

**Abstracts of Platform Talks
(in order of presentation)**

Structure and function of contractile injection systems

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The tail of bacteriophage T4 is a paradigm of a diverse class of complex multicomponent organelles called contractile injection systems. Besides phage tails, this class contains the bacterial Type VI Secretion System (T6SS), Antifeeding Prophages of *Serratia*, *Photorhabdus* Virulence Cassette, Metamorphosis-Associated Contractile arrays of *Pseudoalteromonas luteoviolacea*, R-type pyocins of *Pseudomonas* and similar complexes of *Clostridium*. These systems have a common architecture and functional mechanism that involves a contractile sheath, which resembles a stretched coil spring surrounding a central rigid tube. Upon interaction with the target cell, the sheath contracts and propels the tube through the cell envelope. The event of sheath contraction is coupled to delivery of toxins in T6SS or constitutes a prerequisite for subsequent protein and DNA translocation in phages. The sheath contraction triggering process is controlled by a multicomponent baseplate at the end of the tail. Using cryo-electron microscopy, X-ray crystallography and modeling, we have determined the atomic structure of the 6 MDa baseplate of bacteriophage T4 in two states – in its pre- and post-host cell attachment conformations. This information allowed us to describe – in atomic detail – how the baseplate undergoes its massive structural rearrangement that results in deployment of the short tail fibers and release of the central membrane-piercing spike complex while simultaneously initiating sheath contraction. We have established a minimal composition of the baseplate in all contractile injection systems. This information makes it possible to interpret the structure of other contractile injection systems and deduce the function of their components.

Structures of the single-stranded RNA virus Q β reveal its MurA-binding interface and internalized coat proteins

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In single-stranded RNA bacteriophages (ssRNA phages) a single copy of the maturation protein binds the genomic RNA (gRNA) and is required for attachment of the phage to the host pilus. For the canonical *Allolevivirus* Q β the maturation protein, A₂, has an additional role as the lysis protein, by virtue of its ability to bind and inhibit MurA, the enzyme that catalyzes the first step in peptidoglycan biosynthesis. Using single-particle cryo-electron microscopy, we determined that Q β has its gRNA folded into a dominant defined conformation inside the capsid and the outer surface of the β -region in A₂ is the MurA-binding interface. Additionally, by comparing the Q β virion with Q β virus-like particles that lack a maturation protein, we observed a structural rearrangement in the capsid coat proteins that is required to package the viral gRNA in its dominant conformation. Unexpectedly, we found a coat protein dimer sequestered in the interior of the virion. This coat protein dimer binds to the gRNA and interacts with the buried α -region of A₂, suggesting that it is sequestered during the early stage of capsid formation to promote the gRNA condensation required for genome packaging. These internalized coat proteins are not found in a related phage MS2 and are the most asymmetrically arranged major capsid proteins yet observed in virus structures.

1. Gorzelnik KV., Cui Z, Reed CA, Jakana J, Young R, Zhang J. Asymmetric cryo-EM structure of the canonical *Allolevivirus* Q β reveals a single maturation protein and the genomic ssRNA in situ. *Proc Natl Acad Sci USA*. 2016, 113(41):11519-11524.
2. Cui Z, Gorzelnik KV, Chang J, Langlais C, Jakana J, Young R, Zhang J. Structures of Q β virions, virus-like particles, and the Q β -MurA complex reveal internal coat proteins and the mechanism of host lysis *Proc Natl Acad Sci USA*. 2017; doi:10.1073/pnas.1707102114

Mapping Protein-Protein Interactions with Jun-Fos Phage Display and Deep Sequencing

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Protein-protein interactions govern many processes in the cell and identifying and mapping binding interaction sites on proteins can facilitate discovery of inhibitors to block such interactions. Phage display is a powerful affinity enrichment method for identifying proteins or peptides that bind a chosen target molecule. Here we describe the identification of protein domains or peptides involved in protein-protein interactions from a library of inserts derived from randomly fragmented DNA of a plasmid encoding the β -lactamase inhibitory protein (BLIP) and the lac repressor (LacI). A challenge with constructing libraries of genomic or, in this case, plasmid DNA, for display on the gIII capsid protein is that the DNA encoding the protein or peptide to be displayed is normally inserted after the g3p signal sequence and before the mature g3p. For insertion of randomly fragmented DNA, this means that only $\sim 1/18$ of the inserts will be in-frame from the signal peptide through to mature g3p. This has previously been addressed using the jun and fos leucine zipper proteins to allow insertion of randomly fragmented inserts at the 3' end of the fos zipper, thereby reducing the number of clones that encode out-of-frame peptides. A difficulty with this system is that expression of the jun and fos leucine zippers reduces the fitness of *E. coli* resulting in the accumulation of clones with the jun-fos-insert region deleted from the plasmid. Here we show that PCR amplification of the insert region from phage libraries that have been affinity enriched for binders to the immobilized target proteins including anti-BLIP antibody, anti-LacI antibody and the TEM-1 β -lactamase, followed by next generation sequencing can overcome the high frequency of deletion clones and identify protein domains and peptides that bind the target and allow mapping of the binding hotspots for the target protein.

The multi-component antirestriction system of phage P1

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Bacterial Type I restriction-modification (R-M) systems present a major barrier to foreign DNA entering the bacterial cell. The temperate phage P1 packages several proteins into the virion that protect the phage DNA from host restriction. Isogenic P1 deletion mutants were used to reconstitute the previously described restriction phenotypes associated with *darA* and *darB*. While P1 Δ *darA* and P1 Δ *darB* produced the expected phenotypes, deletions of adjacent genes *hdf* and *ddrA* also produced *darA*-like phenotypes and deletion of *ulx* produced a *darB*-like phenotype, implicating several new proteins of previously unknown function in the P1 *dar* antirestriction system. Interestingly, disruption of *ddrB* decreased P1's sensitivity to *EcoB* and *EcoK* restriction. Proteomic analysis of purified virions suggests that packaging of antirestriction components into P1 virions follows a distinct pathway that begins with the incorporation of DarA and Hdf and concludes with DarB and Ulx. Electron microscopy analysis showed that *hdf* and *darA* mutants also produce abnormally high proportions of virions with aberrant small heads, which suggests Hdf and DarA play a role in capsid morphogenesis. The P1 antirestriction system is more complex than previously realized and is comprised of multiple proteins including DdrA, DdrB, Hdf, and Ulx in addition to DarA and DarB.

Using phages to study evolution: a retrospective

Jim Bull

The study of evolution is largely retrospective, providing inferences about processes that already happened. The many conveniences of phages allow them to be used to study evolution in real time, providing a forward-based, experimental approach to the discipline. The convenience of phages is not always convenient for the theories being tested however. This talk will describe a few different realms of experimental tests of evolutionary theories and methods: phylogenetics, optimality, and the fate of genomes genetically engineered to be altruistic. This experimental approach provides a humbling perspective on the prospect for predicting evolution.

Title: Does Cell Growth Rate Affect Event Timing in *Escherichia coli*?

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Abstract

A major question in molecular biology is how changes in cell growth rate and size affect timing of cellular events. When cells grow and divide, proteins produced by cells will be diluted unless transcription and translation are up-regulated to compensate for dilution. For cellular events that require a threshold protein concentration for triggering, dilution imposed by cell growth may prevent the proper event timing. Here we investigate the effect of increased growth on event timing in a cellular model of event timing, the lysis of *Escherichia coli* cells by the bacteriophage λ . This virus induces host lysis via the production of the protein, holin. Holin accumulates in the *E. coli* inner membrane until it reaches a critical threshold. At this threshold, holin nucleates to permeabilize the bacterial inner membrane, allowing for the subsequent lysis of the cell. Our mathematical models of the holin lysis process when cell growth rate varies suggest that limitations exist in the ability of phage λ gene expression to compensate for growth-imposed holin dilution, especially when translation rates are low or threshold concentrations required for lysis are high. We test these predictions by observing mean lysis time for a panel of holin mutants when *E. coli* growth rates are manipulated. Our data show that, when holin threshold concentrations required for lysis are high or when translation rates are low, mean lysis time takes a concave up shape with respect to growth rate, confirming the predictions of our model.

Intracellular Competition and Cooperation Mediate Viral Decision-making

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From single cells, whose decisions direct differentiation and disease, to entire organisms, whose collective choices cause populations to flourish or vanish, decision-making profoundly influences the development of life. We choose to deconstruct this complex process by using a simple model system: the bacteriophage lambda lysis/lysogeny paradigm. Lambda propagates either by manufacturing virions and bursting its host to release progeny during lysis or by integrating its viral genome into the host's genome to replicate passively during lysogeny. Little is known about the detailed mechanisms of this subcellular fate selection, partly due to limited resolution of past studies. We constructed single-cell/virus/molecule reporters to probe lambda decision-making at unprecedented resolutions using live-cell microscopy. We find that phages, at the individual DNA level, have distinct interactions following lysis versus lysogeny. Competition ensues during lysis to produce phages that dominate viral propagation, whereas cooperation occurs during lysogeny to promote mutual phage propagation and thus, proliferation of variable viruses. We highlight phage DNA replication as a vital, yet overlooked process, serving as the focus of phage competition. Our data suggest that infection timing and host resource sequestration confer advantages to the eventual victor. Altogether, we conclude that these behaviors optimize evolutionary fitness, where competition allows favored phages to monopolize a given niche, and cooperation encourages genetic diversity, such that unknown niches might be colonized effectively. The apparent simplicity of these bacterial viruses belies their true complexity when observed using the proper approaches, and with this perspective, we will continue to reveal more interesting phenomena of phages.

T7 Internal Head Proteins Interact with the F₁F₀ ATPase to Enhance Phage Growth

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T7 is an obligately lytic member of the *Podoviridae*. As a short-tailed phage, T7 must form its own trans-envelope channel for transport of its genome into the host cell cytoplasm. This channel is created by ejecting three internal head proteins: gp14, gp15, and gp16 into the infected cell. Gp16 is thought to be at least bifunctional in that the N-terminal domain has lytic transglycosylase activity while the C-terminal domain is important for DNA translocation into the infected cell cytoplasm. Specifically, removal of the C-terminal six amino acids prevents DNA internalization. Extragenic suppressors of the truncated gp16 lie in gp15, suggesting that the proteins cooperate in DNA ejection. This process requires the proton motive force and we had proposed that DNA was ratcheted into the cell by a molecular motor, using an analogy with the F₁F₀ ATPase.

We have now found that the ATPase is directly involved in the early steps of T7 infection. Six copies of the enzyme have been visualized by cryo-electron tomography associated with an infecting T7. T7 recruitment of the ATPase requires both the F₁ and the F₀ components.

We have begun to investigate the role of the ATPase in T7 development. It is not essential for phage growth although moderate physiological defects are observed in *atp* mutants. In particular, removal of the C-terminal five amino acids prevents phage growth in *atp* mutants but not isogenic *atp*⁺ strains. We have also isolated extragenic suppressor phage mutants that allow growth in *atp* mutant hosts even though the phage lacks those C-terminal amino acids in gp16. The mutations are being sequenced but we predict that they will also affect gp15, the partner of gp16 in T7 genome ejection.

A Novel Lysis Protein: How PhiKT Disrupts the Outer Membrane

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Double-stranded DNA (dsDNA) phages of Gram-negative hosts typically require three types of lysis proteins: the holins that make holes in the inner membrane, endolysins that degrade the peptidoglycan layer, and spanins that disrupt the outer membrane. Bioinformatic analysis has recently revealed a number of dsDNA phages that do not have spanins. Because spanins are essential for lysis, we investigated how phage PhiKT lyses its host, *E. coli*. Phage PhiKT lacks recognizable spanins, but in conditions that require spanin function, PhiKT caused rapid lysis of the bacterium. The explosive lysis of PhiKT suggests the presence of a spanin equivalent. Plasmid-based expression of the lysis region caused rapid lysis. This region includes three predicted genes: the endolysin, a hypothetical novel gene *gp28*, and the holin. *gp28* encodes a 56 amino acid product that lacks the features required for spanin function. Expression of Gp28 complemented lysis defect in a spanin mutant lysogen. In addition, both in-frame deletions and nonsense mutations of *gp28* stalled lysis at the outer membrane disruption step. Therefore, Gp28 is functioning to disrupt the bacterial outer membrane. Future experimentation is necessary to determine the mechanism of this novel lysis gene.

Predicting the fate of individual *E. coli* cells following phage infection

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The infection of *Escherichia coli* by bacteriophage lambda is a paradigm for the way gene regulation drives cell fate choices. Although the lambda genetic circuitry has been well characterized, we still cannot predict, at the single-cell level, whether an infection will result in cell death (lysis) or viral dormancy and cell survival (lysogeny). This is due to a large degree of cellular heterogeneity: Genetically identical cells, subject to identical conditions, exhibit different cell fate choices. Previous work suggested that much of the heterogeneity is due to undetected factors, which deterministically drive the cellular decision between the two outcomes. Identification of these factors will allow us to better predict the fate of individual cells. By combining single-cell and single-molecule fluorescence microscopy with advanced image and data analysis algorithms, we can detect single DNA and RNA molecules and discrete transcription events in individual cells, and thus can measure gene activity at single-molecule resolution. We have begun to describe the transcription dynamics of critical genes in the lysis/lysogeny decision circuit, and to develop a mathematical model that will guide us in interpreting these experimental results. Next, we plan to use these tools to determine the role of cell-cycle phase, as well as the number and spatial positions of viral genomes, in the decision process. Our study will reveal to what degree the apparent indeterminacy (“noise”) in the lysis/lysogeny decision can be reduced, instead replaced by a deterministic picture of cell fate determination.

The Bacterial Mutagenic Tetrasect and the Evolvable Medicine to Neutralize It's Clinical Impact
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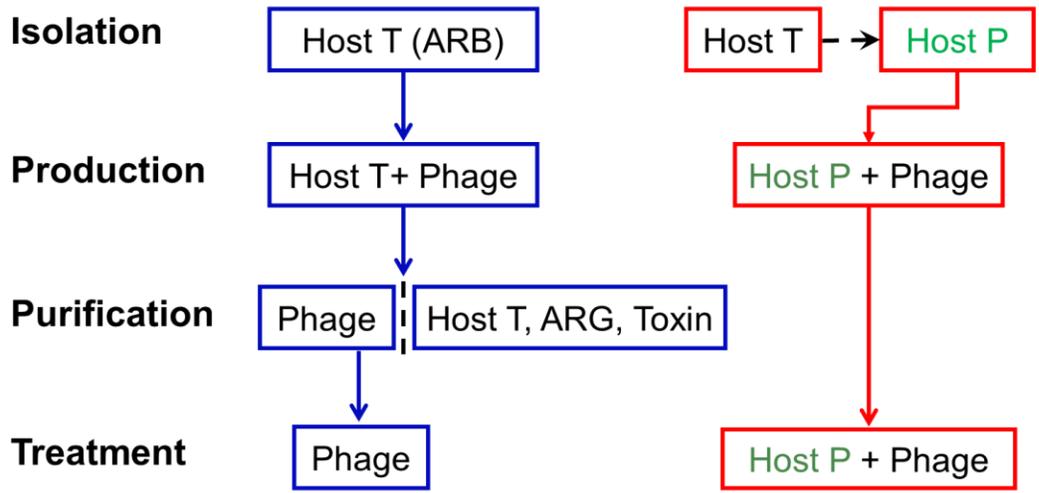
We lie at the anti-microbial precipice. Multi-drug resistance combined with the acquisition of virulence factors has created bacterial superbugs that are deadly for immunocompromised patients. The conventional way by which new antibiotics are developed has failed to keep pace with the rate at which pathogenic bacteria evolve. The solution to this problem will require unconventional approaches that are as rapidly adaptable as the bacteria we seek to target. In this presentation, we discuss the challenges facing the infectious disease community concerning the evolution of drug resistance and how the very inconsistent nature of the bacteriophage might just be the very property needed to overcome the resistance problem. We summarize the state of the science for phage therapy and highlight three aspects of the field that must be addressed to gain widespread acceptance of phage as a commonplace anti-microbial. These three areas include the feasibility of targeting circulating pandemic bacterial strains, the human factors that contribute to phage efficacy in vivo, and the specific clinical problems by which such an approach might prove feasible. Only then will we be able to use the inherent adaptability and diversity of phage to turn the tables on the very processes that harmful bacteria use against us.

Polyvalent Bacteriophage Therapy to Suppress Antibiotic Resistant Bacteria in the Environment

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A universal decline in the effectiveness of antibiotics has revived interest in phage-based biocontrol. However, the scope and efficacy of phage applications in environmental systems have been hurdled due to phage narrow infectious spectrum, infectivity loss during phage delivery, and difficulty of phages to penetrate bacterial biofilms. To facilitate research on polyvalent (broad host-range) phage-host interactions and evaluate their potential as antimicrobial agents, we developed sequential multiple-host approaches for preferential isolation of polyvalent phages. These polyvalent phages showed interspecies or even inter-order infectivity without significant reduction in efficiency of infection. Due to these unique features, polyvalent phages can not only infected multiple problematic bacteria but also be safely and efficiently produced using nonpathogenic fast-growing hosts. When we added the polyvalent phages along with one helper host, polyvalent phages reached greater densities, which offset phage loss during delivery and increased the probability of infection. We then investigated the effect of phage host range on interspecies competition. Without restricted phage diffusion, phage infection and interspecies competition synergistically contributed to bacterial suppression, although polyvalent phages attenuated interspecies competition. Polyvalent phages propagation in multiple bacterial hosts facilitated their penetration and spreading through biofilms, improving targeted microbial control. Overall, polyvalent phages isolated with sequential multiple hosts hold promise for safe production and enhanced microbial control in environmental systems.



(A) Conventional phage biocontrol (B) Modified phage biocontrol

Resistance to ϕ NPV1 is accompanied by daptomycin sensitivity and susceptibility to osmotic stress in *Enterococcus faecalis*

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Enterococcus faecalis is a commensal Gram-positive bacterium that inhabits the human gastrointestinal tract. Treatment of *E. faecalis* infection has proven difficult because of high frequency of resistance to multiple antibiotics. The *epa* gene cluster encodes synthesis of a cell wall rhamnose polysaccharide whose role in resistance to macrophage killing, daptomycin sensitivity, and overall cell wall architecture has been investigated in *E. faecalis*. A previous report indicated that mutations in the *epa* genes also affect sensitivity to phage ϕ NPV1, but no mutant affecting phage reversible adsorption was found. Considering that there has been revived interest in phage therapy which calls for the use of phages utilizing different receptors, it is important that phage receptors are identified. Furthermore, it has been shown in *Pseudomonas aeruginosa* that selection for phage-resistant mutants defective for OprM functionality recovered antibiotic sensitivity, suggesting that phage resistance comes at a cost if the receptor is of physiological significance. In light of these findings, we sought to determine the receptor for ϕ NPV1 for *E. faecalis* OG1RF and the consequences, if any, of phage resistance. We found that point mutations in *epaR* arising spontaneously during ϕ NPV1 infection as well as engineered *epaR* deletion abolish ϕ NPV1 reversible binding to *E. faecalis* OG1RF, resulting in phage resistance. Mutants defective for phage adsorption lack the Epa polymer, suggesting that the Epa polymer is required for ϕ NPV1 adsorption. However, ϕ NPV1-resistant OG1RF strains with *epaR* mutations are more susceptible to daptomycin and are less resistant to osmotic stress than wild-type strains. These results underscore the importance of the Epa polymer as a phage receptor as well as being a player in maintaining osmotic pressure and mediating daptomycin susceptibility in *E. faecalis*.

Phage Therapy Strategy

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A phage therapy success has recently been achieved with an *Acinetobacter baumannii* infection of a US human [1]. This success raises the question of what is optimal phage therapy strategy. The strategy used above is similar to strategy successfully used by the Eliava Institute in Tbilisi, Georgia, former USSR [2].

These strategies include building a stockpile of numerous different lytic phages for each pathogen. For therapy, one constructs a mixture (cocktail) of several different lytic phages, selected from the stockpile. One changes the mixture if bacterial resistance occurs. The scientific subtext includes (a) optimization of procedure for rapidly detecting, purifying, storing and characterizing phages, especially jumbo phages [3-6], such as the increasingly useful, soil-isolated 201phi2-1 [6]. (b) development of informatics for rapid phage characterization, and (c) development of databases for optimally using information and rapidly retrieving phages.

Using lytic phages with long blood lifetime should be part of strategy. Current data with several phages [7, 8] indicate minimally a 2-log phage titer loss from mouse blood between 5 and 60 min. Phage lambda undergoes > 5-log loss in 2 hr and complete loss by two days [9]. A long-life lambda mutant, selected via 10 cycles in mice, had an ~1-log loss per day [7].

We have determined infectivity titer vs. time of wild type, lytic phage T3 in mouse blood after IP injection. Clearance was significantly slower than previously reported for other phages. Significant titer loss was not observed until 3-4 hr, followed by about 1-log loss per day. A possible explanation is that (a) T3 is naturally lytic (unlike lambda), (b) a podovirus (unlike T2 and T4, also short-lived in mouse blood) and (c) isolated from human sewage. Points (a) and (c) suggest that, before isolation, wild type T3 was (unlike lambda) adapted to mammals, primarily humans.

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**Abstracts of Posters
(numbered)**

Poster # 1

Poster title: Functionalized phage nanoparticles: Versatile ultrasensitive reporters for the detection of proteins and viruses

Abstract:

Beyond traditional phage display, viruses have taken up new roles in diverse applications including diagnostics. Phage nanoparticles present monodisperse scaffolds of versatile morphologies, with high colloidal and storage stability, that can accommodate a large range of recognition (antibodies, aptamers, lectins, etc.) and/or reporter (enzymes) elements leading to universal, ultrasensitive, bio-detection reporters. We have been actively pursuing two different approaches to integrate these phage reporters into ultrasensitive assays. In the first scheme, M13 phage particles functionalized with target-specific antibodies serve as recognition elements and their binding to the target can be ultra-sensitively reported through real-time PCR amplification of the phage genome. Thus, we achieved the detection of femtomolar concentrations of a model cancer biomarker (VEGF) in bronchoalveolar lavage fluid. In the second scheme, M13 phage particles functionalized with both target-specific antibodies and enzyme reporters were integrated into an immunochromatographic lateral flow assay (LFA) for the detection of a model virus (MS2), leading to greatly enhanced detection sensitivity (thousand-fold better than a conventional gold nanoparticle LFA with the same antibodies). Since our goal is to build a genuinely useful diagnostic assay, we have been systematically engineering and characterizing these ultra-sensitive phage reporters and integrating them with complex sample matrices, such as blood.

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Structures of Q β virions, virus-like particles, and the Q β -MurA complex reveal internal coat proteins and the mechanism of host lysis

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In single-stranded RNA bacteriophages (ssRNA phages) a single copy of the maturation protein binds the genomic RNA (gRNA) and is required for attachment of the phage to the host pilus. For the canonical Allovivivirus Q β the maturation protein, A2, has an additional role as the lysis protein, by its ability to bind and inhibit MurA, which is involved in peptidoglycan biosynthesis. Here, we determine structures of Q β virions, virus-like particles, and the Q β -MurA complex using single-particle cryoelectron microscopy, at 4.7-Å, 3.3-Å, and 6.1-Å resolutions, respectively. We identified the outer surface of the β -region in A2 as the MurA-binding interface. Moreover, the pattern of MurA mutations that block Q β lysis and the conformational changes of MurA that facilitate A2 binding were found to be due to the intimate fit between A2 and the region encompassing the closed catalytic cleft of substrate-liganded MurA. Additionally, by comparing the Q β virion with Q β virus-like particles that lack a maturation protein, we observed a structural rearrangement in the capsid coat proteins that is required to package the viral gRNA in its dominant conformation. Unexpectedly, we found a coat protein dimer sequestered in the interior of the virion. This coat protein dimer binds to the gRNA and interacts with the buried α -region of A2, suggesting that it is sequestered during the early stage of capsid formation to promote the gRNA condensation required for genome packaging. These internalized coat proteins are the most asymmetrically arranged major capsid proteins yet observed in virus structures.

Evolving Phage for the Treatment of MDR ExPEC Bacteremia

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Multi-drug resistant (MDR) bacteria are increasingly a global problem. One clonal group, *Escherichia coli* sequence type (ST)131, is driving the expansion of antimicrobial resistance worldwide. This pandemic group is part of a diverse collection of pathogens known as Extraintestinal Pathogenic *E. coli* or ExPEC. Immunocompromised patients are at particular risk of ExPEC infections, and chemoprophylaxis with antibiotics potentially selects for resistant strains. Phage therapy is the use of bacterial viruses to treat infections. Previously, we examined the applicability of environmental phage isolates to treat MDR ExPEC in mouse models of bacteremia designed to recapitulate infection types in the clinical setting. One representative phage, ϕ HP3, when tested in murine models of bacteremia, reduced disease severity and ExPEC levels for four distinct strains. Here, we characterized ϕ HP3 using genomic sequence analysis. We find that ϕ HP3 has a GP38 adhesin similar to that of a known phage with broad host range, IP008. This adhesin constructed of four hypervariable repeats surrounded by conserved glycine rich regions is considered to be a region with high potential for plasticity. To test ϕ HP3's adaptability we performed a host-range expansion experiment in physiologically relevant media, human blood. After serial incubation with clinical isolates of ExPEC, an ϕ HP3 phage mixture which previously lysed 25% (2/8) of clinical isolates in human blood, after expansion could lyse 100% (8/8). ϕ HP3 is a phage that is effective at reducing bacterial counts in mouse models of infection but also has the potential to be highly plastic, an advantage that may be necessary to combat evolving pathogenic *E. coli* strains.

Mechanism of the Lysis-lysogeny Decision Making upon Infection by Wild-type Bacteriophage λ Strain: Ur- λ

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Bacteriophage Ur- λ has long thin side tail fibers that are absent from the commonly used laboratory λ strain, λ WT. With the presence of those side tail fibers, Ur- λ has been reported to adsorb significantly faster onto the host cell surface. At the bulk level, we observed that the lysogenization frequency of Ur- λ is higher than that of λ WT over a wide range of average phage input (API) after infecting *E. coli* MG1655 host cells. In our work, we label Ur- λ with a 2-color fluorescence reporter system to visualize the lytic and lysogenic decision made by Ur- λ upon infection of *E. coli* cells. Ur- λ capsid decoration protein gpD is translationally fused with mTurquoise2 fluorescent protein, allowing for the visualization of the lytic pathway. Meanwhile, mKO2 fluorescent protein is transcriptionally fused after λ cI gene, whose product is required for lysogenic establishment and maintenance. Our preliminary results show that the lysogenization frequency of Ur- λ correlates with cell size as λ WT: Shorter cells exhibit higher frequency to lysogenize. Ur- λ has similar lysis time compared with λ WT. Previous research has reported that phages compete within the host cell via DNA ejection timing and host resource. So when we mix Ur- λ and λ WT to do the co-infection, we expect to observe that Ur- λ dominates the overall cell fate, since Ur- λ could eject its DNA earlier owing to its higher absorption rate and start gene expression and DNA replication earlier. However, the preliminary observation suggests that λ WT is dominant over Ur- λ within the population of lytic cells, while Ur- λ dominates the lysogenic pathway. The detailed mechanism of the competition between Ur- λ and λ WT is still under investigation. In summary, this work reveals differences between Ur- λ and λ WT resulting from the side tail fibers in order to further elucidate the underlying mechanism of decision making during the infection by Ur- λ .

Mechanistic Studies of Siphophage Tape Measure Proteins and their role in Bacteriophage Infection

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Bacteriophages are being rediscovered for their therapeutic potential, however, the pathways and mechanisms guiding efficient genome transfer from the phage capsid to the host cytoplasm, a critical step in the phage infection cycle, remain unclear. Phages ensure their viral genome enters the host by traversing the host cell envelope. Phages infecting gram-negative bacteria, therefore, must contest the outer membrane, a periplasmic peptidoglycan meshwork, and the inner membrane to accomplish this task. It is known that phage tail proteins interact with host factors on the outer membrane at the onset of infection, however, the subsequent steps are poorly understood.

Experimental evidence suggests that some phage encoded tape measure proteins (TMPs) play significant roles in gram-negative cell penetration by possessing muralytic domains that degrade the peptidoglycan layer enough for the phage genome to enter the cell. However, for the family of siphophages, phages with long, noncontractile tails, this is not the case, as no known TMPs contain such activity. Thus, the mechanisms governing siphophage genome entry remain elusive. Previous genetic studies utilizing siphophage lambda have demonstrated that the mannose-specific phosphotransferase system (*manXYZ*) on the host inner membrane is required for lambda DNA ejection and thus replication, however, we were unable to replicate these results. Aiming to generally address the mechanism of siphophage genome entry, we isolated two novel ManYZ-dependent N15-like siphophages and isolated suppressors that eliminate ManYZ-dependence. Genomic analysis of the suppressors revealed single mutations exclusively in the TMP genes. Interestingly, each mutation mapped to a single 18 residue alpha-helical region in the TMPs, indicating that this region plays a significant role in ManYZ-dependence. These TMPs will be further characterized to identify possible interacting partners and results will be contrasted with current models describing siphophage genome entry.

Application of Lysis Proteins from Mycobacteriophage D29 to lyse *Rhodococcus opacus* PD631 for Lipid Release

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Lignocellulosic biomass is a renewable resource as a feedstock for biolipids such as triacylglycerols (TAGs) for lipid-based biofuel production. Under nitrogen limited conditions, *Rhodococcus opacus* PD630 (referred as PD630) can accumulate TAGs up to 76% of its cell dry weight. However, conventional solvent-based lipid extraction is costly and requires downstream process, making lipid-based biofuel less competitive than fossil-based fuels.

In an attempt to reduce TAG extraction cost, we explored the feasibility of applying phage lysis proteins to lyse cells. External applications of lysis proteins derived from lytic phages have been shown to hydrolyze peptidoglycan of cell wall and inhibit the growth of gram positive bacteria. Accordingly, we hypothesized that lysis proteins, through external or internal application, can release TAGs from TAG-accumulating *R. opacus*. To test this hypothesis, micobacteriophage D29 lysis genes (*lysA*, *holin*, *lysB*) were cloned into *R. opacus* PD631 (a domesticated strain of PD630). Following induction, PD631 was lysed successfully, confirmed with an observation of decreased optical cell density and present of lactate dehydrogenase, a cytolysis biomarker, in supernatant suggesting compromised of cell membrane. Additionally, the lysis genes were overexpressed from *E. coli*. and the crude lysis enzymes were applied to PD631 externally. Unlike the internal lysis gene expression, only a small fraction of PD631 cells was lysed and cell debris were observed microscopically. The results of this study suggest that application of phage lysis proteins can be used for lysing lipid-producing bacteria and thus has a potential to reduce extraction costs of TAGs.

Molecular function of the prototype unimolecular spanin gp 11 from phage T1

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Spanins are bacteriophage lysis proteins responsible for disruption of outer membrane (OM), the final step of Gram-negative host lysis. The absence of spanins results in a terminal phenotype of fragile spherical cells. The phage T1 employs a unimolecular spanin (US) gp11 that has an N-terminal lipoylation signal and a C-terminal transmembrane domain (TMD). Upon maturation and localization, gp11 ends up as an OM lipoprotein with a C-terminal TMD embedded in the inner membrane (IM), thus connecting both the membranes as a covalent polypeptide chain. Unlike the two component spanins (2CS) encoded by most of other phages including λ , the u-spanins haven't been studied extensively yet. The aim of this research is to investigate the molecular mechanism behind US function and further gain insights into phage lysis as well as the bacterial cellular envelope.

A detailed bioinformatics based, biochemical and genetic approach was used to study gp11. Using the ability of gp11 to complement the λ RzRz1 lysis defect, we showed that both the OM and IM localization signals were true and necessary for gp11 function. Furthermore, 14 lysis-defective single missense mutants, distributed throughout the periplasmic domain of gp11, were isolated from a mutant library created with error-prone PCR mutagenesis. Fluorescence spectroscopy time lapse videos using gp11-gfp showed gp11 accumulating in distinct punctate foci, suggesting localized oligomerization within the peptidoglycan meshwork.

In addition to these findings, our model for gp11 function will be presented. The results from the novel spheroplast fusion assay designed to test our model, indicating gp11 works through the fusion of IM and OM, will also be discussed. Along with the clues about 2CS and US evolutionary differences from bioinformatics, this work has significantly advanced our understanding about gp11 and other US's role in phage lysis.

Investigating *Staphylococcus aureus* Bacteriophages that are Present in Swine Production Environments

Abby Korn

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a major risk to human health, with approximately 94,000 cases occurring annually in the United States. Recent surveys in the United States on the prevalence of MRSA in swine production have found various sequence and *spa* types of MRSA that are harbored by both the animals and production workers. This colonization with MRSA raises concerns for worker safety and overall community health in areas where swine production is prominent. With antibiotic usage being gradually restricted in livestock production, there is a renewed interest in the application of bacteriophages to decolonize swine production environments of MRSA. In this study environmental swabs from 20 different swine production facilities across the United States were collected and evaluated for the presence of *S. aureus* phage by an enrichment procedure. Nineteen of the farms were sampled only once and provided five environmental swabs, while one farm was sampled in depth (30 environmental swabs) at time zero and then six months later.

Out of the 19 facilities surveyed, 12 were positive for *S. aureus* phage yielding 70 total phage isolates. Preliminary analysis of 54 phage isolates to date has shown they fall into 4 distinct groups as determined by restriction digest with enzyme DraI and three additional subgroups when digested with enzyme EcoRI-HF. The first in depth sampling of Farm 16 yielded only one phage positive swab on one *S. aureus* host with only one phage type isolated. However, the second sampling conducted six months later yielded seven phage positive swabs on four *S. aureus* hosts and two different phage types, one that matched the original phage isolate and a new type. Further characterization of each phage will include TEM imaging to determine morphology, genome sequencing, host range and biofilm mass reduction assays.

Post Isolation Differentiation of Mixed *Streptomyces* Phages Thestral and Haizum

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Recently at the University of North Texas, a student isolated a novel *Streptomyces* Phage sample on the bacteria *Streptomyces xanthochromogenes*. This phage sample was given the name Thestral and was sent for routine sequencing. However, the phage was not a single isolate, and two phage sequences were obtained from the single sample. They were originally given the names, Thestral A and Thestral B. Both sequences were predicted to be in the BD2 phage subcluster. Because of requirements of phage databases, a physical sample of each individual phage must be acquired in order to document these samples as new and unique. Techniques of bacteriophage isolation vary among many samples. In order to separate the two phages, plaques made by the phage lysate were chosen and tested using two unique primers, one from each phage. To analyze the PCR product, electrophoresis gels of the product were run. Different sized PCR products indicated the presence of either Thestral A, Thestral B, or a mix of the two. Once identified by their DNA, the phage samples were amplified and collected. After several trials, positive results of both phages were confirmed. Next two pure phage samples were collected; the sequences of Thestral A and B were released to the phage database. Thestral B was renamed as Haizum and added to the phage database. The two phage genomes has a 67% similarity, a high G+C content, and a genome length of about 50,000 basepairs. Their sequence release in the phage database will surely open doors for more study of *Streptomyces* bacteriophage and their applications in science.

Jordyn Michalik

Carbapenem-resistant *Enterobacteriaceae* (CRE) are a group of Gram-negative bacterial pathogens which carry resistance to a large proportion of available antibiotics, and are a significant cause of nosocomial infections. *Klebsiella pneumoniae* carrying the bla_{KPC} carbapenemase gene (KPC+) are of particular concern in the hospital setting among immunocompromised patients, and strains of sequence type (ST) 258 are prevalent in the US, South America and Europe. Bacteriophages, bacterial viruses, have been proposed as a potential alternative form of treatment for infections caused by multidrug-resistant pathogens, including KPC+ *K. pneumoniae*. In this study, we investigate a possible treatment for KPC+ *K. pneumoniae* via a bacteriophage cocktail. Isolation of 27 bacteriophages from various environmental sources resulted in nine bacteriophages which infect the ST258 KPC+ *K. pneumoniae* model strain 39827 as well as 18 others which infect a panel of KPC+ clinical and isolates. Our panel includes 32 *K. pneumoniae* strains representing ST258, ST42, and ST359. We were able to identify, evaluate and characterize candidate phages based on *in vitro* virulence assays, genomic sequencing, transmission electron microscopy and traditional plaque assays. Additionally, we addressed bacterial resistance by isolation of phage-resistant mutants and subsequent isolation of new bacteriophages against these mutants. Characterization of the receptor for these bacteriophages by genomic sequencing of phage-resistant mutants suggests a model of bacterial capsule as a common phage receptor in ST258 KPC+ *K. pneumoniae*; mutational loss of this receptor exposes additional cell surface features that can be recognized by other phages. Growth of the wild-type KPC+ *K. pneumoniae* host was suppressed more strongly by a phage cocktail recognizing different bacterial receptors than by individual phages, and phage-resistant bacterial mutants exhibited growth defects, suggesting a loss of fitness associated with these mutations. These data describe a promising potential treatment for KPC+ *K. pneumoniae*.

Characterizing lysis in ssRNA phages

Lorna Min

Host cell lysis is essential to continued bacteriophage infection and replication. While large, dsDNA phages have evolved strictly timed and regulated systems of three lysis proteins, small ssDNA *Microviridae* and ssRNA *Leviviridae* have evolved single gene lysis (SGL) systems. In the canonical phages ϕ X174 (*Microviridae*), Q β (*Alloleviviridae*), and MS2 (*Leviviridae*), the single lysis proteins E, A₂, and L are amuralytic, inhibiting peptidoglycan (PG) biosynthesis enzymes (E and A₂) or acting through an unknown mechanism (L). In our study of SGL systems, we have elucidated the lysis mechanism of Lys^M. Studying additional SGL systems may help us better understand the evolution of lysis, the lysis mechanism of MS2 L, and the organization of PG biosynthesis. Towards this goal, we sought to isolate novel RNA phages against *Acinetobacter* genospecies 16 and *Escherichia coli* encoding various pili. To select for small phages, we filtered source material through 50 kDa centrifugal filters before enriching; filters allowed MS2 to flow through while retaining T4 on top. Interestingly, six DNA phages specific to *E. coli* expressing the RA1 plasmid were isolated. DNA phages specific to plasmid-encoded structures have not been isolated before. Two RNA phages (Bo.RNA and Lo.RNA) infecting *Acinetobacter* genospecies 16 were also isolated, and the Bo.RNA genome has been sequenced and annotated. Bo.RNA shares 89% sequence homology with AP205, and the capsid structure of Bo.RNA resembles that of AP205.

Visualization of a novel trans-envelope channel during P22 infection and localization of E proteins in the infected cells

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To study how a hydrophilic DNA molecule crosses hydrophobic membranes, P22, a temperate phage of *Salmonella typhimurium*, was examined by an interdisciplinary approach combining phage biology, biochemistry and genetics with *in situ* structural biology. P22 ejects four E proteins (gp7, gp20, gp16, and gp26) upon adsorption. Our preliminary data with wild type P22 show that gp7, gp20, and gp16 form a continuous trans-envelope channel across the cell membranes during infection of *S. typhimurium* minicells. In order to obtain mechanistic and structural understanding of P22 infection initiation and DNA ejection, P22 mutants lacking one or more of these E proteins were produced and examined by a combinatorial study of Cryo-EM, Cryo-ET, and biochemical methods upon adsorption. Our preliminary Cryo-ET data with the triple deletion P22 mutant phage suggest that strong rod-like density with the dimensions of dsDNA fills up the space normally occupied by the E proteins in wild-type. Subsequently, there is no trans-envelope channel present upon infection and no major conformational change at the initial stage of adsorption. The fractionation of P22 infected cells shows that all three E proteins are found inside the cell. The membrane localization of E proteins is under further investigation.

Structure of N-terminal truncated tail fibers from R1 and R2-type pyocins reveals several novel domains and putative LPS binding pockets

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R-type pyocins are P2-like myophage tailocins of *Pseudomonas sp.* Strain sensitivity to pyocins of a given R-type is mediated by tail fiber binding to lipopolysaccharide (LPS). In order to characterize this interaction from a structural perspective, we crystallized and solved the structures of N-terminal truncated, R-type pyocin tail fiber fragments (NTFs) corresponding to a region of dense polymorphism between R-types known to be involved in host recognition.

R1- and R2-type structures revealed that the NTF is a helical trimer composed of three domains: a baseplate proximal head, medial shaft, and distal foot. The globular head and fibrous shaft contain novel structural motifs without sequence or tertiary homology to known proteins. The head domain is formed of mixed, anti-parallel β -sheets and contains aromatic residues that line the oligomeric interface. The shaft domain is a thin fiber (93 x 21Å) with a solvent-free central channel (3.3 Å diameter) situated along the oligomer symmetry axis. The shaft also includes a promiscuous cis-peptide, hexa-histidine divalent metal binding site, an oligomeric interface of aromatic and hydrophobic residues, and regions of staggered, trans-peptide hydrogen bonding. Unlike the head and shaft domains, the foot is composed entirely of a canonical jelly roll fold with structural similarity to a fragment of *A. baumannii* myophage AP22 tail fiber (pdb: 4mtm) and several eukaryotic adhesins.

Modelling R-type polymorphisms onto NTF surface maps elucidated several candidate carbohydrate binding pockets in both the head and foot domains. In R1-type NTF structures, a sugar backbone analogue, glycerol, was observed in a small pocket absent in the R2-type NTF, suggesting that the pocket may be involved in R1-type specific LPS binding. Given what is known about podophage tail spike protein interactions with LPS, we predict that simultaneous carbohydrate binding interactions at several of our identified pockets are required by R-type pyocins for specific adsorption.

Effect of DNA Replication on Bacteriophage Lambda Decision-Making

Qiuyan Shao

Cellular decision-making is defined by a series of biochemical reactions such as gene transcription and translation along a regulatory cascade which can lead to distinct outcomes. Variations in gene copy numbers, often introduced by DNA replication, can have significant effects on the rates of gene expression to change the network output. Yet how DNA replication can affect the decision-making process remains to be investigated. To gain more insights, we study the bacteriophage lambda infection system by asking how viral DNA replication can affect the lysis-lysogeny decision outcomes. The phage lytic-lysogenic choice is determined by a network of regulatory genes expressed early after infection and the decision is enforced by the production of CI for lysogeny, or the accumulation of lytic proteins for lysis. First, we quantitatively measure the transcriptional activity of the key genetic components using single-molecule fluorescence in situ hybridization (smFISH) to see how DNA replication affects gene expression. Combined with live-cell imaging to determine the final cell-fate choice, we reveal that DNA replication is essential for the enforcement of the decision. Distinct DNA dosage effects on gene expression are revealed: *ci* expression, which allows phage commitment to the lysogenic pathway, increases drastically with viral DNA replication, while expression of its regulator, or transcription factor, *cII* is not sensitive to gene dosage changes. Mathematical modeling further shows that living organisms can utilize different network structures to allow different response to variations at the DNA level in order to regulate the decision-making outcomes. Notably, this work also supports that individual phage genomes have the agency to make decisions, but lack the means to realize them, thus highlighting the importance of interactions between intracellular entities, which is partially promoted by DNA replication.

Gene Deletion in Bacteriophage EagleEye Using Electroporated DNA and Exploration of this Application to *Streptomyces spp.*

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Because of the ubiquitous nature of Actinobacteria in soil, it is important to study the genes of bacteriophages that infect these bacteria. A protocol called BRED (Bacteriophage Recombineering with Electroporated DNA) was developed to efficiently delete or modify genes in phages that infect *Mycobacterium spp.* Using this protocol, we have explored deleting gene 84 from mycobacteriophage EagleEye. EagleEye infects *Mycobacterium smegmatis* which has become a rising genetic model due to its relatedness to the disease-causing *M. tuberculosis*. EagleEye has 97 potential genes in its genome with only 34 genes having known functions. Knocking out genes of unknown function in EagleEye will help us determine their importance to the survival of the phage as well as hint towards their unknown function. In addition, we have started to test the compatibility of this protocol with phages coming from the genus *Streptomyces*, another member of the phylum Actinobacteria. Because of the inherent differences between *Mycobacterium* and *Streptomyces* species, namely in their cell wall composition and plasmid vectors, the protocol likely needs to be modified and adjusted to work in *Streptomyces*. Preliminary results have shown that electroporation techniques involving the growth of *Streptomyces* into mycelium and the use of lysozyme have started to make this modified protocol seem more possible.

Dynamics of Filamentous Viral Nanoparticles in Semi-Dilute Polymer Solutions

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Lateral flow assays (LFAs) are the most popular format for point-of-care diagnostics. In an LFA, an analyte of interest binds to reporter particles during capillary flow through an antibody-functionalized membrane. The analyte-reporter complex is subsequently captured on the membrane. The number of captured complexes, determined by detecting the reporters, directly correlates with analyte concentration. In our recent studies, antibody-modified bacteriophage (phage) LFA reporters have shown increased LFA sensitivity compared to traditional gold nanoparticle reporters. Real clinical samples (blood, saliva, etc.), however, are complex solutions containing proteins, saccharides, and other macromolecules as well as cells that will affect phage transport through the membrane, and their effect on phage transport has not been studied. We initially studied phage particle dynamics in polymer solutions of hydrolyzed polyacrylamide of varying concentrations (0.031 to 3.1 g L⁻¹). Fluorescently-dyed M13 filamentous phage, anisotropic particles 6 – 7 nm in diameter and 900 nm in length, were imaged while diffusing in the polymer solutions using fluorescence microscopy, and their dynamics were analyzed using a particle-tracking algorithm. At high polymer concentrations, the diffusion coefficients of phage were greater than those of spherical nanoparticles of similar radius of gyration. The diffusivities of both phage and spherical particles, normalized by their diffusivities in water, were able to be collapsed onto a single curve as a function of a dimensionless length scale, the ratio of the particle radius to the polymer correlation length. The success of this collapse indicates that the smallest length scale (in this case, the radius) controls the diffusive dynamics of phage in these solutions. These results allow us to predict the diffusive behavior of phage in solutions from their size and dimensions. Going forward, we will explore the wide natural diversity of phage morphologies while assessing their transport and binding properties in flow through complex solutions. Our long-term goal is to increase our understanding of phage reporter flow and capture through membranes and to rationally identify better performing phage LFA reporters.

PBSX phagocin: an antimicrobial with adaptable specificity in a modular framework

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PBSX is a temperate bacteriophage mutant of *Bacillus subtilis* 168 incapable of infection while maintaining antimicrobial characteristics with unit efficiency. PBSX-like defective prophages, called phagocins, are induced in the SOS response of various *Bacillus* genera and function as antimicrobial agents capable of targeting species other than the host. Upon attachment, phagocins are thought to dissipate the proton motive force of their target bacterium causing cell death. Disease caused by dysbiosis of the human microbiome and antibiotic-resistant bacteria has promoted research for novel antimicrobials, such as bacteriophage, to address the problems facing antibiotic use. Here we present a PBSX system with modular specificity in the genetically tractable *B. subtilis* 168 as a docile approach to phage therapy. Our data show: *B. subtilis* 168 allows for easy manipulation of the host genome and is perfect for phage production while eliminating endotoxin contamination, G4S and A19V are required together to confer thermoinducibility to Xre previously thought to only require A19V, and tail fiber exchange is sufficient for swapping specificity of PBSX among the observed systems. Future studies will focus on leveraging this adaptable antimicrobial as a specific tool for the remodeling of the human microbiota, specifically targeting those microbes that dominate the immature microbiota (*Bifidobacterium longum*, *Lactobacillus mucosae*, *Streptococcus spp.* and *Clostridium spp.*).

***Campylobacter rectus*: Investigation & Importance of Secretion Systems**

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Abstract:

Campylobacter rectus is an oral anaerobic bacterium commonly found in individuals with periodontitis (gum disease), and associated with negative pregnancy outcomes including pre-term birth and low birth weight. Supportive of a role as an emerging pathogen, *C. rectus* has recently been linked to Barrett's esophagus, appendicitis, bacteremia, and abscesses (both oral & extra-oral). Thus, *C. rectus* virulence factors and their modes of delivery are of increasing interest and importance to understand. Bacterial secretion systems, like type III (T3SS), type IV (T4SS), and type VI (T6SS) are known for their diverse roles in many bacteria including, but not limited to, DNA transport/uptake, protein effector delivery, and host- or bacterial-specific toxin delivery.

Through genome sequencing, genes for T3SS/flagellar, T4SS, and T6SS were found in several *C. rectus* strains, indicating their potential importance to pathogenesis. Mutants were then generated via allelic replacement in ATCC 33238 (sequenced by JCVI) for potential virulence factors, including campylobacter invasion antigen B (CiaB), to investigate its role in *C. rectus* pathogenesis as well as its possible relationship with the *C. rectus* T3SS/flagellar secretion system (Δ *ciaB*). To assess the T4SS (Δ *virB9*) and for the T6SS (Δ *hcp*) mutants were generated. These mutants, along with available wild-type *C. rectus* strains were used to test several hypotheses including 1) CiaB's role in adherence and invasion of host cells (BeWo, HGF-1), 2) DNA uptake ability, i.e. natural transformation (T4SS), and 3) T6SS function via bacterial competition assays. Mutant validation and reference gene identification/standardization was performed to verify the absence of transcriptional polar effects for the deletion mutants and to provide experimental standardization. These results and continuing investigations aim to provide a better understanding of *C. rectus* pathogenesis.

Jennifer Tran

Single-stranded RNA bacteriophages are the smallest viruses known, with genomes around 4000 nucleotides long and 3 to 5 genes. Unlike the canonical double-stranded DNA phages, these viruses have single gene lysis systems, needing only one gene product to kill and lyse bacteria. These lysis genes are highly genetically diverse, with little to no homology from phage to phage, and could provide insight into novel antibiotic targets or new antibiotic mechanisms. There are four paradigm single gene lysis systems: *L* from MS2, *A₂* from Q β , *Lys^M* from M, and *E* from ssDNA phage ϕ X174. The mechanism of *L* remains unknown, but *A₂*, *Lys^M*, and *E* have been shown to target enzymes in the peptidoglycan biosynthesis pathway, causing host lysis. Whereas only 31 ssRNA phages were previously known – some without genomes readily available – hundreds of ssRNA phage genomes were recently isolated from environmental metadata in 2016. Because of varying genetic architectures and lack of sequence similarity, the lysis genes of these newly discovered phage genomes were not identified. We annotated potential lysis genes from these genomes, synthesized candidates, then tested them for function in *Escherichia coli*. From this, two novel lysis genes have been identified, and we are currently characterizing their lytic activity.

Title: Develop and validate a microtiter plate liquid method for testing host range of *Salmonella* bacteriophages and evaluate the antimicrobial capacity of phages against *Salmonella* on a cattle hide model

Authors: Yicheng Xie, Jason J. Gill

Objectives: 1) Develop and validate a microtiter plate liquid assay that could serve as an alternative lytic activity measurement that allows multiple phage-bacterial interactions to be monitored simultaneously to predict host range, virulence and bacterial resistance development in a high throughput format
2) Evaluation of individual and mixed bacteriophages for control of *Salmonella* on a Cattle Hide Model and determine the accuracy of the prediction via the microtiter plate liquid host range assay.

Methods: A panel of 15 lytic phages were tested in a 96-well microtiter plate liquid assay against a panel of 20 *Salmonella* strains with various serovars that has been utilized for host range experiment on traditional agar overlay method. A standardized bacterial inoculum of each *Salmonella* strain of the 20-*Salmonella*-panel were placed into broth medium in 96-well microtiter plates and challenged with concentrations at 10^6 and 10^8 PFU/ml of the panel of 15 lytic phages individually, and growth were observed every 30 minutes for 12 hours in the Tecan Spark® 10M plate reader. Cattle hides obtained from harvest were inoculated with a cattle feedlot isolated *Salmonella* strain and treated with single and mixed bacteriophages, and treated cattle hides were homogenized and plated to observe bacterial reduction.

Results: Host range of the panel of 15 phages obtained via microtiter plate assay is highly variable, ranking from infecting 85% (17/20) to 20% (4/20) of tested strains at phage concentration 10^8 PFU/ml. Dosage effect was found widely across the panel of tested phages.

Salmonella was significantly reduced by single and mixed phages treatments ($n=3$, $p<0.05$), with reductions up to $1.4\log_{10}$ CFU/cm². The prediction of virulence of phage via liquid host range method agreed with the capacity of phage to reduce *Salmonella* loads on cattle hide model.

DECISION MAKING OF BACTERIOPHAGE P1

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Upon infection by bacteriophage P1, a bacterium cell can make the decision for either entering the lytic pathway, where new viruses are produced inside the cell and released to the environment after cell lysis, or entering the lysogenic pathway, so that the virus DNA remains in a dormant state as a unit copy plasmid[1].

Consistent with the literature [2], our bulk experiments show that the lysogenization frequency of P1 against the average phage input (i.e. ratio of phage over cell concentration) follows a Poisson distribution of $N \geq 1$, and the lysogenization frequency can reach 30% under optimal conditions. In contrast, a cell-fate paradigmatic system, bacteriophage lambda, follows a Poisson distribution of $N \geq 2$ indicating the cell requires 2 or more phages to lysogenize a cell [3]. Recently, studies at higher resolution have yielded new insights about the lysis-lysogeny decision making in phage lambda, such as individual phage voting and concurrent development of the lytic and lysogenic pathways within the same cell [4-6]. The gene regulatory network for lysis-lysogeny decision of P1 is fundamentally different from lambda. Here, we ask how different regulatory networks contribute to different cell-fate decision patterns, and how stochasticity plays a role in the decision-making process.

In order to study post-infection decision of P1 at the single-cell/single-phage level, we firstly constructed the mosaic phages by providing fluorescently labeled major capsid protein, in trans. Phage DNA ejection and the establishment of lysogeny can be tracked by the expression of genome modified c1 and cell division. The choices of the lytic pathway are detected through a transcriptional reporter plasmid expressing mTurquoise2 from the late lytic promoter, LP23. Our aim is to create a spatiotemporal map linking the microscopic infection parameters—number and position of infecting phages, cell age and growth rate, etc.—with the eventual decision made.

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