**Significance**

Membrane proteins compose 25-30% of the proteins found within genomes of various organisms (Fagerberg et al., 2010). Proper membrane protein folding is critical for essential biological functions, including cell signaling, ion balance, and gene regulation. Misfolding of membrane proteins has been found to be involved in several human diseases such as Parkinson’s, cystic fibrosis, and cancer (citations). Investigating how these proteins fold is important to better understand the fundamental forces contributing to membrane protein stability as well as why these proteins are necessary for human health.

Membrane protein folding takes place in two distinct steps: First, the protein is translated by the ribosome and transported into the membrane by a protein known as a translocon. The second step of folding occurs after the entire protein has been inserted into the lipid bilayer: As a result of hydrogen bonds, electrostatic interactions, and van der Waals packing, the individual parts of the protein are assembled into a stabilized structure (Popot and Engelman, 1990). However, the extent at which each of these forces is involved in stabilizing membrane proteins is not well understood. Of particular interest is implicit van der Waals packing: tight sidechain packing is a necessary force for the assembly of protein structure. However, it has been difficult to determine whether this sidechain packing plays a more dominant role in membrane protein folding than interactions typically thought of as stabilizing, such as hydrogen bonding, topology, and polar interactions (Zhou et al., 2000; Yano et al., 2002; Johnson et al., 2007). By deducing the importance of packing within membrane proteins, we will have a better understanding of the extent at which van der Waals packing contributes to stability, and thus how packing works in combination with other forces stabilize folded structures.

If membrane protein stability is dependent on van der Waals packing between subunits, then a fine balance with the other packing interactions present within the membrane is necessary for it to stabilize the folded state (citations). Because van der Waals packing results between any atoms in close proximity, packing interactions within membrane proteins include the following: lipid-lipid packing, protein-lipid packing, and protein-protein packing. Previous research has focused specifically on protein-protein packing, with one group in particular suggesting that optimization of packing between sidechains plays a significant role in stabilization of the folded state (Mravic et al., 2019). Our lab has successfully predicted the impact of sidechain packing in the context of a specific dimeric structural motif, and we aim to use this knowledge to design hundreds of sequences with a range of weak to strong sidechain packing to determine the role of packing on stability. In addition, our lab has developed an experimental sort-seq method to analyze dimerization in high-throughput. We will then apply FRET to a subset of our designs to quantify the free energy range at which sidechain packing contributes to stability of the dimeric state. By combining these tools, we will determine and quantify the extent at which sidechain packing is necessary for helix-helix association.

The contribution of van der Waals packing to stabilized structure is one of the large gaps remaining in our understanding of membrane protein folding (Hong et al., 2014). Determining the extent at which sidechain packing impacts the association of membrane protein subunits will give us a better understanding of how membrane proteins assemble to fold stabilized structures. In doing so, it may open up an avenue for future study of the other van der Waals interactions: lipid-lipid packing and lipid-protein packing. By understanding how these forces contribute to stability, we will be closer to obtaining a holistic view of how all forces involved (hydrogen bonding, electrostatic interactions, van der Waals packing) combine to stabilize membrane protein structure. Overall, the proposed work will increase our knowledge of the fundamental rules of membrane protein folding, contributing to our understanding of complex membrane protein mechanisms, such as oligomerization and conformational change. Eventually, this knowledge can be used to design new functional membrane protein structures, advancing the fields of drug design and synthetic biology.

One particular model that has been used to model the association between helices in the folded state is dimerization, a tractable and simple system where a variety of methods have successfully characterized and quantified hydrogen bonding and electrostatic interactions in the membrane (cite). However, unlike these studies where it is possible to make mutations to add or remove these forces, there are no known dimeric structures that rely solely on hydrophobic amino acids for packing. To address this, we will use a high-throughput computational and experimental approach.

Things to mention:

* Difficulty in understanding structure of MPs
  + Maybe compare to soluble?
* Therefore, difficult to understand folding
  + Techniques are not as efficient
    - Expression, pruifcation, solubilization, are all problems with MPs
* VdW is implicit: difficult to study, especially with little structural knowledge
  + Hard to study without context of other forces
* Design is slow: no design in MPs has been done at this scale
* By measuring range that vdW allows for stability, can compare to other forces to determine what might be the driving force in MPs
  + Can lead to more accurate design of MPs or drugs that target MPs
  + Understand why specific folds occur vs others (maybe even why some proteins are able to undergo conformational changes)
  + Start to gain insight into how lipids affect MP folding and association (could they be involved in conformational changes?
  + Random question to think about: What about vdW/other binding interactions outside of the membrane influences binding inside? Why are some single pass MPs promiscuous? Will my research help with that at all?

Methods that have found recent success in analyzing folding include the experimental assay TOXCAT and FRET. …justify each of these (say what each gives)…

However, studying van der Waals packing is not as simple as it seems.

In order to function properly, these proteins fold into stable and functional structures as a result of forces including hydrogen bonding, electrostatic interactions, and van der Waals packing. The extent at which each of these forces contributes to stabilizing the folded state of membrane proteins is not well understood. In particular, van der Waals packing interactions are extremely difficult to study. Van der Waals interactions, or the resulting induced dipole attraction between atoms in close contact, is an implicit force present during protein folding. However, unlike in soluble proteins where the hydrophobic effect drives the formation of tight packing interactions at the protein core,

Why is it important to understand how van der Waals interact in membrane proteins?

OR

Why is it important to understand the forces involved in membrane protein folding?

* What do we understand in soluble protein folding and what has it helped us accomplish?
  + I think it’ll be a little tough to read soluble literature; so maybe just what will membrane protein folding give us? Why is filling the gap important?

However, van der Waals packing interactions are extremely difficult to study. One reason for this is

What I want to say:

* Difficult to study
* Many kinds of interactions to balance folded and unfolded states
  + How do these interactions work? Which is faborable? No way to measurably study and so mostly estimates (I don’t know if I have enough reading on this subject…?)

I think difficulty may be more of an innovation problem; instead, think of why membrane protein folding is important to study

* Why would it be beneficial to understand these forces; what has been done previously (generally; why has it been challenging study? I think I say that here, then emphasize in innovation?), what is the gap that my research is trying to fill, and what can my research be used for/inspire in the future?