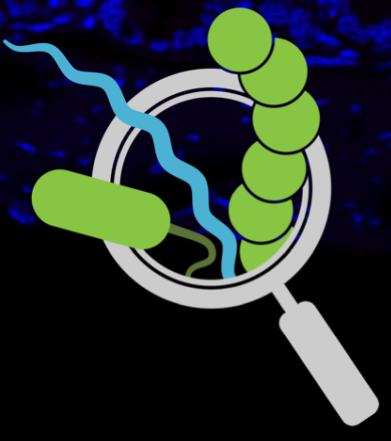
Boston Bacterial Meeting

June 6-7, 2019
Harvard University Science Center



Keynote Speaker:
Denise Monack
Professor of Microbiology and Immunology
Stanford University

Northeast Branch
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2019 Boston Bacterial Meeting

Day 1 - Thursday June 6

8:00 AM	Registration and coffee -- Set up Session I posters	
9:00 AM	Opening Remarks	
I: Regulation		**Session chair: Niels Bradshaw**
9:10 AM	Boyuan Wang	Affinity-based capture and identification of (p)ppGpp-binding proteins
9:30 AM	Jennifer Peña	AlpA controls virulence gene expression in *Pseudomonas aeruginosa* by functioning as an antiterminator
9:50 AM	Satoshi Kimura	Surveying the landscape of tRNA modifications in bacteria by combined tRNA sequencing and RNA mass spectrometry
10:10 AM	*Flash Talks*	Poster #6 Alex (Donghoon) Kang; #34 Samantha Randall; #37 Marianne Grognot
10:20 AM		**Poster Session I - Posters 1-37**
II: Physiology		**Session chair: Christina Faherty**
11:35 AM	Sean Wilson	Understanding the role and regulation of cell wall hydrolases in *Bacillus subtilis*
11:55 AM	Hugo Brandão	RNA polymerases are moving barriers to condensin loop extrusion
12:15 PM	Hoong Chuin Lim	Identification of new components of the RipC-FtsEX cell separation pathway of *Corynebacterineae*
12:40 PM		**Lunch -- Harvard Science Center**
1:15 PM		**Breakout Sessions/Networking**
2:15 PM		Set up Session II posters
2:30 PM		**Special Presentation**
III: Antibiotic susceptibility		**Session chair: Bryan Bryson**
2:40 PM	Samantha Palace	A new mechanism of reduced cephalosporin susceptibility in *Neisseria gonorrhoeae*
3:00 PM	Jenna Wurster	Host hyperglycemia impacts antibiotic efficacy within the murine gut microbiome
3:20 PM	Nathan Hicks	Natural genetic variation in *dnaA* influences antibiotic susceptibility in *Mycobacterium tuberculosis*
3:40 PM	*Flash Talks*	Poster #46 Nili Ostrov; #48 Wenwen Huo; #53 Yu-Cheng Lin
3:50 PM		**Poster Session II - Posters 38-74**
IV: New antimicrobial tools		**Session chair: Kathryn Ramsey**
5:05 PM	Yu Imai	Darobactin, a novel antibiotic selectively kills Gram-negative pathogens
5:25 PM	Eachan Johnson	Large-scale chemical-genetic interaction profiling yields new classes of inhibitors of *Mycobacterium tuberculosis*
5:45 PM	Sarah Bening	Sensitizing tolerant bacteria to beta-lactam antibiotics
6:05 PM		**Networking tables and reception**

2019 Boston Bacterial Meeting

Day 2 - Friday June 7

8:30 AM	Morning Coffee -- Set up Session III posters	
V: Advances in microbial methods		**Session chair: Otto Cordero**
9:00 AM	Max Schubert	Production of ssDNA in vivo enables new types of screens
9:20 AM	Stacie Clark	Mimicking *Yersinia pseudotuberculosis* growth in deep tissue sites using microdroplet technology
9:40 AM	Erika DeBenedictis	Toward the evolution of an exclusively quadruplet-decoding translation system
10:00 AM	*Flash Talks*	Poster #76 Damien Cabral; #96 Jason Lynch
10:10 AM		**Poster Session III - Posters 75-111**
VI: Signaling		**Session chair: Alexandre Bisson**
11:25 AM	Amy Rohlfing	The CspC pseudoprotease regulates germination of *Clostridioides difficile* spores in response to multiple environmental signals
11:45 AM	Adnan Syed	Biofilm formation by *Staphylococcus aureus* is triggered by a drop in the levels of the second messenger cyclic-di-AMP
12:05 PM	Gerardo Cárcamo-Oyarce	Mucins modulate virulence and bacterial competition in *Pseudomonas aeruginosa*
12:30 PM		**Lunch on your own**
1:30 PM		Set up Session IV posters
VII: Pathogenesis		**Session chair: Maha Farhat**
1:50 PM	Fabian Rivera-Chávez	Cholera toxin promotes pathogen acquisition of host-derived nutrients
2:10 PM	Viktoria Betin	Dual RNAseq profiling of *Mycobacterium tuberculosis* infection identifies essential bacterial iron-limitation and redox stress response pathways
2:30 PM	Beau Wager	Identifying *Borrelia burgdorferi* lipoproteins ErpQ and ErpB as novel complement C1 inhibitors
2:50 PM	*Flash Talks*	Poster #115 Ted Chavkin; #124 R Cooper Baer; #128 Matt Henke
3:00 PM		**Poster Session IV - Posters 112-147**
VIII: Microbial communities		**Session chair: Jeffrey Moffitt**
4:15 PM	Marsha Wibowo	Reconstruction of ancient microbial genomes from the human gut
4:35 PM	Cooper Park	Highways of recombination and non-random patterns of donor-recipient relationships in multiple species of bacterial pathogens
4:55 PM	Anthony Ortiz	Massively parallel screening of synthetic microbial communities
5:15 PM		**Keynote Address - Denise Monack**
6:30 PM		**Reception**

2019 Boston Bacterial Meeting

WELCOME!

Dear Participants,

Welcome to the 25th annual Boston Bacterial Meeting! We are looking forward to an incredible two days of scientific presentations and discussions.

Over the past 25 years, BBM has consistently offered an intellectually stimulating and collegial atmosphere for bacteriologists throughout the New England area to discuss cutting-edge scientific research and to share ideas. Although the meeting has continued to grow in size, BBM remains a highlight of many people's scientific year due to our continued commitment to providing an environment that is both welcoming and scientifically rigorous. This is in large part thanks to the BBM Organizing Committee, a group of trainees (primarily graduate students and postdocs) who are passionate about providing a forum for other trainees to present their work and foster collaborations between researchers from all levels, in both academia and industry.

One highlight of BBM every year is the quality of the talks and poster presentations. In response to the continued growth in excellent abstract submissions, we have introduced short-format flash talks to allow more trainees an opportunity to present their work in front of the entire BBM audience. We are also featuring a record number of posters (more than 145!) which will be presented across four smaller sessions designed to allow for more discussion. As in the last several years, BBM will also have a range of breakout sessions on Thursday with a diverse set of panelists who are volunteering their time to participate in discussions spanning scientific topics, career paths, and science outreach.

We are very excited to feature Dr. Denise Monack as our Keynote Speaker this year. Dr. Monack is a Professor of Microbiology and Immunology at Stanford University and Fellow of the American Academy of Microbiology. Her research is an incredible reflection of the diversity of bacterial research presented at BBM. In the course of Dr. Monack's work on *Salmonella typhimurium* and *Francisella tularensis*, her lab has explored a remarkable range of questions spanning individual bacterial molecules, their cognate eukaryotic sensors, and the interactions within bacterial communities that shape disease course.

We are indebted to our faculty sponsors (Tom Bernhardt and Ethan Garner) and meeting founders (Rich Losick, Roberto Kolter, John Mekalanos, and Andrew Wright) and thank them for their continuing guidance. Additionally, we thank our generous sponsors for their financial support! With their sponsorship, we are able to keep BBM affordable and provide scholarships.

On behalf of the BBM2019 Organizing Committee, we thank you for attending BBM! We hope you will find the next two days both intellectually and socially fulfilling.

Nathan Hicks and Miriam Ramliden

Co-chairs, BBM2019

Meeting Announcements

- Please silence your mobile devices before entering the auditorium and breakout sessions.
- No food or drink is permitted in the auditorium or classrooms.
- Recording or photography of oral or poster presentations is prohibited.
- There is an overflow room in Hall A which will project oral presentations.
- Restrooms are located in the basement.
- Poster session judges: please check in at the registration desk to pick up materials.
- Room 109 is available as a lactation room. For additional information or alternate accommodations please inquire at the registration table.
- Please keep your name badge on at all times during the meeting. 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.
- Please direct any questions or concerns to the registration table in front of the auditorium.

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:

BBM Merchandise

In honor of the 25th anniversary of the Boston Bacterial Meeting, BBM merchandise will be on sale at the registration table during lunch breaks!
(Sales will be by credit/debit card only)

Insulated stainless steel travel tumbler
$12

Cotton canvas tote
$6

Keynote Speaker

Denise Monack, Ph.D.

Dr. Denise Monack is a Professor of Microbiology and Immunology in the School of Medicine at Stanford University.

The primary focus of her research is to understand the genetic and molecular mechanisms of bacterial pathogenesis. She is particularly intrigued by host-adapted pathogens that have evolved to persist within hosts for long periods of time. She has developed a mouse model to study mechanisms of asymptomatic persistent Salmonella infections. She uses this model to study pathogen-microbiota interactions and mechanisms of disease tolerance that impact host-to-host transmission.

In addition, her laboratory studies the cytosolic recognition of bacteria. Her lab discovered that two innate immune pathways, type I IFN and the inflammasome, are sequentially linked and that this 2-tiered response is a host gauge of the “danger” level before commitment to host cell death. She has received numerous prestigious awards in microbiology, including The Burroughs Wellcome Fund Recipient in Infectious Disease and is an elected Fellow to the American Academy of Microbiology, the world’s oldest and largest life science organization. She is currently the director of the Training Grant for the Program in Microbiology and Immunology at Stanford.

2019 Boston Bacterial Meeting – Session Chairs

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 been invited by the BBM Organizing Committee to act as session chairs. We encourage everyone to provide these new faculty members with a warm welcome to our Boston-area bacteriology community!

Bryan Bryson
Assistant Professor of Biological Engineering
Massachusetts Institute of Technology

Research in the Bryson Lab focuses on developing a quantitative understanding of the host-pathogen interface during *Mycobacterium tuberculosis* (Mtb) infection. Current projects in the lab include developing engineered Mtb strains that can provide unbiased assessments of phagosome composition, microfluidic tools to assemble synthetic phagosomes for quantitative analysis of Mtb responses to combinatorial stresses, and developing new approaches for studying host-pathogen interactions at the single-cell level.

Christina Faherty
Assistant Professor
Massachusetts General Hospital
Harvard Medical School

My research focuses on the host-pathogen interactions of *Shigella flexneri*, which causes infectious diarrhea by invading the colonic epithelium. By utilizing human-specific culture conditions to mimic small intestinal transit as well as human-specific organoid-derived infection models, the laboratory has identified new virulence factors, improved our understanding of survival and gene regulation during host transit, and expanded the *Shigella* infection paradigm. Our goal is to improve therapeutic development to combat this formidable bacterial pathogen and mitigate the significant global health burden caused by *Shigella*.

2019 Boston Bacterial Meeting – Session Chairs

Maha Farhat
Assistant Professor of Biomedical Informatics
Harvard Medical School

Maha Farhat holds an MD from the McGill University Faculty of Medicine and a MSc in biostatistics from the Harvard Chan School of Public Health. She is also a practicing physician at the Massachusetts General Hospital Division of Pulmonary and Critical Care Medicine. Dr. Farhat's research focuses on the development and application of methods for associating genotype and phenotype in infectious disease pathogens, with a strong emphasis on translation to better diagnostics and surveillance in resource-poor settings. To date, Farhat's work has focused on the pathogen Mycobacterium tuberculosis and spans the spectrum from computational analysis to field studies. She has led or participated in several projects funded by NIH including the NIAID and the BD2K initiative. She is a past Parker B Francis fellow, and a current Burke fellow at the Harvard Institute for Global Health.

Allison Lopatkin
Assistant Professor
Barnard College of Columbia University

My work combines experimental systems microbiology approaches with mathematical modeling to better understand non-genetic selection dynamics that lead to antibiotic resistance in bacterial populations. My doctoral work in Biomedical Engineering at Duke University focused on the propagation of resistance via horizontal gene transfer. As a postdoctoral fellow at MIT/Broad Institute, my research currently focuses on exploiting the cellular metabolic state to potentiate antibiotic lethality. Next year I will be joining the Biology Department at Barnard College of Columbia University; my lab will focus on applying these strategies to increasingly complex systems, e.g., heterogeneous bacterial populations. By elucidating population-level strategies that combat the spread antibiotic resistance, our goal is to both complement and spur novel drug discovery.

2019 Boston Bacterial Meeting – Session Chairs

Jeffrey Moffitt
Assistant Professor
Harvard University
Boston Children's Hospital

The Moffitt laboratory develops and utilizes highly multiplexed imaging methods to perform spatially resolved single-cell transcriptomic and proteomic measurements. In particular, the Moffitt laboratory is using and extending a technique known as multiplexed error robust fluorescence in situ hybridization (MERFISH), an approach that allows hundreds to thousands of different RNA molecules to be imaged and identified within individual cells in fixed tissues slices. These measurements provide a direct measure of the transcriptional profile of individual cells in their native tissue context, allowing cell types to be discovered and identified as well as the organization of these cell types to be mapped. The Moffitt lab is using this method to understand the spatial organization of complex bacterial communities and understand how different host cell types sense and shape the activity of commensal bacterial communities, primarily, in the gut.

2019 Boston Bacterial Meeting – Session Chairs

Otto Cordero
Associate Professor
Massachusetts Institute of Technology

The Cordero lab works on Microbial Community Ecology – microbial interactions, dispersal and community assembly – and its impact on the structure and function of microbial ecosystems. We work on a variety of systems, but our main focus is the community ecology behind the degradation of complex polysaccharides. Polysaccharide degradation is an essential ecosystem process in virtually all environments, from soils and oceans to animal guts, yet very little is known about how microbial interactions impact this fundamental process. A common assumption is that cells consume polysaccharides at rates comparable to the consumption of simple dissolved substrates in a well-mixed systems. In contrast, our work has shown that the degradation of complex polysaccharides is mediated by the assembly of spatially structured microbial communities on particle surfaces, where interactions such as public good mediated cooperation and crossfeeding dictate population dynamics and ecosystem function. Our research integrates quantitative measurements of population dynamics and function, mathematical modeling and genomics. Our goal is to reveal simple organizing principles of community assembly that can be adopted to engineer heterotrophic microbiomes.

2019 Boston Bacterial Meeting – Session Chairs

Niels Bradshaw
Assistant Professor of Biochemistry
Brandeis University

All cells must sense cellular and environmental conditions to appropriately grow, divide, survive, and coordinate their behaviors in communities. Reversible phosphorylation is an important and widespread regulatory mechanism that is executed by kinases and phosphatases to control the activity of target proteins. Phosphatases are direct targets of regulatory inputs and are highly substrate-specific, but the molecular mechanisms that govern phosphatase regulation and specificity are largely unknown. My lab uses structural biology, enzymology, genetics, and cell biology to address how phosphatases are regulated, achieve specificity, and have evolved.

PP2C family protein serine/threonine phosphatases mediate diverse transcriptional responses in bacteria and also function in human signaling pathways. The most widespread role of PP2C phosphatases is control of the general stress response in bacteria, which is important for virulence, antibiotic resistance, and persistence. These phosphatases are ideal systems for determining how phosphatase activity is controlled in response to signals, and how regulation of phosphatases has been diversified by evolution. Additionally, the signals phosphatases directly sense to initiate the general stress response are virtually unknown. We pursue identification of such signals by a combined genetic and biochemical strategy in both model organisms and pathogens. The signals an organism uses to detect stress indicate what is important for survival of that organism in the environment and within communities. These signals will also suggest targets for species-specific antimicrobials and other targeted therapies.

A fundamental question in molecular evolution is how new catalytic activities evolve on pre-existing protein scaffolds. My work revealed that PP2C phosphatases and a family of proteases (including the proteasomal proteases) are structurally and evolutionarily related, and share an allosteric regulatory switch but use divergent catalytic mechanisms. This suggests that allosteric regulatory mechanisms can facilitate the evolution of new catalytic activities and establishes PP2C phosphatases and proteasomal proteases as an experimental system with which to study how such evolution occurs.

2019 Boston Bacterial Meeting – Session Chairs

Alex Bisson
Assistant Professor of Biology
Brandeis University

Archaea are the least microscopically studied microbes, making archaeal cell biology a young field and full of promising discoveries. The Bisson Lab will explore into this alien domain of life to seek for novel cellular behaviors and molecular mechanisms. Among many other projects, we will investigate how archaea sense their environment to make decisions such as to propagate or adapt their cellular shape, to pace the progress of their circadian cell cycle, to mate or compete with surrounding microbes. Our group will focus on interdisciplinary approaches - combining genetics, advanced microscopy, computational analysis, microfluidics, *in vitro* reconstitution systems and -omics - to quantitatively understand these emergent life forms that share evolutionary traits with both bacteria and eukaryotes. The Bisson Lab will officially open its doors in the Fall of 2019 and is recruiting excited and fearless scientists to join Alex in this quest into the unknown.

Boston Bacterial Meeting 2019 – Bacterial Art

In celebration of the 25th anniversary of BBM, the Organizing Committee has commissioned a work of Bacterial Art. The commission was made by the artist Maria Peñil Cobo in collaboration with Dr. Mehmet Berkmen at New England Biolabs using the bacteria *Staphylococcus aureus*, *Nesterenkonia*, *Deinococcus*, and *Bacillus* on LB plates. We will be presenting an image printed on metal of the final BacArt piece at its completion, captured by the photographer Scott Chimileski, and beside it is the original piece sealed in epoxy.

Mehmet is the creative director and Maria is the bacterial artist of BacArt, an outreach organization that works to engage the public and increase their awareness of bacteria in and around us using Bacterial Art workshops.

You can learn more about BacArt at www.bacterialart.com

2019 Boston Bacterial Meeting – Breakout Sessions

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

Antibiotics, Hall A
The fight against antimicrobial resistance is one of the most significant challenges in modern medicine. Hear from leading researchers about the approaches they take to combat resistant microbes, improve diagnostics, and discover new treatments.

Panelists: Yonaton Grad, Kim Lewis, Edward Geisinger

Microbiome, Hall E
While microbiome research is a rapidly growing field, the overall impact of its microbial members on human health and disease remains largely unknown. Hear from leaders in the field on how their research looks to harness the microbiome to treat disease and design therapeutics to improve overall human health.

Panelists: Dan Brownell, Xuesong (Song) He, Hera Vlamakis

Diversity and Inclusion in Science, Room 110
Diversity and inclusion strengthen our scientific community. Our panelists will discuss the importance of creating a more inclusive and a diverse scientific community, and how to facilitate that growth in microbiology.

Panelists: Bruce Birren, Ellise LaMotte, Brianna Pina, Sheila Thomas

Early Careers in Academia, Room 221
How do you become a professor these days? What does academia look like for a young faculty member? Learn more about the academic job market from faculty members starting their careers at both primarily undergraduate institutions and large research universities.

Panelists: Colleen O'Loughlin, Shumin Tan, Niels Bradshaw, Katie Berry

Grant Writing, Room 116
Grantsmanship is an art that is vital to sustaining scientific research. Our panel consists of grant writing experts who have tips for all levels of writers on how to effectively communicate their science and craft a winning grant.

Panelists: Ralph Isberg, Aimee Shen, Christina Faherty

Bacteriophage, more than just lambda, Room 304
Phage are a valuable research tool in fields ranging from microbiology to molecular genetics. Join researchers from both academia and biotech to learn more about how bacteriophages are being repurposed to fight bacterial infections.

Panelists: Peter Weigele, Kathryn Kauffman, Byran Hsu, Sian Owen

2019 Boston Bacterial Meeting – Breakout Sessions

Careers Beyond the Bench, Room 309
What else can you do with a degree in, and a passion for, microbiology? Hear from a variety of bacteriologists who have brought their expertise outside of the lab – from law to consulting to science communication. Learn more about how our panelists discovered their new careers and what steps you can take now to find yours.

Panelists: Minmin Yen (PhagePro), Lisa Hawver (Choate Hall & Stewart), Jennifer Tsang (Addgene), Anne Madden (Lachancea LLC)

Careers in Industry, Room 309A
Explore a career in industry by hearing from professionals in biotech! Panelists will speak about their jobs, the companies they work for, and what you should do to be successful.

Panelists: Geoffry Hannigan (Merck), Andrew Hempstead (Addgene), Brian Meehan (Kaleido)

Food Science, Room 222
Come hungry to learn about the microbes that make our favorite foods and beverages from our panel of microbiologists and food science specialists.

Panelists: Ronn Friedlander, John Gibbons, Pia Sorensen

Science Communication, meet in front of the first floor elevators of the Science Center
Learn more about how to communicate science in an effective and engaging way through a guided tour with Scott Chimileski of the Microbial Life exhibition at the Harvard Museum of Natural History.

Bacterial Art, Room 216
Join Mehmet Berkmen, creative director at Bacterial Art, for a presentation and discussion on bacterial art, its role in history, science, and education, and how we should merge the artificial separation between science and art.

2019 Boston Bacterial Meeting - Oral Presentation Abstracts

1 Affinity-based capture and identification of (p)ppGpp-binding proteins

Boyuan Wang[1] Peng Dai[2] Robert A. Grant[1] Bradley L. Pentelute[2] Michael T. Laub[1, 3]
[1]Department of Biology, Massachusetts Institute of Technology [2]Department of Chemistry, Massachusetts Institute of Technology [3]Howard Hughes Medical Institute

(p)ppGpp is a starvation-induced second messenger universally conserved in bacteria. Upon accumulation, (p)ppGpp interacts with its cellular targets and reprograms bacterial physiology to promote survival over growth. Despite decades of research, comprehensive identification of (p)ppGpp targets from a given organism has remained a major challenge. To address this limitation, we developed photo-crosslinking probes that efficiently capture (p)ppGpp binding proteins in cell lysates for identification by mass spectrometry. Using this strategy, we identified 56 ppGpp-binding proteins in *E. coli* including most previously identified targets. We have validated PurF and Gsk, two enzymes involved in purine nucleotide metabolism, as *bona fide* targets both *in vitro* and *in vivo*. PurF controls the first, dedicated step of *de novo* purine synthesis, while Gsk harbors kinase activity necessary to salvage purine nucleosides. ppGpp potently inhibits both PurF and Gsk, and X-ray crystallography reveals two unprecedented modes of ppGpp-protein interaction. Importantly, by targeting two previously known effectors (Gpt and Hpt), as well as PurF and Gsk, ppGpp inhibits all pathways leading to the synthesis of GTP. Notably, the inhibition of GTP synthesis by (p)ppGpp is conserved in *B. subtilis*, but occurs through a different target, the guanylate kinase Gmk. Collectively, our findings (i) demonstrate the importance of downregulating GTP and ATP production in response to nutrient adversity and (ii) provide a nearly comprehensive list of ppGpp targets for future study.

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

Jennifer Peña[1, 2] Kirsty McFarland[2] Padraig Deighan[3] Tracy Kambara[1, 2] Simon Dove[1, 2]
[1]Harvard Medical School, Boston, MA [2]Division of Infectious Diseases, Boston Children's Hospital [3]Department of Biology, Emmanuel College, Boston, MA

Pseudomonas aeruginosa is an opportunistic human pathogen on the Center for Disease Control's list of top drug resistant threats in the United States. P. aeruginosa has a programmed cell death (PCD) pathway that is linked to the virulence of the organism. This PCD pathway is encoded by the genes alpABCDE and is switched on in a subset of cells in response to DNA damage. The genes alpBCDE encode cell lysis genes, whereas alpA encodes a positive regulator of the lysis genes. Deletion of alpA results in a decrease in bacterial burden in an acute lung infection model. Here we present evidence that AlpA positively regulates target gene expression by functioning as a processive antiterminator rather than as a transcription activator. AlpA specifically functions by recognizing a DNA sequence in target promoters, then directly binding RNA polymerase and allowing it to bypass intrinsic termination sites positioned downstream of target promoters. We show AlpA positively regulates genes in a second operon PA0807-PA0831. This operon does not appear to contribute to cell lysis, however genes in this operon are known to be important for virulence of P. aeruginosa. Lastly, we provide evidence that the activity of AlpA increases when ppGpp levels increase. ppGpp is a small molecule which is known to regulate the stringent response in bacteria additionally ppGpp levels are known to increase when DNA damage occurs. Overall, this study shows that AlpA functions as a processive antiterminator to regulate virulence gene expression in response to DNA damage and intracellular ppGpp levels.

3 Surveying the landscape of tRNA modifications in bacteria by combined tRNA sequencing and RNA mass spectrometry

Satoshi Kimura[1, 2, 3] Peter C. Dedon[4] Matthew K. Waldor[1, 2, 3]
[1]Brigham and Women's Hospital [2]Harvard Medical School [3]HHMI [4]Massachusetts Institute of Technology

tRNAs are heavily decorated with post-transcriptional modifications (tRNA modification) that have pivotal roles in their function as essential adapter molecules for translation. Some modifications are thought to be broadly conserved in the three domains of life whereas other modifications are only found in a narrow range of organisms. However, profiling an organism's tRNA modification is extremely labor intensive and has been accomplished for few species. Here, we took advantage of deep sequencing to map tRNA modifications in *Escherichia coli* and *Vibrio cholerae*. In E. coli, where modifications have been well-characterized, we found that more than half of the known modified tRNA nucleosides resulted in either premature termination or incorporation of mismatched bases during reverse transcription. These RT-derived signatures were present in sequences of V. cholerae's tRNAs and enabled rapid detection of the predicted conserved modifications.

Furthermore, we also observed RT signatures that are specific to *V. cholerae*. RNA mass spectrometry revealed that at least one of these modifications has a novel chemical composition and the pathway for its generation is being investigated. In addition, RT signatures of modification varied according to growth conditions, e.g., log vs stationary phase and during infection, suggesting that growth conditions modulate the abundance and/or chemical composition of tRNA modifications. Collectively, our findings indicate that combining tRNA sequencing with RNA mass spectrometry enables a high throughput means to catalog modification sites in diverse organisms, track how environmental conditions modulate tRNA modification, and provides clues for detection of novel modifications and the biochemical pathways that create them.

4 Understanding the role and regulation of cell wall hydrolases in *Bacillus subtilis*

Sean Wilson[1] Ethan Garner[1]
[1]Department of Molecular and Cellular Biology, Harvard University

Bacteria are encased in a rigid meshwork called the cell wall. In order to grow, bacteria must continuously remodel their cell wall, inserting new material and breaking old bonds. Recent research has explained many of the mechanisms of cell wall insertion, but the regulation of bond breakage is still not understood. The cell wall hydrolases responsible for this breakage act outside of the cell in a dense meshwork, physically removed from most potential mechanisms of regulation. How is their activity controlleddelta

First, we characterized the activity of the hydrolase enzymes. Using fluorescent D-amino acids (FDAAs) to measure wall turnover, we found that there are two classes of hydrolase enzymes with separable functions: one group removes of old material from the wall, and the other breaks bonds in the wall to allow for cell growth. Strikingly, were able to knock out all of the hydrolase genes except those responsible for growth (35 in total) with no change in viability or wall thickness.

Next, we investigated the regulation of these activities, and found that mechanical stress plays a key role. We used osmotic shocks to alter stress on the cell wall, and measured autolysis rates as a proxy for the activity of the hydrolases. These experiments indicate that the enzymes responsible for cell wall removal preferentially cleave material that is not under stress. FDAA and electron microscopy experiments indicate that the growth enzymes are also spatially regulated. We thus propose a stress-based model for regulation of wall thickness and growth.

5 RNA polymerases are moving barriers to condensin loop extrusion

Hugo B. Brandão[1] Xindan Wang[2, 3] Payel Paul[3] Aafke A. van den Berg[4] David Z. Rudner[2] Leonid A. Mirny[1, 4, 5]
[1]Graduate Program in Biophysics, Harvard University, Cambridge [2]Department of Microbiology and Immunobiology, Harvard Medical School, Boston [3]Department of Biology, Indiana University, Bloomington [4]Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge [5]Department of Physics, Massachusetts Institute of Technology (MIT), Cambridge

To separate replicated sister chromatids during mitosis, eukaryotes and prokaryotes have structural maintenance of chromosome (SMC) condensin complexes that were recently shown to organize chromosomes by a process known as DNA loop extrusion. In rapidly dividing bacterial cells, the process of separating sister chromatids occurs concomitantly with ongoing transcription. How transcription interferes with the condensin loop extrusion process is largely unexplored, but recent experiments show that sites of high transcription may directionally affect condensin loop extrusion. We quantitatively investigate different mechanisms of interaction between condensin and elongating RNA polymerases (RNAP) and find that RNAPs are likely steric barriers that can push and interact with condensins. Supported by new Hi-C and ChIP-seq data for cells after transcription inhibition and RNAP degradation, we argue that translocating condensins must bypass transcribing RNAPs within 2 seconds of an encounter at rRNA genes and within 10 seconds at protein coding genes. Thus, while individual RNAPs have little effect on the progress of loop extrusion, long, highly transcribed operons can significantly impede the extrusion process. Our data and quantitative models further suggest that bacterial condensin loop extrusion occurs by two independent, uncoupled motor activities; the motors translocate on DNA in opposing directions and function together to enlarge chromosomal loops, each independently bypassing steric barriers in their path. Our study provides a quantitative link between transcription and 3D genome organization and proposes a mechanism of interactions between SMC complexes and elongating transcription machinery relevant from bacteria to higher eukaryotes.

6 Identification of new components of the RipC-FtsEX cell separation pathway of *Corynebacterineae*

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Several important human pathogens are represented in the Corynebacterineae suborder, including Mycobacterium tuberculosis and Corynebacterium diphtheriae. These bacteria are surrounded by a multilayered cell envelope composed of a cytoplasmic membrane, a peptidoglycan (PG) cell wall, a second polysaccharide layer called the arabinogalactan (AG), and finally an outer membrane-like layer made of mycolic acids. Several anti-tuberculosis drugs target the biogenesis of this complex envelope, but their efficacy is declining due to resistance. New therapies are therefore needed to treat diseases caused by these organisms, and a better understanding of the mechanisms of envelope assembly should aid in their discovery. To this end, we generated the first large-scale library of transposon insertion mutants in the model organism C. glutamicum. Transposon-sequencing was then used to define its essential gene set and identify loci that, when inactivated, confer hypersensitivity to ethambutol (EMB), a drug that targets AG biogenesis. Among the EMBS loci were genes encoding RipC and the FtsEX complex, a PG cleaving enzyme required for proper cell division and its predicted regulator, respectively. Inactivation of the conserved steAB genes (cgp-1603-1604) was also found to confer EMB hypersensitivity and cell division defects. A combination of quantitative microscopy, mutational analysis, and interaction studies using a novel assay that enable examination of protein-protein interactions in the periplasmic space indicate that SteA and SteB form a complex that localizes to the cytokinetic ring to promote cell separation by RipC-FtsEX and may coordinate its PG remodeling activity with the biogenesis of other envelope layers during cell division.

7 A new mechanism of reduced cephalosporin susceptibility in *Neisseria gonorrhoeae*

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Reduced susceptibility to extended-spectrum cephalosporins (ESCs), including ceftriaxone, has emerged in *Neisseria gonorrhoeae*. With no clear next-line agent, this threatens the future of effective treatment for gonorrhea. Most reduced susceptibility to ceftriaxone has been attributed to horizontally-acquired *penA* (PBP2) alleles. However, this mechanism does not explain all observed reduced susceptibility. Indeed, the isolates with the highest-level ESC resistance identified by the Centers for Disease Control and Prevention's surveillance system lack these alleles and other characterized genetic variants known to contribute to reduced cephalosporin susceptibility. Here, we show that *penA*-independent reduced ESC susceptibility has emerged multiple times in clinical *N. gonorrhoeae* isolates through distinct mutations in RNA polymerase components. These mutations result in large-scale transcriptional changes, but do not cause a general drug tolerance phenotype, indicating a cephalosporin-specific mechanism. Among the genes with altered expression profiles in these mutants are those encoding cell wall biosynthesis machinery and the pilus pore protein PilQ, which has been reported to enhance outer membrane permeability. We show that increased PBP1 expression can contribute to decreased ESC susceptibility, likely through enzymatic replacement of ESC-inhibited PBP2 transpeptidation. However, other factors are needed to recapitulate the high-level reduced susceptibility observed in the context of RpoB or RpoD variants. This is the first report of a mechanism of reduced ESC susceptibility in clinical gonococcal isolates that is not reliant on genetic variation in the target PBP. The identification of this resistance mechanism has clear implications for the development of molecular diagnostics for and surveillance of AMR in gonorrhea and highlights the need for continued efforts in understanding the basis for diverse mechanisms of cephalosporin resistance.

8 Host hyperglycemia impacts antibiotic efficacy within the murine gut microbiome

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Insufficient mechanistic explanations still exist for the differential survival observed between bacterial species upon antibiotic exposure in the microbiome despite recent major advances. The microbiome is known to be profoundly sensitive to host metabolism and thus host metabolic state likely plays an important role in the responses of the microbiome to antibiotic perturbation. Specifically, the impact of hyperglycemia remains to be elucidated. 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 metagenomic taxonomic profiling with whole community metatranscriptomics and metabolomics to study the impacts of antibiotic therapy on the murine microbiome. We found that, upon challenge with bactericidal antibiotics, hyperglycemic and normoglycemic mice exhibited marked variation in both taxonomic composition and functional responses. Amoxicillin challenge elicited significantly contrasting transcriptional signatures at the community level that was dependent on host glycemic status. Hyperglycemic communities exhibited vast downregulation of mannose family phosphotransferase system activity, type-II toxin/antitoxin systems, oxidative stress response systems, motility, sporulation, central carbon metabolism, and cell wall biosynthesis. These communities also exhibit upregulation in efflux transporters and known antibiotic response systems. Taken together, these seem to suggest that surviving taxa within hyperglycemic communities exhibit a degree of antibiotic tolerance or desensitization that is driven by differences in host-derived factors, ultimately highlighting the sensitivity of the microbiome to host metabolism.

9 Natural genetic variation in *dnaA* influences antibiotic susceptibility in *Mycobacterium tuberculosis*

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Multidrug resistant Mycobacterium tuberculosis is now globally prevalent, representing over 450,000 newly diagnosed tuberculosis (TB) cases in 2018. This necessitates widespread antibiotic susceptibility testing to effectively treat TB disease. Compared with growth-based phenotypic profiling, nucleic acid-based tests to detect causal resistance variants will dramatically reduce the time to diagnose drug resistance in this slow growing pathogen. However, the genotypic basis of drug resistance is not completely understood. Using a global collection of whole-genome sequenced M. tuberculosis clinical isolates profiled for phenotypic drug resistance, we performed a genome-wide association to identify novel bacterial determinants of antibiotic susceptibility. We found that the conserved bacterial chromosome replication initiator, dnaA, was the target of frequent mutations in clinical isolates, and these mutations were statistically enriched in drug resistant strains. Using oligo-mediated recombineering, we constructed a panel of isogenic dnaA mutants at the native chromosomal locus. In each case, mutation of dnaA did not alter growth kinetics in the absence of antibiotics, however all dnaA mutations conferred low-level resistance to the widely used first-line antibiotic isoniazid. We further found that there was a trend toward delayed bacterial clearance of dnaA mutants in a clinical cohort of patients treated with an isoniazid-containing regimen. Initial characterization of genome-wide dnaA binding sites using ChIP-seq has identified a conserved mycobacterial dnaA binding box that is also under remarkably strong selective pressure in M. tuberculosis. We are now interrogating the role of this locus as we work to identify the molecular mechanism connecting dnaA and isoniazid resistance.

10 Darobactin, a novel antibiotic selectively kills Gram-negative pathogens

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Appearance of drug-resistant pathogens is a serious public health concern. Most currently used antibiotics were isolated from actinomycetes, however, this source has been overmined and novel sources of antibiotics are needed. Enterobacteriaceae *Photorhabdus* contain a number of secondary metabolite biosynthetic gene clusters in their genome. They live symbiotically with nematodes that infect insects and produce antibiotics to protect the food source from other microorganisms. We screened a small set of *Photorhabdus* against *Escherichia coli*, and found the culture extract from *P. temperata* HGB1456 showed inhibitory activity. Active extract was fractionated by HPLC and bioassay-driven purification led to the isolation of an active compound whose structure was elucidated by LC-MS and NMR analysis. The compound, named darobactin, is ribosomally synthesized and post-translationally modified, and has a scaffold of two rings with unusual linker bonds. Darobactin kills *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* at 2-8 μg/ml, with no activity against Gram-positive bacteria and important Gram-negative members of the gut microbiota, Bacteroides. We generated darobactin-resistant mutants from *E. coli* by serial passaging. All mutations clustered around the lateral gate of BamA, an essential outer membrane protein responsible for assembly and insertion of β-barrel proteins into the outer membrane. Interestingly, all isolated mutants contained 2 or 3 mutations in *bamA*. Darobactin has efficacy in animal models of infection caused by colistin-resistant E. coli *and* P. aeruginosa. These results suggest that darobactin is a promising lead antibiotic against Gram-negative pathogens, and based on its unusual spectrum, is unlikely to harm the microbiome.

11 Large-scale chemical-genetic interaction profiling yields new classes of inhibitors of *Mycobacterium tuberculosis*

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With rising antibiotic resistance, new drugs are desperately needed tuberculosis (TB), a complex disease caused by *Mycobacterium tuberculosis* (Mtb) which kills 1.5 million people per year. Combining chemistry and genetics, we developed a new strategy for rapidly identifying many new small molecule inhibitors of Mtb and for elucidating their mechanisms of action (MOA), by performing large-scale chemical screening on pooled genetic libraries containing 100 barcoded strains hypomorphic for essential genes. We created barcoded hypomorphs for 474 of the 625 essential genes in Mtb and developed a multiplexed, whole-cell assay to measure strain abundance. Applying the approach with an activity-enriched, 3226 compound library and an unbiased 47,353 compound library, we characterized 8.5 million chemical-genetic interactions. Using machine learning, we identified 40 novel compounds against known MOAs, including new classes of inhibitors of DNA gyrase, mycolic acid biosynthesis, and folate biosynthesis. By identifying highly specific chemical-genetic interactions, we identified new inhibitors of RNA polymerase and of a novel target, EfpA. Finally, we showed an inhibitor, discovered by screening the hypomorphs, could be optimized by medicinal chemistry to be active against wild-type Mtb. The results demonstrate that this systems chemical biology approach can empower discovery, prioritization, and development of novel antimicrobials.

12 Sensitizing tolerant bacteria to beta-lactam antibiotics

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Antibiotic tolerant bacteria lead to chronic and costly infections in the clinic, and antibiotic tolerance has been shown to facilitate the evolution of antibiotic resistance *in vitro*. Anti-tolerance strategies stimulating central carbon metabolism sensitize tolerant bacteria to some classes of antibiotics, but these metabolism-based approaches have thus far been unsuccessful with beta-lactams, one of our most widely used classes of antimicrobials. Here we present an approach – which combines stimulation of central carbon metabolism and treatment with a D-amino acid – to sensitize tolerant bacteria to beta-lactam antibiotics. We used

stationary phase as a model of antibiotic tolerance, where *Escherichia coli* was not killed by up to 1 mg/ml of the beta-lactam ampicillin. In these conditions, we found that supplementation with D-alanine or D-serine sensitized killing by lower ampicillin concentrations than both their L-isomers and central carbon metabolites such as glucose and pyruvate. This pattern of D-amino acid-enhanced sensitization extended to other D-amino acids when combined with pyruvate, chosen as a central carbon metabolite because both D-alanine and D-serine can be catabolized into pyruvate. Potentiation of antibiotic lethality by D-amino acids was specific to beta-lactam antibiotics and strongest for D-amino acids which can be misincorporated into *E. coli* peptidoglycan. Our anti-tolerance approach was also effective against *Klebsiella pneumoniae* and *Mycobacterium smegmatis*, and could be combined with a beta-lactamase inhibitor to treat simultaneously tolerant and resistant bacteria. Overall, our work suggests that continued study into stimulating bacterial metabolism will broaden the drugs, species, and conditions for which anti-tolerance approaches are available.

13 Production of ssDNA in vivo enables new types of screens

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Next-generation DNA sequencing (NGS) observes tremendous bacterial genetic variation of unknown consequence in the environment, in patient samples, and in the lab. Our ability to create variants and test their impact, however, lags far behind in scale. Existing methods such as Transposon-insertion sequencing (Tn-seq) and CRISPRi create thousands of genotypes in parallel and measure phenotypes using NGS, allowing high-throughput functional annotation of genomes. But what about sequence variationdelta These methods cannot create and test variation because they only create gross loss-of-function changes in genomes, leaving the impact of many mutations unknown and unexplored. Here we demonstrate using Retron elements to create abundant single-stranded DNA (ssDNA) in *E. coli*, resulting in barcoded libraries of specified mutants. We term this method Retron Library Recombineering (RLR), and use it to characterize antibiotic resistance alleles obtained by directed evolution for resistance, or mutations designed by the researcher. We quantify the relative contributions of different alleles toward a resistance phenotype, and explore epistatic interactions that result in high-level resistance. RLR thus precisely characterizes mutations either created by evolution, or intelligently designed to test your hypotheses. Stay tuned: production of ssDNA in vivo may soon be a versatile method of generating barcoded mutant libraries in your favorite bug!

14 Mimicking *Yersinia pseudotuberculosis* growth in deep tissue sites using microdroplet technology

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Bacterial pathogens invade mammalian hosts and localize to deep tissue sites where they can propagate despite the presence of the host immune system. We have probed the dynamics of deep tissue growth by analyzing the colonization of murine spleens by Yersinia pseudotuberculosis (Yptb). *Yptb* microcolony formation involves extracellular clonal clusters of bacteria that are in direct contact with a layer of neutrophils which are, in turn, encased by a layer of macrophages. Within the microcolony, peripheral bacteria upregulate the nitric oxide (NO)-detoxifying protein, Hmp, to detoxify NO gas being produced by the macrophages. Although live animal models provide valuable insight into the spatial organization of bacterial subpopulations and immune cells in tissue, analysis is limited to microscopy of fixed tissue with little opportunity to study the heterogeneity of the observed microcolony subpopulations. To address this, we combined the use of droplet-based microfluidics to support *Yptb* microcolony formation with the incorporation of host innate immune cells to accurately model bacterial-host cell interactions that take place in deep tissue sites. We demonstrate that activated bone marrow-derived macrophages drive peripheral expression of *hmp* in droplet microcolonies, recapitulating the spatial regulation seen in tissue. Now that we have established a novel *in vitro* system, this approach will allow us analyze the dynamics of bacterial growth in these sites, and identify unique difficult-to-detect bacterial subpopulations that respond to tissue-resident immune cells.

15 Toward the evolution of an exclusively quadruplet-decoding translation system

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Incorporating Non-Canonical Amino Acids (NCAAs) into protein therapeutics can increase bioavailability and decrease production costs, providing an avenue to improve all 380 FDA-approved protein therapeutics on the market today. However, current methods for in vivo NCAA incorporation, such as amber or frameshift suppression, can integrate no more than two NCAAs into a single protein due to competition between the endogenous and expanded genetic codes. The goal of this project is to engineer an orthogonal translation system in vivo that exclusively decodes quadruplet codons, enabling production of proteins largely or entirely composed of NCAAs. Toward this end, we have validated a Phage Assisted Continuous Evolution (PACE) approach for evolving tRNA, rRNA, and r-protein components for improved frameshift suppression. We have applied this approach to evolve a several suppressors, including a naively-engineered Serine UAGA suppressor, which improved production of a UAGA-containing luciferase from 2% to 73% in comparison to WT luciferase. We have identified frameshift suppressor tRNAs that are charged endogenously for 13/20 canonical amino acids, and are utilizing massively parallel robotic directed evolution to evolve these for improved efficiency. These efforts constitute the first step toward assembling an orthogonal translation system composed of evolved rRNA, r-proteins, and 20 codon-compatible suppressor tRNAs capable of exclusively implementing a quadruplet codon code.

16 The CspC pseudoprotease regulates germination of *Clostridioides difficile* spores in response to multiple environmental signals

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Clostridiodies difficle is the leading cause of healthcare-associated gastrointestinal disease and is transmitted by the metabolically dormant spore form of the bacteria. *C. difficile* initiates infection when spores germinate in the small intestine in response to specific mammalian bile salts. This is in contrast to most spore-forming bacteria studied to date that sense nutrient germinants using transmembrane germinant receptors to initiate germination. Furthermore, *C. difficile* lacks homologs of transmembrane germinant receptors and genetic evidence has implicated the pseudoprotease, CspC, in directly sensing bile salt germinants. To gain insight into C. difficile's unique germination pathway, we solved the crystal structure of CspC from *C. difficile*. Mutational analysis of CspC guided by this structure led to the identification of mutants that rendered spores either hypo- or hypersensitive to bile salt germinant. Surprisingly, hypersensitive mutants germinated in the absence of bile salt germinant, although to a lesser extent than in the presence of germinant, and were also hypersensitive to amino acid and/or calcium co-germinants that potentiate spore germination. As substitutions at specific residues altered the responsiveness of CspC to bile salt germinant and two biochemically distinct classes of co-germinants, our work indicates that CspC integrates multiple environmental signals to initiate germination. In addition, this work raises the possibility that CspC may function as a critical signaling node rather than as a direct ligand binding receptor.

17 Biofilm formation by *Staphylococcus aureus* is triggered by a drop in the levels of the second messenger cyclic-di-AMP

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The bacterial pathogen Staphylococcus aureus forms multicellular communities known as biofilms in which cells are held together by an extracellular matrix. The matrix consists of repurposed cytoplasmic proteins and extracellular DNA. These communities assemble during growth on medium containing glucose, but the intracellular signal for biofilm formation was unknown. Here we present evidence that biofilm formation is triggered by a drop in the levels of the second messenger cyclic-di-AMP. Previous work identified genes needed for the release of extracellular DNA, including genes for the cyclic-di-AMP phosphodiesterase GdpP, the transcriptional regulator XdrA, and the purine salvage enzyme Apt. Using a cyclic-di-AMP riboswitch biosensor and mass spectrometry, we show that the levels of the second messenger drop during

biofilm formation in a glucose-dependent manner and that the drop is prevented in mutants of all three genes. Importantly, in the case of the XdrA mutant, the block in release of extracellular DNA and biofilm formation was reversed by lowering cyclic-di-AMP levels via overproduction of GdpP. Our findings point to cyclic-di-AMP as the intracellular signal for biofilm formation and the phosphodiesterase as an attractive target for drug development.

18 Mucins modulate virulence and bacterial competition in *Pseudomonas aeruginosa*

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Mucus is a three dimensional hydrogel that houses the majority of the human microbiome. The multifaceted mucous environment sustains host-microbe homeostasis, as well as it preserves a diverse and functional microbial community. The primary structural components of mucus are mucin glycoproteins, which crosslink to form a complex polymer network that surrounds microbes. Little is understood about how this key environmental factor influences microbial phenotypes. To fill this gap in knowledge, we have established an interdisciplinary set of tools, which allow the systematic study of bacterial behavior in mucus-like environments. In this study, we show that natively purified gel-forming mucins suppress key virulence traits in the opportunistic pathogen Pseudomonas aeruginosa, including biofilm formation, siderophore biosynthesis, and toxin secretion, while preventing the bacterium from infecting epithelia. Additionally, mucins environments prevent P. aeruginosa from outcompeting Staphylococcus aureus in co-cultures. Mucins promote bacterial coexistence by altering their physical interactions and by reducing the production of anti-staphylococcal compounds in P. aeruginosa. Our results highlight the role of mucus, and its components, in keeping healthy and diverse microbial communities in the human body

19 Cholera toxin promotes pathogen acquisition of host-derived nutrients

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Vibrio cholerae is the causative agent of cholera, a potentially lethal enteric bacterial infection. Cholera toxin (CT) is required for V. cholerae to cause severe disease and also promotes transmission of the organism in that victims can shed many liters of diarrheal fluid. However, how the pathogen is able to reach such high concentrations in the intestine during infection remains poorly understood. Here we show that CT-mediated disease enhances pathogen growth and induces a distinct V. cholerae transcriptome signature that is indicative an iron-depleted gut niche. We show that V. cholerae heme utilization confers a growth advantage to the pathogen only when CT is produced, which correlated with an increased bioavailability of heme in the gut lumen. CT-induced disease also led to increased luminal concentrations of host-derived long-chain fatty acids (LCFAs) and L-lactate and metabolites. Genetic analysis of V. cholerae suggested that heme and LCFA uptake-dependent growth of V. cholerae occurs during infection but only in a strain capable of producing CT in vivo. We conclude that CT-induced disease creates an iron-depleted metabolic niche in the gut that selectively promotes the explosive growth of this pathogen through acquisition of host-derived nutrients.

20 Dual RNAseq profiling of *Mycobacterium tuberculosis* infection identifies essential bacterial iron-limitation and redox stress response pathways

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Mycobacterium tuberculosis (Mtb) is the leading infectious killer in the world. Key to its pathogenic success is the ability to grow within host innate immune cells, macrophages. Simultaneous profiling of host and pathogen transcription, dual RNAseq, can identify gene networks that mediate disease outcome and reveal new therapeutic targets. However, dual RNAseq profiling of bacterial infections is limited by detection of lowly abundant bacterial mRNA. To gain insights into how Mtb transcriptionally adapts to the intracellular environment we developed Pathogen Hybrid Capture (Path-cap) to perform dual RNAseq on

Mtb infected macrophages. We performed integrated analysis of host and pathogen gene expression during a time course of infection and identified iron limitation as a host-imposed stressor felt by intracellular bacteria late in infection. We then leveraged this dataset and Path-cap methodology to characterize the regulon of a Mtb transcription factor (TF) important for growth in macrophages. Rv0135c regulates distinct pathways during in vitro and intracellular growth highlighting the dangers of extrapolating in vivo relevance from in vitro datasets. Within macrophages, Rv0135c negatively regulates redox genes, yet these regulatory targets do not appear to be important for Mtb infection of naïve macrophages, suggesting that Rv0135c functions to constrain their expression during Mtb intracellular growth. This work links bacterial transcriptional regulation within macrophages to coordinated host cell responses offering new insights into the intracellular mechanisms of Mtb pathogenesis.

21 Identifying *Borrelia burgdorferi* lipoproteins ErpQ and ErpB as novel complement C1 inhibitors

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Borrelia burgdorferi, the causative agent of Lyme borreliosis, encodes a large number of lipoproteins. Although several surface-exposed lipoproteins have been functionally characterized, most of their roles remain unknown. We utilized an epitope-tagged lipoprotein expression library consisting of 80 predicted *B. burgdorferi* surface lipoproteins, each ectopically produced in the high-passage, non-infectious strain B31-e2, which lacks many endogenous plasmids, in whole cell binding assays to identify novel pathogen-host interactions. Strains overexpressing ErpQ and ErpB were discovered to interact with high affinity for human C1, the first component of the classical complement pathway. These strains and recombinant ErpQ and ErpB also specifically bind to the C1r and C1s subunits of the C1 protein complex. The mechanism of binding, inhibition of serum complement activity, and synergy with previously identified *B. burgdorferi* C1 inhibitor, BBK32, for these lipoproteins have all been characterized via multiple biochemical assays and approaches. This discovery furthers our understanding of *B. burgdorferi* immune evasion and the lipoprotein expression library provides a powerful tool for identifying host-pathogen interactions directly on the bacterial surface.

22 Reconstruction of ancient microbial genomes from the human gut

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Studies have indicated that industrialized humans have lost certain gut microbes, and this loss of microbial diversity is associated with chronic diseases. To discover extinct bacterial species, we performed shotgun metagenomic sequencing on 12 human paleofeces aged 2,000 years from Utah and Arizona. This is, to our knowledge, the first unbiased de novo assembly and the largest reconstruction of microbial genomes from paleofeces to date. We reconstructed 398 medium- and high-quality draft genomes, 207 (52.01%) of which are novel species. Phylogenetic analyses showed our novel genomes expanded the bacterial tree of life and formed new phylogenetic branches with large evolutionary distances to presently known bacteria. Additionally, our gene-level analysis supports the hypothesis of extinctions in the modern gut microbiome. At equivalent sequencing depths of 40,000,000 reads, the average number of unique genes in the ancient gut microbiome was 331,648. In contrast, the number for 145 modern stool samples from the Human Microbiome Project was 2.54x lower at 130,437. From metabolic pathway analyses, we found pathways targeted by antibiotics to be lower in abundance in the modern gut, whereas pathways involved in starch utilization were higher in abundance in the modern gut compared to the ancient gut. These are consistent with higher antibiotic usage and starch consumption in modern populations. Our work elucidates the evolutionary history of gut symbionts at the gene, pathway, and genome levels and may lead to discovery of extinct bacteria with the potential to restore human health.

23 Highways of recombination and non-random patterns of donor-recipient relationships in multiple species of bacterial pathogens

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Phenotypic and genetic variation in microbes can take tremendously complex forms even within a single species. An important process that generates this variation is recombination, defined as the re-assortment of DNA between strains of different genomic backgrounds. The rate of recombination of a species is important for estimates of mutation and genomic change, and therefore the capability of a species to respond and adapt to selective pressures. We aim to elucidate the extent in which variation in recombination exists within a microbial species and the factors that drive this variation.

We analyzed the frequency and characteristics of genome-wide recombination in three bacterial species using fastGEAR (Klebsiella pneumoniae, Streptococcus pneumoniae, Yersinia enterocolitica). We also introduce the novel program HERO to detect and visualize the presence of pairs of lineages or strains that exchange DNA more often between them than with others.

We show that heterogeneity and biases in recombination exist among members of the same species, with some pairs of strains linked by recombination highways. Some strains exhibit significantly higher frequencies of DNA donation or receipt, and may vary depending on their gene content. We show that serotype, ecology and geographical proximity can influence these patterns. Hyper-recombinant strains likely act as hubs of gene flow, facilitating the rapid spread of certain genes.

These results demonstrate that recombination in microbial populations and species is a heterogeneous process. Our findings provide valuable insight in developing a coherent model for genome evolution that integrates variation in recombination within and among microbial species.

24 Massively parallel screening of synthetic microbial communities

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Microbial communities have numerous potential applications in biotechnology, agriculture, and medicine. Nevertheless, the limited accuracy with which we can predict interspecies interactions and environmental dependencies hinders efforts to rationally engineer beneficial consortia. Empirical screening is a complementary approach wherein synthetic communities are combinatorially constructed and assayed in high throughput. However, assembling many combinations of microbes is logistically complex and difficult to achieve on a timescale commensurate with microbial growth. Here we introduce the kChip, a droplets-based platform that performs rapid, massively parallel, bottom-up construction and screening of synthetic microbial communities. We first show that the kChip enables phenotypic characterization of microbes across environmental conditions. Next, in a screen of 100,000 multi-species communities comprising up to 19 soil isolates, we identified sets that promote the growth of the model plant symbiont Herbaspirillum frisingense in a manner robust to carbon source variation and the presence of additional species. Broadly, kChip screening can identify multi-species consortia possessing any optically assayable function, including facilitation of biocontrol agents, suppression of pathogens, degradation of recalcitrant substrates, and robustness of these functions to perturbation, with many applications across basic and applied microbial ecology.

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1 Role of RNase J in development of 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. Two recent studies 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. To study the role of RNase J at transcriptomic level, RNAseq 5' end-directed and expression libraries were performed. Our results show that loss of RNase J affects the expression levels of a small number of genes, some of them associated with mycobacterial virulence and pathogenesis. Interestingly, RNase J deletion mutants displayed accumulation of small RNA fragments in some genes, suggesting that RNase J could be involved either in the processing or in the degradation of certain transcripts. Additional experiments to determine the half-life of these fragments revealed that indeed they are more stable in RNase J mutant. These accumulating fragments have strong predicted secondary structure and high G+C content, supporting a model in which RNase J plays a specialized role in degradation of highly structured RNA molecules that are inefficiently degraded by the core RNA degradation machinery.

1. Zhang H et al., 2013, Nature Genetics 45(10), 1255–1260.
2. Hicks et al, 2018, Nat Microbiol, 3(9), 1032-1042.

2 Linking cell physiology to the successional dynamics of particle-associated microbial communities

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Organic particles are a major source of nutrients for heterotrophic marine microbes. Natural communities assemble on particles in successions driven by the enzyme-catalyzed mobilization of polymerized nutrients. We have previously observed that seawater communities assemble with successional dynamics on model marine particles made of polysaccharide hydrogels. The assembly dynamics are highly reproducible, suggesting that deterministic principles underpin community assembly. Although successions can be partly explained in terms of facilitative interactions between polysaccharide degraders and consumers of waste products, the large diversity of degraders that coexist on particles remains puzzling.

We hypothesized that variation in key physiological and behavioral traits among polysaccharide-degrading taxa represent ecological strategies to colonize nutrient patches in a spatially structured environment. To provide initial intuition, we developed a computational model of particle colonization which showed that dispersal rates can enable fast nutrient uptake in structured environments. Moreover, modifying dispersal was sufficient to cause rapid successions among taxa with similar rates of nutrient uptake and growth. We tested these model predictions using synthetic communities made of 2-3 species isolated from natural consortia, for which we measured a suite of phenotypes such as polysaccharide hydrolysis, enzyme secretion, growth rate and affinity for hydrolysis products. Ongoing work suggests that traits do not vary at random, and are constrained by other physiological features of a cell. We hope to use this simple experimental system to predict principles that structure the assembly and function of particle-associated communities.

3 Abscisic Acid-Mediated Signaling is a Nexus of Host Modulation by the *A. tumefaciens* Type VI Secretion System

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In *A. tumefaciens*, as in several other bacterial species, the recently discovered type VI secretion system (T6SS) releases effectors that act as toxins in inter- and intra-bacterial competition. However, the contribution of the *A. tumefaciens* T6SS to virulence is not yet well understood. We discovered that inoculation of *Arabidopsis thaliana* stems with agrobacteria lacking the T6SS resulted in the formation of smaller tumors than infection with wild type bacteria. Ablation of the homologous secretion system in the closely related alpha-proteobacterium *Rhizobium leguminosarum* was previously shown to extend the bacterium's

host range (MPMI 16: 53; 2003). Together, these and other data from our lab led us to postulate that the T6SS promotes bacterial virulence in mature plants by dampening *A. thaliana* defenses, but may elicit host defenses that limit transient transformation of host seedlings. T6SS-dependent differences in host defense-related gene expression and in resistance to subsequent infection by *Pseudomonas syringae* pv. *maculicola* were consistent with this model. Investigations with mutant plant lines provided insights into particular host defense pathways, including the abscisic acid (ABA) hormone pathway, that were modulated by the T6SS. While the T6SS did not appear to alter transcription of the rate-limiting ABA biosynthetic gene, it did impact ABA-regulated gene expression at both early and late time points after infection. Collectively, our data support the hypothesis that ABA signaling is a direct or indirect target of the T6SS and a determinant of mutual host-pathogen tolerance.

4 Exponential decay of viability of *Escherichia coli* in carbon starvation

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While growth of bacteria is well understood and studied, its counterpart the decay of viability in starvation is not. We use the mathematical simplicity of the decay of viability during carbon starvation, a simple exponential function, to uncover how E. coli survives nutrient limitation. We find that viable bacteria feed on the carcasses of perished bacteria. This leads to an interdependence of survival and death which naturally leads to the observed exponential decay of viability. The magnitude of the death rate is set by the maintenance rate and the yield of the biomass recycling. This finding permits quantitative insights into how environments and genes affect bacterial surviva. We exemplified this by a study of the cost of a wasteful enzyme and the benefit of the stress response sigma factor rpoS.

5 Genome Evolution And Recombination in Coagulase-negative Staphylococci Bacteria

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Coagulase-negative *Staphylococcus* (CoNS) are a group of bacteria made up of more than 40 species traditionally considered to be non-pathogenic.1 They were initially defined using diagnostic procedures that aim to differentiate them from the pathogenic *Staphylococcus aureus*. However, many CoNS species now represent major causes of nosocomial infections.2 While these species have been studied morphologically in clinical laboratory experiments, little is known about their evolutionary history. Using 1,285 publicly available genomes from NCBI representing 37 species, we present the phylogenetic relationships and genome characteristics of all known and completely CoNS species. The CoNS group exhibits an open pan-genome with a total of nearly 138,000 genes. The core genomes of the 14 most-represented species contain on average 1,485 genes, with accessory genes totaling as much as 11,326 in one species. We found that recombination within and between species has greatly contributed to the evolutionary history of the group, with a nearly 600,000 recombination events found to have occurred across species, and many more events within species. However, recombination is non-random and even members of the same species do not exhibit uniformity in the frequency and patterns of recombination. For example, a single genome from the *Staphylococcus caprae* has donated to 94% of *Staphylococcus simulans* genomes. Additionally, two *Staphylococcus epidermidis* genomes have donated several hundred sequences to nine other CoNS species. These hyper-donors may represent hubs of gene flow that facilitate the spread of resistance and virulence genes. Our findings shed light on the evolutionary history of a lesser known, but still clinically important group of pathogens and the contributions of recombination to their pathogenicity.

6 Pyoverdine-Dependent Virulence in *P. aeruginosa* Strains Isolated from Cystic Fibrosis Patients

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Pseudomonas aeruginosa is a gram-negative, multidrug-resistant pathogen that frequently causes serious

infections in susceptible populations. Cystic fibrosis patients are particularly burdened by *P. aeruginosa*, as mutations of the cystic fibrosis transmembrane conductance regulator increases the viscosity of the bronchial mucosa, facilitating bacterial colonization within the host. Due to iron starvation that occurs under these conditions, *P. aeruginosa* produces large amounts of the siderophore pyoverdine. In addition to being an effective iron-scavenging molecule, pyoverdine is a key regulator of secreted toxins such as the translational inhibitor exotoxin A. Pyoverdine is critical for full virulence in many mammalian and invertebrate pathogenesis models, including *Caenorhabditis elegans* Liquid Killing.

To further investigate the clinical significance of pyoverdine, we compared the pathogenicity of the highly virulent *P. aeruginosa* PA14 to an isogenic pyoverdine biosynthetic mutant in a murine pneumonia model. Loss of pyoverdine production significantly hampered virulence and limited mortality, demonstrating the importance of pyoverdine in vivo. Furthermore, pyoverdine alone, even in the absence of live pathogen, was sufficient to disrupt iron and mitochondrial homeostasis in *C. elegans*, activating a distinct immune response characteristic of acute iron removal. Based on these findings, we surveyed 69 additional strains of P. aeruginosa *isolated from pediatric cystic fibrosis patients for pyoverdine production and virulence in* C. elegans. We observed a strong correlation between pyoverdine production and host death. Finally, we demonstrated that inhibiting pyoverdine biosynthesis or function was sufficient to ameliorate *P. aeruginosa* infection in two different hosts. Our data demonstrate that pyoverdine is a promising therapeutic target.

7 Investigating a Putative SloR-LivK Partnership in *Streptococcus mutans*

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Streptococcus mutans is an acidogenic member of the human oral microbiota and a primary causative agent of dental caries. This work focuses on the 25kDa SloR metalloregulator, a bifunctional regulator of essential metal ion transport and virulence-associated genes in *S. mutans*. We identified "Class I" genes in *S. mutans* that are subject to SloR repression, and "Class II"-type gene transcription that is enhanced by SloR. Herein, a Bacterial Adenylate Cyclase Two Hybrid approach was coupled with co-immunoprecipitation experiments to identify SloR binding partners that might facilitate Class II-type gene activation. Several potential SloR binding proteins were elucidated, including a 41kDa stress response protein, LivK. Functional similarities between SloR and LivK are consistent with their predicted interaction. LivK is annotated as an ABC-type transporter that contributes to the *S. mutans* acid and stress tolerance responses. Differential *livK* expression in the *S. mutans* UA159 wild-type transcriptome compared with that of its isogenic SloR-deficient mutant indicates that *livK* is SloR-modulated. BACTH experiments are ongoing to confirm SloR-LivK binding in E. coli, as are PCR ligation mutagenesis experiments to generate a LivK insertion-deletion mutant. The *S. mutans livK* mutant will be characterized in 54Mn uptake assays, and in H2O2 and pH challenge experiments to elucidate livK's predicted role in metal ion homeostasis and stress tolerance. An improved understanding of SloR-mediated gene regulation and the binding partnerships that may guide it can inform rational drug design that targets SloR as an anti-caries therapeutic.

8 mRNA degradation rates are coupled to metabolic status in mycobacteria

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Mycobacterium tuberculosis (MTB) is the causative agent of tuberculosis, a significant cause of human mortality. MTB exists in non-growing states during infection, e.g. during carbon starvation and hypoxia stress, and its capacity to enter, maintain and exit from non-growing states is critical for its survival. To better understand the biology of non-growing mycobacteria, we sought to investigate the mechanisms by which mRNA half-life is regulated.

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. Using *M. smegmatis* as a model, we found that mRNA half-life is responsive to energy stress, with carbon starvation and hypoxia causing global mRNA stabilization. Moreover, our findings suggest that mRNA stabilization cannot be explained by the stringent response, mRNA abundance or RNase levels, but that it is linked to metabolic status. Indeed, drugs with opposing effects on cellular energy status led to contrasting mRNA half-lives, which could not be explained by intracellular ATP levels alone. Taken together, our work suggests that the main driver of mRNA stability is energy metabolism rather than growth status.

9 *Gardnerella Vaginalis* Causing Pulmonary Infection in Young Adult: A Novel Case

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Gardnerella vaginalis is an anaerobic, gram-variable bacterium primarily found in vaginal microflora of women. Previous reports of G. vaginalis cultured in men are few and have primarily been limited to the gastrointestinal and genitourinary tract. Few reports of G. vaginalis causing severe infections have been reported in the literature, including septicemia and two cases of perinephric abscess. There has been one previously reported case of G. vaginalis causing pulmonary complications that occurred in a male alcohol abuser. In our case review, we aim to demonstrate an unusual source of a pulmonary infection and highlight the importance of proper microbial isolation to guide treatment. Our patient is a young male who presented following multiple gunshot wounds including one to his head causing an intracranial hemorrhage, hydrocephalus, and a dural sinus thrombosis. His hospital course was complicated by a decline in neurological status treated with a craniotomy and external drain placement and multiple pulmonary infections. During his fever work-ups, he found to have G. vaginalis on mini-bronchoalveolar lavage and was subsequently treated with metronidazole. After treating his G. vaginalis pneumonia and other infectious sources, namely Haemaphilus influenzae and coagulase-negative staphylococcus pneumonias, his fevers and leukocytosis resolved and he was successfully discharged to a rehabilitation facility for neurologic recovery. To our knowledge, this is the second reported case of G. vaginalis isolated from a pulmonary culture and the first in a previously healthy, immunocompetent young male outside of the urinary tract.

10 Core and accessory genome evolution of *Cronobacter sakazakii*, an emerging opportunistic pathogen in neonates

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Cronobacter sakazakii is an emerging opportunistic bacterial pathogenknown to cause neonatal and pediatric infections, including meningitis, necrotizing enterocolitis and bacteremia. Multiple disease outbreaks of *C. sakazakii* have been documented in many countries in the past few decades, yet little is known of its genomic diversity and evolutionary history. We analyzed the pan-genome characteristics and phylogenetic relationships of 297 genomes of C. sakazakii *and related* Cronobacter *species to understand its success as a pathogen. The* C. sakazakii pan-genome contains more than 23,454 orthologous gene clusters, and approximately 14% of these genes constitutes the core genome. A remarkably large part of the accessory genome is composed of 10,460 strain-specific genes. *C. sakazakii* exhibits high gene diversity between strains. We find that the species has diversified into multiple distinct lineages, which can be distinguished in their core genome sequences and accessory gene content. Results show that the diversification of C. sakazakii *has likely been shaped by a history of widespread recombination among members of the species as well as with other species of* Cronobacter. However, the impact of recombination on *C. sakazakii* genomes varies considerably among strains. These findings provide crucial insights into the contributions of inter-strain variation and variable recombination patterns in the evolution and pathogenicity of an emerging pathogen that cause fatal diseases to the most vulnerable members of the human population.

11 Genetic code expansion in *Bacillus subtilis*

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Encoding nonstandard amino acids (nsAAs) into proteins allows for expansion of the genetic code beyond the standard 20 amino acids for probing, labelling, or controlling proteins in a minimally disruptive manner. However, these tools have been mostly unavailable in many bacterial model systems, such as the primary gram-positive model organism, Bacillus subtilis. Here we describe the use of several classes of genome-integrated synthetases to incorporate many different nsAAs into proteins in B. subtilis, including nsAAs used for biorthogonal labelling, fluorescence imaging and photo-crosslinking. We also demonstrate a nsAA-dialable protein expression system in this bacterium. The expression of a target gene can be enhanced 50-fold when nsAAs are added and up to 1000s-fold when combined with a transcriptional inducer. Unlike E. coli nsAA systems, where nsAAs are not incorporated into native UAG codons even before recoding

efforts, B. subtilis nsAA systems incorporate nsAAs into many genomic proteins at native UAG codons. This feature presents both challenges and opportunities for follow-up work in B. subtilis nsAA research. The general and effective expansion of nsAA technology to B. subtilis can facilitate our understanding of cell biology in this bacterium and industrial protein production of nsAA-containing proteins.

12 Elucidating pathogen/commensal dichotomy of *Corynebacterium glucuronolyticum* in the male urogenital system: insights from clinical data and species' genetic virulence signatures

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Corynebacteirum glucuronolyticum (*C. glucuronolyticum*) is a coryneform bacterial species of uncertain pathogenicity; however, it is increasingly being recognized as a potential causative agent of various clinical entities in the male urogenital tract. Our research has shown its proclivity to cause acute symptomatic urethritis in men, but also to resurface as a cause of sustained urethritis in male patients previously treated for *Chlamydia trachomatis* infection. Since the latter phenomenon has to be differentiated from a simple post-treatment overgrowth of a colonizing agent, the term 'uncloaking urethritis' has been introduced to the literature based on this research. We have also shown that the resolution of the infection in the male urogenital system correlates with statistically significant improvement of spermatozoa vitality, but also with lower numbers of neck and mid-piece defects. Conversely, sperm count, spermatozoa motility and other morphological traits were not influenced by the treatment and subsequent microbiological clearance. Regarding its antibiotic susceptibility, our research demonstrated low susceptibility of *C. glucuronolyticum* to tetracycline and clindamycin, and a trend towards ciprofloxacin resistance. When genetic markers are appraised, it is observed how C. glucuronolyticum *harbors* srtA *and* srtC genes absent in many other coryneform bacteria. Such presence of specific sortase/SpaA-type pili gene clusters (alongside specific type VII secretion system and, potentially, the Allee effect) can explain why this species demonstrates colonizing abilities comparable to some other, well-established urogenital pathogens. To firmly corroborate species' pathogenic potential, future approaches will be based on genomic profiling and a wide array of structure-function studies.

13 SloR Degradation by the Clp Protease System and its Role in the *S. mutans* Oxidative Stress Response

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Streptococcus mutans is a member of the human oral microbiota and the primary causative agent of dental caries. This study investigates post-translational control of a 25kDa SloR metalloregulator by a Clp protease complex in *S. mutans*. Previously, we described a role for SloR in repressing the *S. mutans* oxidative stress response, and in de-repressing an SpxA activator of this response in the presence of a H2O2 stressor. Consistent with these findings are the results of qRT-PCR studies that support suppression of *sloR* transcription in the presence of H2O2. Interestingly, Western blots support SloR accumulation in *S. mutans clp* mutants, consistent with SloR depletion by the Clp protease system. Taken together, we propose that SloR is subject to Clp degradation under conditions of oxidative stress, and that a direct SloR-Clp interaction mediates de-repression of the *S. mutans* oxidative stress response. We performed Western blots that reveal decreasing SloR concentrations after 30 minutes of *S. mutans* exposure to H2O2, and increasing ClpX levels after only 15 minutes of exposure to the stressor. These observations implicate the ClpX ATPase in SloR degradation. A bacterial adenylate cyclase two hybrid (BACTH) approach further supports SloR-ClpX binding, as do the results of coimmunoprecipitation studies that reveal up to six SloR binding partners in the anti-SloR pulldown, including SloR. In summary, the *S. mutans* SloR metalloregulator is subject to post-translational Clp-mediated degradation that involves a direct interaction between SloR and the ClpX ATPase.

14 All-*trans*-retinoic acid augments macrophage control and survival 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. Meanwhile, disappointments in vaccine and drug development have highlighted an expansive gap in our understanding of host control of tuberculosis infection. The role of vitamin A in immunity to tuberculosis was recently affirmed by epidemiological association. This builds on a body of literature suggesting a host-directed antimicrobial effect of the transcriptionally active metabolite of vitamin A, all-*trans*-retinoic acid (ATRA). We found that ATRA is effective in increasing bacterial control during *Mycobacterium tuberculosis* infection of human primary monocyte-derived macrophages, especially when compared to a wide array of other immune modulators. This augmented control is independent of a direct antibacterial effect, and is characterized by reduced host macrophage death. Other compounds targeting the ATRA receptor have a range of impacts on macrophage control of *M. tuberculosis* infection. This supports a chemical genetic approach that can be paired with bacterial gene essentiality analysis to elucidate mechanisms of ATRA-mediated cellular immunity against bacterial infection.

15 Role of Non-Canonical Mutations in *Mycobacterium tuberculosis* Altered Drug Susceptibility

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Mycobacterium tuberculosis, the causative agent of tuberculosis, remains a global health issue, with an estimated one third of the world's population latently infected with the bacterium. With rising drug resistance in tuberculosis, understanding the determinants of altered drug susceptibility is of paramount importance. To identify novel mechanisms of drug susceptibility, we analyzed a set of 710 clinical isolates of M. tuberculosis, including both drug resistant and drug sensitive strains. We identified polymorphic genes that are likely to be indicative of functional mutations causing a change in drug susceptibility. Included in this set are some genes already known to be associated with drug resistance including rpoB and gyrA. A previous genome wide association study (GWAS) using these strains also highlighted mutations in prpR, a transcriptional regulator, which we later found to play a role in drug tolerance phenotypes. Additional genes of interest, such as Rv0010c and Rv3260c (whiB2), were also found to be highly polymorphic. Interestingly, whiB2, a transcriptional regulator, has previously been identified as being associated with drug resistance, specifically in the case of ethionamide and isoniazid. Here, I aim to understand the importance of these mutations in the susceptibility to different drug treatments. Further, I also use a library of prpR mutants to investigate the effect of these mutations in the context of several new drugs being administered for tuberculosis treatment.

16 Characterizing the molecular mechanisms for how strain identity regulates social swarming behavior in the bacterium *Proteus mirabilis*

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Microbes use cell-cell communication to coordinate virulence-associated population behaviors. I am interested in how individual cells integrate these signals to produce emergent population behaviors. The opportunistic pathogen Proteus mirabilis exhibits collective swarm motility, which is associated with human disease but also with macroscale surface migration. During swarm expansion of P. mirabilis, identity information is exchanged between adjacent cells. Identity information regulates the extent of swarm expansion. Remaining unknown is which intermediate subcellular pathway(s) might connect the received identity signal to a resulting change in the emergent macroscopic behavior. To identify potential factors, I looked for the spontaneous emergence of mutant strains that bypass identity-based swarm regulation. Over twelve independent experiments, I isolated seven mutant strains with disruptions in a predicted serine transporter. This transporter is well-conserved across gammaproteobacteria and has not yet been studied in P. mirabilis. We have since confirmed that this transporter is required for identity-based regulation by constructing a clean deletion mutant strain for behavioral and physiological assays. We have subsequently shown that

serine metabolism is also a regulator of swarm expansion. We propose that intercellular identity signaling taps into central metabolism, and through this, is able to regulate collective swarm expansion. Central metabolism may act as a conserved integrator of intercellular signals in the regulation of other collective virulence-associated behaviors.

17 Identification of an essential mycobacterial polar growth factor involved in LM/LAM transport

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Mycobacteria grow and divide differently compared to well-studied model bacterial organisms. For example, instead of inserting new cell wall material along their side walls, they insert material at their poles. In addition, they do not encode obvious homologs to well-conserved cell growth and division proteins. Moreover, while mycobacteria are classified as Gram positive, their cell envelope structure differs dramatically from other organisms in this taxon, as they possess an outer-membrane rich in long-chain fatty acids. A better understanding of the unique factors that synthesize this unusual structure could lead to therapeutics that target pathogenic mycobacteria, like Mycobacterium tuberculosis. Here, we identify and characterize an essential gene of previously unknown function predicted to be associated with mycobacterial polar growth factors. We establish that ms0317 is essential: its transcriptional depletion leads to loss of polar growth, disruption of the outer membrane, and cell death. Surprisingly, we find that depletion of ms0317 leads to accumulation of LM/LAM in the periplasm. LM/LAM are large glycolipids abundant in cell envelope, which have been extensively studied in relation to infection, but their role in bacterial physiology is not well-understood. Taken together our data suggest that MS-0317 is involved in the transport of LM/LAM to the surface of mycobacteria, and that correct localization of LM/LAM is important for polar growth and the structural integrity of the mycobacterial cell envelope.

18 Rv0500A is a novel transcriptional regulator of *Mycobacterium tuberculosis* response to ionic cues

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Successful host colonization by *Mycobacterium tuberculosis* (Mtb) requires that the bacteria sense and respond to myriad environmental signals present in the heterogeneous milieu *in vivo*, which include ionic cues such as pH and chloride (Cl-). While adaptation to local environmental cues is a critical aspect of Mtb infection, much remains unknown about how it integrates these disparate signals into a coordinated response. Through a transposon-based screen utilizing our unique fluorescent pH and Cl–responsive reporter Mtb strain, we have identified Rv0500A as a novel master transcriptional regulator of Mtb response to the ionic cues of pH, Cl-, and potassium (K+). Inactivation of Rv0500A enhances the response of Mtb to acidic pH, high [Cl-], and low [K+], suggesting its function as a repressor. Electrophoretic mobility shift assays with purified recombinant Rv0500A demonstrate that it binds directly to the promoter of *rv2390c*, a gene whose expression is specifically upregulated during Mtb exposure to acidic pH and high [Cl-]. Using an inducible *rv0500A* over-expression Mtb strain, we find that over-expression of *rv0500A* adversely affects bacterial growth characteristics, with filamentation and a decrease in the bacterium's ability to respond to pH and Cl-, both in broth and during macrophage infection. Attenuation of host colonization upon overexpression of *rv0500A* is further observed in a murine infection model. Ongoing work is focused on understanding the global transcriptional role of Rv0500A, and on combining our fluorescent Mtb reporters with modulation of Rv0500A levels to delineate the relationships between bacterial environmental response and heterogeneity in infection outcome.

19 Withdrawn

20 Oral immunization with a probiotic cholera vaccine induces broad protective immunity against *Vibrio cholerae* colonization and disease in mice

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Oral cholera vaccines (OCVs) are being increasingly employed, but current killed formulations generally require multiple doses and lack efficacy in young children. We recently developed a new live-attenuated OCV candidate (HaitiV) derived from a *Vibrio cholerae* strain isolated during the 2010 Haiti cholera epidemic. HaitiV exhibited an unexpected probiotic-like activity in infant rabbits, preventing intestinal colonization and disease by wild-type V. cholerae before the onset of adaptive immunity. However, it remained unknown whether HaitiV would behave similarly to other OCVs to stimulate adaptive immunity against *V. cholerae*. Here, we orally immunized adult germ-free female mice to test HaitiV's immunogenicity. HaitiV safely and stably colonized vaccinated mice and induced known adaptive immune correlates of cholera protection within 14 days of administration. Pups born to immunized mice were protected against lethal challenges of both homologous and heterologous *V. cholerae* strains. Cross-fostering experiments revealed that protection was not dependent on vaccine colonization in or transmission to the pups. These findings demonstrate the protective immunogenicity of HaitiV and support its development as a new tool for limiting cholera.

21 Genetic interruption of *Chlamydia trachomatis* gene CT166 using group II intron technology

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Chlamydia trachomatis is a sexually-transmitted, obligate, intracellular bacterial pathogen that is classified into multiple serovars. When infections with C. trachomatis serovar D (Ct-D) in the female genital tract are left untreated, they can cause immunopathology that drives severe disease sequelae including pelvic inflammatory disease and ectopic pregnancy. We and others have previously shown that Ct-D causes more cytotoxicity in cell culture and more immunopathology in mouse models of genital tract infection when compared to an alternative serovar, L2 (Ct-L2). We hypothesize that Ct-D expresses virulence factors that are not present in the Ct-L2 genome, contributing to the increased virulence associated with infection. Through sequence analysis, we identified putative virulence factors found in the Ct-D genome, which are lacking in the Ct-L2 genome. One of these putative virulence factors, CT166, has significant amino acid sequence homology to the Clostridium difficile cytotoxins TcdA/B, with both the suspected Ct-D and Clostridial cytotoxins sharing a glucotransferase motif. We are in the process of creating targeted mutants in CT166 using the TargeTron^{TM} Gene Knockout system. The TargeTron^{TM} system, which employs a group II intron to interrupt gene expression, has been shown to be one of the few methods for producing targeted genetic mutations in Chlamydia. We are modifying this method for use in Ct-D with the goal of producing stable mutants that lack CT166 expression. These mutants will be used in ongoing work to test whether CT166 is necessary to drive Ct-D's observed cytotoxicity in cell culture and immunopathology in mouse models of infection.

22 Conserved interacting components of the conjugation machinery of *Bacillus subtilis*

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Conjugation, or mating, is the transfer of DNA from one bacterial cell to another via specialized DNA translocation channels classified as Type IV secretion systems (T4SSs). T4SSs are versatile multi-protein complexes that span the bacterial cell envelope. They play a fundamental role in horizontal gene transfer, contributing to bacterial genetic diversity, metabolism, symbiosis, and the spread of antibiotic resistance. Our goal is to gain a greater understanding of the conjugation machinery of Gram-positive bacteria using the integrative and conjugative element ICE*Bs1* of *Bacillus subtilis* as a model system. We have investigated two conserved components of the ICE*Bs1* T4SS multi-protein complex, the ConE ATPase and the transmembrane protein ConB. Here, we show that ConB and ConE oligomerize and interact with each other,

and that the interaction is functionally important. Crosslinking studies and Blue Native PAGE analysis of purified ConE and ConB revealed the formation of oligomers *in vitro*. Bacterial two-hybrid analysis reveals that the two proteins interact with both themselves and each other *in vivo*. Finally, we have discovered that both ConB and ConE localize to the membrane, predominantly at the cell poles. While the single-pass membrane protein ConB localizes properly in the absence of other T4SS components, the peripheral membrane protein ConE's localization is dependent on the presence of ConB. Thus, ConE is likely recruited or maintained at the membrane through its interaction with ConB. Altogether, our results provide new insights into the interactions of these conserved components of the DNA translocation channel found in Gram-positive bacteria.

23 Z ring assembly is regulated by FtsZ filament binding proteins

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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 constricts as the cell divides, through a mechanism which remains poorly understood. Recently, we have shown that FtsZ filaments treadmill around the division site, and that these dynamics are of key significance in bacterial cell division.

Now, we investigate whether and how FtsZ filament assembly and treadmilling dynamics are regulated. Of particular interest are the FtsZ binding proteins, which are known to bind directly to FtsZ at the division site, and which have been proposed to regulate both FtsZ dynamics and bundling in vitro. We ask whether and how FtsZ binding proteins control filament structure and/or dynamics during the bacterial cell cycle. To investigate this, we use live-cell imaging to characterize the morphology of individual FtsZ filaments and of the Z ring, and to measure the lifetimes of single FtsZ monomers as a precise quantitative reporter of FtsZ's treadmilling dynamics. Surprisingly, we find that the FtsZ binding proteins do not regulate FtsZ filament kinetics directly, but rather mediate Z ring assembly through filament bundling. Functional regulation of filaments by bundling is a common feature of eukaryotic cytoskeletal systems, but has rarely been seen in bacteria. We propose that this regulated FtsZ filament bundling is a prerequisite for normal Z ring formation and cytokinesis.

24 Potassium response and homeostasis in *Mycobacterium tuberculosis* modulates environmental adaptation and is important for host colonization

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Sensing and response to the immediate environment is required for bacteria to adapt and successfully colonize its host. We had previously discovered that *Mycobacterium tuberculosis* (Mtb) responds to pH and chloride (Cl-) as synergistic cues, suggesting that abundant ions may serve as important environmental signals during host colonization by Mtb. Potassium (K+) is the most abundant intracellular cation in both mammalian and bacterial cells, and we now show that Mtb has a unique transcriptional response to [K+], with both distinct and shared regulatory factors controlling the bacterial response to K+, Cl-, and pH. We find that unlike in lysosomes, K+ accumulates within the maturing macrophage phagosome. Using a K+-responsive Mtb reporter, we further demonstrate that the bacteria-containing macrophage phagosomes are not K+-limiting. Disruption of bacterial K+ homeostasis by deletion of the Mtb Trk K+ uptake system dampens the bacterial responses to pH and Cl-, independent of effects on maintenance of intrabacterial pH and membrane potential. In accord with this disruption in environmental response, the Mtb Trk K+ uptake system mutant is attenuated in host colonization, both in primary bone marrow-derived macrophages and in vivo in a murine model. Our findings reveal the close relationships between bacterial ionic homeostasis and environmental ionic response, and highlight the role that abundant ions can play during host colonization. We are continuing to expand our suite of fluorescent reporter Mtb strains to interrogate how local ionic response by Mtb influences heterogeneity in infection and treatment outcome.

25 Restriction-modification system and horizontal gene transfer

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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 (HGT). 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-modification systems involved in protection against exogenous DNA entrance. In addition, this region was suggested to be involved in site-specific HGT.

We developed a conjugal transfer system of chromosomal DNA to characterize basal and enzyme-stimulated RecA-independent gene transfer of the ICR between laboratory descendants of one natural isolate of E. coli and Salmonella enterica sv Typhimurium LT2 descendants harbouring various restriction-modification phenotype. As part of the groundwork, we determined the sequences of an isolate of the model organism S. typhimurium LT2 and of the multiply restriction-deficient hybrid strain often used for molecular genetic constructions (LB5000). Comparison of the LB5000 genes that determine the restriction activities SenLT2I (LT, StyLT in the early literature), SenLT2II (SA, StySA) and SenLT2III (SB, StySB) with those of LT2 permitted us to identify the mutations that potentially result in restriction-deficiency in the hybrid. We could especially highlight and study the BREX-like architecture of the StySA system.

26 Probing the molecular mechanism of ProQ-RNA 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 that is often facilitated by RNA-chaperone proteins. ProQ has recently been identified as a global RNA-binding protein that binds to dozens of sRNAs and hundreds of mRNAs in multiple proteobacteria; this observation has 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 regulatory RNAs, 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 genetically detect ProQ-RNA interactions. In the B3H assay, ProQ is fused to RNA polymerase (RNAP) and a hybrid RNA of interest is tethered upstream of a test promoter. Interaction of ProQ with the RNA stabilizes the binding of RNAP to the promoter and activates reporter-gene transcription. We have detected B3H interactions of ProQ with several of its RNA partners and identified the conserved N-terminal-domain (NTD) as the primary site for RNA binding. Further, we have identified point mutations in ProQ that disrupt its RNA interaction without altering ProQ expression levels. Current efforts are focused on screening for additional ProQ point mutations to locate the binding interface(s) for the sRNAs and mRNAs with which it interacts as well as exploring the structure and sequences of RNA that are required for ProQ interaction.

27 Characterizing Hfq-RNA interactions using a bacterial three-hybrid assay

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Non-coding small RNAs (sRNAs) contribute to bacterial biofilm formation, host interactions, and virulence, but there is much we still do not understand about their molecular mechanisms. Hfq is the best-studied bacterial RNA chaperone protein and has become the paradigmatic example for how RNA-binding proteins facilitate mRNA regulation by sRNAs. Here we use a recently developed bacterial three-hybrid (B3H) assay to explore the mechanisms of Hfq interactions with its RNA substrates. Our results suggest that differences in charge of non-conserved residues of Hfq result in species-specific binding preferences for different sRNAs. This finding suggests a model where RNAs have distinct mechanisms for Hfq binding that take advantage of the electrostatic character of rim-surface residues. We also demonstrate the potential of this assay to detect Hfq's interactions with its mRNA targets in addition to its capacity to detect Hfq-sRNA interactions, and show data suggesting that B3H results correlate with in vitro binding affinities. Using this assay, we hope to establish foundational knowledge of the mechanisms by which Hfq regulates its RNA targets.

28 Achieving species-level resolution from 16S rRNA gene short-read data using a high-resolution training set with the RDP naïve Bayesian Classifier

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Microbiome studies must achieve species-level resolution for clinical relevance, since both harmless and pathogenic species of the same genus are often native to a body site. Moreover, there is a need for large-scale molecular epidemiological studies of the microbiota of thousands of humans to gain clinically useful insights. The cost of which is feasible with 16S rRNA gene-based short-read sequencing. Therefore, we developed a microbiota analysis pipeline that achieves species-level resolution from 16S rRNA gene short-read sequences. We focused on bacterial microbiota of the human aerodigestive tract (nasal passages, sinuses, throat, esophagus, and mouth) because it has the potential to reveal new insights for promoting human health. We first overcame technical limitations and successfully Illumina sequenced the 16S rRNA gene V1-V3 region, the most informative for classifying bacteria native to the human aerodigestive tract. We parsed sequences into high-resolution Amplicon Sequence Variants (ASVs) using Minimum Entropy Decomposition (MED) or Divisive Amplicon Denoising Algorithm (DADA2). To accomplish accurate and optimally informative taxonomic assignment to these ASVs, we generated a high-resolution V1-V3 region training set from our actively curated, and comprehensive, expanded Human Oral Microbiome Database (eHOMD) for use with the Ribosomal Database Project naïve Bayesian Classifier. We also generated a full-length eHOMD 16S rRNA gene training set to analyze PacBio-sequenced data, which we used to validate the representation of species in our training sets. Our approach facilitates species/supraspecies taxonomic assignment to ASVs derived from both short-read and full-length 16S rRNA gene sequences, enhancing the utility of 16S rRNA gene sequencing.

29 Investigation of a *Chlamydia trachomatis* virus-like particle (VLP) vaccine candidate

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Chlamydia trachomatis (Ct) is an obligate intracellular pathogen and so relies on infection of epithelial cells to proliferate. The infectious form of Ct's biphasic life cycle, called an elementary body (EB), uses a type III secretion system (T3SS) to facilitate infection. We hypothesize that a vaccine targeting the T3SS protein Ct584 will block Ct infection in the female genital tract. To test this, we designed vaccines against two predicted surface epitopes of Ct584: residues 70-77 and 154-164. These epitopes were used to create 3 virus-like particle (VLP) vaccines, two constructed with residues 70-77 or 154-164, and a third with a mixture of the two, each conjugated to a qbeta capsid. A negative control vaccine consisted of a bare qbeta capsid. Vaccines were administered intramuscularly in female C57Bl6 mice 3 times at 3 week intervals, followed by transcervical Ct challenge. Sera was collected post immunization and pre challenge to measure antibody responses with ELISA. Bacterial burden was measured in the uterus 3 days post challenge. Two independent vaccine trials, n=5/vaccine and n=20/vaccine, were performed. We found a significant decrease in Ct burden between vaccine and control groups in the first but not the second trial. Both trials elicited high titer serum antibody to Ct584 epitopes. These results suggest that Ct584 VLPs are capable of stimulating robust antibody production but do not provide reproducible defense against Ct infection in the uterus. Future studies may investigate the protective effect of other Ct584 epitopes.

30 Investigating the structure and function of a novel bacterial anti-virulence factor

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Francisella tularensis is a highly infectious intracellular human pathogen. How *F. tularensis* regulates expression of genes important for replication in macrophage, a key host niche, is still incompletely understood.

One transcription factor critical for virulence is the response regulator PmrA. This transcription factor is necessary for virulence primarily because it functions to repress expression of PriM (PmrA-repressed-inhibitor of intramacrophage growth). Although how PriM functions to inhibit virulence is still unknown, we have defined the crystal structure of the PriM protein and identified several structural elements that may contribute to its function. We have generated cells that produce PriM with distinct mutations to purposefully disrupt these structural elements. To determine how the structure of PriM contributes to its function, we are assessing the ability of these cells to survive within macrophage. Our goal is to understand how the anti-virulence factor PriM functions at the molecular level; bacterially-encoded anti-virulence pathways may be targets for future therapeutics.

31 The Role of Flagella in Attachment of *Salmonella enterica* on Red Leaf Lettuce

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Salmonella enterica is a Gram-negative bacterium that is the causative agent of Salmonellosis, the leading cause of hospitalizations due to foodborne illnesses in the United States. The objective of this research was to determine the role of flagellar components in attachment, colonization, and persistence of *S. enterica* on red leaf lettuce. Flagellar genes *fliB* (flagellin, phase 1), *fliC* (flagellin, phase 2), *flgK* (flagellar hook gene), and *fljB* (flagellin methyltransferase) were targeted for replacement with antibiotic resistance cassettes in several *S. enterica* serovars using lambda Red homologous recombineering. By deleting the *flgK* gene, *S. enterica* will no longer assemble a flagellar filament. We hypothesize that this will impair the ability of these strains to attach to red leaf lettuce. Antibiotic resistance cassettes were successfully amplified for all genes and transformed into *S. enterica* serovars by way of electroporation. Potential mutants were screened by PCR amplification of the target DNA region. To date, deletion mutations in *fliB*, and *flgK* have been obtained for *S.* Typhimurium. Phenotypic assays are underway in order to determine the effect of gene deletion on swimming and swarming motility. In order to test the effects of these mutations on interactions with red leaf lettuce, wild-type and mutant cells will be spotted onto lettuce, and the levels of attachment, colonization, and persistence will be determined. By understanding the role of the flagellum components in plant-bacterium interactions, interventions can be developed to interfere with these interactions and subsequently decrease the frequency of Salmonellosis cases worldwide.

32 Motility can drive spatial exclusion and promote coexistence in bacterial populations

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Bacterial cells have the ability to explore their surroundings by swimming and get a sense if life is getting better or worse[1]. They can form actively swimming (i.e. motile) communities and compete for space and nutrients with cohabitant microbial populations. Despite the ubiquity of motile phenotypes, the role of active swimming in bacterial coexistence has remained unrecognized to date. In this study, we investigated the motility-driven competition for resources between two strains resulting in strong negative frequency-dependent-selection, i.e. each strain becomes fitter than the other when low in frequency. The observed lack of competitive exclusion is a direct consequence of the active segregation: few fast movers can forage and rapidly colonize in virgin territories of the habitat whereas few fast-grower cells can proliferate at the initial contact position. Our findings revealed a fundamental mechanism shaping bacterial communities: motility and growth rate differences alone can drive coexistence and promote bacterial diversity. The coexistence emerging from a motility-growth trade-off provides an explanation for the variety of bacterial motility phenotypes found in nature and raises new questions on the ecological role of motility in the formation of diverse bacterial communities.

[1]Berg, H.C., 2008. E. coli in Motion.

33 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 competition and niche overlap. We are also exploring how environmental factors such as pH, temperature, nutrient availability, succession, and spatial heterogeneity impact community composition. Thus far, our data suggests that shifts in environmental conditions (e.g., pH) has a significant effect on species interactions and community assembly.

34 Investigating the Role of DNA Methylation in *Mycobacterium smegmatis*

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Mycobacterium tuberculosis is a pathogenic bacterium of great medical relevance, causing disease in millions of people annually and living dormant in many more. During infection, *M. tuberculosis* is exposed to harsh environmental conditions that require it to utilize adaptive mechanisms to survive. One such mechanism is DNA methylation. In *M. tuberculosis*, DNA methylation has been observed to influence gene expression and fitness in hypoxia. To further understand the role of DNA methylation in mycobacteria, we knocked down the expression of the methyltransferase MamA in *Mycobacterium smegmatis*. Expression of *mamA* was repressed using the CRISPR interference knockdown system, which utilizes a modified version of CRISPR-Cas9 to block transcription of *mamA*. The cells with reduced expression of *mamA* demonstrated growth cessation, elongated cell bodies, reduced distribution of DNA, and abnormal septation within cells. These results suggest that MamA could play a role in DNA replication and/or division. Further investigation into the state and localization of M. smegmatis' *DNA during depletion of* mamA may provide more information on how MamA influences cell growth and division.

35 Visualizing CD4+ T cell response to *Chlamydia trachomatis*

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Given the importance of CD4+ T cells in clearing *Chlamydia trachomatis* (Ct) infection, we have begun to utilize two-photon microscopy to visualize antigen specific CD4+ T cells in the murine female genital tract. Two-photon microscopy allows us to image the entire infected genital tract, eliminating the need for traditional tissue sectioning. Using fluorescent Ct specific CD4+ transgenic T cells (NR1 T cells) that recognize the Ct protein Cta1, we found that NR1 T cells cluster in different areas of the genital tract. These clusters correlate with levels of Ct, confirming that NR1 T cells home to sites within the genital tract that contain antigen. We also find that endogenous CD4+ and CD8+ T cells localize to the same sites that contain Ct, and that these sections correlate with higher levels of the cytokine interferon-gamma (IFNg). We have discovered that IFNg production by NR1 T cells is not required for localization to sites in the genital tract containing Ct. However, IFNg production by NR1 T cells is necessary to protect mice against infection. Ongoing experiments include using live imaging to track NR1 T cells *in vivo* and visualizing the interactions between dendritic cells and NR1 T cells in the genital tract. These experiments demonstrate the power of two-photon microscopy to study the T cell response to *Chlamydia* infection *in vivo* and our results will further define how T cells home to barrier sites and respond to a mucosal pathogen.

36 Broadening 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 (Multiplex 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 both improving the efficiency of recombineering (specifically: oligo-mediated genomic editing) and for easily moving recombineering technology into new host organisms. We demonstrate the efficacy of the method by improving editing efficiency in E. coli, Lactococcus lactis, and Mycobacterium smegmatis. In so doing, we gained insight into the interactions that are important between SSAPs, which mediate recombineering at the replication fork, and bacterial host proteins. We then use this knowledge to lay out a strategy for quickly maximizing recombineering efficiency in a novel bacterium, and we demonstrate this capability by developing efficient editing in Agrobacterium tumefaciens and Pseudomonas aeruginosa, two organisms for which recombineering has not yet been reported. Widely-available oligo-mediated genetic editing promises to radically accelerate our study of microbiology.

37 Navigating Complex Environments With A Dual Flagellar Architecture

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Most chemotaxis work has focused on *E. coli* in liquid media, and much less attention has been devoted to species with different flagellar architectures or environments that more closely mimic the complexity of natural habitats. Here, we address how pathogens with a dual flagellar architecture, consisting of a single polar flagellum and conditionally expressed lateral flagella, navigate complex environments.

Using our new chemotaxis assay combining a recent high-throughput 3D tracking method (Taute et al., Nat. Commun. 2015) with microfluidically created chemical gradients, we quantify chemotactic performance and identify the underlying behavioral mechanisms in flagellar mutants of pathogen *Vibrio alginolyticus* navigating liquid environments as well as viscous media or agar hydrogels.

We show that lateral flagella expressed in addition to the polar flagellum lower the chemotactic drift speed in liquid environments but increase it in dense hydrogels and certain viscous media. Trajectory analysis reveals distinct underlying mechanisms: in liquid medium, lateral flagella lower the swimming speed (Kawagishi et al., J. Bact. 1995), whereas it increases it at higher viscosity in certain polymer media. By contrast, inside hydrogels, where swimming is interrupted by extended "trapping" events (Wolfe and Berg, *PNAS* 1989), lateral flagella maintain a similar average swimming speed, but achieve a higher chemotactic drift speed by decreasing the trapping probability.

Our findings expand the significance of lateral flagella beyond their long-recognized role in swarming (McCarter et al., Cell 1988) to motility in a large range of complex environments and showcase the power of high-throughput 3D tracking for unraveling chemotactic mechanisms.

38 Bacteria: Beauty, not the Beast

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Microbes were the first living things to inhabit this planet and have shaped every aspect of our ecosystems for the past 4 billion years. They represent one of the largest biomasses, the most biodiversity and are essential for recycling key elements of our planet and for human health. They are everywhere and all over our bodies and yet are invisible to our eyes. We neither see them nor notice them. Usually, we interact with them only in the context of disease and rot. This imbalanced and misinformed relationship that humans have with their own microbial community causes fear and disgust. Our core mission is to change the relationship humans have with microbes by engaging the public with bacterial art. Using the universal language of art, we would like to: 1) bring the invisible to the visible, 2) familiarize the public with the beauty of bacteria, and 3) engage the public, providing a deeper understanding and awareness of the microbial communities in and around us. Here, we describe a bacterial art workshop we conducted at Suffolk University to teach undergraduate science majors about the beauty of bacteria. We show the general method and student artistic pieces.

39 The nasal commensal *Dolosigranulum pigrum* exhibits stable synteny and highly similar strain circulation over a 20-year span

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The harmless Gram-positive bacterium *Dolosigranulum pigrum* has emerged in multiple systems-level studies of human nasal microbiota as positively associated with health. For example, *D. pigrum*, often in conjunction with *Corynebacterium* species, is negatively associated with the pathogen *Streptococcus pneumonia* in the nasal microbiota of young children. Whereas, in adult nostril microbiota, D. pigrum *is negatively associated with the pathogen* Staphylococcus aureus*. Being free of nasal colonization by these pathogens decreases the risk of invasive infection by each at distant body sites. Together* D. pigrum's harmlessness and negative associations with pathogen colonization make it a candidate for future therapeutic investigation. Here, we performed a comparative genomic analysis of 28 distinct *D. pigrum* strains collected across 20 years. Phylogenetic analysis showed that one third of the terminal clades contain strains collected 20 years apart indicating that highly similar strains have circulated in the population across this time. Mauve alignment of 19 closed genomes representing two thirds of the terminal clades revealed highly conserved chromosomal synteny. With 28 strain genomes, the *D. pigrum* core genome is approaching a plateau of 1102 coding sequences (CDS) while the pangenome remains open with 3700 CDS. Compared to the core genome, the *D. pigrum* accessory genome exhibited functional enrichment for gene clusters involved in carbohydrate transport and metabolism and defense against mobile genetic elements. Our results highlight the stable circulation of highly similar strains across 20 years and a high degree of chromosomal synteny across the phylogeny of 28 strains of the health-associated nasal bacterium *D. pigrum*.

40 *Staphylococcus aureus* extracellular vesicles: more than just a novel secretory pathway

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Release of extracellular vesicles (EVs) represents a novel secretory pathway for microbes to secret and transport virulence determinants into host cells. Staphylococcus aureus secretes EVs that package multiple bacterial products, including cytosolic, surface, and membrane proteins, polysaccharides, and secreted pore-forming toxins. Many of these components have been shown to play roles in bacterial virulence. However, the biogenesis of EVs and their downstream effects on host cells are poorly characterized. Staphylococcal alpha-type phenol-soluble modulins promote EV biogenesis by disrupting the bacterial membrane, whereas peptidoglycan cross linking and autolysin activity modulate EV production by altering the permeability of the cell wall. S. aureus EVs are cytolytic for a variety of mammalian cells, and they trigger NLRP3 inflammasome activation in human macrophages, inducing maturation and secretion of proinflammatory cytokines interleukin-1 β(IL-1β) and interleukin-18 (IL-18). EV-associated cytolytic toxins are critical for NLRP3 inflammasome activation but not for TLR2 signaling. EV-associated lipoproteins not only signal TLR2 to initiate the priming step of NLRP3 inflammasome activation but also modulate EV biogenesis and the toxin content of S. aureus EVs. We show that EVs purified from a S. aureus mutant that is genetically engineered to express detoxified cytolysins are immunogenic in mice, elicit cytolysin-neutralizing antibodies, and protect the animals in a lethal sepsis model. Our studies lend a better understanding of S. aureus EV biogenesis, provide novel insights into the role of EVs in staphylococcal pathogenesis, and highlight the usefulness of EVs as a vaccine platform against S. aureus infections.

41 Polysaccharide chain length modulates mycobacterial cell stiffness and periplasm size

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Bacterial polysaccharides are critical for numerous processes, including immune evasion, virulence, and physiology. Polysaccharide chain length can influence immunomodulation; however, its impact on bacterial physiology is unclear. We probed the importance of the length of the mycobacterial galactan, a linear polysaccharide of about 30 residues. The galactan covalently bridges cell envelope layers with the outermost cell-wall linkage point occurring at residue twelve. We found that reducing galactan chain length compromises fitness and alters cell morphology. Polysaccharide truncation increased hydrophobic antibiotic permeability and altered cell shape. Systematic variation of galactan chain length revealed it is a determinant of periplasm size. Our findings reveal that cell envelope glycan chain length can directly impact cellular physiology and show that mycobacterial glycans, and not proteins, influence periplasm size.

42 High-content analysis of protein localization in *Mycobacterium smegmatis*

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Mycobacteria, a genus of pathogenic and environmental microbes, are defined by asymmetric growth and an unusually thick, waxy cell wall. These characteristics have been shown to play central roles in mycobacteria's ability to withstand drug exposure and cause human disease. As such, it is necessary to investigate the molecular determinants underlying the formation and maintenance of the cell wall and its asymmetrical kinetics. Here, we used a library of over 1000 fluorescently tagged highly conserved proteins to examine protein localization in Mycobacterium smegmatis (Msm), a prototypical mycobacterial strain. We applied automated wide-field microscopy techniques to generate a large dataset of still images containing nearly 800 original characterizations of mycobacterial protein localization patterns. Using a customized image analysis pipeline, we captured cell size and protein distribution of a single cell across a population. We found that proteins with known localization could be easily identified. In addition, we discovered several new patterns of protein localization. Moreover, combining cell length with changes in localization allows us to simulate re-localization during the cell cycle. Using this, we are reconstructing temporal re-localization using a single time point with fixed cells.

43 Determining the *Streptococcus pneumoniae* Essentialome and Virulome at a Pan-Genome Wide Scale

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Streptococcus pneumoniae is frequently carried in the human nasopharynx, but it can trigger severe disease when it disseminates to the lungs or blood. Prevention and infection control of *S. pneumoniae* is achieved by vaccines and antibiotic treatment. However, strains not included in current vaccines are becoming more prevalent, along with an increase in antibiotic resistant infections as well, creating a need for new antimicrobial strategies. An essential gene is indispensable for bacterial propagation, and thus a drug inactivating its function will stop growth. This is why the identification of a pathogen's essentialome (i.e. the set of essential genes in a defined genome or group of genomes) is an attractive approach for the identification of new drug targets. In addition to genes essential for general growth, the genes required for colonization and infection (i.e. the virulome) are also attractive targets for vaccine or drug therapies. We have used genomics approaches including Tn-Seq to collect a robust and high-confidence functional data set that describes essential and infection required genes in *S. pneumoniae* on a pan-genome wide scale. Considerable limitations are imposed by the genetic background of a strain; meaning that some genes are not essential or required for virulence in every strain. Thus, we considered in detail the genetics of the bacterium in the context of its pan-genome. By integrating our results in functional networks, we have been able to identify several processes that could eventually be used as scaffolds for the design of novel antibacterial therapies effective at the species-wide level.

44 A *Shigella* type 3 effector protein co-opts host inositol pyrophosphates for activity

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Shigella spp. cause diarrhea by invading human intestinal epithelial cells. Effector proteins delivered into target host cells by the *Shigella* type 3 secretion system modulate host signaling pathways and processes in a manner that promotes infection. The effector OspB activates mTOR, the central cellular regulator of growth and metabolism, and potentiates mTOR inhibition by rapamycin. The net effect of OspB on cell monolayers is cellular proliferation at infectious foci. To gain insights into the mechanism by which OspB renders mTOR hypersensitive to inhibition by rapamycin, we employ *in silico* analyses to identify putative catalytic residues of OspB and show that a conserved cysteine-histidine dyad is required for OspB activity. In a screen of an over-expression library in *Saccharomyces cerevisiae*, we identify a dependency of OspB activity on inositol pyrophosphates, a class of eukaryotic secondary messengers that are distinct from the inositol phosphates known to act as cofactors for bacterial cysteine proteases. We show that inositol pyrophosphates are required for OspB activity not only in yeast, but also in mammalian cells - the first demonstration of inositol pyrophosphates being required for virulence of a bacterial pathogen *in vivo*.

45 Single-molecule imaging reveals distinct subcomplexes of the *Bacillus subtilis* division machinery

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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 multiple factors (the divisome) necessary for division. Z-ring constriction and the corresponding synthesis of cell wall between daughter cells are essential for cell division; FtsZ treadmilling is required for both. But what are the specific functions of each component of the divisome, and how do they collectively contribute to cell divisiondelta Motions of divisome components were imaged using HaloTag fusions to each protein, labelled with Janelia Fluor dyes. We characterize divisome members as either remaining immobile with FtsZ subunits, or moving around the division site. This latter group contains cell wall transglycosylase FtsW and a subset of non-enzymatic proteins (DivIB, DivIC, and FtsL) which interact with one another but have unknown function. All four proteins move at the division site with velocities comparable to the previously characterized cell wall transpeptidase Pbp2B, and these directional motions require cell wall synthesis. We propose that these proteins move around the cell in complex in a manner dependent on treadmilling FtsZ filaments. Characterization of molecular interactions between this moving complex and treadmilling FtsZ filaments is ongoing.

46 Functional genomics of the rapidly replicating bacterium *Vibrio natriegens* by CRISPRi

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The fast-growing Gram-negative bacterium *Vibrio natriegens* is an attractive microbial system for molecular biology and biotechnology due to its remarkably short generation time and metabolic prowess. However, efforts to uncover and utilize the mechanisms underlying its rapid growth are hampered by the scarcity of functional genomic data. Here, we develop a pooled genome-wide clustered regularly interspaced short palindromic repeats (CRISPR) interference (CRISPRi) screen to identify a minimal set of genes required for rapid wild-type growth. Targeting 4,565 (99.7%) of predicted protein-coding genes, our screen uncovered core genes comprising putative essential and growth-supporting genes that are enriched for respiratory pathways. We found that 96% of core genes were located on the larger chromosome 1, with growth-neutral duplicates of core genes located primarily on chromosome 2. Our screen also refines metabolic pathway annotations by distinguishing functional biosynthetic enzymes from those predicted on the basis of compar-

ative genomics. This work provides a broadly applicable platform for high-throughput functional genomics to accelerate biological studies and engineering of *V. natriegens*.

47 Discovering small molecules that affect template-switch mutagenesis in *Escherichia coli*

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Errors in DNA replication and repair in bacteria can result in genome instability, which can lead to cell death or bacterial evolution. Inverted repeats can form secondary structures, such as hairpins, which is a major hindrance to DNA replication. One type of repeated DNA sequence is a quasipalindrome (QP). A QP is an imperfect inverted repeat that has been shown to be a mutational hotspot. Mutations in QP regions result from template-switch events during DNA replication. Template switching occurs when DNA polymerase stalls at a QP region and switches from using the template strand to using the nascent strand for DNA synthesis. The polymerase switches back to using the template strand after incorporating mutations that result in the QP becoming a palindrome with complete complementarity. Since it is not fully understood how template-switch events are stimulated or repressed, we designed a high-throughput screen to discover drugs that affect these events. QP reporters were engineered in the *E. coli lacZ* gene to allow us to specifically study template switch events. We tested 700 compounds from the NIH Clinical Collection through a disk diffusion assay. 10 positive hits were identified. One of the hits was azidothymidine (AZT), a DNA chain terminator. The other nine were found to be fluoroquinolone antibiotics, which induce DNA-protein crosslinks. This work shows that our screen is effective in identifying small molecules that affect template-switch events. We are currently assessing more small molecule libraries and plan on applying this method to study other types of mutations.

48 Evolution of fluoroquinolone resistance in a murine model of *Acinetobacter baumanii* pneumonia in the presence and absence of immune suppression

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The emergence of multi-drug resistant *Acinetobacter baumannii* has raised great concern in the clinic and is a global challenge for physicians. In particular, the formerly effective fluoroquinolone antibiotics are now useless, as most clinical *A. baumannii* strains show high level resistance to this drug class. In this study, we aimed to identify the evolutionary pathways during disease that lead to fluoroquinolone resistance in presence or absence of neutrophils to determine how resistance arises in the presence of antibiotic therapy. The *A. baumannii* ATCC type strain 17978 was consecutively passaged via oropharyngeal inoculation in ciprofloxacin-treated mice, either in the presence or absence of neutrophil depletion by cyclophosphamide. After fifteen passages, the majority of bacteria in the neutrophil-depleted animals had acquired resistance mutations, whereas in the presence of neutrophils, the yields of drug resistant mutants were no more the 1% of the population. Using whole genome sequencing of the pools that arose after passage, we detected two-step genomic mutations associated with the increased resistance in immunosuppressed model. The first step always conferred mutations that increased persistence in the presence of the drug, without marked increase in tolerance. Subsequent steps provided frank resistance. The identified mutations were further compared with a larger collection of *A. baumannii* genomes from PATRIC database to evaluate their clinical relevance. Based on these results, we conclude that *in vivo* development of ciprofloxacin resistance strongly correlates with immunosuppression. Future work will focus on the interaction between neutrophils and A. baumannii *using* in vitro tissue culture models.

49 What can cranberry juice do for you? The gut microbiome of women with recurrent urinary tract infections in a placebo-controlled trial of daily cranberry juice consumption

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Urinary tract infections (UTIs) affect 15 million women each year in the United States, with 20% of patients experiencing frequent recurrent UTIs (rUTIs). A recent placebo-controlled clinical trial found a 40% decrease in reported symptoms of UTI in women who suffer from rUTI that consumed cranberry juice daily for six months. Using metagenomic sequencing on a subset of this clinical trial cohort, we sought to assess the impact of cranberry consumption on the gut microbiome, a reservoir for UTI-causing pathogens, including *E. coli*, which causes 70% of UTIs. We determined that neither overall microbial composition nor relative abundance of *E. coli* changed significantly after cranberry consumption. However, one unnamed *Flavonifractor* species decreased significantly compared to placebo. The *Flavonifractor* genus was named for its ability to degrade flavonoids, which have been suggested to block E. coli *binding to epithelial cells based on* in vitro experiments. However, comparative genomics analysis suggested that this *Flavonifractor* does not have any known flavonoid-degrading enzymes. More recently, new literature has suggested *Flavonifractor* may alter the host nervous system, being implicated in multiple mental health disorders. In a combined analysis of whole metagenomic shotgun sequencing and comparative genomics, we found that genes involved in the transport of various substrates were unique to this *Flavonifractor*. Interestingly, two of these genes have the potential to act on neuroactive compounds in the gut-brain axis, tryptophan and kynurenine. Our results suggest further studies are warranted to assess the role of *Flavonifractor* in health and wellbeing in the context of recurrent UTI.

50 Modeling the impact of bacterial contact-dependent growth inhibition on community dynamics

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Contact-dependent growth inhibition (CDI) is one example of how cells within microbial communities inhibit other cells. With direct contact, the CDI cell injects a toxin into target cells to inhibit their growth, while its own kin is protected from harm by producing an immunity protein that neutralizes the CDI toxin. 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 inhibition in community dynamics. We aim to describe how the relative ratio and density of CDI cells compared to target cells impacts the inhibition of a target cell. For example, it is unclear if a high density of CDI cells will continue to inhibit the target or if they will instead self-contact repeatedly, using energy to produce the immunity protein and replenish the toxin. This would offer the target some relief from the inhibition. 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 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. Modeling CDI will facilitate future efforts in simulating microbial communities.

51 Identifying the molecular mechanism of aflatoxin degradation by *Rhodococcus* species

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Aflatoxins are secondary fungal metabolites that contribute to major foodborne diseases in both humans and animals. The ability to degrade aflatoxins from common feed commodities will improve health standards as well as the economic drain inflicted by toxin food contamination. It has been shown that two bacterial species, *Rhodococcus erythropolis* and *Rhodococcus pyridinivorans*, possess the ability to degrade aflatoxins through the secretion of unknown extracellular enzymes. However, a lack of knowledge about the mechanisms of aflatoxin degradation has hampered the efforts to improve them.

In this study, we aim to uncover the molecular mechanism behind the degradation potential of R. erythropolis *and* R. pyridinivorans. Taking advantage of the native fluorescence of aflatoxin, we have developed an assay to quantify the rate of enzyme release and have observed the highest levels of degradation during stationary growth phase, suggesting enzyme release is under regulation. To identify what genes are involved in enzyme production, release, and regulation, a short-term experimental evolution was conducted

selecting for variants reaching stationary phase quicker. At different times within 100 generations, we isolated variants exhibiting different degrees of growth rate and enzyme production. Phenotyping isolates using our fluorescence assay, we ranked them based on enzyme release rate. Using whole-genome sequencing, we will correlate mutations within isolates to their enzyme release rates, allowing for identification of genes contributing to the release of aflatoxin-degrading enzymes. Identifying the molecular mechanism of aflatoxin degradation in R. erythropolis *and* R. pyridinivorans will enable targeted engineering of these strains to improve their degradation performance.

52 Impact of population structure in the design of RNA-based diagnostics for antibiotic resistance in *Neisseria gonorrhoeae*

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Quantitative assessment of antibiotic-responsive RNA transcripts holds promise for a rapid point of care (POC) diagnostic tool for antimicrobial susceptibility testing (AST). These assays aim to distinguish susceptible and resistant isolates by transcriptional differences upon drug exposure. However, an often-overlooked dimension of designing these tests is that the genetic diversity within a species may yield differential transcriptional regulation independent of resistance phenotype. Here, we use a phylogenetically diverse panel of Neisseria gonorrhoeae and transcriptome profiling coupled with RT-qPCR to test this hypothesis, to identify azithromycin responsive transcripts and evaluate their potential diagnostic value, and to evaluate previously reported diagnostic markers for ciprofloxacin resistance (porB and rpmB). Transcriptome profiling confirmed evidence of population structure in transcriptional response to azithromycin. Taking this population structure into account, we found azithromycin-responsive transcripts overrepresented in susceptible strains compared to resistant strains, and selected four candidate diagnostic transcripts (rpsO, rplN, omp3, and NGO1079) that were the most significantly differentially regulated between phenotypes across drug exposure. RNA signatures for these markers categorically predicted resistance in 19/20 cases, with the one incorrect categorical assignment for an isolate at the threshold of reduced susceptibility. Finally, we found that porB and rpmB expression were not uniformly diagnostic of ciprofloxacin resistance in a panel of isolates with unbiased phylogenetic sampling. Overall, our results suggest that RNA signatures as a diagnostic tool are promising for future POC diagnostics; however, development and testing should consider representative genetic diversity of the target pathogen.

53 High throughput discovery of microorganisms containing non-canonical DNA nucleotides

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Microorganisms use a variety of strategies to protect themselves against infection including the bacterial restriction-modification and CRISPR-Cas systems. Bacteriophages such as T4 phage, which contain glycosylated hydroxy-methylcytosines, can harbor diverse DNA base modifications to evade host immunity. So far no systematic investigation of the number of organisms with modified DNA has been conducted on a microbiome. Here, we present a novel metagenomic sequencing approach to specifically identify organisms harboring modified cytosines in their genome. Applied to a sewage treatment plant microbiome, we uncovered phages with non-canonical DNA nucleotides. Further analysis of their gene content reveals pathways predicted to be implicated in novel cytosine base modifications.

54 Microbial succession and safety during ripening of Canastra Cheese in Brazil

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Canastra artisanal cheese is made from raw milk by small farmers in Serra da Canastra region in Brazil. The production process employs fermentation by an endogenous culture called pingo originated from the whey collected from the previous day's production. Since it is a product made from raw milk, it is important

to understand its microbial communities and to control potential foodborne pathogens during the 22 days ripening period required by legislation. This work analyzed the microbial communities and microbiological safety indicators of cheese from 3 rural properties during ripening. Total coliforms, Escherichia coli and Staphylococcus coagulase positive were counted on Petrifilm® plates (3M). DNA extraction was carried out by E.Z.N.A.® Soil DNA and Amplicon libraries for the V4 region of 16S rDNA and ITS1 region were prepared according to the EMP protocol. The pH analyzes were measured directly on the product by HI99163 phmeter. The study showed that the pH changed throughout time and all properties reached the satisfactory microbiological limits before 22 days. The lactic acid bacteria prevailed during ripening, where Streptococcus and Lactococcus genera were the most relatively abundant in two rural proprieties while Streptococcus dominated in one property during ripening. Genera like Corynebacterium, Lactobacillus, Leuconostoc, Weissella also appeared in some samples in less abundance. The study allowed the understanding of the microbiota of this artisanal cheese and showed that the adequacy to the microbiological parameters required by the legislation before 22 days of ripening indicates that farmers have good hygienic sanitary conditions during production.

55 Characterization of *Vibrio cholerae* Motility Using High-Throughput 3D Tracking

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Motility and chemotaxis are known to contribute to pathogenicity in many bacterial species [Matilla and Krell, FEMS Microbiol. Rev., 2018], but the underlying mechanisms are mostly unknown. We strive to characterize motility behavior in *Vibrio cholerae*, the causative agent of cholera, both in liquid environments as well as in complex ones more closely mimicking the host, and identify adaptations relevant to pathogenicity. *V. cholerae* swims with a single polar flagellum. Other species with this flagellar architecture have been shown to exhibit so-called "run-reverse-flick" motility, consisting of linear segments (runs) interrupted by turns alternating in magnitude between 180 (reversals) and a smaller value (flicks) [Xie et al., PNAS, 2011]. Standard 2D microscopy studies have thus far failed to determine whether *V. cholerae* shows flicks. 3D bacterial tracking, by contrast, yields accurate turning angle measurements and long trajectories with multiple turning events, thus enabling a comprehensive analysis of motility behavior. Here we present first steps in the characterization of *V. cholerae* motility in liquids using a recent high-throughput 3D tracking method [Taute et al., Nat. Commun., 2015]. We typically acquire more than 4,000 individual 3D trajectories in 10 minutes. We aim to extend our work to motility behavior in more complex environments as well as chemotaxis. An understanding of *V. cholerae* navigation strategies could provide insight into the early stages of infection.

56 The *Shigella* Type 3 Secretion System effector IpaC interactions with the cell-cell adhesion protein β-catenin to promote spread

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A subset of bacterial pathogens invade into the cytoplasm of eukaryotic cells. A key step in the propagation of these cytoplasmic-dwelling pathogens is direct cell-to-cell spread, a multistep process by which bacteria move from the cytoplasm of one cell to the cytoplasm of an adjacent cell. The host and bacterial proteins that are required for spread are poorly understood. The intracellular pathogen *Shigella* requires direct cell-to-cell spread to cause disease and the *Shigella* type 3 secretion system effector IpaC is required for this process.

We previously found that interactions between IpaC and intermediate filaments are necessary for efficient bacterial invasion into eukaryotic cells. Here we tested the role of intermediate filaments in spread and, surprisingly, found them to be dispensable to this process. However, we identified a critical function of IpaC during spread that is dependent upon the C-terminal domain of the protein. *Shigella* expressing IpaC with a mutation in this domain (R362W) formed plaques 4-fold smaller in area than bacteria expressing WT IpaC. Using live microscopy, we identified that *Shigella* expressing this mutant IpaC exhibit normal actin-based motility but are defective in protrusion formation. Through a protein interaction platform assay in yeast, we found IpaC interacts with the eukaryotic cell-cell adhesion protein β-catenin and that the c-terminal domain of IpaC is required for this interaction. Other cytoplasmic-dwelling bacteria employ virulence proteins that interact with cell-adhesion proteins to reduce membrane tension and promote spread. We hypothesize that

IpaC may serve a similar role for *Shigella* spread.

57 Dual host processes regulate the type 3 secretion system translocon pore

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More than 30 bacterial pathogens require a type 3 secretion system (T3SS) for virulence. The T3SS translocon pore is delivered into the plasma membrane of the eukaryotic cell by the T3SS upon host cell contact. The translocon pore is essential for T3SS activity; it is the conduit through which bacterial virulence proteins are delivered into the eukaryotic cell by the T3SS, and it is a platform onto which the T3SS attaches (docks) to the host cell. Although essential for T3SS function, the molecular events that regulate translocon pore activity are poorly defined. *Shigella* spp. cause diarrheal illness and require a T3SS for virulence. Here, we used the *Shigella* T3SS translocon pore as a model to generate insights into the role of translocon pore during T3SS activity. We identified two distinct host cell processes regulate translocon pore function. Host intermediate filaments interact with the C-terminal region of the translocon pore protein IpaC and induced a conformational change to IpaC that enabled the T3SS to attach (dock) onto the translocon pore. Host actin polymerization induced distinct conformational changes to the translocon pore, which were associated with T3SS mediated virulence protein secretion. The IpaC putative coiled-coil domain was dispensable for docking but required for the actin dependent effects on T3SS secretion. Together, these data identified T3SS docking and secretion are regulated by distinct host cell process that require unique regions of IpaC. They define a novel mechanism by which T3SS function is regulated through host protein interactions with the translocon pore.

58 Plasma membrane domains orchestrate the bacterial peptidoglycan synthesis

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The cell wall peptidoglycan is an essential mesh of polysaccharides crosslinked by short peptides that encase the bacterial cell and protects it from turgor pressure lysis. It is ubiquitous in all bacteria and its synthesis is an effective antibiotic target. Albeit the diverse modes of growth bacteria employ, the assembly of this biopolymer occurs in close association with the plasma membrane. Nevertheless, a higher order organization of this process has not yet been described. Mycobacteria is a relevant group of microbes that includes the causative agent of tuberculosis. In these bacteria, intracellular membrane domains comprise biochemically and spatially distinct regions within their conventional plasma membrane. We aimed to investigate whether peptidoglycan synthesis could be organized within these compartments. We found that lipid-linked peptidoglycan precursors are made intracellularly in these domains and then trafficked to the conventional plasma membrane for their final insertion into the cell wall. Disruption of the plasma membrane disorganizes the distribution of key enzymes in the cell and stops peptidoglycan assembly. This effect remained true for other Gram-positive and Gram-negative bacteria. Our data suggest that membrane compartmentalization is essential for bacteria to synthetize peptidoglycan.

59 Studies on the *E. coli* Stress Response Regulator RssB - a Structural Approach

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The ability to detect various stressors and react upon them is a crucial process for all organisms. In Escherichia coli this response is orchestrated by the RNA polymerase δ-subunit δS, which turns on a large regulon, including genes important for virulence and antibiotic resistance. Due to its key role, δS is itself tightly controlled - during exponential growth δS levels are low due to its degradation by the ClpXP machinery, but rapidly increase upon exposure to stress or during the stationary phase.

δS degradation is dependent on the adaptor protein RssB which facilitates delivery of δS and its loading onto ClpXP. RssB is an atypical response regulator and consists of an N-terminal conserved receiver and a C-terminal effector domain that are connected by a flexible linker. Unlike with other members of the response regulator family, the effector domain of RssB is involved in protein-protein rather than protein-DNA interactions ,and phosphorylation is not critical for RssB activity As a consequence, regulation of δS proteolysis is not achieved though phosphorylation, but mainly through the use of RssB inhibitors, collectively coined anti-adaptors. These are activated by specific stress signals and bind to RssB to prevent δS loading onto ClpXP. Here we present the first crystal structure of a complex between RssB and an anti-adaptor, the DNA-damage inducible IraD, which in combination with in vivo and in vitro data demonstrate that the plasticity of the inter-domain RssB linker plays a crucial role in regulating δS degradation.

60 Investigation of the role of the Tol-Pal system in *E. coli* cell separation

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During cell division, bacteria have to simultaneously synthesize a peptidoglycan septum and constrict the inner membrane (IM). Furthermore, in order for the daughter cells to separate, the shared PG septum has to be split, producing distinct new cell poles for both cells. Gram-negative bacteria, such as E. coli, face an additional challenge to also constrict the outer membrane (OM). The processes of septal generation, septal hydrolysis, and IM and OM constriction appear to be highly temporally coordinated, and it has been proposed that the coordination of OM invagination with the alterations of the other cell envelope layers relies primarily on the Tol-Pal system. All five components of the Tol-Pal system localize to the septum during cell division, and their loss results in a chaining phenotype wherein the daughter cells fail to separate despite completion of IM invagination. Here, we report a genetic analysis of E. coli suppressor mutants capable of surviving in the absence of Tol-Pal under conditions that are normally restrictive for growth of the Δtol-pal strain. Our results suggest a previously unappreciated role for the Tol-Pal system in promoting septal PG splitting.

61 High-Throughput Discovery of Mobile and Intrinsic Resistance Factors in *Enterococcus faecalis* using TnSeq

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Enterococci emerged as commensal gut inhabitants of early terrestrial animals after diverging from an aquatic ancestor and gaining a selective advantage in the Paleozoic era. Today, the pathogenic species *Enterococcus faecalis* is one of the leading causes of nosocomial infections, likely due to their intrinsic resistance to many antibiotics, antiseptics, and surface disinfectants. However, the mechanisms underlying the species' intrinsic hardiness remains largely unknown, hampering efforts to prevent their rise and spread within hospital settings. Using the TnSeq (Transposon Sequencing) high-throughput, sequence-based screening approach in the hospital-adapted *E. faecalis* MMH594 strain, we identified 92 genes that contribute to both intrinsic and acquired resistance of *Enterococcus* to various (n= 10) antibiotics applied at sub-inhibitory levels. Interestingly, when placed within a phylogenomic framework along with other hospital and non-hospital associated strains of E. faecalis *and other species from the* Enterococcus genus, we observed two main patterns among experimentally validated resistance genes: i) association with mobile elements enriched in hospital-adapted lineages and ii) ubiquity to all enterococci including commensal species rarely associated with humans. The former suggests recent adaptation to the hospital environment through horizontal gene transfer, while the latter leads us to hypothesize that ancestral, intrinsic enterococcal genes, that were instrumental in enabling the emergence and adaptation of the genus from marine associated microbes to life on land, are now also advantageous in the modern hospital.

62 Development of a MoClo based cloning system for *Acinetobacter baumannii*

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Acinetobacter baumannii is gram-negative, opportunistic and multi-drug resistant pathogen. *A. baumannii* is the leading cause of hospital associated infections. The organism is known to grow and survive in unfavorable conditions and is responsible for a variety of infections. *A. baumannii* has been established as a lab organism throughout the years though an easy cloning system for the organism has not been yet developed. All current systems are based on inefficient standard restriction enzyme type II cloning. In this work we show the development of a system utilizing a different sub-type of restriction enzymes, II-S, which cut outside of their recognition site. This system permits to construct recombinant molecules in a fast, efficient and parallelized manner. It will allow the expression of up to 20 transcriptional units and whole pathways that can be cloned and expressed in *A. baumannii*. We demonstrate the efficiency of the system by comparing a wide-range of promoter expression strength and demonstrate multiple examples of applications in the construction of different reporter strains using the system under development.

63 Mucins reduce biofilm formation of *Klebsiella pneumoniae*

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Antimicrobial resistance is a growing threat, with an estimated 10 million people per year dying of related infections by 2050. Our approach to antimicrobial resistance is inspired by a natural component of the human body that can inhibit infections: mucus, a hydrogel that serves as a protective barrier and houses our microbiome. Mucins, large glycoproteins that give mucus its gel-forming structure, decrease biofilm formation and overall virulence in certain opportunistic pathogens. Here, we demonstrate that mucins reduce biofilm formation by the often multi-drug resistant and hypervirulent opportunistic pathogen Klebsiella pneumoniae and present our ongoing work on identifying its mechanism of influence. By understanding the mechanism of this protection, we hope to engineer new natural virulence-attenuating therapeutics against this bacterium.

64 Engineering Synthetic Biotic Medicines for the Treatment of Cancer

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At Synlogic we apply synthetic biology to non-pathogenic bacteria (E. coli Nissle) to develop "Synthetic Biotic medicines" which perform and deliver critical therapeutic functions to treat diseases throughout the body. Our synthetic biology platform allows us to design bacterial strains capable of executing metabolic conversions (production or consumption of metabolites), secretion of proteins (chemokines, cytokines, enzymes) and secretion or display of ligands and single-chain Fv (scFv) molecules to trigger or disrupt ligand-receptor interactions.

Here we demonstrate the utility of engineered E. coli Nissle as a multifunctional, therapeutic platform technology for the modulation of immune responses in cancer. By monitoring bacterial abundance following intratumoral injection we show that Nissle specifically colonizes syngeneic murine tumors and persists for up to 15 days in immunocompetent hosts. Using a GFP reporter system, we show the capabilities of engineered Nissle to respond to various inputs, both small molecules and environmental, for specific control of gene expression. Finally, we show three approaches for engineering additional immunomodulatory functionalities into Nissle, including the consumption of immune suppressive metabolites, the secretion of biologically active cytokines and the production of potent pathogen-associated immune agonists. These engineered strains show anti-tumor activity in a variety of syngeneic mouse models, as single agents or in combination with checkpoint inhibitors.

Taken together, these results establish our synthetic biology-based platform as a versatile system for the localized and sustained modulation of the tumor microenvironment, and support the development of Synthetic BioticTM medicines as a novel approach for the treatment of cancer.

65 Saccharibacteria and their fascinating biology

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The oral cavity contains many previously uncultivated bacteria. Some of these organisms are predicted to have small genomes and depend on other bacteria for nutrients/growth factors. Numerous efforts have been made to bring these elusive organisms in culture and we have only recently succeeded with a few isolates of Saccharibacteria (TM7). The goal was to develop a method to allow coculture of TM7s with their host/s and understand their lifecycle.

Subgingival dental plaque sample was obtained from a healthy donor and filtered through a 0.2-micron pore size filter, followed by high-speed centrifugation to pellet the TM7 cells. Pelleted cells were tested for infectivity towards multiple bacterial hosts. Only one of them, Pseudopropionibacterium propionicum (F0700) supported growth of PM004. A 1:10 culture of P. propionicum in a modified Trypticase Soy broth (mTSBY) was infected with PM004 cells and incubated at 37°C. Samples were drawn at 4, 8, 24 and 48 hours and imaged with 16S rRNA FISH probes. FISH imaging showed that the number of PM004 cells increased over 24 hours and killed majority of the P. propionicum at 48 hours.

Based on the results obtained so far, we hypothesized a parasitic lifecycle for PM004, which has a reduced genome, that allows for attachment of the TM7 cell to the host surface, entry of the TM7 cell/material inside the host and killing of the host bacterium. Our findings will allow study of other TM7 and CPR organisms and can reveal many more clues to allow cultivation of other CPR members.

66 Determining how ICEBs1, a mobile genetic element in *Bacillus subtilis*, recognizes substrates for transfer during conjugation

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Integrative and conjugative elements (ICEs) are mobile genetic elements that reside in a bacterial host chromosome. Upon activation, these elements excise, circularize, and can transfer to a neighboring cell via the element-encoded conjugation machinery, a type-IV secretion system (T4SS). ICE-encoded T4SSs recognize and transfer a nucleoprotein complex consisting of their cognate relaxase covalently attached to a linear, single-stranded DNA form of the ICE. An element-encoded coupling protein is responsible for recognizing the secretion substrate and delivering it to the conjugation machinery for transport. However little is known about how ICEs residing in Gram-positive hosts recognize substrates for transfer. We have been using ICEBs1 of Bacillus subtilis as a model to investigate this question. In addition to transferring itself, ICEBs1 is also able to transfer Tn916, as well as mobilize several plasmids lacking their own conjugation machinery. We suspect that ICEBs1's coupling protein is able to recognize the corresponding relaxases of these elements. In an effort to understand how these relaxases are recognized for transfer, we are working to identify ICEBs1 mutant relaxases defective in their ability to be transferred. Importantly, we must decouple the relaxase's functions for DNA processing and replication from its functions for recognition. We have been attempting both targeted and random mutagenesis approaches to identify the desired mutant(s). If such a mutant is identified, future work will attempt to extend the identified translocation motif to the other identified transfer substrates, as well as try to determine which region(s) of the coupling protein are important for this recognition.

67 StrainGE: A new tool for dissecting bacterial communities at the strain level for low abundance species

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Identifying individual strains and their genomic variation within complex samples is critical to both follow the dynamics of an infection and differentiate pathogens from commensals. Current tools to obtain strain level information from whole metagenomic shotgun (WMS) sequencing are limited to marker genes, do not provide SNP level resolution, or are not sensitive enough to detect strains of low abundance species. To address these shortcomings, we have developed StrainGE, a suite of tools that includes 1) StrainGST: a k-mer based tool to identify the closest reference genome to each strain present in a complex metagenomic sample, at coverages as low as 0.1x; and 2) StrainGR: an alignment-based tool that analyzes WMS data aligned to the reference genome(s) identified by StrainGST to identify genomic variation within the predicted strains. We validated our tool using mock communities of known composition, as well as by

comparing predictions based on WMS data with whole genome sequencing of strains isolated from the same samples. StrainGE is currently being used to obtain high resolution views of uropathogens in the context of recurrent urinary tract infections.

68 Diversification of *Cutibacterium acnes* across human skin

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The roles of migration and selection in generating diversity across space within the human microbiome are relatively underexplored, particularly at the sub-species level. The microbiome of human sebaceous skin (e.g. facial skin) is ideal for studying spatial structure due to the ease of acquiring samples across different sites. Here, we use selective culturing and whole-genome sequencing to investigate colonization of *Cutibacterium acnes*, the dominant member of the sebaceous skin microbiome in healthy people. We sequence the whole genomes of 800 *C. acnes* isolates originating from 17 individuals, including specimens both from the skin surface and from within sebaceous follicles (skin pores). We observe simultaneous diversification of multiple coexisting *C. acnes* lineages on individual subjects. On certain body sites (e.g. back), one lineage can dominate the *C. acnes* community on one individual, yet a different lineage can dominate the same site on another individual. This finding suggests that geographic factors rather than selective advantages drive the observed spatial structure, and that barriers to migration or establishment limit the distribution of *C. acnes* lineages across body sites. Furthermore, we find mutations (SNPs) that are only present on *C. acnes* isolates originating from the same skin pore, suggesting that individual pores are ecologically isolated from one another. This island-like nature of pores will enable replicate colonization experiments on single individuals. This work illustrates how whole genome evolutionary interference can operate on fine spatial scales and can illuminate colonization dynamics of a single species.

69 Genetic selections for the discovery of new reductases and oxidases of methionine

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Oxidation of methionine to methionine sulfoxide (MSO) is considered an oxidative damage that can lead to protein disfunction or degradation. However, oxidized methionine can also be "repaired" by enzymes called methionine sulfoxide reductases (MSR). MSRs are conserved from prokaryotes to mammals and represent one of the largest groups of reductases devoted to revert oxidative damage. Considering that only few chemical oxidants target methionine, particularly hydrogen peroxide and hypochlorous acid, a mayor question in the field is how methionine is oxidized in vivo. A major breakthrough was the discovery of vertebrate-specific enzymes MICALs, large multidomain proteins that oxidize conserved methionines on actin affecting its polymerization. The discovery MICAL's indicated the existence of enzymes that can oxidize methionine. The objective of our work is to identify new oxidases of methionine in a prokaryotic genetic selections and screen libraries of environmental and microbiome genomic DNA. Selection were done on an E. coli strain lacking five MSRs (δ5) that can't grow on minimal media when MSO is provided instead of methionine. When libraries are transformed on that strain, a growth recovery on MSO will capture any functional MSR capable of reducing MSO to methionine. To screen for methionine oxidases, we developed a synthetic lethality screen forcing δ5 to retain a plasmid carrying an MSRs when a potential oxidase is expressed from plasmid. The hits we obtained will allow us to identify the building blocks of methionine sulfoxide-based signaling and to speculate on the conservation and evolution of methionine redox signalling.

70 Characterization of microbiome stability using composition-independent diversity analyses

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A key function of the gut microbiome is to protect the host from gastrointestinal pathogens. Antibiotic

disruption can compromise this function. The ecological definition of "stability" is the ability of a community to resist disruption and/or to return to equilibrium. This project strives to measure microbiome stability in the context of antibiotic perturbation to characterize the effects of microbiome manipulation on this important property. Using a mouse model, we disrupted the gut microbiome with a high, intermediate or low dose of the antibiotic clindamycin. We used 16s rRNA DNA sequencing and QIIME analysis before and after clindamycin treatment to define the microbiome at equilibrium and in response to disruption. We used composition-independent metrics to determine the diversity of the microbiome such that the definition of a healthy and stable community was applicable regardless of seasonal or source differences in starting composition. We identified a differential response of the microbiome to the different doses of clindamycin and a threshold dose for disruption of a healthy microbiome. Correlation network analysis revealed key nodes of low abundance genera that are susceptible to clindamycin disruption and highly correlated with other members of the gut microbiome. This work will allow us to assess whether changes to the composition of the stable microbiome will affect the ability of the microbiome to protect the host from disease. Ongoing studies examine the effect of the commensal fungus *Candida albicans* on stability of the gut microbiome measured by its response to clindamycin treatment.

71 Impacts of Genotoxic Agents on Ribosomal Mutations in the *rpsE* Gene of *Bacillus subtilis*

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yet their efficacy is waning due to the increase in antibiotic resistance. Resistance can be intrinsic to a bacterial population, or can arise through mutations that lead to decreased antibiotic susceptibility. This type of acquired resistance can develop rapidly in environments in which bacteria encounter genotoxic agents such as antibiotics, reactive oxygen species, and UV radiation. These agents cause DNA damage through different mechanisms which manifest themselves in a variety of mutations. It is imperative to understand what types of mutations might be expected from different types of DNA damage in order to predict the development of potential mutations and combat the increase in antibiotic resistant infections. Using the rpsE gene of Bacillus subtilis as a model, we investigate the spectrum of spectinomycin resistance-conferring mutations that result from exposure to a variety of genotoxic agents. Using a novel next-generation sequencing approach we profiled 4,500 resistant isolates generated with a variety of stressors. Remarkably, we found that several genotoxic agents induce a unique set of mutations. Our results reveal that each genotoxic agent tested has a unique profile of mutations, suggesting that specific regions of genomic DNA are targeted by each agent. These results increase our understanding of how DNA damaging agents may lead to antibiotic resistance and allow us to predict what type of DNA mutations may arise from specific treatments.

72 Transposon-insertion sequencing screens unveil requirements for *Escherichia coli* O157:H7 growth and intestinal colonization

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Enterohemorrhagic Escherichia coli O157:H7 (EHEC) are important food-borne pathogens that colonize the colon. Transposon-insertion sequencing (TIS) was used to identify bacterial genes required for EHEC growth in vitro and in vivo in the infant rabbit colon. Similar TIS-based protocols were used to compare the in vitro growth requirements of EHEC with commensal E. coli K12. Surprisingly, many loci contribute to EHEC's but not to K12's growth in vitro, suggesting that gene acquisition during EHEC evolution has heightened the pathogen's reliance on certain metabolic processes that are dispensable for K12. For analysis of in vivo TIS data, a new approach that accommodates the presence of experimental bottlenecks was developed. The analysis revealed that the EHEC LEE-encoded T3SS is required for growth in the rabbit colon, but only a few effectors for this apparatus were found to be critical for in vivo fitness. Numerous mutants not previously associated with EHEC survival/growth in vivo also appeared attenuated in vivo, and a subset of putative in vivo fitness factors were validated. Some were found to contribute to efficient type-three secretion while others, promote EHEC resistance to stresses encountered in vivo including low pH and bile. cvpA, which is also required for intestinal growth of several other enteric pathogens, proved to be required for EHEC resistance to deoxycholate. Collectively, our findings provide a comprehensive framework for understanding EHEC growth in the intestine.

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

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Gastrointestinal inflammatory and infectious diseases are major sources of morbidity and mortality worldwide. Their high disease burden reflects, in part, the limited efficacy and off-target effects associated with current therapies. In response to these limitations, we have outfitted *E. coli* Nissle 1917 (EcN), a probiotic used for preventing flares in patients with ulcerative colitis, with a protein secretion system that enables the targeted delivery of therapeutic agents to sites of disease. Type III secretion systems (T3SSs) are nanomachines used by many pathogenic Gram-negative bacteria to inject virulence proteins into the cytosol of host cells. Using homologous recombination-based approaches, we transferred the genes coding for the *Shigella flexneri* T3SS, but none of its secreted virulence proteins, into EcN, creating EcN-T3SS. We further modified the system to secrete proteins into the gut lumen rather than into cells. For therapeutic payloads, we have developed variants of nanobodies that, once secreted, bind to and inhibit bacterial toxins. EcN-T3SS has been engineered to constitutively secrete nanobodies into its surroundings and maintains all of the genetic modifications in the absence of any selection. EcN-T3SS stably colonizes and remains secretion-competent within the intestines of C57BL/6J mice, even in the absence of antibiotic pretreatment, for at least 9 days. We are currently testing EcN-T3SS in mouse models of enteric infections. This designer probiotic platform can be modified to secrete a diversity of nanobodies targeting bacterial toxins and pro-inflammatory cytokines, and as such has the potential to be developed into a novel therapeutic paradigm for intestine-based diseases.

74 The cyclic-di-GMP phosphodiesterase VieA degrades cyclic-GMP-AMP in *Vibrio cholerae*

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Bacteria encode myriad proteins to synthesize, degrade, and signal through cyclic-di-nucleotide second messengers. To date most research has focused on cyclic-di-GMP (cdG) and its role in cellular processes. However, it was recently discovered the human pathogen Vibrio cholerae produces a second cyclic dinucleotide similar in structure to cdG: cyclic-GMP-AMP (cGAMP). cGAMP was shown to be important for virulence and it was recently demonstrated cGAMP directly binds and activates the phospholipase CapV. To gain further insight on the functions of cGAMP and its regulation, we performed a transposon screen in a CapV-DncV overexpression strain to identify suppressors that relieve the toxicity caused by over-activation of CapV. From this screen, we found that over-production of the well-studied cdG phosphodiesterase VieA suppresses CapV-DncV mediated toxicity. We showed that VieA breaks down cGAMP in vitro and prevents cGAMP activation of CapV. VieA is the first example of an EAL phosphodiesterase degrading cGAMP and our results suggest that there is a larger interplay between regulation of c-di-GMP and cGAMP than previously understood, underscoring the need to better understand the interactions between different second messengers.

75 *Staphylococcus aureus* Biofilms Cleared from in vitro Catheters by Pulse dosing of Oxacillin

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Mature biofilms are extremely tolerant to antibiotic treatment, and significantly contribute to treatment failure of chronic infections. We hypothesized that dormant bacteria in biofilms are the major cause of tolerance, and that repeated pulse cycles of antibiotic presence and absence could activate dormant cells and sensitize the biofilm. A novel tractable and economical in vitro flow system was developed to expose catheter segments carrying mature *Staphylococcus aureus* (HG003) biofilms to simulated in vivo pharmacokinetic profiles of oxacillin. The biofilms were tolerant to continuous oxacillin, with 7 days treatment at 100x the planktonic MIC removing only 1 – 2 log of bacteria. In contrast, if the starting concentration of

100x MIC was given as a pulse regimen, diluted over time with a half-life of 1.3 h (mouse PK) and given every 12 h, the biofilm was decreased by 5 – 6 log by day 7. Providing a regular break from the antibiotic caused greater antibiotic susceptibility. Decreasing or increasing the starting concentration or the dosing interval gave a U-shaped dose-response curve. The effective pulse window had flexibility of 3 – 4 h. If the break from antibiotic is too long, the biofilm repopulates between doses. If the pulse window is too narrow bacteria resuscitation and sensitization is prevented or sub-optimal. Flow chambers were used to examine *S. aureus* biofilms with confocal microscopy. An inducible GFP strain revealed that transcriptional activity is highly variable across a biofilm, with many cells displaying low transcriptional activity. Fluorescent dead stains revealed the kinetics of oxacillin killing in the biofilm. Biofilm killing of *S. aureus* by oxacillin can be enhanced by providing an optimal pulse regimen.

76 Potential Role of Polysaccharide Utilization in the Response of the Gut Microbiome to Antibiotics

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Clinical usage of antibiotics is known to have profound effects on the commensal bacteria that constitute the gut microbiome, leading to large shifts in bacterial counts, community structure, and diversity. While many studies have identified changes in composition or functional potential in response to antibiotic therapy, none have measured changes in transcriptional activity. We hypothesize that the induction of tolerance mechanisms related to bacterial metabolism mediates toxicity to antibiotic exposure within the microbiome. Using species-level metagenomics, we found that amoxicillin treatment dramatically increased the relative abundance of *Bacteroides* species, despite reducing most species. Using metatranscriptomics, we observed that antibiotics generally decreased the expression of key metabolic pathways at the whole-community level. Despite the apparent reduction in metabolic activity, we were able to detect unique drug- and species-specific changes in gene expression in response to antibiotics. Most notably, our data suggests that amoxicillin induces the expression of genes involved in starch utilization within *Bacteroides*. To determine if starch utilization could represent a potential tolerance mechanism, we tested the susceptibility of *Bacteroides* species grown on various carbon sources to amoxicillin *in vitro*. We observed that growth on polysaccharides dramatically decreased susceptibility of *Bacteroides* to amoxicillin while supplementation with monosaccharides sensitized *Bacteroides*. Lastly, we found that we were able to reduce the expansion of *Bacteroides* during amoxicillin therapy within the microbiome through dietary supplementation with glucose. These results suggest that diet may have a profound impact on the response of the gut microbiota to antibiotic therapy, specifically as it relates to carbohydrate composition.

77 Model-guided design of engineered *E. coli* strains biosynthesizing a non-standard amino acid

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Cross-feeding of essential metabolites is pervasive in natural microbial communities. However, the exchanged metabolites are costly to synthesize (e.g., amino acids), cross-feeding is vulnerable to invasion by non-producers (i.e., cheaters), which do not contribute to the pool of shared metabolites and just reap the benefit of those produced by others. One possible avenue toward the design of robust synthetic microbial cross-feeding is to "encrypt" these metabolic channels through the exchange of small molecules that are artificial, i.e., not a component of the natural cellular milieu. Toward addressing this goal, we describe an integrated effort that combines genome-scale modeling of metabolism and synthetic biology approaches to design engineered microbes capable of overproducing a nonstandard amino acid. Specifically, we first constructed an engineered *E. coli* strain that is capable of biosynthesizing the nonstandard amino acid p-aminophenylalanine (pAF) through incorporating a heterologous gene cluster. Next, we used a genome-scale metabolic network model of *E. coli* to systematically rewire the "native" metabolism of the host *E. coli* strain to further accommodate pAF biosynthesis. We identified a set of targeted interventions, including gene up-regulations, down-regulations, and deletions that permit enhanced pAF production *in silico*. These computationally identified metabolic interventions are then being used to experimentally construct

a pAF-overproducing *E. coli* strain. This study paves the way for constructing encrypted microbial cross-feeding mediated by the exchange of nonstandard amino acids and, more broadly, establishes systems biology modeling to guide the design of synthetic microbial consortia.

78 The hunt for a hydrolase: flagellar construction in *Bacillus subtilis*

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While the cell wall of bacteria is necessary to protect the cell from osmotic stress in their surroundings, the inherit rigid nature of the cell wall also poses a physical barrier to trans-envelope machinery assembly. Many Gram-positive bacteria like *Bacillus subtilis* encode in their genome hydrolases which are enzymes that can cleave the bonds of the peptidoglycan (PG) cell wall and have been implicated in remodeling the PG to allow for construction of nano-machines. One of these nano-machines is the flagellum, which must be assembled through the PG in order to allow cells to propel through their environment. We have taken a reverse genetics approach to determine if any of the known and annotated hydrolases found in the *B. subtilis* genome are necessary for flagellar construction. Knockout strains were constructed in each of the hydrolases and the strains were assayed for motility. Furthermore, we have used a forward genetics approach by using transposon mutagenesis and high-throughput sequencing (Tnseq) to identify all genes that are required for motility in *B. subtilis*. Candidate genes previously not known to affect motility were validated by directed knock-out mutation to determine whether they had a motility defect. Candidate non-motile strains were tested for their ability to construct flagella. At present, we have not been able to identify a predicted hydrolase needed for flagellar synthesis. Our findings suggest that the traditional models for hydrolase assisted nano-machine assembly established for Gram-negative bacteria might not hold true in *B. subtilis* and therefore will present some alternative working hypotheses.

79 CRISPRi-Tn-Seq: A genome-wide high-throughput tool for bacterial essential and nonessential genetic interaction mapping

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Genome-wide genetic-interaction maps (GIMs) illustrate the relationships between genes in a genome and can be leveraged to better understand biological processes and identify drug targets. Tn-Seq can be used to generate GIMs by combining transposon mutagenesis in the background of a deletion mutant, however such GIMs do not contain essential genes, as deletion mutants cannot be made for essential genes. Here we develop CRISPRi-Tn-Seq, a fusion of CRISPRi and Tn-Seq, that can reconstruct bacterial GIMs including essential genes.

A Streptococcus pneumoniae strain was constructed that carries the dcas9 gene under a Plac promoter controlled by IPTG. A set of sgRNAs, which target individual essential genes involved in different biological processes, were introduced individually under a P3 constitutive promoter, generating target-specific CRISPRi strains. Transposon mutant libraries were constructed for 12 CRISPRi strains and libraries were grown in the absence and presence of a sub-lethal concentration of IPTG, after cultures were analyzed by qPCR and Tn-Seq. Decreased growth and reduced target gene expression were observed with increased concentrations of IPTG in growth and qPCR studies, respectively, confirming the successful design of the CRISPRi strains. From Tn-Seq data, genome-wide fitness changes were calculated by comparing libraries grown in the presence (target knockdown) and absence of IPTG , which identified target-specific nonessential gene sets with increased or decreased fitness, indicating a unique interaction of nonessential genes with corresponding essential genes, demonstrating the potential of CRISPRi-Tn-Seq to reconstruct a GIM. Identified connections were mapped in a genetic-interaction network, which are further studied to elucidate the mechanistic and functional connections between genes.

80 Bacterial cell wall modification by glycolipid probes

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Despite the ubiquity and importance of glycans in biology, methods to probe their structures in cells are limited. The structures of mammalian glycans can be perturbed through metabolic incorporation, in which non-natural sugars are taken up by cells, converted to nucleotide-sugar intermediates, and incorporated via biosynthetic pathways. Applications of metabolic incorporation in bacteria are complicated by the diversity of pathways bacteria use to acquire, metabolize, and catabolize monosaccharide intermediates. We sought a strategy to alter glycan structure that avoids intracellular processing; our plan is to use lipid-linked glycans, intermediates directly used in glycan biosynthesis. We generated synthetic arabinofuranosyl phospholipids to test this strategy in Corynebacterium glutamicum and Mycobacterium smegmatis, organisms that serve as models of Mycobacterium tuberculosis. Using a C. glutamicum mutant that lacks arabinan, we identified glycolipid donors whose addition restores cell wall arabinan, demonstrating that non-natural glycolipids can serve as biosynthetic intermediates and function in chemical complementation. Our lipid-based probe could rescue wild type cells treated with an inhibitor of cell wall biosynthesis. Our current focus is on the incorporation of glycolipid derivatives with chemical handles for labeling such that bacterial glycans can be captured and visualized. Specifically, we have generated the first probe to label arabinose-containing structures in mycobacteria and corynebacteria.

81 Multi-omic profiling and machine learning approaches for predictive modeling of bacterial survival outcomes; towards the development of personalized diagnostics for infectious diseases

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Bacterial pathogens experience various types of stress (e.g. the immune system, antibiotics) and the manner in which they respond determines whether they will survive. Predictions on whether a pathogen will thrive in the host or successfully evade antibiotic treatment, could thus have tremendous value in clinical diagnostics and prognostics. However, it is unknown what type of data and models are necessary to enable such predictions. We present a substantial experimental dataset containing the multi-omic profiles of different human pathogens to various stresses. We trained a set of models, that vary in their complexity and the amount and type of data required, that can accurately predict the survival outcome of a bacterium. The simplest model is a small gene panel that can distinguish survival success from failure in *Streptococcus pneumoniae*. However, since gene panels can be strain or condition specific, we implement a model based on the total extent of perturbation in the transcriptome, i.e. entropy. We demonstrate that entropy is predictive of survival in pathogenic bacteria, independent of species or (antibiotic) stress. Entropy also correlates with an antibiotic's MIC, indicating that it can be used in antibiotic susceptibility testing. Moreover, the inclusion of multiple omics data-types and a more sophisticated machine learning model, reduces ambiguity in survival predictions. This work forms a gateway towards novel infectious disease diagnostics and prognostics, which we are exploring by the model's application to predict the outcome of an infection or the probability of relapsing based on a strain's antibiotic susceptibility and treatment strategy.

82 Chemical-genetic interactions of essential *Mycobacterium tuberculosis* systems

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Tuberculosis (TB) is one of the most prevalent infectious diseases in human history. Approximately a third of the world's population has been exposed to *Mycobacterium tuberculosis* (*Mtb*), the causative agent of TB, with more than 1 million deaths annually. TB must be treated with combinations of antibiotics to minimize the emergence of resistance but despite this complex regimen, TB cure requires months of therapy and multiple drug resistant (MDR) strains continue to emerge. Therefore new strategies are needed to both accelerate cure and treat MDR infections. The requirement for multi-drug therapy represents both a limitation and an opportunity. While combining agents can dramatically increase efficacy, predicting which potential new drugs will produce a synergistic effect in the relevant environmental conditions remains a challenge. Here we utilize conditional *Mtb* mutants of essential genes to investigate antibiotic-genetic interactions that occur during infection and compare with relevant *in vitro* conditions such as different carbon sources. Synergies identified *in vivo* were found *in vitro* in both context independent and dependent manner, with specific synergies found during cholesterol and fatty acid growth conditions. By creating an atlas of chemical-genetic interactions of essential genes during infection, we can identify new synergies to

be exploited for drug development that will improve current antibiotic efficacies and treatment timelines for patients.

83 ATP Regulates Functional Interactions of the Highly Conserved Cell Division Protein FtsA in *Escherichia coli*

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Bacteria divide through the process of binary fission, which occurs at the septum and is facilitated by a dynamic protein complex called the divisome. In *Escherichia coli*, the essential and highly conserved actin-like ATPase FtsA directly remodels phospholipid (PL) vesicle architecture and tethers FtsZ polymers to the inner membrane to establish the FtsZ-ring and coordinate constriction. FtsA rapidly hydrolyzes ATP in the presence of PLs in vitro. To further investigate the relationship between ATP hydrolysis, PL vesicle remodeling and FtsZ interaction, we performed site-directed mutagenesis to engineer substitution mutations in the FtsA active site. We identified several residues in the ATP-binding site that are important for rapid ATP hydrolysis by FtsA including E14 and Y375. FtsA mutant proteins FtsA(E14R) and FtsA(Y375A) are both defective for ATP hydrolysis in vitro. In PL-recruitment assays, FtsA(E14R) binds to PLs, similar to wild type FtsA, however, FtsA(Y375A) is defective for recruitment to PLs. While FtsA(E14R) engages phospholipids, it is defective for ATP-dependent remodeling of PL vesicles. To determine if FtsA mutant proteins insert a C-terminal membrane targeting sequence (MTS) into the PL bilayer, we performed fluorescence resonance energy transfer assays using a lipophilic acceptor. We observed that FtsA(E14R) is not defective for insertion of the MTS into the PL bilayer. These results show that ATP hydrolysis mutants of FtsA are defective for PL engagement and remodeling, which suggests that ATP utilization is critical for regulation of FtsA functional interactions.

84 Development of a Robust Biofilm Assay of *S. pneumoniae* to study adaptive evolution and the emergence of antibiotic resistance.

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Bacteria grow in at least two different states: 1) planktonically as free-swimming single cells; and 2) as biofilms in complex organized bacterial communities embedded in an extracellular matrix of polymeric substances. Bacterial biofilms are known to diminish the effectiveness of antibiotics, posing a challenge for successful clearance in a clinical setting. Additionally, bacterial adaptive evolution has been shown to differ between bacteria growing planktonically or in a biofilm. Since biofilms are important during different stages of (establishing) an infection it is critical to study bacteria in the context of a biofilm. Biofilm formation, maintenance and recalcitrance have been studied in many species but little is known for the opportunistic pathogen *Streptococcus pneumoniae*. Here we develop a method to create a reliable, consistent and long-term biofilm assay, which allows for examining S. pneumoniae's biofilm population dynamics. The major advantage of this novel method is that the biofilm can be maintained and reconstituted indefinitely, rather than hours/days (as in previously published assays). This allows for adaptive evolution experiments to be carried out for the first time with *S. pneumoniae* in biofilms, enabling the monitoring of the emergence of antibiotic resistance. Using our biofilm assay, we have successfully adapted multiple parallel *S. pneumoniae* populations to two different antibiotics over a period of 40 days. We aim to uncover how *S. pneumoniae* undergoes adaptive diversification in biofilms in contrast to planktonic growth. As such, we intend to establish a more realistic model to study bacterial adaptation towards antibiotic resistance during an infection

85 Identification and *a priori* machine learning driven predictions of antibiotic resistance in the bacterial pathogen *Streptococcus pneumoniae*

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The acquisition of antibiotic resistance limits our ability to fight bacterial infections, causing millions of fatalities each year. In addition to disrupting specific targets, antibiotics induce genome-wide stress responses

that are influenced by the bacterial metabolic status and genetic background. When the experienced stress is maintained over many generations, a bacterial population acquires adaptive mutations, which are generally localized in genetic regions that resolve the experienced stress. However, it is unclear whether the emergence of antibiotic resistance in a bacterial population is predictable, and what types of data would be necessary to make accurate predictions. Here we apply experimental evolution, genome-wide profiling techniques, next-generation sequencing, and machine learning to study strain-specific responses and adaptive evolution of the pathogen ()Streptococcus pneumoniae() to different antibiotics. The evolution studies reveal conserved adaptive mechanisms among different strains, where similar adaptive mutations arose on antibiotic-specific targets and/or efflux pumps. Surprisingly, kanamycin-adapted populations display strain-specific mechanisms that have not been described before. Moreover, antibiotic cross-resistance tests show that kanamycin-adapted strains are resistant not only to other aminoglycosides but also to different classes of antibiotic in a strain-specific fashion. Finally, by combining multi-omics data that accurately captures genome-wide stress with machine learning, we design an approach that allows for the prediction of adaptive mutations under antibiotic selection, highlighting that adaptive evolution has deterministic components that are partially predictable. Further improvements of this predictive model may lead to novel hypotheses regarding the involvement of uncharacterized or off-target genes in adaptive evolution, and antibiotic cross-resistance.

86 Integrative elements as drivers of genome plasticity in the marine cyanobacterium *Prochlorococcus*

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Prochlorococcus is the smallest and numerically most abundant cyanobacterium in the oceans, singlehandedly responsible for 10% of global phytoplankton productivity. Its physiological and genomic diversity has been described in cultures and in the wild, revealing a large pan-genome containing hypervariable genomic islands that are linked to niche differentiation.

Our objective is to understand how genomic islands form, how they function, and how they evolve within the Prochlorococcus pan-genome. This will help us unveil some of the evolutionary principles that shape the self-assembly and resilience of the global Prochlorococcus population 'collective'.

We performed a comparative genomics study of 600 Prochlorococcus genomes revealing the presence of integrative elements as the primary drivers of genomic island evolution. These integrative elements carry diverse sets of metabolic gene cargo that undergo rapid rearrangements among cells in culture. Most of them carry primase enzymes, and we show evidence of their ability to replicate within cells, offering a mechanism for the high recombination rates observed in island regions. We then analyzed the transcription of 8 integrase genes in 4 different strains, showing that most integrative elements are tightly regulated and activated under severe DNA damage conditions. We are now complementing these results by uncovering the precise sources of DNA damage that may induce their activity in the natural environment, such as phage infection or chronic UV exposure.

Overall, our results suggest that genomic islands function as adaptive tools in the Prochlorococcus 'collective', such that when facing severe environmental stress leading to DNA damage, cells trigger genomic island shuffling to produce genetic innovation.

87 Recognition and repair of DNA mismatches by the *Mycobacterium smegmatis* NucS protein in vivo

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The mismatch repair (MMR) system is essential to maintain replication fidelity in both Eukarya and Prokarya. The MutS/MutL protein families correct mismatches at the replication fork to maintain genome stability. Despite lacking the mutS/mutL genes, Mycobacterium tuberculosis exhibits a spontaneous mutation rate similar to MMR-bearing bacteria, suggesting the existence of an alternative MMR system. The EndoMS/NucS protein from actinobacterium Corynebacterium glutamicum is a recently discovered mismatch-specific endonuclease that binds to and corrects mismatches in vitro. However, the purified NucS homolog from Mycobacterium smegmatis was recently shown not to bind mismatches in vitro. We sought to examine this discrepancy. We constructed an M. smegmatis δnusS strain and performed complementation analyses. We also measured recognition and repair of mismatches by NucS in the bacterial cell by using

RecET-promoted oligo-mediated recombineering to deliver mismatched oligos to the replication fork. The mutator phenotype of an M. smegmatis δnucS strain could be complemented by expression of wild type NucS, but not a NucS protein deleted of its last five amino acids (NucS δCtmer), a region in C. glutamicum NucS important for binding to B-clamp. In our recombineering experiments, oligos that delivered G/G, G/T, T/T mismatches to the defective HygR gene were repaired, but not other types of mismatches. This is consistent with mismatches repaired by NucS in C. glutamicum. Our study demonstrates that M. smegmatis NucS is required for spontaneous mutation avoidance, reveals the types of mismatches that are recognized in vivo, and shows that interaction of NucS with the replication B-clamp (DnaN) likely occurs.

88 Taxonomic and functional profiling of the microbiome of the eastern oyster, *Crassostrea virginica*

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As a suspension feeding animal, the eastern oyster, Crassostrea virginica, filters up to 50 gallons of water a day and thus has a high encounter rate with the diverse microbial communities represented in the surrounding seawater. While the microbiomes of oysters are distinct from the free-living communities in the water column or sediment, significant challenges remain in understanding the complex host-microbiota-environment interactions that shape the function and composition of the microbiome of different host tissues. As an initial step towards addressing these challenge, in this research we examined the function and distribution of tissue-specific microbiota among individual oysters. 16S rRNA gene sequencing was used to profile the digestive tract, gill, mantle, shell, hemolymph, and pallial fluid microbial communities, where a number of core taxa were identified along with a subset of taxa showing differential abundance upon infection by a protozoan pathogen Perkinsus marinus. Functional studies of the digestive tract core microbiome was performed using shotgun metagenomic sequencing, where over 200 metagenomic bins were determined, including two high quality metagenome assembled genomes (MAGs) representing uncultured strains of Mollicutes and Chlamydiae. Detailed functional analysis of the MAGs revealed genes involved in potential host associations, with machinery in the Mollicutes MAG mediating binding to the host epithelium layers and genes in the Chlamydiae MAG mediating interactions with the host immune system. Further study of eastern oyster-microbe interactions will enable our understanding of the role of microbes in host physiology, health, and population dynamics.

89 Regulated gene drives conservation and degradation glycine riboswitch aptamers

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Riboswitches are RNA motifs found within the 5' UTR of bacterial mRNAs that regulate expression of metabolic genes in response to a ligand. Canonical riboswitches are comprised of one ligand-binding domain (aptamer) and one expression platform, which undergoes conformational changes affecting expression in response to aptamer-ligand binding. The glycine riboswitch is unique because it is found in both the canonical conformation (singleton) and with two aptamers regulating one expression platform (tandem). While this tandem conformation is well documented, it is not clear how/why it arose and became conserved across bacteria. To investigate this, we use phylogenetic and graph clustering techniques to study the conservation of the tandem glycine riboswitch and their relation to singleton glycine riboswitches.

Our investigation reveals that the gene being regulated by a riboswitch (genomic context) effects which tandem glycine-binding aptamer is more highly conserved: the first aptamer is more highly conserved in riboswitches regulating the glycine cleavage system (GCV), while the second is more highly conserved in those regulating transport proteins (TP). Our analysis also reveals that singleton riboswitches are more similar to the first or second tandem aptamer based on genomic context: singletons regulating GCV are more similar to the first aptamer of tandems regulating GCV, while singletons regulating TP are more similar to the second aptamer of tandems regulating TP. Taken together, these findings paint a picture of tandem glycine riboswitches degrading into functional singletons, with the genomic context dictating which glycine-binding aptamer is conserved.

90 Multi-omic stress response signatures are explorable with ShinyOmics, and define underlying bacterial survival success and failure

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Pathogenic bacteria experience a multitude of stress factors when they colonize a human host and establish an infection. Whether the pathogen can be eliminated by the host immune system or antimicrobial treatment, depends largely on whether it is sensitive to these stresses. In order to identify patterns that distinguish stress-sensitive from -insensitive strains, and different types of stress, we generated a substantial experimental dataset consisting of six human pathogens under nine antibiotics and two non-antibiotic conditions. By profiling the temporal transcriptional response of Streptococcus pneumoniae upon antibiotic exposure or nutrient depletion, distinct patterns emerge that characterize both the mechanism of action (MOA) of the stress, and the stress-sensitivity of the pathogen. The MOA of an antibiotic can be accurately inferred from the differential expression of a small set of genes. On the transcriptome level, while a stress-sensitive strain tends to trigger a pronounced and functionally random response involving down-regulation of essential genes; a stress-insensitive strain triggers few expression changes with functional enrichment. Importantly, the difference in the extents of transcriptional disruption between sensitive and insensitive strains is a generalizable feature across six bacterial species under various antibiotic and non-antibiotic conditions. Additionally, we develop ShinyOmics, a web-based application that enables interactive and dynamic visualization of all experimental data. Lastly, the omic signatures underlying different bacterial survival outcomes identified in this work can be implemented in predictive modeling of survival outcomes, which provides an exciting opportunity to improve infectious diseases prognostics.

91 The gut and SMS microbiomes of Northern Pike (*Esox lucius*) are distinctly unique and divergent from the environment

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The fresh-water fish microbiome has important applications for both fisheries and ecology. Aqueous environment serves as a unique avenue for challenge by free-floating microbes, however little is known about the impacts of this surrounding microbiota on piscine communities. To better understand the establishment and perseverance of microbial communities exposed to the natural environment we compared communities on the skin mucosal surface (SMS) and the gut of Northern Pike (Esox lucius), to their surrounding water. We collected samples from 8 sites along the Chochocouane River, in Quebec Canada and analyzed microbial composition via 16S rRNA sequencing. Our findings reveal a profile similar to other carnivorous fish, characterized by a lower diversity in the gut compared to the SMS and a large proportion of Cetobacterium, a genus previously linked to carnivorous species. Robust taxonomic differences were found between the SMS, gut and water communities indicating a divergence between the microbiomes. Communities found in the SMS were more similar to the water than the gut, however, at lower taxonomic levels we found the communities were in fact highly distinct with less than 30% shared OTUs between the SMS and water. Overall, our results suggest the establishment of distinct communities across various parts of the fish and a separation from microbes in the surrounding waters.

92 *Bacillus subtilis* TerC Family Proteins Contribute to Manganese Homeostasis

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Manganese (Mn) is an essential element throughout most of the tree of life and is required for the virulence of many Gram-positive pathogens. MntR is a divalent manganese (Mn(II)) responsive DNA-binding protein that is critical for Mn(II) homeostasis. Recent results have expanded the scope of the MntR regulon and demonstrate that it functions as a transcriptional activator for expression of two CDF Mn(II) efflux pumps, MneP and MneS. Mutants lacking either *mntR* or both *mneP* and *mneS*, are extremely sensitive to Mn(II) intoxication. Here, we have explored the functional redundancy between these two Mn(II) efflux pumps, and three other proteins implicated in resistance to Mn(II) intoxication: YybP, YkoY, and YceF. YybP is a small, protein of unknown function, whereas YkoY is a member of the TerC family of membrane

proteins. Genetic studies have revealed that the expression of a second TerC homolog (which is not controlled by either the yybP-ykoY riboswitch or MntR), YceF, also confers Mn(II) resistance to *B. subtilis*. We have generated a set of strains lacking these five Mn(II) tolerance proteins in various combinations to assess their functional overlap. Using ICP-MS to monitor the impact of single and multiple mutations on intracellular metal ion concentrations, we find that *yceF* and *ykoY* contribute to Mn(II) export, either directly or indirectly. More generally, TerC family proteins are widespread in Bacteria, including pathogens and therefore they may impact the ability of cells to resist metal-mediated intoxication mechanisms relevant during host-pathogen interactions.

93 Bacterial cytological profiling provides a tool to track the frequency of antibiotic resistance within an adapting population in *S. pneumoniae*

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Streptococcus pneumoniae is the leading cause of community acquired pneumonia and meningitis. Despite effective vaccines against this pathogen, antibacterial resistance continues to emerge, limiting treatment options for some strains. Resistance in this and other pathogenic bacteria has increased the need to discover new antimicrobials. Bacterial cytological profiling (BCP) has been shown to be a rapid and low-cost means of determining the mechanism of action (MOA) of antimicrobials identified by drug screens based on comparing morphological changes induced by the drug to a reference model. Previously, antibiotic response models have been reported for *E. coli*, *B. subtilis*, and *A. baumannii*. Here we present reference models for *S. pneumoniae* strains TIGR4 and 19F, and demonstrate the use of BCP as a tool for determining the frequency of mutants in an adapting population. We found these *S. pneumoniae* models to be equally effective in determining the MOA of blinded antimicrobials with high specificity despite attenuated morphological changes compared to other species. BCP detects differences in response to antibiotics on a per cell basis, thus we evaluated its ability to track resistance frequencies as a population was experimentally adapted to levofloxacin. We found that BCP was able to discriminate levofloxacin sensitivity within adapting populations which corresponded with the mutant frequency determined by whole genome sequencing. This shows that BCP can serve as rapid and low-cost means of tracking adaptive populations that complements whole genome sequencing.

94 Antimicrobial peptides as probes to study microbial communities

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To understand the functional role of different members of microbial communities, it is essential to observe and manipulate select subpopulations within the community while minimizing off-target effects. While significant effort has been expended in building and using bottom-up representative models of natural communities, our ability to observe and manipulate natural microbial communities from the top-down is severely limited. Further, since microbial communities are not homogeneous mixtures of different species, biogeographic locations of different species are key to understanding functional roles and relationships. For example, within the human gut microbiota, aerotolerant Lactobacillales are dominant in the small intestines whereas anaerobic Bacteroidales dominate the colon. Current techniques to profile biogeographic locations of constituents rely on sequencing and are unable to provide high-resolution non-destructive maps of microbial community heterogeneity. Further, there are currently no methods to manipulate select subpopulations within mixed species communities. Thus, there is significant need to develop methods that allow observation and manipulation of microbial communities. In this talk, we will present an innovative solution to current limitations using the antimicrobial peptide as a probe for specific bacterial species within a model synthetic microbial community. We use a well-studied antimicrobial peptide pediocin PA-1 to demonstrate this technology. We partially purified the bacteriocin and conjugated it to a fluorophore, and then used the pediocin fluorophore conjugate to assess its specificity and quantitate affinity to a diverse population of bacterial species. We additionally were capable of in situ labelling and modulating of a synthetic microbial community and could selectively purify labelled species.

95 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. We sought to understand mRNA degradation machinery in mycobacteria by investigating the role of RNase E, an enzyme involved in both mRNA degradation and selective mRNA stabilization in *E. coli*. Moreover, RNase E is reported to interact physically with proteins coordinated in mRNA degradation. Using the non-pathogenic model specie *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. 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. In order to explore interacting proteins of RNase E in *M. smegmatis*, we did pull down assays and LC-MS/MS with a FLAG-tag RNase E strain and found several candidate proteins. To map the interacting sites of RNase E with candidate proteins, we constructed strains with truncated versions of RNase E. We are expecting to have a big picture of mRNA degradation machinery in Mycobacterium. Currently, we are performing RNA-seq to measure mRNA stability transcriptome-wide in the context of RNase E repression to further define its contribution to mRNA metabolism.

96 Development of designer probiotics for targeted delivery of immunomodulatory payloads

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New drug delivery platforms that target the delivery of therapeutics to sites of disease are needed in order to limit off-target effects. Here we developed a probiotic E. coli-based strategy capable of directly secreting therapeutic proteins into the gut lumen. Our chosen chassis E. coli Nissle 1917 (EcN) is a human probiotic with a proven safety record in human subjects. To enable EcN to secrete heterologous proteins into the gut, we engineered it to encode a type III secretion system modified to secrete proteins into its surroundings rather than into host cells. We established that fusion of a type III secretion sequence to nanobodies (heavy chain only antibodies) is sufficient to generate variants that are recognized as secreted substrates by the modified strain, referred to as T3EcN. However, we find that monovalent nanobodies exhibit markedly different secretion levels, despite sharing highly conserved structural domains. We successfully improved nanobody secretion without affecting activity by constructing homodimers and adding both linkers with greater flexibility and hinge amino acid regions. To enable antibiotic-free selection and constitutive secretion in vivo, we placed an anti-TNF-α nanobody under the control of a constitutive promoter and introduced it into an antibiotic-free selection plasmid that complements an auxotrophic variant of T3EcN. We find that this strain stably colonizes the C57/BL6 mouse gut and remains secretion competent. Ongoing studies will determine the therapeutic efficacy of this strain in a dextran sodium sulfate model of inflammatory bowel disease and provide proof of principle for this novel targeted drug delivery platform.

97 Regulatory Network Analysis Identifies a New Role for MpeR in Gonococcal Resistance to Oxidative Stress

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhea, a high morbidity disease with approximately 78 million annual cases worldwide. During infection N. gonorrhoeae encounters immune cells that express oxidative innate immune defenses. N. gonorrhoeae adapts to this environment by tight control of gene expression via DNA binding regulators such as Fur, a global transcriptional regulator responding to several conditions including iron and oxidative stress. Fur has also been shown to control additional regulatory proteins including MpeR, which plays a role in antibiotic resistance. Recent high-throughput sequencing studies and gene co-expression network analysis of N. gonorrhoeae suggest that

MpeR also plays a role in oxidative stress responses. We demonstrated induction of mpeR expression in response to hydrogen peroxide (H2O2) treatment in wild-type strain F62. A N. gonorrhoeae isogenic mpeR mutant strain was more resistant to H2O2 as compared to F62. Despite this induction of mpeR, qRT-PCR results demonstrate de-repression of an MpeR-repressed gene bacterioferritin known to play a protective role in oxidative stress, in both the wild-type and mutant strains, suggesting that the repressive role of MpeR can be relaxed even as the transcription of the gene is increased. Ongoing studies are aimed at determining the mechanistic details of the role of MpeR in oxidative stress responses and at defining the comprehensive MpeR transcriptional regulatory network expressed during iron and oxidative stress. Given the role of MpeR in antibiotic resistance, the correlation between H2O2 resistance and antibiotic susceptibility will also be explored.

98 Colonization resistance: insights from a mediator-explicit model of microbial communities

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Human associated microbiota protects the host from colonization of pathogens, a property known as colonization resistance. Controlling and restructuring the microbiota as a preventative or treatment measure requires developing predictive models about how communities respond to interventions. In particular, how does the network of interactions among our resident microbes confers resistance to invadersdelta Such predictive modeling of microbial communities has been challenging. On one hand, traditional ecological network representations based on species abundances appear to be inadequate for modeling microbial communities. On the other hand, acquiring the information to build mechanistic representations often appears to be a daunting feat. Here we characterize simple communities to formulate empirically justified assumptions for modeling microbial interactions through chemical mediators. We use this model to predict how intercellular interactions contribute to species coexistence and how such communities may prevent the colonization of new invaders. We study two main questions. (1) What features of the resident community allow it to resist invasiondelta (2) How the properties of an invader (e.g. its basal fitness) and its interactions with resident microbes determine the efficiency of invasion. We outline how these predictions can be tested in the future using simple synthetic communities.

99 Proteomic and metabolomic characterization of the syntrophic interactions between TM7 phylotype and *Actinomyces odontolyticus* using minimal media

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Species of the candidate phylum Saccharibacteria (formally known as TM7) are ubiquitous members of human microbiome from Neanderthal to modern human and have consistently been found associated with mucosal infection, particularly increasing in abundance with gingivitis severity and in subjects with periodontal diseases. However, their physiology, lifestyle and role in health and diseases remained elusive due to their recalcitrance to in vitro cultivation until we recently isolated the first cultivated representative, strain TM7x from human oral cavity. TM7x is an obligate epibiont of the oral Actinomyces odontolyticus strain XH001 and displays unusual manifestations of both symbiotic and virulent parasitic behavior towards its host. To study possible exchange of nutrients, we developed a cultivation strategy that includes a growth phase in rich medium (BHI) and an incubation phase in minimal medium (VMX) to enable the study of their lifestyle under defined conditions. Our data shows that compared to host strain alone, the symbiotic interaction with TM7x leads to much reduced intracellular arginine and increased citrulline levels in XH001. This is consistent with our genomic finding that, while equipped with much reduced genome and lacking numerous biosynthetic pathways, TM7x encodes arginine deiminase (ADI) pathway which is missing in its host XH001's enzymatic portfolio for arginine metabolism. Our data suggested that, while metabolically heavily relying on its host, TM7x could offer its host certain benefit, such as promoting arginine metabolism via its encoded ADI pathway. In the future study, we will further investigate metabolic interaction between the two partners and its impact on their physiology. Altogether, the study of syntrophic interaction partners in minimal media offers a convenient strategy to study their metabolic crosstalk, a territory that has been little explored thus far by mass spectrometry-based proteomics and metabolomics.

100 Rational design of anti-TB drug combinations

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Tuberculosis (TB) remains a major cause of death worldwide. TB is difficult to treat because the bacteria occupy lesions that create pockets of persisters. The standard drug regimen is six months of combination therapy with four antibiotics. We critically need shorter and more effective multidrug regimens using a new set of antibiotics that are active against drug resistant strains. Building a new regimen is challenging because drugs combinations are often more or less potent than expected based on single-drug behaviors. A path to optimizing multidrug therapies must therefore consider combinations early in regimen development. We have recently developed a platform (diagonal measurement of n-way drug interactions; DiaMOND) that uses a geometric optimization of the traditional checkerboard assay to efficiently measure drug combination efficacies and interactions. Here, we take advantage of DiaMOND's efficacy to make systematically assay pairwise and high-order (more than 2 drugs) combinations among ten TB drugs. To improve our ability to model pharmacodynamics in lesions using *in vitro* measurement, our measurements are made in six *in vitro* conditions that model specific stressors encountered during infection. Our preliminary data reveal that drug interactions and combination effects are strongly dependent on growth condition. Using clinically tested combinations among this drug set, we are able to rank-order combinations into their clinical efficacies using heuristics from *in vitro* DiaMOND data. Our long-term goal is to develop a data-driven pipeline that fully accounts for the potential of the rich combination space and prioritize combinations for testing *in vivo*.

101 Impact of *Haemophilus parainfluenzae* and Streptococci Interactions on Community Structure

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Haemophilus parainfluenzae is a member of the *Pasteurellaceae* family that is abundant in human supragingival plaque. Sequence data indicates that H. parainfluenzae *positively correlates with several* Streptococcus species, including hydrogen peroxide-producing *Streptococcus mitis*. Recent microscopy data confirms that H. parainfluenzae *is associated with* Streptococcus sp. but seemingly excluded from "Hedgehog" structures that are composed of a consortium of bacteria, including *S. mitis*, that decorate the tooth surface. Using a reductionist approach, we discovered that S. mitis *growth is stimulated by* H. parainfluenzae, while S. mitis *significantly reduces the growth of* H. parainfluenzae. *Coculture experiments suggest that hydrogen peroxide production by* S. mitis *explains the exclusion of* H. parainfluenzae from these structures. In contrast to most aerobic bacterial species, catalase appears to play a minor role in the oxidative stress response of *H. parainfluenzae*. Anaerobically, where hydrogen peroxide is not produced, S. mitis *unexpectedly enhanced the growth of* H. parainfluenzae. Transcriptome (RNASeq) analyses under these conditions suggest the cooperative utilization of complex carbohydrates by H. parainfluenzae *and* S. mitis as well as increased expression of adhesins. Together these observations may explain mechanisms underlying the spatial arrangement of these species in supragingival plaque and provide an insight into the conditions that dictate these arrangements.

102 Isolation and identification of antibiotic- and biofilm-producing bacterial extremophiles from the Atacama Desert and Patagonia Region

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The Atacama Desert (the driest in the world) and the Patagonia region in Chile are hostile environments for life. Despite previous beliefs, bacteria thrive in these locations regardless of the inhospitable conditions. As such, the study of bacteria isolated from these regions is key to understanding bacterial adaptation to extreme environments. Environmental samples including plant matter, water, and soil were collected from 22 diverse locations within the Atacama Desert and the Patagonia. 16S rRNA gene sequencing was performed directly on soil samples to identify the bacterial taxa, diversity, and abundance in each sample. As expected, the highest abundance and diversity was identified in the locations rich in plant life with *Actinobacteria* and *Proteobacteria* representing the most dominant phyla. Bacteria were also cultured from the samples and over 180 pure isolates were obtained encompassing 71 unique bacterial species, many of which are putatively

associated to the plant rhizosphere. The following functional assays were carried out: colony morphology to record changes in shape, size, or pigment formation, antibiotic production against *Escherichia coli* MG1665, and biofilm development. We found that most colonies produced pigments, 47/153 isolates had antibiotic activity against *E. coli*, and not surprisingly, 92% of isolates assayed formed biofilms. Of note is a particular isolate identified as *Janthinobacrterium sp.* (91% identity) that produces a novel deep purple floating biofilm. The functional characterization of these isolates will permit us to learn about adaptive bacterial strategies in harsh environments and about their possible use in agriculture or in biotechnology.

103 Analysis of nascent transcripts bound by RsmA reveals connections to Hfq in *Pseudomonas aeruginosa*

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Bacterial regulatory RNA binding proteins (RBPs) often function as translational repressors by binding at or near the ribosome binding site on target mRNAs. In the opportunistic human pathogen *Pseudomonas aeruginosa* two of the best studied RBPs are Hfq and RsmA. Hfq is a widely conserved RNA binding chaperone that - among other functions - facilitates the binding of small non-coding RNAs to target mRNA species. In *P. aeruginosa*, Hfq has been documented to play a critical role in multiple pathways, including metabolic regulation and virulence gene expression. RsmA is a central player in the Gac/Rsm pathway; a multi-protein signaling network that mediates the transition between planktonic and biofilm growth phases. Previously, our lab demonstrated that Hfq functions co-transcriptionally by associating with 600 nascent transcripts. We have since determined that RsmA also binds target mRNAs co-transcriptionally. Interestingly, there is significant overlap between the mRNAs targeted by Hfq and those of RsmA, suggesting that Hfq and RsmA may coregulate a subset of target mRNAs. Follow-up analyses have confirmed that RsmA and Hfq do indeed work together at multiple target transcripts to regulate key aspects of *Pseudomonas* biology, including several important virulence factors.

104 Elucidating how the putative *Clostridioides difficile* germinant receptor, CspC, integrates distinct co-germinant signals.

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Clostridioides difficile is a Gram-positive anaerobic bacterium that causes 500,000 gastrointestinal infections each year in the U.S., generating $4 billion in excess medical costs. Germination of *C. difficile* spores into toxin-producing vegetative cells in the gut is critical for disease to occur. *C. difficile* spores germinate in response to specific bile acids in the mammalian gut, and a pseudoprotease, CspC, was identified as the receptor for these bile acid germinants. However, recent work from our lab challenges the evidence implicating CspC as the germinant receptor. Our structure-guided mutational analyses indicate that CspC integrates signals from not only bile acid germinants but also from two classes of co-germinants: amino acids and cations. Specifically, we found that a D429K substitution in CspC increased the sensitivity of spores to calcium, while an R456G substitution increased sensitivity to amino acids. Since the mechanism by which CspC integrates these signals remains unknown, we tested whether CspC directly interacts with co-germinants. In thermal shift assays, we detected CspC binding to calcium but not to amino acids. Interestingly, the D429K substitution does not increase the affinity of CspC for calcium, suggesting that it transduces the calcium signal rather than directly binding calcium. To gain insight into signal conveyance through CspC we used intra-protein epistasis analyses and assessed the how the nature of the residues at positions 429 and 456 influences sensitivity to calcium and amino acids, respectively. These mutant analyses will provide insight into the inter- or intra-protein conduits through which co-germinants signal in *C. difficile* spores.

105 The gut-bladder axis: gut microbiome dynamics and *E. coli* transmission in women with recurrent urinary tract infections

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Urinary tract infections (UTIs) are common, costly, and frequently recurrent. The majority of UTIs are caused by uropathogenic *Escherichia coli* (UPEC), which can reside asymptomatically in the gut of otherwise healthy individuals, but may ascend into the bladder through the urethra. This induces an over-exuberant inflammatory response resulting in pain, increased frequency and urgency in urination, and sometimes recurrent disease (rUTI). Despite the importance of this gut-bladder axis, the population dynamics of UPEC resident in the gut around the time of UTIs remain poorly understood. Further, it is unknown to what extent the structure of the gut microbiome influences UPEC colonization and UTI susceptibility. To address these questions, we conducted a longitudinal study of women with and without a history of rUTI using metagenomic techniques on host fecal and urine samples. We identified differences in the structure and diversity of the gut microbiota that typified rUTI history, indicating that the gut environments of women with rUTI are fundamentally different than healthy controls. Further, *E. coli* strain-level analyses indicated that rUTIs are frequently caused by strains from specific phylogroups which are also better adapted to stable colonization of the gut, indicating that fitness in the gut is linked to infectivity of the bladder in humans. Overall, our work suggests that an atypical microbiome composition with less strain diversity is associated with a history rUTI in women, and that the gut environments of women with frequent rUTI may provide a rich reservoir enabling UPEC transmission to the bladder.

106 Bacterial cGAS-like Enzymes at the Host-Pathogen Interface

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Humans and bacteria synthesize cyclic dinucleotides that act as second messengers to control diverse cellular processes. Cyclic GMP–AMP (2′3′cGAMP) is synthesized in humans by cGAS and 3′3′cGAMP is synthesized in *Vibrio cholerae* by DncV. cGAS and DncV are 10% identical at the amino acid level but share remarkable structural homology and a conserved nucleotidyltransferase enzyme architecture. Here we report that cGAS / DncV-like nucleotidyltransferases (CD-NTases) comprise a large, unappreciated subfamily of structurally homologous enzymes that are present in nearly every bacterial phyla, and that many of these enzymes synthesize novel nucleotide second messengers. Bioinformatic analysis of greater than 5,600 CD-NTases demonstrated that these genes are distributed within mobile genetic elements and cluster into 8 specific clades. To determine the nucleotide product of each CD-NTase clade, we purified 66 enzymes and performed a biochemical screen. CD-NTases use both purine and pyrimidine nucleotides to synthesize an exceptionally diverse range of cyclic dinucleotides. CD-NTase products are not restricted to dinucleotides and also include an unexpected class of cyclic trinucleotide compounds.

Cyclic dinucleotides have emerged as potent stimulators of the mammalian innate immune system and are pathogen associated molecular patterns. Biochemical and cellular analysis of novel signaling nucleotides demonstrated that these molecules activate distinct host receptors that control the type I interferon and NF-κB pathways. We hypothesize that the exceptional biochemical diversity of CD-NTase products may enable both pathogens and commensal microbiota to modulate their interactions with animal and plant hosts.

107 Induction of the Ap4A Alarmone in Response to Aminoglycoside Exposure Increases Susceptibility to Aminoglycosides in *Pseudomonas aeruginosa*

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The rapid rise in antibiotic-resistant bacteria is one of the greatest challenges facing the field of microbiology. Drug resistant pathogens may soon become one of the leading causes of death worldwide. Among the potential candidates to combat infectious diseases are the aminoglycosides, a class of bactericidal antibiotics which often retain activity against multidrug-resistant strains. However, they are nephrotoxic and ototoxic to humans at the currently effective dosages, which restricts suitability in the clinic. Previous

work has shown that exposure to aminoglycosides leads to the production of the alarmone Ap4A in *E. coli*. This elevation of Ap4A generates an increased susceptibility to aminoglycosides. We sought to characterize this susceptibility in *Pseudomonas aeruginosa*, a nosocomial, drug-resistant pathogen. We developed strains with elevated Ap4A levels both by overexpressing lysS, which synthesizes Ap4A, and deleting apaH, which degrades Ap4A. Deletion of apaH proved to cause higher susceptibility to killing by kanamycin, tobramycin, and gentamycin than their wildtype counterparts. We believe that aminoglycoside-driven tRNA mismatching leads to mistranslated protein products and the formation of ROS. This then increases Ap4A levels through induction of lysS and increases the aminoglycoside susceptibility of the cell. Future *in vivo* studies will be performed to investigate the colonization of Ap4A-modified strains and test if this increased aminoglycoside susceptibility is observed in infection. This work may lead to the identification and development of an aminoglycoside potentiator, enabling the effective *in vivo* killing of *Pseudomonas* at lower, safer aminoglycoside dosages.

108 Low Density Polyethylene Degradation by *Pseudomonas putida* and Marine Heterotrophic Bacteria

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Low-density polyethylene (LDPE) is one of the most common plastics due to its low cost, flexibility, and unique material properties. Unfortunately, these unique mechanical properties cause it to be highly resistant to biodegradation. This has led to widespread pollution as these plastics can take centuries to degrade and are often not recycled. This project investigates the degradation of LDPE using the bacterium Pseudomonas putida (KT2440) and explores how this degradation can be enhanced by co-culturing different bacterial species. The degradation of LDPE was measured by weight loss of the LDPE sample and optical density was used to determine bacterial growth. It was found that Pseudomonas putida is able to utilize LDPE powder as a sole carbon source. Additionally, we have curated several key steps in the pathway that Pseudomas putida uses to breakdown LDPE. Using bioinformatic analyses we determined the main enzymes responsible for degradation: Alk-B (alkane monooxygenase), Alk-T (rubredoxin reductase) and Alk-F/G (rubredoxin I and II). With this information, we searched for these genes in the genomes of marine microbes and similar bacteria and found that bacteria with these genes were often known to degrade plastic. From this, we can make hypotheses about which other microbes have the ability to degrade plastics based on their gene components. Our research presents the first steps to finding a feasible way to use bacteria to solve the plastic pollution problem. Further research needs to be done to optimize this process and make it applicable on a wider scope.

109 Identification of substrates targeted by ADPribosyl-dependent ubiquitination *via* the *Legionella pneumophila* Sde family

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Legionella Pneumophila is a Gram-negative bacterium that causes Legionnaire's disease, which is initiated by growth within alveolar macrophages. Previously, our lab discovered that a tubular ER protein, Reticulon 4 is associated with *Legionella* infection in macrophages during early stages of infection, forming detergent-resistant protein aggregates. It was also found that this rearrangement was promoted by the *Legionella* Sde family, which ADPribysolates ubiquitin (ADPR-Ub), and then transferred the ADPR-Ub to Rtn4, independently of the Ub system.

I have been working on identifying other substrates that are ubiquitinated by the Sde family during infection, dependent on the ADPribosylation mechanisms and whether there are specific amino acid sites on substrates being recognized by Sde proteins. Surprisingly, we discovered that the HA peptide is a Sde substrate which is an excellent easy system to start with. We successfully identified that Tyr2 on HA peptide, was a novel ubiquitination site. To identify all the Sde-mediated substrates during infection, I generated a "Click"(alkyne) ubiquitin that can be used by Sde proteins to modify potential substrates, and allows covalent linkage to azide beads so that I can determine Sde substrates by mass spectrometry. I successfully identified a number of hit proteins. Interestingly there appears to be significant targeting of translation

initiation factors, namely eIF3B, 5, 4B and 4H. I was able to verify those hits in both *in vitro* and infected cells. Currently I am investigating the influence of Sde proteins on host cell translation pathway during infection.

110 *Sinorhizobium meliloti* Histidine Kinases DivL and CckA Regulate the Cell Cycle During Symbiosis

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Sinorhizobium meliloti is a Gram-negative and nitrogen-fixing soil bacteria capable of forming a symbiotic relationship with legumes. Upon host colonization, S. meliloti alters its cell cycle resulting in endoreduplication followed by terminal differentiation into a non-reproductive G0 phase. The histidine kinase CbrA has been shown to play a key role in both CtrA-dependent cell cycle regulation and symbiosis. While the δcbrA is not able to form competent nitrogen-fixing nodules with its host, there are rare suppressors that were isolated from phenotypically wild type nodules. These symbiosis suppressors were identified through whole genome sequencing and found to have single base pair substitutions in the CtrA signaling pathway components DivL and CckA. Via complementation, all alleles were found to be recessive. In addition to suppressing the symbiosis defect of δcbrA, the mutations suppress many free-living cell cycle defects, including aberrant cell morphology. Suppression of both free-living and symbiotic phenotypes point towards restoration of the CtrA signaling pathway. Mutations at both loci are being studied using genetic and biochemical assays to define how these symbiosis suppressor alleles function in phosphoryl transfer and the regulation of CtrA.

111 Epigenetic regulation of *Clostridioides difficile* sporulation by a conserved orphan DNA methyltransferase

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Clostridioides difficile is a leading cause of antibiotic-associated diarrhea worldwide. As a strict anaerobe, spore formation is essential for *C. difficile* to survive exit from the host and transmit disease. Like other spore-forming organisms, *C. difficile* uses the master transcriptional activator, Spo0A, and four sporulation-specific sigma factors to control the transcriptional program that ultimately leads to the production of dormant spores. Notably, only a subset of C. difficile cells initiate spore formation during infection, and the mechanisms that control this decision remain poorly characterized. Using RNA-Seq and mutational analyses, we have determined that an orphan DNA methyltransferase, CamA, enhances the number of spores produced by *C. difficile* by acting downstream of Spo0A activation. qRT-PCR and fluorescence microscopy using morphological stains and fluorescent transcriptional reporters indicate that the activation of the early-stage sporulation-specific sigma factor, SigF, is diminished in a camA deletion mutant. We are currently using targeted mutagenesis to identify specific methylation sites that affect the frequency of SigF activation. Since the reduced spore formation observed in a *camA* mutant coincides with a reduced ability to persist in a mouse colonization model relative to wild type, our analyses implicate DNA methylation as a novel mechanism for controlling *C. difficile* disease transmission.

112 DncV/cGAS-like enzymes synthesize cyclic oligonucleotides to activate adjacently-encoded effector proteins

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Cyclic dinucleotides (CDNs) act as second messengers in bacteria and coordinate important functions that can contribute virulence. Dinucleotide cyclase in *Vibrio* (DncV) is an enzyme that produces a CDN involved in the pathogenesis of *Vibrio cholerae*. Surprisingly, there is a structural homolog of DncV in

metazoans called cyclic GMP-AMP synthase (cGAS) that synthesizes a CDN upon cytosolic dsDNA recognition, thereby activating the innate immune response. cGAS and DncV are members of a large family of proteins called cGAS/DncV-like nucleotidyltransferases (CD-NTases), which synthesize a diverse array of CDNs. CD-NTases are newly discovered, poorly characterized, and a separate protein family from canonical CDN synthases. CD-NTase genes are commonly encoded on mobile genetic elements in conserved operons containing effector-like genes, which are predicted to be involved in biological conflict and cell death. We predict that effector enzymes are activated by their cognate CDN. Here we coexpressed newly discovered CD-NTases with their adjacently encoded putative effectors in *Escherichia coli* and screened for phenotypes. We demonstrate that CD-NTases are able to activate both cognate and foreign effectors. In addition, we identified residues that are responsible for CD-NTase activation. These results suggest there are undiscovered mechanisms that bacteria use to coordinate growth inhibition. We hypothesize that CD-NTase coordinated growth inhibition confers a selective advantage because these genes are widely distributed throughout the mobilome of the bacterial kingdom. We hope to understand the specific function of CD-NTases and their effectors by further investigating the modularity of these CD-NTase operons.

113 Regulation of DnaA and replication in *Sinorhizobium meliloti*

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DnaA is a conserved essential protein in bacteria that acts as the chromosomal replication initiator. DnaA is known to bind to the origin of replication, unwinding the DNA, and recruits the replisome to begin replication. In the alpha-proteobacterium Caulobacter crescentus, DnaA binding at the chromosomal origin is inhibited by the master cell cycle regulator CtrA. Upon binding the origin, CtrA silences DNA replication in swarmer cells to ensure replicative daughter cell asymmetry. Sinorhizobium meliloti has a similar cell cycle, including the initiation of replication only once per cell division and asymmetric replicative daughter cell fate. In S. meliloti it was shown through ChIP-seq that CtrA does not bind to the Cori, although CtrA-depletion does result in a polyploidy defect suggesting that it may have an indirect role in regulating DNA replication initiation. A stress response sRNA, EcpR1, negatively regulates dnaA, however it is not understood how DnaA is regulated to effect asymmetric replicative daughter cell fate during normal growth. Importantly, S. meliloti is a plant symbiont that undergoes endoreduplication while inside the host. This endoreduplication phenotype can be induced in free-living cells by plant NCR peptides, which also repress dnaA and ctrA expression. However, it is unclear what role regulation of DnaA plays in symbiosis. By placing chromosomal dnaA under a taurine-controlled promoter, we can control expression of the essential gene allowing for the synchronization of cells in G1 phase. Overexpression and loss of function studies with V5-tagged DnaA will reveal mechanisms involved in replication initiation in S. meliloti.

114 Cell wall hydrolases of Gram-positive conjugative elements appear to act on the cell wall of both the donor and the recipient in a mating pair

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Gram-positive conjugative elements encode cell wall hydrolases that are required for efficient transfer. Although these hydrolases are presumed to be necessary to degrade the cell walls of both the donor and the recipient in a mating pair, this has not been conclusively demonstrated. Here we have evidence to suggest that Gram-positive element-encoded cell wall hydrolases do indeed act on both the donor and recipient cell wall. We created cell wall-less L-form strains of *Bacillus subtilis* which contain the alleles needed to be inducible donors or recipients of the integrative and conjugative element ICE*Bs1*. We observed conjugation machinery-dependent transfer of ICE*Bs1* from cell wall-less L-form donors to L-form recipients. ICE*Bs1* encodes the cell wall hydrolase *cwlT* which is required for transfer between a walled donor and a walled recipient, but we found that *cwlT* was dispensable for transfer in matings between L-forms. We also observed conjugation-dependent ICE*Bs1* transfer from L-form donors to walled recipients, as well as transfer from walled donors to L-form recipients; in both cases we found that *cwlT* was dispensable for transfer. These findings suggest a role for *cwlT* in degrading both the donor cell wall and the recipient cell wall in a mating pair. The mechanism by which a Δ*cwlT* ICE*Bs1* mutant can bypass a single cell wall will be further explored.

115 A Gut Microbe Isolated from Elite Athletes that Improves Athletic Performance in Mice

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The human gut microbiome encodes a vast metabolic repertoire, yet it is unknown whether it can impact exercise. We performed a longitudinal metagenomic analysis on runners in the 2015 Boston Marathon. The strongest microbiome feature enriched post-marathon was an increase in the abundance of the bacterial genus Veillonella. In laboratory mice inoculated with a Veillonella atypica isolate, exhaustive treadmill runtime was increased by 13% in an AB/BA crossover trial. V. atypica utilizes lactate as its sole carbon source and produces the short-chain fatty acid propionate. Using 13C3-labeled lactate we demonstrate that serum lactate crosses into the lumen of the gut. We also show that intrarectal instillation of propionate is sufficient to reproduce the increased treadmill runtime performance observed with V. atypica gavage. These studies reveal that V. atypica improves runtime via its metabolic conversion of exercise-induced lactate into propionate, identifying a natural, microbiome-encoded enzymatic process that facilitates exercise.

116 Engineering the Natural Competence Machinery of *Bacillus subtilis*

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Bacillus subtilis is naturally competent, meaning it can take-up exogenous DNA and integrate it into its genome. In fact, it can take up multiple pieces of DNA at once in a process called co-transformation. By using pairs of genetic markers, we have assessed the co-transformation frequency of *B. subtilis* str. 168 to be 5%. Improved co-transformation frequencies could enable multiplex genome editing by natural transformation (MuGENT) and help further develop *B. subtilis* as a platform for synthetic biology. Therefore, our goal is to engineer an ultra-competent strain of *B. subtilis* with sufficiently high co-transformation frequencies to enable 3 edits simultaneously. First, we assessed the co-transformation frequencies of two strains that were previously engineered for improved transformation efficiency by overexpression of the competence regulator ComK – strains SCK6 and Reg19. Second, we have reworked a previously developed CRISPR-Cas9 toolkit to enable precise scarless genetic modifications in *B. subtilis*. Third, after identifying candidate gene-deletions that would potentially increase co-transformation frequencies we used the CRISPR-Cas9 toolkit to target these genes for deletion and have profiled their effect on co-transformation frequencies. We have also developed an RBS library for 14 genes that play a role in competence; we plan to use this library to favorably modulate expression levels of competence machinery during a directed evolution campaign targeting an ultra-competent strain of *B. subtilis* with increased co-transformation frequencies. If successful, this work will enable rapid engineering of *B. subtilis* for numerous biotechnology applications.

117 Using Comparative Genomics to Understand Functional Capacities of Nasal Commensal *Corynebacterium*

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Accumulating evidence from multiple studies points to the potential for a beneficial role of commensal Corynebacterium species in the microbiota of the human nasal passages. Either alone, or in the presence of other nasal commensals like Dolosigranulum pigrum, Corynebacterium may protect their human host from colonization by Staphylococcus aureus and/or Streptococcus pneumoniae. Therefore, it is important to elucidate the genomic content of nasal Corynebacterium species to understand their functional capacities. Towards achieving this, we collected 544 isolates of Corynebacterium species from the nostrils of 343 volunteers in the Greater Boston area in 2017 and 2018. The distribution of these isolates was concordant with the four predominant nasal Corynebacterium species we detected in a recent species-level reanalysis of the nostril 16S rRNA gene data from 210 Human Microbiome Project participants. We sequenced the genome of 96 isolates distributed across the four common nasal species (C. pseudodiphtheriticum, C. accolens, C. tuberculostearicum, and C. propinquum) plus C. macginleyi using the Illumina platform. Here, we present a preliminary analysis of these genomes, including for each species a determination of the upper bound of the core genome, and functions, and a lower bound for the accessory genome, along with its func-

tional enrichments. This work provides new insights into the biology of the human-associated commensal Corynebacterium species and contributes genomic data key for future metagenomic and metatranscriptomic analysis of the human nasal microbiome.

118 Identification of the Achilles heel of high-level fluoroquinolone resistance in *Acinetobacter baumannii*

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Acinetobacter baummannii is one of the ESKAPE bacterial pathogens associated with a growing number of nosocomial diseases that are untreatable with most clinically useful antibiotics. The increased occurrence of multidrug resistant (MDR) bacteria demonstrates a need to identify new strategies for combating these pathogens. We are focusing on the molecular basis of fluoroquinolone resistance, in the hope of identifying strategies to eliminate drug resistant mutants from the population. We hypothesize that the acquisition of high-level fluoroquinolone resistance (FQR) creates new targets that make these mutants particularly vulnerable for elimination using novel therapeutics. We are pursuing this goal by identifying proteins that protect against stress caused by acquired FQ resistance. High-level FQR is associated with the acquisition of three mutations, two of which result in lowered drug affinity for the targets DNA gyrase (*gyrA*) and topoisomerase IV (*parC*), along with a third mutation that upregulates one of three known resistance-nodulation-division (RND) efflux pumps. Most known clinical isolates in the PATRIC database have mutations in these targets. Using high-density transposon mutagenesis, we have been identifying transposon mutants that show lowered fitness specifically in pump-overexpressing strains compared to strains with normal levels of expression.

119 Hybridization-based targeted capture of bacterial mRNA for single-cell paired host-pathogen transcriptional analysis

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Dual RNA-seq profiling of host and pathogen during bacterial infections is limited by detection of lowly abundant of bacterial mRNA. We report an efficient, accurate, and reproducible method, Pathogen Hybrid Capture (Path-cap), to enrich for bacterial mRNA derived transcripts from dual RNA-seq libraries using transcriptome-specific probes. Path-cap addresses both the differential RNA content of the host relative to the pathogen and the overwhelming abundance of uninformative structural RNAs (rRNA, tRNA) of both species. By sequencing libraries before (pre-Path-cap) and after (post-Path-cap) enrichment, we achieve dual transcriptional profiling of host and pathogen, respectively, from the same sample. We demonstrate that Path-Cap enables study of single host cells infected by few (1-3) bacteria in a model of epithelial cells infected by *Pseudomonas aeruginosa*. Importantly, Path-cap increases the number of unique reads detected for bacterial transcripts allowing for accurate gene expression quantification of hundreds of bacterial genes in single infected cells. Path-cap is adaptable to the study of a range of bacterial pathogens and, more generally, to low-abundance species in mixed populations.

120 Droplet Tn-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 variety of stresses including nutrient limitation, the host immune system and antibiotics. We lack a comprehensive understanding of how bacterial 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) utilizes pooled libraries of transposon mutants to untangle the genetic complexity of a phenotype by accurately calculating mutant growth rates on a genome-wide scale. The pooled batch culture of insertion mutants makes Tn-Seq a powerful technique, however it is unclear how interactions between pooled mutants affect the fitness of individual mutants. To address this question, we developed droplet Tn-Seq (dTn-Seq) by combining Tn-Seq with droplet-based microfluidics, 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 significantly different fitness when cultured as single cells compared to pooled culture. Moreover, we describe for several mutants how their phenotype depends on community structure, including: 1) enzymes involved in breaking down host glycoproteins, 2) a hydrolase in a novel function in bacterial competence and regulated cell death, 3) capsule genes necessary for planktonic growth, and 4) an ionic membrane channel and its role in protection against neutrophil elastase. Additionally, we show how dTn-Seq can be used to study bacterial cell-cell signaling, host-cell-bacteria interactions and can be combined with FACS. Thereby dTn-Seq identifies novel phenotypes previously masked by pooled mutant batch culture making it a valuable extension of Tn-Seq.

121 How can unsuccessful invaders drive long-term shifts in community statedelta

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The stability of virtually all microbial communities is frequently challenged by the arrival of new individuals that could potentially invade the system. This urges for a deeper understanding of how invasions can interfere the dynamics of microbial communities. I will present a bistable model system to study the dynamics between alternative stable states in microbial ecosystems. By introducing additional species into the system, we observed induced transitions between stable states. Interestingly, in many cases the invading species did not survive in the final community state, making these species what we call a "transient invader." This suggests that short-term invasions (such as infections) could be a common mechanism driving transitions between stable states in microbial communities.

122 The relationship of the gut microbiome, environmental exposures, and neurocognitive development in infants and children

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The human gut and its resident microbes are directly linked to the central nervous system through the gut-brain axis, yet the particulars of how the microbiome influences neurocognitive development and to what extent these effects are mediated by other environmental exposures are not well established. The brain and the gut microbiome undergo many drastic changes in early childhood with the gut microbiome being integral to the healthy development of many other body systems, such as the immune system. To investigate the role of the gut microbiome in human neurocognitive development in the context of environmental factors, we sampled fecal matter from 318 unique subjects (221 children, 105 mothers, 8 mother-child dyads) and sequenced the metagenomes to produce taxonomic and functional profiles. We also used neurodevelopmental outcomes such as white matter microstructure integrity, myelination, grey matter volume, and functional connectivity evaluated by quantitative MRI methods. Metagenomic and cognitive data were compared with other clinical covariates such as delivery mode (vaginal or cesarean), feeding behavior (breast milk or formula), and the children's age. Among clinical covariates, delivery mode and breastfeeding behavior had large effects on the microbiome, explaining up to 4.1% and 6.3% of microbial taxonomic variation respectively (P 0.001, PERMANOVA). Brain structural development was also linked to the microbiome, with 3.6% of taxonomic variation explained by the ratio of white matter to gray matter as measured by MRI (P 0.001, PERMANOVA). Multivariate linear modeling identified several taxa that were strongly correlated with increased white matter to gray matter ratio after controlling for age, mode of delivery and breastfeeding history.This research represents the first look at a larger longitudinal study into how the gut microbiome may affect neurocognitive development in children and how these effects may be mediated by environment factors.

123 Treatment of recurrent *Clostridium difficile* infection with SER-109 increases the concentration of secondary bile acids in a dose dependent manner

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C. difficile infection recurs when dormant spores germinate in the dysbiotic gut, facilitated by an increase of 1°vs 2°bile acids. SER-109, an ecology of bacterial spores purified from stool of healthy donors, is an investigational first-in-class microbiome therapeutic intended to facilitate microbiome restoration and reduce risk of recurrent *C. difficile* (rCDI). Rapid engraftment of spore-forming species is associated with higher doses of SER-109 in our dose-ranging Phase 1b study (Ph1b) and with reduced rCDI in our Phase 2 trial (Ph2). Ph1b subjects who received a higher dose (1.5x108 SporQ) had significantly higher spore-forming species richness after 1 week than subjects who received a low dose (1.5x108 SporQ; p = 0.017). Spore-forming species richness was comparable in Ph1b patients receiving a low dose and non-recurrent Ph2 patients, who received the same mean dose. Ph1b high-dose subjects had a significantly higher concentration of 2°bile acids than Ph1b low-dose subjects and non-recurrent Ph2 subjects (p=0.036, p0.001 respectively). A high dose (3x108 SporQ x 3 days) suppressed recurrence in a gut model of rCDI; a single dose did not. Higher doses of SER-109 are significantly associated with 1) higher spore-forming species richness, 2) concentrations of secondary bile acids, and 3) prevention of recurrence in a gut model of CDI. These results suggest that SER-109 in the Ph2 trial was biologically active and that a dose increase may optimize efficacy. A Phase 3 study of SER-109 to reduce rCDI, which includes an increase in dose titer and frequency, is underway.

124 Discovery of a Novel Progesterone Inducible Bacterial Transcription Factor for Use as a Biosensor

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In order to survive and grow in their environments, bacteria must be able to sense and respond to chemicals in their environments. The proteins responsible for this biorecognition make promising targets for integration into biosensors, devices that transduce the presence of small molecules into a human-readable output. Transcription factors are one class of biosensing element that bacteria have evolved, and make promising targets for development into sensors for biological small molecules. Many bacteria catabolize physiologically important steroids as a sole or primary carbon source, but the transcription factors responsible for pathway regulation are only partially understood. We have developed a novel screening strategy to mine bacteria for transcription factors that sense target analytes. We used our strategy to identify and biophysically characterize a novel allosteric transcription factor with primary inducibility to progesterone. We will describe our genomic screening platform as well as the results of quantitative characterization of this transcription factor's DNA and ligand binding properties. As part of a larger collaboration we also developed this transcription factor into an optical biosensor device. Together, these results provide proof of concept for discovery and development of transcription factor biosensors from microbes.

125 Isolation of *Prevotella copri* from HIV-infected cohorts in North America and Sub-Saharan Africa

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Prevotella copri is a prominent member of the fecal microbiota associated with important disease phenotypes including HIV and rheumatoid arthritis, but there are few genome-sequenced *P. copri* isolates, and none from Africa or the Americas. We sought to develop methods for systematic isolation of *P. copri* from HIV-infected and uninfected individuals in North American and Africa. We performed isolation from

37 frozen fecal samples, including 8 HIV+ and 8 HIV- samples from an urban cohort in Boston and 10 HIV+ and 11 HIV- samples from a mixed-rural cohort in Mbarara, Uganda. Under anaerobic conditions, we plated dilutions of each sample on Laked Blood Kanamycin Vancomycin (LKV) agar to select for *Prevotella* and *Bacteroides* and picked unique colony morphologies for isolation. To phenotypically identify *P. copri*, we re-streaked isolates onto Bacteroides Bile Esculin (BBE) agar. We hypothesized that isolates displaying pigmentation without growth on BBE were likely *P. copri*, as *P. copri* hydrolyzes esculin but does not grow in high bile concentrations. Preliminary sequence-based identification was performed using 16S Sanger sequencing, followed by genome-sequencing on Illumina NextSeq and species identification using alignment to universal single copy marker genes. Of 616 colonies picked for isolation, 109 were identified as *P. copri*, with 40 unique strains from 20 samples - 14 from Boston and 6 from Uganda. The phenotype of pigmentation without growth on BBE was 87% sensitive and 96% specific for *P. copri*. In summary, we developed methods to efficiently isolate fecal *P. copri* in order to perform genomic analysis assessing potential geographic subspecies segregation.

126 LexA3 alters the number and appearance of RecA-GFP structures before and after UV treatment

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The SOS response is the archetypal system for DNA-damage inducible responses. LexA is the transcriptional repressor which regulates approximately 40 genes in Escherichia coli. RecA binds ssDNA produced as a consequence of DNA damage. This facilitates the auto-cleavage of LexA and induces the SOS Response. In this work, we tested the hypothesis that LexA could also affect RecA structure similar to other SOS proteins. We used LexA3, an uncleavable form of LexA so it would not be cleaved and degraded. This allows us to measure any change that might occur with LexA. We used the number and shape of RecA-GFP structures as an assay. In lexA+ cells, RecA-GFP structures form in about 15% of the cells forming a mixture of circular and linear forms, 50% and 25% of the population respectively. The remaining structures are oblong, neither linear nor circular. In lexA3 cells, the number of structures increases 2-fold and now 70% are circular and less than 5% linear. In lexA+ cells, nearly every cell has a RecA-GFP structure 10 minutes after UV exposure. These structures start out circular and by 90 minutes are mostly linear. In lexA3 cells, almost all cells have a circular structure at 10 minutes like wild type, but they do not make the transition to the linear form at 90 minutes. We hypothesize that LexA3 stabilizes the circular structures, possibly in the presynaptic form, preventing the transition to linear form that maybe necessary for the completion of the DNA repair after UV treatment.

127 Identification of a TetR-like repressor involved in the regulation of error-prone DNA polymerases in *Acinetobacter baumannii*

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Current knowledge about bacterial DNA damage responses (DDR) is based on *Escherichia coli*, where the global SOS repressor, LexA, controls genes involved in the response to DNA damage. *Acinetobacter baumannii* is an emerging opportunistic pathogen able to quickly acquire antibiotic resistances and survive desiccation better than other bacteria. Remarkably, *A. baumannii* does not have a LexA homologue and as a result, there is much to learn about the *A. baumannii* gene network in response to DNA damage and environmental stress. Clearly, *A. baumannii* is adept at surviving harsh environments, and we have previously shown that *A. baumannii* acquires antibiotic resistances due to activities controlled by the DDR.

We have evidence suggesting that there are multiple regulators involved in the induction of the *A. baumannii* DDR and that there are multiple regulatory layers involved in its regulation. In this work, we have identified a transcriptional regulator of error-prone DNA polymerases, the activities responsible for mutagenesis in response to DNA damage. Through a forward genetic screen, we have found that when a TetR-like protein is inactivated, there is deregulation of the expression of several genes that encode error-prone DNA polymerases. Here, we refer to the TetR-like regulator as EppR (Error Prone DNA Polymerase Regulator), and we show that it binds to the promoter region of several genes encoding error-prone. Our data is consistent with EppR playing a role in the *A. baumannii* DDR as a repressor of expression of error-prone DNA polymerases.

128 Discovery of an inflammatory polysaccharide from a Crohn's disease-associated gut microbe

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Crohn's disease is an inflammatory disease of the human digestive tract. While there are implications of host genetics and diet in disease, imbalances in members of the gut microbial community are strongly correlated with disease status. The gram-positive commensal organism *Ruminococcus gnavus* is among those most strongly, and consistently associated with disease. To establish a molecular mechanism between R. gnavus and Crohn's disease, R. gnavus was grown in monoculture in a defined medium and screened for molecules that activate host immune response in dendritic cells.

From this screen, a potent inflammatory complex polysaccharide has been discovered which induces secretion of inflammatory cytokines in a TLR4-dependent manner. The structure of this polysaccharide was determined by NMR on the native material and derivatives, and while not previously reported, it resembles cell-wall polysaccharides of other pathobiont species in various human diseases. A potential biosynthetic gene cluster has been identified, which will facilitate further investigations of biosynthesis and clinical expression of this inflammatory polysaccharide. Additionally, a mouse model of inflammatory bowel disease is being used to determine the effects of R. gnavus and its purified immunomodulator on disease initiation, severity, and progression. This work will expand our knowledge of how a member of the gut microbiome modulates the immune system and its relevance for IBD, and may also identify potential avenues for therapeutic intervention.

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129 Peptidoglycan fragments stimulate proliferation of quiescent uropathogenic *Escherichia coli*

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Many bacteria enter into a slow or non-growing state, which helps the cells survive conditions of environmental stress. The decision to resume growth usually occurs after the environmental stress has subsided and may be stimulated through the detection of one or more external signals. Here, we show that two strains of uropathogenic *Escherichia coli* (UPEC), including the prototypic strain CFT073 and the multidrug resistant pandemic clone JJ1886, enter into a non-proliferative, quiescent state when plated at low cell density on glucose minimal agar. Cells remain quiescent but viable for extended incubations. Although neither strain is auxotrophic for lysine, L-lysine can prevent and reverse quiescence by stimulating proliferation. Accordingly, deletion of the major lysine importer *lysP*, as well as the transcriptional regulator *argP*, which positively regulates transcription of *lysP*, inhibits the stimulatory activity of lysine. We also observed that actively growing *E. coli* secrete one or more lysine-independent signals that stimulate proliferation of quiescent cells. Peptidoglycan fragments are known to be secreted by actively growing cells, therefore we tested if *E. coli* peptidoglycan also stimulates proliferation of quiescent cells. We observed robust growth in the presence of peptidoglycan, suggesting that the peptidoglycan fragments function as a signal to prevent quiescence and stimulate proliferation. Together, these results suggest that the decision by UPEC to proliferate or become quiescent may be controlled by external signals including peptidoglycan. Moreover, the *in vitro* quiescent state demonstrated here may be similar to the quiescent intracellular reservoirs that occur during urinary tract infections.

130 A chemostat system to study the lung microbiome in a diseased model

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The lung microbiome may play a role in many common diseases including COPD, pneumonia and allergies where increased carbon dioxide levels may be present. In this project, a chemostat system was developed to study the interaction of resident lung microbes and how a diseased condition may impact their populations. Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Streptococcus pyogenes, Streptococcus pneumoniae and Klebsiella pneumoniae were cultivated together in a chemostat containing defined lung medium with and without increased carbon dioxide. Populations shifted

from an E. faecalis dominated culture to that of S. aureus and S. epidermidis when carbon dioxide levels increased. This model system offers a method to study pathogens of the lungs in a controlled setting and potentially could be used to complement in vivo studies

131 A Machine Learning Bioinformatics Tool for Predicting Natural Product Antimicrobial Activity

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We developed a machine learning-based bioinformatics tool that predicts bacterial natural product bioactivity using the natural product's biosynthetic gene cluster. We generated a training dataset of natural product biosynthetic gene clusters with known bioactivities and created a mathematical representation of the gene clusters using the number of times various protein features occurred. These features included PFAM domains, biosynthetic proteins, and genes with similarity to known resistance genes. We trained three commonly used classifiers, logistic regression, support vector machines, and random forests, on binary classification problems to predict the presence or absence of certain bioactivities. For this initial study we focused on different antimicrobial activities. All classifiers performed well, with balanced accuracies of at least 57%. A classifier for antibacterial activity performed especially well with an accuracy of 79%. Our tool will allow researchers to take advantage of the increasing amount of genetic data to prioritize bacteria that are most likely to produce natural products with desirable activities. We also determined that some biosynthetic genes are highly associated with antibacterial or antifungal activity, linking molecular features to function.

132 Bacterial Co-culture Inhibits *S. sanguinis* Hydrogen Peroxide Production via Diffusible Signal

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We previously identified a bacterial consortium of Staphylococcus saprophyticus, Streptococcus infantis, *and* Streptococcus sanguinis *that uses regulated H2O2 production for oral cavity colonization resistance.* S. infantis *normally inhibits* S. sanguinis' H2O2 production. Detection of an invader, *Escherichia coli*, triggers signal cascades resulting in de-repression of H2O2 production. This study aimed to elucidate the underlying regulatory mechanisms for H2O2 production in *S. sanguinis.*

Kanamycin-resistant S. sanguinis was grown with S. infantis, and CFU was monitored to determine if S. infantis inhibits S. sanguinis growth. Catalase/peroxidase activity of S. infantis was measured. S. sanguinis, with an spxB promoter-controlled luciferase reporter gene, was used to study the impact of the presence of S. infantis on spxB expression. Soft agar overlay assays were conducted to control for nutritional competition and isolate the co-cultured species. Along with S. infantis, a variety of other oral and non-oral bacterial species were tested.

Co-cultivation with S. infantis abolished H2O2 production in *S. sanguinis.* Our results showed that S. infantis *does not kill* S. sanguinis when grown in co-culture. *S. infantis* tested negative for catalase/peroxidase activity. Furthermore, S. sanguinis *bearing an* spxB-luc reporter gene displayed greatly reduced luciferase activity in the presence of *S. infantis* and its spent media. Additionally, the soft agar assays show that S. infantis *still inhibits* S. sanguinis' H2O2 production without physical contact or nutritional competition.

Together these data suggest that S. infantis *inhibits* S. sanguinis H2O2 production via diffusible signal(s), which could result in downregulation of *spxB* gene expression through a yet-to-be determined pathway.

133 Molecular mechanisms of transcriptional regulation by DnaA, the replication initiator, 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) to form a helical nucleofilament, promote DNA unwinding, and recruit replication machinery. DnaA, a AAA+ ATPase, is active for replication initiation and binds more cooperatively to oriC when ATP-bound than when ADP-bound. While DnaA must be in its ATP-bound form in order to initiate replication, initiation is regulated by anti-cooperativity proteins in B. subtilis, rather than by modulating the

nucleotide bound state, as in E. coli. DnaA also functions as a transcription factor, playing an important role in regulating its own transcription and in the response to replication stress. We established an in vitro system to dissect the molecular mechanisms by which B. subtilis DnaA affects transcription. We identified six genomic loci in B. subtilis that are directly bound by DnaA in vivo and in vitro, containing nine promoters regulated by DnaA. The number and arrangement of DnaA binding sites within each promoter is variable and determines how cooperatively DnaA binds. We asked whether DnaA-ADP is able to regulate transcription as well as DnaA-ATP. Surprisingly, we found that DnaA-ADP is active for repression, although it loses cooperativity. We also asked whether YabA, a negative regulator of replication initiation, negatively regulates DnaA's role as a transcription factor. Our current model is that DnaA is negatively regulated by YabA at highly cooperative promoters, but not at less cooperative promoters. Similarly, the nucleotide bound by DnaA has a larger effect at highly cooperative promoters.

134 A New Suite of Allelic Exchange Vectors for the Scarless Modification of Proteobacterial Genomes

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Despite the advent of new techniques for genetic engineering of bacteria, allelic exchange through homologous recombination remains an important tool for genetic analysis. Currently, sacB-based vector systems are often used for allelic exchange, but counter-selection escape, which prevents isolation of cells with the desired mutation, limits its utility. To circumvent this limitation, we engineered a series of "pTOX" allelic exchange vectors. Each plasmid encodes one of a set of inducible toxins, chosen for their potential utility in a wide range of medically important Proteobacteria. A codon-optimized rhaS transcriptional activator with a strong synthetic ribosome binding site enables tight toxin induction even in organisms lacking an endogenous rhamnose regulon. Expression of the blue amilCP or magenta tsPurple non-fluorescent chromoproteins facilitates monitoring of successful single- and double-crossover events using these vectors. The versatility of these vectors was demonstrated by deleting genes in Serratia marcescens, Escherichia coli O157:H7, Enterobacter cloacae, and Shigella flexneri. Finally, pTOX was used to characterize the impact of disruption of all combinations of the 3 orthologous S. marcescens peptidoglycan amidohydrolases on chromosomal ampC beta-lactamase activity and corresponding beta-lactam antibiotic resistance. Mutation of multiple amidohydrolases was necessary for high level ampC derepression and beta-lactam resistance. These data suggest why beta-lactam resistance may emerge during treatment less frequently in S. marcescens than in other AmpC-producing pathogens like E. cloacae. Collectively, our findings suggest that the pTOX vectors should be broadly useful for genetic engineering of Gram-negative bacteria.

135 Trehalose cycling promotes energy-efficient mycomembrane remodeling in nutrient-limited mycobacteria.

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The mycobacterial cell envelope counteracts environmental, immune and antibiotic insults. In particular, the mycomembrane layer of the envelope is a key permeability barrier. We find that there is cell-wide mycomembrane remodeling in nutrient-starved, non-replicating mycobacteria concomitant with a decrease in cell permeability. Remodeling is supported by cycling of trehalose, a non-mammalian glycan that shuttles fatty acid mycolates to the mycomembrane. In the absence of trehalose recycling, mycomembrane remodeling still occurs but mycobacteria experience redox stress. Redox stress is accompanied by enhanced oxygen consumption and lower ATP levels. We show that redox stress from depletion of the trehalose pool is suppressed in a mutant that is unable to synthesize the glycan *de novo*. As synthesis requires more ATP than recycling, our data suggest that trehalose recycling alleviates the energetic burden of mycomembrane remodeling. Loss of trehalose recycling attenuates *M. tuberculosis* during infection and makes the bacterium more susceptible to a variety of drugs. Targeting recycling pathways may be a new paradigm for sensitizing non-growing bacteria to host and antibiotic stress.

136 Mutational analysis of a putative sensor loop in essential *Escherichia coli* cell division protein FtsA

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Bacterial cell division is a highly conserved pathway across many prokaryotes. The key conserved component is FtsZ, a tubulin homolog which directs assembly of the divisome in *Escherichia coli* and most other prokaryotes. The FtsZ polymers, which assemble into the Z-ring at the site of cell division, are tethered to the inner face of the cytoplasmic membrane via FtsA, another highly conserved division protein. FtsA is an ATPase that shares structural homology with actin. Yeast actin has previously been shown to detect nucleotide occupancy through a sensor region adjacent to the nucleotide binding site. Accordingly, bacterial actin homologs display considerable conformational flexibility across different nucleotide-bound states. Here, we investigate FtsA ATP hydrolysis to determine how this activity is related to polymerization, phospholipid binding and reorganization and contact with FtsZ. To determine if residues in the putative FtsA sensor loop are necessary for protein function in vitro, we purified and characterized FtsA with mutagenized residues in the loop region, including FtsA(K86A) which is defective for ATP hydrolysis, and FtsA(S84L). While cells expressing Gfp-FtsA fusions containing the substitutions show fluorescent Z-rings, a functional assay using an *ftsA* temperature-sensitive strain suggests that FtsA(S84L) is defective for function. These studies will provide biochemical insight into nucleotide-dependent sensing and functional interactions of FtsA during cell division.

137 Phenotypic Profiling of *Mycobacterium tuberculosis* in Response to Drugs

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Tuberculosis remains difficult to cure. The standard regimen that lasts for a minimum of six months with multidrug therapy. This arduous therapy is necessary to prevent drug resistance and to ensure clearance of persisters (drug tolerant subpopulations). We critically need to develop more effective drug regimens. To accelerate the design of optimized therapies, we aim to develop a pipeline to rapidly identify pathways of action for new drugs and drug combinations. One of the ways that bacteria respond to drugs is through morphological changes such as changes in cell size and nucleoid shape. In other bacterial species, these pathways of drug can be rapidly determined by classifying cells by changes across a suite of morphological profiling as measured by whole-cell high-content imaging (called bacterial cytological profiling; BCP). We and others have found that different drugs elicit different cellular morphologies at the single-cell level in Mycobacterium tuberculosis (Mtb). We have overcome technical challenges posed by the unique growth properties of Mtb to develop a BCP platform for Mtb. Using BCP, we have categorized phenotypic responses of Mtb cells treated with drugs based on the molecular pathway targeted by the drug. Current ongoing studies with environmentally stressed Mtb cells will provide further insight into how different physiological states influence the response of the bacteria to antibiotics. We anticipate that cytological profiling will be an important tool for rationally designing TB therapy by identifying drugs and drug combinations that target novel pathways early in discovery.

138 Exploring the mechanism of lipid transport to the outer membrane of *Mycobacteria* in the LprG-Rv1410c pathway

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Mycobacterium tuberculosis (Mtb) infects one third of the global population and approximately 18% of TB treated cases are due to multiple drug-resistant strains. In recent years, considerable advances in understanding the molecular bases of *Mtb* pathogenicity and virulence have been made. One noteworthy contribution has been the identification of essential genes for *Mtb* survival, including the gene that codes for LprG. Loss of function of LprG and the co-cistronic integral membrane transporter Rv1410c results in *Mtb* strains that exhibit attenuated growth and virulence in the mouse model of infection. Since LprG-Rv1410c are both critical for *Mtb* virulence, the biochemical characterization of LprG functionality should improve our understanding of a potential drug target for *Mtb*. Thus far, we have established LprG as a lipid-binding protein that can transfer triacylglyceride (TAG) between lipid membranes. In this work we

focus on (a) the expression and purification of native acylated LprG for further validation of LprG TAG transfer activity using our previously established vesicle-based transfer assay and (b) the utilization of a spheroplast-based assay in the *Mtb* surrogate *Mycobacterium smegmatis* mc2155 to create a native lipid environment for confirmation of LprG TAG transfer activity. The spheroplast system will also be used to explore the functions and relationship between Rv1410c and LprG, towards building a first model of lipid transport to the mycomembrane.

139 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 and LprG is required for Mtb virulence in mouse models. Given the importance of LprG in Mtb virulence and physiological functions, we hypothesize that this lipid transport protein is a potential pharmacological target. To identify inhibitors of LprG, I conducted a computational virtual screening of 300,000 compounds to identify potential lead compounds. The binding affinities for top hit compounds to LprG were determined in vitro and the antimicrobial activity and target specificity of the highest-affinity compounds were characterized in mycobacteria. We successfully confirmed one compound that inhibited mycobacterial growth in an LprG-dependent manner. Similarity search and structure-activity relationship (SAR) analysis are currently in progress for better understanding the molecular basis of inhibitor binding and to discover more specific inhibitors. 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.

140 Peptidoglycan labeling of *Mycobacterium tuberculosis* to track metabolic heterogeneity

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Metabolic heterogeneity of Mycobacterium tuberculosis (Mtb) in vivo may contribute to variability in antibiotic response. Tracking the metabolic status of different Mtb subpopulations has the potential to improve treatment regimens, diagnostics and monitoring. Chemical probes such as fluorescent D-amino acids (FDAAs) provide readouts for bacterial peptidoglycan (PG) metabolism. We hypothesized that they also report Mtb's overall metabolic status. In the present work we utilized existing FDAAs and synthesized new ones to differentiate Mtb metabolic status in broth culture and in host cells. We used a double auxotroph strain ΔleuD ΔpanCD to mimic three different states viz. (a) non-replicating but viable (b) replicating and viable (c) nonreplicating and nonviable. We found two FDAAs (HADA and NADA) incorporate in PG of replicating and viable bacilli but another (RADA) labels all the three groups. We also investigated whether FDAAs could discriminate intra- and extracellular subpopulations of Mtb. For this, we developed a cell culture model for intracellular, replicating and non-replicating Mtb by infecting macrophages with ΔleuD ΔpanCD in presence or absence of leucine/pantothenate. We identified one set of probes that incorporated into Mtb both in broth and in macrophages, and a second set that labeled Mtb only in broth. Investigation whether first set of probes labels only replicating bacilli is on going Delineation of metabolic status for both intracellular and extracellular Mtb is the first step toward deploying FDAAs in vivo.

141 Designing effective combination therapies against *M. abscessus*

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Mycobacterium abscessus infection is the third most common Nontuberculous mycobacteria (NTM) infection. M. abscessus, a rapidly growing bacteria, is responsible for a wide range of disease in both im-

munocompromised and immunocompetent patients. Hence, the pathogen plays a critical role for the rapid rise of mortality rates caused by NTM agents in recent years. There are limited pharmacological options to treat M.abscessus as it is intrinsically resistant to different classes of antibiotics. It is therefore important that we expand the current treatment landscape of M.abscessus infections while prioritizing the potential of antimicrobial combination therapy. Due to the lack of systemized and feasible methods to measure higher order drug interactions, combination therapy has been limited in its effectiveness to discover new potential targets in NTM. To overcome this hurdle, we utilized a new platform (diagonal measurement of n-way drug interactions; DiaMOND) that allows us to measure the efficacies and interactions of higher order drug combinations in M.abscessus. Using fractional inhibitory concentrations (FICs) derived from our drug interaction model, we can classify combinations as synergistic, additive, and antagonistic. Our preliminary data suggests the emergence of new synergistic combination of drugs that can be prioritized in drug development and clinical trials. We hope to relate our in vitro measurements to in vivo conditions to better harness the power of combination therapy in the long-term.

142 Cutaneous infection caused by *Stenotrophomonas maltophilia* after Mohs Micrographic Surgery for Squamous Cell Carcinoma In Situ in an immunocompromised patient with Diffuse Large B Cell lymphoma

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S. maltophilia is a multi-drug resistant opportunistic pathogen rarely found in humans. Here, we present the first known reported case of cutaneous S. maltophilia infection in an elderly patient with Diffuse Large B Cell Lymphoma (DLBCL).

An 80-year-old Caucasian male with sleep apnea on CPAP and DLBCL status post therapy with rituximab, cyclophosphamide, doxorubicin, vincristine, Urelumab, Zevalin and prednisone presented to Dermatology clinic for excision of squamous cell carcinoma of his left pretibial leg. He underwent Mohs Micrographic Surgery of the lesion followed by complex linear closure. Over the next few days, the patient reported increased pain, bleeding and pus in the pretibial wound. Examination on post-operative day 9 was consistent with wound dehiscense complicated by local infection. Wound culture grew Staphylococcus aureus and Stenotrophomonas maltophilia. He was subsequently started on a 14 day course of doxycycline, followed with our wound care clinic and his infection resolved.

S. maltophilia has a high affinity for aquatic environments and most reported infections are associated with catheters, breathing tubes and lines. Previous reported cases of S. maltophilia causing infections in humans are few and mostly pulmonary, cardiac, ocular and gastrointestinal infections. However, our patient presented with cutaneous infection and did not exposure to aquatic environments nor any invasive lines. However, he was immunocompromised as a result of his DCLBL and subsequent treatment. As such, we propose that S. maltophilia should be considered in post-operative evaluations of immunocompromised patients.

143 Characterizing a novel dynamically unstable actin in the archaeon *Halobacterium salinarum*

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In the halophilic-archaea field, there are known tubulin homologs that control essential processes like cell division and cell shape. However, there is currently a lack of known actin filaments in haloarchaea, which sets a unique case in cell biology. Many organisms across all domains contain both sets of homologs, which can determine cell shape, segregate DNA, or help with cell division. So, the Garner lab carefully searched for actin candidates in *H. salinarum* and discovered one putative homolog, Haloactin. To understand Haloactin's potential function, the dynamics and localization of Haloactin-mNeonGreen in live cells was observed. The protein dynamics was very similar to that of dynamically unstable eukaryotic microtubules; the Haloactin filaments appear to nucleate from both poles of the cell, polymerize toward the center of the cell, and randomly depolymerize back to the pole. This data will be analyzed to determine the *in vivo* kinetics (polymerization, depolymerization, catastrophe rate based on filament length) of the polymer. Furthermore, we will perturb dynamics and analyze this perturbed state using titratable ATPase deficient Haloactin mutants. In addition, since this is the first dynamically unstable filament discovered in archaea and the second dynamically unstable actin across all domains, the in vitro kinetics of Haloactin polymerization will also be studied. The Haloactin has been purified from E. coli. so far. With this purified protein, the underlying

biochemical and kinetic basis of dynamic instability can be better understood.

144 Insights into antitubercular mechanism of action through the study of small molecule intrabacterial metabolism

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Tuberculosis, caused by *Mycobacterium tuberculosis* (*Mtb*), kills 1.5 million people annually. The rise of multi-drug resistant *Mtb* has to lead to an urgent need for new antibiotics with novel mechanism of actions. Antitubercular agents suffer intrabacterial metabolism resulting in either activation and/or detoxification, and perturbation of xenobiotic metabolism via genetic mutations can cause drug resistance. We have developed and optimized a methodology based on liquid-chromatography mass-spectrometry to study intrabacterial drug metabolism (IBDM). This method has been leveraged to study the modes of action of three antituberculars, JSF-2019, JSF-2164 and CD117. JSF-2019 is a novel triazine antitubercular uncovered via a dual-event naïve Bayesian model with in vitro bactericidal activity versus both replicating and non-replicating *Mtb*. IBDM study has revealed dual mechanisms for JSF-2019 as an intrabacterial NO• donor and an InhA inhibitor. The bacteriostatic antitubercular JSF-2164 was discovered via a docking/Bayesian screen in the search for a direct in vitro InhA inhibitor with whole-cell efficacy and low cytotoxicity. JSF-2164 is evidenced to suffer a rapid metabolism to afford two distinct amine metabolites with release of intrabacterial NO•. The antitubercular CD117 is bactericidal in *Mtb* partially due to the InhA inhibition. The intrabacterial metabolism study suggests a two-step detoxification via a sulfide oxidation followed by a cysteine nucleophilic aromatic substitution. Overall, this work places emphasis on intrabacterial metabolism as an approach to mechanistically comprehend antitubercular activity as well as resistance, which provides insights as to the optimization of current antitubercular agents to overcome drug resistance.

145 Mechanisms of metabolic interaction between *Corynebacterium matruchotii* and oral streptococci

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Human supragingival plaque is a polymicrobial biofilm on the tooth surface above the gumline, comprised of ordered structures with spatial associations between specific bacterial taxa. Recent microscopy and microbiome data has suggested that plaque structure is scaffolded by the abundant filamentous bacterium, *Corynebacterium matruchotii*, decorated with *Streptococcus* species including S. mitis *and* S. cristatus. *By comparing mono vs coculture growth we discovered that aerobically* S. mitis *and* S. cristatus *growth is stimulated by* C. matruchotii. Anaerobically, S. mitis *inhibits* C. matruchotii *which could explain one facet of community structure as* Streptococcus *species only decorate* C. matruchotii in the aerobic perimeter of plaque structures. Transcriptome (RNASeq) analyses of these cocultures reveal that *C. matruchotii* increases expression of lactate catabolism genes (lutABC) aerobically with S. mitis *or* S. cristatus *but not anaerobically where its growth is inhibited. We hypothesize that* C. matruchotii *crossfeeds on* Streptococcus-produced lactate which could allow for pH stress relief in the community. Together these findings may explain mechanisms underlying the spatial arrangement of these species in a polymicrobial community. We have constructed a *lutA* knockout mutant in *C. matruchotii*, no longer able to utilize lactate, and are in the process of testing it in coculture with S. cristatus *and* S. mitis. *We hypothesize that* C. matruchotii will be less fit in coculture without the ability to crossfeed on Streptococci-produced lactate and that growth benefit to streptococci will be diminished.

146 *Galleria mellonella* infection reveals *Coxiella burnetii* effector mutants that display altered virulence phenotypes

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To explore the role of Coxiella burnetii secreted bacterial effector proteins during infection of a multicellular host, we monitored survival of Galleria mellonella larvae infected with 68 isogenic Nine Mile Phase

II C. burnetii strains. We found that as expected, CoxIgA, cig57 and cig2 disruption led to reduced virulence in the wax moth larvae. Eight different mutants were hypovirulent and three were hypervirulent when compared to wild-type Coxiella NM II by two measures. Four of the seven hypovirulent strains, including a cig2::Tn mutant, were also at a disadvantage in a competition assay that included co-infection with a wild-type NMII strain. These strains did not demonstrate pronounced replication defects in whole larvae or HeLa cells, indicating that host death phenotypes resulted from differences in host tolerance or from failure of the bacteria to undergo multiple rounds of host cell infection. Two of the hypervirulent strains increased the IMD-dependent antimicrobial peptide response in Drosophila S2 cells. One predicted effector, YebC, appeared to be important for production of infectious progeny in vitro from HeLa cells. In addition, a YebC::tn strain expressing bacterial luciferase was less viable than wild-type during in vivo infection and visualization of Galleria larvae. Thus, the larval infection model has revealed Coxiella effector genes that modulate conserved aspects of the host innate immune system and have specific functions during multicellular host infection.

147 Investigating the Post-Transcriptional Effects of the sigA 5' UTR on Gene Expression

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Mycobacterium tuberculosis (MTB) causes tuberculosis, which sickens over 10 million people per year. Despite harsh environmental stresses inside the human host, MTB is able to survive through adaptation and regulation of its gene expression. MTB accomplishes this in part by regulating its mRNA stability. In *Escherichia coli*, 5' Untranslated Regions (UTRs) have been shown to affect mRNA stability; however, this has yet to be shown in mycobacteria. In both MTB and the non-pathogenic model *Mycobacterium smegmatis*, the essential sigma factor, SigA, has an unstable transcript with a relatively short half-life. We hypothesized that *sigA*'s long 5' UTR caused this instability. To test this, we constructed fluorescent reporters and demonstrated that the *sigA* 5' UTR has a modest effect on expression in *M. smegmatis*, and this effect appears to be mediated by altered translation efficiency. Surprisingly, the first 54 nts of the *sigA* coding sequence substantially decreased expression, and this effect appeared to be attributable to reduced transcription and/or reduced mRNA stability.