Helix–helix interaction patterns in membrane proteins

Dieter Langosch[1], Jana R. Herrmann[1], Stephanie Unterreitmeier[1] and Angelika Fuchs[2]

[1]Department für biowissenschaftliche Grundlagen, Technische Universität München and Center for Integrated Protein Science (CIPS[M]), Freising, Germany
[2]Department für biowissenschaftliche Grundlagen, Technische Universität München, Freising, Germany

Abstract

Membrane-spanning α-helices represent major sites of protein–protein interaction in membrane protein oligomerization and folding. As such, these interactions may be of exquisite specificity. Specificity often rests on a complex interplay of different types of residues forming the helix–helix interfaces via dense packing and different non-covalent forces, including van der Waal's forces, hydrogen bonding, charge–charge interactions, and aromatic interactions. These interfaces often contain complex residue motifs where the contribution of constituent amino acids depends on the context of the surrounding sequence. Moreover, transmembrane helix–helix interactions are increasingly recognized as being dynamic and dependent on the functional state of a given protein.

Abbreviations: GpA, glycophorin A; H-bond, hydrogen bond; TMD, transmembrane domain.

1 Introduction

Studying membrane protein structure and assembly has made it clear that interactions and dynamics of α-helical transmembrane domains (TMDs) play a crucial role in their folding, oligomeric assembly, and function. Various aspects around this topic have been covered by excellent recent reviews (Fleming 2000; Popot and Engelman 2000; Shai 2001; Ubarretxena-Belandia and Engelman 2001; Arkin 2002; Helms 2002; Langosch et al. 2002; Chamberlain et al. 2003; DeGrado et al. 2003; Schneider

Corresponding author: Dieter Langosch, Department für biowissenschaftliche Grundlagen, Technische Universität München, Weihenstephaner Berg 3, and Munich Center For Integrated Protein Science (CIPS[M]), 85354 Freising, Germany (E-mail: langosch@tum.de)

2004; Seelig 2004; Bowie 2005; MacKenzie 2006; Matthews et al. 2006; Rath et al. 2007, 2009; MacKenzie and Fleming 2008; Moore et al. 2008; Slivka et al. 2008; Langosch and Arkin 2009).

The importance of transmembrane helix–helix interactions for membrane protein folding was originally indicated by showing that the polytopic light-sensor bacteriorhodopsin could be split proteolytically into several fragments, which could subsequently be reassembled to functional protein (Popot et al. 1986; Ozawa et al. 1997). A role for TMD–TMD interactions in the non-covalent assembly of single-spanning, or bitopic, membrane proteins was demonstrated when the TMD of the major erythrocyte membrane protein glycophorin A (GpA) formed dimers on SDS gels with exquisite sequence-specificity (Bormann et al. 1989; Lemmon et al. 1992a,b). These findings were conceptualized in the two-stage model. In the first stage, transmembrane α-helices are membrane-integrated independent from each others and assemble via sequence-specific helix–helix interactions in the second stage (Popot and Engelman 1990, 2000).

TMD–TMD assembly results in distinct patterns of residue conservation during evolution. Specifically, TMDs of bitopic proteins are more conserved than the remainder of the protein and conservation is stronger at one side of the helix (Zviling et al. 2007). With polytopic proteins, sequence variation is higher where TMD helices face the lipid bilayer than at helix–helix interfaces (Samatey et al. 1995; Stevens and Arkin 2001). Further, single-spanning membrane proteins are more tolerant to mutation in comparison to multi-spanning proteins, where most TMDs contact multiple helices (Jones et al. 1994a,b). Together, this reflects conservation of amino acids at the sites of TMD–TMD packing and highlights their importance for specific interaction. Analyzing high-resolution structures of polytopic proteins showed preferential orientation of aliphatic residue types (Ile, Leu, Phe, and Val) toward the lipid phase while polar residues tend to participate in helix–helix interfaces (Liang et al. 2005). Small and hydroxylated residues (Gly, Ala, Ser, and Thr) prefer regions of high packing density (Adamian and Liang 2001). Neighboring pairs of residues with a high propensity of occurrence include Gly pairs, pairs of an aromatic residue and a basic residue (e.g., Trp–Arg, Trp–His, and Tyr–Lys), of polar non-ionizable residues (e.g., Asn–Asn, Gln–Asn, and Ser–Ser), of two ionizable residues, and of one ionizable residue and a residue with a carboxamide side chain (e.g., Asp–Asn, Javadpour et al. 1999; Adamian and Liang 2001). These contact potentials clearly point at a rich diversity of molecular forces within transmembrane helix–helix interfaces discussed in detail below. They also hint at the mechanisms that provide sequence-specificity of interaction. Nevertheless, we currently only have a rudimentary understanding of the mechanisms that ensure specificity of TMD–TMD interactions and avoidance of promiscuous ones. In addition, it is clear that these

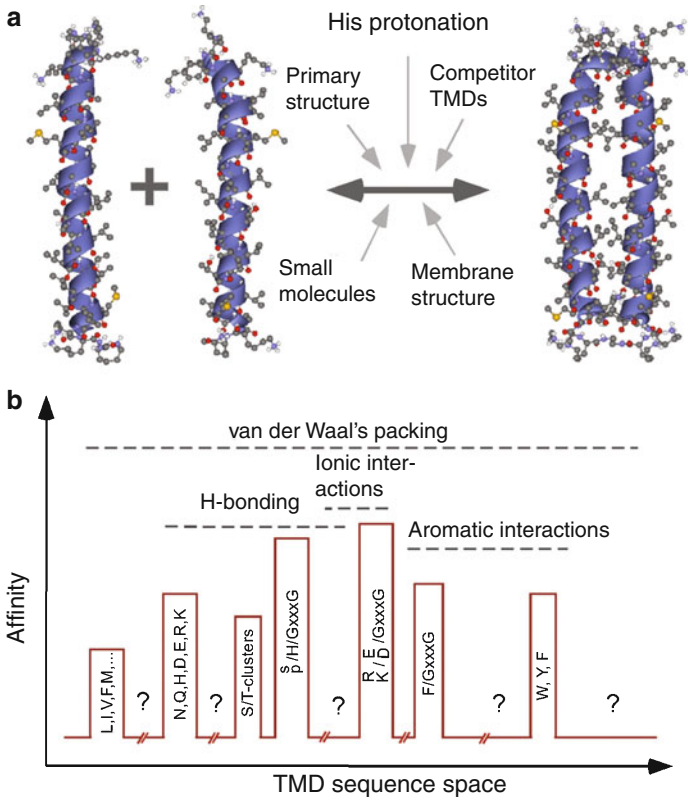


Fig. 1. Molecular basis of transmembrane helix–helix assembly. (**a**) Overview of factors that are known to influence TMD–TMD interaction. (**b**) A simplified depiction of how different types of interfacial residues and motifs might be distributed in TMD sequence space. The baseline of the distribution corresponds to low-affinity non-specific interactions; peak heights are crude estimates based on published data of model cases. Single letter designation for amino acids is used, s and p refer to small and polar, respectively. Part a is modified after Fig. 1 in Langosch and Arkin (2009).

interactions are frequently regulatable by expression of competitor sequences, side-chain protonation, lipid bilayer structure, small molecules, etc. (Fig. 1a). Regulating reversible interactions within the membrane is likely to be essential for regulation of protein function. Also, certain TMDs exhibit more than one interface in a complex, rendering it janus-headed (Rath et al. 2006; Barwe et al. 2007).

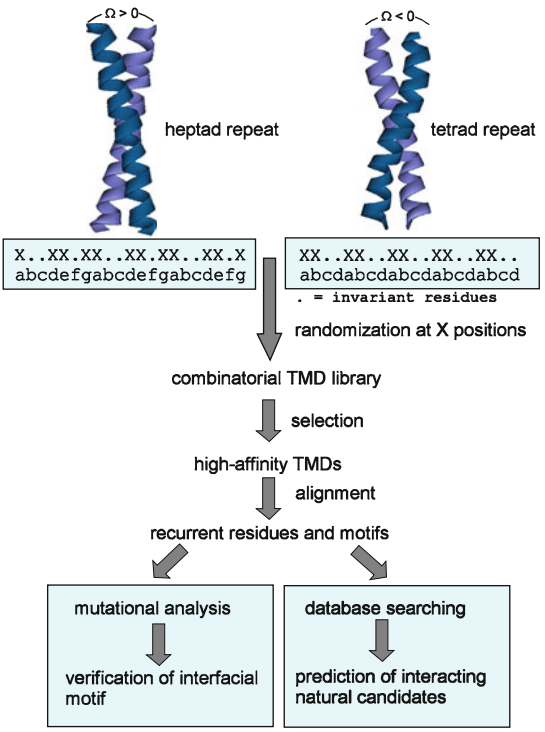
2 Technical approaches to identify transmembrane helix–helix interfaces

High-resolution membrane protein structures, and by implication of TMD–TMD interfaces, are experimentally investigated mostly by X-ray crystallography which

has revealed the structures of about 210 unique polytopic membrane proteins (*http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html*) that currently account for only ~2% of all protein structures. Progress has been slower with bitopic proteins. While the structure of the GpA TMD dimer has already been solved over 10 years ago by NMR studies in detergent (MacKenzie et al. 1997) and later in membranes (Smith et al. 2001), about half a dozen NMR structures have been presented more recently and X-ray crystallography has solved one of them (Oxenoid and Chou 2005; Call et al. 2006; Bocharov et al. 2007, 2008a,b; Schnell and Chou 2008; Stouffer et al. 2008; Lau et al. 2009; Sato et al. 2009; Stein et al. 2009; Wang et al. 2009; Yang et al. 2009).

High-resolution structures provide detailed insight into protein–protein interfaces but do not necessarily identify the most critical residues that may form "hotspots" of interaction within them. Patterns of interfacial amino acids have also been identified by biochemical and biophysical methods that measure non-covalent TMD–TMD assembly coupled to point mutagenesis. Assembly may be examined by gel shift assays, analytical ultracentrifugation, fluorescence resonance transfer, and disulfide exchange in detergent or membranes (reviewed in: Ridder and Langosch 2005; MacKenzie 2006; Merzlyakov et al. 2007; Fleming 2008; Merzlyakov and Hristova 2008). In addition, genetic approaches have been developed where interaction is monitored in a natural membrane environment. These genetic approaches allow investigation of candidate TMDs. In addition, they also permit exploration of TMD–TMD interfaces in a systematic ab initio approach by selection of self-interacting TMDs from combinatorial libraries of randomized hydrophobic sequences (Russ and Engelman 2000; Gurezka and Langosch 2001; Dawson et al. 2002; Ridder and Langosch 2005; Unterreitmeier et al. 2007; Herrmann et al. 2009, 2010). Selection of high-affinity TMDs requires an experimental system where their interaction results in a selectable phenotype. The ToxR transcription activator system has been developed for this purpose (Langosch et al. 1996) and exploits the fact that self-interaction of ToxR-embedded TMDs within the inner membrane of expressing *Escherichia coli* reporter strains enhances expression of chloramphenicol resistance. The ToxR system exists in two versions used for library screening for homotypic interactions, TOXCAT (Russ and Engelman 1999) and POSSYCCAT (Gurezka and Langosch 2001). It has been modified to investigate heterotypic interactions in a dominant-negative fashion (Lindner and Langosch 2006; Yin et al. 2007; Herrmann et al. 2009). The beauty of the library screening approach is that interfacial consensus motifs emerge from alignments of selected sequences and can be verified by mutational analysis and reconstruction on neutral host sequences. Moreover, searching homology-purged databases can reveal whether or not a given motif is overrepresented in natural TMDs. Overrepresented motifs are likely to infer a

a



b

interfacial motif:	variant aa	invariant aa	reference
pa..Ga..Ga..T	G, A, V, L, I, S, T, P, A	L	1
.[S,p]..aG..aG..a	G, A, V, L, I, S, T, P, A	A	1
....F..G...G....	A, C, F, G, I, L, M, R, S, T, V, W	A	2
.[S,p]..[S,p]H......G...G	all natural residues	L	3
....DR......G...G	all natural residues	L	4
.....R.E....G...G	all natural residues	L	4
....S..SS..T	A, T, S, F, V, L, I, P	L	5
...S...SS..T	A, T, S, F, V, L, I, P	L	5
W......W......W.	all natural residues	A	6

p = polar (S, T)
a = aliphatic (L, I, V)
s = small (G, A, S)
. = residues outside consensus motif

Fig. 2. Approaches and outcomes in screening combinatorial libraries for high-affinity TMDs. (**a**) Outline of library construction and screening. The outcome of individual screens depends on whether tetrad or heptad motifs are randomized, on the hydrophobicity of invariant amino acids, and on the complement of codons used for the variant ones. (**b**) Recurrent motifs as identified from different libraries where different interfacial residue patterns had been randomized with different sets of amino acids on different invariant host backgrounds. Ω = helix/helix crossing angle; aa = amino acid. The presence of GxxxG motifs in high-affinity TMDs suggests that the corresponding helix–helix pairs have negative crossing angles, even though a heptad-repeat pattern underlying left-handed pairs had been randomized. References: 1 – Russ and Engelman (2000); 2 – Unterreitmeier et al. (2007); 3 – Herrmann et al. (2009); 4 – Herrmann et al. (2010); 5 – Dawson et al. (2002); 6 – Ridder et al. 2005). Modified after Langosch and Arkin (2009), Fig. 2.

functional advantage for the proteins in question, for example via stronger oligomer formation. Also, database searching leads to testable predictions of related motifs in natural membrane proteins. Figure 2a illustrates the general strategy, while Fig. 2b summarizes the results obtained so far.

3 Structure of transmembrane helix–helix interfaces

The structure of TMD–TMD interfaces is both defined by the geometry of side-chain packing and by more focal forces, like hydrogen bonding (H-bonding), charge–charge interactions, and aromatic interactions. These different forces frequently cooperate to form complex interfaces that exhibit high degrees of sequence-specificity. As a result, the role of individual amino acids tends to be highly dependent on the context of the surrounding structure. In the following, those different forces are discussed separately for the sake of simplicity. Figure1b summarizes how different interfacial amino acid motifs may be distributed in sequence space.

3.1 Amino acid side-chain packing

Due to packing constraints, the long axes of soluble or transmembrane helix–helix pairs usually adopt either positive or negative crossing angles (Chothia 1984; Bowie 1997). In a simplified model, interfacial residues of pairs with positive crossing angles, also termed left-handed pairs, follow a $[a..de.g]_n$ heptad-repeat pattern (where lower case letters represent residue positions) reminiscent of leucine zippers where side chains of one helix form "knobs" that pack into "holes" of the opposite helix surface. The interfaces of pairs characterized by negative angles, or right-handed pairs, correspond to a $[ab..]_n$ tetrad repeat where side-chain packing is less regular than in the "knobs-into-holes" model (Langosch and Heringa 1998; Langosch et al. 2002, Fig. 2a). Accordingly, a recent rigorous structural classification of TMD–TMD pairs from polytopic proteins revealed that about 2/3 of them fall into only four structural clusters, i.e., antiparallel and parallel helices with a limited range of crossing angles that is dictated by the nature of side-chain interactions (Walters and deGrado 2006). This suggests a limited conformation space for TMD–TMD pairs, as predicted based on geometrical considerations (Oberai et al. 2006). However, it has to be borne in mind that the remaining third of these pairs correspond to additional conformations with more varied crossing angles and irregularities in helix structures (mostly wide or tight helical turns that are often associated with kinks, Lehnert et al. 2004). The same broad structural classification seems to hold true for TMD–TMD assemblies from bitopic proteins as indicated by high-resolution structures (MacKenzie et al. 1997; Smith et al. 2001; Call et al. 2006; Bocharov et al. 2007, 2008b; Schnell and Chou 2008; Stouffer et al. 2008) and scanning mutagenesis

(Laage and Langosch 1997; Li et al. 2004b; Ruan et al. 2004a,b; Sulistijo and MacKenzie 2006; Dews and MacKenzie 2007).

The formation of well-packed interfaces is supported by non-directional van der Waal's forces. Albeit weak, undirectional, and strongly dependent on distance, van der Waal's interactions apply to any type of side-chain atom and accumulate over an entire well-packed interface. As such, they are suited to support interaction of TMDs composed of aliphatic residues, such as oligo-Leu helices (Gurezka et al. 1999; Mall et al. 2001; Ash et al. 2004). Similarly, TMDs containing only Leu, Ile, Val, Met, and Phe arranged in a heptad-repeat pattern tend to self-interact with little sequence-specificity, yet are overrepresented in natural bitopic membrane proteins (Gurezka and Langosch 2001). The NMR structure of the ErbB2 TMD (pdb code: 2jwa) dimer provides an example where an interface is primarily composed of non-polar side chains plus a Gly residue at the site of closest contact. The observation that fluorinated interfaces enhance interaction of TMD helices (Naarmann et al. 2006) could be explained by polarization of neighboring side-chain atoms by fluorine.

3.2 GxxxG motifs

GxxxG motifs exist in many TMDs and can induce their interaction. As their function appears to result from different physical forces, they are discussed in this separate chapter. A GxxxG motif has first been seen when interfacial residues of the GpA TMD–TMD homodimer (Lemmon et al. 1992a,b, 1994; Langosch et al. 1996; Fleming et al. 1997; Fisher et al. 1999; Russ and Engelman 1999; Fleming and Engelman 2001; Doura and Fleming 2004; Doura et al. 2004) were mapped by mutagenesis. Identification of the GxxxG motif as such was originally based on the observation that changing the residue spacing between both Gly residues affects dimerization and GxxxG induces self-interaction of model TMDs (Brosig and Langosch 1998). The GpA TMD dimer exhibits a negative crossing angle as implied by molecular modeling (Treutlein et al. 1992; Adams et al. 1996) and confirmed by NMR studies (MacKenzie et al. 1997; Smith et al. 2001). The contribution of GxxxG to an interface is apparently driven by a complex mixture of attractive forces and entropic factors (MacKenzie and Engelman 1998). It has been suggested that it leads to formation of a flat helix surface that maximizes van der Waal's interactions and that the loss of side-chain entropy upon association is minimal for Gly (Russ and Engelman 2000). Moreover, the Gly residues reduce the distance between the helix axes and thus may facilitate hydrogen bond formation between their C_α-hydrogens and the backbone carbonyl of the partner helix (Senes et al. 2001a). The early work on GpA TMD assembly was particularly rewarding since the GxxxG motif and degenerate versions thereof (designated "smallxxxsmall" or "GxxxG-like" with Gly

exchanged for Ala, Ser, Cys, etc.), were later found in many other TMDs, including those of syndecans (Asundi and Carey 1995; Dews and MacKenzie 2007), members of the BNIP family (Sulistijo and MacKenzie 2006, 2009; Bocharov et al. 2007), protein tyrosine phosphatases (Chin et al. 2005), viral envelope proteins (Miyauchi et al. 2005; Arbely et al. 2006), growth factor receptors (Mendrola et al. 2002; Bocharov et al. 2008b), integrins (Gottschalk et al. 2002; Schneider and Engelman 2004; Lin et al. 2006a; Slivka et al. 2008; Wegener and Campbell 2008), and the Alzheimer precursor protein (Kim et al. 2005; Munter et al. 2007; Gorman et al. 2008) where it occurs in tandem.

Screening combinatorial TMD libraries where a tetrad repeat pattern had been randomized yielded high-affinity GxxxG motifs in more than 80% of all isolates (Russ and Engelman 2000), thus underpinning the role of this motif in TMD–TMD interactions. Indeed, database searching identified the GxxxG motif as the most prevalent pair-wise motif in TMDs (Arkin and Brünger 1998; Senes et al. 2000; Unterreitmeier et al. 2007). Overrepresentation of GxxxG relative to statistical expectation demonstrates that its presence supports protein function in evolution. At the same time, the fact that 12.5% of TMDs from non-homologous bitopic proteins contain at least one GxxxG motif (Senes et al. 2000; Unterreitmeier et al. 2007) suggests that mechanisms must have evolved to prevent promiscuous interaction of TMDs with GxxxG. Indeed, the mere presence of such motifs does not reliably predict high-affinity interaction. This is exemplified by the fact that GxxxG present within the ErbB2 receptor TMD lies outside the interface, which extends only over the N-terminal half of the helix (Bocharov et al. 2008b). Indeed, the N-terminal half self-associates with slightly higher propensity than the C-terminal half. It was suggested that interaction of the former one stabilizes the active state of the receptor while the latter one forms an interface in the inactive state (Escher et al. 2009). Further, GxxxG is highly effective within the contexts of oligo-Met and oligo-Val sequences (Brosig and Langosch 1998), but not within either an oligo-Leu TMD, a number of randomized TMDs (Unterreitmeier et al. 2007) or the M13 major coat protein TMD (Johnson et al. 2006). To avoid promiscuous homo- and heterotypic interactions, the impact of GxxxG depends on sequence context (Melnyk et al. 2004). This is underpinned by the finding that the interaction energy of the GpA TMD varies over a wide range after mutation of the sequence surrounding GxxxG (Doura et al. 2004). High-affinity TMDs holding GxxxG may therefore be regarded as islands in GxxxG sequence space. Screening combinatorial TMD libraries has identified some of these islands by showing that GxxxG can form high-affinity interfaces with appropriately spaced Phe (Unterreitmeier et al. 2007), clusters of His and polar/small residues (Herrmann et al. 2009), or ionizable residues (Herrmann et al. 2010) as described below.

3.3 Hydrogen bonding

The role of H-bonds in TMD–TMD interfaces is discussed controversially. On one hand, polar residues were inferred to form extensive H-bond connections that enhance packing between the TMDs of polytopic membrane proteins (Adamian and Liang 2002). Also, Asn and Gln residues strongly promote self-interaction of model (Choma et al. 2000; Zhou et al. 2000, 2001; Gratkowski et al. 2001; Ruan et al. 2004b) or natural (Ruan et al. 2004a) TMDs. These polar residues are thought to form strong interhelical H-bonds within the apolar millieu of lipid bilayers. Apart from hydroxylated side chains and carboxamides, homotypic interaction of model TMDs is also promoted by ionizable residues, including Asp, Glu, His (Gratkowski et al. 2001; Zhou et al. 2001; Sal-Man et al. 2004), Lys, and Arg (Johnson et al. 2007), which may also be attributed to H-bond formation in the absence of an oppositely charged residue on the partner helix.

On the other hand, recent studies suggest only modest stabilization of a bitopic model TMD–TMD interface by H-bonds (North et al. 2006) and H-bonds seem to contribute little toward stability of bacteriorhodopsin in SDS micelles (Joh et al. 2008; see Grigoryan and Degrado 2008 for a discussion of these results). Apart from H-bonds contributed by polar side-chains, it has been proposed that the C_α–H group is capable of participating in H-bonding (Senes et al. 2001b) since the marginal polarity of the C_α proton might be sufficient to serve as an H-bond donor in a highly hydrophobic environment. However, the effect upon stability of a single C_α–H$\cdots$O=C bond in bacteriorhodopsin was estimated by mutagenesis in detergent micelles to be insignificant (Yohannan et al. 2004) and the enthalpy of a similar H-bond in GpA is relatively small (0.88 kcal/mol; Arbely and Arkin 2004) compared to an H-bond extending from a polar side chain (~2–3 kcal/mol).

Thus, the extent to which an H-bond contributes to the stability of a given interface may critically depend on its structural environment. One example underscoring this notion is the finding that high-affinity TMDs isolated from a combinatorial library were enriched for His residues which were frequently accompanied by Gly, Ser, and/or Thr residues at positions i-4 and i-1 relative to His (Herrmann et al. 2009). Mutational analyses confirmed the importance of these residues in homotypic interaction. Probing heterotypic interactions indicated that His residues interact in trans with hydroxylated residues suggesting that hydrogen bonds and possibly aromatic interactions stabilize the interface. Interestingly, the sequences with the highest affinities contained a C-terminal GxxxG motif which results in a [G/S/T]xx[G/S/T]HxxxxxxGxxxG consensus pattern. Reconstruction of minimal interaction motifs on an oligo-Leu sequence supported the idea that His is part of a H-bonded node that may be brought into register by a distant GxxxG (Herrmann

et al. 2009). Isolated His residues support the assembly of model TMDs much less efficiently (Zhou et al. 2001; Herrmann et al. 2009). This exemplifies one case where precise geometric positioning, apparently accomplished here by GxxxG, may be required for optimal stabilization of H-bonds at a distant site. Database searching yielded only few candidate TMDs holding this motif. One of them corresponds to the previously well-investigated BNIP3 TMD. BNIP3 is a Bcl-2 family pro-apoptotic protein that initiates hypoxia-induced cell death. The BNIP3 TMD forms a homodimer characterized by the motif SHxxAxxxGxxxG (Sulistijo et al. 2003; Sulistijo and MacKenzie 2006) and its NMR structure confirmed these interfacial residues in the right-handed pair of helices (Bocharov et al. 2007; Sulistijo and Mackenzie 2009). The BNIP3 TMD–TMD interface thus corresponds to one variant of the consensus motif identified in a library screen. Apart from stabilizing interaction of bitopic subunits, His is also important in interfaces between the helices of polytopic proteins as residue triplets containing His and Ser or Thr are strongly overrepresented there (Adamian et al. 2003).

The context dependence of H-bonds in TMD–TMD interfaces is also supported by the formation of interfaces containing Ser/Thr-clusters (Dawson et al. 2002) and QxxS-motifs (Sal-Man et al. 2005). Self-interacting TMDs with predominant SxxSSxxT and SxxxSSxxT motifs were isolated from a combinatorial library and point mutagenesis showed the requirement of a cooperative network of interhelical H-bonds while single Ser or Thr residues did not promote interaction (Dawson et al. 2002). A QxxS motif was found essential for homodimerization of the bacterial Tar-1 protein and is significantly overrepresented in a bacterial TMD database suggesting its wide-spread role in homodimerization (Sal-Man et al. 2005).

3.4 Charge–charge interactions

Early evidence for charge–charge, or ionic, interactions between TMDs came from studies that probed the location of helices within the membrane. There, pairs of positively charged Lys and negatively charged Asp residues one helical turn apart placed a model helix deeper in the membrane than other spacings of the two residues (Chin and von Heijne 2000). On the other hand, heterotypic interaction of a pair of helices containing either Glu or Lys within an oligo-Leu host sequence did not exceed that of homotypic interaction in liposomal membranes (Shigematsu et al. 2002). The contribution of ionic interactions to oligomeric assembly was also tested for a few natural proteins. One well-investigated system corresponds to the T-cell receptor complex that is composed of single-span subunits. Three basic residues are found in the TMDs of the αβ heterodimeric receptor while a pair of acidic residues is present in the TMDs of each of the three associated CD3γε, CD3δε, and ζζ signaling homodimers. Assembly of the complete oligomer rests on interaction of one basic resi-

due of the central αβ receptor with a pair of acidic residues within any of the signaling modules. Precise geometrical positioning of oppositely charged TMD residues is required for T-cell receptor complex assembly. The underlying TMD–TMD interaction is highly residue specific as Arg and Lys of the αβ receptor heterodimer or Asp and Glu of the associated signaling modules cannot be exchanged for other residues of the same charge without loss of assembly competence (Call and Wucherpfennig 2007). The solvent NMR structure of the ζζ homodimeric signaling module provides some clues as to the structural basis of specificity. In this isolated pair of subunits, helix–helix interaction is stabilized by a disulfide bond and the interface contains a H-bond between Tyr and Thr residues. In addition, one Asp side-chain oxygen of each helix forms an interhelical hydrogen bond to a carbonyl of the opposing strand while one seems to be available for interaction with a basic residue of the receptor. The presence of structural water within the ζζ interface may precisely orient the ionizable side-chains within a network of H-bonds and thus explain residue specificity in charge–charge interaction (Call et al. 2006). A triad of basic and acidic residues also appears to drive assembly of a number of other activating immune receptors (Call and Wucherpfennig 2007). Ionic TMD–TMD interactions can be dynamic, such as in activation of voltage-activated ion channels. There, the sliding helix model posits that sequential formation of ion pairs between Arg residues of the S4 TMD with acidic residues of different surrounding TMDs stabilizes S4 in the membrane and permits its voltage-triggered movement (Zhang et al. 2007; DeCaen et al. 2008).

A set of high-affinity TMDs was recently isolated from a combinatorial library whose members contain both basic and acidic residues at certain positions (Herrmann et al. 2010). The invariant Leu-based host employed here is apparently hydrophobic enough to maintain polar residues within the membrane (Lew et al. 2000; Hessa et al. 2005). A detailed analysis of representative sequences indicated that ionic forces between appropriately spaced basic and acidic residues seem to be essential for interaction. Specifically, an ionizable residue at position i can interact with another one at position i – 1, i + 2, or i + 3. It is quite likely that additional productive combinations exist. Context dependence of these interfacial residues is again apparent since a C-terminal GxxxG starting at i + 7 is essential for high-affinity interaction and neighboring Ser, Cys, Tyr, or His residues contribute to the interfaces. Similar to the polar/His node discussed above, pre-orientation of the helices via interaction of the GxxxG motif may ensure precise geometrical positioning required for charge–charge interaction. Database searching yielded only few TMDs whose potential self-interaction is suggested by a pair of appropriately spaced ionizable residues in combination with GxxxG. However, hundreds of natural TMDs contain either a basic or an acidic residue plus GxxxG. The majority of the latter motifs was

overrepresented and might thus enter heterotypic interactions provided the spatio-temporal co-expression of the respective proteins (Herrmann et al. 2010). In addition to this cooperation of charged residues and GxxxG, it is clear that mechanisms not relying on GxxxG motifs must exist that allow for formation of salt-bridges between the TMDs of those natural proteins where experiment has clearly identified them, like the T-cell receptor.

3.5 Aromatic interactions

Evidence for aromatic interactions between TMDs was originally provided by the frequent interfacial positioning of Trp, Tyr, and Phe in polytopic proteins (Langosch and Heringa 1998; Adamian and Liang 2001; Adamian et al. 2003). Experimentally, a library screen showed that Trp residues of high-affinity TMDs prevailed at *g* positions of the randomized heptad motif. Mutation of Trp residues reduced self-interaction and grafting Trp residues onto artificial TMDs strongly enhanced their affinity (Ridder et al. 2005). A contribution of aromatic residues is also implied by the overabundance of WxxW and YxxY motifs in bacterial TMDs and mutational analysis of one candidate TMD that belongs to the cholera toxin secretion protein EpsM confirmed that WxxW, YxxW, WxxY, YxxY, and single Trp residues support its self-interaction (Sal-Man et al. 2007). A stabilizing role of aromatic–aromatic interactions was also seen when Phe, Tyr, and Trp promoted interaction of model TMDs. Further, cation–π interactions between aromatics and Arg, Lys, or His residues on the partner helix can lead to even higher TMD–TMD affinities (Johnson et al. 2007). In another study, a stabilizing role was observed for Phe when located at the i-3 position of GxxxG of high-affinity TMDs as isolated from a combinatorial library, thus yielding FxxGxxxG motifs (Unterreitmeier et al. 2007). This motif, and a number of analogs with different Phe/GxxxG spacings, is overrepresented in TMDs of natural bitopic membrane proteins. Within the framework of an oligo-Met host, only FxxGxxxG (present in >200 natural TMDs) self-interacted more strongly than GxxxG; thus, other overrepresented variants, such as FGxxxG, GxxFG, GxxxGF, GxxxGxF, GxxxGxxF, and GxxxGxxxF (>1300 natural TMDs) might support heterotypic interactions. It is currently not clear how Phe and GxxxG cooperate to form a helix–helix interface. The role of GxxxG might be to orient the Phe residues such as to promote aromatic–aromatic interactions. Alternatively, the Phe residue could interact with the backbone at a Gly of GxxxG of the partner helix via a C_α–H$\cdots\pi$ interaction known to be prevalent in soluble protein cores (Brandl et al. 2001). Albeit weak, these C_α–H$\cdots\pi$ interactions could be stabilized by the low dielectric environment of membranes as discussed above. A noteworthy observation is that the efficiencies by which the different aromatics stabilized TMD–TMD interactions

in these studies vary widely. While Trp, but not Tyr, promoted interaction of a library isolate where GxxxG was absent (Ridder et al. 2005), self-interaction of certain model TMDs followed the order Phe>Tyr ≈ Trp (Johnson et al. 2007), and only Phe is effective at the –3 position of GxxxG (Unterreitmeier et al. 2007). Therefore, the mechanism and efficiency by which aromatics can induce helix–helix interactions seems to be strongly dependent on the surrounding structure.

4 Dynamic TMD–TMD interactions

TMD–TMD interfaces have been discussed above in the conceptual framework of static structures that are stabilized by mixtures of different non-covalent forces. This picture is appropriate in cases where TMDs mediate kinetically stable subunit oligomerization. It is clear, however, that these interactions may be reversible on time scales that are relevant for biological function. Thus, formation of a TMD–TMD interface appears to depend on the functional state of certain proteins, such as in signal transduction after ligand binding. TMDs may interact reversibly by translational movement within the bilayer plane, rotate relative to each others, or undergo even

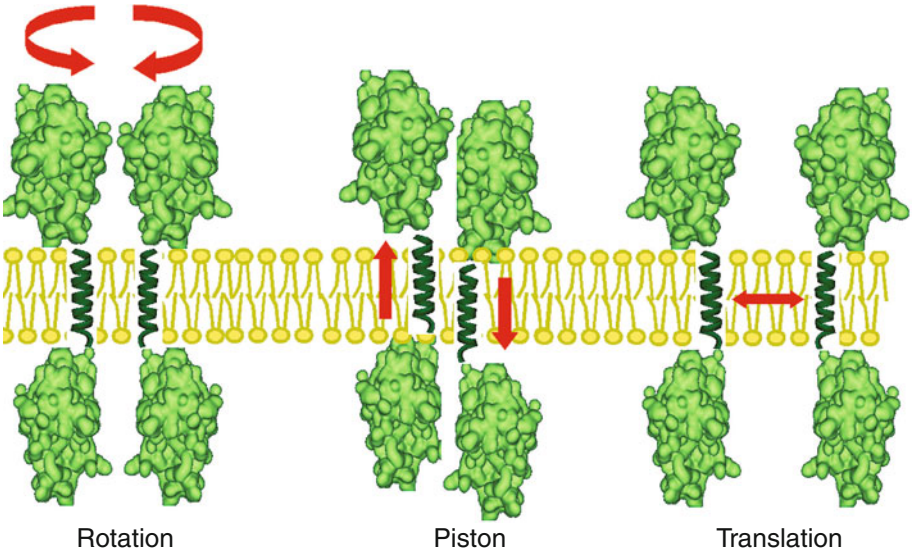


Fig. 3. Dynamics of membrane-embedded protein domains. The activation of bitopic proteins upon binding of soluble ligands to extracellular domains has been proposed to involve the reorientation of transmembrane helices relative to each others about their long axes, reversible association/dissociation, and piston movements. Modified after this figure in Langosch and Arkin (2009).

piston motions (Fig. 3; Matthews et al. 2006; Moore et al. 2008). A few model proteins will be discussed here to illustrate the point. Reversible interactions involving translational movement are proposed to regulate the adhesive function of integrins (Gottschalk and Kessler 2002; Luo and Springer 2006). There, heterotypic TMD–TMD interactions between a set of α and β subunits (Gottschalk and Kessler 2004a,b; Schneider and Engelman 2004; Lin et al. 2006b; Lau et al. 2009) are thought to be displaced in favor of homotypic interaction (Li et al. 2004b) during activation (Li et al. 2004a). Rotation of TMDs relative to each other is a concept that appears to supersede the more traditional idea of ligand-induced dimerization of growth factor receptors. There is now substantial evidence that these receptors can exist as preformed dimers that are stabilized by TMD–TMD interactions. Receptor activation seems to involve TMD rotation in response to ligand-binding to extracellular domains, in case of erythropoietin (Seubert et al. 2003), epidermal growth factor (Moriki et al. 2001), and growth hormone (Brown et al. 2005) receptors. Interestingly, the arrangement of TMDs can also be influenced by direct binding of hydrophobic ligands. For example, the thrombopoietin receptor was activated by a synthetic compound that required a TMD His residue (Nakamura et al. 2006; Kim et al. 2007). Also, modeling studies suggest that the TMD of the ErbB2 tyrosine kinase is able to rotate to adopt two alternate dimerization motifs, thereby controlling the activity of the protein (Fleishman et al. 2002). This view is largely compatible with the idea that upon receptor activation helix–helix interaction moves from the N-terminal helix half to the C-terminal half (Escher et al. 2009). Changing the electrostatics between TMDs is another way to change their orientation relative to each other. The homotetrameric M2 protein from influenza A forms a proton channel, which is activated by lowering the pH. Its TM-helices cross each other at positive angles as indicated by earlier functional (Pinto et al. 1997), biochemical (Bauer et al. 1999), and modeling (Dieckmann and DeGrado 1997) work. The high-resolution structures which have been solved recently (Schnell and Chou 2008; Stouffer et al. 2008) suggest that His protonation promotes channel gating, although it still remains unknown how exactly a pH change opens the pore. Linear and 2D-IR spectroscopic studies have provided evidence that is consistent with a rotation of the helices about their long axes upon pH change (Manor et al. 2009). This rotational change is on the order of one amino acid register and may provide a molecular picture of channel gating.

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