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Review

The gramicidin ion channel: A model membrane protein

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Abstract

The linear peptide gramicidin forms prototypical ion channels specific for monovalent cations and has been extensively used to study the organization, dynamics and function of membrane-spanning channels. In recent times, the availability of crystal structures of complex ion channels has challenged the role of gramicidin as a model membrane protein and ion channel. This review focuses on the suitability of gramicidin as a model membrane protein in general, and the information gained from gramicidin to understand lipid–protein interactions in particular. Special emphasis is given to the role and orientation of tryptophan residues in channel structure and function and recent spectroscopic approaches that have highlighted the organization and dynamics of the channel in membrane and membrane-mimetic media.

Keywords: Gramicidin; Ion Channel; Tryptophan; Membrane Interface; Lipid–protein interaction

Contents

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPhPC, 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2 dioleoyl-*sn*-glycero-3-phosphocholine; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phosphoserine; GMO, glycerylmonoolein; LPC, lysophosphatidylcholine; REES, red edge excitation shift; SDS, sodium dodecyl sulfate

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1. Introduction

Ion channels are transmembrane proteins that regulate ionic permeability in cell membranes. They represent an important class of molecules due to their ability to serve as key elements in signaling and sensing pathways and to connect the inside of the cell to its outside in a selective fashion. They are crucial for normal functioning of cells and defective ion channels are implicated in a number of diseases collectively known as channelopathies [1,2]. Mutations in ion channels underlie diseases such as cystic fibrosis [3], renal disorders, certain types of hypertension, osteoporosis [4], and conditions such as deafness [5] and reduced sleep [6]. The recent successes in crystallographic analyses of ion channels starting with the KcsA potassium channel [7], the chloride channel [8] and more recently the voltage gated potassium channel [9] have provided exciting molecular insights to ion channel function and structure [10]. However, it is becoming increasingly clear that static crystallographic structures of membrane proteins may not always provide accurate representations of channel function [11–13]. Due to this ambiguity in the structural analysis of ion channel function, simple models of ion channels continue to provide useful information to understand and characterize more complex systems [14].

The linear gramicidins are a family of prototypical channel formers that have been extensively used to study organization, dynamics and function of membrane-spanning channels [14,15]. They are linear pentadecapeptide antibiotics with a molecular weight of ~1900. Gramicidins are produced by the soil bacterium *Bacillus brevis*, and consist of alternating L- and D-amino acids [16]. They form well-defined cation-selective ion channels in model membranes [17] with conductance of the order of $\sim 10^7$ ions per second. Due to their small size, ready availability and the relative ease with which chemical modifications can be performed, gramicidins serve as excellent models for transmembrane channels. The natural mixture of gramicidins, often denoted as gramicidin A′ [termed gramicidin D (after René Dubos, who originally discovered gramicidin) in older literature], consists of ~85% of gramicidin A, which has four tryptophan residues at positions 9, 11, 13, and 15 (see Fig. 1). Gramicidin A′ is readily available commercially and is fluorescent, due to the presence of tryptophan residues [18]. It has one of the most hydrophobic sequences known [19] and has very low solubility in aqueous solution ($\sim 5 \times 10^{-7}$ M) [20].

Gramicidins were first identified as an antibiotic produced by a culture of *B. brevis* isolated from soil samples by Dubos [21,22]. Gramicidin was the first antibiotic to be used clinically

HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-**L-Trp**-D-Leu-**L-Trp**-D-Leu-**L-Trp**-D-Leu-**L-Trp**-$NHCH_2CH_2OH$

Fig. 1. Amino acid sequence of gramicidin A. Note that all amino acid side chains are either hydrophobic (Ala, Leu, Val) or amphipathic (Trp). In addition, the -NH_2 and -COOH termini are blocked making the sequence unusually hydrophobic. Alternating D-amino acid residues are underlined and the tryptophan residues are highlighted.

(in 1939) and its success in clinical settings stimulated research into the practical application of other drugs such as penicillin [23,24]. Gramicidin is produced during sporulation shortly after the production of another antibiotic, tyrocidine [25]. The concentration of gramicidin in the bacterial cell is known to reach up to 1 M during sporulation. Its function in *B. brevis* is not known, but it is believed to play a role in gene regulation during the shift from vegetative growth to sporulation and has been shown to inhibit *Escherichia coli* RNA polymerase [25].

Interestingly, the amino acid sequence of gramicidin consists of alternating L- and D-amino acids [16], in sharp contrast to most proteins which contain exclusively L-amino acids [26]. Gramicidin is synthesized by non-ribosomal multienzyme complexes, and the conversion of L- to D- amino acids takes place during the biosynthesis. As mentioned earlier, the natural mixture of gramicidins contains predominantly gramicidin A (85%). The other peptides, gramicidin B and C differ in the nature of the aromatic residue at position 11, where Trp (in A) is replaced by Phe and Tyr, respectively. Further, Val at position 1 is replaced by Ile in about 5 to 20% molecules. In spite of the alternating sequence of L–D chirality generally not encountered in naturally occurring peptides and proteins, gramicidin represents a useful model for realistic determination of conformational preference of proteins in a membrane environment. This is due to the fact that the dihedral angle combinations generated in the conformation space by various gramicidin conformations are allowed according to the Ramachandran plot [27]. The focus of this review is to highlight the organization and dynamics of the gramicidin ion channel in membranes and its suitability as a model membrane protein. It should be mentioned here that this review does not cover in detail the significant advances made in recent years on the molecular modeling of gramicidin conformation and free energy profiles or in continuum electrostatics. These issues are covered in great detail in several recent reports [14,28–31].

2. Gramicidin conformation in membranes

The unique sequence of alternating L- and D- chirality renders gramicidin sensitive to the environment in which it is placed. Gramicidin therefore adopts a wide range of environment-dependent conformations. Two major folding motifs have been identified for gramicidin in various media: (i) the single stranded helical dimer (the channel form) [32], and (ii) the double stranded intertwined helix (collectively known as the non-channel form) [33,34]. Interestingly, while double helical conformations are predominant in organic solvents, the conformation of the membrane-bound form of gramicidin is markedly different [35]. Ever since its initial isolation, a number of crystalline forms of gramicidin from a variety of organic solvents in the presence and absence of ions have been reported [36–41]. However gramicidin crystals have proved to be recalcitrant to X-ray analysis methods (possibly as a result of its intermediate size–too large for direct small molecule methods and too small for replacement methods traditionally used for macromolecules) and rather limited structural data have been reported (see [42] for a detailed review). It is instructive to note

that the reported double helical structures are mostly inconsistent with channel activity and have long narrow pores that cannot accommodate an ion [42]. However, the structure reported in the presence of cesium chloride clearly shows a fatter helix with a diameter large enough to accommodate an ion [39].

Functional analysis of channel activity revealed the first details of the molecular organization of gramicidin in membranes. Channel activity was clearly shown to require transmembrane association using charged analogues of gramicidin [43]. While C-terminal charged analogues produced conducting channel (when applied symmetrically to black lipid membranes), N-terminal charged analogues did not form conducting channels when applied to black lipid membranes. This indicated the involvement of the N-terminus in dimerization. In addition, simultaneous conductance and fluorescence measurements in black lipid membranes showed that the conducting unit is a dimer [44]. These functional characteristics fitted well with the single stranded helical dimer formed by the head-to-head dimerization of (π^6_{LD}) helices. The $\pi_{(LD)}$ helix was proposed by Urry [32] on the basis of molecular modeling, in which consecutive peptide carbonyl groups alternate direction with respect to the helix axis. The nomenclature of these structures was later changed to β-helices to better reflect the type of hydrogen bonding present, which is in fact the same as that of β-sheets [45]. Due to alternating L- and D- residues, all side chains project from the same side of the peptide strand, which becomes the outside when the β-strand is coiled into a helix. The interior of the channel is formed by the polar peptide backbone and side chains project outward in contact with neighboring lipid fatty acyl chains and help in modulating the channel conductance (see Fig. 2a). This dimer form is stabilized by fifteen intramolecular and six intermolecular hydrogen bonds and the channel is gated by the association and dissociation of monomers [46]. The size of the channel pore (diameter ~4 Å) is large enough to accommodate the passage of monovalent cations [47]. Ion selectivity is observed in the order $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ and divalent cations such as Ca^{+2} block the channel [48]. The length of the dimer is ~26 Å and is the same order of magnitude as the hydrophobic part of a lipid bilayer [25].

In the channel conformation, the carboxy terminus of the peptide is exposed to the membrane interface and the amino terminus is buried in the lipid bilayer. As discussed above, the high-resolution crystal structures available for gramicidin conformations in organic solvents are inconsistent with channel function. The first well-resolved structure of gramicidin in a membrane-mimetic environment was obtained by solution NMR [49]. Later, the high-resolution structure of membrane-bound gramicidin A in a lipid bilayer was deduced by solid-state NMR [50] and refined by molecular dynamics simulations [51]. Other conformations, which can be collectively termed as non-channel conformations, have been shown to exist in membranes with polyunsaturated lipids [52], and in membranes with increased acyl chain lengths [53–55].

The detailed molecular mechanism of gramicidin adopting various conformations in membranes is not known. However, it has been reported that the initial conformation that gramicidin adopts when incorporated into membranes is dependent on the nature of the solvent in which it was dissolved prior to incorporation in membranes [53,56]. Thus, when gramicidin is dissolved in solvents such as chloroform/methanol, benzene/methanol, or ethanol before incorporation into membranes, it tends to adopt a non-channel conformation. Upon sonication and incubation at 65 °C, this is converted to the characteristic channel conformation. On the other hand, if gramicidin is dissolved in solvents such as trifluoroethanol (TFE) prior to membrane incorporation, it appears to adopt the channel conformation even without sonication and incubation at elevated temperature. Gramicidin conformation in membranes therefore appears to be dependent on its solvent history [56]. In membranes, the single stranded $\beta^{6.3}$ dimer channel conformation is

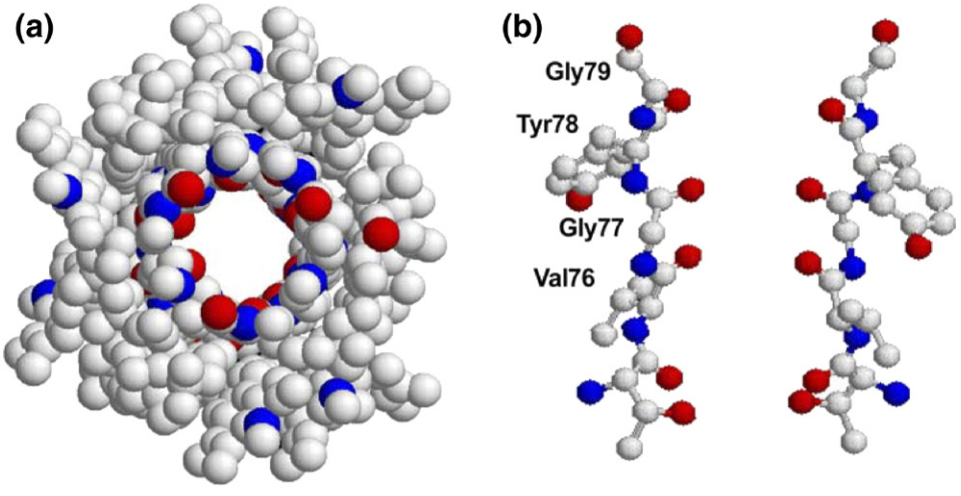

Fig. 2. (a) Top view of the gramicidin channel as a space-filling model (color code: white, carbon atoms; blue, nitrogen atoms; red, oxygen atoms) made using RASMOL ver. 2.7.2.1 [214] and using coordinates from PDB 1MAG. Note how the alternating L–D arrangement allows all amino acid side chains to project outward from the channel lumen and the channel lumen is lined by the peptide backbone. (b) Structure of the selectivity filter (residues 75–79) of the KcsA K^+ channel. Two subunits are shown in ball-and-stick representation (made as in (a), color code: same as in (a)) using coordinates from PDB 1BL8. Note that all amino acid side chains extend outward from the selectivity filter.

the most preferred (thermodynamically stable) conformation [53]. The rate of interconversion from the non-channel to the channel form can be modulated by sonication and prolonged incubation at elevated temperatures [53,56].

3. Gramicidin tryptophans at the membrane interface

The interfacial localization of the gramicidin tryptophan residues in the channel conformation is an essential aspect of gramicidin conformation and function in membranes [15]. In membrane proteins, tryptophan residues are not uniformly distributed along the membrane axis and tend to be preferentially clustered at the membrane interface. This is due to the fact that the experimentally determined interfacial hydrophobicity of tryptophan is the highest among the naturally occurring amino acid residues [57]. While tryptophan has the polar NH group which is capable of forming hydrogen bonds, it also has the largest nonpolar accessible surface area among the naturally occurring amino acids [58]. Due to its aromaticity, the tryptophan residue is capable of π–π interactions and weakly polar interactions [59,60]. This amphipathic character of tryptophan gives rise to its unique hydrogen bonding property and ability to function through long range electrostatic interaction [61,62]. The preferential location of tryptophan residues at the membrane interface is thought to be due to the aromaticity of the indole moiety and the overall amphipathic nature of tryptophan [63]. The thermodynamically preferred channel conformation of gramicidin highlights this essential aspect of membrane protein conformation. Interestingly in the double helical non-channel conformations, the tryptophan residues are distributed across the membrane axis (see Fig. 3) making this conformation thermodynamically unstable. Importantly, the gramicidin analogue in which all the four tryptophan residues are replaced by phenylalanines, which are more hydrophobic and cannot act as hydrogen bond donors, appears to preferentially adopt the alternate antiparallel double stranded helical dimer conformation [64–66]. In the absence of tryptophan residues, the non-channel conformation therefore becomes the preferred conformation. Interestingly, the channel conformation is the preferred conformation even in membrane-mimetic environments such as micelles [49,67,68] and reverse micelles [69,70].

4. Synergestic approaches can reveal structure–function relationships

The gramicidin channel is characterized by rectangular current steps of defined amplitudes in planar lipid bilayers [17]. The ability to synthesize gramicidin incorporating specific modifications has enhanced the utility of gramicidin as a model ion channel to understand specific structure–function relationships [71]. This includes gramicidins labeled with various isotopes which have greatly advanced NMR studies of this peptide to study specific backbone and side chain conformations [50,72,73]. Hybrid channel formation (using gramicidin analogues that form functionally distinct channels) has been used to test the structural equivalence of different gramicidin analogues [74,75]. Negatively charged analogues of gramicidin [43] were used to form distinct hybrid channels with native gramicidin to show that the functional channel is formed by a transmembrane dimer with no lateral associations [76]. Surprisingly, even though the sequence of gramicidin is extremely hydrophobic, it is relatively impermeant in membrane bilayers (i.e., it does not rapidly flip to the other membrane leaflet), and low channel activity is observed when gramicidin is added asymmetrically to membrane bilayers (black lipid membranes) [46]. This is probably due to the high tryptophan content at the C-terminus (see Fig. 1) that could prevent transmembrane flipping. As discussed earlier, tryptophan is an amphipathic amino acid with a marked preference for the membrane interface. The burial of tryptophan residues in the hydrophobic core of the membrane is therefore relatively

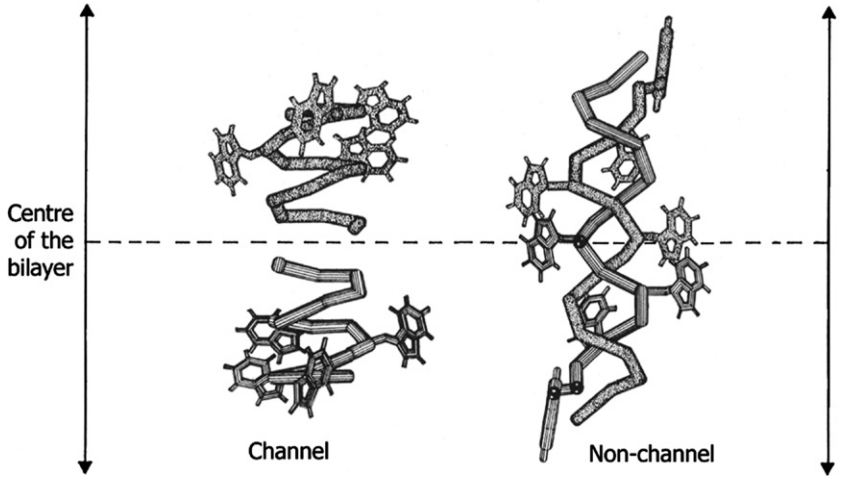

Fig. 3. A schematic representation of the non-channel and channel conformations of gramicidin indicating the location of tryptophan residues in a membrane bilayer. The membrane axis is represented by double headed arrows and the center of the bilayer is marked by a dotted line. Note that, in the channel conformation, tryptophan residues are clustered toward the membrane interface whereas in the non-channel conformation tryptophan residues are distributed along the membrane axis (from [165]).

unfavorable [63]. Interestingly, theoretical calculations predict a large free energy barrier of ~35 kcal/mol for the vertical insertion of gramicidin dimers in the channel form into a membrane bilayer [77].

Detailed analysis of the contribution of specific amino acids to channel function has been possible due to a synergistic combination of functional and spectroscopic studies. The C-terminal tryptophan residues play an important role in channel conductance and gramicidin structure (discussed in detail later). While the tryptophan residues are known to be important for channel function and structure, the intervening D-Leu spacer residues (residues 10, 12, and 14) have also been implicated in maintaining ion conductance [78,79]. Semi-conservative substitutions of the D-Leu residues with residues such as D-Ala, D-Val or D-Ile were found to have surprisingly large effects on channel structure and function. All the substituted analogues were found to occur in heterogeneous conformations (both double and single stranded) in membranes and had significantly lower conductance as compared to native gramicidin [78]. In view of the importance of the orientation of tryptophan dipoles in channel conductance, these results point to a complex interaction between the aromatic and aliphatic side chains that can influence backbone folding and ion entry. In addition, D-Leu-10 stabilizes optimum orientations of Trp-9 in membrane bilayers and therefore substitutions at this position alter channel conductance without affecting the basic fold of the peptide backbone [79].

While C-terminal residues play important roles in ion entry and channel conductance, the residues at the N-terminal junction influence the formation of the transbilayer channel. Using simultaneous conductance and fluorescence measurements, Veatch et al. [44] showed that the dimerization constant of gramicidin in black lipid membranes is $\sim 2\times 10^{13}$ mol^{-1} cm^2. As mentioned earlier, charge at the N-terminus is not tolerated for channel function [43]. The dimeric channel is stabilized by at least six hydrogen bonds at the N-termini [46] and channel gating is thought to occur when at least two hydrogen bonds are broken [80]. Substitutions or deletions at N-terminal positions therefore interfere with the formation of the conducting dimer [81,82]. Introduction of a dipolar F_6Val-1 at the N-terminus leads to the formation of homodimeric channels with reduced conductance and lifetime as compared to gramicidin A channels [74]. However, heterodimers of this analogue and gramicidin A were found to have much lower conductance than either homodimer rather than the expected intermediate conductance. The heterodimeric channels were found to show voltage-dependent transitions from a low to a high conductance state and are therefore voltage gated [83]. Interestingly, these heterodimeric channels are voltage gated in the absence of any fixed charge. In addition, gramicidins with positively charged amino groups tethered to the C-terminus via carbamate linkers, also show voltage-dependent gating but to a weaker extent [84].

N-linked gramicidin analogues have been synthesized to form long-lived gramicidin channels with tunable conductance properties [85–89]. The open state of these dimers is several orders of magnitude longer than in native gramicidin channels. Of particular interest are the dioxolane linked gramicidin analogues that retain the β helicity of the dimers [87–89]. These analogues provide the ability to study the effects of different chemical groups (that may be hosted on the dioxolane linker) on channel activity without significantly perturbing channel structure [90]. In addition, the linked dimer has a single sharp conductance state, unlike native gramicidin, that may have subconductance states depending on monomer–monomer association. This greatly facilitates the analysis and interpretation of single channel data [91,92]. The two chiral carbons in the dioxolane linker permit the synthesis of two distinct diastereomers and the stereochemistry of the linker (namely, SS and RR) form ion channels with distinct properties. While the SS dimer retains the β helicity of the dimer, the linker could obstruct the center of the channel leading to an increased energetic barrier to ion transport in the RR dimer [87]. In line with this structural prediction, channels formed by the RR dimer showed brief channel closures to zero conductance (flickers) that were thought to be a result of the rotation of the RR dioxoloane inside the pore of the channel [88,93]. This possibility was later challenged in studies that used a retinal group attached to the dioxolane linker [94]. Since the rotation of the dioxolane linker would be significantly hindered by the bulky hydrophobic retinal group, flickers in the RR channel should be eliminated if linker rotation is the sole cause. However, both SS and RR channels showed flicker activity with the retinal group. Channel properties and flicker behavior were shown to be related to the nature of the permeating ion in both SS and RR dimeric channels rather than to the nature of the linker group [94]. Such flicker behavior is observed even in native gramicidin channels [95,96] and was initially thought to be solely a result of dimer dissociation. The frequency of flickers is found to be dependent on the thickness of the host bilayer [95]. Recently, it was shown that the flicker frequency in the dioxolane linked dimers (both SS and RR), which do not dissociate, is dependent on the thickness of the bilayer [97] and is attenuated in thinner bilayers. Based on these results, it was hypothesized that the flickering activity was essentially a consequence of bilayer deformation and caused due to undulations of the bilayer that could obliterate the channel mouth leading to brief closures (i.e., flickers) [97].

5. Tryptophans in ion channel function

The C-terminal tryptophan residues of gramicidin have been shown to be an integral component of channel activity and the thermodynamically preferred conformation of gramicidin in membrane environments. Gramicidin is a very hydrophobic peptide and the four C-terminal tryptophan residues (in gramicidin A) are the only amphipathic moieties in the sequence. As discussed earlier, tryptophan has a distinctive amino acid side chain with unique molecular properties among the naturally occurring amino acids. The membrane interface seeking properties of tryptophan and the oriented dipole moments of the tryptophan side chain influence gramicidin structure and function. Replacement of gramicidin tryptophans with other aromatic side chains such as napthylalanine,

phenylalanine or tyrosine [61,98–100] has been found to reduce gramicidin channel activity. The natural gramicidin variants, gramicidin A, B, and C which differ at position 11 (see earlier) show distinctive channel properties [101]. In addition, photolysis of tryptophan by ultraviolet irradiation [102–105] or chemical modification by an oxidizing agent such as N-bromosuccinimide [106] leads to reduction in cation conductivity.

Trp→Phe substitutions represent an interesting case since they are semi-conservative. While both Trp and Phe are aromatic, Phe is nonpolar and does not function as a hydrogen bond donor like Trp (through the indole –NH). Importantly, while the Phe side chain does not have a dipole moment, the Trp side chain has a dipole moment of ~2D [107]. In a careful analysis of cumulative Trp→Phe substitutions, it was shown that the single channel conductance of substituted gramicidins decreases as a function of the number of Trp residues substituted by Phe [100]. The specific position of the Phe substitution was shown to be important for the effect on channel conduction. For example, in case of monosubstituted channels, conductance decreases as Phe is moved from position 15 to 9 (and *vice versa* for the single tryptophan of trisubstituted analogues). Interestingly, channel lifetimes (i.e., the average duration of the open channel) are not specifically affected by Phe substitutions. However, the trisubstituted analogue with a single Trp at position 11 shows a dramatically reduced channel lifetime [100]. These results suggest that the predominant effect of Trp→Phe substitutions is the removal of electrostatic interactions between the indole dipole and the permeant ion. In addition, orientation and mobility of gramicidin tryptophans (and hence, indole dipole moments) in membrane environments would be an important structural feature of gramicidin.

If long-range electrostatic interactions are important for gramicidin conduction, Trp modifications that modulate the indole dipole moment without affecting the hydrogen bonding ability of tryptophan should alter channel conductance. Fluorinated tryptophans represent a useful class of Trp analogues, that can be used to alter the spectral properties of tryptophan [108,109] and the magnitude of the indole dipole moment [107]. In 5F-Trp, fluorination at position 5 in the indole side chain results in an increased dipole moment of 3.6D (as compared to 2.1D for indole) with a similar orientation as the dipole of indole [107]. Since the removal of a dipole due to Trp→Phe substitution decreases channel conductance, 5F-Trp analogues should exhibit increased conductance as compared to native gramicidin [62,110]. This was shown to be the case for a gramicidin analogue with 5F-Trp at position 11 [62]. Significantly, a doubly substituted analogue (5F-Trp-9 and Phe-11) was found to have increased conductance as compared to an analogue with a single Trp→Phe (Phe-11) substitution. A series of detailed reports by Busath, Cross and co-workers used both computational and experimental data to establish the relationship between fluorination and gramicidin channel ion and proton conductance [110–116]. An early anomaly in the fluorination induced effect on gramicidin conductance was the observation that the 5F-Trp-13 analogue showed increased ion conductance in diphytanoylphosphatidylcholine (DPhPC) bilayers but not in glycerylmonoolein bilayers (GMO) [110]. The effects of fluorination of Trp-13 on ion conduction were shown to be a result of an increased rate constant of translocation due to a free energy change in the translocation barrier and an increase in first-ion binding affinity due to a decrease in the first-ion exit rate constant. The 5F-Trp-13 analogue therefore shows increased conductance in DPhPC bilayers where the high interfacial dipole makes translocation rate limiting and decreased conductance in GMO where ion exit or entry is rate limiting [111]. Interfacial dipole modulating agents such as phloretin and 6-ketocholestanol provide a convenient tool to delineate the specific effects of dipole potential on gramicidin conductance [117]. The specific effect of the interfacial dipole potential on gramicidin conductance in DPhPC bilayers was further verified utilizing such agents [118]. In addition, it was predicted that the specific position of fluorination (i.e., 6F-, 5F-, or 4F-Trp) which alters the orientation and magnitude of the indole dipole moment would influence the effect of the substitution on channel activity [119]. This was shown to be largely accurate for most 6F- and 5F-Trp analogues of gramicidin. However, anomalies for 6F analogues indicate the possibility of interaction with interfacial water [114]. Importantly, solid-state NMR shows that fluorination of Trp side chains has little effect on side chain positions [120].

Keeping in mind the interfacial propensity of tryptophan residues, and the overall hydrophobicity of the gramicidin sequence, Trp→Phe conversions should result in altered interactions with the membrane environment and therefore possible changes in backbone structure. NMR and size exclusion chromatography have shown that the gramicidin analogue in which all four tryptophan residues are replaced by phenylalanines appears to preferentially adopt the alternate antiparallel double stranded helical dimer conformation [64,66]. In the absence of any tryptophan residues, the double stranded helical dimer non-channel conformation becomes the energetically favored state in the membrane. However, the naturally occurring variants of gramicidin, gramicidin B and C (with Trp→Phe, Tyr substitutions at position 11, respectively) have been shown to be structurally similar to gramicidin A [67]. The mono-substituted Trp→Phe analogues (at positions 11, 13, 15) have also been shown to have backbone conformations close to the native sequence [121].

It should be noted that functional studies using heterodimer channel formation have indicated that substituted gramicidin sequences with variable Trp→Phe replacements form structurally equivalent channels with native gramicidin [100]. However, functional approaches selectively study only that fraction of the peptide population that forms functional channels and do not provide any information about the non-conducting fraction of molecules. While it is possible that conducting channels formed by altered sequences would be structurally similar to native channels, it may not reflect the average conformation of the altered sequence in membranes. Channel-forming potency is a measure of the gramicidin concentration (in the aqueous solution) required for a given channel appearance rate [100] and would therefore account for partitioning of the membrane adsorbed gramicidin among various conformers. While the

channels formed by the tri-substituted analogues are qualitatively similar to native channels, channel-forming potency is found to decrease twenty-fold. This suggests the possibility of a population of non-channel conformations in the membrane in addition to the subset that forms conducting channels. Interestingly, it has recently been shown using detailed fluorescence spectroscopic approaches that the single tryptophan analogues of gramicidin predominantly adopt non-channel conformations in membranes (Chattopadhyay, A., Rawat, S.S., Greathouse, D.V., Kelkar, D.A., and Koeppe, R.E., unpublished observations).

6. Tryptophan orientations in channel activity

As discussed earlier, side chain orientations of gramicidin tryptophan residues would play an important role in channel activity [113]. The additive effect of replacing tryptophan residues by Phe on channel activity suggests that the orientation of the dipole moment of each tryptophan is similar. Solid-state NMR analysis of specifically labeled Trp residues has shown that the dipole moment of each indole ring is parallel with the channel axis and oriented to lower the potential energy barrier for cation transit at the bilayer centre [122,123]. However, later work showed that Trp-9 (which is the most buried of the gramicidin tryptophan residues) may be exposed to the hydrophilic surface as evidenced by hydrogen exchange [124]. Interestingly, the indole rings of gramicidin tryptophans have been shown to be relatively immobile [18,73,122,123,125–127], possibly due to hydrogen bonding with lipid carbonyl oxygens [128]. Molecular dynamics simulations have shown this to be particularly true for Trp-13 which forms a long-lasting hydrogen bond to a phospholipid oxygen [126]. In addition, the relatively immobile nature of gramicidin tryptophans is supported by the relatively long ($\sim 2\times 10^{-7}$ s) correlation times obtained from deuterium NMR [129]. However, it has been suggested that Trp-9 is relatively more mobile than the other Trp side chains and high-resolution structures differ in the orientation of Trp-9 relative to Trp-15 [50,67]. Calculations suggest that changes in the rotameric state of Trp-9 could give rise to energy differences of the order of ~ 1 kcal/mol for an ion in the pore [125]. Further, the Trp dipoles have been shown to stabilize the cations near the binding sites near the channel entrance and reduce the potential energy barrier at the bilayer centre [124]. Precise knowledge of the orientation of Trp side chains is important since the rate of ion movement depends on the energetics of ion-channel interactions and ~ 1.3 kcal/mol difference in energy could result in a ten-fold change in ion flux. Interestingly, the implicit flexibility of the Trp side chain conformation in gramicidin has been tested using molecular dynamics simulations in vacuum [130]. These studies show that the Trp side chains move quite freely from one conformation to another and behave like surface residue side chains. The lipid environment would favor specific Trp orientations and therefore stabilize such orientations. A recent NMR analysis used a fluorinated Trp (Trp-13 and -15) to determine the influence of the membrane phase on the orientation of gramicidin tryptophan residues [131]. This work shows a slight change in Trp side chain conformation upon the phase transition (from gel to fluid phase) possibly due to a change in bilayer thickness.

Considering that the $\beta^{6.3}$ helix would have 6.3 residues per turn, any residue (residue i) would be spatially close to the seventh residue ($i+6$) in the sequence. An interaction between the Trp-9 and Trp-15 indole groups is therefore possible due to their close proximity in space. However, high-resolution solution NMR structures of gramicidin in sodium dodecyl sulphate (SDS) micelles showed that Trp-9 and Trp-15 do not interact through space [49,67] while solid-state NMR (in oriented DMPC bilayers) has shown the Trp-9 and Trp-15 indole rings to be stacked [50,72]. In addition, fluorescence spectroscopy suggested the presence of aromatic-aromatic stacking interactions in membrane environments on the nanosecond timescale [18]. Importantly recent fluorescence spectroscopy results indicate that the stacking interactions of Trp-9 and Trp-15 may be dependent on the curvature of the host assembly [68]. Therefore, relatively planar rod-shaped micelles or oriented bilayers (as used in solid-state NMR) could favor aromatic-aromatic stacking interactions of Trp-9 and Trp-15, while in highly curved spherical SDS micelles, orientations of Trp-9 away from Trp-15 are preferred. Recent reports have compared the conformations of Trp-9 during molecular dynamics simulation in DMPC and in vacuum, when the high-resolution NMR structures of gramicidin reported from SDS micelles and DMPC bilayers were used as the starting conformations [113,125]. Importantly in the simulation in DMPC environments showed that the side chain of Trp-9 was found to be more mobile than Trp-11, -13, or -15, and undergoes spontaneous transitions between orientations of the DMPC (stacked) and SDS (non-stacked) structures. It has been suggested that a mixture of rotameric states for Trp-9 would better reproduce experimental results. A mixture of rotameric states with 80% of the SDS rotamer and 20% of the DMPC rotamer was found to best reproduce NMR side chain observables. Interestingly, simulations of gramicidin Trp rotameric states in vacuum showed that stacking conformations are strongly disfavored, suggesting a specific role for the lipid environment in the possible stabilization of such a conformation [130].

7. Lipid–protein interactions influence gramicidin conformation and function

The membrane lipid environment is a strong modulator of membrane protein structure and function. A large portion of a membrane protein remains in contact with the membrane lipid environment. This raises the obvious possibility that the membrane could be an important modulator of membrane protein structure and function. It is becoming increasingly clear that subtle properties of the membrane environment such as membrane thickness and order, besides lipid composition, influence membrane protein function [132–135]. Due to its relative simplicity, gramicidin has been a testing ground to elucidate the fundamental principles of lipid–protein interactions, and membrane protein structure and function [25,136–138].

Early work by Killian and others has clearly demonstrated the influence of gramicidin on membrane phase change [25]. Interestingly, the micellar organization of lysophosphatidylcholine (LPC) has been shown to convert to a bilayer type organization in the presence of gramicidin [139,140]. This bilayer forming ability has been shown to depend on the solvent history of the peptide and therefore on gramicidin conformation [141]. Channel conformation is therefore important for the formation of bilayers with LPC. The channel conformation is hour glass-shaped due to the presence of bulky tryptophan residues at the C-terminus (i.e., the monomer is cone-shaped) [142]. This shape complimentarity with cone-shaped LPC molecules leads to the bilayer type organization of LPC in the presence of gramicidin. The bilayer inducing ability of gramicidin was found to be reduced in the case of gramicidin B and C (with phenylalanine and tyrosine respectively at position 11 instead of tryptophan), pointing out the critical role of tryptophan residues in the interaction of gramicidin with such organized assemblies [143]. In addition, gramicidin has been shown to promote hexagonal (H_{II}) phases when present at high concentrations in bilayer-forming lipids such as DOPC [144,145] and in natural membranes [146,147]. The formation of the H_{II} phase is associated with peptide aggregation, and upon phase separation the hexagonal phase has been found to be enriched in gramicidin as compared to the lamellar phase [148–150]. Interestingly, the concentration of gramicidin required to induce the H_{II} phase is found to be related to the acyl chain thickness of the membrane and is found to decrease with increasing membrane thickness [144,151,152]. More importantly, a mismatch between the peptide length and membrane thickness was found to be essential for the induction of the H_{II} phase [153]. While gramicidin can induce H_{II} phase in DOPC membranes, long gramicidin analogues could induce H_{II} phase only in thicker bilayers. The H_{II} phase forming property of gramicidin is also associated with gramicidin induced vesicular fusion [154].

The intrinsic tryptophan fluorescence of gramicidin offers a convenient tool to monitor gramicidin organization and dynamics and its interaction with the membrane environment. Spectroscopic approaches in general, and fluorescence spectroscopic approaches in particular, have become very useful for analyses of membrane protein dynamics [155]. For gramicidin, CD spectroscopy has served as a valuable tool to distinguish the channel conformation from other forms [53,56,136,156]. Further, size exclusion chromatography has provided useful information about the equilibrium ratio of single stranded and double stranded conformations existing in various environments [66,157]. The intrinsic tryptophan fluorescence of gramicidin offers a convenient tool to monitor gramicidin conformation and organization. In particular, the well documented sensitivity of tryptophan fluorescence to environmental factors such as polarity and mobility [158,159] makes tryptophan fluorescence a valuable tool in probing of membrane protein structure and dynamics. In addition, the possibility of obtaining information at low lipid to peptide ratios makes fluorescence studies particularly advantageous, keeping in mind the lipid phase modulating properties of gramicidin at high concentrations (discussed above).

Novel and sensitive fluorescence approaches such as the red edge excitation shift have been very useful in delineating the organization and dynamics of gramicidin tryptophan residues in various membrane and membrane-mimetic media [18,68,70,127,156,160–166]. A shift in the wavelength of maximum fluorescence emission toward higher wavelengths, caused by a shift in the excitation wavelength toward the red edge of the absorption band, is termed red edge excitation shift (REES) [167–169]. This effect is mostly observed with polar fluorophores in motionally restricted environments such as viscous solutions or condensed phases where the dipolar relaxation time for the solvent shell around a fluorophore is comparable to or longer than its fluorescence lifetime. REES arises from slow rates of solvent relaxation (reorientation) around an excited state fluorophore, which depends on the motional restriction imposed on the solvent molecules in the immediate vicinity of the fluorophore. The unique feature of REES is that while other fluorescence techniques yield information about the fluorophore itself, REES provides information about the relative rates of solvent relaxation which is not possible to obtain by other techniques. The phenomenon of REES, in conjunction with time-resolved fluorescence spectroscopic parameters such as wavelength-dependent fluorescence lifetimes and time-resolved emission spectra, were utilized to probe the dynamics of the tryptophan residues in the gramicidin channel conformation in membranes [18]. These results pointed out the motional restriction experienced by the tryptophans at the peptide–lipid interface of the gramicidin channel and the heterogeneous environments experienced by the tryptophan residues. Interestingly, such heterogeneous environments for gramicidin tryptophans were also reported in membrane-mimetic reverse micellar media [70]. More importantly, REES and related fluorescence approaches have been used to distinguish between the channel and non-channel conformations of gramicidin in membranes [127,165]. REES of gramicidin is therefore sensitive to the conformation adopted by the peptide in the membrane. Such a detailed characterization of gramicidin [126,165] in functional and non-functional conformations has provided a convenient spectroscopic handle to identify these conformations. This information can therefore be further utilized to directly study

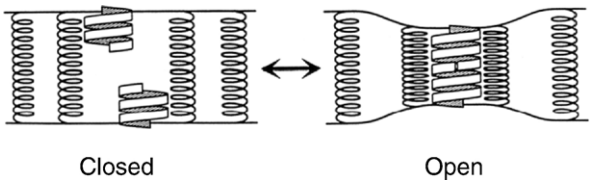

Fig. 4. A schematic representation of gramicidin channel gating by the formation and dissociation of transmembrane dimers. The average membrane thickness is more than the length of the functional dimer. Channel formation is therefore associated with membrane deformation. Membrane lipids are approximated as springs and membrane deformation is described by a spring constant that includes deformation due to compression/expansion, distortion and increased monolayer curvature (adapted from [190] with permission from Elsevier).

the influence of the membrane (or membrane-mimetic) environment on the conformation of gramicidin. For example, structural transitions can be induced in charged micelles by increasing the ionic strength of the medium [170]. The organization and dynamics of the functionally important tryptophan residues of gramicidin in spherical and rod-shaped micelles utilizing a combination of wavelength-selective fluorescence and related fluorescence approaches have been studied. The results show that gramicidin conformation and dynamics is sensitive to the structural transition and deformation of the host assembly [68]. Very recently, the conformation-dependent fluorescence of gramicidin was used to monitor conformational changes due to hydrophobic mismatch in saturated membrane bilayers [55].

8. Membrane deformation and ion channel function: gramicidin as a molecular force transducer

Gramicidin channel gating is known to occur by a well-defined conformational change, *viz*., the formation and dissociation of a transmembrane dimer [46] (see Fig. 4). The conformational change involves the breaking of hydrogen bonds at the monomer interface and lateral movement of the monomer [80]. Such a conformational change implies changes at the lipid–protein interface that could be coupled to modifications in the immediate lipid environment. The effective hydrophobic thickness of the functional gramicidin dimer is considered to be ~22 Å [171], based on the dependence of mean channel lifetime on the hydrocarbon thickness (as determined by capacitance measurements) of the supporting membrane. In membranes with increased surface tension or increased membrane thickness, there is a sharp reduction in mean channel lifetime. Since the hydrophobic thickness of gramicidin is less than the thickness of the membrane used for functional measurements [172], gramicidin channel formation is almost always associated with a reversible local membrane deformation [173,174]. The energetic cost of membrane deformation associated with channel formation is therefore a primary factor governing gramicidin channel function.

Modification of the material properties of the membrane (such as monolayer curvature, thickness and membrane stiffness) would affect membrane deformation and therefore gramicidin channel lifetime (i.e., stability of the functional dimer). Monolayer membrane curvature can be modulated by the addition of agents such as detergents and lysophospholipids. It has been shown that increased gramicidin channel lifetimes correlate with an increase in positive membrane curvature due to the addition of non-physiological detergents [175], lysophospholipids [176] and lipids such as DOPS under conditions of reduced electrostatic repulsion [177]. In addition, when gramicidin analogues of reduced lengths are used, the potency of lysophospholipids to increase channel activity (as a result of increased gramicidin dimerization) is enhanced [176]. As a result of the gating mechanism of gramicidin channels, gramicidin may also be considered to be a mechanosensitive channel i.e., a mechanically gated channel [178]. Interestingly, the response of gramicidin to mechanical stress (stretch activation or inactivation) is dependent on the thickness of the membrane. Therefore, gramicidin functions as a stretch activated channel in thinner membranes while in thicker membranes it is inactivated by membrane stretch [179].

It has been well established that membrane material properties are extremely sensitive to cholesterol content [180]. Cholesterol (at high mol%) increases bilayer cohesion and reduces membrane compressibility in fluid membranes [181] thereby increasing the free energy cost of membrane deformation [182]. In addition, cholesterol is known to increase membrane thickness [183,184]. As a result of these effects of cholesterol on membrane properties, cholesterol is found to decrease gramicidin channel activity [172,185–187]. Gramicidin activity has been found to reduce in membranes of increased thickness [171]; however, this is also associated with a conformational change to non-conducting dimers [54,55,188]. In addition, mini-gramicidins (with 11 amino acids instead of 15) have been shown to function better in shorter membranes as compared to gramicidin A [189]. The energetic cost of the membrane deformation involved in channel gating has been quantified in terms of a spring constant that accounts for all modes of membrane deformation and scales according to the size of the protein [190]. Interestingly, deformation energies calculated this way, could be as large as 2–4 kcal/mol, comparable to the effects of point mutations on protein function [191]. The dependence of channel dimerization on the free energy of membrane deformation has been utilized to obtain a measure of the energetic cost of membrane deformation [192]. Gramicidin channel activity can therefore be used as a reporter to delineate the effect of membrane-active compounds and treatments on membrane material properties and predict how such treatments may influence membrane protein function under physiological conditions. For example, gramicidin has been utilized as a molecular force transducer to describe the mechanism of action of genistein, an isoflavinoid frequently used as a tool to alter ion channel function [193]. Genistein is utilized as a generic tyrosine kinase inhibitor and is thought to alter the function of a range of ion channels due to inhibition of phosphorylation. However, genistein was found to inhibit gramicidin channel activity due to its affect on bilayer elastic properties as a result of membrane partitioning [193] ruling out a general role for tyrosine kinase inhibition in ion channel function. Such bilayer-mediated mechanisms for the action of modulators of membrane protein function are being increasingly recognized [194–196].

9. Gramicidin is a model membrane protein and ion channel

The structural features that make gramicidin channels so unique raise the question whether gramicidin channels are appropriate models for real ion channels. Fortunately, the elucidation of the crystal structure of the *Streptomyces lividans* K^+ channel (KcsA) [7] in molecular detail has provided an opportunity to evaluate the features of the gramicidin channel that are shared with other channels from natural sources. This has led to the rather interesting finding that gramicidin channels

share important structural features with real ion channels [197]. An essential feature of ion channels is the ability to select for specific ions using a selectivity filter. Remarkably, both the gramicidin channel and the selectivity filter of the KcsA K^+ channel are lined by the polar carbonyls of the peptide backbone and ion selectivity in both cases arises due to backbone interactions with ions [198,199]. While such interactions are possible in the gramicidin channel due to alternating L–D chirality (Fig. 2a), KcsA utilizes two highly conserved glycine residues in the selectivity filter (Fig. 2b). The choice of glycine is obvious since it is achiral, and has a larger allowed region in the Ramachandran plot, and so effectively it can behave as a D-amino acid. A recent report [200] confirms and extends this notion by pointing out that the two absolutely conserved glycine residues in the KcsA K^+ channel, which are essential in the K^+-selectivity filter, serve as surrogate D-amino acids, reminiscent of the D-amino acids found in the gramicidin channel. Interestingly, addition of regularly placed D-amino acids in a synthetic membrane spanning sequence has been reported to convert the porin-like channel formed by the sequence to a cation-selective gramicidin-like channel [201].

As discussed previously, gramicidin represents a useful model for realistic determination of conformational preference of proteins in a membrane environment. The conformational preferences of membrane-bound gramicidin are therefore not unique to it. The conformational change in membrane-bound gramicidin from the non-functional non-channel conformations to the functional channel conformation [127,202] is a consequence of the preference of tryptophan residues to reside at the membrane interface [57]. Therefore, while gramicidin is a unique peptide in sequence and structure, its membrane-bound conformation is influenced by lipid–protein interactions that are universal in nature. We can consider the property of conformational transition of gramicidin between functional and non-functional states, to be analogous to the conformational changes that occur during the switch between 'on' and 'off' states during the activation of membrane-bound receptors and channels. This is exemplified by a recent example from the KirBac1.1 channel [203]. These authors showed that, analogous to what is observed in the case of gramicidin, localization of aromatic amino acids in the membrane interior could result in a closed conformation in case of the KirBac 1.1 channel.

The utility of the membrane interface anchoring property of aromatic residues becomes more evident when the transition from the closed to activated state is dependent on the vertical displacement (or tilt) of a transmembrane helix in the membrane axis. The signaling helix of the aspartate receptor of bacterial chemotaxis functions by a 'piston-type' sliding toward the cytoplasm upon ligand binding. Mutagenesis studies have revealed that the position of aromatic amino acids in the signaling helix in relation to the membrane interface is critical to the switch between 'on' and 'off' states [204,205]. Importantly, the substitution of aromatic residues near the membrane interface was found to stabilize specific signaling states, possibly due to modulation of anchoring at the membrane interface. Interestingly, mechanosensitive channels such as MscL are very useful systems to understand the role of membrane anchoring residues in protein function since the gating transition for this channel utilizes an 'iris-like' mechanism that involves extensive changes in transmembrane helix tilt. It has been shown that while capping of a specific transmembrane helix by aromatic residues can slow channel gating leading to partial loss of function [206], channel function can be partially restored by modulation of the distance between aromatic caps. Altering lipid–protein interactions at the membrane interface is therefore found to directly compromise MscL function.

10. Conclusions and future perspectives

Knowledge of membrane protein structure is crucial for understanding membrane function [207]. Since the elucidation of the first membrane protein structure ~20 years ago [208], there have been several reports of high-resolution crystallographic structures of physiologically relevant membrane proteins [7,9,209,210]. However, despite these recent successes and predictions of an explosion of membrane protein structures in the future [211,212], information obtained from crystallographic data is necessarily static. The utility of such static membrane protein structures in the interpretation of functional mechanisms is limited [11,12,213] by the inherent disorder and fluctuation of the membrane environment. Relatively simple model membrane proteins such as gramicidin are therefore invaluable tools to understand membrane protein function and organization.

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References

[1] E.C. Cooper, L.Y. Jan, Ion channel genes and human neurological disease: recent progress, prospects and challenges, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4759–4766.
[2] T.J. Jentsch, C.A. Hübner, J.C. Fuhrmann, Ion channels: function unravelled by dysfunction, Nat. Cell Biol. 6 (2004) 1039–1047.
[3] M.J. Stutts, C.M. Canessa, J.C. Olsen, M. Hamrick, J.A. Cohn, B.C. Rossier, RC. Boucher, CFTR as a cAMP-dependent regulator of sodium channels, Science 269 (1995) 847–850.
[4] U. Kornak, D. Kasper, M.R. Bösl, E. Kaiser, M. Schweizer, A. Schulz, W. Friedrich, G. Delling, T.J. Jentsch, Loss of the ClC-7 chloride channel leads to osteoporosis in mice and man, Cell 104 (2001) 205–215.
[5] T. Kharkovets, J.-P. Hardelin, S. Safieddine, M. Schweizer, A. El-Amraoui, C. Petit, T.J. Jentsch, KCNQ4, a K^+ channel mutated in a

form of dominant deafness, is expressed in the inner ear and the central auditory pathway, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4333–4338.
[6] C. Cirelli, D. Bushey, S. Hill, R. Kreber, B. Ganetzky, G. Tononi, Reduced sleep in *Drosophila Shaker* mutants, Nature 434 (2005) 1087–1092.
[7] D.A. Doyle, J.M. Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, R. MacKinnon, The structure of the potassium channel: molecular basis of K+ conduction and selectivity, Science 280 (1998) 69–77.
[8] R. Dutzler, E.B. Campbell, M. Cadene, B.T. Chait, R. MacKinnon, X-ray structure of a ClC chloride channel at 3.0 Å reveals the molecular basis of anion selectivity, Nature 415 (2002) 287–294.
[9] Y. Jiang, A. Lee, J. Chen, V. Ruta, M. Cadene, B.T. Chait, R. MacKinnon, X-ray structure of a voltage-dependent K^+ channel, Nature 423 (2003) 33–41.
[10] R. MacKinnon, Nobel lecture. Potassium channels and the atomic basis of selective ion conduction, Biosci. Rep. 24 (2004) 75–100.
[11] M.B. Jackson, Advances in ion channel structure, Trends Neurosci. 24 (2004) 291.
[12] F. Bezanilla, The voltage-sensor structure in a voltage-gated channel, Trends. Biochem. Sci. 30 (2005) 166–168.
[13] C. Gonzalez, F.J. Morera, E. Rosenmann, O. Alvarez, R. Latorre, S3b amino acid residues do not shuttle across the bilayer in voltage-dependent Shaker K^+ channels, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 5020–5025.
[14] G.V. Miloshevsky, P.C. Jordan, Permeation in ion channels: the interplay of structure and theory, Trends Neurosci. 27 (2004) 308–314.
[15] R.E. Koeppe, O.S. Andersen, Engineering the gramicidin channel, Annu. Rev. Biophys. Biomol. Struct. 25 (1996) 231–258.
[16] R. Sarges, B. Witkop, The structure of valine- and isoleucine-gramicidin A, J. Am. Chem. Soc. 87 (1965) 2011–2020.
[17] S.B. Hladky, D.A. Haydon, Ion transfer across lipid membranes in the presence of gramicidin A: I. Studies of the unit conductance channel, Biochim. Biophys. Acta 274 (1972) 294–312.
[18] S. Mukherjee, A. Chattopadhyay, Motionally restricted tryptophan environments at the peptide–lipid interface of gramicidin channels, Biochemistry 33 (1994) 5089–5097.
[19] J.P. Segrest, R.J. Feldman, Membrane proteins: amino acid sequence and membrane penetration, J. Mol. Biol. 87 (1974) 853–858.
[20] G. Kemp, K.A. Jacobson, C.E. Wennera, Solution and interfacial properties of gramicidin pertinent to its effect on membranes, Biochim. Biophys. Acta 255 (1972) 493–501.
[21] R.J. Dubos, C. Cattaneo, Studies on a bactericidal agent extracted from a soil bacillus: III. Preparation and activity of a protein-free fraction, J. Exp. Med. 70 (1939) 249–256.
[22] R.D. Hotchkiss, R.J. Dubos, Bactericidal fractions from an aerobic sporulating bacillus, J. Biol. Chem. 136 (1940) 803–804.
[23] C.L. Moberg, Z.A. Cohn, René Jules Dubos, Sci. Am. 264 (1991) 66–74.
[24] H.L. Van Epps, René Dubos: unearthing antibiotics, J. Exp. Med. 203 (2006) 259.
[25] J.A. Killian, Gramicidin and gramicidin–lipid interactions, Biochim. Biophys. Acta 1113 (1992) 391–425.
[26] J.B.O. Mitchell, J. Smith, D-amino acid residues in peptides and proteins, Proteins 50 (2003) 563–571.
[27] O.S. Andersen, G. Saberwal, D.V. Greathouse, R.E. Koeppe, Gramicidin channels a solvable membrane–protein folding problem, Ind. J. Biochem. Biophys. 33 (1996) 331–342.
[28] S. Edwards, B. Corry, S. Kuyucak, S.-H. Chung, Continuum electrostatics fails to describe ion permeation in the gramicidin channel, Biophy. J. 83 (2002) 1348–1360.
[29] S.-H. Chung, S. Kuyucak, Recent advances in ion channel research, Biochim. Biophys. Acta 1565 (2002) 267–286.
[30] B. Roux, Computational studies of the gramicidin channel, Acc. Chem. Res. 35 (2002) 366–375.
[31] T.W. Allen, O.S. Andersen, B. Roux, Molecular dynamics—potential of mean force calculations as a tool for understanding ion permeation and selectivity in narrow channels, Biophys. Chem. 124 (2006) 251–267.
[32] D.W. Urry, The gramicidin A transmembrane channel: a proposed $\pi_{(l,d)}$ helix, Proc. Natl. Acad. Sci. U. S. A. 68 (1971) 672–676.
[33] G.N. Ramachandran, R. Chandrasekaran, Conformation of peptide chains containing both L- and D-residues: part I—helical structures with alternating L- and D-residues with special reference to the LD-ribbon and the LD-helices, Indian J. Biochem. Biophys. 9 (1972) 1–11.
[34] W.R. Veatch, E.T. Fossel, E.R. Blout, The conformation of gramicidin A, Biochemistry 13 (1974) 5249–5256.
[35] B.A. Wallace, Gramicidin channels and pores, Annu. Rev. Biophys. Biophys. Chem. 19 (1990) 127–157.
[36] R.D. Hotchkiss, R.J. Dubos, Fractionation of the bactericidal agent from cultures of a soil bacillus, J. Biol. Chem. 132 (1940) 791–792.
[37] R.E. Koeppe, K.O. Hodgson, L. Stryer, Helical channels in crystals of gramicidin a and of a cesium–gramicidin a complex: an X-ray diffraction study, J. Mol. Biol. 121 (1978) 41–54.
[38] R.E. Koeppe, J.M. Berg, K.O. Hodgson, L. Stryer, Gramicidin a crystals contain two cation binding sites per channel, Nature 279 (1979) 723–725.
[39] B.A. Wallace, K. Ravikumar, The gramicidin pore: crystal structure of a cesium complex, Science 241 (1988) 182–187.
[40] D.A. Langs, Three-dimensional structure at 0.86 Å of the uncomplexed form of the transmembrane ion channel peptide gramicidin A, Science 241 (1988) 188–191.
[41] D.A. Langs, G.D. Smith, C. Courseille, G. Précigoux, M. Hospital, Monoclinic uncomplexed double-stranded, antiparallel, left-handed $\beta^{5.6}$-helix ($\uparrow\downarrow\beta^{5.6}$) structure of gramicidin A: alternate patterns of helical association and deformation, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 5345–5349.
[42] B.A. Wallace, Crystallographic studies of a transmembrane ion channel, gramicidin A, Prog. Biophys. Mol. Biol. 57 (1992) 59–69.
[43] E. Bamberg, H.J. Apell, H. Alpes, Structure of the gramicidin A channel: discrimination between the $\pi_{L,D}$ and the β helix by electrical measurements with lipid bilayer membranes, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 2402–2406.
[44] W.R. Veatch, R. Mathies, M. Eisenberg, L. Stryer, Simultaneous fluorescence and conductance studies of planar bilayer membranes containing a highly active and fluorescent analog of gramicidin A, J. Mol. Biol. 99 (1975) 75–92.
[45] C.M. Venkatachalam, D.W. Urry, Theoretical conformational analysis of the gramicidin A transmembrane channel: I. Helix sense and energetics of head-to-head dimerization, J. Comp. Chem. 4 (1983) 461–469.
[46] A.M. O'Connell, R.E. Koeppe, O.S. Andersen, Kinetics of gramicidin channel formation in lipid bilayers: transmembrane monomer association, Science 250 (1990) 1256–1259.
[47] D.W. Urry, M.C. Goodall, J.D. Glickson, D.F. Mayers, The gramicidin A transmembrane channel: characteristics of head-to-head dimerized $\pi_{(L,D)}$ helices, Proc. Natl. Acad. Sci. U. S. A. 68 (1971) 1907–1911.
[48] V.B. Myers, D.A. Haydon, Ion transfer across lipid membranes in the presence of gramicidin A.II. The ion selectivity, Biochim. Biophys. Acta 274 (1972) 313–322.
[49] A.S. Arseniev, I.L. Barsukov, V.F. Bystrov, A.L. Lomize, Y.A. Ovchinnikov, ^{1}H-NMR study of gramicidin A transmembrane ion channel. Head-to-head right-handed, single stranded helices, FEBS Lett. 186 (1985) 168–174.
[50] R.R. Ketchem, W. Hu, T.A. Cross, High-resolution conformation of gramicidin A in a lipid bilayer by solid-state NMR, Science 261 (1993) 1457–1460.
[51] R.R. Ketchem, B. Roux, T.A. Cross, High-resolution polypeptide structure in a lamellar phase lipid environment from solid state NMR derived orientational constraints, Structure 5 (1997) 1655–1669.
[52] S.V. Sychev, L.I. Barsukov, V.T. Ivanov, The double $\pi\pi$5.6 helix of gramicidin A predominates in unsaturated lipid membranes, Eur. Biophys. J. 22 (1993) 279–288.
[53] J.A. Killian, K.U. Prasad, D. Hains, D.W. Urry, The membrane as an environment of minimal interconversion. A circular dichroism study on the solvent dependence of the conformational behavior of gramicidin in diacylphosphatidylcholine model membranes, Biochemistry 27 (1988) 4848–4855.
[54] M. Zein, R. Winter, Effect of temperature, pressure and lipid acyl chain

length on the structure and phase behaviour of phospholipid-gramicidin bilayers, Phys. Chem. Chem. Phys. 2 (2000) 4545–4551.
[55] D.A. Kelkar, A. Chattopadhyay, Modulation of gramicidin channel conformation and organization by hydrophobic mismatch in saturated phosphatidylcholine bilayers, Biochim. Biophys. Acta 1768 (2007) 1103–1113.
[56] P.V. LoGrasso, F. Moll, T.A. Cross, Solvent history dependence of gramicidin A conformations in hydrated lipid bilayers, Biophys. J. 54 (1988) 259–267.
[57] W.C. Wimley, S.H. White, Experimentally determined hydrophobicity scale for proteins at membrane interfaces, Nat. Struct. Biol. 3 (1996) 842–848.
[58] C. Chothia, The nature of the accessible and buried surfaces in proteins, J. Mol. Biol. 105 (1976) 1–14.
[59] S.K. Burley, G.A. Petsko, Aromatic–aromatic interaction: a mechanism of protein structure stabilization, Science 229 (1985) 23–28.
[60] S.K. Burley, G.A. Petsko, Weakly polar interactions in proteins, Adv. Protein Chem. 39 (1988) 125–189.
[61] V. Fonseca, P. Daumas, L. Ranjalahy-Rasoloarijao, F. Heitz, R. Lazaro, Y. Trudelle, O.S. Andersen, Gramicidin channels that have no tryptophan residues, Biochemistry 31 (1992) 5340–5350.
[62] O.S. Andersen, D.V. Greathouse, L.L. Providence, M.D. Becker, R.E. Koeppe, Importance of tryptophan dipoles for protein function: 5-fluorination of tryptophans in gramicidin A channels, J. Am. Chem. Soc. 120 (1998) 5142–5146.
[63] W.-M. Yau, W.C. Wimley, K. Gawrisch, S.H. White, The preference of tryptophan for membrane interfaces, Biochemistry 37 (1998) 14713–14718.
[64] M. Cotten, F. Xu, T.A. Cross, Protein stability and conformational rearrangements in lipid bilayers: linear gramicidin, a model system, Biophys. J. 73 (1997) 614–623.
[65] D. Salom, M.C. Baño, L. Braco, C. Abad, HPLC demonstration that an all Trp→Phe replacement in gramicidin A results in a conformational rearrangement from beta-helical monomer to double-stranded dimer in model membranes, Biochem. Biophys. Res. Commun. 209 (1995) 466–473.
[66] D. Salom, E. Pérez-Payá, J. Pascal, C. Abad, Environment- and sequence-dependent modulation of the double-stranded to single-stranded conformational transition of gramicidin A in membranes, Biochemistry 37 (1998) 14279–14291.
[67] L.E. Townsley, W.A. Tucker, S. Sham, J.F. Hinton, Structures of gramicidin A, B, and C incorporated into sodium dodecyl sulfate micelles, Biochemistry 40 (2001) 11676–11686.
[68] S.S. Rawat, D.A. Kelkar, A. Chattopadhyay, Effect of structural transition of the host assembly on dynamics of an ion channel peptide: a fluorescence approach, Biophys. J. 89 (2005) 3049–3058.
[69] D. Salom, C. Abad, L. Braco, Characterization of gramicidin A in an inverted micellar environment. A combined high-performance liquid chromatographic and spectroscopic study, Biochemistry 31 (1992) 8072–8079.
[70] D.A. Kelkar, A. Chattopadhyay, Effect of graded hydration on the organization and dynamics of an ion channel: a fluorescence approach, Biophys. J. 88 (2005) 1070–1080.
[71] D.V. Greathouse, R.E. Koeppe, L.L. Providence, S. Shobana, O.S. Andersen, Design and characterization of gramicidin channels, Methods Enzymol. 294 (1999) 525–550.
[72] W. Hu, T.A. Cross, Tryptophan hydrogen bonding and electric dipole moments: functional roles in the gramicidin channel and implications for membrane proteins, Biochemistry 34 (1995) 14147–14155.
[73] R.E. Koeppe, H. Sun, P.C.A. van der Wel, E.M. Scherer, P. Pulay, D.V. Greathouse, Combined experimental/theoretical refinement of indole ring geometry using deuterium magnetic resonance and ab initio calculations, J. Am. Chem. Soc. 125 (2003) 12268–12276.
[74] J.T. Durkin, R.E. Koeppe, O.S. Andersen, Energetics of gramicidin hybrid channel formation as a test for structural equivalence. Side-chain substitutions in the native sequence, J. Mol. Biol. 211 (1990) 221–234.
[75] J.T. Durkin, L.L. Providence, R.E. Koeppe, O.S. Andersen, Formation of non-$\beta^{6.3}$-helical gramicidin channels between sequence-substituted gramicidin analogues, Biophys. J. 62 (1992) 145–159.
[76] A.S. Cifu, R.E. Koeppe, O.S. Andersen, On the supramolecular organization of gramicidin channels. The elementary conducting unit is a dimer, Biophys. J. 61 (1992) 189–203.
[77] S. Bransburg-Zabary, A. Kessel, M. Gutman, N. Ben-Tal, Stability of an ion channel in lipid bilayers: implicit solvent model calculations with gramicidin, Biochemistry 41 (2002) 6946–6954.
[78] A.R. Jude, D.V. Greathouse, R.E. Koeppe, L.L. Providence, O.S. Andersen, Modulation of gramicidin channel structure and function by the aliphatic "spacer" residues 10, 12, and 14 between the tryptophans, Biochemistry 38 (1999) 1030–1039.
[79] R.E. Koeppe, J. Hatchett, A.R. Jude, L.L. Providence, O.S. Andersen, D.V. Greathouse, Neighboring aliphatic/aromatic side chain interactions between residues 9 and 10 in gramicidin channels, Biochemistry 39 (2000) 2235–2242.
[80] G.V. Miloshevsky, P.C. Jordan, Gating gramicidin channels in lipid bilayers: reaction coordinates and the mechanism of dissociation, Biophys. J. 86 (2004) 92–104.
[81] J.T. Durkin, L.L. Providence, R.E. Koeppe, O.S. Andersen, Energetics of heterodimer formation among gramicidin analogues with an NH2-terminal addition or deletion. Consequences of missing a residue at the join in the channel, J. Mol. Biol. 231 (1993) 1102–1121.
[82] A.R. Jude, D.V. Greathouse, M.C. Leister, R.E. Koeppe, Steric interactions of valines 1, 5, and 7 in [valine 5, D-alanine 8] gramicidin A channels, Biophys. J. 77 (1999) 1927–1935.
[83] S. Oiki, R.E. Koeppe, O.S. Andersen, Voltage-dependent gating of an asymmetric gramicidin channel, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 2121–2125.
[84] G.A. Woolley, V. Zunic, J. Karanicolas, A.S.I. Jaikaran, A.V. Starostin, Voltage-dependent behavior of a ball-and-chain gramicidin channel, Biophys. J. 73 (1997) 2465–2475.
[85] E. Bamberg, K. Janko, The action of a carbonsuboxide dimerized gramicidin A on lipid bilayer membranes, Biochim. Biophys. Acta 465 (1977) 486–499.
[86] V.S. Rudnev, L.N. Ermishkin, L.A. Fonina, Y.G. Rovin, The dependence of the conductance and lifetime of gramicidin channels on the thickness and tension of lipid bilayers, Biochim. Biophys. Acta 642 (1981) 196–202.
[87] C.J. Stankovic, S.H. Heinemann, J.M. Delfino, F.J. Sigworth, S.L. Schreiber, Transmembrane channels based on tartaric acid-gramicidin A hybrids, Science 244 (1989) 813–817.
[88] C.J. Stankovic, S.H. Heinemann, S.L. Schreiber, Immobilizing the gate of a tartaric acid-gramicidin A hybrid channel molecule by rational design, J. Am. Chem. Soc. 112 (1990) 3702–3704.
[89] C.J. Stankovic, S.H. Heinemann, S.L. Schreiber, Photo-modulated ion channels based on covalently linked gramicidins, Biochim. Biophys. Acta 1061 (1991) 163–170.
[90] S. Cukierman, Flying protons in linked gramicidin A channels, Israel, J. Chem. 39 (1999) 419–426.
[91] S. Cukierman, E.P. Quigley, D.S. Crumrine, Proton conduction in gramicidin A and in its dioxolane-linked dimer in different lipid bilayers, Biophys. J. 73 (1997) 2489–2502.
[92] E.P. Quigley, P. Quigley, S. Cukierman, D.S. Crumrine, The conduction of protons in different stereoisomers of dioxolane-linked gramicidin A channels, Biophys. J. 77 (1999) 2479–2491.
[93] S. Crouzy, T.B. Woolf, B. Roux, A molecular dynamics study of gating in dioxolane-linked gramicidin A channels, Biophys. J. 67 (1994) 1370–1386.
[94] K.M. Armstrong, E.P. Quigley, P. Quigley, D.S. Crumrine, S. Cukierman, Covalently linked gramicidin channels: effects of linker hydrophobicity and alkaline metals on different stereoisomers, Biophys. J. 80 (2001) 1810–1818.
[95] A. Ring, Brief closures of gramicidin A channels in lipid bilayer membranes, Biochim. Biophvs. Acta 856 (1986) 646–653.
[96] F.J. Sigworth, D.W. Urry, K.U. Prasad, Open channel noise. III. High-resolution recordings show rapid current fluctuations in gramicidin A and four chemical analogues, Biophys. J. 52 (1987) 1055–1064.

[97] K.M. Armstrong, S. Cukierman, On the origin of closing flickers in gramicidin channels: a new hypothesis, Biophys. J. 82 (2002) 1329–1337.
[98] K.U. Prasad, T.L. Trapane, D. Busath, G. Szabo, D.W. Urry, Synthesis and characterization of (1–13C) Phe9 gramicidin A. Effects of side chain variations, Int. J. Pept. Protein Res. 22 (1983) 341–347.
[99] P. Daumas, F. Heitz, L. Ranjalahy-Rasoloarijao, R. Lazaro, Gramicidin A analogs: influence of the substitution of the tryptophans by naphthylalanines, Biochimie 71 (1989) 77–81.
[100] M.D. Becker, D.V. Greathouse, R.E. Koeppe, O.S. Andersen, Amino acid sequence modulation of gramicidin channel function: effects of tryptophan-to-phenylalanine substitutions on the single-channel conductance and duration, Biochemistry 30 (1991) 8830–8839.
[101] E. Bamberg, K. Noda, E. Gross, P. Lauger, Single-channel parameters of gramicidin A, B, and C, Biochim. Biophys. Acta 419 (1976) 223–228.
[102] D.D. Busath, R.C. Waldbillig, Photolysis of gramicidin A channels in lipid bilayers, Biochim. Biophys. Acta 736 (1983) 28–38.
[103] D. Jones, E. Hayon, D. Busath, Tryptophan photolysis is responsible for gramicidin-channel inactivation by ultraviolet light, Biochim. Biophys. Acta 861 (1986) 62–66.
[104] D.D. Busath, E. Hayon, Ultraviolet flash photolysis of gramicidin-doped lipid bilayers, Biochim. Biophys. Acta 944 (1988) 73–78.
[105] M. Strässle, G. Stark, M. Wilhelm, P. Daumas, F. Heitz, R. Lazaro, Radiolysis and photolysis of ion channels formed by analogues of gramicidin A with a varying number of tryptophan residues, Biochim. Biophys. Acta 980 (1989) 305–314.
[106] A.A. Sobko, M.A. Vigasina, T.I. Rokitskaya, E.A. Kotova, S.D. Zakharov, W.A. Cramer, Y.N. Antonenko, Chemical and photochemical modification of colicin E1 and gramicidin A in bilayer lipid membranes, J. Membr. Biol. 199 (2004) 51–62.
[107] H. Weiler-Feilchenfeld, A. Pullman, H. Berthod, C. Giessner-Prettre, Experimental and quantum-chemical studies of the dipole moments of quinoline and indole, J. Mol. Struct. 6 (1970) 297–304.
[108] J.B.A. Ross, A.G. Szabo, C.W.V. Hogue, Enhancement of protein spectra with tryptophan analogs: fluorescence spectroscopy of protein–protein and protein–nucleic acid interactions, Methods Enzymol. 278 (1997) 151–190.
[109] J.B.A. Ross, E. Rusinova, L.A. Luck, K.W. Rousslang, Spectral enhancement of proteins by *in vivo* incorporation of tryptophan analogues, in: J.R. Lakowicz (Ed.), Topics in Fluorescence Spectroscopy, Volume 6: Protein Fluorescence, Kluwer Academic/Plenum Publishers, New York, 2000, pp. 17–42.
[110] D.D. Busath, C.D. Thulin, R.W. Hendershot, L.R. Phillips, P. Maughan, C.D. Cole, N.C. Bingham, S. Morrison, L.C. Baird, R.J. Hendershot, M. Cotten, T.A. Cross, Noncontact dipole effects on channel permeation. I. Experiments with (5F-indole)Trp^{13} gramicidin A channels, Biophys. J. 75 (1998) 2830–2844.
[111] N. Thompson, G. Thompson, C.D. Cole, M. Cotten, T.A. Cross, D.D. Busath, Noncontact dipole effects on channel permeation: IV. Kinetic model of 5F-Trp$_{13}$ gramicidin A currents, Biophys. J. 81 (2001) 1245–1254.
[112] L.R. Phillips, C.D. Cole, R.J. Hendershot, M. Cotten, T.A. Cross, D.D. Busath, Noncontact dipole effects on channel permeation: III. Anomalous proton conductance effects in gramicidin, Biophys. J. 77 (1999) 2492–2501.
[113] A.E. Dorigo, D.G. Anderson, D.D. Busath, Noncontact dipole effects on channel permeation: II. Trp conformations and dipole potentials in gramicidin A, Biophsy. J. 76 (1999) 1897–1908.
[114] C.D. Cole, A.S. Frost, N. Thompson, T.A. M.Cotten, D.D. Cross, Noncontact dipole effects on channel permeation. VI. 5F- and 6F-Trp gramicidin channel currents, Biophys. J. 83 (2002) 1974–1986.
[115] J.A. Gowen, J.C. Markham, S.E. Morrison, T.A. Cross, D.D. Busath, E.J. Mapes, M.F. Schumaker, The role of Trp side chains in tuning single proton conduction through gramicidin channels, Biophys. J. 83 (2002) 880–898.
[116] D.G. Anderson, R.B. Shirts, T.A. Cross, D.D. Busath, Noncontact dipole effects on channel permeation: V. Computed potentials for fluorinated gramicidin, Biophys. J. 81 (2001) 1255–1264.
[117] T.I. Rokitskaya, Y.N. Antonenko, E.A. Kotova, Effect of the dipole potential of a bilayer lipid membrane on gramicidin channel dissociation kinetics, Biophys. J. 73 (1997) 850–854.
[118] R.L. Duffin, M.P. Garrett, K.B. Flake, J.D. Durrant, D.D. Busath, Modulation of lipid bilayer interfacial dipole potential by phloretin, RH421, and 6-ketocholestanol as probed by gramicidin channel conductance, Langmuir 19 (2003) 3561.
[119] D.G. Anderson, R.B. Shirts, T.A. Cross, D.D. Busath, Noncontact dipole effects on channel permeation: V. Computed potentials for fluorinated gramicidin, Biophys. J. 81 (2001) 1255–1264.
[120] M. Cotten, C. Tian, D.D. Busath, R.B. Shirts, T.A. Cross, Modulating dipoles for structure–function correlations in the gramicidin A channel, Biochemistry 38 (1999) 9185–9197.
[121] J.B. Jordan, P.L. Easton, J.F. Hinton, Effects of phenylalanine substitutions in gramicidin A on the kinetics of channel formation in vesicles and channel structure in SDS micelles, Biophys. J. 88 (2005) 224–234.
[122] W. Hu, K.-C. Lee, T.A. Cross, Tryptophans in membrane proteins: indole ring orientations and functional implications in the gramicidin channel, Biochemistry 32 (1993) 7035–7047.
[123] W. Hu, N.D. Lazo, T.A. Cross, Tryptophan dynamics and structural refinement in a lipid bilayer environment: solid state NMR of the gramicidin channel, Biochemistry 34 (1995) 14138–14146.
[124] W. Hu, T.A. Cross, Tryptophan hydrogen bonding and electric dipole moments: functional roles in the gramicidin channel and implications for membrane proteins, Biochemistry 34 (1995) 14147–14155.
[125] T.W. Allen, O.S. Andersen, B. Roux, Structure of gramicidin A in a lipid bilayer environment determined using molecular dynamics simulations and solid-state NMR data, J. Am. Chem. Soc. 125 (2003) 9868–9877.
[126] S.W. Chiu, S. Subramaniam, E. Jakobsson, Simulation study of a gramicidin/lipid bilayer system in excess water and lipid: I. Structure of the molecular complex, Biophys. J. 76 (1999) 1929–1938.
[127] S.S. Rawat, D.A. Kelkar, A. Chattopadhyay, Monitoring gramicidin conformations in membranes: a fluorescence approach, Biophys. J. 87 (2004) 831–843.
[128] T.B. Woolf, B. Roux, Structure, energetics, and dynamics of lipid–protein interactions: a molecular dynamics study of the gramicidin A channel in a DMPC bilayer, Proteins 24 (1996) 92–114.
[129] P.M. Macdonald, J. Seelig, Dynamic properties of gramicidin A in phospholipid membranes, Biochemistry 27 (1988) 2357–2364.
[130] N.C. Bingham, N.E.C. Smith, T.A. Cross, D.D. Busath, Molecular dynamics simulations of trp side-chain conformational flexibility in the gramicidin A channel, Biopolymers 71 (2003) 593–600.
[131] S.L. Grage, J. Wang, T.A. Cross, A.S. Ulrich, Solid-state ^{19}F-NMR analysis of ^{19}F-labeled tryptophan in gramicidin A in oriented membranes, Biophys. J. 83 (2002) 3336–3350.
[132] K. Burger, G. Gimpl, F. Fahrenholz, Regulation of receptor function by cholesterol, Cell. Mol. Life Sci. 57 (2000) 1577–1592.
[133] A.G. Lee, Lipid–protein interactions in biological membranes: a structural perspective, Biochim. Biophys. Acta 1612 (2003) 1–40.
[134] A.G. Lee, How lipids affect the activities of integral membrane proteins, Biochim. Biophys. Acta 1666 (2004) 62–87.
[135] T.J. Pucadyil, A. Chattopadhyay, Role of cholesterol in the function and organization of G-protein coupled receptors, Prog. Lipid Res. 45 (2006) 295–333.
[136] G.A. Woolley, B.A. Wallace, Membrane protein structure: lessons from gramicidin, in: S.H. White (Ed.), Membrane Protein Structure: Experimental Approaches, Oxford Univ. Press, New York, 1994, pp. 314–334.
[137] D.A. Kelkar, A. Chattopadhyay, Membrane interfacial localization of aromatic amino acids and membrane protein function, J. Biosci. 31 (2006) 297–302.
[138] U. Hollerbach, D.P. Chen, D.D. Busath, B. Eisenberg, Predicting function from structure using the Poisson–Nernst–Planck equations: sodium current in the gramicidin A channel, Langmuir 16 (2000) 5509–5514.
[139] I. Pasqual-Ronchetti, A. Spisni, E. Casali, L. Masotti, D.W. Urry, Gramicidin A induces lysolecithin to form bilayers, Biosci. Rep. 3 (1983) 127–133.
[140] J.A. Killian, B. de Kruijff, C.J. van Echteld, A.J. Verkleij, J. Leunissen-

Bijvelt, J. de Gier, Mixtures of gramicidin and lysophosphatidylcholine form lamellar structures, Biochim. Biophys. Acta 728 (1983) 141–144.
[141] J.A. Killian, D.W. Urry, Conformation of gramicidin in relation to its ability to form bilayers with lysophosphatidylcholine, Biochemistry 27 (1988) 7295–7301.
[142] R. Brasseur, V. Cabiaux, J.A. Killian, B. de Kruijff, J.M. Ruysschaert, Orientation of gramicidin A at the lysophosphatidylcholine/water interface: a semi-empirical conformational analysis, Biochim. Biophys. Acta 855 (1986) 317–324.
[143] F.J. Aranda, J.A. Killian, B. de Kruijff, Importance of the tryptophans of gramicidin for its lipid structure modulating activity in lysophosphatidylcholine and phosphatidylethanolamine model membranes. A comparative study employing gramicidin analogs and a synthetic α-helical hydrophobic polypeptide, Biochim. Biophys. Acta 901 (1987) 217–228.
[144] C.J.A. van Echteld, B. de Kruijff, A.J. Verkleij, J. Leunissen-Bijvelt, J. de Gier, Gramicidin induces the formation of non-bilayer structures in phosphatidylcholine dispersions in a fatty acid chain length dependent way, Biochim. Biophys. Acta 692 (1982) 126–138.
[145] J.A. Szule, R.P. Rand, The effects of gramicidin on the structure of phospholipid assemblies, Biophys. J. 85 (2003) 1702–1712.
[146] H. Tournois, J. Leunissen-Bijvelt, C.W. Haest, J. de Gier, B. de Kruijff, Gramicidin-induced hexagonal HII phase formation in erythrocyte membranes, Biochemistry 26 (1987) 6613–6621.
[147] H. Tournois, U. Henseleit, J. deGier, B. de Kruijff, C.W. Haest, Relationship between gramicidin conformation dependent induction of phospholipid transbilayer movement and hexagonal HII phase formation in erythrocyte membranes, Biochim. Biophys. Acta 946 (1988) 173–177.
[148] J.A. Killian, B. de Kruijff, Importance of hydration for gramicidin-induced hexagonal HII phase formation in dioleoylphosphatidylcholine model membranes, Biochemistry 24 (1985) 7890–7898.
[149] J.A. Killian, K.N.J. Burger, B. de Kruijff, Phase separation and hexagonal HII phase formation by gramicidins A, B and C in dioleoylphosphatidylcholine model membranes. A study on the role of the tryptophan residues, Biochim. Biophys. Acta 897 (1987) 269–284.
[150] A.R. Dibble, G.W. Feigenson, Detection of coexisting fluid phospholipid phases by equilibrium Ca^{2+} binding: peptide-poor L_α and peptide-rich HII phase coexistence in gramicidin A′/phospholipid dispersions, Biochemistry 33 (1994) 12945–12953.
[151] P.I. Watnick, S.I. Chan, P. Dea, Hydrophobic mismatch in gramicidin A′/lecithin systems, Biochemistry 29 (1990) 6215–6621.
[152] B.A. Cornell, L.E. Weir, F. Separovic, The effect of gramicidin A on phospholipid bilayers, Eur. Biophys. J. 16 (1988) 113–119.
[153] J.A. Killian, K.U. Prasad, D.W. Urry, B. de Kruijff, A mismatch between the length of gramicidin and the lipid acyl chains is a prerequisite of HII phase formation in phosphatidylcholine model membranes, Biochim. Biophys. Acta 978 (1989) 341–345.
[154] H. Tuornois, C.H. Fabrie, K.N. Burger, J. Mandersloot, P. Hilgers, H. van Dalen, J. de Gier, B. de Kruijff, Gramicidin A induced fusion of large unilamellar dioleoylphosphatidylcholine vesicles and its relation to the induction of type II nonbilayer structures, Biochemistry 29 (1990) 8297–8307.
[155] A. Chattopadhyay, H. Raghuraman, Application of fluorescence spectroscopy to membrane protein structure and dynamics, Curr. Sci. 87 (2004) 175–180.
[156] K.J. Cox, C. Ho, J.V. Lombardi, C.D. Stubbs, Gramicidin conformational studies with mixed-chain unsaturated phospholipid bilayer systems, Biochemistry 31 (1992) 1112–1118.
[157] M.C. Baño, L. Braco, C. Abad, Conformational transitions of gramicidin A in phospholipid model membranes. A high-performance liquid chromatography assessment, Biochemistry 30 (1991) 886–894.
[158] E.P. Kirby, R.F. Steiner, Influence of solvent and temperature upon the fluorescence of indole derivatives, J. Phys. Chem. 74 (1970) 4480–4490.
[159] J.M. Beechem, L. Brand, Time-resolved fluorescence of proteins, Annu. Rev. Biochem. 54 (1985) 43–71.
[160] P. Cavatorta, A. Spisni, E. Casali, L. Lindner, L. Masotti, D.W. Urry, Intermolecular interactions of gramicidin A′ transmembrane channels incorporated into lysophosphatidylcholine lipid systems, Biochim. Biophys. Acta 689 (1982) 113–120.
[161] S.F. Scarlata, The effects of viscosity on gramicidin tryptophan rotational motion, Biophys. J. 54 (1988) 1149–1157.
[162] S.F. Scarlata, Effect of increased chain packing on gramicidin–lipid interactions, Biochemistry 30 (1991) 9853–9859.
[163] J.-M. Camaleño-Delango, X.K. Zhao, J.H. Fendler, Intrinsic gramicidin fluorescence lifetimes in bilayer lipid membranes and in vesicles, Can. J. Chem. 68 (1990) 888–896.
[164] J. Shobini, A.K. Mishra, N. Chandra, Conformation of gramicidin-A in CTAB micellar media, J. Photochem. Photobiol., B 70 (2003) 117–124.
[165] D.A. Kelkar, A. Chattopadhyay, Monitoring ion channel conformations in membranes utilizing a novel dual fluorescence quenching approach, Biochem. Biophys. Res. Commun. 343 (2006) 483–488.
[166] A. Chattopadhyay, S.S. Rawat, Wavelength-selective fluorescence in ion channels formed by gramicidin A in membranes, J. Chem. Sci. 119 (2007) 135–140.
[167] A.P. Demchenko, The red-edge effects: 30 years of exploration, Luminescence 17 (2002) 19–42.
[168] A. Chattopadhyay, Exploring membrane organization and dynamics by the wavelength-selective fluorescence approach, Chem. Phys. Lipids 122 (2003) 3–17.
[169] H. Raghuraman, D.A. Kelkar, A. Chattopadhyay, Novel insights into protein structure and dynamics utilizing the red edge excitation shift approach, in: C.D. Geddes, J.R. Lakowicz (Eds.), Reviews in Fluorescence 2005, vol. 2, Springer, New York, 2005, pp. 199–222.
[170] S.S. Rawat, A. Chattopadhyay, Structural transition in the micellar assembly: a fluorescence study, J. Fluoresc. 9 (1999) 233–244.
[171] J.R. Elliott, D. Needham, J.P. Dilger, D.A. Haydon, The effects of bilayer thickness and tension on gramicidin single-channel lifetime, Biochim. Biophys. Acta 735 (1983) 95–103.
[172] J.A. Lundbæk, P. Birn, J. Girshman, A.J. Hansen, O.S. Andersen, Membrane stiffness and channel function, Biochemistry 35 (1996) 3825–3830.
[173] H.W. Huang, Deformation free energy of bilayer membrane and its effect on gramicidin channel lifetime, Biophys. J. 50 (1986) 1061–1070.
[174] P. Helfrich, E. Jakobsson, Calculation of deformation energies and conformations in lipid membranes containing gramicidin channels, Biophys. J. 57 (1990) 1075–1084.
[175] J.A. Lundbæk, P. Birn, A.J. Hansen, R. Søgaard, C. Nielsen, J. Girshman, M.J. Bruno, S.E. Tape, J. Egebjerg, D.V. Greathouse, G.L. Mattice, R.E. Koeppe, O.S. Andersen, Regulation of sodium channel function by bilayer elasticity: the importance of hydrophobic coupling. Effects of micelle-forming amphiphiles and cholesterol, J. Gen. Physiol. 123 (2004) 599–621.
[176] J.A. Lundbæk, O.S. Andersen, Lysophospholipids modulate channel function by altering the mechanical properties of lipid bilayers, J. Gen. Physiol. 104 (1996) 645–673.
[177] J.A. Lundbæk, A.M. Maer, O.S. Andersen, Lipid bilayer electrostatic energy, curvature stress, and assembly of gramicidin channels, Biochemistry 36 (1997) 5695–5701.
[178] O.P. Hamill, B. Martinac, Molecular basis of mechanotransduction in living cells, Physiol. Rev. 81 (2001) 685–740.
[179] B. Martinac, O.P. Hamill, Gramicidin A channels switch between stretch activation and stretch inactivation depending on bilayer thickness, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4308–4312.
[180] D. Needham, R.S. Nunn, Elastic deformation and failure of lipid bilayer membranes containing cholesterol, Biophys. J. 58 (1990) 997–1009.
[181] D. Needham, Cohesion and permeability of lipid bilayer vesicles, in: S.A. Simon, E.A. Disalvo (Eds.), Permeability and Stability of Bilayers, CRC Press, Boca Raton, FL, 1995, pp. 49–76.
[182] J.A. Lundbæk, O.S. Andersen, T. Werge, C. Nielsen, Cholesterol-induced protein sorting: an analysis of energetic feasibility, Biophys. J. 84 (2003) 2080–2089.
[183] S.A. Simon, T.J. McIntosh, R. Latorre, Influence of cholesterol on water penetration into bilayers, Science 216 (1982) 65–67.
[184] F.A. Nezil, M. Bloom, Combined influence of cholesterol and synthetic

amphiphillic peptides upon bilayer thickness in model membranes, Biophys. J. 61 (1992) 1176–1182.
[185] J.R. Elliott, D. Needham, J.P. Dilger, O. Brandt, D.A. Haydon, A quantitative explanation of the effects of some alcohols on gramicidin single-channel lifetime, Biochim. Biophys. Acta 814 (1985) 401–404.
[186] L.V. Schagina, K. Blaskó, A.E. Grinfeldt, Y.E. Korchev, A.A. Lev, Cholesterol-dependent gramicidin A channel inactivation in red blood cell membranes and lipid bilayer membranes, Biochim. Biophys. Acta 978 (1989) 145–150.
[187] L.V. Schagina, Y.E. Korchev, A.E. Grinfeldt, A.A. Lev, K. Blaskó, Sterol specific inactivation of gramicidin A induced membrane cation permeability, Biochim. Biophys. Acta 1109 (1992) 91–96.
[188] N. Mobashery, C. Nielsen, O.S. Andersen, The conformational preference of gramicidin channels is a function of lipid bilayer thickness, FEBS Lett. 412 (1997) 15–20.
[189] H.-D. Arndt, A. Knoll, U. Koert, Synthesis of minigramicidin ion channels and test of their hydrophobic match with the membrane, ChemBioChem 2 (2001) 221–222.
[190] O.S. Andersen, C. Nielsen, A.M. Maer, J.A. Lundbæk, M. Goulian, R.E. Koeppe, Ion channels as tools to monitor lipid bilayer–membrane protein interactions: gramicidin channels as molecular force transducers, Methods Enzymol. 294 (1999) 208–224.
[191] C. Nielsen, M. Goulian, O.S. Andersen, Energetics of inclusion-induced bilayer deformations, Biophys. J. 74 (1998) 1966–1983.
[192] J.A. Lundbæk, O.S. Andersen, Spring constants for channel-induced lipid bilayer deformations. Estimates using gramicidin channels, Biophys. J. 76 (1999) 889–895.
[193] T.-C. Hwang, R.E. Koeppe, O.S. Andersen, Genistein can modulate channel function by a phosphorylation-independent mechanism: importance of hydrophobic mismatch and bilayer mechanics, Biochemistry 42 (2003) 13646–13658.
[194] L. Liu, T. Yang, M.J. Bruno, O.S. Andersen, Voltage-gated ion channels in nociceptors: modulation by cGMP, J. Neurophysiol. 92 (2004) 2323–2332.
[195] T.M. Suchyna, S.E. Tape, R.E. Koeppe, O.S. Andersen, F. Sachs, P.A. Gottlieb, Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers, Nature 430 (2004) 235–240.
[196] M.L. Garcia, Gate expectations, Nature 430 (2004) 153–155.
[197] A. Chattopadhyay, D.A. Kelkar, Ion channels and D-amino acids, J. Biosci. 30 (2005) 147–149.
[198] B.A. Wallace, Common structural features in gramicidin and other ion channels, BioEssays 22 (2000) 227–234.
[199] B.A. Wallace, X-ray crystallographic structures of gramicidin and their relation to the *Streptomyces lividans* potassium channel structure, Novartis Foundation Symposium 225, Gramicidin and related ion channel forming peptides, Wiley, Chichester, 1999, pp. 23–37.
[200] F.I. Valiyaveetil, M. Sekedat, R. MacKinnon, T.W. Muir, Glycine as a D-amino acid surrogate in the K^+-selectivity filter, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17045–17049.
[201] J. Thundimadathil, R.W. Roeske, L. Guo, Conversion of a porin-like peptide channel into a gramicidin-like channel by glycine to D-alanine substitutions, Biophys. J. 90 (2006) 947–955.
[202] S. Arumugam, S. Pascal, C.L. North, W. Hu, K.-C. Lee, M. Cotten, R.R. Ketchem, F. Xu, M. Brenneman, F. Kovacs, F. Tian, A. Wang, S. Huo, T.A. Cross, Conformational trapping in a membrane environment: a regulatory mechanism for protein activity? Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5872–5876.
[203] C. Domene, S. Vemparala, M.L. Klein, C. Vénien-Bryan, D.A. Doyle, Role of aromatic localization in the gating process of a potassium channel, Biophys. J. 90 (2006) L01–L03.
[204] A.S. Miller, J.J. Falke, Side chains at the membrane–water interface modulate the signaling state of a transmembrane receptor, Biochemistry 43 (2004) 1763–1770.
[205] R.R. Draheim, A.F. Bormans, R.Z. Lai, M.D. Manson, Tryptophan residues flanking the second transmembrane helix (TM2) set the signaling state of the Tar chemoreceptor, Biochemistry 44 (2005) 1268–1277.
[206] C.S. Chiang, L. Shirinian, S. Sukharev, Capping transmembrane helices of MscL with aromatic residues changes channel response to membrane stretch, Biochemistry 44 (2005) 12589–12597.
[207] R. MacKinnon, G. von Heijne, Membranes, Curr. Opin. Struct. Biol. 16 (2006) 431.
[208] J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, X-ray structure analysis of a membrane protein complex. Electron density map at 3 Å resolution and a model of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis*, Nature 318 (1985) 618–624.
[209] H. Sui, B.-G. Han, J.K. Lee, P. Walian, B.K. Jap, Structural basis of water-specific transport through the AQP1 water channel, Nature 414 (2001) 872–878.
[210] J. Abramson, I. Smirnova, V. Kasho, G. Verner, H.R. Kaback, S. Iwata, Structure and mechanism of the lactose permease of *Escherichia coli*, Science 301 (2003) 610–615.
[211] J.U. Bowie, Are we destined to repeat history? Curr. Opin. Struct. Biol. 10 (2000) 435–437.
[212] S.H. White, The progress of membrane protein structure determination, Protein Sci. 13 (2004) 1948–1949.
[213] G. Miller, The puzzling portrait of a pore, Science 300 (2003) 2020–2022.
[214] R.A. Sayle, E.J. Milner-White, RasMol: biomolecular graphics for all, Trends Biochem. Sci. 20 (1995) 374–376.