Optimization of mini-Fluorescence Activating Protein (mFAP) Brightness with GFP-mimetic chromophore DFHBI

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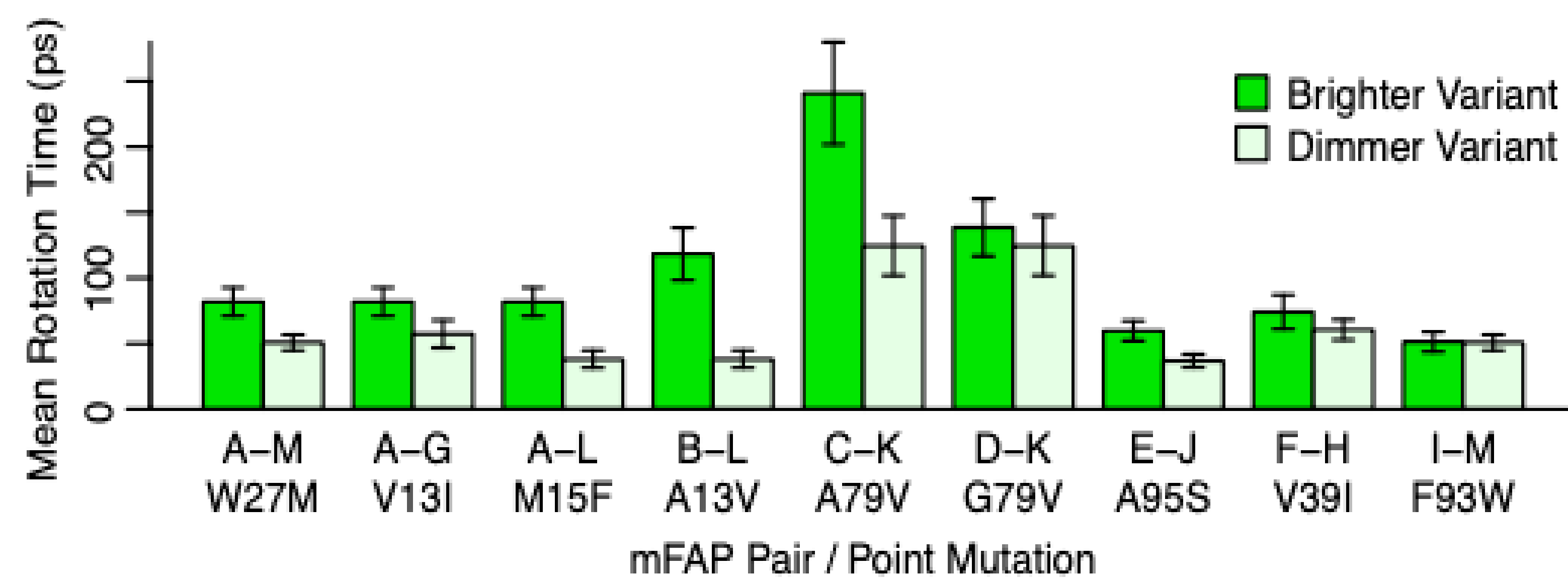Wesleyan University, Middletown, CT 06459

Background

- Artificial proteins can be useful in antiviral, enzymatic or clinical fieldwork

- The mini–Fluorescence Activating Protein (**mFAP**) which binds to a small, fluorescent molecule known as 3,5-difluoro-4-hydroxybenzylidene imidazolinone (**DFHBI**)

- Upon binding, it **stabilizes the chromophore** in a planar-Z conformation, enabling it to fluoresce
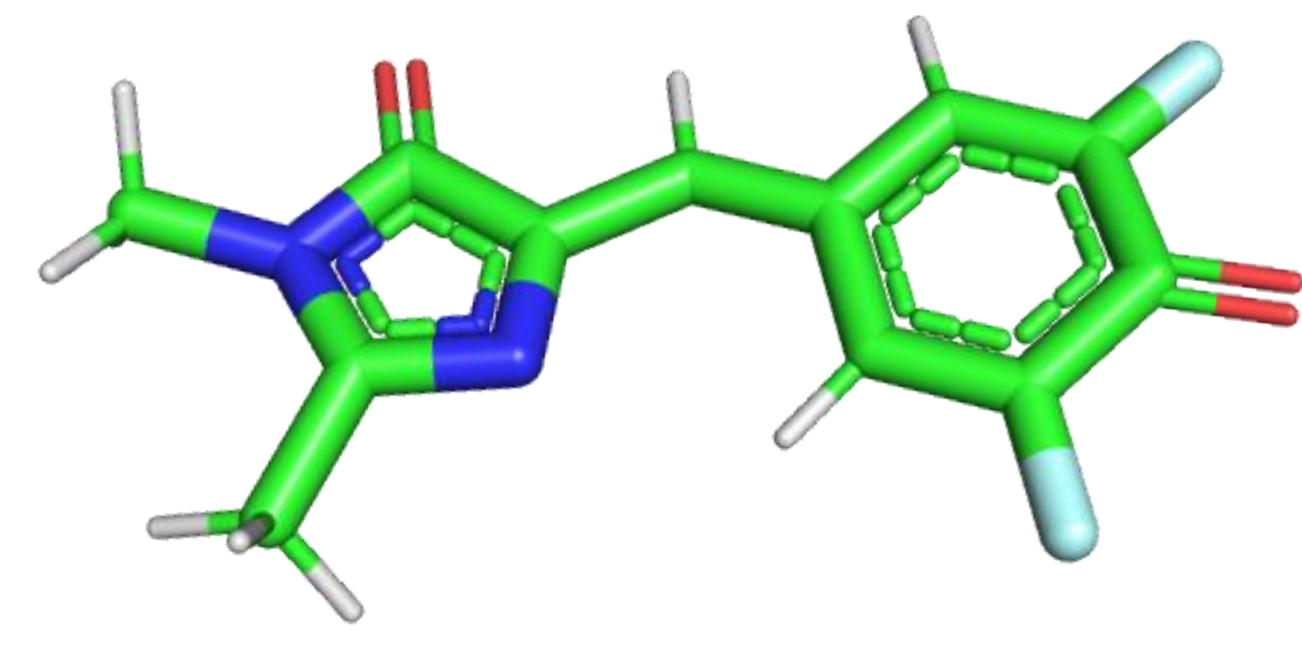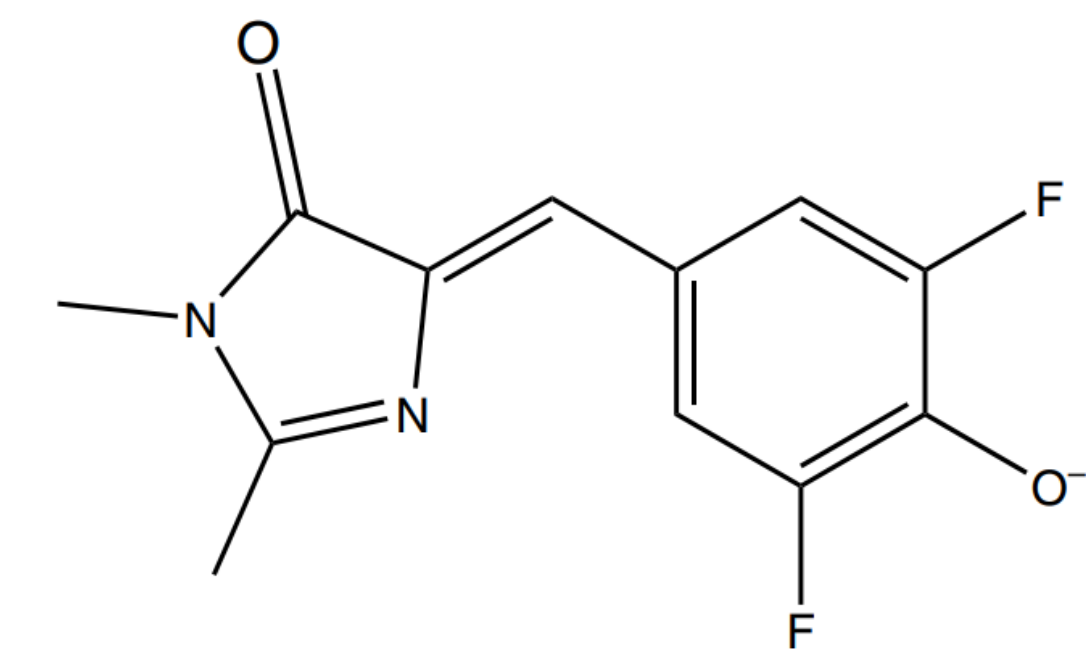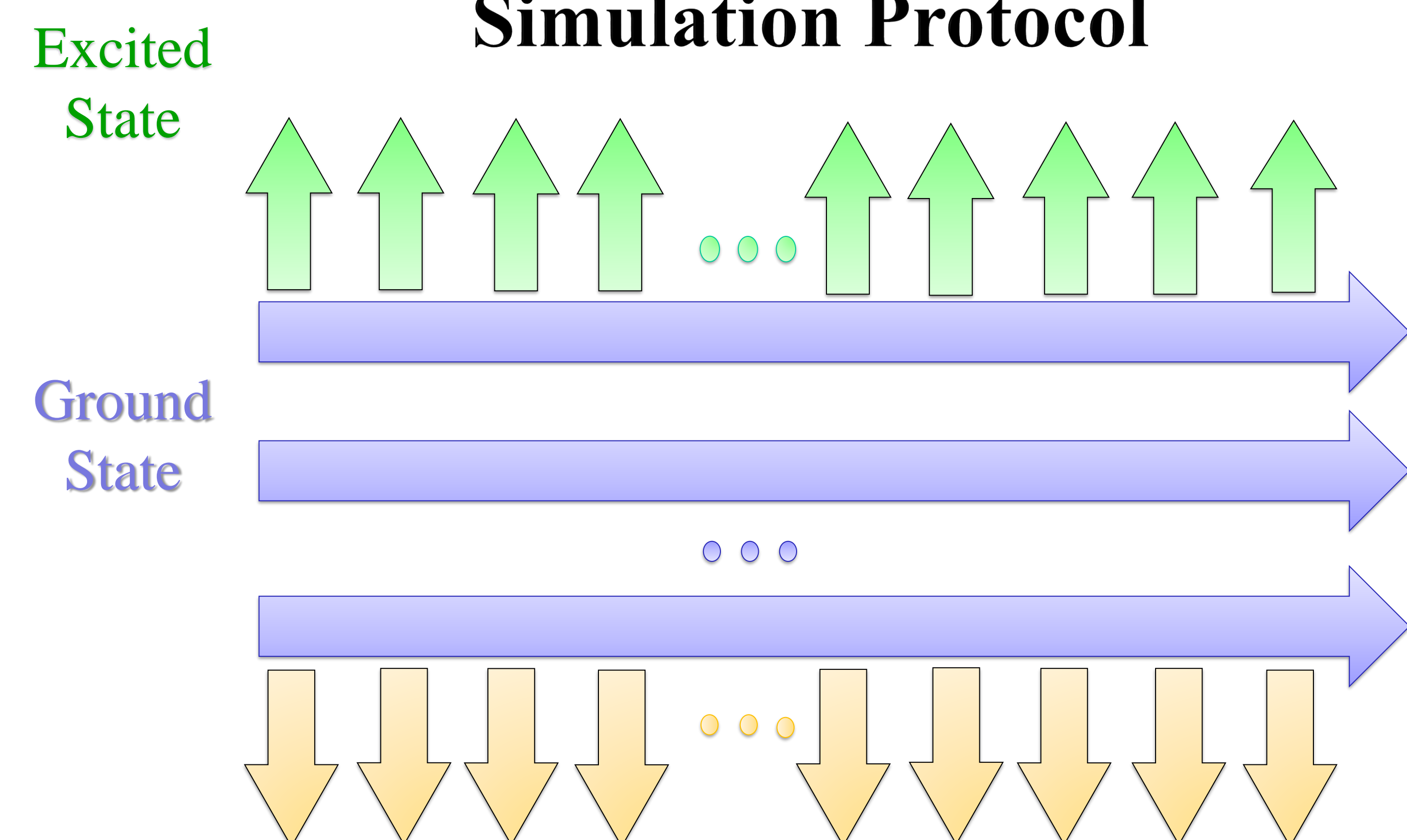
Chemical Structure   Cartoon Structure

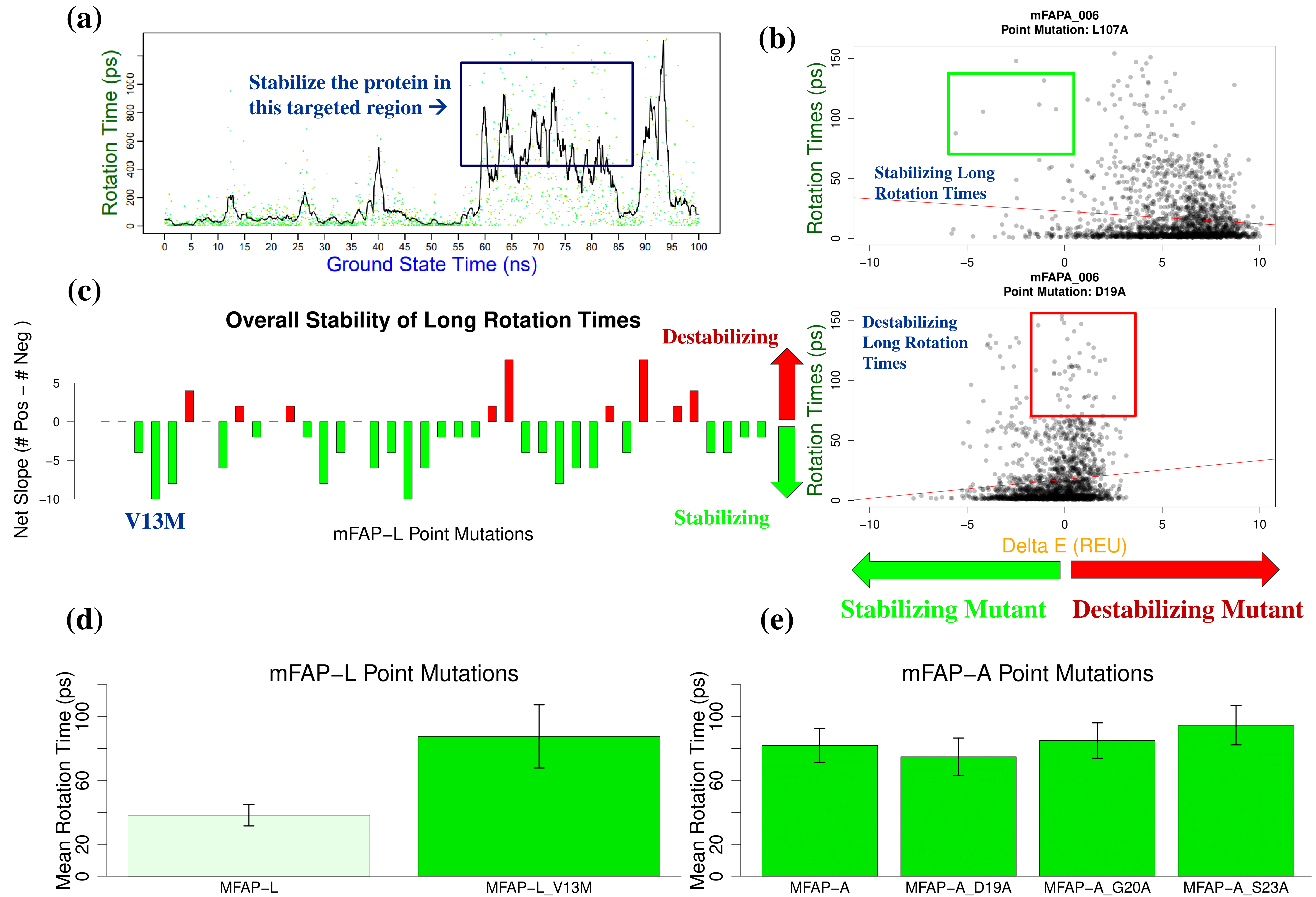## Molecular Dynamic (MD) Simulations Predicts Fluorescence

Simulation Protocol

Excited State

Ground State

PyRosetta Mutations

Results

(a) Stabilize the protein in this targeted region →

(b) mFAPA_006 Point Mutation: L107A — Stabilizing Long Rotation Times

(c) **Overall Stability of Long Rotation Times** — Destabilizing / Stabilizing — V13M — mFAP–L Point Mutations

mFAPA_006 Point Mutation: D19A — Destabilizing Long Rotation Times

Delta E (REU) — Stabilizing Mutant / Destabilizing Mutant

(d) mFAP–L Point Mutations

(e) mFAP–A Point Mutations

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References

1. Klima, J.C., Doyle, L.A., Lee, J.D. et al. Incorporation of sensing modalities into de novo designed fluorescence-activating proteins. Nat Commun **12,** 856 (2021). https://doi.org/10.1038/s41467-020-18911-w

2. 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

3. Hostetter, E., Keys, J., Poon, I., Nguyen, J., Nite, J., Molecular Modeling and Design Class, Hoyos, C. J., Smith, C. A. Prediction of fluorophore brightness in designed mini fluorescence activating proteins. Manuscript in preparation.

4. M.J. Abraham, D. van der Spoel, E. Lindahl, B. Hess, and the Gromacs development team, GROMACS User Manual version 2019, http://www.gromacs.org