Biochimica et Biophysica Acta 1818 (2012) 963–973

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Transmembrane helix–helix interactions are modulated by the sequence context and by lipid bilayer properties ☆

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ARTICLE INFO

Article history:
Received 14 June 2011
Received in revised form 19 July 2011
Accepted 24 July 2011
Available online 31 July 2011

Keywords:
Membrane protein
Helix dimer
GxxxG
Hydrogen bond
Lateral pressure
Hydrophobic thickness

ABSTRACT

Folding of polytopic transmembrane proteins involves interactions of individual transmembrane helices, and multiple TM helix–helix interactions need to be controlled and aligned to result in the final TM protein structure. While defined interaction motifs, such as the GxxxG motif, might be critically involved in transmembrane helix–helix interactions, the sequence context as well as lipid bilayer properties significantly modulate the strength of a sequence specific transmembrane helix–helix interaction. Structures of 11 transmembrane helix dimers have been described today, and the influence of the sequence context as well as of the detergent and lipid environment on a sequence specific dimerization is discussed in light of the available structural information. This article is part of a Special Issue entitled: Protein Folding in Membranes.

1. Interaction of TM helices

Folding of polytopic TM proteins involves interactions of individual TM helices, and multiple TM helix–helix interactions need to be controlled and aligned to result in the final TM protein structure. Most TM α-helices insert individually (or in small entities) into a lipid bilayer, they might interact to form helix dimers and subsequent interactions eventually do result in formation of the final higher ordered oligomeric structure. Thus, individual helix–helix interactions and TM helix dimer formation are keys for folding of polytopic membrane proteins. This conceptional simplification of the folding pathway has already been summarized two decades ago in the two-stage model of membrane protein folding [1]. However, in polytopic TM proteins, neighboring TM helices, bound cofactors or soluble domains might stabilize a given helix–helix interaction and the structure and stability of larger TM proteins will thus be determined by multiple short- and long-range interactions [2]. Defined interactions, involving packing interactions, hydrogen bonding, aromatic interactions and salt bridges, can determine sequence specific packing of TM helices in single-span as well as in polytopic, multi-span TM proteins [3,4]. Since it is difficult to define contributions of an individual TM helix–helix interaction to folding of larger polytopic TM proteins [5], analyzing and understanding the structural basis for interactions of simple single-span TM proteins that form TM helix dimers will eventually also allow a proper description of more complex TM proteins' folding pathways [6]. Furthermore, determining sequence specificity in TM helix–helix interactions is also crucial to identify and eventually modify structural rearrangements involved in processes, such as TM signal transduction, channel activities or membrane fusion [7,8]. Thus, structural analyzes of selected (simple) TM helix dimers can serve as an excellent starting point to analyze and quantitatively describe folding pathways and structural dynamics of larger proteins, where additional folding events, such as the formation of re-entry loops, cofactor binding or even repositioning of peptide stretches, are part of a more complex folding pathway. Individual single span TM proteins are most likely in the local free energy minima and they adopt nearly canonical helical structures and rotamer orientations of the side chains within a membrane [9]. However, for higher ordered oligomeric TM structures it is not certain that these fold into a single (low-energy) structure, as structural rearrangements are of functional importance, which might already be true for simple TM helix-dimers (as further discussed below).

Today, structures of 11 TM helix homo- or hetero-dimers have been solved mainly by NMR spectroscopy (Table 1 and Fig. 1). Analyzing the structure, assembly and dynamics of such simple TM helix dimers will help to understand interactions of individual TM helices on the level of amino acids and/or amino acid motifs. Following lateral association of individually stable TM helices within a biological membrane provides the opportunity to analyze structural and energetic contributions of e.g. individual amino acids or amino acid motifs to α-helical membrane protein folding as well as to

Abbreviations: GpA, glycophorin A; TM, transmembrane; NMR, nuclear magnetic resonance spectroscopy; DMPC, Dimyristoylphosphatidylcholine; DOPC, 1,2-Dioleoyl-sn-glycero-3-phosphocholine

☆ This article is part of a Special Issue entitled: Protein Folding in Membranes.

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doi:10.1016/j.bbamem.2011.07.035

Table 1
Structures and properties of TM helix dimers solved in recent years. Small residues involved in a GxxxG-like motif are highlighted in bold.

Protein	PDB	Method	Amino acids involved in TM helix packing	Crossing angle	Ref.
GpA	1afo	NMR	^{75}LIxx**G**Vxx**G**VxxT^{87}	−40	[11]
ErbB2	2jwa	NMR	^{651}L**T**xxI**S**AxV**G**I^{661}	−41	[40]
ErbB1/ErbB2	2ks1	NMR	ErbB1: ^{646}IxT**G**MxG**A**xLLxxV^{659} ErbB2: ^{652}**T**xxI**S**AxV**G**IxLV^{664}	−46	[41]
ErbB3	2l9u	NMR	^{49}IxxLVxIFxxLxxxFLxxR^{67}	+24	[48]
αIIbβ3	2k9j	NMR	αIIb:^{972}**G**xxx**G**xxLL^{980} β3: ^{700}VMxxxILxxG^{709}	−25	[43]
EphA1	2k1k	NMR	^{550}**A**Vxx**G**Lxx**G**AxxLL^{563}	−39 to −48	[45]
EphA2	2k9y	NMR	^{535}LAxIGxxAVxxVVxLVxxxxxFF^{557}	+15	[49]
BNIP3	2j5d	NMR	^{161}FxxxFxxxLxxSHxx**A**xxx**G**xx**I**G^{184}	−45	[46]
BNIP3	2ka1, 2ka2	NMR	^{172}SHxx**A**Ixx**G**Ixx**G**^{184}	−34	[47]
ζζ	2hac	NMR	^{2}CxxxDxxLxxYxxxLTxxFxxV^{23}	+23	[50]
DAP12	2l34	NMR	^{9}LxxIVxxDxxLT^{20}	+20	[51]
Sx1A/Syb2	3hd7	X-ray	Syb2:^{95}MxxILxxIxxxIxxIIxxY^{113} Sx1A: ^{268}IxxCxxILxxIxxxT^{282}	+18	[52]

describe structural dynamics in TM helix–helix interactions. Besides sequence-specific TM helix–helix interactions, the lipid environment might be critically involved in inducing and/or stabilizing defined structures of TM helix bundles. Energetically it is highly unfavorable to unfold a TM peptide within a membrane environment, and e.g. disrupting the backbone H-bonds of a 20 amino-acid-long peptide would cost approximately 80 kcal/mol [10]. Within a biological membrane, individual TM helices might interact to form a thermodynamically stable structure. However, as a membrane is highly dynamic and far away from equilibrium in vivo, the highly dynamic lipid environment might locally induce or stabilize one out of several possible TM structures.

2. Sequence-specific TM helix–helix interactions

2.1. The beauty of simple structures

The first structure of a TM helix dimer was reported in 1997 [11]. The structure of the homo-dimeric glycophorin A (GpA) TM domain was solved by NMR spectroscopy in micelles. Several years earlier biochemical analyzes have indicated that the single TM helix of human GpA is able to form a strong dimer in vivo as well as in vitro [12]. Interaction of the GpA TM helix has been analyzed in great detail, and the amino acid motif ^{75}LIxxGVxxGVxxT^{87} has been determined in an extensive mutational study to be key for dimerization [13]. Based on the mutational data, the homo-dimeric GpA structure has subsequently been modeled [14], and the later solved NMR structure has essentially confirmed the modeled structure and the special role of the interaction motif [11]. The core of the GpA interaction interface is the GxxxG-motif. The small side chain of the G residue might allow some structural flexibility of the TM helix, and two small residues in a distance of four allow two helices to closely pack and thereby Van der Waals interactions of neighboring amino acids as well as hydrogen bond formation are promoted. The two G residues cause a void on the surface of one helix, which in the case of GpA is filled by the adjacent V side chains of the interacting helix (Fig. 1A). This “knobs into hole” packing is a prominent way to facilitate tight helix–helix interactions [15]. Furthermore, it has been suggested that due to the close distance of the two adjacent TM helices, C_α atoms of G residues form hydrogen bonds to backbone carbonyl oxygens on the adjacent TM helix [16], which can stabilize the TM helix dimer with 0.9 kcal/mol [17].

Besides few other thus far identified interaction motifs, the GxxxG motif and variations of it are still the most prominent interaction motif identified up to the present day [18,19]. This motif provides a framework for helix–helix interactions and is highly overrepresented in TM helices of membrane proteins [20,21]. However, as the GxxxG-motif only provides a framework, the strength of a given interaction is critically mediated by surrounding amino acids, and a motif of two small amino acids in a distance of four (GxxxG-like motif) alone might not be sufficient to mediate a specific TM helix–helix interaction and additional interactions are required [22–24], as visible in the determined structures of TM helix dimers.

In the original NMR structure of the GpA TM helix dimer solved in micelles, the two helices form a right-handed helix dimer with a crossing angle of ~40° [11]. Van der Waals interactions were monitored between residues I76–L75, V80–G79/2G83, and V84–G83/2T87, whereby the first amino acid is located on one helix and the second on the interacting helix. However, the structure of the GpA helix dimer has subsequently been solved by solid state NMR spectroscopy in lipid bilayer environments, and here the helix crossing angle was determined to be ~35° and additional inter-helical interactions have been described [25–27]. Most importantly, a hydrogen bond in between T^{87} and a backbone carbonyl oxygen of the adjacent helix has been identified, which has not been identified in the solution state NMR structure [27]. While a hydrogen bond of a side chain hydroxyl group might contribute to the stability of a helix dimer by about 0.5 kcal/mol [28], Van der Waals interactions are most likely sufficient to stabilize GpA in micelles, as many mutations of T^{87} only result in a slight destabilization of the helix dimer [13,29,30]. Noteworthy, the solid state NMR structure of the GpA helix dimer has been solved in two different lipid bilayers, DMPC and DOPC, which have hydrophobic thicknesses of about 23 and 27 Å, respectively [31]. Although the thickness of the hydrophobic bilayer core differs substantially, the determined structures were essentially identical [25–27], indicating that the structure of the GpA dimer is not dynamic, which is supported by MD simulations [32,33]. Thus, GpA exists in a simple monomer-dimer equilibrium. However, dynamic structures of TM helix dimers and structural rearrangements are believed to be essential for the physiological function of several TM proteins.

In recent years, motifs of two small residues in a distance of four have been shown to be involved in formation and stabilization of many TM helix–helix interactions, such as integrins or ErbB receptor tyrosine kinases [19,34–36], and in some cases the structures of these TM helix dimers have been solved. Interestingly, within the TM domain of most human ErbB receptor tyrosine kinases, two GxxxG-like motifs are conserved and it has been suggested that interaction mediated by one motif represents the active state, whereas interaction mediated by the second interaction motif corresponds to the receptor inactive state [37–39]. Thus, receptor function depends on the TM dimer alternating in a switch-like fashion between two structures, which are stabilized by either of the two GxxxG-like motifs [34,39]. The structure of the active ErbB2 TM conformation has been solved by NMR spectroscopy in the presence of lipids [40], and ErbB2 TM helix homo-dimerization is mediated by overlapping GxxxG-like (glycine zipper) motifs (Fig. 1B). In contrast to GpA, interaction of the ErbB2 TM domain is mediated and stabilized by the more polar amino acids motif ^{652}TxxxSxxxG^{660}, where the small residues tightly pack and additionally form hydrogen bonds. This rather polar interaction interface is shielded from the hydrophobic lipid bilayer by homo-dimerization as well as by the hydrophobic residues L^{653}, V^{654} and L^{657}, which additionally stabilize the helix dimer by Van der Waals packing interactions. A second putative interaction surface, which involves the motif ^{668}GxxxG^{672}, was not part of the contact surface seen in the NMR structure, and this motif faces to the bilayer. Nevertheless, as this motif is also able to mediate TM helix–helix interactions within biological membranes [37], it has been suggested that in a full-length protein soluble domains stabilize helix–helix interactions mediated by the second interaction motif and thereby stabilize the receptor inactive state. Ligand binding releases a tether in

the receptor binding domain and the structure of the TM helix dimer switches into the thermodynamically more favored structure seen in the NMR analysis. A structural switch, by which ErbB2 is stabilized by either one of the two GxxxG-like motifs, has been suggested to be critically involved in ErbB2 signaling [34,39].

Some residues involved in homo-dimerization of ErbB2 are also involved in formation of ErbB1/ErbB2 hetero-dimers [41]. On the ErbB1 TM helix, the amino acids ^{646}IxTGMxGAxLLxxV^{660} are involved in close TM helix–helix packing (Fig. 1C). The small residues T^{648}, G^{649}, G^{652} and A^{653} favor close packing of the two helices and the more hydrophobic residues of the interaction surface mediate and stabilize the dimer by Van der Waals contacts. In the ErbB1/ErbB2 hetero-dimer the amino acids of the ErbB2 TM helix, which are involved in formation of the ErbB2 homo-dimer, are extended and the interacting surface is formed by the residues ^{652}TxxISAxVGIxLV^{664}. Thus, while the core of the dimerization motif, which involves T^{652}, S^{656} and G^{660}, has remained constant, the residues additionally involved in stabilizing the respective ErbB2 homo- or ErbB1/ErbB2 hetero-dimeric structures, differ. This observation nicely illustrates that while a GxxxG-like motif can provide a framework for defined helix–helix interactions and might be involved in stabilizing a given TM helix structure, the motif not necessarily supports formation of

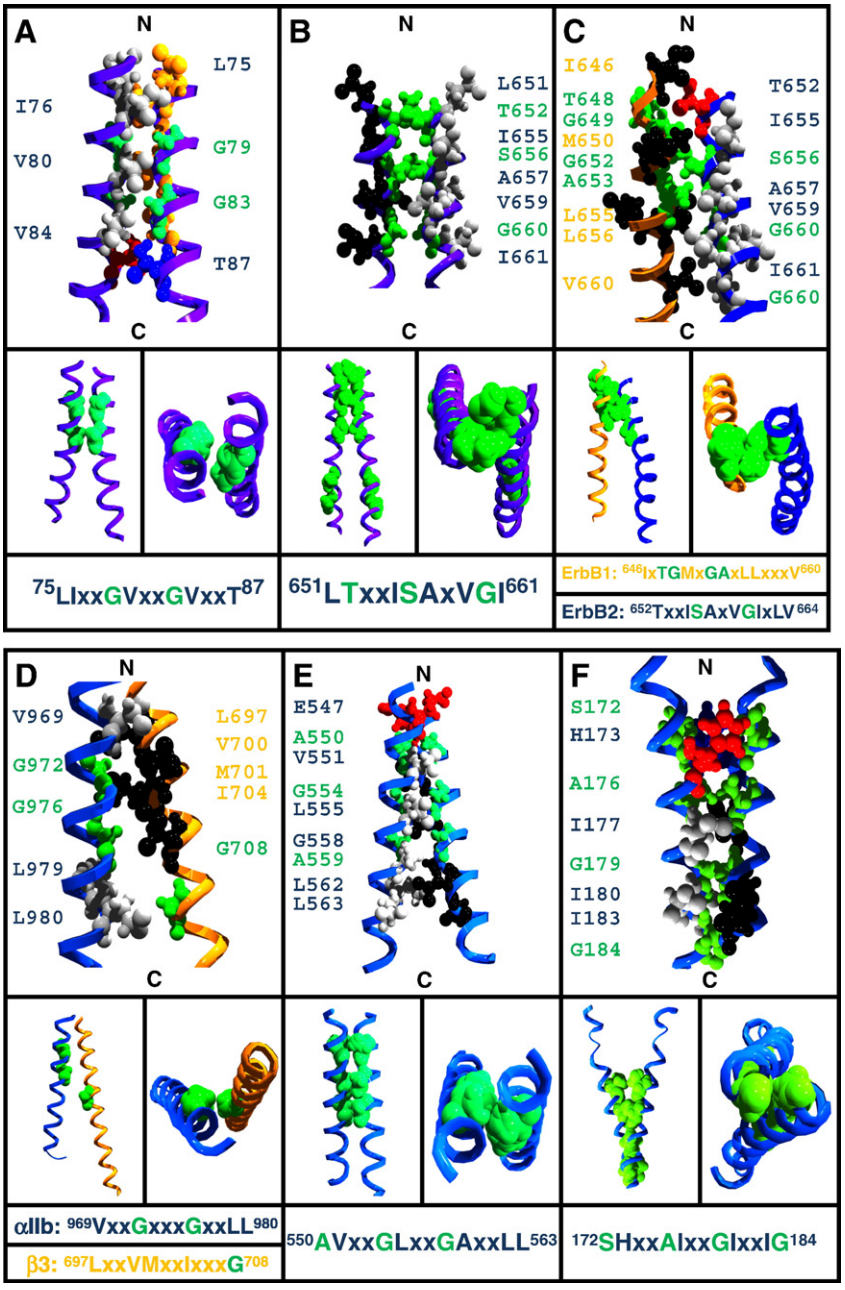

Fig. 1. 3D structures of the TM helix dimers discussed in the text. In each figure an overview of the helix-dimer is shown in the lower left (side view) and lower right (top view). The helices are oriented with the N-terminus upwards and the GxxxG or GxxxG like motifs are shown in Van der Waals representation to demonstrate tight packing. In panels B, E and J GxxxG-like motifs, which are not involved in dimerization, are shown as well. The large figure shows the helix–helix region which is involved in the interaction, and the Van der Waals radius has been decreased for clarity. Residues shown in green are part of a GxxxG (−like) motif and residues shown in gray, black or orange are involved in helix packing. Polar and aromatic residues are shown in red and blue. (A) glycophorin A, (B) ErbB2, (C) ErbB1/2, (D) integrin αIIb β3, (E) Eph1, (F) BNIP3, (G) ErbB3, (H) Eph2, (I) ξξ, (J) DAP12, (K) synaptobrevin 2 (Syb2) and syntaxin 1A (Sy1A) heterodimer.

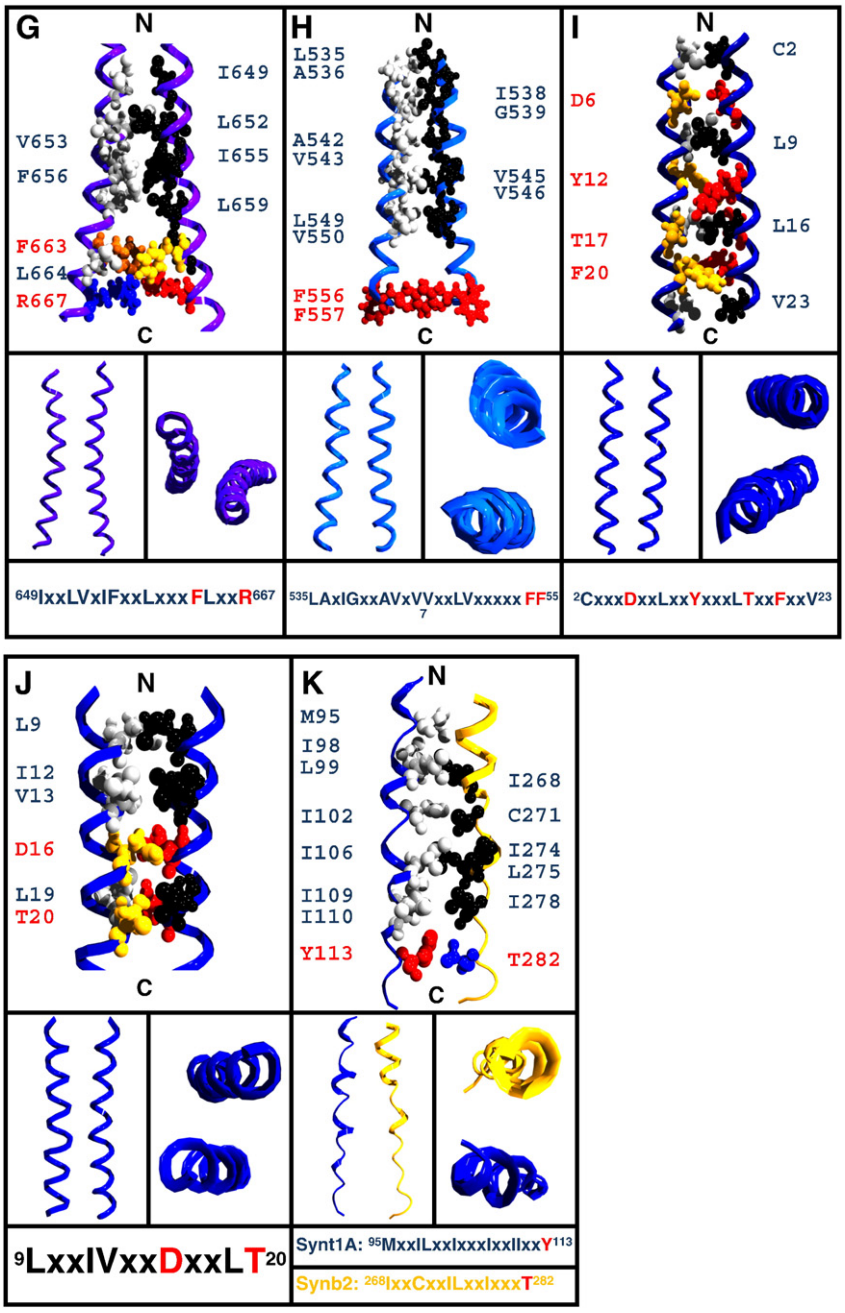

Fig. 1(*continued*).

only a single TM oligomer structure. Surrounding amino acids and the interacting helix determine the specificity and stability of a dynamic helix–helix interaction. Furthermore, the ErbB2 homo- and hetero-dimer structures illustrate that it might be complicated to define a single interaction surface within a given TM helix and multiple interacting helical surfaces might exist in parallel.

Integrin signaling from the inside of the cell toward the outside through a heterodimeric α/β complex was suggested to involve dissociation of the coiled–coiled TM domain [35]. TM helix–helix interactions of integrin TM domains have been shown to be highly dynamic, and involvement of GxxxG-like motifs, which are conserved in the amino acid sequences of integrin TM domains, has been suggested [36,42]. In the NMR structure of the integrin αIIb and β3TM domain hetero-dimer, the TM helices tightly interact with a crossing angle of 30° [43]. The interaction is mainly stabilized by Van der Waals packing interactions (Fig. 1D). On the αIIb TM helix, the small residues ^{972}GxxxG^{976} are involved in dimerization, whereby only the small residue G^{708} of the adjacent β3 helix is involved in distinct packing interactions of the N-terminal TM helix part. The complementary faces of the two interacting TM helices interact in a "knobs into holes" -like fashion and thereby form an extended interface with strong Van der Waals packing interactions, and residues ^{972}GxxxGxxLL^{980} of the αIIb TM and residues ^{700}VMxxILxxG^{708} of the β3 TM domain are directly involved in interhelical contacts. As the helices are tightly packed, mutations in the helix interface easily led to a disruption of the dimer interface [44]. However, other amino acids surrounding these residues also appear to contribute to packing. In the C-terminal TM region the helix-dimer appears to be also stabilized

by stacking interactions of the amino acid F^{992} on the two interacting TM helices as well as by a D-R electrostatic interaction in the juxtamembrane region of the helix dimer.

Electrostatic interactions in the N-terminus of the human EphA1 TM domain are also critically involved in controlling the structure of the Eph1 TM helix dimer [45]. The EphA1 dimer is stabilized by a glycine zipper motif ^{550}AxxxGxxxG^{558} as well as by electrostatic interactions of E^{547} (Fig. 1E). Importantly, at low pH, E^{547} is protonated and uncharged, whereas deprotonation of E^{547} at more basic pH values results in partial unfolding of the helix and in a structural rearrangement of interhelical hydrogen bonds [45]. Under such conditions a second dimer structure, which utilizes a second GxxxG-like motif (^{560}AxxxG^{564}), is partly populated. Thus, the structure of the EphA1 TM helix dimer might be in a dynamic equilibrium and sensitive to the local pH. It is intriguing to speculate that EphA1 serves as a pH sensor, sensing changes in the local environment and in the membrane surface potential, and the local pH induces a structural transition of the TM helix dimer structure.

A pH-sensitive dimer structure has also been reported for the BNIP3 TM peptide [46,47]. The energetically most stable structure is stabilized by three small residues organized in a tandem GxxxG-like glycine-zipper motif, which allow close helix–helix contacts involving C_α-hydrogen bonding as well as close Van der Waals packing of bulkier side chains against the small residues on the adjacent helix monomer [46]. The identified dimerization interface includes the amino acids ^{161}FxxxFxxxLxxSHxxAxxxGxxIG^{184} and involves the two polar amino acids S^{172} and H^{173} (Fig. 1F). The glycine-zipper motif properly aligns the side chains of these two residues in such a way that an interhelical S–H hydrogen bond forms, which stabilizes the dimeric BNIP3 structure. Sulistijo and MacKenzie have suggested that the dimeric structure of the BNIP3 TM helix is modulated by the detergent/lipid environment, indicating that more than one dimeric structure exists [47]. Indeed, in Bocharaov et al. a second less populated dimeric structure has been identified, and MD simulations have indicated that protonation of H^{173} results in loss of the hydrogen bond to S^{168} on the adjacent helix [46]. Furthermore, upon protonation of H^{173}, the side chains of S^{172} as well as of H^{173} turn more toward the lipid polar head group region, and accessibility of these two residues to water increases dramatically, possibly leading to an increased water permeability of the membrane. Thus, the identified H–S tether might also be a pH sensor triggering the structure and function of the helix dimer.

The above discussed observations suggest that stabilization of the available (right-handed) structures of TM helix dimers typically involves GxxxG-like motifs at the contact surface. Furthermore, structural analyzes indicate that defined amino acids as well as structural dynamics might modulate the GxxxG-mediated interaction propensity of TM helices.

While several of the dimeric TM peptide structures are stabilized by GxxxG-like motifs, the existence of such a motif does not per se define a specific interaction surface. ErbB1 and ErbB2 appear to interact via GxxxG-like motifs, whereas the recently resolved NMR structure of a third ErbB-family member, ErbB3, indicates that TM helix dimerization is mediated by another motif [48], although a GxxxG-like motif is also conserved in the ErbB3 TM region and has been shown to be able to mediate and stabilize TM helix interactions [37]. In the left-handed ErbB3 structure, an extended and rather hydrophobic amino acid motif ^{49}IxxLVxIFxxLxxxFLxxR^{67} is involved in dimer formation and stabilization (Fig. 1G) [47]. Residues 49–59 are in close contact and stabilize the helix dimer by Van der Waals packing interactions. The aromatic ring of F^{63} stabilizes the dimer by stacking interactions as well as by π-cation interactions with the guanidine group of R^{67}. In contrast to the ErbB2/ErbB2 and ErbB1/ErbB2 dimers, the area of the helix–helix contact surface is significantly larger in case of the ErbB3 homo-dimer. The increased surface area together with the F-stacking and π-cation interactions highly stabilize the dimer structure. While ErbB3, just as all ErbB family members, contains a conserved GxxxG-like motif in the TM domain, experimental data had already indicated that the ErbB3 TM helix dimer might be stabilized by another amino acid motif [37], as later supported by the NMR structure. Since structural rearrangements have been suggested to be important for TM signaling of ErbB TM domains, it is still likely that the ErbB3 GxxxG-like motif stabilizes another, energetically less favored, TM helix dimer structure [34]. Intriguingly, as ErbB3 is the only member of the human ErbB family having an inactive kinase domain, the different dimerization mode of ErbB3 might reflects physiological differences in signal transduction and receptor function [34].

Similar to the structures of the ErbB hetero- and homo-dimers, the EphA1 and EphA2 TM helix dimers are also differently stabilized. The EphA1 TM helix dimer is stabilized by an amino acid motif involving a glycine zipper (compare above), whereas EphA2 helix dimerization is mediated by the extended motif ^{535}LAxIGx-xAVxVVxxLVxxxxxFF^{557}, which involves amino acids located along the entire EphA2 TM helix (Fig. 1H) [49]. The bulky side chains of L^{535}, I^{438} and V^{543} pack tightly into voids on the adjacent helix formed by A^{536}, G^{539} and A^{542}. The two F side chains stabilize the dimer via π-stacking and the hydrophobic side chains of V^{543}, V^{545}, V^{546}, L^{549} and V^{550} interact and stabilize the dimer by Van der Waals packing interactions. While the solved EphA2 structure represents a left-handed helix dimer, the EphA1 TM helix forms a right-handed helix dimer stabilized by a C-terminally located dimerization motif (compare above). The geometry of the TM helix dimer is most likely affected by the properties of the surrounding membranes, as different thicknesses of the lipid bilayer appear to induce and/or stabilize different dimeric EphA2 structures, which are stabilized by alternative dimerization motifs and contact surfaces on the helix [49]. A structural rearrangement would result in a switch from a right-handed structure, as seen in the case of EphA1, to a left-handed structure, as seen in the case of EphA2, and different dimeric structures might correlate with signaling competent and incompetent structures.

The ζζ TM homo-dimer, which is a subunit of the human T-cell receptor complex, is mainly stabilized by polar interactions, and the residues ^{6}DxxLxxYxxxxTxxF^{20} are critically involved in dimer formation and stabilization (Fig. 1I) [50]. The hydroxyl hydrogen of Y^{12} hydrogen bonds to the hydroxyl oxygen of T^{17} on the adjacent helix, and the resulting two interhelical hydrogen bonds are located at the edges of the dimer interface. D^6 forms hydrogen bonds to the adjacent helix and mutation to other polar amino acids severely reduced the dimerization propensity of the ζζ peptide [50]. The only hydrophobic residue L^9 is most likely involved in Van der Waals packing interactions, which further stabilize the TM helix dimer. Interestingly, the ζζ structure was solved in mixed detergent micelles containing DPC and SDS in a ratio of 5:1. SDS was needed to monitor a proper NMR spectrum and reduction of the DPC/SDS ratio to 10:1 or higher resulted in unspecific aggregation. Thus, the detergent environment was critical for formation of the proper ζζ dimer structure.

A pair of acidic residues is also essential for homo-dimerization of the DAP12 TM peptide, another component of the human T-cell receptor complex [51]. The DAP12 TM helix also forms a left-handed homo-dimer, which is stabilized by the amino acid motif ^{9}LxxIVxxDxxLT^{20} (Fig. 1J). The hydrophobic residues are in close enough contact to form favorable Van der Waals interactions. The two D^{16} residues of the interacting helices form interhelical hydrogen bonds, which are vital for stabilizing the TM helix dimer structure. Noteworthy, while the DAP12 TM helix contains a highly conserved GxxxG-like motif, in the NMR structure this motif is not involved in interhelical packing and the G residues were located outside the contact surface. Since immunoreceptor activation involves structural rearrangements in the TM region, the highly conserved GxxxG motif is possibly involved in other TM interactions [51].

Similarly, while the TM domain of human synaptobrevin 2 also contains a GxxxG-like motif, these residues are not involved in the helix–helix interactions seen in the NMR structure [52]. The left-handed TM helix hetero-dimer of synaptobrevin 2 and syntaxin 1A is stabilized by Van der Waals interactions involving the residues ^{95}MxxILxxIxxxIxxIIxxY^{113} on the synaptobrevin and ^{268}IxxCxxILx-xIxxxT^{282} on the syntaxin TM helix (Fig. 1K). The structure of the TM helix-dimer has been solved in different detergent environments resulting in formation of two different space groups and, more importantly, the structure of the helix-dimers slightly differed [52]. Different interhelical contacts possibly have a function during membrane fusion reactions.

2.2. Summary I: The sequence context modulates GxxxG-mediated TM helix–helix interactions

While in recent years several amino acid motifs have been described to be involved in mediating and stabilizing TM helix–helix interactions, the GxxxG-motif and variations of this is still the most prominent and best characterized interaction motif. Originally identified in TM domains of receptor tyrosin kinases [53], GxxxG-mediated interaction of TM helices was mostly studied in the framework of the human GpA TM helix. While GpA forms a rather stable TM helix dimer, the sequence context highly determines the thermodynamic stability of a GxxxG-mediated TM helix dimer and, as e.g. observed in case of ErbB3 or synaptobrevin 2 (see above), a GxxxG-like motive might not even be involved in TM helix–helix interactions.

In the following we briefly summarize factors identified in the structural analyzes, which are involved in controlling the stability of a given GxxxG-stabilized TM helix dimer (Fig. 2).

1. Interactions of soluble domains might hinder formation of a thermodynamically favored TM helix oligomer, as e.g. suggested in the case of human ErbB receptors.
2. The sequence context surrounding a GxxxG (−like) motif determines the potential of two helices to form a TM helix dimer, as two helices have to pack closely and the geometry of the interacting helical surfaces needs to be aligned, so that two helices can pack in a “knobs into hole” -like fashion. Thus, all amino acids involved in helix–helix contact determine the strength and crossing angle of the helix-dimer. Van der Waals interactions of amino acids surrounding a GxxxG (−like) motif are important for formation and stabilization of a given structure.
3. Hydrogen bonds might stabilize the structure of a helix dimer, formation of which involves a GxxxG (−like) motif. Hydrogen bonds might form in between C_α hydrogen atoms and backbone carbonyls on an interacting helix, side chains of the less polar amino acids' side chains of S, T, Y or H might be involved, and hydrogen bonding might even involve highly polar residues, such as D or E. Reversible protonation of H and E might trigger structural rearrangements of TM helix dimer structures.
4. π-stacking interactions of the aromatic amino acid side chains can also stabilize and/or modulate a structure formed by a GxxxG (−like) interaction motif.

3. TM helix oligomers, lipids and detergents

3.1. Modulation of TM protein structures by detergent and lipid properties

Sequence specific dimerization of individual TM helices can also be significantly influenced by the lipid or detergent environment. In several of the above described structural studies it has been recognized that the detergent environment severely influences the dimerization propensity of TM helices and the structure of a TM helix dimer. In case of the ζζ TM helix dimer, a small amount of SDS appeared to be necessary to stabilize a dimeric TM helix structure [50], and SDS has also been used to determine the structure of the DAP12 TM helix dimer [51]. While several TM helix oligomers form SDS-stable structure, SDS is typically considered to be a rather harsh detergent. Thus, the described observation might be surprising, and typically milder detergents are used to stabilize TM helix structures. However, SDS can have a dual effect on membrane proteins: on one hand it is a denaturing detergent, which in case of the ζζ dimer might avoid unspecific peptide aggregation, whereas on the other hand the negatively charged SDS head group attracts protons in its close vicinity and therefore the local pH on the surface of mixed micelles containing SDS, is significantly more acidic [54]. A pH sensitive dimer has been reported for the TM cytochrome b_{559}′ as well as for the structure of the BNIP3 dimer, where protonation of a single His residue has been suggested to alter the dimer structure [46,54]. In a lipid bilayer the local lipid environment surrounding a BNIP3 TM helix oligomer might create a local pH gradient and the actual structure of the TM helix dimer is controlled by moving BNIP3 in or out of defined lipid domains having local pH differences. Similarly to SDS in mixed micelles, lipids with negatively charged head groups might concentrate protons locally.

Based on the structure of the GpA TM dimer solved in DPC micelles, dimerization is mainly guided by the GxxxG motif, whereas no hydrogen bond has been identified [11]. In contrast, the structure of a GpA dimer solved in lipid bilayers by solid-state NMR suggested formation of a hydrogen bond in between the T side chain and a

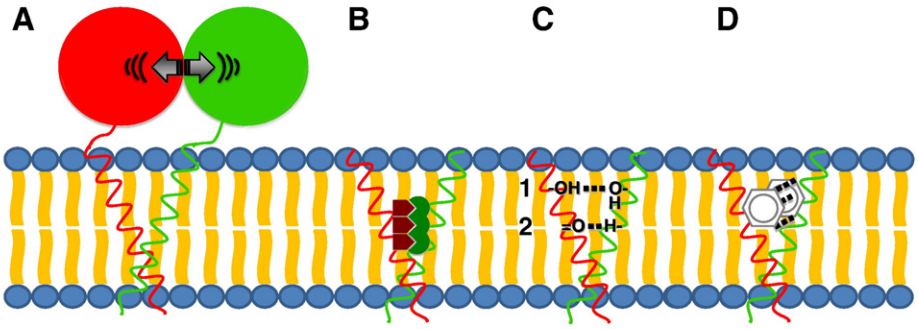

Fig. 2. Modulation of GxxxG-mediated helix–helix interactions by the sequence context. Interactions might be modulated by (A) the soluble domains, (B) defined packing interactions, (C) hydrogen bond formation or (D) π-stacking interaction between aromatic side chains. For details see the text.

backbone carbonyl oxygen on the adjacent helix [27], suggesting a direct impact of the detergent/lipid environment on the structure of the GpA TM helix dimer. Noteworthy, when the GpA structure was analyzed in lipid bilayers possessing different membrane thicknesses, the structure of the dimer was essentially preserved, and thus GpA appears to exist in a simple monomer-dimer equilibrium [25–27]. While the GpA TM helix dimer is stable in low SDS concentrations [55] and can be visualized on SDS-gels after polyacrylamide gel electrophoresis [12], truncated GpA peptides did not form stable dimers in SDS anymore [56]. Interestingly, in this study amino acids, which are located C-terminally to the hydrophobic GpA TM region and are (based on the NMR structures) not involved in dimerization, were clipped off. While the dimerization interface of the GpA TM domain has been mapped in an extensive mutagenesis approach [13], the described observation clearly suggests that interactions of the juxtamembrane part are also essential for formation of a stable GpA TM helix dimer in SDS, and thus it is not (solely) the TM helix–helix interaction which renders the GpA helix dimer SDS stable. Dimerization of GpA in SDS micelles thus depends on at least three types of interactions: sequence-specific TM helix–helix interactions, the juxtamembrane region and the detergent environment. While GpA forms stable dimers in SDS, dimerization of the GpA TM helix was highly abolished in SDS-like detergents with different acyl chain length [57], indicating an influence of a mismatch between the hydrophobic GpA TM helix and the hydrophobic core of the detergent micelles. In line with this, dimerization of helices 3 and 4 of the human CFTR protein directly depends on the detergent acyl chain length [58], and the hetero-dimer was most stable in a detergent having an acyl chain of 9 carbons. While the cross-sectional geometry of the C9-detergent micelle (~23.1 Å) does not match the hydrophobic length of the CFTR TM helices (~31.5 Å), the diameter of the C9-detergent micelle might increase upon insertion of the TM helices, thus favoring optimal helix packing. In the larger hydrophobic core region of micelles formed from detergents having longer acyl chains, helix dissociation might be energetically favored to prevent polar peptide regions from incorporation into the hydrophobic micelle core [58]. Such studies clearly highlight an important role of the detergent/lipid environment for folding and stability of oligomeric TM proteins. As interactions of the GpA TM helix appear to highly depend on the actual detergent environment [59,60], the thermodynamic stability of the helix-dimer within the eukaryotic plasma membrane still has to be established. However, recent data indicate that the interaction strength of the GpA TM helix in a membrane might be lower than currently assumed [61,62].

The local lipid environment and the defined properties of individual lipids might crucially define the structure of a TM helix oligomer. The lipid acyl chains can e.g. vary significantly in their length and thereby determine the actual thickness of a membrane. TM proteins might adjust their individual tilt angle and the oligomeric state, in order to cope with hydrophobic mismatch conditions. Besides varying a TM helix tilt angle, helices might deform the bilayer thickness locally [63]. Simple model TM peptides aggregate unspecifically under hydrophobic mismatch conditions, which already highlights the importance of hydrophobic matching conditions with regard to formation and stabilization of correct oligomeric TM helix structures [64,65]. When sequence-specific dimerization of the GpA TM helix was analyzed in model membranes having increasing bilayer thicknesses, interaction was strongest under hydrophobic matching conditions [62]. Conceptionally, formation of the correct GpA TM helix dimer might require the individual GpA TM helices to align with a correct tilt angle of about ~20°, which is energetically less favorable under hydrophobic mismatch conditions. Thus, if the hydrophobic thickness of the bilayer does not promote formation of a properly tilted TM helix, the monomer-dimer equilibrium will be shifted toward the monomer. Similarly, lateral association of the viral M2 TM peptide into tetramers is modulated by the phospholipid acyl chain length and is stronger in a POPC lipid bilayer under hydrophobic matching conditions [66]. The M2 tetramer adapts to the lipid bilayer thickness by varying the helix tilt from 35° to 15° to minimize the hydrophobic mismatch between the oligomer and the lipid bilayer [67,68]. Thus, as in case of the GpA TM helix, the helix tilt relative to the membrane can lead to favorable helix–helix interactions and can thereby influence the folding and function of membrane proteins. However, as the lipids analyzed in this study also differed in their chemical properties, such as the amount of double bonds, other parameters, such as the lipid order and the fluidity of the membrane, might also have influenced the observed interaction propensities. Beside the thickness of a hydrophobic membrane core, the acyl chain order can indeed dramatically influence the stability of a TM helix dimer. The GpA TM helix dimer is more stable when the acyl chains are ordered, thus when the membrane is less fluid [62]. Cholesterol, which is present in many eukaryotic membranes, has various effects on biological membranes [69]. Cholesterol slightly increases the thickness of a membrane and incorporation of cholesterol results in ordering of the lipid acyl chains. Any change in motional freedom of the lipid fatty acyl chains, for example caused by addition of cholesterol, will lead to a decrease in chain entropy, which also leads to an unfavorable helix–lipid interaction and can drive the self-association of TM helices [62,65,66,70]. On the other hand, decreasing the lipid acyl chain order by addition of the local anesthetic phenyl ethanol affects the interaction propensity of the human GpA as well as of other human receptor tyrosine kinases TM helices significantly [71].

Clearly, the numerous restrictions imposed by the bilayer environment on TM peptides can also explain the bilayer-dependent activity of some multi-helix spanning proteins [72]. It has also been suggested that these lipid bilayer effects on helix–helix interactions could explain the fact that associations of TM helices, as observed in crystal structures of detergent solubilized proteins, are slightly looser compared to packing that would occur in a lipid bilayer. Furthermore, it has been observed that the hydrophobic thicknesses of membranes along the exocytotic pathway increase from the endoplasmic reticulum via the Golgi to the plasma membrane [73–75]. In addition, the lipid composition as well as the cholesterol content of individual membranes within an eukaryotic cell differ substantially [76]. Thus, as it is desirable to have a given membrane protein active only in a defined intracellular membrane system and the membrane proteins encounter very diverse lipid environments after synthesis, activity might be controlled by the actual lipid environment, which induces or stabilizes a signaling competent and/or functional protein structure. Some membrane proteins are active in the plasma membrane and the protein must not become functional en route to the plasma membrane, since this might severely disturb the function of an organelle or even of the whole cell [50,77,78]. Controlling an oligomeric TM protein structure by the local lipid environment might be a powerful mechanism to regulate the activity of membrane proteins.

Concentrating a TM domain within a restricted lipid area and controlling the monomer-dimer equilibrium by the protein-to-lipid ratio might regulate the function of TM proteins within the heterogeneous lipid bilayer environment. Within a given membrane, the local peptide-to-lipid ratio might differ substantially and local lipid domains within a membrane provide local control of TM helix structures. In many of the above described TM dimer structures, structural rearrangements have been described or suggested, which are believed to be important for signaling of the full-length TM proteins. However, while thus far biophysical analyzes on sequence specific TM helix–helix interactions often allow proper description of a single TM helix oligomer structure, such studies typically do not allow describing structural dynamics of a TM helix dimer, which strongly depend on the local membrane environment. The complexity of biological membranes is often not reflected properly by the far too simple detergent environments or model membranes used in most in vitro studies. The phase of a biological membrane exists at a steady state, with quasi-equilibrium at maximum describing local lipid

domains within a given membrane [76]. Different interactions between various membrane components result in small but defined heterogeneity within a membrane plane, and due to phase separation, surface domains of different lipid compositions and physical properties are induced. At the boundaries of such separated lipid phases, the membrane properties change dramatically. Proteins and protein structures can display a clear preference for a certain membrane phase and cells eventually use such abrupt changes to e.g. colocalize or separate certain membrane proteins or membrane protein structures [79]. Vice versa, proteins might also influence the local phase of a membrane by specific protein–lipid interactions.

Besides the sequence context and the membrane thickness, dimerization of the GpA TM helix also depends on the position of the dimerization motif within the TM domain. GxxxG motifs located in the center of the hydrophobic membrane region promote stronger helix–helix interactions than those proximal to TM helix ends [80]. This observation might be explained by the differences in the membrane pressure profile [81–83]. In lipid bilayers the polar lipid head groups are located on the outside surfaces and face an aqueous environment, whereas the hydrophobic acyl chains are directed inwardly. This creates an asymmetry in the transverse direction. Thus, since TM helices are incorporated into the lipid bilayers, they experience pressure modulations directly within the membranes, which might in turn affect helical packing and stability. While at equilibrium the sum of the forces acting on the lipid bilayer is essentially zero [81,82], individual contributions to the total lateral pressure in general will act at different depths. Positive lateral pressures occurring at some depth acting in the plane of the bilayer must therefore be balanced by negative pressures elsewhere. The exact nature of the lateral pressure profile depends on numerous factors, including size, charge and hydration of the lipid head groups, acyl chain length and degree of saturation or branching of the acyl chains and presence of the membrane active compounds that modulate the lipid packing [72,84]. Unfortunately, there is no direct experimental method available to evaluate the lateral pressure profile modification in lipid bilayers. However, the recent advent of pressure-susceptible fluorescent probes emerges as a promising important tool to investigate pressure profiles in the lipid bilayers [85–88].

Besides lateral heterogeneity within a cellular membrane, the different lipid composition within each monolayer of a bilayer membrane structure adds another level of complexity. The bilayer is composed of two lipid leaflets, which differ in their composition in eukaryotic membranes [76] as well as in some prokaryotic membranes [89]. This asymmetry in the lipid distribution is also reflected in the structure of membrane proteins [68]. The inner leaflet of the eukaryotic plasma membrane appears to be more electronegative than the outer leaflet, which e.g. contains highly glycosylated lipids. Differences in the local lipid composition and phase behavior in between the two leaflets of a bilayer might influence the structure and stability of a TM protein, since e.g. interactions within the plane of one leaflet are more stabilized. For example, an asymmetric membrane patch formed by neighboring POPC and POPE require 51 lipids in the POPC leaflet and 64 lipids in the POPE leaflets, so that the average area per lipid in each leaflet reproduces approximately the average area per lipid in corresponding single-component bilayers [90]. However, the asymmetric distribution of the respective lipids between the two membrane leaflets leads to a non-zero membrane potential [90], which might affect TM protein structures differently within a lipid bilayer.

3.2. Summary II: Lipids control the structure of TM helix-dimers and oligomers

Beside defined amino acid motifs, the “solvent” of a TM protein, the lipid bilayer, severely influences the structure and stability of TM helix oligomers. While in several of the solved TM protein crystal structures lipids are tightly bound and thus have to be considered as cofactors [91], most lipids surrounding a TM helix are not tightly bound and might conceptionally be considered as a solvent . The properties of the solvent influence TM helix–helix interactions in several ways (Fig. 3):

1. The thickness of the hydrophobic bilayer core region influences TM helix–helix interactions severely. Whereas hydrophobic matching conditions appear to stabilize sequence specific oligomerization, hydrophobic mismatch might stabilize unspecific TM helix aggregation.

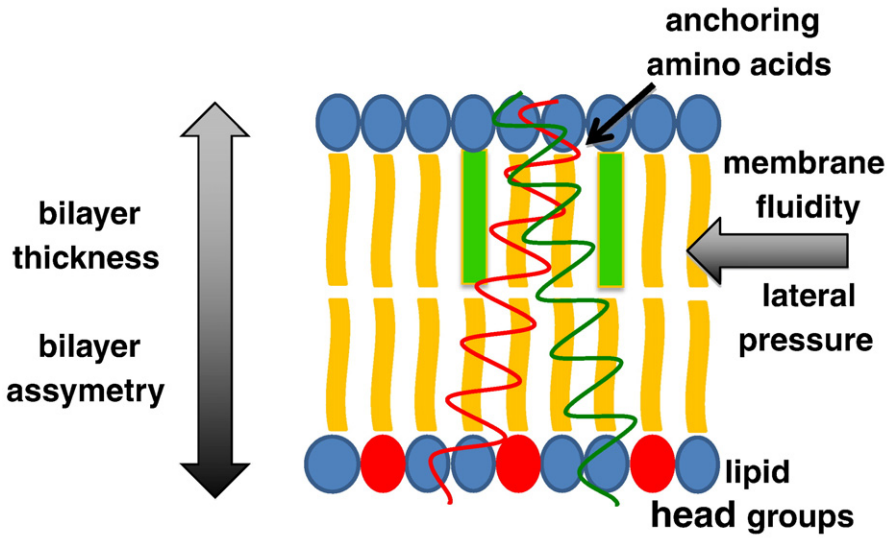

Fig. 3. Lipid bilayer properties and protein–lipid interactions control the structure of TM helix dimers. Bilayer properties, such as the bilayer thickness, bilayer asymmetry, membrane fluidity and the lateral pressure profile of a membrane might influence a TM protein structure. The nature of the lipid head groups, as well as interactions of anchoring amino acids with lipid head groups influence TM helix orientations and structures of TM helix bundles. For details see the text.

2. The acyl chain order and the fluidity of a lipid bilayer directly control the stability of a TM helix oligomer.
3. The nature of a lipid head group can influence the structure and function of a TM protein significantly, e.g. negatively charged lipid head groups might locally attract protons and thereby lower the local pH. The local pH at a membrane stabilizes or destabilizes a given TM oligomeric structure by reversible protonation of individual amino acid side chains.
4. The amino acid side chains of the so-called "anchoring amino acids" R, K, W and Y interact directly with lipids and such interactions might also control the orientation of a single TM helix within a membrane and thereby the structure of a TM helix oligomer.
5. The lateral bilayer asymmetry creates local lipid domains with defined properties. The lipid composition of lipid domains might have a distinct lateral pressure profile, which stabilizes or destabilizes a defined helix–helix interaction.
6. Concentrating defined TM proteins in restricted lipid domains results in local concentration of TM domains and thus alters the lipid-to-protein ratio locally. This might shift a monomer-dimer equilibrium.
7. The two leaflets of a lipid bilayer might have distinct lipid compositions and this asymmetry might control formation and/or stabilization of distinct TM protein structures.

4. Outlook

Dynamic interactions of TM helices are critically involved in different physiological processes, such as signal transduction across membranes or membrane transport processes. Several mutations within TM regions of human TM proteins are described, which lead to severe diseases such as cancer or cystic fibrosis [92,93]. Thus, it will be vital to be able to properly correlate the sequence of a TM domain to the structure, and analyzing only a single TM protein structure most likely does not result in unraveling the structural basis of a given disease.

In many diseases a lipid component is involved and lipid molecules even serve as signaling molecules. However, local release of a distinct lipid or local lipid modification will certainly result in altered lipid properties within a restricted area of the lipid bilayer. This might locally also affect or disturb the structure and function of TM proteins. It has been noticed already two decades ago that e.g. in cancer cells increased fluidity of a membrane significantly correlates with the malignant potential [94,95]. Increased membrane fluidity might activate or inactivate certain TM proteins, resulting in disturbed signaling pathways. In line with this, some drugs are membrane active, and it has been suggested that the ability of a drug to alter a membrane fluidity correlates with the drug activity [96–98].

Solely a detailed analysis as to how a specific sequence context as well as to how the lipid bilayer environment influence the structure and function of a TM protein will result in a complete picture describing dynamics involved in TM protein functions.

Acknowledgments

This work has been supported by grants from the Stiftung Rheinland-Pfalz für Innovation, the Deutsche Forschungsgemeinschaft (SCHN 690/2-3 and GRK 1478), the German-Israeli Foundation, the Centre of Complex Matter (COMATT) and the University of Mainz. We thank Hildegard Pearson for critically reading the manuscript.

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