using the Golden Gate synthetic biology approach we have assembled a series of multigene constructs that contain alternate metabolic pathways to bypass photorespiration. In addition, we designed a screen based approach to test a range of standardized parts (promoters, terminators) in model plants *Arabidopsis thaliana* and *Nicotiana tabacum*. We have successfully transformed in large multigene constructs and have demonstrated detectable gene expression. Using a fluorescence based screen we tested single construct transformed lines for rescue of photorespiratory deficient plants in Arabidopsis. Our results indicate that large multigene constructs containing a metabolic bypass to photorespiration can rescue changes in fluorescence caused by low CO_2 stress in deficient plants. Determining robust photorespiratory bypass constructs can provide insight into next generation crops and our utilization of standard parts and fluorescent screening provide a new tool kit for plant synthetic biology to engineer improvements in photosynthetic efficiency.

161. A Single Plasmid System to Addict Bacteria to Non-Canonical Amino Acids

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Genetic incorporation of non-canonical amino acids (NCAAs) allows the addition of new and novel chemistries to the genetic code. This can be accomplished by using an orthogonal aminoacyl tRNA synthetase:tRNA pair derived from an evolutionarily distant organism to reassign the amber (UAG) stop codon. Unfortunately, global UAG suppression imposes a significant fitness cost on the host cell, leading to deletion or inactivation of the orthogonal translation machinery during serial culture experiments. To solve this problem, and ensure maintenance of functional orthogonal translation machinery, we engineered an essential protein, β-lactamase, to require incorporation of NCAAs. Following library selections with various 3-substituted tyrosine analogues, clones conferring β-lactam resistance were counter-screened to eliminate those active in the presence of common *E. coli* suppressor tRNAs. We show that by iteratively decreasing the number of canonical amino acid solutions, enzymes can be evolved with a strong dependence on NCAA incorporation. An evolved β-lactamase has been shown to maintain orthogonal translation machinery in *E. coli* for more than 250 generations, without detectable escape from NCAA dependence. Unlike other approaches that rely on extensive manipulation of the host genome, this system is compact and broadly compatible. To demonstrate the utility of this approach the sub-circuit containing the addicted lactamase and the orthogonal synthetase and tRNA pair were transferred on a broad host range vector to different bacterial species, including *Shigella* and *Salmonella*, which immediately resulted in the non-revertible addiction of these strains to the NCAA without the alteration of their underlying codes via other synthetic biology approaches. The use of our or similar addiction modules should quickly allow the introduction of NCAAs into a much wider variety of bacterial species, including industrially-relevant fermentation and production strains

162. A Biofilm Free *E. coli* Chassis for Use in Continuous Culture

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Continuous culture provides constant culture conditions enabling better characterization of synthetic circuits as well as constant selective pressures for directed evolution. When characterizing synthetic circuits over generational timescales (tens of minutes to hours), cellular metabolism impacts batch culture conditions, which can act as a disturbance to synthetic circuits and change selective pressures in directed evolutions. In continuous culture, substrate concentrations and metabolic byproducts reach a steady state eliminating the drawbacks of batch culture. However, continuous culture selects strongly for phenotypes that are resistant to washout, such as flocculent yeast or adherent biofilms in *E. coli* [2]. Lab strains of yeast that are resistant to flocculation [3] are readily available, however a strain of *E. coli* that does not form biofilms in continuous culture has not yet been reported. Here, we have developed a five gene/operon *E. coli* knockout strain BF019 that is resistant to biofilm formation in continuous culture. We show that wild type *E. coli* can form disruptive biofilms in as little as 48 hours, while our novel strain remains biofilm free for at least two weeks, after which we ended the experiment. Strain BF019 and our previously reported [4] open-source turbidostat design [5] form a platform enabling characterization of long-term dynamics as well as directed evolution of better substrate utilizers and more tolerant strains required for consolidated bioprocessing.

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163. Metabolic Engineering of *Saccharomyces cerevisiae* for Production of Fatty Acid Short-Chain Alkyl Esters

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Biodiesel is a mixture of fatty acid short-chain alkyl esters obtained via transesterification of vegetable oils with an alcohol. Since methanol is a cheap alcohol, biodiesels are more commonly found as methyl esters. As a more renewable and scalable biodiesel production method, microbes have been genetically engineered to produce fatty acid ethyl esters from biomass. While fatty acid (methyl and ethyl) esters are useful alternative diesel fuels, fatty acid esters with longer chain alcohol moiety, and with branching have better fuel properties. Here, we engineered yeast to produce fatty acid short-chain esters, including ethyl, isobutyl and isoamyl esters using endogenously synthesized fatty acids and alcohols. Two wax ester synthases were identified, cloned and expressed. Both enzymes were found to catalyse the formation of fatty acid short-chain esters, with different alcohol preference. In order to boost the ability of yeast in producing the aforementioned esters, multiple gene disruptions were carried out to increase flux towards fatty acyl-CoAs. In addition, as native production of isobutanol is insufficient and rate-limiting in yeast, isobutanol pathway genes targeted into the mitochondria were overexpressed. By combining these engineering strategies, we successfully engineered yeast metabolism to produce fatty acid short-chain esters, including ethyl, isobutyl and isoamyl esters

164. Unleashing the 21st Amino Acid: Removing the Sequence Constraints of Selenocysteine Incorporation

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Selenocysteine is a rare, naturally occurring amino acid with a mosaic distribution across all domains of life. Unlike canonical amino acids, selenocysteine does not exist freely, and is directly synthesized on its tRNA which is pre-charged with serine. In bacteria, selenocysteine is co-translationally incorporated in response to specific opal stop codons, designated by the presence of a selenocysteine insertion sequence (SECIS) element which recruits the selenocysteine specific elongation factor and charged $tRNA^{Sec}$ needed to reassign the UGA codon. The SECIS element is a stem-loop RNA structure immediately following the UGA codon and forms part of the coding sequence in bacterial selenoproteins. With a low pKa and strong nucleophilicity, the site specific incorporation of selenocysteine has great potential for protein engineering, but the sequence constraints imposed by the adjoining SECIS element severely limit its use. A potential solution to this problem is to interface selenocysteine incorporation with the canonical translation machinery. We developed a novel genetic reporter system which depends on the formation of an essential selenyl-sulfhydryl bond to confer antibiotic resistance, allowing high-throughput screening of $tRNA^{Sec}$ variants compatible with the canonical elongation factor EF-Tu. From a $tRNA^{Sec}$ library containing a variable antideterminant region, which blocks interaction with EF-Tu, we isolated a $tRNA^{Sec}$variant which is compatible with the canonical translation machinery and can suppress amber stop codons to incorporate selenocysteine with high efficiency. Using this evolved $tRNA^{Sec}$ we have produced new recombinant selenoproteins containing structural motifs such as selenyl-sulfhydryl and diselenide bonds.

165. Crispri-Based Metabolic Pathway Balancing and Optimization

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Regardless of the host organism, metabolic pathway balancing and optimization efforts are usually required for high-value chemical production. The transcription and translation levels will vary from gene to gene and the turnover efficiencies of the enzymes and branching and/or competing pathways interfere with the output of the system substantially, which usually deviate the system's output from the optimal. To overcome these problems, robust, universal and high-throughput pathway optimization strategies are always required. Here, we demonstrate extremely versatile CRISPRi/dCas9 regulated platform technique aiming to utilize standardized biological parts that can be applied to multi-enzyme metabolic pathways. First, we maximize the copy number of the genes within *S. cerevisiae* genome by ribosomal DNA integration technique. Then, upon multiplex cloning of various synthetic guide RNA into single vector, we achieve various levels of down-regulations for each gene randomly. From a single transformation plate bearing every single possible combination of down-regulations, we screen and aim to fine-tune the gene expression levels and find the balanced/optimized metabolic pathway towards the end product. Our platform system includes single pot multiplex

cloning system, a derivative of PCR-after-ligation technique for assembly of different synthetic guide RNAs, and characterized standard transcriptional block targets for dCas9, which can be well utilized by any kind of gene of interest, making our platform system more powerful and universal.

166. An Attempt of Transplantation of Carotenoid Genes from Eukaryote to Prokaryote

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Bioproduction of useful chemicals by living organisms would have some problems with productivity. For one of the possible solutions, transplantation of the entire pathway to a well-known host cell, such like *Escherichia coli*, by gene transfer have been considered. Although the genome projects reveals all of gene set in one organism, however, it is empirically obvious that only gathering and transferring gene set for the relevant metabolic pathway isn't enough to perform the pathway in heterologous host. To elucidate possible problem with gene expression beyond the barrier of species, especially between eukaryote and prokaryote, a series of astaxanthin, carotenoid, biosynthesis genes from algae were tried to transfer to *E. coli* as a case study. *Haematococcus lacustris* (=*H. pluvialis*) NIES-144, which is one of the famous algae for astaxanthin production, was rendered for a donor. cDNA of the strain sampled at astaxanthin production phase was subjected to Next Generation Sequencer (NGS) not only to determine the sequence of carotenoid genes but also to evaluate relative expression abundance of these genes. Identified 8 genes for astaxanthin were then assembled into a plasmid with designed artificial operon by OGAB (Ordered Gene Assembly by *Bacillus subtilis*) method according to the gene expression abundance. In parallel with this, we also constructed an allelic carotenoid operon plasmid using bacterial genes obtained from Erwinia. Recombinant *E. coli* of alga operon didn't produce any astaxanthin, while that of bacterial one did. To identify possible problematic gene(s) in the algae operon, we performed to construct a series of chimeric operons comprised from gene(s) of algae and bacteria. Finally we identified that only lycopene synthesis step that is comprised from 3 genes have problem, while other 5 reaction steps than lycopene synthesis well work in *E. coli*. In the presentation, possible reason for defect of lycopene synthesis will be discussed.

167. Low-Cost Healthcare Diagnostics by Directed Evolution of Peptide Receptors in Yeast

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Low cost, point-of-care (POC) diagnostics for specific proteins would have imminently useful applications in resource-poor healthcare, as well as counter-bioterrorism (detecting pathogens), and environmental monitoring (detecting contamination). However, existing technologies for protein detection are either too expensive or too bulky for portability (mass spec) or not suitable for continuous monitoring and have limited analyte possibilities (lateral flow immunoassays, i.e., pregnancy tests). To date, the majority of Synthetic Biology biosensing parts detect analytes in the cytoplasm and nucleus, which preclude most proteins that cannot cross the membrane.

We have developed a directed evolution approach for tuning sensitivity and specificity of G-protein coupled receptors (GPCRs). Nature has already evolved mechanisms for sensitive and specific detection of peptides and proteins using GPCRs. In particular the yeast GPCR, Ste2p, detects the 13 amino acid peptide called a-factor. Evolving Ste2p to recognize different peptide sequences would be a relatively small evolutionary step.

Using a FACS-based high throughput screen, we have evolved receptors that can detect several different peptide sequences, including sequences with homology to important clinical biomarkers for renal failure and tuberculosis. We have shown that we can alter the sensitivity and specificity of a receptor by selecting mutant receptors that respond to low concentrations of ligand (sensitivity) and not respond to non-target ligands until the concentration is very high (specificity). Evolved receptors can discriminate peptides with only one amino acid variation.

This approach should have many useful applications in Synthetic Biology, including: healthcare diagnostics, novel cell-to-cell communication (as cells can be easily engineered to produces any number of peptide sequences), and to reveal sequence-activity relationships (SARs) for GPCRs, a class of drug-target receptors that have been difficult to characterize because of difficulties crystalizing transmembrane receptors.

168. Evolving Around the Genetic Switches and Circuits

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Directed evolution is a powerful strategy to construct complex yet functional systems ranging from genetic switch, regulatory circuits, and metabolic networks. In the last SEED meeting, we developed and tested various types of positive/ negative selection systems to widely meet the demand for improving switching stringency, PoPS values, and rapidity in on/off transition. In this meeting, we would like to

show various applications of our systems ranging from new induction systems to rapidly tuning their device specificities, as well as their gathering into multi-input genetic circuits.

1. Evolving Switches
To create new induction system that strictly meet the demand from industrial sides – self cloning, use of safe and cheap induction reagents, high induction level, and high stringency-, we created plasmid-encoded regulatory systems based on Bet-operon. Due to the need to minimize the interactions with chromosome-encoded Bet operons, the prototype Bet-switches were implemented on high-copy vectors and under highly active promoters, resulting in the 'always-off' behaviour. Random mutagenesis of BetI genes, followed by ON-/ OFF- selection in the presence/ absence of choline, resulted in the isolation of dozens of switches with different properties (PoPS, stringency, IC50, ….etc). Multiple round of directed evolution also (and quickly) isolated various types of Bet-switches with (1) altered ligand specificity and/or (2) reversed phenotypes (where choline acts as co-repressor). In this presentation, we also show the directed evolution of various known switches to 'better' behave' in various context.

2. Two-input gates and circuits
Even the complex and multi-membered circuits ultimately turn-on and -off the gene expression. The switches we created was assembled into the prototype logic gates and subjected to the randomization and systematic on/ off selection in various input states. Almost all of the starter circuits malfunctioned, but we obtained rapidly obtained a series of two-input gates (AND, imply, N-imply, NAND, ….etc) with decent fidelity/ robustness.

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169. Optimization of Cellular Resource Allocation Using Global mRNA Decay

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The behavior of synthetic circuits is shaped by the availability of cellular resources and the context of the host cell as the heterologous pathway relies on host transcription-translation machinery. Global control mechanisms for host gene expression could be used to optimize cellular resource allocation by decoupling the synthetic pathway and the host cell. Inspired by viral strategies that hijack cellular resources using global mRNA decay, we developed a control system to modulate resource allocation between the host and a synthetic pathway. This mechanism exhibits a fast response and can re-distribute ribosomes. A sequence-specific RNA degrading toxin from *E. coli* was used to trigger global mRNA decay while protecting the target gene(s) of interest. Our approach demonstrates a significant enhancement of protected gene expression and relies on the presence of negative feedback loop and specific induction timing. To optimize and understand the system, genome-wide measurements of RNA and protein levels were used to identify critical target genes for protection and provide key insights into the cellular response to the toxin. These measurements revealed a two-phase dynamic behavior in which transcript abundance is significantly reduced followed by a delayed activation in a set of target genes.

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170. Probabilistic Design of RNA Based Genetic Components: Next Generation Rgr Devices

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The current disconnect between designing biological systems and realizing their construction within biological chassis necessitates multiple iterations within the design-build-test engineering framework. It has been demonstrated that by considering genetic components at the level of RNA in terms of structure and free energy it is possible to predict integrated part performance. Recently, Cas9 proteins have been repurposed and extended to be modular transcriptional regulators. To construct genetic networks based on the Cas9 platform it is necessary to both robustly and efficiently express guide RNA (gRNA) and have that gRNA function in an orthogonal and predictable manner. Previous work has efficiently expressed gRNA from robust and well characterized pol II promoters by flanking gRNA with self-cleaving ribozymes (RGRs). Our work will demonstrate how probabilistic non-comparative RNA structure prediction methods as well as kinetic based RNA folding-path simulations can inform the design and implementation of the original RGR framework as well as develop more sophisticated expression devices. Finally, the aforementioned tools are used to develop rules for the predictable and orthogonal implementation of Cas9 based genetic systems.

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171. Interfacing Microbial Styrene Production with a Biocompatible Cyclopropanation Reaction

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Iron(III) phthalocyanine has been identified as a highly active and biocompatible carbene-transfer catalyst capable of cyclopropanating styrene generated by engineered *E. coli* metabolism. The biocompatible reaction is non-toxic to the producing organism and performs alongside cellular styrene output, enabling the near-quantitative production of various phenyl cyclopropanes directly from D-glucose in single vessel fermentations.

172. Implementation of Synthetic Metabolic Pathways for the Production of 2,4-Dihydroxybutyric Acid

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2,4-Dihydroxybutyric acid (DHB) is a molecule with considerable potential as a versatile chemical synthon. Notably, it may serve as a precursor for chemical synthesis of the methionine analogue 2-hydroxy-4-(methylthio)butyrate, thus, targeting a considerable market in animal nutrition. However, petrochemical synthesis of DHB is not economically viable, and no natural metabolic pathways exist for the biochemical production of DHB. We have therefore conceived three synthetic metabolic pathways for the synthesis of DHB starting from the natural metabolites malate or homoserine.

The two pathways departing from malate proceed via activation of malate by phosphorylation or acylation to yield malyl-phosphate or malyl-CoA, respectively. These compounds are then subject to two successive rounds of reduction to yield DHB via the common intermediate malate semialdehyde.

Synthesis of DHB from homoserine requires deamination of homoserine to yield 2-oxo-4-hydroxybutyrate followed by the reduction of the latter to obtain DHB.

The pathways employ several previously unreported enzymatic activities. Those activities were obtained by rational design based on structural and mechanistic knowledge, and/or by screening of candidate enzymes acting on sterically similar cognate substrates.

Each of the individual pathways has its particular thermodynamic and physiological requirements which were met by rational strain design. The pathways were expressed in genetically optimised *Escherichia coli* strains, and production of DHB from glucose was demonstrated.

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173. Role of Positive and Negative Feedback in Dynamic Expression of *E.coli* MarA

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Bacteria can stochastically express stress response genes, allowing a subset of a given population to survive sudden changes to the environment. This 'bet-hedging' approach maximizes fitness in variable environments without overtaxing the cells. The multiple antibiotic resistance activator (MarA) in *Escherichia coli* is a transcriptional activator that can trigger more than 40 downstream genes related to stress response. Preliminary data indicates that MarA's expression is dynamic, using a series of stochastic pulses. We hypothesize that the stochastic pulsing behavior of MarA is driven by the interaction between positive and negative feedback loops controlling the marRAB operon. However, it is not yet clear how the interaction between the two feedback loops affects the pulsing dynamics. Our research goal is to examine how positive and negative feedback regulating marRAB may cause pulsing in MarA expression. To test this, we are constructing systems which contain either only positive or only negative feedback using transversion mutations in the MarR or MarA binding sites in the promoter of the marRAB operon. Single-cell microscopy will be employed to measure the expression of MarA expression levels over time. Previous computational results from our group indicate that pulsing in MarA expression may be caused by the interlinked positive and negative feedback loops. However, with only positive or only negative feedback controlling the marRAB operon, MarA expression will lose the stochastic pulsing behavior, and instead exhibit continuous high or low levels of MarA expression.

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174. Massively Parallel High-Order Combinatorial Genetics by Combigem in Human Cells

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The systematic analysis of combinatorial genetic interactions, which play significant roles in regulating complex biological traits, has been limited in the throughput and order of complexity of genetic combinations that can be studied through current methods. To overcome these bottlenecks and accelerate the study of combinatorial genetics in human systems, we created the CombiGEM (Combinatorial Genetics *En Masse*) technology for the scalable and easy assembly of barcoded combinatorial genetic perturbation libraries. CombiGEM enables multiplexed quantification of all members in a given combinatorial genetic library by using next-generation sequencing technologies. The genetic elements included in CombiGEM libraries can be arbitrary, including gene expression/knockdown constructs, microRNAs, synthetic-biology circuit components, and programmable genome editing tools. We generated high-coverage combinatorial libraries comprising two-wise and three-wise barcoded genetic components in a lentiviral delivery system for efficient and stable genomic integration in human cells. We have validated the entire pipeline for complex genotype-to-phenotype mapping, including combinatorial library assembly, library verification, pooled screening assays with barcode sequencing, computational analysis, and hit validation, in a relevant human cell model of disease. More broadly, our work establishes a powerful platform for the high-throughput profiling of multifactorial genetic combinations that regulate a myriad of phenotypes relevant to biomedical, biotechnology, and basic science applications.

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(DP2 OD008435), the Office of Naval Research, and the Ellison Foundation New Scholar in Aging Award, and the Croucher Foundation.

175. Design Space Exploration of 1,4-Butanediol (BDO) Biosynthesis Pathway in a Cell-Free Transcription-Translation (TX-TL) System

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Current methods for assembling metabolic pathways require a process of repeated trial and error and have a long design-build-test cycle. Further, it remains a challenge to precisely tune enzyme expression levels for maximizing target metabolite production. Recently it was shown that a cell-free transcriptional-translation system (TX-TL) can be used to rapidly prototype novel complex biocircuits as well as metabolic pathways. TX-TL systems allow protein expression from multiple DNA pieces, opening up the possibility of modulating concentrations of DNA encoding individual pathway enzymes and testing the related effect on metabolite production. In this work, we demonstrate TX-TL as a platform for exploring design space of metabolic pathways using a 1,4-BDO biosynthesis pathway. Using TX-TL, we verified enzyme expression and enzyme activity and identified the conversion of 4-hydroxybutyrate to downstream metabolites as a limiting step of the 1,4-BDO pathway. We further tested combinations of various enzyme expression levels and found increasing downstream enzyme expression levels improved 1,4-BDO production.

176. Design and Construction of a Switchable Bistable Switch in Yeast

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The genetic bistable switch is a fundamental building block for engineering synthetic living systems. In synthetic biology, researchers have designed and constructed rudimentary bistable switches in *E. coli* and in mammalian cells. However, it remains challenging to construct a reusable, modular, switchable bistable switch in eukaryotes. Here we describe a design for such a bistable switch in *S. cerevisiae* (yeast), develop a predictive mathematical model, and present experimental results demonstrating the functionality, and in particular, the switchability, of the switch.

177. Anti-Virulent Disruption of Pathogenic Biofilms Using Synthetic Quorum-Based Technology

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The rapid emergence of multi-drug resistant bacteria has accelerated the need for novel therapeutic approaches to counter life-threatening infections. The persistence of bacterial infection is often associated with quorum-sensing-mediated biofilm formation. Thus, the disruption of this signaling circuit presents an attractive anti-virulence strategy. Quorum-quenching lactonases have been reported to be effective disrupters of quorum-sensing circuits. However, there have been very few reports of the effective use of these enzymes in disrupting bacterial biofilm formation. Here, we present the use of Synthetic Quorum-Based Technology to disrupt biofilm formation in a clinically relevant *A. baumannii* S1 strain through the use of an engineered quorum-quenching lactonase. *Acinetobacter baumannii* is a major human pathogen associated with serious hospital-acquired infections, and its virulence is attributed predominantly to the persistence of its biofilms. The engineered lactonase treatment achieved significant *A. baumannii* S1 biofilm reduction. This study also showed the possibility of using engineered quorum-quenching enzymes in future treatment of biofilm-mediated bacterial diseases.

178. Metabolic Engineering of *Saccharomyces cerevisiae* for the Overproduction of Short Branched-Chain Fatty Acids

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Short branched-chain fatty acids (sBCFAs, C4-6) are versatile platform chemicals in high demands because they can be converted to a wide range of valuable products in the manufacturing, pharmaceutical and food industry. For example, their esters are used for the production of plastics, plasticizers, surfactants, textile auxiliaries, fragrances and flavorings. Currently, sBCFAs are mainly synthesized chemically, which can be costly and causes pollution. In order to develop a more economical and environmentally friendly route for sBCFAs production, we engineered *Saccharomyces cerevisiae*, a model eukaryote of industrial significance, for the overproduction of sBCFAs. *S. cerevisiae* natively produces the sBCFAs isobutyric acid, isovaleric acid, and 2-methylbutyric acid from branched-chain amino acids via the Ehrlich pathway. However, the natural production levels of these sBCFAs are very low in *S. cerevisiae*. In order to utilize *S. cerevisiae* as an efficient cell factory for high-level production of our target sBCFAs, we optimized the endogenous Ehrlich pathway for

sBCFA biosynthesis. We achieved overproduction of sBCFA in yeast by overexpressing pathway genes, knocking out genes in competitive pathways, reengineering native transporters and optimizing growth conditions. Our engineered yeast can thus serve as a platform strain for biosynthesis of valuable sBCFA-derived chemicals.

179. Dynamic Control of ERG9 Expression for Improved Amorpha-4,11-Diene Production in *Saccharomyces cerevisiae*

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Background
To achieve high-level production of non-native isoprenoid products, it requires the metabolic flux to be diverted from the production of sterols to the heterologous metabolic reactions. However, there are limited tools for restricting metabolic flux towards ergosterol synthesis. In the present study, we explored dynamic control of ERG9 expression using different ergosterol-responsive promoters to improve the production of non-native isoprenoids.

Results
Several ergosterol-responsive promoters were identified using quantitative real-time PCR (qRT-PCR) analysis in an engineered strain with relatively high mevalonate pathway activity. We found mRNA levels for ERG11, ERG2 and ERG3 expression were significantly lower in the engineered strain over the reference strain BY4742, indicating these genes are transcriptionally down-regulated when ergosterol is in excess. Further replacement of the native ERG9 promoter with these ergosterol-responsive promoters revealed that all engineered strains improved amorpha-4,11-diene by 2~5-fold over the reference strain with ERG9 under its native promoter. The best engineered strain with ERG9 under the control of PERG1 produced amorpha-4,11-diene to a titer around 350 mg/L after 96 h cultivation in shake-flasks.

Conclusions
We envision dynamic control at the branching step using feedback regulation at transcriptional level could serve as a generalized approach for redirecting the metabolic flux towards product-of-interest.

180. Engineering Bacterial Microcompartments to Shield Toxicity during Protein Expression and Purification

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Bacterial microcompartments (BMCs) are “organelles” that are natively produced by bacteria to organize and sequester enzymes in a biosynthetic pathway within the confines of a protein shell. BMCs are thought to shield the toxicity of pathway intermediates and increase reaction efficiency. As such, there is significant interest to engineer BMC shell proteins for biotechnology applications. Here, we express toxic proteins inside BMC shell proteins in *E. coli* as a platform for protein expression and purification. We demonstrate that BMCs can shield toxicity of these proteins during expression. Our results have broad utility to improve cytoplasmic expression of toxic proteins for protein purification applications.

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181. Secure Offline Communication of Short Messages with DNA

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The Internet has revolutionized communication with its great speed and volume but remains vulnerable to security breaches. For applications where security is more important than speed, the offline transfer of data remains vital. Moving beyond pen and paper, DNA is increasingly being used as a medium for information storage and communication. Inspired by one-time pads, considered to be an unbreakable form of encryption, we present a rational strategy for designing individualized keyboards (iKeys) that are amenable to randomization, serve as a linguistic platform for high-density encoding of plaintext into DNA, and achieve the first instance of chromatogram patterning through multiplexed sequencing. We used an iKey in combination with a secret-sharing system we call Multiplexed Sequence Encryption (MuSE) for the secure offline communication of information that is disseminated across multiple DNA strands, but can be extracted in one step. By recreating a World War II communication from Bletchley Park, we demonstrate that watermarks, a key, a message, and a decoy can be written on DNA and the correct information is revealed only if specific strands are co-sequenced. These technologies enable facile encoding and decoding of high-density information within DNA for secure offline communications.

182. Spytag/Spycatcher: Programmable Protein Assembly for Synthetic Biology

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Protein-protein interactions in biology are generally weak and temporary. Yet many applications in synthetic biology require the stable and long-term assembly of proteins post-translationally. Conventional assembly methods that

rely on non-covalent interactions lead to the engineering of unstable and transient molecular architectures that are not ideal for most downstream applications. Here we present the application of the SpyTag/SpyCatcher covalent tagging system for genetically programmable and irreversible engineering of covalent protein architectures both *in vitro* and *in vivo*. We demonstrate covalent protein assembly for engineering living materials using biofilms and nanoparticles that bridge the biotic-abiotic interphase. Furthermore, we demonstrate the intracellular assembly of a resistance marker for the maintenance of dual plasmids with a single origin, for applications in molecular steganography for DNA camouflage.

183. Engineering of an NADPH/Nadp+ Redox Sensor in *Saccharomyces cerevisiae*

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Last two decades have seen an enormous progress in metabolic engineering as an enabling technology for a biosustainable society. By introducing a heterologous pathway into a microbial host and rewiring the carbon fluxes, it is often achievable that a new compound can be produced with improved titer, rate or yield. However, the improvement may be largely limited by non-optimal flux distribution or imbalanced cofactors, including NADPH, a reducing equivalent for the biosynthesis of many economically important chemicals. Due to the technical challenges, analysis of this cofactor can only be performed at a low throughput. To facilitate high-throughput screening of strains with increased reducing equivalent capacity, we developed an NADPH/NADP+ redox sensor in the yeast *Saccharomyces cerevisiae* by exploiting the native oxidative stress defense system, namely, the yeast transcription factor Yap1, and the promoter of its target gene, TRX2. When coupled with yeast enhanced green fluorescent protein (yE*GFP*), our sensor-reporter can generate an output of up to 10-fold increase induced by diamide, a disulfide generating oxidant, as well as by genetic modifications that hamper NADPH production. In this study, we built a sensor-actuator circuit to demonstrate the capability to select strains with better NADPH regeneration capacity. In principle, this approach can be scaled up for screening of a larger mutant library to identify novel target for metabolic engineering. The sensor can also be applied to drive the expression of NADPH-generating enzymes to rescue the cofactor deficiency.

184. Illinois Biological Foundry for Advanced Biomanufacturing (iBioFAB)

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Inspired by the exponential growth of the microelectronic industry, synthetic biologists have been attempting to build biological foundries for rapid prototyping and manufacturing of biological systems for synthesis of bioproducts ranging from chemicals to materials to therapeutic agents. In this talk, I will introduce the Illinois Biological Foundry for Advanced Biomanufacturing (iBioFAB) that we recently established at UIUC, which consists of (i) a computational design framework that automates the design-build-test-analyze cycle and (ii) an integrated automated robotic system that automates DNA cloning, DNA assembly, heterologous expression, and product detection. This iBioFAB features a 6vdegree of freedom articulated robotic arm that travels on a 5-meter-long track and transfers microplates among more than 20 instruments installed on the platform; a sophisticated sample tracking system; and libraries of parts and modules for a variety of platform organisms (see video: http://youtu.be/Hwb735qZIQ). To demonstrate its unprecedented power, the iBioFAB was recently used to synthesize up to 1000 TALENs per day with dramatically reduced cost for large-scale genetic editing application. In addition, the iBioFAB has been used to automate the entire process of the RNA interference (RNAi)-assisted genome evolution (RAGE) method we recently developed for metabolic engineering of *Saccharomyces cerevisiae* (1). Through iterative cycles of creating a library of RNAi induced reduction-of-function mutants coupled with high throughput screening or selection, RAGE can continuously improve target trait(s) by accumulating multiplex beneficial genetic modifications in an evolving yeast genome. Previously, we used the manual RAGE method to improve acetic acid tolerance (1) and the furfural tolerance (2), two key traits for microbial production of chemicals and fuels from cellulosic materials. Recently, we augmented the genetic diversity created by the RAGE method by including a library of cDNA overexpression induced enhancement-of-function mutants and further developed a fully automated modified RAGE method using the iBioFAB. We then used this new method to dramatically improve the glycerol utilization rate and cellulase production level in addition to further increasing the acetic acid tolerance in *S. cerevisiae*.

[1] T. Si, Y. Luo, Z. Bao, and H. Zhao. "RNAi-Assisted Genome Evolution in Saccharomyces
cerevisiae for Complex Phenotype Engineering." ACS Synthetic Biolo gy, DOI: 10.1021/sb500074a.

[2] H. Xiao and H. Zhao. "Genome-wide RNAi Screen Reveals the E3 SUMO-protein Ligase Gene SIZ1 as a Novel Determinant of Furfural Tolerance in *Saccharomyces cerevisiae*." Biotechnology for Biofuels, 7:78 (2014).

186. Upgrading the Alkane Precursor Pool through Thioesterase Engineering

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In recent years microbially produced alkanes have become popular as direct drop-in fuel compounds. In 2013 Howard and colleagues introduced a highly modular alkane production pathway demonstrating that modification of the fatty acid precursor pool led to altered alkane production. However, they were unable to generate medium-length (C5 to C11), branched chain compounds. Here I propose to engineer thioesterase specificity to enable production of medium-length branched free fatty acids that can be converted into respective alkanes. In order to identify residues that are involved in specificity determination we expressed chimeras of CpFatB1 (C8) and CpFatB2 (C14) in *Escherichia coli* and measured their fatty acid profiles. We found that a region spanning 100 amino acids around the proposed binding pocket highly contributes to substrate specificity. We will use the Rosetta Enzyme Design Application to identify mutants that enable branched-chain activity in four different thioesterases (BsTES, C4/6; CpFatB1: C8; UcFatB2: C12 and CpFatB2: C14). Mutated thioesterases will be expressed in a branched-chain fatty acid production strain and free fatty acids will be extracted and measured via GC-MS. This work will amplify the pool of microbial-derived fuel compounds and provide insight into enzyme specificity determination.

187. Design Principles of Protein Organelles

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Cells use structure to catalyze and facilitate the chemical reactions of metabolism. This principle is embodied by bacterial microcompartments, a class of protein-based organelles that are found in nearly 30% of bacterial genomes. More specifically, microcompartments are capsid-like structures composed of thousands of proteins that self-assemble to form an outer shell and encapsulated cargo proteins that form a short metabolic pathway within the inner lumen. Despite knowledge of the component proteins, it is less well understood how these structures self-assemble and function *in vivo*. Here, we describe our efforts to answer these questions using a combination of synthetic expression in heterologous hosts, modeling of the reaction-diffusion process, and biochemical reconstitution of assembly. These results suggest general themes for how encapsulation can improve pathway performance and specific methods for employing microcompartments and other capsid-forming proteins as a means of engineering metabolism and biomaterials.

188. Cell-Free Systems for Synthetic Biology

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Cell-free systems provide a valuable platform for understanding, using, and expanding the capabilities of natural systems. They reduce complexity, remove structural barriers, and do not require the maintenance of cell viability. As a consequence, cell-free systems provide an unprecedented and otherwise unattainable freedom of design to modify and control biological systems. In this presentation, I will describe both top-down and bottom-up efforts to design, construct, and modify biological catalysts involved in protein synthesis. In one example, I will describe our efforts to establish the first crude extract based cell-free protein synthesis (CFPS) platform based on an *Escherichia coli* strain lacking release factor 1 (RF1) and capable of high-level batch expression (>1.5g/L) of pure proteins harboring non-standard amino acids (nsAAs). Our yields outperform the best expression of proteins with single or multiple nsAAs *in vivo* by >10x, which widens the aperture to new diverse applications in functional biomaterials and medically relevant proteins. In another example, I will describe a bottom-up approach for constructing minimal cells *in vitro*. Towards this objective, we show significant progress in building a synthetic ribosome, the most complex of the macromolecular catalysts needed to boot-up self-replication. This goal has been precluded for decades because conventional *E. coli* ribosome reconstitutions are non-physiological, and ribosomes reconstituted with *in vitro* transcribed ribosomal RNA (rRNA) are essentially non-functional. To move beyond previous limitations, we developed an integrated synthesis, assembly, and translation method (called iSAT) that enables efficient one-step co-activation of rRNA transcription, assembly of transcribed rRNA with native ribosomal proteins into *E. coli* ribosomes, and synthesis of functional protein by these ribosomes in a ribosome free S150 extract. A novel feature of iSAT is that it mimics co-transcription of rRNA and ribosome assembly as it occurs *in vivo*. We also developed a ribosome synthesis and evolution method (termed RISE) for the selection of variant ribosomes *in vitro*. We have used RISE, the first purely *in vitro* method for ribosome engineering, to evolve the large ribosomal subunit for altered functionalities. We anticipate that iSAT and RISE will aid studies of ribosome biogenesis, make possible minimal cells, and catalyze a new paradigm for the synthesis and evolution of abiological polymers. Taken together, our complementary approaches are enabling a deeper understanding of why nature's designs work the way they do and opening new frontiers for harnessing a dramatically expanded genetic code for manufacturing novel therapeutics and synthesizing genetically-encoded materials.

189. The Repeatability of Evolutionary Trajectories and Implications for Strain Optimization

Aaron Miller
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Synthetic biologists often liken microbes to chassis in which engineered circuits are placed. From this perspective, the genetic background and various functions of the cell are an unfortunate necessity, and often times a hindrance to the desired product of a given genetic circuit. However, cellular fitness is important for many applications, and much can be gained from improving the background itself. One traditional and unbiased way of doing this is to simply evolve microbes in relevant environments for an extended period of time. We have done this for 96 populations of haploid *S. cerevisiae*, evolved for 300 generations using an array of miniature chemostat bioreactors in glucose, phosphate and sulfate-limited conditions. For this study, we performed inline measurements of fitness every 50 generations by competing the evolving population against a *GFP* expressing ancestral strain. We found that populations grown in both sulfate and phosphate limited conditions reached increased levels of fitness (avg. 15% increased) after just 50 generations. These populations eventually grew up to 60% more fit than the ancestral strain after 300 hundred generations of growth. However, yeast populations grown in glucose-limited media acquired significantly lower increases in fitness (~10% on avg.) after 300 generations of growth. Thus, a given background will be variably adaptable to different environments – however in some cases (sulfate and phosphate limitation for *S. cerevisiae*) one can obtain strains with greatly increased fitness in relatively little time. We have also performed whole genome sequencing of these 96 evolved populations. Our sequencing results show that (1) nutrient transporter genes SUL1, PHO84, and HXT6,7 are frequently amplified or otherwise mutated in sulfate, phosphate, and glucose-limited environments. Frequently recurrent large effect mutations such as nutrient transporter amplifications could be used to guide traditional targeted strain engineering approaches. (2) Hundreds of other mutations are observed in these evolution experiments having varied and unknown effects on fitness. These run the gamut from canonical nutrient sensing/signaling genes to more varied and hard to rationalize mutations across most other gene functions. The large number of mutations and continual increases in fitness throughout 300 generations of evolution in a constant environment suggest that multiple mutations seen in our evolution experiments may be adaptive. The experimental evolution approach can help us bypass our lack of understanding of the additive effect of many mutations and arrive at more fit strains in relatively little time.

190. Optimizing Heterologous Pathways in Mammalian Cells via Competitive Splicing

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The application of synthetic biology in mammalian cells offers great promise for developing new tools for exploring biology and novel therapeutics to treat human disease. However, the dearth of tools for regulating gene expression quantitatively limits the phenotypic landscape that can be explored with engineered genetic circuits. This challenge is particularly evident with efforts to engineer metabolite production, for which the relative expression level of enzymes can impact yield of the final product or result in the formation of unwanted or toxic byproducts. Similarly, from a developmental perspective, nuances in expression level among multiple genes often lead to drastic changes in cell fate. I address this technological gap by engineering alternative 3' splice site selection as a mechanism for regulating and optimizing the relative expression level of multiple genes from a single, polycistronic operon. In doing so, not only have I develop a novel strategy for modulating gene expression, but I will also gain significant insight into the rules that govern RNA splicing in mammalian cells. This technology will specifically be applied to engineer essential amino acid production in human cells and to improve the efficiency of induced pluripotent stem cell conversion during cellular reprogramming, demonstrating the far-reaching capabilities and impact of this modular platform.

191. Autonomous DNA Nanosystem for Interrogating Molecular Interactions

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We describe the use of DNA to create a self-assembling nanosystem for interrogating molecular interactions. The nanosystem contains a rigid supporting frame, a cylindrical central core and a mobile ring that is coaxial with the core. Motion of the ring is influenced by several control elements whose force generating capability is based on the transition of single-stranded DNA to double-stranded DNA. These forces can be directed to act in opposition to adhesive forces between the ring and the frame. We interrogate base stacking adhesion and demonstrate detection of a soluble nucleic acid target using this nanosystem.

192. Small Molecule–Triggered Cas9 Protein with Reduced Off-Target Cleavage

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The RNA-guided endonuclease Cas9 enables simple and efficient genome editing. We have developed Cas9 nucleases that are activated by the cell-permeable small molecule 4-hydroxytamoxifen (4-HT) by inserting a 4-HT-responsive intein at specific positions in Cas9. These intein-Cas9s enable small-molecule control of genome editing, with Cas9 activity being controlled at the post-translational level. In human cells, these conditionally active intein-Cas9s demonstrate improved genome-editing specificity over wild-type Cas9.

Organized by