LUND UNIVERSITY

Effect of hydrophobic modifications in antimicrobial peptides.

Schmidtchen, Artur; Pasupuleti, Mukesh; Malmsten, Martin

Published in:
Advances in Colloid and Interface Science

DOI:
10.1016/j.cis.2013.06.009

2013

Link to publication

Citation for published version (APA):
Schmidtchen, A., Pasupuleti, M., & Malmsten, M. (2013). Effect of hydrophobic modifications in antimicrobial peptides. *Advances in Colloid and Interface Science*. https://doi.org/10.1016/j.cis.2013.06.009

Total number of authors:
3

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Effect of hydrophobic modifications in antimicrobial peptides

Artur Schmidtchen[1], Mukesh Pasupuleti[1], and Martin Malmsten[2,*]

[1]*Section of Dermatology and Venereology, Department of Clinical Sciences, Lund University, SE-221 84 Lund, Sweden*
[2]*Department of Pharmacy, Uppsala University, P.O. Box 580, SE-751 23 Uppsala, Sweden*

*Corresponding author

Key words: AMP, antimicrobial peptide, bacteria, liposome, membrane

Tel.: +46-184714334
Fax.: +46-184714377
Email: martin.malmsten@farmaci.uu.se

Abstract

With increasing resistance development against conventional antibiotics, there is an urgent need to identify novel approaches for infection treatment. Antimicrobial peptides may offer opportunities in this context, hence there has been considerable interest in identification and optimization of such peptides during the last decade in particular, with the long-term aim of developing these to potent and safe therapeutics. In the present overview, focus is placed on hydrophobic modifications of antimicrobial peptides, and how these may provide opportunities to combat also more demanding pathogens, including multi-resistant strains, yet not provoking unacceptable toxic responses. In doing so, physicochemical factors affecting peptide interactions with bacterial and eukaryotic cell membranes are discussed. Throughout, an attempt is made to illustrate how physicochemical studies on model lipid membranes can be correlated to results from bacterial and cell assays, and knowledge from this translated into therapeutic considerations.

1. Introduction

Infectious diseases cause millions of deaths each year and result in tremendous socioeconomic costs. Due to increasing antibiotics resistance, effective therapeutic agents are no longer available for an increasing number of infections. Methicillin- and vancomycin-resistant *Staphylococcus aureus* are well-known examples of this, but also numerous other bacterial strains display extensive antibiotics resistance (1). As a result of this, there is large current interest in alternative therapeutic approaches, including antimicrobial peptides (AMPs) (2-4). AMPs are key components of the innate immune system, forming a first line of defense against invading pathogens. They are ≈10-40 amino acids long, and generally carry a net positive charge (5). Many AMPs contain a substantial fraction of hydrophobic residues, frequently appearing in patterns of 1-2 for every 3-4 residues. Consequently, many AMPs form α-helices, particularly when interacting with lipid membranes. These characteristics are motivated by the mode of action of AMPs. Thus, although AMPs affect bacteria in many different ways, including inhibition of cell wall-, DNA-, RNA-, and protein synthesis, as well as of enzymatic activity, the main mode of action of AMPs is disruption of bacterial membranes (2-4). The walls of Gram-positive bacteria consist of a single lipid membrane surrounded by a thick peptidoglycan envelope, while those of Gram-negative bacteria contain two lipid membranes with an interspaced peptidoglycan layer, the outer membrane containing a high fraction of negatively charged lipopolysaccharide (LPS) (Figure 1). Based primarily on studies using model lipid systems, several mechanisms have been suggested for AMP-induced membrane rupture, including packing disruption by a detergency-like mechanism, or through formation of membrane pores/defects of barrel-stave or toroidal type (Figure 2) (2,3,6,7). Since barrel-stave pores can only be formed by peptide oligomers, for which hydrophobic and charged residues are precisely

spaced to reduce free energy, such pores have only been experimentally demonstrated for a couple of peptides. Toroidal pores, on the other hand, can be formed by a wider range of peptides. After initial binding in the polar headgroup region, the peptide either inserts into the membrane, or induces a positive curvature strain, resulting in the formation of a toroidal pore. Higher peptide densities at the membrane surface may subsequently cause membrane disruption and micellization (6). For some peptides, the chemical potential gradient across the bilayer may also result in transient defects due to peptide translocation across the membrane (8). In addition, lateral expansion of the lipid membrane due to peptide binding to the polar headgroup region allows relaxation of the alkyl chains and causes membrane thinning, further facilitating membrane rupture (9). Also peptide-induced phase transitions and/or lipid segregation may contribute to AMP-induced membrane rupture (10).

A crucial factor for any therapeutically relevant AMP is that it is selective in its membrane interactions, thus efficiently killing bacteria and other microbes, but causing limited damage to human cells. Such membrane selectivity is facilitated by composition differences between human and bacterial membranes. Thus, cholesterol is abundant in human cell membranes (≈40 mol%), but replaced by ergosterol in fungal membranes, and absent in bacteria. There are also considerable differences in phospholipid composition. For example, the outer leaflet of erythrocyte membranes is dominated by zwitterionic phosphatidylcholine and sphingomyelin, rendering it essentially uncharged, while bacteria membranes are rich in anionic lipids (11). In addition, Gram-negative bacteria contain negatively charged LPS (up to 70% of the outer membrane), while Gram-positive bacteria contain lipoteichoic acid. Attempting to take advantage of such compositional differences, considerable efforts have been directed to identifying selective AMPs, e.g., through screening for peptides from various species, through use of combinatorial

libraries and quantitative structure-activity relationships, or through identification of AMPs from endogenous proteins (2-4).

2. Effects of peptide properties on membrane interactions

2.1. Peptide length

In analogy to polyelectrolyte and protein adsorption (12), peptide binding (e.g., to membranes) is expected to decrease with decreasing peptide length due to the increased entropy penalty, per amino acid, on adsorption. For transmembrane pore structures, there is an additional effect of peptide length, as peptides need to match the thickness of the membrane in order to stabilize the pore. There is also a decreased tendency to form ordered secondary structures (notably helices) with decreasing peptide length. Given this, decreased membrane lysis and antimicrobial effect is anticipated with decreasing peptide length. Investigating these effects, Ringstad et al. studied membrane interactions and antimicrobial effects for the peptide series $(AKKARA)_n$ and $(ARKAAKKA)_n$ (13). Indeed, peptide-induced bacterial killing was found to decrease with decreasing peptide length, as was peptide-induced liposome rupture, both effects demonstrated to be due to a decreasing peptide binding to lipid membranes with decreasing peptide length. Analogous effects were observed on truncating the kininogen–derived peptide HKH20 (14). Similarly, Deslouches et al. investigated the length dependence of the antibacterial effect of a base motif peptide and found its lytic activity to decrease with decreasing peptide length (15). Although less comprehensive regarding underlying physicochemical mechanisms, reduced antimicrobial effects on AMP truncation has been found also by various other investigators (2,3). Note however, that there may also be considerable room for selective truncations of longer AMPs without loosing too much activity, in some cases even reaching improved performance of the

truncated variant compared to the initial peptide. For example, several studies have demonstrated that it is possible to truncate the endogenous peptide LL-37 to less than half its length and retain antimicrobial and several other functions (16-18). Ultimately, when truncated to become sufficiently short, however, selective membrane-rupturing capacity is lost.

2.2. Peptide charge

Since bacterial membranes are rich in anionic phospholipids, increasing the AMP positive charge may result in increased peptide adsorption to, and lysis of, bacterial membranes. Exemplifying this for the C3a-derived peptide CNY21, Ringstad et al. found both peptide adsorption and membrane rupture to increase with peptide positive charge, but to be completely lost on elimination of all peptide positive charges (19). In parallel, bactericidal effects were drastically reduced on elimination of the peptide positive net charge. That electrostatics provides the main driving force for membrane disruption in these systems was demonstrated also by a drastic reduction in liposome leakage, peptide adsorption, and bactericidal effect with increasing ionic strength. Through titrating histidine groups, some AMPs furthermore display pronounced pH dependence. For example, replacement of lysine and arginine by histidine in $(AKKARA)_n$ and $(ARKKAAKA)_n$ consensus peptides completely abrogates their antimicrobial activities at neutral pH, i.e., above the pKa of histidine (≈6.0), whereas their antibacterial activity, as well as liposome rupture, is restored at acidic conditions (pH 5.5) (13). In parallel, peptide binding to bacteria and model lipid membranes is significantly enhanced at pH 5.5. Similar pH-dependent antimicrobial activities were demonstrated for the histidine-rich peptide histatin 5 and for peptides derived from histidine-rich regions of high molecular weight kininogen and histidine-rich glycoprotein (20). Similarly, Makovitzki and Shai investigated the pH dependence of lipopeptides with dodecanoic acid-modified LXXLLXXLLXXL peptides (X being H, K, or R)

(21). While the K- and R-containing peptides displayed little pH dependence, the H-containing one showed no antimicrobial activity at pH 7.4 (contrary to the K- and R-containing peptides), but restored activity at pH 5.5. As demonstrated by these and other studies (2,3), it is thus clear that AMP binding to negatively charged membranes increases with peptide net charge, as does peptide-induced membrane rupture. However, as demonstrated by Ringstad et al. for the kininogen-derived peptide HKH20, there is a limit to these charge-induced effects at sufficiently high peptide charge density due to a decreased peptide adsorption and membrane insertion (14). An analogous reduction in bactericidal activity at very high peptide charge was found by Giangaspero et al. (22). As for polyelectrolytes, there is thus a decreased adsorption at high charge densities due to the electrostatic penalty of reducing the intramolecular charge distance on adsorption (12).

It should also be noted here, that while high peptide-membrane charge contrast promotes peptide adsorption to lipid membranes, and frequently also membrane lysis, it may also oppose membrane insertion. For example, increasing the charge in the C3a-derived peptide CNY21 by H->K substitutions was found by electrochemical methods to decrease peptide insertion into DOPC monolayers (23). Similarly, Strömstedt at al. found that membranes rich in anionic headgroups display higher resistance against melittin-induced rupture than zwitterionic ones (24). From fluorescence quenching of the single melittin tryptophan residue by depth-specific doxyl groups, it was demonstrated that the origin of this effect is that melittin penetrates deeper into zwitterionic bilayers, but is electrostatically arrested in the polar headgroup region of anionic ones, particularly at low ionic strength.

2.3. Effect of peptide secondary structure

For a range of AMPs, formation of amphiphilic ordered structures (notably α-helices) has been found to correlate to peptide-induced bacterial killing and liposome disruption (2,3). A peptide displaying pronounced such helix formation on membrane interaction is LL-37 (16). Since LL-37 is susceptible to proteolytic degradation, there has been interest in identifying proteolytically stable and less toxic LL-37 variants. Along this line, Sigurdadottir et al. identified the internal sequence GKE21 as a promising peptide which maintains the bactericidal potency of LL-37, but displays lower toxicity (17). An interesting feature of GKE21 is its ability to form a nearly perfectly amphiphilic helix, where hydrophobic/nonpolar and polar/charged residues are localized on opposite sides. As a consequence of this, helix-induction provides a driving force for peptide binding to, and rupture of, lipid membranes, thus compensating for the effect of truncation and resulting in an overall potency better than that of LL-37. Reducing the helix-related peptide amphiphilicity through selected D-amino acid substitutions in GKE21 was found to result in reduced peptide binding to phospholipid membranes, as well as decreased peptide-induced liposome leakage and bacterial killing (25). Similar effects were demonstrated also for the further truncated EFK17 (18). Also other means for reducing helicity, including proline substitutions, have been found to reduce peptide interaction with phospholipids membranes (26). Furthermore, decreasing AMP helicity, either by D-enantiomers or proline substitutions, has been correlated to decreased cytotoxicity caused by disruption of eukaryotic cell membranes (22). Destabilizing helix formation has therefore been suggested as a way to reduce cytotoxicity of AMPs (27,28). As demonstrated by Strömstedt for EFK17, however, such toxicity reduction due to helix destabilization may ultimately result in reduction or elimination of antimicrobial effects of such peptides, illustrating that a balance is required also from this perspective.

2.4. Peptide hydrophobicity

As discussed above, high AMP adsorption, membrane rupture, and antibacterial effect can sometimes be facilitated by increasing the AMP charge density. However, due to electrostatic screening, highly charged and hydrophilic peptides loose much of their effect at high ionic strength (e.g., at physiological conditions). Furthermore, many important pathogens have a low surface potential, which may be reduced or even reversed, e.g., by alanine modification of lipoteichoic acid, lysine modification of phosphatidylglycerol, and aminoarabinose modifications in lipopolysaccharide (29). By increasing AMP hydrophobicity, sensitivity to these effects can be reduced. For example, Ringstad et al. investigated selected L substitutions in the C3a-derived peptide CNY21, and found both liposome leakage and bactericidal effect of this peptide to increase with increased hydrophobicity (19,23). This, in turn, was demonstrated to be due to a combined effect of increased adsorption and facilitated membrane insertion of the more hydrophobic peptide variant. Similarly, Cornut et al. investigated K/L peptides, and found membrane lysis to increase with peptide hydrophobicity (30). Hydrophobicity was furthermore demonstrated by Dathe et al. to be of critical importance for KLA peptide activity against Gram-positive bacteria (31), and has also been found to be a key parameter for antimicrobial effect in a number of quantitative structure-activity-relation investigations (2,3,28). However, since membrane binding for very hydrophobic peptides results from poor solvency rather than from peptide-membrane interactions, binding occurs irrespectively of membrane composition, resulting in lysis of both bacteria and human cells. For example, K/L peptides with high hydrophobicity:charge ratios have been found to be even more hemolytic than the bee venom melittin (30). Thus, AMP hydrophobicity can only be used to some extent to boost peptide potency without loosing selectivity.

3. End-tagging peptides with hydrophobic moieties

Since membrane rupture scales with peptide incorporation into membranes, hydrophobic modifications represent a frequently used approach to enhance peptide binding to, and rupture of, bacterial membranes, also for low-charged pathogens at electrostatically highly screened conditions at physiological electrolyte concentrations, as well as in the presence of serum, blood, or other anionic scavengers. However, with hydrophobic point mutations, not only mean hydrophobicity changes, but also hydrophobicity-dependent conformational transitions, which complicate the interaction with both lipid membranes and other bacterial wall components. Furthermore, point mutations may have detrimental consequences for the chemical and enzymatic stability of AMPs, as well as for functions other than membrane-targeted bactericidal affects, including effects on coagulation, complement, and cytokine production. An alternative to sequence-specific point mutations with hydrophobic amino acids is therefore hydrophobic end-modification of AMPs, which may be achieved, e.g., by attachment of an acyl group or a hydrophobic amino acid stretch to the template peptide.

3.1. Acyl-containing lipopeptides

Extensively investigated in the context of hydrophobically end-modified AMPs are lipopeptides. These consist of a linear or a cyclic peptide sequence of either positive or negative charge, with a hydrophobic moiety, e.g., a sterol or a fatty acid, covalently attached (32-35). As for non-modified AMPs, bacterial membranes constitute the main target of lipopeptides. Demonstrating this for a series of acylated (C_{12}-C_{16}) short-chain peptides (KAAK, KLLK, KKKK, KGGK, GIGK, and KKEK), Makovitsky et al. found a clear correlation between bactericidal effect and membrane disruption in model liposomes (Figure 3a) (36). In analogy to hydrophobic point

mutations, the acyl chain in lipopeptides results in an increased membrane binding and disruption, as well as in increased antimicrobial activity, increasing with the length of the acyl moiety (32,33). Investigating related effects for $C_{14}K_n$ peptides, Jia et al. found that the adsorption of these peptides increased with increasing mean hydrophobicity (Figure 3b) (35). This is primarily related to an increased lipopeptide binding and membrane insertion for longer acyl moieties, but has also been suggested, e.g., by Avrahami and Shai, to be an effect of the longer acyl chains promoting lipopeptide oligomerization (33). However, self-assembly has also been found to limit membrane interactions and antimicrobial effects of lipopeptides. For example, Chu-Kung et al. investigated effects of the length of fatty acids conjugated to peptide AKK, and found the antimicrobial activity to increase with fatty acid length, but also that antimicrobial activity is lost when the minimal active concentration is higher than the critical micelle concentration (cmc) of the lipopeptide (37). In parallel, it was found that at concentrations above cmc, solution self-assembly inhibits peptide membrane binding and antimicrobial effect (Figure 4).

Due to the long acyl chains, commonly investigated acyl-containing lipopeptides are exceedingly efficient in their ability to insert into lipid membranes, largely irrespectively of the composition of the latter. Demonstrating this for fengomycin (Figure 5a), Eeman et al. found this peptide to display very high expulsion pressure also for cholesterol-containing (mammalian-mimicking) and cholesterol/fatty acid-containing (stratum corneum-mimicking) membranes (Figure 5b) (38). As a result of this, fengomycin inserts readily into both bacterial and mammalian membranes and causes their destabilization. Further demonstrating the efficient, but non-selective, membrane insertion of lipopeptides, Malina and Shai found hemolysis caused by acylated L_6K_6 to be very high for longer acyl chains (Figure 5c) (39). Indeed, this lack of selectivity causes many

lipopeptides to display substantial toxicity, which has restricted their use to local applications, and to severe indications for which other antibiotics are uneffective, e.g., multi-resistant *P. aeruginosa* infections in cystic fibrosis (32).

As for conformational changes, these depend to some extent on the AMP end-modified. For example, Avrahami and Shai investigated magainin variants modified with fatty acids, and found helix induction both on interaction with phospholipid membranes and on conjugation of the peptide to the fatty acid moiety (34). Similar results were obtained for AKK peptides modified by fatty acid chains by Chu-Kung et al. (37). In contrast to these readily helix-forming peptides, however, disordered peptides display limited secondary structure induction also on acyl modification, exemplified, e.g., by results from Avrahami and Shai on palmitic acid-modified disordered peptides (34).

3.2. End-tagging with aromatic amino acids

Somewhat similar to lipopeptides, end-tagging of AMPs with hydrophobic amino acid stretches offers an interesting approach to achieve high, but *selective*, AMP activity (40). Although a number of hydrophobic amino acids may be used as end-tags, W and F are particularly interesting in this context. These bulky and polarizable residues have an affinity to interfaces, and are frequently located close to the polar headgroup region in phospholipid membranes. In the case of antimicrobial peptides, examples of this have been provided, e.g., by Glukhov et al., who found membrane insertion of KKKKKKAAXAAWAAXAA (X being W or F) of 2.5-8Å (41). Similarly, Li et al., investigated aurein 1.2 analogs and found their F residues to localize 2-5Å below the polar headgroup region (42). Through this, W/F residues are able to act as an anchor for the peptide, resulting in increased bactericidal effects and salt resistance. For example, W/F-

tagging was found by Schmidtchen et al. to promote binding of the kininogen-derived peptide GKH17 to bacteria, as well as subsequent permeabilization (40). While bacterial killing by the non-tagged template peptide GKH17 was essentially non-observable at high ionic strength, it increased strongly with the length of the hydrophobic tag (Figure 6a). In parallel, tagged GKH17 caused release of intracellular material of bacteria, also at high ionic strength, which increased with the length of the hydrophobic tag (Figure 6b). The biological relevance of these effects was demonstrated *ex vivo* and *in vivo* in porcine *S. aureus* skin infection models. In agreement with these findings, the binding of GKH17-WWW was much higher than that of GKH17 at bacteria-mimicking DOPE/DOPG membranes, as was peptide-induced liposome leakage. Mirroring the low eukaryotic cell toxicity of these peptides, peptide-induced leakage of liposomes formed by cell-mimicking membranes (DOPC/cholesterol) was low, as was peptide binding to the corresponding supported bilayers (40).

In contrast to poorly soluble AMPs and lipopeptides with long alkyl chains, W/F end-tags thus *contribute* to AMP selectivity between bacterial and human membranes. Demonstrating this further, Figure 7 shows antimicrobial and cell toxicity data for the peptide GRR10W5N (GRR10WWWWW-NH_2). As shown, this peptide displays potent antimicrobial potency against both Gram-positive *S. aureus*, Gram-negative *E. coli* and *P. aeruginosa*, and the fungi *C. albicans* and *C. parapsilosis*, also at physiological ionic strength. As demonstrated most clearly for the fungi *C. albicans* and *C. parapsilosis*, GRR10W4N displays stronger, or much stronger, inhibitory effects than both best-in class benchmarks omiganan and LL-37. At the same time, however, toxicity of GRR10W4N to human cells is limited, with very low hemolysis up to 120 μM, as well as low toxicity towards human keratinocytes. To further demonstrate selectivity

between bacteria and erythrocytes, citrate-blood was supplemented with either *S. aureus* or *P. aeruginosa*, whereafter peptide was added, and hemolysis and bacteria viable count assays performed on the same sample. Also in such experiments, GRR10W4N was found to display potent antimicrobial effects against *S. aureus* and *P. aeruginosa*, but with hemolysis on the same level as the background negative control (43).

For a peptide to be able to insert into the phospholipid membrane, it must overcome the cohesive energy of the latter. Particularly for bulky groups such as W and F, which require substantial area expansion, insertion into membranes containing cholesterol (known to condense lipid bilayers (44)) is energetically costly. Consequently, the adsorption of tagged peptides is reduced at cholesterol-containing membranes, as is peptide-induced membrane rupture (43). This difference in membrane interactions in the presence and absence of cholesterol, together with that due to the charge difference between the anionic and zwitterionic membranes, contributes to the selectivity between bacteria and human cells. Illustrating this, Figure 8 shows results by Schmidtchen et al. for GRR10W5N, demonstrating potent leakage induction of anionic DOPE/DOPG liposomes, but very limited leakage from DOPC/cholesterol ones (43). In parallel, a higher peptide binding occurs to anionic membranes as compared to zwitterionic ones (Figure 8b), with cholesterol content and membrane charge density moderating membrane insertion (Figure 8c). A good agreement between these findings and those on antimicrobial effects and cell toxicity of this peptide (Figure 7) was thus obtained. As further demonstrated in the same investigation, membrane selectivity for this type of peptides is at least partly generalizable, since similar results on liposome leakage induction was observed when replacing the original sequence GRRPRPRPRPWWWW with another charged group (GKKPKPKPKPWWWW), another

aromatic amino acid end-tag (GRRPRPRPRPFFFFF), or a similar but different peptide sequence (KNKGKKNGKHWWW).

Finally, we note that in contrast to lipopeptides containing moderate to long alkyl chains, self-assembly seems not to influence membrane interaction of such W/F-tagged peptides. As for surfactants containing aromatic tails, the bulky and polarizable nature of W and F precludes self-assembly, resulting in low aggregation numbers and high critical aggregation concentrations, thus limiting the relative importance of the aggregated state for membrane insertion and defect formation (40).

Since cost of goods is a limiting factor for AMPs in many indications, reducing peptide length is of major importance in the development of AMPs towards novel therapeutics. In addition, chemical degradation and modifications of AMPs scales with peptide length, as does sensitivity to endoprotease degradation, again pointing to the importance of reducing peptide length. Since AMP activity generally decreases with peptide length, hydrophobic modifications have attracted attention in this context. However, as discussed above, short acyl-modified lipopeptides often display poor selectivity and resulting toxicity. In contrast, W-tagging offers opportunities for efficient and selective ultra-short AMPs. Focusing on the kininogen-derived peptide KNK10 and truncations thereof, Pasupuleti et al. found W-tagging to result in boosting of bactericidal effect against both Gram-negative and Gram-positive bacteria, allowing potency and salt resistance to be maintained down to 4-7 amino acids in the hydrophilic template peptide (Figure 9) (45). Although end-tagging resulted in increased eukaryotic cell permeabilization at low ionic strength, the latter was insignificant at physiological ionic strength and in the presence of serum. The high bactericidal potency of the tagged peptides correlated to a high degree of bacteria binding and

resulting bacterial wall rupture. Analogously, tagging enhanced peptide-induced rupture of anionic liposomes.

W-tagged peptides offer opportunities to combat also difficult pathogens. Demonstrating this, Pasupuleti et al. investigated antimicrobial effects and membrane interactions for *P. aeruginosa*, a Gram-negative bacteria and an opportunistic pathogen under a range of conditions (46). *P. aeruginosa* infections are common both in out-patient settings and in hospital environments, where antibiotics-resistant strains are frequently observed. A spectrum of diseases involves *P. aeruginosa*, including otitis, keratitis, chronic leg ulcers, postoperative and burn wound infections, cystic fibrosis, and pneumonia. New bactericidal agents against *P. aeruginosa* are therefore needed. However, the use of AMPs in this context is not trivial, since *P. aeruginosa* is able to excrete AMP-scavenging exopolysaccharides, as well as proteolytic enzymes, in defense against AMPs. Nevertheless, as demonstrated by Pasupuleti et al., W-tagging yields increased AMP potency against *P. aeruginosa*, irrespective of exopolysaccharide and bacterial protease production. For the kininogen-derived peptides GKH17 and KNK10, potency increased with tag length, correlating to more efficient bacterial wall and vesicle rupture, and to more pronounced LPS binding. End-tag effects remained at high electrolyte concentration and in the presence of plasma or anionic macromolecular scavengers. The tagged peptides furthermore displayed stability against *P. aeruginosa* elastase, and were potent *ex vivo*, both in a contact lens model and in a skin wound model (Figure 10).

Through W/F end-tags, which drive selective membrane binding, insertion, and lysis, the remainder of the peptide sequence can be more freely selected to accommodate for other desirable properties, including stability against proteolytic degradation. Apart from *Pseudomonas*

elastase discussed above, W-tagged peptides have been demonstrated to display excellent stability against human leukocyte elastase, as well as staphylococcal aureolysin and V8 proteinase (40). In parallel, these peptides have been demonstrated to be potent against a range of Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* clinical isolates, also in the presence of human plasma and blood, but at maintained low toxicity against mammalian cells. In addition, the peptide RRPRPRPRPWWWW-NH_2 was demonstrated to be effective against a range of "superbugs", including vancomycin-resistant enterococci, multi-drug resistant *P. aeruginosa*, and methicillin-resistant *S. aureus* (MRSA) (47). Thus, W/F-tagging of the cationic sequence RRPRPRPRP generates highly selective AMPs with potent activity against multi-resistant bacteria and efficiency in *ex vivo* wound infection models.

In addition to selectivity between bacteria and eukaryotic cells, W/F-tagged peptides display selectivity between different eukaryotic cells, of interest for antifungal therapeutics. The basis for this is that while sterols are found in a wide range of membranes from various species, these differ with respect to sterol identity. Thus, while ergosterol and lanosterol are present in the membranes of fungi, protozoa, and insects, cholesterol dominates in mammalian membranes. Although these sterols are structurally quite similar, their effects on membranes differ. Thus, cholesterol is particularly potent in increasing lipid order in membranes while maintaining fluidity, and provides large mechanical cohesion to membranes (48). Due to their bulky and polarizable nature W/F-tagged peptides are therefore sensitive to sterol identity as demonstrated, e.g., by Schmidtchen et al. (43). As exemplified for GRR10W4N, such peptides display potent antifungal effects against *Candida albicans* and *Candida parapsilosis*, but simultaneously low hemolysis and toxicity to epithelial cells (Figure 7). In parallel, peptide-induced liposome rupture is substantial for (“fungi-mimicking”) DOPC/ergosterol liposomes, but largely absent in

DOPC/cholesterol liposomes, despite comparable peptide adsorption and DOPC/cholesterol and DOPC/ergosterol lipid bilayers (Figure 8). Thus, the higher dilatational elasticity of the cholesterol-containing systems prevents peptide insertion, and therefore also membrane lysis.

Although dealing with receptor interactions rather than antimicrobial effects, Ember et al. found that hydrophobic end-tagging increased the biological potency of short C3a peptides (49). In line with effects of W-tagging on the antimicrobial effects, discussed above, potency of these peptides increased with the number of terminal W residues, W being more efficient than I in increasing peptide biological activity. Although membrane interactions played a role, W-tagging notably promoted specific peptide binding to the C3a receptor. End-tagging by W/F stretches may thus potentially offer a way to promote also more specific peptide docking processes.

Finally, we note that W/F-tagging, while generally applicable, typically has to be "tuned" to the template peptide, e.g., for achieving the desired effect/toxicity profile, or for optimizing membrane interactions. Such tuning is readily done, however, through varying the length of the end-tag, and/or by W->F substitutions. The finer details of such tuning on membrane interactions was investigated by Strömstedt et al., when studying the interplay between electrostatic and hydrophobic interactions for end-tagged heptamers of lysine (K7) and arginine (R7) (50). Both K7 and R7 were found to be lytic against *E. coli*, which correlated to the binding of these peptides to bilayers formed by *E. coli* phospholipids, and corresponding liposome leakage. When W-tagging these peptides, substantial increase in peptide adsorption, membrane lysis, and bacterial killing was observed, also at high ionic strength. Strikingly, the order of membrane lytic potential of the cationic peptides investigated was reversed when tagged, suggesting that tagged and untagged peptides act by different lytic mechanisms, which to some extent counterbalance

each other. Thus, while the untagged peptides act by generating negative curvature strain in the phospholipid membrane, the tagged peptides cause positive curvature strain.

8. Summary and outlook

Although AMPs affect bacteria in numerous ways, their main mode of action is the disruption of bacterial membranes. Consequently, there is generally good correlation between bacteria killing, bacteria lysis, liposome lysis, and peptide adsorption to lipid membranes, providing studies with model lipid systems relevance translating also to the biological situation. Numerous parameters are of importance for AMP-membrane interactions, including peptide length, charge (distribution), hydrophobicity (distribution), and secondary structure. Of particular focus in the present overview, end-tagging with hydrophobic amino acid stretches, notably W and F, provides a facile and flexible approach of general applicability, by which AMP potency can be boosted without causing parallel toxicity effects. W/F-tagging is also applicable for a spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, as well as fungi. This includes also a wide range of "superbugs", including vancomycin-resistant enterococci, multi-drug resistant *P. aeruginosa*, and methicillin-resistant *S. aureus* (MRSA). Through tag composition and/or length, potency and toxicity can be tuned, e.g., depending on whether the peptide is to be applied in an environment containing serum or not, or depending on the relative need for antimicrobial potency and limited toxicity. This flexibility is attractive from a therapeutic perspective, since it allows AMPs to be tagged to fit the conditions of the indication at hand. Particularly for AMPs not sensitive to infection-related proteolysis, the finding that hydrophobic tagging may be achieved without affecting stability against proteolytic degradation, also opens up applications characterized by high proteolytic activity, such as infected wounds,

eye infections, and cystic fibrosis. Importantly, hydrophobic tagging may be applied to a broad range of AMPs, particularly polar and highly charged ones. As exemplified, the approach also holds promise for enhancing biological peptide activities in a broader perspective.

Acknowledgement

This work was financed by the Swedish Research Council (projects 2012-1842 and 521-2009-3378, 7480).

References

1) G.L. French, Clinical impact and relevance of antibiotic resistance, Adv. Drug Delivery Rev. 75 (2005) 1514-1527.

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

3) M. Pasupuleti, A. Schmidtchen, and M. Malmsten, Antimicrobial peptides: key components of the innate immune system, Crit. Rev. Biotechnol. 32 (2012) 143-171.

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

5) The Antimicrobial Peptide Database. http://aps.unmc.edu/AP/.

6) M.N. Melo, R. Ferre, and M.A. Castanho, Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations, Nat. Rev. Micro. 7 (2009) 245-250.

7) Y. Shai, Mode of action of membrane active antimicrobial peptides, Biopolymers 66 (2002) 236-248.

8) A. Pokorny, and P.F.F. Almeida, Kinetics of dye efflux and lipid flip-flop induced by δ-lysin in phospholipid vesicles and the mechanism of graded release by amphipathic, α-helical peptides, Biochemistry 43 (2004) 8846-8857.

9) A. Mecke, D.-K. Lee, A. Ramamoorthy, B.G. Orr, and M.M. Banaszak Holl, Membrane thinning due to antimicrobial peptide binding: an atomic force microscopy study of MSI-78 in lipid bilayers, Biophys. J. 89 (2005) 4043-4050.

10) R. El Jastimi and M. Lafleur, Nisin promotes the formation of non-lamellar inverted phases in unsaturated phosphatidylethanolamines, Biochim. Biophys. Acta 1418 (1999) 97-105.

11) E.J.J. Lugtenberg and R. Peters, Distribution of lipids in cytoplasmic and outer membranes of *Escherichia coli* K12, Biochim. Biophys. Acta, 441 (1976) 38-47.

12) G.J. Fleer, M.A. Cohen Stuart, J.M.H.M. Scheutjens, T. Cosgrove, and B. Vincent, Polymers at interfaces, Chapman & Hall, London, 1993.

13) L. Ringstad, A. Schmidtchen, and M. Malmsten, Effect of peptide length on the interaction between consensus peptides and DOPC/DOPA bilayers, Langmuir 22 (2006) 5042-5050.

14) L. Ringstad, L. Kacprzyk, A. Schmidtchen, and M. Malmsten, Effects of topology, length, and charge on the activity of a kininogen-derived peptide on lipid membranes and bacteria, Biochim. Biophys. Acta 1768 (2007) 715-727.

15) B. Deslouches, S.M. Phadke, V. Lazarevic, M. Cascio, K. Islam, R.C. Montelaro, and T.A. Mietzner, De novo generation of cationic antimicrobial peptides: influence of length and tryptophan substitution on antimicrobial activity, Antimicrob. Agents Chemother. 49 (2005) 316-322.

16) U.H. Durr, U.S. Sudheendra, and A. Ramamoorthy, LL-37, the only human member of the cathelicidin family of antimicrobial peptides, Biochim. Biophys. Acta 1758 (2006) 1408-1425.

17) T. Sigurdadottir, P. Andersson, M. Davoudi, M. Malmsten, A. Schmidtchen, and M. Bodelsson, In silico identification and biological evaluation of antimicrobial peptides based on human cathelicidin LL-37, Antimicrob. Agents. Chemother. 50 (2006) 2983-2989.

18) A.A. Strömstedt, M. Pasupuleti, A. Schmidtchen, and M. Malmsten, Evaluation of strategies for improving proteolytic resistance of antimicrobial peptides by using variants of EFK17, an internal segment of LL-37, Antimicrob. Agents Chemother. 53 (2009) 593-602.

19) L. Ringstad, E. Andersson Nordahl, A. Schmidtchen, and M. Malmsten, Composition effect on peptide interaction with lipids and bacteria: variants of C3a peptide CNY21, Biophys. J. 92 (2007) 87-98.

20) L. Kacprzyk, V. Rydengård, M. Mörgelin, M. Davoudi, M. Pasupuleti, M. Malmsten, and A. Schmidtchen, Antimicrobial activity of histidine-rich peptides is dependent on acidic conditions, Biophys. Biochim. Acta 1768 (2007), 2667-2680.

21) A. Makovitzki, and Y. Shai, pH-Dependent antifungal lipopeptides and their plausible mode of action. Biochemistry 44 (2005) 9775-9784.

22) A. Giangaspero, L. Sandri, and A. Tossi, Amphipatic α helical antimicrobial peptides: a systematic study of the effects of structural and physical properties on biological activity, Eur. J. Biochem. 268 (2001) 5589-5600.

23) L. Ringstad, E. Protopapa, B. Lindholm-Sethson, A. Schmidtchen, A. Nelson, and M. Malmsten, An electrochemical study into the interaction between complement-derived peptides and DOPC mono- and bilayers, Langmuir 24 (2008) 208-216.

24) A.A. Strömstedt, P. Wessman, L. Ringstad, K. Edwards, and M. Malmsten, Effect of lipid headgroup composition on the interaction between melittin and lipid bilayers, J. Colloid Interface Sci. 311 (2007) 56-69.

25) L. Ringstad, A. Schmidtchen, and M. Malmsten, Effects of single amino acid substitutions on peptide interaction with lipid membranes and bacteria – variants of GKE21, an internal sequence from human LL-37, Colloids Surf. A 354 (2010) 65-71.

26) V.V. Andrushchenko, H. J. Vogel, and E. J. Prenner, Interactions of tryptophan-rich cathelicidin antimicrobial peptides with model membranes studied by differential scanning calorimetry, Biochim. Biophys. Acta 1768 (2007) 2447-2458.

27) I. Zelezetsky, S. Pacor, U. Pag, N. Papo, Y. Shai, H. G. Sahl, and A. Tossi, Controlled alteration of the shape and conformational stability of α-helical cell-lytic peptides: effect on mode of action and cell specificity, Biochem. J. 390 (2005) 177-188.

28) M. Pasupuleti, B. Walse, B. Svensson, M. Malmsten, and A. Schmidtchen, Rational design of antimicrobial C3a analogues with enhanced effects against Staphylococci using an integrated structure and function-based approach, Biochemistry 47 (2008), 9057-9070.

29) V. Nizet, Antimicrobial peptide resistance mechanisms of human bacterial pathogens, Curr. Issues Mol. Biol. 8 (2006), 11-26.

30) I. Cornut, K. Büttner, J.-L. Dasseaux, and J. Dufourcq, The amphipathic α-helix concept: application to the de novo design of ideally amphipathic Leu, Lys peptides with hemolytic activity higher than melittin, FEBS Letts. 349 (1994) 29-33.

31) M. Dathe, T. Wieprecht, H. Nikolenko, L. Handel, W.L. Maloy, D.L. MacDonald, M. Beyermann, and M. Bienert, Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides, FEBS Letts. 403 (1997) 208-212.

32) R. Jerala, Synthetic lipopeptides: a novel class of anti-infectives, Expert Opin. Investig. Drugs 16 (2007) 1159-1169.

33) D. Avrahami and Y. Shai, Conjugation of a magainin analogue with lipophilic acids controls hydrophobicity, solution assembly, and cell selectivity, Biochemistry 41 (2002) 2254-2263.

34) D. Avrahami, and Y. Shai, A new group of antifungal and antibacterial lipopeptides derived from non-membrane peptides conjugated to palmitic acid, J. Biol. Chem. 279 (2004) 12277-12285.

35) D. Jia, K. Tao, J. Wang, C. Wang, W. Zhao, M. Yaseen, H. Xu, G. Que, J.R.P. Webster, and J.R. Lu, Dynamic adsorption and structure of interfacial bilayers adsorbed from lipopeptide surfactants at the hydrophilic silicon/water interface: effects of the headgroup length, Langmuir (2011) 8798-8809.

36) A. Makovitzki, D. Avrahami, and Y. Shai, Ultrashort antibacterial and antifungal lipopeptides, Proc. Natnl. Acad. Sci., 103 (2006) 15997-16002.

37) A.F. Chu-Kung, R. Nguyen, K.N. Bozelli, and M. Tirrell, Chain length dependence of antimicrobial peptide-fatty acid conjugate activity, J. Colloid Interface Sci. 345 (2010) 160-167.

38) M. Eeman, G. Francius, Y.F. Dufrene, K. Nott, M. Paquot, and M. Deleu, Effect of cholesterol and fatty acids on the molecular interactions of fengycin with stratum corneum mimicking lipid monolayers, Langmuir 25 (2009) 3029-3039.

39) A. Malina, and Y. Shai, Conjugation of fatty acids with different lengths modulates the antibacterial and antifungal activity of a cationic biologically inactive peptide, Biochem. J. 390 (2005) 695-702.

40) A. Schmidtchen, M. Pasupuleti, M. Mörgelin, M. Davoudi, J. Alenfall, A. Chalupka, and M. Malmsten, Boosting antimicrobial peptides by hydrophobic oligopeptide end-tags, J. Biol. Chem. 284 (2009) 17584-17594.

41) E. Glukhov, M. Stark, L.L. Burrow, and C.M. Deber, Basis for selectivity of cationic antimicrobial peptides for bacterial versus mammalian membranes, J. Biol. Chem. 280 (2005) 33960-33967.

42) X. Li, Y. Li, A. Peterkofsky, and G. Wang, NMR studies of aurein 1.2 analogs, Biochim. Biophys. Acta 1758 (2006) 1203-1214.

43) A. Schmidtchen, L. Ringstad, G. Kasetty, H. Mizuno, M.W. Rutland, and M. Malmsten, Membrane selectivity by W-tagging of antimicrobial peptides, Biochim. Biophys. Acta 1808 (2011) 1081-1091.

44) O.G. Mouritsen and M.J. Zuckermann, What’s so special about cholesterol?, Lipids 39 (2004) 1101-1113.

45) M. Pasupuleti, A. Schmidtchen, A. Chalupka, L. Ringstad, and M. Malmsten, End-tagging of ultra-short antimicrobial peptides by W/F stretches to facilitate bacterial killing, PLoS One 4 (2009) e5285, 1-9.

46) M. Pasupuleti, A. Chalupka, M. Mörgelin, A. Schmidtchen, and M. Malmsten, Tryptophan end-tagging of antimicrobial peptides for increased potency against *Pseudomonas aeruginosa*, Biochim. Biophys. Acta 1790 (2009) 800-808.

47) M. Malmsten, G. Kasetty, M. Pasupuleti, J. Alenfall, and A. Schmidtchen, Highly selective end-tagged antimicrobial peptides derived from PRELP, PLoS One 6 (2011) e16400, 1-13.

48) J. Henriksen, A.C. Rowat, E. Brief, Y.W. Hsueh, J.L. Thewalt, M.J. Zuckermann, and J.H. Ipsen, Universal behavior of membranes with sterols, Biophys. J. 90 (2006) 1639-1649.

49) J.A. Ember, N.L., Johansen, and T.E. Hugli, Designing synthetic superagonists of C3a anaphylatoxin, Biochemistry 30 (1991) 3103-3612.

50) A.A. Strömstedt, M. Pasupuleti, A. Schmidtchen, and M. Malmsten, Oligotryptophan-tagged antimicrobial peptides and the role of the cationic sequence, Biophys. Biochim. Acta 1788 (2009) 1916-1923.

Figure Captions

Figure 1. Schematic illustration of the cell walls of Gram-negative (a) and Gram-positive (b) bacteria.

Figure 2. Schematic illustration of different modes of AMP interaction with lipid membranes.

Figure 3. (a) Relationship between lipopeptide activity against *S. aureus*, and their ability to induce liposome leakage. Activities were normalized with those of the most potent peptide. Results are shown for C_{16}-KAAK (open square), C_{14}-KAAK (open diamond), C_{12}-KAAK (open circle), C_{16}-KLLK (reversed open triangle), C_{14}-KLLK (filled triangle), C_{12}-KLLK (filled diamond), C_{16}-KKKK (open square), C_{16}-KGGK (open diamond), C_{16}-GIGK (filled star circle), and C_{16}-KKEK (open star circle) (36). (b) Adsorption of $C_{14}K_n$ lipopeptides at the silica/water interface from water, pH 6.5. Results are shown for $C_{14}K_1$ (open circles), $C_{14}K_2$ (filled diamonds), $C_{14}K_3$ (filled squares), and $C_{14}K_1$ (open triangles) (35).

Figure 4. Influence of lipopeptide self-assembly on antimicrobial effect, given as minimum bactericidal concentration, of fatty acid-conjugated AKK peptides as a function of tail length for *E. coli* DH5α (diamonds), *E. coli* ML-35 (circles), and *S. epidermidis* (squares). The dashed line represents the onset of lipopeptide micellization. (37)

Figure 5. (a) Structure of fengomycin. (b) Surface pressure increase following fengomycin addition (0.5 μM) as a function of initial surface pressure for monolayers formed by C_{24}-ceramide (N-lignoceroyl-D-sphingosine) (open circles), C_{24}-ceramide/cholesterol (3/1 mol/mol)

(filled circles), C_{24}-ceramide/cholesterol (2/1 mol/mol) (open squares), and C_{24}-ceramide/cholesterol/lignoceric acid (1/1/1 mol/mol) (filled squares), the latter mimicking *stratum corneum* lipids (38). (c) Lipopeptides frequently display hemolysis and toxicity against eukaryotic cells, which decreases with acyl chain length. Results are shown for (D)-L_6K_6-C_{14} and (D)-L_6K_6-C_{16} (upper curves), while lower curves are for (D)-L_6K_6, (D)-L_6K_6-C_{10} and (D)-L_6K_6-C_{12} (39).

Figure 6. (a) Effect of W-tagging of GKH17 on its antimicrobial activity, as assessed by viable count assay, against *S. aureus* in 10 mM Tris, pH 7.4, 0.15 M NaCl (upper panel) or the same buffer containing 20% human plasma (lower panel). (b) Effect of tag length on bacteria permeabilization. *S. aureus* was incubated with GKH17 and the indicated end-tagged variants (all at 30 µM), and analysed with electron microscopy. As can be seen, increasing the W-tag length increases bacteria wall permeabilization (40).

Figure 7. (a) Antimicrobial effect, as determined by radial diffusion assay (RDA; the larger the inhibition zone, the more potent the peptide) in 10 mM Tris, pH 7.4, with additional 150 mM NaCl, of GRR10W4N against Gram-positive *S. aureus*, Gram-negative *P. aeruginosa* and *E. coli*, and fungi *C. albicans* and *C. parapsilosis* clinical isolates. (b) Cytotoxicity of GRR10W4N, monitored by LDH release (left), MTT assay (middle) and hemolysis (right). For comparison, data for the control peptides GRR10, GRR10N, LL-37, and omiganan are included as well. As can be seen, the W-tagged peptide displays potent broad-spectrum antimicrobial effects at low maintained cytotoxicity. (43)

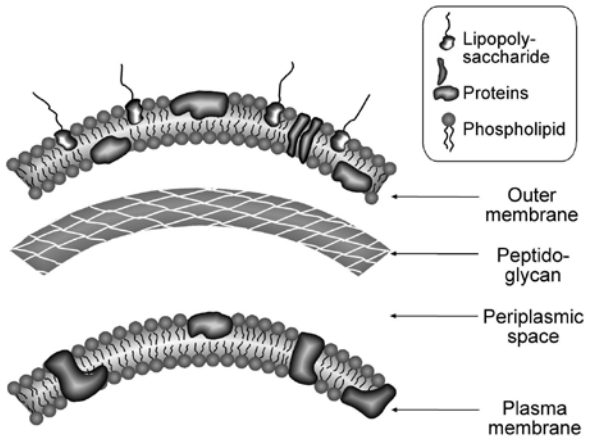
Figure 8. (a) Peptide-induced liposome leakage for GRR10W4N (GRRPRPRPRPWWWW-NH_2) at 10 mM Tris, pH 7.4. Shown in (b) and (c) is the adsorption of the same peptide to supported lipid bilayers, as well as insertion of GRR10W4N to DOPC, DOPC/cholesterol, and DOPE/DOPG monolayers from Tris buffer, pH 7.4, at a surface pressure prior to peptide addition of 30 mN/m (43).

Figure 9. Effects of truncation on antimicrobial and cytotoxic effects of W-tagged peptides. Antibacterial activity was assessed by RDA in the presence and absence of 0.15 M NaCl against *E. coli* and *S. aureus*. Shown also are effects of the peptides on human erythrocytes in the hemolysis assay (45).

Figure 10. Activities of GKH17 and GKH17WWWWW in *P. aeruginosa ex vivo* infection models. (a) Contact lenses were cut into 4 pieces, with bacterial suspension placed on each piece and incubated for 2 h. After peptide incubation and rinsing, the number of bacteria (CFU) was determined. (b) Activities of peptides in an *ex vivo* skin infection model, in which punch biopies were made to pig skin, and the epidermal parts removed, leaving a dermal wound. The wounded area was infected by adding *P. aeruginosa* (clinical chronic ulcer isolate). After an incubation time of 2 h, peptides were applied and incubated for 4 h. Skin biopsies were taken, and CFU determined (46).

Figure 1.

(a) Gram-negative



(b) Gram-positive

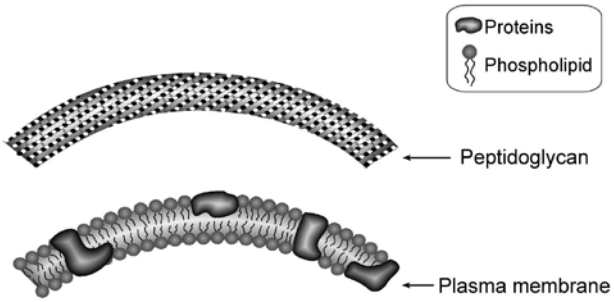


Figure 2.

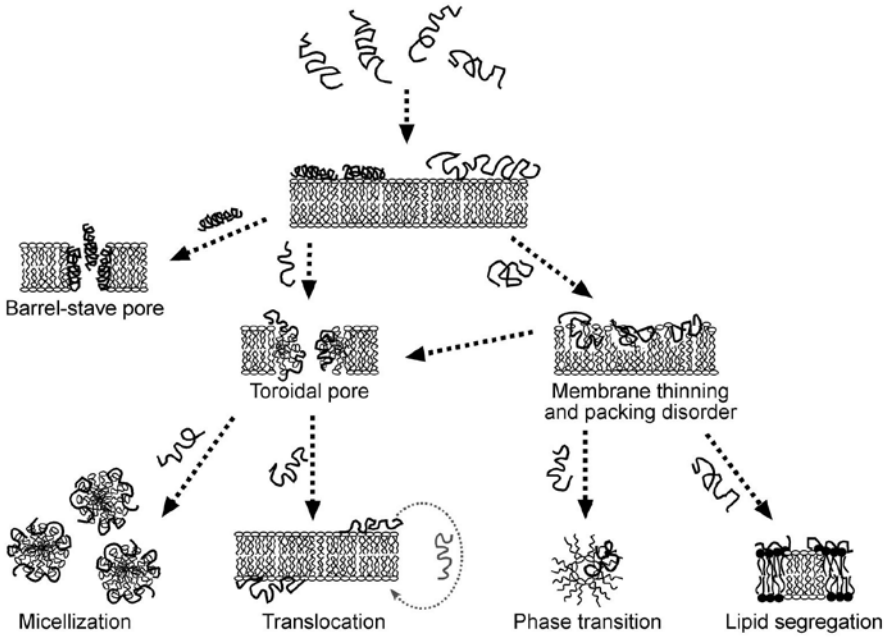
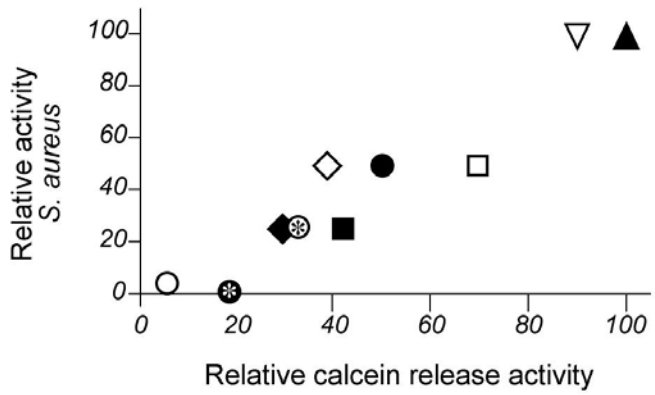

Barrel-stave pore
Toroidal pore
Membrane thinning
and packing disorder
Micellization
Translocation
Phase transition
Lipid segregation

Figure 3.

(a)


Relative activity
S. aureus
100
80
60
40
20
0
0
20
40
60
80
100
Relative calcein release activity

(b)

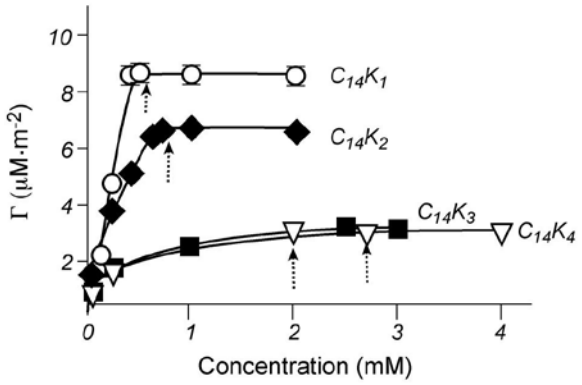

Γ (μM·m-2)
10
8
6
4
2
0
1
2
3
4
Concentration (mM)
C14K1
C14K2
C14K3
C14K4

Figure 4.

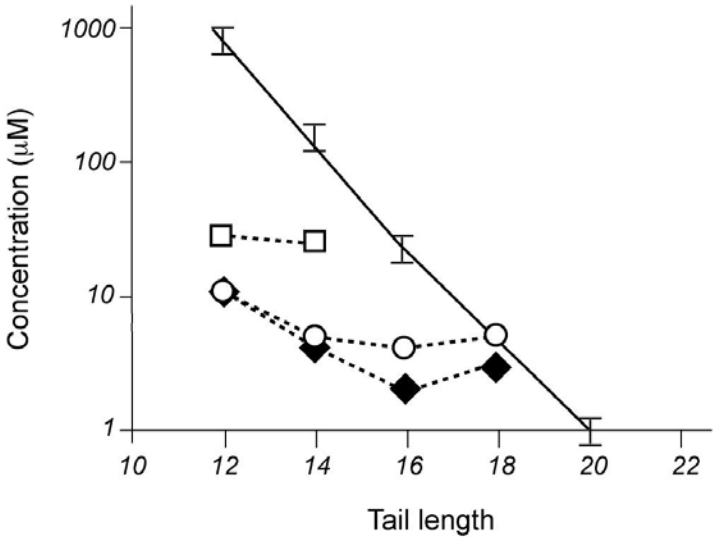
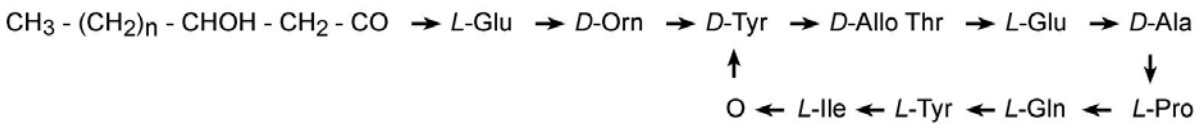
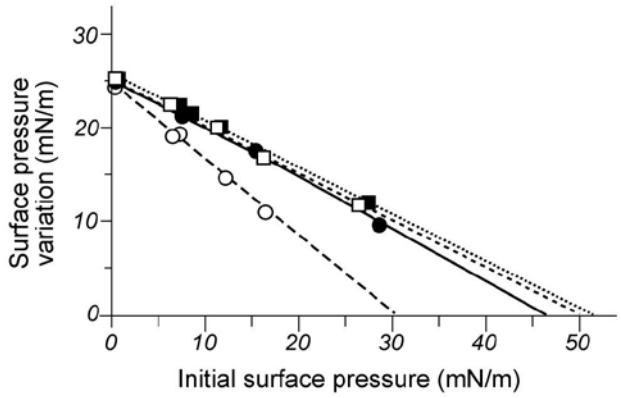


Figure 5.

(a)


CH3 - (CH2)n - CHOH - CH2 - CO
L-Glu
D-Orn
D-Tyr
D-Allo Thr
L-Glu
D-Ala
L-Pro
L-Gln
L-Tyr
L-Ile
O

(b)


Surface pressure variation (mN/m)
Initial surface pressure (mN/m)
0
10
20
30
40
50

(c)

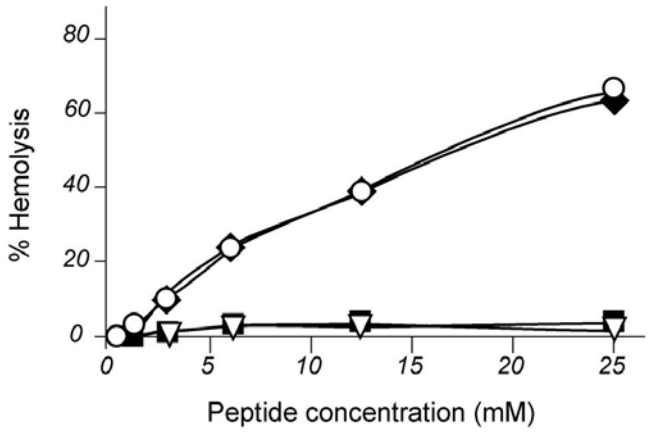
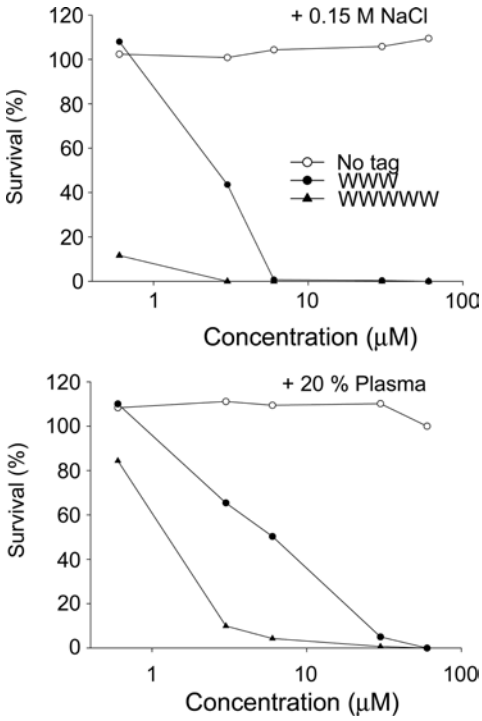

% Hemolysis
Peptide concentration (mM)
0
20
40
60
80
5
10
15
20
25

Figure 6.

(a)


+ 0.15 M NaCl
Survival (%)
120
100
80
60
40
20
0
No tag
WWW
WWWWW
1
10
100
Concentration (μM)
+ 20 % Plasma
Survival (%)
120
100
80
60
40
20
0
1
10
100
Concentration (μM)

(b)

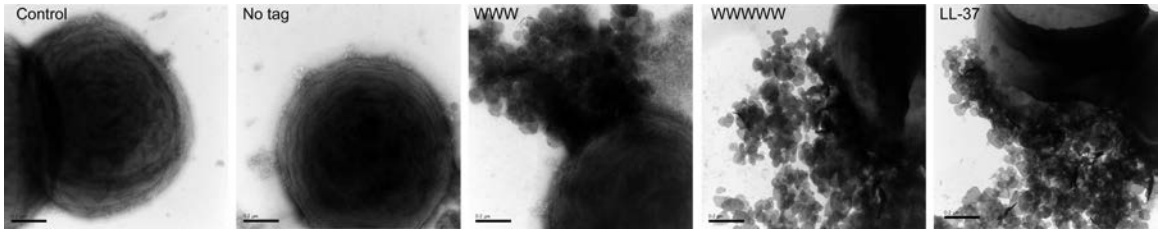
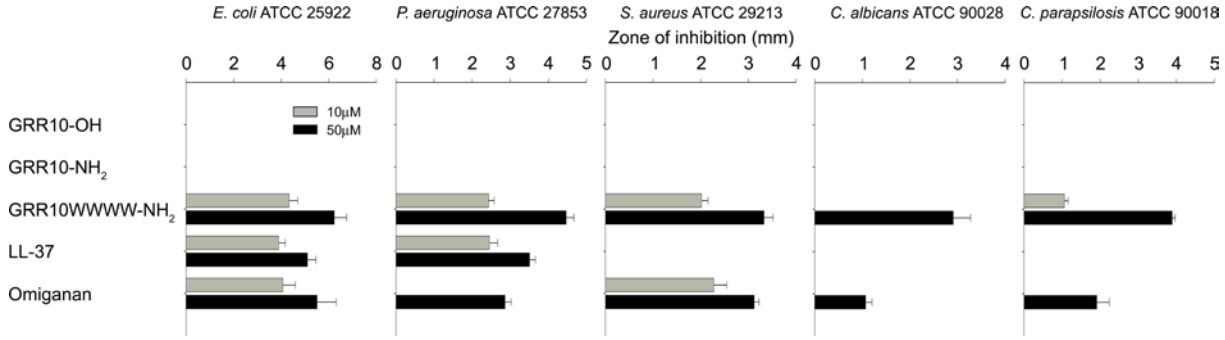

Control
No tag
WWW
WWWWW
LL-37

Figure 7.

(a)


E. coli ATCC 25922
P. aeruginosa ATCC 27853
S. aureus ATCC 29213
C. albicans ATCC 90028
C. parapsilosis ATCC 90018
Zone of inhibition (mm)
10μM
50μM
GRR10-OH
GRR10-NH2
GRR10WWWW-NH2
LL-37
Omiganan

(b)

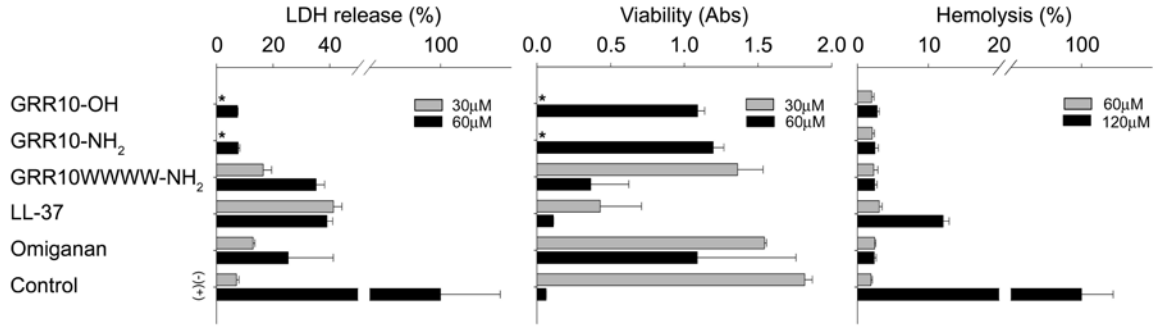
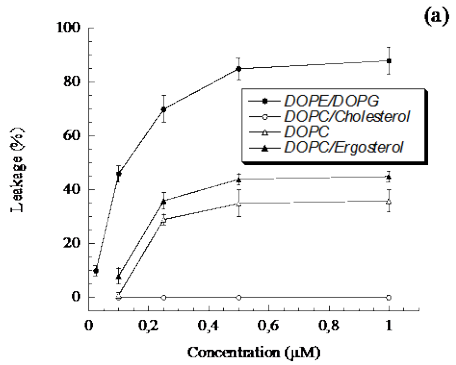
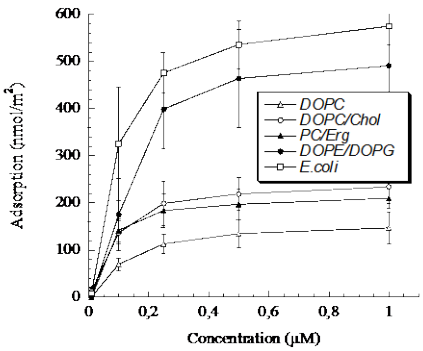

LDH release (%)
Viability (Abs)
Hemolysis (%)
30μM
60μM
60μM
120μM
GRR10-OH
GRR10-NH2
GRR10WWWW-NH2
LL-37
Omiganan
Control
(+)(-)

Figure 8.

(a)


(a)
Leakage (%)
Concentration (μM)
DOPE/DOPG
DOPC/Cholesterol
DOPC
DOPC/Ergosterol

(b)


Adsorption (nmol/m²)
Concentration (μM)
DOPC
DOPC/Chol
PC/Erg
DOPE/DOPG
E.coli

(c)

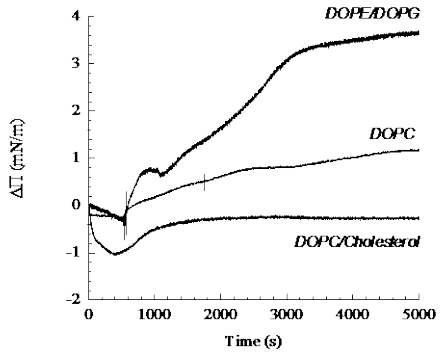

ΔΠ (mN/m)
Time (s)
DOPE/DOPG
DOPC
DOPC/Cholesterol

Figure 9.

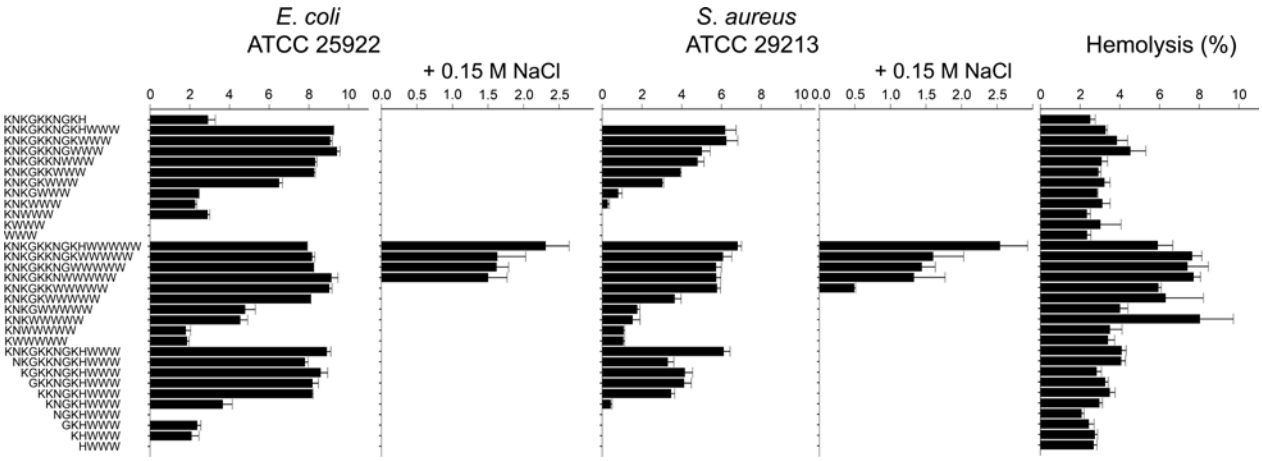
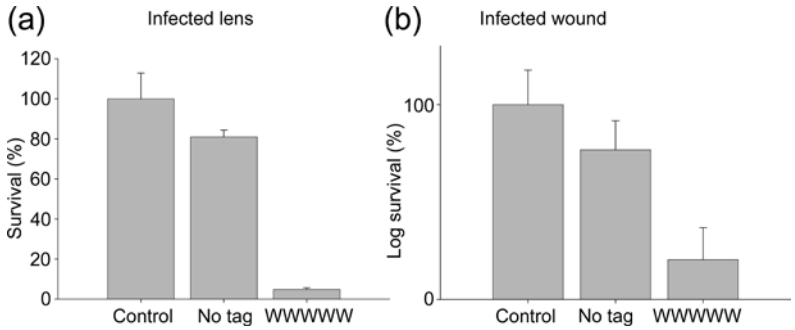

E. coli
ATCC 25922
+ 0.15 M NaCl
S. aureus
ATCC 29213
+ 0.15 M NaCl
Hemolysis (%)
KNKGKKNGKH
KNKGKKNGKHWWW
KNKGKKNGKWWW
KNKGKKNGWWW
KNKGKKNWWW
KNKGKKWWW
KNKGKWWW
KNKGWWW
KNKWWW
KNWWW
KWWW
WWW
KNKGKKNGKHWWWWW
KNKGKKNGKWWWWW
KNKGKKNGWWWWW
KNKGKKNWWWWW
KNKGKKWWWWW
KNKGKWWWWW
KNKGWWWWW
KNKWWWWW
KNWWWWW
KWWWWW
KNKGKKNGKHWWW
NKGKKNGKHWWW
KGKKNGKHWWW
GKKNGKHWWW
KKNGKHWWW
KNGKHWWW
NGKHWWW
GKHWWW
KHWWW
HWWW

Figure 10.


(a)
Infected lens
Survival (%)
120
100
80
60
40
20
0
Control
No tag
WWWWW
(b)
Infected wound
Log survival (%)
100
0
Control
No tag
WWWWW