**Innovation**

Studying membrane protein folding is a difficult challenge. Membrane proteins are difficult to express in yields high enough for biophysical experiments. In addition, purification and solubilization of these proteins often leads to aggregation or unfolding (cite). Finally, it is difficult to control and monitor folding within the bilayer, which is necessary to analyze the process of folding and the forces involved. One force that the field has found the most difficulty in assessing is van der Waals packing. Van der Waals packing interactions, or the resulting induced dipole attraction between atoms in close contact, is an implicit force present during protein folding. When mutagenizing polar or aromatic groups to determine the impact of hydrogen bonding or electrostatic interactions on folding, previous studies were unable to take into account the changes in van der Waals packing within the core of the protein (cite papers cited in Mravic). Using a combination of *in silico*, *in vivo*, and *in vitro* methods, I aim to address our lack of understanding of this force by determining the strength of apolar sidechain packing in dimers, a simple and tractable model system for membrane protein folding (Mackenzie and Engelman, 1998; Russ and Engelman, 1999).

To analyze the impact of tight sidechain packing on dimerization, I will need a set of sequences that dimerize at different levels based solely on the amount of packing. However, there are no known structures of membrane protein dimers that are composed solely of apolar amino acids. To address this, I am planning to use large scale computational design to engineer dimeric sequences with varying degrees of stabilities solely based on sidechain packing. A membrane protein design study at this extent has not yet been accomplished, as previous design studies typically result in structural and functional confirmation of one well designed protein (Joh et al., 2014; Lu et al., 2018; Mravic et al., 2018). However, my lab has previously used similar computational methods to accurately predict energy and structure from sequence in hundreds of membrane dimers, ensuring that we have a high level of understanding sequence, structure, and energy relationships that can be used to assess how changes in sidechain packing will impact dimer stability (Mueller et al., 2014; Anderson et al. 2017). Thus, with computational algorithms being common in both prediction and design, we have the ability to engineer of dimers with an assortment of sidechain packing.

Although it will be impossible to determine the structural accuracy of every design, if our sequences dimerize as expected *in vivo*, we will have data to support our hypothesis that packing contributes to membrane protein association. In addition, we have recently developed high-throughput sort-seq, a method that measures the dimerization propensity of our proteins based on fluorescence as a readout of association strength. This method will be used to complement my large-scale design, allowing me to test hundreds of designed constructs at a time for their dimerization propensity. If our expected designed stabilities are recapitulated in sort-seq, then we will gain a better understanding of the extent at which apolar sidechain packing plays a role in stabilizing helix-helix association. However, the goal of my project is to determine the strength of apolar sidechain packing. Because sort-seq does not measure free energies, I will take a subset of weak to strong dimers and determine differences in their thermodynamic stability using *in vitro* FRET. With FRET, I will quantify the extent at which apolar sidechain packing can influence association of two helices. Overall, my research will give insight into the extent at which fundamental sidechain packing is necessary for folding to occur, something that has not yet been done despite the importance of van der Waals packing to membrane protein folding.

Put this in significance…?

Previous research on redesign of phospholamban suggests that apolar sidechain packing is enough to stabilize and fold the structure of this pentameric protein. However, other effects (sequence? Lipid effects?) … . In addition, the relative strength of sidechain packing within this structure was not determined, demonstrating a gap in the strength of these tandem vdW interactions that would allow us to compare to others and determine if these are stabilizing (fix this sentence: knowing total strength of vdW allows to compare to H-bonding and other interactions to tell which force may act as a driving force).

- Why is your research something that hasn’t yet been done and what will it give you? How do your specific techniques aid you in accomplishing your goal?

Specifically want to understand protein-protein vdW interactions-that is the crux of my project’s pitch

I think for this section, I can take a bit from Mravic 2019; take their narrative about how forces have been studied, but not vdW, explain why vdW haven’t been explored very well, then say why my project will be able to do so

Intro- similar to Mravic

Second-what are my methods and what do they accomplish that other people couldn’t

Conclude with how it answers the question and opens up the field for further research

Efforts have been made to study hydrogen bonding (cite) and polar interactions, but each of these is difficult to characterize strength of without knowing vdW effect. Because vdW is implicit and present in a variety of ways, it is difficult to determine the extent that each part stabilizes unfolded vs folded state.

What do I want to say next:

* So far, research for characterizing hydrogen bonding, topology, and electrostatics has been done
* However, van der Waals packing is implicit and has not been studied
* My research aims to specifically study the effect of van der Waals packing on stabilization of proteins
  + By limiting to hydrophobic AAs, it may be possible to determine the extent of vdW packing on stability