NAI VIRUS FOCUS GROUP FIELD TRIP TO MONO AND MAMMOTH LAKES, CALIFORNIA
June 22-25, 2004

Baruch S. Blumberg and Kenneth Stedman
Co-chairs of the Group

07.07.04

The first Field Trip of NAVIFOG was conducted at Mono Lake and the Long Valley caldera region near Mammoth Lakes in eastern California, June 22 - 25, 2004. There were 16 participants of whom 2 were funded from sources other than NAI. (See Table). The participants from the East and West (other than CA) assembled in Reno, Nevada on 06.21.04 from whence they drove in convoy to Mammoth Lake, CA on 06.22.04 where we were joined by the California contingent. The party included 9 investigators (including the co-chairs), 5 graduate students/assistants, a photographer/videographer, and a logistic and travel support person.

THE WORKSHOP. 06.22.04

On arrival at the village of Mammoth Lakes a Workshop was held at the assembly room of the Shilo Inn. The program of the workshop and the abstracts are appended. The presentations, in general reflected the interests of the investigators and their students, and a discussion of their plans for the present Field Trip. Several of the investigators noted the small amount of data on the viruses of geothermal sites including those of high and low pH and various degrees of salinity.

Kepner plans to address the question of the role of the diverse viruses on carbon metabolism and the ecology of the geothermal sites. He collected large amounts of hot springs water sample, concentrated the material and made it available for others to use in their studies. The goal of Mead (and Rohwer) is to collect bacteriophage and the phages of archaea, as well as the bacteria and archaea in which they reside to construct metagenomic libraries for sequence analysis and expression studies. They will add this to previous collections from Little Hot Creek in the caldera region to understand the diversity of the phage and their gene expression.

Nadya Morales, a graduate student with Paul Turner at Yale (who did not attend the field trip), plans to study the microbial ecology to understand the community structure of extreme organisms and investigate the effect of the phages on the systems biology of the site. They are interested in the possible medical uses of phages to “attack” bacteria in circumstances where antibiotics are ineffective. This is an interesting medical technique used before antibiotics were available and is now being reconsidered at a time when some antibiotics are losing their effectiveness. It points to the possible role of extreme bacteria and phages as human, plant, or animal pathogens, a subject that has the focus group may consider in the future. They will use predator-prey models to understand the observed phenomenon.

Wegley and Rohwer will study phages as predators of bacteria and archaea under different conditions of desiccation, vacuum, temperature, and pH. They will perform metagenomic analyses in sections of Little Hot Creek from which they have sampled in the past to study possible changes in the composition of the population over time. Steward and Brum will study the distribution, heterogeneity and molecular characteristics of the free viruses of the sampled sites looking for novel morphologies and molecular characteristics. This is an underlying theme of the Field Trip, that is to discover, describe, and classify the many different kinds of viruses in these extreme environments of which very little is known.

Sullivan and his colleagues presented a more focused study involving the lateral transfer of photosynthetic genes to and from the cyanobacterium *Prochlorococcus* and the study of the role of the double-stranded DNA viruses, *Myroviridae* and *Podoviradae* that infect the cyanobacterium. Through genomic techniques they will investigate the role of the phages in the differentiation of their hosts.

Voigt's research is in the new area of synthetic biology, that is, the molecular construction of organisms that can perform therapeutic and other functions in humans and other species. For example they are developing a *Salmonella* strain as an intravenous cancer therapy that can target cancer cells to destroy them with the hope that this will eliminate or slow down the activity of the cancer. They now wish to study the use of viruses to modify the designs or, by themselves, to provide a therapeutic modality.

Young has had extensive experience studying the viruses in Yellowstone high temperature acidic environments. In collaboration with Stedman and Mead he proposes to sample related organisms in the neutral/basic geothermal sites on the field trip environment. He also is interested in synthetic biology and the formulation of crafted bacteria and other microorganisms that can be used for medical and other purposes. In particular they have developed techniques of using viral shells as "reaction vessels" for potential nano-medical devices and medications.

Hambly video-taped the field activities with the goal of creating a ten-minute documentary available for educational and other purposes. This film and additional footage will also provide documentary support to define the site collections for reference in future field trips in the immediate and distant future. The video will be placed on the NAVIFOG and NAI websites. Still photographs to further document the field sites and the participants will also be made available. These will be linked to the GPS location records for each of the collection sites.

OTHER MEETINGS.

All the participants met nightly, during dinner and afterward at the assembly rooms in the Motel. The work of the day, details of the research, plans for the subsequent collections and the general subject of astrobiology and "astrovirology" were discussed during these meetings. The significance of viruses as an early form of life, perhaps in the postulated RNA world that preceded the contemporary DNA world was a central theme of the discussions.

COLLECTION SITES 06.23-24.04

During the next two days the party was broken into two groups to facilitate virus collection at several sites on Mono Lake and in the Long Valley caldera. Each of the groups was given the opportunity to sample at each of the sites. Paoha Island in Mono Lake is reachable only by boat. Access to the island in summer is restricted. We were assisted in the sampling on the island by Robert S. Jellison of the nearby UC Sierra Nevada Aquatic Research Laboratory (SNARL), who very kindly ferried the parties to the island in the SNARL Boston Whaler. He devoted many hours to transport and guide the collection parties and we are very grateful to him for his help. The island, shimmering white in the nearly constant sun, has a strange and complex geology. It is the result of an up thrust about 200 years ago (dated from Paiute Indian observations) and includes sediment lake bottom that gives the island its white color, pumice from the volcanic eruptions, the early basaltic rock, and the characteristic *tufa* stacks of Mono Lake. They are formed when the calcium-containing waters of the tributaries that feed the lake, encounter the carbonate-rich lake water to precipitate out as calcium carbonate columns. The tufa columns were starkly revealed when the lake water level dropped after the early 1940s when the inflowing creeks were diverted to supply water for Los Angeles and southern California. The hot springs bubble up at the edges of the lake and immediately inland on the island. Multiple samples were taken at these sites. Their exact locations and descriptions will be given in the final reports.

Hot Springs Creek and Little Hot Springs Creek are located in very attractive settings south of Mono Lake. There are several alkalaine, neutral and acidic high temperature springs that are located in and adjacent to both creeks. On Hot Springs Creek, fording the stream allowed access to these sites. GPS locations will be provided in the final report.

Several members of the party attempted, unsuccessfully, to locate hot springs in a swamp near Dechambeau Ranch. Shortly after we departed the site a lightning strike emanating from a passing storm struck the ground about 100 meters from our location and started a rapidly spreading brush fire.

CONCLUSION.

The Field trip collections have been, in our view, successful. Many specimens were collected and several of these have already been processed and distributed to members of the field trip. Different groups will work on the same specimens thereby unifying the projects. We have asked the participants to submit a report on their previous and projected research by August 1, 2004. These will be reviewed by the co-chairs and sent to NAI Central later in the summer. We expect that the scientific reports will be published over the next few months (and years) and we will request that the authors acknowledge their funding by NAI.

We hope to have yearly field trips and plan to apply to NAI Central again for support. There were several discussions about possible future field sites. They should be sites of interest for environmental virology, accessible, and not costly to visit. Among the sites suggested were, Mt. Lassen CA, Iceland, Costa Rica, and St. Lucia, all with interesting geothermal sites where viruses have not been extensively studied or studied at all. We may also have an interim meeting in a venue that would not require extra travel (i.e., at an astrobiology meeting). On subsequent field trips we hope to include geologists, geochemists, paleontologists, and other disciplines as well as biologists.

We believe that NAVIFOG can be an important means to move forward the field of environmental virology in extreme locations. We discussed the possibility of formulating a program project grant application. Several of the investigators spoke of the biomedical relevance of their research; these aspects could be developed at a subsequent meeting and could be the basis for a grant application. We have attracted several outstanding workers in the field and, what may be more important, young scientists who are excited about the possibilities of this strange new domain that is so important to our understanding of how life began and if it exists elsewhere.

Participants

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16	Anselm Levskaya	

7/13/04
(Chris Voight, and Anselm Levskaya did not require NAI funding))

1. Portland State University. Co – chair,
2. Portland State University. Travel and logistics support
3. Fox Chase Cancer Center. Co – chair,
4. San Diego State U. .
5. San Diego State U. Rohwer Assistant/Grad Student
6. University of Hawaii
7. University of Hawaii. Steward Graduate Student
8. Montana State University.

9. Marist College.
10. Woods Hole - MIT
11. U. of British Columbia. Videographer and photographer
12. Lucigen, Inc. .
13. Yale Paul Turner grad student
14. Portland State U. Stedman grad student
15. U. C. San Francisco.
16. U. C. San Francisco. Voigt Grad student

Workshop Program:

NAI-ViFoG Mono-Mammoth Workshop Schedule:

1:00-1:30 Ken Stedman/Baruch Blumberg: Welcome/Introductions/Goals

1:30-2:00 Emma Hambly: Production of video archive and supporting website.

2:00-2:30 Bob Jellison: Mono Lake and Local Hot Springs

2:30-3:00 Ray Kepner: Effect of Mono Lake and Hot Springs Creek phage on bacterial community carbon metabolism.

3:00-3:30 David Mead :Great Balls of Fire: Bacteriophage and Microbial Diversity of Boiling Thermal Pools

3:30-4:00 Nadya Morales: Haloarchaea Viral Community Dynamics

4:00-4:30 Forest Rohwer: Phage Communities in Hot Springs

4:30-5:00 Grieg Steward: Morphological and biochemical characterization of viral communities in extreme environments.

5:00-5:30 Matt Sullivan: Lateral transfer of photosynthetic genes to and from Prochlorococcus viruses

5:30-6:00 Chris Voigt: Synthetic Biology

6:00-6:30 Mark Young: Searching for Crenarchaeal Viruses

ABSTRACTS:

Phage Communities in Hot Springs

Linda Wegley and Forest Rohwer San Diego State University

In extreme environments such as hot springs, phage are the only known microbial predators. We have preformed the first studies of prokaryotic and phage community dynamics in these environments. Phage were abundant in hot springs, reaching concentrations of several million viruses per milliliter. Hot spring phage particles were resistant to shifts to lower temperatures, possibly facilitating DNA transfer out of these extreme environments. The phage were actively produced, with a population turnover time of 1-2 days. Phage-mediated microbial mortality was significant, making phage lysis an important component of hot spring microbial food webs. Together, these results show that phage exert an important influence on microbial community structure and energy flow in extreme thermal environments. In addition to community dynamics, we are now measuring phage diversity in hot springs. To do this, DNA from complete hot spring phage communities has been isolate, cloned, and sequenced. These metagenomic analyses show the these phage are very novel and diverse[1].

At the NAI-Virus Focus Group (NAVIFOG) Workshop/Field Trip we will follow up on the above experiments in 3 ways.

1) Measure survival rates of phage exposed to desiccation and vacuum, as well as temperature and pH changes. For these studies, phage communities will be isolated from both extreme (Little Hot Creek, Mono Lake) and non-extreme environments (e.g., Mammoth Lake). This will provide insight into how well phage might survive the rigors of space travel.

2) Our earlier studies of hot spring phage community dynamics did not address the relative importance of temperate versus lytic behavior. Therefore, we propose to perform filtering and *in situ* incubation experiments to measure the relative importance of these two processes in hot springs (and possibly Mono Lake).

3) Gather DNA and RNA samples for metagenomic analyses. So far, our metagenomic analyses of hot spring phage communities only consists of one time point. Therefore, we will collect nucleic acid samples for comparison purposes.

We will bring tangential flow filtration units with us, as well as supplies and tools for sample collection and preservation.

[1]All of the metagenomic analyses has been done in collaboration with David Mead and Tom Schoenfeld of Lucigen Corporation (Madison).

Great Balls of Fire: Bacteriophage and Microbial Diversity of Boiling Thermal Pools

David Mead [1], Ronald Godiska[1], Phillip Brumm[1], Forest Rohwer [2], Thomas Schoenfeld[1]

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Abstract

Boiling thermal pools are unique ecosystems to study microbial ecology, biogeochemistry and evolution, because they are physically isolated from other ecosystems, they are relatively biologically simple, and they are among the most hostile environments known. The water column of boiling hot springs represents an extreme environment that has not been previously explored for viruses or microbes. Planktonic organisms found in the water column potentially originate from significantly higher temperatures and pressures than those found in surface sediments. For example, water temperatures as high as 238 C have been measured in shallow drill holes (< 330 m) in Yellowstone National Park. Bacteriophage and host cells emanating from this formidable environment have important implications for a number of disciplines.

Constructing genomic libraries from extreme environmental niches is challenging due to the low abundance of microbes and bacteriophage; for example, as little as 10,000 cells per ml are found in thermal aquifers, yielding only picogram amounts of DNA. Lucigen has developed methods to make complex gene libraries from anonymous DNA sequences, starting with less than a picogram of purified material. Improved sampling methods and our NanoClone, Single Cell Genomics and CloneSmart technologies have allowed construction of complex community genomic libraries from very limited samples of directly isolated microbial and viral DNA. Limited 16S rRNA sequencing of NanoClone libraries made from Bath hot spring has revealed at least 20 distinct Bacteria and Archaea, many without significant similarity to cultivated microbes. Sequence analysis of approximately 5,000 reads from a bacteriophage metagenomic library derived from Little Hot Creek also shows limited similarity to the NCBI database and a surprising degree of diversity. Comparative data analysis of phage DNA from four different thermal pools will provide a unique glimpse into the diversity and community makeup of a unique set of environments.

The goal of this field trip is to collect additional bacteriophage and microbial samples from new hot springs to construct metagenomic libraries for sequence analysis and expression studies. Analysis of the diversity of bacteriophage genes in different thermal pools from geographically similar and isolated sites will begin to answer important questions about the ecology of hot springs. We will use methods similar to those employed by Breitbart et al. (Genomic analysis of an uncultured marine viral community. Proceedings of the National Academy USA 99:14250-14255, 2002). D Mead will collect samples on this trip and they will be processed at Lucigen for phage and

microbial expression analysis (T Schoenfeld, R Godiska, P Brumm). Data analysis will be performed by F Rohwer.

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ABSTRACT
Morphological and biochemical characterization of viral communities in extreme environments.

Viruses are the most diverse and numerous microbes in aquatic systems, but their ecology in extreme environments remains poorly studied compared to oceans and freshwater lakes. Extreme environments are likely to harbor many novel viruses. Although differences in viral assemblages among diverse environments is proximally controlled by the host presence and diversity, lytic viruses must also be adapted to survive as free virions in the chemical and physical conditions of their particular habitat. Comparisons among diverse environments may thus reveal themes in the evolution, adaptation, and diversity of viruses.

We propose to obtain an overview of the characteristics of the free virions within hot springs and /or Mono Lake to seek novel morphologies and relate these to physical properties and biochemical composition. Viruses would be harvested by filtration and ultrafiltration in the field. In the lab the viruses in the concentrate would be subjected to 2-dimensional physical fractionation to determine the range of biophysical and biochemical properties present in the assemblages. The viral concentrates will be fractionated in a CsCl equilibrium density gradient. Each CsCl fraction will then be separated in a second dimension by ion exchange chromatography. Fractions will be examined by transmission electron microscopy to document morphologies and analyzed to determine the associated nucleic acid size and type (RNA vs DNA). Given sufficient starting material, additional analyses could also be performed on the archived viral fractions (biochemical composition, genome sequence, proteome analysis).

Searching for Crenarchaeal Viruses

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Other Scientists involved in project: Ken Stedman (PSU), David Mead (Lucigen, Madison WI), and Frank Roberto (DOE-INEEL).

Hypothesis & Goals: We hypothesize that viruses related to novel viruses replication in Crenarchacta hosts in found in Yellowstone's high temperature thermal environments will also be present in the high temperature environments located in the Mammoth Lakes region of eastern California. A comparative analysis of such viruses, we hope, will provide insights into the diversity and evolutionary history of these unusual viruses. We are also interested to determine if related viruses are also present in more neutral/basic represented in the Mammoth Lakes region. The goal of this trip will be to collect appropriate samples for isolation of viruses.

Techniques to be used: Both aerobic and anaerobic field sample from water and soil samples > 80C will be collected from each sampling site. Approximately 50 ml or 50 g samples will be collected from each site. Back in the laboratory, total DNA will extracted from each sample which will be used as templates for PCR-based detection of SSV-, SIRV-, SIFV-, STIV-, and AFV-like viruses. In addition, we will attempt to establish both aerobic and anaerobic enrichment cultures from all field samples. These cultures will be directly screen for virus like particles by transmission electron microscopy. All materials and results will be available to all interested parties.

TITLE: Effect of Mono Lake and Hot Springs Creek phage on bacterial community carbon metabolism.

NAME: Ray Kepner, Marist College

Investigators have demonstrated that Mono Lake contains an abundant & molecularly diverse viral assemblage (*e.g.*, PFGE work of Jiang, Steward *et al.*), while others (Stedman *et al*.) have worked with viruses of thermophilic *Sulfolobus spp.* microbes more typical of acidic hydrothermal systems. Little is known regarding how either lytic, lysogenic, or other types of phage influence ecosystem function in either Mono Lake or nearby thermal waters of the Long Valley caldera. Perhaps phage play a demonstrably important role in regulating the physiological capabilities of the community as a whole.

I propose a simple, preliminary study designed to assess the relative impact of viruses on carbon metabolism by both aerobic Mono Lake bacteria and by other aerobic thermophilic bacteria (*e.g.*, those from Hot Springs Creek). "Natural" bacterial assemblage carbon-source utilization patterns will be compared to those of induced (mitomycin-C) and virus-spiked bacterial assemblages. It is hypothesized that bacterial community diversity, with respect to sources of utilizable carbon, will be reduced upon exposure to both a) higher concentrations of potentially-lytic phage occurring naturally in the water, and b) excised prophage induced by the mito-C treatment.

The proposed fieldwork would involve:

1. Collection of small-volume (1.0 L) water samples with standard membrane filtration to remove most eukaryotic organisms
2. Field concentration and storage of the viral-sized fraction from large-volume (10-100 L) water samples by tangential-flow filtration (30 kDa)
3. Return of concentrates (*i.e.*, retentates) and water samples to Marist College

Back at the lab we would:

4. Enumerate viruses (as virus-like particles) in both small-volume and retentate samples by epifluorescence microscopy following SYBR-Gold staining
5. Induce prophage in small-volume subsamples by addition of 1 g mL^{-1} mito-C
6. Inoculate 96-well BiOLOG EcoPlates™ with: a) untreated "natural" water samples, b) mito-C induced water samples, and c) water samples spiked with viral concentrate (*i.e,*. TFF retentate) to increase viral density by 100
7. Incubate inoculated plates at *in situ* temperatures and analyze plates in a microplate reader (590 nm) at defined time intervals
8. Statistical analysis of data would be by PCA and specific changes in utilizable carbon-sources, should they occur, would be noted.

Viral concentrate and other samples, and data collected as part of this exercise will be available to all other NAVIFOG Workshop/Field Trip participants. Results from the proposed work will add to our knowledge regarding the ecological importance of viruses. Wonderful advances have been made in understanding the diversity of phage genotypes. This work would add a tiny bit of "What are they doing?" onto to our increasing knowledge of "Who's there?" with respect to viruses in extreme environments.

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An important goal in microbial ecology is to understand community structure, and the ways in which community dynamics and diversity can differ through time and in space. Environmental heterogeneity should play a key role, due to its proposed influence on the evolution of niche breadth, on the quantity of genetic variance within populations and on patterns of species diversity across landscapes. Although the archaea comprise one of the three major domains of life, relatively little is known of their natural community structure or their phages. In the proposed work, we predict that temporal and spatial changes in the salinity concentrations of solar salterns will strongly impact the profile of resident haloarchaea communities and that variation in haloarchaeal diversity will simultaneously affect halophage diversity due to differing host availability. The pressing need for antibiotic alternatives is great and many advocate a return to studying and developing phage use as therapeutic and prophylactic treatment. Efforts to model the population dynamics of host-phage interactions using computer simulations are worthwhile. But these studies cannot capture the complexities of the population and evolutionary dynamics of host/phage interactions in controlled laboratory environments, much less those occurring in heterogeneous habitats such as within infected hosts. This research proposes to study how environmental heterogeneity affect host-phage dynamics using haloarcahea and halophages as a model.

Abstract of application to participate in NAI-Virus Focus Group (NAVIFOG) Workshop/Field Trip, Mammoth Lakes, California, 22-24 June 2004.

Production of video archive and supporting website.
Emma Hambly

The primary aim of this project is to document the work of the NAI-Virus Focus Group and the workshop/ field trip to Mammoth Lakes as a short (ca. 10 minute) film. The film will be available to be deposited on the NASA Astrobiology Institute website video archive and to all workshop attendees for use in their educational activities. Further, it is hoped that the film, or another edit of the same material, will be commercially transmitted on television. The film will primarily be aimed at a mid to high level undergraduate audience and the broader scientific community, although it is hoped that it will also be comprehensible to interested members of the general public and high school students.

More specifically the film will comprise an introduction to astrovirology through an overview of the work of the members of the NAI–Virus Focus Group. This will include consideration of the scientific questions posed by the NASA Astrobiology Institute and the individual investigators. It will include the aims of studying viruses in extreme environments, the potential roles of viruses in the origin and evolution of life on earth, and discussions of what it is currently possible to address directly, and some of the experimental limitations of these studies.

Interviews with individual investigators will be recorded through the course of the workshop, to fit into a planned film outline. Specific questions that are being addressed will be distributed to participants shortly before the workshop in order to allow some time for their consideration.

The workshop and field trip footage, and possibly some laboratory scenes, will be interlaced within this framework in order to show the reality of sampling and experimentation in this environment. This portion of the film will be relatively unplanned, in the hope of maintaining some of the spontaneity of fieldwork in the final film.

In support of the film still photographs and information assimilated from the NAI-Virus Focus Group members, not only the field trip participants, will be deposited on a website (it would seem appropriate that this would be the NAI-Virus Focus Group website). The film and supporting media will be designed to encourage education and understanding of current issues in astrovirology-related virus research by the public and the wider scientific community, and to enhance interdisciplinary knowledge within the field.

The film and website will be available to go on-line three months after completion of the workshop / field trip. The copyrights of all still photographs, video footage and film imagery, and website design work collected and undertaken in this process will remain the property of Emma Hambly.

M.B. Sullivan[1], D. Lindell[2], Z. Johnson[2], J.A. Lee[2], A. Tolonen[1], F. Rohwer[3], S.W. Chisholm[2]

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Lateral transfer of photosynthetic genes to and from *Prochlorococcus* viruses

Here we report the presence of genes central to oxygenic photosynthesis in three representatives of two families of double-stranded DNA viruses (*Myoviridae*, *Podoviridae*) known to infect the oceanic cyanobacterium, *Prochlorococcus*. All three viruses (phage) contain genes which encode a core reaction center protein (D1) and a high-light inducible protein (HLIP) that are associated with the photosynthetic membrane. In addition, each myovirus encodes further proteins including a second core reaction center protein (D2) in one and two photosynthetic electron transport proteins (plastocyanin and ferredoxin) in the other. All of the genes encoding these proteins are full-length, conserved and clustered in each genome suggesting a functional role that affords an adaptive advantage during phage infection. We hypothesize that these genes allow host photosynthesis to continue until lysis. Molecular phylogenies of phage-encoded D1, D2 and HLIP protein sequences suggests that these genes are of common ancestry to those from *Prochlorococcus* and were horizontally transferred between host and phage. Interestingly, phylogenetic inference suggests the D1 and D2 proteins were transferred multiple times from host to phage, while phylogenetic and clustering analyses with the highly divergent HLIP multigene family suggests that these genes have been acquired multiple times from their host cells, but after a period of evolution in the phage have been laterally transferred back from phage to host. In addition to the proposed functional role for these phage-encoded functional genes, we hypothesize that through lateral gene transfer these *Prochlorococcus* phage are also driving the niche differentiation of their hosts.

Voigt Abstract

Therapeutic bacteria have the potential to treat a wide range of disease. Their intrinsic ability to preferentially target and replicate within tumor masses has been exploited to develop a *Salmonella* strain as an intravenous anti-cancer therapy, which is currently in phase I clinical trials (Toso *et al*., *J. Clin. Oncology*, 2002). Existing bacterial therapeutics rely on using a pathogenic strain where the virulence is attenuated via gene knockouts. In this talk, I will describe our efforts to synthetically design a non-pathogenic strain of *E. coli* to specifically target and destroy cancerous cells. This requires the engineering of a bacterial touch sensor that can distinguish between healthy and cancerous cells, the introduction of virulence systems such as the Type III secretion apparatus, and the introduction of robust regulation to wire it all together. To facilitate this process, we are also developing computational models of the regulatory network controlling *Salmonella* pathogenesis. In addition, I will describe bioinformatic tools to predict which circuits and components out of the growing database of sequenced bacterial genomes are likely to function robustly when transferred into a new host background. These techniques will broadly aid the forward design of bacteria for applications in synthetic biology.

A natural extension of this work is to study viruses, both as the primary vector of the therapeutic as well as a means to identify novel circuits and components that will function robustly in a bacterial host background. In particular, bacteriophage often contain circuitry that can be introduced and programmed in a broad range of hosts. They can also be vectors promoting the hyperevolution of genes that produce a benefit to the bacteria and may be involved in gene transfer between different trees of life. For example, Salmonella contains many lysogenic phage that encode secreted virulence factors, including a modified human gene that perturbs a signaling relay involved in apoptosis.

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