Introduction to the Membrane Protein Reviews: The Interplay of Structure, Dynamics, and Environment in Membrane Protein Function

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Abstract

In our review, we introduce an organizational scheme for membrane protein function. It is the relationship between structure, dynamics, and environment that endows the membrane and its constituents with remarkable sensitivity and robustness. Our understanding begins with landmark advances like those presented in the following chapters. Membrane proteins are notoriously difficult to study, and so the work presented here on the ADP/ATP carrier [Nury et al. (2)], rhodopsin [Palczewski (24)], and the cytochrome b_6f complex [Cramer et al. (35)] represents incredible progress in this now blossoming field.

INTRODUCTION

Membrane protein structure determination is proceeding at an exciting pace, driven by the hope that structures can connect decades of biochemical and biophysical observations to protein function and mechanism. The reviews that follow demonstrate that structures are capable of resolving long-standing mysteries while suggesting new questions and opening new areas of scientific discovery. As science as a whole unravels increasingly complex and finely tuned functionality, can structure determination continue to keep pace, or will new methodologies and perspectives be required? The ultimate test will be to build robust molecular-level models capable of predicting the functional outcome of any arbitrary structural perturbation. The creation of such models will require adopting a broad perspective, one that takes into account dynamic deviations from static structures as well as the influence of the membrane environment.

It is helpful to establish a paradigm that categorizes and organizes the relevant contributors to function. Structures should, and will, act as the nucleation points for this organization. This is because understanding mechanisms is ultimately a matter of chemistry, and one cannot invoke chemical ideas of the mechanism without knowing where the atoms are. As we detail in the discussion that follows, evolution has exploited three categories of molecular-level organization in order to achieve efficient and diverse membrane protein functions: structure, molecular dynamics, and environmental constraints. As suggested schematically in **Figure 1**, they are inextricably linked, each influencing the other, collectively dictating membrane protein function. By recasting the structure-function relationship in this way, we suggest that a comprehensive view of membrane protein function may be more readily achieved.

STRUCTURE

Any modern model for predicting function must start with structure. As demonstrated in the following reviews, static structures can elegantly illuminate the structure-function relationship. Collectively, the articles detail a series of new features only now visible in the increasingly high-resolution structures. The emergence of these elements, which include protein oligomerization and complexation, lipidation, glycosylation, and the presence of structural waters, suggests that future models may need to account for a high level of complexity and structural variability.

Oligomerization is increasingly seen as a common motif in membrane proteins (1), and dimerization in particular is thought to be the rule for each of the three proteins discussed below. As the authors point out, distinguishing between dimers and higher order oligomers is far from trivial. Although this difficulty may be primarily due to experimental limitations such as dissociation by detergent, it may also reflect true biological variability: Perhaps there is a functionally

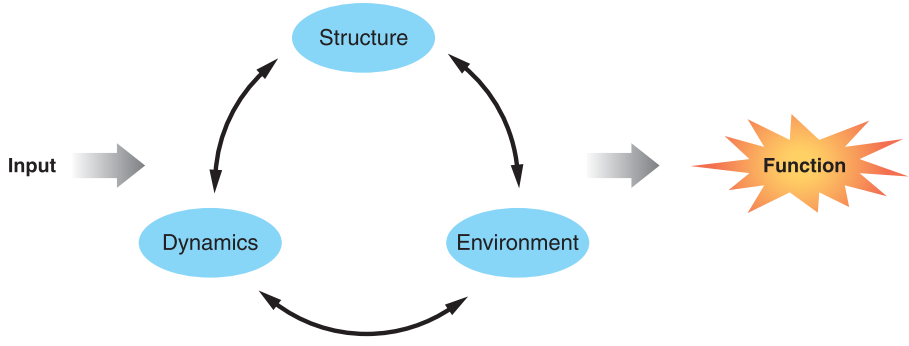

Figure 1
The three categories of molecular-level organization needed to achieve efficient and diverse membrane protein functions.

relevant equilibrium distribution of *n*-mers within cell membranes. This possibility suggests an investigation into the evolutionary origin of oligomerization. We suggest four distinct, though not exclusive, explanations. First, in most studied cases, oligomerization serves a functional role, and this function most likely drove its evolution. For example, the cytochrome b_6f dimer interface is thought to form an electron transfer bridge for "cross talk" between the two monomers. In the case of rhodopsin, dimerization is thought to regulate G-protein coupling. Second, stability of newly evolved proteins must have been critical, and oligomerization may provide an efficient way to select for stabilizing mutants. In the case of a homodimer, for example, a single mutation at the interface could be twice as efficient in stabilizing the protein compared with a single mutation in a monomer. Similarly, a third explanation is that formation of oligomeric structures can augment genetic efficiency. For example, in the several ion channel structures we now know, identical subunits surround the ion pathway, requiring the coding of only a single unit to form a larger structure. As a fourth possible explanation, we speculate that if dense packing of membrane proteins is evolutionarily important for optimizing functional output per unit area of membrane, then the evolution of oligomeric interfaces might have been a structural adaptation to support high packing density while minimizing energetically unfavorable protein-protein contacts. Evolution would then have built upon this through adaptations that functionalized the interface.

Like oligomerization, the presence of strongly bound lipids and specific sites for water molecules found in recent high-resolution structures suggests a potentially large degree of structural, and hence functional, variability. What is their role in structural stability and what is their functional significance? In the case of b_6f, structural lipids are suggested either to be stabilizers, acting as "structural struts," or functional, "imposing restraints on protein dynamics." In the case of the ADP/ATP carrier, removal of structural cardiolipin molecules, as Nury et al. (2) point out, leads to a 20% decrease in protein activity. How dynamic is the lipid binding? Should these lipids be considered as ligands or as parts of the structure? In some cases it seems that the lipid requirement is not specific, but in others it is. Imagine the impact if it is found that these interactions with lipids are a highly regulated and ubiquitous cellular phenomenon. Similar questions can and should be asked for the fascinating case of structural waters, as well as for all posttranslational modifications, including glycosylation. The potential combinatorial explosion because of variable glycosylation patterns on the surface of membrane proteins underscores our contention, as we now discuss, that the size of structure space may be staggeringly large if all variations have functional relevance.

WHY DYNAMICS?

In many cases, a protein must undergo a dynamic conformational transition between discrete structural states to carry out its function. Such transitions, for example from state A to state B, involve a change in the thermodynamic free energy of the system, $\Delta G_{A\rightarrow B}$. The ADP/ATP carrier protein located in the mitochondrial membrane, discussed by Nury et al. (2), is a good example. The authors point to the "induced transition fit" (ITF) mechanism to explain the dynamics of the protein. In this mechanism, described by Klingenberg (3), the membrane protein exists in multiple, discrete conformations, with the metabolite only binding perfectly to the highest energy state. The energy of this transition state is then utilized in triggering further conformational changes necessary for metabolite release. The total free energy change of such a process is the sum over all intermediate states:

$$\Delta G_{A\rightarrow B} = \sum_{i=1}^{N-1} \Delta G_{i\rightarrow i+1},$$

with $i = 1$ corresponding to state A, $i = N - 1$ corresponding to state B, and N commonly assumed to be a finite and reasonably small number of states.

Just how many relevant energy states exist for any given mechanism? Five such states are suggested in the case of the ITF mechanism. In the case of ion channels, models built from gating behavior suggest an even larger number (4). If one thinks in terms of a multidimensional free energy landscape for transitions in proteins, then these discrete states are found either in local energy minima (intermediates) or maxima (transition states). However, the energy wells corresponding to the minima may be quite broad, and transitions between them may follow multiple paths. Each of these paths may, in fact, be populated with an extremely large number of functionally pertinent conformational substates. Such an idea is supported by the case of calmodulin, wherein analysis of disorder in the crystal structure has suggested that the protein "may sample a quasi-continuous spectrum of conformations" (5). Similarly, a very large number of paths between any two states are theoretically possible, although the accessible number is likely diminished by environmental conditions (see below). This all suggests that the overall free energy change may also be expressed as

$$\Delta G_{A\to B} = \sum_{j=1}^{N'-1} \Delta G'_{j\to j+1} = \sum_{k=1}^{N''-1} \Delta G''_{k\to k+1} = \cdots.$$

Which path is taken determines the work required in effecting a transition and thus has important consequences for protein efficiency and function. Could path choice be a variable parameter in cellular control, for example, through concerted variations in the proteins' surroundings (i.e., the membrane)?

Clearly, given the relevance of conformational states to function, single structures can only partially describe a mechanism. The range of available energy states along a transition path may be exploited by proteins for tuning function. How do we investigate the transition path? Is it possible to predict, solely from two static structures, the relevant path between the two states? The problem is especially complicated because structural dynamics span a range of frequencies, from low (large conformational changes or domain movements, more traditionally associated with function) to high (side-chain rotameric isomerizations, which may, for example, play an important role in electron transport proteins, which are otherwise relatively immobile). There is, however, significant progress being made in the area of computational biology that portends a numerical solution to this problem, although further improvements in the computational representation of chemistry are needed. For example, molecular dynamics simulations generally provide information about high-frequency fluctuations (6), and normal mode analysis can help predict the lower frequency paths (7). More sophisticated computational methods known as path sampling have been shown to yield highly detailed transition path information for simple systems (8–12), and more recently for biomolecules (13–16).

ENVIRONMENT

As we suggested above, a high degree of complexity exists owing to structural variability and dynamic transition paths. A third factor that contributes to complexity is the membrane environment. Structure determination generally requires isolation of the protein from the remainder of the biological system. However, any proper thermodynamic analysis must include all relevant components of the system and must pay particularly close attention to boundaries where energy is exchanged. The contribution of the membrane environment therefore deserves consideration in analysis of function. We have noted the

multiplicity of available conformational transition paths, and that path choice determines the trade-off between energy spent and work done. The number of available paths, along with the paths themselves, must be altered by physical constraints placed on the protein by the membrane environment. Therefore, isolation of the protein from its native membrane provides only a partial story.

The membrane consists of, among other things, lipids and a multitude of proteins. In addition to severely biasing protein conformational states and the paths between them (17), the membrane itself is capable of storing energy through conformational flexibility of its own (18, 19). It has been clearly established that lipid composition varies profoundly in different membranes (20). Additionally, single membranes can be highly heterogeneous, as in the case of "lipid rafts," the functional manifestation of lipid domains known for decades to exist in synthetic mixtures (21–23). Sustaining such diversity, for example, through variable lipid composition, may cost the cell valuable resources, so specific functional rationales should be examined. Furthermore, biophysical measurements have established that physical properties of membranes, such as curvature elastic stress, profoundly affect the efficiency of membrane proteins. As one landmark example, a specific conformational transition in rhodopsin, the molecule discussed by Palczewki (24), is favored by the highly curved reverse hexagonal phase, rather than the standard lamellar phase (25). It seems likely that cells might take advantage of this type of specificity to tune function by genetically regulating their membrane-specific constituent lipid populations. Other significant membrane properties that are now appreciated as influencing structure and function of membrane proteins are, among others, lateral tension (26, 27), hydrophobic matching (28–32), and electrostatics (33, 34). By modifying these properties, cells have afforded themselves a highly tunable molecular environment and thus, we suggest, have gained incredibly fine control over protein function.

Molecular function underlies all of biology, from the processing of input in the simplest organisms to higher consciousness in humans. How intricate must the molecular machinery be to support such a diverse and elegant world? It is, of course, natural that we think in terms of discrete states. Primarily, it makes investigation of structure-function more tractable. Additionally, experimental techniques such as crystallization lend themselves to thinking in terms of single, or averaged, structures. The perspective we offer here poses a challenge to modern biologists given the immensity of combinatorial possibilities it suggests. It is hard to imagine how to begin to study this complexity; however, it is a challenge worth pursuing. If we seek a molecular-level explanation of phenomena as mystifying as consciousness, then our suggestion of near infinite combinatorial complexity seems less of a stretch. Clearly, the mere existence of complexity does not prove its evolutionary or functional significance. The important tasks are to digest the complexity and find the simplifications that remain true to the biology. We suggest that computational methods, along with increased experimental resolution, both spatially and temporally, should facilitate this effort. The progress reflected in the reviews that follow suggests that we are well on our way.

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LITERATURE CITED

1. Engelman DM. 2005. *Nature* 438:578–80
2. Nury AH, Dahout-Gonzalez C, Trézéguet V, Lauquin GJM, Brandolin G, Pebay-Peyroula E. 2006. *Annu. Rev. Biochem*. 75:713–41
3. Klingenberg M. 2005. *Biochemistry* 24:8563–70
4. Imredy JP, Yue DT. 1994. *Neuron* 12:1301–18
5. Wilson MA, Brunger AT. 2000. *J. Mol. Biol.* 301:1237–56
6. Crozier PS, Stevens MJ, Forrest LR, Woolf TB. 2005. *J. Mol. Biol.* 333:493–514
7. Valadie H, Lacapcre JJ, Sanejouand YH, Etchebest C. 2003. *J. Mol. Biol.* 332:657–74
8. Huber GA, Kim S. 1996. *Biophys. J.* 70:97–110
9. Pratt LR. 1986. *J. Chem. Phys*. 85:5045–48
10. Woolf TB. 1998. *Chem. Phys. Lett.* 289:433–41
11. Dellago C, Bolhuis PG, Csajka FS, Chandler D. 1998. *J. Chem. Phys.* 108:1964–77
12. Zuckerman DM, Woolf TB. 1999. *J. Chem. Phys.* 111:9475–84
13. Zuckerman DM. 2004. *J. Phys. Chem. B.* 108(16):5127–37
14. Bolhuis PG. 2005. *Biophys. J.* 88(1):50–61
15. Arora K, Schlick T. 2005 *J. Phys. Chem. B.* 109(11):5358–5367
16. Tobi D, Bahar I. 2005. *Proc. Nat. Acad. Sci.* 102(52):18908–13
17. Nanda H, Sachs JN, Petrache HI, Woolf TB. 2005. *J. Chem. Theory Comput.* 1:375–88
18. Mouritsen OG, Bloom M. 1993. *Annu. Rev. Biophys. Biomol. Struct.* 22:145–71
19. Hamill OP, Martinac B. 2001. *Physiol. Rev.* 81:685–740
20. Wenk MR, De Camilli P. 2004. *Proc. Natl. Acad. Sci. USA* 101:8262–69
21. Jain MK, White HB III. 1977. *Adv. Lipid Res.* 15:1–60
22. Klausner RD, Kleinfeld AM, Hoover RL, Karnovsky MJ. 1980. *J. Biol. Chem.* 255:1286–95
23. Simons K, van Meer G. 1988. *Biochemistry* 27:6197–202
24. Palczewki K. 2006. *Annu. Rev. Biochem*. 75:743–67
25. Brown MF. 1994. *Chem. Phys. Lipids* 73:159–80
26. Cantor RS. 1999. *Chem. Phys. Lipids* 101:45–56
27. Carrillo-Tripp M, Feller SE. 2005. *Biochemistry* 44:10164–69
28. Lee AG. 2003. *Biochim. Biophys. Acta* 1612:1–40
29. Williamson IM, Alvis SJ, East JM, Lee AG. 2002. *Biophys. J.* 83:2026–38
30. de Planque MRR, Killian JA. 2003. *Mol. Membr. Biol.* 20:271–84
31. Tieleman DP, Forrest LR, Sansom MSP, Berendsen HJC. 1998. *Biochemistry* 37:17554–61
32. Petrache HI, Zuckerman DM, Sachs JN, Killian JA, Koeppe RE, Woolf TB. 2002. *Langmuir* 18:1340–51
33. Aguilella VM, Bezrukov SM. 2001. *Eur. Biophys. J. Biophys. Lett.* 30:233–41
34. Jensen MO, Mouritsen OG. 2004. *Biochim. Biophys. Acta* 1666:205–26
35. Cramer WA, Zhang H, Yan J, Kurisu G, Smith JL. 2006. *Annu. Rev. Biochem.* 75:769–790

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