
HOW HYDROGEN BONDS SHAPE MEMBRANE PROTEIN STRUCTURE

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ABSTRACT

The energetic cost of partitioning peptide bonds into membrane bilayers is prohibitive unless the peptide bonds participate in hydrogen bonds. However, even then there is a significant free energy penalty for dehydrating the peptide bonds that can only be overcome by favorable hydrophobic interactions. Membrane protein structure formation is thus dominated by hydrogen bonding interactions, which is the subject of this review.

I. INTRODUCTION

Two things are paramount in shaping the structure of membrane proteins (MPs): the energetics of peptide bond dehydration and the structure of the cell membrane lipid bilayer. The high energetic cost of dehydrating the peptide bond, as when transferring it to a nonaqueous phase (Liu and Bolen, 1995), causes it to dominate structure formation. For example, the only permissible transmembrane structural motifs of MPs are α -helices and β -barrels because internal H-bonding lowers this cost. What complicates protein structure formation of MPs is the anisotropy and chemical heterogeneity of lipid bilayers at the atomic level. This is the subject of the first part of this review. It provides a structural context for understanding the energetics of hydrogen bond formation, which is the subject of the second part of the review.

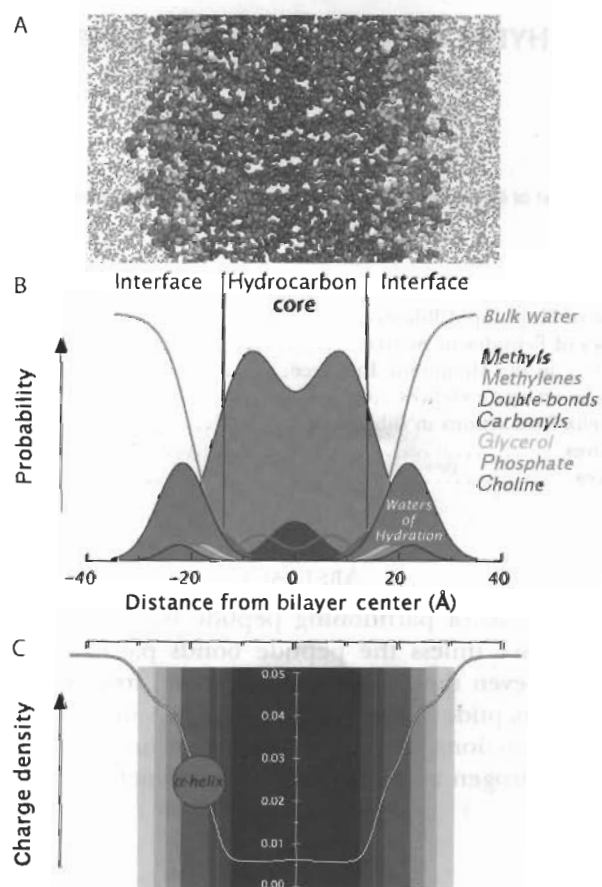


FIG. 1. The liquid-crystalline structure of a fluid dioleoylphosphatidylcholine (DOPC) bilayer. (A) Molecular graphics image of DOPC taken from a molecular dynamics simulation by Ryan Benz, UC Irvine. Adapted from White *et al.* (2005). The color scheme for the component groups (carbonyls, phosphates, water, etc.) is given in B. The image was prepared by S. White using VMD (Humphrey *et al.*, 1996). (B) Liquid-crystallographic structure of a fluid DOPC lipid bilayer (Wiener and White, 1992). The “structure” of the bilayer is composed of a collection of transbilayer Gaussian probability distribution functions representing the lipid components that account for the entire contents of the bilayer unit cell. Areas under the curves correspond to the number of constituent groups per lipid represented by the distributions (1 phosphate, 2 carbonyls, 4 methyls, etc.). The widths of the Gaussians measure the thermal motions of the lipid components and are simply related to crystallographic B factors (Hristova *et al.*, 1999, 2001; Wiener and White, 1991). The thermal motion of the bilayer is extreme: lipid-component B factors are typically $\sim 150 \text{ \AA}^2$, compared to $\sim 30 \text{ \AA}^2$ for atoms in protein crystals. (C) Polarity profile (yellow curve) of the DOPC bilayer (see earlier discussion) computed from the absolute values of atomic partial charges (White and Wimley, 1998).

II. STRUCTURE OF FLUID LIPID BILAYERS

Because membranes must be in a fluid state for normal cell function, only the structure of fluid (L_{α} -phase) bilayers is relevant to understanding how membranes mold proteins. However, atomic resolution images of fluid membranes are precluded due to their high thermal disorder (Fig. 1A). Nevertheless, fundamental and useful structural information can be obtained from multilamellar bilayers (liquid crystals) dispersed in water or deposited on surfaces (Nagle and Tristram-Nagle, 2000, 2001; Petrache *et al.*, 1998; Tristram-Nagle *et al.*, 1998). Their one-dimensional crystallinity perpendicular to the bilayer plane allows the distribution of matter along the bilayer normal to be determined by combined X-ray and neutron diffraction measurements [liquid crystallography; reviewed by White and Wiener (1995, 1996)]. The resulting “structure” consists of a collection of time-averaged probability distribution curves of water and lipid component groups (carbonyls, phosphates, etc.), representing projections of three-dimensional motions onto the bilayer normal. Figure 1B shows the liquid crystallographic structure of an L_{α} -phase dioleoylphosphatidylcholine (DOPC) bilayer (Wiener and White, 1992).

Three features of this structure are important. First, the widths of the probability densities reveal the great thermal disorder of fluid membranes. Second, the combined thermal thicknesses of the interfaces (defined by the distribution of the waters of hydration) are about equal to the 30-Å thickness of the HC. The thermal thickness of a single interface (~ 15 Å) can easily accommodate an α -helix parallel to the membrane plane. The common cartoons of bilayers that assign a diminutive thickness to the bilayer interfaces (IFs) are thus misleading. Third, the thermally disordered IFs are highly heterogeneous chemically. A polypeptide chain in an IF must experience dramatic variations in environmental polarity over a short distance due to the steep changes in chemical composition, as illustrated by the yellow curve in Fig. 1C (White and Wimley, 1998). As the regions of first contact, IFs are especially important in the folding and insertion of nonconstitutive MPs, such as diphtheria toxin (Ladokhin *et al.*, 2004; Rosconi *et al.*, 2004), and to the activity of surface-binding enzymes, such as phospholipases (Bollinger *et al.*, 2004; Frazier *et al.*, 2002; Gelb *et al.*, 1999).

The end-on view in B of an α -helix with a diameter of ~ 10 Å—typical for MP helices (Bowie, 1997)—shows the approximate location of the helical axes of the amphipathic-helix peptides Ac-18A-NH₂ (Hristova *et al.*, 1999) and melittin (Hristova *et al.*, 2001), as determined by a novel, absolute-scale X-ray diffraction method [reviewed by White and Hristova (2000)]. B and C have been adapted from reviews by White and Wimley (1994, 1998, 1999) and White *et al.* (2005). (See Color Insert.)

Experimentally determined bilayer structures such as the one in Fig. 1B are essential for understanding thermodynamic measurements of peptide–bilayer interactions at the molecular level. Recent extensions of the liquid crystallographic methods to bilayers containing peptides such as melittin (Hristova *et al.*, 2001) and other amphipathic peptides (Hristova *et al.*, 1999) make this a practical possibility. However, there are numerous other X-ray and neutron diffraction approaches that provide important information about the molecular interactions of peptides with lipid bilayers (Bradshaw *et al.*, 1998, 2000; Chen *et al.*, 2003; He *et al.*, 1996; Heller *et al.*, 2000; Weiss *et al.*, 2003; Yang *et al.*, 2000). Molecular dynamics (MD) simulations of bilayers (Feller, 2000; Forrest and Sansom, 2000; Pastor, 1994; Tieleman *et al.*, 1997) (Fig. 1A) are rapidly becoming an essential structural tool for examining lipid–protein interactions at atomic scales (Bernèche and Roux, 2001; Deol *et al.*, 2004; Feller *et al.*, 2003; Freitas *et al.*, 2005; Tieleman *et al.*, 2002; Tobias, 2001; Zhu *et al.*, 2004). The future offers the prospect of combining bilayer diffraction data with MD simulations in order to arrive at experimentally validated MD simulations of fluid lipid bilayers (Benz *et al.*, 2005). This approach should allow one to convert the static one-dimensional images obtained by diffraction (Fig. 1B) into dynamic, three-dimensional structures for examining peptide–lipid interactions in atomic detail.

III. ENERGETICS OF PEPTIDES IN BILAYERS

Experimental exploration of the stability of intact MPs is problematic due to their general insolubility. One approach to stability is to “divide and conquer” by studying the membrane interactions of fragments of MPs, that is, peptides. Because MPs are equilibrium structures, one is free to describe the interactions by any convenient set of thermodynamic pathways that is experimentally accessible, irrespective of the biological synthetic pathway. One particularly useful set of pathways is the so-called four-step model (White and Wimley, 1999) (Fig. 2), which is a logical combination of the early three-step model of Jacobs and White (1989) and the two-stage model of Popot and Engelman (1990) in which TM helices are first “established” across the membrane and then assembled into functional structures [helix association; reviewed by Curran and Engelman (2003)]. Although these pathways do not mirror the actual biological assembly process of MPs, they are nevertheless useful for guiding biological experiments, because they provide a thermodynamic context within which biological processes must proceed.

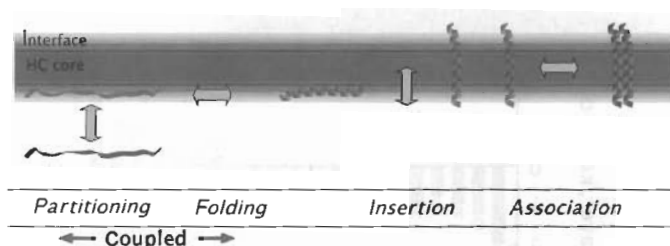


FIG. 2. Energetics of peptide interactions with lipid bilayers. Schematic representation of the shaping of protein structure through polypeptide–bilayer interactions. This figure is based on the four-step thermodynamic cycle of White and Wimley (1999) for describing the partitioning, folding, insertion, and association of α -helical polypeptides. The aqueous insolubility of membrane proteins, folded or unfolded, precludes direct determinations of interaction-free energies. The only route to understanding the energetics of MP stability is through studies of small, water-soluble peptides (Ladokhin and White, 1999; Wimley and White, 1996, 2000; Wimley *et al.*, 1998) (see Figs. 3 and 4). The association of TM helices is driven primarily by van der Waals interactions, giving rise to knob-into-hole packing (Arkin *et al.*, 1996; Fleming *et al.*, 1997; MacKenzie and Engelman, 1998; MacKenzie *et al.*, 1997). The GxxxG motif is especially important in helix–helix interactions in membranes (Russ and Engelman, 2000; Senes *et al.*, 2000). Interhelical H-bonds also stabilize membrane proteins, but no more so than in soluble proteins (Adamian and Liang, 2002; Gimpelev *et al.*, 2004). Figure adapted from White and Wimley (1999) and White *et al.* (2001).

A. Folding in the Membrane Interface

In the four-step model, the free energy reference state is taken as the unfolded protein in an IF. However, this state cannot actually be achieved with MPs because of insolubility nor can it be achieved with small non-constitutive membrane-active peptides, such as melittin, because binding usually induces secondary structure (partitioning folding coupling, see later). It can be defined for phosphatidylcholine (PC) interfaces by means of an experiment-based interfacial free energy (hydrophobicity) scale (Wimley and White, 1996) derived from partitioning into POPC bilayers of tri- and pentapeptides (Jacobs and White, 1989; Wimley and White, 1996) that have no secondary structure in the aqueous or interfacial phases. This scale (Fig. 3A), which includes the peptide bonds as well as the side chains, allows calculation of the virtual free energy of transfer of an unfolded chain into an IF. For peptides that cannot form regular secondary structure, such as the antimicrobial peptide indolicidin, the scale predicts observed free energies of transfer with remarkable accuracy (Ladokhin and White, 2001; Hristova and White, 2005). This validates it for computing virtual partitioning free energies of proteins into PC IFs. Similar scales are needed for other lipids and lipid mixtures.

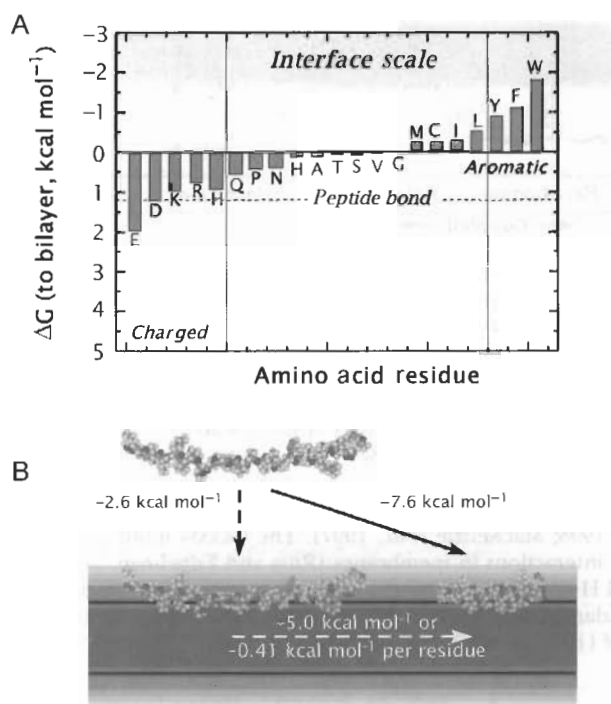


FIG. 3. Energetics of protein folding at bilayer interfaces. (A) The Wimley-White (WW) whole-residue interfacial hydrophobicity scale determined from measurements of the partitioning of short peptides into phosphatidylcholine vesicles (Wimley and White, 1996). (B) Energetics of secondary structure formation by melittin at the bilayer interface (Ladokhin and White, 1999). Unfolded peptides are driven toward the folded state in the IF because hydrogen bond formation lowers the cost of peptide bond partitioning dramatically, which is the dominant determinant of whole-residue partitioning. The free energy reduction accompanying secondary structure formation by melittin is ~ 0.4 kcal mol⁻¹ per residue (Ladokhin and White, 1999; Wimley *et al.*, 1998), but may be as low as 0.1 kcal mol⁻¹ for other peptides (Wieprecht *et al.*, 1999a). Although small, such changes in aggregate can be large. For example, the folding of 12 residues of 26-residue melittin into an α -helical conformation causes the folded state to be favored over the unfolded state by ~ 5 kcal mol⁻¹. To put this number in perspective, the ratio of folded to unfolded peptide is ~ 4700 . Figure adapted from reviews by White and Wimley (1999) and White (2003).

The high cost of interfacial partitioning of the peptide bond (Wimley and White, 1996), 1.2 kcal mol⁻¹, explains the origin of partitioning-folding coupling and also explains why the interface is a potent catalyst of secondary structure formation. For interfacial β -sheet formation, Wimley

et al. (1998) showed that H-bond formation reduces the cost of peptide partitioning by about $0.5 \text{ kcal mol}^{-1}$ per peptide bond. The folding of melittin into an amphipathic α helix on POPC membranes involves a per-residue reduction of about $0.4 \text{ kcal mol}^{-1}$ (Ladokhin and White, 1999) (Fig. 3B). The folding of other peptides may involve smaller per-residue values (Li *et al.*, 2003; Wieprecht *et al.*, 1999b). The cumulative effect of these relatively small per-residue free energy reductions can be very large when tens or hundreds of residues are involved.

B. Transmembrane Helices

The energetics of TM helix stability also depends critically on the partitioning cost of peptide bonds (Fig. 4). Determination of the energetics of TM α -helix insertion, which is necessary for predicting structure, is difficult because nonpolar helices tend to aggregate in both aqueous and interfacial phases (Wimley and White, 2000). The broad energetic issues are clear (Roseman, 1988), however. Computational studies (Ben-Tal *et al.*, 1996, 1997) suggest that the transfer-free energy ΔG_{CONH} of a non-H-bonded peptide bond from water to alkane is $6.4 \text{ kcal mol}^{-1}$, compared to only $2.1 \text{ kcal mol}^{-1}$ for the transfer-free energy ΔG_{Hbond} of an H-bonded peptide bond. The per-residue free energy cost of disrupting H-bonds in a membrane is therefore about 4 kcal mol^{-1} . A twenty-residue TM helix would thus cost 80 kcal mol^{-1} to unfold within a membrane, which explains why unfolded polypeptide chains cannot exist in a transmembrane configuration.

As discussed in detail elsewhere (Jayasinghe *et al.*, 2001a; White *et al.*, 2001), ΔG_{Hbond} sets the threshold for transmembrane stability as well as the so-called decision level in hydrophathy plots (White, 1994). The free energy of transfer of nonpolar side chains dramatically favors helix insertion, whereas the transfer cost of the helical backbone dramatically disfavors insertion. For example (White *et al.*, 2001), the favorable (hydrophobic effect) free energy for the insertion of the single membrane-spanning helix of glycoporphin A (Segrest *et al.*, 1972) is estimated to be $-36 \text{ kcal mol}^{-1}$, whereas the cost ΔG_{bb} of dehydrating the helix backbone is 26 kcal mol^{-1} (Fig. 4B). The net free energy ΔG_{TM} favoring insertion is thus $-10 \text{ kcal mol}^{-1}$. As is common in so many biological equilibria, the free energy minimum is the small difference of two relatively large opposing energetic terms. Uncertainties in the per-residue cost of backbone insertion will have a major effect on estimates of TM helix stability, the interpretation of hydrophathy plots, and the establishment of the minimum value of side chain hydrophobicity required for stability. An uncertainty of 0.5 kcal

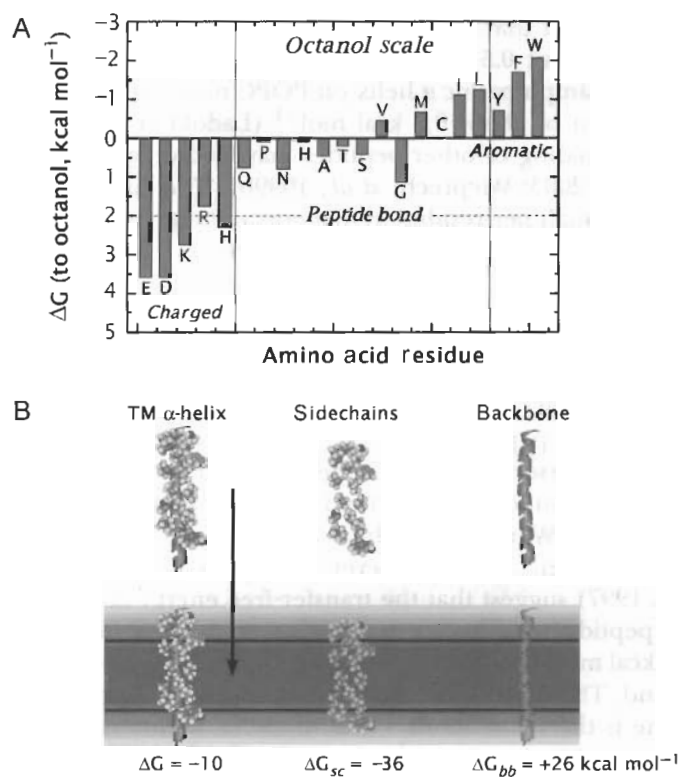


FIG. 4. Energetics of transmembrane (TM) helix stability. (A) The WW whole-residue octanol hydrophobicity scale determined from the partitioning of short peptides into *n*-octanol (Wimley *et al.*, 1996a) that predicts the stability of transmembrane helices (Jayasinghe *et al.*, 2001a). Free energy values along the abscissa are ordered in the same manner as in Fig. 3. (B) The energetics of transmembrane helix stability based on the work of Wimley and White (2000) and Jayasinghe *et al.* (2001a). Estimated relative free energy contributions of the side chains (ΔG_{sc}) and backbone (ΔG_{bb}) to the helix-insertion energetics of glycoporphin A (Segrest *et al.*, 1972). The net side chain contribution (relative to glycine) was computed using the *n*-octanol hydrophobicity scale of Wimley *et al.* (1996a). The per-residue cost of partitioning a polyglycine α -helix is 1.15 kcal mol⁻¹ (Jayasinghe *et al.*, 2001a). Figures adapted from reviews by White *et al.* (2001) and White (2003).

mol⁻¹, for example, would cause an uncertainty of about 10 kcal mol⁻¹ in ΔG_{TM} !

What is the most likely estimate of ΔG_{Hbond} ? The practical number is the cost $\Delta G_{glycyl}^{helix}$ of transferring a single glycyl unit of a polyglycine α -helix into the bilayer HC. Electrostatic calculations and the octanol partitioning study

of Wimley *et al.* (1996a) suggested that $\Delta G_{\text{glycyl}}^{\text{helix}} = 1.25 \text{ kcal mol}^{-1}$, which is the basis for the calculation of ΔG_{bb} . The cost of transferring a random-coil glycyl unit into *n*-octanol (Wimley *et al.*, 1996a) is $1.15 \text{ kcal mol}^{-1}$, which suggested that the *n*-octanol whole residue hydrophobicity scale (White and Wimley, 1999) (Fig. 4A) derived from partitioning data of Wimley *et al.* (1996a) might be a good measure of $\Delta G_{\text{glycyl}}^{\text{helix}}$. This hypothesis was borne out by a study (Jayasinghe *et al.*, 2001a) of known TM helices cataloged in the MPtopo database of MPs of known topology (Jayasinghe *et al.*, 2001b), accessible via the World Wide Web at <http://blanco.biomol.uci.edu/mptopo>. This study showed that $1.15 \text{ kcal mol}^{-1}$ is indeed the best estimate of $\Delta G_{\text{glycyl}}^{\text{helix}}$. Using this value, TM helices for MPs of known three-dimensional structure could be identified with high accuracy in the 2001 edition of MPtopo. This scale also includes free energy values for protonated and deprotonated forms of Asp, Glu, and His. In addition, Wimley *et al.* (1996b) determined the free energies of partitioning salt bridges into octanol, which are believed to be good estimates for partitioning into membranes (Jayasinghe *et al.*, 2001a). This has led to the augmented Wimley–White (aWW) hydrophobicity scale (Jayasinghe *et al.*, 2001a), which forms the basis for a useful hydropathy-based tool, MPEx, for analyzing MP protein stability. MPEx is available over the world wide web at <http://blanco.biomol.uci.edu/mpex>. The WW experiment-based whole residue hydrophobicity scales (Jayasinghe *et al.*, 2001a; Wimley and White, 1996; Wimley *et al.*, 1996a), shown in Figs. 3A and 4A, provide a solid starting point for understanding the physical stability of MPs and a connection between physical biochemistry and biology (Hessa *et al.*, 2005; White, 2003).

IV. HELIX–HELIX INTERACTIONS IN BILAYERS

The hydrophobic effect is generally considered to be the major driving force for compacting soluble proteins (Dill, 1990), but it cannot be the force driving compaction (association) of TM α -helices. Because the hydrophobic effect arises solely from dehydration of a nonpolar surface (Tanford, 1973), it is expended after helices are established across the membrane. Helix association is most likely driven primarily by van der Waals forces, more specifically the London dispersion force [reviewed by White and Wimley (1999) and Popot and Engelman (2000)], but hydrogen bonding can also be important.

Extensive work (Fleming *et al.*, 1997; Lemmon *et al.*, 1992, 1994; MacKenzie and Engelman, 1998; MacKenzie *et al.*, 1997) on dimer formation of glycophorin A in detergents revealed that knob-into-hole packing allows more efficient packing between helices than between helices and

lipids. Tight, knob-into-hole packing has been found to be a general characteristic of helical bundle MPs as well (Bowie, 1997; Langosch and Heringa, 1998). For glycoporphin A dimerization, knob-into-hole packing is facilitated by the GxxxG motif, in which the glycines permit close approach of the helices. The substitution of larger residues for glycine prevents the close approach, and hence dimerization (Fleming *et al.*, 1997; Lemmon *et al.*, 1992; MacKenzie and Engelman, 1998). The so-called TOX-CAT method (Russ and Engelman, 1999) has made it possible to sample the amino acid motifs preferred in helix-helix association in biological membranes by using randomized sequence libraries (Russ and Engelman, 2000). The GxxxG motif is among a significant number of motifs that permit close packing. A statistical survey of MP sequences disclosed that these motifs are very common in membrane proteins (Senes *et al.*, 2000). Although evidence for the importance of this motif in helix-helix interactions is compelling, more recent work suggests that GxxxG alone is not always sufficient for dimerization (Kobus and Fleming, 2005) and that sequence context can affect the strength of dimerization (Doura and Fleming, 2004). Dimerization studies of glycoporphin in detergent micelles (Fleming *et al.*, 1997) do not permit the absolute free energy of association to be determined because of the large free energy changes associated with micelle stability. However, estimates (White and Wimley, 1999) suggest 1 to 5 kcal mol⁻¹ as the free energy cost of separating a helix from a helix bundle within the bilayer environment.

Because the energetic cost of breaking H-bonds within the bilayer HC is so high (see earlier discussion), H-bonding between α -helices should provide a strong stabilizing force for helix association. This is borne out by studies of synthetic TM peptides designed to hydrogen bond to one another (Choma *et al.*, 2000; Zhou *et al.*, 2000). Such interactions are likely to be particularly important in the association of transmembrane signaling proteins (Smith *et al.*, 1996). However, lacking the specificity of knobs-into-hole packing, generalized interhelical H-bonds could be hazardous because of their tendency to cause promiscuous aggregation (Popot and Engelman, 2000). A close examination (Rees *et al.*, 1989; Yeates *et al.*, 1987) of one of the early crystallographic MP structures, the photosynthetic reaction center of *Rhodobacter sphaeroides*, revealed that H-bonds between secondary structure elements were rare and salt bridges nonexistent, except for coordination among four histidines on TM helices with the heme iron. These observations have generally held true for subsequent structures (Bowie, 1997; Cramer *et al.*, 1992; Langosch and Heringa, 1998; Wallin *et al.*, 1997). Recent surveys of interactions between pairs of helices in membrane proteins (Adamian and Liang, 2002; Gimpelev *et al.*, 2004) indicate an average of about one H-bond per pair of helices, with a range

of about one to five per pair. These H-bonds are split about equally between side chain–side chain H-bonds and side chain–backbone H-bonds. Structurally homologous helices in soluble proteins have similar statistics, except that the percentage of side chain–side chain H-bonds is higher (~70%) (Gimpelev *et al.*, 2004).

V. PERSPECTIVES

The lipid bilayer presents a complex environment for the folding and stability of membrane proteins. The hydrophobic effect and Coulombic attraction drive unfolded and partially folded peptides into the bilayer interface where they experience an environment intermediate between water and pure hydrocarbon. Even though this environment is “wet,” the burial of peptide bonds is energetically costly. Hydrogen bonding can reduce the cost, and secondary structure is the result. For soluble proteins, the hydrophobic effect (Tanford, 1973) is the dominant force driving the initial collapse of an unfolded chain toward the folded state—a collapse that is opposed by the unfavorable cost of desolvating the secondary structure (Yang *et al.*, 1992). This desolvation cost is equally important in the overall energetics of membrane protein stability, but is not as apparent because of the complex translocón machinery that manages cotranslational insertion of transmembrane helices [reviewed by Johnson and van Waes (1999), Dalbey and von Heijne (2002), von Heijne (2003), and White and von Heijne (2004)]. The importance of the desolvation penalty becomes apparent from the strong correlation between a biological hydrophobicity scale determined using a microsomal *in vitro* MP expression system (Hessa *et al.*, 2005) and the Wimley–White octanol scale (White and Wimley, 1999). The biological machinery of MP assembly thus does its job by using the fundamental principles outlined in this chapter.

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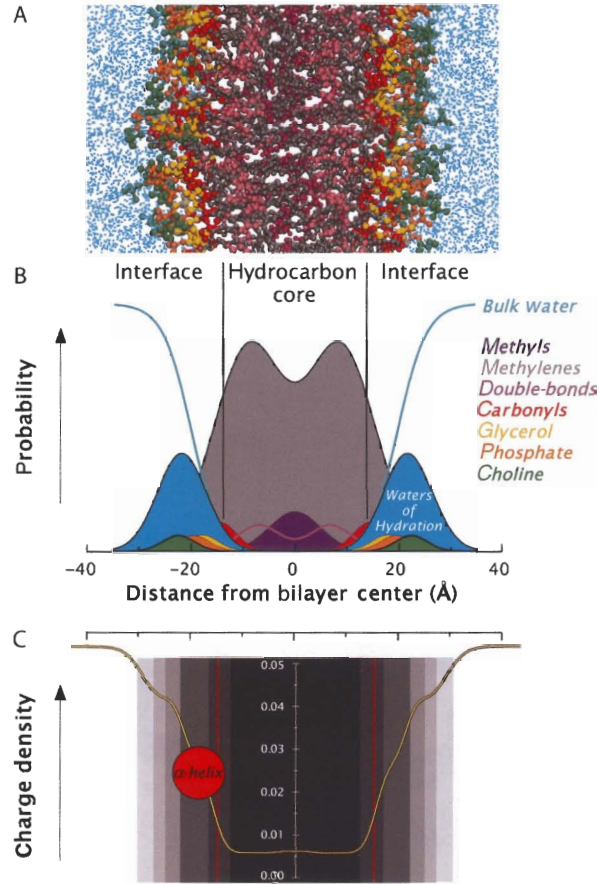
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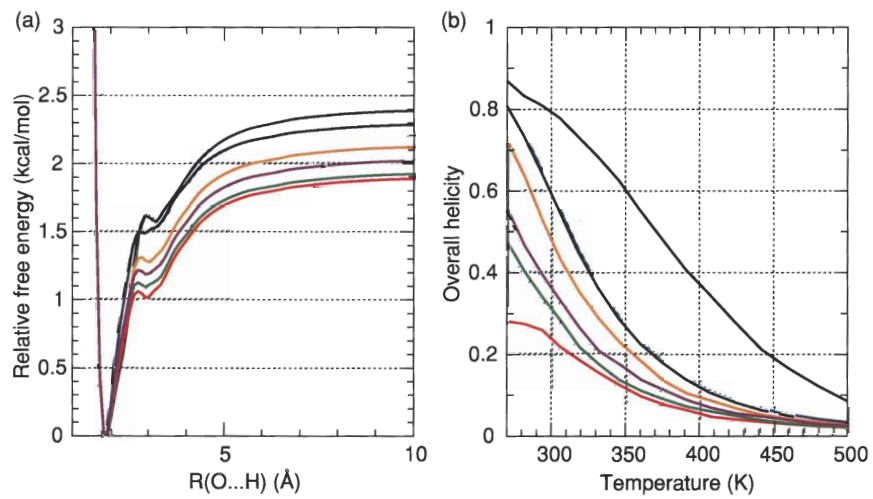
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WHITE, CHAPTER 6, FIG. 1. (See Legend in Text.)



IM ET AL., CHAPTER 7, FIG. 3. (See Legend in Text.)