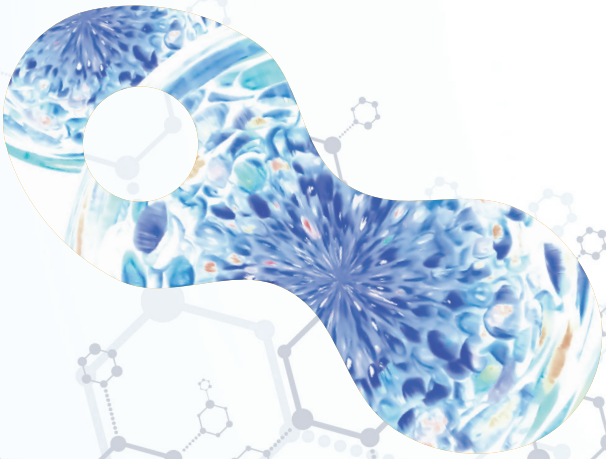
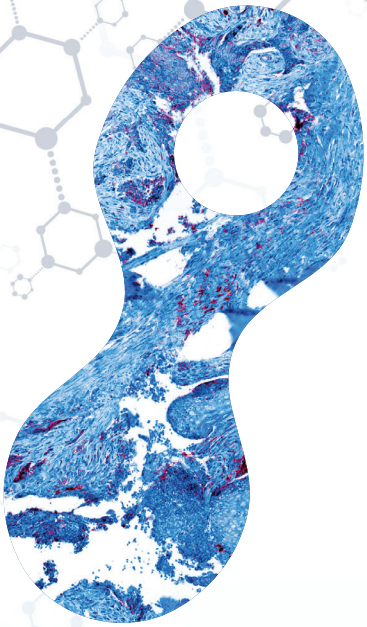
International Conference MICROBIOME ENGINEERING

JOSEPH B. MARTIN CONFERENCE CENTER
BOSTON, MA • NOVEMBER 4-6, 2018





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WELCOME ADDRESS

Greetings!

We are thrilled to welcome you to the first International Conference on Microbiome Engineering being held at the Joseph B. Martin Conference Center in Boston, MA on November 4-6, 2018.

Microbiomes consist of diverse microbial communities and exist in all types of ecosystems. Anomalies in microbiomes can disturb the balance of the ecosystem, resulting in diseases and disorders. Microbiome engineering attempts to mitigate these occurrences by modifying structures of the microbiota to restore ecological balance. Because of this, microbiome engineering has been instrumental in improving human health and agriculture productivity.

ICME 2018 explores the importance and applications of microbiome engineering. This conference brings in experts in industry and academia worldwide to discuss the challenges seen in microbiome engineering and the future of the field.

We are pleased to host many esteemed speakers. There will be keynote addresses from Elhanan Borenstein (University of Washington) and Pamela Silver (Harvard Medical School) as well as presentations from invited speakers who will introduce each session topic. Invited speakers include: Cynthia Collins, Arolyn Conwill, Claire Duvallet, Almut Heinken, Tami Lieberman, Paul Miller, Mark Mimee, Michelle O'Malley, Jeff Tabor, and Harris Wang.

Along with this incredible line-up, ICME 2018 will be hosting a panel discussion on the role of engineers in the microbiome field. Panelists include: Vanni Bucci, Timothy Lu, Costas Maranas, and Ophelia Venturelli.

Please plan to attend our poster session and reception on Sunday; it will be a great opportunity to network and collaborate with new peers over their presented work.

Finally, we would like to thank the Organizing Committee for their integral role in shaping and developing this conference and you for attending the conference. This conference is made possible by the participants and we hope you find your experience enjoyable and enriching.

Sincerely,

Vanni Bucci
Assistant Professor
University of Massachusetts Dartmouth

Stephanie Culler
Co-Founder and Chief Executive Officer
Persephone Biome

Timothy Lu
Associate Professor
Massachusetts Institute of Technology

Bernat Olle
Chief Executive Officer
Vedanta Biosciences

CONFERENCE ORGANIZERS

Conference Co-Chairs

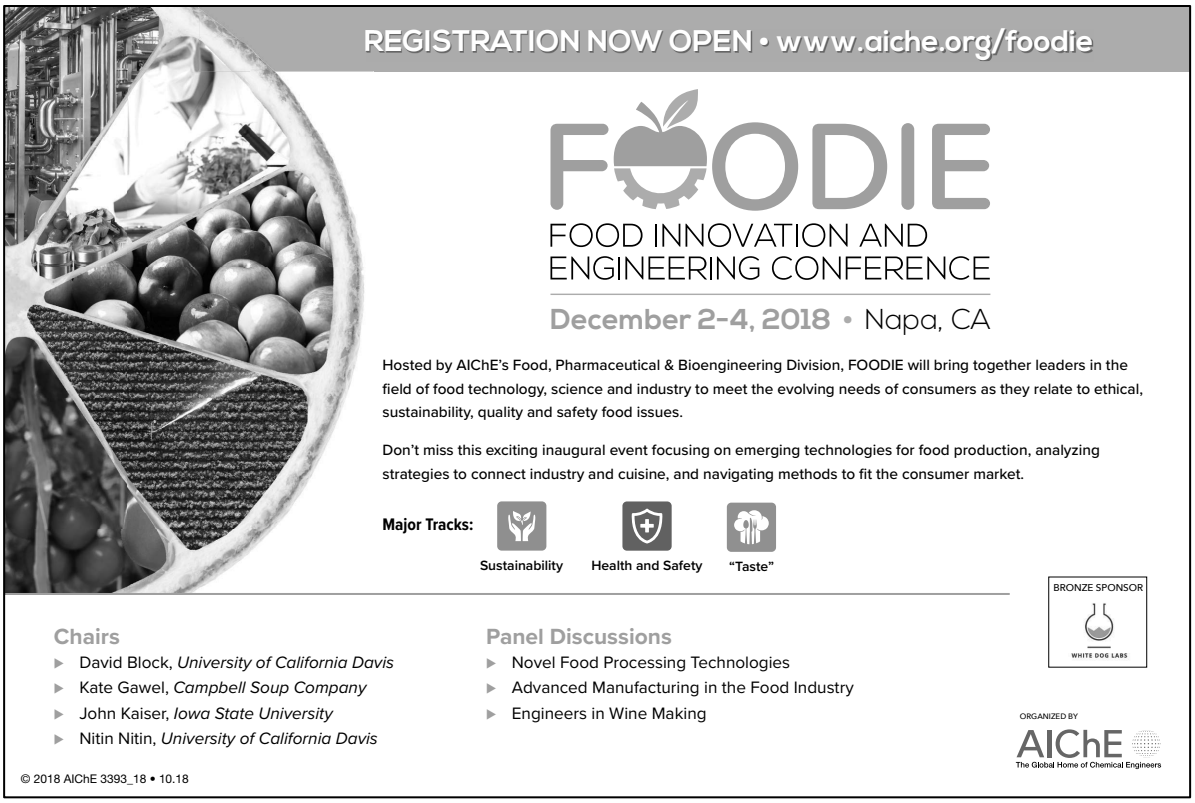
Vanni Bucci, *University of Massachusetts Dartmouth*
Stephanie Culler, *Persephone Biome*
Timothy Lu, *Massachusetts Institute of Technology*
Bernat Olle, *Vedanta Biosciences*

Organizing Committee

Elhanan Borenstein, *University of Washington*
James Collins, *Massachusetts Institute of Technology*
Jeff Gore, *Massachusetts Institute of Technology*
Costas Maranas, *Pennsylvania State University*
Daniel Segrè, *Boston University*
Jeff Tabor, *Rice University*
Ophelia Venturelli, *University of Wisconsin-Madison*

TECHNICAL PROGRAM

Sunday, November 4th	
1:00 PM to 3:00 PM	REGISTRATION
1:00 PM to 1:45 PM	POSTER SET-UP
1:45 PM to 2:00 PM	WELCOMING REMARKS
2:00 PM to 3:00 PM	**PANEL DISCUSSION: The role of engineers in the microbiome field**
3:00 PM to 4:00 PM	**SESSION 1: From genome-scale to community-scale models**
3:00 PM to 3:15 PM	Scalable Tools for Metabolic Modeling of Microbial Communities with Application to the Gut Microbiota **Siu Hung Joshua Chan**, *Colorado State University*
3:15 PM to 3:30 PM	Examination of metabolic interactions between phototrophs and their symbiotic microbiome **Ali Navid**, *Lawrence Livermore National Laboratory*
3:30 PM to 3:45 PM	Building Metabolic Models of Human Microbiota to Probe Community Composition and Diversity **Michael A. Henson**, *University of Massachusetts Amherst*
3:45 PM to 4:00 PM	Miniaturized Next-Generation Sequencing for Microbiome and Metagenomics **Jefferson Lai**, *Labcyte, Inc.*
4:00 PM to 4:30 PM	BREAK and POSTER SET-UP
4:30 PM to 5:30 PM	**Poster Session and Reception**



Monday, November 5th	
8:30 AM to 12:00 PM	REGISTRATION
9:15 AM to 9:30 AM	OPENING REMARKS
9:30 AM to 10:15 AM	KEYNOTE SPEAKER: Designing Gut Microbes for Diagnostics and Therapeutics **Pamela Silver**, *Harvard Medical School*
10:15 AM to 12:45 PM	**SESSION 2: Engineering and modeling of microbial communities I**
10:15 AM to 10:45 AM	INVITED SPEAKER: Engineering Synthetic Microbial Consortia Inspired Bythe Herbivore Rumen **Michelle O'Malley,** *University of California Santa Barbara*
10:45 AM to 11:00 AM	Spurious Associations in Microbiome Studies **Rajita Menon**, *Vedanta Biosciences*
11:00 AM to 11:45 AM	BREAK
11:45 AM to 12:15 PM	INVITED SPEAKER: Oscillatory Population Dynamics in a Bacterial Cross-Protection Mutualism **Arolyn Conwill**, *Massachusetts Institute of Technology*
12:15 PM to 12:30 PM	An Integrated Multi-Scale Modeling Approach for 13c Metabolic Flux Analysis in Microbial Communities **Maciek R. Antoniewicz**, *University of Delaware*
12:30 PM to 12:45 PM	Spatial Metagenomic Characterization of Microbial Biogeography in the Gut **Ravi U. Sheth**, *Columbia University*
12:45 PM to 2:15 PM	LUNCH
2:15 PM to 3:15 PM	**SESSION 2: Engineering and modeling of microbial communities II**
2:15 PM to 2:45 PM	INVITED SPEAKER: **Tami Lieberman,** *Massachusetts Institute of Technology*
2:45 PM to 3:00 PM	Mechanical Forces and Constraints Shape the Gut Microbiota **Carolina Tropini**, *Stanford University*
3:00 PM to 3:15 PM	Multi-Species Co-Culture Platform to Enable Spatial Analysis of Microbial Communication **Christopher A. Vaiana**, *Massachusetts Institute for Technology*
3:15 PM to 5:15 PM	**SESSION 3: Computational models for design**
3:15 PM to 3:45 PM	INVITED SPEAKER: Personalized Modeling of the Host-Gut Microbiome Axis and Its Role in Multifactorial Diseases **Almut Heinken,** *University of Luxembourg*
3:45 PM to 4:00 PM	Systematic Dissection of Sequence Elements Controlling sigma70 Promoters Using a Genomically-Encoded Multiplexed Reporter Assay **Guillaume Urtecho**, *University of California Los Angeles*
4:00 PM to 4:30 PM	BREAK
4:30 PM to 5:00 PM	INVITED SPEAKER: Framework for Rational Donor Selection in Fecal Microbiota Transplant Clinical Trials **Claire Duvallet**, *Massachusetts Institute of Technology*
5:00 PM to 5:15 PM	Engineering of *Lactobacillus Reuteri* As a Biotherapeutic Delivery System **Laura Ortiz-Vélez**, *Baylor College of Medicine*

Tuesday, November 6th	
8:30 AM to 12:00 PM	REGISTRATION
9:00 AM to 9:15 AM	OPENING REMARKS
9:15 AM to 10:00 AM	KEYNOTE SPEAKER: **Elhanan Borenstein**, *University of Washington*
10:00 AM to 12:45 PM	**SESSION 4: Engineering of the organism and tools to manipulate it I**
10:00 AM to 10:30 AM	INVITED SPEAKER: **Cynthia Collins**, *Rensselaer Polytechnic Institute*
10:30 AM to 10:45 AM	Probiotic Associated Therapeutic Curli Hybrids (PATCH) **Pichet Praveschotinunt**, *Harvard University*
10:45 AM to 11:00 AM	Utilizing CRISPR-Based Genome Editing for Microbiome Engineering **Jonathan W. Kotula**, *Caribou Biosciences*
11:00 AM to 11:30 AM	BREAK
11:30 AM to 12:00 PM	INVITED SPEAKER: Next-Gen Genome Engineering Tools for the Gut Microbiome **Harris Wang**, *Columbia University*
12:00 PM to 12:15 PM	A Stealth-Based Approach to Evade Restriction-Modification Systems during Genetic Engineering: Lessons from Bacteriophage **Christopher D. Johnston**, *The Forsyth Institute*
12:15 PM to 12:30 PM	A Modular Platform for Antibody-Targeted Bacterial Delivery **Ava M. Vargason**, *University of North Carolina*
12:30 PM to 12:45 PM	Multiplexed Characterization of Regulatory Components in Diverse Bacterial Cell-Free Expression Systems **Nathan Johns**, *Columbia University Medical Center*
12:45 PM to 2:00 PM	LUNCH
2:00 PM to 4:45 PM	**SESSION 4: Engineering of the organism and tools to manipulate it II**
2:00 PM to 2:30 PM	INVITED SPEAKER: Genetic Technologies to Engineer and Understand the Microbiome **Mark Mimee**, *Massachusetts Institute of Technology*
2:30 PM to 3:00 PM	INVITED SPEAKER: Repurposing Bacterial Two-Component Systems As Sensors for Microbiome Applications **Jeff Tabor**, *Rice University*
3:00 PM to 3:15 PM	Living Materials in the Gut **Neel Joshi**, *Harvard University*
3:15 PM to 4:00 PM	BREAK
4:00 PM to 4:30 PM	INVITED SPEAKER: Engineering of Probiotic E. coli to TREAT Phenylketonuria Using a NOVEL Drug Discovery Paradigm **Paul Miller**, *Synlogic*
4:30 PM to 4:45 PM	Applying Advanced Synthetic Engineering Capabilities for Developing Phage Cocktail **Lior Zelcbuch**, *BiomX*
4:45 PM to 5:00 PM	CLOSING REMARKS
5:00 PM to 6:00 PM	POSTER TEAR-DOWN

POSTER TITLES

1. **A High-Throughput Genetics Approach Reveals Underlying Complexity of Salmonella Phage-Host Interactions.**
Benjamin A. Adler[1,2], *Crystal Y. Zhong*[3], *Adam M. Deutschbauer*[4], *Vivek Mutalik*[4], *and Adam P. Arkin*[5]
(1)Bioengineering, University of California, Berkeley, Berkeley, CA, (2)UC Berkeley-UCSF Graduate Program in Bioengineering, Berkeley, CA, (3)Bioengineering, University of California, Berkeley, Berkeley, (4)Lawrence Berkeley National Lab, Berkeley, CA, (5)Bioengineering, UC Berkeley, Berkeley, CA

2. **Development and Characterization of a Genetically Engineered *E. coli* nissle for the Treatment of Phenylketonuria.**
Mark Charbonneau
Synlogic Inc, Cambridge, MA

3. **Elucidating and Modeling Interactions of Inulin-Consuming Consortia Derived from Consecutive Passages of Human Gut Microbiome.**
Ming-Hsu Chen[1], *Tianming Yao*[1], *Doraiswami Ramkrishna*[2], *and Stephen Lindemann*[1]
(1)Food Science, Purdue University, Lafayette, IN, (2)Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN

4. **Quantifying Metabolite Cross-Feeding through 13C Metabolic Flux Analysis: A Case Study Using E. coli and Salmonella Grown in Co-Culture.**
Michael Dahle *and Maciek Antoniewicz*
Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

5. **Engineering Altruism in Nitrogen Self-Sufficient Cocultures of *Azotobacter Vinelandii* and *E. coli*.**
Camil A. C. Diaz *and Maciek R. Antoniewicz*
Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

6. **Gene Therapy for the Microbiome: Reprogramming Bacteria in Situ with Engineered Phage.**
Jasmine Edelstein[1], *Timothy Jacobs*[2], *Carlos Cruz-Teran*[3], *and Samuel Lai*[4]
(1)Biomedical Engineering, University of North Carolina - Chapel Hill, Chapel Hill, NC, (2)University of North Carolina - Chapel Hill, Chapel Hill, NC, (3)University of North Carolina - Chapel Hill, Raleigh, NC, (4)Pharmacoengineering and Molecular Pharmaceutics, University of North Carolina - Chapel Hill, Chapel Hill, NC

7. **Isolation and Genomic Analysis of Resistant Starch-Degrading Human Gut Microorganism, *Bifidobacterium Adolescentis* P2P3.**
Dong-Hyun Jung[1], *Ga-Young Kim*[1], *Dong-Ho Seo*[2], *Young-Do Nam*[2], *and Cheon-Seok Park*[1]
(1)Graduate School of Biotechnology, Kyung Hee University, Yongin, Korea, Republic of (South), (2)Korea Food Research Institute, Wanju, Korea, Republic of (South)

8. **Linking Microbial Taxonomic and Metabolomic Profiles: A Case Study Using Stool Samples from Patients with Colorectal Adenomas.**
Minsuk Kim[1,2], *Jun Chen*[1,3], *Vanessa Hale*[1,2,4], *Emily Vogtmann*[5], *Rashmi Sinha*[5], *Jaeyun Sung*[1,2], *and Nicholas Chia*[1,2]
(1)Center for Individualized Medicine, Mayo Clinic, Rochester, MN, (2)Department of Surgery, Mayo Clinic, Rochester, MN, (3)Department of Health Sciences Research, Mayo Clinic, Rochester, MN, (4)Department of Veterinary Preventive Medicine, The Ohio State University College of Veterinary Medicine, Columbus, OH, (5)Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD

9. **Assessment of 16S rRNA Amplicon Analysis Bias By Sequencing Platform.**
Seil Kim *and Changwoo Park*
KRISS, Daejeon, Korea, Republic of (South)

10. **Utilizing CRISPR-Based Genome Editing for Microbiome Engineering.**
Jonathan W Kotula*, Joel D Berry, Stephen Smith, Agnes Oromi-Bosch, and Steven B. Kanner*
Caribou Biosciences, Berkeley, CA

11. **Enabling Member Coexistence and Programming Community Composition in Synthetic Microbial Communities Via Temperature Regulation.**
Adam Krieger
Cellular and Molecular Biology Ph.D. Program, University of Michigan, Ann Arbor, MI

12. **Identifying Regulators of Microbiome-Encoded Bile Acid Metabolism.**
Daniel Lachance[1], *Naisi Li*[2], *and Neelendu Dey*[2,3]
(1)Molecular Engineering, University of Washington, Seattle, WA, (2)Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA, (3)Gastroenterology, University of Washington, Seattle, WA

13. **Miniaturized Next-Generation Sequencing for Microbiome and Metagenomics.**
Jefferson Lai*,* ***Jared Bailey****, and* ***John Lesnick***
170 Rose Orchard Way, Labcyte Inc, San Jose, CA

14. **Using Evolver to Construct and Characterize Microbial Communities in Controlled Dynamic Environments.**
Christopher P. Mancuso*, Brandon G. Wong, and Ahmad S. Khalil*
Biomedical Engineering, Boston University, Boston, MA

15. **Prebiotic Control of Engineered Probiotics.**
Fatima Enam, Yanfen Bai, Logan Ryerson, and ***Thomas J. Mansell***
Chemical and Biological Engineering, Iowa State University, Ames, IA

16. **Cell-Free Metabolic Engineering for the Exploration of Cryptic Lasso Peptide Natural Products in the *Populus* root-Associated Microbiome.**
Benjamin P. Mohr[1,2], *Patricia M. Blair*[1], *Richard J. Giannone*[3], *Dale A. Pelletier*[1], *Robert L. Hettich*[3], *and Mitchel J. Doktycz*[1,2]
(1)Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, (2)Bredesen Center for Interdisciplinary Research, University of Tennessee, Knoxville, TN, (3)Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

17. **Application of Catechol Microcins As Antimicrobial Peptides for the Prevention of Enteric Disease.**
Jacob Palmer[1], *Emma Piattelli*[2], *Benedikt Mortzfeld*[1], *Beth McCormick*[3], *Mark Silby*[2], *Christopher Brigham*[4], *and Vanni Bucci*[1]
(1)Bioengineering, University of Massachusetts Dartmouth, North Dartmouth, MA, (2)Biology, University of Massachusetts Dartmouth, North Dartmouth, MA, (3)Microbiology and Physiological Systems, University of Massachusetts Medical, Worcester, MA, (4)Interdisciplinary Engineering, Wentworth Institute of Technology, Boston, MA

18. **Functional Microbiome Design for Agile and Expedient Manufacturing.**
Matthew Perisin
Biotechnology Branch, Army Research Laboratory, Adelphi, MD

19. **Modeling Community Growth and Diversity of the Chronic Wound Microbiota.**
Poonam Phalak *and Michael A. Henson Department of Chemical Engineering, University of Massachusetts Amherst, Amherst, MA*

20. **Characterization of Microbial Anticipatory Responses in the Mammalian Gut.**
Navneet Rai*, Minseung Kim, and Ilias Tagkopoulos*
Computer Science & Genome Center, University of California - Davis, Davis, CA

21. **A Model Culture System for the *in Vitro* Human Colonic Microbiota of Ulcerative Colitis.**
Kengo Sasaki *and Akihiko Kondo Graduate School of Science, Technology and Innovation, Kobe University, Kobe, Japan*

22. **Cargo Transport Enables the Spatial Engineering of a Microbial Community.**
Abhishek Shrivastava[1,2,3], *Visha Patel*[1], *Yisha Tang*[1], *Susan C. Yost*[3], *Floyd E. Dewhirst*[3], *and Howard C. Berg*[1,2]
(1)Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, (2)The Rowland Institute at Harvard, Cambridge, MA, (3)The Forsyth Institute, Cambridge, MA

23. **Autonomous Regulation of Bacterial Co-Cultures Based on the AI-2 Quorum Sensing Signal.**
Kristina Stephens*, Maria Pozo, Chen-Yu Tsao, Pricila Hauk, and William Bentley Fischell Department of Bioengineering, University of Maryland, College Park, MD*

24. **Engineering Soil- and Gut-Adapted Bacteria for Expression of Antimicrobial Gene Clusters in Their Respective Microbiomes.**
Alexander Triassi[1] *and Christopher A. Voigt*[2]
(1)Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, (2)Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

25. **Examining the Human Infant Gut Using Genome-Scale Models.**
Patrick F. Suthers*, Debolina Sarkar, and Costas Maranas*
Department of Chemical Engineering, The Pennsylvania State University, University Park, PA

26. ***In Vitro* Discovery of Novel Biomarkers As Indicators of Toxicant Exposure in the Human Gut.**
David Walsh III
Bioengineering Systems & Technologies, MIT-Lincoln Laboratory, Lexington, MA

27. **Massively Parallel Transcriptional Measurements in Cell-Free Systems from Diverse Bacteria.**
Sung Sun Yim[1], *Nathan Johns*[2], *and Harris H. Wang*[3]
(1)Systems biology, Columbia University, New York, NY, (2)Systems Biology, Columbia University Medical Center, New York, NY, (3)Department of Systems Biology, Columbia University, New York, NY

28. **Metagenomic Engineering of the Mammalian Gut Microbiome *in Situ*.**
Carlotta Ronda, ***Sway Chen***, *Vitor Cabral, and Harris H. Wang*
Department of Systems Biology, Columbia University, New York, NY

29. **Signatures of Exposure in the Skin Microbiome.**
Kristin Loomis, *David Karig, Bryan Brensinger, Craig Howser, Kianna Blount, and Joshua Wolfe*
Research and Exploratory Development, Johns Hopkins University Applied Physics Laboratory, Laurel, MD

30. **Evaluation of Engineered Microbe Persistence and Function of Using a Simplified Polymicrobial Gut Community.**
Steven Arcidiacono[1], *Laurel A. Doherty*[1], *Jordan Whitman*[2], *Ida Pantoja-Feliciano*[1], *Michael Goodson*[3], *Amy M. Ehrenworth Breedon*[3,4], *Scott A. Walper*[5], *Joseph R. Spangler*[6], *and Jason W. Soares*[1]
(1)U.S. Army Natick Soldier Research Development and Engineering Center, Natick, MA, (2)NSRDEC, Natick, MA, (3)711th Human Performance Wing, Air Force Research Laboratory, Wright-Patterson Air Force Base, OH, (4)UES, Inc., Dayton, OH, (5)Naval Research Laboratory, Washington, DC, (6)National Research Council, Washington, DC

KEYNOTE AND INVITED SPEAKER BIOGRAPHIES

Keynote Speakers Biographies

Elhanan Borenstein
University of Washington
Elhanan Borenstein is an associate professor of Genome Sciences at the University of Washington, with an adjunct position in the Department of Computer Science and engineering. He is also an external professor at the Santa Fe Institute for complexity science. Dr. Borenstein received his PhD in computer science from Tel-Aviv University, Israel, and held a joint postdoctoral fellowship at the Department of Biology in Stanford and at the Santa Fe Institute. He also has extensive professional experience in the hi-tech industry, where he held top management positions in several hi-tech companies.

Dr. Borenstein integrates metagenomic data with methods inspired by systems biology, in-silico modeling, network theory, machine-learning, and statistical inference to develop a variety of computational frameworks for studying the human microbiome. His work focuses on reconstructing predictive, systems-level models of the microbiome and on developing novel computational methods for integrative, multi-omic analysis of microbiome data, aiming to provide a better principled understanding of the microbiome and its role in human health and to inform microbiome-based therapy efforts.

Dr. Borenstein is the recipient of various awards including the Alfred P. Sloan Fellowship and the NIH New Innovator Award.

Pamela Silver
Harvard Medical School
Pamela Silver is the Elliot T and Onie H Adams Professor of Biochemistry and Systems Biology in the Department of Systems Biology at Harvard Medical School. She is also a member of the Harvard University Wyss Institute for Biologically Inspired Engineering. Her group combines lessons from Nature to the design of new organisms for both discovery and applications. She received her BS in Chemistry from the University of California, Santa Cruz and PhD in Biochemistry from the University of California, Los Angeles. She was a Postdoctoral Fellow at Harvard University where she was a Fellow of the American Cancer Society and The Medical Foundation.

Her work has been recognized by an Established Investigator of the American Heart Association, a Research Scholar of the March of Dimes, an NSF Presidential Young Investigator Award, a Claudia Adams Barr Investigator, an NIH MERIT award, a Fellow of the Radcliffe Institute, top ten innovations by the World Economic Forum and election to the American Academy of Arts and Sciences. She has served on numerous editorial boards, was the Editor of Molecular Biology of the Cell, has served on the Council of the American Society for Cell Biology and on the Committee for Women in Cell Biology, presented to members of Congress and was a co-founder of Karyopharm Therapeutics (NASDAQ:KPTI) that makes novel anti-cancer drugs and other biotech companies. In her free time, she enjoys sailboat racing and running.

Invited Speaker Biographies

Cynthia Collins
Rensselaer Polytechnic Institute
Cynthia Collins joined the Department of Chemical and Biological Engineering at RPI in March 2008 as an assistant professor. Cynthia grew up in Winnipeg, Manitoba, Canada. She obtained her Honours B.Sc. in Chemistry and Biochemistry from the University of Toronto in 2000, and her Ph.D. in Biochemistry and Molecular Biophysics from Caltech in 2006. She subsequently completed a postdoctoral fellowship in Michael Suretteís lab at the University of Calgary, where she was the recipient of a prestigious Alberta Ingenuity Post-Doctoral Fellowship.

Research:
Communities of microorganisms are ubiquitous in nature and play important roles in processes that directly impact human life, from environmental remediation, wastewater treatment and assistance in food digestion to biofouling, biofilm-related corrosion, and hospital-acquired infections. The Collins Lab focuses on fundamental and applied aspects of microbial consortia and combines multiscale modeling of biological networks (from gene to protein to organism to community), metabolic and biochemical engineering, synthetic biology and engineered cell-cell communication with the complexities of coexisting communities of bacteria. Applications range from engineering biosensors to bioprocessing, bioremediation and bio-energy production, and may also include the development of therapeutics that specifically target the balance between good and bad bacteria in the human body.

Arolyn Conwill
Massachusetts Institute of Technology
Arolyn Conwill received her PhD in Physics from MIT in February 2018, and she received her BA in Physics from Pomona College in 2010.

During graduate school, Arolyn's research as part of the Gore Lab used experimentally tractable laboratory microcosms to explore how interactions between individuals drive the evolution and ecology of communities. Her first project demonstrated that a bacterial cross-protection mutualism can arise from cooperative antibiotic resistance, allowing for survival in multi-drug environments (Yurtsev*, Conwill*, et al, PNAS, 2016). She then studied range expansion patterns in discrete-space and discrete-time systems (i.e. patchy environments and periodic growth cycles) and explored population dynamics and alternative stable states in multi-species communities of marine bacterial isolates. For more information, please visit the Gore Lab website at http://www.gorelab.org/index.html.

Arolyn has recently joined the Lieberman Lab at MIT as a postdoc.

Claire Duvallet
Massachusetts Institute of Technology
Claire is currently a PhD student in the Biological Engineering department at MIT in Eric Alm's lab, where she studies the relationship between the human microbiome and health and disease. She's is interested in using various physiological data to predict disease states, both on the individualized and broader community levels.

Claire graduated from Columbia University's Biomedical Engineering department, where she worked as an undergraduate research assistant in Samuel Sia's lab on a project developing an integrated microfluidic device to diagnose multi-drug resistant M. tuberculosis in developing countries.

Claire's research is motivated by her interest in harnessing large, untargeted biological data to better understand, predict, and monitor human disease. In particular, she uses increasingly

available non-invasive, stool-derived microbiome data to better understand gut microbial community shifts in disease, with the goal of informing microbiome-based therapeutic and diagnostic research efforts. Additionally, Claire studies biomarkers in sewage to predict and track disease outbreaks, identify emerging antimicrobial resistance, and extract information about a city's overall health.

Almut Heinken
University of Luxembourg
Almut Heinken specializes in systems biology of the human gut microbiome with the particular focus on personalized host-diet-microbiome interactions in health and disease states.

We have recently published a compendium of manually curated reconstructions of >800 human gut microbes, AGORA (Magnusdottir, Heinken et al., Nature Biotechnology 2016). Moreover, we have developed a COBRA Toolbox extension that enables the prediction of microbe-microbe interactions and individual-specific microbial community fluxes (Baldini, Heinken et al., bioRxiv 2018). These resources allow for the construction of personalized gut microbiome models by mapping metagenomic samples onto the reference set of AGORA genomes. I have constructed personalized microbiome models for ~200 human individuals including a cohort of pediatric inflammatory bowel disease patients and control children (Heinken et al., bioRxiv 2017). I simulated the bile acid metabolism profiles in each microbiome and found that the profiles of IBD patients were distinct. Finally, we have also combined personalized microbiome models with a multi-organ model of human metabolism, which enables us to model whole body host-microbe cross-talk (Thiele et al, bioRxiv 2018).

Tami Lieberman
Massachusetts Institute of Technology
Tami Lieberman joined the MIT faculty in January 2018. She leads a computational and experimental research group focused on uncovering the principles governing colonization, niche range, and personalization in the human microbiome.

Tami trained in molecular biology and mathematics at Northwestern University, where she conducted research in the laboratory of Jon Widom and was funded by a Barry M. Goldwater Scholarship. She then earned a Ph.D. in Systems Biology from Harvard University, where she conducted research in Roy Kishony's laboratory. During her graduate research, Tami developed new genomic approaches for understanding how bacteria evolve during infections of individual people. As a postdoc in Eric Alm's lab at MIT, she further developed and applied these genomic approaches to understand the microbes that colonize us during health. Tami has also made contributions to our understanding of antibiotic resistance, including the co-invention of a new platform for visualizing evolution in real time. Her work has been covered in the popular press, including online coverage from The Atlantic, The Wall Street Journal, National Geographic, The Boston Globe, and ArsTechnia.

Research
Rational microbial-based therapies have the potential to treat a wide range of diseases and promote wellness. However, we remain severely limited in our ability to employ such therapies, as we cannot predict which bacterial strains have the potential to stably colonize an individual. My lab seeks to close this knowledge gap, developing an understanding of how individual species and strains behave in the human microbiome—including the selective pressures they face, niche ranges, survival strategies, and the degree to which they adapt to individual people.

A key approach in the lab is evolutionary retrospective studies of commensal species at the whole-genome level. We leverage the mutations that bacteria accumulate during colonization of individual people and

evolutionary inference methods to infer past migrations within and across body sites, selective pressures faced by bacteria in vivo, and the molecular strategies used to adapt to these pressures. Crucially, these inferences can be performed without longitudinal studies, because bacterial strains diversify within hosts to form co-existing lineages that preserve a record of their natural history within the host. Other favored approaches include high-throughput culturing and experiments, computational tool development, and interrogation of spatial structure. When possible, we focus on the human environment, in order to rapidly translate discoveries from these complex ecosystems.

In the long term, we are optimistic that these studies will help us predict which therapeutic strains and co-administered treatments (prebiotics) provide the most potential for colonization, understand the degree to which each person's microbiome requires personalized treatment, and predict how the future evolution of a strain will impact its community and host.

Paul Miller
Synlogic
Dr. Paul Miller joined Synlogic in 2014 as chief scientific officer and is accountable for all aspects of discovery research and platform expansion at the company. Previously, Paul was the vice president of infection biology at AstraZeneca, where he was responsible for the early discovery portfolio and strategy while also leading several external collaborations. Prior to Astra-Zeneca, Paul was the chief scientific officer for antibacterial research at Pfizer, leading discovery teams that produced eight drug development candidates, provided critical research support for several successful marketed antibiotics including Zithromax and Zyvox, and also successfully advanced a novel oxazolidinone (sutezolid) for tuberculosis into Phase II studies. A microbial geneticist by training, Paul began his professional career at the Warner-Lambert Company in Ann Arbor, Michigan, where he integrated modern molecular-genetic approaches into a traditional antibacterial drug discovery program and established novel target discovery projects. His work there led to new insights into the mechanisms by which bacteria sense and respond to antibiotics and other environmental agents. Paul received his Ph.D. in microbiology and immunology from the Albany Medical College, and conducted post-doctoral studies at the National Institutes of Health. He has also served as a member of the Institute of Medicine's Forum on Microbial Threats.

Mark Mimee
Massachusetts Institute of Technology
Mark Mimee PhD is a Group Leader in the Lu Lab, which is affiliated with the Biological Engineering Department, the Research Laboratory of Electronics, and the Synthetic Biology Center at MIT. He obtained his B.Sc. in Microbiology & Immunology at McGill University and completed his PhD under the supervision of Timothy K. Lu in the Microbiology Program at MIT, during which, he was an HHMI International Student Fellow as well as a Qualcomm Innovation Fellow. His research focuses on developing fundamental technologies to facilitate microbiome engineering. These strategies include using natural and modified bacteriophages to remodel microbial communities, genetically engineered commensal organisms to modulate host immunity, and interfacing bacterial biosensors with microelectronics to detect biomarkers in the gut. Together, these approaches enable both basic and translational research on elucidating host-microbe interactions to address major unmet clinical needs.

Michelle O'Malley
University of California, Santa Barbara
Michelle A. O'Malley earned a B.S. in Chemical Engineering and Biomedical Engineering from Carnegie Mellon University in 2004. She holds a PhD in Chemical Engineering from the University of Delaware in 2009, where she worked with Prof. Anne Robinson to engineer overproduction of membrane proteins in yeast. O'Malley was a USDA-NIFA postdoctoral fellow in the Department of Biology at MIT, where she developed new strategies for cellulosic biofuel production. She joined the Chemical Engineering faculty at UC-Santa Barbara in 2012, and her research group engineers protein synthesis within anaerobes and consortia for sustainable chemical production, bioremediation, and natural product discovery. O'Malley was named one of the 35 Top Innovators Under 35 by MIT Technology Review in 2015, and is the recipient of the Presidential Early Career Award for Scientists and Engineers (PECASE), a DOE Early Career Award, an NSF CAREER award, the Camille Dreyfus Teacher-Scholar Award, an ACS PMSE Young Investigator Award, an ACS WCC "Rising Star" Award, and a Hellman Faculty Fellowship.

Research
The O'Malley Lab works at the interface of engineering and biology to engineer microbes and consortia with novel functions. We are especially interested in deciphering how "unwieldy" microbes in the environment perform extraordinary tasks - many of these microbes have no available genomic sequence and are exceptionally difficult to manipulate. We seek a better understanding of how proteins are synthesized by cells, and how their three-dimensional structure informs their function would enhance our ability to engineer proteins (and cellular expression platforms) for diverse biomedical and biotechnology applications. To address these issues, our approach combines classical cell biology tools with cutting-edge technologies (genome sequencing, RNAseq, cellular reprogramming) that are rooted in the core biological sciences to interrogate and engineer molecular mechanisms that underlie protein production in eukaryotic cells. In addition, we rely on biophysical methods to elucidate protein-protein contacts, with the aim of controlling these interactions both in vivo and in vitro. Systems of interest to us have broad applicability to bioenergy and sustainability, as well as to drug development and detection.

Jeffrey Tabor
Rice University
Jeff Tabor builds synthetic signaling circuits to engineer biological behaviors such as multicellular pattern formation and social interactions. Tabor takes an engineering approach by using cellular sensors and synthetic gene circuits to control genes of interest in tractable model organisms. Because these control systems are constructed in a step-wise fashion, they are amenable to rigorous characterization and optimization. This allows the development of well-parameterized mathematical models that increase the predictability of the design process in synthetic biology. Reprogramming how cells respond to their environment and interact with one another is of interest to basic science and has broad biomedical and industrial applications.

Research
The tools of modern molecular biology allow the DNA of living organisms to be rapidly rewritten, and this in turn allows unnatural biological behaviors to be engineered. The Tabor lab takes a synthetic biological approach to studying how population-level phenomena, such as multicellular pattern formation and cooperation, are coordinated by the underlying gene regulatory networks. By constructing synthetic gene regulatory networks and linking cells together with artificial communication systems they aim to understand the rules by which a sequence of DNA can encode a population-level process. Also, by evolving their engineered gene circuits in the laboratory they can ask not only how biology works but why certain biological control systems are preferred

over others. The study of biological 'design principles' has broad applications in science, medicine and biotechnology.

Harris Wang

Columbia University
Harris Wang is an Assistant Professor at Columbia University jointly appointed in the Department of Systems Biology and the Department of Pathology and Cell Biology. Dr. Wang received his B.S. degrees in Mathematics and Physics from MIT and his Ph.D. in Biophysics from Harvard University. His research group develops platform genomic technologies to characterize and engineer microbial communities that colonize various environments such as the mammalian gut, towards synthetic biology applications to deliver and actuate novel functions in complex ecosystems in situ. Dr. Wang is an Investigator of the Burroughs Wellcome Fund and the recipient of numerous awards, including the NIH Director's Early Independence Award, NSF CAREER, Sloan Research Fellowship, ONR Young Investigator and was named in Forbes' 30 Under 30 in Science. Dr. Wang is a recipient of the Presidential Early Career Award for Scientists and Engineers (PECASE), which is "the highest honor bestowed by the United States Government on science and engineering professionals in the early stages of their independent research careers."

Panelist Biographies

Vanni Bucci
University of Massachusetts Dartmouth
Dr. Vanni Bucci received his Bachelor of Science degree in Environmental Engineering at the University of Florence in 2006. He then moved to the Hellweger Lab at Northeastern University in Boston to pursue a Master of Science and Doctorate degrees in Civil Engineering in 2008 and 2010, respectively. Dr. Bucci spent the period ranging from September 2010 to July 2013 in the Xavier Lab in the Department of Computational Biology at Memorial Sloan-Kettering Cancer Center in NYC.

Dr. Bucci's research spans across the fields of computational and systems biology, mathematical modeling, microbial ecology, evolutionary biology, genomics and metagenomics. The goal is to understand what mechanisms are responsible for the temporal and spatial dynamics of complex biological systems and develop tools to predict them.

Timothy Lu
Massachusetts Institute of Technology
Timothy K. Lu, M.D., Ph.D. is an Assistant Professor leading the Synthetic Biology Group in the Department of Electrical Engineering and Computer Science and Department of Biological Engineering at MIT. Tim received his S.B. And M.Eng. in Electrical Engineering and Computer Science at MIT and completed his M.D./Ph.D. training in the Harvard-MIT Health Sciences and Technology Program. He is a core member of the Synthetic Biology Center at MIT, Associate Member at the Broad Institute of MIT and Harvard, and co-founder of Sample6 Technologies. He is also affiliated with the MIT CSBi Program, the MIT Microbiology Program, and the Harvard Biophysics Program.

Tim has pioneered new approaches to combat infectious diseases with synthetic biology, encode memory in the DNA of living cells, and perform both digital and analog computation in biological systems. His group's research focuses on engineering fundamental technologies to enable the scalable design of biological systems and on applying synthetic biology to solve medical and industrial problems. Tim is a recipient of the Henry L. and Grace Doherty Professorship, NIH New Innovator Award, Lemelson-MIT Student Prize for Invention, Army Young Investigator Award, Ellison New Scholar in Aging Award, and Presidential Early Career Award for Scientists and Engineers (PECASE).

Costas Maranas
Pennsylvania State University
Dr. Costas D. Maranas (b. 1967) is the Donald B. Broughton Professor in the Department of Chemical Engineering at The Pennsylvania State University. He received his Diploma in Chemical Engineering from the Aristotle University, Greece in 1990 and a Ph.D. in Chemical Engineering from Princeton University in 1995. He has been in the faculty of the department of Chemical Engineering at Penn State since 1995. He is the recipient of the Allan P. Colburn Award for Excellence in Publications by a Young Member of AIChE (2002), the Outstanding Young Investigator Award of the Computing and Systems Technology AIChE Division (2006), the S.V. Sotirchos Lectureship at 6th Panhellenic Chemical Engineering Conference (2007), the Penn State Engineering Alumni Society (PSEAS) Premier Research Award (2016) and Outstanding Research Award in (2012). He is a member of a number of journal Editorial Boards including PLOS Computational Biology, BMC Systems Biology, Biotechnology Journal and Metabolic Engineering. He is a Fellow of the American Institute of Medical and Biological Engineering (AIMBE). He is a member of advisory/steering committees for PNNL/EMSL

and EcoCyc and the “Use Inspired Research” Lead in the Center for Bioenergy Innovation (CBI) DOE center.

The C. Maranas group develops and deploys computational framework informed by systems engineering and mathematical optimization to understand, analyze and redesign metabolism and proteins. Research interests include: Computational protein design; enzyme and antibody engineering; reconstruction, curation and analysis of metabolic networks; computational strain design and synthetic biology; metabolism of photosynthetic organisms; metabolism of obligatory anaerobes; modeling of microbial communities; optimization theory and algorithms. He has co-authored over 160 refereed journal publications including a textbook on “Optimization Methods in Metabolic Networks” (2016). He has supervised 29 PhD theses with many group alumni occupying leading positions in industry and academia.

Bernat Olle
Vedanta Biosciences
Dr. Olle is a co-founder and Chief Executive Officer of Vedanta Biosciences. He has been a member of the founding teams of several companies of the PureTech portfolio and served as a member of the Board of Directors of Vedanta Biosciences and Follica Biosciences.

In 2013 Dr. Olle was named "Innovator of the Year" in MIT Technology Review Spain's "Innovators under 35" awards. He completed his doctoral work at the Chemical Engineering Department at MIT, where he developed a novel method for large-scale bacterial culture. During his graduate work, Dr. Olle was awarded the "la Caixa" fellowship. Dr. Olle received his B.S. in Chemical Engineering from Universitat Rovira i Virgili, in the Republic of Catalonia, his M.S. and PhD. in Chemical Engineering Practice from MIT, and his M.B.A. from the MIT Sloan School of Management. He has published his work in journals including Nature and Nature Biotechnology.

Ophelia Venturelli
University of Wisconsin-Madison
Ophelia S. Venturelli is an Assistant Professor at the University of Wisconsin-Madison. She finisehed her Postdoctorial Fellow and the University of California Berkeley, obtained her PhD from the California Institute of Technology, and completed her Bachelor's in Science at Stanford University.

The Venturelli lab aims to extract evolutionary design principles of networks and synthetic ecologies using a combination of experiment, computation and theory. They seek to elucidate the network design principles that underlie dynamic responses of microbial populations and ecosystems in response to complex environmental inputs, and develop methods to construct predictable feedback control systems for targeted manipulation of microbial function, dynamics and fitness. Their work combines concepts from control theory, information theory, game theory, nonlinear dynamical systems, and multi-objective optimization with multiplexed measurements and perturbations of single cells and populations.

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Sunday, November 4

SESSION 1: FROM GENOME-SCALE TO COMMUNITY-SCALE MODELS

Scalable Tools for Metabolic Modeling of Microbial Communities with Application to the Gut Microbiota.

Siu Hung Joshua Chan[1] *and Costas Maranas*[2]
(1)Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO,
(2)Department of Chemical Engineering, The Pennsylvania State University, University Park, PA

Extending genome-scale metabolic modeling to microbial communities is a promising way to unravel community interactions. To this end, standardized models, new modeling elements and algorithms scalable to an increasing number of community members are required. We present three tools that address these challenges. First, an unstandardized biomass reaction of any organism in a community that produces biomass with a molecular weight different from the standard 1 g/mmol introduces a systematic error. We developed a computational procedure for checking the biomass weight to eliminate the error. 42 of 64 examined models show >5% discrepancies in biomass weights. We demonstrated that the discrepancies could cause disproportionately larger errors in the predicted community composition.
Second, little emphasis was placed on the need to impose a time-averaged constant growth rate across all members to ensure co-existence and stability. Without this constraint, faster growing organisms will ultimately displace other microbes. We introduced the SteadyCom framework for predicting community metabolism consistent with the steady-state requirement. SteadyCom is scalable to the number of organisms and compatible with flux variability analysis (FVA). It can predict an abundance profile with good agreement to experimental gut microbiota. We further incorporated SteadyCom into a dynamic framework to model the spatially differential microbiota along the intestinal tract. The model can capture the essential features of the experimental spatial distribution of anaerobes vs. aerobes.
Third, to effectively perform FVA in the absence of thermodynamically infeasible cycles in community models, we devised a method termed localized loop-less FVA (lll-FVA). We identified the fewest constraints required and put forth the concept of localized loop-less constraints. The computational time is reduced by 10-150 times compared to the original formulation and by 4-20 times compared to the currently fastest method. lll-FVA offers a scalable strategy for loopless flux calculations for large multi-compartment/multi-organism models (e.g., $>10^4$ reactions).

Examination of metabolic interactions between phototrophs and their symbiotic microbiome

Ali Navid
Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA

For the efficient conversion of sunlight into energy dense fuels, large-scale culturing of microalgae is a promising renewable source. To engineer efficient and robust biofuel-producing algal systems, we need to examine the interactions between microalgae and the bacteria in their surrounding environments, and how these interactions affect the reliability of biofuel production. Here we report on our progress examining the metabolic interactions between the diatom *Phaeodactylum tricornutum* and some of the bacteria that occupy its phycosphere.

P. tricornutum is a long-studied model for phytoplankton ecology, physiology and evolution. It also has a potential role in microalgal biofuel production. Important with regards to engineering, *P. tricornutum* is genetically tractable, with a fully sequenced genome, and published genome-scale constraint-based metabolic models. We isolated a set of algal phycosphere bacterial cultures from natural and cultivated pond samples. We found that *P. tricornutum* co-cultured with phycosphere bacteria often had higher Carbon fixation rates and appeared to support more attached bacteria than other algae. We collected 15 phycosphere-associated bactarial strains in single cultures, from the bacterial families that are known to dominate algal pond communities. We examined the effect of these strains on *P. tricornutum* growth under different conditions, and sequenced the genome of those bacteria that most profoundly affected the algal phenotype. When *Algoriphagus ARW1R1* it is co-cultured with *P. tricornutum*, the algal lipid production is significantly enhanced. To asses the nature of the metabolic interactions between these two organisms, we generated a draft metabolic model of the bacterium and using dynamic Flux balance analysis and a published model of *P. tricornutum*, examined the spectrum of possible interactions between them. We identified a set of interactions that could explain the observed behavior. However, our results seem to indicate that the interaction is highly dependent on the biochemical composition of the growth medium.

Building Metabolic Models of Human Microbiota to Probe Community Composition and Diversity.
Michael A. Henson[1]*, Poonam Phalak*[1]*, and George O'Toole*[2]
(1)Department of Chemical Engineering, University of Massachusetts Amherst, Amherst, MA,
(2)Microbiology and Immunology, Dartmouth University, Hanover, NH

Metabolic modeling of human microbiota has emerged as an important in silico tool for investigating community composition as well as species interactions. While yielding useful insights into community behavior, most microbiota models developed to date have been limited with respect to the number of species included and/or the metabolic interactions allowed. We have developed several large-scale community models based on combining the vast array of genome-scale metabolic reconstructions developed with the AGORA pipeline with the computational efficiency of the SteadyCom community modeling tool. AGORA allows the modeled bacterial species to be chosen based on the most abundant genera in the human microbiome of interest rather than by the availability of curated reconstructions, which remains limited to a few dozen species. Here we illustrate the application of our community modeling framework to bacterial microbiota in the cystic fibrosis (CF) lung. A 15 species model was developed from 16S sequence data reporting the most abundant genera in sputum collected from 30 adult CF patients. The model was constrained by specifying the community uptake rates of 82 metabolites and the species uptake rates of putative crossfed amino acids and metabolic byproducts. By performing hundred of simulations with randomized community uptake rates to simulate patient heterogeneity, we were able to rationalize the frequent domination of the community by *Pseudomonas* and *Streptococcus* as well as infrequent domination by *Burkholderia* or Enterobacteriaceae. The co-domination of *Pseudomonas* and *Streptococcus* was predicted to occur in patients with relatively low amino acid and high oxygen levels and was driven by crossfeeding of acetate, formate, lactate and threonine.

Miniaturized Next-Generation Sequencing for Microbiome and Metagenomics.
Jefferson Lai**, **Jared Bailey**, and **John Lesnick
170 Rose Orchard Way, Labcyte Inc, San Jose, CA

Our understanding of the role of the human microbiome in health and disease has been growing rapidly in recent years. Next-generation sequencing technology has enabled vast applications and analyses in both microbiome and metagenomics. As researchers continue to deepen our understanding of how microbiome impacts health, as biologists explore the metagenomic space, our tools and analyses need to scale accordingly. In this presentation, we demonstrate miniaturized library preparation of 16S rRNA amplicons and Nextera XT whole genomes for time and cost efficient workflows by utilizing the Labcyte® Echo® 525 Liquid Handler. We show miniaturized reactions produce high-quality data, while using typically one-tenth the volumes described in protocols. This allows researchers to expand and accelerate projects and developments in an efficient manner.

Monday, November 5

KEYNOTE SPEAKER

Designing Gut Microbes for Diagnostics and Therapeutics
Pamela Silver
Harvard Medical School, Systems Biology, Cambridge MA

The engineering of Biology presents vast opportunities for therapeutic design, diagnosis, prevention of disease and solutions to environmental problems. We use what we know from Nature to engineer systems with predictable behaviors. We also seek to discover new natural strategies to then re-engineer. Here, I will present concepts and experiments that address how we approach these problems in a systematic way. In one instance, we engineer components of the gut microbiome to act as both diagnostics and therapeutics. In doing so, we have been able to explore the inflamed gut. We can engineer the same bacteria to secrete toxins that could result in localized killing of pathogens and to act in a communal manner. Lastly, we consider issues around how to safely and effectively deploy engineered probiotics.

SESSION 2: ENGINEERING AND MODELING OF MICROBIAL COMMUNITIES

INVITED SPEAKER

Engineering Synthetic Microbial Consortia Inspired Bythe Herbivore Rumen.
Michelle A. O'Malley
Department of Chemical Engineering, University of California, Santa Barbara, Santa Barbara, CA

Anaerobic microbes evolved to 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) and build synthetic consortia to uncover their interdependencies. Initially, we tracked the development of enrichment cultures from goat fecal pellets grown on four types of substrates: alfalfa stem, bagasse, reed canary grass, and xylan over several generations. We tested the hypothesis that the composition of these enriched consortia would stabilize to match the metabolic requirements needed to degrade each substrate. Metagenomic sequencing of the 16S rRNA (prokaryotes), 18S rRNA (eukaryotes), and ITS (fungi) population within the consortia revealed strong specialization of the microbes during selection, suggesting that the membership of each culture tuned to match the substrate. Using these natural systems as inspiration, synthetic consortia of fungi, bacteria, and methanogens were combined in culture and tested for stability and substrate hydrolysis. In nearly all cases, synthetic consortia demonstrated faster and more complete degradation of cellulosic substrates, as well as a wider range of utilized substrates compared to monocultures. We will further discuss the roles that interwoven metabolism and secondary metabolites play on microbial consortia dynamics, both in natural and synthetic systems. Overall, the stable microbial consortia we identified here directed the formation of synthetic, interdependent communities via a bottom up approach to compartmentalize biomass-degradation and bioproduct formation.

Spurious Associations in Microbiome Studies.

Rajita Menon[1]*, Vivek Ramanan*[2]*, and Kirill Korolev*[3]

(1)Vedanta Biosciences, Cambridge, MA, (2)Swarthmore College, Swarthmore, PA, (3)Physics, Boston University, Boston, MA

Microbiota contribute to many dimensions of host phenotype, including disease. To link specific microbes to specific phenotypes, microbiome-wide association studies compare microbial abundances between two groups of samples. Abundance differences, however, reflect not only direct associations with the phenotype, but also indirect effects due to microbial interactions. We found that microbial interactions could easily generate a large number of spurious associations that provide no mechanistic insight. Using techniques from statistical physics, we developed a method (DAA) to remove indirect associations and applied it to the largest dataset on pediatric inflammatory bowel disease. Our method corrected the inflation of p-values in standard association tests and showed that only a small subset of associations is directly linked to the disease. Direct associations had a much higher accuracy in separating cases from controls and pointed to immunomodulation, butyrate production, and the brain-gut axis as important factors in the etiology of inflammatory bowel disease.

INVITED SPEAKER

Oscillatory Population Dynamics in a Bacterial Cross-Protection Mutualism.

Arolyn Conwill[1] *and Jeff Gore*[2]

(1)Institute for Medical Engineering and Science (IMES), Massachusetts Institute of Technology, Cambridge, MA, (2)Department of Physics, Massachusetts Institute of Technology, Cambridge, MA

Cooperation between microbes can enable microbial communities to survive in harsh environments. Enzymatic deactivation of antibiotics, a common mechanism of antibiotic resistance in bacteria, is a cooperative behavior that can allow resistant cells to protect sensitive cells from antibiotics. Understanding how bacterial populations survive antibiotic exposure is important both clinically and ecologically, yet the implications of cooperative antibiotic deactivation on both population and

evolutionary dynamics remain poorly understood, particularly in the presence of more than one antibiotic. Here, we show that two *Escherichia coli* strains can form an effective cross-protection mutualism, protecting each other in the presence of two antibiotics (ampicillin and chloramphenicol) so that the co-culture can survive in antibiotic concentrations that inhibit growth of either strain alone. Moreover, we find that daily dilutions of the co-culture lead to large oscillations in the relative abundance of the two strains, with the ratio of abundances varying by nearly four orders of magnitude over the course of the three-day period of the oscillation. A simple mechanistic model is consistent with our experimental results. Furthermore, migration between population patches in a spatially structured environment can change the period of population oscillations, influencing the mutualism's ability to survive in a harsh environment or expand into new territory. Together, these results suggest that the interplay between ecological interactions and spatial structure may have important consequences for microbial communities, particularly in cases where there are underlying population oscillations.

An Integrated Multi-Scale Modeling Approach for ^{13}c Metabolic Flux Analysis in Microbial Communities.

Maciek R. Antoniewicz
Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

Syntrophy, or cross-feeding, is the co-existence of two or more microbes whereby one feeds off the products of the other. Recently, we have developed an integrated multi-scale flux modeling approach that allows us, for the first time, to dissect interactions in microbial communities using ^{13}C tracers. Specifically, to quantify metabolism and identify cross-feeding interactions we have developed a compartmental multi-scale ^{13}C metabolic flux analysis (^{13}C-comMFA) approach that quantifies metabolic fluxes for multiple cell populations in microbial communities without separation of cells or proteins. In this presentation, I will illustrate our investigations of metabolic interactions between *E. coli* auxotrophs that are unable to grow on glucose in minimal medium by themselves, but can grow on glucose when cultured together. Using our novel ^{13}C-comMFA flux analysis tool we have quantified metabolic interactions (i.e. metabolite cross-feeding) in four distinct synthetic *E. coli* co-cultures. We also applied adaptive laboratory evolution to elucidate how syntrophic interactions evolve and are strengthened through adaptive co-evolution of co-cultures. Overall, the methods we have developed for studying microbial communities enable a broad new area of investigations, allowing us and others to dissect complex microbial systems that are of significant importance in biology but cannot be investigated with current tools. More broadly, by better understanding syntrophic relationships at the genetic, molecular, cellular and systems levels we are generating new knowledge on microbial syntrophy that enables us to ensemble synergistic interactions in engineered microbial communities for novel applications.

Spatial Metagenomic Characterization of Microbial Biogeography in the Gut.

Ravi U. Sheth *and Harris H. Wang*
Department of Systems Biology, Columbia University, New York, NY

Many microbial communities, including the mammalian gut microbiome, display intricate spatial organization at microscopic scales. Mapping this bacterial spatial structuring enables the delineation of fundamental ecological processes and interactions within the community. Understanding these behaviors is crucial to rational design of microbiome perturbations, such as dietary changes or administration of defined microbial consortia. However, current approaches have a limited capacity to measure the spatial organization of natural microbiota composed of hundreds of species. We have

developed “spatial metagenomics”, a framework to dissect the organization of a microbiome at micron-scale spatial resolution and metagenomic depth through nucleic acid “plot sampling”. Intact microbiome samples are immobilized within a gel matrix and subjected to cryo-fracturing to generate clusters of co-localized cells, and the identities and abundances of taxa present in these clusters are determined via droplet-based encapsulation and deep sequencing. Analysis of thousands of microbiome clusters from the mouse intestine across three distinct regions revealed heterogeneous microbial distributions with positive and negative co-associations between specific taxa. In addition, we identified taxa with distinct spatial organization, such as *Lachnospiraceae* which forms self-aggregating clusters in the cecum. Together, our results reveal fascinating distributions of locally structured microbes at anatomically distinct compartments along the mammalian gut. Spatial metagenomics constitutes a powerful and new culture-independent technique to rapidly understand complex microbiota ecology and to better develop rational perturbations of microbiomes.

INVITED SPEAKER

Tami Lieberman
Massachusetts Institute of Technology

Mechanical Forces and Constraints Shape the Gut Microbiota.

Carolina Tropini *and Justin Sonnenburg*
Stanford University, Stanford, CA

The consortium of microbes living in and on our bodies is intimately connected with human biology, and deeply influenced by abiotic factors such as osmolality, pH, or temperature. Despite incredible gains in describing this community, and emerging knowledge of the mechanisms linking it to human health, understanding the basic physical properties and responses of this ecosystem has been comparatively neglected. Importantly, the physical environment plays an essential role in determining which species may thrive in a healthy or diseased host, and strongly affects the success of community assembly and engineering.
In our studies we assessed the resilience of the gut ecosystem to osmotic perturbation at multiple length and time scales (Tropini et al, in press, Cell). Osmotic stress caused consistent, lasting changes to human and mouse microbiotas in a mouse model, leading to extinction of highly abundant taxa, and expansion of less prevalent members. We further found that the repopulation of extinct microbiota members was dependent on re-equilibration of the gut environment, rather than the residing microbiota community.
These findings demonstrate that even mild osmotic disturbance can cause lasting changes to the microbiota and host, and lay the foundation for engineering communities resilient to common perturbations.

Multi-Species Co-Culture Platform to Enable Spatial Analysis of Microbial Communication.
Christopher A. Vaiana[1]*, Aya G. Halawi*[2]*, Christopher A. Voigt*[3]*, and Cullen R. Buie*[4]
(1)Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, (2)Massachusetts Institute of Technology, Cambridge, MA, (3)Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, (4)Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA

Microbes interact within a "social network" to accomplish tasks that are not possible in isolated monoculture. Spatially interacting microbes often form complex patterns that improve survival and function of the group. Understanding how natural communities interact will enable synthetic biologists to engineer designer communities that can accomplish higher order tasks. Co-culture studies often rely on chemical diffusion on agar plates or between two chambers separated by porous membranes. Herein, we demonstrate a multi-well porous hydrogel platform that enables bacterial multispecies co-culture. The hydrogel physically isolates individual cultures while allowing media and small molecule exchange. Recovery of liquid culture wells after growth can be fed into existing bio-analysis pipelines such as cytometry, mass spectroscopy, and sequencing to obtain spatially resolved information. We use this growth chamber to demonstrate diffusion-driven spatial communication between engineered quorum sender-receiver pairs. We are also developing protocols to study the co-culture of human skin microbiome derived communities that include *C. acnes* and *S. epidermidis*. Overall, we present the unique capability of spatially-resolved liquid multi-species co-culture.

SESSION 3: COMPUTATIONAL MODELS FOR DESIGN

INVITED SPEAKER

Personalized Modeling of the Host-Gut Microbiome Axis and Its Role in Multifactorial Diseases.
Almut Heinken[1]*, Laurent Heirendt*[1]*, Federico Baldini*[1]*, Ronan M.T. Fleming*[2]*, and Ines Thiele*[1]
(1)Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg, (2)Leiden Academic Centre for Drug Research, Leiden University, Leiden, Netherlands

The human gut microbiome plays an important role in human health. It has been implicated in the etiology of complex, multifactorial diseases such as type II diabetes and inflammatory bowel diseases. To gain insight into the role of the microbiome in disease states, we have developed a constraint based modeling framework that enables the personalized, mechanistic prediction of the human-gut microbiome-diet axis.
Recently, we published AGORA, a resource of curated genome-scale metabolic reconstructions for 773 common gut microbial strains. We retrieved strain-level relative abundances from metagenomics data from a cohort of pediatric inflammatory bowel disease (IBD) patients with and without dysbiosis and healthy control children (108 samples in total) and constructed a personalized microbiome model for each sample using the corresponding AGORA reconstructions. Subsequently, we predicted for each microbiome model the quantitative biosynthesis potential for all secreted metabolites as well as the strain-level contributions to each metabolite in each individual microbiome. The computed metabolic profiles of IBD patient microbiomes with dysbiosis were distinct from both IBD microbiomes without dysbiosis and healthy control microbiomes. The predicted amino acid secretion profiles agreed well with fecal metabolomics data from the same individuals.
In another study, we used metagenomics data and metadata from 149 healthy adults to individually parametrize a combined model of the human whole-body model, Harvey, and personalized gut

microbiome models. We computed the neurotransmitter production potential of the 149 individuals in the presence and the absence of a microbiome and found it to be significantly increased due to microbial activity. The combined multi-scale model thus allows for the computation of the gut-brain axis.
In summary, we have developed resources and tools that allow for the mechanistic prediction of individual-specific host-microbiome-diet interactions. Future applications include the prediction of potential therapeutic interventions targeting the microbiome.

Systematic Dissection of Sequence Elements Controlling sigma70 Promoters Using a Genomically-Encoded Multiplexed Reporter Assay.

Guillaume Urtecho[1]*, Arielle D. Tripp*[2]*, Kimberly Insigne*[3]*, Hwangbeom Kim*[4]*, and Sriram Kosuri*[5]
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Promoters are the key drivers of gene expression and are largely responsible for the regulation of cellular responses to time and environment. In *E. coli* , decades of studies have revealed most, if not all, of the sequence elements necessary to encode promoter function. Despite our knowledge of these motifs, it is still not possible to predict the strength and regulation of a promoter from primary sequence alone. Here we develop a novel multiplexed assay to study promoter function in *E. coli* by building a site-specific genomic recombination-mediated cassette exchange (RMCE) system that allows for the facile construction and testing of large libraries of genetic designs integrated into precise genomic locations. We build and test a library of 10,898 σ70 promoter variants consisting of all combinations of a set of eight -35 elements, eight -10 elements, three UP elements, eight spacers, and eight backgrounds. We find that the -35 and -10 sequence elements can explain approximately 74% of the variance in promoter strength within our dataset using a simple log-linear statistical model. Neural network models can explain greater than 95% of the variance in our dataset, and show the increased power is due to nonlinear interactions of other elements such as the spacer, background, and UP elements. Ultimately, this work has presented a novel platform to rapidly characterize genomically-encoded *E. coli* promoters and may facilitate the development of more generalized *E. coli* promoter models.

INVITED SPEAKER

Framework for Rational Donor Selection in Fecal Microbiota Transplant Clinical Trials.

Claire Duvallet[1]*, Caroline Zellmer*[2]*, Pratik Panchal*[2]*, Shrish Budree*[2]*, Majdi Osman*[2]*, and Eric Alm*[1,3]
(1)Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, (2)OpenBiome, (3)Center for Microbiome Informatics and Therapeutics

Fecal Microbiota Transplantation (FMT) is the transfer of healthy gut bacteria through whole stool into an individual with a diseased microbiome. Early clinical successes combined with emerging research linking the microbiome to many diseases are driving enthusiasm for FMT as a clinical treatment for complex conditions. However, preliminary trials in new indications have yielded mixed results and suggest that heterogeneity in donor stool may play a role in patient response. Thus, as FMT expands to complex diseases, clinical trials with randomly-selected donors may fail because an ineffective donor was chosen rather than because FMT is not appropriate for the indication. Here, we describe a framework to guide rational donor selection to increase the likelihood that FMT clinical trials will

succeed. We argue that the mechanism by which the microbiome is hypothesized to be associated with a given indication should inform how donors are selected for FMT trials, and we describe different disease models which may underlie microbiome-mediated conditions. We describe strategies to rationally select donors for each type of disease model, and provide examples based on previously published FMT trials and ongoing investigations. Finally, we discuss considerations involved in performing discovery-based retrospective research after an FMT clinical trial concludes. Employing rational donor selection for FMT trials will thus ensure that microbiome science reaches its potential to impact patients and lead to development of microbiome-based therapeutics swiftly and efficiently.

Engineering of *Lactobacillus Reuteri* As a Biotherapeutic Delivery System.

Laura Ortiz-Vélez[1]*, Laura Schaefer*[1]*, Annie Goodwin*[2]*, Min-Shan Chen*[3]*, Noah Shroyer*[4]*, James Ferrara*[5]*, Jeff Tabor*[6]*, and Robert Britton*[7]

(1)Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, (2)Gastroenterology, Baylor College of Medicine, Houston, TX, (3)Baylor College of Medicine, Houston, TX, (4)partment of Department of Medicine Section of Gastroenterology and Hepatology, Baylor College of Medicine, Houston, TX, (5)Pediatric Hematology-Oncology, The Mount Sinai Hospital, New York, (6)Department of Bioengineering, Rice University, Houston, TX, (7)Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

Our ability to engineer biologically relevant microorganisms to fight diseases of the gastrointestinal tract (GIT) is exponentially increasing. *Lactobacillus reuteri* 6475 (*LR*) is a probiotic strain with desirable features for therapeutic delivery purposes due to its adaptation to the human GIT and remarkable safety profile. Our goal is to develop a robust platform for efficient delivery of therapeutic proteins to the GIT by engineering LR to precisely secrete proteins with therapeutic purposes such the human cytokine interleukin-22 (IL-22). This cytokine has proven to confer colonization resistance against enteric pathogens in mice and significantly improve wound healing in murine diabetic models. IL-22 delivered by LR is being explored as a therapeutic tool in response to diseases such as graft versus host disease and wound-healing disorders. First, to achieve precise control of protein production, we designed and developed libraries of gene expression tools in LR, generating a ribosomal binding site (RBS) (1000 fold increase) and promoter library (10,000 fold increase) for the expression of green fluorescent protein. Next, to generate a strain secreting active IL-22 we explored using several signal peptides to improve signal peptide cleavage and reduce its proteolysis, which yielded an *LR* strain producing up to 2µg/ml of IL-22. The bioactivity of IL-22 has been confirmed *in vitro* through the induction of IL-10 in colo205 colonic cells and through the production of antimicrobial peptides from human enteroids. IL-22 secreted *LR* also stimulates expression of intestinal Reg3γ in mice, demonstrating its *in vivo* activity. In conclusion, we have been able to generate an *LR* that produces active IL-22, and we are currently working on evaluating its therapeutic value in a mouse model of epithelial injury.

Tuesday, November 6

KEYNOTE SPEAKER

Elhanan Borenstein
University of Washington

SESSION 4: ENGINEERING OF THE ORGANISM AND TOOLS TO MANIPULATE IT

INVITED SPEAKER

Cynthia Collins
Rensselaer Polytechnic Institute

Probiotic Associated Therapeutic Curli Hybrids (PATCH).

Pichet Praveschotinunt[1], *Anna Duraj-Thatte*[1], *Ilia Gelfat*[2], *Franziska Bahl*[3], *David B. Chou*[4], *and Neel Joshi*[5]
(1)School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, (2)John A. Paulson School Of Engineering And Applied Sciences, Harvard University, Cambridge, MA, (3)Albert Ludwigs University of Freiburg, Freiburg im Breisgau, Germany, (4)Wyss Institute for Biologically Inspired Engineering, Boston, MA, (5)Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA

There is an unmet need for new treatment methods for inflammatory bowel disease (IBD) that can reliably maintain remission without leading to detrimental side effects. The use of probiotic bacteria has been utilized as an alternative treatment of IBD albeit with low efficacy. We genetically engineered *Escherichia coli* Nissle 1917 (EcN), a widely used probiotic strain with moderate therapeutic efficacy against colitis, to create an anti-inflammatory fibrous matrix *in situ* inside the gastrointestinal tract. This matrix consists of EcN-produced curli nanofibers displaying trefoil factors (TFFs) domains, derived from a family of human cytokines that promote intestinal barrier function and epithelial restitution. We confirmed that engineered EcN was able to secrete the curli-fused TFFs *in vitro* and *in vivo* within the mouse gut, and that the enabling genetic programming did not alter EcN's non-pathogenic nature. We observed an enhanced protective effect of EcN expressing curli-fused TFFs against dextran sodium sulfate (DSS) induced colitis in a murine model when compared to wild-type EcN. We associated the observed therapeutic effects with barrier function reinforcement and immunomodulation induced by the engineered matrix. This work sets the foundation for the development of a novel therapeutic platform for not only IBD, but also other gut related diseases, in which the *in situ* production of a therapeutic protein matrix from probiotic bacteria can be exploited.

Utilizing CRISPR-Based Genome Editing for Microbiome Engineering (oral).
***Jonathan W Kotula**, Joel D Berry, Stephen Smith, Agnes Oromi-Bosch, and Steven B. Kanner*
Caribou Biosciences, Berkeley, CA

Microbial Engineering, Caribou Biosciences, Berkeley, CA
Interactions between the microbiota of the gastrointestinal tract and the host significantly impact systemic processes and may alter disease progression. The combined metabolic potential of the trillions of microbes that live within the human gastrointestinal tract is on par with metabolic organs such as the liver. This microbial metabolic organ impacts everything ingested from food to therapeutics, altering the nutrients absorbed, the efficacy of drugs, and systemic signal transduction pathways. New studies demonstrating associations between microbiome composition and disease state are being added to the literature at a rapid pace. The majority of these publications identify microorganisms that correlate with specific outcomes through increasingly advanced sequencing techniques. Demonstrating causation requires a means to test the hypotheses generated from association studies with genetic experiments. Currently established tools and protocols do not enable the facile genetic manipulation of a majority of these implicated organisms. At Caribou Biosciences, we are leveraging our experience and expertise with CRISPR systems to develop tools and protocols for performing reliable and robust genomic modifications in microorganisms of the microbiome. These tools enable the rapid evaluation of association hypotheses and will identify causative metabolic pathways, which could lead to the identification of therapeutic engineering targets. We are employing a mix of strategies that include CRISPR-based recombination and selection, CRISPRi-mediated metabolic pathway engineering, and phage engineering.

INVITED SPEAKER

Next-Gen Genome Engineering Tools for the Gut Microbiome.
Harris Wang
Systems Biology, Columbia University, New York, NY

Recent advances in genome engineering have led to a modernized toolbox for studying basic microbial physiology and altering their metabolism in bioindustrial settings. However, complex multi-species communities that naturally exist in open and changing environments are still challenging to study and manipulate, including the mammalian gut microbiome. This talk discusses emerging tools to program the whole gut microbiome. First, I describe an *in situ* microbiome engineering platform using directed horizontal gene transfer to deliver new traits into the native gut microbiota of a living animal. New bacteria chasses from the mammalian gut can be genetically enhanced as better personalized probiotics. Second, using metagenomic mining, *de novo* DNA synthesis, and deep-sequencing, I describe our efforts to generate libraries of new genetic parts for a variety of bacteria. These parts can be used to leverage natural differences in bacterial gene-expression capacities to program species-selective circuits that function in specified members of a complex microbiome. Finally, I describe a new tool to maintain the sequence integrity of engineered genetic elements and provide biocontainment of recombinant DNA from unintended release into the environment. Integration of these methods will lead to next-generation capabilities to manipulate, control, and contain engineered genetic circuitry and modified cells in the gut microbiome for many emerging applications.

A Stealth-Based Approach to Evade Restriction-Modification Systems during Genetic Engineering: Lessons from Bacteriophage.

Christopher D. Johnston*, Sean Cotton, Susan R. Rittling, Jacqueline R. Starr, Gary Borisy, Floyd Dewhirst, and Katherine P. Lemon*
The Forsyth Institute, Cambridge, MA

Restriction-modification systems hinder the use of genetic approaches in the vast majority of bacteria and exhibit strain-level variation. Inspired by a bacteriophage anti-restriction mechanism, we developed a systematic approach for directed evolution of genetic tools to evade bacterial restriction-modification (RM) systems using stealth-by-engineering. In this process, we determine the genome and methylome of a bacterial strain and use this information to define the bacterium's RM target motifs. We then synonymously eliminate RM targets from the nucleotide sequence of a genetic tool *in silico*, synthesize an RM-silent 'SyngenicDNA' tool and propagate the tool as novel minicircle plasmids, termed SyMPL tools, before transformation. Using SyngenicDNA and SyMPL tools, we achieved a profound, >100,000-fold, improvement in the transformation of a clinically relevant USA300 strain of *Staphylococcus aureus* demonstrating the efficacy of these approaches for evading RM systems. The SyngenicDNA and SyMPL approaches are effective, flexible, and should be broadly applicable. We expect these will facilitate a new era of microbial genetics free of the restraints of restriction-modification barriers.

A Modular Platform for Antibody-Targeted Bacterial Delivery.

Ava M. Vargason *and Aaron C. Anselmo*
Pharmaceutical Sciences, University of North Carolina, Chapel Hill, NC

Bacteria-based therapies such as drug-producing genetically engineered bacteria, consortiums of spores, fecal transplants, and probiotics hold promise for the treatment of various enteric diseases (including *Clostridium difficile* infection, *Vibrio cholerae* infection, and inflammatory bowel disease). Current research has demonstrated that interactions between therapeutic bacteria, pathogens and the host environment can dictate the efficacy of these bacteria-based therapies. This includes both direct and indirect interactions, such as cell-to-cell contact, pathogen niche exclusion, resource competition, and the secretion of antimicrobial molecules. Despite the importance of bacteria-host interactions, advanced strategies to modulate them are lacking and, as such, approaches to engineer bacterial cells to interact with the host environment in specific ways are warranted. Here, we describe the development of a modular approach to enable direct binding of therapeutic bacteria to specific receptors encountered in the GI tract. Three bacterial strains – *Escherichia coli, Bacillus coagulans,* and *Lactobacillus casei* – were functionalized with antibodies specific for intestinal cells. We show that functionalization does not impair bacteria growth or viability and facilitates enhanced binding to targeted surfaces under both static and microfluidic conditions. Using a model antibody against inflammation, anti-CD54, we additionally show that this platform can be used to increase binding to intestinal cells by over 3-fold *in vitro* as compared to a non-targeted control. This cell surface engineering approach can be broadly applied to existing therapeutics, including spores and consortiums, without modification. As direct conjugation only alters the cell exterior, it remains complementary with current genetic engineering strategies while being immune to their challenges, such as safety concerns and novel plasmid development. Targeting of bacterial cells can potentially enable spatiotemporal control of therapeutic bacteria in the GI tract, increasing their concentration and residence time at sites of disease (e.g. enteric inflammation) or infection (e.g. *Salmonella* or *enteropathogenic E. coli*) for improved efficacy.

Multiplexed Characterization of Regulatory Components in Diverse Bacterial Cell-Free Expression Systems.

Nathan Johns[1]*, Sung Sun Yim*[1]*, and Harris H. Wang*[2]
(1)Systems Biology, Columbia University Medical Center, New York, NY, (2)Department of Systems Biology, Columbia University, New York, NY

Expansion of synthetic biology approaches to new organisms requires development of genetic and regulatory tools used for construction of functional genetic circuits. Cell-free expression systems dramatically simplify the prototyping of genetic designs *in vitro*. However, the small number of simultaneous measurements that can be made by using color or fluorescence as readout limits the application of these systems to understand the complex behavior of regulatory sequences. Here we devised a method to measure gene expression levels from thousands of regulatory sequences in a single reaction by sequencing, and applied this method to active cell-free transcription systems developed from ten phylogenetically and ecologically diverse bacterial species. Interspecies analysis of transcriptional profiles from diverse regulatory sequences revealed functional differences in gene expression that could be predictively modeled. We anticipate that this multiplexed approach using cell-free expression systems will expand the capacity for genetic circuit prototyping in new bacterial chassis.

INVITED SPEAKER

Genetic Technologies to Engineer and Understand the Microbiome.

Mark Mimee
Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge, MA

The microbes that inhabit the human body are integral to human health and disease: from inflammatory bowel disease to autism, metabolic syndrome to cancer. Due to its high connectivity with human physiology, manipulation of the microbiota has therapeutic potential across multiple physiological axes. Here, I will discuss synthetic biology strategies that can be applied to engineer and better understand the microbiota, including genetic modification of commensal organisms, bacteriophage engineering, and micro-bio-electronic sensors to monitor gastrointestinal health. These efforts set the stage for basic mechanistic studies of host-microbe interaction, as well as translational efforts to advance cellular and viral microbiome therapies to the clinic.

INVITED SPEAKER

Repurposing Bacterial Two-Component Systems As Sensors for Microbiome Applications.

Jeffrey J. Tabor
Department of Bioengineering, Rice University, Houston, TX

Bacterial two-component systems (TCS) are the largest family of signal transduction pathways in nature, and a treasure trove of sensors for synthetic biology applications such as the engineering of diagnostic and therapeutic gut bacteria. However, most TCSs remain uncharacterized or difficult to harness as sensors. Major challenges include the fact that most TCS output promoters are unknown, cross-regulated, or silent in heterologous host organisms, and that TCS input detection thresholds are often mismatched with application needs. We have developed a series of synthetic biology technologies to overcome these limitations, and are now utilizing TCSs for a range of microbiome applications. In particular, we have developed a general method to rewire TCSs to synthetic output promoters by

swapping the DNA binding domains (DBDs) of response regulator proteins. We have shown that DBD swapping enables TCSs to be functionally ported to new hosts where they are otherwise silent, eliminates unwanted cross-regulation, and facilitates discovery of TCS inputs. Furthermore, we have developed a novel method called "TCS tuning" whereby we introduce mutations that specifically alter the phosphatase activity of sensor kinases to tune the detection thresholds over two orders of magnitude while leaving the major sensory function in-tact. Finally, we have demonstrated the use of TCS sensors to engineer diagnostic gut bacteria in animals and are now performing trials with human samples.

Living Materials in the Gut.

Neel Joshi[1]*, Anna Duraj-Thatte*[2]*, Pichet Praveschotinunt*[2]*, Noémie-Manuelle Dorval Courchesne*[1]*, and Peter Nguyen*[3]

(1)Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, (2)School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, (3)Wyss Institute of Biologically Inspired Engineering, Harvard University, Cambridge, MA

Recent efforts to use living microbes as therapeutics have uncovered several new challenges in rationally manipulating cellular behavior. Compared to laboratory conditions, host organisms are an inhospitable environment where small changes in organismal fitness or behavior could dramatically affect factors like residence time, trafficking, and metabolic function. We have developed tools to control the microbial delivery of therapeutic proteins and to study microbial localizations in vivo. The talk will focus on a platform for the creation of a living material, capable of forming a nanofiber network in situ and persisting in the colon for extended periods. The material is in a hydrogel form that facilitates delivery to the gut, where it self-renews and serves as a mucin mimetic. This is enabled by the secretion of self-assembling fiber networks that can be tailored to adhere to specific tissues and dampen inflammatory processes. The talk will also explore applications of this platform in therapeutic delivery and non-invasive microbial tracking in mouse models.

INVITED SPEAKER

Engineering of Probiotic *E. coli* to TREAT Phenylketonuria Using a NOVEL Drug Discovery Paradigm.

Paul Miller

Synlogic Inc, Cambridge, MA

The fields of synthetic biology and microbiome research have developed significantly over the last decade. The convergence of these disciplines is enabling the development of new therapeutic strategies using engineered microbes that operate from within the host as living medicines. Inborn errors of metabolism represent candidate diseases for these therapeutics, particularly where a toxic metabolite causing a syndrome is accessible from the intestinal lumen. Phenylketonuria (PKU), a rare inherited disease caused by a defect in phenylalanine hydroxylase (PAH) activity, is one such disease and is characterized by the accumulation of systemic phenylalanine (Phe) leading to severe neurological deficits unless patients are placed on a strict low-Phe diet. As an alternative treatment, *Escherichia coli* Nissle (EcN), a well-characterized probiotic, was genetically modified to efficiently import and degrade Phe, resulting in the candidate strain SYNB1618. The coupled expression of a Phe transporter with a cytoplasmically-expressed Phe ammonia lyase (PAL) in SYNB1618 allowed rapid conversion of Phe into trans-cinnamic acid (TCA), which was further metabolized by the host to hippuric acid (HA) and excreted

quantitatively in the urine. Experiments conducted in the *enu2-/-*PKU mouse showed that the oral administration of SYNB1618 significantly reduced blood Phe levels in a model of recirculating Phe. Decreases in circulating Phe levels were associated with proportional increases in urinary HA, confirming that Phe metabolism was caused by the engineered pathway in SYNB1618. Subsequent studies showed that SYNB1618 is similarly operative in non-human primates, providing an important translational link to inform human clinical studies. Recently, biomarker data from safety and tolerability studies in healthy volunteers provided clear evidence of engineered strain function in humans in a dose-responsive fashion, confirming the predictive value of the drug discovery cascade used to select SYNB1618. Further evaluation of SYNB1618 as a potential therapy for the treatment of PKU is therefore warranted.

Applying Advanced Synthetic Engineering Capabilities for Developing Phage Cocktail.

Lior Zelcbuch
BiomX, Ness Ziona, Israel

BiomX is a microbiome drug discovery company developing customized phage therapies that seek and destroy harmful bacteria in chronic diseases such as IBD, liver disease and cancer. We discover and validate proprietary bacterial targets and customize our natural and engineered phage compositions against these targets. In this talk we will present how we apply synthetic biology capabilities that include “reprogramming” of lysogenic (dormant) phage to a strictly lytic (active) mode, and the expansion of the phage host range to both achieve eradication of a wider array of bacterial strains and overcome bacterial resistance.

POSTER SESSION

A High-Throughput Genetics Approach Reveals Underlying Complexity of Salmonella Phage-Host Interactions.

Benjamin A. Adler[1,2]*, Crystal Y. Zhong*[3]*, Adam M. Deutschbauer*[4]*, Vivek Mutalik*[4]*, and Adam P. Arkin*[5]
(1)Bioengineering, University of California, Berkeley, Berkeley, CA, (2)UC Berkeley-UCSF Graduate Program in Bioengineering, Berkeley, CA, (3)Bioengineering, University of California, Berkeley, Berkeley, (4)Lawrence Berkeley National Lab, Berkeley, CA, (5)Bioengineering, UC Berkeley, Berkeley, CA

Salmonella species comprise a chronic threat to human health, with over 1.2 million infections and 450 deaths occurring in the US per year due to foodborne Salmonella infection. With the rise of antibiotic resistant infections and no vaccine to foodborne infections, it is a health imperative to develop alternative prevention and treatment strategies. Here, Salmonella bacteriophages present a specific, efficacious mode to combat Salmonella infection. By treating raw foods with phage, suppliers can prevent Salmonella infection from occurring in the human host. Alternatively, by treating infected individuals, Salmonella phages present an avenue for therapy. However, phage-host interactions remain poorly understood in the genomics era, slowing widespread practice. Here, we employed high-throughput, functional analyses to obtain an in-depth perspective of the phage-host interactions between a model Salmonella species, *Salmonella enterica serovar Typhimurium* (*S. typhimurium*) and its phages. To rapidly assess genetic contributions to phage sensitivity, we created a 66,000 member randomly barcoded transposon (RB-TnSeq) library in *S. typhimurium LT2 MS1868*. Using this library, we compared fitness across four of its industrially-adopted biocontrol phages. Despite 3 of these 4 phages binding directly to the lipopolysaccharide (LPS) layer, this approach yielded structural-level insight into the specific recognition elements of the host LPS layer. In addition, we discovered multiple other unreported modes of resistance, ranging from central metabolism to ion flow, indicative of the many complex interactions that occur between phage and their hosts. The high throughput genetic approach between Salmonella and biocontrol phages employed here, elucidates a deeper understanding of host physiology and its interactions with its phages. This work additionally reveals greater depth into the failure modes of Salmonella phage, which will enable better rational design of phage-based biocontrol treatments.

Development and Characterization of a Genetically Engineered *E. coli* nissle for the Treatment of Phenylketonuria.

Mark Charbonneau
Synlogic Inc, Cambridge, MA

Phenylketonuria is a genetic disease characterized by the inability to metabolize phenylalanine (Phe), which can result in neurotoxicity. To provide a potential alternative to a protein-restrictive diet, we engineered a strain of *Escherichia coli* Nissle 1917 to express genes encoding two distinct Phe metabolizing enzymes, L-amino acid deaminase (LAAD) and phenylalanine ammonia lyase (PAL), with the latter under the control of an anaerobically-induced promoter to drive activity in the mammalian intestinal tract. To characterize the metabolic activity of the synthetic strain, SYNB1618, we developed an *in vitro* simulation (IVS) model to recapitulate physiological parameters of the human upper gastrointestinal tract. IVS studies of SYNB1618 revealed a temporal response to simulated upper gastrointestinal transit that included increased expression of the Phe transporter, *pheP*, suggesting a predisposition to metabolize luminal Phe. We also demonstrated that SYNB1618 consumes Phe over a

period of several hours and confirmed that the products of Phe metabolized by SYNB1618 include phenylpyruvic acid (PPA; produced by LAAD) and *trans*-cinnamic acid (TCA; produced by PAL). Administration of SYNB1618 in the context of a phenylketonuria mouse model ($Pah^{enu2/enu2}$) reduced blood Phe concentration by 38% compared to administration of unmodified *E. coli* Nissle. In mice and primates, TCA was quantitatively metabolized to hippuric acid (HA) and excreted in the urine, enabling the use of HA as a predictive biomarker of strain activity. In healthy cynomolgus monkeys, administration of SYNB1618 resulted in dose-dependent increases in urinary and serum HA, as well as other relevant serum metabolites. In addition, dosing cynomolgus non-human primates (NHPs) with SYNB1618 inhibited increases in serum Phe after an oral Phe challenge. These studies indicate that SYNB1618 can impact Phe metabolism in mice and monkeys, demonstrating potential for the treatment of metabolic disorders with genetically engineered bacteria.

Elucidating and Modeling Interactions of Inulin-Consuming Consortia Derived from Consecutive Passages of Human Gut Microbiome.

Ming-Hsu Chen[1]*, Tianming Yao*[1]*, Doraiswami Ramkrishna*[2]*, and Stephen Lindemann*[1]
(1)Food Science, Purdue University, Lafayette, IN, (2)Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN

Inulin-type fructans, a plant storage carbohydrate, has been industrially manufactured and utilized as a prebiotic food ingredient and supplement. In the human gastrointestinal tract, inulin resists host digestive enzymes but can be utilized by colonic microorganisms. While the bifidogenic effect of inulin has been validated, the identity and the number of gut bacterial types that are involved in inulin digestion are still not clear.
In this study, we used a consecutive passage approach to identify and characterize inulin-consuming bacteria and their interactions. Batch fecal fermentations were conducted in Balch-type tubes using two phosphate buffered media, either fortified with 200 µm amino acids and 1% ATCC vitamin supplement or not fortified. The cultures were passaged daily 6 times at a 1:100 ratio to remove the non-substrate consuming population. Cells were collected from the final consortia and their compositions were examined using 16S rRNA gene sequencing. The results demonstrated that *Klebsiella pneumoniae* (85% of the read total) dominated the inulin-consuming consortia in the absence of nutrient fortification; whereas, when exogenous vitamins and amino acids were present, an *Escherichia* sp. (38%) and *Bifidobacterium dentium* strain (60%) became dominant. Strains matching these three bacterial species were further isolated from microbial consortia using selective MacConkey and MRS media; their substrate consuming capabilities were corroborated using media containing inulin in pure culture fermentation. To describe the ecology of inulin consumption by microbiota, we formulated a mathematical growth model to describe interactions between inulin-consuming organisms and the inulin substrate under multiple nutrient environmental conditions.

Quantifying Metabolite Cross-Feeding through 13C Metabolic Flux Analysis: A Case Study Using E. coli and Salmonella Grown in Co-Culture.

Michael Dahle *and Maciek Antoniewicz*
Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

Identifying and quantifying metabolite exchange is crucial to understanding cooperation and exploitation in microbial ecology. In this work, the full metabolic flux profiles of an auxotrophic E. coli and a cooperative Salmonella enterica were determined, both in isolation and in co-culture. In this

model co-culture developed by the Harcombe lab, the methionine-auxotrophic E. coli cleaves lactose, providing glucose and galactose to Salmonella. In turn, the Salmonella excretes excess methionine that is taken up by E. coli. Metabolic flux analysis using ^{13}C-labeled tracers correctly identified which metabolites were exchanged, while this was not possible by only measuring concentrations of nutrients in the media. We show that both species prefer consuming glucose when present in high concentrations. However, the rate of lactose cleavage keeps the glucose and galactose concentrations in a range wherein glucose and galactose are co-utilized at identical rates. In this work, co-cultures were grown in two different experimental setups: 1) bioreactors and 2) transwell plates. The latter setup allowed us to elucidate the effect of diffusion on co-culture performance. Lastly, we compared metabolic profiles for each monoculture grown on glucose or galactose to the metabolic profiles in co-culture. Knowing only the biomass composition of each species and the combined biomass ^{13}C-labeling, we used our recently developed co-culture methodology to simultaneously resolve the ratio of Salmonella to E. coli and the full metabolic profile for each species.

Engineering Altruism in Nitrogen Self-Sufficient Cocultures of *Azotobacter Vinelandii* and *E. coli*.
Camil A. C. Diaz *and Maciek R. Antoniewicz*
Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

Cocultures are attractive microcosms for studying metabolic interactions in a controlled system. Cocultures involving diazotrophs, or nitrogen-fixing organisms, are of particular interest for both agricultural and industrial applications. While diazotrophs like *Azotobacter vinelandii* have successfully been engineered to produce significant levels of ammonium, these strains also exhibit a high substrate demand and overall inefficient carbon metabolism. Thus, a major challenge in developing stable cocultures with nitrogen-fixers is to identify suitable partners that can meet these high substrate demands while receiving a limited supply of ammonium in return.
Through the manipulation of four key enzymes in the central carbon metabolism of *E. coli*, we engineered a strain capable of converting xylose into glucose. By performing ^{13}C-metabolic flux analysis (^{13}C-MFA), we determined that 34% of the carbon imported by this strain as xylose was ultimately exported as glucose. Furthermore, through adaptive evolution, we further developed a strain that could continue to export glucose even under complete nitrogen starvation. Whole-genome next-generation sequencing enabled us to identify the mutation responsible for the emergence of this altruistic behavior. We are currently targeting additional genes in central metabolism and catabolite regulation to further enhance the glucose output of our *E. coli* strain.
Whereas neither *E. coli* nor *A. vinelandii* can grow independently when provided only xylose and atmospheric nitrogen, we have demonstrated that the coculture of our engineered strains nearly triples in cell density over 24 hours through the active sharing of carbon and nitrogen. Furthermore, by performing ^{13}C-coculture-MFA, we have been able to identify additional cross-fed nutrients that are not otherwise secreted by either organism when grown in isolation. Future work will employ adaptive evolution of our coculture to not only maximize cross-feeding but identify additional traits that enable enhanced symbiosis.

Gene Therapy for the Microbiome: Reprogramming Bacteria in Situ with Engineered Phage.

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Colonizing microbes outnumber human cells, offering substantially more genetic diversity than the human genome. Given the flexibility in target selection and significant impact on host health, the microbiome warrants consideration as a target for gene therapy. To date, there is no effective method to precisely tune the microbiome on a genetic level. Current approaches to microbiome manipulation (antibiotics, lytic phage therapy, probiotics) either lack specificity or durability. We propose engineered lysogenic phage as a novel platform to durably reprogram specific commensals. Lysogenic phage are bacterial viruses that propagate either by hijacking the host's machinery to produce new phage particles (lytic cycle) or by integrating their genetic material into the host genome (lysogenic cycle). By embedding a transgene in the phage genome, we can exploit the lysogenic cycle to transfer the transgene to the bacterial host and enable constitutive expression. Depending on the design of the transgene, we can convert commensals into drug factories, in situ biosensors, or even mucosal vaccine platforms. Initial studies with three clinically relevant genera (Streptococcus, Lactobacillus, Escherichia) indicate that engineered lysogenic phage can durably mediate transgene expression and transfer, as demonstrated using transgene cassettes coding for antibiotic resistance or a nanoluciferase reporter. Ultimately, engineered lysogenic phage has the potential to be a disruptive tool for microbiome modification, enabling us to reprogram our own bacteria to treat disease.

Isolation and Genomic Analysis of Resistant Starch-Degrading Human Gut Microorganism, *Bifidobacterium Adolescentis* P2P3.

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Resistant starch (RS) is a kind of starch, which is not degraded by human digestive enzymes but fermented by gut microbiota. It confers many benefits to human health through the production of short-chain fatty acids (SCFAs). The purposes of this study were 1) to isolate RS-degrading human gut bacteria, 2) to characterize the RS degradation property of the isolated microorganism, and 3) to analyze the whole genome of an RS-degrading human gut bacterium. As a result, *Bifidobacterium adolescentis* P2P3, which can utilize high amylose corn starch (HACS) granules up to 63.3%, was isolated from human feces. *B. adolescentis* P2P3 has an ability to degrade commercially available RS, HM 260, HM 958, NV 330, VF 1490, and VF 2470 by 37.3%, 69.6%. 44.5%, 51,6%, and 54,4%, respectively. This result indicates that *B. adolescentis* P2P3 is a strong RS-degrader in the human gut. The genome of *B. adolescentis* P2P3 is composed of one circular sequence, which is a 2,202,982 bp chromosome with 59.4% GC content. A total of 1,847 genes were identified in the genome, including 1,777 protein-coding genes, 70 RNA genes, and 62 pseudogenes. Four sets of full-length rRNA genes, including 5S, 16S, and 23S, were placed in the genome, and 54 tRNA genes and three non-coding RNA genes were identified. The genes of *B. adolescentis* P2P3 encoding amylolytic enzymes active on α-glucan substrates such as glycogen phosphorylase, 4-α-glucanotransferase, glycogen debranching enzyme, α-amylase, 1,4-α-glucan branching enzyme, glycosidase, pullulanase, α-1, 4-glucan-maltose-1-phosphate maltosyltransferase, and α-glucosidase. In addition, *B. adolescentis* P2P3 demonstrated the ability to stimulate secretion of

Th1 type cytokines from mouse macrophage *in vitro* that was not shown in other *B. adolescentis.* These results suggested that *B. adolescentis* P2P3 is a useful probiotic candidate not only utilizing RS but also having immunomodulatory activity.

Linking Microbial Taxonomic and Metabolomic Profiles: A Case Study Using Stool Samples from Patients with Colorectal Adenomas.

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In recent years, many different human diseases have been shown to be associated with microbiota dysbiosis, and this has spurred numerous studies into developing microbiota-based treatments. However, mechanisms by which microbiota affect host health and disease remain largely unknown, hindering the development of novel therapies. As a means to shed a better light on the underlying mechanisms, multi-omics approaches, e.g. simultaneous microbial taxonomic and metabolomic profiling, have been gaining popularity in microbiome studies. Herein, we analyzed fecal microbial taxonomic and metabolomic profiles of patients with and without colorectal adenomas to reveal possible mechanistic roles of the gut microbiota in colorectal adenoma-carcinoma sequence. We employed various statistical methods to find metabolomic signatures and microbe-metabolite inter-relationships associated with early events of colorectal cancer pathogenesis. We found that lipid metabolites, such as secondary bile acids, sphingolipids and polyunsaturated fatty acids, were significantly enriched in patients with adenomas compared to those without adenomas. Interestingly, some of these metabolic signatures are known to be associated with the colorectal cancer development. A strong inter-omic relationship between the taxonomic and metabolomic data, regardless of presence or absence of adenomas, was observed; this could imply that certain features of the gut microbiota and its chemical microenvironment are tightly intertwined. At the level of individual microbe-metabolite pairs, OTUs belonging to the uncultivated genus *Oscillospira* (phylum Firmicutes) strongly correlated with many different metabolites, including some of the lipid metabolites found to be enriched in patients with adenomas. However, relying solely on statistical analyses, it is hard to tell whether the genus *Oscillospira* is a true and major microbial contributor for the observed cancer-related metabolic signatures. Therefore, this study highlights the needs of novel computational approaches, e.g. genome-scale metabolic modeling, for better inference of true mechanistic taxon-metabolite links, which in turn can be useful information for microbiome engineering.

Assessment of 16S rRNA Amplicon Analysis Bias By Sequencing Platform.

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Microbial community analysis based on 16S rRNA were used widely in the various fields. However there are various sequencing platforms and the microbial profile from different platforms showed different results. The primer sites are also related with amplicon bias. The 16S rRNA gene amplicon with different

primer site showed different result although the sequencing was carried out using same sequencing platforms.
In this study, the sequence reads and microbial profiles were assessed by various way. Four mock community were made with various bacterial genomic DNA and amplified with various pairs of primers. The amplicon were sequenced using Miseq (Illumina), PGM (Thermofisher), Sequel (Pacific bioscience), and MINion (Oxford nanopore). The sequencing error of individual reads were varied by platforms. The sequence reads from Miseq and PGM showed high accuracy. Sequels and Minion also showed good accuracy. These results indicated that recently introduced 600 bp sequencing of PGM can be an excellent alternative for 454 pyrosequecing (Roche) which was widely used previously in the field of metagenomics. The result of microbial profiles from different platforms were calibrated using internal standards. The adjusted microbial profiles showed that the calibration of the microbial profile results from different sequencing platforms can improve the accuracy of microbial profiles and make these microbial profiles from different sequencing platforms compatible with each other.

Utilizing CRISPR-Based Genome Editing for Microbiome Engineering.
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Interactions between the microbiota of the gastrointestinal tract and the host significantly impact systemic processes and may alter disease progression. The combined metabolic potential of the trillions of microbes that live within the human gastrointestinal tract is on par with metabolic organs such as the liver. This microbial metabolic organ impacts everything ingested from food to therapeutics, altering the nutrients absorbed, the efficacy of drugs, and systemic signal transduction pathways. New studies demonstrating associations between microbiome composition and disease state are being added to the literature at a rapid pace. The majority of these publications identify microorganisms that correlate with specific outcomes through increasingly advanced sequencing techniques. Demonstrating causation requires a means to test the hypotheses generated from association studies with genetic experiments. Currently established tools and protocols do not enable the facile genetic manipulation of a majority of these implicated organisms. At Caribou Biosciences, we are leveraging our experience and expertise with CRISPR systems to develop tools and protocols for performing reliable and robust genomic modifications in microorganisms of the microbiome. These tools enable the rapid evaluation of association hypotheses and will identify causative metabolic pathways, which could lead to the identification of therapeutic engineering targets. We are employing a mix of strategies that include CRISPR-based recombination and selection, CRISPRi-mediated metabolic pathway engineering, and phage engineering.

Enabling Member Coexistence and Programming Community Composition in Synthetic Microbial Communities Via Temperature Regulation.
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Synthetic microbial consortia are increasingly being used in biotechnological applications. Features of microbial consortia which enable execution of complex tasks include communication among species via metabolite cross-feeding and signaling molecules, division of labor, and specialization. Synthetic microbial communities therefore offer significant potential advantages compared to single species

systems for numerous applications such as biosynthesis of target compounds through complex pathways.
However, population dynamics, inter-species interactions, and differing ecological niches of resident microorganisms result in several additional layers of complexity that must be addressed to achieve effective and robust synthetic microbial communities. One of the most fundamental challenges is regulation of community composition. At the most basic level, maintaining coexistence of resident community members is required to enable the desired community level functionality. Additionally, community composition often needs to be tuned to optimize overall functionality. This type of microbial community manipulation has not been fully utilized in synthetic biology applications, likely due in part to a lack of available tools.
Here we develop temperature regulation as a general tool to enable coexistence and control community composition in synthetic microbial communities. We demonstrate that rationally selected constant temperature regimes can be used to enable coexistence of species from distinct thermal niches. Furthermore, cycling temperature regimes can be used to regulate relative species abundance in microbial communities. We employ mathematical modeling to design various cycling temperature regimes for desired community compositions and related features. Finally, we interpret our observations within the theoretical ecological framework regarding species coexistence developed over the last century to create a model which is capable of accurately predicting coexistence or competitive exclusion from empirical parameters. In the future we plan to develop a feedback device capable of automated composition control.

Identifying Regulators of Microbiome-Encoded Bile Acid Metabolism.

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Bile acids comprise metabolites at the host-microbial interface: they are synthesized by the host and modified by gut bacteria, beginning with deconjugation by microbiome-encoded bile salt hydrolases (BSH). Bile acids have varied physiologic effects; thus, identifying regulators of bacterial bile acid metabolism (BAM) offers a promising strategy for developing novel clinical approaches with wide-ranging applications. We previously observed that BLAST predictions based on presence of homologs to published BSH sequences in the genomes of strains grown in monoculture predicted BSH activity correctly 85% of the time in vitro. We hypothesized that interspecific interactions are critical in regulating BAM. To test this hypothesis, we generated in silico BAM predictions in mock bacterial communities: the genomes of 42 gut bacterial type strains were annotated using a reference database containing all known BAM genes/enzyme sequences; sum totals of BAM enzymes encoded by the metagenomes of thousands of consortia were calculated; finally, an in silico simulation (that assumed starting bile acid substrates tauro-β-muricholic acid and taurocholic acid, and expression of all metagenome-encoded BAM genes) predicted resulting bile acid pools. To test a subset of these predictions, we adopted our in vitro functional assay, which we developed for characterizing BSH activity of individual strains in monoculture, to characterize BAM in multiple strains that were anaerobically co-cultured in media containing primary bile acid substrates; resulting bile acids were characterized by mass spectrometry. We found that metagenome-based in silico predictions of BAM were imperfect and varied in accuracy by bile acid species, ranging from 38% (deoxycholic acid) to 89% (β-muricholic acid); BSH activity (i.e., deconjugation) was correctly predicted in 67% of test cases — evidencing our incomplete understanding of pertinent genetics/regulation. A comparative genomic

analysis revealed candidate BSH regulators representing diverse metabolic pathways. Together, these data suggest that BAM interaction networks are more extensive than previously imagined.

Miniaturized Next-Generation Sequencing for Microbiome and Metagenomics.
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Our understanding of the role of the human microbiome in health and disease has been growing rapidly in recent years. Next-generation sequencing technology has enabled vast applications and analyses in both microbiome and metagenomics. As researchers continue to deepen our understanding of how microbiome impacts health, as biologists explore the metagenomic space, our tools and analyses need to scale accordingly. In this presentation, we demonstrate miniaturized library preparation of 16S rRNA amplicons and Nextera XT whole genomes for time and cost efficient workflows by utilizing the Labcyte® Echo® 525 Liquid Handler. We show miniaturized reactions produce high-quality data, while using typically one-tenth the volumes described in protocols. This allows researchers to expand and accelerate projects and developments in an efficient manner.

Using Evolver to Construct and Characterize Microbial Communities in Controlled Dynamic Environments.
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Microbiomes are adapted to the diverse and dynamic conditions of natural environments; there is a need for tools for characterizing microbiomes in laboratory conditions that reflect this complexity. While automated culture devices, such as bioreactors, enable highly-controlled experiments, reconfiguring such systems for different growth conditions can be challenging, particularly when scaling to high-throughput. We developed eVOLVER, a DIY automated culture platform that can be easily configured to conduct a wide variety of microbial growth experiments in a highly scalable manner. eVOLVER implements real-time monitoring and feedback control over culture parameters (e.g. culture density, temperature, media composition) across an array of independent cultures for long-term experiments (>100 hours). Designed with inexpensive, open-source hardware and software, the system can be scaled to hundreds of vessels and is easily modified to accommodate custom experimental needs. eVOLVER is well-suited for studies of microbial communities for several reasons: 1) its scalability is critical for microbiome applications requiring high-throughput replicate cultures; 2) it operates robustly at population sizes and timescales needed for ecological studies; and 3) eVOLVER interfaces with novel millifluidic devices we developed, (inspired by large-scale integration in electronics and microfluidics) to implement complex fluidic routines (e.g. multiplexed media routing or transfer of liquid between cultures) in continuous culture. We applied these devices to carry out vial-to-vial transfers for biofilm prevention, necessary for long-term culture of undomesticated bacteria. More broadly, by mixing parallel cultures in an automated manner, microbial consortia could be constructed in real-time or tested for invasibility. I have applied eVOLVER to construct an 8-species synthetic consortium and a natural microcosm from soil bacteria. Using eVOLVER to impose dynamic growth conditions, I am able to track changes in species composition over time and characterize the response of these communities to fluctuating environments.

Prebiotic Control of Engineered Probiotics.

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The synbiotic relationship between human milk oligosaccharides (HMOs) and probiotic Bifidobacteria exemplifies prebiotic control of microbial community dynamics. Inspired by this example, we have engineered the well-known probiotic, *E. coli* Nissle, to metabolize HMOs and used this metabolism to control population dynamics and protein expression in mixed cultures of E. coli. We accomplish this using a unique whole-cell biosensor which provides linkage-specific, quantitative detection of various HMOs (Enam and Mansell, *Cell Chemical Biology*, 2018). Addition of these complex substrates to synthetic microbial consortia orthogonally controls growth rate or protein expression of particular strains. In addition, we performed further metabolic engineering on our probiotic, enabling production of short-chain fatty acids from HMOs as sole carbon sources, recapitulating an important function of the infant gut microbiota. Finally, we present a high-throughput, sequencing-independent method of tracking the dynamics of an engineered probiotic in mixed culture. This work lays the groundwork for the application of directed evolution to biosynthesis of complex carbohydrates as well as the prebiotic manipulation of population dynamics in natural and engineered microbial communities.

Cell-Free Metabolic Engineering for the Exploration of Cryptic Lasso Peptide Natural Products in the *Populus* root-Associated Microbiome.

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Interactions between plant roots and their microbiomes have been shown to promote desirable agricultural traits including drought tolerance, disease resistance and biomass yield. The natural products expressed by organisms within this environment influence microbiome composition and mediate microbial interactions with the plant and other microbes. However, natural product biosynthesis often depends on environmental conditions. These natural conditions can be difficult to determine and recreate in the laboratory, which impedes elucidation of their biochemical functions and their use for microbiome engineering. Cell-free systems are tools that can be used to produce cryptic and low abundance natural products as limitations due to resource competition from endogenous pathways, concerns of cell viability, toxic products, purification from biomass, and genetic tractability are minimized. Combining cell-free protein synthesis with enrichment of post-translation tailoring enzymes establishes a novel platform for the production and screening of ribosomally-synthesized and post-translationally modified peptide (RiPP) natural products. Biosynthetic gene clusters (BGCs) for lasso peptide RiPPs are well represented in the root-associated microbiome of model tree *Populus deltoides*. The BGC architecture and predicted products differ across organisms, but most products do not have described activity. Current progress is presented on the use of cell-free metabolic engineering to produce and characterize the lasso peptide natural products of the *Populus deltoides* soil microbiome.

Application of Catechol Microcins As Antimicrobial Peptides for the Prevention of Enteric Disease.

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Prokaryotic antimicrobial peptides are abundant in nature, and serve producing strains as a type of chemical warfare with neighboring cells. While small molecule antimicrobials produced by bacteria have been exploited for decades as traditional antibiotics, antimicrobial peptides as therapeutic agents have gone largely underutilized and underexplored. Microcins provide an example of one such class of antimicrobial peptides, characterized as ribosomally synthesized, relatively small proteins that are actively secreted into the extracellular milieu. Microcin H47 (MccH47) is a 5.6KDa class IIb microcin, first identified by Laviña *et. al*. 1990, produced and processed by the *mch* gene cluster in some *E. coli* strains, most notably *E. coli* Nissle 1917, a widely used engineered probiotic chassis strain. Interestingly, due to its high degree of hydrophobicity, MccH47 has been notably difficult to purify, and therefore all efforts to determine inhibitory activity have come from live producing strains using a variety of methodologies that have led to conflicting reports of MccH47's efficacy against many potential targets. Therefore, we developed vectors capable of overproduction in order to clearly demonstrate efficacy against *Salmonella* species, as well as other members of Enterobacteriaceae. Additionally, as Class IIb microcins are largely understudied, we developed a modular two-vector system to rapidly develop microcin producing vectors in order to test their efficacy against clinically relevant enteric pathogens.

Functional Microbiome Design for Agile and Expedient Manufacturing.

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To win in the deep operational environment, the Army will need to be more adaptive and expeditionary with less logistic demand. Readily available resources such as food waste and vegetation provide waste streams from which to generate energy and materials at the point of need. These substrates are poor candidates for chemical conversion to useful products; however, microbes such as the inhabitants of human and ruminant guts can ferment a wide range of nutrients present in these waste streams to commodity/specialty chemicals. Typical industrial production from these microbes involves stable culture conditions, consistent feedstock composition, and monocultures. This type of production is unrealistic in Army environments in which there are variable inputs depending on available resources and variable product needs depending on mission requirements. To address this challenge, we design functional microbiomes, multi-species consortia capable of metabolizing a wide range of substrates to produce a diverse range of useful products. We hypothesize that consortia will outperform monocultures for waste to commodity chemical production in Army relevant conditions. Here, we first computationally assess the ability of consortia vs. single organisms to convert food waste to commodity chemicals. The combination of genome-scale metabolic models with flux-balance analysis predicts that every organism analyzed can benefit from interactions with another microbe, as evidenced by increased biomass fluxes in co-culture vs. monoculture. Furthermore, microbe combinations result in emergent or increased commodity chemical production. To assess consortia vs monocultures for production from lignocellulosic feedstocks, we experimentally combined ruminant gut fungi with *Clostridium acetobutylicum*. This combination demonstrated synergistic growth and fermented lignocellulose to

useful outputs such as butanol. Overall, both human gut co-culture predictions and ruminant co-culture fermentations demonstrate that the whole is indeed greater than the sum of the parts and there is great potential for functional microbiomes for agile and expedient manufacturing.

Modeling Community Growth and Diversity of the Chronic Wound Microbiota.
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Chronic wounds are often colonized by polymicrobial communities which prevent effective treatment and timely healing. An estimated 2% of the U.S. population (6 million people) have a non-healing chronic wound with treatment costing more than $25 billion per year. Therefore, improved understanding of the wound microbiome plays a critical role in characterizing the bioburden and designing effective therapies. An important attribute of the wound microbiota is high species diversity, which provides system robustness through overlapping and redundant metabolic capabilities. The gradual loss of bacterial diversity has been associated with a highly inflamed wound environment and poor treatment outcomes. We formulated an *in silico* community model of the chronic wound microbiota by combining genome-scale metabolic reconstructions of 19 representative species from the AGORA database to explore the relationship between species diversity and community growth within the SteadyCom computational framework. Model predictions of species abundances were compared to 16S rDNA pyrosequencing data collected for 2,963 chronic wound patients in a published study. Our model was able to predict the high prevalence of the genera *Staphylococcus* and *Pseudomonas* and provide insights into the metabolite cross-feeding relationships that promoted their co-dominance. When the model was constrained to reproduce measured percentages of patients infected by each modeled genus, abundance predictions were in good agreement with the 16S data. Therefore, our model provided new insights into metabolic interactions in the chronic wound microbiome and provides a computational tool to interrogate community behavior.

Characterization of Microbial Anticipatory Responses in the Mammalian Gut.
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Gut microbiota constitutes the largest microbial population of the human microbiota. Recent studies have identified several of its functions, including host nutrient metabolism, drug metabolism, and immunomodulation. Interestingly, diet alone can contribute to more than 50% of the variation observed in the gut microbiota, a key component of various health and/or disease states. In the gut, dietary carbon sources are catabolized in a predefined order, which may allow for a microorganism to adapt and anticipate the upcoming carbon source based on the preceding one, a behavior also observed in various microbes. In the present study, we investigated the existence of natural anticipatory behavior in *Escherichia coli*, a well-known resident of the gut*,* by exposing the bacterium to sequential carbon sequences. For this experiment, we chose seven carbon sources that have spatial concentration gradients within the intestine, can be metabolized by *E. coli* and haven not been transcriptionally profiled before. To screen for anticipatory responses, we first grew *E. coli* in single carbon sources and quantified the expression of carbon catabolic genes by performing transcriptional profiling measurements, and subsequent validation by RT-PCR. Interestingly, several cases of anticipatory responses were observed, including cross-repression, cross-activation, symmetric and asymmetric regulations. Our results show that anticipatory behavior is a natural phenomenon in gut microbiota and

microbiota has capability to develop either a positive or a negative anticipatory response depending on the metabolic benefits. Present study warrants further characterization to understand the genetic and metabolic basis.

A Model Culture System for the *in Vitro* Human Colonic Microbiota of Ulcerative Colitis.

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Perturbation in gut commensal microbial communities are known as dysbiosis and are predicted to adversely affect the immune response, leading to numerous immune-mediated inflammatory diseases. Recent 16S rRNA and metagenomics community profiling studies of gut microbiota have linked dysbiosis to ulcerative colitis (UC), an idiopathic relapsing disorder of the colon. The incidence of UC is now increasing rapidly worldwide. However, the disease pathogenesis is not fully understood.
In vitro model that mimics the human colonic microbiota is desirable for developing treatment strategies targeting the colonic microbiota in UC patients. We, therefore, developed the model culture system for the *in vitro* human colonic microbiota of UC. 16S rRNA sequencing confirmed that UC models developed from the faecal inoculum successfully maintained the bacterial species richness and diversity of original UC faeces. UC models largely reproduced the microbial components, although not completely, and successfully maintained distinct clusters from healthy subjects (HS) observed by direct faecal analysis. The relative abundance of bacteria belonging to family *Lachnospiraceae* was significantly decreased in the UC models compared to in HS, as observed in the faeces. Our system detected significantly lower butyrogenesis in the UC models compared to those in HS, correlating with the decreased abundance of family *Lachnospiraceae*. Interestingly, the relative abundance of *Lachnospiraceae* did not correlate with disease activity (defined as partial Mayo score). These data suggested that a reduced *Lachnopiraceae* level remained in UC patients despite altered disease activity. Our findings may partly explain why UC can relapse irrespective of the induction of remission. In addition, we tested administration of the probiotic strain. Our model detects deregulation in the intestinal environment in UC patients and may be useful for simulating the effect of probiotics.

Cargo Transport Enables the Spatial Engineering of a Microbial Community.

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The human microbiome is an assemblage of diverse bacteria that interact with one another to form communities. Bacteria in a given community are arranged in a three-dimensional matrix with many degrees of freedom. Snapshots of the community display well-defined structures, but the steps required for their assembly are not understood. Here, we show that this construction can be carried out with the help of gliding bacteria of the phyla bacteroidetes. Gliding is defined as the motion of cells over a solid or semi-solid surface without the necessity of growth or the aid of pili or flagella. We focus on *Capnocytophaga gingivalis* which is a gliding bacteroidetes abundant in the human oral microbiome. Particle Image Velocimetry of gas bubbles carried by fluid flow shows that swarms of gliding bacteroidetes are layered, with cells in the upper layers moving more rapidly than those in the lower layers. Thus, cells glide on top of one another and help engineer the community architecture in 3D. Cells of non-motile bacterial species attach to the surface of gliding bacteroidetes and are propelled as cargo.

The cargo cell moves along the length of a gliding cell, looping from one pole to the other. Multi-color fluorescent spectral imaging of cells of different live but non-motile bacterial species abundant in the human microbiome reveals their long-range transport in a polymicrobial community. A swarm of gliding bacteroidetes transports some non-motile bacterial species more efficiently than others and helps engineer the spatial organization of a polymicrobial community.

Autonomous Regulation of Bacterial Co-Cultures Based on the AI-2 Quorum Sensing Signal.
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The human microbiome has been shown to be a complex environment that has a critical effect on human health. This has led to interest in engineering microbes or small consortia (composed of subpopulations working cooperatively) that are able to respond to signals in the GI tract in order to, for instance, flag a disease state or produce a therapeutic. One signal of interest that is likely important in the GI tract is the "universal" quorum sensing signal, autoinducer-2 (AI-2). AI-2 has been shown to influence the composition of the murine microbiome after antibiotic treatment [1] and has also been shown to affect gene expression in epithelial cells [2]. Here, we designed a system where the composition of two synthetic populations of *E. coli* is autonomously regulated in response to the level of AI-2 in the environment. To construct our co-culture system, we used an *E. coli* cell line whose growth rate is modulated by the level of the autoinducer-1 (AI-1) quorum sensing signal. This strain contains the gene for the phosphotransferase system protein HPr, involved in sugar uptake, under the AI-1 inducible *lasI* promoter. A second *E.* coli strain, the "translator" cell line, produces AI-1 in response to AI-2. When the two cell lines are cultured together, the "translator" strain senses the level of the environmental cue AI-2 and subsequently regulates composition of the co-culture. We further show that a simple mathematical model developed using data from the individual strains can be used to predict behavior of the co-culture over a range of AI-2 concentrations and initial cell populations. We envision this or similar strategies could be used to design sophisticated multi-strain microbial systems for treatment of disease or modulation of the microbiome.
[1] Thompson et al. *Cell Rep.* 10, 1861–1871 (2015).
[2] Zargar et al. *mBio* 6, e00025-15 (2015).

Engineering Soil- and Gut-Adapted Bacteria for Expression of Antimicrobial Gene Clusters in Their Respective Microbiomes.
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Due to the rise in antimicrobial resistance, there is great demand for new methods of eliminating pathogenic bacteria from the microbiomes that they disrupt. Producing antimicrobial compounds from commensal bacteria within these microbiomes can localize the compounds to the site of infection and increase treatment efficacy. In order to express antimicrobial compounds from soil and gut bacteria, we have developed a tool for transferring gene clusters from an easily engineerable strain of bacteria (*Bacillus subtilis*) into previously undomesticated bacteria. Utilizing this tool, we have demonstrated killing of *Clostridium difficile* by engineered soil and gut bacteria through production of Thuricin CD. This work demonstrates a pipeline for production of gene clusters with therapeutic potential from application-relevant bacteria.

Examining the Human Infant Gut Using Genome-Scale Models.

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Diet highly impacts human health, in part by modulating and affecting gut microbiome composition. In this study, we examine the colonization process of the human infant gut microbiome using genome-scale metabolic models using representative organisms. These individual models are ensured to have a molecular weight of 1 g/mmol in order to avoid introducing a systematic error when evaluating organisms in a community. The SteadyCom framework is used to impose a time-averaged constant growth rate across all members. We further examine the influence that arises from changes in free amino acids content of breast milk during colostrum, transition and mature stages of lactation.

In Vitro **Discovery of Novel Biomarkers As Indicators of Toxicant Exposure in the Human Gut.**

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A lack of methods to rapidly test how the microbiome responds to and potentially mediates the effects of exposure to toxicants has drastically slowed down development of potential therapies, mitigation strategies, and identification of exposure biomarkers. The interactions between environmental toxicant exposures and human health and performance are complex. Understanding this complexity may require understanding the role that the microbiome plays in mediating many of these interactions. MIT Lincoln Laboratory has leveraged its expertise in engineering, modeling, and prototyping to develop an Artificial Gut (ArtGut), which focuses on oxygen gradients and the role of mucus for physiologically relevant co-culture of the human gut microbiome. The near-term goal is to study links between environmental exposures, the metabolome, and Parkinson's disease. The ArtGut will be used to assess the effects of different toxicants on the metabolome by enabling incubation of human fecal samples (acquired through collaborators, either healthy or diagnosed with Parkinson's disease) with chemicals theorized to have a causative link to Parkinson's disease. The effluent from this ArtGut-enabled incubation will then be used to test the connections between the metabolome and development of Parkinson 's disease, through a collaboration with neurodegenerative disease expert, Dr. Steven Finkbeiner (UCSF). We will examine how the metabolism of pesticides and other mitochondrial toxins associated with Parkinson's impact nerve growth and development using fecal microbiome samples from both Parkinson patients and healthy controls. Initial results indicate that a physiologically-realistic oxygen gradient can be established and maintained inside an ArtGut *in vitro* system while the device remains in ambient (aerobic) conditions. A mucus layer can also be created and maintained within the ArtGut device and preliminary results indicate that differences in local oxygen concentration change the mechanical properties of the mucus, as expected.

Massively Parallel Transcriptional Measurements in Cell-Free Systems from Diverse Bacteria.
Sung Sun Yim[1]*, Nathan Johns*[2]*, and Harris H. Wang*[3]
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Precise tuning of gene expression levels is crucial for engineering predictably behaving genetic circuits. Our current understanding of how regulatory sequences control gene expression levels remains limited for most bacterial species. Cell-free expression systems greatly simplify prototyping of genetic designs *in vitro*. However, the small number of simultaneous measurements that can be made using reporter fluorescence as readouts limits the scale at which biological parts can be characterized. Here we devised a method to measure expression levels from thousands of regulatory sequences in single cell-free reactions using oligo library DNA synthesis and targeted deep sequencing of RNA and DNA. This multiplexed approach was highly robust and corresponded well with *in vivo* measurements in *E. coli*. We further applied this approach in active cell-free transcription systems developed from ten diverse bacterial species, enabling comparison of sequence-function relationships across hosts and predictive modeling of transcriptional activation. We anticipate that this multiplexed approach using cell-free expression systems will expand the capacity for genetic circuit prototyping in new bacterial chassis.

Metagenomic Engineering of the Mammalian Gut Microbiome *in Situ*.
Carlotta Ronda, ***Sway Chen****, Vitor Cabral, and Harris H. Wang*
Department of Systems Biology, Columbia University, New York, NY

The gut microbiome plays essential roles in mammalian homeostasis and disease and has been extensively cataloged using DNA sequencing. However, the inability to cultivate and genetically alter most gut microbes in vitro has hindered our understanding of these organisms' biology and potential for use in biotechnology applications. To overcome these challenges, we developed a platform -- Metagenomic Alteration of Gut microbiome by In situ Conjugation (MAGIC) -- to genetically modify gut microbes in their native habitat. Using engineered horizontal gene transfer systems, we demonstrated in situ gene transfer inside live mice to deliver new genetic traits into the existing gut microbiota. Furthermore, we identified and isolated bacteria from the mouse gut microbiome that were amenable to genetic manipulation and then redeployed them back into the gut as host-optimized probiotics that could also mediate secondary gene transfer. This in situ metagenomic engineering approach enables the accelerated development of new microbial chasses for biotechnological applications and the introduction of novel capabilities into established microbial communities with minimal disruption to their native milieu.

Signatures of Exposure in the Skin Microbiome.
Kristin Loomis*, David Karig, Bryan Brensinger, Craig Howser, Kianna Blount, and Joshua Wolfe*
Research and Exploratory Development, Johns Hopkins University Applied Physics Laboratory, Laurel, MD

The presence of some taxa within the skin microbiota change over time. This variance may be explained, in part, by exposure of the microbiome to novel environments, materials, or chemicals. The full spectrum of mechanisms that drive these changes is not fully understood. Gaining insight into how microbiome communities change will further our understanding of the structure of the skin microbiome, enabling diagnostic or forensic analysis of an individual's skin microbiome as well as directed efforts for modulation of an individual's microbiome. We are investigating how an environmental exposure affects

community structure by evaluating the response of human skin commensal isolates to a model environmental exposure—the pesticide chlorpyrifos. The influence of chlorpyrifos on skin bacteria is being assessed both in monoculture studies as well as in synthetic microbiome communities. In monoculture experiments, we established that skin commensals exhibit a spectrum of growth responses to chlorpyrifos: some isolates grow faster and others do not grow at all. Additionally, we identified that a number of skin commensals have the capacity to utilize chlorpyrifos as an energy source, and thus could potentially alter local concentrations of the chemical upon an individual's cutaneous exposure. We assessed how synthetic microbiome communities are altered following chlorpyrifos exposures by culturing microbes on 3D *in vitro* skin tissue to simulate the skin environment. We observe that chlorpyrifos exposure alters composition of synthetic skin microbiome communities. Three days following chlorpyrifos exposure, the synthetic community contained a decreased relative abundance of the common skin bacteria *Acinetobacter* and *Staphylococcus* as well as an increase in the chlorpyrifos-surviving bacteria *Arthrobacter* and *Bacillus*. Our findings shed light on how an external perturbation influences microbiome community compositions. This work may inform eventual efforts using the skin microbiome to diagnose an individual's environmental exposures as well as efforts to modulate skin microbiome communities.

Evaluation of Engineered Microbe Persistence and Function of Using a Simplified Polymicrobial Gut Community.

Steven Arcidiacono[1], *Laurel A. Doherty*[1], *Jordan Whitman*[2], *Ida Pantoja-Feliciano*[1], *Michael Goodson*[3], *Amy M. Ehrenworth Breedon*[3,4], *Scott A. Walper*[5], *Joseph R. Spangler*[6], *and Jason W. Soares*[1]
(1)U.S. Army Natick Soldier Research Development and Engineering Center, Natick, MA, (2)NSRDEC, Natick, MA, (3)711th Human Performance Wing, Air Force Research Laboratory, Wright-Patterson Air Force Base, OH, (4)UES, Inc., Dayton, OH, (5)Naval Research Laboratory, Washington, DC, (6)National Research Council, Washington, DC

There is great interest in using synthetic biology to engineer organisms with novel or improved function to add to the human gut microbial community; including engineering probiotics for increased beneficial function. Synthetic biology generally employs the design-build-test-learn approach to generate genetic circuits, which are then placed into a microbial chassis. Engineered probiotics are developed and tested in monoculture under aerobic and ideal pH/nutrient conditions. However, in contrast to monoculture, characterizing engineered organism behavior within complex microbial community can be challenging. *In vitro* fermentation models using simplified microbial communities address this challenge by reducing complexity and allowing the study of organism persistence and function within more tailored microbial community dynamics. Furthermore, evidence is emerging that simplified microbial communities can facilitate understanding of population dynamics that translate to bacterial metabolism behavior in more complex communities. Additionally, *in vitro* models have increased experimental capacity to allow rapid evaluation of multiple parameters to generate knowledge that could then inform animal studies. Here, simplified communities were utilized to investigate the probiotic bacteria *Escherichia coli* Nissle, engineered to produce plasmid-encoded Green Fluorescent Protein (GFP). A 4-member polymicrobial community was designed to increase complexity and challenge *E. coli Nissle* metabolic persistence and GFP reporter function. *E. coli* Nissile-GFP growth remained unchanged as community members were added; however, while GFP plasmid remained stable, GFP output was adversely effected as community complexity increased. Persistence and function of engineered *Lactobacillus plantarum*-GFP tested with a similar 4-way co-culture showed comparable functional effects. An *in vitro* fermentation engineered probiotic test bed can provide critical knowledge for circuit design feedback and functional validation prior to higher fidelity testing within animals or humans.

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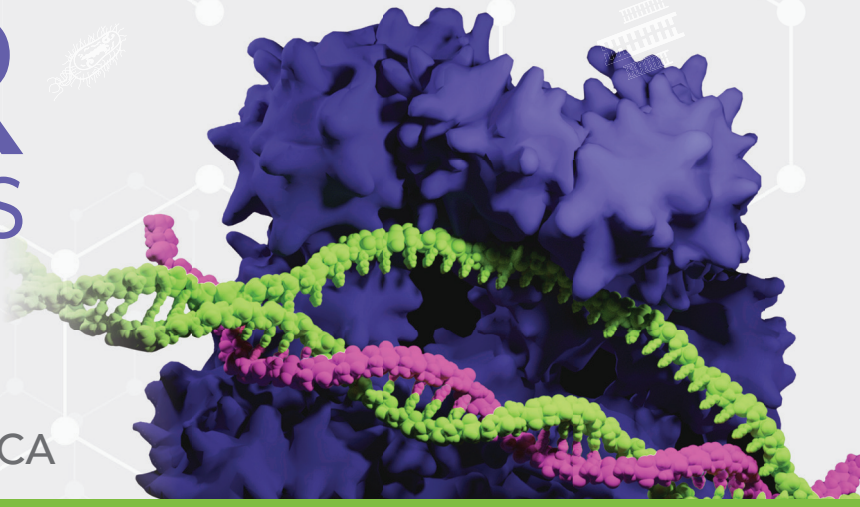

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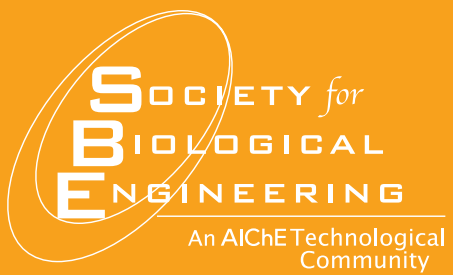

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