



Published in final edited form as:

Curr Opin Chem Biol. 2017 June ; 38: 87–96. doi:10.1016/j.cbpa.2017.03.014.

Host Defense Antimicrobial Peptides as Antibiotics: Design and Application Strategies

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Abstract

This review deals with the design and application strategies of new antibiotics based on naturally occurring antimicrobial peptides (AMPs). The initial candidate can be designed based on three-dimensional structure or selected from a library of peptides from natural or laboratory sources followed by optimization via structure-activity relationship studies. There are also advanced application strategies such as induction of AMP expression from host cells by various factors (e.g., metals, amino acids, vitamin D and sunlight), the use of engineered probiotic bacteria to deliver peptides, the design of prodrug and peptide conjugates to improve specific targeting. In addition, combined uses of newly developed AMPs with existing antimicrobial agents may provide a practical avenue for effective management of antibiotic-resistant bacteria (superbugs, including biofilm). Finally, we highlight AMPs already in use or under clinical trials.

1. Antimicrobial peptides to combat resistant bacteria

There are now global voices calling for solutions to the antibiotic resistance problem. At the moment, key pathogens such as *Clostridium difficile* and *Staphylococcus aureus* cause over 25,000 deaths per year [1]. The deaths due to *C. difficile* increased from almost none in 1989 to 2.4 per 100,000 populations in 2007 [2]. In addition, the total deaths from methicillin-resistant *S. aureus* (MRSA) are now comparable to those caused by HIV-1 [3]. It is projected that 10 million people could die of infectious diseases by 2050 if effective measures had not been taken [4]. Such a devastating picture should never become a reality. Therefore, efforts are now being made to achieve a proper use of existing antibiotics on one hand, and to develop new alternatives on the other.

Antimicrobial peptides (AMPs) are important candidates for developing novel antibiotics. They are expressed by the host to eliminate invading pathogens and boost immune response

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[5–10]. Such beneficial effects of AMPs are determined by their physical properties: short (<50 amino acids), cationic (average net charge +3), and having an average hydrophobic content of 42% [11,12]. Some representatives and their properties are provided in Table 1. The cationicity and hydrophobicity of these peptides are two critical elements for generating the frequently observed amphipathic structure (Figure 1a), which enables cationic AMPs to preferentially target anionic bacterial membranes (Figure 1b) rich in phosphatidylglycerols (PGs) [9,10,13,14]. In contrast, human cell membranes are dominated by zwitterionic phospholipids (PCs) and cholesterol (Figure 1c). Such a membrane composition difference is believed to be one of the major reasons why AMPs are selective [9,13]. The membrane-bound peptides appear to exert their effects via multiple mechanisms. For example, human cathelicidin LL-37 peptides can cause anionic lipid clustering, permeate bacterial membranes, and even damage the membranes [14,15] via carpet/toroidal models [16,17]. In addition, different AMPs can work by different mechanisms. While proline-rich peptides, with a low hydrophobicity of 23% on average [11], target bacterial ribosomes [18,19], lantibiotics and cyclotides can both bind to phosphatidylethanolamines (PEs) [20–22]. The expression of multiple AMPs may not be redundant. Rather, these peptides may work synergistically for optimal outcomes [10]. Finally, AMPs can also boost immune response to further clear invading pathogens [6,7]. All of these mechanisms make it difficult for pathogens to develop resistance. Indeed, some AMPs are already in use and Figure 1d depicts the timeline of their applications. This article highlights current design and application strategies for antimicrobial peptides. Our discussion focuses on the most recent achievements published during 2015–2016. Some important AMP discoveries reported in 2014 can be found in our recent review article [20].

2. Discovery and design strategies of antimicrobial peptides

Two general strategies are currently in use to obtain a peptide lead. First, a starting candidate can be identified from a peptide library. Second, a peptide candidate can be designed based on the three-dimensional (3D) structure of a target molecule or the peptide itself.

2.1. Library screening

To identify a peptide template, a library of candidates, either from natural or man-made sources, can be screened or searched. Naturally occurring AMPs (currently over 2,800), isolated from the three life domains, are registered in the Antimicrobial Peptide Database (APD) [23]. Such natural peptides appear to have good activity against pathogens, but poor toxicity to host cells [24,25]. By screening the APD database, we have obtained potent antibacterial and anti-HIV peptides [26,27]. One can also use the database filters to design new peptides. For example, frequently occurring amino acids derived from the database are sufficient to design antimicrobial peptides. In addition, most abundant peptide motifs can be searched and combined into new AMPs [23]. Remarkably, it is possible to derive from the database all the parameters needed for *ab initio* design of a potent peptide against MRSA [25*]. Here a set of AMPs with known activity against Gram-positive bacteria were utilized as templates to extract the most probable peptide parameters such as peptide length, net charge, hydrophobic content and structure. Interestingly, the database-derived parameters have been successfully utilized to synthesize small molecules that closely mimic the

database designed peptide [28]. Useful peptides have also been screened directly from uncultivable bacteria by using the i-chip technology, allowing Ling et al. to identify teixobactin as a potentially new peptide antibiotic [29**]. As a different approach, Mongui et al. tried to obtain novel anti-malarial peptides from metagenomic libraries containing candidates originated from diverse microbial communities [30].

Likewise, combinatorial libraries can be used. Masuda et al. screened the library of collagen-like triple-helical peptides to identify RO-A, which is active against *Escherichia coli* and *Bacillus subtilis*. The RO-A peptide has stability in human serum and low cytotoxicity to mammalian cells [31]. Chew et al. screened a phage displayed peptide library to identify a novel antiviral peptide gg-ww against dengue virus serotype 2 [32]. In addition, a high-throughput screening system was used to identify a peptoid K15 with modest efficacy against drug resistant pathogens [33].

2.2. Structure-based design

Structure-based design (also called rational design) has been a cornerstone technique for development of traditional antibiotics. In this approach, the 3D structure of the target and drug molecules are normally harnessed to glean the needed clues for molecular design. Interestingly, the major antimicrobial region of human cathelicidin LL-37 for membrane binding was identified via structural studies by NMR spectroscopy [34]. Moreover, the 3D structure of the LL-37 peptide was utilized to enhance antibacterial potency against MRSA [35**].

Not all AMPs target bacterial membranes, however. Proline-rich AMPs (PrAMPs) have recently been shown to bind either the 70S or 50S subunit of ribosomes of Gram-negative bacteria [18,19,36]. Gagnon et al. [37] confirmed this mechanism by determining the crystal structures of several PrAMPs, such as Bac7₁₋₃₅ and pyrrolicorin (Table 1) in complex with a ribosome. The N-terminal twelve residues of Onc112 are critical for partially occluding the ribosomal exit tunnel and simultaneously overlapping the aminoacyl (A) site, preventing elongation after initiation [38,39]. Furthermore, both potency and activity spectrum of oncocin 18 is enhanced by systematically replacing each amino acid with other 19 residues [40]. Based on the structural information, Goldbach et al. have combined the two types of ribosomal binding PrAMPs by bridging Api137 (an apidaecin analog binding to 50S) and Onc112 (an oncocin binding to 70S) via esterification to ethylene glycol, leading to increased antimicrobial activity against *Pseudomonas aeruginosa* and *E. coli* [41*]. Hence, PrAMPs are a promising class of AMPs as a new type of antibiotics. Indeed, Onc72 shows *in vivo* efficacy comparable to meropenem, although the *in vitro* activity of meropenem is 44-fold higher [42].

3. Application strategies of antimicrobial peptides

Not all AMPs possess all the required properties (e.g., potency, selectivity, stability, and easy production) for direct uses as antibiotics and additional engineering is usually required to make them drug-like. Alternatively, AMPs may be applied indirectly by inducing gene expression from host cells or by engineering probiotic bacteria as an AMP delivery vehicle.

3.1. Prodrug

The majority of AMPs are gene-encoded and expressed as a precursor protein to neutralize the potential harmful effects of the AMP on the host. The AMP will be released by a protease. A construct that is designed to be similar to the AMP precursor is called prodrug here. This construct may offer advantages such as minimized cytotoxicity and enhanced protease stability. The prodrug form named P-dpMtx, consisting of an anionic peptide, a cephalosporin antibiotic linker, and a delivery peptide (dpMtx), is very effective in clearing mycobacterium residing within the macrophages [43]. Forde et al. described the optimization of the AMP prodrug model for cystic fibrosis (CF) to produce pro-WMR, a peptide with greatly reduced cytotoxicity. The bactericidal activity of pro-WMR is restored in neutrophil elastase-rich bronchoalveolar lavage fluid from the CF patients [44].

The prodrug concept can also be utilized to achieve specific pathogen targeting [45]. Here a prodrug construct can contain a recognition moiety (e.g., antibody), a protease-sensitive peptide linker, and an effector molecule (e.g. rifalogue). Thus, intracellular *S. aureus* is effectively eliminated by an antibody–antibiotic conjugate [46**]. In another interesting experiment, kanamycin plus a cell penetration peptide (P14LRR) can release the antibiotic in a reduced cellular environment, leading to effective clearing intracellular pathogens (*Mycobacterium tuberculosis*) within macrophages. It is also effective in vivo against Salmonella in a *Caenorhabditis elegans* model [47*].

3.2. Conjugation to improve potency or specific targeting

AMP properties may also be improved via conjugation, where two components are merged into one. Different from the prodrug design, *there is no cleavage site in such conjugates*. The fluoroquinolone antibiotic (levofloxacin) conjugated to a 10-residue CAMP Pep-4 shows enhanced antimicrobial activity even in the presence of an increased salt concentration. However, the antibiotic counterpart is not responsible for the mechanism of action, which was found to be peptide driven through disruption of bacterial membrane integrity [48]. Furthermore, the nonantibiotic moiety dithiocarbamate, conjugated to a cell penetrating peptide, is highly active against a wide range of pathogens, especially *S. aureus* [49]. Hyperbranched polyglycerol conjugated to aurein 2.2, or conjugation of an AMP to polyphosphoester, yields peptides with better biocompatibility and antimicrobial activity [50–51].

Bacterial targeting and detection of peptide library fragments against *Listeria monocytogenes* was achieved by binding with the C-terminal fragment (GEAFSAGVHRLANG) of leucocin A, a class IIa bacteriocin [52]. Persister cells of *E. coli* and *S. aureus* are effectively targeted by pentobra (cell penetrating peptide conjugated to tobramycin) up to 4–6 logs better than the individual aminoglycoside, tobramycin [53]. Further, chloramphenicol attached to UBI_{29–41} peptide using a glutaric anhydride linker is selectively targeted at the infection sites with *S. aureus* and *E. coli*, and demonstrated efficacy in both in vitro and in vivo mouse models. It also shows reduced toxicity to normal cells [54]. In another study, acridine conjugation to a nuclear localization sequence converts it into a potent broad spectrum AMP, with membrane disruption and DNA binding mechanisms of action [55].

3.3. Combined uses of AMPs with existing antimicrobial agents

Synergy between AMPs could be a fundamental defense strategy to keep different life forms healthy [56*]. In current clinical practice, two or more drugs are frequently used in combination to improve the treatment outcomes. Naturally, a combined use of new AMPs with existing antibiotics can extend the lifetime of traditional antibiotics and reduce the amount of the peptide needed for treatment. This is important considering that the cost of production is a major hurdle in the development of peptide therapeutics.

There are numerous examples demonstrating the advantages of combined use. For instance, synergy between polymyxin and carbapenems or rifampicin can efficiently suppress the development of polymyxin resistance [57]. Also, plectasin NZ2114 is synergistic with new antibiotics like teicoplanin, moenomycin, and dalbavancin against VanA-type vancomycin-resistant *Enterococcus faecalis* [58]. In an important unorthodox combination, colistin with fusidic acid is excellent against multidrug-resistant *Acinetobacter baumannii* infections [59]. Interestingly, select peptidomimetics are highly active in human blood plasma against a wide range of bacteria. The synergism may result from complement proteins and/or clotting factors [60]. Further, a single combined dose of oligo-acyl-lysyls (OAKs) with rifampin also imparts increased survival chances from 10–20% to 60% against *Klebsiella* infected mice [61]. In addition, the combination of a lipopeptide bacillomycin D with antifungal amphotericin B leads to excellent antibiofilm and wound-healing properties against *Candida albicans* [62]. Inexpensive compounds (e.g., ZnCl₂, NaF, or EDTA) can also be used as synergistic elements to improve peptide activity [63]. The antimicrobial cell wall hydrolases is synergistic with daptomycin in a murine model of *S. aureus* bacteremia [64].

Combined uses of AMPs with antibiotics can also enhance their antibiofilm potency [20]. For instance, mercedin (formerly known as 17BIPHE2), a human LL-37 derived peptide, can disrupt the biofilms of *S. aureus* USA300 under laboratory conditions [65], but is unable to do so in the case of *P. aeruginosa*. This may result from the complex nature of biofilms with a protective biopolymer coating for the bacterial communities. The preformed biofilms, however, can be dispersed when mercedin is used in combination with existing antibiotics (unpublished).

3.4. Induction of AMP expression in host cells

The observation that AMPs are induced in host cells upon pathogen invasion offers a novel strategy to combat infections. Instead of administering antibiotics to treat infected patients, doctors may use a proper agent to induce AMP expression at a desired site and at the right time. At present, multiple factors, ranging from amino acids to sunlight, are documented to stimulate the expression of AMPs (reviewed in [66]). The connection between light therapy and the human LL-37 expression provides new insight into this therapy for tuberculosis [67]. In addition, butyrates can work synergistically with vitamin D to induce additional AMPs. Recently, Ottosson et al. [68] found new analogs aroylated phenylenediamines that can induce even more peptides. Importantly, in vivo efficacy of this approach has been demonstrated in a rabbit model for Shigellosis. In the same vein, the maggot therapy is likely related to the expression and secretion of AMPs such as lucifensin into infected wounds by fly larvae that promote healing [69].

3.5. Engineered probiotic bacteria

AMPs may also be expressed by creating an expression vector for use in probiotic bacteria. Multiple lactic acid bacteria (LAB) are regarded as safe by the FDA [70]. Many of these bacteria are symbiotic with their hosts. They confer benefits such as the upregulation of anti-inflammatory pathways, the down-regulation of pro-inflammatory cytokine pathways [19,71], protection from enteric pathogens via H₂O₂ release and species-specific bacteriocin production [72], and attenuation of virulence factor expression by such pathogens [73]. Therefore, LAB are highly desirable as carriers for delivery of AMPs against specific human pathogens that colonize the human intestinal tract [72]. The development of plasmid vectors with AMP insertions should consider an appropriate origin of replication, a co-expressed immunity gene against the inserted AMP, a fused secretory signal recognized by specific ATP-binding cassette (ABC) transporters (or by the more widely used Sec secretion pathway), and a means of inducing expression, if the engineered construct does not enable constitutive expression. A promising construct was made by Themsakul et al. who created an expression secretion vector using new signal peptides (SPs) from *Lactobacillus casei* ATCC334. The three SPs they chose were used to make green fluorescent protein (GFP) fusions, with the CwhSP showing the highest amount of GFP secretion [74]. CwhSP was then fused and used to express and secrete the M2e:HBc (matrix 2 protein fused to Hepatitis B core antigen) protein in a pLC plasmid. Another LAB vector was made by Jiminez et al. [75] who fused the Sec dependent signal peptide, Usp45, to the enterocin A and the *entA* immunity gene. Although these systems are promising, there are hurdles to overcome. One such obstacle is that of lateral transfer of these plasmids to other members of the gut microbial community. Another issue stems from unintentional microbiotic imbalances that may be created through administration of these therapeutic LAB, causing unexpected health issues. For example, some commensal bacteria aid in eliciting an immune response through intercellular communication via vesicular release of microbial factors [76]. Prolonged release of AMPs by engineered probiotics may kill commensal bacteria, and thus mitigate their beneficial effects. Nevertheless, as many as seven AMPs have been expressed in a recently reported modular peptide expression system pMPES [77]. In addition, commensal *Enterococcus faecalis* strain has been engineered to express bacteriocin-21 (a variant enterocin AS-48) via a plasmid with impaired bacterial conjugation ability to de-colonize vancomycin-resistant enterococci in the mouse gut [78**,79].

4. Peptide antibiotics and concluding remarks

After the discovery of human lysozyme, the first antimicrobial protein, in 1922 by Alexander Fleming (refer to the AMP discovery timeline at <http://aps.unmc.edu/AP/timeline.php>), multiple peptides have been discovered and used (Figure 1d). Some of the peptides in Table 2 are assembled by a multienzyme system. Bacterial gramicidin is the first peptide antibiotic used to treat wound infections [80]. Daptomycin is another FDA-approved AMP for treating Gram-positive bacterial infections [81], adding another example to the list of antimicrobial peptides in clinical use (Table 2). In addition, nisin is the first ribosomally synthesized AMP widely utilized as a food preservative in over 50 countries [82]. Interestingly, these AMPs (Table 1 and its footnote) fall into the four unified peptide classes

proposed by Wang [83], implying that a wide range of peptides has a potential to become a new antibiotic.

By utilizing the design and application strategies in Figure 2, as well as expanding the search space in nature, additional AMPs are anticipated to find practical uses. The prodrug and peptide conjugates are elegant constructions that can improve drug specificity and potency at the expense of cost. Cell-specific and timely expression of AMPs from the host by inducing agents such as light and vitamin D, or via the delivery of engineered probiotic bacteria will be an important antimicrobial strategy actively under development. Finally, understanding the mechanism of commensal bacterial survival or pathogenic bacterial resistance to antibiotics, including AMPs, may provide new clue to the development of novel antibiotics [20]. Indeed, additional antibacterial or antifungal peptides are currently under clinical trials (see select examples in Table 2).

In summary, we predict that future peptide antibiotic research will at least cover the following three aspects: First, new treatment possibilities for existing AMPs will be further explored [84]. Second, new AMPs may be developed by utilizing the strategies highlighted in Figure 2 [10] and our search for new peptide candidates from nature will continue. Third, new AMPs and existing antimicrobial agents may be used in combination to better combat antibiotic-resistant bacteria (i.e., superbugs), especially those in the biofilm form [20]. Cost is regarded as a limiting factor in developing peptide antibiotics. With the development of personalized medicine, however, it may not be always required to produce AMPs at a large scale, thereby removing the cost issue as well.

Acknowledgments

We are grateful to the NIH funding support (R01 AI105147 and R03 AI128230) to GW. DZ gratefully acknowledges UGC, Govt. of India for the Raman Postdoctoral Fellowship (F.No. 5–133/2016 (IC)).

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** Papers of outstanding interest

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Highlights

1. Antibiotic resistance is a serious problem of our era.
2. Antimicrobial peptides are important templates for developing new antibiotics.
3. Both peptide discovery and application strategies are described.
4. Some AMPs are already in use and more are under development.

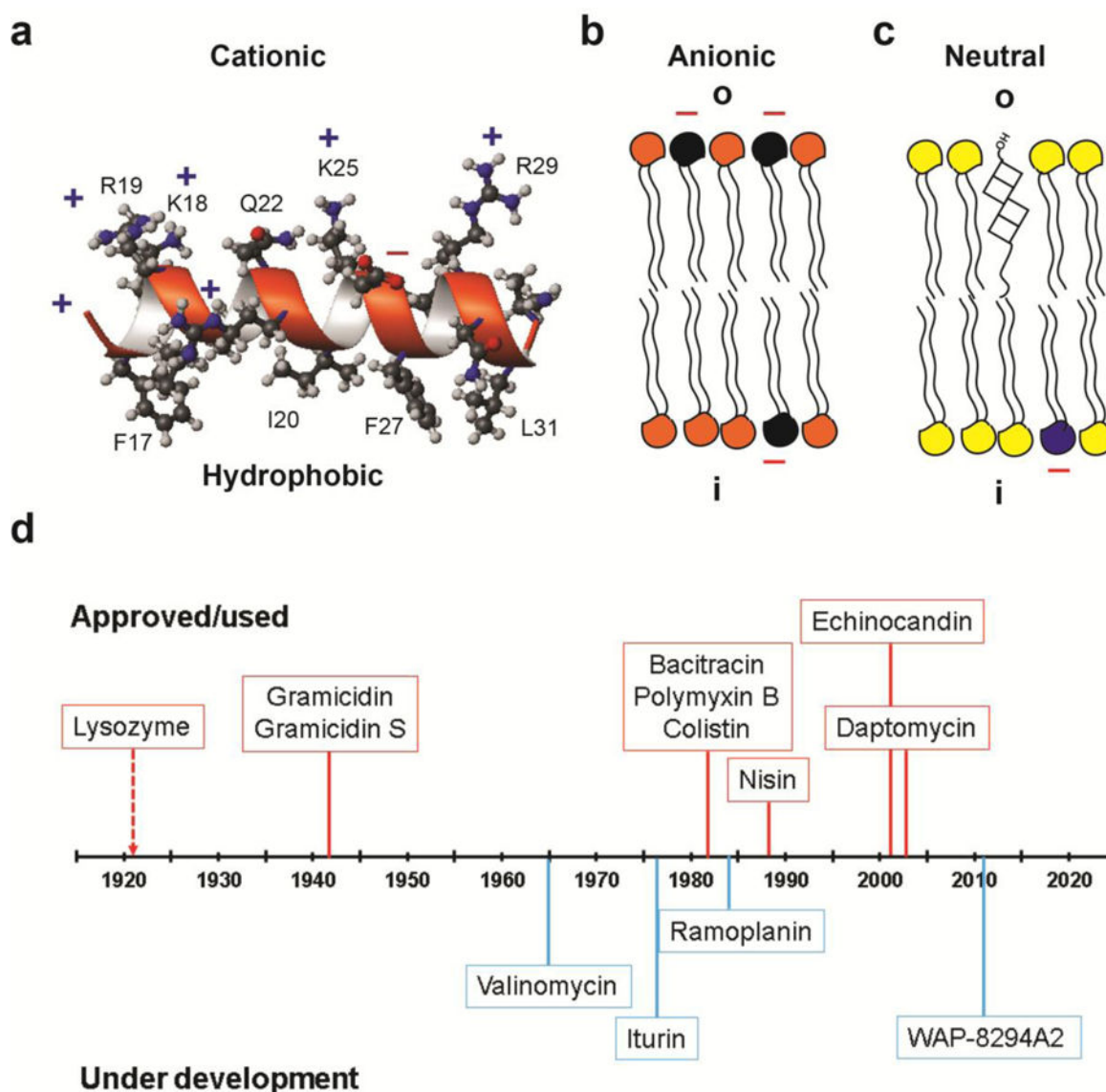


Figure 1. Antimicrobial peptides (a–c) and their timeline of applications (d)

The amphipathic and cationic antimicrobial peptide (a) preferentially binds to anionic bacterial membranes (b) rather than mammalian cell membranes, consisting of phosphatidylcholines (yellow) and cholesterol (square) with minor anionic lipid (blue) in the inner leaf (i) of the bilayer (c). The NMR structure of the major antimicrobial region (residues 17–32) of human cathelicidin LL-37 (a) is used to illustrate the amphipathic feature [14]. In the cartoon view of bacterial inner membranes (b), *E. coli* is assumed with 30% anionic phosphatidylglycerols (black) and 70% phosphatidylethanolamines (orange). (d) Select AMPs in use (orange, top) or under clinical trials (blue, bottom) are depicted. Further examples can be viewed in Table 2.

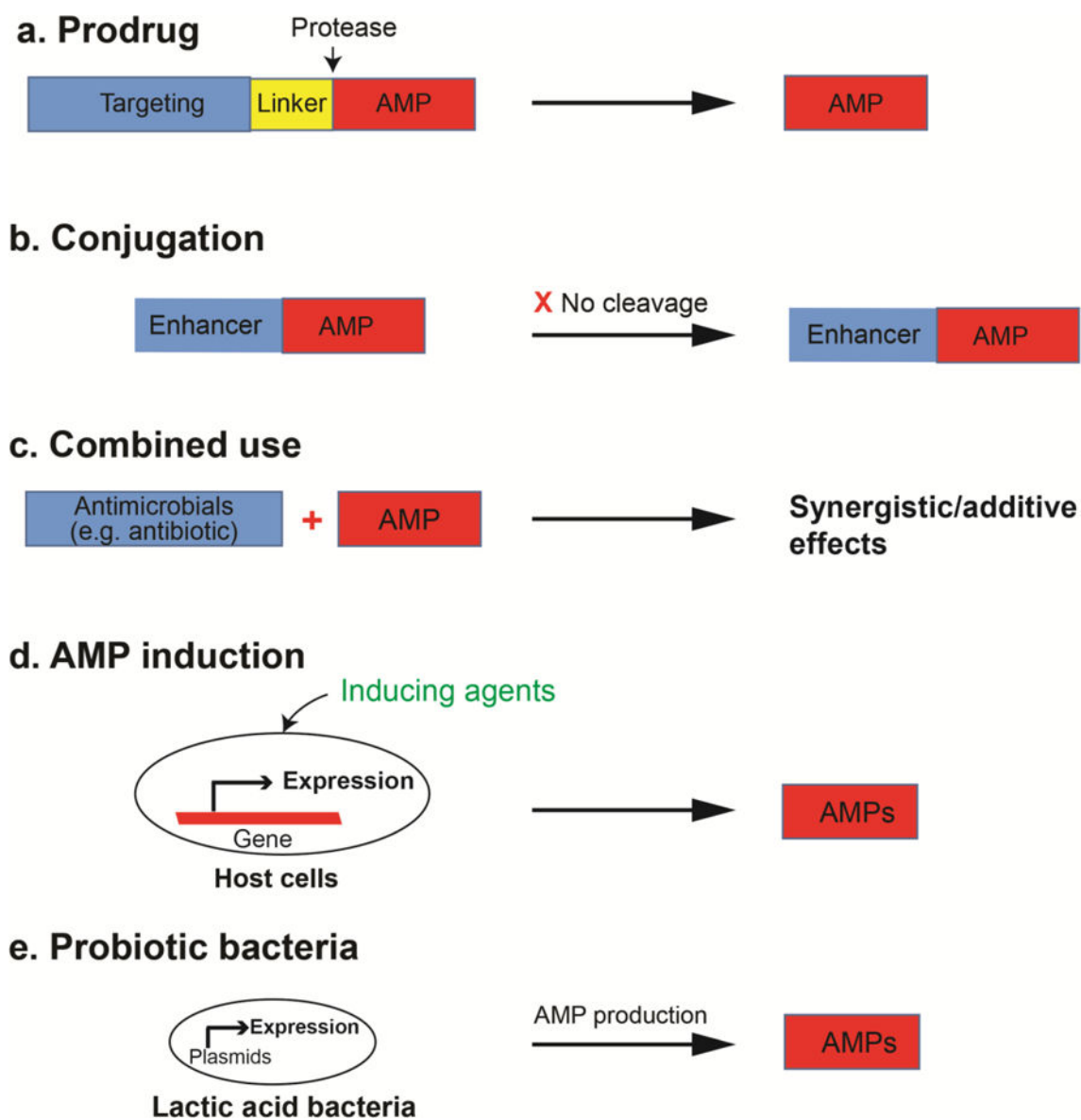


Figure 2. Application strategies of antimicrobial peptides

(a) prodrug; (b) conjugation; (c) combined use; (d) induction from the host; and (e) the use of probiotic bacteria to deliver and express the needed antimicrobial peptides. See the text for further details.

Table 1

Select antimicrobial peptides, amino acid sequences and properties¹

Class	Peptide	Amino acid sequence	Length	Net charge	Pho	Boman index	Source	APD ID
I	Gramicidin A	VGALAVVVWLWLWLW	15	0	93%	-3.3	Bacteria	499
	Magainin 2	GIGKFLHSAKKFKGKAFVGEIMNS	23	+3	43%	0.41	Amphibians	144
	LL-37 (cathelicidin)	LLGDFFRKSKEKIGKEFKRIVQRIKFLRNLVPRTES	37	+6	35%	3.0	human	310
II	Pyrrhocortin (P α -AMP)	VDKGSYLPRPTPPRIYNRN	20	+3	15%	3.2	insects	170
	Nisin A (lantibiotic)	ITSISLCTPGCKTGALMGCNMTATCHSIHYSK	34	+3	44%	0.37	Bacteri	205
	HNP1 (α -defensin)	ACYCRIPACIAGERRYGTCTI YQGRLWAFCC	30	+3	53%	1.1	Human	176
	TAP (β -defensin)	NPVSCVRNK GICVPRCPGSMKQIGTCVGRVAKCCRKK	38	+9	42%	1.7	Bovine	235
	Plectasin	GFGCNGPWD EDDMQCHNH CKSIKGYKGGYCAKGGFVCKCY	40	+1	32%	1.4	Fungi	549
III	Colistin	XTXXKLLXXT	10	+6	20%	2.9	Bacteria	2204
	Daptomycin	WNDTGKDADGSEY	13	-3	15%	3.5	Bacteria	2203
	Microcin J25 (bactertocin)	VGIGTFESYGGGAGHVPEYF	21	-1	33%	-0.64	Bacteria	480
IV	Alamethicin (peptaibol)	PBABAQBYBGLBPVBBEQ	18	-1	66%	-0.68	Fungi	2197
	Gramicidin S	VKLPVKLFP	10	2	60%	-1.3	Bacteria	2243
	Subtilosin A (bactertocin)	NKGCATCSIGAAACLVDPDPDFEIAAGATGLFLWG	35	-2	51%	-0.46	bacteria	928
	Kalata B1 (cyclotide)	GLPVCGETCVGGTCNTPG CTCSWPVCTRN	29	0	37%	0.67	Plants	729
	RTD-1 (θ -defensin)	GFRCRLCRRGVCRICICTR	18	5	55%	2.8	Monkey	445

¹Data taken from the APD (<http://aps.ummc.edu/AP>), where Pho = hydrophobic%, B = α -aminoisobutyric acid and X = 2,4-diaminobutanoic acid. Boman index was originally called protein-binding potential by Boman [5]. Additional information, including the original reference, can be found in the APD. A unified peptide classification has been proposed [83] and adopted in the APD. This classification is based on the covalent bonding patterns of polypeptides. Class I (or UCLL) contains all linear peptides where both the backbone and sidechains may be modified but do not lead to a loop structure. Class II (or UCSB) contains peptides with a sidechain-sidechain connection, irrespective of intra or inter-connections. Class III (or UCSB) peptides must contain a covalent bond between backbone and sidechain, while class IV (or UCBB) consists of circular peptides due to a bond between backbone and backbone [83].

Table 2

Select antimicrobial peptides in use or under development

Class	Peptide	Source	Activity spectrum	Mechanism of action	Approval/Use	Ref
I	Gramicidin	<i>Bacillus brevis</i>	Gram +/- pathogens	Forms an ion channel in membranes.	Ophthalmic use as solutions/drops (1940s)	[85]
II	Nisin	<i>Lactococcus lactis</i>	Gram +pathogens	Binds lipid II and inhibits cell wall synthesis.	Food preservative (1969, WHO; 1988, FDA)	[86]
III	Bacitracin	<i>Bacillus subtilis</i>	Gram +/- pathogens	Inhibits cell wall and peptidoglycan synthesis.	Powder form (before 1982)	[87]
III	Polymyxin B	<i>Bacillus polymyxa</i>	Gram - pathogens (e.g., <i>P. aeruginosa</i>)	Damages membranes	Aerosporin injection (before 1982); endotoxin removing (2003)	[88]
III	Colistin, or polymyxin E	<i>Paenibacillus polymyxa</i>	Pseudomonas and Acinetobacter sp.	Damages membranes	Suspension/drops (1982)	[89]
III	Daptomycin	<i>Streptomyces roseosporus</i> .	Gram+ MRSA	Damages membranes	Treat complicated skin infection (2003, FDA).	[90]
III	Echinocandin ²	<i>Glarea lozoyensis</i> ; <i>Coleophoma empedrae</i> ; <i>A. nidulans</i>	Fungi (<i>C. albicans</i>)	Inhibits the synthesis of glucan and cell wall.	FDA approved	[91]
IV	Gramicidin S	<i>Bacillus brevis</i>	Gram +/- pathogens	Disrupts the outer membrane.	Wound infections (1942, Soviet Union)	[92]
I	Pexiganan (Magainin)	<i>Xenopus laevis</i>	Diabetic foot ulcers	Membranes	Phase III	[93]
I	Omgiganan (indolicidin)	<i>Bos taurus</i>	Rosacea	Membrane/DNA	Phase II	[94]
I	OP-145 (LL-37)	<i>Homo sapiens</i>	Chronic middle ear infection	Membranes	Phase I/II	[94]
I	PXL01 (lactoferricin)	<i>Homo sapiens</i>	Prevention of postsurgical adhesion formation	Repressing proinflammatory cytokine secretion and promoting fibrinolysis.	Phase II	[94]
I	PAC-113 (histatin 3)	<i>Homo sapiens</i>	Oral candidiasis	Membranes/Inhibiting cytokine production.	Phase II	[94]
II	Iseganan/IB-367 (protegrin-1)	<i>Sus scrofa</i>	Oral mucositis	Membranes	Phase III	[94]
II	Novexatin/NP-213 (defensin)	<i>Homo sapiens</i>	Fungal nail infection	Membrane lysis	Phase II	[94]
III	Iturin A (lipopeptide)	<i>Bacillus subtilis</i>	Fungi; G +/- bacteria	Forms ion conducting pores in membranes.	Preclinical	[95]
III	Ramoplanin	<i>Actinoplanes sp. ATCC 33076</i> .	Gram+ bacteria	Inhibits cell wall synthesis.	VRE (Phase III); <i>C. difficile</i> (Phase II).	[96]
III	WAP-8294A2 (Lotifibcin)	Lysobacter sp.	Gram+ bacteria (VRE and MRSA)	Interacts with membranes.	Systemic infection (Phase I/II)	[97]
IV	Valinomycin	<i>Streptomyces tsusimaensis</i> ; <i>S. fulvissimus</i>	Gram+ bacteria; Fungi (<i>C. albicans</i>)	Forms a K+ ion channel in membranes.	Not available	[98]

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¹Data from http://www.accessdata.fda.gov/scripts/cder/ob/search_product.cfm; <https://clinicaltrials.gov/>; <https://pubchem.ncbi.nlm.nih.gov/>; <http://aps.unmc.edu/AP>.

²The FDA-approved echinocandins are semisynthetic lipopeptides derived from caspofungin, micafungin, and anidulafungin.