Chapter 1: Introduction

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* 1. ****Introduction to membrane proteins****

Membrane proteins (MP) are a vast category of proteins found tethered to the cellular membrane. MPs comprise 25-30% of the proteins found within protein-coding genes of various organisms (Fagerberg et al., 2010). Some are found bound to the edges of the membrane (peripheral) while others are embedded into the membrane (integral). This membrane is a bilayer composed of hydrophobic small molecules known as lipids, separating the cell from the outside environment. Integral MPs receive and orchestrate interactions between cells and the outside environment, relaying sensory information (G-protein coupled receptors), regulating pH and ion balance (Na+-K+ pumps), and promoting signaling cascades to regulate expression of important genes (receptor tyrosine kinases). However, the hydrophobic nature of this lipid bilayer environment results in a complex network of interactions responsible for MP folding and stability. Misfolding of MPs affects not only the aforementioned interactions but has been found to be involved in several human diseases such as Parkinson’s, cystic fibrosis, and cancer (Sanders and Myers, 2004; Gregersen et al., 2006). To fully understand how MPs are involved in essential biological functions and to combat the progression of diseases, it is necessary to learn how and why MPs reach their folded state.

However, studying MP folding is inherently a difficult challenge because of their hydrophobic nature. MPs are difficult to express in high yields for biophysical experiments, and purification and solubilization of these proteins often lead to aggregation or unfolding (Carpenter et al., 2008). Additionally, proteins fold differently depending on the composition of lipids in the bilayer, a daunting task to replicate these biological conditions either *in vitro* or *in silico*. To combat these challenges, much of the research studying membrane protein folding is focused on combining *in vitro* or *in silico* studies with live cell research, aiming to explore and understand the impact of biophysical forces governing the folding process in a natural environment.

My graduate work focuses on developing an *in silico* algorithm to design novel single-pass membrane proteins known as homodimers, then using high-throughput experiments to test and validate my computational models. In this introduction, I expand on the forces found to be involved in membrane protein association, with a particular focus on the lack of knowledge on the impact of van der Waals packing. I then introduce current experimental and computational methods that inspired my research. I then highlight an important sequence and structural motif GASright, an important control for my research in developing information on van der Waals packing.

* 1. ****Forces involved in membrane protein folding and association****

Folding of integral transmembrane (TM) MPs involves a variety of energetic constraints due to the hydrophobic nature of the lipid bilayer. First, MPs must be translated and inserted properly into the membrane. This is accomplished through the aid of the translocon, a protein that in conjunction with the ribosome properly translates MPs into the proper orientation in the bilayer. However, once the protein is embedded into the membrane, the hydrophobicity at the core of the bilayer results in an energetic penalty for any unpaired hydrogen bond donors and acceptors (Marinko et al., 2019; Popot & Engelman, 1990, 2000). This forces TMs, composed of backbone containing oxygen and nitrogen atoms, to conform to standard secondary structures with backbone amide protons and carbonyl oxygens satisfying the membranes lack of hydrogen bonding. These TMs can be separated into two groups: beta barrels, large proteins composed of repeating layers of beta sheets, and alpha helices, proteins twisted in a coil like helix structure. My research focuses on the latter group of proteins.

As alpha helices are threaded by the translocon into the membrane, the process to bring the MP to its folded state is regulated by a distribution of hydrogen bonds, weak polar and electrostatic interactions, and van der Waals forces between the unfolded and folded states. Hydrogen bonding regulates secondary structure within TM insertion, but hydrogen bonding between polar amino acids on opposing helices have been shown to drive membrane protein folding (Johnson et al., 2007; Yano et al., 2002; Zhou et al., 2001). Additionally, electrostatic interactions between positively charged residues (Lys) and electronegative ring amino acids (Tyr, Trp, Phe) results in association between helices (Johnson et al., 2007). However, not many studies have solely accounted for van der Waals forces between atoms coming in close contact.

* 1. ****The importance of van der Waals packing****

Even if hydrogen bonding or polar interactions play a significant stabilizing role, because van der Waals occurs between any nonbonded atoms in close contact, it is a necessary force that is always present within the folded state. This means that van der Waals packing is essential for folding, but the extent at which packing can be a driving force for membrane protein folding is unclear (expand on this, maybe bring up that image that Alessandro has).

The contribution of van der Waals packing to membrane protein folding can be broken down into three distinct interactions: lipid-lipid packing, lipid-protein packing, and protein-protein packing. Lipid-lipid packing involves the interactions between individual lipid molecules that are necessary to keep the bilayer assembled. Lipid-protein packing occurs between these lipid molecules and parts of proteins exposed to the lipid environment. Protein-protein (or sidechain) packing focuses on the stability gained between individual or portions of proteins in close contact. Each of these forces plays a role in the ability for a protein to be stabilized in the bilayer. When an individual protein subunit is inserted into the membrane, it must destabilize the lipid-lipid packing with more favorable lipid-protein packing interactions. For protein-protein packing to occur, these newly formed lipid-protein interactions must be destabilized for a more favorable combination of protein-protein packing and lipid-lipid packing. Protein-protein (or sidechain) packing, is a technically feasible starting point because of the ability to manipulate sequences and determine changes in stability due to mutation.

Previous research has demonstrated that disruption of sidechain packing within the core of bacteriorhodopsin destabilizes protein structure (Faham et al., 2004; Joh et al., 2009). In addition, a recent study using MP design has shown that optimized sidechain packing can stabilize the folded state of the 5-helix bundle protein phospholamban (Mravic et al., 2019). Although these studies suggest that sidechain packing plays a significant role in stabilizing MP structure, there has not been much investigation of the energetic contribution of packing outside of individual MP systems. My research aims to characterize and quantify the extent to which sidechain packing is a driving force for MP folding for the general population of MP structures.

* 1. ****GASright****

The GASright is one of the most prevalent sequence and structural motifs found in TM proteins (Walters & DeGrado, 2006). GAS is an acronym for the three amino acids typically found in the sequence: Glycine, Alanine, and Serine. These small residues define the interface of the motif (G/A/S)xxx(G/A/S), resulting in a short interhelical distance between TM helices. Right originates from the structural features in which TM helices associate with a right-handed crossing angle. Additionally, it is frequently found in a variety of biological systems involved with immunology, metabolism, and cancer. Due to its potential importance in medical applications as well as its well-defined sequence and structural features, GASright proteins have been used as a simple and tractable system to further understand forces governing TM association.

The GASright motif’s unique sequence and defined structure has been shown to permit an uncommon structural feature. The short interhelical distance allows TM backbones to come in close contact, forming a network of weak hydrogen bonds where donors are Cα carbons and acceptors are carbonyl oxygens on the opposite helix (Cα–H∙∙∙O=C, or Cα–H bonds). Carbon atoms are not commonly associated with hydrogen bond donors because carbon is a less electronegative atom than other donors nitrogen and oxygen. However, these carbon atoms are found near electronegative withdrawing groups on the peptide backbone, increasing their electronegativity. Estimates from quantum mechanics calculations suggest that the stabilizing energy of an Cα–H bond may contribute one third to one half of that of an N—H donor in vacuum (Scheiner et al., 2001; Vargas et al., 2000). Anderson et al. utilized a combination of computational structure prediction and the experimental assay TOXCAT to determine the influence of this network of Cα–H bonds. By predicting and analyzing GASright motifs found in natural sequences using *in vivo* TOXCAT, they showed that structures with more Cα–H bonds had a higher stability in the dimer state (Anderson et al., 2017). Additionally, Díaz-Vázquez et al. measured the free energy of association of GASright structures using *in vitro* FRET, concluding that the thermodynamic stability of GASright proteins is well correlated with the *in vivo* TOXCAT (Díaz Vázquez et al., 2023). Both studies posit that the primary forces involved in GASright association are a combination of hydrogen bonding and van der Waals packing. Because the goal of my research is to understand the extent to which packing can drive proteins to associate, this motif is an excellent control to evaluate my designed sequences against for their propensity to associate using solely van der Waals packing.

* 1. ****Studying membrane protein folding and structure****

The complex nature of the lipid bilayer makes it difficult to directly study the forces involved in MP folding. Many groups focus on utilizing a combination of experimental and computational tools to estimate the contribution that these biophysical forces have on MP stability. In particular, thermodynamic experiments return results that can be relayed into information regarding the stability of proteins in specific conditions. This thermodynamic information can be used to assess and validate computational models, which can be further implemented to design and engineer novel proteins. In this section, I will summarize different experimental and computational approaches that have been used to study the association and folding of single pass TMs, including the ability to solve and predict MP structures using both experimental and computational methods.

****1.5.1 Structural methods****

In 1995, the protein data bank (PDB) was established to collaborate and share protein structures globally online. This tool allows researchers to deposit structures alongside literature, allowing people from around the world to access their findings. Initially, proteins structures were solved primarily using x-ray crystallography. X-ray crystallography has contributed to solving ~80% of MP structures (Kermani, 2021). However, producing crystals at high resolution remains a difficulty in crystallography. To bypass the need to crystallize proteins, many other MP structures have been solved by nuclear magnetic resonance (NMR). In particular, solid-state NMR has bypassed the need for detergents in crystallography, obtaining structures of MPs less than 50 residues within lipid bilayers or nanodiscs (Liang & Tamm, 2016).

More recently cryo-EM has been used to solve MP structures. In addition to bilayers and nanodiscs, it is possible to solubilize and obtain the structures of MPs within detergents, saposin-lipoprotein nanoparticles, amphipols, and peptidiscs (Januliene & Moeller, 2021). This technique allows MP structures to be studied in a large variety of different environments, giving researchers the ability to study alternative structures of these proteins by changing the solubilization conditions. Despite these advancements in MP structural characterization, many of these efforts take years to determine conditions that yield publishable results in high resolution. MPs make up only 4.6% of structures deposited in the PDB (April 2024; PDB). Lacking structures for MPs to assess and study, researchers focus on using other methods to study MP folding and association.

1.5.2 Quantitative methods

To determine changes in folding or association states of proteins, many groups take an *in vitro* approach using fluorescence. One popular approach is Förster resonance energy transfer (FRET). In FRET, fluorescent proteins that emit different wavelengths of light, or fluorophores, are first attached to two individual proteins. The emission spectrum of the donor fluorophore overlaps with the excitation spectrum of the acceptor fluorophore. If the proteins associate, then the donor fluorophore transfers its energy to the acceptor fluorophore, resulting in emission of the acceptor fluorophore. Conversely, if there is no association, there will only be donor fluorescence. Using FRET, researchers have determined the thermodynamics of association for a variety of proteins, including GpA (Díaz Vázquez et al., 2023; Fisher et al., 1999; Maeda et al., 2018; You et al., 2005).

Mass spectrometry (MS) of MPs is a growing field, with more groups using it to study stoichiometry and dynamics of protein association. MPs are ionized within MS compatible detergent solutions, and spectra analyzed to assess the homogeneity of the MP in solution (Laganowsky et al., 2013). However, MS is traditionally used to analyze larger protein complexes. Native MS is a complementary approach that is able to analyze a larger range of proteins, including small TMs. This method preserves noncovalent interactions in MPs by using non-denaturing ionization conditions, with aqueous buffers near physiological pH at lower temperatures and voltages. By determining the composition and abundance of distinct complexes, MS is able to return interaction data reflecting MP oligomeric states (Keener et al., 2021).

Sedimentation equilibrium analytical ultra-centrifugation (SE-AUC) is a technique that has been used to study the association of TM helices by solubilizing MPs in detergent and centrifuging the solution at high speeds. By analyzing the concentration gradients that form at different centrifugation speeds, researchers can calculate the equilibrium constant for various oligomeric states. Association energetics were calculated for multiple mutants of GpA, determining that differences in association were due to the loss of van der Waals packing (Doura & Fleming, 2004; Fleming et al., 1997; Fleming & Engelman, 2001). However, these *in vitro* techniques rely on producing large quantities of MPs and solubilizing them in appropriate solutions for each application, which can alter MP structure. Alternative experiments rely on *in vivo* approaches to study association and folding of MPs.

1.5.3 Genetic reporter assays

Genetic reporter assays have been utilized to investigate the folding and association of MPs in their natural environment. Cells express MPs of interest fused to a DNA binding domain that can either inhibit or promote transcription of a reporter gene. GALLEX is a two-hybrid system where TMs are fused to DNA binding domain LexA. Association of the TMs results in repression of the β-galactosidase gene (Schneider & Engelman, 2003). Another reporter assay known as TOXCAT has also been used to study TM helix-helix interactions. The TM of interest is fused to ToxR, a dimeric transcription factor, and maltose binding protein to determine proper insertion. When the TMs associate, ToxR dimerizes and promotes the expression of chloramphenicol acetyltransferase (CAT). TOXCAT has been utilized to study the association of GpA and a variety of its mutants to better understand the impact of hydrogen bonding and van der Waals packing on association (Anderson et al., 2017; Russ & Engelman, 1999)

Recently, TOXCAT has been adapted into a high-throughput assay TOXGREEN. The gene for CAT has been replaced with green fluorescent protein (GFP), allowing fluorescent readings to be used to assess the association levels of the MPs of interest (Armstrong & Senes, 2016). The development of this assay allows it to be utilized in high-throughput applications such as cell sorting, where a library of TMs can be expressed, sorted, and sequenced through next generation sequencing (NGS). These results can then quantified to determine the relative association propensities for each protein present in the library. I utilized this technique to quantify the association for the proteins that I computationally designed to determine the impact of van der Waals packing on association.

1.5.4 Computational methods

In conjunction with structural determination, computational methods have been created to evaluate previously solved MP structures. Many methods look to further understand the MP folding by developing energetic terms that aim to estimate the thermodynamics of association. The Baker Lab at University of Washington are experts in this area, utilizing known energetic and structural information for the development of the software suite Rosetta. Rosetta houses a variety of energy functions and prediction tools for soluble environments, including the ability to dock or design proteins *de novo*.

The original Rosetta energy functions have been adapted to accommodate predicting helical TMs within the membrane environment. The updated functions include an energy term that models the membrane into layers classified defining atoms as water-exposed, polar, interface, and hydrophobic (White & Wimley, 1999; Yarov-Yarovoy, Schonbrun, et al., 2006). Successful predictions determined structural details in MPs associated with voltage sensing and gating mechanisms (Vargas et al., 2012; Yarov-Yarovoy, Baker, et al., 2006). Recently, RosettaMP was developed to enhance the functionality of MP prediction which includes modeling TM helices *de novo* from sequence, a representation of the membrane bilayer, and the ability to transform a protein into the membrane (Koehler Leman et al., 2017). These tools increase the accessibility to MP structure, improving the ability to visualize and predict structures of MPs that have not yet been solved. Simultaneously, energetic predictions enhance the knowledge in the field by allowing researchers to assess structural mutations *in silico* prior to testing with *in vitro* or *in vivo* experiments.

Another computational tool for membrane proteins is the Molecular Software Library, or MSL. Similar to Rosetta, MSL houses a variety of tools to perform MP structure prediction. These tools include the ability to transform proteins in space, mutate specific residues, extract geometric information from known structures, and predict the structure and energetics of an input sequence. Additionally, it has been used to develop the CATM algorithm using two energy terms parameterized by CHARMM: hydrogen bonding and van der Waals packing. The CATM algorithm has been used to predict the structures of both known and unknown TM dimers, and experimental studies have shown that it accurately predicts the energetics of association (Anderson et al., 2017; Díaz Vázquez et al., 2023; Mueller et al., 2014). My research adapts the CATM algorithm to design structures with high levels of van der Waals packing in the absence of hydrogen bonding to assess the extent at which packing can drive MP association.

Other methods for predicting interactions between MPs focus on predicting the topology or docking of individual TM helices. OCTOPUS predicts TM topology using a combination of Markov models and neural networks (Viklund & Elofsson, 2008). PREDDIMER utilizes a novel surface-based modeling approach to predict and screen TM dimers for conformation heterogeneity (Polyansky et al., 2012). EVFold uses evolutionary based structural restraints to refine the docked structures (Braun T et al., 2015). TMDOCK uses an all-atom model for helices, inserting them in the membrane and outputting a structure alongside a predicted ΔG of insertion and ΔG of association (Lomize & Pogozheva, 2017). Each of these methods was adapted into a webserver for online use, where users can input the sequence and additional information to guide the process.

Most notably, at the 2020 Critical Assessment of Structure Prediction (CASP) conference, Google’s Deepmind introduced the machine learning model AlphaFold. AlphaFold utilizes a combination of neural networks and training on multiple sequence alignments (MSAs) and solved protein structures to predict unknown structures to near atomic precision with a 95% confidence interval (Jumper et al., 2021). Shortly afterward, David Baker’s group introduced RoseTTAFold, improving on the Rosetta prediction by incorporating a similar architecture to AlphaFold, with the addition of a third track network that connects sequence, residue-residue distances, and atomic coordinates (Baek et al., 2021). Each of these methods drastically improve the ability to predict unknown protein structures using information from previously studied and solved proteins. However, there are limits to how well these prediction algorithms work. AlphaFold struggles to predict proteins with <30 homologs in their MSAs, and accuracy decreases for protein structures dependent on other protein-protein interactions, while RoseTTAFold has difficulty predicting higher-order oligomers (Agard et al., 2022). The disparity between soluble proteins and MPs in the PDB contributes to these limitations, making small TM proteins difficult to predict.

1.5.5 Working at the experimental and computational interface

With the rise of computational technologies and experimental throughput over my time in graduate school, a lot of research is now being done at the interface between computation and experiments. The development of AlphaFold and RoseTTAFold allows researchers to predict structures of their proteins, making informed decisions on potential residues to mutate to see an impact in the structure or function (cites). With the ability to analyze data in high throughput using computation, there is a rising boon for complementary high-throughput experiments. My research is at the interface of computational and experimental design, utilizing computational tools to predict and create homodimer structures with novel sequences to be tested with a complementary high-throughput assay.

In Chapter 3, I will further detail studies at the experimental and computational interface. I cite studies that inspired and enhanced both the experiments and computational analysis within my research and describe the methods for my computational design and analysis at length.

* 1. ****Thesis overview****

My graduate research focused on using computational protein design in combination with high throughput assays to determine the extent at which van der Waals packing contributes to membrane protein association and folding. Prior research on the impact of packing to the folded state of membrane proteins honed-in on singular systems, and I aimed to expand this knowledge to a larger variety of membrane protein structures.

**In Chapter 2**, I present the majority of my graduate school work to be published in the near future. In this paper, I determined that van der Waals packing is a weak driving force that leads to association of MP homodimers. I data mined the PDB for all solved MP structures to determine the best structures for computational design, developed a protein design algorithm and designed 1000s of proteins, and assessed the ability of proteins designed with solely van der Waals packing for their ability to associate using a high-throughput assay. I found that my designed proteins in regions outside of the GASright mildly associate in comparison to GASrights that associate according to both hydrogen bonding and van der Waals packing.

**In Chapter 3**, I discuss research at the interface of experiments and computation in detail, citing the inspirations for data mining, the design algorithm, and the analysis that led to the conclusions found in my paper.

**In Chapter 4**, I describe a variety of future directions for my protein design project, expanding on what can be improved upon and how I would design proteins with the tools available today.

**In Chapter 5**, I share a collaboration with the SciFun program at UW-Madison, detailing my PhD journey through a chapter written for the public. I summarize the goals of my research in leyman’s terms while reflecting on the lessons that graduate school imparted onto me, giving my fully transparent thoughts on how my research affected my physical, emotional, and mental well-being. I showcase a bit of creativity in sharing these thoughts on how science and research has helped me grow during my time in graduate school.

* 1. ****References****

Agard, D. A., Bowman, G. R., DeGrado, W., Dokholyan, N. V., & Zhou, H. X. (2022). Solution of the protein structure prediction problem at last: crucial innovations and next frontiers. *Fac Rev*, *11*, 38. <https://doi.org/10.12703/r-01-0000020>

Anderson, S. M., Mueller, B. K., Lange, E. J., & Senes, A. (2017). Combination of Cα-H Hydrogen Bonds and van der Waals Packing Modulates the Stability of GxxxG-Mediated Dimers in Membranes. *J Am Chem Soc*, *139*(44), 15774-15783. <https://doi.org/10.1021/jacs.7b07505>

Armstrong, C. R., & Senes, A. (2016). Screening for transmembrane association in divisome proteins using TOXGREEN, a high-throughput variant of the TOXCAT assay. *Biochim Biophys Acta*, *1858*(11), 2573-2583. <https://doi.org/10.1016/j.bbamem.2016.07.008>

Baek, M., DiMaio, F., Anishchenko, I., Dauparas, J., Ovchinnikov, S., Lee, G. R., . . . Baker, D. (2021). Accurate prediction of protein structures and interactions using a three-track neural network. *Science*, *373*(6557), 871-876. <https://doi.org/10.1126/science.abj8754>

Braun T, Koehler Leman J, & OF, L. (2015). **Combining Evolutionary Information and an Iterative Sampling Strategy for Accurate Protein Structure Prediction**. In: PLOS Computational Biology.

Doura, A. K., & Fleming, K. G. (2004). Complex interactions at the helix-helix interface stabilize the glycophorin A transmembrane dimer. *J Mol Biol*, *343*(5), 1487-1497. <https://doi.org/10.1016/j.jmb.2004.09.011>

Díaz Vázquez, G., Cui, Q., & Senes, A. (2023). Thermodynamic analysis of the GAS. *Biophys J*, *122*(1), 143-155. <https://doi.org/10.1016/j.bpj.2022.11.018>

Fisher, L. E., Engelman, D. M., & Sturgis, J. N. (1999). Detergents modulate dimerization, but not helicity, of the glycophorin A transmembrane domain. *J Mol Biol*, *293*(3), 639-651. <https://doi.org/10.1006/jmbi.1999.3126>

Fleming, K. G., Ackerman, A. L., & Engelman, D. M. (1997). The effect of point mutations on the free energy of transmembrane alpha-helix dimerization. *J Mol Biol*, *272*(2), 266-275. <https://doi.org/10.1006/jmbi.1997.1236>

Fleming, K. G., & Engelman, D. M. (2001). Specificity in transmembrane helix-helix interactions can define a hierarchy of stability for sequence variants. *Proc Natl Acad Sci U S A*, *98*(25), 14340-14344. <https://doi.org/10.1073/pnas.251367498>

Januliene, D., & Moeller, A. (2021). Single-Particle Cryo-EM of Membrane Proteins. *Methods Mol Biol*, *2302*, 153-178. <https://doi.org/10.1007/978-1-0716-1394-8_9>

Johnson, R. M., Hecht, K., & Deber, C. M. (2007). Aromatic and cation-pi interactions enhance helix-helix association in a membrane environment. *Biochemistry*, *46*(32), 9208-9214. <https://doi.org/10.1021/bi7008773>

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., . . . Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, *596*(7873), 583-589. <https://doi.org/10.1038/s41586-021-03819-2>

Keener, J. E., Zhang, G., & Marty, M. T. (2021). Native Mass Spectrometry of Membrane Proteins. *Anal Chem*, *93*(1), 583-597. <https://doi.org/10.1021/acs.analchem.0c04342>

Kermani, A. A. (2021). A guide to membrane protein X-ray crystallography. *FEBS J*, *288*(20), 5788-5804. <https://doi.org/10.1111/febs.15676>

Koehler Leman, J., Mueller, B. K., & Gray, J. J. (2017). Expanding the toolkit for membrane protein modeling in Rosetta. *Bioinformatics*, *33*(5), 754-756. <https://doi.org/10.1093/bioinformatics/btw716>

Laganowsky, A., Reading, E., Hopper, J. T., & Robinson, C. V. (2013). Mass spectrometry of intact membrane protein complexes. *Nat Protoc*, *8*(4), 639-651. <https://doi.org/10.1038/nprot.2013.024>

Liang, B., & Tamm, L. K. (2016). NMR as a tool to investigate the structure, dynamics and function of membrane proteins. *Nat Struct Mol Biol*, *23*(6), 468-474. <https://doi.org/10.1038/nsmb.3226>

Lomize, A. L., & Pogozheva, I. D. (2017). TMDOCK: An Energy-Based Method for Modeling α-Helical Dimers in Membranes. *J Mol Biol*, *429*(3), 390-398. <https://doi.org/10.1016/j.jmb.2016.09.005>

Maeda, R., Sato, T., Okamoto, K., Yanagawa, M., & Sako, Y. (2018). Lipid-Protein Interplay in Dimerization of Juxtamembrane Domains of Epidermal Growth Factor Receptor. *Biophys J*, *114*(4), 893-903. <https://doi.org/10.1016/j.bpj.2017.12.029>

Marinko, J. T., Huang, H., Penn, W. D., Capra, J. A., Schlebach, J. P., & Sanders, C. R. (2019). Folding and Misfolding of Human Membrane Proteins in Health and Disease: From Single Molecules to Cellular Proteostasis. *Chem Rev*, *119*(9), 5537-5606. <https://doi.org/10.1021/acs.chemrev.8b00532>

Mueller, B. K., Subramaniam, S., & Senes, A. (2014). A frequent, GxxxG-mediated, transmembrane association motif is optimized for the formation of interhelical Cα-H hydrogen bonds. *Proc Natl Acad Sci U S A*, *111*(10), E888-895. <https://doi.org/10.1073/pnas.1319944111>

Polyansky, A. A., Volynsky, P. E., & Efremov, R. G. (2012). Multistate organization of transmembrane helical protein dimers governed by the host membrane. *J Am Chem Soc*, *134*(35), 14390-14400. <https://doi.org/10.1021/ja303483k>

Popot, J. L., & Engelman, D. M. (1990). Membrane protein folding and oligomerization: the two-stage model. *Biochemistry*, *29*(17), 4031-4037. <https://doi.org/10.1021/bi00469a001>

Popot, J. L., & Engelman, D. M. (2000). Helical membrane protein folding, stability, and evolution. *Annu Rev Biochem*, *69*, 881-922. <https://doi.org/10.1146/annurev.biochem.69.1.881>

Russ, W. P., & Engelman, D. M. (1999). TOXCAT: a measure of transmembrane helix association in a biological membrane. *Proc Natl Acad Sci U S A*, *96*(3), 863-868. <https://doi.org/10.1073/pnas.96.3.863>

Scheiner, S., Kar, T., & Gu, Y. (2001). Strength of the Calpha H..O hydrogen bond of amino acid residues. *J Biol Chem*, *276*(13), 9832-9837. <https://doi.org/10.1074/jbc.M010770200>

Schneider, D., & Engelman, D. M. (2003). GALLEX, a measurement of heterologous association of transmembrane helices in a biological membrane. *J Biol Chem*, *278*(5), 3105-3111. <https://doi.org/10.1074/jbc.M206287200>

Vargas, E., Yarov-Yarovoy, V., Khalili-Araghi, F., Catterall, W. A., Klein, M. L., Tarek, M., . . . Roux, B. (2012). An emerging consensus on voltage-dependent gating from computational modeling and molecular dynamics simulations. *J Gen Physiol*, *140*(6), 587-594. <https://doi.org/10.1085/jgp.201210873>

Vargas, R., Garza, J., Dixon, a. D. A., & Hay, B. P. (2000). How Strong Is the Cα−H···OC Hydrogen Bond? *Journal of the American Chemical Society*, *122*, 4750-4755.

Viklund, H., & Elofsson, A. (2008). OCTOPUS: improving topology prediction by two-track ANN-based preference scores and an extended topological grammar. *Bioinformatics*, *24*(15), 1662-1668. <https://doi.org/10.1093/bioinformatics/btn221>

Walters, R. F., & DeGrado, W. F. (2006). Helix-packing motifs in membrane proteins. *Proc Natl Acad Sci U S A*, *103*(37), 13658-13663. <https://doi.org/10.1073/pnas.0605878103>

White, S. H., & Wimley, W. C. (1999). Membrane protein folding and stability: physical principles. *Annu Rev Biophys Biomol Struct*, *28*, 319-365. <https://doi.org/10.1146/annurev.biophys.28.1.319>

Yano, Y., Takemoto, T., Kobayashi, S., Yasui, H., Sakurai, H., Ohashi, W., . . . Matsuzaki, K. (2002). Topological stability and self-association of a completely hydrophobic model transmembrane helix in lipid bilayers. *Biochemistry*, *41*(9), 3073-3080. <https://doi.org/10.1021/bi011161y>

Yarov-Yarovoy, V., Baker, D., & Catterall, W. A. (2006). Voltage sensor conformations in the open and closed states in ROSETTA structural models of K(+) channels. *Proc Natl Acad Sci U S A*, *103*(19), 7292-7297. <https://doi.org/10.1073/pnas.0602350103>

Yarov-Yarovoy, V., Schonbrun, J., & Baker, D. (2006). Multipass membrane protein structure prediction using Rosetta. *Proteins*, *62*(4), 1010-1025. <https://doi.org/10.1002/prot.20817>

You, M., Li, E., Wimley, W. C., & Hristova, K. (2005). Forster resonance energy transfer in liposomes: measurements of transmembrane helix dimerization in the native bilayer environment. *Anal Biochem*, *340*(1), 154-164. <https://doi.org/10.1016/j.ab.2005.01.035>

Zhou, F. X., Merianos, H. J., Brunger, A. T., & Engelman, D. M. (2001). Polar residues drive association of polyleucine transmembrane helices. *Proc Natl Acad Sci U S A*, *98*(5), 2250-2255. <https://doi.org/10.1073/pnas.041593698>