Excited state QM/MM dynamics of a BLUF photoreceptor

Joshua Goings and Sharon Hammes-Schiffer

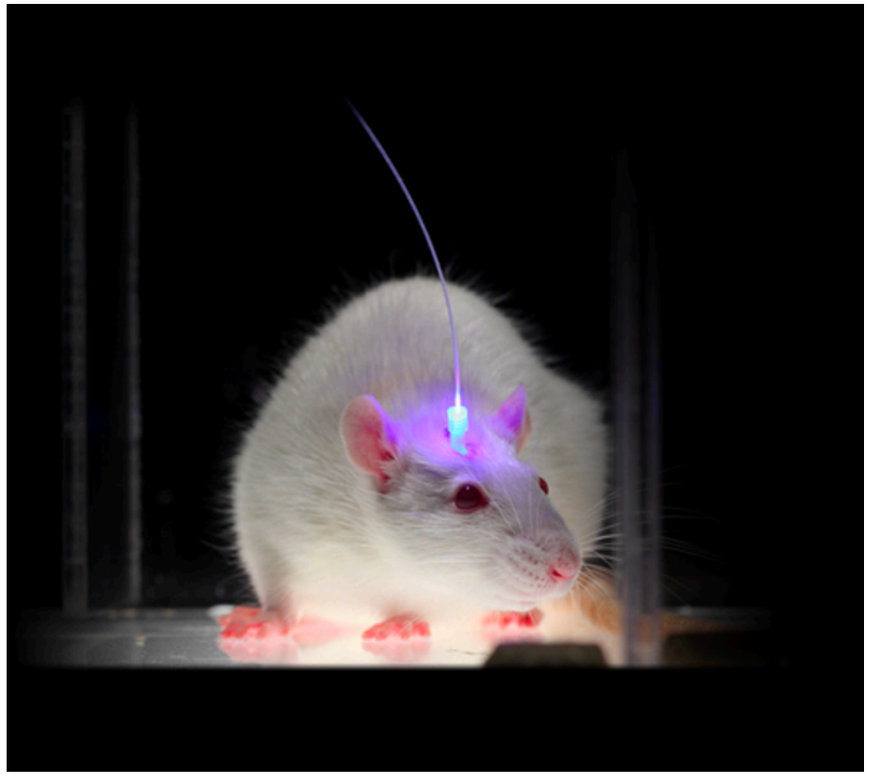
COMP: Sunday, August 25, 2019, 8:30 AM

Yale

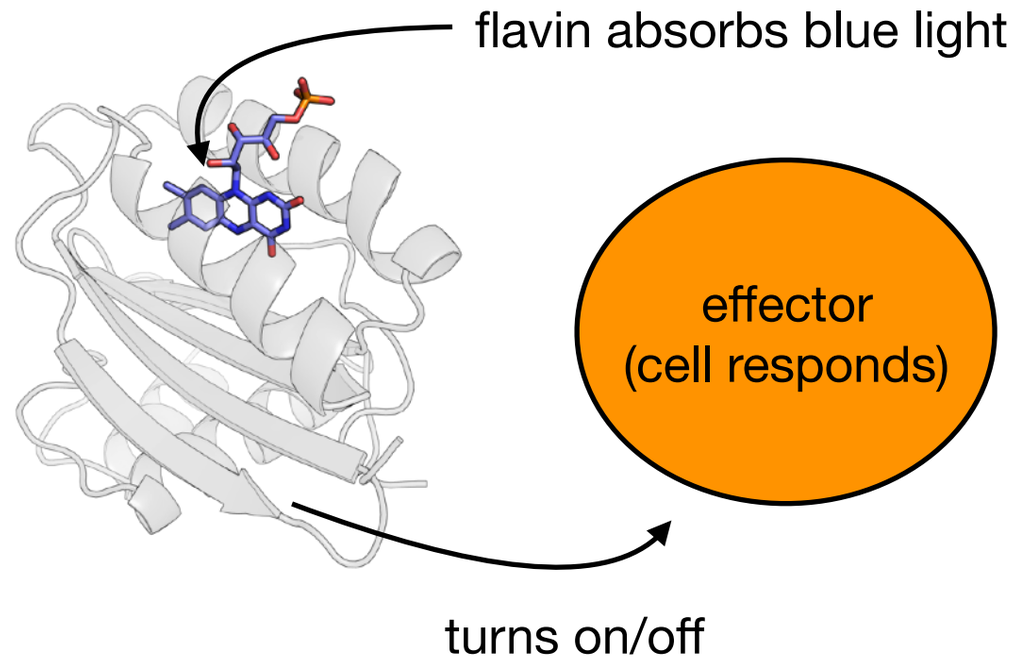
BLUF Photoreceptor

BLUF: blue light using flavin

optogenetics: use light to control cells in living tissue



Source: Getty's Open Content Program (John B. Carnett / Getty Images)



BLUF, coupled to an effector,
causes changes under blue light
(neuron fires, catalyze a reaction, etc.)

It's a blue light sensitive switch.

https://spectrum.ieee.org/tech-talk/biomedical/devices/lasers-switch-memories-from-bad-to-good

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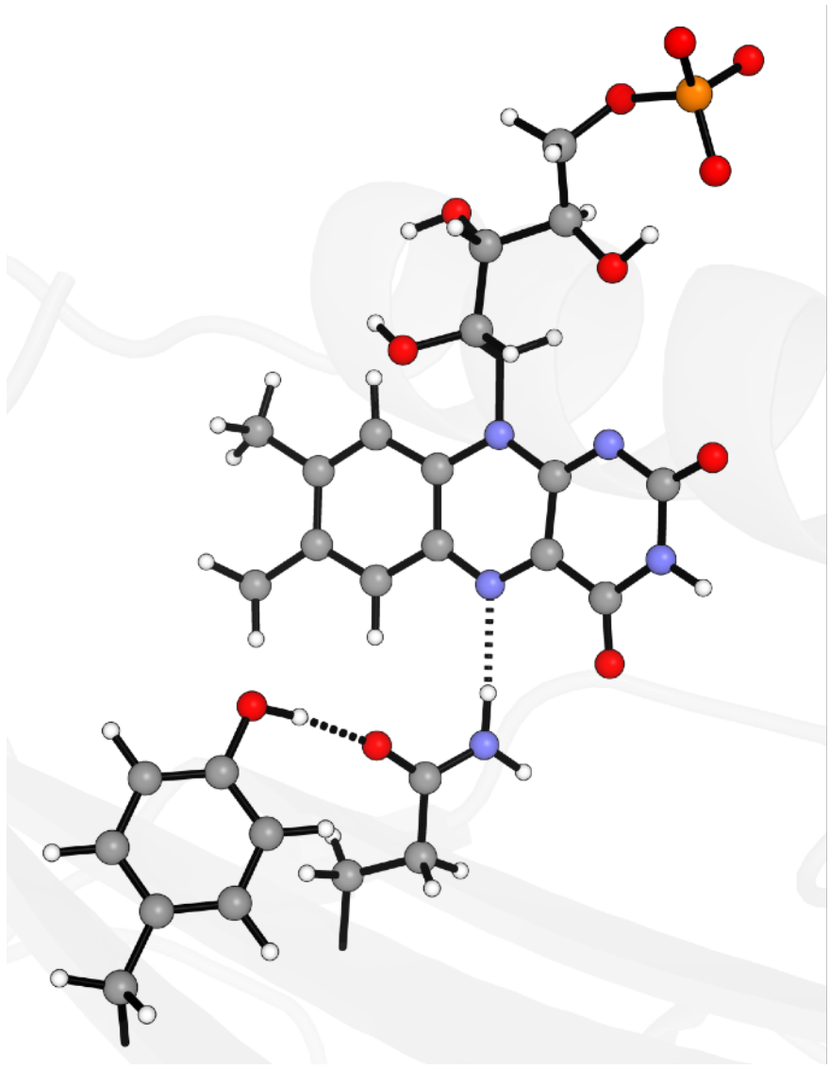

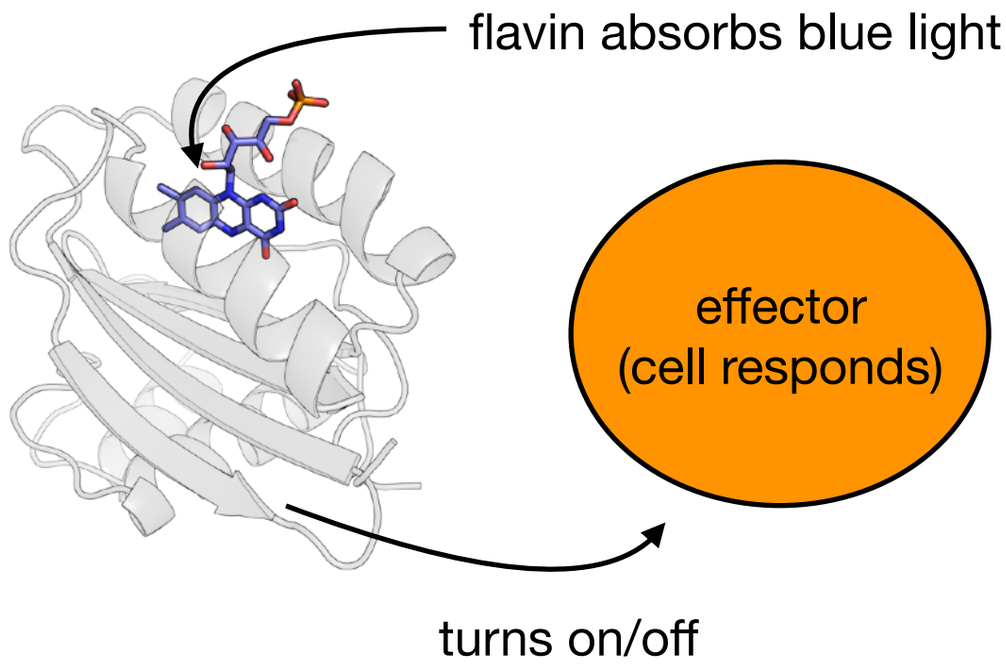

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Model for biological photoinduced PCET

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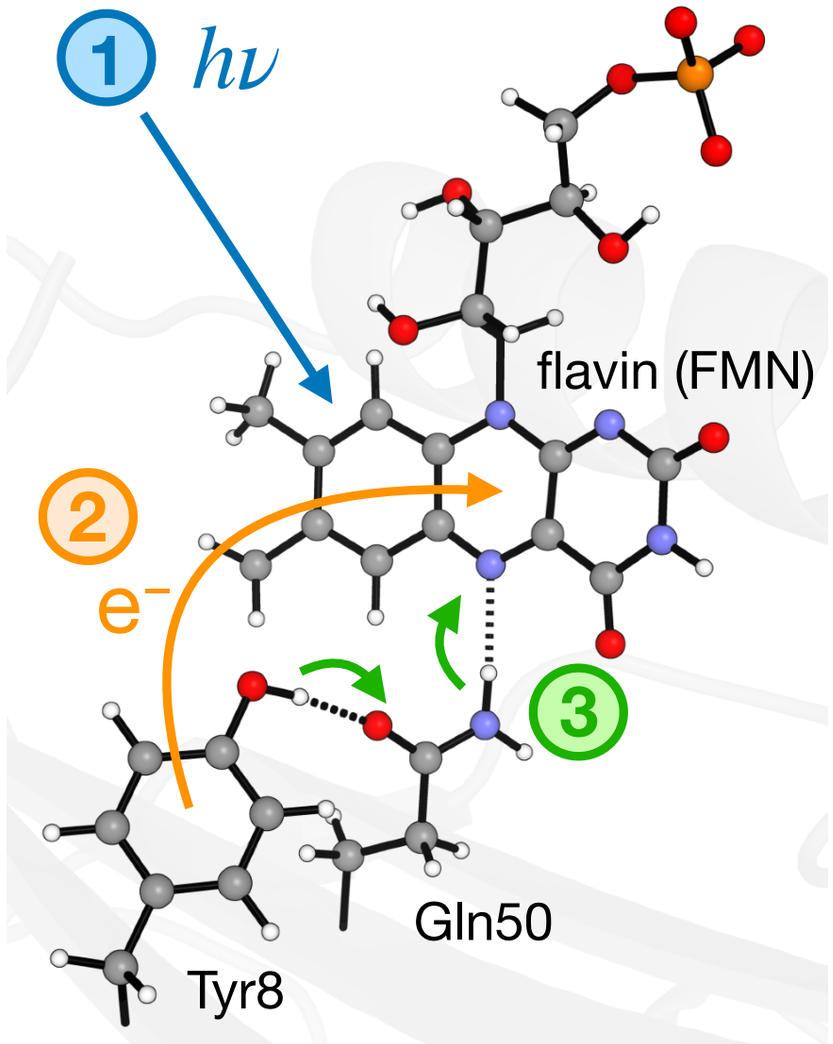

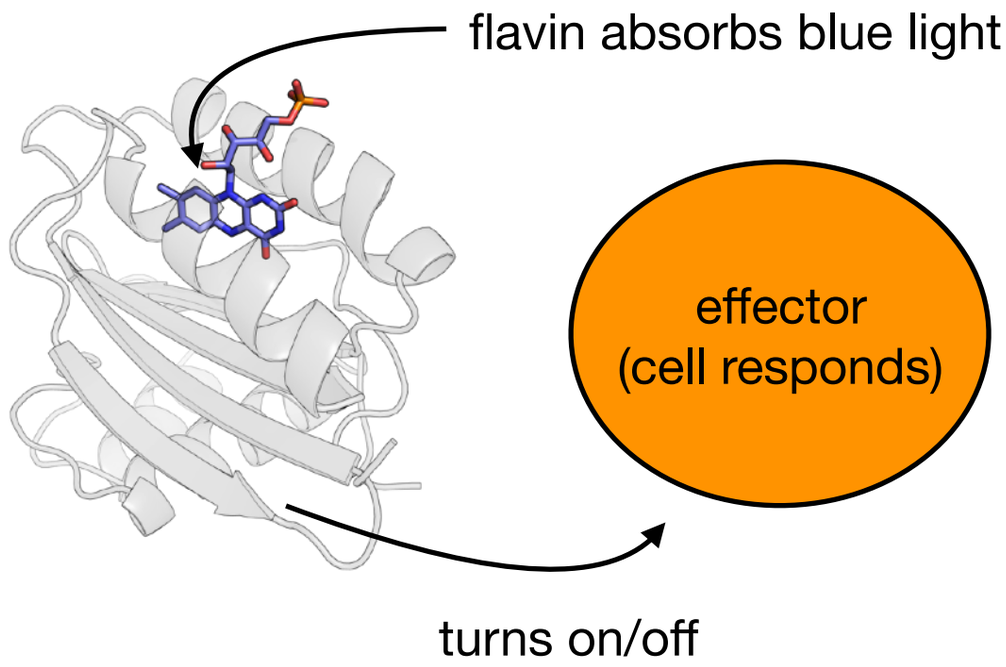

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Model for biological photoinduced PCET

Slr1694 BLUF Photocycle

1. Flavin (FMN) absorbs blue light, form FMN*

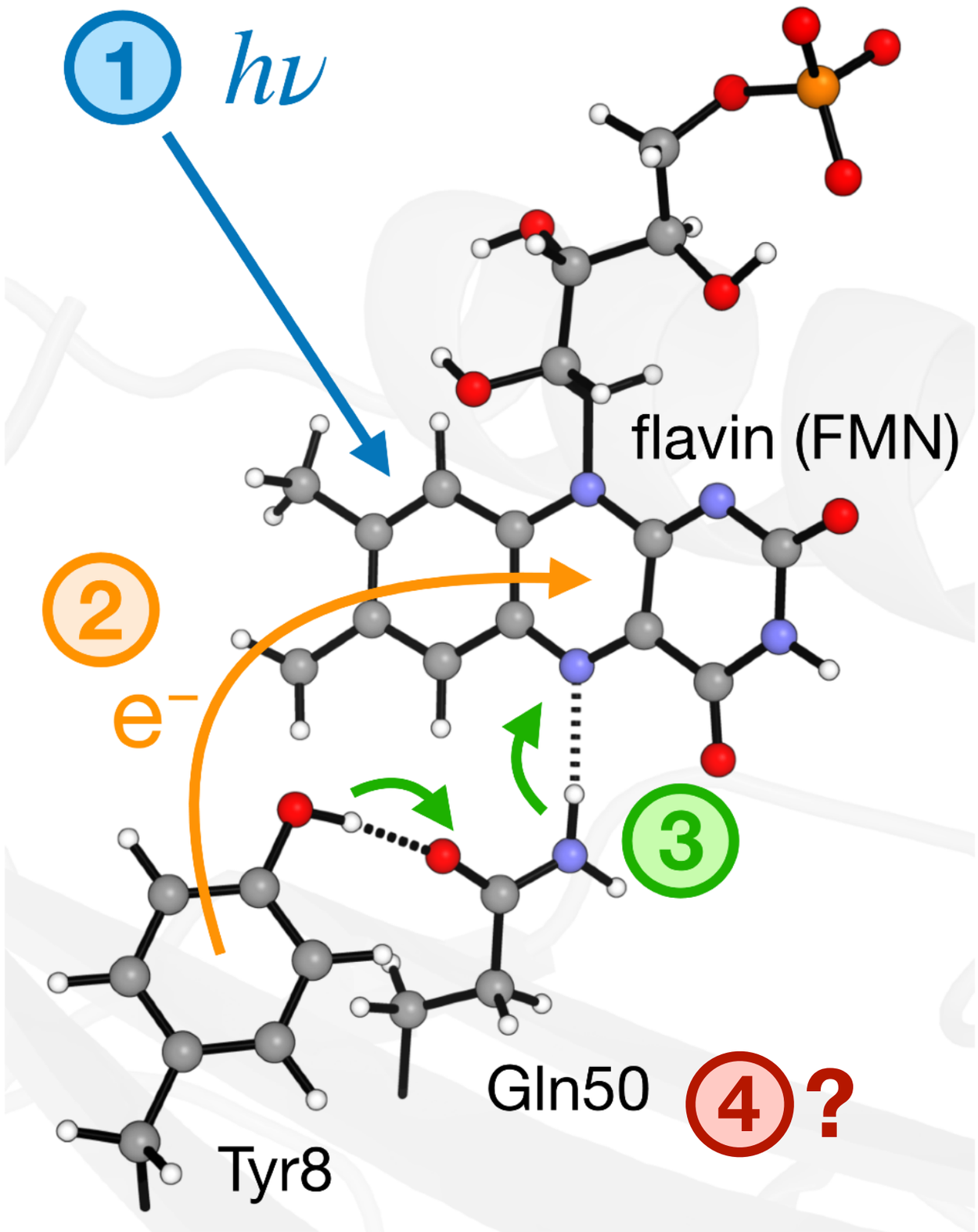
2. Electron transfer from Tyr to FMN*, form $FMN^{\bullet -}$ **(7 ps)**

3. Proton transfer from Tyr to $FMN^{\bullet -}$, form $FMNH^{\bullet}$ **(6 ps)**

4. Relaxation to light-adapted state, back to FMN **(65 ps)**

But, what are changes in the active site?

How can non-equilibrium excited state QM/MM dynamics help understand photocycle?



Gauden, M.; van Stokkum, I. H. M.; Key, J. M.; Lührs, D. C.; van Grondelle, R.; Hegemann, P.; Kennis, J. T. M. *PNAS.* **2006.**

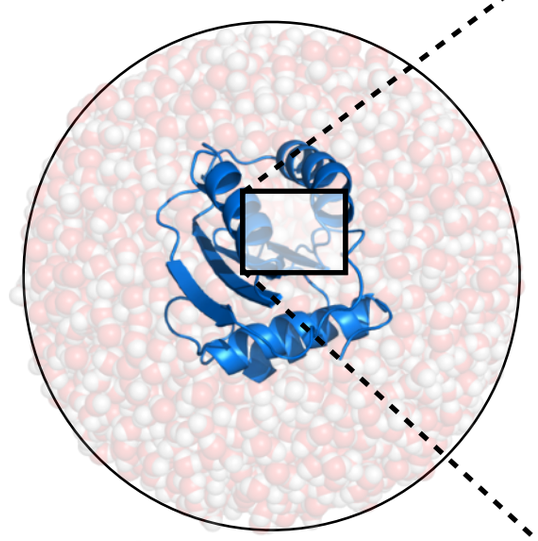
Excited State QM/MM Dynamics

How can excited state QM/MM dynamics help understand photocycle?

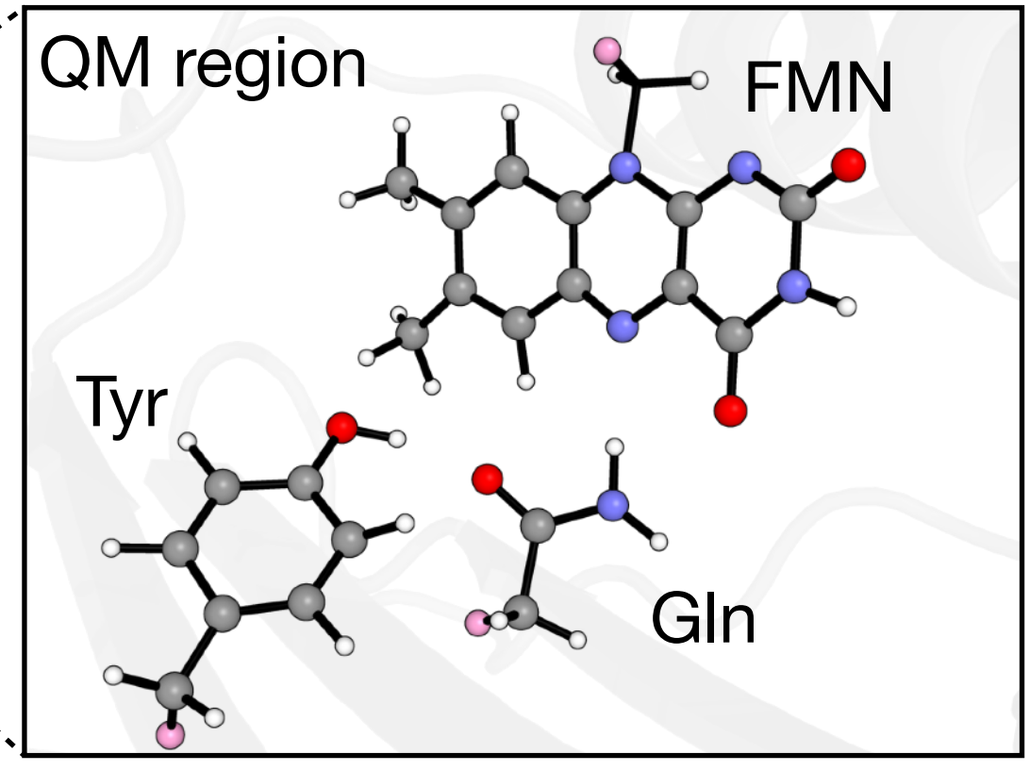
Simulate non-equilibrium excited state QM/MM dynamics directly for BLUF Slr1694 starting from locally excited flavin, LE state

CHARMM + Q-Chem

MM:
CHARMM36 FF
QM:
TDA-TDDFT
LRC-ωPBEh
6-31G* basis



full protein
+ solvent (36 Å radius)



link atoms in pink

Tamm-Dancoff Approximation (TDA) to avoid potential triplet instabilities
LRC-ωPBEh chosen after extensive benchmarking vs CASSCF+NEVPT2

Benchmarking DFT vs CASSCF+NEVPT2

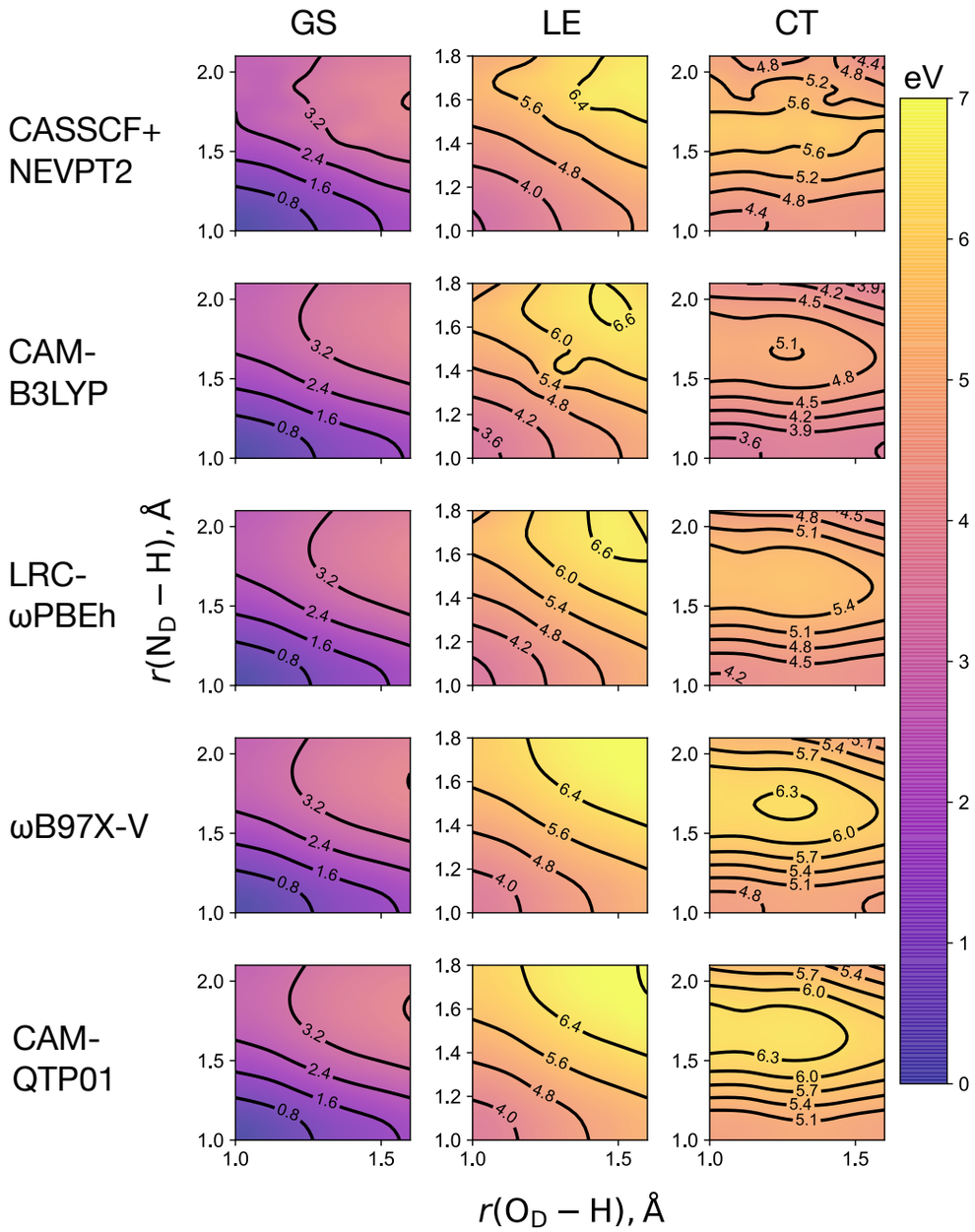
Compare PES of double proton transfer with respect to CASSCF+NEVPT2 reference for states of interest (GS, LE, CT)

Error in functional with respect to CASSCF+NEVPT2

State	LE			CT		
Functional	Max Error	Mean Error	RMSD	Max Error	Mean Error	RMSD
LRC-ωPBEh	-0.74	0.16	0.26	-0.57	-0.27	0.31
CAM-B3LYP	0.79	0.18	0.29	-0.96	-0.69	0.71
ωB97X-V	0.97	0.49	0.51	0.93	0.51	0.54
CAM-QTP01	1.03	0.55	0.57	1.08	0.65	0.67

*includes all grid points, units in eV

- LRC-ωPBEh gave the best agreement
- CAM-B3LYP underestimates CT
- CAM-QTP01 and ωB97X-V tend to overestimate both LE and CT states



Sayfutyarova, E. R.; Goings, J. J.; Hammes-Schiffer, S. Electron-Coupled Double Proton Transfer in the Slr1694 BLUF Photoreceptor: A Multireference Electronic Structure Study. *J. Phys. Chem. B* **2019.**

Local heating with QM/MM relaxation[1]

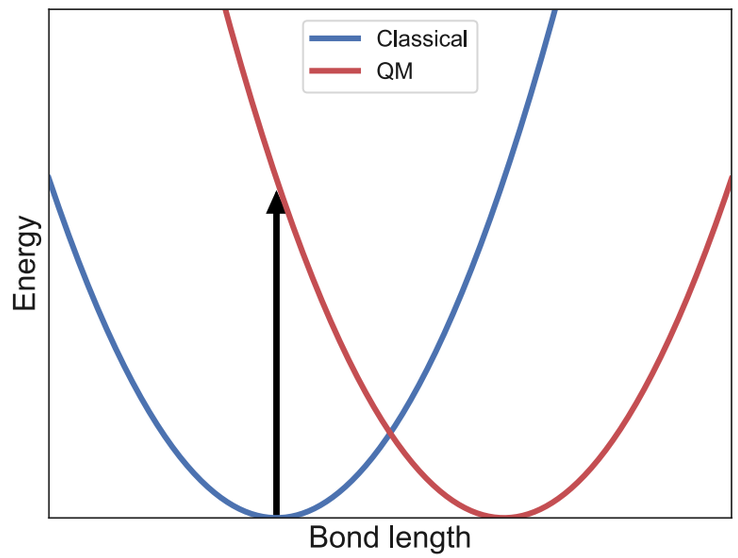
QM/MM with excited states can be very sensitive to initial conditions

Zero-point energy (ZPE)

problem: classical MD has no ZPE, but sampling from Wigner distribution impractical for proteins

Shift from classical to QM Hamiltonian

problem: shifting from classical to QM/MM can add additional energy



[1]Mai, S.; Gattuso, H.; Monari, A.; González, L. *Front Chem* **2018**, *6*, 495.

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solution: mimic ZPE by adding additional thermal energy to QM region (heat QM to 530 K from 300 K)

$$E(\mathrm{ZPE}) = E(\mathrm{thermal})$$
$$= Nk_BT$$
$$\implies T = E(\mathrm{ZPE})/Nk_B$$

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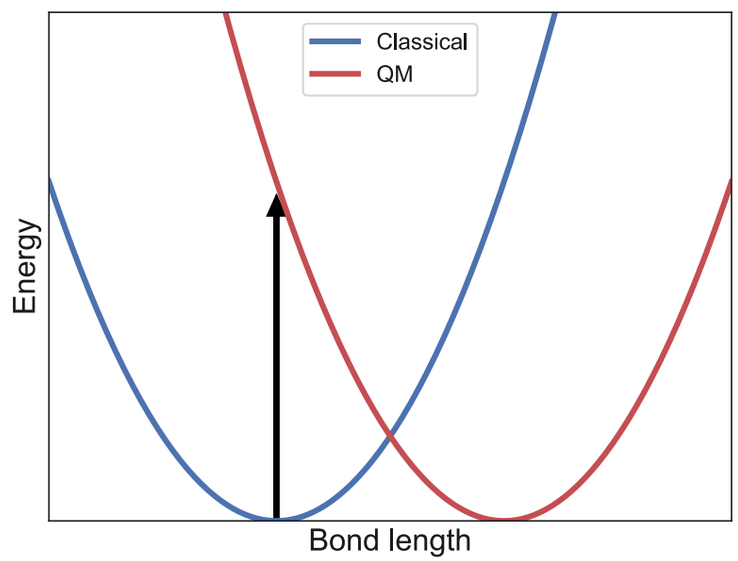

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Shift from classical to QM Hamiltonian

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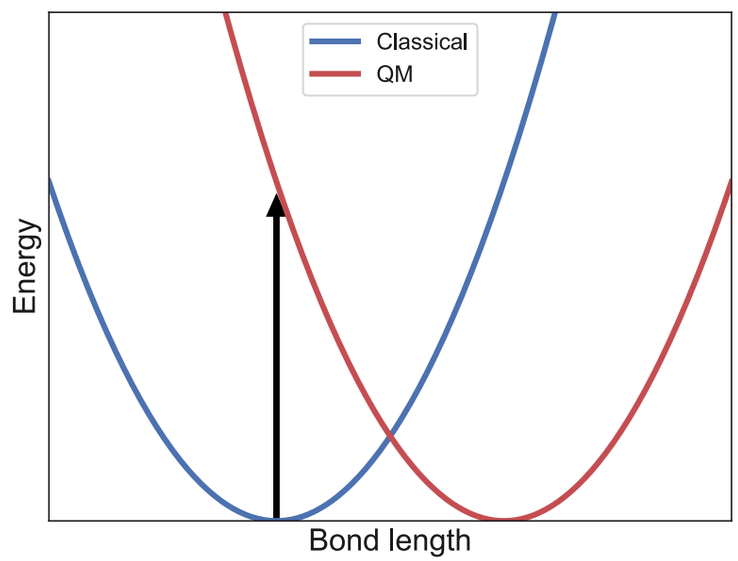
solution: let system relax for a short period after heating to dissipate energy and move closer to equilibrium bond lengths, etc.



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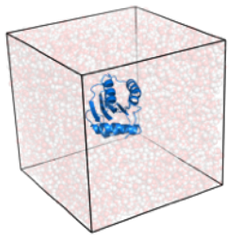
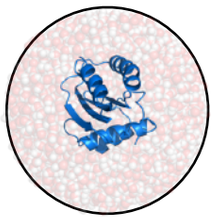
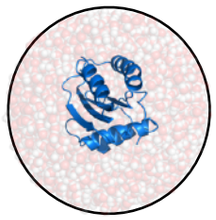
System preparation







equilib.

20 ns

equilib.

500 ps

production MD (5 ns)

50 ps

Heat QM region
(530 K, MM frozen)

20 ps

MM region relaxation
(QM region frozen)

100 fs

full QM/MM relaxation
(nothing frozen)

100 fs

Sample every 50 ps along production
(100 initial configurations)

Final QM region temperature: 561 ± 58 K
Final MM region temperature: 298 ± 7 K

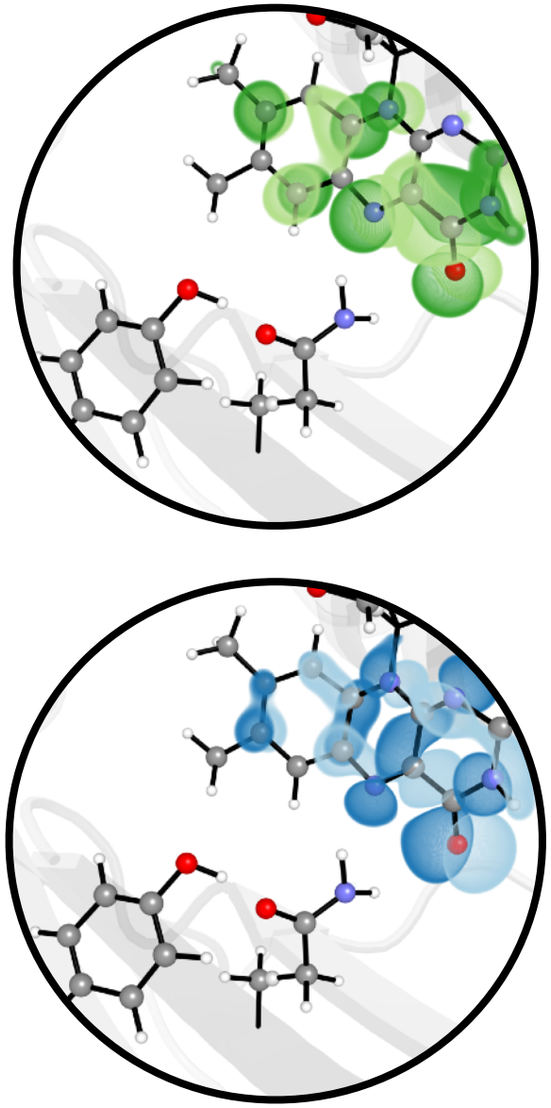
Figure and schema adapted from Mai, S.; Gattuso, H.; Monari, A.; González, L. *Front. Chem.* **2018.**

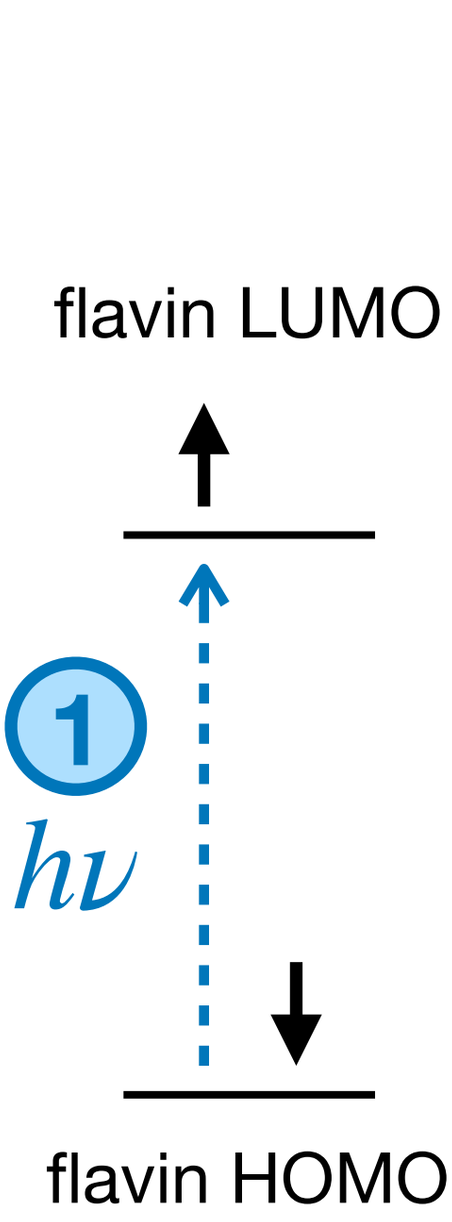
① Excited State QM/MM Dynamics

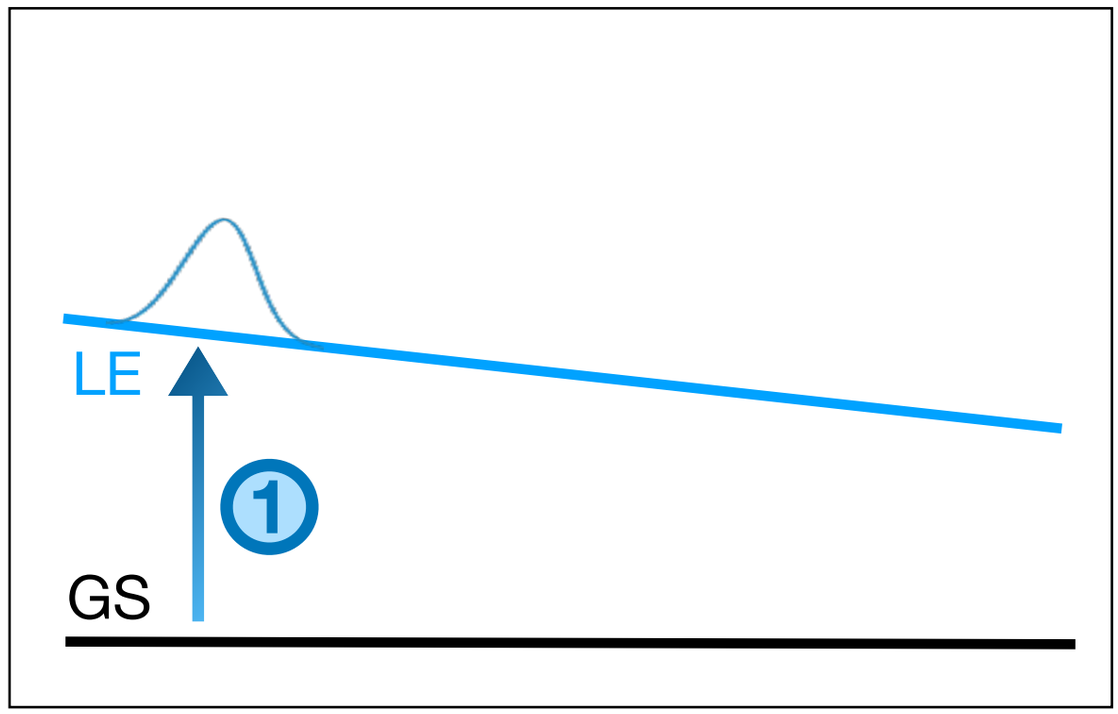
How can excited state QM/MM dynamics help understand photocycle?

Simulate excited state QM/MM dynamics directly for BLUF Slr1694

100 trajectories, start with population on S_1 (locally excited flavin, LE state)







photocycle progression

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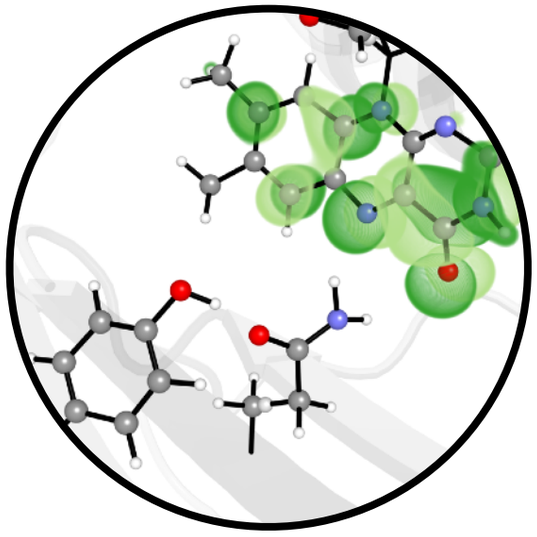
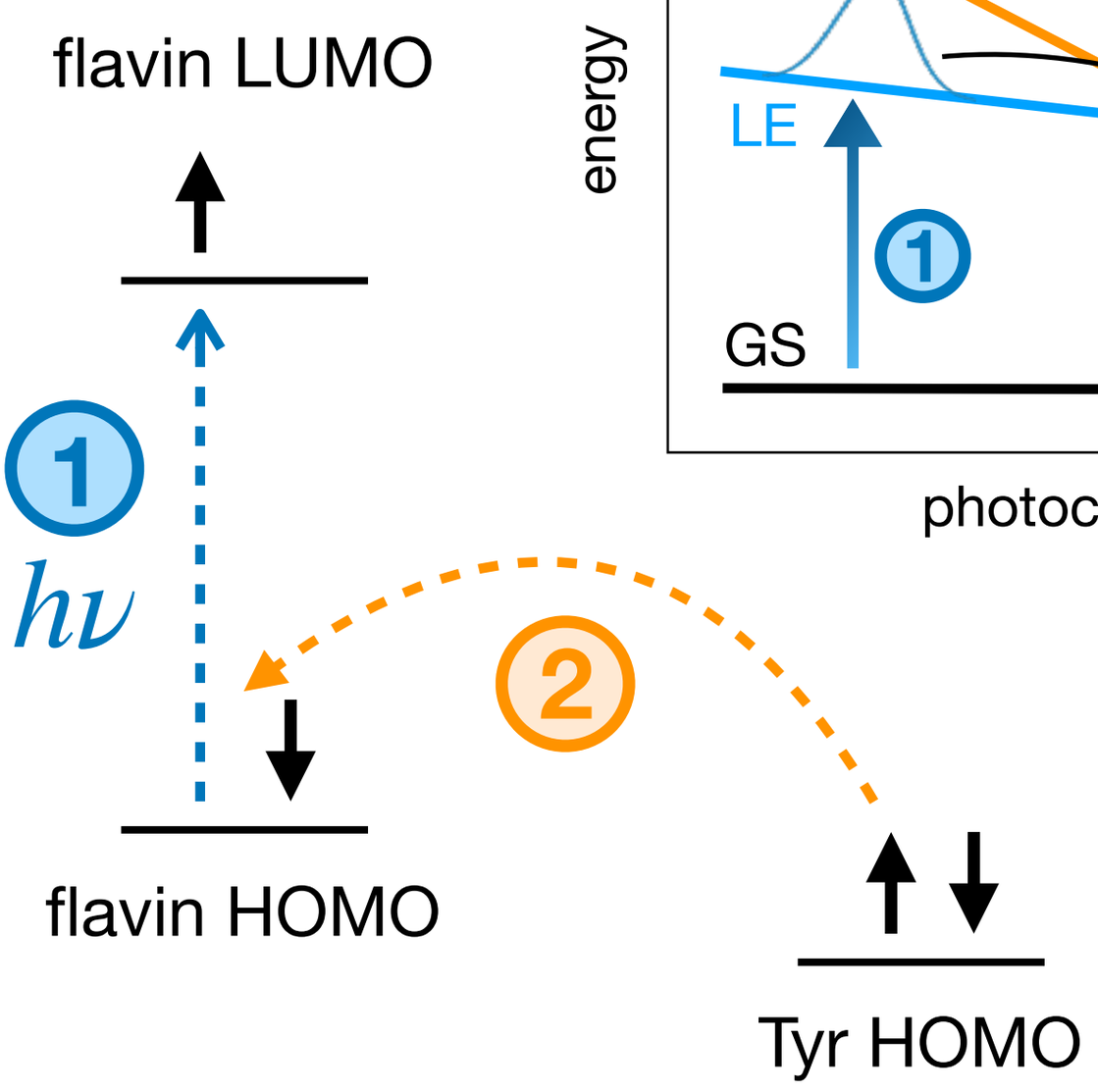
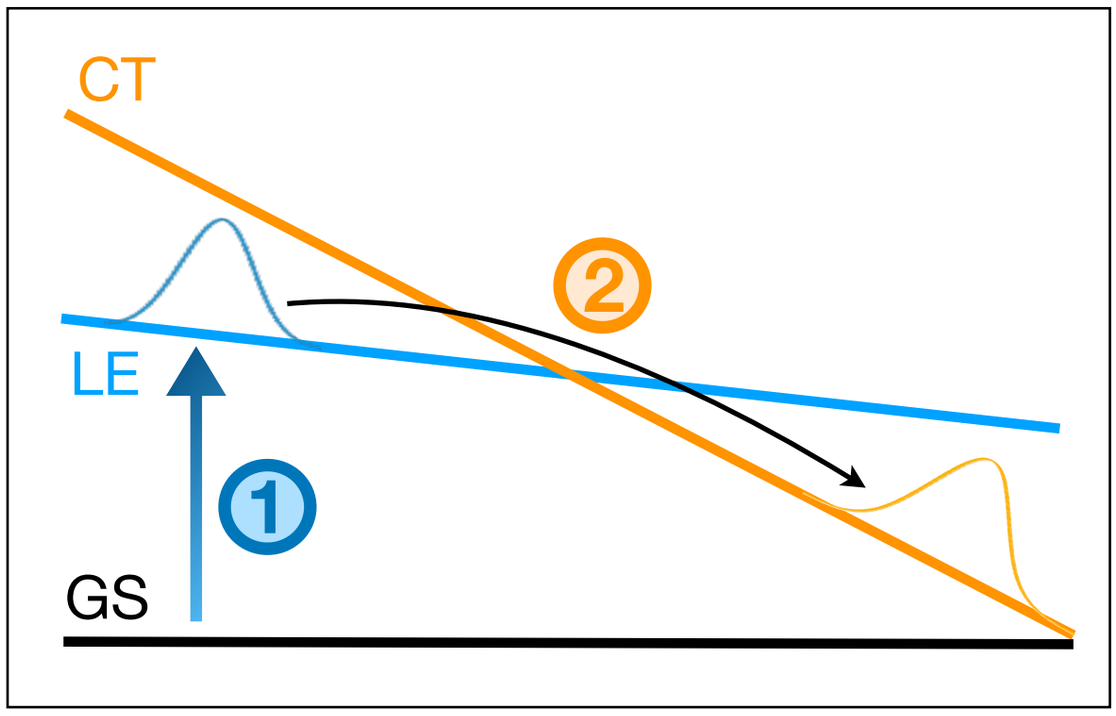
CT state *always* higher energy

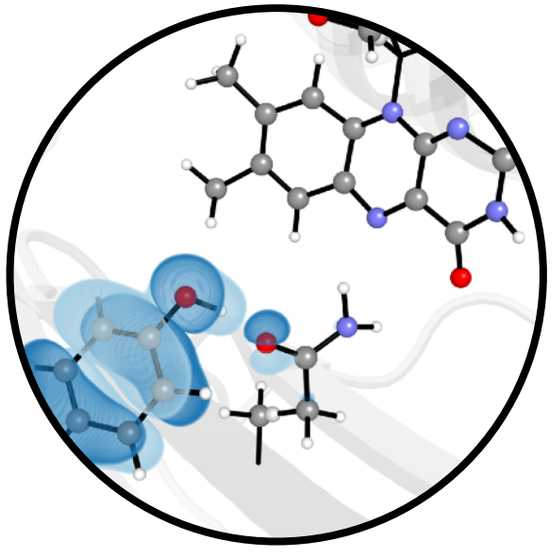
Must cross below LE for pop. transfer







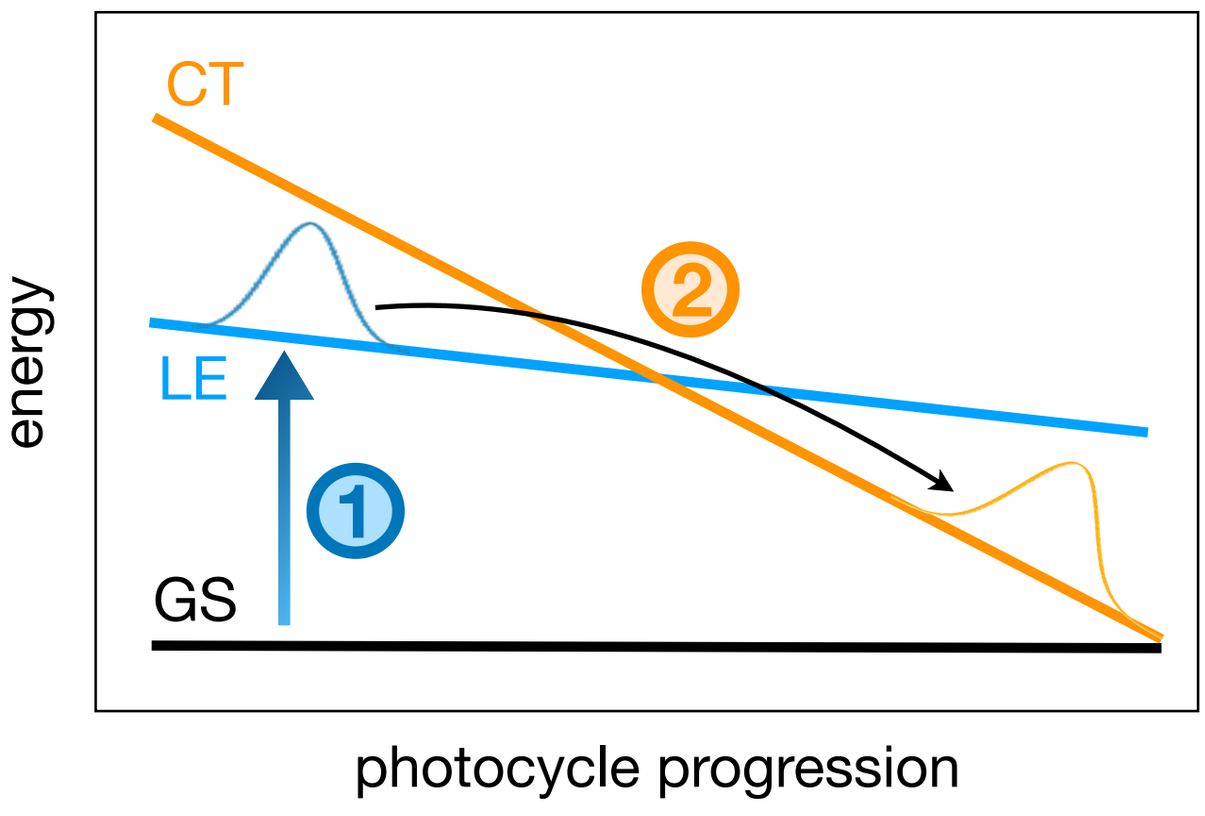
photocycle progression

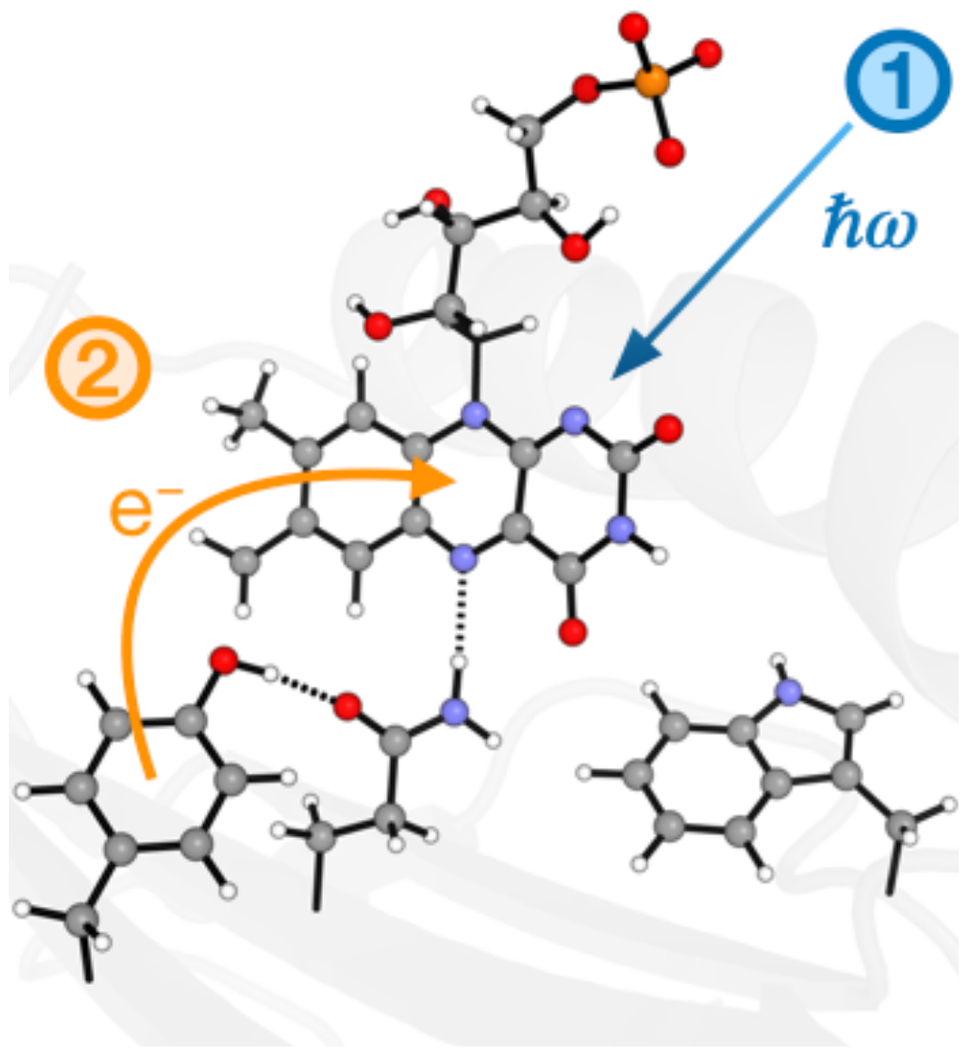


② Electron Transfer

Over the course of the dynamics, we follow S_1 adiabatically, but look for trajectories where the **excited state character changes to charge transfer**.

How many trajectories show charge transfer, how long does it take, and what physical factors are involved?





② Electron Transfer

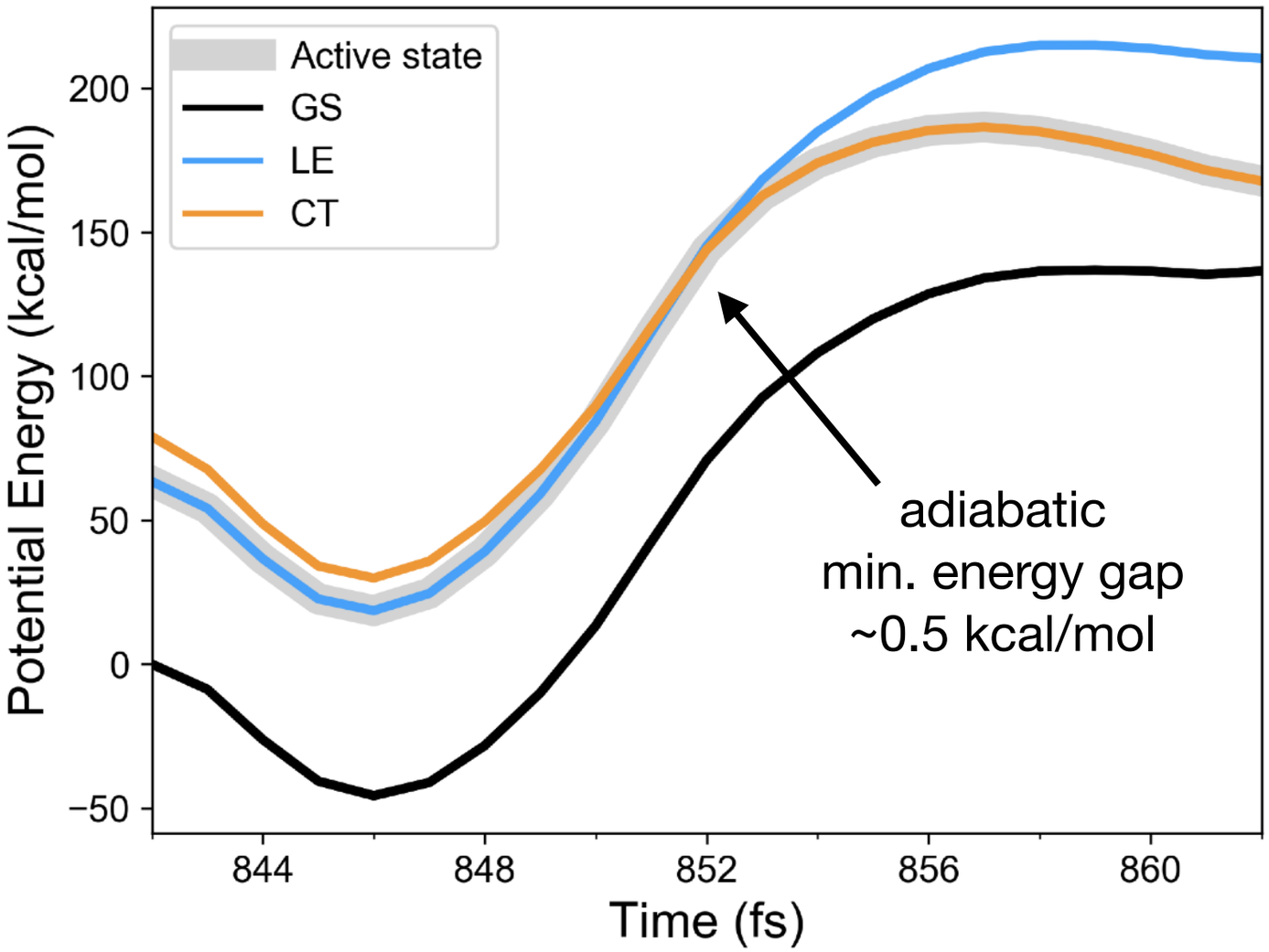
We observe charge transfer for **9 out of 100 trajectories** (within 6 ps).

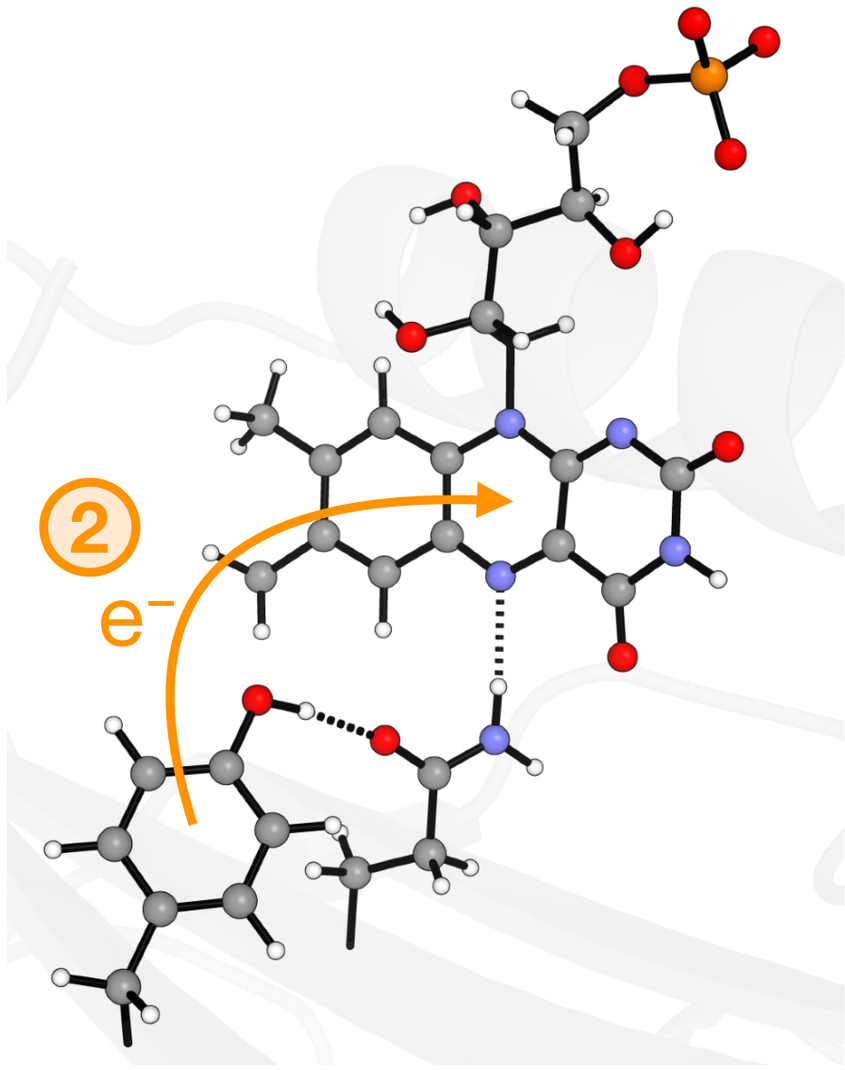
For every trajectory, S_1 is initially of flavin locally excited (LE) character.

The character of S_1 changes from LE to CT at the avoided crossing.

Neglecting nonadiabatic effects may be one reason for fast CT.

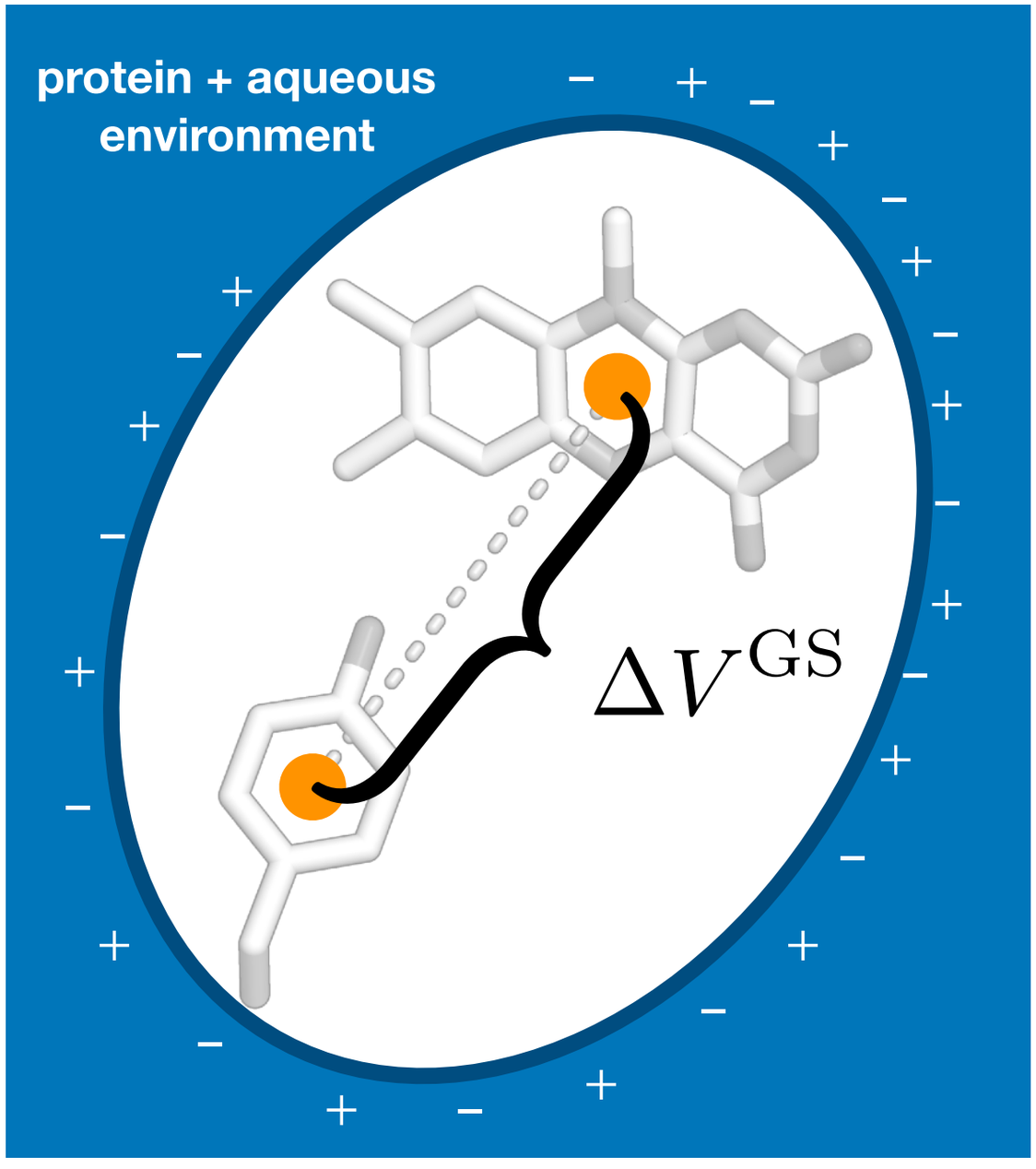
But, what drives charge transfer?





② Electron Transfer

How does protein environment affect CT?



1. Measure electrostatic potential due to environment at Tyr and FMN

$$\Phi(r_i) = \frac{1}{4\pi\epsilon_0} \sum_j \frac{q_j}{r_{ij}}$$

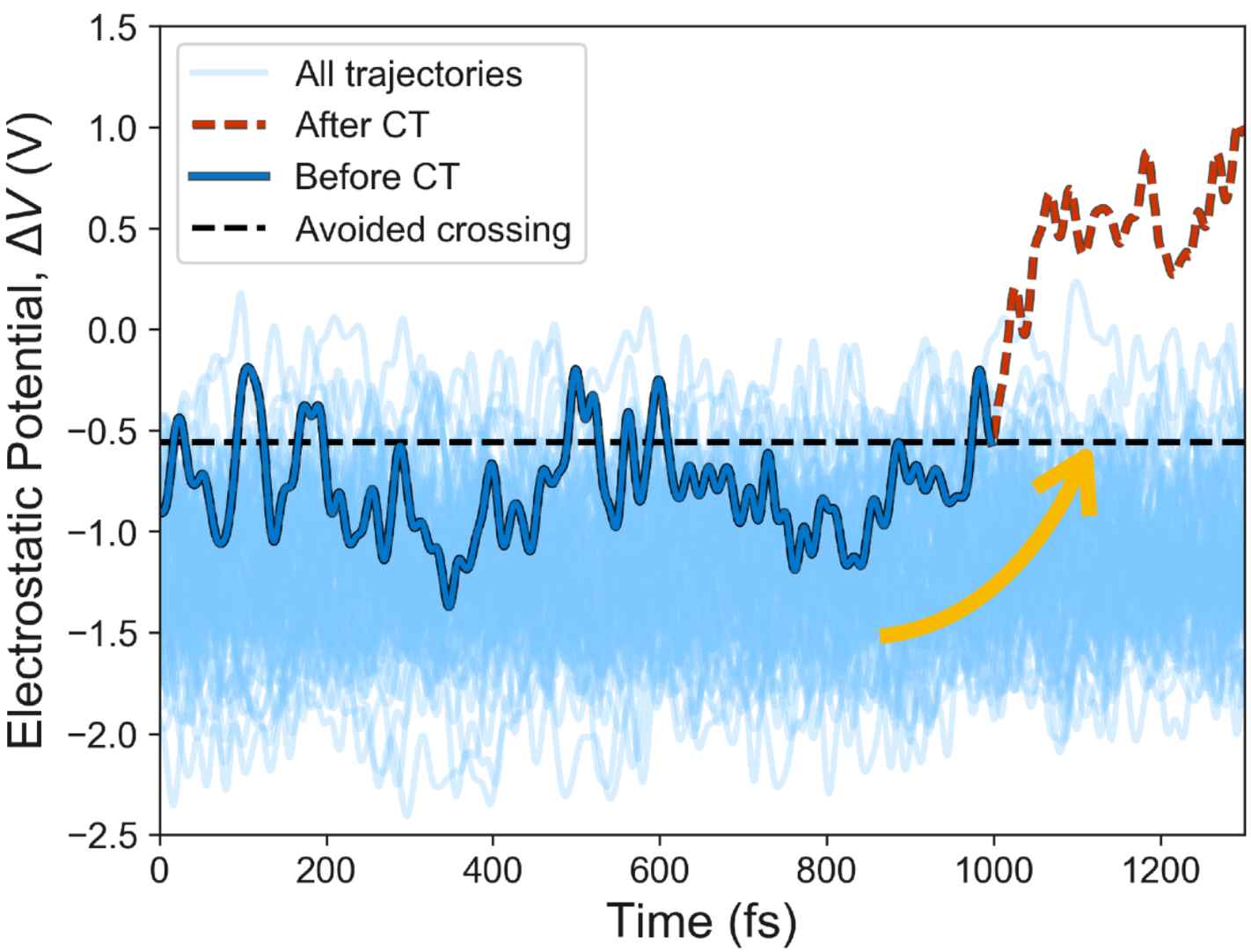
2. Compute potential difference between FMN and Tyr

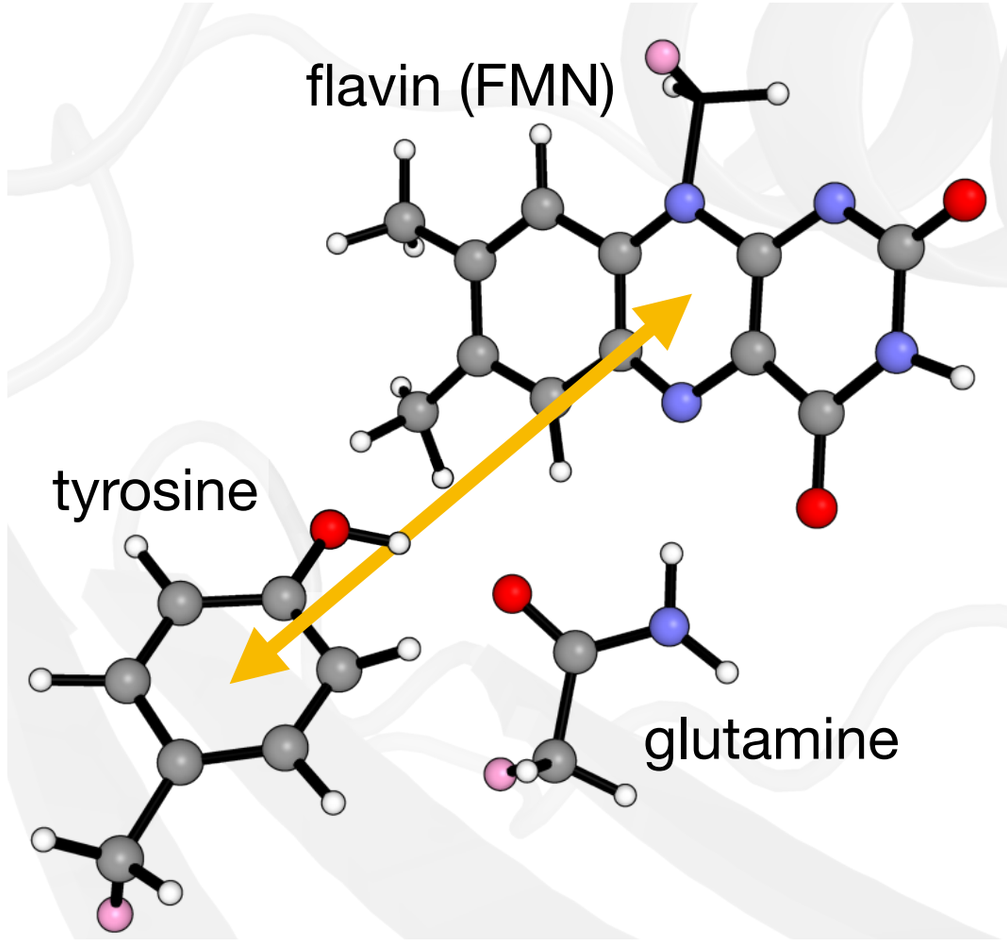
$$\Delta V = \Phi(r_{\mathrm{FMN}}) - \Phi(r_{\mathrm{Tyr}})$$

3. More positive $\Delta V(t)$ stabilizes CT

② Electron Transfer

How does protein environment affect CT?

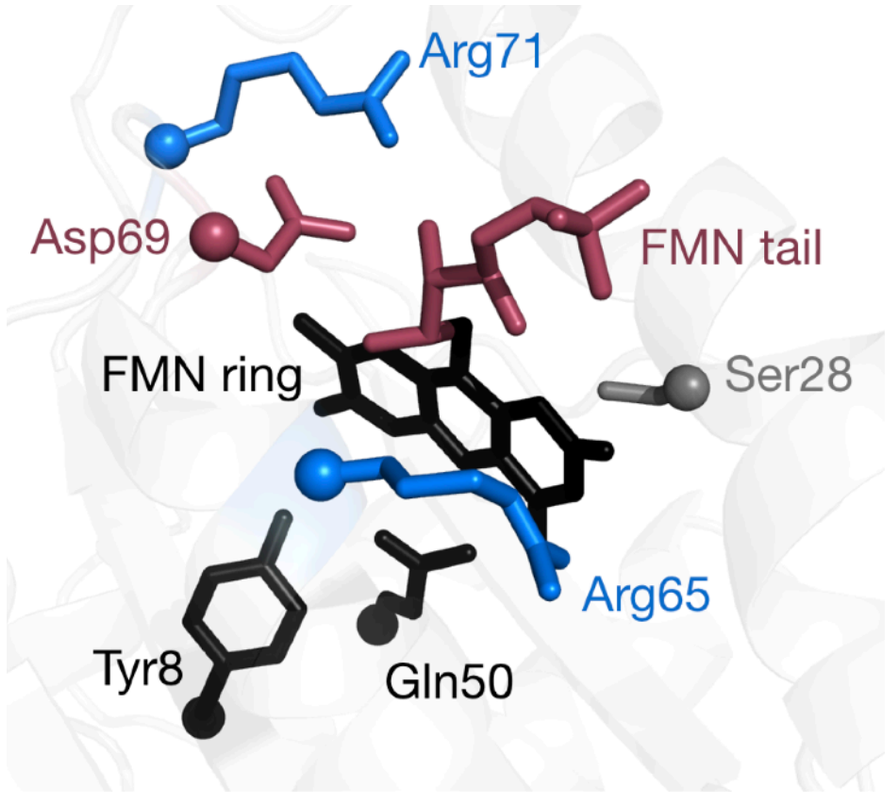


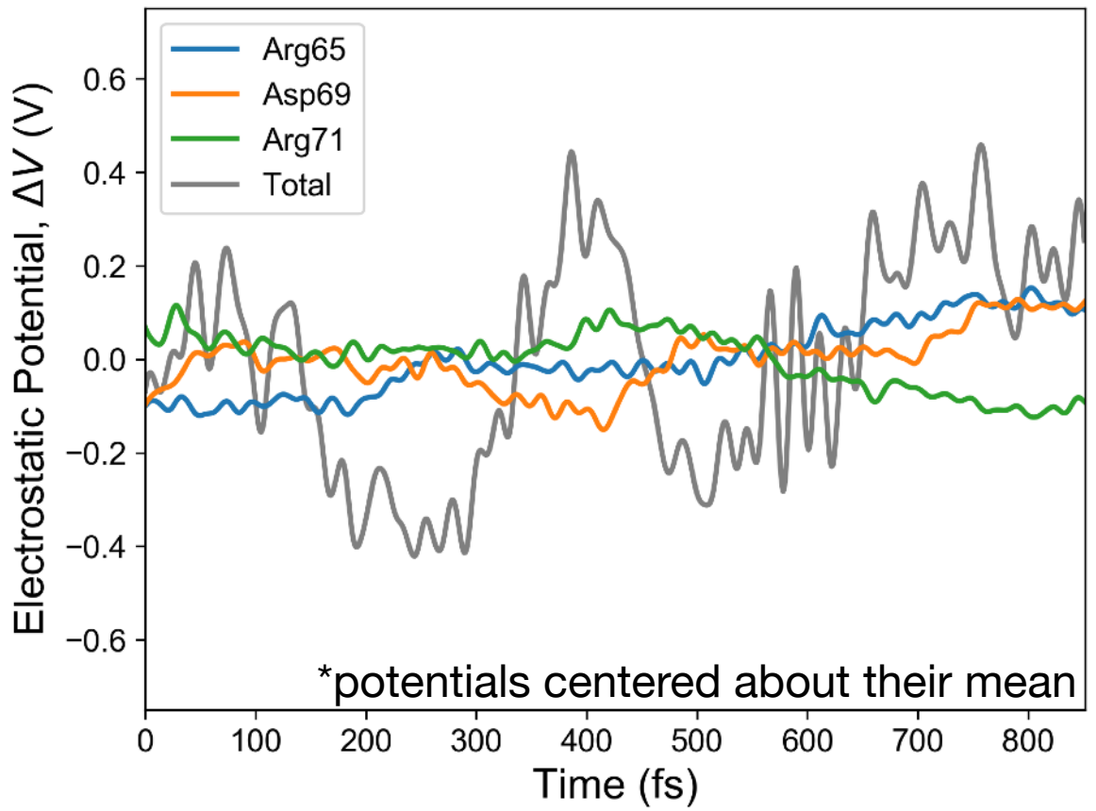


Electrostatic potential increases prior to charge transfer

More positive ΔV
→ lower energy of CT state

② Electron Transfer





What residues influence charge transfer?

Electrostatic potential is additive, so we can decompose into contributions by individual residue; look for residues with greatest variance

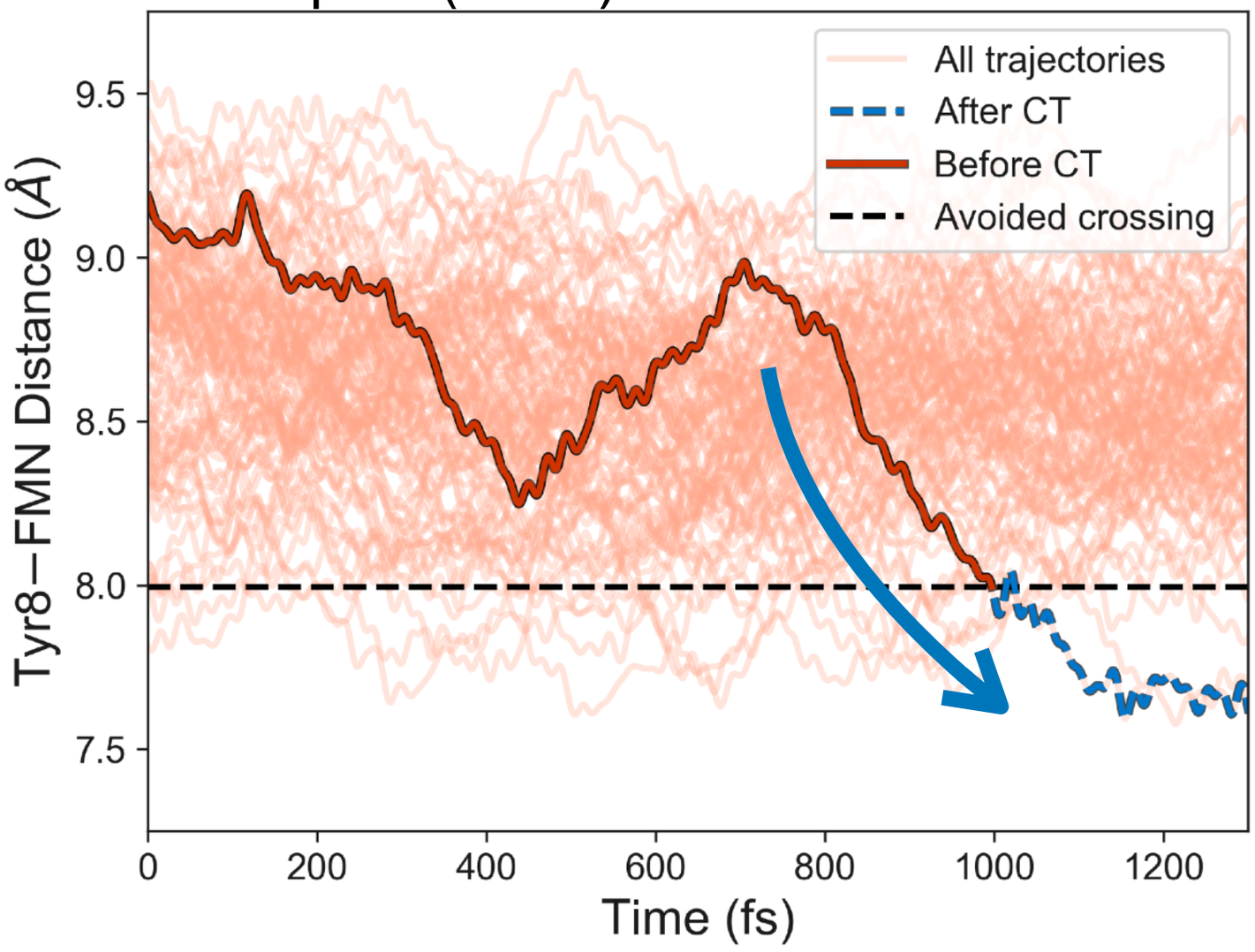
Flexible charged groups around the flavin binding pocket:
positive and negative (or polar)

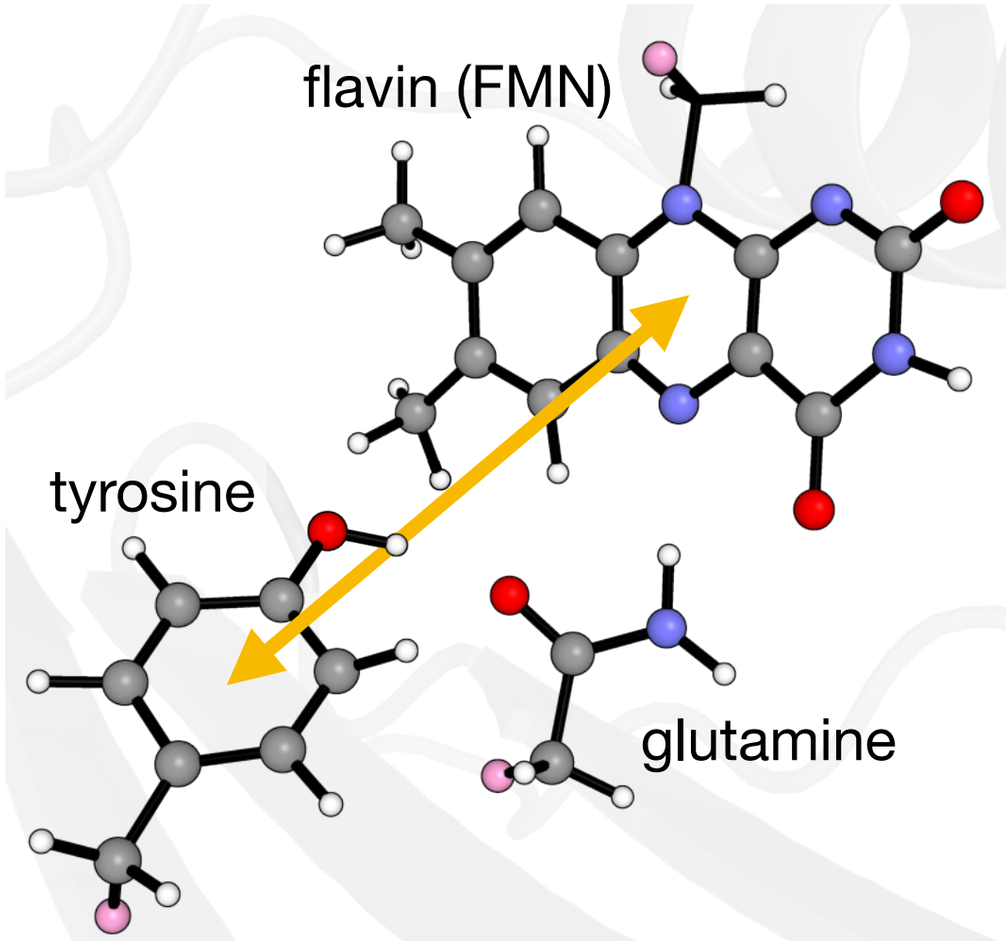
Moving net positive charge toward flavin stabilizes CT

② Electron Transfer

But electrostatics are not only governing factor

Energy of charge transfer state depends on the distance between electron donor (Tyr) and acceptor (flavin)



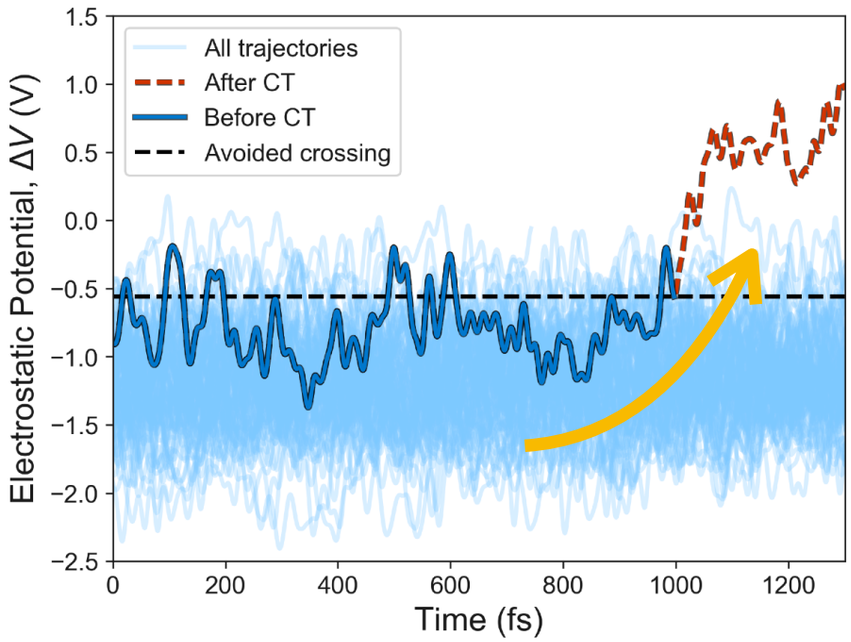


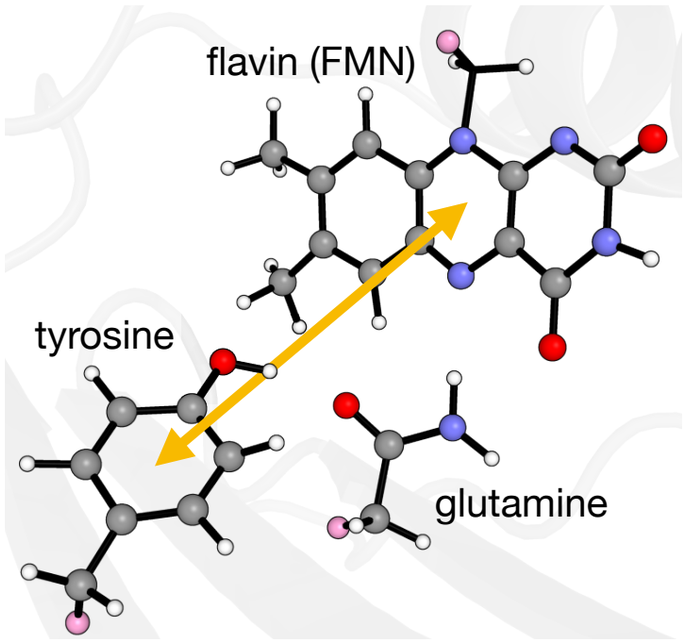
Donor-acceptor distance decreases prior to CT

Closer donor-acceptor distance → lower energy of CT state

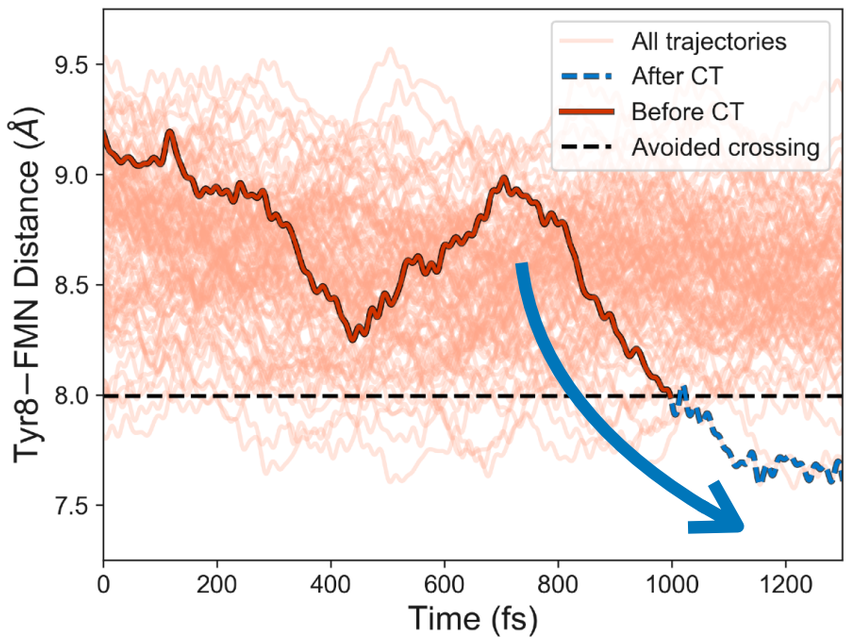
② Electron Transfer

changes in electrostatic potential



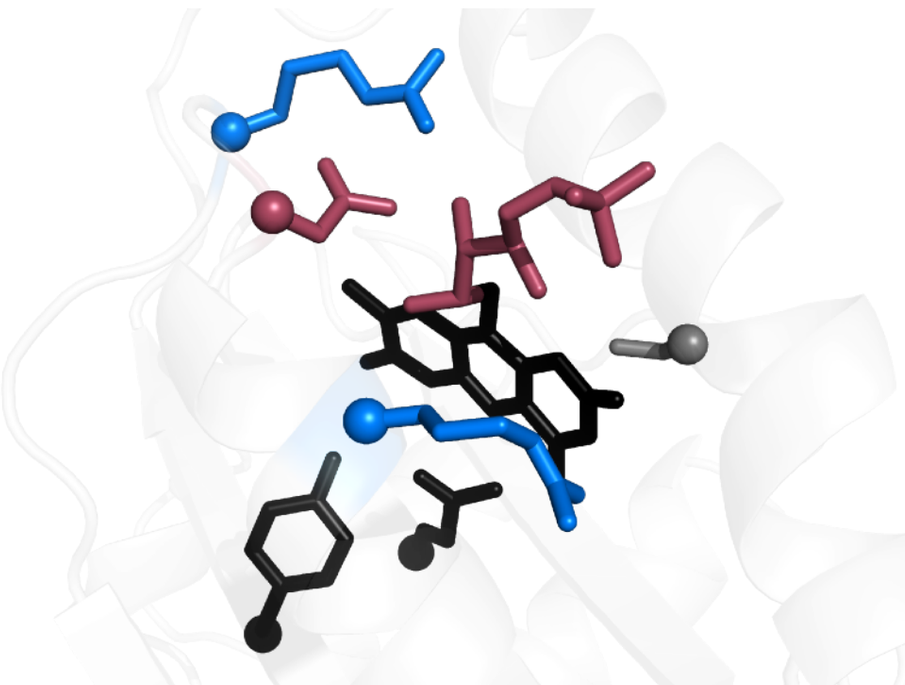


changes in donor-acceptor distance



Non-equilibrium response of the protein surrounding the active site can lead to conditions suitable for electron transfer to the flavin

The radical pair $Tyr^{\bullet+}/FMN^{\bullet-}$ drives subsequent proton transfer to neutralize the anionic flavin



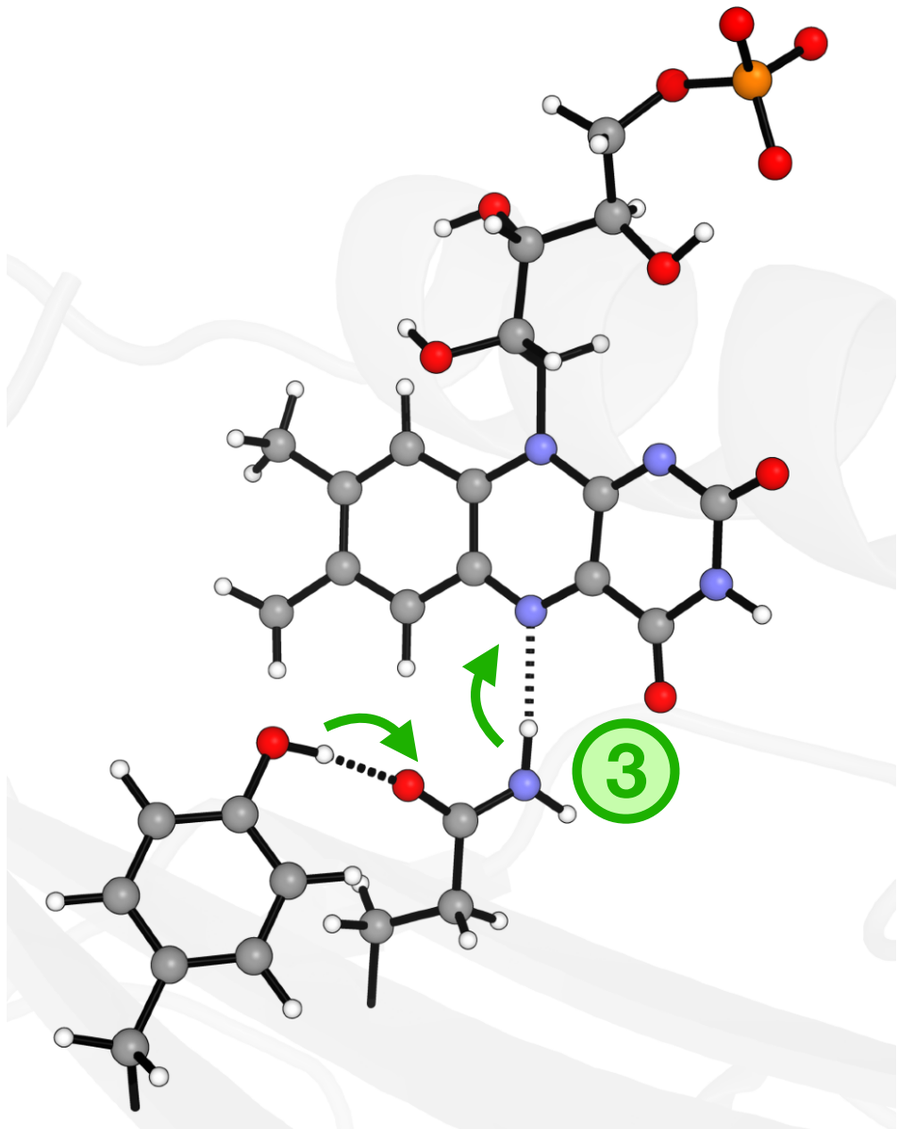
3 Proton Transfer

$Tyr^{\bullet+}/FMN^{\bullet-}$ radical pair drives proton transfer

Proton transfer from Tyr begins rapidly (20—200 fs) after charge transfer — on the order of a few hydrogen oscillations

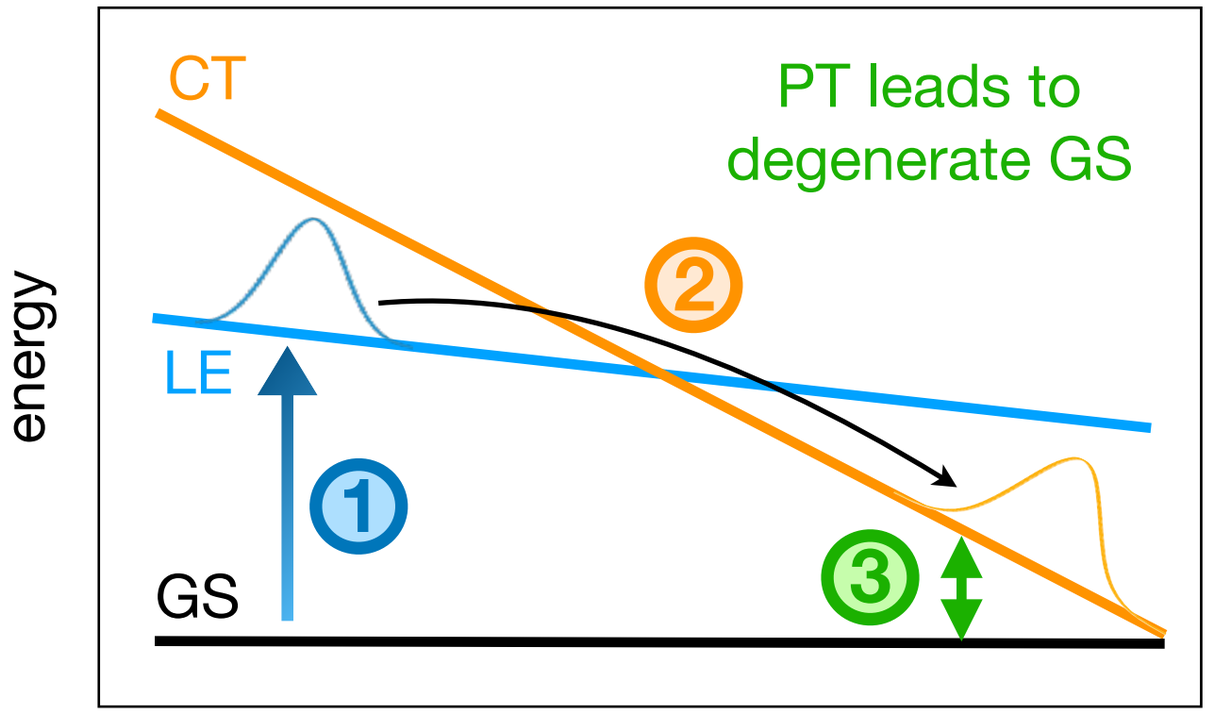
Experimentally, tyrosine pK_a changes from 10 to -2 upon oxidation, so first PT is favorable

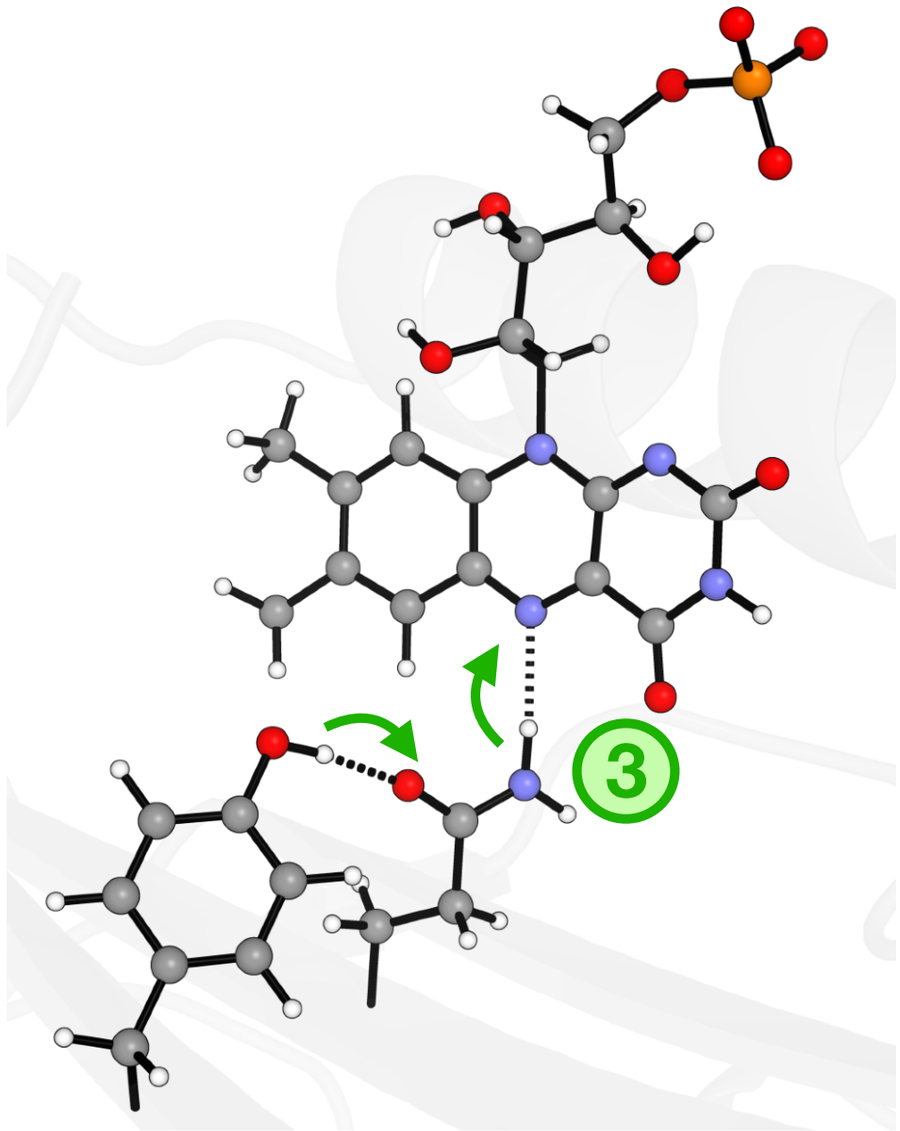
But, the transfer of protons is correlated with the formation of diradical character in the ground state



③ Proton Transfer

Ground state becomes diradical, which requires multireference methods



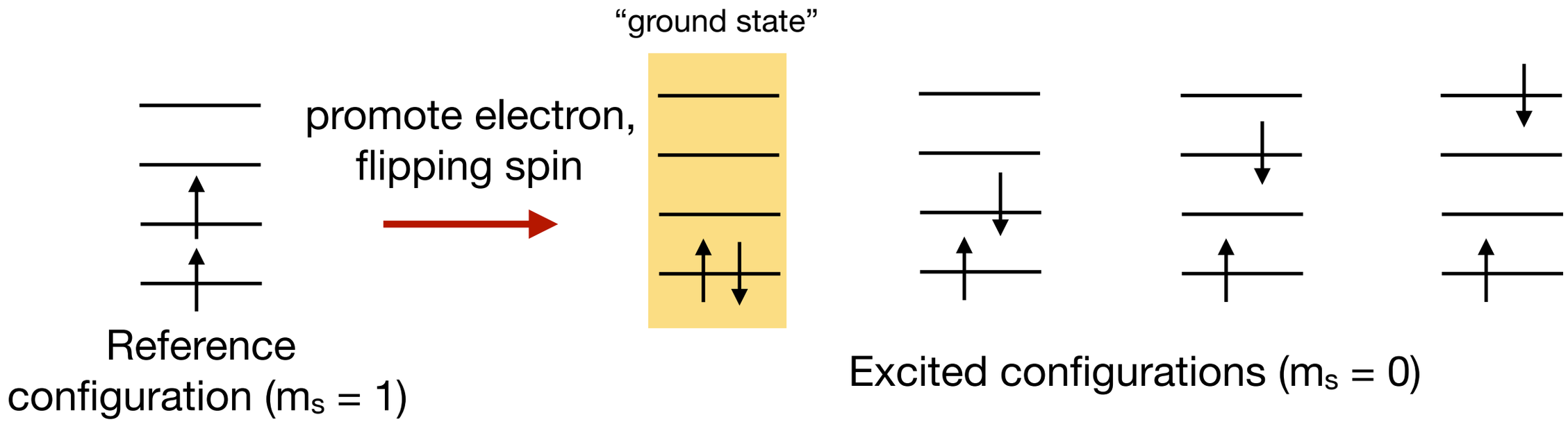


TDA-TDDFT no longer suitable!

3 Proton Transfer

Spin-flip (SF)-TDA-TDDFT: Designed to treat ground and excited states on equal footing.

Useful in treating radicals, open shell species, and conical intersections.



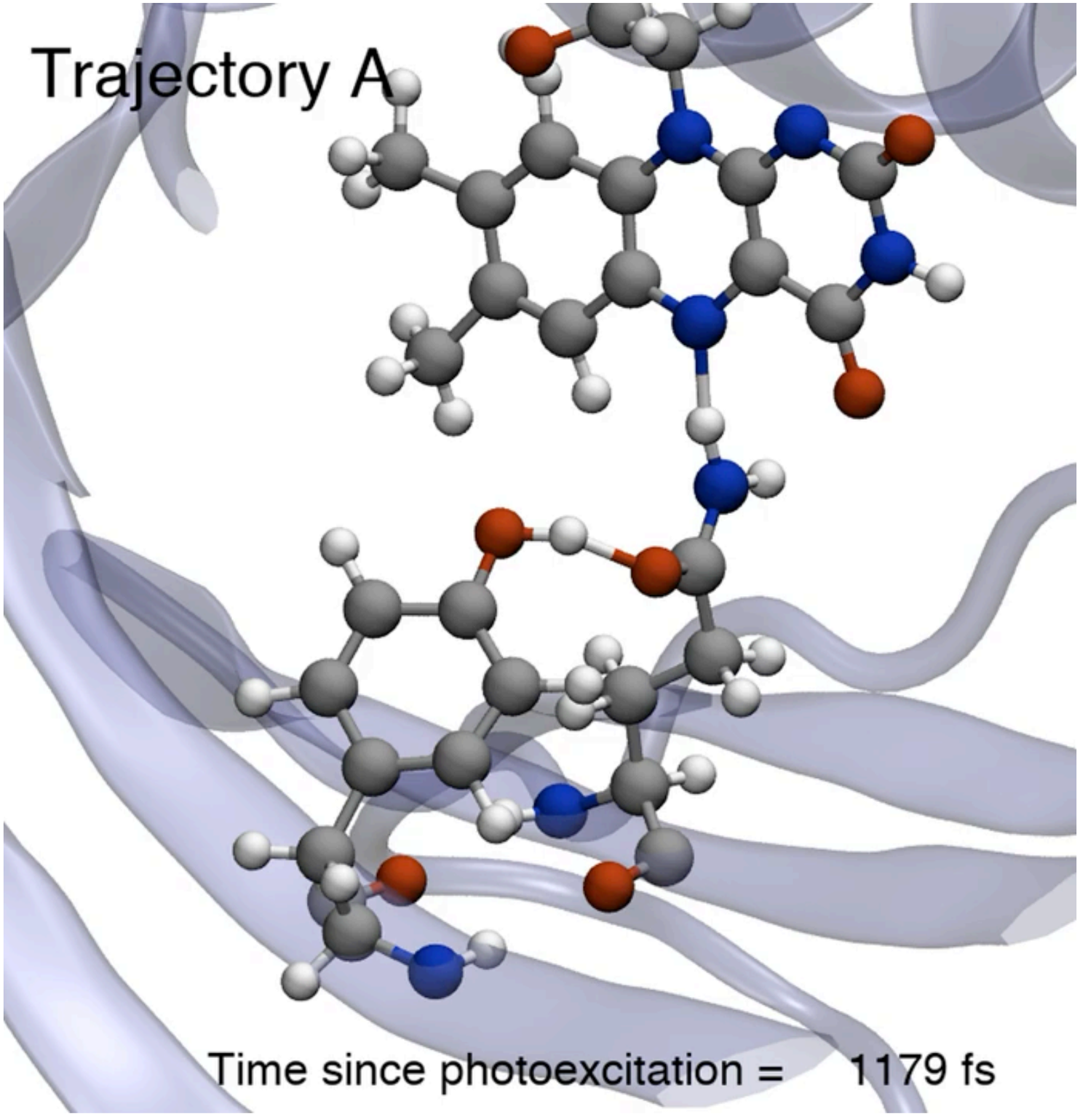
The ground state shows up as the first "excited state."

So, when reference state fails, pick up where TDA-TDDFT left off but use spin-flip approach — qualitative, but allows us insight into potential mechanism.

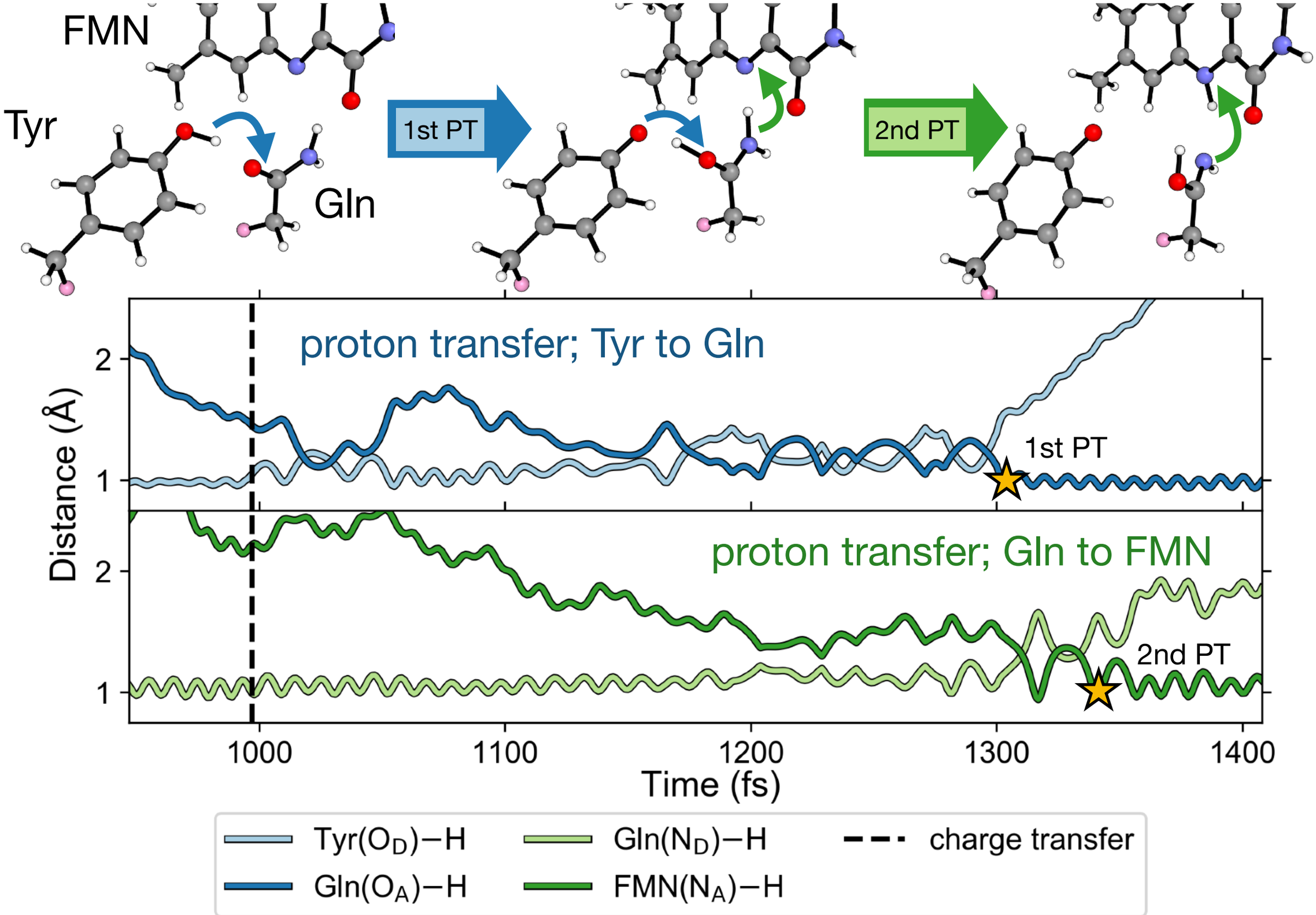
Shao, Y.; Head-Gordon, M.; Krylov, A. I. The Spin–flip Approach within Time-Dependent Density Functional Theory: Theory and Applications to Diradicals. *JCP.* **2003**, *118* (11), 4807–4818.

③

Proton Transfer



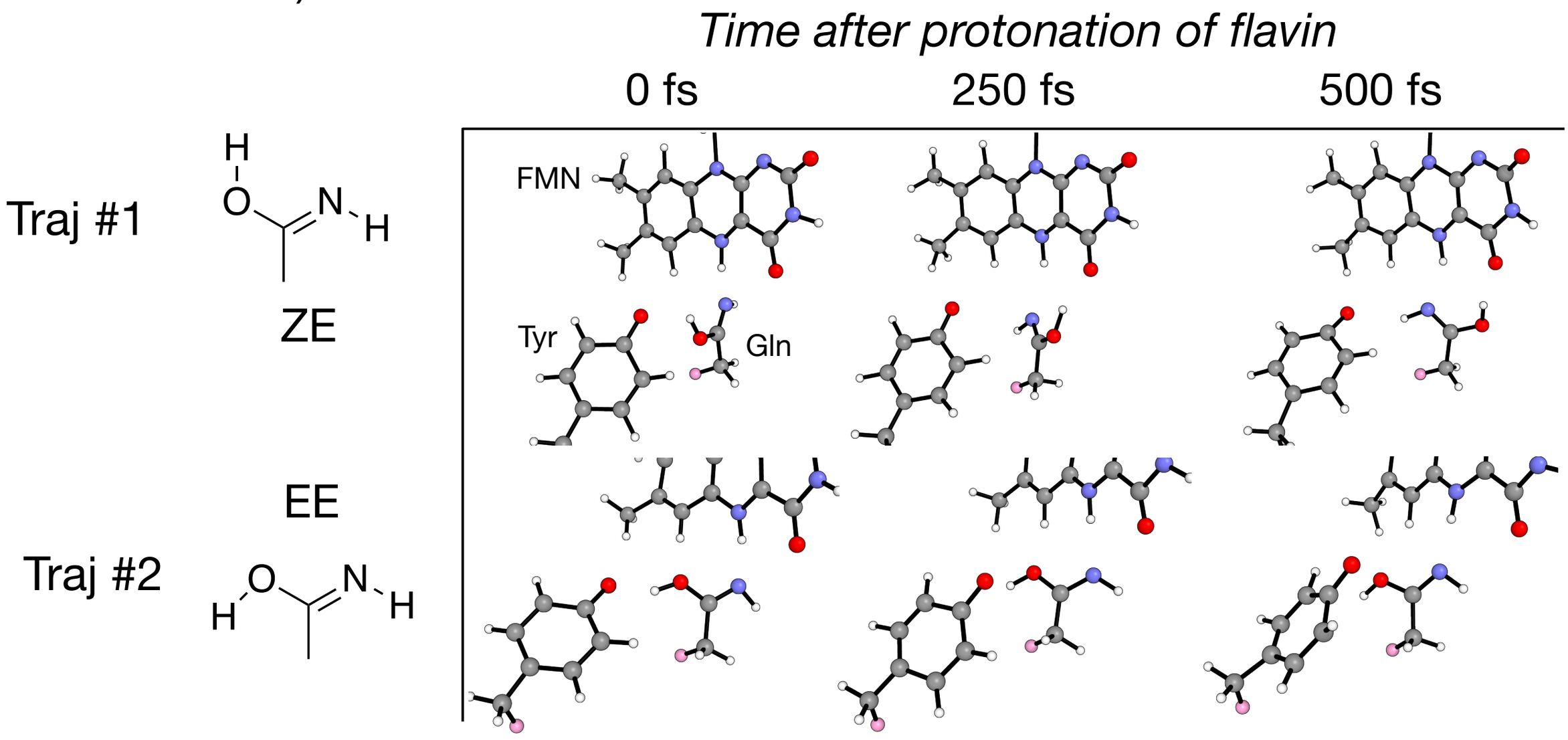
③ Proton Transfer



④ Hydrogen Bond Rearrangement

After protonation of flavin, glutamine exists as one of two imidic acid tautomers

Fast glutamine rotation if ZE tautomer, no motion observed for EE
(on this timescale)



Recent similar arguments for BLUF domain BlrB:

Domratcheva, T.; *et al. Sci. Rep.* **2016**. Grigorenko, B. L.; *et al. Phys. Chem. Chem. Phys.* **2018**.

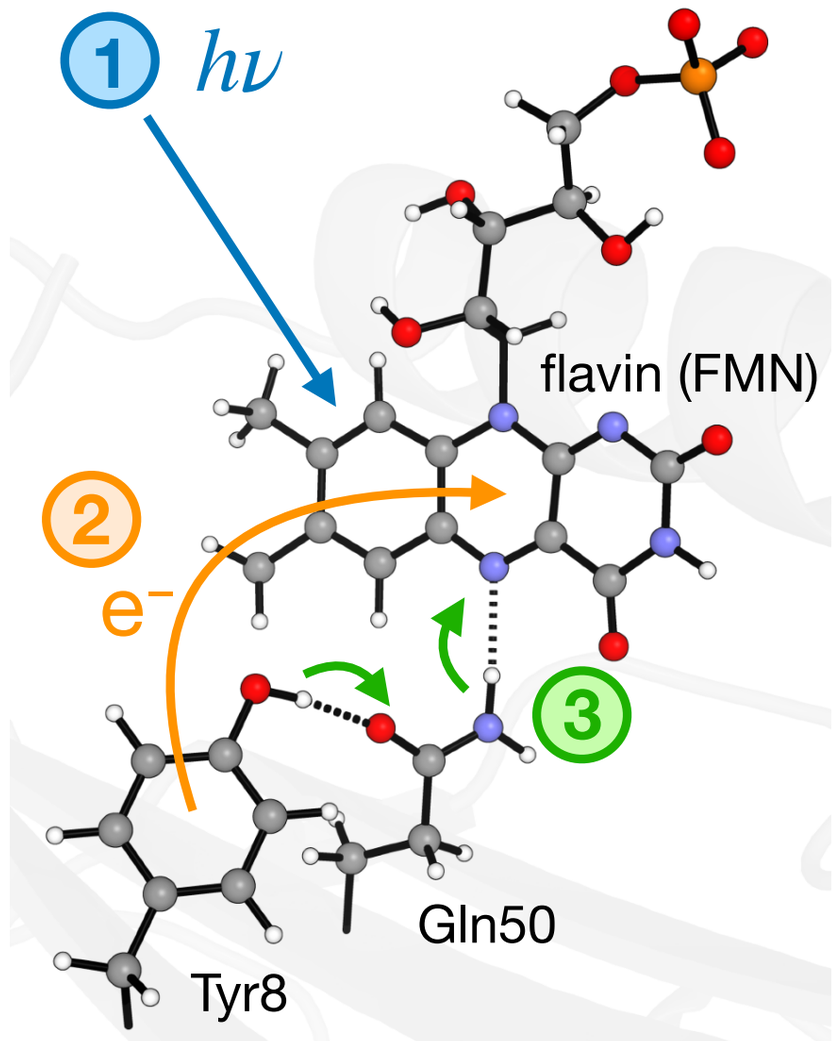
Summary

1. Direct simulation of non-equilibrium excited state QM/MM dynamics for Slr1694 BLUF

2. Electron transfer to flavin driven by protein reorganization and electrostatics

3. $Tyr^{\bullet+}/FMN^{\bullet-}$ radical pair drives proton transfer

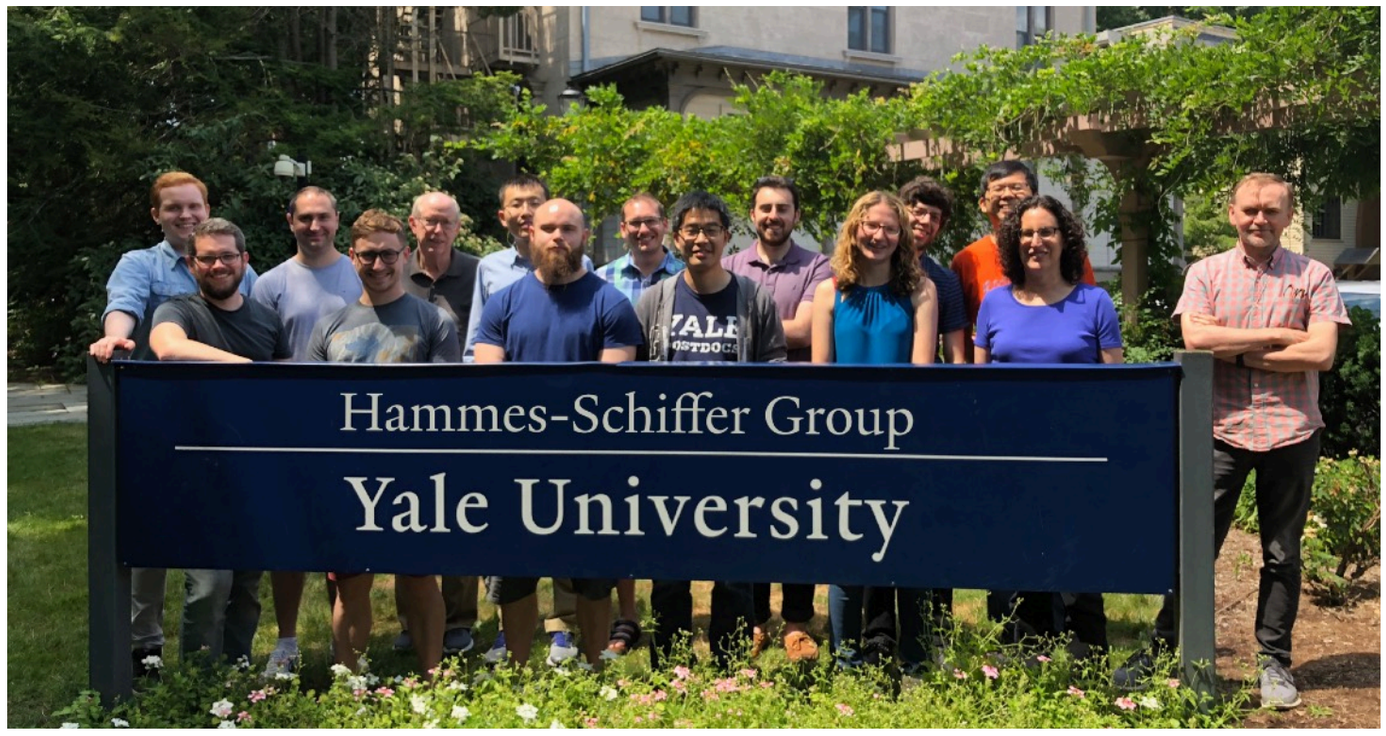
4. Direct dynamical support for Gln tautomerization + rotation mechanism in Slr1694 photocycle



Acknowledgments

Thank you!

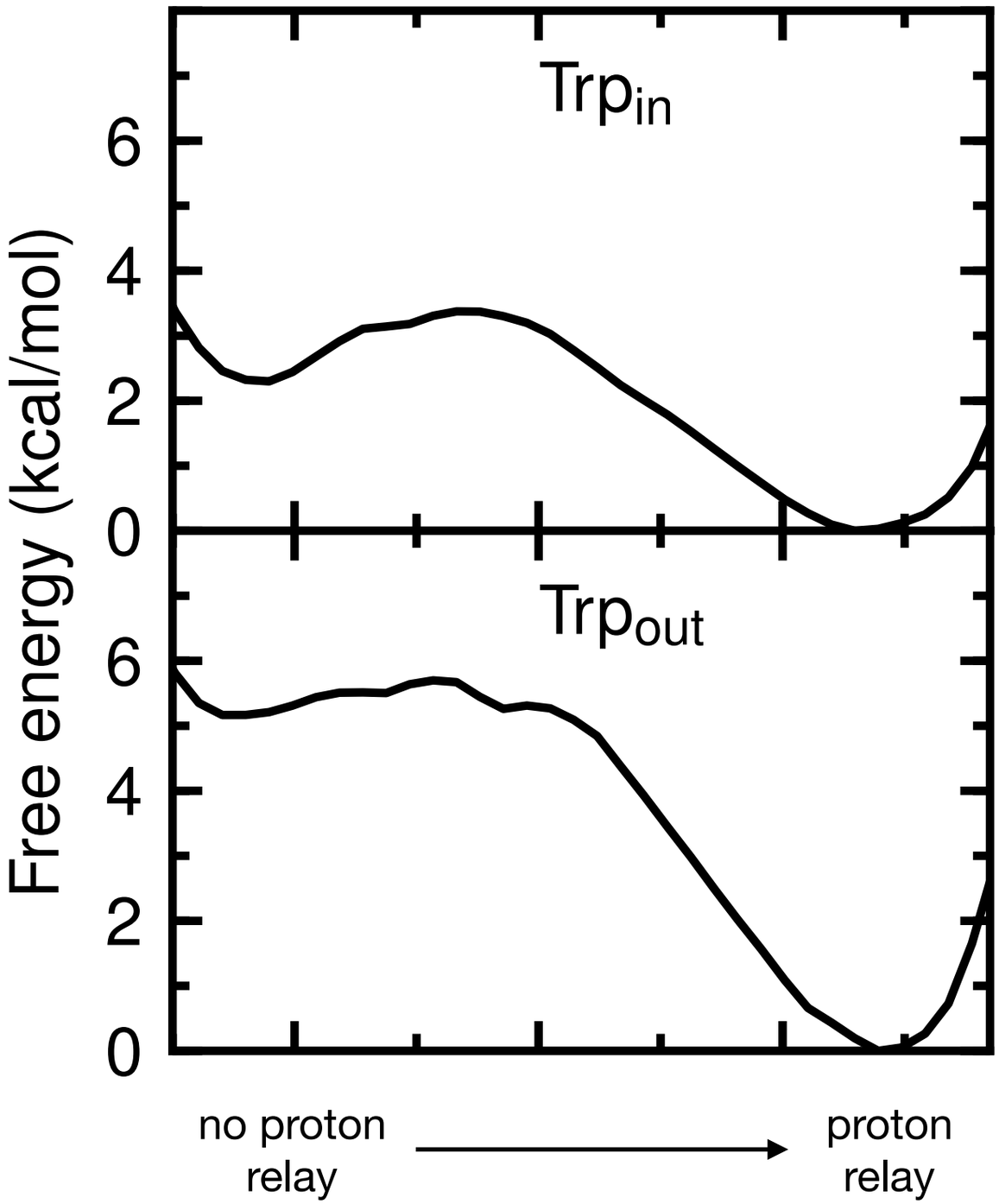
CASSCF+NEVPT2 benchmarking data:
Elvira Sayfutyarova



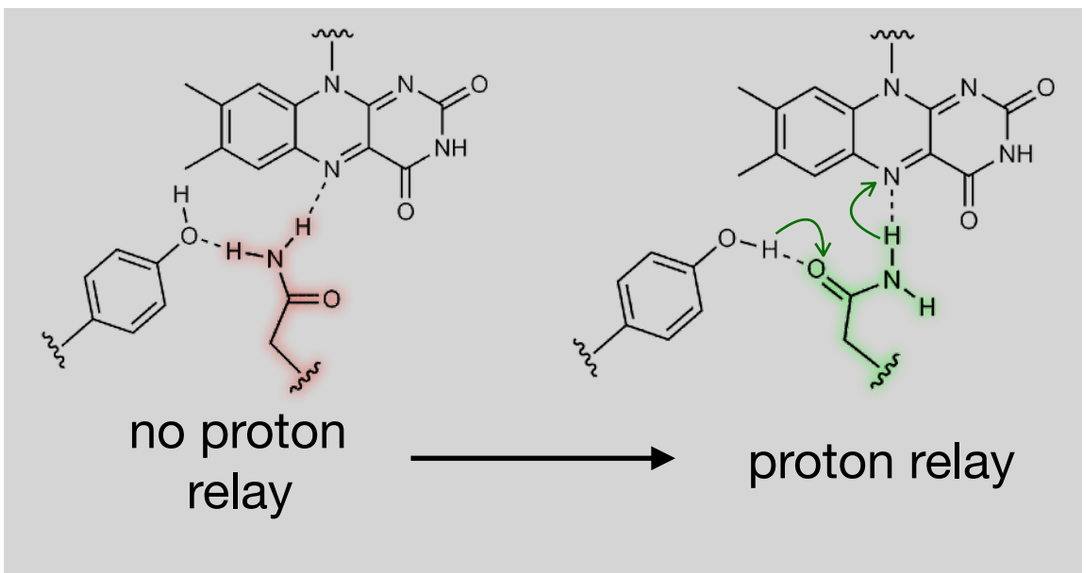
Funding:
AFOSR,
XSEDE (Comet @ SDSC)

XSEDE
Extreme Science and Engineering Discovery Environment

Gln50: proton relay favored in Slr1694



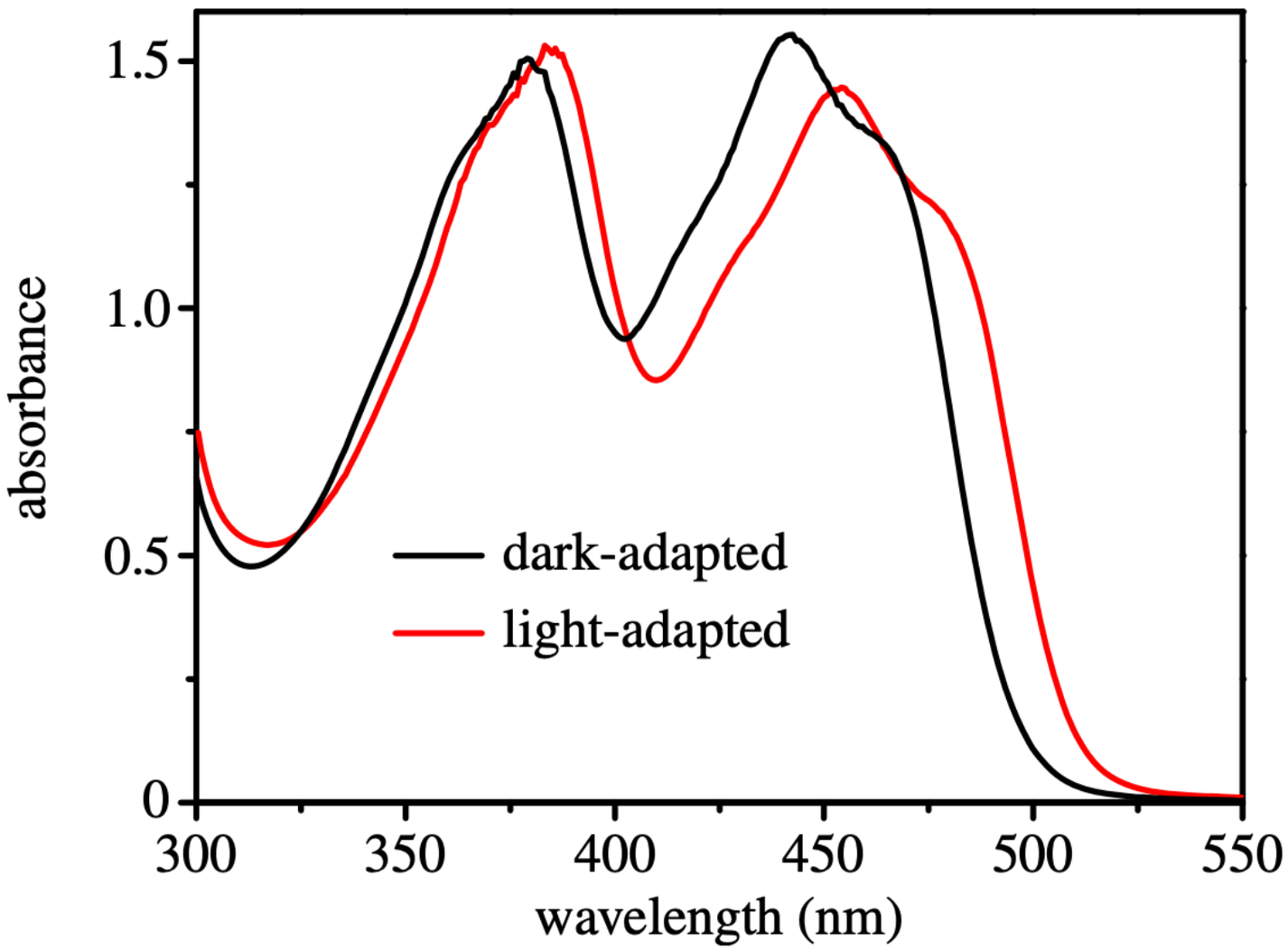
- Crystal structure does not provide unambiguous assignment of Gln orientation
- Umbrella sampling used to explore free energy changes along Gln rotation
- Proton relay always favored regardless of Trp$_{in}$ or Trp$_{out}$



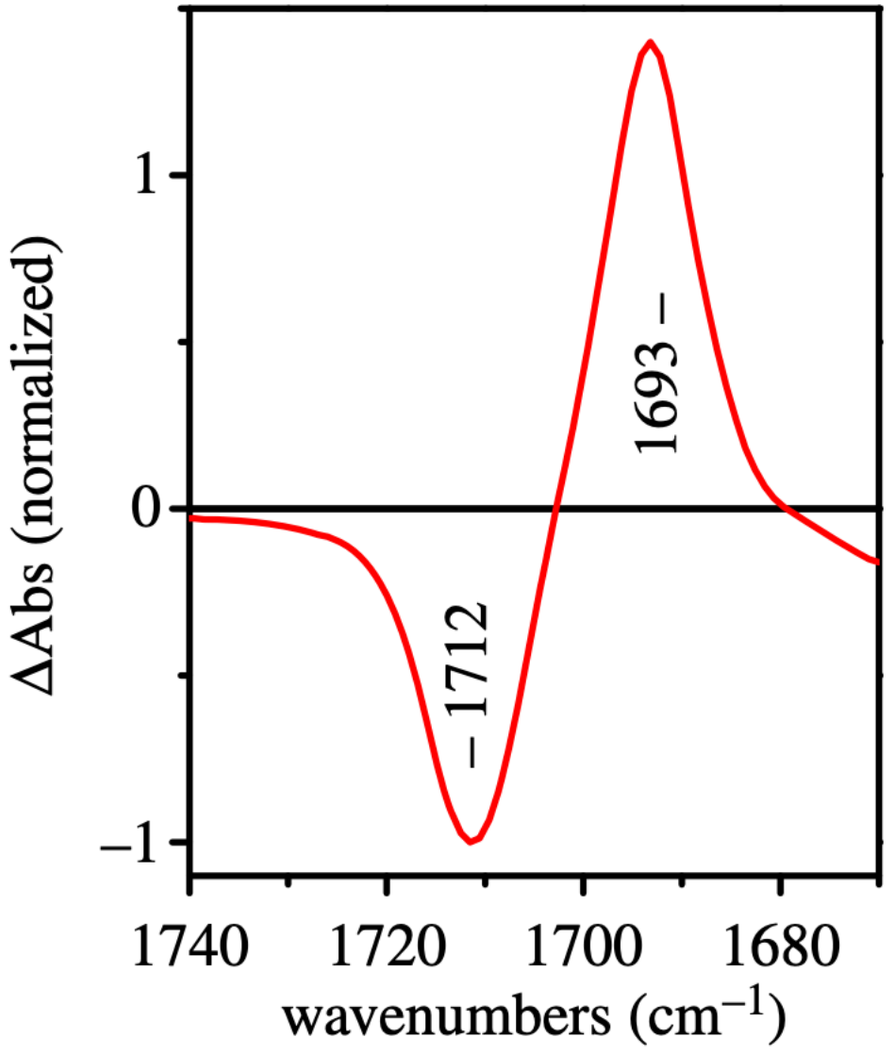
Goings, J. J.; Reinhardt, C. R.; Hammes-Schiffer, S. Propensity for Proton Relay and Electrostatic Impact of Protein Reorganization in Slr1694 BLUF Photoreceptor. *J. Am. Chem. Soc.* **2018.**

Experimental Slr1694 BLUF Photocycle

red-shifted flavin
absorption spectrum



red-shifted flavin
C_4=O FT-IR



Suggest changes in hydrogen bonding network around the flavin

Kennis, J. T. M.; Mathes, T. Molecular Eyes: Proteins That Transform Light into Biological Information. *Interface Focus* **2013**.