Computational investigation of tryptophan fluorescence in DPO4 mutants through classical approaches

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3rd ICTP-SAIFR Symposium on Current Topics in Molecular Biophysics (CTMB3)

Tryptophan fluorescence

Tryptophan fluorescence is highly sensitive to the molecular environment, so it is widely used to investigate the structure and dynamics of proteins.

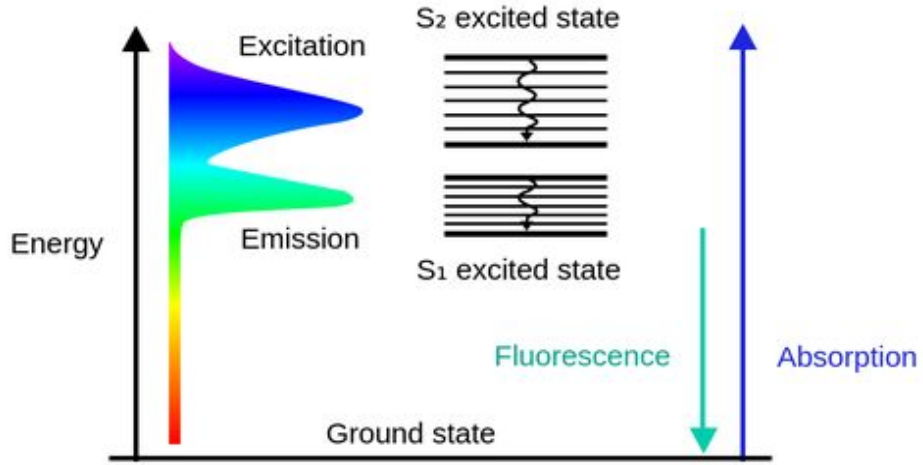


Image: Labster Theory pages

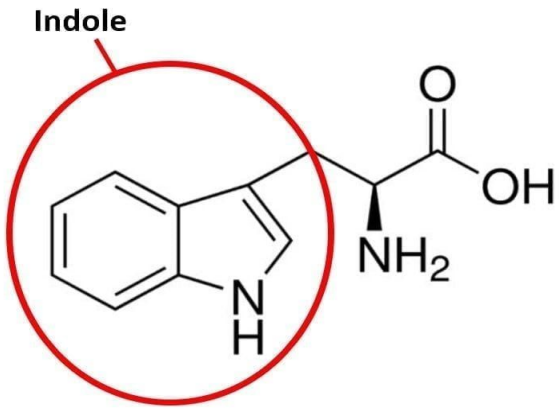


Image: Edinburgh Instruments

Tryptophan fluorescence

Non-radioactive decay pathways are difficult to predict, and usually require quantum methods.

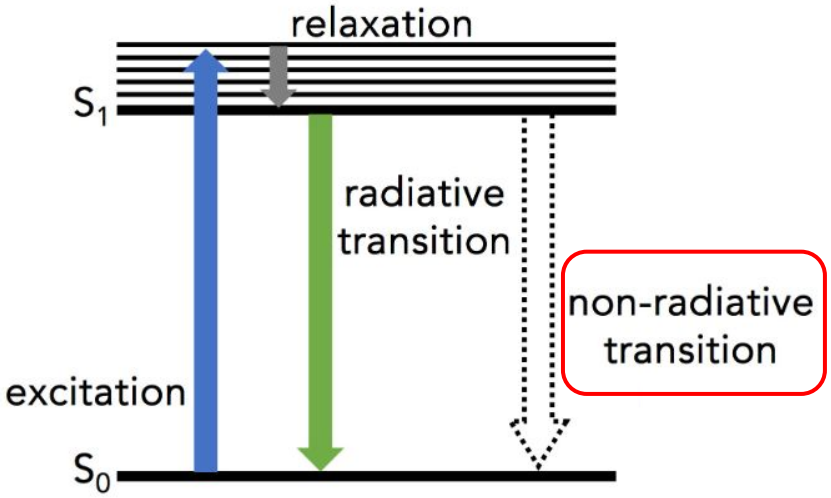


Image: Edinburgh Instruments

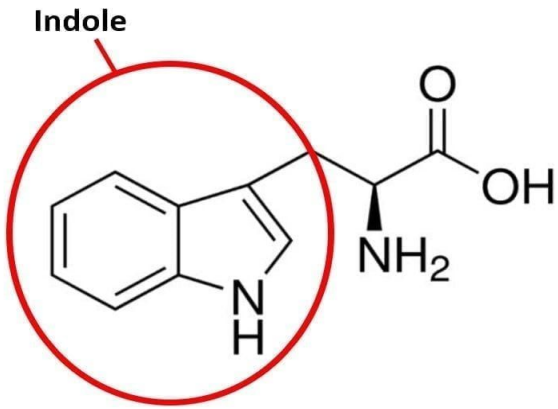
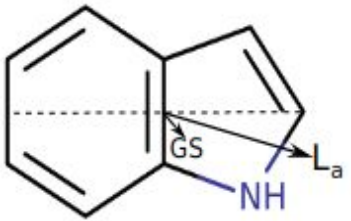


Image: Edinburgh Instruments

Dipole moments of the TRP indole group

Orientation of the ground state (GS) and La dipoles of indole and correlation between experimental and calculated λ_{em}, according to a hybrid QM/MM model (Vivian & Callis, 2001. Biophys. J.)



indole

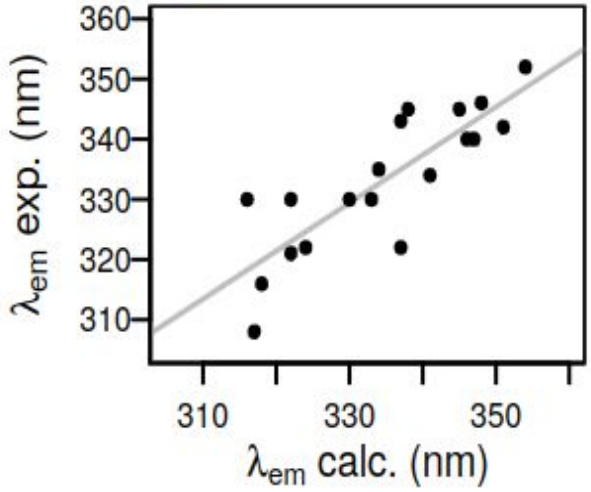
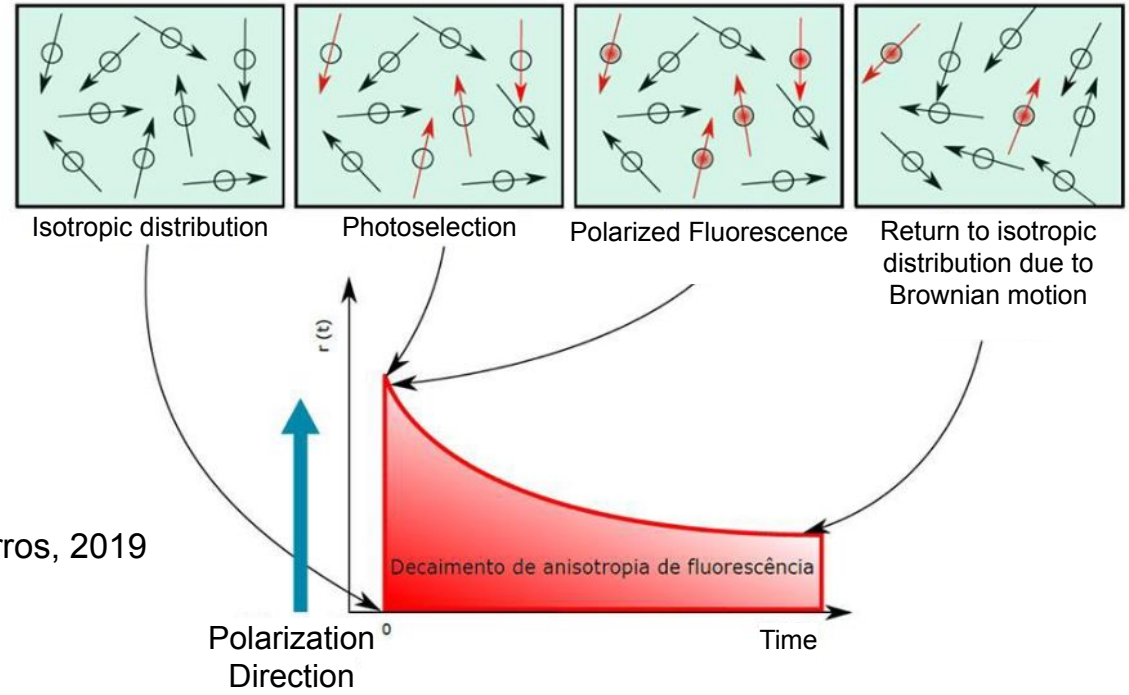


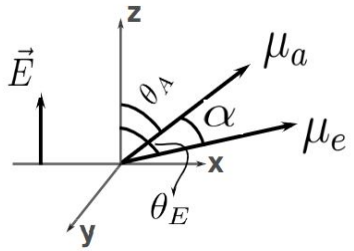
Image: Ayme, 2019

Fluorescence anisotropy

Anisotropy experiments track the orientation of the chromophore transition dipole after excitation.



Barros, 2019

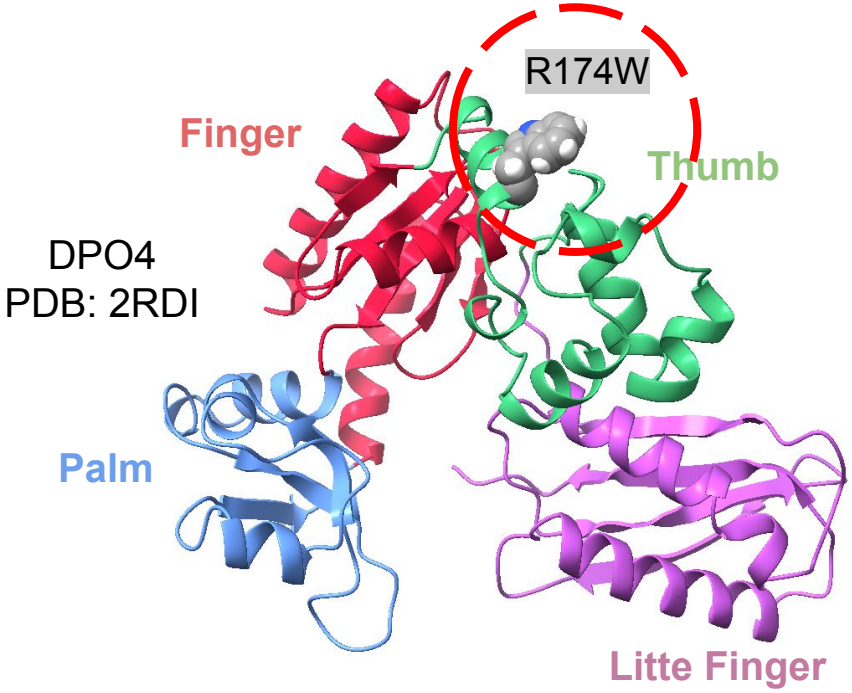


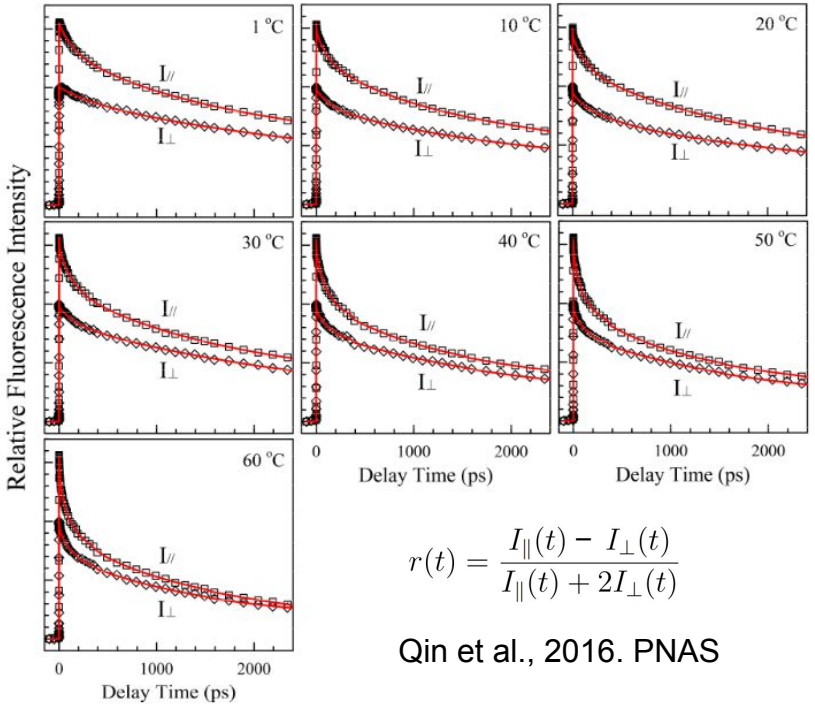
Anisotropy can be obtained classically through the correlation function:

$$r(t) = \frac{2}{5} \langle P_2[\mu_a(0)\mu_e(t)] \rangle$$

The target enzyme: DPO4

Anisotropy data for the R176W mutant of DOP4 at various temperatures



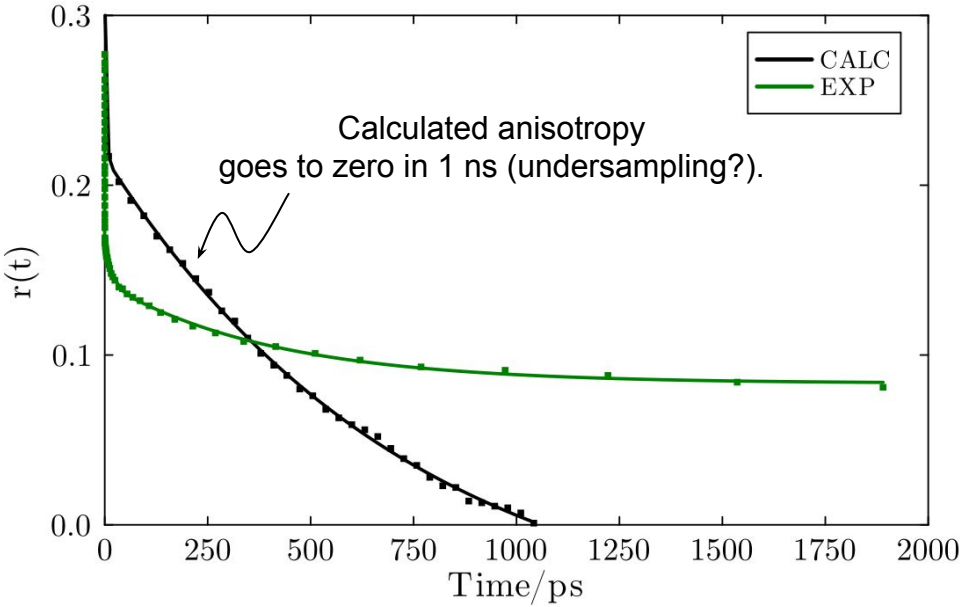


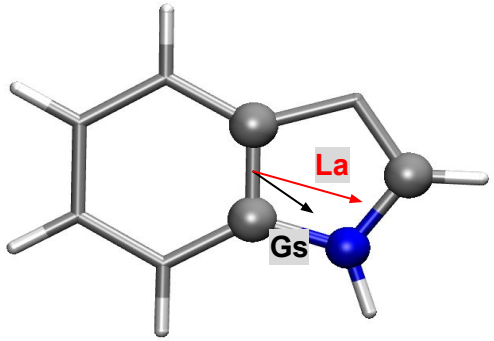
$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)}$$

Qin et al., 2016. PNAS

Anisotropy calculations

Calculated vs. experimental anisotropy of the W176 indole group

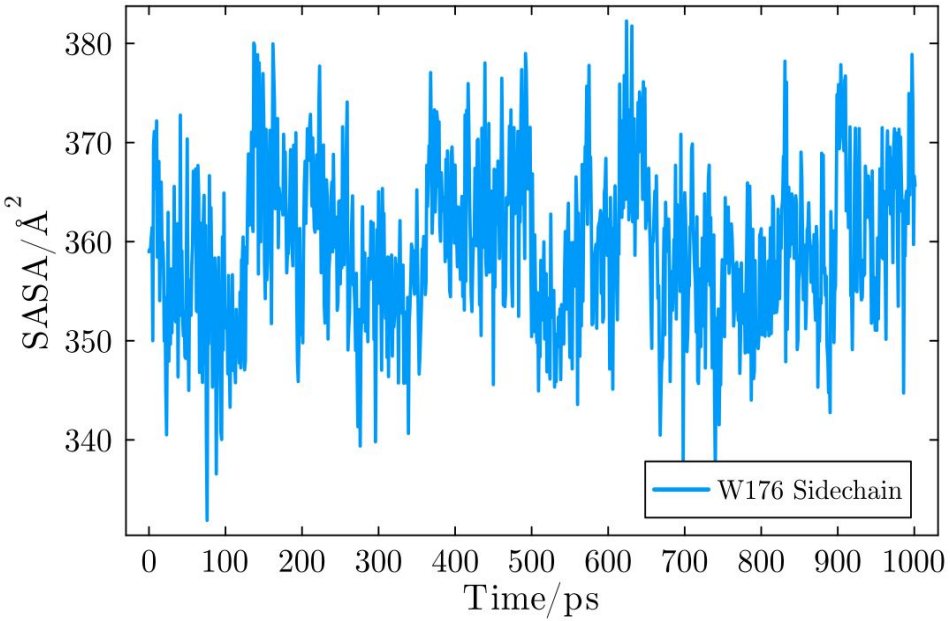


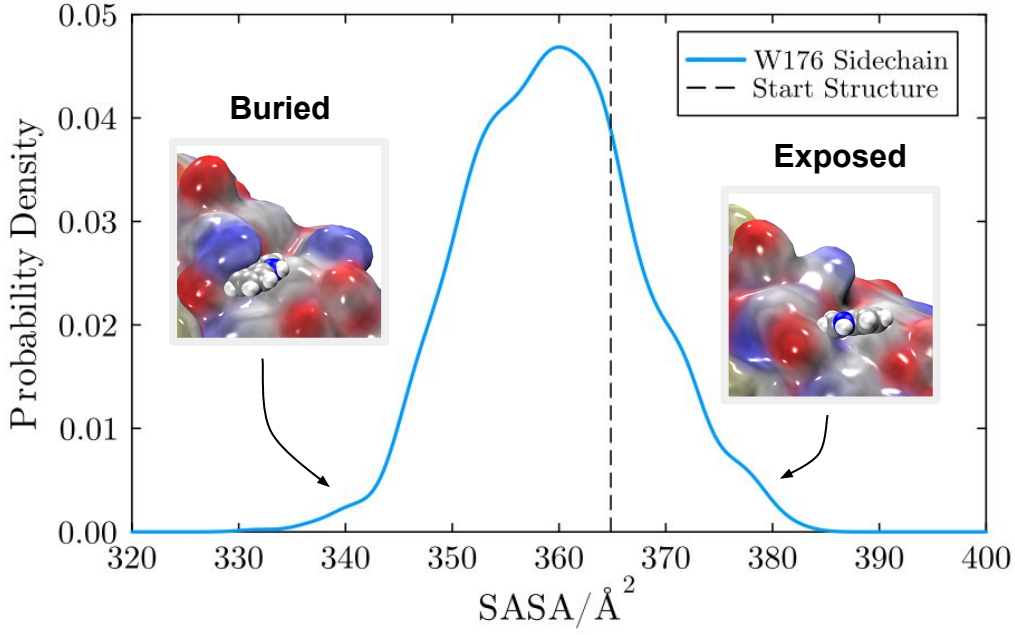


tryptophan indole

Exposure to the solvent of the indole

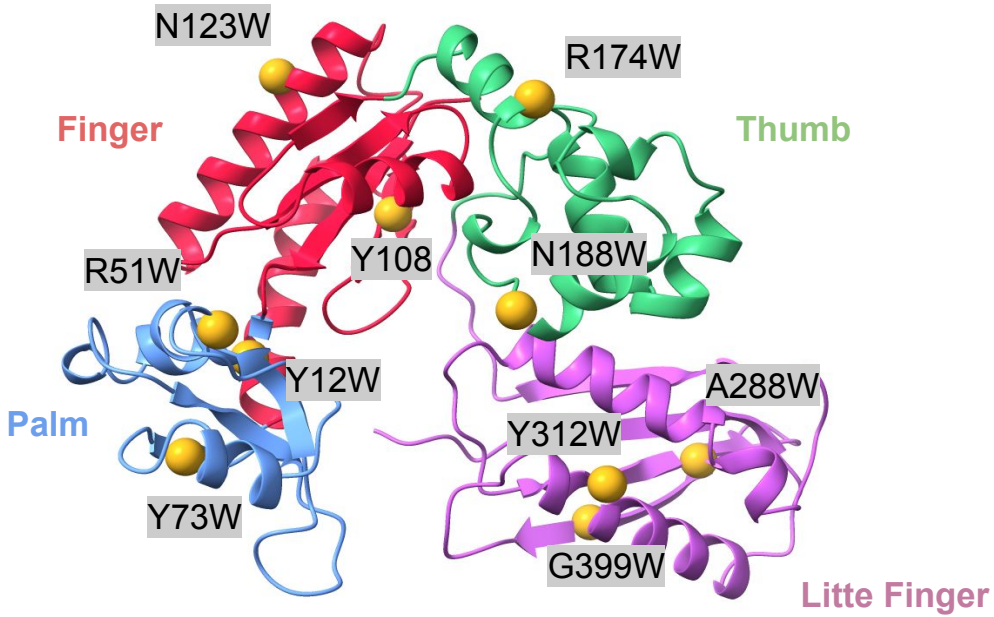
Is the TRP conformation representative of experimental anisotropy decay?

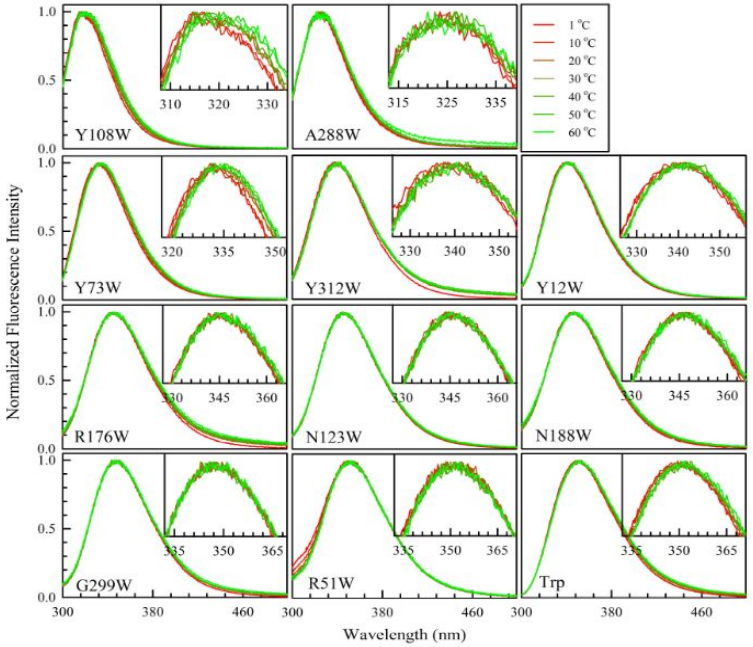




Experimental spectra of DPP4 mutants

Fluorescent spectra for various DPO4 mutants at different temperatures (1-60 ºC)

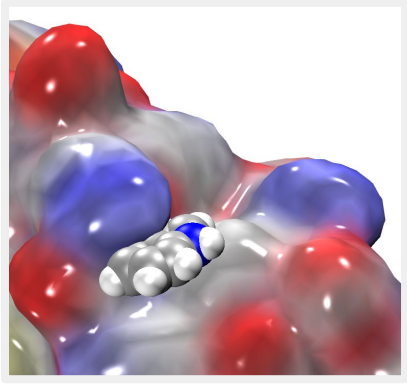




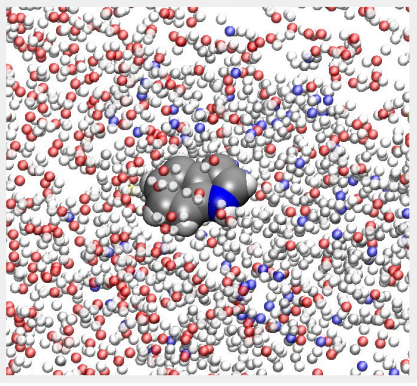
Qin et al., 2016. PNAS

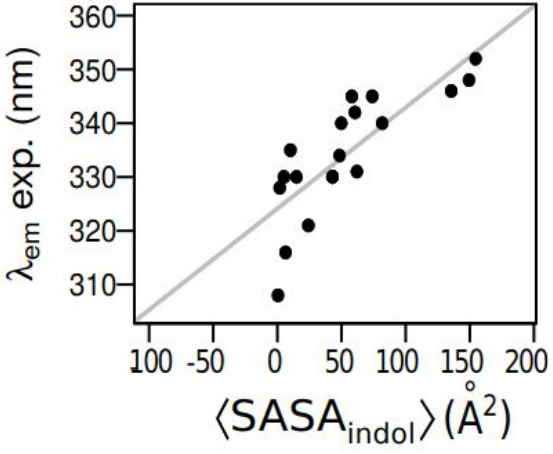
Parametric models to compute tryptophan emission wavelengths

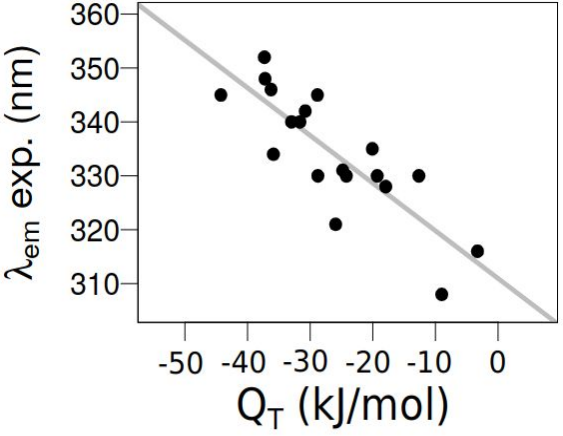
SASA



Electrostatic interaction







Data: Ayme, 2019

Phases of the project

1. **PDO4 R176W mutant anisotropy:** establish a simulation and analysis protocol for reproducing the experimental decay of anisotropy in water;
2. **Parametric models:** employ the experimental spectra of different DOP4 mutants to formulate a parametric model (based on SASA, electrostatic field, etc.) to compute λ_{em};
3. **Cossolvents:** investigate the DP4O in the presence of cossolvents, combining solution thermodynamics and spectroscopy analysis.

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FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

Thank you!

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