Chapter 3: Computational Methodology

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3.1 Introduction

* Talk about the importance of computation through my time in grad school, catching up, and how there are good tools to do so
* Reference papers that seem to do a good job mixing in computation with the biology simplifying for learners
* Emphasize the importance of computation due to high-throughput data analysis and AI

3.2 Structure prediction tools

In conjunction with structural determination, computational methods have been invented to evaluate previously solved MP structures. Many methods look to further understand MP folding by establishing energetic terms that aim to estimate the thermodynamics of association.

3.2.1 Rosetta

David Baker’s Lab at University of Washington are experts in this area, utilizing known energetic and structural information to develop the software suite Rosetta. Rosetta houses a variety of energy functions and prediction tools for soluble environments, including the ability to dock or design proteins *de novo*. The original Rosetta energy functions have been adapted to accommodate predicting helical TMs within the membrane environment. The updated functions include an energy term that models the membrane into layers classified defining atoms as water-exposed, polar, interface, and hydrophobic (White & Wimley, 1999; Yarov-Yarovoy, Schonbrun, et al., 2006). Successful predictions determined structural details in MPs associated with voltage sensing and gating mechanisms (Vargas et al., 2012; Yarov-Yarovoy, Baker, et al., 2006). Recently, RosettaMP was developed to enhance the functionality of MP prediction which includes modeling TM helices *de novo* from sequence, a representation of the membrane bilayer, and the ability to transform a protein into the membrane (Koehler Leman et al., 2017). These tools increase the accessibility to MP structure, improving the ability to visualize and predict structures of MPs that have not yet been solved. Simultaneously, energetic predictions enhance the knowledge in the field by allowing researchers to assess structural mutations *in silico* prior to testing with *in vitro* or *in vivo* experiments.

3.2.2 Molecular Software Library

Another computational tool for membrane proteins is the Molecular Software Library, or MSL (Kulp et al., 2012). Similar to Rosetta, MSL houses a variety of tools to perform MP structure prediction. These tools include the ability to transform proteins in space, mutate specific residues, extract geometric information from known structures, and predict the structure and energetics of an input sequence. Additionally, it has been used to develop the CATM algorithm using two energy terms parameterized by CHARMM: hydrogen bonding and van der Waals packing. The CATM algorithm has been applied to predict the structures of both known and unknown TM dimers, and experimental studies have shown that it accurately predicts the energetics of association (Anderson et al., 2017; Díaz Vázquez et al., 2023; Mueller et al., 2014). My research adapts the CATM algorithm to design structures with strong van der Waals packing in the absence of hydrogen bonding to assess the extent at which packing can drive MP association. I further detail how I used MSL to design TM homodimers in section 3.3.

3.2.3 Docking Algorithms

Other methods for predicting interactions between MPs focus on predicting the topology or docking of individual TM helices. OCTOPUS predicts TM topology using a combination of Markov models and neural networks (Viklund & Elofsson, 2008). HADDOCK can apply experimental knowledge of the interface region between proteins to refine docking (de Vries et al., 2010; Dominguez et al., 2003). PREDDIMER utilizes a novel surface-based modeling approach to predict and screen TM dimers for conformation heterogeneity (Polyansky et al., 2012). EVFold uses evolutionary based structural restraints to refine their docked structures (Braun T et al., 2015). TMDOCK uses an all-atom model for helices, inserting them in the membrane and outputting a structure alongside a predicted ΔG of insertion and ΔG of association (Lomize & Pogozheva, 2017). Each of these methods was adapted into a webserver for online use, where users can input the sequence and additional information to guide the process.

3.2.4 Machine learning tools

Most notably, at the 2020 Critical Assessment of Structure Prediction (CASP) conference, Google’s Deepmind introduced the machine learning model AlphaFold. AlphaFold utilizes a combination of neural networks and training on multiple sequence alignments (MSAs) and solved protein structures to predict unknown structures to near atomic precision with a 95% confidence interval (Jumper et al., 2021). Shortly afterward, David Baker’s group introduced RoseTTAFold, improving on the Rosetta prediction by incorporating a similar architecture to AlphaFold, with the addition of a third track network that connects sequence, residue-residue distances, and atomic coordinates (Baek et al., 2021). Each of these methods drastically improved the ability to predict unknown protein structures using information from previously studied and solved proteins. With increasing interest in using these technologies, multiple free webservers have been established to enhance access to these advanced protein prediction algorithms (Mirdita et al., 2022; Roberts et al., 2024). However, there are limits to how well these prediction algorithms work. AlphaFold struggles to predict proteins with <30 homologs in their MSAs, and accuracy decreases for protein structures dependent on other protein-protein interactions, while RoseTTAFold has difficulty predicting higher-order oligomers (Agard et al., 2022). The disparity between soluble proteins and MPs in the PDB contributes to these limitations, making small TM proteins difficult to predict.

3.3 Protein Design Algorithm

Intro paragraph about design and what my program does generally, with schematic

3.3.1 Analysis of membrane protein PDBs

Utilize MSL to extract geometries from MP PDBs from PISCES: http://dunbrack.fccc.edu/PISCES.php

Analyze geometries: KDE plot

3.3.2 Defining the Interface

Initially used SASA to determine the interface

* Lots of different interfaces, even though only 8 positions
* Too many variables to unpack in the initial data

Take subset of those geometries and define interfaces: Visual inspection of range of geometries and the stripe plots? What were those about?

3.3.3 Developing the energy terms

Introduce the already given energy terms from CATM

Self and pair baseline energies: define and talk about why this was needed to be done (calculating the energy for every single iteration of a sequence took forever)

* Polyleucine sequence, iterate through all AAs at each position, calculate the energy between the two, 1000 times or more?, average to get the baseline, show the plot that says it’s a good estimate of monomer energy
  + Did not use all AAs, just a subset of the AAs expected to be used in the search, so would need to rerun this with other AAs if interested in a more expansive list

Sequence Entropy term: entropy to get the sequence to a somewhat reasonable facsimile of the distribution of AAs

* Show the image of the comparison between distributions (both from extracted MPs and the liu 2002 paper)

3.3.4 Sequence Search

Randomly pick a position and change the AA, optimize the rotamers, measure the energy, compare to monomer, get all the energies, keep the best sequence, then go to the next iteration

Monte Carlo

3.3.5 Backbone Refinement

Inspired by Rosetta backbone iterative refinements: change the backbone for each given sequence, then redesign on the new backbone

One final iterative refinement step with Monte Carlo procedurally decreasing changes

* Paired with code that reads in pdb structures and does local structure repacks
  + Optimized by decreasing the repack distances in accordance with the monte carlo, making a very refined repack (similar to Rosetta)
* How to utilize the information from this paper to make the code better at design

3.3.6 Mutating the interface

Add in the code that needs to be used here: mutating all of the interfacial positions to a given AA and measuring the energy

* Reads in the PDB and the interfacial positions

3.4 Analysis

* Python scripts built for ease of use and how to use them all detailed here, probably with images
  + ngsReconstruction
  + runAllAnalysis
  + toxgreenAnalyzer

3.5 Conclusion

* Talk about ways to improve the molecular software library and/or to transition some of these scripts to something more usable like Rosetta/Alphafold/how to make this easy to use
  + Creating a webserver was a pipe dream I had but never got the skill to do it

3.6 References

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