

Abstract

Bacteriophage therapy is a niche antimicrobial technique that has recently gained significant interest due to the emergence and rapid spread of multidrug-resistant bacterial pathogens. Unlike many chemical antibiotics, which can be active against a broad spectrum of bacterial targets, phages often need to be assembled into polyvalent cocktails consisting of ten or more unique phage isolates to achieve a level of breadth that is consistent with other modern antimicrobials. Although previous studies have documented several cases of synergistic and antagonistic interactions between competing phage strains, little is known about how prevalent these interactions are, or about how significantly they influence the efficacy of therapeutic cocktails. Here, I undertake a systematic *in vitro* characterization of pairwise interactions among a set of naturally-isolated phages using high-throughput combinatorial growth profiling. Notably, these experiments reveal a diverse array of synergistic and antagonistic phage interactions, suggesting that a rational cocktail design framework that optimizes these interaction networks (“iNets”) could improve the antimicrobial efficacy of phage cocktails. Using a simple regression model incorporating the results of pairwise phage screening experiments, I thus identify higher-order cocktails that minimize mutual antagonism and maximize synergy. This work demonstrates that phage interactions are important mediators of phage cocktail efficacy and illustrates the power of interaction-centric optimization platforms in enhancing the efficacy of polyvalent phage preparations.

Introduction

The emergence and rapid spread of multidrug-resistant bacterial pathogens suggests that a “post-antibiotic era” is approaching (CDC, 2013; Kåhrström, 2013). Antibiotic resistant infections account for roughly 23,000 deaths per year in the United States and 25,000 in Europe (CDC, 2013; Group, 2009), and the direct economic burden of these infections is estimated to exceed \$55 billion in the U.S. alone (Smith & Coast, 2013). In addition, industrial investment in antibiotic research and development has declined steadily due to diminished corporate returns resulting from antimicrobial resistance (AMR)-related government restrictions (Power, 2006) on top of rising costs due to a dearth of novel drug targets (Butler, Blaskovich, & Cooper, 2013; Power, 2006; Projan, 2003). Efforts to curb the spread of resistance have primarily been concentrated in the discovery of new antibiotic classes as well as public awareness campaigns promoting the responsible use of antibiotic drugs (CDC, 2013). However, despite these efforts, AMR is predicted to worsen in the coming decades, perhaps even to surpass cancer as a leading cause of mortality by 2050 (O’Neil, 2014).

Motivated in part by the difficulty to discover new antibiotic classes, there has recently been a revival of interest in alternatives to traditional chemical antibiotics. Biomolecular approaches (biologics)—most notably, antimicrobial peptides and bacteriophages (bactericidal viruses)—are of particular interest due to their natural abundance and diversity (Lakshmaiah Narayana & Chen, 2015). In addition, screening for biologics is often higher throughput than antibiotic discovery, making isolation procedures much more economical (Lakshmaiah Narayana & Chen, 2015; Loc-Carrillo & Abedon, 2011). A recent spike in novel classes of antimicrobial biologics (Bishop et al., 2017; Tucker et al., 2017) suggests that there remains a high degree of untapped potential for future development in this area.

One of the most promising of the antimicrobial biologics is the use of bactericidal viruses—bacteriophages—as antimicrobial products. Phage therapy offers a number of potential advantages over traditional antibiotic therapy.

- Phages are remarkably abundant and diverse in the immediate environment, meaning that phage isolation is often a trivial procedure. It is thought that all living bacteria are affected by phage predation in the environment (Suttle, 2005).
- Clinical phage preparations exhibit “auto-dosing”, a phenomenon in which therapeutic doses *increase* throughout the infection cycle due to natural phage replication (Stephen & Cameron, 2010). Phages’ ability to replicate improves the pharmacodynamic properties of phage-based antimicrobials.
- Phage resistance is considered significantly more manageable than antibiotic resistance. This is because phages “coevolve” with their hosts to overcome resistant phenotypes, so ineffectual phages (i.e. those that are no longer active in the face of resistance) can be easily “reactivated” via simple laboratory evolution experiments (Betts, Vasse, Kaltz, & Hochberg, 2013; Torres-Barcelo & Hochberg, 2016).
- Finally, phages exhibit remarkably narrow target specificity, often evolved to infect only a handful of bacterial strains (Hyman & Abedon, 2010; Skurnik, Pajunen, & Kiljunen, 2007). In contrast to broad-spectrum antibiotics that target a wide range of species—often including favorable human microsybionts—phages are capable of waging pinpoint attacks on pathogenic bacteria while leaving mutualists unaffected. As a result, phage therapy consistently yields fewer side effects than antibiotic therapy (Carlton, 1999).

But the high target specificity of therapeutic phages is, from a clinical standpoint, both a positive and a negative. On one hand, it confers an ability to eliminate pathogens without harming mutualists; however, it also limits the spectrum of pathogenic hosts that any particular phage can conceivably treat. For this reason, phage-based antimicrobial preparations have classically taken the form of polyvalent phage cocktails containing between two and 50 or more distinct phage isolates (Chan, Abedon, & Loc-Carrillo, 2013). Well-designed cocktails have broader host ranges than monovalent phage preparations because cocktails complement the host ranges of all phages contained within. Although cocktails are more likely to harm

favorable human microflora (in a similar manner as broad-spectrum antibiotics), the broadened collective host range of cocktails is often preferable in clinical settings because it eliminates the need for strain-level knowledge about each infection. Whereas single-phage preparations need to be personalized for each patient, cocktails are much more generalizable.

The process of designing and constructing phage cocktails has classically assumed a standardized approach, passed down from early pioneers in microbiology (Chan et al., 2013; Kutateladze & Adamia, 2008). Starting from panels of highly virulent phage candidates, the set of phage candidates chosen for use in a cocktail is that which maximizes their collective host range. Newer cocktail design strategies enhance cocktails by slowing the arrival of resistant mutants (Gu et al., 2012). Notably, little effort has been devoted

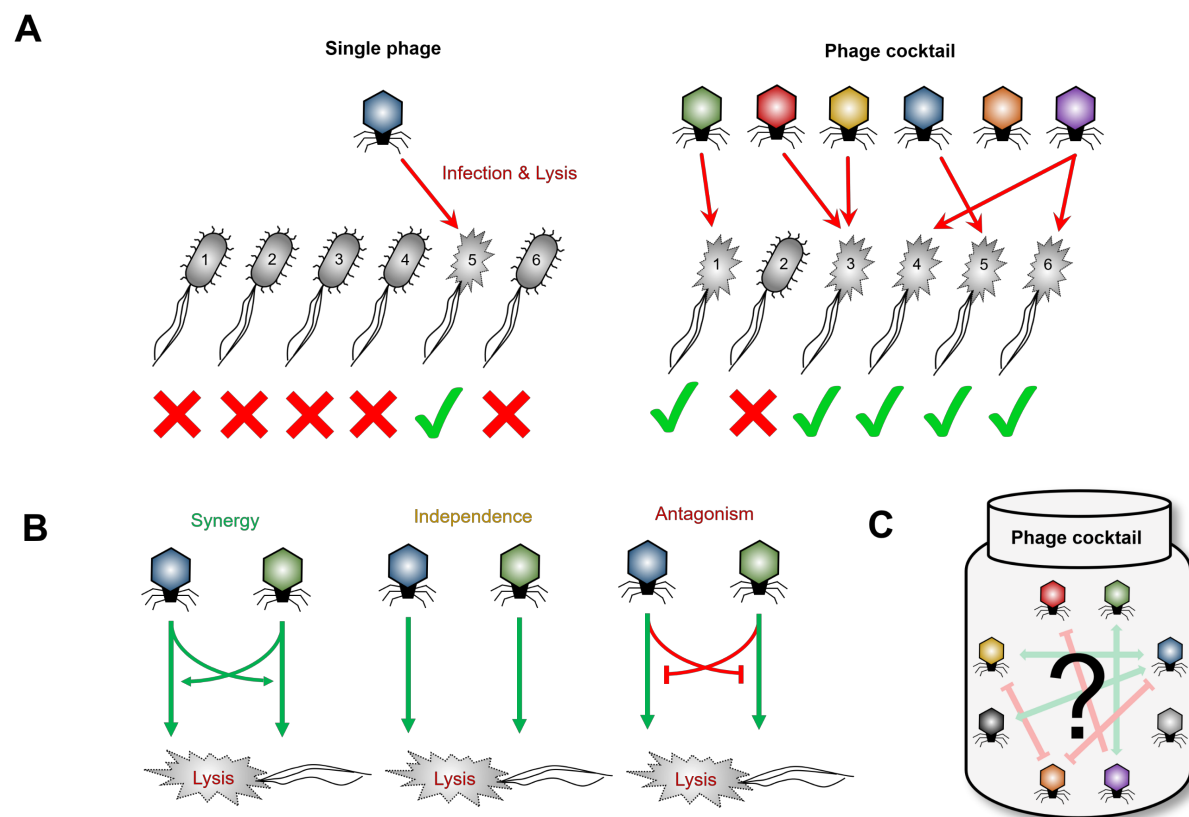


Fig. 1. Bacteriophage cocktails. (A) Cocktails overcome the narrow host specificity of bacteriophages and are thus more generalizable antimicrobial preparations. (B) Competing phages within a cocktail will assume any of three states: synergy, independence, or antagonism. (C) I hypothesize that complex networks of synergy, independence, and antagonism exist within phage cocktails, and that these interactions could influence the overall antimicrobial efficacy of a cocktail.

to rationally designing cocktails on the basis of collective therapeutic efficacy or potency (beyond starting with maximally virulent phage candidates), especially through the lens of phage interactions.

Molecular-scale interactions between coexisting phages are rarely considered whilst designing phage cocktails, because such interactions have historically been considered rare and/or negligible. However, there exist a number of reported examples of nontrivial phage interactions (Ghisotti, Zangrossi, & Sironi, 1983; Hattman & Hofschneider, 1967; Johnson, Widner, Xin, & Feiss, 1991; Lindahl, Sironi, Bialy, & Calendar, 1970). Despite these examples, little effort has been devoted to better understanding how prevalent phage interactions are, as well as if they appreciably influence the therapeutic efficacy of phage cocktails. In this study, I delved to address this shortcoming by asking whether phages from natural or commercial sources influence antimicrobial efficacy when combined *in vitro*. To do this, I first assembled a novel phage panel against a clinically-relevant pathogen (*Burkholderia cepacia*) by isolating phages from the environment. I then utilized an automated high-throughput screening platform to culture *B. cepacia* in the presence of two phages at a time (in a pairwise fashion) and compared the cell inhibition of the combination treatments to those of the corresponding single-phage assays. These data then enabled direct inferences of synergy and antagonism for all phage pairs in my library. I hypothesized that this experiment would reveal a complex network of phage-to-phage interactions (iNet) which significantly influences antimicrobial efficacy.

I next suggested that optimizing iNets—i.e. selectively removing antagonistic interactions and retaining synergistic interactions—could be a high-potential avenue for improving the antimicrobial efficacy of phage cocktails. Because my experimental platform was limited to pairwise data (in other words, it would become impossible to exhaustively test all n -membered cocktails), I first sought a quantitative method to predict the antimicrobial efficacy of n -membered cocktails using knowledge of two-membered cocktails. Borrowing from previous studies on combination therapy optimization (Wang et al., 2015; Wood, Nishida, Sontag, & Cluzel, 2012; Zimmer, Katzir, Dekel, Mayo, & Alon, 2016), I implemented a regression algorithm which I hypothesized would be able to effectively predict the cell inhibition of any cocktail

assembled using my *B. cepacia* phage panel. I further hypothesized that this algorithm would allow me to identify improved cocktails with optimized iNets, laying the foundation for a novel interaction-based optimization strategy for bacteriophage cocktails.

Results

A novel phage library against *B. cepacia*

To better understand the interactions that exist between phages, I first delved to isolate a novel library of phages against the multidrug-resistant pathogen *Burkholderia cepacia*. After undergoing phage enrichments using sewage water samples taken from three wastewater treatment plants in the Durham/Chapel Hill area (see Methods for in-depth isolation procedure), I isolated a total of 15 novel phages with diverse characteristics (Table S1). Host range analyses revealed that these phages exhibited varying host generality and were able to successfully form plaques on confluent lawns of anywhere between one and fourteen *B. cepacia* strains each (of 22 originally tested). These phages formed primarily non-haloed pinhole plaques on bacterial lawns, suggesting an abundance of large, non-LPS-degrading phages. In addition, four of the fifteen phages formed turbid plaques on all *B. cepacia* hosts, indicating possible abortive infection by the bacteria or lysis from without (Abedon, 2011). Finally, timecourse infection experiments revealed that all fifteen phages in this library were able to inhibit the growth of *B. cepacia* in liquid culture (Fig. 2), indicating that these phages represent fifteen novel antimicrobial agents against multidrug-resistant *B. cepacia*.

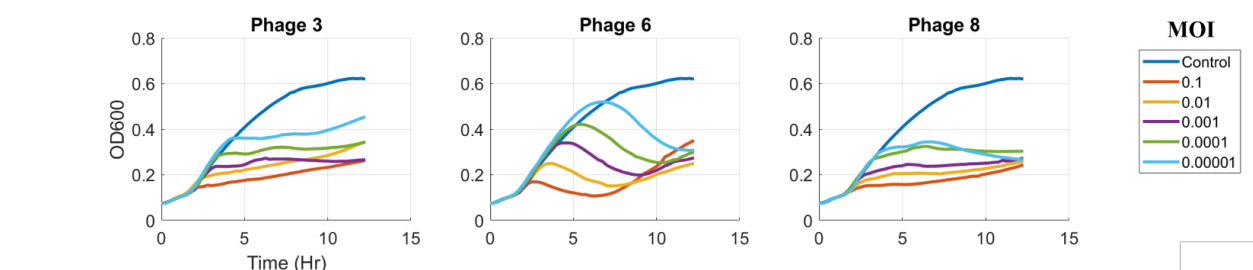


Fig 2. Representative lysis profiles for novel *B. cepacia* phages isolated during this project. All fifteen novel phages can effectively inhibit *B. cepacia* in liquid culture.

An automated, high-throughput screening platform for studying phage interactions

In order to more efficiently quantify phage interactions, I designed and implemented a high-throughput screening platform capable of testing more than 12,000 cocktail candidates in a single run. This level of throughput is necessary for combinatorial screens, because the number of experiments necessary to exhaustively test the interactions in a library of drug candidates increases exponentially with the number of drugs in the library. Due to the large number of necessary experiments, this platform emphasized a “hands-off” design which integrated a Matlab-based protocol generator, an automated liquid handler, a plate-transferring system, a photometric plate reader, and a battery of software tools to assist in analysis (Fig. S2).

Phages exhibit complex networks of synergy and antagonism *in vitro*.

I hypothesized that networks of phage interactions within cocktails influence antimicrobial efficacy. To test this, I began by assessing between-phage interactions within my newly-isolated *B. cepacia* phage library. I leveraged my automated screening platform to undertake a series of pairwise culturing experiments, in which a *B. cepacia* strain was exposed to two distinct phages at a time in a pairwise fashion (Fig. 3A). The resulting lysis curves were documented and compared to similar curves produced by single-phage infection assays, and synergy/antagonism was captured in a metric called the combination index (CI). CI values above 1 represent synergy and CI values below 1 represent antagonism.

My pairwise phage infection experiment revealed a surprising amount of diversity in pairwise interactions within my library of *B. cepacia* phages (Fig. 3B). Although many interactions clustered around a CI of 1 (representing independence), indicating that these phages did not significantly interact *in vitro*, most phage pairs veered into the realms of either synergy or antagonism. This result suggests that interaction-blind cocktails—such has been the *status quo* in the field of phage therapy for more than 100 years—are likely

to be non-optimal, because antagonistic interactions that limit the efficacy of the cocktail are likely to exist within them.

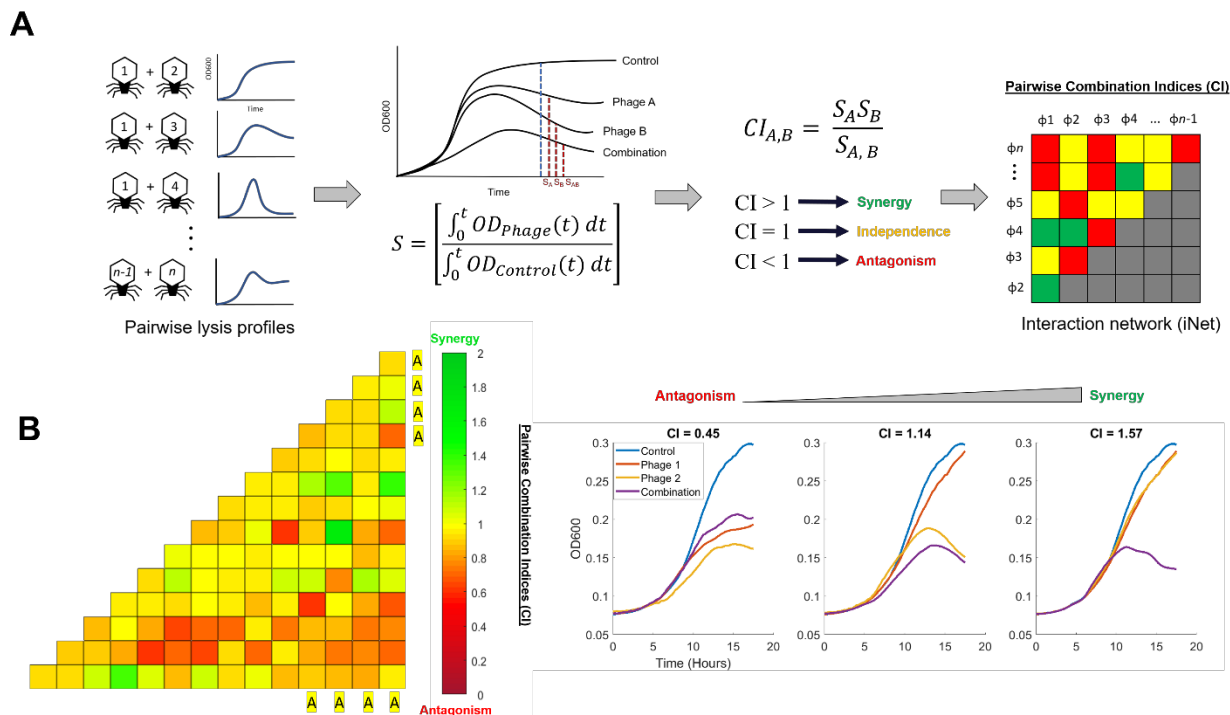


Fig. 3. Characterizing bacteriophage interaction networks (iNets). (A) To quantify phage interactions, I utilize pairwise *in vitro* infection assays. Two phages at a time are allowed to infect each host strain overnight, and OD600 measurements are obtained using a photometric plate reader. These data can then be used to derive a combination index (CI), a quantitative metric capturing the strength and direction (i.e. synergy vs. antagonism) of an interaction between two phages. Calculation of CI relies on cell survival (S ; a metric for phage efficacy), which involves an integration of OD curves over an entire timecourse to control for heterogenous lysis profiles. Pairwise CI values can then be tabulated to form a graphical representation of an interaction network (iNet). (B) Using a panel containing novel *B. cepacia* phages as well as four antibiotics of varying drug targets (marked with “A”), these experiments reveal a surprising diversity of interactions. This result suggests that an iNet optimization platform could improve the efficacy of polyvalent phage cocktails. (Bottom-right) Illustrative examples of timecourse experiments that allow for inferences of synergy and antagonism.

Interaction networks (iNets) can be optimized to improve phage cocktail efficacy

I next delved to develop a framework with which to leverage interaction networks (iNets) to design more effective phage cocktails (Fig 4A). Because exhaustive screening of all possible higher-order cocktails would be experimentally intractable, I first sought to validate a method to infer the effects of higher-order cocktails using pairwise interaction data. Using a regression model demonstrated previously, I first simulated the antimicrobial efficacies of all possible higher-order cocktails containing phages in my *B. cepacia* phage library. Sorting the outputs and plotting in barplot format (Fig. 4B), we can see that cocktails

are predicted to exhibit dramatic differences in antimicrobial efficacy based on iNet characteristics. Plotting the predicted efficacy of the full, naïve cocktail (i.e. the cocktail containing all phages and antibiotics screened in Fig. 3B), I found that this cocktail is far from the most effective; in fact, it is ranked 14,729 out of 16,000 in my simulation. This suggests that there is significant room for optimization based on phage interactions. I next sought to validate my quantitative platform experimentally by selecting six cocktails and testing their efficacies (Fig. 4C). Although the algorithm in its current form is a non-perfect estimator for higher-order cocktail efficacy, I did achieve a positive correlation between predicted and actual efficacy, implying that this computational method could be useful for identifying the most effective cocktail candidates.

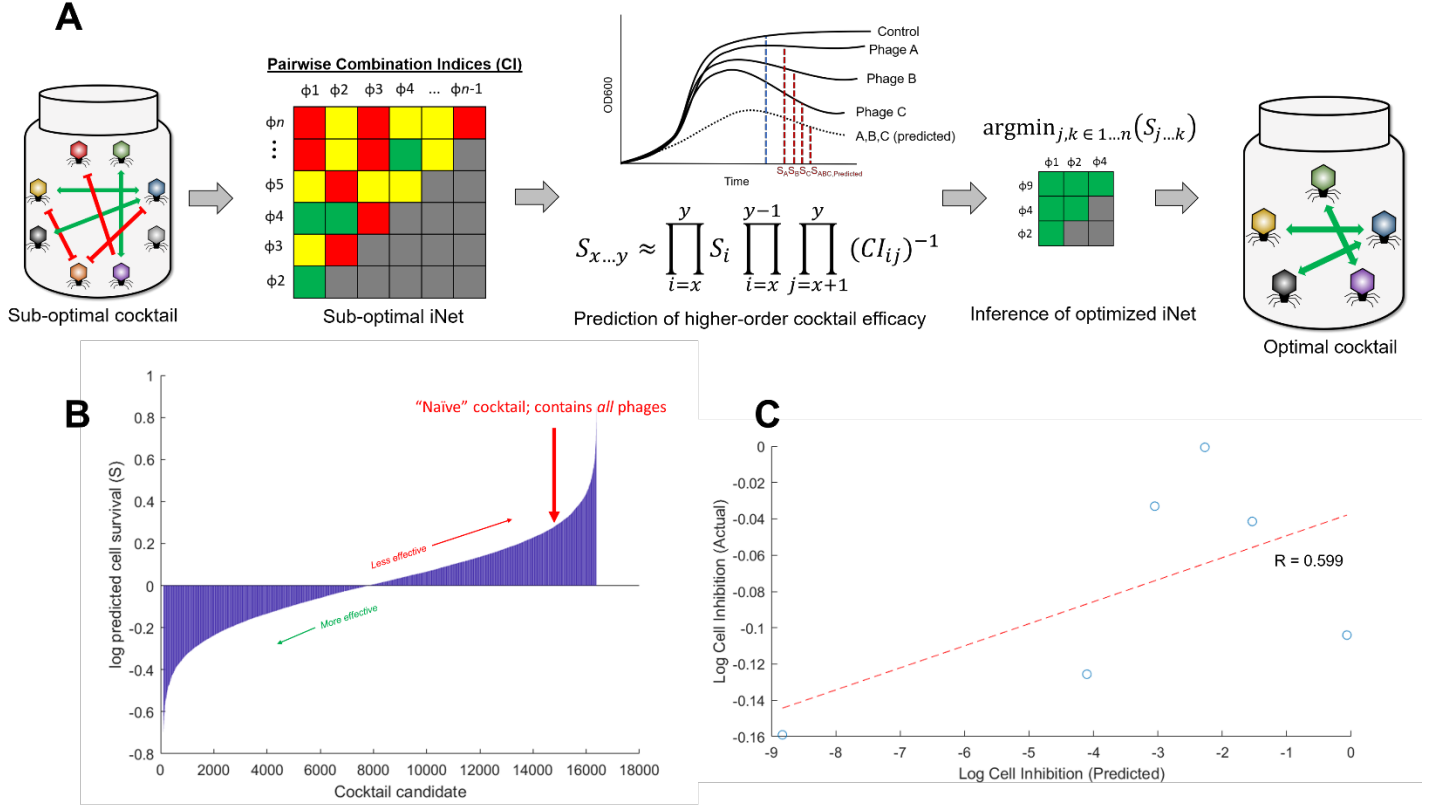


Fig 4. iNet optimization. iNets can be optimized to increase the potency of polyvalent bacteriophage cocktails, as well as antibiotic combination therapies, over randomly-generated cocktails. (A) Schematic of proposed iNet optimization procedure for developing personalized bacteriophage cocktails with maximized net synergy. A non-optimal phage cocktail first undergoes a pairwise synergy screen (as in figure 3) to produce an iNet. Combination indices in this iNet are then used to predict the efficacy of all possible higher-order cocktail configurations. Finally, the higher-order candidate that maximizes cocktail efficacy is chosen. (B) Predicted antimicrobial efficacies of higher-order cocktails were generated using the regression model. Sixteen thousand cocktails were randomly selected from the pool of all possible cocktails ($n=8,388,607$) and the predicted S values were plotted. Note that the fully-comprehensive cocktail (before optimization) falls far to the right (rank 14,729), suggesting that there is much room for improvement. Six cocktails were then selected from this distribution and screened experimentally (C) to validate the predictive power of the regression model. The positive correlation suggests that iNets can be optimized to improve cocktail efficacy.

Discussion

Previous phage cocktail development procedures have not taken phage interactions into account because such interactions were assumed to be rare and/or negligible. This study demonstrates that networks of interactions among competing phages in polyvalent cocktails are significant mediators of antimicrobial efficacy and should be considered whilst designing such cocktails. The discovery of abundant antagonistic interactions among wild phages suggests that previous phage cocktails, which were largely designed to extend target specificity, may contain incompatible phages which limit their overall antimicrobial efficacy. My work thus suggests that large, interaction-blind phage cocktails—which have constituted the *status quo* of clinical phage therapy for a century—are likely to be non-optimal and should be revisited.

In addition, my iNet optimization procedure represents a novel pipeline with which to optimize combination therapies involving phages. Like previous optimization platforms (Wood et al., 2012; Zimmer et al., 2016), my approach overcomes the scalability problem associated with combinatorial screening because it collapses the antimicrobial efficacy of complex cocktails down to a set of pairwise interactions, which are significantly more tractable when screening in the laboratory. In addition, my optimization approach is blind to the mechanism of action of the antimicrobial mixture being tested, allowing for the development of cocktails containing diverse phages of varying modes of infection. This perk also suggests that it could be used to optimize combination therapies involving other drug classes (i.e. non-phages), or even mixtures between different drug classes. For example, this method could leverage phage-antibiotic synergy (PAS)—a phenomenon which has previously only been identified on a case-by-case basis (Comeau, Tétart, Trojet, Prère, & Krisch, 2007; Kamal & Dennis, 2015)—to develop highly-synergistic combination therapies involving both phages and small-molecule antibiotics. This could aid in the incorporation of phage therapy into the current antimicrobial industry in the U.S., which has historically been dominated by antibiotics.

Finally, drug cocktail optimization approaches like the one presented here could herald a new general approach to antimicrobial therapy—one which incorporates information about each patient’s individual pathogen strain. Because phages likely interact in a host-specific manner (i.e. synergistic phages in one host background could antagonize one another in a different host background, and vice versa), iNet optimization is largely pathogen-specific, and thus patient-specific. This method therefore underscores a new basis for personalized medicine, a concept which has gained significant traction over the past several decades (Jameson & Longo, 2015).

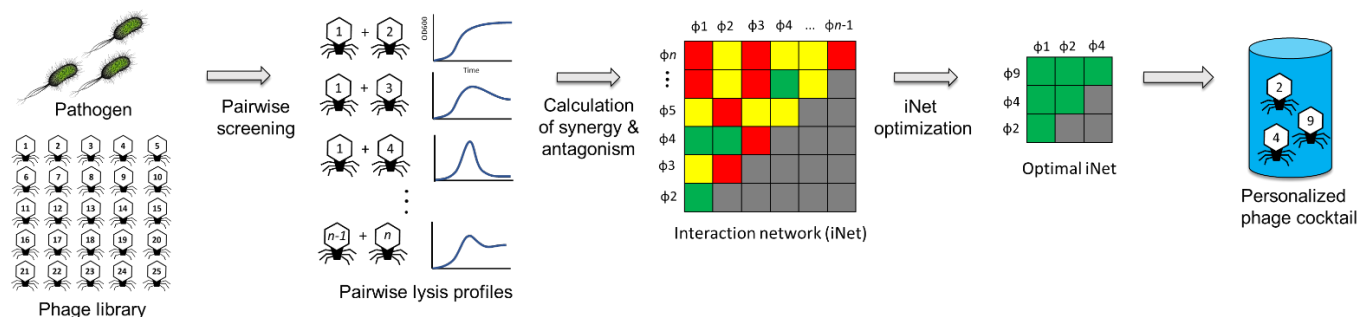


Fig. 5. Proposed pipeline for the development of personalized phage preparations using iNet optimization. Pathogens of interest are screened against libraries of previously-isolated phage particles in a pairwise fashion. Using the resulting lysis profiles, phage/phage interactions can be inferred, after which optimal higher-order combinations can be computed. This results in “designer” cocktails containing optimized iNets.

Finally, my discovery of diverse phage-to-phage interactions also poses significant implications for our understanding of microbial ecology. Phages are thought to be the most abundant and diverse biological entities on the planet (Suttle, 2005), and phage predation is a vital force for global carbon turnover by serving as a major cell fate for bacterial populations, one of the most significant carbon reservoirs (Suttle, 2005; Weinbauer, Chen, & Wilhelm, 2011; Wilhelm & Suttle, 1999). The forces that mediate the efficacy of phage predation in natural environments, therefore, is of great ecological significance. This study suggests that phage-to-phage interactions should be considered whilst studying the ecological interplay between bacteria and their viral parasites and raises new questions into how

significantly such interactions affect natural microbial population structures, carbon turnover, and global climate.

Future Directions

My iNet optimization approach, in its current form, is purely based on laboratory screening and does not incorporate any information about mechanisms of action. Although my mechanism-free approach allows me to identify clinically effective combination therapies containing agents from diverse drug classes, the user will be left without any novel biological insights about the molecular interactions that account for synergy and antagonism. I thus envision this platform as a valuable *first step* in studying the mechanisms of drug interactions, in that it can identify drugs or sets of drugs that may be interesting to dissect further on a mechanistic level. Thus, one future direction for this work could be to examine the sets of phages in my *B. cepacia* phage library that exhibited significant interactions, and to undergo a more detailed characterization of the cellular and molecular mediators of these interactions. Such experiments could help to provide a biological interpretation of the iNets I uncovered experimentally and could even lead to novel approaches with which to engineer iNets for specific applications.

In the future I also intend to expand the generality of my iNet optimization procedure. Because this work was contained to *B. cepacia*, it is currently unknown whether this approach could be applied to other pathogens. I will thus assemble phage libraries against other clinically-relevant pathogens—such as multidrug-resistant *Enterobacteriaceae* or MRSA—and attempt to develop optimized phage cocktails using iNet optimization.

Finally, my platform optimized cocktails solely on the basis of cell inhibition, but this is only one of many factors that might be considered whilst designing a combination therapy. The next version of my optimization platform could incorporate other information, such as the speed of resistance accumulation, the length of the latency period before cell inhibition occurs, the number of drugs in the

cocktail, and the relative doses of each drug (my current approach assumes all drugs are added at equal doses). Incorporating these additional factors into the optimization algorithm may enable the development of even more finely-tuned combination therapies.

Materials & Methods

Phages, strains & growth conditions

Novel *B. cepacia* phages were isolated from influent sewage water samples taken from three wastewater treatment plants in the Research Triangle region of North Carolina (Mason Farm Wastewater Treatment Plant, Chapel Hill, NC; North Durham Water Reclamation Facility, Durham, NC; South Durham Water Reclamation Facility, Durham, NC). These samples were pooled before undergoing phage enrichments via standard procedures (Van Twest & Kropinski, 2009). Clonal phage preparations were isolated from mixed enrichments by serially picking plaques showing distinct morphologies on a confluent bacterial lawn. High-titer phage stocks ($> 10^9$ PFU/mL) were prepared from clonal phage preparations using plate lysates (also according to standard procedures) and were stored in tryptic soy broth (TSB; Sigma-Aldrich 22092) with 1% chloroform. A total of 10 *B. cepacia* complex (BCC) strains, including 5 *B. multivorans*, 3 *B. conocephala*, and 2 *B. vietnamiensis* strains—all of which are recent cystic fibrosis pulmonary isolates (isolated either from sputum or airway aspirates)—were used as hosts during phage isolation. Overnight BCC cultures were prepared by inoculating 2 mL of TSB with a single colony and allowing the cells to grow for 16 h at 37°C with agitation (to roughly 4×10^9 CFU/mL).

See table S1 in the supplemental information for more detailed information about strains and phages.

Pairwise synergy screens

To screen for antimicrobial synergy among phages, I utilized combinatorial pairwise phage infection experiments. Overnight cultures underwent a 100-fold dilution before being infected with two phages to an MOI of 0.001 per phage (multiplicities were kept low to avoid complete cell lysis). Cultures were grown overnight with shaking at 30°C, and OD600 measurements were taken at intervals of 15 minutes. Results of these experiments were compared with a negative control (no phage) as well as single-phage baselines (i.e. a repeat of the growth assay with a single phage infection).

To exhaustively screen all phages within each strain background in a pairwise manner, I implemented a high-throughput experimental platform capable of running over 9,000 growth experiments in parallel (fig. S2). My platform integrates experimental setup, execution, and analysis; first, plate maps were generated and converted to a format compatible with the Formulatrix Mantis liquid handler system; the Mantis then filled 96-, 384-, or 1536-well plates with the specified assays; growth profiles were then generated using a Tecan Infinite M200 plate reader, with plate parallelization supported by a Tecan Freedom EVO robotic system; finally, results were analyzed and interaction networks (iNets) generated. The software tools I developed for generating plate maps and Mantis protocols, as well as generating iNets, can be found here: <https://github.com/jkreitz/cmb-phage-screening>. This platform was used during both iNet characterization and optimization.

Quantifying synergy and constructing iNets

In accordance with previous literature (Fouquier & Guedj, 2015), I define synergy as the state in which two phages exert a combined effect that is beyond independence (Bliss, 1939). Using cell survival (S ; approximated using OD600) as a metric of phage efficacy, I thus define a combination index (CI) which captures the strength of an interaction into a single metric. When computing cell survival, I integrate the OD curve over the entire experimental timecourse in order to account for heterogeneous lysis profiles.

$$S = \left[\frac{\int_0^t OD_{Phage}(t) dt}{\int_0^t OD_{Control}(t) dt} \right] \quad (1)$$

$$CI_{A,B} = \frac{S_A S_B}{S_{A+B}} \quad (2)$$

Under this model, the null hypothesis would be a scenario in which two phages A and B exhibit a CI of 1; in other words, they exert antimicrobial effects independently of one another. However, phage pairs with CI greater than 1 exhibit synergy—the cells exhibit less survival than would be expected given the null hypothesis of independence—and phage pairs with CI less than 1 exhibit antagonism.

iNet optimization

To estimate the efficacy of higher-order cocktails using knowledge of pairwise interactions (represented as an iNet), I used a simple regression model (Wang et al., 2015). Regression was used in place of a physical model in order to expand the application space of this optimization approach; in other words, this regression model is blind to mechanisms of action and therefore remains applicable to virtually any drug type. This regression model contains two primary components: single-order terms (represented by single-phage efficacies) and combination terms (captured by the inverse of CI values). This model ignores higher-order terms, however previous studies have found such terms to be negligible compared to first- and second-order terms (Al-Shyoukh et al., 2011; Wood et al., 2012). This model (eq. 3) then simplifies to a simple relation between the efficacies of single phages and the interactions between those phages (eq. 4).

$$\log(S_{a...b}) \approx \sum_a^b \textcolor{teal}{b_i x_i} + \sum_a^{b-1} \sum_{a+1}^b \textcolor{violet}{c_{ij} x_i x_j} \quad (3)$$

First-order terms
Combination terms

$$S_{a...b} \approx \frac{\textcolor{teal}{S_a} \dots \textcolor{teal}{S_b}}{\textcolor{violet}{CI_{a,a+1}} \dots \textcolor{violet}{CI_{b-1,b}}} \quad (4)$$

Given a set of n phages and $\binom{n}{2}$ CI values, a round of iNet optimization would thus involve predicting and storing survival (S) values for $\binom{n}{2} + \binom{n}{3} + \dots + \binom{n}{n}$ higher-order cocktails, and simply selecting the cocktail candidates that minimized S. In other words, one aims to select phages $j...k$ such that

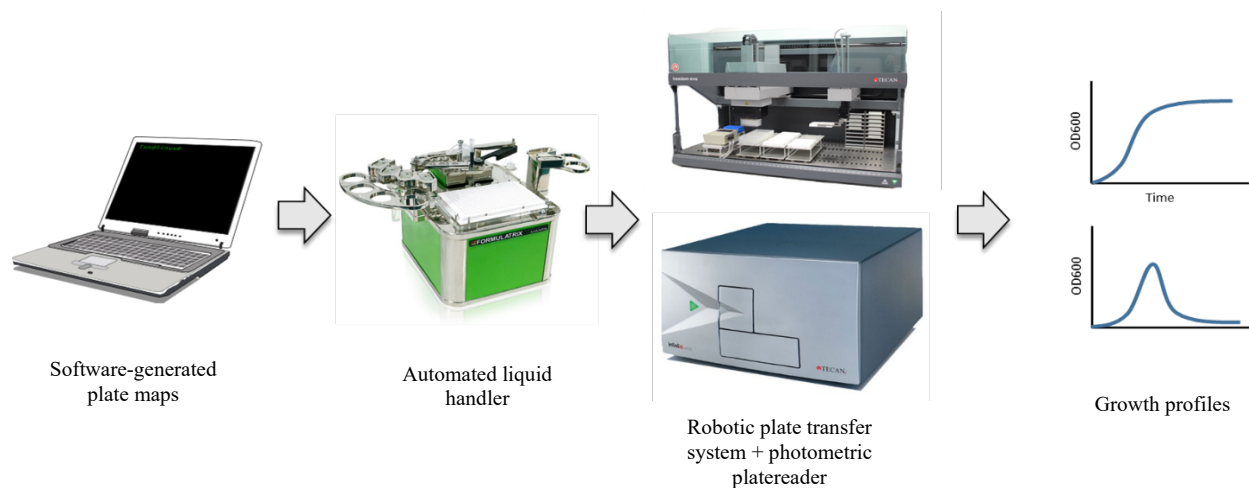
$$\operatorname{argmin}_{j,k \in 1...n} (S_{j...k}). \quad (5)$$

Supplemental Materials

Table S1. Novel *B. cepacia* phage library used during cocktail optimization experiments. All phages were isolated from influent sewage water from wastewater treatment plants in the Durham-Chapel Hill area. Note: plaque-mutants are identified with asterisks.

Designation	Original host strain	Host range (% strains infected)	Plaque morphology
ΦBcep1	<i>B. multivorans</i> C130A	42	1 mm pinhole
ΦBcep2	<i>B. multivorans</i> C130C	52	0.25 mm pinhole
ΦBcep4	<i>B. multivorans</i> AU34941	5	0.1 mm pinhole
ΦBcep5	<i>B. multivorans</i> AU34941	33	1.5 mm pinhole
ΦBcep8	<i>B. multivorans</i> AU34905	66	0.1 mm pinhole, turbid
ΦBcep9	<i>B. vietnamiensis</i> AU34701	5	0.2 mm pinhole
ΦBcep9-2*	<i>B. vietnamiensis</i> AU34701	Not tested	0.6 mm pinhole/0.2mm halo
ΦBcep10	<i>B. vietnamiensis</i> AU34747	5	1 mm pinhole
ΦBcep10-2*	<i>B. vietnamiensis</i> AU34747	5	1.3 mm pinhole
ΦBcep10-3*	<i>B. vietnamiensis</i> AU34747	5	2 mm pinhole
ΦBcep11	<i>B. multivorans</i> AU34924	33	0.2 mm pinhole, turbid
ΦBcep12	<i>B. cenocepacia</i> AU34124	5	0.2 mm pinhole, turbid
ΦBcep12-2*	<i>B. cenocepacia</i> AU34124	5	0.5 mm pinhole
ΦBcep12-3*	<i>B. cenocepacia</i> AU34124	5	0.9 mm pinhole
ΦBcep13	<i>B. cenocepacia</i> AU34124	Not tested	0.8 mm pinhole
ΦBcep14	<i>B. cenocepacia</i> AU33928	66	0.15 mm pinhole, turbid
ΦBcep15	<i>B. cenocepacia</i> AU34108	24	0.25 mm pinhole, turbid
ΦBcep17	<i>B. cenocepacia</i> AU34124	Not tested	0.2 mm pinhole, turbid
ΦBcep18	<i>B. cenocepacia</i> AU34124	5	0.2 mm pinhole

Table S2. Outline of the screening platform developed during this project to assist in combinatorial phage infection assays. It emphasized automation to reduce technical variability and to increase throughput.



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