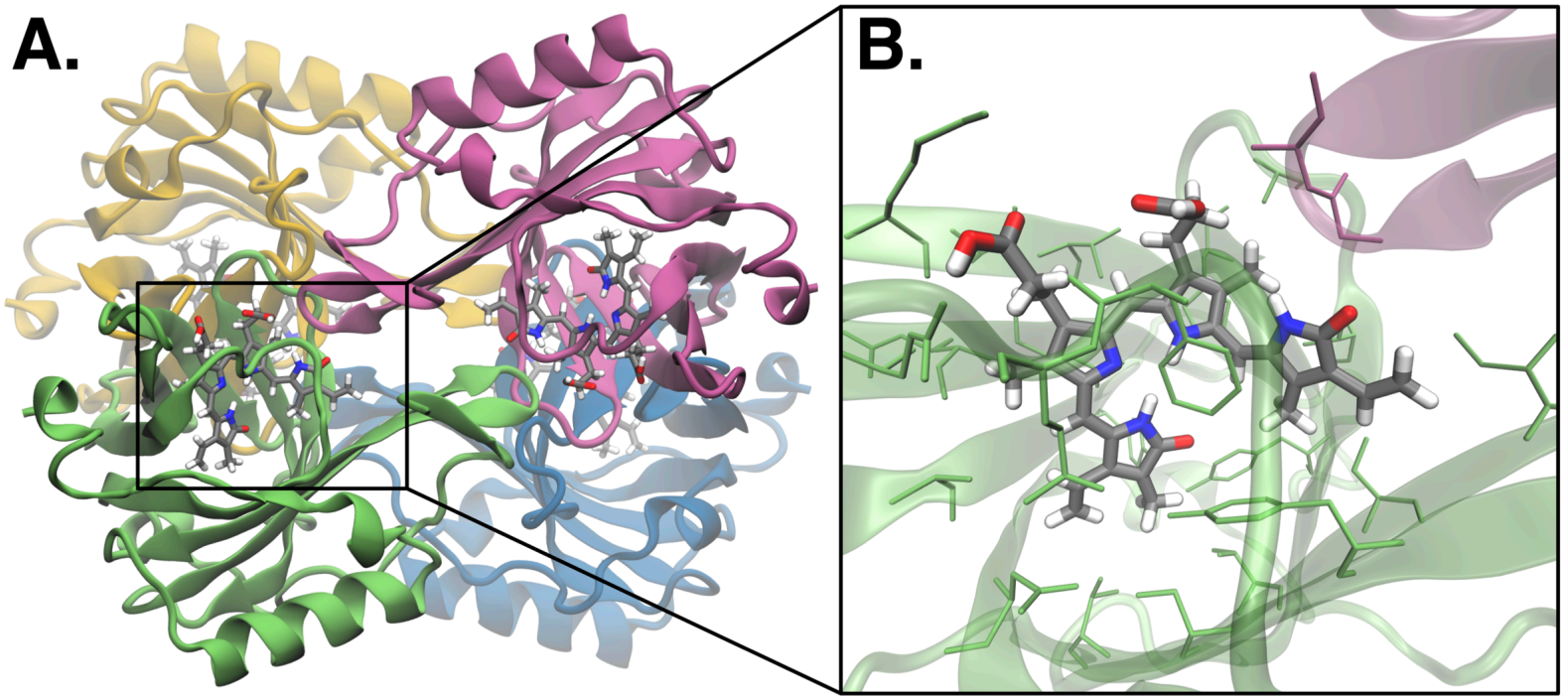
The Propionic Chains of Biliverdin Influence Oligomerization in Sandercyanin

Eleftherios Mainas, Ph.D.

outline of paper

- intro & motivation
- CpHMD model of the two propionic tails
- microstate model
- protonation dependent dynamics
- absorption spectra

🐟 protein - SFP/BLA complex

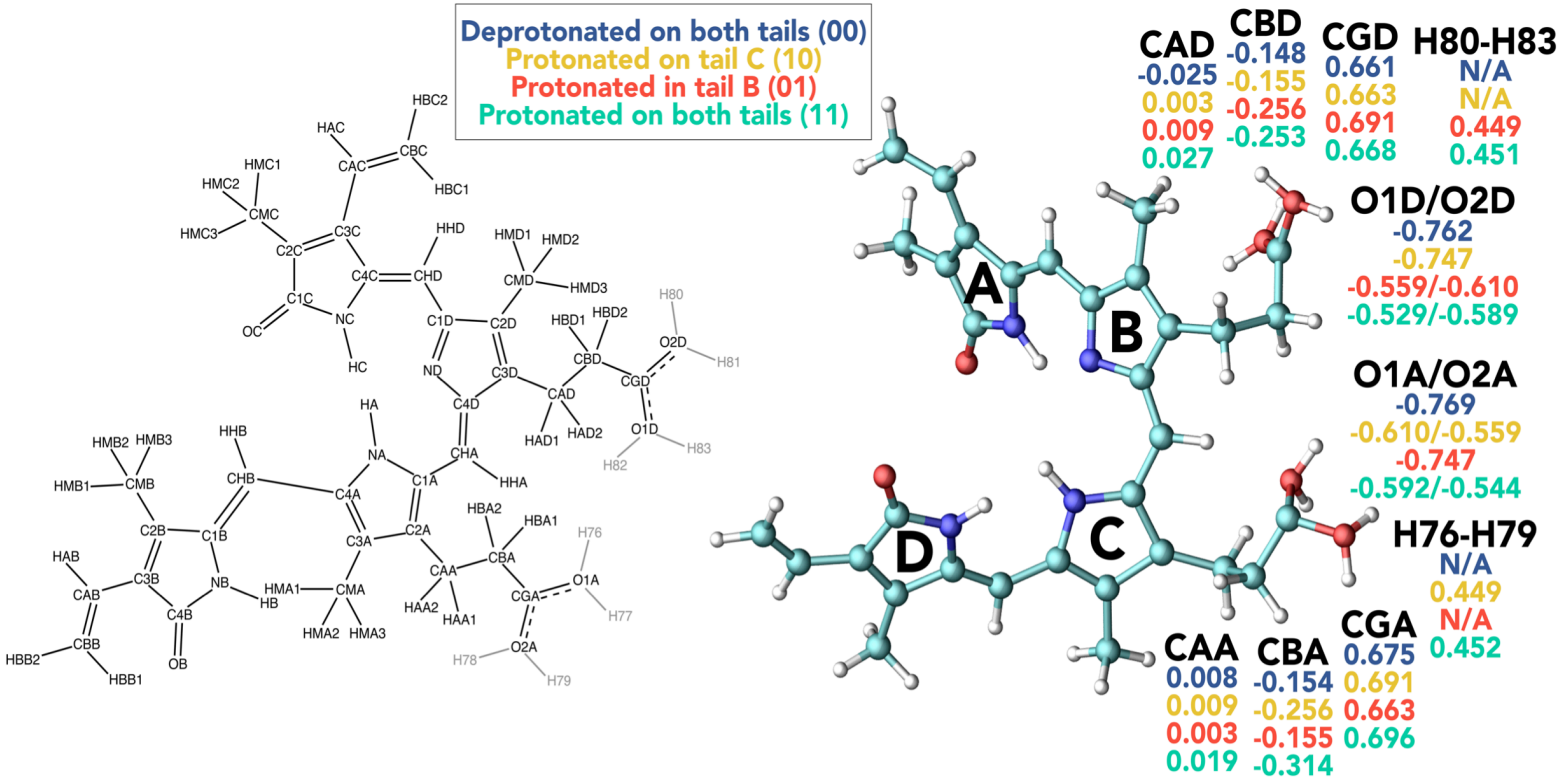


- Tetramer (4 biliverdins) - *[Ghosh et al., PNAS, 2016]*
- monomeric variants & preserve spec properties

propionic tails

- protagonist of the "interactome" of the SFP-SFP interface
- protonation state? what is the pK_a?
- interplay between protonation/conformation/dynamics
- intramolecular/intermolecular h-bonding, pi-pi stacking and other interactions

CpHMD model



- MD in implicit solvent/chain-clustering/RESP fitting

microstate model

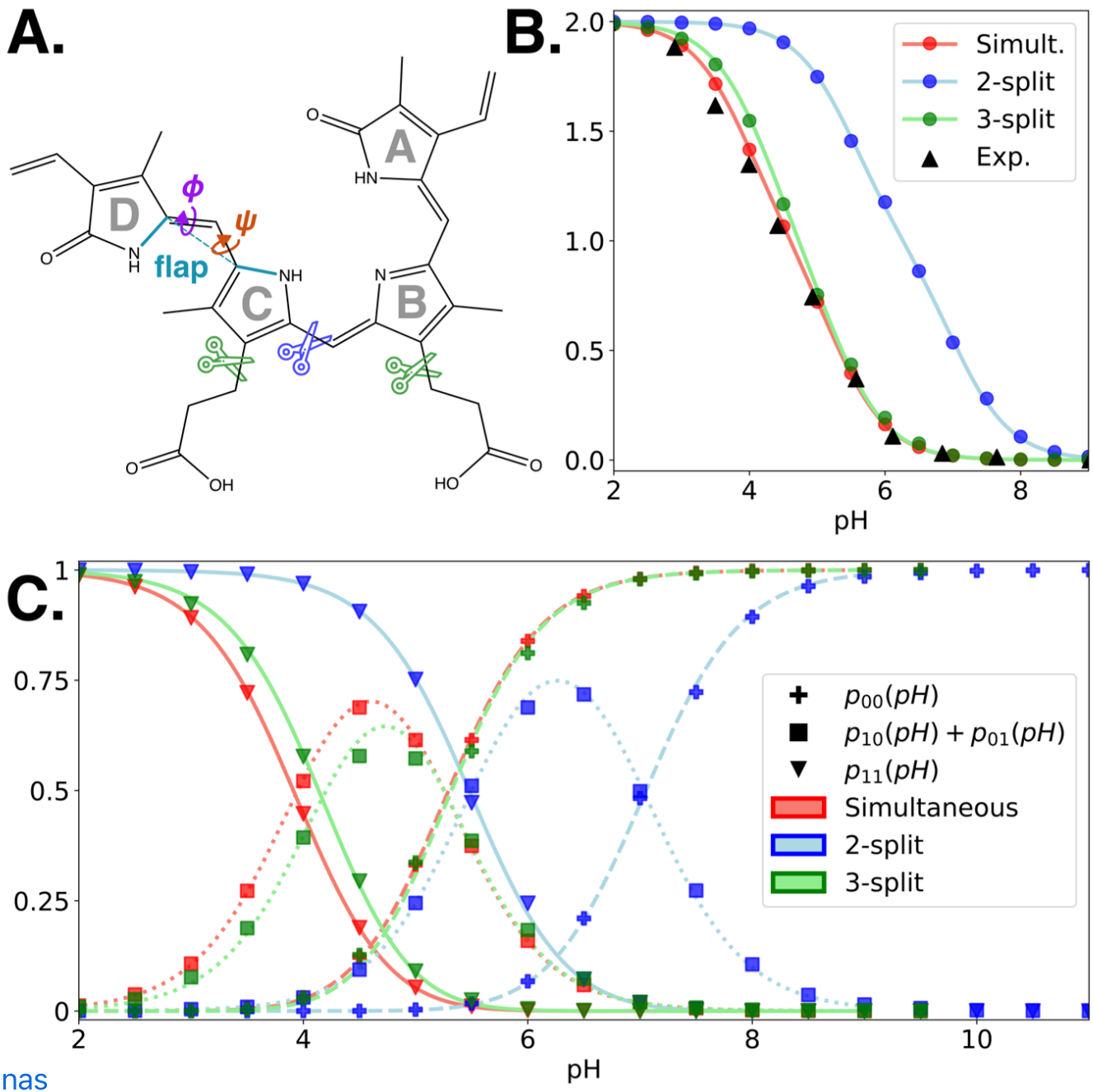
$$p_{00}(pH) = \frac{1}{\Xi(pH)} \quad (1)$$

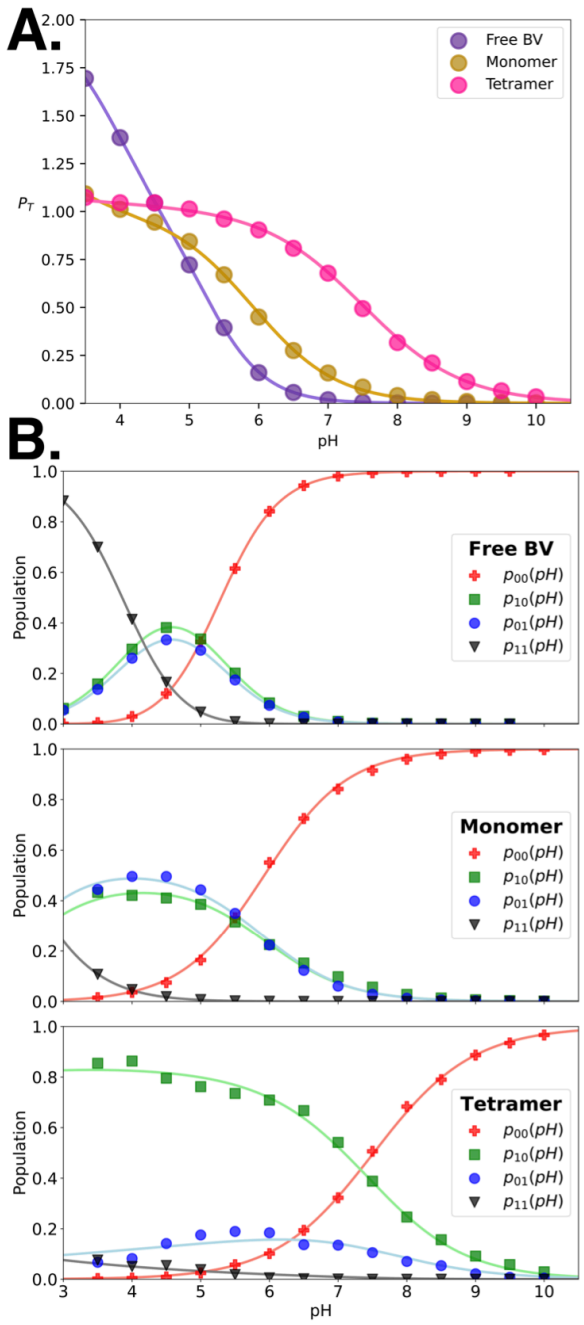
$$p_{10}(pH) = \frac{10^{pK_{10}-pH}}{\Xi(pH)} \quad (2)$$

$$p_{01}(pH) = \frac{10^{pK_{01}-pH}}{\Xi(pH)} \quad (3)$$

$$p_{11}(pH) = \frac{10^{pK_{10}+pK_{01}-W-2pH}}{\Xi(pH)} \quad (4)$$

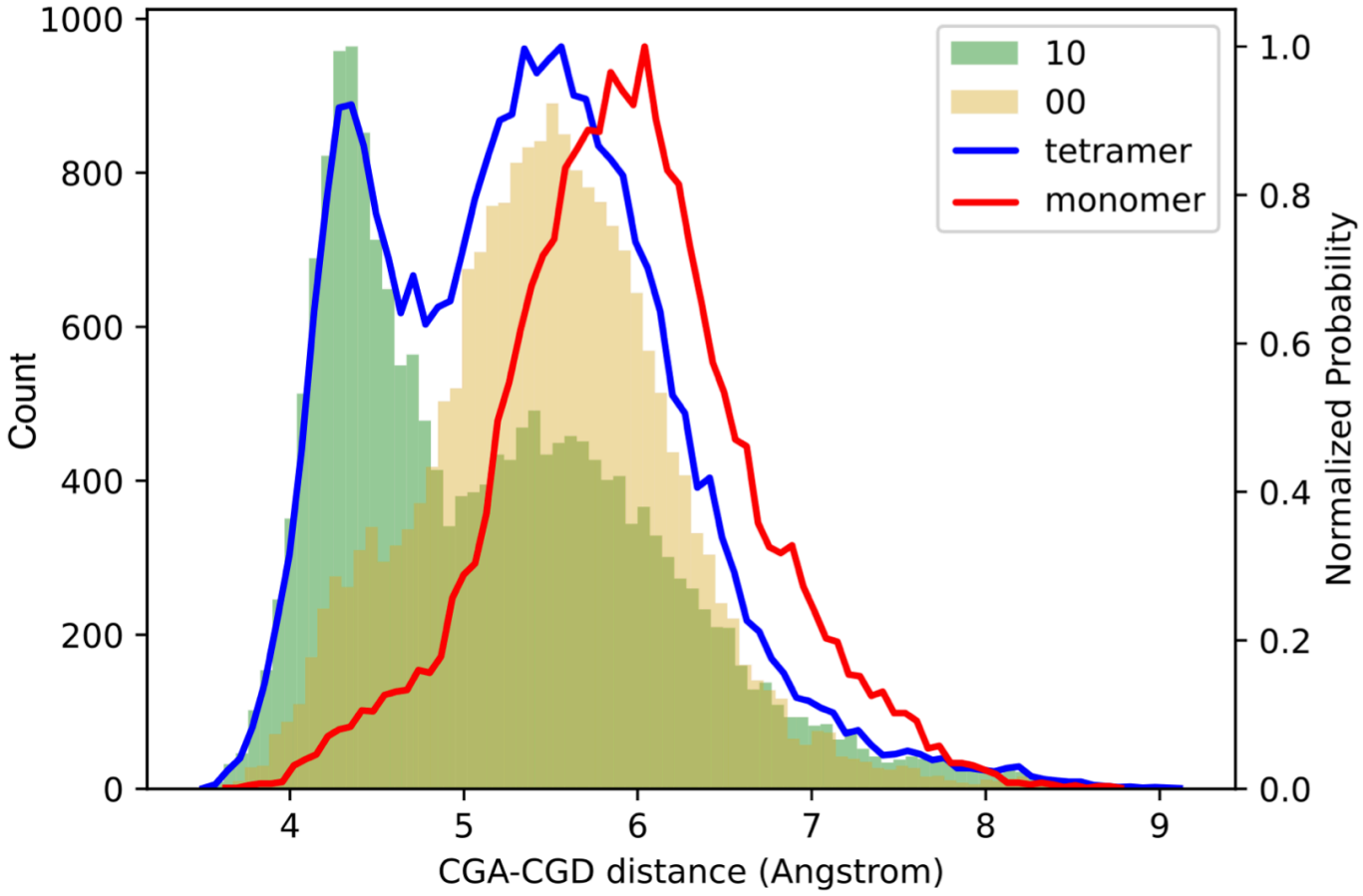
Ullmann et al., JPCB, 2003 and *Bombarda et al., JPCB, 2010*


A.
B.
C.
D
A
B
C
flap
ϕ
ψ
Simult.
2-split
3-split
Exp.
pH
$p_{00}(pH)$
$p_{10}(pH) + p_{01}(pH)$
$p_{11}(pH)$
Simultaneous
2-split
3-split



- water and monomer - symmetry
- tetramer - no symmetry
- p10 dominates
- also: spot the kinetic trap
- why is tetramer so different?

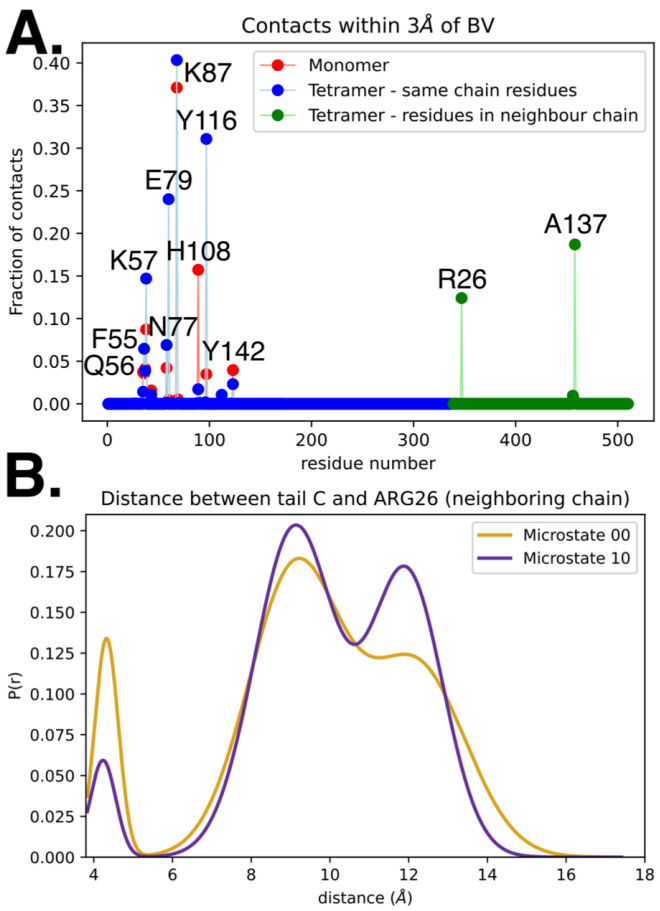
surprising h-boding

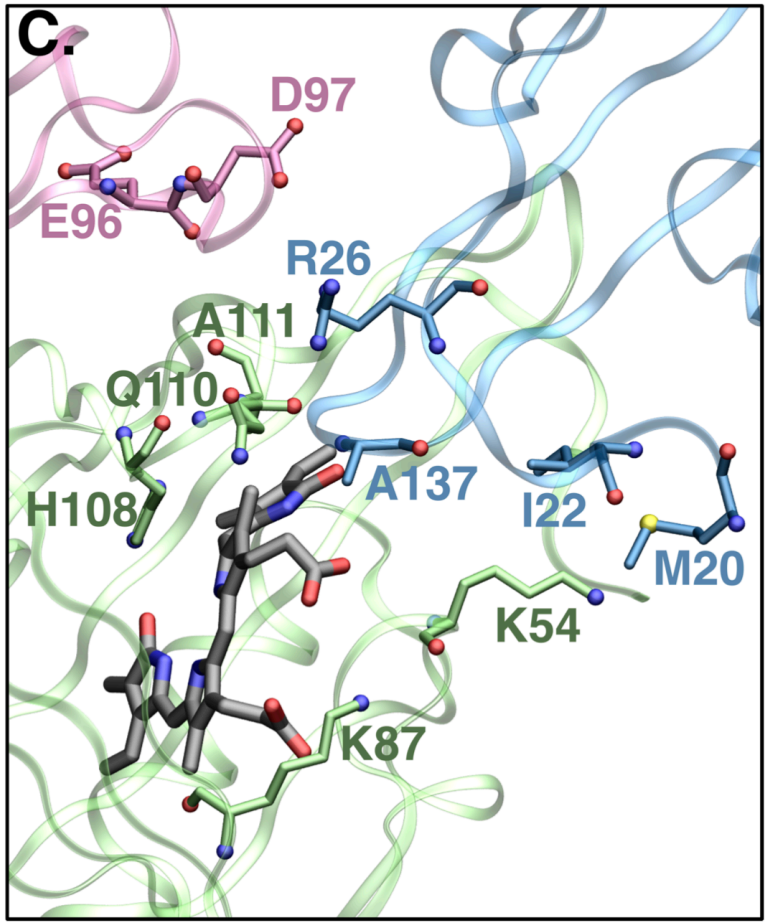


tetrameric packing

- first hydration shell around tail C loses on average ~1.5 water molecules
- tight packing of BV and h-bonding between chains
- average tail distance of 4.3 Å vs 5.9 Å
- Neighboring SFP unit provides hydrophobic residues to the left chain
- Right chain is exposed to polar residues and water

tetrameric environment

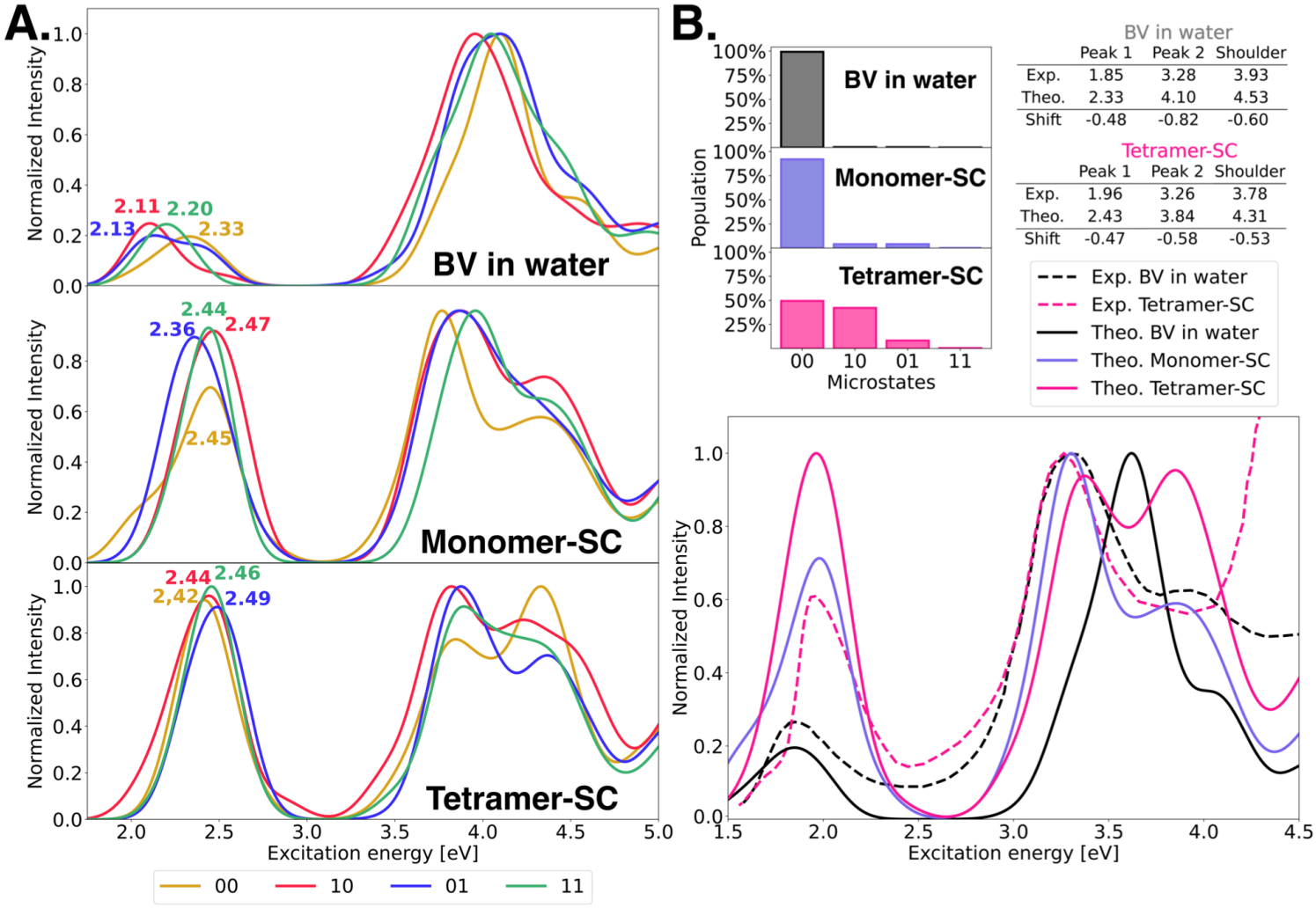




targeted mutations

- disrupt oligomerization while having minimal impact on the fluorescent quantum yield of SC
- Hybrid mutations (tighter BV packing without oligomerization)
- our paper suggests: ARG26, LYS54, ASP96, GLU97, ALA111 and ALA137

spectroscopy



what is next?

- long unbiased MD of point-mutated proteins (Noureen and Shaena). We should talk BV FF/protonation states/setup!
- calculation of protein-protein binding free energy from unbiased MD
- dimer-dimer binding happens in long time-scales
- enhanced sampling is needed

predict complex formation

- statmech (Molecular Dynamics)
- docking (Rosetta - 1/2 Nobel 2024)
- deep learning (AlphaFold - 1/2 Nobel 2024)

statmech for ABFE 😢 or RBFE 😄

- enhanced sampling (MetaDynamics, Gaussian accelerated MD, T-REMD)
- biased MD (Umbrella Sampling, Steered MD)
- Coarse Grained MD (Sirah FF, Martini FF)
- endpoint free energy methods (mmPBSA or mmGBSA) - tool in AMBER
- alchemical methods (FEP, TI or BAR)

plan

- quantity we wanna calculate (ABFE/RBFE)
- mini-lit review on methods
- PPI-GaMD, MiaoLab@UNC *[Wang et al., JCTC, 2022]*
- workshop on 28th of October

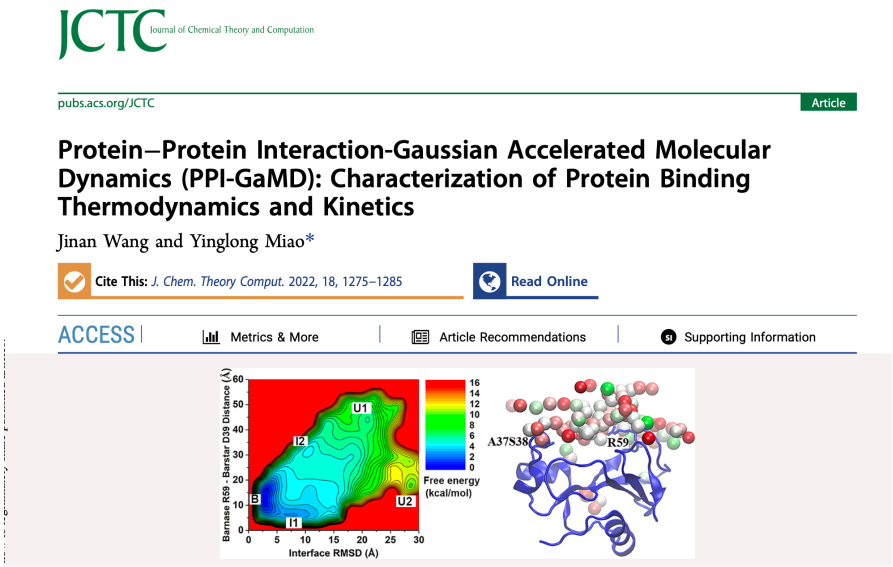
4. Protein-protein interaction-Gaussian accelerated molecular dynamics (PPI-GaMD)

Protein-protein interactions (PPIs) play key roles in many fundamental biological processes such as cellular signaling and immune responses. However, it has proven challenging to simulate repetitive protein association and dissociation in order to calculate binding free energies and kinetics of PPIs, due to long biological timescales and complex protein dynamics. To address this challenge, we have developed a new computational approach to all-atom simulations of PPIs based on GaMD. The method, termed "PPI-GaMD", selectively boosts interaction potential energy between protein partners to facilitate their slow dissociation. Meanwhile, another boost potential is applied to the remaining potential energy of the entire system to effectively model the protein's flexibility and rebinding. PPI-GaMD has been demonstrated on a model system of the ribonuclease barnase interactions with its inhibitor barstar. Six independent 2 μs PPI-GaMD simulations have captured repetitive barstar dissociation and rebinding events, which enable calculations of the protein binding thermodynamics and kinetics simultaneously. The calculated binding free energies and kinetic rate constants agree well with the experimental data. Furthermore, PPI-GaMD simulations have provided mechanistic insights into barstar binding to barnase, which involve long-range electrostatic interactions and multiple binding pathways, being consistent with previous experimental and computational findings of this model system. In summary, PPI-GaMD provides a highly efficient and easy-to-use approach for binding free energy and kinetics calculations of PPIs.

Reference

Wang, J. and Miao, Y*, (2022) Protein-protein interaction-Gaussian accelerated molecular dynamics (PPI-GaMD): Characterization of protein binding thermodynamics and kinetics. Journal of Chemical Theory and Computation, 18(3):1275-1285. (Abstract | PDF)

method I like



- unconstrained enhanced sampling
- no requirement of predefined RC or CVs
- kinetics
- barnase–barstar:

$$\Delta G_{sim} = -17.79\ \mathrm{kcal/mol}$$

$$\Delta G_{exp} = -18.90\ \mathrm{kcal/mol}$$

ideas?