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Designing Mimics of Membrane Active Proteins

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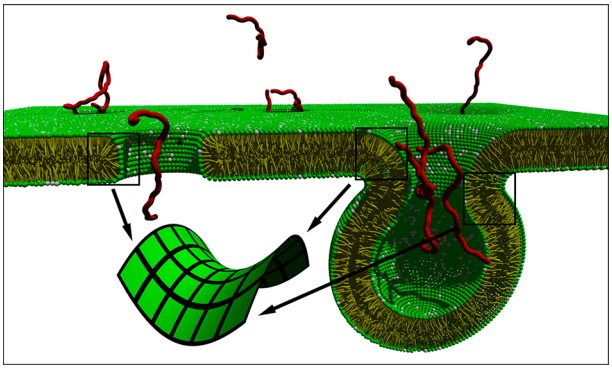
CONSPECTUS



As a semipermeable barrier that controls the flux of biomolecules in and out the cell, the plasma membrane is critical in cell function and survival. Many proteins interact with the plasma membrane and modulate its physiology. Within this large landscape of membrane-active molecules, researchers have focused significant attention on two specific classes of peptides, antimicrobial peptides (AMPs) and cell penetrating peptides (CPPs), because of their unique properties.

In this Account, we describe our efforts over the last decade to build and understand synthetic mimics of antimicrobial peptides (SMAMPs). These endeavors represent one specific example of a much larger effort to understand how synthetic molecules interact with and manipulate the plasma membrane.

Using both defined molecular weight oligomers and easier to produce, but heterogeneous, polymers, we have generated scaffolds with biological potency exceeding that of the natural analogues. One of these compounds has progressed through a phase II clinical trial for pan-staph infections. Modern biophysical assays have highlighted the interplay between the synthetic scaffold and lipid composition: a negative Gaussian curvature is required both for pore formation and for the initiation of endosome creation. Although work remains to better resolve the complexity of this interplay between lipids, other bilayer components, and the scaffolds, significant new insights have been discovered. These results point to the importance of considering the various aspects of permeation and how these are related to "pore formation".

More recently, our efforts have expanded toward protein transduction domains, or mimics of cell penetrating peptides. Using a combination of unique molecular scaffolds and guanidinium-rich side chains, we have produced an array of polymers with robust membrane (and delivery) activity. In this new area, researchers are just beginning to understand the fundamental interactions between these new scaffolds and the plasma membrane. Negative Gaussian curvature is also important in these systems, but the detailed relationships between molecular structure, self-assembly with lipids, and translocation will require more investigation. It has become clear that the combination of molecular design, biophysical models, and biological evaluation provides a robust approach to the generation and study of novel proteinomimetics.

Introduction

The plasma membrane constitutes a semipermeable barrier which controls the flux of biomolecules in and out the cell. It has a fundamental role in cell function and survival. If its integrity is compromised, for example by the formation of large and permanent pores, it will result in cell death. Many proteins interact with the plasma membrane and modulate its physiology. Within this large landscape of membrane-active molecules, two specific classes of peptides, antimicrobial peptides (AMPs) and cell penetrating peptides (CPPs), have received significant attention due to their unique properties.[1,2]

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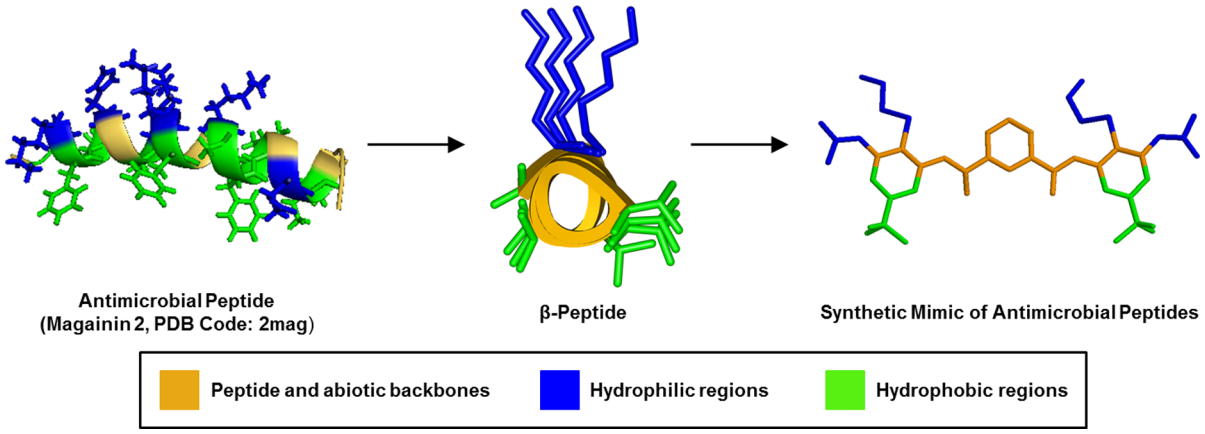

FIGURE 1. From AMPs to SMAMPs.

Although AMPs and CPPs share structural and functional aspects, they have mainly existed as separate literatures until recently.[3−5] Both consist of short sequences that are net cationic. Almost all AMPs have significant hydrophobic residues, or domains, while CPPs may not. Another difference has been the biological assays, which evaluate most AMPs for their antibacterial and hemolytic activities while CPPs studies tend to focus on mammalian cell translocation. Because the detailed mechanisms of membrane activity are complex and remain under investigation, it is difficult to say that any specific peptide, regardless of sequence, follows a mechanism consistent with an AMP or CPP. Future studies will surely provide important insight in this area. In this Account, we will describe structural and functional aspects of synthetic mimics of AMPs and CPPs developed in our group.

AMPs and Their Synthetic Mimics

AMPs, isolated from organisms across the phylogenetic spectrum, are an important part of the innate immune system.[6] Despite the diversity observed in AMP sequences, one hallmark is their facially amphiphilic (FA) topologies that appear crucial for membrane activity and antimicrobial properties (Figure 1). Although the exact mechanisms of membrane permeation are still not fully understood, it is thought that electrostatic interactions facilitate association with the anionic bacterial membrane and hydrophobic interactions promote pore formation and cell death.[7] The differences in membrane composition between bacteria and eukaryotes is important to AMPs selectivity. Bacterial membranes are rich in negative intrinsic curvature (NIC) lipids, such as phosphatidylethanolamine (PE) in Gram-negative and cardiolipin (CL) in Gram-positive bacteria, which play a critical role in pore formation since they facilitate the negative-curvature circumferential barrels typical of transmembrane pores.[8]

The ability to bind and control the integrity of phospholipid membranes is closely tied to the FA topology of AMPs. Over the past decade, their unique molecular architectures inspired the design of novel synthetic mimics of AMPs (SMAMPs) with tunable structural features.[9−11] β-Peptides, a class of polyamides, have been shown to adopt a variety of secondary structures analogous to those of proteins. DeGrado and co-workers designed a series of amphiphilic, helical β-peptides to mimic natural membrane-active peptides.[12] In particular, a series of β^3-peptides showed reasonable antibacterial activity and selectivity (HC_{50}/MIC) > 100 for *E. coli* versus mammalian cells. Structure−function correlation studies provided important information about how the FA topology was related to their activities. The difference in vesicle leakage kinetics suggested that chain length might affect the bilayer disruption mechanisms. These initial studies, along with similar work by Gellman[13] and Seebach,[14] provided a useful guide for designing synthetic molecules and demonstrated that the α-helix was not essential for activity. However, these early designs still adopted overall FA secondary structures, with large surface areas of amphiphilic topology. Therefore, it remained unknown whether an inherent secondary structure was critical for activity until the first oligomers and polymers were prepared.[15]

Nonpeptidic Oligomeric SMAMPs. Motived by this question and by the desire to mimic the functions of biomolecules, our research group developed a series of novel FA synthetic polymers based on *meta*-phenylene ethynylene (*m*PE) backbone.[16] This was one of the first attempts to produce synthetic mimics with a completely abiogenic backbone and no intramolecular H-bonding. These *m*PE polymers were found to be good mimics of AMPs, highlighting the importance of amphiphilicity, rather than peptide structure, on bioactivity.[17,18] It was also possible to reduce the

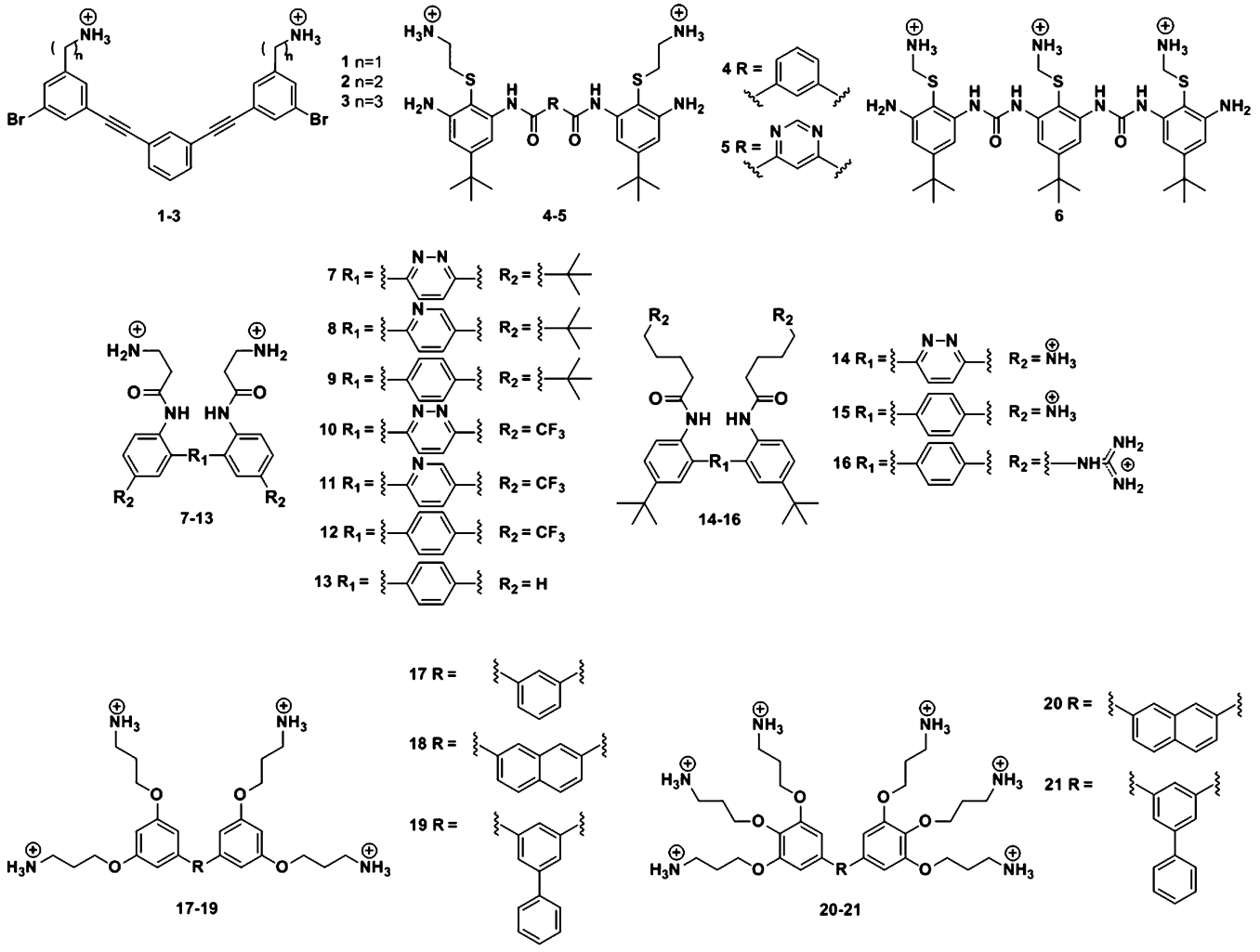

FIGURE 2. Oligomeric SMAMPs.

molecular weight (MW) leading to triaryl *m*PE SMAMPs (**1**−**3**) which were even more potent and selective than the polymeric counterparts (Figure 2).[19] SMAMP **2** showed outstanding broad spectrum antibacterial activity and low toxicity, measured as the minimum inhibitory concentration (MIC $\sim$ 0.1 μg/mL against *E. coli*, 0.2 μg/mL against *S. aureus*) and hemolytic concentration (HC_{50} = 75 μg/mL), respectively.[19,20]

Extensive biophysical studies with this series of SMAMPs, including small-angle X-ray scattering (SAXS), dye release assays, solid-state NMR, and patch-clamp experiments, were performed to evaluate the interaction between these SMAMPs and membranes. The ability of **2** to modulate the self-assembly and morphology of model membranes was studied in detail.[8,20] SAXS data showed **2** restructured membranes, inducing an inverted hexagonal phase (H_{II}) with 3 nm water channels in PE/PG (phosphatidylglycerol) model vesicles, but only when the PE lipid content in the membrane

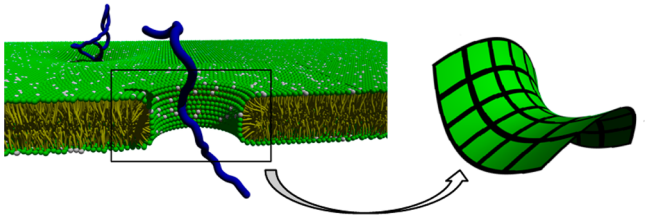

FIGURE 3. Illustration of saddle-splay membrane curvature induced by SMAMPs.

was above a minimum threshold of 64%.[20] The H_{II} phase is important for the generation of negative Gaussian membrane curvature (or saddle-splay), topologically required for pore formation (Figure 3).[8] This indicates that the membrane activity of **2** critically depends on the concentration of the NIC PE lipid present in the membrane. This could also explain the selectivity of **2** because Gram-negative bacteria contain much higher volume fractions of NIC lipids than mammalian cells. In the Gram-positive bacteria model

membrane, CL, in the presence of divalent metal cations (Ca^{2+} or Mg^{2+}), acts as an NIC lipid and H_{II} phases are induced by the presence of **2**.[21] An extensive dye leakage study supported the SAXS findings indicating that these simpler biophysical assays can be used as a screening tool for more detailed and time-intensive experiments.[22,23]

Another class of FA SMAMPs, with a conformationally stiff arylamide backbone, was also reported.[15] For the arylamide oligomers, the conformational rigidity was derived from intramolecular H-bonding between the amide groups in the backbone and the thioether function in the side chains. Replacement of the central benzene ring (**4**) with pyrimidine (**5**) led to additional intramolecular H-bonding, resulting in a structure with greater rotational restriction, and enhanced antimicrobial activity (12 μg/mL and 0.8 μg/mL against *E. coli,* respectively).[24] Within this arylamide series, it was shown that increased conformational stiffness led to excellent antimicrobial activity (10^5 reduction in viable CFU of *S. aureus*) in a mouse model.[25]

Molecular dynamics (MD) simulations revealed the preferential positions of the SMAMPs with respect to the bilayer. The simulations in *n*-octane/water and PC/water environments showed that arylamide oligomers based on **4** rapidly reached the interface with the charged side chains interacting with the charged phospholipid head groups and water, while the hydrophobic groups remained buried between the lipid tails (Figure 4).[15,26] The primary driving force for insertion was hydrophobicity. MD simulations also showed that the preferential orientation of the arylamide oligomers was perpendicular to the bilayer normal, which was later supported experimentally.[27] This particular orientation appears to maximize amphiphilic interactions. These computational studies have both verified and developed important SMAMP design principles. For instance, a semirigid backbone is apparently not an absolute requirement for optimal activity, provided that the SMAMPs can assemble into well-defined amphiphilic conformations in the heterogeneous lipid bilayer environment.

To better investigate the role of backbone flexibility, several series of aromatic oligomers based on urea and triaryl scaffolds with intramolecular H-bonds between the rings were designed. Biological activity similar to **5** was observed for the urea-based oligomer **6**, which had a completely locked conformation.[28] However, when the same principle was applied to the triaryl series (**7**–**16**), the opposite trend was observed.[29] This suggested that the potency of the SMAMP was not the effect of one parameter alone but the result of a proper coordination between the number of positive charges, amphiphilicity, and hydrophobicity of the

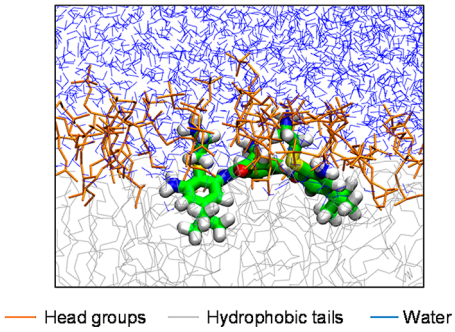

FIGURE 4. Equilibrium conformation of a SMAMP in a hydrated PC lipid environment.

molecules. Without knowing the detailed mechanisms of action, challenges arise in understanding structure–activity relationships. For example, within this series, the overall hydrophobicity had a greater impact than the conformational rigidity of the molecules. Modifications of the polar and nonpolar side chains led to the same conclusion, since the molecules carrying the more hydrophobic *tert*-butyl groups (**7**–**9**) were more active.

To further explore the influence of charge and hydrophobicity on SMAMP activity, a new series of molecules was synthesized containing four or six cationic charges and three different central rings: benzene (**17**), naphthalene (**18**, **20**), and phenylbenzene (**19**, **21**).[30] By increasing the hydrophobicity and the number of cationic charges, the compounds became more potent and selective. **20** was one of the best candidates, with a selectivity of >200 for both *E. coli* and *S. aureus*. These results confirmed the importance of fine-tuning the overall hydrophobicity and total number of cationic charges in order to improve the biological activities of SMAMPs.

Although not directly related to pore formation, **20** also showed immunomodulatory activities similar to AMPs. Mounting evidence shows AMPs modulate the immune system. This SMAMP stimulated pro- and anti-inflammatory cytokines (TNF, IL-6, and IL-10) as well as induced murine chemokine production (CXCL1). The ability of **20** to further capture the immunomodulator properties of AMPs demonstrates the importance of developing these mimics. The potential discovery of novel scaffolds that control the immune system is tremendous.[31] Thus, **20** is a promising model for the design and application of dual-functional SMAMPs.[32]

Polymers as SMAMPs. An important extension in the field of SMAMPs was the introduction of oligomeric and polymeric SMAMPs. The fact that biomimetic activity could be obtained from molecules of variable and less defined MWs, which deviate more from the original peptide sequences, greatly expanded our fundamental knowledge

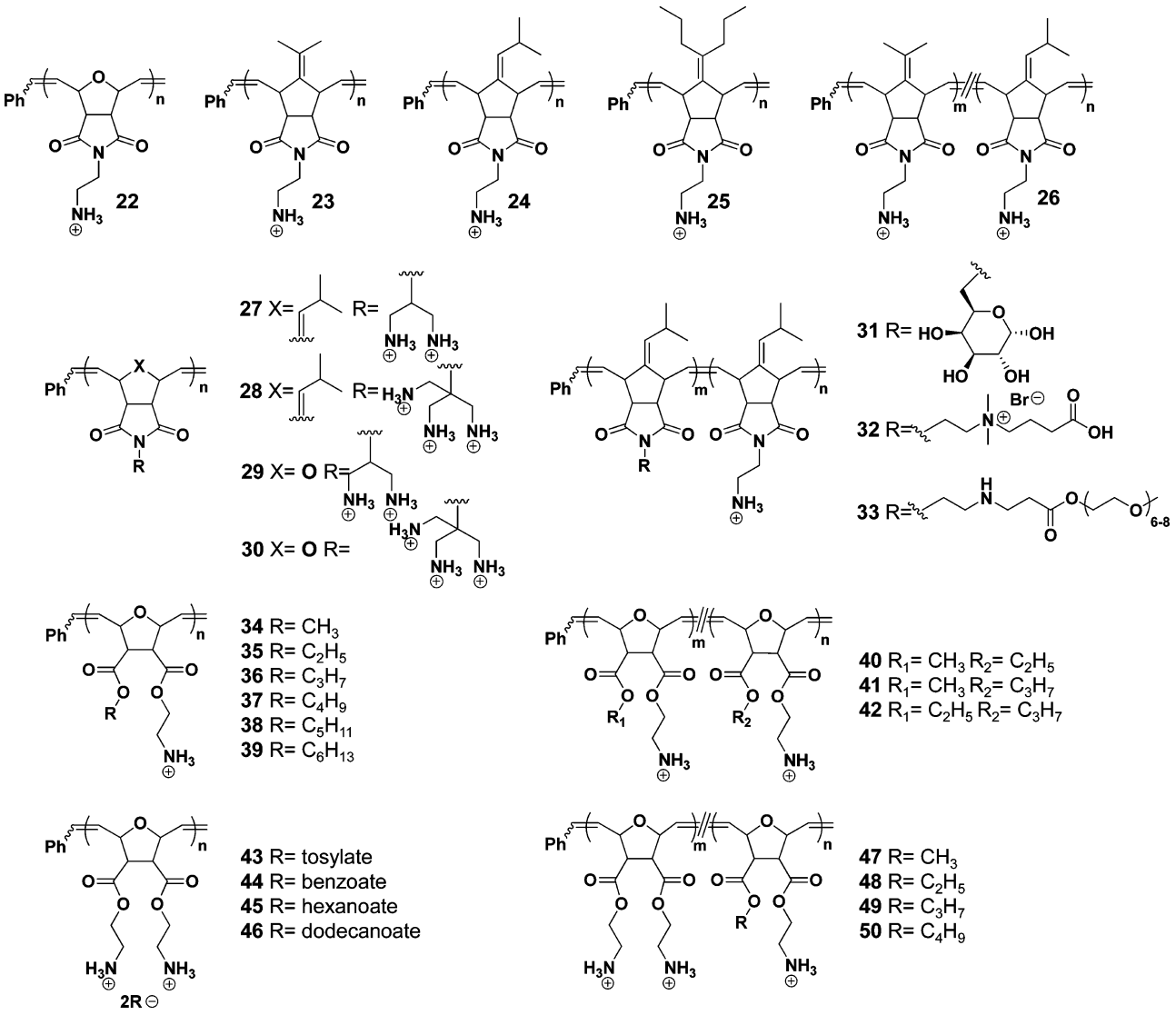

FIGURE 5. Antimicrobial polymers. Values for *n* and *m* are detailed in the text.

and design capabilities. The initial work on oligomeric systems as nonbiological AMPs mimics was conducted with aromatic scaffolds.[15−17] Their activity against several bacterial strains demonstrated their broad spectrum; however, these polymers were also found to be hemolytic, likely due to the significant hydrophobicity of the aromatic groups.

Therefore, we developed aliphatic polymeric SMAMPs based on polynorbornene backbones obtained by ring-opening metathesis polymerization (ROMP). The ease, efficiency, and control of ROMP as well as its high functional group tolerance made this synthetic platform advantageous for our purposes.

The first series of such antimicrobial ROMP polymers (**22**−**25**, Figure 5) included primary ammonium groups opposite a variety of hydrophobic side chains.[33] The effects of hydrophobicity as well as the MW were studied in terms of MIC (*E. coli*, *B. subtilis*) and HC_{50}. Four polymers with M_n = 10 000 g/mol were first screened to determine antimicrobial activity. A general trend of increasing hemolytic and antimicrobial activity with increasing hydrophobicity (**22**−**24**) was observed, with **22** and **25** performing poorly due to low activity and high hemolysis, respectively. Among these four antimicrobial polymers, no significant changes to MIC or HC_{50} values were observed as a function of MW, the only time this has been true to date for ROMP-synthesized SMAMPs.

Given the intermediate properties of **23** and **24**, several random copolymers were synthesized using different monomer ratios in an effort to design a polymer that was both nonhemolytic and antimicrobial. A copolymer consisting primarily of monomers from **23** was found to have a selectivity of >100. This value demonstrates the versatility and tunability for this series of polymers.

An alternate approach to reducing the toxicity of **24** was to increase hydrophilicity. The ammonium density on **24** was increased to either two or three amines per residue (**27**–**28**).[34] These SMAMPs maintained antimicrobial activity (MIC ~ 30 μg/mL for *E. coli*, ~50 μg/mL for *S. aureus*) accompanied by a nearly 1000-fold decrease in hemolysis, yielding selectivities of nearly 100. In addition to changing the ammonium density, the monomer used in **24** was copolymerized with other hydrophilic monomers, yielding polymers **31**–**33**.[35] In order to reduce hemolysis, large ratios of the hydrophilic comonomer were required, which also decreased the antimicrobial activity. This decrease was attributed to an overall decrease in positive charge, an important characteristic of active SMAMPs. One exception to this was **32** which had a 1:1 ratio of the two monomers. This polymer displayed low hemolysis (HC_{50} = 1500 μg/mL) and moderate antimicrobial activity, resulting in selectivities of 10 for *E. coli* and 7.5 for *S. aureus*.

The "second" generation of antimicrobial oxanorbornene-based polymers (**34**–**42**) expanded the control of hydrophobicity and charge by introducing difunctionalized, diester monomers.[36] It was found that homopolymers **34**–**39** displayed different biological activities at varying MWs, with lower MWs affording higher activity. **36** was selected due to its high activity, and a series of low MW oligomers (DP = 2–7) was synthesized to determine the effect of MW on biological activity. While selectivities toward *E. coli* remained similar across the series, those toward *S. aureus* dropped steadily from 280 (dimer) to >0.25 (M_n = 10 000 g/mol). This trend was attributed to larger SMAMPs becoming trapped within the murein layer of Gram-positive bacteria, decreasing their activity.

Copolymers were synthesized combining monomers used in **34** (nonactive, nonhemolytic) with those used in **36** (active, hemolytic). Copolymers were also made using monomers in **35**, as it displayed the highest selectivities among the homopolymers. Of these, **41** demonstrated the best selectivities toward *S. aureus* (>533) at all ratios tested (9:1, 1:1, and 1:9 of monomers in **34**:**36**) while remaining relatively inactive (selectivity = 10) toward *E. coli*. This difference was shown to be an effect of the double membrane present in Gram-negative bacteria rather than an issue of membrane composition.[37]

An alternative approach to controlling hydrophobicity examined the counterion. A series of diamine homopolymers coupled with counterions **43**–**46** were synthesized, and it was determined that increasing the counterion size/hydrophobicity lowered antimicrobial activity due to strong polymer–counterion complexation, decreasing the membrane activity of the polymer.[38]

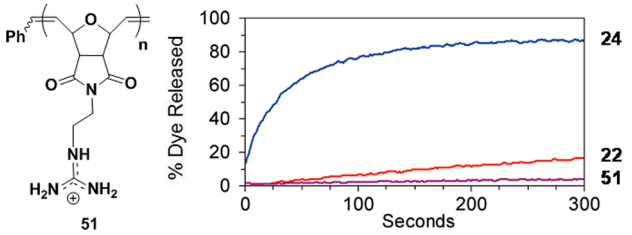

FIGURE 6. PGON structure and its membrane activity compared to its analogues **22** and **24** as measured in PE/PG vesicle dye release assay.

Similar to charge density studies performed on imide based SMAMPs (**27**–**30**), the diamine monomer was copolymerized with previously reported monomers to yield polymers **47**–**50**. Of these copolymers, **47**, with a ratio of 9:1 methyl/amine:diamine, showed the highest selectivity (650) toward *S. aureus*. On the contrary, **49** and **50** showed good activity against *E. coli* at high ratios of hydrophobic monomer due to the polymers' increased abilities to disrupt the membrane via hydrophobic interactions. This study demonstrated the difference between Gram-positive and Gram-negative membrane disruption mechanisms as well as the ability to design selective SMAMPs by tuning the polymer compositions.

PGON: A Non-Membrane-Disruptive SMAMP. Although most AMPs and their synthetic mimics show direct action on the membrane, some, like buforin, also have intracellular targets;[39] however, any intracellular target still requires these molecules to transverse the lipid membrane. Polyguanidinium oxanorbornene (PGON, **51**, Figure 6), a guanidine-functionalized version of **22**, appears to fall within this category. The introduction of the guanidine functionality instead of amines led to totally new, unique membrane interactions. To the best of our knowledge, this was the first guanidine-containing, polymeric SMAMP. The presence of this group gave PGON significant activity against both Gram-positive and Gram-negative bacteria (MICs of 6 μg/mL for *E. coli* and 12 μg/mL for *S. aureus*) while remaining nonhemolytic.[40] When tested in a dye release assay, PGON was clearly different from the many analogues previously studied. Even though highly bactericidal, it was found not to disrupt membranes of bacterial-like PE/PG vesicles (Figure 6). Cell staining confirmed a lack of membrane disruption, suggesting a different mechanism for killing bacteria.

The peculiar membrane activity of PGON and its chemical similarity with CPPs, such as polyarginine and TAT, suggested it may be an effective membrane transporter in addition to being antimicrobial. As anticipated, PGON indeed facilitated release of specific fluorescent dyes from PC vesicles without the help of an external activator like pyrenebutyrate.[41] External activators

are normally bulky, aromatic groups that provide the necessary hydrophobicity to aid polyarginines in efficient membrane activity. None of the PGON derivatives studied (DP = 5−41) required an activator, presumably due to the inherent hydrophobicity of the polyoxanorbornene backbone compared to peptides. Moreover, the membrane activity appeared to correlate in a nonlinear manner to the degree of polymerization, with longer polymers performing better than short ones ($EC_{50} = 2 \times 10^{-8}$ M for DP = 41; $EC_{50} = 3 \times 10^{-6}$ M for DP = 5),[41] in agreement with the behavior of well-known peptide systems such as polyarginine. The DP-dependence of PGON membrane activity was also explored by SAXS.[42] PGON derivatives were able to generate negative Gaussian curvature in PE-rich membranes, with a maximum induction at intermediate polymer length (DP = 14).

The unique membrane interaction properties of PGON are most likely due to the guanidine side chain. Many membrane-active peptides and proteins, such as the TAT peptide or the amphipatic α- and θ-defensins, display a stronger cell-membrane interaction with arginine over lysine.[43] As suggested by Wender and others, one of the advantages of arginine over lysine is its ability to form stable bidentate hydrogen bonds with phosphate and sulfate anions.[44] More recently, quantum mechanical (QM) calculations showed that both guanidinium and amine groups are able to coordinate two phosphate groups together, with the same complexation energy (ca. 160 kcal/mol).[42,45] However, because of the bidentate H-bonding ability and the planar Y-shape of the guanidinium groups, arginine side chains are induced to lay on the bilayer instead of staying perpendicular as in the case of lysine. Moreover, they are able to stack in a "face to face" conformation, in the case of polyarginine, and still coordinate two phosphate groups each even at a distance less than 5 Å. This creates a steric hindrance on the membrane and a lipid head crowding that generates a negative Gaussian curvature.[46−49] As shown by QM calculations, curvature generation by PGON is also sensitive to the guanidinium group spacing, since an increase from 3.6 Å in polyarginine to 5.8 Å in PGON caused a 22% decrease in the maximum induced negative Gaussian curvature.

Introduction of the guanidine groups in oxanorbornene polymers resulted in unique membrane activity, making PGON both a good SMAMP with high antimicrobial activity and low cytotoxicity, and a good CPP-like membrane transporter. This unique membrane activity of guanidine-rich polyoxanorbornenes motivated our group to explore more carefully polymer designs that mimics arginine-rich CPPs.

CPPs and Their Synthetic Mimics

The field of CPPs started two decades ago when HIV-1 TAT, a small nuclear trans-activator of transcription protein, was shown to readily cross the cellular membrane and localize into the nucleus of many cell lines.[50,51] It was then determined that this activity was the result of a small, cation-rich domain between amino acids 49−57 (RKKRRQRRR).[52,53] This sequence was referred to as a PTD (Protein Transduction Domain). Synthetic variants, mostly peptides composed exclusively of arginine residues, such as polyarginine, outperformed TAT and entered cells in a length-dependent fashion.[43,54] The most active range was found to be between 5 and 17 arginine residues. Since then, β-peptides and several other molecular scaffolds (unique MWs) were richly decorated with guanidine functionality and showed similar internalization properties.[44,55] In addition to TAT, in 1991, Antennapedia, a *Drosophila* homeoprotein widely studied since the 1980s, was found to be readily internalized by cells.[56] Extensive studies demonstrated that the third helix, amino acids 43−58 (RQIKIWFQWRRMKWKK), was required for internalization, leading to the development of *penetratin*.[57,58] Although this CPP contains arginine and lysine residues like Tat_{49-57}, it also contains hydrophobic residues, which are critical for its cellular uptake. This expanded the design and suggested the importance of hydrophobic residues for efficient cellular uptake.

Since then, the study of peptide sequences has expanded tremendously in the search for PTDs. These include Transportan (GWTLNS-AGYLLGKINLKALAALAKKIL-NH_2), which is a fusion between the neuropeptide galanin-1−13 and wasp venom peptide mastoparan, first reported in 1998, and Pep-1 (KETWWETWWTEWSQPKKKRKV-cya), which is the fusion of the lysine-rich NLS from Simian Virus 40 large T antigen and a tryptophan-rich sequence linked by the SQP sequence, first reported in 2001.[59,60] It is worth noting that few CPPs have FA topologies but tend toward more block-type (linear segregation along the backbone) arrangements (Pep-1, Transportan, MPG, etc.).

Inspired by the abilities of these native and chimeric proteins to translocate membranes, our group aimed to develop a series of nonpeptidic, synthetic CPP mimics (CPPMs) that captured these unique features (Figure 7). Kiessling and Wender/Hedrick/Waymouth have also independently reported polymeric CPPMs.[53,61−63] Much like our antimicrobial polymers, we utilized ROMP since it is fast, efficient and yields polymers with low PDIs. In addition to homopolymers and random copolymers, the living nature of this synthetic platform has allowed for the synthesis of block copolymers. Moreover, a wider range

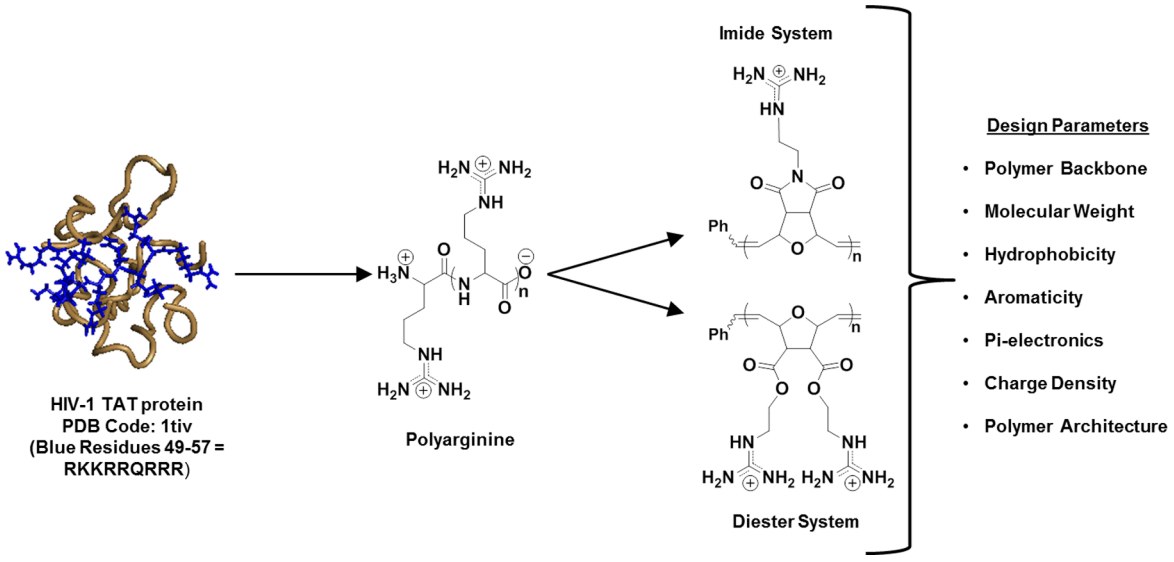

FIGURE 7. From CPPs to CPPMs.

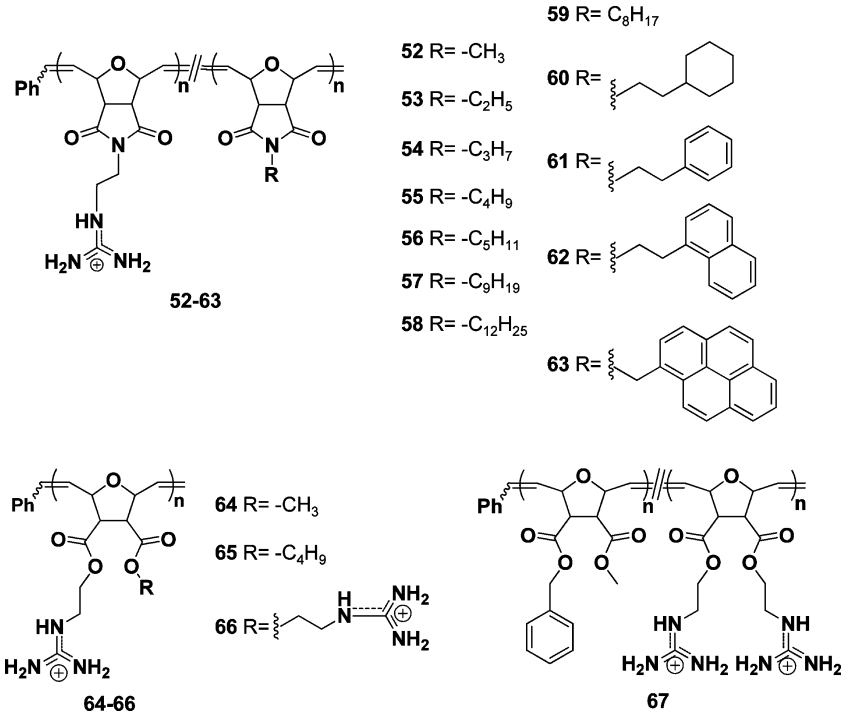

FIGURE 8. Oxanorbornene-based CPPMs.

of synthetic variations became available compared to native proteins or peptides, which is important for easy structural and physicochemical optimizations.

Based on previous work, which indicated that supramolecular hydrophobic activators enhance membrane activity of polyarginine, we aimed to directly incorporate these activators into CPPMs. Guanidine-containing monomers were copolymerized with a series of increasingly hydrophobic monomers ranging from methyl to dodecyl alkyl chains (**52**–**58**, Figure 8).[64] The activity of this series, compared to PGON, was significantly improved. The butyl-containing molecule **55** showed the best activity (EC_{50} = 0.003 μM compared to 6.4 μM of **52**), while polymers containing longer alkyl chains showed lower activities, due to poor solubility. This structure–activity study additionally demonstrated that "neutralization" of the guanidinium cationic charge by hydrophobic counteranions is not required for activity.

Knowing that aromatic amino acids are present in both membrane proteins and many CPP sequences, like *penetratin* and *Pep-1*, and that the best activators are also aromatic,[65] we further investigated the role of aromaticity in CPPMs. A series of CPPMs (**59**–**63**) was designed to compare linear and cyclic aliphatic side chains versus aromatic side chains.[66] Among the series, the aromatic **61** was the most active (EC_{50} = 4.3 nM) while also being the least hydrophobic of the series according to HPLC RTs. This suggested that aromaticity may indeed play a special role in CPP activity.

Besides the imide-based series, we also developed the diester synthetic system (Figure 7), which easily allowed doubling of the functional group density and offered the opportunity to vary two side chains independently. Homopolymers containing one guanidine functionality and one hydrophobic group (methyl or butyl alky chains) per repeat unit (**64**, **65**) were synthesized and tested. Vesicle studies showed that the polymers behaved similar to the imide system (**52**–**58**). In addition, polymers **64** and **66** of various MWs were evaluated in vitro for cellular uptake with HEK293T, CHO, and Jurkat T cells.[67] The two series of polymers studied were different based on the presence of a methyl group (**64**) and the density of guanidine groups (double for **66**). Both polymers were able to function as CPPMs, and **64** with 9 guanidine groups outperformed the control peptide **R9**. The fact that **64** and **66**, with 12 and 18 guanidinium groups, respectively, showed similar internalization efficiencies in HEK293T suggests that not only the number, but also the guanidine density has an effect in this cell line.

Expanding these preliminary reports, a block copolymer (**67**) with a total DP = 10 and hydrophobic to hydrophilic ratio of 1:1 was synthesized to mimic the best features of Tat_{49-57} and Pep-1. This polymer was compared to **66** (DP = 9) for siRNA delivery against *hNOTCH1* into Jurkat and human peripheral blood mononuclear cells (PBMCs).[68] **67** outperformed **66** and reduced *hNOTCH1* expression by 50%. This is a substantial knockdown considering *hNOTCH1* is a highly regulated gene and not a reporter gene.

Outlook

Using AMPs and CPPs as case studies, it has been possible to build synthetic mimics of the natural systems using simple, synthetic building blocks. This is essential for numerous reasons including the fact that it provides new model systems for understanding fundamental mechanisms. The larger toolbox of synthetic chemistry also enabled us to eliminate detrimental features of peptides leading to clinical development of a novel antibiotic. It would be reasonable to expect similar results in the CPPM field. Finally, these mimics are leading to new insight on polymer-membrane assemblies which are expected to have important implications in manipulating cell biology.

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BIOGRAPHICAL INFORMATION

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Gregory N. Tew was trained in Chemistry, Materials Science, and Biophysics before joining the Faculty at PSE in 2001. Since then he has received a number of awards including the PECASE, currently serves as Chair of the ACS Polymer Chemistry Division, and is a 2013 ACS Fellow. It remains a pleasure to work with a diverse group of talented students interested in complex and challenging scientific questions.

FOOTNOTES

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The authors declare no competing financial interest.

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