Designing antimicrobial peptides: form follows function

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Abstract | Multidrug-resistant bacteria are a severe threat to public health. Conventional antibiotics are becoming increasingly ineffective as a result of resistance, and it is imperative to find new antibacterial strategies. Natural antimicrobials, known as host defence peptides or antimicrobial peptides, defend host organisms against microbes but most have modest direct antibiotic activity. Enhanced variants have been developed using straightforward design and optimization strategies and are being tested clinically. Here, we describe advanced computer-assisted design strategies that address the difficult problem of relating primary sequence to peptide structure, and are delivering more potent, cost-effective, broad-spectrum peptides as potential next-generation antibiotics.

Non-opsonic phagocytosis
A host defence mechanism mediated by leukocyte receptors that recognize corresponding bacterial cell surface adhesins.

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Antimicrobial peptides (AMPs) are produced by multicellular organisms as a defence mechanism against competing pathogenic microbes[1,2]. Pioneering studies have led to the discovery of various types of these 'host defence peptides' — including defensins[3], cecropins[4], magainins[5] and cathelicidins[6] — with remarkably different structures and bioactivity profiles[7]. Extensive research has led to the realization that these bioactive peptides do not merely act as direct antimicrobial agents but also represent important effectors and regulators of the innate immune system that are able to profoundly modulate the immune response through a range of activities, including increasing chemokine production and release by immune and epithelial cells, enhancing wound healing and angiogenesis, exerting pro- and anti-apoptotic effects on different immune cell types, as well as having adjuvant activity in promoting adaptive immunity[2,8–10].

In addition to their direct (although sometimes weak) antimicrobial activities, AMPs have additional antimicrobial effects as they are able to suppress biofilm formation and induce the dissolution of existing biofilms[11], chemotactically attract phagocytes and mediate non-opsonic phagocytosis[12–14]. Some natural AMPs, like porcine protegrin, exhibit strong antimicrobial effects but in general the activity of AMPs is improved by their increased concentrations in phagocyte granules, the crypts of the intestine and near degranulating phagocytes[15–17]. *In vitro* studies have revealed that direct antimicrobial activity is not limited to the previously suggested mechanisms of membrane and/or cell rupture but instead extends to interference with membrane-associated biosynthesis, macromolecular synthesis in the cytoplasm and metabolic functions[2,18,19].

It has been suggested that the term 'antimicrobial peptide' should be used when direct antimicrobial activity is being examined, and the term 'host defence peptide' should be used when referring to anti-infective activity that enhances or modulates the host immune response to infectious agents[20]. Here, we review the current status of computer-assisted peptide design, with particular focus on the *in silico* generation of novel AMPs. We concentrate on describing the optimization of direct antimicrobial activity, as optimization studies on host defence peptides and their synthetic innate defence regulator counterparts are still in their infancy.

Our awareness of natural antimicrobials — namely, the "existence of antimicrobial substances in blood, leucocytes and lymphatic tissue"[21] — dates back to the late nineteenth century[22]. The concept that low-molecular-weight antimicrobial proteins were important in immunity was first highlighted by studies on human phagocytes[23] and *Hyalophora cecropia* moths[24]. Currently, the most potent natural peptides known to exhibit antimicrobial activity are the β-hairpin peptides typified by the horseshoe crab polyphemusin I ($RRWC_1FRVC_2YRGFC_2YRKC_1R$; where the equivalently numbered cysteine residues form cystine disulphide bridges)[25,26] and pig protegrin ($RGGRLC_1YC_2RRRFC_2VC_1VGR$)[27,28]. Polyphemusin I inhibits the growth of various Gram-positive and Gram-negative bacteria as well as *Candida albicans* at a minimal inhibitory concentration (MIC) of around 0.5–1 μg per ml[29]. However, polyphemusin I does not protect against

Pseudomonas aeruginosa infections in cyclophosphamide-treated (neutropenic) mice, it exhibits haemolytic activity at higher concentrations, and a protegrin derivative — IB-367 (iseganan) — failed in Phase III clinical trials of oral mucositis[29–31].

Most of the natural cationic peptides are much less (~10–100-fold) active than these AMPs and are strongly antagonized by physiological concentrations of mono- and divalent cations as well as polyanionic polymers like glycosaminoglycans and mucin[32]. Thus, new design approaches are required that enable the identification of more cost-effective sequences (that is, smaller sequences without post-translational modifications) that are highly active, have broad-spectrum activity without associated toxicities, good pharmacokinetics and a desired selectivity profile.

The challenge of bacterial resistance

Presumably, bacteria have been exposed to AMPs for millions of years and, with the exception of a few species (such as *Burkholderia* spp.)[33], widespread resistance has not been reported, making them a potential treasure trove of starting points for rational, focused antimicrobial drug design. In most instances natural AMPs do not appear to be highly optimized for direct antimicrobial activity, and it is likely that multiple modestly active peptides with concomitant immunomodulatory activities work effectively in combination and/or when induced or delivered to sites of infection[34]. Drug developers are not limited to such considerations and can strive to develop AMPs with an optimized specific function or target.

Modern antibiotics have a considerably limited number of macromolecular targets, often essential bacterial proteins, and are subject to severe and growing resistance problems[35–37]. Notably, the development of resistance against AMPs has occurred to a much lesser degree as they usually work by attacking multiple hydrophobic and/or polyanionic targets[38,39]. Thus, it is difficult to obtain mutants that are resistant to AMPs, and training methods — for example, multiple passages with half the MIC of AMPs — are usually required for the development of any resistance[40].

Mechanisms of resistance to AMPs include cell surface modification (which can occur adaptively through the two-component regulator PhoPQ, for example), external trapping of AMPs, active efflux of AMPs, proteolytic degradation, as well as the suppression of host pathways by the pathogen for the production of AMPs[39,41]. These challenges are usually met by screening peptides for activity against intact bacteria using conventional bacterial growth media that contain physiological salt concentrations, and by counterscreening for lack of mammalian cell toxicity. The development of computational alternatives to this experimental selection protocol is aiding the elimination of poor candidate peptides at very early stages of development.

In addition, the realization that small-molecule drugs tend to interact with multiple macromolecular targets has profoundly changed drug design[42–44]. Consequently, chemogenomics and 'systems' views have evolved as methods for rational drug discovery and compound library design, and target profile prediction that specifically addresses undesired off-target activity is now possible for drug-like compounds[45–47]. So-called kernel methods have recently been added to the set of algorithms that seem to be particularly suited for the purpose of identifying drug–target interaction pairs[48]. These methodological advances have not yet been transferred to peptide design but have the potential to boost the design of AMPs with desired selective antibacterial activity.

Mechanism of action of AMPs

A profound understanding of the molecular mechanisms responsible for the direct antibacterial activity of AMPs will enable the development of improved predictive models, and several mechanisms of action have been proposed[49–51]. Indisputably, AMPs must interact with membranes as part of their direct antibacterial mechanism (or mechanisms) of action, leading to membrane perturbation, disruption of membrane-associated physiological events such as cell wall biosynthesis or cell division, and/or translocation across the membrane to interact with cytoplasmic targets (FIG. 1). It is generally assumed that the positively charged AMP initially interacts with the negatively charged lipid head groups of the outer surface of the cytoplasmic membrane. The peptide is then inserted, in an approximately parallel orientation to the bilayer, into the outer leaflet of the cytoplasmic membrane lipid bilayer, leading to the displacement of lipids.

Possible alterations in membrane structure, including thinning, pore formation, altered curvature, modified electrostatics and localized perturbations, may result in the reorientation of peptide molecules in the membrane. Finally, the peptides may translocate through the membrane and diffuse into the cytoplasm to reach intracellular targets. These basic mechanisms certainly explain many aspects of the observed antibacterial activity of AMPs but they do not increase our understanding of the overall number of fundamentally different mechanisms by which AMPs interact with the bilayer, or the relative importance of membrane rupture, graded leakage and non-membrane mechanisms. Moreover, they do not answer the important question of whether membrane binding represents the sole basis of AMP selectivity[52]. Further modelling analyses of peptides and lipids will be required to achieve significant progress in rational AMP design and engineering based on membrane interactions.

The interactions of an AMP with the membrane cannot be explained by a particular sequential amino-acid pattern or motif; rather, they originate from a combination of physicochemical and structural features[8] including size, residue composition, overall charge, secondary structure, hydrophobicity and amphiphilic character[53]. There has been considerable discussion, based largely on model membrane studies, about how AMPs exhibit strong preferences for specific membrane compositions and seemingly prefer membranes that: contain comparatively large fractions of anionic lipids, such as phosphatidylglycerol and cardiolipin; maintain a high electrical potential gradient; and lack cholesterol, such as bacterial

Cyclophosphamide-treated (neutropenic) mice
Animals lacking neutrophil granulocytes. Neutrophils represent a primary defence against bacterial infections, partially through phagocytosis and neutrophil extracellular traps containing high local concentrations of antibacterial agents.

Chemogenomics
A drug discovery approach that uses high-throughput technology to study the genomic effect of chemical compounds on target families, thereby complementing disease area- and single-target-oriented concepts.

Kernel methods
A class of algorithms for computational pattern analysis. Kernel functions allow the detection of nonlinear relationships among data without having to fit a nonlinear model (known as a Kernel trick).

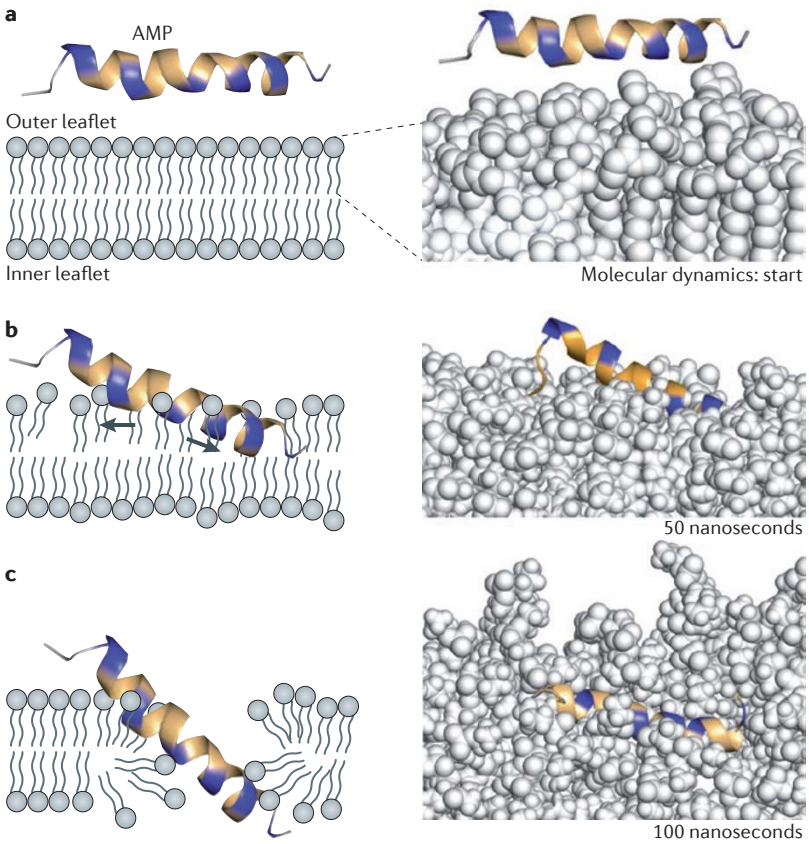


Figure 1 | **Potential mechanism of membrane disruption and/or translocation by antimicrobial peptides.** The antimicrobial peptide (AMP) is represented as a ribbon diagram, with positively charged residues indicated in blue (other residues are shown in yellow). The initial stages include membrane attachment (part **a**) and insertion into the outer leaflet (part **b**). Not all AMPs actually insert into and disrupt the membrane (part **c**) or form pores. Some peptides are too short to span the bilayer (for example, the cyclic decapeptide gramicidin S). A high peptide concentration leads to increased membrane curvature and facilitates pore formation[192] or translocation across the membrane. A suggested feature of the mode of action of AMP is to stretch, disorder and thin the outer leaflet of the membrane (part **b**). The panels on the right illustrate hypothetical mechanisms of the AMP–membrane interaction that can be studied by molecular dynamics simulations. Here, spinigerin (Protein Data Bank[193] ID: 1ZRV[194]) was simulated in explicit water with a POPE (1-palmitoyl-2-oleoyl-phosphaethanolamine) membrane bilayer model using the NAMD v2.7b2 software[195] and the CHARMM22 force field[196].

membranes. For example, bacterial membranes possess a comparatively large fraction (up to 20 mole percent) of negatively charged lipids and maintain high electrical potential gradients (a transmembrane potential ($\Delta\psi$) of approximately –120 mV) that attract positively charged substances like AMPs, whereas the membranes of plant cells and animal cells are enriched in cholesterol and lipids, have no net charge and maintain weak $\Delta\psi$[1].

To assess AMP-induced membrane tropism, a protocol for surface plasmon resonance has been devised that involves using membrane compositions that mimic those of mammalian and microbial cells[53]. A membrane-based surface plasmon resonance sensor chip has also been described that can detect a binding event and monitor the actual membrane-disturbing effect of the peptides[54]. However, the actual *in vivo* situation is likely to be more complex as membranes are not just simple passive bilayer structures as assumed in this surface plasmon resonance protocol and in model membrane studies; rather, they contain domains that may include hexagonal structures, minor lipid species, embedded proteins and glycolipids, and may differ in fluidity, fatty acid chain composition, as well as transmembrane $\Delta\psi$ and ΔpH gradients[49,55].

This does not mean that model membrane systems have no value; however, any predictions that come from such systems cannot automatically be transferred to the *in vivo* setting. For example, the human cathelicidin AMP LL-37 has an absolute tropism for model membranes of bacterial-like composition[56] but its antibacterial activity is largely restricted by physiological conditions[57]. LL-37 is freely translocated across eukaryotic membranes, in a similar manner to cell-penetrating peptides, and this translocation is obligatory for its immunomodulatory function of chemokine induction[58,59].

Preference for a certain membrane or classes of membranes is likely to be important in peptide activity. This probably requires nascent or induced (via peptide–membrane interaction) structural features of AMPs, rather than mere electrostatic attraction due to opposing charges or hydrophobic interactions. Further evidence for this concept stems from extensive studies performed with the cell-penetrating peptide penetratin (also known as the protein transduction domain), a 16-residue cationic peptide (RQIKIWFQNRRMKWKK-amide) corresponding to the third helix of the homeodomain of the antennapedia protein[60], which has been patented as a carrier peptide (or a cargo carrier) for drug delivery into cells[61]. The mutation of a single residue (W6F) abolished the membrane-transfer properties of penetratin, indicating that lipid binding per se might be insufficient for AMP activity[62]. Similarly a W2G mutation in cecropin, an AMP constituting a major part of cell-free immunity in insects[63], nearly abolished antibacterial activity[64].

There are many similarities between cell-penetrating peptides and AMPs. Both exhibit antimicrobial effects and can carry passenger molecules into the cell; in fact, the well-studied peptide LL-37 can be translocated into eukaryotic cells at concentrations below those required to kill bacteria (at the equivalent divalent cation concentrations)[65]. However, it is worth noting that AMPs are considered to have the ability to pass across bacterial membranes without requiring a transport apparatus, whereas cell-penetrating peptides are thought to be internalized primarily by active endocytosis[66], perhaps pointing to a fundamental difference in how peptides access prokaryotic and eukaryotic cells.

AMPs feature a remarkable variety of structural motifs[67]. FIGURE 2 presents an alternative to the traditional structural classification of peptides with some degree of antimicrobial activity (and for which experimentally determined structures are available). To obtain an overview of their structural diversity, we clustered the peptides according to their backbone structure in solution (data obtained from nuclear magnetic resonance

Surface plasmon resonance
A detection principle based on an electron charge density wave phenomenon at the surface of a metallic film for label-free determination of the mass concentration on the film and the kinetics of a receptor–ligand interaction.

Figure 2 | **Clustering of antimicrobial peptides according to backbone torsion angles.** Representatives of each cluster formed are shown as ribbon diagrams with secondary structure elements highlighted (helices are shown in turquoise, and strands are shown in magenta). We selected 135 antimicrobial peptides (AMPs) from the updated Antimicrobial Peptide Database[76], for which nuclear magnetic resonance solution structures are available from the Protein Data Bank. Each peptide structure was represented by a 16-dimensional vector coding for the prevalence of backbone torsion angles (Φ and ψ), which are characteristic of peptide structure, and clustered using Ward's method[197]. As a result, AMPs with similar backbone folds were grouped together. This automated, fine-grained classification provides a basis for the rational design of novel AMPs with desired structural features. All calculations were performed using Matlab R2010b (The MathWorks Inc., Natick, USA).

spectroscopy). These peptides have very different folds, which suggests that elements of their secondary structure can be used as a means for classification[68]. Furthermore, these peptides have to integrate, interact with and/or pass a specific membrane, but the mechanisms by which they do this are unclear. Extracting the discriminating features, however delicately tuned, among these groups of membrane-interacting peptides will help us understand the functional aspects of AMPs and enable the optimization of activity and 'rational' structure-based peptide design.

AMP databases

As natural AMPs are largely derived from gene-coded sequences, bioinformatics methods have been applied to create databases of known AMPs as well as tools to specifically predict AMPs from genomes that have not yet been annotated. For example, the AMPer resource was developed with the aim of classifying natural AMPs, as well as predicting novel AMPs in the bovine genome using hidden Markov models[69,70]. Recently, feature-extraction methods have been applied to the Collection of Antimicrobial Peptides[71], and alignment-based AMP detection was performed[72]. Another example is the ANTIMIC collection, which contains approximately 1,700 known and putative AMP sequences[73]. One of the original data resources, the Antimicrobial Sequences Database, was created more than 14 years ago and there are now at least 21 AMP repositories in different stages of activity and maintenance[74]. The Antimicrobial Peptides Database (APD)[75], and later the updated APD2 (REF. 76), is a valuable resource of AMP sequences. This well-maintained database offers various options for convenient searches in different phylogenetic kingdoms. In recent years, the discovery of AMPs from plants and marine organisms has further augmented the variety of anti-infective peptides with biotechnological and pharmaceutical potential[77,78].

Despite many convenient database analysis options, one may doubt whether studies across AMPs that are structurally widely dissimilar will be fruitful for understanding AMP activity in general, given their potentially diverse mechanisms of action. To address this issue and enable the development of improved data resources, synthetic peptide design will help to unravel the underlying structure–activity relationships (SARs). Like databases of small, drug-like bioactive compounds, AMP databases provide an indispensable knowledge base for both qualitative and quantitative activity prediction models[79–81]. Knowledge-based computational approaches have already led to the design of synthetic AMPs, such as the adepantins (automatically designed peptide antibiotics)[82], and allowed the systematic mining of genomic expressed sequence tag data aimed at the discovery of hitherto undescribed natural AMP sequences[83].

Synthetic AMPs

To develop AMPs into therapeutics, several objectives must be met. As an anti-infective treatment, an AMP must be active against the pathogen of interest and have low toxicity at the therapeutic dose (that is, it must have a high therapeutic index). Recent research on AMPs has focused on methods to search through the constellation of known or predicted peptide sequences — either empirically or computationally — for peptides with desired properties, and these approaches are continually evolving. We distinguish three research approaches: modification of known AMP sequences (known as templates) with limited computational input; rigorous biophysical modelling to understand peptide activity; and virtual screening. We outline these methodologies and data requirements below.

Template-based studies. Studies of AMP activity based on a template AMP with known activity seek to identify peptides with greater antimicrobial activity or reduced toxicity based on altered amino-acid sequences[84]. These studies have often systematically — but incompletely — changed a single amino acid in the peptide to identify amino acids and positions that are important for activity. Cecropin, magainin, protegrin and lactoferricin have all been used as AMP templates. In most cases, the source of

Hidden Markov model
A probabilistic method to learn temporal and sequential patterns based on observations. This model can be used for amino-acid sequence analysis and prediction.

their activity has been investigated by examining peptide variants that were designed on the basis of the general — but somewhat limited — concept that charge and amphiphilicity are crucial to peptide activity[85–87]. Despite their apparent limitations, such studies have shed light on the importance of specific amino acids and residue positions to peptide activity.

It is evident, however, that such 'local' approaches fail to account for interactions between amino acids that influence the global three-dimensional conformation of the peptide[88]. Using high-throughput peptide synthesis on arrays, coupled with a high-throughput and rapid luminescence-based assay for bacterial killing[89], it has been possible to carry out a complete substitution study including all single amino-acid changes to bactenecin 2A, a linearized version of bovine bactenecin[90,91]. Overall, it is clear when comparing two amino-acid substitution studies that the substitutions favouring activity vary according to the template sequence, and analogous substitutions may well have substantially different effects at different positions in the primary sequence. In other words, the effect of a particular residue substitution is context-dependent[92].

Possibly the most intuitive attempt to model AMPs based on natural peptide sequences was a linguistic model in which sequences in one-letter amino-acid codes were considered as 'text' and 'formal grammar rules' were applied to identify text patterns in naturally occurring peptides[93]. When novel peptides were constructed based on this linguistic model but selected to be dissimilar to natural AMPs, they were found to be superior to similar peptides with a shuffled amino-acid sequence, demonstrating that this method did indeed identify functionally relevant patterns or motifs. Out of the 40 peptides that were designed, four exhibited activity against *Escherichia coli* or *Bacillus cereus* at concentrations below 64 μg per ml; the most potent peptide designed through this approach, D28, had an MIC against *Staphylococcus aureus* of 4–8 μg per ml and MICs against *E. coli* of 64 μg per ml. Although these values fall far short of the most potent natural peptides and even the peptides used in the training sets, this study elegantly demonstrated how innovative computer-based modelling could support peptide design.

Several studies have chosen templates that are based on small amino acids or reduced numbers of amino acids. As early as 1992, AMPs consisting only of leucine and lysine residues were synthesized, which were active against both Gram-negative and Gram-positive bacteria[94]. Short peptides acting as AMPs were designed *de novo* that consisted of only tryptophan, leucine and lysine residues, and the positioning of tryptophan residues and sequence length on antimicrobial activity was investigated[95]. This study also succeeded in designing peptide sequences that are capable of forming amphipathic helices, using only arginine and valine as prototype residues.

In another project, the generic template sequence acetyl-C-HBHB(P)HBH-GSG-HBHB(P)HBH-C-amide (where B corresponds to a cationic residue; H corresponds to a hydrophobic residue; and P corresponds to a polar residue) served as the starting point for the design of anti-endotoxic peptides using chemoinformatics methods[96]. Following a similar template approach, simplistic cationic amphiphilic peptides have been devised and shown to have membrane-lytic effects against *Bacillus subtilis* and *C. albicans* by scanning electron microscopy[85]. Notably, the replacement of very hydrophobic residues in the apolar face of the amphipathic helix with weakly hydrophobic residues decreased the lytic effect of the peptide on erythrocytes, demonstrating that excessive hydrophobicity in AMPs increases undesirable erythrocyte lysis[97].

Recently, a template-based design approach has been presented, introducing the concept of specificity determinants to achieve membrane selectivity[98]. This design concept involves the use of positively charged amino-acid residues in the centre of the non-polar face of amphipathic α-helical AMPs to enhance the peptide's selectivity between eukaryotic and prokaryotic membranes. Starting from a known broad-spectrum AMP, the systematic alteration of residues led to reduced haemolytic activity and improved therapeutic indices against the targets *Acinetobacter baumannii* and *P. aeruginosa*. These results demonstrated that a reduction in haemolytic activity can be achieved using computer-assisted drug design without losing antimicrobial activity, and that both charge distribution and structural features have a delicate role in the balance between membrane recognition and the selectivity of AMPs.

In 2009, the design of an AMP that exhibits considerable selectivity for both *P. aeruginosa* and *Streptococcus mutans* was reported[99]. This effect was accomplished by fusing a nonspecific widespread AMP with a specifically targeted AMP. One domain of the chimeric peptide was proposed to harbour the killing function, whereas the other led to selectivity. Forming chimaeras from two active compounds is a popular concept in computer-assisted drug design, and is used by the software tools TOPAS[100] and BREED[101]. However, this approach may not always be applicable to peptides, as it does not automatically take into account structural folding (or refolding) that might involve the two supposed domains.

Another important strategy for enhancing AMP activity is the addition of acyl moieties, as these can provide the necessary hydrophobic domains for making short peptides[102–104]. For example, the aminolauryl-acylated AMP sequence ALWKTLLKKVLKA exhibits improved bactericidal properties and is less prone to degradation by plasma proteases than the unmodified peptide[42].

Biophysical studies. In contrast to template-based design methods in which peptides are treated as a 'text' formed by amino acid 'letters' bearing individual properties like charge and hydrophobicity values, biophysically motivated modelling studies aim to understand AMP activity and design improved variants by examining peptide structures in hydrophobic environments or by modelling peptides at the atomic level. Examples of these types of studies include molecular modelling based on free energy perturbation, molecular dynamics simulation as well as thermodynamic calculations of the interactions

Free energy perturbation
A method based on statistical dynamics that breaks down the physical problem of calculating free energy differences (ΔG^0) for the formation of large receptor–ligand complexes into several smaller steps that are feasible to calculate.

Molecular dynamics
Simulations that compute conformations and physical movements of molecules over a simulated period of time. Physically motivated force fields in different surroundings (for example, in a vacuum or water box) are often used to compute interaction energies between particles.

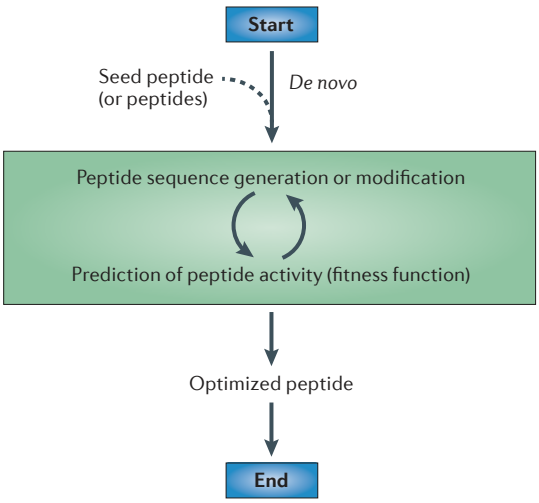


Figure 3 | **Computer-assisted molecular design cycle.** Peptide design starts from scratch (*de novo*) or from known peptides that have a desired activity (also termed 'seed peptides'). In an iterative process new peptides are generated *in silico* using alternating variation-selection operators. A 'fitness function', often a machine-learning model, guides the design towards regions in sequence space that contain residue sequences with a higher predicted biological activity.

of peptides with membranes[105]. Such computationally demanding exercises have been successfully applied to drug design and optimization[106,107], and are also increasingly being applied to peptide design. The AMPs bactenecin[108] and indolicidin[109] are examples of known peptides that have been used as templates for structure-guided design.

Conversely, porcine protegrin[110] was investigated using molecular dynamics methods. Protegrin is thought to act primarily by causing membrane disruption as a result of pore formation (at a concentration of approximately 1 μg per ml), and it is amenable to computational modelling because of its β-hairpin structure[111]. Although the precise details of the mechanism of action of protegrin are largely unknown, the model of protegrin activity suggests that the following mechanisms are involved: electrostatic attraction to the anionic membrane; dimerization; insertion of protegrin into the membrane; and the formation of large aggregates that lead to transmembrane pores and a consequent lethal flux of ions from the cytoplasm (FIG. 1).

Molecular dynamics simulations may involve representing each atom of the peptide, surrounding solvent and portions of the membrane; the value of a model is inevitably limited by the complexity of natural systems as simplifications have to be made in computational approaches. For example, one can restrict the number of peptide atoms represented or replace the solvent by a continuous medium (known as an implicit solvent model). Interactions between atoms or coarser-grained sites are typically calculated based on Coulomb and van der Waals forces as well as bond interactions using methods derived from a consistent empirical force field. Simulations and experimental confirmation are often restricted to peptides interacting with micelles or lipid bilayers as surrogates for the bacterial membrane. Phenomena such as lipid bilayer thinning and conductance induction, as well as the biological effects of pores, have emerged from these studies, and potentially relevant hydrogen-bonding sites have been suggested[112–114].

Even though molecular dynamics simulation of the AMP–membrane interaction can provide a working hypothesis, one has to bear in mind that by using the currently available technology the initial conditions for simulation (including the conformation of pore formation) must be well defined, and practical simulation times might be too short to allow spontaneous pore formation to be observed — if indeed this occurs biologically. Simulation studies are often interpreted by a disordered toroidal pore model but they tend to demonstrate[115] that only a very small number of peptides (as few as one or two) are oriented in a perpendicular manner to the membrane, causing substantial membrane perturbation, and these peptides do not tend to cluster (which is possibly more consistent with an aggregate model of an AMP–membrane interaction)[116]. Nevertheless, molecular dynamics simulation was successfully applied in the design of ovispirin[117] and indolicidin[118] analogues, and has led to the development of peptides with a twofold improvement in antimicrobial activity and a tenfold decrease in haemolytic activity.

Virtual screening studies. Virtual screening methods can be used when exhaustive synthesis and testing is prohibitively expensive and biology-assisted techniques such as phage display[119] cannot be applied[120]. These approaches have the advantage of having only a few *a priori* assumptions, as they seek to impute peptide structures based on primary sequences. In contrast to computational simulation studies, virtual screening studies do not necessarily attempt to create models with immediately and easily interpretable outcomes. Instead, numerical methods are used to determine quantifiable properties of peptides (descriptors) — such as charge and hydrophobicity — from the primary structure and physicochemical characteristics of the peptides, and they are used to relate such properties to the biological activities of the peptides using SAR models. Virtual screening studies — more specifically, quantitative SAR (QSAR) models — apply numerical analyses to describe the relationship between these descriptors as input variables and biological activity as the output variable. Many papers have been published on the statistical analyses and machine-learning methodologies that may be used for this purpose[121,122].

The most important aspect of computer-assisted AMP design (FIG. 3) is the accurate estimation or prediction of desired biological activity from the primary amino-acid sequence alone. Since the 1980s, computational QSAR models for peptides have been used as a guide for activity prediction and sequence optimization for several biological activities (TABLE 1). In the 1990s,

Disordered toroidal pore
A model of the interaction of an antimicrobial peptide with lipid membranes. According to this model the peptides forming the pore lack structural organization and have no defined orientation.

Table 1 | **Selection of computer-based peptide design concepts and approaches**

Year	Fitness function	Design strategy	Description	Refs
1994 (and 1998)	Artificial neural network	Evolutionary algorithm	Peptides are generated *de novo* by a simulated molecular evolution process so that they maximize a neural network-scoring function value	92,187, 201,202
2004	Fold stability, energy-based probabilities	Amino-acid replacement	The 'inverse folding problem' is solved to identify novel peptide sequences that are compatible with backbone templates of reference peptides	203
2005 (and 2006)	Docking simulation	Evolutionary algorithm, heuristic	A docking score is calculated as an estimation of peptide–receptor interaction energy, and serves as a fitness function for an evolutionary algorithm	204,205
2006	Regular grammar	Linguistic model	New antimicrobial peptides (AMPs) are generated based on grammatical rules derived from naturally occurring AMPs	93
2007	Docking simulation	Genetic algorithm	Peptides are docked and then altered by mutation or crossover	206
2007	Experimentally optimized alignment matrix	Random sequence generation	A scoring matrix is systematically altered based on substitutions that approve the differentiation between strong and weak binders	207
2007	Artificial neural network	Ant colony optimization algorithm	Automated peptide design is performed *de novo* using adaptive positional weight matrices	208
2008	Hidden Markov model (HMM)	Random sequence generation	An HMM model is used to identify and classify AMPs based on natural proteins in the UniProt database	70
2008	Molecular dynamics simulation	Evolutionary algorithm	The algorithm searches for stable parts of a peptide by generating point mutants, which are evaluated in molecular dynamics simulations	209
2009	Artificial neural network	Random sequence generation	The algorithm ranks a large set of random sequences according to a neural network model that had been trained for AMP recognition	149,150
2011	Artificial neural network	Genetic algorithm	Automated peptide design is performed *de novo* using a genetic algorithm for AMP sequence generation	151

machine-learning methods — specifically artificial neural networks[123] — replaced the more traditional regression functions for peptide QSAR models. Currently, a common computer-based design approach involves combining a sophisticated activity estimator with a technique that enables stochastic optimization (that is, involving random iterative processes to overcome issues associated with experimental noise).

Evolutionary algorithms, particularly evolution strategies and genetic algorithms, in which the process of evolution is performed *in silico* through successive generations of mutations, deletions, sequence shuffling and so on, have also been used to search for peptides with improved activity[124–126]. Stochastic approaches are suited for peptide optimization in a vast search space, particularly for long sequences for which deterministic methods are prohibitive. Although some implementations of evolutionary algorithms suffer from certain well-known computational inefficiencies (for example, a dependency on parameter initialization, partially insufficient sampling and premature convergence)[127], they have proven their applicability in many practical studies[128,129]; this can be attributed to their overall robustness to experimental noise and optimization involving many solutions that are locally optimal.

Notably, for each molecular design problem there is a best-suited combination of the size of a screening compound library (the number of peptides synthesized and tested at a time) and the number of iterative synthesize-and-test rounds required, with the aim of keeping experimental efforts minimal[130]. The overall concept is to utilize the results that are obtained by activity testing or prediction to influence the decision as to which peptides should be designed, generated and tested in the next cycle. Such a 'memory' concept is characteristic of adaptive systems and essential for successful navigation in a large sequence space[131,132] (BOX 1).

Molecular descriptors and QSAR models

In general, peptide modelling is guided by the same concepts as small-molecule drug design, particularly to account for the underlying pharmacophore and molecular shape features that are relevant for an observed or desirable activity[133,134]. Modelling peptides using molecular descriptors that account for these features is not a new approach. In 1987 a set of three descriptors (*z*1, *z*2 and *z*3) was proposed, based on a principal component analysis of 29 largely empirical properties such as molecular weight, pK (the logarithm of the dissociation constant), pI (the isoelectric point), nuclear magnetic resonance

Artificial neural networks
Estimators of universal function that are modelled loosely on nervous systems. They can be used to find patterns in sequence data and for modelling structure–activity relationships.

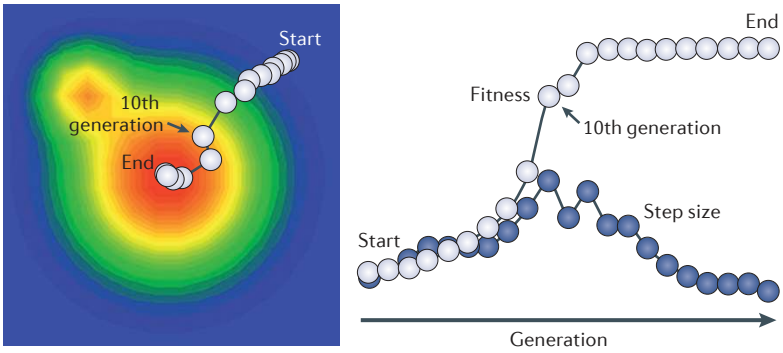
Box 1 | **Principles of adaptive peptide design**

Evolutionary algorithms are based on the iterative generation of potential solutions (x_i) to a problem and the selection of the best solution according to a (usually) multimodal fitness function, defined as $f(x_i)$[186]. In peptide design, the underlying fitness landscape is either provided by a determination of the candidate peptides using actual biochemical activity, or by computing a structure–activity landscape. The ideal measure of 'fitness' is an experimental response. If sufficient training data are available, $f(x_i)$ can be expressed by a machine-learning model[187]. To find the best solution in the fitness landscape containing A^n peptides (where A is the number of different building blocks, and *n* is the peptide length), the population of solutions (peptides) must be able to adapt to the local ruggedness of the landscape so that the search does not prematurely converge in areas of low fitness. Such a desired behaviour can be achieved using so-called strategy parameters — that is, variables that capture the progress made within a population and are used for projecting a promising search path for the next generation.

A straightforward way to perform an informed search is illustrated in the figure. A simple evolution strategy[188] was implemented to find the global optimum in a virtual fitness landscape. For clarity, in this case a mathematical function serves as the fitness estimator $f(x_i)$ and is a substitute for an experimental activity assay. For each of the 50 simulated generations, ten offspring (x_1 to x_{10}) were bred from a single parent (indicated by the arrow, x^p, as shown in the left panel of the figure). Their spread around the location of the parent approximates a normal distribution with width σ (known as step size), which serves as the adaptive strategy parameter in this algorithm. This factor is a property of the current parent — that is, the best among the set of offspring solutions, *x*, from the previous generation. In every new generation, a new population (*x*) will be created so that its position in the search space satisfies a localized distribution with width σ and center x^p. As only the best solution (and its associated individual σ value) is picked as the next parent in the subsequent selection step, over time small values of σ will evolve in rugged parts of the fitness landscape or near an optimum point (leading to small steps in the search space), whereas large values of σ will dominate in flat, plateau-like areas (leading to large steps in the search space). It is important to realize that σ adapts to the local form of the fitness landscape so that optimal progress of the search takes place, without any predefined cooling schedule, operator intervention or external control.

Different implementations of evolutionary optimization use different mechanisms for adaptation[189,190], and the adaptive step-size concept presented here is a characteristic of evolution strategies, which — in the case of peptide design — work directly on molecular properties such as residue polarity, side-chain volume, hydrophobic moment, and so on. By contrast, genetic algorithms first code molecular properties in the form of bit strings representing a peptide's genotype, and then apply mutation and recombination operations to the bit strings to generate offspring[191].

The figure provides an example of evolutionary optimization. A mathematical function (indicated by the contour plot on the left) served as a fitness function that had to be optimized by an evolution strategy. The size of the population was 10 individuals (search agents), and a single parent (elitism) was selected in each of the 20 simulated generations. After 10 generations the search converged close to the global optimum (as indicated by the trend line joining the grey points below). Note that the distribution of the population is adaptive (step size, σ, represented as the blue curve on the right). In terms of molecular design, the σ value corresponds to compound diversity.



measurements and chromatographic indices[135]. Studies examining peptides based on lactoferricin successfully used the *z*-descriptors as well as other descriptor sets[136–139]. Other early studies of AMPs using chemoinformatics were based on protegrin[140,141].

The most straightforward molecular representations in terms of computed descriptors provide a quantification of whole-molecule properties — such as partial charge, hydrophobicity and amphiphilicity — and are relatively intuitive. However, arrays of descriptors have been developed that lack such clear interpretations and intuitive understanding. These include descriptors that are calculated based on theoretical models, such as van der Waals surface area and hardness (akin to the energy required to remove the outermost electron), as well as properties that are experimentally measured, such as retention time in a given chromatography column, pI, octanol–water partition fraction or circular dichroism. Commercial software packages are available that offer hundreds of descriptors of the molecular nature of molecules, and are often customized specifically to the type of molecule (for example, they can be specific for small compounds versus peptides and proteins)[142].

Nevertheless, to some extent the computational approaches pursued in peptide design have been uncoupled from those used in small-molecule drug design. This might be due to the comparably higher molecular weight, increased flexibility and abundance of repetitive pharmacophoric features (such as the amide backbone) of peptides. The choice of descriptors has often been made based on a prior understanding of the physical attributes that give rise to the activity of the peptide, but ideally descriptors can and should be automatically selected during numerical analysis, through a method termed 'feature selection'[143,144].

Regardless of the numerical method used, most algorithms require at least as many distinct peptides with measured activity as the number of parameters matched by the method. For simplistic methods this is not an onerous requirement (for example, using only two parameters can fit activity to a linear method). However, more recent studies on modelling AMP activity have led to models based on machine-learning methods that involve simultaneously fitting tens to hundreds of parameters depending on the configuration. Many descriptors are available for modelling AMPs, and a substantial number of modelling methods have been applied to find QSAR functions that predict AMP activity (reviewed in REF. 145).

Complex prediction models using machine-learning methods often require a large number of measured values of AMP activity to fit the correspondingly large set of parameters. Solid-phase synthesis and high-throughput screening of large peptide arrays has become common practice in drug discovery[146]. For peptide lengths of up to 6 or 7 residues, full combinatorial arrays have been only marginally practically feasible, resulting in a sequence space containing 20^n peptides for the 20 natural amino acids (if $n = 6$, $20^6 = 6.4 \times 10^7$; if $n = 7$, $20^7 = 1.28 \times 10^9$); owing to potential crosslinking, oxidation, poor aqueous solubility and synthetic issues, cysteine and methionine residues as well as very hydrophobic sequences are usually excluded from these arrays.

Measurements of MIC, which must be made using low-throughput methods (overnight incubation with dilutions of AMPs), have restricted the size of data sets available for modelling AMP activity. Surrogate measures of bacterial killing, such as lipid vesicle experiments[147] or the diminished energy-dependent luminescence of bacteria constitutively expressing luciferase[90], have been used to develop faster assays for peptide activity. By replacing MIC values with such surrogate measures[148], and combining these with high-throughput analysis and relatively inexpensive peptide array synthesis on cellulose sheets, a set of peptides with more than 1,400 distinct sequences was assayed for activity[149,150]. Initially, two iterative sets of randomized peptide sequences were synthesized and tested for antibacterial activity against *P. aeruginosa*. These peptide sequences were biased according to the amino-acid composition of the most active peptides, which were especially rich in lysine, arginine, tryptophan and hydrophobic amino acids.

Based on these data, several artificial neural networks were trained to recognize potent peptides using the measured peptide activities and 44 calculated descriptors of the peptides based on the properties of the amino-acid sequences (rather than the sequence per se). To maximize the use of the data, a set of crossvalidated neural networks was used rather than a single neural network. A consensus of this set of neural networks was used to predict the ranked activities of nearly 100,000 virtual (that is, generated *in silico)* peptides. A total of 200 peptides, classified into four levels of activity (assessed by comparison with the bactenecin 2A control), were synthesized and used for validation. Of the novel peptides predicted to be highly active compared to the bactenecin 2A control, 94% were found to be highly active. Of the novel peptides predicted to have low activity, all were found to have low activity. Interestingly, when MIC values were measured against clinical pathogens with demonstrated drug resistance, many synthetic peptides had MIC values <10 μM and several had MIC values <1 μM, which is equivalent to the best peptides described in the literature, despite being significantly shorter (nine residues long) than any natural peptide (natural peptides can be >12 residues long, but are usually >18 residues long).

It is also notable that peptides with similar overall properties, such as hydrophobicity or charge, can have dramatically different levels of activity. For example, the peptides KRWWKWIRW and KRWWKWWRR demonstrated the highest antibacterial activity, whereas peptides with a very similar residue composition — for example, WHGVRWWKW, WVKVWKYTW, WVRFVYRYW and AIRRWRIRK — were ranked in the least active 30% of peptides and found to be virtually inactive. These findings suggest that there are common physicochemical features shared by AMPs, presumably in the context of their three-dimensional structures. Some of the features found in the most active AMP sequences are consecutive pairs of tryptophan residues and interspersed arginine and/or lysine residues; these residues might aid in the general interfacial affinity of these AMPs for lipid membranes or in their translocation across lipid membranes (FIG. 4).

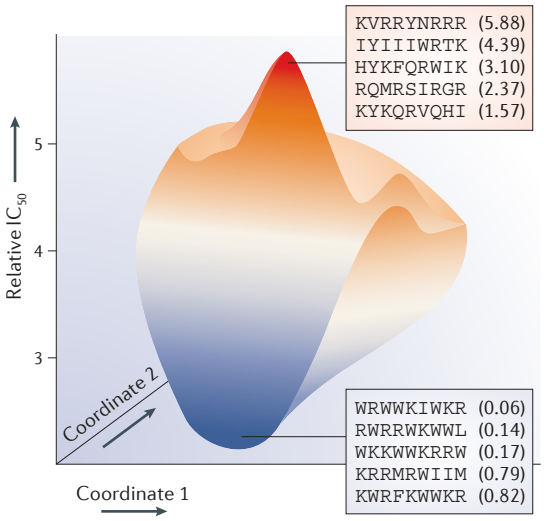


Figure 4 | **Artificial fitness landscape spanned by synthetic peptides.** There is a visible separation between only moderately active peptides (red) and potent antimicrobial peptides (AMPs) (blue). Selected peptide sequences are shown together with their relative IC_{50} (half-maximal inhibitory concentration) values indicating their antibacterial activity against *Pseudomonas aeruginosa* in a reporter gene assay[151]. Many active peptides are strongly enriched in tryptophan and arginine residues. For computation and visualization of the structure–activity landscape, each nonapeptide was represented by 9 × 19 properties (principal component scores computed from a set of 434 physicochemical amino-acid properties)[123,198] and projected to two new coordinates using stochastic proximity embedding[199]. The continuous landscape was generated using the software tool LiSARD[200].

Evolutionary search methods for peptide sequences allow for a guided search in the sequence space, in which peptide sequences are varied to achieve improvements in a 'fitness landscape' — an analogy for visualizing how good (or well matched) numerical solutions are in the space of possible parameter settings (BOX 1). This concept of peptide design was first applied to signal peptides[88,92]. Recently, the methodology has been adapted to generate potent synthetic AMPs. Using trained neural network models as an estimate of peptide 'fitness', a genetic algorithm was used to find AMPs with greater efficiency in a preliminary computational screening[151].

Owing to the limited number of studies that have used machine-learning models for peptide design, we feel that an assessment of their relative performance and practical applicability would be premature. Nevertheless, pioneering concept studies have already demonstrated that novel, short AMP sequences with substantial biological activity can be obtained using adaptive computer-based design. It is safe to say that machine-learning applications hold substantial promise for peptide design in general, and not just for finding novel membrane-interacting peptides like AMPs.

Hydrophobic moment
The hydrophobicity of a peptide measured for different angles of rotation per residue.

Signal peptides
Typically amino-terminal stretches of amino-acid residues that are required for the targeting of proteins to their intra- and extracellular destination.

Table 2 | **Selected host defence peptides in drug development**

Name	Sequence	Company	Description	Application	Trial phase	Comments	Clinical trial identifiers and further information
Pexiganan acetate (MSI 78)	GIGKFLKK AKKFGKAF VKILKK	MacroChem	Synthetic analogue of magainin 2 derived from frog skin	Topical antibiotic	III	No advantage demonstrated over existing therapies	NCT00563433 and NCT00563394
Omiganan (MX-226/ MBI-226)	ILRWPW WPWRRK	Migenix/ BioWest therapeutics	Synthetic cationic peptide derived from indolicidin	Topical antiseptic, prevention of catheter infections	III	Missed primary end point (infections) but achieved secondary end points of microbiologically confirmed infections and catheter colonization	NCT00027248 and NCT00231153
Omiganan (CLS001)	ILRWPW WPWRRK	Cutanea Life Sciences/ Migenix	Synthetic cationic peptide derived from indolicidin	Severe acne and rosacea; anti-inflammatory	II/III	Significant efficacy in Phase II trials for both indications; in Phase III trials	NCT00608959
Iseganan (IB-367)	RGGLCY CRGRFC VCVGR	Ardea Biosciences	Synthetic 17-mer peptide derived from protegrin 1	Oral mucositis in patients undergoing radiation therapy	III	No advantage demonstrated over existing therapies	NCT00022373
hLF1–11	GRRRRS VQWCA	AM-Pharma	Cationic peptide fragment comprising amino-terminal amino acids 1–11 of human lactoferricin	Bacteraemia and fungal infections in immuno-compromised haematopoetic stem cell transplant recipients	I/II	Significant efficacy observed in Phase I trials; mechanism of action appears to be immunomodulatory rather than antibiotic; Phase II trials initiated after a long delay	NCT00509938
XOMA 629	KLFR-(D-naphtho -Ala)-QAK-(D-naphtho -Ala)	Xoma	Derivative of bactericidal permeability-increasing protein	Impetigo	IIa	No Phase IIa results available (trial started in July 2008)	XOMA website
PAC-113	AKRHHG YKRKFH	Pacgen Biopharma-ceuticals	Synthetic 12-mer peptide derived from histatin 3 and histatin 5	Oral candidiasis	IIb	Phase IIb results (announced June 2008): 34% increase in primary end point efficacy level; Phase III trial not initiated	NCT00659971
CZEN-002	$(CKPV)_2$	Zengen	Dimeric octamer derived from α-melanocyte-stimulating hormone	Vulvovaginal candidiasis; anti-inflammatory	IIb	Positive efficacy results announced; Phase IIb trial is a dose-ranging study	US Patent application serial number 09/535066
IMX942	KSRIVPA IPVSLL	Inimex	Synthetic cationic peptide derived from IDR1 and bactenecin	Nosocomial infection, febrile neutropenia	Ia	Phase Ia trial completed in 2009; no Phase II trial announced yet	Inimex Pharmaceuticals website
OP-145	IGKEFK RIVERIK RFLREL VRPLR	OctoPlus; Leiden University, The Netherlands	Synthetic 24-mer peptide derived from LL-37 for binding to lipopoly-saccharides or lipoteichoic acid	Chronic bacterial middle ear infection	II (com-pleted)	Clinical proof-of-efficacy in Phase II trials; no Phase III trials proposed yet	ISRCTN84220089
Ghrelin[210]	GSSFLSPE HQRVQQ RKESKKPP AKLQPR	University of Miyazaki, Japan; Papworth Hospital, Cambridge, UK	Endogenous host-defence peptide	Airway inflammation, chronic respiratory infection and cystic fibrosis	II	Peptide hormone that suppresses neutrophil-dominant inflammation in airways of patients with chronic respiratory infection	JPRN-UMIN000002599, JPRN-UMIN000001598 and NCT00763477

Table 2 (cont.) | **Selected host defence peptides in drug development**

Name	Sequence	Company	Description	Application	Trial phase	Comments	Clinical trial identifiers and further information
PMX-30063	Structure not disclosed	PolyMedix	Arylamide oligomer mimetic of a defensin	Acute bacterial skin infections caused by *Staphylococcus* spp.	II	Mimetic rather than peptide; currently in Phase II trials	NCT01211470; PolyMedix website
Delmitide (RDP58)[211]	RXXXRX XXGY (X = norleucine)	Genzyme	Semisynthetic D-amino acid decapeptide derived from HLA class I B2702	Inflammatory bowel disease	II (completed)	A protease-resistant, D-amino acid-containing peptide with similar efficacy to asacol; attempting to improve activity through formulation	Genzyme website; ISRCTN84220089
Plectasin[212]	GFGC$_1$NG PWDEDD MQC$_2$HNH C$_3$KSIKGYK GGYC$_1$AKG GFVC$_2$KC$_3$Y)	Novozymes	Fungal defensin; candidate in development is an amino-acid substitiution variant	Bacterial diseases	Pre-clinical	Excellent efficacy demonstrated in animal models	Novozymes website
HB1345	Decanoyl-KFKWPW	Helix BioMedix	Synthetic lipohexapeptide	Acne; broad-spectrum antibiotic	Pre-clinical	Looks promising as this is a very small lipopeptide	Helix BioMedix website

HLA, human leukocyte antigen; IDR1, innate defence regulator 1; LL-37, human cathelicidin antimicrobial peptide LL-37.

AMPs as drugs: challenges and solutions

Since 2000, 20 new antibiotics have been launched and approximately 40 compounds are currently in active clinical development[152]. Several synthetic AMPs have entered clinical trials and at least 15 peptides or mimetics are in (or have completed) clinical trials as antimicrobial or immunomodulatory agents (TABLE 2). In a Phase I/II trial, the AMP hLF1–11 (which is composed of amino-terminal amino acids 1–11 of human lactoferrin) was found to be safe and well tolerated when delivered intravenously[153]. By contrast, a protegrin derivative, iseganan, failed to demonstrate significant efficacy in the clinic[30,154].

Two peptides have demonstrated efficacy in Phase III clinical trials but have not yet been approved. Pexiganan[155], a derivative of magainin, showed equivalence to an oral fluoroquinolone for foot ulcer infections in patients with diabetes but was deemed non-approvable by the US Food and Drug Administration, although there is evidence it might resurface in clinical trials. Omiganan (MBI-226), an analogue of indolicidin, has been proven to be capable of significantly reducing catheter colonization and microbiologically confirmed tunnel infections during catheterization (ClinicalTrials.gov identifier: NCT00608959), and in Phase II trials it exhibited anti-inflammatory activity against the non-infectious skin condition rosacea. Both of these peptides are first-generation peptides that were devised by template-based design. Overall, the peptides that are currently in the clinic offer fascinating alternatives to standard therapies and indicate that synthetic peptides are an active and promising area of research. AMP-coated devices represent another promising application, although the reduction in antimicrobial activity by the tethering of the peptide to solid supports must be overcome[156]. SAR studies have demonstrated that tethered peptides are nearly 100-fold less active (on a molecular weight basis) than their soluble counterparts[157].

Despite several attempts to develop AMPs as antibiotics, the reasons why synthetic AMPs have not progressed more successfully through the clinic include the cost of goods, their lability to proteolytic degradation, and their unknown toxicology profile when administered systemically[2]. Each of these factors can be addressed by the peptide design approaches described above in combination with advanced chemoinformatics tools[158,159]. For example, the cost of goods can be addressed by making smaller peptides, and machine-learning approaches have already delivered highly active, broad-spectrum peptides that work systemically in animals. The liability to degradation by proteases in the body can be addressed using D-amino acids, non-natural amino-acid analogues, mimetics with different backbone structures or appropriate formulations[160,161].

The toxicology of AMPs can typically be addressed by making a plethora of highly active sequences and testing these for lack of toxicity in animals and/or using formulations that mask the peptides — for example, liposomal formulations[2,162]. Although it has become common to investigate the haemolytic toxicity of AMPs[163], it is evident that reliable computational toxicology prediction will be necessary to improve design algorithms that explicitly consider crucial preclinical toxicological end points for AMPs. Taking into consideration the multifactorial nature of toxicology and current lack of large sets of published standardized toxicology data for AMPs, some machine-learning methods and alerting tools have been devised

Deep-ultraviolet resonance Raman spectroscopy
A biophysical technique for sensitive secondary-structure detection in peptides and proteins, and for confident discrimination between secondary structure types.

that seem to be suited for this task[164–166]. Multidimensional design techniques have been devised and were first applied to combinatorial optimization and drug discovery[167–172]. Computer-assisted peptide design and peptide-mimetics design coupled with *in silico* pharmacology will undoubtedly benefit from these methodological advances[173,174].

A recent and extensive review of the field of peptide mimetics provides an overview of various peptide-to-drug design approaches[175]. In many cases these design principles are analogous to those described above and will benefit from prior experiences in this arena with natural peptides[176,177]. For example, the reported effects of secondary-structure disruption or modification of D-amino-acid replacements in AMPs suggest that secondary-structure preference and biological activity are not directly coupled[178]. Furthermore, methylation has been shown to fine-tune the haemolytic activity of a cecropin A–melittin-derived helical AMP (KWKLFKKIGAVLKVL-amide)[179] without significantly affecting the secondary structure of the AMP. These studies also suggest that helix formation in at least part of this chimeric AMP, together with ionic interactions with the bacterial membrane, is mandatory for direct antimicrobial activity.

There are apparent similarities in amino-acid composition (with the exception of positively charged residues) between aggregation-prone regions of proteins and AMPs, and it has recently been shown that amyloid-forming peptides can be turned into membrane-disrupting AMPs by placing cationic amino acids at selected residue positions so that the mutated peptides possess the ability to adopt amphiphilic structures[180]. Such experiments provide a rationale for linking molecular structure with direct antimicrobial activity in peptides that have been designed *de novo*. The apparent preferences of AMPs for certain membranes could be triggered by differential membrane lipid compositions[181], and methodological advances in structure determination will aid future investigations of the dynamic behaviour of AMP structure at the membrane–solvent interface; this was recently demonstrated using deep-ultraviolet resonance Raman spectroscopy of a model helical peptide embedded in a membrane-mimetic environment[182].

Given these considerations, how likely is it that AMP-like compounds will succeed in delivering their therapeutic potential? There are clear precedents for cationic peptides having clinical efficacy[183]; the cationic lipopeptide polymyxin is the last-resort drug for treating multiresistant *Pseudomonas* spp. and *Acinetobacter* spp. infections, the cyclic cationic peptide gramicidin S is highly used in topical ointments and eye drops, and the cationic lantibiotic nisin is an approved food additive in Europe[184]. Thus, in our opinion, the increasing availability and use of innovative computer-assisted design strategies has considerable potential to boost the discovery of next-generation therapeutic peptides and peptide mimetics as anti-infectives not only for targeting bacteria that have become resistant to existing antibiotics but also for targeting disease-causing protozoa, helminths, insects and fungi[185].

1. Zasloff, M. Antimicrobial peptides of multicellular organisms. *Nature* **415**, 389–395 (2002). **Together with reference 19, this paper gives a recommended overview of the mechanisms of action of AMPs.**
2. Hancock R. E. W. & Sahl H. G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotech*. **24**, 1551–1557 (2006).
3. Lehrer, R. I. Primate defensins. *Nature Rev. Microbiol*. **2**, 727–738 (2004).
4. Boman, H. G. Innate immunity and the normal microflora. *Immunol. Rev*. **173**, 5–16 (2000).
5. Berkowitz, B. A., Bevins, C. L. & Zasloff, M. A. Magainins: a new family of membrane-active host defense peptides. *Biochem. Pharmacol*. **39**, 625–629 (1990).
6. Zanetti, M. The role of cathelicidins in the innate host defenses of mammals. *Curr. Issues Mol. Biol*. **7**, 179–196 (2005).
7. Wang, G. (ed.) *Antimicrobial Peptides: Discovery, Design and Novel Therapeutic Strategies. Advances in Molecular and Cellular Microbiology* 18th edn (CABI Publishing, Cambridge, Massachusetts, 2010).
8. Boman, H. G. Antibacterial peptides: basic facts and emerging concepts. *J. Intern. Med*. **254**, 197–215 (2003). **This is a must-read paper on the history of AMPs and the significance of their discovery for modern medicine.**
9. Zasloff, M. Antibiotic peptides as mediators of innate immunity. *Curr. Opin. Immunol*. **4**, 3–7 (1992).
10. Hoskin, D. W. & Ramamoorthy, A. Studies on anticancer activities of antimicrobial peptides. *Biochim. Biophys. Acta* **1778**, 357–375 (2008).
11. Sullivan, R. *et al.* Clinical efficacy of a specifically targeted antimicrobial peptide mouth rinse: targeted elimination of *Streptococcus mutans* and prevention of demineralization. *Caries Res.* **45**, 415–428 (2011).
12. Chung, Y. S. & Kocks, C. Recognition of pathogenic microbes by the *Drosophila* phagocytic pattern recognition receptor Eater. *J. Biol. Chem*. **286**, 26524–26532 (2011).
13. Alalwani, S. M. *et al.* The antimicrobial peptide LL-37 modulates the inflammatory and host defense response of human neutrophils. *Eur. J. Immunol*. **40**, 1118–1126 (2010).
14. van der Does, A. M. *et al.* Antimicrobial peptide hLF1–11 directs granulocyte-macrophage colony-stimulating factor-driven monocyte differentiation toward macrophages with enhanced recognition and clearance of pathogens. *Antimicrob. Agents Chemother*. **54**, 811–816 (2010).
15. Tomasinsig, L., Scocchi, M., Di Loreto, C., Artico, D. & Zanetti, M. Inducible expression of an antimicrobial peptide of the innate immunity in polymorphonuclear leukocytes. *J. Leukoc. Biol*. **72**, 1003–1010 (2002).
16. Lehrer, R. I. & Ganz, T. Cathelicidins: a family of endogenous antimicrobial peptides. *Curr. Opin. Hematol*. **9**, 18–22 (2002).
17. Melo, M. N., Ferre, R., Castanho, M. A. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nature Rev. Microbiol*. **7**, 245–250 (2009).
18. Yeung, A. T. Y., Gellatly, S. L. & Hancock, R. E. W. Multifunctional cationic host-defence peptides and clinical applications. *Cell. Mol. Life Sci*. **68**, 2161–2176 (2011). **This review covers the various clinical applications of cationic peptides — from antimicrobial to immunomodulatory — and describes the clinical stage of current candidate peptides in development.**
19. Brogden, K. A. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature Rev. Microbiol*. **3**, 238–250 (2005).
20. Mayer, M. L., Easton, D. M. & Hancock, R. E. W. in *Antimicrobial Peptides: Discovery, Design and Novel Therapeutic Strategies*. *Advances in Molecular and Cellular Microbiology* 18 edn (ed. Wang, G.) 195–220 (CABI Publishing, Cambridge, Massachusetts, 2010).
21. Skarnes, R. C. & Watson, D. W. Antimicrobial factors of normal tissues and fluids. *Bacteriol. Rev*. **4**, 273–294 (1957).
22. Nuttall, G. Experimente über die bakterien feindlichen Einflüsse des tierischen Körpers. *Z. Hyg. Infektionskr*. **4**, 353–394 (1888).
23. Spitznagel, J. K. Antibiotic proteins of human neutrophils. *J. Clin. Invest*. **86**, 1381–1386 (1990).
24. Merrifield, R. B., Vizioli, L. D. & Boman, H. G. Synthesis of the antibacterial peptide cecropin A (1–33). *Biochemistry* **21**, 5020–5031 (1982).
25. Miyata, T. *et al.* Antimicrobial peptides, isolated from horseshoe crab hemocytes, tachyplesin II, and polyphemusins I and II: chemical structures and biological activity. *J. Biochem*. **106**, 663–668 (1989).
26. Powers, J. P., Rozek, A. & Hancock, R. E. Structure–activity relationships for the β-hairpin cationic antimicrobial peptide polyphemusin I. *Biochim. Biophys. Acta* **1698**, 239–250 (2004).
27. Zhao, C., Liu, L. & Lehrer, R. I. Identification of a new member of the protegrin family by cDNA cloning. *FEBS Lett*. **346**, 285–288 (1994).
28. Cole, A. M. & Waring, A. J. The role of defensins in lung biology and therapy. *Am. J. Respir. Med*. **1**, 249–259 (2002).
29. Zhang, L. *et al.* Interaction of polyphemusin I and structural analogs with bacterial membranes, lipopolysaccharide, and lipid monolayers. *Biochemistry* **39**, 14504–14514 (2000).
30. Trotti, A. *et al.* A multinational, randomized Phase III trial of iseganan HCl oral solution for reducing the severity of oral mucositis in patients receiving radiotherapy for head-and-neck malignancy. *Int. J. Radiat. Oncol. Biol. Phys*. **58**, 674–681 (2004).
31. Kollef, M. *et al.* A randomized double-blind trial of iseganan in prevention of ventilator-associated pneumonia. *Am. J. Respir. Crit. Care Med*. **173**, 91–97 (2006).
32. Hancock, R. E. W. & Lehrer, R. I. Cationic peptides: a new source of antibiotics. *Trends Biotechnol*. **16**, 82–88 (1998).
33. Loutet, S. A. & Valvano, M. A. Extreme antimicrobial peptide and polymyxin B resistance in the genus *Burkholderia*. *Front. Microbiol*. **2**, 159 (2011).
34. Franco, O. L. Peptide promiscuity: an evolutionary concept for plant defense. *FEBS Lett*. **585**, 995–1000 (2011).

35. Smith, P. A. & Romesberg, F. E. Combating bacteria and drug resistance by inhibiting mechanisms of persistence and adaptation. *Nature Chem. Biol.* **3**, 549–556 (2007).
36. Falconer, S. B., Czarny, T. L. & Brown, E. D. Antibiotics as probes of biological complexity. *Nature Chem. Biol.* **7**, 415–423 (2011).
37. Fabbretti, A., Gualerzi, C. O. & Brandi, L. How to cope with the quest for new antibiotics. *FEBS Lett.* **585**, 1673–1681 (2011).
38. Friedrich, C. L., Rozek, A., Patrzykat, A. & Hancock, R. E. W. Structure and mechanism of action of an indolicidin peptide derivative with improved activity against Gram-positive bacteria. *J. Biol. Chem.* **276**, 24015–24022 (2001).
39. Peschel, A. & Sahl, H. G. The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nature Rev. Microbiol.* **4**, 529–536 (2006).
40. Perron, G. G., Zasloff, M. & Bell, G. Experimental evolution of resistance to an antimicrobial peptide. *Proc. Biol. Sci.* **273**, 251–256 (2006).
41. Nizet, V. Antimicrobial peptide resistance mechanisms of human bacterial pathogens. *Curr. Issues Mol. Biol.* **8**, 11–26 (2006).
42. Overington, J. P., Al-Lazikani, B. & Hopkins, A. L. How many drug targets are there? *Nature Rev. Drug Discov.* **5**, 993–996 (2006).
43. Yildirim, M. A., Goh, K. I., Cusick, M. E., Barabási, A. L. & Vidal, M. Drug–target network. *Nature Biotech.* **25**, 1119–1126 (2007).
44. Leach, A. R. & Hann, M. M. Molecular complexity and fragment-based drug discovery: ten years on. *Curr. Opin. Chem. Biol.* **15**, 489–496 (2011).
45. Rognan, D. Chemogenomic approaches to rational drug design. *Br. J. Pharmacol.* **152**, 38–52 (2007).
46. Balakin, K. V., Ivanenkov, Y. A. & Savchuk, N. P. Compound library design for target families. *Methods Mol. Biol.* **575**, 21–46 (2009).
47. Scheiber, J. *et al.* Gaining insight into off-target mediated effects of drug candidates with a comprehensive systems chemical biology analysis. *J. Chem. Inf. Model.* **49**, 308–317 (2009).
48. van Laarhoven, T., Nabuurs, S. B. & Marchiori, E. Gaussian interaction profile kernels for predicting drug–target interaction. *Bioinformatics* **27**, 3036–3043 (2011).
49. Shai, Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochim. Biophys. Acta* **1462**, 55–70 (1999).
50. Fuertes, G., Giménez, D., Esteban-Martín, S., Sánchez-Muñoz, O. L. & Salgado, J. A lipocentric view of peptide-induced pores. *Eur. Biophys. J.* **40**, 399–415 (2011).
51. Lohner, K. New strategies for novel antibiotics: peptides targeting bacterial cell membranes. *Gen. Physiol. Biophys.* **28**, 105–116 (2009).
52. Wimley, W. C. & Hristova, K. Antimicrobial peptides: successes, challenges and unanswered questions. *J. Membr. Biol.* **239**, 27–34 (2011).
This is a critical review on the current state of AMP design and a discussion on specific open questions that should be addressed in the future.
53. Hall, K., Mozsolits, H. & Aguilar, M. I. Surface plasmon resonance analysis of antimicrobial peptide-membrane interactions: affinity and mechansim of action. *Lett. Pept. Sci.* **10**, 475–485 (2004).
54. Hiep, H. M. *et al.* Label-free detection of melittin binding to a membrane using electrochemical-localized surface plasmon resonance. *Anal. Chem.* **80**, 1859–1864 (2008).
55. Epand, R. M. & Epand, R. F. Bacterial membrane lipids in the action of antimicrobial agents. *J. Pept. Sci.* **17**, 298–305 (2011).
56. Neville, F. *et al.* Lipid headgroup discrimination by antimicrobial peptide LL-37: insight into mechanism of action. *Biophys. J.* **90**, 1275–1287 (2006).
57. Bowdish, D. M. E. *et al.* Impact of LL-37 on anti-infective immunity. *J. Leukoc. Biol.* **77**, 451–459 (2005).
58. Lau, Y. E. *et al.* Interaction and cellular localization of the human host defence peptide, LL-37, with lung epithelial cells. *Infect. Immun.* **73**, 583–591 (2005).
This study demonstrated that uptake into cells is essential for the chemokine-inducing properties of LL-37 and other cationic peptides, changing the paradigm that such peptides were selective because they were not taken up into eukaryotic cells.
59. Mookherjee, N. *et al.* Intracellular receptor for human host defence peptide LL-37 in monocytes. *J. Immunol.* **183**, 2688–2696 (2009).
60. Prochiantz, A. Homeodomain-derived peptides: in and out of the cells. *Ann. NY Acad. Sci.* **886**, 172–179 (1999).
61. Lee, S. H. *et al. De novo* generation of short antimicrobial peptides with simple amino acid composition. *Regul. Pept.* **166**, 36–41 (2011).
62. Dom, G. *et al.* Cellular uptake of Antennapedia Penetratin peptides is a two-step process in which phase transfer precedes a tryptophan-dependent translocation. *Nucleic Acids Res.* **31**, 556–561 (2003).
63. Boman, H. G. & Hultmark, D. Cell-free immunity in insects. *Annu. Rev. Microbiol.* **41**, 103–126 (1987).
64. Andreu, D., Merrifield, R. B., Steiner, H. & Boman, H. G. N-terminal analogues of cecropin A: synthesis, antibacterial activity, and conformational properties. *Biochemistry* **24**, 1683–1688 (1985).
65. Sandgren, S. *et al.* The human antimicrobial peptide LL-37 transfers extracellular DNA plasmid to the nuclear compartment of mammalian cells via lipid rafts and proteoglycan-dependent endocytosis. *J. Biol. Chem.* **279**, 17951–17956 (2004).
This is a breakthrough paper that describes how LL-37 acts as a cell-penetrating peptide and thus shares physicochemical properties with this group of peptides.
66. El-Andaloussi, S., Holm, T. & Langel, U. Cell-penetrating peptides: mechanisms and applications. *Curr. Pharm. Des.* **11**, 3597–3611 (2005).
67. Epand, R. M. & Vogel, H. J. Diversity of antimicrobial peptides and their mechanisms of action. *Biochim. Biophys. Acta* **1462**, 11–28 (1999).
68. Powers, J. P. & Hancock, R. E. W. The relationship between peptide structure and antibacterial activity. *Peptides* **24**, 1681–1691 (2003).
69. Fjell, C. D., Hancock, R. E. W. & Cherkasov, A. AMPer: a database and an automated discovery tool for antimicrobial peptides. *Bioinformatics* **23**, 1148–1155 (2007).
70. Fjell, C. D. *et al.* Identification of novel host defense peptides and the absence of α-defensins in the bovine genome. *Proteins* **73**, 420–430 (2008).
71. Thomas, S., Karnik, S., Barai, R. S., Jayaraman, V. K. & Idicula-Thomas, S. CAMP: a useful resource for research on antimicrobial peptides. *Nucleic Acids Res.* **38**, D774–D780 (2010).
72. Wang, P. *et al.* Prediction of antimicrobial peptides based on sequence alignment and feature selection methods. *PLoS ONE* **6**, e18476 (2011).
73. Brahmachary, M. *et al.* ANTIMIC: a database of antimicrobial sequences. *Nucleic Acids Res.* **32**, D586–D589 (2004).
74. Hammami, R. & Fliss, I. Current trends in antimicrobial agent research: chemo- and bioinformatics approaches. *Drug Discov. Today* **15**, 540–546 (2010).
75. Wang, Z. & Wang, G. APD: the antimicrobial peptide database. *Nucleic Acids Res.* **32**, D590–D592 (2004).
76. Wang, G., Li, X. & Wang, Z. APD2: the updated antimicrobial peptide database and its application in peptide design. *Nucleic Acids Res.* **37**, D933–D937 (2009).
This is the most comprehensive AMP database resource.
77. Benko-Iseppon, A. M. *et al.* Overview on plant antimicrobial peptides. *Curr. Protein Pept. Sci.* **11**, 181–188 (2010).
78. Hughes, C. C. & Fenical, W. Antibacterials from the sea. *Chem. Eur. J.* **16**, 12512–12525 (2010).
79. de Azevedo, W. F. Jr & Dias, R. Computational methods for calculation of ligand-binding affinity. *Curr. Drug Targets* **9**, 1031–1039 (2008).
80. Timmers, L. F., Pauli, I., Caceres, R. A. & de Azevedo, W. F. Jr. Drug-binding databases. *Curr. Drug Targets* **9**, 1092–1099 (2008).
81. Langer, T., Hoffmann, R., Bryant, S. & Lesur, B. Hit finding: towards 'smarter' approaches. *Curr. Opin. Pharmacol.* **9**, 589–593 (2009).
82. Juretić, D., Vukicević, D., Ilić, N., Antcheva, N. & Tossi, A. Computational design of highly selective antimicrobial peptides. *J. Chem. Inf. Model.* **49**, 2873–2882 (2009).
83. Juretić, D. *et al.* Knowledge-based computational methods for identifying or designing novel, non-homologous antimicrobial peptides. *Eur. Biophys. J.* **40**, 371–385 (2011).
84. Robinson, J. A. Protein epitope mimetics as anti-infectives. *Curr. Opin. Chem. Biol.* **15**, 379–386 (2011).
85. Wiradharma, N. *et al.* Synthetic cationic amphiphilic α-helical peptides as antimicrobial agents. *Biomaterials* **32**, 2204–2212 (2011).
86. Huang, Y., Huang, J. & Chen, Y. α-helical cationic antimicrobial peptides: relationships of structure and function. *Protein Cell* **1**, 143–152 (2010).
87. Pag, U. *et al.* Analysis of *in vitro* activities and modes of action of synthetic antimicrobial peptides derived from an α-helical 'sequence template'. *J. Antimicrob. Chemother.* **61**, 341–352 (2008).
88. Wrede, P. & Schneider, G. (eds) *Concepts in Protein Engineering and Design* 281–317 (Walter-de-Gruyter, Berlin, New York, 1994).
89. Tapia, V. E., Ay, B. & Volkmer, R. Exploring and profiling protein function with peptide arrays. *Methods Mol. Biol.* **570**, 3–17 (2009).
90. Hilpert, K., Volkmer-Engert, R., Walter, T. & Hancock, R. E. W. High-throughput generation of small antibacterial peptides with improved activity. *Nature Biotech.* **23**, 1008–1012 (2005).
91. Hilpert, K. *et al.* Sequence requirements and a novel optimization strategy for short antimicrobial peptides. *Chem. Biol.* **13**, 1101–1107 (2006).
92. Schneider, G., Schuchhardt, J. & Wrede, P. Artificial neural networks and simulated molecular evolution are potential tools for sequence-oriented protein design. *Comput. Appl. Biosci.* **10**, 635–645 (1994).
93. Loose, C., Jensen, K., Rigoutsos, I. & Stephanopoulos, G. A linguistic model for the rational design of antimicrobial peptides. *Nature* **443**, 867–869 (2006).
This paper presents a creative bioinformatics concept for a new perspective on amino-acid sequence description and design.
94. Blondelle, S. E. & Houghten, R. A. Design of model amphipathic peptides having potent antimicrobial activities. *Biochemistry* **31**, 12688–12694 (1992).
This landmark paper used library-based combinatorial design methods to create small active peptides.
95. Deslouches, B. *et al. De novo* generation of cationic antimicrobial peptides: influence of length and tryptophan substitution on antimicrobial activity. *Antimicrob. Agents Chemother.* **49**, 316–322 (2005).
96. Frecer, V., Ho, B. & Ding, J. L. *De novo* design of potent antimicrobial peptides. *Antimicrob. Agents Chemother.* **48**, 3349–3357 (2004).
97. Kondejewski, L. H. *et al.* Dissociation of antimicrobial and hemolytic activities in cyclic peptide diastereomers by systematic alterations in amphipathicity. *J. Biol. Chem.* **274**, 13181–13192 (1999).
98. Jiang, Z., Vasil, A. I., Gera, L., Vasil, M. L. & Hodges, R. S. Rational design of α-helical antimicrobial peptides to target Gram-negative pathogens, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: utilization of charge, 'specificity determinants,' total hydrophobicity, hydrophobe type and location as design parameters to improve the therapeutic ratio. *Chem. Biol. Drug Des.* **77**, 225–240 (2011).
99. He, J., Anderson, M. H., Shi, W. & Eckert, R. Design and activity of a 'dual-targeted' antimicrobial peptide. *Int. J. Antimicrob. Agents* **33**, 532–537 (2009).
100. Schneider, G., Lee, M. L., Stahl, M. & Schneider, P. *De novo* design of molecular architectures by evolutionary assembly of drug-derived building blocks. *J. Comput. Aided Mol. Des.* **14**, 487–494 (2000).
101. Pierce, A. C., Rao, G. & Bemis, G. W. BREED: generating novel inhibitors through hybridization of known ligands. Application to CDK2, p38, and HIV protease. *J. Med. Chem.* **47**, 2768–2775 (2004).
102. Radzishevsky, I. S. *et al.* Effects of acyl versus aminoacyl conjugation on the properties of antimicrobial peptides. *Antimicrob. Agents Chemother.* **49**, 2412–2420 (2005).
103. Serrano, G. N., Zhanel, G. G. & Schweizer, F. Antibacterial activity of ultrashort cationic lipo-β-peptides. *Antimicrob. Agents Chemother.* **53**, 2215–2217 (2009).
104. Avrahami, D. & Shai, Y. A new group of antifungal and antibacterial lipopeptides derived from non-membrane active peptides conjugated to palmitic acid. *J. Biol. Chem.* **279**, 12277–12285 (2004).
This study introduced acylation as a strategy to enhance the activity of AMPs.
105. Mátyus, E., Kandt, C. & Tieleman, D. P. Computer simulation of antimicrobial peptides. *Curr. Med. Chem.* **14**, 2789–2798 (2007).

106. Jorgensen, W. L. Efficient drug lead discovery and optimization. *Acc. Chem. Res.* **42**, 724–733 (2009).
107. Schneider, G. & Fechner, U. Computer-based *de novo* design of drug-like molecules. *Nature Rev. Drug Discov.* **4**, 649–663 (2005).
108. Wu, M. & Hancock, R. E. W. Improved derivatives of bactenecin, a cyclic dodecameric antimicrobial cationic peptide. *Antimicrob. Agents Chemother.* **43**, 1274–1276 (1999).
109. Rozek, A., Powers, J. P., Friedrich, C. L. & Hancock, R. E. W. Structure-based design of an indolicidin peptide analogue with increased protease stability. *Biochemistry* **42**, 14130–14138 (2003).
110. Bolintineanu, D. S. & Kaznessis, Y. N. Computational studies of protegrin antimicrobial peptides: a review. *Peptides* **32**, 188–201 (2011).
111. Fahrner, R. L. *et al.* Solution structure of protegrin-1, a broad-spectrum antimicrobial peptide from porcine leukocytes. *J. Chem. Biol.* **3**, 543–550 (1996).
112. Bond, P. J. & Khalid, S. Antimicrobial and cell-penetrating peptides: structure, assembly and mechanisms of membrane lysis via atomistic and coarse-grained molecular dynamics simulations. *Protein Pept. Lett.* **17**, 1313–1327 (2010).
113. Wang, Q., Hong, G., Johnson, G. R., Pachter, R. & Cheung, M. S. Biophysical properties of membrane-active peptides based on micelle modeling: a case study of cell-penetrating and antimicrobial peptides. *J. Phys. Chem. B* **114**, 13726–13735 (2010).
114. Romo, T. D., Bradney, L. A., Greathouse, D. V. & Grossfield, A. Membrane binding of an acyl-lactoferricin B antimicrobial peptide from solid-state NMR experiments and molecular dynamics simulations. *Biochim. Biophys. Acta* **1808**, 2019–2030 (2011).
115. Sengupta, D., Leontiadou, H., Mark, A. E. & Marrink, S. J. Toroidal pores formed by antimicrobial peptides show significant disorder. *Biochim. Biophys. Acta* **1778**, 2308–2317 (2008).
116. Mani, R. *et al.* Membrane-dependent oligomeric structure and pore formation of a β-hairpin antimicrobial peptide in lipid bilayers from solid-state NMR. *Proc. Natl Acad. Sci. USA* **103**, 16242–16247 (2006).
117. Khandelia, H. & Kaznessis, Y. N. Molecular dynamics simulations of helical antimicrobial peptides in SDS micelles: what do point mutations achieve? *Peptides* **26**, 2037–2049 (2005).
This study demonstrates the use of molecular dynamics simulations to define how point mutations influence peptide–membrane interactions.
118. Tsai, C. W. *et al.* Coupling molecular dynamics simulations with experiments for the rational design of indolicidin-analogous antimicrobial peptides. *J. Mol. Biol.* **392**, 837–854 (2009).
119. Pande, J., Szewczyk, M. M. & Grover, A. K. Phage display: concept, innovations, applications and future. *Biotechnol. Adv.* **28**, 849–858 (2010).
120. Schneider, G. & Böhm, H. J. Virtual screening and fast automated docking methods. *Drug Discov. Today* **7**, 64–70 (2002).
121. Engel, T. Basic overview of chemoinformatics. *J. Chem. Inf. Model.* **46**, 2267–2277 (2006).
122. Schneider, G. & Baringhaus, K. H. *Molecular Design: Concepts and Applications*. (Wiley-VCH, Weinheim, 2008).
123. Schneider, G. & Wrede, P. Artificial neural networks for computer-based molecular design. *Prog. Biophys. Mol. Biol.* **70**, 175–222 (1998).
124. Klyger, Y. Computational approaches to therapeutic peptide discovery. *Biopolymers* **94**, 701–710 (2010).
125. Hiss, J. A., Hartenfeller, M. & Schneider, G. Concepts and applications of "natural computing" techniques in *de novo* drug and peptide design. *Curr. Pharm. Des.* **16**, 1656–1665 (2010).
This is an authoritative review of stochastic optimization methods for peptide and drug design, highlighting nature-inspired algorithms.
126. Hohm, T., Limbourg, P. & Hoffmann, D. A multi-objective evolutionary method for the design of peptidic mimotopes. *J. Comput. Biol.* **13**, 113–125 (2006).
127. Belda, I., Madurga, S., Tarragó, T., Llorà, X. & Giralt, E. Evolutionary computation and multimodal search: a good combination to tackle molecular diversity in the field of peptide design. *Mol. Divers.* **11**, 7–21 (2007).
128. Foster, J. A. Evolutionary computation. *Nature Rev. Genet.* **2**, 428–436 (2001).
129. Schneider, G., Schuchhardt, J. & Wrede, P. Development of simple fitness landscapes for peptides by artificial neural filter systems. *Biol. Cybern.* **73**, 245–254 (1995).
130. Schneider, G. & Schüller, A. Adaptive combinatorial design of focused compound libraries. *Methods Mol. Biol.* **572**, 135–147 (2009).
131. Hartenfeller, M. & Schneider, G. *De novo* drug design. *Methods Mol. Biol.* **672**, 299–323 (2011).
132. Schneider, G. *et al.* Voyages to the (un)known: adaptive design of bioactive compounds. *Trends Biotechnol.* **27**, 18–26 (2009).
133. Kortagere, S., Krasowski, M. D. & Ekins, S. The importance of discerning shape in molecular pharmacology. *Trends Pharmacol. Sci.* **30**, 138–147 (2009).
134. Ebalunode, J. O., Zheng, W. & Tropsha, A. Application of QSAR and shape pharmacophore modeling approaches for targeted chemical library design. *Methods Mol. Biol.* **685**, 111–133 (2011).
135. Hellberg, S., Sjostrom, M., Skagerberg, B. & Wold, S. Peptide quantitative structure–activity relationships, a multivariate approach. *J. Med. Chem.* **30**, 1126–1135 (1987).
136. Lejon, T., Strom, M. B. & Svendsen, J. S. Antibiotic activity of pentadecapeptides modelled from amino acid descriptors. *J. Pept. Sci.* **7**, 74–81 (2001).
137. Lejon, T., Stiberg, T., Strom, M. B. & Svendsen, J. S. Prediction of antibiotic activity and synthesis of new pentadecapeptides based on lactoferricins. *J. Pept. Sci.* **10**, 329–335 (2004).
138. Strøm, M. B., Stensen, W., Svendsen, J. S. & Rekdal, O. Increased antibacterial activity of 15-residue murine lactoferricin derivatives. *J. Pept. Res.* **57**, 127–139 (2001).
139. Jenssen, H., Gutteberg, T. J. & Lejon, T. Modeling of anti-HSV activity of lactoferricin analogues using amino acid descriptors. *J. Pept. Sci.* **11**, 97–103 (2005).
140. Frecer, V. QSAR analysis of antimicrobial and haemolytic effects of cyclic cationic antimicrobial peptides derived from protegrin-1. *Bioorg. Med. Chem.* **14**, 6065–6074 (2006).
141. Ostberg, N. & Kaznessis, Y. Protegrin structure–activity relationships: using homology models of synthetic sequences to determine structural characteristics important for activity. *Peptides* **26**, 197–206 (2004).
142. Todeschini, R. & Consonni, V. *Handbook of Molecular Descriptors* (Wiley-VCH, Weinheim, 2002).
143. Walters, W. P. & Goldman, B. B. Feature selection in quantitative structure-activity relationships. *Curr. Opin. Drug Discov. Devel.* **8**, 329–333 (2005).
144. González, M. P., Terán, C., Saíz-Urra, L. & Teijeira, M. Variable selection methods in QSAR: an overview. *Curr. Top. Med. Chem.* **8**, 1606–1627 (2008).
145. Jenssen, H. Descriptors for antimicrobial peptides. *Expert Opin. Drug Discov.* **6**, 171–184 (2011).
146. Marasco, D., Perretta, G., Sabatella, M. & Ruvo, M. Past and future perspectives of synthetic peptide libraries. *Curr. Protein Pept. Sci.* **9**, 447–467 (2008).
147. Wimley, W. C. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem. Biol.* **5**, 905–917 (2010).
148. Lewenza, S. *et al.* Construction of a mini-Tn5-luxCDABE mutant library in *Pseudomonas aeruginosa* PAO1: a tool for identifying differentially regulated genes. *Genome Res.* **15**, 583–589 (2005).
149. Cherkasov, A. *et al.* Use of artificial intelligence in the design of small peptide antibiotics effective against a broad spectrum of highly antibiotic-resistant superbugs. *ACS Chem. Biol.* **4**, 65–74 (2009).
This study used peptide array methods to make thousands of nine-amino-acid peptides that were then used to train neural network QSAR methods, enabling the identification and synthesis of peptides with broad-spectrum activity that were effective in systemic models of infections.
150. Fjell, C. D. *et al.* Identification of novel antibacterial peptides by chemoinformatics and machine learning. *J. Med. Chem.* **52**, 2006–2015 (2009).
151. Fjell, C. D., Jenssen, H., Cheung, W. A., Hancock, R. E. W. & Cherkasov, A. Optimization of antibacterial peptides by genetic algorithms and cheminformatics. *Chem. Biol. Drug. Des.* **77**, 48–56 (2011).
152. Butler, M. S. & Cooper, M. A. Antibiotics in the clinical pipeline in 2011. *J. Antibiot.* **64**, 413–425 (2011).
153. Velden, W. J. F. M. V. D., van Iersel, T. M. P., Blijlevens, N. M. A. & Donnelly, J. P. Safety and tolerability of the antimicrobial peptide human lactoferrin 1–11 (hLF1–11). *BMC Med.* **7**, 44 (2009).
154. Giles, F. J. *et al.* A Phase III, randomized, double-blind, placebo-controlled, study of iseganan for the reduction of stomatitis in patients receiving stomatotoxic chemotherapy. *Leuk. Res.* **28**, 559–565 (2004).
155. Lipsky, B. A., Holroyd, K. J. & Zasloff, M. Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. *Clin. Infect. Dis.* **47**, 1537–1545 (2008).
156. Onaizi, S. A. & Leong, S. S. Tethering antimicrobial peptides: current status and potential challenges. *Biotechnol. Adv.* **29**, 67–74 (2011).
157. Hilpert, K. *et al.* Screening and characterization of surface-tethered cationic peptides for antimicrobial activity. *Chem. Biol.* **16**, 58–69 (2009).
158. van de Waterbeemd, H. & Gifford, E. ADMET *in silico* modelling: towards prediction paradise? *Nature Rev. Drug Discov.* **2**, 192–204 (2003).
159. Zhou, J. Z. Chemoinformatics and library design. *Methods Mol. Biol.* **685**, 27–52 (2011).
160. Choudhary, A. & Raines, R. T. An evaluation of peptide-bond isosteres. *Chembiochem.* **12**, 1801–1807 (2011).
161. Wipf, P., Xiao, J. & Stephenson, C. R. Peptide-like molecules (PLMs): a journey from peptide bond isosteres to Gramicidin S mimetics and mitochondrial targeting agents. *Chimia* **63**, 764–775 (2009).
162. Desai, T. R., Wong, J. P., Hancock, R. E. W. & Finlay, W. H. A novel approach to the pulmonary delivery of liposomes in dry powder form to eliminate the deleterious effect of milling. *J. Pharm. Sci.* **91**, 482–491 (2002).
163. Robinson, J. A. *et al.* Properties and structure–activity studies of cyclic β-hairpin peptidomimetics based on the cationic antimicrobial peptide protegrin I. *Bioorg. Med. Chem.* **13**, 2055–2064 (2005).
164. Yang, C., Valerio, L. G. Jr & Arvidson, K. B. Computational toxicology approaches at the US Food and Drug Administration. *Altern. Lab. Anim.* **37**, 523–531 (2009).
165. Dearden, J. C. *In silico* prediction of ADMET properties: how far have we come? *Expert Opin. Drug Metab. Toxicol.* **3**, 635–639 (2007).
166. Valerio, L. G. Jr. *In silico* toxicology for the pharmaceutical sciences. *Toxicol. Appl. Pharmacol.* **241**, 356–370 (2009).
167. Agrafiotis, D. K. Multiobjective optimization of combinatorial libraries. *Mol. Divers.* **5**, 209–230 (2002).
168. Gillet, V. J. New directions in library design and analysis. *Curr. Opin. Chem. Biol.* **12**, 372–378 (2008).
169. Lill, M. A. Multi-dimensional QSAR in drug discovery. *Drug Discov. Today* **12**, 1013–1017 (2007).
170. Prado-Prado, F. J. *et al.* Unified QSAR approach to antimicrobials. 4. Multi-target QSAR modeling and comparative multi-distance study of the giant components of antiviral drug–drug complex networks. *Bioorg. Med. Chem.* **17**, 569–575 (2009).
171. Nicolaou, C. A., Apostolakis, J. & Pattichis, C. S. *De novo* drug design using multiobjective evolutionary graphs. *J. Chem. Inf. Model.* **49**, 295–307 (2009).
172. Fischer, J. R., Lessel, U. & Rarey, M. LoFT: similarity-driven multiobjective focused library design. *J. Chem. Inf. Model.* **50**, 1–21 (2010).
173. Ekins, S., Mestres, J. & Testa, B. *In silico* pharmacology for drug discovery: applications to targets and beyond. *Br. J. Pharmacol.* **152**, 21–37 (2007).
174. Pirogova, E., Istivan. T., Gan, E. & Cosic, I. Advances in methods for therapeutic peptide discovery, design and development. *Curr. Pharm. Biotechnol.* **12**, 1117–1127 (2011).
175. Liskamp, R. M., Rijkers, D. T., Kruijtzer, J. A. & Kemmink, J. Peptides and proteins as a continuing exciting source of inspiration for peptidomimetics. *Chembiochem.* **12**, 1626–1653 (2011).
176. Mason, J. M. Design and development of peptides and peptide mimetics as antagonists for therapeutic intervention. *Future Med. Chem.* **2**, 1813–1822 (2010).
177. Matsuzaki, K. Control of cell selectivity of antimicrobial peptides. *Biochim. Biophys. Acta* **1788**, 1687–1692 (2009).
178. Rathinakumar, R., Walkenhorst, W. F. & Wimley, W. C. Broad-spectrum antimicrobial peptides by rational combinatorial design and high-throughput screening: the importance of interfacial activity. *J. Am. Chem. Soc.* **131**, 7609–7617 (2009).

179. Díaz, M. D. *et al.* Structural framework for the modulation of the activity of the hybrid antibiotic peptide cecropin A-melittin [CA(1–7)M(2–9)] by N^{ε}-lysine trimethylation. *Chembiochem.* **12**, 2177–2183 (2011).
180. Torrent, M., Valle, J., Nogués, M. V., Boix, E. & Andreu, D. The generation of antimicrobial peptide activity: a trade-off between charge and aggregation? *Angew. Chem. Int. Ed. Engl.* **123**, 10686–10689 (2011).
This is an elegant study linking the structural features of peptides with antimicrobial activity.
181. Epand, R. F., Mor, A. & Epand, R. M. Lipid complexes with cationic peptides and OAKs; their role in antimicrobial action and in the delivery of antimicrobial agents. *Cell. Mol. Life Sci.* **68**, 2177–2188 (2011).
182. Halsey, C. M. *et al.* Simultaneous observation of peptide backbone lipid solvation and α-helical structure by deep-UV resonance Raman spectroscopy. *Chembiochem.* **12**, 2125–2128 (2011).
183. Gordon, Y. J., Romanowski, E. G., McDermott, A. M. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr. Eye Res.* **30**, 505–515 (2005).
184. Hancock, R. E. W. Cationic antimicrobial peptides: towards clinical application. *Exp. Opin. Invest. Drugs* **9**, 1723–1729 (2000).
185. Sakata, T. & Winzeler, E. A. Genomics, systems biology and drug development for infectious diseases. *Mol. Biosyst.* **3**, 841–848 (2007).
186. Koza, J. R. *Genetic Programming: On the Programming of Computers by Means of Natural Selection (Complex Adaptive Systems)* (MIT Press, Cambridge, Massachusetts, 1993).
187. Schneider, G. & Wrede, P. The rational design of amino acid sequences by artificial neural networks and simulated molecular evolution: *de novo* design of an idealized leader peptidase cleavage site. *Biophys. J.* **66**, 335–344 (1994).
188. Rechenberg, I. *Evolutionsstrategie: Optimierung Technischer Systeme Nach Prinzipien der Biologischen Evolution* (Frommann-Holzboog, Stuttgart, 1973).
189. Kaufmann, S. A. *The Origins of Order: Self-Organization and Selection in Evolution* (Oxford University Press, New York, 1993).
190. Schwefel, H. P. *Evolution and Optimum Seeking* (Wiley & Sons, New York, 1995).
191. Holland J. H. *Adaptation in Natural and Artificial Systems: An Introductory Analysis with Applications to Biology, Control and Artificial Intelligence (Complex Adaptive Systems)* (MIT Press, Cambridge, 1992).
192. Huang, H. W. Molecular mechanism of antimicrobial peptides: the origin of cooperativity. *Biochim. Biophys. Acta* **1758**, 1292–1302 (2006).
193. Berman, H. M. *et al.* The Protein Data Bank. *Nucleic Acids Res.* **28**, 235–242 (2000).
194. Landon, C., Meudal, H., Boulanger, N., Bulet, P. & Vovelle, F. Solution structures of stomoxyn and spinigerin, two insect antimicrobial peptides with an α-helical conformation. *Biopolymers* **81**, 92–103 (2006).
195. Phillips, J. C. *et al.* Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **26**, 1781–1802 (2005).
196. Brooks, R. R. *et al.* CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *J. Comp. Chem.* **4**, 187–217 (1983).
197. Ward, J. H. Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* **58**, 236–244 (1963).
198. Tomii, K. & Kanehisa, M. Analysis of amino acid indices and mutation matrices for sequence comparison and structure prediction of proteins. *Protein Eng.* **9**, 27–36 (1996).
199. Agrafiotis, D. K. *et al.* Stochastic proximity embedding: methods and applications. *Mol. Inf.* **29**, 758–770 (2010).
200. Reutlinger, M. *et al.* Visualization of adaptive structure–activity relationship landscapes in drug discovery. *Angew. Chem. Int. Ed.* **50**, 1–5 (2011).
201. Wrede, P. *et al.* Peptide design aided by neural networks: biological activity of artificial signal peptidase I cleavage sites. *Biochemistry* **37**, 3588–3593 (1998).
202. Schneider, G. *et al.* Peptide design by artificial neural networks and computer-based evolutionary search. *Proc. Natl Acad. Sci. USA* **95**, 12179–12184 (1998).
Together with reference 187, this study provided new strategies for applying computational machine-learning methods to the design of peptides using virtually any quantifiable measurement of peptide activity.
203. Klepeis, J. L., Floudas, C. A., Morikis, D., Tsokos, C. G. & Lambris, J. D. Design of peptide analogues with improved activity using a novel *de novo* protein design approach. *Ind. Eng. Chem. Res.* **43**, 3817–3826 (2004).
204. Belda, I. *et al.* ENPDA: an evolutionary structure-based *de novo* peptide design algorithm. *J. Comput. Aided Mol. Des.* **19**, 585–601 (2005).
205. Belda, I., Llorà, X. & Giralt, E. Evolutionary algorithms and *de novo* peptide design. *Soft Comput.* **10**, 295–304 (2006).
206. Yagi, Y., Terada, K., Noma, T., Ikebukuro, K. & Sode, K. *In silico* panning for a non-competitive peptide inhibitor. *BMC Bioinformatics* **8**, 11 (2007).
207. Oren, E. E. *et al.* A novel knowledge-based approach to design inorganic-binding peptides. *Bioinformatics* **23**, 2816–2822 (2007).
208. Hiss, J. A. *et al.* Design of MHC I stabilizing peptides by agent-based exploration of sequence space. *Protein Eng. Des. Sel.* **20**, 99–108 (2007).
209. Gronwald, W., Hohm, T. & Hoffmann, D. Evolutionary pareto-optimization of stably folding peptides. *BMC Bioinformatics* **9**, 109 (2008).
210. Kodama, T., Ashitani, J., Matsumoto, N., Kangawa, K. & Nakazato, M. Ghrelin treatment suppresses neutrophil-dominant inflammation in airways of patients with chronic respiratory infection. *Pulm. Pharmacol. Ther.* **21**, 774–779 (2008).
211. Travis, S. *et al.* RDP58 is a novel and potentially effective oral therapy for ulcerative colitis. *Inflamm. Bowel Dis.* **11**, 713–719 (2005).
212. Kristensen, H. H. *et al.* Plectasin, a fungal defensin, targets the bacterial cell wall precursor lipid II. *Science* **328**, 1168–1172 (2010).
This was a paradigm-shifting paper regarding a recombinantly made fungal defensin with promising preclinical data and *in vivo* efficacy. This study demonstrated that potent AMPs do not need to disrupt lipid membranes but can work by inhibiting cell wall biosynthesis.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.

FURTHER INFORMATION
Robert E. W. Hancock's homepage: http://www.cmdr.ubc.ca/bobh
Gisbert Schneider's homepage: http://www.modlab.ethz.ch
AMPer — A Database And An Automated Discovery Tool For Gene-Encoded Antimicrobial Peptides: http://marray.cmdr.ubc.ca/cgi-bin/amp.pl
The Antimicrobial Sequences Database: http://www.bbcm.univ.trieste.it/~tossi/amsdb.html
The Antimicrobial Peptide Database and Analysis System: http://aps.unmc.edu/AP/main.html
BACTIBASE Database: http://bactibase.pfba-lab-tun.org
CAMP — Collection of Antimicrobial Peptides: http://www.bicnirrh.res.in/antimicrobial
ClinicalTrials.gov website: http://clinicaltrials.gov
COPE — Cytokines & Cells Online Pathfinder Encyclopedia: http://www.copewithcytokines.org
DEFENSINS Knowledgebase: http://defensins.bii.a-star.edu.sg
Genzyme website — RDP58 (delmitide) partnering overview: http://www.genzyme.com/corp/licensing/RDP58_Non-Confidential_Overview_01Mar2007.pdf
Helix BioMedix website (anti-infective program): http://helixbiomedix.com/antiinfective.html
Inimex Pharmaceuticals website: http://www.inimexpharma.com/prod_tech_profile.html
Novozymes website (27 May 2010 press release): http://www.novozymes.com/en/news/news-archive/Pages/45873.aspx
PenBase: http://penbase.immunaqua.com
Peptaibol Database: http://peptaibol.cryst.bbk.ac.uk/home.shtml
PhytAMP — A database dedicated to plant antimicrobial peptides: http://phytamp.pfba-lab-tun.org
PolyMedix website — PMX-30063 Antibiotic Factsheet: http://www.polymedix.com/pdf/PMX-30063factsheet.pdf
Recombinantly Produced Antimicrobial Peptides Database: http://faculty.ist.unomaha.edu/chen/rapd
XOMA website (24 July 2008 press release): http://www.xoma.com/company/news-events/press-releases/index.cfm?releaseID=324334

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