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Current Opinion in Biotechnology

Enhancing phage therapy through synthetic biology and genome engineering

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The antimicrobial and therapeutic efficacy of bacteriophages is currently limited, mostly due to rapid emergence of phage-resistance and the inability of most phage isolates to bind and infect a broad range of clinical strains. Here, we discuss how phage therapy can be improved through recent advances in genetic engineering. First, we outline how receptor-binding proteins and their relevant structural domains are engineered to redirect phage specificity and to avoid resistance. Next, we summarize how phages are reprogrammed as prokaryotic gene therapy vectors that deliver antimicrobial 'payload' proteins, such as sequence-specific nucleases, to target defined cells within complex microbiomes. Finally, we delineate big data- and novel artificial intelligence-driven approaches that may guide the design of improved synthetic phage in the future.

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Current Opinion in Biotechnology 2021, **68**:151–159

This review comes from a themed issue on **Nanobiotechnology – phage therapy**

Edited by **Martin Loessner** and **Rob Lavigne**

https://doi.org/10.1016/j.copbio.2020.11.003

Introduction

In recent decades, the widespread and often poorly regulated use of antibiotics in medicine and agriculture has escalated the emergence of antibiotic-resistant pathogens [1]. The increasing burden placed on health care systems by these multidrug-resistant (MDR) microorganisms, alongside the simultaneous decline in pharmaceutical companies developing and stockpiling novel antibiotics [2], presents a dire threat to continued human prosperity [3]. As a result, there is rekindled interest in the use of phages as potential antimicrobial therapeutics [4], underlined by a series of recent successful compassionate use cases and upcoming randomized clinical trials (e.g. NCT03808103 and NCT04191148) [5,6]. However, *in vitro* and clinical data suggest that the efficacy of phage therapy using natural phage may be limited due to the rapid selection of phage resistant bacteria [6,7], due to immunogenicity as a consequence of prolonged treatment [8], and also because the host ranges of natural phage isolates rarely cover all clinically relevant pathogenic strains.

Advances in synthetic biology, where engineering principles are utilized to design biological systems from interchangeable parts, and the increasing pace of phage discovery may enable the augmentation of natural phage properties to overcome many of these shortcomings. Recent studies describing naturally evolved phage systems to broaden host range [9] and evade host defenses such as CRISPR-Cas [10,11], suggest that phage genomes contain a treasure trove of natural antimicrobial mechanisms and products to add to the synthetic biology toolbox. Here, we review the phage engineering techniques used to integrate such components into *designer phages* that possess enhanced properties over their naturally occurring counterparts, such as altered/broadened host ranges and the ability to transduce therapeutic payload genes to defined targets within the microbiota. We also give a speculative outlook on the future use of artificial intelligence and machine learning approaches to design the next generation of phage-based therapeutics.

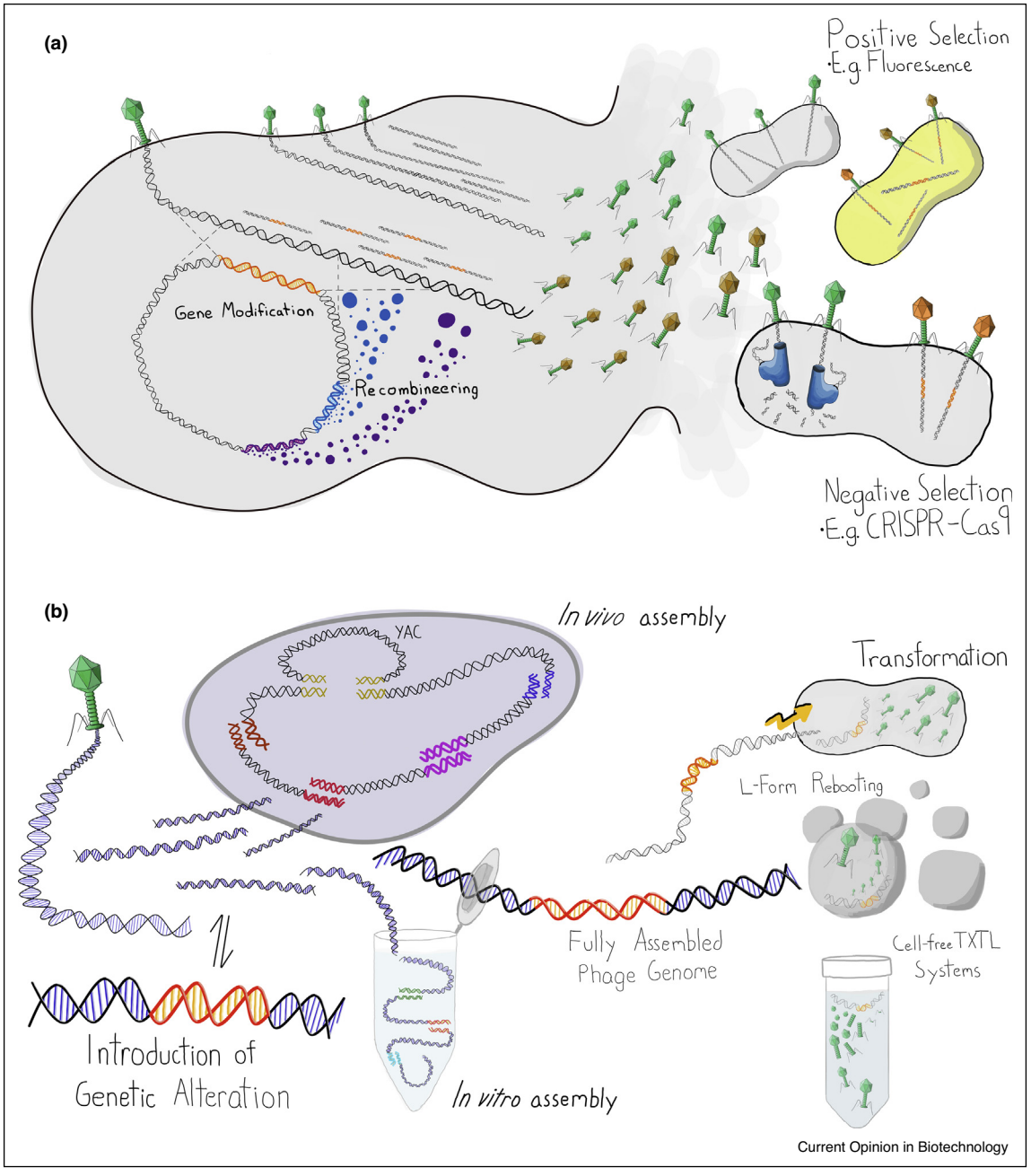
Phage genome engineering methods

The stable integration of temperate phage genomes into host chromosomes results in a resident prophage, and generally enables virus manipulation via the same methodologies as those for bacterial genomes. In contrast, strictly lytic (virulent) phages require specialized genome engineering methods, which can all be classified into two broad conceptual groups: i) homologous recombination (HR), and ii) genome rebooting, that is, the activation of exogenously assembled, synthetic phage DNA.

Homologous recombination

Recombination-based approaches (Figure 1a), where the phage genome undergoes HR-driven allelic exchange

Figure 1



Phage genome engineering methods. **(a)** Homologous recombination is often used to engineer phage genomes. In *recombineering*, heterologous recombination proteins are expressed within the cell, thereby increasing recombination frequency and protecting the recombination template from intracellular degradation. Both positive selection (e.g. fluorescence markers) and negative selection (e.g. CRISPR-Cas9 targeted against the wild-type phage) can be used for downstream selection of engineered phage particles. **(b)** Phage genomes can also be assembled from synthetic DNA fragments to introduce desired genetic alterations. Both *in vivo* (e.g. TAR-cloning) and *in vitro* (e.g. Gibson) assembly techniques are used. The fully assembled phage genome is *rebooted* to produce fully functional phage particles either in Gram-negative bacteria following electrotransformation, or using cell-free transcription-translation (TXTL) systems. Phage genomes of Gram-positive bacteria are rebooted in cell-wall deficient L-form bacteria after PEG-assisted transformation.

with a cytoplasmic editing template during infection, are the most commonly used methods of phage genome engineering. However, with respect to strictly lytic phage, these methods are limited by low natural recombination frequencies and require extensive screening to obtain progeny phages with the desired mutations [12–14].

Recombination efficiency has been significantly improved by co-opting the natural recombination systems of temperate phages in a method termed recombineering. Expression of phage recombination proteins (e.g. lambda Red and Rac RecE/RecT) within the recombination host protects the editing template from degradation and facilitates annealing with the injected phage genome (*in vivo* recombineering), thereby increasing recombination frequency and reducing the homology arm length requirement [15]. The identification of RecE/RecT homologs in mycobacteriophage Che9c expanded these techniques to Gram-positive bacteria and enabled the bacteriophage recombineering of electroporated DNA (BRED) technique, in which the phage genome and editing templates are co-electroporated into recombineering hosts [16].

HR-based editing techniques have also been improved through the use of positive and negative selection of progeny phages. The insertion of reporter genes (e.g. luciferase or fluorescent proteins) or phage-specific marker genes (e.g. *trxA* or *cmk* in coliphages) into phage genomes facilitates rapid positive selection of recombinant phages, despite low recombination frequencies [13,17,18]. Recently, bacterial CRISPR-Cas systems have been adapted as a mechanism for the negative selection of unmodified phage progeny, effectively enriching rare mutants from recombinant phage lysates. To date, type I-E, II-A, and III-A CRISPR systems have been used to select for recombinant phages targeting Gram-negative and Gram-positive hosts [19].

Genome rebooting

To address the problem that phage gene products may be toxic to their bacterial host, synthetic methods for genome assembly outside of the natural bacterial hosts have been developed. These techniques rely on the assembly of small to medium-size DNA fragments into full-length phage genomes through transformation-associated recombination (TAR) or *in vitro* enzymatic assembly (Gibson assembly) followed by transformation into competent bacterial hosts for rebooting and assembly into mutant phage particles (Figure 1b). Phage genome assembly from synthetic DNA fragments enables flexible engineering to introduce mutations, deletions, or insertions at any genomic locus, scales easily for genetic library construction, and eliminates the need to select against wild-type sequences.

Jaschke *et al.* [20] first demonstrated the efficacy of such approaches to reconstruct and archive the ΦX174 phage genome in yeast. The small 5.4 kb ΦX174 genome could be assembled directly from PCR products and synthetic fragments into a yeast artificial chromosome through TAR, released through restriction enzyme digestion, and rebooted in *Escherichia coli* cells. Ando *et al.* [21] utilized similar approaches to modulate the host range of several T7-like phages to target various Gram-negative pathogens (see below). Because of the requirement for high transformation efficiency and access to the *E. coli* molecular machinery, these approaches were initially limited to phages infecting Gram-negative hosts. Recently, cell wall-deficient L-form bacteria have been developed as effective Gram-positive phage rebooting hosts following genome transformation using polyethylene glycol. Kilcher *et al.* [22•] demonstrated cross-genus rebooting of various Gibson-assembled and wild-type phage genomes in L-form *Listeria monocytogenes*, suggesting that this approach may be broadly applicable for phages infecting Gram-positive bacteria. This technique was subsequently used to construct reporter phages [23] and to modify the host-range of a *Listeria* phage (see below) [24••].

New synthetic biology techniques to reboot phage genomes outside host cells eliminate the need for DNA transformation and enable the synthesis of phages infecting unknown or undomesticated hosts. High yields of self-assembling MS2, T4, T7, and ΦX174 phage particles have been generated in a test tube from a small quantity of phage DNA and optimized *E. coli* extracts, via cell-free transcription-translation (TXTL) systems [25–27]. Although genetic engineering has in the past been limited to phages infecting well-studied laboratory hosts, recent high-throughput screens for recombination protein homologs [28] and the development of TXTL systems [29] from additional bacteria is expected to expand future phage engineering to new bacterial hosts (e.g. *Vibrio*, *Streptomyces*, *Bacillus*, and *Pseudomonas* species).

Designer phage applications

The techniques outlined above have been used to create genetically modified phages for use in bacterial diagnostics, therapeutics, and drug delivery [30,31]. Below, we will highlight efforts to engineer designer phages with tunable host range and enhanced payload delivery to develop the next generation of phage therapy.

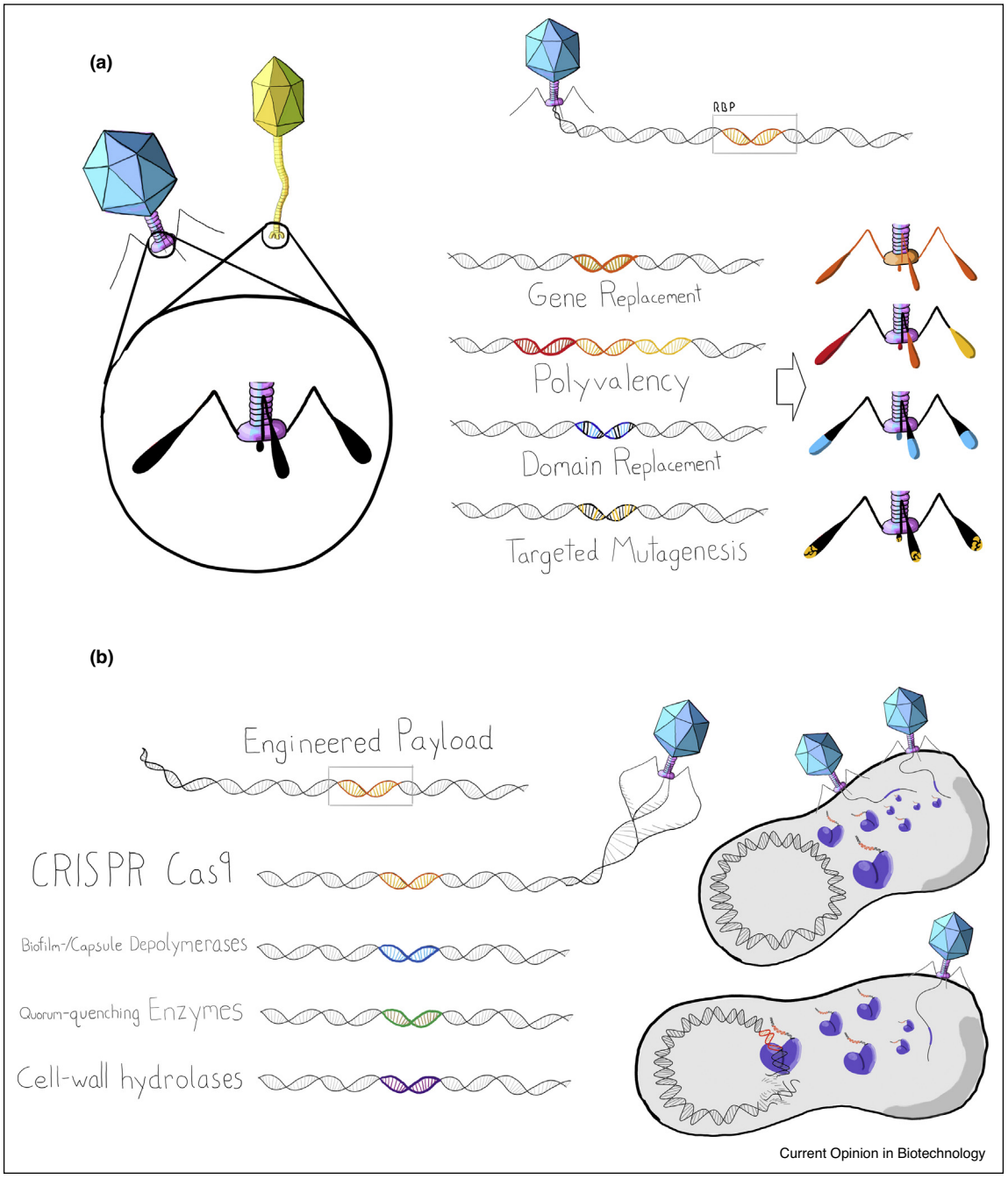
Programming host specificity

Phage host specificity is a double-edged sword: although phages can selectively infect and kill host bacteria within complex microbial communities, individual phages often lack sufficient host range to target all strains responsible for clinical infections [9]. Multi-phage cocktails can target various pathogenic strains and prevent the proliferation of phage-resistant mutants; however, the isolation and characterization of the constituent phages is time-consuming and requires laborious regulatory approval [32]. To ameliorate these limitations, synthetic biologists are now

implementing design principles from the natural host range expansion mechanisms of broad-host-range phages for the scalable synthesis of modular phages, creating a tunable host range based upon previously validated phage scaffolds (Figure 2a).

The primary host-range determinants are receptor-binding proteins (RBPs) located either at the distal end of the phage adsorption apparatus (the baseplate), or at the tip of the tail fibers, which mediate the interaction with carbohydrate or protein receptors at the host cell surface.

Figure 2



Designer phage applications. **(a)** Host specificity can be (re-)programmed by altering the phage receptor binding modules. Genetic alterations of these modules may be introduced at different scales, ranging from full gene replacements (e.g. tail or RBP swapping) to individual point mutations (e.g. amino acid exchanges in distal loops at the host-RBP interface). **(b)** *Payload engineering* refers to 'arming' the phage with genes and properties aimed at increasing antimicrobial activity. For example, including CRISPR-Cas9 as a genetic payload can introduce intracellular sequence-specific toxicity whereas phage-encoded biofilm depolymerases target extracellular bacterial polymers in the vicinity of the lysed host cell.

Broad-host-range phages have evolved strategies to expand or switch host range, including: i) RBP allele and domain exchange, ii) targeted RBP diversification in hypervariable regions, and iii) polyvalent RBP virions [9].

Rational engineering of phage host range has so far been accomplished by tail fiber exchange via HR between closely related phages in the T2, T4, and T7 families [12,14,33–37]. Ando *et al.* expanded these techniques to a broad range of T7-like phages using a yeast-based genome rebooting strategy in order to create phage scaffolds with modular exchange of those tail components dictating the host range [21]. Whereas exchanging complete or partial T7 phage scaffold tail fibers (gp17) with those of closely related phages effectively modulated host range, the creation of viable phage hybrids between *Escherichia* phage T7 and *Klebsiella* phage K11 required the exchange of the complete tail apparatus (gp11, 12, and 17), thus also demonstrating certain limits of RBP modularity.

Most mature RBPs are involved in multiple protein-carbohydrate and protein-protein interactions with the host cell receptor as well as with structural phage tail proteins. These interactions are required to maintain structural integrity, to mediate receptor recognition, and to initiate and orchestrate receptor-mediated structural rearrangements. It is therefore not an easy task to modify RBPs without losing infectivity. Domain shuffling techniques have been developed to create chimeric RBPs by exchanging the globular receptor-binding domains located in the C-terminal domain (CTD) of phage tail fibers, while leaving the N-terminal domain required for interactions with the phage tail intact [21,24••,33,34].

Recently, high-throughput RBP diversification strategies have been implemented to screen for phage RBP mutants with shifted or expanded host ranges. Yehl *et al.* [38••] used a targeted mutagenesis approach inspired by antibody engineering to generate functional diversity within unstructured loops located at the RBP-host cell interface of Enterobacteria phage T3. The resulting synthetic 'phagebody' libraries (10^7 variants) contained individual phages with expanded host range and suppressed the evolution of phage resistance. Yosef and co-workers integrated tail/RBP allelic exchange and iterative cycles of targeted mutagenesis to engineer the host ranges of phage particles for optimized DNA transduction and payload delivery (see below) into new bacterial targets [39••]. These techniques rapidly increase RBP diversity at a pace unachievable through natural evolution and help to create synthetic phage populations one step ahead of their bacterial hosts in the co-evolutionary arms race.

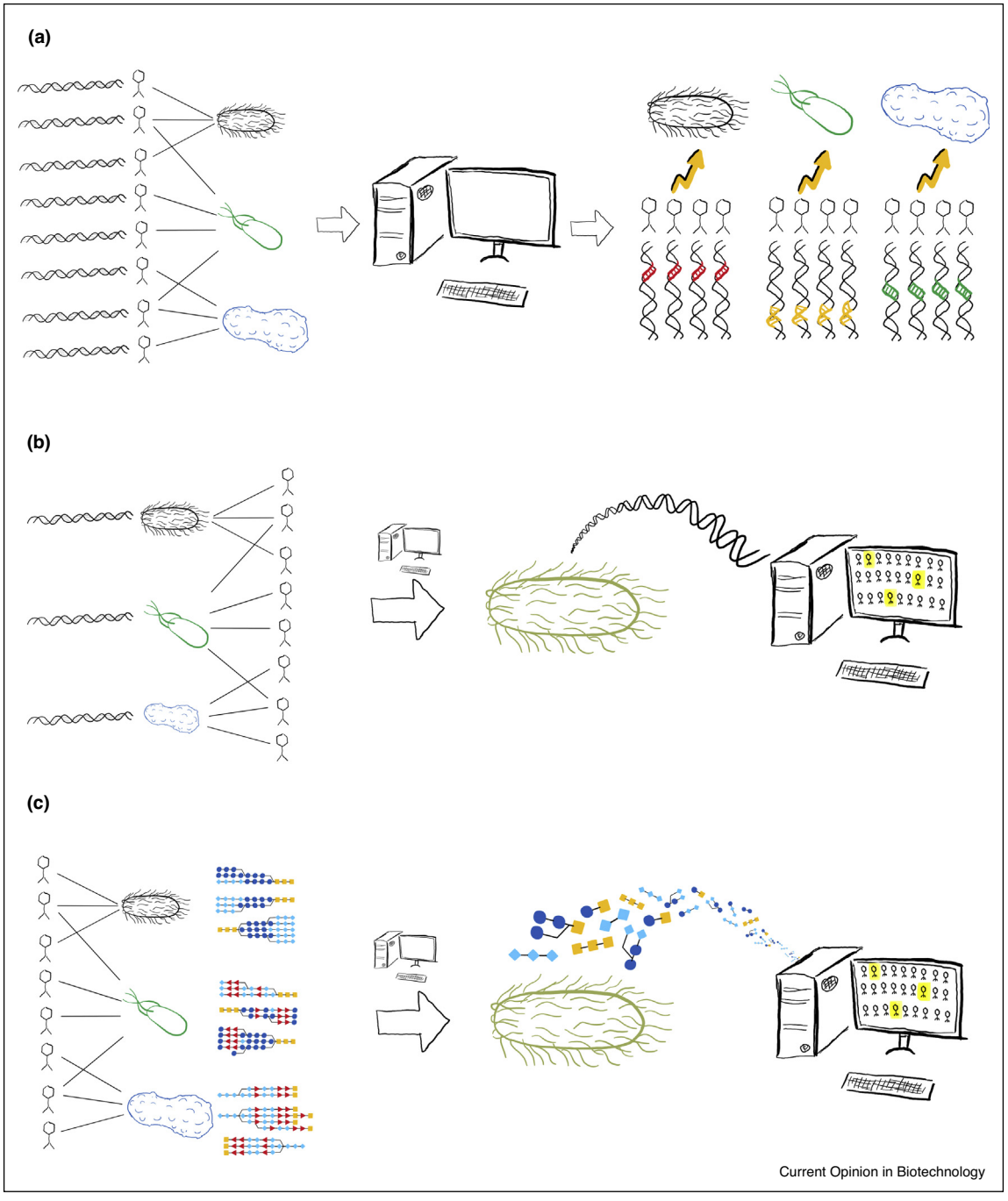
Dunne *et al.* combined RBP and lysogeny engineering to reprogram the temperate, narrow-range *Listeria* siphovirus PSA into a strictly lytic phage with broadened host specificity [48]. Akin to the T7 phagebody approach, the PSA host range was shifted via RBP diversification using targeted mutagenic PCR and subsequent rebooting of genome libraries in *Listeria* L-form cells. Additionally, a second *rbp* allele targeting a different *Listeria* serovar was inserted into the PSA genome to generate a synthetic phage featuring polyvalent host cell binding. Finally, the elucidation of the RBP CTD crystal structure enabled the structure-guided design of chimeric RBPs. Globular, C-terminal affinity domains identified in sequenced *Listeria* genomes were swapped at conserved helical bundle motifs connecting the CTD to the baseplate. This approach generated viable chimeric phages, whose modified and expanded host-ranges were predicted through globular domain phylogeny and the glycotype of the donor lysogen.

It is evident that all these novel rational design principles depend on a detailed understanding of the molecular interactions dictating individual phage-host interactions. Thus, high-throughput tools to identify phage RBPs and their conserved structural domains are required to rapidly engineer modular phages at sufficient scale for therapy.

Delivering Cas nucleases as antimicrobial payloads

Besides engineering host range, phage antimicrobial activity can be enhanced or modulated through the *in-situ* production of heterologous proteins, often described as genetic 'payloads' (Figure 2b) [30]. For example, phages have been engineered to deliver biofilm-depolymerases and capsule-depolymerases [40,41], quorum-quenching enzymes [42], and cell wall hydrolases with cross-genus lytic activity [22•]. Infected cells produce and release these proteins upon host cell lysis where they act on target cells or substrates in their vicinity. Another group of payload proteins that have recently gained much attention are CRISPR-Cas nucleases. Here, the phage serves as a target cell-specific vector to deliver a programmable Cas nuclease toxin, effectively creating a nucleotide sequence-specific antimicrobial. Two initial landmark studies describe the use of non-replicative phagemids that target antimicrobial resistance- or virulence genes in *E. coli* [43] and *Staphylococcus aureus* [44], using sequence-specific crRNA and the type II-A effector nuclease Cas9. Phagemid delivery enabled selective removal of resistant or virulent bacterial sub-populations and can be employed to immunize against plasmids containing antibiotic-resistance genes. The same pathogens were later also targeted by engineered temperate phages delivering Cas9 or Cas3 (type I-E) effectors [45,46]. Very recently, Selle *et al.* presented a slightly modified approach that makes use of conserved type I-B CRISPR-Cas systems present in most *Clostridium difficile* genomes [47••]. Based on the temperate phage ΦCD24-2, the authors engineered a set of phage derivatives that delivered host-targeting crRNA as a toxin and/or feature genomic deletions that convert the phage lifestyle from temperate to virulent. Both

Figure 3



Artificial intelligence-driven phage research. The figure outlines different applications of machine learning (ML) and artificial intelligence (AI) to phage engineering. **(a)** Given a novel phage sequence, a ML model can by trained on phage whole-genome sequences as features and corresponding host range information as classification labels. This approach could enable the prediction of phage-host interactions and provide insights into potential genetic elements dictating host range. **(b)** Similarly, by combining bacterial whole-genome sequences with information on phage infectivity, an AI algorithm could be trained to identify a selection of phages likely to infect a novel pathogen. **(c)** By associating bacterial surface glycans, that is, using (partial) surface glycan structures as input data, with information on phage infectivity, the model could predict phage binding to a host cell surface.

life-style conversion and crRNA-mediated genome targeting improved killing *in vitro* and in a *C. difficile* mouse infection model. Similarly, Lam et. al used engineered, Cas9-delivering M13 phagemids to selectively deplete one of two *E. coli* strains competitively colonizing the gut of mice [48]. These two studies highlight the potential applicability of engineered phage therapy for gastrointestinal targeting and *in vivo* microbiome engineering.

Outlook and conclusions

The advent of big data and the pioneering of novel machine learning (ML) methodologies constitute a major technological advancement in biological and medical research. Whereas the rational engineering efforts described above have been modeled on natural phage systems, synthetic biology approaches integrating recent advancements in ML may further boost phage therapy as a viable clinical treatment.

Research combining 'omics with ML-powered bioinformatics is already being widely used in cancer and other biomedical fields [49]. These methods have thus far been limited in phage therapeutics, barring a handful of publications [50,51,52•,53], an inattention most likely due to the scarcity of data available from online resources. As it stands, the most abundant dataset for any class of phage is an Actinobacteriophage collection comprising more than 3400 complete genome sequences (The Actinobacteriophage Database; URL: https://phagesdb.org). One trending approach is to utilize phage and bacteria whole-genome sequences from such databases, along with corresponding experimentally determined host-range information, to predict virus-host interactions (Figure 3a,b). While the Actinobacteriophage collection covers a sequence space of sufficient size for ML applications, the vast majority of these phages were isolated on a single bacterial species (*Mycobacterium smegmatis*), which drastically reduces its applicability. Generally, comprehensive and reliable host-range data is difficult to obtain as it requires labor-intensive experimentation as well as standardized protocols and scoring systems that allow comparing host range data from different laboratories. Negative interaction data (i.e. bacterial hosts not infected by a given phage) is particularly underrepresented, a limitation that could be solved only by implementing complex data augmentation methods [54] or by making unvalidated assumptions regarding phage specificity.

Just as sequence-based, ML-driven phage research is on the rise, so are the inferences and predictions of biological processes being made on the basis of structural data. In addition to utilizing host-range information to infer phage sensitivity based on predicted protein-protein interactions [52•], recent advances in glycan research are similarly promising. Glycans make up a large portion of the bacterial cell surface and often function as phage attachment ligands and receptors. Two recent studies by Bojar *et al.* [55,56] use natural language processing to create a comprehensive database of glycan structures with associated phage-binding information readily available and infer the evolutionary relationships of the bacterial species [57,58]. This opens an interesting angle for AI-driven phage research. Analogous to using host-range information as a classification label, these types of experimental data could very well serve as structural input data to predict phage-host interaction (Figure 3c).

To fully develop the untapped potential of phage therapy, the authors firmly believe that an interdisciplinary approach is required. Besides genome engineering, synthetic biology, structure-guided design, and machine learning, this includes a plethora of other fields such as drug formulation and administration, pharmacokinetics/-dynamics, and immunology. When coupled with a non-prohibitive regulatory framework for the manufacturing and application of phage-based biologics, this integrative approach paints a carefully optimistic picture of a future with targeted phage therapeutics effective against multi-drug-resistant pathogens.

Conflict of interest statement

T.K.L. is a co-founder of Senti Biosciences, Synlogic, Engine Biosciences, Tango Therapeutics, Corvium, BiomX, and Eligo Biosciences. T.K.L. also holds financial interests in nest.bio, Ampliphi, IndieBio, MedicusTek, Quark Biosciences, and Personal Genomics. M.J.L holds financial interests in Micreos.

CRediT authorship contribution statement

Bryan R Lenneman: Conceptualization, Writing - original draft, Writing - review & editing. **Jonas Fernbach:** Conceptualization, Visualization, Writing - original draft, Writing - review & editing. **Martin J Loessner:** Writing - review & editing, Funding acquisition. **Timothy K Lu:** Writing - review & editing, Funding acquisition. **Samuel Kilcher:** Conceptualization, Writing - original draft, Writing - review & editing, Funding acquisition.

Acknowledgements

We would like to thank Karen Pepper for critical review and revising the manuscript. Funding: S.K. is funded by the Swiss National Science Foundation (grant PZ00P3_174108). B.R.L is funded through the National Institutes of Health [grant 5-R33-AI121669-04]. T.K.L. is supported through the National Institutes of Health [grants 1DP2OD008435, 1P50GM098792, 1R01EB017755], the Defense Threat Reduction Agency [grant HDTRA1-14-1-0007], and the Institute for Soldier Nanotechnologies [contract W911NF-13-D-0001].

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

- • of special interest
- •• of outstanding interest

1. Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N: **A review on antibiotic resistance: alarm bells are ringing**. *Cureus* 2017, **9**:e1403.

2. Piddock LJ: **The crisis of no new antibiotics–what is the way forward?** *Lancet Infect Dis* 2012, **12**:249-253.

3. Adegoke AA, Faleye AC, Singh G, Stenstrom TA: **Antibiotic resistant superbugs: assessment of the interrelationship of occurrence in clinical settings and environmental niches**. *Molecules* 2016, **22**.

4. Hesse S, Adhya S: **Phage therapy in the twenty-first century: facing the decline of the antibiotic era; is it finally time for the age of the phage?** *Annu Rev Microbiol* 2019, **73**:155-174.

5. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K, Gilmour KC, Soothill J, Jacobs-Sera D, Schooley RT *et al.*: **Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus***. *Nat Med* 2019, **25**:730-733.

6. Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, Barr JJ, Reed SL, Rohwer F, Benler S *et al.*: **Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection**. *Antimicrob Agents Chemother* 2017, **61**.

7. Rostol JT, Marraffini L: **(Ph)ighting phages: how bacteria resist their parasites**. *Cell Host Microbe* 2019, **25**:184-194.

8. Krut O, Bekeredjian-Ding I: **Contribution of the immune response to phage therapy**. *J Immunol* 2018, **200**:3037-3044.

9. de Jonge PA, Nobrega FL, Brouns SJJ, Dutilh BE: **Molecular and evolutionary determinants of bacteriophage host range**. *Trends Microbiol* 2019, **27**:51-63.

10. Mendoza SD, Nieweglowska ES, Govindarajan S, Leon LM, Berry JD, Tiwari A, Chaikeeratisak V, Pogliano J, Agard DA, Bondy-Denomy J: **A bacteriophage nucleus-like compartment shields DNA from CRISPR nucleases**. *Nature* 2020, **577**:244-248.

11. Pawluk A, Davidson AR, Maxwell KL: **Anti-CRISPR: discovery, mechanism and function**. *Nat Rev Microbiol* 2018, **16**:12-17.

12. Le S, He X, Tan Y, Huang G, Zhang L, Lux R, Shi W, Hu F: **Mapping the tail fiber as the receptor binding protein responsible for differential host specificity of *Pseudomonas aeruginosa* bacteriophages PaP1 and JG004**. *PLoS One* 2013, **8**:e68562.

13. Loessner MJ, Rees CE, Stewart GS, Scherer S: **Construction of luciferase reporter bacteriophage A511::luxAB for rapid and sensitive detection of viable Listeria cells**. *Appl Environ Microbiol* 1996, **62**:1133-1140.

14. Mahichi F, Synnott AJ, Yamamichi K, Osada T, Tanji Y: **Site-specific recombination of T2 phage using IP008 long tail fiber genes provides a targeted method for expanding host range while retaining lytic activity**. *FEMS Microbiol Lett* 2009, **295**:211-217.

15. Marinelli LJ, Hatfull GF, Piuri M: **Recombineering: a powerful tool for modification of bacteriophage genomes**. *Bacteriophage* 2012, **2**:5-14.

16. Marinelli LJ, Piuri M, Swigonova Z, Balachandran A, Oldfield LM, van Kessel JC, Hatfull GF: **BRED: a simple and powerful tool for constructing mutant and recombinant bacteriophage genomes**. *PLoS One* 2008, **3** e3957.

17. Oda M, Morita M, Unno H, Tanji Y: **Rapid detection of *Escherichia coli* O157:H7 by using green fluorescent protein-labeled PP01 bacteriophage**. *Appl Environ Microbiol* 2004, **70**:527-534.

18. Qimron U, Marintcheva B, Tabor S, Richardson CC: **Genomewide screens for *Escherichia coli* genes affecting growth of T7 bacteriophage**. *Proc Natl Acad Sci U S A* 2006, **103**:19039-19044.

19. Hatoum-Aslan A: **Phage genetic engineering using CRISPR(-)Cas systems**. *Viruses* 2018, **10**.

20. Jaschke PR, Lieberman EK, Rodriguez J, Sierra A, Endy D: **A fully decompressed synthetic bacteriophage oX174 genome assembled and archived in yeast**. *Virology* 2012, **434**:278-284.

21. Ando H, Lemire S, Pires DP, Lu TK: **Engineering modular viral scaffolds for targeted bacterial population editing**. *Cell Syst* 2015, **1**:187-196.

22. • Kilcher S, Studer P, Muessner C, Klumpp J, Loessner MJ: **Cross-genus rebooting of custom-made, synthetic bacteriophage genomes in L-form bacteria**. *Proc Natl Acad Sci U S A* 2018, **115**:567-572.
By using cell wall-deficient bacteria as surrogate hosts for genome activation, this study expands the synthetic genome rebooting approach to several phages that infect Gram-positive host bacteria.

23. Meile S, Sarbach A, Du J, Schuppler M, Saez C, Loessner MJ, Kilcher S: **Engineered reporter phages for rapid bioluminescence-based detection and differentiation of viable listeria cells**. *Appl Environ Microbiol* 2020, **86**.

24. •• Dunne M, Rupf B, Tala M, Qabrati X, Ernst P, Shen Y, Sumrall E, Heeb L, Pluckthun A, Loessner MJ *et al.*: **Reprogramming bacteriophage host range through structure-guided design of chimeric receptor binding proteins**. *Cell Rep* 2019, **29**:1336-1350 e1334.
This is the first study to demonstrate targeted and structure-guided host range engineering in a non T7-like phage targeting a Gram-positive pathogen. The authors use structure-guided RBP chimera design and targeted *rbp* mutagenesis screens to modify *Listeria* phage host range.

25. Bundy BC, Franciszkowicz MJ, Swartz JR: ***Escherichia coli*-based cell-free synthesis of virus-like particles**. *Biotechnol Bioeng* 2008, **100**:28-37.

26. Rustad M, Eastlund A, Jardine P, Noireaux V: **Cell-free TXTL synthesis of infectious bacteriophage T4 in a single test tube reaction**. *Synth Biol* 2018, **3**.

27. Rustad M, Eastlund A, Marshall R, Jardine P, Noireaux V: **Synthesis of infectious bacteriophages in an *E. coli*-based cell-free expression system**. *J Vis Exp* 2017.

28. Wannier TM, Nyerges A, Kuchwara HM, Czikkely M, Balogh D, Filsinger GT, Borders NC, Gregg CJ, Lajoie MJ, Rios X *et al.*: **Improved bacterial recombineering by parallelized protein discovery**. *Proc Natl Acad Sci U S A* 2020, **117**:13689-13698.

29. Noireaux V, Liu AP: **The new age of cell-free biology**. *Annu Rev Biomed Eng* 2020, **22**:51-77.

30. Kilcher S, Loessner MJ: **Engineering bacteriophages as versatile biologics**. *Trends Microbiol* 2019, **27**:355-367.

31. Lemire S, Yehl KM, Lu TK: **Phage-based applications in synthetic biology**. *Annu Rev Virol* 2018, **5**:453-476.

32. Chan BK, Abedon ST, Loc-Carrillo C: **Phage cocktails and the future of phage therapy**. *Future Microbiol* 2013, **8**:769-783.

33. Chen M, Zhang L, Abdelgader SA, Yu L, Xu J, Yao H, Lu C, Zhang W: **Alterations in gp37 expand the host range of a T4-like phage**. *Appl Environ Microbiol* 2017, **83**.

34. Lin TY, Lo YH, Tseng PW, Chang SF, Lin YT, Chen TS: **A T3 and T7 recombinant phage acquires efficient adsorption and a broader host range**. *PLoS One* 2012, **7**:e30954.

35. Pouillot F, Blois H, Iris F: **Genetically engineered virulent phage banks in the detection and control of emergent pathogenic bacteria**. *Biosecur Bioterror* 2010, **8**:155-169.

36. Trojet SN, Caumont-Sarcos A, Perrody E, Comeau AM, Krisch HM: **The gp38 adhesins of the T4 superfamily: a complex modular determinant of the phage's host specificity**. *Genome Biol Evol* 2011, **3**:674-686.

37. Yoichi M, Abe M, Miyanaga K, Unno H, Tanji Y: **Alteration of tail fiber protein gp38 enables T2 phage to infect *Escherichia coli* O157:H7**. *J Biotechnol* 2005, **115**:101-107.

38. •• Yehl K, Lemire S, Yang AC, Ando H, Mimee M, Torres MT, de la Fuente-Nunez C, Lu TK: **Engineering phage host-range and suppressing bacterial resistance through phage tail fiber mutagenesis**. *Cell* 2019, **179**:459-469 e459.
This work utilized a structure-guided rational design approach to introduce diversity to the host-range-determining regions of phage T3 tail fibers. The resulting phage libraries demonstrated altered host range profiles and suppressed the emergence of phage-resistant bacterial mutants.

39. •• Yosef I, Goren MG, Globus R, Molshanski-Mor S, Qimron U: **Extending the host range of bacteriophage particles for DNA transduction**. *Mol Cell* 2017, **66**:721-728 e723.

A T7 tail exchange strategy is employed to modify the range of target cells that can be transduced by non-replicative virions. In addition, the authors use their own targeted evolution approach to enhance transduction efficiencies.

40. Born Y, Fieseler L, Thony V, Leimer N, Duffy B, Loessner MJ: **Engineering of bacteriophages Y2::dpoL1-C and Y2::luxAB for efficient control and rapid detection of the fire blight pathogen, *Erwinia amylovora***. *Appl Environ Microbiol* 2017, **83**.

41. Lu TK, Collins JJ: **Dispersing biofilms with engineered enzymatic bacteriophage**. *Proc Natl Acad Sci U S A* 2007, **104**:11197-11202.

42. Pei R, Lamas-Samanamud GR: **Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes**. *Appl Environ Microbiol* 2014, **80**:5340-5348.

43. Citorik RJ, Mimee M, Lu TK: **Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases**. *Nat Biotechnol* 2014, **32**:1141-1145.

44. Bikard D, Euler CW, Jiang W, Nussenzweig PM, Goldberg GW, Duportet X, Fischetti VA, Marraffini LA: **Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials**. *Nat Biotechnol* 2014, **32**:1146-1150.

45. Park JY, Moon BY, Park JW, Thornton JA, Park YH, Seo KS: **Genetic engineering of a temperate phage-based delivery system for CRISPR/Cas9 antimicrobials against *Staphylococcus aureus***. *Sci Rep* 2017, **7**:44929.

46. Yosef I, Manor M, Kiro R, Qimron U: **Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria**. *Proc Natl Acad Sci U S A* 2015, **112**:7267-7272.

47. •• Selle K, Fletcher JR, Tuson H, Schmitt DS, McMillan L, Vridhambal GS, Rivera AJ, Montgomery SA, Fortier LC, Barrangou R *et al.*: **In vivo targeting of *Clostridioides difficile* using phage-delivered CRISPR-Cas3 antimicrobials**. *mBio* 2020, **11**.

Using a mouse model of *C. difficile* infection, Selle *et al.* demonstrate how combined CRISPR-Cas arming and phage lifestyle conversion enhance therapeutic efficacy, prevent the generation and expansion of phage-resistant lysogens, and reduce tissue damage in the host.

48. Lam KN, Spanogiannopoulos P, Soto-Perez P, Alexander M, Nalley MJ, Bisanz JE, Yu FB, Turnbaugh PJ: **Phage-delivered CRISPR-Cas9 for strain-specific depletion and genomic deletions in the gut microbiota**. *bioRxiv* 2020. 2020.2007.2009.193847.

49. Landhuis E: **Deep learning takes on tumours**. *Nature* 2020, **580**:551-553.

50. Amgarten D, Braga LPP, da Silva AM, Setubal JC: **MARVEL, a tool for prediction of bacteriophage sequences in metagenomic bins**. *Front Genet* 2018, **9**:304.

51. Auslander N, Gussow AB, Benler S, Wolf YI, Koonin EV: **Seeker: alignment-free identification of bacteriophage genomes by deep learning**. *bioRxiv* 2020. 2020.2004.2004.025783.

52. • Leite DMC, Brochet X, Resch G, Que YA, Neves A, Pena-Reyes C: **Computational prediction of inter-species relationships through omics data analysis and machine learning**. *BMC Bioinformatics* 2018, **19**:420.

This work demonstrates the application of supervised machine learning algorithms on phage sequence data to make phage-host interaction predictions.

53. Tynecki P, Guziński A, Kazimierczak J, Jadczuk M, Dastych J, Onisko A: **PhageAI - bacteriophage life cycle recognition with machine learning and natural language processing**. *bioRxiv* 2020. 2020.2007.2011.198606.

54. Taylor L, Nitschke G: **Improving deep learning with generic data augmentation**. *IEEE Symposium Series on Computational Intelligence (SSCI)* 2018:1542-1547.

55. Bojar D, Camacho DM, Collins JJ: **Using natural language processing to learn the grammar of glycans**. *bioRxiv* 2020. 2020.2001.2010.902114.

56. Bojar D, Powers RK, Camacho DM, Collins JJ: **SweetOrigins: extracting evolutionary information from glycans**. *bioRxiv* 2020. 2020.2004.2008.031948.

57. Bielmann R, Habann M, Eugster MR, Lurz R, Calendar R, Klumpp J, Loessner MJ: **Receptor binding proteins of Listeria monocytogenes bacteriophages A118 and P35 recognize serovar-specific teichoic acids**. *Virology* 2015, **477**:110-118.

58. Briers Y, Schmelcher M, Loessner MJ, Hendrix J, Engelborghs Y, Volckaert G, Lavigne R: **The high-affinity peptidoglycan binding domain of Pseudomonas phage endolysin KZ144**. *Biochem Biophys Res Commun* 2009, **383**:187-191.