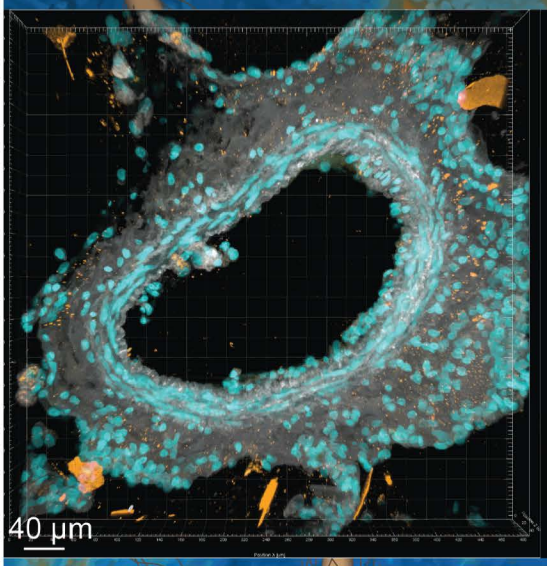
Boston Bacterial Meeting

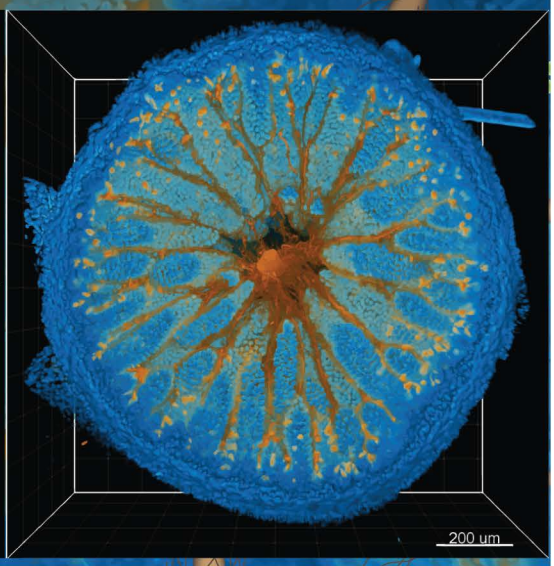
May 31 - June 1, 2018
Harvard University Science Center



Keynote Speaker:

Dianne K. Newman, PhD

Caltech



2018 Boston Bacterial Meeting - Schedule and Introduction

Thursday, May 31

8:00 AM	Registration and Breakfast	
9:00 AM	Opening Remarks	
Session I: Motility		**Moderator: Becky Lamason**
9:10 AM	Shanice Webster	The type IV pili alignment complex, surface sensing, and cyclic-di-GMP signaling in early biofilm formation
9:30 AM	Marianne Grognot	High throughput 3D tracking of bacterial chemotaxis in complex environments
9:50 AM	Miles Duncan	*Vibrio cholerae* motility exerts drag force to impede attack by the bacterial predator *Bdellovibrio bacteriovorus*
10:10 AM	Sophie Robitaille	Experimental directed evolution of a swarming motility-defective mutant of *Pseudomonas aeruginosa* leads to regained motility
10:30 AM	Coffee Break	
Session II: Antibiotics and Resistance		**Moderator: Hesper Rego**
11:00 AM	Wilma Neumann	The siderophore enterobactin targets ciprofloxacin to Gram-negative pathogens
11:20 AM	Gleb Pishchany	Amycomicin: an antibiotic that isn't
11:40 AM	Sylvie Manuse	Phenotypic variation of intracellular ATP concentration impacts persister formation in *E. coli* at a single cell level
12:00 PM	Cristina Peñaranda	AlgR controls in vivo persistence of *Pseudomonas aeruginosa*
12:20 PM	Lunch and Breakout Session 1	
12:40 PM	Breakout Sessions Begin	
1:30 PM	Networking	
Session III: Stress Response Mechanisms and Systems Biology		**Moderator: Cheryl Andam**
1:40 PM	Rilee Zeinert	A legacy role for DNA binding of Lon protects against genotoxic stress
2:00 PM	Michelle Bellerose	*Mycobacterium tuberculosis* mechanisms for survival under antibiotic pressure
2:20 PM	Karen Zhu	Forecasting bacterial survival-success and adaptive evolution through multi-omics approaches, network analyses and machine learning
2:40 PM	Monica Guo	A bacterial chromosome structuring protein binds overtwisted DNA to stimulate type II topoisomerases and enable DNA replication
3:00 PM	Coffee Break	
Session IV: Microbial Ecology		**Moderator: Carey Nadell**
3:30 PM	Batbileg Bor	A glimpse into the secret lifestyle of TM7x as a CPR organism
3:50 PM	Audrey-Anne Durand	The symbiotic complex of *Dendroctonus simplex*: implications on the beetle tree attack and life cycle
4:10 PM	Cathleen Schlundt	Spatial structure in the "Plastisphere": Imaging bacterial communities on plastic marine debris using CLASI-FISH
4:30 PM	Steven Biller	Heterotroph interactions alter *Prochlorococcus* transcriptome dynamics during extended periods of darkness
Poster Session I / Day 1 Reception		
5:00 PM	Poster Session IA: Posters 1-73 (odds) and 75	
6:00 – 7:00 PM	Poster Session IB: Posters 1-73 (evens) and 82, 88, 100	

Friday, June 1

8:30 AM	Morning Coffee	
Session V: Host-Pathogen		**Moderator: Scarlet Shell**
9:00 AM	Jen Helble	Two-photon imaging of *Chlamydia* infection reveals host immune-cell migration to mucosal surfaces
9:20 AM	Sally Demirdjian	PIP3 induces phagocytosis of non-motile *Pseudomonas aeruginosa*
9:40 AM	David VanInsberghe	Diarrheal illnesses trigger *Clostridium difficile* colonization in the human gut
10:00 AM	Troy Hubbard	Re-engineering the cholera pathogen yields a probiotic agent that rapidly protects against cholera
10:20 AM	Coffee Break	
Session VI: Microbial Communities		**Moderator: Michelle Larsen**
10:40 AM	Sajjad Hossain	Discovery of a novel nitric oxide responsive pathway and a putative nitric oxide sensor (NosP)
11:00 AM	Murray Tipping	Self recognition signals induce antibiotic tolerance in the opportunistic pathogen *Proteus mirabilis*
11:20 AM	Kristin Loomis	Carbon source utilization of the skin Microbiota
11:40 AM	Abhishek Shrivastava	Cargo transport shapes the spatial organization of a microbial community
12:00 PM	Lunch and Breakout Sessions	
12:20 PM	Breakout Sessions Begin	
1:10 PM	Networking	
Session VII: Microbial Engineering		**Moderator: Chris Johnston**
1:20 PM	Bo Yan	SMRT-Cappable-seq reveals the complex operome of bacteria
1:40 PM	Jeffrey Wagner	Live and let die: molecular kill switches in *Mycobacterium tuberculosis* with applications to vaccine development
2:00 PM	Jacob Palmer	Engineered probiotic for the inhibition of *Salmonella* via tetrathionate-induced production of microcin H47
Coffee and Poster Session II		
2:20 PM	Poster Session IIA: Posters 74-146 (odds)	
3:20 PM	Poster Session IIB: Posters 74-146 (evens), 32	
Session VIII: Morphogenesis and Cell Surface		**Moderator: Frédéric Veyrier**
4:20 PM	Katherine Wu	Mycobacterial HtrA is required to detoxify a putative cell wall muramidase
4:40 PM	Oscar Diaz	The *Clostridium difficile* lipoprotein GerS is required for cortex modification and thus spore germination.
5:00 PM	Florence Caro	Lipoprotein transport system knockdown reveals a novel cell death trajectory in *V. cholerae*
5:20 PM	**Keynote Address: Dianne Newman**	
6:30 - 8:30 PM	Day 2 Reception	

2018 Boston Bacterial Meeting – Schedule and Introduction

WELCOME!

Dear Participants,

On behalf of the BBM2018 Organizing Committee, we thank you for attending the 24th Boston Bacterial Meeting! Over the past 24 years, the BBM has offered an intellectually stimulating and collegial atmosphere for bacteriologists throughout the New England area to discuss cutting-edge science, share ideas, and highlight the work of early-career researchers. The BBM is proud to provide a forum that fosters collaborations between researchers from all levels, within both academia and industry.

The BBM has grown significantly since its inception—this year, we have expanded the conference to two full days. The past few years have seen record-breaking participation from researchers both near and far. Given the success and demand of the interactive breakout sessions, we have also expanded the number of offerings to occur on both days. This year also brings a record-breaking number of undergraduate students in attendance. Although the BBM has undergone many changes over the last 24 years, one crucial element has remained consistent: our commitment to the highest quality research. This is particularly evident in the 30 talks and over 140 posters that will be presented at this year's meeting.

This year we are excited to feature Dr. Dianne Newman as our Keynote Speaker. Dr. Newman is the Gordon M. Binder/Amgen Professor of Biology and Geobiology at the California Institute of Technology (Caltech) and a 2016 MacArthur Foundation Fellow. Prior to her tenure at Caltech, Dr. Newman participated in BBM in its early years, as a postdoctoral fellow in the Kolter lab. We are delighted to welcome back Dr. Newman and hear about her ongoing work in molecular geomicrobiology.

The BBM would not be possible without the efforts of the Organizing Committee, a highly motivated and enthusiastic group of graduate students and post-docs dedicated to the success of this meeting. We are also indebted to our faculty sponsors Ethan Garner and Tom Bernhardt and meeting founders Rich Losick, Roberto Kolter, John Mekalanos, and Andrew Wright; we thank them for their continuing guidance. Additionally, we thank our generous industry sponsors and the NIH, for the financial support that helps make this meeting possible!

Finally, we thank all of you for your participation and support of Boston Bacterial Meeting. We hope you will find the next two days both intellectually and socially fulfilling.

Thank you again for joining us!

Sora Kim and Max Schubert

Co-chairs, BBM2018

Meeting Announcements

- Please silence your mobile devices during the talks.
- No food or drink allowed in the classrooms or auditorium.
- For people attending breakout sessions, after picking up lunch, you may eat your packed meal right before your session starts or after it ends.
- For poster session judges, please check in at the registration desk to pick up materials.
- To submit your vote for popular choice poster, write down the number of your nominated poster and place it in the ballot box.
- To maintain confidentiality of unpublished data, recording or photography of oral and poster presentations is prohibited without prior permission of presenters.
- Please keep your name badge on you at all times during the conference. At the end of the conference, please help us recycle the empty plastic name badge holders by placing them in the designated boxes or by leaving them at the registration desk.
- Restrooms are located in the basement.
- Room 109 is available as a lactation room but does not have a pump or fridge.

A portion of the funding for this year's conference was made possible by grant R13AI138540 from the National Institute of Allergy and Infectious Diseases. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

Additional funding generously provided by:

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Keynote Speaker

Dianne K. Newman, Ph.D.

Dianne K. Newman is a 2016 MacArthur Foundation Fellow and the Gordon M. Binder/Amgen Professor of Biology and Geobiology in the Divisions of Biology and Biological Engineering and Geological and Planetary Sciences at the California Institute of Technology (Caltech).

Dr. Newman's research examines the relationships between bacterial metabolism, geochemistry, and infection. Her work has revealed how bacteria can mobilize arsenate in arsenic respiration and how anoxygenic phototrophs utilize ferrous iron during photosynthesis. She is a pioneer in molecular geomicrobiology who has contributed widely to understanding microbe-mineral interactions in the context of evolution of life on Earth. More recent studies by the Newman lab have expanded their investigations to bacterial communities in chronic infections, such as elucidating the role of phenazine redox cycling in the survival of *Pseudomonas aeruginosa* within the lungs of cystic fibrosis patients.

Dr. Newman received her Ph.D. in Civil and Environmental Engineering from the Massachusetts Institute of Technology and was a postdoctoral fellow in the Department of Microbiology and Molecular Genetics at Harvard Medical School. She became a faculty member at Caltech in 2000 and was appointed as a Howard Hughes Medical Institute Member from 2005-2016. She is a fellow of the American Academy of Microbiology and serves as an editor of mBio and eLife. Her awards have included the National Academy of Sciences Award in Molecular Biology (2016), the NIH Director's Transformative Research Award (2012), the Eli Lilly and Company-Elanco Research Award from the American Society of Microbiology (2008), an Office of Naval Research Young Investigator award, and the David and Lucile Packard Fellowship in Science and Engineering.

This profile information was adapted from: http://www.bbe.caltech.edu/content/dianne-k-newman

2018 Boston Bacterial Meeting – New Faculty

As part of BBM's goal to foster interactions and collaborations between various individuals and their institutions, we would like to highlight the new members of our research community who have recently begun their appointments. We encourage everyone to provide these new faculty members with a warm welcome to our Boston-area bacteriology community!

Christopher Johnston
Assistant Research Investigator
The Forsyth Institute, Cambridge, MA
cjohnston@forsyth.org

Research in my lab is focused on overturning the restrictive paradigm of genetic intractability in microbiology by creating broadly applicable methodologies to allow genetic engineering of virtually any cultivable bacterial species; massively expediting fundamental examinations of microbes relevant to health and disease. In 2017, I received an 'NIH Director's Transformative Research Award' to support this effort. The long-term goal of my research is to generate a deeper functional understanding of 'what bacteria are capable of doing' and 'how they are doing it', and then utilize that information to engineer novel therapeutics and the next generation of microbe-based technologies for application in human medicine and bioengineering. Using synthetic microbiology, my lab will explore bacterial epigenetics, virulence, and the development of bacteriophage-based therapeutics.

Carey Nadell
Assistant Professor of Biological Sciences
Dartmouth College, Hanover, NH
carey.d.nadell@dartmouth.edu

Dr. Nadell works at the interface of theoretical ecology, evolutionary biology, microbiology, and molecular genetics to uncover fundamental driving forces of bacterial community structure. Bacteria often live in groups, or biofilms, in which they envelop themselves with a secreted polymer matrix, and where cells cooperate and compete with each other using a broad spectrum of interactive behaviors. These interactions are central to how bacteria evolve, and how they impact the world around them. The Nadell lab currently has three main lines of research: 1) understanding how the mechanisms of biofilm production, and especially extracellular matrix secretion, influence the micro-ecology of cell-cell interactions and population dynamics; 2) understanding how bacteria-phage interactions impact biofilm community assembly; and 3) the application of ecological and evolutionary theory tailored to biofilm environments. In pursuing these goals the lab uses concepts and approaches from many disciplines, with particular emphasis on spatial simulations

of biofilm population dynamics and confocal microscopy of experimental biofilms cultivated using microfluidic devices.

Rebecca Lamason
Assistant Professor, Department of Biology
Massachusetts Institute of Technology
rlamason@mit.edu

The Lamason lab investigates how diverse bacterial pathogens reprogram host machinery during infection. We focus on two intracellular bacterial pathogens, *Rickettsia parkeri* and *Listeria monocytogenes*, and explore how these pathogens move through our tissues using a process called cell-to-cell spread. Both pathogens can invade host cells, hijack the host's actin cytoskeleton to promote cytosolic motility, and spread into neighboring cells. Despite the importance of this process for virulence, little is known about the key bacterial and host factors required for cell-to-cell spread. We have discovered that these pathogens spread throughout our tissues by targeting host intercellular communication pathways (e.g. endocytosis, adhesion, membrane remodeling) and manipulating different force-generating machinery. We are continuing to explore the molecular underpinnings of spread by combining genetic, cell biological, biochemical and biophysical approaches to determine how bacteria manipulate host cell processes to promote spread. Ultimately, our goal is to reveal exciting mechanisms of host-pathogen interactions in order to gain fundamental insights into pathogenesis and host cell biology.

Scarlet Shell
Assistant Professor, Department of Biology and Biotechnology
Worcester Polytechnic Institute
sshell@wpi.edu

How does *Mycobacterium tuberculosis* adapt to stress and regulate its physiology over both short and long timescales to maximize survival in the harsh environment of the human host? Stress response is intimately linked to the regulation of gene expression. To learn how *M. tuberculosis* tolerates the challenges of infection, we need to understand the regulatory processes that control mycobacterial stress response systems. In the Shell lab, we approach this problem from multiple angles, using both *M. tuberculosis* and its avirulent relative *M. smegmatis* to interrogate the fundamental biology of mycobacterial gene regulation and stress response.

Regulation of transcript abundance is controlled by both transcription and RNA degradation. We combine transcriptome-wide and focused approaches to understand the contributions of both of these processes to physiological adaptations to stress, revealing the mechanisms mycobacteria use to tolerate stressors such as hypoxia, reactive oxygen species, and antibiotics.

Cheryl Andam
Assistant Professor of Microbial Ecology
University of New Hampshire, Durham, NH
Cheryl.Andam@unh.edu

The genomic history of prokaryotic organismal lineages is marked by extensive horizontal gene transfer (HGT) between groups of organisms at all taxonomic levels. HGT enables organisms to acquire pre-existing adaptive characters from other organisms, regardless of phylogenetic distance. Thus, instead of genetic traits within lineages always emerging gradually through successive mutations and selection, evolution is accelerated as a parallel process, where inventions made in different lineages can come together in a single cell through HGT. In microbial populations, HGT and recombination (the acquisition of genetic material through genetic exchange of similar DNA sequences) are important processes that can lead to extensive genetic diversity in terms of gene content and allelic variation within a species. While puzzling, within-species heterogeneity in microbes is not uncommon, but the underlying factors that drive this variation remains unclear.

My lab is interested in understanding the processes that contribute to the generation and maintenance of genetic variation in microbial populations. To address this, we study “populations of genomes” that represent clusters of close relatives within and between environments. My lab works closely with the New Hamphire Veterinary Diagnostic Laboratory in studying different *Staphylococcus* species from pets, livestock animals and wildlife. Specifically, we study differences in antibiotic resistance, host adaptation, prevalence of specific lineages in the population and geographical spread. We also study the genomic diversity of antibiotic-producing *Streptomyces* bacteria from diverse habitats and hosts, including bats, earthworms and insects. *Streptomyces* is known as the major source of naturally-derived antibiotics and many pharmaceutically relevant compounds (e.g., antifungals, antitumor, antihelminths, antiprotozoans, immunosuppressants).

2018 Boston Bacterial Meeting – New Faculty

Michelle Larsen
Assistant Professor of Microbiology & Immunology
Albert Einstein College of Medicine
Bronx, NY
michelle.larsen@einstein.yu.edu

Michelle H. Larsen, PhD, is an Assistant Professor of Microbiology & Immunology at the Albert of Einstein College of Medicine. Her doctoral work with Dr. David Figurski at Columbia University on drug-resistance of broad-host-range bacterial plasmids sparked an interest in applying bacterial genetics to infectious disease. As a post-doctoral fellow in the laboratory of Dr. William Jacobs, Jr., she studied bacterial replication and drug resistance of *Mycobacterium tuberculosis.* Those post-doctoral studies were foundational for developing research and teaching projects that include TB vaccine development, transmission and pathogenesis of XDR-TB, biomarkers of difficult to treat patients (i.e. MDR-TB/HIV and XDR-TB), HIV vaccine development, livestock and wildlife tuberculosis, "Many Hosts of Mycobacteria" meeting series, and training of young scientists in sub-Saharan Africa for TB research.

Hesper Rego
Assistant Professor of Microbial Pathogenesis
Yale School of Medicine
New Haven, CT
hesper.rego@yale.edu

For a bacterial infection to linger after antibiotic treatment only a small handful of bacteria need to remain. What is different about these cells from the ones that were easily killed? How do these differences arise? The goal of my lab is to answer these questions, which could lead to therapeutics designed to treat bacterial infections faster and more completely.

In model bacteria, like *Escherichia coli*, much of the focus has been on stochastic mechanisms that underlie cells switching into rare drug tolerant states. However, stochasticity is just one way of generating diversity and many bacterial pathogens take additional routes to variability. In mycobacteria, a genus that includes the human pathogen *Mycobacterium tuberculosis*, asymmetric cell division is a major source of variability. Every time a cell divides it produces daughters with different sizes, growth rates, and susceptibilities to antibiotics. We have recently discovered that this process is genetically encoded; that is, deletion of a single gene, specific to the mycobacterial genus, can collapse much of the heterogeneity created through mycobacterial cell division. Importantly, deletion of this gene – lamA – in *Mycobacterium tuberculosis* results in a population of cells that is killed faster and more completely by a variety of antibiotics. Current projects in my lab include: working out the molecular mechanism of LamA; defining the consequences of LamA-mediated heterogeneity in the context of infection; and discovering other sources of variability, especially metabolic heterogeneity, in single mycobacterial cells.

2018 Boston Bacterial Meeting – New Faculty

Frédéric J. Veyrier, Ph.D.
Professor, INRS-Institut Armand-Frappier
Université du Québec, Laval, Québec, Canada
frederic.veyrier@iaf.inrs.ca

There are at least ten times more bacteria in our body than human cells. Evolution has forced the emergence of several mechanisms that allowed the maintenance and the multiplication of these bacteria in the human microbiome (skin, respiratory, urinary and digestive tracts). In some cases, these symbionts evolved parasitic-type of symbiosis and are responsible for numerous deadly diseases (sepsis, meningitis, pneumonia among others). They represent the world's leading causes of death particularly in the current era of global acquisition of antibiotic resistances. To adapt to the human microbiome, these symbiotic bacteria have undergone a stepwise adaptation through genetic alterations where only permissive changes are selected. Using high throughput sequencing, it is now possible to identify these genetic modifications by performing genomic comparisons with non-symbiotic related species and use these data as the basis of biological experiments that aim at understanding their consequences.

The expertise of our group is now well established in the field of bacterial evolution. In the next four years, we will focus the major part of our research efforts to describe the mechanisms and the consequences of the bacterial cell-wall alteration during establishment of the host-bacteria symbiosis using different models of obligate symbionts such as *Neisseria meningitidis* and *N. gonorrhoeae, Mycobacterium tuberculosis* or accidental pathogens such as *Leptospira interrogans*. Knowing what was necessary for the bacteria to reside, at the outset, in the different human body's ecosystems, is crucial to understand pathogenesis mechanisms and decisive to find innovative and originally new treatment avenues.

2018 Boston Bacterial Meeting – Breakout Sessions

Lunchtime sessions will be moderated by an expert in the field and will include a short informative overview and time for discussion and questions. Breakout sessions have been expanded to both days of BBM, please note on which day the session occurs.

Thursday Sessions

Antibiotic Resistance and Tolerance, Hall E
Adaptive and innate responses provide bacteria with a variety of survival mechanisms in the face of antibiotic stress. Hear the latest on the frontiers of research on bacterial responses to antibiotics.

Panelists: Kim Lewis, Edward Geisinger, Peter Belenky

Little Signals, Big Outcomes, Room 110
Bacteria contain complex, elegant signaling networks that allow them to thrive in a variety of niches. Come hear a discussion on the molecular mechanisms bacteria use to interact with each other and their environment.

Panelists: Michael Laub, Jon Clardy

Careers in Industry, Room 216
Explore a career in industry by hearing from professionals in biotech! Panelists will speak about their jobs, companies, and what you should do to be successful.

Panelists: Brian Meehan (Kaleido Biosciences), Lauren Fitch (Clarivate Analytics), Geoffrey Hannigan (Merck), Vincent Isabella (Synlogic)

The Age of 'Omics, Room 221
Come discuss high-throughput techniques with experts in the fields of metabolomics, transposon sequencing, and microbiome profiling.

Panelists: Tim van Opijnen, Aleksandar Kostic, Julian Avila

How to Win Grants and Influence People, Room 222
In an increasingly competitive funding environment it is essential to write a grant that stands out from the rest. Here we've assembled a winning team of grant writers to talk about writing strategies and grantsmanship that apply to all levels of writers

Panelists: Christina Faherty, Karine Gibbs, Linden Hu, Emily Balskus

Synthetic Biology, Room 304
With the progress in molecular tools, our ability to engineer biology is increasingly limited only by our imagination. Synthetic biology allows for manipulation and utilization of biological systems in unique and often unconventional ways. Come join our panel for a creative conversation on the field at large.

Panelists: Kristala Jones Prather, Lisa Goers, Robert Brewster

2018 Boston Bacterial Meeting – Breakout Sessions

The Host-Pathogen Arms Race, Room 309
Two competing interests, one environment. An action-packed discussion on the myriad ways pathogens fight for their niche and sometimes win.

Panelists: Clare Smith, Cammie Lesser, Tami Lieberman, Brian Russo

Heterogeneity in Bacterial Populations, Room 309a
Your bacteria may be clones, but it doesn't mean they act the same. Here we look at the interesting differences that exist within populations and the remarkable outcomes these discrepancies can produce.

Panelists: Maha Farhat, Bill Hanage, Johan Paulsson

Friday Sessions

Scientific Taste, Hall E
A conversation with keynote speaker Dianne Newman on the question of "What is scientific taste and how subjective is it?". This is a question that can impact students/postdocs/faculty in different ways, from how to pick a research problem, to how to present results for a paper or a proposal, to how to give an effective job talk.

Panelist: Dianne Newman (Keynote Speaker)

Single Cell Techniques, Room 110
From microscopy to quantitative -omics, we now have unprecendented tools to understanding microbes down to the single cell level. Hear from experts on the development and use of single cell techniques.

Panelists: Ethan Garner, Bryan Bryson, Bree Aldridge

Environmental Microbiology, Room 216
Despite being the dominant organisms on earth, we know remarkably little about microbes in the environment. Join our panel to learn about the approaches being taken to breach this gap and study the diversity and interactions of environmental microbes at the individual and population level.

Panelists: Martin Polz, Slava Epstein

Building a Diverse Community of Microbiologists, Room 221
Diversity in STEM profits us all. How do we check our implicit bias and actively build a supportive and diverse community of microbiologists? Hear stories and practical ideas from experienced diversity campaigners.

Panelist: Joanne Kamens (Executive Director of Addgene), Leslie McClain (Instructor at MIT), Whitney Henry (JCC Postdoctoral Fellow at Whitehead Institute), Steven Lopez (Assistant Professor at Northeastern)

2018 Boston Bacterial Meeting – Breakout Sessions

Communication and Public Engagement, Room 222
Have you wondered how best to effectively engage audiences with your science? Effective communication is key! Gain insights from science communication & public engagement experts here!

Panelists: Scott Chimileski (Harvard Museum of Natural History), Katherine Wu (Harvard SITN), Tyler Ford (Addgene Outreach), Morgan Thompson (Harvard Medical School)

Biofilms, Room 304
Delve into the vast, but microscopic world of biofilms in this panel. Their complex nature, including how they form & disperse, and other questions, will be explored here!

Panelists: Yunrong Chai, Neel Joshi, George O'Toole, Katharina Ribbeck

The Microbiome, Room 309
Hear perspectives on the human oral and gut microbiomes, their members, disease states, influences on other systems, and potential therapeutic approaches.

Panelists: Henry Haiser, Xuesong He, Philip Strandwitz

Careers Beyond the Bench, Room 309a
A variety of careers await scientists beyond the bench. Come hear from our speakers who have explored and enjoyed success outside the realms of academia and research industries.

Panelists: April Pawluk (Editor at Cell Press), Rachel Yunck (Technology Specialist a Clark + Elbing), Kate Steblenko (Quality Manager at Jack's Abby Brewing), Garima Bhardwaj (Sales at STEMCELL Technologies), Danielle Gutelius Andruchow (Science Teacher)

2018 Boston Bacterial Meeting – Oral Presentations

Thursday, 9:10 AM – 10:30AM – Session I, Motility

1. **The type IV pili alignment complex, surface sensing, and cyclic-di-GMP signaling in early biofilm formation**
   Shanice S. Webster; Amy E. Baker; Yun Luo; George A. O'Toole
   shanice.s.webster.gr@dartmouth.edu
2. **High throughput 3D tracking of bacterial chemotaxis in complex environments**
   Marianne Grognot and Katja Taute
   grognot@fas.harvard.edu
3. ***Vibrio cholerae* motility exerts drag force to impede attack by the bacterial predator *Bdellovibrio bacteriovorus***
   Miles C. Duncan; John C. Forbes; Y Nguyen; Lauren M. Shull; Rebecca K. Gillette; David W. Lazinski; Afsar Ali; Robert M. Q. Shanks; Daniel E. Kadouri; Andrew Camilli
   miles.duncan@tufts.edu
4. **Experimental directed evolution of a swarming motility-defective mutant of *Pseudomonas aeruginosa* leads to regained motility**
   Sophie Robitaille and Eric Déziel
   sophie.robitaille@iaf.inrs.ca

Thursday, 11:00 AM – 12:20 PM – Session II, Antibiotics and Resistance

5. **The siderophore enterobactin targets ciprofloxacin to Gram-negative pathogens**
   Wilma Neumann; Martina Sassone-Corsi; Manuela Raffatellu; Elizabeth M. Nolan
   wneumann@mit.edu
6. **Amycomicin: an antibiotic that isn't**
   Gleb Pishchany; Emily Mevers; Sula Ndousse-Fetter; Camila R. Paludo; Eduardo A. Silva-Junior; Jon Clardy; Roberto Kolter
   gleb_pishchany@hms.harvard.edu
7. **Phenotypic variation of intracellular ATP concentration impacts persister formation in *E. coli* at a single cell level**
   Sylvie Manuse; Yue Shan; Silvia Canas Duarte; Wei-Sheng Sun; Hirotada Mori; Johan Paulsson; Kim Lewis
   sylvie.manuse@gmail.com
8. **AlgR controls *in vivo* persistence of *Pseudomonas aeruginosa***
   Cristina Penaranda; Deb Hung
   penaranda@molbio.mgh.harvard.edu

Thursday, 1:40 PM – 3:00 PM – Session III, Stress Response Mechanisms and Systems Biology

9. **A legacy role for DNA binding of Lon protects against genotoxic stress**
   Rilee Zeinert; Jing Liu; Qiyuan Yang; Yunguang Du; Cole M. Haynes; Peter Chien
   Rzeinert@umass.edu
10. ***Mycobacterium tuberculosis* mechanisms for survival under antibiotic pressure**
    Michelle Bellerose; Clare Smith; Caitlin Moss; Richard Baker; Seung-Hun Baek; Christopher Sassetti
    Michelle.Bellerose@umassmed.edu
11. **Forecasting bacterial survival-success and adaptive evolution through multi-omics approaches, network analyses and machine learning**
    Karen Zhu; Defne Surujon; Aidan Pavao; José Bento; Tim van Opijnen
    zhuzd@bc.edu

2018 Boston Bacterial Meeting – Oral Presentations

12. **A bacterial chromosome structuring protein binds overtwisted DNA to stimulate type II topoisomerases and enable DNA replication**
Monica S. Guo; Diane L. Haakonsen; Wenjie Zeng; Maria A. Schumacher; Michael T. Laub
msguo@mit.edu

Thursday, 3:30 – 5:00 PM – Session IV, Microbial Ecology

13. **A glimpse into the secret lifestyle of TM7x as a CPR organism**
Batbileg Bor, Lujia Cen, Thao T. To, Jefferey S. McLean, Xuesong He and Wenyuan Shi
bbor@forsyth.org

14. **The symbiotic complex of *Dendroctonus simplex*: implications on the beetle tree attack and life cycle**
Audrey-Anne Durand; Philippe Constant; Eric Déziel; Claude Guertin
audrey-anne.durand@iaf.inrs.ca

15. **Spatial structure in the "Plastisphere": Imaging bacterial communities on plastic marine debris using CLASI-FISH**
Cathleen Schlundt; Jessica L. Mark Welch; Erik R. Zettler; Linda A. Amaral-Zettler
cschlundt@mbl.edu

16. **Heterotroph interactions alter *Prochlorococcus* transcriptome dynamics during extended periods of darkness**
Steven J. Biller; Allison Coe; Sara E. Roggensack; Sallie W. Chisholm
sbiller@mit.edu

Friday, 9:00 AM – 10:20 AM – Session V, Host-Pathogen

17. **Two-photon imaging of *Chlamydia* infection reveals host immune-cell migration to mucosal surfaces**
Jennifer D. Helble; Rodrigo J. Gonzalez; Ulrich H. von Andrian; Michael N. Starnbach
jhelble@g.harvard.edu

18. **PIP3 induces phagocytosis of non-motile *Pseudomonas aeruginosa***
Sally Demirdjian; Daniel Hopkins; Hector Sanchez; Michael Libre; Brent Berwin
sally.a.demirdjian.gr@dartmouth.edu

19. **Diarrheal illnesses trigger *Clostridium difficile* colonization in the human gut**
David VanInsberghe; Bernard Varian; Susan Erdman; Martin Polz
davevan@mit.edu

20. **Re-engineering the cholera pathogen yields a probiotic agent that rapidly protects against cholera**
Troy P. Hubbard; Gabriel Billings; Tobias Dörr; Brandon Sit; Alyson R. Warr; Carole J. Kuehl; Minsik Kim; Fernanda Delgado; John J. Mekalanos; Joseph A. Lewnard; Matthew K. Waldor
troyhubbard@fas.harvard.edu

Friday, 10:40 AM – 12:00 PM – Session VI, Microbial Communities

21. **Discovery of a novel nitric oxide responsive pathway and a putative nitric oxide sensor (NosP)**
Sajjad Hossain and Elizabeth M. Boon
saj.hossain@gmail.com

22. **Self recognition signals induce antibiotic tolerance in the opportunistic pathogen *Proteus mirabilis***
Murray J. Tipping and Karine A Gibbs
mtipping@fas.harvard.edu

23. **Carbon source utilization of the skin Microbiota**
Kristin Loomis; Bryan Brensinger; David Karig

kristin.loomis@jhuapl.edu

24. **Cargo transport shapes the spatial organization of a microbial community**
Abhishek Shrivastava; Visha K. Patel; Yisha Tang; Susan Connolly Yost; Floyd E. Dewhirst; Howard C. Berg
ashrivastava@fas.harvard.edu

Friday, 1:20 PM – 2:20 PM – Session VII, Microbial Engineering

25. **SMRT-Cappable-seq reveals the complex operome of bacteria**
Bo Yan; Matthew Boitano; Tyson Clark; Laurence Ettwiller
yan@neb.com

26. **Live and let die: molecular kill switches in *Mycobacterium tuberculosis* with applications to vaccine development**
Jeffrey C. Wagner; Michelle Gardner; Jacob L. Fohtung; Flavio A. Franchina; Theodore Mellors; Aniek Lotterman; Matthew Zimmerman; Dirk Schnappinger; Véronique Dartois; Jane E. Hill; Sarah M. Fortune; Eric J. Rubin
jwagner@hsph.harvard.edu

27. **Engineered probiotic for the inhibition of *Salmonella* via tetrathionate-induced production of microcin H47**
Jacob Palmer; Emma Piattelli; Beth McCormick; Christopher Brigham; Mark Silby; Vanni Bucci
jpalmer4@umassd.edu

Friday, 4:20 PM – 5:20 PM – Session VIII, Morphogenesis and Cell Surface

28. **Mycobacterial HtrA is required to detoxify a putative cell wall muramidase**
Katherine J. Wu; Cara C. Boutte; Thomas R. Ioerger; Eric J. Rubin
katherinejwu@g.harvard.edu

29. **The *Clostridium difficile* lipoprotein GerS is required for cortex modification and thus spore germination.**
Oscar Diaz; Cameron Sayer; David Popham; Aimee Shen
oscar_r.diaz@tufts.edu

30. **Lipoprotein transport system knockdown reveals a novel cell death trajectory in *V. cholera***
Florence Caro, Nicole M. Place, John J. Mekalanos
florencecaro@gmail.com

2018 Boston Bacterial Meeting - Oral Presentation Abstracts

1 The type IV pili alignment complex, surface sensing, and cyclic-di-GMP signaling in early biofilm formation

Shanice S. Webster[1], Amy E. Baker[1], Yun Luo[1], George A. O'Toole[1]
[1]Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA

Most microbes do not exist as planktonic cells but are found in association with surfaces in structured communities known as biofilms. Biofilm formation is characterized by bacterial cells first detecting that they have engaged a surface, then committing to surface attachment, a process known as irreversible attachment. In the model organism, *Pseudomonas aeruginosa*, we have shown that once cell-to-substratum contact is detected, the bacterial nucleotide molecule, cyclic-di-GMP is upregulated, which results in establishing irreversible attachment. This upregulation in levels of cyclic-di-GMP is modulated by the type IV pili (T4P) alignment complex proteins (PilMNOP) and the diguanylate cyclase, SadC. Here we establish a model wherein the T4P alignment complex protein, PilO, modulates cyclic-di-GMP by regulating the activity of SadC. Using bacterial two hybrid, and cyclic-di-GMP quantification and genetic studies, we show the PilO interacts with SadC and that this interaction stimulates cyclic-di-GMP production, ultimately reducing flagellar motility. Thus, the alignment complex provides a means to coordinate pilus and flagellar function. The T4P alignment complex has been known for its structural role and requirement for efficient T4P pili assembly. We highlight a novel function of the T4P alignment complex in surface sensing and motility coordination, which ultimately promotes the transition to a biofilm lifestyle.

2 High throughput 3D tracking of bacterial chemotaxis in complex environments

Marianne Grognot[1] and Katja Taute[1]
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Chemotaxis has been studied extensively in the peritrichously flagellated model organism *E. coli* but much less attention has been devoted to the diversity of motility patterns displayed by other organisms with different flagellar architectures or to motility in viscous or porous environments that more closely mimic the complexity of natural habitats. We strive to understand how flagellar architecture and environmental properties relate to the resulting bacterial motility pattern, how bacteria achieve chemotaxis with these motility patterns, and how motility patterns might be adapted to specific environments. Current chemotaxis assays are either only at the population scale, qualitative or low throughput, in 2D and/or relying on theoretical models for known flagellar architectures. Here we present a simple yet powerful chemotaxis assay combining a recent high throughput 3D tracking method [Taute *et al.*, Nat. Comm. 2015] with a microfluidically created chemical gradient. We demonstrate that we can directly determine chemotactic drift velocities in different types of environments while simultaneously resolving each individual's 3D motility patterns, enabling unprecedented access to a mechanistic understanding and comparison of chemotactic mechanisms. We highlight the application of our technique to flagellar mutants of the marine bacterium *Vibrio alginolyticus* which conditionally expresses lateral flagella in addition to a single polar flagellum, and show that the chemotactic drift speed depends on both the flagellar architecture and the physical properties of the environment. This work highlights the role of lateral flagella for swimming motility in complex media, in addition to their known role in attaching and swarming on surfaces.

3 *Vibrio cholerae* motility exerts drag force to impede attack by the bacterial predator *Bdellovibrio bacteriovorus*

Miles C. Duncan[1], John C. Forbes[2], Y Nguyen[1], Lauren M. Shull[1], Rebecca K. Gillette[1], David W. Lazinski[1], Afsar Ali[3, 4], Robert M. Q. Shanks[5], Daniel E. Kadouri[6], Andrew Camilli[1]
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The bacterial predator *Bdellovibrio bacteriovorus* is evolved to attack and kill other bacteria, including the human intestinal pathogen *Vibrio cholerae*. Though *B. bacteriovorus* exhibit a broad prey range, little is known about the genetic determinants of prey resistance and sensitivity. Here we perform a genetic screen (Tn-seq) on *V. cholerae* and identify five pathways contributing to predation susceptibility. We find that

the essential virulence regulators ToxR/S increase susceptibility to predation, as mutants of these genes are more resistant to predation. We observe by flow cytometry that lipopolysaccharide provides a critical role in defense, as mutants lacking O-antigen are rapidly attacked by predatory *B. bacteriovorus*. We also demonstrate that while non-motile prey mutants are bound at the same rate as motile prey, *B. bacteriovorus* rounds the non-motile prey into spherical bdelloplasts more quickly. Using a polymer solution of ficoll to alter media viscosity, we find that when *B. bacteriovorus* attacks motile *V. cholerae*, increased drag forces slow its ability to prey. These results provide insights into key prey resistance mechanisms, and may be useful in the application of *B. bacteriovorus* in treating infections.

4 Experimental directed evolution of a swarming motility-defective mutant of *Pseudomonas aeruginosa* leads to regained motility

Sophie Robitaille[1] and Eric Déziel[1]
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Bacteria can live on surfaces or thrive as free-swimming cells in the planktonic state. One type of motility used by bacteria to colonize a surface is swarming. It consists in a coordinated group of bacterial cells moving on a surface. For a functional swarming motility phenotype, the bacterial cells need to express a functional flagellar function, and produce a surface-active agent, to sustain the movement on the surface. We use *Pseudomonas aeruginosa*, an opportunistic bacterium, to better understand this type of motility. Specifically, we have been investigating a mutant in *hptB*, a regulatory gene important for the switch between the motile and non-motile states that has an important swarming motility defect, even though it has a functional flagellum and produces surfactants. To identify the unknown factor necessary for swarming motility missing in this mutant, an experimental evolution experiment was performed to select spontaneous mutants displaying a recovery of the wildtype swarming motility phenotype. After several rounds of swarming and selection of evolved clones, mutants displaying restored swarming were obtained. Complete genome sequencing of a few evolved mutants revealed the emergence of spontaneous mutations in quorum sensing genes. While a wildtype swarming phenotype was regained, most bacteria in the colony do not seem to have acquired a mutation and only a small proportion of the mutants resulting are responsible for the restoration of the swarming phenotype. This suggests that the missing element allowing recovery of the normal swarming phenotype must be a public good available to all bacteria in the group.

5 The Siderophore Enterobactin Targets Ciprofloxacin to Gram-negative Pathogens

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Siderophores are Fe(III)-chelating secondary metabolites that are secreted by bacteria for iron acquisition. Siderophore-utilizing bacteria express dedicated membrane transporters for the uptake of ferric siderophores that can be hijacked for the targeted delivery of antibiotics to pathogens. We report that a conjugate, Ent–Cipro, composed of the siderophore enterobactin (Ent) and the broad-spectrum antibiotic ciprofloxacin exhibits selective antibacterial activity against *Escherichia coli* that express the pathogen-associated *iroA* gene cluster. Our studies demonstrate that conjugation of the siderophore to ciprofloxacin generates an inactive prodrug and guides the antibiotic into the cytoplasm of bacteria that express the Ent uptake machinery (FepABCDG). Intracellular hydrolysis of the siderophore restores the activity of the antibiotic. We show that this hydrolysis requires IroD, a cytoplasmic Ent hydrolase that is expressed only by *E. coli* that harbor the iroA gene cluster and are predominantly pathogenic. EntCipro is one of the rst reported synthetic siderophoreantibiotic conjugate carrying a cytoplasmic antibiotic that exhibits antibacterial activity comparable to the unmodied antibiotic against Gram-negative pathogens, including uropathogenic *E. coli* (UTI89, CFT073). This work highlights that the siderophore uptake and processing machinery can be leveraged for the targeted delivery of cytoplasmic antibiotics, and provides an approach to convert broad-spectrum antibiotics into narrow-spectrum antibiotics. Narrow-spectrum antibacterial approaches are important strategies that can limit the spread of antibiotic resistance and preserve the vitally important microbiota of the human host during antibiotic therapy. This work was supported by the NIH (1R21AI126465, 1R01AI114625) and the German National Academy of Sciences Leopoldina (postdoctoral fellowship LPDS-2015-08 to W.N.).

6 Amycomicin: an antibiotic that isn't

Gleb Pishchany[1], Emily Mevers[2], Sula Ndousse-Fetter[1], Camila R. Paludo[1, 2, 3], Eduardo A. Silva-Junior[2, 3],

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In the environment, bacteria continuously compete and interact with each other. The primary mode of microbial intra- and interspecific communication is through secreted small molecules. The genomes of soil-dwelling bacteria contain numerous biosynthetic gene clusters predicted to produce yet-to-be-discovered small molecules. Many of these clusters remain silent under standard laboratory conditions and are upregulated by interspecies interactions. Some of the secreted products are sought after for their potential antimicrobial properties, however, their true ecological functions remain largely unknown. Using this paradigm, we have been searching for novel antimicrobials produced in multispecies communities. In this way, we identified amycomicin (named after its producer *Amycolatopsis* sp. AA4), a natural product that is induced by interspecies interactions and is bactericidal against *Staphylococcus aureus* at nanomolar concentrations. We solved the structure of amycomicin, identified the genes responsible for its biosynthesis, and determined its mode of action. In *S. aureus*, amycomicin inhibits fatty acid biosynthesis. Interestingly, soil bacteria are resistant to amycomicin. Instead, amycomicin perturbs membrane fatty acid composition and alters the developmental pathways of soil bacteria without killing them in a manner that may be beneficial to *Amycolatopsis*. Therefore, amycomicin while being potently lethal against a human pathogen may have a subtler role in interspecies interactions that play out in soil environments.

7 Phenotypic variation of intracellular ATP concentration impacts persister formation in *E. coli* at a single cell level

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Persisters are a small population of dormant bacteria that can survive high doses of antibiotic treatment, and that largely contribute to the recalcitrance of chronic bacterial infections. Toxin-antitoxin systems and the stringent response have been proposed to contribute to persister formation. Interestingly, recent work from our group shows that persister formation is associated both in *Staphylococcus aureus* and *Escherichia coli* with a drop of ATP which results in a decrease in the activity of antibiotic targets. This suggests that a decrease in ATP is a common underlying mechanism of persister formation in bacteria. We proposed that stochastic noise in protein abundance of energy producing components such as those of the TCA cycle would lead to this drop in ATP and to persister formation. We investigated this hypothesis in *E. coli* by using a combination of fluorescent reporters and single cell approaches such as cell sorting and fluorescent microscopy. By expressing a fluorescent reporter called QUEEN in *E. coli*, we were able to examinate ATP level in single cells by ratiometric fluorescence measurement. We identified a small population of cells in growing *E. coli* that are dormant, low ATP, not lysing when challenged by carbenicillin, and which we suspected to be persisters. By using fluorescent translational fusions of energy producing enzymes, we then identified cells in a growing culture with low abundance of several TCA enzymes that were more tolerant to antibiotic treatment. We found that the mutation of those corresponding TCA genes was impacting both intracellular ATP concentration and persister level. Altogether, our work suggests that cell-to-cell variation in metabolic proteins abundance leads to intracellular ATP depletion that plays a major role for persister formation. Ongoing work is focused on directly linking both metabolic proteins abundance and ATP level in the same cell using time lapse microscopy.

8 AlgR controls *in vivo* persistence of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (PsA) is one of the most virulent opportunistic human pathogens and is often highly resistant to antibiotic therapy. Although it is traditionally considered an extracellular pathogen, PsA has been shown to invade and persist inside mammalian cells. We hypothesize that this ability to persist intracellularly allows bacteria to become antibiotic insensitive, leading to the formation of reservoirs for recurrent infections. We have developed an *in vitro* infection model where we identified that the

two-component response regulator AlgR is required for infection of human bladder epithelial cells. We demonstrate that AlgR controls invasion, via pilus expression, and intracellular persistence, via an unknown mechanism. Furthermore, we show that although its cognate histidine kinase, AlgZ, is also required, AlgR phosphorylation is not required for persistence. RNA profiling of host cells infected with WT PsA shows induction of hallmark TNF-alpha signaling via NFK-b, suggesting that even though host cells recognize infecting bacteria, they are not able to kill them. Finally, we show that AlgR is also required in an *in vivo* model of murine urinary tract infection, demonstrating that the *in vitro* model correlates with chronic infections in a whole animal model. Our results advance our understanding of the requirements for *in vivo* bacterial persistence and identify a potential target for the development of novel antibiotics.

9 A legacy role for DNA binding of Lon protects against genotoxic stress

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The energy dependent quality control protease Lon was originally characterized as a DNA binding protein, yet the physiological consequence of DNA binding is not understood. Here we use the alpha-proteobacteria Caulobacter crescentus to show that the DNA binding ability of Lon is critical for DNA damage tolerance. *In vitro*, DNA can directly activate or inhibit Lon activity depending on the sequence and can also promote degradation of DNA binding proteins by Lon in an adaptor-like manner. Bacteria expressing a DNA-binding deficient Lon variant are phenotypically wildtype with respect to normal growth and response to proteotoxic stresses, but are sensitive to genotoxic stresses arising from either genetic changes or chemical mutagens. Disrupting Lon binding to mitochondria genomes also results in sensitivity to DNA damage but otherwise maintained normal mitochondrial function, consistent with the bacterial ancestry of this organelle. We propose that clearance of persistent proteins from DNA by the Lon protease is an important component of the DNA damage response that originated in free-living alpha-proteobacteria and has been preserved during the endosymbiotic transition to mitochondria.

10 *Mycobacterium tuberculosis* mechanisms for survival under antibiotic pressure

Michelle Bellerose[1], Clare Smith[1], Caitlin Moss[2], Richard Baker[1], Seung-Hun Baek[3], Christopher Sassetti[1]

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Mycobacterium tuberculosis (Mtb) assumes a phenotypically drug-tolerant state during infection, which contributes to both the relative inefficacy of antibiotics and to the development of drug resistance. To probe mechanisms underlying drug tolerance, we performed a comprehensive genetic screen for Mtb mutants with altered susceptibility to antibiotics during infection. Mice were infected with a saturated Mtb transposon mutant library and then treated with the 4 first-line TB drugs alone and in combination. Following *in vivo* selection, we observed mutants that were both over- and under-represented. Mutants more susceptible to treatment identified mechanisms of tolerance, whereas mutants less susceptible provided insights into the development of resistance. These chemical-genetic interactions identified genes that alter susceptibility to individual drugs, as well as genes that alter efficacy of all antibiotics tested. Genes in these groups are involved in a range of functions, such as cell wall formation, DNA binding, efflux pumps, and metabolism. For example, glycerol metabolism mutants were less susceptible to treatment in all conditions. We found that similar glycerol catabolic mutations are common in extensively drug-resistant clinical Mtb isolates, indicating that loss of glycerol utilization may enhance phenotypic drug tolerance and could play a role in development of resistance. The better understanding of Mtb tolerance and response to antimicrobials gained by this genome-wide approach may identify targets for novel treatment strategies.

11 Forecasting bacterial survival-success and adaptive evolution through multi-omics approaches, network analyses and machine learning

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Pathogenic bacteria experience various types of stress during an infection, including the immune system and antibiotics. Therefore, whether a pathogen establishes an infection and/or evolves antibiotic resistance largely depends on its survival-success while enduring and overcoming these stress factors. Previously we showed, to our own surprise, that in response to environmental stress, phenotypic (Tn-Seq) and transcriptional responses (RNA-seq) are largely presented by non-overlapping network patterns. However, detailed analyses revealed specific network patterns that could be indicative of whether a bacterium is familiar with a stress and as a consequence whether it may survive under that stress. By generating genome-wide temporal RNA-Seq and Tn-Seq data-sets and developing new network analyses we show here that distinct response-patterns indeed have important predictive value; i.e. stress-sensitive strains display very different network trajectories compared to stress insensitive or stress-adapted strains. For instance, in the presence of antibiotics a sensitive strain displays a chaotic network trajectory specific for survival-failure while an antibiotic resistant strain is typified by a coordinated network trajectory defining survival-success. Importantly, we show that this approach can also be applied to in vivo data from Pseudomonas aeruginosa and predict survival-success in the host. Lastly, by implementing a machine learning approach we distinguish where in the genome adaptive innovation occurs to overcome antibiotic-stress, thereby enabling the forecasting of the emergence of resistance. This work thus has important value as a potentially new diagnostics approach that can aid in determining the most effective antibiotic treatment and forecast resistance and/or immune escape.

12 A bacterial chromosome structuring protein binds overtwisted DNA to stimulate type II topoisomerases and enable DNA replication

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When DNA is unwound during replication, it becomes overly twisted and can form positive supercoils in front of the translocating DNA polymerase. Unless removed or dissipated, this superhelical tension can impede replication elongation. Topoisomerases, including gyrase and topoisomerase IV in bacteria, are required to relax positive supercoils ahead of DNA polymerase, but may not be sufficient for replication. Here, we find that GapR, a chromosome structuring protein in *Caulobacter crescentus*, is required to complete DNA replication. GapR associates *in vivo* with chromosomal DNA that is positively supercoiled, and our biochemical and structural studies demonstrate that GapR forms a dimer of dimers that fully encircles overtwisted DNA. Further, we show that GapR stimulates gyrase and topo IV to relax positive supercoils, thereby enabling DNA replication. Analogous chromosome structuring proteins that locate to the overtwisted DNA in front of replication forks may be present in other organisms, similarly helping to recruit and stimulate topoisomerases during DNA replication.

13 A Glimpse Into the Secret Lifestyle of TM7x as a CPR Organism

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The Candidate Phyla Radiation (CPR) organisms (70 bacterial phyla) contribute a large diversity to the domain bacteria and may heavily influence bacterial ecology at large due to the predicted symbiotic life style of its constituents. However, their ecological role and pathogenic potential is largely unknown due to their recalcitrance to cultivation. TM7x, isolated from the human oral cavity, is thus far the only cultivated representative of the entire CPR group. TM7x, an obligate epiparasite, lives on the surface of its host, the *A. odontolyticus strain*, XH001. Using TM7x/XH001 as a model CPR/host system, we revealed unusual TM7x trophic behavior. When we infected a host that never seen TM7x before (naïve) with isolated TM7x, the TM7x initiated social predation as seen by 50 TM7x decorating a single host, leading to death of the host. Small portion of the hosts did survive however, forming a long-term stable relationship with TM7x. This stably growing co-culture consisted of three sub-populations: TM7x-infected XH001, uninfected XH001, and host-free TM7x. The infected hosts had reduced cell growth compared to uninfected hosts in the same culture, suggesting a parasitic castration behavior, where the parasite keeps its host alive or uninfected to benefit from a long life. The XH001 cells that are no longer susceptible to the social predation of TM7x had genetic mutations in multiple loci, including transporters as well as regulatory genes. These TM7x facilitated changes likely contribute to naïve XH001's transformation from TM7x's prey to its host, thereby

results in TM7x's lifestyle from predation to parasitic castration.

14 The symbiotic complex of *Dendroctonus simplex*: implications on the beetle tree attack and life cycle

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The eastern larch beetle (*Dendroctonus simplex* Le Conte) is recognized as a serious destructive forest pest in Canada and the USA. Under epidemic conditions, this insect can attack healthy trees, causing severe damages to larch stands. *Dendroctonus* species are considered as holobionts, as they engage in multipartite interactions with diverse microorganisms (bacteria, filamentous fungi, and yeasts). This microbiota is important for many physiological processes of the insect, such as nutrition and protection against antagonistic microorganisms. Furthermore, these microorganisms play a key role in the beetle's attack, as they are responsible for the detoxification of the subcortical environment and weaken the tree's defense mechanisms. Still, the exact implication of the eastern larch beetle microbiota in the colonization success and development remains unknown. We have thoroughly analyzed the bacterial and fungal microbiota of this beetle pest throughout its ontogeny (pioneer adults, larvae and pupae). Additionally, both beetle's microenvironments (ecto- and endomicrobiota) were fully dissected. A successional microbial assemblage was identified throughout the beetle life cycle, reflecting the beetle's requirements to thrive under the bark. Our results indicate that a symbiotic association between the beetle and some of these microorganisms takes place and that this symbiotic complex is helping the insect to colonize its host tree and survive the conditions encountered. Possible functions for these microorganisms are proposed.

15 Spatial structure in the "Plastisphere": Imaging bacterial communities on plastic marine debris using CLASI-FISH

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Plastic marine debris (PMD) is an increasing anthropogenic pollutant in the global ocean. Its effects on marine animals due to entanglement or ingestion are well known, but the interaction of PMD and bacteria is comparatively understudied. To visualize and study bacterial communities on PMD, we applied CLASI-FISH (Combinatorial Labeling and Spectral Imaging – Fluorescence In Situ Hybridization) to investigate the composition and spatial distribution of microbial communities on PMD. We designed oligonucleotide probes targeting major bacterial groups commonly found attached to PMD and validated these probes using pure cultures. We then designed and validated a set of 7 probes, each labeled with a distinct fluorophore and targeting all Bacteria, Alpha- and Gamma-Proteobacteria, marine *Bacteroidetes*, *Vibrionaceae*, *Rhodobacteraceae*, and Alteromonadaceae. In this nested probe set design, most bacterial targets are expected to hybridize with two or more probes, increasing confidence in microbial identification. We applied this probe set to microplastic sampled in coastal North Atlantic water in controlled colonization experiments. Using fluorescence spectral imaging and linear unmixing to differentiate the 7 fluorophores, as well as up to 3 different autofluorescence spectra, we simultaneously identified and visualized these microbes and their spatial distributions on single pieces of PMD. Microbial communities were heterogeneous, with direct spatial interaction among distantly related bacteria and between bacteria and eukaryotes such as diatoms. These results indicate that CLASI-FISH can be used to investigate biofilm development and succession on PMD at micrometer scales.

16 Heterotroph interactions alter *Prochlorococcus* transcriptome dynamics during extended periods of darkness

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Microbes evolve within complex ecological communities where biotic interactions impact both individual cells and the local environment as a whole. In this study, we examined how cellular regulation in the globally abundant marine cyanobacterium *Prochlorococcus* is influenced by a co-occurring heterotrophic bacterium,

Alteromonas macleodii, under different light conditions. We followed the transcriptome of *Prochlorococcus*, grown either alone or in co-culture, across a diel light:dark cycle as well as under the stress of extended darkness – a condition that cells would experience when mixed below the sunlit upper layer of the ocean. More *Prochlorococcus* transcripts exhibited 24-hour periodic oscillations in co-culture than in pure culture, both over the normal diel cycle and following the shift to extended darkness. This demonstrates that biotic interactions, and not just light, can affect timing mechanisms in *Prochlorococcus*, which lacks a self-sustaining circadian oscillator. The transcriptomes of replicate pure cultures of *Prochlorococcus* lost their synchrony within 5 hours of extended darkness, and reflected changes in stress responses and metabolic functions consistent with growth cessation. By contrast, when grown with *Alteromonas*, replicate *Prochlorococcus* transcriptomes tracked each other for at least 13 hours in the dark and showed signs of continued biosynthetic and metabolic activity. The transcriptome patterns suggest that the heterotroph may be providing energy or essential biosynthetic substrates to *Prochlorococcus* in the form of organic compounds, sustaining this autotroph when deprived of solar energy. Our findings reveal conditions where mixotrophic metabolism may benefit marine cyanobacteria and highlight new impacts of community interactions on basic *Prochlorococcus* cellular processes.

17 Two-photon imaging of *Chlamydia* infection reveals host immune-cell migration to mucosal surfaces

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Recent advances in *Chlamydia* genetics have resulted in the development of fluorescently labeled strains of both the human pathogen *Chlamydia trachomatis* and the mouse adapted species *Chlamydia muridarum*. Using these fluorescent strains, we have been able to image infection dynamics *in vivo* for the first time using two-photon microscopy. Two-photon microscopy allows us to image the entire genital tract without the need for tissue sectioning, resulting in 3-D renderings that we can use to track the invading bacteria and responding host cells simultaneously. We discovered that in a murine transcervical model of infection, *C. muridarum* infection leads to a significantly higher number of infected epithelial cells compared to *C. trachomatis* infection, likely signifying differential invasion efficiencies between the strains. We have also utilized fluorescent CD4+ transgenic T cells (NR1 cells) that recognize the dominant *C. trachomatis* antigen to image the *C. trachomatis*-specific T cell response to infection in mice. We have confirmed that NR1 cells can home to the genital tract during *C. trachomatis* infection and that they cluster together in different areas of the genital tract. Ongoing experiments include adoptive transfer studies to investigate whether NR1 T cell clustering is due to localized inflammatory signals or presence of antigen. These experiments demonstrate the power of two-photon microscopy to study *Chlamydia* pathogenesis *in vivo* and the results will further define how T cells can home to barrier sites and respond to a mucosal pathogen.

18 PIP3 Induces Phagocytosis of Non-Motile *Pseudomonas aeruginosa*

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Pathogenic bacteria that establish chronic infections in the lungs of immunocompromised patients frequently undergo adaptation or selection for traits that are advantageous for their growth and survival. Clinical isolates of *Pseudomonas aeruginosa*, a gram-negative, opportunistic bacterial pathogen, exhibit a temporal transition from a motile to a non-motile phenotype through loss of flagellar motility during the course of chronic infection. This progressive loss of motility is associated with increased resistance to both antibiotic and immune clearance. We have previously shown that loss of bacterial motility enables *P. aeruginosa* to evade phagocytic clearance both *in vitro* and in vivo and fails to activate the PI3K/Akt -dependent phagocytic pathway. Therefore, we tested the hypothesis that clearance of phagocytosis-resistant bacteria could be induced by exogenously pre-treating innate immune cells with the Akt activating molecule phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). Here we demonstrate that PIP3 induces the uptake of non-motile *P. aeruginosa* 25-fold by primary human neutrophils and this effect is phenocopied with the use of murine phagocytes. However, surprisingly, mechanistic studies revealed that the induction of phagocytosis by PIP3 is because polyphosphoinositides promote bacterial binding by the phagocytes rather than Akt activation. Moreover, this induction was selective, since the uptake of other non-motile gram-negative, but not gram-positive bacteria, can also be induced by PIP3. Since there is currently no treatment that effectively eradicates chronic *P. aeruginosa* infections, these findings identify a methodology by which to induce

clearance of non-motile pathogenic bacteria and provide novel insights into the endogenous determinants of phagocytic recognition of *P. aeruginosa*.

19 Diarrheal illnesses trigger *Clostridium difficile* colonization in the human gut

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Although it is widely held that *Clostridium difficile* is an antibiotic and hospital associated pathogen, recent evidence indicates that this is an insufficient depiction of the risks and reservoirs. In fact, the rate of infection continues to rise despite the implementation of patient isolation, increased hospital sanitation, and antibiotic stewardship protocols. A common thread that links all known major risk factors of infection is that they are all associated with gastrointestinal disturbances, but the relationship with *C. difficile* colonization has never been tested directly. Here we show that disturbances caused by increased intestinal flow-rate trigger susceptibility to *C. difficile* colonization. We first detected *C. difficile* blooms in human gut microbiome surveys following *Salmonella* and *Vibrio cholerae* associated diarrheal disease that remained untreated with antibiotics. Carriers remained colonized for year-long time scales, but *C. difficile* abundance was highly variable over time, where short one-to-two day periods of increased shedding and weeks where C. difficile was undetectable were common. To directly test the importance of increasing intestinal flow-rate on susceptibility to colonization, we developed a mouse model of *C. difficile* colonization. As mice ingested increasing quantities of laxatives while being exposed to avirulent *C. difficile* spores, more mice experienced blooms of *C. difficile* in their feces, while control groups experience none. Our results suggest that the likelihood of colonization is highest in the days immediately following acute disturbances. Therefore, the recovery period after diarrheal illnesses could be an important target for preventing transmission and lowering the incidence of infection.

20 Re-engineering the cholera pathogen yields a probiotic agent that rapidly protects against cholera

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Outbreaks of cholera, a rapidly fatal diarrheal disease, often spread explosively. The efficacy of reactive vaccination campaigns - deploying *Vibrio cholerae* vaccines during epidemics - is partially limited by the time required for vaccine recipients to develop adaptive immunity. We created HaitiV, a live attenuated cholera vaccine candidate, by deleting diarrheagenic factors from a recent clinical isolate of *V. cholerae* and incorporating safeguards against vaccine reversion. We demonstrate that administration of HaitiV 24hrs prior to lethal challenge with wild-type *V. cholerae* reduced intestinal colonization by the wild-type strain, slowed disease progression, and reduced mortality in an infant rabbit model of cholera. HaitiV-mediated protection required viable vaccine, and rapid protection kinetics are not consistent with development of adaptive immunity. These features suggest that HaitiV mediates probiotic-like protection from cholera, a mechanism that is not known to be elicited by traditional vaccines. Mathematical modeling indicates that an intervention that works at the speed of HaitiV-mediated protection could improve the public health impact of reactive vaccination.

21 Discovery of a Novel Nitric Oxide Responsive Pathway and a Putative Nitric Oxide Sensor (NosP)

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In nature, most bacteria live in surface attached communities called bacterial biofilms. Community living is beneficial to the bacteria, as it offers heightened resistance to environmental stresses due to the production of a protective exopolymeric matrix. Bacterial biofilms can be beneficial to us as is the case

with those found in our gastrointestinal tracks that help us digest food particles. However, biofilms can pose a major threat to human health when they are comprised of pathogenic bacteria that cause chronic infections. In order for bacteria to switch between a free-swimming and biofilm state, they rely on responding to various environmental stimuli, including nitric oxide (NO), a diatomic gas molecule that has been shown to modulate biofilm formation in many bacteria. In some bacteria, NO is sensed by the H-NOX (heme nitric oxide/oxygen binding protein) protein, a homologue of the mammalian nitric oxide sensor sGC (soluble guanylate cyclase). However, many bacteria that respond to NO, including the opportunistic pathogen *Pseudomonas aeruginosa*, lack an *hnoX* gene; therefore, suggesting the presence of an alternative NO sensing protein. Here we present the discovery of a novel NO sensing protein (NosP). We demonstrate that *P. aeruginosa* NosP is able to ligate to NO via the ferrous iron of a heme cofactor. By disrupting a NosP associated histidine kinase, we illustrate a defect in NO-mediated biofilm dispersal in *P. aeruginosa*. Further, we investigate the pattern of NosP and NosP associated effector protein signaling biochemically in *P. aeruginosa*, *Vibrio cholerae*, and *Legionella pneumophila*. Considering all our findings, we propose that NosP is a primary NO sensor which shares no sequence homology to the H-NOX proteins. Thus, for the first time, we highlight a novel NO signaling pathway in bacteria, providing a strong foundation for future research.

22 Self recognition signals induce antibiotic tolerance in the opportunistic pathogen *Proteus mirabilis*

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Cells in swarms of the opportunistic pathogen *Proteus mirabilis* can distinguish self from nonself: in swarms of two different strains, one strain can non-lethally exclude the other from the expanding colony edge. This territorial exclusion is mediated by the Ids locus, which uses a contact-dependent system to transfer the identity protein IdsD to neighboring cells. Here we show that IdsD transfer induces nonself cells to enter a previously unknown state incompatible with swarming, characterized by reduced expression of genes associated with protein synthesis, virulence and motility. We demonstrate that this lifestyle change also allows cells deemed nonself to tolerate previously lethal concentrations of antibiotics. We probe the dynamics and timescales of entry into this state and explore how varying the numbers and types of social encounters during swarming affects the emergence of territorial exclusion. We propose a model in which Ids acts as a policing mechanism, effectively enforcing clonality in advancing swarm fronts.

23 Carbon Source Utilization of the Skin Microbiota

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The metabolic activity of the skin microbiota influences the chemical composition of the skin surface, contributing to the health of our skin. Additionally, the metabolic activity of the skin microbiota leads to the secretion of volatile organic compounds, which can attract or repel disease-carrying mosquitos. However, the carbon sources utilized by individual skin taxa are not well characterized. Here we have developed an assay to experimentally determine how commensal microbes utilize carbon sources that are present in healthy human skin. We have developed an assay, inspired by the commercially available Biolog microbial characterization plates, to assess how skin commensals utilize 73 unique carbon sources that are relevant to healthy human skin, including sweat and daily hygiene products. We are using this assay to assess the carbon source utilization of microbes in our recently acquired collection of skin isolates. This skin isolate collection is sourced from 17 healthy human subjects and comprises 800 different bacterial and fungal strains. We have selected 50 of these isolates representative of the skin microbiota and have found wide variations in their patterns of carbon source utilization. Every tested carbon source in our assay was utilized by at least one skin taxa, and no two taxa were found to have identical carbon source utilization patterns. We are currently analyzing this data to assess how well phylogenetic relationships explain carbon source utilization patterns. We expect this data could be used to develop strategies for modulation of the skin microbiome based on metabolic profiles.

24 Cargo Transport Shapes the Spatial Organization of a Microbial Community

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The human microbiome is an assemblage of diverse bacteria that interact with one another to design a community. Rapid availability of nutrition and protection from antibiotics are advantages of a specific spatial niche within a microbial community. The mechanisms that drive microbial interactions and guide the architecture of microbial communities via collective motion are unclear. Bacteria of the genus *Capnocytophaga* are abundant in human oral microbial communities and have the genes for bacterial Type IX Secretion System (T9SS) and gliding motility. Via a mobile cell surface adhesin SprB, single gliding cells were able to attach to 7 abundant non-motile bacterial species of the human microbiome. The attached non-motile bacteria were propelled as 'cargo' along the length of a gliding cell. Multi-color fluorescent spectral imaging of live bacterial cells within a polymicrobial community showed long range transport of non-motile cargo bacteria by a collectively moving swarm. Tracking of fluid flow patterns via gas bubbles, and fluorescently labeled single cells showed hierarchy within a swarm. Our results suggest that gliding bacteria from the human microbiome synchronize the transport of non-motile microbes to form pre-defined spatial structures. We find that some non-motile microbes of the community use this mode of public transport more efficiently than others.

25 SMRT-Cappable-seq reveals the complex operome of bacteria

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Current methods for genome-wide analysis of gene expression depend on short-read sequencing. Therefore it is difficult to identify the bacterial operon structure since the phasing of transcripts start and end is challenging. Additionally, *in vivo* processing of transcripts confounds the accurate identification of the 5' and 3'ends of operons. Here we describe SMRT-Cappable-seq as the first experimental methodology to identify operon structures genome-wide in prokaryotes. It combines the isolation of unfragmented primary transcripts with single-molecule long read sequencing. Applied to *E. coli*, SMRT-Cappable-seq results in an unprecedented definition of the transcriptome with 34 percent of the known operons being extended by at least one gene, and identifies a total of 2300 operons from which around 900 are novel. Furthermore, our result reveals a pervasive read-through of previous experimentally validated transcription termination sites. Termination read-through represents a powerful strategy to control gene expression. Taken together this technology presents an invaluable resource for the study of operon, gene network and regulation in bacteria.

26 Live and Let Die: Molecular Kill Switches in *Mycobacterium tuberculosis* with Applications to Vaccine Development

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Human challenge studies utilizing model strains are a powerful tool for the development of effective treatments and vaccinations for infectious disease. Due to the lengthy required treatment time, high rate of relapse, and difficulty in quantitative assessment of bacterial load, no human challenge strain currently exists for the pathogen *Mycobacterium tuberculosis*. What is needed is an effective kill switch or set of kill switches which could effectively eliminate the bacteria from a human host at the end of trial without the need for an antibiotic course. Such a strain could also have applications as a vaccine strain itself by allowing the host a longer time to generate adaptive immunity than currently existing strains. Towards this end, we have adapted a trimethoprim responsive destabilization tag for use in mycobacteria. Utilizing genetic engineering techniques, this tag can be inserted at the N-terminus of a essential proteins in the M. tuberculosis genome. Such strains survive when trimethoprim is present to stabilize the protein and die when trimethoprim is absent, in essence converting an antibiotic to probiotic. In addition, we have utilized an orthogonal system for the incorporation of a non-canonical amino acids (NAAs) into essential proteins. We have developed a strain of *Mycobacterium smegmatis* that is dependent on the supplementation of the NAA for survival. Further, this strain will grow for multiple generations in the absence of the NAA before dying, creating a "molecular fuse" which would be essential for measuring treatment outcomes. By using multiple

kill switches in combination we can drive the escape rate to an estimated 10pwr-14 escapes/generation and have the potential to be used as a human challenge strain or a genetically engineered vaccine strain.

27 Engineered Probiotic for the Inhibition of *Salmonella* Via Tetrathionate-Induced Production of Microcin H47

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Complications arising from antibiotic-resistant bacteria are becoming a major issue in modern medicine. Members of drug-resistant *Enterobacteriaceae* spp. include opportunistic pathogens that are among the leading causes of morbidity and mortality worldwide, and outgrowth of these bacteria is considered a strong indicator of intestinal dysbiosis. Microcins (small antimicrobial peptides) produced by some gut commensals have the potential to cure these conditions by inhibiting a narrow range of organisms and have been proposed as a viable alternative to antibiotic treatment. Here we develop a genetically engineered probiotic to inhibit *Salmonella* spp. when in an environment with tetrathionate, a molecule produced in the mammalian inflamed gut during the course of a *Salmonella* infection. We developed a plasmid-based system which conferred the ability to detect and utilize tetrathionate, while at the same time secreting microcin H47 (MccH47). We transferred this plasmid-based system to *Escherichia coli* and demonstrated the ability of the engineered strain to inhibit growth of *Salmonella* in anaerobic conditions while in the presence of tetrathionate, with no evidence of MccH47 production in the absence of tetrathionate. In direct competition assays between engineered *E. coli* and *S. Typhimurium*, the engineered probiotic had a significant increase in fitness in the presence of tetrathionate as compared to the absence of tetrathionate. In this work, we have demonstrated the ability to engineer a strain of *E. coli* capable of using an environmental signal indicative of intestinal inflammation as an inducing molecule, resulting in production of a microcin capable of inhibiting the organism potentially responsible for the inflammation.

28 Mycobacterial HtrA is required to detoxify a putative cell wall muramidase

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Tuberculosis is a leading cause of death worldwide, infecting a third of the global population. While *Mycobacterium tuberculosis* must withstand numerous and diverse stresses in harsh host environments, many of the mechanisms underlying Mtb's ability to main homeostasis remain elusive. The periplasmic protease HtrA has been predicted to be essential in mycobacteria, but is required only under stress in nearly all other bacteria; thus, we hypothesized HtrA has a novel function in mycobacterial physiology. To test this, we confirmed HtrA essentiality in *Mycobacterium smegmatis* and found it is only essential in the presence of a potentially hyperactive cell wall regulation pathway defined by *ami3*, which encodes a putative cell wall muramidase, and *pmt*, which encodes a protein O-mannosyltransferase. Under normal circumstances, HtrA inhibits the activity of fully active, O-mannosylated Ami3 through degradation. However, in the absence of HtrA, Ami3 becomes dysregulated, accumulating and compromising cell wall integrity. Loss of either *ami3* or *pmt* is sufficient to relieve the essentiality of *htrA*, and the toxicity of Ami3 is dependent on its catalytic activity. Thus, HtrA represents an unusual case of conditional essentiality dictated by gene content rather than by environmental conditions. Our work describes a novel mechanism of mycobacterial homeostasis and a previously undescribed role for an essential HtrA homolog.

29 The *Clostridium difficile* lipoprotein GerS is required for cortex modification and thus spore germination.

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Clostridium difficile is a Gram-positive, spore-forming bacterium that is a leading cause of antibiotic-associated diarrhea. *C. difficile* infections begin when its metabolically dormant spores germinate to form toxin-producing vegetative cells. Successful spore germination depends on the degradation of the cortex, a thick layer of modified peptidoglycan that maintains dormancy. Cortex hydrolysis is mediated by the SleC cortex hydrolase, which is thought to recognize the cortex-specific modification, muramic delta-lactam. *C. difficile* cortex hydrolysis also depends on the *Peptostreptococcaceae*-specific lipoprotein GerS for unknown reasons. In this study, we tested whether GerS regulates production of muramic delta-lactam and thus controls SleC's ability to recognize its cortex substrate. By comparing the muropeptide profiles of *gerS* spores to spores lacking either CwlD or PdaA, which mediate cortex modification in *Bacillus subtilis*, we determined that *C. difficile* GerS, CwlD, and PdaA are all required to generate muramic delta-lactam. Both GerS and CwlD were needed to cleave the peptide side chains from N-acetylmuramic acid, suggesting that these two factors act in concert. Consistent with this hypothesis, biochemical analyses revealed that GerS and CwlD directly interact and that CwlD modulates GerS incorporation into mature spores. Since *gerS*, *cwlD*, and *pdaA* spores exhibited equivalent germination defects, our results indicate that *C. difficile* spore germination depends on cortex-specific modifications, reveal GerS as a novel regulator of these processes, and highlight additional differences in the regulation of spore germination in *C. difficile* relative to *B. subtilis* and other spore-forming organisms.

30 Lipoprotein transport system knockdown reveals a novel cell death trajectory in *V. cholerae*

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Essential gene products are attractive antibiotic targets because their functions are necessary for the life of a cell and they are often conserved. Unlike their non-essential counterparts, essential genes are poorly studied *in vivo* because they are refractory to knockout and require genetic or chemical complementation for growth. Here we screened a set of putative essential genes for growth inhibition using CRIPSR-interference (CRISPRi) knockdown in the human pathogen *V. cholerae* and identify the lol system as a vulnerable regulator of membrane biogenesis. The lol system is a central hub for directing lipoproteins from the inner to the outer membrane with many of these lipoproteins coordinating their own essential processes. Lol knockdown was bactericidal in both exponential and stationary phase and resulted in a unique form of cell death. We observed that after lol depletion, cells undergo elaborate membrane dynamics in which the periplasm extrudes into a bubble encased by outer membrane, specifically at plasmolysis sites. Our work reveals a novel cell death pathway and identifies the lol system as an ideal drug target, which is conserved in Gram negative bacterial pathogens.

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1 Cell Shape Perturbations Alter Lethality of Aminoglycoside Treatment

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Antibiotic combinations are used clinically to improve treatment efficacy and counter antibiotic resistance. In particular, ribosome targeting aminoglycoside antibiotics are frequently combined with the cell wall synthesis inhibiting beta-lactams. Despite this frequent usage, we do not fully understand the mechanism of synergy between these classes which makes designing effective combination treatments challenging. Here we measure killing of *E. coli* by combinations of aminoglycosides with a panel of beta-lactams that have distinct target specificities and resulting effects on cell shape. We find that beta-lactams that induce sphere formation are antagonistic with aminoglycosides, while beta-lactams that inhibit cell division and lead to filamentation are synergistic. We have tested this model with other classes of small molecules affecting cell shape and observed similar shape-dependent outcomes when combined with aminoglycosides, including synergy between aminoglycosides and the DNA-damaging quinolone antibiotics, which block cell division through activation of the SOS response. We are currently testing other genetic and biochemical perturbations altering cell shape to investigate the generality of and mechanisms underlying this model. Collectively, this work suggests that the efficacy of aminoglycoside combinations with different beta-lactams reflects, in part, differing effects of the beta-lactam on cell shape, and that screening small molecule libraries for effects on cell shape may identify novel adjuvants for aminoglycoside antibiotics.

2 Growth conditions determine cost of bearing a mobile genetic element in *Bacillus subtilis*

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Mobile genetic elements are important drivers of bacterial evolution by horizontal gene transfer. Several strains of subtilis contain a mobile genetic element, ICEBs1, which transfers between cells by conjugation. ICEBs1 is regulated by cell-cell signaling and self-sensing, which favors induction of the element at high cellular densities but low relative abundance of ICEBs1+ cells. We have found that in biofilms containing a mix of cells with and without ICEBs1, ICEBs1 increases in abundance in the population through conjugation and a selective advantage provided to its host cells. This fitness benefit requires induction of ICEBs1 and only occurs when the ICEBs1+ cells are inoculated as the minority in the population. In contrast, when co-cultures of cells with and without ICEBs1 are grown in liquid media, the ICEBs1+ cells have a selective disadvantage. This fitness burden also requires ICEBs1 induction and is stronger when the ICEBs1+ cells are inoculated at low relative abundances. We hypothesize that the cost of ICEBs1 in liquid culture is due to constitutive induction of the element due to dilution of its inhibitory peptide throughout the culture volume. In biofilms, the inhibitory peptide builds up locally, shutting down ICEBs1 induction after a period of activation. We hypothesize that the cost of ICEBs1 comes from its plasmid-like replication within the host cell following induction, and that some gene or genes within ICEBs1 provide a selective advantage to the host.

3 Urogenital schistosomiasis is associated with signatures of microbiome dysbiosis in Nigerian adolescents

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Urogenital schistosomiasis is a neglected tropical disease that affects millions in the developing world, particularly in Africa. It is caused by the parasite *Schistosoma haematobium*, which lives in the vasculature surrounding the bladder and produces eggs which cause significant host pathology. Previous work has shown that helminth infection can have dramatic impacts on the immune system and some may alter the composition of the intestinal microbiome. Alterations in microbiome composition or function can have significant impacts on human health, particularly when such changes lead to dysbiosis, inflammation, or pathobiont blooms. Therefore, we sought to determine whether infection with *S. haematobium* can impact the gut microbiome. We sequenced the V4 hypervariable region of the 16S rDNA of the faecal bacteria of *S. haematobium*-infected and -uninfected adolescents from Kebbi State, Nigeria, to analyze differences in the diversity or taxonomic composition of the gut microbiome. We found differential community composition in the microbiota of the groups, driven by decreases in the abundance of Firmicutes and increases in the abundance of Proteobacteria in the infected cohort. More specifically, we saw changes in various taxa that

were similar to alterations seen in patients with dysbiosis, such as increases in *Desulfovibrio* and decreases in *Clostridiales*. Changes to specific genera were confirmed with qPCR, and functional potential analysis indicated an enrichment in urease genes, which has been linked to inflammation. Overall, our results indicate that *S. haematobium* infection in adolescents is associated with pervasive changes in the gut microbiota that may be indicative of dysbiosis.

4 Macrophage phenotypic state impacts the response to and outcome of infection with *Mycobacterium tuberculosis*

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Despite the widespread availability of first-line antibiotics, tuberculosis remains the leading worldwide cause of death due to an infectious disease. There is dire need for new drugs and vaccines to target its causative agent, *Mycobacterium tuberculosis* (Mtb), but disappointments in vaccine development have highlighted an expansive gap in our understanding of host control of Mtb infection. Recent work has shown that Mtb survival and replication can be restricted at a local level by functionally distinct lesions and cells, suggesting that heterogeneous immune states affect infection outcome. Differential polarization of monocyte-derived macrophages (MDM) may contribute to this variability. To test this, we have polarized human MDM *in vitro* with the cytokines M-CSF or GM-CSF and identified multifaceted functional divergence between these macrophage states—in terms of immune responses to purified stimulants and live Mtb, the ability to restrict Mtb replication, and macrophage foam cell phenotype. These differences inform an investigation of how different macrophage phenotypes impact the requirements for bacterial survival during infection, which will begin to uncover the mechanisms of sterilizing immunity to Mtb.

5 Characterizing transcriptional dynamics of *Mycobacterium tuberculosis* infection with dual RNAseq

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Mycobacterium tuberculosis (Mtb) is the leading infectious killer in the world. Key to its pathogenic success is the ability to establish a replicative niche within host innate immune cells, macrophages. Simultaneous profiling of host and bacterial transcriptomes (dual RNAseq) can identify transcriptional networks that mediate the host-pathogen interaction, revealing new therapeutic targets. To gain insights into how Mtb transcriptionally adapts to the intracellular macrophage environment we developed a method to perform dual RNAseq on Mtb infected macrophages. We apply dual RNAseq to characterize a novel Mtb transcription factor (TF) important for intracellular replication. Upon phagocytosis by macrophages, Mtb permeabilizes its containing phagosome leading to activation of the host type I IFN response. However, a mutant of this TF fails to activate the type I IFN response. Previously, secretion of EsxA by the bacterial type VII secretion system, ESX-1, has been proposed to permeabilize the Mtb containing phagosome. Surprisingly, this TF mutant secretes EsxA *in vitro* and does not differentially expresses the ESX-1 operon in macrophages. Though, it up-regulates a homologous secretion system, ESX-5, during infection. We show that structural components of ESX-5 affect Mtb membrane permeability, which is supported by our finding that that this mutant displays membrane permeability defects. We are currently investigating the role of ESX-5 and ESX-1 system regulation in Mtb membrane permeability and phagosomal escape within the host cell. Ultimately, linking bacterial transcriptional regulation within macrophages to corresponding host cell responses will offer new insights into the intracellular mechanisms of Mtb pathogenesis.

6 Exploring *Acetobacter-Lactobacillus* Interactions in the *Drosophila Gut*

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The gut microbiome is an important contributor to animal health and homeostasis. *Drosophila* is an excellent model system for studying the gut microbiota and its interactions with the host. In particular,

Drosophila has a simplified gut microbiome compared to other animals, containing between five and twenty members instead of tens or hundreds. *Acetobacter pasteurianus*, *Lactobacillus brevis*, and *Lactobacillus plantarum* are bacterial species commonly found in the fly gut. The interactions between these species confer greater benefit to the host than mono-association with any one species. Our data indicates that gut colonization by *A. pasterianus* is enhanced by the presence of *Lactobacillus* spp. This growth enhancement is also observed in liquid and solid media, and on fly food. Our results suggest that *Lactobacillus* spp. improve the growth environment for *A. pasteurianus* across many different conditions. Additionally, the different *Lactobacillus* species have slightly different effects on the growth enhancement of *Acetobacter* and are themselves enhanced when in co-culture. Further work will determine the specificity of this interaction and identify the mechanisms underlying the growth promotion. Specifically, we are exploring the metabolites and genes that are differently regulated when *Acetobacter* and *Lactobacillus* are grown in mono- and co-culture, and whether other bacteria are able to also enhance *Acetobacter* growth. Our long-term goal is to understand the different microbial and host factors that leads to successful establishment of the microbiome and to explore their long-term impacts on the function and evolution of the gut bacterial community.

7 CD59 Indirectly Supports Type III Secretion System Function in *Yersinia pseudotuberculosis*

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The type III secretion system (T3SS) is a highly conserved virulence-associated complex found in the envelope of many Gram-negative bacteria that translocates substrate proteins into host cells through channels formed in the host membrane. In the gastrointestinal human pathogen *Yersinia pseudotuberculosis* (Yptb), the translocated Yop proteins function to misregulate the innate immune response and disrupt cell signaling. Previous RNA interference (RNAi) screens identified mammalian proteins important for the function of YopE, a GAP protein that inactivates host Rho-family GTPases. Among the top hits was the GPI-linked surface protein CD59, which is localized in lipid rafts and has been shown to act as a receptor for several bacterial pore forming toxins including intermedilysin (ILY). This work aims to reveal the role of CD59 in T3SS-mediated Yersinia infection. CD59 CRISPR-KO 293T cells revealed that host cells lacking CD59 impair T3SS function as demonstrated by decreased translocation and pore formation. To determine if CD59 is a direct receptor, mammalian cells were pretreated with a pore deficient ILY mutant that binds CD59 and then infected with Yptb. Blocking CD59 did not impair T3S, indicating CD59 has an indirect role in T3SS function. Stable cell lines expressing low, WT, and high CD59 levels were established to assess rescue of the KO. Interestingly, cells with low and high CD59 expression exhibited resistance to pore formation during Yptb infection, while WT expression remained most susceptible. This evidence suggests CD59 may have a role in maintaining a specific lipid raft composition critical for Yptb T3SS function.

8 Topology of the Type 3 Secretion System Translocon Pore Defined at Pore Formation

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Shigella are intracellular pathogens and a leading cause of diarrhea worldwide. *Shigella* establish infection by invading into colonic cells. They require a protein delivery system known as a type 3 secretion system (T3SS) to cause disease. The T3SS, which is conserved among 30 pathogens, injects effector proteins into eukaryotic cells. T3SS effectors remodel cellular processes to promote disease. The injection of effectors depends on docking of the T3SS on the plasma membrane. The T3SS delivers bacterial proteins that form a pore in the host membrane, onto which the T3SS apparatus then docks. Docking activates secretion of effectors through the pore that connects the bacterial cytoplasm with the eukaryotic cytosol. Thus, docking is a critical step in pathogenesis. Docking of the T3SS requires interaction of the pore with host intermediate filaments (Russo et al., Nature Microbiology 2016). In the absence of intermediate filaments, the pore assembles but the T3SS is not activated. Here we tested whether intermediate filaments altered the conformation of the pore in the membrane. To do so, we stabilized the conformation of the pore as it is formed using disulfide bond crosslinking. We found that at the time of pore formation, disulfide bonds efficiently form at all tested residues irrespective of intermediate filaments. Moreover, crosslinking the pore at the time of infection restricts docking of the T3SS onto the pore. These data support a model in which the pore inserts in a docking-incompetent conformation and interacts with intermediate filaments

to under a change to support docking.

9 Investigating host-pathogen interactions of *Yersinia pseudotuberculosis* in the intestine using enteroid monolayers

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The enteric bacterial pathogen, *Yersinia pseudotuberculosis* (Yptb), causes acute intestinal infection and young children are a particularly vulnerable population. Although many studies have described various Yptb factors required for colonizing intestinal tissues in mice, we do not fully understand the detailed molecular host-pathogen interactions that occur between Yptb and human hosts. *In vitro* cell culture systems provide a means of visualizing host-pathogen interactions, however they are often used with immortalized cell lines that do not fully mimic the microenvironment and diversity of cells found in the intestine. To improve physiological relevance to the intestine, we have employed enteroids that can be cultured to express the four major subpopulations of cells found in the intestine. Using enteroids seeded as monolayers on transwells, we have found that during infection, Yptb binds sparsely to monolayers that lack M cells. When M cells are present in the monolayer, we detect clusters of Yptb bound preferentially to M cells. Further, relative IL-8 mRNA is upregulated by enteroid monolayers after 5 hour infection with Yptb compared to uninfected monolayers, which triggers neutrophil migration. Ongoing studies seek to identify bacterial and host factors involved in Yptb binding, colonization, and damage to enteroid derived monolayers. Addition of immune cells and microbiota will enable us to study the effects that these physiologically relevant intestinal features have on enteric infection.

10 Identification and Characterization of Human IgG and IgA Monoclonal Antibodies for Immunoprophylaxis Against Enterotoxigenic *Escherichia coli* Infection

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Background. Enterotoxigenic *Escherichia coli* (ETEC) cause significant diarrheal illness in infants in developing world and travelers to endemic countries including military personnel. ETEC infection of the host involves colonization of the small intestinal epithelium and toxin secretion leading to watery diarrhea. There is currently no vaccine licensed to prevent ETEC. CFA/I is one of the most common colonizing factor antigens (CFAs). The CFA/I adhesion subunit, CfaE, is required for ETEC adhesion to host intestinal cells. Human antibodies against CfaE have potential to block colonization of ETEC and serve as a potent immunoprophylactic against ETEC-related diarrhea. Methods. Mice transgenic for human immunoglobulin genes were immunized with CfaE to generate a panel of human monoclonal IgG1 antibodies (HuMAbs). The most potent IgG1 identified in the *in vitro* functional assays were selected and isotype switched to secretory IgA (sIgA) and tested in colonization assays in animals via oral administration. Results. Over 300 unique anti-CfaE IgG1 HuMabs were identified. The lead IgG1 anti-CfaE HuMAbs completely inhibited hemagglutination and blocked adhesion of ETEC to Caco-2 cells. Epitope mapping studies revealed that HuMAbs recognized epitopes in the N-terminal domain of CfaE near the putative receptor binding site. Oral administration of anti-CfaE antibodies in either IgG or sIgA isotypes inhibited intestinal colonization in mice challenged with ETEC. Two to four log decrease of colony forming units was observed as compared to irrelevant isotype controls. Conclusions. We identified a panel of fully human monoclonal antibodies against CfaE adhesion domain that can be potentially employed to prevent ETEC-related diarrhea.

11 A synthetic approach to investigate *Shigella*-induced macrophage cell death

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The cytosol of mammalian cells involved in the host innate immune response, including macrophages and intestinal epithelial cells, contains sensors that, upon activation by microbial-derived molecules, trigger the

activation of inflammasomes. This results in the maturation and secretion of pro-inflammatory cytokines and cell death. During an infection, *Shigella*, the causative agents of bacillary dysentery, traverse the epithelial layer and are engulfed by macrophage cells. Once internalised, the intracytoplasmic *Shigella* trigger rapid macrophage cell death, resulting in the release of bacteria that invade the basal lateral surface of nearby epithelial cells and subsequently spread throughout the colonic epithelium. *Shigella*, and many other Gram-negative pathogens, use a type III secretion system (T3SS) to directly inject proteins, known as effectors, into the cytosol of the mammalian host cells. *Shigella* effectors act to modulate host pathways, but the targets of many effectors remain poorly characterized, as traditional top-down approaches using knock-out strains are hindered by functional redundancy of effectors. By introducing a minimal but functional T3SS into T3SS-free strains of *Shigella* and laboratory strains of *E. coli* our group has developed a novel bottom-up platform to investigate the roles of individual *Shigella* T3SS components and effectors in specific steps of pathogenesis. Using these *Shigella* models, as well as macrophage knockout lines of various inflammasome components, we will address questions about how pathogens are detected inside infected human cells, why *Shigella* induce cell death of macrophages, but not epithelial cells, and how *Shigella* can use effector proteins to manipulate host cell behaviour.

12 T2Bacteria® Panel: A rapid, culture independent assay for identification of sepsis causative agents

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There are 1.35M cases of sepsis a year, costing $20B and a 30% mortality rate. The time to result for blood culture, the standard for diagnosis, delays patient treatment; which increases patient mortality. A culture independent assay requires sensitivity at clinically relevant titers while maintaining specificity and performance in the presence of interferents. T2 Biosystems developed the T2Bacteria Panel to identify the most common causes of sepsis and provide rapid, actionable data to physicians. Here we discuss data demonstrating analytical sensitivity and specificity, the effect of interferents on assay performance, competitive inhibition, and performance at clinically relevant titer levels. For these studies healthy donor blood was spiked with target bacterial species at clinically relevant concentrations. For analytical sensitivity, samples were tested across multiple sample preps and reagent lots with a criteria of 95% positivity rate. Specificity was determined using an initial *in silico* screen, followed by testing of clinically relevant species. Interfering substances, both endogenous and exogenous, were selected based on their potential presence in patient specimens and tested at high physiological concentrations. Contrived sensitivity was determined for each species in individual donor blood samples at clinically relevant titers. Our studies show that the T2Bacteria Panel provides species identification direct from blood. The combination of target-specific primers and probes provides specificity to prevent cross-reactivity with non-target species. Contrived sample data demonstrates a sensitivity of 91% for targets and a specificity of 97%. These data demonstrate that the T2Bacteria Panel provides a sensitive diagnostic for clinicians to provide effective therapy.

13 Targeting of nascent transcripts by Hfq and Crc mediates translational silencing

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Hfq is a key post-transcriptional regulator in bacteria, associating with RNAs to influence diverse cellular processes including growth, virulence, and catabolism. We performed a modified chromatin immunoprecipitation protocol followed by high-throughput sequencing (ChIP-Seq) to show that Hfq associates with hundreds of genes, but only when transcription is actively occurring. This suggests that Hfq functions in a co-transcriptional manner, associating with target transcripts as they are being synthesized. Among the genes that are associated with Hfq are several known targets of Crc, a translational repressor that regulates carbon catabolism in *Pseudomonas aeruginosa*. Crc, like Hfq, also appears to associate with its targets co-transcriptionally. Our ChIP-Seq data identified several genes not previously known to be regulated by Hfq and Crc. In addition, we found that Crc and Hfq influence each other's ability to associate with nascent transcripts. Combining ChIP with transcription inhibition may be a broadly useful strategy in studying the co-transcriptional activities of RNA binding proteins. Targeting transcripts as they are being synthesized represents an efficient, and perhaps common, mechanism for post-transcriptional regulators to exert their

regulatory effects.

14 A novel PriA mutant sequesters PriB away from the replication fork

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In *Escherichia coli*, PriA helicase recognizes abandoned replication forks and directs reloading of the replisome onto the lagging strand coated with the single-stranded DNA-binding protein (SSB). The cell governs this essential process via two major and functionally redundant pathways, PriA-PriB and PriA-PriC, and a minor PriC pathway. *priA* null mutants are poorly viable, have high levels of SOS expression and chromosome partitioning defects. Despite recent advances in understanding of the structural mechanisms underlying the replication restart, very little is known about how PriA is recruited onto the fork. Crystallographic studies have revealed that SSB binds to an evolutionarily conserved site on PriA. *In vitro*, a missense mutation R697A disrupts PriA-SSB interaction. In this study, we tested its significance *in vivo*. A *priA341* (R697A) mutation was placed on the chromosome (as well as two other mutations affecting the SSB-binding pocket). The *priA341* mutation alone was not sufficient to perturb cellular phenotype and had only little effect in a *priB* mutant strain. Overproduction of PriA341 in a *priC* background, however, led to a *priA* null phenotype. To explain this phenotype, we suggest that PriA341 has a higher affinity to PriB than the wild type PriA. Thus, when PriA341 is overproduced it acts as a sink for PriB, sequestering it away from the replication fork. Additional experiments testing this hypothesis will be presented.

15 An alternative ClpP isoform modulates Clp protease activity during stationary-phase growth in *P. aeruginosa*

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Clp proteases are central to regulated protein degradation in bacteria and thus provide an important mechanism for controlling bacterial physiology. Clp proteases are composed of one of several hexameric AAA+ protein unfoldases in complex with the 14-subunit compartmentalized protease ClpP, thus directly coupling substrate unfolding to degradation. Although most bacteria contain a single ClpP homolog, some species, including the opportunistic pathogen *P. aeruginosa*, contain two ClpP paralogs (*clpP1* and *clpP2*). We recently identified distinct requirements for *clpP1* and *clpP2* in biofilm development and virulence factor production. Additionally, although ClpP1 is constitutively expressed, ClpP2 protein expression is restricted to stationary phase. These data suggest that although substrate selection is performed by the Clp unfoldase, alternative assemblies of ClpP may also specify Clp protease complexes with different biological roles. To investigate a mechanistic basis for such differentiation, we compared the activities of ClpP1 and ClpP2 *in vitro*. ClpP1 is fully active as a homo-oligomer; in contrast, ClpP2 is only active in a ClpP1/P2 hetero-complex. ClpP1/P2 complexes exhibited a slower rate of peptide hydrolysis than ClpP1 complexes, but an equivalent rate of proteolysis when in complex with AAA+ ATPases. Our biochemical data and structure of a ClpP2 homo-complex also indicated that ClpP1/P2 complexes have only a single binding site for a AAA+ unfoldase, in contrast to the two sites present in ClpP1 complexes. We are currently pursuing a structure of the hetero-complex and testing how the different biochemical parameters of ClpP1/P2 hetero-complexes may contribute to the formation and pathogenicity of *P. aeruginosa* biofilms.

16 Impact of interspecies interactions on microbial coexistence

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Many important microbial functions happen within communities of interacting species. Explaining how species with disparate intrinsic fitness can stably coexist is important for a wide range of applications: maintaining natural ecosystems, engineering industrial communities, or managing persistent infections. Previous studies of coexistence have primarily abstracted interactions to the ecological level and avoided interaction mechanisms. Here we study coexistence in a mathematical model that explicitly incorporates chemicals (such as metabolites and waste products) that mediate interactions among microbes. We use simple experimental case studies in a variety of conditions to constrain the assumptions of our model. By simulating

many instances of enrichment in this model, we search for properties of enriched communities that could explain species coexistence. We show that interactions that facilitate growth are strongly favored in enriched communities. Among negative influences, we find that self-restraint, such as production of self-inhibiting waste, contributes to coexistence, whereas inhibition of other species disrupts coexistence. Importantly, coexistence is enhanced when interactions are mediated via *depletable* chemicals (i.e. those that get consumed or degraded by cells) compared to when interactions are mediated via *reusable* chemicals that remain in the environment. Our findings underscore the importance of incorporating the mechanisms of microbial interaction in predicting coexistence.

17 Costless metabolic secretions as drivers of interspecies interactions in microbial ecosystems

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Metabolic exchange can mediate beneficial interactions among microbes, helping explain diversity in microbial communities. These interactions are often assumed to involve a fitness cost, prompting questions on how cooperative phenotypes can be stable and withstand the emergence of cheaters. Here we use genome-scale models of metabolism to investigate whether a radically different scenario, the pervasive release of costless metabolites (i.e. those that cause no fitness cost to the producing organism), can serve as a prominent mechanism for inter-microbial interactions. By carrying out over 1 million pairwise growth simulations for 14 microbial species in a combinatorial assortment of environmental conditions, we find that there is indeed a large space of metabolites that can be secreted at no cost, which can generate ample cross-feeding opportunities. In addition to providing an atlas of putative costless interdependencies, our modeling also demonstrates that oxygen availability significantly enhances mutualistic interactions by providing more opportunities for metabolic exchange through costless metabolites, resulting in an over-representation of specific ecological network motifs. In addition to helping explain natural diversity, we show how the exchange of costless metabolites can facilitate the engineering of stable synthetic microbial consortia.

18 Point mutations in the RNA polymerase holoenzyme specifically reduce susceptibility to third-generation cephalosporins in clinical isolates of *Neisseria gonorrhoeae*

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Widespread antimicrobial resistance in Neisseria gonorrhoeae has limited the effective treatment options. Cephalosporins remain one of the few classes of antibiotics recommended for gonococcal infections, but reduced susceptibility to the third-generation cephalosporins, including ceftriaxone, has emerged. Most reduced susceptibility to ceftriaxone is caused by an alternative *penA* (PBP2) allele. However, the isolates with the highest-level ceftriaxone resistance identified by the Centers for Disease Control and Prevention's surveillance system lack this allele as well as other characterized genetic contributors to reduced cephalosporin susceptibility. To identify the genetic basis of resistance in these isolates, we employed an undirected transformation strategy. From three of these strains, we isolated two different mutations in the major housekeeping sigma factor *rpoD* and one in the RNA polymerase subunit *rpoB* that each independently conferred ceftriaxone resistance. The resistance caused by these changes is not a general tolerance response: these mutations neither changed the growth rate *in vitro* nor altered susceptibility to other classes of antibiotics (including penicillin). These mutations have a genetic background dependency, as we succeeded in using these mutations to achieve ceftriaxone resistance in gonococcal strains from some phylogenetic lineages but not from others. This genetic background dependence may explain the failure of previous approaches to identify RNA polymerase holoenzyme mutations as a potential mechanism for cephalosporin resistance, and may also prove instrumental in elucidating the mechanism by which altered RNAP alleles result in cephalosporin resistance.

19 The Type VI Secretion System Regulator A Activated Chemotaxis at the Expense of Virulence Factor Expression

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Vibrio cholerae contains a Type VI Secretion System (T6SS) that delivers toxic effector proteins across the membrane of a target cell. In the seventh pandemic strain of O1 El Tor C6706, the T6SS is repressed by quorum sensing and the uncharacterized protein VC0070 (TsrA) *in vitro*. To expand our knowledge of TsrA and its regulon, we conducted an RNASeq experiment to measure the transcriptome of a *tsrA* mutant and wild type. In the TsrA mutant, we detected an up-regulation of T6SS operons and virulence factors such as the toxin co-regulated pilus (tcp) and cholera toxin. We also detected a repression of chemotaxis genes in the mutant. The chemotaxis genes found to be significantly regulated were not those essential for chemotaxis, in standard conditions, but the TsrA mutant does show impaired chemotaxis ability. The TsrA regulon not only represses virulence factor expression, but also suggests a mechanism to activate chemotaxis genes. This inverse correlation of virulence factor expression and chemotaxis regulated by TsrA for C6706 has potential to provide insight into *V. cholerae* colonization and pathogenesis.

20 A host-induced conformational change activates the type 3 secretion system

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Type 3 secretion systems (T3SSs) are encoded by 30 bacterial pathogens and are required for their virulence. T3SSs deliver bacterial effector proteins into the cytosol of host cells, where the effector proteins reprogram normal cellular processes and establish a niche for the pathogen. How T3SSs are activated for secretion is incompletely understood. Host contact activates the T3SS to form the translocon pore in the host plasma membrane, the T3SS then docks to the extracellular face of the translocon pore and delivers the effector proteins through the translocon pore. Rather than a passive role, we showed an active role of the translocon pore for T3SS activity. Host cytoskeletal proteins, known as intermediate filaments, interact with the translocon pore and promote docking by unknown mechanisms. Here an approach was developed to investigate the translocon pore in the host plasma membrane during bacterial infection of cells. The topology of the translocon pore was compared in the presence of intermediate filaments to the absence of intermediate filaments using the substituted cysteine accessibility method (SCAM) in combination with cellular fractionation. Docking was associated with increased accessibility of residues at the extracellular face of the translocon pore, where T3SS is predicted to interact with the translocon pore. Our data support a model in which intermediate filaments cause conformational changes to the translocon pore to support docking. This is the first report to identify a host protein that induces a conformational change in the T3SS, which is associated with T3SS activation

21 Role of hydrolases in flagellar construction and stability in *Bacillus subtilis*

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Bacterial murein hydrolases are proteins that remodel peptidoglycan (PG), a mesh like structure that protects the cells from high internal osmotic pressure. As PG mechanically protects the cell, it must be rigid but also needs to be flexible enough to be remodeled in order for the cell to grow, divide and insert macromolecular machines. The flagellum is one of these macromolecular machines, which must be inserted through the PG during the assembly process. Flagellar insertion and stability is aided by hydrolases which are capable of cleaving the bonds in the PG. CwlQ a poorly studied hydrolase in *Bacillus subtilis* that is induced by elements of the main flagellar synthesis operon and is required for flagellar stability and motility. Preliminary data has shown that expression of CwlQ is under the control of an alternative sigma factor, D, a regulatory protein important for flagellar construction. A *cwlQ* mutant has a motility defect and fluorescence microscopy has revealed that mutant cells do not have a morphology defect but that the flagella appear to break from the cell body, suggesting the motility defect is due to flagellar instability. In a forward genetic approach transposon mutagenesis and high throughput sequencing was performed to find other hydrolases/proteins that are necessary for flagellar insertion and stability. These experiments will allow us to determine what proteins are required for flagellar stability and how flagellar stability is related to fundamental aspects of bacterial cell envelope synthesis and turnover.

22 dropletTn-Seq combines microfluidics with Tn-Seq to identify genome-wide complex single-cell phenotypes

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Pathogenic bacteria have evolved mechanisms to overcome a wide variety of environmental disturbances including starvation, the host immune system and antibiotics. We lack a comprehensive understanding of how pathogens are able to survive such diverse stresses partially due to the complexity of uncovering the genetic origin of a phenotype. Transposon insertion sequencing (Tn-Seq), a technique developed by our lab, utilizes pooled libraries of transposon insertion mutants to untangle the genetic complexity of a phenotype by accurately calculating mutant growth rates on a genome-wide scale. The pooling and batch culture of insertion mutants is one characteristic that makes Tn-Seq so powerful, however it is unclear how interactions between mutants in a pool affect the fitness of individual mutants. To address this question, we have developed a new approach by combining Tn-Seq with droplet-based microfluidics (dTn-Seq), which allows for culturing of single-cell mutants within individual environments on a genome-wide scale. We show that approximately 2-5% of mutants have different growth fitness when cultured as single cells compared to batch culture. Moreover, we explain in detail for several genes how their complex phenotypes are dependent on community structure. These genes include ones that code for enzymes involved in breaking down the host alpha-1-acid glycoprotein, and we discovered a novel involvement of the peptidoglycan hydrolase lytB in bacterial competence and regulated cell death. These results demonstrate that dTn-Seq can accurately identify novel mutant phenotypes previously masked by pooled mutant batch culture more quickly and cheaply compared to alternative methods such as ordered mutant libraries.

23 Mapping LexA-RecA Interactions

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The SOS response is the archetypal example of a DNA damage response. This type of response is critical for genomic integrity in all organisms. In *Escherichia coli*, the SOS response consists of a transcriptional repressor, LexA, and a sensor, RecA. DNA damage causes the production of ssDNA. RecA then binds to the ssDNA forming a RecA-DNA helical filament. LexA interacts with this filament in its major groove. This increases LexA's rate of auto-proteolysis, lowering the amount of LexA in the cell and de-repressing the SOS regulon. The points of contacts between RecA and LexA in this key reaction are unknown. To determine residues in LexA that were important for this interaction, we devised a genetic selection. Relying on the observation that dsDNA and LexA compete for binding in the major groove of the RecA-DNA filament, we overproduced an uncleaveable form of LexA, called LexA3 (G85D), which would compete with dsDNA binding *in vivo* such that the strain would be unable to recombine DNA. *Dam* mutants accumulate double strand breaks and require recombination for growth; hence LexA3 overproduction would be lethal in *dam* mutants. Thirteen new missense mutations were isolated in *lexA* using this method. These are now being separated from *lexA3* and placed on the *E. coli* chromosome in the place of wildtype *lexA*. This poster will describe the position of these amino acids changes in the crystal structure of LexA, our progress in the phenotypic characterization of these mutants and our model for RecA-LexA interactions.

24 Expanding the Host Specificity of MAGE

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Genome editing capabilities in *Escherichia coli* have been accelerated over the past two decades with the development of recombineering and MAGE (Multiplexed Automatable Genome Engineering). The promise of recombineering technology, however, has not been fully realized, as it has been difficult to port into even closely related bacterial species. Successes have been minor (comparatively low efficiencies), sporadic, and have involved significant investment of time to screen candidate single-stranded annealing proteins (SSAPs). Here we present a method for more easily moving recombineering technology into new host organisms, demonstrating its efficacy in diverse bacteria. We present a better understanding of the interactions that are important between SSAPs, which mediate recombineering at the replication fork, and bacterial host proteins. We further lay out a strategy for improving recombineering efficiency in a novel bacterium.

25 Tracking human gut resistome dynamics using metagenomic shotgun and 10x linked read sequence data

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The human gut microbiome is an important reservoir of antibiotic resistance, and factors affecting the composition of resistance elements (the resistome) are still poorly understood. Furthermore, while antibiotic resistance genes (ARGs) can be detected with short read sequencing data, identifying their chromosomal or plasmid host is challenging, especially for species at low abundance. We quantified resistome dynamics over the course of a year in a cohort of 28 women with and without recurrent urinary tract infections (UTIs), an infection accounting for 10% of all US antibiotic prescriptions. We sequenced stool samples using both Illumina and 10x Genomics platforms in order to determine both the impact of antibiotic use on the resistome and to explore the genomic context of ARGs. All study participants harbored resistance to multiple classes of antibiotics; the most common were beta-lactam and tetracycline. In 11/14 antibiotic classes, the burden of antibiotic resistance was higher for patients who reported antibiotic use during the study vs. those who did not, and we observed perturbations in the resistome associated with UTIs and antibiotic treatment. Antibiotic exposure was significantly associated with an increase in the abundance of genes conferring resistance to the administered drug ($p=0.035$). Levels of many specific ARGs were strongly correlated, suggesting coexistence on the same chromosome or plasmid. Indeed, metagenomic assembly revealed some correlated ARGs to be present on the same contig. Using 10x linked read data, assemblies revealed contextual information to annotate and categorize ARGs, even for relatively low abundance species such as *Escherichia coli*.

26 Impacts of Acute Hyperglycemia on the Response of the Murine Microbiome to Antibiotic Exposure

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Despite major advancements in microbiome research over the past decade, the data are still insufficient to mechanistically explain why specific bacteria within a microbial community are negatively impacted by antibiotics while others are not. Additionally, it is unknown how host-dependent factors influence the microbiome's response to antibiotic therapy. Recent work has demonstrated that the microbiome is profoundly sensitive to host metabolism, and thus, it is likely that host metabolic state plays an important role in the responses of the microbiome to antibiotic perturbation. Interestingly, the impacts of hyperglycemia on these responses have not been extensively studied despite knowledge that metabolic syndromes are associated with a drastically altered microbiome and reduced antibiotic efficacy. We hypothesize that the host's metabolic state can impact antimicrobial susceptibility in the microbiome by activating both tolerance and resistance pathways related to, or regulated by, microbial metabolism. Using a streptozotocin-induced model of acute hyperglycemia, we combined 16S rRNA taxonomic profiling with whole community metatranscriptomics to study the impacts of antibiotic therapy on the murine microbiome. We found that, upon exposure to either beta-lactam or fluoroquinolone antibiotics, hyperglycemic and normoglycemic mice exhibited noticeable variation in taxonomic composition and responses. This both highlights microbiome sensitivity to host-related factors and suggests that host glycemic status might attribute to observed variations of antimicrobial responses of the microbiome.

27 Human calprotectin induces iron starvation and inhibits phenazine production in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that is responsible for severe infections in immunocompromised individuals and cystic fibrosis (CF) patients. Over an infection time course, *P. aeruginosa* becomes the predominant pathogen of an initially diverse CF lung microbiome, and persists

for decades as a chronic infection. Pathogen sensing by the lung epithelium induces a pro-inflammatory response, and unresolved infection over extended periods of time leads to an extreme inflammatory state. As part of this response, neutrophils that are recruited to an infection site exhaust their first lines of defense (phagocytosis and granule release) and resort to the formation of neutrophil extracellular traps (NETs). As the most abundant protein in the cytoplasm of neutrophils, the antimicrobial metal-sequestering protein calprotectin (CP, heterooligomer of S100A8 and S100A9) is released during NET formation, and accumulates in the lungs of CF patients as infection persists. Human CP exhibits antimicrobial activity attributed to its ability to withhold transition metal ions at two binding sites: a His3Asp motif (site 1) and a His6 motif (site 2). Previous work has focused on the zinc and manganese sequestration by CP. Recent work in the Nolan lab has uncovered high-affinity ferrous iron chelation at the versatile His6 site. Here we examine the implications of metal sequestration by CP for *P. aeruginosa* physiology. Our investigation demonstrates that CP inhibits pseudomonal iron uptake, induces an iron starvation response, and inhibits phenazine production, the latter of which is indicative of altered pseudomonal virulence.

28 Shared mycobacterial strategies for undermining innate immune recognition

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Tuberculosis is a major threat to global public health, having risen to be the leading cause of death by infection. A key feature of mycobacterial infection is the ability of the causative bacterium, *Mycobacterium tuberculosis* (Mtb), to survive within macrophages; the ways in which Mtb evades a protective innate immune response that could clear infection still remain opaque. The Mtb ESX-1 protein secretion system has previously been shown to be essential for permeabilizing Mtb-containing phagosomes and triggering the macrophage Type I IFN response. In recent work, we found that the Mtb outer lipid phthiodimycocerosate (PDIM) is similarly required for phagosome permeabilization and downstream induction of type I IFNs. In our current work, we describe a second shared phenotype between ESX-1 and PDIM mutants: both generate an enhanced macrophage NF-kB-dependent pro-inflammatory response to infection. We find that this response is entirely dependent on both MYD88 and TLR2, and that the signal requires uptake into phagosomes. The enhanced inflammation observed in response to infection with PDIM or ESX-1 mutants is entirely independent of cytosolic PRR pathways known to be activated by Mtb infection. Collectively, our work demonstrates a new, shared functionality between PDIM and ESX-1 in blunting a productive TLR2-dependent macrophage inflammatory response to Mtb infection.

29 *Edwardsiella piscicida* enters non-phagocytic cells via macropinocytosis and caveolin-dependent endocytosis in a cholesterol-, and dynamin-dependent manner

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Invading into non-phagocytic cells is a key step for pathogenic bacteria to establish infection *in vivo*. *Edwardsiella piscicida* (*E. piscicida*, previously known as *Edwardsiella tarda*) is an important pathogen which could infect a wide range of host from fish to human. Recent work has demonstrated that *E. piscicida* could invade into multiple non-phagocytic cells, but the internalization mechanism remains elusive. Here we employed HeLa cells as a non-phagocytic cell model to investigate the endocytic strategy of a pathogenic *E. piscicida* strain EIB202. By a combination of fluorescence microscope and transmission electron microscope, EIB202 was showed to induce obvious membrane ruffles and F-actin rearrangements in HeLa cells upon bacterial infection. Further, the internalization of EIB202 significantly depended on activity of Na+/H+ exchangers and multi signaling events related to macropinocytosis. These data proved that *E. piscicida* utilized host macropinocytosis pathway to invade into HeLa cells. Moreover, the uptake process of EIB202 was also showed to be caveolin, dynamin and cholesterol-dependent, but clathrin-independent. These data signified the involvement of multiple endocytic mechanisms in the internalization of *E. piscicida*. In addition, *E. piscicida* was showed to move into early endosomes labeled with Rab5 after entry. In conclusion, our work revealed that *E. piscicida* enters HeLa cells via a macropinocytosis and caveolin-dependent endocytosis with the involvement of cholesterol and dynamin, providing us a better understanding of how *E. piscicida* interacts with non-phagocytic cells.

30 Environmental controls on extracellular vesicle production by marine microbes

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Extracellular vesicles are produced by diverse bacterial taxa in environments ranging from the human body to the global oceans. These small, membrane-bound particles (100nm diameter) represent a versatile cellular secretion mechanism, and can contain biomolecules such as proteins, DNA, RNA, and small metabolites. Extracellular vesicles have been implicated in numerous biological processes including quorum sensing, horizontal gene transfer, pathogenicity, and phage defense. Due to their potentially diverse functions and bioactive cargo, vesicles likely play important roles mediating microbial interactions and the transport of cellular material between cells. Vesicles are abundant in marine environments, but their distribution varies throughout the water column, likely reflecting variation in vesicle production, degradation, and uptake rates. To begin to explore the factors influencing this pattern, we measured vesicle release rates in cultured representatives of globally abundant marine microbial taxa, including *Cyanobacteria*, *Proteobacteria*, and *Bacteroidetes*, using nanoparticle tracking analysis. We found that vesicle production varies among different taxa grown under identical conditions. While there was some relationship between vesicle production rates and cell size, the data suggested that other environmental factors influence vesicle production as well. To explore this, we studied the effects of light and temperature on vesicle release in multiple strains of the globally abundant marine cyanobacterium *Prochlorococcus*. We found that *Prochlorococcus* cells exhibit order-of-magnitude differences in vesicle production rates as a function of changes in both temperature and light intensity. These findings support a combination of physiological and environmental drivers as contributing to the distribution of extracellular vesicles in the oceans.

31 Novel Two-Step Method for Isolation and Enrichment of New *Leptotrichia* Species

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Out of 688 bacterial species inhabiting the human oral cavity, 224 taxa remain as-yet-uncultivated. *Leptotrichia* species are Gram-negative, anaerobic, saccharolytic rods. They are opportunistic pathogens associated with several diseases and isolated from both immunocompromised as well as immunocompetent subjects. *L. buccalis* was the first of six species to be cultivated from human dental plaque. Previously, 11 species had been cultured (6 named and 5 unnamed). The whole genomes of 9/11 cultured *Leptotrichia* sp. have been obtained. Nine additional oral *Leptotrichia* sp. remain to be cultivated (HOT 217, 219, 218, 221, 223, 392, 417, 463, 909). The objective of this study is to culture uncultivated *Leptotrichia* sp. either in isolation or in co-culture. From a 35-subject-9-site study, plaque samples from subject/sites with high levels of *Leptotrichia* sp. were cultured in modified versions of previously published media, i.e. brain heart infusion (BHI) with yeast extract (Y), hemin (H), and 1.4-dihydroxy-2-naphthoic acid (D). Here we report a two-step isolation protocol for *Leptotrichia* sp. The initial isolation plate has BHIYHD agar with crystal violet, nicotinamide adenine dinucleotide (NAD), fetal calf serum and erythromycin and predominantly isolates *Leptotrichia* sp., and few other bacteria. The second step was growth on an enrichment plate BHIYHD with sheep blood, mucin and NAD which allowed robust growth and expansion of the *Leptotrichia* sp. DNA was isolated for16S rRNA sequencing analysis, and assembled to confirm identity. Using the above culture techniques we successfully isolated 4 out of the 9 uncultivated *Leptotrichia* sp. and are currently isolating additional species.

32 Shedding Light on Oral Microbial Dark Matter: Quorum Sensing Modulates the Epibiotic-Parasitic Relationship between *Actinomyces odontolyticus* subspecies *actinosynbacter* strain (XH001) and its epibiont, a TM7 phylotype (TM7x)

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Recent metagenomic analyses illuminated an entire domain of life, denoted Candidate Phyla Radiation (CPR), with over 35 phyla constituting 15% of the bacterial domain. As the only cultivated representative of the CPR group, Saccharibacteria, TM7x, was co-isolated with its bacterial basibont host, *Actinomyces odontolyticus* subsp. *Actinosynbacter* strain, XH001. *Actinomyces* and *Saccharibacteria* are associated with multiple human systemic diseases, notably periodontitis. Additionally, *Actinomyces* species are early microbial colonizers in the oral cavity and the relationship between XH001 with TM7x may influence the oral microbiota composition and pathogenesis of disease. Transcriptomic analysis (RNA-seq) revealed a set of differentially regulated genes within XH001 when associated with TM7x as compared to parasite-free XH001. The highest upregulated gene, an *lsrB* orthologue, the receptor for the auto inducer (AI) 2 signaling molecule. This study involved creating a genetic manipulation system for XH001 to elucidate the role of AI-2-mediated quorum sensing in the epibiotic-parasitic relationship between XH001 and TM7x. We created XH001*lsrB* and XH001*luxS* single deletion mutants and subjected them to the following phenotypic analyses: growth kinetics, biofilm formation capacity, and quantification of the AI-2 signaling molecule. *lsrB* upregulation was confirmed and XH001*lsrB* and XH001*luxS* single deletion mutants were successfully verified. Phenotypic analyses provided data suggesting the association with TM7x enhances wildtype XH001's biofilm formation capability. Confocal microscopy determined biofilm formation deficiencies in TM7x associated XH001*lsrB* and XH001*luxS*. Furthermore, XH001*luxS* displays an ablated AI-2 production signal compared to wildtype. Current studies involve rescuing XH001*lsrB* and XH001*luxS* mutants and further molecular characterization of AI-2 signaling in XH001.

33 Linking cellular regulation in *Prochlorococcus* to global niche partitioning in heterotrophs

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Dissolved organic carbon is a major component of the ocean carbon cycle, and microbial activity is the primary factor underpinning its composition and distribution. Major challenges are to unravel the pathways involved in the production and consumption of organic carbon, the distribution of pathways across microbial taxa, and the mechanisms controlling their flux. We used comparative metabolomics and phylogenomics to examine the evolution of organic carbon production in the highly abundant phytoplankter *Prochlorococcus*. We find that unexpectedly high levels of thymidine are exported by *Prochlorococcus*, and that export is greater in more recently diverging strains. Indeed, over the course of our experiments, the total accumulated extracellular thymidine exceeds the total amount of thymidine incorporated into DNA. In addition, we find that *Prochlorococcus* cells excrete significant amounts of adenine and guanine under P-limited relative to P-replete conditions, which we interpret as an increased pressure to scavenge intracellular phosphate. To understand the ecological consequences of nucleotide export by *Prochlorococcus*, we further examined the genomes of the highly abundant co-occurring heterotrophic bacterial groups SAR11, SAR86 and SAR116. We find widespread genetic capacity to recycle purines, but not thymidine, in SAR11 and SAR116 and widespread genetic capacity to recycle thymidine, but not purines, in SAR86. This suggests niche partitioning due the evolution of pathways involved in the cross-feeding among these abundant oceanic microbes. We end by discussing evidence that excretion of nucleosides and nucleobases reflects cellular regulatory processes in *Prochlorococcus*, possibly related to its cell cycle.

34 Microbial Metabolism is a Major Determinant of Antibiotic Susceptibility Within the Microbiome

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The rise of antibiotic resistance has severely hampered the efficacy of our current antibiotic arsenal. To increase the efficacy of existing antimicrobial drugs, it is essential that we understand how antibiotics impact both the pathogenic and beneficial members of the microbiome. The goal of this project is to profile the effects of antibiotics on the function of the total microbial community *in vivo* to provide mechanistic insight into the impacts of therapy on the microbiome. We hypothesize that the induction of tolerance mechanisms, particularly those related to the metabolic state of the bacteria, mediate toxicity to antibiotic exposure in susceptible members of the microbiome. Using traditional 16S rRNA amplicon sequencing, we demonstrate that three different classes of antibiotics (beta-lactams, fluoroquinolones, and tetracyclines)

cause distinct taxonomic shifts and reduce diversity within the gut microbiota of mice. Additionally, we utilized metagenomic and metatranscriptomic analysis to characterize the functional changes elicited by each class of antibiotics. We demonstrate that each antibiotic elicits unique metabolic changes among specific members of the microbiome and in the population as a whole. Furthermore, our data suggests the the resulting communities are less metabolically active following antibiotic treatment. Together, these data suggest that metabolic activity may be a determinant of antibiotic susceptibility within the gut microbiome and that depressing metabolic state may serve a tolerance mechanism *in vivo*.

35 Polymicrobial communities in mucus environments

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Human beings coexist in a delicate equilibrium with a vast microbial ecosystem that profoundly influences human health and disease. Substantial efforts are currently focused on identifying the microbiota composition and its association with health and disease states. However, little is known about host-produced factors that influence microbe-microbe interactions, and specifically prevent bacterial competition and keep diversity within the microbial community. Here we need *in vitro* systems, which faithfully reflect the physiological habitat of the microbiome, and thus, enable a characterization of potential key factors of microbiome regulation. One interesting factor to consider is the mucus covering epithelial surfaces. We have established an interdisciplinary set of tools, which allow the detailed study of bacterial interactions in mucus-like environments, such as: i) a biochemical strategy to purify and reconstitute the key structural components from complex mucus, ii) fluorescence imaging to monitor live mixed bacterial communities in mucus-like gels, iii) a quantitative method to analyze small molecules produce by microbes, as well as their binding to, and diffusion through, mucin networks. With these tools in place, we studied the influence of mucus, and specifically its gel-forming mucin polymers, in bacterial interactions between the human pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We have found that mucins prevent *P. aeruginosa* from outcompeting *S. aureus* in co-cultures by altering the community structure of these microbes in mixed communities, and by reducing the production of anti-staphylococcal compounds by *P. aeruginosa*. Our results highlight the role of mucus in keeping diverse microbial communities in the human body.

36 Exploration of pinning and pulsed invasion in a spatially expanding bacterial mutualism

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Range expansions occur when a population expands in space due to dispersal or growth. In nature, these expansions can result from environmental changes, introduction of invasive species, or evolutionary adaptations that enable a population to exist in previously unoccupied territory. Theoretical work has shown that fragmented habitats and seasonal growth (discrete space and discrete time) can result in pinning and pulsed invasions in expanding populations with an Allee effect (when average individual fitness increases with population size or density). These dynamics are distinct from constant velocity invasion fronts observed in continuous systems. We probe these phenomena in an experimental model system consisting of a bacterial mutualism inhabiting discrete population patches and subject to periodic growth. Preliminary experimental and modeling results indicate that these phenomena can occur in our system. For low migration between nearest-neighbor population patches, the mutualism cannot expand, meaning that the population is pinned. For high migration between population patches, the mutualism can expand at both constant and pulsed speeds, depending on the migration rate and the initial population relative abundances. Overall, this work highlights how range expansions can progress differently in discrete systems.

37 Mechanisms of probiotic inhibition of the periodontal pathogen *Porphyromonas gingivalis*

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Despite a growing interest in using probiotic micro-organisms to prevent disease, very little is understood about the mechanisms by which probiotics exert their action. We are interested in understanding the interactions between *Porphyromonas gingivalis* (Pg), an important pathogen implicated in the development of

periodontitis, and probiotic bacteria such as *Lactobacillus*. We isolated several probiotic strains of *Lactobacillus delbrueckii* from dairy products and examined their ability to inhibit Pg growth *in vitro*. We employed agar overlays and spot plating to test for inhibition of Pg. We identified *L. delbrueckii* strains that inhibit Pg growth *in vitro*. There is strain specificity in the inhibition as some, but not all, strains of *L. delbrueckii* inhibit Pg growth. Whole genome sequencing of inhibitory and non-inhibitory strains of *L. delbrueckii* revealed significant genetic differences. Testing of *L. delbrueckii* supernatants and lysates to characterize the mechanism of inhibition suggest that the inhibitory molecule is a heat-labile, protease-sensitive protein with a molecular weight greater than 10 kDa. Its activity is abolished by treatment with catalase and sodium sulfite suggesting the protein may be a hydrogen peroxide-producing, flavin-dependent oxidase. We have partially purified the inhibitory protein(s) from *L. delbrueckii* lysates using ammonium sulfate precipitation, anion exchange chromatography, and gel filtration chromatography and identified several oxidases as being enriched in inhibitory fractions. Our results underscore the importance of strain selection, not simply species selection, in understanding microbial interactions. Specific *L. delbrueckii* strains or their products may be effective in the treatment and prevention of Pg-associated periodontal disease.

38 Species Interactions of the Nasal Microbiota: Microbial community management using an ecological network representation

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In an era of rising antibiotic resistance, the medical field has turned towards microbial-based therapies (e.g., microbiota transplants) as a viable treatment option. Early applications of such therapies have shown great promise. However, there remains a large gap in knowledge regarding what strategies would work best to manipulate the complex, dynamic ecosystem of human microbiota. Objective: To develop an ecological network for a model community (based on human nasal microbiota) to contribute to the design of microbial-based therapies. Hypothesis: A network-based approach will enable us to identify key driver species and influential environmental factors involved in restructuring a microbial community. Results: We have focused on species interactions of the nasal microbiota. The nasal cavity is a reservoir to diverse phyla, including commensal and pathogenic bacteria. Previous work has demonstrated the importance of interspecies interactions in shaping the composition of this community, and a number of commensal-produced compounds that inhibit the colonization of pathogens, such as *Staphylococcus aureus*, have been identified. Using *in vitro* experiments, we characterize how species within this community interact and compile a corresponding network of interspecies interactions for the community. Specifically, we show that these interactions cannot simply be explained by resource utilization and niche overlap. Furthermore, we find that most species tested are sensitive to fluctuations in pH and that a significant number of interspecies interactions are due to species-driven alteration of environmental pH. Overall, our goal is to better understand the impact of interspecies interactions on the response of nasal microbiota to potential pathogens.

39 Modeling bacterial contact-dependent growth inhibition

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Microbial communities are comprised of species exerting facilitatory and/or inhibitory influences on one other. These interactions shape the community by encouraging (e.g. supplying metabolites) or hindering (e.g. releasing inhibitory byproducts) the growth of other species. Contact-dependent growth inhibition (CDI) is an example of cells negatively influencing target cells. Upon direct contact, the CDI cell injects a toxin into target cells to inhibit their growth. CDI cells also produce an immunity protein to neutralize the CDI toxin, preventing self-harm or harm to other closely related members of the community. Even though the molecular machinery of CDI has been studied in detail, the impact on population dynamics has not been well described. We model CDI to elucidate the role of negative interactions in community dynamics. To inform our model, we use two-strain communities comprised of an *Escherichia coli* strain that exerts CDI and a target *E. coli* K12 strain. We label these two strains with different fluorescent markers to quantitatively measure how the populations and their spatial distributions change over time. Using the fluorescence quantification, we investigate how the community is influenced by CDI at varying ratios and densities. From this data, we quantify the impact of CDI cells on target cells and construct mathematical models to describe this impact. We use an agent-based model for spatial environments and a population-level model for well-mixed environments. These models will assist in predicting and extrapolating CDI impact in both well-mixed and spatial environments. Modeling CDI will also facilitate future efforts in simulating microbial communities.

40 Gastrointestinal tract microbiome dynamics following treatment with SER-109, an investigational oral microbiome therapeutic to reduce the recurrence of *Clostridium difficile* infection (CDI)

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Recurrence of CDI occurs within a few weeks after treatment due to antibiotic-induced dysbiosis. SER-109, an investigational, first-in-class microbiome drug, was designed to sustain a clinical response through microbiome restoration. In an open-label Phase 1b (Ph1b) trial of SER-109 for prevention of recurrent CDI, 26 of 30 subjects did not recur following treatment. In a Phase 2 (Ph2) double-blind controlled trial of SER-109 (n=59) vs placebo (n=30), no significant difference was observed in the proportions of subjects with recurrence (44.1% vs 53.3%, respectively). We contrast gut microbiome changes among subjects in both trials to understand differences in clinical outcomes observed 8-weeks after dosing, and the impact of treatment on carriage of antibiotic resistance associated bacteria. Using 16Sv4 and whole metagenomic shotgun (WMS) sequencing, we observed significantly greater richness of commensal spore-former species in subjects treated with SER-109 compared to PBO at weeks 1 and 4 post-treatment (p=0.008, p=0.044), consistent with drug engraftment. The number of spore-forming species at 1 week was significantly greater in non-recurrent subjects vs recurrent subjects with a positive EIA toxin test (p=0.011). We identified 10 spore-former species that were significantly more prevalent in both SER-109 and non-recurrent subjects. In comparison to Ph1 subjects, SER-109 engraftment was significantly reduced and delayed in Ph2 subjects. Moreover, Ph1 subjects who received higher doses of SER-109 than that used in Ph2 had increased levels of engraftment. In patients with recurrent CDI and dysbiosis, a focused spore-based therapeutic approach leads to engraftment of SER-109 strains. In addition, microbiome signatures of engraftment were associated with a favorable clinical outcome. Although SER-109 was biologically active, a higher dose may improve the rate and degree of microbiome repair.

41 Analysis of co-transcriptional RNA processing patterns in *E. coli*

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RNA processing of bacterial transcripts contributes to shaping cellular gene expression. Endonucleolytic cleavages carried out by endonucleases alter stability and/or translatability of transcripts through decay of the message and in some cases generation of stable transcript isoforms by cleaving specific processing sites. Considering the distinct localization of the main endonuclease RNase E in *Escherichia coli* at the membrane and transcription in the center of the cell, it is unclear how RNA processing dynamics are coordinated with transcription and translation. We seek to investigate when specific RNA processing events occur during gene expression. To elucidate RNA processing pattern associated with transcription globally in *E. coli*, we biochemically isolate nascent RNAs as part of the transcription elongation complex by immunoprecipitation of tagged RNA polymerase. Nascent transcripts are sequenced using end-enriched RNA sequencing (Rend-seq) (Lalanne et al., Cell 2018), with which we can quantify the levels of transcripts with 5' ends originating from both transcription start sites and endonucleolytic RNA cleavages. Validating the enrichment of nascent transcripts in our preparation, we find that transcripts with short half-lives are enriched in this fraction relative to the input and recapitulate previously mapped transcription pause sites. Our analysis of known processing sites suggests that most processing occurs co-transcriptionally. This approach opens the door to assess competition and timing between the different steps in gene expression. Lalanne *et al.*, Cell 2018 Mar 23. Evolutionary Convergence of Pathway-Specific Enzyme Expression Stoichiometry.

42 Dynamic subcomplexes of the *Bacillus subtilis* divisome

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The tubulin homolog FtsZ is integral to bacterial cytokinesis and FtsZ polymers localize in a ring (the Z-ring) at future division sites, where they recruit other factors creating a complex (termed the divisome) to divide the cell. Recent work in our lab demonstrated that Z-ring constriction and corresponding septal PG synthesis is driven by the directional treadmilling of FtsZ. But how does each other component of the divisome move: do they move along with the processive PG synthesis enzymes or remain immobile with

FtsZ monomers? Motions of divisome components were imaged using HaloTag fusions to each protein, labelled with Janelia Fluor dyes. We have observed and characterized subsets of stationary and moving division proteins. Moreover, the velocities of the moving proteins are correlated, with motion dependent on cell wall synthesis. These data suggest a subcomplex of directionally moving proteins at the division site, with movement limited by FtsZ treadmilling and dependent on cell wall synthesis.

43 Modulation of the host immune response by mycobacterial surface molecules

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Tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), in spite of being one of the oldest human diseases, remains the leading cause of death by a single infectious agent. Outcomes of interactions between Mtb and the human host that result in clinical disease are complex and involve both host and bacterial factors. Mtb manages to survive in macrophages, innate immune cells that theoretically should both kill invading pathogens and prime an adaptive immune response. Failure of macrophages to mount an effective antimicrobial response to Mtb arguably begins the moment that macrophages "see" the bacterium, through interactions between macrophage Pattern Recognition Receptors (PRRs) and bacterial Pathogen Associated Molecular Patterns (PAMPs). The study of PRR/PAMP interactions classically involves minimalist systems: purified PAMPs interacting with either innate immune cells or cell lines engineered to express only pieces of innate immune signaling pathways. The use of such minimalist systems has elucidated much of what we understand about innate immune signaling. These discoveries translate well to classes of bacteria such as Gram-negative rods, which express a single highly inflammatory, dominant PAMP on their surfaces. For Mtb, the use of minimalist systems has led to the description of dozens of PAMPs, none of which are highly inflammatory or dominant on the bacterial surface. The specific PAMP/PRR interactions that are most relevant in the context of interactions between intact host cells and Mtb are unknown. More broadly, the ways that recognition of multiple subdominant surface PAMPs sum to the observed macrophage response to infection with complex bacterial pathogens remains poorly understood. In this work, we take a bacterial genetic approach to understand how individual Mtb PAMPs within the context of the intact bacterium contribute to the observed macrophage response to infection.

44 The Induction of Natural Transformation in *B. subtilis* is Modulated by Genotoxic Stress

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Horizontal gene transfer (HGT) is an important mechanism that promotes rapid evolution in microbial communities. Understanding how environmental conditions impact HGT in microorganisms can identify factors that lead to the spread of virulence and antibiotic resistance genes. Natural transformation is a form of HGT that allows bacteria to take up and incorporate extracellular double stranded DNA. Competence machinery utilized for the import of DNA is typically induced by cellular state or external stimuli. Following import, DNA is recombined into the genome in a RecA-dependent fashion. We hypothesize that elevated RecA plays a central role in elevating rates of natural transformation. This is important because genotoxic agents have been shown to induce RecA through activation of cellular stress responses. Here we investigate the links between genotoxic stress, RecA induction, and changes in the rate of natural transformation in *Bacillus subtilis*. In order to investigate the role of RecA in natural transformation, we have also developed an artificially inducible RecA system in *B. subtilis* and show that RecA induction is linked to increased natural transformation. We find that while genotoxic agents uniformly induce RecA, they have divergent impacts on natural transformation. Specifically, we find that mitomycin C robustly induces transformation, while genotoxic ciprofloxacin inhibits transformation. We utilize RNA-Seq to explain this dichotomy and link transformation efficiency to RecA induction. These results show that environmental genotoxic stressors could have profound impacts on the spread of virulence and resistance via natural transformation.

45 Inhibition of DNA Replication Initiation in *E. coli* by the Alarmone (p)ppGpp Through Reduction in Supercoiling

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The stringent response is the classic response bacteria mount in response to a variety of environmental stresses, especially various forms of starvation. During stringency, the cell produces large quantities of the nucleotide alarmone (p)ppGpp, affecting many aspects of cell physiology, including reprogramming transcription, potentiating protein translation, and inhibiting new rounds of DNA replication. Despite (p)ppGpp having long been known to inhibit DNA replication initiation in *E. coli*, the mechanism of action has remained elusive, having been suggested to work through decreasing DnaA levels or modulated by the sequestration factor SeqA. Here we suggest that (p)ppGpp prevents new rounds of DNA replication in *E. coli* by modulating the supercoiling status and structure of the origin (*oriC*) due to reduced transcription, and not through modulation of DnaA levels. Modulation of transcription around *oriC* with a (p)ppGpp-insensitive promoter suppresses (p)ppgpp's ability to inhibit new rounds of DNA replication. We show that an increase of global negative supercoiling by inhibition of TopA relieves inhibition and suggest that increased negative supercoiling in the absence of SeqA is responsible for continued replication in the *seqA* mutant. Finally, we demonstrate the importance of proper replication regulation by demonstrating a defect in recovery from stringency in *seqA* cells.

46 MinC N- and C-domain interactions regulate *Escherichia coli* FtsZ assembly, division site selection and MinD-dependent oscillation

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The *E. coli* Min system, including MinC, MinD, and MinE, regulates the site of cell division by antagonizing FtsZ-ring assembly at the cell poles. MinD oscillates between the cell poles through MinE-driven cycles of membrane association and dissociation. MinC inhibits FtsZ polymerization and also oscillates *in vivo*, directed by the interaction with MinD. To better understand how MinC interacts with FtsZ and MinD, we generated a library of strains containing mutagenized *minC* at the native locus on the chromosome and screened for cell division defects *in vivo*. Mutations mapping to both the MinC N- and C-domains were associated with cell length defects, suggesting that division site selection is impaired. Using *in vitro* assays, we also showed that MinC destabilizes dynamic FtsZ-GTP polymers; however, several MinC mutant proteins were defective. Altogether, we identified two distinct regions on the surface of MinC that are important for the FtsZ interaction: one on the cleft in the N-domain, and an additional site on the C-domain near the MinD interaction site. Introducing a mutation at either site also leads to slow oscillation of Gfp-MinC *in vivo*. These results suggest that the direct interaction with FtsZ modifies the MinC oscillation cycle *in vivo*.

47 *Chlamydia trachomatis* infection drives sustained expression of chemokines *CXCL9*, *CXCL10*, and *CXCL11* in the female genital tract

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Chlamydia trachomatis is an obligate intracellular bacterial pathogen that is the most common reportable sexually-transmitted infection in the United States. Infection with *Chlamydia trachomatis* serovar D in the female genital tract causes an influx of inflammatory cells that drive immunopathology. Infection with a different *C. trachomatis* serovar, L2, does not recruit these cells or cause immunopathology. To determine what recruits inflammatory cells to *C. trachomatis*-infected tissue, we compared the expression of 700 inflammation-related genes early after infection with *C. trachomatis* serovar D or serovar L2. We observed an upregulation of the chemokines CXCL9, CXCL10, and CXCL11, which signal through a common receptor, CXCR3. We hypothesized that infection with *C. trachomatis* serovar D compared to L2 and mock infected controls would induce higher levels of CXCL9-11 expression that would increase over time. To test this hypothesis, we measured expression levels of CXCL9-11 in mock-infected, L2-infected, and D-infected mice on days 2, 6, and 9 post-infection. We found an increase in expression of all three chemokines over time, as well as significantly more *CXCL9* and *CXCL10* in *C. trachomatis* serovar D-infected vs. serovar L2-infected mice, as predicted. These results show that the node of interaction between CXCL9/10/11 and their common receptor, CXCR3, is involved in driving *C. trachomatis* serovar D immunopathology.

48 Exploiting reporter strains to illuminate *Mycobacterium tuberculosis*-host interactions: Potassium as a novel cue

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Mycobacterium tuberculosis (Mtb) remains a major global health burden, causing $\tilde{1}$.7 million deaths per year. We previously discovered that Mtb responds synergistically to chloride (Cl-) and pH, as cues to the immune status of its host. This raised the intriguing concept of abundant ions as important environmental signals, and we have now uncovered potassium (K+) as another abundant ion that can critically impact colonization by Mtb. RNA-seq analysis indicates that the transcriptional response of Mtb to low K+ levels is distinct and does not track simply with response to other ionic signals or osmolarity. However, crosstalk between Mtb response to different abundant ion signals is present, as shown by (i) identification of a shared novel regulatory factor that influences Mtb response to K^+, Cl^-, and pH, and (ii) the altered transcriptional response to increased external [Cl^-] and low pH exhibited by a Mtb mutant deficient in K^+ uptake. We have developed a fluorescent K^+-sensitive reporter Mtb strain, and find that intraphagosomal [K^+] increases during macrophage phagosomal maturation. Importantly, Mtb mutants in K^+ transport are attenuated in their ability to colonize the host. Our findings illustrate how Mtb integrates sensing and response to multiple linked signals for effective host colonization. We are exploiting our novel fluorescent reporter Mtb strains, in combination with thick tissue imaging methods, to enable *in vivo* examination of how environmental cues may (i) regulate Mtb replication and impact disease progression, and (ii) be vital drivers of the marked bacterial population heterogeneity observed during Mtb infection.

49 Essential amino acid synthesis as a therapeutic target for *Klebsiella pneumoniae* infections

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As one of the ESKAPE pathogens, the CDC recently declared that two antibiotic-resistant strains of *Klebsiella pneumoniae*, CRE (Carbapenem Resistant Enterobacteriaceae) and ESBL (Extended-Spectrum beta-Lactamase producing), represent urgent and serious threats to public health in the United States, respectively. A high-throughput transposon mutagenesis screen identified fifty genes important for *K. pneumoniae* virulence in both immunocompetent and neutropenic mouse lung infections, including many of those involved in essential amino acid biosynthesis. One of these, *aroA*, encodes 5-enoylpyruvyl shikimate phosphate synthase (EPSP synthase) and is essential for synthesizing aromatic amino acid. We found that the small molecule glyphosate, a competitive inhibitor of AroA, can be used to target this pathway. *K. pneumoniae* is sensitive to glyphosate *in vitro* and exposure to glyphosate lowers the bacterial burden of lung infections in immunocompetent mice. While glyphosate-resistant point mutations in *aroA* have been well-studied biochemically, the physiology of glyphosate resistance in *K. pneumoniae* is unknown. We found that the rate of spontaneous glyphosate resistance in *K. pneumoniae* is very low. Of ten spontaneous glyphosate resistant mutants, none contain mutations in *aroA* or its upstream regulatory regions. We will use whole genome sequencing to identify and further characterize other genes involved in glyphosate resistance. As *aroA* and the shikimate pathway do not exist in mammals, this presents a potential therapeutic target for *K. pneumoniae* infections in both healthy and immunocompromised patients. Given the ease with which *K. pneumoniae* acquires antibiotic resistance, understanding common mechanisms of glyphosate resistance is important for potential therapeutic use.

50 Competitive genomic screening of drug resistant and drug susceptible mutants using *Escherichia coli* barcode deletion library

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Drug resistance is a major worldwide health problem, demanding new approaches to overcome. Advancements in NGS technology have opened up new ways to study drug resistance using high-throughput approaches. We recently established an *E. coli* single-gene deletion library (Otsuka et. al., 2015; PMID

25399415), in which each deletion is marked with a unique 20-nucleotide molecular barcode. The availability of this library has allowed us to assess each mutant's competitive growth advantage or disadvantage by pooling the mutants and analyzing the population using Bar-seq. Here we report results from analyzing our barcoded *E. coli* deletion library for antibiotic-resistant and susceptible mutants under competitive growth conditions. Our mutant library was first grown with sub-inhibitory concentrations, IC50, of drugs to identify susceptible mutants, then at inhibitory concentrations up to 4 x IC90 to identify resistant mutants. In our initial studies, we found 202 candidates for susceptible mutants and 56 candidates for resistant mutants. Candidates showing increased susceptibility to ciprofloxacin and tetracycline included *mrcB*, *pal*, *tolQRA*, *acrB*, and *tolC* mutants. Candidates showing increased resistance to ciprofloxacin included several in lipopolysaccharide (LPS) biosynthesis (*rfaC*, *rfaE*, *rfaF*, *rfaG* and *rfaQ*). Candidates showing increased resistance to tetracycline included ones in DNA replication (*recJ*, *ruvA* and *ruvC*).

51 Differential Gene Expression in the Symbiotic Northern Star Coral, *Astrangia poculata*, Under Oxidative Stress

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In tropical corals, the mutualism with *Symbiodinium* supplies up to 95% of the coral's energetic needs. However, oxygenic photosynthesis increases the production of reactive oxygen species in the host coral's cells. So, symbiotic cnidarians face another source of oxidative stress not faced by aposymbionts. While elevated temperatures can cause corals to lose their *Symbiodinium*, the proximate cause of thermal bleaching has been shown to be oxidative stress. We determined how the coral-*Symbiodinium* symbiosis impacts the oxidative stress response by isolating symbiotic state of the facultatively symbiotic coral *Astrangia poculata* and its *Symbiodinium*. RNAseq experiments revealed significant differences between symbiotic and aposymbiotic (in the same control or treatment state) or control vs H2O2 exposed individuals of the same symbiotic state, some of which appear related to the resilience of corals exposed to oxidative stress.

52 The role of *Shewanella oneidensis* Hfq in the oxidative stress and short term starvation responses

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Bacterial small RNAs (sRNAs) are regulatory molecules that contribute to adaptive gene expression in response to stress. Hfq, an RNA chaperone, has been broadly implicated in sRNA function in Gram-negative bacteria. We are investigating the role of Hfq in the adaptive physiology of the dissimilatory metal reducing bacterium *Shewanella oneidensis*, including oxidative stress and short-term starvation stress. The *hfq* mutant is hypersensitive to lethal doses of hydrogen peroxide. However, pretreatment with sub-lethal doses of peroxide prior to lethal dose challenge rescues the peroxide hypersensitivity. This suggests that the *hfq* mutant is able to adapt to oxidative stress, but does so more slowly than wild type. Our data indicate that there is no difference in the kinetics or magnitude of adaptive catalase production following peroxide treatment. We are currently testing the hypothesis that defects in repair of 8-oxoguanine, the primary DNA lesion formed by hydrogen peroxide damage, is responsible for the hydrogen peroxide hypersensitivity of the *hfq* mutant. Loss of *hfq* also results in a compete loss of colony forming units after short-term starvation in late stationary phase cultures. Cell-free media conditioned to stationary phase by growth of *S. oneidensis* kills both wild type and *hfq* mutant cells, but the *hfq* mutant is more sensitive to this killing. Preliminary data suggest that this death event requires oxygen, as anaerobic conditions are protective. Additionally, the severity of this death phenotype is a function of nutrient density. We are currently investigating the relationship between this death phenotype and known stationary phase regulatory systems.

53 Controlled expression of the periplasmic protein LmeA regulates the abundance of lipomannan and lipoarabinomannan in *Mycobacterium smegmatis*

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The mycobacterial cell envelope is a thick multilayered complex composed of an outer membrane, arabinogalactan layer, peptidoglycan layer and plasma membrane. Of specific interest are the key glycolipids, phosphatidylinositol mannosides (PIMs), lipomannan (LM), and lipoarabinomannan (LAM), which are im-

munomodulatory and essential for growth in *Mycobacterium tuberculosis*. The mannosyltransferases, MptA and MptC, are required for mature LM biosynthesis. Recently, we discovered a phospholipid binding protein, LmeA, which is involved in maturation of LM and is likely localized in the periplasmic space in *Mycobacterium smegmatis*. Another study indicated that the *M. tuberculosis* ortholog of LmeA is induced upon infection, suggesting that the expression of LmeA is tightly regulated. Inspired by these observations, here we examined if changes in the expression level of LmeA has an impact on LM/LAM biosynthesis and cell envelope integrity. We established an *M. smegmatis* strain overexpressing the HA epitope-tagged LmeA (LmeA-HA OE), a deletion of *lmeA* (*lmeA*), and its complemented strain. Interestingly, overexpression of LmeA-HA resulted in an increased abundance of both LM and LAM. To start dissecting how LmeA impacts the abundance of LM/LAM, we examined if overexpression of LmeA-HA impacts the protein levels of the LM/LAM mannosyltransferases. We found that the level of MptA was significantly higher in the LmeA OE strains. Additionally, MptA levels were reduced more significantly in *lmeA* during stationary than in wild type. Given the roles of LM/LAM in the cell envelope integrity, we are currently exploring the impact of LmeA expression on antibiotic sensitivity.

54 Predicting species-wide virulence for *Streptococcus pneumoniae*: a bacterial pathogen with a large pan-genome

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Streptococcus pneumoniae is a natural inhabitant of the human nasopharynx, but it can trigger severe disease when it disseminates to other sites like the lungs or blood. Its success in colonizing or invading these different sites relies on interactions that are not completely understood, and are often thought to be primarily dependent on the capsule. In addition to high strain-to-strain variability in capsule composition, *S. pneumoniae* has a large pan-genome, where two random strains on average differ by more than 200 genes. We hypothesize that non-capsule related genetic determinants play an important role in colonization and invasion, and this importance may change depending on the genetic background of different strains. To elucidate this complexity, we are performing a comprehensive functional study of *S. pneumoniae*-host interaction, for which we carefully selected 33 strains that cover 77% of the pan-genome and 15 different capsule serotypes. We have set-out to characterize these 33 isolates by determining in detail the genetic elements important for attachment to human airway epithelial cell cultures, colonization of the nasopharynx, and induction of pneumonia in mice. To achieve this we apply the cutting-edge tool transposon insertion sequencing (Tn-Seq). Our latest results show that our isolates have: 1) widely different virulence levels independent of the capsule serotype and 2) shared and strain-unique genetic requirements for growth, attachment, colonization and invasion. Our final aim is to integrate all the genetic and phenotypic data to create a *S. pneumoniae* species-wide model that is able to predict the main phenotypes for non-characterized strains.

55 The Effect of YopH on Signal Transduction Pathways and Antimicrobial Responses in Neutrophils During *Yersinia pseudotuberculosis* Infection

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YopH is a critical tyrosine phosphatase that is translocated into neutrophils (PMNs) by a Type III secretion system during *Yersinia* infection. Previous work in our lab has shown YopH blocks signaling from SLP-76, SKAP2, PRAM1, Vav and PLC2 proteins, that function downstream of different receptors in PMNs. Because these proteins are known to play critical roles in mediating reactive oxygen species (ROS) production in PMNs, we infected gp91phox$^{-/-}$ mice and found that the growth of a *yopH* mutant was significantly restored in absence of ROS in both spleen and lung. To dissect the signal transduction pathways that YopH inactivates, we infected SKAP2$^{-/-}$ and SLP-76 defective mice. In the absence of SKAP2, the growth of a *yopH* mutant was partially restored in spleen of infected mice indicating that SKAP2 is an important yet not the only target of YopH. By contrast, growth of the *yopH* mutant was not restored in SLP-76 defective mice. Importantly, in BMPMNs, YopH completely blocked ROS production after stimulation with pRGD, IgG immune complex, and fMLP. These findings suggest that YopH inactivates either shared proteins or multiple distinct targets downstream of integrin, ITAM-coupled and GPCR receptors to block

ROS production. We are currently examining whether the SKAP2 and SLP-76 regulatory hubs involved in mounting antimicrobial responses downstream of these receptors play overlapping and redundant roles or whether there are SKAP2 and SLP-76 independent pathways inactivated by YopH. This work will provide mechanistic insights into critical signaling pathways that mediate PMNs antimicrobial functions that are dismantled by *Yersinia*.

56 Investigating *Vibrio cholerae* mucin metabolism with transposon-insertion sequencing

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A subset of colonization factors in the intestinal bacterial pathogen *Vibrio cholerae* is required for the bacterium to liberate, transport and utilize nutrients in the small bowel. A major source of nutrients in the intestine are mucins, a family of heavily O-glycosylated oligomeric proteins responsible for the gel-like structure of intestinal mucus. *V. cholerae* can use mucins as a nutrient *in vitro* and localizes to intestinal mucus *in vivo*, but the factors mediating these processes have not been comprehensively investigated. Using a *V. cholerae* clinical outbreak isolate, we designed and executed a high-throughput transposon-insertion sequencing (TIS) screen to identify pathogen genes that contribute to mucin metabolism. The screen yielded several hits in genes with known connections to mucin metabolism in other pathogens. Furthermore, we discovered several annotated and unannotated loci that were subsequently shown to contribute to mucin metabolism *in vitro*. Our results indicate that *V. cholerae* utilizes both oligopeptide and sugar components of mucin to enable growth and that usage of this complex nutrient may influence diverse aspects of bacterial physiology.

57 Regulation of Z ring dynamics in bacterial cytokinesis

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Cell division in bacteria is orchestrated by a group of proteins that work together to carry out cytokinesis and synthesize new cell wall at the division site. Filaments of FtsZ, a bacterial homolog of tubulin, form a "Z ring" at the middle of the cell that recruits other cell division proteins and constricts as the cell divides. While many of these proteins have been identified, the mechanisms of cell division remain poorly understood. Recently, it has become clear that protein dynamics in the divisome play a key role in the cytokinesis process. Using TIRF microscopy, we have shown that FtsZ filaments treadmill around the division site and elucidated the significance of these dynamics in bacterial cell division. Now, we are investigating whether and how FtsZ filament assembly and dynamics are regulated by the cell to ensure proper division. Specifically, such regulation could be accomplished by proteins in the divisome whose proposed function is to bind to FtsZ filaments and modulate their stability; such proteins could alter both filament structure and treadmilling dynamics. First, we introduce single molecule lifetime measurements as a quantitative metric of FtsZ's treadmilling dynamics which is independent of both spatial resolution and filament density. We combine these measurements with 3D Structured Illumination Microscopy (3D-SIM) to probe the structure of the Z ring with high spatial resolution. Finally, we use these assays to ask whether and how proteins that bind to FtsZ control filament structure and stability during the bacterial cell cycle.

58 Increasing the survival of dormant *M. tuberculosis*

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Currently, *M. tuberculosis* is the deadliest pathogen. One of the reasons is its increased ability to persist in a non-replicating, metabolically quiescent and drug tolerant state that nonetheless allows it to regrow and

potentially trigger active tuberculosis. In order to understand which bacterial factors are crucial for latency we worked with an *in vitro* carbon-starvation model that renders *M. tuberculosis* antibiotic tolerant and resembles the non-replicating or latent state *in vivo*. We performed high throughput genomic screens and transposon insertion sequencing using a library that covers the entirety of the non-essential genome. Among the most interesting hits was *rv1363c* an Mce associated protein that is conserved among many Mycobacterial species and most likely plays a role in fatty acid metabolism. A deletion of the gene decreases fitness during carbon-starvation, though its over-expression enhances survival dramatically. In addition, *rv1363c* over-expressers react much faster and more uniformly to favorable conditions after starvation, lacking the lag phase observed in wildtype cells. A combination of Transcriptomics and Metabolomics suggests that *rv1363c* links fatty acid metabolism with sigma factor cascades and leads thus to global changes within the bacterial cell. *rv1363c* over-expressers also show higher tolerance as well as slower kill kinetics to rifampin during carbon-starvation. We believe our work helps to improve our understanding of regulatory processes in dormant *M. tuberculosis* cells and might even have the potential to shift drug tolerance during dormancy.

59 Analysis of Gonococcal Regulatory Networks Reveals Gender-Specific Gene Clusters Linked to Human Infection

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhea, a high morbidity disease with approximately 106 million annual cases worldwide. While males are typically symptomatic, females are often asymptomatic, and untreated infection can lead to complications including pelvic inflammatory disease, ectopic pregnancy, and infertility. Like other human pathogens, DNA binding regulators allow this organism to adapt to the environments encountered during infection, i.e. low pH and varying oxygen and iron levels, by altering gene expression patterns. We recently demonstrated that an iron-responsive regulator, Fur, can also control additional regulatory proteins, extending its transcriptional control. To gain an overarching view of multiple regulatory pathways in *N. gonorrhoeae*, we collected a large set of RNA-Seq data, including data from human genital tract infection, and inferred a network of 967 nodes (genes) with nearly 2000 edges (instances of gene expression coordination) between them using the Context Likelihood of Relatedness program. We identified 18 known DNA binding regulators with edges to other genes in the network that also respond to infection of the human genital tract in a gender-specific manner, including those regulated by Fur. These analyses are some of the first to infer a global network using -omics data, which can be a powerful tool for generating a systems-level view of biological systems and understanding the molecular mechanisms of pathogenesis. This will be crucial for the development of new drug treatments for gonorrhea, a pressing need as *N. gonorrhoeae* becomes more antibiotic resistant in the future.

60 Microbial Dynamics Along A Traditional Fermented Beverage

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Pulque is a mexican traditional alcoholic beverage produced by the spontaneous fermentation of the Agave sap known as *Aguamiel*. Bacteria like *Lactobacillus* and *Leuconostoc* have been isolated from Pulque and characterized as probiotic and exopolysaccharide (EPS) producers, respectively. *Saccharomyces* strains have also isolated. Thus, *Pulque* harbors microorganisms with biotechnological potential. We studied the dynamics throughout *Pulque* fermentation in controlled laboratory conditions to identify changes in the microbial composition, their metabolic capacity and micron-scale structure of the community. *Aguamiel* and *Pulque* were recollected from a local producer in the town of Huitzilac, state of Morelos, Mexico. Fermentation was carried through for 6 hours. For taxonomic and functional annotation, we performed metagenomic shotgun sequencing. Metabolites were analyzed by HPLC. For imaging, fluorescence *in situ* hybridization was performed on samples of *Aguamiel* and *Pulque* using probes targeting Bacteria and specific groups identified by the sequencing data. *Acinetobacter* was the major genus in *Aguamiel* followed by *Leuconostoc*.

This was confirmed by microscopy where characteristic acini-like structures were observed. Ethanologenic bacteria *Zymomonas mobilis* and yeast *Saccharomyces cerevisiae* were enriched throughout *Pulque* fermentation which correlated with an increase in ethanol content. *Leuconostoc*, the EPS-producer, did not change its abundance along the fermentation although fructo-oligosaccharides did increase. This suggests that EPS are produced under ethanol stress or to retain carbon source. Differences in micron-scale structure in *Pulque* were also observed. We conclude that *Pulque* fermentation is a dynamic process with changes in the microbial composition, synthesis of fructo-oligosaccharides and production of ethanol, lactic- and acetic acid.

61 Expression and Functional Characterization of Human Monoclonal Antibodies Against *Klebsiella pneumoniae*

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The rise in hospital transmissions of multidrug antibiotic resistant *Klebsiella pneumoniae* has become a significant concern, with the CDC classifying the threat as urgent as of 2013. Given the challenge of developing new antibiotics, one promising alternative is the development of monoclonal antibodies (mAbs) for use as preventive or post-infection treatment of antibiotic resistant *K. pneumoniae*. A total of 60 out of 260 paired VH and VL gene sequences isolated from B cells from patients with no history of infection were selected for cloning and expression. The antibodies chosen included both IgGs and IgAs as well as kappas and lambdas representing diverse gene families. In a whole killed cell ELISA against either a wild type (WT) or a nonencapsulated (mutant) strain, 5 mAbs were reactive against only the WT and 37 were reactive against both the WT and mutant strains. Eleven mAbs that displayed no reactivity in the killed cell format were found to be reactive against the WT and/or mutant strains using a live cell ELISA approach. While none of the mAbs tested was able to mediate complement dependent bactericidal activity, 5 mAbs were able to mediate killing via opsonophagocytosis. Of note, one of these (258, an IgA mAb) was specific for the mutant strain, suggesting that it targeted a non-capsular antigen that may be conserved across isolates. Overall, the results of this study support the potential for development of therapeutic antibodies for use against *K. pneumoniae* as well as other antibiotic-resistant Gram-negative bacterial pathogens.

62 Mucin glycans suppress virulence traits of *Pseudomonas aeruginosa*

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The natural mucus barrier is capable of supporting the coexistence of a diversity of microbes while preventing infection. The microbe-taming power of mucus stems in part from mucin glycoproteins, which are the primary gel-forming components of mucus. In this study, we develop an *in vitro* model using native purified mucin to identify the mechanisms by which the mucus environment prevents bacterial virulence. Using RNA-sequencing, we measured transcriptional changes in the opportunistic pathogen *Pseudomonas aeruginosa* following growth in the presence of mucin. This work shows that intact native mucins are capable of providing a dual mechanism for virulence control, suppressing surface attachment and the expression of several virulence pathways including quorum sensing, siderophore biosynthesis, and the type III secretion system. The suppression of key virulence traits and concomitant reduction in host cell cytotoxicity illustrate the importance of healthy mucins in promoting a host-compatible physiology of potentially pathogenic microbes like *P. aeruginosa*. Furthermore, the ability of mucin, but not other polymer solutions, to manipulate *P. aeruginosa* physiology suggests that specific biochemical attributes present in mucins may serve as a host-derived regulatory signal. To test the signaling potential of mucin-associated glycans, these sugars were isolated from the mucin protein backbone and added to culture media. Transcriptional studies of *P. aeruginosa* exposed to even small amounts of mucin glycans reveal a similar downregulation in the expression of virulence pathways, revealing that these sugars are an important bioactive component of the mucus barrier and play a key role in host defense.

63 The role of RNase E in mRNA metabolism in *Mycobacterium smegmatis*

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Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb). A major challenge in TB treatment is the high tolerance of Mtb to various types of stress, including antibiotics and hypoxia. We hypothesize that adaptation to stress conditions involves both transcriptional and post-transcriptional regulatory mechanisms. mRNA cleavage may regulate gene expression post-transcriptionally by affecting the stability (half-life) of mRNA. Multiple ribonucleases (RNases) may perform endonucleolytic cleavages of primary mRNAs producing fragments with different stabilities and thus differentially alter transcript abundance. We sought to understand the role of mRNA cleavage in mycobacteria by investigating the role of RNase E, an enzyme involved in both mRNA degradation and selective mRNA stabilization in *E. coli*. Using the non-pathogenic model species *Mycobacterium smegmatis*, we constructed inducible repression systems to knock down RNase E. Repression of RNase E slows growth, consistent with the prediction that RNase E is an essential gene, and also results in decreased mRNA cleavage at the esx-1 operon, which plays vital roles in conjugation in *M. smegmatis* as well as infection of macrophages by Mtb. Altered relative transcripts abundances also demonstrated a complex role of RNase E in both stabilizing and destabilizing portions of this transcript. We also examined the half-lives of several mRNAs and found that most were increased when RNase E was repressed, suggesting that RNase E plays a global role in mRNA degradation. Moreover, we constructed endogenous FLAG-tagged RNase E strain and processed *in vivo* pull down assay and LC-MS/MS to identify RNase E-associated proteins. Further, we plan to map cleavage sites and measure mRNA stability transcriptome-wide in the context of RNase E repression to further define its contribution to mRNA metabolism.

64 *Pseudomonas* aeruginosa swarming colonies are protected from antibiotics by a dead cell physical barrier

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Pseudomonas aeruginosa is an opportunistic pathogen causing various infections among immunocompromised individuals and cystic fibrosis patients. It is also well known for its ability to perform different types of motilities. *Swarming* motility is considered a social phenomenon carried out by a bacterial colony and can be observed on semi-solid surfaces. *Swarming* requires at least two essential features: a functional flagellum and production of a wetting agent (rhamnolipids). The study of bacterial multicellularity is currently a topic of high interest, largely because of the involvement of the biofilm mode of growth in antibiotic tolerance. Although *swarmer* cells display elevated resistance to various antibiotics, the mechanism(s) behind this adaptation in *P. aeruginosa* remains poorly understood. Various concentrations of the antibiotic tobramycin were used to measure the surface coverage ratio of a *swarmer* colony of *P. aeruginosa* strain PA14. These tests led to the observation of an important feature of these colonies: the enlargement of the dendritic width correlating with augmenting tobramycin concentrations. Our current hypothesis is that the dendritic arms width is larger because a protective wall composed of dead cells accumulates at the edge, shielding the metabolically active bacteria inside the colony. This would suggest that some bacteria are sacrificed for the better outcome of the entire colony. Imaging analyses were performed and methods of identification of the dead cells are underway. The study of spatial organization of bacteria in a *swarming* colony is useful for our understanding of social phenomenon among bacteria and the benefits it provides to these communities.

65 Bacteria of genus *Vibrio* colonize the esophagus of the cuttlefish (*Sepia officinalis*)

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Micron-scale biogeography shapes the interaction of microorganisms with their environment and host. We studied micron-scale structure of the microbiome of the cuttlefish (*Sepia officinalis*) using a combination of imaging and massively-parallel tag sequencing. Samples from esophagus, intestine and cecum of 9 cuttlefish were collected and divided for sequencing and imaging. Tissues for imaging were fixed in 2% paraformaldehyde, embedded in methacrylate, and sectioned to 5 um. Fluorescence in situ hybridization was performed

using probes targeting bacteria, gamma-proteobacteria, the family *Vibrionaceae*, and the most abundant species in the sequencing data; fluorophore-labeled wheat germ agglutinin was used to localize mucus and DAPI was used to detect nucleic acids. Samples were imaged on a laser-scanning confocal microscope with a spectral detector and processed by linear unmixing. DNA extraction and sequencing of the 16S rRNA gene were performed using standard protocols. Sequencing data revealed a highly simplified community in the esophagus, dominated by a single OTU that showed 97% identity to genus *Vibrio*. The second most abundant OTU was identical to numerous species from the genus *Photobacterium*. FISH revealed a striking organization of these bacteria in the esophagus of cuttlefish, distributed in a layer lining the interior and associated with mucus. Imaging with specific probes confirmed the identity of these bacteria as *Vibrionaceae*. In the stomach and cecum, these bacteria are present not in a layer but in lower density throughout the lumen. We conclude that bacteria belonging to the *Vibrionaceae* are the major symbiont of the cuttlefish *Sepia officinalis*.

66 A diverse collection of skin microbiota to study the human skin microbiome

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Human skin is colonized by diverse microbial organisms which can impact our interaction with the environment and our skin health. The composition of the microbial community differs between skin sites which can vary in moisture, pH, temperature, salinity and oil content. To enable the study of microbes on our skin, we isolated 800 organisms from the skin of healthy human subjects. Specifically, organisms were collected from the forehead, forearm, and antecubital fossa (inner elbow) of 17 healthy human subjects. We used diverse isolation conditions which included anaerobic growth, 10% salt, and nutrient poor media. Our collection is dominated by representatives from *Staphylococcus* and *Micrococcus*, with additional representation from 25 families and 38 genera. Further, we isolated organisms from 14 genera of fungi with both yeast and mold isolates. This collection will enable the study of interactions between human skin and microbial colonists.

67 Suppression of DNA replication overinitiation in *Bacillus subtilis* through lowered levels of the replicative helicase, DnaC

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In bacteria, DNA replication is initiated by DnaA, a AAA+ ATPase. ATP-bound DnaA binds cooperatively to the origin, causes open-complex formation, and recruits the replication machinery. In *Bacillus subtilis*, DNA replication initiation is primarily controlled by regulating the activity of DnaA through several proteins, including YabA. YabA limits cooperative binding of DnaA-ATP at the origin and *yabA* results in overinitiation. When *yabA* is combined with an overactive mutant of *dnaA*, *dnaA1*, the double mutant has an additive effect on replication initiation. Under fast growth conditions *yabA dnaA1* is synthetic lethal due to severe overinitiation leading to replication fork collapse and the SOS response. In order to determine the cells' mechanisms to overcome this replication stress, we isolated independent suppressors and screened for those that did not have mutations in *dnaA*. One class of suppressors that came up 1/3 of the time contained mutations in the promoter region of the replicative helicase, *dnaC*. These suppressors all had a decrease in the levels of helicase protein and a decrease in DNA replication initiation. Decreased levels of helicase in an otherwise wild type background were sufficient to lower replication initiation under fast growth conditions but not under slower growth conditions. These data indicate that under high initiation conditions, decreased levels of the replicative helicase are sufficient to limit DNA replication initiation. We are working on classifying more of the suppressors to develop a better understanding of different mechanisms *B. subtilis* uses to deal with overinitiation and replication stress.

68 Exclusion System of the Mobile Genetic Element ICE*Bs1*

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Horizontal gene transfer, including transfer of mobile genetic elements such as conjugative plasmids and integrative and conjugative elements (ICEs), is a major force driving microbial evolution, and an important mechanism for spreading genes involved in antibiotic resistance, symbiosis, and metabolism. Many of these

elements also encode regulatory mechanisms to prevent redundant transfer, including a broad variety of exclusion systems. Most conjugative plasmids contain one exclusion system, and exclusion has also been documented in gram-negative ICEs. The gram-positive ICE*Bs1* of *Bacillus subtilis* has an exclusion mechanism, in which an ICE*Bs1* gene encoding a putative lipoprotein specifically targets the ICE*Bs1* conjugation machinery, to prevent conjugative DNA transfer. A screen for exclusion-resistant ICE*Bs1* mutants yielded three independent exclusion-resistant, transfer-competent mutations within the conjugation machinery, and the target protein was further confirmed by homolog swaps with the related element ICE*Bat1* from *Bacillus atrophaeus*. Identification of the ICE*Bs1* exclusion gene and its target protein in the ICE*Bs1* conjugation machinery represents the first exclusion system to be reported and characterized in a gram-positive ICE, and is an important step in exploring the structure and function of the Gram-positive conjugation machinery.

69 Investigating a novel regulation on a checkpoint protein Sda that is essential for biofilm formation and sporulation in *Bacillus subtilis*

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Bacteria have evolved various protection mechanisms, such as biofilm formation and sporulation, to help them adapt to and survive extreme environments. In the soil bacterium *Bacillus subtilis*, Sda is a "checkpoint" protein that plays a critical role in sporulation and biofilm formation by regulating the phosphotransfer from the histidine kinase KinA that culminates in phosphorylation of the transcription factor Spo0A, a master regulator for both sporulation and biofilm formation. Recent studies have shown a serine codon hierarchy-based novel signaling mechanism for biofilm formation in *B. subtilis*. Interestingly, the sda gene has a much higher percentage of serine codons (12%) compared to the genome average (5%) in *B. subtilis* and a biased distribution of different serine codons. We hypothesize that the serine codon hierarchy-based signaling mechanism also regulates Sda in that translation of Sda will slow down during nutrient limitation at the onset of biofilm formation and sporulation. To test our hypothesis, we constructed six Sda point mutants with synonymous substitutions in three TCN serine codons. We designed biofilm and sporulation competition assays to determine the impact of those synonymous changes in serine codons. We found that the Sda mutant with AGC substitutions outcompeted other mutants and became the dominant strain after several rounds of competition in both biofilm and sporulation assays. Our data indicates that synonymously switching serine codons in *sda* gene can delay biofilm formation and sporulation. This study suggests that this codon-based serine sensing mechanism may be widely present in *B. subtilis*.

70 Bacterial genome-wide association reveals clinically prevalent mutations conferring multidrug tolerance in *Mycobacterium tuberculosis*

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The emergence of antibiotic resistant *Mycobacterium tuberculosis* (Mtb) threatens to undermine improvements in tuberculosis control achieved through the widespread adoption of effective combination drug therapy. The subsequent identification of resistance-conferring bacterial mutations, however, has led to the development of rapid molecular diagnostics that can help guide appropriate regimen selection for patients. Nevertheless, some fraction of resistant isolates defies genetic explanation with currently known resistance-determining loci. Further, antibiotic resistance is only one of several mechanisms by which bacteria can undermine effective treatment, where delayed clearance in the form of antibiotic tolerance, has been identified in clinical pathogen populations. Here we performed a bacterial genome-wide association study looking for Mtb mutations correlated with antibiotic resistance in a large cohort of clinical Mtb isolates from the 2007 nationwide survey of drug resistant tuberculosis in China. We identify most known drug resistance-conferring loci and several novel loci, including the central-carbon metabolism regulator *prpR* and the chromosome replication regulator *dnaA*. We demonstrate that prevalent *prpR* mutations alter propionate metabolism resulting in increased tolerance to multiple unrelated antibiotics in a carbon-source dependent manner. Importantly, this tolerance is readily observed during intracellular infection of human macrophages, which are the major replicative niche of Mtb during natural infection. Preliminary analysis of dnaA mutants

also revealed increased antibiotic tolerance, suggesting that previously unidentified tolerance mutations may impact treatment efficacy and often precede the evolution of high-level resistance in Mtb populations.

71 The role of RNase J in drug resistance in mycobacteria

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis. Emergence of multidrug-resistant (MDR) strains has become a major health challenge worldwide. A recent study reported a set of MDR isolates carrying biased mutations in genes previously undescribed. Among these genes is Rv2752c, encoding ribonuclease RNase J. We found that RNase J mutants have altered susceptibility to a subset of drugs, including the two first-line ones rifampicin and isoniazid. To study the role of RNase J at genomic level, RNAseq 5' end-directed and expression libraries using *M. smegmatis* and Mtb were performed. Our results show that loss of RNase J affects the expression levels of a small number of genes. Interestingly, RNase J is strongly linked to the regulation of PE/PPE-like genes, suggesting that RNase J could be involved in mycobacterial virulence and pathogenesis. Genes involved in the synthesis of phthiocerol dimycocerosates and other essential components of the cell wall, were also affected by loss of RNase J. Finally, lacking of RNase J produced the accumulation of small RNA fragments in some genes, suggesting that RNase J could be involved either in the processing or degradation of certain transcripts. Additional experiments to determine the half-life of these fragments in absence of transcription revealed that they are more stable in RNase J mutant. These unprocessed fragments have a strong secondary structure and high G+C content, suggesting that this nuclease could be involved in degradation of highly structured RNA molecules that can not be processed by RNase E, the major RNase in mycobacteria.

72 Identifying Mechanisms That Dictate Spatial Organization of the Human Oral Microbiota

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Human supragingival plaque is a complex polymicrobial biofilm on the tooth surface above the gumline, comprised of ordered reproducible structures with spatial associations between specific bacterial taxa. In healthy subjects these communities harbor a low abundance of opportunistic pathogens whose numbers increase during oral infection. Our lab seeks to determine mechanistic interactions between healthy plaque microbiota to find ways to prevent or diminish growth and/or virulence of opportunistic pathogens. Recent microscopy and microbiome data has revealed that plaque structure is scaffolded by the abundant, filamentous *Corynebacterium matruchotii* bacterium decorated with *Streptococcus* species including *S. mitis* and *S. cristatus*. These data also indicate a high number of *Haemophilus parainfluenzae* adjacent to plaque structures and adhered to *Streptococcus*. Using a reductionist approach comparing mono vs cocultures we discovered that aerobically *S. mitis* and *S. cristatus* growth is stimulated by *C. matruchotii* and that *S. mitis* strongly inhibits *H. parainfluenzae* growth. Anaerobically, *S. mitis* inhibits *C. matruchotii* while enhancing *H. parainfluenzae* growth and these results can explain community structure as *Streptococcus* species only decorate *C. matruchotii* in the aerobic perimeter of plaque scaffolds. Transcriptome (RNASeq) analyses of these cultures indicate that *S. mitis*-produced bacteriocin may suppress *H. parainfluenzae* growth aerobically but not anaerobically where *H. parainfluenzae* expresses surface adhesins, hypothetically to adhere to *S. mitis*. *C. matruchotii* shows increased expression of lactate catabolism genes aerobically with *Streptococcus* but not anaerobically where its growth is inhibited. Together these findings may explain mechanisms underlying the spatial arrangement of these species in a polymicrobial community.

73 Carbapenemase-carrying plasmid found in three different bacterial genera in a single hospital over a nine-year span

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Carbapenem-resistant *Enterobacteriaceae* (CRE) cause life-threatening infections that are difficult, if not impossible to treat. In order to understand carbapenem resistance and track its spread, we have collected and sequenced almost 300 CRE isolates from four hospitals (three in Boston) since 2012, including a small collection of retrospective isolates dating back to 2007. During a two-month period in 2017, nearly identical carbapenem-resistant *Citrobacter freundii* were isolated from four different patients at one Boston-area hospital. All isolates had a distinctive resistance pattern and harbored a notable arrangement of the Tn4401(which carries the carbapenem-resistance gene *KPC*) and Tn5403 transposons. Comparisons to a global collection of 216,999 assembled bacterial genomes stored at NCBI revealed that this arrangement was found exclusively in CRE isolated from our study with all but one (1 of 12) isolated from patients from within the same hospital. Strikingly, these isolates, collected over a nine year period, represented three distinct bacterial genera, suggesting that this unique element is highly mobilizable among bacterial genera and persistent within a single hospital. Using a combination of Illumina and Oxford Nanopore sequencing, we obtained virtually finished genome assemblies, which are revealing highly dynamic interactions among two plasmids harboring this unique KPC-carrying transposon structure and the bacterial host chromosome.

74 Interrogating Macromolecular Interactions at Biological Membranes

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Cellular membranes create a fascinating physicochemical environment that expands the metabolic repertoire. Reactions localized in this environment are catalyzed by membrane proteins that can assemble into supramolecular complexes, channeling intermediates and increasing the efficiency of cellular pathways. The N-linked protein glycosylation (pgl) pathway of the human pathogen *Campylobacter jejuni* is a relevant example of a membrane-associated pathway. It involves seven membrane-associated proteins that coordinate assembly of a well-defined polyprenyl-diphosphate-heptasaccharide, which is ultimately transferred to the side chain of asparagine residues in target proteins. Previous work supports the hypothesis that these enzymes form a supramolecular complex. Studying the organization and dynamics of membrane complexes *in vitro*, including the pgl pathway, remains a Herculean task. In particular, the requirement for detergent solubilization to investigate membrane proteins may disrupt native interactions. We focus on complementing the current approaches by integrating two methods: (i) the highly efficient site-specific labeling of the bicyclononyne-modified pyrrolysine amino acid introduced via unnatural amino acid mutagenesis and (ii) the detergent-free membrane protein solubilization based on the styrene-maleic acid copolymer (SMA copolymer). Critically, the SMA copolymer solubilizes membrane proteins together with a shell of native phospholipids, generating discoidal nanoparticles. Once solubilized, the membrane proteins are labeled and visualized using single-molecule imaging, demonstrating the presence of individual proteins in lipoparticles. We have evaluated and established an approach that is both rapid and generalizable providing a stable population of uniquely-labeled membrane proteins for quantitative fluorescence-based studies.

75 Genome-driven evolutionary game theory helps understand the rise of metabolic interdependencies in microbial communities

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Metabolite exchanges in microbial communities give rise to inter-species dependencies that govern ecosystem diversity and stability. It is unclear, however, how these metabolic interdependencies emerge and subsist in natural microbial communities. Here we address this question by integrating evolutionary game theory and genome-wide network models of metabolism. Specifically, we use microbial fitness values estimated by metabolic models to infer evolutionarily stable interactions in multi-species microbial "games". We first validated our approach using a well-characterized yeast cheater-cooperator system. We next performed over 80,000 *in silico* experiments to infer how metabolic interdependencies mediated by amino acid leakage in *Escherichia coli* vary across 189 amino acid pairs. While most pairs showed shared patterns of inter-species interactions, multiple deviations are caused by pleiotropy and epistasis in metabolism. Furthermore, simulated invasion experiments revealed possible evolutionary paths to obligate cross-feeding. Our study provides genomically driven insight into the rise of metabolic interdependencies in microbial communities,

with implications for microbiome research and synthetic ecology.

76 Unusual association between transposase and lanthipeptide genes in *Prochlorococcus* - A new form of diversifying recombinationdelta

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Lanthipeptides are ribosomally derived peptides that undergo extensive post-translational modification, giving them complex polycyclic structures. Prochlorosins are a subgroup of lanthipeptides produced by certain strains of the globally abundant marine picocyanobacteria *Prochlorococcus* and *Synechococcus*. Instead of synthesizing a few lanthipeptides with defined structures as is typical for bacteria, *Prochlorococcus* strains can produce a cocktail of up to 29 prochlorosins with remarkable structural diversity from prochlorosin precursor peptides, ProcA. ProcA genes have a highly-conserved leader region followed by a highly variable core region which is processed by a single highly versatile prochlorosin lanthionine synthetase. Although the vast majority of lanthipeptides are bactericidal, the function of prochlorosins is unknown. In this work, we present evidence for an unusual association between a single-stranded DNA transposase and prochlorosin genes in *Prochlorococcus* genomes, that we propose is helping to drive the establishment of structural diversity. The transposase belongs to the IS200/IS605 insertion sequence family, that carries single-stranded DNA intermediates and recognizes hairpin structures, which we identified as potential link to the prochlorosin genes. We show that the transposase is active and able to promote the lateral transfer of ProcA through phage particles, providing a vector to generate the multiplicity and diversity in the pool of prochlorosin genes. By combining the characteristics of these single-stranded DNA transposases and the unusual prochlorosin gene diversity, we propose a novel mechanism of diversifying recombination in the bacterial genome.

77 All that can be regulated shall be: How transcriptional control drives bacterial cell cycle progression

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Every cell undergoes transcriptional changes as it progresses through its cell cycle. The cell must balance metabolic need and energy requirements in a manner that is not only efficient but also amenable to changes in environmental conditions. In this study, we ask how different aspects of *M. tuberculosis* (Mtb) physiology are driven by cell cycle dependent transcriptional changes. A *dnaA* cold sensitive strain of Mtb provides us the opportunity to study global transcriptional changes in synchronously replicating populations. We performed RNA-Seq analysis of Mtb as it goes through two cell cycles. We hypothesized that periodic fluctuations in gene expression could be a plausible mechanism to maintain thermodynamic efficiency. Hence, we imposed a sinusoidal function on our data to extract genes with periodic expression. We identified ~400 periodic genes, an unprecedented 10% of the genome. We find that proteins required for new growth pole biogenesis are sequentially transcribed, i.e, the proteins required early peak in expression early as well. We timed the septal arrival of the proteins coded by these genes and discovered that their localization dynamics are mirrored, and potentially dictated, by their expression dynamics. We find that anabolism of nucleosides coincides with the DNA replication phase of the cell cycle, with the intriguing exception of guanosine whose synthesis coinicides with cell division. We are in the process of testing our hypothesis that an enhanced *ftsZ* driven GTP requirement dictates this unique regulation. In summary, we predict that genes are transcribed only when (or just before) they are needed and exquisite mechanisms are in place to ensure this regulation.

78 Selection for proteins that overcome heat-induced lethality of *degP* strain

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The periplasmic protease/chaperone DegP (HtrA) plays a key role in the quality control of many proteins in the periplasm of *E. coli*. Proteins that fail to fold in the periplasm can be proteolysed, while others are chaperoned to their native folded state by DegP. In a *degP* strain, *E. coli* is unable to survive the protein

folding stress at elevated temperatures. Utilizing this phenotype, we developed a plasmid-based selection of suppression of heat-induced lethality in a *degP* strain. Plasmid libraries of various prokaryotic genomes were screened for proteins that overcame heat-induced lethality. Initial hits indicate novel mechanisms of overcoming periplasmic stress such as the periplasmic expression of a cytoplasmic GrpE homolog and the cytoplasmic expression of an unknown protein.

79 RNA-Seq Enrichment Reveals Novel Mucosal Colonization Factors in the Human Gut Bacteria

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Gut microbiome changes have been linked to many diseases, including diabetes, inflammatory bowel disease, and mental illness. However, little is known about how gut microbiota inhabit a variety of microenvironments within the gastrointestinal tract and, in particular, how a subset of this community specializes in colonizing the epithelial surface. Previously, commensal colonization factors (CCFs) were identified in *Bacteroides fragilis* that are necessary for colonization of the mucus and crypts of the host gut. However, overabundance of host RNA restrain the exploration of bacterial transcriptome within host-associated niches. To overcome this difficulty, we used hybrid selection to enrich for bacterial RNA, and performed RNA-Seq to compare the transcriptome of *B. fragilis* in the colon lumen, mucus and tissue of mono-colonized mice. Hybrid selection increased reads mapping to the *B. fragilis* genome by 48 and 154-fold in mucus and tissue, allowing for high fidelity comparisons across sample sites. In the mucus and tissue, *B. fragilis* up-regulated many genes involved in protein synthesis, indicating these bacteria are active and thriving in the mucosal niche. Rather than broad changes in metabolic pathways, a specific sulfatase and glycanase were highly induced in mucus and tissue. Mutants in these individual genes exhibited a mucus-specific defect in colonization of the mouse colon, which was disadvantageous during competitive colonization experiments. Furthermore, the mucosal glycanase was required for *B. fragilis*-mediated immunomodulation and protection from colitis. Therefore, colonization of the colon mucus is mutually beneficial for this bacterial symbiont and its mammalian host.

80 Tn-Seq Screen reveals metabolic genes important for oxidative stress survival in *P. gingivalis*

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Tolerance of oxidative stress is an essential function of the oral pathogen, *Porphyromonas gingivalis* (PG), for survival in the human mouth. PG is an obligate anaerobe and exposure to atmospheric oxygen for extended periods of time causes the formation of damaging oxygen radicals. Exposure to hydrogen peroxide, a reactive oxygen species (ROS), also causes the formation of oxygen radicals in PG. The genetic basis of oxidative stress tolerance in PG is partially known, and not all of the elements involved have been characterized. We used Tn-seq, a method of mining the bacterial genome for genes that are involved in various processes, to discover previously unidentified players in the oxidative stress tolerance machinery in PG. We exposed a transposon mutant library to atmospheric air or hydrogen peroxide and used a scoring system to analyze the sequencing data for loss of function mutations that appear to cause oxidative stress sensitivity. Several metabolic and biosynthesis genes, including two we have designated HutU and FacL, were identified as previously uncharacterized contributors to oxidative stress tolerance in PG.

81 How a transcript calls for help: LepA-mediated regulation of porin synthesis in mycobacteria

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Across all kingdoms of life, ribosomes require the activity of GTPases to aid in protein production. Within

the proteome of any species, certain proteins, such as membrane proteins, require the delicate tuning of translation rate through ribosome-associated GTPases. Elongation factor 4, or LepA, is a ribosome-associated GTPase conserved across bacteria, mitochondria, and chloroplasts. Yet, its physiological function at the ribosome remains controversial. Structural data indicates that LepA could function as a ribosome – back-translocase, relieving ribosomes stalled during translation. Alternative data suggests that LepA functions during translational initiation. A transposon-screen in our lab indicated that a *lepA* mutant had altered drug tolerance in *M. smegmatis*, suggesting that LepA might be controlling translation of proteins involved in this phenotype. Using whole-cell proteomics and RNA-sequencing to discover proteins whose translation is controlled by LepA, we show that LepA regulates translation of mycobacterial porins. Specifically, LepA improves translation of an mRNA motif within the coding sequence of the porin MspA. We hypothesize that this mRNA motif may stall the ribosome, subsequently requiring LepA activity. LepA regulation of MspA influences the permeability of the mycobacterial cell membrane, and disruption of this regulation results in the drug tolerance phenotype of the *lepA* mutant. In conclusion, we have demonstrated that in mycobacteria, LepA maintains membrane homeostasis through control of translation rate at the ribosome.

82 Coordination of antibiotic resistance and growth by an atypical two-component system and the nitrogen phosphotransferase system

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As single-celled organisms, bacteria possess a wide array of sensory systems that detect and respond to diverse environmental conditions and stimuli. *Pseudomonas aeruginosa*, which is a ubiquitous environmental bacterium and also a frequent cause of opportunistic human infections. It has long been appreciated that *P. aeruginosa* is able to survive and replicate under a wide variety of environmental conditions, including the lungs of patients afflicted with Cystic Fibrosis (CF). Indeed, *P. aeruginosa* is a leading cause of morbidity and mortality in CF patients. Coupled with a rapid rise in antibiotic resistance amongst bacterial pathogens, including *P. aeruginosa*, novel insights into how these organisms sense and respond to diverse environmental stimuli, such as exposure to antibiotics, is needed. This study focuses on the characterization of an atypical two-component regulatory system required for resistance to aminoglycoside antibiotics. Strains deleted for the gene encoding either component demonstrate a 1,000-fold increase in sensitivity to the aminoglycoside tobramycin, a front-line therapeutic option for CF *P. aeruginosa* infections. Using a variety of bacterial genetics approaches, we have identified a link to the Nitrogen PhosphoTransferase System (PTS-Ntr), suggesting that this pathway may be critical for coordinating external environmental cues with the intracellular metabolic state of the bacterial cell.

83 DNA as a source of phosphorus: insights from microbial community dynamics in nutrient-supplemented experiments

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Extracellular DNA (eDNA) is naked DNA found in many environments, and could be used by microorganisms as a nutrient source. Hypersaline environments harbor high concentrations of eDNA and are home to communities of halophilic microorganisms. However, it remains poorly understood which of these microorganisms can use eDNA and under what environmental conditions. In this study, we investigated how growth rate and composition of a halophilic microbial community change under different nutrient limitations, and how nutrient availability and community composition affect the eDNA pool. To address these questions, we extracted a natural microbial community of a saltern, and incubated it under different combinations of carbon, nitrogen, phosphorus, and eDNA. The community composition was tracked using the rpoB gene as a molecular marker. We found that under carbon or nitrogen starvation, two genera in the community compete for the limited nutrients. Providing both carbon and nitrogen (but not phosphorus) increased overall growth of the community, indicating that either chromosomal DNA or eDNA available in the environment may serve as a source of phosphorus. A dramatic shift in the community composition was also observed, suggesting that only some community members could utilize these potential phosphorus sources. Provision of eDNA in addition to carbon and nitrogen led to even better growth of some community members, indicating that eDNA may indeed serve as a source of phosphorus.

84 Systematic analysis of the *Escherichia coli*-phage T4 interaction as a host-parasite model system

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The phage T4 has a remarkable history as an important model system that helped to lay the foundation of molecular biology in mid-20th century. Yet, nearly one-half of genes still have an unknown function. We hypothesize that many of these genes have functions specific for interaction with its host. Accordingly, the interaction of T4 with *E. coli* can serve as model system to identify targets important for parasite-host relationships. To elucidate interactions and survival strategies of phage T4 with *E. coli*, we and T4 phage, we used the *E. coli* single-gene deletion mutant library, the Keio collection, to conduct genome-wide screens to identify mutants that inhibit phage T4 growth by spotting tests on *E. coli* in microplate assays (n=3). More than 30 candidates showing T4 growth inhibition were found. Interestingly, *E. coli* mutants deleted of cryptic phage genes (*ymfE, ymfO, ymfP, and ymfR*) inhibited phage T4 growth. Mutants deleted of these *ymf* genes showed smaller plaque sizes and a lower efficiency of plating (EOP) compared to wild-type *E. coli* K-12 BW25113 (the parent of the Keio collection). Some mutants showed a mucoid phenotype in the spotting test. The crystal violet biofilm assay showed that the *nsrR*, *ydfK*, *ymfR*, and *ECS078* mutants increased the amount of biofilm, suggesting that extracellular polymeric substances may affect T4 phage growth. Also, the *glyA* mutant (which lacks an enzyme for conversion of glycine to serine) showed decreased phage adsorption efficiency compared to wild-type, suggesting a role for GlyA in lipopolysaccharide biosynthesis. One-step growth experiments showed that *greA*, *ybeY*, and *ychF*, which may be involved in transcription or translation, decreased burst size and growth rate of phage T4.

85 Bacterial growth physiology limits the rate translation termination

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The *in vivo* kinetics of the various steps of mRNA translation depend on the concentration of associated protein factors. However, what limits the expression levels of translation factors and the resulting reaction rates is generally unknown. Focusing on translation termination, we describe a coarse-grained model of the relationship between release factor (RF) abundance and ribosome usage. We show that bacterial RFs are not expressed at saturating levels for termination rates, but instead at the model-predicted optima for efficient ribosome usage as required by bacterial growth physiology. Targeted perturbations of RF expression in *Bacillus subtilis* combined with high resolution growth and translational profiling confirmed two key model predictions across different scales: (1) the protein abundance fitness landscape macroscopically and (2) ribosome queuing at stop codons microscopically. These results highlight the requirement for precisely tuned protein levels due to a trade-off between reaction rates and proteome allocation.

86 Alternate pathways to type I IFNs in macrophages infected with *Mycobacterium tuberculosis*

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Tuberculosis remains a leading cause of death globally; a fundamental understanding of the host-pathogen interactions that allow disease to be established and progress would facilitate the development of new approaches to preventing or treating disease. Failure to sterilize infection arguably begins the moment that macrophages fail to "see" the causative bacterium, *Mycobacterium tuberculosis* (Mtb), in a way that triggers a protective response. Although Mtb is theoretically recognized by multiple macrophage pathogen recognition receptors (PRRs), the specific molecular interactions that are most salient in the context of infection remain unclear. The cytosolic PRR cGAS, which binds double-stranded DNA, has previously been shown to be required for induction of the type I IFN response to Mtb. According to the current model, following Mtb-mediated phagosome permeabilization, Mtb genomic DNA binds cGAS, triggering the type I IFN response. Several aspects of this model are unclear, including how mycobacteria remain viable after exporting their DNA and a recent report linking mitochondrial damage with the macrophage type I IFN response to infection with Mtb clinical isolates. In addition, we find that macrophage lipid metabolism upon infection

with Mtb is critically important for induction of type I IFNs; this effect cannot be explained within the current model. Here we propose and test an alternate model for induction of the macrophage type I IFN response to Mtb infection.

87 Investigating the Role of DinB in R-loop Resolution

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As the replication fork progresses through the DNA template, it may encounter many obstacles, one of which is the RNA transcription machinery. This encounter occurs because both processes utilize the same template and synthesize at different rates. The encounter of the two machineries results in replication-transcription collisions (RTCs). Failure to resolve these collisions leads to replication fork stalling and eventually cell death. One of the events required to resolve RTCs is the removal of RNA: DNA hybrids, known as R-loops. In *E. coli*, RNase H1, coded by *rnhA*, mediates the resolution of R-loops by cleaving the RNA strand on RNA: DNA hybrids. If not resolved, R-loops cause genomic instability in the form of double-stranded breaks through the collapse of the replication fork and accumulation of DNA lesions. In *E. coli*, failure to resolve R-loops lead to induction of the SOS response. Among the first genes to be upregulated is dinB, encoding DNA polymerase IV (DinB), becoming the most abundant DNA polymerase in the cell (2500 nM). DinB main role is to synthesize DNA past lesions in the template. There is evidence to support the notion that DinB is involved in homologous recombination, a mechanism used to repair double-stranded breaks where it outcompetes other polymerases for the recombination intermediate and synthesizes DNA from it. Due to this, DinB may also be involved in the resolution of R-loops. Here we investigate the possible role of DinB in R-loop resolution in a rnhA-deletion strain.

88 Mutation of a Diguanylate Cyclase in Environmental Isolates of Fluorescent Pseudomonads

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Pseudomonas fluorescens is a biofilm-forming rhizobacteria. In *Pseudomonas fluorescens* Pf0-1, diguanylate cyclase GcbC synthesizes messenger molecule c-di-GMP and interacts with adhesive proteins to regulate surface attachment. Though other fluorescent Pseudomonads form biofilms, it is unknown whether they utilize similar mechanisms to Pf0-1. In this study, two fluorescent Pseudomonads containing *gcbC* orthologs were isolated from the rhizosphere and evaluated for biofilm formation. GcbC orthologs were mutated using bacterial conjugation, and biofilm formation was reassessed. Mutant strains with a defective *gcbC* display changes in phenotype and biofilm formation. This study explores mutations to biofilm-related genes and methods of biofilm assessment in Pseudomonas.

89 Molecular Mechanisms of Transcriptional Regulation by DnaA in *Bacillus subtilis*

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DnaA is the replication initiator in bacteria. It binds cooperatively to DnaA boxes in the origin of replication (*oriC*), promotes DNA unwinding, and recruits replication machinery. DnaA also functions as a transcription factor, playing an important role in regulating its own transcription and in the response to replication stress. DnaA, a AAA+ ATPase, is active for replication initiation and binds more cooperatively to *oriC* when ATP-bound than when ADP-bound. In contrast, little is known about its activity as a transcription factor. Because DnaA is an essential gene, it has been difficult to study its effect on transcription *in vivo*. We therefore established an *in vitro* system to dissect the molecular mechanisms by which DnaA affects transcription. We identified two promoters that are directly bound and repressed by DnaA *in vivo* and *in vitro*: *PywcL* and *PtrmE*. Each of these promoters contains DnaA boxes both upstream and downstream of the transcription start site (TSS). In this system, addition of purified DnaA represses transcription specifically and in a dose-dependent manner. We have shown that the DnaA box arrays upstream of the TSS in *PywlC* and *PtrmE* are sufficient to repress transcription. We have also shown that DnaA-ATP represses transcription from these upstream arrays at lower concentrations than DnaA-ADP. Our current model is that DnaA binds these promoters using the same cooperative mechanism by which it binds *oriC*, assembling a helical filament that occludes the core promoter to repress transcription initiation.

90 Shape, form and dynamics of bacterial swarms and biofilms.

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Collective microbial swarming and biofilm colonization on hydrated surfaces are important in many clinical settings, such as in the contamination of implants, catheters and during chronic infections. What are the physical constraints that set the limits to the structure and dynamics in expanding microbial colonies? To address this question, we develop a single unified multiphase framework that couples geometry, fluid flow, mechanical stresses, nutrient and osmolyte transport with localized cell growth and biomass production. Our model describes steady-state swarm expansion as fluid-mediated, and governed by osmolyte production and fluid exudation from the substrate. In contrast, transient biofilm colonization is nutrient-transport mediated, and governed by localized zones of exopolysaccharide production that drives expansion. Our unified framework allows us to explain a range of recent experimental observations associated with the shape, form and dynamics of *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* swarms and biofilms in terms of underlying mechanical and physical forces. In this manner, we demonstrate how hydrodynamics and transport serve as key physical constraints in regulating biological organization and function in microbial communities.

91 Nonstandard amino acid incorporation in *Bacillus subtilis*

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Non-standard amino acids (nsAAs) are useful experimental and practical tools for probing, labelling, or controlling proteins in a minimally disruptive manner. However, these tools have been mostly unavailable in many bacterial model systems, especially the primary gram-positive model organism, *Bacillus subtilis*. Here we describe successful introduction of several aminoacyl-tRNA synthetases derived from the *Methanococcus jannaschii* tyrosyl-tRNA synthetase to incorporate nsAAs into proteins in *B. subtilis*, including nsAAs used for bio-orthogonal labelling, biocontainment, and photo-crosslinking. Additionally, we use a combination of traditional transcriptional induction and nsAA-mediated translational control to tightly regulate protein expression. Our dialable system is capable of a 700-fold increase in protein signal upon induction. The expansion of nsAA technology to *B. subtilis* should present new experimental options for bacterial researchers.

92 Defined mutant library of *E. piscicida*

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A defined mutant library (DML) is a powerful resource for functional genomics research. By using a modified Mariner transposon harboring promoterless egfp and constitutively expressed mCherry, we constructed and characterized a near-saturating transposon insertion library with 20,668 identified unique mutants (5.04 mutants/gene) in *Edwardsiella piscicida* strain EIB202, a highly infectious pathogen colonizing macrophages and plaguing the aquaculture industries. At least one representative insertion mutagenesis could be assigned to 2,806 distinct genes, accounting for 78.0% of all predicted ORFs, thus defining the putatively essential gene set for *in vitro* growth of the bacterium. The composition of defined subset libraries and transposon insertion sequencing enabled explore the conditional essential gene sets when grown under conditions representing key stages of the bacterial life cycle *in vivo* and *in vitro* of the natural host turbot. Besides the mosaic distribution of the various conditional essential genes in the chromosome, clustered genes associated to type III/VI secretion systems (T3/6SS), LPS, and NADH are found and validated to be essential for *in vivo* colonization of a fish, nevertheless they are only required for specific *in vitro* condition(s). The screens unexpectedly identified T3SS important during growth in prolonged cold marine conditions.

93 Evaluating conservation of cross-species antibiotic stress response pathways

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Antibiotic resistance can be driven by selective pressures on genes responding to the direct target of an antibiotic or involve genes indirectly targeted. The phenotypic response of bacteria to a particular stressor can be assessed by colony growth screens of ordered mutants or more recently by transposon insertion sequencing (Tn-Seq). While we and others have evaluated phenotypic responses for individual bacteria and strains, it is not clear if phylogenetically diverse pathogenic bacteria respond similarly to the same antibiotic stress. To address this question, we combined our Tn-Seq fitness datasets for *Streptococcus pneumoniae* and *Acinetobacter baumannii* with published fitness datasets for *Escherichia coli* and *Pseudomonas aeruginosa*. We evaluated the phenotypic response to ciprofloxacin, and also gentamycin and azithromycin. Conserved responses were identified by overlaying genes demonstrating a phenotype to these antibiotics on two orthology frameworks generated by OMA (Orthologous MAtrix) and PATRIC (Pathosystems Resource Integration Center). For ciprofloxacin, only one gene, *recN*, was found to be orthologous and conserved among all 4 strains, but more closely related species were found to share a greater number of genes. All species showed enrichment for genes involved in DNA replication and repair for their individual responsive genes, consistent with ciprofloxacin mechanism of action as a DNA synthesis inhibitor. However, responsive genes not involved in DNA replication and repair were not well conserved between species. Our data suggest that while small number of similar genes may respond to the primary target of an antibiotic across species, the indirectly responsive genes are highly variable.

94 Identifying new gene regulatory mechanisms of the Gac/Rsm pathway in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a ubiquitous environmental bacterium mainly recognized for its innate resilience to antimicrobial treatments and the acute and chronic infections it causes in human. The reason for this resilience is simple: *P. aeruginosa* possesses a relatively large genome encoding many intrinsic antibiotic resistance mechanisms, along with a plethora of regulation genes to better adapt to its environment. The main lifestyle modulating system, responsible for the acute or chronic infection outcome, is the Gac/Rsm pathway. Briefly, the Gac two-component system comprised of sensor kinase GacS and response regulator GacA activates the transcription of the small non-coding RNAs *RsmY* and *RsmZ*, which then bind to, and sequester, the post-transcriptional regulator RsmA. The level of available of RsmA then dictates the main lifestyle (motile or sessile) of the population, favoring acute or chronic infections, respectively. We recently revealed the existence of a new regulatory pathway activating specifically *RsmZ* when the bacteria are grown on surfaces, vs planktonically. Using a random mutagenesis screening with a Tn*5* transposon, we are uncovering genes involved in this surface-only overexpression of *RsmZ*. Until now, various genes have been identified including *fgtA*, a flagellar glycosyltransferase, suggesting a role in mechanosensing of surfaces. Better understanding of the surface lifestyle of *P. aeruginosa* is critical to further comprehend the complex regulatory mechanisms involved in the pathogenesis of this important opportunistic human pathogen.

95 Regulation of mRNA stability in mycobacteria as an adaptive response to stress conditions

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis, a significant cause of human mortality. Mtb is a successful pathogen due in part to its ability to survive stress conditions by entering non-growing states. In these low-metabolic states, Mtb can develop antibiotic resistance, making its adaptation to stress crucial for survival.Numerous bacteria have been shown to reduce their rates of mRNA turnover under stress. While the existence of this response appears to be conserved in bacteria, the mechanisms underlying mRNA stabilization remain unknown. To identify the mechanisms that govern mRNA stabilization in mycobacteria, we used the non-pathogenic model *Mycobacterium smegmatis*. We found that mRNA half-lives were longer in hypoxia and carbon starvation compared to log phase. Interestingly, hypoxia-adapted cultures that were re-exposed to oxygen for two minutes displayed increased transcription of some genes accompanied by dramatic shortening of mRNA half-lives. Furthermore, experiments using a translational inhibitor in re-aerated hypoxia cultures suggested that the increased mRNA turnover occurs in absence of protein synthesis. Hence, we hypothesize that metabolic changes during growth cessation impact the activity of degradation proteins, increasing mRNA stability. However, other mechanisms may also be involved. For example, we found that in *M. smegmatis* total RNA levels remain similar in non-growing states,

while mRNA levels are significantly reduced. Thus, mRNA stability could result from ribosomes masking RNase cleavage sites. Together, our data are consistent with a model in which mRNA stability is controlled at the level of RNase activity and/or accessibility of transcripts to the RNA degradation machinery.

96 *Pseudomonas chlororaphis* as a new model for shikimic acid prodution

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Shikimic acid is a high-value metabolite and can be naturally produce by plants and bacteria. Usually, shikimic acid is used for the production of antiviral drugs (essentially oseltamivir), cosmetics, dietary supplements, anticoagulant and many others. Currently, the main source of this compound is the Chinese star anise (*Illicium verum*) but the yields and efficiency are very low, for these reason, different groups have modified strains of *E. coli* and recently, *Corynebacterium glutamicum* to overproduce this metabolite. Our group propose *Pseudomonas chlororaphis* as a potential producer for this compound because of its natural characteristics such as a resistance to stressful conditions, an ability to metabolize different carbon sources and facilities to produce compounds derived from the shikimate pathway. Until now, we have done some genetical modification in this strain to maked it capable for acumulate shikimate, aditionally we have direct the carbon flux to shikimic acid pathway. We have tested these strains in batch fermentation using rich medium with glucose or glycerol as a carbon source noting differences in growth and production between them. Glycerol, which is cheaper than glucose have demostrated to be a better carbon source obteining a higher biomass and yields up to 0.24 mmol/L

97 Illuminating Translation Through Synthetic Biology and Directed Evolution

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In nature, fundamental biological phenomena that are central to cellular life are inherently hindered from probing and interrogation, as these dynamic systems cannot be easily decoupled from immediate artifactual disruptions throughout the living cell. One such case is the ribosome, a colossal multi-component protein factory that functions as the nexus for cellular information and signaling events, integrating nutrient availability with growth dynamics and resource allocation. Despite decades of research, this biomolecular assembly remains superficially understood and underexplored, owing to the difficulty associated with decoupling the translational apparatus from cellular viability. Using engineered orthogonal transcription-translation networks, we can robustly monitor ribosome activity in living cells, overcoming challenges associated with ribosomal manipulation *in vivo* and providing a framework for high-throughput ribosomal interrogation. Our ongoing work has built upon this platform to study various parameters of ribosomal function, including the evolution of the modern ribosome, dissection of putative processivity-fidelity relationships during translation, experimental validation of ribosomal divergence across prokaryotes, and prediction of ribosome-small molecule interactions. This work has the potential to extend our understanding of key factors governing ribosomal function and dynamics, providing potentially general design paradigms for manipulating translation *in vivo*.

98 Exploring potential inhibitors of LprG towards a better understanding of lipid transport mechanisms in *Mycobacteria*

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Tuberculosis (TB) is the 2nd deadliest infectious disease worldwide and kills approximately 1.2 million people per year. The public health crisis posed by TB requires new therapeutic strategies. Current front-line drugs such as isoniazid (INH) and ethambutol (ETB) kill the causative bacterium, *Mycobacterium tuberculosis* (Mtb), by inhibiting the biosynthesis of essential cell wall components. New chemical agents targeting proteins that transport cell wall components may act synergistically or additively with existing drugs to improve efficacy and lower the rate of resistance. Our previous studies have implicated Mtb lipoprotein LprG in lipid transport between the inner and outer membranes. Also LprG is required for Mtb virulence in mouse models, suggesting this lipid transport mechanism as a potential drug target. In my research, a set of 350,000 compounds were screened using DOCK, a molecular docking program, to predict the binding modes

and associated energies between small molecules and a crystallographically defined protein structure. 48 compounds were purchased for biological assay. The dissociation constants of the leading compounds were identified by a competition assay using a fluorescent lipid analogue and several compounds with low M Kd were found. Furthermore, the specificity of the top compound is proved by showing its growth inhibition to wild type *Mycobacterium smegmatis* (Msmeg) but not *lprG*. These studies will promote our understanding of the roles of LprG in lipid transport, as well as the potential validation of lipid transport as a drug target.

99 A universal tradeoff between bacterial growth and adaptation

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The rate of cell growth is widely recognized as a crucial fitness measure, and proteome allocation has been established as a critical determinant of steady-state growth rate for bacteria. Yet increasing evidence suggests objectives other than growth rate also play key roles in proteome allocation. The rate of adaptation is another physiological characteristic that is crucial in ecology and evolution, especially for microorganisms coping with frequently changing environments. Although tradeoffs between phenotypes have often been invoked, concrete examples offering systematic, quantitative analysis of such tradeoffs are rare. Here, we report the occurrence of an obligatory tradeoff between growth and adaptability for *E. coli*, upon abruptly shifting a growing culture from a preferred carbon source (e.g., glucose) to fermentation products (e.g., acetate). Such transitions, which are common for enteric bacteria, are often accompanied by multi-hour lag before growth resumption. For these transitions, the inverse lag time (a measure of adaptability) was found to exhibit a remarkable linear decline with increasing pre-shift growth rates, approaching zero (infinite lag) at the maximum growth rate. Metabolomic analysis revealed that the long lags result from the depletion of key metabolites due to the sudden reversal of central carbon metabolic flux imposed by these nutrient shifts. The metabolic data led us to a model of sequential flux limitation which not only explained the observed universal tradeoff between growth and adaptability, but also generated many other quantitative predictions that we validated experimentally. In particular, the existence of this obligatory tradeoff limits the benefits of fast growth and provides a quantitative criterion for selecting growth rates based on the expected abundances of substrates in the environment.

100 Balancing kinetic and thermodynamic barriers to isomerization catalysis in probiotic *Lactobacillus plantarum.*

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Food-safe probiotic *Lactobacillus plantarum* is a promising non-model platform bacterium for production of nutraceuticals using metabolic engineering. Biosynthesis D-tagatose, a low-caloric sugar-substitute with anti-glycemic properties, has been reported using various engineered bacteria expressing L-arabinose isomerase. However, low productivity remains a barrier to economical production of this sweetener. Strategies to improve productivity have relied on enzyme engineering to improve kinetic properties toward substrate D-galactose. However, the primary limitation to productivity is not kinetics, but thermodynamics since isomerization of D-galactose to D-tagatose is only mildly favorable. Resultantly, whole cell biocatalysts that disproportionally partition substrate and product across a membrane can circumvent this thermodynamic limitation. Unfortunately, this thermodynamic advantage results in a kinetic penalty due to transport limitations. In this work, we use the mesophilic and acid tolerant L-arabinose isomerase from *Lactobacillus sakei* in *Lactobacillus plantarum* as a model system to study D-tagatose production. We confirmed that D-tagatose production was thermodynamically limited in cell-free lysates and transport-limited during whole-cell catalysis. Next, we focused on improving productivity through an investigation and subsequent mitigation of membrane transport barriers. We explored, in detail, cellular engineering strategies including surface display, overexpression of native and non-native sugar transporters, and cell permeabilization techniques to achieve a superior whole-cell biocatalyst for D-tagatose production compared to those reported in

literature. Through this investigation, were able to circumvent the thermodynamic conversion barrier while maintaining high reaction rates. This work provides novel insights and demonstrates new tools to guide engineering efforts in probiotic *Lactobacillus plantarum* as well as other Gram-positive bacteria.

101 The potential of *Burkholderia cepacia* complex to produce 4-hydroxy-3-methyl-2-alkylquinolines

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Burkholderia cepacia complex (Bcc) comprises of more than 20 ubiquitous and versatile species, including both plant-beneficial and pathogen groups. A few *Burkholderia* species, are already known to release 4-hydroxy-3-methyl-2-alkylquinolines (HMAQs), molecules analogous to the *Pseudomonas* quinoline signal (PQS), a quorum sensing autoinducer produced by *Pseudomonas aeruginosa*. An initial assessment was performed using liquid chromatography coupled to mass spectrometry, on production of five HMAQ and 4-hydroxy-2-alkylquinolines (HAQ) families by various environmental and clinical Bcc strains. The data revealed that some strains, belonging to at least seven different Bcc species produce HMAQs. Moreover, our initial results show that clinical strains are more likely to produce HMAQs than environmental ones. Also, we have studied the distribution of the *hmqABCDEFG* operon, encoding the enzymes involved in the biosynthesis of HMAQ, in the Bcc group. Based on the 593 available genomic sequence data, this operon is not found in all Bcc species and, furthermore, not even all strains in a given species have the operon. For instance, almost all strains of nine species are positive while none of the 30 sequenced strains of *B. cenocepacia* species encode the capacity of synthesizing these metabolites. Moreover, we have looked for the synteny of the genes surrounding the *hmqABCDEFG* operon. For some species, the operon seems to have been deleted or replaced by other genes. Future studies of the regulation of the Hmq system and the role of HMAQs are underway. This knowledge should provide a better understanding of pathogenic *Burkholderia* strains.

102 Probiotic *E. coli* outfitted with a protein delivery system for *in situ* delivery of therapeutic payloads in the gut

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New approaches are needed for the delivery of therapeutic agents to sites of disease to improve efficacy and limit off-target side effects. Here we describe our efforts to reengineer the type III secretion system (T3SS) of *Shigella flexneri* into a programmable protein-delivery system. T3SSs are nanomachines used by many pathogenic bacteria to inject virulence proteins into the cytosol of host cells. Previous work in our laboratory led to the development of a non-pathogenic strain of *Escherichia coli* that encodes a functional *Shigella* T3SS, but none of its secreted virulence proteins. In order to transform the *Shigella* T3SS into a therapeutic agent for the delivery of proteins, we have transferred this nanomachine into *E. coli* Nissle 1917, a probiotic strain with a well-established human safety profile. For its use in the gut lumen, we have modified the T3SS to secrete into its surroundings as opposed to host cells. We have engineered single domain antibody-based neutralizing agents (VNAs) to be recognized and secreted by the T3SS. Constitutive expression of the system allows VNA secretion into the medium at levels of approximately 5 mg/L and toxin neutralization assays have shown that VNAs remain active once secreted. We are currently working to improve the stability of the secretor strain for its use in animal models of disease. Once established, this designer probiotic platform can be used to secrete a diversity of VNAs targeting essential bacterial toxins and pro-inflammatory cytokines, with the potential of becoming a novel therapeutic paradigm for intestinal infections and inflammation disorders.

103 Evidence for a sugar intermediate as a novel cell wall peptidoglycan inhibitor in *Bacillus subtilis*

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Galactosemia is a potentially fatal disease arising from the inability to properly metabolize galactose. Caused

by a mutation or deficiency in the highly conserved Leloir pathway, galactosemia has been shown to affect all domains of life with the molecular basis of toxicity largely unknown. It has recently been shown in the model bacterium *Bacillus subtilis* that a deletion of *galE*, the final gene in the Leloir pathway, causes a buildup of an intermediate sugar metabolite uridine-diphosphate-galactose (UDP-Gal) and a toxicity phenotype characterized by cell shape abnormality and rapid cell lysis. Herein, we show that this accumulation of UDP-Gal causes an inhibition of peptidoglycan synthesis, the main component of bacterial cell walls. We demonstrate that this inhibition is due to a depletion of Lipid II levels, the precursor of peptidoglycan, synthesized by the essential protein MurG. This toxicity is partially alleviated by overexpression of MurG in *B. subtilis* as well as other organisms. We identify a previously uncharacterized step to the Leloir pathway, involved in glucose metabolism and essential for UDP-Gal production, and provide evidence of a galactose uptake mechanism in *B. subtilis*. Finally, we show that toxicity is not due to depletion of essential nucleotide sugar but instead accumulation of UDP-Gal. Given this, we propose that toxicity associated with UDP-Gal accumulation is due to inhibition of the essential glycosyltransferase and suggest that this mechanism affects multiple organisms, providing insight into toxicity associated with galactosemia.

104 Electron Transport Chain Perturbations Alter Isoniazid Efficacy in *Mycobacterium tuberculosis*

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Isoniazid (INH) is a first-line antitubercular agent, used to treat latent tuberculosis and in combination therapy for active tuberculosis. INH is efficacious only against actively growing cultures of *Mycobacterium tuberculosis*, and virtually ineffective against static cultures. Here we present a system-wide analysis to identify metabolic processes that contribute to altered INH susceptibility in *Mycobacterium tuberculosis*. We first performed a nutrient counter-screen to probe and quantify changes to INH sensitivity. Then, we leveraged metabolic modelling to predict the underlying processes contributing to sensitization. Increased in ATP Synthase activity was the most significant predictor of INH sensitization. We tested this prediction with a mycobacterial ATP-synthase inhibitor, Bedaquiline, in combination with INH, and confirmed that limiting ATP Synthase activity antagonizes INH lethality within the first week of treatment. However, at later time points the combination lead to a significant drop in viable bacteria, which was maintained over 30 days, without the emergence of resistance. Currently, we are biochemically targeting other components of the electron transport chain and proton motive force in combination with INH to investigate the underlying mechanism of this interaction.

105 Understanding the role RNA regulators play in *Streptococcus pneumoniae* organismal fitness

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Antibiotic resistant bacteria are a growing public health concern that has necessitated a search for novel antimicrobial targets. Bacterial RNA regulators have proven to be effective targets due to their complex structures, regulation of essential processes, and divergence from eukaryotic regulatory mechanisms. However, current knowledge of the roles these regulators play in organismal fitness, and the mechanisms by which they evolve to compensate for fitness defects, is inadequate. If we hope to preserve these regulators as antibiotic targets, it is essential to add to our understanding of the selective pressures generated by targeting them. In order to determine which regulators play an important role in organismal fitness, we inactivated a number of specific structured RNA regulators in *Streptococcus pneumoniae*, a pathogen considered a serious threat due to the prevalence of strains fully resistant to clinically relevant antibiotics. By assessing the fitness cost of inactivating these regulators under a variety of culture conditions, and within a mouse model of infection, we have identified regulators that play a crucial role in colonization and disease induction. The regulators we found to be important for infection and pathogenesis in mice, such as the pyrR RNA and the FMN riboswitch, could make robust targets for antibiotics. Moreover, regulators found to be essential for successful host infection and organismal survival provide promising sites for exploring how the organism is able to compensate for the loss of regulation, and ultimately evolve resistance.

106 Isolation, characterization, and molecular modeling of ComA mutants that bypass activation by its cognate histidine kinase ComP provides a novel mechanism for ComA activation

during the quorum response in *Bacillus subtilis*

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Quorum sensing is a process used by bacteria to coordinate biological processes with population density. In *Bacillus subtilis*, ComA is the master transcriptional regulator of quorum response genes. ComA is activated by phosphorylation via its cognate histidine kinase, ComP, resulting in increased transcriptional activation of target genes. To gain insight into the molecular mechanism(s) of ComA activation, we developed a genetic screen, identifying 38 single point mutations of ComA that bypass phosphorylation by ComP. Interestingly, no phosphomimetic substitutions were identified in the conserved aspartic acid at position 55 that have been found with other response regulators. Rather, all ComA mutants identified in our screen appear to be phosphorylated at D55 by small molecule phosphate donors like acetyl phosphate, or by a yet to be identified histidine kinase, further illustrating the unique mechanism of ComA activation. Computational modeling was performed using the ComA mutants and the VraR protein from *Staphylococcus aureus*. Our model predicts that transition of ComA from an inactive to active conformation occurs via a molecular hinge creating a pocket between alpha helices 1 and 4 that exposes a hydrophobic region required for ComA oligomerization on DNA. Mutants of ComA were isolated that mimic the active conformation with enhanced hydrophobicity and volume within the helix 1-4 patch or decreased hydrophobicity and volume in the patch comprised of helices 3 and 5, opposite the hinge. Taken together, this work provides new insight into the molecular mechanisms of activation of quorum response two component regulatory systems.

107 Genetic suppression of *hfq* colony growth defect reveals a *mexT*-dependent toxic pathway in *Pseudomonas aeruginosa*

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In the pathogenic bacterium, *Pseudomonas aeruginosa*, the RNA chaperone Hfq is important for growth. Here we demonstrate that the deletion of *hfq* causes a severe colony size defect on rich medium. Using forward genetic screens we have identified mutations that suppress this growth defect. Loss-of-function mutations in *mexT*, a gene encoding a LysR-like transcription activator, and MexT-activated genes, *mexEF* and *PA1942*, were among the most frequently isolated mutants that restore growth to *hfq* cells. The previously uncharacterized gene, *PA1942*, is acutely toxic upon ectopic expression in wild-type cells. The toxicity of *PA1942* is reduced in the absence of the *mexEF* and *gshA* genes, suggesting these genes participate in a toxic interaction.

108 Rapid turnover of viral defense genes drives clonal dynamics in marine microbes

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Viruses are key members of all ecosystems and exert top-down control of microbial populations. By selecting for resistance, viruses are thought to be major drivers of the fine-scale diversity observed among closely related microorganisms. In a 93-day time-series of marine *Vibrio* and co-occurring phages, we identify two nearly clonal populations of *V. lentus* (differentiated by 25 SNPs in the core genome) that are exclusively killed by specific phages. Using transposon-mutagenesis, gene knockouts, and comparative genomics, we show that hosts have identical receptors, but differ in phage-defense islands. The distribution of these islands reflects the hosts' phage predation profiles, suggesting these genes mediate viral specificity. These defense islands are strikingly diverse, containing restriction-modification systems, recently discovered anti-phage genes, and additional unannotated loci shared among subsets of islands. Moreover, we find highly diverse defense islands correlated to phage predation profiles across all other *Vibrio* species analyzed. We further show by quantifying viral and bacterial clonal abundance across the time-series that the different hosts undergo correlated fluctuations at near constant 5-fold difference in abundance. These differences appear to be driven by phage properties since phages show an inverse abundance profile suggesting that phages directly control host abundance even at low concentrations in the ocean. Our results overall show that phage defense islands are the most rapidly varying genetic islands, displaying astoundingly high diversity and turnover, and are responsible for clonal-level diversification of bacterial populations.

109 Understanding the Human Skin Microbiome through Integration of Metagenomics, Bioinformatics, Spatial Ecology, and Synthetic Biology

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Human skin is home to 10^{12} microbial cells comprising over 200 genera. A quantitative understanding of skin microbe communities across a range of spatial scales will lead to new approaches for identification of disease risk, facilitate prediction of disease outcomes, and spur the development of new tools for disease prevention. We aim to achieve such an understanding by analyzing the physical, chemical, and behavioral traits impacting the skin environment and then determining the role that these traits play in maintenance and disruption of complex skin microbiomes. One goal is to uncover the forces shaping interpersonal variation. For this goal, we measure skin physiology and administer detailed questionnaires in conjunction with microbiome sampling. Another goal is to examine fine-scale organization, e.g. patterns in species occurrence or co-occurrence within a single body site. For both goals, we augment sequencing studies a variety of *in vitro* analyses to determine mechanistic explanations for observed patterns from human sampling. In particular, using a library of skin commensal isolates, we study microbe-environment, microbe-microbe, and microbe-host interactions.

110 Exploring Lon-related pathways using a Phenotypic Suppressor Screen

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Regulated proteolysis is an essential cellular process that involves the degradation of both damaged as well as regulatory proteins. Protein degradation in bacteria is carried out by energy-dependent proteases, such as Lon and ClpXP, that utilize the power of ATP hydrolysis to recognize, unfold, and degrade substrates. Deficiencies in these proteases leads to defects in viability and virulence. The Lon protease is an ATP-dependent homo-oligomer composed of an ATPase as well as peptidase domain encoded on a single polypeptide. Bacteria deficient in Lon are sensitive to DNA damage, are more filamentous and, in the case of the Gram-negative bacterium *Caulobacter crescentus*, are less motile than their wild type counterparts. We can take advantage of these phenotypes to identify suppressors that can rescue the phenotypes associated with bacteria lacking Lon. Using *C. crescentus* as a model organism, we employed a phenotypic screen to identify suppressors that restore the motility defects in *lon*. The goal of the screen was to identify novel Lon interactions. Using the screen, we identified an allele of ClpX, the ATPase component of the ClpXP protease, that rescues motility as well as other *lon* phenotypes. Intriguingly, this novel allele of ClpX is now able to degrade DnaA *in vivo* whereas wildtype ClpX cannot. These studies suggest that there is an interplay between bacterial proteases, such that if one is eliminated, the others are mobilized to potentially take on new roles in the cell.

111 Studying the mechanisms of interactions among oral microorganisms in a synthetic community

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The oral cavity hosts a diverse microbial community which is crucial to human health. Yet, representing this community as a network of interactions has been a challenge, because of the multitude of species and complexity of their environment. Here we aim to reconstruct a network of interactions between a selected group of oral bacteria in an *in vitro* experimental setting. We used 9 species isolated from the oral cavity of healthy individuals, which included representative species from genera *Streptococcus*, *Actinomyces*, *Rothia*, and *Neisseria*. To characterize interactions, we grew species in the supernatant of themselves and other species under several conditions. We specifically varied the initial amount of nutrient in the supernatant (BHI, 50% or 100%), supplemented supernatants with fresh medium (at 50% or 100%), and compared pH-buffered versus non-buffered media. Surveying the results showed a variety of interaction types. In several cases, growth rate and carrying capacity were lower than what was expected from a simple null model of nutrient availability, suggesting an additional inhibitory effect. We also observed examples in which growth rate and carrying capacity improved when some species were exposed to supernatants of other species, sug-

gesting a facilitative interaction. Importantly, the environmental pH impacted the interaction types. Many species lowered the pH in the non-buffered condition (range 5.8-6.1, from an initial pH of 7.2), which we showed to negatively impact growth by testing species in fresh medium with pH values ranging from 4.5 to 8.0. Future work will investigate whether a model built on supernatant experiments successfully predicts community dynamics.

112 Machine Learning Methods to Evolve Novel Antibacterial Therapeutic Strategies

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With drug-resistant bacterial infections as a global health pandemic, the need for novel antibacterial therapeutic strategies is crucial. These strategies are derived in many cases from the study of the pertinent bacteria with high-value chemical tools. Significant challenges in chemical tool and drug discovery include (a) discovering small molecules with the antibacterial activity of interest and (b) optimizing these entities to display the other desirable properties of a drug-like molecule. Our lab applies novel machine learning techniques to aid the chemical tool and drug discovery processes. With these methods, we can dramatically decrease the time it takes to discover and then optimize a small molecule antibacterial with the desired biological activity profile. We will discuss our efforts with machine learning models to discover new chemotypes for antibacterial agents against drug-resistant bacteria and also pursue compound optimization for critical properties such as mammalian cell cytotoxicity, aqueous solubility, and mouse liver microsomal stability.

113 A fermenter model system to study the lung microbiome

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The lung microbiome consists of a complex variety of microorganisms including bacteria, fungi, and viruses. These commensal organisms often provide a barrier to pathogenic infections. This project involved the development of a fermenter-based system to study the dynamics of lung-associated bacteria during normal physiological conditions and with challenge. To do this, a medium was developed that simulated lung fluid, and then the growth rates of eight lung-associated bacteria in batch culture were determined. The bacteria were then cultivated together to study their competition for nutrients. Since *Klebsiella pneumoniae* initially dominated the system, growth conditions were optimized to allow other organisms to grow. The study was repeated with the introduction of a pathogen and reduced oxygen to mimic lung dysfunction.

114 AlpA controls virulence gene expression in *Pseudomonas aeruginosa* by functioning as an antiterminator

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Pseudomonas aeruginosa is a Gram-negative bacterium, an opportunistic human pathogen, and is on the Center for Disease Control's list of top drug resistant threats in the United States. *P. aeruginosa* encodes a programed cell death pathway (PCD) that is positively regulated by AlpA. *alpA* is expressed in response to DNA damage. Deletion of *alpA* prevents the expression of lysis genes and decreases the ability of *P. aeruginosa* to colonize mice in an acute lung infection model. Here we present evidence that AlpA regulates lysis gene expression by directly binding RNAP and allowing it to bypass intrinsic termination sites positioned downstream of target promoters. AlpA thus appears to function as a processive antiterminator to regulate virulence gene expression in response to DNA damage.

115 Clock-like divergence and domain-wide convergence in bacterial phenotypic evolution

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Understanding the evolutionary processes that shape prokaryotic functional diversity starts with the comparison of microbial phenotypic traits. However, the study of phenotypic evolution is complicated due to

a paucity of standardized experimental data. The use of *in silico* models of bacterial metabolic networks alleviates this problem by allowing the inference of phenotypes from annotated genomes. We have applied constraints based analyses of metabolic networks to predict and compare phenotypic profiles of hundreds of bacterial species, and to observe patterns of phenotypic similarity over times that span a few million to billions of years of evolutionary divergence. We find that bacterial phenotypic similarity is poorly correlated with divergence time within closely related strains. However, a regular, exponential-like decay of phenotypic similarity is seen as species diverge over hundreds of millions of years. The results, which are validated by available experimental data, are reminiscent of the molecular clock in protein evolution. Similar to how different proteins evolve at different rates, different phenotypic properties show distinct divergence patterns. Interestingly, while individual species usually account for a small fraction of all possible phenotypes, higher-level taxonomic groups span large swaths of phenotypic space and converge to similar trait frequencies upon billions of years of evolution. These results are in agreement with evolutionary simulations spanning trillions of generations. Finally, we observe remarkably similar phenotypic evolutionary rates across distant and independent taxa, suggesting common evolutionary patterns and metabolic network properties across long-lived bacterial clades.

116 MenG, the demethylmenaquinone methyltransferase, is an essential enzyme associated with the intracellular membrane domain in *Mycobacterium smegmatis*

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Menaquinone (MK) is an essential electron carrier in mycobacteria. The final three steps of its biosynthesis take place in the plasma membrane. First, the prenyltransferase MenA forms demethylmenaquinone (DMK). Second, the methyltransferase MenG modifies DMK, forming MK. Finally, the MK polyprenyl lipid is reduced by MenJ to become the mature molecule, MK (II-H2). We recently reported that *Mycobacterium smegmatis* has an intracellular membrane domain (IMD), which is distinct from the conventional plasma membrane tightly associated with cell wall (PM-CW). Proteomic analysis revealed a variety of enzymes enriched in the IMD, including MenG and MenJ. Therefore, we hypothesize that part of the MK biosynthesis takes place in the IMD. In this study, we first examined the subcellular localization of MenG and MenJ by sucrose density gradient fractionation, and confirmed that these two enzymes are specifically associated with the IMD. In contrast, we found that MenA is associated with the PM-CW, suggesting that the MK biosynthesis is spatially segregated within the membrane. To test the physiological significance of the IMD-associated enzyme MenG, we attempted to delete the gene *menG*, but the deletion was not successful unless an extra copy of this gene was introduced prior to deleting the endogenous copy. We then constructed a MenG knockdown strain, and showed a decrease in the MenG protein level after anhydrotetracycline (ATC) addition, which coincides with a significant level of growth retardation. We are currently testing if the level of MK after MenG knockdown, and its impact on cellular ATP levels and oxygen consumption rate.

117 An activation pathway governs cell wall polymerization by a the rod system in *Escherichia coli*

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Cell elongation in rod-shaped bacteria is mediated by the Rod system, a morphogenic complex that uses the actin-like MreB protein to spatially control cell wall assembly by the glycan polymerase RodA and crosslinking enzyme PBP2. Using *Escherichia coli* as a model system, we identified a PBP2 variant that promotes Rod system function when essential accessory components of the machinery are inactivated. Furthermore, the altered PBP2 was shown to hyperactivate cell wall synthesis *in vivo* and to stimulate the polymerase activity of RodA-PBP2 complexes *in vitro*. Our results define an activation pathway for the machinery in which PBP2 senses Rod complex formation and stimulates RodA in a manner that couples cell wall polymerization and crosslinking and prevents the toxic accumulation of uncrosslinked glycans. Cell wall biogenesis by the related enzymes of the cytokinetic ring is likely to be controlled by a similar activation pathway.

118 The Making of a Pathogen: Exploring the Uncharted Diversity of *Enterococcus*

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Multidrug resistant *Enterococcus* have become one of the leading causes of hospital acquired infections despite their origins tracing back to commensal gut inhabitants of early terrestrial animals (Lebreton et al. *Cell* 2017). In a new investigation, we identified a genetic marker that cleanly distinguishes enterococcal species and used it to scour a large and globally diverse collection of enterococci recovered from 2,000 animal specimens obtained through an extensive network of scientists and adventurers. Within our collection - gathered from the guts of Formosan subterranean termites to fresh feces of elephants in Uganda and arctic foxes in Greenland - we identified 20 novel enterococcal species, expanding the number of species within this genus by nearly 50%. Comparative genomics analyses of our enlarged set of enterococcal species are providing new insights into the environments encountered by these species' ancestors, including apparent fluctuations in the availability of branched chain amino acids, that appear critical in the evolution of a pathogenic enterococcal lineage. We have also extended our comparative analysis to include species from within the closely related *Streptococcus* genus. Results from this work are reshaping our view of the origins of the *streptococci*.

119 Pan-genome clustering and machine learning Predicts Antibiotic Susceptibility in *Streptococcus pneumoniae*

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Streptococcus pneumoniae, a bacterium that is both a nasopharyngeal commensal and respiratory pathogen, has thousands of different strains with vast phenotypic heterogeneity in antibiotic tolerance and infectivity level. The average genome of a given strain is about 2 Mbp containing 2,100 genes. However, any two strains may differ by the presence or absence of several hundred genes, making it difficult to properly interpret strain-specific phenomena. Here we apply existing software packages in addition to several tried-and-true clustering methods to establish a functional correspondence across all genes in *S. pneumoniae*. We optimize the number of clusters and find that normalized spectral clustering outperforms other algorithms, yielding a pan-genome (collection of all genes in the species) of 4061 genes, and a core genome (genes shared by all strains) of 1241 genes. We show through the functional annotation of each gene cluster that strains have similar composition in terms of higher-order gene function. Despite this, we can use the gene presence-absence data to make inferences on strain-specific phenotypes. We use machine learning to train and test classifiers that can predict antibiotic susceptibility with high accuracy. Our study suggests that, through pan-genome wide analysis of pathogenic species, it is possible to select antibiotic treatments that are most likely to be effective for a new clinical strain. Furthermore, this workflow could be used for analyzing pan-genome wide data from a large number of strains for any pathogenic species with intra-species variability.

120 Identifying mechanisms of phage-host interactions in the Nahant Collection

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The Polz Lab maintains the Nahant Collection, which consists of 243 *Vibrio* strains challenged by 241 unique phage, all with sequenced genomes. This is the largest phylogenetically-resolved host range cross test available to date. These host strains match to 19 populations that coexist but ecologically differentiated, and the phage fall into around 18 phylogenetically distinct groups, with diverse infection strategies and morphologies. This rich dataset offers the opportunity to glean mechanistic insights from sequencing data, however doing so comes with a few challenges. (1) While the diverse population structure of phage and hosts is an interesting feature of the data, it means, however, that statistical independence does not apply. Ignoring phylogenetic relationships can result in relevant signals being drowned out by spurious correlations. (2) A model of infection should capture the generally lock-and-key nature of infection specificities (for example, a specific methylase may evade only a specific restriction modification system). Here in order

to simplify computation, we first fit marginal models that account for phylogenetic confounding to screen for genes of interest. Then we build a multivariate model with statistical interaction terms that, in some sense, represent putative interactions of host and phage genes. This multivariate model also allow us to view the problem from an infection prediction perspective. And finally, in order to enhance interpretability of the model as well as the explorability of the data, we are in the process of writing a graphical web application that anyone interested in the Nahant Collection will be able to access.

121 Constraints on horizontal gene acquisition in bacteria: comparing intralineage and intergeneric crosses

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Bacteria assemble genome islands, which code for niche-adaptive functions, by RecA-independent mechanisms that remain obscure and play a major role in horizontal gene transfer. Of particular interest are regions not mobilized by site-specific recombinases or known transposases, such as the Immigration Control Region (ICR). This region is highly enriched for variable sequence-specific restriction enzymes (defence against nucleic acid invasion). We have developed a conjugal transfer system of chromosomal DNA to characterize basal and enzyme-stimulated RecA-independent gene transfer of the ICR in between laboratory descendants of one natural isolate of *E. coli*. The basal intralineage events move very large (60 kb-2 Mb) segments, replacing recipient DNA with donor sequence. Interestingly, in our preliminary results in crosses with the restriction-deficient hybrid *Salmonella enterica* sv Typhimurium hybrid strain, we identify two distinct properties: an increased fraction of events are additions, and shorter segments predominate. As part of the groundwork for these intergeneric experiments, we have determined the sequences of this multiply restriction-deficient hybrid strain, of *S. enterica* sv Typhimurium LT7 and of an isolate of the model organism *S. typhimurium* LT2 obtained from the Segall laboratory. Comparison of the LT2 genes that determine the restriction activities SenLT2I (LT, StyLT in the early literature), SenLT2II (SA, StySA) and SenLT2III (SB, StySB) with those of LT7 and the restrictionless host will be presented. The genetic and molecular composition SenLT2II (SA) in particular will be addressed, since it has not been reported before. Variation contributed by the four inducible prophages of LT2 will also be outlined.

122 Keeping identity in check: how the *Proteus mirabilis* self-identity protein IdsD is regulated before transport to a neighboring cell

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Bacteria use transported proteins to exchange information that influences behaviors such as macroscale population migration. Much remains unknown about such cell-to-cell signaling. Here we addressed how protein signals are regulated before transport using the *Proteus mirabilis* self-identity protein, IdsD, which is secreted by a type VI secretion system (T6SS). Intriguingly, IdsD only interacts with its known self-identity binding partner, IdsE, after transport. Using genetic, biochemical, and cell biological assays, we found that a separate protein, IdsC, functions to chaperone IdsD before transport. IdsC belongs to a conserved family of predicted chaperone proteins. The molecular mechanism of this family, or other T6SS-associated chaperones, is not yet fully examined. We have elucidated that IdsC binds IdsD to regulate IdsD protein levels, localization, and secretion. We posit that IdsC also prevents IdsD-IdsE interactions before transport and propose that in doing so IdsC ensures that the communication of self-identity occurs between neighboring cells.

123 Cell-based screen for discovering lipopolysaccharide biogenesis inhibitors

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New drugs are needed to treat Gram-negative bacterial infections. These bacteria are protected by an outer membrane which prevents many antibiotics from reaching their cellular targets. The outer leaflet of the outer membrane contains lipopolysaccharide (LPS) which is responsible for creating this permeability barrier. Interfering with LPS biogenesis affects bacterial viability. We developed a cell-based screen that identifies inhibitors of LPS biosynthesis and transport by exploiting the non-essentiality of this pathway in *Acinetobacter*. We used this screen to find an inhibitor of MsbA, an ATP-dependent flippase that translocates LPS across the inner membrane. Treatment with the inhibitor caused mislocalization of LPS to the cell interior. The discovery of an MsbA inhibitor, which is universally conserved in all Gram-negative bacteria, validates MsbA as an antibacterial target. Because our cell-based screen reports on the function of the entire LPS biogenesis pathway, it could be used to identify compounds that inhibit other targets in the pathway, which can provide insights into vulnerabilities of the Gram-negative cell envelope.

124 Evolution of Optimum Growth Temperature in Thermotogae

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Life is found in a wide range of temperature conditions, from the extreme cold to the extreme heat. Yet every organism has evolved to survive only in a limited temperature interval. While some cellular and genomic features that contribute to survival at high and low temperatures are known, detailed mechanisms behind both microbial response to changing temperatures and adaptation to new growth temperatures remain to be discovered. The bacterial phylum Thermotogae is an excellent system for studying the evolution of temperature preference. The phylum comprises ubiquitous deep-biosphere organisms isolated from terrestrial and marine settings, and includes representative taxa that have been grown in temperatures from 20C to 90C. Previous research indicated that the last common ancestor of Thermotogae was likely a hyperthermophile, and that preference for high temperature was lost in some genera over time. A recent discovery of a new organism, *Mesoaciditoga lauensis*, placed the assumption of Thermotogae's hyperthermophilic ancestor into question. Although *M. lauensis* is currently the deepest-branching Thermotogae lineage, it is a moderate thermophile. Utilizing sequences from 53 Thermotogae with known optimal growth temperatures, we inferred the optimal growth temperatures of the phylum's ancestral nodes. We used both direct inference of growth temperature at the internal nodes of the Thermotogae phylogeny, and indirect deduction from sequence composition metrics known to correlate with growth temperature. Our analysis indicates that the Thermotogae last common ancestor is a thermophile, and that evolution of both hyperthermophily and mesophily within this phylum is secondary.

125 Multicellular Development and the DNA Damage Response in the Opportunistic Pathogen *Acinetobacter baumannii*

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Our evidence shows that an *Acinetobacter baumannii* strain lacking a functional DNA damage response (i.e. lacking *recA*) forms robust biofilms compared to its parental strain. This led us to examine the mechanism by which the DNA damage response (DDR) regulates biofilm formation in *A. baumannii*. We started our analysis by scanning the genome of *A. baumannii* 17978 for proteins containing motifs that are ubiquitous in biofilm development, focusing on GGDEF-containing cyclic di-GMP cyclases and on EAL-containing phosphodiesterases. We then focused on those that were also part of the DDR. This analysis was followed by a forward genetic screen using random transposon mutagenesis of a chromosomally encoded DDR reporter, and a novel *in vitro* enrichment step, allowing us to find mutants proficient at forming biofilms that were also deficient in the DNA damage response. The top 12 biofilm-forming mutants, based on a quantitative adherence assay using crystal violet, were chosen for whole genome sequencing identify the insertion site. One of the robust biofilm forming mutants has the transposon inserted in a GGDEF/EAL motif containing protein previously identified *in silico*. We constructed an independent knockout of the transposon insertion gene and confirmed its robust biofilm forming phenotype. We are currently studying this gene product further to confirm its role in the development of biofilm formation in *A. baumannii*. Through a combination of bioinformatics and genetics we have putatively identified a novel gene product involved in both the

regulation of biofilm development and the DDR in *A. baumannii*, a clinically relevant organism.

126 Increasing fitness cost in resistant bacteria to reduce antibiotic resistance

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Antibiotic resistance is generally associated with a fitness deficit resulting from the burden of maintaining resistance machinery. The additional cost suggests that resistant bacteria could be outcompeted by susceptible bacteria when the usage of antibiotic is reduced, thereby reducing the resistance in a population. However, several experimental and theoretical studies have shown that the process of reducing resistance by exploiting the fitness cost of resistant bacteria will be very slow because the fitness cost is too small to direct an efficient evolution towards antibiotic susceptibility. In this research, we seek to investigate the process of reducing resistance conferred by MarA (Multiple Antibiotic Resistance Activator) in *E. coli* by maximizing the fitness cost in resistant cells. To accelerate the process of reversing resistance, we tested the application of the MarA inducer salicylate and its effects on increasing burden in resistant bacteria. Furthermore, we studied the sensitivity of resistance level to various population compositions of resistant bacteria. We constructed a quantitative fitness landscape of resistant and susceptible cells in salicylate and showed that decreasing the proportion of MarA-conferred resistant cells will reduce the population's resistance to antibiotics. This research lends insight into strategies of reversing resistance of a population by maximizing the fitness cost in resistant bacteria.

127 Maturing Mycobacterial Peptidoglycan Requires Non-canonical Crosslinks to Maintain Shape

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In most well studied rod-shaped bacteria, peptidoglycan is primarily crosslinked by penicillin binding proteins (PBPs). However, in mycobacteria, L,D-transpeptidase (LDT)-mediated crosslinks are highly abundant. To elucidate the role of these unusual crosslinks, we characterized mycobacterial cells lacking all LDTs. We find that LDT-mediated crosslinks are required for rod shape maintenance specifically at sites of aging cell wall, a byproduct of polar elongation. Asymmetric polar growth leads to a non-uniform distribution of these two types of crosslinks in a single cell. Consequently, in the absence of LDT-mediated crosslinks, PBP-catalyzed crosslinks become more important. Because of this, (Mycobacterium tuberculosis) (Mtb) is more rapidly killed using a combination of drugs capable of PBP- and LDT- inhibition. Thus, knowledge about the single-cell distribution of drug targets can be exploited to more effectively treat this pathogen.

128 Structure-function analysis of the *Escherichia coli* lytic transglycosylase MltG

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Peptidoglycan (PG) synthesis is an essential process in bacteria that has been intensely studied for decades. Despite this scrutiny, the processes that determine glycan strand length and mediate glycan strand termination have yet to be fully elucidated. Recent work by Yunck et al. (2016) revealed that the novel lytic transglycosylase (LT) MltG might act as a glycan strand terminase in *Escherichia coli*. However, further characterization of MltG by Yunck et al. was limited due to inability to identify a tractable phenotype of *mltG*. As a result, it remains unclear what roles the transmembrane domain, LysM domain, and YceG-like/catalytic domain of MltG play in its function. Here we report the discovery of an easily tractable *mltG*

phenotype and the results of domain analysis of MltG performed using this phenotype.

129 Phospholipids regulate the interactions between essential bacterial cell division proteins FtsA and FtsZ

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The cell division protein FtsA is an actin-like ATPase that is highly conserved among prokaryotes and essential in *Escherichia coli*. FtsA contains a C-terminal membrane targeting sequence (MTS) and directly recruits FtsZ polymers to the inner membrane to establish the Z-ring and coordinate constriction. FtsA directly reorganizes phospholipid (PL) architecture and remodels dynamic FtsZ polymers. To determine if PL engagement by FtsA modifies the interaction with FtsZ, we tested if purified FtsA and a mutant protein lacking the MTS (FtsAMTS) bind to PLs and recruit FtsZ. In sedimentation assays, we observed that FtsA binds PLs with and without ATP. FtsAMTS is unable to bind PLs when ATP is omitted; however, it is recruited in the presence of ATP suggesting that binding PL is ATP-dependent. We observed that FtsAMTS recruits FtsZ to PLs, indicating that interaction between FtsZ and FtsAMTS is maintained. FtsZ polymerizes with GTP, addition of FtsA stoichiometrically inhibits FtsZ polymerization. Interestingly, FtsAMTS is defective for disassembly of FtsZ polymers, suggesting that the MTS of FtsA may regulate FtsA activity and conformation. To determine if there are large conformational differences between FtsA and FtsAMTS, we performed transmission electron microscopy of FtsA and FtsAMTS. We observed FtsAMTS forms long protein polymers, greater than 100 nm, in the presence of ATP. Together, these results suggest that FtsA has a secondary, ATP-dependent, membrane interaction and that the MTS may regulate FtsZ remodeling by FtsA. These studies provide biochemical insight into a highly conserved interaction during early prokaryotic cell division.

130 Lysine regulates quorum-dependent growth and quiescence of uropathogenic *Escherichia coli*

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Uropathogenic *Escherichia coli* (UPEC) strains cause urinary tract infections (UTIs). After successful antibiotic treatment, many patients experience infection recurrence within 12 months, suggesting there may be a reservoir *in vivo* that is not susceptible to antibiotic. In addition to forming persister cells, we show that the UPEC strain CFT073, and 27% of clinical UPEC isolates tested, also exhibit density-dependent growth on glucose minimal agar. When cultured at low density (106 CFU), cells survive but become quiescent. In this state, cells continue to elongate for up to 24 h and become filamentous. We show that quiescence is both prevented and reversed by urine, toothpicked actively growing *E. coli* cells, cell-free supernatant, and a mixture of lysine and methionine. To determine if lysine import is required to promote growth at low density, we deleted the major lysine importer *lysP*, as well as the transcriptional regulator *argP*. The deletion strains remain quiescent at low density but fail to grow upon addition of lysine and methionine, suggesting that lysine import is critical for either preventing or reversing quiescence. However, the addition of toothpicked actively growing *E. coli* to quiescent *lysP* cells stimulates growth, suggesting there is another signal secreted by *E. coli* that promotes growth. These results show that growth of quiescent UPEC cells requires a quorum and multiple signals, including lysine, which either prevents or reverses quiescence, overcome the density requirement. The quiescent state of UPEC, described here, may also be important for establishing reservoirs *in vivo* that contribute to recurrent UTI infection.

131 Membrane partitioning of mycobacterial peptidoglycan synthesis

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Antibiotics that target peptidoglycan are potent weapons against many bacterial infections but have been of limited utility against tuberculosis. The specter of drug resistance has revitalized interest in alternative strategies to halt *Mycobacterium tuberculosis* peptidoglycan synthesis. The peptidoglycan polymer is built next to and with precursors from the plasma membrane. While it is known that the synthetic pathway spans both cytoplasmic and periplasmic compartments, lateral organization within plasma membrane is

an emerging concept. In mycobacteria, including *M. tuberculosis*, intracellular membrane domains (IMD) comprise biochemically and spatially distinct regions within the conventional plasma membrane. We find that MurG, an essential, membrane-bound synthase for peptidoglycan precursors, is enriched in the IMD but the sequentially-acting flippase MurJ and extracellular polymerases such as PonA1 localize to the conventional plasma membrane. Lipid-linked precursors are detectable throughout the plasma membrane in wildtype bacteria but accumulate in the IMD upon depletion of MurG or MurJ. By contrast, inhibition of peptidoglycan polymerization or depletion of a negative regulator of MurJ alters the distribution of precursors within, but not between, different regions of the plasma membrane. Our data suggest that lipid-linked precursors are made in the IMD but flipped and polymerized in the conventional plasma membrane. We hypothesize that membrane partitioning enables efficient precursor synthesis and precise insertion of these molecules into the growing peptidoglycan polymer. In support, disruption of pathway partitioning results in mislocalized peptidoglycan synthesis and, eventually, mycobacterial cell death. Focal targeting of the IMD may be a new strategy for disrupting *M. tuberculosis* cell wall synthesis.

132 Lipomannan and Lipoarabinomannan are essential structural components of the mycobacterial cell envelope

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The mycobacterial cell wall is multi-layered and complex. It is comprised of covalently-linked layers of peptidoglycan, arabinogalactan, and mycolic acids. Large glycolipids, lipomannan and lipoarbabinmanan (LM/LAM), are also abundant. While LM/LAM stimulate the immune system during mycobacterial infections, they also exist in non-pathogenic mycobacterial species, suggesting a role in fundamental bacterial cell physiology. Gene deletions in the biosynthetic pathway of LM/LAM do not result in their complete absence, making it difficult to decipher the function of these glycolipids in mycobacterial physiology. Here we characterize an essential gene of unknown function that is highly conserved in mycobacteria and related bacteria. We show that depletion of this gene, *ms0317*, leads to a severe drop in both LM and LAM levels, loss of cell wall integrity and, eventually, cell lysis. MS-0317 is a member of the division and elongation complexes. It dynamically localizes to sites of new cell wall synthesis, and MS0317-depleted cells no longer elongate. Surprisingly, by cryo-electron microscopy we find that depletion results in severe fraying and disorganization of the cell envelope. Together, these results suggest that LM/LAM are essential for polar growth and promote structural organization of the mycobacterial cell envelope.

133 Cell Fate Determination in Biofilms of *Bacillus subtilis*

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Biofilms are multicellular bacterial communities embedded in a self-produced extracellular matrix. These communities feature physiologically distinct cell types that express unique patterns of genes. The soil bacterium *Bacillus subtilis* is well-studied for biofilm formation, and for genetic competence— a transient state marked by enhanced genetic transformability. While previous research has addressed competence development in shaking cultures, little is known about the regulations and function of competence development within biofilms. However, putative evidence suggests an essential biofilm gene *sinI* is repressed by the master competence regulator ComK, suggesting competence is exclusive from matrix production. Here, a dual-labeled reporter strain revealed that competence arises in only a miniscule fraction of cells in a population, and that ComK induction drastically reduces expression of biofilm genes. We further provide a physiological basis for competence/matrix exclusivity by showing that both the biofilm matrix and the characteristic chaining phenotype of matrix cells inhibit transformation. Finally, it is known that the key competence gene *comS* is embedded within an operon for synthesis of the lipopeptide antibiotic surfactin. However, the reason for this peculiar genetic organization has not been addressed. Here, we demonstrate that surfactin kills the closely- related *Bacillus cereus*, and allows competent *B. subtilis* to take up its genomic DNA . We propose that surfactin serves an additional role as a "parricide" molecule by providing a source of extracellular DNA for transformation. This research lends insight into the composition of bacterial communities, and proposes a novel role for surfactin in interspecies killing and horizontal gene transfer.

134 Investigating the role of translesion DNA polymerases on template-switch mutagenesis in *Escherichia coli*

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Errors in DNA replication and repair can result in mutagenesis and genome instability. Naturally occurring repeat sequences have been shown to adopt secondary DNA structures which cause replication stalling. One type of repeated DNA sequence is a quasipalindrome (QP). A QP is an imperfect inverted repeat and a mutational hotspot. Mutations in QP regions result from template-switch events during DNA replication. These events "perfect" the palindrome through the formation of a hairpin structure. Previous research in *Escherichia coli* has shown that the two major 3' ssDNA exonucleases, Exo I and Exo VII, are involved in preventing hairpin structures from forming. In addition, the three translesion synthesis (TLS) DNA polymerases in *E. coli* have been found to be involved in replicating past QPs when the major replicative polymerase stalls. Using engineered QP reporters in the *lacZ* gene, we show that the TLS polymerases have a redundant role in preventing TSM on both the leading and lagging strands during replication. This effect is only seen when at least one of the 3' ssDNA exonucleases is absent and therefore hairpins are forming more frequently at the QP site. In addition, we find that overproduction of one of the TLS polymerases, Pol IV, causes a substantial increase in TSM on both the leading and lagging strands, even when Exo I and Exo VII are present. This work suggests that the overall abundance of the TLS polymerases and the 3' ssDNA exonucleases are important for regulating the rate of TSM.

135 Discovering the Maltose Binding Protein epitope tag for *Escherichia coli* expressed proteins.

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Maltose Binding Protein (MBP) is used in recombinant protein expression as an affinity and solubility tag. The anti-MBP monoclonal antibody B48 binds tightly and has no cross-reactivity to other proteins in an *E. coli* lysate. For all these criteria, the MBP tag provides a useful epitope for fusion proteins expressed in *E. coli*. The co-crystal structure of MBP bound to its antibody was defined and four amino acids of MBP were characterized for the binding interaction. Fusion of various fragments of MBP to the glutathione S-transferase protein was engineered in order to identify the smallest fragment still recognized by the anti-MBP antibody. Further engineering of the epitope to stabilize and minimize the tag will be discussed.

136 Characterization of ConQ, a key ATPase required for bacterial mating

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Bacterial conjugation, also known as mating, is the transfer of DNA from a donor cell into a recipient cell. The transfer of DNA occurs through a specialized channel, referred to as a Type IV Secretion System (T4SS). These membrane-embedded multi-protein complexes transport DNA and/or proteins across the bacterial cell envelope. Conjugative T4SSs may move genes involved in metabolism, symbiosis, pathogenesis, or antibiotic resistance. The conjugation machinery of the conjugative element ICE*Bs1* of the bacterium *Bacillus subtilis* is composed of the proteins ConB, ConC, ConD, ConE, ConG, CwlT, and ConQ. Our research is focused on purifying and characterizing ConQ, a putative coupling protein with two transmembrane segments and a cytoplasmic ATPase domain. Coupling proteins generally are hexameric ATPases that function in recruiting and targeting the conjugative DNA to the DNA translocation channel. In order to overcome prior aggregation problems, we fused seven different solubility tags to the cytoplasmic domain of ConQ and then screened for solubility. We found that His6-SlyD-ConQ was expressed well and soluble. We subsequently purified it, along with three different ATPase motif mutants, using nickel affinity chromatography. Blue native PAGE indicates that purified His6-SlydD-ConQ is a mixture of oligomers, and that the mutations in the ATPase motifs do not alter the oligomerization state. Future studies will investigate whether purified ConQ binds DNA and/or hydrolyzes ATP *in vitro*.

137 Elucidating the role of a NosP signaling pathway in regulating c-di-GMP concentration and biofilm formation in *Burkholderia thailandensis*.

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Bacteria use two-component (His-Asp) phosphorelay signaling to sense and respond to stimuli, and regulate various processes such biofilm formation. Biofilms are extremely tolerant to antibiotics, and develop when bacteria aggregate in a self-secreted exopolysaccharide matrix. Nitric oxide (NO), a small diatomic gas, has been shown to mediate biofilm dispersal in many bacteria through ligation to H-NOX proteins. Not all NO responsive bacteria, however, code for an H-NOX domain. Recently, our lab has discovered a novel NO sensing protein (NosP), which is predicted to be co-cistronic with two-component signaling networks that regulate biofilm formation. Therefore, in this work, we use biochemistry to invesigate, characterize and elucidate how a NosP-associated two-component signal transduction pathway participates in the cyclic-di-GMP signaling and therefore biofilm regulation in *Burkholderia thailandensis*. We find that NosP can undergo heme-dependent NO ligation and that NahK functions as a histidine kinase *in vitro* that can engage in phosphorelay signaling with NarR. We therefore hypothesize that NosP is an NO sensing hemoprotein that regulates the autokinase activity of NahK and phosphate flux NarR, thus resulting in an NO dependent modulation of intracellular c-di-GMP levels, and therefore biofilm formation in *Burkholderia thailandensis*.

138 Probing the molecular mechanism of ProQ-sRNA interactions using a bacterial three-hybrid assay

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In bacteria, small RNAs (sRNAs) play important roles in gene regulation; sRNAs can regulate the translation and stability of target mRNAs via imperfect base pairing. These sRNA-mRNA interactions are often facilitated by protein chaperones. Recently, ProQ has been shown to bind to dozens of *Salmonella* sRNAs and mRNAs, and to facilitate base pairing between the mRNA hupA and sRNA RaiZ with the effect of regulating hupA expression. These observations have led to the proposal that ProQ may act as a widespread regulator of bacterial gene expression. Our goal is to understand the molecular mechanisms of ProQ's interaction with sRNAs, mapping the amino acids on ProQ's surface and nucleotides of RNAs that contribute to binding and regulation by using a bacterial three-hybrid (B3H) assay to detect ProQ-RNA interactions genetically. In the B3H assay, ProQ is fused to RNA polymerase (RNAP) and a hybrid RNA containing an sRNA or mRNA of interest is tethered to a DNA sequence upstream of a test promoter. Interaction of ProQ with the RNA stabilizes the binding of RNAP to the test promoter and activates transcription of a reporter gene. We have detected preliminary B3H interactions of ProQ with several RNA partners, and are using this assay to screen for mutants in ProQ that alter its RNA interactions to locate the binding interface(s) for the sRNAs and mRNAs with which it interacts.

139 Mucus and mucin biopolymers reduce efficacy of antibiotics against *Pseudomonas aeruginosa*

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Mucus is a biopolymer gel that covers all wet epithelia in the human body. While the mucus barrier protects against a variety of agents, it can also serve as the host environment for pathogenic microbes that can cause serious infections. The impact of mucus on the efficacy of antibiotics used to treat bacterial infections is poorly characterized. We investigated the efficacy of polymyxin and fluoroquinolone antibiotics against the opportunistic mucosal pathogen *Pseudomonas aeruginosa* in unpurified human and porcine mucus and in purified mucin biopolymer environments. We show that mucus reduces the efficacy of polymyxin and fluoroquinolone antibiotics against *Pseudomonas aeruginosa*. Mucin biopolymers, the gel-forming components of mucus, are a primary contributor to this effect and thus are a strong candidate model environment for more accurate *in vitro* antimicrobial assessment. Our findings highlight that inclusion of components of the infection environment, like mucin, is an important consideration when evaluating antimicrobial efficacy *in vitro*.

140 Exploring the gut-bladder axis: a longitudinal study of the gut microbiome of women with recurrent UTIs

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Urinary tract infections (UTIs) are common, costly, frequently recurrent, and often caused by uropathogenic *Escherichia coli* (UPEC). UPEC are known to asymptomatically colonize the gut of healthy adults. They are able to access the bladder and cause disease after being shed in feces, colonizing the periurethral area, and ascending through the urethra. Unlike most gut commensals, *E. coli* are aerobic and able to expand during periods of inflammation by gaining access to nutrients and niche space due to inflammation-mediated reshaping of the gut. However, it is unknown how UPEC respond to the altered immune environment and gut microbiota or if these changes in the gut influence UTI susceptibility. To address these gaps in knowledge, we conducted a longitudinal study of women with and without history of rUTIs using a combination of multi-omics technology and analysis of host immune status. Among our preliminary findings, the gut microbiotas of women with rUTIs were less diverse than those of healthy controls and had decreased abundances of bacteria normally associated with healthy guts. We also observed blooms of *E. coli* in the guts of women at the onset of UTI that were genetically similar to the strains isolated from the urine. Further, we found that women with rUTIs had different baseline immune states than healthy controls, and that these differences may be associated with maintenance of the gut epithelium barrier. Taken together, these data provide a more comprehensive view of the gut-bladder axis and foundational insights needed to disentangle microbiota-pathogen-immune system interactions in UTI.

141 Metagenome-assembled genomes reveal previously unrecognized levels of bacterial diversity in the oral microbiome

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Humans and their microbes are highly integrated, with microbiome composition an essential aspect of human health. The human tongue microbiome represents a good test bed for inquiries into microbial community organization given its diverse, stable community and clinical relevance. New developments in deriving metagenome-assembled genomes (MAGs) from whole-community sequencing now allow direct investigation of the environment, avoiding culture biases. Short-read metagenomic data can also be recruited back to a pangenome of cultured isolates or MAGs. We studied the genomic composition of the tongue microbiome by sequencing bulk community DNA from the tongues of 19 individuals sampled 4-7 times over a 2 week time course. 400 high-quality bacterial MAGs were obtained in total. Pangenomic analysis demonstrated that MAGs clustered into species groups while revealing significant differences between MAGs and cultured representatives. Based on short-read data from our metagenomes and the Human Microbiome Project (HMP), cultured genotypes were environmentally rare in either dataset, if at all present, while MAGs were more prevalent and abundant in both datasets. Oral genera which had both well-defined MAGs and sufficient genomes in existing databases were analyzed, including *Rothia*, *Prevotella*, and *Capnocytophaga*. Each MAG contributed tens to hundreds of novel, environmentally-relevant genes, identified as phage and other mobile genetic elements, surface receptors, iron uptake and utilization, multidrug efflux pumps, and carbon metabolism, suggesting that the uncultured microbiome may employ different ecological strategies than their cultured relatives. The oral microbiome clearly harbors a vast amount of environmental diversity, indicating previously underappreciated ecological and evolutionary processes at work.

142 How bacteria pop

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Membrane lysis, or rupture, is a cell death pathway in bacteria frequently caused by cell wall-targeting antibiotics. Although several studies have clarified some biochemical mechanisms of antibiotic action, a physical understanding of the processes leading to lysis remains lacking. Here we model the dynamics of membrane bulging and lysis in *Escherichia coli*, where it has been observed that the formation of an initial bulge after cell wall digestion occurred on a characteristic timescale as fast as 100 ms and the growth of the bulge occurred on a slower characteristic timescale of 10-100 s. We show that bulging can be energetically favorable due to the relaxation of the entropic and stretching energies of the inner membrane, cell wall, and outer membrane and that experimentally observed bulge shapes are consistent with model predictions. We then show that our model predicts lysis dynamics for cells under stabilizing and destabilizing perturbations. Our results elucidate the physics of membrane bulging and lysis and may have implications for cellular morphogenesis and antibiotic discovery.

143 Random Sequences Rapidly Evolve into *De Novo* Promoters

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How new functions arise *de novo* is a fundamental question in evolution. We studied *de novo* evolution of promoters in *Escherichia coli* by replacing the lac promoter with various random sequences of the same size (100bp) and evolving the cells in the presence of lactose. We find that 60% of random sequences can evolve expression comparable to the wild-type with only one mutation, and that 10% of random sequences can serve as active promoters even without evolution. Such a short mutational distance between random sequences and active promoters may improve the evolvability, yet may also lead to accidental promoters inside genes that interfere with normal expression. Indeed, our bioinformatic analyses indicate that *E. coli* was under selection to reduce accidental promoters inside genes by avoiding promoter-like sequences. We suggest that a low threshold for functionality balanced by selection against undesired targets can increase the evolvability by making new beneficial features more accessible.

144 The development of new chemical tools to study quorum sensing systems in Gram-negative bacteria

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Anti-virulence therapeutic approaches that focus on infection rather than growth are less prone to resistance development, and thus, serve as attractive antimicrobial agents. Quorum Sensing (QS) systems control virulence in a variety of pathogens. In the QS systems of Gram-negative bacteria, a LuxI-type synthase produces an N-acyl-L-homoserine-lactone (AHL) chemical signal which binds to a LuxR-type receptor at high population density to coordinate group behavior. These receptors are difficult to characterize *in vitro* because of their poor stability without their native signal; however, the receptor found in *E. coli*, SdiA, is uniquely well behaved *in vitro*. Unfortunately, a dearth of chemical modulators exist for studying ligand binding to this receptor. Herein, I evaluated the activity profiles of a library of approximately 150 non-native small molecules using a cell-based reporter of SdiA activity which unveiled a collection of potent agonists and antagonists of QS in *E.coli*. In addition, phenotypic acid resistance assays further confirmed the SdiA modulating activities of this new collection of compounds. These results provide chemical tools for studying ligand binding in SdiA, thus, providing new tools to study other QS receptors and inferring chemical insight instrumental to the future development of anti-virulence agents.

145 Characterization and Engineering of a Membrane Enzyme for Biosynthetic Production of New Polymers

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Poly amino acid synthetases are a small group of enzymes capable of catalyzing the linkage of amino acids independently from ribosomes. Among these, the membrane-associated poly-gamma-glutamic acid

(PGA) synthetase found in many *Bacillus* species can simultaneously polymerize and secrete long polymers of glutamic acid (1000 kDa) by linking alpha amine to the adjacent gamma carboxylate. Our work aims to change the specificity of this enzyme and catalyze the polymerization of different organic compounds, creating a microbial strain that can convert renewable substrates into polymeric materials that are currently produced only by chemical synthesis. First, we have heterologously expressed the PGA synthase subunits (B, C, A and E) in *Escherichia coli* and developed a semi-quantitative assay for fast detection of the polymer. Instead of randomly changing the substrate specificity of the enzyme, we also tried to elucidate some more information about its cell localization, structure, and reaction to foster our future work in building a mutational library. Sequence alignments show promising similarities with other peptide ligases such as *murE* and *folC*. This will help us identify and characterize residues that are relevant for the enzymatic activity. Tagged fusions of the enzyme with fluorescent proteins also indicate its presence at the inner membrane of *E. coli*. Succeeding, we would like to use some of the discovered putative sites for mutagenesis and asses for activity with the substrate. If successful, this research project will develop in the end a new microbial production platform for polymers with promising industrial applications.

146 Discovery of a novel antitubercular with a dual mechanism of action

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Tuberculosis, caused by *Mycobacterium tuberculosis*, kills 1.5 million people annually. The rise of multi-drug resistant *M. tuberculosis* has led to an urgent need for new antibacterials with novel mechanisms of action. To address this issue, we have leveraged a dual-event Bayesian model to rediscover a triazine antitubercular (JSF-2019). JSF-2019 exhibits bactericidal activity against both replicating and non-replicating *M. tuberculosis* along with low mammalian cell toxicity. JSF-2019 has demonstrated a dual mechanism of action. It induces RNS in *M. tuberculosis* via intracellular nitric oxide release dependent on one or more nitroreductases utilizing F420H2 as a cofactor. Formation of a des-nitro metabolite, JSF-2026, is concurrent with nitric oxide release. JSF-2019 and JSF-2026 demonstrate inhibitory activity of InhA, a vulnerable and essential FAS-II enzyme. This work provides an example of apparent polypharmacology, and highlights a novel antitubercular JSF-2019 with utility versus multi-drug resistant *M. tuberculosis* by circumventing current clinical resistance to isoniazid and pretomanid rooted in their activation requirements.

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