Modifying a Mini Protein with Two Conformational States to Instead Adopt Only One Conformation

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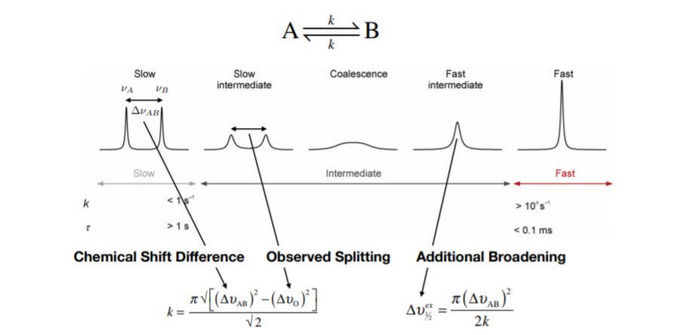
Introduction

Created by Rockland et al. using Rosetta, EHEE_rd2_0005 is a computationally designed mini protein of 40 residues. Exhibiting little structural change at 95°C or in solution of up to 4M GuHCl, EHEE_rd2_0005 is extremely thermodynamically stable.[1]

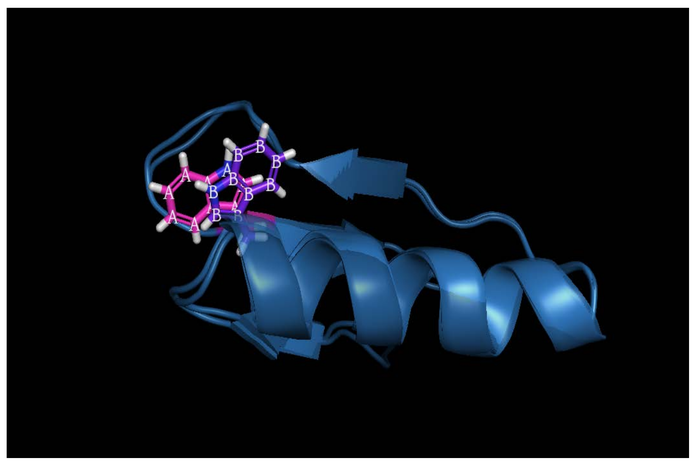
Our lab had previously attempted to analyze this mini protein using NMR spectroscopy, but our members were unable to assign several NMR peaks because of peak overlap. We suspected that this problematic peak overlap was due to the intermediate-rate dynamic exchange of our TRP-35 residue between two equally preferred conformations. However, there is still a possibility that partial dimerization is responsible for this effect.

Dynamic Exchange Regimes



Dynamic exchange of tryptophan can be particularly problematic in NMR peak assignment because its aromatic ring current effects, combined with its large size, strongly influences the chemical shifts of neighboring nuclei.

In order to identify effective strategies for controlling conformational isomerism in proteins, we tested various single-point mutations that would not create drastic changes in the protein backbone structure.



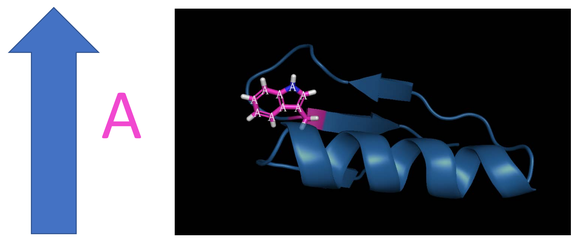
The Two Conformations of TRP-35: "State A" and "State B"

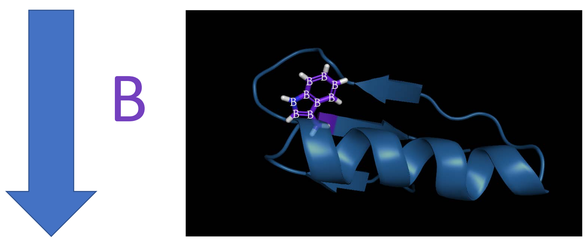
Dry Lab

A) Exhaustive yet Imprecise Single-Point Mutation Scan

Rosetta Commons

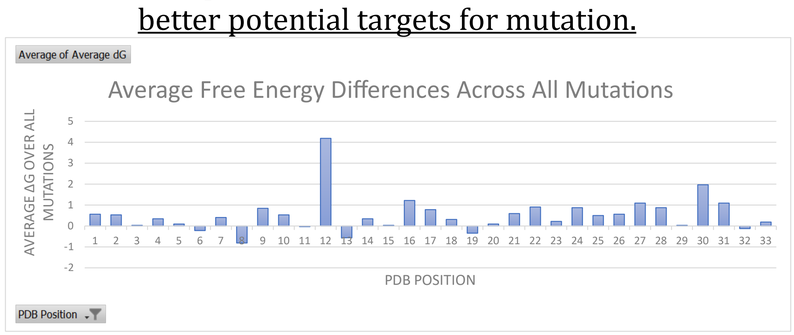
While we first chose mutations based only on the hypothetical attractive and repulsive forces in which the new side chains may participate, we later began to use Rosetta to help us choose mutations.





Our Rosetta data suggested that it is easier to mutate our protein to push the tryptophan ring into the "state A" conformation. This strategy's effectiveness is likely due to the strong secondary structure's ability to hold sterically large sidechains in place, which can push the TRP35 sidechain outward.

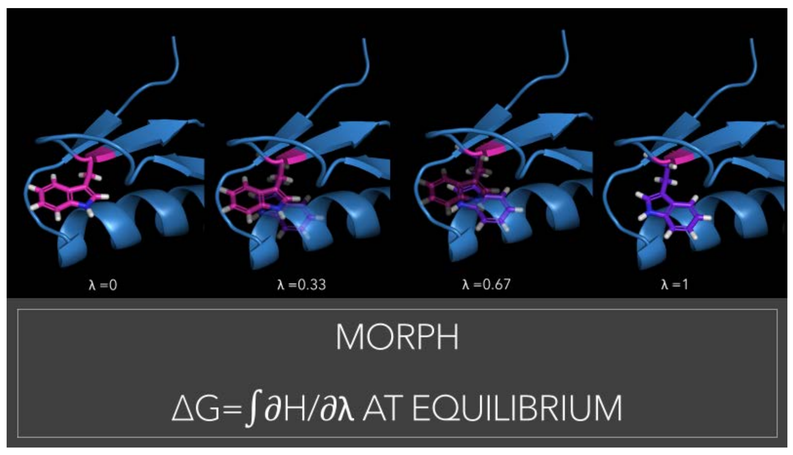
Rosetta also helped us determine which residues would be better potential targets for mutation.

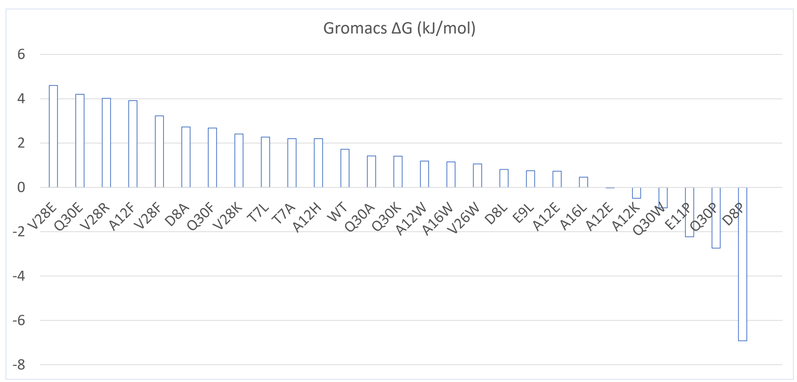


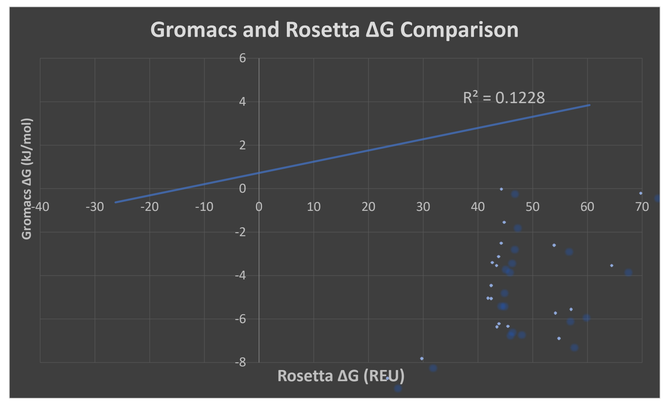
B) Slower but More Accurate Molecular Dynamics Simulations

GROMACS
FAST. FLEXIBLE. FREE.

Using Gromacs for our molecular dynamics simulations, we measured the effectiveness of each of our mutations by measuring the free energy differences of the two conformations with alchemical morph simulations.





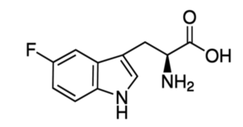


Despite its use in guiding our mutation strategies, Rosetta was a poor predictor of specific mutations' free energy values when compared to Gromacs.

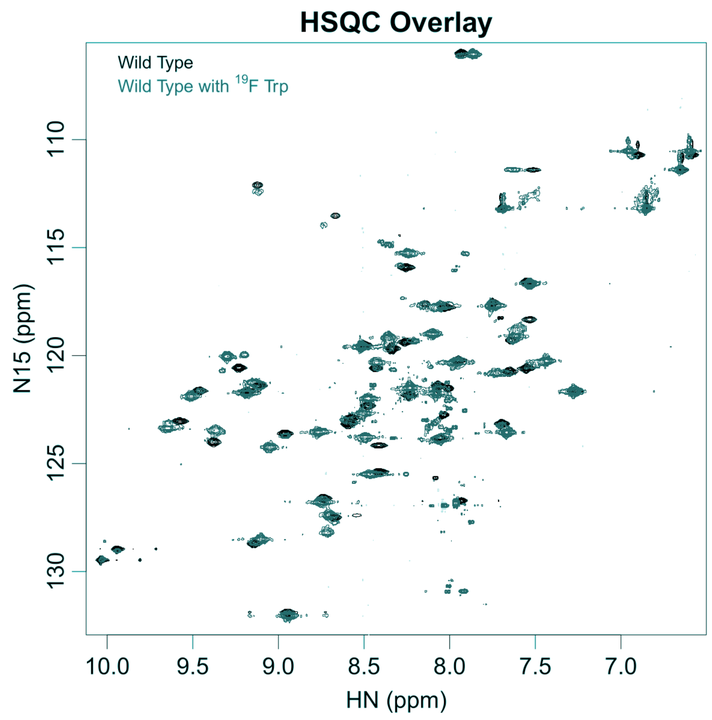
Wet Lab Validation

We have been working to validate our computational work by synthesizing our most promising mutations and analyzing them using NMR.

One technique we are planning to use is the introduction of a fluorine atom into the tryptophan sidechain. By doing so, we can use 1D ^{19}F NMR to find additional evidence that the peak multiplicity is caused by dynamic exchange between the two conformational states, not by partial dimerization.



5-Fluoro-tryptophan



Acknowledgements

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- Josh Dudley for his help in synthesizing my protein and obtaining my NMR spectra
- Professor Smith for guiding me through this project

References

1. Rocklin, G. J., Chidyausiku, T. M., Goreshnik, I., Ford, A., Houliston, S., Lemak, A., . . . Baker, D. (2017). Global analysis of protein folding using massively parallel design, synthesis, and testing. *Science, 357*(6347), 168-175. doi:10.1126/science.aan0693