proteins. This project seeks to determine which amino acids are necessary or present in the transmembrane segment as well as the juxtamembrane segments. It also seeks to better characterize the physical orientation of the fibroblast growth factor receptor transmembrane segment in the membrane and how the amino acids are oriented within the helix. An exclusive database of transmembrane proteins and juxtamembrane domains was created to search for trends, homology, and potential phosphorylation sites. Even though only a very limited amount of homology was found, the transmembrane segment from the fibroblast growth factor receptor will be used as a model and synthesized and characterized through a variety of biophysical techniques such as multidimensional NMR spectroscopy, circular dichroism, and fluorescence spectroscopy.

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An Empirical Scoring Function for the Transmembrane Helical Protein Assembly

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We present a coarse-grained scoring energy able to identify near-native transmembrane (TM) helices pairs of an ensemble generated by two different strategies: Rigid Body Monte Carlo simulations and a collision detection algorithm (Seetharaman et al., work in progress). We test the scoring energy on a set of four known native transmembrane helix pairs. Near native structures are identified with C_α root mean squared deviation (RMSD) lower than 3 Å. This empirical energy function is based on a knowledge based potential obtained from a representative set of globular protein structures. We compensate packing differences of globular and helical membrane protein structures by adding a residue solvent accessible area energy term based on a membrane partition scale obtained from the membrane insertion by the Sec translocon (Hessa et al., Nature 450, 1026-1030, 2007). In addition, we strengthen the interactions among small and polar amino acids that improve the scoring of topologically correct near-native structures. We conclude that our scoring energy function favors near-native conformations of TM dimers without structural knowledge extracted from the yet small set of known membrane protein structures. This proposed method circumvents intensive membrane protein molecular dynamics simulations opening the possibility of further refinement of near-native TM structures through atomistic MD simulations.

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Structure-Functional Insight into Transmembrane Helix Dimerization by Protein Engineering, Molecular Modeling and Heteronuclear NMR Spectroscopy

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The interaction between transmembrane helices is of a great interest because it directly determines biological activity of membrane proteins. Either destroying or enhancing such interactions can result in many diseases related to dysfunction of different tissues in human body. One of the most common forms of membrane proteins is a dimer containing two membrane-spanning helices associating laterally to form a tight complex. Development of new types of drugs targeting membrane proteins requires precise structural information about this class of objects. Recent development of protein engineering, optical spectroscopy, molecular modeling and heteronuclear NMR techniques made it possible studies of the nature and mechanisms of important helix-helix interactions inside the membrane mimicking supramolecular complexes. Using a robust strategy we investigated recombinant transmembrane fragments from different families of bitopic membrane proteins including receptor tyrosine kinases, amyloid precursor and pro-apoptotic proteins, which play important roles in normal and pathological conditions of human organism by providing cell signaling, maintaining cellular homeostasis and controlling cell fate. We characterized thermodynamics of transmembrane helix association, diverse helix-helix packing interfaces and obtained detailed atomistic picture of the intra- and intermolecular (protein-protein, protein-lipid and protein-water) interactions, that along with the available biochemical data provided useful insights into the membrane protein functioning in norma and pathology.

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Transmembrane Helix-Helix Interactions in the Human Single-Span Membrane Proteome

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Most integral membrane proteins form non-covalent functional complexes that are frequently supported by sequence-specific interaction of transmembrane helices [1]. It has been suggested that non-covalent membrane protein multimerization may substitute for the frequently observed multi-domain organization of soluble proteins [2,3]. Here, we aligned human single-span membrane proteins with orthologs from other eukaryotes and examined the sidedness of transmembrane helices. We find that almost half of the human single-span membrane proteins possess a transmembrane helix with unilateral conservation. We propose unilateral conservation in most cases to indicate the presence of a helix-helix interface as well as the strength of interaction since it correlates well with experimentally determined self-affinities. This suggests that unilateral conservation is a good predictor of homotypic TMD interaction and underlines that transmembrane helix-helix interactions significantly contribute to protein assembly in the human single-span membrane proteome.

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Self-Association of Transmembrane Domains of ErbB2 Receptors in Cholesterol-Containing Membranes

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The transmembrane domain of ErbB2 receptors presents two separate GxxxG motifs that are proposed to be connected to stability and activity of the dimer. Recently developed parallel Monte Carlo methods are employed to study the association of ErbB2 TM domains in cholesterol-containing membranes with coarse-grained models that retain a level of amino-acid specificity. Extensive sampling along separation between the two helices shows that GxxxG motifs play a critical role during the recognition stage. In pure phospholipid bilayers association occurs by contacts formed at the C-terminus promoted by the presence of phenylalanine residues. Helices subsequently rotate to eventually form a stable dimer favored by lipid entropic contributions. In contrast, at intermediate cholesterol concentrations a different pathway is followed that involves dimers with a weaker interface towards the N-terminus. However, at high cholesterol content, a switch towards the C-terminus is observed with an overall non-monotonic change of the dimerization affinity. This conformational switch modulated by cholesterol has important implications on the thermodynamic, structural and kinetic characteristics of helix-helix association in lipid membranes.

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Assembling the Transmembrane Domain of Vpu from HIV-1

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Vpu from HIV-1is an 81 amino acid monotopic viral membrane protein involved in the amplification of viral replication. Vpu is identified to down regulate membrane proteins of the host e.g. CD4, CD74, CD317 and BST-2/Tetherin. Based on the findings that Vpu exhibits channel activity especially when reconstituted into lipid membranes the protein is also proposed to act as a viral channel forming protein (VCP) in vivo. How Vpu is supposed to form the channel is unknown.