

Membrane proteins subunits associate to fold into biologically essential structures as a result of forces including hydrogen bonding, weak polar interactions, and van der Waals packing. After a membrane protein is inserted into the membrane, a combination of these forces stabilizes the folded state. Previous research has investigated the impact of hydrogen bonding and weak polar interactions on membrane protein association, however, the contribution of van der Waals packing to association in the folded state remains poorly understood. Van der Waals packing is a complex set of diverse interactions comprised of lipid-lipid packing, lipid-protein packing, and protein-protein packing, which collectively stabilize both the unfolded and folded states. Each of these interactions must be taken into account to understand the contribution of packing to membrane protein association in the folded state, but prior research has found difficulty isolating these interactions from each other and from other stabilizing forces. Literature suggests that protein-protein packing, or sidechain packing, plays a significant role in membrane protein stability, demonstrating that optimized sidechain packing alone can stabilize the folded state. However, the relative contribution of this force to the stable structure of a membrane protein has not yet been determined. My work aims to control for other stabilizing forces to study the extent at which sidechain packing can stabilize membrane protein structure. To investigate this question, I will use helical dimers which are a simple and tractable model system for the association of membrane protein subunits during folding. Using established computational design techniques, I will engineer dimeric sequences with an array of sidechain packing energies to determine how much packing is necessary for stability of the folded state. Using a high throughput in vivo assay that combines fluorescence activated cell sorting and next generation sequencing, I will screen the dimerization propensity of each construct, to identify correlations between computational and experimental stability. Then, I will make point mutants on my designed structures and measure changes in thermodynamic stability using in vitro FRET. This will allow me to confirm the correlation seen between sidechain packing and dimerization propensity in sort-seq and estimate the free energies of my designed constructs. This work will provide a better understanding of how sidechain packing can facilitate and stabilize membrane protein association and addresses a crucial knowledge gap in our understanding of membrane protein biology.