

Perspectives on Membrane Protein Insertion, Protein–Bilayer Interactions, and Amino Acid Side Hydrophobicity

The purpose of the Perspectives in General Physiology is to provide a forum where scientific uncertainties or controversies can be discussed in an authoritative, yet open manner.

The Perspectives are solicited by the editors, often based on recommendations by members of the editorial advisory board. To frame the issue, two or more experts are invited to present brief points of view on the problem, which are published consecutively in the Journal. The comments and opinions expressed in the Perspectives are those of the authors and not necessarily those of the Editors or the Editorial Advisory Board. The Perspectives are accompanied by a few editorial paragraphs that introduce the problem—and invite the submission of comments, in the form of letters to the editor, which are published in a single, predetermined issue (usually three months after publication of the Perspective). After the letters to the editor have been published, further responses are limited to full manuscripts.

In this issue of the Journal, Gunnar von Heijne (Stockholm University, Stockholm, Sweden), Richard Wolfenden (University of North Carolina, Chapel Hill, NC), Stephen H. White (University of California at Irvine, CA), and Justin L. MacCallum, W.F. Drew Bennett and D. Peter Tieleman (University of Calgary, Calgary, Canada) provide different perspectives on the membrane insertion of bilayer-spanning α helices, protein–bilayer interactions, and the role of amino acid side chain hydrophobicity.

The importance of amino acid side chain hydrophobicity (and solvent water) for protein folding (Kauzmann, W. 1959. *Adv. Protein Chem.* 14:1–63) and membrane insertion (Kyte, J., and R.F. Doolittle. 1982. *J. Mol. Biol.* 157:105–132) was appreciated long ago, but it has proven difficult to establish a consensus hydrophobicity scale, as evidenced by the many scales that have been proposed (e.g., Cornette, J.L., K.B. Cease, H. Margalit, J.L. Spouge, J.A. Berzofsky, and C. DeLisi. 1987. *J. Mol. Biol.* 195:659–685). This may not be too surprising; hydrophobicity scales appropriate for globular proteins, for example, could differ from hydrophobicity scales appropriate for bilayer-spanning transmembrane (TM) proteins. The difficulties persist, however, even in the case of TM segments, where different hydrophobicity scales differ in their ability to predict TM segments (Zhao, G., and E. London. 2006. *Protein Sci.* 15:1987–2001). Is the notion of a single hydrophobicity scale simply untenable?

The differences among hydrophobicity scales pertain primarily to the aromatic amino acid residues, especially tryptophan and tyrosine, and the charged

amino acid residues. The former tend to localize near the bilayer/solution interface, where the tryptophan NH and tyrosine OH can form hydrogen bonds with polar groups, including water, at the interface. The latter were long considered to be excluded from being in direct contact with the bilayer hydrophobic core. This dogma was challenged by the determination of the KvAP potassium channel structure (Jiang, Y., A. Lee, J. Chen, V. Ruta, M. Cadene, B.T. Chait, and R. MacKinnon. 2003. *Nature.* 423:33–41) and biotin-avidin trapping experiments (Jiang, Y., V. Ruta, J. Chen, A. Lee, and R. MacKinnon. 2003. *Nature.* 423:42–48), which together led to the suggestion that the arginine-rich S4 segment might be at the channel/bilayer interface, an arrangement that prompted a reevaluation of the prevailing orthodoxy and its origins. In trying to shed some light on this issue, we have at our disposal the (incomplete) information from thermodynamic partition experiments of amino acids between different solvents, empirical hydrophobicity scales designed to predict TM helices, biological experiments on protein translation, and atomistic computer simulations. The available information is somewhat disparate, which complicate matters further.

As a preamble, studies on the lipid bilayer permeability to small nonelectrolytes long ago established that the permeability coefficients conform to predictions based on the solubility-diffusion mechanism, in which the permeability coefficients scale with the solutes' partition coefficient between water and some appropriate nonpolar solute (Orbach, E., and A. Finkelstein. 1980. *J. Gen. Physiol.* 75:427–436; Walter, A., and J. Gutknecht. 1986. *J. Membrane Biol.* 90:207–217). The choice of nonpolar solute is, in part, a matter of taste (olive oil tastes better than octanol; Finkelstein, A., personal communication). To a first approximation, the solubility-diffusion mechanism also accounts for the bilayer's impermeability to small charged solutes, even though electrostatic interactions between a bilayer-embedded charge and the adjacent aqueous solutions would cause the charge to be attracted to the bilayer/solution interface and maybe deform the liquid-crystalline bilayer (Parsegian, A. 1969. *Nature.* 221:844–846). Not only would the bilayer/solution interface undergo thermal fluctuations, the magnitude of the fluctuations could be modulated by bilayer-embedded charges, including charges on bilayer-spanning proteins. Moreover, even if C_{α} of a charged amino acid were “buried” in the bilayer, the charged group itself might “snorkel” to the surface to be (partially) solvated by polar groups (Segrest, J.P., H. De Loof,

J.G. Dohlman, C.G. Brouillette, and G.M. Anantharamaiah. 1990. *Proteins*. 8:103–117). Thus, though the solubility-diffusion model constitutes a first-order description of the bilayer barrier properties, it is incomplete. Similarly, though we all tend to depict lipid bilayers as (mathematically) plane sheets and bilayer-spanning α helices as smooth cylinders with well-defined amino acid side chain placements, the limitations in this description for understanding more complex questions were established, if not always fully appreciated, long ago.

To further complicate matters, integral membrane proteins are inserted into the lipid bilayer by a cotranslational process in which the nascent peptide strand is threaded through the translocon in the ER (or bacterial plasma) membrane for then to fold into an α -helix and, eventually, exit laterally to the bilayer to become a bilayer-spanning segment. Key “decisions” in this process may take place in an environment with physico-chemical properties very different from those of the bilayer hydrophobic core. It therefore is not obvious whether the energetic considerations pertaining to the biosynthetic bilayer insertion would be identical to the energetic considerations pertaining to membrane protein conformational changes or other posttranslational/insertion events. In this series of Perspectives, Gunnar

von Heijne describes an elegant approach to determine a “biological” hydrophobicity scale that underlies the events in the translocon. Then Richard Wolfenden and Stephen H. White each summarize different approaches to establish “physico-chemical” hydrophobicity scales. Finally, D. Peter Tieleman and colleagues summarize insights derived from computer simulations on amino acid side chain analogues.

Letters to the editor related to these Perspectives will be published in the August 2007 issue of the *Journal of General Physiology*. Letters to the editor should be received no later than Monday, June 25, 2007, in order to allow for editorial review. The letters may be no longer than two printed pages (approximately six double-spaced pages) and will be subject to editorial review. They may contain no more than one figure, no more than 15 references, and no significant references to unpublished work. Letters should be prepared according to the Journal’s instructions and can be submitted electronically at www.jgp.org, or as an e-mail attachment to jgp@mail.rockefeller.edu.

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