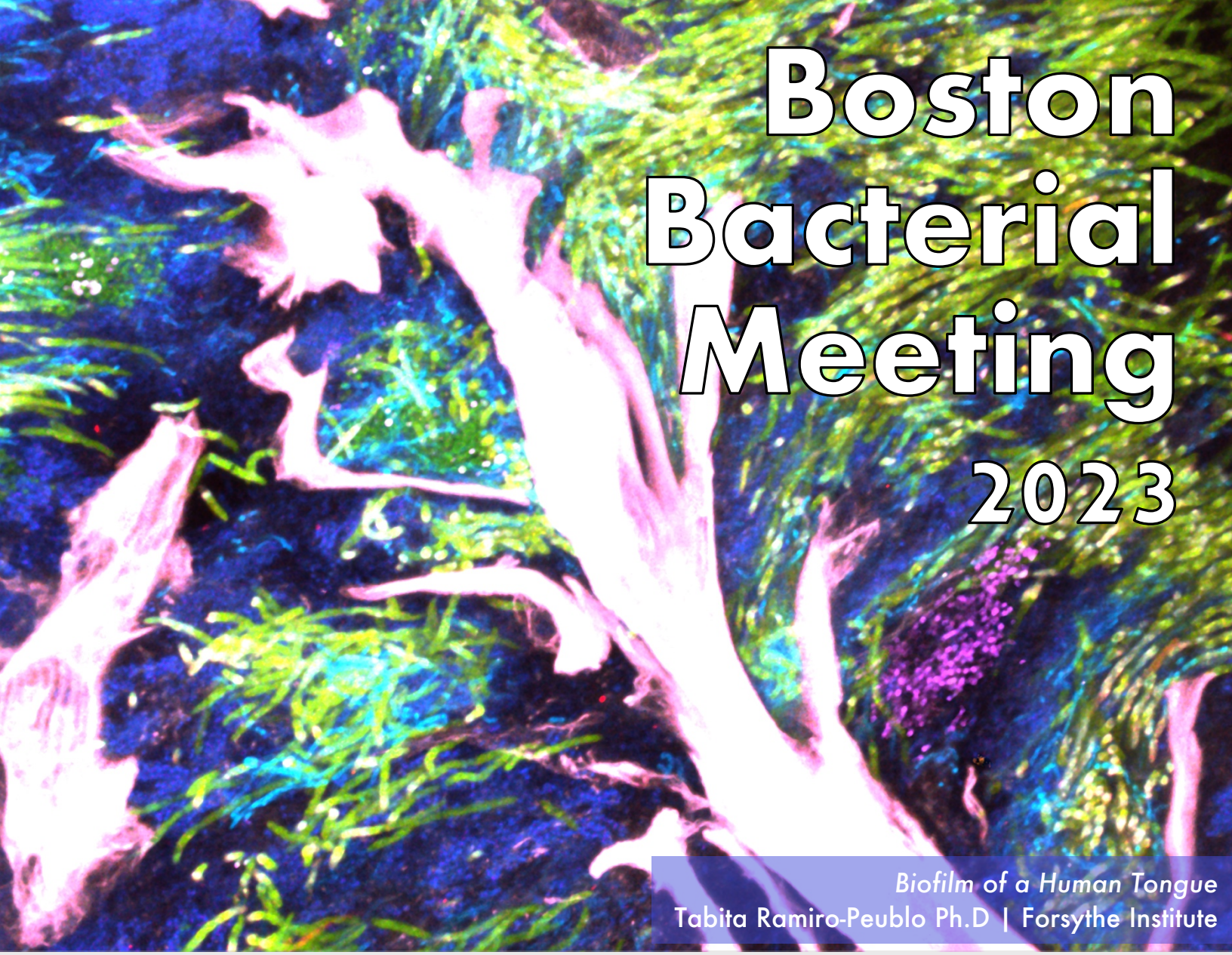
Boston Bacterial Meeting 2023



Biofilm of a Human Tongue
Tabita Ramiro-Peublo Ph.D | Forsythe Institute

KEYNOTE SPEAKER:

Kumaran S. Ramamurthi, Ph.D

Senior Investigator & Deputy Chief, Laboratory of Molecular Biology
National Cancer Institute, National Institute of Health

June 12 – 13, 2023
Harvard Science Center

Microbial Machines
SERES THERAPEUTICS
NEW ENGLAND BioLabs Inc.
HMS/HSPH GPiB Graduate Program in Bacteriology
THE COOP HARVARD www.thecoop.com
CONAGEN MAKING *IMPOSSIBLE* POSSIBLE
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CONTENTS

MONDAY, JUNE 12

8:15 – 9:00	Breakfast and Check-in	
9:00	Opening Remarks	
I.	ANTIBIOTIC PERSISTENCE & RESISTANCE	Session Chair: Thea Brennan-Krohn
9:10 - 9:30	Jamie H. Corro State University of New York at Albany	Ribosome hibernation factor induces isoniazid persisters of *Mycobacterium tuberculosis* in neutrophil-rich lung lesions.
9:30 - 9:50	Jason Wittenbach Day Zero Diagnostics	Keynome *g*AST: a machine learning system for predicting antimicrobial resistance phenotypes from whole-genome sequencing
9:50 - 10:10	Alexis Jaramillo Cartagena Broad Institute	RarA is a transcription factor in *Klebsiella pneumoniae* that confers extreme levels of resistance to different antibiotic classes in clinical isolates
10:15 - 11:15	Poster Session I (1-37)	
II.	BACTERIAL PHYSIOLOGY	Session Chair: Lindsey Richelle Fernandez Backman
11:20 - 11:40	Elayne M. Fivenson Harvard University	A role for the Gram-negative outer membrane in bacterial shape determination
11:40 - 12:00	Suyen Espinoza Boston College	Genome-wide identification of genetic requirements of *Streptococcus pneumoniae* biofilms
12:00 - 12:20	Luis Gutierrez Harvard University	A High-Throughput Essential-gene CRISPRi Screen Using Time-Lapse Microscopy Reveals Insights on Nucleoid Morphology and Division Regulation in *E. coli*
12:20 - 01:20	Lunch (catered)	
01:20 - 02:05	Breakout Sessions I-IV (see abstract book)	
02:15 - 03:20	KEYNOTE ADDRESS: Kumaran S. Ramamurthi Synthetic bacteria deliver the goods (to cancer cells)	
03:25 - 04:25	Poster Session II (38-74)	
III.	STRESS RESPONSE	Session Chair: Roby Bhattacharyya
04:30 - 04:50	Katherine Cotton Johns Hopkins University	Understanding phenotypic responses to doxycycline and pathways that lead to bacterial death versus survival
04:50 - 05:10	Justyne Ogdahl University of Massachusetts Amherst	DNA dependent regulation of the *Caulobacter crescentus* Protease Lon
05:10 - 05:30	Rachael Workman Sparklin Johns Hopkins University	CRISPR-Cas auto-regulator tracr-L balances CRISPR costs and benefits and can be de-repressed by phage infection
05:30	End of Day 1	

TUESDAY, JUNE 13

8:15 - 9:00	Breakfast	
9:00 - 9:05	Day 2 Opening	
IV.	**MICROBIOME & MICROBIAL COMMUNITIES**	**Session Chair: Andrea Etter**
9:05 - 9:25	Megan D. McCurry Harvard University	Gut bacteria convert glucocorticoids into progestins
9:25 - 9:45	Mariia Taguer Merck Research Labs	Spatial recovery of the gut microbiome after antibiotics perturbation
9:45 - 10:05	Gavin A Kuziel Boston Children's Hospital	Functional diversification of plant small molecules by the gut microbiome tunes intestinal homeostasis
10:10 - 11:10	Poster Session III (75-111)	
V.	**NEW METHODS IN MICROBIOLOGY**	**Session Chair: Jeffrey Moffitt**
11:15 - 11:35	Bryan R. Lenneman Massachusetts General Hospital	Engineering novel bacteriophage-based biotherapeutics for antimicrobial-resistant pathogens
11:35 - 11:55	Heidi Arjes Concerto Biosciences	Discovery of an *S. aureus*-pacifying microbial ensemble via massively parallel measurement of millions of microbial interactions
11:55 - 12:15	Neetika Jaisinghani Stony Brook University	Compartment specific cell wall labeling in mycobacteria used to identify cell wall proteins informs hierarchical secretion mechanism in Type VII secretion systems
12:15 - 02:00	Lunch (not catered)	
02:00 - 02:45	Breakout Sessions V-VIII (see abstract book)	
02:55 - 03:55	Poster Session IV (112-147)	
VI.	**BACTERIAL PATHOGENESIS**	**Session Chair: Melis Anahtar**
04:00 - 04:20	Molly Sargen Harvard University	Pathogens within Pathogens: Prophage Competition in Intramacrophage *Salmonella*
04:20 - 04:40	Allen Sanderlin Massachusetts Institute of Technology	Needle in a haystack: cell-selective secretomics reveal a novel bacterial effector that interacts with the eukaryotic translocon
04:40 - 05:00	Emily R. Goering Harvard University	Defining Kynurenine Pathway Control of *Salmonella Typhimurium*
05:00 - 05:20	Awards and Closing Remarks	
05:30 - 07:00	Reception (catered)	
07:00	End of BBM 2023	

Welcome to BBM 2023!

A message from our co-chairs

Dear Participants,

Welcome to the Boston Bacterial Meeting! For 29 years, BBM has offered an intellectually stimulating and collegial atmosphere. Bacteriologists throughout the New England area and its surroundings join to discuss cutting-edge scientific research and share ideas.

We are so pleased to see many of you again this year and want to welcome all of our new participants! We are thrilled to have two fantastic days of scientific presentations and discussions. This year, we have four poster sessions, two breakout sessions, and a networking reception at the end of the second day. We hope these spaces facilitate networking and collaborations among trainees and foster new mentor-mentee relationships.

BBM is made possible by our passionate volunteer Organizing Committee, which comprises graduate students, postdocs, and other early career scientists. We are very thankful to be surrounded by such a dedicated group, as all of their time, energy, and endeavors make this conference possible.

We are also delighted to welcome Dr. Kumaran Ramamurthi as our keynote speaker this year. Dr. Ramamurthi is a Senior Investigator and Deputy Chief of the Laboratory of Molecular Biology at the National Cancer Institute. Dr. Ramamurthi's research incorporates cytological, biochemical, genetic, biophysical, and computational techniques to study protein localization during morphogenetic processes in *Bacillus subtilis* and *Staphylococcus aureus*. This multidisciplinary blend has recently led to the development of synthetic bacterial cells that mimic bacterial spores and show promise as drug delivery vehicles. Please join us in welcoming Dr. Ramamurthi to BBM, as we look forward to hearing about his current work.

We are indebted to our faculty sponsors (Drs. Tom Bernhardt and Ethan Garner) and meeting founders (Drs. Rich Losick, Roberto Kolter, John Mekalanos, and Andrew Wright). We thank them for their continuing guidance, time, and support. We would also like to thank our generous sponsors for their contributions.

On behalf of the entire Organizing Committee, we would like to thank you for joining us at the 29th annual Boston Bacterial Meeting. We hope you will find the next two days both scientifically and socially stimulating.

Sincerely,

Alam García-Heredia and Shailab Shrestha

BBM 2023 Co-chairs

Meeting Announcements

- Please silence your mobile devices before entering the auditorium and breakout sessions.
- Respect data confidentiality! Photography or recording of any research is strictly prohibited without the explicit approval of the presenter.
- There is an overflow room in Hall A which will project oral presentations.
- Food and drink are permitted in the main auditorium but not in the classrooms.
- A Mother's Room is available in Room 304 of the Harvard Science Center. Please ask a member of the Organizing Committee if you require any assistance surrounding its use.
- BBM maintains a strict stance against harassment in any form. Any individuals engaging in harassing behavior will be subject to immediate removal from the meeting and be banned from future meetings. Please review our Discrimination and Harassment Policy for more information.
- Please keep your name badge on during the meeting. At the end of the conference, please help us recycle the empty plastic name badge holders by leaving them at the registration desk.
- Use the hashtag #BBM2023 on Twitter when discussing this year's meeting!

Organizing Committee

2023 Co-Chairs

Alam García-Heredia
Postdoctoral Fellow | MIT

Shailab Shreshta
Graduate Student | Tufts

Molly Sargen
Graduate Student | Harvard
Registration, Catering

Alexis Jaramillo Cartagena
Research Fellow | Broad Institute
Breakout Sessions, Outreach

Irem Ozkan
Graduate Student | Boston College
Breakout Sessions

Laurent Dubois
Graduate Student | Harvard
Facilities, Registration

Pola Kuhn
Graduate Student | Tufts
Catering, Abstract Book, Website

Liz Tan
Graduate Student | Tufts
Breakout Session, Outreach

Jenna Wurster
Scientist | Seres Therapeutics
Poster Design, Fundraising

Suyen Miranda Espinoza
Graduate Student | Boston College
Fundraising

Kathy Suarez
Graduate Student | Harvard
Fundraising, Breakout Sessions

Defne Surujon
Research Scientist | Day Zero Diagnostic
Facilities, Abstract Book

Carolina Santamaria
Graduate Student | UMass Amherst
Fundraising

Andres Florez
Postdoctoral Fellow | Harvard
Breakout Sessions, Outreach

Yishak Woldetsadik
Graduate Student | Tufts
Catering, Poster Design

Anna Brogan
Graduate Student | Harvard
Abstract Book

Kyle Gibbs
Postdoctoral Fellow | Tufts
Registration d

Wanassa Beroual
Postdoctoral Fellow | Harvard
Fundraising, Registration

Irina Shlosman
Graduate Student | Harvard
Facilities

Anastacia Parks
Postdoctoral Fellow | Harvard
Registration, Fundraisingx

Apostolos Liakopoulos
Postdoctoral Fellow | Harvard
Facilities, Outreach

Johnathan Kepple
Graduate Student | Harvard
Fundraising, Registration

Quinlan Furumo
Graduate Student | Boston College
Website

Federico Rosconi
Research Scientist | Boston College
Website

BBM's Stance on Discrimination, Racism, and Harassment

We, the BBM Organizing Committee, are dedicated to providing a safe, respectful, and professional space for scientists of all backgrounds to share their research. To this end, harassment based on an individual's race, gender, religion, sexual orientation, or any other legally protected characteristic will not be tolerated at the meeting. In addition, we take a strict stance against sexual harassment, which is defined as behavior of a sexual nature that is unwelcome and offensive to the person or persons it is targeted toward. As a regular recipient of NIH funding, we operate under Title IX procedures. If a participant feels they have been harassed, we encourage them to report this to the Organizing Committee, and the matter will be investigated discreetly and confidentially. Any participants engaging in discriminatory or harassing behavior will be subjected to disciplinary measures up to and including being removed from the conference.

We stand firmly in continued solidarity with the Black Lives Matter movement. We condemn the longstanding tradition of anti-Asian violence in the United States and the continued harm brought upon Latinx and Indigenous communities throughout the country. Ultimately, dismantling systems of oppression will require an intersectional framework, and we, the BBM Organizing Committee, maintain our commitment to anti-racism and to promoting equity in all its forms.

While this work is not new, a hard line on anti-racism is essential to our efforts to build a more equitable community where all can thrive. The past two years of extensive participation in protests across the US have underscored how critical it is to speak out and take action. Through silence and inaction, we perpetuate the prejudice and violence that have been part of the daily lives of our BIPOC colleagues and friends, both inside and out of the science community.

As a result of the COVID-19 pandemic, we as scientists have increased visibility, bolstered public trust, and a magnified voice. We have the means to augment underrepresented voices. As scientists, employers, educators, science communicators, and clinicians, we have the power to implement real change by challenging injustice and prejudice in our labs, classrooms, and clinics. At BBM, we strive to promote inclusivity and diversity. To this end, we are committing to the following:

- Offering a dedicated space for discussion on race and representation in the sciences by holding a breakout session on Diversity in STEM.
- Continuing to offer our "Underrepresented Minorities in Biomedical Sciences Scholarship" which waives the registration fee.
- Expanding our scholarship to include a Travel Award to help underrepresented minority microbiologists attend our meeting. All proceeds from our online store will go towards furnishing this award for next year. If you or your company wish to sponsor this endeavor, contact us at bbm@hms.harvard.edu.

We acknowledge that there is still work to be done to ensure equity, diversity, and representation both at BBM and in the scientific community. We have provided multiple resources on our website so that you, too, can participate. It is on all of us, especially those of us who have the privilege to speak out to fight against injustice. This message is also an invitation for comments and suggestions from our participants on how the Organizing Committee can practice good allyship as BBM organizers and ways we can promote BIPOC and underrepresented voices. We are here to listen and act together and to ensure that we all enjoy the same privileges and basic human rights.

In Solidarity,
The 2023 BBM Organizing Committee

Keynote Speaker

Kumaran S. Ramamurthi, Ph.D

Senior Investigator & Deputy Chief
Laboratory of Molecular Biology
National Cancer Institute, National Institute of Health

Kumaran Ramamurthi is a Senior Investigator and Deputy Chief of the Laboratory of Molecular Biology at the National Cancer Institute. Dr. Ramamurthi received his Ph.D. in Molecular Biology from the University of California, Los Angeles (UCLA), where he studied virulence protein secretion in pathogenic bacteria under Olaf Schneewind. He joined the laboratory of Richard Losick at Harvard University as a Ruth L. Kirschstein National Research Service Award Postdoctoral Fellow to study mechanisms driving the subcellular localization of bacterial proteins. His research was among the first to establish a geometric cue model for protein localization, whereby certain “shape-sensing” proteins localize to specific subcellular sites by recognizing membrane curvature.

After completing his postdoctoral fellowship, Dr. Ramamurthi started his lab at the NIH in 2009 as a Tenure Track Investigator. He was promoted to Senior Investigator and received the National Cancer Institute Director’s Award in 2016. At the NIH, Dr. Ramamurthi continues to apply a multidisciplinary approach to solving fundamental cell biological problems. His lab incorporates cytological, biochemical, genetic, biophysical, and computational techniques to study protein localization during morphogenetic processes in *Bacillus subtilis* and *Staphylococcus aureus*. His lab has identified and characterized novel factors driving spore formation and cell division in *B. subtilis* and *S. aureus*, respectively. Excitingly, his group’s studies of these fundamental processes have recently led to the development of synthetic bacterial cells that mimic bacterial spores and show promise as drug delivery vehicles. Please visit his NCI website profile for more information on his research and publications.

Beyond his research, Dr. Ramamurthi’s dedication to academic service and science education is evidenced by his involvement with the American Society for Microbiology (ASM). He has served on the Program Committee for the annual ASM Microbe Meeting since 2013 and co-chaired the World Microbe Forum in 2021 and the Microbe Meeting in 2022. Furthermore, he is the Co-Director of the NIH-Johns Hopkins University Graduate Partnership Program and a member of the NIH Equity Committee, which reviews hiring and promotion procedures to enhance diversity and inclusion in the Intramural Research Program of the NIH. He currently serves on the editorial boards of The Journal of Biological Chemistry and Microbiology and Molecular Biology Reviews.

2023 BBM Session Chairs

Andrea Etter, Ph.D.
Assistant Professor
Nutrition and Food Sciences Department
The University of Vermont

Andrea Etter is a food microbiologist and assistant professor at the University of Vermont. She grew up on a homestead in rural WI and earned her BS in Biology from Maranatha Baptist University and her PhD in Food Microbiology from Purdue University. Her doctoral research focused on understanding *Listeria monocytogenes* colonization of grocery store delis, and on whether bacterial stress tolerance played a role in a large outbreak of *Salmonella* Heidelberg. Her work on *Salmonella* Heidelberg was funded by a USDA NIFA AFRI Predoctoral fellowship.

Andrea's work at UVM studies colonization strategies of *L. monocytogenes* from artisan cheese facilities, the ecology and antimicrobial resistance of *Salmonella enterica* and *Campylobacter jejuni* in small-scale farming, including backyard poultry, and understanding the potential role of bacterial stress tolerance in additional foodborne outbreaks. She enjoys mentoring undergraduate and graduate researchers in research, especially inviting and encouraging the participation of students from underrepresented backgrounds. She also enjoys introducing students to the joy of microbiology and food safety through her courses, including *Global Food Safety* and *Deadly Foods: outbreak investigation.*

Roby P. Bhattacharyya, M.D., Ph.D.
Associate Member | Broad Institute
Infectious Disease and Microbiome Program
Assistant Professor of Medicine | MGH Division of Infectious Diseases
Assistant Professor | Harvard Medical School

Roby Bhattacharyya MD PhD is an Assistant Professor of Medicine in the Infectious Diseases Division at Massachusetts General Hospital and Harvard Medical School and an Associate Member at the Broad Institute in the Infectious Disease and Microbiome Program. He leads a research laboratory at the Broad focusing on antimicrobial resistance, sepsis, transcriptional profiling of pathogens and patients, and molecular diagnostic development. He is also a practicing infectious disease physician at MGH with particular clinical interest in antibiotic-resistant infections and COVID-19. Roby grew up in the Chicago area, received his MD and PhD in Biochemistry and Molecular Biology from the University of California at San Francisco, did Internal Medicine residency, chief residency, and Infectious Disease fellowship training at MGH, and a postdoctoral fellowship in Deb Hung's lab at the Broad.

Thea Brennan-Krohn, M.D., D (ABMM)

Attending Physician | Division of Infectious Diseases |
Boston Children's Hospital
Faculty | Clinical Microbiology | Beth Israel Deaconess Medical Center
Assistant Professor of Pediatrics and Pathology | Harvard Medical School

Dr. Brennan-Krohn is a physician-scientist whose research is focused on novel approaches to diagnosis and treatment of multidrug-resistant gram-negative bacterial infections. She completed a Pediatrics Residency at the Boston Combined Residency Program, a Pediatric Infectious Diseases fellowship at Boston Children's Hospital, and a Clinical Microbiology fellowship at Beth Israel Deaconess Medical Center (BIDMC). Her post-doctoral fellowship work, in the lab of James Kirby, involved investigation of antimicrobial synergy to identify regimens in which antibiotic activity could be restored when drugs were used in combination against multidrug-resistant organisms. In addition to research, she spends time seeing patients on the Infectious Diseases service at Boston Children's Hospital and serving as an attending physician in the Clinical Microbiology Laboratory at BIDMC.

The Brennan-Krohn laboratory at BIDMC is dedicated to carrying out translational research investigating the effects of antibiotic exposure on gram-negative bacteria, with the goal of identifying optimal treatment approaches for different types of complex, drug-resistant infections. Particular areas of interest include investigation of the activity of antibiotics against *Burkholderia cepacia* complex (Bcc) organisms in artificial sputum medium to understand activity in the lungs of people with cystic fibrosis, a high-throughput screen of antibiotic potentiators for Bcc, and studies of the mechanisms of activity and of bacterial resistance to novel non-β-lactam β-lactamase inhibitors.

Melis Anahtar, M.D., Ph.D.

Clinical Microbiologist | Massachusetts General Hospital
Research Fellow | Broad Institute
Co-founder and Consultant | Day Zero Diagnostics

Melis Anahtar, MD, PhD is a clinical microbiologist and co-founder of Day Zero Diagnostics, a Boston-based healthcare startup that is developing a genomic sequencing-based rapid diagnostic for bacterial infections. She received her SB in Mechanical Engineering from MIT, followed by an MSc in Immunology from Oxford University on a Rhodes Scholarship, and an MD and PhD in Immunology from Harvard Medical School. She completed her residency in Clinical Pathology and fellowship in Medical Microbiology at Massachusetts General Hospital. Her research focus is on understanding and combating antimicrobial resistance in bacteria using genomics and machine learning. She is currently working in Dr. James Collins' lab to discover novel antibiotics for drug-resistant *Neisseria gonorrhoeae*.

Jeffrey R. Moffitt, Ph.D.

Assistant Professor | Department of Microbiology,
Harvard Medical School
Investigator | Program in Cellular in Molecular Medicine,
Boston Children's Hospital
Associate Member | Broad Institute

Dr. Jeffrey Moffitt received his PhD in Physics from the University of California Berkeley under the direction of Dr. Carlos Bustamante. During his PhD he developed novel a high-resolution optical tweezers with Angstrom-scale sensitivity, and he used this to follow the basepair scaling packaging of a viral genome into a bacteriophage capsid. He received postdoctoral training from Dr. Xiaowei Zhuang at Harvard University, where he developed methods for transcriptome-scale imaging in single cells. In particular, he was a co-inventor of the spatially resolved single-cell transcriptomics approach known as MERFISH. Dr. Moffitt is an Investigator in the Program in Cellular and Molecular Medicine at Boston Children's Hospital and an Assistant Professor in the Department of Microbiology at Harvard Medical School. His laboratory is developing new spatially resolved methods for single-cell 'omics and using these tools to discover and map cells in a wide range of tissues. His laboratory is supported by a Pew Biomedical Scholar Award, the Chan Zuckerberg Initiative, and the NIH.

Lindsey R. F. Backman, Ph.D.

Valhalla Whitehead Fellow
Whitehead Institute for Biomedical Research

Lindsey Richelle Fernandez Backman is a new Valhalla Fellow at the Whitehead Institute for Biomedical Research. Lindsey earned her BS in Chemistry from the University of Florida in 2015 and her Ph.D. in Chemistry from the Massachusetts Institute of Technology in 2022. Lindsey was first introduced to the MIT community as an undergraduate when she participated in the MIT Summer Research Program in Biology (MSRP-Bio) and Howard Hughes Medical Institute (HHMI) EXROP, which enabled her to do a summer research internship in Prof. Cathy Drennan's lab. She returned to complete her Ph.D. in the Drennan lab, where she worked on structurally and biochemically characterizing new glycyl radical enzymes abundant in the human gut microbiome. In graduate school, Lindsey benefited from multiple programs that provided funding and a supportive community, including the HHMI Gilliam Fellows Program, which helps students from underrepresented backgrounds in STEM pursue academic careers. Lindsey is passionate about making academia and science a more inclusive space, especially for scientists from historically excluded backgrounds. She has participated in many initiatives to help create supportive academic environments, including being a member of the MIT Chemistry DEI Committee, helping to co-found the MIT Latinx Graduate Student Association (LGSA), and actively mentoring undergraduate students, especially through the MSRP-Bio program.

In her independent career, Backman is interested in exploring bacterial competition within the human microbiome. With the global increase of antibiotic-resistant pathogens, there is a critical need for developing new antibiotics; understanding the strategies that pathogens employ to outcompete commensal bacteria and proliferate could lead to novel therapeutic targets. The Backman lab will utilize structural, biochemical, and genetic methods to identify and interrogate strategies that enable anaerobic pathogens to thrive amidst oxidative stress. Such protective measures are particularly important for pathogens and disease-associated bacteria, as host immune and inflammatory response pathways are associated with high levels of oxidative stress. Lindsey is excited to begin building her team and tackling these questions!

Breakout Sessions

Breakout sessions will be facilitated by moderators who will open the floor for questions from the audience after a short introduction and Q&A with the panelists.

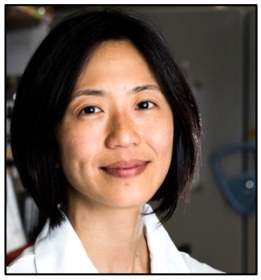
Day 1

June 12th, 2023 1:10-2:00pm

Diversity, Equity, and Inclusion in STEM (Room B10)

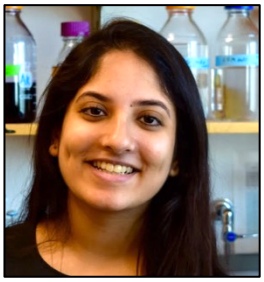
What kinds of barriers do underrepresented communities face in science? How can we promote inclusivity and support among our fellow scientists? Hear from advocates of diversity in STEM, how to open up discussions on DEI topics, and what we can do to help build a diverse and supportive scientific community.

Kyra Taylor
Moderator
Engineer
Seres Therapeutics



Aimee Shen, Ph.D.
Associate Professor of Molecular Biology and Microbiology
Tufts School of Medicine

Karina Gonzalez, Ph.D.
Associate Dean
Office of DEI
Yale University



Dia Ghose
Graduate Student
Department of Biology
MIT

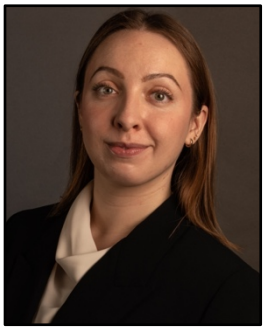
Andrew Henderson, Ph.D.
Professor of Medicine and Microbiology
PREP Director
Boston University

Careers Beyond the Bench (Hall A)

Careers in microbiology extend beyond the research performed in academia and industry. Scientists play vital roles in patent law, intellectual property, venture capital, science outreach, public policy, and much more. Join our panelists from a variety of these fields to learn more about their transitions from the bench.

Sergio Lopez
Moderator
Broad Institute

Alba Jiménez Asins, Ph.D.
Associate
Flagship Pioneering



Emily Foster, Ph.D.
Technology Specialist
Lando & Anastasi

Andrews Akwasi Agbieke
President and Co-founder
Sena Institute of Technology

Danika Khong, Ph.D.
CEO/Co-founder
Scismic

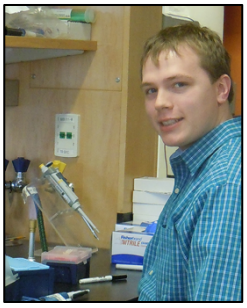
Antibiotic Resistance & Beyond (Hall D)

Due to the surge of antibiotic-resistant microbes and the inefficacy of current antimicrobial therapies, treating bacterial infections has become exceptionally challenging in the last decade. We invite you to join a breakout session that goes beyond antibiotic resistance and explores tolerance, persistence, antibiotic discovery platforms, alternative therapies, and much more.

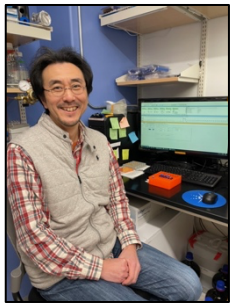
Elizabeth Tan
Moderator
Ph.D. Candidate
Tufts University

Roberto Molina-Quiroz, Ph.D.
Core Deputy Director
Molecular Biology and Microbiology
Tufts University

Andrea Etter, Ph.D.
Assistant Professor
College of Agriculture and Life Sciences
University of Vermont



Trever Smith, Ph.D.
Postdoctoral Fellow
Tufts University



Akira Iinishi, Ph.D.
Principal Research Scientist
Northeastern University

Synthetic Microbiology (Hall E)

How do scientists reimagine nature to develop therapeutics, biofuels, and beyond? Designing microbes has become an essential tool with a multitude of applications in industrial and medical research. Learn about the vast possibilities engineered microbes offer from experts in the field.

Elizabeth Libby, Ph.D.
Moderator
Assistant Professor,
Bioengineering
Northeastern University

Mary Dunlop, Ph.D.
Associate Professor,
Department of Biomedical Engineering
Boston University

Lauren Andrews, Ph.D.
Assistant Professor,
Chemical Engineering
UMass Amherst

Daniel Segrè, Ph.D.
Professor of Biology,
Bioinformatics, and Biomedical Engineering
Boston University

Rebecca Sherbo, Ph.D.
Assistant Professor,
Chemistry and Chemical Biology, and Chemical Engineering
Northeastern University

Day 2

June 13th, 2023 1:10-2:00pm

Careers in Industry (Hall E)

Many scientists continue on to fruitful careers at pharmaceutical and biotechnology companies after their academic training, and others even start their own companies! Hear from our panelists and their experiences starting a company, joining an early-stage startup, and working for an established pharma/biotech company as you plan your career's next steps.

Andres Florez
Moderator
Postdoctoral Fellow
Harvard Medical School

Analise Reeves, Ph.D.
Head of Synthetic Biology
Synlogic

Marika Ziesack, Ph.D.
Co-founder and
chief technology officer
Circe Bioscience

Felix Wong, Ph.D.
Co-founder
Integrated Biosciences

Jenna Wurster, Ph.D.
Senior Scientist
Seres Therapeutics

Maier Avendaño, Ph.D.
co-founder and
Director of Platform
Invaio Sciences

Food Microbiology, Brewing, & Bacteria Art (Hall A)

From producing raw materials to creating the delicious final product, the formation of many foods and beverages requires microbial activity. Join us to discuss organisms and processes that make certain treats taste and look just right, as well as the foes that could threaten flavor and quality.

Jennifer Tsang, Ph.D.
Moderator
Freelance Science
Communicator

Mehmet Berkmen, Ph.D.
Senior Scientist
Bacterial Artist
New England Biolabs

Lori Koziol, Ph.D.
Professor
New England College

Jeff Rowe
Brewmaster
Idle Hands Craft Ales

Computational Microbiology (Hall B)

How can we bridge the gap between bench scientists and bioinformaticians? Join us for a session highlighting cutting-edge sequencing techniques, bioinformatics methods, and statistics algorithms. Discuss how to move the field of sequencing and bioinformatics beyond description and quantitation and toward prediction and diagnostic/therapeutic applications.

Irem Ozkan
Moderator
Ph.D. Candidate
Boston College

An-Ni Chang, Ph.D.
Research Fellow
Biological Engineering Dept.
MIT

Babak Momeni, Ph.D.
Associate Professor
Mathematical Modelling of Biological Systems.
Boston College

Emma Briars, Ph.D.
Computational Biology Research Scientists
Day Zero Diagnostics

Xochiti Morgan, Ph.D.
Microbiome Analysis Core Director
Senior Research Scientist
Huttenhower Lab

Host-Pathogen Interactions (Hall D)

Two competing interests in one environment. Join us for a multidisciplinary discussion with experts at the intersection of microbiology and immunology to understand why some microbes persist within a host while others are effectively cleared

David Roach, M.D, MBA
Moderator
Infectious Diseases Fellow
Mass General Brigham

Rebecca Lamason, Ph.D.
Assistant Professor
Dept. of Biology
MIT

Jonathan Livny, Ph.D.
Senior Group Leader
Microbial 'Omics Core
Broad Institute

Ralph Isberg, Ph.D.
Professor
Molecular Biology & Microbiology
Tufts University School of Medicine

Dr. Michal Tal
Principal Scientist
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2023 Boston Bacterial Meeting - Oral Presentation Abstracts

1 Ribosome hibernation factor induces isoniazid persisters of Mycobacterium tuberculosis in neutrophil-rich lung lesions.

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The current treatment for tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), consists of a months-long multi-drug regimen. The extended regimen is necessitated by a small subpopulation of Mtb bacilli called persisters, which acquire phenotypic resistance to antibiotics. Where and how these bacilli persist in hosts remain unknown. We previously discovered that zinc limitation, a condition prevalent in chronic TB lung lesions, induces ribosome hibernation in Mtb. Ribosome hibernation involves inactivation and stabilization of non-translating ribosomes by mycobacterial protein Y (Mpy). Exploiting our molecular understanding of the Mpy recruitment process, we constructed a reporter strain to visualize Mtb bacilli with a physiological condition inducing ribosome hibernation. Using time-lapse imaging of the reporter cells on a microfluidics platform and flow cytometry, we observed that cells with induced ribosome hibernation acquire viable but non-replicating state in a Mpy-dependent manner, suggesting that ribosome hibernation protects Mtb from drugs like isoniazid that target growing bacilli. The frequency of Mtb cells expressing the reporter signal increased from 1.0% to 25% upon isoniazid treatment of infected mice, and the change was Mpy-dependent. Moreover, these isoniazid persisters were harbored in acellular spaces surrounded by neutrophils. In summary, our findings begin to develop a molecular understanding of the linkage between ribosome hibernation and antibiotic persistence in zinc-starved TB lesions, which are likely produced by neutrophils.

2 Keynome gAST: a machine learning system for predicting antimicrobial resistance phenotypes from whole-genome sequencing

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Traditional antimicrobial susceptibility testing (AST) provides a direct observation of a pathogen's antimicrobial resistance/susceptibility (AMR/S) phenotype, which is a critical piece of information in targeting appropriate therapy to patients with bacterial infections. Unfortunately, these tests rely on culturing the bacteria from blood and then observing cell growth (or lack thereof) and can take days to complete – time which a critically ill patient may not have. Recent advances in molecular diagnostics offer a quicker turn-around based on targeted amplification of known resistance markers in the pathogen DNA. But such techniques are limited to detecting only the most well-characterized resistance markers in the most widely-studied pathogens, thus severely limiting the negative predictive value of such tests. Here we review the current state of diagnostics for bacterial bloodstream infections and present Keynome *g*AST (genomic AST) – a machine learning (ML) system for predicting AMR/S phenotype from pathogen whole-genome sequencing data derived directly from whole blood via an ultra-high pathogen DNA enrichment process. The ML models at the heart of the system are trained on MicrohmDB – our extensive dataset of paired pathogen genomes and traditional AST results. The models learn genomic signatures that are useful for predicting AMR/S phenotype, even for species and drugs where resistance mechanisms are not yet well characterized. We show Keynome *g*AST can achieve >90% agreement with traditional AST (>95% for strongly susceptible/resistant samples) on 100 contrived direct-from-blood samples across a panel of >40 pathogen-drug combinations, and demonstrate its superiority to resistance marker based approaches.

3 RarA is a transcription factor in Klebsiella pneumoniae that confers extreme levels of resistance to different antibiotic classes in clinical isolates

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We characterized a *Klebsiella pneumoniae* bacterial strain (MGH83) isolated at a Boston hospital with unusually

extreme levels of antibiotic resistance. In vitro evolution yielded different MGH83 derivatives that became more antibiotic susceptible. Whole-genome sequencing (WGS) revealed mutations in one transcription factor called RarA coincided with the loss of resistance to multiple antibiotics, including quinolones, tetracyclines, nitrofurans, sulfonamides, and carbapenems. We showed that disruption of the putative transcriptional repressor of RarA resulted in RarA overexpression in many clinical isolates. We cloned RarA and transformed it into RarA-mutant derivatives, which restored the parental level of antibiotic resistance. We built a structural protein model of RarA that revealed how acquired point mutations interfere with its ability to dimerize and bind promoter DNA. We observed that RarA upregulates at least two different efflux pump systems while suppressing the expression of antibiotic-permeable porins, which helps explain its extreme levels of antibiotic resistance. We identified another "K. pneumoniae" isolate, MGH97, with even higher RarA expression. This strain was in vitro evolved, and a large chromosomal deletion occurred in the rarA locus. Interestingly, the parental MGH97 typically grows very slowly, but its RarA-mutant derivatives grow at normal rates. This suggests that while RarA offers an advantage in the presence of antibiotics, it also imposes a high fitness cost. Lastly, we used a CRISPR-based genome editing approach to knock out RarA and confirmed that knocking out RarA decreases antibiotic resistance. This work shows how transcription factors can intrinsically potentiate resistance without additional events like horizontal gene transfer.

4 A role for the Gram-negative outer membrane in bacterial shape determination

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The cell envelope of Gram-negative bacteria consists of three distinct layers: the cytoplasmic membrane, a cell wall made of peptidoglycan (PG), and an asymmetric outer membrane (OM) composed of phospholipid in the inner leaflet and lipopolysaccharide (LPS) glycolipid in the outer leaflet. The PG layer has long been thought to be the major structural component of the envelope protecting cells from osmotic lysis and providing them with their characteristic shape. In recent years, the OM has also been shown to be a load-bearing layer of the cell surface that fortifies cells against internal turgor pressure. However, whether the OM also plays a role in morphogenesis has remained unclear. Here, we report that changes in LPS synthesis or modification predicted to strengthen the OM can suppress the growth and shape defects of *Escherichia coli* mutants with reduced activity in a conserved PG synthesis machine called the Rod system (elongasome) that is responsible for cell elongation and shape determination. Evidence is presented that OM fortification in the shape mutants restores the ability of MreB cytoskeletal filaments to properly orient the synthesis of new cell wall material by the Rod system. Our results are therefore consistent with a role for the OM in the propagation of rod shape during growth in addition to its well-known function as a diffusion barrier promoting the intrinsic antibiotic resistance of Gram-negative bacteria.

5 Genome-wide identification of genetic requirements of Streptococcus pneumoniae biofilms

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Streptococcus pneumoniae, a common human pathogen, causes biofilm-associated infections. The life cycle of a biofilm begins when planktonic cells attach to a surface. These surface-attached cells generate extracellular polymeric substances that provide anchoring and protection. Eventually, dispersed cells detach from mature biofilms and seek new surfaces for colonization. Understanding the genetic mechanisms underlying the *S. pneumoniae* biofilm cycle is crucial for developing strategies to prevent and treat infections caused by this pathogen.

Here, we developed a long-term *in vitro* assay to grow *S. pneumoniae* biofilms and monitor the genetic requirements of the different biofilm stages over time. We first showed that phenotypes between planktonic, surface-attached, and dispersal cells are strikingly different. For instance, dispersal cells exhibit increased virulence and antibiotic tolerance. Transposon sequencing (Tn-Seq) and experimental evolution experiments demonstrated that capsule biosynthesis, homeostasis of the second messengers cAMP and (p)ppGpp, and nucleotide metabolism are crucial factors for biofilm formation and maintenance. Site-directed knockouts' characterization validated Tn-Seq results and showed how the studied genes influence surface-attached and dispersal cells' phenotypes.

Traditionally, infection and antibiotic tolerance studies have been conducted with planktonic cells, neglecting the potential differences between cell states generated from biofilms. In this work, in addition to identifying the genes involved in *S. pneumoniae* biofilm life cycle, we present a reliable method for obtaining surface-attached

and dispersal cells with different virulence and antibiotic tolerance. *In vivo* studies using inocula obtained by this approach could better resemble an actual infection since organ-to-organ and host-to-host dissemination probably involve biofilm-generated cells.

6 A High-Throughput Essential-gene CRISPRi Screen Using Time-Lapse Microscopy Reveals Insights on Nucleoid Morphology and Division Regulation in E. coli

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DNA replication, segregation, cell division, and growth are fundamental processes that must be coordinated in all living beings, including bacteria. Understanding how these processes are regulated is a long-standing question in microbiology. Time-lapse microscopy has given insights into how these processes are regulated; however, this technique has not been amenable to high-throughput screening. We present a novel method that enables the screening of genetic libraries using time-lapse microscopy. We used this method to evaluate the effect of a CRISPRi knockdown library of essential genes on *E. coli* growth, cell, and nucleoid morphology. We observed condensation of nucleoids along the short axis for knockdowns of genes involved in Sec-dependent secretion, providing direct genetic evidence for the influence of co-translational secretion on nucleoid topology. Additionally, by examining nucleoids in filamentous cells, we were able to determine in which cases division defects were anticipated by non-segregating nucleoids, allowing for systematic classification of these variants into division-related and segregation/replication-related categories. Based on this we proposed a list of potential direct division regulators. Finally, suppression of ribosomal genes yields diverse nucleoid structures; most ribosomal genes provoke compaction, while *rplL* and *rplJ*, trigger decondensation. In *relA*-deficient cells, *rplL* and *rplJ* knockdowns result in compact nucleoids, highlighting the stringent response's distinct role in nucleoid morphology alterations during ribosomal disturbances. The high sensitivity, throughput, exhaustivity, and diversity of phenotypes that can be assessed with our approach will enable the discovery and characterization of mechanisms regulating essential biological processes beyond what we can study using existing methods.

7 Understanding phenotypic responses to doxycycline and pathways that lead to bacterial death versus survival

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An underappreciated cause of antibiotic treatment failure is antibiotic tolerance, where phenotypic changes in response to stress allow bacteria to survive antibiotic treatment. To understand what transcriptional changes induce tolerance, we preformed RNA-seq on our model bacterium, *Yersinia pseudotuberculosis*. We found that bacteria downregulate the gene, *tusB*, in response to doxycycline, a bacteriostatic antibiotic that targets the ribosome. TusB promotes a tRNA modification that prevents misreading of glutamate, glutamine, and lysine codons, a proofreading activity thought to slow translation. We hypothesized downregulation of *tusB* allows for rapid translation of proteins and promotes survival during doxycycline stress. Interestingly, we found deletion of *tusB* does promote survival whereas overexpression promotes death, allowing doxycycline to have bactericidal activity. To understand whether *tusB* overexpression impacts ribosome speed, we performed ribosome profiling. Interestingly, we found that ribosomes stall preferentially at glutamate, glutamine, and lysine codons when *tusB* is deleted, but there is no effect when *tusB* is overexpressed. This suggests that translational speed is not responsible for the bactericidal activity of doxycycline. Rather, ribosome pausing correlates with antibiotic tolerance. Our current model is that loss of the tRNA modification decreases translation efficiency and induces a slowed-growth phenotype and tolerance. To further determine the impact of *tusB* deletion and overexpression on doxycycline susceptibility, we are taking a global approach to detect transcriptional and proteomic changes using RNA-seq and mass spectrometry. We predict that identification of pathways impacting bacterial survival will highlight a novel target to elicit bacterial death and increase the efficacy of doxycycline treatment.

8 DNA dependent regulation of the Caulobacter crescentus Protease Lon

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Regulated protein degradation is essential for protein homeostasis but must be tightly controlled as it is an irreversible process. AAA+ proteases like Lon are responsible for most of the protein degradation in the cell. In eukaryotes, deletion of Lon is embryonic lethal and defects in mitochondrial Lon result in deficits to mitochondrial DNA maintenance and stress adaptation. In bacteria loss of Lon results in growth defects, lowered motility, and a diminished stress response. While Lon is known to bind to DNA, it is not known how DNA binding affects Lon activity. Here we find that Lon's DNA binding can affect both proteolytic function and ATP hydrolysis activity depending on sequence and topology. Double stranded DNA acts as a scaffold to recruit Lon to DNA, where it clears DNA-bound proteins during genotoxic stress. Bacterial Lon binds single-stranded DNA more tightly than double stranded DNA and this interaction results in and increase in ATP hydrolysis, changes in proteolytic activation and nucleotide affinity. These effects are sequence dependent. DNA binding data and mutational analysis of the putative DNA binding domain suggest Lon can adopt multiple activation states. Together these data inform on a model of Lon adopting various states to regulate proteolysis and potentiate overall cellular proteostasis

9 CRISPR-Cas auto-regulator tracr-L balances CRISPR costs and benefits and can be de-repressed by phage infection

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CRISPR-Cas systems must discriminate self from non-self nucleic acids to provide bacteria with adaptive immunity against foreign agents, including bacteriophages. However, they are not perfect discriminators, and CRISPR-Cas hyperactivity can lead to lethal autoimmune events as well as exert other fitness costs. Therefore, regulation of CRISPR-Cas is likely beneficial to maximize targeting of foreign agents while avoiding autoimmunity. However, little is known about how type II CRISPR systems are controlled. We conducted a genetic screen for regulators of *Streptococcus pyogenes* CRISPR-Cas and discovered the novel CRISPR-Cas auto-regulatory RNA, tracr-L. *We found that while deletion of* tracr-L increased Cas operon expression by 50-fold and immunity by 3000-fold, it carried a substantial fitness cost for the mutant cells, revealing that the tracr-L repressor helps mitigate costs associated with maintenance of CRISPR-Cas. We then speculated that there might be physiological conditions, cues, and/or host pathways which naturally de-repress tracr-L to induce CRISPR-Cas expression. Indeed, we discovered that phages themselves can provide an inducing signal. Many phages encode anti-CRISPRs (Acrs) that inhibit Cas9 targeting and cleavage. We show that many Cas9 Acrs also inhibit Cas9:tracr-L repression and lead to an increase in Cas9 expression. We hypothesize that this new wave of Cas9 synthesis could help bacteria survive Acr-phage infection. These data suggest that tracr-L functions not only to repress autoimmunity but also as a phage infection sensor and anti-anti-CRISPR mechanism.

10 Gut bacteria convert glucocorticoids into progestins

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Steroid hormones regulate fundamental physiological processes, including metabolic activity, sexual characteristics, reproduction, and behavior. Recent studies suggest that there are correlations between gut bacteria, host functions, and sex- and stress-related steroid hormones. However, the causal relationships between these three entities remain unclear. Earlier studies performed before the advent of modern genetic and microbiological tools demonstrated that gut bacteria can 21-dehydroxylate corticosteroids to form progestins, thereby converting one class of steroids into another with different biological effects on the host. However, the bacterial strains, genes, and enzymes responsible for this activity have yet to be determined. In these studies, we reconstituted this progestin production in both healthy human feces and in specific strains of the gut bacterium *Eggerthella lenta* in co-culture with *Escherichia coli* Nissle 1917 and other hydrogen-producing gut bacteria. We determined the *E. lenta* genes responsible for 21-dehydroxylation through a combination of comparative genomics and homologous expression. Furthermore, we demonstrated that while both healthy human and specific pathogen-free (SPF) mouse feces contain 21-dehydroxylated products, germ-free (GF) mouse feces lack these metabolites, suggesting that gut bacteria contribute to the production of progestins in vivo. Some of the progestins produced by *E. lenta* and other prevalent Actinobacteria are neuroactive steroids, including the drugs brexanolone, an FDA-approved drug used to treat postpartum depression, and sepranolone, which is in Phase II clinical trials to treat Tourette's syndrome. Therefore, our enhanced understanding of this conversion of corticoids to progestins has potential

importance for human health and disease, especially pregnancy and women's health.

11 Spatial recovery of the gut microbiome after antibiotics perturbation

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Antibiotics disrupt the gut microbiome in an antibiotic- and individual-specific manner, with prolonged microbiome disruptions leading to increased risk of immunological and metabolic diseases. A deep understanding of these antibiotic-mediated microbiome disruptions is critical for understanding the differences in response and identifying characteristics of resilient communities. Much of the work in the field to date focuses on sequencing-based approaches, which while valuable, fail to capture spatial and contextual information that can provide additional insight into antibiotic-mediated microbiome disruptions and resulting disease.

Here, we employ a highly multiplexed imaging method to study the spatial organization of bacterial species at the micron level: high-phylogenetic-resolution microbiome mapping by fluorescence in-situ hybridization (HiPR-FISH). We used HiPR-FISH to study the spatial disruption and recovery of the murine gut microbiota in response to two antibiotics: ampicillin and vancomycin. Paired and longitudinal within-cage sampling allow us to characterize disruption and recovery in a spatial manner. Co-localization analysis is used to determine the degree of spatial disturbance and is compared between antibiotic type and GI location.

By studying the effects of both ampicillin and vancomycin, we identify generalized species interactions that lead to resilient microbiomes, as well as interactions that are specific to the perturbation. Lastly, by including both the cecum and the distal colon, resiliency at the GI locational level is studied and the cecum's emerging role as a microbial reservoir during perturbation is characterized. Altogether this approach furthers our understanding of microbiome recovery to antibiotic-mediated disruption.

12 Functional diversification of plant small molecules by the gut microbiome tunes intestinal homeostasis

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The interaction between diet and the gut microbiome is instrumental in affecting host health and disease. Dietary carbohydrates shape the microbiome as a carbon source and affect host physiology via microbial products of carbohydrate fermentation. In contrast, little is known about the interaction between the microbiome and "dietary dark matter", the countless, chemically-diverse plant secondary metabolites, phytochemicals, that we consume. Here, we asked whether phytochemicals are transformed by gut bacteria, and if products of phytochemical catabolism affect health. We first identified broad phytochemical catabolism across diverse gut bacterial species. The Bacteroidales exhibited an enhanced capacity for phytochemical catabolism. Genetic dissection of this catabolism in two prevalent and abundant *Bacteroides* species, *Bacteroides ovatus* (*Bo*) and *Bacteroides uniformis* (*Bu*), identified two divergent metabolic systems. Whereas *Bo* harbored a non-specific, generalist system for phytochemical and disaccharide catabolism, *Bu* harbored a multiple-locus catabolic system in which some loci were selective for phytochemicals and not chemically-similar disaccharides. Furthermore, we demonstrated that microbial phytochemical catabolism expands the chemical diversity of diet by generating metabolites with distinct bioactivities from their parent molecules. In a colitis model, mice were protected when treated with *Bu* (but not mutant *Bu* unable to catabolize phytochemicals) and the ancient medicinal phytochemical salicin. This suggests saligenin, salicin's metabolite, is the active anti-inflammatory agent. This protection was unique to salicin, as mice treated with *Bu* and arbutin, a salicin analog, were not protected. Our findings highlight new mechanistic insights into microbiome-dependent transformation of phytochemicals and the effects of these metabolic transformations on host homeostasis.

13 Engineering novel bacteriophage-based biotherapeutics for antimicrobial-resistant pathogens

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The accelerating incidence of antimicrobial-resistant (AMR) infections, coupled with the dwindling antibiotic

discovery pipeline, represents a worldwide public health emergency. Infectious diarrheal diseases caused by enteric bacterial pathogens are the fourth leading cause of in children under five years of age. Despite decades of research, effective vaccines against these pathogens are either nonexistent or limited, a challenge exacerbated by alarming rates of antibiotic resistance. In fact, the World Health Organization has designated these bacteria as AMR priority pathogens for which new therapies are urgently needed. To address this urgent medical need, we aim to develop and evaluate bacteriophages (phages) as pathogen-specific therapeutics for the treatment of AMR enteric pathogens. Here, we have used the tools of synthetic biology to enhance the efficacy and pathogen specificity of our previously characterized *Shigella flexneri*-specific lytic phage -2457T. We engineered a -2457T-derived phagemid system to efficiently package exogenous plasmids encoding CRISPR-Cas9 nucleases targeting conserved *S. flexneri* virulence factors into -2457T phage particles. CRISPR-Cas9 antimicrobial payload delivery to recipient cells resulted in sequence-specific targeting of pathogens without harming commensal bacteria. We are currently expanding -2457T host range through tail fiber engineering to target multiple Shigella species and testing our engineered phages in our novel human intestinal organoid-derived epithelial monolayer (HIODEM) model to evaluate their therapeutic efficacy in human-specific infection conditions. Thus, we have generated a -2457T-based pre-clinical biotherapeutic candidate targeting *Shigella* as proof-of-principle to create a phage engineering platform for the delivery of programmable, sequence-specific antimicrobial payloads to combat the rising threat of AMR pathogens.

14 Discovery of an S. aureus-pacifying microbial ensemble via massively parallel measurement of millions of microbial interactions

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The microbiome industry endeavors to restore damaged microbial communities in, on, and around our bodies. Identifying products that can effectively achieve this goal is arduous as the complex networks of microbial interactions that drive community dynamics remain largely unmapped. To address this issue, Concerto Biosciences developed kChip, an ultra-high throughput platform that directly measures millions of microbial interactions. Using kChip data, we map microbial interaction networks to reveal "ensembles"—defined groups of microbes that shepherd damaged microbial communities to a more healthy state. We used kChip to identify an ensemble of healthy skin bacteria that corrects the microbial deficiency underlying atopic dermatitis, a painful inflammatory skin disorder. Atopic dermatitis flares are elicited and exacerbated by virulent *Staphylococcus aureus*, which secretes proteases that disrupt the skin barrier and toxins that aggravate inflammation. To identify skin microbiome components that can modulate *S. aureus* virulence, we created a biobank of skin-dwelling bacteria and used kChip to measure the impact of ¿6 million defined microbial communities on *S. aureus* virulence gene expression. From these millions of interaction measurements, we constructed the first ever skin microbiome interactome—a map of how the skin microbiome regulates *S. aureus* virulence—and identified ensembles of microbes that completely suppress *S. aureus* virulence. We validated that these ensembles placate *S. aureus* through in vitro and ex vivo experiments and have selected an ensemble for development into a novel topical live biotherapeutic product we call Ensemble No.2 (ENS-002).

15 Compartment specific cell wall labeling in mycobacteria used to identify cell wall proteins informs hierarchical secretion mechanism in Type VII secretion systems

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Mycobacteria being multi-compartment organisms have a peculiar cell wall which plays a key role in interactions with the environment and act as a selective barrier. The cell wall contains specialized protein complexes that aide the import and export of nutrients, proteins, lipids and virulence factors thereby playing a key role in survival and pathogenesis. Accurately identifying cell wall proteins is an important step in assigning function, especially as homologues from many related organisms are absent in mycobacteria. Efforts to separate these compartments and identify the proteins in the cell wall have either been non-specific, as with sedimentation-based techniques that are performed on lysates, or low throughput, as with techniques that localize a specific protein. Both approaches fail to give a broad and accurate picture of the cell wall proteome. Here we show that

compartment specific protein tagging by the engineered peroxidase APEX2 within live Mycobacterium tuberculosis enables the accurate identification of the cytosolic and cell wall proteomes. This strategy provides the first direct evidence that substrates of virulence-associated Type VII proteins secretion systems (ESX) are exposed to the periplasmic environment. These results support the recently proposed model for ESX-1 secretion beyond the inner membrane, in which substrates support their own secretion through the periplasm. Using APEX2-mediated protein tagging in ESX-1 substrate knockout strains, we are now determining the hierarchy of ESX substrate occupancy within the cell wall, towards a more detailed model of secretion.

16 Pathogens within Pathogens: Prophage Competition in Intramacrophage Salmonella

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Persisters are a subpopulation of transiently nongrowing bacteria capable of surviving antimicrobial attacks from antibiotics and the immune system and eventually resuming growth. Bacterial persistence contributes to antibiotic treatment failure and the relapse of many recalcitrant infections. Many pathogens, including Salmonella enterica, Mycobacterium tuberculosis, and *Staphylococcus aureus*, form persisters within macrophages where they survive for extended periods of time and subvert host immune defenses. Marker-frequency analysis sequencing (MFA-seq) of Salmonella persisters revealed prophage replication in intramacrophage *Salmonella* persisters. Remarkably, despite prophage induction, intramacrophage *Salmonella* avoid prophage-induced lysis. We characterized the mechanism through which *Salmonella* avoids prophage-induced lysis and found a prophage-encoded antiphage-like system acting through tRNA cleavage. tRNA both blocks prophage-induced lysis and triggers prolonged growth arrest. Growth arrest contributes to the persistence of *Salmonella* in macrophages improving antibiotic survival and increasing the potential for infection relapse. Our findings reveal a multi-layered host-pathogen feud whereby prophage-prophage competition influences growth of *Salmonella* and its interaction with host immune cells. This implicates a role for prophages in pathogenicity beyond mobilization of prophage-encoded virulence factors. Critically, understanding the molecular mechanisms of *Salmonella* growth arrest in macrophages, provides insight to how we can resensitize persisters to antibiotics and limit infection relapse.

17 Needle in a haystack: cell-selective secretomics reveal a novel bacterial effector that interacts with the eukaryotic translocon

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Rickettsia spp. are obligate intracellular bacterial pathogens that pose a growing threat to human health. Nevertheless, their strict reliance on a host cell niche has hindered investigation of the molecular mechanisms driving rickettsial infection. *Rickettsia* spp. secrete effectors to hijack host machinery and remodel their intracellular environment, but only a few rickettsial effectors and their host cell targets are known. Therefore, we implemented bioorthogonal non-canonical amino acid tagging (BONCAT) in *Rickettsia parkeri* to selectively label, enrich, and identify effectors secreted during infection. Our screen represents the first use of BONCAT in an obligate intracellular bacterium and more than doubles the list of experimentally-validated effectors known for *R. parkeri*. The novel secreted rickettsial factors (Srfs) we identified include several hypothetical proteins that are unique to the *Rickettsia* genus. We demonstrate that one such effector, SrfD, localizes to the endoplasmic reticulum where it interacts with the core eukaryotic translocon component Sec61. Moreover, we show that SrfD inhibits the host secretory pathway through its interaction with Sec61. By characterizing these novel effectors and how they subvert host cell processes, we hope to improve our understanding of the host-pathogen interface and the bacterial factors governing rickettsial pathogenesis.

18 Defining Kynurenine Pathway Control of Salmonella Typhimurium Infection

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Antibiotic-resistant bacterial infections remain a persistent cause of hospitalization and death around the world. In a whole organism screen for compounds that improve host health during infection, we found that exogenous application of the kynurenine pathway metabolite 3-hydroxy-kynurenine (3-HK) rescued zebrafish larvae from

lethal *Salmonella* Typhimurium infection. Exogenous 3-HK did not act like a typical antibiotic in that it did not inhibit bacterial growth *in vitro* but did restrict bacterial growth *in vivo*. Further analysis of endogenous production of 3-HK through the kynurenine pathway revealed that kynurenine 3-monooxygenase (Kmo), the enzyme responsible for catabolism of kynurenine to 3-HK, is required for the normal immune response to systemic *Salmonella* Typhimurium infection. Inhibition of Kmo and the endogenous production of 3-HK resulted in decreased survival to sub-lethal infection. Sensitivity to infection upon Kmo inhibition correlated with more rapid expansion of bacterial burden. However, whole organism inflammatory signatures were not impaired, as Kmo inhibition enhanced ROS production and pro-inflammatory cytokine induction, which did not correlate with bacterial control. While macrophage cell numbers are not altered following Kmo inhibition, their ability to control intracellular bacterial replication is impaired. Infected macrophages with Kmo inhibition display signatures of impaired lysosomal acidification through RNA sequencing and through functional analysis with a pH-sensitive dye. Taken together, this work provides a novel mechanism by which decreasing endogenous 3-HK production through Kmo inhibition alters lysosomal function to promote intracellular bacterial replication in macrophages, leading to increased whole organism bacterial load.

Poster Presentation List

Poster Session I: June 12, 10:15-11:15 AM

1	**Eleanor Young**	Deploying Go-PhAST-R on Clinical Samples
2	**Irem Ozkan**	The impact of subinhibitory levels of translation inhibiting antibiotics on transcription initiation and termination sites in *Streptococcus pneumoniae*
3	**Rebecca McSweeney**	Investigating the genetic modulators of observed vancomycin susceptibility in *Neisseria gonorrhoeae* isolates
4	**Emily McNutt**	A genetic selection for identifying enzymes that metabolize modified nucleosides
5	**Thais Harder de Palma**	Absolute and conditionally essential genes of *Haemophilus parainfluenzae*
6	**Benjamin Koleske**	Screening the PE/PPE secreted effectors of *M. tuberculosis* to uncover novel virulence-promoting genes
7	**Britta Goncarovs**	Engineering Synthetic Biotics to Secrete Therapeutic Proteins
8	**Kevin Barthes**	A functional metagenomic screen for antiphage defense systems
9	**Desiree Sukhram**	Development of Synthetic RNA thermometers for biocontainment genetic circuits
10	**Arvie Grace Masibag**	Membrane vesicles as microbial delivery systems for mediating competition in pathogen-probiont interactions
11	**Alicia Egea Weiss**	Use of morphological profiling in *B. burgdorferi* to investigate environmental stress
12	**Samuel Verzino**	Development of Whole-Cell Biosensors For Early Detection Of Oral Squamous Cell Carcinoma
13	**Yasha Ektefaie**	Deep learning for antibiotic discovery in *Mycobacterium tuberculosis*
14	**Elizabeth Tan**	Using transposon-sequencing to assess phage gene contributions for fitness in the virulent phage ICP1 during the predation of *Vibrio cholerae*
15	**Samantha Lindberg**	A Genome-wide CRISPRi Screen Identifies Drivers of Antibody-mediated Agglutination in *Salmonella* Typhimurium
16	**Abigail Dzordzorme**	Distinct Nanoparticle Surface Chemistry Influences Early *P. aeruginosa* Biofilm Association
17	**Mark Young**	Pangenome hybrid capture increases coverage of *Escherichia coli* within stool metagenomes, enabling transcriptomic analyses of *E. coli* within the rUTI gut microbiome
18	**Alicia Mendoza**	Understanding how Histidine Kinase, NahK, Influences Biofilm Formation and Quorum Sensing in *Pseudomonas aeruginosa*
19	**Amanda Adams**	Bacteria-virus interactions in the vaginal microbiome reduce herpes virus infectivity

20	**Nicole Reinhold-Larsson**	Identifying *Chlamydia trachomatis* virulence factors that inhibit adaptive immunity
21	**Pooja Uchil**	Host Factors Engaged in the Repair of the Mtb Phagosome Membrane
22	**Nick Peterson**	Non-canonical pattern recognition of a pathogen-derived metabolite by a nuclear hormone receptor identifies virulent bacteria in *C. elegans*
23	**Pathricia Leus**	Identifying neutrophil effector functions targeted by *Yersinia pseudotuberculosis* Yops
24	**Jennie Ruelas Castillo**	The heme oxygenase-1 metalloporphyrin inhibitor stannsoporfin enhances the activity of a novel regimen for multidrug-resistant tuberculosis in a murine model
25	**Shuying Xu**	*S. pneumoniae* pneumolysin promotes airway barrier breach by triggering neutrophil transepithelial migration and elastase release
26	**Lia Parkin**	Defining LprG-Rv1410c mediated lipid transport through the mycobacterial cell envelope
27	**Aseem Palande**	Identifying the *Mycobacterium tuberculosis* surface proteome using proximity labeling in live cells
28	**Andres Herrera-Tequia**	Posttranslational regulation of the leucine-responsive regulatory protein (Lrp) from *E. coli*
29	**Merlin Brychcy**	The Impact of *Acinetobacter baumannii* Vanillate Catabolism on Biofilms
30	**Jason Denoncourt**	Inhibition of disulfide bond formation enzymes potentiates bacteriophage killing of *Acinetobacter baumannii*
31	**Ibukun John Abulude**	New interaction network among hypothetical proteins may serve important role in *Bdellovibrio bacteriovorus* predation
32	**Amy Rohlfing**	Variations in sporulation conditions and purification methods affect sensitivity to germinants in *Clostridioides difficile* spores
33	**Daigo Inoyama**	A Preclinical Drug Candidate Targeting *Mycobacterium tuberculosis* KasA
34	**Celena Gwin**	Spatial segregation of metabolic processes underlies cellular asymmetry in mycobacteria
35	**Sierra Schmidt**	Investigating the autoregulation of a ribosomal protein in the pathogen *Francisella tularensis*
36	**Natalia Quinones-Olvera**	Diverse and abundant viruses exploit conjugative plasmids
37	**Saumya Bandyopadhyay**	*Legionella* employ a cell surface signaling system to maintain replication vacuole integrity

Poster Session II: June 12, 3:25-4:25 PM

38	**Justin Bae**	Investigating heterogeneous regrowth of *Salmonella* persisters inside macrophages using spatially resolved, single-cell transcriptomics
39	**Rebecca Korn**	Exploring the relationship between sequence and antibiotic resistance with FMN riboswitch fitness landscapes

40	**Yishak Woldetsadik**	CRISPR/Cas mediated evolutionary arms race between *Vibrio cholerae* and ICP1 bacteriophage
41	**Allison Scott**	Understanding the role of gene family expansion in *R. parkeri*'s type IV secretion system
42	**Matteo Luban**	A unique CRASP (complement regulator-acquiring surface protein) from *Borrelia afzelii* possesses a distinct pattern from other members of its identified paralogous pfam54 gene family
43	**Katerina Roth**	Genomic Analysis of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus suci* reveals genetic differences that could contribute to differences in spoilage potential
44	**Quinn Furumo**	PIPETS: A Novel 3'-seq Analysis Method for the Identification of Transcription Termination Signal
45	**Tommy Tashjian**	Proteomic survey of the DNA damage response in *Caulobacter crescentus* reveals evidence of post-transcriptional control
46	**Natalie Murphy**	Design of a propeptide-based antimicrobial for *Vibrio cholerae*
47	**Berent Aldikacti**	Phenotypic fitness landscapes in *Caulobacter* during proteotoxic stresses
48	**Andrew Barnett**	Experimental characterization of the diversity of gene transfer agents in alphaproteobacteria
49	**Yunha Hwang**	Genomic language model predicts protein co-regulation and function
50	**Sosie Yorki**	Metagenomic assembly to track antibiotic resistance genes in international traveler stool
51	**Rachel Bargabos**	Small molecule produced by *Photorhabdus* kills bacteria by blocking ubiquinone synthesis
52	**Hyerim Ban**	Elucidating the transferability of CRISPRi systems across model and non-model *E. coli* strains
53	**Devin Lloyd**	Exploring the Regulation of Biofilm Formation in *Acinetobacter baumannii*
54	**Jacob Baker**	Community assembly dynamics in the human skin microbiome
55	**Michael Zulch**	How Many Microbes Can a Plant Support?
56	**Samantha Wellington Miranda**	Evolution and function of bacterial communication systems in polymicrobial communities
57	**Ananyaa Welling**	Characterizing the role of neutrophil extracellular traps in *Staphylococcus aureus* kidney infection
58	**Kender Poore**	Characterization of an Unknown Virulence Gene in *Shigella flexneri*
59	**Juan Hernandez-Bird**	Applying the Mouse Pneumonia Model of *Acinetobacter baumannii* for the Evolution and Characterization of Colistin-Selected Mutants.
60	**Katherine Dailey**	Dissemination of barcoded uropathogenic *Escherichia coli* in a murine model of urinary tract infection
61	**Brandon Lee**	The Impact of Age on Lyme Disease-Associated Atopic Dermatitis

62	**Alexandra Grote**	Adaptive mechanisms of chronic salmonellosis in humans
63	**Brandon Sit**	New biology from an old antigen: a role for the 17kDa surface lipoprotein in rickettsial cell envelope maintenance
64	**Aditya Bandekar**	Cell division principles and mechanisms of ceftriaxone resistance in *Neisseria gonorrhoeae*
65	**Anna Seminara**	Characterization of the microbial ergothioneine transporter EgtUV in the gastric pathogen *Helicobacter pylori*
66	**Christina Rothenberger**	Investigating a Predicted Fimbriae-forming Lipoprotein in *Porphyromonas gingivalis*
67	**Ellen Madden**	Cell division protein ZapE links the division machinery to central carbon metabolism in pathogenic and non-pathogenic strains of *Escherichia coli*
68	**Mark Soo**	A novel control system links cell wall stress to outer membrane synthesis in *Acinetobacter baumannii*
69	**Ella Messner**	Identifying Genes that Regulate Gene Transfer Agent Synthesis and Release in *Caulobacter crescentus*
70	**Morgan McNellis**	Pseudoprotease-mediated regulation of germinant sensing in *C. difficile*
71	**Caitlin Wiafe-Kwakye**	Investigating the impact of prophages on bacterial fitness of Group B *Streptococcus*
72	**Kuldeepkumar R Gupta**	An essential periplasmic protein coordinates lipid trafficking and is required for asymmetric polar growth in mycobacteria
73	**Jinna Bai**	Global transcriptional control of membrane lipid biosynthesis and metabolism in *A. baumannii*
74	**Duncan Kountz**	The structure and proposed biosynthesis of an elusive bacterial pigment associated with efficient cellulose digestion

Poster Session III: June 13, 10:10-11:10 AM

75	**Lisa-Marie Nisbett**	Exploring the mechanism of lipid transport to the outer membrane of mycobacteria in the LprG-Rv1410c pathway
76	**Jade Law**	Analysis of BfmRS essentiality in multidrug-resistant *Acinetobacter baumannii*
77	**Daniel Banas**	Antibiotic tolerance in populations of non-proliferating and intracellular uropathogenic *E. coli*
78	**Ari Sarfatis**	Highly Multiplexed Spatial Transcriptomics in Bacteria
79	**Audrey Ory**	Improving the genetic tractability of *Xanthobacter autotrophicus* for use as a point-of-need food production platform
80	**Sydney Edwards**	Use of a Graph Theoretic Approach to Identify and Study a Novel Transcriptional Regulator in *Shigella*
81	**Danielle Peterson**	Accurate species identification and antibiotic susceptibility prediction (AST) for multiple pathogens in contrived polymicrobial blood samples using whole genome Oxford Nanopore sequencing

82	**Sophie Bodrog**	Applying droplet Tn-Seq to identify metallophore transport systems in *Yersinia pestis*
83	**Abigail Miller**	Developing a Fluorescence-activated Cell Sorting Method to Evaluate Ribosomal Cis-Regulatory RNAs
84	**Margaret Hill**	Outer Membrane Vesicles Isolated from *Pseudoalteromonas rubra* Carry Prodiginine Antibiotics as Cargo
85	**Sunhee Bae**	Lectin-seq: uncovering the determinants of host-microbe interactions
86	**Nathan Hill**	*Escherichia coli* Surface Display of *Borrelia burgdorferi* Lipoproteins
87	**Michelle Naegeli**	Rapid Emergence of Resistance against Direct Antimicrobial Activity of Avibactam in Gram-Negative Pathogens
88	**Courtney Price**	*Bacillus subtilis* biofilm induction by diverse carbon sources
89	**Kindra Becker**	Selective *in vivo* tagging of mycobacteria surface proteins with a cell-impermeable fluorescent substrate
90	**Celia Souque**	Ecology & evolution of a plasmid population
91	**Rosalind Xu**	A Single-Cell Spatial Atlas of the Mouse Gut Reveals Spatial- and Microbiota-Dependent Sensation
92	**Maximillian Soltysiak**	Food and biomass from air, water, salt, and electricity
93	**Monica Wei**	Mining the Pig Skin Microbiome for Antimicrobial Products
94	**Karthik Hullahalli**	Innate immune responses yield tissue-specific bottlenecks that scale with pathogen dose
95	**Kristina Kelley**	The Microbiota-derived Metabolite TDCA May Contribute to the Pathogenesis of Pediatric Celiac Disease
96	**Talia Greenstein**	Granuloma-specific design of drug combinations for tuberculosis treatment
97	**Jon McGinn**	Dissecting the genetic networks underlying host subversion during *Rickettsia* infection
98	**Siavash Valafar**	Continuing Evolution in *Mycobacterium tuberculosis* result in Evasion of Molecular Diagnostics but provide prognostic markers for Isoniazid (INH) Resistance
99	**Nicholas DiBenedetto**	Investigating the role of epithelium-associated populations of *Clostridioides difficile* as a determinant of disease severity
100	**Isabel Sakarin**	Rv3802c function in mycobacterial cell envelope remodeling, barrier properties, and antibiotic susceptibility
101	**Natalie Sontag**	Investigating the interplay between serine/threonine protein kinases and two-component systems in *Mycobacterium tuberculosis* environmental response
102	**Pola Kuhn**	DNA methylation promotes *Clostridioides difficile* sporulation by enhancing transcription of a gene encoding the cell fate determinant SpoIIE
103	**Kubra Yigit**	Lon protease is important for the adaptation to low oxygen and the regulation of heme biosynthesis in *Caulobacter crescentus*

104	**Samantha Palace**	Rapid evolution to serum resistance through phase variation in *Neisseria gonorrhoeae*
105	**Colby Ferreira**	FtsA-FtsN direct interactions support assembly of translateral divisome complexes de novo
106	**Natalia Quirk**	Uncovering novel regulators of the *Mycobacterium tuberculosis* nitric oxide (NO) response
107	**Mimi Kay**	An Alternative Model of Error Prone Polymerase Recruitment in Post-replicative DNA repair
108	**Christian Loyo**	A gene in the integrative and conjugative element ICE*Bs1* confers phage defense in *Bacillus subtilis*
109	**Takehiro Kado**	Bilayer-Intrinsic and -extrinsic structure to separate mycobacterial plasma membrane into domains
110	**Kerry McGowen**	DNA damage as an *in vivo* mechanism of action of pretomanid in *Mycobacterium tuberculosis*
111	**Anne McCabe**	Exploring the mechanisms of collateral sensitivity to evolved Gentamicin resistance in *Klebsiella pneumoniae*

Poster Session IV: June 14, 2:55-3:55 PM

112	**Keiichiro Mukai**	Analysis of molecular mechanisms underlying concentration-dependent positive effects of the antibiotic lincomycin on *Streptomyces coelicolor* A3(2)
113	**Amina Bradley**	Investigating Biosynthetic Gene Cluster Activation in *Bacilli*
114	**Yun Tian**	Gene silencing through CRISPR interference in *Borrelia burgdorferi*
115	**Bharathi Sundaresh**	Bacterial genomic profiling reveals novel routes to complement evasion and targeted therapeutic strategies
116	**Julie McDonald**	A Versatile Plasmid-based System for Continuous Evolution in Bacterial Hosts
117	**Nicoletta Collison**	*Streptococcus pneumoniae* transcription factor fitness quantification using transcriptional regulator-induced phenotype screens
118	**Emily MacLeod**	Development of a Culture-Free Diagnostic for Urosepsis Leveraging Whole Genome Sequencing and Machine Learning
119	**Annie Wen**	Development of the First Inducible CRISPRi System in *Rickettsia* to Knockdown Gene Expression
120	**Patricia Romanos**	Inhibition of NE release as a novel therapeutic target to prevent systemic disease during *S. pneumoniae* infection
121	**Doug Kenny**	Engineering Synthetic Biotics to Secrete Therapeutic Proteins and Small Molecules for the Treatment of Intestinal Inflammation
122	**Irene Lepori**	Screening small molecule permeation across the mycobacterial outer membrane
123	**Dora Mahecic**	Elucidating plasmid copy number control with super-resolution microscopy
124	**Patrick Woida**	Proximity-based proteomics as a tool to dissect the bacterial-host interface during *Listeria* cell-to-cell spread

125	**Angela Dao**	Microbiome Avatar Colitis Mouse Model - A Translational Tool For In Vivo Characterization of Heterogeneity in Stool Samples From Patients With Inflammatory Bowel Disease (IBD)
126	**Christopher O'Connell**	*Pseudomonas* spp. are prevalent in the microbial communities in maple sap
127	**Nora Pyenson**	Community ecology of phage on a single bacterial host
128	**Aanya Subramaniam**	MIT Mucosal and Systemic Signatures Triggered by Responses to Infectious Organisms (MAESTRO) Clinical Study
129	**Dezmond Cole**	Investigating the role of cell shape in the host interactions of *Rickettsia parkeri*
130	**Renuka Ramanathan**	Redox regulation of host ribosomal proteins during *Helicobacter pylori* infection
131	**Aditi Kannan**	The diversity and dynamics of vaginal *Lactobacillus crispatus* prophages
132	**Deepak Chouhan**	Saccharibacteria decreases *Actinomyces* induced innate immune response
133	**Danielle Guercio**	Characterizing the role of NahK in Anaerobic Respiration Regulation in *Pseudomonas aeruginosa*
134	**Vania Lopez Ruiz**	Potentiating immune responses to microbial pathogens by innate-like T cells via non-classical antigen presentation in *Xenopus laevis*
135	**Julia Ryan**	Evolution of growth-enhancing mutations in *Mycolicibacterium smegmatis* strains deleted for a small RNA and an RNA binding protein
136	**Lucas Guzman**	Determining the role of cyclic-di-GMP in enhanced biofilm formation in *Vibrio cholerae* and *Paracoccus aminovorans* cocultures
137	**Daniel Eaton**	An Essentialome-Wide CRISPRi Screen for Cell Cycle Defects with Barcoded Live-Cell Microscopy
138	**Satoshi Kimura**	A tRNA modification in *Mycobacterium tuberculosis* facilitates optimal intracellular growth
139	**Kimberly Barker**	Magnesium-Dependent Regulation of Cell-Cycle-Dependent Proteolysis in *Caulobacter crescentus*
140	**Audrey Randall**	Determining if *Parabacteroides distasonis* requires HPRT to accelerate diabetes and activate insulin B:9-23-specific T-cells
141	**Janan Wang**	Elucidating Lon Protease Biological Functions Through the Use of Overexpression Vectors in *Acinetobacter baumannii*
142	**Ololade Gbadebo**	Membrane Vesicles from the Marine Bacterium *Pseudoalteromonas piscicida* Deliver Antimicrobial Cargo
143	**Malavika Prithviraj**	Membrane-intrinsic Factors that Control the Dynamics of Mycobacterial Membrane Compartments
144	**Emily Chu**	Transcriptional Profiling of *Desemzia incerta* to Explore Colony Morphology Differences
145	**Aaron Love**	Specific Codons Control Cellular Resources and Fitness
146	**Anna Green**	Integrating protein 3D structure with mutation data identifies proteins under selection in *Mycobacterium tuberculosis*
147	**Yanqi Wu**	Single-cell growth rate of heterotrophic bacterioplankton in natural seawater

2023 Boston Bacterial Meeting - Poster Presentation Abstracts

1 Deploying Go-PhAST-R on Clinical Samples

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Antimicrobial resistance (AMR) infections worsens patient outcomes. Traditional AMR diagnostics rely on growth in the presence of antibiotics, delaying appropriate therapy. Recently, we introduced Go-PhAST-R (combined genotypic and phenotypic AST through RNA detection). This assay uses the NanoString RNA hybridization platform that enables AMR detection by looking at fold change in antibiotic responsive transcripts that distinguish susceptible from resistant isolates. Concurrently, it looks for genotypic markers of resistance to provide relevant epidemiological data. In this study, we sought to demonstrate the validity of the assay in a clinical context: that isolates from patient samples would demonstrate transcriptome patterns correlating to susceptible and resistant phenotypes. Broth from consecutive positive blood cultures with E. coli (n = 27) and K. pneumoniae (n = 16) were assayed. We used squared projected distance (SPD) calculations to estimate similarity to samples with known MICs. We identified 5 samples expressing CTX-M15 and 1 sample expressing KPC, all displaying extensive beta-lactam resistance. SPD separated E. coli samples into S and NS with 96.3% categorical agreement, 0.6% major error rate, 0% very major error rate, and 4.8% minor error rate. Genotypic detection of KPC and SPD separation of K. pneumoniae achieved 98.1% categorical agreement, 0% rate of both major errors and very major errors, and a 1.9% minor error rate. In a clinical context, GoPhAST-R maintained the advantages of quick, minimal sample preparation and concordant genotype and phenotype results. Go-PhAST-R has the potential to decrease turnaround time for antibiotic susceptibility testing and improve patient outcomes.

2 The impact of subinhibitory levels of translation inhibiting antibiotics on transcription initiation and termination sites in Streptococcus pneumoniae

Irem Ozkan[1] Indu Warrier[2] Tim vanOpijnen[1,3] Michelle M. Meyer[1]
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The widespread overuse of antibiotics selects for resistance within bacterial populations at antibiotic concentrations far below those used in the clinic, thus significantly contributing to the rise of antibiotic resistant strains. Therefore, assessing the impact of subinhibitory antibiotics concentrations provides a view into the emergence of antimicrobial resistance. In many bacterial pathogens transcription and translation are tightly coupled. However, recent literature highlights that RNA polymerase and ribosome movements are disjointed in gram-positive bacteria, resulting in fundamentally different transcriptional regulation. To examine how subinhibitory concentrations of 3 different translation inhibiting antibiotics impact the gene expression, we performed RNA-seq on populations of *Streptococcus pneumoniae*. Our findings demonstrate that tetracycline and chloramphenicol, which respectively act on 30S and 50S ribosomal subunits for translation inhibition, result in diverse changes to cellular functionality. Meanwhile, kasugamycin, which inhibits translation initiation, displayed behavior similar to that of tetracycline. We subsequently performed 5'- and 3'-end sequencing on the chloramphenicol and kasugamycin treated samples. From our 3'-end sequencing data, it is apparent that transcription termination is only moderately affected by translation inhibition. However, altered termination sites were more likely to be found inside a gene compared to those that are constant. The 5'-end sequencing data revealed that 25% of the primary transcription start sites were retained in all conditions. Taken together, our results indicate that inhibition of translation only causes a moderate change in the transcriptional landscape, further questioning the stringency of coupled transcription and translation in gram positive bacteria.

3 Investigating the genetic modulators of observed vancomycin susceptibility in Neisseria gonorrhoeae isolates

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The obligate human pathogen *Neisseria gonorrhoeae* differs from other Gram-negative bacteria in that a subset of isolates is susceptible to vancomycin. The true prevalence of vancomycin-susceptible *N. gonorrhoeae* is not known, because vancomycin is routinely included in media used to culture *N. gonorrhoeae* from patient

specimens. Variant alleles of the efflux pump *mtr* and the major porin *porB* have been reported to influence N. gonorrhoeae's vancomycin susceptibility, but the full set of genetic modulators of vancomycin resistance is not known. To identify genetic factors associated with vancomycin resistance, we measured the vancomycin minimum inhibitory concentrations (MICs) of 212 genetically diverse *N. gonorrhoeae* isolates, then performed a genome-wide association study (GWAS). We also cultured susceptible strains to investigate their growth on Thayer-Martin media and performed transformations to identify loci that confer vancomycin resistance. We identified 14 vancomycin-susceptible isolates, approximately 6.6% of isolates tested. Neither *mtr* nor *porB* alleles were significantly associated with vancomycin MIC by GWAS; known variants in these loci explain less than 40% of variation in this phenotype. Seven of the fourteen vancomycin-susceptible strains grew on Thayer-Martin agar with 3 µg/mL vancomycin, but susceptible isolates from patients with disseminated gonococcal infection—which are often cultured from patient blood specimens on non-selective media—did not. This variation by infection site in ability to grow on vancomycin-containing media possibly occurs because of some previous specimen collection on selective media. While *mtr* and *porB* explain some variation in vancomycin MIC, other genetic factors contribute to vancomycin susceptibility in *N. gonorrhoeae*.

4 A genetic selection for identifying enzymes that metabolize modified nucleosides

Emily McNutt[1] James Eaglesham[1] Mehmet Berkmen[1]
[1]New England Biolabs

RNA modifications are found in all domains of life with over 170 modifications identified to date. The functional role of the majority of these modifications is not well understood. To identify the role these modifications play *in vivo* it is essential to identify where they occur within the transcriptome. Enzymes that can selectively remove modifications from RNA, called 'eraser' enzymes, can be used in conjunction with high-throughput sequencing to map RNA modifications. Only a handful of eraser enzymes have been discovered thus far. To identify new eraser enzymes, we have developed a genetic selection system that uses auxotrophic strains of *Escherichia coli* deficient in either purine or pyrimidine biosynthesis. These auxotrophic strains were used to screen for the ability of *E. coli* to grow on minimal media supplemented with a modified nucleoside as the sole source of purines or pyrimidines. If *E. coli* is unable to grow on the provided modified nucleoside, genomic DNA libraries can be used to select for enzymes from other species that restore growth and remove the modification. If *E. coli* can grow on the modified nucleoside, then a native enzyme is capable of metabolizing that modification. Thus far, we have shown that *E. coli* is able to grow on N6-methyladenosine (m6A) as its sole purine source. Additionally, we show that the *E. coli* adenosine deaminase, Add, is essential for growth on m6A. Using this genetic approach, we aim to identify both novel eraser enzymes and illuminate the metabolism of modified nucleosides in *E. coli*.

5 Absolute and conditionally essential genes of Haemophilus parainfluenzae

Thais Harder de Palma[*,1] Morgan McPartland[*,1] Matthew Ramsey[1]
[1]University of Rhode Island

Haemophilus parainfluenzae (Hp), a Gram-negative bacterium, is a prevalent and abundant member of the oral microbiome, and it is considered an "habitat generalist". HHaemophilus parainfluenzae is also a HACEK organism which are oral microorganisms that can cause infective endocarditis. Currently, it is unknown which genes *Hp* requires to survive. Very little is also known about which gene products provide *Hp* with the ability to survive in the human bloodstream and cause distal infection. To identify these factors, we first determined the absolute essential genes of two strains of *Hp* (ATCC 33392 and the oral isolate EL1) using Tn-seq. Data show that there are 503 (25%) genes absolute essential for ATCC 33392, and 531(26%) genes for EL1. We also identified 427 genes that are absolute essential for both strains tested. These genes are associated with genetic information processing, carbohydrates, protein, and energy metabolism. We then identified genes required for survival in active serum complement finding that 13 (0.6%) genes are conditionally essential for the ATCC strain and, 27 (1.3%) for EL1. Among the genes that are conditionally essential for serum survival in the ATCC and EL1 strains, we observed genes associated with syntheses of surface lipopolysaccharide (LPS) which was previously demonstrated to confer complement resistance in other species. These results bring new information about the absolute and conditionally essential gene products of *Hp* that can be used to help find new possible drug targets to fight *Hp* infections as well as reveal new mechanisms of complement resistance.

6 Screening the PE/PPE secreted effectors of M. tuberculosis to uncover novel virulence-promoting genes

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Mycobacterium tuberculosis (M.tb) remains the leading cause of infectious disease mortality, with 10 million active cases and 1.5 million annual deaths globally. Although M.tb infection prompts highly inflammatory granulomatous lesions in the lung, the bacterium is not eradicated by the immune cell infiltrate. Instead, M.tb uses numerous virulence factors to facilitate growth within macrophages. In this work, we examined substrates of ESX-5, a prolific type VII secretion system unique to slow-growing pathogenic mycobacteria. These secretory substrates, the PE and PPE family proteins, have evolutionarily expanded from 11 members in the commensal *M. smegmatis* to 167 members in the M.tb H37Rv reference strain, suggesting they serve critical functions during a pathogenic lifestyle. Certain PE/PPE genes have been implicated in immune-relevant functions, including TLR signaling, apoptosis inhibition, and NF-κB activation, though most remain understudied. We conducted a pooled screen of 81 M.tb strains with single transposon insertions in PE/PPE genes, all without in vitro growth defects, to identify mutants that demonstrate in vivo fitness costs. Bacterial genomes were harvested from the lungs of aerosol-infected BALB/c mice at acute (1 week) and subacute (3 week) timepoints and compared to the composition of the initial pool (day 1). Deep sequencing demonstrated significant decreases in the prevalence of 20 strains over time; these findings were highly reproducible across animals (n=10 per timepoint) and validated several PE/PPE genes previously found to contribute to M.tb pathogenesis. Hence, our screen of PE/PPE genes in a well-validated animal lung model has produced many hits for candidate virulence-promoting factors.

7 Engineering Synthetic Biotics to Secrete Therapeutic Proteins

Britta Goncarovs[1] JR Gao[1] Chun-Cheih Chao[1] Jenny Shu[1] Doug Kenny[1] Isabel Masteika[1] Jaclyn Thompson[1] Dave Hava[1] Analise Reeves[1]
[1]Synlogic Therapeutics

Synlogic's synthetic biology platform allows the engineering of probiotic strains with versatile modalities, including secretion of proteins with immunomodulatory functions. Here we demonstrate the utility of our platform to secrete single or multiple bioactive effectors through several types of distinct secretion mechanisms. Synthetic biotics can be engineered to enhance secretion of human cytokines through modifications to the outer membrane architecture. A strain designed to secrete the notoriously insoluble cytokine, human IL2 (hIL2), secreted high levels of protein upon addition of solubilization tags and targeted mutagenesis while maintaining activity. We show that multi-protein membrane spanning secretion systems heterologous to EcN can be engineered to secrete hIL2 and a hIgA-GLP2 fusion protein. Additionally, we found that modifications to the growth process development of the secretion chassis were sufficient to improve secreted cargo yields, which can be relevant for scaling up for commercial processes. Synthetic biotics can be engineered to simultaneously secrete multiple cargo with the potential to mitigate inflammatory signaling. A strain engineered with a dual-cytokine secreting capability and a second strain designed to secrete hIL2 while producing a STING agonist illustrate the versatility and potential to combine multiple modalities into a therapeutic chassis. Finally, we show that the colon effluent of mice dosed with a strain engineered to secrete an anti-TNFalpha nanobody can neutralize TNFalpha activity.

Together, these data detail a variety of options for secreting effectors from a synthetic biotic chassis and provide the basis for developing synthetic biotics to target pathologies in the gut.

8 A functional metagenomic screen for antiphage defense systems

Kevin Barthes[1,2] Léa Chuzel[1] Yan-Jiun Lee[1] Samantha Fossa[1] Peter Weigele[1] Chris Taron[1] Mehmet Berkmen[1] James Eaglesham[1]
[1]New England Biolabs (Ipswich, MA) [2]École Supérieure de Biotechnologie de Strasbourg (ESBS, University of Strasbourg, France)

Bacteria and phages have been fighting and co-evolving for billions of years, engaging in an arms race that has led to the evolution of sophisticated and diverse antiphage defense systems. Only a few defense systems are well-studied including Restriction-Modification and CRISPR-Cas, both of which have led to the development of incredible biotechnological tools. In the past few years, a handful of studies have uncovered a plethora of new defense systems conferring immunity to an assortment of phages in E. coli *or* B. subtilis. Most newly discovered bacterial immune systems have been uncovered using powerful bioinformatic approaches to identify genes of unknown function which exist within defense islands. Here, we develop an arrayed genetic screening method to identify new antiphage defense systems which function in *E. coli* using the NEB fosmid library collection: a diverse 100,000+ clones resource containing metagenomic DNA from hot springs to the human gut microbiome. A partial screen of the NEB human gut microbiome library (11,500 clones) using T4 bacteriophage identified 250 hit clones with varying degrees of phage resistance and differential sensitivity to phage DNA modifications.

Future work will encompass screens of the entire library collection using diverse phages, and will provide insight into the classes, mechanisms, and phylogenetic distribution of defense systems capable of restricting phages of *E. coli*.

9 Development of Synthetic RNA thermometers for biocontainment genetic circuits

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RNA thermometers (RNATs) are non-coding RNA structures that can control gene expression by altering their structure in response to changes in temperature. Frequently found in the 5' UTR region of mRNA, these temperature-responsive structures can sequester the ribosome binding site at non-permissive temperatures, thus preventing translation of the transcript. The most compact, effective RNATs possess a single hairpin-like structure displaying a small number of mismatched base pairs in the stem. This secondary structure unravels at permissive temperatures and thus enables the access of ribosomes to the RBS upstream of the regulated gene. RNATs can be found in nature or can be specifically designed for synthetic biology applications.

To build a proof-of-concept biocontainment tool (kill-switch) that will trigger cell death in bacteria when grown at non-permissive temperatures, we are leveraging synthetic RNATs to control expression of the cognate methyltransferase (antitoxin) of a restriction-modification system to counteract the constitutively expressed restriction endonuclease (toxin) when grown at or above 37°C. When the host strain is grown at 37°C or above, the hairpin of the RNATs straightens enabling translation of the antitoxin, which methylates cut sites for the toxin and prevents self-destruction. In colder temperatures, protection is lost, and the host genome is shredded, thus killing the host cell.

In this communication we present the development of synthetic RNATs as well as their ability to differentially express the antitoxin in response to temperature changes. The utilization of kill-switches based on RNATs presents a versatile tool in biocontainment, protection of intellectual property, and metabolic engineering.

10 Membrane vesicles as microbial delivery systems for mediating competition in pathogen-probiont interactions

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Membrane vesicles (MVs) are nanosized single- or double-membraned particles produced by bacteria through blebbing and, for some, explosive cell rupture. MVs may contain biologically active components, such as lipopolysaccharides (LPS), phospholipids, and outer membrane proteins (OMPs), as well as periplasmic components, allowing MVs to be involved in diverse cellular processes such as cell-to-cell communication, stress responses, antimicrobial resistance, horizontal gene transfer, and immune evasion. MVs can also package virulence factors and may function as delivery vesicles, contributing to host colonization and infection-associated pathology. In this study, MVs were isolated and purified from the marine bacteria *Pseudoalteromonas piscicida* JC3, *Vibrio coralliilyticus* RE22, *Vibrio parahaemolyticus* PSU5579, and *Phaeobacter inhibens* S4. *Vibrio* strains RE22 and PSU5579 are notable for their ability to cause disease outbreaks in oyster and shrimp aquaculture systems, respectively. JC3 and S4 are putative probiotic strains that show promise as microbial additives to mitigate host infections. The MVs were subjected to an array of in vitro assays to assess their possible roles in microbial interactions. MVs were found to demonstrate various biological effects, including antibacterial activity, alteration of biofilm formation, and iron-binding properties, suggesting their role in microbial competition and nutrient acquisition. The results demonstrate that MVs produced by phylogenetically diverse marine bacteria exhibit ecological roles for mediating bacterial and environmental interactions.

11 Use of morphological profiling in B. burgdorferi to investigate environmental stress

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Lyme Disease (LD) is a vector-borne illness caused by the bacterial pathogen Borrelia burgdorferi (Bb), transmitted through the bite of an infected Ixodes spp. tick. LD is treatable with antibiotics if diagnosed early enough in infection. However, left untreated, LD can result in a wide array of clinical manifestations, such as antibiotic-refractory forms of arthritis and infections of the heart or central nervous system. While many studies have focused on the mechanisms that contribute to the transmission of Bb to a vertebrate host during tick feeding, less is known about how subpopulations of Bb form to ensure both successful transmission from and persistence within the feeding tick vector. Because of cell heterogeneity, Bb single cells in bulk culture respond differently to environmental signals. How these subpopulations are formed remains poorly understood due to the lack of tools to study Bb at a single-cell level. To address this gap, we developed an imaging-based pipeline to measure morphological differences between individual Bb cells and optimized fluorescence imaging protocols that report on differences in physiological state. Bb is adapted to different environmental conditions, such as temperature, media composition, and drug treatment, mimicking the conditions of Bb life cycle. We anticipate that these studies will lead to future investigations utilizing small molecules to dysregulate Bb subpopulation response to environmental cues and prevent transmission or persistence of Bb in the tick vector.

12 Development of Whole-Cell Biosensors For Early Detection Of Oral Squamous Cell Carcinoma

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The construction of sensors for early detection of disease biomarkers has enormous potential for the future of preventive medicine. Based on an array of synthetic biology tools, we can assemble whole-cell biosensors (WCBs) guided by the innate information cascades that cells undergo *in vivo* to process the presence in their environment of a small soluble molecule of interest. In our approach we treat biological parts as modular pieces to create new transcription factors (TFs) so that, if a protein capable of binding a disease biomarker can be identified in available databases, a new chimeric TF capable of modulating the expression of a reporter gene can be generated and become the cornerstone of a WCB for said biomarker. The proof-of-concept that we propose in this communication consists of the detection of neuraminic acid (Neu5Ac), a salivary biomarker associated to oral squamous cell carcinoma (OSCC), a cancer that accounts for 80% of all malignant neoplasms of the oral cavity. Accurate and timely diagnosis is crucial in preventing progression to advanced disease stages and in improving survival rates of OSCC. Here we described the functionality of a preliminary bacterial strain capable of detecting Neu5Ac at concentrations equivalent to those found in the saliva of an adult, as well as the complexities confronted during its construction. Our ultimate aim is the creation of portable devices housing living cells that express our synthetic genetic circuits. Salivary samples can then be obtained from patients *in situ* at a dental clinic, followed by rapid detection of the presence of biomarkers. This technology has the potential to transform the future of diagnostics and personalized medicine. While our particular biosensor will serve to aid in the early detection of OSCC, we consider it an initial leap towards the assembly of a platform that can be applied to clinical screening panels for a multitude of other medical conditions.

13 Deep learning for antibiotic discovery in Mycobacterium Tuberculosis

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Traditional methods of antibiotic discovery are slow and costly. One novel machine learning (ML) model, the deep message passing neural network (DMPNN), can predict the antibiotic inhibitory effect of compounds in silico to E.coli. In this work, we apply and extend the DMPNN and test its ability to 'transfer' learn potential novel antibiotics to another pathogen. We focus on M. tuberculosis (Mtb): an obligate pathogenic bacterium genetically and phenotypically distant from E.coli and responsible for the most infectious disease deaths globally. To benchmark our models, we utilized a dataset of 1000 molecular fragments tested for growth inhibition of Mtb in whole cell screens and a list of 12 compounds in current clinical use for Mtb and 27 other antibiotics active against other bacteria but not Mtb as positive and negative controls respectively. We trained and tested a DMPNN on this benchmark and found that although the method achieves high AUC (0.86) on the fragment dataset and high negative control accuracy (0.96), it achieved limited positive control accuracy (0.42). To improve model generalization and circumvent small dataset size, we use self-supervised learning to pretrain a DMPNN on a large dataset of unlabeled molecules. We found pretraining to improve AUC by 0.051 and the positive control accuracy by 0.17. Using ML we were able to achieve high AUC and specificity in predicting growth inhibition in Tuberculosis. Our results emphasize the potential of in-silico screening in antibiotic discovery for Mtb and other pathogens.

14 Using transposon-sequencing to assess phage gene contributions for fitness in the virulent phage ICP1 during the predation of Vibrio cholerae

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Bacteriophages are diverse, abundant predators of bacterial hosts and represent promising therapeutic antibacterial agents. However, the current tools available to alter and study the genomes of virulent phages are limited, making it difficult to assess which factors are important for phage applications. For example, high-throughput genetic methods to systematically characterize phage gene functions are currently unavailable. Moreover, most phage genomes remain unsatisfactorily annotated. To address this, we describe the development of a novel application of high-throughput transposon-insertion sequencing (Tn-seq) to interrogate the essentiality and fitness contributions of phage genes in different host and environmental contexts. We constructed a nearly saturated phage transposon mutant library in the virulent phage ICP1, a persistent predator of the human pathogen, *Vibrio cholerae*. Of the three known virulent cholera phages which replicate in the gut during cholera infections, ICP1 uniquely preys on Vibrio cholerae in its native estuarine environments and during early stationary phase. The majority of its predicted 255 genes are of unknown function. To identify ICP1 genes that confer fitness under different conditions, we passaged the Tn library in either exponential-growth host (EG) or stationary-growth host (SG) grown in either estuary-like minimal media (EMM) or rich media (LB). Assessment of these phage populations via Tn-seq revealed many essential genes, dispensable genes, and a handful of conditional genes. We are currently validating and characterizing several putative conditional genes. Overall, our work represents an advance in methods for assessing virulent phage gene function on a genome-wide scale.

15 A Genome-wide CRISPRi Screen Identifies Drivers of Antibody-mediated Agglutination in Salmonella Typhimurium

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Secretory IgA (SIgA) represents the first line of defense at mucosal surfaces to protect against invasive microorganisms such as the globally prevalent enteric pathogen *Salmonella* Typhimurium (STm). SIgA mediates a key effector function known as immune exclusion, a process in which invading bacteria are aggregated via antibody cross-linking, encased in mucus, and then cleared from the intestinal tract via peristalsis. While immune exclusion can confer protection in vivo, the underlying mechanism of IgA-mediated bacterial agglutination, including the role of bacterial gene expression in this process, remains unknown. In this study, we implemented the anti-lipopolysaccharide monoclonal IgA Sal4 in a genome-wide CRISPR interference (CRISPRi) screen to identify genes that actively promote bacterial agglutination in response to antibody exposure. We found that repeated Sal4 treatment enriched for single guide RNAs targeting genes linked to cyclic-di-GMP metabolism, virulence, fimbriae, stress response signaling, and outer membrane composition. These findings indicate that repressed

expression of these individual genes promotes antibody evasion and resistance to Sal4-mediated agglutination. Studies are currently underway to determine the role of each identified gene in the response to Sal4 binding, and to ultimately characterize the mechanism by which Sal4 IgA promotes agglutination of STm.

16 Distinct Nanoparticle Surface Chemistry Influences Early P. aeruginosa Biofilm Association

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The bacterium, *Pseudomonas aeruginosa*, is a global health threat due to high levels of intrinsic multi-drug resistance through biofilm formation that slows the penetration of antibiotics, allowing the bacteria to gain drug tolerance. Biofilms are composed primarily of an extracellular polymeric substance (EPS) matrix that contains the exopolysaccharides: pel, psl, and alginate. Each of these exopolysaccharides has its own chemistries, yet there is little understanding of the interactions between the exopolysaccharides and particles used to eradicate biofilms. In this study, we used nine distinct layered nanoparticles, seven mutant *P. aeruginosa* strains with inducible exopolysaccharide production, and five mucoidal patient-derived *P. aeruginosa* strains to probe how different nanoparticle surface chemistries and biofilm compositions affect penetration. To answer this question, we synthesized uniform, layered nanoparticles using the layer-by-layer electrostatic adsorption technology and imbued nanoparticles with distinct surface chemistries, varying hydrophobicity, and charged functional groups. We then quantified particle-biofilm interactions by using transwell assays and confocal microscopy. The transwell assays showed reduced penetration with increased biofilm production but showed few differences between strains with similar biofilm production. Our confocal microscopy results showed that biofilms with increased alginate production associated more strongly with carboxylated nanoparticles, as opposed to sulfated nanoparticles. We further probed these differences using synthetic polymers with carboxylated and sulfated functional groups, and again showed this increased association with carboxylated particles. We were also able to reproduce this trend in our patient-derived strains. These results provide criteria for the design of antimicrobial nanocarriers with improved penetration and drug delivery through biofilms.

17 Pangenome hybrid capture increases coverage of Escherichia coli within stool metagenomes, enabling transcriptomic analyses of E. coli within the rUTI gut microbiome

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Clinically important microbes are often found at low abundances within complex communities, presenting a challenge for their investigation. We developed a hybrid capture methodology for enrichment of entire bacterial species pangenomes from metagenomic samples. Using a probe set that we designed to efficiently tile across the *Escherichia coli* pangenome, we enriched *E. coli* DNA 40-fold and RNA 23-fold from human stool samples, without bias in breadth or depth of coverage. Applied to stool samples from a clinical study of women with recurrent urinary tract infections (rUTI), our probe set provided sufficient coverage of the *E. coli* pangenome for differential expression testing of thousands of genes. In prior work, we hypothesized that rUTI is typified by gastrointestinal inflammation, which is accompanied by the release of reactive oxygen and nitrogen species that are co-opted by facultative anaerobic bacteria for respiratory growth. Accordingly, we observed that, in comparison to that of healthy women, *E. coli* carried by women with rUTI history showed increased expression of genes involved in respiratory metabolism and protein synthesis. We also used enriched *E. coli* DNA from stool samples to profile the orientation of the invertible promoter controlling expression of type I fimbriae, an essential UTI virulence factor. Similar proportions of *E. coli* in rUTI and healthy samples had active promoters, suggesting that expression of type I fimbriae in stool does not predict uropathogenesis. Together, these analyses demonstrate the breadth of data and gene-level resolution provided by pangenome hybrid capture.

18 Understanding how Histidine Kinase, NahK, Influences Biofilm Formation and Quorum Sensing in Pseudomonas aeruginosa.

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Pseudomonas aeruginosa is a Gram-negative bacterium that establishes biofilms as part of its pathogenicity. The most at-risk patients are those on ventilators, a problem intensified by increased ventilator use during the Sars-Cov-2 pandemic. Because prevalent *P. aeruginosa* strains are becoming more multidrug resistant, it is essential that we understand the underlying molecular mechanisms of these infections.

The major molecular network that controls *Pseudomonas* biofilm formation is the GacS Multi-Kinase Network (MKN). This MKN is responsible for the switch between a motile, acute infection and a biofilm, chronic infection. The regulation of this switch is critical as the bacteria can seamlessly transition between each state in vivo to cause infection. However, the details driving this switch are unknown. This network is implicated in many essential processes including quorum-sensing, antibiotic resistance, metabolism, and virulence.

My goal is to understand the distinct function of histidine kinase, NahK, in the GacS MKN. My preliminary data shows that a *P. aeruginosa nahK* deletion strain significantly increases exotoxin (pyocyanin) and *Pseudomonas* quinolone (PQS) production, suggesting NahK has an important regulatory role in controlling expression of virulence factors that was previously unknown. Here, we have shown that NahK has a major effect on quorum sensing, disrupting how and when these signals activate. I plan to investigate how NahK signals through the GacS MKN to control quorum sensing and how that leads to an upregulation of biofilm formation machinery and virulence.

19 Bacteria-virus interactions in the vaginal microbiome reduce herpes virus infectivity

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The vaginal microbiome is an important determinant of host health and the first barrier encountered by sexually transmitted pathogens during infection. Among the vaginal microbiome, Lactobacilli are associated with reduced susceptibility to viral infection, but the mechanisms by which various Lactobacilli strains reduce viral infectivity remain poorly understood. Using a collection of human vaginal microbial strains, we show that the prominent vaginal strain, *Lactobacillus crispatus* reduces infectivity of sexually transmitted pathogen Herpes Simplex Virus (HSV). Reduction of HSV infectivity is species specific, with *L. crispatus* reducing infection and disease better than gut-associated *L. reuteri*. Active cell metabolism is not required as UV-killed *L. crispatus* retain the ability to reduce herpes infection. Since one of the most abundant structures on the outside of the *L. crispatus* cell is peptidoglycan, we assessed whether peptidoglycan could reduce HSV infection. We found that commercial peptidoglycan from multiple bacteria reduced herpes infection *in vitro* and *in vivo* in a mouse model of genital herpes infection in a TLR-2 independent manner. Cleavage of the glycosidic linkages in the peptidoglycan chain with lysozyme restored virus infectivity *in vitro* and *in vivo* suggesting that antiviral effects are dependent on longer peptidoglycan chains. Current studies aim to determine the mechanisms by which peptidoglycan reduces HSV infectivity with a focus on HSV host cell entry and the host immune system. Such results provide a greater understanding of the ways that the vaginal microbiome serves as a barrier to infection and why some vaginal communities promote better antiviral protection than others.

20 Identifying Chlamydia trachomatis virulence factors that inhibit adaptive immunity

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Chlamydia trachomatis is an obligate intracellular bacterial pathogen that if left untreated, can cause chronic infections and reproductive harm. Additionally, repeat infections are common. Chronic and repeat infections result from a failure of adaptive immunity which is critical to understand to develop an effective vaccine. CD8 T cells are normally integral for controlling intracellular pathogen infections, but during *C. trachomatis* infection, the CD8 T cell response is significantly impaired at least in part due to upregulation of the immunoinhibitory ligand PD-L1 during infection. To gain a deeper understanding of the pathogen side of the host-pathogen interface, we are currently focusing on identifying the *C. trachomatis* gene(s) responsible for upregulating PD-L1 which we hypothesize is required for downmodulating the CD8 T cell response. To identify candidate genes, we are employing two complimentary screening approaches in parallel. The first is creating and screening a lentiviral

library containing individual *C. trachomatis* genes, testing the ability of individual bacterial genes to upregulate PD-L1. The second approach is based on a fortuitous phenotype in HeLa cells where only *Chlamydia muridarum* and not *C. trachomatis* is capable of upregulating PD-L1. By using a C. trachomatis/C. muridarum chimera collection developed by collaborators, we will identify portions of the *C. muridarum* chromosome that are sufficient to upregulate PD-L1. In pre-liminary chimera screening results, we have already identified 4 hits. We plan to validate gene candidates identified in our screens and to create and use loss of function mutants for further *in vivo* studies.

21 Host Factors Engaged in the Repair of the Mtb Phagosome Membrane

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Tuberculosis (TB), caused by the respiratory pathogen Mycobacterium tuberculosis (Mtb), is one of the leading causes of death worldwide. Upon infection, Mtb is taken up into phagosomes of alveolar macrophages. The molecular interactions that establish the balance of the intracellular host-pathogen standoff have been a subject of significant interest in the field. Mtb is known to prevent phagosome maturation and thereby evade degradation. Growing evidence suggests that damaging the phagosomal membrane is a second critical function for Mtb survival in macrophages and intracellular pathogenesis. Two key Mtb virulence factors, the virulence lipid PDIM and the type VII protein secretion system EsxA, contribute to phagosomal membrane damage. The host factors that repair Mtb-induced phagosomal membrane damage and constrain the bacterium are less well known. The host ESCRT system is one factor shown to promote repair of Mtb-damaged membranes, but we reasoned that additional host factors likely contribute. To identify such factors, the lab previously performed a genome wide CRISPR screen was performed to identify candidate genes required to repair Mtb-mediated phagosomal membrane damage. Identified hits included the phosphoinositide kinase PIKFYVE, the cystic fibrosis transmembrane conductance regulator CFTR, and the solute carrier family member SLC11A1. We are currently generating and validating monoclonal CRISPR knockouts of each of these candidate genes to test for a role in Mtb infection and membrane damage repair. We are simultaneously using chemical inhibitors as an orthogonal approach. Preliminary results suggest that inhibition of PIKFYVE during Mtb infection leads to enhanced phagosomal membrane damage. Ongoing investigation aims to define the molecular role that these genes play in maintaining the integrity of the Mtb-containing phagosome.

22 Non-canonical pattern recognition of a pathogen-derived metabolite by a nuclear hormone receptor identifies virulent bacteria in C. elegans

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Distinguishing infectious pathogens from harmless microorganisms is essential for animal health. The mechanisms used to identify infectious microbes are not fully understood, particularly in metazoan hosts that eat bacteria as their food source. Here, we characterized a non-canonical pattern recognition system in *Caenorhabditis elegans* that assesses the relative threat of virulent *P. aeruginosa* to activate innate immunity. We discovered that the innate immune response in *C. elegans* was triggered by phenazine-1-carboxamide (PCN), a toxic metabolite produced by pathogenic strains of *Pseudomonas aeruginosa*. We identified nuclear hormone receptor NHR-86/HNF4 as the PCN sensor in *C. elegans* and validated that PCN bound to the ligand-binding domain of NHR-86/HNF4. Activation of NHR-86/HNF4 by PCN directly engaged a transcriptional program in intestinal epithelial cells that protected against *P. aeruginosa*. Thus, a bacterial metabolite is a pattern of pathogenesis surveilled by nematodes to identify a pathogen among its bacterial diet.

23 Identifying neutrophil effector functions targeted by Yersinia pseudotuberculosis Yops

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The extracellular pathogen, Yersinia pseudotuberculosis (Yptb), is swarmed by neutrophils during tissue infection. Previous work has shown that *Yptb* inactivates key antimicrobial functions of neutrophils through T3SS-injected Yop effector proteins. YopH, a potent tyrosine phosphatase, is critical for inhibiting neutrophil ROS production and degranulation. We recently identified SKAP2 as one of the key targets of YopH, where inactivation of SKAP2 results in inhibition of ROS production but not degranulation. Importantly, deletion of SKAP2 only partially restored the growth of a *yopH* mutant in mouse infections, indicating that critical SKAP2-independent pathways are still at play. However, the YopH-targeted proteins that are independent of SKAP2 are unknown. Because neutrophils are terminally differentiated and short lived, they are challenging to genetically manipulate and study *in vitro*. Using an engineered immortalized myeloid progenitor cell line (MP), we can terminally differentiate MPs into neutrophils (MP-PMNs) to study ROS production, degranulation, and NETosis. Here, we use Cas9-expressing MP cells to generate 40 knock outs of known and hypothesized Yop targets, which can readily be differentiated into KO-MP-PMN's. Using this small library of knockouts, we employed a flow cytometry based assay to monitor neutrophil degranulation and activation. Thus far, we have identified various genes required for integrin-induced neutrophil degranulation. These genes will be assessed further to study the impact of gene deletions on neutrophil effector functions after *Yptb* infection. This work will continue to elucidate the signaling pathways disrupted by various Yops and enable us to dissect the interdependencies of neutrophil effector functions during tissue infection.

24 The heme oxygenase-1 metalloporphyrin inhibitor stannsoporfin enhances the activity of a novel regimen for multidrug-resistant tuberculosis in a murine model

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The emergence of multidrug-resistant tuberculosis (MDR TB) strains pose significant challenges to global TB control efforts. Host-directed therapies (HDT) offer a novel approach for the clearance of *Mycobacterium tuberculosis* (*Mtb*) infection by enhancing host immune responses. Inhibition of Heme oxygenase-1 (HO-1) with Tin-protoporphyrin IX (SnPP) reduced the bacterial load when co-administered with the standard regimen for drug-susceptible TB. Here we evaluated the adjunctive tuberculocidal activity of a novel HO-1 inhibitor, stannsoporfin (SnMP), in combination with a novel MDR TB regimen containing TBAJ-876 (S), pretomanid (Pa), and TBI-223 (O)) (collectively, SPaO) in the standard mouse model of TB. After 4 weeks of treatment, SPaO + SnMP 5 mg/kg had a 0.69 logfold greater decrease in CFU compared to SPaO alone. After two weeks of treatment mRNA expression of pro-inflammatory cytokines were regulated, and the M1 CD38 macrophage marker was upregulated by SnMP therapy. To determine the sterilizing activity of SnMP adjunctive therapy for the relapse setting in BALB/c mice, we treated *Mtb*-infected mice for five or six weeks and assessed relapse three months post-treatment. After six weeks of treatment, SPaO + SnMP 10 mg/kg had a 0.78 log-fold greater decrease in lung CFU compared to SPaO alone. There's no difference in relapse rates between the two treatment groups following 6 weeks of treatment, but mice receiving SPaO + SnMP 10 mg/kg had the lowest lung CFU upon relapse. Stannsoporfin appears to be a promising adjunctive HDT, with the potential for shortening curative treatment for drug-susceptible and drug-resistant TB.

25 S. pneumoniae pneumolysin promotes airway barrier breach by triggering neutrophil transepithelial migration and elastase release

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Streptococcus pneumoniae (Spn), the most common cause of community-acquired bacterial pneumonia, has a mortality rate that increases three-fold when infection breaches the respiratory epithelial barrier and enters the bloodstream, enhancing the risk of septicemia and meningitis. Pneumolysin (PLY), a cholesterol-dependent pore-forming cytolysin, is a major virulence factor produced by virtually all Spn isolates. We previously showed

that PLY triggers 12-lipoxygenase- (12-Lox-) dependent epithelial cell production of the PMN chemoattractant hepoxilin A3 (HXA3). Here, we found that during pulmonary Spn challenge, PLY promoted acute pulmonary inflammation, increased lung permeability to FITC-dextran, and facilitated systemic bacterial spread. Comparison of wild-type and PLY-deficient Spn strains revealed that PLY promotes disruption of stem cell-derived air-liquid interphase (ALI) respiratory epithelial cultures and translocation of Spn in a manner dependent on 12-Lox and PMN transepithelial migration. PMN transmigration, epithelial disruption, and bacterial translocation after apical infection of 12-Lox deficient ALIs was rescued by a chemotactic gradient of HXA3-containing supernatant or purified HXA3 methyl ester. Notably, a non-eicosanoid chemoattractant fMLP elicited PMN migration but did not promote epithelial disruption or bacterial translocation. PMNs primed with HXA3 but not fMLP showed elevated neutrophil elastase (NE) release in response to Spn, and blockade of NE secretion or activity with pharmacological inhibitors protected against bacterial translocation *in vitro* and bacteremia *in vivo*. Thus, HXA3-activated PMN transmigration and NE release drive barrier breach during infection by PLY-expressing Spn, events that can be targeted for inhibition to curtail systemic disease during pneumococcal pneumonia.

26 Defining LprG-Rv1410c mediated lipid transport through the mycobacterial cell envelope

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The mycobacterial cell wall includes an outer membrane. However, compared to gram negative bacteria, very little is understood about transport systems involved in outer membrane biogenesis in mycobacteria. Uncovering these pathways is expected to yield potential drug targets and establish unique precedents for transport mechanisms within the bacterial cell wall. Here, we focus on the LprG-Rv1410c pathway, which is highly conserved across mycobacteria, required for virulence, and associated with triacylglyceride (TAG) transport. The individual roles of LprG and Rv1410c in lipid transport are not yet defined. We hypothesize that the integral membrane protein Rv1410c transports TAG across the plasma membrane to the periplasmic lipid-binding lipoprotein LprG. We predict Rv1410c functions by extracting TAG from the outer leaflet, or alternatively, by flipping TAG across the plasma membrane. To examine how LprG and Rv1410c impact TAG transport, we are using spheroplast-based assays to obtain plasma membrane leaflet-specific information on how LprG and Rv1410c each impact TAG localization. Using these tools, we will define the nature of lipid transport events mediated by LprG and Rv1410c towards establishing a general paradigm for lipid transport to the mycobacterial outer membrane.

27 Identifying the Mycobacterium tuberculosis surface proteome using proximity labeling in live cells

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Tuberculosis is a top ranking cause of human mortality that is caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*). *Mtb* is an intracellular pathogen that can infect various host cell types. Based on the severity and complexity of host imposed and/or drug induced stresses, *Mtb* can maintain viability by modulating intrabacterial biochemical pathways. In order to understand such adaptive responses in *Mtb*, it is essential to identify cell surface localized proteins responsible for sensing environmental cues. Although the overall architecture and composition of the mycobacterial cell wall have been studied, the proteins in the outermost layer of the cell wall (also called mycomembrane) remain a mystery because they are difficult to identify. Peroxidase-mediated proximity labeling is a well-established technique for identifying proteins in subcellular compartments. We have previously shown successful application of this technique to identify cytosol or periplasm localized proteins in mycobacteria. In this work, we focus on identifying surface proteins in *Mtb* through (a) genetic fusion of an engineered ascorbate peroxidase (APEX2) to a known outer membrane protein in order to facilitate localization of APEX2 to the cell surface or (b) *in trans* labeling of surface proteins by horseradish peroxidase. Surface-specific labeling will be validated using known markers for the different subcellular compartments. The labeled proteins will be identified using mass spectrometry to reveal surface localized proteins in *Mtb*. The surface proteome is expected to yield candidate proteins that can be investigated for their roles in essential processes such as environmental sensing during adaptive responses in *Mtb*.

28 Posttranslational regulation of the leucine-responsive regulatory protein (Lrp) from E. coli

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Lrp is one of the seven global transcriptional regulators in *E. coli*, where it regulates many genes important for cell survival and motility as well as genes involved in virulence and amino acid metabolism and transport. Some Lrp-regulated virulence operons include the *LEE* and *Fim* operons present in enteropathogenic and uropathogenic *E. coli* strains, respectively, which are important for facilitating bacterial attachment and host colonization. To date most studies of Lrp regulation have been focused on transcriptional, translational, or effector binding regulation; however, little is known about the regulation by posttranslational modifications of this protein. Several whole acetylproteome and phosphoproteome studies have identified Lrp as one of the many targets of acetylation and phosphorylation in *E. coli*; however, it remains unexplored the physiological conditions in which these post-translational modifications are carried out, the mechanisms underlying them, and the biological effects in terms of downstream gene regulation that arise from them. To gain more insights into the posttranslational regulation of Lrp, we immunoprecipitated Lrp from *E. coli* and explored the landscape of posttranslational modifications on this protein. Our findings allowed us to identify that in addition to acetylation and phosphorylation, Lrp is modified by arginine mono-methylation at residue 48 on the DNA-binding domain. Genome-integrated R48K, R48F, R48A, and R48Q Lrp mutants display a growth defect under conditions selective for proper Lrp function suggesting that R48 plays an important role in Lrp function. This study constitutes a first step into characterizing the posttranslational regulation of this important master regulator in *E. coli*.

29 The Impact of Acinetobacter baumannii Vanillate Catabolism on Biofilms

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Acinetobacter baumannii is a nosocomial pathogen that easily acquires antibiotic resistances. It often colonizes medical equipment by forming biofilms, clusters of bacteria that are difficult to eradicate, through mechanisms that are not yet fully understood. *A. baumannii* biofilm attachment on abiotic surfaces is based on an archaic pilus encoded by the csu operon. Vanillate, a plant derived phenolic compound exhibiting antimicrobial activity, can be utilized as a carbon source through ligninolytic pathways in multiple Acinetobacter species. Previous research has shown that the vanillate catabolism genes in *A. baumannii* are only activated during biofilm formation. We reasoned that there may be a functional relationship between biofilm attachment and the catabolism of vanillate. In this study, we provide new insights into this connection by investigating the regulation of the genes involved. VanR is a transcriptional repressor of the vanillate catabolism genes in which addition of vanillate derepresses these genes. Surprisingly, an *A. baumannii* strain lacking VanR, Δ vanR, shows a prominent biofilm phenotype suggesting that VanR may also repress *A. baumannii* attachment. We identified a VanR binding motif in the Csu pili promoter and showed that purified VanR binds to this promoter in vitro by electrophoretic mobility shift assay (EMSA). Moreover, addition of vanillate to the growth medium resulted in prominent biofilms, providing further support to the notion that vanillate derepresses not only the genes responsible for its catabolism but also the Csu pili genes, which mediate surface attachment. Overall, this study reveals a novel regulatory connection between seemingly unrelated processes, shedding light on cell attachment, a key step in biofilm formation.

30 Inhibition of disulfide bond formation enzymes potentiates bacteriophage killing of Acinetobacter baumannii

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Multidrug-resistant infections due to *Acinetobacter baumannii* are a major public health threat. These infections have high incidence in intensive care units and cause serious hospital-acquired diseases, including sepsis. Bacteriophages have emerged as a potential alternative therapy against *A. baumannii* infections that are resistant to standard antibiotics. Mechanisms that could enhance the efficacy of phage treatment against these bacteria were largely unknown. To address this problem, we combined phage challenge with bacterial Tn-seq, and identified mutations that increased the sensitivity of *A. baumannii* to attack by a lytic phage, Loki. Mutations knocking out the disulfide bond formation enzyme, DsbA, led to the most dramatic hypersusceptibility to phage. To determine the impact of these mutations across different *A. baumannii* strains, we constructed a Δ *dsbA* deletion in laboratory strain 17978 as well as a multidrug-resistant clinical isolate, BAA-1790, and found that each mutant showed phage hypersensitivity. We determined that Δ *dsbA* leads to increased phage virulence in two ways: it increases phage adsorption as well as post-adsorption replication. These changes occur because Δ *dsbA* activates a global regulator in *A. baumannii*, BfmRS, which enhances the amounts of the phage receptor, capsule, and promotes intracellular phage replication. We hypothesized that chemical inhibitors of bacterial

disulfide bond formation would mimic the genetic results. We found that pyridazinone inhibitors of bacterial disulfide bond formation indeed potentiated killing of *A. baumannii* by phage Loki. This work demonstrates that phage sensitivity is enhanced through interruption of disulfide bond formation and provides new perspectives for improving future anti-*A. baumannii* phage therapy.

31 New interaction network among hypothetical proteins may serve important role in Bdellovibrio bacteriovorus predation

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Bdellovibrio bacteriovorus can kill Gram-negative bacteria including multidrug resistant pathogens and is being studied for its potential as an alternative to antibiotics. However, despite a well-established life cycle, the molecular mechanisms underlying Bdellovibrio-prey interaction remain unclear. Many genes encoding hypothetical proteins in *B. bacteriovorus* have been found to be essential for predation, but very little is known about their functions. Here, by analysis of completely sequenced genomes of four *B. bacteriovorus* strains, HD100, 109J, Tiberius and SSB, we identified 3133 *B. bacteriovorus* core proteins, of which 843 are hypothetical. In comparison with experimentally established predation genes, a priority set of 23 predation-essential hypothetical proteins were identified. In-silico analysis showed that Bd0075 protein interacts with at least five members from the priority set. We have experimentally confirmed the interaction between Bd0075 and Bd0474 while work is in progress on the others. Bd0075 may function as a protein hub and its interactome may serve a novel function in B. bacteriovorus predation. The exploration on *B. bacteriovorus* hypothetical proteins could help us to better understand this group of bacteria and maximize their potential as novel antimicrobials.

32 Variations in sporulation conditions and purification methods affect sensitivity to germinants in Clostridioides difficile spores

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Clostridioides difficile is an opportunistic bacterial pathogen that causes a severe gastrointestinal infection in individuals with a compromised microbiome or weakened immune system. As *C. difficile* is an obligate anaerobe, it relies on the metabolically dormant and aerotolerant spore form to transmit infection. After ingestion, spores must exit the dormant state through the process of germination to initiate infection. Germination occurs in response to germinant sensing of cholate-derived bile salts and amino acids or divalent cations in the small intestine. Research into how spores sense these diverse signals has been complicated by a lack of consensus in the literature on the concentration of germinant signals required to initiate germination. A factor contributing to this variability in germinant sensitivity may be the variety of methods used to generate and purify spores in the lab. We examined whether the media (SMC vs mix of SMC and BHI) used to induce sporulation and the spore purification method effect sensitivity to the germinants taurocholate and glycine. Spores generated on the SMC/BHI mixed media had a small, but reproducible increase in sensitivity to taurocholate and glycine. A more pronounced effect was seen with differences in purification methods. Spores purified by a 7-day incubation in water had a marked increase in sensitivity to taurocholate compared to spores isolated with multiple water washes. Understanding how conditions during *C. difficile* sporulation and spore isolation contribute to sensitivity to germination signals will help standardize the field and may help elucidate mechanisms of germinant sensing.

33 A Preclinical Drug Candidate Targeting Mycobacterium tuberculosis KasA

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A significant drug discovery opportunity exists for Mycobacterium tuberculosis β-ketoacyl-ACP synthase (KasA) inhibitors with in vivo potency. A structure-based approach was leveraged to evolve an existing indazole KasA inhibitor. This afforded JSF-3285 with a 30-fold enhancement in mouse plasma exposure. Biochemical, genetic, and X-ray studies are supportive of JSF-3285 potently modulating KasA. JSF-3285 offers substantial activity in both sub-acute and chronic mouse models of infection. In the chronic infection model, a minimum effective dose is 5 mg/kg once-daily oral. Furthermore, JSF-3285 can augment the efficacy of existing tuberculosis drugs in combination. JSF-3285 constitutes a promising pre-clinical candidate for tuberculosis.

34 Spatial segregation of metabolic processes underlies cellular asymmetry in mycobacteria

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Mycobacteria grow asymmetrically from their poles and divide off center, creating a heterogeneous population of cells that varies in size, growth rate, and antibiotic susceptibility. The deletion of a single gene conserved exclusively in mycobacteria, known as *lamA*, is capable of abrogating a significant amount of this heterogeneity. We set out to characterize this protein of unknown function using a combination of genetics, biochemistry, and super-resolution microscopy. We find that the localization of LamA is highly dynamic between the poles and side walls of the cell. Congruent with a presupposed function in cell growth, we show LamA helps maintain the uneven distribution of cell envelope proteins including Wag31, MmpL3, and PgfA. Interestingly, we also found that LamA functionally interacts with proteins in the electron transport chain, despite these proteins being excluded from the tips of the cell. We demonstrate that deletion of *lamA* leads to more uniformity in the localization of respiratory complexes, membrane potential, and ATP concentration between daughter cells. Together, our data support a model in which LamA functions to spatially and temporally coordinate two essential systems: cell growth and respiration, and argues that LamA provides a unique target for the development of anti-tubercular therapeutics.

35 Investigating the autoregulation of a ribosomal protein in the pathogen Francisella tularensis

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Francisella tularensis is a highly infectious, intracellular human pathogen that can cause fatal disease. The *F. tularensis* type VI secretion system is an essential virulence factor required for survival in host cells, including survival in what are considered a key niche, macrophage. We have determined that ribosome composition influences production of the *F. tularensis* type VI secretion system and virulence. Ribosomes containing one of the three homologs for the small ribosomal subunit protein bS21, bS21-2, positively control key virulence genes and intramacrophage replication. However, the mechanisms that control and coordinate production of bS21-2 and the other bS21 homologs, bS21-1 and bS21-3, are unknown. We have found that bS21-2 negatively regulates its own production, as the presence of bS21-2 leads to significant reductions in abundance of its transcript, *rpsU2*. Further, we have found that the 5′untranslated region (UTR) of *rpsU2* is sufficient for this bS21-2-mediated repression. Production of bS21-2 appears to be tightly controlled by bS21 levels in the cell, as both bS21-1 and bS21-3 also negatively regulate bS21-2 transcript abundance. In contrast, bS21-1 and bS21-3 do not affect their own production. Thus, the bS21-mediated regulation of bS21-2 appears to be unique among the three homologs. This suggests that *F. tularensis* integrates multiple signals into a regulatory network to control the appropriate production of each bS21 homolog. This regulatory network in turn may control ribosomal heterogeneity and virulence gene expression.

36 Diverse and abundant viruses exploit conjugative plasmids

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Viruses exert profound evolutionary pressure on bacteria by interacting with receptors on the cell surface to

initiate infection. While the majority of bacterial viruses, phages, use chromosomally-encoded cell surface structures as receptors, plasmid dependent-phages exploit plasmid-encoded conjugation proteins, making their host range dependent on horizontal transfer of the plasmid. Despite their unique biology and biotechnological significance, only a small number of plasmid-dependent phages have been characterized. Here we systematically search for new plasmid-dependent phages using a targeted discovery platform, and find that they are in fact common and abundant in nature, and vastly unexplored in terms of their genetic diversity. Plasmid-dependent tectiviruses have highly conserved genetic architecture but show profound differences in their host range which do not reflect bacterial phylogeny. Finally, we show that plasmid-dependent tectiviruses are missed by metaviromic analyses, showing the continued importance of culture-based phage discovery. Taken together, these results indicate plasmid-dependent phages play an unappreciated evolutionary role in constraining horizontal gene transfer.

37 Legionella employ a cell surface signaling system to maintain replication vacuole integrity

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The respiratory pathogen *Legionella* causes life-threatening pneumonia known as Legionnaires' disease. *Legionella* replicate in alveolar macrophages within a membrane bound compartment called the *Legionella* containing vacuole (LCV). Maintaining LCV membrane integrity is critical for evading recognition by host immune surveillance systems and *Legionella* replication. *Legionella* accomplish this by translocating bacterial proteins termed as effectors into the host cell that modulate host cell vesicle trafficking and ER dynamics. Recently, we identified a *Legionella* CSS system that, akin to effectors, is also important for LCV stability. Cell surface signaling (CSS) systems function in the bacterial cell wall allowing them to monitor their environment and activate adaptive responses. The observation that a CSS system protects against LCV destabilization suggests a possible role in monitoring the impact of effector functions within the host cell and/or membrane integrity from within the LCV. In contrast to canonical CSS systems, the *Legionella* system appears to consist of outer and inner membrane proteins connected by a diffusible FecR domain-containing transducer protein, indicating a novel spatial arrangement and composition. Activation of the CSS leads to production of the second messenger cAMP that likely regulates activity of a protease to elicit an adaptive response. While most well-characterized CSS systems function in iron/siderophore acquisition and plant-bacterium symbiosis, the identification a CSS system employed by an intracellular pathogen to ensure LCV stability and in turn, intracellular replication, expands the role for CSS systems in bacterial pathogenesis

38 Investigating heterogeneous regrowth of Salmonella persisters inside macrophages using spatially resolved, single-cell transcriptomics

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Salmonella infections are prone to antibiotic treatment failure and relapse, which have been attributed to transiently nongrowing cells known as persisters. Upon phagocytosis by macrophages, *Salmonella* forms growth-arrested persisters that not only survive antibiotic killing but also secrete host-reprogramming effectors and repair DNA. We have shown that *Salmonella* is locked into the nongrowing state by macrophage-produced nitric oxide (NO). As NO production declines, persisters heterogeneously resume growth within the macrophage by incompletely understood mechanisms. Intriguingly, a high probability of regrowth is associated with regional DNA duplications that are found only in a subset of persisters. Therefore, alleviation of NO toxicity and DNA duplications could be important for persister regrowth, but their molecular mechanism and the remaining requirements for regrowth remain a knowledge gap. I hypothesize that diverse transcription-level mechanisms in macrophages and Salmonella control persister regrowth. Expecting metabolism-wide changes in macrophages and *Salmonella* during regrowth, I will leverage multiplex error-robust fluorescent *in situ* hybridization (MERFISH), a cutting-edge spatial transcriptomics technology, to test my hypothesis across many host and bacterial genes. In Aims 1 and 2, I will use MERFISH and genetic perturbations to characterize host and bacterial factors that control regrowth, verified by flow cytometry. In Aim 3, I will pair MERFISH with DNA FISH to determine the role of the persister-specific DNA duplications in bacterial transcription and regrowth. Through these aims, I will establish the use of imaging-based spatial transcriptomics for host-pathogen systems and investigate the determinants of *Salmonella* regrowth.

39 Exploring the relationship between sequence and antibiotic resistance with FMN riboswitch fitness landscapes

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The rise of multidrug resistant bacteria has led to increased deaths from microbial infections. To help treat these infections, exploring new drug targets and optimizing antibiotics is critical. Labeled as a serious threat by the CDC in 2013, *Streptococcus pneumoniae* is an important pathogen to study and target with antibiotics in new ways. The flavin mononucleotide (FMN) riboswitches in *S. pneumoniae* are promising antibiotic targets. FMN is an essential cofactor that must either be synthesized or acquired by the organism. Mutations to FMN aptamers in related organisms such as *Staphylococcus aureus* confer resistance to the antibacterial compounds roseoflavin and ribocil-C. To understand the role that mutations to the riboswitch play in mediating resistance in *S. pneumoniae*, we constructed fitness landscapes for the FMN riboswitch regulating transcription of the riboflavin transporter under a variety of conditions including the presence and absence of riboflavin and in the presence of antibiotics. To accomplish this, we generated an FMN riboswitch mutant strain library, and assessed the fitness of each strain using high-throughput amplicon sequencing. We determined the frequency of single nucleotide mutations within the riboswitch enabling antibiotic resistance or increased susceptibility. Our preliminary results suggest strong selective action at the base of the unusually extended terminator and in the binding region for the antiterminator in the presence of roseoflavin. Additionally, mutations to the extended P3 stem appear to be detrimental to fitness in the presence of roseoflavin.

40 CRISPR/Cas mediated evolutionary arms race between Vibrio cholerae and ICP1 bacteriophage

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Vibrio cholerae resides in estuaries and the human intestine where it encounters virulent bacteriophages (phages) that prey on it. Bacteria are continually evolving anti-phage mechanisms and in response phage are continually evolving ways to overcome these barriers. In *V. cholerae*, anti-phage defenses include PLE (phage inducible chromosomal island like element) and CRISPR/Cas systems (clustered regularly interspaced short palindromic repeats/CRISPR associated protein). PLE is found in current El Tor biotype strains while CRISPR/Cas is lacking. In contrast, classical biotype strains, which for largely unknown reasons became extinct in the second half of the 20th century, possessed a Type 1-E CRISPR/Cas system and one type of PLE. Virulent phage ICP1, encodes a Type 1-F CRISPR/Cas system to overcome the PLE through targeting protospacers in PLE sequences. This arms race has resulted in the diversification of PLE elements, of which more than four types have been found thus far in El Tor biotype strains. However, our knowledge on the presumed arms race mediated by the CRISPR/Cas systems of classical biotype strains and ICP1 is limited since the biotype is extinct in nature. To study this, we propose to pit classical biotype *V. cholerae* and ICP1 against each other in vitro and in an animal model of cholera. First, we tested the CRISPR/Cas activity of fifteen classical strains using a plasmid targeting assay and found a range of activities from low to high. Next, we sought to identify an ICP1 isolate than can plaque on classical strains. Using a panel of eight ICP1 strains, four of which have CRISPR/Cas, only one ICP1 isolate lacking CRISPR/Cas, plaques on most classical strains. Additionally, only one CRISPR/Cas containing ICP1 isolate can plaque on two different classical strains. However, these latter two strains have a severe growth defect and are avirulent. We are currently investigating why the healthy growing, virulent classical biotype strains are resistant to ICP1. We hypothesize that they contain an anti-phage defense system that is lacking in other classical or El Tor biotype strains. We are using multiple approaches, including transposon screening, to identify the hypothesized phage resistance genes in these classical strains.

41 Understanding the role of gene family expansion in R. parkeri's type IV secretion system

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In recent years, there has been a dramatic increase in cases of tick-borne diseases, many of which are caused by bacteria of the order Rickettsiales. As obligate intracellular pathogens, the Rickettsiales rely on a unique type IV secretion system (T4SS) to secrete protein effectors and manipulate host cell processes. Despite massive genome reduction within the order, the rickettsial T4SS has undergone significant gene-family expansion, resulting in multiple genes encoding divergent paralogs of certain subunits. We hypothesize that these paralogs serve distinct functions throughout *Rickettsia* spp.'s dynamic, multi-step, intracellular life cycle. Here, we use *Rickettsia parkeri* as a model to investigate the function and assembly of five highly diverse paralogs of the inner membrane channel subunit, VirB6, which has been shown in other bacteria to contact effectors during translocation and to regulate T4SS activity. Through transposon mutant analysis, we have found that one of the VirB6 paralogs (RvhB6e)

promotes invasion of host cells. RvhB6e also contains a C-terminal extension that is exposed on the bacterial surface during and immediately following host cell invasion, consistent with this paralog's role in early infection. Ongoing work is leveraging biochemical and imaging assays to investigate RvhB6e's mechanism of action, as well as the broader interactions and assembly patterns of the other RvhB6 paralogs. By studying these unique T4SS paralogs, we hope to uncover new T4SS functions and better understand the host-pathogen interactions that support infections by *Rickettsia* species.

42 A unique CRASP (complement regulator-acquiring surface protein) from Borrelia afzelii possesses a distinct pattern from other members of its identified paralogous pfam54 gene family

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Lyme disease is caused by a family of spirochetes known as Borrelia burgdorferi *sensu lato* including *Borrelia burgdorferi*, *Borrelia afzelii*, and *Borrelia garinii*. Lyme disease is a multi-stage disease that can vary significantly in presentation and severity. Of importance is the unusually high number of plasmids within *Borrelia* (>20) which have been extensively shown to contribute to pathogenesis and disease heterogeneity by coding for over 80 surface exposed lipoproteins involved in the evasion of host-immune systems and adherence to extracellular tissues. Although many lipoprotein-immune system interactions have been identified, most lipoproteins remain uncharacterized. We scanned the *Borrelia* pan genome and identified a lipoprotein, found in *Borrelia afzelii* and associated with strains that cause acrodermatitis chronicum atrophicans, with significant BLAST homology to the factor-H binding family pfam54. We cloned this ORF, generically termed BL078, into a high-passage strain of *B. Burgdorferi* optimized for surface display. After confirming expression and surface display via western blotting and flow cytometry, we tested its binding affinity to factor H by far-western immunoblotting and *in vitro* cell adherence assays. Preliminary binding data has shown that unlike many other members of the pfam54 family, BL078 does not bind factor H. We hypothesize that BL078 may be implicated in other immune escape mechanisms like the formation of the terminal complement membrane attack complex (MAC), and/or by adhesion to the extracellular matrix (ECM). We plan further experiments including serum killing and binding assays to other complement factors/ECMs to characterize the role of BL078 in Lyme disease pathogenesis.

43 Genomic Analysis of Alicyclobacillus acidoterrestris and Alicyclobacillus suci reveals genetic differences that could contribute to differences in spoilage potential

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Food spoilage-relevant bacteria *Alicyclobacillus acidoterrestris* and *A. suci* produce the metabolite guaiacol and are closely related, but there is a gap in understanding the genetic differences between these species. The purpose of this study was to: (1) identify genes unique to either A. acidoterrestris *or* A. suci and (2) assess potential single locus PCR targets appropriate for differentiating isolates of these species. A genome wide association study (GWAS) was performed on 34 A. suci *and* A. acidoterrestris assemblies using prokka v1.14.5, panaroo v1.3.0, and Scoary v1.6.16. Genes with p-values ¡4.5E-10 were assigned to Clusters of Orthologous Gene (COG) categories using EggNOG-mapper v2. For the second objective, the 16S, *gyrB*, and *rpoB* gene sequences were extracted from the WGS data of 31 *Alicyclobacillus* representing 9 species using a custom BLAST search. RAxML phylogenetic trees (1,000 BS) were constructed for each gene. The GWAS identified 194 genes unique to A. acidoterrestris *and 161 genes unique to* A. suci. The COG analysis found these genes were involved in amino acid, ion, and carbohydrate transport and metabolism; transcription; and energy. *A. acidoterrestris* was found to have additional, unique genes involved in cell cycle and motility. The genes unique to either A. acidoterrestris *or* A. suci reveal patterns in metabolite transfer and metabolism, which has implications for spoilage mitigation strategies. The phylogenies generated from the *rpoB* and *16S* gene sequences showed clear clustering by species. By contrast, the phylogeny generated from the *gyrB* gene sequence did not well differentiate between A. acidoterrestris *and* A. suci.

44 PIPETS: A Novel 3'-seq Analysis Method for the Identification of Transcription Termination Signal

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Transcription termination in bacteria is critical for regulating responses to environmental conditions. There-

fore, it is critical to understand how different experimental conditions alter termination and thus gene expression. 3 prime end-sequencing (3'-seq) allows for the identification of exact positions of transcript termination and how those positions change under stresses. While there is an increasing amount of 3'-seq data available, there are few analysis methods which use statistically robust approaches to garner results. In order to better analyze 3'-seq data, we developed PIPETS (Poisson Identification of PEaks from Term-Seq data) which uses a Poisson Distribution test and a sliding window framework to increase sensitivity and provide statistically robust results. Additionally, unlike other currently available 3'-seq analysis methods, PIPETS analyzes reads in both genic and intergenic regions. When PIPETS was compared to two previously used methods on the same data, PIPETS identified more than double the significant positions, more than 50% of which were found in genic regions. We used PIPETS to analyze two additional 3'-seq datasets generated in the lab, one from *Streptococcus pneumoniae* and one from *Acinetobacter baumannii*. We are applying PIPETS to additional data sets in the literature that were analyzed with older methodologies to determine if the qualitative conclusions of these works remain valid. Our findings illustrate the importance of providing statistically robust analysis methods to draw conclusions on an ever-growing pool of sequencing data.

45 Proteomic survey of the DNA damage response in Caulobacter crescentus reveals evidence of post-transcriptional control

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The bacterial DNA damage response controls the response to DNA damage from exogenous and endogenous sources. *Caulobacter crescentus* encodes two distinct DNA damage regulons, both controlled by transcriptional regulators that sense and respond to ssDNA accumulation. Very little is known other mechanisms of control over the DNA damage response. In *Caulobacter*, proteolysis by the AAA+ protease is central to regulation of critical cellular systems like cell cycle progression and DNA synthesis, so we aimed to characterize the role of proteolysis and post-translational regulation in the regulation of DNA damage response proteins.

We used a quantitative tandem mass tagging proteomics to observe protein abundance upon mitomycin C treatment. As expected, we found that transcriptional regulation was the likely driver for induction of many of the proteins that were upregulated after DNA damage (e.g. known DNA damage response factors like UvrA, RecA, ImuA, and RuvB). However, we also identified proteins that are upregulated upon DNA damage in a transcription-independent manner. One of these is a homolog of the *E. coli* protein YaaA, which is involved in *E. coli*'s response to oxidative stress.

To investigate the role of the Lon protease in the DNA damage response, we performed a survey in a Δ *lon* strain. This revealed that induction of the DNA damage response at the protein level is dampened in these strains, possibly explaining why this strain is sensitive to DNA damage.

This quantitative proteomics approach has allowed us to identify potential non-transcriptional regulation of the DNA damage response.

46 Design of a propeptide-based antimicrobial for Vibrio cholerae

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Antimicrobial peptides (AMPs) have potent bactericidal activity and are ubiquitous in nature, particularly in the immune defenses of multicellular eukaryotes. AMPs are highly diverse and have a degree of specificity for bacterial cell membranes, making them appealing tools for drug development. In addition to the need for novel antibiotics, there is a growing emphasis on selectivity in antimicrobial strategies that preserve commensal microbes in complex communities, like the gut microbiota. We previously identified a secreted serine protease produced by the cholera pathogen *Vibrio cholerae*, IvaP, that is active in a rabbit model of cholera and in human choleric stool. IvaP contains a propeptide domain, I9, that binds to, and is cleaved by IvaP when supplied to the protease *in trans*. We harnessed the IvaP–I9 interaction to develop a cleavage-activated, propeptide-based biosensor consisting of an environment-sensitive dye covalently linked to the purified I9 propeptide domain. This proof-of-concept probe enabled selective detection of *V. cholerae* in mixed microbial communities with limited off-targeted activation by human intestinal cells. We are now adapting the biosensor scaffold into a cleavage-activated prodrug by covalently linking various AMPs to I9 using thiol-based bioconjugation chemistry. Given that many bacteria encode secreted proteases with I9 domains, our prodrug design should be broadly applicable to other pathogens.

47 Iron sequestration by murine calprotectin induces starvation and alters virulence in Pseudomonas aeruginosa

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Protein quality control (PQC) is critical for cell homeostasis affecting human diseases and pathogenic bacteria virulence. A network of molecular chaperones and proteases determines the fate of unfolded or misfolded proteins formed in response to various conditions. While the individual chaperones and proteases of the PQC network are well-studied, there are many unknowns regarding our knowledge on the network level. To address this, we have utilized a multiplexed reverse genetic screen approach, transposon insertion sequencing technology. We have identified changes in fitness landscapes from five different mutagenesis libraries under three different proteotoxic stresses. By employing a novel computational pipeline, we find that combination of stresses that are quantitatively dissimilar in the context of the fitness landscape generates a synergistic fitness defect. Furthermore, when we look at previously identified transcriptomic stress regulons, we find redundancies in fitness level that are only recapitulated when a specific part of the PQC network is perturbed. Finally, we find new genes important for the heat stress response that synergize with known disaggregation factors.

48 Experimental characterization of the diversity of gene transfer agents in alphaproteobacteria

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Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that is responsible for severe infections in immunocompromised individuals. Pathogen sensing by the host induces a pro-inflammatory response which involves neutrophil release of the antimicrobial metal-sequestering protein calprotectin (CP, heterooligomer of S100A8 and S100A9). Murine models of infectious disease have advanced understanding of the physiological functions of CP and its ability to compete with microbes for essential metal nutrients. Despite this extensive work, our knowledge of how murine CP (mCP) sequesters metals and its impacts on P. aeruginosa physiology are limited. Here, we demonstrate that mCP displays antibacterial activity against P. aeruginosa. We further show that mCP prevents Fe uptake and affects multiple Fe-dependent processes in P. aeruginosa. In particular, mCP induces pyoverdine production, inhibits antR translation, and inhibits phenazine production by P. aeruginosa. Thus, our work demonstrates that mCP induces Fe-starvation and Fe-sparing responses in P. aeruginosa and therefore affects the virulence of the organism. We attribute these effects to high-affinity Fe chelation at the versatile hexahistidine (His6) site of mCP. This work is relevant for understanding the function and physiological consequences of mCP and how the mammalian host and bacterial pathogen complete for essential metal nutrients.

49 Genomic language model predicts protein co-regulation and function

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Deciphering the relationship between a gene and its genomic context is fundamental to understanding and engineering biological systems. Machine learning has shown promise in learning latent relationships underlying the sequence-structure-function paradigm from massive protein sequence datasets; However, to date, limited attempts have been made in extending this continuum to include higher order genomic context information. Evolutionary processes dictate the specificity of genomic contexts in which a gene is found across phylogenetic distances, and these emergent genomic patterns can be leveraged to uncover functional relationships between gene products. Here, we trained a genomic language model (gLM) on millions of metagenomic scaffolds to learn the latent functional and regulatory relationships between genes. gLM learns contextualized protein embeddings that capture the genomic context as well as the protein sequence itself, and appears to encode biologically meaningful and functionally relevant information (e.g. phylogeny, enzymatic function). Our analysis of the attention patterns demonstrates that gLM is learning co-regulated functional modules (i.e. operons). Our findings illustrate that gLM's unsupervised deep learning of the metagenomic corpus is an effective and promising approach to encode functional semantics and regulatory syntax of genes in their genomic contexts and uncover complex relationships between genes in a genomic region.

50 Metagenomic assembly to track antibiotic resistance genes in international traveler stool

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We recently carried out an in-depth controlled comparative analysis of short read (SR), long read (LR), and hybrid (HY) assembly to pinpoint the taxonomic origin and genomic context of antibiotic resistance genes (ARGs) in low-abundance species within stool metagenomes. We found that the optimal approach is goal-dependent *e.g.*, LR assembly is optimal for determining gene context around ARGs, but is outperformed by SR and HY assembly in producing highly accurate ARGs. Using a marker-based approach to identify ARGs from unassembled SR data generated from international traveler stool, we found that travelers acquire ARGs at high rates, but the persistence, context, and species of origin of these ARGs remained unknown. In a pilot study, we used SR and HY metagenomic assembly to examine ARGs and their genomic context in longitudinally collected stool samples from two travelers before and up to a year after their return from international travel. I will show how travel changes the gut resistome, highlighting examples of ARG acquisition and persistence, and demonstrating the power of metagenomic assembly to reveal ARG dynamics in complex communities.

51 Small molecule produced by Photorhabdus kills bacteria by blocking ubiquinone synthesis

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The current need for novel antibiotics is at an all-time high. However, the drug discovery pipeline is plagued with issues such as lack of new antibiotic producers and a deficiency of novel antimicrobial scaffolds. Additionally, antibiotic resistant Gram-negative bacteria account for the majority of species in the CDC's 2019 Antibiotic Resistance Threats Report, as no new class of Gram-negative acting antibiotics has been developed since the 1960's. By examining under-explored antibiotic producers, *Photorhabdus* and *Xenorhabdus*, we maximized our chances of novel compound discovery. Use of a counter-screening method that employs a Gram-positive bacterium allowed us to prioritize candidates based on selectivity for Gram-negative bacteria and removes cytotoxic junk. Our improved screening methods resulted in identification of a compound from a *Photorhabdus* strain not previously known to produce an antibiotic. This compound, ADC181, has strong activity against several strains of *E. coli* (MIC of 1 µg/mL) and *E. cloacae* (0.5 µg/mL), as well as moderate to weak activity against A. baumannii, K. pneumoniae, and *B. cenocepacia*. Additionally, ADC181 shows no cytotoxicity against human cell lines, and is well tolerated by mice. We determined the structure of ADC181 and found that it is benzisoxazole, a known natural product. By selecting for resistant mutants and generating metabolomics profiles, we determined the target of ADC181 to be 4-hydroxybenzoate octaprenyltransferase (UbiA), a non-essential member of the ubiquinone biosynthesis pathway. ADC181 apparently kills bacteria by leading to the accumulation of a toxic metabolite. This compound adds another antibiotic to the growing set in *Photorhabdus* bacteria.

52 Elucidating the transferability of CRISPRi systems across model and non-model E. coli strains

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CRISPR interference (CRISPRi) is a gene regulation technique that offers many advantages for programmable transcriptional control, including ease of design and precise DNA targeting. Although widely used for transcriptional repression in bacteria, the relative performance of various CRISPRi systems among diverse bacterial strains remains unknown. Limited CRISPRi systems have been extensively studied in an array of bacteria, and few have used standardized quantification of activity that allows for direct comparison among strains, including the less studied non-model bacterial strains. Here, we sought to investigate the transferability of three CRISPRi systems to both model and non-model strains of *E. coli*, the laboratory strain (MG1655) and three clinically-relevant strains (probiotic Nissle 1917, uropathogenic CFT073, and uropathogenic UMN026). We used standard methods to characterize the CRISPRi systems and quantified their activity in each strain for libraries of guide RNAs targeting different strands and locations on the DNA, showing a wide range of repression and activity. We also quantified

the relative growth inhibition (i.e. toxicity) due to dCas protein expression across these strains, which is widely reported for the most prevalent dCas9 protein (from Streptococcus pyogenes). We observed that Lb-dCas12a protein has the least toxicity among these CRISPRi systems and *E. coli* strains. We further present a multiplexing strategy to improve repression. Based on these rules, we provide design rules and recommendations for the use of CRISPRi in these diverse *E. coli*, which can be utilized in a variety of applications.

53 Exploring the Regulation of Biofilm Formation in Acinetobacter baumannii

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Acinetobacter baumannii is a pathogen that can form biofilms – multicellular communities of bacteria within a self-made environment of various proteins, sugars, and nucleic acids. These biofilms protect the bacteria from stressors such as antibiotics and desiccation, which causes difficulty in treatment and prevention of illnesses caused by this pathogen. It has been shown that Lon protease promotes biofilm formation in *A. baumannii*. Additionally, Lon transcriptionally downregulates SurA1, a surface protein involved in biofilm formation and virulence. In accordance with Lon's ability to bind to DNA in other species of bacteria, we believe that Lon is affecting SurA1's expression levels by binding to DNA in *A. baumannii*, either by degrading a transcriptional activator or directly acting as a repressor. To determine the relationship between Lon, SurA1, and other transcription factors within the cell, we used an assay that combines key features of CRISPRi and ChIP-seq procedures. By programming a CRISPRi system to cause transcriptional interference on the *surA1* and *lon* ORFs, we were able to position Cas9 to immunoprecipitate the complex. This would allow us to pull down any proteins bound to these genes' promoter regions and identify them via mass spectrometry. By identifying the proteins controlling Lon and SurA1 expression and better defining the relationship between these proteins and others, we can find a more complete picture of biofilm formation in *A. baumannii*. Additionally, we have shown that Lon and SurA1 are transcriptionally upregulated in several conditions that exert stress on cells.

54 Community assembly dynamics in the human skin microbiome

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The commensal bacteria C. acnes *and* S. epidermidis are the most abundant species on human skin globally. Although we know that adults can carry multiple stable strains of both, we do not know when we acquire these strains or from whom they are acquired. Here we describe the formation of the adult-type communities of C. acnes *and* S. epidermidis in humans by observing strain-level transmission dynamics within and across nuclear families over time. We pair single-isolate genomes (N=4,305) and metagenomes (N=528) with new algorithms to analyze the diversity of individual peoples' microbiomes at high within-species resolution. We discover two distinct community types through which humans transition during development, and that the formation of the adult-type community is characterized by the horizontal acquisition of new *C. acnes* strains from sources outside the nuclear family. Additionally, we observe distinct clades of *C. acnes* which are more abundant on certain age groups and exhibit clade-specific patterns of sharing between parents and children. Characterizing the natural formation of microbiome communities is necessary to understanding their interaction with humans, and designing ways to manipulate microbiome composition. This work analyzes transmission at multiple levels of intraspecies resolution for mechanistic understanding of microbial community formation.

55 How Many Microbes Can a Plant Support

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Photosynthetic plants are the primary conduits by which energy enters the biosphere and supports most non-photosynthesizing organisms from bacteria to mammals. The plant-bacteria relationship is defined by a producer-consumer model wherein the plant is the primary producer of energy through photosynthesis and bacteria are primary consumers of that energy. Energy flows unidirectionally starting with the conversion of light to chemical energy in the chloroplasts, the energy is then transferred to the rest of the plant where a majority of it is

consumed in the synthesis of biomass. Eventually it's released via the roots into the surrounding environment, finally becoming available to bacteria. Understanding the quantitative relationship between a plant and its rhizobial bacteria is difficult as the size difference between the two organisms span several orders of magnitude. To overcome this, we take a unique approach to measure the quantitative relationship between the energy producer, including the plant and its chloroplasts, and the bacterial consumer by using small individual biospheres which support the growth of a single *Arabidopsis thaliana* plant which in turn provides carbon necessary for the growth of *Bacillus subtilis*. We then use qPCR to enumerate the plant cells, chloroplast, and bacteria in each biosphere to resolve a ratio between the three which can then be used to answer the question "how many microbes can a plant supportdelta"

56 Evolution and function of bacterial communication systems in polymicrobial communities

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Though comprised of single-celled organisms, bacterial communities are greater than the sum of their parts. Many bacteria communicate and cooperate with kin using a form of cell-cell signaling called quorum sensing (QS). Moreover, bacteria typically live in polymicrobial communities where interspecies interactions, such as through QS cross talk, can have large impacts on bacteria and their hosts. Hundreds of QS systems with diverse properties have been identified, but how these systems evolved from a presumed common ancestor and how they interact in polymicrobial settings are open questions. Our ability to understand the evolution and function of the diversity in QS systems is limited by our inability to predict signaling sensitivity and selectivity from amino acid sequence. To address this problem, we used a computational covariation approach to identify the amino acids responsible for signal selectivity in the model QS system LasI-LasR from the pathogen *Pseudomonas aeruginosa*. Through this work, we observed that the receptor, LasR, has not evolved to maximal signal sensitivity and that variants of both LasI and LasR tend to be less selective than wildtype. Based on these observations, we hypothesize that QS systems evolve to maximize fitness by balancing signal sensitivity and selectivity. Taking advantage of hyper- and hypo-sensitive LasR variants, we examine the costs and benefits of altered signaling sensitivity. Our findings provide insight into the selective pressures on bacterial communication systems and lay the foundation for a better understanding how signaling systems function in complex, polymicrobial communities.

57 Characterizing the role of neutrophil extracellular traps in Staphylococcus aureus kidney infection

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Staphylococcus aureus is a major cause of skin and soft tissue infections. Bacterial growth at these sites can promote bloodstream access and colonization of the kidney, where *S. aureus* forms abscesses. Kidney abscesses contain a central Staphylococcal Abscess Community (SAC) comprised of extracellular bacteria, surrounded by a fibrin layer, and layers of neutrophils. It is known that *S. aureus* virulence factors are required to form abscesses, but it remains unclear whether these are expressed uniformly or heterogeneously. It is also unclear how neutrophil extracellular traps (NETs) influence abscess formation and infection progression, and whether specific bacterial virulence factors, such as toxins, promote NET formation in the kidney. To investigate the heterogeneity of *S. aureus* and its interplay with neutrophils, we established a mouse model of kidney infection. Using immunofluorescence microscopy and fluorescent *S. aureus* reporter strains, we determined that bacteria in contact with host neutrophils had a different toxin expression profile than those within the interior of the SAC. We also found widespread NETosis associated with irregular bacterial clusters within the kidney, but not surrounding kidney abscesses. The NET-bacterial complexes were consistently found around the collecting tubule and renal pelvis of the kidney. It is unknown if this is part of the host response to contain infection or a result of bacterial manipulation of host defenses. Further studies with bacterial deletion strains and transgenic mouse lines lacking NET formation will highlight the role of host proteins and bacterial toxins in NETosis, and the subsequent consequences of these interactions on infection.

58 Characterization of an Unknown Virulence Gene in Shigella flexneri

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Infection with the bacterial pathogen *Shigella flexneri* is one of the largest causes of diarrheal death across the world. Extensive antimicrobial resistance and no effective vaccine limit treatment options and highlight the importance of improving our understanding of pathogenesis. As *S. flexneri* traverses the digestive system to infect the colonic epithelium, exposure to bile salts (BS) in the small intestine serves as a signal for increased virulence, including activation of the Type-III Secretion System (T3SS) that injects bacterial proteins directly into the host cell cytoplasm for invasion. RNA-sequencing revealed a gene encoding an unknown protein was significantly induced in the presence of BS. A deletion mutant was constructed, in which the mutation showed no effect on *S. flexneri* growth or BS resistance. To examine effects on colonic infection, we measured T3SS function and ability of the mutant to invade HT-29 cells relative to wild type (WT) bacteria following bacterial subculturing in BS to mimic small intestinal transit. First, T3SS function was measured by monitoring protein secretion, and the mutant displayed increased secretion. Second, infection analyses indicated increased invasion of the mutant. We are currently performing competition assays with an equal ratio of WT and the mutant to determine the competitive index and if the mutant preferentially invades HT-29 cells. Combined, the analyses indicate a hypervirulence phenotype of the mutant. Future analyses will determine the role of this unknown protein, which we hypothesize regulates virulence and invasion so phenotype *S. flexneri* can successfully establish infection inside colonic cells.

59 Applying the Mouse Pneumonia Model of Acinetobacter baumannii for the Evolution and Characterization of Colistin-Selected Mutants.

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Antibiotic resistant infections in healthcare settings caused by the ESKAPE group of pathogens are responsible for more than a million deaths per year. Belonging to this group is *Acinetobacter baumannii* (AB), a Gram-negative opportunistic pathogen. Traditionally, selections for drug resistance have been performed in culture medium, which allows the acquisition of mutations that can occur at a steep fitness cost. To overcome this shortcoming, we have adapted the AB mouse pneumonia model to simulate the conditions under which drug resistance most likely emerges. To this end, colistin-resistant mutations were isolated after performing 16 sequential lung infections in both immunocompetent and neutropenic mice. Although colistin resistance mutants were selected under both conditions, they appeared after fewer passages and achieved 100

60 Dissemination of barcoded uropathogenic Escherichia coli in a murine model of urinary tract infection

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Uropathogenic *Escherichia coli* (UPEC) is a major cause of urinary tract infections (UTIs). UTI-associated bacteria have the capacity to transmit to other sites in the body and lead to severe diseases, such as sepsis. However, the dynamics of pathogen dissemination from the urinary tract and underlying mechanisms for systemic spread are largely uncharacterized. Here, using barcoded clinical isolates of UPEC, we characterized pathogen dissemination in a murine model of UTI. We quantified infection bottlenecks at multiple sites of the urinary tract including urine, bladder, and kidneys one-day following infection. Despite the direct inoculation of millions of organisms into the bladder, an average of less than 0.05% of the inoculum survived and replicated to cause UTI, revealing a very tight bottleneck to infection. In some mice, ascending infection led to the establishment of a population in the kidneys which continually seeded the bladder and the urine. Surprisingly, the most prevalent sites of dissemination were reproductive organs, including the uterus and vagina. The source of bacteria within reproductive organs was dependent on inoculum size; at higher doses, bacteria from the inoculum were able to directly colonize the uterus without infection of the bladder. Our findings reveal that the dissemination of bacteria during UTI is dependent on the infectious dose and suggest that inoculum size is a critical parameter controlling UPEC dissemination in murine UTI models. Together, this work advances our understanding of the dissemination of UPEC during UTI within the urogenital tract.

61 The Impact of Age on Lyme Disease-Associated Atopic Dermatitis

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Borrelia burgdorferi are bacteria that cause Lyme disease (LD) after infecting a susceptible host. Immune responses to the bacteria are highly variable and host specific, both in mice and humans. The murine substrain, C3H/HeJ, is a frequently utilized model for LD because it develops two key features of the human infection. The murine tail skin shares critical features with human skin, and could provide an important and underutilized tool to probe dermatological manifestations of the infection. Age and sex-matched C3H/HeJ mice aged 5-8 weeks, 1 year, or 2 years were infected with 105 *B. burgdorferi* for a minimum of 8 weeks, up to a maximum of 2 years. Features of the skin were evaluated by gross examination and histology. Key features of atopic dermatitis were present in infected mice after 1 year of age, including ulceration, hemorrhaging, flaking, hair loss, and dark lesions as well as spongiosis and acanthosis. Furthermore, mice infected with *B. burgdorferi* containing luciferase were studied over a 6-month long time course using an In Vivo Imaging System, and were found to have significant infection in their tails. We then identified 5,248 individuals with LD and 17,233 with atopic dermatitis in FinnGen and find that also in human epidemiological data LD associates with atopic dermatitis (OR = 1.91 [1.68 -2.37], P ¡ 2e-16). We demonstrate the onset of murine atopic dermatitis with LD, which is further exacerbated by host age at time of infection, and likewise report a similar association in humans.

62 Adaptive mechanisms of chronic salmonellosis in humans

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Salmonella enterica can establish chronic infections, evading the host immune system and killing by antibiotics, providing a reservoir for transmission to new hosts. Non-typhoidal serovars (NTS) of *S. enterica* have been linked to an estimated 93 million cases and 155,000 deaths annually, yet little is understood about the bacterial and host factors, and their interplay, that enable chronic infections. I hypothesize that establishment of chronic infections involves genomic adaptations in NTS that influence the host-pathogen interactions in a way that enables the pathogen to persist long-term. Using whole genome sequencing of longitudinal isolates from all persistent, culture-confirmed salmonellosis cases that occurred in Israel over 17 years, I found a striking enrichment of genetic variation that accrued over time in global virulence regulators in persistent isolates, including overwhelmingly in genes encoding the BarA/SirA two-component system. I used comparative RNA-Seq analysis to show that distinct, naturally occurring mutations in barA or sirA led to significant downregulation of virulence-associated genes encoded in the *Salmonella* Pathogenicity Islands (SPIs) 1 and 4, critical for host cell invasion and the production of enteritis. In the salmonellosis mouse model, persistent isolates with barA/sirA mutations displayed attenuated virulence *in vivo* compared to their wild-type counterparts. Taken together, these data suggest that selection of mutations in key virulence regulators plays a fundamental role in establishing persistent salmonellosis in humans. Defining the adaptations underlying chronic infections will enable the development of more effective strategies to combat a wide variety of chronic bacterial infections.

63 New biology from an old antigen: a role for the 17kDa surface lipoprotein in rickettsial cell envelope maintenance

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Pathogens in the genus *Rickettsia* are obligate intracellular bacteria that can cause an array of mild to life-threatening arthropod-borne vascular diseases in humans. *Rickettsia* encode shared and distinct cell surface protein antigens that provoke mammalian immune responses. Although most major *Rickettsia* antigens have been identified for decades, our knowledge of their contributions to infection and pathogen cell biology is limited. One such factor is the 17kDa surface lipoprotein (also called *hrtA*), which was one of the earliest cloned *Rickettsia* genes. The *hrtA* locus is widely used in rickettsial genotyping and diagnostic studies, but the precise biological function of HrtA is unknown. Phenotypic characterization of an R. parkeri *transposon mutant strain harboring an insertion in* hrtA revealed a marked in vitro infection defect in bacterial growth. We found that bacteria lacking *hrtA* have striking cell shape defects, including a bipolar phenotype suggestive of impaired cell division. The predicted structure of HrtA contains a folded C-terminal domain with structural resemblance to bacterial pseudopilus components and OmpA-like domains, hinting that HrtA binds to specific extracellular structures and/or the cell wall. Our data are consistent with a model where HrtA functions similarly to other Gram-negative outer membrane lipoproteins like Pal to stabilize the cell envelope. Improved understanding of rickettsial cell surface

antigen biology could potentiate the use of these factors as components in vaccines and diagnostic assays for these emerging pathogens.

64 Cell division principles and mechanisms of ceftriaxone resistance in Neisseria gonorrhoeae

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Extensive antibiotic resistance in the Gram-negative diplococcus *Neisseria gonorrhoeae* (Ng) has resulted in only one remaining drug, ceftriaxone, recommended for treatment of infections. Ceftriaxone resistance (CROR) is primarily mediated by target-site variants in *penA*, which codes for Penicillin-Binding Protein 2, a peptidoglycan transpeptidase that functions during cell division. With the emergence and international spread of CROR strains, it is critical to understand cell division associated mechanisms of resistance and how Ng mitigates the fitness costs incurred by resistance mutations. Here, we probed the mechanisms of Ng division and used microscopy to determine the impact of CROR on cellular morphology and of a mutation in the TCA cycle enzyme, aconitase hydratase (*acnB*) that compensates for resistance-promoted fitness costs. Live cell imaging revealed that successive division planes were oriented perpendicularly and were overlapped in timing, such that assembly of a second division plane began before the first resolved. CROR mutants harboring resistance-conferring *penA* alleles were less fit compared to susceptible counterparts in vitro and showed abnormalities in cell size, septal thickness and interdivision time. Introduction of a compensatory mutation in *acnB* (*acnB*G348D), previously identified as a compensatory mutation alleviating the fitness costs incurred by the *penA41* allele in a mouse model of gonorrhea, restored single cell size and interdivision time, and led to faster bulk doubling times, as compared to strains with the *penA41* allele alone. This work details cellular consequences of CROR and how Ng maintains fitness in the face of antibiotic pressure through acquisition of resistance and compensatory mutations.

65 Characterization of the microbial ergothioneine transporter EgtUV in the gastric pathogen Helicobacter pylori

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Low-molecular-weight (LMW) thiols are small-molecule antioxidants that maintain redox homeostasis. Ergothioneine (EGT) is an LMW thiol synthesized by some bacteria and fungi and imported by mammalian cells. The gastric pathogen *Helicobacter pylori* encodes a widely conserved microbial EGT transporter, *egtUV*. EgtUV is an ABC transporter with fused permease and solute-binding domains (SBD) and an ATPase. The basis for the high affinity and specificity of the SBD for EGT is unknown. We therefore solved crystal structures of the EgtU SBD in free and ligand-bound forms. A conserved aromatic residue network common to betaine SBDs is required for EGT-binding, and we also identified residues which may differentiate EGT-binding proteins. We are now investigating how EGT import protects *H. pylori* against physiologically relevant bleach stress. This work provides novel mechanistic insights into how EGT import promotes microbial redox homeostasis.

66 Investigating a Predicted Fimbriae-forming Lipoprotein in Porphyromonas gingivalis

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Our studies have shown that oral anaerobe Porphyromonas gingivalis (Pg) can surface translocate, and one of the most highly upregulated genes during the initial stages of this process is PG1881, a predicted fimbriae-forming lipoprotein. PG1881 has been detected on outer membrane vesicles (OMVs) and may be selectively carried on sphingolipid-containing OMVs. Data show that PG1881 is citrullinated by PPAD, an enzyme that converts arginine to citrulline within peptides, modifying protein structure and function. The objective of this study is to further characterize PG1881 and investigate its function in the context of surface translocation. Deletion mutants were generated in strain W83 and strain W50. Western analysis that measured PG1881 levels on OMVs isolated from the parent strain W83, PG1881 mutant, a sphingolipid-null mutant, and a PPAD mutant

showed that PG1881 is enriched on Pg OMVs, yet not detected on OMVs isolated from the sphingolipid-null or PPAD mutant. To observe surface translocation, W50 and the PG1881 mutant were stabbed to the bottom of soft agar plates and incubated anaerobically. The extracellular matrix of surface translocating cells was examined by fluorescent staining as well as by transmission electron microscopy (TEM). Fluorescent staining revealed that the extracellular matrix is structurally distinct in the mutant and TEM images suggest this may be attributed to differences in OMV properties. Overall, the data show that PG1881 localizes to SL-OMVs and the release of PG1881 containing OMVs is influenced by PPAD activity. Furthermore, the data indicate that PG1881 plays a role in the biogenesis and properties of OMVs.

67 Cell division protein ZapE links the division machinery to central carbon metabolism in pathogenic and non-pathogenic strains of Escherichia coli

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Uropathogenic *E. coli* (UPEC) is the causative agent of most urinary tract infections. Many UPEC strains, including CFT073, can enter a metabolite-dependent, non-proliferative state of dormancy, called quiescence, when cultured in vitro. During infection, UPEC are thought to form quiescent intracellular reservoirs in bladder epithelial cells, which may help them evade antibiotics. In a minitransposon screen to identify Tn5 mutants that are defective for quiescence, we discovered that the cell division regulator ZapE, which is important for division in *E. coli* during periods of stress, is essential for UPEC to enter quiescence. Entry into quiescence also requires a complete TCA cycle, and deletion of *sdhA*, which encodes a subunit of succinate dehydrogenase (SDH), inhibits quiescence and promotes proliferation. Here, we used bacterial two-hybrid assays (BACTH) to show that ZapE directly interacts with SDH subunits, including SdhC. ZapE also engages FtsZ and FtsN, therefore we tested if FtsN and SdhC interact. We discovered these interactions are conserved using genes derived from the non-pathogenic *E. coli* K-12 strain, MG1655. We also demonstrate that ZapG, a cell division protein involved in septal peptidoglycan synthesis, interacts with ZapE from CFT073. To elucidate the molecular determinants for these interactions, we engineered fusion proteins to contain truncated variants of ZapE, SdhC, and ZapG and tested them in the BACTH assay. Together, our results demonstrate that there is crosstalk between the cell division machinery and the TCA cycle, which is mediated by ZapE, and that these interactions may underlie the metabolite-dependent, quiescent state in UPEC.

68 A novel control system links cell wall stress to outer membrane synthesis in Acinetobacter baumannii

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Acinetobacter baumannii is a Gram-negative pathogen notorious for antibiotic-resistant infections. Its envelope is critical to multidrug resistance. How *A. baumannii* coordinates synthesis of envelope components, including a lipooligosaccharide (LOS) outer membrane and peptidoglycan cell wall, is poorly understood. Previous studies revealed that knockout of a peptidoglycan hydrolase, PbpG, led to decreased LOS, suggesting a role for a cell wall enzyme in outer membrane synthesis.

To investigate how PbpG affects LOS synthesis, we mapped its genetic interactions. We found that *pbpG* is synthetic lethal with rpoE-Ab *and* rseP-Ab, homologs of key *E. coli* σE system components. RpoE-Ab or RseP-Ab deficiency blocks the LOS decrease by Δ pbpG, suggesting that A. baumannii's σE pathway is what modulates LOS synthesis in response to cell wall lesions. *lpxC* overexpression is also synthetic lethal with Δ pbpG, suggesting that constant LOS production is toxic to Δ pbpG and that σE is a detoxifier. To identify targets of σE regulation mediating detoxification, we isolated suppressors of the above synthetic lethals. A major site of suppression was LirL, a poorly-characterized lipoprotein. Δ lirL was sufficient to reduce LOS, mirroring Δ pbpG. Δ pbpG *and* rpoE *overexpression each caused* lirL down-regulation.

These findings suggest a model in which Δ pbpG causes LOS assembly stress, which is sensed by σE. The resulting detoxifying response is to down-regulate *lirL*, which in turn lowers LOS synthesis. Further investigation will address how LirL modulates LOS synthesis, and how cell wall lesions affect LOS assembly. This work sheds light on a new control circuit maintaining the protective *A. baumannii* envelope.

69 Identifying Genes that Regulate Gene Transfer Agent Synthesis and Release in Caulobacter crescentus

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The α-proteobacterium *Caulobacter crescentus* produces gene transfer agents (GTAs), phage-like particles that horizontally transfer genetic information. GTA-producing cells synthesize capsids, package them with fragments of genomic DNA, and then lyse, killing the cell and releasing the GTA particles, which proceed to transfer the packaged DNA to recipient cells. In *C. crescentus*, *gafYZ* have been identified as the direct activators of GTA synthesis and the transcription factor *rogA* is known to repress *gafYZ*, but much remains unknown about the regulation of GTA synthesis. Even under *gafYZ* overexpression, *C. crescentus* only produces GTAs in the stationary phase. The mechanisms responsible for this cell-density dependence have not been explained.

To further elucidate the mechanisms that regulate GTA synthesis and release in *C. crescentus*, we established a mutagenesis technique to identify mutants that rescue GTA lethality. Induction of GTA production via *gafYZ* overexpression led to a greater than 10,000-fold decrease in cell viability. Transposon mutagenesis during GTA production revealed 24 potential mutants that rescue lethality. From these mutants we have identified several genes that allow for survival under *gafYZ* overexpression when interrupted, and may therefore play a role in GTA regulation, synthesis, or release. Further investigation of mutants is ongoing and may reveal additional mechanisms controlling this understudied form of horizontal gene transfer.

70 Pseudoprotease-mediated regulation of germinant sensing in C. difficile

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Clostridioides difficile infection begins when metabolically dormant spores encounter germinants in the vertebrate gut and initiate the process of germination. This process is essential for *C. difficile* to produce vegetative cells that release toxins and cause disease. *C. difficile* germination differs from that of most spore-forming bacteria. First, *C. difficile* spores germinate in response to bile acid germinants combined with amino acid and/or Ca2+ co-germinants. Second, while most spore-formers sense germinants through highly conserved transmembrane receptors, *C. difficile* does not encode these receptors and instead uses soluble pseudoproteases (CspA and CspC) to sense co-germinants and germinants, respectively. CspA and CspC then go on to activate CspB, which activates a lytic enzyme responsible for the degrading the protective cortex layer of the spore. While CspA and CspC clearly play key roles in regulating germination initiation, the mechanism by which they integrate (co-)germinant signals is poorly understood. We recently discovered that CspC and CspA form a heterodimer, while CspA forms a homodimer. By solving the crystal structure of the CspA homodimer, we identified residues that regulate CspA homodimerization. Mutation of these CspA homodimerization residues promotes CspA:CspC heterodimerization in vitro and reduces the sensitivity of *C. difficile* spores to bile acid germinant. These biochemical and genetic analyses suggest that CspC and CspA integrate (co-)germinant signals using a "Partner-Swap" model in which (co-)germinants encountered in the gut destabilize the CspC:CspA heterodimer and promote CspA homodimerization. Liberated CspC may then modulate activity of CspB, allowing for subsequent germination of the spore.

71 Investigating the impact of prophages on bacterial fitness of Group B Streptococcus

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Prophages are viral genomes that become integrated into bacterial genomes, and they are known to improve bacterial colonization, adaptation, and ecological fitness, providing a greater opportunity for pathogenic bacteria to spread and cause infection. Group B *Streptococcus* (GBS), a common bacterium found in pregnant women that can cause severe infections in neonates, typically carries one or more prophages, but their precise role in pathogen fitness and virulence remains unclear. Although detecting maternal colonization and administering antibiotics during labor can prevent early-onset GBS disease in neonates, antibiotics negatively affect newborns' microbiota, leading to complications like gastrointestinal disorders and immune system dysregulation. Therefore, alternative therapeutic measures are necessary to improve maternal and neonatal outcomes. By understanding the role of prophages in GBS virulence, we provide new opportunities to develop alternative treatments. Bioinformatic analysis of 49 clinical isolates of GBS identified 42 prophages present in their genomes, which can be classified into 5 clusters based on their genomic content, indicating differences in the genetic makeup of the prophages. Further investigation of a hypervirulent GBS strain, found that the only prophage present provides a competitive advantage to the bacterium, possibly by enabling it to better compete for nutrients or resist other

bacterial species. Whole-genome transcriptome analysis showed that loss of the prophage significantly altered the expression of 20.3% of GBS genes. These results provide insight into the role that prophages play in the disease pathogenesis of GBS and ultimately may lead to the identification of potential targets for alternative therapeutic approaches

72 An essential periplasmic protein coordinates lipid trafficking and is required for asymmetric polar growth in mycobacteria

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Mycobacteria, including the human pathogen Mycobacterium tuberculosis, grow by inserting new cell wall material at their poles. This process and that of division are asymmetric, producing a phenotypically heterogeneous population of cells that respond non-uniformly to stress (Aldridge et al., 2012; Rego et al., 2017). Surprisingly, deletion of a single gene – lamA – leads to more symmetry, and to a population of cells that is more uniformly killed by antibiotics (Rego et al., 2017). How does LamA create asymmetrydelta Here, using a combination of quantitative time-lapse imaging, bacterial genetics, and lipid profiling, we find that LamA recruits essential proteins involved in cell wall synthesis to one side of the cell – the old pole. One of these proteins, MSMEG-0317, here renamed PgfA, was of unknown function. We show that PgfA is a periplasmic protein that interacts with MmpL3, an essential transporter that flips mycolic acids in the form of trehalose monomycolate (TMM), across the plasma membrane. PgfA interacts with a TMM analog suggesting a direct role in TMM transport. Yet our data point to a broader function as well, as cells with altered PgfA levels have differences in the abundance of other lipids and are differentially reliant on those lipids for survival. Overexpression of PgfA, but not MmpL3, restores growth at the old poles in cells missing lamA. Together, our results suggest that PgfA is a key determinant of polar growth and cell envelope composition in mycobacteria, and that the LamA-mediated recruitment of this protein to one side of the cell is a required step in the establishment of cellular asymmetry.

73 Global transcriptional control of membrane lipid biosynthesis and metabolism in A. baumannii

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Acinetobacter baumannii is a global threat for its ability to develop antibiotic resistance to a wide range of antibiotics and is listed as the highest priority for research and discovery of new antibiotics by the WHO. Biosynthesis, metabolism, and homeostasis of fatty acids and membrane lipids is critical to bacterial viability, antibiotic resistance, and adaptation to changing environments. Bacteria have evolved complex mechanisms to control these processes, including transcriptional regulation. However, *A. baumannii* lacks orthologs of the known fatty acid transcriptional regulators from other species, and how lipid synthesis is regulated in *A. baumannii* is still unknown. In this research, we identify an AraC-family protein named AlmC (for Acinetobacter Lipid Metabolism Control) as a global transcriptional regulator of fatty acid biosynthesis and multiple additional metabolic pathways in *A. baumannii*. AlmC is essential for growth. RNA-seq and ChIP-seq data show that AlmC is a bifunctional regulator, directly activating expression of multiple genes responsible for fatty acid and membrane lipid synthesis, and repressing genes mediating glyoxylate shunt. Partial depletion of AlmC greatly hypersensitizes *A. baumannii* to the fatty acid synthesis inhibitor cerulenin and triclosan. It also increases susceptibility to the hydrophobic antibiotic rifampicin and leads to structural changes in lipopolysaccharide, indicating dependence on AlmC for outer membrane integrity. Future work will define the structure and ligands of AlmC to better understand bacterial control of lipid metabolism and to develop ways to target this process for blocking *A. baumannii* infections.

74 The structure and proposed biosynthesis of an elusive bacterial pigment associated with efficient cellulose digestion

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Anerobic degradation of cellulose, the most abundant polymer on the planet, has major impacts on production of biofuels/biocommodities, agriculture, climate change, and carbon cycling. Primary fermenters specializing in anaerobic cellulose degradation adhere to the surface of the insoluble cellulose substrate. Interestingly, many bacterial cellulose fermenting specialists produce a yellow cellulose-binding pigment ("flavoaffinin"), likely of polyketide origin, that appears to be linked with efficiency of cellulose degradation. We purified and solved the structures of two of the four flavoaffinin congeners from Hungateiclostridium thermocellum, both of which had an indolyl-dienelactone head group linked to an arylpolyene tail. Comparative genomics linked to targeted comparative metabolomics permitted identification of a plausible flavoaffinin BGC in H. thermocellum and close relatives within Clostridium cluster III. We provisionally named this BGC the faf gene cluster. Surprisingly, the faf gene cluster is not present in Ruminococcus sensu stricto spp. (from Clostridium cluster IV) that also produce flavoaffinins. Again, comparative genomics/metabolomics permitted identification of a likely flavoaffinin BGC in Ruminococcus sp. (the fla gene cluster), phylogenetically unrelated to the faf gene cluster. This suggests that flavoaffinin biosynthesis has arisen by convergent evolution—specifically in anaerobic cellulose fermenters—at least twice and predicts an important function for flavoaffinin in the specialized cellulose fermentation niche. The function of the flavoaffinins is unknown, but we favor the hypothesis that flavoaffinins are contact-dependent antimicrobials that are deposited on the cellulose surface so as to specifically target competing cellulose fermenters with a concentrated poisonous dose.

75 Exploring the mechanism of lipid transport to the outer membrane of mycobacteria in the LprG-Rv1410c pathway

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Mycobacterium tuberculosis (*M.tb*), the causative agent of tuberculosis (Tb) in humans, is the leading cause of death from a single infectious agent worldwide. Additionally, the continued emergence and spread of multidrug-resistant TB further demonstrates the urgent need for novel therapeutic approaches against *M.tb*. The drug resistance of *M.tb* is due in part to a well-armored outer membrane composed of chemically diverse and extremely hydrophobic complex lipids. To build the outer membrane, *M.tb* requires dedicated transport machinery to shuttle these lipids across the aqueous periplasm and deliver them to the mycobacterial outer membrane. Despite their importance, the identity and mechanism of these transport systems remain largely unknown. My work primarily focuses on a highly conserved pathway defined by two proteins, LprG and Rv1410c, that have been implicated in lipid transport to the outer membrane. In recent years, our lab has demonstrated that LprG interacts with Ag85A, an enzyme that synthesizes the outer membrane glycolipid trehalose dimycolate (TDM) from trehalose monomycolate (TMM). TDM is of particular interest as it is important for virulence in *M.tb*. Using the non-pathogenic surrogate *Mycobacterium smegmatis* (*M.sm*) and detailed lipid analyses, my results implicate these proteins in regulating the transport of TDM to the outer membrane of mycobacteria. Current work focuses on testing the hypothesis that cross-talk between the LprG-Rv1410c and Ag85A pathways facilitates outer membrane biogenesis in mycobacteria by modulating how TDM is transported to the outer membrane.

76 Analysis of BfmRS essentiality in multidrug-resistant Acinetobacter baumannii

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Acinetobacter baumannii is a pressing public health threat due to its extensive drug resistance and ability to infect critically ill patients. The impressive drug resistance of *A. baumannii* is facilitated by acquisition of large plasmids carrying resistance genes. The shortage of effective antibiotics makes uncovering new mechanisms of resistance and vulnerability in *A. baumannii* increasingly important. The two-component system, BfmRS, is established as the master regulator of intrinsic resistance in the bacteria. Moreover, Tn-seq data suggest BfmRS may also be essential for viability in some clinical isolates, but not others, in the absence of antibiotics. Whether *A. baumannii* depends on BfmRS for growth, and the strain-specific nature of this potential dependence, are not known. To address these problems, we used CRISPRi and gene deletions to show that strain AB5075 depends on BfmRS for growth. We used these platforms to develop a genetic selection for suppressors bypassing BfmRS dependence. We found that some suppressor mutations mapped to chromosomal loci, including *clpA* and ribosomal genes. Other suppressors, however, had a range of large deletions or point mutations in AB5075's large resistance plasmid, p1. Almost all the p1 mutations involved the same gene, encoding a hypothetical protein and located near the plasmid conjugation machinery. We showed that BfmRS transcriptionally represses this gene. Since plasmids vary by strain, these results suggest that resistance plasmids may serve as specific sources of toxicity in *A. baumannii*, requiring mollification by BfmRS. Further work will test this model and define the

function of the novel *A. baumannii* protein.

77 Antibiotic tolerance in populations of non-proliferating and intracellular uropathogenic E. coli

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Bacterial antibiotic resistance is of growing concern within the medical community and, with few new antibiotics in development, there is a growing threat to public health. Uropathogenic *Escherichia coli* (UPEC) are the leading cause of urinary tract infections (UTIs). Infection occurs when UPEC colonize the epithelial cells that line the bladder, and approximately a quarter of patients experience reoccurring UTIs (rUTIs) after antibiotic treatment. This may be attributed to some UPEC populations entering a non-proliferative, or quiescent state that allows them to evade antibiotic treatment and persist in bladder cells. We have shown that cells from the endemic UPEC ST-73 lineage CFT073 are unable to proliferate when cultured at a low cell density on glucose minimal agar, and instead they enter a dormant state, called quiescence. We show that quiescent UPEC cells are tolerant to a wide range of antibiotics, including cephalexin, trimethoprim, and fosfomycin. Molecular cues that reverse quiescence and promote proliferation, including the amino acids L-Lys and L-Met and the metabolite succinate, restore sensitivity to antibiotics. We developed an invasion assay to monitor intracellular populations of UPEC in cultured human bladder epithelial cells and observed that UPEC cells survive intracellularly for up to 72 hours. We are currently evaluating antibiotic tolerance of intracellular UPEC cells to determine which antibiotics are most effective. Our work aims to understand why UPEC associated with rUTIs are resilient to antibiotic treatment.

78 Highly Multiplexed Spatial Transcriptomics in Bacteria

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Spatial transcriptomics enables the localization, identification, and quantification of thousands of RNA species in their native spatial context. In mammalian systems, this powerful tool has offered new insights into tissue architecture, cell identity, and transcriptional programs. Bacterial communities can also exhibit strong spatial architecture and single-cell heterogeneity, and, thus, a wide range of questions in microbiology would benefit from spatial transcriptomics. Unfortunately, the application of such methods to prokaryotes has been hampered by the small size of bacteria and the extreme density of their transcriptome.

Here, we overcome these challenges to extend MERFISH–a highly multiplexed single molecule RNA FISH technology–to bacteria. Building on recent developments in expansion microscopy we leveraged MERFISH to profile the expression of hundreds to thousands of bacterial operons in single cells. To demonstrate the potential of this technique, we characterized E. coli sub-populations that arise during a canonical metabolic perturbation, mapped the intracellular organization of the E. coli transcriptome, and profiled the modulation of B. thetaiotaomicron gene expression in situ in the mouse gut. Collectively, our results underscore how bacterial MERFISH can be employed to address biological questions ranging from subcellular localization, to subpopulation heterogeneity, to spatially-modulated bacterial transcription in complex environments.

79 Improving the genetic tractability of Xanthobacter autotrophicus for use as a point-of-need food production platform

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As the world faces greater food insecurity in the face of anthropogenic climate change, the development of point-of-need food production systems is of utmost importance. Firstly, agriculture at present requires significant land use. Additionally, food distribution is both logistically and energetically intensive. Here, we explore *Xanthobacter autotrophicus* as a potential platform for point-of-need food production. *X. autotrophicus* is an attractive candidate for such an application due to its ability to sustain growth on nitrogen, oxygen, and carbon dioxide from the air, hydrogen from water splitting, and salts. However, *X. autotrophicus* grows slowly and requires a higher percentage of carbon dioxide than is available in the air. Thus, the organism must be genetically engineered or evolved before it can be employed for efficient food production. However, there has been little work done to develop genetic tools for *X. autotrophicus*. Here, we present a novel modular Golden Gate tool system for constructing plasmids for *X. autotrophicus*. Using this system, we systematically tested and quantified promoter strength with a fluorescent signal readout. Additionally, we tested CRISPR gene editing in the organism to further expand the genetic toolbox available to researchers working with the organism.

80 Use of a Graph Theoretic Approach to Identify and Study a Novel Transcriptional Regulator in Shigella

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Shigella flexneri is a facultative anaerobic bacterial pathogen that invades the colonic epithelium and causes shigellosis, a severe disease that affects over 180 million patients annually and is characterized by fever, vomiting, and watery and/or bloody diarrhea. During transit through the small intestine, *Shigella* resists bile salts and forms a biofilm in the presence of bile salts and glucose that is held together by an exopolymeric substance (EPS) matrix. Upon entry into the colon, *Shigella* disperses this biofilm and infects the colonic epithelium. Past RNA-sequencing analysis from our lab identified genes induced and repressed in the presence of bile salts. Here, we present LambdaCC, a novel optimization method that clusters co-expressed genes, to predict regulatory gene networks from RNA-sequencing data. LambdaCC identified a unique pattern of differentially expressed genes of similar function clustering alongside seemingly unrelated genes in bile salts conditions, including clustering chromosomal genes and genes encoded on Shigella's large virulence plasmid required for pathogenesis. We then used LambdaCC to predict transcriptional regulators induced in bile salts and constructed a knockout mutant of one predicted regulator, *yhjC*, as proof-of-concept. To investigate the effects of this mutation, we examined the growth, biofilm formation, and EPS matrix production of Δ *yhjC*. The mutation did not affect growth without or with bile salts but did affect biofilm formation and EPS production in a glucose-dependent manner, as predicted by LambdaCC. This work provides an experimentally validated framework to study gene expression changes, identify transcriptional regulators, and help improve our understanding of *Shigella* pathogenesis.

81 Accurate species identification and antibiotic susceptibility prediction (AST) for multiple pathogens in contrived polymicrobial blood samples using whole genome Oxford Nanopore sequencing

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Polymicrobial infections – defined broadly as infection by multiple microorganisms – are associated with increased morbidity, length of hospital stay and duration of intensive care. These infections are typically challenging to diagnose with standard blood culture systems. In addition, because microbes can share resistance genes, molecular methods can struggle to attribute resistance to the correct species when multiple are present.

Day Zero Diagnostics® is developing a diagnostic for identifying bacteria in bloodstream infections using ultra-high pathogen enrichment (Blood2Bac™) followed by whole genome sequencing of bacteria on the Oxford Nanopore Technologies (ONT) platform. Using Keynome® ID, our taxonomic identification algorithm, species identification from whole blood can be done in hours, compared to blood culture, which can often take days. Previously, we have demonstrated that we can accurately identify pathogens in monomicrobial blood samples; here, we tested Keynome® ID on a set of 15 contrived polymicrobial blood samples where 5 pathogen pairs were spiked in at clinically relevant bacterial loads (1-13 CFU/mL). This work showcases our ability to detect all organisms in contrived polymicrobial blood samples with 100% sensitivity and 100% specificity. Furthermore, we show that Keynome® gAST, our proprietary machine learning algorithm that predicts antibiotic resistance, achieves 92.22% categorical agreement in these challenging samples.

We show that the Keynome® pipeline is able to robustly identify polymicrobial species directly from a blood sample, allowing accurate predictions of antibiotic resistance phenotypes for all tested species present in polymicrobial blood samples. These advancements in our diagnostic will potentially allow clinicians to treat patients more quickly, reduce the use of unnecessary antibiotics and improve health-outcomes.

82 Applying droplet Tn-Seq to identify metallophore transport systems in Yersinia pestis.

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Pneumonic plague is an acute disease caused by the Gram-negative reemerging pathogen, *Yersinia pestis*. The host's ability to actively sequester key biometals(e.g. iron, zinc and manganese), essential for bacterial growth, is an important barrier to bacterial infection(nutrition immunity). Metallophores are essential virulence factors that facilitate bacterial pathogens' ability to overcome nutritional immunity. *Y. pestis* secretes the metallophore Yersiniabactin, capable of chelating iron and zinc in a biometal-limited environment. The role of Yersiniabactin in the iron import system of *Y. pestis* has been previously described. However, the complete system for zinc import and export is unknown. Here, we apply microfluidic single-cell encapsulation, and droplet Tn-Seq to identify transport systems under a zinc-limited environment. As Yersiniabactin is exported into the extracellular milieu, mutants with a defect in this system may be able to 'cheat' and exploit the Yersiniabactin made by others. Thus, it is necessary to isolate transposon-mutants in a single-cell droplet environment to better resolve these complex phenotypes. The inner membrane ABC-transporter *Znu*ABC system is required during *in vitro* growth of *Y. pestis* in submicromolar zinc levels. To force the bacteria to use the Yersiniabactin system, six transposon insertion libraries were generated on a Δ *znu*BC background. The genes y0703 (RND efflux permease) and y0704 (RND efflux TolC) belong to the operon AcrAB-RND efflux and showed a fitness defect. The growth response of the *Y. pestis* Δ *znu*BC/*acr*AB/*tol*C mutant under zinc-limiting conditions suggests that *Y. pestis* has a second high-affinity zinc transporter system. To elucidate this, six transposon insertion libraries were generated on aΔ *znu*BC/*acr*AB/*tol*C background. These library mutants are secluded in droples and cultured in media with varying zinc availability, followed by sequencing.

83 Developing a Fluorescence-activated Cell Sorting Method to Evaluate Ribosomal Cis-Regulatory RNAs

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The Fluorescence-activated Cell Sorting (FACS) based assay under development aims to improve upon existing methods of assaying the function of cis-regulatory RNAs, with a goal of sensitive, high-throughput assays for various regulatory RNAs. The cis-regulatory RNA being studied for assay development, the S15 ribosomal leader, is a structured region in the 5'-UTR that regulates translation of the S15 ribosomal protein and is a key regulator of ribosomal synthesis. S15 leader mutant libraries with a Green Fluorescent Protein (GFP) expression system are cultured into S15 knockout strains, sorted through flow cytometry, and the resulting populations sequenced. Locations of mutations in relation to RNA structure will then be analyzed to evaluate how altered shape affects functionality. This FACS method allows for assessment of large mutant libraries and analysis of the effects of sequence and structural changes on protein synthesis. Previous research in the Meyer Lab indicated that cells cultured into microcolony droplets using microfluidics provided amplified and more sensitive GFP signals, but this technique came with significant bottlenecking of mutant populations. Flow cytometry results in free, single cells indicate that an improved GFP expression system, along with changed gating strategies, adequately amplify the fluorescent signal to differentiate between leader variants, avoiding the bottleneck of previous microfluidics assays. Further development of this regulatory assay indicates that a dsRed cassette, originally inserted for microcolony normalization, does not have deleterious impacts on the current GFP assay which does not require such normalization. This FACS-based sorting method is currently being applied to large cis-regulatory RNA libraries.

84 Outer Membrane Vesicles Isolated from Pseudoalteromonas rubra Carry Prodiginine Antibiotics as Cargo

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Astrangia poculata, the northern star coral and official state coral of Rhode Island, is a model organism for the study of microbial-coral interactions due to the ease with which it can be cultivated under laboratory conditions. Coral larvae are thought to settle and metamorphose on substrates, such as crustose coralline algae (CCA), based on their microbial community composition and their associated chemical cues. Strains of Pseudoalteromonas rubra isolated from CCA have been shown to induce larval settlement of tropical corals. We hypothesize that P. rubra induces the settlement of A. poculata larvae by producing specialized metabolites packaged within membrane vesicles (MVs). In this study, we investigated MVs produced by P. rubra strains

KB1 and CH007, which were isolated from CCA in A. poculata coral culture tanks at Roger Williams University. MVs were isolated by ultracentrifugation, lyophilized, and extracted with organic solvents. Analysis by UHPLC tandem mass spectrometry revealed that P. rubra MVs contain prodiginines, a class of red-pigmented specialized metabolites with diverse biological activities, including antimicrobial and algicidal activity, toxigenicity, and immunosuppressive properties. P. rubra MVs further demonstrated antimicrobial activities against Vibrio parahaemolyticus PSU5579, a problematic marine invertebrate pathogen. These results suggest a role for MVs from P. rubra as a delivery mechanism for mediating microbial and coral interactions.

85 Lectin-seq: uncovering the determinants of host-microbe interactions

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Soluble human lectins are critical components of innate immunity. Genetic models suggest lectins influence host-resident microbiota, but their specificity for commensal and mutualist species is understudied. Elucidating lectins' roles in regulating microbiota requires an understanding of which microbial species they bind within native communities. Here, we describe Lectin-SEQ, a generalizable workflow to characterize human lectin recognition. We applied our approach to human fecal microbiota using the soluble mannose-binding lectin (MBL) and intelectin-1 (hItln1). Our results reveal hItln1 exhibits strain-specific binding to a broad range of commensal species, while MBL showed high specificity for select species. To investigate the genetic basis of the observed lectin specificity, we surveyed the binding of hItln1 and MBL to strain isolates of commensal species. Genomic analysis of lectin-bound isolates against un-bound isolates will identify genes involved in glycan synthesis that leads to unique glycan structures and subsequently to differential lectin binding. Our findings show that hItln1 and MBL recognize divergent groups of microbes and demonstrate the utility of our method for characterizing interactions between lectins and commensal microbes.

86 Escherichia coli Surface Display of Borrelia burgdorferi Lipoproteins

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Lyme disease, caused by the bacteria *Borrelia burgdorferi*, is the most common vector-borne illness in the U.S. Due to the slow growth and low transformation efficiency of *B. burgdorferi*, as well as the complexity of its genome, which includes over 20 difficult-to-manipulate plasmids, *B. burgdorferi* presents experimental challenges. Adding to the complexity is the variation in severity and tissue tropism across different cases of Lyme disease. Many plasmid-borne surface lipoproteins produced by the bacteria have roles in tissue adhesion and/or immune evasion; greater understanding of these proteins will improve understanding of host infection and bacterial survival, as well as the diversity of disease presentation. There have been extensive efforts to systematically assess binding functions of suspected surface lipoproteins; however, these efforts are hampered by the unique biology and many plasmids of *B. burgdorferi*.

To overcome these challenges, we adapted an *Escherichia coli* surface display system to heterologously present *B. burgdorferi* lipoproteins. This system, an application of the widely used INP surface display system, utilizes ice nucleation protein from *Pseudomonas syringae* as a membrane linker, and an IPTG-inducible T7 promoter, allowing for successful, inducible surface display of *B. burgdorferi* lipoproteins, while also recapitulating known binding interactions. This work presents a proof-of-concept application of the system with well-characterized lipoprotein CspA, testing the specific binding activity of surface-expressed CspA to its known binding target, Factor H. We plan to use E. coli *display of* B. burgdorferi surface lipoproteins to perform high-throughput screening of many uncharacterized lipoproteins to identify their binding partners.

87 Rapid Emergence of Resistance against Direct Antimicrobial Activity of Avibactam in Gram-Negative Pathogens

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Background: Avibactam is a DBO and a non-beta-lactam beta-lactamase inhibitor. Three new DBOs, zidebactam, nacubactam, and durlobactam, are currently being developed and have strong direct antimicrobial activity, through inhibition of penicillin-binding protein 2 (PBP2). Due to numerous mutational targets involving compensatory mutations that upregulate the stringent response, resistance to PBP2-targeting drugs is known to rapidly appear.

Methods: Minimal inhibitory concentrations (MIC) were assessed for 74 strains. Resistance mutation frequency was tested and persistence of resistance was monitored by serial subculture on antibiotic-free media. The fitness of avibactam-resistant cells vs. avibactam-susceptible cells was investigated using a growth fitness assay. Hybrid short- and long-read whole-genome sequencing was carried out on 16 avibactam-resistant *E. coli* isolates.

Results: Avibactam showed direct activity against most *Enterobacterales* strains (MIC50 = 16 ug/mL). Resistance frequency was high, at >10-6 at 8-16X MIC, and was maintained at $\geq$ 16X MIC for >10 days of subculture on antibiotic-free media. Avibactam-resistant cells had impaired fitness in a growth assay. Mutations in avibactam-resistant strains were diverse and displayed remarkable overlap with mutations known to cause resistance to other PBP2-targeting drugs, including mutations in tRNA synthetase inhibitors.

Conclusion: Avibactam had direct antimicrobial activity against most *Enterobacterales* isolates, and while it is less potent than newer DBOs, it may be an effective surrogate for in vitro studies. Resistance occurred rapidly, persisted in the absence of selective pressure, and displayed a mutational basis resembling that seen in other PBP2-targeting drugs.

88 Bacillus subtilis biofilm induction by diverse carbon sources

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Bacillus subtilis is a gram-positive, ubiquitous soil microorganism that promotes plant growth and is a model used to study mechanisms of biofilm formation and sporulation. Biofilm formation in *B. subtilis* is induced by the plant products pectin, xylan, and arabinogalactan. However, the mechanism of such induction, as well as the influence of other carbon sources on *B. subtilis* biofilm formation, is poorly characterized. D-gluconic acid, the oxidized form of D-glucose found in fruits, induces *B. subtilis* biofilm formation and sporulation in media that otherwise represses both processes. Expression of the secondary metabolite subtilosin is also induced by D-gluconic acid. Interestingly, subtilosin is known to respond to starvation and oxygen limitation (nitrite), and is regulated by Spo0A, the master regulator of biofilm formation in *B. subtilis*. A genetic screen of B. subtilis *identified iron homeostasis and surfactin as key intermediaries in the* B. subtilis response to D-gluconic acid. Follow-up assays identified iron, but not nitrite, as a suppressor of D-gluconic acid-induced sporulation. These data demonstrate that carbon metabolism is intricately linked to biofilm formation and sporulation in *B. subtilis*, likely via an iron-regulated mechanism.

89 Selective in vivo tagging of mycobacteria surface proteins with a cell-impermeable fluorescent substrate

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is the leading lethal infectious disease worldwide. The rise of multidrug- and extensively drug- resistant cases of TB reveal a pressing need for the development of novel drugs of increased efficacy against Mtb. While targeting biosynthesis of the mycobacterial cell wall is an established strategy in the treatment of TB, little is understood about the export and import processes within the mycobacterial cell envelope. Crucially, the mycobacterial cell surface proteome is not well elucidated. Previously, we successfully used proximity labeling by APEX2, an engineered peroxidase, to tag proteins in a compartment-specific manner. To identify proteins at the cell surface, we have genetically encoded APEX2 as a fusion to an outer membrane protein found in *M. smegmatis*, MspA. Unfortunately, labeling with

biotin-phenol, a common APEX2 substrate, reveals that the MspA-APEX2 construct is not exclusive to the cell surface but also present in the cell wall. To address this issue, cell-impermeable substrates are needed. Relying on the reactivity of isothiocyanate with primary amines, we employ 5-fluorescein isothiocyanate to synthesize the necessary phenol substrate now predicted to react only with APEX2 located at the cell surface. The development of this and other cell impermeable substrates will allow the accurate identification of mycobacterial cell surface proteins.. We expect this proteome to help elucidate export and import processes in mycobacteria and facilitate the identification of potential new drug targets.

90 Ecology and evolution of a plasmid population

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Tackling the challenge created by antibiotic resistance requires understanding the mechanisms driving its spread. Plasmids are crucial drivers of antibiotic resistance in bacteria population, as they allow the transfer of resistance genes across bacterial species and environments through conjugation, but their dynamics in large scale clinical populations remain understudied. Using a dataset consisting of all E. coli isolates collected over a year in one healthcare network, with both genotypic and phenotypic data of more than 2000 samples, we investigated its plasmids ecology and evolution. First, more than 80We then investigated the hypothesis that this effect may be due to inter-plasmid competition. As plasmids of similar incompatibility type cannot stably co-exist within a cell, a successful antibiotic sensitive plasmid could hinder the spread of resistance gene carrying plasmids of the same type. The prevalence and dynamics of resistance genes in a bacteria population may therefore be impacted not only by the selection pressure generated by antibiotic use but also by the interactions between their plasmid vectors, even the ones that do not carry resistance.

91 A Single-Cell Spatial Atlas of the Mouse Gut Reveals Spatial- and Microbiota-Dependent Sensation

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The gastrointestinal tract (GI) is a remarkably complex environment comprising a diverse array of cell types, microbes, and bioactive small molecules. To shed new light on how the gut is organized and senses its environment, we constructed a spatially resolved single-cell atlas of the mouse lower digestive tract using MERFISH with a specific focus on the thousands of receptors involved in small molecule sensation and microbial pattern recognition. We identified expected and novel cell types, charted their spatial organization across four GI regions, discovered receptor expression gradients cued by spatial location, and, overall, revealed divergent sensing across cell types, regions, and spatial contexts. In addition, we charted the remodeling of these features that occurs in the absence of the microbiome. Our atlas opens a new window into the molecular and cellular organization of gut sensation, which, given the pharmacological relevance of many receptors, may provide a clinically relevant resource.

92 Food and biomass from air, water, salt, and electricity

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To meet the demands of modern life for a growing population, we need a more energy-efficient, sustainable method to produce food and commodity products. Recent advancements have led toward the microbial production of biomass to generate feedstocks and products while using less land and water than agriculture to do so. However, the majority of the species used in these processes are still reliant on energy-intensive carbon and nitrogen sources for growth. Xanthobacter is one microbe that can take carbon dioxide, dinitrogen gas, and hydrogen gas from the air and grow autotrophically in just water and a few heavy metal salts. Thus, using Xanthobacter as a chassis, there is unique potential for the sustainable production of biomass, food, and genetically engineered commodity products. Here, we describe the establishment and improvement of a synthetic biology toolbox for the efficient genetic engineering of Xanthobacter and the use of parallel continuous evolution devices to improve its growth.

93 Mining the Pig Skin Microbiome for Antimicrobial Products

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a leading cause of infections by antibiotic resistant organisms in the US, requiring the development of new antibiotics against MRSA and other drug resistant pathogens. The skin is the most common site for MRSA infection; MRSA also colonizes the skin and nasal passages, contributing to community spread. This motivated us to consider the skin microbiome as a potential source of novel antimicrobials, as skin commensals may be enriched for competitive interactions against MRSA. We screened > 7000 bacterial isolates from pig skin, an established model system for skin, and identified 30 unique bacterial species that inhibit MRSA in vitro. We further found the novel skin commensal *Desemzia incerta* reduces MRSA colonization efficiency by 60% in a murine skin model. Furthermore, we find that *D. incerta*'santimicrobial activity can be isolated from cell free supernatant. This activity is sensitive to digestion by proteases suggesting that *D. incerta* antimicrobial properties occur through a secreted protein. Analysis of the *D. incerta* genome shows little homology to known antimicrobial biosynthetic genes clusters, suggesting that this antimicrobial molecule is novel. We developed a protein purification strategy that combines centrifugal filtration, liquid chromatography, and comparative proteomics to identify 27 candidate proteins potentially related to the observed antibiotic activity. Of particular interest is a peptidoglycan hydrolase homologue; proteins in this family have previously been suggested to have antibiotic properties. Further efforts will focus on narrowing our candidate protein list, examining the antibiotic mechanism of action, and exploring porcine models of skin colonization.

94 Innate immune responses yield tissue-specific bottlenecks that scale with pathogen dose

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To cause infection, pathogens must overcome bottlenecks imposed by barriers in the host immune system. These bottlenecks restrict the inoculum and are critical determinants of whether pathogen exposure result in infection and disease. Infection bottlenecks therefore reflect the effectiveness of immune barriers. Here, using a model of *Escherichia coli* systemic infection, we quantify bottlenecks that scale with the size of the inoculum, suggesting that the potency of innate immune responses varies with pathogen dose, a concept we term "dose scaling". We show that dose scaling is tissue specific and dependent on the LPS receptor TLR4. Furthermore, spiking the inoculum with killed bacteria can alter bottlenecks in manner predicted by scaling behavior. Therefore, dose scaling in this model is due to sensing of pathogen molecules rather than interactions between host and live bacteria. We propose that dose scaling quantitatively links innate immunity with infection bottlenecks and is an important framework for understanding how dose governs the outcome of pathogen exposure.

95 The Microbiota-derived Metabolite TDCA May Contribute to the Pathogenesis of Pediatric Celiac Disease

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Celiac Disease (CD) is an autoimmune condition defined by small intestinal inflammation that leads to villous atrophy in genetically predisposed individuals. Those who develop celiac disease are predisposed by the presence of the HLA-DQ2 *or* HLA-DQ8 haplotypes. 20-40% of the general population possess these alleles, however only 1-2% of the population develops celiac disease. This indicates that genetics alone are not responsible for gluten intolerance and that there are additional environmental factors at play in the pathogenesis of the disease, such as the gut microbiome. Recently, we conducted a prospective cohort study using fecal and plasma samples obtained from children at ages 2.5 and 5 years, before the onset of celiac disease (CD progressors). We found that CD progressors have an altered gut microbiome, plasma metabolome and cytokine profile years before diagnosis. In addition, IgA sequencing determined that these children have an increased IgA response and identified unique

microbial targets of IgA in the gut. One metabolite found to have increased two-fold in CD progressors is taurodeoxycholic acid (TDCA), a secondary bile acid that is produced by some gut microbes. Treating C57BL/B6 mice with TDCA caused the development of villous atrophy in the small intestine as well as upregulated CD4+ T-cells in intraepithelial lymphocytes (IELs) and natural killer cells in the lamina propria (LP). TDCA treatment upregulated the expression of the immunoregulatory proteins Qa-1 and NKG2D on T-cells. Thus, the microbiota-derived metabolite TDCA contributes to intestinal inflammation and may contribute to the pathogenesis of CD.

96 Granuloma-specific design of drug combinations for tuberculosis treatment

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Infection with *Mycobacterium tuberculosis* results in multiple types of granulomas. The granuloma environments and structural differences determine response to drug treatment, and different environments elicit varied levels of drug tolerance. Therefore, multidrug therapy is needed to target bacteria residing in different granulomas. To optimize drug combinations, granuloma-specific responses to drug combinations must be considered early in drug regimen development. It is logistically impossible to screen every drug combination using animal models; instead, we propose to improve a platform that uses computational tools to model *in vivo* responses based on *in vitro* measurement of drug combination responses. We have developed three complex *in vitro* models for drug combination screens to capture the drug-tolerant bacterial states induced in the host and incorporate three granuloma-specific drug access scenarios in our experimental design. Using these models, we have measured bactericidal dose response to >100 drug combinations. Granuloma-specific outcomes from drug treatment experiments in marmosets inform how different drug treatments target the various lesion types *in vivo*. Using this ground truth, we are constructing mathematical models using our *in vitro* data to learn which bacterial states drive different responses to treatment in different lesion types and, therefore, which bacterial states and locations must be modeled to design the best treatment regimens that will target the bacteria most difficult to access and kill. By leveraging these *in vitro* and computational tools, we aim to establish *in vitro* screening methods to model different drug-tolerant bacterial subpopulations and to prioritize treatment-shortening combinations for *in vivo* testing.

97 Dissecting the genetic networks underlying host subversion during Rickettsia infection

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Obligate intracellular bacterial pathogens have evolved sophisticated, tailored mechanisms to sense and manipulate the host cell environment that they depend on for survival. However, we lack many of the necessary tools to study them at a mechanistic level, leaving many fundamental questions about their unique biology and pathogenesis unanswered. One striking example is the obligate intracellular bacterial pathogen *Rickettsia parkeri*, which is remarkable for its ability to invade, reside within, and directly spread between eukaryotic host cells in ways that are distinct from other bacterial pathogens. To gain insights into the genetic networks that underlie the rickettsial intracellular life cycle, we have developed a new RNA sequencing pipeline to precisely measure gene expression during infection. Using this RNA-seq pipeline, we have profiled transcriptional changes in *R. parkeri* across human cell infection. Notably, we observed differential expression of more than half of rickettsial coding sequences over 48 hours of infection. Also, we have used RNA-seq to characterize novel regulators of rickettsial gene expression, including uncovering a potential gene regulatory pathway that promotes host cell invasion. Further development of tools to complement and build upon the insights gained from the RNA-seq pipeline is currently underway. Together, these experiments have great potential to advance our understanding of bacterial gene regulatory mechanisms and host-pathogen interactions.

98 Continuing Evolution in Mycobacterium tuberculosis result in Evasion of Molecular Diagnostics but provide prognostic markers for Isoniazid (INH) Resistance

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Tuberculosis (TB) causes nearly 1.5 million deaths annually. Molecular Diagnostics (mDx) is rapidly becoming an integral component of global TB control. Uncommon mechanisms of resistance escape detection and undermine outbreak containment efforts. This is a systematic review of published articles reporting INH-resistance-conferring mutations from 2013 through 2019. Fifty-two articles were included describing 9,306 clinical isolates (5,804 INHR, 3,502 INHS) from 31 countries. The three most frequently mutated loci continue to be katG315 (4,271), inhA-15 (787), and inhA-8 (106). However, the diagnostic value of inhA-8 is far lower than previously thought, only appearing in 25 (0.4

99 Investigating the role of epithelium-associated populations of Clostridioides difficile as a determinant of disease severity

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Clostridioides difficile is an anaerobic, spore-forming pathogen that is the most common cause of healthcare-associated infections in the United States. *C. difficile* is transmitted by spores through the fecal-oral route, which, upon reaching the colon germinate into metabolically active, toxin-producing vegetative cells. Surprisingly, despite toxins being essential for causing damage to the host, *C. difficile* toxin levels and biomass do not correlate with disease severity in a murine infection model. Previous work indicates that *C. difficile* exists in two subpopulations during murine infection, with most found in the gut lumen and a subset located near the colonic epithelium. Recent analyses suggest that this epithelium-associated sub-population may be the primary driver of disease severity and thus explain the lack of correlation between toxin levels and biomass in feces and clinical outcomes. However, what roles these epithelium-associated populations play in modulating disease severity during *C. difficile* infection remains unclear. My work focuses on testing the hypothesis that the spatial distribution of *C. difficile* within the colon is a key factor in determining disease severity. To address this hypothesis, I have constructed fluorescent reporter strains to visualize the spatial distribution of different *C. difficile* subpopulations and toxin gene expression within colonic microenvironments in a murine infection model. Additionally, to identify genetic factors in *C. difficile* that determine disease severity, I plan to investigate the roles of various candidate genes that have been implicated in driving *C. difficile* association with the gut epithelium. Completion of this work will provide critical information on the role of epithelium-associated sub-populations of *C. difficile* in modulating disease severity and the genetic factors that influence this spatial distribution.

100 Rv3802c function in mycobacterial cell envelope remodeling, barrier properties, and antibiotic susceptibility

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A quarter of the world's population is infected with Mycobacterium tuberculosis, the causative agent of tuberculosis (TB). Mycobacteria are intrinsically resistant to many common antibiotics, partly due to the unique structure and composition of the mycobacterial cell envelope. The mycobacterial cell envelope contains more layers than other bacterial cell envelopes, including an outer member composed mainly of essential, large, hydrophobic lipids called mycolic acids (MAs). MAs are thought to grant the mycomembrane with exceptionally low fluidity, and the cell envelope has low permeability to many solutes, including antibiotics. To fight TB, we need therapeutic strategies that overcome the unique barrier properties of the mycobacterial cell envelope. However, a major gap in knowledge has slowed development of effective antibiotics: We lack a systematic understanding of how the functions of cell envelope proteins affect cell envelope permeability, membrane fluidity, and importantly, resistance to different antibiotics. I plan to address this gap in knowledge by using Rv3802c – an essential cell envelope protein – as an archetype to study the connection between biological function, membrane properties, and intrinsic antibiotic resistance. I hypothesize that Rv3802c remodels the cell envelope to allow for the deposition of new cell envelope material, and that depletion of Rv3802c will decrease membrane fluidity and permeability to solutes with shared molecular properties. I will (1) characterize the biological function of Rv3802c and (2) determine Rv3802c's role in membrane barrier properties and antibiotic resistance. Ultimately, this work will help pave the way for the rational design of novel antibiotics and synergistic drug combinations.

101 Investigating the interplay between serine/threonine protein kinases and two-component systems in Mycobacterium tuberculosis environmental response

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Successful growth and host colonization by *Mycobacterium tuberculosis* (Mtb) requires appropriate bacterial response and adaptation to the variety of signals it encounters throughout infection, including pH, chloride (Cl-), nitric oxide (NO), and hypoxia. To respond to these environmental signals, Mtb utilizes signal transduction regulatory mechanisms such as serine/threonine protein kinases (STPKs) and two-component systems (TCSs). Mtb possesses similar numbers of STPKs (11) and TCSs (12), but if and how these two regulatory systems work together to enable Mtb environmental adaptation remains poorly understood. By generating site-directed mutations at putative STPK phosphorylation sites on key response regulators, we have discovered that STPK phosphorylation of TCSs broadly affects Mtb growth and environmental response. Strikingly for example, a STPK phosphoablative point mutant of DosR, the response regulator that is part of the hypoxia and NO/hypoxia-responsive TCS DosRS, fails to induce DosR regulon genes upon Mtb exposure to NO. Mycobacterial protein fragment complementation assays reveal specific STPK-TCS interactions, with a subset of STPKs exhibiting interactions with multiple TCS response regulators. While the importance of STPKs and TCSs on Mtb environmental response have been independently recognized, our results provide evidence for the importance of STPK regulation on TCS function and Mtb adaptation. Current work is focused on understanding: (i) the mechanism(s) underlying STPK regulation of TCS activity, and (ii) how STPK regulation of TCSs may serve to coordinate activity of multiple TCS systems, enabling integration of Mtb response to different environmental cues.

102 DNA methylation promotes Clostridioides difficile sporulation by enhancing transcription of a gene encoding the cell fate determinant SpoIIE

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Many bacteria use DNA methylation as a mechanism to epigenetically regulate diverse cellular processes such as chromosome replication, DNA repair, and transcriptional activation. This post-replicative modification can result in phase-variable gene expression, leading to phenotypic cell variants with distinct functions or cellular fates. While recent advances in methylome sequencing have revealed that DNA methylation is ubiquitous in bacteria, the exact mechanism by which it epigenetically regulates phenotypic heterogeneity remains unclear in most cases. We recently determined that methylation by the *Clostridioides difficile*-specific DNA methyltransferase CamA promotes spore formation. Since the formation of aerotolerant spores is critical for this enteric pathogen and obligate anaerobe to transmit disease, we sought to elucidate the mechanism by which DNA methylation enhances sporulation. Using transcriptional reporters, RNA-Seq, and qRT-PCR, we show that CamA promotes the transcription of *spoIIE*, which encodes a regulator of asymmetric division that also functions to activate the earliest acting sporulation-specific sigma factor σF. Notably, the inactivation of a single CamA methylation site in *spoIIE*'s promoter region reduces both *spoIIE* expression and the frequency of sporulation. Since the methylation site overlaps with the binding site of the master transcriptional regulator Spo0A, which directly activates *spoIIE* transcription, we propose a model in which DNA methylation increases the binding affinity of Spo0A for the *spoIIE* promoter. Given that SpoIIE activation commits *B. subtilis* cells to completing sporulation, our data suggest a mechanism by which CamA-mediated methylation regulates cell fate and enhances disease transmission.

103 Lon protease is important for the adaptation to low oxygen and the regulation of heme biosynthesis in Caulobacter crescentus

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The FixLJ pathway in Caulobacter crescentus is involved in regulating gene expression in response to changes in oxygen levels. The FixLJ two-component response system comprises two feedback regulators: FixK and FixT. FixK, by turning on the expression of the genes encoding FixL and FixJ, functions as the positive feedback regulator. In turn, FixK also activates the transcription of the *fixT* gene by binding to its promoter region, resulting in the production of the FixT protein. The accumulation of FixT protein leads to inhibition of the autophos-

phorylation of FixL, establishing a negative feedback loop that enables the system to shut down in response to elevated FixT levels. Interestingly, the activity of FixT is also regulated by the Lon protease, which degrades FixT in the presence of excess oxygen. This degradation is prevented under anaerobic conditions, allowing FixT to accumulate.

To understand the relationship between FixT and the Lon protease, we generated strains lacking fixT in both wild type and Δ lon background. Based on our results, the overabundance of FixT caused by the loss of Lon protease results in decreased heme biosynthesis and red pigmentation and deletion of *fixT* rescues this effect. To address whether this suppression extends to other Δ *lon* phenotypes we first investigated the effects of *fixT* deletion on cell growth. As expected, we saw that cells lacking Lon accumulate less cell mass upon entry to stationary phase which was not restored upon deletion of *fixT* and in fact, we found substantially reduced fitness of Δ *lon* Δ *fixT* compared to Δ *lon*. Consistent with the RNA-seq results, we also found that *fixK* promoter activity is two-fold lower in Δ *lon* cells compared to wild-type in aerobic conditions. Deletion of *fixT* increased PfixK activity three-fold in wildtype strains and six-fold in Δ *lon* strains, resulting in the same higher steady-state promoter activity for both strains. In anaerobic conditions, *fixK* expression increases as expected and the difference in expression between wild-type and Δ *lon* cells is less. Similar to the aerobic condition, loss of *fixT* results in loss of differences between wild-type and Δ *lon* cells. We conclude that the ability of FixT to inhibit the FixLJ pathway and downregulate *fixK* expression is retained even in the presence of oxygen and is lon-dependent. Conversely, the effect role of Lon protease in the regulation of *fixK* expression is diminished when the oxygen levels are depleted.

104 Rapid evolution to serum resistance through phase variation in Neisseria gonorrhoeae

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Neisseria gonorrhoeae, the causative agent of gonorrhea, has evolved to evade killing by complement, but the degree of complement resistance varies among clinical isolates. While some genetic contributors to serum resistance have been identified, our understanding of the genetic basis of complement resistance in *N. gonorrhoeae* is incomplete, hindered by the involvement of repetitive and phase-variable loci in complement resistance. Using conditional genome-wide association studies (GWAS), we evaluated genetic contributors to complement resistance in a panel of 157 diverse *N. gonorrhoeae* strains and performed in vitro evolution in normal human serum for nine genetically diverse complement-sensitive *N. gonorrhoeae* clinical isolates. We then analyzed the genomes and transcriptomic profiles of resistant strains, including long-read genome sequencing to resolve phase-variable loci, to determine the mechanisms of complement resistance that emerged. GWAS revealed the major porin *porB* as the main contributor to complement resistance, in agreement with previous reports. In vitro evolution experiments found that phase variation of *opa* genes, a family of 9-11 homologous genes per gonococcal genome, are associated with complement resistance. Loss of all Opa proteins was previously known to reduce complement resistance, but the long-read sequencing in this work allowed us to determine that complement resistance is associated with expression of specific *opa* paralogs. We also observed that evolved complement resistance varied in different batches of pooled human serum, suggesting that the efficacy of this resistance mechanism varies with antibody repertoire. Future work will focus on mechanistically exploring the role of different Opa proteins in resistance to human complement.

105 FtsA-FtsN direct interactions support assembly of translateral divisome complexes de novo

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In Escherichia coli, the protein FtsZ promotes the formation of a division septum at the cell center and assembles into a large protein structure called the Z-ring. Coordination between FtsZ in the ring and other division proteins such as FtsA, which anchors FtsZ to the cytoplasmic membrane and FtsN, a bitopic membrane protein ending in a peptidoglycan binding SPOR (sporulation-related repeat) domain, direct peptidoglycan (PG) synthesis. Interactions between FtsA and FtsN may play a role in initiating septal PG synthesis. Gfp fusion proteins containing full length FtsN, or the cytoplasmic domain (1-32), localize to midcell. However, a substitution mutation in FtsN, D5N, mapping to a putative FtsA-interaction site, impairs localization of Gfp-FtsN1-32, but not Gfp-FtsN. To investigate the FtsA-FtsN interaction further, we monitored direct interactions in a bacterial two-hybrid assay. We observed that both FtsN and FtsN(D5N) strongly interact with FtsA. Substitution mutations in FtsA also fail to disrupt the direct interaction. Since FtsA and FtsN interact in vivo, we purified both proteins to test if we could assemble stable complexes in vitro. We inserted FtsN into phospholipid vesicles and observed recruitment of a truncated variant of FtsA, FtsA(Δ MTS), which was enhanced with ATP. We also

tested if FtsN binds to peptidoglycan via the SPOR domain. We observed that both FtsN and FtsN(D5N) recruit FtsA(Δ MTS) to peptidoglycan. Peptidoglycan-bound FtsN-FtsA complexes also recruit FtsZ. Assembly of this translateral complex will allow us to evaluate stoichiometry, architecture, regions of interaction, and additional division proteins to further understand the biochemical mechanism of division.

106 Uncovering novel regulators of the Mycobacterium tuberculosis nitric oxide (NO) response

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During infection, Mycobacterium tuberculosis (Mtb) encounters heterogeneous environmental signals, including nitric oxide (NO), iron limitation, and hypoxia. The NO/hypoxia responses of Mtb have been studied predominantly in the context of their co-regulation, as both signals drive Mtb into an adaptive non-replicating state mediated by the DosRS(T) two-component system. However, Mtb also encounters NO independently of hypoxia, provoking widespread gene expression changes beyond that which overlaps with the hypoxia response. It is therefore likely that there are as yet unidentified regulators of the Mtb NO response, and thus our understanding of the regulatory network underlying Mtb adaptation to NO stress remains incomplete. Here, using a fluorescent reporter Mtb strain responsive to NO but not hypoxia, we find that Mtb mounts an additive transcriptional response to the simultaneous presence of NO and iron limitation. With a luciferase version of this reporter, we have carried out a transcription factor (TF) overexpression screen to identify novel regulators of the Mtb NO response, distinct from the well-characterized DosRS(T) pathway. Preliminary results have revealed several TFs whose overexpression increased reporter luminescence upon NO exposure above levels observed with an empty vector control, supporting the existence of novel NO response regulators. Current work is focused on validating these hits and characterizing the functional role of these TFs in the response of Mtb to NO, hypoxia, and iron limitation, and in host colonization. We anticipate that these studies will reveal new connections between the NO response and other aspects of Mtb cell biology, beyond the hypoxia response.

107 An Alternative Model of Error Prone Polymerase Recruitment in Post-replicative DNA repair

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DNA damage can significantly disrupt the protein machinery which carries out high-fidelity replication. Cells utilize error prone polymerases to tolerate this damage. Often, in *E. coli*, repriming of replication occurs downstream of DNA lesions, leaving long stretches of ssDNA. Pol V, a highly mutagenic Y-family DNA polymerase, is subsequently employed to fill in these gaps. Understanding the basis for Pol V-mediated mutagenesis has implications for antimicrobial resistance and drug treatment for bacterial pathogens, including the causative agent of tuberculosis. The prevailing mechanistic understanding posits that the RecA recombinase mediates both localization and activation of Pol V. Based on prior research suggesting a key role for ssDNA binding protein (SSB) in the bacterial damage response, we propose that SSB mediates localization of Pol V to the gap, enabling RecA-mediated activation. To study this, we identified novel Pol V separation-of-function mutations. Through genetic, biochemical, and imaging analysis of these mutants, we aim to parse out the mechanisms of recruitment and activation of this polymerase, which contributes to the emergence of antimicrobial resistance.

108 A gene in the integrative and conjugative element ICEBs1 confers phage defense in Bacillus subtilis

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Many bacteria harbor genes that provide protection (defense) against predation by bacteriophages. These phage defense genes are often found in mobile genetic elements, including integrative and conjugative elements (ICEs). The integrative and conjugative element ICE*Bs1* in *Bacillus subtilis* harbors a phage defense gene, *spbK*, that prevents the spread of the temperate phage SPβ of *B. subtilis* to other cells. SpbK works through an abortive infection mechanism, wherein SpbK-mediated cell death prevents the production of viable phage particles. SpbK-mediated cell death is activated by expression of the putative phage portal protein YonE of SPβ. The SpbK protein contains a Toll-interleukin-1 receptor (TIR) domain. Consistent with the known activity of some TIR-domain-containing proteins, we found that SpbK causes degradation of NAD+ in cells and this degradation is stimulated

by YonE. Thus, SpbK acts as an NADase, degrading NAD+ and causing cell death prior to the production of progeny phage. Experiments are underway to determine the molecular interactions and functions between SpbK and YonE during the activation of SpbK-mediated killing. This work will provide a deeper understanding of the mechanisms that host bacteria employ to prevent the spread of phage by recognizing specific phage products to activate defense mechanisms.

109 Bilayer-Intrinsic and -extrinsic structure to separate mycobacterial plasma membrane into domains

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Lateral partitioning of proteins and lipids shapes membrane function. In model membranes, partitioning can be influenced by lipid composition and interactions with other membranes, but the mechanism by which bacteria partition their membrane is largely unknown. The plasma membrane of *Mycobacterium smegmatis* can be spatially and biochemically partitioned into intracellular membrane domains (IMD) and the conventional plasma membrane associating with cell wall (PM-CW). IMD and PM-CW were mixed by the fluidizing agents such as benzyl alcohol and dibucaine. By characterizing the membrane separation upon fluidizer treatment and removal, we established the reversible membrane-departitioning model to study *de novo* membrane partitioning *in vivo*. By investigating mutants that were not able to recover from fluidizer treatments, we showed that PonA2 and Cfa promoted membrane partitioning and cell growth of *M. smegmatis*. PonA2 is a bifunctional cell wall synthase and Cfa is a methyltransferase for the synthesis of major membrane phospholipids containing a C19:0 monomethyl-branched stearic acid. Those data suggest that membrane partitioning of *M. smegmatis* relies on its phospholipids composition and bilayer-extrinsic structures. We also identified the outer membrane (also called the mycomembrane) synthase FbpA as a factor that enables *M. smegmatis* to withstand membrane fluidization. Together with our published data, these preliminary results support a model in which intrinsic and extrinsic membrane components separate the plasma membrane into functional membrane domains.

110 DNA damage as an in vivo mechanism of action of pretomanid in Mycobacterium tuberculosis

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Mycobacterium tuberculosis (Mtb) is responsible for more than one million deaths annually, and Mtb treatment is complicated by the growing incidence of multi-drug (MDR) and extensively-drug resistant (XDR) Mtb. Pretomanid is a new antibiotic recently approved to treat non-responsive MDR- and XDR-Mtb infections. Pretomanid is a prodrug that is converted by an Mtb enzyme into reactive metabolites, reactive nitrogen species and nitric oxide (NO). The pleiotropic effects of such a reactive drug have precluded the elucidation of pretomanid's mechanism of action. Further, Mtb resides predominantly within the phagolysosome of alveolar macrophages, an environment abundant in reactive metabolites that may alter pretomanid's effects. We performed a genome-wide screen in a mouse model of Mtb infection to identify genes required upon pretomanid treatment. Unexpectedly, each member of the nucleotide excision DNA repair (NER) pathway was required for Mtb survival in pretomanid-treated mice, suggesting that pretomanid may damage Mtb DNA. The central NER pathway member, *uvrB*, was essential for Mtb to survive pretomanid treatment when residing within macrophages, but not under *in vitro* culturing conditions. *In vitro* addition of exogenous nitric oxide (NO) to imitate the NO produced by macrophages synergized with pretomanid in *uvrB* knockouts, suggesting that NO production within the macrophage is responsible for the *in vivo* requirement for DNA repair. In support of this model, we found that pretomanid treatment causes nitrosative DNA damage in a *uvrB*-dependent manner. Together, these results suggest that pretomanid's *in vivo* mechanism of action involves damaging Mtb DNA, and that the NER pathway is critical for Mtb survival under pretomanid treatment.

111 Exploring the mechanisms of collateral sensitivity to evolved Gentamicin resistance in Klebsiella pneumoniae

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Klebsiella pneumoniae is a Gram-negative, opportunistic pathogen that causes urinary tract infections, pneumonias, and septicemia, especially in hospitalized and immunocompromised patients. *K. pneumoniae* frequently carries antibiotic resistance plasmids and multidrug-resistant strains including Extended-spectrum Beta Lactamases (ESBL) or Carbapenemase (CRE) producing strains are considered urgent public health threats worldwide. With a lack of new drugs in the antibiotic pipeline, combination therapies repurposing existing drugs can limit the emergence and spread of additional antibiotic resistance. Identifying effective combinations requires understanding the underlying mechanisms; we are particularly interested in mechanisms of collateral sensitivity-where resistance to one drug increases susceptibility to another-which may underpin these combinations. Using a panel of lab strain, drug-sensitive *K. pneumoniae* that have been evolved to be resistant to high levels of gentamicin, we have identified mutants that have increased resistance to gentamicin but show increased susceptibility to other clinically-relevant antibiotics including antimicrobial peptide colistin and a variety of cephalosporins. We successfully isolated mutants with different physiological and resistance phenotypes which will allow us to explore multiple mechanisms behind effective combination therapies. Whole genome sequencing has identified mutations in multiple metabolic, stress response, and protein synthesis pathways that may be involved in these phenotypes. These pathways are not well-studied in *K. pneumoniae* and will provide mechanistic insight into how common antibiotic resistance mechanisms can be exploited to design effective combination therapies.

112 Analysis of molecular mechanisms underlying concentration-dependent positive effects of the antibiotic lincomycin on Streptomyces coelicolor A3(2)

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Antibiotics are originally defined as chemical compounds produced by a microorganism that inhibit the growth of others. Meanwhile, antibiotics can exert concentration-dependent positive effects on the growth and secondary metabolism of bacteria. The ribosome-targeting antibiotic lincomycin has the effect of secondary metabolic activation in the model actinomycete *Streptomyces coelicolor* A3(2) in a concentration-dependent manner, as opposed to its translational inhibitory effects 1). Here, the dynamic modification of ribosomes in the presence of lincomycin was investigated, and the underlying ribosomal modification factors were explored to better understand the mechanistic basis for such concentration-dependent positive effects of the antibiotic lincomycin on *S. coelicolor* A3(2). *S. coelicolor* A3(2) cells were grown with lincomycin at a concentration that activates secondary metabolism, and total RNA and proteins were subjected to transcriptional and ribosome profiling analyses, respectively. Our results indicate that lincomycin stimulates the expression of various genes under the control of the WblC transcription factor, involved in lincomycin resistance and translation, to rescue ribosome activity stalled by lincomycin. Ribosome profiling analysis revealed that the reconstructed ribosomes were dynamic and stable even in the late growth phase. Positive alterations to the ribosome, both in terms of quality and quantity, can be beneficial for translating secondary metabolite biosynthetic enzymes. Interestingly, proteasome disassembly was enhanced in the presence of lincomycin, reducing proteasomal proteolytic activity. Although further analysis is necessary, these findings suggest that reduced proteasome activity may contribute to ribosome stabilization in the presence of lincomycin. 1) Yu Imai et al. Appl and Environ Microbiol, 2015

113 Investigating Biosynthetic Gene Cluster Activation in Bacilli

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Microbial specialized metabolites are structurally complex natural products that have been a rich source of therapeutics, including antibiotics, for decades. These diverse molecules are often employed in bacterial signaling and provide microbes with a selective advantage in competitive environments. The machinery required to man-

ufacture specialized metabolites is encoded by biosynthetic gene clusters (BGCs). Most BGCs are 'cryptic', or not expressed, under standard laboratory conditions, leaving a vast repertoire of untapped biosynthetic potential. The objective of this work is to exploit bacterial coculture to identify conditions that activate cryptic BGCs in *Bacilli*, a biosynthetically underexplored genus. To achieve this goal, I will use a combinatorial approach leveraging bioinformatics (to prioritize *Bacilli* with high biosynthetic potential), transcriptomics (to determine which BGCs are cryptic in monoculture and activated and thus expressed in coculture), and metabolomics (to investigate metabolites that may act as activators of cryptic BGCs). I will also cultivate strains under a variety of conditions to activate cryptic BGCs that may be resource dependent. To identify bioactive compounds, strains with high antagonism towards other bacteria (termed "super-antagonizers") will be cultivated in coculture to elicit specialized metabolite production. I will use bioassay-guided fractionation of these microbial extracts to identify bioactive metabolites from super-antagonizer cocultures and liquid chromatography-mass spectrometry(MS)/MS to identify coculture-specific metabolites that appear to be uncharacterized. If unknown bioactive metabolites are identified, I will work to elucidate their chemical structures using MS and nuclear magnetic resonance (NMR). Together, this work will uncover methods to illicit antibiotic production from previously untapped sources.

114 Gene silencing through CRISPR interference in Borrelia burgdorferi

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Borrelia burgdorferi, the causative agent of Lyme disease, is the most prevalent tick-borne pathogen threatening human health in the Eastern United States. The megabase-sized complex *Borrelia* genome (1.5M base pair) includes a linear chromosome DNA, twelve linear plasmids and nine circular plasmids from representative B31 strain. Many putative encoded proteins have no known BLAST homology to annotated proteins, complicating the study of *Borrelia* genomics. As a result, the gene function of large parts of the *B. burgdorferi* genome remain underexplored. To address this, we adapted CRISPR interference (CRISPRi) technology using a recently-reported system with an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible dcas9 to silence the chromosomally-located flagellin gene (*flaB*) and a plasmid-borne green fluorescence protein gene (GFP) in *Borrelia* B31e2 strain. We measured expression levels of *flaB*, *gfp*, and other targeted transcripts. The *B. burgdorferi* flagellum confers both the bacterium's characteristic spiral shape and motility and silencing of *flaB* resulted in morphologically straight *Borrelia* with impaired mobility. We quantified reductions in *flaB* mRNA transcript levels and FlaB protein levels. Silencing of *gfp* showed the loss or decrease of green fluorescence in *B. burgdorferi* by fluorescence microscopy. In summary, we have adapted a recently reported CRISPR interference (CRISPRi) technology for use in our laboratory and demonstrated its utility for programmable gene silencing of both the chromosome genes and plasmid genes in *Borrelia*. This CRISPRi platform will facilitate the construction of B. burgdorferi *CRISPRi lipoprotein libraries and accelerate the understanding on* Borrelia lipoprotein function in pathogen-host interaction such as adherence, invasion, and immune evasion.

115 Bacterial genomic profiling reveals novel routes to complement evasion and targeted therapeutic strategies

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Complement activation is a critical first line of defense against bacterial pathogens. Individuals with complement deficiencies are more sensitive to invasive infections by gram-positive pathogens such as *Streptococcus pneumoniae*. During this process, bacteria are first opsonized, or marked, for phagocytosis by the major complement protein C3 which triggers recognition and engulfment by the neutrophils recruited to the site of infection. Activation of the innate receptors on the surface of neutrophils initiates antimicrobial mechanisms, ultimately leading to the destruction of the bacterial pathogen. *S. pneumoniae* has numerous mechanisms to avoid C3 complement deposition; however, we currently possess a limited understanding of them. Combining an in vitro C3 deposition assay, FACS selection, and Tn-Seq, we developed a robust method to obtain a high-resolution, genome-wide understanding of the surface proteins and associated molecular pathways used by *S. pneumoniae* to avoid complement deposition. Our data show that *S. pneumoniae* strain TIGR4 has multiple uncharacterized gene products that impede C3 deposition on the bacterial cell surface beyond the well-studied polysaccharide capsule, choline-binding proteins, and surface exoglycosidases. In vivo protection studies demonstrate that directed antibodies against two identified proteins reduce the bacterial burden to non-lethal levels. In conclusion,

we identified and validated newly identified *S. pneumoniae* surface proteins involved in complement evasion, and we demonstrated these proteins are suitable targets for developing targeted antimicrobial therapies.

116 A Versatile Plasmid-based System for Continuous Evolution in Bacterial Hosts

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Enzyme directed evolution is a foundational strategy for advancements in medicine, agriculture, and sustainability. However, most directed evolution campaigns rely on generation of *in vitro* DNA libraries, which are limited in sequence exploration and by transformation efficiency into various bacterial species. We have developed MutaT7, a plasmid-encoded inducible chimeric protein that produces targeted mutations in a gene of interest, thereby enabling *in vivo* evolution. We have tested this system in *E. coli* for evolution of several proteins, uncovering targeted mutations that enable antibiotic resistance and enhance enzyme kinetics. Thus, when paired with a stringent selection couple, MutaT7-based evolution is a powerful tool for continuous *in vivo* evolution of target proteins. We plan to expand this system to biotechnologically-useful bacterial species with low transformation efficiencies, such as *Cupriavidus necator*.

117 Streptococcus pneumoniae transcription factor fitness quantification using transcriptional regulator-induced phenotype screens

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Streptococcus pneumoniae is a Gram-positive, facultative pathogen responsible for infecting two million people in the United States and killing 300,000 children globally each year. *S. pneumoniae* is capable of migrating to secondary locations throughout the body leading to a number of Invasive Pneumococcal Diseases (IPDs) in the lungs, brain, ear, eye, blood and bone. Additionally, *S. pneumoniae* is becoming increasingly antibiotic-resistant, with more than 30% of its infections resistant to one or more clinically relevant antibiotics. In order to survive in vastly different chemical environments, *S. pneumoniae* must be constantly regulating its gene expression. Here, we aim to use transcriptional regulator-induced phenotype (TRIP) screens, as a high-throughput technique, to quantify the fitness of every transcription factor simultaneously, under a variety of conditions. We constructed and cloned Transcription Factor Induction (TFI) strains for each transcription factor in the *S. pneumoniae* strain TIGR4, downstream of an IPTG-inducible promoter with an HA-tag and a unique DNA barcode. Then, we pooled all TFI strains together and induced TF overexpression in many different conditions, including a variety of antibiotic and nutritional stresses. This TRIP screen allow us to simultaneously determine the fitness of each TFI strain via barcode sequencing. The different TRIP screen combinations are used to identify the most phenotypically-important TFs in *S. pneumoniae* infections and antibiotic response. These results reveal, for the first time in this pathogen, TF involvement under specific stresses.

118 Development of a Culture-Free Diagnostic for Urosepsis Leveraging Whole Genome Sequencing and Machine Learning

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Complicated urinary tract infections (cUTIs) can be life-threatening and occur most often in hospitalized patients. There are 2.8 million cases of cUTIs in the U.S. each year, with over 20% of cases progressing to urosepsis, causing nearly 150,000 deaths annually. Effective clinical management of cUTIs requires rapid identification (ID) of causative pathogens and reliable antibiotic susceptibility tests. Urine culture, the current gold-standard, exhausts vital turn-around time from specimen collection to actionable information for treatment. Day Zero Diagnostics (DZD) developed DZD-UroSeq to address the need for a culture-free diagnostic, leveraging ultra-high enrichment of pathogen DNA, whole-genome sequencing (WGS), and machine learning to deliver high resolution species ID with antimicrobial resistance and susceptibility profiling from patient urine in under 6 hours. A total of 7 clinically relevant pathogen species found to cause cUTIs and urosepsis were assessed using

DZD-UroSeq. To model infection, urine was contrived with white blood cells and bacteria at 10^5 CFU/mL. We report over 99.99% enrichment of bacterial DNA for 7/7 target species in contrived samples. Coupling WGS with our Keynome® ID algorithm for pathogen ID, we achieve a total accuracy of 99%. Using our Keynome® gAST method, specialized machine learning to detect determinants of antimicrobial resistance or susceptibility and report a genomic assessment of antibiotic sensitivity, DZD-UroSeq averaged greater than 90% accuracy of prediction models for 4 bacterial species across 27 bug/drug combinations. Preliminary data demonstrates that within 6 hours DZD-UroSeq delivers accurate pathogen ID and gAST directly from urine samples.

119 Development of the First Inducible CRISPRi System in Rickettsia to Knockdown Gene Expression

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Rickettsia parkeri is an obligate intracellular bacterium transmitted to humans by arthropod vectors and causes a mild vascular disease. Due to its unique niche inside a host cell, it has been difficult to decipher how rickettsia regulate transmission and pathogenicity. Understanding the complex, underlying genetics that govern rickettsia-host interactions has been limited due to the lack of available tools that enable targeted manipulation of rickettsial genes. To overcome this challenge, we are developing an inducible-CRISPRi system compatible with *Rickettsia parkeri*. We have screened four different dCas9s and demonstrated for the first time that all four dCas9s were expressed upon the addition of the inducer anhydrotetracycline. Furthermore, we have used these systems to knockdown the expression of genes encoding rifampicin resistance and the virulence gene sca2 to demonstrate the system's efficacy. The development of a CRISPRi system expands the repertoire of needed genetic tools so that we can better understand how rickettsia infect eukaryotic cells to cause disease.

120 Inhibition of NE release as a novel therapeutic target to prevent systemic disease during S. pneumoniae infection

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Streptococcus pneumoniae (SPN) is the leading cause of community-acquired pneumonia. From the lungs it can spread to the blood to cause bacteremia. This bacterial translocation is associated with a three-fold increase in mortality rate. A virulence factor proposed to promote bacterial escape from the lung is pneumolysin (PLY). PLY triggers the epithelial production of Hepoxilin A3 (HXA3), a potent PMN chemoattractant. HXA3 has been shown to disrupt epithelial barriers and promote bacterial translocation by mediating PMN infiltration to the lung. Previous work in the lab showed that activation of PMNs by HXA3 increased neutrophil elastase (NE) release from azurophilic granules in response to *S. pneumoniae*. Given the broad substrate specificity of the enzyme, we hypothesized that NE release facilitates epithelial barrier disruption and bacterial escape from the lungs. We tested this hypothesis in an *in vitro model* that mimics key aspects of respiratory epithelium. We seeded isolated mouse basal stem cells on Transwell filters using an air-liquid interface (ALI) to obtain fully differentiated lung epithelium. To model the disease, we infected the apical side of the monolayer and introduced PMNs to the basolateral compartment. To target the transmigrating PMNs, we added to the apical side the pharmacological inhibitor Nexinhibit20 (Nex), which inhibits exocytosis of azurophilic granules. Nex treatment protected the epithelial barrier; Strep-induced barrier disruption was diminished 2-fold and bacterial translocation showed a 1-log reduction. These results indicate that pharmacological inhibition of NE release may serve as a novel therapeutic target to prevent systemic disease during S. pneumoniae infection.

121 Engineering Synthetic Biotics to Secrete Therapeutic Proteins and Small Molecules for the Treatment of Intestinal Inflammation

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Synthetic Biotic medicines are live, non-pathogenic E. coli Nissle (EcN) bacteria designed with drug-like properties. Here we demonstrate the utility of our platform to produce and secrete proteins *in vivo* using environmental promotors and show that we can produce anti-inflammatory small molecules in the gut environment directly

impacting inflammation. To demonstrate the ability of our platform to produce and secrete proteins in the intestines, we engineered a strain of EcN to secrete NanoLuc (NLuc) under environmentally inducible expression systems and confirmed that EcN was able to secrete NLuc both *in vitro*, in a gut-on-chip system, and *in vivo*, in a mouse intestine. We then engineered a strain of EcN to secrete the anti-inflammatory cytokine IL-22, which when given to mice was able induced IL-22 dependent gene expression changes. To evaluate the ability of our platform to produce meaningful levels of anti-inflammatory metabolites *in vivo*, we generated a strain of EcN that could produce IAA, an AHR agonist we demonstrated is able to maintain intestinal barrier integrity in human epithelial monolayer treated with inflammatory cytokines. We further showed that this engineered strain can secrete bioactive AHR ligands in the mouse intestine leading to decreased disease in DSS models of IBD by impacting both gut barrier integrity and host gene expression. Together our data demonstrates that our engineered Synthetic Biotic Medicines are active in the GI tract of mice and offer a novel approach to locally deliver both proteins and small molecules to reduce inflammation and enhance mucosal healing in IBD.

122 Screening small molecule permeation across the mycobacterial outer membrane

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Infections from *Mycobacterium tuberculosis* (Mtb) and nontuberculous mycobacteria (NTB) such as *Mycobacterium abscessus* represent a great challenge for public health. One of the reasons for the notorious drug recalcitrance for this class of bacteria is the presence of a complex and waxy outer membrane, the mycomembrane, outside their cell wall which acts as an additional barrier to drug penetration. Understanding the chemical motifs that control small molecule permeation through mycomembrane has the potential to guide antibiotic design for mycobacteria but has been limited by the lack of high-throughput methods to assess permeation. We developed a flow cytometry-based assay that allows quantification of small test molecule permeation through the outer membrane of the important human pathogen *Mycobacterium tuberculosis*. The assay consists of the (i) metabolic tagging of bacterial peptidoglycan, cell envelope layer that sits directly beneath the outer membrane, (ii) bioorthogonal ligation of test molecules followed by (iii) a fluorescent labeling chase step to quantify their permeation. Our assay lays the foundation for medicinal chemistry efforts to circumvent mycomembrane barrier through the comprehensive understanding of molecular determinants of permeability.

123 Elucidating plasmid copy number control with super-resolution microscopy

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Bacterial plasmids are major sources of antibiotic resistance in hospitals, and invaluable tools in biotechnology. They are considered as minimal molecular machines for processes like DNA replication and segregation, and under strong evolutionary pressure to minimise fluctuations around their average copy numbers. Hence, all plasmids autoregulate their own replication through negative feedback between plasmid-encoded regulatory molecules. However, plasmid copy number measurements have so far been dominated by bulk assays tracking average responses, whereas reliable methods to quantify fluctuations in copy numbers in single cells have been lacking. This has made it impossible to evaluate the molecular control mechanisms against the phenotype they are selected to affect. We combine recent developments in plasmid labelling with state-of-the-art super-resolution imaging to study the copy number control of the model plasmid mini-R1. Live-cell super-resolution imaging captures the spatial organization and temporal dynamics of plasmid copies in single *E. coli* cells, enabling a quantitative evaluation of the copy number control machinery and providing experimental tests for putative control principles. These results show that mini-R1 copy numbers are tightly regulated, with a spatial distribution that is better-than-random and enriched at cell poles. Furthermore, changes in plasmid copy numbers during the cell cycle and across generations provide experimental plasmid replication control curves at a single-cell level, supporting a hyperbolic control model of plasmid replication. Finally, ongoing work aims to correlate the measured plasmid copy numbers to the expression of plasmid-encoded control molecules in single cells, to better understand how such tight and robust control is achieved molecularly.

124 Proximity-based proteomics as a tool to dissect the bacterial-host interface during Listeria cell-to-cell spread

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Listeria monocytogenes is an intracellular bacterial pathogen that promotes its own uptake into host cells. Once in the cytosol, *L. monocytogenes* uses actin-based motility to move through the cytosol, push against the cell membrane, and spread into a neighboring cell. This process of cell-to-cell spread forces the bacteria to recruit host factors to reshape the host membrane, form intracellular protrusions, and resolve them into a double membrane vacuole in the neighboring cell cytosol. Cell-to-cell spread allows the pathogen to disseminate through human tissue and cause severe disease. However, despite being a critical virulence mechanism, the complement of host factors recruited by listeria to sites of cell-to-cell spread is unknown. We adapted the biotin ligase split-TurboID to identify the complete array of host factors recruited to these intercellular protrusions. We fused the N-terminal domain of TurboID to the bacterial surface and localized the C-terminal domain to the host cell plasma membrane. Bacterial interaction with the membrane during spread reconstituted the two halves and biotinylated proteins within bacterial protrusions. Using this approach, we have identified host factors that *L. monocytogenes* interacts with at the membrane, including proteins previously implicated as regulators of spread, such as p120-catenin. Future studies will examine their specific roles during cell-to-cell spread, providing mechanistic insight into this unique virulence strategy. Additionally, by changing the localization of the C-terminal domain of TurboID, we are using this approach to identify host factors *L. monocytogenes* interacts with at every stage of its intracellular life cycle and provide insight into how the bacterial-host interactome regulates virulence.

125 Microbiome Avatar Colitis Mouse Model - A Translational Tool For In Vivo Characterization of Heterogeneity in Stool Samples From Patients With Inflammatory Bowel Disease (IBD)

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A spectrum of taxonomic and functional diversity of the gastrointestinal microbiome is observed among patients with IBD resembling that of healthy individuals to distinctly altered microbiome composition and function. In Phase 1b and 2b studies of patients with mild-to-moderate Ulcerative Colitis (UC), we observed microbiome compositional and metabolic changes in a subset of patients treated with an investigational microbiome therapeutic. Based on these clinical observations, we developed a microbiome avatar IL-10 knockout (Il10-/-) mouse model – mice colonized with fecal microbiota transplant (FMT) from IBD patients to assess the relationship between microbiome disruption and immunomodulatory responses. To generate IBD FMTs, stool collected from IBD patients (UC and Crohn's Disease) were characterized using whole metagenome sequencing (WMS) and metabolomics; we then selected stool samples to capture a range of dysbiosis seen in IBD patients based on: a) a β-diversity-based dysbiosis score defined as the median Bray-Curtis distance to a healthy reference cohort, b) Enterobacteriaceae family abundance, and c) the ratio of secondary-to-primary bile acids. We selected stools with variable dysbiotic characteristics to colonize GF Il10-/-. The severity of intestinal inflammation was assessed by multiple methods, including fecal lipocalin-2, fecal metabolomics, gene expression analysis, and flow cytometric characterization of colonic immune cell populations. Here we show there is microbial heterogeneity in patients with IBD, where the microbial disruption potentially has a causative role in disease-relevant inflammatory activity. Our microbiome avatar Il10-/- model is a translational preclinical tool to further develop clinically relevant interventional models and assess microbial features associated with host inflammatory responses.

126 Pseudomonas spp. are prevalent in the microbial communities in maple sap

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Maple syrup is produced by harvesting the sap of the sugar maple tree (*Acer saccharum*) and boiling extensively to concentrate the sugar. During this process, microbes can colonize the sap and change the metabolites that are present in the sap, which in turn leads to different colors and flavors in the final maple syrup product. While some of the community members in these microbiomes have previously been characterized, little is known about how interactions between these microbes might influence community composition or metabolite production. Therefore, we have conducted a metagenomic survey across multiple sites in the northeastern United States and determined that *Pseudomonas* spp. are widespread in maple sap communities. Additionally, we have isolated *Pseudomonas* spp. from maple sap and assessed interactions with other members of the sap microbial community in both growth and transcriptomic assays. Taken together, our work demonstrates the prevalence of one genus across maple sap microbiomes and demonstrates some of the ways that this genus interacts with other

members of the sap microbial community.

127 Community ecology of phage on a single bacterial host

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Phage are thought to be the most diverse and abundant organisms on earth. Yet, we lack a clear understanding of how this diversity is maintained because, as parasites, phages are expected to be limited by host diversity. Here, we identify a process by which phage diversity can outstrip the diversity of their bacterial hosts. We generated and passaged diverse phage communities on a single strain of the bacterium Escherichia coli across 12 days. To our surprise, in all cases, multiple distinct lytic phages were maintained across the whole experiment. Ecological experiments revealed negative frequency dependence that enables different phage types to coexist on a single bacterial host. Moreover, we find that these phage communities display ecological networks of interaction, akin of bacterial or macroscopic communities. Finally, we show that phage are able to niche separate upon a single host population due to the phenotypic heterogeneity of bacteria, thus providing a mechanism for coexistence. Our work reveals a community ecology of phages at the scale of single bacterial hosts.

128 MIT Mucosal and Systemic Signatures Triggered by Responses to Infectious Organisms (MAESTRO) Clinical Study

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Borrelia burgdorferi is a bacterial spirochete that causes Lyme disease (LD). Patients with LD exhibit a high degree of variability in clinical presentation and disease severity, suggesting individual immune responses may drive this heterogeneity. Unfortunately, even after receiving a correct diagnosis and standard of care antibiotic treatment, approximately 10% of people will experience persistent symptoms. This is similar to observations with Long COVID, which has significant overlap in symptom presentation as chronic LD. Currently, there are no known biomarkers that can distinguish between acute and chronic LD, or predict which acute LD patients will return to health. We have found an antibody signature that can distinguish acute LD patients who continued to have chronic symptoms after treatment from those who returned to health. With that, we have designed a clinical study, MAESTRO, to test the accuracy of our ratiometric antibody score and to create an extensive biobank to investigate differences between acute and chronic LD, compared to Long COVID and healthy controls. Through the MIT Center for Clinical and Translational Research, we will collect blood, saliva, and urine samples, as well as optional sweat, throat swabs, and vaginal/menstrual samples. Biological specimen sampling will be paired with cognitive, eye, and hypermobility testing. We will also follow the acute LD participants who experience symptoms post-treatment, as well as matched participants from the other cohorts, longitudinally with follow-up visits at 6 and 12 months. These data will allow us to test our antibody ratio as a biomarker for predicting recovery from LD.

129 Investigating the role of cell shape in the host interactions of Rickettsia parkeri

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The Spotted Fever Group (SFG) of the obligate intracellular bacteria genus *Rickettsia* is responsible for life-threatening diseases in humans and animals worldwide. Despite their significance to public health, fundamental aspects of rickettsial biology remain elusive due to the challenges imposed by their obligate intracellular lifestyles. Recent studies have highlighted uncharacterized mechanisms that may have arisen through adaptation to intracellular life. Our preliminary data suggests that bacterial cell morphology, may play a critical role in the pathogenesis of *R. parkeri*, a tractable model for SFG species. We are currently testing the hypothesis that the normal rod shape of *R. parkeri* is required for its pathogenesis by assessing the impact of morphological perturbation on three major infection events: internalization, actin-based motility, and cell-to-cell spread. These findings may provide insight into the pathogenesis of SFG bacteria and inform the development of new treatments for rickettsial diseases.

130 Redox regulation of host ribosomal proteins during Helicobacter pylori infection

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Helicobacter pylori infection is commonly associated with several gastric pathologies including gastritis, peptic ulcer disease, and gastric adenocarcinoma. *H. pylori* has evolved various mechanisms to evade the host innate immune response, and as a result, these bacteria can thrive for decades in the gastric mucosa. Chronic infection elicits the continuous generation of reactive oxygen species (ROS) by neutrophils and gastric epithelial cells. Elevated levels of ROS can damage macromolecules, and previous studies have focused on how ROS mediate DNA damage during infection. However, ROS can also generate oxidative post-translational modifications on host proteins that regulate important cellular functions. In particular, protein thiols are a major cellular target of ROS that exhibit decreased reactivity following oxidation. Our lab previously performed a chemical proteomic screen to identify cysteines with reduced reactivity in human gastric cancer cells infected with *H. pylori*. Two cysteines from host ribosomal proteins, Cys125 of uL14 and Cys77 of eS27, were among the top hits of our screen. Since ribosomes are essential for protein synthesis and cell growth, and host translation inhibition is a well-characterized response to infection by other pathogens, we hypothesize that *H. pylori* infection may dysregulate host ribosome biogenesis through an oxidation-dependent mechanism. Through a combination of polysome profiling, protein translation, and nucleolar stress assays, we are characterizing infection-induced changes in the cellular localization and interactions of host ribosomal proteins, as well as the specific contributions of uL14 Cys125 and eS27 Cys77 to these phenotypes. Overall, this work will provide insight into how redox regulation of host ribosomal proteins affects their function and expand our understanding of host cellular processes that are modulated by *H. pylori* infection.

131 The diversity and dynamics of vaginal Lactobacillus crispatus prophages

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In the vaginal microbiome, *Lactobacillus crispatus* dominance is correlated with beneficial health outcomes, including lower rates of sexually transmitted infections and preterm birth. However, little is known about the degree or drivers of intraspecies diversity within *L. crispatus*. One prominent driver of strain-level diversity in natural microbial populations is prophage presence. While many species belonging to the *Lactobacillus* genus are known to harbor active prophages, the diversity and dynamics of prophages shaping the *L. crispatus* species remains unexplored. Here, we used in-house and existing prophage search algorithms to search 394 genomes of vaginal *L. crispatus* strains isolated from healthy donors from the United States and South Africa. We found >92% of strains harbor at least one putative prophage, with > 73% harboring more than one putative prophage. Focusing in on high-quality, closed genomes of 15 unique vaginal *L. crispatus* strains, we found 3-12 complete, putative prophages in each strain. Upon mitomycin-C and natural induction, isolation, and sequencing, we identified 37 active prophages. We observed 57% of the prophages induced with mitomycin-C were also naturally induced, suggesting spontaneous induction is common. Using comparative genomics, we found that several nearly-identical prophages are shared across distantly-related strains and, interestingly, that specific prophages are found in strains from different geographies. Our findings indicate that diverse prophages are abundant and active in health-associated strains of *L. crispatus*. Moving forward, we aim to determine the rates of transfer and the host-ranges of these prophages, ultimately to better understand the ecology and evolution of vaginal microbiomes.

132 Saccharibacteria decreases Actinomyces induced innate immune response

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Several oral *Actinomyces* species (spp.) serve as a host for Saccharibacteria (TM7), a recently cultured phyla of ultra-small bacteria. Clinical findings suggest a high prevalence and abundance of TM7 bacteria in periodontitis and other oral inflammatory diseases but the immunological importance of TM7 is still understudied. In this study, we aimed to understand the interaction of Saccharibacteria strain TM7x and its host bacteria (*Scchaalia odontolytica* strain XH001) with oral epithelial cells (TIGK, NOK-SI and HGEPp) in the context of innate immu-

nity. Our findings suggest that the XH001 induce strong cytokine (IL8 and GRO-a) responses in oral epithelial cells, while TM7x alone did not (immune inert). Although microscopy images show strong evidence of TM7x attaching to gingival epithelial cells compared to XH001. Using the HEK293/hTLR2 cells, we also determined that the XH001 stimulate cytokines in a TLR2-dependent manner. This was further supported by inhibition of XH001 cytokine response using TLR2 specific antibody. Interestingly, the addition of TM7x to XH001 reduced cytokine response compared to XH001 alone, suggesting that TM7x may alter the host bacterial immunity or inhibit the TRL2 receptor. Supporting the prior hypothesis, TM7x-exposed but TM7x-free XH001 host bacteria induced less cytokine compared to naïve XH001 that was not exposed to TM7x. Finally, testing the cytokine response in TM7x host and non-host bacteria illustrated that TM7x can reduce cytokine response only in its host bacteria. This study emphasizes the importance of *Actinomyces* spp. in oral inflammatory diseases and the potential of TM7 bacteria in reducing inflammation in various inflammatory diseases.

133 Characterizing the role of NahK in Anaerobic Respiration Regulation in Pseudomonas aeruginosa.

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The lungs of patients with Cystic Fibrosis are supportive of anaerobic bacterial growth within mucoid layers which leads to chronic infections while damaging epithelial tissue. *P. aeruginosa*(PA) are a major contributor to these infections. PA thrive in microaerobic conditions, are multidrug resistant, secrete exotoxins, and readily form biofilms. Biofilms form when bacteria adhere to surfaces and secrete extracellular polymeric matrices, resulting in environmental barriers. One method for treating biofilms is targeting pathways that contribute to bacteria dispersal out of the matrix. This reduces protection from antibiotics or immune system defenses. Nitric oxide(NO) was previously shown to trigger biofilm dispersal, although the mechanism is poorly understood. Our lab demonstrated that in PA, a NO sensing protein(NosP) is necessary for triggering NO-mediated biofilm dispersal by inhibiting its associated histidine kinase(NahK). NosP and NahK are part of a multi-kinase network that regulate transitions between biofilm and planktonic states partially through quorum sensing(QS). Preliminary experiments found that Δ nahk overproduces pyocyanin, which is excreted partially to support respiration in the anaerobic interior of a biofilm. Under low oxygen conditions, PA can support respiration by conducting denitrification using a QS regulated system of reductases where NO is an intermediate product. NO is toxic to the cell at high concentrations. It binds to macromolecules, resulting in oxidative damage. We hypothesize NahK modulates anaerobic metabolism in response NO. My preliminary results suggest a role for NahK on biofilm formation, cell morphology and misregulation of denitrification machinery due to changes in QS signaling.

134 Potentiating immune responses to microbial pathogens by innate-like T cells via non-classical antigen presentation in Xenopus laevis

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MHC-I-like restricted innate-like T cells (iT cells) can respond rapidly against pathogens without requiring expansion like conventional T cells restricted by classical MHC class I (class Ia). Since MHC-I-like molecules are non-polymorphic, they are great candidates for immunotherapy. XNC4 is an MHC-I-like molecule in the amphibian *Xenopus laevis* that confers resistance to a major pathogen of aquatic vertebrates, the non-TB mycobacteria, M. marinum (Mm). Mm *is also a model organism for* M. tuberculosis, the causing agent of tuberculosis in humans. *Xenopus* tadpoles are class Ia-deficient and thus ideally suited to investigate MHC-I-like function. Furthermore, unlike mice, tadpoles have prominent iT cell populations. Owing preliminary data suggesting that XNC4 binds long peptides (11-15mer) like human HLA-F, we hypothesize that XNC4 presents long mycobacteria-derived peptides in a TAP- and beta2 microglobulin (b2m)-independent manner. Contrastingly, co-immunoprecipitation data suggests that XNC10, another MHC-I-like molecule, associates with b2-microglobulin (b2m). Accordingly, we co-transfected tagged recombinant XNC4 or XNC10 with or without *b2m* into mammalian cell lines to assess the b2m requirements for cell surface expression. We are now generating a Lentiviral-VSV hybrid system to allow transduction of XNC4 and XNC10 with higher efficiency. To investigate the peptide processing requirement for XNC4 and XNC10 surface expression, we will disrupt by CRISPR/Cas9 genome editing the Antigen Peptide Transporter 1 (TAP1) necessary for peptide transport into the endoplasmic reticulum. Elucidating the mechanism of XNC4 antigen presentation will contribute to better defining iT cell activation and direct new therapeutic strategies to combat TB and nonTB infections.

135 Evolution of growth-enhancing mutations in Mycolicibacterium smegmatis strains deleted for a small RNA and an RNA binding protein

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Knowledge of RNA biology in mycobacteria, the group of bacteria including the causative agent of tuberculosis, is lacking compared to better-studied bacteria such as Escherichia coli. B11 is an sRNA involved in regulation of gene expression and virulence, and KhpB is an RNA-binding protein that has recently begun to be characterized in some firmicutes and proteobacteria. Mycolicibacterium smegmatis, a non-pathogenic mycobacterial model organism, has a notable growth defect when lacking B11 or KhpB. Suppressor mutants were evolved by regularly diluting Δ b11 or Δ khpB cultures until growth rates increased, and whole-genome sequencing (WGS) was performed to identify compensatory mutations. Suppressor strains have an enhanced growth rate and a round colony morphology. Mutations in a fatty acid desaturase and an ABC transporter were common between the Δ b11 and Δ khpB suppressors, possibly contributing to the shared phenotypes. The Δ khpB suppressors had a high number of mutations, confounding the prediction of which mutations affect growth rate by compensating alleviating for a khpB-specific defect or by another mechanism. Moreover, a predicted B11 target, msmeg-1389, which is thought to be transcribed together with msmeg-1388, was mutated in some of the Δ b11 suppressors. Both genes were determined by RNAseq to be overexpressed in Δ b11 knockout. Since msmeg-1388 has a B11 binding site in its RBS region which may repress translation. We therefore predict that overexpression of *msmeg-1389 may be toxic and hypothesize that the mutation found in some* Δ b11 suppressors mitigates this toxicity. Our work in characterizing KhpB, as well as exploring its potential relationship with B11, is still ongoing.

136 Determining the role of cyclic-di-GMP in enhanced biofilm formation in Vibrio cholerae and Paracoccus aminovorans cocultures

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Recent studies suggest that host colonization of the bacterial pathogen Vibrio cholerae (Vc), which causes the diarrheal disease cholera, is influenced by its interactions with the gut microbiota. Particularly, the commensal bacterium Paracoccus aminovorans (Pa) *has been shown to enhance* Vc *biofilm formation* in vitro and intestinal colonization in a mouse model. Cyclic di-GMP (cdG) is the key molecule that governs *Vc* biofilm formation, although its role in this interspecies relationship is unknown. We hypothesize that phosphodiesterases (PDEs), enzymes that degrade intracellular cdG, play a critical role in facilitating this synergistic effect between *Vc* and *Pa*. In the present study, we examine the effect of overexpression of a selected set of PDEs in the enhancement of *Vc* biofilm formation in the presence of *Pa* utilizing established biofilm assays. Preliminary results suggest that overexpressing particular PDEs decreases biofilm formation in Vc-Pa *coculture but not in* Vc monoculture, supporting the hypothesis that altered cdG levels and PDE activity are linked to increased *Vc* biofilm formation, and potentially colonization, in the presence of *Pa*.

137 An Essentialome-Wide CRISPRi Screen for Cell Cycle Defects with Barcoded Live-Cell Microscopy

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Cells coordinate growth, division, and DNA replication to ensure stable reproduction, an especially challenging task in fast generating bacteria. Over the past few decades, advances in microscopy have enabled direct observation of the cell cycle machinery and its dynamics, leading to several models of cell cycle control. However, the genetic details of these models remain unclear, as nearly every critical player in reproduction is essential, making standard genetic perturbations lethal. To quantify essential gene effects on cell cycle dynamics, we developed a barcoding method coupling large genetic libraries with single-cell time-lapse microscopy in a microfluidic device. We applied this technology to screen for cell cycle defects in a 30k sgRNA CRISPRi library targeting all essential genes in *E. coli* with varied knockdown strength. We classify >300 genes according to their single-cell growth dynamics. Using a fluorescent nucleoid reporter, we refined the roles of variants with division defects

into those impacting division through or downstream of replication. Through this approach, we elucidated a key role for the GTPase activity of the ribosome in modulating both DNA replication and cell division. We showed that nucleoid topology, replication status and Z-ring assembly are sensitive to perturbations of ribosome GTPase activity and propose a mechanism whereby RelA-dependent conversion of GTP into ppGpp provides a metabolic sink for cell cycle control during translation downshifts. We believe that our unbiased approach to screening essential genes will further reveal a broad array of regulatory players that would otherwise be impossible to screen and characterize using existing methods.

138 A tRNA modification in Mycobacterium tuberculosis facilitates optimal intracellular growth

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Diverse chemical modifications fine-tune the function and metabolism of tRNA. Although tRNA modification is universal in all kingdoms of life, profiles of modifications, their functions, and physiological roles have not been elucidated in most organisms including the human pathogen, *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis. To identify physiologically important modifications, we surveyed the tRNA of *Mtb*, using tRNA sequencing (tRNA-seq) and genome-mining. Homology searches identified 18 candidate tRNA modifying enzymes that are predicted to create 13 tRNA modifications across all tRNA species. Reverse transcription-derived error signatures in tRNA-seq predicted the sites and presence of 9 modifications. Several chemical treatments prior to tRNA-seq expanded the number of predictable modifications. Deletion of *Mtb* genes encoding two modifying enzymes, TruB and MnmA, eliminated their respective tRNA modifications, validating the presence of modified sites in tRNA species. Furthermore, the absence of *mnmA* attenuated *Mtb* growth in macrophages, suggesting that MnmA-dependent tRNA uridine sulfation contributes to *Mtb* intracellular growth. Our results lay the foundation for unveiling the roles of tRNA modifications in *Mtb* pathogenesis and developing new therapeutics against tuberculosis.

139 Magnesium-Dependent Regulation of Cell-Cycle-Dependent Proteolysis in Caulobacter crescentus

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In *Caulobacter crescentus*, progression through the cell cycle is regulated by the AAA+ protease ClpXP, and there are several classes of cell-cycle substrates that require adaptors in order to be degraded. CpdR, a single domain-response regulator, binds the N-terminal domain of ClpXP and primes the protease for degradation of downstream factors (Lau et al., 2015). The ability of CpdR to bind ClpX is regulated by its phosphorylation state. In the unphosphorylated state, CpdR binds ClpXP and guides its localization to the cell pole during the swarmer to stalked transition, where CpdR is mediates degradation of substrates such as PdeA. Phosphorylation of response regulator receiver domains requires magnesium as a cofactor to stabilize the phosphorylated aspartate and reciprocally, phosphorylated receiver domains bind magnesium more effectively. Through a screen, we found that mutation of Mg-binding residues increased CpdR activity even when dephosphorylated, suggesting a model where phosphorylation of CpdR stabilizes a Mg-bound state which is blocked for ClpX binding. Modeling of this interface with Collabfold is also consistent with this hypothesis. In this work, we directly test the role of magnesium in CpdR priming of ClpXP to show that magnesium may play a regulatory role in CpdR-mediate degradation, and thus binding to ClpX.

140 Determining if Parabacteroides distasonis requires HPRT to accelerate diabetes and activate insulin B:9-23-specific T-cells

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Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the destruction of pancreatic, insulin-producing beta cells by autoreactive T-cells. The gut microbiota and certain human leukocyte antigen (HLA-DQ8) alleles influence T1D onset. Autoantibodies develop against insulin, the peptide of amino acids 9-23 of the insulin B chain (insB:9-23) is one of the most commonly recognized epitopes. InsB:9-23 cross-reacted with amino acids 4-18 of hypoxanthine phosphoribosyl transferase (HPRT:4-18) which originates from a gut commensal bacterium, *P. distasonis*. Previous studies have shown molecular mimicry is pathogenic where microbial proteins mimicked

host proteins leading to cross-reactivity and the induction of disease-like symptoms. Similarly, *P. distasonis* accelerated diabetes in non-obese diabetic (NOD) mice. We hypothesize *P. distasonis* accelerates diabetes via molecular mimicry of insB:9-23 by HPRT and HPRT independently accelerates diabetes. To prove molecular mimicry is why *P. distasonis* accelerated diabetes, a mutagenized HPRT gene and recombinant HPRT will be generated. They will be used for HPRT (wild-type or mutant) treatment experiments and *P. distasonis* (mutant or wild-type) colonization experiments in mice. Changes in the infiltration of pancreatic islets and diabetes incidence will be assessed by histological brightfield microscopy and by measuring glycemia levels. Antigen presentation, T-cell composition and activation will be assessed with flow cytometry and enzyme-linked immunospot (ELISpot) assays to see how each treatment affected the CD8+ T-cells at the site of infiltration, the pancreas, and to assess cross-reactivity of experimental HPRT-bound splenic antigen-presenting cells (APC) with an insB:9-23-specific T-cell line.

141 Elucidating Lon Protease Biological Functions Through the Use of Overexpression Vectors in Acinetobacter baumannii

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Acinetobacter baumannii is a Gram-negative nosocomial opportunistic pathogen able to form robust biofilms allowing it to survive in harsh environments and desiccation. It is commonly associated with its ability to rapidly gain antibiotic resistance. These factors contribute to the high level of concern surrounding *A. baumannii* in hospital acquired infections, especially in immunocompromised patients. Previous studies have shown the Lon Protease having an influential role in multiple biological functions in *A. baumannii*, including biofilm formation, UV sensitivity, motility, and membrane morphology. The deletion of *lon* produces a striking decrease in biofilm formation as well as a large transcriptional upregulation of two genes: *SurA1* and *A1S-2230*, both of which are hypothesized to localize to the cell surface. A preliminary macrophage phagocytosis assay has shown an increased sensitivity in the Δ Lon strain. We hypothesized that the upregulated genes have a role in contributing to certain characteristics observed in the Δ Lon strain and have begun to find evidence that supports this. Here we show the effects of overexpression of these genes in *A. baumannii* on human serum sensitivity, biofilm formation, antibiotic resistance, cell morphology, and motility. In addition to testing the role of these proteins, we have also continued work attempting to identify the cause of their overexpression when cells lack *lon* using ChIP-Seq to identify potential novel transcription factors targeted by *lon*. Lon Protease's multifaceted function appears to rely at least in part on its ability to regulate the surface proteins SurA1 and A1S-2230, potentially by targeted degradation of a transcription factor.

142 Membrane Vesicles from the Marine Bacterium Pseudoalteromonas piscicida Deliver Antimicrobial Cargo

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All cells produce extracellular vesicles, making them one of the fundamental products of life. Bacteria produce membrane vesicles (MVs) that are usually within the size range of 20 – 200 nm in diameter and are composed of lipids, proteins, and small molecule cargoes. MVs likely serve important roles in inter- and intra-species, as well as bacteria-environment, interactions. In this study, we conducted a metabolomic investigation of MVs produced by the marine bacterium *Pseudoalteromonas piscicida* JC3. This bacterium demonstrates strong antibiosis toward the marine pathogen *Vibrio parahaemolyticus* under co-culture conditions. We hypothesized that MVs produced by JC3 played a role in this antagonistic interaction. Nanoparticles were harvested and purified by repeated ultracentrifugation from 48-hour, cell-free supernatants of *P. piscicida* JC3. Transmission electron microscopy revealed vesicles with a spherical shape and a single membrane, consistent with outer membrane vesicles. Dynamic light scattering analysis showed that the MVs possessed an average diameter of 188 nm. Suspensions of the MVs in HEPES buffer limited the growth of *Vibrio parahaemolyticus* cultivated on agar plates. Chemical extracts were next generated from lyophilized MVs and analyzed by UHPLC-MS/MS. Multiple congeners of lipopeptides known as bromoalterochromides were identified within the *P. piscicida* MV extracts, suggesting a potential role for these specialized metabolites in microbial interactions. Previous studies have identified bromoalterochromides as antibiotics with activity against *Vibrio* pathogens. These findings suggest that MVs produced by *P. piscicida* JC3 serve as delivery mechanisms for specialized metabolites and that these vesicles play a role in microbial competition.

143 Membrane-intrinsic Factors that Control the Dynamics of Mycobacterial Membrane Compartments

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Mycobacteria is a causative agent of diseases worldwide based in part on their unusual cell envelope, which acts as a barrier to antibiotics and host immunity. Additionally, mycobacterial membranes have evolved structural adaptations to combat various stressors. The intracellular membrane domain (IMD) is a laterally discrete region of mycobacterial plasma membrane, enriched in the subpolar region of the rod-shaped cell. Here we report genome-wide transposon sequencing to discover the controllers of membrane compartmentalization in *Mycobacterium smegmatis*. The putative gene *cfa* showed the most significant effect on recovery from dibucaine, which disrupts subpolar IMD enrichment. Enzymatic analysis of Cfa and lipidomic analysis of a *cfa* deletion mutant (Δ cfa) demonstrated that Cfa as an essential S-adenosyl-L-methionine-dependent methyltransferase for the synthesis of major membrane phospholipids containing a C19:0 monomethyl-branched stearic acid, also known as tuberculostearic acid (TBSA). Δ *cfa* displayed delayed restoration of subpolar IMD and delayed outgrowth after bacteriostatic dibucaine treatment. In contrast, membrane permeability and proton gradient formation were unaffected in Δ *cfa*, suggesting the physiological significance of TBSA in controlling lateral membrane partitioning in mycobacteria. More recently, we found that dibucaine-induced delocalization of subpolar IMD is an active process dependent on a proton gradient. We are currently testing the roles of the electron transport chain enzymes in the lateral membrane dynamics.

144 Transcriptional Profiling of Desemzia incerta to Explore Colony Morphology Differences

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Methicillin-resistant *Staphylococcus aureus* (MRSA), can colonize the skin and cause skin infections, creating a significant healthcare burden. We used a porcine skin model to further investigate potential isolates with antimicrobial activity towards MRSA. Pig skin is histologically similar to human skin but contains a diverse microbiome. A total of 7,700 isolates were collected, where 249 bacterial isolates, compromising 30 unique bacterial species, were found to inhibit MRSA. We focus on *Desemzia incerta*, an understudied species. We find that *D.incerta* exhibits temperature-dependent colony morphologies (domes and craters). To investigate the transcriptional cause of these different colony morphologies, we performed RNA-seq on crater and dome *D.incerta* colonies. FastQC was used to perform quality control of sequenced reads. Kallisto was used for memory-efficient mapping of the raw reads. An annotated *D.incerta* genome was used to help align and determine abundance of our raw reads.The limma R package was used to measure differential gene expression. The p-value threshold used to filter the differentially expressed genes was 0.11. The top five hits were fliC, cheR, cheB, mcpC, and cheA. These genes have functions related to the flagellum; fliC is responsible for the flagellar filament structural protein, and the *che* operon is essential for changing swimming direction and flagellar rotation. As the p-value used was relatively high, the DESeq2 package will be used to conduct a more sensitive investigation. In the future, we aim to address whether dome and crater colonies have varying antimicrobial activity.

145 Specific Codons Control Cellular Resources and Fitness

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As biotechnology research progresses from simply overexpressing proteins to creating intricate metabolic pathways, gene circuits, and complex phenotypes, harmonizing gene expression with the resource demands of a host organism has become essential. The degenerate genetic code provides an opportunity to allocate cellular tRNA and ribosomal resources optimally between host and heterologous protein expression. 18 of the 20 natural amino acids can be encoded with multiple codons, which provides biological engineers with a large combinatorial design space that can be unpredictable. Through our research, we aim to improve the predictability and robustness of genetic engineering in microbes by systematically determining optimal codon bias schemes.

We investigate how the partitioning of translational resources, specifically through incorporating dissimilar codon usage bias (CUB), can drastically alter expression of proteins and reduce the burden on gene expression systems. We assayed genetic resource competition both in vitro and in vivo using novel gene designs that

isolate translation elongation from other variables. We find that codon bias can trans-regulate gene expression of competing heterologous and endogenous genes, yielding profitable or catastrophic design options. By isolating individual codons experimentally, we correlate specific codon usage patterns with genetic burden, and derive novel coding schemes for multi-gene expression. These empirically derived coding schemes based on a new codon adaptation index enable the design of harmonious multi-gene expression systems while avoiding catastrophic cellular burden. Our research enables improved gene design in E. coli, and provides a framework for optimizing codon bias in other organisms as well.

146 Integrating protein 3D structure with mutation data identifies proteins under selection in Mycobacterium tuberculosis

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Mycobacterium tuberculosis is the causative agent of tuberculosis, a globally prevalent and deadly disease that is increasingly resistant to antibiotics. Identifying regions of the *M. tuberculosis* genome under positive selection would uncover important features of pathogen evolution and pinpoint potential drug targets.

Here, we detect positive selection in *M. tuberculosis* by integrating mutation data with protein 3D structure to boost signal for rare mutations that are clustered in protein structures, and identify functionally relevant regions in proteins under selection. We generate a dataset of structural annotation for 98% of *M. tuberculosis* proteins by combining solved structures with machine learning-based predictions, alongside a dataset of over 470,000 unique mutation events (non-synonymous SNPs and short indels) reconstructed across a phylogeny of 31,428 genomes. We develop a new approach called the GeO score, first validating our approach on a positive control dataset of computationally generated clustered mutations, achieving ROC AUC of .91 at distinguishing clustered cases from randomly generated controls. Applying our method to the entire genome, we find 305 proteins with significant clustering, including selection in known antibiotic-resistance conferring proteins, as well as proteins involved in host adaptation. We also identify a number of metabolic enzymes of interest for further investigation. This study provides a useful basis for future studies of *M. tuberculosis* adaptation, as well as providing a framework for analysis of bacterial genome evolution guided by knowledge of protein structure.

147 Single-cell growth rate of heterotrophic bacterioplankton in natural seawater

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Marine heterotrophic bacteria play a crucial role in the biogeochemical cycles of the ocean by decomposing and recycling organic matter. Recent efforts have highlighted the enormous co-existing genetic and functional diversity of marine microbes. However, our understanding of *in situ* growth rates, especially at fine levels of taxonomic resolution, is hindered by both technical limitations and low nutrient concentrations in natural seawater. In this study, we developed a highly sensitive, microfluidic single-cell mass sensor called suspended microchannel resonator (SMR) which can measure single-cell instantaneous growth by continuous mass measurements and thereby mass accumulation within a short window. The system can achieve a mass resolution of sub-femtograms and specific growth rate resolution of $<$0.1 per day. The high precision allows for the measurement of growth rates for different bacteria species in unamended natural seawater, as well as their growth rate response to nutrient amendment. We find that *in situ* growth rates in the same seawater sample vary from species to species and the fastest growing species can grow at an average specific growth rate of 5 per day. Different species also responds differently to the nutrient amendments in the same seawater sample. For example, two *Vibrio* (*Gammaproteobacteria*) strains are shown to be carbon limited and a *Celeribacter* (*Alphaproteobacteria*) strain co-limited by more than one type of nutrient or auxotrophic for a micronutrient. These results provide precise estimates for *in situ* growth and biomass production within the bacterioplankton community and reveal species-to-species heterogeneity from inhabiting the same local environmental conditions.

Lunch Options In Harvard Square

Note: Lunch will be provided only on June 12, 2023. Below are our suggestions (with links) for some local options for lunch for June 14, 2023.

Mexican/Latin American
Felipe's Taqueria
Orinoco Harvard Square
El Jefe's Taqueria

Vegan/Vegetarian
Life Alive Organic Cafe
Vegan Bowls For All
Veggie Grill

Gastropub/American
Alden & Harlow
Charlie's Kitchen
Grendel's Den Restaurant & Bar
Harvest
Henrietta's Table
Mr. Bartley's Burger Cottage
Russell House Tavern

Quick Eats
Bon Me
Clover Food Lab
DIG
OTTO
Shake Shack
Sweetgreen
Tasty Burger

Asian
Bosso Ramen Tavern
Hokkaido Ramen
Le's Vietnamese
The Maharaja
Pokeworks

Cafes
Bluestone Lane
Flour Bakery
Pavement Coffehouse
Tatte Bakery and Cafe

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