2021 Microbial Systems Initiative Virtual Symposium
9 a.m.–12:30 p.m., March 24–26
Wednesday, March 24
9:00 a.m. Welcome Remarks – Cari Vanderpool, MSI Director

Session 1. Host-Microbiome Interactions
Session chair: Joseph Sanfilippo
9:10 a.m. Quantifying Interactions between Gut Microbiota and Intestinal Parasites
Chris Gaulke, Assistant Professor, Pathobiology
9:30 a.m. Psychological stress mediates intestinal epithelial cell-gut microbiota communication
Jacob Allen, Assistant Professor, Kinesiology & Community Health
9:50 a.m. Prenatal origins of neurodevelopmental dysfunction: the intersection of maternal microbes and fetal microglia
Adrienne Antonson, Assistant Professor, Animal Sciences
10:10 a.m. Break / Breakout rooms with session 1 speakers

Session 2. Cancer and the Microbiome
Session chair: Chris Gaulke
10:30 a.m. Bile acid signaling: a missing link between metabolome and microbiome?
Sayee Anakk, Associate Professor, Molecular & Integrative Physiology
10:50 a.m. Completion of the epi-bile acid pathway
Jason Ridlon, Assistant Professor, Animal Sciences
11:10 a.m. Engineering antibody fragments and commensal microbes to improve accessibility of cancer treatment
Vince Kelly, Graduate Student, Bioengineering
11:30 a.m. Break / Breakout rooms with session 2 speakers / MicroERA networking

11:45 a.m. Concurrent discussions and networking
- Resources room (main room)
  Facilitator: Sharon Donovan
  - Fuming Pan, Rodent Gnotobiotic Facility
  - Alvaro Hernandez, DNA Services
  - Chris Fields, HPCBio
- Mentoring room 1 – Success in Grad School
  Kristen Farley (facilitator), Rebecca Batstone, Shannon Sirk
- Mentoring room 2 – Career Transitions
  Adrienne Antonson (facilitator), James Imlay, Cari Vanderpool
- Networking room
Thursday, March 25

Session 3. Infectious Disease Dynamics
Session chair: Asma Hatoum

9:00 a.m. Socioeconomic status and its association with COVID-19 incidence and related mortality in Santiago, Chile
Pamela Martinez, Assistant Professor, Microbiology and Statistics

9:20 a.m. Antigenic evolution of influenza virus in the presence of functional constraints
Nicholas Wu, Assistant Professor, Biochemistry

9:40 a.m. Honey bee virus causes context-dependent changes in host social behavior
Adam Dolezal, Assistant Professor, Entomology

10:00 a.m. Break / Breakout rooms with session 3 speakers

Session 4. Host-Virus Interactions
Session chair: Pamela Martinez

10:15 a.m. A unique mode of anti-phage immunity performed by a single multifunctional enzyme
Asma Hatoum, Assistant Professor, Microbiology

10:35 a.m. The evolution of transmission mode in mu-like viruses of Pseudomonas aeruginosa
Laura Suttenfield, Graduate Student, Microbiology

10:55 a.m. Engineered decoy receptors for SARS-CoV-2
Erik Procko, Assistant Professor, Biochemistry

11:15 a.m. Break / Breakout rooms with session 4 speakers

11:30 a.m. Lightning talks
Room 1
11:30 a.m. Taylor Chen
Physiological Change of Salmonella Under Polyamine Stress

11:40 a.m. Anshika Gupta
Chronic exposure to low micromolar doses of peroxide can cause substantial DNA damage

11:50 a.m. Sumiti Vinayak
Genetics and cellular biology of the diarrheal pathogen, Cryptosporidium parvum
12:00 p.m.  Myung Whan Oh  
_Deletion Analysis of Streptococcus pneumoniae Delayed Genes_

Room 2  
11:30 a.m.  Nic Handy  
_Accessory Module Domains in Bacterial AB Toxins_

11:40 a.m.  Tongyu Liu  
_Variation in influenza virus neuraminidase activity shapes the antigenic evolution of hemagglutinin_

11:50 a.m.  Joel Rivera Cardona  
_Induction of immune response by reassortment between H1N1 and H3N2_

12:00 p.m.  Ami Seeger  
_Co-opting Mitochondrial Dynamics as a Novel Antimicrobial Strategy against Mitochondrial-Targeting Pathogens_

12:10 p.m.  Ipshita Upadhayay  
_Cholera multiepitope fusion antigen induces protective antibodies against Vibrio cholera_

Room 3  
11:30 a.m.  Noah Hutchinson  
_Effects of Broad-spectrum Antibiotic Administration and Germ Free Status on Running Wheel Behavior and Exercise Adaptation in Mice_

11:40 a.m.  Heng-An Lin  
_The effect of foliar fungicide on Septoria brown spot and phyllosphere microbiome on soybean_

11:50 a.m.  Beatriz Penalver Bernabe  
_Secondary Bile Acids and Saturated Fatty Acids are Increased in Antenatal Depression_

12:00 p.m.  Anqi Zhao  
_Brassica Vegetables Enhance Microbial Transformation of Glucosinolates to Bioactive Isothiocyanates and Beyond_

12:10 p.m.  Amanda Adams  
_A Novel Family of RNA Binding Proteins Regulate Polysaccharide Metabolism in Human Gut Bacteroidetes_

Room 4  
11:30 a.m.  Yu-Heng Deng  
_Self-Locomotive Antimicrobial Microbubbler for Active Biofilm Removal_
11:40 a.m. Veronika Dubinkina
Ecology-guided prediction of cross-feeding interactions in the human gut microbiome

11:50 a.m. Conghui Huang
Effects of corrosion inhibitors on biofilm structural and mechanical properties and microbial risk

12:00 p.m. Yoon Jeong
Elucidating Dynamic Cancer Cell Response with A Model of Multi-layered Bacterial Beads

12:10 p.m. Suyue Lyu
Engineering Bacteroides thetaiotaomicron to Secrete Heterologous Therapeutic Protein
Friday, March 26

Session 5. Microbial Cell Biology  
*Session chair: Jacob Allen*

9:00 a.m.  It’s all about communication: coordinating the bacterial cell cycle  
*Paola Mera, Assistant Professor, Microbiology*

9:20 a.m.  Bacteria in fluid flow  
*Joseph Sanfilippo, Assistant Professor, Biochemistry*

9:20 a.m.  Probing birth and death of mRNAs in bacteria using single-molecule microscopy  
*Sangjin Kim, Assistant Professor, Physics*

10:00 a.m.  Break / Breakout rooms with session 5 speakers

Session 6. Genomics and Eco-evolution of Multi-scale Symbioses (GEMS)  
*Session chair: Paola Mera*

10:15 a.m.  GEMS: Genomics and Eco-evolution of Multi-scale Symbiosis  
*Katy Heath, Associate Professor, Plant Biology*

10:35 a.m.  The soil microbiome is a shared symbiosis  
*Anthony Yannarell, Associate Professor, Natural Resources & Environmental Sciences*

10:55 a.m.  Genomic signatures of host adaptation in a protective symbiont of the honey bee  
*Irene Newton, Associate Professor, Biology, Indiana University*

11:15 a.m.  Break / Breakout rooms with session 6 speakers

11:30 a.m.  **Panel discussion: “Lessons from the Pandemic”**  
*Panelists: Jessica Brinkworth, Chris Brooke, Paul Hergenrother, Rebecca Smith*

12:30 p.m.  Closing Remarks
Mentoring Room 1: Success in Grad School Discussion Questions

Panelists: Kristen Farley (facilitator), Rebecca Batstone, Shannon Sirk

1. **Apply for fellowships** – Earning a fellowship during your time in graduate school is an excellent indicator to future employers that you have and the skills to convey the importance of your research in an impactful way. Are there any fellowships that you would like to apply for or have applied for in the past? Did you receive any feedback on your fellowship proposals?

2. **Start preparing for your career early** – When you become a senior graduate student, your committee and advisor will ask you what you are thinking about your career goals. It is a good idea to decide early on, if possible, what you would like to pursue as your future career in science. Have you decided on your scientific career goal(s)? If so, how did you make the decision?

3. **Staying organized** – Can you think of a time when being disorganized put you in a bad position? How did that experience feel? Conversely, can you think of a time when being organized paid off? Did this experience feel different?

4. **Time management** – Can you think of a time when an external distraction got you off task at work? Have you made any changes to minimize external distractions while you are working?

5. **Work-life balance** – Do you have any hobbies that help you maintain work-life balance? Do you reasonably limit your time at work?

6. **Ask for help** – Struggling with an experiment? Can’t get your gene of interest cloned? Talk to someone! Your committee members, PI, labmates, colleagues in other labs, and other PIs in your department, on campus, or external are all great sources of knowledge and help for experiment ideas, troubleshooting, protocols, etc. Most are nice and helpful! Do you have an example of an occasion when reaching out to another scientist helped you overcome an obstacle in the lab?

7. **Make and reach your goals** – Learn how to set reasonable goals and achieve them! Do you have an example of how goal-setting has impacted your academic career?

8. **Networking** – Networking can be intimidating, but it is necessary for success in many cases! How do you feel about networking? Do you have any tips to make networking easier?
Mentoring Room 2: Career Transitions Discussion Questions

Panelists: Adrienne Antonson (facilitator), Cari Vanderpool, Jim Imlay

1. **Career steps in academia** – Is there a typical time period during your postdoc research that you should begin applying for positions in academia? Are your research advisor and other researchers in your department some of the primary sources of help during the application and interview processes for academic faculty positions or is there an external office as well that you can reach out to (example: The Graduate College for graduate students)? How long is the process to secure a faculty position from application submission to job offer?

2. **Transition from academia to industry** – What are the major differences between working in industry versus academia? Do you know a scientist who was trained or previously worked in an academic research setting who successfully transitioned to an industry position? How did the transition go for them?

3. **Applying for academic and industry positions** – What should I really try to highlight on my CV and other application documents to make my application stand out for an academic position? What about for an industry position? Is the number of publications the most important factor that determines if you get the position or not?

4. **How to find your next position** – How did you begin your job search? Can social media be helpful for finding job postings/advertisements? Do you know of other good resources for the job search?

5. **The interview process** – What is the interview process like for an academic faculty position? Does it differ from the interview process for an industry position? How long should you take to prepare for the interview process?
Physiological Change of Salmonella Under Polyamine Stress

The transport of polyamines, such as cadaverine and putrescine, is essential for cell physiology and stress response in *Salmonella*. PaeA, an inner membrane protein, has been characterized as a cadaverine/putrescine exporter. In stationary phase, paeA mutants are sensitive to external cadaverine at pH > 8. The mechanism of toxicity is unknown. By isolating spontaneous mutants of paeA that survive in the presence of cadaverine, we have identified a list of suppressive genes, namely, rpoS, kdpD, corA, barA, yeaG, topA, and clpA. To unravel how they play a role in suppressing, we reconstructed the mutations in wild type and paeA background. We also created complete deletions of each of the genes. The deletion of corA, barA, yeaG, topA, or clpA was found to be suppressive, while only single-nucleotide mutations in rpoS, kdpD, and corA suppressed, suggesting gain of function mutations. Using tests of epistasis, we also conclude that both barA and clpA mutations act by increasing the degradation of cadaverine. Further characterization of the corA mutations and other magnesium transporters led us to speculate that intracellular magnesium concentration affects the sensitivity of *Salmonella* to polyamine, and the homeostasis between magnesium and polyamine is crucial to the survival of *Salmonella*. 
Chronic exposure to low micromolar doses of peroxide can cause substantial DNA damage

*Escherichia coli* (*E. coli*) inhabits the oxic-anoxic interface of the human gut. Indirect evidence indicates that at this location it experiences low micromolar concentrations of extracellular hydrogen peroxide (H$_2$O$_2$). Prior studies determined that millimolar doses of H$_2$O$_2$ exert only a moderate effect on the survival of *E. coli*, suggesting that low micromolar doses of H$_2$O$_2$ would have a minuscule effect. However, the induction of DNA repair enzymes at micromolar doses of peroxide suggests that natural concentrations of H$_2$O$_2$ are a threat to DNA. To test this hypothesis, I am studying the regulation of a DNA repair enzyme, exonuclease III (Exo III), and its impact on cell fitness in H$_2$O$_2$ scavenging-deficient mutants (Hpx$^-$). Peroxide measurements have shown that Hpx$^-$ mutants accumulate about 1 μM intracellular H$_2$O$_2$ on aeration which can be sustained for a prolonged period of time. My data show that in this situation OxyR mediates Exo III induction, implying that 1 μM H$_2$O$_2$ can cause DNA damage. Indeed, when Hpx$^-$ Exo III mutants are aerated, they form filaments and also show survival defects, suggesting the presence of significant DNA damage. It is the protracted exposure to low micromolar H$_2$O$_2$ which has a disproportionate effect on DNA damage. The induction of the SOS regulon is also critical for cell fitness at physiological doses of H$_2$O$_2$. Using an Hpx$^-$ mutant, we have also identified endonuclease III as the primary glycosylase that is important in dealing with oxidized DNA lesions.
Cryptosporidium parvum, a protozoan parasite, is a leading cause of diarrheal disease and mortality in young children and neonatal calves. Currently, there is no effective drug or vaccine available to treat or prevent cryptosporidiosis. Progress in the development of novel therapies has been stymied due to lack of understanding of the parasite biology; partly due to lack of genetic tools, inability to grow the parasite in the laboratory, and poor animal infection models. To overcome these challenges, we have pioneered CRISPR/Cas9 genome editing system to genetically manipulate Cryptosporidium, and an immunocompromised mouse infection model to propagate stable transgenic parasites. The key advantage of this genetic system is that the entire life cycle of the parasite comprising of asexual and sexual stages can be completed in the mouse intestine, thus allowing us to unravel parasite cell biology and answer key questions related to host-pathogen interactions and disease pathogenesis. We are employing a combination of genetics and cellular biology approaches to uncover the molecular signaling mechanisms that orchestrate transition of developmental stages for successful completion of the Cryptosporidium life cycle. Using genetically modified parasites and super-resolution structured illumination microscopy, we have defined the localization and expression dynamics of two signaling kinases during male and female gametes (sexual stage) development. Genetic ablation of the male-specific kinase resulted in decreased infectivity, and reduction in oocyst shedding in the mouse infection model. More functional studies are underway to further unravel the mechanistic function of these kinases in Cryptosporidium virulence, host-pathogen interactions and disease transmission.
Deletion Analysis of Streptococcus pneumoniae Delayed Genes

One of the major characteristics of *Streptococcus pneumoniae* (pneumococcus) is its competence regulon, which is crucial for its natural genetic transformation. The integration of exogenous single stranded (ss)DNA into the pneumococcal genome results in genetic plasticity, allowing pneumococcus highly adaptable to certain environmental stresses including antibiotic treatments. Whether the pneumococcal competence development plays an equally important role in virulence and fitness during infection is poorly understood. There are four distinct expression profiles in competence genes that are commonly referred in context of pneumococcal infection: early, late, delayed gene induction and repression. Among the 24 ‘early’ genes are the functionally redundant *comX1* and *comX2*, encoding the alternative sigma factor ComX that in return, activates the transcription of approximately 80 ‘late’ genes during the competence state, some of which we have previously shown to be important for pneumococcal pneumonia and bacteremia infections. We are currently focusing on the 19 genes that exhibit the delayed expression pattern during competence induction. Apart from the putative functions and that some of these delayed genes are largely unaffected by the inactivation of ComX, the mechanisms of regulation of these genes and their role in pneumococcal disease pathogenesis have not been elucidated. We have performed genetic deletion and genetic epistatic studies to characterize the functions of these genes and their contributions to pneumococcal virulence and fitness in mouse models of bacteremia and acute pneumonia.
Accessory Module Domains in Bacterial AB Toxins

Large macromolecule therapeutics have an assortment of potential biomedical and research applications, ranging from delivery of therapeutic biologics to modulation of microbiomes. However, they lack an efficient method for reaching their intracellular targets. One potential solution is the use of AB type bacterial toxins that contain modular domains capable of transporting their toxic cargo into the cytosol of mammalian cells. *Pasteurella multocida* toxin (PMT) contains an N-terminal delivery vehicle motif (PMT-N) that can be found in a variety of toxin sequences from many pathogenic bacteria, including the cytotoxic necrotizing factor (CNF) family of toxins. This PMT-N delivery vehicle is found attached to a number of different types of cargos, along with a variable accessory module that has been shown to be required for delivery of the PMT catalytic domain. Here, to study how the accessory modules influence the delivery vehicle’s ability to deliver cargo of other toxins in this group, we constructed a series of chimeras swapping functional domains among PMT, CNF1, CNF3, and CNFy with and without their native accessory modules. We found that the CNF catalytic cargo also required the accessory module in order to reach the cytosol in a cell-based SRE-luciferase assay. These findings indicate that the accessory modules of this group of toxins facilitate the efficient cytosolic delivery of native and heterologous cargos normally excluded from entering host cells.
Variation in influenza virus neuraminidase activity shapes the antigenic evolution of hemagglutinin

Influenza A viruses (IAV) pose enormous health risks and economic burdens on both individuals and society, despite the huge efforts made on vaccines. Antigenic drift is the greatest challenge to vaccines’ efficacy and to the prediction of the next season’s predominant strains. However, very little is known about the mechanism of IAV antigenic evolution. During adaptation, IAVs need to establish a balance between hemagglutinin (HA) receptor-binding avidity and the receptor-destroying activity of neuraminidase (NA). Thus, we hypothesize that variation in NA activity plays a critical role in determining the genetic pathways of HA antigenic evolution. Our preliminary result shows that the viruses with reduced NA activity generate distinct escape variants under a monoclonal antibody selection, suggesting the potential effect of NA activity on HA antigenic evolution. Further study will be performed on selections by other monoclonal antibodies and serum, determining the HA avidity of the escape variants and their effects on viral fitness. Our study will expand our mechanistic understanding of IAV antigenic drift and improve our ability to predict IAV antigenic properties more accurately.
Induction of immune response by reassortment between H1N1 and H3N2

The adaptation of influenza A virus (IAV) from animals to humans has originated significant outbreaks such as the 1918 flu pandemic that resulted in close to 50 million deaths. During this adaptation process, IAV goes through reassortment, which is the packaging of gene segments from different strains into one viral particle. This recombination can further the function of multiple viral factors, such as its ability to suppress or antagonize the innate immune response. Furthermore, reassortment can influence viral fitness by either increasing its ability to adapt and transmit to a new host or decreasing its replication and frequencies in the populations. Interestingly, reassortment between the pandemic strains H1N1 and seasonal strains H3N2 have not been frequently identified in humans. However, studies have shown that the progeny produced by this reassortment can significantly decrease viral fitness by the inability to produce functional viral complexes such as the RNA polymerase. The hypothesis is that IAV H1N1 and H3N2 reassortment is driven primarily by intersegment interaction that is not restricted to the polymerase segments. Preliminary data has shown that viral particles that packaged segment from both H1N1 and H3N2 induce higher expression of genes associated with the host’s immune response. Suggesting that reassortment can disrupt IAV intersegment interactions affecting viral fitness and increasing the host’s antiviral response by mechanisms that are not well understood.
Ami Seeger  
Graduate Student, Steven Blanke Lab, Microbiology

**Co-opting Mitochondrial Dynamics as a Novel Antimicrobial Strategy against Mitochondrial-Targeting Pathogens**

Host humoral immune responses effectively neutralize bacterial protein toxins in the extracellular environment, but provide no protection once toxins have breached the plasma membrane barrier and accessed the interior of host cells. However, strategies evolved within host cells to sense and respond to the modulatory activities of intracellular-acting toxins are poorly understood. The channel-forming protein exotoxin VacA, secreted by human gastric pathogen *Helicobacter pylori (Hp)*, is taken up into host cells and targets the inner mitochondrial membrane, resulting in mitochondrial dysfunction, characterized by the depolarization of the mitochondrial membrane and decrease in the cellular energy levels. While cells exposed to high VacA concentrations are reported to undergo cell death, cells intoxicated with limited VacA exposure survive disruption of mitochondrial homeostasis, suggesting that intoxicated cells employ poorly understood strategies to rescue cells from the mitochondrial damaging action of VacA. Our findings revealed evidence suggesting of the existing host mechanism that counteract the extent of the VacA-mediated mitochondrial damage, in order to facilitate the restoration of the mitochondrial homeostasis and to promote cell survival. Mitochondrial dynamics that is activated by the presence of VacA is found to be involved in counteracting the mitochondrial-damaging toxin action, demonstrated by the attenuated mitochondrial functional restoration in cells that are unable to undergo mitochondrial fission. Additionally, we find that the activation of mitochondrial dynamics is induced by the host cellular sensing of the VacA-mediated energy deficiency, through the activation of an energy regulator AMP-activated protein kinase. The inhibition of cellular energy sensing dampens mitochondrial functional recovery, indicating a causal relationship between the sensing of VacA-mediated cellular energy reduction and the activation of a mitochondrial restoration response. Together, these results support a model in which the normal physiological process of mitochondrial dynamics is co-opted as antimicrobial strategy for responding to *Hp*-mediated disruption of metabolic homeostasis through the action of VacA, and in an efforts to promote cell survival during *Hp* infection.
Cholera multiepitope fusion antigen induces protective antibodies against Vibrio cholera

Cholera remains a major public health threat particularly in Southeast Asia and sub-Saharan Africa. It is estimated that *Vibrio cholerae* infection is responsible 21,000 - 143,000 deaths annually. In this study, we applied novel epitope- and structure-based vaccinology platform multi-epitope fusion antigen (MEFA) to construct a multivalent cholera MEFA immunogen, characterized MEFA immunogenicity and MEFA-induced antibody neutralization activities, and assessed the potential application of cholera MEFA for development of a safe and broadly effective subunit vaccine for cholera. By using the MEFA platform, we selected the strongly immunogenic and adjuvantic flagellin B subunit as the backbone and then constructed cholera MEFA that presented the immunodominant and conserved epitopes of seven virulence factors from *V. cholerae* serotypes Ogawa and Inaba and biotypes classical and El tor (cholera toxin-CT, toxin coregulated pilus A-TcpA, Sialidase, Hemolysin A-HlyA, FlaB, FlaC and FlaD) and mimicked native antigenicity of each epitope. Mice IM immunized with cholera MEFA, with or without adjuvant dmLT (double mutant of *E. coli* heat labile toxin), developed strong IgG antibody responses to each virulence factor antigen. Importantly, anti-mouse antisera showed significant inhibition against the adherence of different *Vibrio cholerae* strains to Caco-2 and neutralization activity against CT enterotoxicity. Cholera MEFA also proved to be protective in adult rabbit *Vibrio cholerae* challenge model. Results from this study indicated that this cholera MEFA is broadly immunogenic and induces neutralizing antibodies, and suggested cholera MEFA potential application for development of effective vaccines against cholera.
Effects of Broad-spectrum Antibiotic Administration and Germ Free Status on Running Wheel Behavior and Exercise Adaptation in Mice

The gut microbiota modulates many physiologic processes. Regular endurance exercise alone alters the composition of the gut microbiome and metabolome. We hypothesized that microbes contribute to endurance training adaptations and tested this by depleting microbes using broad spectrum antibiotics (ABX) and using germ free (GF) mice. We sought to determine if wheel running behavior or adaptations were affected by ABX or GF status. In a 2 x 3 design (wheel/sedentary, control/ABX/GF), male and female C57Bl/6 mice ran for six weeks to induce endurance adaptations and wheel running was monitored. ABX mice received ABX in their water three days prior to and throughout the intervention. ABX mice received ABX in their water three days prior to and throughout the intervention. There were no differences in food intake between control and ABX groups, but a slight increase in water intake in ABX mice. Wheel running tended to reduce body weight, significantly reduced perigonadal fat pad weight, and increased relative heart weight in control and ABX, and improved time to fatigue on a treadmill test in all groups. These effects were not altered by ABX, but were significantly reduced by GF status. Notably, SED GF mice displayed increased fatigability in comparison to sedentary controls. ABX and GF status increased cecum weight and tended to improve glucose tolerance. ABX did not significantly affect wheel running behavior, but GF status significantly reduced daily running distance. We conclude that ABX does not affect wheel running and may be used to test whether microbial depletion alters endurance exercise-induced training adaptations. Also, GF status affects locomotor motivation reflected by reduced voluntary wheel running.
The effect of foliar fungicide on Septoria brown spot and phyllosphere microbiome on soybean

Septoria brown spot, caused by Septoria glycines, is the most prevalent soybean foliar disease in Illinois and often co-occurs with other diseases, such as Cercospora leaf blight and frogeye leaf spot. Foliar fungicide applications during the reproductive stage is a common method to control these diseases. However, the application of foliar fungicide does not always result in yield increase, and the effect of the fungicide applications on the phyllosphere fungal community needs further understanding. In this study, we used DNA metabarcoding to understand the dynamics between \textit{Septoria} and non-target species using samples collected from inoculated and fungicide-treated plots. Full-length ITS and partial LSU were sequenced using oxford nanopore technology. The cultivars had a significant effect on the fungal communities at the V4 stage. Ten fungi were identified as core components of the leaves. Although possible interactions were identified between \textit{Septoria} and other fungal organisms, the inoculation treatment did not significantly impact the entire communities according to the $\alpha$ and $\beta$ diversity analyses. At the R5 stage, the fungicide application was an essential factor that shaped the fungal communities. From the relative abundance analysis, the fungicide treatment significantly decreased the proportion of most fungi, but the proportion of \textit{Bipolaris}, and \textit{Diaporthe} increased. In this study, we demonstrated that metabarcoding could be a tool to quantify the effect of fungicide on target and non-target organisms. Understanding the impact of fungicide on the phyllosphere microbiome dynamics is necessary to develop better management practices.
Secondary Bile Acids and Saturated Fatty Acids are Increased in Antenatal Depression

Background: Antenatal depression (AD), depression during pregnancy, is common (10-20%) and have negative consequences for mother and infants, including preterm birth. An underexplored mechanism that could contribute to the onset of antenatal depression is the microbiome-gut-brain axis (MGBA). Aim: Identify the associations between elevated symptoms of depression during pregnancy and quantifiable characteristics of the MGBA. Method: We recruited 78 pregnant women at less than 16 gestational weeks, and we followed them at 28 gestational weeks. At each visit, participants answered a battery of mental health questionnaires and provided fecal and blood samples. Gut microbial composition, immune activity and serum metabolomics were characterized with 16S rRNA sequencing, Luminex and LC/MS/MS respectively. Results: Participants were 55% Black and 30% Latina with a 22% rate of antenatal depression (greater than the national average). *Lactobacillus* presented lower abundance in the first trimester and *F. prausnitzii* species in both first and second trimesters in women with AD. An analysis of the host’s inflammatory and systemic metabolism showed that women who developed AD had higher serum concentrations of proinflammatory cytokines (e.g., IL-17A, IFN-γ) and that the concentrations of microbial-dependent metabolites in plasma, e.g., saturated fatty acids and secondary bile acids increased with AD levels. Conclusions: MGBA is dynamically altered during the antenatal period and distinct in women with AD versus those without. Our results showed the potential of the MGBA to diagnose and predict the onset of antenatal depression.
Brassica Vegetables Enhance Microbial Transformation of Glucosinolates to Bioactive Isothiocyanates and Beyond

Brassica vegetables are rich in glucosinolates (GSLs), which, upon hydrolysis, produce bioactive isothiocyanates (ITCs). Previously, we showed changes in cecal microbiota composition and a greater production of ITCs by the rat cecal microbiota after four days of broccoli feeding. Recently, we determined that a 4-day feeding of cooked kale caused a similar effect in significantly enhancing ITC production by the rat cecal microbiota. These findings suggest that consumption of GSL rich vegetables enhances microbial transformation of GSLs to ITCs, which may further enhance the health benefits relative to ITCs to the host. Beside their activities on cancer prevention, ITCs also contribute to the characteristic bitter and pungent flavor of brassica vegetables. The recent findings of the expression of bitter taste receptors (T2Rs) in the gut attracted our interests in investigating the interaction between ITCs and intestinal T2Rs. The activation of intestinal T2Rs was linked with improving glucose homeostasis, mainly through inducing the secretion of gut hormones. We hypothesized that GSLs and/or ITCs may interact with the intestinal T2Rs and induce the secretion of glucagon-like peptide-1 (GLP-1). Our preliminary data showed that allyl isothiocyanate (AITC) is more potent at inducing GLP-1 secretion from an enteroendocrine cell line compared to its parent GSL, sinigrin. Other ITCs, including benzyl isothiocyanate and sulforaphane, also increased GLP-1 secretion. Further investigation of the underlying mechanism showed AITC induces GLP-1 secretion, potentially through T2R pathways, mediated by IP3, TRPA1, and extracellular Ca2+. This study may provide a novel therapeutic mechanism explaining brassica vegetables improving glucose homeostasis.
Human gut microbiome composition is constantly changing, and diet is a major driver of these changes. Gut microbial species that persist in mammalian hosts for long periods of time must possess mechanisms for sensing and adapting to nutrient shifts to avoid being outcompeted. Global regulatory mechanisms mediated by RNA binding proteins (RBPs) that govern responses to nutrient shifts have been characterized in Proteobacteria and Firmicutes but remain undiscovered in the Bacteroidetes. We report the identification of RBPs that are broadly distributed across the Bacteroidetes, with many genomes encoding multiple copies. Genes encoding these RBPs are highly expressed in many Bacteroides species. A purified RBP, RbpB, from *Bacteroides thetaiotaomicron* binds to single-stranded RNA in vitro with an affinity similar to other characterized regulatory RBPs. *B. thetaiotaomicron* mutants lacking RBPs show dramatic shifts in expression of polysaccharide utilization and capsular polysaccharide loci, suggesting that these RBPs may act as global regulators of polysaccharide metabolism. A *B. thetaiotaomicron* mutant lacking RbpB shows a growth defect on dietary sugars belonging to the Raffinose Family of Oligosaccharides (RFOs). The ΔrbpB mutant had reduced expression of BT1871, encoding a predicted RFO-degrading melibiase, compared to the wild-type strain. Mutation of BT1871 confirmed that the enzyme it encodes is essential for growth on melibiose and promotes growth on the RFOs raffinose and stachyose. Our data reveal that RbpB is required for optimal expression of BT1871 and other polysaccharide-related genes, suggesting that we have identified an important new family of global regulatory proteins in the Bacteroidetes.
Self-Locomotive Antimicrobial Microbubbler for Active Biofilm Removal

Biofilms are communities of bacterial cells that can cause a variety of healthcare problems. In particular, over 80% of microbial infections in human body is associated with bacterial biofilms, according to a survey from the US National Institutes of Health (NIH). However, traditional antibiotics treatments are not effective enough to remove the wound biofilm because the extracellular polymeric substances (EPS) can impede the diffusion of antibiotics into biofilm. In particular, the bacterial cells residing beneath the biofouling layer are 100 to 1,000 times more resistant to chemicals than planktonic bacterial cells. In this talk, I will introduce a unique method to remove biofilms using self-locomotive antimicrobial microbubbler (SLAM). The micro-sized SLAM made of MnO₂ and hollow-shaped diatom cylinder can self-propel and swarm to form microbubbles in H₂O₂ solution. Those microbubbles generated from SLAM can further deform biofilms while expanding and abrade the biofilms through the cycle of bubble burst and regrowth.
Ecology-guided prediction of cross-feeding interactions in the human gut microbiome

Understanding a complex microbial ecosystem such as the human gut microbiome requires information about both microbial species and the metabolites they produce and secrete. These metabolites are exchanged via a large network of cross-feeding interactions and are crucial for predicting the functional state of the microbiome. However, till date, we only have information for a part of this network, limited by experimental throughput. Here, we propose an ecology-based computational method, GutCP, using which we predict hundreds of new experimentally untested cross-feeding interactions in the human gut microbiome. GutCP utilizes a mechanistic model of the gut microbiome with the explicit exchange of metabolites and their effects on the growth of microbial species. To build GutCP, we combine metagenomic and metabolomic measurements from the gut microbiome with optimization techniques from machine learning. Close to 65% of the cross-feeding interactions predicted by GutCP are supported by evidence from genome annotations, which we provide for experimental testing. Our method has the potential to greatly improve existing models of the human gut microbiome, as well as our ability to predict the metabolic profile of the gut.
Effects of corrosion inhibitors on biofilm structural and mechanical properties and microbial risk

Biofilms, a common environmental niche, play an important role in opportunistic pathogen survival and propagation in drinking water distribution systems (DWDS). Understanding the effects of biofilm structural and mechanical properties, which can influence biofilm cohesiveness and detachment under physical stress, is important for biofilm and biofilm-associated pathogens risk control. Corrosion inhibitors, which are commonly added into drinking water due to aging DWDS to reduce lead and copper level in drinking water, are usually phosphate-based and can provide nutrients to microorganisms grown inside premise plumbing systems. To investigate the role of silicate, and tin (common and experimental corrosion inhibitors) on mechanical and structural properties of biofilms, we characterize the structure of biofilms grown from groundwater amended with these corrosion inhibitors over 6 months by optical coherence tomography (OCT) and determine their mechanical properties by nanoindentation. Compared to tin biofilms, silicate biofilms were significantly thicker and more porous. Based on the pore network modeling (PNM), more pores and channels were detected in silicate biofilms than tin biofilms. Our results showed that thicker and more porous biofilms (silicate biofilms) were less resistant to deformation than thinner and denser biofilms (tin biofilms). The findings of this study can aid in the biofilm-associated pathogens risk management.
Elucidating Dynamic Cancer Cell Response with A Model of Multi-layered Bacterial Beads

We hypothesize that certain bacterial species interact with cancer cells through extracellular signals and play a key role in altering the micro-environment of tumor and surrounding tissues toward a milieu of other signals to affect tumor growth and/or dynamic cellular responses. Axenic models are generally considered to be the gold standard for in vivo studies of the intestinal microbiome. However, maintaining germ-free mice requires specialized facilities and skilled personnel and is expensive thus making these models inaccessible to many researchers. Furthermore, no specific standardized models exist to date to evaluate host-microbiome communications utilizing 3D coculture models to evaluate the interaction between the microbiome and host, cancer cells or microbe-microbe communications. We have developed a unique multi-layered hydrogel confinement platform for long-term monitoring and in vitro co-culturing to bridge the knowledge gap between the role of microbiome on cancer. The hydrogel modalities allow for bacterial colonization with heterogenous microbial spatial distribution under confinement. Stratified confinement with multilayered characteristics for microorganisms have excellent potential to develop biologically relevant heterogenous environment to study cell to cell communication. In addition, selected bacteria can be recapitulated in hydrogels and co-cultured with cancer cells using the co-cultivation platforms complemented with high performance spectroscopic approaches. We believe our studies will be an effective avenue to organize cells into sophisticated and stratified structures to study intra and multicellular communication to evaluate multicellular interactions in a 3D microenvironment.
Engineering *Bacteroides thetaiotaomicron* to Secrete Heterologous Therapeutic Protein

Aim: The aim of this work is to engineer a prevalent and abundant gut commensal bacterium, *Bacteroides thetaiotaomicron*, to secrete antibodies for novel therapies for intestinal diseases.

Methods and Results: To facilitate engineering of *B. thetaiotaomicron*, a Golden Gate Assembly system was established to enable rapid construction of plasmids with a variety of regulating genetic parts. Using this plasmid system, we are testing the secretion ability of 11 signal peptides fused with 3 different antibody cargos. We have identified 3 signal peptides that lead to different levels of secretion. We are also testing the hemolysin secretion system as an alternative secretion pathway. In the future, we will optimize the wild type strain by overexpressing Sec secretion system components to increase the secretion level.

Conclusions: *B. thetaiotaomicron* was successfully engineered to secrete two single domain antibodies and an IgG light chain fragment. Significance and Impact: This is the first report on successful engineering of *B. thetaiotaomicron* to secrete heterologous protein. This delivery strategy avoids the costs of in vitro production and purification of therapeutic protein, and it also increases the bioavailability by circumventing significant degradation of protein drugs in the stomach and duodenum. The secretion of active therapeutic protein in *B. thetaiotaomicron* could lead to development of low-cost long-term therapies for chronic intestinal diseases.