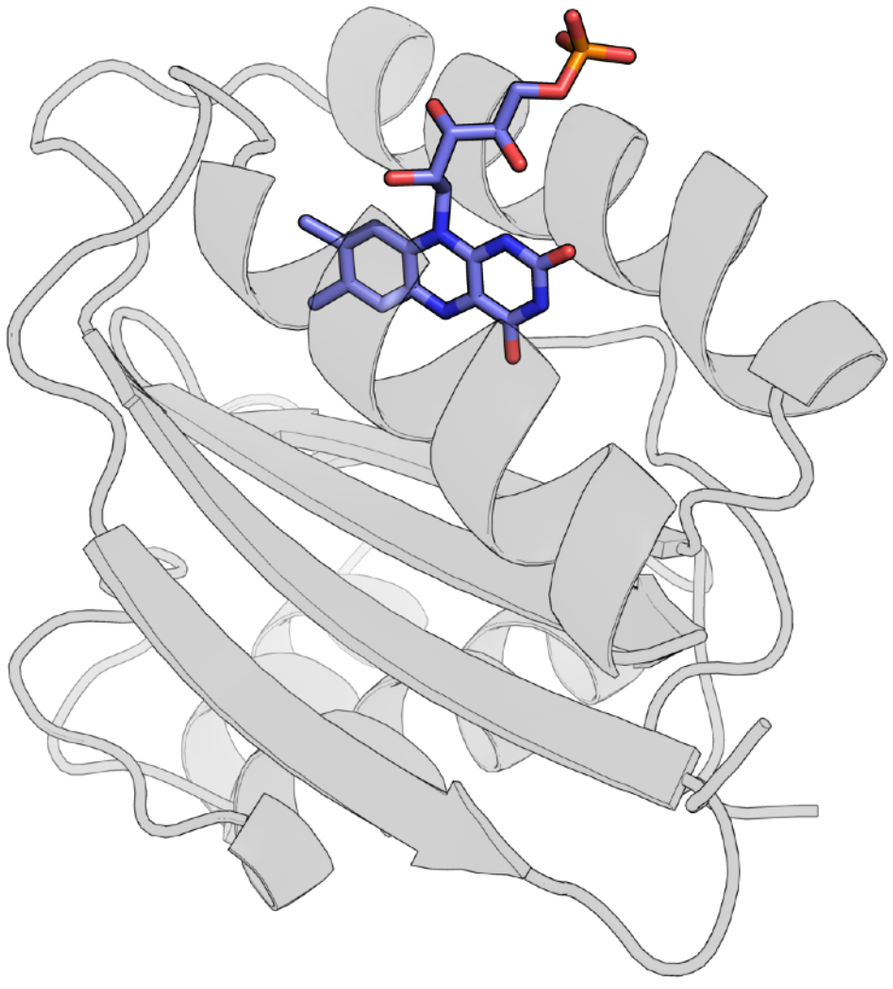
Impact of Protein Environment on Slr1694 BLUF Photoreceptor

Joshua Goings
October 4, 1:00 pm

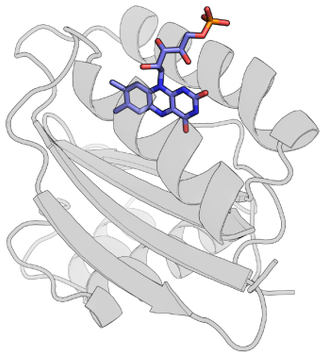
Photoreceptor domain BLUF



***B**lue **L**ight **U**sing **F**lavin*

BLUF uses a flavin chromophore to convert a light signal into a biological response.

Photoreceptor domain BLUF



BLUF

Blue light using flavin

BLUF uses a flavin to convert a light signal into a biological response.

Found in photosynthetic bacteria, but also some single-celled eukaryotes.

Subudhi, Sanjukta, et al. Int J Hydrogen Energ. 41.32 (2016): 13979-13985.

Ferjani, Ali, et al. Plant physiol. 131.4 (2003): 1628-1637.

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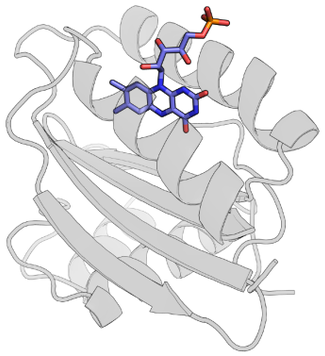
Photoreceptor domain BLUF



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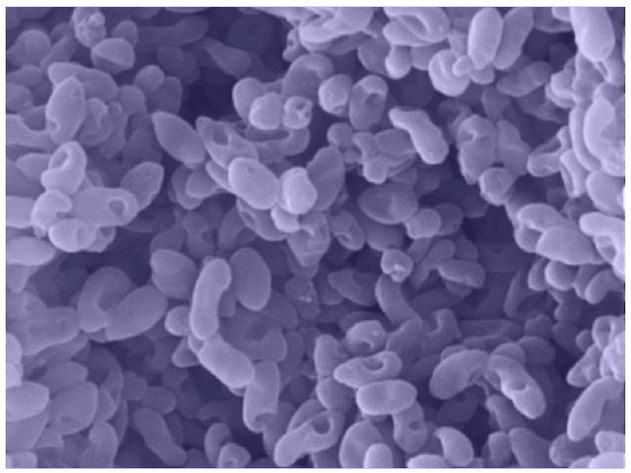
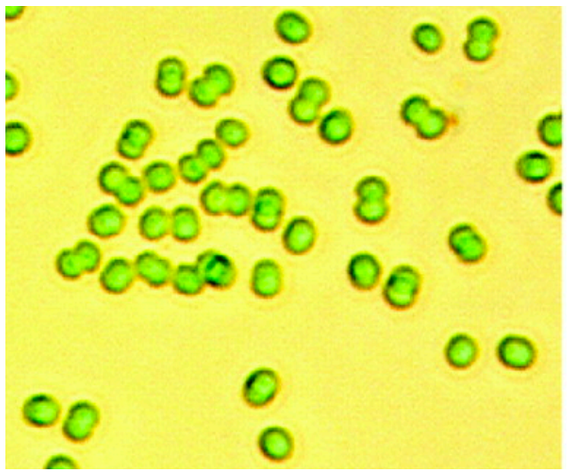
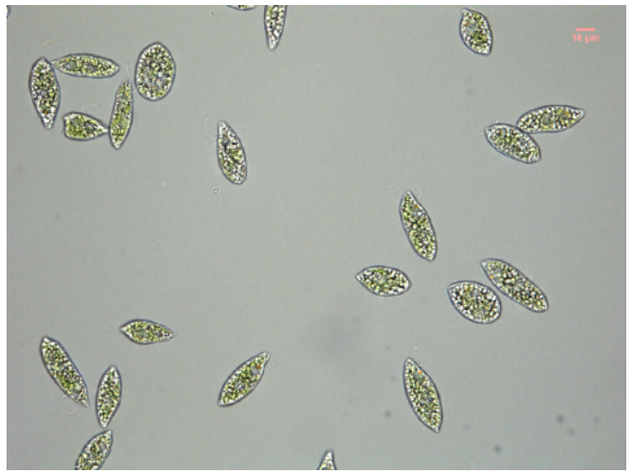


Purple Bacteria
R. sphaeroides



Cyanobacteria
S. sp. PCC6803



Algae
E. gracilis

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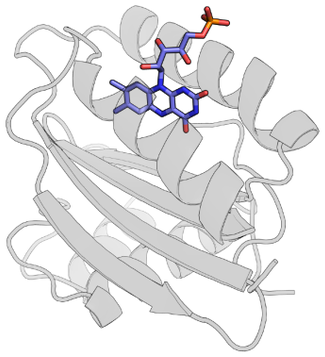

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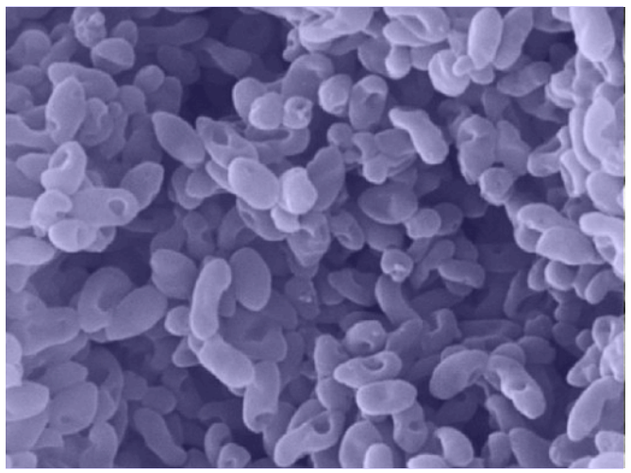

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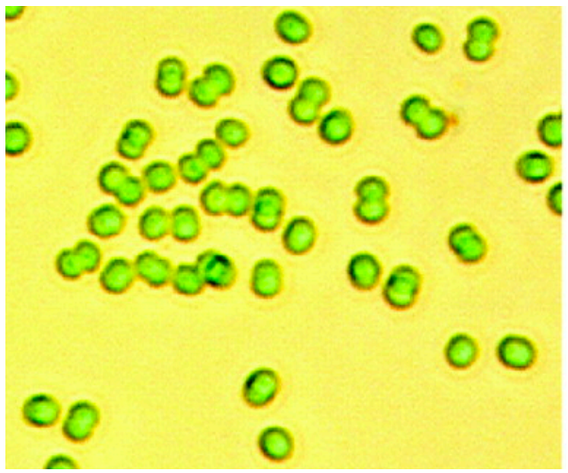

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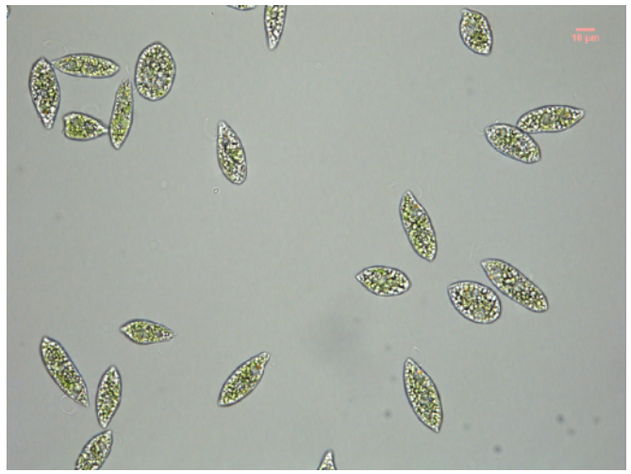

Algae
E. gracilis

AppA	**Slr1694**	**PAC**
Photosynthetic gene expression	Positive phototaxis	Synthesize cAMP

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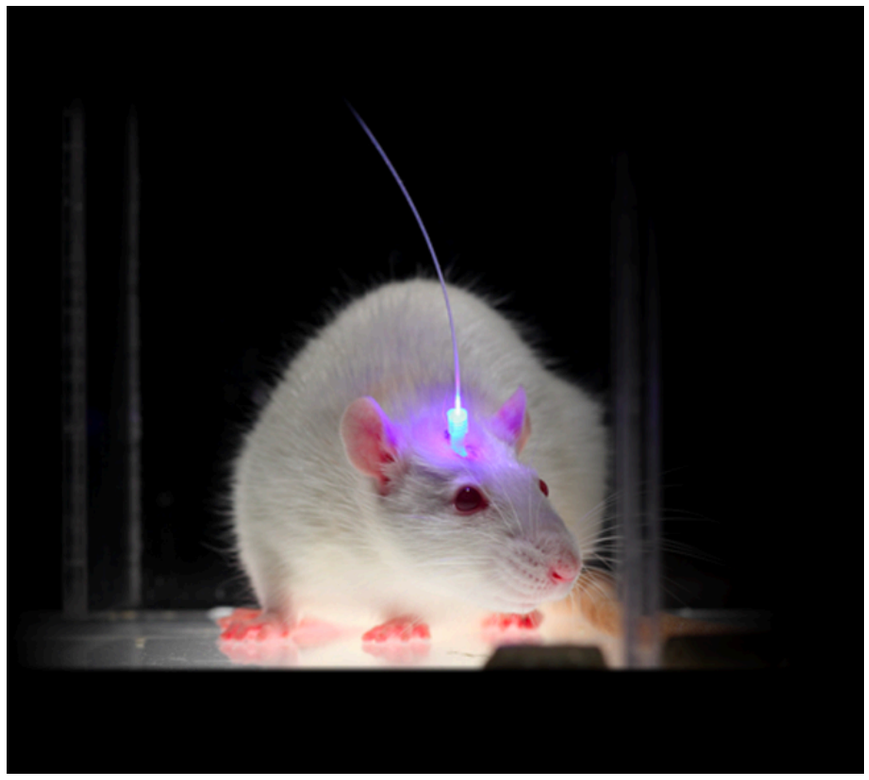
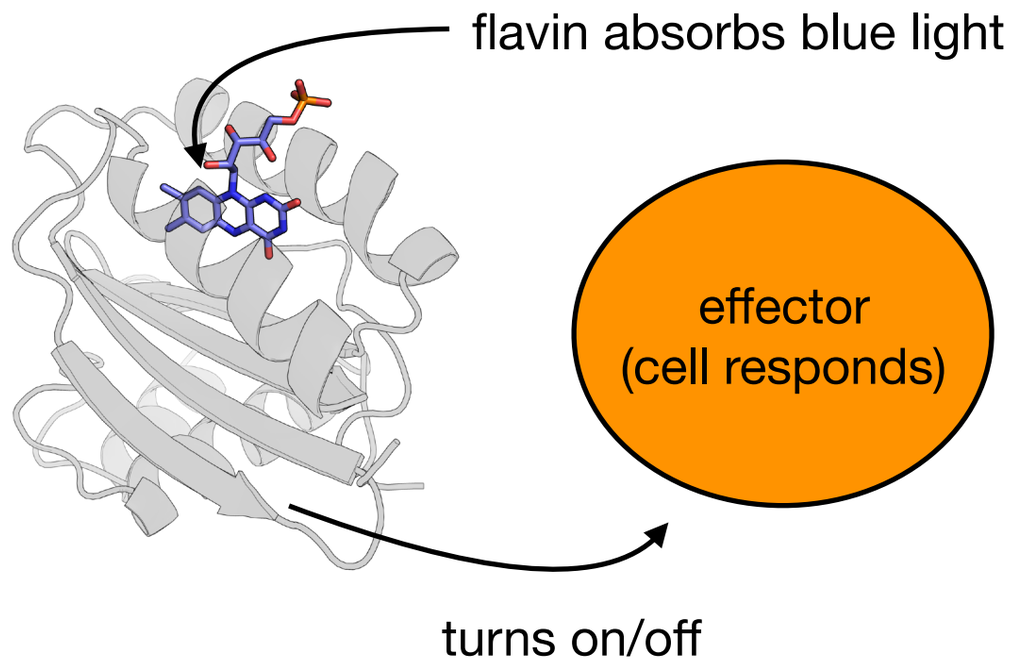
Using BLUF for optogenetics

optogenetics: use light to control cells in living tissue



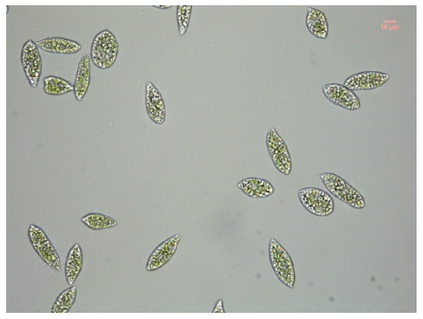
Source: Getty's Open Content Program (John B. Carnett / Getty Images)



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

It's a blue light sensitive switch.

Using BLUF for optogenetics



Algae
E. gracilis

Insert PAC into neurons of target organism
Blue-light illumination causes increase in cAMP

PAC
Photoactivated **a**denylyl **c**yclase

BLUF + AC

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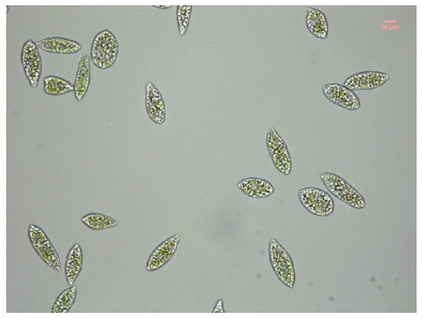
Using BLUF for optogenetics



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BLUF + AC

Fruit fly
D. melanogaster
(Schroder-Lang, *et al.* 2007)

Sea slug
A. kurodai
(Nagahama, *et al.* 2007)

Roundworm
C. elegans
(Weissenberger, *et al.* 2011)

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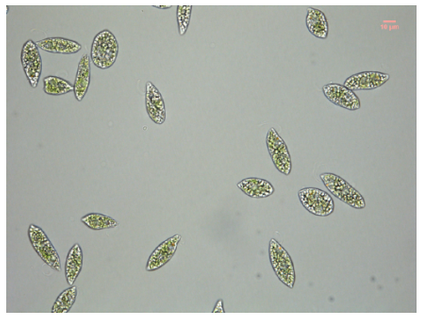
Using BLUF for optogenetics



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E. gracilis

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Blue-light illumination causes increase in cAMP

PAC
Photoactivated **a**denylyl **c**yclase
BLUF + AC

Fruit fly
D. melanogaster
(Schroder-Lang, *et al.* 2007)

- induce hyperactivity
- decrease grooming behavior

Sea slug
A. kurodai
(Nagahama, *et al.* 2007)

- changes neuron spike width and amplitude
- implies changes in synaptic strength (learning)

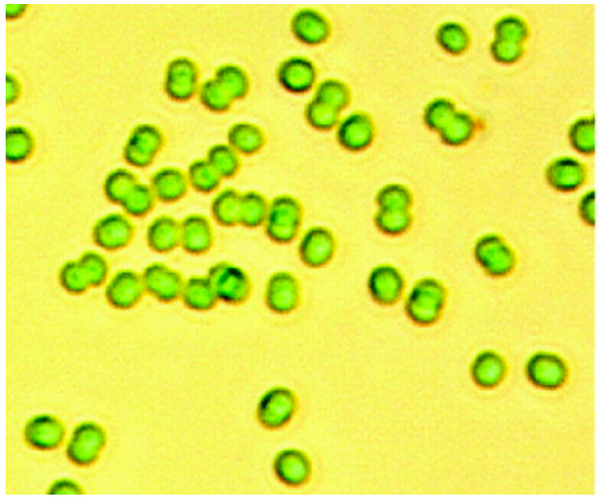
Roundworm
C. elegans
(Weissenberger, *et al.* 2011)

- increased swimming frequency
- increased rate of locomotion

If we want to engineer novel optogenetic function…

…how does the BLUF signaling mechanism work?

BLUF domain Slr1694 (PDB 2HFN)



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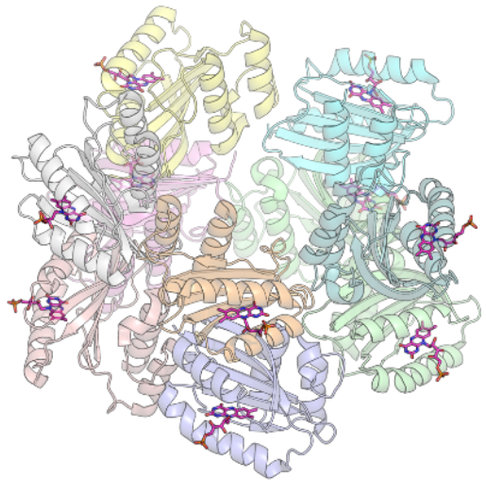
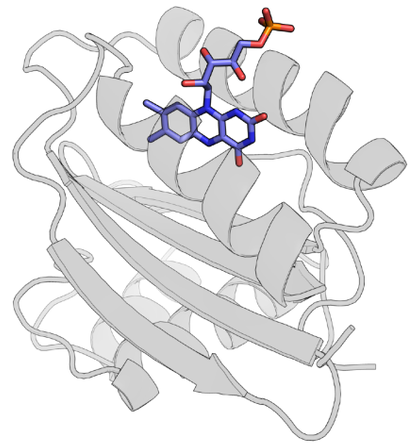


2HFN decamer
1.8 Å resolution



2HFN monomer
chain A

Interesting choice to study because:

1. *We have a crystal structure, and know the conserved residues*

2. *Spectroscopically well-characterized*

   Photocycle progresses through metastable flavin radical intermediates.
   Good for validation of any computational work (e.g. milestones)

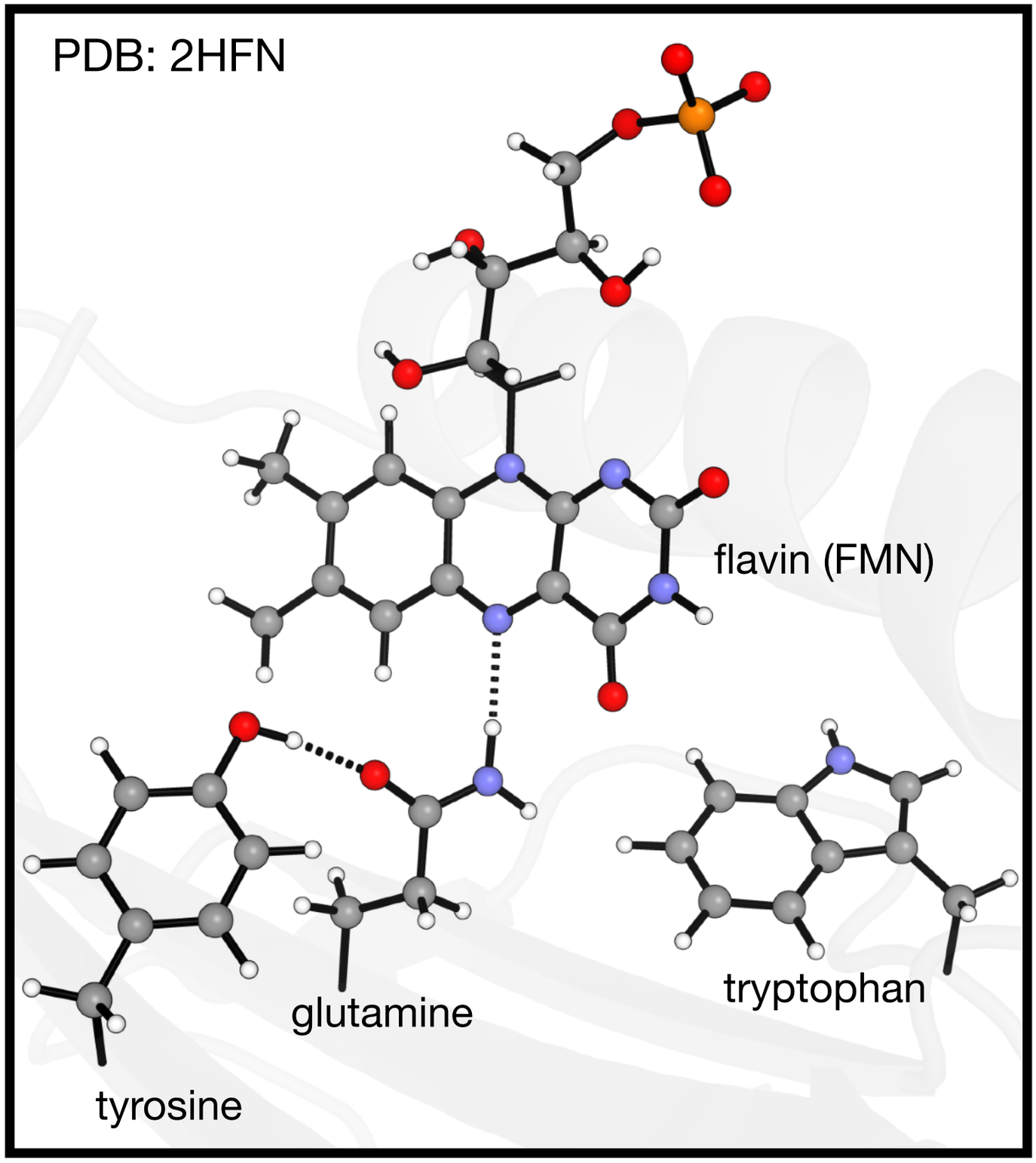
3. *Fast formation of light-adapted (“on”) state (< 1 ns)*

   Any computational dynamics will be much cheaper!

No consensus on mechanism

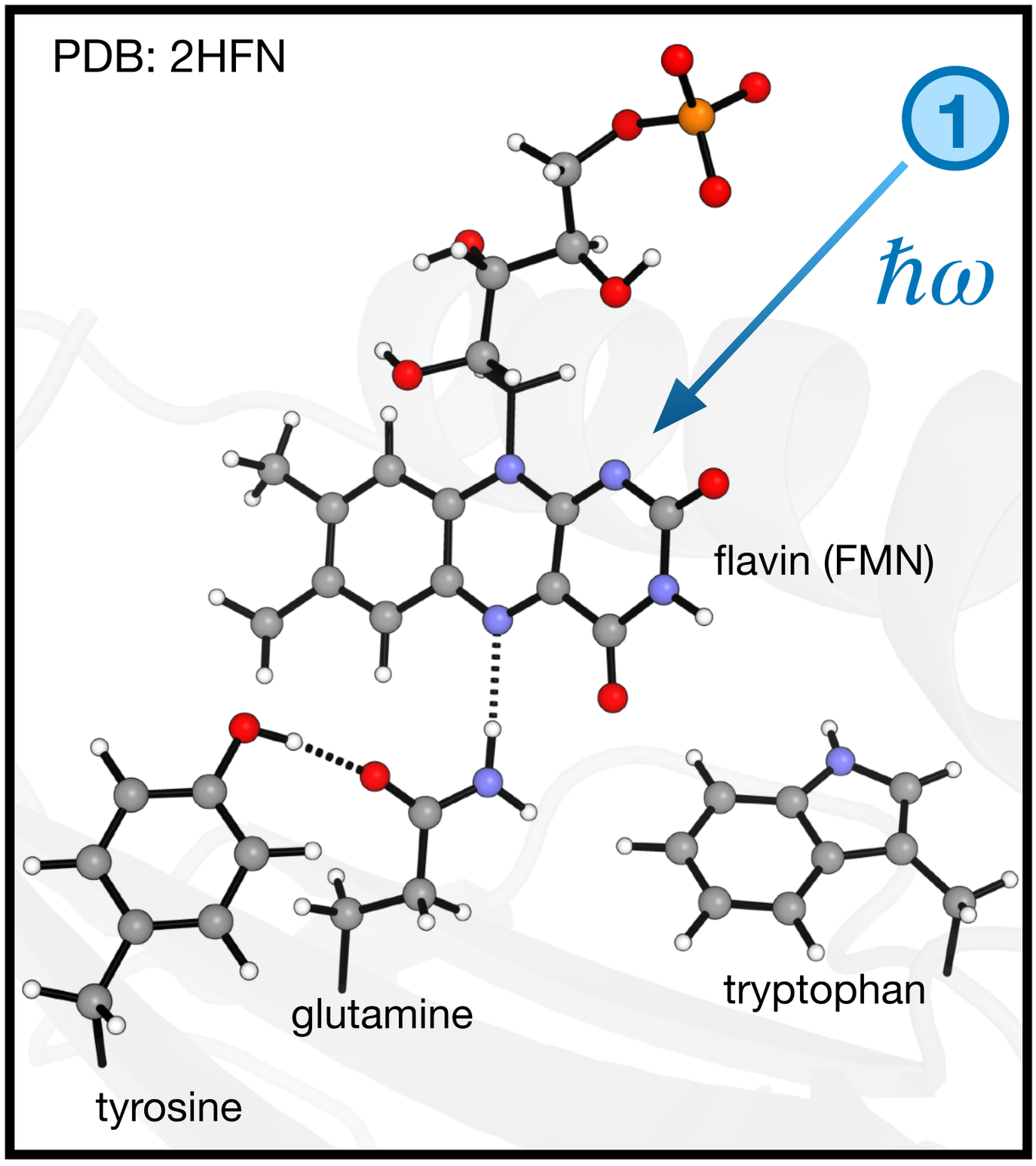
Ferjani, Ali, et al. Plant physiol. 131.4 (2003): 1628-1637.

A rough outline of the Slr1694 photocycle



A rough outline of the Slr1694 photocycle

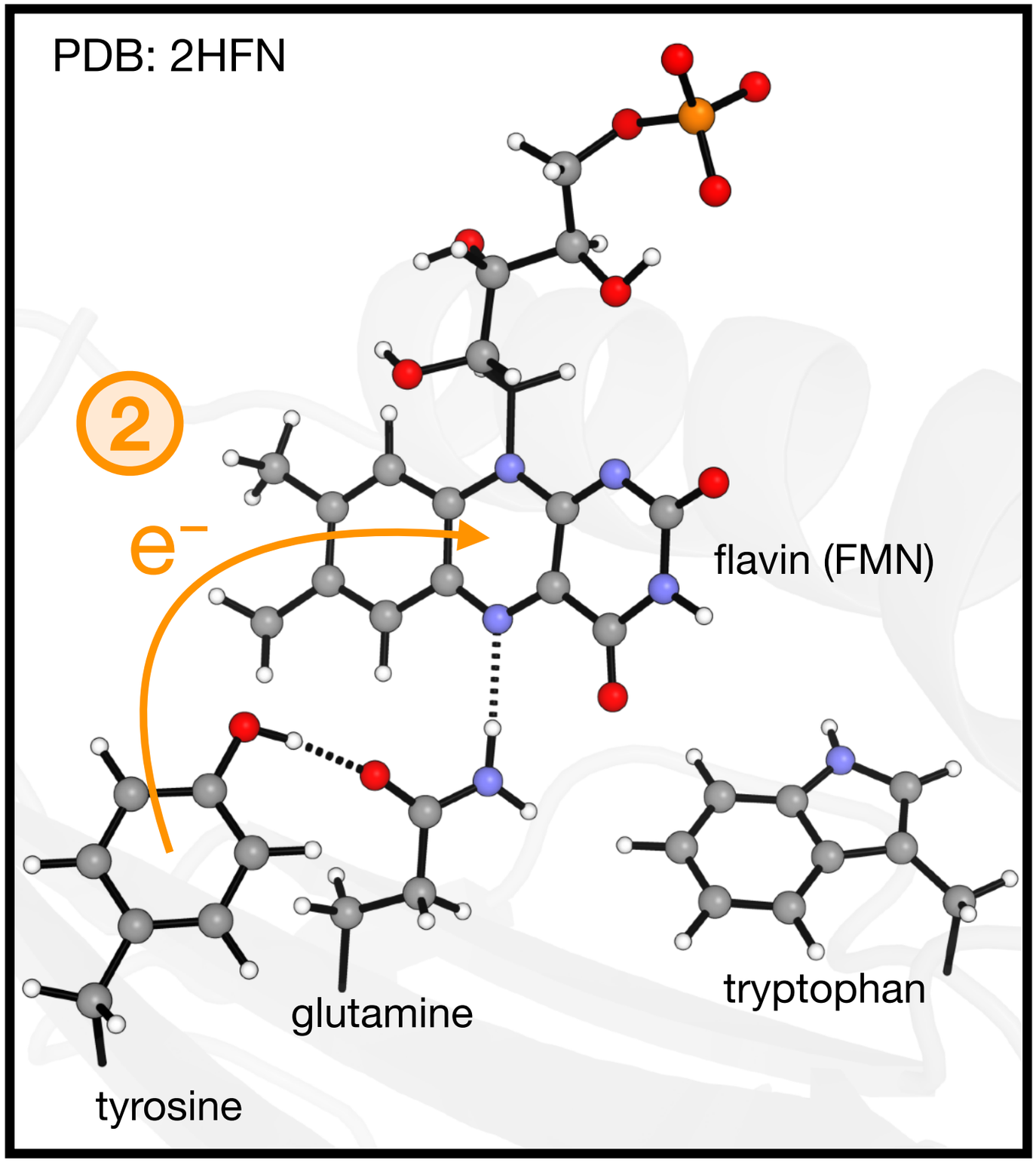
1. Flavin (FMN) absorbs blue light, form FMN*



A rough outline of the Slr1694 photocycle

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

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



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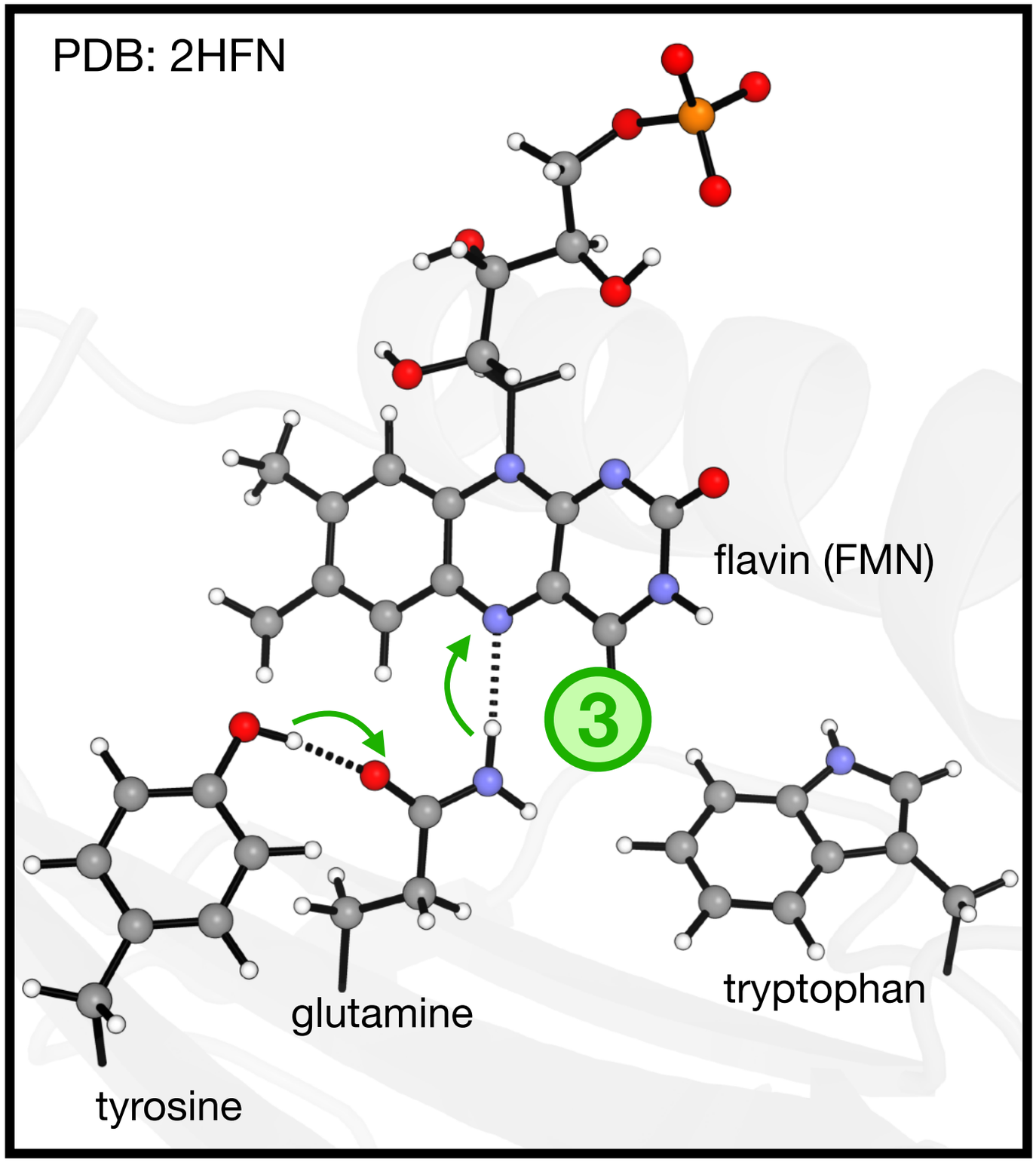

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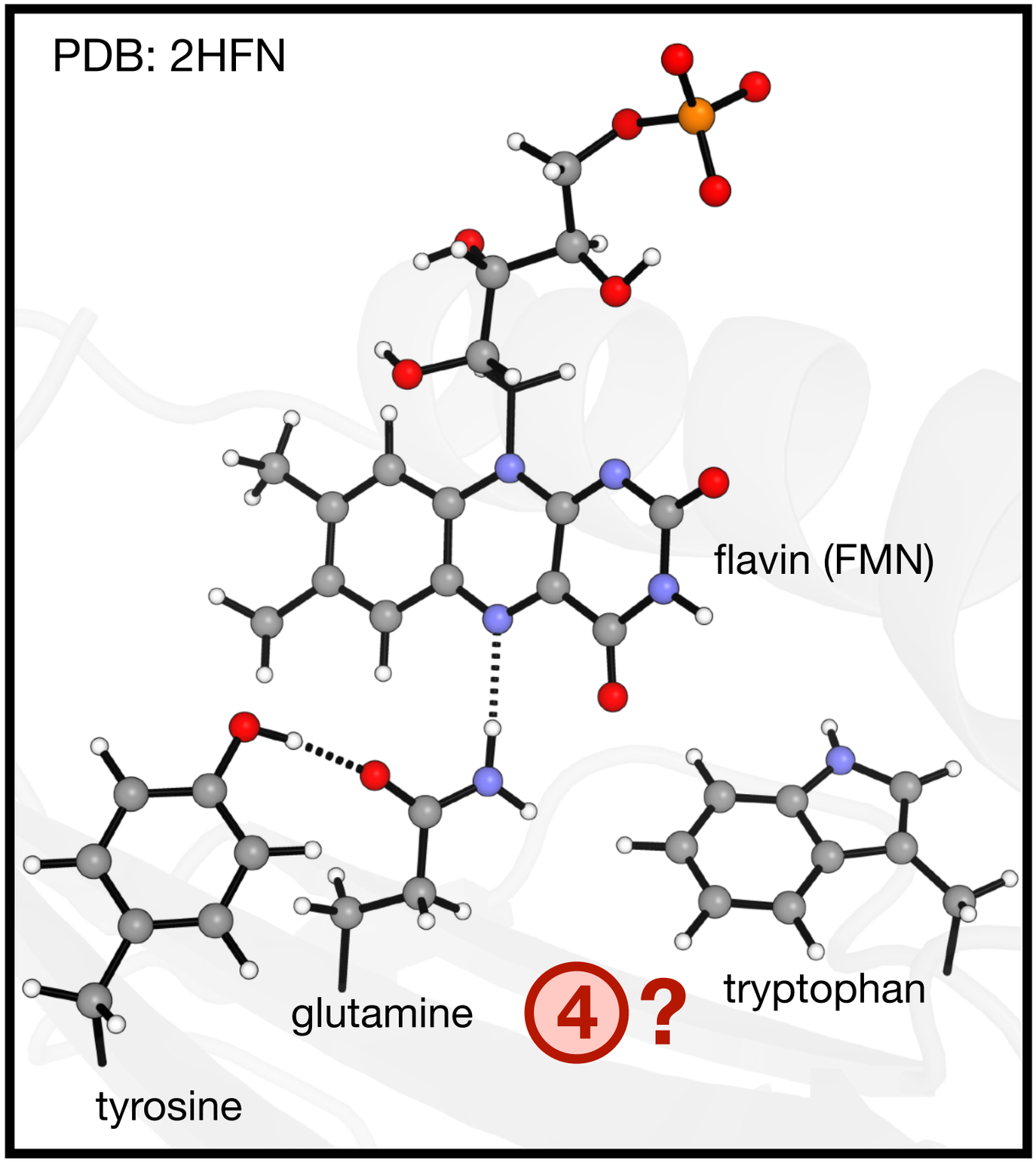
4. Relaxation to light-adapted state, back to FMN **(65 ps)**



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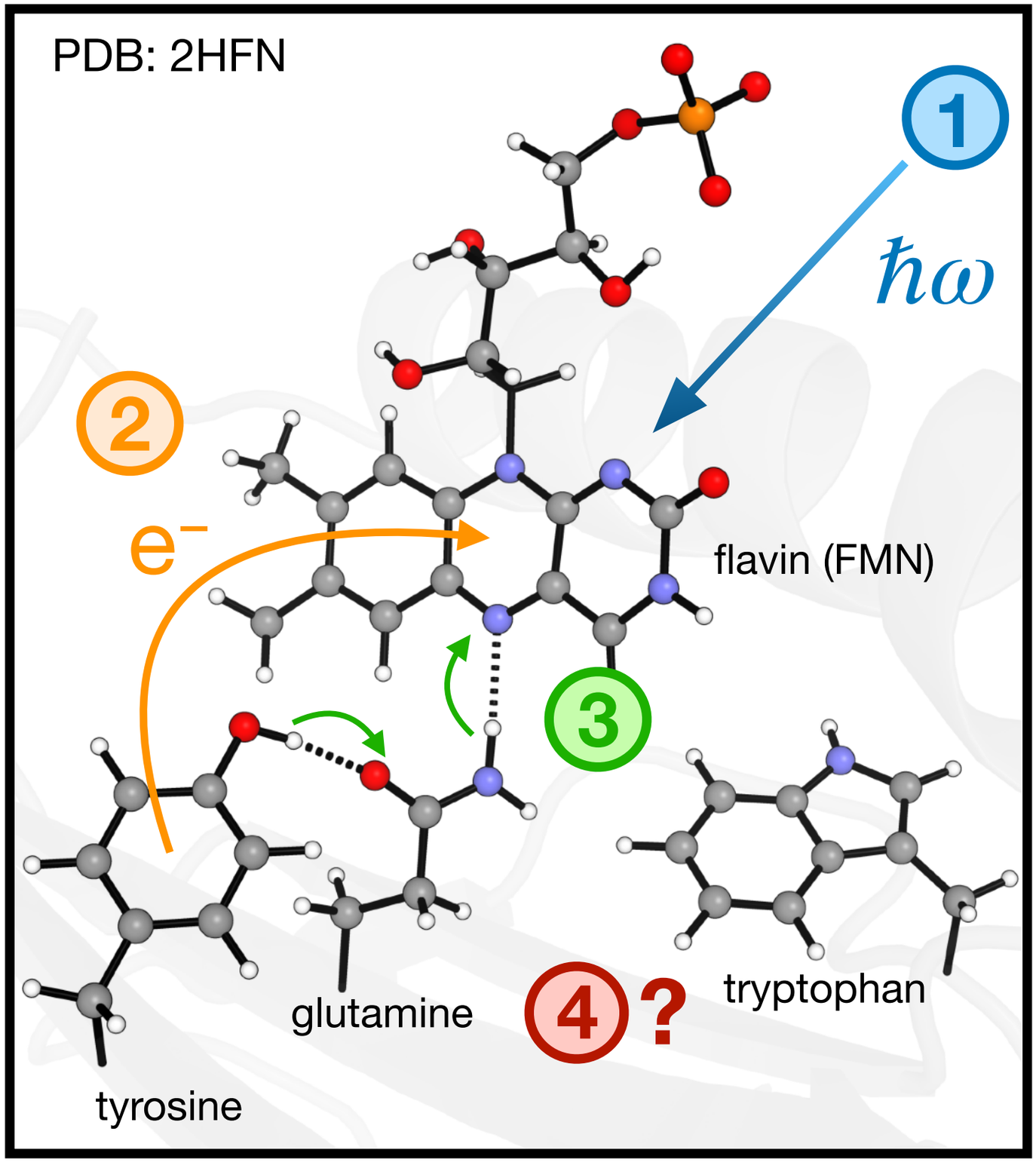
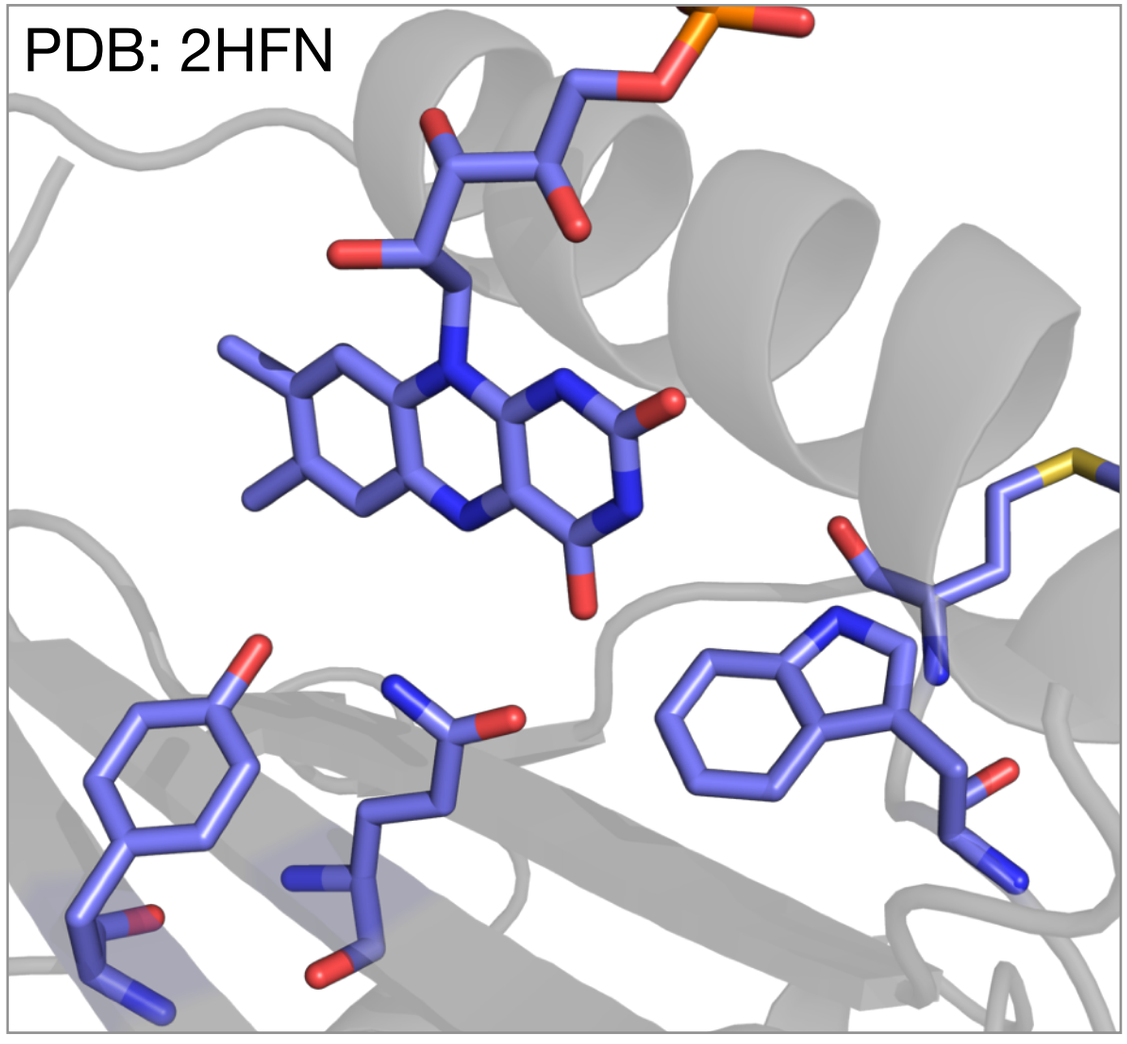


Molecular dynamics could help fill in the gaps in the photocycle…

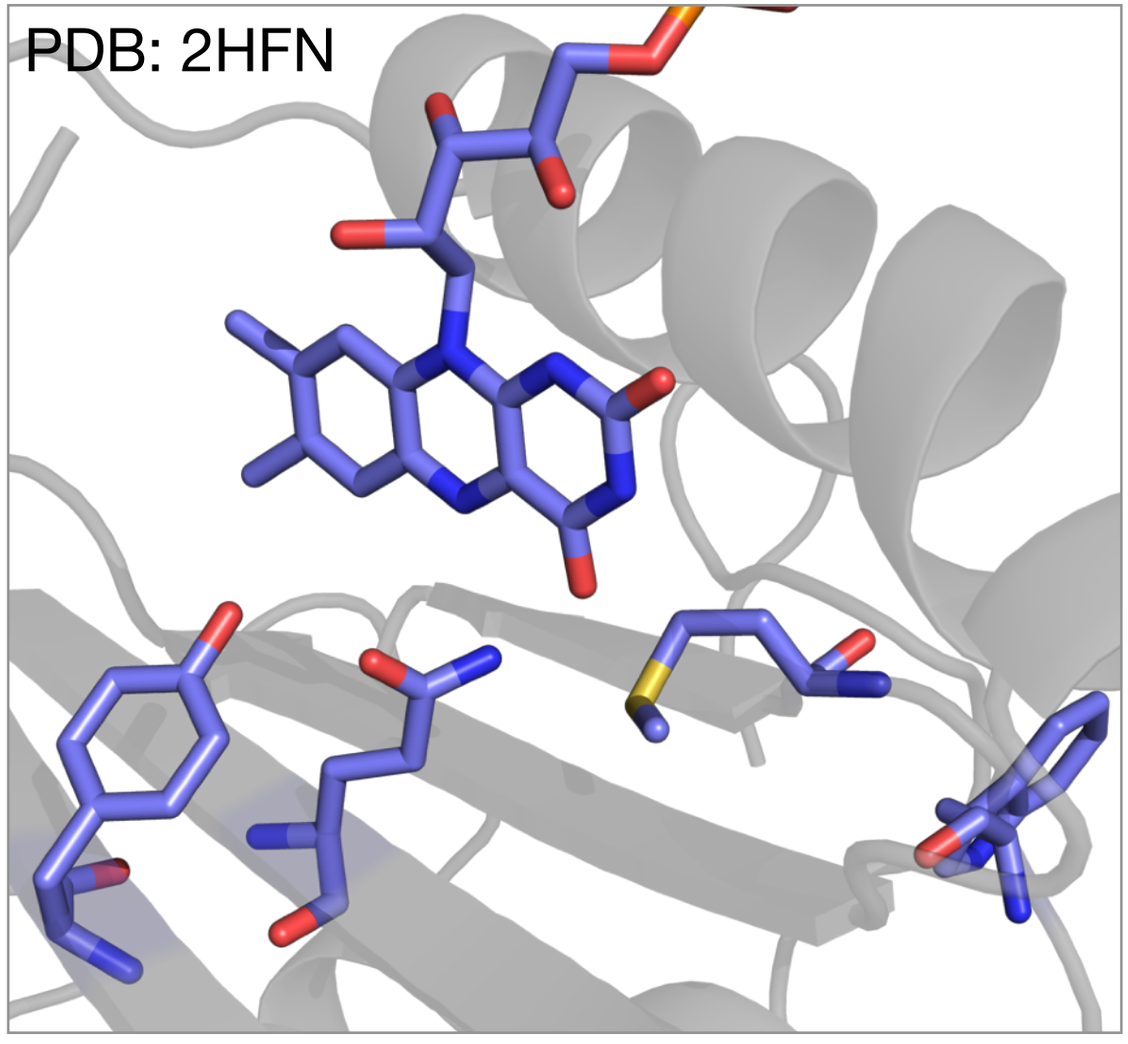
…but the crystal structure doesn’t give unambiguous dark-adapted state.

Crystal structure shows ambiguity

What is the biologically relevant configuration of the active site?



chain D

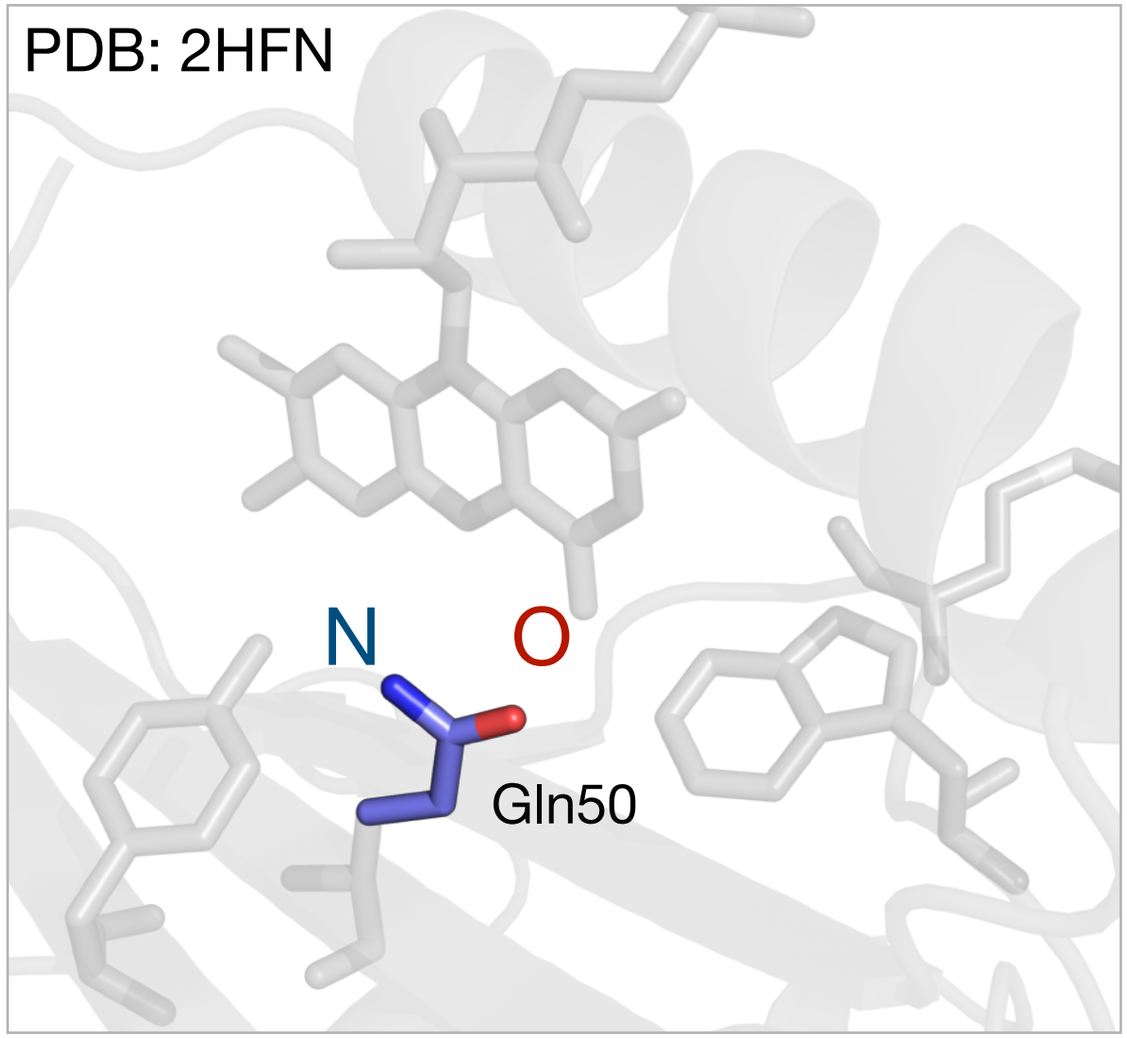


chain A

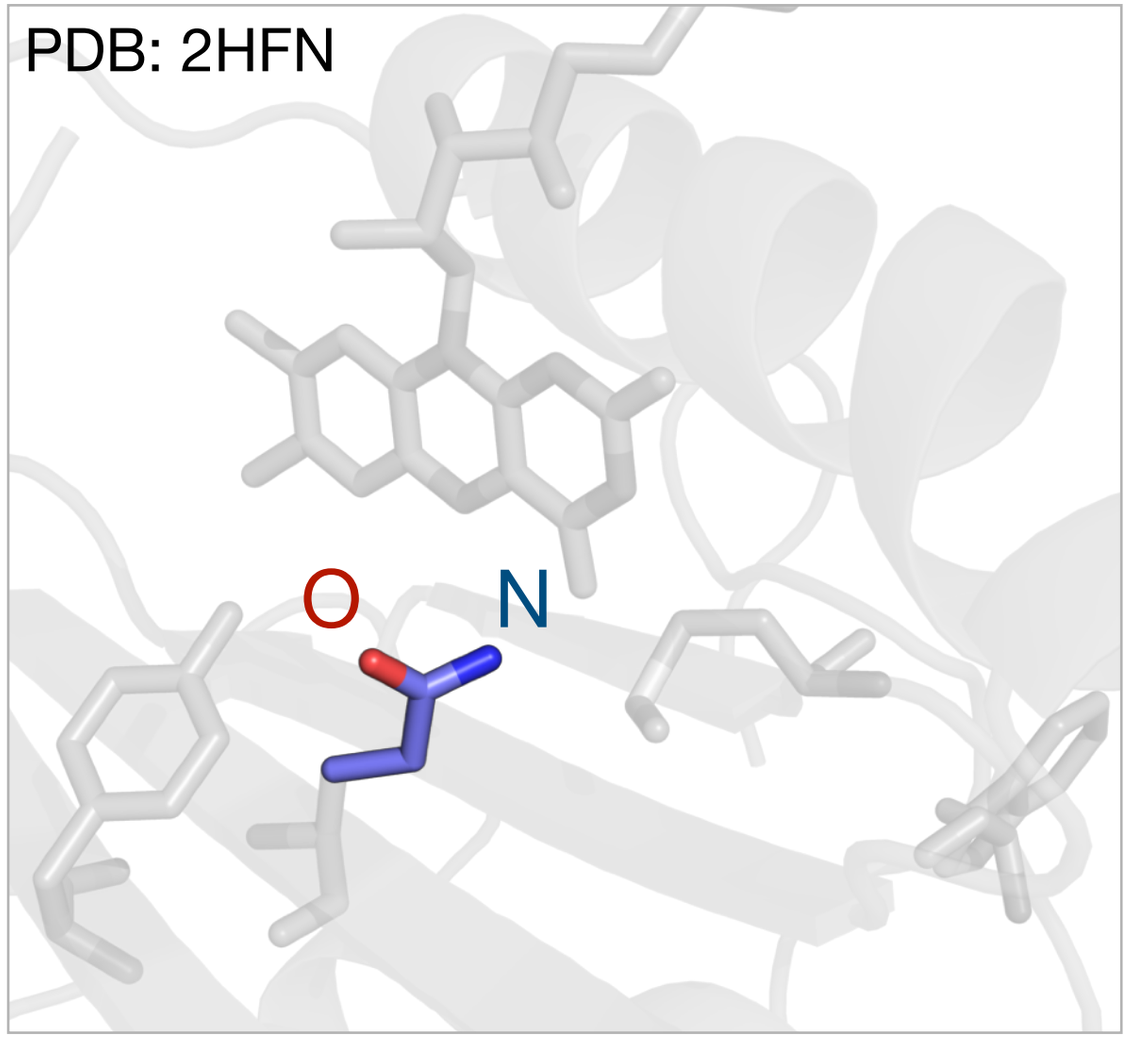
Same crystal structure, monomers differ on key residue location.

Crystal structure shows ambiguity

What is the preferred orientation of Gln50?



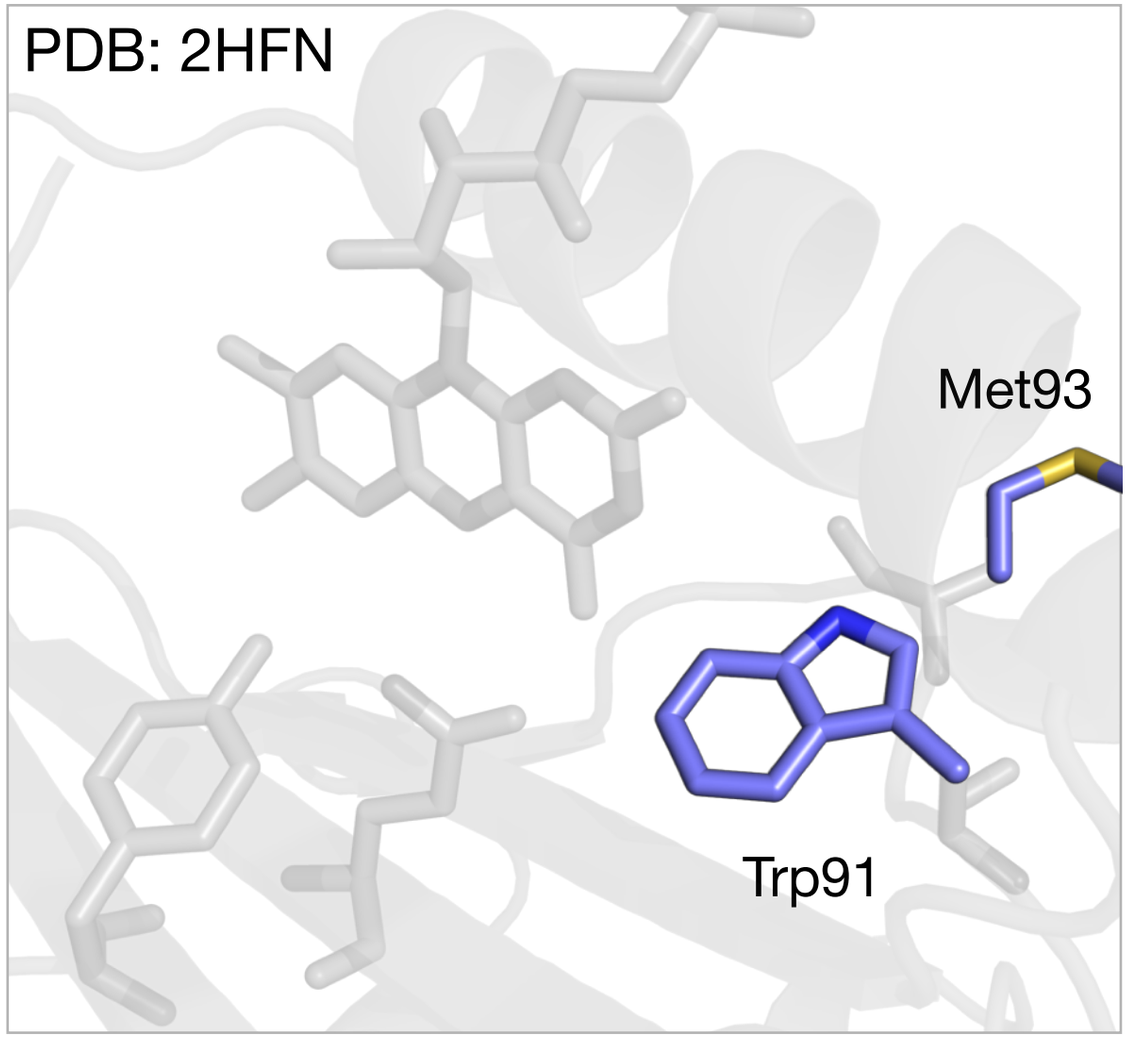
chain D



chain A

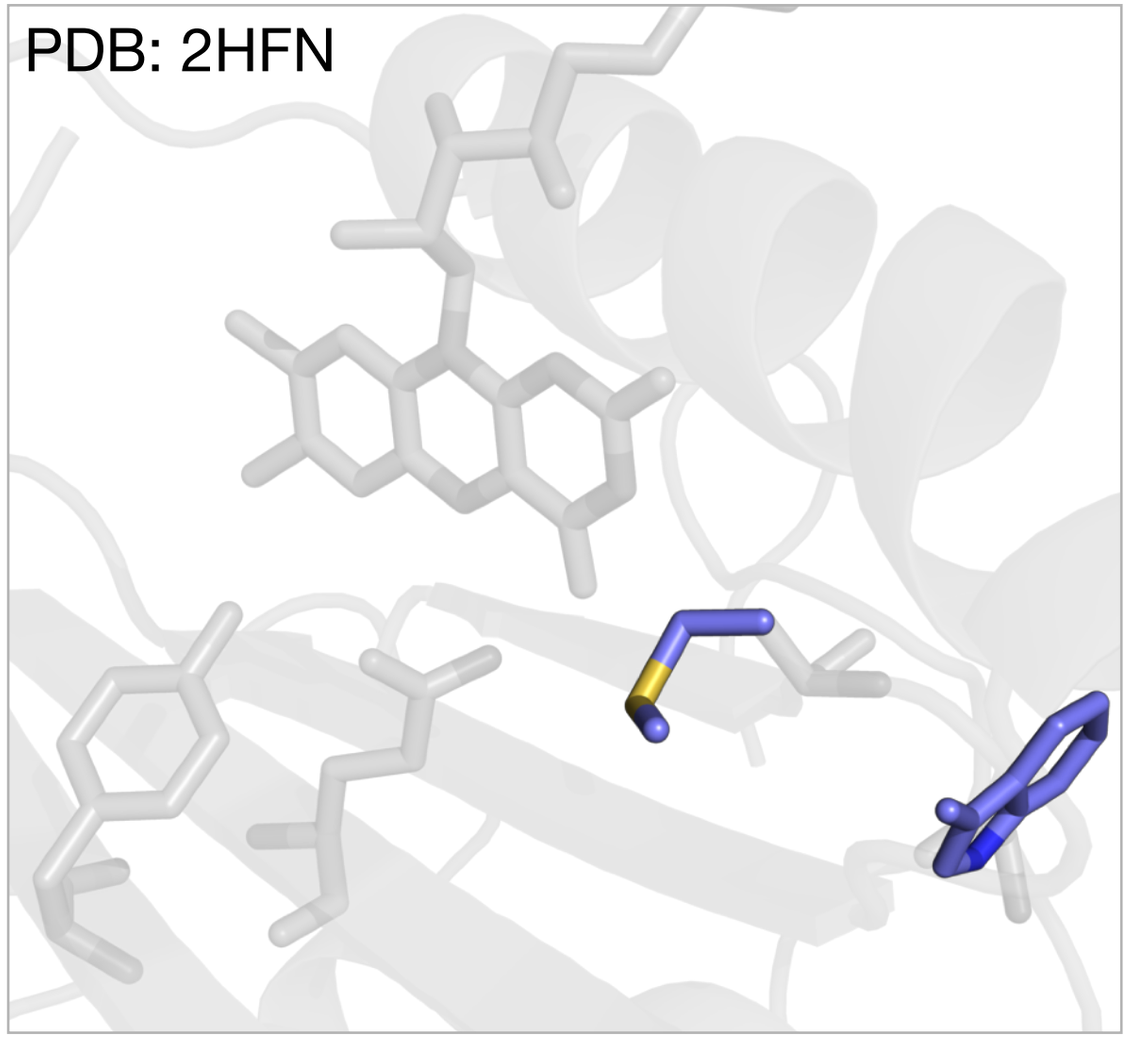
Crystal structure shows ambiguity

What is the preferred orientation of Trp91 (and Met93)?



chain D

“Trp_{in}”



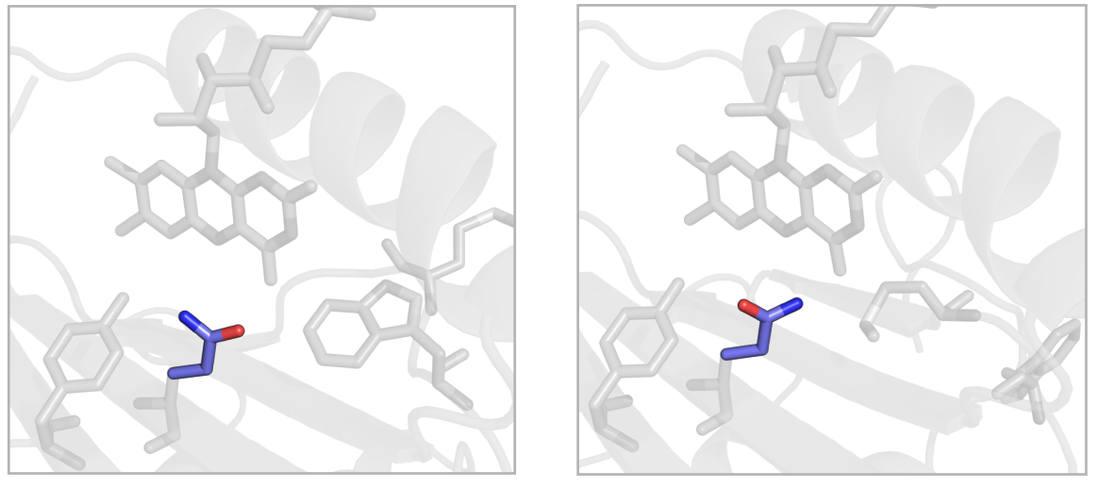
chain A

“Trp_{out}”

Crystal structure shows ambiguity

To understand the photocycle, we must understand the nature of the dark-adapted state.

We use **enhanced sampling molecular dynamics** methods to compute free energy profiles between conformations.



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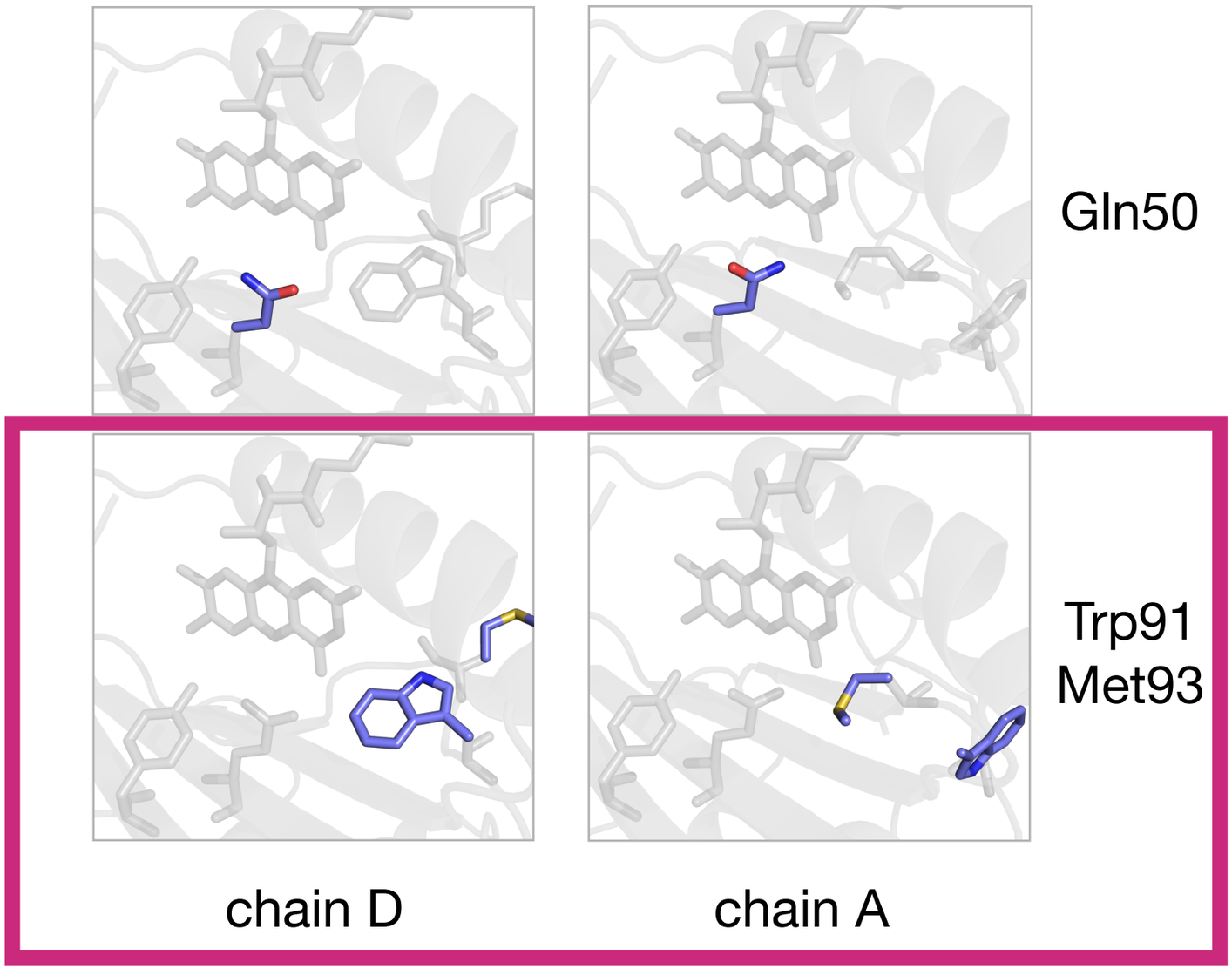
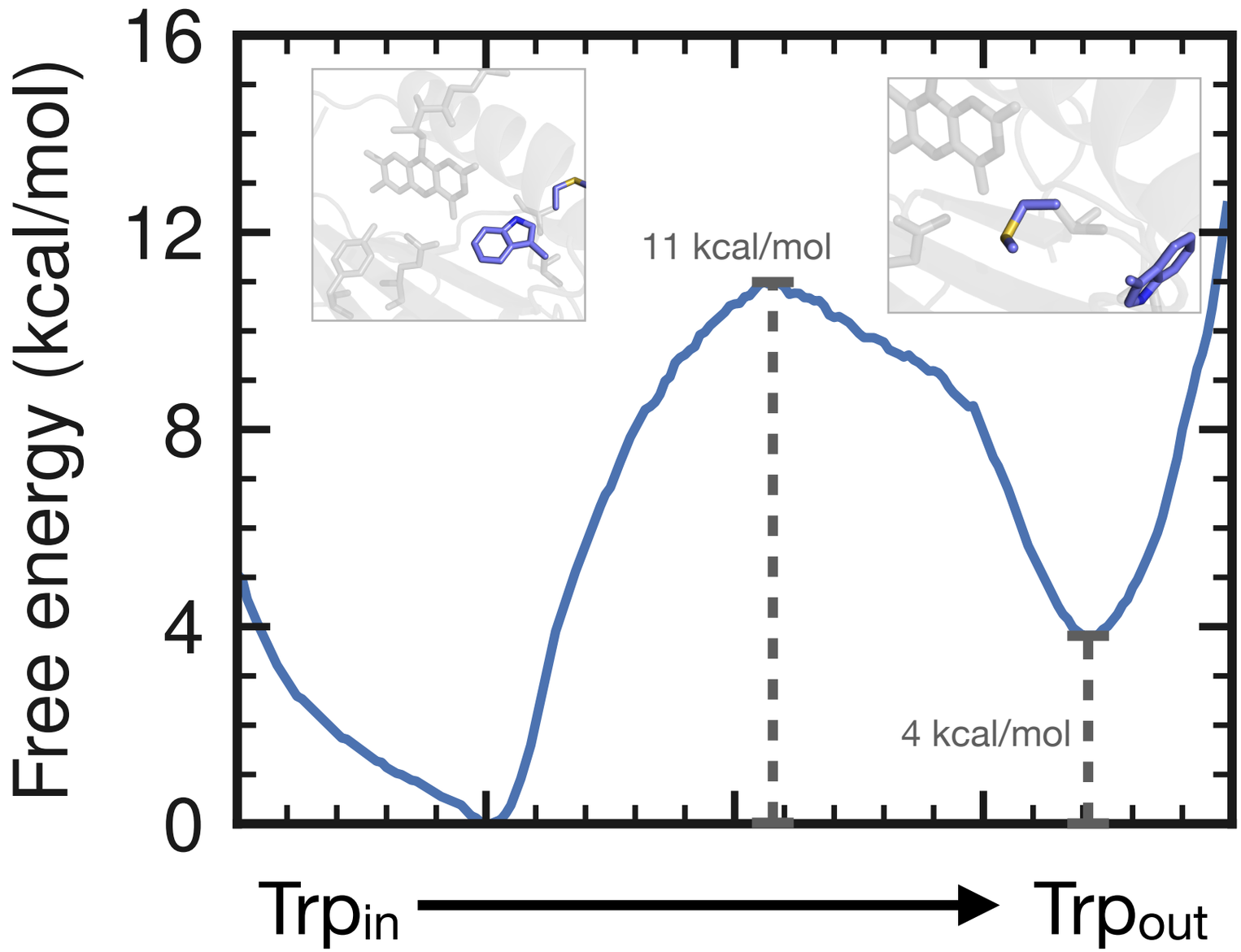


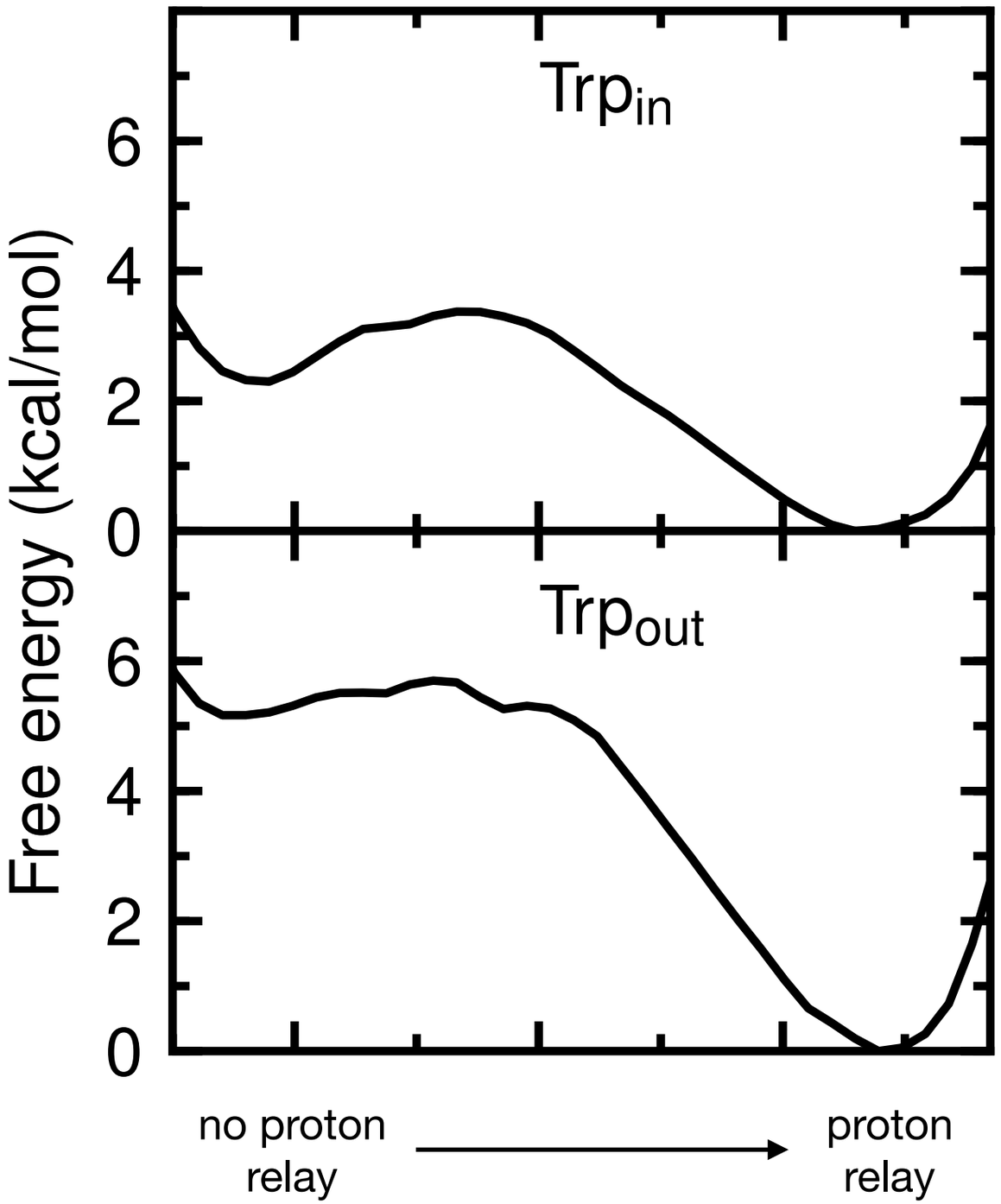
We will look at Trp/Met conformation first

Trp91 predominately in the active site

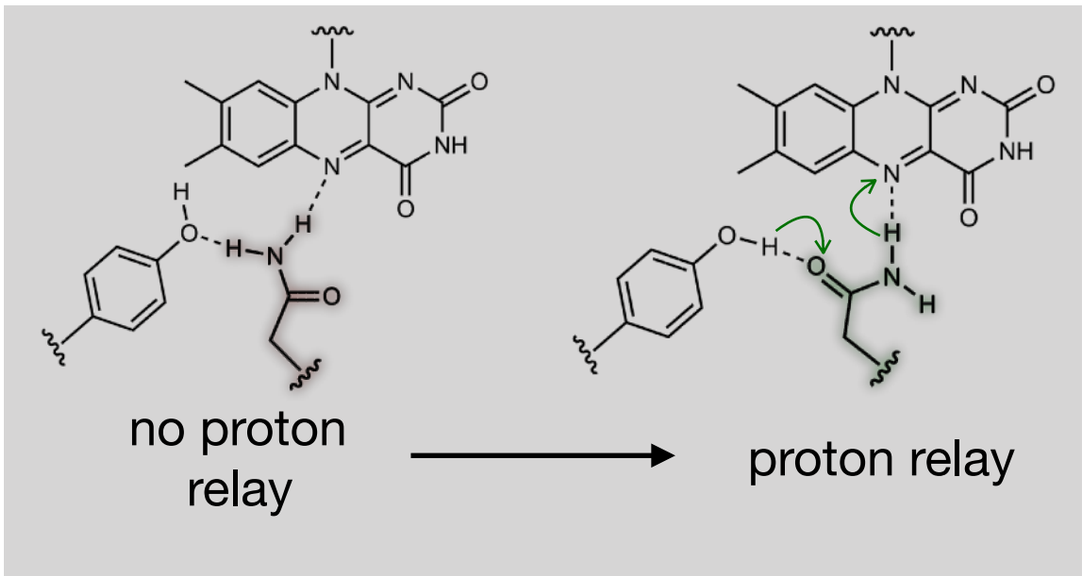


- Slr1694 can be in both Trp$_{in}$ and Trp$_{out}$ in crystal structures
- Adaptively-biased path optimization with CHARMM36 force field on S_0
- On S_0, BLUF can interconvert Trp$_{in}$ and Trp$_{out}$ on ~ 100 μs timescale
- Trp$_{in}$ favored by 4 kcal/mol

Gln50: proton relay favored in Slr1694



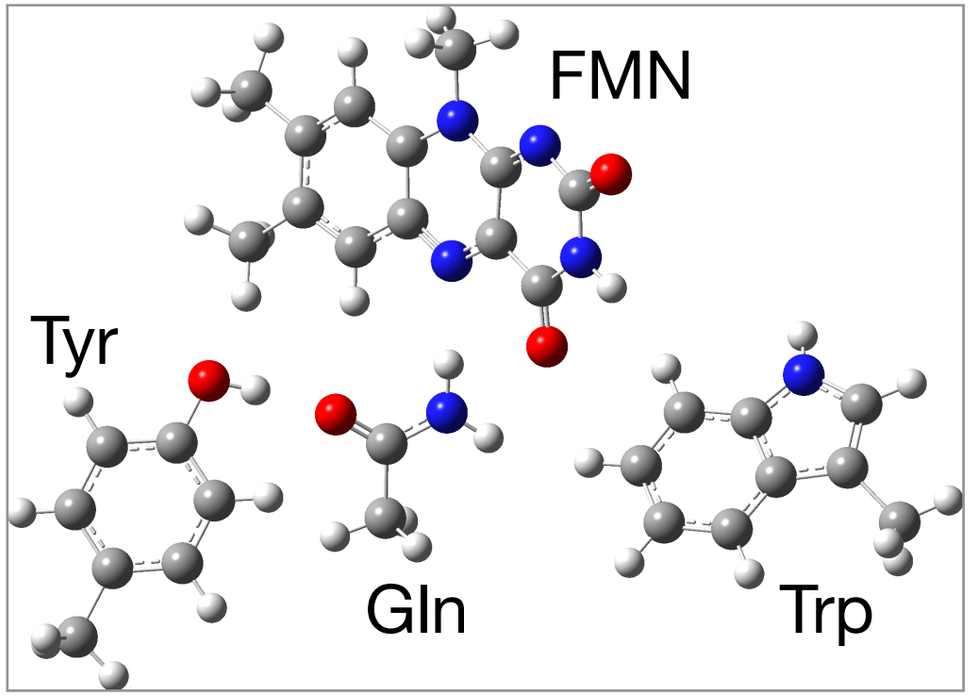
- Crystal structure does not provide unambiguous assignment of Gln orientation
- Umbrella sampling used to explore free energy changes along Gln rotation
- Proton relay always favored regardless of Trp$_{in}$ or Trp$_{out}$



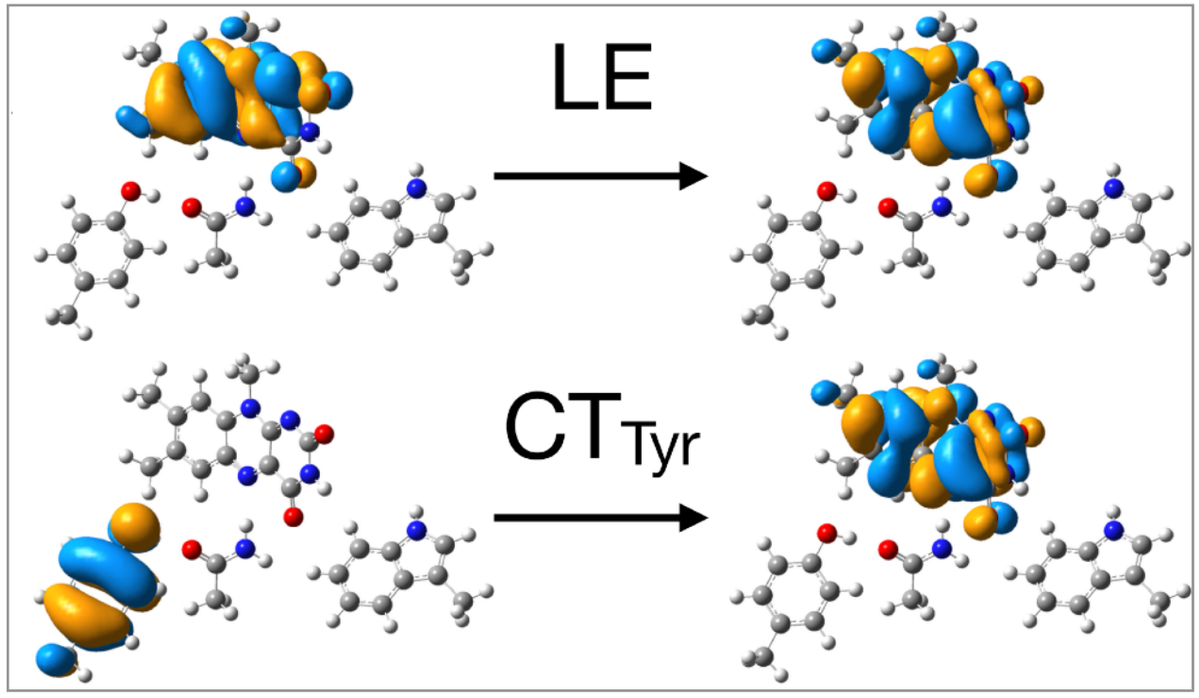
Conformational free energies are useful, but how do they impact excited states?

TDDFT of active site in protein environment

In photocycle, LE state must cross with CT state to drive electron transfer.
How do conformational changes impact the electronic energy level ordering?



QM active site model



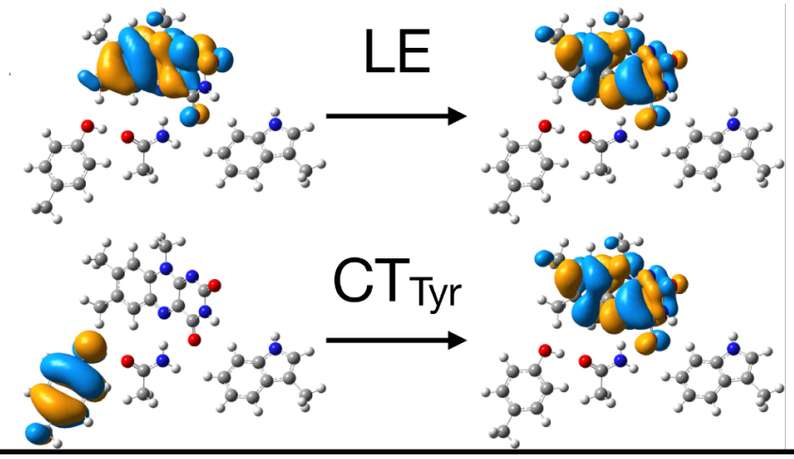
Natural transition orbitals for locally excited (LE) and charge transfer (CT) states

- Protein MM optimization after 20 ns NPT (300K, 1atm) molecular dynamics
- TDDFT/CAM-B3LYP/6-31+G** with electrostatic embedding of QM active site within protein and aqueous environment

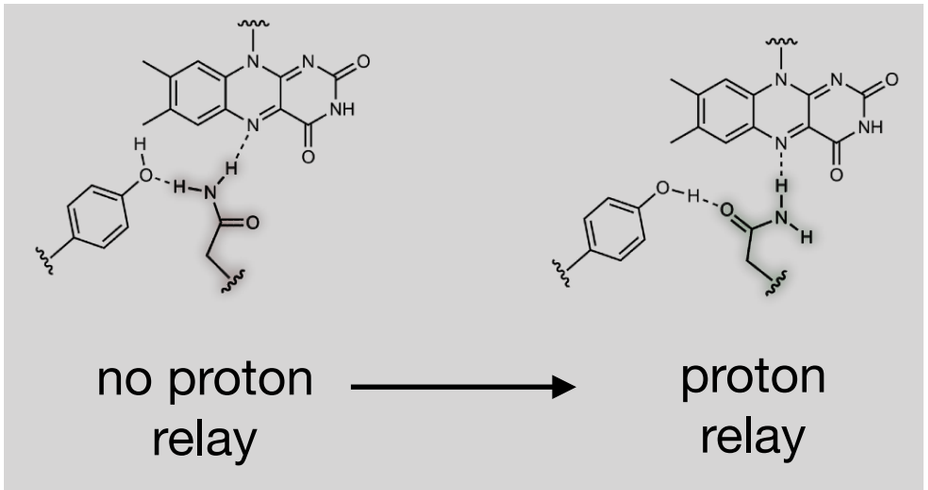
Proton relay facilitates charge transfer to flavin

Excitation energies (eV) of active site embedded in protein and aqueous environment obtained from ground state trajectories

	no proton relay	proton relay
LE	3.00	2.99
CT	4.42	3.68
CT−LE gap ΔE	1.42	0.69



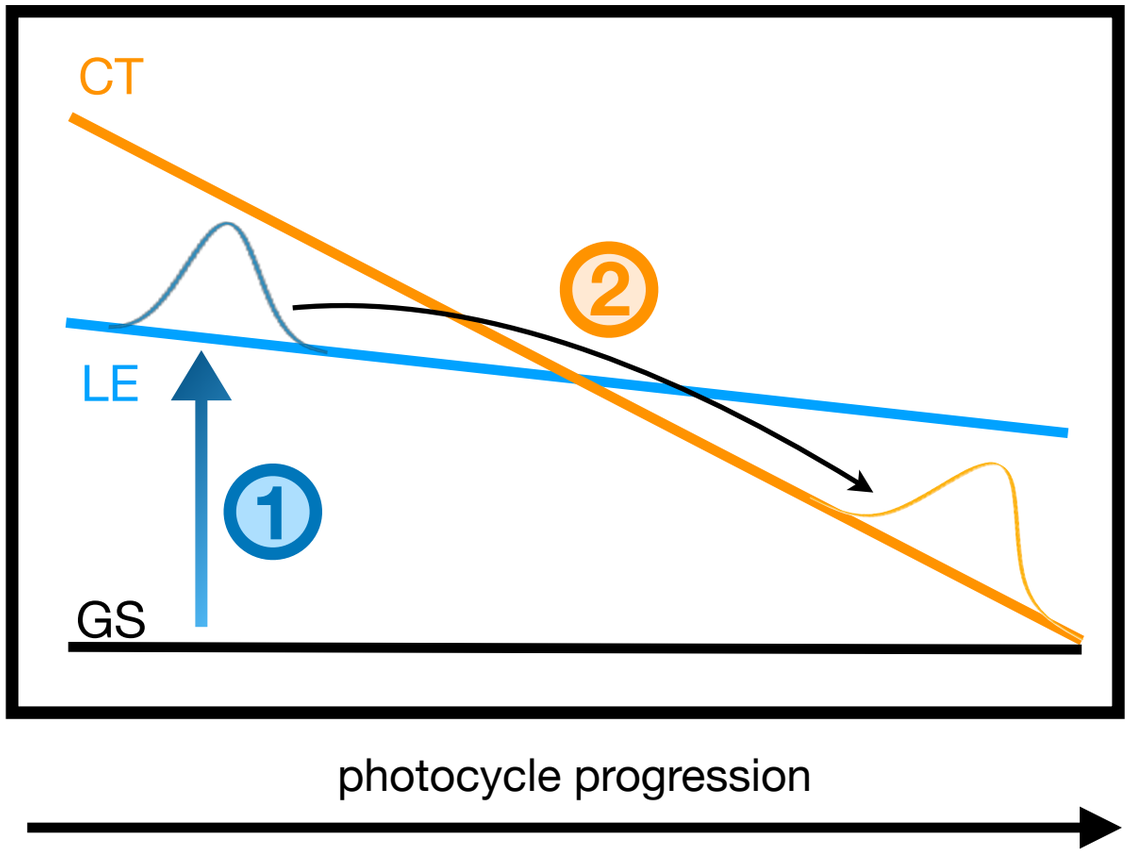
- Initial photoexcitation to LE state (only state with non-zero oscillator strength)
- Excitation energies for CT states depend strongly on active site Gln conformation
- LE states relatively insensitive to conformational changes

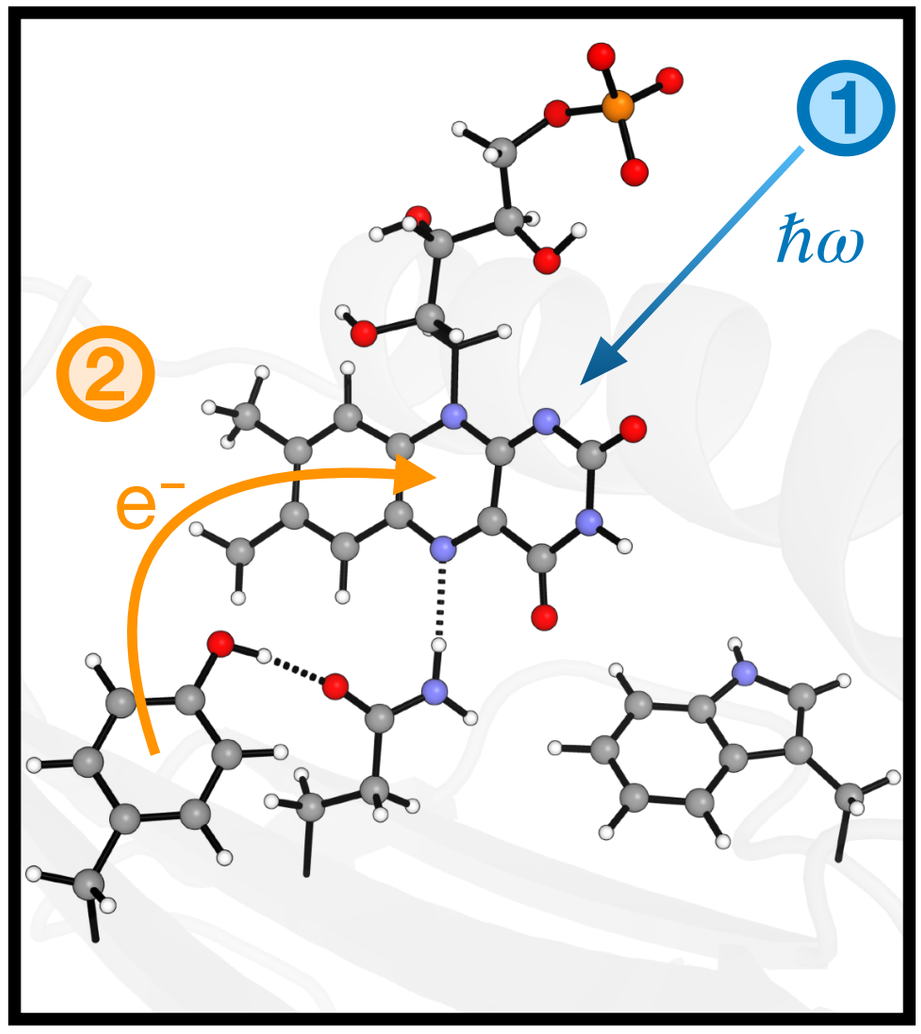


LE states still always lower energy than CT states — how do they cross?

Problem: CT states still above LE states

For the photocycle to progress, we must cross to the CT state — how?





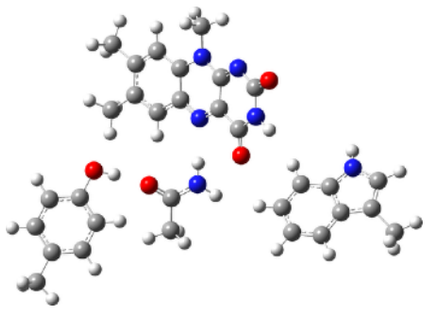
- Obviously a good question for nonadiabatic dynamics methods, but…
- Would be very interesting to find a BLUF conformation with the energy order swapped. Hint at possible mechanism?

	no proton relay	proton relay
LE	3.00	2.99
CT	4.42	3.68
CT−LE gap ΔE	1.42	0.69

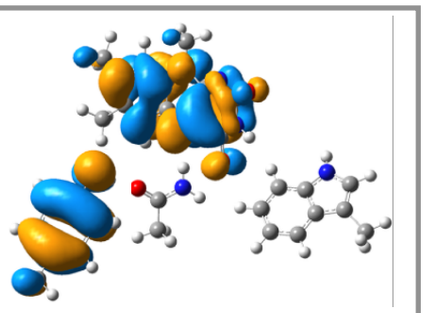
(Excitation energies in eV)

Finding conformations that stabilize CT

QM active site cluster model



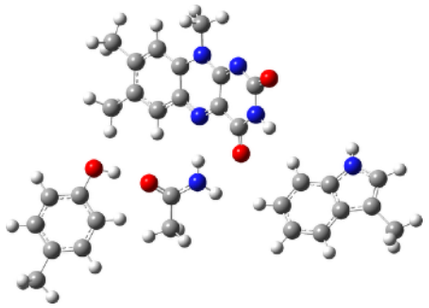
(1)



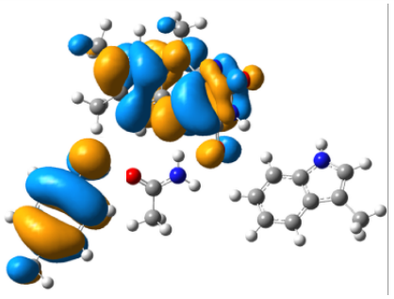
gas-phase TDDFT

Finding conformations that stabilize CT

QM active site cluster model



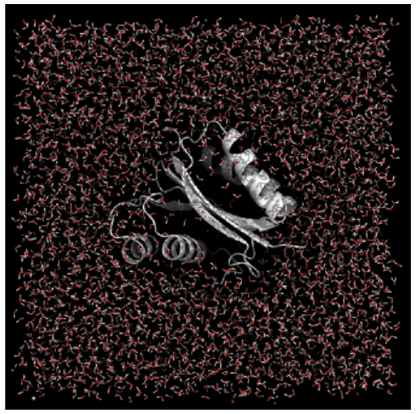
(1)



gas-phase TDDFT

(2)

modify classical force field with new partial charges

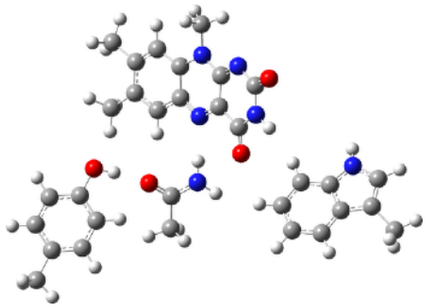


run classical MD with modified charges

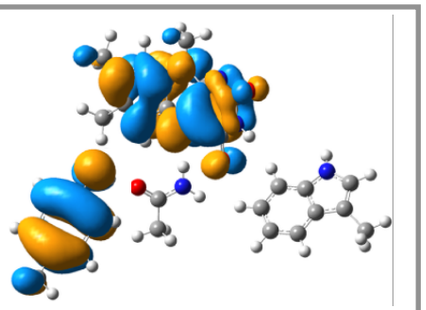
(3)

Finding conformations that stabilize CT

QM active site cluster model



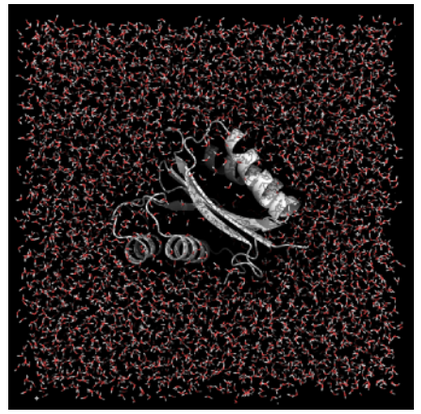
(1)



gas-phase TDDFT

(2)

modify classical force field with new partial charges

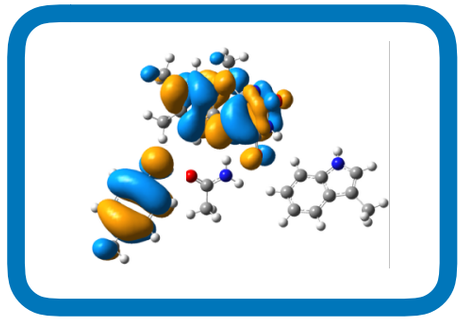


run classical MD with modified charges

(3)

with new conformation in hand, re-create QM/MM model

(4)



embedded TDDFT

Note any modified classical charges now in QM region, so any changes due to conformation alone

(5) **Analyze if/how energy levels shift due to new geometry**

Protein reorganization can induce charge transfer

Use protein environments equilibrated on CT states to find conformations that stabilize the CT state.

Excitation energies (eV) of active site embedded in protein and aqueous environment obtained from ground and excited state trajectories

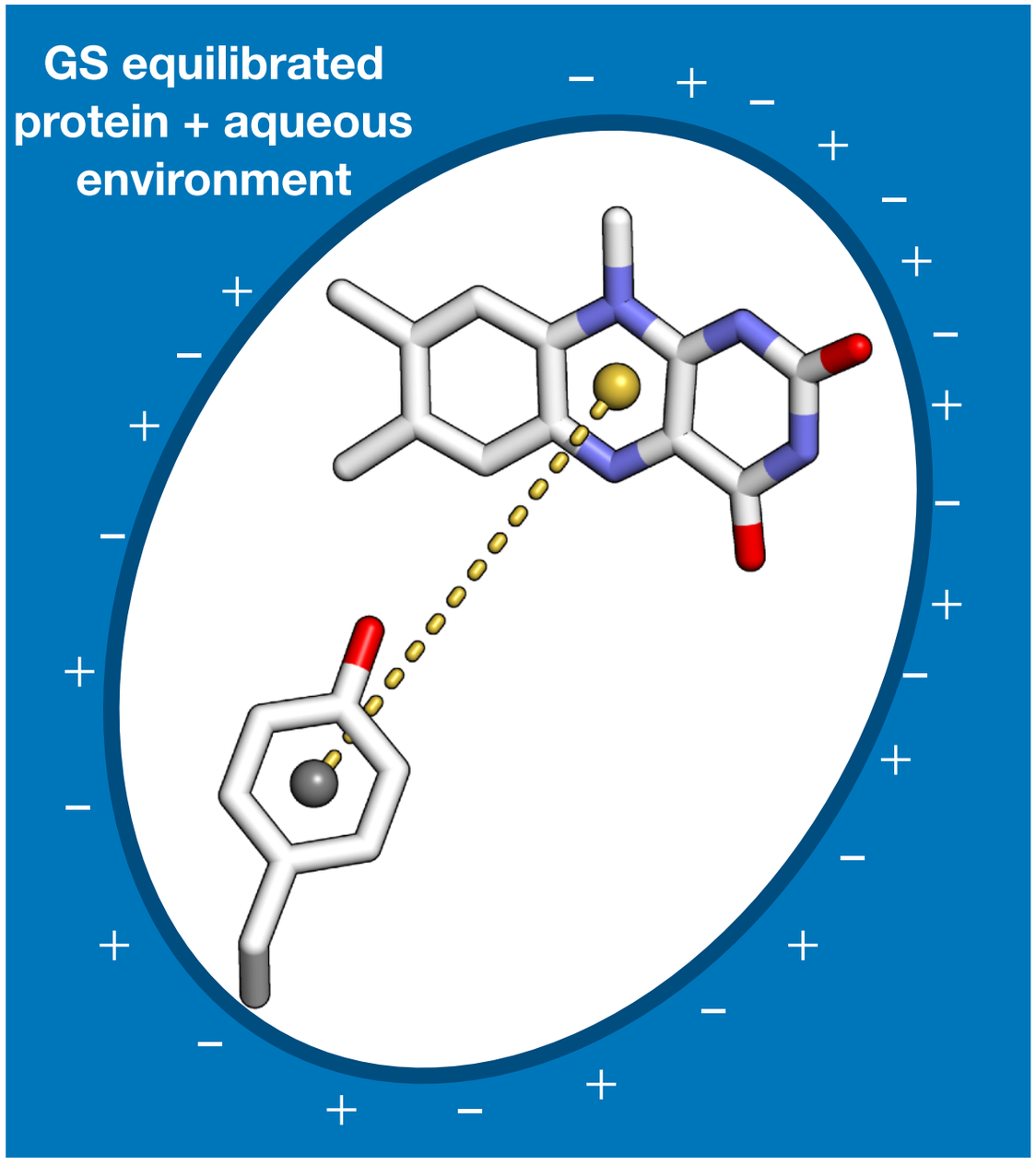
	GS conf.	LE conf.	CT conf.
LE	2.99	2.95	2.87
CT	3.68	3.77	1.71
CT−LE gap ΔE	0.69	0.82	-1.16

- As expected, the conformation obtained from the CT state trajectory appears to markedly stabilize CT state. We can replicate for other independent conformations.
- CT stabilization goes away if we exclude electrostatic environment (e.g. gas phase)

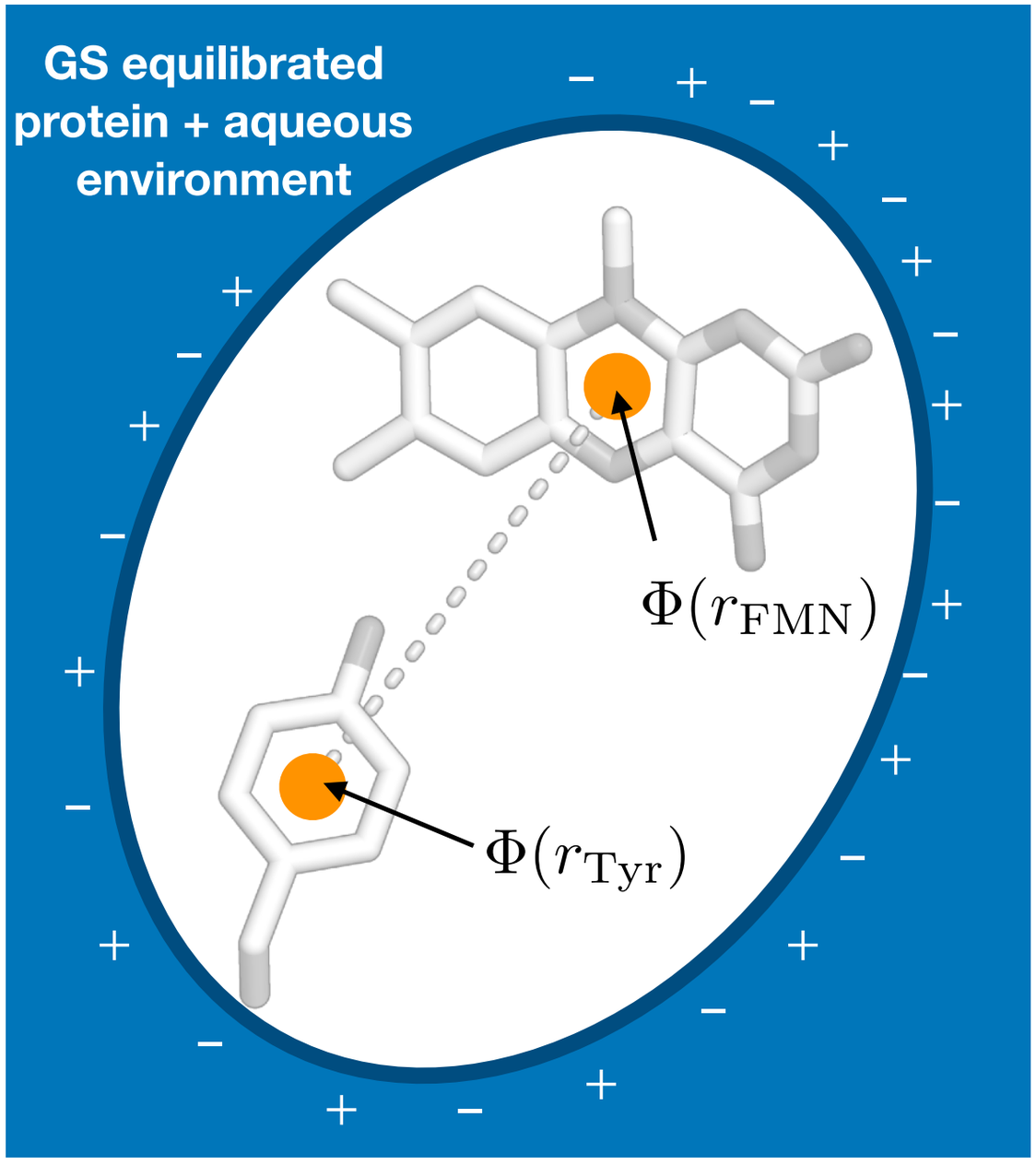
What conformational change is leading to the stabilized CT state?

Protein reorganization can induce charge transfer

How does protein environment stabilize CT?



Protein reorganization can induce charge transfer

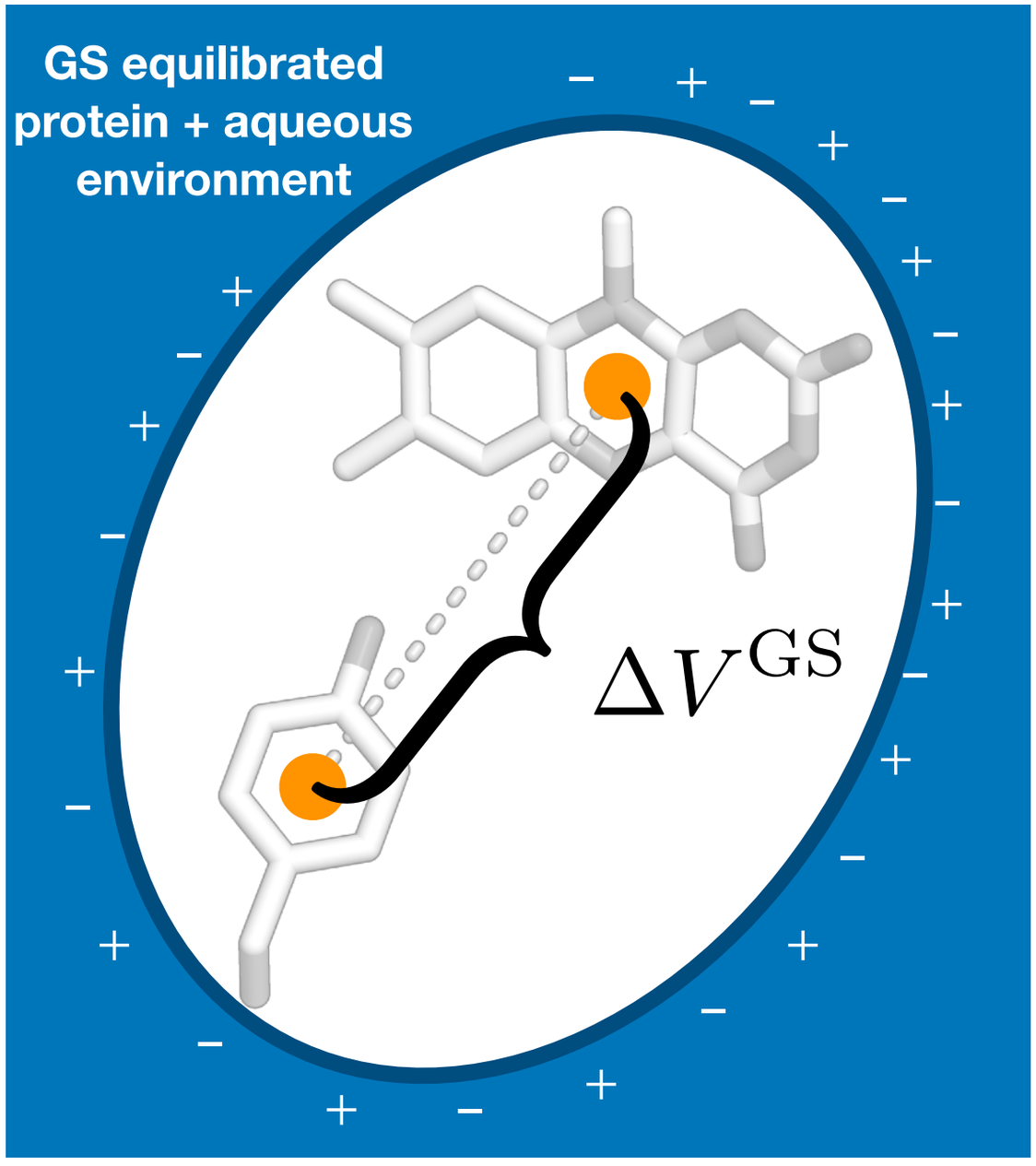


How does protein environment stabilize CT?

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

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

Protein reorganization can induce charge transfer



How does protein environment stabilize CT?

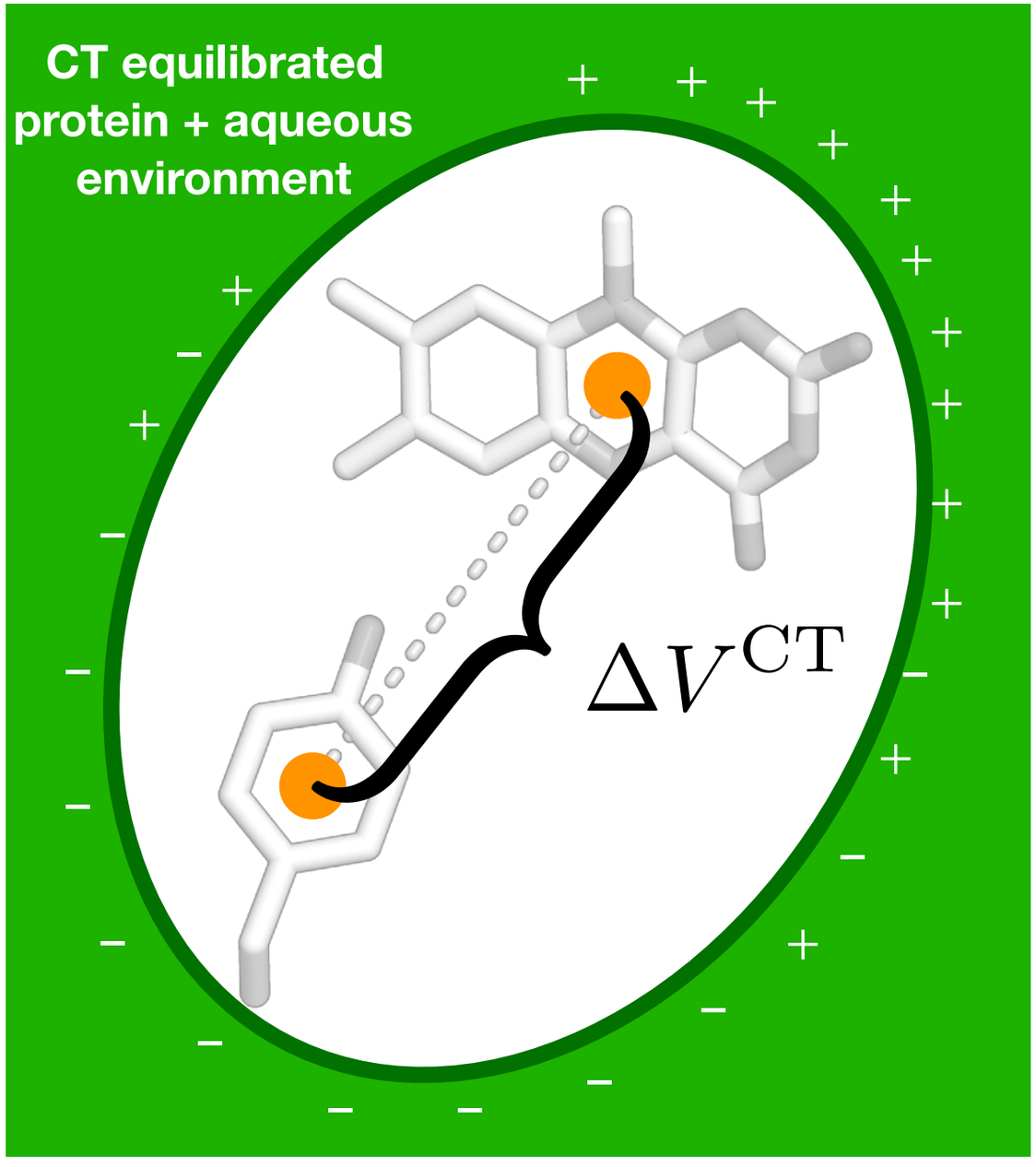
1. Measure electrostatic potential due to environment at Tyr and FMN

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

2. Compute potential difference between FMN and Tyr

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

Protein reorganization can induce charge transfer



How does protein environment stabilize CT?

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

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

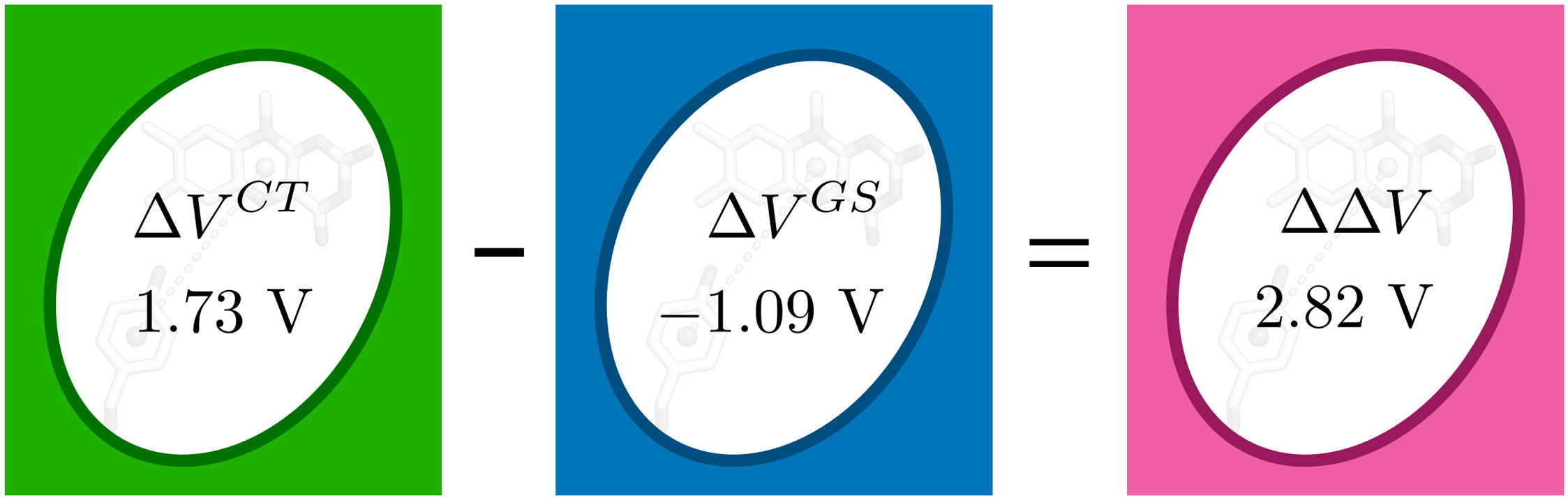
2. Compute potential difference between FMN and Tyr

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

3. Perform for both CT and GS. The difference tells how much CT environment stabilizes CT state over GS environment.

$$\Delta\Delta V = \Delta V^{\mathrm{CT}} - \Delta V^{\mathrm{GS}}$$

Protein reorganization can induce charge transfer



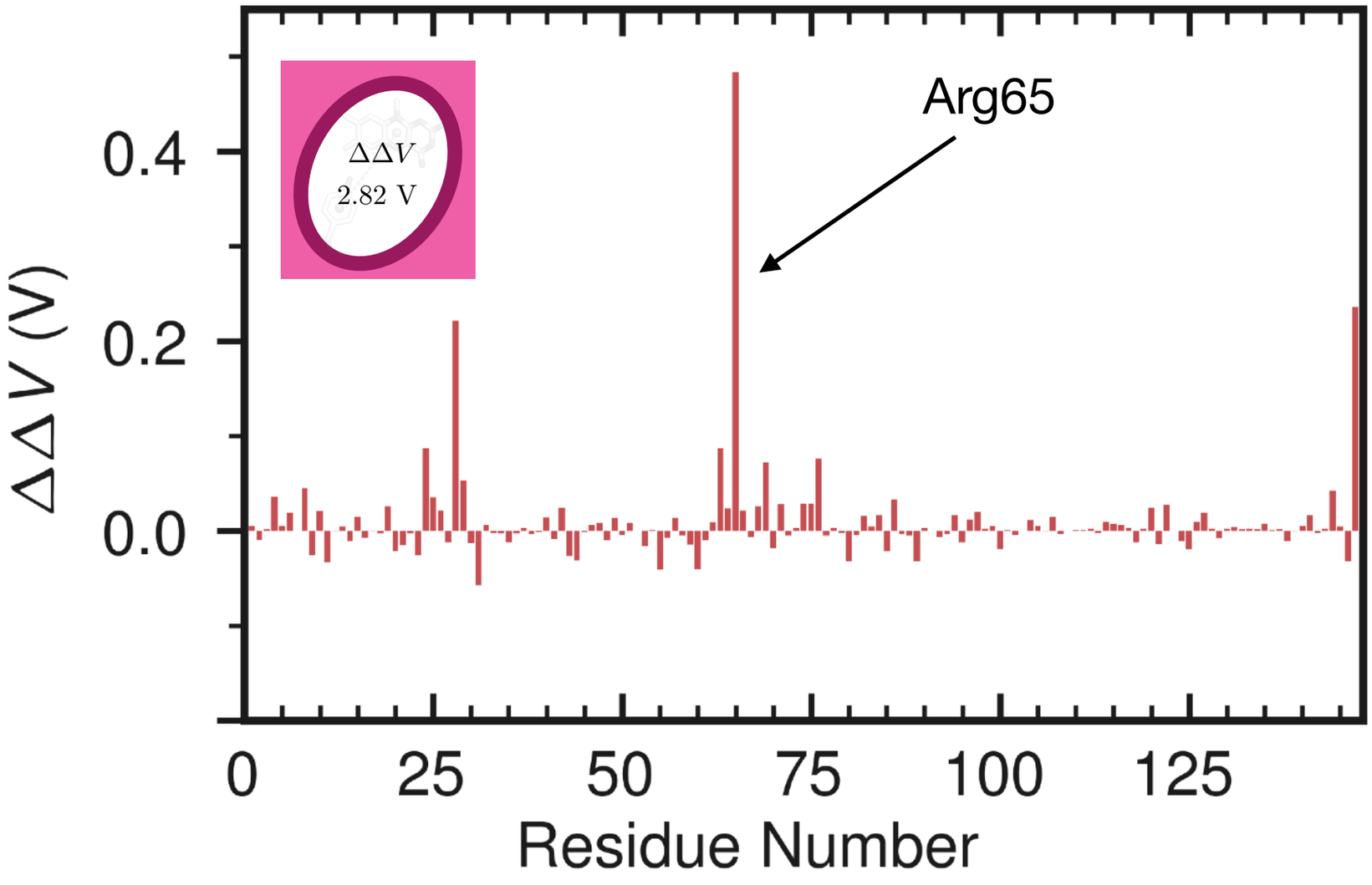
CT favorable environment

CT unfavorable environment

net CT favorable environment change

Many ways the protein can reorganize. What is going on in this case?

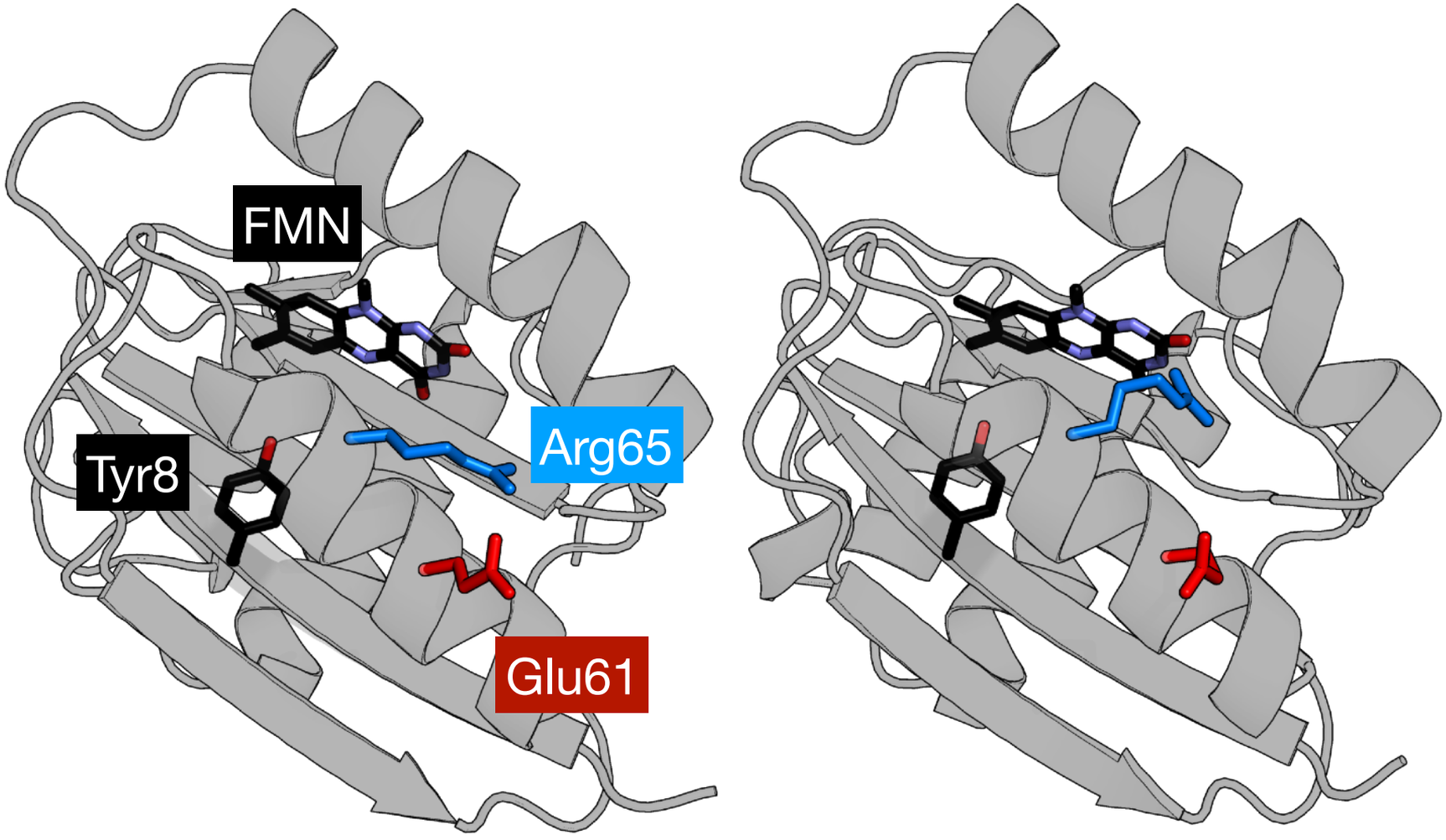
Individual residue contribution to CT stabilization



Potential difference is additive, so we can decompose into contributions by individual residue

Here we will look at the contribution by Arg65

Arg65 contribution to CT stabilization



GS conformation CT conformation

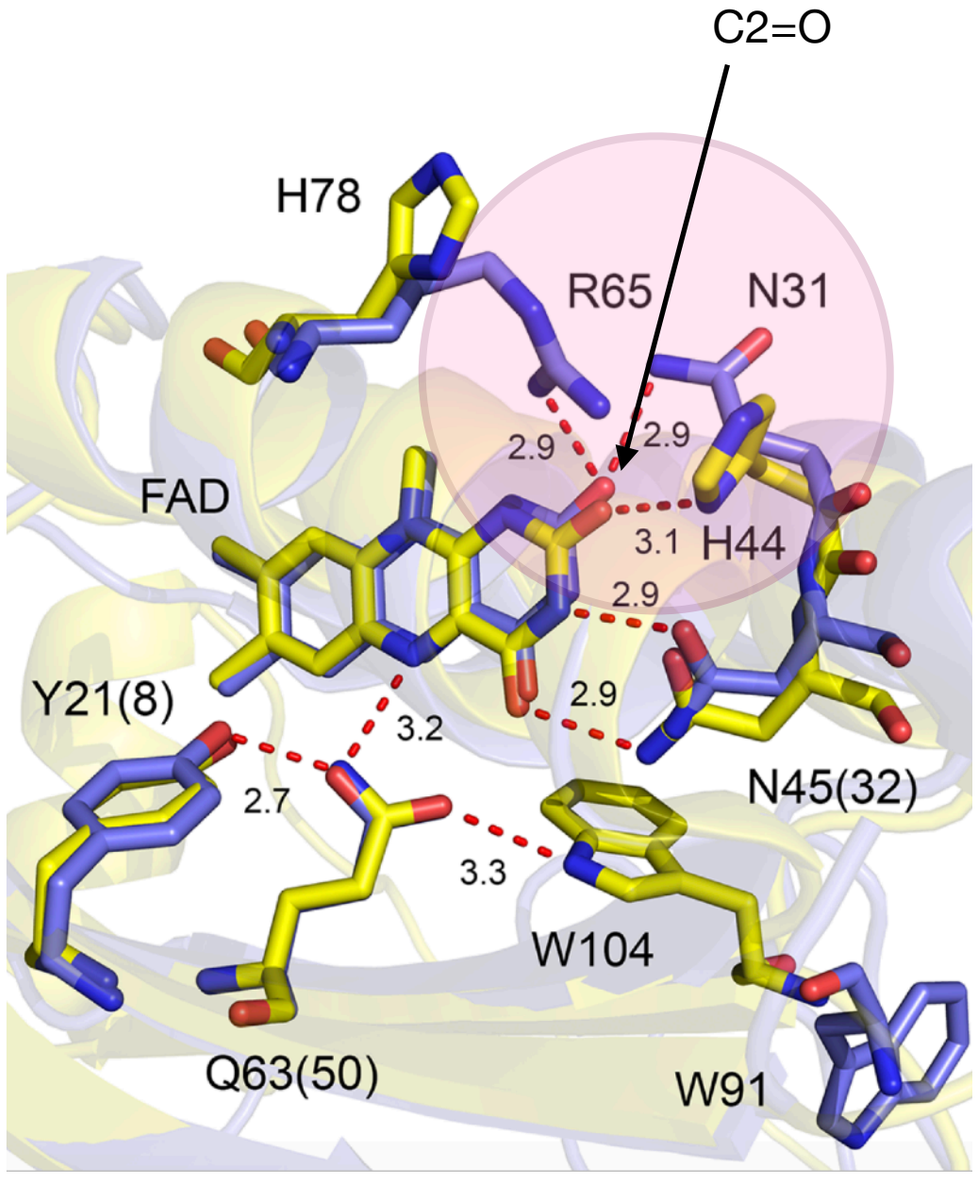
Arg65 (positively charged) swings toward flavin,
stabilizing potential negative charge buildup on the flavin

Interesting connection to experiment

Gil, *et al.* point out that BLUF proteins with two hydrogen bonds to C2=O correlate with radical formation.

We see Arg65 facilitating charge transfer to form flavin radical intermediates

Pursuing the impact of changes in the flavin binding pocket may be a fruitful line of research



AppA (yellow) and Slr1694 (blue) crystal structures

Gil, A. A. et al. Photoactivation of the BLUF Protein PixD Probed by the Site-Specific Incorporation of Fluorotyrosine Residues. J. Am. Chem. Soc. 2017, 139 (41), 14638–14648.

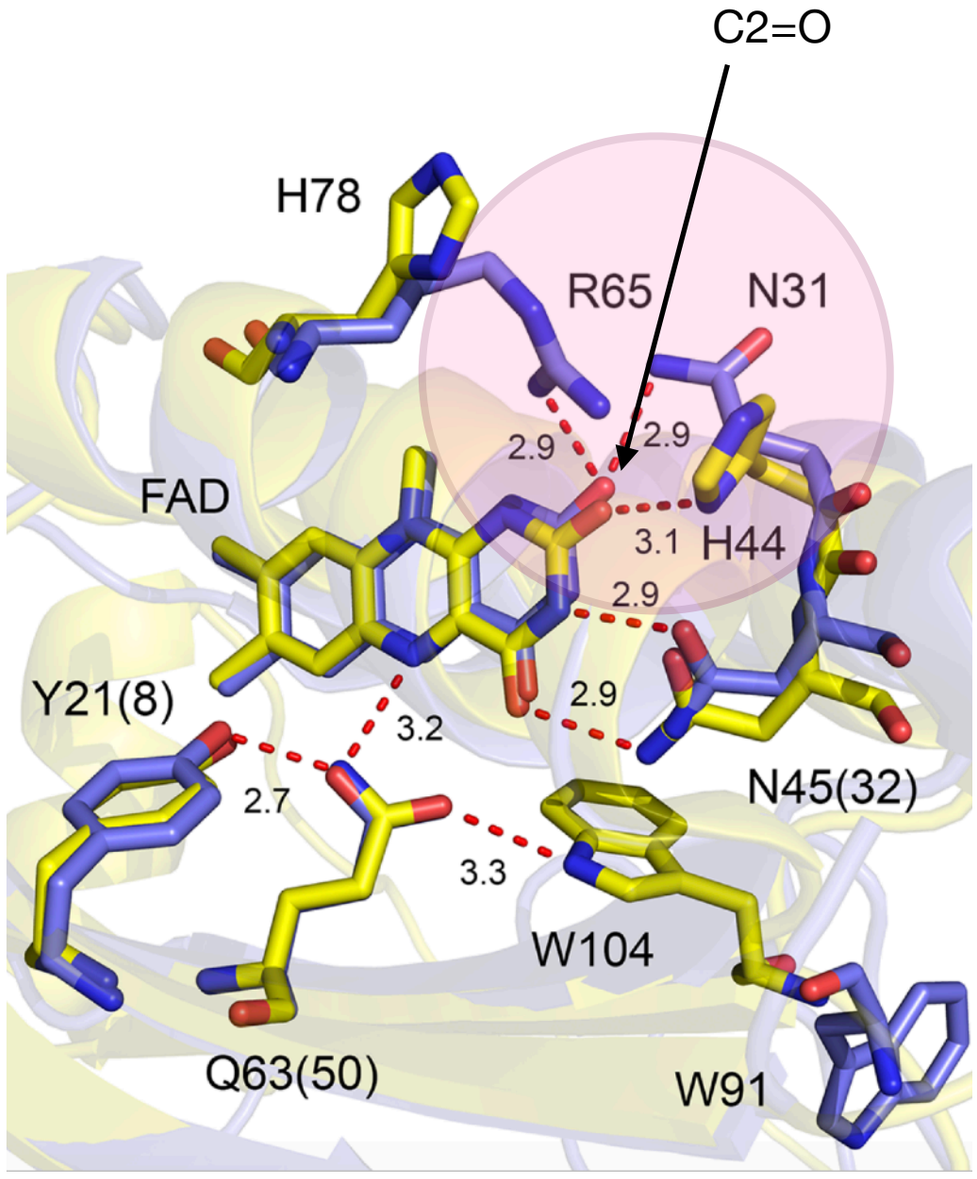
Interesting connection to experiment

Gil, *et al.* point out that BLUF proteins with two hydrogen bonds to C2=O correlate with radical formation.

We see Arg65 facilitating charge transfer to form flavin radical intermediates

Pursuing the impact of changes in the flavin binding pocket may be a fruitful line of research

BLUF photocycle dynamics are strongly influenced by changes in the protein environment.



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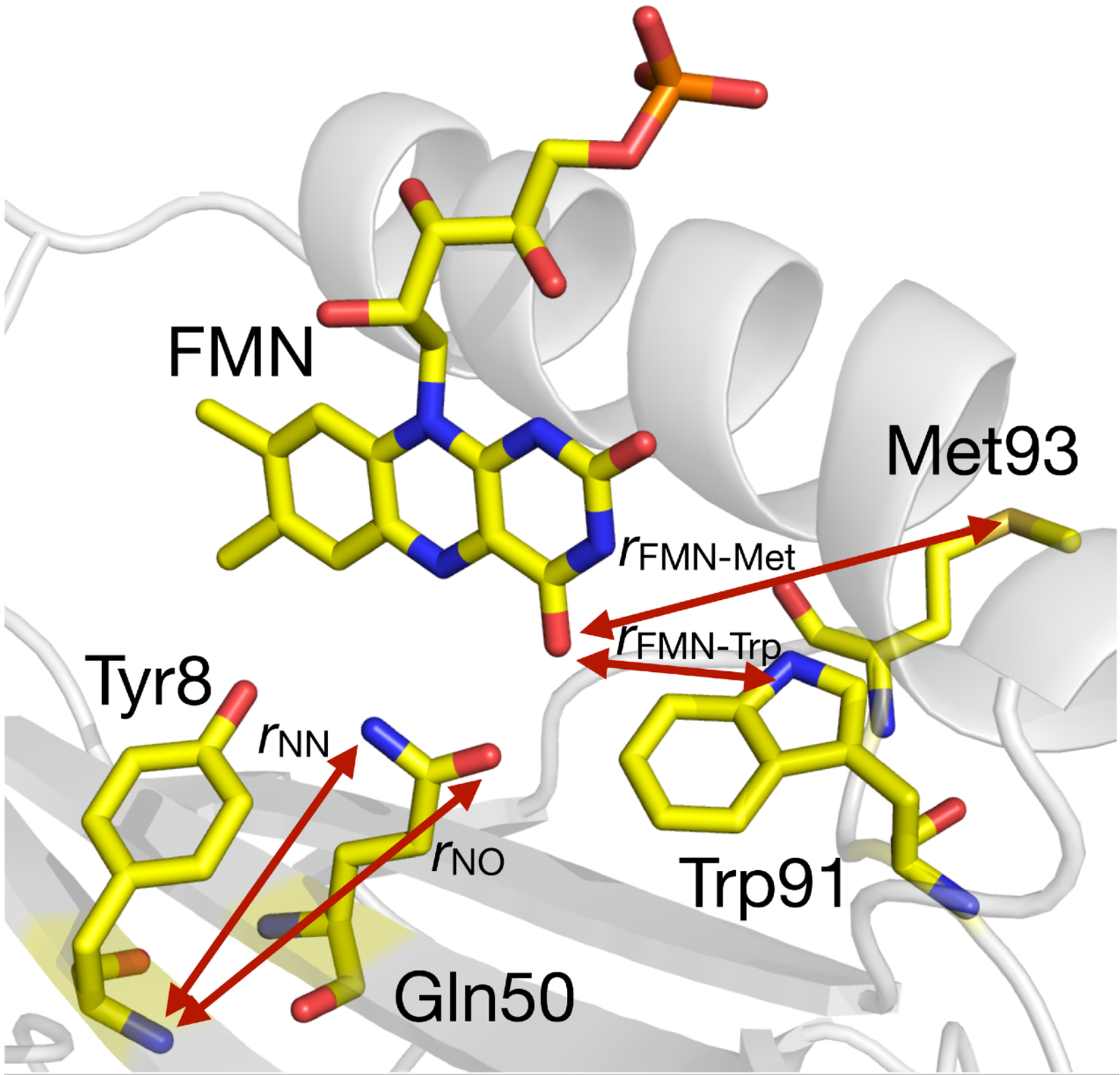
Thank you!

Acknowledgments

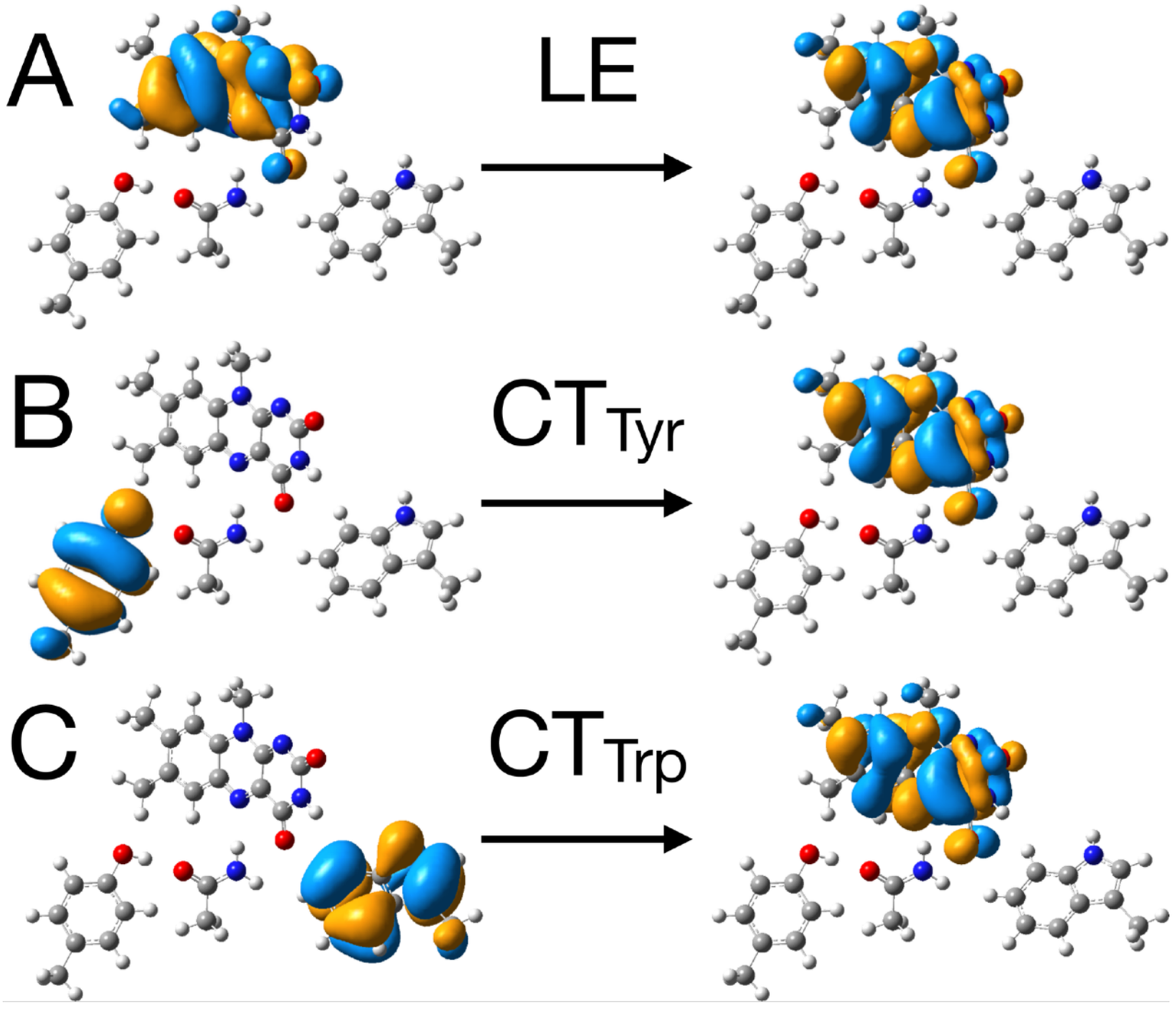
- Prof. Sharon Hammes-Schiffer
- Clorice Reinhardt
- Alexander Soudakov
- Puja Goyal
- Archit Vasan

BLUE WATERS

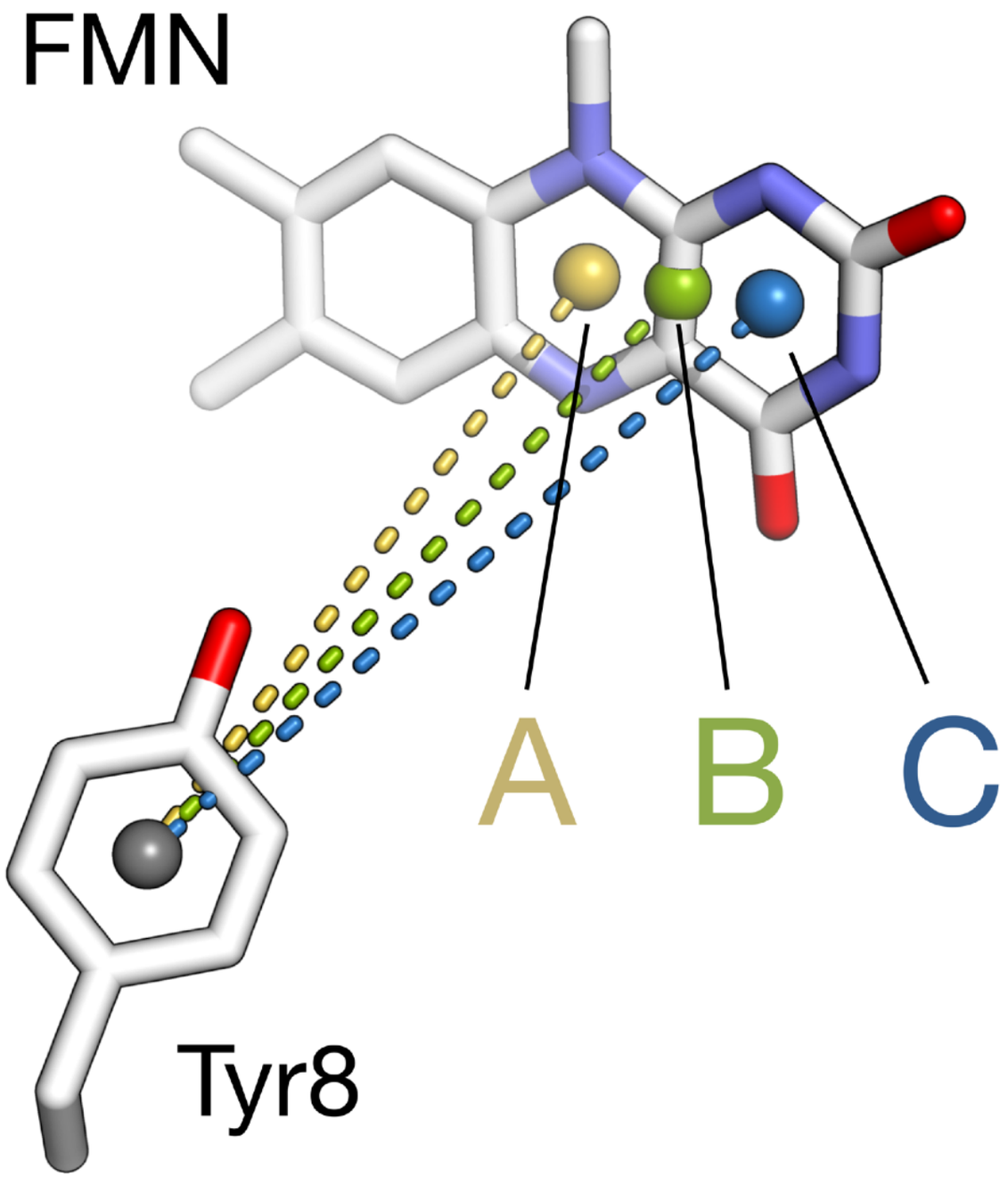
Free-energy reaction coordinates



Natural transition orbitals



Different definitions of $\Delta\Delta V$



Yields nearly identical results, regardless of location

Proton relay facilitates charge transfer to flavin

Table 1. Excitation Energies in eV from TDDFT/CAM-B3LYP/6-31+G** Calculations of Active Site of Slr1694 including Protein and Aqueous Environment Obtained from Ground State Trajectories[a,b]

	$Trp_{in}(NH_{in})$		$Trp_{in}(NH_{out})$	
	no proton relay	proton relay	no proton relay	proton relay
LE	3.05 (2.99)	2.98 (2.95)	3.00 (3.00)	2.99 (2.93)
CT_{Tyr}	4.09 (3.95)	3.25 (3.36)	4.42 (4.25)	3.68 (3.17)
CT_{Trp}	3.52 (2.15)	3.43 (3.25)	3.46 (2.80)	3.58 (3.17)
CT_{Tyr}-LE gap ΔE	1.04 (0.96)	0.27 (0.41)	1.42 (1.25)	0.69 (0.24)
CT_{Trp}-LE gap ΔE	0.47 (-0.84)	0.45 (0.30)	0.46 (-0.20)	0.59 (0.24)

[a] The active site is shown in Figure 5, and the protein and aqueous environment was included with electrostatic embedding. The gas phase results are given in parentheses. The conformations were obtained from MD trajectories propagated in the GS, followed by energy minimization.

Protein reorganization can induce charge transfer

Table 2. Excitation Energies in eV from TDDFT/CAM-B3LYP/6-31+G** Calculations of Active Site of Slr1694 including Protein and Aqueous Environment Obtained from Excited State Trajectories[a, b]

	$Trp_{in}(NH_{in})$ / proton relay		$Trp_{in}(NH_{out})$ / proton relay	
	LE conf.	CT conf.	LE conf.	CT conf.
LE	2.90 (2.94)	2.89 (2.92)	2.95 (2.93)	2.87 (2.91)
CT_{Tyr}	3.33 (3.31)	1.71 (3.10)	3.77 (3.31)	1.71 (3.15)
CT_{Trp}	3.64 (3.35)	3.16 (3.17)	3.83 (3.48)	2.15 (3.17)
CT_{Tyr}-LE gap ΔE	0.43 (0.37)	-1.18 (0.18)	0.82 (0.38)	-1.16 (0.24)
CT_{Trp}-LE gap ΔE	0.74 (0.41)	0.27 (0.25)	0.88 (0.26)	-0.72 (0.26)

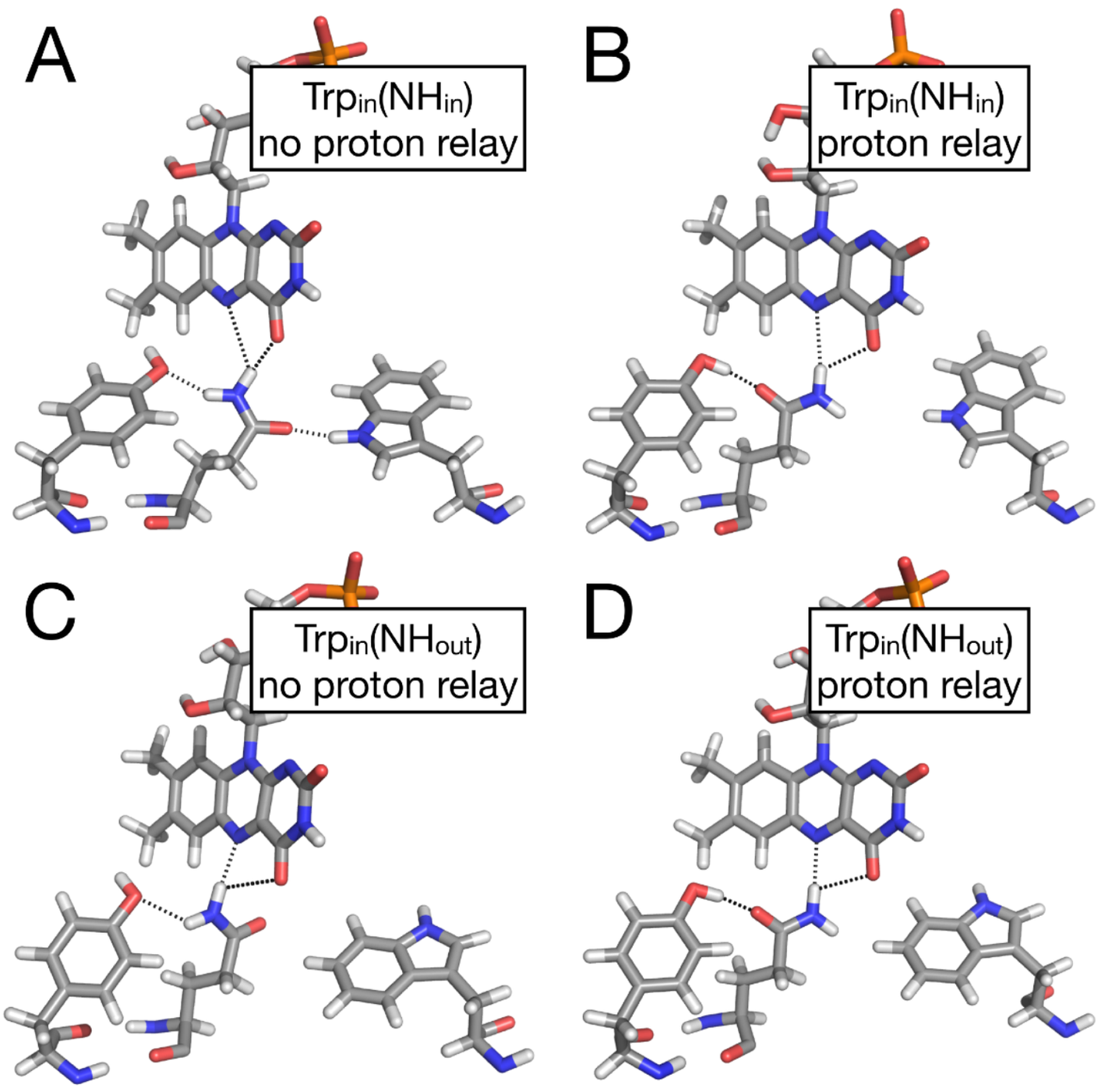
[a] The active site is shown in Figure 5, and the protein and aqueous environment was included with electrostatic embedding. The gas phase results are given in parentheses. The conformations (conf.) were obtained from MD trajectories in the LE and CT_{Tyr} state, followed by energy minimization.

Potential differences for both Slr1694 conformations

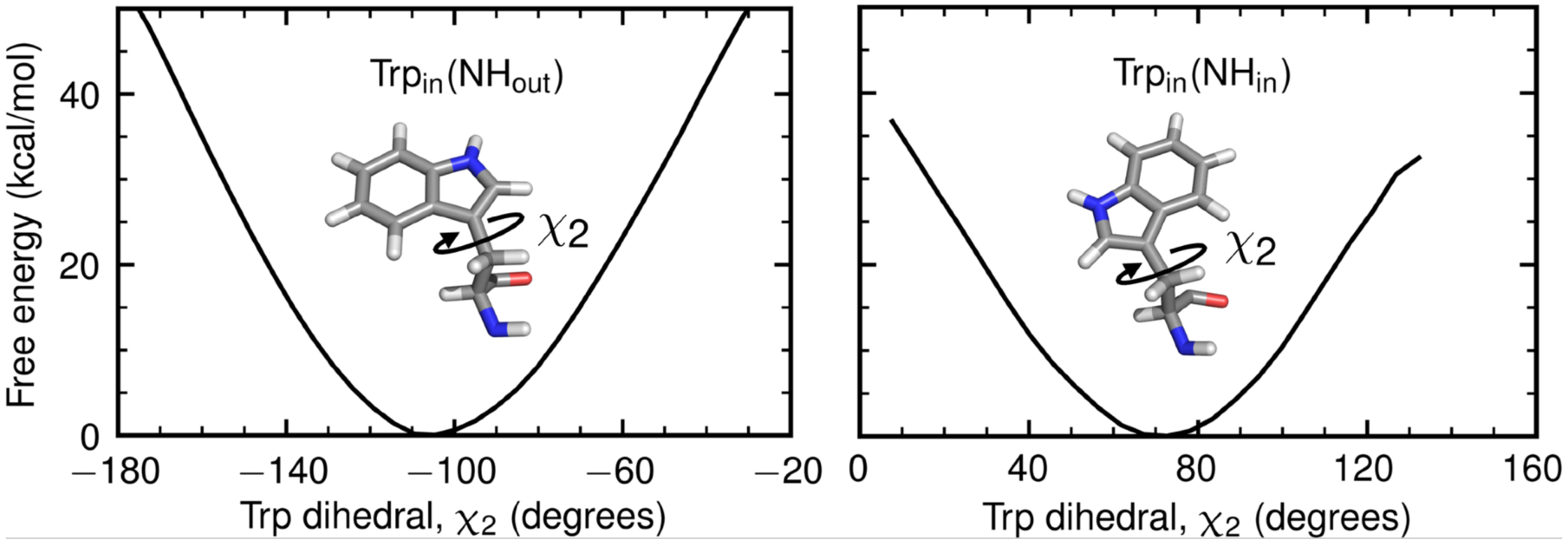
Table 3. Electrostatic Potential Differences in Volts Between Tyr and FMN Due to Electrostatic Environment

	$Trp_{in}(NH_{in})$	$Trp_{in}(NH_{out})$
ΔV^{GS}	0.01	−1.09
ΔV^{CT}	1.63	1.73
$\Delta\Delta V$	1.62	2.82

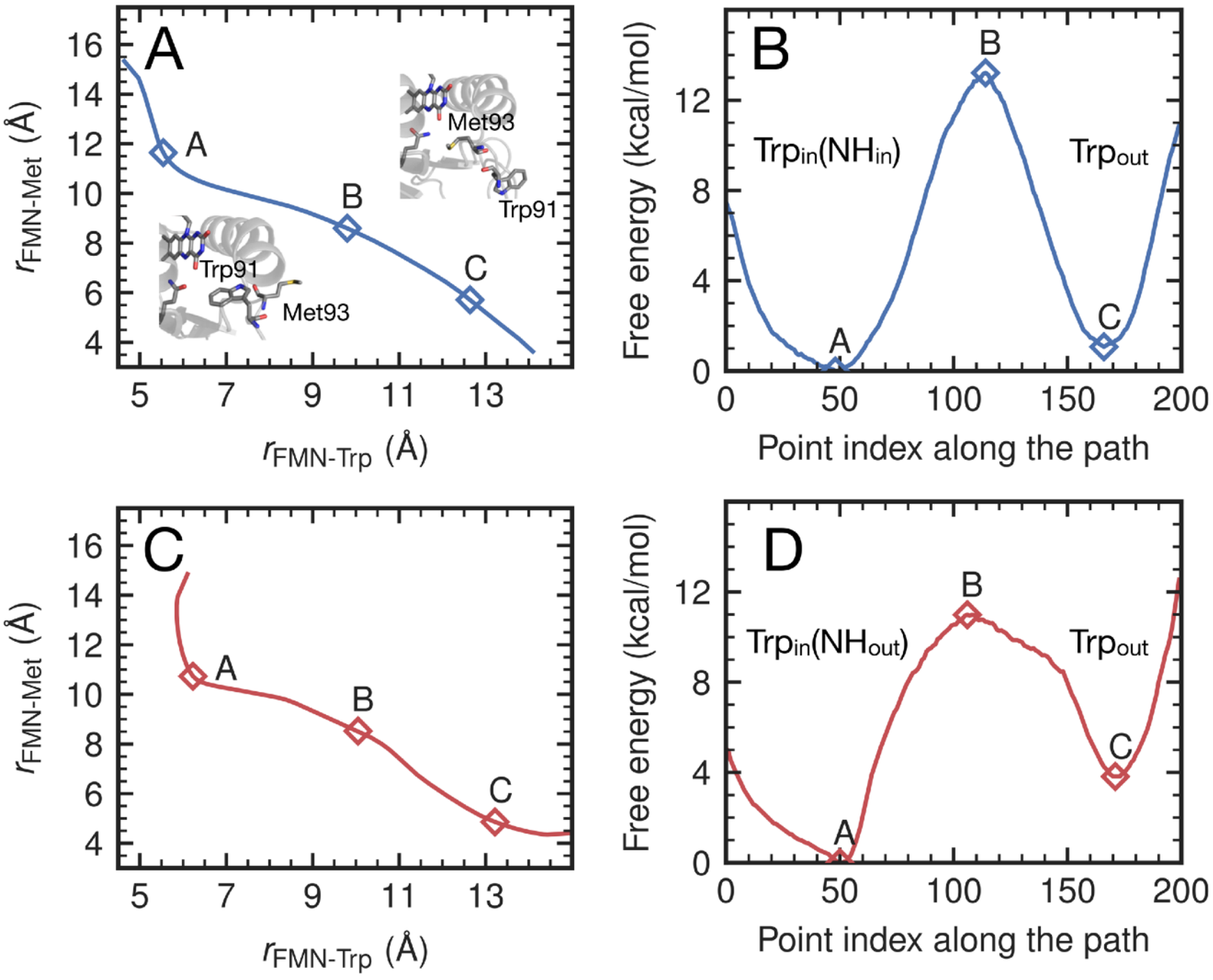
Slr1694 active site conformations



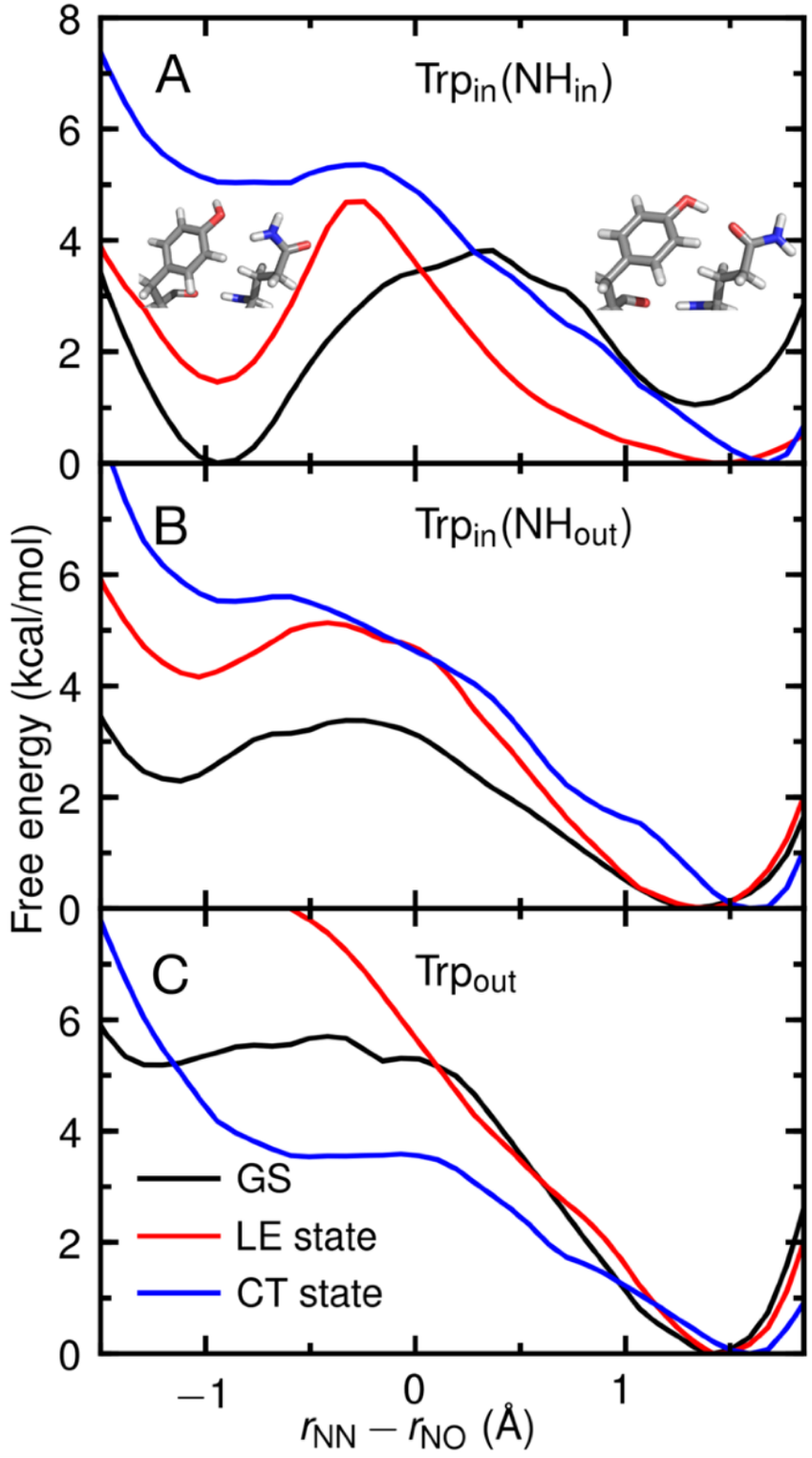
Trp_{in} freedom to rotate internally



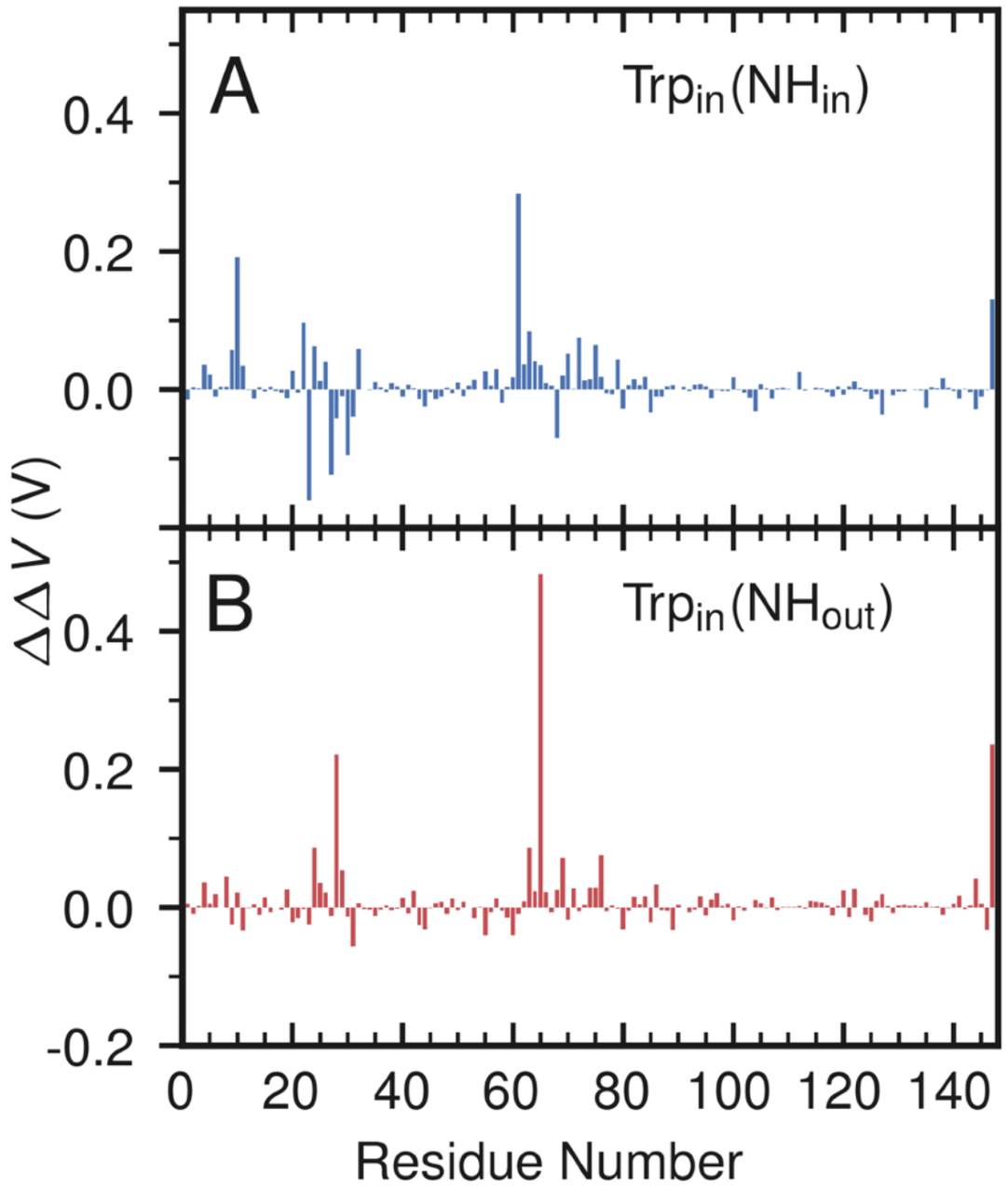
Trp/Met ABPO for two Slr1694 Trp_{in} conformers



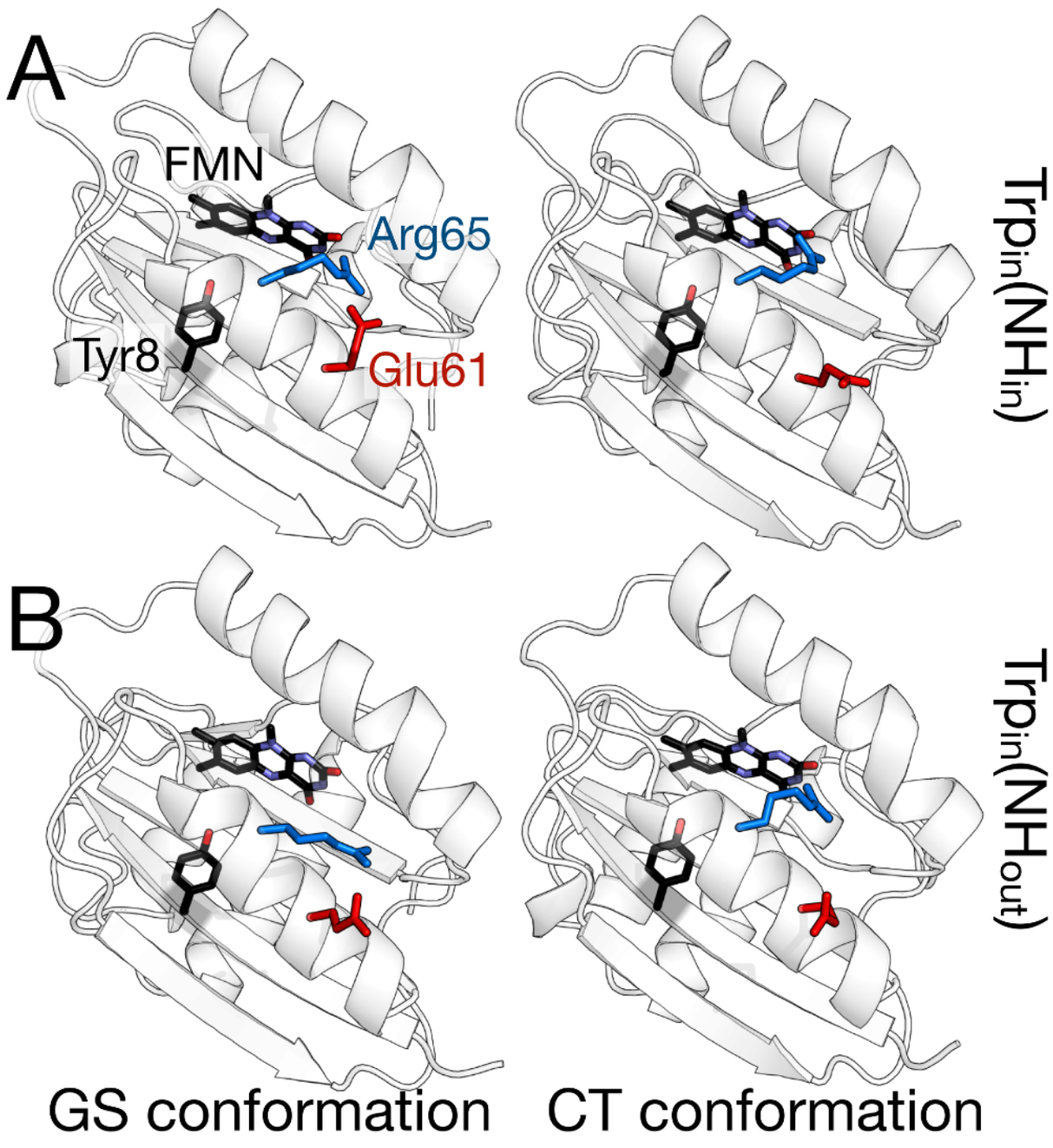
Proton relay for three Slr1694 Trp_{in} conformers



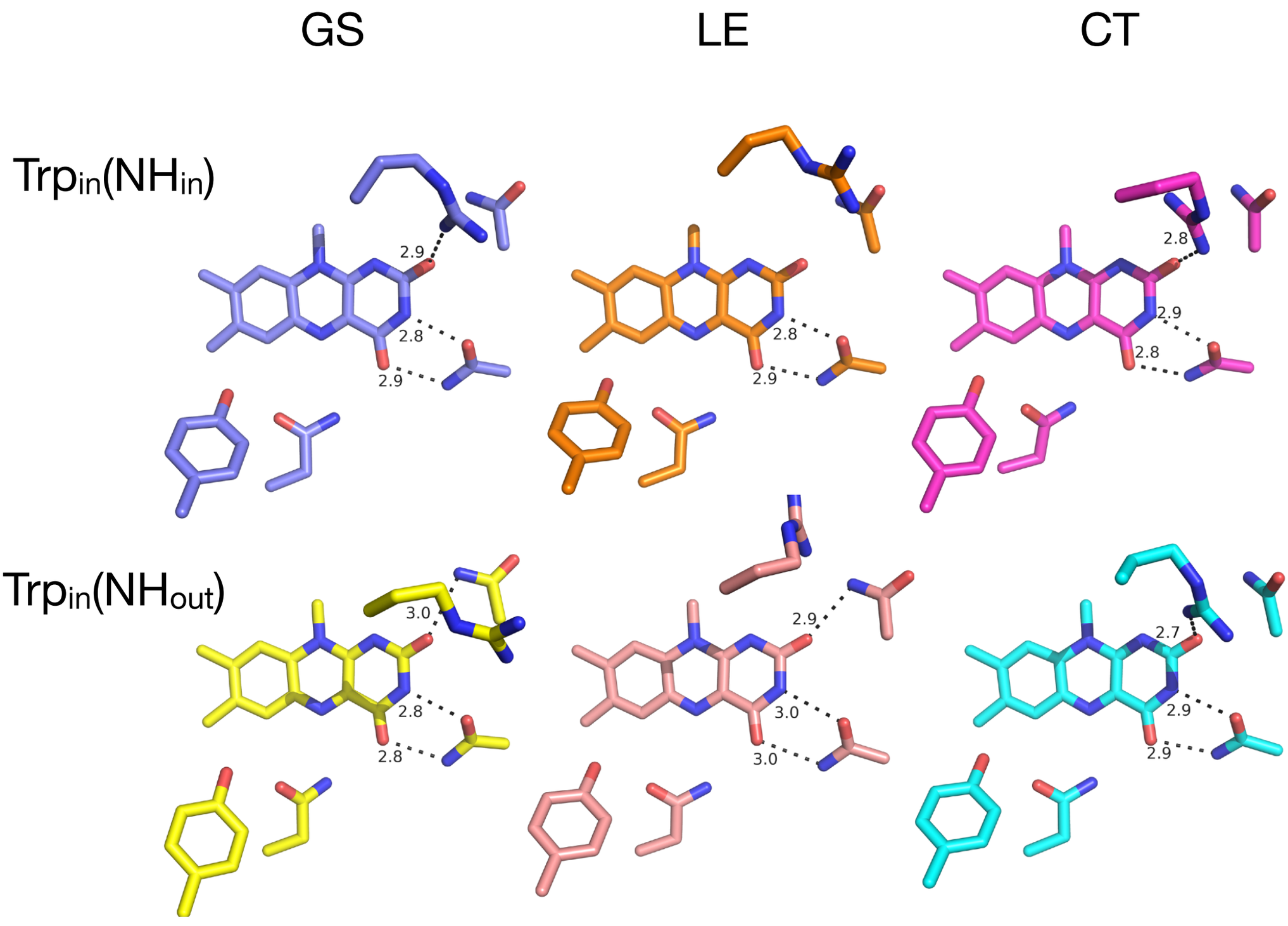
Individual residue contribution to CT stabilization for both Trp_{in} conformers



Arg65 and Glu61 contribution to CT stabilization



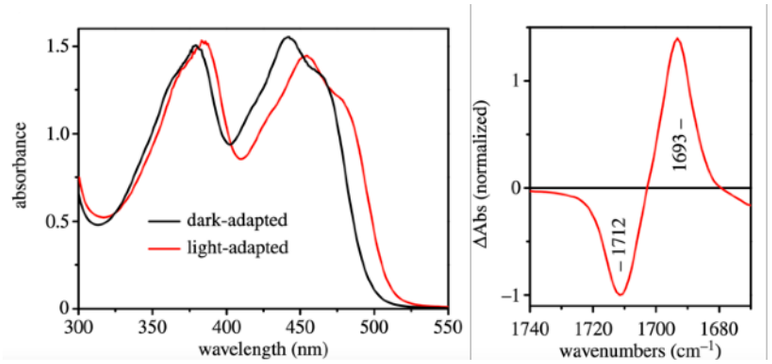
Binding pocket changes in snapshots

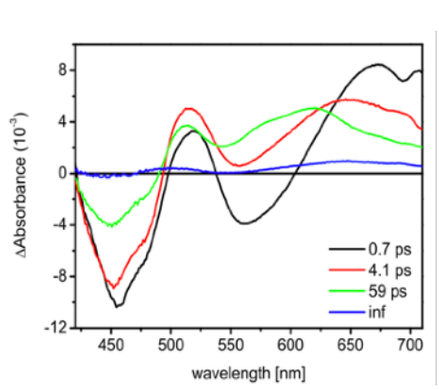


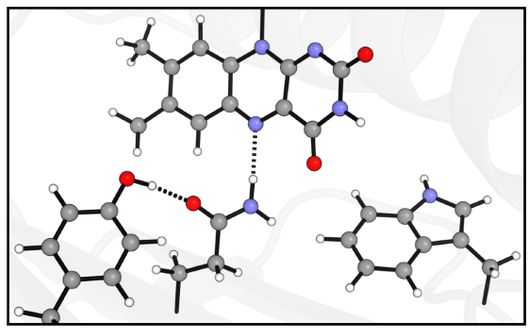
Focus on BLUF Slr1694: what we know

Fast formation of light-adapted state (< 1 ns) — good for dynamics!

Radical intermediates well-characterized — good for validation!







1. <u>Environment around flavin changes</u>
dark-vs-light adapted states ("off-vs-on")
10-15 nm red-shift in absorption spectra
20 cm^{-1} red shift in C4=O stretch of flavin

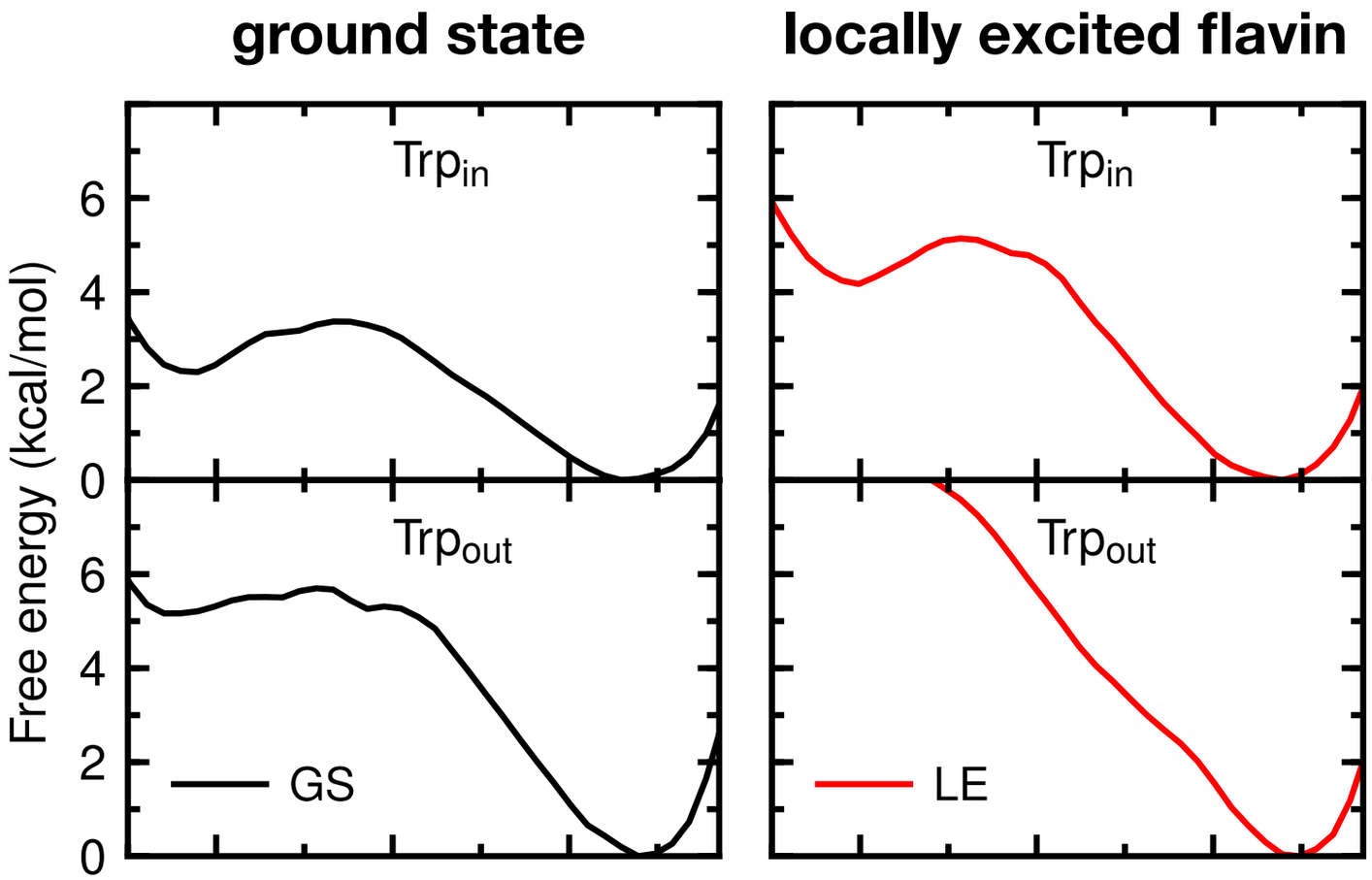
2. <u>Progresses via flavin redox intermediates</u>
Time-resolved spectroscopy implicates a photoinduced proton-coupled electron-transfer (PI-PCET) mechanism

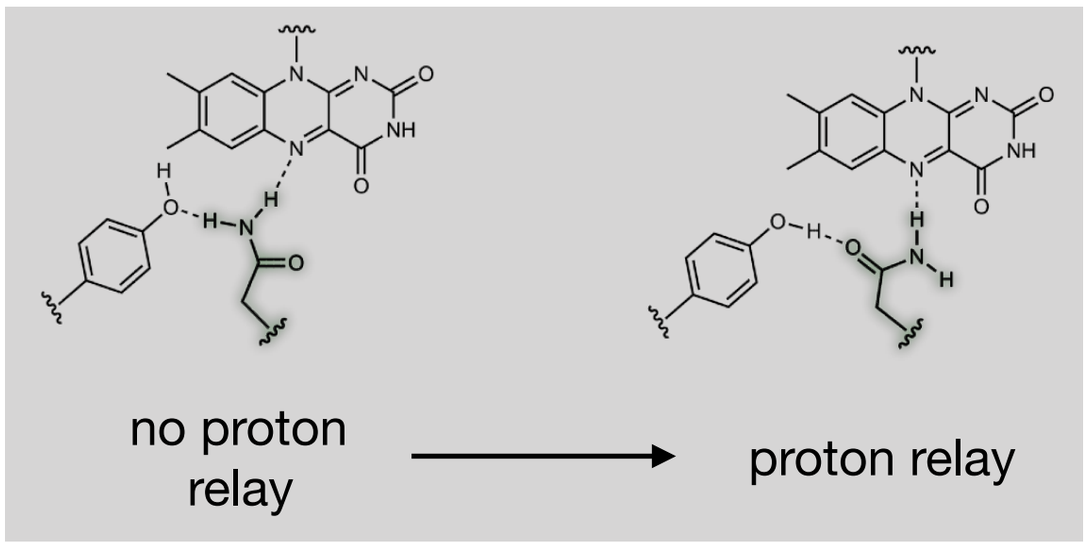
3. <u>Several key residues required</u>
Mutation studies implicate flavin, Tyr, Gln, Trp, Met (more on this next slide)

Currently no consensus on mechanism.

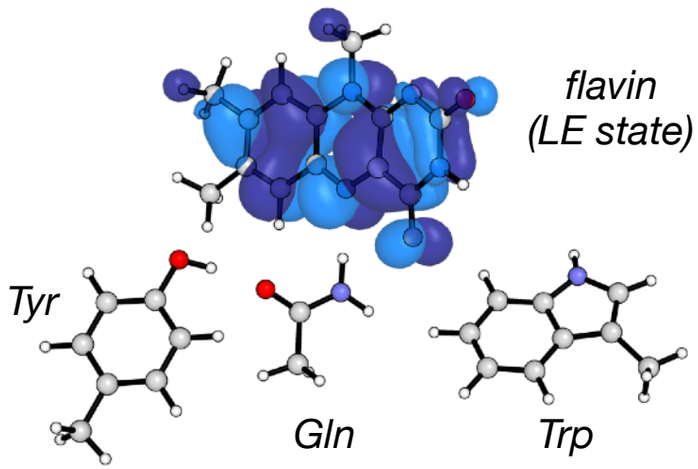
Source: Kennis, J. T. M.; Mathes, T. Molecular Eyes: Proteins That Transform Light into Biological Information. Interface Focus ***2013****, 3 (5), 20130005.*
Source: Mathes, T .et al.. Hydrogen Bond Switching among Flavin and Amino Acids Determines the Nature of Proton-Coupled Electron Transfer in BLUF Photoreceptors. JPCL ***2012****, 3 (2), 203–208.*

Flavin excitation promotes proton relay





Umbrella sampling with modified partial charges to reflect locally excited flavin



Modify force field with partial charges taken from cluster TDDFT/ CAM-B3LYP

Similar results for charge transfer state between Tyr and flavin (CT_{Tyr}) — proton relay strongly preferred