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Promising antimicrobial agents designed from natural peptide templates

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ABSTRACT

Treatment of infectious diseases is a paramount healthcare issue as the number of multidrug resistant pathogens rise rendering our aging small-molecule antibiotics ineffective. Innovation and discovery in new molecular species that are active against novel targets is vital to meet the challenges of resistance development. The ability of host-defense, or antimicrobial, peptides (AMPs) to selectively target the harmful microbial membrane over that of a host's is a unique characteristic making these innate immune effectors promising candidates to fill the growing therapeutic void. Despite nearly two decades of active research into their selective mechanism against pathogens, few peptides have been found suitable for pharmaceutical applications. Fundamental structure–activity principles underlying the physiochemical properties of AMPs have guided the development and design of synthetic alternatives to peptide-based drugs. Here we first review work in understanding the mechanism and membrane selectivity of AMPs as it provides a good basis for the interpretation of other membrane-active agents as the same physical and chemical driving forces are at work. Recent advances in the rational design of synthetic mimics of antimicrobial peptides (SMAMPs) will also be discussed. Emphasis is placed on the paradigm shift that a rigid secondary structure is not required for the membrane-disruptive ability of SMAMPs.

1. Introduction

1.1. Antibiotic resistance necessitates innovation in new pharmaceuticals

Alexander Fleming's happenstance discovery in 1928 of the compound later known as penicillin [1], would prove to be a life-saving resource against the public health burden of microbial induced illness. Oxford researchers Howard Florey and Ernest Chain, who later shared the 1945 Nobel Prize in Medicine with Fleming, took his work one step further using the *penicllium* mold isolate to treat bacterial infections both in live mice and humans with remarkable success [2]. The potential of penicillin as a drug to save millions of lives was soon realized as it became mass produced and widely available during wartime 1940s. Fleming's Nobel Lecture heeds caution to the wide spread use of this new class of drug stating: "I would like to sound one note of warning. Penicillin is to all intents and purposes non-poisonous so there is no need to worry about giving an overdose and poisoning the patient. There may be a danger, though, in underdosage. It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same has occasionally happened in the body" [3]. An impending health crisis indeed looms as multidrug resistant pathogens rise and emphasis on antibiotic research and development declines.

Various targets within microbial biosynthetic pathways serve as points of inhibition for small-molecule antimicrobial agents to

Abbreviations: AFM, atomic force microscopy; AMP, antimicrobial peptide; CD, circular dichroism; CL, cardiolipin; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphatidylcholine; Di(16:1)PE, 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphatidylethanol amine; Di(18:1-Δ9-trans)PE, 1,2-dielaidoyl-*sn*-glycero-3-phosphatidylethanol amine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylserine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3- phosphatidylethanolamine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylglycerol; d-DPPG, deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylglycerol; DSC, differential scanning calorimetry; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine; GIXD, grazing incidence X-ray diffraction; GUV, giant unilamellar vesicle; HC_{50}, hemolytic concentration; MD, molecular dynamics; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; NMR, nuclear magnetic resonance; OCD, oriented circular dichroism; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3- phosphatidylethanolamine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol; PS, phosphatidylserine; RBC, red blood cell; SAXS, small-angle X-ray scattering; SFG, sum frequency generation; SMAMPs, synthetic mimics of antimicrobial peptides; SUV, small unilamellar vesicle; XR, X-ray reflectivity.

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impede the virulence of pathogens (Fig. 1). Though these mechanisms yield therapeutic specificity, the ease with which pathogens mutate coupled with excessive use of these drugs selects for microbes that have some intrinsic or acquired mechanism of resistance. Multidrug resistant mechanisms typically rely on drug inactivation through enzyme-mediated degradation/modification, target site mutation, and reduced drug accumulation owing to limited uptake or enhanced efflux [4]. Although these resistant species are initially rare (for example, 1 in 10^8), in the continuing presence of the selecting drug the resistant bacteria become more populous than their dying neighbors [5]. For instance, within a year of the introduction of first-generation penicillins, resistant *Staphylococcus aureus* strains were identified, and a decade later, β-lactam resistance had spurred the development and introduction of methicillin [5]. The useful lifetime of methicillin was soon cut short with the growing occurrence of MRSA strains in hospitalized patients during the 1960s [6]. Treatment then relied on the drug of last resort, vancomycin. It is estimated that over 90,000 invasive MRSA infections occur annually in the United States [6] with health care costs from antibiotic-resistant infections estimated at more than $20 billion per year [7]. Clinically important microbes that are rapidly developing resistance to available treatments include bacteria that cause pneumonia, ear infections, and meningitis (e.g. *Streptococcus pneumonia*), skin, bone, lung, and bloodstream infections (e.g. *Staphylococcus aureus*), urinary tract infections (e.g. *Escherichia coli*), foodborne infections (e.g. *Salmonella* or *E. coli*), and infections transmitted in healthcare settings (e.g. enterococci, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Klebsiella* species). Antibiotic resistance has proven to be a matter of time rather than a matter of if.

1.2. Innovation from natural peptide-based templates

Antibiotic natural products display rich and varied architectural scaffolds, densely decorated with functional groups to provide specific interaction with, and recognition by, targets in pathogenic species (Fig. 1). By precedent, the development of new antimicrobial drugs has extensively relied on the re-engineering of these archetypical scaffolds with minor modifications to maintain potency against conventional inhibition targets outlined in Fig. 1. The approval of new anti-infectives by the US Food and Drug Administration has dramatically fallen in the past two decades (Fig. 1 in Ref. [8]) contributing to a growing pharmacological void. To meet the challenges of resistance development there is a need for innovation and discovery in new molecular entities that are active against novel targets. Just as generations of medicinal chemists have mined natural product scaffolds for new small-molecule drug candidates, nature again can serve as a muse to propel innovation in a new class of antimicrobial agents that show both low susceptibility to multidrug resistant mechanisms and high activity against a wide range of microorganisms.

Peptides as host-defense effectors were first discovered in the humoral immune system of silk moths (*Hyalophora cecropia*) [9]. Today over a thousand of these AMPs have been identified and found to be an ubiquitous innate immune response in many living organisms, including invertebrates, vertebrates, and plants. Upon infection AMPs target a wide range of bacterial, fungal, and even viral species. Rather than recognizing cell membrane expressed targets, that are easily mutative and hence confer resistance, nature has evolved AMPs to exploit the fragility of cellular membranes by disrupting the barrier function of a target pathogen's membrane.

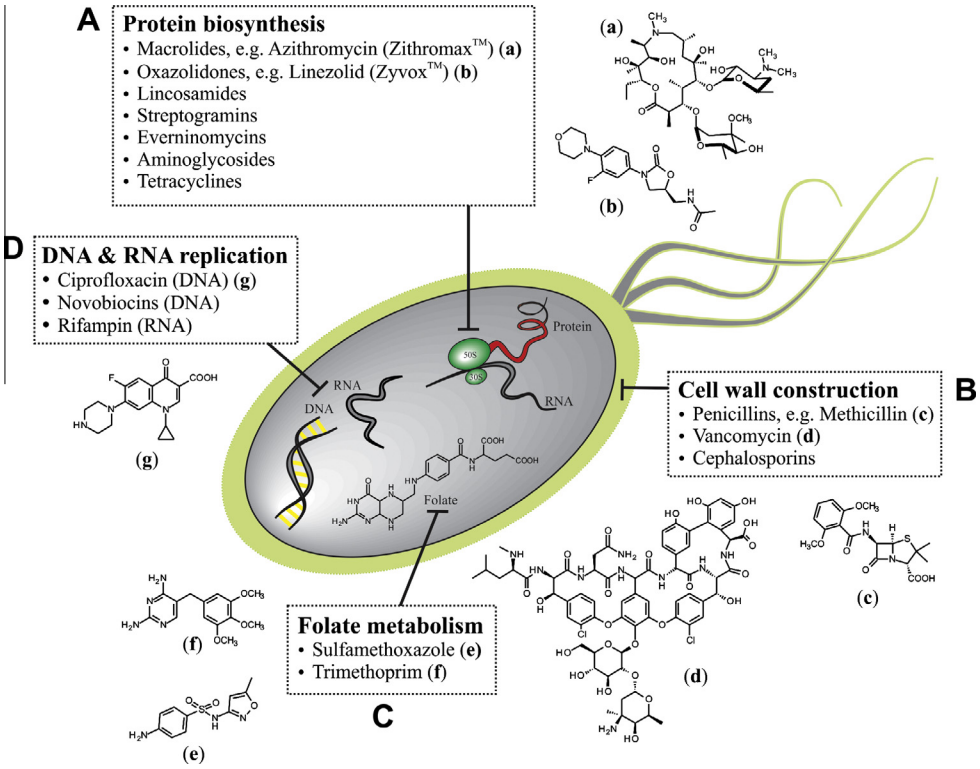

Fig. 1. Principal bacterial targets for antibiotic action. (A) Protein biosynthesis: antibiotics in this class commonly bind to the bacterial ribosome impeding translation. (B) Cell wall construction: bacteria have a protective cell wall composed of strands of peptidoglycan, a polymer comprised of alternating *N*-acetylglucosamine and *N*-acetyl-muramic acid hexoses. Inter-strand cross-linking is achieved through an amide bond formation between short peptide chains on the individual peptidoglycan strands. Antibiotics such as penicillin and vancomycin prevent cross-linking of strands, thereby weakening the structural integrity of the cell wall. (C) Folate metabolism: antibiotics such as sulfamethoxazole and trimethoprim block steps in the metabolism of folate, a precursor in the synthesis of thymine, which in turn is an essential component in DNA. (D) DNA and RNA replication: ciprofloxacin and novobiocins bind to DNA gyrase preventing the enzyme from relieving strain as the DNA double helix is being unwound by helicases. Rifampin prevents the attachment of RNA polymerase to DNA, inhibiting transcription. The antibiotics are correlated with their chemical structures by the bold-faced numbers in parentheses. This figure was inspired by the material presented in Ref. [5].

Although a diversity of sequences and structures with limited homology exist between organismal species, a fundamental structural principle unites all classes of AMPs – the ability of the molecule to adopt a well-defined amphipathic shape in which groups of hydrophobic and cationic amino acids are spatially arranged in distinct regions of the molecule. Membrane binding is facilitated by an electrostatic interaction between these predominately cationic peptides and the bacterial membrane surface which is heavily populated with negatively charged phospholipid head groups. Upon adsorption of the peptide, the amphiphilic secondary structure of AMPs favorably allows the insertion of lipophilic residues into the membrane. Eventually disruption through increased permeation towards molecular and ionic compounds occurs through transmembrane pore formation. "Despite their ancient lineage, AMPs have remained effective defensive weapons, confounding the general belief that bacteria, fungi, and viruses can and will develop resistance to any conceivable substance [10]." These natural peptides are less than ideal drug candidates due to toxicity concerns, low bioavailability following dosage, and high production costs. Structure–activity studies have shown that various parameters such as stability and adoption of a secondary structure, balance of hydrophobic-to-hydrophilic residues, net charge, and supramolecular organization are critical to the lytic activity of AMPs. Sequence-specific oligomers that mimic critical aspects of AMPs have been designed to overcome the limitations of peptide based drugs.

This review begins by looking at AMPs by delving into their mechanism of interaction and their discriminatory behavior believed to be based upon a membrane compositional difference between host and pathogen. Emphasis is placed on providing recent experimental evidence that sheds light on the underlining physiochemical principles of AMP activity as the same parameters guide the structural design of synthetic materials that mimic AMPs and their membrane activity. Close attention will be given to how lipid compositions effect SMAMP activity in the representative classes discussed. Though a rigid secondary structure is crucial for AMP activity, recent work will be highlighted where synthesis of sequence-random SMAMPs that adopt irregular amphiphilic structures yield both selective and active polymers.

2. Antimicrobial peptides

AMPs defend a wide array of living species, with representatives such as amoeboid protozoa, plants, insects, amphibians, and mammals from pathogenic infection. The primary amino acid sequences of the peptides discussed in this review are provided in Table 1. These small host-defense molecules (<100 amino acid residues in size) of the nonspecific innate immune system serve to complement the highly specific but relatively slow adaptive immune system. The gene-encoded peptides may be constitutively expressed or inducibly expressed in response to pathogenic stimuli and are often localized to specific cell or tissue types in the organism most susceptible to infection, such as mucosal epithelia and the skin [10]. Localization primes the peptides to mobilize shortly after infection and act rapidly to neutralize a broad range of microorganisms. As effective self-defense weapons AMPs exhibit a threshold concentration for activity, called the lethal or minimum inhibitory concentration (MIC), below which no effect is observed. The MIC is the minimum amount of a compound required to inhibit bacterial growth by 90–100%. Typically these lethal concentrations are in the micromolar range against microbes and one to two orders of magnitude higher against cells of the host organism. The standard measure of toxicity against eukaryotic host cells is the concentration at which 50% of RBCs lyse, also known as the hemolytic concentration. Without this threshold concentration, AMPs would not be able to discriminate cell type and would harm all cells at any dosage.

2.1. AMPs are membrane-active molecules

Rather than recognizing cell membrane expressed protein targets AMPs induce selective membrane lytic activity against microbes causing depolarization, leakage, and finally cell death [10]. The activity of D-amino acid containing analogs exhibited a similar effectiveness to their native all-L peptide counterparts and thus demonstrated a non-stereospecific interaction between peptide and membrane [11–13]. Electron microscopy of bacterial species exposed to protegrin-1 (PG-1) [14], gramacidin S [15], and the α-helical peptidyl-glycylleucine-carboxyamide peptide (PGLa) [15] for instance revealed that the outer membrane is greatly expanded and the morphology critically altered with numerous folds, protrusions, and mesosomes observed. Recent work from the Belcher lab has utilized high-speed AFM to probe in real time the interaction dynamics of the AMP CM15 on individual *E. coli* cells with nanometer resolution [16]. Live cell imaging with AFM and fluorescence microscopy were used to simultaneously correlate AMP-induced cell surface morphological changes with cell death using a fluorescent indicator of cell viability [16]. These experiments exemplified the ability of AMPs to mount a swift response to pathogenic stimuli. Presumably the interaction was mediated through a two-stage process consisting of an incubation period, lasting from seconds to minutes, followed by an execution stage, in which 50% of the damage was completed in less than one minute [16]. Preferential adsorption of the peptide on the negatively charged bacterial surface drove accumulation until a critical concentration was reached, by which the activity of the peptide engaged and membrane disruption commenced.

Initially an AMP adsorbs at the hydrophilic–hydrophobic interface between the polar headgroup region and the hydrocarbon region of the bilayer. In this process electrostatic interaction can be viewed as playing a regulatory role in target cell selectivity since bacterial membranes include substantial amounts of negatively charged phospholipids whereas mammalian cell membranes are largely zwitterionic. When the peptide interacts with the lipid headgroups, the added cross-sectional area of the adsorbed peptide would be matched by a corresponding increase in the area of the hydrocarbon region. Lipid monolayers prove to be advantageous systems for binding studies of AMPs since their composition and packing density can be directly controlled to mimic the outer leaflet of a particular cellular membrane [17]. Powerful interface-sensitive techniques such as GIXD and specular XR are complementary tools uniquely fit to probe both the in-plane changes of lipid packing structure and changes in the density and thickness of thin layers upon peptide-membrane association, respectively [18]. These X-ray scattering techniques have been utilized to study the perturbative effect upon binding of PG-1 [14,19] and LL-37 [20] to lipid monolayers. The XR data indicated that both peptides adsorb and insert more readily into DPPG monolayers as compared to counterpart monolayers comprised of zwitterionic DPPC and DPPE lipids. Introduction of the peptides to the subphase resulted in complete disappearance of Bragg peak signatures for an ordered structure in DPPG monolayers, whereas less disruption was seen in the presence of DPPC and DPPE monolayers [14,19,20]. A loss in lipid layer crystallinity resulted from changes in the aliphatic chain conformation from *trans* to *gauche*, increasing the cross-sectional area of the lipid chain to accommodate the addition of peptide. Epiflouresence microscopy additionally confirmed that peptide incorporation had a fluidizing effect on the lipid film, disordering the lipid tailgroup packing as evident by a decrease in the fractional area occupied by the liquid-condensed phase [14,20,21]. Interfacial studies of the ovine α-helical peptide SMAP-29 [22] and the antimicrobial frog

Table 1
Primary sequences of peptides presented in this review

Alamethicin[a]	Ac-[1]Aib-Pro-Aib-Ala-[5]Aib-Ala-Gln-Aib-Val-[10]Aib-Gly-Leu-Aib-Pro-[15]Val-Aib-Aib-Gln-Gln-[20]Phl
Aurein 1.2	[1]Gly-Leu-Phe-Asp-[5]Ile-Ile-Lys-Lys-Ile-[10]Ala-Glu-Ser-Phe-NH_2
Dermaseptin	[1]Ala-Leu-Trp-Lys-[5]Thr-Met-Leu-Lys-Lys-[10]Leu-Gly-Thr-Met-Ala-[15]Leu-His-Ala-Gly-Lys-[20]Ala-Ala-Leu-Gly-Ala-[25]Ala-Ala-Asp-Thr-Ile-[30]Ser-Gln-Gly-Thr-Gln
Gramacidin S[a,b]	cyclo([1]Val-Orn-Leu-D-Phe-[5]Pro-[10]Pro-D-Phe-Leu-Orn-Val)
Indolicidin	[1]Ile-Leu-Pro-Trp-[5]Lys-Trp-Pro-Trp-Trp-[10]Pro-Trp-Arg-Arg
LL-37	[1]Leu-Leu-Gly-Asp-[5]Phe-Phe-Arg-Lys-Ser-[10]Lys-Glu-Lys-Ile-Gly-[15]Lys-Glu-Phe-Lys-Arg-[20]Ile-Val-Gln-Arg-Ile-[25]Lys-Asp-Phe-Leu-Arg-[30]Asn-Leu-Val-Pro-Arg-[35]Thr-Glu-Ser
Magainin-2	[1]Gly-Ile-Gly-Lys-[5]Phe-Leu-His-Ser-Ala-[10]Lys-Lys-Phe-Gly-Lys-[15]Ala-Phe-Val-Gly-Glu-[20]Ile-Met-Asn-Ser
MSI-78, A Magainin analogue	[1]Gly-Ile-Gly-Lys-[5]Phe-Leu-Lys-Lys-Ala-[10]Lys-Lys-Phe-Gly-Lys-[15]Ala-Phe-Val-Lys-Ile-[20]Leu-Lys-Lys-NH_2
Melittin	[1]Gly-Ile-Gly-Ala-[5]Val-Leu-Lys-Val-Leu-[10]Thr-Thr-Gly-Leu-Pro-[15]Ala-Leu-Ile-Ser-Trp-[20]Ile-Lys-Arg-Lys-Arg-[25]Gln-Gln-NH_2
MSI-103, A Melittin analogue	[1]Lys-Ile-Ala-Gly-[5]Lys-Ile-Ala-Lys-Ile-[10]Ala-Gly-Lys-Ile-Ala-[15]Lys-Ile-Ala-Gly-Lys-[20]Ile-Ala-NH_2
PGLa	[1]Gly-Met-Ala-Ser-[5]Lys-Ala-Gly-Ala-Ile-[10]Ala-Gly-Lys-Ile-Ala-[15]Lys-Val-Ala-Leu-Lys-[20]Ala-Leu-NH_2
PG-1[b,c]	[1]Arg-Gly-Gly-Arg-[5]Leu-Cys-Tyr-Cys-Arg-[10]Arg, H_2N-Arg-Gly-Val-[15]Cys-Val-Cys-Phe-Arg
RTD-1[b,c]	[1]Gly-Phe-Cys-Arg-[5]Cys-Leu-Cys-Arg-Arg, Arg-Thr-Cys-[15]Ile-Cys-Arg-Cys-Val-[10]Gly
SMAP-29	[1]Arg-Gly-Leu-Arg-[5]Arg-Leu-Gly-Arg-Lys-[10]Ile-Ala-His-Gly-Val-[15]Lys-Lys-Tyr-Gly-Pro-[20]Thr-Val-Leu-Arg-Ile-[25]Ile-Arg-Ile-Ala-Gly

The *N*- and *C*-terminal protecting group abbreviations are Ac- for acetyl- and NH_2 carboxyamide respectively.
[a] The following abbreviations are used for the non-standard residues: Aib, α-aminoisobutyric acid; Orn, ornithine; Phl, L-phenylalaninol.
[b] Solid lines indicate residue connections in cyclic and β-hairpin structures.
[c] Dilsulfide bonds between cysteine residues are indicated by dotted lines.

skin peptide PGLa [23] reinforced the results seen with PG-1 [14,19] and LL-37 [20] suggesting a common perturbative behavior upon AMP adsorption to the membrane surface.

2.2. Orientation within the membrane determines an inactive vs. active state

Molecular arrangement within the membrane consequently defines the level of peptide activity, as a specific orientation positions opposing faces of the amphipathic structure to different extents towards the hydrophobic bilayer interior or to the polar lipid/water interface. Initially when the peptides embed in the outer leaflet of the membrane they lie parallel with the membrane surface, defined by the long molecular axis in amphipathic α-helices and β-strands, in a so called *S*-state. The adsorbed peptides at the membrane–water interface in effect form a two-dimensional gas, with each molecule having a finite area requirement that gives rise to a bulk lateral surface pressure [24,25]. As the number of adsorbates increases, the lateral pressure may become large enough to provide the free energy necessary to overcome the activation barrier for peptide insertion resultant from a positive membrane edge tension [24]. The peptide insertion state, called an *I*-state, has both faces of the molecule positioned within the slab of the bilayer such that the molecular axis is perpendicular to the plane of the membrane. Though an isolated peptide molecule would not be stable in this alignment, interaction with other peptide monomers to form an oligomeric pore would avoid any unfavorable exposures of the hydrophobic lipid chains to the external aqueous environment. Expanding on established theory by Minton and others on Gibbs adsorption of proteinaceous species to planar surfaces [26,27], Heimburg's theoretical framework described the membrane-bound behavior of peptides as a distribution between a *S*- and *I*-state that correctly captured the concentration-dependent transition between the states observed in experiment [24]. For instance at low concentration the energetic cost of insertion is high and the peptides remained interfacially bound in an inactive *S*-state and no transmembrane pores were formed; however, once a critical peptide-to-lipid molar ratio (P/L^*) was reached the chemical potential of the adsorbed species overcame the edge tension necessary for insertion. Assumption of the *I*-state as an oligomeric pore structure gave rise to a sharp transition between conformers that accurately modeled the experimentally observed threshold concentration for activity in bactericidal assays [24].

Huang and coworkers have similarly portrayed the action of AMPs as a two-state model [28] but instead described the free energy of adsorption as an energy balance between the negative binding energy and the positive elastic energy cost for bilayer deformation [25]. When the peptide partitions asymmetrically into the external leaflet the *S*-state peptide does not fill the volume at the level of the acyl chains and therefore the peptide acts as a spacer at the lipid headgroup region; consequently, the surrounding hydrocarbon moieties fill the additional space by decreasing the order parameter of the lipid acyl chains [29]. It has been previously discussed in this review that a lipid monolayer with an embedded peptide indeed shows acyl chain packing perturbation. When a peptide partitions on the surface of a bilayer, the volume of the lipid acyl chains, to first approximation, remains constant during an order–disorder transition; therefore, the increase in the cross-sectional area per lipid correlates to the decrease in the hydrophobic thickness [30]. Elastic theory predicts that the bilayer deformation energy is proportional to the square of the amplitude of the thinning [30–32]. Huang and coworkers have provided extensive X-ray diffraction evidence that while in the *S*-state α-helical peptides such as alamethicin [33,34], melittin [34], and magainin-2 [35] and β-hairpin peptides such as PG-1 [36] ubiquitously cause membrane thinning in direct proportion to the peptide concentration. Using a phase-separated DOPC/DSPC solid-supported phospholipid bilayer as a metric for detecting height change upon peptide interaction, Shaw et al. directly visualized with AFM membrane thinning in gel-phase DSPC domains with

the tryptophan-rich AMP, indolicidin [37]. Moreover, studies involving a synthetically derived AMP from magainin-2, MSI-78, with DMPC supported bilayers revealed that membrane thickness was non-uniformly reduced over the entire bilayer area where distinct domains were thinned by approximately 1 nm [38].

A vast collection of experimental evidence supports the strong influence the *P*/*L* ratio has on the orientation of AMPs with respect to the membrane. OCD spectra of peptides in a multilamellar array of bilayers yield the alignment state of the peptides within the membrane [39]. Particularly α-helical AMPs are well suited for OCD measurements as the absorption of the CD band near 208 nm depends on the alignment between the electrical field component and the transition dipole moment of the π–π^* electronic transition in the amide chromophores in a helix. The presence or absence of a negative band near 208 nm is therefore a discriminator for surface or transmembrane helix orientation, respectively. Many α-helical AMPs such as alamethicin [32,34,40–43], melittin [43–45], magainin [46], and PGLa [47] have universally been shown to undergo a concentration-dependent transition. Though no theoretical basis has yet been developed for interpreting OCD spectra of secondary structures other than α-helices [47], OCD has been applied in a qualitative manner to show the β-hairpin peptide PG-1 [48] and the rhesus theta defensin-1 (RTD-1) [49], an 18-residue peptide with a cyclic structure cross-linked by three disulfide linkages, display two-state orientational behavior that is concentration dependent. Solid-state NMR analysis of macroscopically oriented membranes is an additional powerful technique for yielding angular information and oligomerization states of membrane active peptides through the anisotropic chemical shift or dipolar couplings of isotope labels [50]. Beyond the archetypal ^{2}H, ^{13}C, and ^{15}N isotope labels, the recent incorporation of fluorinated amino acid side chains into peptide primary sequences has provided more comprehensive pictures of the membrane-associated state of peptides as the ^{19}F nucleus has a higher sensitivity and does not suffer from a natural-abundance background [51]. Ulrich and coworkers have applied this technique to probe, for instance, the synergistic formation of a stable heterodimeric transmembrane pore by PGLa and magainin-2. Alone, PGLa below a *P*/*L* ratio of 1/50 to 1/100 (in DMPC) adopted a helix alignment nearly parallel to the bilayer surface with a tilt angle (measured between the helix axis and the membrane normal) of $\tau \sim 98°$ [52]. Above this threshold concentration the tilt angle changed to $\tau \sim 126°$ and the peptide was obliquely immersed in the bilayer with a tilted orientation [52]. However, when the peptide was combined with magainin-2, PGLa tilted much earlier and was able to insert fully in the membrane adopting an *I*-state [52,53].

2.3. Models of AMP membrane action: pore-formation to detergent-like behavior

Above a P/L^* ratio the peptides begin to insert into the membrane and oligomerize presumably as bilayer spanning pores (Fig. 2A); evidence for which has been provided, for instance, by in-plane neutron diffraction [54–57]. Much effort has been placed on determining the structure of the pores as this configuration is largely believed to be the active state by which AMPs kill target cells. The first model of peptide-induced pore formation was proposed by Baumann and Mueller in 1974 to account for the conductance induced by alamethicin in lipid membranes [58]. In their model the alamethicin helices associate to form a bundle with a central aqueous lumen, like a barrel made of peptide staves. The multiple discrete conducting states were nicely attributed to the barrel-stave model in which the conductance state of the pore could be changed when a single monomer joined or leaved the aggregate [44]. Recently Huang and coworkers used X-ray diffraction to directly visualize the structure for the first time of alamethicin-induced pores (Fig. 2B) [59]. Through use of a brominated lipid, multiwavelength anomalous diffraction at the bromine *K* edge was employed to single out the bromine atoms alone amongst the electron density milieu of the surrounding lipids and peptides [59]. Reconstruction of the bromine electron density profile unambiguously showed that indeed the alamethicin pore was of the barrel-stave type consisting of about eight monomers and an inner diameter roughly 1.8 nm in size [59].

Transmembrane peptide pore structures have been explicitly, or sometimes implicitly, described in the literature by a barrel-stave model ever since its introduction. This model was later revised and a new pore structure, toroidal pore, proposed in order to explain the rapid lipid flip–flop coupled with pore formation by the α-helical magainin peptide [60]. The membrane-bound peptides in the proposed toroidal pore (Fig. 2A) force the outer leaflet to bend continuously and fuse with the inner leaflet providing validation for the rapid diffusivity of lipids across leaflets as seen in experiment. A lipid-spacer region of length corresponding to several lipid head group diameters separates the peptides lining the pore. In retrospect this model appears quite natural in view of the strong electrostatic repulsion that would result between highly charged peptides had they been arranged in a barrel-stave fashion. Structural evidence for such lipid-based pores has been recently shown for the peptide segment derived from the pore-forming domain of Bax, an apoptosis-regulating protein (Fig. 2B) [61].

The presence of water-filled cavities resembling the toroidal construct has been implicated for such peptides as PG-1, magainin-2, and melittin; however, recent findings suggested that the well-ordered structure originally proposed by Huang is amenable to revision. The Hong lab has employed solid-state NMR spectroscopy to probe the oligomeric structure of PG-1 while associated in bacterial mimicking membranes comprised of POPE and POPG. Their results suggested that contrary to the canonical model of a toroidal pore, in which the peptides line the lipid pore as monomers, PG-1 instead formed a beta-barrel structure composed of parallel-oriented dimers with tightly associated C strand interfaces [62]. Lipids do form a portion of the pore rim as ^{31}P lineshapes of aligned bilayers indicated a high nonlamellar fraction. The multimeric PG-1 complex was estimated to surround a water pore of ~2.1 nm most likely containing four to five dimer subunits [62]. Using high-resolution AFM, Capone et al. have indeed supported the above results [62] showing that the most probable number of subunits was three or four in a DOPS/POPE membrane [63]. The size distribution of the inner pore diameters was fit to a Gaussian distribution and found to be centered at 2.07 nm, correlating well with the solid-state NMR results discussed before [63].

Melittin and magainin-2 have both been hypothesized to form toroidal pores as well, and as a consequence presumed to operate mechanistically in a similar fashion. However, fluorescent dye leakage assays performed with vesicles have indicated that this is not the case. These experiments reported an all-or-none release of vesicular contents induced by magainin-2 [64,65] whereas melittin induced a graded dye release [66]. Almeida and Pokorny have thoroughly described the two modes of dye release and have provided additional peptide examples that exhibit these leakage modes [67]. Briefly, their description initially has the peptide binding and accumulating on the vesicle surface whereby a mass imbalance across the bilayer creates a membrane tension. After this common initial step, the two modes diverge. In the all-or-none case, peptide-stabilized pores are formed, though a highly organized structure as depicted in an idealized toroidal pore is not necessary. As the lifetime of the pore is long, the entire content of the vesicle is released. On the other hand when the graded mechanism occurs AMPs insert into the membrane with higher probability, transiently permeating the membrane and causing a gradual efflux of dye. As the peptides translocate across the bilayer, a mass balance across the membrane

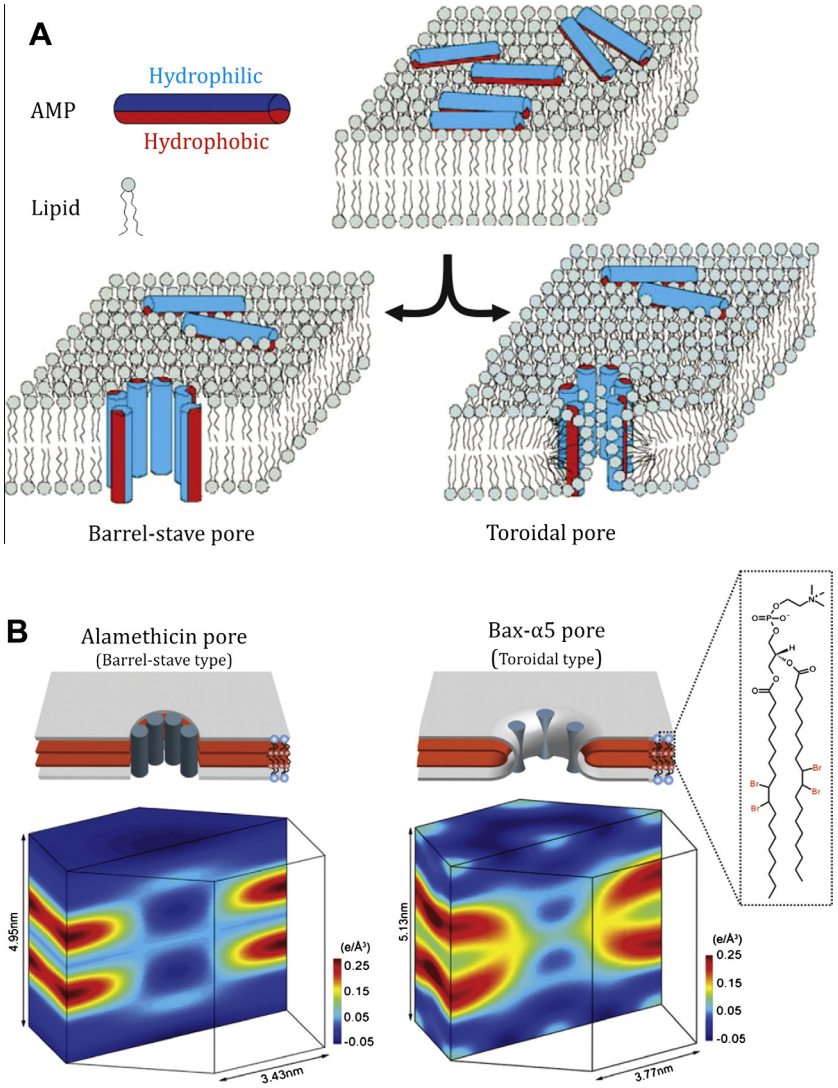

Fig. 2. Proposed pore structures for host defense peptides. (A) At low *P*/*L* ratios, the peptides initially remain bound at the membrane interface in a *S*-state. Above a critical concentration, the peptides insert into the membrane and oligomerize to form bilayer-spanning pores either as the barrel-stave or toroidal type. In the case of toroidal pores, positive curvature strain is induced on the membrane as evident by the orientational change of the lipids surrounding the peptides. Reprinted and modified with permission from Ref. [167]. (B) The normalized electron density distributions of Br atoms present on 1,2-di-(9,10-dibromo)stearoyl-*sn*-glycero-3-phosphocholine lipid molecules were used to reconstruct the pore structures induced by alamethicin and Bax-α5, confirming the barrel-stave and toroidal pore structures. Reprinted and modified with permission from Ref. [61]. Copyright 2008 National Academy of Sciences, USA.

is achieved and the rate of efflux diminishes and eventually ceases. Using coarse-grained MD simulations, Santo and Berkowitz have investigated the difference between magainin-2 and melittin in the self-assembly and possible pore formation in DPPC membranes, obtaining results consistent with dye efflux experiments [68]. The simulations showed that a large number of magainin-2 peptides aggregate at the pore rim in a rather disordered transmembrane fashion, bending the lipids such that the phosphate groups face the pore interior. The large "disordered" toroidal pores were permeated extensively by water reconciling the all-or-none efflux observed in experiments. Conversely, melittin aggregation size was small in comparison to that of magainin-2 and well-defined water channels lined by lipid head groups were never formed. Interestingly, melittin molecules often adopted a U-shaped conformation most likely instigated by the presence of a proline residue. This conformation tended to attach the positively charged terminal ends of the molecule within the same membrane leaflet; in effect, few melittin molecules ever spanned the entire bilayer thickness and were thus unable to drive curvature strain as effectively as magainin-2.

Contrary to the mechanisms described previously in which equilibrium pore structures of a well-defined size are formed, AMPs that disrupt cellular membranes through a non-pore mechanism, known as the carpet model (Fig. 3), instead remain in contact with the lipid headgroup throughout their entire process of membrane permeation and never insert into the hydrophobic core of the bilayer [69,70]. In this model, peptides accumulate on the target membrane surface and extensively cover it in a carpet-like manner. The resulting expansion of the outer leaflet induces strain between the two bilayer leaflets and eventually the tension is released through breakdown of the lipid membrane. Membrane integrity loss may begin through transient defect formation in the bilayer causing translocation of peptides to the interior. Disintegration escalates at high peptide concentrations as micellization occurs. The carpet model was proposed for the first time to describe the mode of action of dermaseptin, an amphipathic α-helix rich in lysines resembling magainins for which an inserted state has been demonstrated [70]. Peptides too short to span the entire length of the bilayer, such as aurein 1.2, are believed to disintegrate lipid membranes through this mechanism [71].

Recent experimental work has shown that the toroidal and carpet models of AMP action may be considered special cases of an overall detergent type interaction between peptide and membrane. Amongst the toroidal pore and carpet models the interaction between peptide and membrane results broadly in a decrease in the order parameter of the lipid acyl chains and induction of considerable curvature strain which in many aspects is reminiscent of detergent–lipid interactions. Detergent solubilization of phospholipid membranes is based on the intercalation of these molecules into the bilayer resulting in a variety of self-assembled structures beyond lamellar bilayers; consequently, detergent solubilization can be approximately described as a transition between different macroscopic assemblies (perforated bilayers, bicelles, micelles, etc.) as a result of compositional changes [72,73]. The supramolecular structures adopted as a consequence of amphiphile intercalation are largely based on the molecular shape of the lipids and the amphiphiles. In order to rationalize the detergent–lipid interactions a simple model that explains the lipid macroscopic phase preference has been suggested in which the lipid molecules have been described by cylinders, cones, truncated cones (wedges), or inverted truncated cones which assemble into macromolecular structures [74]. Following the molecular shape concept a detergent occupies the space of a cone with a positive spontaneous curvature ($c_0 > 0$) where the polar headgroup occupies considerably more space relative to its hydrophobic tail; consequently, detergents form aggregates with positive curvature such as micelles [74]. When an amphipathic peptide partitions into the membrane interface, the peptide does not fill the volume at the level of the acyl chains and therefore the peptide acts as spacers at the lipid headgroup region [72,73]. Consequently the surrounding hydrocarbon moieties compensate by filling the additional space with increased chain interdigitation and the membrane consequently suffers positive curvature strain [72,73]. The resulting phases reflect the energetically most favorable three-dimensional assembly of detergent–lipid or peptide–lipid mixtures.

The detergent-like properties of AMPs has recently been implicated to the function of PG-1, in which pore formation was observed to be a small part of a much more complex array of lipid–peptide structures as seen in Fig. 4A [75,76]. Solid-supported phospholipid bilayer patches were utilized by Lam et al. as a metric for gauging the interfacial activity of PG-1. Bilayer patches in the absence of peptide assumed compact, circular shapes and displayed smooth contours [75–77]. The equilibrium shape of the bilayer patches are attributable to a high interfacial line tension, resultant from an energetic penality exacted to self-assemble lipid molecules at the highly curved cap at the bilayer edge [77,78]. Fusing the outer leaflet with the inner leaflet at the bilayer rim incures steric crowding of the acyl chains but favorably shields the hydrocarbon chains from aqueous exposure [77,78]. Conversely, in the presence of peptide, observed changes in the membrane edge to a rugged and more extended shape can be attributed to a line tension reduction caused by peptide adsorption. The amphiphilic nature of the peptide renderes it line-active; its presence therefore stabilized the edge and hence afforded the formation of a more extended bilayer edge upon its adsorption. An analogous scenario was the one-dimensional Gibbs adsorption of detergents, such as Tween 20, to tension-induced pores in vesicles, where their adsorption increased the stability and lifetime of the pores [79]. Indeed when the bulk PG-1 concentration was increased, the edge of the bilayer appeared to be more susceptible to PG-1 adsorption as compared to the lamellar region of the patch, indicating a reduction in the line tension. The lamellar interior of the patch was further compromised with bilayer-spanning pores and eventually wormlike micelles which extensively thinned the patch from the original DMPC bilayer thickness. Fig. 4B illustrates a classic amphiphilic system involving a binary mixture of short- and long-chain lipids such as DHPC and DMPC that show comparable assemblages to those observed with PG-1 and DMPC. DHPC is line active and hence stabilizes edges of bicelles, elongated (ribbon or wormlike micelle), or porous aggregates [80–82]. The detergent-like behavior of antimicrobial peptides with different secondary structure has recently been implicated as part of their overall disruption mechanism. For instance, stabilization and growth of nanometer-size pores observed in simulations of magainin has been similarly attributed to the induction and stabilization of lipid curvature in "disordered toroidal pores" [68,83]. Through solid-state NMR spectroscopy a shift in the ^{31}P resonance indicating magnetic alignment of the membranes with magainin has been attributed to the formation of disk-like bicelles, perforated membranes, and worm-like micelles [84]. Additionally, the temporin and gomesin peptides showed tubular structures at high-bulk concentrations indicative of a detergent-like interaction [85,86]. The toroidal and carpet models previously described may hence be special cases within a complicated diagram describing the morphological plasticity of peptide-lipid assemblies that are dependent on parameters such as temperature, pH, lipid composition, salt concentration, and cholesterol content.

2.4. The selectivity of AMPs is based on membrane compositional differences

One of the most important characteristics of AMPs is their ability to selectively target the harmful microbial membrane over that of the host cell. This discriminatory behavior is believed to strongly depend on the chemical and structural properties of the lipids that make up the cell membrane. Membrane compositional differences would retard or enhance the efficacy of the peptide activity by affecting the threshold concentration necessary for the onset of disruption. Prokaryotic and eukaryotic cells have distinct membrane lipid compositions; for instance, the bacterial outer membrane include substantial amounts of negatively charged phospholipids such as PG and CL whereas mammalian cell membranes are comprised mainly of PC, PE, sphingomyelin, and cholesterol which are charge neutral at physiological pH. Furthermore, gram-negative bacteria include substantial amounts of the polyanionic molecule lipopolysaccharide in their external monolayer of their outer membrane. Naively, the selectivity of AMPs can be based on the electrostatic attraction of these predominately cationic peptides for the bacterial membrane surface heavily populated with negatively charged lipid components. For example, the antimicrobial peptides PG-1 [14,21], LL-37 [20,87], SMAP-29 [22,87], and PGLa [23] were shown to insert more readily into anionic monolayer films composed of either DPPG or lipid A. Additionally, highly charged AMPs, when bound to a membrane surface, clustered anionic lipids away from zwitterionic ones for charge balance purposes [88–90]. A purely electrostatic argument fails to explain the selectivity of negatively charged AMPs such as dermcidin [91]. Though the hemolytic activity is an order of magnitude higher than the MIC against pathogens, differences amongst several mammalian RBCs demonstrate that AMP selectivity is a more complex interaction than that suggested by a simple electrostatic argument. Case in point, PG-1 exhibited hemolytic activity toward human erythrocytes at high concentrations, but had little effects towards those of goat or sheep [92]. Effects from factors like membrane fluidity, lipid head group identity, and lipid acyl chain length may further explain how the behavior of AMPs is tuned to selectively disrupt bacterial membranes over those of mammals.

Based upon the large difference in the amount of PE present in bacterial membranes over that of eukaryotic membranes (*E. coli* inner membrane ~77% PE vs. human RBC ~22% PE; Ref. [93]), Wong and coworkers have postulated that membranes rich in lipids intrinsically displaying negative curvatures ($c_0 < 0$) enhance the efficacy of AMP disruption through favorable promotion of

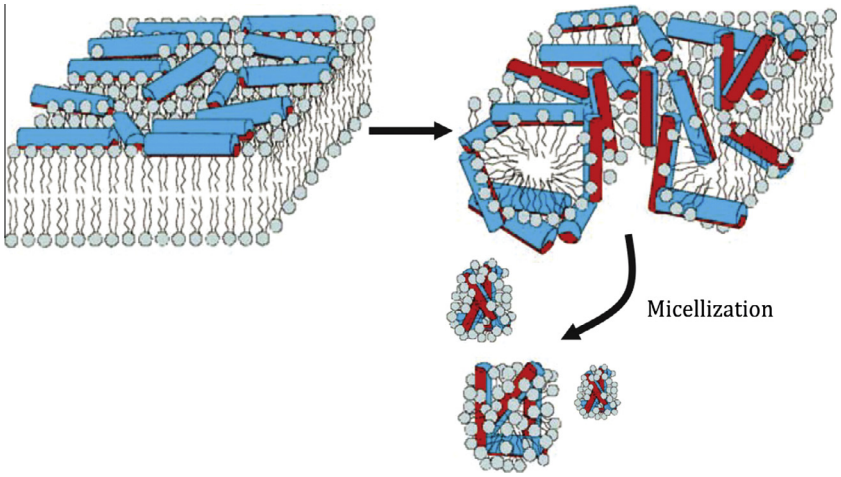

Fig. 3. Carpet model of membrane disruption. Peptides in this model do not oligomerize to form pores of a discrete size. Instead, the peptides initially bind to and cover the surface of the membrane. As strain is imposed on the bilayer, membrane permeation results in eventual micellization. Reprinted and modified with permission from Ref. [167].

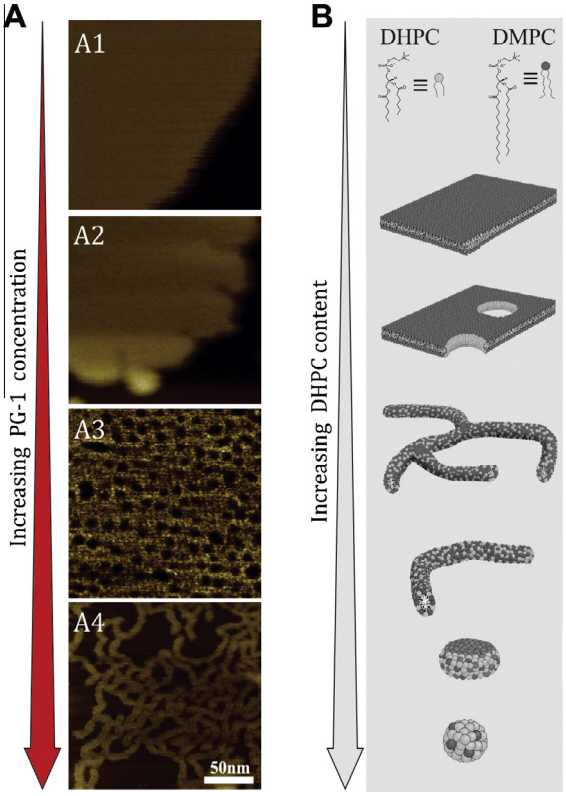

Fig. 4. Antimicrobial action as detergents. (A) Protegrin-1 induced morphological changes of DMPC bilayer patches at 30 °C imaged with AFM. Initially the bilayer edge exhibits a smooth contour dictated by the line tension of the system (A1). Adsorption of the peptide to the bilayer patch induces a stable extended boundary (A2). Noticeable pore formation is evident in the lamellar core of the patch; though the depth of the defects cannot be accurately measured due to the physical size of the AFM (radius of curvature is ~10 nm) (A3). Wormlike micelle structures develop through the entire patch exhibiting an average width of 9 nm (A4). Reprinted and modified with permission from Ref. [76]. (B) The mild detergent DHPC is also line active and when mixed with lipids preferentially occupies the edge in the mixed lipid assembly. The structural transformations observed in the DMPC/DHPC system share strikingly similar characteristics with the DMPC/PG-1 system. With increasing content of DHPC, lamellar structural transformations occur giving way to perforated bilayers, wormlike micelle formation, and eventually onto spherical micelles at high DHPC content. Reprinted and modified with permission from Ref. [80].

saddle-splay ("negative Gaussian") curved topologies [94]. Negative Gaussian curvatures are commonly seen in detergent solubilization of membranes and encompass processes such as pore formation, blebbing, budding, and vesiculation. A free energy penalty is exacted for their formation as the Gaussian curvature modulus in the Helfrich Hamiltonian is negative [95]. However, the addition of lipids with a negative spontaneous curvature, such as those with PE head groups, modifies the Gaussian modulus towards positive values and thereby lowers the free energy barrier towards the formation of structures containing saddle-splay. SAXS was employed to detect the onset of a bicontinuous cubic phase, a membranous phase rich in negative Gaussian curvature, upon introduction of AMPs of the defensin family. Results showed that the arginine-rich defensin peptides induced saddle-splay membrane curvature in a manner that depends on target membrane lipid composition. Since saddle-splay topologies have negative and positive curvatures in orthogonal directions, it is possible that lipids with positive spontaneous curvatures can aid in AMP activity as specific peptides will display asymmetry in the degree of positive and negative curvatures generated. For instance, there is evidence with magainin and melittin that lipids with positive spontaneous curvature instead promoted the formation of pores [96,97]. In addition, Ulrich and coworkers saw that the designer analog of PGLa, MSI-103, was preferentially in a surface bound state in membranes containing DOPE rather than in a membrane inserted state [98]. Upon utilizing membranes comprising of a higher amount of positively curved lipids, MSI-103 changed its orientation to an obliquely immersed tilted state [98]. Debate between which lipid species, positive or negative, is better at promoting AMP-induced membrane disruption is gratuitous when in fact they both can contribute.

Cholesterol, while absent in bacterial membranes, is a major constituent in mammalian cell membranes and is of special interest as a regulator of membrane properties for selectivity purposes in the action of AMPs. For instance the presence of cholesterol has been shown to protect human erythrocytes against magainin-2 [99]. Foremost cholesterol modulates membrane fluidity by influencing the organization of surrounding lipids through changes in their available area and ordering [100]. As first described by Leathes in 1925, the addition of cholesterol in a lipid system is one of non-ideal mixing in which the area per molecule is much lower compared with ideal mixing estimates [101]. The ordering effect cholesterol imparts on the surrounding lipids molecularly originates from re-orientation of the lipid tails to configurations in which the tilt and number of gauche conformations are reduced

(order parameter increased); consequently, the phospholipids are orientated more perpendicular to the membrane plane and the bilayer becomes thicker [102–105]. As a result, the membrane mechanically stiffens, making it more difficult for AMPs to insert and subsequently deform the membrane through curvature-induced strain. This interaction elevates the threshold concentration for insertion and as a consequence the peptides transition to a more surface bound *S*-state (tilt angle decreases) [62,98,106]. The capability of cholesterol to stabilize a bilayer is believed to account for the selectivity of AMPs as its incorporation in mammalian cell membranes can reduce AMPs activity by shifting the disruption susceptibility of the bilayer to a higher peptide dosage regime.

3. SMAMPs: synthetic mimics of antimicrobial peptides

AMPs have many desirable features of a novel class of antibiotics since they show low susceptibility to multidrug resistant mechanisms, combine selective activity against a broad-spectrum of microorganisms, and rapidly mount a response before pathogens have time to multiply. Additionally, the physical nature of membrane disruption makes the development of new drug-resistant strains improbable as profound alterations in membrane composition would be needed to diminish AMP activity and confer effective resistance. Thus AMPs are attractive to fill a pharmacological void caused by the growing number of resistant strains to traditional forms of antibiotic treatments such as penicillin, methicillin, and vancomycin. Despite their popularity, only a few peptides have been found suitable for pharmaceutical applications and are often employed as topical rather than intravenous treatments (see Ref. [107] for a review of current peptide-based drugs on the market and in clinical trials). AMPs are less than ideal drug candidates namely because of the following disadvantages: (1) their susceptibility to proteolytic degradation; (2) poor tissue distribution; (3) toxicity concerns resulting from high dosage concentrations necessary for *in vivo* effectiveness; and (4) the cost of synthesizing peptides is much higher compared to that of conventional small-molecule antibiotics. Structure–activity studies aimed at better understanding the chemical and biophysical attributes that influence the lytic activity of AMPs have inspired synthetic chemists and material scientists to rationally design unnatural, sequence-specific oligomers that mimic the critical characteristics of AMPs. Synthetic mimics of antimicrobial peptides (SMAMPs) include a broad family of molecular entities that are based upon the structure and function of AMPs but whose backbone is not solely based on that of α-amino acids. Typical classes of SMAMPs will be briefly discussed with particular emphasis on how fundamental structure–activity studies on AMPs have influenced the development and design of these synthetic alternatives to peptide-based drugs.

3.1. Importance of mimicking secondary structure

An essential attribute to the activity of AMPs is their adoption of amphiphilic secondary structures that organizes groups of hydrophobic and hydrophilic amino acid residues in spatially arranged domains. This segregation allows AMPs to adopt orientations within the membrane that favorably allow apolar residues to bury into the bilayer core whereby curvature-induced disruption can be enacted. Fig. 5 depicts the well-defined amphiphilic structures adopted by magainin-2 and PG-1. Due to the highly cationic nature of the peptides, those species that fold into α-helices exhibit a random coil structure in aqueous solution from the electrostatic repulsion between charged side chains. Indeed, CD studies have shown, for example, that magainin and indolicidin are unstructured in solution but adopted an α-helical structure upon binding to lipid membranes or detergent micelles [108,109]. Chen et al. suggested that stabilization of the α-helical content of magainin while in solution would enhance the peptide interaction with lipid membranes and hence increase its antimicrobial activity. Substitution of glycine residues with helix promoting alanines improved the antimicrobial activity, though selectivity was inadvertently lowered as the mutants showed increased ability to lyse human RBCs [108]. Complementary results by Everett et al. showed that placement of a helix-breaking proline residue near the middle of the magainin helix (position 10 or 11) substantially reduced the antimicrobial activity [110]. The disulfide bonds present in the secondary structures of β-sheet peptides, such as PG-1 and human defensins, confer conformational rigidity in solution and are vital for their membrane activity; deletion of these covalent linkages greatly diminished their membrane lytic activity [111–113]. Moreover, Hong and coworkers have shown that structural rigidity of PG-1 plays a more important role than the overall net charge in determining its activity [114]. They reported that a PG-1 analog with diminished charge (+3 instead of +6 in the wild type) induced disorder in a POPC:POPG (3:1) lipid system nearly identical to that of the wild type peptide (70% fractional disorder induced by the

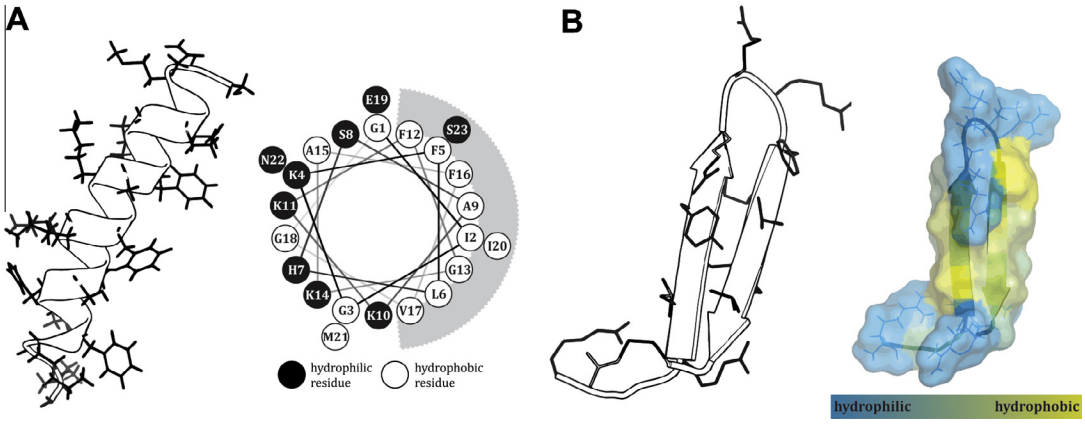

Fig. 5. Polar and apolar residues are segregated in AMPs to form amphilphilic structures. (A) The helical wheel representation for magainin-2 identifies a hydrophobic face on the α-helix as indicated by the shaded area. (B) The rigid structure of PG-1 localizes its positively charged arginine groups at the *N*- and *C*- termini and the β-hairpin turn, consequently surrounding a central hydrophobic core. Molecular representations of the peptides were generated in PyMOL using the PDB files 2MAG and 1PG1 for magainin-2 and PG-1 respectively. The hydrophobic density of PG-1 was rendered as a colored surface based on the hydrophobicity scale derived by Black and Mould (Analytical Biochemistry 1991;193:72–82). The gradation of color from blue to yellow signifies a transition form more to less hydrophilic.

mutant vs. 74% fractional disorder induced by the wild type) as measured by ^{31}P NMR spectra of oriented bilayers. Conversely, a PG-1 analog devoid of disulfide bonds had the lowest fractional disorder at 45%. Presumably the removal of the disulfide bonds rendered the PG-1 mutant structureless and confined it to the membrane surface; absence of a well-defined amphipathic structure negated its ability to insert in the bilayer and cause disruption. The examples presented indicate that a strong correlation exists between the membrane-disruptive ability of an AMP and the conformation it adopts.

3.2. β-Amino acid oligomers

Similar to the global amphipathic structures formed by AMPs, the group of William F. DeGrado first reported the de novo design of antibacterial β-peptides, short polyamides constructed of β-amino acids (where the amino group is bonded to the β carbon rather than the α carbon) that can be made to fold into turns, helices, and sheet-like structures (Fig. 6) [115]. The structural motifs adopted by β-peptides have been thoroughly reviewed in Refs. [116–118]. Since the 14- and 12-helices have precise residue periodicity regarding their hydrogen bonding pattern, polar and apolar side chains can be easily arranged such that they segregate to opposite sides of the helix forming a facially amphipathic molecule upon membrane association. Secondary structure stabilization can be encouraged through proper placement of salt-bridge forming residues [119] and disulfide clamps [120]. Construction of these polymers using unnatural amino acids have the added bonus of being chemically stable and more resistant to enzymatic degradation, which has plagued AMPs as potential drug candidates for years. A series of tripeptide constructs of varying lengths were prepared with the hydrophobic residues β^3-hLeu and/or β^3-hVal and the positively charged β^3-hLys to interrogate their chain-length dependent propensity for 14-helix adoption and their biological activity (Fig. 6C, compounds **1** and **2**) [115]. Similar to α-helical AMPs, the β-peptides adopted a helix conformation upon membrane binding as illustrated in Fig. 6D. A minimum of 9–12 residues was found necessary for secondary structure formation that coincided with the minimal length necessary for their biological activity. Peptides **1** and **2** were found to be highly active against *E. coli* cells, but were additionally found to lyse human erythrocytes at a HC_{50} of 80 nM compared to 0.5 μM for melittin. The authors speculated that the lack of selectivity might be due to excessive hydrophobicity. The ability of natural AMPs to discriminate bacterial rather than mammalian cells for lysis depends on a delicate balance of hydrophobicity, size, secondary structure formation, and charge distribution [121,122]. Particularly crucial is the hydrophobicity of the peptide. If the peptide is too polar they have little affinity for bacterial membranes and if they are too hydrophobic they fail to discriminate between mammalian and bacterial targets. In an effort to improve the lack of antimicrobial selectivity of their previous constructs [115], Liu and DeGrado synthesized a second series of analogs in which the less hydrophobic β^3-hAla was substituted for β^3-hLeu or β^3-hVal (Fig. 6C, compound **3**) [123]. The resulting peptides displayed potencies and selectivities comparable to those of natural AMPs such as magainin. Compound **3** in addition showed a striking ability to bind to and disrupt negatively charged PS liposomes while neutral PC membranes were unaffected.

Paralleling the work done by DeGrado and coworkers, the Gellman group similarly developed an antimicrobial 17-mer β-peptide (β-17) inspired by the natural AMP magainin [124]. This oligomer (Fig. 7A, compound **4**) was composed of residues constrained by five-membered rings, (*R*,*R*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) and (3*R*,4*S*)-*trans*-4-aminopyrrolidine-3-carboxylic acid (APC), that strongly promoted the adoption of a global amphiphilic 12-helix (Fig. 7B) with all polar APC residues on one side of the helix and all apolar ACPC residues on the other (Fig. 7C). Screening of the antibacterial and hemolytic capabilities of **4** with that of a synthetic magainin derivative revealed comparable antibacterial activities while **4** was less hemolytic. Additional

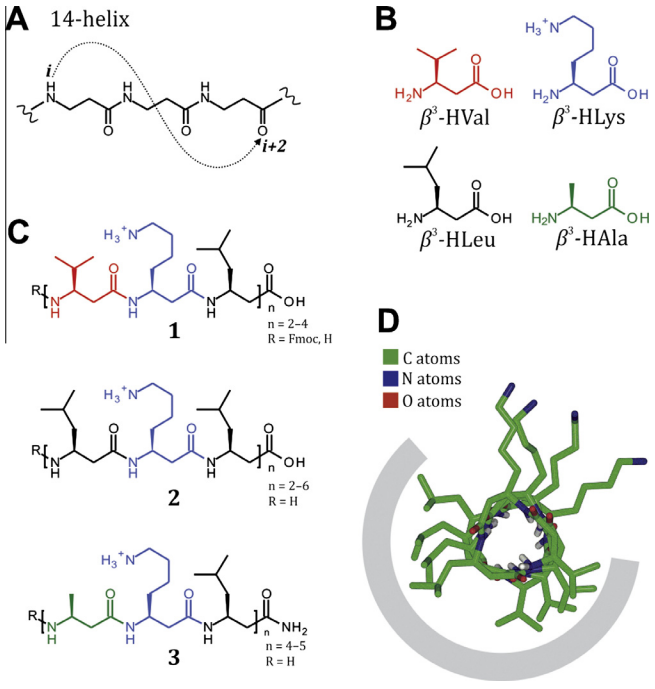

Fig. 6. Amphiphilic helix-forming oligomers of β-amino acids. (A) Adoption of a 14-helix conformation is characterized by an intercatenated 14-membered ring formed by the hydrogen bonding between an amide proton at residue position *i* and a main chain carbonyl at residue position *i* + 2. (B) Structures of the C^3 substituted β-amino acid monomers. (C) Representative antimicrobial polyamides constructed with the monomers depicted in B. (D) Membrane binding facilitates the adoption of an amphiphilic helix with a hydrophobic face indicated by the shaded area.

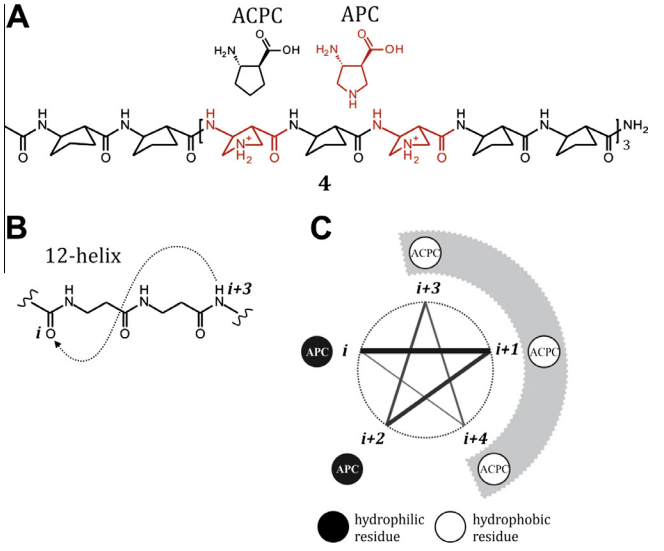

Fig. 7. Structure of β-17. (A) Chemical structure of β-17. (B) The constrained cyclopentane-containing amino acids bias for the adoption of a 12-helix, a conformation stabilized by a series of hydrogen bonds between a main chain carbonyl at residue position *i* and an amide proton at residue position *i* + 3 forming an intercatenated 12-membered ring. (C) Axial projection of the β-peptide 12-helix depicting the pentad repeat (APC, ACPC, APC, ACPC, ACPC) that results in an amphiphilic conformation.

studies in which flexible acyclic residues had been incorporated into the peptides showed that increasing the helical propensity had little effect on the antimicrobial activity [125].

Using DSC and dye leakage experiments Epand et al. investigated the interaction of compound **4** with various lipid systems and found that it was able to induce nonbilayer phases and promoted negative curvature in bilayer membranes [126]. Using magainin-2 as a control to compare against **4**, the authors showed that both peptides lysed anionic liposomes with greater potency as compared to zwitterionic ones; however, where magainin-2 exhibited greater lysis with membranes containing PG rather than PS [96], the authors found that **4** had no preference. The effect of **4** on the bilayer-to-hexagonal phase transition temperature (T_H) of Di(16:1)PE was used as a measure for the propensity of the peptide to induce membrane curvature. Since **4** lowered the T_H of Di(16:1)PE (T_H = 43 °C), the compound therefore destabilized the bilayer phase and facilitated the conversion to a more highly curved nonlamellar structure. This result contrasted that obtained with magainin-2 in which the peptide increased T_H and was posited to thus induce positive membrane curvature in the formation of toroidal pores [96]. In addition, the authors explored the impact of the lipid intrinsic curvature on the ability of **4** to lyse vesicles. DOPE was used as a lipid species having more intrinsic negative curvature whereas Di(18:1-Δ9-trans)PE was used as a lipid species having lower intrinsic negative curvature (more positive intrinsic curvature). Results showed that **4** had a reduced activity against vesicles comprised of Di(18:1-Δ9-trans)PE:DOPG compared to DOPE:DOPG; whereas, magainin-2 caused greater leakage from vesicles comprised of Di(18:1-Δ9-trans)PE:DOPG. These results supported findings obtained with DSC and led the authors to conclude that magainin-2 and **4** mechanistically impart their membrane disruption differently. Epand et al. later explored the membrane interaction of 14 homologs and found that they could cause rapid lipid flip–flop in anionic PC:PG lipid vesicles, suggesting these peptides might be able to form pores [127].

3.3. Arylamide oligomers

Various amino-based synthetic analogs of AMPs have been designed as candidates for biomimetic drugs; however, these relatively high molecular weight entities are rather closely related to their wild type cousins and hence can be expected to suffer from many of the limitations inherent to that class of compounds. Tew and DeGrado departed from these amino-based biomimetic compounds and developed a simpler synthetic SMAMP scaffold, of low molecular weight, based upon the repetition of an arylamide unit (Fig. 8). The platform utilized hydrogen bonding to produce conformationally rigid backbones lacking a formal amphipathic secondary structure [128]. Computational studies on arylamide **5** showed that both aromatic conjugation and intramolecular hydrogen bonds between the alkyl-thioether and the *meta* positioned amide controlled the conformation around the aryl carbon–nitrogen bonds, consequently lending to the planarity of the backbone [129,130]. The calculations aided in the refinement of subsequent arylamide oligomers, yielding several potent and more highly selective SMAMPs through variable decoration of the benzene rings with different polar and apolar side chains [131,132]. Switching the central benzene group in **5** to a pyrimidine ring as in compound **6**, brought about additional intramolecular hydrogen bonds to the construct, thereby improving the antibacterial activity 10-fold (the pyrimidine construct had a MIC of ~1 μM while the benzene ring analog had a MIC of ~18 μM) [133]. Rigidification of the backbone proved to preserve the facial amphiphilicity of the molecule, a criterion which mirrors that of natural AMPs and *β*-peptides. Indeed, simulations showed that these arylamides are surface active (in an *n*-octane/water system) and orient their functionalized benzene rings roughly perpendicular to the interface normal, maximizing both polar and apolar interactions [128,131]. Such a conformation is believed to enable favorable binding to the lipid bilayer/water interface.

Experimental techniques such as SFG vibrational spectroscopy [134,135], solid-state NMR [136], and GIXD [137] have proven useful in comparing and contrasting interactions between arylamide oligomers and cell mimicking membranes to those of natural AMPs. SFG vibrational studies were advantageous in monitoring the level of membrane perturbation upon arylamide association. Through asymmetric deuteration of a solid-supported lipid bilayer (proximal leaflet DPPG, distal leaflet d-DPPG) independent monitoring of the C–H and C–D stretching ranges could be performed simultaneously to observe induced disruption from one leaflet to the other. At oligomer concentrations well below the MIC value (0.8 μg/mL), arylamide **6** was localized to the distal leaflet causing increased disorder in the lipid acyl chains, as measured by a decrease in the symmetric stretching intensity of the terminal CD_3 groups of the aliphatic chains, while disruption did not extend to the proximal leaflet. Ivankin et al. [137] similarly showed with GIXD that upon membrane association of **7**, an analogous arylamide to **6** studied by Chen et al. [134], complete disappearance of Bragg peak signatures for an ordered structure in DPPG monolayers occurred. Disruption was concentration dependent and eventually spanned across the bilayer, though pore formation was not expected as the rigid amphiphilic molecules are too short to span the bilayer and instead remain associated at the membrane/water interface. Coarse-grained MD simulations have suggested that these oligomers operate cooperatively to cross the membrane core to the opposite leaflet and occasionally align to provide a pathway for water permeation [138]. The *in silico* results posed that the arylamide oligomers imparted a more subtle

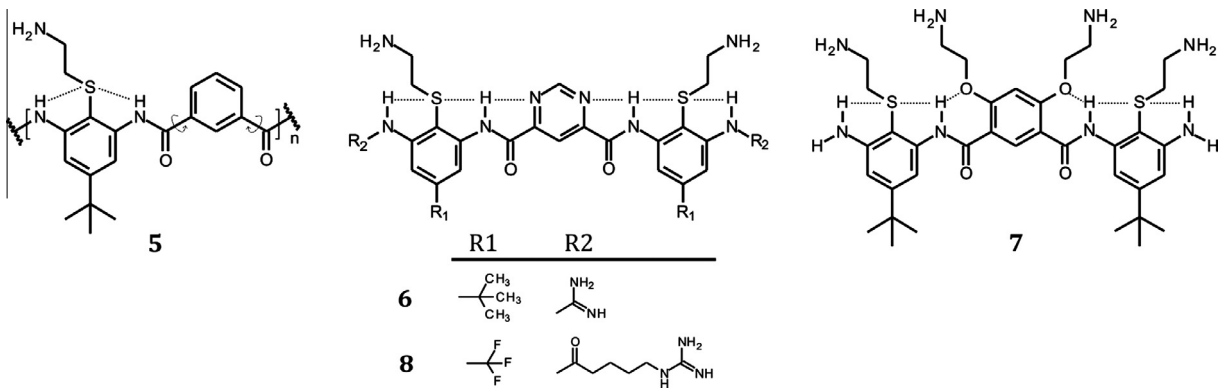

Fig. 8. Chemical structure of facially amphiphilic arylamide oligomers.

perturbation on the membrane integrity as compared to that of natural AMPs. Solid-state NMR tuned to ^{19}F and ^{31}P nuclei resonances was used to determine the orientation, depth of insertion, and mobility of a side-chain fluorinated arylamide, compound **8** [136]. The results showed that the oligomer inserted and preferentially sat just below the lipid headgroups with the molecular plane tilted either 20° or 30° from the membrane normal of neutral and anionic bilayers, respectively. These orientations were similar to those measured by SFG vibrational spectroscopy [134,135]. Since the molecular plane is nearly perpendicular in orientation with respect to the bilayer surface, the molecule inserted in a "knife-like" fashion. Such a conformation is believed to enable favorable binding to the lipid bilayer/water interface, but due to the stiffness of the backbone an insertion state may be improbable. Moreover, the molecule was found to undergo fast uniaxial diffusion with rates greater than 10^5 s^{-1}, suggesting that spinning the molecule has the potential to perturb and destabilize a large area of the membrane. Rather than imparting curvature strain on the membrane that permanently damages the lipid bilayer integrity, the arylamides instead create transient defects through which cell electrical depolarization could occur.

3.4. Phenylene ethynylenes

Tew and coworkers developed the novel *meta*-phenylene ethynylene (*m*-PE) constructs, depicted in Fig. 9, that are completely built upon an abiotic hydrocarbon-based backbone lacking proteolytic amide bonds and chiral centers [139–141]. Poly(*m*-PE)s patterned with primary amines and different length alkyl nonpolar groups adopted highly extended and facially amphiphilic structures as elucidated by Langmuir monolayer studies at the air–water interface [142]. These surface-active molecules proved to be potent membrane disrupting agents as they were able to induce calcein leakage from anionic lipid vesicles in a concentration-dependent manner [139] and thus offer a promising ability to mimic the activity and selectivity of natural AMPs and other synthetic constructs. Large molecular weight poly(*m*-PE)s decorated with pentoxy side chains, such as **9**, were found to be highly hydrophobic and thus showed no antibacterial activity as result from poor solubility [140]. A shortened backbone coupled with the removal of the pentoxy side chains greatly improved the antibacterial and selective properties of the polymers. For example the potent magainin analog MSI-78 has an average MIC value of 12 μg/mL (*E. coli*) and HC_{50} value of 120 μg/mL, whereas polymers such as **9** were found to have a MIC value of 50 μg/mL (*E. coli*) and a HC_{50} value of 540 μg/mL [140]. The potency and selectivity of this class of SMAMPs was remarkably increased with the synthesis of **10** as it displayed a MIC value of 0.1 μg/mL (*E. coli*) and a HC_{50} value of 88 μg/mL, showing a 880-fold selectivity (HC_{50}/MIC) of prokaryotes over eukaryotes [141]. Additionally, **10** was readily active against methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococci* and displayed extremely low susceptibility towards resistance development [141].

A combination of various experimental techniques has helped to understand the detailed interaction behavior of phenylene ethynylenes with membranes. Using XR and GIXD measurements, Ishitsuka et al. was able to provide molecular-level information to explain the non-existent antibacterial and highly active behavior of **9** and **10**, respectively [143]. Results from XR revealed that both molecules insert into the membrane and localize themselves in the polar headgroup region and part of the nonpolar tailgroup. While **9** and **10** were both found to insert more readily into and disrupt the lateral packing of lipids in DPPG monolayers, **10** exhibited a higher insertion and disruption capacity in DPPC monolayers as compared to **9**. These insertion/disruption differences between the two compounds are in good agreement with findings from *in vitro* cellular assays performed by Arnt et al. [140].

Tew and coworkers recently used vesicles containing an entrapped fluorescent dye to probe the effect of lipid composition on the activity of a series of phenylene ethynylene homologs through the extent with which the polymers were able to induce vesicular leakage [144]. The vesicles contained 20 mol% negatively charged phospholipids (DOPG or DOPS) with the remaining amount comprised of a zwitterionic phospholipid (DOPE or DOPC). The DOPE/DOPG composition was used to emulate the bacterial membrane while the DOPC/DOPS system was selected as it more closely resembled the mammalian RBC membrane. Compound **10** was able to induce high leakage from DOPE/DOPG vesicles (~75%) whereas leakage was reduced by roughly a factor of 5 when vesicles were composed of either DOPC/DOPS or DOPC/DOPG. The vesicle leakage results clearly showed that lipid composition rather than the charge of the lipid constituent affected the activity of the polymer, with the presence of PE particularly enhancing the disruption capability of **10**.

Yang et al. used SAXS to show that the negative intrinsic curvature of DOPE is an important factor in determining the potent selective behavior of **10** [145]. When **10** was introduced to a bulk suspension of DOPG/DOPE/DOPC SUVs, an inverted hexagonal phase (H_{II}) was induced only above a minimum threshold concentration of 64 mol% DOPE. The authors postulated that the ability of **10** to restructure a large ensemble of membranes critically depended on the presence of lipids with negative intrinsic curvatures. In the case when **10** was tested against single GUVs, pores were presumably formed in the plane of the membrane that released entrapped fluorescent markers of a select size. Clearly an H_{II} phase does not structural match that of a pore, but since a pore requires the generation of negative Guassian curvature, the authors postulated that lipids having negative intrinsic curvature will be essential in the formation of such structures. Since bacterial membranes contain a large amount of PE compared to that of mammalian membranes, Yang et al. believed this compositional difference helps explain the selectivity of **10** in a similar regard to the

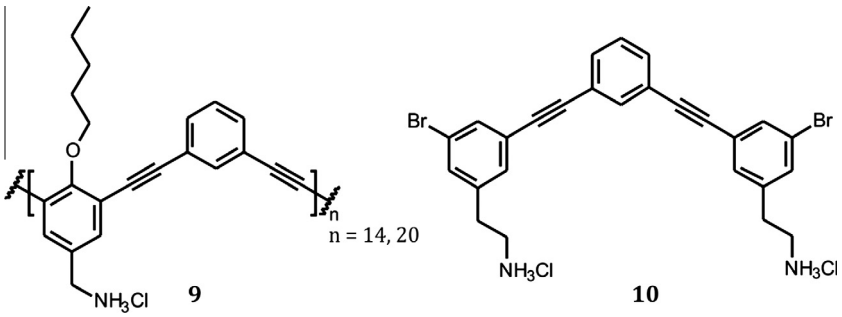

Fig. 9. Phenylene ethynylene constructs.

selectivity suggested for AMPs [94]. This notion helps explain the potent activity against gram-negative bacteria such as *E. coli* as this species contains PE lipids, but it does not explain the activity of **10** against gram-positive bacteria, such as *S. aureus*, as this species has no PE lipid present in its membrane [146]. Som et al. speculated that the ability of CL to adjust its intrinsic curvature from $c_0 \sim 0$ in the presence of monovalent salts to $c_0 < 0$ in the presence of divalent cations accounted for **10's** activity against gram-positive bacteria [146]. The authors have shown that when divalent Mg^{2+} or Ca^{2+} cations are introduced to a sample of **10** and CL containing vesicles, leakage of entrapped calcein readily occurred (~60%). Without the cations, leakage induced by **10** is reduced to 5%. SAXS shows that divalent cations are able to enhance the tendency of **10** to induce the formation of a H_{II} phase in a DOPG/CL SUV suspension. Indeed intrinsic membrane curvature appears to be a mechanism for the antimicrobial specificity of phyenlene ethynylenes.

3.5. Adoption of a defined amphiphilic secondary structure is not a prerequisite for antimicrobial activity

The attribute that adoption of an ordered and globally amphiphilic secondary structure by natural AMPs as a necessity for their antimicrobial activity has guided the rational design of polymeric mimics for years. The SMAMPs discussed thus far are synthesized in a step-by-step fashion according to this criterion such that sequences of hydrophilic and hydrophobic subunits ultimately form a well-defined conformation with delineated polar and apolar faces critical for their action. Though appearing to be an ubiquitous trait underpinning all sequence-specific oligomers, new evidence highlights that nature does not constrain itself to this condition. For instance, the Santos lab has actively studied the membrane mechanism of a recombinant N-terminal fragment of a bactericidal/permeability-increasing protein, known as $rBPI_{21}$, that is currently in phase III clinical trials for treatment of meningococcal sepsis [147]. Interestingly, Domingues et al. have found that upon preferential interaction with negatively charged phospholipid vesicles, $rBPI_{21}$ loses its conformational rigidity as a disulfide bond breaks. The transition from a folded state to an unfolded state lets the protein obtain an irregular extended conformation that subsequently allows its Trp residues to bury within the hydrophobic core of the bilayer, causing membrane aggregation as its pertinent bactericidal mechanism. The loss of $rBPI_{21}$'s predominately β-sheet structure through course of its perturbation mechanism is at odds with AMPs that require a defined secondary structure for their activity. Additionally, Oren and Shai have reported synthetic amphiphilic peptides designed to have conformational constraints placed on their tendency to form α-helices (e.g., 33% D residues; N-to-C cyclization) were still able to exhibit antimicrobial activity and little hemolytic activity [148]. The notion that a more irregular conformation can still result in global amphiphilicity and impart antimicrobial activity represents a significant shift in our understanding of the structure–activity relationships among antimicrobial agents.

Averting the rigid confines of distinct structure adoption as a rationale design criterion, far easier synthetic approaches may be implemented that usher in new active antimicrobial agents that are potentially less expensive to prepare as compared to the more laborious synthesis of sequence-specific SMAMPs. Mimics with a random sequence may adopt irregular amphiphilic structures that yield both selective and active polymers (Fig. 10A). The ease with which the hydrophobic-to-hydrophilic balance can be tuned in random-sequence polymers makes them especially advantageous as antimicrobial active entities. Tunability in canonical triblock copolymers (poloxamers) composed of hydrophobic poly-(propylene oxide) and hydrophilic poly-(ethylene oxide) easily switched the nature of their membrane interaction from a membrane sealant to a membrane permeabilizer at high degrees of hydrophilicity and hydrophobicity, respectively [149,150]. As a primary example, this synthesis rationale has been applied in the Gellman lab to prepare nylon-3 based random copolymers as functional mimics of AMPs via ring-opening copolymerization of β-lactams (Fig. 10B, compound **11**) [151]. Variation in the percentage of cationic subunits present resulted in optimized polymers with appreciably low MIC values and very weak hemolytic activity orders of magnitude higher (>800 μg/mL) [151]. Studies with model vesicles composed of anionic lipids with compositions mimicking those of gram-negative or gram-positive bacteria showed preferential

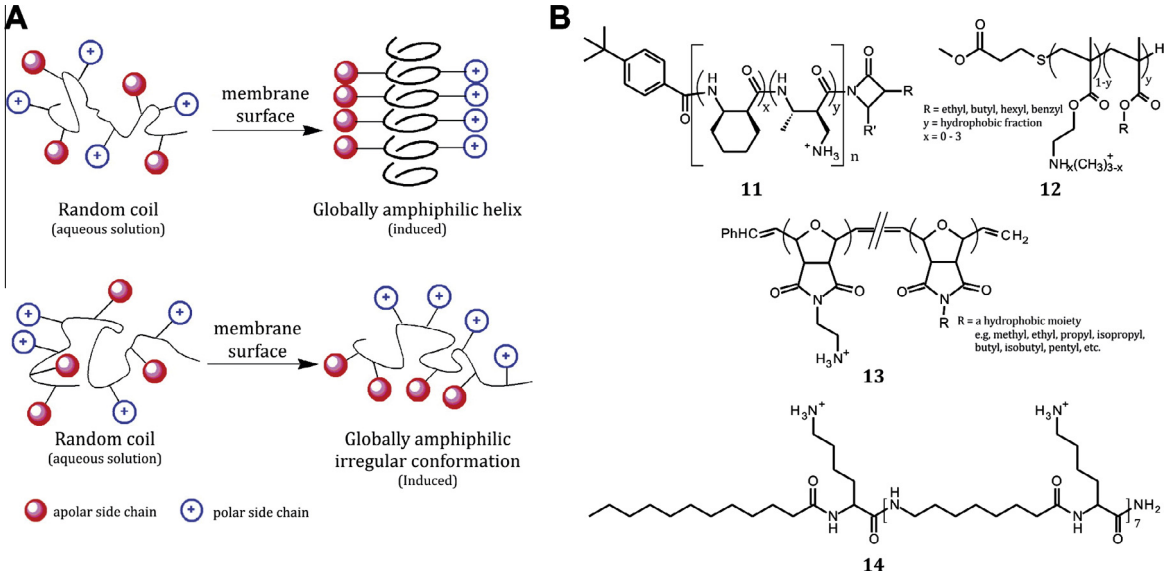

Fig. 10. Averting the rigid confines of distinct structure adoption as a rationale design criterion. (A) The activity of natural AMPs and their synthetic analogs has relied on the adoption of an ordered and globally amphiphilic secondary structure upon contact with a pathogen's membrane. Alternatively, random-sequence polymers that remain active and selective antimicrobial agents can be explained through the induction of amphiphilic yet irregular conformations in the presence of a membrane. Reprinted and modified with permission from Ref. [151]. Copyright 2007 American Chemical Society. (B) Representative selective antimicrobial copolymers that presumably adopt irregular conformations.

activity of the random β-lactam polymers whereas zwitterionic vesicles were left undisrupted, highlighting the selective behavior of the polymers for bacterial rather than mammalian cells [152]. Mechanistically, the polymers were believed to segregate lipids in mixtures, resulting in the formation of domains by which boundary defects allowed the leakage of vesicle contents [152]. Additional polymeric constructs displaying selective antimicrobial activities have been designed based on flexible backbones and random amphiphilic sequences such as polymethacrylates (Fig. 10B, compound **12**) [153–155] and polynorbornenes (Fig. 10B, compound **13**) [156–158]; though not fully discussed herein, readers interested in these polymers are directed towards a recent review by Engler et al. [159]. Interestingly, simulations of random polymethacrylate copolymers have shown that upon interaction with a bilayer interface, the flexible backbone allowed for the adoption of a globally amphiphilic yet irregular conformation [154].

Taking a more reductionist approach to the construction of an oligomer that prevents any adoption of a defined structure, Mor and coworkers developed a dermaseptin inspired SMAMP having a simple linear sequence of alternating acyl chains and cationic lysine residues termed oligo-AKs (OAKs), where A represents an acyl chain of variable length and K is the polar lysine residue [160]. Readers are encouraged to look at a recent review on this novel molecular construct [161]. The choice of acyl chains as a subunit was advantageous in that it allowed for a systematic tool to control the molecular hydrophobicity (by changing the acyl chain length) and provided ample rotational freedom about the carbon atoms to hinder formation of a defined secondary conformation. OAKs constructed of acyl chain lengths of four and eight carbons were found unstructured in buffer and remained so upon interaction with liposomes as measured by CD spectroscopy [160]. Additionally the butyl- and octyl-based OAKs were quite selective and displayed HC_{50} values approximately two orders of magnitude higher in concentration as compared to the MIC values ($HC_{50} \sim 150$ μM). When the hydrophobic content was increased, lauryl-based OAKs were found to be far less selective and aggregated with CD spectral bands indicative of stable folds similar to those in α-helices and β-sheets. The structure–activity results indicated that if rigid structures were hydrophobic enough they would be able to interact with RBC membranes in a manner that causes hemolysis. This mirrors many findings when the hydrophobicity of AMPs was increased, they lost all ability to discriminate between bacterial and mammalian cells, lowering the threshold concentration for hemolysis. Consequently, conformational rigidity of a defined secondary structure is not necessary for the membrane disruptive activity of these SMAMPs. Ivankin et al. utilized GIXD to unequivocally show that **14**, a flexible octyl-based OAK lacking any defined secondary structure, was able to exert a powerful membrane-disruptive capability on DPPG and lipid A monolayers similarly to the rigid arylamide **7** [137]. Interestingly, where **14** could completely disrupt the in-plane order of lipid A monolayers, natural AMPs such as PG-1 [14,19] and LL-37 [20,87] could not. The unstructured OAK was able to insert in the hydrocarbon region of lipid A, a lipid having a large cross-sectional area, to an extent similar to its insertion into DPPG, which has a smaller cross-sectional area. The authors speculated that the flexibility of **14** gave it the ability to accommodate itself into interstitial lipid spaces of various sizes more easily as compared to the conformationally more rigid AMPs.

4. Summary and future outlook

The fight against dangerous microbes is increasingly more difficult as many species have developed resistance to antibiotics. AMPs have received attention in recent years as an alternative to traditional receptor-specific antibiotics based on the nature of their interaction with the cell membrane. Elucidating the non-stereospecific interaction between AMPs and membranes is important for the future development of more robust antimicrobial agents in which pathogens do not rapidly develop immunity. In this review, we first focused on examining the interaction of AMPs with model membrane systems in an effort to understand the underlying selectivity and mechanism of their biocidal activity. The rationale being to emphasize key physiochemical parameters active in AMP behavior that are additionally at play in synthetically designed mimics. Though obvious electrostatic interactions are important to an extent for determining AMP selectivity, through modulating the initial adsorption of the peptide to lipid membranes, compositional differences in cholesterol and PE lipid content amongst various organisms' cell membranes have shown to alter a membrane's susceptibly to AMP disruption, either favorably or unfavorably, providing further insight into their discriminatory ability. While previous investigations have uniformly considered AMPs as static pore formers, we have provided evidence that suggests that their membrane perturbing mechanism is much more complex with dynamic structural transformations that are dependent upon the relative ratio of lipid and peptide. Only by exploring a wide spectrum of PG-1 concentrations was it possible to show that PG-1 formed structures other than pores and that those self-assembled structures were similar to those formed be mixed detergent–lipid systems. Exploration of a large phase space that encompasses parameters such as temperature, pH, lipid composition, and salt concentration. might reveal similar results for other peptides, suggesting a unifying description for their mechanism. The simple comparison to detergents places importance on the overall amphiphilicity of the peptides; that a delicate balance of the hydrophobic-to-hydrophilic ratio is an over-arching physical property unifying these peptides with such disparate molecular structures, sequences, and sizes. Evidence provided herein has shown that adoption of a well-defined molecular conformation is not essential for antimicrobial activity. For instance, a peptide was found to unfold upon membrane association in order to assume an amphiphilic structure active against microbes. Additionally, sequence-random copolymers that adopted irregular conformations when in contact with membranes were yet found to display selective antimicrobial behavior. These examples support that the overall amphiphilic nature of these species is a tunable parameter with which to create selective antimicrobial materials while strict adherence to adoption of a well-defined structure is not necessary.

We have shown that as membrane-active agents, AMPs are generally accepted to perturb the integrity of a target cellular membrane as their primary active mechanism. The perturbation whether it be from discrete pore formation or a detergent-based disruption ultimately leads to a loss of transmembrane electrochemical gradients, thus killing a pathogenic cell *in vivo*. Decades of intensive investigations on model systems have provided mechanistic details on the molecular events that take place during the course of their activity. Broadly the perturbation the peptides exert involves curvature strain on the membrane, explained here through a molecular shape argument in which peptides act as wedges. The simplified model reflects the overall amphiphilic shape of the peptide, but must additionally take into account the charge and hydrogen-bonding interactions present between the peptide and surrounding lipids. For instance, the differing hydrogen bonding capabilities between arginine and lysine residues have been postulated in the ability with which they induce membrane curvature [94,162]. As arginine residues can hydrogen bond multiple lipids simultaneously, they therefore generate curvature more readily as compared to the monodentate hydrogen bonds lysine residues form with the phosphate on a single lipid headgroup. However, an interesting discrepancy arises when one compares

AMPs to cell-penetrating peptides (CPPs). Both peptide species are similarly amphiphilic and highly charged with polar arginine and lysine residues. Where AMPs have evolved to irreversibly disrupt membranes, CPPs translocate across a membrane in a non-disruptive manner for the purpose of cargo delivery [163]. Though both peptide species construct their primary sequences with similar polar and apolar residues, how do such contrasting activities emerge? With no sequence homology existing within each peptide class, is there a unifying criterion with which curvature-generating arginine and lysine residues are modulated with hydrophobes to shift peptide activity from one extreme to another? Questions such as these continue to stimulate and necessitate further research in the mechanism by which AMPs interact with membranes.

The development and design of synthetic alternatives to peptide-based drugs have been reviewed with particular emphasis placed on their interaction with lipid membranes. Clear experimental evidence has shown that the activity of SMAMPs is lipid dependent and correctly captures the selective behavior exhibited by natural AMPs. Numerous biophysical techniques have been used to shed light on the molecular details of their interactions with membranes resulting in a consensus that these compounds induce localized disorder upon binding. However, much remains unclear on their mechanism of interaction, especially at concentrations above their MIC value. The threshold concentration for AMP activity has been explained by the transition to a pore-forming state where the peptides cooperatively insert and span the thickness of the bilayer. A pore forming state for SMAMPs on the other hand remains inconclusive. Where vesicle leakage and X-ray scattering experiments have suggested such a PE dependent structure, NMR studies on the other hand have shown that low molecular weight SMAMPs, such as the arylamide oligomers, are too short to span a bilayer and remain interfacially bound disturbing the lipid packing through fast uniaxial diffusion. Continued efforts must be made in elucidating the mechanistic steps in SMAMP-membrane interaction, especially in regards to the sequence-random polymers where little structural investigations have been performed. It is clear that the activity of these polymers and future constructs must be optimized to determine the correct hydrophobic-to-hydrophilic balance to elicit selective antimicrobial behavior. The structure of the charged moieties present in poly(methacrylate) polymers (primary, tertiary, and quaternary ammonium groups) and poly(norobornene) polymers (primary amine and guanidinium), for example, have been varied to investigate their antimicrobial and hemolytic activity [159]. Modulation of their activity as the chemical structure of the charged group was varied suggested that the capacity with which the polar moiety is able to hydrogen bond is crucial to the polymer's ability to perturb a membrane and mirrors the difference between lysine's and arginine's proposed ability to induce membrane curvature. Further studies in elucidating how specific hydrogen bonding interactions are at work in the membrane activity of AMPs and SMAMPs should be conducted.

SMAMPs have greater applicability as antimicrobial agents for a variety of biomedical uses based upon their resistance to biodegradation and the ease with which they can be produced in larger quantities at a relatively low cost. SMAMP **10**, for instance, is a promising candidate for intravenous treatments as it has shown to cause no resistance development and exhibits strong potency against MRSA and vancomycin-resistant *E. faecium*, *E. faecalis*, and *S. pneumonia* [141]. Though not previously discussed in this review, AMPs such as dermaseptin and indolicidin, in addition to having membrane activity, have intracellular targets inhibiting DNA, RNA, and protein synthesis [164,165]. SMAMP **10** was shown by Diamond and coworkers to bind and retard the mobility of both single- and double-stranded DNA in a concentration-dependent fashion [166]. The mechanistic duality promises to be a unique route with which material scientists can design future antimicrobial scaffolds.

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