

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
1. REPORT DATE (DD-MM-YYYY) 05-11-2021		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 18-Nov-2020 - 17-Mar-2021	
4. TITLE AND SUBTITLE Final Report: Synthetic Biology Approaches to Improve Human and Environmental Health			5a. CONTRACT NUMBER W911NF-21-1-0010		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER 611102		
6. AUTHORS			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES New York Academy of Sciences 2 East 63rd Street New York, NY 10021 -7210			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) 77595-LS-CF.1		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Sonya Dougal
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 212-298-8630

RPPR Final Report
as of 08-Dec-2021

Agency Code: 21XD

Proposal Number: 77595LSCF

Agreement Number: W911NF-21-1-0010

INVESTIGATOR(S):

Name: Alexis Komor
Email: akomor@ucsd.edu
Phone Number: 8582462608
Principal: N

Name: Irene Chen
Email: chen@chem.ucsb.edu
Phone Number: 8058938364
Principal: N

Name: Farren Isaacs
Email: farren.isaacs@yale.edu
Phone Number: 2034323783
Principal: N

Name: Jennifer Costley
Email: jcostley@nyas.org
Phone Number: 2122988675
Principal: N

Name: Neal Devaraj
Email: ndevaraj@ucsd.edu
Phone Number: 8585349539
Principal: N

Name: Neha P. Kamat
Email: nkamat@northwestern.edu
Phone Number: 8474671213
Principal: N

Name: Reshma Shetty
Email: reshma@ginkgobioworks.com
Phone Number: 8774225362
Principal: N

Name: Sara Donnelly
Email: sdonnelly@nyas.org
Phone Number: 2122988642
Principal: N

Name: Sonya Dougal
Email: sdougal@nyas.org
Phone Number: 2122988630
Principal: Y

Organization: **New York Academy of Sciences**

Address: 2 East 63rd Street, New York, NY 100217210

Country: USA

DUNS Number: 075232751

EIN: 131773640

Report Date: 17-Jun-2021

Date Received: 05-Nov-2021

Final Report for Period Beginning 18-Nov-2020 and Ending 17-Mar-2021

Title: Synthetic Biology Approaches to Improve Human and Environmental Health

Begin Performance Period: 18-Nov-2020

End Performance Period: 17-Mar-2021

RPPR Final Report

as of 08-Dec-2021

Report Term: 0-Other

Submitted By: Megan Prescott

Email: mprescott@nyas.org

Phone: (121) 229-886008628

Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 0

STEM Participants: 42

Major Goals: The goals of this two-day conference are to strongly emphasize foundational cellular and genetic tools that are applicable to a broad range of scientific questions and uses. There will be significant discussion of artificial cells including top-down and bottom-up approaches for their design and generation. Moreover, the core principles needed to engineer biological pathways and novel signaling circuits to perform specific functions are a key focus of this meeting. This symposium will foster knowledge sharing and collaboration between scientists from diverse fields including genetics, chemical biology, synthetic biology and environmental science to advance our understanding of the evolution of biological regulatory mechanisms, which is central to our ability to both comprehend our world and to rationally design functional biological assemblies with desirable properties. These topics will be covered in the context of a broad range of applications including the sustainable production of therapeutics, novel materials, food products and biofuels, all of which promise to positively affect the health and performance of soldiers, and the broader population.

The projected outcomes of this event are:

- (1) To review the latest advances in the development of synthetic cells and their utility in interrogating fundamental biological processes of significance to human health and the environment.
- (2) To describe novel methods to design and program synthetic biological signaling circuits for production of complex chemicals of critical importance in medicine, and sustainable food, fuel and materials production.
- (3) To assess synthetic biology approaches in diagnostics, disease control, and environmental toxicology including discussion of gene drives, and whole cell and cell-free biosensors.
- (4) To highlight key ethical issues in synthetic biology via an interactive panel discussion.

Accomplishments: On November 18-19th, 2020, the New York Academy of Sciences (NYAS) presented a one day symposium titled "Synthetic Biology Approaches to Improve Human and Environmental Health" (www.nyas.org/SynBio2020). We are pleased to report that the multi-disciplinary, virtual conference was a great success. This meeting had a total of 112 registrants, who assembled to discuss innovative research in the field of synthetic biology, with the aim of highlighting the latest developments in the tools and technologies central to this area, and their broad applicability to medicine, environmental toxicology and the future of sustainable living. To this end, a diverse group of attendees and speakers contributed unique ideas and perspectives with the goal of forging new connections between academic and industry scientists working in this arena. A mobile meeting app, which includes networking capabilities, also facilitated the development of new collaborations between participants. In addition to the 18 invited experts who presented innovative findings, the agenda also included three short talks, competitively selected from submitted abstracts. A virtual poster session gave a platform to young researchers in the field to share their work. Two interactive panels, "Ethical Issues in Synthetic Biology" and "Current and Future Challenges in Synthetic Biology", facilitated discussion and spotlighted important areas of broad interest to the field.

To assess the Academy's success in delivering a conference program that met the overall objectives and goals of the meeting, conference participants were asked to complete an Attendee Evaluation Questionnaire following the conclusion of the event. Highlights of the report include:

90.91% of survey respondents indicated that the Conference met their personal learning goals.

78.57% rated the symposium as above average in the landscape

On average, respondents scored their satisfaction with the conference as 4.36/5

A summary of the results of the Attendee Evaluation Questionnaire is attached in the "uploads" section of this report.

RPPR Final Report
as of 08-Dec-2021

Training Opportunities: A poster session and three short talks allowed emerging researchers to showcase their work.

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Co-Investigator

Participant: Sara Donnelly

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: PD/PI

Participant: Sonya Dougal

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Co-Investigator

Participant: Neal Devaraj

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Co-Investigator

Participant: Irene Chen

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Co-Investigator

Participant: Neha P. Kamat

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Co-Investigator

Participant: Alexis Komor

Person Months Worked: 4.00

Project Contribution:

Funding Support:

RPPR Final Report
as of 08-Dec-2021

National Academy Member: N

Participant Type: Co-Investigator

Participant: Reshma Shetty

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Co-Investigator

Participant: Farren Isaacs

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Co-Investigator

Participant: Jennifer Costley

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Other Professional

Participant: Angela Belcher

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Other Professional

Participant: Ethan Bier

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Other Professional

Participant: Jef Boeke

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Other Professional

Participant: Ethan Bier

Person Months Worked: 4.00

Project Contribution:

National Academy Member: N

Funding Support:

RPPR Final Report
as of 08-Dec-2021

Participant Type: Other Professional
Participant: James Carothers
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: James Collins
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Neal Devaraj
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Farren Isaacs
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Gregory Kaebnick
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Wendell Lim
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Julius B. Lucks
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

RPPR Final Report
as of 08-Dec-2021

Participant Type: Other Professional
Participant: Stephen Mayfield
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Michelle O'Malley
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Petra Schwillie
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Smita Shankar
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Reshma Shetty
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Pamela Silver
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Christina Smolke
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Emma Frow

RPPR Final Report
as of 08-Dec-2021

Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Aditya Kunjapur
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

Participant Type: Other Professional
Participant: Javier Santos-Moreno
Person Months Worked: 4.00
Project Contribution:
National Academy Member: N

Funding Support:

RPPR Final Report
as of 08-Dec-2021

Partners

American Institute for Medical and Biological Engineering
Washington, DC USA

0
Promotional Partner

Biomedical Engineering Society
Landover, MD USA

0
Promotional Partner

Checkerspot
Alameda, CA USA

0
Promotional Partner

IEEE Engineering in Medicine & Biology Society
Piscataway, NJ USA

0
Promotional Partner

Synthetic Biology Gordon Research Conference
Waterville Valley, NH USA

0
Promotional Partner

Sherlock Biosciences
Boston, MA USA

1
\$5,000

Novartis
Hancock, NJ USA

1
\$5,000

I certify that the information in the report is complete and accurate:

Signature: Megan Prescott

Signature Date: 11/5/21 4:50PM

Agenda

Wednesday

November 18, 2020

10:30 AM

Introduction and Welcome Remarks

10:40 AM

Keynote Presentation: Harnessing Synthetic Biology to Fight Pathogens

Speaker

James Collins, PhD

Massachusetts Institute of Technology

Session 1: Minimal Systems to Probe Biological Behavior

11:10 AM

Karyotype Engineering and Other Ways to Torment Genomes

Speaker

Jef Boeke, PhD

NYU Langone School of Medicine

- Abstract

Rapid advances in DNA synthesis techniques have made it possible to engineer diverse genomic elements, pathways, and whole genomes, providing new insights into design and analysis of systems. The synthetic yeast genome project, Sc2.0 is well on its way with the 16 synthetic *Saccharomyces cerevisiae* chromosomes now >99% completed by a global team. The synthetic genome features several systemic modifications, including TAG/TAA stop-codon swaps, deletion of subtelomeric regions, introns, tRNA genes, transposons and silent mating loci. Strategically placed loxP sites enable genome restructuring using an inducible evolution system termed SCRaMbLE which can generate millions of derived variant genomes with predictable structures leading to complex genotypes and phenotypes. The fully synthetic yeast genome provides a new kind of combinatorial genetics based on variations in gene content and copy number. Remarkably, the 3D structures of synthetic and native chromosomes are very similar despite the substantial number of changes introduced.

We recently completely engineered the yeast karyotype, by systematically fusing pairs of telomeres and deleting single centromeres, thus generating an isogenic series of yeast ranging from $n=16$ to $n=2$. These strains show reproductive isolation and a massively altered 3D genome structure, but are surprisingly “normal” and show high fitness. We have also developed a method that allows us to move megabase segments to distant locations in the genome in a single step, again with surprisingly little impact on fitness.

Finally, we have automated our big DNA synthesis pipeline (the GenomeFoundry@ISG), opening the door to parallelized big DNA assembly, including assembly of human genomic regions of 100 kb along with multiple designer synthetic variants thereof. We can precision deliver such segments to stem and cancer cells, and use these methods to dissect genomic “dark matter”, perform transplants of specific human genomic regions to animal genomes, and endow human cells with new capabilities.

11:40 AM

Designing Minimal Cell Division

Speaker

Petra Schwille, PhD

Max-Planck Institute of Biochemistry

- Abstract

One of the hallmarks of cell theory is the recognition that any life form, consisting of one or more living cells, results from another living cell. Although this insight was a huge step towards making biology a scientific discipline, and freeing the concept of life from metaphysics and speculation, it left us with a fundamental riddle – how did the first cell, how did life as such originate? Using bottom-up synthetic biology of simplistic reconstituted systems, we attempt to identify the fundamental principles of how chemical systems acquire, one by one, the particular features and functions of living systems. Our particular emphasis is on the origin of cell division, and how we could build a minimal system that is able to divide autonomously.

12:10 PM

Break

12:20 PM

Engineering Synthetic Genetic Elements for Mobilization and Expression in Diverse Organisms

Speaker

Farren Isaacs, PhD

Yale University

Session 2: Data Blitz Talks

12:50 PM

Synthetic Auxotrophy Remains Stable After Continuous Evolution and in Co-culture with Mammalian Cells

Speaker

Aditya M. Kunjapur, PhD

University of Delaware

- Abstract

Understanding the evolutionary stability and possible context-dependence of biological containment techniques is critical as engineered microbes are increasingly under consideration for applications beyond biomanufacturing. While batch cultures of synthetic auxotrophic *Escherichia coli* previously exhibited undetectable escape throughout 14 days of monitoring, the long-term effectiveness of synthetic auxotrophy is unknown. Here, we report automated continuous evolution of a synthetic auxotroph using custom chemostats that supply a decreasing concentration of essential biphenylalanine (BipA). After 100 days of evolution in three separate trials, populations exhibit no observable escape and are capable of normal growth rates at 10-fold lower BipA concentration than the ancestral synthetic auxotroph. Allelic reconstruction of three proteins implicated in small molecule transport reveals their contribution to increased fitness at low BipA concentrations. Mutations do not appear in orthogonal translation machinery nor in synthetic auxotrophic markers. Based on its evolutionary stability, we introduce the progenitor synthetic auxotroph directly to mammalian cell culture. We observe containment of bacteria without detrimental effects on HEK293T cells. Overall, our findings reveal that synthetic auxotrophy is effective on timescales and in contexts that enable diverse applications.

1:05 PM

Multistable and Dynamic CRISPRi-based Synthetic Circuits

Speaker

Javier Santos-Moreno, PhD

University of Lausanne

- Abstract

One of the greatest challenges of synthetic biology is how to accurately control living organisms so that they perform desired (novel) tasks. Synthetic gene circuits provide such control knobs, and constitute invaluable tools for the realization of the next generation of biological devices that will tackle health and environmental challenges of the 21st century. However, most synthetic circuits so far were based on transcription factors, which suffer from inherent constraints that limit their applicability, such as insufficient modularity, orthogonality and programmability. Alternatively, CRISPRi has emerged as a powerful tool for creating synthetic circuits, both in prokaryotes and eukaryotes; yet, its lack of cooperativity has been pointed out as a potential obstacle for dynamic or multistable synthetic circuit construction. Here we use CRISPRi to build a synthetic oscillator ("CRISPRi-oscillator"), bistable network (toggle switch) and stripe pattern-forming incoherent feed-forward loop. Our circuit designs, conceived to feature high predictability and orthogonality, as well as low metabolic burden and context-dependency, allow us to achieve robust behaviors in *E. coli* populations. Mathematical modeling suggests that unspecific binding in CRISPRi is essential to establish multistability. Our work demonstrates the wide applicability of CRISPRi in synthetic circuits and paves the way for engineering more complex synthetic networks, boosted by the advantages of CRISPR technology.

1:20 PM

Break and Virtual Poster Session

Session 3: Panel Discussion

Session Chairperson

Gregory Kaebnick, PhD, The Hastings Center

2:05 PM

Ethical Questions in Synthetic Biology

Speakers

Ethan Bier, PhD

University of California, San Diego

Emma Frow, PhD

Arizona State University

Pamela Silver, PhD

Harvard Medical School

Session 4: Foundational Technologies for Cellular Engineering

2:50 PM

Engineering Multi-Gene CRISPRa/i Programs for Bacteria and Synthetic Cell Systems

Speaker

James Carothers, PhD

University of Washington

3:20 PM

Designing Biology

Speaker

Reshma Shetty, PhD

Ginkgo Bioworks

3:50 PM

Break

4:00 PM

Lipid Sponge Droplets as Programmable Synthetic Organelles

Speaker

Neal Devaraj, PhD

University of California, San Diego

4:30 PM

Keynote - New Horizons in Synthetic Biology

Speaker

Pamela Silver, PhD

Harvard Medical School

5:00 PM

Day 1 Closing Remarks and Adjourn

Thursday

November 19, 2020

10:30 AM

Welcome Back Remarks

10:40 AM

Keynote - Learning to Program Cellular Machines: Harnessing Cells to Treat Disease, Build Tissues, and Elucidate Design Principles

Speaker

Wendell Lim, PhD

University of California, San Francisco

Session 5: Synthetic Biology in Human Health and Disease

11:10 AM

Active Genetics Comes Alive

Speaker

Ethan Bier, PhD

University of California, San Diego

- Abstract

Active genetic elements are transmitted during reproduction at greater than expected Mendelian frequencies. Such "super-Mendelian" inheritance can be used for a variety of applications including: gene-drive systems for disseminating beneficial traits throughout populations (e.g., spreading immunizing factors that prevent mosquitoes from transmitting malarial parasites), reversing insecticide resistance, devising elements that can eliminate or inactivate gene drives, creating active genetic modifications that facilitate breeding by bypassing constraints imposed by independent assortment and linkage, and development of self-amplifying systems in bacteria to scrub antibiotic resistance factors from the environment or potentially from patients with chronic bacterial infections, and engineering next-generation genetic circuits for synthetic biology.

11:40 AM

Break

11:50 AM

Biological Systems to Synthesize Complex Therapeutics

Speaker

Christina Smolke, PhD

Stanford University

12:20 PM

Harnessing Gut Microbes to Turn Waste into Energy

Speaker

Michelle O'Malley, PhD

University of California, Santa Barbara

[+ Abstract](#)

12:50 PM

Multiplexed Biosensors for Enhanced Bacteria Tropism in vivo

Speaker

Tiffany Chien

Columbia University

[- Abstract](#)

Engineering microbes spurs biotechnological innovations across disciplines ranging from environmental monitoring to medical therapeutics. However, translating these technologies requires stringent control mechanisms to confine bacterial growth within defined regions of interest. Here, we engineer enhanced bacterial tropism with genetic circuits that couple bacterial sensing and growth in response to physiological signatures in vivo. Specifically, we construct oxygen, pH, and lactate biosensors with tunable features for activation at distinct physiological concentrations. We use these biosensors to control the expression of essential genes, which results in shifts in bacterial growth distributions towards upper or lower regions of the gastrointestinal tract in vivo, following physiological gradients of oxygen and pH. Additionally, in a syngeneic mouse tumor model, multiplexing hypoxia and lactate biosensors with an AND logic-gate architecture reduces bacterial off-target colonization by 100-fold. Together, these results demonstrate a synthetic biology approach to enable precision localization of bacteria to specified organ niches

1:05 PM

Break and Virtual Poster Session

Session 6: Panel Discussion

Session Chairperson

Neal Devaraj, PhD, University of California, San Diego

2:05 PM

Current and Future Challenges in Synthetic Biology

Speakers

Angela Belcher, PhD

Massachusetts Institute of Technology

James Collins, PhD

Massachusetts Institute of Technology

Christina Smolke, PhD

Stanford University

Session 7: Driving Innovation with Synthetic Biology

2:50 PM

Engineering *Pichia pastoris* to Make the Impossible Burger Possible

Speaker

Smita Shankar, PhD

Impossible Foods

- Abstract

Using animals as a cheap way to make meat has brought us to the brink of environmental collapse. At Impossible Foods, we're trying to reduce the impact of animal farming on the planet by creating a delicious burger made entirely out of plant materials. A key driver of flavor in beef is heme, a molecule found in animal muscle in the form of the hemoprotein myoglobin. We have engineered the methylotrophic yeast *Pichia pastoris* to produce a similar plant hemoprotein called Leghemoglobin, which is found naturally in soybean root nodules. Leghemoglobin is the "magic meatless ingredient" in Impossible beef.

In the last few decades, *Pichia pastoris* has been used for production of several heterologous proteins of industrial relevance. In my talk, I will share a few examples of novel, rational strain engineering approaches that have led to improved expression of Leghemoglobin. I will highlight how an integrated approach to strain development, fermentation development, analytics and downstream processing enabled successful production of Leghemoglobin at commercial scale, leading to product commercialization and market expansion.

3:20 PM

What is in Your Water? Giving Individuals the Power to Monitor their Environments with Cell-Free Synthetic Biology.

Speaker

Julius B. Lucks, PhD

Northwestern University

- Abstract

Poor water quality affects over two billion people across the globe. While we can't often see or taste water contaminants, it turns out bacteria can. Here I will present our latest research on developing a 'pregnancy test for water' - a cheap, fast and reliable approach that allows anyone, anywhere to detect if their water is contaminated. Our approach builds off of advances in cell-free synthetic biology—extracting the machinery of natural organisms to perform their functions but in test tube reactions instead of living cells. Using the principles of synthetic biology, we can 'rewire' natural biosensors to produce visible signals when specific contaminants are present in a water sample. The addition of synthetic genetic circuits optimizes these reactions to detect contaminants with high degrees of sensitivity and specificity. Finally, the ability to freeze dry these reactions allows them to be easily stored and transported without cold chain, which has enabled us to demonstrate their utility in the field. This work is creating a new approach to water quality monitoring that promises to dramatically increase the scale at which we can monitor the health of ourselves and our environment.

3:50 PM

Break

4:00 PM

Engineering Algae for Sustainable Materials and Food Production

Speaker

Stephen Mayfield, PhD

University of California, San Diego

4:30 PM

Keynote- Talk title to be confirmed

Speaker

Angela Belcher, PhD

Massachusetts Institute of Technology

5:00 PM

Closing Remarks

5:05 PM

Adjourn

Speaker Abstracts

Active Genetics Come Alive

Ethan Bier, University of California, San Diego

Active genetic elements are transmitted during reproduction at greater than expected Mendelian frequencies. Such "super-Mendelian" inheritance can be used for a variety of applications including: gene-drive systems for disseminating beneficial traits throughout populations (e.g., spreading

immunizing factors that prevent mosquitoes from transmitting malarial parasites), reversing insecticide resistance, devising elements that can eliminate or inactivate gene drives, creating active genetic modifications that facilitate breeding by bypassing constraints imposed by independent assortment and linkage, and development of self-amplifying systems in bacteria to scrub antibiotic resistance factors from the environment or potentially from patients with chronic bacterial infections, and engineering next-generation genetic circuits for synthetic biology

Karyotype engineering and other ways to torment genomes

Jef D. Boeke and Laboratory, Institute for Systems Genetics, NYU Langone Health

Rapid advances in DNA synthesis techniques have made it possible to engineer diverse genomic elements, pathways, and whole genomes, providing new insights into design and analysis of systems. The synthetic yeast genome project, Sc2.0 is well on its way with the 16 synthetic *Saccharomyces cerevisiae* chromosomes now >99% completed by a global team. The synthetic genome features several systemic modifications, including TAG/TAA stop-codon swaps, deletion of subtelomeric regions, introns, tRNA genes, transposons and silent mating loci. Strategically placed loxP sites enable genome restructuring using an inducible evolution system termed SCRaMBLE which can generate millions of derived variant genomes with predictable structures leading to complex genotypes and phenotypes. The fully synthetic yeast genome provides a new kind of combinatorial genetics based on variations in gene content and copy number. Remarkably, the 3D structures of synthetic and native chromosomes are very similar despite the substantial number of changes introduced. We recently completely engineered the yeast karyotype, by systematically fusing pairs of telomeres and deleting single centromeres, thus generating an isogenic series of yeast ranging from $n=16$ to $n=2$. These strains show reproductive isolation and a massively altered 3D genome structure, but are surprisingly “normal” and show high fitness. We have also developed a method that allows us to move megabase segments to distant locations in the genome in a single step, again with surprisingly little impact on fitness. Finally, we have automated our big DNA synthesis pipeline (the GenomeFoundry@ISG), opening the door to parallelized big DNA assembly, including assembly of human genomic regions of 100 kb along with multiple designer synthetic variants thereof. We can precision deliver such segments to stem and cancer cells, and use these methods to dissect genomic “dark matter”, perform transplants of specific human genomic regions to animal genomes, and endow human cells with new capabilities.

Membrane-Based Synthetic Cell Systems for Plant Natural Product Biosynthesis

James M. Carothers, PhD, University of Washington

Synthetic cell systems (SCSs) engineered through bottom-up construction offer great promise as platforms for advanced applications in health and the environment. We are creating SCSs as platforms for consistent, high-yield plant natural product drug biosynthesis. One of the central challenges is that many of the plant enzymes catalyzing key steps in these plant-derived drug biosynthetic pathways (e.g. P450s and dioxygenases) are transmembrane proteins that are notoriously difficult to express. Our approach is to engineer membrane-based, cell-free transcription-translation (TXTL) systems with phospholipid 'flavors' tailored to emulate plant cell environments. We are currently focused on developing genetic control systems that permit the implementation of multi-gene pathways and the regulated self-assembly of membrane-based SCSs from phospholipid components produced by the TXTL system itself. In my talk, I will present results and outline a vision for the scalable production of medically-important, plant-derived drugs using self-assembled, membrane-based SCSs.

What is in your water? Giving individuals the power to monitor their environments with cell-free synthetic biology.

Julius B. Lucks, Center for Synthetic Biology, Northwestern University

Poor water quality affects over two billion people across the globe. While we can't often see or taste water contaminants, it turns out bacteria can. Here I will present our latest research on developing a 'pregnancy test for water' - a cheap, fast and reliable approach that allows anyone, anywhere to detect if their water is contaminated. Our approach builds off of advances in cell-free synthetic biology – extracting the machinery of natural organisms to perform their functions but in test tube reactions instead of living cells. Using the principles of synthetic biology, we can 'rewire' natural biosensors to produce visible signals when specific contaminants are present in a water sample. The addition of synthetic genetic circuits optimizes these reactions to detect contaminants with high degrees of sensitivity and specificity. Finally, the ability to freeze dry these reactions allows them to be easily stored and transported without cold chain, which has enabled us to demonstrate their utility in the field. This work is creating a new approach to water quality monitoring that promises to dramatically increase the scale at which we can monitor the health of ourselves and our environment.

Engineering Synthetic Microbial Consortia Inspired by the Herbivore Rumen

Michelle A. O'Malley, University of California, Santa Barbara

Anaerobic microbes work together in complex communities that decompose and recycle carbon biomass throughout the Earth – from our guts to landfills and compost piles. Despite their importance, little information exists to parse the role of each microbial member within their dynamic community. To address these knowledge gaps, we pioneered new techniques to isolate anaerobes from biomass-rich environments (e.g. guts and fecal materials of herbivores), characterize their shared metabolism, and build synthetic microbiomes to drive biomass to renewable chemicals. Herbivore fecal samples were challenged by different types of biomass during cultivation to identify important microbial partnerships; 10 billion metagenomic reads spread across 402 enrichment samples tracked biological diversity as the cultures converged to a set of stable microorganisms. Nearly 200,000 carbohydrate active enzymes were identified from the fecal samples, and 724 genomes were assembled for previously uncultured microbes. Surprisingly, consortia dominated by anaerobic fungi generated more than twice the amount of methane compared to prokaryotic consortia, suggesting that fungi accelerate biomass breakdown and methane release in herbivores. Overall, our analysis points to natural compartmentalization between anaerobes as a means to degrade crude biomass, which can be exploited to harness nature's microbes for sustainable chemical production using synthetic systems.

Designing Minimal Cell Division

Petra Schwille, Max Planck Institute of Biochemistry

One of the hallmarks of cell theory is the recognition that any life form, consisting of one or more living cells, results from another living cell. Although this insight was a huge step towards making biology a scientific discipline, and freeing the concept of life from metaphysics and speculation, it left us with a fundamental riddle – how did the first cell, how did life as such originate? Using bottom-up synthetic biology of simplistic reconstituted systems, we attempt to identify the fundamental principles of how

chemical systems acquire, one by one, the particular features and functions of living systems. Our particular emphasis is on the origin of cell division, and how we could build a minimal system that is able to divide autonomously

Engineering Pichia pastoris to make the Impossible burger possible

Smita Shankar Impossible Foods Inc. Redwood City CA

Using animals as a cheap way to make meat has brought us to the brink of environmental collapse. At Impossible foods, we're trying to reduce the impact of animal farming on the planet by creating a delicious burger made entirely out of plant materials. A key driver of flavor in beef is heme, a molecule found in animal muscle in the form of the hemoprotein myoglobin. We have engineered the methylotrophic yeast *Pichia pastoris* to produce a similar plant hemoprotein called Leghemoglobin, which is found naturally in soybean root nodules. Leghemoglobin is the "magic meatless ingredient" in Impossible beef. In the last few decades, *Pichia pastoris* has been used for production of several heterologous proteins of industrial relevance. In my talk, I will share a few examples of novel, rational strain engineering approaches that have led to improved expression of Leghemoglobin. I will highlight how an integrated approach to strain development, fermentation development, analytics and downstream processing enabled successful production of Leghemoglobin at commercial scale, leading to product commercialization and market expansion.

Short Talk Abstracts

Synthetic Auxotrophy Remains Stable After Continuous Evolution and in Co-culture with Mammalian Cells

Aditya M. Kunjapur, University of Delaware

Understanding the evolutionary stability and possible context-dependence of biological containment techniques is critical as engineered microbes are increasingly under consideration for applications beyond biomanufacturing. While batch cultures of synthetic auxotrophic *Escherichia coli* previously exhibited undetectable escape throughout 14 days of monitoring, the long-term effectiveness of synthetic auxotrophy is unknown. Here, we report automated continuous evolution of a synthetic auxotroph using custom chemostats that supply a decreasing concentration of essential biphenylalanine (BipA). After 100 days of evolution in three separate trials, populations exhibit no observable escape and are capable of normal growth rates at 10-fold lower BipA concentration than the ancestral synthetic auxotroph. Allelic reconstruction of three proteins implicated in small molecule transport reveals their contribution to increased fitness at low BipA concentrations. Mutations do not appear in orthogonal translation machinery nor in synthetic auxotrophic markers. Based on its evolutionary stability, we introduce the progenitor synthetic auxotroph directly to mammalian cell culture. We observe containment of bacteria without detrimental effects on HEK293T cells. Overall, our findings reveal that synthetic auxotrophy is effective on timescales and in contexts that enable diverse applications.

Multistable and Dynamic CRISPRi-based Synthetic Circuits

Javier Santos-Moreno, University of Lausanne

One of the greatest challenges of synthetic biology is how to accurately control living organisms so that they perform desired (novel) tasks. Synthetic gene circuits provide such control knobs, and constitute invaluable tools for the realization of the next generation of biological devices that will tackle health and environmental challenges of the 21st century. However, most synthetic circuits so far were based on transcription factors, which suffer from inherent constraints that limit their applicability, such as insufficient modularity, orthogonality and programmability. Alternatively, CRISPRi has emerged as a

powerful tool for creating synthetic circuits, both in prokaryotes and eukaryotes; yet, its lack of cooperativity has been pointed out as a potential obstacle for dynamic or multistable synthetic circuit construction. Here we use CRISPRi to build a synthetic oscillator (“CRISPRlator”), bistable network (toggle switch) and stripe pattern-forming incoherent feed-forward loop. Our circuit designs, conceived to feature high predictability and orthogonality, as well as low metabolic burden and context-dependency, allow us to achieve robust behaviors in *E. coli* populations. Mathematical modeling suggests that unspecific binding in CRISPRi is essential to establish multistability. Our work demonstrates the wide applicability of CRISPRi in synthetic circuits and paves the way for engineering more complex synthetic networks, boosted by the advantages of CRISPR technology.

Multiplexed Biosensors for Enhanced Bacteria Tropism in vivo

Tiffany Chien, Columbia University

Engineering microbes spurs biotechnological innovations across disciplines ranging from environmental monitoring to medical therapeutics. However, translating these technologies requires stringent control mechanisms to confine bacterial growth within defined regions of interest. Here, we engineer enhanced bacterial tropism with genetic circuits that couple bacterial sensing and growth in response to physiological signatures in vivo. Specifically, we construct oxygen, pH, and lactate biosensors with tunable features for activation at distinct physiological concentrations. We use these biosensors to control the expression of essential genes, which results in shifts in bacterial growth distributions towards upper or lower regions of the gastrointestinal tract in vivo, following physiological gradients of oxygen and pH. Additionally, in a syngeneic mouse tumor model, multiplexing hypoxia and lactate biosensors with an AND logic-gate architecture reduces bacterial off-target colonization by 100-fold. Together, these results demonstrate a synthetic biology approach to enable precision localization of bacteria to specified organ niches

Synthetic Biology Approaches to Improve Human and Environmental Health November 18-19, 2020: 10:30 AM – 5.00 PM

On November 18-19th, 2020, the New York Academy of Sciences (NYAS) presented a one day symposium titled “**Synthetic Biology Approaches to Improve Human and Environmental Health**” (www.nyas.org/SynBio2020). We are pleased to report that the multi-disciplinary, virtual conference was a great success. This meeting had a total of **112 registrants**, who assembled to discuss innovative research in the field of synthetic biology, with the aim of highlighting the latest developments in the tools and technologies central to this area, and their broad applicability to medicine, environmental toxicology and the future of sustainable living. To this end, a diverse group of attendees and speakers contributed unique ideas and perspectives with the goal of forging new connections between academic and industry scientists working in this arena. A mobile meeting app, which includes networking capabilities, also facilitated the development of new collaborations between participants. In addition to the **18 invited experts** who presented innovative findings, the agenda also included **three short talks**, competitively selected from submitted abstracts. A **virtual poster** session gave a platform to young researchers in the field to share their work. **Two interactive panels**, “Ethical Issues in Synthetic Biology” and Current and Future Challenges in Synthetic Biology”, facilitated discussion and spotlighted important areas of broad interest to the field.

To assess the Academy’s success in delivering a conference program that met the overall objectives and goals of the meeting, conference participants were asked to complete an Attendee Evaluation Questionnaire following the conclusion of the event. Highlights of the report include:

- **90.91%** of survey respondents indicated that the Conference met their personal learning goals.
- **78.57%** rated the symposium as **above average** in the landscape
- On average, respondents scored their satisfaction with the conference as **4.36/5**

A summary of the results of the Attendee Evaluation Questionnaire is below.

Demographics:

Please Describe Your Occupation	%
Academic Researcher	28.57
Physician/Clinician	0.00
Student/Postdoc/Medical Fellow	42.86
Industry R&D	0.00
Industry Other	7.14
Academic (Non-Research)	14.29
Not-for-Profit	0.00
Government	0.00
Press	7.14
Other	0.00

What is the highest degree that you have received?	%
PhD	57.14
MD, PhD	14.29
MD	7.14
MS/MA	14.29
BS/BA	0.00
Other Graduate Degree	0.00
Currently a Student	14.29
Other	0.00

NOTE: This reflects survey respondent demographics. n=14

Event Evaluation:

How you would rate your experience with this symposium?	Weighted Average (max. 5)
Overall Virtual Symposium Quality	4.36
Virtual Symposium Format	4.21
Coverage of the Topic	4.36
Poster Session Format	4
Knowledge, Ability, and Responsiveness of Academy Staff	4.33
Quality of Audio and Visual Support	4.43

How did this symposium compare to other similar symposia or conferences that you have attended?	% of Respondents
Above Average	78.57%
Average	7.14%
Below Average	14.29%

If above or below average, please explain:

- NYAS is very well organized for these events. I would appreciate further notices when the date of the webinar approaches
- Really well organized. Excellent participants.
- This was a very well organized meeting with an interesting mix of excellent talks.
- Great interactive panels with good questions.

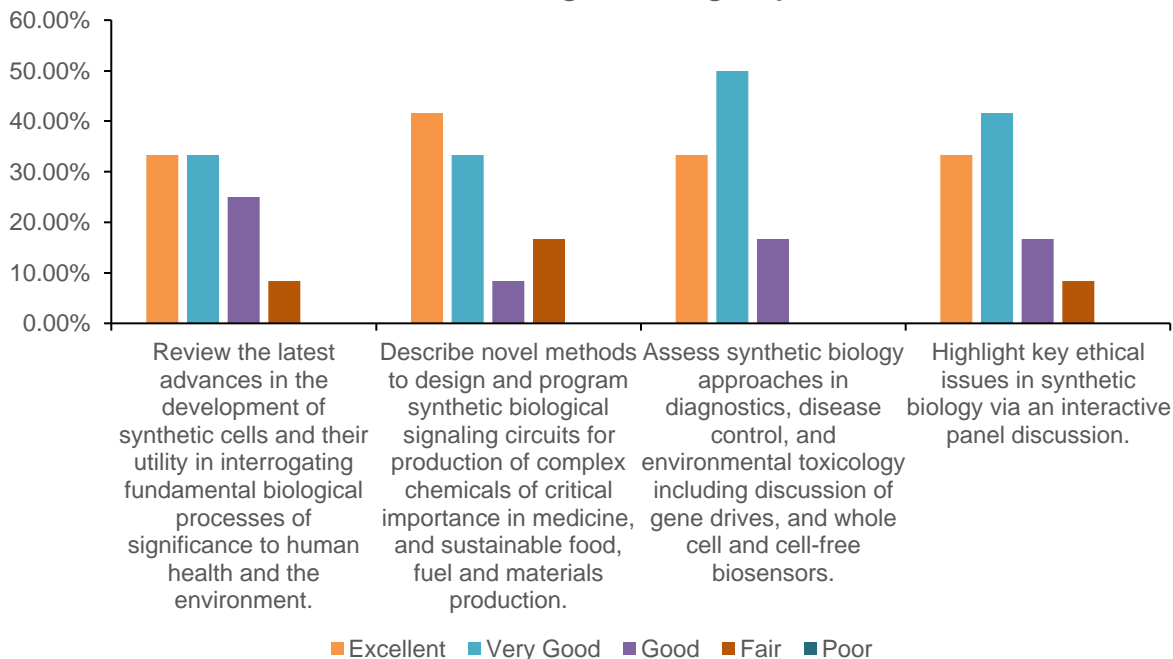
Learning Goals & Outcomes

Did the symposium meet your personal learning goals?	% of Respondents
Yes	90.91
No	9.09
If no, please explain.	
-	

What changes to your research plans, clinical practice, and/or policy goals do you intend to make in light of new information learned at this symposium?

- NA
- None so far.
- Nothing specific, but overall symposium has opened my mind to some future studies.
- I am not sure I understand the question. I learned a great deal from the meeting, but it did not directly impact my research plan. Maybe down the road?
- I think I want to end up in the synthetic biology arena, now that my field of knowledge regarding this booming area of research is expanding, mostly due to this symposium.
- I am not going to change anything right now but new knowledge always helps you with your journey.
- Unclear for now.
- None.
- Apply for more short talks.

In your opinion, to what extent did this symposium fulfill the following learning objectives



Content Evaluation

What did you enjoy most about this symposium?

- The diversity of talk topics around the field.
- Everything.
- Discussion about future uses of synthetic biology
- The energy of discovery.
- The plethora of interest research occurring in this field was really exciting and inspiring!
- That there were lots of talks.

Please rate the overall quality of each presentation. Please only rate presentations which you attended.	Weighted Average (max. 4)
James Collins, PhD, Massachusetts Institute of Technology: <i>Harnessing Synthetic Biology to Fight Pathogens</i>	4.75
Jef Boeke, PhD, NYU Langone School of Medicine: <i>Form and Function of Extensively Engineered Yeast Genomes</i>	4.25
Petra Schwille, PhD, Max-Planck Institute of Biochemistry: <i>Designing Minimal Cell Division</i>	4.38
Farren Isaacs, PhD, Yale University: <i>Engineering Synthetic Genetic Elements for Mobilization and Expression in Diverse Organisms</i>	4
Aditya M. Kunjapur, PhD, University of Delaware: <i>Synthetic Auxotrophy Remains Stable After Continuous Evolution and in Co-culture with Mammalian Cells</i>	4
Javier Santos-Moreno, PhD, University of Lausanne: <i>Multistable and Dynamic CRISPRi-based Synthetic Circuits</i>	4.14
Panel Discussion: Ethical Questions in Synthetic Biology	3.83
James Carothers, PhD, University of Washington: <i>Membrane-Based Synthetic Cell Systems for Plant Natural Product Biosynthesis</i>	4.33
Reshma Shetty, PhD, Ginkgo Bioworks: <i>Designing Biology</i>	3.88
Neal Devaraj, PhD, University of California, San Diego: <i>Lipid Sponge Droplets as Programmable Synthetic Organelles</i>	4.38
Pamela Silver, PhD, Harvard Medical School: <i>New Horizons in Synthetic Biology</i>	4.56
Wendell Lim, PhD, University of California, San Francisco: <i>Learning to Program Cellular Machines: Harnessing Cells to Treat Disease, Build Tissues, and Elucidate Design Principles</i>	4.56
Ethan Bier, PhD, University of California, San Diego: <i>Active Genetics Comes Alive</i>	3.88
Christina Smolke, PhD, Stanford University: <i>Biological Systems to Synthesize Complex Therapeutics</i>	4.56
Michelle O'Malley, PhD, University of California, Santa Barbara: <i>Harnessing Gut Microbes to Turn Waste into Energy</i>	4.56
Tiffany Chien, Columbia University: <i>Multiplexed Biosensors for Enhanced Bacteria Tropism in vivo</i>	4.13
Panel Discussion: Current and Future Challenges in Synthetic Biology	4.13
Smita Shankar, PhD, Impossible Foods: <i>Engineering Pichia pastoris to Make the Impossible Burger Possible</i>	4.2
Julius B. Lucks, PhD, Northwestern University: <i>What is in Your Water? Giving Individuals the Power to Monitor their Environments with Cell-Free Synthetic Biology.</i>	4.44
Stephen Mayfield, PhD, University of California, San Diego: <i>Engineering Algae for Sustainable Materials and Food Production</i>	4.1

Angela Belcher, PhD, Massachusetts Institute of Technology:
Title to Be Announced

4.33

Are there any additional topics that you would have liked to see included in this symposium? Please explain below.

- Wound healing with biomaterials.
 - Biomaterial assembly.
 - Regulations for Synthetic Biology.
-

Any Additional Feedback?

- 1) Break-out sessions to discuss the panel discussion topics
 - 2) Poster format was... difficult to follow
 - Send explicit guidelines for Zoom attendance for all members/signees.
 - When viewing people's slides in fullscreen, the zoom banner along the bottom covered up the bottom of their slides.
-