Membrane Protein Insertion: The Biology–Physics Nexus

Stephen H. White

Department of Physiology and Biophysics, University of California, Irvine, CA 92697

The Journal of General Physiology

Introduction

Membrane proteins are a greasy lot. Thrown into water without detergents, they form intractable aggregates, which minimize exposure of their nonpolar transmembrane surfaces to water. Aggregation would be their certain fate if membrane proteins were translated on the ribosome in the manner of soluble proteins. But this fate is avoided: the elongating polypeptide segment from the ribosome exit tunnel (Voss et al., 2006) passes into the membrane-dwelling translocon assembly whose architecture permits selected segments to enter the membrane bilayer to become transmembrane (TM) helices (for review see White and von Heijne, 2004, 2005; von Heijne, 2006). The principles underlying this selection process are beginning to emerge from our recent studies of the recognition of transmembrane helices by the ER translocon (Hessa et al., 2005a; Meindl-Beinker et al., 2006). As Gunnar von Heijne discusses in his Perspective (p. 353), one outcome of our experiments was a "biological" hydrophobicity scale that describes the apparent free energy of insertion (ΔG_{app}) of each of the 20 natural amino acids when located in the center of a hydrophobic TM helix. This biological scale correlated strongly with the so-called Wimley-White (1999) physical hydrophobicity scales, suggesting that, somehow, the translocon selection of TM segments follows general thermodynamic principles that underlie partitioning of peptides between water and *n*-octanol (Wimley et al., 1996). The primary goal of this Perspective is to explore this intriguing nexus between biology and physical chemistry.

One of the motivations for this series of Perspectives is to gain deeper insights into the connection between the biological hydrophobicity scale and physical scales, especially the Wimley-White (1999) octanol scale. Our current understanding of membranes, hydrophobicity, and membrane protein stability originates from the Fluid Mosaic Membrane Model (Singer and Nicolson, 1972). I therefore begin by reviewing the origins of this conceptual model, which is built upon the fundamental idea that hydrophobic molecules (e.g., membrane proteins) partition between water and immiscible nonpolar phases (e.g., lipid bilayers). Because this concept arose before our understanding of the structure and dynamics of fluid bilayers was complete, I next discuss the modern view of lipid bilayer structure and dynamics, which is essential for connecting the biological and physical scales. Implicit in the motivation for this Perspectives series is the apparent ability of the translocon to insert charge-containing TM helices across the ER membrane. I discuss how this might work in the light of the modern view of bilayer structure. This sets the stage for considering why the biological hydrophobicity scale correlates so strongly with the Wimley-White octanol scale. I attempt to provide an explanation. To begin to connect the biological and physical hydrophobicity scales, I then discuss insights into translocon function obtained from molecular dynamics (MD) simulations of SecY/Sec61 heterotrimer in lipid bilayers. I argue that the heterotrimer is ideally suited to serve as a thermodynamic switch that determines whether or not a polypeptide segment passes into the lipid bilayer as a TM segment. Finally, I consider whether TM helix recognition by the translocon is equivalent to direct partitioning of helices between water and lipid bilayer.

The Fluid Mosaic Membrane Concept

Hydrophobic solutes partition strongly from water into immiscible nonpolar phases. This simple principle is so obvious and compelling that we have long thought of membranes as thin, immiscible lipid (bilayer) phases into which lipid-soluble molecules readily partition (Overton, 1895; Gorter and Grendel, 1925). John Singer, inspired by the germinal paper of Kauzmann (1959) on the role of hydrophobicity in protein stability, extended this thinking to membrane proteins (Lenard and Singer, 1966; Singer, 1971; Singer and Nicolson, 1972). His now famous and still influential cartoon of a transmembrane protein in a lipid bilayer (Fig. 1 A) spawned myriad papers on the identification of transmembrane segments by hydrophobicity analysis, beginning with Segrest and Feldman (1974). At about the same time, Nozaki and Tanford's (1971) studies of amino acid hydrophobicity and Tanford's (1973) classic book on the hydrophobic effect stimulated critical investigations of the partitioning of amino acid side chains

Correspondence to Stephen White: stephen.white@uci.edu

Abbreviations used in this paper: DOPC, dioleoylphosphatidylcholine; MD, molecular dynamics; TM, transmembrane.

J. Gen. Physiol. © The Rockefeller University Press $15.00
Volume 129 Number 5 May 2007 363–369
http://www.jgp.org/cgi/doi/10.1085/jgp.200709741

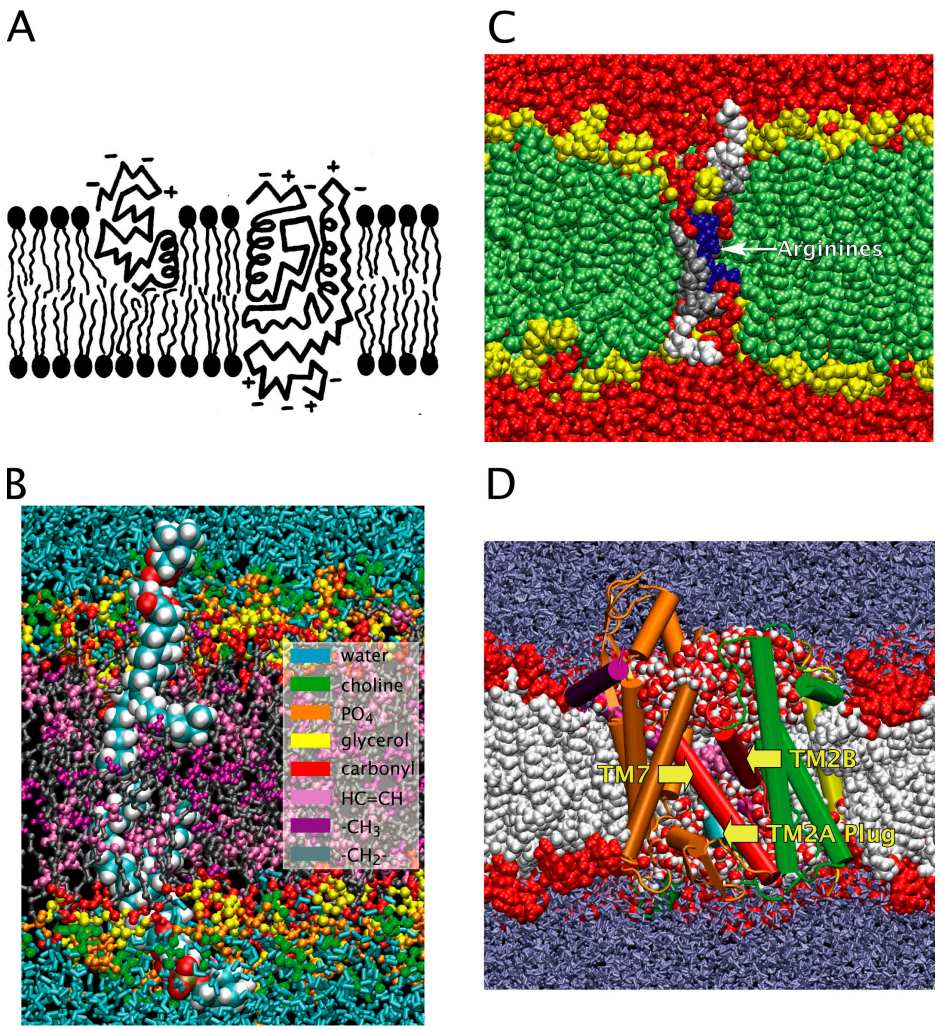

Figure 1. Proteins in lipid bilayers. (A) The Fluid Mosaic Concept of Singer and Nicolson (1972) showing "proteins" penetrating deeply into (left) or across the lipid bilayer. This concept treats the lipid bilayer primarily as a thin hydrocarbon slab. Image reprinted from Singer and Nicolson (1972) with permission from AAAS. (B) Snapshot from a molecular dynamics simulation (Benz et al., 2005) of a dioleoylphosphatidylcholine (DOPC) bilayer. It stands in stark contrast to the bilayer shown in A. The component groups represented by the ball and stick representations of the lipids are indicated by the color scale. Two of the DOPC molecules are shown in CPK format. This image reveals that the headgroup region of the bilayer accounts for a significant fraction of the total thermal thickness of the bilayer, emphasizing the likely importance of the headgroups in lipid–protein interactions. (C) Snapshot of a molecular dynamics simulation of a model KvAP S4 helix in a POPC bilayer (Freites et al., 2005). The S4 helix has the same sequence used in the translocon-assisted insertion of S4 across the ER membrane (Hessa et al., 2005b): GGPG-LGLFRLVRLL-RFLRILLII-GPGG. Color code: red, water; yellow, phosphates; green, alkyl chains; gray, S4 hydrophobic residues; white, GGPG– and –GPGG flanks; blue, arginines. The S4 peptide is stabilized by phosphates acting as counter-ions and a hydrogen-bonded network of waters that penetrate into the alkyl chain region. The bilayer is highly distorted in the vicinity of S4, due to the flexibility and adaptability of the phospholipid molecules. (D) Snapshot from a molecular dynamics simulation of the SecY heterotrimer (Van den Berg et al., 2004) embedded in a POPC bilayer (White and von Heijne, 2005). This image, from an MD trajectory provided by Dr. Alfredo Freites, shows that the SecY heterotrimer (translocon) allows polypeptide chains passing through its pore to sample the water/bilayer/protein environment, suggesting that the movement of the polypeptide into the bilayer is equivalent to a partitioning event. Color code: blue-gray, water; red, lipid headgroups; white, acyl chains; red/white CPK representations, waters in translocon "hourglass;" pink, hydrophobic residues forming the so-called isoleucine ring that separates the upper and lower halves of the hourglass. The images in B and C were created using Visual Molecular Dynamics (VMD) (Humphrey et al., 1996).

into nonpolar phases. Among the most influential of these were those of Wolfenden and colleagues, directed toward establishing biologically relevant hydrophobicity scales (Wolfenden and Lewis, 1976; Wolfenden et al., 1981; Radzicka and Wolfenden, 1988).

These classic papers and a critical analysis of the energetics of charge insertion into bilayers (Parsegian, 1969) led to a strong focus on the solvent properties of the bilayer hydrocarbon interior. Although useful for many purposes, the properties of the hydrocarbon interior alone could not readily accommodate the suggestion of MacKinnon and colleagues that the charge-bearing voltage-sensing paddle of voltage-gated K^+ channels might be exposed directly to the lipid bilayer (Jiang et al., 2003a,b). Nor could these properties accommodate our observation that the arginine-rich KvAP S4 helix of the voltage sensor can be inserted across the ER membrane with high efficiency (Hessa et al., 2005b).

Lipid Bilayer Structure

The publication of the Fluid Mosaic Model (Singer and Nicolson, 1972) gave impetus for many years to extensive physical studies of lipid bilayers, summarized authoritatively by Small (1986). The first steps toward detailed and quantitatively useful bilayer structural models began with the advent of neutron diffraction methods for studying the transbilayer distribution of lipid component groups (Büldt et al., 1978; Büldt et al.,

1979; Zaccai et al., 1979) and MD simulation methods for visualizing fluid-bilayer dynamics (van der Ploeg and Berendsen, 1982, 1983; Pastor et al., 1991; Heller et al., 1993). Wiener and White (1992) built upon the early successes of neutron diffraction by developing a method for the joint refinement of x-ray and neutron diffraction data that yielded quantitatively useful structural models of a fluid bilayer. These models consisted of time-averaged transbilayer distributions of all the lipid structural components. The most important conclusions were that (a) the hydrated headgroups of fluid lipid bilayers account for 50% of the bilayer thickness and (b) the headgroup/hydrocarbon boundary is a region of "tumultuous chemical heterogeneity because of the thermal motion of the bilayer" (Wiener and White, 1992).

The shortcoming of diffraction-based models of fluid bilayers is that they are literally one dimensional; the transbilayer component-group distributions are time-averaged projections of the unit cell contents onto an axis normal to the membrane plane. This is a consequence of lamellar (one-dimensional) diffraction from oriented arrays of lipid bilayers (Franks and Levine, 1981). It is now possible to use these one-dimensional data to arrive at experimentally validated MD simulations that provide accurate three-dimensional, dynamic structures (Benz et al., 2005, 2006; Kucerka et al., 2005; Klauda et al., 2006). An image of a single frame of an MD simulation of the dioleoylphosphatidylcholine (DOPC) bilayer studied by Wiener and White (1992) is shown in Fig. 1 B. The high thermal disorder and the extent of the headgroup regions are apparent in this image. A reasonable a priori conclusion is that the headgroup interactions with MPs are likely to be significant. Indeed, it is now clear that headgroup phosphates are essential for ion channel gating, presumably through arginine–phosphate interactions in the case of KvAP (Schmidt et al., 2006).

The modern view of bilayer structure, exemplified by Fig. 1 B, provides a basis for understanding an intriguing discovery from our study of the recognition of TM helices by the ER translocon (Hessa et al., 2005a); ΔG_{app} for some residues, notably charged or aromatic ones, depended strongly on position within the TM helix, becoming more favorable near helix ends. This is reminiscent of the statistical preferences of aromatic and charged residues in membrane proteins for the membrane headgroup region (Schiffer et al., 1992; Wallin et al., 1997; Seshadri et al., 1998; Killian and von Heijne, 2000; Chamberlain et al., 2004; Ulmschneider et al., 2005). The position dependence of ΔG_{app} suggested that the translocon allows TM helices to enter the bilayer in a manner that optimizes the positioning of the helices to account for the thermodynamic preferences of hydrophobic residues for nonpolar phases, aromatic residues for the bilayer interface (Yau et al., 1998), and charged residues for "snorkeling" (Segrest et al., 1974) into the zwitterionic lipid headgroups. The simplest interpretation of our results was that elongating polypeptide segments passing through the translocon are selected for diversion into the lipid bilayer by thermodynamic partitioning between the translocon channel and the lipid bilayer, as proposed by Heinrich et al. (2000). This idea and the structure of fluid lipid bilayers help explain the surprising observation that the ER translocon can insert the KvAP S4 helix and its four arginines across the ER membrane with high efficiency.

Charge-bearing Transmembrane Helices in Lipid Bilayers

To gain insights into the ability of the translocon to insert the KvAP S4 voltage-sensor helix across the ER membrane (Hessa et al., 2005b), Freites et al. (2005) performed an all-atom molecular dynamics simulation of an S4 helix across a POPC bilayer. Assuming that S4 was stable across the membrane during the simulation (it was), the objective was to understand how the lipid bilayer could accommodate S4 in a transmembrane configuration. The simulation did not address the question of thermodynamic equilibrium between the inserted and noninserted states for two reasons: the tens-of-nanosecond time scale of all-atom simulations are impractical for this purpose and the structure and location of the noninserted population of S4 peptides in the Hessa et al. (2005b) experiment is unknown, although it might be surface bound, as observed in model membrane systems (Halsall and Dempsey, 1999; Mattila et al., 1999). Indeed, a recent coarse-grained simulation of the assembly of Kv sensors suggests an equilibrium between surface-bound and transmembrane states with a $\Delta G_{app} \approx 0$, as in the Hessa et al. (2005b) experiment.

Fig. 1 C shows one frame from the Freites et al. (2005) simulation. This simulation disclosed that the four arginines of S4 could be stabilized deep within the hydrocarbon core, because the lipid phosphate groups act as counter-ions for the arginines. In addition, the lipids apparently rearrange around S4 to optimize hydration of the arginines through hydrogen-bonded chains of water that dip into the hydrocarbon core. Concerns about the physical reality of the MD simulation in this dramatic case are quieted by direct physical measurements, which show that the S4 helix can be stably inserted across oriented lipid bilayers formed from simple mixtures of a synthetic S4 peptide with phospholipids (Fernández-Vidal, M., F. Castro-Román, and S.H. White. 2006. *Biophys. J.* 90:241a; Castro-Román, F., M. Fernandez-Vidal, M. Mihailescu, and S.H. White. 2007. *Biophys. J.* 92:294a).

Of course, the interactions of the entire sensor (helices S1–S4) with phospholipids are more complex than those of S4 alone, as shown in a recent MD simulations of the KvAP sensor in lipid bilayers (Freites et al., 2006;

Bond and Sansom, 2007). But phosphate–arginine interactions were still found to be important for the stabilization of the sensor. Are these interactions peculiar to arginine? When S4 arginines are mutated to lysines one at a time (Aggarwal and MacKinnon, 1996) or even to MTS-trimethylammonium reagents (Ahern and Horn, 2005), K^+-channel gating works just fine. It is not known if all four arginines can be replaced simultaneously by arginines, although a recent report indicates that voltage gating is not entirely lost when three of four arginines in the voltage sensor of *shaker* are replaced by glutamines (Voets, T., A. Janssens, and B. Nilius. 2007. *Biophys. J.* 92:125a). In any case, the insertion of the charge-bearing TM helices in the studies by Hessa et al. (2005a,b) can be reasonably explained by a combination of charged-residue snorkeling and local lipid rearrangements in the immediate vicinity of the charge-bearing helices.

Peptide Partitioning into *n*-Octanol

When the complexity of the lipid bilayer and the possibility of headgroup interactions with polar and charged amino acids are taken into account, the relevance of hydrophobicity scales based upon partitioning of amino acid side chains into purely nonpolar phases such as cyclohexane becomes doubtful. The presence of a modest polar group in the "nonpolar" phase, such as the hydroxyl of *n*-octanol, can profoundly affect partitioning free energies. For example, the free energy of transfer of arginine from water to octanol is only $\sim$2 kcal mol^{-1} (Wimley et al., 1996) rather than the value of $\sim$15 kcal mol^{-1} observed for water-to-cyclohexane transfer (Radzicka and Wolfenden, 1988). It is significant that the Wimley-White octanol-based whole-residue hydrophobicity scale (Wimley et al., 1996) provides very accurate detection of TM helices, including those carrying charged residues, in hydrophobicity plots of MPs (Jayasinghe et al., 2001). There are two likely reasons for this success. First, the Wimley-White scale is a whole-residue scale, because it includes the energetic cost of partitioning H-bonded peptide bonds. Second, water-saturated *n*-octanol has a micellar structure that allows the octanol and water molecules to adapt readily to both polar and nonpolar molecular groups as necessary to minimize the free energy of the system (Franks et al., 1993). The phospholipids in a bilayer in excess water seem to have a molecular adaptability similar to octanol in water-saturated octanol solutions. But there is a major difference: cell membrane phospholipids are thermodynamically constrained to a bilayer configuration that provides, in simple terms, a transmembrane "polarity gradient" that is not feasible in an octanol micelle. This gradient underlies the preference of aromatic and charged residues for interfacial positions in TM helices and provides a basis for understanding the position dependence of the ΔG_{app} values for these residues.

If hydrated octanol cannot geometrically mimic the polarity gradient of bilayers, how can hydrophobicity values derived from octanol–water partitioning predict TM helices with considerable accuracy? One can only speculate about the answer to this question. A simple explanation may be a convergence of physical chemistry and protein evolution. One can suppose that octanol's molecular adaptability allows optimization of a residue's local environment that yields a free energy that is similar, perhaps by coincidence, to the optimal local environment of the residue in a lipid bilayer. For aromatic and charged residues, the optimal local environment is the membrane interface. If TM helices have evolved to optimize the local environment of each residue using position dependence as a tool, then the average partitioning free energy of a helix reported by octanol could match the partitioning free energy of a helix computed from the biological scale with position dependence taken into account.

The Translocon as a Thermodynamic Switch

These considerations suggest how bulk-phase octanol measurements could provide a useful hydrophobicity scale for membrane proteins in lipid bilayers, and why the biological (Hessa et al., 2005a) and the octanol scales (White and Wimley, 1999) are similar. Taken at face value, the close correlation between the scales implies that translocon-assisted insertion involves the equivalent of a partitioning measurement. Indeed, Rapoport and colleagues proposed several years ago that movement of polypeptide segments out of the translocon involved partitioning between translocon and bilayer (Heinrich et al., 2000). That this is a real possibility becomes evident from MD simulations of the SecY heterotrimer (Van den Berg et al., 2004) embedded in a lipid bilayer (White and von Heijne, 2005; Gumbart and Schulten, 2006; Haider et al., 2006). Fig. 1 D shows a snapshot from one such simulation.

The crystallographic structure of Van den Berg et al. (2004) revealed that (a) the translocon tunnel is hourglass shaped and probably filled with water and (b) polypeptides traversing a translocon could exit into the bilayer only between the so-called gate helices (TM2B and TM7, Fig. 1 D). A close look at this region in the simulations reveals, as surmised from the x-ray structure, that the tunnel is filled with hundreds of water molecules. The top and bottom halves of the hourglass are separated by a ring of hydrophobic side chains (mostly isoleucine) through which the elongating peptide must pass. Just beyond the TM2B and TM7 helices are the phospholipids, which in the MD simulations are pounding at the gate. The translocon aqueous pore and its guardian gate helices are geometrically well suited for allowing a translocating chain to sample, through thermal fluctuations, both the aqueous environment of the translocon pore and the heterogenous environment

of the lipid bilayer. Because polypeptide chains elongate (Goder and Spiess, 2003) at a rate of about five residues per second, much slower than the thermal fluctuations of the bilayer and translocon, there is likely to be ample time for partitioning events to occur. Given the intimate spatial relationship between translocon and bilayer, one can readily imagine how the translocon can "set up" a transiting KvAP S4 segment for release into the bilayer.

The translocon thus seems well adapted to the task of optimizing the positioning of TM helices across ER membranes through a partitioning process at the protein/water/lipid interface provided by the translocon. Many billions of polypeptide chains pass through the ER translocons during the expression of a single Lep construct in the Hessa et al. (2005a) experiments, which means that the probability of diverting a segment into the bilayer is accurately defined by the experiment. The insertion efficiency, represented by ΔG_{app}, measures this probability. From this point of view, the translocon acts as a thermodynamic switch in the sense that diversion of a polypeptide segment from the water-filled translocon channel into the bilayer is dictated by partitioning probabilities of the sort one measures in a bulk-phase partitioning experiment.

TM Helix Recognition by the Translocon and Water-to-Bilayer Partitioning

The recognition of TM helices by the translocon is most simply explained as a partitioning process between the translocon and the membrane bilayer. But is this partitioning quantitatively equivalent to direct water-to-bilayer partitioning? If it is, then the ΔG_{app} values will equal the free energy values obtained from direct water-to-bilayer partitioning experiments or simulations. The answer hinges in part on the equivalence of the water-filled translocon tunnel to bulk water. Considering the structural and chemical complexity of the translocon–bilayer complex (Fig. 1 D), complete equivalence seems unlikely. Another issue is timing. Cheng and Gilmore (2006) have found for *Saccharomyces cerevisiae* that elongation of nascent polypeptides occurs more rapidly than translocon gating, which raises the possibility of significant aqueous exposure of TM segments before membrane insertion by the translocon. The free energy difference between a chain in the translocon and in the water-exposed state becomes a critical issue in such a case.

Yet another issue is the environment of TM helices within the membrane. Is it equivalent to that of a pure bilayer? If, for example, the emergent TM helices interacted with other TM proteins, then they could not be considered to be in a pure bilayer environment. We recently examined the possibility that the engineered H-helix in leader peptidase (Lep) used for the determination of ΔG_{app} might interact strongly with the native H1 and H2 helices of Lep (Meindl-Beinker et al., 2006). To study this question, we replaced H2 with constructs (H2') whose amino acid sequences could be modified to include residues with strong hydrogen-bonding potential (Asp or Asn). Significant effects on the insertion of H were observed only when there was the possibility of two interhelix (H2'⇔H) Asn–Asn or Asp–Asp interactions. But even in these cases only a moderate effect on ΔG_{app} was observed. These results suggest that changes in local environment due to helix–helix interactions are not of first-order concern in comparisons of biological and physical hydrophobicity scales.

Perspective

An important feature of fluid lipid bilayers is the two interfacial regions that together account for about 50% of the total thermal thickness of the bilayer. The ability of the bilayer to accommodate the KvAP S4 segment as a transmembrane helix attests to the importance the interfacial headgroups in lipid interactions with membrane proteins. Translocons embedded in bilayers seem well adapted to the task of inserting TM segments across the bilayer in a manner that optimizes side chain interactions with the hydrocarbon core and interfaces. They seem to behave as thermodynamic switches that divert nascent polypeptides into the bilayer by a thermodynamic partitioning process. The probability of diversion depends on the free energy of the nascent chain in the bilayer relative to the free energy in the translocon. Because the translocon channel is water filled, the membrane insertion free energy must be closely related to the free energy of helix insertion that could be determined by direct water-to-bilayer partitioning. This is apparent from the close correlation of physical hydrophobicity scales to the biological scale described by ΔG_{app}. However, because of the complex and currently ill-defined environment of the translocon channel, it is not clear that ΔG_{app} will be exactly equal in all cases to free energies determined from direct water-to-bilayer partitioning experiments or simulations. The thermodynamic comparison may be further complicated by the fact that translocon gating (switching) may be slower than nascent chain elongation. Given these complications, the close correlation between the Wimley-White octanol scale and the biological scale is intriguing. One possibility is that octanol's molecular adaptability allows optimization of a residue's local environment that is similar to the optimal local environment of the residue at a specific position in a lipid bilayer. If TM helices have evolved to optimize the local environment of each residue through their positions in the helix, then the average partitioning free energy of a helix reported by octanol would be similar to the partitioning free energy of a helix computed from the biological scale with position dependence taken into account.

I thank Michael Myers for editorial assistance and the TEMPO group for stimulating discussions.

This work was carried out through the support of research grants from the National Institute for General Medical Sciences and the NIH National Center for Research Resources.

REFERENCES

Aggarwal, S.K., and R. MacKinnon. 1996. Contribution of the S4 segment to gating charge in the *Shaker* K^+ channel. *Neuron.* 16:1169–1177.

Ahern, C.A., and R. Horn. 2005. Focused electric field across the voltage sensor of potassium channels. *Neuron.* 48:25–29.

Benz, R.W., F. Castro-Román, D.J. Tobias, and S.H. White. 2005. Experimental validation of molecular dynamics simulations of lipid bilayers: a new approach. *Biophys. J.* 88:805–817.

Benz, R.W., H. Nanda, F. Castro-Román, S.H. White, and D.J. Tobias. 2006. Diffraction-based density restraints for membrane and membrane/protein molecular dynamics simulations. *Biophys. J.* 91:3617–3629.

Bond, P.J., and M.S. Sansom. 2007. Bilayer deformation by the Kv channel voltage sensor domain revealed by self-assembly simulations. *Proc. Natl. Acad. Sci. USA.* 104:2631–2636.

Büldt, G., H.U. Gally, A. Seelig, J. Seelig, and G. Zaccai. 1978. Neutron diffraction studies on selectively deuterated phospholipid bilayers. *Nature.* 271:182–184.

Büldt, G., H.U. Gally, J. Seelig, and G. Zaccai. 1979. Neutron diffraction studies on phosphatidylcholine model membranes. I. Head group conformation. *J. Mol. Biol.* 134:673–691.

Chamberlain, A.K., Y. Lee, S. Kim, and J.U. Bowie. 2004. Snorkeling preferences foster an amino acid composition bias in transmembrane helices. *J. Mol. Biol.* 339:471–479.

Cheng, Z., and R. Gilmore. 2006. Slow translocon gating causes cytosolic exposure of transmembrane and lumenal domains during membrane protein integration. *Nat. Struct. Mol. Biol.* 13:930–936.

Franks, N.P., M.H. Abraham, and W.R. Lieb. 1993. Molecular organization of liquid *n*-octanol: an x-ray diffraction analysis. *J. Pharm. Sci.* 82:466–470.

Franks, N.P., and Y.K. Levine. 1981. Low-angle X-ray diffraction. *In* Membrane Spectroscopy. E. Grell, editor. Springer-Verlag, Berlin. 437–487.

Freites, J.A., D.J. Tobias, G. von Heijne, and S.H. White. 2005. Interface connections of a transmembrane voltage sensor. *Proc. Natl. Acad. Sci. USA.* 102:15059–15064.

Freites, J.A., D.J. Tobias, and S.H. White. 2006. A voltage-sensor water pore. *Biophys. J.* 91:L90–L92.

Goder, V., and M. Spiess. 2003. Molecular mechanism of signal-sequence orientation in the endoplasmic reticulum. *EMBO J.* 22:3645–3653.

Gorter, E., and F. Grendel. 1925. On bimolecular layers of lipoids on the chromocytes of the blood. *J. Exp. Med.* 41:439–443.

Gumbart, J., and K. Schulten. 2006. Molecular dynamics studies of the archaeal translocon. *Biophys. J.* 90:2356–2367.

Haider, S., B.A. Hall, and M.S.P. Sansom. 2006. Simulations of a protein translocation pore: SecY. *Biochemistry.* 45:13018–13024.

Halsall, A., and C.E. Dempsey. 1999. Intrinsic helical propensities and stable secondary structure in a membrane-bound fragment (S4) of the Shaker potassium channel. *J. Mol. Biol.* 293:901–915.

Heinrich, S.U., W. Mothes, J. Brunner, and T.A. Rapoport. 2000. The Sec61p complex mediates the integration of a membrane protein by allowing lipid partitioning of the transmembrane domain. *Cell.* 102:233–244.

Heller, H., M. Schaefer, and K. Schulten. 1993. Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid-crystal phases. *J. Physiol. Chem.* 97:8343–8360.

Hessa, T., H. Kim, K. Bihlmaier, C. Lundin, J. Boekel, H. Andersson, I.M. Nilsson, S.H. White, and G. von Heijne. 2005a. Recognition of transmembrane helices by the endoplasmic reticulum translocon. *Nature.* 433:377–381.

Hessa, T., S.H. White, and G. von Heijne. 2005b. Membrane insertion of a potassium channel voltage sensor. *Science.* 307:1427.

Humphrey, W., W. Dalke, and K. Schulten. 1996. VMD: visual molecular dynamics. *J. Mol. Graph.* 14:33–38.

Jayasinghe, S., K. Hristova, and S.H. White. 2001. Energetics, stability, and prediction of transmembrane helices. *J. Mol. Biol.* 312:927–934.

Jiang, Y., A. Lee, J.Y. Chen, V. Ruta, M. Cadene, B.T. Chait, and R. MacKinnon. 2003a. X-ray structure of a voltage-dependent K^+ channel. *Nature.* 423:33–41.

Jiang, Y., V. Ruta, J.Y. Chen, A. Lee, and R. MacKinnon. 2003b. The principle of gating charge movement in a voltage-dependent K^+ channel. *Nature.* 423:42–48.

Kauzmann, W. 1959. Some factors in the interpretation of protein denaturation. *Adv. Protein Chem.* 14:1–63.

Killian, J.A., and G. von Heijne. 2000. How proteins adapt to a membrane-water interface. *Trends Biochem. Sci.* 25:429–434.

Klauda, J.B., N. Kucerka, B.R. Brooks, R.W. Pastor, and J.F. Nagle. 2006. Simulation-based methods for interpreting x-ray data from lipid bilayers. *Biophys. J.* 90:2796–2807.

Kucerka, N., Y.F. Liu, N. Chu, H.I. Petrache, S. Tristram-Nagle, and J.F. Nagle. 2005. Structure of fully hydrated fluid phase DMPC and DLPC lipid bilayers using x-ray scattering from oriented multilamellar arrays and from unilamellar vesicles. *Biophys. J.* 88:2626–2637.

Lenard, J., and S.J. Singer. 1966. Protein conformation in cell membrane preparations as studied by optical rotatory dispersion and circular dichroism. *Proc. Natl. Acad. Sci. USA.* 56:1828–1835.

Mattila, K., R. Kinder, and B. Bechinger. 1999. The alignment of a voltage-sensing peptide in dodecylphosphocholine micelles and in oriented lipid bilayers by nuclear magnetic resonance and molecular modeling. *Biophys. J.* 77:2102–2113.

Meindl-Beinker, N.M., C. Lundin, I. Nilsson, S.H. White, and G. Von Heijne. 2006. Asn- and Asp-mediated interactions between transmembrane helices during translocon-mediated membrane protein assembly. *EMBO Rep.* 7:1111–1116.

Nozaki, Y., and C. Tanford. 1971. The solubility of amino acids and two glycine peptides in aqueous ethanol and dioxane solutions. Establishment of a hydrophobicity scale. *J. Biol. Chem.* 246:2211–2217.

Overton, E. 1895. On the osmotic properties of living plants and animals. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich.* 40:159–201.

Parsegian, A. 1969. Energy of an ion crossing a low dielectric membrane: solutions to four relevant electrostatic problems. *Nature.* 221:844–846.

Pastor, R.W., R.M. Venable, and M. Karplus. 1991. Model for the structure of the lipid bilayer. *Proc. Natl. Acad. Sci. USA.* 88:892–896.

Radzicka, A., and R. Wolfenden. 1988. Comparing the polarities of the amino acids: side-chain distribution coefficients between the vapor phase, cyclohexane, 1-octanol, and neutral aqueous solution. *Biochemistry.* 27:1664–1670.

Schiffer, M., C.H. Chang, and F.J. Stevens. 1992. The functions of tryptophan residues in membrane proteins. *Protein Eng.* 5:213–214.

Schmidt, D., Q.-X. Jiang, and R. MacKinnon. 2006. Phospholipids and the origin of cationic gating charges in voltage sensors. *Nature.* 444:775–779.

Segrest, J.P., and R.J. Feldman. 1974. Membrane proteins: amino acid sequence and membrane penetration. *J. Mol. Biol.* 87:853–858.

Segrest, J.P., R.L. Jackson, J.D. Morrisett, and A.M. Gotto Jr. 1974. A molecular theory for lipid-protein interactions in the plasma lipoproteins. *FEBS Lett.* 38:247–253.

Seshadri, K., R. Garemyr, E. Wallin, G. von Heijne, and A. Elofsson. 1998. Architecture of β-barrel membrane proteins: analysis of trimeric porins. *Protein Sci.* 7:2026–2032.

Singer, S.J. 1971. The molecular organization of biological membranes. In Structure and Function of Biological Membranes. L.I. Rothfield, editor. Academic Press, New York. 145–222.

Singer, S.J., and G.L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science.* 175:720–731.

Small, D.M. 1986. The Physical Chemistry of Lipids. Plenum Press, New York. 672 pp.

Tanford, C. 1973. The Hydrophobic Effect: Formation of Micelles and Biological Membranes. Volume 1. John Wiley & Sons, New York. 200 pp.

Ulmschneider, M.B., M.S.P. Sansom, and A. Di Nola. 2005. Properties of integral membrane protein structures: derivation of an implicit membrane potential. *Proteins.* 59:252–265.

Van den Berg, B., W.M. Clemons Jr., I. Collinson, Y. Modis, E. Hartmann, S.C. Harrison, and T.A. Rapoport. 2004. X-ray structure of a protein-conducting channel. *Nature.* 427:36–44.

van der Ploeg, P., and H.J.C. Berendsen. 1982. Molecular dynamics simulation of a bilayer membrane. *J. Chem. Physiol.* 76:3271–3276.

van der Ploeg, P., and H.J.C. Berendsen. 1983. Molecular dynamics of a bilayer membrane. *Mol. Physiol.* 49:233–248.

von Heijne, G. 2006. Membrane-protein topology. *Nat. Rev. Mol. Cell Biol.* 7:909–918.

Voss, N.R., M. Gerstein, T.A. Steitz, and P.B. Moore. 2006. The geometry of the ribosomal polypeptide exit tunnel. *J. Mol. Biol.* 360:893–906.

Wallin, E., T. Tsukihara, S. Yoshikawa, G. von Heijne, and A. Elofsson. 1997. Architecture of helix bundle membrane proteins: an analysis of cytochrome *c* oxidase from bovine mitochondria. *Protein Sci.* 6:808–815.

White, S.H., and G. von Heijne. 2004. The machinery of membrane protein assembly. *Curr. Opin. Struct. Biol.* 14:397–404.

White, S.H., and G. von Heijne. 2005. Transmembrane helices before, during, and after insertion. *Curr. Opin. Struct. Biol.* 15:378–386.

White, S.H., and W.C. Wimley. 1999. Membrane protein folding and stability: physical principles. *Annu. Rev. Biophys. Biomol. Struct.* 28:319–365.

Wiener, M.C., and S.H. White. 1992. Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of x-ray and neutron diffraction data. III. Complete structure. *Biophys. J.* 61:434–447.

Wimley, W.C., T.P. Creamer, and S.H. White. 1996. Solvation energies of amino acid sidechains and backbone in a family of host-guest pentapeptides. *Biochemistry.* 35:5109–5124.

Wolfenden, R., L. Andersson, P.M. Cullis, and C.C.B. Southgate. 1981. Affinities of amino acid side chains for solvent water. *Biochemistry.* 20:849–855.

Wolfenden, R., and C.A. Lewis Jr. 1976. A vapor phase analysis of the hydrophobic effect. *J. Theor. Biol.* 59:231–235.

Yau, W.-M., W.C. Wimley, K. Gawrisch, and S.H. White. 1998. The preference of tryptophan for membrane interfaces. *Biochemistry.* 37:14713–14718.

Zaccai, G., G. Büldt, A. Seelig, and J. Seelig. 1979. Neutron diffraction studies on phosphatidylcholine model membranes. II. Chain conformation and segmental disorder. *J. Mol. Biol.* 134:693–706.