Structural Features in Mini-Fluorescence Activating Proteins affecting Chromophore Bond Rotation Times

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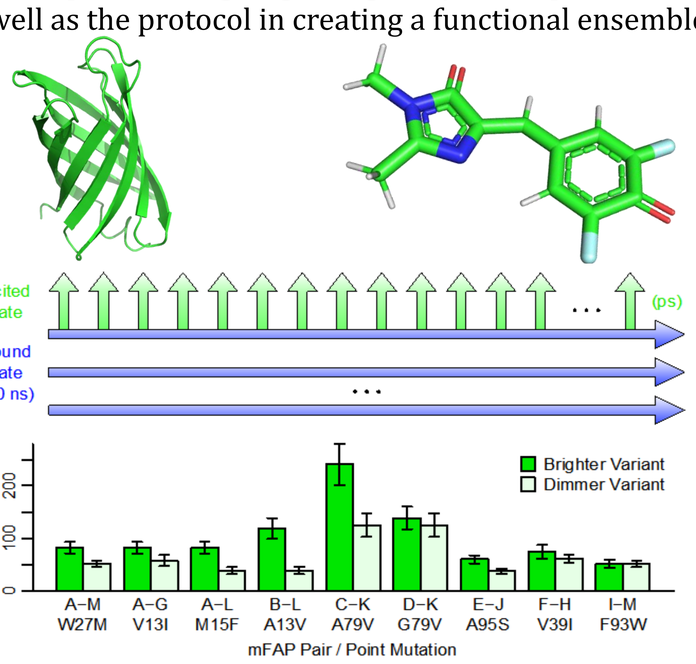
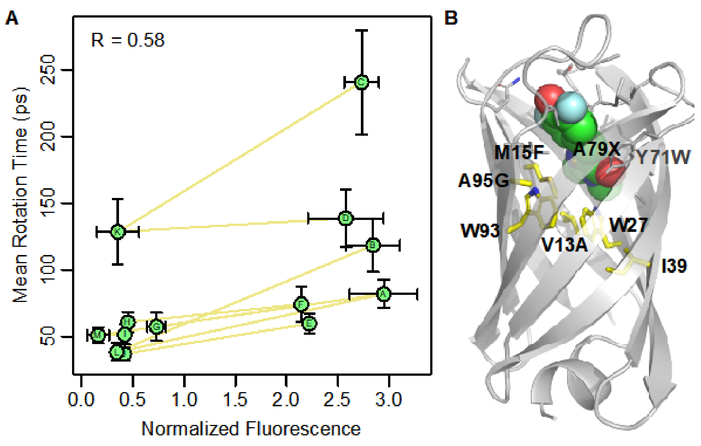
Introduction

- Designing proteins from scratch has been on the forefront of structural biology and can lead to key designs in enzymes within industrial, environmental and clinical fieldwork.
- Dou et al (2018) successfully engineered the first de novo designed fluorescence-activating β-barrel (mFAP) which binds to a small, green fluorescent-derivative known as 3,5-difluoro-4-hydroxybenzylidene imidazolinone(DFHBI).
- This protein-ligand experiment elucidates many general principles in designing a singular β-sheet protein as well as the protocol in creating a functional ensemble.



- Throughout the 100ns ground state simulation, an excited state simulation branched off every 50ps, creating 2000 snapshots per simulation

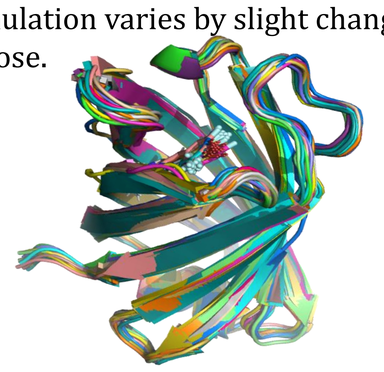


Central Question

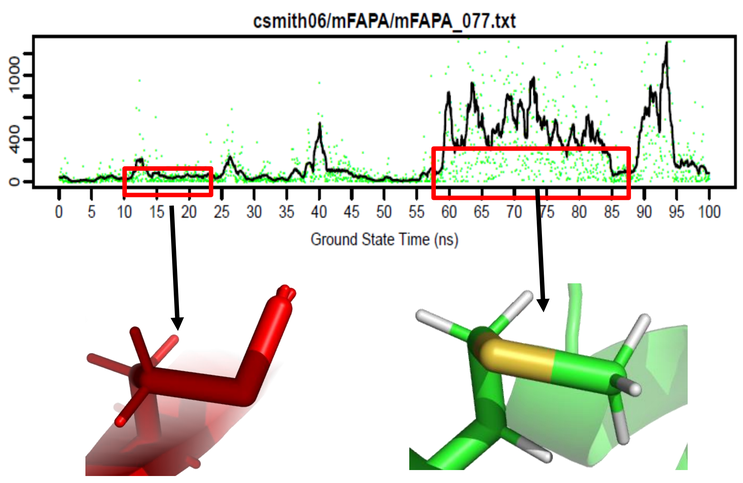
- Are there structural features that affect chromophore bond rotation times?

mFAPA MD Simulations

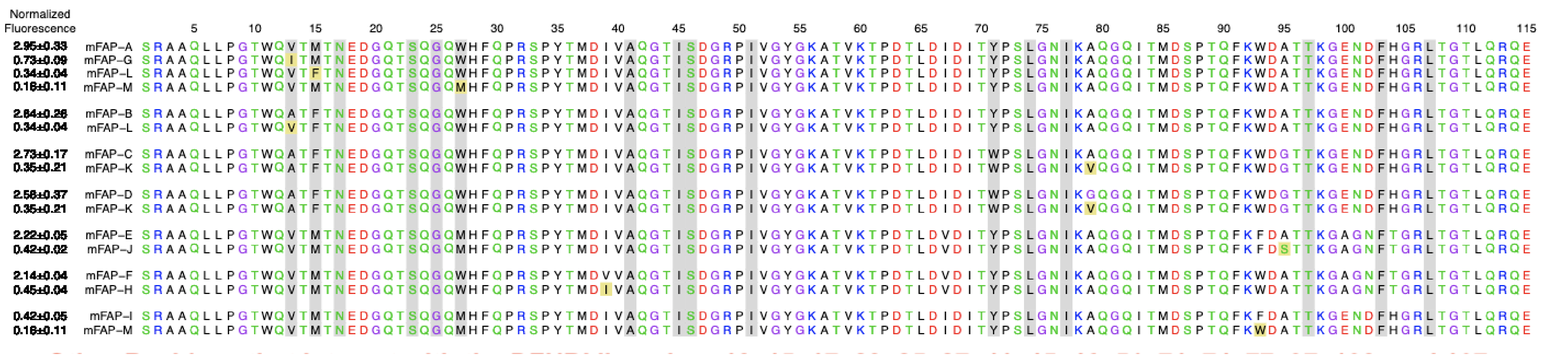
- Generating hypotheses of what significant features affected chromophore bond rotation times were done through visual analysis in Pymol.
- There are 20 simulations to observe
  - Each simulation varies by slight change DFHBI pose.



Notable Structural Features



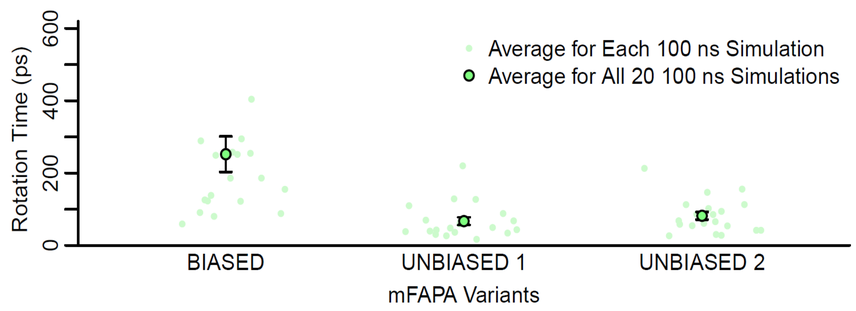
- Initial observations were done by looking at simulations with higher bond rotation times compared to others.
- Methionine 15 was found to be interesting in simulation 077 where it looked like it correlated with higher mean rotation times whenever it was in a g+,g+,g+ rotameric state.



Methodology

- The mFAPA-Met15 biased simulation was done using Gromacs. The process utilized 200 different DFHBI poses followed by the first repacking and minimizing mFAP models. After the minimization, the mFAP model was repacked again. When running the ground state simulation, a dihedral restraint itp file was included to ensure it stays at the ideal chi angle.
- Running the excited state simulations were done on the High-Performance Computing Cluster (HPCC) at Wesleyan.
- Data visualization was primarily done in R/Rstudio where additional hypotheses were generated.
- Gromacs 2019 was also used in generating spliced trajectory files, average atomic fluctuation pdbs and for the Met-15 dihedral restraint.
- Pymol v2.4 was used to primarily observe the 100ns MD simulations

Results



- Met-15 was restrained in a g+,g+,g+ rotamer conformation throughout the entire ground state simulation.
- Most of the simulations experienced an increase in chromophore bond rotation times compared to unbiased 1 and unbiased 2.

Conclusion

- It appears biasing Methionine 15 into a rotameric state of a g+,g+,g+ increased the mean rotation time by almost double compared to other mFAPA ensembles.
- Although the bias simulation results indicates an increase in chromophore bond rotation time, the mechanisms in which influencing the rotamer into that state is unknown.

Future Directions

- Run additional bias simulations to find significant changes in chromophore bond rotation times
- Perform a partial least squares regression to find the linear mode that shows the highest co-variance of bond rotation times
- Validate the bias simulation by creating possible mutations within mFAP models that can lead to the rotameric state of Met-15 in g+,g+,g+
- Re-run the dihedral restraint simulation using Rosetta constraint file and compare the results with the Gromacs only biased simulation.

References and Acknowledgement

Dou, J., Vorobieva, A.A., Sheffler, W. *et al.* De novo design of a fluorescence-activating β-barrel. *Nature* **561,** 485–491 (2018). https://doi.org/10.1038/s41586-018-0509-0

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