On the Electrostatics of Cell-Membrane Recognition: From Natural Antibiotics to Rigid Push–Pull Rods

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Abstract: The question why pore-forming, α-helical natural antibiotics are not toxic is discussed within the general context of the interaction of electrostatically asymmetric "nanorods" with neutral, anionic, and polarized bilayer membranes. We suggest that simplification of the structural complexity of natural systems will be necessary to ultimately define the involved subtle balance between constructive and destructive electrostatic interactions.

Keywords: antibiotics · ion channels · membranes · molecular recognition · peptidomimetics

Introduction

Pore formation in cell membranes by extracellular agents is usually fatal. Many pore-forming toxins isolated from plants and animals illustrate the powerful use of this mechanism in nature. It is thus intriguing to learn that certain antibacterial components of the innate immunity of human, animals, and plants (i.e., "natural antibiotics") utilize this seemingly deadly mechanism to selectively permeabilize the cytoplasmic membranes of Gram-positive and Gram-negative bacteria.[1] In particular, the capacity of natural antibiotics to withstand microbial resistance despite their use over thousands of years implies that selective pore formation in bacterial cell membranes may be a "biomimetic" strategy to bypass resistance.[2] The central question underlying the mode of action of natural antibiotics, however, is still open: "How, exactly, can pore-forming molecules differentiate between cell membranes?" or, on the macroscopic level: "Why are pore-forming natural antibiotics not toxic?"

At least four distinct mechanisms are conceivable for cell-membrane recognition by extracellular agents. Specific binding to constituents that are unique for the targeted membrane is best exemplified by the recognition of ergosterol by the clinically used antifungal agent amphotericin B.[3] The three other mechanisms are conceptually different: they focus on the recognition of unique physical properties of the targeted biomembrane, namely on their thickness,[4] charge, and potential (Figure 1). A combination of the last two recognition mechanisms has been suspected to account for the specific activity of natural antibiotics in the cytoplasmic membranes of bacteria, because they are characterized by anionic surfaces and unusually high, inside negative membrane potentials around -200 mV.[1, 5, 6]

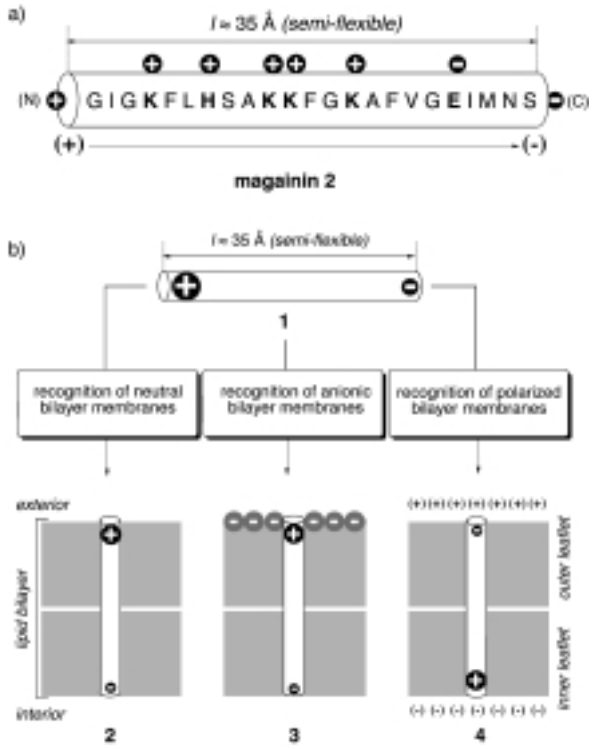

Figure 1. a) Sequence, topology, and electrostatic asymmetry of the α-helical natural antibiotic magainin 2. b) Simplified general structure of electrostatically asymmetric rods **1** and their possible recognition neutral bilayers in host–guest complex **2**, of anionic membrane surfaces by constructive electrostatic interactions in host–guest complex **3** and of polarized membrane by constructive electrostatic interactions in host–guest complex **4**.

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The electrostatic principles underlying recognition and depolarization of bacterial membranes by natural antibiotics, however, are practically unknown despite considerable efforts in academia and industry over the past few years. In this concept article, we will elaborate on our impression that these difficulties to elucidate the electrostatics of cell-membrane recognition are directly related to the elusive structural complexity of natural systems (Figure 2). This view will be further refined by discussing novel insights on mechanistic, methodological, electrostatic, and structural aspects of bilayer membrane recognition with particular emphasis on the interaction of the membrane potential with the dipole moment of asymmetric molecular rods. From this discussion, we will then try to extract guidelines for the design of future "universal probes" that fulfill all prerequisites needed to ultimately define the specific electrostatic interactions of asymmetric "nanorods" with neutral, anionic, or polarized biomembranes. With a permanent eye on this specific aim, the selection from a remarkable body of recent literature and the discussion of the chosen studies are necessarily subjective. Interested readers may, however, obtain access to more comprehensive, detailed, and objective information through the bibliography.

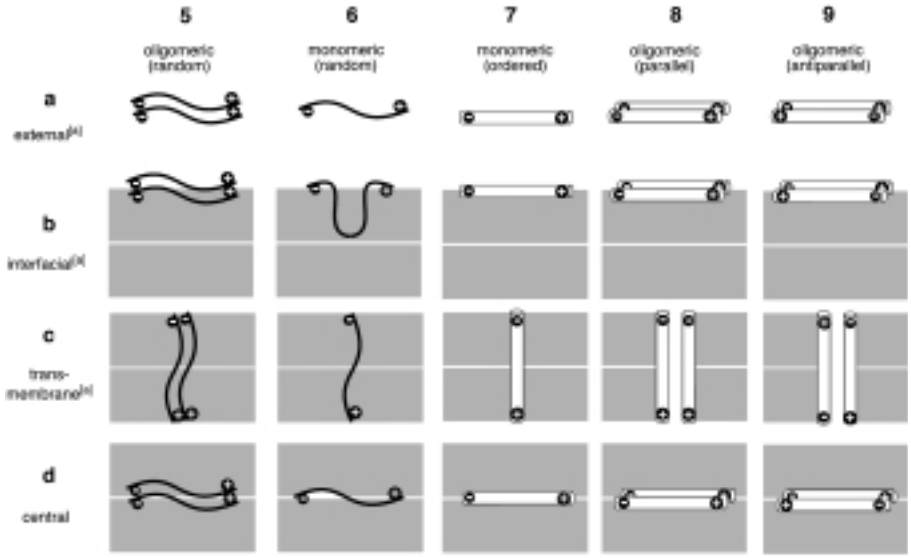

Figure 2. Structural complexity of external guests in bilayer hosts classified by location [external (**a**), interfacial (**b**), transmembrane (**c**), and central (**d**)], conformation [random (**5**, **6**), ordered (**7**, **8**, **9**)] and aggregation [monomeric (**6**, **7**), oligomeric (**5**, **8**, **9**)]. In principle, all these structures are in equilibrium. [a] Structures that are duplicated in the case of membrane asymmetry with the exception of **9c**.[10]

Discussion

Mechanistic considerations: "The paradigm of natural antibiotics" is magainin 2 (Figure 1a).[7, 8] In its α-helical conformation, magainin 2 may be considered as a semi-flexible, amphiphilic "nanorod" with a length that roughly matches the thickness of the hydrophobic core of biomembranes. The anionic charges of magainin 2 are located near the C terminus of the α-helical rod, while the center and N terminus are cationic. This axial charge separation coincides with the direction of the dipole moment of the α helix, which originates from uniform alignment of the dipoles of the backbone amides. Fixed permanent charges and the backbone dipole therefore create considerable electrostatic asymmetry along the long axis of α-helical magainin 2 (see simplified structure **1** in Figure 1b).[9]

The complexes formed by electrostatically asymmetric α-helical nanorod guests in bilayer hosts can conceivably be classified by rod location (Figure 2a–d), conformation, and aggregation (structures **5**–**9**, Figure 2).[10] For magainin 2, host–guest complexes with disordered and ordered, monomeric and oligomeric, external, interfacial, and transmembrane rods have been shown or proposed to be involved in the formation of active pores (**6a**, **6b**–**9b**, **7c**–**9c**). The most likely active pore structure of magainin 2 is thought to be a transmembrane parallel oligomer (**8c**) of transient nature; active interfacial structures (**6b**–**9b**) have been suggested as well.[7, 8] Such likely active host–guest complexes of magainin 2 and other α-helical rods with bilayer hosts are in *concentration-dependent* equilibrium with inactive structures.[7, 8, 10] The effects of anionic membrane surfaces and membrane polarization on this situation (i.e., the structural basis of cell-membrane recognition by natural antibiotics) are thus inherently complex and difficult, if not impossible, to define. For this reason, we felt that the isolated discussion of the possible effects of membrane charge and potential on the simplest possible transmembrane host–guest complex (**7c**, Figure 2) may be more productive (Figure 1b).

In neutral and unpolarized membranes, the driving force for transmembrane orientation is hydrophobic matching of the length of rod guest **1** with the thickness of the bilayer host in host–guest complex **2**.[4] Terminal charges may stabilize this transmembrane orientation by electrostatic interactions at the membrane/water interface. The formation of complex **2** with bolaamphiphilic guests seems, however, energetically more demanding, because it requires charge translocation across the hydrophobic core of the membrane. Thus, bolaamphiphilic guests possibly remain at the membrane/water interface (Figure 2b). On the other hand, this increased activation energy will also hinder dissociation of an established complex **2** (Figure 1 and Figure 2c). For electrostatically asymmetric guests **1**, the less charged terminus can be expected to cross the membrane and end up at the internal interface in host–guest complex **2**.

Constructive ionic interactions would further stabilize the transmembrane orientation of the asymmetric rod **1** in lipid bilayers with negatively charged surface (Figure 1, **3**). These

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interactions at the external interface may account, therefore, for the recognition of anionic membranes in the presence of neutral ones by cationic rods.[11]

In polarized membranes, the alignment of electrostatically asymmetric nanorods to the membrane potential may be the dominant driving force for transmembrane orientation. (As discussed later on, there is practically no direct structural evidence for this fundamental effect of membrane potentials.) Host–guest complex **4** exemplifies the biologically most significant situation with inside negative membrane potentials. In this case, the cationic terminus is expected at the internal interface. For the overall cationic rods of biological importance, this implies that the terminus of higher charge may cross the hydrophobic core of the bilayer. Therefore, the activation energy for complex formation and dissociation may be considerable. However, complex formation, but not dissociation, should be facilitated by energy gains from the favorable alignment of the axial-rod dipole to the membrane potential. Thus, the unusually strong inside negative potentials found in bacteria could, in principle, facilitate formation *and* practically inhibit dissociation of host–guest complex **4**, while the weaker membrane potentials of mammalian cell membranes may not offer enough energy for transmembrane charge translocation.[12]

Favorable alignment to the negative potential together with initial electrostatic attraction to the negative membrane surface may thus account for the recognition of strongly polarized bacterial membranes by asymmetric cationic rods **1**. The isolated comparison of the hypothetical host–guest complexes **4** and **3**, however, is more puzzling and practically unexplored. Namely, both complexes may be stabilized by specific electrostatic interactions between rod guest and bilayer host, but the relative rod orientation in host–guest complex **4** is opposite to that in host–guest complex **3**. This apparently competing situation applies for the orientation of asymmetric rods in anionic *and* polarized (bacterial) membranes as well. Specifically, membrane polarization may destabilize complex **3** because of the unfavorable alignment of rod dipole and potential. The presence of an anionic outer membrane surface, on the other hand, may destabilize complex **4** because of destructive electrostatic interactions with the anionic rod terminus. The recognition and depolarization of anionic, highly polarized bacterial membranes by membrane-spanning asymmetric rods that form ion channels (pores) will ultimately depend on a subtle balance of the involved electrostatics, that is, rod asymmetry and charge on the one hand and membrane potential and surface charge on the other.

As mentioned in the introduction, these electrostatic principles that determine cell-membrane recognition are poorly understood. We have suggested that one explanation for this situation is an eventually misleading influence of the involved structural complexity on conclusions drawn from the observed activities of asymmetric rods. For instance, the expected presence of transient structures and concentration-dependent multiple equilibria suggests that relevant structure determination must be done in a time-dependent manner at biologically significant concentrations. Moreover, modifications of the electrostatic nature of α-helical asymmetric nanorods are likely to result in undesired structural changes. The most troublesome effects are possible destabilization of α helices (or reduction of amine pK_a's) by destructive electrostatic interactions between fixed charges and the α-helical dipole.[13] Unlimited variability of the electrostatic asymmetry of nanorods without undesired conformational (and subsequent supramolecular) consequences, however, seems imperative for precise elucidation of the electrostatics of cell-membrane recognition. Ideally, one would like to work with asymmetric rods that follow the simple mechanisms in Figure 1 without any of the structural complications depicted Figure 2. In the following we will therefore try to define the prerequisites of "universal probes" that would, at least on paper, be compatible with this ultimate objective.

Methodological considerations: For the reasons discussed in the preceding paragraphs, the determination of the orientation of molecules in lipid bilayers *at biologically relevant concentrations* is essential for studying the effect of membrane potentials on electronically asymmetric rods (around 1 μM or less). The following examples demonstrate that, in our view, fluorescence depth quenching is the most suitable method for this not trivial purpose.

The orientation of "the paradigm of natural antibiotics" in bilayer membranes has been elucidated by fluorescence depth quenching (Figure 3).[8] For this purpose, Matsuzaki and coworkers prepared three analogues of magainin 2 with the fluorescent tryptophans in position 5 (5-W-), 12 (12-W-), and 16 (16-W-magainin 2). The emission intensities of these W-tags were measured in membranes containing quenchers Q at defined distance d_Q from the center of the bilayer (Figure 3a). The observed relative efficiency of a quencher Q at a distance d_Q from the center of the bilayer is directly proportional to the average distance d_{QF} between fluorophore F and quencher Q. Comparison of the efficiencies of (at least) two quenchers Q_1 and Q_2 with different d_Q gives d_F, the distance of the fluorophore F from the center of the bilayer. Comparison of the d_F values of (at least) two fluorophores F_1 and F_2 at a defined distance d_{FF} with respect to the molecular rod then gives its orientation in the bilayer. For magainin, identical d_F values of 8–10 Å for 5-W-, 12-W-, and 16-W-magainin were consistent with interfacial location (**7b**–**9b**, Figure 2) in anionic, unpolarized bilayer membranes. Although no studies in polarized membranes were reported, this work illustrates the prerequisites for orientational studies by fluorescence depth quenching, that is, the presence of (at least) one fluorophore F_1 near the terminus and one fluorophore F_2 near the middle of the molecular rod (Figure 3a).

Pioneering orientational studies in polarized membranes have been performed with lantibiotics.[14] These commercially used, cationic natural antibiotics, which also function by permeabilizing the membrane, may be considered as asymmetric "rods" rigidified by the presence of α,β-didehydro amino acids and multiple cycles along a highly unusual peptide backbone. Epilancin K7, a lanthibiotic with random-coil conformation in water and increased α-helical structure in "membrane-like" environments,[14b] contains a central tyrosine suitable for fluorescence depth quenching (Figure 3).

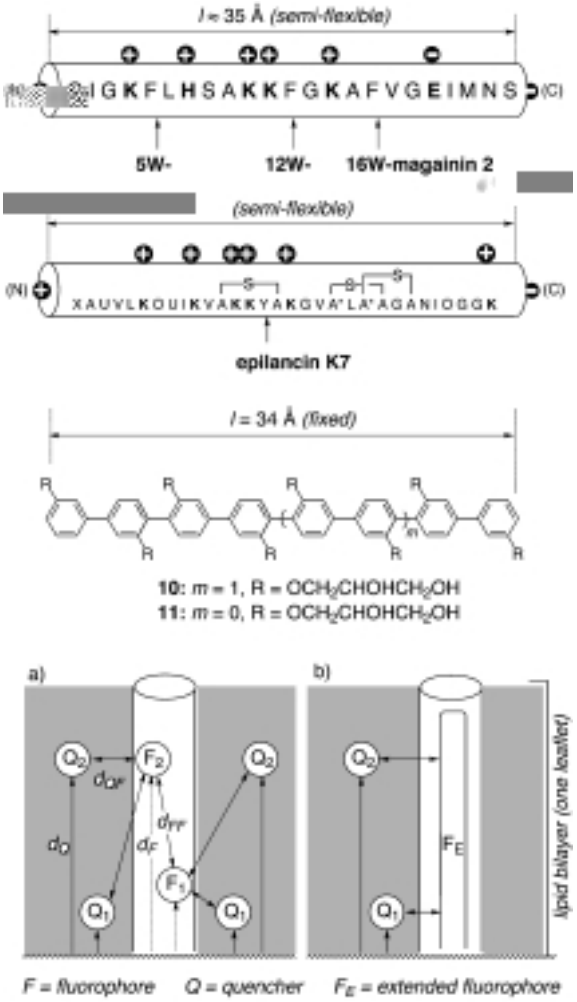

Figure 3. Examples of α-helical natural antibiotics (magainin 2), lantibiotics (epilancin K7), and oligo(*p*-phenylene)s that were subjected to orientational studies in lipid bilayers by distance-dependent depth quenching of a) multiple fluorophores of precise location or b) one extended fluorophore. Abbreviations: A*-S-A, β-methylanthionine; A-S-A, lanthionine; U, α,β-didehydroalanine; O, α,β-didehydrobutyrine; X, unknown.

Very little quenching was observed in unpolarized neutral membranes that contained quenchers with different d_Q. Application of an inside negative membrane potential, however, caused efficient quenching that disappeared within about five seconds—about the time needed for the pore-forming lantibiotic to depolarize the model membrane. This finding provided unique direct evidence that an inside negative membrane potential indeed pulls the center of epilancin K7 from the surface (conformation **5b**–**9b**, Figure 2) to the center of the membrane. Although additional terminal labeling of epilancin K7 would be needed to differentiate between transmembrane (**5c**–**9c**) and central locations (**5d**–**9d**), this study demonstrates that transient structures in polarized vesicles are detectable at biologically relevant concentrations by fluorescence-depth-quenching techniques.

Depth-quenching experiments in our lab indicated that orientational studies may be simplified by using extended fluorophores such as oligo(*p*-phenylene)s **10** and **11** (Figure 3b).[15] The following simple trends were deduced from unambiguous experimental evidence: About identical quenching of fluorescent rods by quencher Q_1 near the center and Q_2 near the interface (i.e., $d_{Q1F} \approx d_{Q2F}$) proves transmembrane orientation (**5c**–**9c**); $d_{Q1F} > d_{Q2F}$ consequently indicates central (**5d**–**9d**) and $d_{Q1F} < d_{Q2F}$ interfacial locations (**5b**–**9b**). In good agreement with results from W-tagged α-helical rods,[4a] transmembrane orientations consistently coincided with hydrophobic matching (e.g., **10**). Hydrophobic mismatch of the membrane core with regard to truncated oligo(*p*-phenylene) guests ($l < 27$ Å, e.g., **11**) did not affect rod binding, but resulted in central (**7d**) and interfacial orientations (**7b**);[15c] increasing mismatch with regard to lengthened rods ($l > 39$ Å) caused poor guest binding (**7a**–**9a**).[4d]

Electrostatic considerations: Comparison of the rod activities in response to the variation of the electrostatic asymmetry is evidently the practical approach toward insights on cell-membrane recognition by constructive electrostatic interactions. An inspired study on this topic from the Merrifield laboratory describes successful conversion of melittin, the principal toxin of the bee venom, into an antimicrobial peptide of identical amino acid composition.[16] Electrostatic analysis of the α-helical melittin rod **12** shows accumulation of the charged residues at the negative end of the α-helical dipole (Figure 4). This situation is opposite to that in

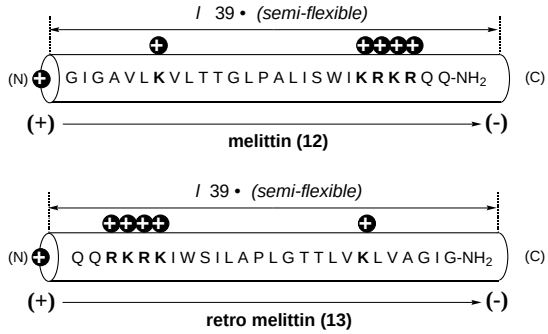

Figure 4. Sequence, topology, and electrostatic asymmetry of the (mainly) α-helical bee-toxin melittin (**12**) and antibacterial retro melittin (**13**).

magainin 2, in which the cationic residues are closer to the positive end of the α-helical dipole (Figure 1). It was argued that inversion of the α-helical dipole in retro melittin **13** may minimize the cytotoxicity of melittin, but maintain its antibiotic activity. Indeed, hemolysis by retro melittin **13** and its enantiomeric and N-acetylated analogues was reduced relative to that of melittin **12**, while their ability to kill Gram-positive and -negative bacteria remained unchanged.

To possibly correlate these results with increased capacity of retro melittin to recognize polarized membranes, the changes in the conductivity of planar or "black" lipid membranes (BLMs) in response to externally applied voltage were investigated. Indeed, slightly higher voltages were apparently needed to induce pore formation with retro melittin compared with melittin. The authors conclude that fixed permanent charges and α-helical dipole need to be considered independently, and that the orientation of the α-

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helical dipole is of particular importance for the recognition of membrane potentials. The most likely active structure of melittin and its analogues is thought to be a parallel oligomer (**8c**, Figure 2).

Recent studies by DeGrado, Lear, and co-workers are in (partial) support of this conclusion.[17] The three studied peptides differ in electrostatic asymmetry ranging from an α-helical dipole only for the uncharged peptide **14** to additional terminal charges for cation **15** and anion **16** (Figure 5). The

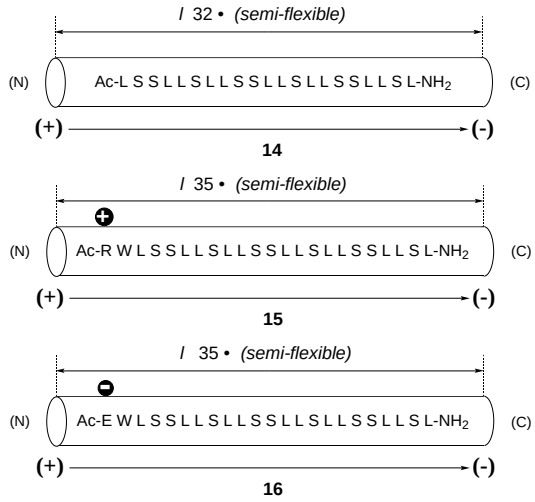

Figure 5. Sequence, topology, and electrostatic asymmetry of DeGrado and Lears neutral (**14**), anionic (**15**), and cationic (**16**) α-helical peptide models.

charge residues near the partial dipole charge of $\approx +0.5$ in **14** results in formal electrostatic *symmetry* for **16** and doubled asymmetry for **15**. Apparently, the formation of ion channels in neutral BLMs by all peptides was facilitated by an applied voltage that corresponded to an inside positive potential. The ion-conducting properties of the ion channel formed by **16**, on the other hand, were nearly independent of the external voltage, while enhanced voltage sensitivity was seen for **15** compared with **14**. These results clearly illustrate that two modes of activation of electronically asymmetric rods in polarized membranes may apply, that is, the population of the active channels and/or the activity of the open channel can increase. They further indicate that the latter is governed by the *overall* electrostatic asymmetry, while alignment of the rod dipole with membrane potential appears indeed particularly important for pore formation in polarized membranes as suggested by Merrifield.[16] Fluorescence depth quenching with W-tagged analogues of **14** suggested interfacial orientation in unpolarized membranes (**7b**–**9b**, Figure 2);[17c] active parallel oligomers of **14**–**16** are expected in polarized membranes (**8c**, Figure 2).

Structural considerations: Ironically, the apparently unique importance of their axial dipole disfavors α-helical peptides as universal probes for elucidation of the electrostatics of cell-membrane-recognition mechanisms, because it can not be varied. In view of the conformational, suprastructural, and orientational complexity of pores formed by α-helical peptide rods in general (Figure 2), the development of artificial ion channels has attracted much attention.[18] Synthetic pores that exhibit electrostatic asymmetry along a membrane-spanning scaffold, however, are still very rare and very novel.[18a, 18h, 19–25]

The presumably best characterized example is Fyles' macrocyclic bolaamphiphile **17** (Figure 6).[19] Ion channel

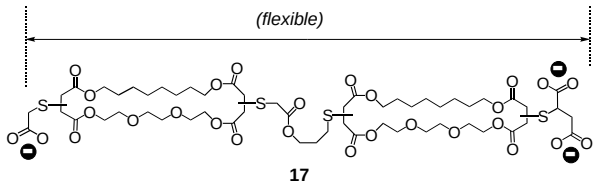

Figure 6. Structure of the electrostatically asymmetric macrocycle **17** studied in the group of Fyles.

formation by the flexible anion **17** was induced by inside positive membrane potentials. The formation of membrane-spanning parallel oligomers (**8c**, Figure 2) was deduced from nonlinear concentration dependence of this activity. The observed disappearance of voltage dependence with time was explained by slow transformation of the active parallel oligomers **8c** into electrostatically symmetric, antiparallel oligomers **9c** in polarized membranes. Partial protonation and replacement of the bis-carboxylate terminus in **17** by a neutral carbohydrate residue[20] resulted in near voltage independence. These findings were consistent with facilitated flip-flop due to charge reduction to yield symmetric antiparallel oligomers **9c**. In summary, Fyles study is of high significance for the understanding of the electrostatics of cell-membrane recognition, because it demonstrated with unprecedented clarity that totally synthetic, membrane-spanning ionophores can exhibit functional properties that were considered unique for α-helical peptides until recently.

The development of novel intramolecular ion-conducting pathways along membrane-spanning nanorods may significantly contribute to the simplification of the structural complexity of natural antibiotics. Toward this end, a clever combination of the best-understood synthetic ion-conducting pathway[18] with the best-understood natural scaffold has been conceived by Voyer and co-workers.[21] Namely, crown-ether residues have been placed on top of each other to provide an intramolecular ion-conducting pathway along one monomeric α-helix **18** (Figure 7).[22] Qualitative importance of high electrostatic asymmetry in zwitterion **18** for function was subsequently deduced from the reduced activity of partially or completely uncharged analogues in BLMs.[22a]

Almost identical intramolecular ion-conducting pathways are present in ionophore **19**, but the α-helical nanorod in **18** is replaced by an octi(*p*-phenylene) of similar length (Figure 7).[23] Electrostatic asymmetry along the oligo(*p*-phenylene) scaffold is introduced by a π donor at one and a π acceptor at the other rod terminus. Push–pull rod **19** depolarized neutral bilayer membranes with inside negative potential, while the corresponding pull–pull rod with two cyano termini was almost inactive in polarized membranes. The presence of an active transmembrane monomer **7c** (Figure 2) was supported by linear concentration dependence

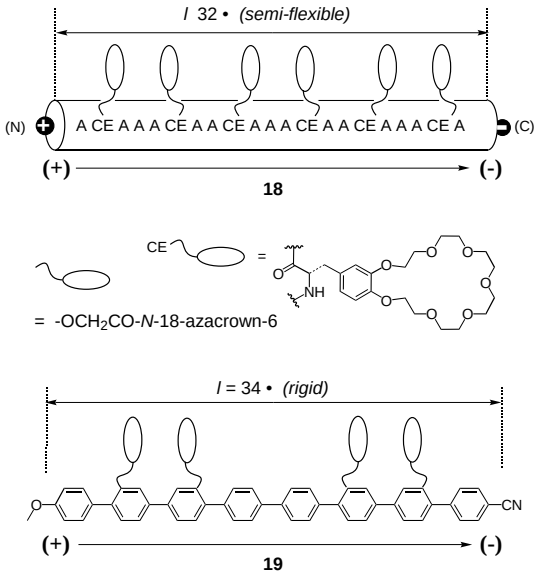

Figure 7. Structures of electrostatically asymmetric synthetic oligomers with crown-ether-based unimolecular ion-conducting pathways along α-helical peptide (**18**) and octi(*p*-phenylene) scaffolds (**19**).

of activity and extensive orientational studies with related oligo(*p*-phenylene)s by fluorescence depth quenching (see above).

An important general advantage of rigid octi(*p*-phenylene) compared with α-helical scaffolds is illustrated by the direct conversion of the push – push rod **20** into push – pull rod **21** by partial sulfide oxidation (Figure 8).[24] Namely, the molecular

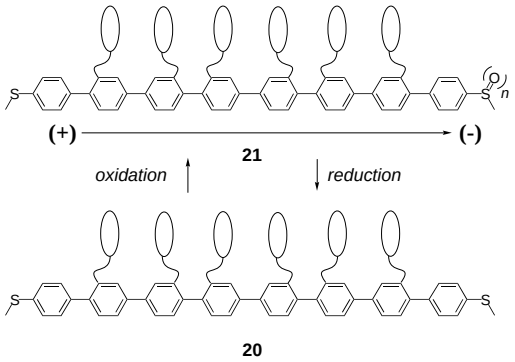

Figure 8. Unimolecular octa(*p*-phenylene) ion channels with variable axial dipole moment.

dipole along the rigid-rod scaffold can, *in principle*, be switched on and off in a controlled manner.[25] Thus, the use of rigid push – pull rods provides the so far unique variability of a permanently oriented dipole parallel to the long axis of a membrane-spanning scaffold, which seems crucial for elucidation of the importance of the alignment of dipole to potential for the recognition of polarized membranes.

Conclusion

Significant insights on isolated aspects involved in the electrostatics of bilayer membrane recognition by asymmetric rods have been obtained from the studies discussed above. These results may serve as (optional) guidelines for the design of future "universal probes" for comprehensive assessment of all involved parameters by direct experimental comparison. The following prerequisites deserve attention.

On the methodological side, experimental evidence was provided that even *transient* orientational changes in *polarized* membranes are detectable with confidence and at biologically significant concentrations by fluorescent depth quenching.[14] This implies that "universal probes" equipped with permanent central *and* terminal (or extended)[15] fluorophores will be compatible with relevant orientational studies.

Progress toward structural and mechanistic simplicity has been made by constructing unimolecular ion-conducting pathways along electrostatically asymmetric rods.[21–24] The use of one of several available unimolecular ion-conducting pathways[18] in "universal probes" seems desirable to minimize supramolecular complexity of the system (structures **5**, **8**, and **9** in Figure 2). To eliminate conformational complexity (structures **5** and **6** in Figure 2), the use of rigid nanorods may be a third important prerequisite for "universal probes". Total axial rigidity is so far unique for oligo(*p*-phenylene) push – pull rods.[23, 24]

Systematic variation of the electrostatic asymmetry of such "universal probes" may ultimately reveal the subtle electrostatic fine-tuning underlying cell-membrane-recognition mechanisms. Variability of the number of charges near the rod termini is guaranteed in all discussed models. In clear contrast, facile variability of the magnitude of the axial dipole in the context of roughly identical overall structures has been demonstrated for rigid push – pull rods only.[23, 24]

In summary, by reviewing the development from natural antibiotics to rigid push – pull rods, several prerequisites for "universal probes" for the study of the electrostatics of cell-membrane recognition have been identified. A rigid-rod molecule of about 3.5 nm length with permanent read-outs for structure and activity as well as variable axial dipole and terminal charges may contain all that is needed. None of the reviewed models fulfill all of these prerequisites. The insights gained from these model studies on isolated aspects of cell-membrane recognition, however, provide a solid basis for progress in an exciting research area of high importance for future public health.

Note added in proof: Two highly relevant reports have appeared in the literature after completion of this article:

1. A clever approach to reduce the conformational complexity of α-helical nanorods in biomembranes based on the use of more stable 3_1 helix formed by β-peptide scaffolds has been introduced.[26]
2. Ion-channel formation by oligo-(*R*)-3-hydroxybutyrates,[12g] a flexible, asymmetric and anionic scaffold, was shown to be voltage sensitive when bound to symmetric, anionic polyphosphates.[27]

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[1] a) J. E. Gabay, *Science* **1994**, *264*, 373, other articles in this special issue of *Science*, and references cited therein; b) J. Harder, J. Bartels, E. Christophers, J.-M. Schröder, *Nature* **1997**, *387*, 861.

[2] The danger of possibly excessive clinical use of an apparently natural defense mechanism has been noticed (C. F. Inglehearn, *Nature* **1997**, *388*, 416).

[3] J. Bolard, *Biochim. Biophys. Acta* **1986**, *864*, 257; see also refs. [8b, 14b].

[4] For recent examples on the importance of hydrophobic matching for cell-membrane recognition, see: a) J. Ren, S. Lew, Z. Wang, E. London, *Biochemistry* **1997**, *36*, 10213; b) S. Otto, M. Osifshin, S. L. Regen, *J. Am. Chem. Soc.* **1999**, *121*, 7276; c) J. A. Killian, *Biochim. Biophys. Acta* **1998**, *1376*, 401; for speculative implications of the usefulness of hydrophobic matching for the development of antimycobacterials, see: d) B. Ghebremariam, V. Sidorov, S. Matile, *Tetrahedron Lett.* **1999**, *40*, 1445.

[5] Similar properties originating from the presence of the unique anionic lipid A in the outer leaflet and large anions in the periplasm characterize the additional outer membrane of Gram-negative bacteria.[1a, 8b, 11a]

[6] Throughout this article, the term "inside negative (or positive)" is used to describe the membrane potential. This indicates that positive (or negative) potential is applied to the side at which the sample is added (outside or *cis* side) relative to the other side (*trans* side) of the membrane.

[7] a) T. Wieprecht, M. Beyermann, J. Seelig, *Biochemistry* **1999**, *38*, 10377; b) E. Jo, J. Blazyk, J. M. Boggs, *Biochemistry* **1998**, *37*, 13791; c) B. Bechinger, *J. Membrane Biol.* **1997**, *156*, 197; d) T. Wieprecht, M. Dathe, M. Beyermann, E. Krause, W. L. Maloy, D. L. MacDonald, M. Bienert, M. *Biochemistry* **1997**, *36*, 6124.

[8] a) K. Matsuzaki, O. Murase, H. Tokuda, S. Funakoshi, N. Fujii, K. Miyajima, *Biochemistry* **1994**, *33*, 3342; for other selected reports on this series, see for example: b) K. Matsuzaki, K. Sugishita, K. Miyajima, *FEBS Lett.* **1999**, *449*, 221; c) K. Matsuzaki, *Biochim. Biophys. Acta* **1998**, *1376*, 391.

[9] For natural, pore-forming antibiotics with β-sheet conformation, see, for example: S. H. White, W. C. Wimley, *Curr. Opin. Struct. Biol.* **1995**, *5*, 521, and ref. [1b].

[10] The representation in Figure 2 is simplified and, for example, does not include tilted and partial guest insertion. Compare, for example: P. J. Schanck, L. Lins, R. Brasseur, *J. Theor. Biol.* **1999**, *198*, 173.

[11] For recent examples on the interaction of hydrophobic cations other than nanorods with anionic bilayer membranes, see: a) C. Li, L. P. Budge, C. D. Driscoll, B. M. Willardson, G. W. Allman, P. B. Savage, *J. Am. Chem. Soc.* **1999**, *121*, 931; b) M. Merritt, M. Lanier, G. Deng, S. L. Regen, *J. Am. Chem. Soc.* **1998**, *120*, 8494.

[12] For the interaction of hydrophobic cations other than nanorods with polarized bilayer membranes, see: a) L. D. Mayer, M. B. Bally, M. J. Hope, P. R. Cullis, *J. Biol. Chem.* **1985**, *260*, 802; b) L. D. Mayer, M. B. Bally, M. J. Hope, P. R. Cullis, *Biochim. Biophys. Acta* **1985**, *816*, 294.

[13] a) K. Ma, E. L. Clancy, Y. Zhang, D. G. Ray, K. Wollenberg, M. G. Zagorsky, *J. Am. Chem. Soc.* **1999**, *121*, 8698; b) K. Johnson, R. K. Allemann, H. Widmer, S. A. Benner, *Nature* **1993**, *365*, 530.

[14] a) A. J. M. Driessen, H. W. van den Hooven, W. Kuiper, M. van de Kamp, H.-G. Sahl, R. N. H. Konings, W. N. Konings, *Biochemistry* **1995**, *34*, 1606; b) M. van de Kamp, L. M. Horstink, H. W. van den Hooven, R. N. H. Konings, C. W. Hilbers, A. Frey, H.-G. Sahl, J. W. Metzger, F. J. M. van de Ven, *Eur. J. Biochem.* **1995**, *227*, 757; the inhibition of potential-induced epilancin K7 reorientation by anionic membrane surfaces was supported later on by quenching experiments with W-tagged mutants of the lantibiotic nisin: c) E. Breukink, C. van Kraaj, A. van Dalen, R. A. Demel, R. J. Siezen, B. de Kruijff, O. P. Kuipers, *Biochemistry* **1998**, *37*, 8153; for current references on lantibiotics, see, for example: d) E. Breukink, I. Wiedemann, C. van Kraaij, O. P. Kuipers, H.-G. Stahl, B. de Kruijff, *Science* **1999**, *286*, 2361.

[15] a) L. A. Weiss, N. Sakai, B. Ghebremariam, C. Ni, S. Matile, *J. Am. Chem. Soc.* **1997**, *119*, 12142; For other selected reports on this series, see: b) N. Sakai, C. Ni, S. M. Bezrukov, S. Matile, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2743; c) C. Ni, S. Matile, *Chem. Commun.* **1998**, 755; d) N. Sakai, K. C. Brennan, L. A. Weiss, S. Matile, *J. Am. Chem. Soc.* **1997**, *119*, 8726.

[16] P. Juvvadi, S. Vunnam, R. B. Merrifield, *J. Am. Chem. Soc.* **1996**, *118*, 8989.

[17] a) J. D. Lear, J. P. Schneider, P. K. Kienker, W. F. DeGrado, *J. Am. Chem. Soc.* **1997**, *119*, 3212; for other selected reports on this series, see: b) G. R. Dieckmann, J. D. Lear, Q. Zhong, M. L. Klein, W. F. DeGrado, K. A. Sharp, *Biophys. J.* **1999**, *76*, 618; c) L. A. Chung, J. D. Lear, W. F. DeGrado, *Biochemistry* **1992**, *31*, 6608.

[18] Reviews on nonpeptide ion channels and channel models: a) U. Koert, *Chem. Unserer Zeit* **1997**, *31*, 20; b) G. W. Gokel, O. Murillo, *Acc. Chem. Res.* **1996**, *29*, 425; c) T. M. Fyles, W. F. van Straaten-Nijenhuis, in *Comprehensive Supramolecular Chemistry, Vol. 10* (Ed.: D. N. Reinhouldt), Elsevier, Oxford, **1996**, p. 53; d) N. Voyer, *Top. Curr. Chem.* **1996**, *184*, 1; some more recent references: e) S. Otto, M. Osifchin, S. L. Regen, *J. Am. Chem. Soc.* **1999**, *121*, 10440; f) Z. Qi, M. Sokabe, K. Donowaki, H. Ishida, *Biophys. J.* **1999**, *76*, 631; g) E. Abel, E. M. Maguire, O. Murillo, I. Suzuki, S. L. DeWall, G. W. Gokel, *J. Am. Chem. Soc.* **1999**, *121*, 9043; h) M. G. Fritz, P. Walde, D. Seebach, *Macromolecules* **1999**, *32*, 574; i) J. de Mendoza, F. Cuevas, P. Prados, E. S. Meadows, G. W. Gokel, *Angew. Chem.* **1998**, *110*, 1650; *Angew. Chem. Int. Ed.* **1998**, *37*, 1534.

[19] T. M. Fyles, D. Loock, X. Zhou, *J. Am. Chem. Soc.* **1998**, *120*, 2997.

[20] T. M. Fyles, D. Loock, W. F. van Straaten-Nijenhuis, X. Zhou, *J. Org. Chem.* **1996**, *61*, 8866.

[21] a) N. Voyer, L. Potvin, E. Rousseau, *J. Chem. Soc. Perkin Trans. 2* **1997**, 1469; b) J.-C. Meillon, N. Voyer, *Angew. Chem.* **1997**, *109*, 1004; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 967.

[22] The alternative route to intramolecular ion-conduction pathways (i.e., covalent assembly of all α-helices of an active parallel bundle **8c** by a unifying template) was realized earlier: a) M. Mutter, K.-H. Altmann, G. Tuchscherer, *Tetrahedron* **1988**, *44*, 771; b) A. Grove, M. Mutter, J. E. Rivier, M. Montal, *J. Am. Chem. Soc.* **1993**, *115*, 5919; for template-assembled α-helix bundles with model α-helices similar to **14**, see: K. S. Åkerfeldt, R. M. Kim, D. Camac, J. T. Groves, J. D. Lear, W. F. DeGrado, *J. Am. Chem. Soc.* **1992**, *114*, 9656; for recent progress in the field of template-assembled α-helix bundles, see: A. R. Mezo, J. C. Sherman, *J. Am. Chem. Soc.* **1999**, *121*, 8983; this productive approach is not discussed in the context of this article because is seems incompatible with facile variability of electrostatic asymmetry of nanorods.

[23] J.-Y. Winum, S. Matile, *J. Am. Chem. Soc.* **1999**, *121*, 7961.

[24] F. Robert, J.-Y. Winum, N. Sakai, D. Gerard, S. Matile, *Org. Lett.* **2000**, *2*, 37.

[25] A new approach to redox-active, centrosymmetric artificial channels has been proposed very recently: C. D. Hall, G. J. Kirkovits, A. C. Hall, *Chem. Commun.* **1999**, 1897; for possible conformational changes of α-helical rods caused by redox-switches, see: H. L. Schenck, G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 12487.

[26] Y. Hamuro, J. P. Schneider, W. F. DeGrado, *J. Am. Chem. Soc.* **1999**, *121*, 12200.

[27] S. Das, P. Kurcok, Z. Jedlinski, R. N. Reusch, *Macromolecules* **1999**, *32*, 8781.