Assessing van der Waals packing as a driving force in membrane protein folding

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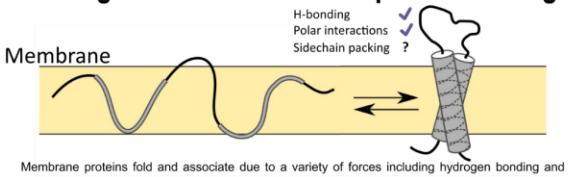
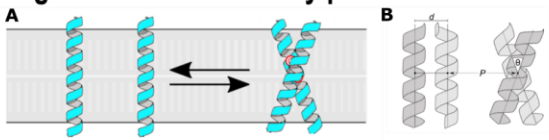
Driving forces in membrane protein folding



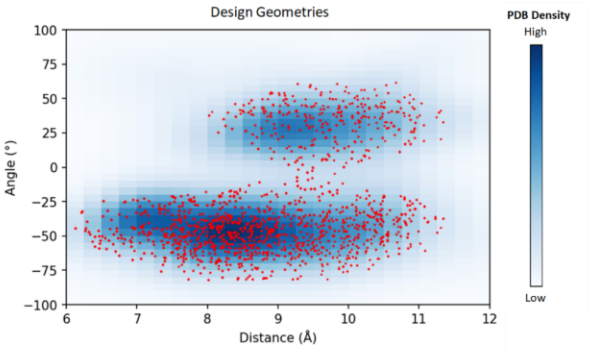
Membrane proteins fold and associate due to a variety of forces including hydrogen bonding and polar sidechain interactions. However, many helix-helix interactions are made up of solely non-polar sidechains, but despite this, the extent at which van der Waals packing between sidechains acts as a driving force in a variety of membrane protein systems has not yet been determined [1, 2].

Using dimerization to study protein association



A) Dimerization is a well studied model that has been used to investigate the impact of both hydrogen bonding and polar interactions [3, 4]. I aim to use this dimerization model to determine the extent at which van der Waals packing between sidechains acts as a driving force. B) Two geometries that are essential for influencing van der Waals packing are the distance (d) and the crossing angle (θ). I will focus on these geometric terms to explore a large variety of native membrane protein systems, aiming to gain a better understanding of how van der Waals packing imapcts folding and association in many membrane protein systems.

Datamining the PDB for design geometries

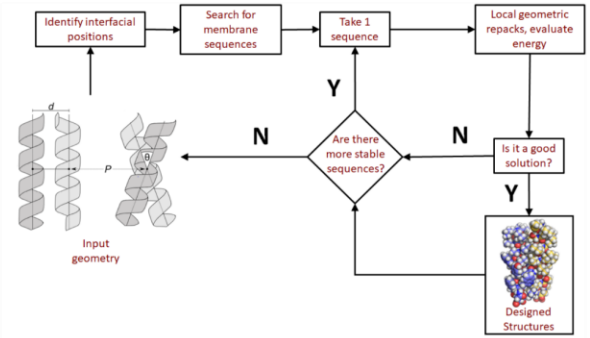


To determine the best geometries for designing membrane protein dimers, I searched through the PDB for all nonredundant membrane proteins that contained two helices in close contact. I plotted the density distribution of these geometries (blue) in order to find the geometries that occured most often in membrane proteins, expecting these geometries to be the most amenable to protein design [5]. I sampled these geometries by their density (red) and input them into my protein design program, allowing me to design more sequences in regions of higher density than lower density. This allows me to test the designability of different geometries based on how well represented they are in native membrane proteins.

References

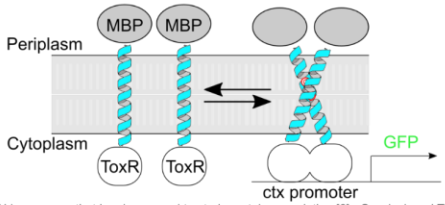
[1] Faham, S., Yang, D., Bare, E., Yohannan, S., Whitelegge, J.P., and Bowie, J.U. (2004). Side-chain Contributions to Membrane Protein Structure and Stability. Journal of Molecular Biology 335, 297–305. [2] Mravic, M., Thomaston, J.L., Tucker, M., Solomon, P.E., Liu, L., and DeGrado, W.F. (2019). Packing of apolar side chains enables accurate design of highly stable membrane proteins. Science 363, 1418–1423. [3] Russ, W.P., and Engelman, D.M. (1999). TOXCAT: A measure of transmembrane helix association in a biological membrane. Proc Natl Acad Sci U S A 96, 863–868. [4] Zhou, F.X., Merianos, H.J., Brunger, A.T., and Engelman, D.M. (2001). Polar residues drive association of polyleucine transmembrane helices. PNAS 98, 2250–2255. [5] Zhou, J., and Grigoryan, G. (2015). Rapid search for tertiary fragments reveals protein sequence–structure relationships. Protein Science 24, 508–524. [6] Armstrong, C.R., and Senes, A. (2016). Screening for transmembrane association in divisome proteins using TOXGREEN, a high-throughput variant of the TOXCAT assay. Biochim Biophys Acta 1858, 2573–2583.

Protein Design Strategy



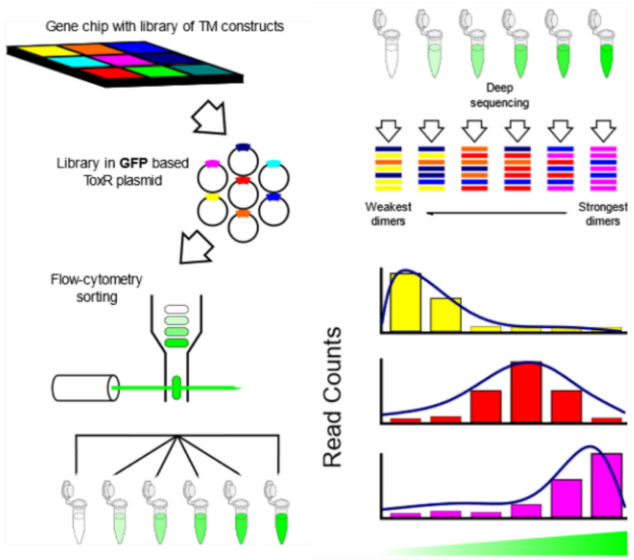
Using well known algorithms and energetic terms, I developed a computational program to design membrane protein dimers. My program reads in an input geometry extracted from the PDB datamining, finds interfacial positions, and then specifically searches for sequences differences in van der Waals packing. With this strategy, I was able to design 6710 sequences with a range of van der Waals packing energies. I will express and test these sequences using a high-throughput *in vivo* dimerization assay, expecting sequences with low van der Waals energies to associate less, and high van der Waals packing energies to associate more.

TOXGREEN: method to screen TM association



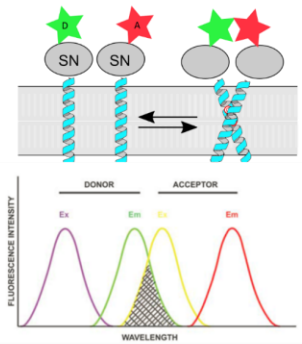
TOXGREEN is an assay that has been used to study protein association [6]. Our designed TMs are expressed in *E. coli* fused to a dimeric transcription factor, ToxR. When our helices dimerize, ToxR dimerizes as well, leading to the expression of green fluorescent protein (GFP). This assay allows us to measure differences in association between each of our designed proteins based on the fluorescence output.

Sort-seq: a high-throughput method to evaluate impact of van der Waals on dimerization

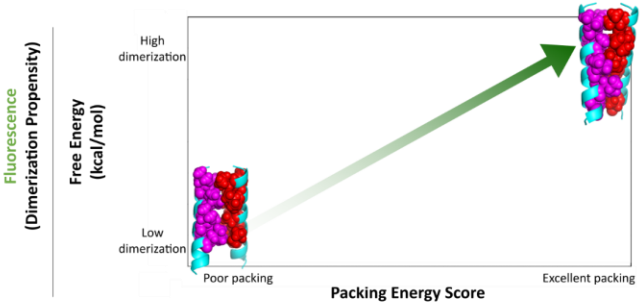


To complement my high-throughput design method, my lab has combined fluorescence activated cell sorting (FACS) with next generation sequencing (NGS) into a process called Sort-Seq. Through FACS, we are able to assess the dimerization propensity of our designed TM sequences by their fluorescence intensity from the TOXGREEN fluorescence. Cells expressing these TMs are separated into bins based on their fluorescence and are sent for deep sequencing. The fluorescence profile is then reconstructed based on the number of sequences per bin, allowing us to distinguish between the strength of our designed dimers, and thus the overall impact of van der Waals packing on dimerization.

Future Work: Quantification of stability using FRET



In order to further quantify the impact of van der Waals packing, on membrane protein folding and association, I am planning to use Förster resonance energy transfer (FRET). To prepare our samples for FRET, we express our proteins as a fusion protein to staphyloccocal nuclease (SN). We are then able to label these fusion proteins with a donor and acceptor fluorophore pair. By monitoring the shift from donor to acceptor fluorescence, we will be able to calculate the free energy for each of our designed sequences.



Overall, since both of our methods are evaluating dimerization propensity, we expect our results in both Sort-Seq and FRET to correlate well to our packing energy score computed for each design. Using this simple and tractable dimerization based model, this work will give us a better understanding on how van der Waals packing impacts membrane protein folding and association.