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Review

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Bacteriophage engineering: from serendipitous hunting to rational design in therapeutics and microbiome modulation

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Abstract: Phage engineering has transitioned from rudimentary genetic modifications into a highly sophisticated discipline that is capable of generating bespoke therapeutic agents. This review systematically evaluates the modern engineering toolbox in this field, spanning high-efficiency CRISPR-Cas systems (Cas9, Cas12, and Cas3) and novel recombitrons designed for counter-selection-free multiplex editing. Building upon these precise editing capabilities, we explore the paradigm shift toward de novo genome writing and artificial intelligence (AI)-driven design (e.g., Evo), while offering a critical assessment of current bottlenecks, such as the reported 5.3% experimental success rate and training data biases. Beyond genomic alterations, we explore phage display and chemical conjugation as parallel strategies for functionalization. Furthermore, we analyze the 'lytic paradox' in microbiome modulation—specifically regarding the restoration of short-chain fatty acid (SCFA) production—and outline essential biocontainment frameworks, such as synthetic 'kill-switches,' that are necessary for clinical translation. Ultimately, the convergence of synthetic biology and AI is poised to catalyze next-generation solutions against antimicrobial resistance and metabolic disorders.

Key words: Phage engineering; Synthetic biology; CRISPR-Cas; Genome rebooting; Therapeutic development

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1 Introduction

The global crisis of antimicrobial resistance (AMR) demands innovative therapeutic strategies that can bypass conventional antibiotic mechanisms (Fowoyo, 2024). Phages—viruses that specifically infect and kill bacteria—have re-emerged as a promising alternative; their natural specificity, self-replicating nature at the infection site, and ability to evolve alongside the development of bacterial resistance offer unique advantages (Nobrega *et al.*, 2015). Nonetheless, the therapeutic application of natural phages is often hampered by their inherent limitations: narrow host ranges, the potential for lysogeny, rapid clearance by the immune system, and the intrinsic variability of natural isolates (Lin *et al.*, 2022).

Phage engineering aims to transform these natural cell predators into programmable, precision therapeutics by systematically overcoming these drawbacks. This field has progressed dramatically, moving from the opportunistic use of environmental isolates to rational design and construction. This advancement has been fueled

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by parallel advances in molecular biology, synthetic biology and nanotechnology (Brown *et al.*, 2017; Lenneman *et al.*, 2021). While early efforts relied on low-efficiency traditional homologous recombination, the integration of CRISPR-Cas systems has recently enabled high-efficiency, site-specific genetic modifications. Engineered phages targeting pathogens such as *Salmonella* and *E. coli* have demonstrated not only strain-specific killing but also sophisticated functional reprogramming (Mitsunaka *et al.*, 2022; Khambhati *et al.*, 2023). Their applications extend beyond simple gene knockout; for instance, recent studies have utilized engineered phages to achieve the sustained *in situ* production and delivery of functional proteins within the mammalian gut, successfully enabling precise base editing of the gut bacterial genome, providing direct evidence for targeted therapy in metabolic diseases. This lays a solid foundation for disease intervention, microbiome modulation, and metabolic health optimization (Baker *et al.*, 2026).

To systematically navigate these advanced editing capabilities, an iterative 'Design-Build-Test' framework (analytics for metabolic engineering) has been increasingly adopted. In its first phase, researchers leverage genomic data and generative AI to plan genetic modifications *in silico*.

A prime example of this paradigm shift is the use of genome-scale language models like Evo, which enables the *de novo* generation of functional phage sequences from semantic descriptions. However, as a nascent technology, AI-driven design faces critical hurdles: the experimental success rate is currently approximately 5.3% and there is significant "proximity bias" within training datasets that are skewed toward Proteobacteria phages (Nguyen *et al.*, 2024; King *et al.*, 2025). This limited success is often attributed to subtle genomic imbalances—such as disrupted gene dosage or metabolic burdens—that current generative models may not yet fully predict (King, *et al.*, 2025). Despite these bottlenecks, this design-centric approach sets the stage for the subsequent 'Build' phase, where theoretical sequences are transformed into physical genetic entities.

2 Precision Phage Engineering via CRISPR-Cas Systems

As phage engineering transitions from computational design to physical implementation, the 'Build' phase relies heavily on high-precision genomic tools. CRISPR-Cas systems have redefined this landscape by enabling site-specific modifications that are essential for translating *in silico* designs into functional biological entities. Given the diverse requirements of different engineering goals, selecting the appropriate CRISPR-Cas system is critical (Alessa *et al.*, 2025; Baker, *et al.*, 2026). Cas9 (Type II), which generates blunt-end double-strand breaks, remains the most widely adopted tool for general editing tasks. In contrast, Cas12 (Type V) recognizes T-rich PAM sequences and produces staggered cuts, which often results in superior insertion efficiency in specific phage genomes. Conversely, Cas3 (Type I) possesses processive exonuclease activity, enabling large-scale genomic deletions, which is advantageous for disrupting host resistance genes or virulence factors. Furthermore, RNA-targeting systems like Cas13 provide a novel dimension for dynamic intervention without permanently altering the genomic background (Brown, *et al.*, 2017).

More recently, the integration of CRISPR with reverse-transcriptase-based editing systems has further expanded the scope of phage engineering. For instance, retron-derived reverse transcriptases utilize a non-coding RNA template to facilitate the continuous production of multicopy single-stranded DNA (msDNA) donors during phage replication, allowing counter-selection-free, multiplex genome modifications in phages such as λ, T7 and T5. The CRISPR-reverse transcriptase fusion system (recombitron) approach supports the installation of multiple edits—including single-base substitutions, insertions, and deletions—across distant loci on individual phage genomes, achieving up to 99% editing efficiency in λ phage without counter-selection. However, the reported high editing efficiency must be rigorously validated through downstream analytical steps. Typically, this involves harvesting phage plaques from the editing experiment and subjecting them to plaque polymerase chain reaction (PCR) using primers flanking the target locus. Amplicons from randomly selected plaques (ranging from 29 to 55 per experiment depending on the degree of multiplexing) are then subjected to Sanger sequencing to confirm the precise integration of the desired mutations and to quantify the proportion of

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edited versus wild-type genomes (Fishman *et al.*, 2025). For multiplex edits or to assess the purity of a phage population, next-generation sequencing of the entire phage genome provides a comprehensive view of editing outcomes and potential off-target effects. Such advances highlight the potential of CRISPR-reverse transcriptase fusion systems and accelerate the development of bespoke phage therapeutics (Isaev *et al.*, 2022; Fishman, *et al.*, 2025).

The transition from conventional recombination to high-efficiency CRISPR-based systems has dramatically improved the precision and efficiency of phage genome editing, as illustrated in Fig. 1 and summarized in Supplementary Table S1. Building upon these advanced editing capabilities, the field is now advancing toward a new paradigm: *de novo* genome writing and computational design. This shift from 'editing' existing templates to 'writing' entirely synthetic genomes—facilitated by the convergence of synthetic biology and AI—represents a fundamental paradigm change that allows overcoming natural genetic constraints.

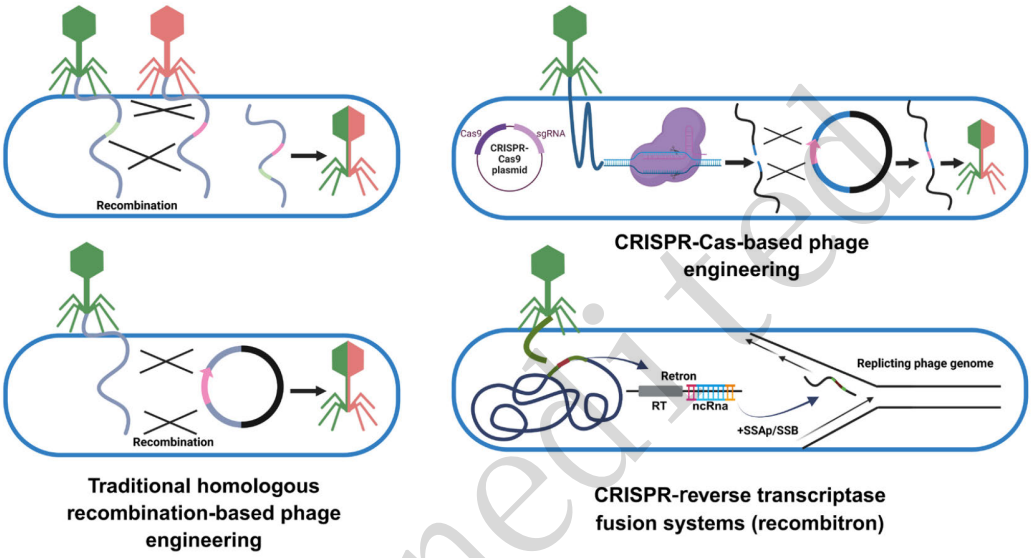

Fig. 1 | Schematic overview of two major strategies for phage genome editing.
(I) Conventional homologous recombination-based editing **(Left)**: this modality relies on the host recombination machinery. Edits can be achieved either by **(Top)** co-infection of a host cell with two parental phages, allowing recombination between their genomes, or **(Bottom)** using a recombination plasmid carrying homologous arms and the desired modification, which recombines with the invading phage genome. Editing efficiency is generally low.
(II) The novel CRISPR-Cas-based phage editing system **(Right)**. **(Top)** Editing mediated by a plasmid expressing the CRISPR-Cas9 system in the host. sgRNA guides Cas9 to generate a double-strand break at a specific locus in the phage genome, and then the host's homologous recombination machinery uses an exogenously supplied repair template to precisely integrate the target sequence, thereby efficiently generating edited progeny phages. **(Bottom)** The recombitron utilizes the Retron system to continuously produce single-stranded DNA donors during phage replication, enabling counter-selection-free, multiplex genome editing in phages such as λ, T7 and T5, with up to 99% editing efficiency in the λ phage.
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3 Synthetic Biology: From Editing to Writing Phages

To translate the aforementioned *in silico* designs into functional phages, the 'Build' phase leverages both precise genome editing and bottom-up synthetic assembly. This transition from editing existing templates to 'writing' entirely synthetic genomes represents a fundamental paradigm shift (Kilcher and Loessner, 2019; Alessa, *et al.*, 2025).

3.1 In Vitro Genome Assembly

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Synthetic biology has enabled a major leap in genome editing to *de novo* genome writing. By chemically synthesizing DNA fragments and assembling them into full phage genomes through efficient techniques such as Gibson Assembly, researchers can freely delete, insert or replace multiple genes in a single step (Kulkarni *et al.*, 2025). The use of highly purified DNA as input eliminates wild-type background, thereby avoiding laborious screening processes (Kilcher *et al.*, 2018).

3.2 Genome Rebooting

For naked DNA genomes assembled *in vitro* that are non-infectious, in order to become functional, replication-competent phages, they must undergo "rebooting" within a cellular or cell-like environment. Traditionally, synthetic genomes are introduced into permissive bacterial hosts via electroporation, where the host's internal machinery reconstitutes viable phage particles (Lenneman, *et al.*, 2021). To overcome the transformation barriers of Gram-positive bacteria, such as *Staphylococcus* and *Bacillus*, researchers have utilized cell-wall-deficient L-form bacteria as universal rebooting chassis. This approach significantly improves genome uptake and has remarkably enabled the cross-genus rebooting of phages from *Listeria*, *Staphylococcus* and *Bacillus*, greatly expanding the versatility of synthetic platforms (Costa *et al.*, 2024; Lyu *et al.*, 2025). More recently, cell-free transcription–translation (TXTL) systems have emerged, allowing phages to be generated directly from synthetic templates *in vitro*. This host-independent approach paves the way for rapid, on-demand, standardized phage production (Kilcher, *et al.*, 2018).

Despite this progress, significant efficiency bottlenecks remain. For example, L-form bacteria are limited by extremely slow growth rates and a dependence on complex media, hindering high-throughput applications (Kilcher, *et al.*, 2018). Similarly, TXTL technology is still in its developmental infancy (Alessa, *et al.*, 2025); for large phage genomes, these systems often suffer from rapid biochemical resource depletion and a lack of standardized protocols for downstream purification and quality control. Consequently, scholars in the field are actively exploring optimized standardization methods to achieve more robust production. A conceptualized workflow is presented in Figure 2.

Regardless of the rebooting method employed, the resulting synthetic progeny must undergo rigorous characterization to ensure functional fidelity. This includes the confirmation of infectivity through plaque assays and analyzing plaque morphology, followed by determining one-step growth curves to assess latent periods and burst sizes (Liu *et al.*, 2024). Finally, the host range of the rebooted phage must be verified against a diverse panel of bacterial strains to ensure that the designed specificity is maintained and to rule out any unintended genomic or phenotypic alterations (Ipoutcha *et al.*, 2024).

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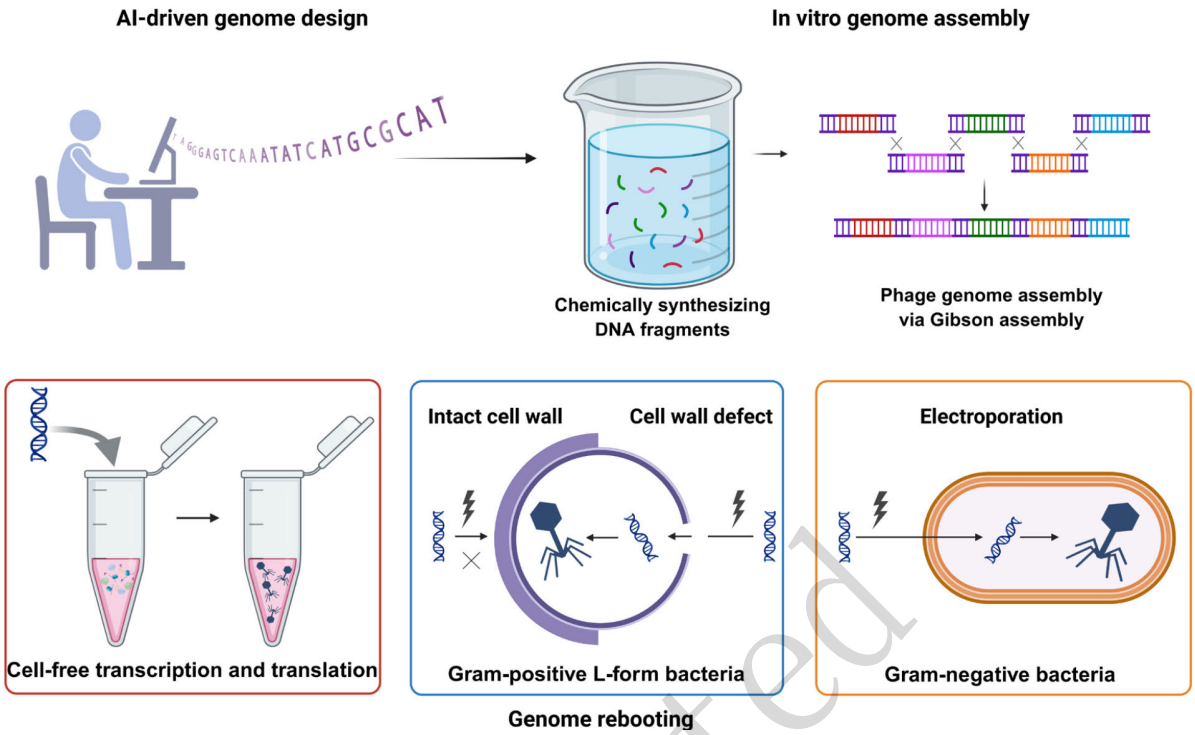

Fig. 2 | De novo synthesis and rebooting of phage genomes.
The schematic illustrates a workflow for constructing functional phages using synthetic biology approaches.
(Top) Genome design and assembly. Functional descriptions are input into a large-scale foundation model (*e.g.*, Evo) to generate complete phage genome sequences *de novo*. DNA fragments representing different regions of the phage genome are chemically synthesized and subsequently ligated into a complete linear genome using *in vitro* assembly methods such as Gibson Assembly.
(Bottom) Genome rebooting. The synthetic genome is converted into infectious phage particles via three main approaches: **(1) electroporation,** introducing the genome into readily transformable host cells, typically Gram-negative bacteria; **(2) L-form bacteria,** where cell wall-deficient forms of Gram-positive bacteria facilitate genome uptake, replication and assembly; and **(3) cell-free systems,** in which the genome serves as a template for *in vitro* transcription and translation, producing all phage proteins and assembling infectious particles, eliminating the need for living cells.
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4 Phage Display and Chemical Modification: Endowing Phages with New Functionalities

4.1 Phage Display

Phage display technology enables the fusion of exogenous peptides or proteins to phage coat proteins, enabling the incorporation of functional molecules into the phage surface. This approach not only allows the high-throughput identification of ligands with strong affinity and specificity for targets such as cancer cells, membrane receptors or extracellular proteins but also extends applicability to diverse biomedical applications including biomarker discovery, targeted drug delivery, tumor therapy, and even blood–brain barrier crossing (Chen *et al.*, 2021; Wang *et al.*, 2025). For example, peptides identified by phage display can selectively target tumor vasculature, microenvironments or cancer-specific receptors, thereby enabling precise recognition and intervention. Moreover, phage display has facilitated the discovery of peptide inhibitors of protein–protein interactions, including covalent cyclic inhibitors with high selectivity, offering powerful tools for drug discovery and molecular probe development (Carmody *et al.*, 2021; Allen *et al.*, 2022).

Despite these successes, phage display is not without its technical challenges. For instance, artifactual enrichment can occur during biopanning due to genomic deletions in the phage vector, such as those observed in M13KE-based libraries. These mutants can acquire a propagation advantage, leading to their unintended selection regardless of their binding affinity to the target (Kamstrup Sell *et al.*, 2022).

4.2 Chemical Modification

In addition to genetic approaches, chemical modification provides another powerful avenue for phage functionalization. Functional molecules such as antibiotics, AMPs, or photosensitizers can be covalently attached to phage capsids, generating hybrid antimicrobials that combine the targeting specificity of phages with the potent activity of small molecules (Adam *et al.*, 2022; Ulfo *et al.*, 2022). For instance, M13 phages conjugated with the photosensitizer Rose Bengal and engineered for epidermal growth factor receptor (EGFR) targeting exhibit potent anticancer effects at low doses, highlighting the synergistic potential of phage-based photodynamic therapy (Banerjee *et al.*, 2010). Similarly, multivalent ligands or antimicrobial agents conjugated to phage capsids can enhance host-pathogen binding affinity and antimicrobial activity (Carrico *et al.*, 2012; Dunne *et al.*, 2019). Beyond the therapeutic effects, chemical conjugation also enables the integration of imaging probes, targeting ligands and therapeutic agents into multifunctional phage-based nanoplatforms, thereby achieving true "theranostics." (Dunne *et al.*, 2021). However, the success of chemical conjugation requires confirmation through both analytical and functional assays. Techniques such as mass spectrometry, fluorescence labeling coupled with gel electrophoresis, or enzyme-linked immunosorbent assay (ELISA) using antibodies against the conjugated moiety can be employed to quantify modification efficiency and confirm covalent attachment (Bernard and Francis, 2014). Crucially, the functionality of the modified phage must be validated. This includes testing the retained infectivity, assessing targeted binding to specific cells (*e.g.*, via flow cytometry or confocal microscopy), and directly measuring the enhanced therapeutic efficacy (*e.g.*, antibacterial or antitumor activity) in relevant *in vitro* models. Overall, the two primary avenues for phage functionalization are genetic display and chemical conjugation, as summarized in Figure 3.

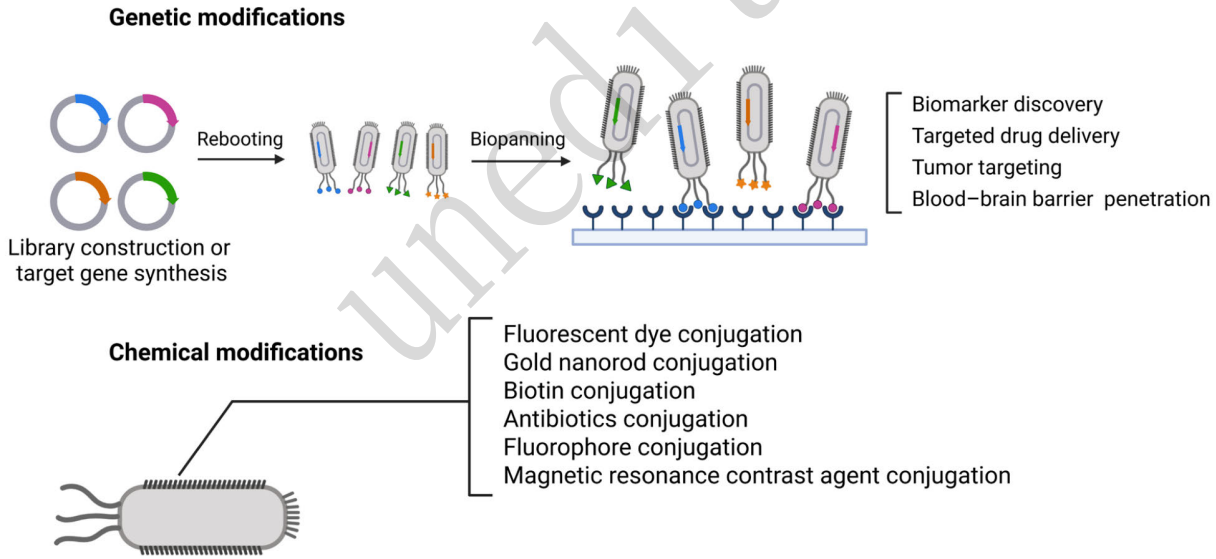

Fig. 3 | Two major strategies for phage functionalization: genetic and chemical modifications.
(Top) Genetic modifications. Phage display is used to generate a library of phages presenting diverse functional peptides or antibodies. Plasmid libraries encoding target genes are combined with genome rebooting to produce phages displaying the desired moieties. Subsequent biopanning allows the selection of phages that specifically bind defined targets, such as tumor cell surface receptors. Genetically engineered phages have broad applications in biomarker discovery, targeted drug delivery, tumor therapy, and enabling drugs to cross the blood–brain barrier.
(Bottom) Chemical modifications. Functional molecules are covalently or non-covalently attached to phage capsid proteins using chemical methods, providing an alternative route for phage functionalization.
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5 Overcoming Natural Barriers: Engineering Phages to Enhance Therapeutic Properties

By leveraging the aforementioned engineering toolbox, researchers are now able to systematically address the key limitations of natural phages in therapeutic applications. Expanding host range, enhancing bactericidal potency, and ensuring biosafety form a "strategic triangle" for rationally designing ideal phage therapeutics. These three objectives are complementary and synergistic, collectively driving the transition of phages from natural products to efficient and safe precision therapeutics.

5.1 Core Role of Receptor-Binding Proteins (RBPs)

Phage host specificity is primarily determined by RBPs located on the tail structure, which recognize specific bacterial surface receptors such as LPS or outer membrane proteins. Consequently, RBP engineering is considered the most direct and effective strategy to modulate phage host range (Latka *et al.*, 2019; Yehl *et al.*, 2019; Huss *et al.*, 2021; Latka *et al.*, 2021). Through a structure-guided chimeric RBP design, modular gene exchange, directed evolution, and high-throughput mutational screening, it is now possible to achieve precise adaptation of phages to different bacterial serotypes or species, enabling predictable and controllable host range expansion. For instance, RBP modular swapping has been shown to effectively modify or extend the host spectra of phages targeting *Listeria*, *E. coli*, *Klebsiella*, and *Salmonella* (Takeuchi *et al.*, 2016; Latka, *et al.*, 2019; Plattner *et al.*, 2019; Krusche *et al.*, 2025). Some phages even carry multiple RBPs, conferring broad or multi-host recognition capabilities (Yehl, *et al.*, 2019; Boeckaerts *et al.*, 2021; Klumpp *et al.*, 2023). In the future, integrating high-throughput screening, AI-based predictive models, and synthetic biology techniques will likely enable more efficient and controllable RBP-driven host range reprogramming (Park *et al.*, 2017; Selle *et al.*, 2020).

5.2 Enhancing Bactericidal Potency: "Arming" Phages

“Armed" phages refer to those engineered not only to lyse host bacteria but also to deliver additional antimicrobial effectors, thereby significantly boosting bactericidal activity and overcoming the limitations of conventional phage therapy. Specifically, phages can serve as delivery vehicles for gene-editing tools, such as CRISPR/Cas systems, to achieve precise cleavage of bacterial genomes. This approach can effectively eliminate multidrug-resistant pathogens including *Staphylococcus aureus*, *E. coli* or *Clostridioides difficile*, and suppress the dissemination of resistance genes (Yosef *et al.*, 2015; Meile *et al.*, 2022; Du *et al.*, 2023; Fa-Arun *et al.*, 2023). Additionally, phages can be engineered to carry bacteriocins, cell wall hydrolases, and other antimicrobial proteins that are co-released upon host lysis, further enhancing killing efficiency and reducing the likelihood of resistance emergence (Chen *et al.*, 2019; Jin *et al.*, 2023).

Advanced strategies also involve decorating phage surfaces with functional nanomaterials (*e.g.*, nanozymes or silver nanoparticles). These are activated within the infection microenvironment to release reactive oxygen species or metal ions, effectively disrupting biofilms and enhancing the clearance of multidrug-resistant bacteria (Chen, *et al.*, 2021; Szymczak *et al.*, 2024).

5.3 Precision Modulation of Gut Microbiota

Through leveraging the above functionalities, engineered phages are developed as targeted delivery vehicles to modulate gut bacterial functions. By incorporating gene-editing systems or synthetic regulatory elements, phages can efficiently introduce CRISPR/Cas tools into specific bacterial hosts, enabling gene silencing, knockout or functional enhancement. This approach enables precise control over bacterial metabolic pathways, attenuates pathogenicity, and suppresses resistance. Ultimately, it helps reshape the gut microbial ecosystem and improve health outcomes (Gencay *et al.*, 2024; Kamm and Beisel, 2024; Mostafavi Abdolmaleky and Zhou, 2024).

Dietary composition exerts profound effects on gut microbiota and is increasingly recognized as a central determinant of metabolic disorders. Dysbiosis promotes oxidative stress, inflammation and intestinal barrier dysfunction, facilitating the systemic dissemination of harmful metabolites and aggravating obesity, insulin resistance, dyslipidemia, and hypertension (Croci *et al.*, 2021; Li *et al.*, 2022; Sun *et al.*, 2022; Du *et al.*, 2025).

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Fiber deficiency, in particular, was shown to reduce the abundance of cellulose-degrading bacteria (*e.g.*, certain species within *Firmicutes* and *Bacteroidetes*) and their associated enzymatic activities, thereby impairing polysaccharide utilization *(Fischer et al., 2020; Martin-Gallausiaux et al.*, 2021; Rous *et al.*, 2025). This leads to a diminished production of SCFAs, critical metabolites that sustain epithelial energy supply, reinforce barrier integrity, and modulate immune and inflammatory pathways through G-protein-coupled receptors and epigenetic regulation *(Baxter et al.*, 2019; Liu *et al.*, 2021; Van Der Hee and Wells, 2021; Liang *et al.*, 2023).

Emerging approaches include the use of engineered phages as precision genetic vehicles to enhance microbiota metabolic capacity. By delivering key enzyme genes (*e.g.*, *but*, *buk*, *pta*, *ack*, *pct*) and polysaccharide-utilization genes (*e.g.*, β-glucosidase, fucose metabolism genes) to targeted bacteria, phages may restore fiber fermentation, increase SCFA production, and ameliorate diet- or disease-driven metabolic imbalance (Tsukuda *et al.*, 2021; Frolova *et al.*, 2022; Wu *et al.*, 2023). Compared with probiotics, phage-based approaches offer advantages in biosafety, manufacturing standardization, and host tolerance, while avoiding the risk of antibiotic resistance gene transfer and opportunistic infections (Aguirre-Ezkauriatza *et al.*, 2010; Shkoporov *et al.*, 2019; Yu *et al.*, 2023). However, implementing phage-mediated enzyme delivery for SCFA restoration (Baker, *et al.*, 2026), as explored in "Sustained in Situ Protein Production and Release in the Mammalian Gut by an Engineered Bacteriophage", involves overcoming complex biological hurdles. First, achieving absolute targeting specificity is paramount. As highlighted in "Phages to the Rescue: In Situ Editing of the Gut Microbiota", engineered phages must be precisely tuned via RBP modification to avoid unintended dysbiosis among commensals (Kamm and Beisel, 2024). Second, the kinetics of delivery presents a 'lytic paradox.' While acute release via lysis mimics the targeted reduction strategies seen in "Engineered Phage with Antibacterial CRISPR–Cas Selectively Reduce *E. coli* Burden in Mice", sustained SCFA production may require non-lytic systems, increasing the risk of chronic host immunogenicity (Gencay, *et al.*, 2024). Furthermore, addressing the potential for horizontal gene transfer of synthetic metabolic payloads remains a critical safety requirement for clinical translation. Collectively, engineered phages represent a promising avenue for restoring microbiota functionality, re-establishing SCFA homeostasis, and mitigating metabolic disease progression.

6 Conclusion and Future Perspectives

The field of phage engineering is evolving rapidly, with synthetic biology tools enabling unprecedented control over phage design and function (Peng *et al.*, 2025). The integration of CRISPR-Cas systems has revolutionized genome editing, while *de novo* synthesis and cell-free rebooting platforms are paving the way for fully synthetic phages tailored for specific applications (Nethery *et al.*, 2022). These advancements are critical for overcoming the historical limitations of phage therapy.

Looking ahead, several emerging trends are poised to shape the next generation of phage-based therapeutics. First, the use of artificial intelligence and machine learning for predicting RBP-receptor interactions and optimizing phage host range will accelerate the design process (Boeckaerts, et al., 2021; Gao et al., 2026). Second, the development of universal chassis systems, including L-form bacteria and TXTL platforms, will streamline the production of synthetic phages for diverse bacterial hosts (Kilcher, *et al.*, 2018; Garenne *et al.*, 2021; Liyanagedera *et al.*, 2022). Third, the convergence of phage display and chemical modification will enable the creation of multifunctional "smart" phages capable of targeted drug delivery, imaging, and real-time monitoring of therapeutic response (Emencheta *et al.*, 2023; Ullrich *et al.*, 2024).

In the context of microbiome modulation, engineered phages offer a novel strategy for the precision editing of gut bacterial communities without broad-spectrum disruption. This approach holds particular promise for metabolic diseases, where the phage-mediated delivery of SCFA-producing enzymes could restore gut homeostasis and alleviate systemic inflammation. However, challenges remain in ensuring the stability, specificity and safety of these constructs *in vivo* (Gencay, *et al.*, 2024).

Regulatory and manufacturing hurdles also need to be addressed. The lack of standardized protocols for

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phage production, purification and quality control has so far impeded large-scale clinical adoption (Pires *et al.*, 2016; Nair and Khairnar, 2019). Unlike natural phages, which are often administered under compassionate use programs or handled as traditional biologics, engineered phages—especially those incorporating recombinant DNA—are increasingly categorized as Genetically Modified Organisms (GMOs) or Advanced Therapy Medicinal Products (ATMPs) (Pires, *et al.*, 2016). This classification subjects them to more stringent oversight by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Consequently, clinical translation requires not only standard pharmacological validation but also rigorous environmental risk assessments and specialized frameworks to evaluate the issues of genetic stability and potential horizontal gene transfer (Nair and Khairnar, 2019).

The regulatory framework for engineered phages is still evolving, with questions surrounding long-term safety, environmental impact, and potential off-target effects. Addressing these safety concerns requires the integration of robust environmental containment strategies into the phage design phase. One promising approach is nutritional biocontainment, where the engineered phage is rendered dependent on synthetic, non-canonical amino acids (ncAAs) that are unavailable in natural settings, effectively restricting viral replication to the controlled therapeutic environment (Mitsunaka, *et al.*, 2022). Synthetic 'kill-switches' can also be engineered using genetic toggle switches or logic gates that induce genome degradation upon exposure to extra-intestinal environmental cues, such as temperature shifts or the absence of specific host-derived metabolites (Gencay, *et al.*, 2024). Furthermore, genomic recoding of the phage sequence to match only specialized, engineered host strains provides an ultimate layer of biological isolation, preventing the functional horizontal transfer of synthetic payloads to the broader environmental microbiota. Implementing such multi-layered safeguards is essential for meeting the stringent quality control and safety standards required for broad clinical application (Pires, *et al.*, 2016; Nair and Khairnar, 2019).

In summary, phage engineering represents a paradigm shift in our approach to combating bacterial infections and modulating complex microbial ecosystems. By leveraging the tools of synthetic biology, researchers are transforming phages into programmable therapeutics with enhanced capabilities. As the field continues to advance, interdisciplinary collaboration between biologists, engineers and clinicians will be essential to realize the full potential of engineered phages in medicine and beyond.

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Electronic supplementary materials

Supplementary material (Table S1) associated with this article can be found in the online version.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Author contributions

Conceptualization: A.F.; Writing – Original Draft: S.X., X.F., G.W.,S.H.and Z.W.; Writing – Review & editing: all authors. Supervision: A.F.All authors are expected to uphold research integrity and share responsibility for the final publication.

Compliance with ethics guidelines

Conflict of Interest: Shujie XU, Xianglin FEI, Guangyao WANG, Shijiao HUAN, Ziyue WU, and Aikun FU declare that they have no conflict of interest. Ethics Statement: This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

During the preparation of this work, the authors used ChatGPT in order to improve language and readability. After using this

tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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