The vast structural diversity of antimicrobial peptides

Johannes Koehbach[1] and David J. Craik[1,*]

[1]*Institute for Molecular Bioscience, The University of Queensland, 4072 Brisbane QLD, Australia*

[*]*Correspondence:* d.craik@imb.uq.edu.au (D.J. Craik)

Keywords

cysteine-rich, cyclic peptides, drug design

Abstract

Antimicrobial peptides (AMPs) occur in all kingdoms of life and are integral to host defense. They have diverse structures and target a variety of organisms, both by non-specific membrane interactions or via specific targets. Here we discuss the structures of AMPs from the four main classes currently recognized, i.e. peptides with (i) α-helical; (ii) β-sheet; (iii) αβ; and (iv) non-αβ elements as well as the growing pool of complex topologies including various post-translational modifications. We propose to group these latter peptides into a fifth class of AMPs. Such peptides exhibit high stability and amenability to chemical engineering, making them of interest for the development of novel antimicrobial agents. Advances and challenges in the development of these peptides towards therapeutic leads are presented.

AMPs – a diverse, but unifying strategy for defense

Antimicrobial resistance has been identified as a major threat to public health and without immediate and global action the world is headed for a dangerous post-antibiotic era [1]. Thus, there is an urgent need for the development of novel antibiotic drugs to treat infectious diseases. In contrast to the rising numbers of multi drug resistant pathogens the rate of discovery of novel drug candidates is dwindling [2]. In this regard antimicrobial peptides (AMP) are a promising class of bioactive compounds that have attracted increasing attention over recent years. Their broad spectrum of activities extends beyond the killing of bacteria and fungi, with several AMPs also exhibiting antiviral [3], antiparasitic [4] or anticancer activities [5]. Furthermore, their multifaceted mechanisms-of-action potentially reduce their susceptibility to suffer from microbial resistance [6].

Starting in the 1980s, at a time when the numbers of novel antibacterial agents started to drop, the field of AMP research gained momentum when several novel examples were independently discovered across different species. These included peptides such as the cecropins from insects [7], the magainins from amphibians [8] and the mammalian defensins [9] to name a few. Since then a plethora of AMPs has been identified from all kingdoms of life, from bacteria to fungi to plants and animals. It is not only evident that these peptides play an integral part of an organism's innate defense machinery, but their variety also makes them a rich source for the discovery of potential novel drug leads. Their distribution among virtually all living organisms is complemented by their structural variety and range of antimicrobial activities. In this review we highlight this vast structural diversity of antimicrobial peptides. As well as providing a brief overview of the structures of well-known classes of AMPs, we introduce the growing class of structurally complex AMP topologies, in particular, cyclic and cysteine-rich defense peptides. We discuss recent progress and challenges in the characterization and development of peptide-based antibacterial molecules.

Structural classes of antimicrobial peptides

The ubiquitous presence and vast diversity of naturally-occurring peptidic antimicrobial compounds make their classification a non-trivial task. Although they can be grouped based on their source organisms or activity spectrum, they are most commonly classified based on their structural properties. It is also worth noting that AMPs can be of ribosomal or non-ribosomal origin, thus providing another alternative classification scheme based on their biosynthetic mechanism. Indeed, many peptide antibiotics that are in clinical use, e.g. gramicidins, polymyxins, bacitracins, or glycopeptides such as vancomycin or teicoplanin, are of non-ribosomal origin. Their complex biosynthetic pathways lead to a diversity of structures involving a variety of unusual amino acids and further modifications [10]. Whereas non-ribosomal synthesized AMPs are typically restricted to bacteria and fungi, gene-encoded AMPs are found in all kingdoms of life and these are the focus of this review. They are derived from larger precursor proteins via proteolytic processing and further post-translational modifications, as are many other ribosomal and post-translational modified peptides [11].

AMPs are typically relatively short, i.e. fewer than 100 amino acids in size, and incorporate mostly cationic, hydrophobic and amphipathic properties. Despite these common characteristics they are highly diverse with respect to their primary, secondary and tertiary (three-dimensional) structures (Table 1 and Figure 1). Based on the presence or absence of the two key secondary structure elements of **α-helices** and **β-sheets** (see Glossary), AMPs are commonly divided into four major classes [12-16], i.e. (i) linear α-helical peptides; (ii) linear extended structures (devoid of α- or β- elements but typically rich in one particular amino acid such as Gly, Arg, Trp or Pro); (iii) β-sheet containing peptides, often stabilized with one or more **disulfide bonds;** and (iv) peptides involving α- and β-elements (Figure 1). In the last two decades there have been increasing reports of cyclic **and** disulfide-rich AMPs (e.g. θ-defensins or cyclotides) as well as AMPs with more complex topologies including,

for example, lasso peptides or **thioether** bridged structures. To facilitate the structural classification of ribosomal AMPs, we propose to group these peptides into a fifth category of 'topologically complex' AMPs.

Insert Figure 1.

Insert Table 1.

Linear α-helical peptides

The largest and best-studied group of AMPs adopt α-helical conformations. Several hundred different sequences have been identified from natural sources and a multitude of synthetic analogues further expands the size and diversity of this class [17]. Prominent examples of α-helical AMPs are from insects, including the honey bee venom component melittin and various cecropins [7]. Other well-known examples are the frog magainins [8], and the mammalian cathelicidins, including the human peptide LL-37 [18, 19]. While the helical structures are often preformed in solution for many of these peptides, some peptides have been identified whose α-helical structures are enhanced upon contact with target membranes [20]. A combination of helix length, content and orientation of charged and hydrophobic residues leads to a variety of helical structures that account for the broad range of observed activities of this class of peptides. In some cases the α-helical motif can be combined with other structural domains, as recently seen in an α-helical AMP identified as part of a modular spider toxin, OtTx1a, which is reported to combine an inhibitor cystine knot domain and an AMP domain in a single molecule [21].

Linear peptides rich in particular amino acids ('extended' or non-α/β element containing AMPs)

Some AMPs do not adopt any particular three-dimensional structure, either in solution or upon contact with membranes and are hence referred to as extended linear structures. Devoid of α-helices

or β-sheets these peptides are often rich in one type of amino acid, typically glycine, proline, tryptophan, or histidine. Prominent examples include abaecin from honeybees [22], bovine indolicidin [23], human histatins [24], and hymenoptaecins from various insects [25]. Another particularly interesting subclass are proline-arginine rich AMPs, with several examples from insects, e.g. apidaecins, drosocin or pyrrhocoricin and the mammalian PR-39 [26]. The latter peptide adopts a PP-II helical conformation [27].

β-sheet containing peptides

The third group of AMPs encompasses peptides with topologies that include β-sheets. Many of these peptides are further stabilized with one or more disulfide bonds. Based on their cysteine content and structural characteristics, they can be grouped into (i) β-hairpin peptides or (ii) α-defensin peptides. Examples of β-hairpin peptides with varying numbers of disulfide bonds are thanatin from insects (one SS bond) [28], protegrin-1 from porcine leukocytes [29], polymephusin and tachyplesin from horseshoe crabs [30] and gomesin from a tarantula spider (two SS bonds) [31], to name a few. Defensin peptides span multiple structural classes. The α-defensins are a subgroup that comprise three antiparallel β-strands which are linked with disulfide bonds in a 'trans'- arrangement, i.e. the two disulfide bonds from the terminal β-strand point in opposite directions and link two different elements. These peptides have a conserved salt-bridge, which is required for proper folding and proteolytic stability, but is not essential for antimicrobial activity [32]. Recent reports of the α-defensin-type peptides rattusin [33] and the HBD-5 dimer [34] further expand the structural diversity of α-defensin peptides. These peptides have been suggested to adopt C_2-symmetrical structures formed via intermolecular cysteine bridges, although the presence of these structures *in vivo* has yet to be confirmed.

Peptides with α- and β- structural elements

Other classes of defensin (besides the α-defensins) often have structures containing both helical and β-sheet elements of secondary structure. Defensins are not only found within humans and other mammals, but have been described in various invertebrate species, and plants. Their sub-classification is based on the different arrangements of their three to five disulfide bonds. Like the human α-defensins, the β-defensins also display a *trans* arrangement of disulfide bonds but contain an additional α-helix. In contrast to human peptides, plant and invertebrate defensins fall into the class of *cis*- defensins, i.e. peptides with two disulfide bonds linking the same α-helix with the same terminal β-strand in a parallel arrangement. *Cis*-defensins where the third disulfide bond connects the N-terminal loop with the second β-sheet are known as cysteine-stabilized αβ defensins (CSαβ) [35]. This motif is also found in several scorpion toxins that are potent ion channel blockers, suggesting an evolutionary link of a common structural motif used for defense [36]. A recent detailed review about defensins, with a particular focus on structural and evolutionary aspects is highly recommended [37].

Cyclic and other unusual or complex peptide topologies

A common feature of the four classes of AMPs described above is that they comprise a topologically linear peptide backbone, albeit in most cases folded into a defined three-dimensional shape. To deal with increasing numbers of newly discovered AMPs with cyclic and other complex topologies we propose here to group peptides containing these complex topologies together as a fifth main class of AMPs. Further sub-classification may then be based on the nature of the peptide's cyclic topology, i.e. 'head-to-tail' or 'head-to-sidechain' and the nature of the crosslinks such as disulfide or thioether bridges if present (Figure 2).

Insert Figure 2.

One group of backbone cyclized peptides that lack additional crosslinks are the cyclic bacteriocins [38]. These peptides are ~6kDa in size and even though their primary sequences do not share high homology, their NMR structures display remarkably similar arrangements, comprising four or five α-helices for carnocyclin A, enterocin NKR-5-3B and enterocin AS-48, respectively [39-41]. Most other backbone-cyclized AMPs contain additional constraints such as disulfide bonds or thioether bridges. For example, two families of backbone cyclized peptides with three stabilizing disulfide bonds are the mammalian θ-defensins (Box 1) and the plant cyclotides (Box 2). θ-defensins are relatively short peptides (e.g. RTD-1 has only 18 residues) and have a simple laddered arrangement of three parallel disulfide bonds. In contrast, the plant cyclotides are almost double in size (around 30 residues) and display a complex knotted topology of their three disulfide bonds. Despite their vastly differing disulfide connectivities, both classes of cyclic tri-disulfide peptides display remarkable stability compared to their linear counterparts.

Insert Box 1.

Insert Box 2.

Besides head-to-tail cyclized backbones, other unusual structural features within AMPs include examples of cyclic motifs that result from the presence of thioether bridges, or from a link between an amino acid side-chain and the peptide backbone. In the latter case many examples exhibit an unusual threaded topology known as a lariat knot or lasso [42]. Within such structures a macrolactam cycle forms between the N-terminus and the carboxylic acid side-chain of a glutamate or aspartate residue which is threaded by the C-terminal tail sequence. Bulky residues sterically lock the threaded tail in place and the structures (see Figure 2) are in some cases further stabilized by one or more disulfide bonds [43]. Advanced genome-mining techniques are continuously expanding the diversity of this class of AMPs, opening up new avenues to exploit their structural and functional diversity

174 further [43]. Recent progress in the field of lasso peptides is described in two recommended review
175 articles [42, 44].

176 A wide range of other post-translational modifications (PTMs) further expand the structural
177 variety of antimicrobial peptides. PTMs include thioether bridges such as in lanthibiotics and
178 sactibiotics, or glycosylation of cysteine residues found within glycocins. For example, the structure
179 of the prototypic lanthibiotic nisin A contains five lanthionine rings and two dehydro-alanines and
180 one dehydro-butyrine residue and adopts various turns upon binding to its target, lipid II [45]. The
181 sactibiotic subtilosin A displays rare sulphur to α-carbon bonds involving D-amino acid residues [46]
182 and also has a head-to-tail cyclic backbone. Finally, glycocins are disulfide-bridged peptides that
183 have sugar moieties linked to an additional cysteine residue, which are important for their
184 antimicrobial activity [47]. The structure of sublancin 168 exhibits two α-helical segments that are
185 linked by two disulfide bonds. The S-linked glucose is attached to the cysteine in the loop region
186 between the two helices [48] .

187 This structural diversity of naturally-occurring AMPs has been further expanded in recent
188 years using advanced synthetic chemistry techniques. For example, recent progress in polymeric
189 AMP design, including peptide dendrimers [49] or nanoparticles [50] adds another dimension to
190 peptide structures to be exploited as novel antimicrobial agents.

191

192 **Linking structure and function – the multiple mode-of-actions of AMPs**

193 AMPs have traditionally been defined as molecules that exhibit direct antimicrobial effects. However,
194 it has now become apparent that several AMPs not only act directly as antimicrobial agents, but are
195 able also to modulate the innate immune system of the host organism. To better account for these
196 immunomodulatory activities, these peptides are referred to as host defense peptides or HDPs. One
197 of the best studied HDPs is the human cathelicidin LL37 [19]. A plethora of analogues have been
198 designed and, reflecting their role, these peptides are referred to as innate defense regulator peptides

[18]. The complex immunomodulatory effects of HDPs have recently been reviewed elsewhere [51]. For completeness we note that AMPs have been reported to have a wide range of antifungal, antiviral and anticancer activities, targeted either at membranes or intracellular targets [3, 5, 52]. Here we focus on describing how the structure of AMPs is linked to their antibacterial functions and modes of action (Table 2).

Insert Table 2.

Direct antimicrobial activity is thought to be closely related to a peptides' structural and biophysical properties. The overall high positive net charge (Table 1) and amphipathicity can explain high affinity for membranes, in particular, for negatively charged bacterial cell walls. Several models have been described that involve binding and insertion of peptides into lipid bilayers that lead to the formation of pores or other forms of disruption or permeabilization of the target membranes [14]. In particular, α-helical peptides have been reported to exhibit their effects via such membrane pore-forming mechanisms. However, α-helical and strongly membranolytic peptides often display undesired toxic effects, such as hemolytic activity. Extensive work on α-helical peptides such as the human LL-37 peptide aimed to identify compounds that retain antimicrobial activity but without associated cytotoxic effects. Subtle changes in the ratio between hydrophobicity and charges can confer drastic changes to a peptides' activity profile. For example, early SAR studies revealed that a 12mer of LL-37, known as KR-12 is the minimal sequence required for its antibacterial activity, without the unwanted cytotoxicity of the 13mer FK-13 [53]. A recent mutagenesis study further highlighted the consequences of subtle changes to the ratio of charge and hydrophobicity of KR-12 [54].

Membrane interaction is not exclusive to α-helical peptides. In fact, most AMPs display high affinity for microbial membranes resulting from their overall amphipathic nature. Thus, it is not

surprising that peptides from other structural classes such as defensins or cyclotides also exhibit potent membranolytic effects [55, 56]. Nevertheless, it is becoming more apparent that membrane-active AMPs often act on specific targets rather than via unspecific pore formation. Recent work on cyclotides, for example, has demonstrated the importance of specific interactions with phosphatidylethanolamine lipids for the binding of, and thus activity of, these peptides [57-59].

Another prominent membranous target for AMPs is lipid II, a key precursor in bacterial cell wall synthesis. Lipid II can be inhibited by various structural classes of AMPs, including lanthibiotics [45] or various defensin-type peptides [60, 61]. With regard to the latter peptides it is worth noting that structurally closely related CSαβ toxins found in scorpion venom show potent activity at potassium channels [36]. Overall, activity at membranes is a common strategy to fight invading pathogens or predators via multiple distinct pathways.

Even though membrane interactions are a major factor contributing to the activity of many AMPs, there are increasing reports of AMPs that have antimicrobial effects without disrupting bacterial membranes and instead act on intracellular targets. Such peptides that can cross membranes without disruption are of broad interest and may be also exploited for applications beyond antimicrobial activity. Table 2 includes examples of these AMPs that exhibit their direct antimicrobial activity without membranolytic effects. Interestingly, a study comparing the effects of the α-helical peptides buforin II and magainin 2 found that despite high structural similarity, these peptides exhibit their activity via two different mechanisms. Whereas magainin acts via membranolytic effects, buforin II was found to bind to RNA and DNA [62]. Similarly, proline-rich AMPs such as pyrrhocoricin or drosicin can act on different components of the bacterial ribosomes as well as the chaperone-assisted folding via inhibition of DnaK [63-65]. The bacterial lasso peptide microcin J25 was found to have different modes of action depending on the organism. It inhibits the bacterial RNA polymerase [66], but was also found to disrupt membranes in *Salmonella* species [67]. Other described targets for AMPs include, for example, the 20S proteasome, which is inhibited by the

proline-arginine rich PR-39 [68]. With regard to intracellular activity, recent reports of cyclotides and other cell-penetrating peptides provide new impetus to engineer AMPs with reduced membranolytic effects and the potential to modulate intracellular targets or protein-protein interactions [69, 70].

Constrained topologies such as cyclic or disulfide rich peptides provide excellent starting points for drug development approaches. A key advantage is their increased stability compared to linear counterparts. Additionally, their amenability to peptide engineering while retaining their overall three-dimensional structure makes them particularly useful for structure-activity studies and rational AMP design. For example, a valuable structural motif that has been recently exploited for engineering of antimicrobial activity is the β-hairpin. A recent study generated peptidomimetics based on protegrin-1 and identified a novel antibacterial mechanism of action via inhibition of the cell wall protein LptD [71]. Similarly, the β-hairpin peptide gomesin was used as a template to successfully engineer highly potent and stable analogues by introducing a cyclized backbone and optimizing amphipathic properties [72].

Concluding Remarks and Future Perspectives

The field of AMP research is highly active, but several challenges have impeded the development of AMPs as therapeutic agents (see Outstanding Questions). Although there are several AMPs in clinical development, their route of delivery is almost exclusively via topical administration [73, 74]. Recent progress to overcome the typically low proteolytic stability and lack of oral bioavailability of peptides using cyclic and disulfide-rich scaffolds could open new avenues for the development of much needed antimicrobial agents [75]. Another challenge is the complexity of linking AMP structures to their activities and targets. In fact, one structure can have activity at multiple targets, and similarly one target can be affected by peptides from different structural classes (Table 2). Such multi-targetting does have some advantages though, and AMPs that target multiple sites might be less likely to suffer from bacterial resistance, although this requires further investigation [76].

An additional problem in AMP progression to the clinic is the translation between *in vitro* and *in vivo* activities. For example, the peptide A3-APO has been reported to have relatively modest activities when tested *in vitro,* but had potent effects in various *in vivo* models [77]. These discrepancies can result from significant differences in the environments that peptides and pathogens are exposed to under differing assay conditions. Furthermore, as outlined earlier, many AMPs display their activity mainly via potent immunomodulatory effects rather than direct killing and thus data from *in vitro* testing may not reflect a peptides' true *in vivo* activity.

Increased knowledge of the structural diversity of AMPs provides a deeper understanding of how AMP structure correlates with observed activities and modes-of-action. Importantly, this establishes the basis for rational peptide design efforts, which are assisted by continuous advances in both computational as well as synthetic methods. Of particular interest, is recent progress in the design of constrained peptides that promises to accelerate the generation of novel lead compounds [78]. In this regard naturally-occurring cyclic and disulfide rich antimicrobial peptides as described in this article are excellent starting points for drug development. Their amenability to mutagenesis and peptide engineering has already resulted in numerous compounds with improved bioactivities and reduced cytotoxic effects [72, 79, 80]. In addition, the increased power and accuracy of bioinformatics methods and molecular dynamics simulations [81] can help in the prediction of the antimicrobial activity [82] and mechanism-of-action and further aid in rational peptide analogue design [83]. Overall developments in the field over recent years provides confidence that research efforts using cyclic and disulfide-rich peptides may lead to the development of much needed novel antimicrobial agents.

Acknowledgements

Work on our laboratory on antimicrobial peptides is supported by a grant from the Australian Research Council (DP150100443) and from the Clive and Vera Ramaciotti Foundation. DJC is an Australian Research Council Laureate Fellow (FL150100146).

References

1 Sugden, R., *et al.* (2016) Combatting antimicrobial resistance globally. *Nat. Microbiol.* 1, 16187

2 Cole, S.T. (2014) Who will develop new antibacterial agents? *Phil. Trans. R. Soc. B Biol. Sci.* 369, 20130430

3 Marcocci, M.E., *et al.* (2018) The Amphibian Antimicrobial Peptide Temporin B Inhibits In Vitro Herpes Simplex Virus 1 Infection. *Antimicrob. Agents Chemother.* 62, e02367-02317

4 Carter, V., *et al.* (2013) Killer bee molecules: antimicrobial peptides as effector molecules to target sporogonic stages of Plasmodium. *PLoS Pathog.* 9, e1003790

5 Felício, M.R., *et al.* (2017) Peptides with Dual Antimicrobial and Anticancer Activities. *Front. Chem.* 5, 5

6 Guilhelmelli, F., *et al.* (2013) Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. *Front. Microbiol.* 4, 353

7 Steiner, H., *et al.* (1981) Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature* 292, 246-248

8 Zasloff, M. (1987) Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proc. Natl. Acad. Sci. U. S. A.* 84, 5449-5453

9 Lehrer, R.I., *et al.* (1991) Defensins: endogenous antibiotic peptides of animal cells. *Cell* 64, 229-230

10 Payne, J.A., *et al.* (2016) Diversity of nature's assembly lines - recent discoveries in non-ribosomal peptide synthesis. *Mol. Biosyst.* 13, 9-22

11 Arnison, P.G., *et al.* (2013) Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature. *Nat. Prod. Rep.* 30, 108-160

12 Brogden, K.A. (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.* 3, 238-250

13 Hancock, R.E.W. and Sahl, H.-G. (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol.* 24, 1551-1557

14 Nguyen, L.T., *et al.* (2011) The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol.* 29, 464-472

15 Wang, G. (2015) Improved methods for classification, prediction, and design of antimicrobial peptides. *Methods Mol. Biol.* 1268, 43-66

16 Mojsoska, B. and Jenssen, H. (2015) Peptides and Peptidomimetics for Antimicrobial Drug Design. *Pharmaceuticals (Basel)* 8, 366-415

17 Wang, G., *et al.* (2016) APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Res.* 44, D1087-D1093

18 Choi, K.Y., *et al.* (2012) Cationic host defence peptides: multifaceted role in immune modulation and inflammation. *J. Innate Immun.* 4, 361-370

19 Xhindoli, D., *et al.* (2016) The human cathelicidin LL-37--A pore-forming antibacterial peptide and host-cell modulator. *Biochim. Biophys. Acta* 1858, 546-566

20 Porcelli, F., *et al.* (2013) On the Role of NMR Spectroscopy for Characterization of Antimicrobial Peptides. In *Membrane Proteins: Folding, Association, and Design* (Ghirlanda, G. and Senes, A., eds), pp. 159-180, Humana Press

21 Nadezhdin, K.D., *et al.* (2017) Modular toxin from the lynx spider Oxyopes takobius: Structure of spiderine domains in solution and membrane-mimicking environment. *Protein Sci.* 26, 611-616

22 Casteels, P., *et al.* (1990) Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (Apis mellifera). *Eur. J. Biochem.* 187, 381-386

348 23 Selsted, M.E., *et al.* (1992) Indolicidin, a novel bactericidal tridecapeptide amide from neutrophils. *J. Biol.*
349 *Chem.* 267, 4292-4295

350 24 Oppenheim, F.G., *et al.* (1988) Histatins, a novel family of histidine-rich proteins in human parotid
351 secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. *J. Biol.*
352 *Chem.* 263, 7472-7477

353 25 Casteels, P., *et al.* (1993) Functional and chemical characterization of Hymenoptaecin, an antibacterial
354 polypeptide that is infection-inducible in the honeybee (Apis mellifera). *J. Biol. Chem.* 268, 7044-7054

355 26 Graf, M., *et al.* (2017) Proline-rich antimicrobial peptides targeting protein synthesis. *Nat. Prod. Rep.* 34,
356 702-711

357 27 Cabiaux, V., *et al.* (1994) Secondary structure and membrane interaction of PR-39, a Pro+Arg-rich
358 antibacterial peptide. *Eur. J. Biochem.* 224, 1019-1027

359 28 Mandard, N., *et al.* (1998) Solution structure of thanatin, a potent bactericidal and fungicidal insect peptide,
360 determined from proton two-dimensional nuclear magnetic resonance data. *Eur. J. Biochem.* 256, 404-410

361 29 Kokryakov, V.N., *et al.* (1993) Protegrins: leukocyte antimicrobial peptides that combine features of
362 corticostatic defensins and tachyplesins. *FEBS Lett.* 327, 231-236

363 30 Miyata, T., *et al.* (1989) Antimicrobial peptides, isolated from horseshoe crab hemocytes, tachyplesin II,
364 and polyphemusins I and II: chemical structures and biological activity. *J. Biochem.* 106, 663-668

365 31 Mandard, N., *et al.* (2002) The solution structure of gomesin, an antimicrobial cysteine-rich peptide from
366 the spider. *Eur. J. Biochem.* 269, 1190-1198

367 32 Andersson, H.S., *et al.* (2012) The alpha-defensin salt-bridge induces backbone stability to facilitate folding
368 and confer proteolytic resistance. *Amino Acids* 43, 1471-1483

369 33 Min, H.J., *et al.* (2017) Rattusin structure reveals a novel defensin scaffold formed by intermolecular
370 disulfide exchanges. *Sci. Rep.* 7, 45282

34 Wommack, A.J., *et al.* (2014) Discovery and characterization of a disulfide-locked C(2)-symmetric defensin peptide. *J. Am. Chem. Soc.* 136, 13494-13497

35 Koehbach, J. (2017) Structure-Activity Relationships of Insect Defensins. *Front Chem* 5, 45

36 Zhu, S., *et al.* (2014) Experimental conversion of a defensin into a neurotoxin: implications for origin of toxic function. *Mol. Biol. Evol.* 31, 546-559

37 Shafee, T.M., *et al.* (2017) Convergent evolution of defensin sequence, structure and function. *Cell. Mol. Life Sci.* 74, 663-682

38 Gabrielsen, C., *et al.* (2014) Circular Bacteriocins: Biosynthesis and Mode of Action. *Appl. Environ. Microbiol.* 80, 6854-6862

39 Gonzalez, C., *et al.* (2000) Bacteriocin AS-48, a microbial cyclic polypeptide structurally and functionally related to mammalian NK-lysin. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11221-11226

40 Martin-Visscher, L.A., *et al.* (2009) The three-dimensional structure of carnocyclin A reveals that many circular bacteriocins share a common structural motif. *J. Biol. Chem.* 284, 28674-28681

41 Himeno, K., *et al.* (2015) Identification, Characterization, and Three-Dimensional Structure of the Novel Circular Bacteriocin, Enterocin NKR-5-3B, from Enterococcus faecium. *Biochemistry* 54, 4863-4876

42 Hegemann, J.D., *et al.* (2015) Lasso peptides: an intriguing class of bacterial natural products. *Acc. Chem. Res.* 48, 1909-1919

43 Tietz, J.I., *et al.* (2017) A new genome-mining tool redefines the lasso peptide biosynthetic landscape. *Nat. Chem. Biol.* 13, 470-478

44 Martin-Gomez, H. and Tulla-Puche, J. (2018) Lasso peptides: chemical approaches and structural elucidation. *Org. Biomol. Chem.* 16, 5065-5080

45 Hsu, S.-T.D., *et al.* (2004) The nisin–lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. *Nat. Struct. Mol. Biol.* 11, 963-967

46 Kawulka, K.E., *et al.* (2004) Structure of Subtilosin A, a Cyclic Antimicrobial Peptide from Bacillus subtilis with Unusual Sulfur to α-Carbon Cross-Links: Formation and Reduction of α-Thio-α-Amino Acid Derivatives. *Biochemistry* 43, 3385-3395

47 Amso, Z., *et al.* (2018) Total chemical synthesis of glycocin F and analogues: S-glycosylation confers improved antimicrobial activity. *Chem. Sci.* 9, 1686-1691

48 Garcia De Gonzalo, C.V., *et al.* (2014) NMR Structure of the S-Linked Glycopeptide Sublancin 168. *ACS Chem. Biol.* 9, 796-801

49 Siriwardena, T.N., *et al.* (2018) Lipidated Peptide Dendrimers Killing Multidrug-Resistant Bacteria. *J. Am. Chem. Soc.* 140, 423-432

50 Lam, S.J., *et al.* (2016) Combating multidrug-resistant Gram-negative bacteria with structurally nanoengineered antimicrobial peptide polymers. *Nat. Microbiol.* 1, 16162

51 Hancock, R.E., *et al.* (2016) The immunology of host defence peptides: beyond antimicrobial activity. *Nat. Rev. Immunol.* 16, 321-334

52 Kerenga, B.K., *et al.* (2019) Salt-Tolerant Antifungal and Antibacterial Activities of the Corn Defensin ZmD32. *Front. Microbiol.* 10, 795

53 Li, X., *et al.* (2006) Solution Structures of Human LL-37 Fragments and NMR-Based Identification of a Minimal Membrane-Targeting Antimicrobial and Anticancer Region. *J. Am. Chem. Soc.* 128, 5776-5785

54 Gunasekera, S., *et al.* (2018) Alanine and Lysine Scans of the LL-37-Derived Peptide Fragment KR-12 Reveal Key Residues for Antimicrobial Activity. *ChemBioChem* 19, 931-939

55 Järvå, M., *et al.* (2018) X-ray structure of a carpet-like antimicrobial defensin–phospholipid membrane disruption complex. *Nat. Commun.* 9, 1962

415 56 Huang, Y.H., *et al.* (2009) The biological activity of the prototypic cyclotide kalata b1 is modulated by the
416 formation of multimeric pores. *J. Biol. Chem.* 284, 20699-20707

417 57 Strömstedt, A.A., *et al.* (2016) Selective membrane disruption by the cyclotide kalata B7: complex ions
418 and essential functional groups in the phosphatidylethanolamine binding pocket. *Biochim. Biophys. Acta* 1858,
419 1317-1327

420 58 Troeira Henriques, S. and Craik, D.J. (2017) Cyclotide Structure and Function: The Role of Membrane
421 Binding and Permeation. *Biochemistry* 56, 669-682

422 59 Grage, S.L., *et al.* (2017) Orientation and Location of the Cyclotide Kalata B1 in Lipid Bilayers Revealed
423 by Solid-State NMR. *Biophys. J.* 112, 630-642

424 60 Schneider, T., *et al.* (2010) Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II.
425 *Science* 328, 1168-1172

426 61 de Leeuw, E., *et al.* (2010) Functional interaction of human neutrophil peptide-1 with the cell wall precursor
427 lipid II. *FEBS Lett.* 584, 1543-1548

428 62 Park, C.B., *et al.* (1998) Mechanism of action of the antimicrobial peptide buforin II: buforin II kills
429 microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem. Biophys. Res.*
430 *Commun.* 244, 253-257

431 63 Roy, R.N., *et al.* (2015) The mechanism of inhibition of protein synthesis by the proline-rich peptide
432 oncocin. *Nat. Struct. Mol. Biol.* 22, 466-469

433 64 Krizsan, A., *et al.* (2015) Short Proline-Rich Antimicrobial Peptides Inhibit Either the Bacterial 70S
434 Ribosome or the Assembly of its Large 50S Subunit. *ChemBioChem* 16, 2304-2308

435 65 Kragol, G., *et al.* (2001) The antibacterial peptide pyrrhocoricin inhibits the ATPase actions of DnaK and
436 prevents chaperone-assisted protein folding. *Biochemistry* 40, 3016-3026

66 Yuzenkova, J., *et al.* (2002) Mutations of bacterial RNA polymerase leading to resistance to microcin j25. *J. Biol. Chem.* 277, 50867-50875

67 Rintoul, M.R., *et al.* (2001) The antibacterial action of microcin J25: evidence for disruption of cytoplasmic membrane energization in Salmonella newport. *FEMS Microbiol. Lett.* 204, 265-270

68 Anbanandam, A., *et al.* (2008) Molecular basis for proline- and arginine-rich peptide inhibition of proteasome. *J. Mol. Biol.* 384, 219-227

69 D'Souza, C., *et al.* (2014) Structural parameters modulating the cellular uptake of disulfide-rich cyclic cell-penetrating peptides: MCoTI-II and SFTI-1. *Eur. J. Med. Chem.* 88, 10-18

70 Guidotti, G., *et al.* (2017) Cell-Penetrating Peptides: From Basic Research to Clinics. *Trends Pharmacol. Sci.* 38, 406-424

71 Srinivas, N., *et al.* (2010) Peptidomimetic antibiotics target outer-membrane biogenesis in Pseudomonas aeruginosa. *Science* 327, 1010-1013

72 Troeira Henriques, S., *et al.* (2017) Redesigned Spider Peptide with Improved Antimicrobial and Anticancer Properties. *ACS Chem. Biol.* 12, 2324-2334

73 Mahlapuu, M., *et al.* (2016) Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. *Front. Cell. Infect. Microbiol.* 6, 194

74 Kosikowska, P. and Lesner, A. (2016) Antimicrobial peptides (AMPs) as drug candidates: a patent review (2003–2015). *Expert Opin. Ther. Pat.* 26, 689-702

75 Wang, C.K., *et al.* (2014) Rational design and synthesis of an orally bioavailable peptide guided by NMR amide temperature coefficients. *Proc. Natl. Acad. Sci. U. S. A.* 111, 17504-17509

76 Andersson, D.I., *et al.* (2016) Mechanisms and consequences of bacterial resistance to antimicrobial peptides. *Drug Resist. Updat.* 26, 43-57

77 Ostorhazi, E., *et al.* (2011) Broad-spectrum antimicrobial efficacy of peptide A3-APO in mouse models of multidrug-resistant wound and lung infections cannot be explained by in vitro activity against the pathogens involved. *Int. J. Antimicrob. Agents* 37, 480-484

78 Bhardwaj, G., *et al.* (2016) Accurate de novo design of hyperstable constrained peptides. *Nature* 538, 329-335

79 Gao, B. and Zhu, S. (2014) An insect defensin-derived beta-hairpin peptide with enhanced antibacterial activity. *ACS Chem. Biol.* 9, 405-413

80 Wang, C.K. and Craik, D.J. (2018) Designing macrocyclic disulfide-rich peptides for biotechnological applications. *Nat. Chem. Biol.* 14, 417-427

81 Ulmschneider, J.P. and Ulmschneider, M.B. (2018) Molecular Dynamics Simulations Are Redefining Our View of Peptides Interacting with Biological Membranes. *Acc. Chem. Res.* 51, 1106-1116

82 Liu, S., *et al.* (2018) Novel 3D Structure Based Model for Activity Prediction and Design of Antimicrobial Peptides. *Sci. Rep.* 8, 11189

83 Di Bonaventura, I., *et al.* (2017) Chemical space guided discovery of antimicrobial bridged bicyclic peptides against Pseudomonas aeruginosa and its biofilms. *Chem. Sci.* 8, 6784-6798

84 Wang, G. (2008) Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *J. Biol. Chem.* 283, 32637-32643

85 Rozek, A., *et al.* (2000) Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles. *Biochemistry* 39, 15765-15774

86 Cornet, B., *et al.* (1995) Refined three-dimensional solution structure of insect defensin A. *Structure* 3, 435-448

87 Rosengren, K.J., *et al.* (2003) Twists, knots, and rings in proteins. Structural definition of the cyclotide framework. *J. Biol. Chem.* 278, 8606-8616

88 Rosengren, K.J., *et al.* (2003) Microcin J25 Has a Threaded Sidechain-to-Backbone Ring Structure and Not a Head-to-Tail Cyclized Backbone. *J. Am. Chem. Soc.* 125, 12464-12474

89 Conibear, A.C., *et al.* (2012) Structural characterization of the cyclic cystine ladder motif of theta-defensins. *Biochemistry* 51, 9718-9726

90 Poth, A.G., *et al.* (2011) Discovery of an unusual biosynthetic origin for circular proteins in legumes. *Proc. Natl. Acad. Sci. U. S. A.* 108, 10127-10132

91 Goransson, U., *et al.* (2009) The conserved glu in the cyclotide cycloviolacin O2 has a key structural role. *ChemBioChem* 10, 2354-2360

92 Felizmenio-Quimio, M.E., *et al.* (2001) Circular proteins in plants: solution structure of a novel macrocyclic trypsin inhibitor from Momordica cochinchinensis. *J. Biol. Chem.* 276, 22875-22882

93 Conibear, A.C., *et al.* (2014) Insights into the molecular flexibility of theta-defensins by NMR relaxation analysis. *J. Phys. Chem. B* 118, 14257-14266

94 Tang, Y.-Q., *et al.* (1999) A Cyclic Antimicrobial Peptide Produced in Primate Leukocytes by the Ligation of Two Truncated α-Defensins. *Science* 286, 498-502

95 Schaal, J.B., *et al.* (2012) Rhesus Macaque Theta Defensins Suppress Inflammatory Cytokines and Enhance Survival in Mouse Models of Bacteremic Sepsis. *PLoS One* 7, e51337

96 Cole, A.M., *et al.* (2002) Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. *Proc. Natl. Acad. Sci. U. S. A.* 99, 1813-1818

97 Conibear, A.C., *et al.* (2013) The cyclic cystine ladder in theta-defensins is important for structure and stability, but not antibacterial activity. *J. Biol. Chem.* 288, 10830-10840

98 Abuja, P.M., *et al.* (2004) The cyclic antimicrobial peptide RTD-1 induces stabilized lipid–peptide domains more efficiently than its open-chain analogue. *FEBS Lett.* 566, 301-306

99 Sable, R., *et al.* (2016) Constrained Cyclic Peptides as Immunomodulatory Inhibitors of the CD2:CD58 Protein-Protein Interaction. *ACS Chem. Biol.* 11, 2366-2374

100 Conibear, A.C., *et al.* (2014) The cyclic cystine ladder of theta-defensins as a stable, bifunctional scaffold: A proof-of-concept study using the integrin-binding RGD motif. *ChemBioChem* 15, 451-459

101 Craik, D.J., *et al.* (1999) Plant cyclotides: A unique family of cyclic and knotted proteins that defines the cyclic cystine knot structural motif. *J. Mol. Biol.* 294, 1327-1336

102 Weidmann, J. and Craik, D.J. (2016) Discovery, structure, function, and applications of cyclotides: circular proteins from plants. *J. Exp. Bot.* 67, 4801-4812

103 Hellinger, R., *et al.* (2015) Peptidomics of Circular Cysteine-Rich Plant Peptides: Analysis of the Diversity of Cyclotides from Viola tricolor by Transcriptome and Proteome Mining. *J. Proteome Res.* 14, 4851-4862

104 Colgrave, M.L., *et al.* (2008) The anthelmintic activity of the cyclotides: natural variants with enhanced activity. *ChemBioChem* 9, 1939-1945

105 Plan, M.R., *et al.* (2008) Backbone cyclised peptides from plants show molluscicidal activity against the rice pest Pomacea canaliculata (golden apple snail). *J. Agric. Food Chem.* 56, 5237-5241

106 Craik, D.J. and Du, J. (2017) Cyclotides as drug design scaffolds. *Curr. Opin. Chem. Biol.* 38, 8-16

107 Gudmundsson, G.H., *et al.* (1996) The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur. J. Biochem.* 238, 325-332

108 Zahn, M., *et al.* (2013) Structural studies on the forward and reverse binding modes of peptides to the chaperone DnaK. *J. Mol. Biol.* 425, 2463-2479

109 Rosengren, K.J., *et al.* (2004) Cyclization of pyrrhocoricin retains structural elements crucial for the antimicrobial activity of the native peptide. *Biopolymers* 76, 446-458

110 Lambert, J., *et al.* (1989) Insect immunity: isolation from immune blood of the dipteran Phormia terranovae of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. *Proc. Natl. Acad. Sci. U. S. A.* 86, 262-266

111 Hoover, D.M., *et al.* (2001) The Structure of Human β-Defensin-1: new insights into structural properties of β-defensins. *J. Biol. Chem.* 276, 39021-39026

112 Saether, O., *et al.* (1995) Elucidation of the primary and three-dimensional structure of the uterotonic polypeptide kalata B1. *Biochemistry* 34, 4147-4158

113 Rosengren, K.J., *et al.* (2003) Microcin J25 has a threaded sidechain-to-backbone ring structure and not a head-to-tail cyclized backbone. *J. Am. Chem. Soc.* 125, 12464-12474

114 Yonezawa, A., *et al.* (1992) Binding of tachyplesin I to DNA revealed by footprinting analysis: significant contribution of secondary structure to DNA binding and implication for biological action. *Biochemistry* 31, 2998-3004

Figure Legends

Figure 1. Common structural classes of naturally-occurring AMPs.

Representative examples from the common main structural classes of AMPs. (a) LL-37 adopts a typical α-helical conformation in the presence of micelles (PDB ID: 2K6O) [84]. (b) 'Extended' AMPs, such as indolicidin typically do not adopt well-defined three-dimensional conformations (1G89) [85]. (c) β-sheet containing peptides are typically stabilized by varying numbers of disulfide bonds such as, for example, the spider-derived β-hairpin peptide gomesin (1KFP) [31]. (d) Peptides such as the insect CSαβ-defensin phormicin (1ICA) [86] contain both α-helical and β-sheet elements. Cartoon representations of NMR solution structures highlight secondary structural elements, i.e. α-helices (orange), β-sheets (green) and disulfide bonds (yellow).

Figure 2. Diversity of AMPs with cyclic and complex topologies.

Backbone cyclized AMPs include (a) peptides devoid of cysteines such as the bacteriocin AS-48 (PDB ID: 1E68) [39], (b) the prototypic cyclic-cystine knot peptide kalata B1 (1NB1) [87] and (c) the sactibiotic subtilosin A (1PXQ) [46] which contains three thioether bonds. (d) Lasso peptides such as microcin J25 (1Q71) [88], have a head to sidechain cycle (magenta) threaded by the C-terminal tail and sterically locked in place by bulky residues (cyan). (e) The glycocin sublancin 168 (2MIJ) [48] carries an unusual S-linked glucose (magenta) attached via a cysteine residue (cyan). (f) The lanthibiothic nisin A (1WCO) [45] has five thioether bonds (one lanthionine and four methyllanthionine bridges) and post translationally modified dehydro-alanine and dehydro-butyrine residues (cyan). Cartoon representations of NMR solution structures highlight secondary structural elements, i.e. α-helices (orange), β-sheets (green), disulfide bonds (yellow) and thioether bridges (red).

Figure legends for figures in text boxes.

Figure I. NMR solution structure of RTD-1.

Cartoon representation of the cyclic cystine ladder motif of rhesus theta defensin-1 (PDB ID: 2LYF) [89]. β-strands (green) and disulfide bonds (yellow) are highlighted.

Figure II. NMR solution structures of the three subclasses of cyclotides.

Cartoon representation of the CCK motif found in plant cyclotides. (a) The insecticidal Moebius cyclotide CterM (PDB ID: 2LAM) [90] and (b) the antibacterial bracelet cyclotide cycloviolacin O2 (PDB ID: 2KNM) [91] exhibit high affinity for phosphatidylethanolamine membranes, whereas (c) the Momordica trypsin inhibitor cyclotide MCoTI-II (PDB ID: 1IB9) [92] is an excellent grafting scaffold. α-helices (orange), β-strands (green) and disulfide bonds (yellow) are highlighted.

Glossary

α-helix: a coiled arrangement of an amino acid chain where backbone N-H residues form hydrogen bonds with C=O of the residue four positions earlier in the sequence

β-sheet: parallel or antiparallel arrangement of β-strands where backbone N-H residues from one strand form hydrogen bonds with the C=O of the next strand. If two strands are linked by a short sequence of ~2-5 residues (often containing a turn inducing amino acid such as glycine or proline) the motif is known as β-hairpin.

Disulfide bond: a covalent bond between two sulfur atoms from cysteine residues. Disulfide bonds play an important role in the folding and stability of many peptides and proteins.

Post-translational modification (PTM): after ribosomal translation, bioactive peptides and proteins can undergo further maturation processes, which are often essential for their activity. Common PTMs involve modifications of amino acids (e.g. acetylation, hydroxylation, methylation or amidation) or the attachment of carbohydrate (glycosylation) or lipid moieties (lipidation).

Thioether: a crosslink between two amino acids, usually formed between the sulphur of a cysteine residue and the α-carbon of a serine or a threonine residue. Within lanthibiotics, thioether bonds are resulting from dehydration of serine and threonine residues and subsequent crosslinking to give respective (methyl) lanthionine bridges.

Text Boxes

Box 1. Mammalian Θ-defensins

Structurally resembling the Greek letter theta, Θ-defensins are smaller than the other two classes of mammalian defensins and only comprise 18 residues. The parallel arrangement of three disulfide bridges is known as a cyclic cystine ladder motif [89] (Figure I). NMR analyses of the dynamics of Θ-defensins characterized them as comprising two rigid β-strands linked by two flexible turn regions [93]. Θ-defensins have potent broad-spectrum activity against Gram-positive and Gram-negative bacteria as well as fungi [94] and have also been reported to have immunoregulatory activity [95]. The human Θ-defensin retrocyclin, which is naturally present only as a pseudogene, was found, when chemically synthesized, to exhibit potent anti-HIV activity [96]. Notably, acyclic analogues showed significantly reduced activity [94]. Although disulfide bonds were found not to be essential for its antimicrobial activity, removal of disulfide bonds leads to unstructured peptides with reduced proteolytic stability [97]. Their mechanism of antimicrobial activity is thought to result from binding to anionic membranes [98]. Besides their intrinsic activities, small size, stability and amenability to peptide engineering, Θ-defensins are attractive scaffolds for peptide drug development beyond antimicrobial activity. Indeed, several engineering approaches have exploited the plasticity of the cyclic cysteine ladder to stabilize bioactive epitopes [99] and even shown its potential to develop of bifunctional compounds [100].

Box 2. Plant cyclotides

Cyclotides are a large family of plant-derived miniproteins of about 30 amino acids. Similar to mammalian Θ-defensins, cyclotides contain three disulfide bonds and a head-to-tail cyclic backbone. However, in contrast to the simple laddered arrangement of disulfide bonds within Θ-defensins, cyclotides have evolved a uniquely complex and knotted topology known as a cyclic cysteine knot (CCK). In addition to this cystine knot motif, where two disulfide bonds are threaded by a third, cyclotides are backbone cyclized and thus display unique stability [101]. Based on distinct sequence characteristics they can be divided into Moebius and bracelet families, as well as the family of cyclic trypsin inhibitors [102] (Figure II). Within Moebius cyclotides a *cis*-proline induces a 180° twist in the peptide backbone, whereas within bracelet cyclotides all residues are in a trans- orientation. The third category encompasses trypsin inhibitors found in *Momordica spp.* and their sequences do not share much homology to the other two cyclotide subfamilies. Due to their naturally occurring diversity they are often regarded as a natural combinatorial peptide library and it is estimated that the total number of different peptides surpasses several tens of thousands of sequences [103]. Beside this natural diversity, their amenability to peptide synthesis and mutagenesis highlights the structural plasticity of cyclotides and expands the structural space cyclotides can adopt. Their natural function is thought to be part of the plant's immune system and indeed, cyclotides exhibit potent activity against several relevant plant pests, including nematodes, [104], snails [105] or insects [90]. Although their antimicrobial activity against human pathogens is still disputed, Moebius and bracelet cyclotides share key features of several other classes of AMPs, which is an amphipathic structure and a high affinity for phospholipid bilayers [58]. Notably, cyclic trypsin inhibitors do not bind to membranes but have been proven to be an excellent scaffold for the stabilization of bioactive peptide epitopes [106].

Tables

Table 1. Sequences of selected AMPs from the five major structural classes of antimicrobial peptides

Peptide (source)[a]	Sequence	#AA	Net charge	Comment	Ref.
Linear α-helical peptides					
LL-37 (human)	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	37	+6	Prototypical human cathelicidin, Host Defense Peptide	[107]
Magainin-2 (amphibian)	GIGKFLHSAKKFGKAFVGEIMNS	23	+3	Prototypical α-helical AMP	[8]
Linear 'extended' peptides rich in particular amino acids					
Indolicidin (bovine)	ILPWKWPWWPWRR-NH_2	13	+4	Trp-rich, structure bound to DPC and SDS micelles	[85]
Drosocin (drosophila)	GKPRPYSPRPTSHPRPIRV	19	+5	Pro-rich, Ser/Thr: o-glycosylated, DnaK bound drosocin fragment	[108]
Pyrrhocoricin (sap sucking bug)	VDKGSYLPRPTPPRPIYNRN	20	+3	Pro-rich, Thr o-glycosylated, unstructured apart from turns	[109]
PR-39 (pig)	RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFP-NH_2	39	+10	Proline and arginine rich, adopts PP-II helix	[27]

β-sheet containing peptides

Protegrin-1 (porcine)	RGGRLCYCRRRFCVCVGR-NH_2	18	+7	β-hairpin peptide stabilized by two disulfide bonds	[29]
HD-5 dimer[b] (human)	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR | | RCCLRYLRGSIECVGSLSERTACRGTRCYCTA	64	+8	C_2-symmetrical defensin dimer	[34]
Rattusin[b] (rodent)	LRVRRTLQCSCRRVCRNTCSCIRLSRSTYAS | | | | | SAYTSRSLRICSCTNRCVRRCSCQLTRRVRL	62	+16	C_2-symmetrical defensin dimer	[33]

Peptides with α- and β- structural elements

Phormicin (insect)	ATCDLLSGTGINHSACAAHCLLRGNRGGYCNGKGVCVCRN	40	+3	Prototypic CSαβ-insect defensin	[110]
HBD-1 (human)	DHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK	36	+4	Human β-defensin 1	[111]

Cyclic peptides and other complex topologies

Bacteriocin AS-48 (bacteria)	cyclo-MAKEFGIPAAVAGTVLNVVEAGGWVTTIVSILTAV GSGGLSLLAAAGRESIKAYLKKEIKKKGKRAVIAW	70	+6	Five α-helices with cyclic backbone, no further crosslinks or other PTMs	[39]

kalata B1 (plant)	cyclo-GLPVCGETCVGGTCNTPGCTCSWPVCTRN	29	0	Prototypic cyclotide with cyclic cystine knot motif	[112]
RTD- 1 (mammalian)	cyclo-RCICTRGFCRCLCRRGVC	18	+5	Three disulfide bonds in a laddered arrangement	[89]
Subtilosin A[c] (bacteria)	cyclo-NKGCATCSIGAACLVDGPIPDfEIAGAtGLfGLWG	35	-1	Cyclic sactibiotic with three sulfur to α-carbon bridges	[46]
Microcin J25[d] (bacteria)	GGAGHVPEYFVGIGTPISFYG	21	-1	Head-to-sidechain cycle threaded by C-terminal tail	[113]
Nisin-A[e] (bacteria)	IDhbSIDhaLCTPGCKTGALMGCNMKTATCHCSIHVDhaK	34	+3	Highly post-translationally modified lanthibiotic	[45]
Sublancin 168[f] (bacteria)	GLGKAQCAALWLQCASGGTIGCGGGAVACQNYRQFCR	37	+3	S-glycosylated via cysteine residue	[48]

[a]selected peptides include prototypical representatives of each structural class, classification adapted from previous literature (α, non-αβ, β, αβ) [15] to account for increased number of cyclic structures as described in this article, [b]presence in vivo yet to be confirmed, [c]post-translational modifications include three sulfur to α-carbon bridges involving D-amino acids (pairs of colored residues), [d]macrolactam ring between N-terminal amino acid and glutamic acid side chain (underlined), [e]post-translational modifications include dehydro-alanine (Dha) and dehydro-butyric acid (Dhb) residues as well as lanthionine (underlined) and four methyllanthionine bridges (pairs of colored residues), [f]S-glycosylated cysteine residue is underlined.

Table 2. Mode-of-action and targets of selected AMPs

Peptide		Mechanism/target	Ref.
Linear α-helical peptides			
LL37	α-helix	Membrane permeabilization via toroidal pore, various host-defence and immunomodulatory activities	[19]
Magainin 2	α-helix	Membrane permeabilization via toroidal pore	[62]
Buforin II	α-helix	Binding and inhibition of RNA and DNA	[62]
Linear 'extended' peptides rich in particular amino acids			
Drosocin	Pro-rich	Inhibition of protein translation via binding to 50S or 70S ribosomal subunits	[64]
Pyrrhocoricin	Pro-rich	Binding and inhibition of bacterial chaperone DnaK	[65]
β-sheet containing peptides			
Tachyplesin	β-hairpin	Binding and inhibition of DNA	[114]
HNP-1	α-defensin	Inhibition of cell-wall biosynthesis via binding to lipid II	[61]
Peptides with α- and β- structural elements			
Plectasin	Defensin (fungal)	Inhibition of cell-wall biosynthesis via binding to lipid II	[60]
NaD1	Defensin (plant)	Binding to phosphatidic acid and membrane permeabilization via carpet-like structures	[55]
Cyclic peptides and other complex topologies			
Nisin-A	lanthibiotic	Inhibition of cell-wall biosynthesis via binding to lipid II	[45]
Microcin J25	lasso peptide	Inhibition of RNA-polymerase, membranolytic	[66, 67]
Kalata B1	cyclotide	Binding to phosphatidylethanolamine lipids	[57, 58]

Highlights

- Antimicrobial peptides are ubiquitously expressed among all kingdoms of life and represent an integral part of an organism's immunity.
- AMPs exhibit a wide range of classical structural motifs and are currently grouped into four categories based on the presence of absence of key structural elements such as α-helices, β-sheets and various turns and loops.
- Based on increasing reports of more complex structures such as disulfide-rich, cyclic or lasso peptides as well as other PTMs we propose to add a fifth structural group of AMPs accounting for these complex peptide topologies.
- AMPs exhibit multiple modes-of-action, including interactions with biological membranes as well as activity at specific extra- and intracellular targets.
- Advances in peptide synthesis and structural characterization methodologies have increased our understanding of AMP structure-activity relationships and provide a means to tackle the current antibiotic crisis.

Outstanding Questions

While advances in solid-phase chemistry to make structurally simple AMP classes have occurred over the last decade, complex peptide topologies that include a variety of PTMs still represent significant challenges for synthetic approaches. Can efficient methods be developed to allow access to the vast structural diversity of post-translationally modified AMPs?

Topical application is the most common route of AMP administration, with oral administration limited by the typically low metabolic stability of peptides. Can the increased stability of cyclic and other constrained peptide topologies as well as peptide engineering overcome this limitation?

Several AMPs display remarkably different activities between *in vitro* minimal inhibitory concentration assays and animal models, confounding screening results and slowing down peptide drug development. How can this poor correlation of observed *in vitro* and *in vivo* antimicrobial activities be successfully overcome?

Figures

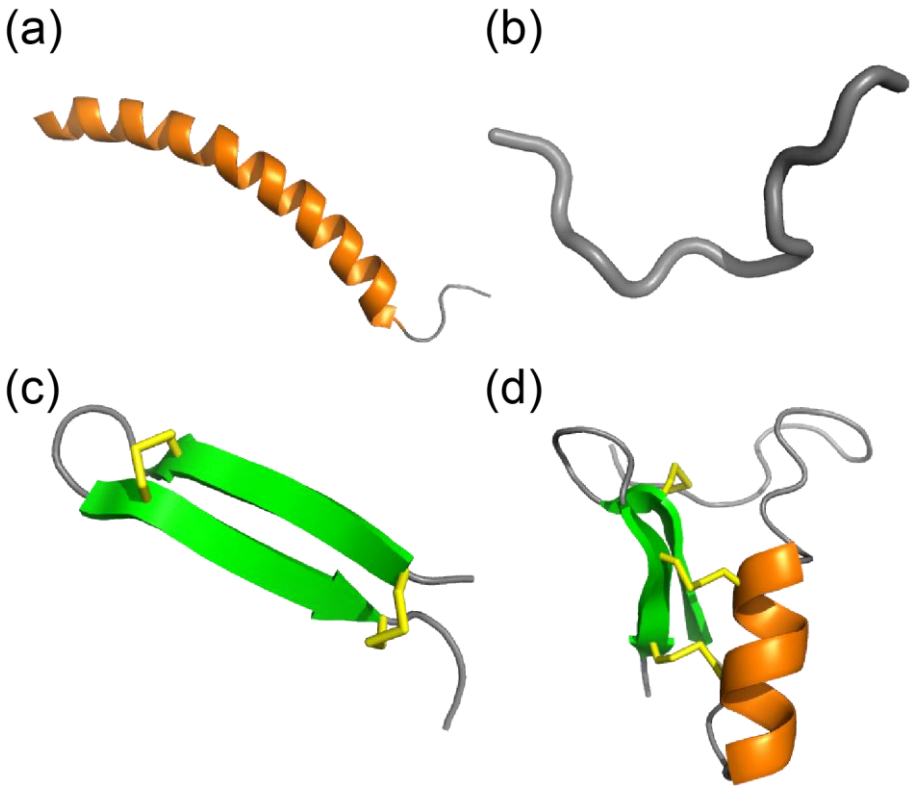

Figure 1.

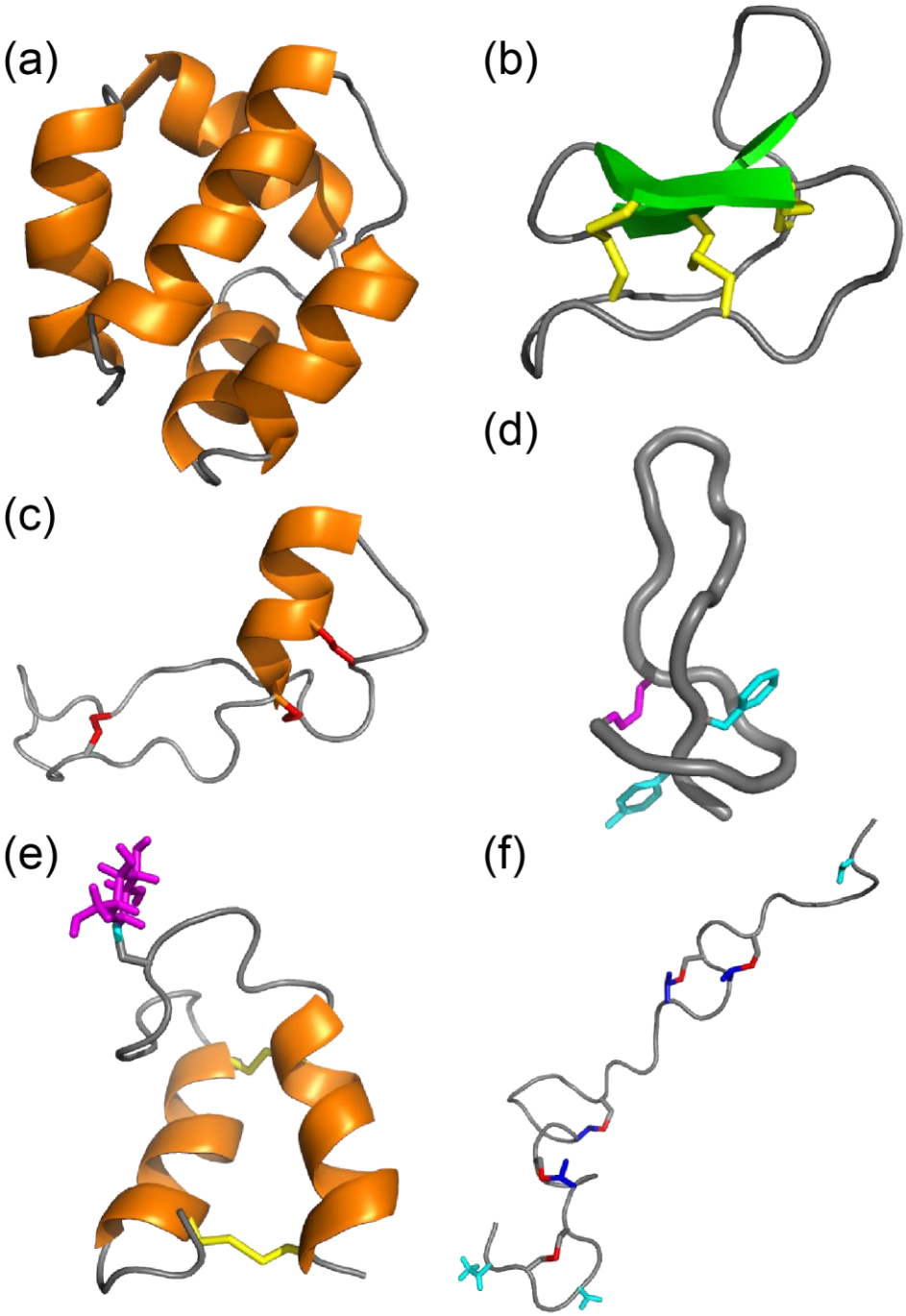

Figure 2.

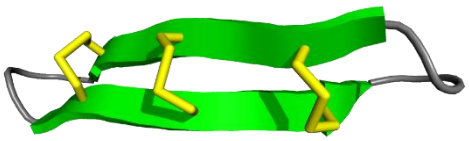

Figure I.

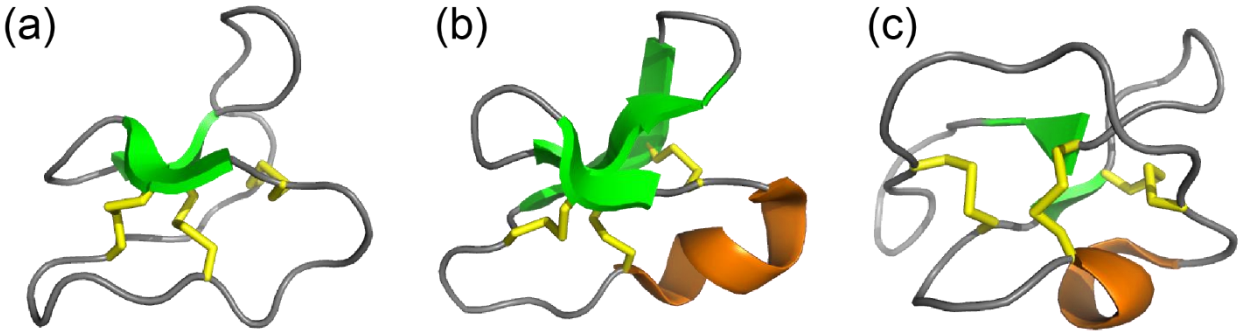

Figure II.