

Helical membrane proteins: diversity of functions in the context of simple architecture

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During the past year, research on helical membrane proteins has brought insights into the use of deviations from canonical α -helical conformation to support function and the further investigation of the sequestration of protein regions from the lipid bilayer to enhance these structural alternatives. Also, the structural roles of polar sidechains, the identification of motifs in helix interactions and the significance of certain topologies on a genome-wide scale have been further explored.

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Abbreviations

GpA glycophorin A
GPCR G-protein-coupled receptor
PDB Protein Data Bank

Introduction

A membrane protein is exposed to a heterogeneous environment, where the transmembrane regions are embedded in a phospholipid bilayer and the extramembrane domains are surrounded by water. The water-exposed polypeptide can adopt a diverse array of folds, whereas the physical and chemical constraints imposed by the lipid bilayer appear to restrict the structural diversity of the embedded protein domain (for recent reviews on membrane protein structure and folding, see [1–5]). All membrane protein structures solved to date show that transmembrane domains fold as either single α helices, bundles of α helices or β strands assembled in β barrels (Figure 1). In this review, we will focus on α -helical membrane proteins.

The folding of α -helical membrane proteins has been conceptualized as a two-stage process. Initially, hydrophobic polypeptide segments form independently stable transmembrane α helices across the membrane and can be regarded as domains. Subsequently, these helical domains assemble laterally to form the native protein [1,3]. Underlying this model is the postulate that one possible approach towards understanding helical membrane protein structure is to analyze the structure of individual transmembrane helices and, subsequently, the interactions and motifs that drive their association. Here, we review emerging themes regarding the structure of transmembrane α helices and their interactions. As revealed by the membrane protein structures published in the past year, deviations from the canonical α helix are relatively common and occur mostly to

fulfill certain important functional roles. Furthermore, the relevance of polar interactions between transmembrane helices is becoming clearer from structural as well as biophysical evidence. We also explore the transmembrane helix–helix packing motifs that have recently become more evident. Finally, we discuss the occurrence of predominant helical membrane protein folds, as revealed by genome-wide analysis of membrane proteins in the past year.

Deviations from the 'canonical' structure of transmembrane α helices have functional consequences

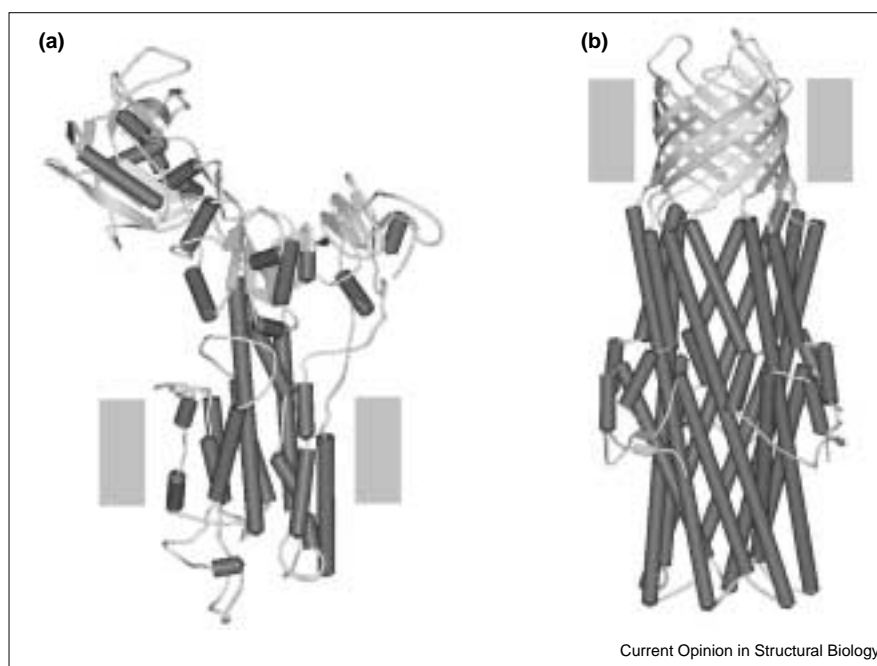
Current membrane protein topology prediction methods achieve over 75% accuracy in predicting the full topologies of α -helical membrane proteins [6,7]. Using these methods, in combination with experimental approaches, a large database of predicted transmembrane domains has been generated. Statistical analysis of these databases reveals that, on average, transmembrane helices are hydrophobic and composed of approximately 20–30 amino acids. Their central region is rich in aliphatic residues and phenylalanines, with short border regions enriched in tryptophan and tyrosine [8,9]. This view of the composition of a typical transmembrane helix was reinforced when recent three-dimensional structures of membrane proteins were analyzed [10,11]. From a structural perspective, the elucidated structures reveal that, in a typical transmembrane helix, hydrogen bonds are intrahelical, with interactions between residue i and residue $i + 4$. However, deviations in helix conformation, length and composition do occur. Recently determined structures provide interesting examples of some of these deviations.

A π -bulge, which is a deformation from a regular α -helical conformation, arises when backbone hydrogen bonds occur between residue i and residue $i + 5$ (Figure 2). In the recently determined structure of the light-driven anion pump halorhodopsin, helices E and G are distorted by π -bulges [12]. The high-resolution structure of the homologous light-driven proton pump bacteriorhodopsin also reveals a π -bulge in helix G [13•]. Another interesting type of deformation is helix unwinding, examples of which can be observed in two transmembrane helices (M4 and M6) of the calcium pump of the sarcoplasmic reticulum [14•]. Finally, proline-induced kinks are often observed in structures, examples of which are found in helices E and C of bacteriorhodopsin [13•], in helix 6 of the G-protein-coupled receptor (GPCR) rhodopsin [15••] and in one of the helices of the fumarate reductase from bacteria [16,17].

Structurally speaking, these distortions in α -helicity have at least two consequences: local conformational instability

Figure 1

Architecture of α -helical and β -barrel membrane proteins. (a) An example of the structure of an α -helical membrane protein – the calcium ATPase of skeletal muscle sarcoplasmic reticulum ([14[•]]; PDB entry code 1EUL). (b) An example of the structure of a β -barrel membrane protein – TolC from the outer membrane of *E. coli* ([59]; PDB entry code 1EK9). The membrane is schematically represented by gray blocks. Note the relatively simple architecture of the membrane-embedded domains compared with the extramembrane regions.



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due to deviations from ideal α -helical geometry; and the exposure of one or more backbone carbonyl groups (i.e. unsatisfied hydrogen-bond acceptors) arising from disruptions in the backbone hydrogen-bonding pattern of the α helix. What could be the functional consequences of local conformational instability in α helices? An attractive possibility is that they create a ‘hot-spot’ in the protein for conformational changes to occur. This possibility is supported by a plethora of new information on structural changes during the bacteriorhodopsin photocycle. In brief, the emerging picture is that the isomerization from all-*trans* to 13-*cis* retinal affects not only the sidechain conformations of Lys216 and other amino acid residues involved in retinal binding, but also backbone conformations involving the π -bulge within helix G (reviewed in [18]). Furthermore, helix C has also been shown to undergo deformations that are coupled to proton transport [19[•]]. It is reasonable to expect that, in the case of halorhodopsin, similar conformational changes will be involved in anion transport. Conformational changes involving proline hinges have also been invoked as a mechanism for signal transduction through membranes. Specifically, in GPCRs and ion channels, proline hinges are suggested to act as molecular switches in transmembrane-helix-mediated signaling (reviewed in [20[•]]).

Exposed backbone carbonyl groups, on the other hand, can participate in polar interactions. Such interactions include: hydrogen bonding to a cofactor, as in bacteriorhodopsin, in which the carbonyl participates in the hydrogen-bond network that binds the Schiff base [13[•]]; binding of an ion, as shown in the calcium pump of the sarcoplasmic reticulum,

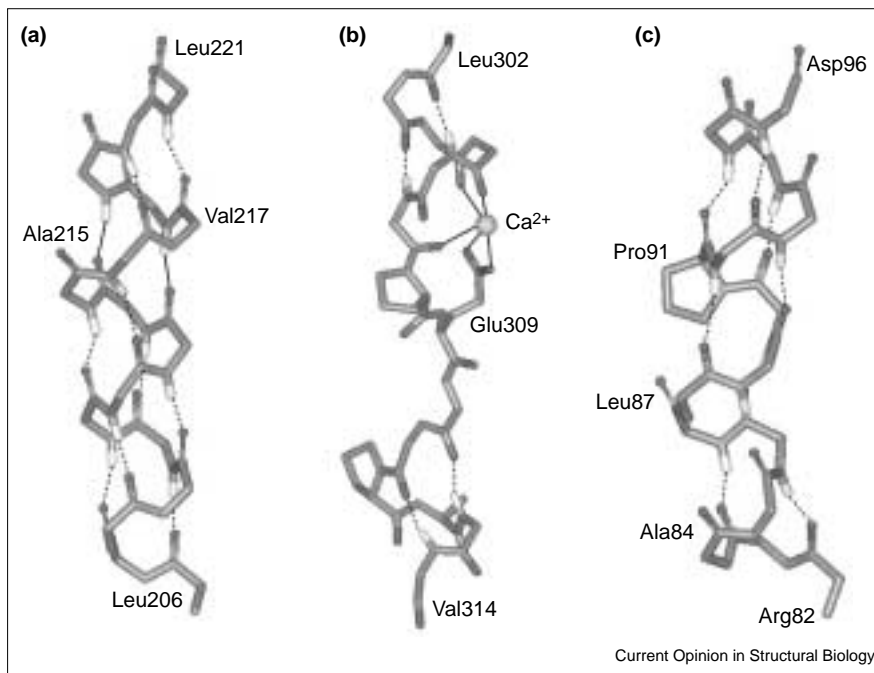
in which several backbone carbonyls coordinate the bound calcium [14[•]]; hydrogen bonding to water molecules, as in bacteriorhodopsin [13[•]]; and interhelical hydrogen-bond formation, as suggested by molecular dynamics simulations of the neu/Erb-2 transmembrane heterodimer [21[•]]. It should be noted that, because the partition of polar amino acid sidechains into the membrane core is more strongly unfavorable [1], the possibility of creating polar interactions with backbone carbonyls confers functional diversity on membrane proteins at a relatively low energetic cost.

Several examples of similar distortions in α -helicity can be found in other transmembrane proteins [3,20[•],22]. The expanding database of membrane protein structures and the fact that helical transmembrane domains are readily predicted and their dynamics can, nowadays, be studied using molecular dynamics simulations [23] strongly suggest that an increasing number of these types of distortions in α -helicity will be discovered and their relation to function rationalized.

Sequestering protein regions from the bilayer to enhance structural diversity

The lipid bilayer restricts the structural diversity of transmembrane domains in membrane proteins. There are provisions, however, that mitigate the bilayer constraints. The recently determined structures of two members of the aquaporin family, human aquaporin-1 and the glycerol facilitator of *Escherichia coli*, have revealed a previously unknown structural feature (Figure 3): two half-helices with their N-terminal ends pointing to each other in the center of the membrane [24^{••},25^{••}]. In principle, placing

Figure 2



Disruptions in the canonical α -helical conformation. (a) π -Bulge in helix G of bacteriorhodopsin ([13 \bullet]; PDB entry code 1C3W). The π -bulge at Ala215 causes the peptide plane between Ala215 and Lys216 to tilt away from the helix axis, locally disturbing the α -helical hydrogen-bonding pattern. The π -helical (i, i + 5) hydrogen bonds are shown by solid lines. Note that the carbonyl group of Ala215 does not participate in backbone hydrogen bonding. (b) Unwinding of helix M4 in the calcium ATPase of the sarcoplasmic reticulum ([14 \bullet]; PDB entry code 1EUL). Note that the unwinding exposes several backbone carbonyl groups. Some of these carbonyls are involved in the coordination of calcium (denoted by solid lines). (c) Proline kink in helix C of bacteriorhodopsin ([13 \bullet]; PDB entry code 1C3W). Note the increased spacing of the backbone below the proline and the carbonyl of Leu87 devoid of hydrogen bond. The canonical α -helical (i, i + 4) hydrogen bonds are denoted by dashed lines.

the end of a helix in a bilayer interior is energetically unfavorable because of the partial charge induced by the net separation of charge at the helix ends [26]. In both structures, however, this problem is circumvented because the N-terminal ends face the central hydrophilic pore, away from the lipids. The functional role of this motif is not clear. In the case of aquaporin, the authors speculate that the positive electrostatic field generated by the ends of the half-helices orients the transported water molecule [24 $\bullet\bullet$]. An analogous case has been observed for the KcsA potassium channel, in which the presence of the conducting pore, formed as a result of tetramerization, enables short polar helices to place their ends near the center of the bilayer [27]. These short helices have been proposed to be part of the constriction pore and create a region of electrostatic compensation for the charge on the K⁺ ion [28]. The outlined examples emphasize an increasingly recurrent feature in membrane protein organization, that is, the use of β barrels and rings of α helices as barriers that enable regions of the protein to be placed in a hydrophilic environment shielded from the lipid bilayer. This permits a wider scope of structure for the sequestered regions. Another illustration of such organization is observed in the siderophore receptor from the outer membrane of *E. coli*, in which an entire soluble domain occupies the hydrophilic lumen of the β barrel [29].

Another possible strategy to mitigate bilayer constraints is oligomerization to create protein–protein interfaces sequestered from lipids. In both aquaporin-1 and the glycerol facilitator, two transmembrane helices (2 and 6) that are not long enough to span the bilayer are positioned near

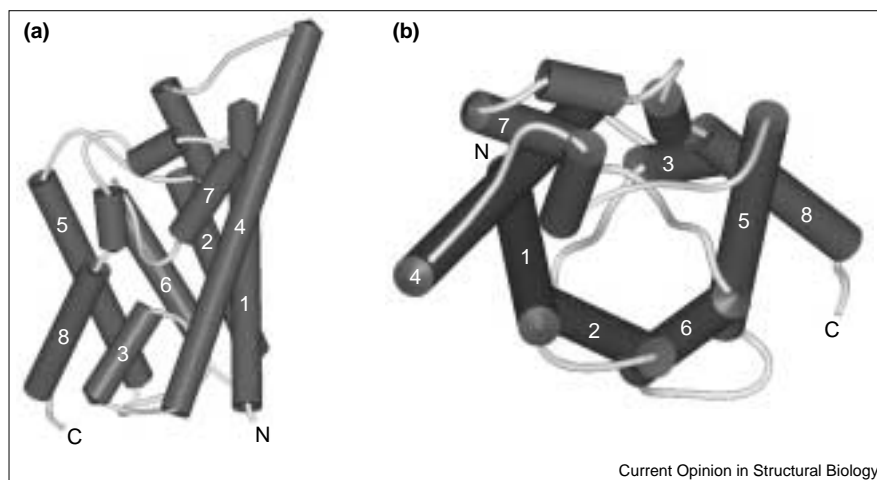
the fourfold axis of the tetramer [24 $\bullet\bullet$,25 $\bullet\bullet$]. The functional role of these helices is not clear, although in the glycerol facilitator they have been proposed to be part of a central hydrophobic channel at the tetramer interface. It should be emphasized that, although membrane proteins are, in many cases, oligomeric, the role of oligomerization is not well understood. It is tempting to propose that, in some cases, oligomerization might allow a wider scope of structural diversity at the oligomerization interface.

Polar residues contribute to membrane protein folding and stability

It is well established that strong polar residues, including glutamine, asparagine, histidine, aspartic acid, glutamic acid, arginine and lysine, are poorly represented in transmembrane segments (total frequency less than 5% compared with 22% in water-soluble regions) [30,31]. These compositional biases can be rationalized in view of the energetic penalty of partitioning polar residues into the low dielectric medium of the hydrocarbon region of the membrane [1]. In general, polar residues appear to be less mutable when they occur in transmembrane segments, as opposed to their counterparts in the extramembranous (water-soluble) regions between transmembrane domains, suggesting conserved structural or functional roles [31,32]. There are now several cases in which a clear functional role for a polar residue in the transmembrane region of a membrane protein has been established. From a structural perspective, however, their role has only recently begun to appear. Of special relevance is the recent progress in understanding interhelical hydrogen bonds.

Figure 3

Architecture of the glycerol facilitator of *E. coli*. (a) Side view of the glycerol facilitator monomer away from the tetramer interface ([25••]; PDB entry code 1FX8). Note the two half-helices (3 and 7) that meet at the center of the bilayer. (b) View of the monomer from the periplasm. Note the channel in the middle of the structure, as well as helices 6 and 2, which are located at the tetramer interface. The helices are numbered for clarity.



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Using model transmembrane helices, two independent studies have shown that an asparagine residue located in the middle of a transmembrane sequence can drive strong transmembrane helix homo-oligomerization [33••,34••]. Because substitution of the asparagine by a nonpolar residue abolished oligomer formation, it was concluded that the presence of the asparagine was the necessary requirement for oligomerization. Further structural characterization using NMR spectroscopy suggested that interhelical hydrogen bonding of the polar residue is the source of oligomer stability [33••]. It is only recently that interhelical hydrogen bonds have been observed in membrane protein structures. At least one hydrogen bond exists between each pair of adjacent helices within the monomer of bacteriorhodopsin [13•]. In the recently solved structure of the GPCR rhodopsin [15••], several polar residues were found to participate in hydrogen bonds between transmembrane helices (Figure 4). Hydrogen bonds have also been observed in the oligomerization of β -barrel proteins, emphasizing their possible role in quaternary structure formation [35].

Recent genetic and biophysical studies of membrane proteins have also identified polar residues that might participate in polar interactions between transmembrane helices. Lactose permease from *E. coli*, for example, retains its function when mutated at any position except for six. These absolutely necessary residues are polar, supposedly buried and close in space, raising the possibility of their participation in interhelical hydrogen bonds (reviewed in [36,37]). Voltage-gated Na^+ , K^+ and Ca^{2+} channels have highly conserved polar residues in transmembrane helices S2–S4 that might be involved in voltage sensing or transmembrane helix association [38]. The outlined examples emphasize the possibility of key interhelical hydrogen bonds becoming a theme in membrane protein structure.

What could be their structural role? An interesting possibility is that they contribute to the stabilization of

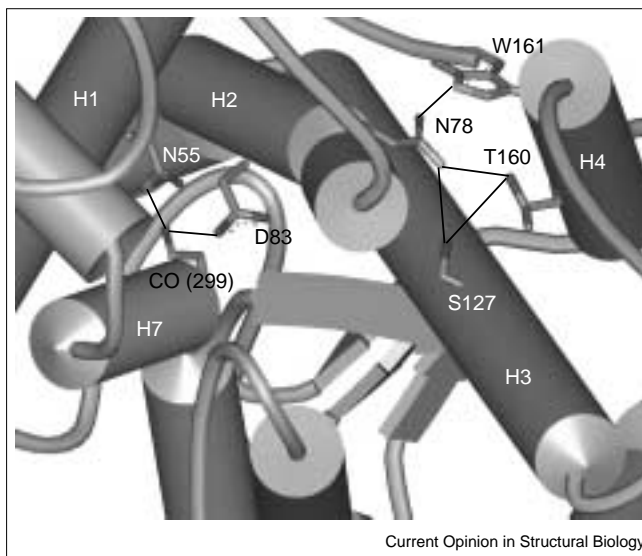
membrane protein structure. For example, there is evidence suggesting that GPCRs are maintained in a stable inactive conformation by strong intramolecular interactions [39,40] in order to suppress background signaling. In the specific case of rhodopsin, such stabilization could arise, as suggested by Palczewski *et al.* [15••], because of the interhelical hydrogen bonds observed in the structure. It should be noted that the residues involved in interhelical hydrogen bonding in rhodopsin appear to be highly conserved among GPCRs, underscoring the possibility that hydrogen bonds between transmembrane helices are a recurrent theme contributing to the stabilization of this superfamily of essential integral membrane proteins.

Finally, it is tempting to suggest that, whenever a polar residue is found towards the center of the transmembrane helix in single-span membrane proteins, the chances of oligomerization are high. Such oligomerization could have deleterious consequences. For example, the single mutation of a valine to a glutamic acid in the transmembrane domain of the neu oncogene results in dimerization and constitutive activation of this protein, leading to abnormal cell proliferation [41]. It was later determined that dimerization was induced as a consequence of interhelical hydrogen bonding between glutamic acid residues [42]. On the other hand, transmembrane domain oligomerization mediated by interhelical hydrogen bonds could be an important mechanism in single-span membrane protein function.

Interaction motifs in transmembrane helix association

As in soluble proteins, motifs can usefully inform us of relations between membrane protein sequences and structures. Considerable insight into this subject has been provided by systematic studies on the glycophorin A (GpA) transmembrane helix dimer (reviewed in [43]). Initially, using mutagenesis experiments, Lemmon *et al.* [43] identified a seven-residue motif (LIXXGVXXGVXXT) responsible for the

Figure 4



Interhelical hydrogen bonding in rhodopsin. A detail of the hydrogen-bonding network between transmembrane helices in bovine rhodopsin ([15^{••}]; PDB entry code 1F88). The hydrogen bonds are mediated by highly conserved asparagines, N55 and N78, connecting helices H1, H2 and H7, and helices H2, H3 and H4, respectively.

specific dimerization of the GpA transmembrane helices. The subsequent NMR structure of the transmembrane dimer confirmed the location of the motif at the dimer interface and revealed that the dimer was stabilized by close van der Waals packing of preformed interfaces [44]. It was later observed that, in a polymethionine background, the GxxxG motif was sufficient to mediate helix oligomerization [45]. The general implications of the lessons learned from the GpA dimer have recently begun to emerge.

Using a reporter system that monitors transmembrane helix oligomerization in membranes, a number of transmembrane domains exhibiting high-affinity homo-oligomerization were selected from a randomized sequence library [46[•]]. Most sequences contained the GxxxG packing motif with some variations, such as the substitution of glycine by small amino acid residues. Interestingly, a statistical analysis of amino acid patterns in a large database of transmembrane sequences also showed the GxxxG pattern, in conjunction with β -branched amino acid residues at neighboring positions, to be the most over-represented in the database [47[•]]. These complementary studies raise the possibility that GpA-type motifs might be a recurrent theme in mediating transmembrane helix interactions. Support for this hypothesis is provided by the recently solved structure of aquaporin-1, in which highly conserved glycine residues distributed in a GxxxGxxxA and AxxxGxxxA sequence pattern have been observed at contact sites between helices [24^{••}]. Analogous patterns have also been found in the structure of the glycerol facilitator from *E. coli* [25^{••}]. In addition, mutagenesis studies have demonstrated the involvement of the GxxxG motif in

transmembrane helix oligomerization of viral envelope proteins of the M13 and hepatitis C viruses [48,49].

Further support for the involvement of glycine in transmembrane helix association has been provided by two studies on the nature of amino acid residues at helix-helix contacts in the structures of membrane proteins [30,50[•]]. The authors found that small hydrophobic residues, such as glycine and alanine, are often found at buried positions between transmembrane helices and suggested that these residues are significant contributors to the association of transmembrane helices.

Currently, the GxxxG motif is the only extensively characterized example of a transmembrane-helix-packing motif. Recently, however, Langosch and co-workers [51] have identified a set of homo-oligomerizing transmembrane proteins that contain a heptad motif of leucine residues, which appear to mediate transmembrane helix association. Further research will be needed to assess the general implications of these findings.

Predominant helical membrane protein folds

The recent avalanche of genome sequence information, combined with the reliability of membrane protein topology prediction methods, enables the study of membrane proteins on a genome-wide scale. These studies estimate that α -helical membrane proteins comprise 20–30% of all genes encoded in the genomes of most eukaryotic, eubacterial and archaean organisms [52,53]. The classification of membrane protein sequences from different organisms according to topology and function has also provided interesting insights. In archaea, eubacteria and plants, membrane proteins with 4, 10 and 12 transmembrane domains (all with the N terminus facing the cytoplasm) are dominant, whereas 4 and 7 transmembrane domain proteins appear to be more common in yeast and in higher eukaryotes [52,54–56]. These findings suggest that, in an analogous manner to soluble proteins, predominant protein folds occur in helical membrane proteins. In addition, a correlation between the number of transmembrane domains and functional processes in the organisms has been revealed. Organisms with many identifiable transport systems have greater numbers of membrane proteins with more than seven transmembrane domains [57^{••},58], whereas multicellular organisms have a greater proportion of seven transmembrane proteins belonging presumably to the GPCR family [54,56].

Conclusions

The increasing number of solved membrane protein structures and genome-wide analysis studies have led to new insights and the revisiting of old concepts. We are now reaching a new phase in which detailed structure/function studies on membrane proteins can be accomplished. These studies should clarify a fascinating question. How can a simple arrangement of hydrophobic helices support the functional diversity of helical membrane proteins?

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. White SH, Wimley WC: **Membrane protein folding and stability: physical principles.** *Annu Rev Biophys Biomol Struct* 1999, **28**:319-365.
 2. von Heijne G: **Recent advances in the understanding of membrane protein assembly and structure.** *Q Rev Biophys* 1999, **32**:285-307.
 3. Popot JL, Engelman DM: **Helical membrane protein folding, stability, and evolution.** *Annu Rev Biochem* 2000, **69**:881-922.
 4. Schulz GE: **β -Barrel membrane proteins.** *Curr Opin Struct Biol* 2000, **10**:443-447.
 5. Koebnik R, Locher KP, van Gelder P: **Structure and function of bacterial outer membrane proteins: barrels in a nutshell.** *Mol Microbiol* 2000, **37**:239-253.
 6. Sonnhammer EL, von Heijne G, Krogh A: **A hidden Markov model for predicting transmembrane helices in protein sequences.** *Proc Int Conf Intell Syst Mol Biol* 1998, **6**:175-182.
 7. Tusnady GE, Simon I: **Principles governing amino acid composition of integral membrane proteins: application to topology prediction.** *J Mol Biol* 1998, **283**:489-506.
 8. Landolt-Marticorena C, Williams KA, Deber CM, Reichmeier RAF: **Non-random distribution of amino acids in the transmembrane segments of human type I single span membrane proteins.** *J Mol Biol* 1993, **229**:602-608.
 9. Arkin IT, Brunger AT: **Statistical analysis of predicted transmembrane α -helices.** *Biochim Biophys Acta* 1998, **1429**:113-128.
 10. Bowie JU: **Helix packing in membrane proteins.** *J Mol Biol* 1997, **272**:780-789.
 11. Wallin E, Tsukihara T, Yoshikawa S, von Heijne G, Elofsson A: **Architecture of helix bundle membrane proteins: an analysis of cytochrome c oxidase from bovine mitochondria.** *Protein Sci* 1997, **6**:808-815.
 12. Kolbe M, Besir H, Essen LO, Oesterhelt D: **Structure of the light-driven chloride pump halorhodopsin at 1.8 Å resolution.** *Science* 2000, **288**:1390-1396.
 13. Luecke H, Schobert B, Richter HT, Cartailler JP, Lanyi JK: **Structure of bacteriorhodopsin at 1.55 Å resolution.** *J Mol Biol* 1999, **291**:899-911.
- This study presents the high-resolution structure of the light-driven pump bacteriorhodopsin and marks the limit of the resolution that can nowadays be achieved for a membrane protein. Such a resolution permits the unambiguous identification of structural details, such as deformations in the α -helical conformation. The protein was crystallized out of lipidic cubic phases, a crystallization method developed by Landau and co-workers that has had great impact on membrane protein structure elucidation.
14. Toyoshima C, Nakasako M, Nomura H, Ogawa H: **Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution.** *Nature* 2000, **405**:647-655.
- The calcium ATPase transports calcium across membranes against a concentration gradient. The structure reveals 10 transmembrane helices and large extramembrane domains involved in ATP hydrolysis and activity regulation. Two of the transmembrane helices are partially unwound and are involved in the coordination of two calcium ions.
15. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE *et al.*: **Crystal structure of rhodopsin: a G protein-coupled receptor.** *Science* 2000, **289**:739-745.
- G-protein-coupled receptors (GPCRs) are the target of most drug therapies worldwide and are subject to intensive research both in industry and in academia. This study presents, for the first time, the structure of a GPCR.

Although the regions of the protein that supposedly interact with G proteins are not well defined, the structure provides an invaluable platform to rationalize the function of GPCRs from a structural perspective.

16. Iverson TM, Luna-Chavez C, Cecchini G, Rees DC: **Structure of *Escherichia coli* fumarate reductase respiratory complex.** *Science* 1999, **284**:1961-1966.
 17. Lancaster CRD, Kroger A, Auer M, Michel H: **Structure of fumarate reductase from *Wolinella succinogenes* at 2.2 Å resolution.** *Nature* 1999, **402**:377-385.
 18. Luecke H: **Atomic resolution structures of bacteriorhodopsin photocycle intermediates: the role of discrete water molecules in the function of this light-driven ion pump.** *Biochim Biophys Acta* 2000, **1460**:133-156.
 19. Royant A, Edman K, Ursby T, Pebay-Peyroula E, Landau EM, Neutze R: **Helix deformation is coupled to vectorial proton transport in the photocycle of bacteriorhodopsin.** *Nature* 2000, **406**:645-648.
- This study presents a structural 'snapshot' of bacteriorhodopsin during the photocycle. This work is an example of the level of structural detail that can be achieved in structure/function studies of membrane proteins.
20. Sansom MSP, Weinstein H: **Hinges, swivels and switches: the role of prolines in signalling via transmembrane helices.** *Trends Pharmacol Sci* 2000, **21**:445-451.
- This review provides a plausible explanation for the role of helix hinges and kinks in the function of cellular receptors.
21. Sajot N, Genest M: **Structure prediction of the dimeric neu/ErbB-2 transmembrane domain from multi-nanosecond molecular dynamics simulations.** *Eur Biophys J* 2000, **28**:648-662.
- This study emphasizes the idea that, with the support of good mutagenesis data, the structure of small transmembrane helix oligomers can be reasonably predicted. Furthermore, it provides evidence for the role of exposed backbone carbonyl groups in transmembrane helix association.
22. Duneau JP, Garnier N, Genest M: **Insight into signal transduction: structural alterations in transmembrane helices probed by multi-ns molecular dynamics simulations.** *J Biomol Struct Dyn* 1997, **15**:555-572.
 23. Forrest LR, Sansom MSP: **Membrane simulations: bigger and better.** *Curr Opin Struct Biol* 2000, **10**:174-181.
 24. Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y: **Structural determinants of water permeation through aquaporin-1.** *Nature* 2000, **407**:599-605.
- The aquaporin family of proteins is involved in the permeation of hydrophilic molecules across membranes. This study presents the structure of human aquaporin-1. The structure reveals a new membrane protein fold and a possible mechanism for the permeation of water.
25. Fu D, Libson A, Miercke LJ, Weitzman C, Nollert P, Krucinski J, Stroud RM: **Structure of a glycerol-conducting channel and the basis for its selectivity.** *Science* 2000, **290**:481-486.
- This study reveals the structure of the glycerol facilitator of *E. coli*. The structure is similar to that of aquaporin [24**], but it has been solved at considerably higher resolution. Interestingly, three glycerol molecules can be observed aligned in the interior of the transport channel, providing structural evidence for protein-glycerol interactions during transport.
26. Ben-Tal N, Ben-Shaul A, Nicholls A, Honig B: **Free-energy determinants of α -helix insertion into lipid bilayers.** *Biophys J* 1996, **70**:1803-1812.
 27. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R: **The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity.** *Science* 1998, **280**:69-77.
 28. Roux B, MacKinnon R: **The cavity and pore helices in the KcsA K⁺ channel: electrostatic stabilization of monovalent cations.** *Science* 1999, **285**:100-102.
 29. Locher KP, Rees B, Koebnik R, Mitschler A, Moulinier L, Rosenbusch JP, Moras D: **Transmembrane signaling across the ligand-gated FhuA receptor: crystal structures of free and ferrichrome-bound states reveal allosteric changes.** *Cell* 1998, **95**:771-778.
 30. Eilers M, Shekar SC, Shieh T, Smith SO, Fleming PJ: **Internal packing of helical membrane proteins.** *Proc Natl Acad Sci USA* 2000, **97**:5796-5801.
 31. Tourasse NJ, Li W-H: **Selective constraints, amino acid composition, and the rate of protein evolution.** *Mol Biol Evol* 2000, **17**:656-664.

32. Jones DT, Taylor WR, Thornton JM: **A mutation data matrix for transmembrane proteins.** *FEBS Lett* 1994, **339**:269-275.
33. Zhou FX, Cocco MJ, Russ WP, Brunger AT, Engelman DM:
 •• **Interhelical hydrogen bonding drives strong interactions in membrane proteins.** *Nat Struct Biol* 2000, **7**:154-160.
 This work emphasizes the possibilities of studying structural themes in membrane proteins using designed transmembrane helices. The authors show that hydrogen bonds between asparagine residues can mediate strong transmembrane helix association. This work is of general importance as it provides support for the idea that polar residues contribute to the stabilization of membrane protein structure.
34. Choma C, Gratkowski H, Lear JD, DeGrado WF: **A membrane-soluble analogue of the two-stranded coiled coil from GCN4.** *Nat Struct Biol* 2000, **7**:161-166.
 This is an independent study that uses a similar approach to that described in [33••] and reaches analogous conclusions.
35. Snijder HJ, Ubarretxena-Belandia I, Blaauw M, Kalk KH, Verheij HM, Egmond MR, Dekker N, Dijkstra BW: **Structural evidence for dimerization-regulated activation of an integral membrane phospholipase.** *Nature* 1999, **401**:717-721.
36. Frillingos S, Sahin-Toth M, Wu J, Kaback HR: **Cys-scanning mutagenesis: a novel approach to structure function relationships in polytopic membrane proteins.** *FASEB J* 1998, **12**:1281-1299.
37. Zhao M, Zen KC, Hubbell WL, Kaback HR: **Proximity between Glu126 and Arg144 in the lactose permease of *Escherichia coli*.** *Biochemistry* 1999, **38**:7407-7412.
38. Bezanilla F: **The voltage sensor in voltage-dependent ion channels.** *Physiol Rev* 2000, **80**:555-592.
39. Honig B, Ebrey T, Callender RH, Dinur U, Ottolenghi M: **Photoisomerization, energy storage, and charge separation: a model for light energy transduction in visual pigments and bacteriorhodopsin.** *Proc Natl Acad Sci USA* 1979, **76**:2503-2507.
40. Gether U, Kobilka BK: **G protein-coupled receptors.** *J Biol Chem* 1998, **273**:17979-17982.
41. Weiner DB, Liu J, Cohen JA, Williams WV, Greene MI: **A point mutation in the neu oncogene mimics ligand induction of receptor aggregation.** *Nature* 1989, **339**:230-231.
42. Smith SO, Smith CS, Bormann BJ: **Strong hydrogen bonding interactions involving a buried glutamic acid in the transmembrane sequence of the neu/erbB-2 receptor.** *Nat Struct Biol* 1996, **3**:252-258.
43. Lemmon MA, MacKenzie KR, Arkin IT, Engelman DM: **Transmembrane α -helix interactions in folding and oligomerisation of integral membrane proteins.** In *Membrane Protein Assembly*. Edited by von Heijne G. New York: Springer-Verlag; 1997:3-24.
44. MacKenzie KR, Prestegard JH, Engelman DM: **A transmembrane helix dimer: structure and implications.** *Science* 1997, **276**:131-133.
45. Brosig B, Langosch D: **The dimerization motif of the glycoporphin A transmembrane segment in membranes: importance of glycine residues.** *Protein Sci* 1998, **7**:1052-1056.
46. Russ WP, Engelman DM: **The GxxxG motif: a framework for transmembrane helix-helix association.** *J Mol Biol* 2000, **296**:911-919.
 Several groups have demonstrated the advantages of using genetic screens to study transmembrane helix association. Here, a library of randomized sequences that oligomerize in the *E. coli* inner membrane was created. From these sequences, motifs that drive parallel transmembrane helix homo-oligomerization could be identified. The use of this type of genetic approach extends the range of tools at our disposal to study membrane proteins in their native environment.
47. Senes A, Gerstein M, Engelman DM: **Statistical analysis of amino acid patterns in transmembrane helices: the GxxxG motif occurs frequently and in association with beta-branched residues at neighboring positions.** *J Mol Biol* 2000, **296**:921-936.
 Statistical analysis of predicted transmembrane helices is a powerful approach to identify sequence patterns. Using a large database of transmembrane sequences, a series of over-represented patterns was identified. Interestingly, these patterns resemble the helix interaction motifs identified in [46•].
48. Wang D, Deber CM: **Peptide mimics of the M13 coat protein transmembrane segment.** *J Biol Chem* 2000, **275**:16155-16159.
49. Op de Beeck A, Montserret R, Duvet S, Cocquerel L, Cacan R, Barberot B, le Maire M, Penin F, Dubuisson J: **The transmembrane domains of hepatitis C virus envelope glycoproteins E1 and E2 play a major role in heterodimerization.** *J Biol Chem* 2000, **275**:31428-31437.
50. Javadpour MM, Eilers M, Groesbeek M, Smith OS: **Helix packing in polytopic membrane proteins: role of glycine in transmembrane helix association.** *Biophys J* 1999, **77**:1609-1618.
 This study provides insight into the nature of the amino acid residues at protein interfaces in membrane proteins, as observed from the analysis of several membrane protein structures.
51. Gurezka R, Laage R, Brosig B, Langosch D: **A heptad motif of leucine residues found in membrane proteins can drive self-assembly of artificial transmembrane segments.** *J Biol Chem* 1999, **274**:9265-9270.
52. Wallin E, von Heijne G: **Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms.** *Protein Sci* 1998, **7**:1029-1038.
53. Stevens TJ, Arkin IT: **Do more complex organisms have a greater proportion of membrane proteins in their genomes?** *Proteins* 2000, **39**:417-420.
54. Jones DT: **Do transmembrane protein superfolds exist?** *FEBS Lett* 1998, **423**:281-285.
55. Devoto A, Piffanelli P, Nilsson I, Wallin E, Panstruga R, von Heijne G, Schulze-Lefert P: **Topology, subcellular localization, and sequence diversity of the Mlo family in plants.** *J Biol Chem* 1999, **274**:34993-35004.
56. Remm M, Sonnhammer E: **Classification of transmembrane protein families in the *Caenorhabditis elegans* genome and identification of human orthologs.** *Genome Res* 2000, **10**:1679-1689.
57. Paulsen IT, Nguyen L, Sliwinski MK, Rabus R, Saier MH Jr: **Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes.** *J Mol Biol* 2000, **301**:75-100.
 This study presents a comprehensive analysis of solute transport systems encoded in the genomes of several prokaryotic organisms.
58. Kihara D, Kanehisa M: **Tandem clusters of membrane proteins in complete genome sequences.** *Genome Res* 2000, **10**:731-743.
59. Koronakis V, Sharff A, Koronakis E, Luisi B, Hughes C: **Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export.** *Nature* 2000, **405**:914-919.