EBRC Engineering Biology Research Consortium

Spring 2019 Retreat Program

March 22 – 23, 2019

Boston University

www.ebrc.org

RETREAT AGENDA

Friday, March 22, 2019

EBRC Session (Ladd Room - HAW 202)	
8:00 AM	**Arrival & Registration** *Continental Breakfast Available* (Rotunda - HAW 291) *Poster Setup* (Living Room - HAW 224)
9:00 AM	**WELCOME TO THE EBRC RETREAT (Ladd Room - HAW 202)**
9:15 AM	**SESSION 1: RESEARCH TALKS - "Plants & Pests"** **Chair: Andrew Ellington (University of Texas)** • Christopher Voigt (MIT): "Transfer and Control of Nitrogenase" (25 min) • Sarabeth Buckley (Harvard Medical School and Boston University): "Microbial Production of Plant Root Enhancer from CO2" (15 min) • Federico Martin (Colorado State University): "Manipulation of transcriptional cis-regulatory modules (CRMs) to improve disease resistance in rice" (15 min) • Mike Smanski (University of Minnesota): "Engineering Species-like Barriers to Sexual Reproduction" (25 min)

10:30 AM	**EBRC UPDATES**
	• State of the EBRC (Doug Friedman)
	• Individual Membership Update (Mary Dunlop)
	• EBRC Working Group Updates
	  ○ Education (Danielle Tullman-Ercek)
	  ○ Security (James Diggans)
	  ○ Policy & International Engagement (Rick Johnson)

11:10 AM	**BREAK (Rotunda - HAW 291)**
11:30 AM	**SESSION 2: RESEARCH TALKS - "Communication"** **Chair: James Diggans (Twist Bioscience)** • Jessica Terrell (Army Research Laboratory): "Electrogenetic design for bidirectional communication at a bioelectronics interface" (25 min) • Kasia Dinkeloo (University of Texas, Austin): "Engineering modular plant-to-plant communication" (15 min) • James Parkin (Caltech): "Long-distance coordination in synthetic bacterial consortia through active cell-cell signal propagation" (15 min) • John Sexton (Rice University): "Multiplexing cell-cell communication" (15 min)
12:45 PM	**LUNCH (Rotunda - HAW 291)**

1:45 PM	**SESSION 3: RESEARCH TALKS - "Tools"** **Chair: Kathryn Brink (Tabor Lab, Rice)** • Elisa Franco (UCLA): "Dynamic control of responsive DNA and RNA scaffolds" (25 min) • Jordan Villa (University of Texas, Austin): "In vivo studies of riboswitch conformations for synthetic biology tools" (15 min) • Matthew Amrofell (Washington University in St. Louis): "Developing RNA regulators that enable predictable and tunable control of gene expression" (15 min) • Scott Walper (Naval Research Laboratory): "Building a Molecular Toolbox for *Lactobacillus* Species" (25 min)
3:30 PM	**ROADMAP PRESENTATION** **Chair: Emily Aurand** Representatives of the Roadmapping Working Group will present the history, scope, and content of the Roadmap and the plans for review, release, and roll-out. EBRC members are requested and encouraged to provide feedback on the Roadmap content. • Jay Keasling (University of California, Berkeley), Working Group Chair • Ute Galm (Zymergen) • James Carothers (University of Washington) • Pamela Peralta-Yahya (Georgia Tech)
4:15 PM	**BREAK (Rotunda - HAW 291)**

	PIs and Industry Members	**Students & Postdocs (Ladd Room - HAW 202)**
4:30 PM	**EBRC Working Group Meetings** • Technical Research Roadmapping (Computer Room - off Rotunda) • Education (HAW325) • Security (Brookline Room) • Policy & International Engagement (HAW324)	**SPA Workshop: "Assessing your Research for Security Implications"** • Facilitated by Clem Fortman
5:45 PM		**EBRC Student & Postdoc Association Business Meeting** • Cassandra Barrett & the SPA Board
5:50 PM		**Prepare for Poster Session / Break**

6:00 PM	• Poster Session (Living Room - HAW 224) • Odd-numbered Poster Presentation Time: 6:05 - 6:50 • Even-numbered Poster Presentation Time: 7:00 - 7:45
8:00 PM	**Adjourn for Day 1**
8:30 PM	**SPA-Hosted Social Event** The Boston University Pub | 225 Bay State Rd, Boston, MA 02115 All participants are encouraged to attend! Don't forget to bring your ID.

Saturday, March 23, 2019

EBRC Session (Ladd Room - HAW 202)	
8:00 AM	**Day 2 Arrival** *Continental Breakfast Available (Rotunda - HAW 291)*
9:00 AM	**WELCOME TO DAY 2**
9:05 AM	**SESSION 4: RESEARCH TALKS - "Therapeutics"** **Chair: Karmella Haynes (Emory)** • Yvonne Chen (UCLA): "Engineering Next-Generation T Cells for Cancer Immunotherapy" (25 min) • Christopher Stach (University of Minnesota): "Synthetic biology platform for modifying glycosylation of biopharmaceuticals" (15 min) • Suzie Hsu (University of Minnesota): "Genetic Design and Optimization of Natural Product Biosynthesis in Streptomyces" (15 min) • Nigel Mouncey (DOE Joint Genome Institute): "New Voyages to Explore the Natural Product Galaxy" (15 min)
10:20 AM	**KEYNOTE ADDRESS:** **Chair: Doug Friedman** • Chris Hassell (US Dept of Defense): "Biodefense in the Age of Synthetic Biology."
10:50 AM	**BREAK (Rotunda - HAW 291)**
11:10 AM	**SESSION 5: RESEARCH TALKS - "Security"** **Chair: Clem Fortman** • Jacqueline Fletcher (Oklahoma State University): "Agricultural Promise (and Peril?) in the Age of Synthetic Biology" (30 min) • Nathan Hillson (Berkeley Lab): "DNA Synthesis Science at the DOE Joint Genome Institute: Biosecurity Sequence Screening and Broader Aspects Review" (20 min) • Natasha Bajema (National Defense University): "Biological Weapons in the Digital Age: Understanding the Future Landscape" (20 min)
12:15 PM	**LUNCH (Rotunda - HAW 291)**

1:15 PM	**SESSION 6: RESEARCH TALKS - "Engineering Biology in Society"** **Moderator: Doug Friedman** • Laurie Zoloth (University of Chicago): "The Ethics of the First Choice: Research and Public Voice" (5 min) • Megan Palmer (Stanford): "Managing Problems & People at the Technology and 'Policy' Interface" (5 min) • Ken Oye (MIT): "Assessing and Addressing Risks: A Brief Lesson from the Past, Plan for the Future" (5 min) • Emma Frow (Arizona State): "Experiments in Governing Synthetic Biology" (5 min)
2:15 PM	**BREAK (Rotunda - HAW 291)**
2:30 PM	**SESSION 7: RESEARCH TALKS - "Synthesis"** **Chair: Rick Johnson (GlobalHelix, LLC)** • Maneesh Gupta (US Air Force Research Laboratory): "Bacterially-produced melanin as biomaterials" (25 min) • Gordon Rix (University of California, Irvine): "Continuous directed evolution of tryptophan synthase toward production of unnatural aromatic amino acids" (15 min) • Kamil Gedeon (RPI): "Co-Culture of Engineered *Escherichia coli* for Violacein Production from Hemicellulosic Sugars" (15 min) • Vanessa Varaljay (UES / AFRL): "Phloroglucinol Tri-Service Effort" (15 min)
3:45 PM **4:00 PM**	**CLOSING REMARKS** **ADJOURNMENT**

EBRC TECHNICAL RESEARCH ROADMAP

Engineering Biology: A Research Roadmap for the Future Bioeconomy

The EBRC Roadmap is a critical assessment of the current status and potential of engineering biology. It is intended to provide researchers and other stakeholders (including government funders) with a compelling set of technical challenges and opportunities in the near and long term. Our ongoing roadmapping process was initiated in response to the recommendations put forth in the 2015 National Academies report, Industrialization of Biology, and at the request of the National Science Foundation and other US government stakeholders, including the National Science & Technology Council. With this inaugural release of the Roadmap, EBRC will provide a "go-to" resource for engineering biology research and related endeavors.

Working with the broader EBRC community, the Technical Roadmapping Working Group led the development of the roadmap scope and content. Collective insight and input was gleaned from more than 70 leading scientists and engineers - from the working group members to academic, industry, and student members from both EBRC and the broader research community. Over the past year, the working group held five workshops and countless teleconferences to develop the content and engage discussion around the roadmap. The result is a collaborative effort of the engineering biology research community and represents the community's vision for the future of the field.

The matrixed framework of the Roadmap focuses on the development and advancement of tools and technologies in engineering biology and their potential applications and impact. The Roadmap considers challenges, bottlenecks, and other limitations existing in research and development and application objectives toward achieving societal goals and overcoming existing challenges. The four technical themes represent a bottom-up approach focusing on tool and technology advancements and innovations to move the field forward, while the five application and impact sectors are a top-down look at how engineering biology might be used to address and overcome national and global challenges. The current draft focuses exclusively on the technical elements of engineering biology advancement. Ethical, policy, security, and education considerations are included in an ongoing effort that will further elaborate these areas of research and impact.

We need your feedback on this draft! The Roadmapping Working Group is currently seeking additional contribution, content review, and feedback from EBRC members on the roadmap content, found at www.ebrc.org/2019roadmap. We'd like to ensure that the roadmap reflects your vision of the field -- Have we identified the most important technical breakthroughs and transformative tools and technologies? Have we missed anything significant? Are the early milestones we've identified attainable and are the later milestones sufficiently visionary and aspirational? And will the engineering biology achievements we've identified meaningfully help to solve the societal challenges? Please provide your feedback via email to roadmap@ebrc.org.

This content will be shared until APRIL 22, 2019, so please submit your comments by that time to ensure they're incorporated into the final version.

Requests and recommendations for your feedback:

- Please provide the title of the Roadmap section (i.e. the document title) and indicate the page number(s) to which you are commenting or would like to add content (including references). The more explicit you are, the easier it is for us to incorporate your ideas.
- Feedback can include suggestions and/or ideas for future content.
- You may use the above questions as framing for your feedback, but all comments (did you catch a typo?) are appreciated.
- Emily and/or the Working Group may respond to your feedback with further request for contribution or clarification.
- Alternatively to email, you can also ask questions and provide feedback by calling Emily at 501.871.3272, ext. 4.

Thank you for your help in making the 2019 Roadmap reflective of our community and expertise!

KEYNOTE

"Biodefense in the Age of Synthetic Biology"

Chris Hassell, PhD
Deputy Assistant Secretary of Defense for Chemical and Biological Defense
US Department of Defense

As the Department of Defense's Chemical and Biological Defense Program looks at the potential concerns posed by advanced biological threats emerging in the future, it also sees opportunities presented by engineering biology to defend and protect its forces. Sound analyses and outreach to academia, industry and international partners are cornerstones of this mitigation strategy. Potential opportunities exist to advance mitigation capabilities across detection, decontamination, diagnostics, and platforms for the development and manufacturing of medical treatments.

RESEARCH TALK ABSTRACTS

SESSION 1: PLANTS AND PESTS
Chair: Andrew Ellington, University of Texas

"Transfer and Control of Nitrogenase"

Chris Voigt, MIT

I will describe our recent, unpublished work in engineering self-fertilizing cereals. This includes the development of sensors and circuits for non-model soil bacterium (e.g., *Azorhizobium*) that respond to root exudates by turning on complex, oxygen tolerant nitrogenase pathways. I will also describe work to create an in vitro TXTL system for chloroplasts and the rapid prototyping of nif pathways prior to plant engineering.

"Microbial Production of Plant Root Enhancer from CO2"

Sarabeth Buckley, Harvard Medical School and Boston University (Silver Lab)

In the face of climate change, we must develop a variety of creative ways to sequester carbon. We are attempting to use an artificial photosynthesis system to produce a more sustainable carbon-based fertilizer or root enhancer to increase crop yields and decrease required traditional fertilizer application. The molecule is lipochitooligosaccharides (LCO), which functions natively as a signal molecule produced by *Bradyrhizobium japonicum* that has been found to enhance root growth and therefore overall growth. In this project we have engineered *Ralstonia eutropha,* a species capable of lithotrophic growth in an artificial photosynthesis system, to produce LCOs and measured the production rate. We have detected LCOs using a standard with HPLC and LCMS and are comparing it to LCOs produced by B. japonicum. The product was then applied to the seeds of various plants to test for a growth and germination effect. If produced successfully, this would be a viable method to help decrease CO 2 concentrations by increasing biological drawdown of carbon and displacing carbon intensive nitrogen based fertilizers while using CO 2 to make useful products in the form of fertilizer and food.

"Manipulation of transcriptional cis-regulatory modules (CRMs) to improve disease resistance in rice"

Federico Martin, Colorado State University (Leach Lab)

Defense response (DR) genes are often members of gene families and are associated with genomic regions that act as disease resistance QTLs. Identifying and increasing the number of QTLs has the potential to improve plant defense responses against diverse pathogens and stabilizing disease resistance in the field. However, trait complexity and lack of reliable genetic markers often makes the identification and transferring of favorable genes within QTLs a slow and daunting process. Multiple reports have shown that not all DR genes within a QTL are equally active during the defense response; mainly due to their transcriptional regulation. Transcriptional regulation of DR genes is influenced by the repertoire of cis-regulatory elements found in their promoter regions. We hypothesize that presence of unique groups of cis- regulatory elements, called cis-regulatory modules (CRMs), can determine the effectiveness and strength of DR genes in their response to pathogen attacks. Through the integration of data from multiple gene expression studies, we identified a series of well conserved CRMs associated with DR genes genome-wide, indicating a broad transcriptional response against diseases. Initial analyses indicate that the presence/absence of certain CRMs can influence temporal expression of DR genes. Using genome editing technologies, we are testing the possibility of improving CRM content in susceptible cultivars to enhance resistance and potentially use these signature modules as reliable genetic markers to improve breeding practices.

"Engineering Species-like Barriers to Sexual Reproduction"

Mike Smanski, University of Minnesota

We introduce a novel approach to engineer a genetic barrier to sexual reproduction between otherwise compatible populations. Programmable transcription factors drive lethal gene expression in hybrid offspring following undesired mating events. In this talk, I describe the technology, demonstrate a proof-of-concept in yeast, and share recent progress in translating the approach to insects with applications for pest control.

SESSION 2: COMMUNICATION

Chair: James Diggans, Twist Bioscience

"Electrogenetic design for bidirectional communication at a bioelectronics interface"

Jessica Terrell, Army Research Laboratory

Toward bioelectronics technology, there is a compelling opportunity to integrate biological systems with electronic devices, which have become increasingly networked and ubiquitous. However, these two entities are dissimilar in composition and information processing mechanism. Biology is organically composed and often uses molecular recognition for signal response, while electronics are typically inorganic and operate by electron flow. This work presents an approach to interface genetically programmed bacteria with electronics as a biohybrid system, addressing both challenges of physical coupling by self-assembly and communication compatibility using electrochemically-active molecular cues. First, bacteria express a peptide tailored for inorganics, which is fused to an outer membrane protein for surface display. In this way, the bacteria can selectively assemble onto an electrode surface.

Then, the cells feature a simple, modular genetic circuit for electrochemically-activated gene expression. Specifically, in a voltage-dependent manner, a charged electrode biases the oxidation state of redox-active signal molecules. This, in turn, influences gene expression based on redox-

specific recognition of the molecular cue. Further, the electrogenetic cells are demonstrated to provide multiple outputs in response to electrochemical stimulation. The cells serve as bioelectonic transducers by 1) relaying the voltage input as small molecule quorum sensing signals to other cell populations and 2) providing redox-active molecular feedback to the electronic infrastructure, which is again detectable through electrochemical analysis. Because the cells are positioned directly on the electrode, this electrochemical signal exchange can occur locally across the interface. This work demonstrates bioelectronic communication where redox-active molecules serve as interconvertible signals that carry electrons between electrodes and simultaneously allow for molecular recognition events within cells. The combination of voltage-mediated redox control and electrogenetic response establishes a bioelectronics dialogue. Bioelectronics connectivity with synthetic biology may yield future applications for programmable biohybrid devices with utility in ecological settings, wearable interfaces, and in vivo environments.

"Engineering modular plant-to-plant communication"

Kasia Dinkeloo, University of Texas, Austin (Ellington Lab)

The ability to engineer plants using the tools of synthetic biology is of increasing importance for solving agricultural problems, and for adapting plants to novel uses. Under the aegis of a DARPA proposal, a collaboration between five labs has begun to engineer plant-to- plant communication using volatile organic compounds (VOCs). By modifying and enhancing natural hormone pathways in plants we have been able to demonstrate synthetic communication. In particular, we have engineered a modular ethylene sensor, and identified modular promoter structures that are responsive to methyl salicylate and methyl jasmonate. When these are cloned adjacent to reporter genes, gas-dependent production of signals can be observed. In parallel, we have determined that carbon flux through the normal pathway for the production of the volatile ethylene may be limited, and have been able to generate transgenic plants with enhanced ethylene production. When ethylene 'senders' are aligned with ethylene 'receivers,' true plant-to-plant communication can be observed. Along the way, we have developed an extensive new tool kit that allows for the establishment of an Orthogonal Control System (OCS) in plants that operates on top of extant plant regulatory and metabolic systems. We have for the first time created wholly orthogonal transcription factors using dCas9:VP64 as a transcription factor, and shown that these can activate completely artificial promoters. While these demonstrations have so far been shown in model plant species (*Nicotiana*, *Arabidopsis*), in parallel we have undertaken an extensive effort to demonstrate that constructs developed using the OCS and common VOC components can be transported into new species, and to this end have made great progress in 'taming' a non-model plant, the common dandelion, *Taraxacum*. Ultimately, by funneling engineered sensor 'inputs' through VOC communication channel to appropriate reporter 'outputs' it should be possible to allow fields of plants to better serve as self-sentinels against pests and other environmental incursions.

"Long-distance coordination in synthetic bacterial consortia through active cell-cell signal propagation"

James Parkin, Caltech (Murray Lab)

A synthetic cell-cell signaling circuit should ideally be (1) metabolically lightweight, (2) insulated from endogenous gene networks, and (3) excitable rather than oscillatory or bistable. To accomplish these three features, we propose a synchronized pulse-generating circuit based on the design of published synchronized oscillators. This communication module employs a pulse generator built using Lux-type quorum sensing components and an IFFL transcriptional circuit. Both the input and output of this module are AHLs, the quorum sensing signaling molecule. Cells bearing this module therefore act as an excitable medium, producing a pulse of AHL when

stimulated by exogenous AHL. Using simulation and microscopy, we demonstrate how this circuit enables traveling pulses of AHL production through microcolonies growing in two dimensions. Traveling pulses achieve cell-cell communication at longer distances than can be achieved by diffusion of signal from sender to receiver cells and may permit more sophisticated coordination in synthetic consortia. We will present ongoing efforts to demonstrate an example of sophisticated coordination, a two-input time-dependent AND gate, constructed from two orthogonal, long-distance pulsatile signaling circuits. This circuit would enable the consortium as a whole to sense the coincidence of a combination of environmental signals when it would be impossible for any individual cell to do so.

"Multiplexing cell-cell communication"

John Sexton, Rice University (Tabor Lab)

The engineering of advanced multicellular behaviors, such as programmed tissue differentiation, requires individual cells to communicate multiple aspects of physiological information. Unfortunately, few cell-cell communication systems have been developed for synthetic biology. Here, we engineer a genetically-encoded channel selector that enables a single communication system to transmit multiple intercellular conversations. Our design comprises multiplexer and demultiplexer sub-circuits constructed from 12 CRISPRi-based transcriptional logic gates, an acyl homoserine lactone-based communication module, and three inducible promoters. Experimentally-parameterized mathematical models of the sub-components accurately predict the steady state and dynamical behavior of the complete system. Multiplexed cell-cell communication has applications in synthetic development, metabolic engineering, and other areas requiring the coordination of multiple pathways amongst a community of cells.

SESSION 3: TOOLS

Chair: Kathryn Brink, Rice (Tabor Lab)

"Dynamic control of responsive DNA and RNA scaffolds"

Elisa Franco, UCLA

Biological systems reconfigure their shape at the level of single cells, tissues, and organs in response to external stimuli, for a variety of purposes such as growth, development, and self-repair. Cell shape reconfiguration is accomplished by directing molecular materials (such as cytoskeletal proteins) through molecular circuits which sense, process, and transmit information from the environment. Although they are often organized in a modular fashion, these cellular pathways are still too complex to be directly embedded in a biosynthetic material. An alternative route is offered by nucleic acid nanotechnology: sensors, logic, and dynamic circuits [1, 2, 3, 4], and a variety of nanostructures [5, 6, 7] have been successfully demonstrated. We aim at recapitulating the cellular organization of dynamic biomaterials in a synthetic system where assembly and disassembly of nucleic acid scaffolds is controlled by dynamic nucleic acid inputs and circuits.

We have built DNA and RNA self-assembling nanotubes that can respond to a variety of inputs that include other nucleic acid molecules, pH, and enzymes. These nanotubes assemble from nanoscale tiles, and are some of the largest known nucleic acid nanostructured assemblies [5]: they reach tens of microns in length and present mechanical properties similar to actin filaments and microtubules. We have engineered DNA [8, 9] and RNA tiles [10, 11] to include actuation domains to which nucleic acid inputs can bind thereby triggering assembly or disassembly. For example, we added a single-stranded overhang to the tile binding domains, or

sticky-ends, to promote disassembly when an "invader" strand is added in solution. Removal of the "invader" species allows the nanotubes to reassemble isothermally, making it possible to control growth reversibly. We have shown that it is possible to control nanotube assembly and disassembly with nucleic acid inputs directly added in solution [8], with a nucleic acid pH sensor [9], and with synthetic transcriptional systems [12]; in particular, we showed that nanotube length can be controlled with an autonomous molecular oscillator [8, 3, 13, 14]. We have also designed hybrid RNA-DNA nanotubes whose assembly and disassembly can be directly controlled by enzymes that produce and degrade RNA.

These dynamic assembly processes can be modeled quantitatively with a coarse- grained model of differential equations capturing the nanotube length distributions [15]. These responsive nucleic acid structures could be used as scaffolds for dynamic heterogeneous materials [16], and as scaffolds for artificial protocells. Current studies are aimed at engineering these nanotube systems for resilience in the cellular environment [17] and encapsulation in droplets and vesicles.

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"In vivo studies of riboswitch conformations for synthetic biology tools"

Jordan Villa, University of Texas, Austin

Riboswitches are located in the untranslated regions of mRNAs and regulate mRNA transcription/translation upon binding a particular ligand. The ability of riboswitches to adopt distinct structural confirmations upon binding specific ligands enables them as useful regulators of bacterial gene expression. In fact, this specificity to certain metabolites coupled with the ability to regulate the corresponding coding RNA makes riboswitches excellent synthetic biology tools, such as switches or biosensors. However, the utilization of riboswitches has been hindered by the lack of design principles for transportability to ensure proper riboswitch function in a diverse set of organisms and conditions. Additionally, discovery of new riboswitches or new ligands for existing riboswitches is often time consuming and can easily yield nontransportable regulators. Considering the importance of conformational changes in riboswitch function, we aim to characterize the conformational changes that occur upon ligand binding in a model synthetic theophylline riboswitch via the in vivo RNA Structural Sensing System (iRS 3) that determines RNA accessibility in vivo using a fluorescent reporter. Additionally, we are optimizing this system for multiple organisms, including *Deinococcus radiodurans*, where structural characterization and discovery of riboswitches has been limited thus far, despite the possible applications based on this organism's resistance to oxidative stresses. Upon validation of our in vivo system to detect changes in riboswitch structural conformations, we propose to utilize this structural technique to screen for

new target ligands in vivo by finding compounds that result in conformational changes indicative of regulatory function.

"Developing RNA regulators that enable predictable and tunable control of gene expression"

Matthew Amrofell, Washington University in St. Louis

RNA regulators are highly useful parts in a synthetic biologist's toolbox. These regulators are modular, generally exhibit low metabolic burdens, and can orthogonally control gene expression. Furthermore, RNA-based systems can either repress (e.g., using antisense RNAs [asRNA]) or activate (e.g., using toehold switches) gene expression. While previous efforts have demonstrated the utility of regulatory RNAs in complex genetic circuits, their widespread implementation remains limited due to their unpredictable behavior in different cellular environments. To address this problem, we recently developed a simple multivariate model that predicts asRNA-mediated repression. We have tested 434 different strain-asRNA combinations, validating the predictive model in multiple target genes (including both endogenous and heterologous genes) and in multiple biotechnologically-important organisms (including both Gram-positive and Gram-negative bacteria). In addition, we demonstrated a novel way to tune outputs of toehold switches. Specifically, using computational tools, we were able to rationally design and build a 2-input AND gate and multiple tunable ultrasensitive switches (with apparent Hill coefficients from 2.8 to 9.5), expanding the utility of toehold switches. Our work will enable predictable and tunable control of gene expression through RNA regulators.

"Building a Molecular Toolbox for Lactobacillus Species"

Scott Walper, Naval Research Laboratory

The continued innovation of molecular biology tools has allowed microbial engineering as a potential solution to critical problems to become a realistic possibility. Considering the scope of problems to be addressed by microbial engineering, lab strains such as *E. coli* K12 can limit application due to growth and survival characteristics. *Lactobacillus* species, on the other hand, can be more attractive candidates for eventual human use as they are generally regarded as safe (GRAS) commensals. Using rational plasmid design and published proteomic data, we wanted to develop a broad host-range shuttle vector for *Lactobacillus* to demonstrate the feasibility of engineering commensals. Different replicons, selective markers, and promoters were assembled as shuttle-vector candidates to function in multiple genera and species. The most stable combinations contained the pWV01 replicon or a truncated version with dual antibiotic selection markers to increase the efficiency of transitioning between species. Plasmid function was determined by assessing superfold GFP (sfGFP) expression from native promoter candidates identified by previously published proteomic data, and promoter strength was determined over time by fluorescence and RT-qPCR methods. Five promoters with varying expression levels in *L. plantarum* were identified, and the best of which was tested in other *Lactobacillus* species. Plasmid stability was tested by culturing without antibiotic selection, and some species were able to maintain plasmids for 11 days in such conditions. Our functional shuttle vectors can be used to engineer *Lactobacillus* species, among others. The assembly method and dual antibiotic selection facilitate a user-friendly and cost-effective protocol with a short turnaround time. In addition, plasmid stability of this magnitude offers the potential to observe long-term results without relying on antibiotics or genome editing techniques.

SESSION 4: RESEARCH TALKS - "Therapeutics"

Chair: Karmella Haynes, Emory

"Engineering Next-Generation T Cells for Cancer Immunotherapy"

Yvonne Chen, UCLA

The adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has demonstrated clinical efficacy in the treatment of advanced cancers, with anti-CD19 CAR-T cells achieving up to 90% complete remission among patients with relapsed B-cell malignancies. However, challenges such as antigen escape and immunosuppression limit the long-term efficacy of adoptive T-cell therapy. Here, I will discuss the development of next-generation T cells that can target multiple cancer antigens and resist immunosuppression, thereby increasing the robustness of therapeutic T cells against tumor defense mechanisms. Specifically, I will discuss the development of multi-input receptors and T cells that can interrogate intracellular antigens. I will also discuss the engineering of T cells that can effectively convert TGF-beta from a potent immunosuppressive cytokine into a T-cell stimulant. This presentation will highlight the potential of synthetic biology in generating novel mammalian cell systems with multifunctional outputs for therapeutic applications.

"Synthetic biology platform for modifying glycosylation of biopharmaceuticals"

Christopher Stach, University of Minnesota

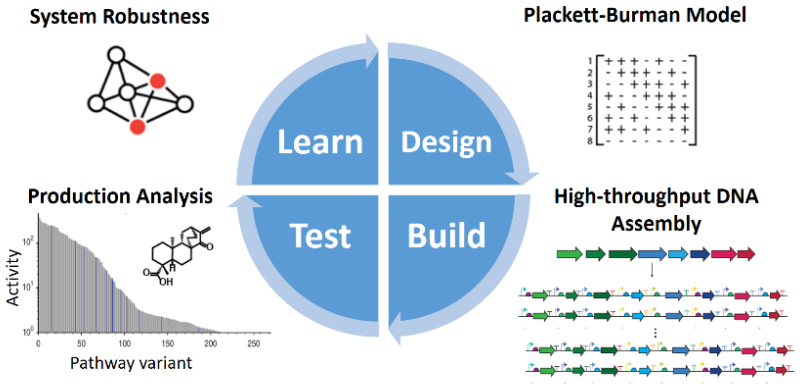
Chinese Hamster Ovary cells are used for industrial production of protein-based therapeutics (i.e. 'biologics'), but systems-level genetic engineering of beneficial traits is slow, difficult, and empirically-guided. We exploited systems- and synthetic biology approaches to design, build, and screen multi-gene constructs that rationally perturb the post-translational glycosylation of a secreted Immunoglobulin G (IgG) towards high galactose incorporation. We constructed a total of 23 clonal transgenic cell pools that express single or three-gene glycoengineering cassettes. The IgG glycan profile for each cell line was characterized by liquid chromatography and compared to known standards. We rationally increased fraction of bi-galactosylated glycan from 11.7% to 61.9% and simultaneously decreased the glycan heterogeneity on the secreted IgG. By bridging systems and synthetic biology, our approach allows for rapid hypothesis testing and quantification of synergistic behavior from genetic perturbations.

"Genetic Design and Optimization of Natural Product Biosynthesis in Streptomyces"

Suzie Hsu, University of Minnesota

Natural products, also known as secondary metabolites, are an invaluable source for high-value chemicals, many of which have important medicinal properties. These important natural products are usually difficult to synthesize chemically, and only scarce amount can be extracted from scarce amount in their natural biological sources. Hence, there has been an increasing attention to control the genetics and the biosynthesis of natural products in native or engineered organisms. I will present a synthetic biology platform for constructing, controlling and optimizing biosynthetic gene clusters via heterologous expression, a technique commonly used to express targeted genes in a more stable and genetically accessible host (Fig. A). For a model system, I used a high-throughput DNA assembly pipeline to build mini library of a completely synthetic eight-gene pathway to produce a late-stage intermediate of serofendic acid, a potent mammalian neuroprotectant, in a bacterial heterologous host *Streptomyces albidoflavus* J1074. We learned that relative expression level of individual genes matters to the final production titer, identified the a novel P450 monooxygenase required for the tailoring reaction, and isolated two new shunt metabolites. Next,

we further attempted to optimize the production titer of serofendic acid late stage intermediate by increasing precursor supply via methylerythritol phosphate (MEP) pathway. The second iteration of synthetic gene cluster (SGC) library design aimed to perturb the relative expression level of eight genes encoding MEP pathway. Preliminary analysis showed that the production titer of late-stage intermediate of serofendic acid was improved from 50 mg/L to over 500 mg/L from the library. The third iteration of SGC library design of MEP pathway was rationally guided by Plackett-Burman (PB) design, a multivariate design principle which allows screening of large number of variables while minimizing the number of tests. This will allow for efficient and rational sampling of the metabolic landscape of MEP pathway. With PB design, all 125 prototypes of the eight-gene pathway have been constructed and they are currently being screened. Over 2.5 megabases of DNA were manually fabricated into meaningful pathway library designs via the high-throughput DNA assembly pipeline.



"New Voyages to Explore the Natural Product Galaxy"

Nigel Mouncey, DOE Joint Genome Institute

Natural products are a large family of diverse and complex chemical entities that have roles in both primary and secondary metabolism, and today ~23,000 natural products have been characterized. Natural products are incredibly important molecules for mankind with uses as antibiotics, antifungals, antitumour and antiparasitic products and in agriculture as products for crop protection and animal health. We are seeing a resurgence of activity in exploring natural products for a wide range of applications, due to not only increasing antibiotic resistance, but the advent of next-gen genome sequencing and new technologies to investigate natural product biosynthesis. At the JGI, we are developing new tools and processes for identification of novel biosynthetic gene clusters from isolate and metagenomes, and complementing these with a suite of new experimental platforms to access the products of these clusters. I will showcase these approaches and discuss their integration to discover and explore the roles of novel natural products.

SESSION 5: SECURITY

Chair: Clem Fortman

"Agricultural Promise (and Peril?) in the Age of Synthetic Biology"

Jacqueline Fletcher, Oklahoma State University

The Decadal Vision for the future of plant sciences (1) points to the need, before the year 2050, to be able to harness plant systems science for the production of food, feed and fiber to support an anticipated global population of 10 billion, and to do so in the face of declining agricultural land area, climate change, and the need for environmental preservation. Continued incremental advances in crop improvement will be insufficient to meet these goals. The application of synthetic

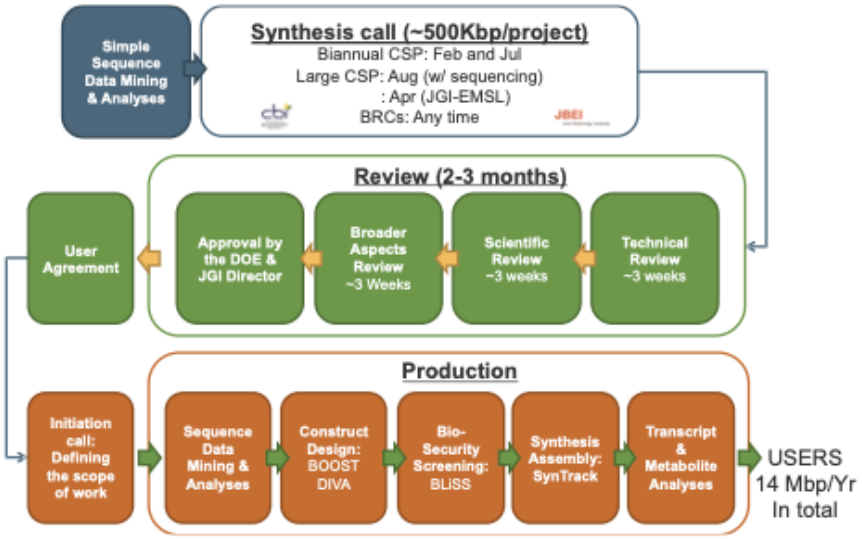
biology technology to crop improvement combines principles of engineering with mathematical analysis and modeling to the design, production and distribution of previously unimagined plant-derived food, feed, fiber, biofuels and building materials as well as plant-produced chemicals and drugs. The process will involve directed modifications to, or creation of, completely new plant biochemical pathways and of the regulatory systems that control their expression. Although applications of synthetic biology are more challenging in plants than in microbial systems due to the complexity of plant metabolism, uncertainty about evolutionary changes in plant regulatory systems, and significant public resistance to plant products produced using synthetic biology techniques, significant milestones have been met in the development of specific enhancements in plants. However, in addition to the potential for adding value and solving problems in agriculture, these powerful approaches to plant improvement present the possibility of new threats or technological mis-use, creating new vulnerabilities in agricultural biosecurity.

1) Baldwin, I., C. Benning, A. Burke, A. Caicedo, N. Carpita, M. Dillworth, R. Horsch, T. Kutchan, R. Last, S. Mackenzie, R. Riley, P. Ronald, J. Schmitt, P. Schnable, D. Stanzione, D. Stern, and C. Taylor. 2013. Plant Science Decadal Vision: An Urgent Call to Action. www.plantsummit.wordpress.com.

"DNA Synthesis Science at the DOE Joint Genome Institute: Biosecurity Sequence Screening and Broader Aspects Review"

Nathan Hillson, Berkeley Lab

Beyond DNA sequencing, the U.S. Department of Energy's Joint Genome Institute (JGI) now offers its User Community a variety of other resources, including DNA Synthesis. This talk will focus on two integral components of JGI's DNA Synthesis Science Program, namely 1) biosecurity sequence screening and 2) broader aspects review. The JGI's Black-List Sequence Screening (BLiSS) software tool implements the U.S. Health and Human Services' guidance for providers of synthetic double-stranded DNA. Recently, the JGI has extended BLiSS beyond this "best-match" guidance to also screen against curated sequence databases (of, for example, viral sequences that may not be present on select agents or commerce control lists), and to counter-screen (to automate the identification of false positive hits) against sequences likely to be involved in basic cellular functions not related to pathogenicity. In addition to biosecurity sequence screening, following technical feasibility and scientific merit review, all JGI DNA Synthesis Science projects additionally undergo a broader aspects review process, in which three reviewers with complementary domain expertise (e.g. policy/governance, ethics, legal, biosecurity) evaluate each project for its broader aspects and implications. Both BLiSS and the broader aspects review process will be described, and their corresponding process metrics data presented.



"Biological Weapons in the Digital Age: Understanding the Future Landscape"

Natasha Bajema, National Defense University

Is synthetic biology transforming the life sciences into a branch of information technology? What does that mean for the threat of biological weapons? For several decades, exponential growth in information and communication technology—a function of expanded capacity of microchips, falling costs of computing power and data storage, miniaturization of electronics, and the rise of the Internet connecting everything together—has produced the trend toward the digitization of everything--home-made plastic guns, DIY drone designs, nuclear power plant parts, jet engine parts for commercial aircraft, missile parts, rolodexes of social connections, and even genomes of living organisms. Digitization involves more than simply converting physical matter into bits, it also entails making physical objects smart, automating manual processes using digital technologies, or allowing automated systems to be remotely controlled over the Internet. As more electronic devices become smart, they not only consume and generate digital information, they are also exposed to a host of cyber-vulnerabilities that have plagued computing devices for decades. The cyber-physical interface facilitates easy transfer of information and makes technologies capable of having physical impacts through digital pathways. The phenomenon of digitization—i.e., conversion and movement of information between the physical and digital worlds—promises to exert significant impacts on the biological weapons space in the near future through a new species of emerging technologies including artificial intelligence, advanced robotics, synthetic biology and additive manufacturing.

SESSION 6: ENGINEERING BIOLOGY IN SOCIETY

Moderator: Doug Friedman

"The Ethics of the First Choice: Research and Public Voice"

Laurie Zoloth, University of Chicago

The call for public review of innovative scientific projects usually emerges after the basic research is conceptualized and the framing of the science settled. However, this was not always the case. In 1815, Britain's most important scientist, discoverer of 4 separate elements, could be found deep in a coal mine on the northeast coast of England, looking for a way to use his understanding of gases to make a technology for some of the poorest of his fellow citizens. His research choices were driven by the public desire, a process that was standard of mid-19th century science. What does this history teach scientists who are committed to public conversation about engineering biology? This talk will argue that public support for science ought to begin with a consideration of the first targets of research.

"Managing Problems & People at the Technology and 'Policy' Interface"

Megan Palmer, Stanford

"Assessing and Addressing Risks: A Brief Lesson from the Past, Plan for the Future"

Ken Oye, MIT

"Experiments in Governing Synthetic Biology"

Emma Frow, Arizona State

SESSION 7: SYNTHESIS

Chair: Rick Johnson, GlobalHelix, LLC

"Bacterially-produced melanin as biomaterials"

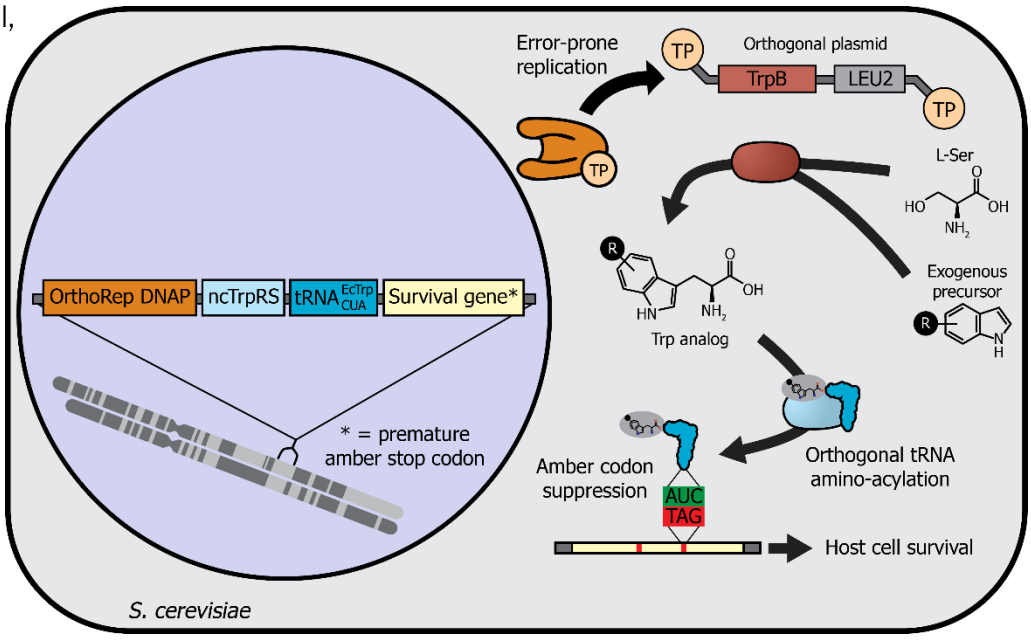
Maneesh Gupta, US Air Force Research Laboratory

Melanin, a highly-coordinated polymeric nanoparticle found in natural pigments, has many intriguing properties including protecting organisms from ultra-violet radiation, ability to scavenge reactive species and metal ions, and reported electrical conductivity. Melanin particles isolated from naturally pigmented sources are biologically templated with pre-defined shapes that dictate the optical and protective properties of the melanin assemblies. The tyrosinase enzyme is known to be involved in the biosynthesis of melanin, which lends credence to the use of a synthetic biology approach to engineer tyrosinase for melanin production. In this work, Bacillus megaterium tyrosinase was engineered and expressed recombinantly in *Escherichia coli*. Two forms of tyrosinase fusions were designed to be either secreted through the curli export system or anchored on the cell surfaces. Using these systems, small melanin nanoparticles were able to be produced. In addition, melanin ghosts templated by *E. coli* cells were synthesized and characterized. Thus the tyrosinase expression, secretion and display capability that will be presented here represents a platform capable of allowing for the production colloidal melanin nanoparticles as well as melanin functionalized surfaces. Future work will investigate tuning the optical and electrical properties of the melanin assemblies through control of size and shape of the superstructure.

"Continuous directed evolution of tryptophan synthase toward production of unnatural aromatic amino acids"

Gordon Rix, University of California, Irvine

Unnatural derivatives of tryptophan and tyrosine are highly valuable commodity chemicals: they provide a chiral building block starting point in the synthesis of a variety of chemical compounds, such as antitumor drugs 1, 2 and have broad applications in synthetic peptides. 3, 4, 5 However, current synthesis methods for unnatural tryptophan and tyrosine derivatives are expensive and environmentally harmful, as they commonly require organic solvents and heavy metal catalysts. 6 To address this, Romney et al. used directed evolution of TrpB, a subunit of the bi-functional enzyme complex tryptophan synthase, to develop enzymes capable of coupling indole derivatives to L-serine to generate stereospecific non-canonical tryptophan



analogs (ncTrp). 7 Access to improved catalytic efficiencies and expanded substrate scope is likely achievable through continuous directed evolution, in which desirable enzyme variants may be evolved entirely in vivo, without the need for screening methods.

Orthogonal Replication (OrthoRep), developed by Ravikumar et al. is a continuous in vivo directed evolution approach in S. cerevisiae. 8 Utilizing a highly error-prone DNA polymerase that exclusively replicates a cytoplasmically-localized linear plasmid, diversity is continuously generated in a user-defined gene without increasing the rate of genomic mutation, and mutants with desired function can be selected via growth conditions. Application of this system in the evolution of TrpB to carry out non-native catalysis requires selection pressure for this function. Such a selection scheme can be achieved through use of expanded genetic codes that accommodate the desired non-canonical amino acid (Fig. 1).

We carried out OrthoRep-mediated evolution of TrpB toward standalone, native function in yeast across fourteen independent replicates. When supplied with the necessary substrates, evolved enzymes are capable of complementing a tryptophan synthase deletion. Additionally, we demonstrate coupling between in vivo production of a tryptophan analog by TrpB and host cell survival through amber codon suppression in a selectable marker. Evolution experiments utilizing this system to evolve TrpB toward production of 5-azido-tryptophan are currently underway.

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3. Talukder, P. et al. Cyanotryptophans as Novel Fluorescent Probes for Studying Protein Conformational Changes and DNA – Protein Interaction. (2015). doi:10.1021/acs.biochem.5b01085

4. Yu, Y. et al. De fi ning the Role of Tyrosine and Rational Tuning of Oxidase Activity by Genetic Incorporation of Unnatural Tyrosine Analogs. 33, (2015).

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7. Romney, D. K., Murciano-Calles, J., Wehrmüller, J. E. & Arnold, F. H. Unlocking Reactivity of TrpB: A General Biocatalytic Platform for Synthesis of Tryptophan Analogues. J. Am. Chem. Soc. 139, 10769–10776 (2017).

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"Co-Culture of Engineered Escherichia coli for Violacein Production from Hemicellulosic Sugars"

Kamil Gedeon, RPI

Hemicellulosic biomass is an abundant waste material that can serve as an ideal carbon source in the process of generating high value chemicals. The hydrolysis of hemicellulose results primarily in glucose and xylose, two sugars that *Escherichia coli* can use for both growth and product formation. Due to catabolic repression, wild-type *E. coli* will preferentially use glucose over xylose, resulting in sequential and inefficient use of the total available carbon in mixed-sugar systems. To overcome this obstacle, we propose the co-culture growth of a wild-type *E. coli* strain that preferentially uses glucose with a high- density achieving xylose-specialist strain. The xylose-specialist strain includes several gene deletions in its glucose metabolism pathway. Both strains were engineered to generate violacein, a purple pigment with antibiotic properties. Because the strains are predicted to behave as specialists for glucose or xylose under mixed-sugar conditions, we tested whether the co-culture would address the inefficiencies observed with the wild-type strain alone. We have observed that the co-culture of a wild-type glucose-preferential strain with a xylose-specialist strain increases the final titer of violacein and achieves higher total carbon consumption in mixed sugar systems than the wild-type strain alone. We will also describe ongoing efforts to

characterize and optimize co-culture growth and violacein production. This work has the potential to generate an economically relevant novel platform for the co-utilization of sugar mixtures to produce target chemicals.

"Phloroglucinol Tri-Service Effort"

Vanessa Varaljay, UES / AFRL

Phloroglucinol (PG) is a precursor to TATB which is an energetic chemical of interest to the DoD. PG is part of the 'Pressure Test' as selected by DoD stakeholders for the MIT-Broad Foundry and is a compelling test case for the Tri-Service Synthetic Biology for Military Environment (SBME) research efforts. The Tri-Service Laboratories approached the PG single 'challenge problem' by leveraging SBME capabilities such as strain engineering capabilities, strain banks, and rapid prototyping of paper-based sensors. The AFRL has a 2,000+ member military aircraft and fuel isolate library enriched for Pseudomonads, which are known to harbor PG synthesizing enzymes known as PhlD. The AFRL team has designed selective PhlD primers for screening Pseudomonad isolates for diverse and potentially more active PhlD enzymes than found in current literature. The NRL team is taking advantage of *Marinobacter* CP1's (SBME chassis) ability to accumulate significant amounts of the PG precursor malonyl-CoA. *Marinobacter* will be genetically engineered to produce PG through a combination of genome editing to divert carbon to malonyl-CoA and the subsequent conversion to PG through the addition of a plasmid- expressed PhlD. The ARL team is harnessing the robust SBME chassis *Clostridium acetobutylicum* to model and optimize its capability to produce significant amounts of the PG precursor malonyl-CoA with plasmid-expressed PhlD. ARL has determined that mixed sugar inputs yield optimal PG production. Additionally, ARL will construct a living PG sensor in soil microorganisms using ICE technology obtained from the Voigt laboratory. The ECBC team and Northwestern University collaborators have developed and optimized a paper-based, cell-free PG sensor. The paper-based PG detection system will be used in field-deployable conditions where the sensor would be triggered by PG, resulting in rapid reporting of the presence of PG. The MIT-Broad Foundry has optimized PG production in the model chassis E. coli and has built a PG genetic sensor for rapid screening. The PG Tri-Service collaborative efforts in biological and cell-free systems will result in optimized PG production and detection, invaluable for DoD and synthetic biology applications.

Posters

1 **Stefan Tekel, Arizona State University**
"A Transient Reporter for Editing Enrichment (TREE) enables detection and isolation of single base edited cell lines"

2 **Antje Kruger, Northwestern University**
"A Clostridia Cell-free Platform Facilitating Fast Prototyping of Metabolic Pathways"

3 **Giulio Chiesa, Boston University**
"A genetic circuit to detect and react to protein aggregation in a neuronal cell model"

4 **Sean Halper, Pennsylvania State University**
"A machine learning pipeline to predict synthesis success"

5 **Ye Chen, MIT**
"Automated Circuit Design in Saccharomyces cerevisiae"

6 **Ariel Langevin, Boston University**
"Bacterial fate depends on introduction rate of antibiotics and population composition"

7 **Parry Grewal, UC Berkeley**
"Bioproduction of a betalain color palette in Saccharomyces cerevisiae"

8 **Wesley Marques, MIT**
"Biosprospecting inositol oxidases (MIOX) for improved glucaric acid production in Saccharomyces cerevisiae"

9 **Kevin Fox and Cynthia Ni, MIT**
"Carbohydrate biosensors and strain engineering in E. coli for food waste utilization"

10 **Paul Carlson, Cornell University**
"Using In-cell SHAPE-Seq to Characterize a De Novo-Designed RNA Regulator"

11 ***Yan Zhang, Georgia Institute of Technology***
"Cell-Free System in Aqueous Two-Phase Enables Multiplexing of Small Molecule and Nucleic Acids from a Single Sample"

12 **Igor Medintz, U.S. Naval Research Laboratory**
"Channeled Biocatalysis in Self-Assembled Nanoparticle Enzyme Cascades"

13 **Andrew Hou, UCLA**
"Characterization of a transforming growth factor-β–responsive chimeric antigen receptor for anti-tumor immunity"

14 **Katherine Warfel, Northwestern University**
"Characterization of Membrane-bound Components in Cell-Free Glycosylation Systems"

15 **Adam Silverman, Northwestern University**
"Combining enzymatic bioconversion with inducible transcriptional regulators for versatile and rapid cell-free molecular sensing"

16 **Hailey Edelstein, Northwestern University**
"COMET: A toolkit for composing customizable genetic programs in mammalian cells"

17 **Jeremy Primus, Colorado State University**
"Computational Tools for Directed Promoter Tuning in Plant Synthetic Biology"

18 **Ania Baetica, UC San Francisco**
"Context Dependence of Biological Circuits"

19 **Jason Fontana, University of Washington**
"Design-driven engineering of CRISPRa components and programs for rapidly optimizing multi-gene metabolic pathways"

20 **Bin Shao, MIT**
"Development of a new measurement standard for genetic circuit design"

21 **Michael Sheets, Boston University**
"Development of an Optogenetically-Controlled Recombinase for E. coli"

22 **Bradley Abramson, JCVI**
"Direct-RNA Sequencing for bacteria and the minimal cell JCVI-Syn3A using Nanopore technology"

23 **Nathan Tague, Boston University**
"Dynamic Control of Antibiotic Resistance Related Genes with Optogenetics"

24 **Nick Mukhitov, MIT**
"Engineered microbial systems for responsive mycelium-based biomaterials."

25 **Jaeyoung (Kirsten) Jung, Northwestern University**
"Engineering a Water Quality Monitoring Biosensor using Regulated in vitro Transcription"

26 **Kok Ann Gan, Boston University**
"Engineering cooperative TF assembly in as a mechanism for complex computation in mammalian cells"

27 **Melissa Klocke, UC Riverside**
"Engineering dynamic nucleic acid nanotubes in cell-sized compartments"

28 **Minhee Park, Boston University**
"Engineering epigenetic regulation using synthetic read-write modules"

29 **Courtney Carlson, UC Irvine**
"Engineering Mammalian Cells to Record Their Own History"

30 **Conor McClune, MIT**
"Engineering orthogonal signaling pathways reveals the sparse occupancy of sequence space"

31 **Benjamin Tickman, University of Washington**
"Engineering predictable input-output relationships in E. coli transcriptional networks using a tunable CRISPRa/i regulatory toolkit"

32 **Laurence Chen, UCLA**
"Evaluating Cytotoxic Potency of Synthetic Granzyme B Variants via Retroviral Cytotoxicity Assay"

33 **Kersh Theva, UC Berkeley**
"Expanding the synthetic capabilities of chemoautotrophic metabolism"

34 **Max Schubert, Harvard Medical School**
"Exploring Genetic Variation Using Retron Library Recombineering (RLR)"

35 **James Lucas, UC San Francisco**
"Fragment-Based Approach for De Novo Ligand Binding Site Design"

36 **Kristina Haslinger, MIT**
"Functional screening of putative O-methyltransferases"

37 **Bon Ikwuagwu, Northwestern University**
"Generation of a Non-Canonical Amino Acid MS2 Fitness Landscape and its Applications"

38 **Suzie Hsu, University of Minnesota-Twin Cities**
"Genetic Optimization of Natural Product Biosynthesis in Streptomyces"

39 **Noam Prywes, UC Berkeley**
"High Throughput Rubisco Biochemistry"

40 **Cassandra Barrett, Arizona State University**
"Improving Cas9 Editing in Mammalian Cells with Synthetic Pioneer Factors"

41 **Venkata Chavali, University of Washington**
"Mathematical Modeling of dynamically-regulated CRISPR-Cas activation and inhibition programs for Rational Design in Metabolic Engineering"

42 **April Miguez, Georgia Institute of Technology**
"Metabolomics Analysis of the Toxic Effects of the Production of Lycopene and Its Precursors"

43 **Frank Cusimano, Columbia University**
"Microbiome Normalization Protocol for Large Scale Animal Oral Probiotic Experiments"

44 **Sung Sun Yim, Columbia University**
"Multiplexed Transcriptional Characterizations in Cell-Free Systems from Diverse Bacteria"

45 **Tyler Toth, MIT**
"Opine sensing in engineered soil bacteria for niche construction and signaling"

46 **Sara Oehmke, Colorado State University**
"Plant Synthetic Biology: Design and Quantification of a Cell Type Specific Synthetic Genetic Circuit"

47 **Widianti Sugianto, Georgia Institute of Technology**
"Production of Plant Natural Products in a Synthetic Cell"

48 **Daniel Anderson, MIT**
"Ratiometric pre-processing circuits enable the rejection of correlated input disturbances"

49 **Lina Gonzalez, MIT**
"Resilient Living Materials Built By Printing Bacterial Spores"

50 **Rob Warden-Rothman, MIT**
"ROUTE: Retrobiosynthetic Design Utilizing High-Throughput Enzyme Characterization"

51 **Kathryn Brink, Rice University**
"Screening for peptide inhibitors of S. typhimurium PhoPQ in E. coli"

52 **Divya Israni, Boston University**
"Synthetic transcriptional regulation for human cell therapy"

53 **Marco Galardini, Boston University**
"Systematic evaluation of conditional gene essentiality changes across four Saccharomyces cerevisiae strains"

54 **Marilyn Lee, US Army ECBC**
"The Performance and Potential of Hydrogel Matrices as an Alternate Reaction Format for Cell-Free Protein Synthesis"

55 **Nicholas Kruyer, Georgia Institute of Technology**
"Understanding Muconic Acid Reductase through Exploration of Substrate Specificity"

56 **Stephanie Robinson, UC Berkeley**
"Using fitness landscapes to identify and characterize a novel position for MS2 peptide insertions"

57 **Matthew Lux, US Army ECBC**
"Sensor development and deployment for defense applications"

58 **Chia Hung, US Air Force Research Laboratory**
"Bacterially-produced melanin as biomaterials"

59 **Ross Jones, MIT**
"Precision control of gene expression in eukaryotic cells using a CRISPR endoribonuclease-based incoherent feedforward loop"

1 Stefan Tekel, Arizona State University

"A Transient Reporter for Editing Enrichment (TREE) enables detection and isolation of single base edited cell lines"

The ability to precisely modify DNA on the human genome is integral for biotechnology and disease modeling. Recent deaminase fused-Cas9 base editing (BE) technologies enable single guide RNA (sgRNA) targeted chromosomal editing in a variety of applications. Base editors are effective at generating single nucleotide changes in some cell lines, but are often inefficient in others, with successful editing difficult to detect. To improve our ability to detect base editing, we developed a blue fluorescent protein (BFP) that converts to enhanced green fluorescent protein (eGFP) upon C-to-T base editing. Our transient reporter for editing enrichment (TREE) enables real time visualization of transfection (BFP) and C-to-T base conversion (GFP). We demonstrate the function of our system in human cells (HEK293s) and generate a HEK293 line that constitutively expresses our BFP reporter. Our fluorescence conversion assay allows for rapid and sensitive detection of different base editor delivery methods. Using dual-targeting of an episomal BFP and a chromosomal site, coupled with fluorescence activated cell sorting (FACS), we enrich for genomically edited populations with up to 80% C-T conversion in HEK293s. Additionally, we demonstrate our editing reporter can be coupled with multiplex sgRNA targeting to enrich for populations containing single base changes at 3 genomic sites simultaneously. Furthermore, our editing reporter enables enrichment of cells with edited disease loci, indicating that our reporter can be used to generate isogenic cell lines harboring disease relevant SNPs. Finally, we demonstrate the function or our editing reporter in a human induced pluripotent stem cells (iPSCs). BFP-to-GFP conversion enables enrichment from undetectable in unsorted populations populations to 69% C-T conversion in GFP-positive populations. We envision this novel blue-to-green fluorescent reporter assisting in detection and enrichment of base editing for basic science, therapeutics, and disease modeling.

2 Antje Kruger, Northwestern University

"A Clostridia Cell-free Platform Facilitating Fast Prototyping of Metabolic Pathways"

Modern world challenges like rapid population growth, rising global living standards and its accompanying increase in energy demand and waste generation necessitate the production of low-cost fuels and high-value compounds from sustainable resources. Microbes can be engineered to produce biofuels, chemicals, materials, and therapeutics. Particularly attractive engineering targets are gas- and food waste-fermenting anaerobes like clostridia strains. Unfortunately, designing, building, and optimizing biosynthetic pathways in clostridia for manufacturing applications remain complex challenges. Cell-free protein synthesis (CFPS) platforms have the potential to accelerate biological design by speeding up design-build-test cycle. Here, we present the development of a clostridia CFPS system. We demonstrate its potential to facilitate rapid studying of metabolic pathway performance and prototyping in vitro. We expect that our new cell-free platform will accelerate engineering clostridia strains that efficiently convert wastes into high-value products.

3 Giulio Chiesa, Boston University

"A genetic circuit to detect and react to protein aggregation in a neuronal cell model"

A common hallmark of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD), is the accumulation of protein aggregates in the brain. Not a single protein species aggregates in each disease, but rather different subsets of proteins. It remains entirely unclear how protein composition influences the aggregation of disease-associated proteins. To better

understand this, new high-throughput techniques are needed that can monitor and quantify aggregation of different types of proteins in living cells. Here, building on our lab's previous work to create genetic sensors of aggregation-prone proteins in yeast cells (Newby et al, Cell 2017), we are designing a genetic circuit capable of detecting protein aggregates in mammalian cells and correspond a genetically encoded output to the state of aggregation of a specific protein. The circuit is composed of: (1) a sensor module, which is a fusion of a synthetic transcription factor (synTF) and the protein of interest, which in this case is TDP-43, a protein whose aggregation is involved in ALS and AD; and (2) an output module, which is a synTF-responsive promoter driving the expression of a reporter or desired output. The circuit functions by sensitively linking the solubility/aggregation state of TDP-43 to downstream expression of the output. In preliminary experiments, we have shown that our genetic circuit can effectively detect TDP-43 aggregation, induced by cell stress or overexpression of TDP-43 aggregation-prone mutants, in both HEK293 and SH-SY5Y neuroblastoma cell lines. This technology could provide a flexible framework for studying the aggregation of multiple disease-relevant proteins, to identify what types of stress trigger the aggregation of multiple proteins in cells, as well as what factors could disassemble aggregates or prevent their formation. Finally, a similar genetic network could be adapted as a versatile gene therapy approach for multiple neurodegenerative diseases.

4 Sean Halper, Pennsylvania State University

"A machine learning pipeline to predict synthesis success"

De novo gene synthesis techniques have revolutionized synthetic biology and metabolic engineering, enabling the rapid construction of novel circuits and the recoding of proteins for a variety of applications. However, not all genes or constructs are well suited for modern synthesis strategies, such as oligonucleotide assembly. Repetitive DNA regions, secondary and tertiary DNA structures, and polymeric nucleotide motifs will disrupt the synthesis process, resulting in mixtures of truncated byproducts, low yields, or complete synthesis failure. However, it remains unclear how combinations of these sequence determinants determine overall synthesis success, motivating the development of predictive models. If DNA synthesis success could be predicted, multi-objective optimization algorithms could design genetic systems for both desired functions as well as DNA synthesis success.

To solve this challenge, we developed the Synthesis Success Calculator, a machine learning algorithm that classifies synthesis success for any given DNA fragment based on biophysically informed features. We carried out rigorous training, cross-validation, and testing of a random forest classifier, achieving an F1 score of 0.9 on 500 unseen DNA sequences. To do this, we first compiled a labeled training set of 1076 synthesized DNA sequences from a variety of internal and public sources, including 375 purposeful negative control sequences and 66 attempted, but unsuccessfully synthesized, DNA sequences. We carried out an exhaustive hyper-parameter grid search, applying 10-fold cross- validation on the training set to ensure our final training yielded the best-possible, most-balanced random forest classifier. We then utilized the trained classifier to assess a variety of natural and synthetic constructs for features that might complicate the DNA synthesis and assembly of these genetic systems, quantifying the importance of sequence determinants and their synergistic effects on synthesis success. The Synthesis Success Calculator's predictions are available stand-alone at https://salislab.net/software. They have also been incorporated into our optimization algorithms for genetic systems, including the Operon Calculator, ELSA Calculator, and Non-Repetitive Parts Calculator, to design genetic systems for both function and synthesis success.

5 Ye Chen, MIT

"Automated Circuit Design in Saccharomyces cerevisiae"

Genetic circuits are widely used in scientific, industrial and therapeutic approaches. In prokaryotic systems, genetic circuits are successfully designed and built by Cello, the genetic circuit design automation platform. However, building genetic circuits in eukaryotic systems are challenging due to the limited sensors and regulatory units. Here, we developed a method to systematically import bacterial transcription factors into Saccharomyces cerevisiae to create a bunch of candidate sensors and NOT gates. We next determined strategies to link these elements together with minimum cross interactions. Finally, we incorporated these novel components and construction strategies with Cello to design DNA sequence. By using this platform, we successfully created several large circuit constructions (6-8 gates, 9–11 regulators, up to 20 regulatory operons) in yeast. We further built an ODE model to investigate the dynamics when input states changed. Our model predicted the intermediate faults during several transfers, which were confirmed in the following experiments. In this study, we demonstrate a general approach of circuit design automation in a novel eukaryotic system. This will highly expand our ability to build complicated circuits across different organisms.

6 Ariel Langevin, Boston University

"Bacterial fate depends on introduction rate of antibiotics and population composition"

Antibiotic resistance has become a major public health concern as bacteria evolve and adapt to evade drugs, leading to recurring infections and overuse of antibiotics. Previously, we have found that the rate of antibiotic addition can compromise the effectiveness of key antibiotic resistance mechanisms, such as multi-drug efflux pumps. For instance, AcrAB-TolC efflux pumps and its homologs, which are ubiquitous in nature, enable the export of antibiotics out of the cell, increasing resistance levels. Using a modular turbidostat, the eVOLVER, we assessed how short-term differences in antibiotic introduction rates affect longer-term bacterial survival, as well as the evolution of drug resistance. We monitored this in different genotypes: in cells that have efflux pumps with their native regulation networks in place, constitutive over-expression of efflux pumps, and in a strain lacking functional efflux pumps. In this work, we identified the significance of these genotypes in how cells respond to different antibiotic introduction rates. Efflux pump expression alters tolerance to antibiotics, as well as the emergence of resistance through mutations. We also studied the response of co-cultured populations using subsets of these strains since the evolutionary patterns are not simply the additive responses of the single species evolution experiments. These results highlight the importance of studying the effect of introduction rates of stress at both the phenotypic and genotypic level.

7 Parry Grewal, UC Berkeley

"Bioproduction of a betalain color palette in Saccharomyces cerevisiae"

Betalains are a family of pigments found exclusively in the plant order Caryophyllales. Of particular interest is the red-violet betanin, commonly obtained from Beta vulgaris (beet) as a food dye. We demonstrate complete microbial production of betanin in Saccharomyces cerevisiae from glucose, an early step towards a fermentation process enabling rapid, on-demand production of this natural dye. A titer of 17 mg/L was achieved, corresponding to a color intensity obtained from 10 g/L of beetroot extract. Further, we expanded the spectrum of betalain colors by condensing betalamic acid with various amines fed to an engineered strain of S. cerevisiae. Our work establishes a

platform for microbial production of betalains of various colors as a potential alternative to land- and resource- intensive agricultural production.

8 Wesley Marques, MIT

"Biosprospecting inositol oxidases (MIOX) for improved glucaric acid production in Saccharomyces cerevisiae"

Glucaric acid is one of the top value added chemicals from biomass according to the 2004 report from the U.S. Department of Energy. The Prather Lab has developed a biosynthetic pathway in E. coli and S. cerevisiae to generate glucaric acid from glucose in four steps, producing titers of up to 2.5 g/L from 10 g/L glucose. The goal of this work is to optimize a glucaric acid production via homologous enzyme identification and screening. Homologs for pathway enzymes were identified from their respective Pfam protein families and selected for evaluation using sequence similarity networks to maximize enzyme diversity while maintaining key catalytic residues. We previously identified that myo-inositol oxygenase (MIOX) low activity is a botleneck on glucaric acid synthesis. MIOX converts myo-inositol to D-glucuronic acid using O2. The MIOX network was constructed with 1600 sequences from Pfam PF05153 (E-value threshold = 120). Node selection was biased toward fungal and plant variants for expected high expression in S. cerevisiae and towards reviewed sequences. 31 MIOX homologs were selected and screened in S. cerevisiae. 23 variants produced glucaric acid with 2 variants producing at same level as best (previous AtMIOX4). 6 variants did not produce under the conditions tested. Next, a new sequence network was constructed based on the glucaric acid titer obtained from the expression of MIOX variants in yeast. From this new network, a new batch of MIOX enxymes will be selected for expression in S. cerevisiae based on aminoacids similarity in the active site and the extension of the N-terminal domain.

9 Kevin Fox and Cynthia Ni, MIT

"Carbohydrate biosensors and strain engineering in E. coli for food waste utilization"

Microbial conversion into value-added products is an economically and environmentally favorable use for food waste. Challenges to achieving this include the heterogeneity of food waste and catabolite repression inhibiting the consumption of multiple carbohydrates simultaneously. In this work, we address these challenges by (1) developing carbohydrate biosensors to respond to the presence of carbohydrates and (2) relaxing the effects of catabolite repression to co-utilize glucose and other carbohydrates.

Naturally occurring, carbohydrate responsive transcription factors (TF) regulate transcription in response to carbohydrate ligands. We engineered a galacturonate biosensor with such a TF and a GFP reporter. We were able to tune the response: increasing promoter strength resulted in higher levels of repression, as did an additional operator site in the gfp promoter.

The phosphorylation state of EIIAGlc dictates secondary carbohydrate uptake. We engineered a strain (named KF) with an EIIAGlc active site mutation that eliminated the enzyme's activity. The mutation enabled uptake and consumption of both glucose and galacturonate, where wild type E. coli does not consume galacturonate in the presence of glucose.

The galacturonate biosensor response dampened upon glucose addition. However, in the KF strain, some of biosensor response was recovered in the presence of glucose.

Future applications of these carbohydrate biosensors and catabolite-repression-negative strain include consolidating multiple carbohydrate biosensors into one strain to respond to more complex carbohydrate mixtures and engineering the sensors to control production pathways.

10 Paul Carlson, Cornell University

"Using In-cell SHAPE-Seq to Characterize a De Novo-Designed RNA Regulator"

The development of completely synthetic (de novo-designed) riboregulators has proceeded rapidly in recent years, enabled by design algorithms like the Nucleic Acid Package (NUPACK). For simple design strategies, a trial-and-error approach is generally sufficient to evaluate the performance of candidate designs in vivo. However, as the complexity of these systems grows, measurements beyond functional characterization will become increasing important to accelerate the design-test-learn cycle. In this work, we use selective 2'-hydroxyl acylation analyzed by primer extension sequencing (SHAPE-Seq) to confirm the design mechanism of a novel synthetic RNA translational repressor design. We then characterize a poorly-performing variant and identify mis-folding events which contribute to its failure mode. Together, these results demonstrate the utility of RNA chemical probing as a central component of the design and testing pipeline of synthetic riboregulators.

11 Yan Zhang, Georgia Institute of Technology

"Cell-Free System in Aqueous Two-Phase Enables Multiplexing of Small Molecule and Nucleic Acids from a Single Sample"

The use of cell-free systems for biomarker detection is becoming increasingly popular due to their simplicity and fast turnaround time. However, most of the current cell-free diagnostic platforms are singleplex assays where a sample containing various analytes is added to a reaction, but a reaction only detects one analyte. In order to assay multiple relevant biomarkers, multiple cell-free reactions would need to be run in parallel using multiple aliquots of the sample, making the process time-consuming and error-prone. Here, we present an approach to combine cell-free reactions with aqueous two-phase systems (ATPS) to multiplex analyte detection from a single sample using compartmentalized cell-free reactions. We employed the phase-separating polymers polyethylene glycol (PEG) and ficoll (FIC) to encapsulate individual cell-free reactions within the FIC-rich reaction phase and added analytes in the PEGrich aqueous phase. We utilized a custom 96-well plate with a variable number of micro-basins (4-Plex or 9-Plex) to separate reaction phase droplets and to allow the aqueous phase to fill up the well. We demonstrated that analytes added in the aqueous phase partition into the reaction phases and elicit reporter protein production; we observed low crosstalk between individual reactions. We showed that a cell-free system combined with ATPS offers a novel multiplexing platform for detecting analytes ranging from small molecule to nucleic acids.

12 Igor Medintz, U.S. Naval Research Laboratory

"Channeled Biocatalysis in Self-Assembled Nanoparticle Enzyme Cascades "

Cell-free synthetic biology relies on exploiting enzymatic activity while also seeking to utilize a minimal number of overall components in the most efficient manner possible. The latter requires that the enzymes in a given biocatalytic reaction scheme ideally engage in concertedsubstrate-product channeling to overcome diffusion limitations. Recent research has revealed that exploiting nanoparticles (NPs) as enzyme display scaffolds can stabilize enzymes and enhance their localized activity suggesting some new approaches towards achieving high catalytic efficiency. Utilizing the enzymes from glycolysis which serve as the starting point for a number of target biosynthetic products in conjunction with luminescent semiconductor nanocrystals or quantum dots (QDs) as a prototypical model system, we show that multienzyme cascades can be self-assembled in a very simple manner to yield QD-enzyme nanoclusters that engage in channeled biocatalysis. The kinetic

flux through these nanoclustered systems is increased by several orders of magnitude compared to controls consisting of the same concentration of enzyme alone. Classical enzymatic experiments provide strong supportive evidence for a channeling mechanism contributing to the observed enhancements in addition to effects from both the aforementioned stabilization and enhancement of key enzyme components. Iterative optimization of the ratio of each enzyme in these nanoclustered systems utilizing numerical simulations allowed the kinetic flux to be enhanced even further. A variety of other factors such as QD size, relative QD shape (rectangular versus spherical), and enzyme-NP assembly order were also shown to influence the magnitude of catalytic turnover in the self- assembled clustered systems. The complexity achievable to such cascaded systems was further evaluated by increasing the number of participating enzymes from 7 up to a 13 enzyme cascade where (di, poly)saccharide precursor substrates were enzymatically processed all the way to a lactic acid product. The results from this study suggest that the benefits inherent to this self-assembled clustered enzyme approach may be readily transferrable to other multienzyme cascades along with providing potential plug-and-play type modularity. These systems are predicted to have broad applicability in enzyme-based biosensing, remediation, and industrial biocatalytic applications.

13 Andrew Hou, UCLA

"Characterization of a transforming growth factor-β–responsive chimeric antigen receptor for anti-tumor immunity"

Adoptive T-cell therapy is a cancer treatment strategy where T cells from a cancer patient are harvested, modified ex vivo to target tumors, and reinfused back into the patient's body. Though remarkably successful against B-cell malignancies, therapeutic efficacy against solid tumors has been more limited. This is in large part due to the fact that solid tumors inhabit a microenvironment that is hostile to immune surveillance, characterized by an abundance of suppressive factors such as transforming growth factor-beta (TGF-β). Recent work has demonstrated that chimeric antigen receptors (CARs) can be engineered to respond to TGF-β, thereby converting an immunosuppressant into a T-cell stimulant. For TGF-β CAR expression to be a clinically viable strategy to augment T-cell immunotherapy, we first sought to address a potential safety concern associated with TGF-β CAR-T cell manufacturing. Specifically, given the well-characterized interplay between suppressive regulatory T cells (Tregs) and TGF-β, we investigated whether presence of a contaminating fraction of Tregs at the onset of CAR-T cell manufacturing might result in counterproductive expansion of Tregs transduced to express the TGF-β CAR. TGF-β CAR-transduced Tregs do not, in fact, preferentially expand over non-Tregs, nor do they exert CAR-mediated immune suppression. Given that Tregs are not a liability, we have begun to assess the ability of the TGF-β CAR to enhance the therapeutic efficacy of T cells targeting solid tumors. Preliminary studies in the murine B16-F10 melanoma model revealed, however, that Pmel-I T cells expressing a TGF-β dominant-negative receptor (DNR), as opposed to a CAR, most effectively treated tumor-bearing mice. We hypothesize that parameters such as relative frequency of T-cell subtypes (e.g., CD4+ vs. CD8+) and targeting modality (e.g., dual CAR versus single-chain, bispecific CAR) may require further optimization to maximize the therapeutic potential of the TGF-β CAR.

14 Katherine Warfel, Northwestern University

"Characterization of Membrane-bound Components in Cell-Free Glycosylation Systems"

Glycosylation is an essential post-translational modification for human health. Recently, cell-free glycosylation systems have been developed for site-specific glycosylation using membrane-bound

oligosaccharyltransferases (OSTs). However, our lack of understanding of these systems at the nano-level has inhibited our ability to engineer diverse and efficient glycosylation systems. In this work, we characterize two cell-free glycosylation systems: (1) one using the N-linked OST, PglB from C. jejuni and (2) one using the O-linked OST, PglO from N. gonorrhoeae. These systems can be generated by expressing the OST enzyme in vivo prior to cell lysis or in vitro, in cell-free reactions using synthetic nanodiscs. We find that when expressed in vivo, glycosylation components are localized in 200 nm lipid vesicles formed during cell lysis. Nanocharacterization techniques enable determination of vesicle size and distribution of glycosylation components. In addition, we demonstrate how tuning vesicle size distribution and concentration improves glycosylation activity for the O-linked OST PglO from N.gonorrhoeae. This work demonstrates the first example of O-linked OST activity in a cell-free system. Furthermore, soluble and active OSTs can be expressed in the cell-free environment following cell lysis in the presence of nanodiscs. Expressing OSTs in vitro allows for precise control over each glycosylation component and enables rapid prototyping and tuning of novel glycosylation systems. This work provides an in-depth analysis of membrane-bound glycosylation components in cell-free systems to enable advances in glycoengineering.

15 Adam Silverman, Northwestern University

"Combining enzymatic bioconversion with inducible transcriptional regulators for versatile and rapid cell-free molecular sensing"

Technological improvements in cell-free gene expression (CFE) technology, including high protein yields, simplified bacterial extract preparation, and the demonstration of shelf stability, have garnered great interest in the use of cell-free systems for biosensing. Yet despite advances in cell-free nucleic acid detection, only a few examples exist in the literature for detecting small molecules, including ions, metals, metabolites, and organic pollutants. To combat this gap, we have developed a generalizable platform for detecting arbitrary small molecules in cell-free reactions, by combining extracts pre-enriched with transcription factors and enzymes at defined and easily optimized ratios. This approach enables enzymatic conversion of the target molecule to a metabolite that is then directly sensed in the same pot. We demonstrate that this platform enables rapid and robust detection of chemical pesticides with fluorescent and colorimetric outputs in cell-free reactions that can be directly assayed from a field environmental sample. We anticipate that this work should have broad consequences for the deployment of cell-free technologies for point-of-need molecular detection.

16 Hailey Edelstein, Northwestern University

"COMET: A toolkit for composing customizable genetic programs in mammalian cells"

Engineering mammalian cells to produce therapeutic proteins or diagnostic readout relies heavily upon technologies for controlling gene expression. Currently, construction of cellular programs is limited to using either native transcription factors (TFs), which are subject to cross-regulation and interference with native cellular functions, or to using a small set of engineered transcriptional regulators, such as tTA and Gal4. Engineering mammalian cells to carry out sophisticated and customizable genetic programs requires a toolkit of multiple orthogonal and well-characterized TFs, which does not yet exist. To address this need, we developed the COmposable Mammalian Elements of Transcription (COMET)—an ensemble of TFs and promoters that enable the design and tuning of gene expression to an extent not previously possible. COMET currently comprises 44 activating and 12 inhibitory zinc-finger protein-based TFs and 81 cognate promoters, combined in a framework that readily accommodates new parts. We perform a rigorous characterization of how the existing parts work in combination and how they can each be tuned to access a dynamic range

of expression levels spanning over three orders of magnitude. The activity of transcription factors can also be temporally and externally controlled via small molecule-induced reconstitution. The high specificity of zinc-finger DNA binding domains makes these transcription factors highly orthogonal and functional in concert, enabling construction of circuits that implement single-layer Boolean logic. We develop a mathematical model that provides mechanistic insights into COMET performance characteristics and can guide the design of new biological functions. Altogether, COMET enables the rapid design and construction of custom genetic programs in mammalian cells.

17 Jeremy Primus, Colorado State University

"Computational Tools for Directed Promoter Tuning in Plant Synthetic Biology"

Plant synthetic biology is an emerging field which could gain significant traction from an expanded library of quantitatively characterized parts. This facilitates precise in silico design and quantitative prediction of dynamic response. Towards this effort, a number of promoter variants were generated from the Nopaline Synthase promoter (PNOS), an Agrobacterium tumefaciens derived constitutive promoter commonly used in plant genetic engineering applications. Promoter variant editing was done by implementing specific nucleotide mutations within the TATA box to perturb the affinity of TATA binding protein (TBP) for the promoter, resulting in modulation of transcriptional activity. These promoter variants were tested in Arabidopsis thaliana protoplasts isolated from leaves and roots. Promoter output was captured via CCD camera with single photon detection in the case of leaf protoplasts (luciferase), and flow cytometry was utilized for root protoplasts (GFP). Results establish the tunability of PNOS genetic output by this approach, yielding variants with expression above and below wildtype levels. This behavior was captured with a thermodynamic model which describes the affected interaction of the promoter and TATA binding protein as it pertains to genetic output. A software was also developed which facilitates semi-automated quantification and analysis of the leaf protoplast data. The combination of the molecular approach with thermodynamic model and analysis software facilitates a high-throughput, scalable workflow that can be utilized for predictable and quantitative promoter tuning. Fine tuning of transcriptional levels becomes critically important when connecting synthetic modules for more complex emergent behaviors.

18 Ania Baetica, UC San Francisco

"Context Dependence of Biological Circuits"

It has been an ongoing scientific debate whether biological parameters are conserved across experimental setups with different media, pH values, and other experimental conditions. Our work explores this question using Bayesian probability as a rigorous framework to assess the biological context of parameters in a model of the cell growth controller in You et al. When this growth controller is uninduced, the E. coli cell population grows to carrying capacity; however, when the circuit is induced, the cell population growth is regulated to remain well below carrying capacity. This growth control controller regulates the E. coli cell population by cell to cell communication using the signaling molecule AHL and by cell death using the bacterial toxin CcdB. To evaluate the context dependence of parameters such as the cell growth rate, the carrying capacity, the AHL degradation rate, the leakiness of AHL, the leakiness of toxin CcdB, and the IPTG induction factor, we collect experimental data from the growth control circuit in two different media, at two different pH values, and with several induction levels. We define a set of possible context dependencies that describe how these parameters may differ with the experimental conditions and we develop mathematical models of the growth controller across the different experimental contexts. We then determine whether these parameters are shared across experimental contexts or whether they are context dependent. For each of these possible context dependencies, we use Bayesian inference to

assess its plausibility and to estimate the parameters of the growth controller. Ultimately, we find that there is significant experimental context dependence in this circuit. Moreover, we also find that the estimated parameter values are sensitive to our assumption of a context relationship.

19 Jason Fontana, University of Washington

"Design-driven engineering of CRISPRa components and programs for rapidly optimizing multi-gene metabolic pathways"

Optimizing metabolic pathways to achieve high production yields often requires fine-tuning the timing and level of the expression of multiple enzymes. Such efforts typically involve exploring a large combinatorial design space. To address this challenge, we are developing dynamically-controlled CRISPR-Cas activation (CRISPRa) tools for E. coli to rapidly implement combinatorial differences in the expression of multiple genes.

We describe a method to generate orthogonal CRISPRa-responsive synthetic promoters that can be selectively activated up to 50-fold by expressing their cognate gRNAs. We computationally designed these promoters to contain unique gRNA target sites and exclude sequences that can bind endogenous regulators. We employed structure-based screening to identify gRNA target sites that avoid misfolding when expressed as a gRNA. We used our promoters to independently regulate the expression of multiple biosynthetic enzymes for the production of the industrial aromatic compound p-amino phenylalanine.

To optimize the expression level of multiple pathway enzymes at once, we need ways to systematically change the expression level at each promoter. We demonstrate that gene expression from our promoters can be tuned by truncating their cognate gRNAs from the 5' end, which will allow the construction of combinatorial libraries of multi-gene expression programs. Last, we show that external inducers and promoters responsive to the state of the cell can be used to dynamically initiate CRISPRa programs and control the timing of gene expression. Together, dynamic initiation of pathway expression and combinatorial optimization of enzyme levels may enable us to rapidly discover new, complex multi-gene CRISPRa programs that improve production yields in metabolic programs.

20 Bin Shao, MIT

"Development of a new measurement standard for genetic circuit design"

Large-scale engineering of biological circuits requires reliable measurements of genetic parts and a deep understanding of interactions between synthetic circuits and the host cell. However, the widespread adoption of non-physical units of measurement makes it difficult to parameterize synthetic devices and cellular context, which hinders the efforts to model multi-component synthetic circuits in a predictable way. Here we present a new measurement standard in which DNA- and RNA- binding proteins fused with spectrally well-separated fluorescent proteins are used to visualize plasmid and mRNA simultaneously. By combining quantitative fluorescence microscopy with a customized image processing pipeline, we are able to quantify promoter copy number, RNA production and protein abundance at the single cell level. This allows us to extract biophysical parameters of genetic devices, including the RNA polymerase flux of a standard promoter in absolute units. We also show that the new measurement standard can be further applied to investigate the transcriptional power of bacteria cells.

21 Michael Sheets, Boston University

"Development of an Optogenetically-Controlled Recombinase for E. coli"

Optogenetics allows direct and programmable control of gene expression. The use of light as a signal instead of small molecules can give precise spatiotemporal control, and can circumvent potential changes in cell state due to media differences and interference with other chemical pathways. We are developing a system that uses a light-activated recombinase in E. coli to dynamically control gene expression. Using split recombinases linked to photoactivatable "Magnet" heterodimers, our system can excise targeted gene segments upon exposure to blue light. This has the potential to expand the bacterial optogenetic toolbox, with particular implications for cellular logic and studying gene networks that interfere with small molecule induction. Our goal is to use this system to activate and inactivate genes involved in quickly-changing conditions, such as transient antibiotic resistance, and investigate how this affects the survival of individual cells. Here, we present progress towards the development of this light-activated recombinase.

22 Bradley Abramson, JCVI

"Direct-RNA Sequencing for bacteria and the minimal cell JCVI-Syn3A using Nanopore technology"

Transcriptomics is widely used to determine expression profiles of various types of organisms from plants to bacteria to the synthetic minimal cell (JCVI-Syn3A). Transcriptomics often requires computational tricks to determine relative expression levels since cDNA synthesis and PCR amplification of the RNA can introduce amplification bias. Here we sequence RNA directly from cells using Oxford Nanopore's direct-RNA long-read sequencing protocol. Comparison of direct-RNA sequencing and Illumina cDNA sequencing show there are differences between the transcriptomic profiles obtained with these methods. Interestingly, long-read sequencing may be able to define transcriptional start and end sites more robustly than Illumina cDNA sequencing.

23 Nathan Tague, Boston University

"Dynamic Control of Antibiotic Resistance Related Genes with Optogenetics"

Temporal fluctuations in gene expression give rise to diverse cellular phenotypes. Optogenetic control of gene expression enables forward engineering of dynamic outputs, which can result in a deeper understanding of the consequences of these temporal fluctuations. For example, temporal fluctuations in genes associated with stress tolerance can cause transient resistance to antibiotics. Furthermore, transient resistance has recently been shown to serve as a stepping stone towards permanent resistance. To study transient resistance dynamics, we are focusing on the multidrug efflux pump AcrAB-TolC, an antibiotic resistance associated gene widespread among Gram-negative bacteria, as a case study. In this project, we are controlling AcrAB-TolC expression optogenetically to study its dynamical behavior. Since the efflux pump is costly to the cell, it was necessary to first engineer optogenetic control with low leakage. Once tight control is established, we propose to study the dynamic relationship between acrAB and mutS, a gene involved in DNA mismatch repair. Additionally, we are working to establish long term, single cell optogenetic control using both microfluidics and a digital micromirror device, which will allow for future closed-loop feedback studies of acrAB and other antibiotic resistance genes. Finally, controlling expression of resistance genes expression optogenetically will inform us about conditions and treatment strategies that minimize the appearance of transient and permanent antibiotic resistance.

24 Nick Mukhitov, MIT

"Engineered microbial systems for responsive mycelium-based biomaterials."

One goal of bioinspired engineering is to generate living and responsive materials that are capable of self-regeneration and execution of programmed functions. The advancement of synthetic biology principles and capabilities has yielded an exciting opportunity for the engineering of complex systems - making the target of engineered living materials attainable. In this work, we set out to engineer the microbiota that inhabits the mycelium-based materials produced by Ecovative Design. The goals of these projects are (1) to engineer microbial systems that can provide responsive attributes and (2) enhance the physical properties of the final material.

25 Jaeyoung (Kirsten) Jung, Northwestern University

"Engineering a Water Quality Monitoring Biosensor using Regulated in vitro Transcription"

Ensuring adequate drinking water quality is essential for human health and for effective allocation of freshwater resources in agriculture, energy, and manufacturing. However, the current state-of-the-art for water quality testing is lacking, as it requires expensive equipment such as ICP-MS, technical expertise to prepare and analyze samples, and can take days to obtain results from off-site labs. While a number of biosensors have been developed to address these problems, these approaches have several limitations. Whole-cell biosensors suffer from long-term genetic instability and are subject to biocontainment regulations. On the other hand, cell-free biosensors that rely on ill-defined cellular extracts are limited by the speed at which a reporter protein is translated and impacted by undesirable batch-batch variability.

Here, we show that minimal, well-defined in vitro transcription reactions can be used to generate visible RNA outputs within minutes and can be regulated using allosteric transcription factors and appropriately designed DNA templates. This platform consists of three core components: (1) a DNA template that encodes a fluorescence-activating RNA, (2) an allosteric transcription factor that regulates transcription of this RNA, and (3) a processive phage RNA polymerase. By eliminating the need for translation in cell-extracts, regulated in vitro transcriptions improves on the speed, cost, and variability associated with whole-cell and cell-free biosensors. This platform is highly modular and compatible with a dozen of transcription regulators, allowing detection of a variety of small molecules including major water contaminants like lead and copper. Combined with freeze-drying techniques, these biosensors meet the demand for rapid, field-deployable and easy-to-use molecular detection for water quality monitoring.

26 Kok Ann Gan, Boston University

"Engineering cooperative TF assembly in as a mechanism for complex computation in mammalian cells"

Observations of the eukaryotic transcriptional regulation system suggest that most of gene transcription is carried out by transcription factors that cooperatively self-assemble into complexes via multivalent interactions. Formation of such complexes underlie non-linear gene regulatory operations. Our lab has previously developed a platform for engineering cooperative assemblies by applying this natural design principle in combination with a model-guided approach to build and embed programmable artificial gene circuits in yeast. We have shown that we can predictably tune gene expression by adjusting the strength and number of interactions in an assembly. Furthermore, these assemblies can be built to manipulate the temporal dynamics of gene expression. Here, we aim to translate this system into mammalian cells by using synthetic biological molecules with well-characterized protein-DNA (zinc-fingers) and protein-protein (protein binders) interactions.

27 Melissa Klocke, UC Riverside

"Engineering dynamic nucleic acid nanotubes in cell-sized compartments"

Programmable, synthetic cells have broad applications in sensing and drug-delivery. Current work in the development of synthetic cell components focuses on on membranes and compartmentalization, and as well on developing the minimum required cellular machinery for synthetic cells to carry out different processes [1, 2]. In native cells, cytoskeletal filaments are a key structure for cell division, motility, and intra-cellular transport. Harnessing these filaments for use in synthetic systems is limited by the complexity of the proteins and processes responsible for the dynamic behavior of the filaments. Synthetic tile-based DNA nanotubes, however, are comparable in length and stiffness to cytoskeletal filaments, but can be engineered to demonstrate dynamic behavior while requiring few reacting components in comparison to the native system [3, 4]. To move towards using DNA nanotubes as cytoskeletons in synthetic systems, their dynamic behavior must be integrated with co-transcriptional circuitry and characterized in compartments.

We demonstrate the characterization of nanotubes that polymerize in water-in-oil droplets [5, 6]. These droplets can be produced very quickly, and serve as a simplified compartmentalization system. While the nanotubes exhibit complex networks during polymerization in confinement, nanotubes mature into a more rigid and aligned morphology in under 24 hours. Ongoing efforts aim at controlling nanotube assembly using in vitro transcriptional networks encapsulated and operating in the droplets.

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28 Minhee Park, Boston University

"Engineering epigenetic regulation using synthetic read-write modules"

Modifications to chromatin play a key role in establishing and perpetuating gene expression patterns that enable cells with identical genomes to exhibit distinct phenotypes. These mechanisms are critical for development and disease, and could provide unique capabilities for synthetic biology. Here, we apply a synthetic biology approach to engineer and understand how chromatin modifications mediate gene expression changes and how these changes can be propagated as epigenetic information. We exploit DNA adenine methylation (m6A), a DNA modification that is rarely found in metazoan genomes, to create a fully synthetic, orthogonal chromatin system in human cells. Specifically, we engineer regulatory components that enable de novo placement of this modification at specific genomic loci and modification-mediated gene regulation changes. We then design regulatory circuits based on the principle of "read-write" positive feedback mechanism to locally reinforce and propagate the modification. Together with a quantitative model of chromatin dynamics, we show these circuits mediate spreading of the modification to regulate genes at a distance and the establishment of long-term epigenetic memory. These minimal circuits are able to program canonical epigenetic functions de novo, conceptually validating ''read-write'' architectures.

We also demonstrate the versatility of our modular system in exploring and engineering additional mechanisms for robust epigenetic memory. Briefly, we engineer read-write modules that

mediate crosstalk between endogenous H3K9me3 and synthetic m6A in fission yeast to study the role for the coupling of positive feedback loops through crosstalk of multiple epigenetic modifications in the robust persistence of epigenetic memory. As such, our work provides a toolkit for investigating models of epigenetic regulation and encoding additional layers of epigenetic information in cells.

29 Courtney Carlson, UC Irvine

"Engineering Mammalian Cells to Record Their Own History"

How does a complex multicellular organism develop from a single cell? Historically, attempts to answer this question have been limited by the lack of techniques that can probe individual cells in opaque heterogeneous tissues, in situ. To overcome this limitation, we have created a molecular memory recording system—Cell HistorY Recording by Ordered iNsertions (CHYRON)—that stores developmental information in the form of continuous insertion mutations at a target synthetic locus in a cell's genome. The latest version of CHYRON utilizes a self-targeting Cas9 nuclease to cut the locus that encodes its own guide RNA, coupled with a template-independent polymerase (TdT) that inserts random nucleotides at the cut site. Repeated rounds of cutting and insertion gradually elongate the CHYRON locus, serving as a long-term "memory" of the cell's exposure to environmental cues (if CHYRON is placed under the control of a user-defined inducible promoter) or cell lineage (if CHYRON is constitutively expressed). Our established system is capable of recording cell history over short time scales, and we are currently implementing improvements to unlock the next level of CHYRON's utility as a developmental biology tool. Here we discuss present-day CHRYON and outline an essential component of our upcoming work—engineering TdT to have unbiased or superbiased nucleotide addition activity. We anticipate our engineered TdT variants will allow CHYRON to maintain functionality over longer time periods and increase the amount of information encoded per editing cycle.

30 Conor McClune, MIT

"Engineering orthogonal signaling pathways reveals the sparse occupancy of sequence space"

When new sensors or signaling pathways are engineered within cells or transferred between genomes they must be insulated from each other, and from endogenous signaling pathways, to prevent unwanted cross-talk. How easily new pathways can be introduced into cells depends on the density and distribution of paralogous pathways in the sequence space defined by their specificity-determining residues. Here, we directly probe the density of sequence space by generating novel two-component signaling proteins in Escherichia coli using cell sorting coupled to deep-sequencing to analyze large libraries designed based on coevolution patterns. We produce 58 new insulated pathways, in which functional kinase-substrate pairs have different specificities than the parent proteins, and demonstrate that several new pairs are orthogonal to all 27 paralogous pathways in E. coli. Additionally, we readily identified sets of 6 novel kinase-substrate pairs that are mutually orthogonal to each other, significantly increasing the two-component signaling capacity of E. coli. These results indicate that sequence space is not densely occupied and that new, insulated pathways can easily arise during evolution or be designed de novo. We demonstrate the latter by engineering a new signaling pathway in E. coli that responds to a plant cytokinin without cross-talk to extant pathways. Our work demonstrates that coevolution-guided mutagenesis and sequence-space mapping can be used to design large sets of orthogonal protein-protein interactions.

31 Benjamin Tickman, University of Washington

"Engineering predictable input-output relationships in E. coli transcriptional networks using a tunable CRISPRa/i regulatory toolkit"

Our goal is to create complex, robust and predictable CRISPR-cas transcriptional networks operating through the regulated expression of gRNAs. Our approach is to develop components that can be assembled into multi-gRNA systems enabling simultaneous activation (CRISPRa) and repression (CRISPRi) of multiple target genes. With our collaborators, we have developed tools for CRISPRa and CRISPRi such that the molecular functions of targeting, action, and strength are decoupled. Briefly, the use of scaffold RNAs (3'extended sgRNAs) displaying protein binding aptamers allows differential recruitment of effector proteins to specific dcas9 complexes. Control over the magnitude of applied regulatory actions is achieved through the use of 5' truncated gRNA sequences which decrease fractional occupancy. Using these new capabilities, we have developed a toolkit comprised of a set of orthogonal synthetic promoters which can be targeted by gRNAs for CRISPRi and CRISPRa. Currently, we are using this toolkit to build an incoherent feed-forward loop (IFFL) to assess the robustness of our component I/O relationships in the context of a larger regulatory program. The immediate outcome of this work will be to generate IFFL circuits that can be used to implement adaptive tuning of gene expression, generate threshold-responsive outputs, or detect fold-change differences in inputs. More broadly, using this circuit, and others, we seek to determine the rules governing I/O relationships in multi-gene CRISPRi/a regulatory programs, enabling construction of increasingly complex genetic circuits with predictable I/O characteristics. Looking forward, we hope our work will decrease the failure rate of prototyped pathways facilitating implementation of increasingly complex genetic programs.

32 Laurence Chen, UCLA

"Evaluating Cytotoxic Potency of Synthetic Granzyme B Variants via Retroviral Cytotoxicity Assay"

While targeted T-cell therapies can potentiate strong anti-tumor immune responses, associated on-target, off-tumor toxicities can lead to life-threatening medical complications. These on-target, off-tumor toxicities stem from T-cell targeting of tumor-associated surface antigens that are also present on healthy cells. To address this bottleneck, our group has previously demonstrated the engineering of synthetic Granzyme B (GrB) molecules, termed Cytoplasmic Oncoprotein VErifier and Response Trigger (COVERT), to selectively unleash cytotoxicity in response to intracellular tumor-associated proteases. We propose to further expand the repertoire of targetable intracellular antigens by engineering synthetic GrB molecules to trigger cytotoxicity in response to non-protease intracellular targets. However, conventional methods to evaluate GrB activity are not conducive to rapid design-build-test cycles needed to rationally engineer synthetic GrB switches. Here, we present a novel cell-based retroviral cytotoxicity assay (RVCA) that facilitates fast and iterative testing of synthetic GrB variants. Using RVCA, we show that GrB is amenable to protein insertions and have identified insertion hotspots for subsequent engineering. Future iterations of GrB switch engineering will be built upon the presented findings and evaluated using RVCA as a method to evaluate synthetic GrB cytotoxicity.

33 Kersh Theva, UC Berkeley

"Expanding the synthetic capabilities of chemoautotrophic metabolism"

The reductive acetyl-CoA pathway is the predominant carbon fixation pathway amongst chemoautotrophic organisms. Central to this pathway is the activity of two unusual metalloenzymes: corrinoid iron-sulfur protein (CFeSP) and acetyl-CoA synthase (ACS). Although

these enzymes have been studied extensively, the method of loading necessary metal cofactors into ACS and CFeSP in vivo has yet to be elucidated. Investigating the maturation of these enzymes could improve our understanding of the assembly of metal centers. Furthermore, understanding these phenomena could allow for reconstitution of the reductive acetyl-CoA pathway in a heterologous host, such as Escherichia coli. This engineering effort could have important industrial implications for the environmentally sustainable production of fuels and polymers from carbon dioxide.

To elucidate the panel of necessary factors required for cofactor loading, I have devised experimental approaches that are based on features of known metalloenzyme maturation machinery to study the reductive acetyl-CoA pathway of the model organism, Moorella thermoacetica. After establishing a list of necessary maturation factors, I will introduce them into E.coli that express CFeSP and ACS to determine the sufficient set of additional genes required for in vivo maturation of these enzymes in a heterologous host.

34 Max Schubert, Harvard Medical School

"Exploring Genetic Variation Using Retron Library Recombineering (RLR)"

Next-generation DNA sequencing (NGS) observes tremendous bacterial genetic variation of unknown consequence in the environment, in patient samples, and in the lab. Our ability to create variants and test their impact, however, lags far behind in scale. Existing methods such as Transposon-insertion sequencing (Tn-seq) and CRISPR knockout (KO) can be used to create thousands of genotypes in parallel and measure their phenotypes using NGS. These tools are the basis of bacterial high-throughput functional genomics, enabling in-depth characterization of genomes. These methods, however, cannot create and test variation occurring naturally, because they only create specific loss-of-function changes in genomes. This lack of precision makes natural sequence variation inaccessible to these methods, and leaves the impact of many natural mutations unknown and unexplored. Here we describe a method for pooled construction of specific mutant E. coli cells, and pooled measurement of their phenotypes, and apply this technique to describe mutations resulting in antibiotic resistance. We are able to determine which mutations lead to antibiotic resistance, and compare the relative impact of mutations. We describe the effective use of genome editing using Retron elements to create in vivo single-stranded DNA (ssDNA), and how libraries of mutations can be explored starting with either synthesized DNA or natural genomic DNA. We are able to determine which mutations observed in a directed evolution experiment lead to high-level antibiotic resistance, in this manner. Rapid exploration of many specific genome variants in this way will enable new understanding of genetic variation observed in nature, and also of variation designed to achieve Synthetic Biology goals.

35 James Lucas, UC San Francisco

"Fragment-Based Approach for De Novo Ligand Binding Site Design"

Protein binding to small molecules is fundamental to many biological processes, yet current methods are unable to predictively design this functionality de novo. A generalizable de novo ligand binding site design method would have broad application creating systems that sense and respond to small molecules for medical diagnostics and synthetic biology. To this end, we developed a method that uses highly parallelizable mixed-integer programming and the Rosetta Molecular Modeling Suite to generate hundreds of thousands of favorable binding sites that are built into proteins to impart desired functionality. We are applying this method to design chemically-induced

protein dimerization systems for (S)-reticuline, a key metabolic intermediate in the benzylisoquinoline alkaloid for opioid biosynthesis.

36 Kristina Haslinger, MIT

"Functional screening of putative O-methyltransferases"

O-methyltransferases (OMTs) acting on small molecules are ubiquitous enzymes involved in various secondary metabolite pathways such as the biosynthesis of bacterial antibiotics, human catecholamine neurotransmitters, plant alkaloids and phenylpropanoids.[1] All members share the highly conserved Rossmann fold and strictly require a S-adenosyl methionine (SAM) cofactor, with some additionally utilizing divalent cations.[2] While a large number of putative OMTs are found in sequence data bases, only few examples are functionally characterized. From a pathway engineering perspective, however, it is crucial to know the substrate and product ranges of the respective enzymes to be able to fully exploit their catalytic power.

This study aims to screen a library of OMTs from various microbes for substrate specificity and product range in order to investigate the correlation of sequence, active site architecture and function. An initial pool of ~16,000 candidate sequences was generated by HMMsearch[3] based on the presence of sequence motifs specific to plant caffeic acid OMTs or promiscuous bacterial OMTs. From this pool ~40 sequences were selected based on homology to characterized enzymes and their sequence similarity to other candidates. The selected genes were synthesized for fast functional screening by cell-free expression and high-throughput activity assays based on SAM consumption. The most promising candidates will then be subjected to more detailed structural and functional characterization. This approach will enable us to identify OMTs that may find application in recombinant biosynthetic pathways.

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37 Bon Ikwuagwu, Northwestern University

"Generation of a Non-Canonical Amino Acid MS2 Fitness Landscape and its Applications"

Virus-like particles are derived from the protein shells of viruses and are attractive nanocarriers because they are biocompatible, uniform in size, and have defined surface chemistries that can be used to attach ligands. Recently our lab constructed a quantitative fitness landscape of all single amino acid mutants of MS2 by using a technique we termed SyMAPS (Systematic Mutation and Assembled Particle Selection). We believe we can expand the single amino acid mutants to non-canonical amino acids (ncaa) as well. This ncaa fitness landscape will find the optimal positions to incorporate a specific ncaa with MS2 and will reveal information on whether a ncaa can out compete canonical amino acids at a specific residue. This landscape will also open up the opportunities to use MS2 as a therapeutic and diagnostic (theranostic) carrier by introducing multiple orthogonal sites of conjugations through ncaa incorporation. In this work, I will describe our efforts toward generating a ncaa MS2 fitness landscape and how we are working towards a theranostic MS2 by first investigating ways to encapsulate nanoparticle MRI imaging agents.

38 Suzie Hsu, University of Minnesota-Twin Cities

"Genetic Optimization of Natural Product Biosynthesis in Streptomyces"

Natural products, also known as specialized metabolites, are an invaluable source for high-value chemicals, many of which have important medicinal properties. These important natural products are usually difficult to synthesize chemically, and only can be extracted from scarce amount in their natural biological sources. Hence, there has been an increasing attention to control the genetics and the biosynthesis of natural products in native or engineered organisms. In this talk, I will present a synthetic biology platform for constructing, controlling and optimizing biosynthetic gene clusters via heterologous expression, a technique commonly used to express targeted genes in a more stable and genetically accessible host. For a model system, I used a high-throughput DNA assembly pipeline to build mini library of a completely synthetic eight-gene pathway to produce a late-stage intermediate of serofendic acid, a potent mammalian neuroprotectant, in a bacterial heterologous host Streptomyces albidoflavus J1074. We learned that relative expression level of individual genes matters to the final production titer, identified the a novel P450 monooxygenase required for the tailoring reaction, and isolated two new shunt metabolites. Next, we further attempted to optimize the production titer of serofendic acid late stage intermediate by increasing precursor supply via methylerythritol phosphate (MEP) pathway. The second iteration of synthetic gene cluster (SGC) library design aimed to perturb the relative expression level of eight genes encoding MEP pathway. Preliminary analysis showed that the production titer of late-stage intermediate of serofendic acid was improved from 50 mg/L to over 500 mg/L from the library. The third iteration of SGC library design of MEP pathway was rationally guided by Plackett-Burman (PB) design, a multivariate design principle which allows screening of large number of variables while minimizing the number of tests. This will allow for efficient and rational sampling of the metabolic landscape of MEP pathway. With PB design, I have built all 125 prototypes of the eight-gene pathway and they are currently being screened. Over 2.5 megabases of DNA were manually fabricated into meaningful pathway library designs via the high-throughput DNA assembly pipeline.

39 Noam Prywes, UC Berkeley

"High Throughput Rubisco Biochemistry"

Carbon fixation through the calvin cycle is the source of most of the organic carbon on Earth. Improvements to the central enzyme performing CO2 fixation in the biosphere, rubisco, can transform agriculture and open new routes towards economically sustainable production of biofuels. Only a tiny subset of the ~50,000 sequenced rubisco genes have been tested by heterologous expression or in vitro biochemical assays. Here we leverage breakthroughs in gene synthesis capabilities and metagenomic data to create a library of diverse, previously uncharacterized rubisco variants to explore the biochemical properties of notable natural rubiscos.

The genes we chose were codon-optimized for heterologous expression, produced by whole-gene synthesis, cloned into overexpression vectors and purified from BL21 E. coli. These proteins will be tested in vitro for all four biochemical parameters: kcat, C, kcat, O, KC and KO. A coupled assay for carboxylation parameters already exists and is readily adapted to 384-well plate format under gas control. Here we develop an analogous assay for testing oxygenation parameters without relying on measuring inhibition of carboxylation.

Once we have tested a representative set of rubiscos from the whole set we intend to improve their function through directed evolution in rubisco-dependent E. coli strains. Promising variants will also be cloned into autotrophic bacterial or tobacco strains in order to test their capacity to replace endogenous rubisco genes in autotrophs.

40 Cassandra Barrett, Arizona State University

"Improving Cas9 Editing in Mammalian Cells with Synthetic Pioneer Factors"

Chromatin contributes to contextual variability in mammalian cell engineering. The ability to control chromatin state allows us to effectively manipulate gene expression and editing in eukaryotic chassis. We have developed a panel of synthetic pioneer factors to control the opening of chromatin in order to enhance the expression of silenced transgenes and improve the efficiency of Cas9-mediated editing in chromatin. Pioneer factors are a unique class of transcriptional activators able to bind DNA even in closed chromatin. Sixteen domains with a variety of functions including post translational histone modification, PolII recruitment, and nucleosome remodeling were fused to a DNA binding domain. To test our synthetic pioneers, we used a doxycycline-inducible system that directly induces PRC2 heterochromatin at a luciferase reporter in HEK293 cells. We challenged this induced heterochromatin with each synthetic pioneer to assess their abilities to enhance expression in closed chromatin as well as increase accessibility to Cas9. We identified a novel active site from the proto-oncogene MYB that increases expression at levels similar to canonical activators such as p65. Transient activation by MYB increases expression for over 12 generations of cell division. While activating synthetic pioneers induce expression in closed chromatin, they failed to induce an accessible chromatin state for CRISPR/Cas9 editing. We found that high levels of transcription after pioneer treatment inhibited Cas9-mediated NHEJ, meaning that different pioneers are needed to induce accessible chromatin. We have previously demonstrated that PRC2-induced heterochromatin significantly reduces Cas9 editing. To reverse this, we have identified other synthetic pioneers in our panel that do not increase expression, but do improve Cas9-mediated editing. We are working to characterize and compare the effect of pioneer treatment on chromatin accessibility and nucleosome occupancy.

41 Venkata Chavali, University of Washington

"Mathematical Modeling of dynamically-regulated CRISPR-Cas activation and inhibition programs for Rational Design in Metabolic Engineering"

Biological systems frequently deploy complex regulatory strategies to optimize native metabolic pathways and ensure robustness to varying environmental and internal conditions. In contrast, existing tools for metabolic engineering remain comparatively limited, relying on just a handful of transcription factors or static regulatory elements such as promoter or RBS libraries. The ability to implement complex responses to metabolites or construct large feedback and feedforward circuits still remains a significant challenge. The development of CRISPR-Cas based gene inhibition and activation (CRISPRi/a) facilitates the generation of large libraries of orthogonal transcription factors that can participate in dynamic regulatory circuits and can be designed to sense metabolite concentrations.

Effective tools for the mathematical modeling of CRISPRi/a circuits is necessary for the rational design of synthetic metabolic pathways involving these components. However, modeling the CRISPRi/a system poses certain unique challenges over traditional regulatory elements , including the role of active CRISPR-Cas complex formation on output kinetics, competition between gRNAs, and the on-off kinetics controlling the interaction between the active complex and the target DNA.

To address these challenges, we present a mathematical model of CRISPRi/a kinetics describing how changes in system design affect the system behavior in predictable ways. We aim to show that our model can be well-characterized through simple experiments involving isolated manipulations of the various model parameters and that the characterization of model components in isolation enables us to predict the behavior of more complex CRISPRi/a circuits.

42 April Miguez, Georgia Institute of Technology

"Metabolomics Analysis of the Toxic Effects of the Production of Lycopene and Its Precursors"

In the field of metabolic engineering (the effort to manipulate cells to produce valuable chemicals), efforts for fine temporal and product specificity control have recently received great attention. Unfortunately, manipulating any given metabolic pathway to create a desired product can indirectly affect other parts of metabolism, making fine-tuning cells a challenge. In recent work, we have designed lycopene-producing E. coli for use as a low-cost diagnostic biosensor. To increase the rate of lycopene production, we heterologously expressed the mevalonate pathway to increase precursor availability. We found that simultaneous induction of these pathways increases lycopene production, but surprisingly, induction of the mevalonate pathway before induction of the lycopene pathway decreases both lycopene production and growth rate. Here, we sought to characterize the metabolic changes the cells may be undergoing during expression of these heterologous pathways using two-dimensional gas chromatography coupled to mass spectrometry. We found that the metabolic impacts of producing non-toxic levels of lycopene are of much smaller magnitude than the metabolic changes inherent to batch growth, and that cells could recover from mevalonate-associated toxicity if lycopene production was not also induced. The metabolites homocysteine and homoserine exhibited profiles that potentially link them to the growth inhibition caused by induction of mevalonate production. Based on this analysis, we predicted that extracellular methionine supplementation would limit mevalonate-associated growth inhibition, and we validated this prediction. This suggests potential future avenues toward engineering increased lycopene biosynthesis, as well as the general utility of metabolomics to inform metabolic engineering.

43 Frank Cusimano, Columbia University

"Microbiome Normalization Protocol for Lart Scale Animal Oral Probiotic Experiments"

With an increasing awareness in the role the gut microbiome plays in health and disease, researchers are now studying the effects of individual bacterial strains on host physiology. Unfortunately, an increase in experimentation has led to low precision and poor reproducibility in many microbiome studies. For large scale animal oral probiotic experiments, we tested different microbiome normalization protocols researching the best techniques to ensure experimental accuracy and reproducibility. For experiments with at least 40 mice, using a seven-day, three-rotation normalization protocol provides the greatest homogeneous starting position for experimentation. By controlling for variables including: location of cages, mouse cages used, method of water delivery, PI cage change only, tool sterilization, and oral liquid sterilization, it is possible to systematically ensure experimental reproducibility with large scale animal oral probiotic experiments.

44 Sung Sun Yim, Columbia University

"Multiplexed Transcriptional Characterizations in Cell-Free Systems from Diverse Bacteria"

Precise tuning of gene expression levels is crucial for engineering predictably behaving genetic circuits. Our current understanding of how regulatory sequences control gene expression levels remains limited for most bacterial species. Cell-free expression systems greatly simplify prototyping of genetic designs in vitro. However, the small number of simultaneous measurements that can be made using reporter genes as readouts limits the scale at which biological parts can be

characterized. Here we devised a method to measure expression levels from thousands of regulatory sequences in single cell-free reactions using oligo library DNA synthesis and targeted deep sequencing of RNA and DNA. This multiplexed approach was highly robust and corresponded well with in vivo measurements in E. coli. We further applied this approach in active cell-free transcription systems developed from ten diverse bacterial species, enabling comparison of sequence-function relationships across hosts and predictive modeling of transcriptional activation. We anticipate that this multiplexed approach using cell-free expression systems will expand the capacity for genetic circuit prototyping in new bacterial chassis.

45 Tyler Toth, MIT

"Opine sensing in engineered soil bacteria for niche construction and signaling"

Interactions between plant roots and microbes in the soil can have positive (e.g. nitrogen fixation) or negative (e.g. crown gall disease) impacts on plant health. We propose taking attributes of these native interactions to make engineerable tools for synthetic biology applications. In this work, we aim to use opines, low-molecular weight amine derivatives, as a signaling molecule between genetically engineered plants and bacteria. We demonstrate the ability to sense, transport, and biosynthesize an opine molecule in genetically tractable bacteria. We also show we can control the expression of the nitrogenase gene cluster using opines. Some rhizobium have the ability to metabolize opines as their sole carbon and nitrogen source and we make steps towards replicating this functionality in a chassis soil bacterium with an ultimate goal of using opines to maintain an association between genetically engineered plants and genetically engineered bacteria.

46 Sara Oehmke, Colorado State University

"Plant Synthetic Biology: Design and Quantification of a Cell Type Specific Synthetic Genetic Circuit"

Synthetic biology can utilize plants as a platform to create genetic circuits that are photosynthetically driven and self-sustainable. Although plants are an excellent platform for downstream applications because of this self-sustainability, issues and unpredictability arise from the innate complexity of multicellularity. The ability to quantitatively control specific cell types can reduce the complexity of the platform, thereby circumventing some of these issues. In recent research, we have developed a genetic circuit that allows us to induce and quantitatively control expression of Arabidopsis thaliana root epidermal cells. Our circuit design uses an externally applied ligand that activates a computationally designed transcriptional response controlling GFP expression. In addition, we also engineered a genetic positive feedback circuit. To quantify the behavior of these circuits we use a Matlab program that removes autofluorescence and computes the mean intensity of optical sections generated on a spinning disk confocal microscope.

Our results indicate that our circuit functions as predicted in that it is highly specific for the cell type (root epidermis), fully controllable with the external ligand, and capable of amplification with the positive feedback circuit. The concepts and components of these circuits can be applied to future designs to produce plants and other multicellular platforms that give the operator greater control over circuit function, thereby producing more predictable outcomes.

47 Widianti Sugianto, Georgia Institute of Technology

"Production of Plant Natural Products in a Synthetic Cell"

Galantamine, an Alzheimer's drug, has low level of accumulation in its native plants (Galanthus, sp.). Large-scale production of plant natural products in traditional heterologous hosts, such as

Escherichia coli and Saccharomyces cerevisiae, presents a major hindrance as overproducing complex plant chemicals in these microbes is not reliable. Moreover, optimization of plant P450 transmembrane protein expression in heterologous host is required. Cell-free expression system (TXTL) with liposomes has been successfully utilized for producing functional transmembrane proteins. Here, we use a TXTL-based system with liposomes to express plant P450 transmembrane proteins in-vitro. The TXTL-based galantamine production would be assessed by quantifying catalytic activities of plant transmembrane P450s. The TXTL system would also be used for rapid prototyping of plant para-ortho P450 for efficient production of N-demethylnarwedine, a galantamine intermediate. Finally, the production of N- demethylnarwedine in a plant-based TXTL system would aid the semi-synthesis of galantamine from inexpensive precursors.

48 Daniel Anderson, MIT

"Ratiometric pre-processing circuits enable the rejection of correlated input disturbances"

Current genetic circuit design principles typically require specific, well-controlled inputs for proper circuit functionality. While this approach has been able to produce large genetic circuits with complex functionalities, the applications of these circuits can be limited by this dependence on specific, absolute input values. Absolute input values can be disturbed for a variety of reasons including environmental variation and sensor crosstalk. Both of the aforementioned examples often exhibit correlated input disturbances, where the disturbance acts in the same manner across all inputs. In this work, we show our progress towards developing a generalizable input pre-processing circuit that can rejected correlated disturbances. We begin by showing that taking the ratios of the disturbed inputs can remove the correlated disturbance. Then, using a model-driven approach, we derive a generalizable genetic circuit mechanism that is capable of responding to the ratios of two inputs. This mechanism is then experimentally demonstrated using dCas9 to enact transcriptional control. Our work provides a generalizable input pre-processing module that can reject correlated input disturbances and output specific, well-controlled absolute values for input into synthetic genetic circuitry. Our input signal pre-processing module can be easily integrated into current genetic circuit design methods to enable the rejection of correlated input disturbances.

49 Lina Gonzalez, MIT

"Resilient Living Materials Built By Printing Bacterial Spores"

A route to advanced multifunctional materials is to embed them with living cells that can perform sensing, chemical production, energy scavenging, and actuation. A challenge in realizing this potential is that the conditions for keeping cells alive are not conducive to materials processing and require a continuous source of water and nutrients. Here, we present a 3D printer that can mix material and cell streams in a novel printhead and build 3D objects (up to 2.5 cm by 1 cm by 1 cm). Hydrogels are printed using 5% agarose, which has a low melting temperature (65oC) consistent with thermophilic cells, a rigid storage modulus (G'= 6.5 x 104), exhibits shear thinning, and can be rapidly hardened upon cooling to preserve structural features. Spores of B. subtilis are printed within the material and germinate on its exterior, including spontaneously in cracks and new surfaces exposed by tears. By introducing genetically engineered bacteria, the materials can sense chemicals (IPTG, xylose, or vanillic acid). Further, we show that the spores are resilient to extreme environmental stresses, including desiccation, solvents (ethanol), high osmolarity (1.5 mM NaCl), 365 nm UV light, and gamma-radiation (2.6 kGy). The construction of 3D printed materials containing spores enables the living functions to be used for applications that require long-term storage, in-field functionality, or exposure to uncertain environmental stresses.

50 Rob Warden-Rothman, MIT

"ROUTE: Retrobiosynthetic Design Utilizing High-Throughput Enzyme Characterization"

We report the development of ROUTE (Retrobiosynthetic Operations Using Tested Enzymes): a suite of computational tools for the forward design of metabolic pathways and large-scale characterization of biosynthetic enzymes. Given a target compound, natural or novel, the tool searches a database of empirically characterized enzymes and returns a DNA sequence capable of producing the compound in vivo. Our tool is agnostic of the enzyme's source organism and pathway, allowing it to combine enzymes in novel ways, opening large chemical spaces of natural and synthetic products. Many of these new compounds would be difficult to produce via organic chemistry but are easily accessible by biosynthesis due to the inherent regio- and stereoselectivity of enzymatic reactions. Literature and database scraping have been used previously to help design biosynthetic schemes, but those tools only suggest which classes of biochemical reactions will be necessary to produce a new compound, leaving the scientist to decide which specific enzymes to include in the final strain design. This is likely because there are few well-characterized enzymes in the databases and an even smaller set that have been tested outside the context of their natural pathways, making it nearly impossible to predict a particular enzyme's ability to carry out a novel reaction based on sequence alone. An empirical approach is necessary for reliable enzyme selection, so we have developed automation pipelines for high-throughput assembly of large pathway libraries and for analyzing their metabolite production by liquid chromatography/mass spectrometry. By combining automation with our novel bioretrosynthesis approach, we have been able to design and detect previously unreported members of the β-arylamine family of small molecules.

51 Kathryn Brink, Rice University

"Screening for peptide inhibitors of S. typhimurium PhoPQ in E. coli"

Antibiotic resistance is an urgent threat, causing 2 million infections each year in the United States alone. In pathogenic bacteria, antibiotic resistance and virulence are often regulated by two-component systems (TCSs), the primary means by which bacteria sense their external environments. Many of these TCSs sense antimicrobial peptides (AMPs), a promising source of novel antibiotics, and in response, activate AMP resistance pathways, hindering the efficacy of these therapeutics. Because of the role that TCSs play in AMP resistance, next-generation peptide therapeutics should either avoid activating these sensors, thereby bypassing AMP resistance pathways, or specifically inhibit them, preventing them from upregulating AMP resistance and virulence genes even under otherwise activating conditions. Here, we aim to identify novel peptide therapeutics that meet these criteria for the TCS PhoPQ, an AMP sensor and global regulator of virulence in many Gram-negative pathogens including Salmonella typhimurium. First, we engineered and ported S. typhimurium PhoPQ to E. coli to enable precise control of PhoP and PhoQ gene expression. We then adapted an existing peptide screening approach to enable high-throughput measurement of peptide-mediated activation and inhibition of PhoPQ. Through preliminary screens, we validated that known PhoQ-activating peptides activated this sensor using our approach. We also identified a putative peptide inhibitor of PhoPQ with potential antivirulence properties. In the future, we will screen PhoPQ against a large peptide library to identify additional therapeutically-relevant peptides and to further elucidate peptide properties required for activation or inhibition of this sensor. By studying PhoPQ-peptide interactions, we will advance our understanding of the biology of a critical regulator of bacterial virulence and discover novel therapeutics for treating antibiotic-resistant infections.

52 Divya Israni, Boston University

"Synthetic transcriptional regulation for human cell therapy"

An enduring goal of synthetic biology is to program human cells to sense and respond to disease in increasingly sophisticated ways. Towards this goal, numerous technological advances have employed orthogonal and engineered regulatory elements to control genetic programs in mammalian cells. However, these components are often incompatible with regulation in a broader therapeutic context due low specificity within the genome, high potential for immunogenicity, and challenges with payload delivery. There remains a need for programmable genetic elements that can both overcome these barriers to therapeutic translation and offer tunable and versatile regulatory profiles in mammalian systems.

Here, we developed a new platform for synthetic transcriptional control poised for diverse gene regulatory programs in human cells. We engineered a class of programmable transcriptional regulators based upon artificial zinc finger domains, DNA-binding domains that are highly advantageous due to their compact size and derivation from native mammalian transcriptional systems. We constructed a library of synthetic transcription factors with binding motifs that are unique and putatively orthogonal to the human genome. Our evaluation of cellular transcriptome response when these synthetic components are expressed in cell lines revealed highly specific on-target and low off-target regulation.

Furthermore, we developed small-molecule controllable versions of our transcription factors that are regulated by safe and/or FDA-approved drugs to achieve dose- and time-dependent control of output gene expression. We then utilized these inducible regulators to precisely modulate the expression of relevant therapeutic agents, including CARs and cytokines, in human cell lines and primary T cells. Taken together, we envision that our synthetic transcriptional regulation platform will facilitate the design of sophisticated programs for a broad array of gene regulation and cell therapy applications.

53 Marco Galardini, Boston University

"Systematic evaluation of conditional gene essentiality changes across four Saccharomyces cerevisiae strains"

Loss-of-function (LoF) mutations associated with disease don't manifest equally in different individuals, a phenomenon known as incomplete penetrance. The impact of the genetic background on incomplete penetrance remains poorly characterized. Here, we systematically assessed the changes in gene deletion phenotypes for 3,786 gene knockouts in four Saccharomyces cerevisiae strains and 38 conditions. We observed 16% to 42% of deletion phenotypes changing between pairs of strains with a small fraction conserved in all strains. Conditions causing higher WT growth differences and the deletion of pleiotropic genes showed above average changes in phenotypes. We further illustrate how these changes affect the interpretation of the impact of genetic variants across 925 yeast isolates. These results show the high degree of genetic background dependencies for LoF phenotypes.

54 Marilyn Lee, US Army ECBC

"The Performance and Potential of Hydrogel Matrices as an Alternate Reaction Format for Cell-Free Protein Synthesis"

Cell-free protein synthesis (CFPS) is an important emerging platform to deploy engineered cellular functions in austere environments. Recent developments have shown that E. coli lysates lyophilized on paper can run genetic circuits upon rehydration, and do not need cold storage. Further

exploration of alternative reaction formats that go beyond the test tube could enable more complex devices powered by CFPS and yield gains in speed and reliability. Hydrogel matrices serve as a support for many bio-engineering applications including cell culture, drug delivery, tissue scaffolding, and bio-printing. The mechanical properties and chemical functional groups displayed by hydrogel materials influence the function of biological systems encapsulated within. In this study, the effects of hydrogel encapsulation on cell-free protein synthesis (CFPS) reactions are explored. The hydrogel encapsulation of E. coli lysates for cell-free protein synthesis yield active, programmable 3D-materials. Protein-based and synthetic hydrogels are tested, and the activity, stability, and longevity of the resulting CFPS gels are assessed. This work expands the CFPS toolbox to enable future development of spatial localization of genetic functionalities, control over the diffusion of bio-molecules, and mechanical actuation output capabilities.

55 Nicholas Kruyer, Georgia Institute of Technology

"Understanding Muconic Acid Reductase through Exploration of Substrate Specificity "

Industrial production of adipic acid relies on toxic petrochemical precursors and catalysts that are large-scale producers of nitrous oxide pollution. Recently, efforts have been made to use biomass-derived feedstocks for microbial production of adipic acid. Some promising feedstocks include lignin-derived monomers, such as catechol and guaiacol, which are funneled to adipic acid through the direct precursor muconic acid. We optimized the production of muconic acid in Escherichia coli by optimizing expression of different catechol 1,2-dioxygenase homologs, heterologous to E. coli. Furthermore, we explored substrate specificity of enzymes recently identified as capable of reducing muconic acid to adipic acid. We explored substrate specificity as a method for gathering information on the structure and mechanism of these under-characterized enzymes. This information can be combined with information from other iron-sulfur cluster characterization techniques to improve the yield and productivity of one of the first fully enzymatic routes from lignin-derived monomers to adipic acid.

56 Stephanie Robinson, UC Berkeley

"Using fitness landscapes to identify and characterize a novel position for MS2 peptide insertions"

Virus-like particles (VLPs) are nanoparticles made of non-infectious viral capsids. For example, the MS2 bacteriophage is a robust VLP and is studied as an imaging and drug delivery system. The MS2 VLP is stable for extended periods of time in heat, is high-yielding with simple recombinant expression, and can encapsulate artificial cargo. Recently, the Francis and Tullman-Ercek groups explored methods of engineering this capsid using protein engineering techniques such as generating an apparent fitness landscape. A fitness landscape generates a fitness score for mutants based on survivability of a genotype after selections such as: capsid assembly, thermostability, and acid sensitivity. This fitness landscape can inform on which residues are tolerated in the protein's assembly and may reveal surprising structural properties. Here, the MS2 fitness landscape was used to identify a highly mutable region in the coat protein known as the FG loop. The mutability and unique structure of the FG loop was used to inform the generation of a tripeptide or (NNK)3 insertion library at the FG loop of the MS2 coat protein. These libraries show that the FG loop is particularly tolerable of residues disruptive to secondary structure, with high localizations of charge, and that can hydrogen bond. These effects dominate capsid assembly to such an extent that when these well-performing residues are grouped with extremely poorly performing residues, capsid assembly is still recovered. Ultimately, this work validates the utility of fitness landscapes in engineering VLPs, and illuminate how properties such as charge, flexibility, and the hydrogen bonding in the FG loop conserve capsid assembly.

57 Matthew Lux, US Army ECBC

"Sensor development and deployment for defense applications"

A major area within synthetic biology that is of interest for defense applications is novel sensing technologies. Natural systems employ sensor elements for huge ranges of analyte categories and with wide variance in performance metrics. Combining naturally occurring systems with engineering tools promises novel sensor systems tailored to targets of interest that meet relevant specifications. Here we describe a consortium of efforts across the US Department of Defense that merge to tackle challenges in sensor development and deployment to meet specific defense needs. In particular, we address defense needs in human performance monitoring, biological agent detection, in-field water quality testing, and explosives detection. In each case, different operational needs (response time, sensitivity, specificity, etc.) and different maturity levels of existing sensors lead to a different set of challenges. Underpinning these application areas is a common approach to deploy the sensors by freeze-drying cell-free systems (CFS) into paper tickets. These tickets are lightweight, cheap, multiplexable, and can be read by eye or by handheld reader. Compared to cell-based sensors, this approach sidesteps major challenges in maintenance of cell viability, cell performance under varying conditions, and concern over release of genetically modified organisms. The work presented here includes both efforts towards key sensors in each operational category and development of the paper ticket platform, and in some cases merges the two into functional detection of molecules of interest on paper.

58 Chia Hung, US Air Force Research Laboratory

"Bacterially-produced melanin as biomaterials"

Melanin, a highly-coordinated polymeric nanoparticle found in natural pigments, has many intriguing properties including protecting organisms from ultra-violet radiation, ability to scavenge reactive species and metal ions, and reported electrical conductivity. Melanin particles isolated from naturally pigmented sources are biologically templated with pre-defined shapes that dictate the optical and protective properties of the melanin assemblies. The tyrosinase enzyme is known to be involved in the biosynthesis of melanin, which lends credence to the use of a synthetic biology approach to engineer tyrosinase for melanin production. In this work, Bacillus megaterium tyrosinase was engineered and expressed recombinantly in Escherichia coli. Two forms of tyrosinase fusions were designed to be either secreted through the curli export system or anchored on the cell surfaces. Using these systems, small melanin nanoparticles were able to be produced. In addition, melanin ghosts templated by E. coli cells were synthesized and characterized. Thus the tyrosinase expression, secretion and display capability that will be presented here represents a platform capable of allowing for the production colloidal melanin nanoparticles as well as melanin functionalized surfaces. Future work will investigate tuning the optical and electrical properties of the melanin assemblies through control of size and shape of the superstructure.

59 Ross Jones, MIT

"Precision control of gene expression in eukaryotic cells using a CRISPR endoribonuclease-based incoherent feedforward loop"

A major objective for studying and engineering biology is to establish precise and predictable control over heterologous gene expression. Previous efforts have successfully linearized and reduced noise in small-molecule induction curves 1,2, reduced or eliminated the effect of gene

copy number on expression levels 3–5, and used quasi-integral control to buffer against ribosome resource competition 6. While these studies have provided great advances for prokaryotic systems, current methods for gene expression stabilization and disturbance rejection in eukaryotes are limited. Here, we introduce a simple CRISPR endoribonuclease (ERN)-based incoherent feedforward loop (iFFL) device, called EIFFL, for precise and predictable control of gene expression. The iFFL structure of EIFFL works by offsetting transcriptional changes with targeted mRNA degradation, which theoretically enables perfect adaptation of output protein expression to pre-translational disturbances affecting both the ERN and the output. To experimentally investigate this adaptation in mammalian cells, we measured the response of EIFFL to ectopic gene copy number variance and depletion of transcriptional resources. We found that the EIFFL output expression is precisely stabilized over several log-decades of transfected DNA copy number input, vastly improving upon previous miRNA-based iFFL designs 3,4. In addition, the set point for output expression can be easily and predictably tuned by modulating the ERN translation rate using upstream open reading frames (uORFs). Finally, we found that EIFFL can effectively buffer output set points ranging over one log-decade against transcriptional resource competition. Together, these results demonstrate that EIFFL can significantly reduce the effects of circuit and host contexts on heterologous gene expression. We expect that this device will show broad utility for precise control of protein expression within eukaryotic systems.

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