Host defense (antimicrobial) peptides

10

Evelyn Sun, Corrie R. Belanger*, Evan F. Haney and Robert E.W. Hancock*
University of British Columbia, Vancouver, BC, Canada

10.1 Overview of host defense peptides

The increasing threat of antibiotic resistance and emergence of multidrug-resistant bacteria in hospital- and community-acquired infections is a growing medical concern. In 2014, the World Health Organization released a global report on antimicrobial resistance emphasizing the increasing threat posed by resistant bacterial, parasitic, viral, and fungal pathogens and suggested that a postantibiotic era may be on the horizon [1]. Subsequently, in 2016 the United Nations recognized the threat posed by antimicrobial resistance to human health, development, and global stability, and committed to foster innovative ways to address this global threat [2]. One promising antiinfective approach is the use of antimicrobial peptides (AMPs). These are short polypeptides found in all species of complex life including plants, insects, crustaceans, and animals (including humans), and are integral components of their innate immune systems [3,4]. Originally appreciated for their direct antimicrobial activity against planktonic bacteria [5], natural AMPs have also been shown to have potent immunomodulatory functions both in vitro and in vivo [5]. Therefore, we prefer to use the term host defense peptide (HDP) to describe these molecules to better reflect the broad range of biological activities that they mediate.

Individual HDPs can exhibit a wide range of activities that are uniquely determined, but often overlapping within a single molecule. These activities encompass various functions including direct antimicrobial activity towards bacteria, viruses, and fungi, antibiofilm activity as well as a variety of immunomodulatory functions. Here we summarize the different types of activities that have been observed for natural and synthetic HDPs, and highlight current and future applications of these multifaceted molecules with a particular emphasis on their potential use as novel antiinfective agents.

* These authors contributed equally to this work.

Peptide Applications in Biomedicine, Biotechnology and Bioengineering.
DOI: http://dx.doi.org/10.1016/B978-0-08-100736-5.00010-7

10.2 General features of HDPs

Natural HDPs are typically 12−50 amino acid residues long, possess a net positive charge (due to an abundance of Arg and Lys residues), and have a substantial proportion of hydrophobic residues (usually >50%) which allows them to fold into amphipathic conformations [6]. The primary and secondary structures of both synthetic and natural HDPs vary widely. Consequently, the relationships between structure of HDPs and their functions are exceptionally complex and of considerable current interest in peptide research, prompting extensive structure−activity relationship studies. Generally, these secondary structures, often induced upon contact with cell membranes, have been used to classify peptide families, though some peptides have multiple structural features and can be associated with more than one category. The majority of HDPs have been classified into four major structural groups defined as α-helical (e.g., magainin, cecropins, and certain cathelicidins), β-stranded (e.g., α- and β-defensins and polyphemusins), extended (e.g., indolicin), and looped structures (e.g., thanatin) [7,8].

The need for new antimicrobial and antiinfective, immunomodulating agents and the increased throughput of new peptide design methods [9] is encouraging the development of HDPs to treat recalcitrant and resistant infections. The different functional categories of peptides and their uses are discussed here.

10.3 Host defense peptides as immunomodulators

Many HDPs were originally found to demonstrate direct antimicrobial activity in dilute medium in vitro, and this direct activity against pathogens was long considered their primary biological role in vivo. However, at the high salt and glycosaminoglycan concentrations normally encountered in vivo, the typical concentrations of natural peptides, such as the human cathelicidin LL-37 that is released from epithelial cells and neutrophils at sites of infection (2−5 μg/mL), have no direct antimicrobial effects [10]. (NB: certain peptides may be present at very high concentrations, e.g., defensins in the intestinal crypts and in the vicinity of degranulating neutrophils, and thus may have meaningful antimicrobial activities.) Importantly, many of these peptides exhibit immunomodulatory activities under in vivo-like conditions (e.g., tissue culture medium) at concentrations that are physiologically meaningful and much lower than those necessary for direct antimicrobial activity [11]. For example, LL-37 can selectively modulate inflammatory responses in macrophages, lung epithelial cells, peripheral blood mononuclear cells (PBMCs), and whole blood leukocytes by dampening Toll-like receptor (TLR) responses, modulating mitogen-activated protein kinase (MAPK) pathways, and tumor necrosis factor (TNF) and interleukin (IL) responses [12,13]. Indeed, many natural HDPs exhibit potent immunomodulatory properties and we consider this to be the primary role of these molecules in vivo [3,4]. In keeping with this, virtually all known immunomodulatory activities have been demonstrated to operate in vivo [4].

Within normal tissues and fluids, HDPs are thought to mainly function as signaling mediators involved in innate immune defenses and their interactions with various immune cells and host molecules allow them to be multifunctional under diverse circumstances, including playing important roles in various diseases and inflammatory conditions [3]. Synthetic peptide mimics termed innate defense regulator (IDR) peptides have also been demonstrated with overlapping activities, although it is becoming increasingly clear that not all peptides have identical activities. Importantly, a peptide named IDR-1 was shown to have absolutely no antimicrobial activity even in dilute medium, but protected against infection in animal models by upregulating immune cell recruitment while dampening proinflammatory responses [14].

Generally, HDPs interact with and act on many different target cell types including monocytes, macrophages, dendritic cells (DCs), epithelial cells, neutrophils, and keratinocytes (Fig. 10.1) [4]. Such peptides can freely translocate across the plasma membrane of cells and/or interact with membrane receptors such as G-protein-coupled receptors, purinorceptor 7 (P2X7), IL receptor CXCR2, or TLRs [14,15]. After membrane translocation, many HDPs bind to intracellular receptors, such as GAPDH or SQSTM1, and stimulate a variety of signal transduction pathways important in the innate immune response including the p38, Erk1/2, and JNK MAP-kinases, NFκB, PI3-kinase, two Src family kinases, TRIF-IRF, TREM pathways, as well as autophagy [12,16]. This signaling can lead to downstream activation of various transcription factors such as NFκB, Creb, IRF4, AP-1, AP-2, Are, E2F1, SP1, Gre, Elk, PPARγ, STAT3, etc., and result in the potential dysregulation of more than 900 host genes directly, and even more indirectly [3,12]. Thus, the action of HDPs, like innate immunity itself, is very complex. It is therefore somewhat expected that HDPs can influence a diverse range of innate immune responses such as selectively modulating innate immunity, dampening bacterially induced proinflammatory cytokines, enhancing the production and release of various chemokines and cytokines, recruiting innate and adaptive immune cells, promoting wound healing, suppressing or increasing apoptosis, influencing angiogenesis, causing mast cell degranulation, and promoting and causing polarization of downstream adaptive immune responses. Some of the known mechanisms of immunomodulatory activity of HDPs are summarized in Fig. 10.1.

10.3.1 Effects of HDPs on inflammatory responses

Inflammation is a natural local response to injury allowing the body to kill foreign microbes and then heal by eliminating the cause of cell damage as well as clearing damaged cells and necrotic tissue [17]. However, excessive inflammation is pathological and dysregulation of inflammation is a feature of virtually every human disease [4]. Inflammation is usually triggered by an event, such as infection or local tissue damage, which stimulates the production of various factors that chemoattract immune cells, promote the loosening of blood vessel walls to ease the passage of immune cells from the blood (diapedesis/extravasation), promote activation of these immune cells, and promote nonopsonic phagocytosis and fibrin

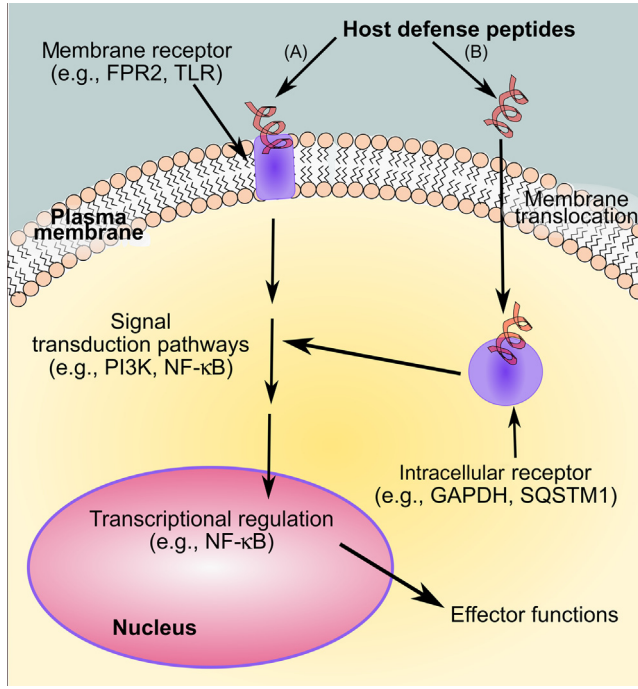

Figure 10.1 Host defense peptides interact with neutrophils or macrophages by (A) triggering membrane receptors such as G-protein-coupled receptor *N*-formyl peptide receptor 2 (FPR2) or Toll-like receptors (TLR) or (B) by spontaneously translocating across the membrane. Intracellularly localized HDPs can target receptors such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and sequestosome 1 (SQSTM1) or signal transduction pathways such as phosphoinositide 3-kinase pathway (PI3K) and nuclear factor-κB pathway (NF-κB), to ultimately alter transcriptional regulation patterns and effect a multitude of pathways in the cell involved in various immunomodulatory functions.

clot formation in an attempt to locally contain any infectious agent. Immune cells involved in inflammatory responses initially include resident tissue macrophages, dendritic cells (DCs), and recruited neutrophils, and subsequently monocytes and lymphocytes. Activation of diverse signaling pathways within these cells leads to the transcription of early response genes encoding for numerous proteins including chemokines, cytokines, acute-phase proteins, cell adhesion molecules, costimulatory molecules, negative feedback proteins, and, of course, HDPs [12].

Natural HDPs, such as LL-37, are neither pro- nor antiinflammatory but rather selectively modulate inflammatory mechanisms. Thus HDPs can enhance inflammation by enhancing the production of chemokines, influencing diapedesis, polarizing macrophage and DC differentiation, and promoting enhanced phagocytosis, or act directly as chemokines attracting neutrophils and other immune cells [4]. At the same time, HDPs exhibit antiinflammatory effects by dampening proinflammatory

cytokine responses through multiple mechanisms, such as inducing antiinflammatory cytokines, MAPK, and PI3-kinase signaling pathway responses, and blocking LPS binding to receptor proteins and LPS-binding protein [12,18].

In attempts to mimic the modulation of innate immunity by natural HDPs, many synthetic peptides have been developed which retain these key biological functions. OPR-145, for example, is a derivative of LL-37 that was designed to maintain the antimicrobial and antiinflammatory activity of LL-37 while increasing the stability against proteolytic degradation. This peptide has been through phase I/II clinical trials for the treatment of otitis media and was found to decrease the production of IL-8 in whole blood samples stimulated by UV-killed *Staphylococcus aureus*, an activity that was proposed to be linked to the peptide's ability to bind bacterial cell wall components and block activation of receptors on macrophages [19].

Another class of synthetic HDPs with inflammatory effects is the IDR peptides. IDR-1, -2, -1018, and -HH2 are derivatives of linear bactenecin, Bac2a, and all have demonstrated in vivo immunomodulatory activity in a variety of animal models of infection and inflammation [14−16,20]. IDR-1 enhanced the production of chemokines involved in clearing *S. aureus*, vancomycin-resistant *Enterococcus* and *Salmonella* infections and suppressed the production of proinflammatory cytokines in mice, while having no direct antibacterial activity [14]. IDR-1002, -1018, and -HH2 were further refined from the same template peptide, and all have been found to have increased antiinfective, antiinflammatory, and wound-healing activity compared to IDR-1 [14−16,20,21]. Indeed, collectively they have shown activity in animal models versus Gram-negative and Gram-positive infections, cerebral malaria, and LPS-hypoxia ischemia-mediated brain injury (a preterm birth model). The role of HDPs/IDRs in pro- and antiinflammatory functions can involve many different pathways and processes, and researchers are actively studying them as antiinfective treatments [22−24]. However, it is worth pointing out that the influence of HDPs on the inflammatory response has also spurred interested in developing these agents as treatments for various inflammatory diseases such as arthritis, chronic obstructive pulmonary disease (COPD), and asthma [25,26].

10.3.2 HDPs can exhibit direct chemoattractant activity

Although many of the anti- or proinflammatory activities of natural and synthetic peptides are linked to their indirect recruitment of immune cells, certain natural HDPs can also directly chemoattract immune cells as observed for LL-37, cathelin-related antimicrobial peptide (CRAMP), and defensins [27−30]. Furthermore, some researchers propose that the structural similarities between chemokines and antimicrobial peptides may help elucidate the structure−function relationships that allows HDPs to have chemoattractant properties. Both are amphipathic cations, and in many cases have shared mechanisms in humans [31]. Defensins, in particular, share various characteristics with chemokines, including size, structure, disulfide bonds, interferon-inducing properties, and overall cationic charge [32]. Human β-defensins 2 and 3, and mouse β-defensin 4 are able to chemoattract keratinocytes; and α-defensins are chemotactic for human monocytes and mast cells [28,33]. Indeed,

structural studies of human β-defensin 2 demonstrate that it shares a CCR6 receptor with chemokine CCL-20 and can induce chemotaxis of dendritic cells and monocytes by targeting the CCR6 and CCR2 receptors, respectively [32].

10.3.3 HDPs promote wound healing and angiogenesis

Wound healing involves multiple steps from inflammation to regeneration [34] and is another complex process that can be enhanced by HDPs. Initially, after an injury to the skin, vascular permeability is increased and platelet and fibrin aggregation occurs. This is quickly followed by the release of several growth factors from platelets that attract neutrophils to the wound and induce inflammation. Eventually, macrophages replace neutrophils as the primary inflammatory cells and debris is removed from the wound followed by reorganization of extracellular matrices [34]. HDPs are often found around oral and cutaneous wounds, and they have been shown to play various roles in promoting wound healing including reducing the bacterial burden, increasing neutrophil and macrophage recruitment, interacting with growth factors, inducing chemotaxis of epithelial cells, promoting production of metalloproteases that restructure the extracellular matrix, and promoting angiogenesis [4,18,35]. Some of the specific processes mediated by HDPs are highlighted below.

During the initial stages of wound repair, inflammation, and proliferation, the production of natural HDPs can be induced in wounded keratinocytes by growth factors [24,36]. The presence of LL-37 at wound sites has been shown to induce migration and proliferation of fibroblasts, human microvascular endothelial cells, and human umbilical vein endothelial cells [36]. Defensins, and particularly human β-defensin 2, promote keratinocyte migration and proliferation through the phosphorylation of the epidermal growth factor (EGF) receptor and activation of STAT1 and STAT3, which are important mediators of immunity, proliferation, apoptosis, and differentiation [33,37].

In the next stages of wound healing, angiogenesis, vascularization, and reepithelialization occur, all of which can be significantly impacted by HDPs [18,24]. For example, HB-107 is a fragment of the α-helical moth peptide, cecropin B, that has no direct antimicrobial activity at physiological concentrations, but can increase leukocyte infiltration and simulate IL-8 secretion from endothelial cells, leading to improved wound repair [23]. Recently, another synthetic peptide, angiogenic peptide 30 (AG-30) and its derivatives, AG-30/5C and SR-0379, were also studied for their angiogenic, wound healing, and antimicrobial functions [38,39]. Experiments in vitro showed that SR-0379 promoted vessel formation, granulated tissue formation, and proliferation of endothelial cells and fibroblasts in rats. SR-0379 was also found to be antiinfective against *Escherichia coli*, *S. aureus*, and *Pseudomonas aeruginosa* in vitro and offered protection against *S. aureus* in rat infection models [39].

The wound-healing properties of natural and synthetic HDPs are proving beneficial in a clinical context as well. For instance, LL-37 treatment enhanced wound healing of hard-to-treat venous leg ulcers compared to placebos in phase I/II randomized clinical trials [22]. Also, the synthetic peptide IDR-1018 significantly improved healing in mouse and pig wounds and had enhanced activity compared to

LL-37 or HB-107. This peptide was found to induce superior wound closure in these models and was less toxic against human keratinocytes and primary human fibroblasts, while having no effect on bacterial colonization [24]. Taken together, it is evident that natural and synthetic HDPs have high potential as wound-healing agents and their inclusion in wound dressings and ointments to prevent wound-associated infections and enhance wound closure are areas of active therapeutic development [40].

10.3.4 The roles of HDPs in autophagy, apoptosis, and oxidative stress

Autophagy is a natural process used by cells to recycle dysfunctional cellular components and preserve cellular energy. This process involves the sequestration of cellular components into vesicles called autophagosomes that fuse with lysosomes to hydrolyze and recycle cytosolic materials, and it relies on signaling pathways and highly conserved *Atg* genes [41]. It is often activated by infections of intracellular organisms such as *Mycobacterium tuberculosis*, and therefore can be considered a part of the innate defense mechanism [6]. Alternatively, cells can undergo apoptosis, which is a process of programmed cell death wherein caspases cleave hundreds of target proteins, cells shrink, bleb, and degrade DNA, RNA, and other cellular material [41]. These two modes of cellular death are believed to be highly interconnected and certain HDPs are known to influence these natural cellular degradation pathways.

Peptide IDR-1018 was demonstrated to rescue the dysfunctional autophagy associated with cystic fibrosis cells and consequently attenuate the typical hyperinflammatory responses exhibited by these cells. In particular, treatment of CF epithelial cells with IDR-1018 abolished the accumulation of LC3 (indicating stalled autophagy) induced by the bacterial inflammatory mediator flagellin. Conversely, LL-37 can induce autophagy in neutrophils through nucleotide scavenging receptor P2X7 and G-protein-coupled receptors, and can promote autophagy by activating Beclin-1 and Atg5 in a vitamin D3-dependent manner [42,43].

Cellular apoptosis can be promoted by LL-37 [44] through the activation of caspases 3 and 9 in the airway epithelium infected with *P. aeruginosa* to promote pathogen clearance [42]. The opposite has also been found in keratinocytes where LL-37 suppresses caspase-3 activity, potentially through upregulation of cyclooxygenase-2 (COX-2) expression, ultimately leading to the upregulation of inhibitor of apoptosis-2 (IAP-2) [45]. Similarly, LL-37 inhibits neutrophil cell death increasing their longevity, while suppressing inflammatory activity [46], although other data indicate the induction of apoptosis in neutrophils [47].

Evidently, the influence of natural HDPs on autophagy and apoptosis is complex and has only recently become appreciated. However, the potential to promote cell death through the use of autophagy or apoptosis inducing synthetic HDPs, could potentially be used to treat infection by promoting clearance of infected (or cancerous) cells, or by triggering connected immunomodulatory signaling pathways.

10.3.5 Modulation of the adaptive immune response by HDPs

Adaptive immunity is an acquired immune response utilizing specialized cells that respond to antigen exposure by rearrangement of specific genes encoding recognition elements and enhancing specific antibody production and cell-mediated immunity. Cells required in cellular immunity include antigen-presenting cells, specialized lymphocytes including helper T-cells (Th1 and Th2) and B-cells involved in antigen-specific recognition and response to eliminate pathogens while retaining immunological memory to prevent reinfection [48]. While their primary role in vivo appears to be modulation of innate immunity, HDPs have been shown to play multiple roles in adaptive immune responses such as promoting adjuvant responses to enhance adaptive immunity by directing immune functions towards Th1, Th2, or mixed Th1/Th2 responses, which depend on the class of helper T-cells activated and aiding in monocyte uptake of antigens [18].

Research has demonstrated that cells overexpressing defensins promote a strong Th1 response and induce cytotoxic T-cells, NK activity, and IL-12 and IFN-γ production in mice, while increasing their protection against leukemia and tumor cells [49]. Alternatively, defensins have been found to promote Th2-type responses by indirectly inducing IL-5 and IL-10 secretion in mice intranasally treated with ovalbumin and human defensins [50]. A synthetic peptide, $KLKL_5KLK$, was also found to induce a Th2-specific activity in response to coinjected antigen ovalbumin in vaccinated mice [51]. However, this same peptide used as an adjuvant in mice vaccinated against *M. tuberculosis* also showed improved and prolonged Th1 responses with increased IFN-γ-producing cells and antigen-specific IgG compared to vaccine without adjuvant [52].

Due to their mixed influences on adaptive immunity, the use of HDPs as adjuvants in vaccine formulations in order to produce balanced cytokine and antibody profiles from both Th1- and Th2-type cells is of increasing interest. For example, the natural peptides, melittin, mouse CRAMP, LL-37, and defensins have all been found to produce mixed Th1 and Th2 responses in adaptive immunity [53–56]. To utilize this effect, Kovacs-Nolan et al. formulated vaccine adjuvant combinations of CpG oligodeoxynucleotides (ODN), polyphosphazine, and indolicidin which showed improved antigen-specific humoral responses and extended cell immune responses in vaccinated cattle [57]. More recently, vaccine formulations containing synthetic IDR peptides IDR-HH2, -HH18, or -1002 along with combinations of CpG ODN and polyphosphazenes were also found to improve adaptive immune responses in mice stimulated with detoxified pertussis toxoid (PTd). Specifically, adjuvant combinations of CpG with peptides exhibited earlier IgG2a responses in both neonatal and adult mice than any of the adjuvants alone and resulted in substantially enhanced, and more protective, Th1/Th2 responses than those to PTd without adjuvant or with traditional adjuvant formulations [58]. As a result of this research multiple IDR peptides are now being developed in adjuvant formulations for cattle vaccines. These examples support the idea that HDPs may play additional roles in mediating the adaptive immune response, expanding their role beyond key components of the innate immune system.

10.4 Direct antimicrobial activities of HDPs

Evidently, as previously mentioned, HDPs were originally studied as antimicrobial peptides (AMPs), and many of them exhibit notable direct antimicrobial activity at high concentrations in vitro and can reduce bacterial burden from pathogenic infections in vivo [59] (although the latter activities may stem in part or whole from immune modulation as discussed earlier). Mechanistically, membrane disruption is probably the most studied mechanism of direct AMP activity. However, over the years, new mechanisms of action have been proposed, such as targeting intracellular processes including synthesis of DNA, RNA, and proteins as well as inhibiting cell wall biosynthesis, cell surface structures, and cell division machinery [60–63]. Modern perspectives view HDPs as highly versatile and multifunctional with the potential to exhibit activity against multiple microbial targets.

10.4.1 Bacterial cell membrane disruption by HDPs

Important differences between microbial and eukaryotic cell surfaces allow AMPs to be selective even in complex environments. Microbial cell surfaces, for instance, are more negatively charged due to the abundance of anionic lipids, whereas eukaryotic surfaces are rich in zwitterionic lipids resulting in an overall reduced negative charge [64]. Therefore, cationic HDPs can distinguish between prokaryotic and eukaryotic cell surfaces through preferential electrostatic interactions [8]. Other features of bacteria such as their high transmembrane electrical potential gradient (oriented internal negative) and lack of cholesterol also favor membrane interaction and/or translocation. After interacting with a target cell surface, HDPs can either directly kill a cell through a lytic mechanism or by entering the cell where they interact with various intracellular targets and inhibit key cellular processes. In our experience virtually all HDPs can disrupt membranes at high enough concentrations but many act at concentrations that do not completely disrupt membranes.

Numerous models have been proposed to describe how peptides disrupt the cell membrane and a brief summary of the most commonly described membrane interaction models is provided here [7,8]. The barrel stave model (Fig. 10.2A) posits that a peptide-lined pore forms in which the hydrophobic face of the peptides directly interacts with the acyl chains in the phospholipid core while the hydrophilic side of the peptides faces towards a water-filled pore. It should be mentioned that the barrel stave model is a highly discussed mechanism of membrane destabilization but is not well supported by the data [65]. The toroidal pore model (Fig. 10.2B) is analogous to the barrel-stave model in that peptides align themselves perpendicular to the membrane with their hydrophobic regions interacting with phospholipid heads and hydrophilic regions facing the pore [7]. However, in this case, the inner and outer leaflets of the phospholipid bilayer curve towards each other and mix, creating a peptide–lipid-lined aqueous pore that allows for leakage of cellular components. Examples of peptides that have been proposed to induce such pores include: magainins, protegrins, and melittin [66–68], although

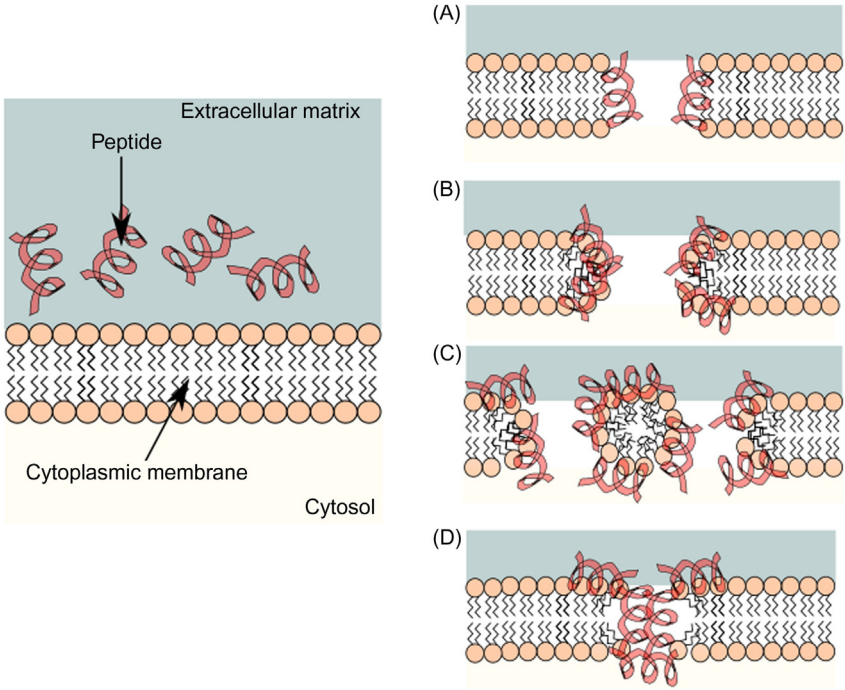

Figure 10.2 Models of peptide-induced membrane disruption or interfacial activity. (A) Barrel-stave model. Peptide forms a pore by inserting perpendicular into the membrane. (B) Toroidal pore model. Peptides form a pore where the hydrophilic regions face the interior of the pore and the hydrophobic regions remain in contact with the phospholipid headgroups. (C) Carpet model. Peptides dissolve the membrane into micelle-like structures in a detergent-like manner. (D) Aggregate model. Peptides aggregate at the membrane surface and reorient to interact with phospholipid acyl chains. This can lead to the formation of informal pores as the peptide aggregates enter the bacterial cell.

molecular dynamic simulations suggest that the actual pore structure is more disordered [69] than the oligomeric form proposed in many cartoon representations. The carpet model is used to describe the activity of HDPs that disrupt the membrane in a detergent-like manner by disintegrating it into micelles resulting in leakage and cell death (Fig. 10.2C) [70]; although this model is largely applicable only at high peptide concentrations [7,8]. Finally, our group proposed the aggregate model (Fig. 10.2D) wherein peptide oligomers aggregate on the surface of a bilayer in a concentration- and voltage-dependent manner [59]. These aggregates interact with the acyl chains of the phospholipid bilayer and can induce the formation of informal and transient aqueous channels that would allow for leakage of cellular component. Importantly, this model can be applied to peptides of all lengths and not only those that are long enough to span the width of the membrane. It also offers a mechanism to describe how some peptides can translocate into cells to reach their intracellular targets [59].

Although models of interfacial activity for peptides such as these have been extensively described in the literature, they are limited to experimentally testable situations in artificial model membrane systems (e.g., vesicles), which poorly reflect natural systems [71]. However, it is generally accepted that membrane interactions with HDPs are a crucial component of their overall mechanism of action and studying these interactions remains an active area of research in the field. In the following sections, we will outline some of the nonmembrane targets of HDPs and examine how they contribute to the overall antibacterial activities of this class of peptides.

10.4.2 Inhibition of cell wall formation by HDPs

The cell wall of Gram-positive bacteria is characterized by a thick mesh of peptidoglycan (PG) that has a major role in cell shape and osmotic stability and is synthesized by enzymes that are targeted by β-lactam antibiotics [72]. PG synthesis involves a cytoplasmic membrane-associated intermediate termed lipid II [73]. Certain HDPs interfere with PG biosynthesis, e.g., by binding of mammalian defensins, fungal defensin plectasin [74], and highly modified bacterially derived lantibiotics like nisin to lipid II [61]. For example, the amide backbone of the N-terminal ring of nisin forms hydrogen bonds with the pyrophosphate moiety of lipid II [61]. This leads to the inhibition of PG synthesis but also triggers the assembly of nisin in the membrane triggering pore formation and the efflux of ions such as K^+ and PO_4^{3-} as well as ATP [75,76]. In addition, by nisin binding to lipid III and lipid IV it interferes with teichoic and lipoteichoic acid biosynthesis [77].

Lantibiotics have been highly studied for their potential uses in food preservation and, indeed, nisin has been commonly used as a preserving agent for many years [78]. Lantibiotics have also garnered interest as potential pharmaceuticals to treat pathogenic infections. Examples include Nai-107 produced by Sentinella Phamaceuticals or MU1140 and OG253 produced by Oragenics [79]. Nai-107 has been shown to be effective against meticillin-resistant *S. aureus* (MRSA) and other multidrug-resistant bacteria and is currently being tested to treat Gram-negative bacterial infections via intravenous administration [80]. MU1140 works well against highly resistant *S. aureus* and *Streptococcus pneumoniae*, and is currently being tested to treat infections by these particular bacterial species [81]. OG253 is a promising lantibiotic that has shown preclinical efficacy against *Clostridium difficile* infections and enteritis in animal models [82].

Synthetic peptides composed of natural amino acids have also been suggested to target the cell envelope of Gram-positive bacteria. A proteomics approach was recently used to examine the shift in protein expression of *Bacillus subtilis* cells treated with a synthetic hexapeptide (RWRWRW-NH_2, called MP196) revealing upregulation of a number of proteins that were representative of cell-envelope stress and energy limitation [83]. Interestingly, the bactericidal activity of MP196 was not related with the formation of pores or ion leakage as found for several other HDPs. Instead, treatment of cells with MP196 caused displacement of cytochrome C from the outer leaflet of the membrane, thereby disrupting the electron transport chain

and limiting energy production within cells. Additionally, MP196 treatment caused the peripheral membrane protein MurG, the enzyme in *B. subtilis* responsible for converting intermediates lipid I to lipid II, to delocalize from the membrane resulting in decreased glucosamine attachment and reduced cell-wall integrity [83]. Thus certain HDPs have the ability to directly inhibit cell wall biosynthesis either by directly interacting with cell-wall precursors or interfering with enzymes and energy sources required to assemble cell wall components. It is worth noting, however, that peptides often have complex mechanisms of action with multiple targets [84].

10.4.3 Antimicrobial HDPs targeting intracellular processes

As mentioned above, most cationic amphipathic peptides will disrupt membranes at sufficiently high concentrations and many "mechanistic" studies do not always examine concentrations around the minimal inhibitory concentrations. In contrast, many HDPs have been shown to enter bacterial cells without excessive membrane disruption, at their minimal effective doses, and to target intracellular processes such as DNA or RNA synthesis, protein translation, protein folding, or various enzymatic reactions [85]. For example, Bac7 is a bovine proline-rich cathelicidin peptide that enters bacteria by interacting with an ABC transporter, SbmA, on the surface of the cell followed by an intracellular interaction with DnaK which is a chaperone for ATPase [86,87]. This, in turn, results in the accumulation of misfolded proteins and reduction of cellular viability [87]. Other peptides that target intracellular processes include the hexapeptide WRWYCR, which targets DNA repair mechanisms by binding to Holliday junctions [88]; buforin II, which diffuses through the plasma membrane to bind RNA and DNA [89]; and polyphemusin, which enters *E. coli* cells without disrupting the membrane [90].

Other peptides both perturb the cytoplasmic membrane and target intracellular processes depending on the concentrations applied. Indolicidin, for example, can cause both membrane depolarization and inhibition of DNA synthesis [91,92], whereas other peptides, including the cathelicidins LL-37 and apidaecin, can inhibit DNA and/or protein synthesis without disrupting the membrane [93]. Additionally, the enzymatic targets of some HDPs can have additional effects benefitting the host aside from directly killing the invading pathogen. For instance, histatin 5, a natural HDP found in saliva, prevents tissue destruction in the mouth by inhibiting the activity of proteases produced by oral bacteria [94].

10.5 Methods of bacterial resistance to HDPs

Although resistance mechanisms to conventional antibiotics are often more readily attained, bacteria are able to develop resistance HDPs as well. There are two major types of resistance mechanisms against peptides: inducible and constitutive (Fig. 10.3). Inducible resistance includes substitutions and modifications like the arabinosaminylation and acylation of membrane molecules, overproduction of

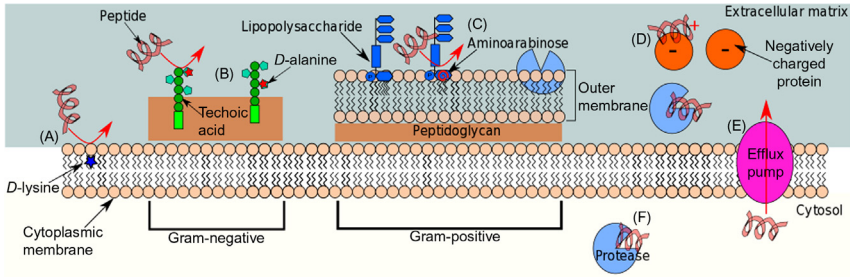

Figure 10.3 Mechanisms of resistance against HDPs. (A) Substituting D-lysine on phospholipid acyl chains. (B) Modifying teichoic acid with D-alanine in Gram-positive bacteria. (C) Modification of LPS by adding aminoarabinose (or glucosamine of phosphoethanolamine) to lipid A of the LPS at the outer membrane of Gram-negative bacteria. (D) Inducing the secretion of negatively charged molecules by host cells into the environment to bind to cationic HDPs. (E) Secreting out HDPs via efflux. (F) Degrading HDPs using intracellular proteases.

proteolytic enzymes or efflux pumps, and modification of intracellular targets. Constitutive resistance includes electrostatic shielding, reductions in membrane potential, and biofilm formation [7].

10.5.1 Bacterial surface remodeling to inhibit binding

Since the initial step in HDP binding to a bacterial cell involves an electrostatic attraction, one way that bacteria have evolved to circumvent this is to remodel surface molecules to reduce their overall negative charge. Teichoic acid, found in the PG layer of Gram-positive bacteria, is normally polyanionic because of its many phosphate groups, but several Gram-positive bacteria can modify teichoic acid by incorporating D-alanine via ester bonds, partially neutralizing surface charge and reducing peptide binding (Fig. 10.2B) [95]. Additionally, the *S. aureus* protein, MprF, a peptide resistance factor, mediates the addition of L-lysine to the phospholipid phosphatidylglycerol components (Fig. 10.1A) in order to reduce the membrane's overall negative charge and affinity for cationic HDPs [96]. Under specific adaptive conditions (e.g., divalent cation deficiency, exposure to HDPs, etc.), Gram-negative bacteria can modify the membrane proximal lipid A moiety of surface lipopolysaccharide molecules by adding L-arabinosamine, glucosamine, or phosphoethanolamine groups to one or both phosphates of the lipid A, effectively reducing the negative charge from the phosphate moiety and preventing peptide uptake via the self-promoted uptake route (Fig. 10.2C) [97–99]. Gram-negatives can also alter the acylation of lipid A through palmitoyl transferase PagP, to confer further resistance [98]. While these mechanisms are adaptive (i.e., they occur due to specific growth conditions and revert when the conditions revert), they can become fixed by mutations that result in constitutive alteration of surface molecules, and the same mechanisms that make cells resistant to the polycationic lipopeptide antibiotic polymyxin generally lead to resistance to HDPs. For example,

chronic infections with *P. aeruginosa* can produce hexa-acylated lipid A molecules that contain palmitate and arabinosamine that make it resistant to certain HDPs [97].

10.5.2 Active efflux and degradation of HDPs

Two commonly used mechanisms by which bacteria resist antibiotics are active expulsion of compounds using efflux pumps and the use of specific enzymes to degrade or inactivate them. These mechanisms are not common for HDPs but have been described for some of these molecules. Examples of HDP efflux mechanisms include (Fig. 10.3E) the MtrCDE efflux system in *Nesseria gonorrhoeae* [100] and the QacA efflux pump in *S. aureus* [101]. In addition proteases and peptidases produced by bacteria can often degrade and inactivate natural HDPs (Fig. 10.3F) [70,102]. For example, the human cathelicidin LL-37 can be degraded by a number of different bacterial proteases including gelatinase from *Enterococcus faecalis*, metalloprotease from *Proteus mirabilis*, and elastase from *P. aeruginosa* [103]. *Salmonella enterica* uses the virulence factor PgtE, an outer-membrane protease, to cleave and inactivate C18G and other synthetic α-helical AMPs [104]. Proteases can be present extracellularly [103], embedded in the outer membrane [104], or intracellular in the case of SapA-mediated degradation of cationic HDPs in *Haemophilus influenzae* [105] (Fig. 10.3F). It is also true that host proteases and peptidases can cause degradation of both synthetic and natural HDPs.

10.5.3 Additional HDP resistance mechanisms

Some bacteria can also have mechanisms to sequester peptides and inhibit their antibacterial activity (Fig. 10.3D). For example, *S. aureus* produces the exoprotein, staphylokinase, which activates host plasminogen to plasmin, a negatively charged enzyme which in turn directly binds to host α-defensins and prevents them from interacting directly with *S. aureus* cells [106]. Conversely, *P. aeruginosa* and *E. faecalis* secrete proteases that degrade proteoglycans triggering the release of anionic glycosaminoglycans, such as negatively charged dermatan sulfate, which bind to and inactivate α-defensins [103]. Bacteria can also interfere with the biosynthesis of HDPs by the host to decrease the effective concentration of HDP present in the vicinity of the pathogen. For example, *Shigella dysenteriae* downregulates the expression of LL-37 and β-defensin 1 during early infection in vitro [107] and *P. aeruginosa* can induce the accumulation of cysteine proteases secreted by macrophages in the airway to degrade β-defensin 2 and β-defensin 3 [108].

From a pharmacological perspective peptide resistance is not very common but these resistance mechanisms show they are possible and create a potential limitation for the future clinical use of peptides. Nevertheless, the discovery of these resistance mechanisms provides insight into HDP mechanisms and this might potentiate the development of novel HDPs. For instance, novel peptide designs can avoid the induction of adaptive resistance mechanisms in bacteria. Therefore, it is crucial to consider existing resistance mechanisms when designing HDPs as novel antimicrobial therapies.

10.6 Antibiofilm activities of HDPs

The term antimicrobial peptides refers to their activity against free-swimming (planktonic) bacteria. However, bacteria in nature often adopt a distinct growth lifestyle as biofilms. Biofilms are multicellular communities of bacteria encased in extracellular polymeric matrices composed of polysaccharides, proteins, and DNA, which allows them to adhere to various surfaces [109,110]. The physiological switch from planktonic to biofilm growth is accompanied by large adaptive changes in gene expression, including genes involved in stress response mechanisms and antibiotic resistance genes, and has been proposed to be the prominent reason that biofilms exhibit such high (10- to 1000-fold) adaptive resistance to antibiotics [109]. Clinically, it has been estimated that up to 65% of all human infections are caused by bacteria growing within biofilms [109] and their intrinsic resistance makes them highly recalcitrant to conventional antiinfective therapies. As a result, biofilms are often associated with various chronic infections such as pneumonia in cystic fibrosis (CF) patients [111], otitis media [112], chronic ulcers and wounds, as well as infections derived from biofilms growing on the surfaces of implanted medical devices and catheters [113]. Due to the tremendous clinical relevance and a paucity of antimicrobial compounds capable of treating biofilm-associated infections, identifying novel antibiofilm agents will be of great interest in the future.

In contrast to conventional antibiotics, many natural and synthetic HDPs are able to prevent biofilm formation as well as possess the ability to kill and eradicate preformed biofilms [114–116]. This phenomenon was first described for human LL-37, which inhibited *P. aeruginosa* biofilms at subinhibitory concentrations in vitro [117]. Subsequently it was found that antibiofilm agents were independently determined compared to antimicrobial activities against planktonic cells, since peptides with preferential activity against one or the other bacterial growth states were identified [118]. Critically antibiofilm peptides exhibit broad-spectrum activity versus Gram-positive and Gram-negative bacteria [114,115,118]. Since these initial observations, a number of other naturally occurring HDPs have been found to exhibit antibiofilm properties as well as including phylloseptin-1 from waxy monkey frogs [119,120], pleurocidin from winter flounder, and human β-defensin 3 [121]. Recently synthetic HDPs have also been identified with even more potent broad-spectrum antibiofilm activity often inhibiting or killing organisms in biofilms at concentrations as low as $\sim 1\ \mu g/mL$. For example, IDR-1018 and the D-enantiomeric peptides DJK-5 and DJK-6, have all been shown to possess potent broad-spectrum activity against preformed biofilms from *P. aeruginosa* and other antibiotic-resistant species [114,115]. Consistent with the lack of relationship between antibiofilm and antimicrobial activity, *Burkholderia cepacia* is completely resistant to the antimicrobial effects of HDPs when growing planktonically but highly susceptible to antibiofilm peptides when growing as biofilms [114,115]. Mechanistically, de la Fuente-Núñez et al. demonstrated that IDR-1018 and the DJK peptides work by intracellular targeting and promoting degradation of the stringent response signaling molecule, ppGpp, which plays an important role in bacterial biofilm formation [114,115].

Recently, synthetic HDPs have also been demonstrated to effectively reduce abscess size in a murine model of chronic, high-density bacterial infections [122]. Though bacteria within an abscess are generally considered to be physiologically different from those found within biofilms, both are strongly dependent on the stringent response, suggesting a strong mechanistic connection between the two growth phenotypes [122]. Importantly, DJK-5 [114], previously shown to possess potent antibiofilm activity in vitro, reduced the severity of abscesses formed by MRSA and *P. aeruginosa* in mice by significantly reducing tissue injury and lesion size by up to fourfold compared to controls [122]. Though this model is not precisely a direct mimic of a biofilm, peptides that can eradicate high-density abscess infections have also been shown to work in nonmammalian biofilm infection models [114].

Although the examples describing the antibiofilm activity of HDPs presented here are encouraging, their relatively high costs have spurred researchers to explore combination therapies of peptides with conventional antibiotics. Synergistic application of HDPs in combination with conventional antibiotics has been successfully demonstrated with the synthetic peptides DJK-5, DJK-6, and IDR-1018 in vitro. In these cases, the application of the synthetic peptide synergistically decreased the concentration of antibiotics such as ciprofloxacin, gentamicin, ceftazidime, and imipenem by up to 64-fold in treating biofilms formed by several clinically relevant pathogens [123,124]. Natural peptides, such as LL-37 and a cecropin−melittin hybrid (CAMA), have also shown synergy in vitro against *P. aeruginosa* biofilms, where combinations with ciprofloxacin decreased biofilm-eradication concentrations by four- and eightfold compared to single antibiotic treatments [125]. Taken together, these findings highlight the potential for HDPs to be used as standalone or adjunctive therapies to conventional antibiotics. Overall, the potent antibiofilm activity exerted by many synthetic HDPs is rapidly emerging as a treatment option for recalcitrant biofilm-associated infections and chronic abscesses.

10.6.1 Biofilm prevention using peptide-coated surfaces

Implanted medical devices, such as catheters or prosthetic valves, are often colonized by bacteria and this can lead to the formation of biofilms that require the removal of these devices and/or can cause severe infections recalcitrant to treatment by conventional antibiotics [126]. In fact, nearly 100,000 deaths per year in the United States are due to medical device-related infections largely due to the notorious (adaptive) antibiotic resistance of biofilms [127]. One possible method of preventing biofilm formation on implanted medical devices is to attach peptides to the surface of these devices either physically through adsorption or chemically through covalent bonding [126,127]. As an example, Lim et al. established CWR11-coated Foley catheters with effective antimicrobial and antibiofilm activities against *S. aureus*, *E. coli*, and *P. aeruginosa* which could prevent infections commonly associated with indwelling devices [126]. Tethering approaches to coat biomedical plastics with peptides have also been used to prevent bacterial adhesion of Gram-negative and Gram-positive organisms [128]. Recently, Yu et al. [128] used

branched polymers with multiple peptide covalent-attachment sites to increase the density of peptides on catheter surfaces. The catheters were tested in a mouse urinary catheter model, and the AMP-conjugated catheters significantly reduced the formation of *P. aeruginosa* biofilms as compared to uncoated catheters [128]. In addition, the peptide, hLf1-11 (Table 10.1), currently being clinically evaluated against nosocomial bacterial and *Candida* infections has been shown to exhibit effective antibiofilm activity against MRSA when covalently tethered to chitosan ultrathin film [151]. Chitosan is a naturally occurring polymer used in several medical devices used for gene therapy and as drug-delivery carriers [152]. Since biofilms play a major role in medical device-related infections, the use of tethered peptides is a promising new application to prevent medical device-related infections.

Ultimately, the immunomodulatory, antimicrobial, and antibiofilm functions of HDPs have the potential to be exploited for many applications to combat bacterial infections and biofilms, as well as modulate serious inflammatory diseases. Furthermore, the ability of many natural and synthetic HDPs to exhibit multiple activities allows them to have a multifaceted effect in many diseases, or synergy with conventional treatments, which makes them ideal candidates for antiinfective therapies. In this context, to improve on current HDP activities and increase future applications, research is focused on actively designing and developing novel synthetic HDPs with improved clinical potential.

10.7 Designing novel HDPs

Synthetic peptide design is of increasing interest as progressively more information on peptide structure and function becomes available. To be therapeutically relevant, the design of a synthetic peptide has to enhance a specific desired activity while exhibiting low cytotoxicity at the therapeutic dose as well as retaining favorable properties such as high solubility and stability. To design such optimized HDPs, three approaches are generally employed: template-based design, biophysical design, and computational design methods [9].

10.7.1 Template-based design

Template-based peptide synthesis relies on a template peptide with known activity and sequence [153]. This template peptide serves as the starting point to design novel peptide sequences in which various biophysical properties are altered such as charge, hydrophobicity, or amphipathicity. Researchers have been able to examine the importance of specific amino acids and residue positions on peptide activity by synthesizing derivatives with altered residues or functional motifs, truncated or deleted regions, or even scrambling the peptide sequence to determine functional residues and structural characteristics that contribute to the desired biological activity. Many peptide derivatives with enhanced antimicrobial activity have been made

Table 10.1 HDPs previously and currently in clinical trials and their proposed application in the context of specific diseases

Peptide name (company)	Clinical indication seeking approval	Clinical status	Most recent clinical trial ID and/or information	References
Omiganan (Cutanea Life Sciences)	Treatment against acne vulgaris, rosacea, genital warts, and vulvar intraepithelial neoplasia	Phase II/III		[129,130]
Surotomycin (MK-4261/CB-315) (Merck)	*Clostridium difficile*-associated diarrhea	Phase III	Clinical trial ID NCT01597505	[131,132]
Brilacidin or PMX-30063 (PolyMedix)	Acute *Staphylococcus aureus* skin infections	Phase II	Clinical Trial ID: NCT01211470	[133,134]
Novexatin/NP213 (NovaBiotics)	Fungal nail infections	Phase IIb		[135,136]
P-113/PAC-113 (Pacgen Biopharmaceuticals)	Gingivitis and oral candidiasis	Phase II	Clinical Trial ID: NCT00659971	[137]
DPK-060 (DermaGen)	Atopic dermatitis and acute external otitis	Phase II	Clinical Trial ID: NCT01447017	[138,139]
LTX-109 (Lytix Biopharma)	Topical treatment of bacterial impetigo	Phase II	Clinical Trial ID: NCT01803035	[140,141]
OP-145 (OctoPlus)	Otitis media: chronic middle ear infection	Phase II	Acquired by Dr Reddys Laboratories in 2013 Clinical Trial ID: ISRCTN84220089; ISRCTN12149720	[19,142]

(CKPV)2/CZEN-002 (Abiogen Pharma)	Treatment of urogenital conditions (vulvovaginal candidiasis)	Phase II	Clinical Trial ID: CN 1867349 A	[143]
Dusquetide/SGX-942/ IMX-942 (Soligenix)	Treatment of oral mucositis	Phase II	Recently passed phase II	[133,144,145]
Synthetic LL-37 (Lipopeptide AB)	Venous leg ulcers	Phase I/II	EU Clinical Trials Register: 2012-002100-41	[22]
Iseganan or IB-367 (Ardea Biosciences)	Oral mucositis for patients undergoing radiation therapy	Phase III	Discontinued as of 2004 due to health risks Clinical Trial ID: NCT0002233	[146,147]
hLf1-11 (Am-Pharma)	Nosocomial infections; systemic *Candida* infections; bone marrow transplantation patients	Phase II	Company suspended trials for strategic reasons Clinical Trial ID: NCT00509938	[68,148,149]
Plectasin NZ2114 (Novozymes)	Endocarditis associated with MRSA	Phase I	Discontinued in 2011	[74,150]

from natural HDPs using this approach, including derivatives of cecropins, magainins, protegrins, bactenicins, and cathelicidins [154–156].

This type of design strategy is often limited by the number of peptides that can be synthesized and screened for activity, however, this problem has been somewhat alleviated through the use of SPOT-synthesized peptide libraries synthesized on cellulose sheets [157]. This technology has allowed researchers to simultaneously evaluate the activity of hundreds of synthetic peptides at a fraction of the cost and has made it feasible to perform complete amino acid substitution screens on known HDP sequences. For example, 228 single amino acid variants of the linear HDP bactenecin, Bac2a, were SPOT-synthesized on cellulose peptide arrays and their antimicrobial activity was assessed against a luminescent *P. aeruginosa* lux strain. This approach permitted the identification of residues that were favored in the Bac2a sequence and that when combined together in next-generation HDP sequences resulted in peptides with improved antibacterial potency [158].

Synthetic HDPs with immunomodulatory activity have also served as templates for novel IDR peptides with potent immunomodulatory properties. IDR-1, -1018, and -1002 were developed from the Bac2a template using substitution methods [14,16,21]. Originally, IDR-1 was designed to contain sequence features that were incompatible with direct antimicrobial activity [14]. IDR-1018 and IDR-1002 were subsequently discovered to have enhanced immunomodulatory functions compared to IDR-1, as well as enhanced antibiofilm properties [16,21,116]. Derivatives of IDR-1002 and IDR-HH2 have also been optimized using SPOT-synthesized peptide arrays and high-throughput screening methods for various biological activities [159], demonstrating that such an approach could be used to further develop these IDR peptides to treat biofilm-associated chronic infections.

10.7.2 Structure-guided design

A second method of peptide design is the structure-guided method which uses models based on peptide structures determined in hydrophobic environments (similar to the structures that would be formed in membranes) and biophysical measurements, rather than properties associated with the primary amino acid sequence. Bactenecin, indolicidin, and protegrin have been investigated using structure-based design, molecular modeling, and biophysical studies in order to increase their broad-spectrum activity, stability, and elucidate mechanistic membrane interactions to improve their bacterial killing [59,160].

Molecular dynamic modeling can extend structure-guided approaches by using simulations to computationally represent the atoms in an HDP molecule and evaluate their interactions with the solvent conditions, membranes, and each other [9,161]. This information can then serve to inform next-generation peptide sequences that maximize these important biophysical interactions. As an example, molecular dynamic simulations were used to design indolicidin analogues with enhanced antimicrobial activity towards *E. coli* by increasing the charge density at the membrane interface by replacing proline with a lysine residue. At the same time, derivatives with decreased hemolytic activity were generated by replacing tryptophan with

phenylalanine to retain rigid but smaller aromatic rings as side chains, and decrease the overall disruption of the eukaryotic membrane interface [162].

10.7.3 Computational modeling of HDPs

Beyond simple structure and template-based modeling, complex computational peptide analysis is being used to model peptide activity based on quantified biological activities and features of the peptide structure. Such an approach is known as quantitative structure−activity relationship (QSAR) studies and uses large numbers of molecular descriptors that describe each individual peptide sequence and tries to model experimentally measured activities such as antibacterial potency using sophisticated machine learning techniques [9]. Feature selection to describe peptide activity and structure is a crucial step in computational modeling and is typically done automatically based on peptide data sets and statistical models of variable selection [163]. Ultimately, with any model used, feature selection is a tradeoff between predictability of the model and minimizing the necessary descriptors used. An ideal model has equal predictability with fewer descriptors to lower the computational workload and make it easier to interpret [164].

Virtual screening and random design of peptides rely on using numerical methods to determine quantifiable peptide descriptors to design and test peptide structures without the need for large high-throughput screening experiments. In 2009, Cherkasov et al. used available chemical biology information of small broad-spectrum peptides and test sets of randomly generated peptides using previously developed QSAR descriptor preferences to create models of antibiotic activity with artificial neural networks (based on previous Bac2a screens) [165]. A library of 100,000 virtual peptide sequences was scored and classified based on the QSAR models and the top 200 peptides with predicted activity were synthesized using SPOT technology and screened against the lux-*Pseudomonas*. Compared to the Bac2a control, 98% of the peptides predicted to have increased activity actually did, and two lead peptides, HHC-10 and HHC-36, were selected for further analysis revealing significant activity against a wide range of multidrug-resistant bacterial strains [165]. Current research is focused on using similar computation approaches to design novel peptides with specific antibiofilm or immunomodulatory properties and to associate these activities with various HDP structural characteristics.

10.8 The future of HDPs: From the bench to the clinic

Numerous preclinical studies, as discussed in this chapter, reveal the overwhelming potential and efficacy associated with the use of HDPs to treat microbial infections either by directly targeting planktonic cells, stimulating the immune system to mediate clearance of the infection, or by targeting complex growth adaptations like biofilms which exhibit broad-spectrum adaptive resistance against antibiotics. From all the studies and examples presented in this chapter, we can appreciate the

prospects of using HDPs in a clinical setting. Table 10.1 presents a nonexhaustive list of synthetic HDPs that are currently being tested for clinical use. Many peptides such as IMX-942 and a synthetic LL-37 peptide are in early stages of the development pipeline, completing phase I or II trials as antiinfectives [22,133,144]. Similarly there are multiple immunomodulatory peptides such as DPK-060 [138] and OP-145 [19] that are currently being tested (phase II) for the treatment of inflammatory ear infections known as otitis media. Although many of the peptides currently undergoing trials are being tested for topical use (e.g., DPK-060, Novexatin, Brilacidin, Omiganan [129]), HDP Surotomycin has reached phase III clinical trials for systemic treatment of *C. difficile* infections in order to prevent and alleviate associated bowel irritability [131]. The promising clinical applications of peptides are highlighted by the many peptides currently in the pipeline. However, challenges still arise that prevent peptides from continuing clinical testing (see notes on Iseganan, hLf1-11, and Plectasin). These challenges and corresponding solutions are discussed below.

10.8.1 Current commercialization challenges and potential solutions

One of the major limitations associated with HDP use is their inherent low stability in serum and their susceptibility to degradation by host proteases. The use of D-amino acids makes the peptides impervious to proteolytic degradation by bacterial or host proteases [60]. Such a strategy has successfully generated protease-resistant LL-37 derivatives as well as the highly active antibiofilm peptides, DJK-5 and DJK-6, and the angiogenic peptide SR-0379 [39,114].

Another major hurdle facing commercialization of peptides is their relatively short shelf-life and potential for degradation during storage. Several solutions for this have been proposed including chemical modification of functional groups within the peptide [166]. In addition, various formulation strategies have been proposed including loading peptides into various nanoparticles or encapsulating peptides into lipid vesicles [167].

To reduce the high costs of synthetic peptidse (up to \$50−400 mg^{-1}), researchers have favored the development of shorter peptides that are typically easier and cheaper to make. Truncation studies on peptides can reduce the number of residues, and often tryptophanyl substitutions at the hydrophobic−hydrophilic interface of the amphipathic helix are incorporated to maximize activity in shorter peptides [6,168]. Additionally, the use of recombinant fusion peptides allows higher yields of soluble proteins and is potentially a more cost-efficient alternative to solid-phase synthesis chemistry [154].

Peptide toxicity, a major issue in the development of HDPs clinically, has been poorly studied but might have limited peptides to being administered topically as opposed to systemically. Indeed, many of the peptides presently in clinical development are seeking approval for infections of the skin or other topical applications (Table 10.1). As an example, Iseganan was a synthetic protegrin analogue that was shown to be effective as an oral rinse to treat oral mucositis [169] but this peptide

failed phase III of clinical trials due to high systemic toxicity when used to treat ventricular-associated pneumonia [170]. Unfortunately, the relatively high levels of peptide needed to exhibit antiinfective activity also resulted in high eukaryotic cell toxicity and hemolysis [171]. Numerous studies have tried to address the issue of toxicity using the design approaches described above and our group previously established that there exists a positive correlation between peptide hydrophobicity and toxicity [172]. Therefore, by replacing amino acids with less hydrophobic residues or interrupting hydrophobic patches with basic groups, it may be possible to alleviate some of the prospective issues associated with nonspecific peptide toxicity [172], although it is unlikely that this generalization holds true for all HDP sequences. Alternatively, peptide toxicity could also be reduced by using specialized drug-delivery systems such as liposome-encapsulated peptides [168].

10.8.2 Final thoughts

Due to rising antibiotic resistance in pathogenic microbes around the world and a significant lack of antibiotics for treatment of multidrug-resistant infections, new treatments are needed that can replace and improve on current antibiotic therapies. HDPs have certain advantages over antibiotics because they have broad-spectrum activity while also employing multiple mechanisms of action, which makes them less likely to induce resistance in bacteria. Furthermore, HDPs have potent immunomodulatory activities, which is a feature that gives peptides a distinct edge over conventional antibiotics in terms of fighting infections and promoting healing [6,14,116]. Given that infections often trigger local inflammatory responses and can occur in the context of wounds, such activities are highly relevant to the treatment of chronic and biofilm infections. Similarly, the ability of these peptides to act against multiple bacterial species represents a potential advantage against mixed infections [6]. Furthermore, studies have found that combinations of HDPs and antibiotics can be used synergistically to treat infections such as biofilms, which allows for lower quantities of both agents to be used and exhibit increased efficacy against the infections being treated [116,123,128]. With improved screening methods and in silico testing, it is apparent that we are making great strides to identify and understand the properties of HDPs that allow them to exert their diverse range of biological activities. Armed with this information, the design of next-generation HDPs with enhanced biological activity profiles is a feasible goal and will permit future applications of HDPs to improve human health.

Acknowledgments

Our peptide research has been generously supported by grants from the Canadian Institutes for Health Research (CIHR) (Funding reference MOP-74493) and by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. C.R.B. received a CIHR Canada Graduate Master's Scholarship and a Cystic Fibrosis Canada Doctoral Studentship Award. R.E.W.H. holds a Canada Research Chair in Health and Genomics and a UBC Killam Professorship.

Abbreviations

ABP	antibiofilm peptide
AMP	antimicrobial peptide
COPD	chronic obstructive pulmonary disease
CRAMP	cathelin-related antimicrobial peptide
DC	dendritic cells
GlcNAc	β-1,4-linked *N*-acetylglucosamine
HDP	host defense peptide
IDR	innate defense regulator
IL	interleukin
LPS	lipopolysaccharide
MAPK	modulating mitogen-activated protein kinase
MRSA	methicillin resistant *Staphylococcus aureus*
MurNAc	*N*-acetylmuramic acid
ODN	oligodeoxynucleotides
PBMC	peripheral blood mononuclear cells
PG	peptidoglycan
PTd	pertussis toxoid
QSAR	quantitative structure−activity relationship.
ROS	reactive oxygen species
STAT	signal transducer and activator of transcription
TLR	Toll-like receptor
TNF	tumor necrosis factor

References

[1] WHO, Antimicrobial Resistance: Global Report on Surveillance, 2014, WHO, Geneva, Switzerland, 2014. <http://www.who.int/drugresistance/documents/surveillancereport/en/> (accessed 17.03.17).

[2] General Assembly of the United Nations, High-level meeting on antimicrobial resistance, September 21, 2016. <http://www.un.org/pga/71/2016/09/21/press-release-hl-meeting-on-antimicrobial-resistance/> (accessed 20.03.17).

[3] R.E.W. Hancock, E.F. Haney, E.E. Gill, The immunology of host defence peptides: beyond antimicrobial activity, Nat. Rev. Immunol. 16 (2016) 321−334.

[4] A.L. Hilchie, K. Wuerth, R.E.W. Hancock, Immune modulation by multifaceted cationic host defense (antimicrobial) peptides, Nat. Chem. Biol. 9 (2013) 761−768.

[5] D. Yang, O. Chertov, J.J. Oppenheim, Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37), J. Leukoc. Biol. 69 (2001) 691−697.

[6] S.C. Mansour, O.M. Pena, R.E.W. Hancock, Host defense peptides: front-line immunomodulators, Trends Immunol. 35 (2014) 443−450.

[7] A.A. Bahar, D. Ren, Antimicrobial peptides, Pharmaceuticals (Basel) 6 (2013) 1543−1575.

[8] L.T. Nguyen, E.F. Haney, H.J. Vogel, The expanding scope of antimicrobial peptide structures and their modes of action, Trends Biotechnol. 29 (2011) 464−472.

[9] C.D. Fjell, J.A. Hiss, R.E.W. Hancock, et al., Designing antimicrobial peptides: form follows function, Nat. Rev. Drug Discov. 11 (2011) 37−51.

[10] D.M.E. Bowdish, D.J. Davidson, Y.E. Lau, et al., Impact of LL-37 on anti-infective immunity, J. Leukoc. Biol. 77 (2005) 451–459.
[11] A. Nijnik, R. Hancock, Host defence peptides: antimicrobial and immunomodulatory activity and potential applications for tackling antibiotic-resistant infections, Emerg. Health Threats J. 2 (2009) e1. Available from: http://dx.doi.org/10.3134/ehtj.09.001.
[12] N. Mookherjee, K.L. Brown, D.M.E. Bowdish, et al., Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37, J. Immunol. 176 (2006) 2455–2464.
[13] S.M. Walters, V.S. Dubey, N.R. Jeffrey, et al., Antibiotic-induced *Porphyromonas gingivalis* LPS release and inhibition of LPS-stimulated cytokines by antimicrobial peptides, Peptides 31 (2010) 1649–1653.
[14] M.G. Scott, E. Dullaghan, N. Mookherjee, et al., An anti-infective peptide that selectively modulates the innate immune response, Nat. Biotechnol. 25 (2007) 465–472.
[15] A.H. Achtman, S. Pilat, C.W. Law, et al., Effective adjunctive therapy by an innate defense regulatory peptide in a preclinical model of severe malaria, Sci. Transl. Med. 4 (2012) 135ra64.
[16] A. Nijnik, L. Madera, S. Ma, et al., Synthetic cationic peptide IDR-1002 provides protection against bacterial infections through chemokine induction and enhanced leukocyte recruitment, J. Immunol. 184 (2010) 2539–2550.
[17] J. Vane, R. Botting, Inflammation and the mechanism of action of anti-inflammatory drugs, FASEB J. 1 (1987) 89–96.
[18] K.A. Brogden, A.M. Bates, C.L. Fischer, Antimicrobial peptides in host defense: functions beyond antimicrobial activity, in: J. Harder, J.-M. Schröder (Eds.), Antimicrobial Peptides, Springer International Publishing, Cham, 2016, pp. 129–146.
[19] N. Malanovic, R. Leber, M. Schmuck, et al., Phospholipid-driven differences determine the action of the synthetic antimicrobial peptide OP-145 on Gram-positive bacterial and mammalian membrane model systems, Biochim. Biophys. Acta 1848 (2015) 2437–2447.
[20] B. Rivas-Santiago, J.E. Castañeda-Delgado, C.E. Rivas Santiago, et al., Ability of innate defence regulator peptides IDR-1002, IDR-HH2 and IDR-1018 to protect against *Mycobacterium tuberculosis* infections in animal models, PLoS One 8 (2013) e59119.
[21] M. Wieczorek, H. Jenssen, J. Kindrachuk, et al., Structural studies of a peptide with immune modulating and direct antimicrobial activity, Chem. Biol. 17 (2010) 970–980.
[22] A. Grönberg, M. Mahlapuu, M. Ståhle, et al., Treatment with LL-37 is safe and effective in enhancing healing of hard-to-heal venous leg ulcers: a randomized, placebo-controlled clinical trial, Wound Repair Regen. 22 (2014) 613–621.
[23] P.H.A. Lee, J.A. Rudisill, K.H. Lin, et al., HB-107, a nonbacteriostatic fragment of the antimicrobial peptide cecropin B, accelerates murine wound repair, Wound Repair Regen. 12 (2004) 351–358.
[24] L. Steinstraesser, T. Koehler, F. Jacobsen, et al., Host defense peptides in wound healing, Mol. Med. 14 (2008) 528–537.
[25] W. Xiao, Y.-P. Hsu, A. Ishizaka, et al., Sputum cathelicidin, urokinase plasminogen activation system components, and cytokines discriminate cystic fibrosis, copd, and asthma inflammation, Chest 128 (2005) 2316–2326.
[26] L.N.Y. Chow, K.-Y. Choi, H. Piyadasa, et al., Human cathelicidin LL-37-derived peptide IG-19 confers protection in a murine model of collagen-induced arthritis, Mol. Immunol. 57 (2014) 86–92.
[27] D. Yang, Q. Chen, A.P. Schmidt, et al., LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor

to chemoattract human peripheral blood neutrophils, monocytes, and T cells, J. Exp. Med. 192 (2000) 1069–1074.
[28] M. Dürr, A. Peschel, Chemokines meet defensins: the merging concepts of chemoattractants and antimicrobial peptides in host defense, Infect. Immun. 70 (2002) 6515–6517.
[29] G.S. Tjabringa, D.K. Ninaber, J.W. Drijfhout, et al., Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors, Int. Arch. Allergy Immunol. 140 (2006) 103–112.
[30] S.-K. Zhang, J.-W. Song, F. Gong, et al., Design of an α-helical antimicrobial peptide with improved cell-selective and potent anti-biofilm activity, Sci. Rep. 6 (2016) 27394.
[31] L.T. Nguyen, H.J. Vogel, Structural perspectives on antimicrobial chemokines, Front. Immunol. 3 (2012) 384. Available from: http://dx.doi.org/10.3389/fimmu.2012.00384.
[32] J.M. Pérez-Cañadillas, A. Zaballos, J. Gutiérrez, et al., NMR solution structure of murine CCL20/MIP-3alpha, a chemokine that specifically chemoattracts immature dendritic cells and lymphocytes through its highly specific interaction with the beta-chemokine receptor CCR6, J. Biol. Chem. 276 (2001) 28372–28379.
[33] F. Niyonsaba, H. Ushio, N. Nakano, et al., Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines, J. Invest. Dermatol. 127 (2007) 594–604.
[34] T. Velnar, T. Bailey, V. Smrkolj, The wound healing process: an overview of the cellular and molecular mechanisms, J. Int. Med. Res. 37 (2009) 1528–1542.
[35] M.P. Caley, V.L.C. Martins, E.A. O'Toole, Metalloproteinases and wound healing, Adv. Wound Care 4 (2015) 225–234.
[36] R. Ramos, J.P. Silva, A.C. Rodrigues, et al., Wound healing activity of the human antimicrobial peptide LL37, Peptides 32 (2011) 1469–1476.
[37] M. Carretero, M.J. Escámez, M. García, et al., In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37, J. Invest. Dermatol. 128 (2008) 223–236.
[38] K. Kanazawa, K. Okumura, H. Ogawa, et al., An antimicrobial peptide with angiogenic properties, AG-30/5C, activates human mast cells through the MAPK and NF-κB pathways, Immunol. Res. 64 (2016) 594–603.
[39] H. Tomioka, H. Nakagami, A. Tenma, et al., Novel anti-microbial peptide SR-0379 accelerates wound healing via the PI3 kinase/Akt/mTOR pathway, PLoS One 9 (2014) e92597.
[40] M.L. Mangoni, A.M. McDermott, M. Zasloff, Antimicrobial peptides and wound healing: biological and therapeutic considerations, Exp. Dermatol. 25 (2016) 167–173.
[41] A. Thorburn, Apoptosis and autophagy: regulatory connections between two supposedly different processes, Apoptosis 13 (2008) 1–9.
[42] Z. Hu, T. Murakami, K. Suzuki, et al., Antimicrobial cathelicidin peptide LL-37 inhibits the LPS/ATP-induced pyroptosis of macrophages by dual mechanism, PLoS One 9 (2014) e85765.
[43] J.-M. Yuk, D.-M. Shin, H.-M. Lee, et al., Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin, Cell Host Microbe 6 (2009) 231–243.
[44] Y.E. Lau, D.M.E. Bowdish, C. Cosseau, et al., Apoptosis of airway epithelial cells: human serum sensitive induction by the cathelicidin LL-37, Am. J. Respir. Cell Mol. Biol. 34 (2006) 399–409.
[45] C.I. Chamorro, G. Weber, A. Grönberg, et al., The human antimicrobial peptide LL-37 suppresses apoptosis in keratinocytes, J. Invest. Dermatol. 129 (2009) 937–944.

[46] P.G. Barlow, Y. Li, T.S. Wilkinson, et al., The human cationic host defense peptide LL-37 mediates contrasting effects on apoptotic pathways in different primary cells of the innate immune system, J. Leukoc. Biol. 80 (2006) 509–520.
[47] S.M. Alalwani, J. Sierigk, C. Herr, et al., The antimicrobial peptide LL-37 modulates the inflammatory and host defense response of human neutrophils, Eur. J. Immunol. 40 (2010) 1118–1126.
[48] J.R. Dunkelberger, W.-C. Song, Complement and its role in innate and adaptive immune responses, Cell Res. 20 (2010) 34–50.
[49] X.-T. Ma, B. Xu, L.-L. An, et al., Vaccine with beta-defensin 2-transduced leukemic cells activates innate and adaptive immunity to elicit potent antileukemia responses, Cancer Res. 66 (2006) 1169–1176.
[50] J.W. Lillard, P.N. Boyaka, O. Chertov, et al., Mechanisms for induction of acquired host immunity by neutrophil peptide defensins, Proc. Natl. Acad. Sci. U S A. 96 (1999) 651–656.
[51] J.H. Fritz, S. Brunner, M.L. Birnstiel, et al., The artificial antimicrobial peptide KLKLLLLLKLK induces predominantly a TH2-type immune response to co-injected antigens, Vaccine 22 (2004) 3274–3284.
[52] M. Li, D.-H. Yu, H. Cai, The synthetic antimicrobial peptide KLKL5KLK enhances the protection and efficacy of the combined DNA vaccine against *Mycobacterium tuberculosis*, DNA Cell Biol. 27 (2008) 405–413.
[53] L.-L. An, Y.-H. Yang, X.-T. Ma, et al., LL-37 enhances adaptive antitumor immune response in a murine model when genetically fused with M-CSFR (J6-1) DNA vaccine, Leuk. Res. 29 (2005) 535–543.
[54] V.W. Bramwell, S. Somavarapu, I. Outschoorn, et al., Adjuvant action of melittin following intranasal immunisation with tetanus and diphtheria toxoids, J. Drug Target. 11 (2003) 525–530.
[55] K. Kurosaka, Q. Chen, F. Yarovinsky, et al., Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant, J. Immunol. 174 (2005) 6257–6265.
[56] S.V. Vemula, O.A.K. Amen, J.M. Katz, et al., Beta-defensin 2 enhances immunogenicity and protection of an adenovirus-based H5N1 influenza vaccine at an early time, Virus Res. 178 (2013) 398–403.
[57] J. Kovacs-Nolan, J.W. Mapletoft, L. Latimer, et al., CpG oligonucleotide, host defense peptide and polyphosphazene act synergistically, inducing long-lasting, balanced immune responses in cattle, Vaccine 27 (2009) 2048–2054.
[58] A. Gracia, M. Polewicz, S.A. Halperin, et al., Antibody responses in adult and neonatal BALB/c mice to immunization with novel *Bordetella pertussis* vaccine formulations, Vaccine 29 (2011) 1595–1604.
[59] M. Wu, R.E. Hancock, Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane, J. Biol. Chem. 274 (1999) 29–35.
[60] R.E.W. Hancock, H.-G. Sahl, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies, Nat. Biotechnol. 24 (2006) 1551–1557.
[61] D. Münch, H.-G. Sahl, Structural variations of the cell wall precursor lipid II in Gram-positive bacteria: impact on binding and efficacy of antimicrobial peptides, Biochim. Biophys. Acta 1848 (2015) 3062–3071.

[62] V.M. Hernández-Rocamora, C. Alfonso, W. Margolin, et al., Evidence that bacteriophage λ kil peptide inhibits bacterial cell division by disrupting FtsZ protofilaments and sequestering protein subunits, J. Biol. Chem. 290 (2015) 20325–20335.
[63] C.-F. Le, C.-M. Fang, S.D. Sekaran, Intracellular targeting mechanisms by antimicrobial peptides, Antimicrob. Agents Chemother. 61 (2017) e02340-16.
[64] K. Boesze-Battaglia, R. Schimmel, Cell membrane lipid composition and distribution: implications for cell function and lessons learned from photoreceptors and platelets, J. Exp. Biol. 200 (1997) 2927–2936.
[65] B. Leitgeb, A. Szekeres, L. Manczinger, et al., The history of alamethicin: a review of the most extensively studied peptaibol, Chem. Biodivers. 4 (2007) 1027–1051.
[66] T. Lazaridis, Y. He, L. Prieto, Membrane interactions and pore formation by the antimicrobial peptide protegrin, Biophys. J. 104 (2013) 633–642.
[67] Y. Tamba, M. Yamazaki, Magainin 2-induced pore formation in the lipid membranes depends on its concentration in the membrane interface, J. Phys. Chem. B. 113 (2009) 4846–4852.
[68] G. van den Bogaart, J.V. Guzmán, J.T. Mika, et al., On the mechanism of pore formation by melittin, J. Biol. Chem. 283 (2008) 33854–33857.
[69] D. Sengupta, H. Leontiadou, A.E. Mark, et al., Toroidal pores formed by antimicrobial peptides show significant disorder, Biochim. Biophys. Acta 1778 (2008) 2308–2317.
[70] F. Guilhelmelli, N. Vilela, P. Albuquerque, et al., Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance, Front. Microbiol. 4 (2013) 353.
[71] W.C. Wimley, Describing the mechanism of antimicrobial peptide action with the interfacial activity model, ACS Chem. Biol. 5 (2010) 905–917.
[72] X. Zeng, J. Lin, Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria, Front. Microbiol. 4 (2013) 128. Available from: http://dx.doi.org/10.3389/fmicb.2013.00128.
[73] D.-J. Scheffers, M.G. Pinho, Bacterial cell wall synthesis: new insights from localization studies, Microbiol. Mol. Biol. Rev. 69 (2005) 585–607.
[74] T. Schneider, T. Kruse, R. Wimmer, et al., Plectasin, a fungal defensin, targets the bacterial cell wall precursor lipid II, Science 328 (2010) 1168–1172.
[75] S.-T. Hsu, E. Breukink, E. Tischenko, et al., The nisin-lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics, Nat. Struct. Mol. Biol. 11 (2004) 963–967.
[76] I. Wiedemann, E. Breukink, C. van Kraaij, et al., Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity, J. Biol. Chem. 276 (2001) 1772–1779.
[77] A. Müller, H. Ulm, K. Reder-Christ, et al., Interaction of type A lantibiotics with undecaprenol-bound cell envelope precursors, Microb. Drug Resist. 18 (2012) 261–270.
[78] J. Cleveland, T.J. Montville, I.F. Nes, et al., Bacteriocins: safe, natural antimicrobials for food preservation, Int. J. Food Microbiol. 71 (2001) 1–20.
[79] G. Gallo, G. Renzone, E. Palazzotto, et al., Elucidating the molecular physiology of lantibiotic NAI-107 production in Microbispora ATCC-PTA-5024, BMC Genomics 17 (2016) 42.
[80] NAICONS, Nai-107 (and related lantibiotics). <http://www.naicons.com/products/nai-107.html> (accessed 17.03.17).
[81] O.G. Ghobrial, H. Derendorf, J.D. Hillman, Pharmacodynamic activity of the lantibiotic MU1140, Int. J. Antimicrob. Agents 33 (2009) 70–74.

[82] OGEN, OG253: Oragenics, Inc. <http://www.oragenics.com/technology-pipeline/lantibiotics/og253> (accessed 17.03.17).
[83] M. Wenzel, A.I. Chiriac, A. Otto, et al., Small cationic antimicrobial peptides delocalize peripheral membrane proteins, Proc. Natl. Acad. Sci. U S A. 111 (2014) E1409–1418.
[84] C.L. Friedrich, D. Moyles, T.J. Beveridge, et al., Antibacterial action of structurally diverse cationic peptides on gram-positive bacteria, Antimicrob. Agents Chemother. 44 (2000) 2086–2092.
[85] J.D.F. Hale, R.E.W. Hancock, Alternative mechanisms of action of cationic antimicrobial peptides on bacteria, Expert Rev. Anti Infect. Ther. 5 (2007) 951–959.
[86] M. Mattiuzzo, A. Bandiera, R. Gennaro, et al., Role of the *Escherichia coli* SbmA in the antimicrobial activity of proline-rich peptides, Mol. Microbiol. 66 (2007) 151–163.
[87] M. Scocchi, C. Lüthy, P. Decarli, et al., The proline-rich antibacterial peptide Bac7 binds to and inhibits *in vitro* the molecular chaperone dnak, Int. J. Pept. Res. Ther. 15 (2009) 147–155.
[88] C.W. Gunderson, J.L. Boldt, R.N. Authement, et al., Peptide wrwycr inhibits the excision of several prophages and traps holliday junctions inside bacteria, J. Bacteriol. 191 (2009) 2169–2176.
[89] C.B. Park, H.S. Kim, S.C. Kim, Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions, Biochem. Biophys. Res. Commun. 244 (1998) 253–257.
[90] J.-P.S. Powers, M.M. Martin, D.L. Goosney, et al., The antimicrobial peptide polyphemusin localizes to the cytoplasm of *Escherichia coli* following treatment, Antimicrob. Agents Chemother. 50 (2006) 1522–1524.
[91] A. Ghosh, R.K. Kar, J. Jana, et al., Indolicidin targets duplex DNA: structural and mechanistic insight through a combination of spectroscopy and microscopy, ChemMedChem 9 (2014) 2052–2058.
[92] Y.H. Nan, K.H. Park, Y. Park, et al., Investigating the effects of positive charge and hydrophobicity on the cell selectivity, mechanism of action and anti-inflammatory activity of a Trp-rich antimicrobial peptide indolicidin, FEMS Microbiol. Lett. 292 (2009) 134–140.
[93] M. Castle, A. Nazarian, S.S. Yi, et al., Lethal effects of apidaecin on *Escherichia coli* involve sequential molecular interactions with diverse targets, J. Biol. Chem. 274 (1999) 32555–32564.
[94] K. Konopka, B. Dorocka-Bobkowska, S. Gebremedhin, et al., Susceptibility of *Candida* biofilms to histatin 5 and fluconazole, Antonie Van Leeuwenhoek 97 (2010) 413–417.
[95] A. Peschel, C. Vuong, M. Otto, et al., The D-alanine residues of *Staphylococcus aureus* teichoic acids alter the susceptibility to vancomycin and the activity of autolytic enzymes, Antimicrob. Agents Chemother. 44 (2000) 2845–2847.
[96] C.M. Ernst, P. Staubitz, N.N. Mishra, et al., The bacterial defensin resistance protein MprF consists of separable domains for lipid lysinylation and antimicrobial peptide repulsion, PLoS Pathog. 5 (2009) e1000660.
[97] R.K. Ernst, K.N. Adams, S.M. Moskowitz, et al., The *Pseudomonas aeruginosa* Lipid a deacylase: selection for expression and loss within the cystic fibrosis airway, J. Bacteriol. 188 (2006) 191–201.
[98] K. Kawasaki, R.K. Ernst, S.I. Miller, Inhibition of *Salmonella enterica* serovar typhimurium lipopolysaccharide deacylation by aminoarabinose membrane modification, J. Bacteriol. 187 (2005) 2448–2457.

[99] A.J. McCoy, H. Liu, T.J. Falla, et al., Identification of *Proteus mirabilis* mutants with increased sensitivity to antimicrobial peptides, Antimicrob. Agents Chemother. 45 (2001) 2030–2037.
[100] A.E. Jerse, N.D. Sharma, A.N. Simms, et al., A gonococcal efflux pump system enhances bacterial survival in a female mouse model of genital tract infection, Infect. Immun. 71 (2003) 5576–5582.
[101] L.I. Kupferwasser, R.A. Skurray, M.H. Brown, et al., Plasmid-mediated resistance to thrombin-induced platelet microbicidal protein in staphylococci: role of the qacA locus, Antimicrob. Agents Chemother. 43 (1999) 2395–2399.
[102] V. Nizet, Antimicrobial peptide resistance mechanisms of human bacterial pathogens, Curr. Issues Mol. Biol. 8 (2006) 11–26.
[103] A. Schmidtchen, I.-M. Frick, E. Andersson, et al., Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37, Mol. Microbiol. 46 (2002) 157–168.
[104] T. Guina, E.C. Yi, H. Wang, et al., A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar typhimurium promotes resistance to alpha-helical antimicrobial peptides, J. Bacteriol. 182 (2000) 4077–4086.
[105] K.M. Mason, F.K. Raffel, W.C. Ray, et al., Heme utilization by nontypeable *Haemophilus influenzae* is essential and dependent on sap transporter function, J. Bacteriol. 193 (2011) 2527–2535.
[106] T. Jin, M. Bokarewa, T. Foster, et al., *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism, J. Immunol. 172 (2004) 1169–1176.
[107] D. Islam, L. Bandholtz, J. Nilsson, et al., Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator, Nat. Med. 7 (2001) 180–185.
[108] C.C. Taggart, C.M. Greene, S.G. Smith, et al., Inactivation of human beta-defensins 2 and 3 by elastolytic cathepsins, J. Immunol. 171 (2003) 931–937.
[109] C. de la Fuente-Núñez, F. Reffuveille, L. Fernández, et al., Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies, Curr. Opin. Microbiol. 16 (2013) 580–589.
[110] K. Poole, Stress responses as determinants of antimicrobial resistance in Gram-negative bacteria, Trends Microbiol. 20 (2012) 227–234.
[111] N. Høiby, O. Ciofu, T. Bjarnsholt, *Pseudomonas aeruginosa* biofilms in cystic fibrosis, Future Microbiol. 5 (2010) 1663–1674.
[112] L.O. Bakaletz, Bacterial biofilms in otitis media: evidence and relevance, Pediatr. Infect. Dis. J. 26 (2007) S17–S19.
[113] R.M. Donlan, Biofilm formation: a clinically relevant microbiological process, Clin. Infect. Dis. 33 (2001) 1387–1392.
[114] C. de la Fuente-Núñez, F. Reffuveille, S.C. Mansour, et al., D-enantiomeric peptides that eradicate wild-type and multidrug-resistant biofilms and protect against lethal *Pseudomonas aeruginosa* infections, Chem. Biol. 22 (2015) 196–205.
[115] C. de la Fuente-Núñez, S.C. Mansour, Z. Wang, et al., Anti-biofilm and immunomodulatory activities of peptides that inhibit biofilms formed by pathogens isolated from cystic fibrosis patients, Antibiotics (Basel) 3 (2014) 509–526.
[116] S.C. Mansour, C. de la Fuente-Núñez, R.E.W. Hancock, Peptide IDR-1018: modulating the immune system and targeting bacterial biofilms to treat antibiotic-resistant bacterial infections, J. Pept. Sci. 21 (2015) 323–329.

[117] J. Overhage, A. Campisano, M. Bains, et al., Human host defense peptide LL-37 prevents bacterial biofilm formation, Infect. Immun. 76 (2008) 4176–4182.
[118] C. de la Fuente-Núñez, V. Korolik, M. Bains, et al., Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide, Antimicrob. Agents Chemother. 56 (2012) 2696–2704.
[119] J.-K. Lee, S.W. Chang, H. Perinpanayagam, et al., Antibacterial efficacy of a human β-defensin-3 peptide on multispecies biofilms, J. Endod. 39 (2013) 1625–1629.
[120] R. Zhang, M. Zhou, L. Wang, et al., Phylloseptin-1 (PSN-1) from *Phyllomedusa sauvagei* skin secretion: a novel broad-spectrum antimicrobial peptide with antibiofilm activity, Mol. Immunol. 47 (2010) 2030–2037.
[121] R. Tao, Z. Tong, Y. Lin, et al., Antimicrobial and antibiofilm activity of pleurocidin against cariogenic microorganisms, Peptides 32 (2011) 1748–1754.
[122] S.C. Mansour, D. Pletzer, C. de la Fuente-Núñez, et al., Bacterial abscess formation is controlled by the stringent stress response and can be targeted therapeutically, EBioMedicine 12 (2016) 219–226.
[123] F. Reffuveille, C. de la Fuente-Núñez, S. Mansour, et al., A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms, Antimicrob. Agents Chemother. 58 (2014) 5363–5371.
[124] S.M. Ribeiro, C. de la Fuente-Núñez, B. Baquir, et al., Antibiofilm peptides increase the susceptibility of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates to β-lactam antibiotics, Antimicrob. Agents Chemother. 59 (2015) 3906–3912.
[125] S. Dosler, E. Karaaslan, Inhibition and destruction of *Pseudomonas aeruginosa* biofilms by antibiotics and antimicrobial peptides, Peptides 62 (2014) 32–37.
[126] K. Lim, R.R.Y. Chua, H. Bow, et al., Development of a catheter functionalized by a polydopamine peptide coating with antimicrobial and antibiofilm properties, Acta Biomater. 15 (2015) 127–138.
[127] S.A. Onaizi, S.S.J. Leong, Tethering antimicrobial peptides: current status and potential challenges, Biotechnol. Adv. 29 (2011) 67–74.
[128] J. Yu, N. Mookherjee, K. Wee, et al., Host defense peptide LL-37, in synergy with inflammatory mediator IL-1beta, augments immune responses by multiple pathways, J. Immunol. 179 (2007) 7684–7691.
[129] E. Rubinchik, D. Dugourd, T. Algara, et al., Antimicrobial and antifungal activities of a novel cationic antimicrobial peptide, omiganan, in experimental skin colonisation models, Int. J. Antimicrob. Agents 34 (2009) 457–461.
[130] T.R. Fritsche, P.R. Rhomberg, H.S. Sader, et al., Antimicrobial activity of omiganan pentahydrochloride against contemporary fungal pathogens responsible for catheter-associated infections, Antimicrob. Agents Chemother. 52 (2008) 1187–1189.
[131] C.T.M. Mascio, L.I. Mortin, K.T. Howland, et al., *In vitro* and *in vivo*characterization of cb-183,315, a novel lipopeptide antibiotic for treatment of *Clostridium difficile*, Antimicrob. Agents Chemother. 56 (2012) 5023–5030.
[132] BioCentury, Surotomycin (MK-4261) (formerly CB-183,315, CB-315). Product Profile. <http://bciq.biocentury.com/products/cb-183315> (accessed 16.03.17).
[133] R.E.W. Hancock, A. Nijnik, D.J. Philpott, Modulating immunity as a therapy for bacterial infections, Nat. Rev. Microbiol. 10 (2012) 243–254.
[134] B. Mensa, G.L. Howell, R. Scott, et al., Comparative mechanistic studies of brilacidin, daptomycin, and the antimicrobial peptide LL16, Antimicrob. Agents Chemother. 58 (2014) 5136–5145.
[135] S. Li, R.R. Breaker, Fluoride enhances the activity of fungicides that destabilize cell membranes, Bioorg. Med. Chem. Lett. 22 (2012) 3317–3322.

[136] NovaBiotics, NP213 (Novexatin®) is a novel cationic antifungal peptide. <http://www.novabiotics.co.uk/pipeline/novexatin-np213> (accessed 17.03.17).
[137] D.M. Rothstein, P. Spacciapoli, L.T. Tran, et al., Anticandida activity is retained in P-113, a 12-amino-acid fragment of histatin 5, Antimicrob. Agents Chemother. 45 (2001) 1367–1373.
[138] L. Boge, H. Bysell, L. Ringstad, et al., Lipid-based liquid crystals as carriers for antimicrobial peptides: phase behavior and antimicrobial effect, Langmuir 32 (2016) 4217–4228.
[139] ClinicalTrials.gov, A study of DPK-060 to investigate clinical safety and efficacy in patients with acute external otitis – full text view – clinicaltrials.gov. <https://clinicaltrials.gov/ct2/show/NCT01447017>, 2017 (accessed 17.03.17).
[140] J. Isaksson, B.O. Brandsdal, M. Engqvist, et al., A synthetic antimicrobial peptidomimetic (LTX 109): stereochemical impact on membrane disruption, J. Med. Chem. 54 (2011) 5786–5795.
[141] A.C. Nilsson, H. Janson, H. Wold, et al., LTX-109 is a novel agent for nasal decolonization of methicillin-resistant and -sensitive *Staphylococcus aureus*, Antimicrob. Agents Chemother. 59 (2015) 145–151.
[142] R. Roukema, OctoPlus proves efficacy of OP-145 in phase II ear infection study, *Marketwire*. <http://www.marketwired.com/press-release/octoplus-proves-efficacy-of-op-145-in-phase-ii-ear-infection-study-883010.htm>, 2008 (accessed 15.03.17).
[143] H. Ji, Y. Zou, J. Duan, et al., The synthetic melanocortin (CKPV)2 exerts anti-fungal and anti-inflammatory effects against *Candida albicans* vaginitis via inducing macrophage M2 polarization, PLoS One 8 (2013) e56004.
[144] A.A. Miller, P.F. Miller, Emerging Trends in Antibacterial Discovery: Answering the Call to Arms, Caister Academic Press, Norfolk, 2011.
[145] Soligenix, SGX942 oral mucositis. <http://www.soligenix.com/pipeline/biotherapeutics/sgx942-oral-mucositis/> (accessed 27.03.17).
[146] O. Cirioni, C. Silvestri, E. Pierpaoli, et al., IB-367 pre-treatment improves the in vivo efficacy of teicoplanin and daptomycin in an animal model of wounds infected with meticillin-resistant *Staphylococcus aureus*, J. Med. Microbiol. 62 (2013) 1552–1558.
[147] D.A. Mosca, M.A. Hurst, W. So, et al., IB-367, a protegrin peptide with in vitro and *in vivo* activities against the microflora associated with oral mucositis, Antimicrob. Agents Chemother. 44 (2000) 1803–1808.
[148] M. Godoy-Gallardo, C. Mas-Moruno, K. Yu, et al., Antibacterial properties of hLf1-11 peptide onto titanium surfaces: a comparison study between silanization and surface initiated polymerization, Biomacromolecules 16 (2015) 483–496.
[149] AM-Pharma, AM-Pharma announces €2.5 million financing | Blog, August 6, 2007. <http://www.am-pharma.com/blog/2007/08/am-pharma-announces-25-million-financing> (accessed 17.03.17).
[150] Y.Q. Xiong, W.A. Hady, A. Deslandes, et al., Efficacy of NZ2114, a novel plectasin-derived cationic antimicrobial peptide antibiotic, in experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*, Antimicrob. Agents Chemother. 55 (2011) 5325–5330.
[151] F. Costa, S. Maia, J. Gomes, et al., Characterization of hLf1-11 immobilization onto chitosan ultrathin films, and its effects on antimicrobial activity, Acta Biomater. 10 (2014) 3513–3521.
[152] M. Bulwan, S. Zapotoczny, M. Nowakowska, Robust "one-component" chitosan-based ultrathin films fabricated using layer-by-layer technique, Soft Matter 5 (2009) 4726–4732.

[153] J.A. Robinson, Protein epitope mimetics as anti-infectives, Curr. Opin. Chem. Biol. 15 (2011) 379–386.
[154] E.F. Haney, R.E.W. Hancock, Peptide design for antimicrobial and immunomodulatory applications, Biopolymers 100 (2013) 572–583.
[155] Y. Huang, J. Huang, Y. Chen, Alpha-helical cationic antimicrobial peptides: relationships of structure and function, Protein Cell 1 (2010) 143–152.
[156] N. Wiradharma, U. Khoe, C.A.E. Hauser, et al., Synthetic cationic amphiphilic α-helical peptides as antimicrobial agents, Biomaterials 32 (2011) 2204–2212.
[157] K. Hilpert, D.F. Winkler, R.E. Hancock, Peptide arrays on cellulose support: SPOT synthesis, a time and cost efficient method for synthesis of large numbers of peptides in a parallel and addressable fashion, Nat. Protoc. 2 (2007) 1333–1349.
[158] K. Hilpert, R. Volkmer-Engert, T. Walter, et al., High-throughput generation of small antibacterial peptides with improved activity, Nat. Biotechnol. 23 (2005) 1008–1012.
[159] E.F. Haney, S. Mansour, A.L. Hilchie, et al., High throughput screening methods for assessing antibiofilm and immunomodulatory activities of synthetic peptides, Peptides 71 (2015) 276–285.
[160] D.S. Bolintineanu, Y.N. Kaznessis, Computational studies of protegrin antimicrobial peptides: a review, Peptides 32 (2011) 188–201.
[161] T.D. Romo, L.A. Bradney, D.V. Greathouse, et al., Membrane binding of an acyllactoferricin B antimicrobial peptide from solid-state NMR experiments and molecular dynamics simulations, Biochim. Biophys. Acta 1808 (2011) 2019–2030.
[162] C.-W. Tsai, N.-Y. Hsu, C.-H. Wang, et al., Coupling molecular dynamics simulations with experiments for the rational design of indolicidin-analogous antimicrobial peptides, J. Mol. Biol. 392 (2009) 837–854.
[163] M.P. Gonzalez, C. Teran, L. Saiz-Urra, et al., Variable selection methods in QSAR: an overview, Curr. Top. Med. Chem. 8 (2008) 1606–1627.
[164] G. Maccari, M.D. Luca, R. Nifosí, et al., Antimicrobial peptides design by evolutionary multiobjective optimization, PLoS Comput. Biol. 9 (2013) e1003212.
[165] A. Cherkasov, K. Hilpert, H. Jenssen, 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 (2009) 65–74.
[166] R. Eckert, Road to clinical efficacy: challenges and novel strategies for antimicrobial peptide development, Future Microbiol. 6 (2011) 635–651.
[167] J.P. da Costa, M. Cova, R. Ferreira, et al., Antimicrobial peptides: an alternative for innovative medicines? Appl. Microbiol. Biotechnol. 99 (2015) 2023–2040.
[168] M.-D. Seo, H.-S. Won, J.-H. Kim, et al., Antimicrobial peptides for therapeutic applications: a review, Molecules 17 (2012) 12276–12286.
[169] A. Trotti, A. Garden, P. Warde, 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 (2004) 674–681.
[170] M. Kollef, D. Pittet, M. Sánchez García, et al., A randomized double-blind trial of iseganan in prevention of ventilator-associated pneumonia, Am. J. Respir. Crit. Care Med. 173 (2006) 91–97.
[171] A.K. Marr, W.J. Gooderham, R.E. Hancock, Antibacterial peptides for therapeutic use: obstacles and realistic outlook, Curr. Opin. Pharmacol. 6 (2006) 468–472.
[172] Y. Chen, C.T. Mant, S.W. Farmer, et al., Rational design of alpha-helical antimicrobial peptides with enhanced activities and specificity/therapeutic index, J. Biol. Chem. 280 (2005) 12316–12329.