EBRC Engineering Biology Research Consortium

2026 Annual Meeting Program

June 2-3, 2026

North Carolina State University

www.ebrc.org

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10 Year Anniversary - 2026 ANNUAL MEETING AGENDA

North Carolina State University
Tally Student Union
2610 Cates Ave
Raleigh, NC 27607

Monday, June 1, 2026

	Industry Session: Advancing the Bioeconomy Industry Ecosystem (Coastal Ballroom)	**Student and Postdoc Association (SPA): Site Visit and Social**
3:30 PM	**Arrival & Registration**	
4:00 PM	**Welcome Remarks** *India Hook Barnard, EBRC*	**SPA Industry Site Visits** *Email helix@ebrc.org for more information*
4:05 PM	**Featured Remarks** *Sec. Lee Lilley will speak to North Carolina's role in building a thriving bioeconomy, including the state's assets in the Research Triangle Park and the opportunities North Carolina is creating for industry to grow and scale.* ***Lee Lilley**, Secretary of Commerce for the State of North Carolina*	

4:20 PM	**Expert Panel** *This panel brings together across the engineering biology ecosystem to share perspectives on how promising ideas move from early-stage research to funded programs and deployable technologies.* *Moderator: India-Hook Barnard, EBRC* **Speakers:** ● **Deepti Tanjore**, Director (Advanced Biofuels and Bioproducts Process Development Unit, Lawrence Berkeley National Laboratory) ● **Ryan Tappel**, Senior Scientist (LanzaTech) ● **Ursula Koniges**, Deputy Program Scientist, Translational Research (National Aeronautics and Space Administration) ● **Bridget Turaga**, Strategic Advisor for Partnerships (NSF Technology, Innovation, and Partnerships Directorate) ● **Gloria Elliott**, Program Manager (Advanced Research Projects Agency for Health) ● *Others TBC, Government Funding Agencies*	
5:00 PM	**Industry Flash Talks** *Industry and academic researchers deliver 2-minute pitches on their work, highlighting emerging research-industry collaborations.* *Moderator: Garrett Dunlap, EBRC*	
5:30 PM	**Closing Remarks** *India Hook Barnard, EBRC*	
5:30 PM	**Industry Member Mixer** *3rd Floor Lounge*	**SPA Social** *Email helix@ebrc.org for more information*

Tuesday, June 2, 2026

Piedmont Mountains Ballroom	
8:00 AM	**Arrival & Registration** *Coffee and tea available*
9:00 AM	**WELCOME TO THE EBRC ANNUAL MEETING** *India Hook-Barnard, EBRC* • EBRC overview and updates
9:20 AM	**SESSION 1: RESEARCH TALKS - Engineering Non-Model Bacteria: Genetic Tools, Pathway Optimization, and High-Throughput Screening for Sustainable Bioproduction** *Moderator: Nils Averesch, University of Florida* • Joey Galindo (Princeton University): Development of genetic tools for metabolic engineering in the extremely thermophilic anaerobic bacterium Anaerocellum bescii • Andrea Garza Elizondo (Oak Ridge National Laboratory): Domesticating Non-Model Bacteria for Biotechnology • Cody Kamoku (Pacific Northwest National Lab): Development of a whole cell biosensor for high throughput screening of two stage bioprocesses • Robert Barlow (University of Delaware): Developing prokaryotic Argonautes (pAgos) as a more flexible platform for base editing
10:20 AM	**BREAK** *Light refreshments are located in room 3285.*
10:40 AM	**SESSION 2: RESEARCH TALKS - Quantitative Synthetic Biology** *Moderator: Ania-Ariadna Baetica, Drexel University* • Glory J. Onajobi (Georgia Institute of Technology): Expanding the Biocomputing Capacity of Transcriptional Programming Wetware • Nicholas Kaplan (University of Washington): Quantifying XNA replication fidelity using nanopore sequencing • Ishita Kumar (Georgia Institute of Technology): Protecting cells at the genetic level and simulating unauthorized access via a biohackathon • Nguyen Tran (Drexel University): Optimizing amplification circuits in the presence of leak
11:40 AM	**EBRC Focus Area Updates** *Garrett Dunlap, EBRC* *Emily Aurand, EBRC* *Becky Mackelprang, EBRC*
12:15 PM	**LUNCH & SPA Networking Event** *Boxed lunch provided in room 3285.*

1:15 PM	**Global Bioeconomy and Innovation Ecosystem** *Moderator: Garrett Dunlap, EBRC*
2:00 PM	**Securing the U.S. Bioeconomy: A Managed Access Framework for Biotechnology Innovation** *Jon Judd, EBRC*
2:10 PM	**AIxEng Bio** *Advances in artificial intelligence and machine learning (AI/ML) have significantly impacted engineering biology research. In this session we will highlight exciting new examples of the interface between AI/ML and engineering biology, identify new opportunities and what is needed to realize them, and discuss how to conduct this research responsibly.* *Moderator: Sebastian Rivera, EBRC* **Panelists:** ● **Gene Olinger**, University of Texas Medical Branch & Galveston National Laboratory ● **David Ross**, National Institute for Standards and Technology ● **Jose Carlos Garcia-Garcia,** Procter & Gamble
3:00 PM	**BREAK** *Light refreshments are located in room 3285.*
3:30 PM	**Student and Postdoc Flash Talks** *Moderator: Megan Olsen, SPA President, Northwestern University*
4:15 PM	**10 Year Anniversary Keynote** *Moderator: India Hook-Barnard, EBRC* **Speakers:** ● **Theresa Good**, National Science Foundation ● **Caitlin Frazer**, National Security Commission on Emerging Biotechnology
5:30 PM	**Poster Session** *Posters in the Coastal Ballroom* *Light refreshments are located in room 3285* ● *Odd*-numbered Poster Presentation Time: **5:30 - 6:30 PM** ● *Even*-numbered Poster Presentation Time: **6:30 - 7:30 PM**
7:30 PM	**ADJOURN**

Wednesday, June 3, 2026

Piedmont Mountains Ballroom	
6:00 AM	**EBRC Fun Run/Walk (Strava route here)** *Meet in front of Residence Inn Raleigh Downtown, 616 S. Salisbury St, Raleigh NC 27601*
8:00 AM	**Day 2 Arrival** *Coffee and tea available*
9:00 AM	**WELCOME TO DAY 2** *India Hook-Barnard, EBRC*
9:05 AM	**Reflections on Life, Synthetic Cells, and Responsible Innovation** *Moderator: John Glass, JCVI*
9:35 AM	**Education and Workforce Pathways in Engineering Biology** *This session will explore what entering the engineering biology and biotechnology workforce looks like today, including the skills and credentials that matter, where education meets industry expectations, and where the opportunities lie to build a more robust talent pipeline.* *Moderator: Julietta Sheng, EBRC* **Panelists:** ● **Meg McSweeney**, Stanford University ● **Philip Gibson**, Georgia Life Sciences ● **Xu Zhang**, Texas A&M University ● **Cameron Kim**, Duke University ● **Janet Standeven**, iGEM, University of Colorado
10:15 AM	**BREAK** *Light refreshments are located in room 3285.*
10:45 AM	**SESSION 3: RESEARCH TALKS - Advances in Understanding Cells That Make Food and What Those Cells Can Do** *Moderator: Ryan Tappel, LanzaTech* ● Sara Stadulis (Cornell University): Pyruvate formate-lyase enables ethanol reduction in pyruvate decarboxylase-deficient Saccharomyces cerevisiae ● Ella Gunady (Princeton University): Designing synthetic communities to promote agricultural resilience during drought ● Curt Chaffin (Good Food Institute): Exploring the research and bioprocessing benefits of food biotech informatics and how to increase data access ● Aditya Sarnaik (Arizona State University): Beyond Proof-of-Concept: A Systems-Integrated Approach to Scalable Biomanufacturing using Photosynthetic Microbes

11:45 AM	**Building a Resilient and Sustainable Agri-food System** *Moderator: Rohan Shirwaiker, Bezos Center for Sustainable Protein at NC State University* **Panelists:** ● **Kelly Smith**, Sable Fermentation ● **Mike Hess**, Novonesis ● **Jon Arizti Sans**, Stanford University
12:30 PM	**LUNCH** *Provided in room 3285.*
1:30 PM	**SPA Updates** *Meagan Olsen, SPA President, Northwestern University*
1:45 PM	**SESSION 4: RESEARCH TALKS - Engineered Biology in Medicine: Innovation Across the Pharmaceutical Pipeline** *Moderator: Kaitlin Dailey, University of Rhode Island* ● Simon d'Oelsnitz (Harvard Medical School): Using Enantioselective Biosensors to Evolve Asymmetric Biocatalysts ● Matthew Carpenter (Rice University): Towards Electrogenetic Control of Drug Activation by a β-glucuronidase ● Yousong Ding (University of Florida): Programmable Microbial Chassis for Living Therapeutics and Natural Product Production ● Chelsea Hu (Texas A&M University): Domesticating Brucella melitensis DvjbR for living bacterial Immunotherapy
2:45 PM	**BREAK** *Light refreshments are located in room 3285.*
3:15 PM	**Tales from the Road: Driving Emerging Biotech Across America** *Moderator: Garrett Dunlap, EBRC* **Panelists:** ● **Meagan Lizarazo**, National Security Commission on Emerging Biotechnology ● **Megan Damico**, North Carolina Biotechnology Center ● **Kerri Dugan**, Applied Research Institute
4:00 PM	**EBRC In Translation LIVE!** *Moderator: Talia Jacobson, University of Florida*
4:45 PM	**CLOSING REMARKS**
5:00 PM	**ADJOURNMENT**

IMPORTANT REMINDERS & CONFIDENTIALITY

Confidentiality

EBRC Antitrust Policy is available at www.ebrc.org/antitrust.

Photo & Video

EBRC will be taking photographs, sound recordings, and videos during this event in the interest of serving the EBRC's mission of research, education, public service, and for promoting the public good. If you wish for your image to not be used, please find an EBRC staff member and let them know as soon as possible.

Professional Courtesy

Please DO have an awesome time and share that with friends and social media. DO NOT share research results or pictures of posters without permission.

JUNE 3 FUN RUN/WALK

Meet EBRC Program Manager, Julietta, in front of The Residence Inn Raleigh Downtown (616 S. Salisbury St., Raleigh, NC 27601) **Wednesday, June 3rd at 6:00am** for a fun run/walk around downtown Raleigh. Additional details and route can be found here; please contact Julietta (julietta@ebrc.org) if you have any questions.

RESEARCH TALK ABSTRACTS

Tuesday, June 2nd

SESSION 1: RESEACH TALKS - "Engineering Non-Model Bacteria: Genetic Tools, Pathway Optimization, and High-Throughput Screening for Sustainable Bioproduction"
Moderator: Nils Averesch (University of Florida)

"Development of genetic tools for metabolic engineering in the extremely thermophilic anaerobic bacterium Anaerocellum bescii"

Joey Galindo (Princeton University)

Thermophilic anaerobic organisms, particularly species that can naturally degrade lignocellulosic biomass, show great promise for next generation bioprocessing. Yet the development of genetic tools to metabolically engineer these non-model organisms remains limited. One major challenge is a lack of reliable reporter systems compatible with the combination of thermophilic and anaerobic growth conditions. Here we describe the development of a straightforward and robust enzymatic reporter system that overcomes these challenges in Anaerocellum (f. Caldicellulosiruptor) bescii, an anaerobic, extremely thermophilic (Topt ~78 °C), lignocellulolytic bacterium. Our method is based on heterologous expression of hyperthermophilic archaeal galactosidases: an α-galactosidase from Pyroccous furiosus (Pfαgal), and a β-galactosidase from Caldivirga maquilingensis (Cmβgal). We show that these reporters produce strong, orthogonal signals on colorimetric substrates at high temperatures (≥90°C) that eliminate background activity from endogenous galactosidases. We then demonstrate the capability of Cmβgal, the stronger of the two reporters, to distinguish differences in levels of expression between two known A. bescii promoter sequences, which we verify through qRT-PCR. Finally, we utilize this system to screen a diverse suite of new native A. bescii promoter sequences. Taken as a whole, we describe an easy to implement enzymatic reporter system that could be readily adapted for use in other thermophilic anaerobic species. We then utilize our reporter system to expand the genetic toolkit of A. bescii with the aim of enabling future metabolic engineering efforts.

Co-authors: Joey Galindo, Hansen Tjo, Archit Srivastava, Jonathan Conway

"Domesticating Non-Model Bacteria for Biotechnology"
Andrea Garza Elizondo (Oak Ridge National Laboratory)

Modern genomics has shown us that nature is rife with diverse bacterial phenotypes that can potentially be leveraged for a variety of applications - biodegradation of wastes, synthesis of petrochemicals and pharmaceuticals, and accumulation of valuable elements. Replicating these phenotypes in our model organisms isn't always feasible or preferred, thus, as our tools improve, engineering non-model bacteria is becoming a popular strategy. This poses the problem of achieving the genetic tractability we've built over decades of E. coli research, in a fraction of the time. Here, I demonstrate the work we have done in the Synthetic Biology section at Oak Ridge National Laboratory to build pipelines that facilitate and accelerate the domestication of non-model bacteria. Our team has designed bioinformatic and genetic methods to identify and evade restriction modification systems in isolates, increasing transformation efficiencies by mimicking an isolate's methylation patterns. Furthermore, we have collected libraries of genetic elements – such as plasmid origins of replication, inducible promoters, and CRISPR-based editing systems – and are leveraging physical automation to test them at high throughput.

Co-authors: Jiwoo Kim, Ilenne Del Valle Kessra, Michael Melesse Vergara, William Alexander, Adam Guss, Kevin Solomon, Mark Blenner, Carrie Eckert

"Development of a whole cell biosensor for high throughput screening of two stage bioprocesses"

Cody Kamoku (Pacific Northwest National Lab)

Efficient development of microbial bioprocesses is limited by the lack of real time, high throughput measurements that accurately report product formation in small volume cultures. To address this issue, we have developed whole cell biosensors to enable continuous plate based quantification of productivity for dicarboxylic acids, citrate and other value added chemicals. The whole cell sensor consists of a fluorescent Pseudomonas putida (SENS) strain engineered to not consume glucose but instead consume the analyte of interest. The developed sensor strains produce a fluorescent signal proportional to the amount of target analyte when grown with supernatants from a bioproduction strain or when grown in a co-culture with a bioproduction strain.

As an immediate testbed, we have evaluated engineered P. putida strains that produce muconic acid or itaconic acid from diverse lignocellulosic carbon sources with our developed SENS strains. Development of a sensor strain for itaconic acid required additional engineering as nitrogen limitation is used to trigger stationary phase production of itaconic acid by the production strains used in this study. In this case, accurate sensing requires that any nitrogen source used to grow the sensor strain should not disrupt induction of the itaconic acid pathway by the production strain. To address this issue, we have developed SENS strains compatible with two stage bioproduction processes driven by nitrogen limitation by introducing orthogonal nitrogen utilization pathways into the sensor strain, thus eliminating potential crosstalk between the two strains.

The developed sensor strains will be used in the future as a basis for an automated workflow for rapidly mapping genotype–environment–phenotype relationships that drive improved bioproduction in different scales ranging from 96 well plates to small scale bioreactor conditions.

Co-authors: Cody Kamoku, Joshua Elmore, Robert Egbert

"Developing prokaryotic Argonautes (pAgos) as a more flexible platform for base editing"

Robert Barlow (University of Delaware)

Base editing is a precise gene editing approach used in human health, crop development, and sustainable biomanufacturing. Base editors induce targeted nucleotide changes by combining a partially inactive Cas protein with a nucleotide deaminase. This reduces indel formation and improves editing efficiency compared to classical gene editing approaches. However, Cas-based systems require a protospacer adjacent motif (PAM) site for target recognition. This constraint limits editing in genomic regions where PAM sequences are scarce or poorly positioned. Prokaryotic Argonautes (pAgos) are programmable DNA endonucleases that offer a more flexible alternative for directing deaminase activity. They use short, approximately twenty-nucleotide, 5'-phosphorylated single-stranded DNA (ssDNA) guides that do not require a PAM site for targeting. Although pAgos have been used for gene editing in several bacterial species, all existing systems rely on exogenously supplied guides or chopping (pseudorandom DNA cleavage of apo-pAgos). To improve ease of use and guide loading efficiency, I engineered mutant T7 RNA polymerases capable of synthesizing 5'-phosphorylated ssDNA guides that are recognized and bound by Clostridium butyricum Argonaute (CbAgo). These systems fully recapitulate native CbAgo target recognition and cleavage. To enable base editing, we fused CbAgo, which only nicks double-stranded targets, to the adenine deaminase ABE8e. I then combined it with our guide generation system. Successful base editing was detected through activation of an antibiotic resistance phenotype and validated with sequencing. These results demonstrate precise editing without PAM dependence, establishing the foundation for a pAgo-based base editing platform. Ultimately, this work demonstrates the utility of pAgos for developing of next-generation gene editing technologies and provides a proof-of-concept pAgo base editor for unrestricted genome manipulation.

Co-authors: Austin Futty, Jasmine Grimsley, Kevin V. Solomon

SESSION 2: RESEARCH TALKS - "Quantitative Synthetic Biology"
Moderator: Ania-Ariadna Baetica (Drexel University)

"Expanding the Biocomputing Capacity of Transcriptional Programming Wetware"
Glory J. Onajobi (Georgia Institute of Technology)

Synthetic biology seeks to engineer cells that can sense, process, and respond to complex environmental signals, enabling applications in a broad range of areas (e.g., biomanufacturing, living therapeutic drug delivery, and biocomputing). However, a major challenge in the field is developing scalable gene circuit design strategies with minimal metabolic burden, particularly as circuit complexity increases. The presented work addresses this challenge by advancing the Transcriptional Programming (T-Pro) wetware platform as a framework for programmable, logic-based control of gene expression. At the core of this approach is the engineering of anti-repressor biosensors, which expands the current regulatory toolkit and enables more flexible and modular circuit design. A computational design strategy is then introduced to translate objective truth tables into compressed genetic circuit architectures, allowing for the efficient implementation of multi-input behaviors within biological parameters. Genetic circuits generated by the software are then constructed using the expanded set of biosensors to demonstrate the effective implementation of these multi-input behaviors. This work establishes a cohesive strategy that links molecular design with system-level engineering, enabling the predictive design of compressed genetic circuits and quantitative performance for higher-state (>3 inputs) biological programming.

Co-authors: Zachary Herde, Junghwan Lee, Yongjoon Yu, Matthew Realff, Corey J. Wilson

"Quantifying XNA replication fidelity using nanopore sequencing"
Nicholas Kaplan (University of Washington)

Expanded genetic alphabets built from unnatural base-pairing xenonucleic acids, or ubp XNAs, are part of an emerging frontier in biotechnology, therapeutics, and synthetic biology. However, their adoption has been constrained by replication fidelities that remain substantially lower than those of standard, natural DNA. We present a method for rapidly and accurately measuring ubp XNA replication fidelity using single-molecule nanopore sequencing. By coupling nanopore sequencing with post hoc machine-learning classifiers, we infer strand-resolved, cycle-dependent fidelity parameters directly from PCR amplification data and place these measurements on a rigorous mathematical footing that accounts for coupled replication dynamics. We apply this approach to three chemically distinct ubp systems—hydrogen-bonding B≡S and P≡Z pairs, and the hydrophobic Ds:Diol-Px pair. Using this method, we demonstrate: (i) rapid screening of reaction conditions that yields >98.6% per-cycle replication fidelity for the B≡S base pair, (ii) single-molecule tracking of replication outcomes in an 'Hachimoji' 8-letter system (ATGCBSPZ), and (iii) first estimates of context-dependent inversion error rates in the hydrophobic Ds≡Px system, revealing inversion frequencies of ≈8.5% in the 5`-pur-Ds-pur-3` context. Together, this approach provides a general route to quantitatively assess polymerase fidelity in expanded genetic alphabets, enabling systematic optimization across chemistries, enzymes, and reaction conditions.

Co-authors: Jayson Sumabat, Jane McKelvey, Jorge Marchand

"Protecting cells at the genetic level and simulating unauthorized access via a biohackathon"
Ishita Kumar (Georgia Institute of Technology)

The protection of high-value cell lines (assets) relies on physical security by limiting access to samples, which remains vulnerable to breach and misuse. We present a cybersecurity-inspired platform that protects biological assets at the genetic level. This technology uses a permutation lock design where an asset can only be decrypted using an authentication code r from a search space composed of n objects on a defined keypad. Here, the genetic asset is designed as a scrambled DNA sequence, and the code is a temporal pattern of small molecules that regulate sets of recombinases that can unscramble a DNA sequence into the desired final sequence. To enhance security, we engineered genetic penalties in the form of multi-toxin circuits that mitigate incorrect code entry and reduce survival to as low as 10^{-8}. In this work, a "blue team" designed and built an encrypted (scrambled) DNA sequence, and a "red team" sought to break the code through an ethical hacking exercise. Two iterations of testing revealed a 0.2% (2 in 990) chance of gaining access to the asset by random search, which is on par with the theoretical goal of 0.1% (1 in 990). These results establish a foundation for safeguarding engineered biological systems against misuse, reverse engineering, and unauthorized replication.

Co-authors: Dowan Kim, Mohamed I. Hassan, Luisa F. Barraza-Vergara, Christopher A. Voigt, and Corey J. Wilson

"Optimizing amplification circuits in the presence of leak"
Nguyen Tran (Drexel University)

To program biochemical circuits, we need molecular amplifiers to propagate signals. However, processes at the molecular scale are difficult to control, and unintended reactions, or "leak", can occur. Understanding how amplification behaves under these conditions is important for designing effective circuits.

In RNA strand displacement systems, one implementation of amplification is the seesaw circuit. To design these amplifiers, we need to understand how the seesaw mechanism behaves in the presence of leak and how amplification can be optimized.

The seesaw circuit forms a biochemical positive feedback loop. In the forward path, an input RNA strand reacts with a DNA gate to produce an output RNA gate, leaving behind a residual gate. In the backward path, a fuel RNA strand recycles this residual gate to regenerate the input, enabling repeated amplification cycles. However, unintended reactions between the gate and fuel can produce output in the absence of input, creating a leak pathway. The rates of fuel-mediated strand displacement and leak together affect the efficiency of this feedback loop and thus the rate of output production. The key challenge is to balance the cost of driving this loop with its amplification performance. To address this, we combine mechanistic modeling with experimental data to simulate, fit, and optimize amplification across varying conditions.

By fitting the model to experimental data using nonlinear least-squares, we show that fuel-induced leak is present, with a normalized root-mean-square error of ~0.1 nM. We then use the model to predict how tuning system parameters affects amplification. In particular, increasing the fuel strand displacement rate within its physical range ($\sim 10^5$ nM^{-1} s^{-1}) maximizes amplification, at the trade-off of an earlier peak response.

This provides a starting point for a plug-and-play workflow to optimize seesaw circuits and other leak-prone biochemical systems.

Co-authors: Nguyen Tran, Ania-Ariadna Baetica, Samuel Schaffter

RESEARCH TALK ABSTRACTS

Wednesday, June 3rd

SESSION 3: RESEARCH TALKS - "Advances in Understanding Cells That Make Food and What Those Cells Can Do"
Moderator: Ryan Tappel (LanzaTech)

"Pyruvate formate-lyase enables ethanol reduction in pyruvate decarboxylase-deficient Saccharomyces cerevisiae"

Sara Stadulis (Cornell University)

Demand for low-alcohol beer and wine has driven interest in engineering Saccharomyces cerevisiae to produce less ethanol during fermentation. Pyruvate decarboxylase (PDC) is an attractive target because it catalyzes the committed step toward ethanol, and S. cerevisiae encodes only three isozymes — far fewer than the many alcohol dehydrogenases that contribute to ethanol production as well as to desirable flavor chemistry. However, PDC also catalyzes the first step in the cell's sole route to cytosolic acetyl-CoA, making it essential for growth. We reasoned that pyruvate formate-lyase (PFL) could serve a dual function in this context: diverting pyruvate away from ethanol while simultaneously generating cytosolic acetyl-CoA through a route independent of PDC. We expressed PFL and its radical SAM activating enzyme from Lactiplantibacillus plantarum in a haploid S288C-derived strain alongside systematic individual and combinatorial deletions of PDC1, PDC5, and PDC6. Across 18 engineered strain backgrounds, PFL expression reduced ethanol production in every background tested, with L. plantarum PFL consistently outperforming an Escherichia coli ortholog. Combining PFL expression with PDC deletions further reduced ethanol yield, though effects were not additive, suggesting compensatory flux through remaining isozymes. Fermentation kinetics were broadly maintained across viable backgrounds. Ongoing work focuses on CRISPR-Cas9 editing of the industrial diploid wine strain EC1118 and tetraploid ale strain Wyeast 1056, with fermentations in grape juice and wort to evaluate ethanol reduction, flavor chemistry, and sensory quality.

Co-authors: Sara Stadulis, Patrick Gibney

"Designing synthetic communities to promote agricultural resilience during drought"
Ella Gunady (Princeton University)

The plant microbiome consists of the microorganisms that interact closely with plants, influencing plant growth and tolerance to environmental stresses such as drought. Understanding these plant-microbe interactions, and how they respond to drought stress, will inform engineering applications in agriculture to increase resilience as climate change causes more severe and frequent periods of drought. Using sequencing data from a field study of corn and soybean grown under drought conditions at Stony Ford Research Station (Princeton, NJ), we have identified bacterial genera that are enriched in the roots of these crops during drought and drought recovery. Leveraging this field data and a new collection of root-associated bacteria from this field site, we screened 49 bacterial isolates in a high-throughput agar assay, using polyethylene glycol to simulate osmotic stress in the model plant Arabidopsis thaliana. We identified 17 strains that promote tolerance to osmotic stress and represent promising candidates for further characterization in Synthetic Communities (SynComs). Including a subset of these beneficial strains, we have constructed an 87-member SynCom that promotes recovery from drought in Arabidopsis. These data are informing ongoing efforts to genetically engineer strains of interest to elucidate mechanisms involved in colonization dynamics and beneficial effects during drought. Understanding the genetic mechanisms of these plant-microbe interactions will enable engineering efforts to develop microbiome-based biotechnologies to better adapt agricultural systems to a changing climate.

Co-authors: Ella F. Gunady, Ting Jiang, Sarina Wen, Kai Harting, Diana Savchyn, Jonathan M. Conway

"Exploring the research and bioprocessing benefits of food biotech informatics and how to increase data access"
Curt Chaffin (Good Food Institute)

Access to high-quality data is key for excellence in AI and biotechnology, especially food biomanufacturing. The world is shifting to intensive, computational research using AI, requiring large amounts of high-quality data. Individual companies and researchers cannot supply such vast data, leading to fragmented data, inefficient redundancy, and intellectual property lockup. This shortage is especially true for innovators attempting to make nutritious, high-protein foods.

This talk will outline the state of data accessibility for engineering biology, notably fermentation-enabled food proteins and their manufacture. It will identify potential applications of Artificial Intelligence for manufacturing, processing, sensory properties, and product formulation, as well as the needed development of data taxonomies and ontologies. This talk will outline public policy mechanisms to increase data accessibility, exploring models and existing policy proposals. Finally, this talk will identify how such efforts can accelerate all engineering biology sectors.

The National Security Commission on Emerging Biotechnology recently concluded that "the federal government can make the down payment for the infrastructure of the scientific ecosystem" (NSCEB 2025). As the Society for Industrial Microbiology and Biotechnology identified in its Precompetitive Knowledge Base Report, there has long been a need for data sharing for biomanufacturing and biotechnology—ranging from "supportive information to product development and commercialization" (SIMB 2024). In addition to existing Congressional proposals (See H.R. 1387, S. 4069), several executive agencies can address data access, including the NIH, DOE, USDA, and NIST. The Good Food Institute has proposed the establishment of a National Food Biotechnology Data Repository. The purpose of the repository will be to support the collection, storage, retrieval, and dissemination of the results of research in the fields of food and agriculture biotechnology.

Co-authors: Adam Leman

"Beyond Proof-of-Concept: A Systems-Integrated Approach to Scalable Biomanufacturing using Photosynthetic Microbes"

Aditya Sarnaik (Arizona State University)

Several publications emerge every year promising phototrophic microbes as the sustainable platforms for production of chemicals and high-value products. Yet, beyond established dietary supplement, pigment and biofertilizer markets, their commercial success remains severely limited. Although algae and cyanobacteria offer compelling possibilities at the forefront of sustainability, their untapped potential is strongly shaped by choice of a suitable host for the target product, robustness during scale-up, biocontainment strategies, efficient downstream integration, and overall process economics. With evolving federal policies re-igniting the commercial applications of photosynthetic microbes and our aim to transform these cell factories into industrial workhorses, efforts have been made to systematically identify and address the critical bottlenecks that have constrained their commercial translation over the past few decades. As sustainability must extend beyond just a platform selection and integrate into every step, we developed a continuous bioprocess framework to produce next-generation sunscreens from cyanobacteria, presenting a practical case study of how biological innovation must be tightly coupled with engineering design to achieve translational viability.

We implemented an incremental scale-up of a cyanobacterial consortium from 1-1000 L, using stepwise exposure strategy. The culture was scaled up from 1-10 L indoor under controlled conditions, followed by 440 L in outdoor green-house, to 800 L in a tubular photobioreactor, and then 1000 L open-raceway pond for direct exposure to the dynamic environment. This stepwise transition enabled effective culture adaptation, improving the production of UVA- as well as UVB-absorbing mycosporine-like amino acids (MAAs, UV-filters) by almost 10-fold due to direct sunlight exposure, along with anti-aging exopolysaccharide. At this scale, a single disc-stacked centrifuge was integrated with cultivation for biomass harvesting, homogenization and fractionation, enabling a streamlined 3-in-1 downstream process, advancing towards commercial scale continuous operation at 14,000 L. In parallel, the study involved identification, purification, stability testing of known as well as novel MAAs, and their formulation. Together, we demonstrate a realistic pathway toward commercially viable photosynthetic biomanufacturing.

Co-authors: Aditya P. Sarnaik, Rocco Mancinelli, John McGowen, David Smernoff, Taylor L. Weiss

SESSION 4: RESEARCH TALKS - “Engineered Biology in Medicine: Innovation Across the Pharmaceutical Pipeline”
Moderator: Kaitlin Dailey (University of Rhode Island)

“Using Enantioselective Biosensors to Evolve Asymmetric Biocatalysts ”
Simon d'Oelsnitz (Harvard Medical School)

Biocatalysts are championed for their exquisite enantioselectivity, but slow chromatographic separations necessary to measure enantiomeric excess can bottleneck their development. To overcome this limitation, we generate enantioselective transcription factors (eTFs) that convert enantiomer-specific analyte concentrations into programmable gene expression outputs. We focused on generating eTFs for screening imine reductases that convert an achiral imine into enantiomeric amines used to manufacture the pharmaceutical Solifenacin. Using a massively parallel reporter assay, we measure dose-response curves for over 300,000 TF variants in response to the imine and chiral amines. Leveraging this dataset, we quantitatively compare the sensitivity, selectivity, and dynamic range of variants generated by random, site-saturation, and shuffling mutagenesis, enabling the isolation of eTFs with exceptional specificity. High-resolution structures of evolved eTFs elucidate how sterics enforce enantioselectivity and charge interactions differentiate the imine from chiral amines. Using two eTFs, we create an ultrahigh-throughput chiral screen, which is used to evolve an imine reductase with inverted enantioselectivity. Finally, to support the generalizability and speed of our approach for practical use, we design a genetic circuit enabling week-scale biosensor evolution and use it to generate eTFs for a chiral precursor to the paralytic drug Cisatracurium. We demonstrate that eTFs enable rapid screening of asymmetric reactions to support pharmaceutical manufacturing innovation.

"Towards Electrogenetic Control of Drug Activation by a β-glucuronidase "
Matthew Carpenter (Rice University)

In the human gut, the pharmacokinetics of therapeutic drugs are sometimes influenced by microbial metabolism [1]. Controlling this microbial activity may enable more precise and dynamic drug dosing. Therapeutic molecules that are inactivated by glucuronidation can be reactivated by the β-glucuronidases of some gut-dwelling microbes [1]. To achieve dynamic modulation of β-glucuronidase activity, we sought to electronically control β-glucuronidase expression in Escherichia coli cells. The native E. coli OxyR transcriptional regulator was previously shown to induce expression of a gene of interest in response to transient electrochemical production of H2O2 [2]. We electrochemically produced H2O2 within a bioelectrochemical system (BES), activating gene expression from an OxyR-regulated promoter. By regulating β-glucuronidase expression with this promoter, we achieved H2O2 induction of β-glucuronidase activity. The performance of this circuit was optimized by tuning the expression level of two genes involved in glucuronide import, gusB and gusC, to maximize β-glucuronidase activity while minimizing cellular burden. Using these engineered microbes within the BES, we evaluated the application of varied electrode potentials to control the release of active xenobiotic molecules from their glucuronidated precursors. This work takes key steps towards bioelectronic manipulation of a microbial enzymatic flux that shapes the availability and activity of pharmaceuticals.

References:

[1] Pellock, S. J. & Redinbo, M. R. (2017). Glucuronides in the Gut: Sugar-Driven Symbioses between Microbe and Host. J. Biol. Chem. 292 (21): 8569-8576. doi:10.1074/jbc.R116.767434.

[2] Terrell, J. L., et al. (2021). Bioelectronic control of a microbial community using surface-assembled electrogenetic cells to route signals. Nat. Nanotechnol. 16: 688-697. doi: 10.1038/s41565-021-00878-4.

Co-authors: Matthew Carpenter, Zoe Folarin, Ellen Shales, Caroline Ajo-Franklin

"Programmable Microbial Chassis for Living Therapeutics and Natural Product Production "
Yousong Ding (University of Florida)

Synthetic biology is transforming medicine by enabling programmable living therapeutics and discovery and production of bioactive small molecules. Natural products remain foundational to modern pharmacology, accounting for ~50% of FDA-approved drugs. Yet most biosynthetic gene clusters (BGCs) uncovered through genome sequencing (>13 million to date) remain uncharacterized or inaccessible due to the cultivation and genetic intractability of native producers. Overcoming this production bottleneck is a central challenge in engineering biology.

Our laboratory develops synthetic biology platforms for activation, refactoring, and heterologous expression of native and engineered BGCs in tractable microbial hosts. I will highlight two recent advances. First, we engineered a gut-native Escherichia coli strain into a programmable living chassis capable of hypoxia sensing and in situ biosynthesis of therapeutic small molecules. By combining transposon-mediated genome insertion with recombineering-based pathway integration, we established a strategy to domesticate undomesticated strains for robust metabolic engineering. This chassis supports production of plant flavonoids and bacterial mycosporine-like amino acids, demonstrating potential for localized small-molecule delivery. Second, we developed a modular, host-agnostic vector system enabling seamless BGC transfer and expression across phylogenetically diverse bacteria, including cyanobacteria, E. coli, and Bacillus. This platform facilitates rapid pathway refactoring and heterologous production of structurally diverse natural products, expanding access to chemically and ecologically distinct metabolite space. Together, these advances illustrate how synthetic biology can convert genomic potential into functional chemical output, advancing programmable microbial therapeutics and scalable natural product discovery.

"Domesticating Brucella melitensis DvjbR for living bacterial Immunotherapy"

Chelsea Hu (Texas A&M Univeristy)

Next-generation bacterial cancer therapeutics will increasingly rely on non-model organisms with intrinsic immunological and tissue-targeting advantages. Brucella melitensis ΔvjbR, an intracellular bacterium, offers a compelling platform owing to its tumor-colonizing capabilities, low immunogenicity, limited pre-existing immunity in the US population, and an established safety profile in non-human primates. However, like many members of the Brucella genus, it has historically been difficult to engineer due to low transformation efficiency and a lack of characterized genetic tools. Realizing its full therapeutic potential therefore demands a dedicated synthetic biology toolkit enabling programmable control of gene expression, growth, and immune engagement in vivo.

To address this need, we developed a comprehensive synthetic biology toolbox for B. melitensis ΔvjbR. We first improved its transformation efficiency from 102 to 106 CFU/µg DNA. Building on this capability, we characterized plasmids with four different replication origins, constructed a promoter library spanning two orders of magnitude in transcription strength, an RBS library, and a transcriptional terminator library, alongside six characterized fluorescent proteins covering the blue-to-red spectrum for gene expression monitoring. To enable genome-level modifications, we implemented a robust editing pipeline based on CRISPR-associated transposase (CAST) systems and generated targeted auxotrophic mutants to enhance safety measures. Building on this toolkit, we developed a Cas9-based kill switch that is selectively activated by sub-therapeutic antibiotic doses, demonstrating complete (100%) elimination efficiency in vitro and ~85% elimination of bacterial cells in mouse liver upon in vivo induction

Together, these tools enable precise and programmable control over gene expression, growth, and therapeutic output in a clinically relevant, immune-evasive chassis. Our work establishes Brucella melitensis ΔvjbR as a next-generation platform for live bacterial cancer therapies and highlights the broader potential of engineering non-model organisms for synthetic biology applications in medicine.

POSTER SESSION LIST

Session 1 (5:30 – 6:30 pm)

1. **Ryan Langevin, Georgia Institute of Technology**
   "Tuning the Response of GPCR-Based Yeast Sensors Using Fluorescent Reporters"
3. **Meagan Olsen, Northwestern University**
   "Improving cell-free gene expression in the era of AI"
5. **Joseph Vath, Georgia Institute of Technology**
   "A Modular Protocell Platform for Modeling Bacterial Communication Networks"
7. **Mair Edwards, University of Florida**
   "Bioengineering of Fiber-Degrading Enzymes to Unlock New Carbon Feedstocks"
9. **Anela Kerber, University of Rhode Island**
   "Reproducible Fluorescent Labeling of Clostridium novyi-NonToxic Spores with Preserved Spore Viability"
11. **Elise Zimmerman, Rice University**
    "RNA-Activated, Species-Specific Killing of Lactobacillus iners to Improve Cervical Cancer Treatment Outcomes"
13. ***Poster Withdrawn***
15. **Wen-Chia Chen, Rice University**
    "From DNA to Electrical Current: Engineering a Living Bioelectronic Sensor for Environmental Surveillance"
17. **Siyuan Feng, Northwestern University**
    "Transcription-induced coacervation accelerates and sensitizes cell-free biosensing"
19. **Chao Liao, Princeton University**
    "Genetic determinants of immunity suppression by the plant-associated commensal Brevundimonas sp. MF374"
21. **Elise Kammerdiener, Oak Ridge National Laboratory**
    "Multilayered gene regulation by RNA thermometers confers improved expression control in bacteria"
23. **Niesha Cantú, North Carolina State University**
    "Biosensor Guided Evolution of Polyketide Synthase Domains"
25. ***Poster Withdrawn***
27. **Efrain Rodriguez Ocasio, University of Wisconsin-Madison**
    "Functional replacement of surfactants with biosurfactants"
29. **Casey O'Brien, Georgia Tech**
    "Upgrading lignin derived aromatics to styrenic monomers for polymerization"

31. Ruchi Singh, University Of Houston
"Magnetic Field Gradient–Induced Mechanical Modulation of Mitotic Spindle Organization and Cytokinesis in Xenopus laevis embryos"

33. Cody Kamoku, Pacific Northwest National Lab
"Development of a whole cell biosensor for high throughput screening of two stage bioprocesses"

35. *Poster Withdrawn*

37. Soor Vora, Georgia Institute of Technology
"Metabolic Characterization Reveals The Importance Of Addition Timing In E. coli-based Cell-Free Expression Systems (CFES)"

39. Anika Gupta, Rice University
"Programmable protein surface display in broad-host-range microbes for microbiome engineering"

41. Apurv Mhatre, Oak Ridge National Laboratory
"Unraveling Soil Microbe Establishment: Leveraging Genetic Mapping to Identify Genetic Factors shaping the Soil Microbiome"

43. Felicia Oentoro, Georgia Tech
"Protein Degradation as a Tool in Cell-free Systems"

45. Michaela Jones, MIT
"A Generalizable Surface Functionalization Platform for Non-Genetic Microbial Engineering"

47. Caitlyn Davis, Georgia Institute of Technology
"Engineering Intelligent Bacteroidaceae (Gut) Consortium"

49. Ryan Boileau, Duke University
"An autonomous system for multi-objective continuous evolution at scale"

51. Pranay Talla, Harvard Medical School
"Engineering Biosensors Responsive to Alkyl and Perfluoroalkyl Acids"

53. John Cheadle, North Carolina State University
"Uncovering Mechanisms of Microbial Persistence in the Maize Root Microbiome using Functional Metagenomic Screening"

55. Shifeng Xu, Georgia Institute of Technology
"Protein Degradation for Improving Limit of Detection of a Cell-Free Biosensor"

57. Rutuja Bhamare, North Carolina Agricultural and Technical State University
"Computational Design and Analysis of Bioreactor Configurations for Efficient Glucose and Xylose Co-Consumption"

59. Kristen Landreville, NC State University
"Public Perceptions and Acceptance of Microbiome Engineering in Built Environments"

61. **Catherine McCarthy, National Informal STEM Education Network (NISE Network)**
"Public engagement with synthetic biology nationwide at museums and science centers"

63. **Shalley Sharma, Emory University**
"A synthetic yeast two-component platform to decode non-linear regulation by XIST A-repeats"

65. **Gabe DeSantis, Georgia Institute of Technology**
"Differential Effects of Buffer Formulation on Cell-Free Lysate Performance"

67. **Aryan Razdan, North Carolina State University**
"Improving the Colonization Properties of the Probiotic Yeast Saccharomyces boulardii via Mucus Glycan Metabolism"

69. **Ya Wen, Princeton University**
"Dual MarR-family Regulation of Indole-3-acetic acid (IAA) Degradation in Variovorax CL014"

71. **Zoe McCready Martin, East Tennessee State University**
"Solving Global Issues Using Plant Synthetic Biology: Making Monoclonal Antibodies Accessible and ETSU iGEM's Crop Nutrition Improvement"

73. **David Ross, NIST**
"A Strategy for Data Collection to Solve the Protein Function Problem"

75. **Ziqi Zheng, Georgia Institute of Technology**
"Toward Predictive Design of Cell-Free Genetic Systems Using Hybrid Modeling"

77. **Emily Heckard, Georgia Tech**
"Portable Glucose Monitor-Based, Field Deployable Sensing"

79. **Isaiah Woodson, Arizona State University**
"Modulating Redox to Enhance CO2 Fixation during E. coli Fermentation for Succinate Production"

81. **Suvin Baek, Georgia Institute of Technology**
"Engineering Low-Leak TLISA Systems Using Peptide Masking"

83. **Rridhisha Kumar, Georgia Institute of Technology**
"Mechanistic ODE Modeling of TLISA to Guide Cell-Free Diagnostic Design"

POSTER SESSION LIST

Session 2 (6:30 – 7:30 pm)

2. **Dipesh Dhakal, University of Florida**
   "Multichassis expression of biosynthetic gene cluster from marine bacteria"
4. **Talia Jacobson, University of Florida**
   "Crystalline Cell Wall Engineering Insights from an Ancient Mannan Synthase"
6. ***Poster Withdrawn***
8. **Anru Tian, Stanford University**
   "Enabling cell-free synthesis of longer human milk oligosaccharides"
10. **Samuel Eastman, Princeton University**
    "Engineering the rhizosphere: optimization of heterologous opine catabolism in plant-associated bacteria"
12. **Amisha Patel, Georgia Institute of Technology**
    "Improving the sensitivy of G-Protein Coupled Receptor Biosensors in S. cerevisiae."
14. **Gavin Williams, NC State University**
    "Biosensor-guided directed evolution of terpene biosynthesis"
16. **Hannah Lwin, University of Delaware**
    "Identifying engineerable species within the microbiomes of plastic-degrading mealworms via RNA barcoding"
18. **Hamed Rastaghi, North Carolina A&T State University**
    "Modeling the impact of intercellular communication on bacterial feedback systems in coupled bioreactors"
20. **Nona Hashemi, North Carolina A&T State University**
    "Linking Gene Networks to Biofilm Structure and Production Across Species"
22. **Anuran Gayen, University of Florida**
    "Plantizing microbial enzymes – can we evolve suicide enzymes to work catalytically?"
24. **Xu Zhang, Texas A&M University**
    "Engineered E. coli as a bioelectronic sensor for environmental detection"
26. **Rixin Zhang, Arizona State University**
    "Integrating recombinase-based feedback and feedforward control for optimal resource decoupling"
28. **Fayrouz Nossir, East Tennessee State University**
    "Directed Evolution to Enhance Phytoene Synthase Enzyme Activity for Metabolic Engineering"

30. Cassidy Oliverio, North Carolina State University
"Development and Application of Genetically-Encoded Biosensors for Terpene Synthetic Biology"

32. Nathan Gladden, Oak Ridge National Laboratory
"Multiplexed genome engineering: Optimizing metabolism of terephthalate"

34. Hannah Noh, Georgia Institute of Technology
"Creating Synthetic Biology Pipelines by Training High School Educators and Students"

36. Jiho Seok, Georgia Institute of Technology
"Programmable AND-gate strategy to reduce false positives in rolling circle amplification"

38. Jon Arizti Sanz, Stanford University
"Building a synthetic biology toolkit for mushroom-forming fungi"

40. Alissa Meo, North Carolina State University
"Development of transcription factor biosensors for high-throughput engineering of agricultural polyketides"

42. Lex Patterson, Vanderbilt University Medical Center
"From Bug to Feature: Harnessing Resource Competition in Transcription Factor Cell-Free Biosensors"

44. Megan McSweeney, Stanford University
"Probing ribosomal RNA homogeneity via multiplex automated genome engineering"

46. Leah Davis, National Oceanic Atmospheric Administration
"Taking Synthetic Biology to the Sea: Engineering Multiplex Cell-Based Biosensors for Marine Biotoxin Detection"

48. Nicole Zhao, Harvard Medical School
"From Promiscuity to Specificity: Multidrug Regulators Enable Predictive and Scalable Biosensing"

50. Quang Luan Dang Tran, Drexel University
"Performance of Metaheuristic Algorithms in Finding Tradeoffs in One- and Two-Species Biological Feedback"

52. Jon Judd, Engineering Biology Research Consortium
"Securing the U.S. Bioeconomy: A Managed Access Framework For Biotechnology Innovation"

54. Natalia Barna, Northwestern University
"Optimization of Mfp5 Production Through T3SS-Mediated Protein Secretion"

56. Bukola Akindipe, North Carolina A&T State University
"Linking Microfluidic Geometry to Microbial Behavior through Hydrodynamic Simulation and Digital Twin Modeling"

58. Akinwunmi Afuape, North Carolina A&T State University
"Whole genome synthesis of Bacteriophage SynPE3 to combat multidrug-resistant Pseudomonas aeruginosa."

60. Karly Liebendorfer, Georgia Institute of Technology
"Redesigning Split β-Galactosidase for Fast-Acting, Sensitive Biosensors"

62. Nandini Kannoju, Arizona State University
"Improving energy flux in a photoautotrophic system through microbial electro-photosynthesis Cyanobacterium"

64. Emily Singer, Princeton University
"Tracking metabolite-mediated plant-microbe interactions in the rhizosphere"

66. Hayeon Park, University of Delaware
"Fluorescence polarization reveals dynamic formation of biomolecular condensates in vitro and in vivo"

68. Cameron Roots, ORISE & USDA
"Mining Bacillus Genomes for Novel and Chimeric Pesticidal Proteins"

70. Kosuke Hamano, miibio, inc.
"Light-controlled Biomanufacturing System"

72. Varaidzo W. Chakafana, Princeton University
"Identifying Bacterial Genes that Support Microbiome-Mediated Drought Resilience in Plants"

74. Pallavi Ramaswamy, Oak Ridge National Laboratory
"Developing a Genome-Scale Pooled CRISPRi Platform to Identify Improved Formate-Utilizing Variants of Cupriavidus necator H16"

76. August Staubus, National Institute of Standards and Technology
"Using high-throughput engineering methods to develop allosteric splicing ribozymes for broad-host range inducible control, genetic recordings, and biosensing"

78. Kelly Smith, Sable Fermentation, Inc.
"Suppression of Spore-forming Contaminants Using a Novel, Bio-based and Selective Sterilant"

80. Thomas Adamo-Schmidt, California Institute of Technology
"The rise of biotechnology beyond conventional containment (BBCC): A policy toolbox for community engagement in BBCC development and deployment"

82. Arren Liu, CAMRI, UMB
"A Conserved Gut Microbial Gene Cluster Mediates Strain-Specific Allergen Degradation"

84. Cassandra Parada, Georgia Institute of Technology
"Protein nanoparticle-encapsulated cell-free systems for mucosal diagnostics"

POSTER ABSTRACTS

1. *"Tuning the Response of GPCR-Based Yeast Sensors Using Fluorescent Reporters"*
Ryan Langevin (Georgia Institute of Technology)

G protein-coupled receptors (GPCRs) are involved in a variety of signaling functions and are key pharmacological targets. High throughput GPCR sensors in yeast are a proven platform for identification of novel GPCR ligands. However, in these sensors, most human GPCRs lead to small increases in signal after activation. Here, we analyze five fluorescent reporter proteins in the context of yeast GPCR-based sensors, with the goal of identifying a reporter that improves the signal of these sensors. YPet, a yellow fluorescent protein, outperforms the other reporters in a serotonin receptor 4-based testbed. YPet also increases the dynamic range of GPCR-based sensors in general and reduces the time to readout from 4 h to 30 min. Identification of YPet as the optimal fluorescent reporter for yeast GPCR-based sensors sets the stage for ultrahigh-throughput single-cell experiments toward the identification of new ligands for known GPCRs, GPCR deorphanization, and GPCR engineering.

Co-authors: Ryan Langevin, McKenna Martin-Downey, Amisha Patel, Haden Archer, Sara J. Davila Severiano, Pamela Peralta-Yahya

2. *"Multichassis expression of biosynthetic gene cluster from marine bacteria"*
Dipesh Dhakal (University of Florida)

Heterologous expression of biosynthetic gene clusters (BGCs) is a powerful strategy in natural product (NP) discovery, yet achieving consistent expression across microbial hosts remains challenging. Here, we developed a vector systems compatible for expression of BGCs from selected marine bacteria in Gram-negative Escherichia coli, Gram-positive Bacillus subtilis, and two model cyanobacterial strains including unicellular Synechocystis PCC 6803 and filamentous Anabaena sp. PCC 7120. Following validation using constitutive and inducible expression of the enhanced yellow fluorescent protein (eYFP), these vectors were applied to express the shinorine and violacein BGCs in all four hosts. Promoter tuning, BGC refactoring, substrate feeding, and inducible control enhanced NP production and mitigated host toxicity. Our results provide versatile expression platforms for probing BGC function and accelerating natural product discovery.

Co-authors: Dipesh Dhakal, Campbell W. Eckhardt, Yujia Jiang, Hendrik Luesch, Yousong Ding*

3. *"Improving cell-free gene expression in the era of AI"*
Meagan Olsen (Northwestern University)

Recombinant proteins underlie a wide variety of biotechnologies, from industrial chemical production to therapeutics and education. Unfortunately, recombinant protein production is more art than science. Not all proteins can be expressed in all organisms, and the production of soluble, properly folded proteins is nontrivial; some estimates suggest upwards of 50% of attempts to express recombinant products fail. Moreover, despite numerous models for predicting soluble recombinant protein expression in cells, most researchers still rely on laborious experimental trial-and-error to identify optimal expression conditions. We seek to transform recombinant protein production technologies by developing the equivalent of a protein printer, supported by high-quality and interoperable datasets that train predictive models of protein expression. The key idea is to create a robust cell-free gene expression platform that can "print" complex proteins with diverse functionality from any sequence provided by a user, similar to how users can readily upload and print polymer-based structures on a 3D printer. To achieve our goal, we first compile over 5,000 historical cell-free protein solubility and yield measurements and use this data to train machine learning classifiers to predict whether a protein sequence will be soluble when expressed in cell-free systems. We then perform an active learning campaign to systematically examine the effects of codon usage, temperature, and presence of helper proteins (e.g., chaperones) on the soluble protein yield of 25 different proteins. This work expands our ability to express challenging proteins and identify improved expression conditions without slow, one-factor-at-a-time optimization efforts for each new protein of interest.

Co-authors: Meagan L. Olsen, Sai Preethi Nakkina, Zachary M. Shaver, Caleb G. Lay, Brett J. Palmero, Amelia F. Perry, Ashty S. Karim, & Michael C. Jewett

4. *"Crystalline Cell Wall Engineering Insights from an Ancient Mannan Synthase"*
Talia Jacobson (University of Florida)

The β-1,4-linked mannans are the most ancient hemicellulosic polymers in plant cell walls and serve as versatile hydrocolloids that define plant biology and support diverse industrial applications, including food and medicine. Despite their importance, the biological function and evolutionary origin of these polysaccharides remain unknown, limiting our ability to tailor their structure in crops and microbial cell factories. Porphyra umbilicalis is an ancient red alga where crystalline mannan replaces cellulose as the load-bearing polysaccharide. Utilizing synthetic yeast and plant chassis, we demonstrate that P. umbilicalis CELLULOSE SYNTHASE-LIKE K1 (PumCSLSK1) encodes an ancient mannan synthase that predates terrestrial plant CSLA. PumCSLK1 localized to intercellular puncta in yeast and produced pure mannan that altered cell wall properties. In planta, overexpression of PumCSLK1 in the seed coat perturbed cell morphology, despite other CSLA/Ks homologs enhancing mannan accumulation and maintaining seed coat structure. Collectively, our findings identify PumCSLK1 as a Golgi-localized ancestral mannan synthase, illuminating the early evolution of mannan biosynthesis. This work highlights the diverse and complex nature of mannans and establishes a fundamental framework for engineering their production and structural design in crops and synthetic biological systems.

Co-authors: Annika Grieß-Osowski, Moni Qiande, Stefanie Clauss, Hannah Zheng, Catalin Voiniciuc

5. *"A Modular Protocell Platform for Modeling Bacterial Communication Networks"*
Joseph Vath (Georgia Institute of Technology)

Efforts across the engineering biology community aim to develop methods for studying biological processes and leveraging them to create bio-inspired technologies. In this work, I describe a collaborative effort to develop a platform for modeling bacterial communication and signaling networks, with the long-term goal of incorporating these systems into multiplex point-of-care (POC) diagnostic tools. The approach uses an aqueous two-phase separation system to generate simplified, membrane-free "protocell" communities capable of modeling bacterial signaling. Within this platform, protocells communicate through intracellular signaling molecules, including cyclic AMP and bis-(3'–5')- cyclic diguanosine, as well as extracellular, density-dependent signals such as homoserine lactone-based quorum-sensing systems and their cognate receptors. To date I have optimized plasmid concentrations encoding signal receptors and GFP reporter constructs regulated by signal-induced transcription to maximize reporter output while minimizing resource competition and plasmid crosstalk. I am now assembling the signaling pathways to transduce a signal to a reporter protocell capable of producing a visible indicator of successful inter-protocell communication. By incorporating multiple protocells with distinct signaling pathways, I can assess potential crosstalk between signaling molecules and noncognate receptors, enabling the design of protocell communities with orthogonal communication networks. To further approximate complex biological responses and facilitate POC implementations, I also intend to integrate these systems using self-assembling fragmented RNA polymerase to achieve multi-input, single-output signal processing. This platform provides a modular and controllable environment for studying microbial communication and developing communication-based biological circuits for applications including diagnostics and biological computation.

Co-authors: Felicia Oentoro, Mark P Styczynski, Brian K Hammer

6. *Poster Withdrawn*

7. *"Bioengineering of Fiber-Degrading Enzymes to Unlock New Carbon Feedstocks"*
Mair Edwards (University of Florida)

Hemicelluloses are a vital class of plant cell wall polysaccharides in foods, materials, and other products and represent a third of plant biomass on our planet. These biomaterials function as structural support, signalling molecules, and to store carbon reserves in certain plant organs. Glycosyl hydrolases (GHs) that degrade plant hemicellulose are natively expressed by plants to modulate growth and development, and by microbes to unleash carbon sources from lignocellulosic biomass. GHs with higher stability and/or activity are required for biofuel and biomaterial production, food and feed processing, and in health and pharmaceutical sectors. Within the plant, GHs function in many processes including germination, growth and development and fruit ripening. In some cases, over expression of wall degrading GHs in planta has yielded promising traits such as faster germination and growth. In others, plants are used as biofactories to produce GHs and aid lignocellulose degradation. My PhD research focuses on engineering GHs to valorize agricultural waste fibers for biomanufacturing and other applications. Mannan-degrading GHs from diverse organisms were expressed in a modular yeast platform and benchmarked using biochemical assays and robot-driven automation. In addition, a highly active GH prototyped in yeast was overexpressed using a suite of promoters in Arabidopsis plants, to evaluate its ability to remodel the cell wall, alter biomass composition, and plant function. We are also integrating AI-driven predictions to further enhance enzyme activity and/or stability, with the goal of generating GHs for faster, more efficient hemicellulose conversion for scalable bioprocesses.

Co-authors: Abigail Lin, Moni Qiande, Wenjun Xie, Cătălin Voiniciuc

8. *"Enabling cell-free synthesis of longer human milk oligosaccharides"*
Anru Tian (Stanford University)

Human milk oligosaccharides (HMOs) are a diverse class of indigestible sugars found uniquely in human breast milk. They are foundational to a healthy gut microbiome and serve as critical nutraceuticals against conditions such as necrotizing enterocolitis. However, the production of long-chain HMOs is currently limited: chemical synthesis is hindered by complex stereochemistry, while traditional microbial fermentation remains cost-prohibitive. To overcome these challenges, we integrate advanced protein design with cell-free biocatalytic methodologies to develop novel enzymatic strategies for long-chain HMO production. Specifically, we focus on the synthesis of two sialylated lacto-N-tetraoses (LSTs) from the commercially available precursor, Lacto-N-tetraose (LNT). Central to this platform is the engineering of transsialidases, a class of glycosyltransferases that bypass the need for expensive nucleoside-activated sugars. Here, we describe a high-throughput pipeline for the rapid evolution of these enzymes. We first developed a 384-well filtration plate manifold assay to screen enzyme variants at scale. Then, we characterized hundreds of wild-type and engineered transsialidases to map comprehensive sequence-to-function landscapes. By integrating these high-throughput datasets with design-driven computational tools—including ProteinMPNN, Evo2, and custom-trained classifiers—this work will establish a predictive framework for enzyme optimization. Ultimately, this approach accelerates the development of highly efficient biocatalysts, paving the way for the scalable, low-cost production of life-saving HMOs.

Co-authors: Eleanor Zhu, Ian Anderson, Justin Siegel, Michael Jewett

9. ***"Reproducible Fluorescent Labeling of Clostridium novyi-NonToxic Spores with Preserved Spore Viability"***

Anela Kerber (University of Rhode Island)

Clostridium novyi-NT is a spore-forming bacteria with strong potential for development as a cancer therapeutic modality through the application of engineering biology principles. However, the structure of its spore coat and how the spore interacts with different environmental conditions remains poorly understood. This knowledge gap limits the development of effective fluorescent imaging techniques – particularly for in vivo applications. Reliable staining of Clostridium novyi-NT spores is critical for pre-clinical characterization and further therapeutic advancements. However, most commercially available fluorescent staining protocols are intended for eukaryotic cells and are poorly suited for bacterial sores due to their complex, multilayered structure, which can limit dye penetration. Here, previously published methods were optimized to stain C. novyi-NT spores with carboxyfluorescein succinimidyl ester (CFSE) with adjustments to dye concentration, incubation conditions, and methodological parameters to ensure consistent fluorescence while preserving spore integrity and germination capacity. Preservation of these capacities directly impacts therapeutic efficacy and are therefore anti-cancer activities. Through development of a low cost, accessible, and reproducible methodology, this approach enables consistent detection and visualization of C. novyi-NT spores and provides a framework adaptable to alternative fluorophores and related spore-forming systems. Ultimately, this undergraduate research project developing alternative C. novyi-NT detection modalities will expand downstream experimental applications and facilitate clinical translation, including therapeutic strategies for metastatic pancreatic cancer and other solid tumors.

Co-authors: Jack G. Stevenson, Caleb P. Hoffman, Caleb J. Bussard, Jessica E. Pullan, Kaitlin M. Dailey

10. ***"Engineering the rhizosphere: optimization of heterologous opine catabolism in plant-associated bacteria"***

Samuel Eastman (Princeton University)

The plant rhizosphere is comprised of many bacteria that compete for host-secreted nutrients and influence the plant host phenotype. Some plant growth promoting rhizobacteria (PGPR) are capable of positively impacting plant growth, defense, and resilience, yet these PGPR are often limited by competitive exclusion by other bacteria. Engineering the rhizosphere to exhibit bias towards beneficial PGPR would be a powerful technology that reduces our reliance on expensive and environmentally impactful agrochemicals. One proposed method to bias the rhizosphere is opines, which are small metabolites secreted by plants infected by the bacterium Agrobacterium tumefaciens that exclusively support growth of the pathogen. However, it is unclear how heterologous opine catabolism would function in PGPR. Here, we examine catabolic operons for two opines, octopine and nopaline, in diverse root-associated bacteria, including the PGPR Pseudomonas simiae WCS417 and Paraburkholderia phytofirmans PsJN. We find that unmodified opine catabolism functions only in a subset of strains and identify de novo mutations that enable or expand opine catabolism. We iteratively design an optimized catabolic operon by modular assembly of native and heterologous components that enables opine catabolism in PsJN. Engineered, octopine-competent PsJN can not only utilize octopine as a sole growth substrate but also utilize octopine to complement arginine auxotrophy and regulate expression of an octopine-inducible GFP reporter. Our results show the constraints to heterologous opine catabolism and demonstrate the feasibility of using opines to bias the plant rhizosphere towards engineered PGPR.

Co-authors: Hansen Tjo, Emily Singer, Jonathan M. Conway

11. *"RNA-Activated, Species-Specific Killing of Lactobacillus iners to Improve Cervical Cancer Treatment Outcomes"*

Elise Zimmerman (Rice University)

Cervical cancer has an overall 5-year survival rate of only 67%. Chemoradiation therapy is the current standard of care, but 20-40% of patients still experience incurable relapse. A recent study found that cervical cancer chemoradiation resistance can be conferred through lactate-induced metabolic rewiring from Lactobacillus iners within the tumor microenvironment. Traditional antibiotics are not a viable option due to collateral microbiome disruption and non-susceptibility. Thus, there is an urgent need for antimicrobial agents with novel mechanisms of action targeting pro-oncogenic targets that are easy to produce, pathogen specific, and with a platform that is simple and customizable. To address this, we have developed a new class of RNA therapeutics. Our platform, called RNA-Activated Protein by Trans-splicing Ribozyme (RAPTR), is a modular ribozyme-based system that encodes a death-inducing protein output that is translated only when spliced into a pathogen-specific target mRNA, enabling the use of the host translational machinery to express the protein output. RAPTRs are concise RNA sequences encoded in a single RNA strand that can be easily incorporated into delivery vectors. We first engineered RAPTR in E. coli and demonstrated its potential applications by targeting virulence genes of pathogenic E. coli. We achieved a killing efficiency of 99.9% against clinically derived pathogenic strains with no observable effect on a non-pathogenic E. coli strain. While RAPTRs performed well against Gram-negative E. coli, we observed that their function in Lactobacillus, a lactic acid producing, Gram-positive bacteria, was significantly reduced. To address this, we performed rational engineering in L. plantarum to improve splicing efficiency over 10-fold. Using this optimized system, we demonstrated RAPTRs can be made to target diverse mRNAs in a sequence-specific manner and identify effective toxin proteins. In our current experiments, we are now creating RAPTRs against L. iners-specific genes to demonstrate strain-selective killing and potential clinical utility.

Co-authors: Elise H. Zimmerman, Pablo C. Okhuysen, Jonathan (Joff) Silberg, James Chappell

12. *"Improving the sensitivy of G-Protein Coupled Receptor Biosensors in S. cerevisiae."*
Amisha Patel (Georgia Institute of Technology)

Rapid construction of GPCR-based sensors in yeast are important to capture a broader range of ligand-GPCR interactions and enable the generation of previously unavailable high-throughput screens important for accelerating the pace of drug discovery. Heterologous coupling of GPCRs in yeast relies on their ability to couple to the yeast Gα subunit, GPA1, for signal transduction. While some studies have shown successful coupling of GPCRs to GPA1or chimeric Gα, our ability to construct high sensitivity sensors for numerous receptors remains constrained. A key challenge in the development of early stage compounds is identifying off target effects given that these compounds will have a weaker affinity for secondary targets, making them difficult to detect. In this work we engineered a chimeric HTR1A improving the yeast sensor's signal from a 9 to 44-fold increase in signal after activation in the presence of serotonin. This improved dynamic range enabled the identification of the anticancer drug, Lenvatinib, as an activator of HTR1A, which is linked to hypertension, high blood pressure and fatigue. We conclude that high sensitivity human GPCR based sensors in yeast can successfully identify off-target effects of known drugs. In the future, a panel of high-sensitivity human GPCR sensors could be generated and used to rapidly evaluate early-stage compounds.

13. *Poster Withdrawn*

14. *"Biosensor-guided directed evolution of terpene biosynthesis"*
Gavin Williams (NC State University)

Engineering terpene biosynthesis remains a major challenge in synthetic biology due to poorly optimized metabolic pathways and limited high-throughput methods to directly link pathway performance to product formation. Here, we present a biosensor-guided platform that integrates genetically encoded transcription factor–based sensors with directed evolution to accelerate the design–build–test–learn cycle for terpene pathway engineering. By engineering the CymR repressor for selective detection of oxidized monoterpenes, including perillyl alcohol and α-terpineol, we enable real-time, in vivo reporting of pathway output. This enabled rapid interrogation of cytochrome P450 libraries, overcoming traditional analytical bottlenecks and allowing identification of improved variants for terpene oxidation. We also expand the approach to include C15-C30 terpenes by evolving generalist terpene biosensors from other transcription factors. This work establishes a generalizable and scalable framework for biosensor-enabled enzyme and pathway optimization, advancing biomanufacturing and the programmable production of high-value molecules.

15. *"From DNA to Electrical Current: Engineering a Living Bioelectronic Sensor for Environmental Surveillance"*

Wen-Chia Chen (Rice University)

Safeguarding human health against rising antibiotic and infectious disease requires localized monitoring of associated DNA signatures. Conventional approaches, such as PCR amplification, are lab-based and slow, while optical biosensors often underperform in complex or opaque environments. In contrast, cell-based DNA bioelectronic sensors generate electrical current that can be detected in complex and opaque environments, making them well-suited for environmental sensing. Nonetheless, the existing bioelectronic sensors focused on small-molecule analytes are not yet capable of detecting environmental DNA (eDNA).
Herein, we engineer V. natriegens to develop cell-based DNA biosensors that couple natural transformation with a bioelectronic readout. Specifically, the bioelectronic readout is coupled with DNA uptake by placing the synthetic EET gene under the control of a repressor. In the absence of target DNA, the EET gene is repressed, preventing the current generation. Upon natural transformation with target DNA, the repressor gene is deleted, restoring EET gene expression and enabling EET. We propose engineering V. natriegens to serve as a bioelectronic sensor in which DNA integration removes a regulatory gene, thereby activating downstream EET expression as a reporter. We demonstrate that the system can detect the presence of target DNA, with 10% homologous recombination sufficient to generate a detectable bioelectrical readout. Going beyond antibiotic resistance genes, the resulting platform will provide a real-time, optics-free approach for detecting DNA sequences in environmental samples, opening applications in hazard monitoring, biothreat detection, and ecological studies.

Co-authors: Wen-Chia Chen, Lily Metsker, Matthew Carpenter, Zach LaTurner, Lauren Stadler and Caroline M. Ajo-Franklin

16. *"Identifying engineerable species within the microbiomes of plastic-degrading mealworms via RNA barcoding"*

Hannah Lwin (University of Delaware)

Plastics, long valued for their durability and versatility, persist in the environment with harmful consequences for both human and ecological health. Their stability also locks away carbon, preventing reuse and maintaining dependence on fossil resources. Recent studies have revealed that gut microbes from several insect systems can degrade, and potentially assimilate, plastic-derived carbon. However, these insect-associated microbial communities are non-model systems with limited information about compatible genetic tools for their study and engineering. To address this gap, we applied a DNA conjugation and RNA barcoding strategy that identifies genetically tractable species and parts for species-specific functional expression. Successful delivery and expression of the system appends a barcode sequence encoding a fluorescent reporter to the 3' end of ribosomal 16S rRNA, enabling direct detection of hosts that receive and express the system. We validated this approach using Gram-negative isolates from plastic-degrading mealworm gut microbiomes and have begun to develop a series of broad host range toolkits that include diverse selectable markers, promoters, and plasmid origins to target larger fractions of the community. This barcoding framework rapidly evaluates genetic accessibility across environmental communities, supporting efforts to optimize microbial contributions to plastics deconstruction and enabling the repurposing of these systems for sustainable biomanufacturing.

17. *"Transcription-induced coacervation accelerates and sensitizes cell-free biosensing"*
Siyuan Feng (Northwestern University)

Cell-free biosensors are powerful platforms that use biological machinery to detect a wide range of chemicals. While inexpensive, modular, and distributable, these systems are constrained by slow readouts, particularly at room temperature and in field matrices. Here, we address these limitations by interfacing cell-free biosensors with complex coacervates, self-assembling phase-separated droplets reported to enhance catalytic processes. We achieve coacervation by mixing two polyelectrolytes, spermine and polyacrylic acid (PAA), into room temperature in vitro transcription (IVT) reactions, generating IVT outputs comparable to that of controls incubated at 37 °C. We then demonstrate that spermine-PAA coacervates are compatible with transcriptional biosensing, integrating with four transcription factor families to enhance the room temperature sensing of six unique ligands. We also show that coacervates can improve the sensitivity of analyte detection. While investigating this system, we discover that phase separation using spermine and PAA in IVT reactions is transcriptionally induced, mirroring native cellular biology. Similar to cellular phase separation, spermine-PAA coacervates enhance reaction kinetics through co-localization of input molecules: here, DNA and RNA polymerase. We lastly show that spermine-PAA coacervates are amenable to single-pot lyophilization with biosensor components, and maintain their signal enhancement of IVT upon rehydration. Lyophilized zinc-sensing reactions with coacervates demonstrate an 85-min reduction in time-to-signal and a 2-fold improvement in sensitivity when rehydrated in lab water. This effect is preserved when rehydrating lyophilized reactions in lake water, which demonstrate a 3.5-hour reduction in time-to-signal with coacervates. We envision that this work contributes to a growing understanding of phase separation in biology and advances the use of membrane-less compartments to enhance cell-free systems.

Co-authors: Siyuan Feng, Rebecca Rasmussen, Antonio Garcia IV, Lauren Clark, Samanvaya Srivastava, Julius B. Lucks

18. *“Modeling the impact of intercellular communication on bacterial feedback systems in coupled bioreactors ”*

Hamed Rastaghi (North Carolina A&T State University)

Intercellular communication is a central mechanism through which microbial populations coordinate collective behaviors, yet its functional impact becomes harder to predict when cells occupy the same environment, as in most conventional microbial bioreactor systems. Coupled bioreactors provide a promising strategy to overcome this limitation by spatially separating populations while maintaining controlled molecular exchange, thereby enabling more precise analysis and design of communication-dependent interactions. In these systems, signal exchange depends not only on genetic circuit architecture but also on transport between compartments, making communication itself a dynamic design variable. Here, we present a modeling framework to investigate how communication strength shapes feedback behavior in spatially distributed microbial populations connected through controlled fluid exchange. Using a simplified sender–receiver system, in which a sender population produces a diffusible quorum-like signal that induces feedback regulation in a receiver population, we examine how variations in inter-reactor transfer alter signal propagation, feedback activation, and the emergence of coordinated responses. The model reveals distinct behavioral regimes, including cases where weak coupling is sufficient to trigger feedback, cases requiring stronger communication to sustain activation, and regimes in which feedback fails because transported signals never reach effective levels. Together, these results show that intercellular communication is not merely a tunable regulatory feature, but a governing constraint on whether distributed feedback systems can function at all. This work establishes a quantitative basis for designing coupled bioreactor platforms in which transport and communication are jointly engineered to achieve predictable multicellular behaviors.

Co-authors: Hamed Rastaghi, Rutuja Bahamare, and Samuel M.D. Oliveira

19. *"Genetic determinants of immunity suppression by the plant-associated commensal Brevundimonas sp. MF374 "*

Chao Liao (Princeton University)

Plants constantly interact with soil microbiomes and employ innate immune systems to detect and respond to pathogens. However, immune activation induces plant growth inhibition. Approximately 40% of commensal rhizosphere bacteria can suppress plant pattern-triggered immunity to alleviate plant growth inhibition. However, the underlying mechanisms remain largely unknown. Brevundimonas sp. MF374, one such strong commensal suppressor, was investigated in this study. A random barcode transposon site sequencing method was used to construct transposon mutant libraries in MF374. Approximately 4,000 mutants were screened using a β-glucuronidase (GUS) reporter assay to identify candidates defective in plant immunity suppression (PIS). Candidate mutants were further validated using normalized GUS and root growth inhibition assays. To confirm the gene function, targeted gene knockouts and complements were generated and evaluated for loss and gain of PIS, respectively, using the same assays. In addition, a GUS assay panel was developed to assess the contribution of bacteria-flg22 interactions to PIS. In this study, we identified tonB, ppiD, and trpEXGDC as causal genes for flg22-triggered PIS by MF374. Our results suggest that MF374 suppresses plant immune activation in part through TonB-dependent import of flg22, with additional contributions from ppiD and trpEXGDC. In addition to flg22, MF374 suppressed AtPEP1-triggered plant immunity, which was modulated by tonB and trpEXGDC. In summary, MF374 suppresses plant immunity by importing elicitors such as flg22, which is largely dependent on the TonB energy transduction system. Because TonB is widely conserved among Gram-negative bacteria, elicitor import may represent a common mechanism employed by commensals for immune suppression in the host. This finding benefits our fundamental understanding of commensals suppressing plant immunity and applications of the commensals to protect plant health.

Co-authors: Dinie Zheng, Anthea Zhang, Jay Georges, Jonathan M. Conway

20. *"Linking Gene Networks to Biofilm Structure and Production Across Species"*
Nona Hashemi (North Carolina A&T State University)

Biofilms are structured microbial communities whose architecture and production behavior emerge from coordinated genetic networks, metabolic pathways, and extracellular matrix dynamics. However, quantitatively linking gene-level interactions to biofilm structure and extracellular polymeric substance (EPS) production remains a major challenge. Here, a multi-scale framework is presented to investigate how variation in species-specific gene networks and metabolic pathways influences biofilm architecture and production outputs. STRING-based protein–protein interaction networks and Gene Ontology enrichment analyses were used to identify key regulatory genes and functional modules governing biofilm development across Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis. These analyses identify shared processes, including adhesion, quorum sensing, EPS synthesis, and stress response, as well as species-specific hubs that may drive differences in biofilm structure and production. This approach allows simultaneous measurement of EPS distribution, cellular organization, and three-dimensional biofilm morphology across development stages. Next, to connect these gene networks to phenotypic outcomes, we use computational modeling to predict biofilm structural organization and EPS production as a function of species composition and genetic modules. These predictions will later be experimentally evaluated using microfluidic biofilm platforms that enable controlled flow conditions and long-term cultivation, combined with time-resolved fluorescence imaging and quantitative image analysis, which are ongoing. We believe that integrating STRING analysis, modeling, and experimental validation can demonstrate that targeted variation in gene networks results in measurable changes in EPS concentration, spatial organization, and biofilm structural heterogeneity. This work will help establish a quantitative framework linking gene-level interactions to emergent biofilm properties, providing a foundation for predictive design and engineering of biofilm-based systems for biotechnological applications.

Co-authors: Mahdi Hasanzadeh, and Samuel M.D. Oliveira

21. *"Multilayered gene regulation by RNA thermometers confers improved expression control in bacteria"*

Elise Kammerdiener (Oak Ridge National Laboratory)

On demand gene expression via inducible promoters is a critical tool for microbial synthetic biology. Yet, many inducible promoters allow some level of background expression without induction. In many experimental contexts, this "leaky" expression is negligible; however, leaky expression of toxic proteins, like Cas9, can have profound effects on the population. Massively parallel Cas9 libraries (e.g. CRISPR-enabled trackable genome engineering (CREATE), CRISPR interference, CRISPR activation) leverage pools of dozens to thousands of gRNAs to evaluate the relative impact of many mutations. If Cas9 is aberrantly active, these pools were expected to become skewed during cell propagation because genomic targeting by Cas9 with specific gRNAs will cause fitness impacts that change the relative abundance of gRNAs overtime. We employed temperature-dependent post-transcriptional regulatory elements called RNA thermometers (RNATs) to reduce leaky Cas9 expression in E. coli, resulting in greatly improved library stability. We then applied multilayered regulation with RNATs to Firmicutes and identify differences in RNATs that influence the efficacy of this strategy across these diverse organisms. Together these suggest that this multilayered regulation strategy should be applicable across bacterial synthetic biology with only limited reengineering.

Co-authors: Elise K. Kammerdiener, Sarah K. Garcia, Margaret K. Bale, Nandhini Ashok, Dawn M. Klingeman, Adam M. Guss, Richard J. Giannone, Robert L. Hettich, Carrie A. Eckert, William G. Alexander

22. *"Plantizing microbial enzymes – can we evolve suicide enzymes to work catalytically?"*

Anuran Gayen (University of Florida)

Continuous directed evolution (CDE) improves enzymes by hypermutating the enzyme gene in vivo, linking enzyme activity to growth, and selecting for growth rate. Members of the THI4 family of thiamin biosynthesis enzymes are prime targets for CDE. Plant THI4s are suicide enzymes with high protein turnover rates and correspondingly high energy costs. Replacing them with efficient bacterial catalytic THI4s could increase biomass yield. However, these bacterial THI4s are oxygen-sensitive and unsuitable for plants. To improve aerotolerance and activity, we used CDE under aerobic conditions in the yeast OrthoRep system on two such bacterial THI4s, which delivered five beneficial mutations to residues in the active-site region. By increasing selection pressure on the improved mutants via reduced expression, we obtained faster-growing populations whose THI4s carried new nonsynonymous and synonymous mutations. Growth improvement was largely attributable to synonymous mutations, demonstrating OrthoRep's power to optimize codon use as well as amino acid sequence.

23. *"Biosensor Guided Evolution of Polyketide Synthase Domains"*
Niesha Cantú (North Carolina State University)

Polyketides are synthesized by huge multi-enzymatic assembly lines called polyketide synthases (PKSs), including clinically relevant antibiotics. Despite their biosynthetic potential, engineering PKSs to produce novel compounds remains challenging due to the stringent substrate specificity and complex protein-protein interactions of key catalytic domains, particularly ketosynthase (KSs). A model system can be created that will allow us to introduce mutations to KS to overcome the specificity of the "acceptor" unit where various diketide SNAC can be fed into a module six plasmid to produce an α-pyrones.

However, there is currently not a high-throughput means to be able to detect successful mutations. Hence, the need to couple the model system with a biosensor, of which AraC was chosen due to it being engineered to successfully detect triacetic acid lactone (TAL) which is structurally similar to an α-pyrone. Preliminary data showed base level detection for the chemically synthesized pyrone. Lastly, in vivo pyrone production was confirmed by LC-MS analysis in which m/z values were consistent to that of the pyrone that increased with diketide SNAC concentration.

Together, these results establish the foundation for a biosensor-guided platform capable of screening PKS mutants with improved tolerance to non-native substrates. This system will enable semi-rational evolution of KS domains and facilitate the engineering of modular PKSs for the production of novel polyketide scaffolds.

24. *"Engineered E. coli as a bioelectronic sensor for environmental detection"*
Xu Zhang (Texas A&M University)

More than 40% of groundwater sources have been reported to be contaminated, posing a severe environmental challenge. Although traditional analytical techniques such as ion chromatography and high-performance liquid chromatography can provide accurate measurements even in complex samples, they require manual sample collection, controlled storage, and long-distance transportation.

Whole-cell biosensors use bacteria as the functional core to convert biological signals into electrical outputs. This approach expands the capability of sensing systems, enabling portable devices and remote environmental monitoring. Here, we developed E. coli–based bioelectronic sensors to address two major limitations of current biosensing technologies: rapid response and multiplex detection. For rapid detection, we constructed an eight-component synthetic electron transport chain that selectively detects thiosulfate, an anion associated with microbial blooms, producing measurable electrical signals within two minutes. We further extended this platform by integrating a protein switch, enabling detection of an endocrine-disrupting compound in urban waterway samples within three minutes. For multiplex detection, we introduced multiple chemical-regulated extracellular electron transfer pathways into a single E. coli chassis as signal channels. One channel is based on the flavin biosynthesis pathway under the control of a cadmium-responsive promoter, while a second channel employs the CymA–Mtr pathway regulated by an arsenite-responsive promoter. We further developed a redox-potential-dependent algorithm that efficiently converts these biological signals into a 2-bit binary output.

These bioelectronic sensors operate robustly in complex environmental water samples, enabling rapid and accurate detection of multiple contaminants. This work establishes a scalable bioelectronic sensing framework for real-time, multiplex environmental monitoring and field-deployable water quality assessment.

We further extended this platform by integrating a protein switch, enabling detection of an endocrine-disrupting compound in urban water sample in three minutes. For the multiplex detection, we introduced multiple chemical-regulated extracellular electron transfer pathways into a single E. coli chassis as signal channels.

25. *Poster Withdrawn*

26. *"Integrating recombinase-based feedback and feedforward control for optimal resource decoupling"*

Rixin Zhang (Arizona State University)

Resource competition disrupts circuit modularity by introducing unintended coupling between otherwise independent gene modules, thereby compromising the functionality of genetic circuits. Although various control strategies have been proposed, their complexity or limited effectiveness has restricted their broader application.

Here, we present the Re-NF-FF controller, a recombinase-based strategy that integrates negative feedback and feedforward regulation through promoter flipping to mitigate resource competition. Both computational modeling and experimental validation demonstrate that the Re-NF-FF controller effectively reduces resource-mediated coupling, thereby improving the robustness and modularity of gene expression.

Furthermore, the tunability of this system enables performance optimization through simple adjustments of recombinase expression levels. Overall, this strategy provides a versatile and easily implementable framework for the design of reliable synthetic biological systems.

Co-authors: Rixin Zhang, Rong Zhang, Xiao-Jun Tian

27. *"Functional replacement of surfactants with biosurfactants"*

Efrain Rodriguez Ocasio (University of Wisconsin-Madison)

A significant challenge in the functional replacement of petrochemicals is the systematic identification of alternative chemical structures that can be produced from renewable sources and meet the functional requirements of the replacement target. In the case of surfactants, there already exists an alternative class of molecules called biosurfactants that share emulsification, solubilization, and surface tension activities with the added benefit of being biodegradable and easily produced by microorganisms. In this work, relevant properties of biosurfactants were predicted from their structure using a previously reported graph neural network architecture trained on the SurfPro database of surfactant properties. The biosurfactant property database was then used to identify biosurfactants suitable for the replacement of common surfactants based on the properties required for their end-use applications in the largest surfactant markets. Once the promising candidates were identified, a modeling framework was developed to evaluate the biological and economic viability of the replacements by integrating genome-scale models with biorefinery process models. The framework uses theoretical yields in a process network populated with process and economic parameters of a biorefinery to rank the candidates based on the cost of production. This modeling framework is intended to explore new potential areas of the bioeconomy and guide the selection of targets to pursue experimentally when experimental yields are not available.

Co-authors: Brian Pfleger, Christos Maravelias

28. *"Directed Evolution to Enhance Phytoene Synthase Enzyme Activity for Metabolic Engineering"*

Fayrouz Nossir (East Tennessee State University)

Carotenoids are essential metabolites in plants for photosynthesis and serve as precursors for vitamin A in the human diet. Vitamin A deficiency remains a global health problem affecting millions of people, particularly children in developing countries, where it can lead to impaired immunity and blindness. Increasing provitamin A carotenoid production in crops has therefore become a major goal of metabolic engineering and agricultural biofortification. Phytoene synthase (PSY), the first committed and rate-limiting enzyme in the carotenoid biosynthesis pathway, represents a key target for improving carotenoid accumulation. In this study, we apply a semi-rational directed evolution strategy to generate variant libraries of mutants to improve the enzymatic activity of tomato PSY2 as the starting point, which has relatively higher activity than other homologs. Mutant PSY2 enzymes were expressed in a bacterial carotenoid-producing system that enables rapid visual screening, where red-colored lycopene accumulation produces distinct color phenotypes that correlate with enzyme activity. Color-based screening can quickly identify the variants with improved activity. The Opentrons Flex liquid-handling platform is being used to automate high-throughput screening, which allows 96 variants to be processed at once. In the next step, machine learning approaches may help model relationships between PSY sequence variation and enzyme activity to guide subsequent rounds of protein engineering. Ultimately, identifying key amino acid residues that enhance PSY activity may enable targeted genome engineering approaches to improve provitamin A carotenoid production in many crops.

Co-authors: Fayrouz Nossir, Sabiha Sultana Sammi, Tianhu Sun

29. *"Upgrading lignin derived aromatics to styrenic monomers for polymerization "*

Casey O'Brien (Georgia Tech)

Lignin, the third most abundant biopolymer on Earth and the largest natural source of aromatic compounds, is severely underutilized and mostly burned for heat. Methods of upgrading depolymerized lignin to value added chemicals are needed to displace petroleum-based products with bio renewable alternatives. The aromatic monomers of lignin, such as p-coumaric acid (pCA) and ferulic acid (FA) can be decarboxylated to 4-vinylphenol (4VP) and 4-vinylguaiacol (4VG), suitable for repolymerization. 4VP and 4VG are commonly produced through free radical emulsion polymerization in industry, however their hydroxyl group can lead to undesirable side reactions that limit polymer chain growth and hinder the formation of useful materials. Enzymatic methylation of these hydroxy groups offers a strategy to render these styrene derivatives compatible with emulsion polymerization while still retaining polar functionality. Here, we engineer a plant methyltransferase for improved methylation of 4VG and 4VP, enabling the production of aromatics suitable for functional polymer materials.

Co-authors: Shaafique Chowdhury, Katie Swanson

30. *"Development and Application of Genetically-Encoded Biosensors for Terpene Synthetic Biology"*

Cassidy Oliverio (North Carolina State University)

Terpenes, also known as isoprenoids, are a large class of natural products with applications in food, health, medicine, and other fields. Despite their widespread use, plant extraction remains the main source of production. With issues such as climate change, crop diseases, and fluctuating global market trends, the future accessibility of many terpenes could be compromised. To alleviate this strain, microbial fermentation platforms have been proposed as greener alternatives; however, low product titers have hindered their commercial-scale application. Directed evolution is a technique in which a given terpene product pathway can be optimized by random mutation of an enzyme in the pathway, generating thousands of samples to screen for a mutant that produces more product. Yet, without a high-throughput means of screening these pathways, directed evolution has been severely underutilized in terpene biosynthesis.

Genetically encoded biosensors, in which the presence of a given analyte triggers the expression of a fluorescent protein in vivo, can be utilized to rapidly screen directed evolution libraries of heterologous biosynthetic pathways, leading to increased product titers without relying on cumbersome analytical methods such as GCMS or LCMS. While biosensors have been developed to detect monoterpenes (C10), there are no such technologies to detect sesquiterpenes (C15). This work addresses this gap by developing a general sesquiterpene biosensor capable of detecting a wide range of sesquiterpenes, thus providing a route for future biosynthetic pathway engineering to improve access to valuable industrial molecules as well as life-saving medications.

Co-authors: Cassidy Oliverio, Gavin Williams

31. *"Magnetic Field Gradient–Induced Mechanical Modulation of Mitotic Spindle Organization and Cytokinesis in Xenopus laevis embryos"*

Ruchi Singh (University Of Houston)

Magnetic field gradients generate forces on biological materials proportional to their magnetic susceptibility, enabling non-contact mechanical manipulation of cellular structures:

F=χV/μ_0 " " ∇(B^2)

Here, we investigate the effects of high-gradient magnetic fields on cytoskeletal dynamics, mitotic spindle organization, and cytokinesis in macrophages and Xenopus laevis embryos. Using a custom-designed magnetic field-gradient system, we demonstrate that applied forces induce cytoskeletal remodeling, cell elongation, and alterations in division symmetry. In embryonic systems, exposure leads to deviations in cleavage patterns, suggesting perturbation of spindle alignment and cytokinetic processes. The presence of iron-based nanoparticles further amplifies these effects, enabling localized force enhancement. These findings establish magnetic field gradients as a novel physical modality for influencing cell division and provide a foundation for future applications in mechanobiology and biomedical device innovation. Cell division is governed by tightly regulated mechanical and biochemical processes. The mitotic spindle ensures accurate chromosome segregation, while cytokinesis physically separates daughter cells through actin-myosin contractility.

Recent advances in mechanobiology highlight the sensitivity of cytoskeletal systems to external physical forces. Magnetic field gradients provide a unique, non-invasive approach to applying such forces. Unlike uniform magnetic fields, gradients generate translational forces capable of acting on intracellular components.

Mechanical forces play a central role in regulating cellular behavior, yet many existing tools for studying mechanotransduction are invasive or lack spatial precision. High gradient magnetic fields (HGMFs) offer a non contact method for applying mechanical forces at the microscale, but their effects on cellular structures remain poorly understood. Using a combination of controlled magnetic exposure, quantitative imaging, and biophysical analysis, this work demonstrates that HGMFs can induce measurable changes in cytoskeletal structure and cell morphology. These findings provide new insight into magneto mechanical interactions and establish a foundation for future biomedical applications that leverage magnetic fields to modulate cell behavior.

Co-authors: Jarek Wosik, Rachael Miller

32. *"Multiplexed genome engineering: Optimizing metabolism of terephthalate"*
Nathan Gladden (Oak Ridge National Laboratory)

Traditional metabolic engineering relies on controlled, strain-by-strain comparisons to quantify genetic elements. While chromosomal integration is preferred for stability and consistent copy number, the Design/Build/Test/Learn cycle is often bottlenecked by the multi-week effort required to construct many genome-edited strains. To address this challenge, we recently developed multiplexed Serine Recombinase-Assisted Genome Engineering (mSAGE). This method enables rapid and precise insertion of multiple DNA cargos into a bacterial genome at exceptionally high efficiency. mSAGE enables construction of large combinatorial libraries directly in the chromosome, and supports rapid exploration of design space through pooled screening.

Waste polyethylene terephthalate (PET) is abundant and low-value, representing a potential feedstock for bioconversion. Pseudomonas putida is an attractive chassis for bioconversion, but does not natively catabolize terephthalate (TPA). In this study, we use mSAGE to optimize the catabolism of TPA in P. putida by constructing a 3-part combinatorial library comprising 12 TPA dioxygenases, 6 associated dehydrogenase components, and 6 candidate TPA transporters (432 total combinations). With a single pooled transformation followed by one week of selective outgrowth, we screened this library at 197x coverage and identified a gene set that increases growth rate on TPA by 37% relative to the prior best pathway. These results point to a more efficient route toward industrial PET depolymerization and upgrading.

As illustrated in this work, mSAGE represents a powerful platform for tackling complex, multi-variable genome engineering problems by enabling fast, pooled discovery of optimal genetic configurations within diverse design spaces.

Co-authors: Nathan E. Gladden, Austin L. Carroll, Adam M. Guss

33. *"Development of a whole cell biosensor for high throughput screening of two stage bioprocesses"*

Cody Kamoku (Pacific Northwest National Lab)

Efficient development of microbial bioprocesses is limited by the lack of real time, high throughput measurements that accurately report product formation in small volume cultures. To address this issue, we have developed whole cell biosensors to enable continuous plate based quantification of productivity for dicarboxylic acids, citrate and other value added chemicals. The whole cell sensor consists of a fluorescent Pseudomonas putida (SENS) strain engineered to not consume glucose but instead consume the analyte of interest. The developed sensor strains produce a fluorescent signal proportional to the amount of target analyte when grown with supernatants from a bioproduction strain or when grown in a co-culture with a bioproduction strain.

As an immediate testbed, we have evaluated engineered P. putida strains that produce dicarboxylic acids from diverse lignocellulosic carbon sources with our developed SENS strains. Development of a sensor strain for itaconic acid required additional engineering as nitrogen limitation is used to trigger stationary phase production of itaconic acid by the production strains used in this study. In this case, accurate sensing requires that any nitrogen source used to grow the sensor strain should not disrupt induction of the itaconic acid pathway by the production strain. To address this issue, we have developed SENS strains compatible with two stage bioproduction processes driven by nitrogen limitation by introducing orthogonal nitrogen utilization pathways into the sensor strain, thus eliminating potential crosstalk between the two strains.

The developed sensor strains will be used in the future as a basis for an automated workflow for rapidly mapping genotype–environment–phenotype relationships that drive improved bioproduction in different scales ranging from 96 well plates to small scale bioreactor conditions.

Co-authors: Cody Kamoku, Jake Bourdages, Sarah Lemmon, Joshua Elmore, Robert Egbert

34. *"Creating Synthetic Biology Pipelines by Training High School Educators and Students"*
Hannah Noh (Georgia Institute of Technology)

The engineering biology sector faces a critical talent gap as the field accelerates toward applications in health, agriculture, and manufacturing. Industry requires a workforce that is biologically literate, design-minded, and career-ready, yet traditional K–12 education rarely exposes students to synthetic biology prior to college. Bridging this gap necessitates intentional, scalable investment in the pipeline that feeds it. However, barriers impede progress, including academic and research professors perceiving such efforts as distracting from real research, limited knowledge of how to engage K-12 educators, and the challenges of hosting minors in laboratory settings.

This presentation describes a multi-pronged approach to K–12 workforce development, anchored in sustained educator professional development. The Frugal Science Academy has led teacher professional development for three consecutive years, training over 30 educators to confidently deliver synthetic biology content in their classrooms. The impact is measurable, with participation in Georgia extracurricular synthetic biology programs growing from 20 students in 2019 to more than 250 in 2026 across Georgia.

These results demonstrate that empowering teachers is the most scalable lever for building a career-ready engineering biology workforce. The authors invite EBRC members, industry partners, and academic institutions to explore collaborations that extend this model, ensuring the next generation of engineers is prepared to meet the evolving needs of the field.

Co-authors: Hannah Noh, Daeun Lee, Janet Standeven, M. Saad Bhamla

35. *Poster Withdrawn*

36. *"Programmable AND-gate strategy to reduce false positives in rolling circle amplification"*

Jiho Seok (Georgia Institute of Technology)

Improving the specificity of biosensors by reducing their false positive rate is a long-standing challenge, particularly for point-of-care (POC) diagnostics. An emerging and potentially impactful class of POC biosensor is those that can detect specific nucleic acid sequences. These sensors often use isothermal amplification of nucleic acid sequences, which is considered suitable for POC use due to its relative simplicity and its lack of requirement for specialized equipment or highly trained personnel. However, isothermal amplification methods are still quite susceptible to false positives, especially those like Rolling Circle Amplification (RCA) that amplify nucleic acids under mild temperature conditions that are more prone to near-target amplification. To address this limitation, we introduced an AND-gate Boolean logic to create Multi-Key RCA, which uses detection of multiple nucleic acid sequences as a prerequisite to amplification. Multi-Key RCA requiring two DNA sequences not only markedly reduced false positives compared to conventional RCA but also achieved high specificity in single-nucleotide polymorphism (SNP) detection. We showed that Multi-Key RCA also can detect RNA and can be expanded to use AND gates with more than two inputs. We integrated Multi-Key RCA with a lateral flow assay to create a user-friendly synthetic biology-based diagnostic platform requiring no specialized equipment, and we showed that this diagnostic can be implemented as a one-pot reaction that integrates all RCA steps and can be lyophilized for eventual long-term storage stability. Overall, this Boolean logic-based synthetic biology approach shows significant promise to reduce false positives in field-deployable nucleic acid biosensors.

37. *"Metabolic Characterization Reveals The Importance Of Addition Timing In E. coli-based Cell-Free Expression Systems (CFES)"*

Soor Vora (Georgia Institute of Technology)

In vivo cell cultures, which can use living cells as biocatalysts, have been a valuable avenue for producing biologic therapeutics. However, they are inherently limited by their viability requirements and cell-line engineering requirements, which add complexity, time, and cost to the therapeutic pipeline from discovery and development all the way to manufacturing. Cell-free expression systems (CFES) use cellular transcription-translation (TX-TL) machinery to express proteins in vitro, and theoretically, the resulting robustness and modularity can significantly improve therapeutic development. However, in current practice, CFES productivity is capped well below the theoretical capacity of the added nucleotides and amino acids as they experience early halts in protein production. Efforts to address this issue are limited by a poor understanding of the components and behavior of cell-free lysates.

Previous work has shown that the metabolic enzymes in cell-free lysates lead to metabolic activity over time that influences cell-free expression. In this study, we established the inflection point of protein expression as a critical timescale for CFES endogenous metabolism. Based on significant metabolic changes at this time, we established the importance of amino acid supplementation timing and showed that a controlled release of amino acids, specifically, replenishment after the inflection point, led to significant improvements in productivity and lifetime. We were also able to establish the importance of supplementation timing for non-amino acid metabolites. In addition to providing further avenues for improving cell-free performance, this study demonstrated a new potential avenue for crude cell-free systems: metabolomics-informed fed-batch supplementation.

Co-authors: Soor Vora, Mark Styczynski

38. *"Building a synthetic biology toolkit for mushroom-forming fungi"*

Jon Arizti Sanz (Stanford University)

Mushroom-forming fungi (Basidiomycetes) represent a vast and underutilized resource for biotechnology, offering transformative potential for sustainable materials, food security, and environmental remediation. Despite this promise, the widespread adoption of these fungi in industrial settings is currently limited by a lack of predictable and standardized tools for genetic engineering. My research work is focused on the development of a comprehensive synthetic biology toolkit for basidiomycetes, with a primary focus on the model organism Coprinopsis cinerea. My research centers on the establishment of a modular suite of tools, including characterized promoters, efficient selectable markers, identified genomic safe harbor loci, and strategies for tissue-specific expression. To demonstrate the utility of this toolkit, we are optimizing the biosynthesis of high-value terpenoids through a combination of genetic engineering and substrate optimization. Together, these advancements provide a scalable framework for the precise manipulation of mushroom-forming fungi, paving the way for the next generation of fungal-based bioproduction.

Co-authors: Peter Allen, Deniz Sinar, Shahar Schwartz, Vayu Hill-Maini

39. *"Programmable protein surface display in broad-host-range microbes for microbiome engineering"*

Anika Gupta (Rice University)

Protein display in microbes – the expression of functional proteins on the surface of cells – is a powerful strategy for the formation of biomaterials, sequestering cell-impermeable molecules, and enabling programmable cell–environment interactions. However, existing approaches are often host-cell specific and largely restricted to model microorganisms, limiting their utility for applications in microbes that make up diverse microbial ecosystems. To address this, we aim to develop a broad-host range and modular surface display platform that operates across taxonomically diverse microbes. The central hypothesis of this work is to leverage proteins encoded by broad-host-range conjugative plasmids that have naturally evolved to be displayed on the surfaces of diverse microbes to serve as scaffolds for carrying protein cargos engineered into their sequences. As a starting point, we focus on TraT, a membrane-localized lipoprotein natively encoded on IncF conjugative plasmids. Following the validation of TraT surface display in the model organism Escherichia coli, we compiled and screened an array of permissive insertion sites within TraT to define a modular architecture for diverse cargo proteins. By elucidating cargo insertion rules for TraT, we aim to create functional, surface-displayed protein fusions for a variety of applications. To broaden the host range of this work, we will characterize TraT homologs across conjugative plasmid families, with the goal of validating community-wide surface display in complex consortia such as wastewater communities. By coupling protein engineering with microbiome engineering, this work establishes a generalizable framework for deploying functional proteins at the interface of engineered and natural microbial ecosystems.

Co-authors: Anika Gupta, James Chappell

40. *"Development of transcription factor biosensors for high-throughput engineering of agricultural polyketides"*

Alissa Meo (North Carolina State University)

Polyketides are a structurally diverse class of natural products produced by multi-enzyme complexes known as polyketide synthases (PKSs). Their use in agriculture is especially critical, given that the Food and Agriculture Organization (FAO) estimates that approximately 40% of global crop losses are due to plant pests and diseases. However, the commercial production of many macrolides remains a major bottleneck due to reliance on fermentation in non-model organisms. While efforts to increase production titers have been pursued, with limited success, the ability to rapidly screen and optimize fermentation conditions or genetic variants remains a major bottleneck.

To this end, developing a high-throughput screening tool for mature polyketide products could help address production issues and allow for faster optimization. We propose that using highly tunable, genetically encoded transcription factor biosensors could facilitate high-throughput screening and avoid lower-throughput methods, such as LC/MS. Yet, transcription factor biosensors are not known for most macrolides of interest, and only a few biosensors have been reported for polyketides. Here, we demonstrate the tunability of a transcription factor-based biosensor and its ability to selectively detect an agriculturally relevant macrolide while discriminating against structurally similar pathway intermediates. Furthermore, we highlight its potential as a powerful tool for rapidly screening large libraries of engineered or chimeric PKS assembly lines, enabling more efficient pathway optimization and accelerating natural product discovery or production.

Co-authors: Alissa Meo, Gavin Williams

41. *"Unraveling Soil Microbe Establishment: Leveraging Genetic Mapping to Identify Genetic Factors shaping the Soil Microbiome"*

Apurv Mhatre (Oak Ridge National Laboratory)

The plant rhizosphere harbors a diverse community of plant growth promoting rhizobacteria (PGPR) that enhances plant health through mechanisms such as nutrient solubilization, phytohormone production, and pathogen suppression. While leveraging microbial communities for improving plant productivity has been a longstanding goal, targeted microbiome engineering has seen limited success due to an incomplete understanding of microbial establishment. Establishment is a critical determinant of rhizosphere colonization and, consequently, microbiome composition, but the genetic factors that govern this process remain poorly characterized.

To address this knowledge gap, we focus on Bacillus velezensis, a Gram-positive rhizobacterium widely used in commercial biostimulants due to its robust plant growth promoting abilities. Although B. velezensis is agriculturally significant, the genetic basis of its establishment in plant-associated environments remains unclear. Our studies demonstrate that different B. velezensis and B. amyloliquefaceins strains exhibit significant variation in their ability to colonize Populus trichocarpa, a biofuel feedstock species. Colony forming unit (CFU) quantification per mg of leaf tissue confirmed distinct differences in colonization efficiency among strains, suggesting that genetic variation plays a crucial role in establishment success. Given this variation, we sought to systematically investigate the genetic factors influencing colonization through a quantitative trait loci (QTL) mapping approach.

QTL mapping, a powerful tool widely used in eukaryotic genetics used to identify genetic regions associated with the varying phenotypes. QTL mapping was further explored and adapted so it can be used in bacterial system to understand genetic determinants associated with phenotypes of interest. Our bacterial QTL mapping approach enables genome wide association studies in non-model bacteria, allowing us to dissect the genetic basis of complex traits such as microbial establishment. This technique relies on recombination between parental strains to generate a diverse mapping population with shuffled alleles, facilitating the identification of genetic loci that influence colonization and survival in environmental settings. As we were working in non model system, we adapted QTL mapping for B. velezensis and explored genome shuffling and natural competence as tools for generating recombinant progeny populations. Specifically, to construct QTL mapping population we used genome shuffling via protoplast fusion and natural competence to recombine B. velezensis GB03 with B. amyloliquefaciens DSM-7, B. velezensis S4, and B. velezensis FZB42. This approach aims to maximize genetic diversity, enabling mapping of loci associated with microbial fitness and colonization potential.

A crucial factor influencing recombination success in genome shuffling is the restriction-modification (RMS) system, which acts as a genetic barrier between strains. Our preliminary findings suggest that if RMS-mediated interference is partially circumvented it can enhance genetic variability in the recombined population. Similarly, as proof of concept, we used B. subtilis progeny population developed by Protoplast fusion using genome shuffling to study complex phenotypes like fungal inhibition and soil extract soluble organic matter survival.

Co-authors: Apurv Mhatre, Oumar Sacko, Ilene del Valle, Delyana Vasileva, Joshua K. Michener

42. *"From Bug to Feature: Harnessing Resource Competition in Transcription Factor Cell-Free Biosensors"*

Lex Patterson (Vanderbilt University Medical Center)

Plasmid crosstalk—off-target impacts on gene expression levels due to the presence of multiple genetic cassettes—complicates the predictable design of multi-plasmid cell-free reactions. While potential underlying mechanisms for crosstalk have been previously investigated and hypothesized, the practical impact of crosstalk on the implementation of cell-free genetic circuits has not been thoroughly examined. Here, we contextualize plasmid crosstalk in genetic circuits by examining its impact on the design and performance of multiple, diverse transcription factor biosensors. Guided by this insight, we harness crosstalk to enhance cell-free biosensor performance. Together, these findings demonstrate that plasmid crosstalk can serve as a useful, tunable knob in the development of cell-free genetic circuits.

Co-authors: Fernanda Piorino*, Sasha Bronovitskiy, Diya Godavarti, and Mark P. Styczynski

43. *"Protein Degradation as a Tool in Cell-free Systems"*

Felicia Oentoro (Georgia Tech)

In vivo protein degradation via proteases has been studied extensively and used in multiple applications, from controlling protein expression levels to implementing biosensors. An increasingly popular alternative chassis for applications like these are cell-free systems (CFS), powerful in vitro tools made from cell lysates that can execute transcription and translation. However, the characterization of proteases in cell-free systems is more limited, likely because cell-free applications often aim to maximize protein expression levels rather than reduce them. Nevertheless, there are many potential beneficial applications of proteases in CFS. As uses of cell-free systems become more complex, proteases could provide a powerful tool to control gene expression. In this work, we explore the impacts of supplementing endogenous and heterologous proteases into a protease-deficient E. coli cell-free extract.

Co-authors: Shifeng Xu, Mark P. Styczynski

44. *"Probing ribosomal RNA homogeneity via multiplex automated genome engineering"*
Megan McSweeney (Stanford University)

The ribosome is a ribonucleoprotein ribozyme responsible for protein synthesis across all domains of life, thereby making it a powerful platform for innovation in biotechnology and synthetic biology. Ribosomes are highly conserved in nature, yet the cellular pool of ribosomes is intrinsically heterogeneous because most organisms contain multiple, unidentical copies of the ribosomal RNA (rRNA) genes. For example, the E. coli genome contains 7 rRNA operons differing in sequence by 1.4%. In vitro expression studies have shown that these sequence differences have substantial impact on protein synthesis, with distinct rRNA pools leading to up to 100% improvements in protein overexpression. However, it is not known how much these differences matter for translation efficiency when genomically encoded. To address this gap, we used multiplex automated genome engineering (MAGE) to develop a suite of strains with varying levels of rRNA homogeneity. In BL21 Star (DE3) specifically, we reverted 134 polymorphisms across six rRNA operons using rRNA operon B as the consensus. Strains were analyzed for fitness, in vivo recombinant protein yields, and cell-free protein synthesis yields from the corresponding lysates. Surprisingly, many of these mutant strains exhibit similar phenotypes to the wild type, highlighting distinctions between protein translation in vivo and in vitro. Looking forward, these strains can expedite engineering efforts towards bottom-up minimal cell construction and serve as powerful chassis to advance our understanding of the ribosome itself.

Co-authors: Megan A. McSweeney and Michael C. Jewett

45. *"A Generalizable Surface Functionalization Platform for Non-Genetic Microbial Engineering"*
Michaela Jones (MIT)

Microbial surface functionalization offers a powerful strategy for modulating bacterial phenotypes without genetic engineering. Here, we use a recently developed method leveraging strain-promoted azide-alkyne click chemistry to modify the surface of diverse microbial species, including human gut anaerobes and ocean microbiome isolates. Our approach preserves bacterial viability and achieves a greater than 50-fold improvement in attachment efficiency relative to comparable surface functionalization strategies. To demonstrate functional utility, we attached a β-lactamase enzyme to confer antibiotic resistance without introducing a resistance gene, observing a 10-fold improvement in enzyme activity as measured by minimal inhibitory concentration. Building on this, we developed a generalizable protein production pipeline featuring a novel tag design with up to four p-azido-phenylalanine residues, enabling attachment of proteins to the surface of functionalized cells. Together, these results establish a versatile, high-viability platform for engineering microbial surfaces with broad applications in synthetic biology and microbiome engineering.

Co-authors: Michaela Jones, Gabriel Vercelli, Rashi Jeeda, Stefany Moreno Gamez, Ariel Furst, Otto Cordero

46. *"Taking Synthetic Biology to the Sea: Engineering Multiplex Cell-Based Biosensors for Marine Biotoxin Detection"*

Leah Davis (National Oceanic Atmospheric Administration)

Synthetic biology has redefined the design of living systems with programmable sensing and response capabilities across medicine, biotechnology, and environmental applications; however, these capabilities have not been widely extended to marine systems. Harmful algal bloom (HAB) toxins continue to threaten marine ecosystems, seafood safety, and public health, while detection strategies remain constrained to conventional analytical and bioassay-based methods that are slow, low-throughput, and inherently limited in multiplexing capability. Here, we present a programmable, cell-based biosensing platform that directly couples toxin recognition to a post-translational output through ligand-dependent reconstitution of split reporter proteins. This design enables rapid signal generation without reliance on native signaling pathways or transcriptional amplification. The modular architecture allows precise tuning of sensitivity and specificity while supporting easily exchangeable sensing components tailored to different biotoxins. This work establishes a new paradigm for biotoxin detection by extending synthetic biology into marine environments and positioning engineered cells as programmable, multiplexed sensors for next-generation environmental monitoring.

Co-authors: Christina Mikulski, Tod Leighfield

47. *"Engineering Intelligent Bacteroidaceae (Gut) Consortium"*

Caitlyn Davis (Georgia Institute of Technology)

The human gut microbiome consists of 1000s of microbes living as a community inside the gastrointestinal (GI) tract. Host-microbe interactions have been shown to influence neural diseases, immunity, gut inflammation, and many more human health outcomes. The web of physical and metabolic interactions that occur within the gut microbiome are complex and difficult to unravel. Genetic tools to systematically study the gut microbiome are underdeveloped. Accordingly, the Wilson lab has developed genetic toolkits (wetware) to create programmable "intelligent" bacteria with the means for decision-making, memory, and communication. In this work, we have engineered a consortium of gut bacteria capable of multi-input decision-making and inheritable synthetic memory via a collection of wetware that we have developed over the last decade. When fully deployed, decision-making and synthetic memory wetware will facilitate unprecedented community-level programming of cell fate and function. Our intelligent consortium of GI bacteria will enable the advanced study and manipulation of microbiome behavior, paving the way for the development of novel future applications.

Co-authors: Caitlyn M. Davis, Leonard C. Rogers, Zachary D. Herde, Sumin Lee, Xiaoyuan Chen, Camilla Hong, Jia Dhingra, and Corey J. Wilson

48. *"From Promiscuity to Specificity: Multidrug Regulators Enable Predictive and Scalable Biosensing"*

Nicole Zhao (Harvard Medical School)

High-throughput discovery of biocatalysts is often limited by low-throughput analytical assays such as HPLC. Transcription factor-based biosensors offer a scalable alternative, but most are ligand-specific and do not generalize across chemical space. Multidrug allosteric transcription factors (aTFs) are often promiscuous binders and represent promising generalist scaffolds for small-molecule sensing. Here, we systematically identify and characterize such generalist aTFs and develop a predictive framework for biosensor engineering. Screening nine multidrug regulators against a diverse small-molecule panel revealed that only a subset exhibited broad responsiveness. Two top-performing aTFs were further profiled against ~4,000 pharmaceutically active compounds, each responding to ~20% of the library with overlapping and distinct ligand sets. These data were used to train a machine learning model that predicts ligand-sensor compatibility from chemical structure. Model-guided selection, combined with directed evolution, enabled the generation of enantioselective biosensors for new targets from these generalist scaffolds. This work establishes promiscuous aTFs as versatile starting points for engineering highly specific biosensors and provides a scalable, predictive platform for fluorescence-based biocatalyst screening.

Co-authors: Nicole Zhao, Artem Gazizov, Pamela A. Silver, Simon d'Oelsnitz

49. *"An autonomous system for multi-objective continuous evolution at scale"*

Ryan Boileau (Duke University)

Natural evolution is high-dimensional; organisms adapt to many pressures at once, across substrates, environments, and genetic backgrounds. Yet most directed evolution methods flatten this landscape to a single selection axis, hiding tradeoffs, and limiting what can be learned. Phage-assisted continuous evolution (PACE) is uniquely suited for multivariate selection because horizontal gene transfer couples genotype to propagation and allows the same phage lineage to traverse different selection environments. In practice, implementing this at scale has been prohibitive because each selection demands its own host culture, and every culture must be held for days to weeks within a narrow, infectable density window using continuously responsive bioreactors. In this work, TurboPRANCE is presented as an open-source, queueable robotic platform that integrates ~200 independently controlled turbidostats with 96 parallel PACE lagoons under closed-loop control. Each turbidostat operates as a fully separate unit that can be equilibrated and initiated on its own schedule, enabling asynchronous starts and sustained operation without intervention. Automated media formulation, programmable dosing, on-deck sterilization, and adaptive scheduling coordinate growth control with the changing needs of the robotic workflow, dynamically adjusting dilution and transfer timing around formulation, sampling, and handling steps to keep each culture at consistent infectable densities despite unpredictable method demands. Cultures can be multiplexed and titrated into lagoons at defined ratios, swapped in and out on a schedule, or kept fully separate across experiments, creating a combinatorial space of selection pressures and programs that is effectively unbounded. Additionally, to enable high-throughput evolutionary tracking that scales with TurboPRANCE, Nanopore long-read sequencing was combined with DeepVariant, a deep learning-based variant caller, enabling population-level tracking of evolving variants. The result is a system that generates high-resolution time-resolvable evolutionary trajectories and large parallel datasets spanning diverse selection regimes, yielding dense, multivariate training data to map and engineer complex fitness landscapes at scale.

Co-authors: Stefan Golas, Qianni Ma, Emma Chory

50. *"Performance of Metaheuristic Algorithms in Finding Tradeoffs in One- and Two-Species Biological Feedback"*

Quang Luan Dang Tran (Drexel University)

Biological feedback mechanisms play a vital role in helping organisms adapt and respond to their changing environments. The changing external environment can affect the cells' biochemical reactions, hence altering their cellular outcomes. In synthetic biology, feedback regulation is crucial for maintaining homeostasis in engineered circuits and for ensuring reliable performance under varying conditions. In designing synthetic feedback circuits, identifying the best biochemical parameter regimes to obtain robust circuit outcomes under changing external conditions is a computationally demanding task. Our previous work analyzed how variations in biochemical parameters affect the performance of circuits with feedback regulation, quantifying the sensitivity of synthetic gene circuits to changes in their reaction rates. This was previously done using a grid search method, which samples the biochemical parameter space at high resolution. However, as circuit complexity increases, this brute-force approach becomes computationally prohibitive.

We aim to minimize the sensitivity of a circuit to variations in multiple of its biochemical rates, which is a multi-objective optimization problem. We benchmark representative algorithms from three major metaheuristic families - Sobol SA from simulated annealing, NSGA-II from genetic algorithms, and EPO from gradient-based method - on two canonical synthetic feedback architectures: negative autoregulation and coupled positive-negative feedback. We evaluate their ability to identify biochemical parameters that yield robust performance, and we compare their computational performance against exhaustive grid search. In both circuits, the three algorithms recover Pareto fronts that are closely aligned with the grid search method, while being up to 21 hours faster; except Sobol SA is slower by 2 hours in negative autoregulation. We also examine the impact of algorithm-specific hyperparameter choices on solution quality and performance.

Co-authors: Nguyen Tran, David Osei, Ania-Ariadna Baetica

51. *"Engineering Biosensors Responsive to Alkyl and Perfluoroalkyl Acids"*
Pranay Talla (Harvard Medical School)

Perfluoroalkyl substances (PFAS) are persistent contaminants that require scalable, low-cost detection methods beyond LC-MS/MS. We developed a whole-cell transcription factor-based biosensor for PFAS detection using a proprietary, engineered repressor transcription factor scaffold with reprogrammed ligand specificity. Semi-rational, machine learning-guided design was applied to generate variants with predicted affinity for long-chain perfluorinated carboxylic acids. We combined two complementary models: a protein language model (ESM), which captures sequence-level patterns from large-scale evolutionary data, and EVmutation, which leverages residue co-variation to preserve structural and functional constraints. Using both enabled exploration of novel sequence space while maintaining protein stability. To enhance sensitivity, we engineered the E. coli host by deleting efflux and fatty acid degradation genes to reduce PFAS export and metabolism. The resulting biosensors achieved low micromolar detection of perfluorononanoic acid, with EC50 values of 10-100µM. When tested with fatty acid analogs, variants showed approximately two-fold higher induction in response to nonanoic acid and decanoic acid compared to shorter chain lengths. These results point to a promising scalable platform for selective PFAS biosensing suitable for environmental monitoring and diagnostic applications.

Co-authors: Pranay Talla, Pamela A. Silver, Simon d'Oelsnitz

52. *"Securing the U.S. Bioeconomy: A Managed Access Framework For Biotechnology Innovation"*
Jon Judd (Engineering Biology Research Consortium)

Federal biological data is fragmented, siloed, and ungoverned at the moment AI makes integration most consequential. This memo proposes three complementary actions: a DOE-led federated pilot program to unify data access across federal repositories, a NIST-developed biological data ontology to enable machine-readable interoperability, and an OSTP/NSTC interagency subcommittee to replace incompatible agency-specific data-use agreements with a unified, tiered framework. Together, these recommendations build the governance infrastructure the U.S. bioeconomy needs to remain competitive and secure.

53. *"Uncovering Mechanisms of Microbial Persistence in the Maize Root Microbiome using Functional Metagenomic Screening"*

John Cheadle (North Carolina State University)

Rhizosphere microbiomes impact plant productivity through nitrogen fixation, nutrient solubilization, protection from pathogens, and stress resilience. For plant-beneficial bacteria, colonization of and proliferation on or in the plant roots is critical to conferring these benefits. Many prior studies investigating the persistence of a microbe in a plant microbiome pursue a loss-of-function approach and only study a single host species and/or microorganism at a time. Here, we expand upon previous work establishing a genetic toolkit for engineering maize root microbes to efficiently transform (10^6 – 10^7 CFUs) Escherichia coli, Pseudomonas putida, and Bacillus subtilis, allowing for expression of many fragments of metagenomic DNA in a diverse set of hosts. We then leverage this functional metagenomic screening approach to identify microbial genes that confer increased maize root persistence without a priori knowledge of the donor organism, allowing for the discovery of new-to-science genes and microbial functions in the maize root niche. In further studies, we will apply this combination of tools to other plant systems under different selective pressures (host genotype, drought stress, presence of microbial neighbors) to identify fitness-enhancing genes that function within or across specific contexts. Improving our understanding of colonization- and persistence-related genes may improve the design of microbial inoculants for sustainable agriculture, which rely on persistence in a plant microbiome to be effective.

Co-authors: John Cheadle, Solomon Samuel, Manuel Kleiner, Omri Finkel, Maggie Wagner, Nathan Crook

54. *"Optimization of Mfp5 Production Through T3SS-Mediated Protein Secretion"*
Natalia Barna (Northwestern University)

Protein-based biomaterials are often implemented in sensors and adhesives. Recombinant DNA technology has made the rapid, high-titer production of these biomaterials possible. Still, production is limited by various shortcomings of microbial expression, such as the formation of intracellular protein aggregates and inclusion bodies, which elicit costly and time-consuming purification processes. Salmonella enterica's Type III Secretion System (T3SS) bypasses these issues, secreting proteins of interest (POIs) directly from the cytosol to the supernatant. One such biomedically relevant POI is Mfp5, a mussel foot protein that allows mussels to attach to solid substrates in aqueous environments. Mfp5 can be repurposed to construct medical sensors or prevent bacterial infections, making its optimized production essential. Currently, Mfp5 has only been synthetically produced using Escherichia coli and the gram-positive bacteria Bacillus subtilis, which features challenges in its secretion pathway, such as protease activity. While S. enterica lacks this issue, previous attempts have shown that its T3SS does not secrete Mfp5. To address this, the Mfp5 construct was engineered with a gene encoding SicP, a native chaperone that directs proteins to the T3SS, and a 2xFLAG epitope tag, enabling successful secretion. Further optimization was attempted by overexpressing hilD and hilA, T3SS transcriptional factors, and knocking out hilE, a T3SS inhibitor, alongside testing the effect of a prgI::S49R mutation in the T3SS needle protein PrgI, a substitution previously shown to enhance secretion in mutagenesis screens. hilD overexpression did not result in Mfp5 secretion. Expression levels, secretion titers, and secretion efficiencies were then compared across wild-type, hilA, ΔhilE, and prgI::S49R strains, with all three modified strains showing higher mean secretion titers and efficiencies relative to wild-type. Future work will explore additional genetic modifications.

Co-authors: Natalia Barna, Jordan Summers, Danielle Tullman-Ercek

55. *"Protein Degradation for Improving Limit of Detection of a Cell-Free Biosensor"*
Shifeng Xu (Georgia Institute of Technology)

The development of low-cost, rapid diagnostics is critical for expanding healthcare access in resource-limited environments. Cell-free biosensors have emerged as a promising solution for point-of-care testing; however, their utility is often constrained by a high limit of detection (LOD). This limitation frequently stems from leak – unintended sensor activation in the absence of the target – which creates a background signal that obscures the detection of low-concentration analytes. To address this, we implemented a background-reduction strategy by tagging the GFP reporter protein with a degradation tag specific to the endogenous E. coli protease ClpXP. By degrading leaky reporter proteins, background noise is reduced to improve the signal-to-noise ratio and fold-change activation of the sensor. We demonstrate this mechanism using TLISA (T7 polymerase Linked ImmunoSensing Assay), a modular, instrument-free platform. Our method aims to enhance the sensitivity of cell-free diagnostic tools towards point-of-care deployment.

Co-authors: Shifeng Xu, Felicia Oentoro, Mark Styczynski

56. *"Linking Microfluidic Geometry to Microbial Behavior through Hydrodynamic Simulation and Digital Twin Modeling"*

Bukola Akindipe (North Carolina A&T State University)

Bacterial populations in natural environments inhabit spatially confined microenvironments where nutrient availability, flow dynamics, and physical barriers vary across micrometer scales. Such variables can affect the growth, communication, and morphology of species and communities across generations. Although multiple studies have assessed the spatiotemporal dynamics of microbial behavior in microfluidic devices, it remains unclear how the geometry of a microenvironment shapes the metabolic behavior of the communities it contains. We propose to investigate this relationship by engineering controlled microfluidic growth chambers, simulating their hydrodynamic environments, and predicting biological outcomes through digital twin modeling at subcellular resolution. The Monolayer Chamber (MC) provides a shared growth space with cell traps, creating three distinct flow zones for direct observation of bacterial competition, while the Monolayer Communication Module (MCM) features paired chambers connected by a micropillar filter that permits molecular diffusion while preventing physical cell crossing. Both architectures were simulated in COMSOL Multiphysics to characterize flow velocity profiles, pressure distributions, shear stress, and diffusion gradients. The resulting hydrodynamic maps serve as environmental inputs for a digital twin model built on the Vivarium 2.0 composable simulation framework that predicts single-cell growth, morphology, and motility from local flow conditions. Simulation results demonstrate the distinct hydrodynamic profiles expected from varying microfluidic architectures. Next, we explored the impact of their features on potential biological outcomes. Experiments on bacterial populations (i.e., more than 100 hours of live E. coli growing in such environments) and their cell division and tracking data were used to validate the predictions made using the digital twins. We believe this integrated design-simulation-prediction pipeline will enable computational evaluation of new chip geometries before fabrication, establishing a generalizable framework for linking microenvironment geometry to microbial behavior.

Co-authors: Bukola A. Akindipe, Tolulope Oyeniran, Hamed Rastaghi, and Samuel M.D. Oliveira

57. *"Computational Design and Analysis of Bioreactor Configurations for Efficient Glucose and Xylose Co-Consumption"*

Rutuja Bhamare (North Carolina Agricultural and Technical State University)

Microbial co-culture systems offer a promising approach for improving the utilization of mixed carbon substrates in bioprocessing applications. Conventional co-culture bioreactors enable metabolic interactions between species (e.g., Saccharomyces cerevisiae and Escherichia coli), supporting division of labor during co-consumed substrate. However, such co-culturing systems often exhibit limitations, including diauxic growth, substrate competition, and accumulation of inhibitory byproducts, which reduce process efficiency and controllability. Dynamic Flux Balance Model (dFBM) is a widely used computational framework for modeling such systems, enabling the simulation of time-dependent metabolic fluxes, substrate uptake, and microbial growth under changing environmental conditions. Here, we propose to reconstruct the dFBM simulations reported by previous studies to investigate substrate consumption dynamics in S. cerevisiae and E. coli co-cultures grown on glucose/xylose sugar mixtures. First, our simulation results successfully reproduced the characteristic diauxic growth behavior reported in the original study. Next, a systematic parameter exploration revealed that higher ethanol concentrations arise from higher glucose uptake rates, oxygen limitation, and insufficient dilution or downstream consumption. Additionally, we expanded the analysis of diauxic timing, which was strongly influenced by substrate-depletion thresholds and oxygen-transfer capacity. Finally, building on this validated framework, we propose exploring the introduction of separate bioreactor configurations as alternatives to improve production yield and eliminate unnecessary growth inhibition and competition. Our preliminary results indicate promising shifts in diauxic timing and reduced ethanol accumulation under specific conditions, suggesting that modified reactor designs may improve substrate utilization efficiency. These findings both validate the modeling framework and highlight the potential of alternative bioreactor configurations for optimizing microbial co-culture performance.

Co-authors: Rutuja S. Bhamare, Hamed Rastaghi, Samuel M.D. Oliveira

58. *"Whole genome synthesis of Bacteriophage SynPE3 to combat multidrug-resistant Pseudomonas aeruginosa."*

Akinwunmi Afuape (North Carolina A&T State University)

Background: Multidrug-resistant pathogens pose a significant global health threat, necessitating innovative therapeutic solutions beyond traditional antibiotics.

Approach: Bacteriophages, viruses that infect bacteria, offer a promising alternative. This study focuses on the redesign and de novo synthesis of the genome from the P. aeruginosa phage PE3 (SynPE3). Method: To this end, we are assembling the SynPE3 genome in a stepwise fashion using 28 synthetic gene fragments (F1-F28) ranging from 0.6 to 2.5kb. The synthetic fragments are assembled into seven "minichunks" using synthetic biology strategies such as Gibson Assembly and Golden Gate Assembly. Finally, the minichunks will be assembled into the complete SynPE3 genome in a bacterial artificial chromosome, rebooted, and tested for infectivity in model P. aeruginosa strains. The modularity of the system will allow efficient modification of the SynPE3 genome to introduce new genes to overcome host resistance and enable infection of other pathogenic P. aeruginosa strains.

Result: To date, we have assembled minichunks F9-12, F13-F16, F17-F20, F21-F24, F25-F28. The assembly of F1-F4, F5-F8, as well as assembly of the complete SynPE3 genome and the modification of the phage genome, will be the focus of future studies.

Conclusion: This innovative workflow not only improves the precision and reproducibility of synthetic phage development, but it also enables scalable, next-generation bacteriophage therapies targeting drug-resistant pathogens.

Co-authors: Madison Pinckney, Nhoaa Powell, Adam Yang, Penelope Stewart, Robert H. Newman

59. *"Public Perceptions and Acceptance of Microbiome Engineering in Built Environments"*
Kristen Landreville (NC State University)

This poster presents data that shows how public perceptions of microbiome engineering in the built environment are shaped by the interaction of audience predispositions, risk perceptions, and indoor spatial context. Using a nationally representative survey of U.S. adults (N = 901) and a theory-driven audience segmentation approach, we identify four distinct groups based on political ideology, science and technology beliefs, trust in governance, and perceptions of physical and values-based risk. We then assess how these segments differ in their comfort with microbiome interventions across nine indoor spaces, ranging from homes and schools to hospitals and industrial settings. Results show that attitudes toward microbiome engineering are not uniform but structured by coherent belief systems and demographic patterns. A key finding is that acceptance varies systematically by environment: respondents are generally more comfortable with interventions in spaces perceived as distant and institutionally controlled, such as factories and wastewater treatment plants, and less comfortable in proximate, identity-relevant spaces such as homes and schools. Skeptical risk-averse conservatives consistently express low support across contexts, while other segments demonstrate context-dependent acceptance. These findings advance a spatially mediated segmentation framework, highlighting that public evaluations of emerging biotechnologies are shaped by both who the audience is and where the technology is applied. The study offers implications for targeted communication, governance design, and responsible deployment of microbiome engineering.

Co-authors: Christopher Cummings, Nourou Barry, Jennifer Kuzma

60. *"Redesigning Split β-Galactosidase for Fast-Acting, Sensitive Biosensors"*
Karly Liebendorfer (Georgia Institute of Technology)

Current reporters used in synthetic biology–based biosensors are poorly suited for point-of-care (POC) translation, suffering from low sensitivity, slow response times, and reliance on specialized equipment or expensive substrates. While colorimetric reporters offer an advantage by enabling direct visual readout, their large size slows biosensor response. To address this, LacZ (β-galactosidase) is typically split into two fragments, allowing the larger fragment to be pre-expressed so that only the smaller fragment must be synthesized upon target detection. However, the split LacZ system used across all biosensors to date was originally designed in the 1970s and has remained largely unchanged, forcing an undesirable trade-off between sensitivity and response time.

Here, we present the first rational redesign of this colorimetric reporter for synthetic biology biosensors. By identifying and screening novel split sites in LacZ, we developed an improved variant that eliminates this trade-off, dramatically reducing response time, increasing sensitivity, and enabling semi-quantitative visual interpretation in under 8 minutes. To demonstrate its modularity, we incorporated this reporter into two existing biosensing platforms, achieving fast, visually distinguishable detection of diverse targets at low concentrations that far surpass conventional reporters, which failed to detect even the highest target concentrations in the same timeframe. This work closes a critical technical gap in biosensor development, bringing synthetic biology–based diagnostics in line with the performance standards required for real-world POC deployment.

Co-authors: Alexandra T. Patterson, Karly M. Liebendorfer, and Mark P. Styczynski

61. *"Public engagement with synthetic biology nationwide at museums and science centers"*

Catherine McCarthy (National Informal STEM Education Network (NISE Network))

The National Informal STEM Education Network (NISE Network) Building with Biology project created conversations in museums among scientists and public audiences about the emerging field of synthetic biology and societal implications.

Synthetic biology uses new techniques combining biology and engineering to make new or modified living things and materials. The field is exploring where biology-based products might provide solutions to a wide diversity of problems in health, energy, and the environment.

The project included informal science educators, researchers, and scientists dedicated to developing innovative resources, practices and processes to build the capacity of the field to use public engagement with science activities. Project participants worked together to extend STEM learning about science, technology, and societal implications through public and scientist dialogue about synthetic biology.

Building with Biology Kit

The Building with Biology kit was designed to help museum and scientist partners engage public audiences in conversations and hands-on activities about the field of synthetic biology and the ways this emerging technology is interconnected with society. The kit contains activities, discussion forums, videos, and professional learning resources designed to engage the public and prepare informal science educators. The kit was created with NSF funding through a collaboration of informal science education institutions and partnering scientists.

Two hundred free physical kits were awarded to locations nationwide in 2016. Building with Biology events and conversations were held nationwide. Many museums and science centers continue to use these resources and some continue to partner with local iGEM teams.

Evaluation reports and Publications

In addition to engaging the public, the project also examined the model of two-way dialogue and learning between public and scientist audiences resulting in several publications about learning and fostering conversations.

Acknowledgements

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62. *"Improving energy flux in a photoautotrophic system through microbial electro-photosynthesis Cyanobacterium"*

Nandini Kannoju (Arizona State University)

Photosystem II (PSII) plays a critical role in photosynthesis by driving the light-dependent splitting of water into protons, electrons, and oxygen (O_2). However, PSII is highly susceptible to photodamage even under moderate light intensities, creating a fundamental bottleneck that limits sustained electron flow and overall photosynthetic productivity. To overcome this limitation, Microbial Electro-Photosynthetic Systems (MEPS) have been developed, enabling the delivery of exogenous electrons directly into the photosynthetic electron transport chain of cyanobacteria lacking PSII through the use of redox mediators and electrodes. Previous studies demonstrated that MEPS in live Synechocystis (ΔpsbB) cells lacking PSII can generate light-dependent current, with current increasing proportionally with light intensity up to 2050 µmol photons m^{-2} s^{-1}. This resulted in an electron delivery rate of up to 113 µmol electrons h^{-1} mg^{-1} chlorophyll and an average current density of 150 A m^{-2} mg^{-1} chlorophyll.

In this study, we extend the application of MEPS to enable D-lactate production in Synechococcus sp. PCC 11901, a fast-growing cyanobacterial strain. PSII activity was eliminated by constructing a ΔpsbB knockout strain, and a D-lactate dehydrogenase variant (gldA101) was integrated at the native psbB locus. As expected, the engineered strain exhibited impaired growth due to the absence of PSII function. However, under mixotrophic conditions with glycerol, improved growth was observed compared to the wild type. Shake flask experiments conducted at a light intensity of 30 µmol photons m^{-2} s^{-1} demonstrated D-lactate production of up to 60 mg/L. The strain was subsequently cultivated in a MEPS reactor supplemented with an electron mediator, and D-lactate production was evaluated across a range of light intensities and CO_2 concentrations. Together, these results demonstrate that coupling metabolic engineering with electro-photosynthetic electron supply enables sustainable, light-driven D-lactate bioproduction and establishes a promising platform for cyanobacterial biomanufacturing.

Co-authors: Nandini Kannoju, Christine Lewis, Jacob Hackney, César I. Torres, Arul M. Varman

63. *"A synthetic yeast two-component platform to decode non-linear regulation by XIST A-repeats"*

Shalley Sharma (Emory University)

The long non-coding RNA XIST mediates gene silencing through modular repeat domains that recruit RNA-binding proteins (RBPs) and chromatin regulators, with the A-repeat initiating silencing and other regions (e.g. B and E) contributing to propagation and stability. Interestingly RNA transcription at the X inactivation center (XiC) appears to be sufficient to support recruitment in cis, and might not rely entirely on tethering mechanisms seen in other RNA scaffold systems. However, the quantitative principles governing how repeat architecture and RBP identity shape this cis-regulatory mechanism remain unclear.

To address this, we developed a synthetic two-component system in Saccharomyces cerevisiae to dissect XIST-RBP interactions in a controlled and programmable context. Synthetic XIST repeat constructs are expressed under an inducible promoter, and candidate RBPSir2 fusions act as recruited silencers. This design models the mammalian XiC, where RNA-protein interactions recruit silencing proteins in cis. We used a proximal URA3 reporter for growth-based quantification of repression.

We screened sequences derived from XIST region A (4xA, 8xA, 12xA) and heterotypic constructs carrying motifs from downstream XIST regions B and E against SHARP and RBM15 RRM domains that are known to interact with A motifs. Growth phenotypes revealed strong non-linear and context-dependent effects. Notably, the 8xA construct produced the strongest silencing, including conditions where cells failed to reach saturation within 36 hours, while both 4xA and 12xA showed reduced activity. Among heterotypic designs, ABE and AEB exhibited enhanced silencing, whereas AAE showed minimal output.

These effects were RBP-dependent, with SHARP domains producing stronger phenotypes than RBM15 variants. Consistent with these observations, in vitro binding assays showed that SHARP RRM3 exhibits the highest affinity for the 8xA configuration compared to 4xA and 12xA constructs. Additional evidence came from HT1080 male fibrosarcoma cells, where GFP reporter repression saturated at 8xA, recapitulating the non-linear behavior observed in yeast.Together, these results show that XIST function is governed by repeat copy number, linear arrangement, and RBP identity, leading to non-linear regulatory behavior and providing a new framework for engineering epigenetic regulatory systems.

Co-authors: Shalley Sharma, Seong Hu Kim, Bryan Eusse, Alisha Jones, Karmella Haynes

64. *"Tracking metabolite-mediated plant-microbe interactions in the rhizosphere"*
Emily Singer (Princeton University)

Enhancing plant growth by utilizing the microbiome while reducing reliance on chemical fertilizers holds the potential of increasing crop yield and biomass production with reduced environmental harm. Plant Growth Promoting Rhizobacteria (PGPR) play an important role in plant growth within the soil microbiome, buffering biotic and abiotic stresses while also providing necessary nutrients to plants. However, their ability to colonize the plant host and persist within the rhizosphere is often inconsistent. Here, we are testing whether metabolite-mediated interactions can be used to influence bacterial persistence in plant associated communities. In this system, we are engineering bacteria to catabolize molecules called opines as a method for biasing persistence of these strains under conditions where opines are available. The bacterial populations that have the opine catabolism plasmids are tracked using WISH-tags, a 120bp sequence integrated into the bacterial genome containing a unique 40bp barcode that can be amplified using universal primers. This system allows tracking and quantification of strain abundance in mixed communities. Across two plant-associated bacterial strains we show that engineered opine catabolism biases communities toward the opine utilizing members under opine-amended conditions. This work provides a platform for studying bacterial dynamics in the rhizosphere and may inform future strategies for improving beneficial plant-microbe interactions.

Co-authors: Emily Singer, Samuel Eastman, Jonathan M. Conway

65. *"Differential Effects of Buffer Formulation on Cell-Free Lysate Performance"*
Gabe DeSantis (Georgia Institute of Technology)

Cell-free expression systems offer an alternative to whole-cell biomanufacturing by eliminating the need for viability maintenance and directing resource utilization toward target molecule production. Lysate-based cell-free systems execute this biosynthesis via the extraction of intracellular machinery from lysed cells. These extract generation procedures use high salt buffers to wash cultures of media components, facilitate handling during lysis and downstream processing, and establish a consistent small molecule profile through dialysis. Buffer formulation has traditionally focused on maintaining a consistent osmotic, ionic, and pH environment for transcriptional and translational machinery throughout the lysate preparation process. However, previous work has demonstrated differential strain response to identical buffer formulations, and variable responses to different buffer formulations from a single strain, suggesting a more significant role for buffer composition in determining lysate performance. Here we show that this buffer effect is evident as far upstream as the post-harvest wash step, with the most pronounced effects occurring during the optional post-lysis runoff step, while buffer salt identity has little effect during dialysis. Buffer formulation affects both translational and transcriptional capacity, with a direct effect on the performance of the T7 RNA polymerase. Metabolomic analysis further indicates that buffer salts influence the endogenous metabolism of these lysates during lysis and runoff, resulting in downstream performance differences. These findings help elucidate critical processes occurring during the extraction preparation, with future work aimed at designing more intentional and cost-effective lysate production methods.

Co-authors: Mark Styczynski

66. *"Fluorescence polarization reveals dynamic formation of biomolecular condensates in vitro and in vivo"*

Hayeon Park (University of Delaware)

Synthetic biomolecular condensates enable programmable control of cellular behavior in response to defined inputs. However, quantitative analysis of phase transitions remains challenging due to limited detection methods. Here, we present a generalizable fluorescence polarization-based assay for monitoring phase transitions both in vitro and in vivo. Using fluorescent protein fusions to diverse intrinsically disordered proteins (IDPs), we demonstrate sensitive detection of condensate formation across multiple phase-separation systems. This method enables real-time quantification of condensate dynamics that are difficult to resolve by conventional imaging alone. Our results establish fluorescence polarization as a versatile platform for investigating the biophysical mechanisms of biomolecular condensates.

Co-authors: Mruthula Rammohan, Ming Hung Yen, Hayeon Park, Kevin V. Solomon

67. *"Improving the Colonization Properties of the Probiotic Yeast Saccharomyces boulardii via Mucus Glycan Metabolism"*

Aryan Razdan (North Carolina State University)

Saccharomyces boulardii is a popular over-the-counter fungal probiotic that can improve the gut health of individuals suffering from gastrointestinal diseases and is also gaining increased attention as a carrier for recombinant functions into the gut. However, its therapeutic efficacy is limited because it cannot persist in the gut for more than 3-5 days post-administration in healthy individuals. Recent work by our group suggests that the gut microbiome competitively inhibits colonization by S. boulardii.

We found that unlike gut commensals, S. boulardii cannot utilize mucin glycans as a food source and cannot adhere to gut mucus. We therefore hypothesized that engineering S. boulardii to metabolize gut mucin glycans would enable it to survive within the resident microbiome, thereby improving its residence time in the gut.

To test this hypothesis, we have imported a metabolic pathway from the gut resident Candida albicans to enable it to utilize the gut mucin glycan N-acetylglucosamine (GlcNAc). This pathway results in the formation of fructose-6-phosphate, which can then be further metabolized, allowing yeast to survive on this mucin glycan as a sole carbon source. Then, we utilized a promoter library screening approach to optimize and improve the growth of S. boulardii on N-acetylglucosamine. Looking ahead, we will test this strain in a mouse model to assess whether GlcNAc metabolism improves the colonization properties of S. boulardii and to determine its potential effects on the native microbiota of antibiotic-treated and wild-type mice.

68. *"Mining Bacillus Genomes for Novel and Chimeric Pesticidal Proteins"*
Cameron Roots (ORISE & USDA)

Bacterial pesticidal proteins represent a diverse group of metabolites that offer practical opportunities for insect control in agriculture. Farmers consistently need new tools because insecticides lose effectiveness as pests adapt and evolve resistance. CRY proteins form one major class of these agents. Bacillus species produce these proteins as crystals during sporulation. We report early results from a large-scale search for new pesticidal proteins in Bacillus. We sequenced 1,440 Bacillus thuringiensis and Bacillus cereus genomes from the NCAUR Culture Collection. Using a hidden Markov model, we found that 728 genomes contain genes with putative CRY domains. This finding increases the number of CRY-domain-containing B. thuringiensis and B. cereus genomes currently recorded at NCBI by ~50%. Our dataset includes 199 documented CRY genes at 95 percent identity and 262 at 30 percent identity. The curated nature of this dataset prompted us to perform a focused search for naturally chimeric CRY proteins, based on their 3 domain structure. We identified 20 three-domain candidates in which at least two domains align best with distinct primary CRY classes, suggesting recombination events. We also identified 146 domains with less than 50 percent identity to known CRY domains, suggesting potentially novel bioactivities. A clearer understanding of the natural landscape of these proteins will support future studies, including ancestral gene reconstruction, structure-guided host range prediction, and targeted recombinant library screens. This study provides an approach to discover novel CRY proteins that can be developed for pest control applications.

Co-authors: Cameron Roots, Christopher Dunlap

69. *"Dual MarR-family Regulation of Indole-3-acetic acid (IAA) Degradation in Variovorax CL014"*

Ya Wen (Princeton University)

Indole-3-acetic acid (IAA) is a major plant auxin hormone involved in root development and plant–microbe interactions. Some rhizosphere bacteria, including Variovorax CL014, can balance auxin levels in the rhizosphere through IAA degradation mediated by the iad operon. Our previous studies showed that the iad operon is regulated by two MarR-family regulators, MarR50 and MarR73. MarR73 has been identified as a transcriptional repressor of the iad operon, while MarR50 also contributes to repression of IAA degradation. The presence of two related repressors regulating the same operon is relatively uncommon and suggests a more complex regulatory mechanism for bacterial auxin catabolism.

In addition to IAA, our physiological assays indicate that other auxin-related compounds, including 1-naphthaleneacetic acid (NAA) and indole-3-pyruvic acid (IPyA), may also interact with the MarR regulatory system and affect downstream iad expression. These results suggest that the auxin degradation system in CL014 may respond to multiple auxin analogs rather than IAA alone.

Although the DNA binding site and repression mechanism of MarR73 have been characterized, the DNA binding sites of MarR50 remain unclear. To characterize DNA regions bound by MarR50, we adapted a 3×FLAG-tag based ChIP-seq workflow for bacterial cells. Cells expressing FLAG-tagged MarR proteins were formaldehyde cross-linked, chemically lysed, and sonicated using Covaris prior to anti-FLAG immunoprecipitation and DNA recovery. Preliminary ChIP-qPCR results showed enrichment at the known MarR73 target region, supporting the feasibility of this workflow for identifying MarR-associated binding regions. Ongoing work focuses on identifying MarR50 binding sites and understanding its role in transcriptional regulation of bacterial IAA degradation.

Co-authors: Ya Wen, Ting Jiang, Jonathan M. Conway

70. *"Light-controlled Biomanufacturing System"*
Kosuke Hamano (miibio, inc.)

miibio, Inc. is a biomanufacturing startup developing optogenetics-based technologies to dynamically regulate intracellular metabolic pathways. Our core innovation lies in the use of photo-switchable proteins, originally invented by our co-founder Dr. Moritoshi Sato at the University of Tokyo, to precisely control enzymatic activity with high temporal resolution.

Conventional bioprocesses for producing compounds that inhibit cell growth rely on static genetic modifications, which often result in suboptimal yields due to the toxicity of target compounds and metabolic burden during fermentation. While chemical inducers are sometimes used to mitigate these issues, they increase production costs. In contrast, miibio's light-controlled platform enables real-time, non-invasive, and cost-effective regulation of gene expression and enzymatic activity using light as an external input, eliminating the need for chemical inducers or complex media adjustments. This approach allows phase-specific optimization of microbial states—such as separating growth and production phases—within a single cultivation process, significantly improving production efficiency and yield.

Our primary focus is the biomanufacturing of phenolic compounds—used as key building blocks for high-performance polymers, preservatives, and pharmaceutical intermediates—as sustainable alternatives to petroleum-derived materials. However, our platform is broadly applicable and can be extended to the production of peptides, enzymes, antibodies, and other high-value biomolecules.

miibio is actively seeking strategic partners and collaborators in the fields of biomanufacturing, chemicals, and pharmaceuticals to co-develop and scale innovative production processes using our optogenetics platform. We welcome opportunities for joint research, licensing, and industrial applications.

71. *"Solving Global Issues Using Plant Synthetic Biology: Making Monoclonal Antibodies Accessible and ETSU iGEM's Crop Nutrition Improvement"*

Zoe McCready Martin (East Tennessee State University)

Plant synthetic biology is an emerging discipline which attains a broad array of significant applications that pose solutions to global issues and hold potential to facilitate worldwide change. The objective of each of the two respective projects, mentored by cross-disciplinary faculty, is to propose solutions to global issues using plants: a medium that is similarly universal. The first project uses molecular pharming, the transformation of plants to express proteins, specifically pharmaceuticals, within plant tissues. The aim of this project is to engineer plants to synthesize monoclonal antibody Rituximab, a life-saving therapeutic for the treatment of leukemia, non-Hodgkin lymphoma, and rheumatoid arthritis, in common tobacco. The success of this project will provide an easy and costless production platform for monoclonal antibody therapeutics, which could benefit patients globally by providing accessible and effective treatments. At ETSU, the first iGEM (International Genetic Engineering Machine) competition team was launched and conducted plant synthetic biology research which holds global impact. The second project, presented by iGEM team in Paris, France in 2025, confronts the issue hidden hunger, or micronutrient deficiencies: a problem not just in developing countries, but globally. This is the dietary sufficiency of calories, but insufficiency of micronutrients. The aim of this project is to enhance a seed crop, soybean, to have increased vitamin content using resources from its own genome and gene editing: therefore, not introducing any foreign genes. Soybeans can synthesize vitamins, but mainly within the leaves, not active in the seed. A deep learning model was utilized to predict what would make these pathways seed-specific, assisting gene editing and directed mutation, which simply accelerates natural process of mutation. This method can not only be used in the soybean but broadly applied in major crops for nutritional enhancement.

Co-authors: Tianhu Sun, Bikram Giri, Aruna Kilaru, Robert Standaert, Anoop Arunagiri

72. *"Identifying Bacterial Genes that Support Microbiome-Mediated Drought Resilience in Plants"*

Varaidzo W. Chakafana (Princeton University)

For every 1 °C increase in temperature, agricultural productivity is expected to decline by approximately 10%, posing a serious threat to crop yields. Many bacteria inhabit the rhizosphere, where their biochemical and molecular activities influence plant growth and development, including the enhancement of plant tolerance to drought stress. PGPR-mediated drought tolerance is associated with increased accumulation of osmolytes, which promotes water retention and uptake, enhanced antioxidant activity that mitigates reactive oxygen species–induced oxidative damage, and improved photosynthetic efficiency, collectively contributing to enhanced plant performance. Illuminating the genes involved in these molecular interactions provides a foundation for targeted bacterial engineering to mitigate the impacts of drought on agriculture. In this study, PGPRs with drought-mitigating potential were selected based on preliminary screening under polyethylene glycol (PEG)-induced drought stress, and the assessment of key plant growth promoting traits. To investigate drought associated genes, this study utilized Random Barcode Transposon Sequencing (RB-TnSeq) to generate genome-wide mutant libraries in selected strains. Escherichia coli donor strains carrying plasmids with uniquely barcoded transposons were conjugated with selected drought-resilience-promoting PGPR strains, enabling random insertional mutagenesis. The resulting random barcode transposon mutant libraries will be sequenced, and their barcode insertion sites will be mapped against their respective wildtype bacterial genomes. Fitness assays will evaluate gene importance based on the change in barcode abundance under drought relevant conditions. Targeted gene knockouts will further validate the importance of candidate genes. Together, this work will identify bacterial genes that can be leveraged to engineer PGPR strains with improved potential to support crop resilience under drought stress.

Co-authors: Ella F. Gunady, Jonathan M. Conway

73. *"A Strategy for Data Collection to Solve the Protein Function Problem"*
David Ross (NIST)

The success of AlphaFold2 and similar AI models in predicting protein structure from sequence has demonstrated the potential revolutionary impact of AI applied to biotechnology. A key ingredient in that success was the protein structure data in the PDB, which took over four decades and $10B to collect. To enable future AIxBio models with similar impact, we need strategies to efficiently collect the appropriate high-quality, large-scale data. To that end, NIST and The Align Foundation have been collaborating with several other labs to develop a strategy and measurement platform to collect large datasets to enable the quantitative prediction of protein function from sequence. We have used the resulting growth-based quantitative sequencing (GROQ-Seq) platform to collect data for a variety of different protein function types at the scale of hundreds of thousands of protein variants per experiment.

In this presentation, we will give an overview of the development and data collection using GROQ-Seq for different types of proteins including allosteric transcription factors, proteases, RNA polymerases, tRNA synthetases, histidine kinases, single-chain antibody fragments, and metabolic enzymes. We will also use examples from the first several GROQ-Seq datasets to highlight the key features of our data collection strategy that provide high value data both for specific scientific and engineering questions as well as for data aggregation and generalized AI model training. Those key features include:
1. Standardization: We utilize automated protocols and a shared synthetic-biology toolkit for scalable data collection at multiple labs and across protein families and function types.
2. Calibration: We employ "protein function calibration ladder" standards to deliver biophysically meaningful data for aggregation across function types.
3. Multi-parametric Assays: We leverage automation to explore the multi-dimensional biophysics of protein function.
4. Data Quality: We implement rigorous data cleaning and a framework for assessing dataset accuracy, precision, dynamic range, and uncertainty.

Co-authors: David Ross, Shwetha Steenivasan, Svetlana Ikonomova, Olga Vasilyeva, Nina Alperovich, James McLellan, Aviv Spinner, Andi Dhroso, Dana Cortade, Erika DeBenedictis, Simon d'Oelsnitz, Anjali Chadha, Lily Nematollahi, Kristen Sheldon

74. *"Developing a Genome-Scale Pooled CRISPRi Platform to Identify Improved Formate-Utilizing Variants of Cupriavidus necator H16"*

Pallavi Ramaswamy (Oak Ridge National Laboratory)

Genome-scale CRISPR interference (CRISPRi) screens enable systematic mapping of bacterial genotypes to phenotypes exhibiting growth under industrially relevant conditions. While these methods are well established in model organisms, comparable toolkits for non-model microbes remain limited. The chemolithoautotroph Cupriavidus necator H16 is a promising chassis for sustainable biomanufacturing due to its ability to convert formate into value-added products including bioplastics, biofuels, and specialty chemicals. However, high-throughput functional genomics tools in this organism are still underdeveloped. Here, we describe the development of a genome-scale CRISPRi platform in wild-type C. necator H16 to support pooled fitness screens for identifying determinants of formate utilization.

To establish a scalable genetic engineering framework in C. necator H16, two native restriction systems were deleted and a multi-site Serine recombinase-Assisted Genome Engineering (SAGE) cassette containing orthogonal attB landing sites was integrated into the chromosome to support iterative, site-specific genomic integration of genetic cargo. A catalytically inactive form of Streptococcus pyogenes Cas9 (Sp-dCas9) was subsequently inserted at the phi370 attB site to enable inducible CRISPRi repression. In parallel, a 62,000-member, pooled oligonucleotide library targeting all annotated genes in C. necator H16 with multiple guide RNAs per gene was designed alongside a set of non-targeting control guides for downstream fitness analyses. Future work will benchmark CRISPRi repression efficiency using guide RNAs targeting a chromosomally integrated reporter gene, followed by pooled library integration into the engineered attB landing sites and competitive fitness screens in minimal medium containing formate, with fructose- and gluconate-based conditions serving as controls.

Together, this work establishes a foundational CRISPRi screening framework for C. necator H16 and provides the genetic infrastructure necessary for systematic interrogation of condition-dependent fitness landscapes in a non-model bacterium relevant to carbon-efficient biomanufacturing.

Co-authors: Pallavi Ramaswamy, Margaret Bales, Ilene Del Valle Kessra, Carrie Eckert, Adam Guss

75. *"Toward Predictive Design of Cell-Free Genetic Systems Using Hybrid Modeling"*
Ziqi Zheng (Georgia Institute of Technology)

Cell-free systems, composed of cell lysate, energy substrate buffer, and template DNA, provide a versatile platform for gene expression by bypassing cellular membrane constraints, enabling efficient energy allocation and simplifying reaction dynamics. These characteristics establish cell-free systems as robust prototyping tools for applications in various fields, including synthetic biology and biomanufacturing. However, because cell-free reactions rely on finite molecular resource pools, multiple genetic components can interact through shared transcriptional and translational machinery, degradation pathways, metabolic burden, and lysate-dependent effects. These forms of crosstalk can generate context-dependent behaviors that are difficult to capture using mechanistic models alone. Here, we present an early-stage hybrid modeling framework for interpreting and predicting crosstalk in cell-free transcription–translation (TXTL) systems. The framework combines a mechanistic ordinary differential equation backbone, representing known TXTL processes, with structured data-driven residual terms that capture systematic deviations between model predictions and observed dynamics. Rather than replacing mechanistic models with black-box predictors, this approach aims to preserve biological interpretability while identifying conditions where known mechanisms are insufficient. Using simulated and preliminary TXTL trajectory data, we evaluate whether hybrid models can improve prediction across genetic construct doses and circuit contexts while revealing residual patterns associated with potential missing mechanisms, such as limiting resource dominance, translational burden, or inhibitory byproduct accumulation. This framework provides a foundation for model-guided hypothesis generation and future perturbation experiments to improve the predictability of cell-free genetic system design.

76. *"Using high-throughput engineering methods to develop allosteric splicing ribozymes for broad-host range inducible control, genetic recordings, and biosensing"*
August Staubus (National Institute of Standards and Technology)

Biosensing through inducible gene expression has proven to be an invaluable biotechnology with a wide range of applications ranging from metabolic engineering to diagnostics. Ideally, a biosensing platform would be modular with respect to both input and output, would exhibit a high dynamic range, and would function robustly in a wide variety of chassis. RNA has been found to be a highly tractable platform for developing these kinds of biotechnologies. RNA devices have found great utility in this area because they have both information storage and catalytic activity, work effectively across diverse hosts, and impose low cellular burden. One of the most complex naturally occurring catalytic RNA molecules is the group I intron, a splicing ribozyme that carries out its own removal from an RNA strand, joining the flanking sequences into one continuous strand. We have leveraged high-throughput domain insertion engineering to develop modular ligand-activated splicing ribozymes (LASRs) that respond to a variety of molecular inputs ranging from nucleosides to metal ions. LASRs can regulate the translation of any protein coding sequence and function in diverse bacterial hosts. Moreover, by coupling LASRs with a genetic recorder, this tool can be used to report on the intracellular concentration of a target chemical within a microbial consortium with a sequence-based readout. Thus, LASRs deliver a versatile biosensing platform for the creation of new diagnostic tools, high-throughput screens or selections, and inducible genetic-control elements for biotechnology.

Co-authors: James Chappell, Ella Ramamurthy, Anika Gupta

77. *"Portable Glucose Monitor-Based, Field Deployable Sensing"*

Emily Heckard (Georgia Tech)

Point-of-care (POC) diagnostics offer the potential to transform healthcare by providing fast, affordable, and accessible testing outside traditional lab settings. Blood glucose monitors (BGMs) exemplify this technology, widely used for measuring glucose levels in individuals with diabetes. These devices are highly accurate and can provide real-time results. However, their use has the potential to extend beyond glucose detection to diagnose various health conditions. To enable this, we have developed a novel method combining BGMs with cell-free expression (CFE) systems. CFE systems are biological platforms that can detect specific substances or produce proteins without living cells. By integrating CFE with BGMs, we can measure a wide range of biomarkers, turning a simple device into a powerful diagnostic tool. While this method is affordable and sensitive, its complexity limits its practical use in resource-limited settings. My objective is to simplify this system, making it user-friendly and deployable in global health applications. I plan to automate the mixing, heating, and reagent delivery steps with a 3D printed device. These advancements will allow for easy, cost-effective diagnostic testing at the point of care, expanding access to vital health testing, particularly in underserved regions.

Co-authors: Emily Heckard, Yan Zhang, Tabitha Rosenbalm, Mike Farrell, and Mark Styczynski

78. *"Suppression of Spore-forming Contaminants Using a Novel, Bio-based and Selective Sterilant"*

Kelly Smith (Sable Fermentation, Inc.)

Contamination during fermentation often leads to culture failure and reduced productivity. A novel, bio-based selective sterilant was evaluated for its ability to control spore-forming contaminants during E. coli fermentation. The sterilant consistently inhibited M. luteus growth at all stages, and control of B. cereus was observed, with improved control when introduced post-exponential phase.

Co-authors: Srujana Koganti, Toni Bucci, Kelly Smith, and Peter Roettger

79. *"Modulating Redox to Enhance CO2 Fixation during E. coli Fermentation for Succinate Production"*

Isaiah Woodson (Arizona State University)

Succinate, a four-carbon (C4) dicarboxylic acid previously identified as a 'Top Value-Added Chemical from Biomass' by the U.S. Department of Energy, can be produced from biomass-derived sugars by metabolically engineered Escherichia coli during anaerobic fermentation primarily via the reductive branch of the TCA cycle (rTCA), a carbon-negative process. In the rTCA cycle, phosphoenolpyruvate (PEP) carboxykinase (Pck) serves as the key CO2-fixing step that converts oxaloacetate (OAA) using PEP, however, when consuming hexose or pentose sugars, engineered E. coli KJ122 must instead reroute a portion of PEP (~29%) through the oxidative branch of the TCA cycle to satisfy an inherent redox imbalance during succinate production. Overcoming an NADH deficit in this way, however, ultimately reduces the theoretical maximum succinate yield by 15% and, more significantly, the total CO2 fixation potential by 67%. Therefore, we have explored alternative strategies for flux control through rTCA through substrate-associated redox modulation. For example, in one case this can be achieved by heterologously expressing hydrogenase and supplying H2 as an electron donor. To this end, we first over-expressed two native hydrogenases in E. coli KJ122, namely Hyd-1 and Hyd-2, and supplied H2 gas in a hollow fiber membrane bioreactor; a strategy that, unfortunately, was ultimately unsuccessful towards improving succinate production performance. Alternatively, we have more recently been exploring the use of polyols (e.g., xylitol, sorbitol) as more reduced sugar substitutes. Specifically, we have engineered multiple E. coli strains to utilize xylitol by expressing a non-specific, NADH-generating sorbitol dehydrogenase (GutB) from Bacillus subtilis. Furthermore, we have explored the effects of additional regulatory associated with xylitol assimilation via the pentose phosphate pathway. To date, we have demonstrated an almost 5-fold increase in biomass growth and xylitol consumption, as well as comparable levels of succinate production relative to using xylose as a control. These results confirm not only the dependency of GutB for xylitol fermentation in E. coli KJ122 but also showcases that the unique redox balancing employed by E. coli KJ122 uniquely enable anaerobic xylitol fermentation. Future efforts will be made to compare performative results of hexose, pentose, and polyol sugar fermentations during scaled-up processes, and to improve xylitol catabolism in E. coli KJ122 through expression of alternative transporters and by engineering of relevant catabolic genes.

Co-authors: Mihyun Lee, Shane Stevens, David Nielsen

80. *"The rise of biotechnology beyond conventional containment (BBCC): A policy toolbox for community engagement in BBCC development and deployment"*

Thomas Adamo-Schmidt (California Institute of Technology)

In October 2025, the Caltech Linde Center for Science, Society, and Policy (LCSSP) gathered researchers, members of industry, and policy experts together for a workshop on biotechnology beyond conventional containment (BBCC).

Where conventional containment refers to controlled environments, a BBCC is any biotechnology that is deployed in an environment where containment and monitoring is not practical or possible. Because BBCCs operate in open systems, they create greater levels of uncertainty than other biotechnologies. This complexity raises the importance of effective policymaking for BBCCs and puts even more pressure on a regulatory system that has struggled to adapt to the fast-growing biotechnology industry. As BBCCs become an increasing reality, policymaking must embrace innovative approaches.

The LCSSP workshop concluded with three key policy needs for the BBCC field: re-evaluation of risk-management, a clearinghouse on stewardship, and an emphasis on community-driven development and deployment.

This poster focuses on the last of those three. BBCCs could benefit human health, agriculture, and wildlife restoration at a time when all three are under increasing threat due to anthropogenic environmental changes. However, successful use of BBCCs requires public endorsement. Biotechnology, and BBCCs in particular, faces apprehension from a public that has historically been overlooked during development and deployment.

Here, we propose an empirically-grounded toolbox for policymakers interested in a collaborative community governance strategy for BBCCs. It emphasizes community engagement and consent across a BBCC's lifespan, analyzed through the lens of various legal frameworks.

We advocate for a reframing of goals to account for environmental health beyond economic growth, adaptive decision-making with input from stakeholder fora, and greater coordination between local and overarching community and governing bodies.

Co-authors: Thomas Adamo-Schmidt, Alejandro Camacho, Callie Chappell, Madelyn Gelpi, Geoffrey Hunt, Riley Taitingfong

81. *"Engineering Low-Leak TLISA Systems Using Peptide Masking"*
Suvin Baek (Georgia Institute of Technology)

Cell-free system-based biosensors have been attracting attention in the diagnostic and biosensor fields due to their advantages, such as ease of storage at room temperature, rapid response speed, and simple prototyping. TLISA (T7 RNA polymerase–linked immunosensing assay) is a cell-free system-based platform can sense protein. TLISA uses split T7 RNA polymerase fragments fused to affinity domains against a target protein. Upon target antigen binding, the polymerase fragments reassemble and activate reporter expression. However, the spontaneous reassembly of split T7 RNA polymerase can generate background leak signals. These leak signals can degrade sensitivity and specificity of the sensor. In this study, we aim to suppress spontaneous reassembly and reduce leak signals by shielding the reassembly interface of T7 RNA polymerase using a peptide masking strategy. We anticipate that this strategy will improve the sensor's dynamic range, enhance the sensitivity of low-concentration biomarker detection, and reduce false positives. Furthermore, this approach is expected to contribute to the future development of programmable cell-free protein sensing platforms and the advancement of point-of-care diagnostic technologies.

82. *"A Conserved Gut Microbial Gene Cluster Mediates Strain-Specific Allergen Degradation "*
Arren Liu (CAMRI, UMB)

Early-life gut microbiome composition and functional capacity are strongly associated with food allergy development, yet the molecular mechanisms underlying microbiome-mediated allergic sensitization remain poorly understood and largely lack experimental validation. We previously showed that failure of peanut protein allergy treatment is associated increased gut microbial peanut protein (PP) metabolism capacity. To identify gut microbial species responsible for PP degradation, we screened 300 isolates from fecal samples of peanut allergic children and identified 20 isolates capable of degrading PPs (ara h 2), the majority of which belonged to a single microbial species. Using whole genome sequencing and comparative genomics, we identified a gene cluster (FA cluster) only shared between PP degraders, but absent in non-degraders, suggesting that gut microbiome's ability to degrade PPs is strain specific. Notably, the FA cluster confers the ability to degrade not only PPs (ara h 2) but also milk (Bos d 11) and egg (Gal d 1) allergens, suggesting a shared microbial food allergen utilization mechanism. To functionally characterize the role of FA cluster in immune sensitization and FA development, we are using targeted mutagenesis to delete FA cluster in an PP-degrading gut microbial isolate. Overall, this work demonstrates that the early-life microbiome can degrade food allergens in a strain-specific manner, potentially influencing the development of food allergies.

Co-authors: Arren J. Liu, Mustafa Özçam

83. *"Mechanistic ODE Modeling of TLISA to Guide Cell-Free Diagnostic Design"*
Rridhisha Kumar (Georgia Institute of Technology)

Cell-Free Biosensors (CFBs) are a significant technology for point-of-care diagnostics, particularly in low-resource areas, however, rational optimization of these systems remains a challenge due to the complexity of coupled biochemical reactions. The T7 RNA Polymerase-Linked Immunosensing Assay (TLISA) is a modular platform that detects protein biomarkers by coupling nanobody-antigen binding to the reassembly of a split T7 RNA polymerase, followed by transcription of a colorimetric reporter. Despite the recent advances in TLISA sensitivity and dynamic range, the system is poorly generalizable across nanobody-antigen pairs and requires multiple rounds of experimental trial and error.

We present an ordinary differential equation (ODE) model of the TLISA reaction cascade to predict diagnostic signal and guide rational design. The model captures key biochemical steps including nanobody-antigen binding, concentration-dependent polymerase reassembly, promoter binding, reporter transcription, and colorimetric output via the LacZ enzyme. Using published dissociation constants and time-course experimental data, we aim to fit model parameters and validate model behavior against empirically observed dose-response curves. We anticipate that the model will capture the hook effect observed at intermediate polymerase fragment concentrations, providing mechanistic insight into this nonlinear phenomenon. Ultimately, sensitivity analysis will be used to identify rate-limiting steps governing the dynamic range, and nanobody-antigen dissociation constants will be varied in silico to predict optimal polymerase fragment and reporter DNA concentrations for a given binder. This work aims to establish a mechanistic framework for TLISA optimization and provide a generalizable approach for ODE-based design of cell-free protein diagnostics.

84. *"Protein nanoparticle-encapsulated cell-free systems for mucosal diagnostics"*
Cassandra Parada (Georgia Institute of Technology)

Cell-free (CF) expression systems are composed of the transcriptional and translational machinery of cells and can be engineered to detect analytes of interest and produce a colorimetric signal in response, making them an emerging technology for point-of-care (POC) diagnostics. Despite these advantages, the application of CF diagnostics to mucus samples is limited due to the degradative enzymes present in mucus, such as proteases and RNases. These enzymes disrupt CF reactions, compromising detection sensitivity and signal output. Although using enzymatic inhibitors can help prevent degradation, they are often expensive and require refrigeration, thereby limiting shelf-life, portability, and accessibility of these biosensors. To address this limitation, we aim to encapsulate CF biosensors withing Elastin-like polypeptide (ELP) protein nanoparticles to protect sensitive reaction components from enzymatic degradation. By encapsulating the CF system, we will create a physical barrier that limits the diffusion of large degradative enzymes while still permitting smaller analytes to diffuse into the reaction environment and be detected. Additionally, we hope to be able to control the permeability properties of the ELP nanoparticles by changing the guest residue in the ELP sequence. This strategy could help expand the use of cell-free diagnostics to mucosal samples while enabling rapid, low-cost, and field-deployable testing in resource-limited settings.

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