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# Antimicrobial peptides in anti-infective and immunomodulatory applications: Mechanisms, challenges, and emerging computational design

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## ABSTRACT

Antimicrobial Peptides (AMPs) evolutionarily conserved effectors of innate immunity, have emerged as multifunctional agents with broad-spectrum antimicrobial activity, immunomodulatory capabilities, and potential applicability in cancer therapy. Despite their functional diversity and biological potency, the clinical translation of AMPs remains constrained by significant challenges, including proteolytic instability, hemolytic toxicity, high production costs, and protracted development pipelines. This review examines how the diverse molecular mechanisms through which AMPs exert antimicrobial effects—including membrane disruption, intracellular targeting, and immunomodulation—are intrinsically linked to their structural diversity and ecological breadth. We critically evaluate engineering strategies that improve developability, including rational sequence modification, nano/targeted delivery, optimized formulations, and combination regimens with antibiotics or bacteriophages. As a complementary perspective, we briefly summarize recent progress in computational prediction and AI-driven screening/design for AMP discovery and multi-objective optimization, while highlighting major limitations such as dataset bias, scarcity of reliable negative data, and experimental validation bottlenecks. Beyond infectious disease, we discuss the impact of AMPs on reshaping the tumor microbiota-immune axis, revealing a dual function in both microbial control and immune regulation within oncogenic contexts. Overall, this review provides a balanced appraisal of evidence and translational pathways for advancing AMP-based therapeutics.

**Keywords:** Antimicrobial peptides; Mechanism; Clinical translation; Computational screening

Antimicrobial resistance (AMR), recognized as a critical threat under the United Nations “One Health” strategic framework (US Centres for Disease Control and Prevention, 2025), is rapidly reshaping the global healthcare landscape, potentially ushering in a post-antibiotic era in which infections may again account for over one-third of global mortality (Sugden et al., 2016). Projections indicate that by 2050, AMR could lead to 10 million deaths annually (O’Neill, 2016). This crisis stems largely from the historical overuse of antibiotics, with evidence demonstrating that even a single course can increase resistance gene abundance in the gut microbiota threefold (de Nies et al., 2022). Persistent antibiotic residues in the environment further exacerbate selective evolutionary pressure on microbial populations, driving resistance through adaptive restoration mechanisms (Larsson et al., 2023). The



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pace of resistance evolution now far exceeds the development of new antimicrobial agents, with legacy classes such as β-lactams and macrolides exhibiting an exponential decline in efficacy. The implications extend beyond human health to threaten animal welfare, agricultural sustainability, and global economic stability (UNEP, 2023), underscoring the urgent need for innovative antimicrobial strategies.

Antimicrobial peptides (AMPs) offer a compelling alternative, characterized by a tri-modal mechanism of action: membrane disruption, interference with intracellular metabolism, and modulation of host immune responses. For example, AMPs derived from amphibian skin penetrate microbial membranes through coordinated electrostatic and hydrophobic interactions, forming transmembrane pores that disrupt microbial intracellular homeostasis (Brogden, 2005) and precisely modulate the infection microenvironment by regulating host immune responses and inhibiting pro-inflammatory factor release (Mookherjee et al., 2020). Although many AMPs act on bacterial envelopes and/or engage multiple targets—features that can make resistance less straightforward than for single-target antibiotics—adaptive responses and resistance/tolerance to AMPs have been well documented. Reported mechanisms include cell-envelope remodeling and surface charge modifications (e.g., teichoic-acid D-alanylation and phospholipid lysinylation that reduce binding of cationic peptides), two-component-system-mediated remodeling of membrane/lipopolysaccharides (LPS) composition, upregulated efflux, secretion of proteases that degrade peptides, and biofilm- and stress-response-associated protection (Andersson et al., 2016). Therefore, statements about lower resistance potential should be framed conditionally and supported by appropriate long-term passaging and/or in vivo evidence, and AMP resistance mechanisms should be considered as design constraints for engineering and combination strategies. As such, AMPs provide a robust theoretical and functional foundation for the development of next-generation anti-infective therapies.

Nonetheless, the clinical translation of AMPs remains constrained by several major technical bottlenecks. First, conventional extraction methods often rely on large-scale biological sourcing and complex purification processes, making production inefficient and costly (Xue et al., 2023). Second, although machine learning models, such as deep neural network-based AMP classifiers, have achieved high predictive performance (96.48% sensitivity and 91.01% accuracy), their current value lies mainly in candidate prioritization and sequence optimization rather than in replacing experimental validation, while the mechanistic determinants of AMP membrane selectivity and immune regulatory timing remain poorly defined. Third, many AMPs remain vulnerable to proteolytic degradation and may face limitations in stability, safety, and bioavailability under physiological conditions (Jiang et al., 2025) Accordingly, only a limited number of AMP candidates have progressed to advanced clinical evaluation for resistant infections (Cresti et al., 2024).

Accordingly, this review is organized around the structure–function relationships and translational engineering of antimicrobial peptides (AMPs) (Figure 1). We first summarize AMP sources and structural diversity along with key physicochemical determinants of activity. We then discuss multimodal mechanisms of action (membrane disruption, intracellular targeting, and host-directed immunomodulation) and representative evidence across antibacterial, antiviral, antifungal, and selected anticancer-related contexts. Next, we review major discovery and optimization strategies, including mass spectrometry/omics-enabled mining, high-throughput display-based selection, rational sequence engineering, and delivery or formulation approaches; within this section, computational prediction and AI-driven screening/design are discussed as emerging complementary toolsets rather than a central theme. Finally, we critically evaluate key barriers to clinical translation (stability, toxicity, pharmacokinetics/delivery, manufacturing, and cost) and outline priorities for future research.

## SOURCES AND STRUCTURAL DIVERSITY OF AMPs

AMPs are evolutionarily conserved bioactive molecules widely distributed across the microbial, plant, and animal kingdoms. According to the Database of Antimicrobial Activity and Structure of Peptides (DBAASP), animals account for approximately 68% of all known AMPs, followed by bacteria (17%), plants (8%), and fungi (7%) (Wang et al., 2024a). Although naturally derived AMPs exhibit potent antimicrobial and immunomodulatory functions, their clinical application has been hindered by poor stability, cytotoxicity, and high production costs. To address these limitations, synthetic AMPs have been developed to enhance functional resilience, structural adaptability, and therapeutic range, encompassing antibacterial, anticancer, and immunomodulatory activities.

Microbially derived AMPs, secreted by both bacteria and fungi, serve as core components of innate immune defense. These peptides exert broad-spectrum antibacterial effects by disrupting pathogen membrane integrity and energy metabolism, enabling bacteria to exert antibacterial activity against phylogenetically similar or distinct microbial competitors (Hassan et al., 2012). For example, Gram-negative bacteria secrete colicins and microcins—narrow-spectrum peptides that selectively target specific Gram-negative strains—whereas Gram-positive bacteria produce bacteriocins such as lantibiotics, which frequently exhibit broader antibacterial spectra (Mihaylova-Garnizova et al., 2024).

Plant-derived AMPs, also known as plant defensins, are expressed in various tissues, including stems, seeds, and leaves. These peptides comprise structurally heterogeneous families such as thaumatin-like proteins, defensins, and ecdysteroid-like peptides (Das et al., 2016), characterized by high cysteine content and extensive disulfide bonds (Kumar et al., 2018), which confer remarkable conformational stability and resistance to proteolysis.

Animal-derived AMPs exhibit remarkable structural heterogeneity and are broadly categorized into three major groups: insect, mammalian, and amphibian peptides. In insects, such as *Drosophila melanogaster*, the 26-amino acid peptide metchnikowin (Mtk) exerts antifungal activity by selectively inhibiting β-1,3-glucan synthase and mitochondrial succinate-CoQ reductase in sensitive fungal species (Stączek et al., 2024). Mammalian AMPs, particularly β-defensins from species including cattle, pigs, dogs, and cats, are important components of mucosal immunity and have been reported to help limit pathogen colonization, support barrier defense, and contribute to host health and resilience. For instance, the bovine β-defensin TAP, isolated from respiratory epithelial tissue, exhibits potent bactericidal activity against major causative agents of bovine pneumonia (Valdez-Miramontes

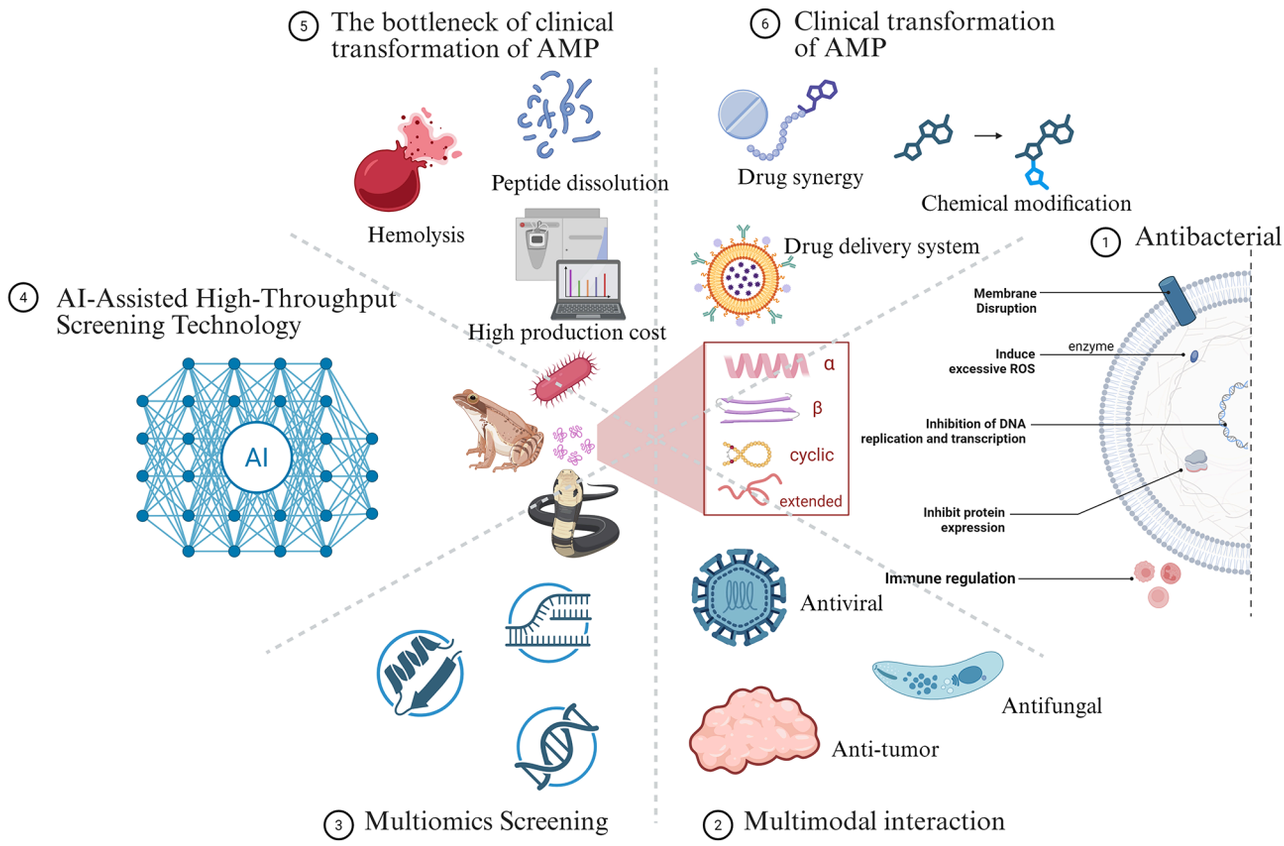


**Figure 1 Overview of AMP research strategies and applications**

This picture elucidates the research progress and translational strategies of AMPs. AMPs exhibit broad-spectrum bioactivity against bacteria, fungi, viruses, and tumors. Notwithstanding their functional diversity and potent biological effects, clinical translation remains constrained by proteolytic instability, hemolytic toxicity, high production costs, and prolonged development cycles. This figure highlights key technologies such as AI-assisted high-throughput screening, multi-omics screening, and multimodal interactions, along with AMP applications in antibacterial, antiviral, antifungal, and anti-tumor therapies. The clinical transformation bottlenecks and challenges in AMP development, including high production costs and peptide dissolution, are also summarized.

et al., 2021). Amphibian AMPs display extraordinary structural and functional diversity, with current classifications encompassing 40 to 100 distinct peptide families. Complex peptide mixtures from *Xenopus laevis*, *Rana nigromaculata*, and *Rana chensinensis* demonstrate broad-spectrum antibacterial activity through membrane-permeabilizing mechanisms (Rollins-Smith, 2023).

Cryptides are functional short peptides released from larger precursor proteins via specific proteolytic cleavage or post-translational processing. In their intact form, these precursors typically lack detectable activity from the embedded cryptic sequences, rendering cryptides a hidden reservoir of bioactive motifs within the proteome. Their generation reveals a dynamic layer of bioactive peptides beyond the canonical proteome—peptides with independent and often unpredictable biological functions that significantly expand the organism's functional molecular repertoire (Autelitano et al., 2006). Growing evidence shows that structural or binding proteins not classically linked to immunity can yield antimicrobial peptide fragments when processed by digestive enzymes, inflammation-associated proteases, or tissue-specific proteolytic systems. These fragments commonly exhibit cationic, amphipathic properties and are enriched in hydrophobic and basic residues—features enabling them to disrupt bacterial membranes or intracellular targets (Pimenta & Lebrun, 2007). Notable examples include lactoferricin, derived from the N-terminus of lactoferrin, which displays potent, well-defined antimicrobial activity often surpassing that of the parent protein (Sinha et al., 2013), and buforin II, a histone H2A-derived peptide whose membrane-translocating ability—and thus antimicrobial efficacy—is governed by structural elements like its proline hinge region (Park et al., 2000). These cases confirm that cryptic antimicrobial peptides not only exist but are amenable to rational design based on defined structure-function relationships.

Advances in synthetic biology and computational modeling have vastly expanded the repertoire of AMP sources. Modern biotechnologies, including site-directed mutagenesis, high-throughput synthetic library screening, probabilistic modeling, *in silico* structural prediction, and phage or ribosome display systems, have greatly enhanced the development of novel AMPs with optimized antibacterial activity, reduced cytotoxicity, and increased stability (Wang et al., 2024a). For example, recent studies have employed computational approaches to predict antimicrobial and cell-penetrating peptides, leading to the synthesis of two novel peptides derived from *Natterins* (de Cena et al., 2025), as well as the development of novel amphipathic AMPs based on reengineered α-helical frameworks (Zhang et al., 2025b). These design strategies effectively addressed key limitations of natural AMPs, including susceptibility to hydrolysis and high cytotoxicity, while enhancing antibacterial activity.

The functional landscape of AMPs is tightly linked to their structural architecture. Based on secondary structure, AMPs

can be categorized into five principal groups: α-helical peptides, β-sheet peptides, αβ-hybrid peptides, extended peptides, and topologically complex peptides (Gani et al., 2025, Mookherjee et al., 2020) (Figure 2). Among these, α-helical and β-sheet structures are among the most promising candidates to replace antibiotics owing to their well-defined amphipathic nature and ability to insert into and disrupt microbial membranes—properties essential to their antimicrobial efficacy.

α-Helical AMPs adopt a helical conformation characterized by a spatial segregation of hydrophobic and hydrophilic residues, a property known as amphipathicity. This amphipathic arrangement typically emerges upon interaction with lipid membranes, membrane-mimetic environments, or through peptide aggregation, facilitating selective insertion into microbial membranes (Kumar et al., 2024). Electrostatic interactions are further enhanced by C-terminal amidation, which increases affinity for negatively charged bacterial membranes (Chen et al., 2023). Hydrophobic residues, such as leucine, alanine, glycine, and lysine, intercalate into lipid bilayers, while hydrophilic regions contribute to the formation of transmembrane pores, disrupting membrane integrity (Bin Hafeez et al., 2021).

β-Sheet AMPs maintain rigid conformations stabilized by intramolecular disulfide bridges, which confer structural integrity even in aqueous environments. These peptides exert antimicrobial activity through β-hairpin motifs that present hydrophobic surfaces capable of associating with fungal sphingolipids, bacterial membranes, and certain viral envelopes (Bucataru & Ciobanasu, 2024), involving surface aggregation and membrane destabilization without the need for significant conformational change.

αβ-Hybrid peptides incorporate both α-helical and β-sheet elements, enabling complex amphipathic topologies. These structures frequently involve disulfide bond-mediated stabilization of α-helices anchored to β-sheets, forming cysteine-stabilized αβ (CSαβ) structures (Dash et al., 2019). This architectural configuration enhances structural stability and optimizes membrane-targeting efficiency.

Extended peptides, classified as non-αβ-expanding AMPs,

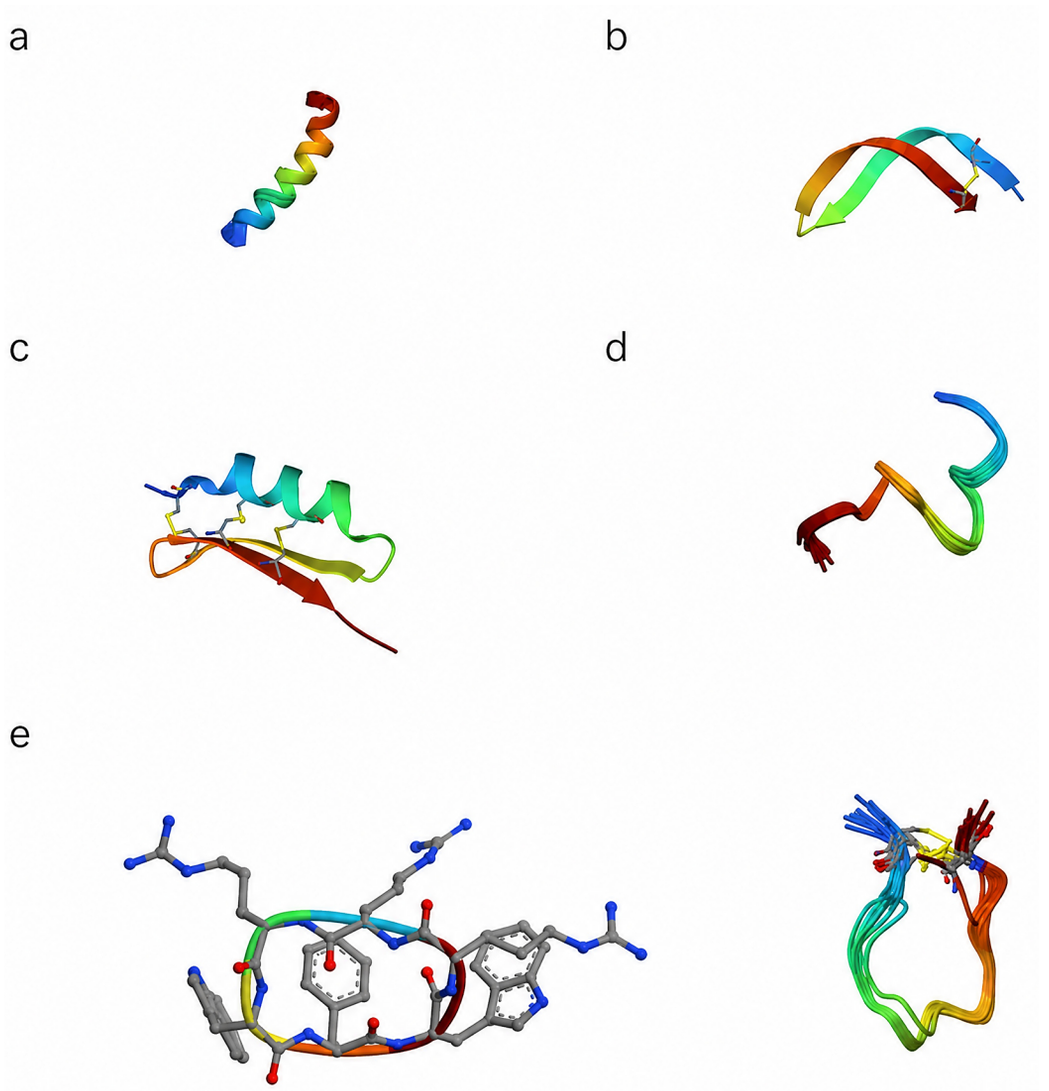


**Figure 2 Representative structural classes of AMPs**

a: α-helical peptide, e.g., alyteserin-1C (PDB: 2L5R); b: β-sheet peptide, e.g., arenicin-1 (PDB: 2JSB); c: αβ-hybrid peptide, e.g., termicin (PDB: 1MM0); d: Extended peptide, e.g., VG16KRKP (PDB: 2MWL); e: cyclic peptide with complex topological features, e.g., RRWFWR (PDB: 2OTQ) and cyclic indolicidin peptide (PDB: 1QX9).

lack defined α-helical or β-sheet secondary structures and instead exhibit flexible, disordered backbones. These peptides are commonly categorized into tryptophan-rich, proline-rich, and glycine-rich subclasses, each associated with distinct mechanisms of antimicrobial action. For instance, KAMP, the first non-αβ-structured AMP identified in humans, compromises bacterial cell envelope integrity and induces pore formation (Lee et al., 2016).

Cyclic peptides, a structurally complex class of AMPs, are characterized by covalently closed backbones that impose conformational rigidity while preserving high charge density and amphipathic organization. This topological constraint enhances their capacity to interact with and destabilize microbial membranes, markedly increasing their pore-forming efficiency and antibacterial activity (Bin Hafeez et al., 2021). Their closed-ring architecture minimizes terminal exposure, thereby reducing protease accessibility and conferring superior serum stability, a critical attribute for sustained therapeutic applications (Falanga et al., 2017). At membrane interfaces, cyclic peptides rapidly fold into β-hairpin conformations within nanoseconds, enabling efficient insertion into lipid bilayers through symmetrically arranged lysine residues that anchor the peptide to the membrane core (Mika et al., 2011). Functionally, cyclic peptides offer a unique combination of high surface area and defined spatial geometry, supporting high affinity and selectivity for target binding. Their compatibility with nanodelivery systems further enhances pharmacokinetic performance by improving peptide stability, bioavailability, and therapeutic specificity (Davani-Davari et al., 2025). Notably, cyclic AMPs have been engineered to selectively target membrane phospholipids of *Borrelia burgdorferi*, demonstrating potent bactericidal activity with minimal cytotoxicity and successful penetration of the blood-brain barrier (Mochnáčová et al., 2025). Beyond direct antimicrobial action, cyclic peptides also exhibit carrier capabilities akin to small molecules. For example, arginine- and tryptophan-based cyclic peptides have been designed to efficiently deliver siRNA to breast cancer cells (Mozaffari et al., 2019). Furthermore, co-administration of cyclic peptides with conventional antibiotics has been shown to synergistically enhance inhibition of multidrug-resistant bacteria (Oh et al., 2014). Thus, cyclic peptides not only exert potent antibacterial efficacy but also serve as efficient and safe carriers for therapeutic agents in infectious and oncologic contexts.

AMPs are typically small molecules, with most exhibiting molecular weights below 10 kDa. Approximately 88% of AMPs consist of fewer than 50 amino acids, while around 10% contain 50 to 100 amino acids. Short-chain peptides (<20 amino acids) typically lack the conformational stability required to maintain defined secondary structure, potentially reducing their antibacterial activity (Chen et al., 2023, Wang, 2022). However, truncated variants of native AMPs display reduced cytotoxicity compared to their full-length counterparts (Thakur et al., 2022), thereby improving safety profiles for therapeutic applications. The antimicrobial function of AMPs frequently depends on a limited subset of key amino acids; targeted modifications of single or multiple residues can substantially alter physicochemical characteristics and modulate efficacy. For instance, modification of tryptophan residue number and position in the peptide DAP-7 has been shown to result in potent antibacterial activity against both antibiotic-sensitive and -resistant strains of *Propionibacterium acnes* (Kim et al., 2025).

Most AMPs (approximately 88%) are cationic, with net charges typically ranging from +2 to +13. They rely on electrostatic attraction to negatively charged components of bacterial membranes to exert a detrimental effect (Bucataru & Ciobanasu, 2024). Modulating cationicity through the inclusion of basic residues, such as lysine, arginine, or histidine, can influence electrostatic interactions and membrane affinity. Insertion of 10 lysine residues into the sequence of magainin-2 has been shown to enhance antibacterial efficiency, although it simultaneously induces hemolytic side effects, highlighting a trade-off between potency, biocompatibility, and clinical safety (Gagat et al., 2024). Hydrophobicity, defined by the percentage of hydrophobic residues within the sequence, governs membrane insertion and translocation efficiency. Moderate hydrophobicity is essential for effective lipid bilayer interaction, while excessive hydrophobicity may provoke nonspecific membrane disruption and induce hemolysis (Zhang et al., 2025b). The functional architecture of AMPs arises from a precise balance between cationic charge and hydrophobicity, which collectively determines amphipathicity—a critical determinant of membrane-targeting capability. Under sufficient positive charge conditions, replacing charged residues with hydrophobic amino acids such as leucine or phenylalanine can further enhance antibacterial efficacy without significantly altering secondary structure (Ganesan et al., 2023). These substitutions have been shown to improve the ability of the peptide to disrupt lipid vesicles and bacterial membranes (Saint Jean et al., 2018), while C-terminal amidation has been shown to increase charge density and strengthen membrane binding capacity.

## MOLECULAR MECHANISMS AND DYNAMIC VISUALIZATION OF AMP ACTION

AMPs have evolved as integral components of innate immunity, executing targeted antimicrobial activity through multifaceted modes of action. Their antibacterial efficacy primarily arises from three mechanistic axes: membrane disruption, metabolic interference, and immunomodulation (Figure 3). This combinatorial approach can improve killing efficiency and may make resistance development less straightforward than with single-target agents. Beyond antibacterial applications, AMPs demonstrate promising antiviral, anticancer, and antifungal activities, highlighting their potential as novel therapeutic agents. To provide a strain-referenced, order-of-magnitude context for commonly reported in vitro potency metrics, Table 1 summarizes QC target MIC ranges for selected clinically used antibiotics and reported MIC values for a small set of representative AMP candidates that have reached advanced preclinical/clinical evaluation. MICs for AMPs can vary substantially with assay conditions (e.g., medium composition and ionic strength, inoculum size, incubation parameters), and physiological environments introduce additional constraints such as proteolytic degradation, serum binding, local pH/salt effects, tissue distribution and clearance, and hemolytic/cytotoxic liabilities that collectively determine exposure and therapeutic window. Loffredo et al. showed that when bacterial density exceeded the standard MIC inoculum of $5\times10^5$ CFU/mL, AMP MIC values increased by 3-fold to more than 100-fold, whereas a clearer plateau was observed only across the $5\times10^1$–$5\times10^5$ CFU/mL range (Loffredo et al., 2021). These data indicate that the apparent antibacterial potency of AMPs is determined not only by peptide sequence, but also by inoculum size and

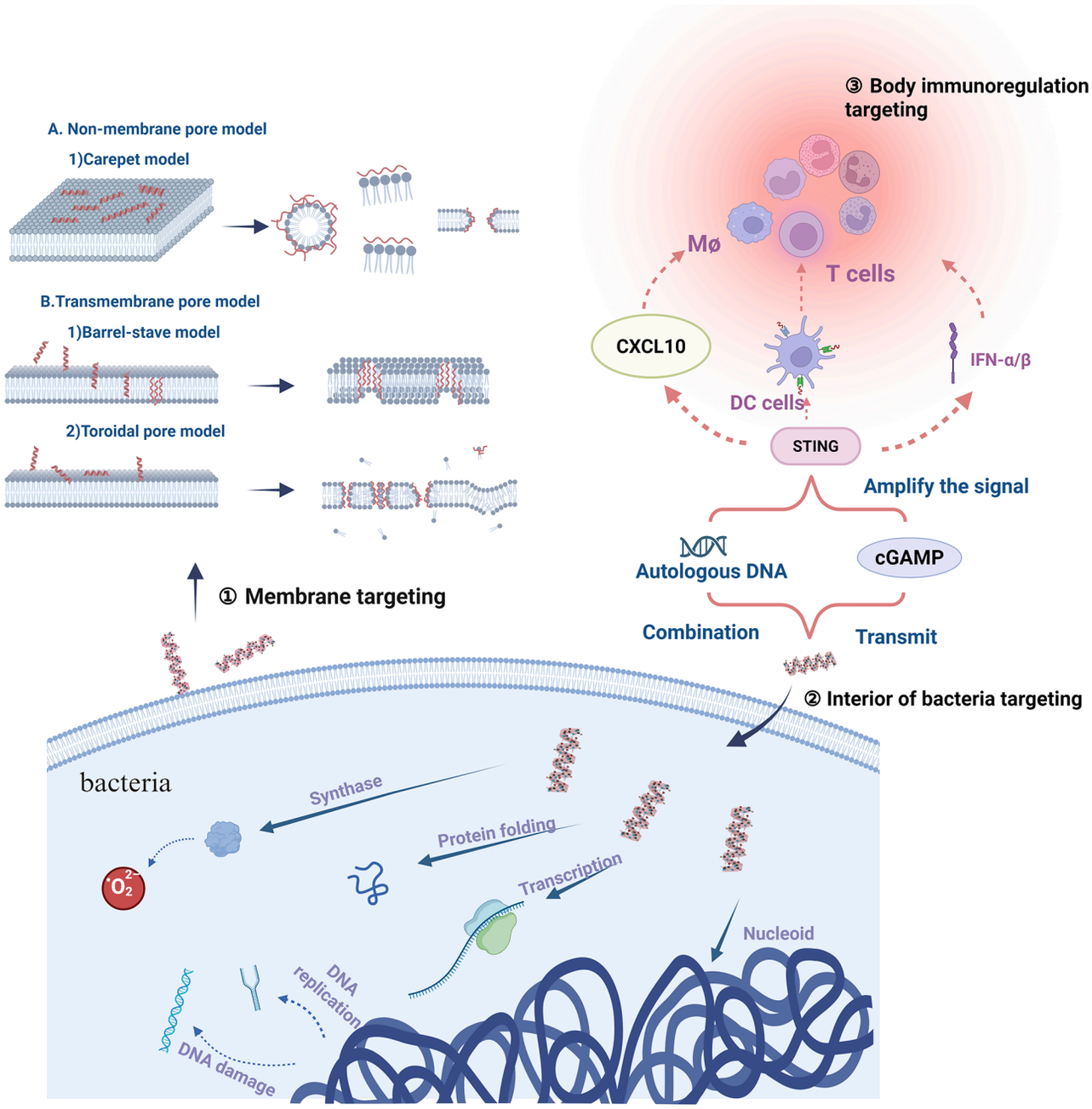


**Figure 3 AMP-mediated membrane disruption and immunoregulatory mechanisms**

Upper panel shows AMPs transmembrane mechanisms, including non-pore carpet model and two pore-forming mechanisms (barrel-stave and toroidal), illustrating membrane disruption. Middle section depicts immune activation via interactions between T cells, dendritic cells, and signaling molecules (STING, NF-κB). Lower panel highlights intracellular effects, including modulation of mitochondrial and ribosomal function by vitamin D and Lp-E/PGE2, as well as enhancement of DNA repair and protein folding.

assay configuration, which substantially limits direct cross-study comparisons. Therefore, cross-study MIC comparisons should be interpreted cautiously, and the table is intended to provide contextual benchmarks rather than strict rank-order potency equivalence between experimental peptides and clinically standardized antibiotics.

As clearly shown in Table 1, conventional clinical antibiotics typically exhibit lower MICs under standardized quality-control conditions, reflecting decades of optimization for in vitro antibacterial efficacy. However, the table also suggests that some AMPs can exhibit measurable antibacterial activity under MIC testing conditions, although their translational potential still requires evaluation beyond in vitro potency. The promise of AMPs is not solely based on theoretical advantages—such as unique mechanisms of action or immunomodulatory properties—but is substantiated by high-quality MIC data confirming their tangible antibacterial activity against key pathogens, including those with drug-resistant profiles. Therefore, with further optimization of delivery strategies, formulations, and safety profiles to expand their in vivo therapeutic window, AMPs hold clear potential as valuable complements to existing antibiotics.

Accordingly, the main advantage of AMPs does not lie in consistently outperforming conventional antibiotics in terms of in vitro MIC values, but in providing an engineerable anti-infective scaffold whose translational value depends on

**Table 1 Comparison of MICs of selected clinically used antibiotics and representative AMPs against reference quality-control strains and/or contemporary clinical isolates**

| Drug/Peptide | Reference strain | MIC (as reported) | source |
|---|---|---|---|
| Cathelicidin-BF | *P. acnes* ATCC6919 | 4.7 μg/mL | (Wang et al., 2011) |
| | *S. aureus* ATCC2592 | 4.7 μg/mL | |
| | *S. epidermidis* 09A3726 | 2.3 μg/mL | |
| Arenicin-3 | *E. coli* ATCC 25922 | 1 μg/mL | (Elliott et al., 2020) |
| | *K. pneumoniae* BAA-2146 | 2 μg/mL | |
| RK22 | *S. aureus* ATCC6538 | 6.25 μg/mL | (Lu et al., 2023) |
| | MRSA11 | 6.25μg/mL | |
| | clinical *S. aureus* isolate SA220823 | 12.5 μg/mL | |
| Vancomycin | *S. aureus* ATCC 29213 | 0.5-2 μg/mL | |
| Colistin | *E. coli* ATCC 25922 | 0.25 μg/ml | (Elliott et al., 2020) |

The MIC range reported for vancomycin corresponds to the EUCAST quality-control (QC) target range for routine MIC testing using the indicated QC strain (Staphylococcus aureus ATCC 29213). In contrast, MIC values for representative AMPs are extracted from individual literature reports and may vary substantially depending on assay conditions, including medium composition, ionic strength, inoculum size, and incubation parameters. Therefore, this table is intended to provide strain-referenced contextual benchmarks rather than strict cross-study rank-order comparisons between experimental AMPs and clinically standardized antibiotics.

simultaneously achieving stability, safety, systemic exposure, and demonstrable in vivo efficacy under physiologically relevant conditions.

**Membrane disruption: targeted disruption of physical barriers**

The primary mode of AMP activity initiates with selective interaction at the bacterial surface. Cationic AMPs exhibit high affinity for negatively charged components such as LPS in Gram-negative bacteria and teichoic acids in Gram-positive cell walls. These electrostatic interactions facilitate rapid peptide localization at the membrane interface (Luo and Song, 2021). In Gram-positive bacteria, the peptidoglycan-rich matrix serves as the initial binding substrate. For instance, teixobactin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) by concurrently targeting lipid I, lipid II, and the precursor lipid III of teichoic acid biosynthesis, compromising cell wall synthesis at multiple points (Karas et al., 2020). Gram-negative bacteria pose additional challenges due to their protective LPS-rich outer membrane (Savitskaya et al., 2023). Effective AMP-mediated activity often requires both LPS destabilization and inhibition of membrane-associated efflux systems. The insect-derived AMP thanatin exemplifies a multifaceted strategy against Gram-negative outer membranes. Initially, thanatin binds electrostatically to LPS, inducing the aggregation of LPS micelles into supramolecular assemblies that disrupt outer membrane architecture (Sinha et al., 2017). Concurrently, thanatin competitively displaces $Ca^{2+}$ ions bound to LPS, disrupting membrane anchoring and inducing LPS release (Dash et al., 2021). In addition to its membrane-disruptive effects, thanatin directly inhibits LPS export by targeting LptA, a key component of the LPS efflux system (Moura et al., 2020), thereby impairing outer membrane function. This coordinated disruption of both membrane integrity and homeostatic maintenance culminates in bacterial lysis and intracellular AMP entry, facilitating further antimicrobial action within the cytoplasmic space.

The membrane-disruptive properties of AMPs constitute the foundation for their rapid and selective bactericidal function. A key factor underlying this selectivity is the differential membrane composition between prokaryotic and eukaryotic cells. Bacterial membranes are enriched in anionic phospholipids, such as dioleoylphosphatidylglycerol (DOPG), which promote strong electrostatic interactions with cationic AMPs. Thermodynamically, AMPs binding to bacterial membranes displaces surface-adsorbed counterions, resulting in increased system entropy. This process is exothermic due to the release of heat, and the overall gain in entropy drives spontaneous membrane association (Halder & Karmakar, 2022).This thermodynamic favorability enhances AMPs selectivity for bacterial membranes while reducing cytotoxicity toward host cells.

AMP-induced pore formation is mechanistically categorized into three principal models: barrel-stave, toroidal pore, and carpet.

The barrel-stave model, although less frequently observed, is exemplified by peptides such as alamethicin (Mihajlovic & Lazaridis, 2010) and hexa-arginine PG-1 (Su et al., 2011). In this process, monomeric AMPs adopt α-helical conformations upon membrane contact and orient perpendicularly to the lipid bilayer. These monomers insert into the hydrophobic membrane core and undergo self-assembly into multimeric helical bundles via hydrophobic side-chain interactions. As oligomerization progresses, the resulting pore diameter increases, enabling ion flux and metabolite leakage (Shai, 1999). Notably, this process is highly concentration-dependent: at low concentrations, AMPs remain monomeric, while at higher concentrations, oligomerization drives pore formation.

In contrast, the toroidal pore model, exemplified by peptides such as melittin (Mihajlovic & Lazaridis, 2010) and magainins (Ludtke et al., 1996), relies on peptide aggregation as a prerequisite for pore formation. Once the peptide-to-lipid (L/P) ratio exceeds a critical threshold, the lipid bilayer bends inward to create transient, ring-like pores (Sengupta et al., 2008). Unlike barrel-stave pores, these toroidal pores are inherently unstable, often expanding through repeated opening and closing cycles or membrane fusion events, which exacerbate membrane damage (Luo & Song, 2021). At elevated L/P ratios, melittin aggregates into surface clusters, inducing asymmetric membrane perturbations that disrupt stress distribution. Concurrently, melittin may adopt a U-shaped conformation as an intermediate transition state during membrane insertion, promoting localized membrane bending and lipid tilt (Hong et al., 2019). Notably, α-helical secondary structures are not essential for toroidal pore formation; instead, symmetrical spatial arrangement of peptides

enhances pore completeness and stability (Irudayam & Berkowitz, 2011). The resulting pores exhibit nanoscale selectivity, permitting leakage of small metabolites (1–2 nm diameter) while restricting larger molecules (Cirac et al., 2011), thereby preserving a balance between bactericidal efficiency and controlled permeability. Recent studies further describe an "avalanche effect" in melittin-induced toroidal pores, wherein the formation of a single pore triggers lipid reorganization and induces sequential pore formation in neighboring membrane regions (Ben Trad et al., 2023), significantly enhancing membrane disruption.

The carpet model, exemplified by peptides such as S-thanatin, involves strong electrostatic interactions between cationic AMPs and anionic phospholipid headgroups, enabling parallel alignment along the lipid bilayer to form a "carpet-like" configuration (Schweigardt et al., 2022). Once a critical peptide concentration is reached, the organized peptide layer destabilizes membrane architecture by inducing lipid phase separation, curvature strain, or micro-fissures, leading to rupture of giant unilamellar vesicles (GUVs). Concurrently, the carpet model generates lateral pressure asymmetry across the lipid bilayer, exacerbating lipid instability and markedly reducing membrane fluidity. These coupled effects drive detergent-like disintegration of the membrane into micelle-like fragments (Kumar et al., 2018, Sinha et al., 2017). For instance, S-thanatin preferentially binds to the hydrophilic surface of lipid membranes, impairing membrane-bound respiration and depleting intracellular potential. Simultaneously, a subset of peptides penetrate the cytoplasmic membrane, facilitating periplasmic leakage and bacteriolysis (Wu et al., 2010). In parallel, evidence suggests that membrane damage may also occur through pore expansion mechanisms in which a single pore can induce lipid reorganization, triggering pore formation in adjacent regions and significantly improving membrane destruction efficiency (Billah et al., 2024).

Microbial biofilms, consisting of complex extracellular matrices including polysaccharides, extracellular DNA, and proteins, form physical barriers that confer drug resistance and hinder treatment, particularly in chronic infections. Antimicrobial peptides disrupt biofilm integrity and inhibit its formation through multiple mechanisms, thereby exerting strong antimicrobial effects (Li et al., 2022). AMPs employ multifactorial strategies to inhibit and disrupt biofilm formation and structures. First, they suppress bacterial quorum sensing (QS) circuits that coordinate bacterial signaling and regulate genes essential for biofilm initiation and maturation. They also disrupt cell surface adhesins and destabilize intercellular aggregates to prevent microbial colonization on host surfaces. Simultaneously, in mature biofilms, AMPs penetrate and degrade matrix components, weakening structural integrity and enhancing pathogen exposure to immune surveillance (Li et al., 2022). For instance, ACP disrupts cell membrane integrity, induces excessive reactive oxygen species (ROS) generation, and reduces mitochondrial membrane potential, thereby inhibiting hyphal growth and biofilm formation in fluconazole-resistant *Candida albicans* (Zou et al., 2024). Similarly, the novel peptide HX-12C selectively permeabilizes mature biofilms of *Staphylococcus aureus* ATCC25923, triggering membrane depolarization, intracellular leakage, and eventual biofilm disintegration (Luo et al., 2022). These broad-spectrum, multi-targeted strategies facilitate direct eradication of biofilm-encased pathogens, offering new therapeutic avenues for managing recalcitrant chronic infections.

**Metabolic disruption: systemic perturbation of biochemical networks**

Upon breaching the bacterial membrane, AMPs initiate a second wave of attack by targeting key intracellular metabolic processes, systematically dismantling intracellular homeostasis. In nucleic acid synthesis, cationic peptides such as indolicidin intercalate into DNA by embedding within major and minor grooves, inhibiting replication and transcription via steric hindrance (K R et al., 2024). Oct-P2, translocated via the Sap transporter, binds to bacterial promoter regions to form DNA-peptide aggregates that suppress bacterial transcription and translation (Chen et al., 2025). In protein synthesis, Tur1A inhibits translation initiation by binding to the ribosomal exit tunnel, blocking the transition from initiation to elongation (Mardirossian et al., 2018). Other AMPs, such as fusaricidin, selectively disrupt glycolytic enzymes like enolase, disrupting ATP production and cellular energy flux. Several AMPs exhibit polypharmacological activity, simultaneously interfering with multiple metabolic nodes. For instance, compound 9b, a harmane-derived AMPs analog, initially accumulates on bacterial surfaces via electrostatic interactions, disrupting membrane structures. Once internalized, it binds lactate dehydrogenase (LDH), suppresses metabolic activity, and triggers excessive ROS production, leading to oxidative damage. Concurrently, it targets DNA integrity by interfering with mismatch repair and homologous recombination pathways, thereby blocking essential genomic repair mechanisms (Liu et al., 2024). Specific cationic AMPs such as W3R6 and its structural analogs demonstrate dual targeting of mycobacterial membranes and cytoplasmic DNA, a mechanism that reduces the likelihood of resistance development (Wang et al., 2023). Similarly, the peptide HJH-3 penetrates bacterial membranes and aggregates in the nucleoid region of *Salmonella*, where it disrupts nucleoid structure, facilitates DNA extrusion through membrane channel damage, and fragments genomic material, ultimately suppressing cell division (Wang et al., 2022a). This multifaceted cascade, encompassing structural destabilization and metabolic suppression, significantly enhances the bactericidal potency of AMPs and represents a powerful strategy to overcome resistance in recalcitrant pathogens.

**Immune modulation: synergistic activation of host defense**

Beyond their direct antimicrobial action, AMPs act as pivotal regulators of host innate immunity, bridging physical defense with immunological coordination. Accumulating evidence indicates that many AMPs, beyond direct microbicidal activity, can modulate host immunity through effects on chemotaxis, cytokine networks, innate receptor signaling, and inflammation resolution (Torres et al., 2025). These peptides interact with surface or intracellular receptors on immune cells, activating complex regulatory networks that facilitate leukocyte recruitment, direct immune cell trafficking, promote phagocytosis, and maintain inflammatory homeostasis via anti-inflammatory mechanisms during infection or inflammation (Gao et al., 2024, Kong et al., 2024). For example, overexpression of TroLEAP-2 significantly enhances macrophage activation and elevates the transcription of non-specific immune-related genes, strengthening early-phase host response (Lei et al., 2020). In parallel, microbial signaling molecules such as c-di-AMP from *Salmonella* activate the

STING/type I interferon pathway, driving dendritic cell maturation and M1 macrophage polarization, ultimately leading to T cell recruitment and heightened antitumor immunity (Huang et al., 2024). The amphibian-derived bioactive peptide FZ1 binds directly to the integrin αvβ3 receptor, activating the FAK-AKT/ERK signaling pathway, thereby promoting angiogenesis, endothelial cell proliferation, migration, and accelerating wound healing in a diabetic wound model (Wang et al., 2025b). When covalently conjugated with the self-assembling peptide RADA16-I, FZ1 significantly enhances its effects on wound healing, epithelial regeneration, angiogenesis, and collagen remodeling (Fu et al., 2026). Another antimicrobial peptide identified in amphibians, Cy_RL-QN15, has been demonstrated to significantly accelerate wound healing and hair follicle regeneration in diabetic mice. Mechanistically, it binds to the Frizzled-7 receptor and activates β-catenin signaling, thereby promoting the proliferation and migration of hair follicle stem cells (Wu et al., 2024). In addition, Cy_RL-QN15 functions as a TLR4 antagonist, modulating immune–epithelial interactions and suppressing MyD88/NF-κB–mediated inflammatory signaling, which consequently facilitates oral ulcer healing (Ru et al., 2025).

The human endogenous defense peptide LL-37 represents a prototypical immunomodulatory AMPs with broad functional versatility. It binds extracellular double-stranded nucleic acids (e.g., dsDNA) and shuttles them into the cytoplasm, where the activation of cGAS–STING-dependent signaling (e.g., NF-κB) induces chemokines (e.g., CXCL10), enhancing immune cell recruitment and amplifying inflammatory responses(Kato et al., 2023). Under septic conditions, LL-37 suppresses macrophage pyroptosis—a form of programmed cell death—to reduce the release of proinflammatory factors such as interleukin-1β (IL-1β), thereby suppressing excessive inflammation. Concurrently, it stimulates neutrophils to release neutrophil extracellular traps (NETs) and antimicrobial ectosomes, enhancing bacterial clearance (Nagaoka et al., 2020). LL-37 also facilitates intercellular transfer of cyclic GMP-AMP from infected to uninfected cells, amplifying STING-mediated interferon signaling to strengthen antiviral immunity (Wei et al., 2022).

The immunomodulatory role of AMPs in host-pathogen interactions further reveals the complexity of dynamic antagonism. For example, *Mycobacterium tuberculosis* inhibits LL-37 function through multiple mechanisms, including secretion of LprE and induction of prostaglandin E2 (PGE2), which together disrupt vitamin D signaling, suppress LL-37 expression, and block autophagy and phagolysosomal fusion, thereby evading immune clearance (Svensson et al., 2016). These immune evasion strategies underscore the critical role of AMPs in host-pathogen dynamics and reveal how their immunoregulatory roles are integral to both containment and clearance of infection.

Through these diverse mechanisms, AMPs demonstrate multifaceted immunomodulatory capabilities in infection, inflammation, and wound healing (Mba & Nweze, 2022, Niyonsaba et al., 2009). Their dual attributes of direct bactericidal activity and signaling regulation provide the host with a highly efficient and precise defense strategy. These findings not only expand current understanding of the complexity of innate immune architecture but also provide a compelling rationale for the development of novel AMP-based immunotherapies targeting complex diseases such as sepsis, tuberculosis, and gastric cancers.

**Expanded applications of AMPs multimodal mechanisms**

Antiviral mechanisms: AMPs exert potent antiviral effects through an integrated set of mechanisms that include direct structural disruption of virions, inhibition of viral replication, and modulation of host immune responses. Firstly, AMPs can block viral entry into host cells by directly targeting viral envelopes or structural proteins. For instance, LL-37 binds to the envelope (E) protein dimers of dengue virus (DENV), disrupting viral membrane fusion and host cell endocytosis (Alagarasu et al., 2017). Similarly, LL-37 interacts with the glycoprotein (GP) of Ebola virus and inhibits cathepsin B-mediated cleavage of GP—exposing the receptor-binding domain, a critical step required for membrane fusion and viral entry (Peskova et al., 2017). In crustaceans, the shrimp-derived peptide ALFPm3 directly targets the envelope and nucleocapsid structure of white spot syndrome virus (WSSV), significantly impairing virion integrity and reducing infectivity (Methatham et al., 2017).

In addition to structural disruption, AMPs also inhibit viral proliferation by binding to viral RNA or disrupting its replication. Given its α-helical structure and cationic charge, LL-37 can bind to viral RNA through electrostatic (Li et al., 2020), thereby blocking RNA release and template formation by generating steric interactions. This inhibition of intracellular replication impedes viral amplification during early infection stages.

AMPs further potentiate antiviral immunity by modulating host immune responses. β-defensins enhance the activity of alveolar macrophages and CD103+ dendritic cells, fostering robust T cell-mediated responses. Concurrently, they inhibit hyperactivation of CD11b+ dendritic cells and neutrophils, maintaining immunological homeostasis and reducing the risk of cytokine storms (LeMessurier et al., 2016). Additionally, LL-37 exerts direct antiviral activity against certain viruses, such as Venezuelan equine encephalitis virus, by physically disrupting viral envelope structures or binding critical viral proteins. It also activates intracellular antiviral signaling pathways (e.g., Toll-like receptors (TLRs) or cGAS-STING pathways), significantly upregulating type I interferon expression. Furthermore, LL-37 recruits immune cells to infection sites, synergistically enhancing the capacity of the host to clear viral infections (Ahmed et al., 2019).

AMPs form a multi-dimensional antiviral defense network via diverse mechanisms, exemplified by the amphibian-derived AMP caerin 1.1 (Ruan et al., 2024). In the early stages of infection, it penetrates and destabilizes the viral membrane, effectively inhibiting replication of porcine reproductive and respiratory syndrome virus (PRRSV). This process may involve interference with early transcriptional steps by targeting viral RNA polymerase. Simultaneously, caerin 1.1 bidirectionally regulates host immunity in a context-dependent manner: it activates innate immune cells, such as macrophages and natural killer cells, to promote pro-inflammatory cytokine and type I interferon release, while also tempering excessive inflammatory responses to prevent immune-mediated cytotoxicity. This tri-layered mechanism—comprising structural disruption, replication blockade, and immune regulation—exemplifies the advanced antiviral potential of AMPs and underscores their promise as multifunctional therapeutic agents in viral disease management.

Antifungal mechanisms: In antifungal therapy, AMPs exert potent fungicidal activity through synergistic, multi-target mechanisms that disrupt structural integrity, impair intracellular metabolism, and destabilize key organelles (Yang et al., 2023a). For instance, MSI-1 binds specifically to glucuronoxylomannan in the capsule of *Cryptococcus neoformans* via electrostatic interactions, significantly increasing membrane fluidity and destabilizing the lipid matrix, resulting in acute membrane disruption and fungal lysis (Ma et al., 2020). Trichosanthin acts enzymatically by activating chitinase and β-1,3-glucanase activity, accelerating degradation of fungal cell wall polymers and compromising cell wall integrity (Li et al., 2024a). Di-K19Hc specifically binds β-1,3-glucan on fungal cell surfaces, initiating membrane invagination, pore formation, and membrane potential disruption, rapidly killing *Candida albicans* (Jang et al., 2006). Cm-p5 exhibits a unique antifungal mechanism by adhering to vesicle surfaces with minimal membrane interaction, bypassing lipid bilayer integration and functioning as a non-pore-forming AMP, although its precise mode of action requires further investigation (Gonzalez-Garcia et al., 2024). Beyond surface disruption, AMPs also engage intracellular targets to inhibit fungal proliferation. For example, CB-M and BP15 bind directly to fungal DNA in *Botrytis cinerea* and *Penicillium italicum*, interfering with replication, transcription, and translation, thereby inhibiting fungal growth (Lei et al., 2025, Yang et al., 2023b). Several AMPs also elicit multifaceted cytotoxic effects. Periplanetasin-4 not only disrupts vacuolar membrane integrity to induce increased permeability and content leakage but also triggers abnormal calcium ion transfer from vacuoles to mitochondria, disrupting mitochondrial metabolism and exacerbating oxidative stress (Lee et al., 2019). Histatin-5, a well-characterized human salivary AMP, inhibits mitochondrial respiratory chain complex I, blocking ATP generation and driving energy collapse (Petruzzelli et al., 2003). Similarly, APT reduces phosphorus uptake and respiratory enzyme activity, including succinate dehydrogenase and malate dehydrogenase, in *Candida albicans*, thereby impairing carbon metabolism and energy supply (Wang et al., 2022b). Notably, many AMPs, such as DvAMPs, exhibit dual functionality, combining membrane disruption with intercellular metabolic interference. These peptides initially compromise the lipid bilayer, forming pores and disrupting membrane potential. Subsequent intracellular accumulation triggers cytoplasmic and mitochondrial calcium overload, mitochondrial membrane depolarization, and ROS accumulation. This cascade culminates in DNA damage, energy collapse, cytochrome c release, and irreversible cell death (Yang et al., 2023a). This multifactorial antifungal strategy confers high potency, low cytotoxicity, and minimal resistance potential, making AMPs particularly valuable in combating recalcitrant infections, including those caused by azole-resistant *Cryptococcus neoformans*.

Antitumor mechanisms: In antitumor therapy, AMPs have emerged as potent anticancer agents with high selectivity and multimodal mechanisms of action. Their tumor-targeting capabilities derive from distinctive biophysical and biochemical features of cancer cell membranes, including elevated surface expression of negatively charged phosphatidylserine (PS), glycosaminoglycans, and increased membrane fluidity. These differences enable AMPs to preferentially bind and disrupt cancer cell membranes while sparing healthy cells (Aghamiri et al., 2021). For example, the fish-derived peptide Pc-pic selectively recognizes PS-enriched outer leaflets on HeLa cancer cell membranes, forming transmembrane pores that disrupt membrane integrity and induce cancer cell death (Zhou et al., 2018). This membrane targeting confers AMPs with natural selectivity toward normal cells; for instance, BmCecA and BmCecD inhibit esophageal cancer cells while exhibiting no toxicity to normal human embryonic kidney 293T cells (Xu et al., 2020).

In addition to direct membrane disruption, AMPs can modulate intracellular processes that are critical to tumor survival and proliferation. For example, temporin-1CEa induces mitochondrial dysfunction by triggering membrane depolarization, cytosolic $Ca^{2+}$ leakage, and excessive ROS generation, thereby exacerbating oxidative stress-driven apoptosis (Wang et al., 2013). Similarly, lipid-modified PA-C1b amplifies mitochondrial-mediated apoptotic signaling by downregulating the anti-apoptotic protein Bcl-2, upregulating the pro-apoptotic protein Bax, and facilitating cytochrome c release, leading to ROS-mediated apoptosis (Guo et al., 2022). Other peptides such as D-LAK inhibit the proliferation and migration of non-small cell lung cancer (NSCLC) cells by arresting the cell cycle in the S phase, potentially through modulation of oncogenic signaling cascades (Patil & Kunda, 2022).

Specifically, temporin-SHa and its analogs have been shown to possess anticancer activity. For instance, temporin-SHa and a growing set of its rationally designed analogs exhibit low-micromolar cytotoxicity against multiple human cancer cell lines together with hallmark features of peptide-induced cell death, including early membrane perturbation, mitochondrial depolarization, reactive-oxygen-species (ROS) accumulation, and caspase-dependent apoptosis (Khan et al., 2022). Building on this scaffold, D-substituted and retro/retro-inverso analogs, as well as lysine-enriched and dendrimeric ($[G10a]_2$/$[G10a]_3$-SHa) architectures, improve proteolytic stability, enhance cationicity and tumor selectivity, and further lower $IC_{50}$ values while preserving amphipathic membrane targeting (Nazir et al., 2024). Recent studies expanded the structure–activity space of SHa derivatives and confirmed their broad-panel activity together with consistent apoptotic readouts, strengthening the mechanistic link between early membrane perturbation and downstream cell-death execution (Khan et al., 2024). Collectively, these data demonstrate that rational engineering of the SHa motif yields AMP leads with improved potency and selectivity for anticancer applications.

AMPs often operate through coordinated multi-target strategies that disrupt cancer cell homeostasis at multiple molecular levels. For instance, KT2 and RT2 peptides precisely target colorectal cancer cells by downregulating key genes involved in the PI3K/AKT/mTOR pathway, thereby inhibiting cell proliferation and invasion. Concurrently, they activate pro-apoptotic factors (e.g., p53, caspase family) and cell cycle arrest factors (e.g., p21), while downregulating anti-apoptotic Bcl-2 and inhibiting X-linked inhibitor of apoptosis protein (XIAP) from blocking caspases (Maijaroen et al., 2018), thereby amplifying apoptotic signaling. This multi-pathway regulatory strategy significantly strengthens therapeutic anticancer efficacy and reduces the potential for resistance development.

The tumor specificity of AMPs also confers unique advantages in combination therapies. NK-2 selectively binds to P-glycoprotein (P-gp)-overexpressing drug-resistant cancer cells, directly lysing them while inhibiting P-gp-mediated drug

efflux, thus restoring sensitivity to chemotherapeutic agents such as doxorubicin (Cirillo et al., 2024). Moreover, AMPs also serve as drug carriers. For example, AK is internalized by cancer cells via clathrin-mediated endocytosis and self-assembles into nanorods, facilitating efficient intracellular drug delivery of therapeutic payloads. AMPs have also been harnessed as immunomodulatory vaccine components: GE33, when co-administered with inactivated tumor cells, stimulates antitumor immunity by modulating macrophage secretion of pro-inflammatory cytokines, including MCP-1, IL-6, and IL-12, and activating both T cells and natural killer cells, without inducing excessive inflammation (Huang et al., 2013).

In summary, AMPs exert antibacterial, antiviral, antifungal, and antitumor activities through a multifaceted set of mechanisms, including membrane disruption, mitochondrial dysfunction, signal pathway inhibition, metabolic interference, and immune modulation. By integrating these effects, AMPs function as a versatile therapeutic platform capable of inducing direct microbial or tumor cell lysis, disrupting intracellular processes, and enhancing host immune responses. Their broad-spectrum activity, low potential for resistance development, and capacity to engage both innate and adaptive immune systems position them as promising candidates for treating infectious diseases and cancer.

## HIGH-THROUGHPUT AND COMPUTATIONAL STRATEGIES FOR AMP DISCOVERY AND OPTIMIZATION

Conventional AMP discovery has largely relied on bioactivity-guided fractionation (BGF), in which peptide-containing extracts are separated stepwise, commonly by chromatographic methods, and the resulting fractions are evaluated using antibacterial and safety assays such as agar diffusion, time-kill analysis, hemolysis testing, and minimum inhibitory concentration (MIC) determination (López-Siles et al., 2025, Yang et al., 2025). Bioactive fractions are then subjected to peptide isolation, structural characterization, and mechanism-oriented evaluation. In practice, AMP mechanisms are commonly investigated using a compact experimental toolbox that includes membrane permeabilization and depolarization assays, liposome leakage experiments, electrophysiological recording, and selected imaging approaches such as fluorescence microscopy, SEM, AFM, or cryo-ET, depending on the biological question and resolution required (Hammond et al., 2021, Saigo et al., 2019, Wang et al., 2015).

While effective, this traditional workflow remains labor-intensive and iterative, relying on repeated cycles of extraction, purification, and bioactivity assessment that can increase cost, prolong timelines, and raise the risk of peptide loss or activity decay. To address these limitations, more recent studies have incorporated high-throughput screening, membrane-interaction profiling, multi-omics analysis, and computationally assisted prediction as complementary tools for improving candidate prioritization and discovery efficiency (Figure 4).

Table 2 summarizes representative studies (2021–2025) reporting computationally assisted identification or design of AMPs with activity against multidrug-resistant (MDR) pathogens. Across these reports, some designed sequences show low homology to known natural AMPs while retaining

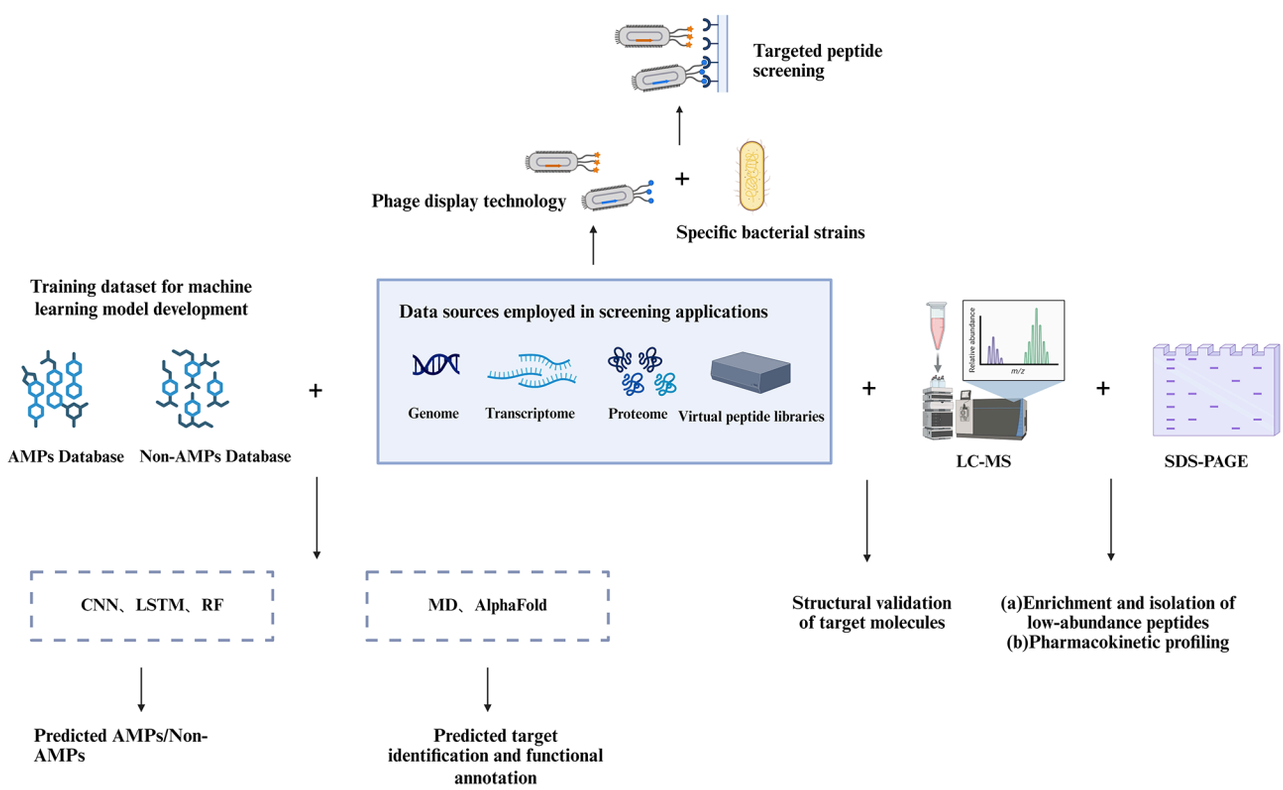


**Figure 4 Multi-omics-driven framework for AMP screening and validation**

Synergistic strategy combining liquid chromatography-mass spectrometry (LC-MS), AI-based algorithms (CNN/LSTM), and phage display platforms leverages integrated multi-omics datasets—including genomics, transcriptomics, and proteomics—to enable high-throughput screening. LC-MS characterizes peptide structure and function, AI algorithms predict and prioritize AMP candidates by integrating omics-derived features, and phage display assays experimentally validate antimicrobial activity.

**Table 2 Recent AI-Guided Discoveries of MDR-Active Antimicrobial Peptides (2024–2025)**

| Study | Year | Methodology | Target MDR Pathogens | Key Findings (AI vs Natural) |
|---|---|---|---|---|
| (Santos-Júnior et al., 2024) | 2024 | Machine-learning predictive mining of global meta/genomes to build AMPSphere (863,498 candidates), followed by synthesize-and-test in vitro/in vivo validation. | Broad MDR panels incl. ESKAPE representatives | AI expanded AMP sequence space beyond natural homologs (low homology to known AMPs) yet retained amphipathic, membrane-targeting motifs; robust in vitro & in vivo activity against MDR strains |
| (Wang et al., 2025c) | 2025 | Generative LLM pipeline (ProteoGPT \ sub-LLMs) designs de novo AMPs, with mechanistic assays confirming membrane depolarization/disruption against MDR strains. | Clinical MDR bacteria (report emphasizes MDR Gram-positives/negatives; e.g., CRAB, MRSA) | Generated low-homology AMPs with potent in vitro/in vivo efficacy; MoA consistent with membrane damage → depolarization; demonstrates efficient exploration of AMP space beyond natural templates. |
| (Wang et al., 2025a) | 2025 | LLM foundation model "AMP-Designer" enables an 11-day design-make-test loop delivering 18 de novo AMPs active against MDR Gram-negative pathogens. | Broad-spectrum MDR Gram-negative bacteria | AI-designed peptides achieved notable potency and breadth with new sequence motifs absent in natural AMPs, while maintaining membrane-targeting features; multiple candidates validated in vitro (and selected in vivo). |

Abbreviations: MDR, multidrug-resistant; CRAB, carbapenem-resistant *Acinetobacter baumannii*; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; LLM, large language model; DMTA, design–make–test–analyze; AMPSphere, a machine-learning–derived non-redundant AMP candidate set; MoA, mechanism of action.

physicochemical features associated with antimicrobial activity, such as amphipathicity and membrane interaction propensity. In several cases, these candidates demonstrated measurable in vitro activity and, where tested, preliminary in vivo efficacy. Collectively, these studies suggest that computational approaches can broaden AMP candidate space, although their biological relevance and translational value still depend on experimental validation.

**Transformation of AMP discovery through advanced mass spectrometry technology**

Mass spectrometry (MS) has become an important tool in AMP discovery by improving the identification and characterization of low-abundance peptides in complex biological matrices (Sutcliffe et al., 2025). In particular, MS combined with cell membrane affinity extraction (CMAE) and liquid chromatography can enrich and identify membrane-active peptides more efficiently than conventional multistep purification workflows; for example, JCpep8 was identified using a CMAE–LC-TOF-MS strategy (Xiao & Zhang, 2012). However, the performance of this approach still depends on effective peptide–membrane interaction during enrichment, and low membrane affinity or extraction efficiency may lead to missed candidates.

In proteomic and peptidomic studies, LC-MS-based workflows have become important tools for identifying AMP candidates, characterizing peptide structures, and, in some cases, inferring potential targets or pharmacokinetic behavior in complex biological matrices (Lin et al., 2025). In practice, these approaches are often combined with upstream fractionation or gel-based separation and, increasingly, with transcriptomic information to improve peptide annotation and reduce false-positive discovery, as illustrated in recent cyclotide and plant peptide screening studies (Turgut et al., 2025). However, their performance still depends on sample preparation quality, protein/peptide separation efficiency, matrix interference control, and reference database completeness, which can limit scalability and the detection of functionally relevant low-abundance peptides.

Mass spectrometry imaging (MSI) adds a spatial dimension to AMP-related research by enabling direct visualization of peptide or peptide-like antimicrobial distribution within tissues (Fang et al., 2024a). For example, MSI tracking of polymyxin B penetration in infected brain tissue has illustrated the utility of this approach for assessing tissue distribution and target-site exposure in complex infection settings (Li et al., 2024b).

Overall, modern MS-based technologies have improved the sensitivity and contextual resolution of AMP discovery and characterization through structural analysis, matrix-aware detection, and, in some cases, spatial mapping. However, their practical application remains limited by sample preprocessing complexity, instrument dependence, and matrix interference. Further progress will depend on more standardized workflows, improved sample handling, and continued advances in high-resolution MS platforms.

**Phage display technology**

Phage display is an important high-throughput platform for AMP discovery and targeting-oriented peptide selection. In this approach, randomized or rationally designed peptide sequences are inserted into bacteriophage coat protein genes, allowing peptides to be displayed on the phage surface and iteratively screened against target molecules without requiring prior peptide purification (Flachbartova et al., 2016). Recent studies have combined phage display with high-throughput sequencing to improve screening efficiency; for example, Hu et al. reported >10-fold efficiency improvement over conventional approaches when screening *Escherichia coli*-targeting candidates from large phage libraries. Phage display has also been adapted for more specialized applications, including target-binding peptide selection and modular antimicrobial design, although downstream activity validation and specificity assessment remain essential (Boyce et al., 2020, Ortiz et al., 2025). Phage display platforms optimize intermodule affinity and specificity, ensuring that AMPs are released exclusively under proteolytic cleavage by pathogen-derived proteases, thereby enhancing targeting precision and biosafety.

Despite its utility, phage display also has several important limitations. Large or structurally complex proteins may be inefficiently displayed on the phage surface, which can reduce library representation and increase the risk of false negatives (Ledsgaard et al., 2022). In addition, non-native protein folding, nonspecific phage adsorption, and uncertainty in

target selection may compromise binding specificity and increase false-positive signals. Because phage display primarily selects peptides with affinity for predefined targets, downstream functional validation and mechanistic assessment remain necessary to confirm antimicrobial relevance and reduce technical bias.

**Synergistic integration of multi-omics and AI in AMP discovery**

The integration of multi-omics data has expanded AMP discovery by enabling joint analysis of genomic, transcriptomic, proteomic, and, where available, metabolomic information across diverse organisms. In practice, these approaches can improve candidate identification and functional annotation beyond what is achievable with single-source screening alone. For example, integrated transcriptomic and proteomic profiling of *Macrothele washanensis* venom glands identified 43 distinct toxin-related proteins (Zhang et al., 2025d), while transcriptome-based screening of 54 amphibian datasets revealed conserved cathelicidin-domain-containing candidates, of which approximately 60% were predicted to display high antimicrobial potential (Varela-Rodríguez et al., 2024). When combined with computational prioritization, multi-omics workflows can help narrow candidate space and support downstream validation, although functional confirmation remains necessary before inferring therapeutic relevance.

Combined transcriptomic and metabolomic profiling can provide useful molecular context for AMP discovery and mechanism-related inference, particularly by linking immune activation, pathway perturbation, and peptide-associated responses during host–pathogen interactions. For example, integrated analyses have associated AMP-related transcriptional changes with immune signaling and metabolic pathway remodeling in infection-relevant settings (Kong et al., 2019, Lian et al., 2024). More broadly, multi-omics approaches can expand candidate discovery in non-model organisms and support functional annotation, while computational prioritization may assist downstream screening and optimization. However, these associations still require direct experimental validation before being translated into mechanistic or therapeutic conclusions.

**AI-driven screening and design of AMPs**

Computational prediction and AI-based methods are increasingly used as complementary tools for AMP discovery and optimization, primarily to support candidate prioritization, sequence refinement, and limited de novo design. In practice, current models are most useful for identifying putative AMPs from omics datasets or synthetic libraries and for assisting multi-parameter optimization of activity-related properties, such as hemolysis, cytotoxicity, or stability. Generative strategies, including protein language models and diffusion-based methods, have further expanded sequence exploration beyond close natural homologs. However, these approaches remain constrained by heterogeneous datasets, scarcity of reliable negative examples, and the strong assay dependence of commonly used in vitro readouts such as MIC. In addition, pharmacologically relevant features, including in vivo stability, immunogenicity, and pharmacokinetic or delivery behavior, remain difficult to infer from sequence information alone. Accordingly, AI outputs should be interpreted mainly as tools for hypothesis generation and candidate ranking, rather than substitutes for standardized experimental validation and translational assessment.

Generative models have advanced AMP research by shifting the focus from screening known-like sequences to the de novo design of novel peptides. In a 2023 *Nature Communications* study, Pandi et al. pretrained a generative model on approximately 1.5 million short peptide sequences and applied transfer learning with around 5 000 known AMPs, followed by MIC regression models for candidate prioritization. The authors generated approximately 500 000 peptide sequences, filtered them to a smaller subset, and experimentally screened 500 candidates using a cell-free protein synthesis platform, ultimately identifying 30 functional de novo AMPs, six of which demonstrated broad-spectrum activity against multidrug-resistant pathogens. Notably, the design-expression-screening workflow was completed within 24 h (Pandi et al., 2023). Similarly, Jin et al. (2025) reported that their AMPGen framework, which combines diffusion-based generation with a discriminator and a target-specific scorer, produced 40 candidates for validation, 38 of which were successfully synthesized, and 31/38 (81.58%) exhibited antibacterial activity. Among these active candidates, 23 had MICs≤25 μM against *E. coli*, and 11 had MICs≤25 μM against *S. aureus* (Jin et al., 2025). These studies highlight that the main progress in generative AI for AMP design lies not just in generating a large number of sequences but in ensuring that the designed peptides retain a sufficiently high hit rate in experimental validation.

Recent advancements in high-throughput sequencing technologies and AI algorithms have provided new opportunities for integrating multi-omics data into AMP discovery workflows. This convergence has the potential to improve traditional pipelines by incorporating sequence-based feature extraction, functional prediction, and experimental validation (Shen et al., 2025). AI-driven platforms, by accelerating computational prioritization, can shorten the AMP development cycle and enhance precision and scalability, though experimental validation remains a critical component of the discovery process.

At the sequence feature analysis level, the TriStack model exemplifies a multi-layered feature fusion strategy that integrates dipeptide composition (DDE), sequence order information (CKSAAP), and physicochemical descriptors within an ensemble learning framework incorporating XGBoost and Random Forest (RF) algorithms (Han et al., 2024). This architecture enhances predictive robustness while elucidating the mechanistic roles of critical amino acid residues through feature importance analysis, effectively addressing the diversity challenges inherent in complex AMP sequences.

To address feature extraction challenges in large-scale datasets, the ESM2 protein language model leverages deep contextual pretraining across vast protein sequences to capture evolutionary signatures and semantic patterns, improving AMP identification accuracy (Zhang et al., 2025c). The PseKRAAC encoding strategy further reduces dimensionality by clustering amino acids based on evolutionary conservation and hydrophobicity, enhancing model training efficiency and prediction sensitivity. In short-peptide prediction, PseKRAAC optimizes computational efficiency by reducing dimensionality, thus improving prediction accuracy in large-scale in silico screening. Integrated with Deep-AmPEP30, a deep learning architecture optimized for short-sequence representation, this approach improves predictive performance (Yan et al., 2020). A multi-

stage machine learning framework has been proposed that incorporates classification, ranking, and regression models to refine peptide candidate pools (Huang et al., 2023). This tiered approach enables efficient identification of high-potency AMPs from large combinatorial libraries with minimized computational demand.

Recent studies suggest that deep-learning-based screening can broaden AMP candidate discovery across taxonomic and environmental sequence spaces and may also support prediction in relatively data-limited settings. To address data scarcity in niche peptide domains, Gao et al. combined the ESM2 protein language model with a low-rank adaptation (LoRA) strategy to fine-tune predictions using only 30 LPS-binding domain (LBD) sequences, identifying active variants and illustrating the potential utility of transfer-style approaches in small-sample AMP-related tasks (Gao et al., 2025). Nevertheless, such models should still be regarded primarily as tools for candidate prioritization, with final assessment depending on experimental validation.

In parallel with screening-based approaches, generative strategies have also been explored for de novo AMP design. For example, an LSTM-based grammar-learning framework generated 6 415 candidate sequences, from which 10 peptides were experimentally validated and reported to show markedly improved antibacterial potency relative to benchmark peptides (Nagarajan et al., 2018). These findings suggest that generative models can expand AMP sequence exploration beyond close natural homologs, although their practical value still depends on downstream experimental confirmation.

However, species-specific prediction remains a substantial challenge, and peptides predicted to be broadly antimicrobial may still show limited activity against individual target pathogens. Accordingly, targeted optimization strategies should be interpreted cautiously and validated in organism-specific experimental systems.

For early-stage AMP discovery, the most established application of machine learning remains classification and prioritization. For example, Medina-Ortiz et al. developed AMP-Detector by integrating protein language models with machine-learning classifiers across 21 peptide bioactivity tasks and reported an average precision above 83%. Using this framework, the authors mined the Peptide Atlas and identified more than 190 000 putative AMPs, while a downstream generative step produced more than 500 additional candidate sequences (Medina-Ortiz et al., 2024). These findings suggest that the immediate value of AI lies mainly in narrowing large sequence spaces into more tractable sets for experimental validation, rather than directly delivering clinically useful peptide drugs.

Beyond conventional descriptor-based models, protein language models (PLMs) and sequence-embedding approaches are becoming increasingly important in AMP prediction. Their main advantage is that they can learn contextual and biochemical sequence patterns directly from large protein corpora without relying exclusively on manually engineered features. At the same time, sequence-only prediction has clear limitations, because AMP activity is closely related to membrane interaction modes, secondary-structure propensity, and conformational behavior. In 2024, Aguilera-Puga and Plisson showed that several preliminary classifiers achieved 86%–88% accuracy (Aguilera-Puga & Plisson, 2024), but that the underlying datasets were enriched for α-helical and coiled structures, leading to better performance for overrepresented structural classes and weaker generalization across the broader AMP structural landscape. To mitigate this issue, they proposed either structure-specific modeling or reducing structural overrepresentation during training. Taken together, these findings suggest that future AI frameworks for AMP discovery should move beyond simple “sequence-to-label” prediction and more explicitly integrate structural features, mechanism-of-action information, and membrane interaction properties to improve both interpretability and generalizability.

Molecular dynamics (MD) simulations serve as useful computational aids for elucidating AMP mechanisms and supporting rational design by providing atomic-level insight into peptide–membrane interactions. For example, MD-guided substitution of lysine with arginine at position 5 in peptide P5 was reported to reduce membrane insertion energy by 18% and lower the MIC from 12.5 μM to 3.3 μM against Gram-negative bacteria (Huynh et al., 2021). These findings suggest that MD is particularly valuable for mechanism-informed sequence refinement, although its predictions still require experimental confirmation.

Taken together, these computational approaches can assist AMP screening and mechanism-informed design, but several important limitations remain. Data scarcity—particularly the lack of reliable negative data related to toxicity, instability, or degradation—can introduce bias and reduce model robustness (Fang et al., 2024b). In addition, MD simulations often rely on simplified membrane models that do not fully capture physiological complexity, while all-atom simulations remain computationally expensive for large-scale peptide–membrane studies (Alpízar-Pedraza et al., 2025). Deep-learning models also face interpretability challenges, which can limit their usefulness for rational sequence optimization. Accordingly, future progress will depend less on model complexity alone than on better datasets, more interpretable frameworks, and tighter integration with experimental validation.

Despite these successes, current AI approaches have notable limitations. Models such as ESM2 and Deep-AmPEP30 achieve high predictive accuracy, but they operate as “black boxes,” making it hard to interpret which peptide features drive activity. They also rely heavily on large, curated datasets of AMP and non-AMP sequences; limited negative examples and data imbalances can bias predictions (Jukič & Bren, 2022). One of the most prominent issues is the lack of reliable negative data: compared with experimentally supported AMP positives, there are very few peptide sequences that are confidently annotated as truly non-antimicrobial, forcing many studies to construct negative datasets by randomly sampling sequences from UniProt or non-secretory proteins. In a systematic benchmark study, Sidorczuk et al. built 660 models using 12 machine-learning architectures and 11 negative-sampling strategies, and showed that model performance could be substantially inflated when training and benchmark sets were generated using the same or similar negative-sampling procedures (Sidorczuk et al., 2022). This finding implies that a considerable fraction of earlier AMP benchmark comparisons may be systematically biased. In addition to the negative-data problem, current AI models still have limited ability to predict clinically relevant features such as proteolytic stability, toxicity, immunogenicity, and in vivo pharmacokinetic behavior. Orsi and Reymond

further showed in 2024 that large language models have promise for AMP activity and toxicity prediction, but their performance remains highly dependent on data source and label quality and is not yet sufficient to replace experimental validation.

## CLINICAL TRANSLATION: KEY BOTTLENECKS AND BREAKTHROUGH STRATEGIES

A major barrier to AMP translation lies in rapid functional loss and insufficient exposure under physiological conditions. In a comparative stability study across blood, plasma, and serum, Böttger et al. found that the apidaecin derivative Api88 retained only 11±6% of the initial peptide after 10 min in freshly prepared mouse serum and was fully degraded by 30 min in all serum samples. By contrast, in fresh whole blood, 88±14% of Api88 remained after 10 min, and 30±14% was still detectable after 30 min, indicating that serum-only assays may not fully reflect peptide behavior in vivo (Böttger et al., 2017). In the same study, the oncocin derivative Onc18 showed a serum half-life of approximately 20–25 min, whereas site-specific substitution with ornithine or D-arginine increased the serum half-life of Onc72 to 3 h and that of Onc112 to more than 8 h. Together, these data show that the major bottleneck in AMP development is not simply whether a peptide has antimicrobial activity, but whether the native sequence can resist rapid proteolysis in complex biological matrices. In practice, substantial gains in stability and systemic exposure often require sequence-level engineering, including residue substitution, incorporation of noncanonical amino acids, or conformational constraint strategies.

Despite their therapeutic promise, peptide-based therapeutics face significant challenges in clinical applications, with low oral bioavailability representing one of the most critical limitations. Peptide stability is significantly compromised due to gastric acidity, proteolytic enzyme degradation, efflux transporters, as well as rapid hepatic and renal clearance (Asif et al., 2024). These factors collectively hinder effective membrane penetration and targeted absorption at therapeutic sites. Furthermore, the inherent hydrophobicity of peptides exacerbates these challenges, promoting aggregation and nonspecific binding *in vivo*, thereby reducing drug utilization efficiency.

Beyond proteolytic degradation, host-cell interactions also impose an important constraint on AMP performance in vivo. Starr et al. reported that bactericidal activity of some AMPs could be severely inhibited after only a few minutes of preincubation with human erythrocytes; in a follow-up study, a broad panel of linear AMPs was shown to undergo degradation in human erythrocyte cytosolic extracts, suggesting that host cells may reduce effective peptide exposure not only through binding but also through cell-associated proteolysis (Starr et al., 2016; Starr & Wimley, 2017). Safety assessment is similarly more complex than a single hemolysis assay would suggest. In an extensive study of 24 peptide-based antimicrobials, Greco et al. measured hemolytic activity over a concentration range of 0.15–150 μM and reported therapeutic indices of approximately 50–500, indicating substantial selectivity relative to bacterial targets. However, when three peptides were subsequently evaluated in acute intravenous toxicity studies in rats, no systemic toxic effects were observed even at doses corresponding to 2–8 ×MIC (Greco et al., 2020). These findings indicate that hemolysis, cytotoxicity, and systemic in vivo toxicity do not follow a simple one-to-one relationship. Therefore, AMP safety should be judged using an integrated framework that includes erythrocyte assays, mammalian cell toxicity, in vivo tolerability, and route-dependent exposure, rather than by overinterpreting any single in vitro toxicity metric.

The low target specificity of peptides similarly restricts their clinical utility, with non-selective membrane disruption potentially inducing severe side effects. Their broad-spectrum mechanism—driven by electrostatic attraction and hydrophobic interactions—can compromise both microbial and host cell membranes, leading to hemolysis, inflammation, and cytotoxicity (Botelho Sampaio de Oliveira et al., 2023). Notably, several amphibian-derived peptides (e.g., frog-derived membrane-active peptides (MAPs)) have been shown to enhance toxin absorption across predator oral epithelia via membrane disruption, triggering systemic toxicity (Raaymakers et al., 2017). These findings highlight the critical challenge of balancing peptide specificity and safety in therapeutic development.

The clinical application of AMPs is further hindered by high production costs, primarily stemming from limitations in conventional manufacturing processes. The linear nature of solid-phase synthesis restricts the efficiency of synthesizing long-chain or structurally complex peptides. Batch production models, low resin loading capacity, and reliance on chromatographic purification significantly escalate production expenses, impeding large-scale manufacturing. The intricate molecular architectures of AMPs (e.g., specific secondary structures) and poor water solubility further complicate downstream separation and purification, necessitating costly chromatographic techniques (Luo et al., 2023). These technical and economic barriers remain critical bottlenecks for industrial-scale AMP production.

To overcome these barriers, a range of multidimensional strategies has been developed, including drug delivery systems, carrier design, and combinational therapies. At the administration level, bypassing gastrointestinal barriers through local, intravenous, or intramuscular delivery significantly improves bioavailability (Botelho Sampaio de Oliveira et al., 2023). Concurrently, the design of protective delivery systems has become a critical area of innovation. Lipid-based nanodelivery systems, such as cubic-phase nanoparticles (Dyett et al., 2021), encapsulate peptides within ordered bilayer structures that protect against proteolysis while leveraging intrinsic membrane activity to augment antimicrobial potency (Yao et al., 2023). Hydrogel-based delivery matrices offer another strategy, enabling localized, sustained release while preserving peptide conformation and shielding against enzymatic degradation and physicochemical stress (Guerrero et al., 2025). Nevertheless, achieving an optimal balance between release kinetics and structural integrity remains a challenge. For example, AMP-OXA immobilized in hydrogel microparticles maintains functional synergy with host defense peptides but the delay in the accumulation of drug concentrations due to its sustained release may weaken the need for rapid sterilization in the early stages of infection (Stepulane et al., 2024).

In targeted delivery strategies, researchers have engineered spatiotemporally responsive systems to enhance AMP localization, activity duration, and safety. One such approach involved the construction of a multifunctional nanoplatform that co-delivered LL-37 with a redox-sensitive cationic polyaminoglycoside (SS-HPT) for chronic skin infections. This

platform functioned as a self-sustaining “AMP factory”, achieving microbial clearance, immune microenvironment modulation, and tissue matrix remodeling through three-dimensional synergistic interactions. The system facilitated wound reprogramming by promoting rapid transition into the inflammatory phase, accelerating resolution of chronic wound infection (Ju et al., 2025). A similar temporal delivery strategy was applied to persistent infection sites using a nanogel that co-encapsulated a nitric oxide donor (SNAP) and an AMP, enabling 24-hour sustained SNAP release that significantly enhanced both antibacterial and anti-biofilm effects (Fasiku et al., 2022). In parallel, stimuli-responsive carriers—such as pH-sensitive nanosystems—enable targeted AMP release by undergoing structural transitions in response to acidic infection microenvironments. Upon exposure to bacterial acidity, these systems convert into liposomes or positively charged vesicles, preventing premature release during circulation and minimizing off-target toxicity to healthy tissues (Gontsarik et al., 2021).

To minimize nonspecific binding and off-target effects, biomimetic design and engineered material carriers have been optimized to improve delivery specificity and pharmacodynamic efficiency. For example, a vancomycin-loaded nanocomplex (VCM-FU-PEP-NP), assembled using electrostatic interactions between fucoidan (FU) and CC-19, achieved high encapsulation efficiency while synergistically enhancing antimicrobial activity, increasing free radical scavenging capacity, and mitigating bacterial toxin-induced cytotoxicity and inflammation (Gafar et al., 2025). In parallel, encapsulating plectasin in poly (lactic-co-glycolic acid) (PLGA) nanoparticles achieved 71%–90% loading efficiency, substantially improving peptide solubility and stability (Bo et al., 2025). These nanoparticles exhibited selective accumulation in airway epithelial cells and THP-1 macrophages, while showing negligible uptake in A549 epithelial cells, thereby concentrating AMPs at infection sites and providing controlled 24-hour release (Water et al., 2015). Furthermore, encapsulation of the vancomycin analogue FU002 in PEGylated liposomes prolonged systemic circulation time and maintained antimicrobial activity against *Staphylococcus* and *Enterococcus* species without observed hepatotoxicity, nephrotoxicity, or erythrocyte toxicity (Werner et al., 2024), underscoring the clinical viability of well-engineered AMP delivery platforms.

At the molecular design level, advanced engineering strategies have been employed to optimize the balance between AMP activity and safety. Approaches such as amino acid substitutions, conformational optimization, and chemical modification have been systematically applied to improve peptide functionality. For example, N-terminal acetylation (e.g., 4b variant of P-α-02-B) has been shown to significantly improve both antimicrobial potency and structural stability, while introducing non-natural high arginine residues further optimizes peptide chain conformational stability (Zhang et al., 2025a). Substitution with D-amino acids provides an alternative pathway for conformational stabilization; substituting phenylalanine with its D-enantiomer (e.g., K-1dF) not only confers resistance to host proteases but also enhances antibacterial efficacy against multidrug-resistant *Acinetobacter baumannii* by modulating hydrophobic moments and spatial conformation (An et al., 2024). Furthermore, repetitive motif design in peptide chains markedly amplifies antimicrobial efficacy. Wang et al. found that the repetitive motif “-KVKF-” enhanced α-helical conformation, thereby strengthening antibacterial activity, while derived peptides 4 and 8 exhibited the highest therapeutic indices, maintaining robust bioactivity under enzymatic, thermal, and serum stress conditions (Wang et al., 2024b).

To improve targeting specificity and mitigate off-target toxicity, phosphorylation-based modifications have emerged as a valuable tool. For example, phosphate-modified W3BipY8-P, generated through selective phosphorylation of lysine or arginine residues, retained comparable antimicrobial activity to the original peptide while significantly extending its half-life and reducing hemolysis and cytotoxicity (Ba et al., 2024). Concurrently, pH-responsive designs demonstrate potential for precise delivery. Amphipathic MAPs modified with histidine/glutamic acid-rich precursors demonstrated pH-sensitive behavior. Given that most sites of bacterial infection are slightly acidic, these peptides selectively inhibit *Escherichia coli* and *Acinetobacter baumannii* in acidic microenvironments (pH 6.0–6.5), while exhibiting minimal cytotoxicity at physiological pH (7.4) (Cheng et al., 2024). Such precision-tuned peptides enable localized activation within infection sites, thereby maximizing antimicrobial efficacy while preserving host tissue integrity.

Emerging antimicrobial strategies suggest that combining AMPs with conventional antibiotics or bacteriophages may improve antibacterial efficacy and, in some contexts, make resistance development less straightforward through multi-target actions. These synergistic effects arise from mechanistically distinct yet complementary modes of action: bacteriophages initiate bacterial lysis by injecting genomic material, whereas AMPs disrupt membrane integrity or interfere with intracellular targets. For example, co-administration of AMP R8K with phage-derived endolysin Lys11 has been shown to enhance *Staphylococcus aureus* membrane permeabilization, increasing bacterial susceptibility to phage lysis (Alisigwe et al., 2025). Further advancements include biomimetic constructs such as Syn-71, an AMP immobilized on nanoparticle surfaces that disrupts bacterial membranes or intracellular targets while combining the adsorption behavior of bacteriophage-like particles, thereby achieving synergy without triggering host immunogenic responses typically associated with natural bacteriophages (Olesk et al., 2024).

The co-administration of AMP with antibiotics offers additional clinical advantages, particularly in combating multidrug-resistant (MDR) pathogens. For instance, Pt5-1c, a phosphomycin derivative, showed potent synergistic activity against biofilm-forming bacteria *in vitro* and *in vivo* when combined with oxacillin or vancomycin (VCM), without inducing antimicrobial resistance even after prolonged exposure (Duan et al., 2021). Witherell et al. further reported that the combination of colistin with OTD-244 reduced colistin MICs by over four-fold in the majority of tested isolates (88%), bypassing MCR-mediated colistin resistance without hemolytic effects (Witherell et al., 2020). Additionally, conjugation of tobramycin to proline-rich PrAMPs via cysteine linkages significantly improved bacterial surface delivery efficiency, enabling faster ribosomal binding of both components and amplifying antibacterial effects through dual-action synergy (Gambato et al., 2023). These multifaceted combination strategies integrate orthogonal antimicrobial mechanisms to establish multi-target, multi-pathway, antimicrobial-therapeutic networks, thereby reducing selective pressure of monotherapy

and offering more robust, resistance-resilient treatment paradigms.

To optimize AMP production, researchers have employed synergistic strategies combining molecular engineering and process innovation to significantly enhance yield efficiency, product quality, and economic viability. At the molecular level, expression-related challenges, such as inclusion body formation, proteolytic degradation, or host toxicity, have been addressed through rational sequence design and structural modulation. For example, the introduction of a C-terminal C-tag to target AMPs (e.g., IMPI) has been shown to effectively reduce inclusion body formation while preserving bioactivity and eliminating the requirement for additional purification steps (Lappöhn et al., 2023). A similar approach was demonstrated with the CC34 tetrameric design, in which four monomeric units were covalently linked to enhance protein stability, minimize degradation in *Pichia pastoris*, and exploit its efficient secretion-based expression system to achieve higher yields (Zhao et al., 2023). These fusion strategies, such as coupling SUMO tags with PR-39 AMPs, have also been applied to mitigate AMP toxicity and aggregation during expression in *Escherichia coli* while enabling high-efficiency purification via His-tag affinity chromatography, streamlining workflows and improving product recovery (Azari et al., 2020).

At the expression system level, researchers have expanded AMP production pathways through platform innovation and process optimization. Cell-free synthesis systems, which operate independently of plasmid insertion or genomic integration (Deo et al., 2022), enable direct expression from plasmids or PCR-amplified templates, significantly reducing culture complexity and maintenance costs while avoiding AMP-induced host cytotoxicity to enhance target protein yields (Cruz & Reyes, 2025). Plant-based expression systems provide another efficient alternative. Directed modification of nuclear or plastid genomes, combined with transient or stable expression strategies, enable efficient and flexible production of AMPs (Nazarian-Firouzabadi et al., 2024). For instance, tobacco plants have been used to precisely express and modify the recombinant peptide roseltide rT1, achieving production costs significantly lower than those associated with chemical synthesis or conventional animal and bacterial cell cultures (Khalifeh-Kandy et al., 2024). Furthermore, optimizing high-cell-density fermentation processes—through precise control of critical parameters such as carbon-to-nitrogen source ratios, dissolved oxygen levels, and temperature—has enabled sustained high cell density maintenance and substantially increased target protein outputs (Zhang et al., 2024).

Notably, within the complex landscape of the tumor microenvironment, cancer cells do not exist in isolation but form dynamic networks involving stromal cells, vasculature, peripheral nerves, immune cells, and diverse microbial communities. Tumor-associated bacteria, such as *Fusobacterium* and *Bacteroides* species enriched in colorectal cancer, exhibit spatial colonization within neoplastic tissue and critically influence tumor progression (Figure 5). Such microorganisms facilitate cancer cell migration by fostering immunosuppressive microenvironments and activating tumor invasion-related pathways (Galeano Niño et al., 2022). Moreover, they influence therapeutic responsiveness by

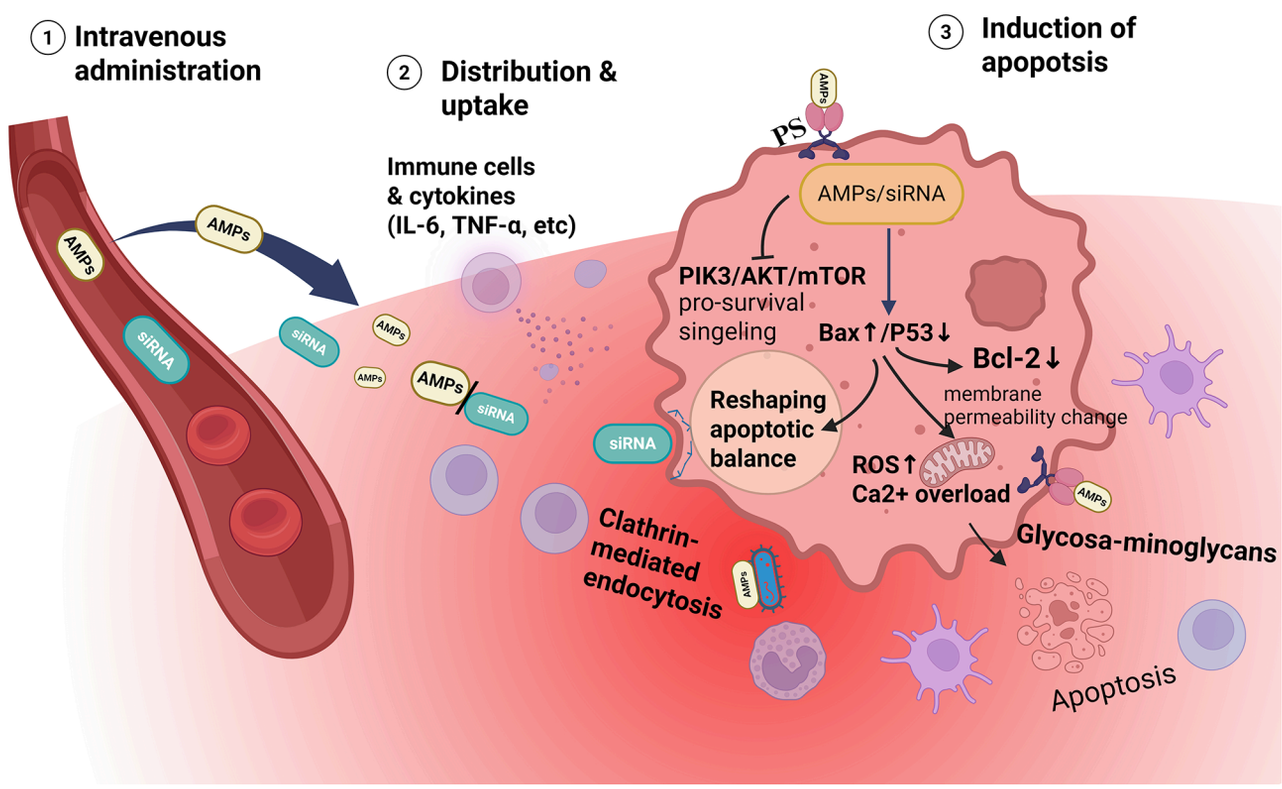


**Figure 5 Multimodal regulation of the tumor microenvironment by AMPs**

AMPs are transported to tumor sites via nanodelivery systems or employed as carriers for siRNA delivery. They inhibit bacterial proliferation, utilize tumor-specific cellular components to target tumor cells, their mitochondria, and related genes, and activate immune cells to regulate cytokines. Through these synergistic mechanisms, AMPs reshape the immune landscape of the tumor microenvironment, effectively suppressing tumor progression.

modulating host immune systems (Wong-Rolle et al., 2021). For example, membrane-based nanovaccines derived from *Fusobacterium nucleatum* have been shown to elicit robust antitumor immune responses and suppress tumor metastasis, with enhanced synergistic effects observed when combined with chemotherapeutic agents (Chen et al., 2024). However, despite these promising advances, the compositional heterogeneity and temporal plasticity of intratumoral microbiota pose significant therapeutic challenges. Notably, while 5-fluorouracil (5-FU) effectively inhibits *Fusobacterium nucleatum*, its cytotoxic activity is markedly attenuated in the presence of *Escherichia coli* due to metabolic interference (LaCourse et al., 2022). During tumor progression, microorganisms play a dual role by dynamically modulating immune responses and the metabolic microenvironment. In early stages, specific microbial communities promote tumorigenesis by inducing immunosuppression and chronic inflammation, whereas in later stages, to promote bacterial growth and survival, microbial metabolites may facilitate the emergence of cancer stem cell-like traits and metastatic adaptation, thereby accelerating malignant progression. These stage-dependent effects underscore the complexity of microbe-host interactions within the evolving tumor ecosystem.

In this context, AMPs have emerged as promising candidates for cancer therapy due to their ability to simultaneously target tumor cells, modulate immune responses, and inhibit tumor-associated bacteria. Beyond their intrinsic cytotoxicity toward malignant cells, AMPs enhance therapeutic efficacy through immunoregulatory and microbiome-modulating functions. For instance, nanofiber-based delivery systems engineered for size-dependent, sequential AMP release significantly reduce Gram-positive and Gram-negative bacterial abundance in the tumor microenvironment, contributing to immune normalization and improved therapeutic outcomes (Zhang et al., 2026). ROS-responsive nanodelivery systems, which exploit the elevated oxidative stress in tumor microenvironments, enable targeted release of cecropin XJ with a 90% release efficiency, thereby maximizing local drug concentration while minimizing systemic toxicity (Pan et al., 2025). Furthermore, the inherent membrane-targeting properties of AMPs have been harnessed for active delivery applications: A9K facilitates efficient delivery of siRNA to cancer cells via clathrin-mediated endocytosis (Cirillo et al., 2024), while chensinin-1b and its lipidated derivative PA-C1b function as vectors, simultaneously delivering therapeutic genes (e.g., p53-eGFP) and exerting direct anticancer effects. These systems have demonstrated potent inhibition of MCF-7 cancer cell proliferation with negligible cytotoxicity toward normal cells (Li et al., 2021), achieving a synergistic integration of gene therapy with targeted anticancer action.

Although modulation of the tumor-associated microbiome offers promising therapeutic potential, its dynamic nature and interindividual variability necessitate further systematic investigation. Microbial community composition within the tumor microenvironment shifts markedly across disease stages, highlighting the need for dynamic monitoring and adaptable intervention strategies. Additionally, anticancer interventions, such as chemotherapy and immunotherapy, may reshape microbial communities, with their impacts on therapeutic efficacy requiring validation through clinical trials. While AMPs have demonstrated early promise in modulating intratumoral microbiota, comprehensive studies are required to clarify their mechanisms of microbial interaction, define optimal strategies for targeting specific taxa, and evaluate long-term safety and efficacy (Ji et al., 2024). Advancing this field will not only enhance mechanistic insights into tumor-microbiome dynamics but also establish a theoretical and practical foundation for the development of precision, microbiota-informed cancer treatment strategies.

## CONCLUSIONS AND PERSPECTIVES

AMPs, as evolutionarily conserved effectors of innate immunity, are widely distributed across biological taxa and exhibit broad application prospects in anti-infective and anticancer therapies. With expanding mechanistic insights, AMPs have transcended the traditional membrane-disruption paradigm, revealing novel modes of action targeting intracellular organelles (e.g., mitochondria), nucleic acids, and immune regulatory pathways. These properties confer distinct advantages in immune modulation, biofilm disruption, and the selective eradication of multidrug-resistant pathogens.

Despite their promise, several critical challenges constrain the translation of AMPs into clinical practice. Although the integration of AI and multi-omics technologies has significantly accelerated AMP screening efficiency and discovery, the black-box nature of current AI models impedes mechanistic interpretation. Most existing prediction algorithms remain biased toward secondary structural features and are limited by the paucity of high-resolution three-dimensional conformations, impairing accurate modeling of dynamic peptide-target interactions. More crucially, the lack of robust negative datasets and incomplete multi-omics profiles from non-model organisms limits both model generalizability and comprehensive exploration of AMP functional diversity. Concurrently, natural AMPs face translational barriers such as high production costs, poor metabolic stability, and off-target hemolytic toxicity, while advanced nanoparticle delivery systems introduce new technical complexities related to biocompatibility, *in vivo* clearance, and regulatory safety thresholds. In oncology, tumor-associated microbiome heterogeneity further complicates AMP efficacy, underscoring the need for spatiotemporally precise regulation of AMP delivery within complex tumor microenvironments. Synergistic integration with immunotherapy requires dynamic monitoring systems and multi-omics-driven threshold models that define optimal therapeutic windows.

To accelerate the de novo discovery of novel AMPs against MDR pathogens and overcome associated bottlenecks, future research must integrate multidisciplinary convergence, focusing on four priorities: advancing interpretable AI by linking sequence-feature-activity via attention/attribution and counterfactual analyses to mitigate black-box behavior; standardizing low-biased data curation through curating hard negatives, harmonizing metadata, and leveraging resources like AMPSphere; building physics-informed modeling by embedding peptide-membrane energetics/electrostatics to enhance extrapolation; and establishing a closed-loop DMTA framework by coupling AI with high-throughput synthesis/phenotyping and active learning. Technologically, integrating molecular dynamics simulations with AI-driven structural prediction enables rational design pipelines. Molecular engineering such as amino acid substitution, conformational refinement, and especially the topological advantages of cyclic AMPs enhances stability and efficacy.

Toxicity is mitigated by machine learning-guided directed evolution to screen low-toxicity variants and site-specific/sustained-release formulations to reduce systemic risks.

On the application front, AMPs are positioned to redefine antimicrobial and anticancer paradigms through synergistic integration with conventional antibiotics, phage therapy, and microbiota-targeted interventions. In oncology, harnessing AMPs to modulate tumor-associated microbial communities and reshape the immune microenvironment, coupled with advanced drug delivery systems, holds promise for developing novel strategies for overcoming immunosuppressive barriers. Their dual role as cytotoxic agents and gene delivery vectors further supports simultaneous tumor cell ablation and local antibacterial release, suppressing therapy-induced secondary infections. In parallel, the immunomodulatory capacity of AMPs offers potential for treating autoimmune and chronic inflammatory disorders. Expanding cross-species multi-omics databases will be essential to broaden candidate peptide libraries and uncover novel functional archetypes. Collectively, these innovations will drive the transformation of AMP research from experimental frameworks into clinically viable therapies, offering dynamic, precision-based solutions to pressing challenges in infectious disease, oncology, and immune regulation.

## SUPPLEMENTARY DATA

Data will be made available on request.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

Y. W.: Writing – original draft, Formal analysis, Writing – review & editing. Y. Y.: Formal analysis, Writing – original draft. Y. Z.: Formal analysis, Investigation, Writing – original draft, Visualization. Z. D.: Formal analysis, Writing – original draft. Mehwish khalid: Validation, Writing – review & editing. *Gang Guo: Conceptualization, Supervision, Investigation, Project administration, Writing – original draft, Writing – review & editing. *R. L.: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. All authors read and approved the final version of the manuscript.

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