BOSTON BACTERIAL MEETING

Image courtesy of the Pael laboratory.

KEYNOTE SPEAKER:

ROBIN PATEL, M.D.

DIRECTOR, INFECTIOUS DISEASES RESEARCH LABORATORY
MAYO CLINIC

JUNE 13 - 14, 2022

HARVARD SCIENCE CENTER

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CONTENTS

AGENDA

Day 1: Monday, June 13

09:00 AM	**Opening Remarks**	

I. MICROBIOME & MICROBIAL COMMUNITIES

Session Chair: Ana Maldonado-Contreras

09:10 AM	Veda Khadka *Massachusetts Institute of Technology*	Application of commensal bacteria following mechanical injury influences tissue repair
09:30 AM	Tabita Ramirez-Puebla *Forsyth Institute*	Improved evaluation of the micron-scale spatial organization of the human tongue dorsum microbiome using plastic sections
09:50 AM	Alan Varghese *New York University*	Modeling multi-cellular behavior in *Salmonella Typhimurium*
10:10 AM	Swathi Penumutchu *Brown University*	Fiber prebiotics alleviate antibiotic-induced gut dysbiosis through modulation of bacterial metabolism
10:30 AM	**Break & Poster Session 1**	

II. MICROBIAL PATHOGENESIS

Session Chair: Amy Rohlfing

11:00 AM	Hannah Trautmann *University of Rhode Island*	Investigating heterogeneous ribosomes and gene expression in the pathogen *Francisella tularensis*
11:20 AM	Matthias Groeschel *Harvard Medical School*	Host-pathogen co-adaptation shapes susceptibility to infection with *M. tuberculosis*
11:40 AM	Gregory Babunovic *Brigham and Women's Hospital*	CRISPR interference reveals that all-*trans*-retinoic acid promotes macrophage control of *Mycobacterium tuberculosis* by limiting bacterial access to cholesterol and propionyl-CoA
12:00 PM	Nadja Leimer *Northeastern University*	A selective antibiotic for Lyme disease
12:20 PM	**Catered Lunch**	
01:30 PM	**KEYNOTE ADDRESS - Robin Patel, M.D.**	
02:30 PM	**Break & Poster Session 2**	

III. TRANSCRIPTIONAL ADAPTATION & STRESS RESPONSES

Session Chair: Shawna Reed

3:15 PM	Elizabeth Hart *Harvard Medical School*	The conserved sigma D envelope stress response monitors mycobacterial envelope integrity
3:35 PM	Valdir Barth *Boston Children's Hospital*	Uncovering cysteine-rich small ORFs in *Mycobacterium tuberculosis* through toxin-mediated ribosome stalling
3:55 PM	Indu Warrier *Boston College*	RNA regulators in *Streptococcus pneumoniae* control metabolism and pathogenesis
4:15 PM	Jeffrey Duncan-Lowey *Yale University*	The *Coxiella* dot/icm effector protein EmcB is a ubiquitin protease that antagonizes RIG-I signaling
4:35 PM	**Break & Poster Session 3**	
5:30 PM	**End of Day 1**	

Day 2: Tuesday, June 14

IV. BACTERIAL PHYSIOLOGY

Session Chair: Josué Flores-Kim

09:00 AM	Constantin (Nick) Takacs *Stanford University*	Novel control of ParA activity and chromosome segregation in vector-borne *Borrelia burgdorferi*, the Lyme disease spirochete.
09:20 AM	Irina Shlosman *Harvard Medical School*	Structural dynamics of PBP2 act as a regulatory switch in peptidoglycan synthesis
09:40 AM	Katherine Hummels *Harvard Medical School*	A regulatory interaction between committed enzymes links lipopolysaccharide and peptidoglycan biogenesis in *Pseudomonas aeruginosa*
10:00 AM	Shailab Shrestha *Tufts University*	*Clostridioides difficile* uses distinct cell division machineries during spore formation and vegetative growth
10:20 AM	**Break & Poster Session 4**	

V. MICROBIAL GENOMICS

Session Chair: Marian Schmidt

11:00 AM	Kevin Yang *New York University*	Functional characterization of the rifampicin binding site
11:20 AM	Saria McKeithen-Mead *Massachusetts Institute of Technology*	Mistimed integration of an integrative and conjugative element leads to death in naïve bacterial hosts
11:40 AM	Brendan O'Hara *Tufts University*	The *Vibrio cholerae* Seventh Pandemic islands act in tandem to defend against a circulating phage
12:00 AM	Anurag Limdi *Harvard Medical School*	Parallel changes in gene essentiality over 50,000 generations of evolution
12:20 PM	**Breakout Session 1**	
1:30 PM	**Lunch (not catered)**	
2:30 PM	**Breakout Session 2**	

VI. INDUSTRY

Session Chair: Minmin Yen

3:30 PM	Alicyn Pearson *T2 Biosystems*	T2Biothreat Panel: A blood culture-independent diagnostic for biothreat pathogens
3:50 PM	Defne Surujon *Day Zero Diagnostics*	Direct bacterial species identification from blood for rapid sepsis diagnostics
4:10 PM	Silvia Caballero *Vedanta Biosciences*	Defined bacterial consortia, a microbiome-based approach to tackle infections with antibiotic-resistant bacteria
4:30 PM	**Closing Remarks**	
4:45 PM	**Reception and Networking**	
6:30 PM	**End of BBM 2022**	

WELCOME TO BBM 2022!

Dear Participants,

Welcome to the 28th annual Boston Bacterial Meeting! We are looking forward to two incredible days of scientific presentations and discussions.

Over the last 28 years, BBM has offered an intellectually stimulating and collegial atmosphere for bacteriologists throughout the New England area to discuss cutting-edge scientific research and share ideas. We are so pleased to see you in person again this year, and want to welcome all of our new participants!

BBM is made possible by our passionate volunteer Organizing Committee which is primarily composed of graduate students and postdocs. Their innovation fosters BBM's continuous growth and we are proud of their dedication and commitment to constant improvement. Our Organizing Committee has worked especially hard to transition this meeting back from its virtual iterations and we thank them for their valiant efforts. This year we are pleased to offer four poster sessions, two breakout sessions, and a networking reception at the end of day 2. We hope that these spaces facilitate networking, collaborations, and foster new mentor-mentee relationships.

While BBM brings together bacteriologists from both academia and industry, in past years the presented oral talks have been heavily academic-centric. This year we hope to expose our attendees to the innovative and exciting work being conducted in industry research space. We are dedicating the last session of the meeting to a selected group of talks from within the biotechnology and pharmaceutical arena. Additionally, many companies have come to BBM with a recruiting team and we encourage you all to meet them and learn more about the non-academic research space.

This year we are excited to feature Dr. Robin Patel as our keynote speaker. Dr. Patel is the Elizabeth P. and Robert E. Allen Professor of Individualized Medicine and the director of the Infectious Disease Research Laboratory, co-director of the Clinical Bacteriology Laboratory, vice chair of education in the Department of Laboratory Medicine and Pathology, and former chair of the Division of Clinical Microbiology at the Mayo Clinic. Dr. Patel is a research powerhouse; her laboratory studies multiple critical components of bacteriological infections in clinical settings and has generated over 500 peer-reviewed publications as well as consistent funding from the National Institutes of Health. Please join us in welcoming Dr. Patel to BBM, as we look forward to hearing about her current work.

We are indebted to our faculty sponsors (Drs. Tom Bernhardt and Ethan Garner) and meeting founders (Drs. Rich Losick, Roberto Kolter, John Mekalanos, and Andrew Wright) and thank them for their continuing guidance. We would also like to thank our generous sponsors who have made this meeting possible. On behalf of the entire Organizing Committee, we would like to thank you for joining us. We hope you will find the next two days both intellectually and socially fulfilling.

Jenna Wurster and Suyen Espinoza

BBM 2022 Co-chairs

MEETING ANNOUNCEMENTS

- Please respect data confidentiality! Photography or recording of any research is strictly prohibited without explicit approval of the presenter.

- Poster sessions, meals, and the networking reception will all be held in the plaza tent located in front of the Harvard Science Center. Be sure to show your badge to gain access to this venue.

- This year we have two breakout session times on Tuesday June 14th. Breakout session locations and times can be found within this abstract book. Please reach out to a member of the Organizing Committee if you require assistance in locating a session location.

- Masking is required at all times within the Harvard Science Center and is optional within the Plaza Tent. Please review the BBM COVID-19 safety policies on our website.

- A Mother's Room is available in Room 304 of the Harvard Science Center. Please ask a member of the Organizing Committee if you require any assistance surrounding its use.

- BBM maintains a strict stance against harassment in any form. Please review our code of conduct. Any individuals engaging in harassing behavior will be subject to immediate removal from the meeting and be banned from future meetings.

- Any use of profanity or obscene imagery will result in immediate removal from the meeting and the individual will be banned from future meetings.

- Please use the hashtag #bostonbacterial22 on Twitter when discussing this year's meeting!

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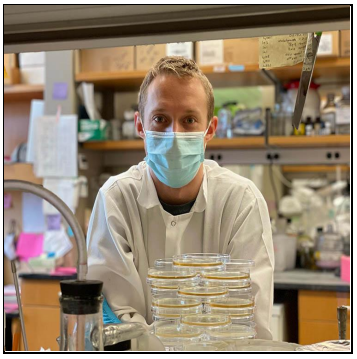

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BBM's Stance on Discrimination, Racism, and Harassment

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

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

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

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

- Offering a dedicated space for discussion on race and representation in the sciences by holding a breakout session on Diversity in STEM.

- Continuing to offer our "Underrepresented Minorities in Biomedical Sciences Scholarship" which waives the registration fee.

- Expanding our scholarship to include a Travel Award to help underrepresented minority microbiologists attend our meeting. All proceeds from our online store will go towards furnishing this award for next year. You can also support our scholarship by donating here. If you or your company wish to sponsor this endeavor, contact us at bbm@hms.harvard.edu.

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

In Solidarity,

The 2022 BBM Organizing Committee

KEYNOTE SPEAKER

Robin Patel, MD

Robin Patel is the Elizabeth P. and Robert E. Allen Professor of Individualized Medicine and the director of the Infectious Disease Research Laboratory, co-director of the Clinical Bacteriology Laboratory, vice chair of education in the Department of Laboratory Medicine and Pathology, and former chair of the Division of Clinical Microbiology, at the Mayo Clinic.

Since the beginning of her tenure at the Mayo Clinic, Dr. Patel has focused her research on multiple critical components of bacteriological infections in clinical settings. Her work primarily focuses on three major areas: (1) improvement of next-generation diagnostics techniques, (2) understanding the inherent biology of periprosthetic biofilm formation, and (3) understanding antibiotic resistance within the clinic. She has published over 500 peer-reviewed publications and is well supported by the National Institutes of Health. Since the start of the SARS-COV-2 pandemic, Dr. Patel has used her expertise in diagnostics to be a prominent voice in scientific communication with the public.

To say Dr. Patel's pedigree is impressive would be an understatement. She received her BA in Chemistry from Princeton University, where she graduated magna cum laude with nomination to Sigma Xi. From there, she obtained her MD from McGill. Afterwards, Dr. Patel completed her internal medicine residency at the Mayo Clinic before completing fellowships in Clinical Microbiology and Infectious Disease where she secured awards from both the Mayo Clinic and the American Society for Microbiology. Since then, she has been significantly involved in setting the standards for diagnostic and clinical care of bacterial infections in the United States, as evidenced by the positions she has held or holds within the American Society for Microbiology (President), American Board of Pathology (Microbiology Test Writing Committee Member), Clinical and Laboratory Standards Institute (Subcommittee on Antimicrobial Susceptibility Testing Voting Member), National Institutes of Allergy and Infectious Diseases (Council Member), National Board of Medical Examiners (Microbiology/Immunology Test Material Development Committee Member and Chair), Journal of Clinical Microbiology (Associate Editor), and Clinical Infectious Diseases (Associate Editor).

In addition, Dr. Patel's continued commitment to mentorship can be translated into a long list of students from around the world; she had dedicated hours of teaching to train the next generation of scientists and MDs. This profile information was adapted from:
https://journals.asm.org/doi/10.1128/JCM.01259-20.

2022 BBM Session Chairs

Josué Flores Kim, Ph.D.

Assistant Professor
Department of Biochemistry and Molecular Biotechnology
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Josué was born and raised in Puerto Rico, where he obtained his undergraduate degree in Industrial Biotechnology at the University of Puerto Rico-Mayagüez campus. He moved to New York City to do his Ph.D. in molecular microbiology at the Vilcek Institute of Graduate Biomedical Sciences at NYU School of Medicine under the guidance of Dr. Andrew J. Darwin. His thesis focused on understanding how signal transduction is achieved in the Phage-shock-protein (Psp) system of *Yersinia enterocolitica*, an extracytoplasmic stress response that is essential for the virulence of several bacteria. For his postdoctoral studies, Josué joined Tom Bernhardt and David Rudner's laboratories in the Microbiology department at Harvard Medical School. The general scope of his postdoc research was to study cell envelope biosynthesis pathways in the human opportunistic respiratory pathogen *Streptococcus pneumoniae*, which he will continue to investigate as an Assistant Professor in the Biochemistry and Molecular Biotechnology (BMB) department. Josué is a member of the Biochemistry and Molecular Biotechnology (BMB) and Immunology and Microbiology Graduate programs, and the Institute for Drug Resistance (IDR).

Ana Maldonado-Contreras, PhD.

Assistant Professor
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Dr Maldonado-Contreras is a clinical translational researcher focused on the intersection of the microbiome, diet, and disease. Her lab investigates how diet manipulates the gut microbiota and how this can be harnessed to improve patient outcomes. Inflammatory Bowel Disease (IBD) imposes a significant burden on healthcare and the economy in the developed world. IBD is characterized by the chronic inflammation of the gastrointestinal tract; however, despite the impact of this disorder, nutrition-based treatments are poorly developed. The IBD anti-inflammatory diet (IBD-AID) is a long-term dietary pattern consisting of probiotic and prebiotic foods to establish a stable community of anti-inflammatory commensal bacteria in the gut, without the need for dietary supplements. Dr Maldonado-Contreras investigates the mechanism of action behind the reduced inflammation displayed by this diet-directed microbiota. Her lab also researches a version of this diet adapted for pregnant women, to help modulate the microbiome of babies and reduce inflammatory

potential. In addition, in partnership with the University of Puerto Rico's Center for IBD and UMass Lowell's Center for Population Health, she is developing culturally tailored behavioral interventions to ameliorate IBD symptoms among Hispanics, a population in which IBD prevalence is rapidly increasing.

Dr Maldonado-Contreras, originally from Venezuela, received her Bachelor's and Master's in Punto Fijo and Caracas, respectively, before completing her PhD in the group of Dr Maria Dominguez-Bello at the University of Puerto Rico. She conducted post-doctoral research at the University of Massachusetts Chan Medical School with Dr Beth McCormick and was awarded a Charles A. King Trust Postdoctoral Research Fellowship. Dr Maldonado-Contreras became a faculty member in the Microbiology and Physiological Systems department at the university in 2015 with support from the Faculty Diversity Scholar Program.

Amy Rohlfing, Ph.D.

Assistant Professor
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Dr. Amy Rohlfing received her Ph.D. in Microbiology and Immunobiology from Harvard University. For her graduate thesis, she studied regulation of virulence gene expression in *Francisella tularensis* in the lab of Dr. Simon Dove at Boston Children's Hospital and Harvard Medical School. She then made the move across Boston for her postdoctoral studies to work with Dr. Aimee Shen at Tufts University School of Medicine to study *Clostridioides difficile* spore germination. During her time at Tufts, Dr. Rohlfing was also able to combine her love of research and teaching as an IRACDA (Institutional Research and Academic Career Development Award) postdoctoral fellow. Dr. Rohlfing is now an Assistant Professor at Saint Anselm College in Manchester, New Hampshire where she splits her time between teaching and doing research with undergraduate students.

The Rohlfing lab studies how *Clostridioides difficile* spores sense germination signals. *C. difficile* is the leading cause of hospital-acquired infections world-wide and the leading cause of gastroenteritis associated death in the United States. As an obligate anaerobe, *C. difficile* relies on its aerotolerant spore form to cause infections. In order to cause disease, the dormant spores must germinate in the small intestine in response to bile salts and nutrient signals (calcium or amino acids) and outgrow into vegetative toxin-producing cells. *C. difficile* lacks the Ger-family receptors common in other spore-forming organisms and instead senses germination signals using two related pseudoproteases. The Rohlfing lab is interested in how these pseudoproteases are able to sense and integrate the small molecule germination signals as well as how variations in environment during spore formation affect the sensitivity of spores to the various germination signals.

Marian L. Schmidt, Ph.D.

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My lab studies the microbial inhabitants of aquatic ecosystems through a combination of field, molecular and computational approaches. Despite their tiny size, microbes are major nutrient recyclers and can have system-level impacts in freshwater ecosystems. They can cause partial or complete deoxygenation of bottom waters or large-scale harmful cyanobacterial blooms, so I am particularly interested in how environmental variation due to climate change impacts the activity, biodiversity, community structure and succession, and genome dynamics of aquatic microbial communities. I also have a special interest in aquatic microhabitats, like particulate organic matter, within these systems, as they tend to be microbial meeting points and have a different ecological and evolutionary trajectory than microbes that live freely in the water.

Shawna C. Reed, Ph. D.

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I am a biologist whose research interests lay in the relationships between eukaryotic cells and their occasional microbial interlopers, having studied HPV, *Rickettsia*, and *Coxiella*. Currently I am an assistant professor at Quinnipiac University, an undergraduate-focused institution in Hamden, CT. I teach a variety of classes, primarily General Microbiology and Cell Culture with a small research team of undergraduate and Master's students. I have hopped across the country from Arizona to Virginia (BS at Randolph-Macon Woman's College) to Maryland (post-bac IRTA at NIH/NIAD) to California (PhD at UC Berkeley, PMB) to Connecticut (Post-doc and CIRTL teaching certificate at Yale University) and I'm happily settled in New Haven, Connecticut.

As a new professor and growing educator, I realized that my own excellent undergraduate professors were using current methods of student-centered, responsive pedagogy two decades ago. I engage in Scholarship of Teaching and Learning (SoTL) research to improve microbiology teaching by integrating primary literature and active learning into the classroom, and by assessing inclusive teaching practices. I am also interested in CURE development for cell biology teaching.

In my research laboratory, I explore the biology of the obligate intracellular bacterium, *Coxiella burnetii.* Coxiella secrete over 140 effector proteins across a parasitophorous vacuole membrane and into the eukaryotic host cell. We are studying these proteins with a goal of generating a complete picture of

the localization and predicted function of all uncharacterized effectors. We hope to understand which pathways and organelles in a eukaryotic cell must be modulated to allow survival of intracellular microbes. Currently we are exploring how one effector protein, CvpG, modulates host cell vesicular trafficking to promote Coxiella growth. We are also researching the development of Coxiella during the infectious life cycle and methods for Coxiella genetic manipulation.

Minmin Yen, Ph.D., MPH

CEO & Co-Founder
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PhagePro is a Boston-based biotechnology company pioneering the use of bacteriophages to prevent bacterial infections and reduce antibiotic resistance in the most vulnerable communities around the world. Under the leadership of its founding Chief Executive Officer Dr. Minmin Yen, Ph.D., MPH, the team is currently optimizing ProphaLytic-Vc (PVC), an orally dosed bacteriophage preparation specifically targeted towards epidemic strains of cholera, for use in real-world settings. In addition, PhagePro is exploring the use of cholera phages to target antibiotic-resistant *Vibrio cholerae*, the causative agent of cholera, and to reduce transmission of antibiotic resistance genes. Cholera remains a significant public health issue in resource-limited areas of the world, where drinking water and sewage are not always separated and treated. An estimated 3-5 million cases of cholera occur each year around the world. At least 100,000 of these cases lead to death. PhagePro is actively working with other cholera experts in the field to provide innovative and broadly effective solutions to this pervasive epidemic, including the world's leading cholera hospital, the International Centre for Diarrhoeal Disease Research, Bangladesh (iccdr,b).

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.

Session I: Career Topic Discussions

June 14, 2022 12:20-1:20pm

Diversity, Equity, and Inclusion in STEM (Room 411)

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

Panelists: Ivan Albino Flores; Bryan Bryson, PhD; Ross Marshall; Katherine Suarez

Careers Beyond the Bench (Hall D)

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

Panelists: Anneke Claypool, PhD; Sandra Dedrick, PhD; Harshita Grover, PhD; Heather Olins, PhD; Martin Strebl-Bantillo, PhD

Careers in Industry/Biotechnology (Hall E)

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

Panelists: Mark Charbonneau, PhD; Jared Kehe, PhD; Sina Mohammadi, PhD; Mimi Yen, PhD, MPH

Mentorship in Science (Room 310)

Effective mentorship requires self-reflection, setting expectations, building trust, and regular review. Learn from seasoned mentors how mentoring evolves, how to foster the next generation of scientists, and how their past experiences have shaped their approach to mentorship in science.

Panelists: Emily Goering; Fatima Hussain, PhD; Aimee Shen, PhD

Food Microbiology and Brewing (B10)

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

Panelists: Charlie Hoxmeier, PhD; Moshe Shemesh, PhD

Session II: Research Topic Discussions

June 14, 2022 2:30-3:30pm

Antibiotic Resistance and Beyond (Hall D)

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

Panelists: Thea Brennan-Krohn, PhD; Andrea Etter, PhD; Kim Lewis, PhD; Eric Stern, PhD

Computational Microbiology (Room 411)

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

Panelists: Bree Aldridge, PhD; Eric Alm, PhD; Jeff Gore, PhD; Mo Kalil, PhD

Host-Pathogen Interactions (B10)

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

Panelists: Sarah Fortune, MD; Rebecca Lamason, PhD; Amanda Prince, PhD; Shumin Tan, PhD;

Synthetic Microbiology (Room 310)

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

Panelists: James Collins, PhD; Dechuan Meng, PhD; Analise Reeves, PhD; Eammon Riley, PhD

Microbiome and Microbial Communities (Hall E)

Do you wonder about the dynamics of microbial communities that impact our environment and our health? Join us for an exciting discussion on microbe-microbe, microbe-host, and microbe-environment interactions.

Panelists: Kevin Bonham, PhD; Esther Miller, PhD; Tu Nguyen, PhD; Andrea Unzueta Martinez PhD

ABSTRACTS FOR ORAL PRESENTATIONS

1

Application of commensal bacteria following mechanical injury influences tissue repair

Veda D. Khadka*,1 Laura Markey*,1 Tami Lieberman[1]
[1]Massachusetts Institute of Technology

The skin is continuously colonized by a diverse microbiota. Understanding the ability of the immune system to tolerate commensal microbes without generating inflammatory responses holds promise for the prevention of infection and development of effective probiotic therapies. *Staphylococcus epidermidis*, one of the most abundant bacterial species on human skin, is generally considered model commensal and potential skin probiotic. However, some *S. epidermidis* strains have been implicated in pathogenesis, and 'commensal-like' *S. epidermidis* strains have produced various results in mouse models of barrier damage.Here, we conducted a mechanical injury model of skin barrier damage and found that damage-concurrent exposure to *S. epidermidis* and other Staphylococci, including mouse commensal species, delays barrier repair. The *S. epidermidis* response involved an overall increase in bulk T-cell populations as well as specific increases in CD8+ and TCR $\gamma\delta$+ cells. Furthermore, the delay in tissue repair was not dependent upon the context of primary exposure. To understand whether this exacerbated inflammatory response was common to all bacteria, we tested the gut probiotic *Lactobacillus reuteri*, which improved the overall rate of healing. Mice were further exposed to a panel of bacteria in the context of a full, minimal, or absent host microbiota; a more complete microbiota greatly aided in improving healing outcomes. Here we present preliminary results from flow cytometry , bulk T-cell and whole-skin transcriptomics to characterize the basis of this response.

2

Improved evaluation of the micron-scale spatial organization of the human tongue dorsum microbiome using plastic sections

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The human tongue has a large surface area with thousands of papillae providing microhabitats for microbial colonization. Bacteria on the tongue dorsum form large consortia of tens to hundreds of microns composed of multiple layers of bacteria organized around a core of epithelial cells. Understanding the spatial organization of these consortia is crucial to decipher the microbe-microbe and host-microbe interactions occurring in the community. Previous work using whole mount preparations of tongue consortia has revealed a patchwork mosaic of bacterial organization. However, a barrier to fully analyzing the structure of the consortia has been their thickness and 3-dimensional complexity. To overcome this barrier, we have investigated an alternative approach; namely, embedding in hydrophilic plastic, sectioning, and 3-D reconstruction. Samples were labeled post-sectioning by hybridization with multiplexed fluorescent oligonucleotide probes and visualized by spectral imaging and linear unmixing. Successive optical and physical sections were spatially registered computationally to enable 3D reconstruction. Proximity analysis was carried out to determine micron-scale spatial associations. With this approach, we detected taxa whose abundance was underestimated in our previous whole mount studies. These included *Prevotella*, *Fusobacterium*, *Leptotrichia*, and bacteria from family Lachnospiraceae. We detected patterns of spatial distribution like *Prevotella* at the periphery and *Leptotrichia* at the core of the consortia. Through three-dimensional reconstruction we observed that the abundance of *Actinomyces* and *Rothia* differed between the core and the edge of the consortium. Our approach effectively provided an improved analysis of the three-dimensional complexity of the human tongue dorsum microbiome.

3

Modeling multi-cellular behavior in *Salmonella* Typhimurium

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Bacteria face diverse stresses in the environment and, sometimes, respond by forming multi-cellular structures such as biofilms. Biofilm formation in the body during infections makes treatment and resolution difficult, as they display increased resistance to nearly all antibiotics. The mechanism of formation of such microbial communities has been heavily studied but there is little information regarding their maturation, maintenance, and dispersal. We recently reported a novel multi-cellular structure formed by *Salmonella* Typhimurium under reducing stress in minimal media, which resembles small (1-cm long) strings. These strings closely mimic biofilms seen in the clinic, in terms of enabling growth and survival under stress, requirement of cellulose for formation and increased resistance to antibiotics. They are, however, free-floating, enabling easy visualization of their formation and maturation using confocal microscopy. Here, utilizing strings as a model for multicellular behavior and economic principles from game theory, we study how multi-cellular structures mature and reach a terminal size. Our models utilize a two-player repeated game, wherein the two decision making players are the outer layer (facing maximum string-inducing stress but minimal nutrient deprivation) and the innermost layer (facing maximal nutrient deprivation but minimal string-inducing stress). Using the game-theoretic models and confocal imaging we predict how multi-cellular bacterial communities decide to mature and show the need for quorum sensing in it. Using the results we obtained, we propose a combination of antibiotics and signaling molecules that activate quorum sensing, as a novel method to increase the sensitivity of biofilms to therapeutics and enable their clearance.

4

Fiber Prebiotics Alleviate Antibiotic-induced Gut Dysbiosis through Modulation of Bacterial Metabolism

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Antibiotic-induced gut dysbiosis is currently a frequent and serious side effect of antibiotic use. Host diet can be a therapeutic target to modulate the structure and function of the microbiome during antibiotic treatment. In this study, we utilize metagenomic and metatranscriptomic sequencing combined with *de novo* gene assembly to elucidate changes to the microbiome during diet modulation and antibiotic treatment. Using a murine model, we found that supplementation of fiber prebiotics alleviates the dysbiotic effect of antibiotics, increasing microbial diversity post-antibiotics. Conversely, glucose supplementation exacerbated the dysbiotic effects of antibiotics. We observe transcriptional signatures of high-energy metabolism in glucose supplemented mice compared to fiber-supplemented mice.Glucose led to increased expression of metabolic reactions with higher redox potential. Increased transcriptional activity of high-energy electron transfers and oxidative metabolism was observed. This suggests that dietary carbon source can affect the energetic environment of the gut leading to selection of higher energy electron acceptors such as oxygen and nitrate. These transcriptomic changes in redox potential also correlated to increased abundance of MAGs containing the high-energy ETC protein Complex I. These data suggest that diet or xenobiotics targeting microbial metabolism in the gut could be an effective tool to protect the microbiome from antibiotic stress.

5

Investigating heterogeneous ribosomes and gene expression in the pathogen *Francisella tularensis*

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Francisella tularensis is a highly infectious and potentially fatal human pathogen that must replicate in macrophage cells during infection. The regulation of specific genes to allow for survival in macrophage is incompletely understood. In prokaryotic and eukaryotic systems, ribosome heterogeneity has been implicated in gene regulation. The *F. tularensis* genome encodes three distinct homologs of the small ribosomal protein bS21, a protein important for translation initiation. This raises the possibility that ribosomes are heterogeneous with respect to bS21 in this pathogen. We determined that wild-type *F. tularensis* cells have heterogeneous ribosomes and each bS21 homolog can be incorporated into actively translating ribosomes. To understand the contribution of each homolog to translation, we have generated cells lacking each bS21 homolog individually and assessed which proteins are differentially abundant. Our work has identified that cells lacking bS21-2 have specific changes in protein abundance that cannot be explained by changes in transcript abundance, indicating that bS21-2 is regulating gene expression at the level of translation. Among the proteins that are positively regulated by bS21-2 are many of the proteins that make up the type VI secretion system, which is absolutely essential for virulence. Additionally, cells lacking bS21-2 have an intramacrophage growth defect. Our research supports a model in which bS21 homologs regulate distinct transcripts, leading to differential translation with downstream impacts on virulence.

6

Host-pathogen co-adaptation shapes susceptibility to infection with *M. tuberculosis*

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The study of evolution of host-pathogen relationships and notably pathogenic phenotypes is fundamental to understanding the emergence and control of infectious diseases epidemics. The obligate human pathogen M. tuberculosis (Mtb) is hypothesized to have co-adapted to specific human populations in the form of ecological specialization and differential transmissibility. Based on Mtb's unique phylo-geography, a group of geographically restricted lineages has been hypothesized to be niche specialists that preferentially infect local human subpopulations. However, the lack of contact tracing data directly measuring transmissibility in cosmopolitan societies with mixing of human and pathogen populations has hindered the study of naturally occurring co-adaptation in Mtb. Here, using a multi-site cohort of tuberculosis index cases with pathogen sequence data and linked social contacts, we show that specialist Mtb lineages L1,L2spec,L3,L4spec,L5,L6 are intrinsically less transmissible than generalist Mtb lineages (L2gen,L4gen). We speculated that the lower transmissibility stems from most exposed contacts in the cohort being allopatric to specialist Mtb lineages. We found that sympatric host-pathogen exposures led more frequently to TB infections, supporting the hypothesis of co-adaption between human subpopulations and Mtb specialist lineages. Furthermore, we found evidence of co-adaptation in macrophage infection models with increased phagocytosis and growth rates in sympatric infections of European and African macrophages. Our results demonstrate that long term co-adaptation between Mtb and humans can be measured today in stable host-pathogen relationships and differential transmission between allopatric and sympatric host-pathogen pairs and helps unravel the driving genotypes of the current epidemic. We anticipate our findings will have implications for public health. If co-adaptation between human and pathogen subpopulations led to increased susceptibility, unraveling the molecular correlates of this relationship can inform rational vaccine design.

7

CRISPR interference reveals that all-*trans*-retinoic acid promotes macrophage control of *Mycobacterium tuberculosis* by limiting bacterial access to cholesterol and propionyl-CoA

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Macrophages are a protective replicative niche for *Mycobacterium tuberculosis* (Mtb) but can kill the infecting bacterium when appropriately activated. To identify mechanisms of clearance, we compared levels of bacterial restriction by human macrophages after treatment with 26 compounds, including some currently in clinical trials for tuberculosis. All-*trans*-retinoic acid (ATRA), an active metabolite of vitamin A, drove the greatest increase in Mtb control. Bacterial clearance was transcriptionally and functionally associated with changes in macrophage cholesterol trafficking and lipid metabolism. To determine how these macrophage changes affected bacterial control, we performed the first Mtb CRISPR interference screen in an infection model, identifying Mtb genes specifically required to survive in ATRA-activated macrophages. These data showed that ATRA treatment starves Mtb of cholesterol and the downstream metabolite propionyl coenzyme A (propionyl-CoA). Supplementation with sources of propionyl-CoA, including cholesterol, abrogated the restrictive effect of ATRA. This work demonstrates that targeting the coupled metabolism of Mtb and the macrophage improves control of infection and that it is possible to genetically map the mode of bacterial death using CRISPR interference.

8

A selective antibiotic for Lyme disease

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Broad-spectrum antibiotics damage the host microbiome and contribute to the development of antibiotic resistance in off-target bacteria. Development of narrow-spectrum antibiotics is therefore highly desirable. With this in mind, we sought to identify compounds acting selectively against *Borreliella burgdorferi*, the causative agent of Lyme disease. The main classes of antibiotics come from actinomycetes, but this source has been largely overmined for broad-spectrum compounds. However, actinomycetes have not been screened against spirochetes. We screened a small library of streptomycetes against *B. burgdorferi*, and counter-screened against *S. aureus*. This differential screen produced hits selective against spirochetes. Unexpectedly, we determined that our best hit is hygromycin A, an old, abandoned antibiotic produced by *Streptomyces hygroscopicus*. The mechanism of selectivity is puzzling because hygromycin A targets the ribosome. We found that hygromycin A is selectively taken up through the essential nucleoside transporter BmpDEFG which is specific to spirochetes. Hygromycin A is efficacious in a mouse model of Lyme disease and clears the pathogens when administered orally. Unlike broad-spectrum compounds, hygromycin A had a minimal effect on the microbiome. This selective compound holds the promise of providing a better therapeutic for Lyme disease. Notably, hygromycin A cleared the infection when it was incorporated into baits. This opens the intriguing possibility of eradicating the disease in the environment.

9

The conserved sigma D envelope stress response monitors mycobacterial envelope integrity

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The bacterial cell envelope functions as the cell's primary defense against changing environmental conditions and chemical threats, such as antibiotics. The transport and assembly of proteins that reside within the outer layers of diderm bacterial species is highly regulated due to their importance for cell viability and their roles in mediating interactions between the intra- and extracellular environment. Gram-positive microbial species in the *Corynebacterineae* suborder contain a protein-rich outer layer called the mycomembrane (MM) composed of primarily mycolic acid that is analogous to the Gram-negative outer membrane. However, despite the diderm nature of *Corynebacterineae* microbes, the pathways that govern outer membrane biogenesis and protein transport in Gram-negative species are not conserved in corynebacterial species. Using a FACS-based Tn-seq screen for mutants defective in the surface exposure of a porin substrate in the model organism *Corynebacterium glutamicum*, we demonstrate that mycomembrane protein transport is regulated by the conserved sigma D envelope stress response. Our screen and subsequent immunoblot analysis have identified the site-1 and site-2 proteases that cleave the associated anti-sigma factor, RsdA. Using a reporter system, we also show that the sigma D pathway responds to defects in corynebacterial cell envelope integrity, including disruption of mycolic acid and arabinogalactan biosynthesis. This work therefore defines a critical envelope stress response that monitors corynebacterial membrane integrity through regulated intramembrane proteolysis in *C. glutamicum* and related organisms, including *M. tuberculosis*.

10

Uncovering cysteine-rich small ORFs in *Mycobacterium tuberculosis* through toxin-mediated ribosome stalling

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The *M. tuberculosis* endoribonuclease toxin, VapC4, specifically cleaves and inactivates tRNA-Cys . This leads to depletion of the pool of tRNA-Cys followed by highly selective ribosome stalling at Cys codons within actively translating mRNAs. This observation enabled application of codon-specific ribosome stalling as a novel tool for reliable detection of new *M. tuberculosis* ORFs. Upon genome mapping of the hundreds of transcripts harboring Cys-stalled ribosomes we unmasked ∼100 unannotated ORFs, of which 75% are <100 amino acids and 50% are small ORFs (sORFs) encoding Cys-rich proteins ≤50 amino acids. Even though these small proteins are notoriously difficult to detect by mass spectrometry because they contain few, if any, trypsin cleavage sites and are often not abundant enough for detection, we identified 18% of these unannotated ORFs in *M. tuberculosis* mass spectrometry datasets. Although we have recently demonstrated that a few of these sORFs function as Cys-responsive attenuators to control translation of downstream genes, other unannotated ORFs encode stable Cys-containing proteins that can also map upstream of a gene in the opposite orientation, overlapping a gene in the opposite orientation, and overlapping a gene in the same orientation. Finally, the sequences of several other ORFs reveal functional clues, e.g. some unannotated ORFs contain zinc-binding motifs and four unannotated sORFs encode novel EsxB-like proteins.

11

RNA regulators in *Streptococcus pneumoniae* control metabolism and pathogenesis

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Riboswitches and other cis-regulatory RNA elements are found in the 5' untranslated region (5'UTR) and control gene expression. Several such bacterial regulators have been shown to play key roles in controlling primary metabolism, but a global view of their importance for organismal fitness and their role in virulence is lacking. To increase our understanding of structured RNA regulators and determine which of them are important for growth and virulence, we inactivated 12 RNA regulators in *Streptococcus pneumoniae*, the causative agent of invasive pneumococcal disease and meningitis. We subsequently assessed their fitness cost under *in vitro* and *in vivo* conditions. Among those displaying significant impacts on fitness in culture and in mouse infection models were two pyrR RNA elements, regulators of pyrimidine nucleotide biosynthesis-genes. To assess the underlying cause of the measured fitness defects, gene expression changes were mapped by performing RNAseq on regulatory mutants in *in vitro* culture conditions. We found that RNA regulatory mutants that displayed significant growth defects showed more global changes in the transcriptome while a smaller defect results in a localized change. Our approach highlights the use of genome-wide sequencing techniques to acquire a global understanding of regulation and its impact on metabolism. Moreover, by incorporating *in vivo* experiments, we also ensure that our findings are likely to have an impact on understanding virulence and mitigating antibiotic resistance.

12

The *Coxiella* dot/icm effector protein EmcB is a ubiquitin protease that antagonizes RIG-I signaling

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Bacterial pathogens employ virulence proteins to subvert host immune responses during infection and promote their replication. We find that *Coxiella burnetii* uses its dot/icm secretion system to prevent activation of the innate immune receptor RIG-I. A screen of *Coxiella* deficient in dot/icm secretion system substrates identified two effector proteins, EmcA and EmcB, required for inhibition of RIG-I signaling. Ectopic expression of EmcB is sufficient to block activation of RIG-I and the related RIG-I-like receptor, MDA5. EmcB expression in cells primed with RIG-I agonist are deficient in the post-translational modification of RIG-I with lysine 63-linked ubiquitin, an essential regulator of RIG-I activation. Biochemical assays show EmcB is a ubiquitin specific protease, and that EmcB deconjugates ubiquitin chains from RIG-I. EmcB contain a core catalytic histidine-cysteine-aspartic acid triad necessary for enzymatic activity, and the ability of EmcB to block RIG-I activation. Our results demonstrate that *Coxiella burnetii* encodes a dot/icm effector protein with specificity towards eukaryotic post-translational modifications and silences host type I interferon signaling.

13

Novel control of ParA activity and chromosome segregation in vector-borne *Borrelia burgdorferi*, the Lyme disease spirochete.

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Borrelia burgdorferi, a spirochete transmitted by ticks, causes Lyme disease, the most prevalent vector-borne infectious disease in North America and Europe. Understanding the bacterium's transmission, infectivity, and pathogenicity has benefited from decades of studies. In contrast, far less knowledge has been acquired on how *B. burgdorferi* accomplishes basic cell biological processes. This work aimed to understand the unusually segmented, 20-piece genome of *B. burgdorferi*. Newly developed genetic tools made possible the visualization of *B. burgdorferi*'s genome. We uncovered that the many genome pieces are polyploid and uniformly distributed within the spirochete cells, both in culture and inside its tick vector. I went on to investigate the mechanism controlling uniform chromosomal origin (*oriC*) spacing in *B. burgdorferi* by focusing on the widely conserved bacterial genome segregation factors ParA and ParB. Surprisingly, *B. burgdorferi*'s ParB lacks a conserved peptide that controls ParA ATPase activity in other bacteria and is not needed for ParA localization. These functions were instead taken over by a novel, likely phage-derived centromere-binding protein, ParZ. ParZ specifically recognizes and binds its own coding region, controls ParA localization and uniform chromosomal origin distribution. ParB further aids *oriC* segregation, likely through recruitment of SMC. This work unveils unexpected diversity in the control of genome organization and segregation and underscores the value of studying non-model organisms.

14

Structural dynamics of PBP2 act as a regulatory switch in peptidoglycan synthesis

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RodA is an essential glycosyltransferase (GT) that functions together with its transpeptidase (TP) partner PBP2 to enable lateral peptidoglycan (PG) synthesis by the Rod complex. Polymerization of glycan chains and their subsequent crosslinking must be precisely coordinated to avoid futile enzymatic cycles and produce a polymer of desired mechanical properties. In line with this mechanistic requirement, PBP2 was previously shown to activate RodA polymerization activity in vitro, suggesting that GT and TP activities are coupled. However, the mechanism of this allosteric activation, including the role of other Rod complex components such as MreC, remains unclear. Using single-molecule FRET and cryo-EM, we show that *E. coli* RodA-PBP2 is dynamic in vitro and adopts a novel conformation in which its TP domain is elevated ("up"). This state is required for Rod complex function in vivo, and likely represents the synthesis-competent state of PBP2. We find that structural opening of the TP domain simultaneously increases the rate of polymerization, indicating that this motion acts as an allosteric switch that couples polymerization and crosslinking. Finally, PBP2 mutants that bypass the requirement for functional MreC exhibit enhanced dynamics, implicating MreC as a major regulator of the PG synthase. Our data support a model in which MreC enhances structural opening of PBP2, activating both GT and TP for concerted PG synthesis. This work quantifies, for the first time, the structural dynamics of an essential cell wall synthase, and offers a mechanistic view of how this motion regulates PG assembly in vivo.

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A regulatory interaction between committed enzymes links lipopolysaccharide and peptidoglycan biogenesis in *Pseudomonas aeruginosa*

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Gram-negative bacteria surround their cytoplasmic membrane with a peptidoglycan (PG) cell wall and an asymmetric outer membrane (OM) with an outer leaflet composed of lipopolysaccharide (LPS). This complex envelope presents a formidable barrier to the entry of drug molecules and is a major determinant of the intrinsic antibiotic resistance of these organisms. The biogenesis pathways that build the surface are also the targets of many of our most effective antibacterial therapies. Understanding the molecular mechanisms underlying the assembly of the Gram-negative envelope therefore promises to aid the development of new treatments effective against the growing problem of drug-resistant infections. Although the individual pathways for PG and OM synthesis and assembly are relatively well characterized, almost nothing is known about how the biogenesis of these essential surface layers is coordinated. Here, we report the discovery of a regulatory interaction between the committed enzymes for the PG and LPS synthesis pathways in the Gram-negative pathogen *Pseudomonas aeruginosa*. We show that the PG synthesis enzyme MurA interacts directly and specifically with the LPS synthesis enzyme LpxC. Moreover, MurA was shown to stimulate LpxC activity in cells and in a purified system. Our results therefore suggest a model in which the assembly of the PG and OM layers is coordinated by linking the activities of the committed enzymes in their respective synthesis pathways.

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Clostridioides difficile uses distinct cell division machineries during spore formation and vegetative growth

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Peptidoglycan synthesis drives major morphological changes during spore formation. These changes include the formation of a polar septum during asymmetric division and the synthesis of a protective peptidoglycan layer known as the cortex. In *Bacillus subtilis*, these two processes are driven by distinct sets of proteins: asymmetric division is mediated by the divisome, a multiprotein complex that also drives vegetative division, while cortex assembly is mediated by the sporulation-specific peptidoglycan synthesizing enzymes SpoVD, a class B penicillin-binding protein (bPBP) transpeptidase, and SpoVE, a SEDS family glycosyltransferase. While we confirm here that SpoVD and SpoVE are critical for cortex synthesis in the major nosocomial pathogen *Clostridioides difficile*, we unexpectedly determined that these peptidoglycan synthases also mediate asymmetric division. Our data indicate that *C. difficile* SpoVD and SpoVE form a unique "polar divisome" with homologs of the highly conserved divisome proteins FtsL, DivIB, and DivIC. Surprisingly, these latter divisome components are dispensable for vegetative division in *C. difficile* and instead function exclusively during asymmetric division. Combined with our finding that *C. difficile* lacks homologs of the divisome-specific SEDS-bPBP peptidoglycan synthases thought to be universally conserved among cell-walled bacteria, *C. difficile*'s medial divisome appears to be distinct from previously characterized bacteria. Indeed, our results suggest that *C. difficile* uses a bifunctional transpeptidase-glycosyltransferase class A PBP as the primary peptidoglycan synthase during vegetative division.

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Functional characterization of the rifampicin binding site

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Rifampicin is a broad-spectrum antibiotic that targets a highly conserved region of bacterial RNA polymerase (RNAP). High resolution structures of the RNAP-rifampicin complex have recently become available and have been instrumental in locating the binding site of rifampicin to within the beta subunit of RNAP (rpoB) where the RNA-DNA transcription bubble forms. In addition, mapping of the binding site has also elucidated rifampicin's unique mechanism of action. Rifampicin blocks the elongation of the nascent RNA chain beyond 2-4 nucleotides through steric hinderance of the RNA exit channel. So far, the quality of x-ray-based structures falls short in guiding the design of next generation, higher affinity rifampicin derivatives. More potent rifampicin derivatives hold the promise of extending the spectrum of susceptible bacteria, shortening treatment time, and reducing side effects associated with administration of rifampicin. We present here an alternative approach for delineating the functional architecture of the rifampicin binding site. Using recent advances in deep mutagenesis, we generate a saturated collection of mutants that span all possible amino acid substitutions in each position of the rifampicin binding site. We identify and characterize a previously unknown class of rpoB mutants hypersensitive to rifampicin. Normally bacteriostatic, rifampicin is bactericidal in these mutants, potentially through a mechanism of DNA damage. We provide a proof of concept for the study of antibiotic binding pockets using saturating mutagenesis and demonstrate that this approach enhances our understanding of cell physiology.

18

Mistimed integration of an integrative and conjugative element leads to death in naïve bacterial hosts

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Integrative and conjugative elements (ICEs) are a major contributor to genome plasticity in prokaryotes and are involved in the spread of antibiotic resistances, alternative carbon metabolism and pathogenesis. ICEs reside integrated in the host chromosome where they are passively propagated. When activated the element excises from the chromosome and transfers as linear single-stranded DNA (ssDNA) through the ICE-encoded conjugation machinery (a type IV secretion system) into a recipient cell. Linear ICE ssDNA circularizes, undergoes second-strand synthesis becoming double-stranded and a suitable substrate for integration into the chromosome. ICEs undergo limited autonomous replication, although unlike conjugative plasmids, integration is required for stable acquisition and propagation. Using the model ICE of Bacillus subtilis, ICEBs1, we found that following transfer ICE integration occurs several generations after transfer to a new host. We bypassed the native regulatory circuit and found that integration occurred earlier than normal and that this early integration caused death of the transconjugants. We found that lethality was due to rolling-circle replication of ICEBs1 that had integrated in the host chromosome, resulting in catastrophic genome instability. We propose that both replication and integration of ICEs is critical in transconjugants and that ICE regulation has evolved to link the cessation of replication to integration of the element through expression of the gene encoding the excisionase, originally thought to only be important for excision in donors.

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The *Vibrio cholerae* Seventh Pandemic islands act in tandem to defend against a circulating phage

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The current circulating pandemic El Tor biotype of *Vibrio cholerae* has persisted for over sixty years and is characterized by its acquisition of two unique genomic islands called the *Vibrio* Seventh Pandemic Islands 1 and 2 (VSP-I and VSP-II). However, the functions of most of the genes on VSP-I and VSP-II are unknown and the advantages realized by El Tor through these two islands are not clear. Recent studies have broadly implicated these two mobile genetic elements with phage defense. Still, protection against phage infection through these islands has not been observed directly in any *V. cholerae* El Tor biotype. Here we report the isolation of a circulating phage from a cholera patient stool sample and demonstrate that propagation of this phage in its native host is inhibited by elements in both VSP-I and VSP-II, providing direct evidence for the role of these genomic islands in phage defense. Moreover, we show that these defense systems are regulated by quorum sensing and active only at certain cell density. Finally, we have isolated phage variants that are resistant to the defense conferred by the VSP islands, illustrating the countermeasures used by phages to evade these defense mechanisms. Together this work demonstrates a functional role for the VSPs in *V. cholerae* and highlights the key regulatory and mechanistic insights that can be gained by studying anti-phage systems in their native contexts.

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Parallel changes in gene essentiality over 50,000 generations of evolution

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Over evolution, bacteria face changing environments which require different sets of genes for survival. As they adapt to a specific constant environment, some genes are modified and lost, which can increase fitness while also modulating the effects of further gene losses. However, whether evolutionary specialization leads to systematic changes in robustness to gene loss is largely unexplored. Here, we compare the effects of insertion mutations in Escherichia coli between ancestral and 12 independently derived strains after 50,000 generations of growth in a simple, uniform environment. We find that epistasis between insertion mutations and genetic background is common, but the overall distribution of fitness effects is largely unchanged. In particular, we see systematic changes in gene essentiality, with more genes becoming essential over evolution than vice versa. The resulting changes often occurred in parallel across the independently evolving populations. A few of the changes in gene essentiality are associated with large structural variations, but most are not. Taken together, our results demonstrate that gene essentiality is a dynamic, evolvable property, and they suggest that changes in gene essentiality are a result of natural selection in this long-term evolution experiment, rather than a mere byproduct of structural changes.

21

Analytical testing of a rapid, direct from patient sample assay for biothreat pathogens using a fully automated assay platform

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The CDC has identified biological agents that pose a national security risk for their ability to cause severe economic and agricultural consequences and high human morbidity and mortality. In the U.S., the low prevalence of biothreat agent infections makes diagnosis difficult and delays initiation of effective therapy. In 2001, the anthrax attacks caused 22 infections with 5 deaths, and in 2021, the accidental contamination of an essential oil with *Burkholderia pseudomallei* caused 4 infections with 2 deaths. These events highlight the need for a rapid, highly sensitive and specific, diagnostic assay to improve patient diagnosis. Here, we discuss the development of a culture-independent molecular diagnostic assay that identifies 6 biothreat organisms (Bacillus anthracis, Burkholderia mallei, Burkholderia pseudomallei, Francisella tularensis, Rickettsia prowazekii, and *Yersinia pestis*) in under 5 hours. To verify assay performance, analytical studies were performed with samples prepared in whole blood using BSL3 bacterial strains. These studies demonstrated that biothreat pathogens can be accurately detected independent of blood culture at titers as low as 2 CFU/mL. Analytical reactivity demonstrated the detection of 5 genetically distinct strains of each organism, and exclusivity testing of near-genetic neighbors and common sepsis-causing pathogens showed assay specificity. Competitive inhibition was not observed for the detection of panel organisms when multiple organisms were present in the sample. These studies demonstrate that this assay may aid clinicians for faster diagnosis, resulting in earlier initiation of effective treatment and improved patient outcomes.

22

Direct bacterial species identification from blood for rapid sepsis diagnostics

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Traditional diagnostic methods for infectious diseases often rely on culture-dependent methods for pathogen identification and antibiotic susceptibility testing, which can take days to weeks. Of all infectious diseases, sepsis is a major cause of mortality worldwide. For septic patients, rapid diagnosis and treatment is critical, as the risk of mortality increases to 82% within the first 12 hours. However, current culture-based diagnostics take days to identify species and antibiotic resistance and as a result force the use of non-specific broad-spectrum antibiotics, which can be both toxic and ineffective Here we present an end-to-end process to perform rapid species identification and antibiotic resistance characterization directly from blood, which could transform the ability of clinicians to treat septic patients with appropriate antibiotics. We demonstrate the accuracy of this process in species identification for a panel of 50 species, assessed via sterile blood spiked-in with known pathogens at various bacterial loads. The samples are enriched for bacteria through the Blood2Bac process, a technology developed by Day Zero Diagnostics which performs ultra-high enrichment of bacterial DNA from blood samples. The resulting DNA is sequenced on the MinION platform, and analyzed with Keynome ID, our algorithm for performing species pathogen identification in mixed human/pathogen samples by measuring breadth of pathogen genome recovered. Keynome ID achieves a micro-averaged sensitivity of 98.8% and specificity of 99.8% in species identification directly from spiked-in blood samples. Keynome ID, when paired with our Blood2Bac enrichment process, may therefore be a highly relevant tool for rapid diagnosis directly from blood of an infecting pathogen for patients with sepsis.

23

DEFINED BACTERIAL CONSORTIA, A MICROBIOME-BASED APPROACH TO TACKLE INFECTIONS WITH ANTIBIOTIC-RESISTANT BACTERIA

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Infections with multidrug-resistant organisms (MDRO) are increasing at an alarming rate in hospitals worldwide. Due to the rapidly growing threat of antibiotic resistance, there is an urgent unmet need for novel therapies to tackle MDRO infections. Intestinal MDRO colonization, resulting from microbiota disruption, frequently precedes infection with the colonizing organism. As such, decolonization strategies including fecal microbiota transplantation (FMT) have shown efficacy at preventing infection following decolonization. Although successful, the efficacy of FMT at decolonizing MDRO is variable and its safety profile questionable. This highlights the need for a uniform, well-characterized microbiome-based product with robust efficacy that can be produced and administered in a standardized manner.Vedanta is developing a defined live biotherapeutic product (LBP) consisting of beneficial gut bacteria to reduce intestinal carriage of multidrug-resistant *Klebsiella pneumoniae* (Kpn) and *Escherichia coli* (Eco) as an infection prevention strategy. We first characterized fecal material from healthy individuals for their ability to suppress Kpn and Eco and identified a donor enriched for activity against both pathogens. Next, we used a series of *in vitro*, *in silico*, and *in vivo* tools to assemble bacterial strains from this donor into LBPs and evaluated their ability to decolonize Kpn and Eco *in vivo*. Of 70 LBPs screened, VE707 showed the greatest decolonization efficacy as demonstrated by a $\geq$3-log reduction in Kpn and Eco fecal levels. Our results show that VE707, a defined bacterial consortium with pathogen-antagonistic properties, was successful at decolonizing Kpn and Eco and it is currently in manufacturing phase.

Poster Session Distributions

Poster Session 1: June 13th 10:30am

#	Abstract title
1	Strain engineering unmasks unstable genomic structural variation in a model gut anaerobe leading to carbohydrate utilization defects
4	Relating single cell heterogeneity to antibiotic tolerance in *E. coli*
8	Dormant spores sense amino acids through the B subunits of their germination receptors
12	Understanding the Correlation between the Gut microbiota and neuropsychiatric disorders
16	Heterologous expression of PoNi_PSU5579 confers partial resistance to T6SS killing by *Vibrio parahaemolyticus* PSU5579
20	Deciphering genetic requirements for *Streptococcus pneumoniae* biofilm formation and maintenance
24	Mechanistic Insights into the Intracellular Survival of *Staphylococcus aureus* in Human Oropharyngeal Keratinocytes
28	Defining Kynurenine Pathway Control of *Salmonella* Typhimurium Infection in Zebrafish Larvae
32	Oxidative stress and DNA damage by a quinone drug
36	The overlap of microbial changes to different mucosal tumor microenvironments
40	Activation of survival state in Coxiella burnetii and Legionella pneumophila coincides with major changes in the cell envelope
44	Identifying *Yersinia pseutoduberculosis* YopH targets in neutrophil degranulation
48	Identification of a key gene in *Pseudomonas aeruginosa* regulating neutrophilic inflammatory signals in infected airways
52	Long read sequencing reveals gene conversion as a diversity generating mechanism in *Mycobacterium tuberculosis*
60	The correlation of the genetic antibiotic resistance markers to antibiotic susceptibility testing (AST)
64	Combining untargeted metabolomics and insertion sequencing to identify colonization determinants in *Phocaeicola vulgatus*
68	Diagnostic for the future: rapid, adjustable, selective and specific electrical detection of pathogens, host-protein biomarkers, and metabolites.
72	The Synthesis of Imines and Their Potential Applications as Antimicrobial Agents
76	Systematic discovery of plasmid-dependent bacteriophages
80	Two broadly conserved families of polyprenyl-phosphate transporters
84	Characterizing the mode of action of an antimycobacterial compound after target identification by activity-based protein profiling
88	Understanding gene family expansion in *R. parkeri*'s type IV secretion system and its role in infection
92	Optogenetic Control of Antibiotic Resistance Genes
96	Lipoarabinomannan regulates septation in *Mycobacterium smegmatis*
100	Blue light dependent protein degradation in *E. coli*
104	Site-specialization of human oral *Actinomyces* and *Schaalia* species
108	Investigating the impact of prophages on bacterial fitness of *Streptococcus agalactiae*
112	The Coxiella effector CvpG modulates fusion of the bacterial vacuole with endolysosomal compartments

Poster Session 2: June 13th 2:30pm

#	Abstract title
2	Towards the identification of surface proteins in *Mycobacteria spp.*
5	Probing the global diversity of shipworm symbionts using genomic, metagenomic and cultivation-based methods
9	A general mechanism for general stress response activation
13	epiXact: bacterial relatedness and outbreak detection pipeline for WGS data
17	Uncovering the interplay between Mycobacterium tuberculosis lipid metabolism, pH response, and potassium homeostasis
21	AdvA forms a critical link between early and late cell division proteins in *Acinetobacter baumannii*
25	PrrA modulates Mycobacterium tuberculosis response to multiple environmental cues and is critically regulated by serine/threonine protein kinases
29	Effect of population size on context-dependent trade-offs of antibiotic resistance
33	Mapping the Transcriptional Regulatory Network of *Streptococcus pneumoniae*
37	Discriminating the mycobacterial cell wall proteome with compartment-specific labelling
41	Genomics of resistance before the age of antibiotics
47	Tuberculosis treatment failure associated with evolution of antibiotic resilience
49	Impact of SER-109, an Investigational Microbiome Therapeutic, on Stool Fatty Acid and Bile Acid Metabolites in a Phase 3 Randomized Trial (ECOSPOR III) for Treatment of Recurrent *Clostridioides difficile* Infection (rCDI)
53	Potential Enhanced Stress Tolerance of *Salmonella enterica* Outbreak-Associated Strains
57	Lectin-Seq: a method to profile lectin-microbe interactions in native communities
61	Mutagenesis of amino acid residues in the active site of FtsA impairs divisome function in *Escherichia coli*
65	Enhanced Synthetic Biochemistry Systems Enabled by Improvements in *Bacillus subtilis* Spore-Display
69	Subinhibitory levels of translation inhibiting antibiotics alter transcriptional regulation in *Streptococcus pneumoniae*
73	Elucidating anaerobe metabolism with HRMAS 13C NMR and genome-scale dynamic flux balance analyses
77	Isolating phages that select for antibiotic susceptibility
81	A functional pan-genome study of *Streptococcus pneumoniae* reveals which genes are species-wide or strain-dependent required for a successful host infection
85	Biochemical screen reveals novel effectors secreted by the obligate intracellular bacterial pathogen *Rickettsia parkeri*
89	Structural and functional characterization of the microbial ergothioneine transporter EgtUV
93	Candidate undecaprenyl phosphate translocases enable conditional microbial fitness and pathogenesis
97	Biotin requirements in *Mycobacterium abscessus* lung infection
101	A genetic analysis of ICP1 a lytic cholera phage: a predator of the gut pathogen *Vibrio cholerae* in an estuary environment
105	Cell wall synthesis and remodeling dynamics determine bacterial division site architecture and cell shape
109	STRUCTURE AND ROLE OF BIDIRECTIONAL HYDROGENASE IN CYANOBACTERIA

Poster session 3: June 13th 4:35pm

#	Abstract title
3	Personalized probiotic therapy to improve gut microbiota maturation in preterm infants
6	The carboxypeptidase DacA1 has a novel role in cell wall turnover homeostasis in Vibrio cholerae
10	Prediction, analysis and expression of a *Coxiella* Type IV effector library
14	Single-molecule tracking of PCNA suggests two distinct pools of chromosomes in polyploid haloarchaea
18	Physiology of unicellular cyanobacteria and their photosynthetic activity
22	Reconstitution of a membrane spanning bacterial division subassembly containing FtsZ, FtsA, and FtsN
26	*Salmonella* persistence in dendritic cells
30	Auxiliary interfaces support the evolution of specific toxin–antitoxin pairing
34	tRNA-acetylating toxins evolve toward narrow substrate specificity
38	Harnessing the Inoculum Effect to Diagnose Molecular Mechanisms of Carbapenem Resistance and Characterization of a *Klebsiella pneumoniae* Transcription Factor involved in Multiple Antibiotic Resistance
42	*S. aureus* Survival and Antibiotic Tolerance in Macrophages
46	Genetic selection on cell size in #Mycobacterium tuberculosis#: Is bigger better?
50	Data-Informed Catastrophic and Harmonious Genetic Codon Bias for Multi-Gene Expression
54	Gut bacteria convert glucocorticoids into progestins
58	Phenotypic variation of growth patterns for *Neisseria gonorrhoeae* isolates
63	A two-plasmid CRISPR/Cas9-based method for rapidly and efficiently generating genetic knockouts in *Mycobacterium abscessus*
66	Exploring the mechanism of lipid transport to the outer membrane of mycobacteria in the LprG-Rv1410c pathway
70	Defining LprG-Rv1410c mediated lipid transport through the mycobacterial cell envelope
74	Recognition and degradation of the MqsA antitoxin by cellular proteases in *Escherichia coli*
78	The *Acinetobacter* virulence-resistance activator BfmR controls a phospho-dependent global regulon
82	Variation in supplemental carbon dioxide requirements defines lineage-specific antibiotic resistance acquisition in *Neisseria gonorrhoeae*
86	Accumulation of DNA by Intramacrophage *Salmonella* Persisters
90	Octopus "taste by touch" chemotactile sensation is mediated by interdomain detection of microbial molecules
94	Exploiting thioether reactivity for labeling mycobacterial glycans
98	Using machine learning to understand the determinants of mRNA stability in mycobacteria
102	Processing of DNA clamp loader subunit DnaX is important in the absence of *Caulobacter* cell division inhibitors
106	*Rickettsia* effector Sca4 may promote cell-to-cell spread through dual interactions with host vinculin and endocytic machinery
110	Comparison of long and short read metagenomic assemblers for recovery of low-abundance species

Poster session 4: June 14th 10:20am

#	Abstract title
7	Exploring the Bacterial Origins of Eukaryotic Riboswitches
11	*Pseudoalteramonas rubra*: A Possible Probiont for Corals and Oyster Larvae
15	Selective intraspecies inhibition by vaginal *Lactobacillus crispatus*-secreted peptides
19	Coculture impacts cellular differentiation in *Bacillus subtilis*
23	Identification of novel biosynthetic gene clusters encoding for ferroptosis inducing secondary metabolites of marine-derived Streptomyces hygroscopicus and S. cacoi
27	Dynamics of Gut Microbiome, IgA Response and Plasma Metabolome in Development of Pediatric Celiac Disease
31	Using Fluorescent Activated Droplet Sorting to Assay Cis-Regulatory RNA Libraries
35	Unraveling infection dynamics with barcodes
39	The role of *Shigella flexneri* effector protein OspC3 in mediating pyroptosis via Caspase-4 binding
43	Exploring the relationship between sequence and antibiotic resistance with FMN riboswitch fitness landscape
45	Human gut bacteria produce Treg-modulating bile acid metabolite
51	Analysis of phototrophic microbial communities that degrade compostable plastic
55	Dissecting the genetic networks underlying host subversion during *Rickettsia* infection
56	Fold or Die: SAS, a Split Antibiotic Selection for Enhancing Protein Solubility
59	Evaluation of the microbiome and resistome of a hamburgers' production line
62	Changes in *E. coli* susceptibility to predation by *Bdellovibrio*
67	DeLTA 2.0: A deep learning pipeline for quantifying single-cell spatial and temporal dynamics
71	Epistasis between *rpoB* and *rpoC* affects susceptibility to rifampicin and **β**-lactam antibiotics
75	The role of tuberculostearic acid in the integrity of mycobacterial plasma membrane
79	Development of SYNB1353, A Synthetic Biotic Engineered to Consume Methionine for the Treatment of Homocystinuria
83	SpoIIE manipulates the cell division polymer network to promote asymmetric division
87	Mapping functional regions of essential bacterial proteins with dominant-negative protein fragments
91	Analyzing the fitness cost of antibiotic resistance to identify targets for combination antimicrobials
95	Unusual characteristics of the bacterial noncoding RNA called *raiA*
99	Genomic profiling reveals novel routes to complement evasion in *Streptococcus pneumoniae*
103	Dimorphic colonies from Klebsiella pneumoniae clinical isolates
107	The Proposed Cloning of Bidirectional Hydrogenase to Enhance Hydrogen Production in Cyanobacteria
111	Probe based enrichment greatly increases coverage of the *E. coli* pangenome in metagenomic sequencing data, exposing regulation of a urovirulence factor

ABSTRACTS FOR POSTER PRESENTATIONS

1

Strain engineering unmasks unstable genomic structural variation in a model gut anaerobe leading to carbohydrate utilization defects

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Bacteroidetes of the human gut play an important role in metabolizing polysaccharides in the diet to support the health of the host and fitness of other microbes. An important determinant of *Bacteroides* species dominance in the gut are their highly dynamic genomes, which undergo structural changes that produce new metabolic functions for adaptation to the changing nutritional landscape and interaction with the host immune system. Common genomic structural changes include duplications, insertions, deletions, and inversions of elements that control surface structures and metabolism. Most work has described the role of genome plasticity in modulating surface structures and antibiotic resistance, but less is known about how structural rearrangements impact polysaccharide metabolism, particularly through control of polysaccharide utilization loci expression and function. Here, we utilize whole genome sequencing to update the genome assemblies of the commonly studied strains *Bacteroides thetaiotaomicron* VPI-5482 and its derivative lacking thymidine kinase (*tdk*). Analysis of these assemblies identified several genomic structural rearrangements with implications for fitness. In particular, a duplication was identified that fuses a 16S rRNA promoter to genes encoding an essential melibiase BT1871 and putative glucosidase BT1872. This duplication is present in VPI-5482 wild-type and *tdk* strains but is frequently lost in *tdk*-derived engineered strains. Loss of the duplication results in growth defects on the dietary sugar melibiose. Overall, these results provide further support for the importance of structural rearrangements in mediating sugar metabolism and indicate an important role for duplication plasticity in influencing polysaccharide utilization.

2

Towards the identification of surface proteins in *Mycobacteria spp.*

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The human pathogen *Mycobacterium tuberculosis* must maintain physiological functions in the restrictive host environment. However the cell surface machinery involved in nutrient uptake, immune evasion, and toxin secretion remains ill-defined. To address this gap in knowledge, we have developed a proximity-labeling tool to enzymatically tag surface proteins. Here we report on progress towards identifying the surface proteome of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* by expressing the labeling enzyme at the cell surface and using cell-impermeable labeling substrates. Our preliminary results indicate that mycobacterial cell-surface proteins may be highly dynamic and responsive to changes caused by different growth media.

3

Personalized probiotic therapy to improve gut microbiota maturation in preterm infants

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Premature birth accounts for about a fifth of neonatal death. A prematurely-born infant, alternatively called a preterm infant, is characterized by a shorter gestation period than normal. As a consequence, a preterm infant suffers from complications of underdeveloped organs. Recent evidence shows that microbial communities of a preterm infant also develop differently. In the case of gut microbiota, neonates go through a slower microbiota maturation. A stunted maturation is correlated with an increased incidence rate of diseases, including the two major causes of death

in preterm infants, Necrotizing Enterocolitis (NEC) and sepsis. To reduce the risk of disease, currently, most hospitals administer a probiotic cocktail to all preterm neonates. However, this one-size-fits-all intervention fails in some cases. We propose to take advantage of the fact that neonate microbiomes cluster into different community types, based on their gut microbiota composition, to design probiotic interventions tailored to specific community types. We use a model of neonate gut microbiota cultured *in silico* to simulate how the microbial composition matures after intervention. The in-silico framework permits a high level of control over the system with a fast run-time. We take advantage of this framework to test a wide-range of possible probiotic mixtures. Our in-silico investigations enable us to identify important factors that determine the success of an intervention, including the bacterial interaction network. The results of this study will inform improved probiotic intervention regimens to reduce the risk of disease in preterm babies.

4

Relating single cell heterogeneity to antibiotic tolerance in *E. coli*

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Antibiotic resistance and recurrent infections are continuously increasing public health concerns. Transient modes of antibiotic tolerance, such as persistence and heteroresistance, are being investigated as sources of recurrent bacterial infections and can allow cells to survive antibiotics long enough to acquire resistance genes or mutations. Heteroresistance allows a subset of cells within an isogenic population to survive antibiotic exposure without a genetic change due to phenotypic variability. Phenotypic variability results from noise in gene expression within an isogenic population and is theorized to be a bet-hedging mechanism in relation to stress response genes. In this project, we investigate the heterogeneity in expression of stress-related genes, compare the pulsatile expression of genes in different pathways, and determine gene expression profiles that increase the probability of surviving antibiotic exposure. To do so, we use dual fluorescent protein reporters and time lapse fluorescence microscopy to correlate the expression patterns of pairs of genes of interest in the same cell over time and with antibiotic exposure. We aim to use this data to better understand the mechanisms underlying heteroresistance.

5

Probing the global diversity of shipworm symbionts using genomic, metagenomic and cultivation-based methods

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Shipworms are marine bivalves that live and feed on wood. Like most xylophagous animals, shipworms rely on symbiotic cellulolytic bacteria to digest wood. Unlike other animals, shipworms lack a conspicuous microbial community in their gut where wood digestion occurs. Instead, the intracellular cellulolytic symbionts reside in their gills and the enzymes that these bacteria produce are selectively transported to the gut. This form of nutritional symbiosis has only been observed in shipworms. In addition to providing lignocellulolytic enzymes for the host, the symbionts have been shown to aid in the acquisition of dietary nitrogen, and potentially supply secondary metabolites to suppress microbial communities from forming in gut. Shipworm gill endosymbionts are unusual in that they are among very few intracellular symbionts that can be grown in pure culture. Here, we conducted cultivation and metagenomic-based approaches to characterize the major gill symbiont communities in several shipworm species. Our data indicate that at least two dozen distinct symbiont species belonging to Family Cellvibrionaceae can be cultivated from a diverse group of shipworm hosts. We show that the symbionts are distributed in a consistent and predicted pattern in wide taxonomic and geographic range of host species, and that they constitute an immense resource for the discovery of new carbohydrate-active enzymes as well unique biosynthetic pathways for secondary metabolite production with potential clinical applications.

6

The carboxypeptidase DacA1 has a novel role in cell wall turnover homeostasis in Vibrio cholerae

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Peptidoglycan (PG) is the main component of the bacterial cell wall. PG provides structural integrity to the cell, protects it from internal osmotic pressure, and determines cell shape and allows cell growth, making it a prime target for antibiotics. While PG synthesis is essential for bacterial growth and survival, a series of PG modifications are required to allow expansion of the sacculus. These modifications are performed by a group of enzymes known as "autolysins" (e.g., endopeptidases, EPs) which cleave almost every bond in the PG structure. How autolysins are regulated to ensure proper growth and morphogenesis is poorly understood. EP activity is essential for bacterial growth, as they cleave the crosslinks between adjacent PG strands so that newly synthesized PG can be incorporated. This activity is presumably carefully regulated, as EP dysregulation results in sacculus degradation and growth arrest or cell death, but EP regulatory mechanisms are not well-understood. Understanding the elements involved in EP regulation will provide insight into possible antibiotic targets that can disrupt the balance between these two processes. Here, we performed transposon insertion sequencing (TnSeq) to uncover novel EP regulation factors in *Vibrio cholerae*. This screen was answered by the carboxypeptidase DacA1 as a novel factor for mitigation of EP toxicity. We also demonstrate that ΔdacA1 PG is largely protected from EP-mediated cleavage, likely due to its high pentapeptide content. Surprisingly, dacA1 is ordinarily essential for viability on LB medium; a dacA1 mutant thus mitigates toxic ShyA activity as much as ShyA activity mitigates ΔdacA1 essentiality. These results demonstrate a complex interplay between architectural PG cues and EP regulation and suggest a novel role for DacA1 in cell wall turnover homeostasis.

7

Exploring the Bacterial Origins of Eukaryotic Riboswitches

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Riboswitches are portions of mRNAs that bind ligands to modulate gene expression and are found in all three domains of life. While a diverse array of riboswitch families exist in bacteria, only one such family is known in eukaryotes, the TPP riboswitch. Understanding the evolution of this riboswitch can give clues into the development of eukaryotic life, as well as insight into the differentiation of protein/DNA based processes from RNA based processes. To determine whether eukaryotic riboswitches are derived from bacteria and what their closest bacterial relatives might be, we used computational approaches to compare TPP riboswitches from a variety of bacterial and eukaryotic sources. Using graph clustering and network analysis approaches, we demonstrated that eukaryotic TPP riboswitches are highly related to those found in cyanobacteria. This evidence supports our hypothesis that eukaryotic riboswitches are derived from the ancient bacteria that later became plastids. We were further able to identify subgroups of TPP riboswitches that seem to represent four distinct subfamilies of the TPP riboswitch that are distinct in both sequence and structure. Further in vitro investigation of these subfamilies is ongoing.

8

Dormant spores sense amino acids through the B subunits of their germination receptors

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In response to starvation, bacterial species in the orders *Bacillales* and *Clostridiales* differentiate into stress-resistant spores. These spores are metabolically inactive and can remain dormant for years, yet will rapidly germinate upon nutrient sensing, leading to food spoilage or life-threatening illness. How dormant spores monitor nutrients in the environment is poorly understood but, in most cases, requires putative receptors embedded in the spore membrane. The prototypical germination receptor GerA of *Bacillus subtilis* consists of three broadly conserved proteins, GerAA, GerAB, and GerAC, all of which are required for spores to germinate in response to L-alanine. Whether this complex directly senses L-alanine and, if so, which protein functions in amino acid recognition have remained unresolved. The B subunit of these complexes are members of the Amino Acid-Polyamine-Organocation (APC) superfamily of transporters, and by using evolutionary co-variation analysis, we provide evidence that GerAB adopts a structure similar to the L-alanine transporter GkApcT, an APC superfamily member. Using the structural model, we analyzed residues in GerAB that are predicted to bind L-alanine. Mutations predicted to disrupt the ligand-binding pocket impair spore germination in response to L-alanine, while mutations in residues predicted to function in L-alanine recognition enable spores to respond to L-leucine or L-serine. Finally, substitutions of bulkier residues at these positions cause constitutive germination. Altogether, these data indicate that the GerAB subunit of the GerA receptor serves as the L-alanine sensor and suggest that B subunits function in nutrient detection in this broadly conserved family of germinant receptors.

9

A general mechanism for general stress response activation

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The general stress response allows bacteria to adapt to changing environments and to control virulence. The most broadly conserved mechanism for initiating the general stress response utilizes a serine/threonine phosphatase to activate an alternative sigma factor. Here we address how the *B. subtilis* phosphatase RsbU senses environmental stress as a model for how these phosphatases are adapted to these signals. RsbU is activated by binding its regulatory protein RsbT, but the mechanism of activation remains to be elucidated. RsbU has three domains, a dimerization domain at the N-terminus that is known to bind RsbT, a linker, and a PP2C family phosphatase domain. Using a combination of unbiased genetic screens, fluorescence anisotropy, and X-ray crystallography, we find that RsbT binding to the RsbU restructures the linker. This promotes binding of an essential metal cofactor in the phosphatase active site, stimulates catalysis, and recruits the substrate. Mutations isolated in an unbiased gain of function genetic screen demonstrate that two alpha-helices that are predicted to make contacts in the active dimer are critical for activation. We propose that this allosteric mechanism is a conserved feature of the related phosphatases that adapts phosphatases to unique signals for general stress signaling in different organisms.

10

Prediction, analysis and expression of a *Coxiella* Type IV effector library

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Coxiella burnetii secretes >150 effectors into the eukaryotic host cell, interacting with all major membrane-bound organelles during infection. Amino acid sequences of effectors lack sequence similarity that could give clues about protein structure or function. Our goal is to predict and experimentally validate the localization of all *Coxiella* effectors, especially those targeting the endomembrane system. We compiled all localization, conservation, and functional characterization data from 13 years of published studies. Next, we eliminated pseudogenized ORFs and those not predicted to be Type IV secreted using S4TE and Bastion. We then predicted the localization of 142 remaining effec-

tor sequences using PredictProtein and TMHMM 2.0. We predicted 71 nuclear, 20 mitochondrial, 49 cytoplasmic, 4 golgi and 2 endoplasmic reticulum-associated proteins. Most PredictProtein scores (68%) had <40% prediction confidence. Compared to published experimental results, we found 33 concordant predictions and 21 discordant. For proteins with a transmembrane motif, experimental localization to a membrane was likely, but not always the same organelle as predicted. We identified 14 uncharacterized effectors that are highly likely to have transmembrane motifs and associate with eukaryotic organelles. We hypothesize these 14 effectors could be novel proteins modulating the development of the *Coxiella*-containing vacuole during infection, and set about to generate ectopic expression plasmids for each one. Five vacuole-associated proteins and two TMHMM predicted ones (CBU0122 and CBU0410) were codon-optimized and cloned into pmClover3-N1. Some membrane localization was observed, but toxicity of overexpressed proteins will necessitate an inducible expression system before the library can be completed.

11

Pseudoalteramonas rubra: A Possible Probiont for Corals and Oyster Larvae

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Mass coral bleaching events are caused by bacterial infection that are becoming more severe as climate change worsens. One of the most prevalent species of bacteria that causes these infections is *Vibrio coralliilyticus*. V. coralliilyticus *strain RE22 is also a known pathogen of several species of oyster larvae including* Crassostrea virginica. An interaction between RE22 and the native microbiome of the temperate coral *Astrangia poculata* has been observed during challenges with wild type and mutant strains of RE22. A red-pigmented bacterium was isolated from sea water previously occupied by *A. poculata*. The isolate is a gram negative motile rod that inhibits the growth of both V. coralliilyticus *and* Vibrio parahaemolyticus. The isolate was identified as *Pseudoalteromonas rubra* by sequence analysis of 16S rRNA. *P. rubra* is a known producer of prodigiosin, a red pigment with antibacterial, antiprotozoal, and antitumor properties. Prodigiosin is a secondary metabolite first discovered in *Serratia marcescens* and has been detected in an array of Gram negative and Gram positive species. In addition to inhibiting bacterial growth, prodigiosin may also inhibit such virulence factors as protease and biofilm formation. Whole cell and extracellular extracts will be examined by LC-MS/MS to identify prodigiosin and other possible antibacterial substances from the isolate. Probiotic activity protecting A. poculata *and oyster larvae against* V. coralliilyticus infection will be presented.

12

Understanding the Correlation between the Gut microbiota and neuropsychiatric disorders

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There is a growing body of evidence demonstrating that differences in the microbial constituents of the gut flora may contribute to the development or progress of neuropsychiatric disorders, particularly when coupled with other known risk factors such as stress. Early life stress (ELS) occurs during the period of brain development and maturation; exposure to ELS at a very young age enhances the likelihood of developing neuropsychiatric disorders. Prior research has demonstrated that female rats are less susceptible to the effects of ELS than their male counterparts. The goal of the current project is to identify differences in the constituents of the gut microbiome following ELS using the maternal separation rodent model. To do this, stool samples from four cohorts (female control, male control, female treated, male treated) were collected and pooled by cohort. A small amount of material was cultured; DNA was extracted from the remainder. The DNA was submitted for 16s ribosomal metagenomic sequencing. While there were no obvious differences in the cultured component of the four cohorts, metagenomic sequencing revealed remarkable differences among the cohorts. Perhaps most striking was the similarities in microbial content between female treated and male control group, which both demonstrated a marked increased abundance of members of the phylum *Verrucamicrobiota* relative to the untreated females and treated males. A heat map for species abundance

also revealed that in treated male rats, there was a marked increase in several different bacterial phylum relative to the other three cohorts. The implications for these findings will be the basis for future investigation.

13

epiXact: bacterial relatedness and outbreak detection pipeline for WGS data

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Hospital acquired infections (HAI) pose a threat to patients and are of increasing concern as antibiotic resistance becomes more widespread. The most accurate way of identifying and tracking transmission involves whole genome sequencing (WGS), and subsequent single nucleotide polymorphism (SNP) analysis across clinical isolates. To enable accurate transmission analysis in a suspected outbreak, we have developed epiXact, a CLIA-certified, rapid lab service that includes WGS and SNP analysis, with a turnaround time of 1-2 days for high resolution characterisation of transmission events. Here, we present a large-scale validation of the epiXact computational pipeline on both simulated and *in vitro* datasets, representing 5 species that span various Gram-stains, genome sizes, and GC-content. epiXact performs quality control on Illumina reads, genome assembly, species and multi-locus sequence type (MLST) identification, and variant calling. Samples are then compared to one another for clonality detection. In simulated data, epiXact was able to detect the correct number of SNPs across isolates with a mean error rate of 2.6 SNPs. In *in vitro* data, epiXact achieved 100% sensitivity and 98.5% specificity in determining clonality, and 100% repeatability. High accuracy in species identification and MLST determination was also observed. We also highlight recent cases where the epiXact service has successfully aided high-stakes hospital outbreak investigations, including *Burkholderia* transmission detection from extracorporeal membrane oxygenation machines, and *Escherichia coli* transmission from a fecal microbiota transplant. The automated epiXact pipeline serves as a powerful tool in HAI transmission analysis, and will facilitate WGS becoming the standard-of-care in infection control.

14

Single-molecule tracking of PCNA suggests two distinct pools of chromosomes in polyploid haloarchaea

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The Archaea are an understudied domain of life, consisting of single-celled microbes with chimeric eukaryotic- and bacterial-like physiology. In particular, archaeal DNA metabolism involves bacterial-like cytosolic, circular chromosomes with defined replication origins that are replicated and repaired using homologs of eukaryotic protein machinery. Thus, applying state-of-the-art molecular biology techniques to archaeal DNA replication may unveil the conserved properties underlying the emergence of eukaryotes from a prokaryotic ancestor. The salt-loving haloarchaea serve as an excellent model for archaeal cell biology, with relatively simple genetic manipulation, growth conditions, and compatibility with standard microbiological tools and techniques. Haloarchaea exhibit extreme genome stability, where they balance multiple replication origins with high rates of recombination and extreme polyploidy. To understand the spatio-temporal dynamics of DNA replication, we generated a suite of cell reporters of DNA replication that can be tracked in real-time using live-cell single-molecule microscopy. A reporter of the beta-clamp PCNA reveals that the amount of DNA synthesis is lower than would be predicted in order to maintain polyploidy. These results suggest that haloarchaea maintain two pools of genomic DNA: those used as templates for new rounds of synthesis, and those that are 'stored' as back-up copies. Future work will aim to define the regulation of these two pools, with a particular focus on how each of the three replication origins (which are preferentially used for replication initiation, but are dispensable for normal growth) influence chromosome copy number and fate.

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Selective intraspecies inhibition by vaginal *Lactobacillus crispatus*-secreted peptides

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Bacterial vaginosis (BV) is characterized by the dominance of diverse, non-*Lactobacillus* anaerobic bacteria in the vagina and is associated with genital inflammation and poor health outcomes such as preterm birth and increased HIV risk. In contrast, *Lactobacillus crispatus* dominance is associated with low inflammation and favorable health outcomes. While there is interest in developing *Lactobacillus*-based therapies for BV, little is known about the degree or drivers of diversity within *L. crispatus*. We investigated antagonistic interactions between vaginal *L. crispatus* strains and found that cell-free supernatants of two strains inhibit growth of 30% of a diverse set of other *L. crispatus* isolates. We identified the inhibitory molecule as heat- and proteinase-sensitive, larger than 30kDa, with concentration dependent effects, suggesting a class-III bacteriocin or other ribosomally synthesized and post-translationally modified peptide (RiPP). All *L. crispatus* genomes we analyzed contain bacteriocin-like regions, and yet comparative genomics identified no previously characterized gene regions unique to the inhibitory strains, suggesting a novel antimicrobial peptide. Previous studies have found robust bacteriocin production by *L. gasseri* that inhibits some BV-associated microbes. These peptides have been proposed as prebiotic treatments for recurrent BV in addition to current treatments of antibiotics or *Lactobacillus* probiotics. However, our finding of peptide-based antagonism between strains of the same species demonstrates potential competition between native and exogenous *L. crispatus*. As we continue to chemically characterize this novel peptide, our work lays the foundation for designing ecologically informed probiotics for vaginal health.

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Heterologous expression of PoNi˙PSU5579 confers partial resistance to T6SS killing by *Vibrio parahaemolyticus* PSU5579

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The Type VI Secretion System (T6SS) contributes to the virulence and fitness of several members within the *Vibrio* genus by allowing these species to deliver toxic effectors to prokaryotic and eukaryotic targets. The apparent significance of T6SS in pathogenicity has led to the discovery and classification of several antibacterial effectors. Many of the effector-encoding genes so far described are paired with an immunity gene that encodes an anti-effector necessary to prevent self-intoxication. One such effector/immunity pair is the Polymorphic Nuclease Effector (PoNe) and its cognate immunity protein (PoNi), first identified by Jana et al. (2019) in *Vibrio parahaemolyticus* 12-297/B. Here we report the presence of the genes encoding PoNe and PoNi in *V. parahaemolyticus* PSU5579 and the experimental validation of PoNe/i activity using an arabinose-inducible expression vector in *E. coli*. Arabinose-induced expression of PoNe-PSU5579 in *E. coli* is lethal, while co-expression of PoNe-PSU5579 and PoNi-PSU5579 is not lethal. Further, we demonstrate that heterologous expression of PoNi-PSU5579 in an E. coli *host confers partial resistance against contact killing by* V. parahaemolyticus PSU5579.

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Uncovering the interplay between Mycobacterium tuberculosis lipid metabolism, pH response, and potassium homeostasis

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB), which remains a leading cause of death globally from an infectious disease. Successful colonization of the host requires Mtb to sense and respond coordinately to disparate environmental cues during infection and adapt its physiology. Among the environmental signals key for Mtb, we have shown that Mtb has a synergistic transcriptional response to pH and Cl-, and that disruption of bacterial potassium (K+) homeostasis (deletion of CeoBC K+ uptake system) impedes Mtb response to pH and Cl- and affects host colonization. Further, a decrease in environmental [K+] significantly dampens Mtb transcriptional response to acidic pH. In contrast, Mtb K+ response is unaffected by changes in environmental pH. Using a reporter-based transcription factor overexpression screen, we have now unexpectedly uncovered a link between Mtb lipid metabolism and its K+ response/homeostasis: (i) overexpression of transcription factors involved in Mtb lipid utilization alter the dampening effect of low external [K+] on Mtb pH response, and (ii) Mtb response to cholesterol is repressed in the ΔceoBC Mtb mutant. While the importance of Mtb ionic cue response and homeostasis and its lipid metabolism are individually recognized, how these facets of Mtb biology are functionally connected remain poorly understood. Our results raise the novel concept of a connection between Mtb lipid metabolism, pH response, and potassium homeostasis. Current work is focused on elucidating how cholesterol may directly interface with the CeoBC K+ uptake system, and understanding the impact of cholesterol and pH on Mtb K+ homeostasis.

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Physiology of unicellular cyanobacteria and their photosynthetic activity

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Society has been growing rapidly, which raises the need for a more sustainable alternative energy and fuels sources. Hydrogen is a potential alternative for fossil fuels, and it is known as the most abundant and most energy dense type of fuel. Hydrogen production could be very expensive. However, properly utilizing cyanobacteria may propose a cheaper option for hydrogen production. Working with Synechocystis sp. strain PCC 6803 may allow further exploration of its photosynthetic potential of cyanobacteria as a source of energy for bioproduction of hydrogen. This specific strain of cyanobacteria was grown in liquid BG-11 medium at a light intensity of 40 µmol of photons m2s1 and 28°C. The liquid cultures were incubated on a rotatory shaker. Optical densities of the cultures were measured at 660 nm. Chlorophyll extraction was done according to the standard protocol. The main goals of this study were (a) to test whether there was correlation between the chlorophyll concentration and the photosynthetic activity of cyanobacterial cultures, and (b) to measure how consistent is its photosynthetic activities, which will contribute towards increasing hydrogen production. Our results indicate the positive correlation of chlorophyll accumulation with photosynthetic activity of cyanobacterial cultures, as well as consistency of photosynthetic processes. As the source of electrons for bidirectional hydrogenase, photosynthetic activity of Synechocystis sp. PCC 6803 is critical for maximizing hydrogen production.

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Coculture impacts cellular differentiation in *Bacillus subtilis*

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Biofilms are collections of microorganisms encased by a self-produced extracellular matrix that acts as a protective barrier from environmental stressors. While many naturally occurring microbes exist as members of multispecies biofilms, the interspecies interactions that mediate biofilm formation in these communities remain minimally explored.

Bacillus subtilis and *Pantoea agglomerans* are soil-dwelling bacteria that form structurally unique biofilms in coculture compared to monoculture. We are using this system to investigate how gene expression and cellular differentiation are altered in mixed biofilms. Using transcriptomics, we determined that many *B. subtilis* genes involved in sporulation and specialized metabolite production were differentially expressed in monoculture and coculture biofilms. We confirmed that *B. subtilis* sporulation gene expression and total spores were reduced in mixed biofilms, but that, over time, sporulation was restored to wild-type levels even in coculture biofilms. Using *B. subtilis* fluorescent transcriptional reporters, we observed increased expression of certain specialized metabolites in the center of coculture biofilms, a region known to be preferentially occupied by *P. agglomerants*. We thus hypothesize that the presence of P. agglomerans *stimulates expression of specific* B. subtilis *specialized metabolites. To identify possible* P. agglomerans *signals activating* B. subtilis specialized metabolite production, we are utilizing transcriptomics and mass spectrometry. We will confirm these potential signals with genetic knockouts in *P. agglomerans*. Taken together, these data indicate that studying cocultures is a productive avenue to identify novel mechanisms that drive the formation of structured microbial communities.

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Deciphering genetic requirements for Streptococcus pneumoniae biofilm formation and maintenance

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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. Biofilm formation and maintenance 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 for the first time allows for monitoring population dynamics in S. pneumoniae's biofilm. 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). As a first step towards characterization, we use confocal microscopy to quantify four distinctive features of biofilm growth and maintenance. We validated our assay using S. pneumoniae strains reported as having high and low biofilm forming index. Our validation results are consistent with previous reported data. Additionally, our data suggest high heterogeneity in the early stages of biofilm formation and a more controlled growth as the biofilm mature over time. Next, we use a transposon mutant library in our biofilm model to uncover the genetic requirements for 1) initial bacterial attachment, 2) expansion and biofilm growth, 3) biofilm maintenance and 4) biofilm dispersal for the first time ever in a high throughput manner. We found a group of genes essential during all four stages of biofilm development as well as some genes specific to each of the stages mentioned above. Our work aims to elucidate genetic requirements for biofilm survival to further establish a targeted treatment in biofilm-related infections.

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AdvA forms a critical link between early and late cell division proteins in *Acinetobacter baumannii*

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Acinetobacter baumannii has shown increasing resistance to many antibiotics, including those considered our last-line of defense treatments. The pathogen has thus been labelled an urgent priority for new drug development. An attractive antimicrobial target is cell division. This process is poorly understood in *Acinetobacter*, however, as these bacteria lack several key enzymes driving division in other Gram-negatives including FtsEX. We previously identified a unique, essential protein, AdvA, that is required for *A. baumannii* cell division. Depletion of the protein causes cell filamentation and death, but its exact role in division is unclear. We hypothesized that AdvA is integral to the assembly and activity of the *Acinetobacter* divisome complex. To test this and characterize AdvA interactions with division proteins, we used two-hybrid analysis in E. coli *as well as fluorescence microscopy analysis in* A. baumannii of

how division proteins depend on AdvA (and vice-versa) for recruitment to Z-rings. We also isolated suppressors that bypass the defects caused by AdvA depletion. We found that AdvA interacts with both early (ZipA) and late (FtsN) division proteins, and is required for the recruitment of late proteins to Z-rings. Suppressors of AdvA essentiality were found to include point mutations mapping to late septum synthesis proteins, FtsB and FtsW. These results support the model that AdvA acts as a key bridge between early and late-stage division proteins that may allow activation of the latter. This work reveals unappreciated aspects of *A. baumannii* cell division that may inform strategies for selectively targeting this fundamental process.

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Reconstitution of a membrane spanning bacterial division subassembly containing FtsZ, FtsA, and FtsN

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Bacterial cells grow and reproduce through coordination of many proteins collectively known as the cell division machinery. This machinery facilitates the division and physical separation of one cell into two identical progeny cells. The components of the cell division machinery are similar across bacterial taxa. In *Escherichia coli*, the cytoskeletal protein FtsZ promotes the formation of a division septum at the cell center and assembles into a large dynamic protein structure called the Z-ring. FtsZ protomers in the Z-ring form large linear polymers that assemble in a head to tail arrangement. Protein interactions between FtsZ in the Z-ring and additional cell division proteins direct peptidoglycan synthesis and facilitate septation of the cell. FtsN, which may trigger this septation event, is a bitopic membrane protein with a small cytoplasmic domain and large periplasmic domain containing a peptidoglycan-binding SPOR domain. FtsN interacts with other cell division proteins on both sides of the cytoplasmic membrane. In the cytoplasm, FtsN interacts with FtsA, an actin homolog, which tethers FtsZ polymers to the membrane. In the periplasm FtsN interacts with the FtsQBL complex and may help to recruit peptidoglycan synthetases such as FtsI and PBP1b. One proposed linear mechanism for activating cell wall synthesis begins with FtsA, which binds to FtsN in the cytoplasm, and causes FtsN to relay information to proteins in the periplasm, such as FtsI and PBP1b, to assemble peptidoglycan. Thus, it is critical that FtsN localizes to the future site of septation to carry out this function. Here we use purified proteins, including FtsZ, FtsA, and FtsN, to reconstitute the membrane-spanning division complex in vitro and assemble a synthetic divisome scaffold. This reconstituted divisome subassembly allows us to probe direct protein interactions, protein conformations, assembly architectures, and nucleotide requirements in the cell division pathway, thus providing novel insight into the mechanics of division.

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Identification of novel biosynthetic gene clusters encoding for ferroptosis inducing secondary metabolites of marine-derived Streptomyces hygroscopicus and S. cacoi

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This project describes an interdisciplinary research study to identify critical biosynthetic gene clusters (BGCs) associated with functional proteins encoding ferroptosis. These proteins are linked to natural products produced by bacterial species such as*Streptomyces*. The discovery of marine-derived*Streptomyces hygroscopicus*and *S. cacoi*in the Stellwagen Bank National Marine Sanctuary using a metagenomic approach has led to this study. *Streptomyces*, a saprophytic actinomycete, can survive in iron-depleted areas by producing secondary metabolites such as polyketides and nonribosomal peptide synthetases (NRPS). The*Streptomycetaceae*family is responsible for nearly 80% of bioactive molecules, with*Streptomyces* species accounting for 39% of microbial metabolites. Prior knowledge of these metabolites supports examining ferroptosis mechanisms associated with anticancer compounds. Ferroptosis is a novel form of regulated cell death characterized by the overaccumulation of iron, causing antioxidant depletion and loss of membrane permeability. Bioinformatics are used to assess cellular processes associated with proteins such as lipoxygenases or non-enzymatic via the Fenton reaction. Conversion from ferric to ferrous iron increases reactive

oxygen species (ROS), causing lipid peroxidation and tumor cell breakdown. This process can disrupt the antiporter system Xc- and decrease intracellular glutathione, resulting in cell death via ferroptosis. Results revealed a relatively high abundance of BGCs, that will facilitate the quantification of functional proteins encoding ferroptosis. The next steps include the development of a natural product pipeline to identify chemotherapeutic agents.

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Mechanistic Insights into the Intracellular Survival of *Staphylococcus aureus* in Human Oropharyngeal Keratinocytes

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The opportunistic pathogen *Staphylococcus aureus* causes a range of infections from superficial skin and soft tissue infections to more serious conditions such as toxic shock syndrome and infective endocarditis. *S. aureus* produces an arsenal of virulence factors that contribute to its intracellular survival in human keratinocytes, many of which are controlled by global virulence factor regulators. The Agr system controls the expression of numerous virulence factors including membrane damaging toxins eg. Hemolysins, leukocidins and the phenol soluble modulins (PSM) and surface adhesins such as Protein A. We found that there was a dramatic reduction in intracellular survival in oropharyngeal keratinocytes in derivatives of *S. aureus* lacking the global regulators Agr and SaeR/S or combinations of virulence factors within their regulons. Investigation of individual gene products revealed that deletion of PSM-αled to a significant reduction in intracellular survival. Immunofluorescence microscopy was complicated by the fact that surface adhesin Protein A binds nonspecifically to most mammalian IgG. We developed an effective method to eliminate this nonspecific binding using recombinant Protein A as a soluble inhibitor. The mechanism by which PSM-αprotects *S. aureus* from intracellular killing may involve enhanced recruitment to autophagosomes. There was no observable difference in the association between bacteria and other intracellular trafficking markers for endosomes (EEA-1), lysosomes (LAMP-1), cytosol (ubiquitin), and endosomal membrane damage (galectin-8). We conclude that PSM-αis required for optimal recruitment of intracellular *S. aureus* to the autophagy pathway and in oropharyngeal keratinocytes, this may represent a key event in intracellular survival.

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PrrA modulates Mycobacterium tuberculosis response to multiple environmental cues and is critically regulated by serine/threonine protein kinases

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The ability of *Mycobacterium tuberculosis* (Mtb) to adapt to its surrounding environment is critical for the bacterium to successfully colonize its host. During infection, Mtb encounters heterogenous microenvironments encompassing differences in signals including pH, chloride (Cl-), nitric oxide (NO), and hypoxia. Transcription factors (TFs) that regulate response to these environmental signals play a pivotal role in enabling Mtb to establish and sustain infection. Indeed, Mtb encodes 214 annotated TFs, yet the biological role of most of these TFs remain unknown. To identify TFs that regulate Mtb response to environmental signals, we exploited our pH and Cl–responsive luciferase reporter Mtb strain in a genetic screen, in combination with an inducible TF overexpression library. This screen has identified the essential TF PrrA, part of the PrrAB two-component system, as a major regulator of Mtb response to not just Cl- and acidic pH, but also NO and hypoxia. Of note, PrrA is phosphorylated by its cognate sensor histidine kinase (PrrB), and also by serine/threonine protein kinases (STPKs) at a distinct second site. We find that STPK phosphorylation of PrrA affected the ability of PrrA to mediate Mtb response to environmental cues, including NO, with a STPK phosphoablative PrrA variant exhibiting a dampened response to NO versus wild type Mtb. NO is known to drive Mtb into growth arrest, and the STPK phosphoablative PrrA variant strongly disrupted the ability of Mtb to enter a non-replicating state upon extended NO exposure. Strikingly, the STPK phosphoablative PrrA

variant was attenuated for host colonization in a murine model of Mtb infection. Together, our results identify PrrA as an essential TF that modulates Mtb response to four important environmental signals, uncovers a critical interplay between STPKs and two-component systems, and sheds light on how environmental response is linked to regulation of Mtb growth.

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Salmonella persistence in dendritic cells

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Persisters are a bacterial subpopulation of non-dividing, metabolically active cells, proposed to serve as both a reservoir for relapsing infection as well as a niche for the accumulation of mutations and the development of genetically resistant clones. It is now well documented that *Salmonella* forms such persister cells in host macrophages, but there is mounting evidence that dendritic cells may serve as a reservoir for persistent *Salmonella* Typhimurium within the mesenteric lymph node. The relevance of dendritic cells more broadly in the context of persistent and relapsing infection remains unclear. Using bone-marrow derived dendritic cells, we have found that dendritic cells indeed induce persistence, that they contain both growing and non-growing bacteria, and a subset of these non-growing bacteria retain metabolic activity. These results could suggest a role for this cell type in vivo, and future experiments will focus on characterizing their role in infection relapse.

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Dynamics of Gut Microbiome, IgA Response and Plasma Metabolome in Development of Pediatric Celiac Disease

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Celiac disease (CD) is an autoimmune disorder triggered by gluten consumption. To identify the role of gut microbes in CD onset, we performed a longitudinal study focusing on two important phases of gut microbiota development at ages 2.5 and 5 (n=16). We obtained samples from children who developed CD during or after the study (CD progressors) and age, sex, and HLA-matched healthy controls. CD progressors had a distinct gut microbiota composition and IgA-sequencing identified unique IgA targets in the gut. Three cytokines, one chemokine, and 19 plasma metabolites were significantly altered in CD progressors at age 5. Feeding C57BL/6J mice with taurodeoxycholic acid (TDCA), a 2-fold increased microbiota-derived metabolite in CD progressors, caused villous atrophy, increased intraepithelial lymphocytes (IELs), CD4+ T-cells, Natural Killer cells, and Qa-1 expression on T-cells while decreasing T-regulatory cells in IELs. Thus, TDCA drives inflammation in the small intestines that potentially contribute to the CD onset.

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Defining Kynurenine Pathway Control of *Salmonella* Typhimurium Infection in Zebrafish Larvae

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Antibiotic-resistant bacterial infections remain a persistent cause of hospitalization and death around the world. In a whole organism screen for anti-infectives, we found that exogenous application of the kynurenine pathway metabolite 3-hydroxy-kynurenine (3-HK) rescued zebrafish larvae from lethal *Salmonella* Typhimurium infection. Exogenous 3-HK did not act like a typical antibiotic in that it did not inhibit bacterial growth *in vitro* but did restrict bacterial growth *in vivo*. Further analysis of endogenous production of 3-HK through the kynurenine pathway revealed that kynurenine 3-monooxygenase (Kmo), the enzyme responsible for catabolism of kynurenine to 3-HK, is required for the normal immune response to systemic *S.* Typhimurium infection. Inhibition of Kmo and the endogenous production of 3-HK resulted in decreased survival to sub-lethal infection. Sensitivity to infection upon Kmo inhibition correlated with more rapid expansion of bacterial burden and, paradoxically, enhanced ROS production and pro-inflammatory cytokine induction. While macrophage cell numbers are not altered following Kmo depletion, their ability to control intracellular bacterial replication is impaired. Additionally, RNA sequencing of infected macrophages with Kmo depletion display signatures of impaired lysosomal function. Taken together, this work provides a novel mechanism by which Kmo and the endogenous production of 3-HK impacts macrophage function.

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Effect of population size on context-dependent trade-offs of antibiotic resistance

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Evolutionary adaptations are generally associated with fitness costs, yet many studies on the costs of antibiotic resistance have failed to identify growth rate deficits in resistant bacteria. Recent work has shown that costs of resistance can manifest as a reduced tolerance of novel environmental conditions. Here, we consider the effects of population size on these context-dependent fitness costs. The relationship between population size and the environmental costs caused by resistance is unclear; it may be that a larger population size means that mutations with lower fitness costs are more likely to arise, or it may be that a larger population size allows for a higher degree of adaptation to specific environments. In this study, we characterize the effect of population size on the growth deficits incurred by chloramphenicol-resistant *Escherichia coli* in novel thermal conditions. We find that when resistance develops in a larger population, resistant bacteria show greater growth deficits when compared to bacteria evolved in smaller populations. This result is consistent with the hypothesis that the observed difference is due to larger populations becoming more tightly adapted to the experimental conditions, causing a greater decrease in their thermal niche breadth; nonetheless, further studies are needed to confirm this hypothesis and integrate this observation into an evolutionary framework. The results of this study give insight into the interplay between two key ecological factors that could influence antibiotic resistance development. More broadly, further characterizing the fitness costs associated with resistance development can help predict scenarios where resistance will arise.

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Auxiliary interfaces support the evolution of specific toxin–antitoxin pairing

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Toxin–antitoxin (TA) systems are a large family of genes implicated in the regulation of bacterial growth and its arrest in response to attacks. These systems encode toxin and antitoxin proteins that pair specifically, even when present in several paralogous copies per genome. *Salmonella enterica* serovar Typhimurium contains three paralogous TacAT systems whose toxins block bacterial translation. We determined the crystal structures of the three TacAT complexes to understand the structural basis of TA neutralization and the evolution of neutralization specificity.

We show that alteration of a discrete structural add-on element on the toxin drives specific recognition by cognate antitoxin contributing to the insulation of the three pairs. Like other TA families, the region supporting TA-specific pairing is key to neutralization. Our work also reveals that additional TA interfaces beside the main neutralization interface increase the safe space for evolution of pairing specificity.

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Using Fluorescent Activated Droplet Sorting to Assay Cis-Regulatory RNA Libraries

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Existing methods of assaying the function of regulatory elements come with significant drawbacks when assaying large libraries. Highly sensitive cell-based assays such as the β-galactosidase assay are labor intensive, difficult to scale up and may lose sensitivity with increased throughput. Fluorescent reporters can be used with fluorescent activated cell sorting (FACS) to increase assay throughput, but sorting small bacterial cells can be challenging. Conversely, *in vitro* methods allow for fast screening of very large RNA libraries, but only select for properties of binding, not regulation. By combining the principles of classic in cell regulatory assays with modern tools, cis-regulatory RNAs can be quickly screened for regulatory activity at a large scale. The assay under development, Fluorescent Activated Droplet Sorting Regulatory Assay (FADSRA), uses microfluidics to encapsulate single cells expressing a fluorescent protein under the control of a cis-regulatory RNA. These cells are cultured into microcolonies within the droplets, and subsequently sorted according to fluorescent signal. Deep amplicon sequencing of the regulatory RNAs can then reveal which sequences can regulate and which cannot. Currently, FADSRA is being developed to create a fitness landscape of the S15 leader to probe sequence-function relationships. The S15 leader is noted for the diversity of regulatory structures across clades. Comparing landscapes between regulator homologs will result in identification of areas both conservation prone and permissive to change, lending further insight into the importance of S15 RNA leader structural motifs and how these ribosomal leaders arise and change.

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Oxidative stress and DNA damage by a quinone drug

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Streptonigrin (SN) is an aminoquinone antibiotic produced by *Streptomyces flocculus* and has been previously used in tumor therapy. This drug is known to cause DNA damage, and it requires both iron and oxygen for its toxicity. Hence it has been proposed that the molecular mechanism for the toxicity of SN is through redox cycling of electrons to oxygen, resulting in the production of reactive oxygen species (ROS). However, we suspect that if ROS were being generated by SN, the cellular oxidative stress response systems could reduce the efficacy of SN. Yet, SN-treated cells die rapidly. Using *E. coli* as a model organism, we have verified the dependence of SN on oxygen and iron for causing DNA damage. However, unlike other quinone drugs (for example, menadione) which elevate intracellular levels of ROS, we do not find any evidence of ROS formation in SN-treated cells. There is no increase in the rate of oxygen consumption, no elevated production of hydrogen peroxide, no induction of ROS defense systems, and no increase in sensitivity of ROS scavenging mutants. Using an in vitro DNA damaging system, we also confirmed that SN-mediated DNA strand breaks occur even in the absence of ROS like hydrogen peroxide, establishing that SN toxicity is not ROS dependent. We suspect that the DNA damage caused by SN could occur through the formation of a high-valence oxidant and/or through a SN-DNA adduct. We are currently examining these hypotheses.

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Mapping the Transcriptional Regulatory Network of *Streptococcus pneumoniae*

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Streptococcus pneumoniae (*S. pneumoniae*) is a Gram-positive, facultative pathogen responsible for killing over 300,000 children globally each year. Along with other bacterial pathogens, *S. pneumoniae* is becoming increasingly antibiotic-resistant, with more than 30% of its infections resistant to one or more clinically relevant antibiotics. Capable of infecting numerous parts of the body, including the lungs, brain, bone and ear, *S. pneumoniae* must be constantly regulating its gene expression to survive these different chemical environments. Similarly, the challenges *S. pneumoniae* faces as a commensal in the nasopharynx are vastly different than the challenges that it faces once it becomes virulent, such as the immune response and antibiotic treatment. However, we still do not have a comprehensive understanding of how *S. pneumoniae* regulates its gene expression. Here, I aim to map the Transcriptional Regulatory Network (TRN) of *S. pneumoniae* by cloning Transcription Factor Induction (TFI) strains for all transcription factors in *S. pneumoniae* strain TIGR4 and subjecting them to a combination of RNAseq and ChIPseq. This will define the regulon of each individual transcription factor and allow us to create a comprehensive TRN by piecing together the regulons of all transcription factors. Single-cell RNAseq data can then be analyzed through the lens of the TRN, identifying how specific transcriptional activity affects phenotypes. Not only that, but knowing the regulatory logic of bacterial pathogens like *S. pneumoniae* will allow us to model their behavior, provide insight for the growing field of synthetic biology and eventually develop next-generation therapies aimed at puppeteering *S. pneumoniae*.

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tRNA-acetylating toxins evolve toward narrow substrate specificity

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Bacterial genomes encode a vast and diverse array of toxin-antitoxin (TA) systems, shown to participate in cell regulation and stress response. Recent work has revealed a family of TA systems in which the toxin acts through the acetylation of tRNAs to disrupt translation and arrest cell growth. We combined phylogenetic analysis with the study of tRNA target specificity to examine the evolutionary trajectory of this toxin family. Our work shows that, while a subset of ancestral toxins has relaxed target specificity toward multiple tRNA species, the majority of these toxins have evolved to specifically target glycine tRNAs. These results indicate a remarkable selective pressure toward the corruption of a single group of tRNA isoacceptors.

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Unraveling infection dynamics with barcodes

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Pathogen population dynamics during infection are critical determinants of infection susceptibility and define patterns of dissemination. However, unveiling these dynamics, particularly host bottleneck sizes and routes of dissemination, is difficult because measuring bacterial burden alone is insufficient to decipher these patterns. Here, we employ a barcoding methodology known as STAMPR that enables the tracking of individual bacterial cells during infection, thereby permitting measurements of bottleneck sizes and dissemination patterns in the host. We demonstrate the use of these tools across three infection contexts: *E. coli* systemic dissemination, *Citrobacter rodentium* intestinal

infection, and *Vibrio cholerae* intestinal colonization in vaccinated animals. During *E. coli* systemic infection, we show that tight bottlenecks and stochastic replication of single clones lead to dramatic variability in bacterial burden. Unexpectedly, dissemination is typically driven by very few bacterial clones that arise from a subset of organs. In the *C. rodentium* intestinal infection model, we found that bottlenecks are surprisingly tight despite the large bacterial burdens in infected animals. The bottleneck is largely microbiota-dependent, and disruption of the microbiota leads to increased founding population sizes that enables infection at very low doses. Furthermore, natural transmission of *C. rodentium* also enables bottleneck evasion without requiring microbiota disruption. In a mouse model of *V. cholerae* intestinal colonization, we discovered that vaccination tightens infection bottlenecks, thereby leading to sterilizing immunity at low doses. Collectively, these results demonstrate that using barcoded bacteria can uncover the hidden dynamics that underlie infections. Furthermore, this work sets the stage for future studies to explore the biological mechanisms that establish and regulate bottlenecks across diverse models of host-microbe interactions.

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The overlap of microbial changes to different mucosal tumor microenvironments

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A hallmark of cancer progression is the Warburg effect, a metabolic switch that results in a reliance on the less efficient aerobic glycolysis. This shift triggers many intracellular and extracellular changes, including excessive production of reactive oxygen species and nucleotides, and the nutrient microenvironment. Such metabolic shifts in the tumoral microenvironment likely result in significant changes in the composition and behavior of proximal microbes. While some work has been done on individual cancers, there has been limited study in the overlap between different cancers of sites that harbor a microbiome. We hypothesize that during cancer progression in the lung, stomach, and cervix, there are recognizable and overlapping patterns of change in tumor environment taxa that likely impact disease progression in cancer. In our analysis, we utilized two 16S RNA-sequencing datasets of non-small cell lung carcinoma, colorectal cancer, and cervical cancer. Then using the DADA2 pipeline and phyloseq visualization methods, we explored the impact each cancer had on the respective organ microbiomes. We found that modulation of the tumoral sites during cancer progression begets corollary changes to the microbiome across the lung, stomach, and cervix. This work highlights the importance of considering local and systemic changes to the microbiome during cancer, as well as potentiating its role tumor dynamics.

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Discriminating the mycobacterial cell wall proteome with compartment-specific labelling

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Mycobacterium tuberculosis, the causative agent of tuberculosis has two lipid membranes, making it a multi-compartment cell comprising the cytoplasm, inner membrane, periplasm and mycobacterial outer membrane. The latter three structures form the cell wall, which contains key processes such as the import and export of nutrients and virulence factors. Efforts to separate these compartments and identify the proteins in the cell wall have either been non-specific, as with sedimentation-based techniques that are performed on lysates, or low throughput, as with techniques that localize a specific protein. Both approaches fail to give a complete and accurate picture of the cell wall proteome. To achieve this goal, we here employ protein biotinylation by engineered peroxidase APEX2. The reaction of the peroxidase with its substrate biotin-phenol produces phenoxyl radicals that biotinylate tyrosine residues on proteins in close proximity, but do not cross lipid membranes. This approach enabled selective detection of known cytoplasmic components such as ribosomal proteins and core metabolic proteins only in the cytoplasm and confirmation of cell wall-resident proteins such as lipid acyltransferases. Importantly, this strategy also provided the first direct evidence that substrates of virulence-associated Type VII proteins secretion systems (ESX) are exposed to the periplasmic environment. This result has implications for the currently unknown mechanism by which ESX systems export proteins beyond the inner membrane.

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Harnessing the Inoculum Effect to Diagnose Molecular Mechanisms of Carbapenem Resistance and Characterization of a *Klebsiella pneumoniae* Transcription Factor involved in Multiple Antibiotic Resistance

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The global spread of carbapenem-resistant Enterobacterales (CRE) presents a major threat to public health as these pathogens are resistant to some of our best antibiotics and are responsible for thousands of deaths. CRE employ two molecular mechanisms of resistance: 1) expression carbapenemases (CPases), which efficiently hydrolyze carbapenems or 2) disruption of porins, which reduces carbapenem influx.We measured carbapenem minimum inhibitory concentrations (MICs) for 103 Enterobacterales isolated from hospitals in Massachusetts and California using broth microdilution assays at 14 inocula spanning four orders of magnitude. We observed the two mechanisms result in distinct profiles; the MICs of CPase-encoding isolates show strong inoculum dependence, whereas the MICs of porin deficient isolates remain largely constant at all inocula. The synergistic action of these mechanisms leads to high-level resistance that we termed "hyper-CRE". We also discovered that in some *Klebsiella pneumoniae* isolates the upregulation of a transcription factor increases resistance to multiple antibiotics including quinolones, tetracyclines, nitrofurans, sulfonamides, and carbapenems. To validate the hyper-CRE phenotype, we employed CRISPR-based gene editing to knock out the major porin in CPase-producing strains, which elevated their carbapenem resistance to hyper-CRE levels.We also determined 18% of our isolates changed susceptibility classification. This is worrisome for the treatment of infections with strains that are deemed "susceptible" via *in vitro* MIC assays but are truly resistant. Overall, our approach demonstrates that measuring MICs at different inoculum can yield crucial diagnostic information about mechanisms of resistance which has important implications for patient care, infection control, and surveillance of emerging CPases.

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The role of *Shigella flexneri* effector protein OspC3 in mediating pyroptosis via Caspase-4 binding

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Shigella is a Gram-negative bacteria that causes bacillary dysentery, a severe inflammatory gastroenteritis. *Shigella* establish a replicative niche within infected colonic epithelial cells. In response to the invasion by *Shigella* and other Gram-negative bacteria, epithelial cells trigger the activation of host cell death via pyroptosis, an inflammatory form of cell death. The pathogenesis of *Shigella* is dependent on a Type 3 Secretion System (T3SS), a complex nanomachine which it uses to deliver 30 proteins, referred to as effectors, directly into the cytosol of host cells. These effector proteins promote the survival and replication of *Shigella* within host cells, including the activation of non-canonical Caspase-4 inflammasomes, which trigger pyroptosis. Shigella encodes three effectors, OspC1, OspC2 and OspC3, that share at least 95% identity. OspC3 has been found to inactivate Caspase-4. By generating reciprocal swaps between OspC3 and OspC2, and human Caspases-4 and Caspase-5, we have identified regions of OspC3 and CASP4 that mediate their binding specificity, enriched in acidic and basic amino acids. Mutations in this region of OspC3, as well as in the P19 domain of Caspase-4, were also used in yeast two-hybrid screening to determine residues important for binding. Mutating 3 charged residues in OspC3 prevented binding to Caspase-4, and mutating charged residues within different predicted secondary structures in Caspase-4 P19 also prevented binding. Our observations, combined with modeling of the recently identified interactions of an OspC3 homolog with CASP7, suggest that two regions of interaction exist between OspC3 and Caspase-4, one of which provides binding specificity.

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Activation of survival state in Coxiella burnetii and Legionella pneumophila coincides with major changes

in the cell envelope

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Coxiella burnetii and *Legionella pneumophila* are two phylogenetically related, Gram-negative, environmentally stable bacterial pathogens with significant public health importance. Central to both pathogens infectious cycle is a biphasic developmental cycle that generates two ultrastructurally distinct morphological variants; one specialized for intracellular replication inside a permissive host, and one specialized for extracellular survival and disease transmission from environmental reservoirs. The developmental variants responsible for extracellular survival exhibit a high degree of intrinsic resistance and stability. Here, we show that differentiation to the survival state correlates with dramatic changes in cell envelope structure and function. Activation of a new mechanism of cell envelope stabilization is observed, where numerous structurally distinct beta-barrel outer membrane (OM) proteins are covalently attached to the peptidoglycan (PG) layer. This also correlates with a significant decrease in OM permeability and changes in peptidoglycan composition and cross-linking. We further show that these structural changes are regulated by the stationary phase sigma factor, RpoS. RpoS regulates the expression of numerous genes including L,D-transpeptidases, key cell envelope remodeling enzymes that are essential for bacterial survival under stress conditions. Collectively, these findings suggest that RpoS-regulated, L,D-transpeptidase-mediated mechanisms of cell envelope remodeling are major determinants of resistance and survival in C. burnetii *and* L. pneumophila.

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Genomics of resistance before the age of antibiotics

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Antibiotic resistant infections are a significant public health concern, and their prevalence has typically been associated with the anthropogenic use of antibacterials. Recent work has complicated this narrative by identifying clinically relevant strains isolated before the age of antibiotics that carry resistance genes. However, it is unclear if these findings are merely one-off occurrences or are representative of a broader trend of pre-existing natural resistance before the age of antibiotics. Here we create a historical database of resistance by merging genome assemblies, annotations, and metadata from the National Collection of Type Cultures. We demonstrate the existence of resistance genes on numerous clinical isolates cultured far before the anthropogenic introduction of antibiotics. We find that not only has overall resistance to particular drug classes has significantly increased since human introduction of a given antibiotic class, but also the mechanisms and genes conferring resistance have changed. In particular, beta-lactamases have experienced increasing diversity since the anthropogenic introduction of beta-lactams. Our results suggest that before the age of antibiotics there already existed a large reservoir of resistance genes circulating in clinically relevant strains, and anthropogenic introduction of antibiotics has merely shifted and refined those pre-existing genes.

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S. aureus Survival and Antibiotic Tolerance in Macrophages

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Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterial pathogen that can cause serious infections in humans. Though traditionally believed to be an extracellular pathogen, *S. aureus* has been shown to infect and survive in both phagocytotic and non-phagocytotic cells. The ability to survive intracellularly is thought to protect *S. aureus* from the host immune system and cell impermeable antibiotics, resulting in difficult-to-treat recurrent infections. To identify mechanisms that permit *S. aureus* to survive in cells, we infected primary murine bone marrow macrophages with *S. aureus* and treated the infected macrophages with cell-permeable antibiotics that target different pathways. We then quantified the surviving intracellular population under each condition. Treatment with oxacillin, an antibi-

otic that targets cell wall biosynthesis in actively growing cells, had a negligible effect on CFU counts compared to the no antibiotic control. In contrast, treatment with rifampicin, which targets both non-growing and growing cells by inhibiting transcription, caused a reduction in CFU count. Taken together, our results suggest that *S. aureus* is non-growing in primary bone marrow macrophages, yet still requires gene expression to survive. To investigate the specific genes and pathways involved in intracellular survival, we will treat macrophages infected with a library of transposon mutants with the previously tested antibiotics and perform TnSeq. Through these experiments, we will elucidate the processes critical for *S. aureus* intracellular survival and lay the groundwork for clinical therapies targeting recurrent infections.

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

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Labeled as a serious threat by the CDC in 2013, *Streptococcus pneumoniae* is an important pathogen to study and target with antibiotics in new ways. The two flavin mononucleotide (FMN) riboswitches in *S. pneumoniae*, one regulating transcription of riboflavin transport and another regulating biosynthesis genes, are promising targets. FMN is an essential cofactor that must either be synthesized or acquired by the organism. Mutations to FMN riboswitches in organisms such as *Staphylococcus aureus* confer resistance to the antibacterial compounds roseoflavin and ribocil-C. To ascertain whether similar mechanisms also confer resistance to *S. pneumoniae*, we raised roseoflavin resistant mutants and found that all strains carried mutations to the FMN riboswitch regulating riboflavin biosynthesis. To more globally understand the role FMN riboswitch mutations play in mediating resistance in *S. pneumoniae*, we are constructing fitness landscapes for the two FMN riboswitches in *S. pneumoniae*. By assessing these landscapes under a variety of conditions including the presence and absence of riboflavin and in the presence of roseoflavin and ribocil-C we hope to determine the full spectrum of mutations that allow resistance. As a pilot experiment we generated an FMN riboswitch mutant strain library, and assessed the fitness of each strain using high-throughput amplicon sequencing. Our preliminary results suggest strong selective action, but our initial sequencing is not sufficiently deep to allow robust analysis of the data, and we are in the process of repeating the sequencing. Our expectation is that this work will lead to better targeting of the FMN riboswitch in the future.

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Identifying *Yersinia pseutoduberculosis* YopH targets in neutrophil degranulation

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The extracellular pathogen, Yersinia pseudotuberculosis (Yptb), is swarmed by neutrophils during tissue infection. Previous work has shown that *Yptb* inactivates key antimicrobial functions of neutrophils through T3SS-injected Yop effector proteins. YopH, a potent tyrosine phosphate, is critical for inhibiting neutrophil ROS production and degranulation. We recently identified SKAP2 as one of the key targets of YopH; inactivation of SKAP2 results in a lack of ROS production but not degranulation. Importantly, deletion of SKAP2 only partially restored the growth of a *yopH* mutant in mouse infections, indicating that critical SKAP2-independent pathways are still at play. However, the YopH-targeted proteins that are independent of SKAP2 are unknown. Because neutrophils are terminally differentiated and short lived, they are challenging to genetically manipulate and study *in vitro*. Using an engineered immortalized myeloid progenitor cell line (MP), we can terminally differentiate MPs into neutrophils (MP-PMNs) to study ROS production and degranulation. We have developed a FACS-based assay to monitor ROS production and degranulation simultaneously in the MP-PMN's and have shown that YopH is sufficient to inhibit both processes. We will identify the YopH-targeted proteins responsible for inactivating degranulation with a targeted approach: (1)

Western Blot analysis to identify phosphorylation status of candidate proteins and (2) deletion of proteins using CRISPR/Cas in the MP cell lines. Neutrophils lacking the ability to generate ROS, degranulate, or both functions will be studied in murine infection. This work will elucidate the signaling pathways disrupted by YopH and enable us to dissect the interdependencies of ROS production and degranulation during tissue infection.

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Human gut bacteria produce Treg-modulating bile acid metabolite

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Primary bile acids discharged into the gut lumen are subject to biotransformation by commensal bacteria and converted into secondary metabolites. These bile acids and bile acid metabolites are signaling molecules that regulate immune homeostasis, including the differentiation of CD4+ T cells into distinct T cell subsets. Previously, we have shown that the bile acid metabolite isoallolithocholic acid (isoalloLCA) enhances the differentiation of anti-inflammatory regulatory T cells (Treg cells). Here, we identify gut bacteria that synthesize isoalloLCA from 3-oxolithocholic acid and uncover a gene cluster responsible for the conversion in members of the abundant human gut bacterial phylum Bacteroidetes. Moreover, the levels of isoalloLCA and its biosynthetic genes are significantly reduced in patients with inflammatory bowel diseases, suggesting that isoalloLCA and its bacterial producers may play a critical role in maintaining immune homeostasis in humans. Our results reveal new ways in which commensal bacteria regulate immune tolerance in the gut, laying the groundwork for potential new therapeutic avenues to control IBD.

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Genetic selection on cell size in *Mycobacterium tuberculosis*: Is bigger better?

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Mycobacterium tuberculosis (*Mtb*) resides in a complex environment with strong host and antibiotic pressure, which drives the emergence of mutations that promote bacterial survival. Using population genomics, reverse genetics, and high-throughput imaging, we identify a network of positively selected variants that have altered cell size in *Mtb*. These variants are found in an essential cell cycle regulatory gene, *dnaA*, an essential transcription factor, *Rv1830* (*resR*), as well as a conserved intergenic region, Rv0010c-Rv0011c. We also find that clinically prevalent genetic variants cause antibiotic resilience, which we define as faster regrowth post antibiotic exposure. To better understand recovery after drug, we performed static and pseudo-time lapse imaging of engineered isogenic strains with clinically relevant mutations. These mutations result in larger cells largely due to increased elongation rate at the mature cell pole. Using IDAPseq, we find that DnaA and ResR both bind to the Rv0010c-Rv0011c intergenic region, implicating this site as a regulator of cellular growth. Preliminary data shows Rv0010c is a small transmembrane protein whose structure resembles those of proteins involved in phosphatidylinositol related pathways. Current work seeks to mechanistically dissect the pathway by which this gene triad controls cell growth dynamics. In conclusion, these findings identify a novel genetic pathway that governs cell size and links cell size control to altered response to drug.

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Tuberculosis treatment failure associated with evolution of antibiotic resilience

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Antibiotics are a cornerstone of medicine, placing bacterial pathogens under intense pressure to evolve new survival mechanisms. Analysis of 51,229 Mycobacterium tuberculosis (Mtb) clinical isolates identified an essential transcriptional regulator, Rv1830 (here named resR) as a frequent target of positive (adaptive) selection. resR mutants do not demonstrate canonical drug resistance or drug tolerance but instead have significantly faster recovery after drug treatment across all antibiotics and combinations tested, a phenotype which we term antibiotic resilience. ResR acts in a regulatory cascade with other growth-controlling transcriptional regulators WhiB2 and WhiA, which are also under positive selection in Mtb clinical isolates. Mutations of these genes are associated with treatment failure and the acquisition of canonical drug resistance.

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Identification of a key gene in *Pseudomonas aeruginosa* regulating neutrophilic inflammatory signals in infected airways

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Pseudomonas aeruginosa is an opportunistic bacterial pathogen that commonly infects the lungs of people with cystic fibrosis. Airway infection with *P. aeruginosa* triggers neutrophil-mediated inflammation, which can severely damage the airway, driving morbidity and mortality.The *P. aeruginosa* (strain PA14) non-redundant transposon insertion mutant library was screened for key genes driving bacterial-induced neutrophil migration. An in-vitro neutrophil trans-epithelial migration assay was used to measure neutrophil migration across epithelium infected with the bacterial mutants. Production of leukotriene B4 (LTB4), an eicosanoid that drives neutrophil chemotaxis, was measured by enzyme-linked immunoassay. Motility, adhesion, and neutrophil chemotaxis assays were performed on the mutants identified. Mutant PA14 23790::MAR2xT7 with a transposon insertion in the *P. aeruginosa leuB* gene showed a significant reduction in neutrophil transmigration compared to wild-type. An in-frame deletion mutant in the *leuB* gene (PA14Δ*leuB*) showed a similar reduction in neutrophil trans-epithelial migration and also a reduction in LTB4 production compared to wild-type. Interestingly, when exogenous leucine, the biosynthetic product of the *leuABCD* operon was supplemented during transmigration assays, neutrophil migration and LTB4 production were restored. PA14Δ*leuB* displayed normal levels of motility and adhesion but showed decreased chemotaxis towards neutrophils as compared to wild-type PA14.These findings suggest that *leuB* plays an important role in the neutrophil response to *P. aeruginosa* infection in the airway, perhaps as a component of a signal peptide that induces neutrophil chemotaxis. Future research is necessary to further elucidate the role of *leuB* in driving airway inflammation and to identify targets for therapeutics to mitigate airway damage.

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Impact of SER-109, an Investigational Microbiome Therapeutic, on Stool Fatty Acid and Bile Acid Metabolites in a Phase 3 Randomized Trial (ECOSPOR III) for Treatment of Recurrent *Clostridioides difficile* Infection (rCDI)

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In ECOSPOR-III, a Phase 3 double-blind, randomized trial, SER-109, an oral investigational microbiome therapeutic composed of purified Firmicutes spores, was superior to placebo in reducing risk of rCDI at week 8 (12% vs 40%, respectively). Compared to placebo, SER-109 led to higher engraftment of dose species at weeks 1, 2 and 8. Here, we evaluated changes in short, medium and branched-chain fatty acids (FAs) and primary and secondary bile acids (BAs), based on the hypothesized roles of these metabolites in inhibiting spore germination and replication of *C. difficile*.Concentrations of FAs and BAs in subjects' stool samples were measured pretreatment ("baseline") and at 1, 2, and 8 weeks by targeted liquid chromatography with tandem mass spectrometry. Two-sided Mann-Whitney U tests were used to determine statistical significance. Baseline concentrations were comparable between the two arms. At week 1, concentrations of butyrate, valerate, hexanoate, and secondary BAs were significantly increased and remained significantly higher at weeks 2 ($p < 0.01$) and 8 ($p < 0.05$) in the SER-109 arm compared to placebo. Conversely, concentrations of primary BAs were significantly reduced at week 1 ($p < 0.05$) in the SER-109 arm compared to placebo. These changes correlated with a greater magnitude of engraftment of SER-109 dose species compared to placebo and were associated with a reduction in rCDI events. These data suggest that mechanisms of action of improved clinical outcomes may include interruption of the two-phase lifecycle of *C. difficile* via increased FA production, along with rapid conversion of primary to secondary BAs.

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Data-Informed Catastrophic and Harmonious Genetic Codon Bias for Multi-Gene Expression

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Expression of genes in microbial hosts forms the foundation of basic research and applied biotechnology. De-coupling non-native gene expression from host resources can improve the outcome of experimental efforts. Few studies to date have investigated the mechanistic underpinnings of resource allocation during translational elongation, which is often mediated by tRNA. The degenerate genetic code provides an opportunity to allocate cellular tRNA and ribosomal resources optimally between host and heterologous protein expression. Through our research, we aim to improve the predictability and robustness of genetic engineering in microbes by systematically determining optimal codon bias schemes.We investigate how the partitioning of microbial translational resources, specifically through allocation of tRNA by incorporating dissimilar codon usage bias (CUB), can drastically alter expression of proteins and reduce burden on gene expression systems. Utilizing nearly identical fluorescent reporters (CFP and YFP), we assayed genetic resource competition both in vitro and in vivo using novel designs that isolate translation elongation from other variables. We find that alternative CUB designs can trans-regulate gene expression of competing heterologous and endogenous genes, yielding profitable or catastrophic design options. By isolating individual codons experimentally, we correlate specific codon usage patterns with genetic burden, and derive novel coding schemes for multi-gene expression. These empirically derived coding schemes based on a new codon adaptation index enable the design of harmonious multi-gene expression systems while avoiding catastrophic cellular burden. More broadly, this research advances our understanding of how biological systems respond to genetic burden and adapt to accommodate the acquisition of new genes.

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Analysis of phototrophic microbial communities that degrade compostable plastic

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The world continues to have growing environmental concerns related to plastics production and recycling. The use of biobased and biodegradable/compostable plastic is a possible solution to make current plastics use more sustainable because of its potential for truly closed-loop processes. We enriched a phototrophic microbial community from compost which degrades poly 3-hydroxybutyrate (PHB)/ 3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV). The

community is anaerobic and forms a red biofilm on the PHB/PHBV pellets and strips. Examination of the biofilm using SEM indicates that the community forms a dense network on the plastic surface that is dominated by a few particular bacterial morphologies. SSU rRNA amplicon sequencing and community metagenomic analysis indicate that the main microbial genera are members of the Bradyrhizobia, Sporomusaceae, and Hyphomicrobiaceae. These community members were found in the metagenomic analysis to contain several potential genes for degradation and synthesis of polyhydroxyalkanoates, as well as nitrogen fixation capability and phototrophy. Study of the microbial community with these metabolisms can provide insight into microbial interactions within biofilms and further development of processes for sustainable use of biobased compostable plastics.

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Long read sequencing reveals gene conversion as a diversity generating mechanism in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis, an airborne infectious disease that kills 1.5 million people every year. Whole genome sequencing has proven to be a useful tool for studying the evolution of Mtb. Due to the limitations of short-read sequencing, researchers commonly exclude 10% of the Mtb genome due to high levels of sequence homology and risk of false positive variant calls. To confidently study these largely obscured regions of the genome, we have used long read sequencing to generate a dataset 158 *M. tuberculosis* clinical isolates with high confidence complete assemblies. We have uncovered regions of extremely elevated nucleotide diversity in regions of the genome that were previously obscured by the limitations of short-read sequencing. Analysis of these patterns of genomic diversity has revealed gene conversion, intrachromosomal recombination between homologous sequences, as the most likely mechanism. The proteins with extremely elevated diversity are strongly enriched for substrates of ESX secretion systems of Mtb. The ESX secretion systems are some of the best studied virulence factors of M. tuberculosis, and several of the secreted proteins have been identified as potent antigens and virulence factors. Understanding how and why these patterns of increased diversity are generated is important because it has the potential to uncover a major driving force in the evolution of gene families that are also believed to play critical roles in Mtb's pathogenic lifestyle.

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Potential Enhanced Stress Tolerance of *Salmonella enterica* Outbreak-Associated Strains

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Non-typhoidal *Salmonella enterica* causes 1.35 million illnesses annually in the United States, with 26,500 hospitalizations and 420 deaths. Previous research found that isolates from the 2013-2014 *Salmonella* Heidelberg outbreak had increased stress tolerance compared to non-outbreak isolates.The objective of this study was to compare attachment capacity and sanitizer tolerance of non-outbreak (NOA) and outbreak associated (OA) isolates of monophasic *Salmonella* to *Salmonella* Heidelberg isolates.We analyzed 21 OA and four NOA isolates using crystal violet assays to test attachment at 24, 72, and 120 hours in 1X and 1/20X TSB at 22°C and 4°C. Minimum inhibitory concentrations (MICs) were determined for bleach and peroxyacetic acid in 1X and 1/20X TSB at 24 hours, 22°C using OD600. Resfinder and PlasmidFinder identified antibiotic resistance elements and plasmids. Statistically significant differences between isolates were determined via analysis of variance with Tukey's HSD test (Padj < 0.05).In 1/20X TSB at 4°C, three *Salmonella* Heidelberg OA, two monophasic OA, and one NOA monophasic isolate attached significantly better than 3 others. At 22°C, five Heidelberg OA, two monophasic OA, and one NOA monophasic isolate attached significantly better than 3 others. In 1X TSB at 4°C and 22°C, only one OA Heidelberg attached significantly better. All NOA monophasic isolates had 1 plasmid. All OA and three NOA monophasic isolates had blaTEM-1B, sul2, and aminoglycoside resistance genes. MICs for bleach, 1X TSB were >200ppm and >100ppm in 1/20X TSB (Padj <

0.01). MICs for PAA, 1X TSB were >175ppm vs 25.9ppm in 1/20X TSB (Padj< 0.01).

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Gut bacteria convert glucocorticoids into progestins

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Steroid hormones regulate fundamental physiological processes, including metabolic activity, sexual characteristics, reproduction, and behavior. Recent studies suggest that there are correlations between gut bacteria, host functions, and sex- and stress-related steroid hormones. However, the causal relationships between these three entities remain unclear. Earlier studies performed before the advent of modern genetic and microbiological tools demonstrated that gut bacteria metabolize steroids. In one important example, literature published prior to 1980 reports that gut bacteria can 21-dehydroxylate corticosteroids to form progestins, thereby converting one class of steroids into another with different biological effects. Given the potential importance of the conversion of corticoids to progestins for human health and disease, we aimed to identify the gut bacteria that convert glucocorticoids into progestins, elucidate the associated biosynthetic pathways responsible for these transformations, and determine whether these molecules are produced by bacteria in vivo. We have reconstituted this activity in both healthy human feces and in specific strains of the gut bacterium *Eggerthella lenta* in co-culture with *Escherichia coli* Nissle 1917. Some of the progestins produced by bacteria are neurosteroids, including allopregnanolone, which is an FDA-approved drug used to treat postpartum depression. Furthermore, we have shown that while both healthy human and specific pathogen-free (SPF) mouse feces contain 21-dehydroxylated products, germ-free (GF) mouse feces lack these metabolites, suggesting that gut bacteria contribute to the production of progestins in vivo. Gaining insight into how human-associated bacteria metabolize steroids may unveil potential therapeutic strategies to improve human health.

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Dissecting the genetic networks underlying host subversion during *Rickettsia* infection

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

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Fold or Die: SAS, a Split Antibiotic Selection for Enhancing Protein Solubility

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Heterologous expression of soluble, natively-folded protein in *Escherichia coli* is a critical step in biochemical and structural biology research, but is frequently limited due to insolubility. Although there are numerous changes that can be made to the protein itself, the expression conditions or to the cells expressing it, there is no one-size fits all approach to enhancing protein solubility. Testing each individual approach is time and labor intensive. In nature, molecular chaperones assist with the native folding of a wide range of proteins across all domains of life, and are frequently used to enhance recombinant protein expression in *E. coli*. To rapidly determine the best chaperone to assist in solubilizing a target we have developed a split antibiotic genetic selection to link protein solubility to cell viability. To this end, we use the hygromycin resistance protein APH(7) from *Streptomyces hygroscopicus*, which has a two-domain architecture. A sandwich construct is created by inserting an insoluble protein between the two domains of APH(7), linking protein cargo solubility to hygromycin resistance. Furthermore, many proteins require expression of multiple chaperones for efficient folding. Using a base library of 42 proteins, we have created a combinatorial chaperone plasmid library with over 12,000 unique combinations. In addition, we have designed a second plasmid library containing more than 270 uncharacterized chaperone homologs with diverse domain architectures. Together, our genetic selection and chaperone libraries will enable us to both enhance expression of proteins recalcitrant to folding, as well as discover and study the biology of new protein-folding factors.

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Lectin-Seq: a method to profile lectin-microbe interactions in native communities

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Soluble human lectins are critical components of innate immunity and genetic models suggest lectins influence commensal microbiota. Lectins recognize microbes by binding cell surface glycans, but their specificity for commensal species is understudied. Elucidating lectins' roles in regulating microbiota requires an understanding of which microbial species they directly bind within native communities. To profile human lectin recognition in native commensal communities, we developed Lectin-Seq. We apply Lectin-Seq to human fecal microbiota using the soluble human proteins mannose-binding lectin (MBL) and intelectin-1 (hItln1). The microbial interaction profiles of MBL and hItln1 are disparate in composition and diversity, suggesting these lectins recognize ligands distributed in distinct patterns across the human microbiota. MBL binding across all samples is highly selective for a small subset of species commonly associated with humans. In contrast, hItln1 binding is more variable, and its interactome encompasses a broader range of lower-abundance species. These findings provide clues to the biological roles of MBL and hItln1 and demonstrate the utility of Lectin-Seq for characterizing interactions between human lectins and commensal microbes in native communities.

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Phenotypic variation of growth patterns for *Neisseria gonorrhoeae* isolates

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Bacteria display a wide range of growth patterns, both within hosts and in the laboratory. Under ideal labora-

tory conditions, the doubling times for different bacterial species range from under ten minutes to over twelve hours, with environmental conditions like incubation temperature and nutrient availability influencing these rates. Genes involved in transcription and metabolic processes also contribute to bacterial growth in different environments, though these effects are often condition-specific. The obligate human pathogen *Neisseria gonorrhoeae* has an average laboratory doubling time of approximately 60 minutes, but the range of growth phenotypes of this species has yet to be characterized. To assess the variation of growth patterns for *N. gonorrhoeae* in standard conditions, we systematically measured the growth phenotypes of a 100-strain panel selected to be representative of the species diversity. We observed replicable variation in traits including carrying capacity and maximum growth rate, with respective means of 0.780 (Standard Deviation 0.095) and 1.023 (SD 0.135). The skewness values for these carrying capacity and maximum growth rate distributions are -0.969 and 1.123, respectively. These data provide a basis for determining the genetic factors that contribute to the variation, as well as a baseline for the range of growth patterns for *N. gonorrhoeae* isolates in complex media, which we can compare to growth in other environments. We can also use this dataset to assess other physiological factors these growth patterns may correlate with, including genome size and antibiotic resistance.

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Evaluation of the microbiome and resistome of a hamburgers' production line

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Meat is a highly perishable food commodity involved in food-borne outbreaks and large economic losses. Otherwise, the extensive use of antibiotics in primary production has resulted in the emergence of antimicrobial-resistant (AMR) bacteria and, consequently, of difficult-to-treat infections. As these AMR bacteria could reach consumers through the food chain, it is necessary to prevent and control their dissemination. One measure might be the design and implementation of new and effective cleaning and disinfection (CandD) procedures, especially against biofilms, which are reservoirs of bacteria with higher resistance. Thus, the main objective of this study was assessing the microbiome and resistome of the poultry meat industry as well as designing strategies to limit the emergence and dissemination of AMR bacteria. First, culturing techniques and metabarcoding were applied to assess the microbiome of different products and surfaces throughout a chicken hamburgers' production line. Culturing techniques allowed the isolation and quantification of the major microbial groups, being *Pseudomonadaceae* the most prevalent; and metabarcoding allowed to determine all microbial communities and their relative abundances at different taxonomic levels, predominating *Pseudomonadaceae* in products, and *Listeriaceae* and *Moraxellaceae* in surfaces. Next, metagenomic is currently being applied to determine all AMR genes present in the same production line. Related to CandD procedures, carvacrol treatments were applied to eradicate mature biofilms formed by an isolated Pseudomonas spp. strain, achieving an inactivation of more than 4 log cycles of biofilm cells. A deep knowledge of food microbiota allows to design measures to prevent and control microbial contamination and AMR dissemination.

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The correlation of the genetic antibiotic resistance markers to antibiotic susceptibility testing (AST)

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Antibiotic resistant infections are a risk for increased patient mortality due to long time-to-results for current diagnostic methods. Results from a 1,500 patient trial demonstrated bacterial blood culture required 51 hours on average for a positive result. Current antibiotic susceptibility testing (AST) requires two additional days for phenotypic results. Studies show delays in effective treatment increases mortality rates by 7.6% every hour. The CDC classifies carbapenem-resistant Enterobacterales (13,000 annual deaths) as urgent threats, and vancomycin-resistant Enterococci and methicillin-resistant *S. aureus* (15,000 annual deaths combined) as serious threats. Identifying resistance markers direct from patient samples may aid clinical decisions, reduce antibiotic use, reduce time to ef-

fective therapy, and improve patient outcomes. 200 antibiotic resistant bacterial strains were genotyped for 13 clinically-relevant resistance genes and screened for antibiotic resistance phenotypes. Results identified correlations between resistance markers and phenotypic resistance and potential aid for clinical treatment. High resistance to vancomycin and piperacillin-tazobactam, first-line antibiotics, were seen in 48 Gram-positive and 69 Gram-negative strains. Presence of Gram-positive markers *vanA* or *vanB* correlated to 100% (28/28) vancomycin resistance and 88% (25/28) ampicillin resistance. In 85 sequenced Gram-negative strains, 35 harbored metallo-β-lactamase (*bla*IMP, *bla*NDM, *bla*VIM), 35 contained extended spectrum β-lactamase (*bla*CTX-M-14, *bla*CTX-M-15), 21 contained AmpC β-lactamase (*bla*DHA, *bla*CMY), and 34 contained carbapenemase (*bla*KPC, *bla*OXA48). These markers correlated to 100% resistance to ampicillin, cefazolin, and cefuroxime in all strains. Together, these data suggest that molecular diagnostics that identify genetic markers may provide clinicians with needed information during a window when therapeutic intervention can improve patient outcomes.

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Mutagenesis of amino acid residues in the active site of FtsA impairs divisome function in *Escherichia coli*

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Cell division in *Escherichia coli* proceeds through polymerization of the conserved tubulin homolog FtsZ to form a ring-like structure, termed the Z-ring, at the site of septation. FtsZ polymers are tethered to the cytoplasmic membrane through a direct interaction with the actin homolog FtsA, which, similar to actin, polymerizes into linear fibers and hydrolyzes ATP. Yeast actin contains a sensor region adjacent to the active site that detects nucleotide occupancy, and actin adopts distinct conformations in the monomeric or filamentous state. Here, we show that mutagenesis of residues located near the FtsA active site impair critical functions including ATP hydrolysis, phospholipid (PL) binding, ATP-dependent vesicle remodeling, and recruitment to the Z-ring in live, dividing cells. Notably, we demonstrate that Ser 84 and Glu 14 are important for vesicle remodeling and magnesium-dependent phospholipid release in vitro, which likely underlies the loss of function by FtsA(E14R) and FtsA(S84L) in vivo. Additionally, we show that FtsA(A188V), an FtsA mutant protein that confers a temperature sensitive growth phenotype in vivo, is defective for ATP hydrolysis across a range of temperatures and for PL binding in vitro. This study provides insight into the importance of ATP interactions by FtsA for its functional activities in vitro and in vivo.

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Changes in E. coli *susceptibility to predation by* Bdellovibrio

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Bacteria vary in their susceptibility to killing by predatory bacteria such as *Bdellovibrio*, but little is known about the mechanisms that govern susceptibility to predation. When we co-cultured E. coli *ML35 with* Bdellovibrio *sp. NC01 we observed* E. coli population reduction at 24hrs but recovery at 48 and 72 hrs, despite the presence of active NC01 predators. In addition, we observed the appearance of an altered colony morphology, which we refer to as "squashed", with the following proportions: 0hrs 0% squashed, 24hrs 54%, 48hrs 78%, and 72hrs 83%. The correlation between E. coli *population recovery and the appearance of squashed* E. coli *prompted two questions. Do squashed* E. coli exhibit reduced susceptibility to killing by NC01, if so, what mechanisms are responsible? We isolated squashed E. coli *and tested their susceptibility to killing by NC01 and* Bdellovibrio *type strain HD100. NC01 produced no squashed* E. coli *prey reduction compared to the control. These results indicate that* E. coli has developed a drastically reduced susceptibility to predation, specifically to NC01. HD100 killing of squashed E. coli *was indistinguishable from HD100 killing of original* E. coli. *The mechanisms governing this change in susceptibility to predation by NC01 are unclear. Initial genomic analysis did not identify point mutations or small indels to explain this phenotype. We are investigating possible explanations including changes in* E. coli gene expression and/or outer surface structures. Understanding mechanisms governing changes in susceptibility to killing are crucial to implement predatory bacteria

as clinical therapies against antibiotic-resistant bacterial pathogens.

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A two-plasmid CRISPR/Cas9-based method for rapidly and efficiently generating genetic knockouts in *Mycobacterium abscessus*

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Mycobacterium abscessus is increasingly known for causing infections that are notoriously difficult to treat, largely due to the large intrinsic arsenal of antibiotic resistance mechanisms. To gain a better understanding of *M. abscessus* biology, pathogenesis and antibiotic resistance mechanisms, tools for genetic manipulation are required. However, existing methods are largely recombination-based and can be suboptimal and tedious. Meanwhile, CRISPR/Cas9 has revolutionized the field of genome editing but has only recently been recently adapted for use in mycobacteria for this purpose. In this study, we report a streamlined and efficient method for rapidly generating gene knockouts in *M. abscessus*. Harnessing the CRISPR1 loci from *Streptococcus thermophilus*, we have developed a two-plasmid workflow that introduces the Cas9 and sgRNA cassettes in separate steps. By targeting genes established for mediating antibiotic resistance, we have shown induction of Cas9 leads to double-strand breaks which are repaired with the formation of indels verified by targeted sequencing. Subsequently, these strains possess increased susceptibility to the respective antibiotics of each resistance gene tested, verifying the generation of functional knockouts. This methodology is also shown to be amenable to generate knockouts in multiple genes sequentially or concurrently, or to induce longer genome deletions by using multiple targeting sgRNAs.

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Combining untargeted metabolomics and insertion sequencing to identify colonization determinants in *Phocaeicola vulgatus*

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Phocaeicola (Bacteroides) vulgatus (Pvu) is a common member of the human gut microbiota and is associated with cardiometabolic disease and inflammatory bowel disease. While Pvu is an effective gut colonizer, fundamental gaps remain in our knowledge of the mechanisms that contribute to its colonization and inflammatory phenotypes. Our goal was to leverage insertion sequencing (INseq), murine colonization studies, and metabolomics to identify key metabolites that promote Pvu gut colonization. A murine colonization screen was used to identify Pvu genes required for in vivo survival when either alone or in competition with mouse microbiota. We prioritized mutants with altered colonization outcomes for metabolomics. Untargeted metabolomics showed that some Pvu INseq mutants demonstrated metabolomic differences from WT when cultivated on agar. Pvu-0777::Tn was particularly interesting due to its depletion when competed in vivo with native mouse microbiota but its enrichment when competing only against other Pvu strains. Pvu-0777::Tn had a metabolome distinct from WT, producing increased levels of fatty amides. Genetic analysis determined that Pvu-0777::Tn harbors an insertion in a putative lipid transport operon unique to Pvu and closely related bacteria. Ongoing work aims to characterize the importance of the genes and metabolic products of this operon in vivo. Additional analyses comparing the metabolomes of other INseq mutants to WT are underway.

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Enhanced Synthetic Biochemistry Systems Enabled by Improvements in *Bacillus subtilis* Spore-Display

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Endospores (or simply, spores) are a highly stable phenotype of bacteria like Bacillus subtilis. Spores have robust tolerance to thermal, pH, proteinase, and solvent challenges and have applications as microparticles or carriers for various applications including vaccines, probiotic delivery, bioremediation, enzyme immobilization, and bioprocessing. For spore-display, a gene of interest is fused to a spore coat protein and, after sporulation, becomes anchored to the spore surface. In comparison to the more commonly used Escherichia coli and yeast-display systems, the surface of B. subtilis spores is less well-characterized. Accordingly, setting up a new spore-display system requires extensive design-build-test cycles to find a functional anchor protein for the display protein. There are at least 44 spore-coat proteins that could potentially act as anchors for spore-display. But only 12 of these have previously been used with enzymes. To improve the modularity of spore-display for use in biotechnology we set out to identify optimal enzyme anchor proteins. In collaboration with the DOE Joint Genome Institute we designed 88 synthetic DNA constructs that would allow us to test the suitability of each of the 44 spore coat proteins to act as either N or C-terminal fusion partners for the tetrameric enzyme beta-glucuronidase (GusA). By characterizing these constructs we have "mapped" the spore spore surface and identified optimal enzyme anchor proteins. We are now completing work on increasing enzyme display density and building complex synthetic biochemistry systems using spore-display.

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

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

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DeLTA 2.0: A deep learning pipeline for quantifying single-cell spatial and temporal dynamics

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Improvements in microscopy software and hardware have dramatically increased the pace of image acquisition, making

analysis a major bottleneck in generating quantitative, single-cell data. Although tools for segmenting and tracking bacteria within time-lapse images exist, most require human input, are specialized to the experimental set up, or lack accuracy. Here, we describe DeLTA 2.0, a purely Python workflow that can rapidly and accurately analyze images of single cells on two-dimensional surfaces to quantify gene expression and cell growth. The algorithm uses deep convolutional neural networks to extract single-cell information from time-lapse images, requiring no human input after training. DeLTA 2.0 retains all the functionality of the original version, which was optimized for bacteria growing in the mother machine microfluidic device, but extends results to two-dimensional growth environments. To showcase this new functionality, we analyze mixed populations of antibiotic resistant and susceptible cells, and also track pole age and growth rate across generations. Overall, DeLTA 2.0 is rapid, with run times of less than 10 minutes for complete movies with hundreds of cells, and is highly accurate, with error rates around 1%, making it a powerful tool for analyzing time-lapse microscopy data.

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Diagnostic for the future: rapid, adjustable, selective and specific electrical detection of pathogens, host-protein biomarkers, and metabolites.

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Outbreaks prevention requires optimized treatment strategies, which involves precise recognition of infectious disease etiologies in a clinical setting. Additionally, real-time and local information of infections at a population level is required to establish effective responses. However, viral and bacterial infections are often clinically indistinguishable. Furthermore, current methods of detection rely on costly laboratory-based techniques that require substantial infrastructure and trained personnel, resulting in long turnaround times. Detection of blood or saliva circulating host-protein signatures provide an opportunity to develop a global diagnostics approach that can assess whether an infection is viral or bacterial or consists of a co-infection. Moreover, wastewater-based epidemiology (WBE) could be an excellent tool for real-time information at the population level. Here we develop G-FET-based biosensors that can be rapidly adapted to diagnose infectious disease etiology by simultaneously detecting specific biomarkers, and determine the pathogen causing the infection. We have developed versatile label-free biosensors that combine G-FETs with a variety of highly specific probes (including ssDNA, RNA, and peptide probes). These biosensors are capable of: 1) selective and specific detection of host-biomarkers in saliva at clinically relevant levels; 2) determine the pathogens causing an infection in less than 5 minutes, at a single cell resolution, and are able to discriminate between antibiotic resistant and sensitive strains; and 3) selective multianalyte detection of different metabolites in wastewater. These biosensors could become a reliable platform for rapid and accurate point-of-care diagnostics of infectious disease, and can be rapidly adjusted to detect a novel pathogen of interest (such as SARS-CoV-2).

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Subinhibitory levels of translation inhibiting antibiotics alter transcriptional regulation in *Streptococcus pneumoniae*

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The widespread overuse of antibiotics selects for resistance within bacterial populations at antibiotic concentrations far below those used in the clinic, thus significantly contributing to the rise of antibiotic resistant strains. Therefore, assessing the impact of subinhibitory antibiotics concentrations provides a view into the emergence of antimicrobial resistance. In many bacterial pathogens transcription and translation are tightly coupled, suggesting that inhibition of translation may have wide-spread impacts on the transcriptome that go beyond a standard stress-response profile. To examine how subinhibitory concentrations of translation inhibiting antibiotics impact the transcriptional landscape, we performed RNA-seq experiments on populations of *Streptococcus pneumoniae* subjected to subinhibitory levels of three different translation inhibiting antibiotics. Our findings demonstrate that tetracycline and chloramphenicol

col, which respectively act on 30S and 50S ribosomal subunits for translation inhibition, result in diverse changes to cellular functionality. Meanwhile, kasugamycin, which inhibits translation initiation, displayed similar behavior to tetracycline. We also demonstrate that two different concentrations (both subinhibitory) of antibiotic induce different alterations to the transcriptional landscape, indicating the importance of exposure levels to the observed phenomenon. To further explore the generalizability of our findings, we established the prevalence of differentially expressed genes across 400 clinically relevant *Streptococcus* strains to show that most impacted genes are distributed widely. Taken together, our results highlight the importance of changes to transcriptional regulation in the presence of translation inhibiting antibiotics, paving the way for further studies to investigate novel regulatory regions in the transcriptional units.

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

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

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Epistasis between *rpoB* and *rpoC* affects susceptibility to rifampicin and β-lactam antibiotics

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Mutations affecting the core subunits of RNA polymerase often arise when bacteria are under strong selective pressure. For example, bacteria acquire rifampicin (RIF) resistance through mutations in *rpoB* (encoding β-subunit of RNAP); and mutations in *rpoC* (encoding for β' subunit) can confer resistance to cefuroxime (CEF), a β-lactam antibiotic. These two drugs synergistically inhibit *Mycobacterium tuberculosis* with possible implications for the use of β-lactams as anti-TB drugs. We observed that this synergy was conserved across many bacteria, including *Bacillus subtilis*. We evolved B. subtilis *in the presence of RIF and CEF and identified* rpoB S487L as conferring resistance to both drugs. This mutation is frequently associated with RIF resistance in MTB clinical isolates. In addition, two other clinically relevant RIF resistant *rpoB* mutants, H482Y and Q469R, were analyzed. The Q469R mutation conferred CEF resistance, although less than S487L, while the H482Y mutation increased CEF sensitivity. Thus, *rpoB* mutations arising with RIF selection may increase or decrease susceptibility to β-lactams. Mutations in *rpoC* often occur as compensatory mutations in *rpoB* mutants. However, the combination of RpoB H482Y (RIFR, CEFS) and RpoC G1122D (CEFR) was synthetic lethal. Moreover, CEF resistance was achieved starting with the *rpoB* H482Y RIFR strain by other mutations in *rpoC*, and RIFR suppressors isolated in a CEFR RpoC G122D strain had an *rpoB* mutation affecting the same amino acid, but with a different substitution, H482Q. Thus, there is an interesting and complex interplay between mutations affecting the two RNAP core subunits, RpoB and RpoC.

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The Synthesis of Imines and Their Potential Applications as Antimicrobial Agents

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Imines have known antibacterial and antifungal properties and coordination of imines to metals is known to enhance these properties. In order to create a library of complexes to study, coordination of different imines to Zn(II) has been accomplished. Imines were synthesized in high yield from amines and aldehydes using microwave irradiation. Microwave synthesis is considered to be an effective and environmentally friendly system. Studies were conducted to determine whether a solvent-free system or water as the solvent produced a higher yield. The imines have been characterized by infrared spectroscopy, proton nuclear magnetic resonance (NMR), and melting point. These complexes have been evaluated against various bacteria to determine their antibacterial activity using minimum inhibitory concentration (MIC) analysis. Bacteria tested include *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Escherichia coli*.

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Elucidating anaerobe metabolism with HRMAS 13C NMR and genome-scale dynamic flux balance analyses

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Anaerobic microbial metabolism drives critical functions within global ecosystems, host-microbiota interactions, and industrial applications, yet remains ill-defined. Here we show a versatile approach to elaborate dynamic metabolism in living cells of the anaerobic pathogen *Clostridioides difficile*. High-Resolution Magic Angle Spinning (HRMAS) Nuclear Magnetic Resonance (NMR) spectroscopy of *C. difficile* grown with uniformly labeled 13C substrates informed dynamic flux balance analysis (dFBA) of the pathogen's genome-scale metabolism. Predictions identified alanine's biosynthesis as a central integration point to support co-occurring amino acid and glycolytic fermentation pathways for energy-generation, maintenance of redox balance, efficient nitrogen handling, and biomass generation. Confirmation of model predictions used the sensitivity of 13C NMR spectroscopy to simultaneously track cellular carbon and nitrogen flow from U-13C glucose and 15N-leucine to form 15N-13C alanine. We illustrate a versatile approach to elaborate complex anaerobic metabolism for clinical, scientific, and industrial applications.

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Recognition and degradation of the MqsA antitoxin by cellular proteases in *Escherichia coli*

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When bacteria experience environmental stress, a series of cellular responses are triggered to stabilize the cellular proteome. One major cellular response in *Escherichia coli* is the enhanced function of the protein quality control network (PQCN). During periods of stress, polypeptides can become misfolded or unfolded. The PQCN can then assist in refolding these proteins into the native state via chaperones, or these misfolded proteins can be targeted for degradation by cytoplasmic proteases such as ClpXP and Lon. A second stress response system includes toxin-antitoxin (TA) systems, such as MqsRA, a type II TA system. Under normal conditions, antitoxins, like MqsA, inhibit the activity of a cognate toxin (MqsR); however, during stress, the antitoxin is degraded by cellular proteases, freeing the toxin to halt cell metabolism or prevent other critical cell functions. In a previous study, we showed that MqsA is degraded by ClpXP in vitro and in vivo (1). Recognition of MqsA by ClpXP occurs via the N-terminal domain of MqsA, and accessibility of the degron is regulated by zinc-coordination. We also mapped the recognition site for MqsA to the N-domain of ClpX and showed that it overlaps with the recognition site of the SspB adaptor protein. Here, we use a rapidly folding eGFP-MqsA chimeric fusion protein in combination with antibiotic chase to monitor turnover of MqsA in vivo via real-time fluorescence. We observed that both Lon and ClpXP are involved in degradation of eGFP-MqsA in unstressed cells. Variants of MqsA that are defective for zinc-coordination, including eGfp-MqsA(1-34), which contains the ClpX degron, and eGfp-MqsA(C3S/C6S), are degraded predominantly by Lon in unstressed cells. These experiments provide a platform to quantitatively monitor MqsA levels in cells under various growth conditions and evaluate contribution of cellular proteases to MqsR toxin activation.1. Vos, M. R., Piraino, B., LaBreck, C. J., Rahmani, N., Trebino, C. E., Schoenle, M., Peti, W., Camberg, J. L., and Page, R. (2022). Degradation of the E. coli antitoxin MqsA by the proteolytic complex ClpXP is regulated by zinc occupancy and oxidation. The Journal of biological chemistry, 298(2), 101557. https://doi.org/10.1016/j.jbc.2021.101557

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The role of tuberculostearic acid in the integrity of mycobacterial plasma membrane

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In mycobacteria, the plasma membrane is compartmentalized to spatiotemporally coordinate biochemical reactions. The intracellular membrane domain (IMD) is a specific region of the plasma membrane enriched in the sub-polar regions of the cell consisting of distinct proteins and lipids. Dibucaine is a membrane fluidizer that disrupts the integrity of mycobacterial plasma membrane and delocalizes the IMD from the poles. A transposon sequencing approach identified genes that are important for the recovery of *Mycobacterium smegmatis* from dibucaine treatment. Among them was *cfa*, a gene that encodes a putative cyclopropane-fatty-acyl-phospholipid synthase proposed to be involved in the synthesis of tuberculostearic acid. Tuberculostearic acid is a characteristic C19:0 mono-methyl branched stearic acid found abundantly in mycobacterial membrane phospholipids. Lipidomics indicated that the *cfa* deletion mutant was deficient in lipids containing tuberculostearic acid and accumulated lipids containing C18:1 mono-unsaturated oleic acid, a precursor to tuberculostearic acid, consistent with the proposed methylation function of *cfa*. Density gradient fractionation indicated Cfa to be associated primarily with the IMD, suggesting that tuberculostearic acid is synthesized in the membrane domain. However, tuberculostearic acids are found ubiquitously in mycobacterial membrane phospholipids, and the cfa mutant only showed a slight shift in the polar enrichment of the IMD. Upon dibucaine treatment, the *cfa* deletion mutant showed decreased fitness and delayed recovery of polar IMD. These observations highlight the critical role of tuberculostearic acids in maintaining the integrity of plasma membrane in mycobacteria.

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Systematic discovery of plasmid-dependent bacteriophages

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Plasmid-dependent bacteriophages are an under-explored group of bacterial viruses that use plasmid-encoded receptors to infect bacteria. Despite their interesting biology, there has not been a systematic search for them or an efficient strategy for their isolation. We developed a method that allows us to efficiently screen for phages that infect bacteria carrying specific conjugative plasmids. With this method, we were able to isolate an unprecedented collection of bacteriophages of the Tectiviridae family, which are broad host range viruses that depend on IncP-type conjugative plasmids. We discovered that these bacteriophages are more common and abundant than previously thought, and our collection expands the known diversity of these phages by an order of magnitude. We are exploring how this special group of phages is able to infect diverse bacterial hosts containing IncP plasmids, and how plasmid-dependent phages shape the evolution of broad-host plasmids.

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Isolating phages that select for antibiotic susceptibility

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As antibiotic resistance rises, bacteriophages (phages for short) are of growing interest to combat resistant pathogens. However, phage therapies are not immune to bacterial resistance. Although this could be seen as a limitation, we can take advantage of the inevitable evolution of phage resistance by choosing phages that direct bacterial evolution towards antibiotic susceptibility. Phage resistance commonly comes about by mutation of the host receptor that the phage uses to initiate infection, and such mutations can then have collateral effects on host cell phenotypes like antibiotic susceptibility. In this project we screened environmental samples for phages that interact with antibiotic resistance mechanisms with the intent to determine what, if any, selective pressure they incur on antibiotic susceptibility. We developed a mixed community plaque assay to identify phages dependent on the TolC multidrug efflux pump for successful infection. After sequencing these phages, we will test the ability of phage resistant bacterial mutants to resist antibiotics as compared to the bacterial ancestor. This project will give us a better understanding of the selective pressure that phages impose on antibiotic resistance in the environment and could be used to inform which phages will make the best therapies in the future.

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The *Acinetobacter* virulence-resistance activator BfmR controls a phospho-dependent global regulon

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The Gram-negative bacterium *Acinetobacter baumannii* has become a threat to human health globally, with few remaining options for effective therapy. To address this problem, more research is needed on what makes *A. baumannii* such an intractable opportunistic pathogen. Previous work has established a two-component system, BfmRS, as an important controller of resistance and virulence in *A. baumannii*. The BfmR protein has homology with OmpR-family DNA-binding response regulators and activates expression of a variety of cell envelope genes. BfmR function is modulated by its cognate sensor kinase, BfmS. How a phosphorylation signal controls the activity of BfmR, and the identity of its direct target genes, are still unknown. Here, we tested the model that phosphorylation activates BfmR, converting it to a state able to bind DNA and enhance resistance and virulence gene transcription. We found that WT bacteria maintain a small fraction of BfmR protein in a phosphorylated state, and that this fraction in-

creases with various mutations that stimulate the system. Phosphorylation depends on a conserved aspartate residue and induces dimerization *in vitro*. Chromatin immunoprecipitation analysis using strains with altered phosphoforms of BfmR allowed identification of its global regulon and binding motif and revealed a nearly complete dependence on phosphorylation for DNA interactions. These results thus uncover the critical role of phosphorylation in BfmR function. Future work will analyze the affinity of BfmR for DNA targets and will dissect the roles of individual BfmR regulon members in effecting BfmRS-mediated control of antibiotic resistance and virulence.

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Development of SYNB1353, A Synthetic Biotic Engineered to Consume Methionine for the Treatment of Homocystinuria

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Homocystinuria (HCU) is an autosomal recessive disorder caused by loss of function of cystathionine beta-synthase, leading to an accumulation of homocysteine in the plasma. When untreated, HCU can cause multisystem clinical manifestations, including thromboembolism, ectopia lentis and intellectual disability. Treatment options for HCU are currently limited due to efficacy and tolerability. Here we present SYNB1353, an engineered strain of the probiotic Escherichia coli Nissle 1917 (EcN) designed to consume methionine in the gut as a novel therapeutic for the treatment of HCU. The strain expresses an importer to internalize methionine and a methionine decarboxylase (MetDC) to degrade methionine into 3-methylthiopropylamine (3-MTP). To optimize the initial prototype design, Ginkgo Bioworks used their proprietary Codebase to generate both metagenomic and protein engineered (PE) libraries of approximately 1000 decarboxylases and nearly 160 importers cloned into EcN. The top performing, optimized enzymes and transporters were ultimately combined and integrated into SYNB1353, which degrades methionine and produces 3-MTP at a significantly greater rate in vitro than wild type EcN. A model of acute homocystinuria was developed by administering a methionine load to overnight fasted nonhuman primates. Dosing with SYNB1353 blunted the increase in plasma methionine and the resulting increase in plasma homocysteine after a methionine challenge compared to wild type EcN, confirming the strain is active and consuming methionine in vivo. Taken together this suggests the potential of SYNB1353 as a therapeutic to treat HCU.

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Two broadly conserved families of polyprenyl-phosphate transporters

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Undecaprenyl-phosphate (Und-P) is the lipid carrier on which virtually all cell surface polymers are built, including peptidoglycan, teichoic acids, and O-antigens of lipopolysaccharide. These UndP-linked precursors are assembled on the inner leaflet of the cytoplasmic membrane, transported across the lipid bilayer, and polymerized or directly transferred to surface polymers, lipids, or proteins. The released UndP must then be flipped to restore the pool of cytoplasmic-facing UndP. The identity of the flippase that catalyzes this transport has eluded identification for decades. Using the antibiotic amphomycin that targets UndP, I discovered two broadly conserved protein families that catalyze UndP transport. One family (UptA) is a member of the DedA superfamily; the other (PopT) contains the domain DUF368. I will present evidence that UptA and PopT family members from diverse gram-positive and gram-negative bacteria can catalyze UndP transport in *Bacillus subtilis*. These flippases are the last undiscovered enzymes in most surface polymer biogenesis pathways and inhibitors of these transporters could potentiate the current arsenal of cell envelope-targeting antibiotics. Finally, characterization of other members of the DedA superfamily revealed that these proteins transport distinct lipid species across the membrane.

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A functional pan-genome study of *Streptococcus pneumoniae* reveals which genes are species-wide or strain-dependent required for a successful host infection

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Despite the introduction of vaccines, *Streptococcus pneumoniae* remains one of the leading bacterial causes of mortality worldwide. New antipneumococcic therapies development needs comprehensive knowledge of the bacterial genes required to infect the host. Like many other pathogens, *S. pneumoniae* is represented by a large pan-genome. We have shown that this genomic diversity strongly influences gene essentiality. Here we demonstrate that the pan-genome also influences the genetic requirements for successful host infection. To highlight this genetic-background effect, we performed in vivo Tn-Seq in a mouse pneumonia model using mutant libraries constructed in 22 phylogenetically diverse strains. We find that 402 and 338 genes are respectively required by at least two strains to colonize the nasopharynx or invade the lungs, and within these, only 63 and 77 genes are respectively required by every strain. These last gene subsets include well-known genes involved in virulence and many non-characterized ones. A deeper analysis of the strain-dependent required genes shows that for lungs invasion, chorismate synthesis and natural competence requirement correlates with the presence or absence of a gene coding for a bacteriocin. These results show that simple mechanisms, e.g., the gain or loss of a single bacteriocin-coding gene, dictate the strategies required by *S. pneumoniae* to survive inside the host.The functional pan-genome approach used in this work reveals novel genetic and host cells-pathogen interactions that shed light on *S. pneumoniae* infection biology and identify which genes are critically important in vivo at the species-wide level. Hence, these genes represent potentially species-wide drug targets.

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Variation in supplemental carbon dioxide requirements defines lineage-specific antibiotic resistance acquisition in *Neisseria gonorrhoeae*

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The evolution of the obligate human pathogen *Neisseria gonorrhoeae* has been shaped by selective pressures from diverse host niche environments as well as antibiotics. The varying prevalence of antibiotic resistance across *N. gonorrhoeae* lineages suggests that underlying metabolic differences may influence the likelihood of acquisition of specific resistance mutations. We hypothesized that the requirement for supplemental CO2, present in approximately half of isolates, reflects one such example of metabolic variation. Here, using a genome-wide association study and experimental investigations, we show that CO2-dependence is attributable to a single substitution in a β-carbonic anhydrase, *canB*. CanB19E is necessary and sufficient for growth in the absence of CO2, and the hypomorphic CanB19G variant confers CO2-dependence. Furthermore, ciprofloxacin resistance is correlated with CanB19G in clinical isolates, and the presence of CanB19G increases the likelihood of acquisition of ciprofloxacin resistance. Together, our results suggest that metabolic variation has impacted the acquisition of fluoroquinolone resistance.

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SpoIIE manipulates the cell division polymer network to promote asymmetric division

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Rod shaped bacteria faithfully produce identical daughter cells by medially positioning the division site. However, *Bacillus subtilis* and related endospore forming organisms shift the division site to one pole to sporulate, leading to different daughter cell types and a morphologically distinct septum. The sporulation-specific protein SpoIIE interacts with the division machinery, promotes polar division site positioning, and is necessary for producing the thin septum characteristic of sporulating cells. Here we address how SpoIIE produces these outcomes. Using a combination of genetics and TIRF microscopy, we discover that SpoIIE allows polar division to occur by 1) inhibiting the Min complex that restricts division site formation to the midcell and 2) modifying the dynamics of the polymeric protein FtsZ. The division machinery consists of two sub-complexes, a treadmilling complex of FtsZ, FtsA, and associated regulatory factors, and a directionally moving complex of cell wall synthases and their regulators. Live cell TIRF microscopy reveals that SpoIIE binds to the treadmilling division complex, but with a longer residence time than previously studied FtsZ-ring regulators or FtsZ itself, suggesting that SpoIIE forms multivalent interactions with FtsZ filaments. Together, these findings suggest a model for how SpoIIE both opposes the function of midcell positioning factors and changes properties of FtsZ treadmilling to relocalize FtsZ-rings and drive changes in septal morphology.

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Characterizing the mode of action of an antimycobacterial compound after target identification by activity-based protein profiling

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The increasing prevalence of multidrug resistant strains of *Mycobacterium tuberculosis* underscores the urgent need for novel anti-tuberculosis (TB) drugs. A major bottleneck in the discovery process for anti-TB drugs is the identification and validation of a compound's targets, which is especially difficult when multiple targets underlie biological activity. Activity-based protein profiling (ABPP) can be used to simultaneously detect all potential targets of a compound. In previous work, our lab has demonstrated the great potential of ABPP in the drug development pipeline. We leveraged phenotypic screening, competitive ABPP, morphological profiling, and a structure-activity series to generate a prioritized list of targets most likely to contribute to the mode of action of our identified inhibitor, AA692. To further demonstrate the applicability of ABPP in drug development, we will determine the contribution of the prioritized targets to the mode of action of AA692. In this study, we focus on the target Rv3802c, an essential *Mtb* protein with a putative role in mycolic acid synthesis. We have generated autoluminescent reporter strains of *Mycobacterium smegmatis* with tunable Rv3802c expression and confirmed that Rv3802c complements the loss of the essential *M. smegmatis* homologue MSMEG-6394. To test whether Rv3802c inhibition contributes to the mode of action of AA692, we will determine how Rv3802c expression affects susceptibility to AA692. This work will provide insight into the mode of action of an inhibitor, but, more importantly, it will contribute to our understanding of how ABPP can be used in the development of novel drugs.

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Biochemical screen reveals novel effectors secreted by the obligate intracellular bacterial pathogen *Rickettsia parkeri*

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Rickettsia spp. are obligate intracellular bacterial pathogens that pose a growing threat to human health. Nevertheless, their strict reliance on a host cell niche has hindered investigation of the molecular mechanisms driving rickettsial infection. It is assumed that *Rickettsia* spp., like other bacterial pathogens, secrete effectors to hijack host machinery and remodel their intracellular environment. However, only a few rickettsial effectors and their host cell targets are known. Therefore, we implemented bio-orthogonal non-canonical amino acid tagging (BONCAT) in *R. parkeri* to selectively label effectors secreted during infection. Coupled with mass spectrometry, this approach enabled

the enrichment and identification of rickettsial effectors from a complex pool of host material. Our screen represents the first use of BONCAT in an obligate intracellular bacterium and nearly doubles the list of secreted effectors known for *R. parkeri*. The novel effectors we identified include several hypothetical proteins that are unique to the *Rickettsia* genus. We hypothesize that these effectors support the rickettsial life cycle by subverting host cell processes. Indeed, we have found that the effectors revealed by our screen localize to a variety of host cell compartments, such as the ER, cytoplasm, and nucleus. By characterizing these effectors and their impacts on the host cell, we hope to improve our understanding of the host-pathogen interface and the bacterial factors governing rickettsial pathogenesis.

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Accumulation of DNA by Intramacrophage *Salmonella* Persisters

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Persisters are a subpopulation of transiently nongrowing bacteria capable of surviving antimicrobial attacks from antibiotics and the immune system and eventually resuming growth. Bacterial persistence contributes to antibiotic treatment failure and the relapse of many recalcitrant infections. Many pathogens, including *Salmonella enterica*, *Mycobacterium tuberculosis*, and *Staphylococcus aureus*, form persisters within macrophages where they survive for extended periods of time. It was shown that although non-growing, *Salmonella* persisters retain the ability to express and inject effector proteins into macrophages leading to interference of the host immune response and supporting persister survival. Strikingly, intramacrophage *Salmonella* persisters also actively synthesize DNA and can accumulate the equivalent of more than four chromosomes worth of DNA. We quantified the regrowth of persisters across a spectrum of DNA content and found that persisters with higher DNA content resumed growth at a higher frequency than those with lower DNA content. Sequencing of the accumulated DNA revealed chromosome amplification and discrete duplication events in persisters. Overall, we found that DNA synthesis despite growth arrest confers a survival advantage to *Salmonella* persisters highlighting the spectrum of unique survival strategies persisters employ during macrophage infection. By improving persister survival, DNA accumulation may potentiate infection relapse and duplication events may provide a route for evolution via the persister state.

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Mapping functional regions of essential bacterial proteins with dominant-negative protein fragments

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Fragments of native proteins can act as dominant negative inhibitors, representing a substantial stress for cells. Massively-parallel measurements of growth inhibition by protein fragments have been used to discover peptide inhibitors and map protein interaction sites. However, the underlying principles governing fragment-based inhibition have thus far remained unclear. Here, we adapt a high-throughput inhibitory fragment assay for use in *Escherichia coli*, applying it to a set of ten essential proteins. This approach yielded single amino acid resolution maps of inhibitory activity, with peaks localized to functionally important interaction sites, including oligomerization interfaces and folding contacts. Leveraging these data, we perform a systematic analysis to uncover principles of fragment-based inhibition. We determine a robust negative correlation between susceptibility to inhibition and cellular protein concentration, demonstrating that inhibitory fragments likely act primarily by titrating native protein interactions. We also characterize a series of trade-offs related to fragment length, showing that shorter peptides allow higher-resolution mapping but suffer from lower activity. We employ an unsupervised statistical analysis to show that the inhibitory activities of protein fragments are largely driven not by generic properties such as charge, hydrophobicity, and secondary structure, but by the more specific characteristics of their bespoke macromolecular interactions. AlphaFold computational modeling of peptide complexes with one protein shows that the inhibitory activity of peptides is associated with their predicted ability to form native-like interactions. Overall, this work demonstrates fundamental

characteristics of inhibitory protein fragment function and provides a foundation for understanding and controlling protein interactions *in vivo*.

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Understanding gene family expansion in *R. parkeri*'s type IV secretion system and its role in infection

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In recent years, there has been a dramatic increase in cases of tick-borne diseases, many of which are caused by bacteria of the order Rickettsiales. As obligate intracellular pathogens, the Rickettsiales rely on a unique type IV secretion system (T4SS) to secrete protein effectors and manipulate host cell processes. Despite massive genome reduction within the order, the rickettsia T4SS has undergone significant gene-family expansion, resulting in multiple genes encoding divergent paralogs of certain subunits. We hypothesize that these paralogs form several distinct T4SS assemblies, each with unique features to help coordinate rickettsia's dynamic, multi-step, intracellular life cycle. Here, we use *Rickettsia parkeri* as a model to investigate the function and assembly of five highly diverse paralogs of the inner membrane channel subunit, VirB6, which has been shown in other bacteria to contact effectors during translocation and to regulate T4SS activity. Through transposon mutant analysis, we have found that one of the VirB6 paralogs (RvhB6e) plays a role in either adhesion to or invasion of host cells. RvhB6e also contains a C-terminal extension with weak homology to an invasion-promoting protein from malaria-causing *Plasmodium yoelii*, consistent with this paralog's role in early infection. Ongoing work is leveraging biochemical and imaging assays to investigate RvhB6e's mechanism of action, as well as the broader interactions and assembly patterns of the other RvhB6 paralogs. By studying these unique T4SS paralogs, we hope to uncover new T4SS functions and better understand the host-pathogen interactions that support infections by Rickettsia species.

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Structural and functional characterization of the microbial ergothioneine transporter EgtUV

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Low-molecular-weight (LMW) thiols are small-molecule antioxidants present in all domains of life that help maintain intracellular redox homeostasis. Ergothioneine (EGT) is an LMW thiol synthesized by fungi and certain bacteria. Mammals acquire EGT through the diet, and EGT accumulates to millimolar levels in mammalian tissues. Our lab has identified the first example of a specific microbial EGT transporter in the gastric pathogen *Helicobacter pylori*, termed EgtUV. EgtUV is an ABC transporter comprised of EgtU, which contains permease and solute-binding domains (SBD), and the ATPase EgtV. The *H. pylori* EgtUV transporter enables import of host-derived EGT and is widely conserved in members of the gut microbiota. The EgtU SBD binds EGT with low-micromolar affinity and high specificity; however, the basis for this ligand preference is unknown. We hypothesize that distinct structural and biochemical features of the EgtU SBD confer its ligand specificity, enabling *H. pylori* to import EGT as a redox regulation mechanism. Here, we present crystal structures of the EgtU SBD in free and ligand-bound forms resolved to 1.8 Å and 3.4 Å, respectively. Notably, these structures exhibit conserved ligand-binding interactions of betaine-binding bacterial and archaeal SBDs, even though EgtU is unable to bind to glycine betaine or structurally similar ligands. By combining sequence and functional information from EgtU homologs, we have identified additional residues that may differentiate EGT-binding proteins. This work will provide a mechanistic understanding of EGT import in diverse microbes with EgtU homologs and may reveal avenues for broadly inhibiting microbial EGT import as an antimicrobial strategy.

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Octopus "taste by touch" chemotactile sensation is mediated by interdomain detection of microbial molecules

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Animals and microorganisms have coevolved to share similar environmental niches and signal to one another in symbiotic relationships. While such interdomain signaling has been appreciated in host-microbiome interactions, whether animals use microbial signaling to explore their external environment is not understood. Octopuses are sensory specialists that use their flexible arms to explore their seafloor surroundings through 'taste by touch' chemotactile sensation. A family of cephalopod-specific chemotactile receptors (CRs) mediates contact-dependent, aquatic chemosensation by detecting a broad range of poorly soluble molecules, terpenes and steroids. Yet, how the octopus distinguishes surfaces of biologically meaningful stimuli, such as prey or eggs, from the rocks and crevices it explores is unknown. Could distinct insoluble chemical signals be present on such surfaces? To ask this question, we are testing whether specific bacterial metabolites from microbiomes of biologically relevant surfaces activate CRs. We have profiled microbiomes from the octopus' environment and cultured 100s of bacterial strains from prey and eggs to identify supernatants which stimulate distinct octopus CRs, as well as arm nerves and behavior. We are now using HPLC-MS to identify specific metabolites which will be leveraged to understand CR biophysical properties, structural evolution, and the coevolution of animal receptors and the biosynthesis of bacterial natural products. We expect these studies will reveal foundational insight regarding how animals sense their environment, which consists largely of microorganisms.

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Analysing the fitness cost of antibiotic resistance to identify targets for combination antimicrobials

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Mutations in the rifampicin (Rif)-binding site of RNA polymerase (RNAP) confer antibiotic resistance and often have global effects on transcription that compromise fitness and stress tolerance of resistant mutants. We suggested that the non-essential genome, through its impact on the bacterial transcription cycle, may represent an untapped source of targets for combination antimicrobial therapies. Using transposon sequencing, we carried out a genome-wide analysis of fitness cost in a clinically common *rpoB* H526Y mutant.. We find that genes whose products enable increased transcription elongation rates compound the fitness costs of resistance whereas genes whose products function in cell wall synthesis and division mitigate it. We validate our findings by showing that the cell wall synthesis and division defects of *rpoB*H526Y result from an increased transcription elongation rate that is further exacerbated by the activity of the uracil salvage pathway and unresponsiveness of the mutant RNAP to the alarmone ppGpp. We applied our findings to identify drugs that inhibit more readily *rpoB*H526Y and other Rif resistant alleles from the same phenotypic class. Thus, genome-wide analysis of fitness cost of antibiotic-resistant mutants should expedite the discovery of new combination therapies and delineate cellular pathways that underlie the molecular mechanisms of cost.

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Optogenetic Control of Antibiotic Resistance Genes

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Antibiotics are a key bacterial control and selection mechanism. Antibiotic resistance genes have long been used to select cells and regulate bacterial populations. However, their use to-date has largely been static, such as a constitutively expressed resistance gene used for plasmid maintenance. A system that provides spatiotemporal control over the expression of antibiotic resistance genes would enable a wide variety of applications, from single-cell selection, to dynamic control of cell survival. Light-inducible antibiotic resistance can also serve as a synthetic system for studying the spread of clinical antibiotic resistance, such as in modeling the acquisition of resistance genes via horizontal gene transfer. Here, we present a novel system that uses blue light-inducible Cre recombinase to regulate the activation of antibiotic resistance genes in E. coli. Because it is straightforward to control the application of light in both time and space, this system can be used to induce antibiotic resistance at a precise time or within a subset of the population. We present our results showing light-activated resistance genes for four antibiotics: ampicillin, kanamycin, chloramphenicol, and tetracycline. Cells grown in blue light survive lethal concentrations of antibiotics, while those grown in the dark do not. We optimized genetic constructs in plasmid-based and chromosomally integrated contexts. As an application, we used time-lapse microscopy to show gene activation dynamics in bacterial populations, characterizing single-cell responses. We envision that these optogenetic tools for controlling antibiotic resistance genes will be broadly useful in synthetic biology and microbiology contexts for precise spatiotemporal control of cell survival.

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Candidate undecaprenyl phosphate translocases enable conditional microbial fitness and pathogenesis

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The microbial cell wall is essential for the maintenance of cell shape and resistance to external stressors. The primary structural component of the cell wall is peptidoglycan (PG), a glycopolymer with peptide crosslinks located outside of the cell membrane. PG biosynthesis and structure are responsive to shifting environmental conditions such as pH, but mechanisms underlying such adaptations are incompletely understood. Precursors of PG and other cell surface glycopolymers are synthesized in the cytoplasm and then delivered across the cell membrane bound to the recyclable lipid carrier undecaprenyl phosphate (C55-P). The transporter protein(s) that return C55-P to the cytosolic face of the cell membrane have been elusive. Here, from *in vivo* high-throughput transposon screens characterizing intestinal colonization determinants of the cholera pathogen *Vibrio cholerae*, we identify the DUF368-containing and DedA transmembrane protein families as C55-P translocases. Gram-negative and -positive bacteria lacking their cognate DUF368-containing protein displayed alkaline-dependent cell wall and viability defects as well as phenotypes consistent with surface accumulation of C55-P. pH-dependent synthetic genetic interactions between DUF368-containing proteins and DedA family members suggest that C55-P transporter requirements are dynamic and determined by environmental inputs. The identification of C55-P translocases fills a key gap in our knowledge of the proteins required for the biogenesis of microbial cell surface polymers. Differential C55-P transporter usage could represent a strategy for optimizing microbial fitness inside and out of the host.

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Exploiting thioether reactivity for labeling mycobacterial glycans

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Prior to the COVID-19 pandemic, *Mycobacterium tuberculosis* (*Mtb*), the etiological agent of tuberculosis, was

the leading cause of death from a single infectious disease worldwide. Mtb cell wall glycans play key roles in mediating infection of host tissues and modulation of the host immune response. However, due to the structural redundancy of glycan components, there are few means by which to site-selectively label and manipulate these glycans in order to study their function in disease pathogenesis.There is therefore a need for new tools to investigate the roles of *Mtb* cell wall glycans. Methylthioxylofuranose (MTX) is monosaccharide appended to the mannose-capped lipoarabinomannan (ManLAM), an antigenic glycolipid in the *Mtb* cell wall that is implicated in inducing the arrest of phagosome maturation and reprogramming of host macrophages towards an anti-inflammatory subtype. MTX contains a unique thioether moiety that is chemically distinct from other functional groups found on glycans and can thus be exploited for selective bioconjugation. Building upon previous work using oxaziridine reagents to label proteins, we developed a small molecule mediated strategy that uses oxaziridines to selectively oxidize the MTX thioether and install a biorthogonal reactive handle. We have validated this approach by labeling purified ManLAM *in vitro*. Furthermore, we have demonstrated that this approach can be used to label and visualize the *Mtb* cell wall *in vivo*. These studies will enable future work investigating dynamic changes to the *Mtb* cell wall during TB infection or antibiotic treatment, helping to identify new opportunities for therapeutic intervention.

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Unusual characteristics of the bacterial noncoding RNA called *raiA*

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Several structured noncoding RNAs (ncRNAs) in bacteria are essential components of major cellular pathways. Recent discoveries of additional ncRNA classes provide new opportunities to explore mechanisms by which RNAs contribute to modern cellular processes. One such ncRNA, named the '*raiA motif*', has been found via bioinformatic analysis in over 500 bacterial species from the Firmicutes and Actinobacteria phyla. Here, we describe the first steps towards establishing the biological and biochemical functions of this highly structured 200-nucleotide ncRNA. Biochemical probing has been used to confirm key features of a sophisticated secondary structure model for *raiA* motif RNAs. Examination of the genetic context of the gene serving as the template for *raiA* motif RNAs potentially provides clues regarding the function of this ncRNA class. Specifically, analysis of the five genes preceding and following the *raiA* motif in bacteria reveals a common synteny with genes coding for the proteins RaiA (ribosomal-associated inhibitor A) and ComFC (competence factor C). Unfortunately, these common gene associations do not immediately reveal the likely biochemical function of the ncRNA. Northern blot expression analysis of *raiA RNA* in *Clostridium acetobutylicum* ATCC 824 reveals that the RNA is produced as an independent transcript, which becomes abundant during exponential growth but not at stationary phase. However, by supplementing the growth medium with glucose, *raiA* motif RNA levels were reestablished to that observed under exponential growth conditions. We are currently working to create a bacterial strain wherein the *raiA* motif RNA has been disrupted to investigate its biological role through phenotype screening.

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Lipoarabinomannan regulates septation in *Mycobacterium smegmatis*

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The growth and division of mycobacteria, which include several clinically relevant pathogens, deviates significantly from that of canonical bacterial models. Despite their Gram-positive ancestry, mycobacteria synthesize and elongate a diderm envelope asymmetrically from the poles, with the old pole elongating faster than the new pole. In addition to being ultrastructurally distinct, the molecular components of the mycobacterial envelope are also evolutionarily

unique, including the abundant lipoglycans lipomannan (LM) and lipoarabinomannan (LAM). LM and LAM are known to modulate host immunity during infection, but their role outside of intracellular survival remains poorly understood, despite their widespread conservation among non-pathogenic and opportunistically-pathogenic mycobacteria. Previously, *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* mutants producing structurally altered LM and LAM were shown to grow slowly under certain conditions and to be more sensitive to antibiotics, suggesting that mycobacterial lipoglycans may support cellular integrity or growth. To test this, we constructed multiple biosynthetic lipoglycan mutants of *M. smegmatis* and determined the effect of each mutation on cell wall biosynthesis, envelope integrity, and division. We found that mutants deficient in LAM, but not LM, fail to maintain cell wall integrity, with envelope deformations specifically associated with septa and new poles, reminiscent of uncontrolled septal hydrolase activity. Conversely, a mutant producing abnormally large LAM formed multiseptated cells, mimicking a characteristic phenotype of septal hydrolase deficient mutants. These results show that LAM is critical for governing daughter cell separation in mycobacteria, and suggest LAM may do so by regulating septal hydrolase activity.

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Biotin requirements in *Mycobacterium abscessus* lung infection

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Mycobacterium abscessus is an emerging pathogen that is resistant to most frontline antibiotics. *M. abscessus* causes lung infection, predominantly in patients with lung disease or structural lung abnormalities. To interrogate the mechanisms required for *M. abscessus* survival in the lung, we have developed a lung infection model using air-liquid interface culture and performed a genetic screen in this setting. Synthesis of the cofactor biotin becomes highly essential in the lung model due to increased intracellular demand for biotin, and pharmacological inhibition of biotin synthesis halts *M. abscessus* proliferation. The heightened need for biotin in the lung environment supports fatty acid remodeling that increases the fluidity of the cell envelope, which promotes survival in alkaline physiological environments. Together, these results indicate that biotin-dependent fatty acid remodeling plays a critical role in pathogenic adaptation to the lung environment and suggest potential therapeutic interventions targeting biotin synthesis and fatty acid metabolism.

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Using machine learning to understand the determinants of mRNA stability in mycobacteria

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As a highly successful pathogen, *M. tuberculosis* is able to infect, survive and proliferate within harsh microenvironments created by human host with the help of global mRNA degradation regulation. While a wide range of mRNA half-lives is observed in mycobacteria, little is known about the mRNA features that control the degradation. This is especially interesting for leadered and leaderless transcripts. Despite features have been identified to be associated with different translation mechanisms, the fairly expected degradation discrepancy of these two types of transcripts is yet fully characterized. Here we developed a computational pipeline using RNAseq and machine learning to identify the features that determine mRNA degradation in mycobacteria. We first calculated mRNA half-lives of the non-pathogenic model *M. smegmatis* using degradation profiles quantified through RNAseq. Then we trained a random forest classifier to identify the mRNA features that are associated with different mRNA half-lives. Our results show that, for both leadered and leaderless transcripts, several types of features including nucleotide and codon

content, secondary structure, ribosome occupancy and other sequence features all contribute to predict the mRNA half-lives but to various extent in either normal or hypoxia condition. However, the specific features that are predictive of half-lives are different for leadered and leaderless transcripts in either condition. We also identified features that are associated with the fold change of stabilization upon transition to hypoxia for leadered and leaderless transcripts. Overall, our results suggest that there are complex regulation mechanisms for mRNA degradation in mycobacteria.

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Genomic profiling reveals novel routes to complement evasion in *Streptococcus pneumoniae*

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Complement activation to recruit neutrophils to the site of inflammation is a critical first line of defense against bacterial pathogens. Individuals with complement deficiencies are more sensitive to invasive infections by gram-positive pathogens such as *Streptococcus pneumoniae*. Triggering the innate receptors on the surface of neutrophils initiates antimicrobial mechanisms, ultimately leading to the destruction of the bacterial pathogen. During this process, bacteria are first opsonized, or marked, for phagocytosis by the major complement protein C3 and/or antibodies which trigger recognition and engulfment by neutrophils. *S. pneumoniae* encodes surface proteins implicated in evading C3 complement deposition, but we currently only possess a limited understanding of these surface-exposed bacterial proteins, and their role during infection clearance or progression. By combining *in vitro* C3 deposition of a transposon mutant library, FACS selection, and Tn-Seq, we obtained a high-resolution, genome-wide understanding of the *S. pneumoniae* surface proteins and associated molecular pathways that are key to facilitating complement-mediated bacterial clearance. Validated data in the S. pneumoniae *strain TIGR4 suggests multiple uncharacterized gene products impede C3 deposition* ex vivo *and* in vivo, beyond the well-studied polysaccharide capsule, choline-binding proteins, and surface exoglycosidases. By combining this set of newly identified and validated surface protein targets with our database of S. pneumoniae *genes critical* in vivo, we generate a network of novel antimicrobial targets that augment the specificity and efficacy of innate immune processes that target the bacterial cell surface.

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Blue light dependent protein degradation in *E. coli*

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Optogenetic systems afford high spatial and temporal control of gene expression, with unique applications such as single-cell perturbation in systems biology and dynamic control in metabolic engineering. Currently, most microbial optogenetic systems function at the transcriptional level, which limits their utility during stationary phase because cells often rely on growth dependent dilution to significantly decrease protein levels. To compliment these approaches, we describe a novel system we have developed for blue light dependent protein degradation in *Escherichia coli* using an engineered version of the *As*LOV2 protein. The light responsive degradation tag is modular, which we demonstrate by using it to control multiple systems such as CRISPRa and endogenous transcription factors. Lastly, we create variants of the degradation tag that change photocycle dynamics. These variants are sensitive to different frequencies of blue light pulsing and could form the basis for single-wavelength, frequency based signal processing.

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A genetic analysis of ICP1 a lytic cholera phage: a predator of the gut pathogen *Vibrio cholerae* in an estuary environment

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Vibrio cholerae, the causative agent of cholera, has a permanent environmental reservoir in estuaries in endemic regions. *V.cholerae* encounters the virulent phage ICP1 in both the aquatic environment and during infection of mammalian hosts. Notably, ICP1 is unique in its predation of early stationary growth phase hosts in estuary. Thus, it is important to characterize the ICP1 lifecycle and the mechanisms that allow ICP1 to disrupt *Vibrio* in estuarine and low nutrient environments. I hypothesize that there are ICP1 genes that specifically confer advantages for predation on *V. cholerae* in estuary and on stationary phase cells. Here, I describe the construction of a nearly saturated transposon mutant library in ICP1. I passaged this library in either exponential-growth host (EG) or early stationary-growth host (SG) cells grown in either estuary-like minimal media(E) or rich media(LB). I performed massively high-throughput transposon-sequencing (Tn-seq) on the passaged phages to identify putative gene functions conferring fitness in E, LB, EG, SG host. Subsequently, mutants will be characterized to determine the function of their defect using quantitative assays for the major steps of the phage life cycle.

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Processing of DNA clamp loader subunit DnaX is important in the absence of *Caulobacter* cell division inhibitors

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The DNA clamp loader is critical to the processivity of DNA replication and the coordination of the leading and lagging strands. In bacteria the major subunit of the clamp loader, DnaX, has two forms: the essential full-length DnaX tau and shorter DnaX gamma. These are conserved across bacterial species and three distinct mechanisms have been found to create them: ribosomal frameshift, transcriptional slippage, and, in *Caulobacter crescentus*, proteolysis. This conservation suggests that DnaX processing is evolutionarily important, but its role remains unknown. To examine this role, we created a strain of *Caulobacter* encoding a nonproteolyzable DnaX(tau-only), which isn't processed into the DnaX gamma form. While there appeared to be selection against the mutation, this strain has no observable growth or morphological defects. We performed transposon sequencing analysis and observed that insertions in the two cell division inhibitors, *sidA* and *didA*, were underrepresented in the dnaX(tau-only) strain compared to wild type. We find that deleting *sidA* in the dnaX(tau-only) background causes a growth defect, cell filamentation, and induction of the DNA damage response. These data suggest a lack of coordination between DNA replication and cell division in the double mutant and that in that cell division inhibitors suppress DNA damage in the absence of DnaX processing. This important observation represents a first step in understanding the 30-year-old question of why the DnaX forms are so highly conserved in bacteria.

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Dimorphic colonies from Klebsiella pneumoniae clinical isolates

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Klebsiella pneumoniae is a growing concern in the healthcare community as carbapenemase-producing Enterobacteriaceae are considered an urgent healthcare threat. *K. pneumoniae* can cause bloodstream infections, pneumonia, wound infections, and meningitis. *K. pneumoniae* is commonly evaluated as clonal populations and rarely examined at the single cell level. However, heterogeneous features of bacterial cells have been previously identified, and the physiological characteristics of subpopulations can differ in terms of biofilm, antimicrobial resistance and virulence. We screened *K. pneumoniae* clinical isolates on selective agar plates and observed that several isolates exhibited dimorphic colonies (gelatinous or solid colonies). We hypothesized that the different colonies would exhibit different properties, such as growth, metabolism, biofilm production, and antibiotic resistance. We grew dimorphic colonies from four clinical isolates in rich medium (BHI) or minimal medium (chemically defined chemical medium: CDMM) and observed no differences in growth between colony types. However, we observed distinct differences between

colony types in terms of swarming and biofilm production. We found that the gelatinous colonies had increased swarming capacity but decreased biofilm production compared to the solid colonies. We next examined the ability of the colonies to use >150 compounds using Biolog Phenotypic microarrays. Interestingly, we did not observe any consistent differences in nutrient utilization between gelatinous colonies and solid colonies. We also screened >48 antibiotics and found that the solid colonies were slightly more sensitive to several antibiotics compared to the gelatinous colonies. These data demonstrate that *K. pneumoniae* can exhibit subpopulations, which potentially increases fitness upon colonization.

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Site-specialization of human oral *Actinomyces* and *Schaalia* species

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Actinomyces species are core members of the healthy human oral microbiome. They are recognized as first colonizers in dental plaque development. The genus *Actinomyces* has been recently subdivided into two genera: *Actinomyces* and *Schaalia*. The oral cavity is made up of multiple habitats harboring specific taxa as shown by culture-dependent and 16S rRNA gene profiling. However, such claims should be tested using more robust methods. The present work aimed to evaluate the site-specialization of *Actinomyces* and *Schaalia* across multiple habitats of the human oral microbiome by combining pangenomics and metagenomics.We identified core and accessory genes, assess the preferred site of species, and resolve genes relevant to each habitat. High-quality genomes representing the diversity of human oral *Actinomyces* (n=55) and *Schaalia* (n=25) were used to build the pangenome and function as bait for read recruitment of metagenomic samples (n=1239). Our findings show that *Actinomyces* and *Schaalia* species are abundant and prevalent in different oral sites of healthy individuals. Most *Actinomyces* species were found in supra- and subgingival plaque; *Schaalia* species were dominant in tongue dorsum, palatine tonsils and throat. We identified strains and genes associated with each habitat.In conclusion, we show that *Actinomyces* and *Schaalia* species have a clear site preference in the oral cavity of healthy humans.Supported by NIH grant DE030136.

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Cell wall synthesis and remodeling dynamics determine bacterial division site architecture and cell shape

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The bacterial division apparatus catalyzes the synthesis and remodeling of septal peptidoglycan (sPG) to build the cell wall layer that fortifies the daughter cell poles. Understanding of this essential process has been limited by the lack of native three-dimensional views of developing septa. Here, we used state-of-the-art cryogenic electron tomography (cryo-ET) and fluorescence microscopy to understand the division site architecture and sPG biogenesis dynamics of the Gram-negative bacterium *Escherichia coli*. Our results uncovered a previously unobserved wedge-like structure of sPG that fortifies the ingrowing septum. Additionally, results with mutant cells altered in the regulation of sPG biogenesis revealed that the architecture of E. coli can be altered to more closely resemble that of other Gram-negative and Gram-positive bacteria, suggesting that a conserved mechanism underlies the formation of different septal morphologies. Furthermore, we found that cell wall remodeling affects the placement and stability of cytokinetic rings and that a competition between the cell elongation and division machineries determines the shape of cell constrictions and the poles they form. Overall, our results highlight how the activity of the division system can be modulated to help generate the diverse array of shapes observed in the bacterial domain.

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Rickettsia effector Sca4 may promote cell-to-cell spread through dual interactions with host vinculin and endocytic machinery

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Rickettsia are obligate intracellular bacteria transmitted to humans by arthropod vectors that cause diseases of the vasculature. Spotted Fever Group (SFG) *Rickettsia* invades endothelial cells where it co-opts host cell processes to grow and spread from cell to cell. SFG member *Rickettsia parkeri* secretes a protein called Surface Cell Antigen 4 (Sca4), which promotes efficient cell-to-cell spread by inhibiting the host protein vinculin at adherens junctions. Using immunoprecipitation-mass spectrometry, we determined that Sca4 also interacts with host clathrin heavy chain and that a segment of the N-terminus of Sca4 is sufficient for this interaction. RNAi-mediated silencing of clathrin expression reduced WT *Rickettsia* cell-to-cell spread, but not the spread of sca4::tn, suggesting that clathrin promotes efficient cell-to-cell spread. During infection, Sca4 localizes to the host plasma membrane but immunofluorescence data show there is no obvious effect on clathrin localization. We hypothesize that the Sca4-clathrin interaction may either facilitate the transport of Sca4 to the host membrane or target a specific clathrin function, such as the uptake of a membrane protein like VE-cadherin. Intriguingly, the interaction between the N terminus of Sca4 and clathrin is conserved across *Rickettsia* species, including in species that do not undergo cell-to-cell spread. Thus, Sca4 could contribute to efficient infection by also targeting other clathrin-dependent host processes that control junctional integrity or immune recognition. By studying the Sca4-clathrin interaction, we hope to increase our understanding of the molecular underpinnings of *Rickettsia* pathogenesis and potentially uncover a new way that pathogens manipulate host endocytic machinery.

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The Proposed Cloning of Bidirectional Hydrogenase to Enhance Hydrogen Production in Cyanobacteria

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Cyanobacteria are photosynthetic microorganisms that produce hydrogen as a waste product using a bidirectional hydrogenase enzyme which takes electrons from the photosynthetic electron transport chain. The hydrogen-producing capability of cyanobacteria is applicable in research for biofuel production and more. Bidirectional hydrogenase works under the direction of the weak promoter *hoxEFUYH*, which is a disposable operon. The light-inducible *psb*AII promoter has increased the expression of genes such as trsA and ccS, which encode for a shuttle protein and aid the construction of surface proteins in cyanobacteria. The *psb*AII promoter has been proven to increase the expression of these genes by 6-18-fold. It is seen that the replacement of *hoxEFUYH* operon promoter with a stronger "λcI857-λpR" promoter system created a TR-hoxEFUYH-hypABCDEF mutant and showed an increase in hydrogenase activity levels by 8-13-fold at 39 C instead of 30 C. Cloning construct for enhancement of hydrogen production in cyanobacteria is based on the pUC19 vector cut with *Hinc*II and *Sfo*I. Then, *Psh*AI/*Afe*I restriction product containing the *Hox* operon is inserted under the chosen *psb*AII promoter. As *psb*AII is regulated by light, this supports the natural photosynthesis of cyanobacteria and enhances the possibility of a variable regulation of hydrogen production. An increase in hydrogen production in cyanobacteria by genetically manipulating the activity of bidirectional hydrogenase has promising applications for green energy and uses in industry.

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Investigating the impact of prophages on bacterial fitness of *Streptococcus agalactiae*

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Most pathogenic bacteria carry prophages, genome integrated bacterial viruses, that encode genes that improve pathogen fitness and virulence resulting in increased dissemination and infection. *Streptococcus agalactiae*, a commensal pathogen, often harbors one or more prophages, but their significance in pathogen fitness and virulence is unknown. *S. agalactiae* is the frequent cause of life-threatening infections in neonates and young infants. However, antibiotic treatment can have long-term negative effects on the microbiota of neonates. By understanding the role of prophages in *S. agalactiae* virulence, we provide new opportunities to develop alternative treatments. CNCTC 10/84 is a hypervirulent neonatal blood isolate carrying a single prophage within the bacterial genome. To understand the prophage contribution to bacterial fitness, we cured CNCTC 10/84 of its prophage (phage-cured) to compare gene expression and fitness with that of the wildtype strain. To examine differences in virulence we used a zebrafish larval model. Co-infection with both strains simultaneously resulted in the wildtype strain having a competitive advantage over the phage-cured strain, demonstrating a phage-encoded advantage. Additionally, whole-genome transcriptome analysis was performed on the wildtype and phage-cured strains to determine if bacterial gene expression is altered by the prophage. The gene expression analysis indicates that loss of the prophage significantly altered expression of 27.3% of *S. agalactiae* genes. These results provide insight into the role that prophages play in the disease pathogenesis of *S. agalactiae* and ultimately may lead to the identification of potential targets for alternative therapeutic approaches.

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STRUCTURE AND ROLE OF BIDIRECTIONAL HYDROGENASE IN CYANOBACTERIA

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With a quickly growing population and dwindling source of fossil fuels, civilization needs an alternative fuel source. One potential alternative to fossil fuels is hydrogen since it is the most energy packed fuel, and, when used in the fuel cell, it only yields water when combined with oxygen. While traditional methods of hydrogen production are expensive and emit high levels of CO2, renewable bio-hydrogen production systems can be carbon neutral and less energy intensive. Cyanobacteria are a diverse group of photoautotrophic prokaryotes that house the ability to carry out oxygenic photosynthesis and are ideal cell factories for hydrogen production. Bioproduction of hydrogen in these species depends on the activity of the bidirectional hydrogenase enzyme that can take electrons from the photosynthetic electron transport chain. Hydrogen evolution by bidirectional hydrogenase requires two components: hydrogenase (HoxYH subunits), and diaphorase (HoxEFU subunits). Hydrogenase maturation and the assembly of the active site require six proteins (encoded by hypABCDEF operon). An endopeptidase (HoxW) is responsible for modifying the C terminus which is necessary for assembly of the active site of bidirectional hydrogenase. Cyanobacterial hydrogenase is inhibited by the increased oxygen concentration in the cells. As bidirectional hydrogenase is the main hydrogen-producing enzyme in these microorganisms, it can be a target for genetic manipulations to increase bioproduction of hydrogen and the tolerance of this enzyme to oxygen.

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Comparison of long and short read metagenomic assemblers for recovery of low-abundance species

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Recent technological and computational advances have made metagenomic assembly a viable approach to achieving high resolution views of complex microbial communities. In previous benchmarking, short-read (SR) metagenomic assemblers had the highest accuracy, long-read (LR) assemblers generated the longest sequences, and hybrid (HY) assemblers balanced length and accuracy. Until now, no benchmarking studies have specifically addressed the ability of assemblers to recover low-abundance organisms, which typify pathogens and other clinically relevant organisms in the gut. We generated synthetic long- and short-read metagenomic datasets by computationally spiking *E. coli* isolate reads into fecal backgrounds at fixed coverage levels, and, using different assemblers, examined *E. coli* scaffolds and

the recovery of antibiotic resistance genes. Then, we assessed how each assembler's performance changed when a competing *E. coli* strain was spiked in. Although SR assemblers recovered more resistance genes with high accuracy, even at low coverages, the increased contiguity in LR assemblies allowed for greater association of *E. coli* genes with their taxonomic origin. HY assemblies identified resistance genes with high accuracy and had lower contiguity than LR assemblies. Our results highlight the power of using long-read sequencing for analysis of low-abundance species in metagenomic samples.

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Probe based enrichment greatly increases coverage of the *E. coli* pangenome in metagenomic sequencing data, exposing regulation of a urovirulence factor

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Clinically important microbes are often found at low abundances (<1%) within complex communities, presenting a challenge for their investigation. In order to gain a high-resolution view of one such gut-associated bacterial species, we developed a set of hybrid capture probes designed to represent the more than 8 million genes of the *Escherichia coli* pangenome. Our approach enriched *E. coli* sequence from stool DNA libraries by an average of approximately 40-fold, and from stool RNA libraries by approximately 23-fold with little to no bias in the breadth or depth of sequencing coverage. As a demonstration of the enhanced resolution provided by this approach, we explored expression of the *fim* operon encoding Type 1 fimbriae, which has a role in invasion of bladder urothelial cells in urinary tract infections (UTIs) and is regulated by an invertible promoter, *fimS*. Using data from libraries enriched with the *E. coli* pangenome probes, we were able to estimate the proportion of *fimS* in the "on" orientation within the gut microbiota of women with and without a history of recurrent UTI, as well as explore strain-level dynamics of *fim* expression. This vignette demonstrates the power of this E. coli *hybrid capture methodology to yield unprecedented insight into* E. coli *dynamics within real world contexts where the genetics of the* E. coli are unknown and their relative abundances are low.

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The Coxiella effector CvpG modulates fusion of the bacterial vacuole with endolysosomal compartments

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Coxiella burnetii secretes at least 140 effector proteins into the eukaryotic host cell, controlling the formation and maturation of the large, fusogenic Coxiella Containing Vacuole (CCV). Six effectors, termed Coxiella Vacuolar Proteins CvpA-F, have been described to modulate the membrane identity, size, and heterotypic and homotypic fusion of the lysosome-derived CCV with other membrane compartments. A previously undescribed Coxiella effector, termed CvpG, is recruited to the CCV membrane when ectopically expressed as a fusion with eGFP, mCherry, or mClover3. CvpG-GFP preferentially localizes to perinuclear, Golgi-associated transferrin-receptor and WGA-positive recycling endosomes in uninfected HeLa cells and remains on TfR1-marked tubules distinct from the Golgi complex after Brefeldin A treatment. Abundant ectopic expression of CvpG-GFP in *Coxiella* infected cells impairs homotypic fusion resulting in smaller, more abundant CCVs. On the other hand, when native *Coxiella* expression of CvpG is disrupted by a transposon, CCVs are enlarged and recruit more of the lysosome marker LAMP1 and the late endosome/TGN-associated Rab9a protein. Compared to cells infected by wild-type *Coxiella*, cvpG::tn infected cells have less TfR1 overall, reduced TfR1 association with the CCV, and more acidic vacuolar pH measured by pHrodo-dextran fluorescence. Finally, CvpG-disrupted strains have decreased virulence in a *Galleria* infection model. Thus, CvpG is a the

seventh Type-IV secreted Coxiella Vacuolar Protein (Cvp) and modulates transferrin recruitment and vesicle fusion leading to proper maturation and maintenance of the mildly acidic Coxiella Containing Vacuole.

LUNCH OPTIONS IN HARVARD SQUARE

Note: Lunch will be provided only on June 13, 2022. Below are our suggestions for some local options for lunch on June 14, 2022

Gastropub/American

Alden & Harlow
Harvest
Russell House Tavern
The Longfellow Bar
Grendel's Den Restaurant & Bar
Henrietta's Table
The Smoke Shop BBQ
Charlie's Kitchen
Boston Burger Company

Cafes

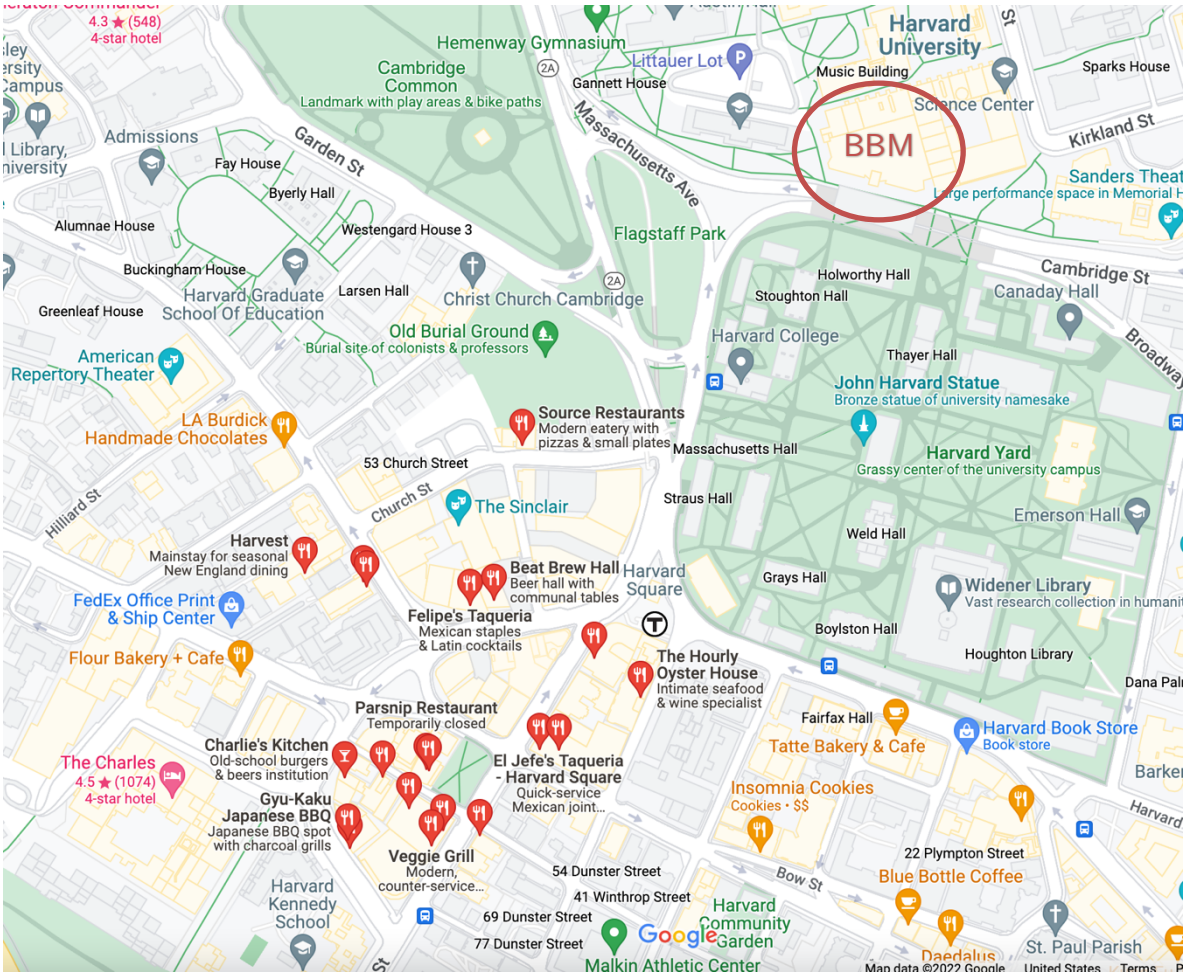
Bluestone Lane
Tatte Bakery and Cafe

Quick Eats

Saloniki Greek
Sweetgreen
Clover Food Lab
OTTO
Shake Shack
Tasty Burger

Asian

Hokkaido Ramen Santouka
Menya Jiro
Pokeworks
The Maharaja
Chutney's Cambridge
Le's Vietnamese Restaurant
Cafe Sushi



THANK YOU FOR ATTENDING BBM 2022!

Feel free to reach out to us at bbm@hms.harvard.edu
if you want to get involved or have any suggestions for next year.