**Specific Aims**

Proper membrane protein folding is necessary for essential biological functions including ion transport across the membrane and cell-cell signaling. Misfolding of membrane proteins often leads to disease phenotypes including growth defects and cancer. In order for membrane proteins to fold properly, individual subunits of the protein associate to stabilize the folded state as a result of forces including hydrogen bonding, electrostatic interactions, and van der Waals packing. In particular, van der Waals packing is extremely important because it is the only implicit force: van der Waals packing, or an attraction between atoms in close contact, is always present in the folded state between sidechains no matter what other forces are involved. Despite the relative importance of packing versus other forces involved in folding, no current membrane protein systems have been identified to specifically study how the strength of packing influences folding. Recently, membrane protein design has been used to determine that optimization of sidechain packing between protein subunits can stabilize protein structure. However, the total contribution of packing to the folded state was not measured. With my research, I propose to characterize and quantify the extent at which sidechain packing is necessary for membrane protein association.

In order to determine the energetic landscape at which sidechain packing contributes to membrane protein association, I aim to characterize a large number of sequences with a gradient of weak to strong sidechain packing to determine if packing strength correlates to association. To do so, I am using a high-throughput approach combining large scale computational design with *in vivo* characterization of association. Using a set of structural geometries and simple energetic terms, my lab has previously predicted structures of thousands of dimeric proteins from sequence alone. With my research, I will use the same algorithms and methods used in prediction to design thousands of sequences with a range of weak to strong packing at the dimer interface. In addition, my lab has developed a novel high-throughput “**sort-seq**” method to measure the dimerization propensity of thousands of transmembrane helices. In this system, dimerization of proteins results in a fluorescence output which we can then **sort** into bins based on fluorescence as a readout of association strength. These can then be sent for next-generation **sequencing** to identify proteins within each bin. After characterizing a range of weak to strong sequences that dimerize, I will take a subset of these sequences and quantify their association stability using *in vitro* FRET. These experiments will allow me to both characterize and quantify the amount of van der Waals packing necessary for membrane protein association.

**Aim 1: High-throughput determination of how sidechain packing affects dimerization**

I will analyze the protein databank for helices in close contact within membrane proteins, searching for common geometries between helices. The more times a geometry appears within our analysis, the more likely that this geometry is optimal for helix-helix interactions. These geometries will be used to design sequences with a range of weak to strong packing which will then be tested using *in vivo* sort-seq. This aim will determine the extent at which optimized protein-protein packing can influence association of two helices.

**Aim 2: Quantification of** **the range at which sidechain packing influences dimerization**

I will quantify the stability of van der Waals dependent association for a subset of dimers from Aim 1. These dimers will range from weak to strong dimerization propensity, allowing me to determine a range at which optimized sidechain packing contributes to stabilizing the dimeric state.

Use your elevator pitch as a template:

Paragraph 1: Why are membrane proteins and specifically van der Waals packing interactions important? Why haven’t they been studied?

Paragraph 2: Why haven’t people been able to study vdW? Is it the techniques? What is your approach for success at your goal?

* Implicit force makes it difficult to study dynamics: no methods for studying folding interactions in larger membrane proteins? Proteins always have polars or hydrogen bonding? Which one of these is it?
* I think it’s both: no dimeric MPs based on solely hydrophobic packing have been characterized in the PDB and dimerization is the best (?) tool to study these folding interactions? What are others?
  + Single molecule FRET
  + SE-AUC
* I think it’s more of a pitch to high-throughput: Why can’t we characterize the landscape in a low throughput manner?
  + It would too difficult to get the entire landscape; how would we know what proteins are going to dimerize really well? Only one paper has said that vdW can drive association; by doing a large scale approach with a variety of geometries, we are more likely guaranteed success.
    - More likely to determine the limits of packing

Significance-Why is your research important to the broader field? Broader background

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?
    - Will my research aid in prediction of structure? I think so; could it be part of the reason that CATM isn’t predicting as accurately as we hoped? Could vdW play a larger role
      * Tough argument to say BECAUSE I say that we’re good at predicting; basically have to spin it so that it says we’ll be better in the end
        + If we understand how vdW work, but not the extent of contribution to stability, some AAs or positions may actually lead to increased stability that we don’t expect (if this is the case, then by implementing changes to our vdW function, we may see a better correlation in predicted structures; better prediction = better prediction of unknown sequences that could be drug targets = more drugs for a variety of systems; make sure to look at Samantha’s data on vdW)
* What does FRET actually give me?
  + Ask Gladys how she explains her project: what does knowing the free energy of a reaction tell you? Or at least how to explain it
  + If single molecule was used instead:
    - What are limitations that would be addressed? What new info would I get? Why might it be a better idea to use bulk FRET rather than single molecule?

Innovation-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?

Simple info about design?

Need detail about Sort-seq (go into Josh’s prelim and steal)

FRET

Sources

Popot and Engelman 1990 (two stage model)

Booth and Curran 1999 (early studies on MPs)

Eilers et al. 2000, Adamian and Liang 2001, Doura et al. 2004, Joh et al. 2009 (importance of vdW in membrane proteins; last two claim similar packing in soluble and membrane, first claims different)

Hong 2014 (the gap in understanding forces involved in MP folding)

Mravic et al. 2019 (literally the previous research)

Sources I need:

* Maybe some biological stuff (signal transduction, cancer, sec translocon, etc.)
* Anything I can find on why membrane protein folding is studied in the context of proteins and not lipids (other than that it’s difficult)

Aim 1

Aim 2

Random thought: think of lipid-protein interaction as something that is important for optimizing protein-protein packing

* Imagine a helix that is tilted in the membrane: AAs will be protruding into lipids at angles that may be less favorable (potentially less entropy of the AA, as well as less enthalpy because unfavorable vdW)
  + By dimerizing, manages to potentially counteract one of these (better vdW packing, more favorable entropy/more acceptable conformations of AAs, or both)