Commentary

Membrane interfacial localization of aromatic amino acids and membrane protein function

Biological membranes are complex assemblies of lipids and proteins that allow cellular compartmentalization and act as the interface through which cells communicate with each other and with the external milieu. It is well known that the interiors of biological membranes are viscous, with an effective viscosity comparable to that of light oil (Edidin 2003). In addition, membranes exhibit a considerable degree of anisotropy along the axis perpendicular to the bilayer. While the center of the bilayer (hydrophobic core) is nearly isotropic, the upper portion, only a few angstroms away toward the membrane surface (membrane interface), is highly ordered (Seelig 1977; Perochon *et al* 1992; White and Wimley 1994; Chattopadhyay 2003). Properties such as polarity, fluidity, segmental motion, ability to form hydrogen bonds and extent of solvent penetration vary in a depth-dependent manner in the membrane. The interfacial region in membranes (see figure 1) is the most important region so far as the dynamics and function of the membrane is concerned. The membrane interface is characterized by unique motional and dielectric characteristics distinct from both the bulk aqueous phase and the more isotropic hydrocarbon-like interior of the membrane. It is a chemically heterogeneous region composed of lipid headgroup, water and portions of the acyl chain. Overall, the interfacial region of the membrane accounts for 50% of the thermal thickness of the bilayer (White and Wimley 1994).

The biological membrane provides a unique backdrop (environment) to membrane-spanning proteins and peptides influencing their structure and function. Membrane-spanning proteins have distinct stretches of hydrophobic amino acids that form the membrane-spanning domain. In addition, it has been observed that aromatic amino acid residues in integral membrane proteins and peptides are not uniformly distributed and that they tend to be localized toward the membrane interface (see figure 1), possibly because they are involved in hydrogen bonding (Ippolito *et al* 1990) with the lipid carbonyl groups or interfacial water molecules. For instance, crystal structures of membrane proteins such as the bacterial KcsA potassium channel (Doyle *et al* 1998), bacteriorhodopsin (Luecke *et al* 1999), maltoporin (Schirmer *et al* 1995), and others show that most aromatic amino acid residues are located in a saddle-like "aromatic belt" around the membrane interfacial region. Statistical studies of sequence databases and available crystal structures of integral membrane proteins also show preferential clustering of aromatic amino acid residues at the membrane interface (Reithmeier 1995; Adamian *et al* 2005). Furthermore, for transmembrane peptides and proteins, aromatic amino acids have been found to be efficient anchors at the membrane interface and to define the hydrophobic length of transmembrane helices (de Planque *et al* 2002). Interestingly, as a result of the efficient anchoring of the sequence at the membrane interface, the introduction of aromatic residues in synthetic channel-forming sequences has been shown to optimize membrane insertion, orientation, and channel activity (Shank *et al* 2006). Importantly, the role of aromatic amino acid residues in maintaining the structure and function of membrane proteins is exemplified by the fact that substitution or deletion of these residues often results in modulation of protein functionality (Becker *et al* 1991; Fonseca *et al* 1992; Miller and Falke 2004; Draheim *et al* 2005).

This preferential localization of aromatic amino acids at the membrane interface could therefore be utilized by membrane proteins as a driving force to stabilize functional membrane-bound conformations leading to switches between 'on' and 'off' states. A well known example of this principle is demonstrated by the prototypical ion channel peptide gramicidin. Gramicidins are pentadecapeptide antibiotics with a molecular weight of ~ 1900. They are produced by the soil bacterium *Bacillus brevis*, and consist of alternating L- and D-amino acids (see figure 2a) (Chattopadhyay and Kelkar 2005). A linear peptide, gramicidin forms ion channels specific for monovalent cations and has been extensively used to study

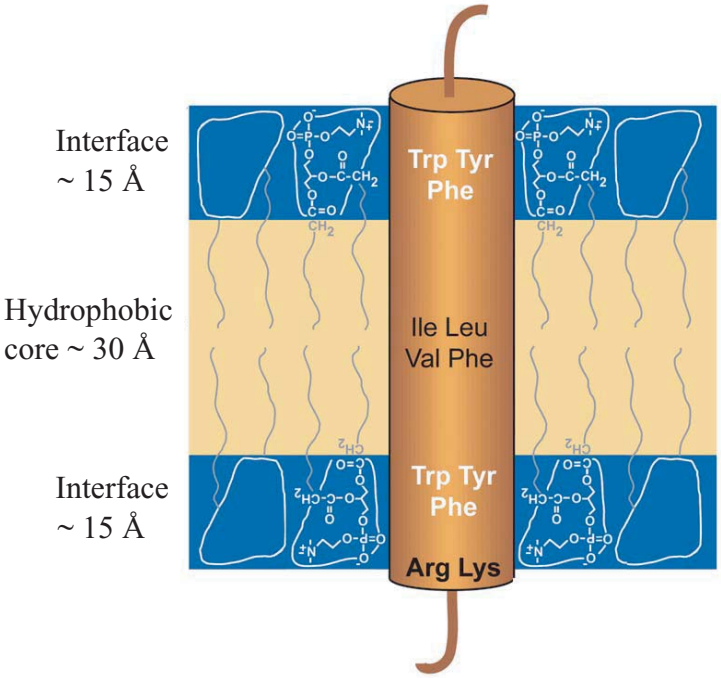

Figure 1. A schematic representation of the membrane bilayer containing a typical transmembrane domain of an integral membrane protein. The membrane lipids shown have two hydrophobic tails with a phosphatidylcholine (PC) headgroup. The preferred locations of various amino acids in relation to the distinct regions of the membrane bilayer are also shown. It should be noted that aromatic amino acid residues are preferentially localized in the membrane interface region, a region characterized by unique organization, dynamics, hydration and functionality. Adapted and modified from Raghuraman *et al* (2005).

the organization, dynamics and function of membrane spanning channels (Killian 1992). The functional transmembrane gramicidin channel is formed by the head-to-head dimerization of $\beta^{6.3}$ helices. This form is stabilized by six intermolecular hydrogen bonds. The channel interior is lined by the polar carbonyl and amide moieties of the peptide backbone, a feature shared with the selectivity filter of the bacterial KcsA potassium channel (Chattopadhyay and Kelkar 2005). An important aspect of this conformation is the membrane interfacial location of the aromatic residues (tryptophan), a common feature of many transmembrane helices (see figure 2b).

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. In contrast to the functional channel form, gramicidin also exists in a nonfunctional 'non-channel' conformation in membranes under certain conditions (Rawat *et al* 2004). The non-channel conformation of gramicidin consists of a double stranded intertwined helix (see figure 2b). Interestingly the functional channel conformation of gramicidin is the most preferred (thermodynamically stable) conformation in membranes. The membrane interface seeking property of tryptophan is implicated in the thermodynamic stability of the single stranded channel form in membranes. The distributions and depths of the gramicidin tryptophans represent major differences between the two forms (shown in figure 2b). The reason that the non-channel conformation is not the thermodynamically stable form in membranes is due to the fact that in this form some of the tryptophan residues are buried in the low dielectric nonpolar region of the membrane which is energetically unfavourable. The preferred localization of the tryptophan residues at the membrane interface has been ascribed to the ability of the tryptophan -NH groups to form hydrogen bonds with the hydrogen bond acceptors near the lipid head groups, one of the strongest candidates for such acceptors being the lipid carbonyls (Ippolito *et al* 1990).

Importantly, 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

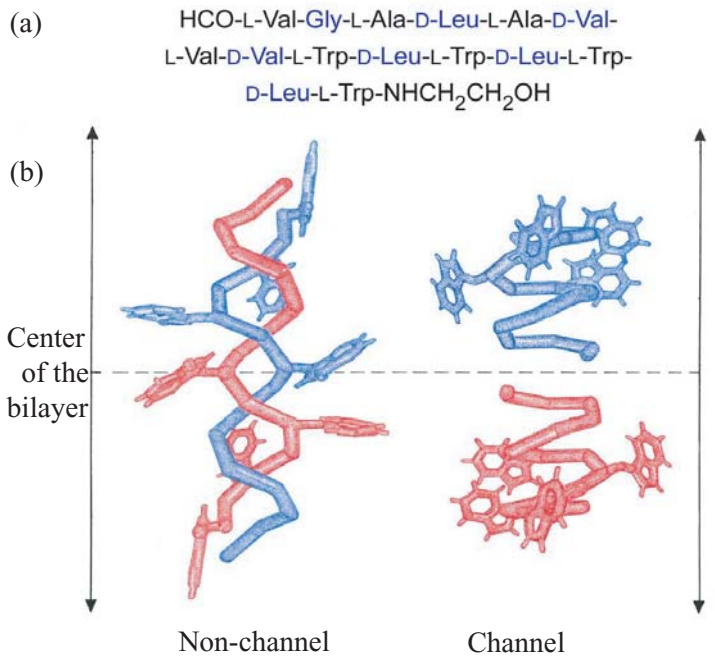

Figure 2. **(a)** Amino acid sequence of gramicidin A. Note that all amino acid side chains are either hydrophobic (Ala, Leu, Val) or amphipathic (Trp). In addition, the -NH_2 and -COOH termini are blocked making the sequence unusually hydrophobic. Alternating D-amino acid residues are shown in blue. **(b)** 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 (modified from Rawat *et al* 2004).

tryptophans with other aromatic side chains (Becker *et al* 1991; Fonseca *et al* 1992) has been found to reduce gramicidin channel activity. In addition, photolysis of tryptophan by ultraviolet irradiation or chemical modification by an oxidizing agent such as N-bromosuccinimide (NBS) has been shown to result in reduced ion conductivity. Significantly, tryptophan modifications that modulate the indole dipole moment without affecting the hydrogen bonding ability of tryptophan are also found to alter channel conductance (Andersen *et al* 1998; Anderson *et al* 2001). The proposition that the tryptophan residues of membrane-bound gramicidin channels are indeed hydrogen bonded to the neighbouring hydrogen bond acceptors is further supported by the fact that 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 non-channel double-stranded helical dimer conformation, and exhibits drastically reduced channel activity (Fonseca *et al* 1992). In the absence of any tryptophan residues, the double stranded helical dimer non-channel conformation becomes the energetically favoured state in the membrane.

The membrane interface anchoring property of aromatic residues becomes particularly useful for membrane protein function 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 (Miller and Falke 2004; Draheim *et al* 2005). 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 (Chiang *et al* 2005), 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.

A very recent example in which the preferential localization of aromatic amino acids at the membrane interface has been shown to be utilized as a means of directing and stabilizing structural changes during conformational transitions within the transmembrane domain is that of an inward rectifying potassium channel (KirBac1.1). Molecular dynamics simulations in the nanosecond timescale showed that the distribution of aromatic residues is significantly different in the open and closed conformation for this protein (Domene *et al* 2006). Interestingly, in the open conformation aromatic residues are clustered at the membrane interface, while in the closed conformation aromatic residues are found to be distributed evenly across the membrane axis (see figure 3a). An analysis of the order parameters obtained from the simulation showed an interesting difference between the open and closed forms of the channel for aromatic amino acid residues (see figure 3b). For a subset of phenylalanine residues that are not buried in the protein matrix, the order parameters exhibit considerable change in the nanosecond timescale in the open conformer while remaining constant in the closed conformer. The orientation of these phenylalanine residues therefore show dramatic variation between the open and closed states. The higher order parameter and fluctuations in the open state (Domene *et al* 2006) are representative of the unique environment and dynamics of the membrane interface (Chattopadhyay 2003). This difference in the location of aromatic amino acids between the closed and open states of KirBac1.1 is an interesting demonstration of membrane proteins utilizing the interfacial localization of aromatic residues as a strategy during conformational

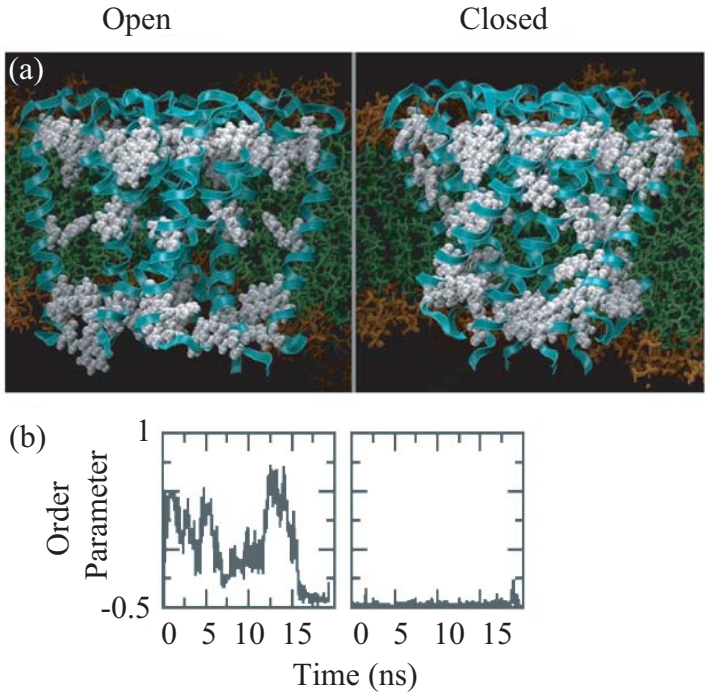

Figure 3. **(a)** Schematic representation of the localization of aromatic amino acids in the membrane for the open and closed states of KirBac1.1. The protein backbone is shown in cyan ribbons and aromatic side chains are displayed in white space filling format. **(b)** Orientation (order parameter) of selected aromatic (phenylalanine) residues in the open and closed conformations of KirBac1.1 as a function of simulation time. Notice the fast fluctuations and higher order parameter in the open state that are absent in the closed state. The higher order parameter observed in the open state is characteristic of membrane interfacial dynamics (see text). The order parameter remains more or less constant in the closed state. Adapted and modified from Domene *et al* (2006) with permission from Biophysical Society.

transitions. In general, phenylalanine residues do not show a preference for the membrane interface and are found to be distributed both at the interface and in the hydrophobic core of the membrane (see figure 1). Surprisingly, this appears to be not true for KirBac1.1. It is the relatively marked membrane interface localization of phenylalanine residues in the open state as compared to the closed state that seems to be involved in guiding the conformational changes in channel gating in this case. Interestingly, since these aromatic residues are highly conserved in the family of potassium channels, their interfacial localization may contribute to the free energy change associated with conformational switches necessary for channel gating. Electron crystallographic structures for the open and closed states of a closely related channel KirBac3.1 (Kuo *et al* 2005) have recently been reported. Future work in this direction may lead to further insights into the role of aromatic residues in the conformational changes involved in the gating of KirBac1.1.

A large class of membrane proteins are involved in the transport of ions and small molecules across membranes. During their function, they are likely to undergo conformational transitions in their transmembrane domains as the channel (or transporter) switches from one functional state to another in response to physiological stimulation. The energetics of interfacial localization of aromatic residues could be a major driving factor in these transitions.

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