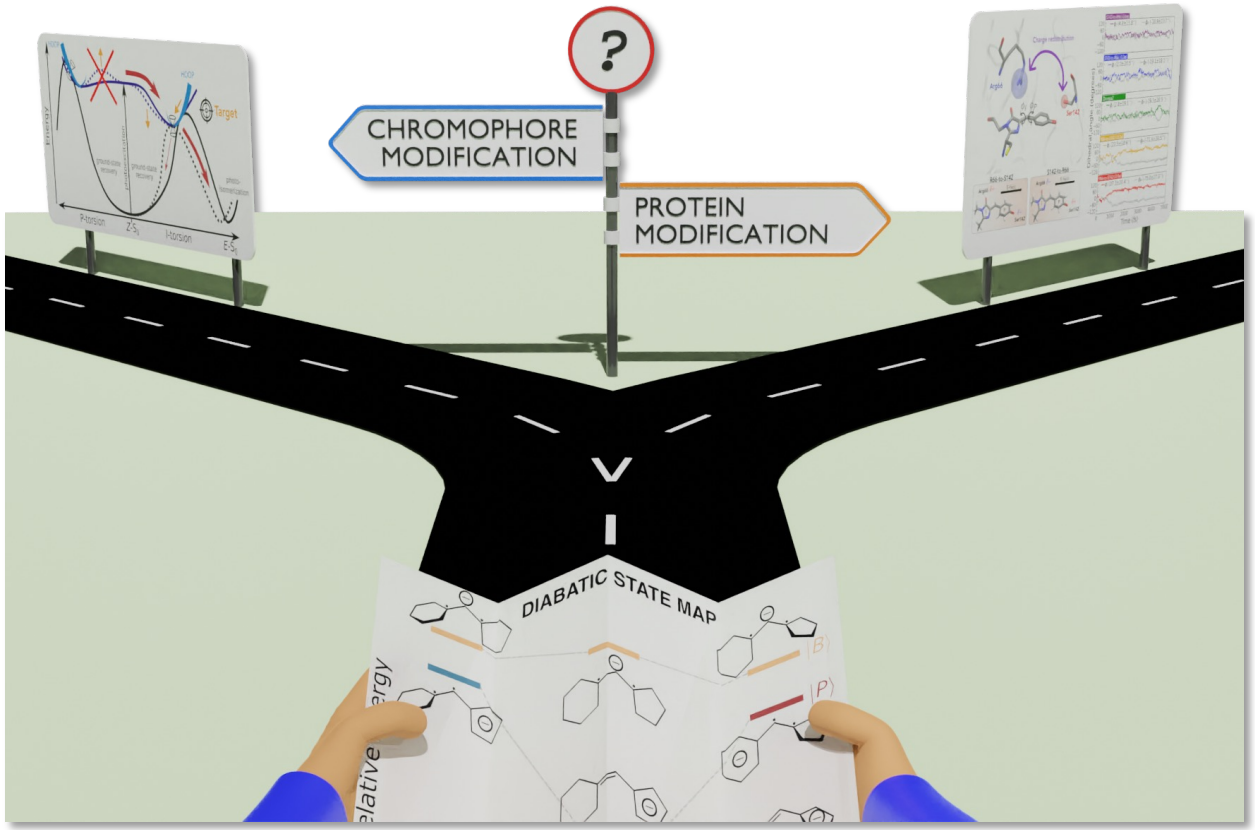
Dimming the lights & switching the twist

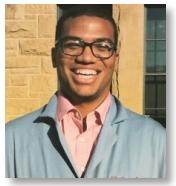
Chemical and environment control of the GFP chromophore



Nanna Holmgaard List
KTH Royal Institute of Technology

VISTA seminar
March 6th 2024

Chey M. Jones
MTZ lab, Stanford
→ *Merck, NJ*



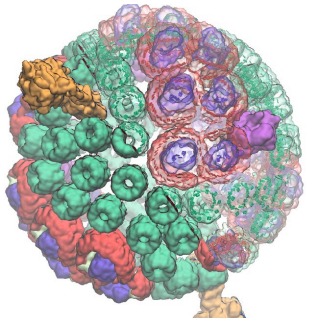
Rafael Couto
KTH

Light in Biology, Energy from light

MOLECULAR DOMAIN

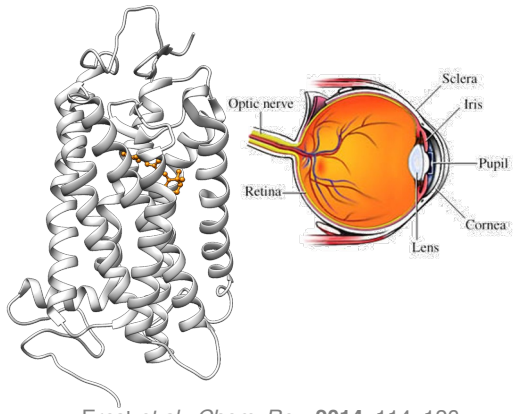
PHOTOBIOLOGY

Photosynthesis



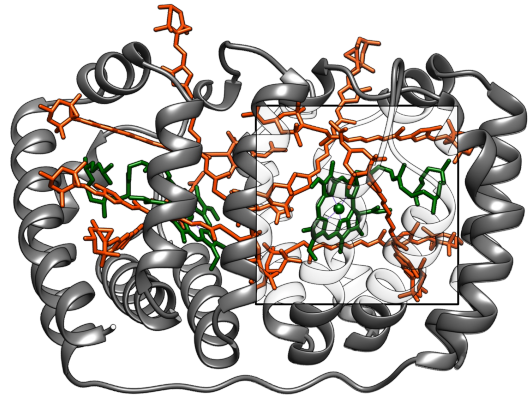
Sener *et al.*, eLife **2016**

Light-sensing to adapt behaviors



Ernst *et al.*, *Chem. Rev.* **2014**, 114, 126

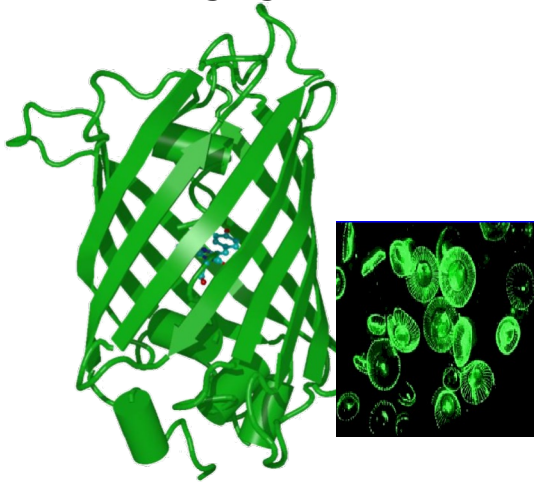
Light-harvesting vs. photoprotection



Horton, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2012**, 367, 3455

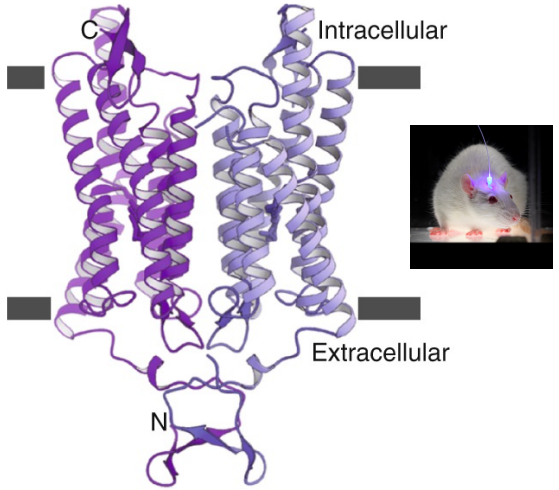
TECHNOLOGY

Imaging



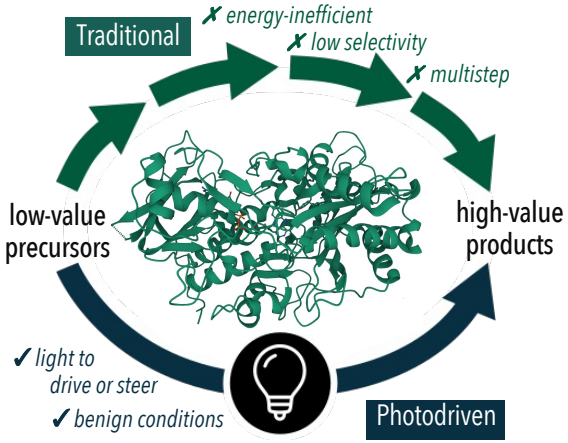
Zimmer, *Chem. Rev.* **2002**, 102, 759
Betzig *et al., Science* **2006**, 313, 1642

Optical control



Deisseroth, *Nat. Methods*, **2011**, 8, 26
Oda et al, *Nat. Comm.* **2018**, 9, 3949

Photobiocatalysis



Harrison *et al., Acc. Chem. Res.* **2022**, 55, 1087

Our dream

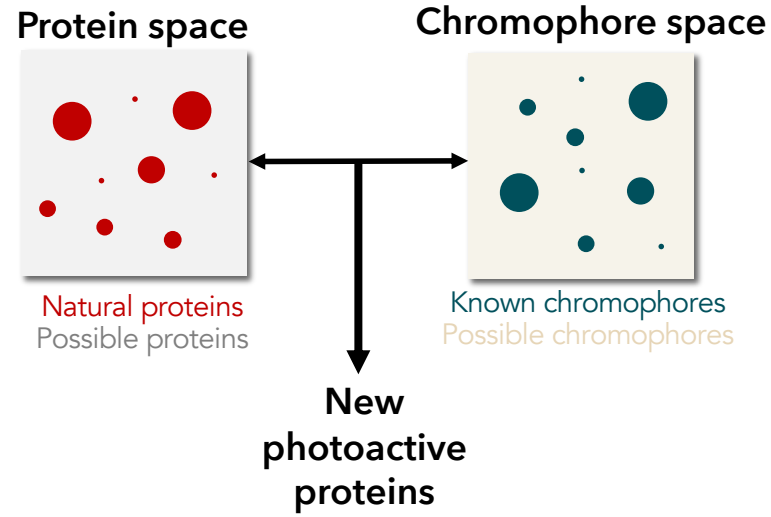
Nature has only explored a small part of protein and chromophore spaces, evolved under biological pressures

Break free of natural protein folds and enable protein design factoring in requisite photofunction

Formidable task! – yet recent breakthroughs in computational de novo protein design strategies*

How might we contribute to this puzzle

1. What does the photofunctional blueprint look like?
2. What are the parameters defining the blueprint?
3. How does a blueprint couple to further multiscale phenomena?



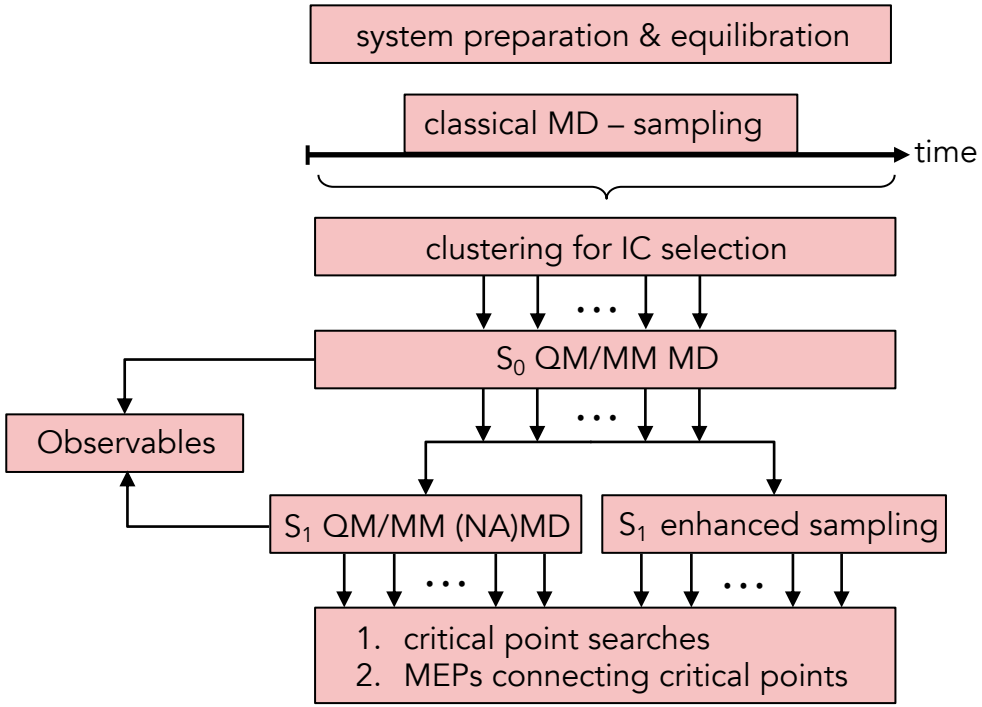
Where to start?

- **If nature provides a template:** Extract blueprints from and across existing systems
- **If the template is not optimal:** Explore photoreactivity bottlenecks and how they may be overcome
- **If much excited-state data is available:** Can we "learn" structure-dynamics-function relations from trends?
- **…**

*enabling, e.g., structure, ensemble and binding motif predictions

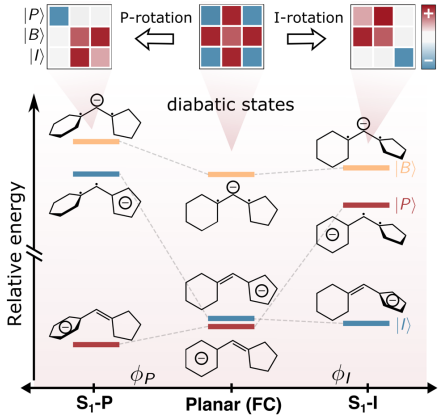
Our current computational toolbox

Multiscale workflow



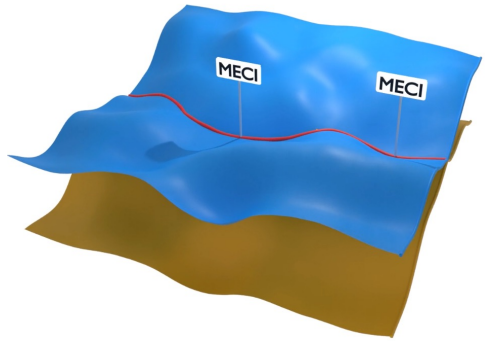
No black-box approach!
Many avenues for further developments!

Quasi-diabatic models



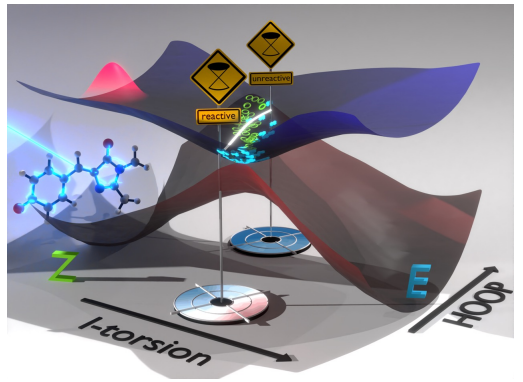
Chemically intuitive electronic basis to assist in design

CI seam exploration



Connecting points on the intersection seam

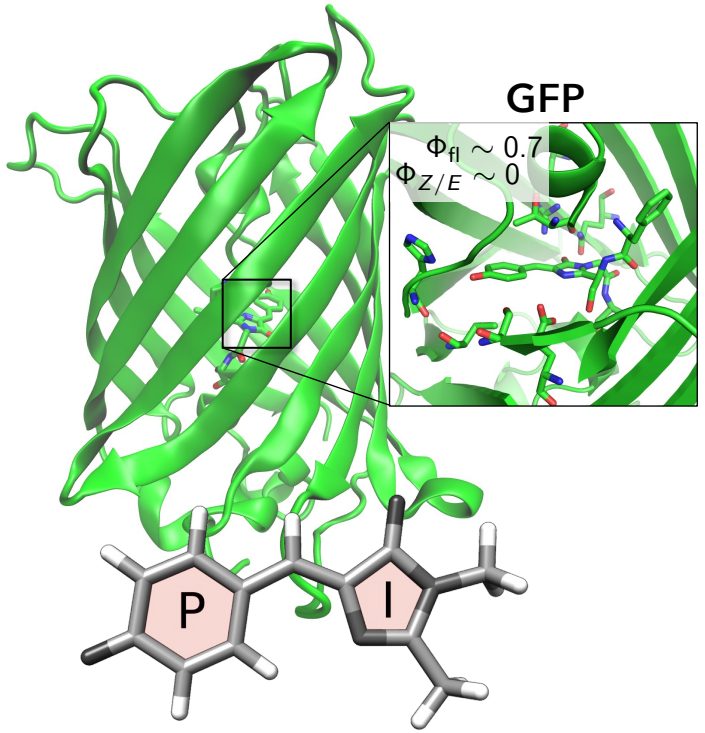
Photoreactivity



Identify factors governing photoreactivity

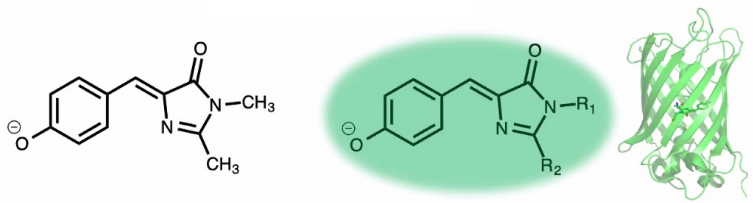
GFP – the monarch of bioimaging

The green fluorescent protein

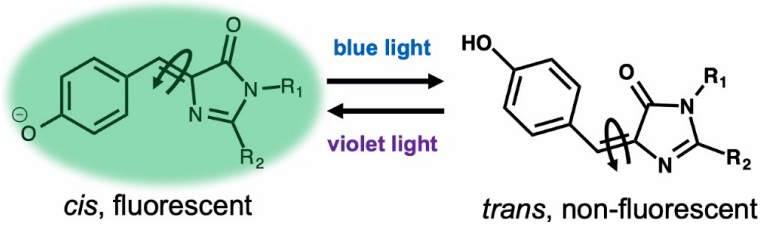


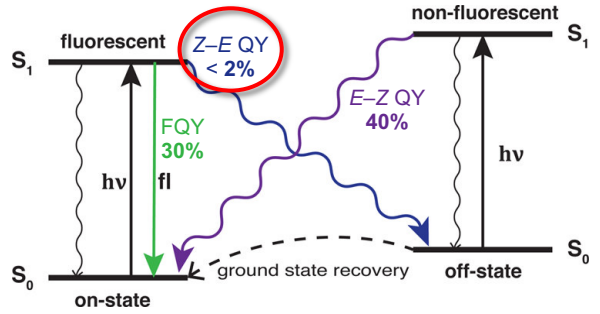
GFP chromophore: HBDI$^-$
(anionic form)

Fluorescence

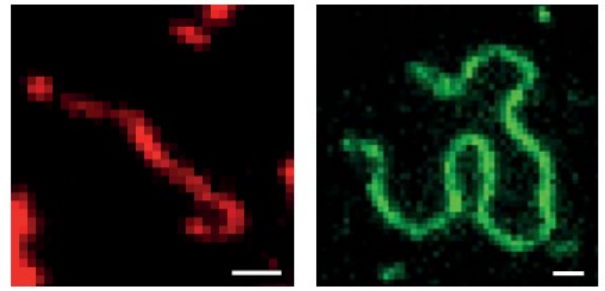


Photoisomerization

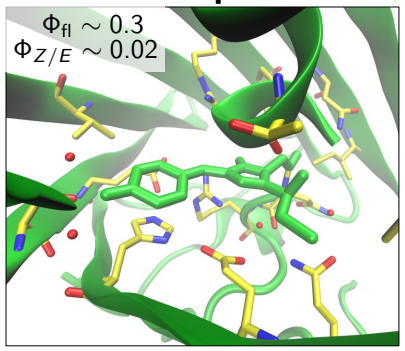




Imaging (passive reporter)



Dronpa2

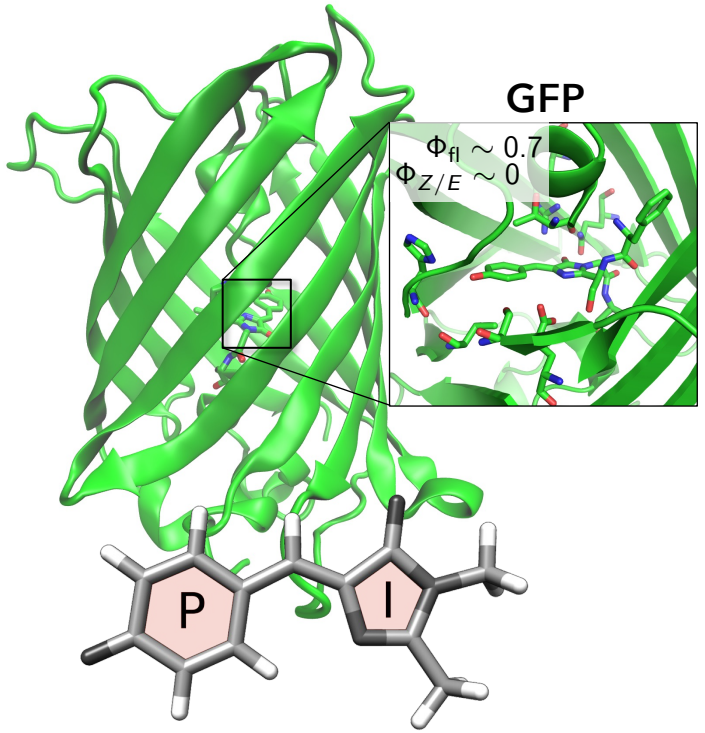


photoswitchable

Adapted from Acharya *et al. Chem. Rev.* **2017**, 117, 758

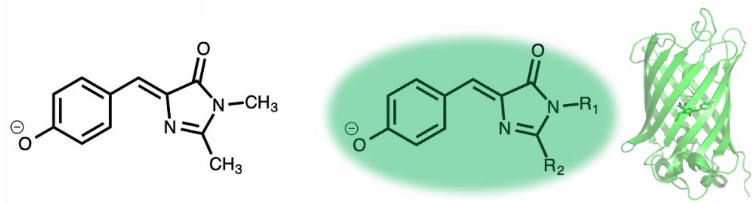
GFP – the monarch of bioimaging

The green fluorescent protein

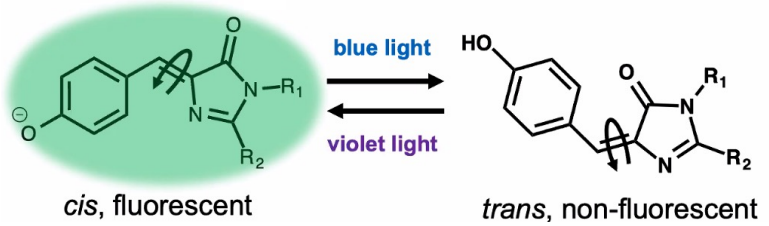


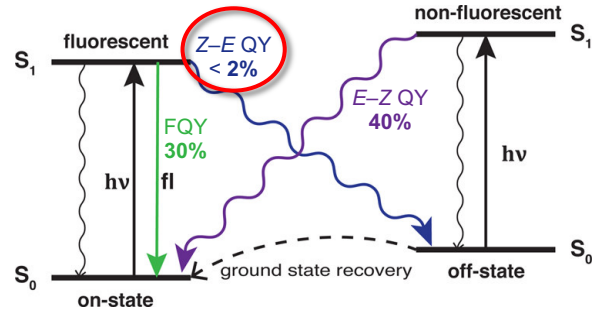
GFP chromophore: HBDI$^-$
(anionic form)

Fluorescence



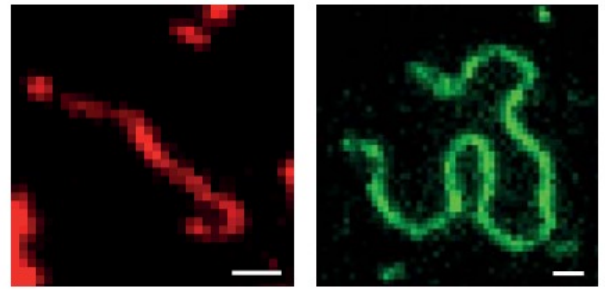
Photoisomerization

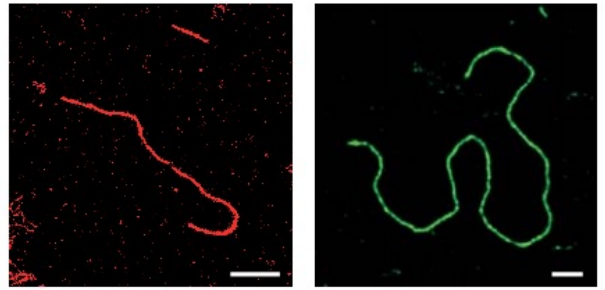




Adapted from Acharya *et al. Chem. Rev.* **2017**, 117, 758

Imaging (passive reporter)





Super resolution

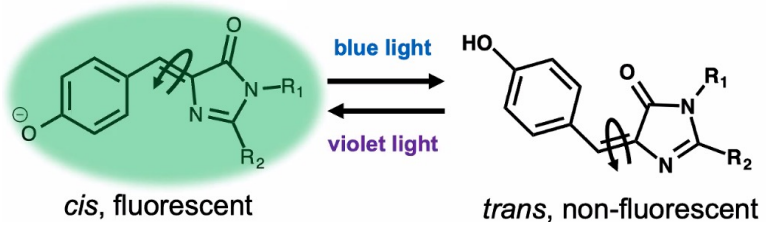
Albertazzi, Meijler *et al. Science* **2014**, 344, 491

GFP – a simple excited-state protein

A "simple" and versatile model system

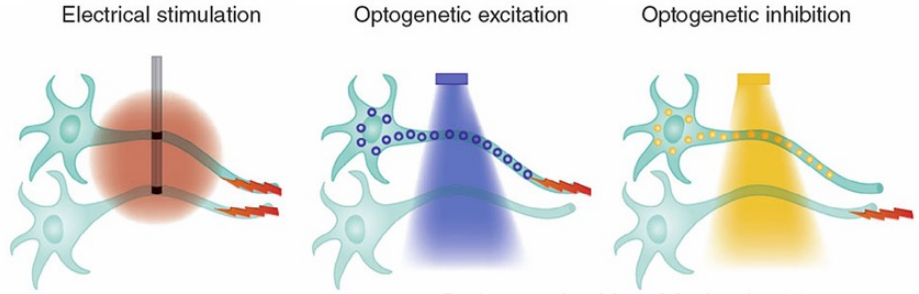
- Multifunction characteristics
  (proton transfer, intramolecular charge-transfer, color-tuning, fluorescence, redox properties, isomerization, …)
- A "friendly" system
  (structurally resilient, crystallizable, purifiable, tunable, tractable simulations, …)

⟹ **understand the link between control variables and photoinduced function**



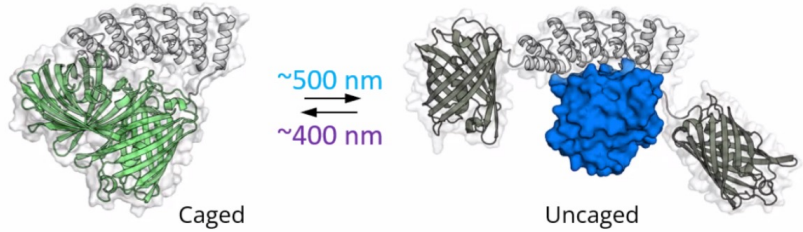
*What does it take to **dim the lights** and **steer the twist** (from Z-to-E)?*

Optogenetics (active control)



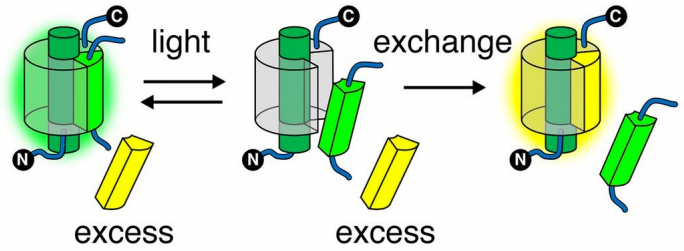
Deisseroth, *Nat. Methods,* **2011**, 8, 26

Non-neural optogenetics



Light-controlled biochemical activity

Zhou, Lin *et al. Science,* **2012**, 338, 810
Westberg, Lin *et al.* BioRxiv **2023**



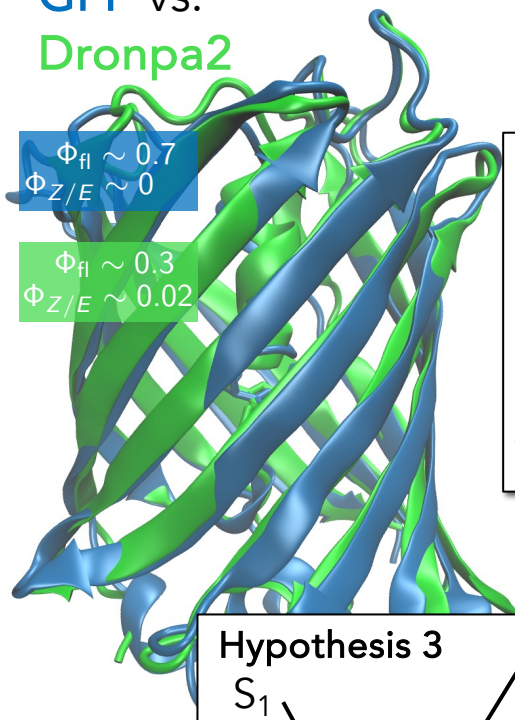
SplitGFPs (strand photodissociation)

Lin, Boxer et al. *PNAS,* **2017**, 114, E2146

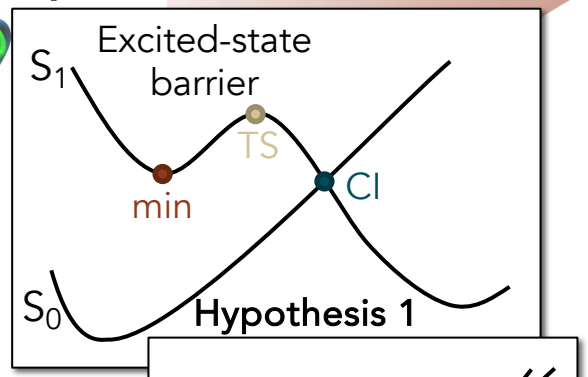
Lin, Boxer *et al. J. Am. Chem. Soc.* **2022**, 144, 9, 3968

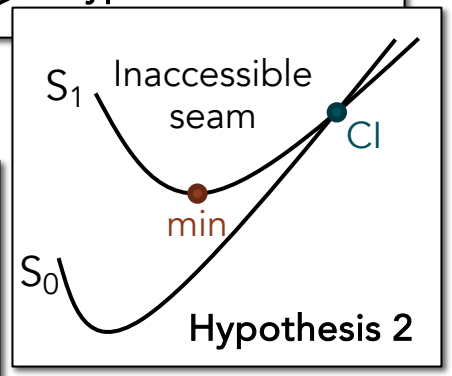
Understanding the existing…

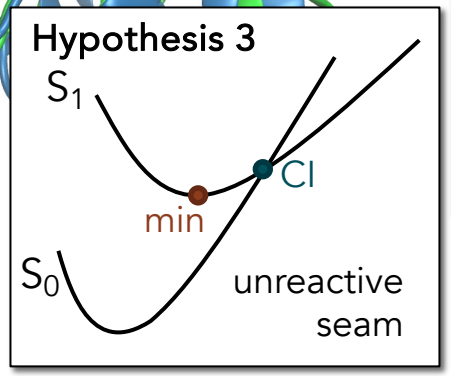
GFP vs.
Dronpa2



Dimmer yet so little photoisomerization?







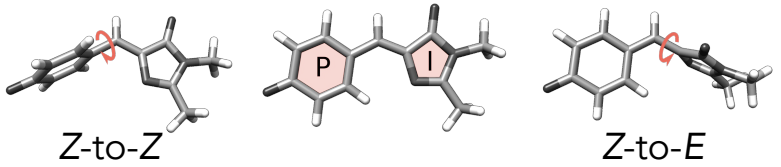
What about outside the protein?

Expt.: ultrafast radiationless decay involving three timescales (300 fs, ~1ps, >10ps)

P-torsion

HBDI$^-$

I-torsion



Z-to-*Z*

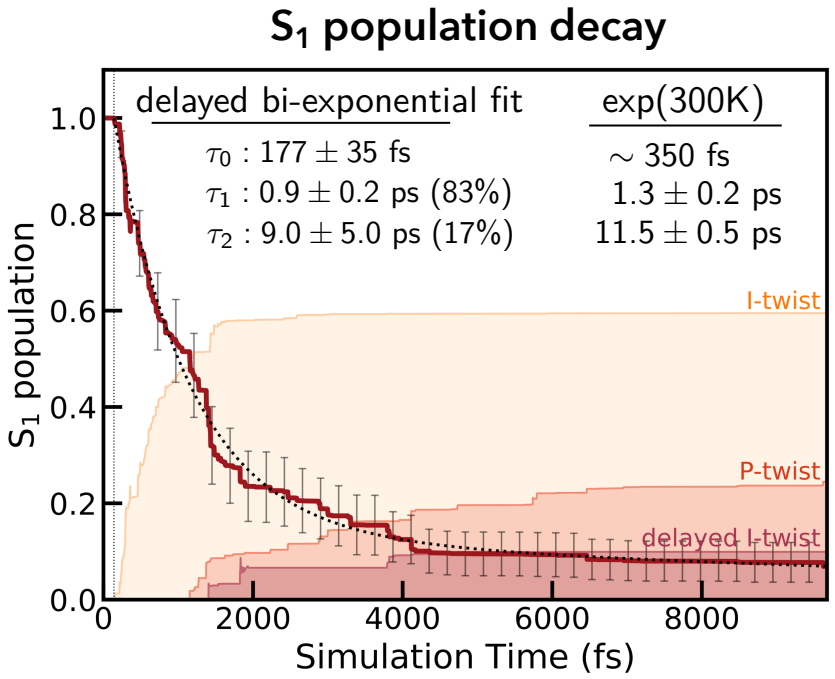
Z-to-*E*

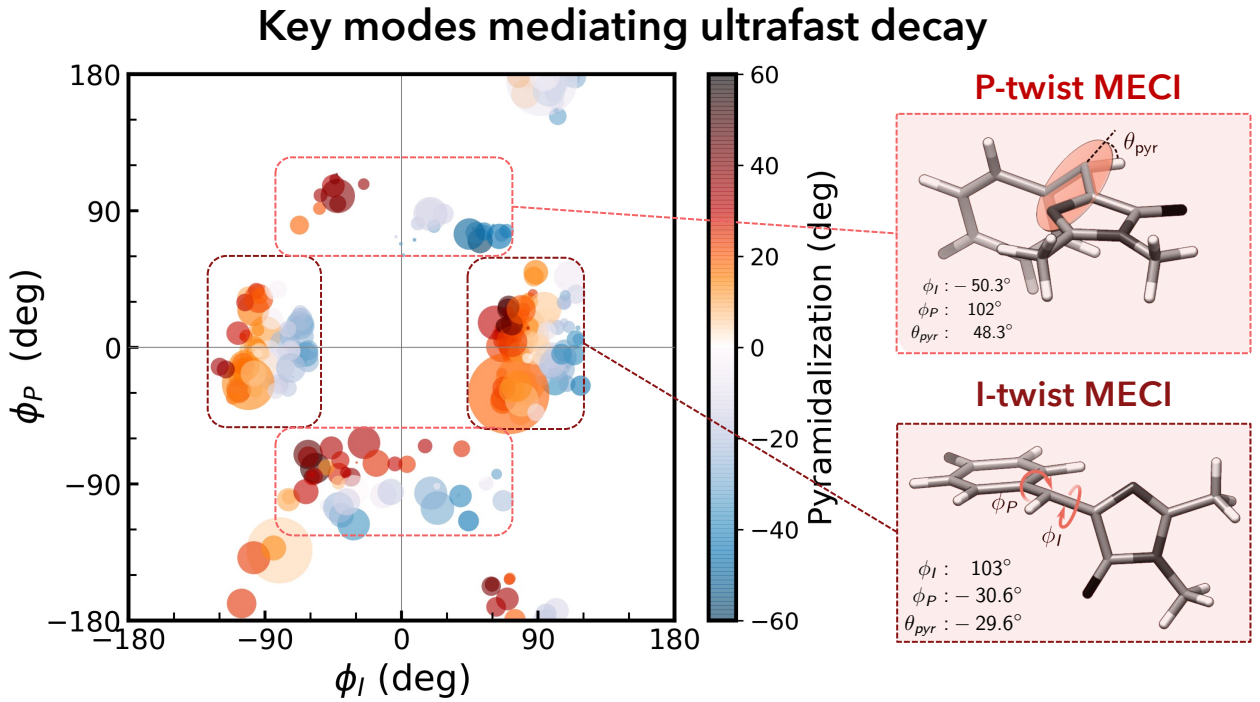
Goals

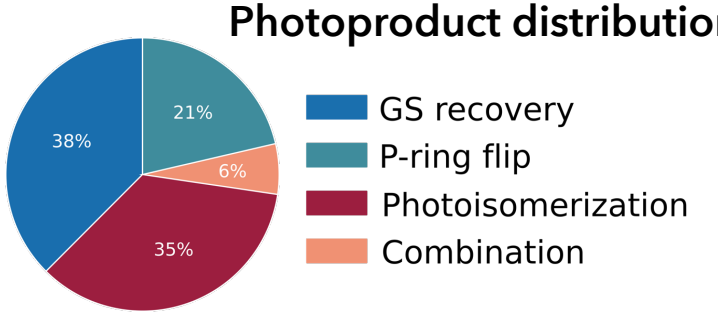
- To what extent does it photoisomerize?
- Any intrinsic bottlenecks to the process?

TR-action: Svendsen, Andersen et al., *J. Am. Chem. Soc.* 2017, 139, 8766
TRPES: Mooney, Verlet *et al.*, *Chem. Sci.* 2013, 4, 921
Carrascosa, Bieske *et al. J. Phys. Chem. Lett.* 2018, 9, 2647

Internal conversion in gas-phase HBDI⁻





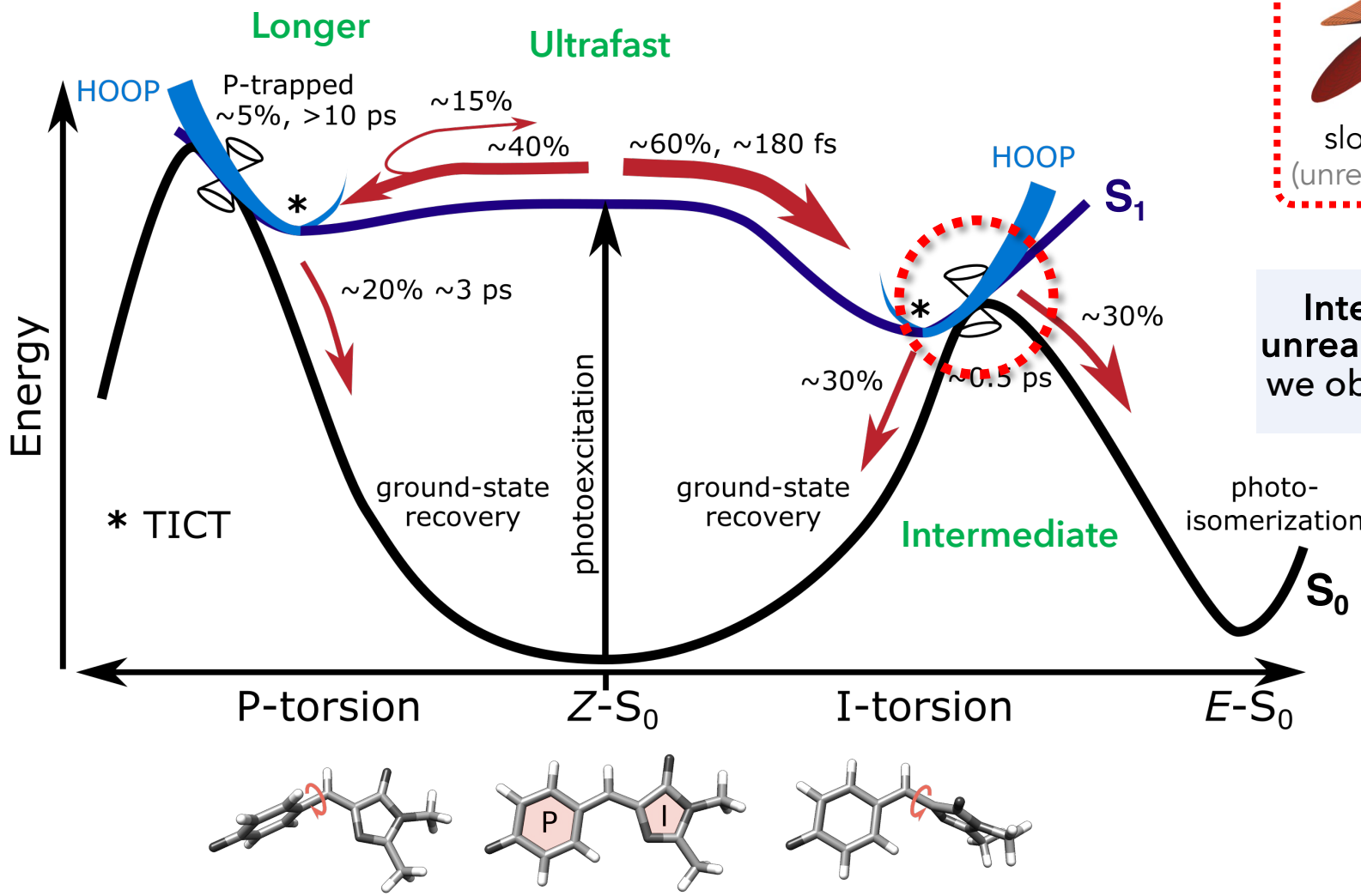


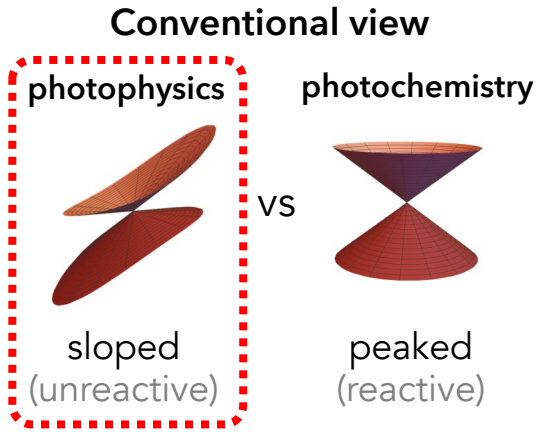
***E*-isomer: ~35%**

NHL, Jones, Martínez, *Chem. Sci.* 2022, 13, 373
TR-action: Svendsen, Andersen *et al.*, *J. Am. Chem. Soc.* 2017, 139, 8766
TRPES: Mooney, Verlet *et al.*, *Chem. Sci.* 2013, 4, 921

AIMS/α(0.64)-SA3-CASSCF(4,3)/6-31G*

Summary of excited-state dynamics





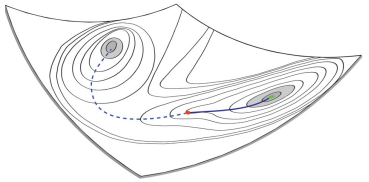
Intersection seam has **unreactive topography yet** we obtain **photoproduct!?**

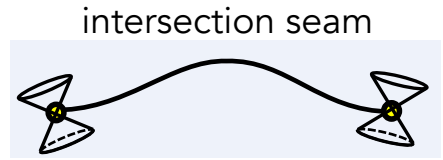
*Twisted intramolecular charge-transfer (TICT)

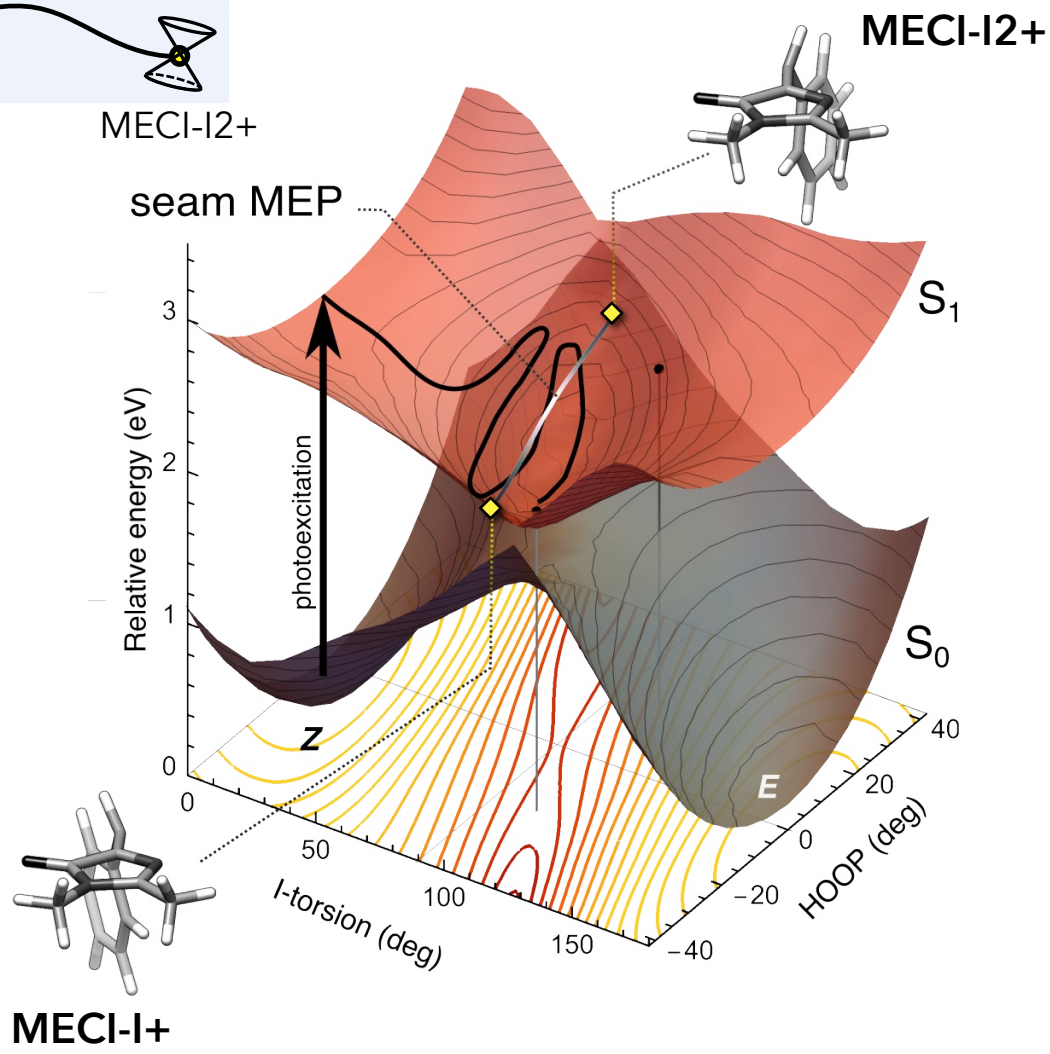
NHL, Jones, Martínez, *Chem. Sci.* 2022, 13, 373

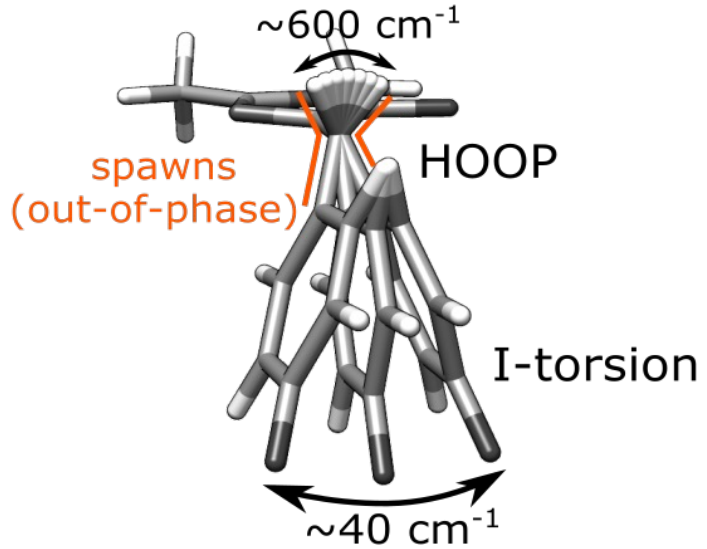
AIMS/α(0.64)-SA3-CASSCF(4,3)/6-31G*

Zooming in on the I-twist intersection seam





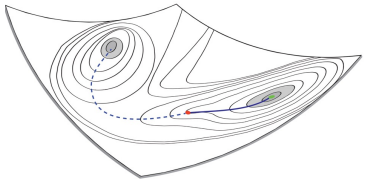




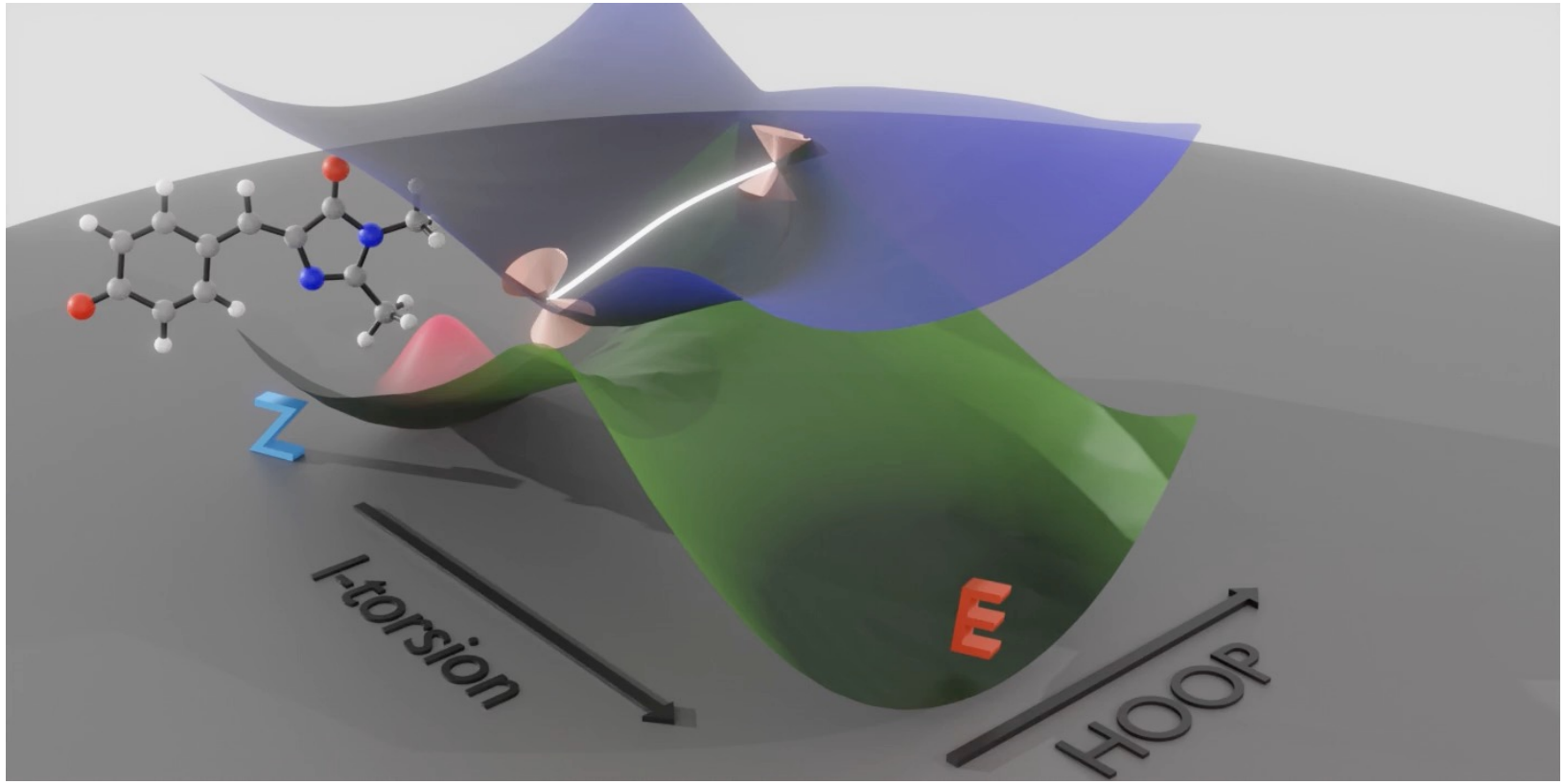
Two *near-enantiomeric* minimum energy conical intersections (MECIs)

NHL, Jones, Martínez, *Chem. Sci.* 2022, 13, 373

Zooming in on the I-twist intersection seam



MECI-I2+



MECI-I+

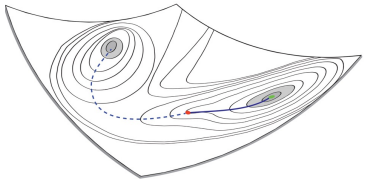
reactive/unreactive ratio

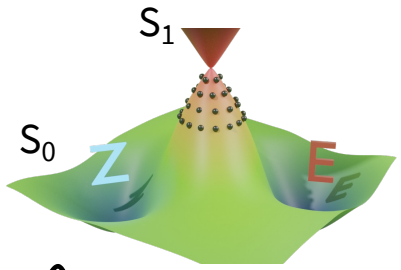
MECI-I+:	~3:1
MECI-I2+:	~1:2

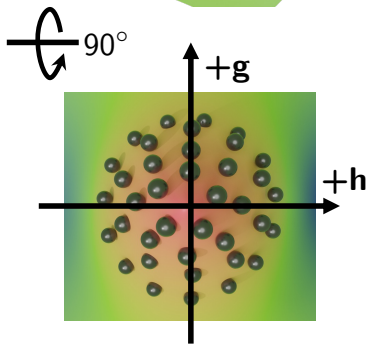
⟹ what is the origin of this difference in photoreactivity?

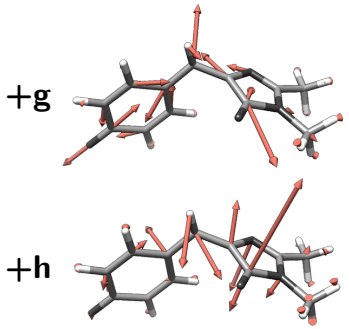
NHL, Jones, Martínez, *Chem. Sci.* 2022, 13, 373

Mapping intrinsic photoreactivity





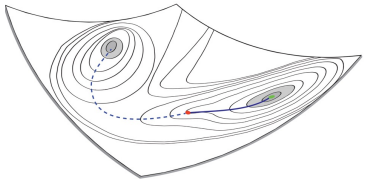


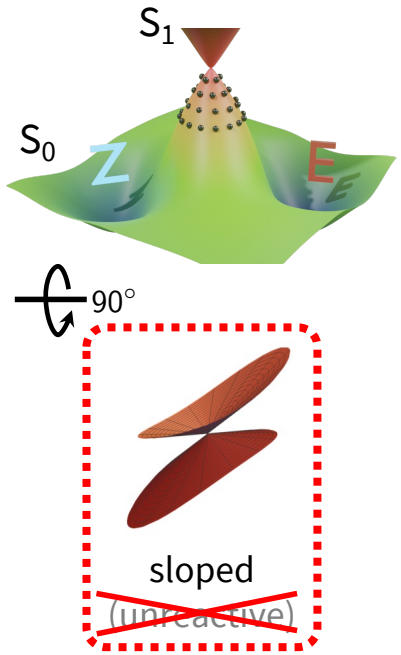


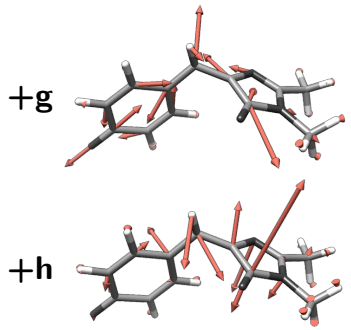
NHL, Jones, Martínez, *Chem. Sci.* 2022, 13, 373
Sellner, Barbatti, Lischka, *J. Chem. Phys.* 2009, 131, 024312

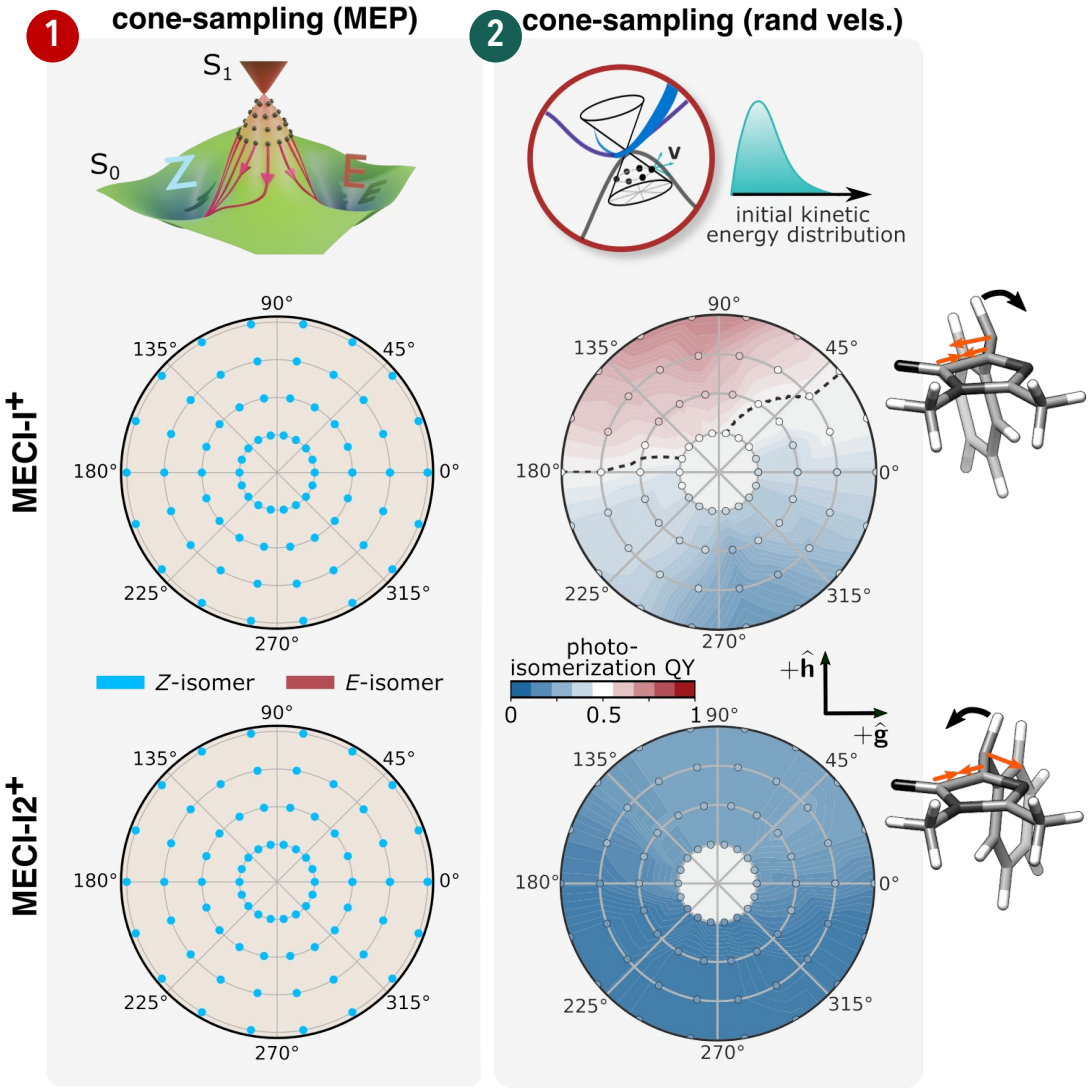
BOMD/α(0.64)-SA3-CASSCF(4,3)/6-31G*

Mapping intrinsic photoreactivity









1. Photophysics only
   ⟹ ground-state recovery

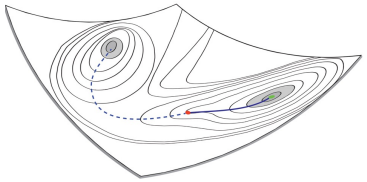
2. Both photophysics and photochemistry
   ⟹ ***Imprint of inertia on the ground state matters***

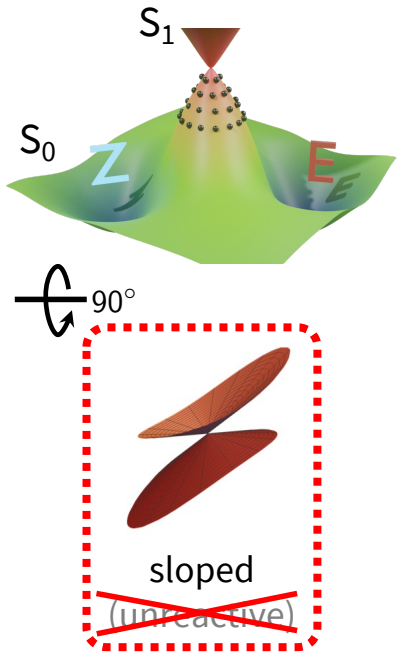
3. Both photophysics and photochemistry
   ⟹ ***Direction and velocity of approach on the excited state matters***

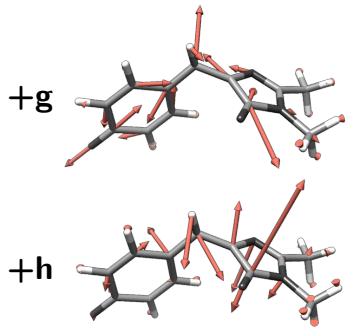
NHL, Jones, Martínez, *Chem. Sci.* 2022, 13, 373
Sellner, Barbatti, Lischka, *J. Chem. Phys.* 2009, 131, 024312

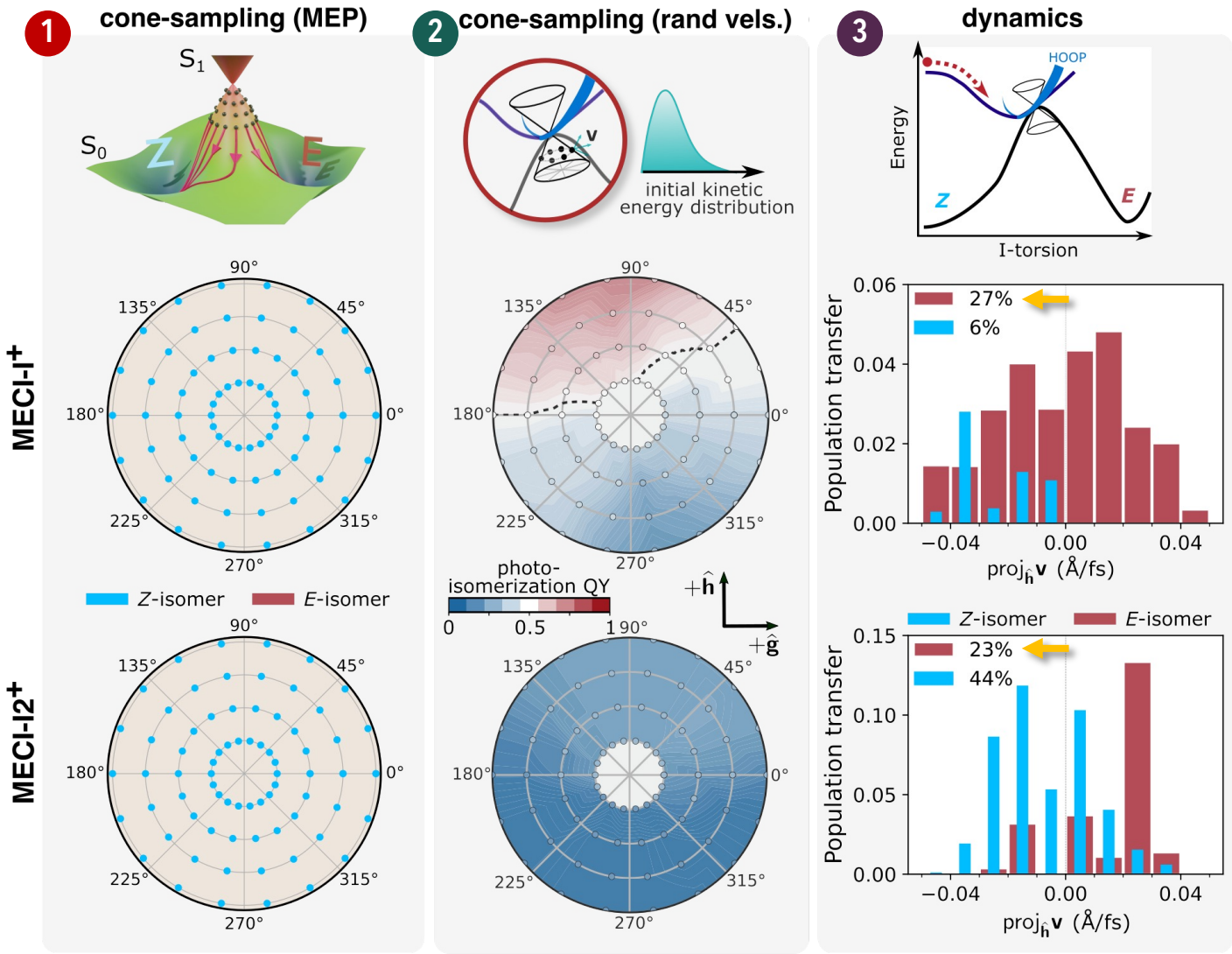
BOMD/α(0.64)-SA3-CASSCF(4,3)/6-31G*

Mapping intrinsic photoreactivity









1 Photophysics only

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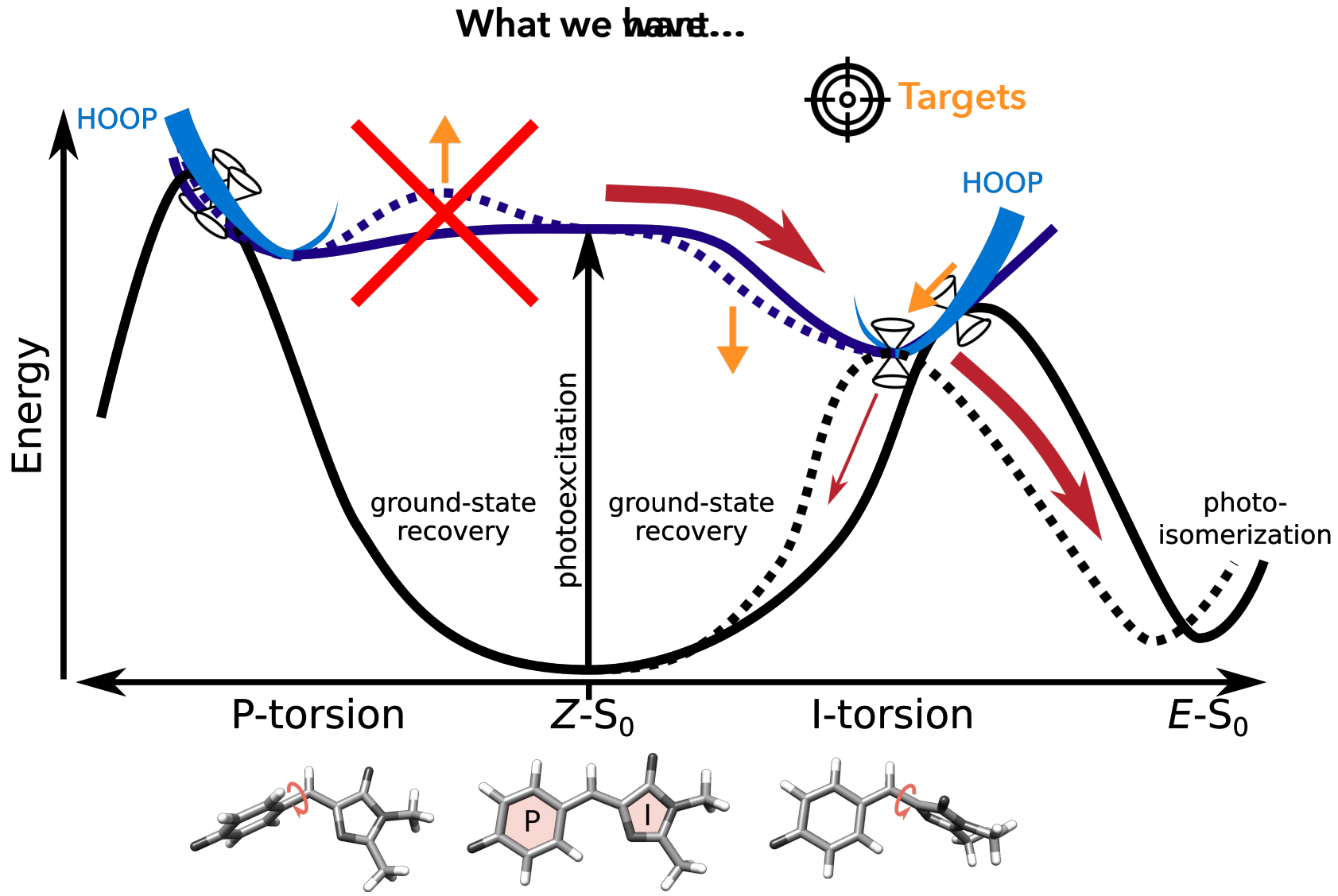
NHL, Jones, Martínez, *Chem. Sci.* 2022, 13, 373
Sellner, Barbatti, Lischka, *J. Chem. Phys.* 2009, 131, 024312

BOMD/α(0.64)-SA3-CASSCF(4,3)/6-31G*

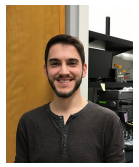
Engineering toward photoswitching



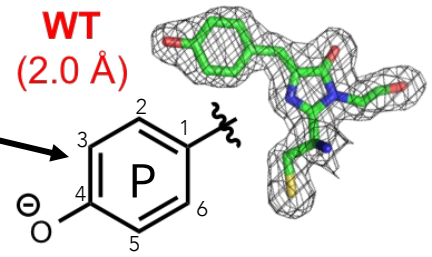
Chemical control of internal conversion?

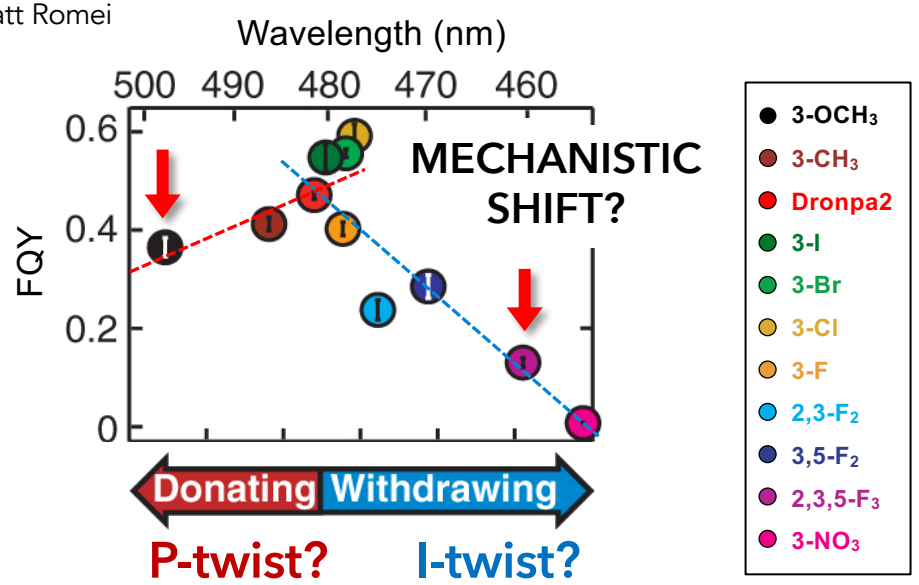
Fluorescence of Dronpa2 variants


Chi-Yun Lin


Matt Romei

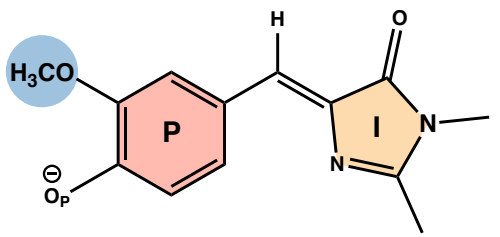
Introducing electron-**withdrawing** and **donating** groups on the P-ring

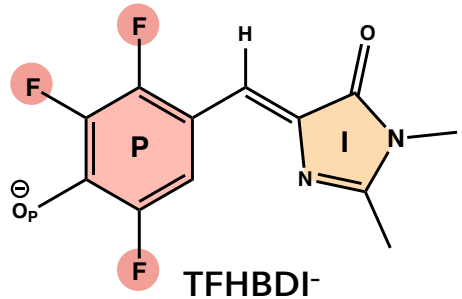




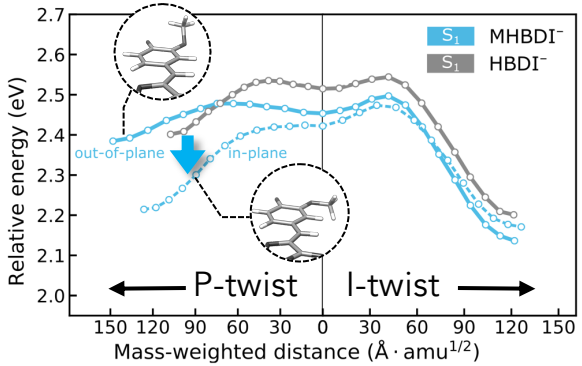
Romei, Lin, Mathews, Boxer, *Science*, 2020, 367, 76

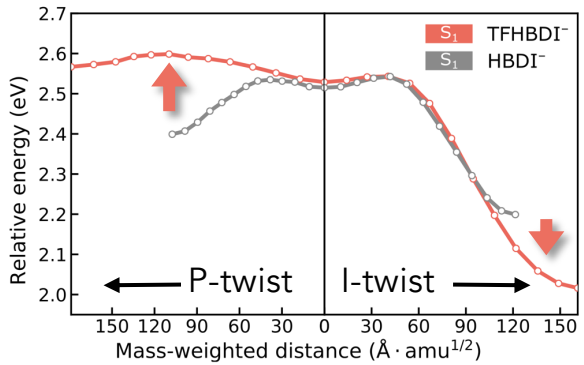
Modified chromophores





Excited-state torsional "barriers"



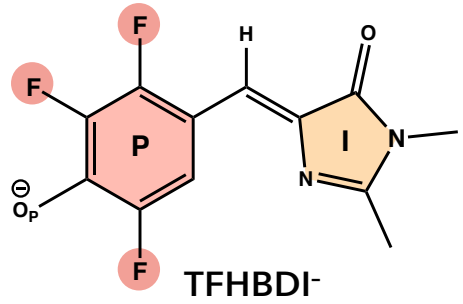


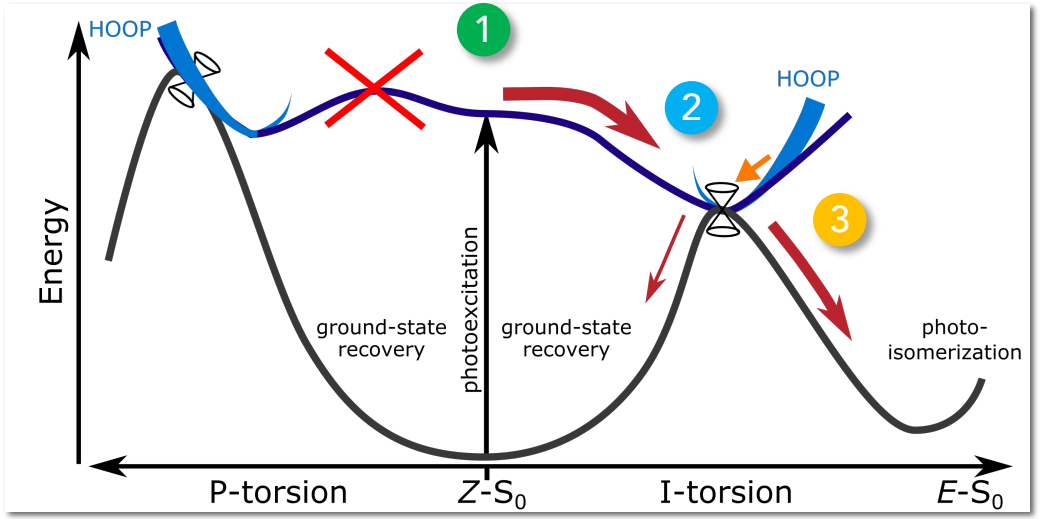
- ⊗ Similar energetics along P/I-twist
- ⊗ MECI-P accessible from the FC point

- ✓ Removes driving force along P-twist
- ✓ Almost isoenergetic S_1-I and MECI-I

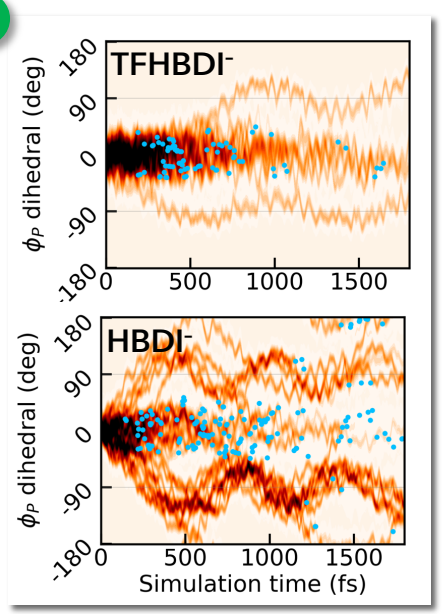
NHL, Jones, Martínez, *Commun. Chem.* 2024, 7, 25 XMS-CASPT2(4,3)/6-31G*//α(0.64)-CASSCF

Strategy to promote photoisomerization



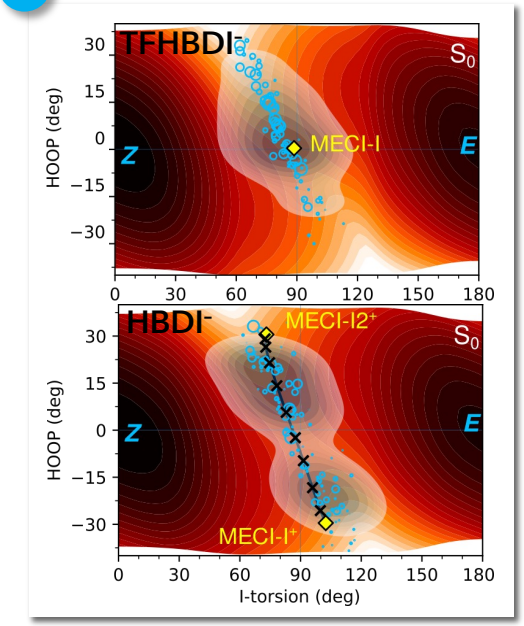


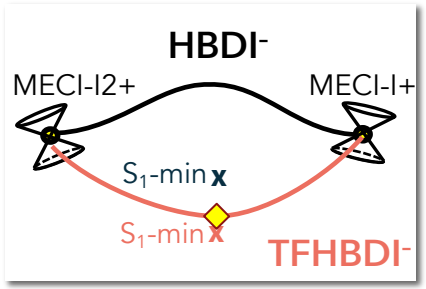
1



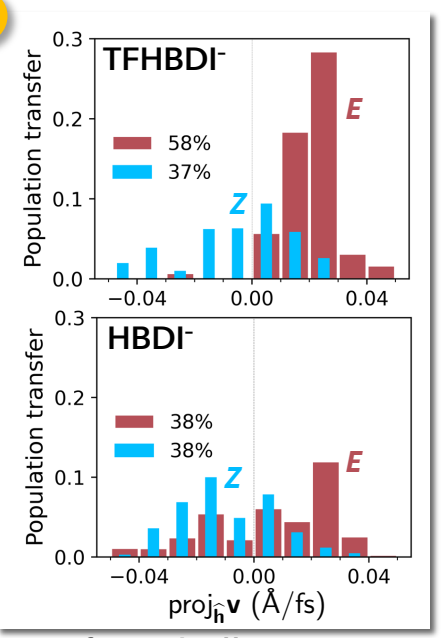
Pathway selectivity

2 Stabilization of I-twist seam





3



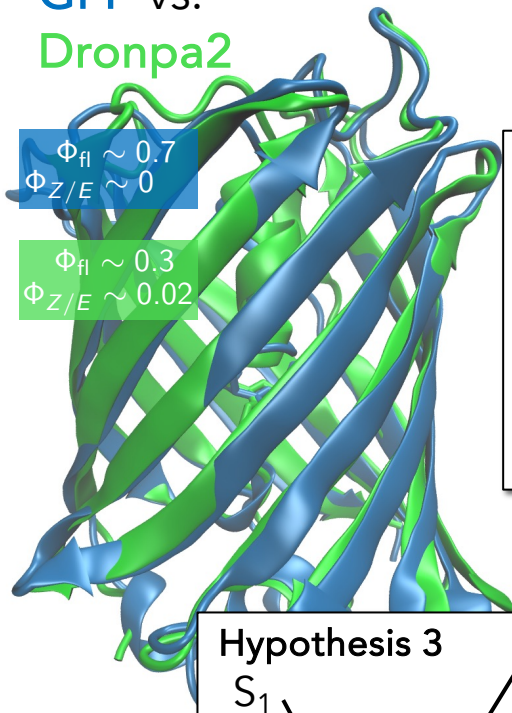
Transfer in ballistic regime

Turning HBDI⁻ into an efficient photoswitch (doubling the PQY)!

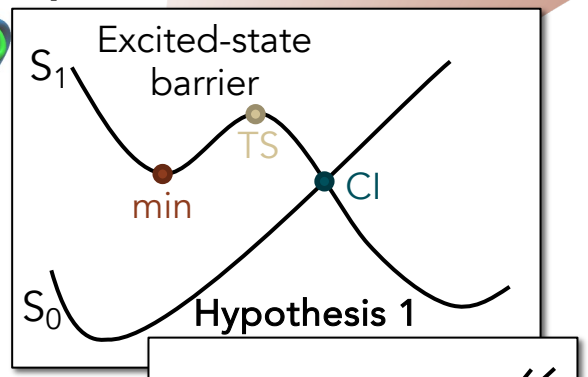
NHL, Jones, Martínez, *Commun. Chem.* 2024, 7, 25

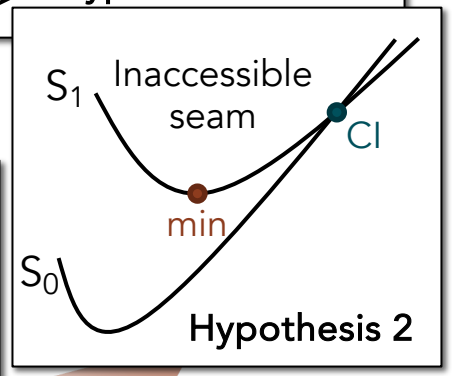
Back to the protein setting

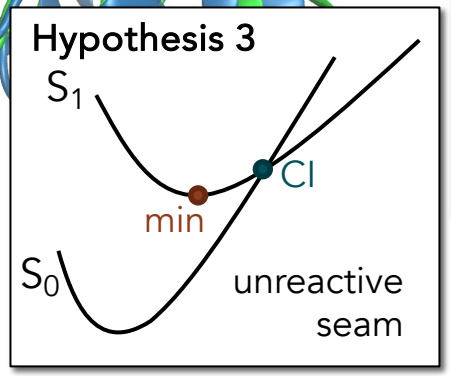
GFP vs.
Dronpa2



Dimmer yet so little photoisomerization?

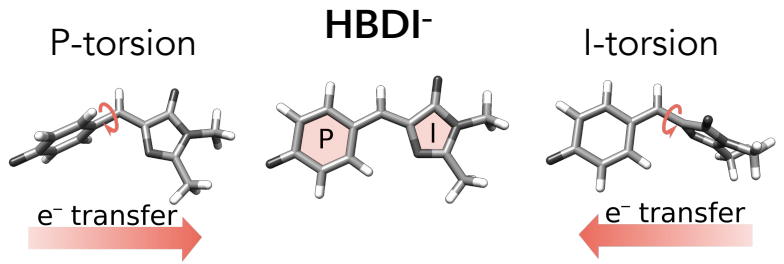






What about outside the protein?

Expt.: ultrafast radiationless decay involving three timescales (300 fs, ~1ps, >10ps)



Goals

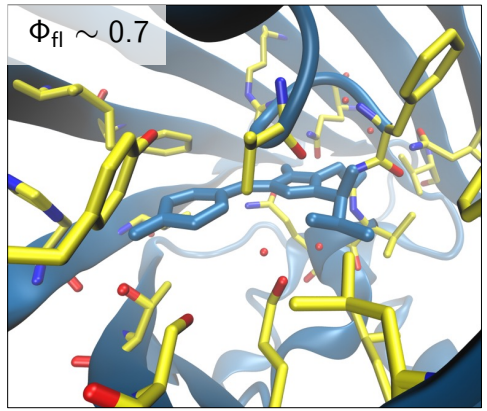
- To what extent does it photoisomerize?
- Any intrinsic bottlenecks to the process?
- Can we connect these findings to the behavior in the protein setting?

TR-action: Svendsen, Andersen et al., *J. Am. Chem. Soc.* 2017, 139, 8766
TRPES: Mooney, Verlet *et al.*, *Chem. Sci.* 2013, 4, 921
Carrascosa, Bieske *et al. J. Phys. Chem. Lett.* 2018, 9, 2647

Dronpa2: Dimmer yet little photoisomerization?

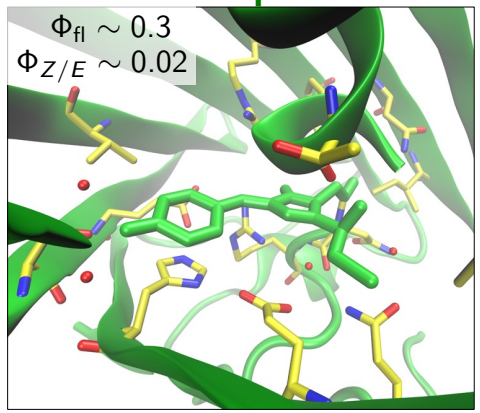
Tight and symmetric landscape that preserves chromophore planarity

GFP



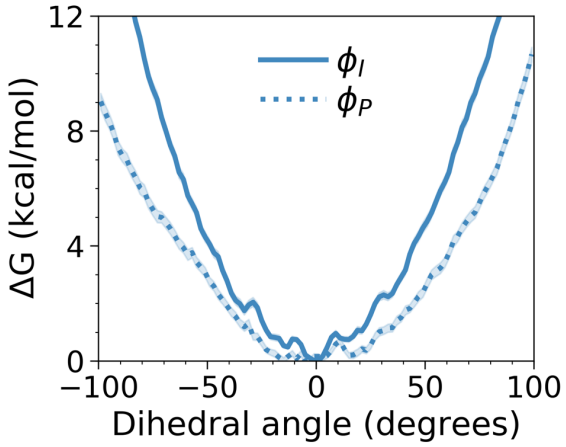
fluorescent

Dronpa2

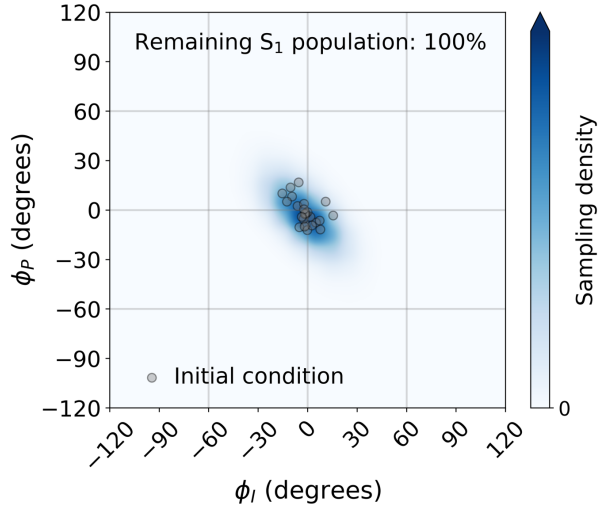


"photoswitchable"

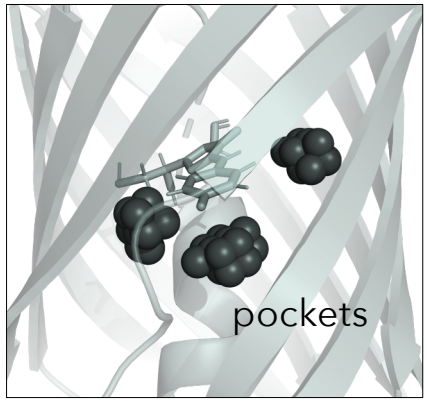
S_1 free-energy curves



QM/MM AIMS dynamics



Sterics

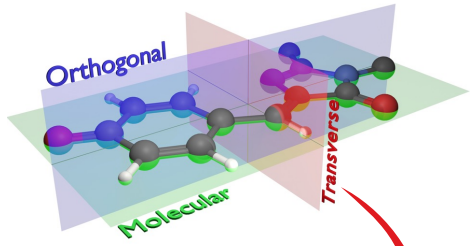


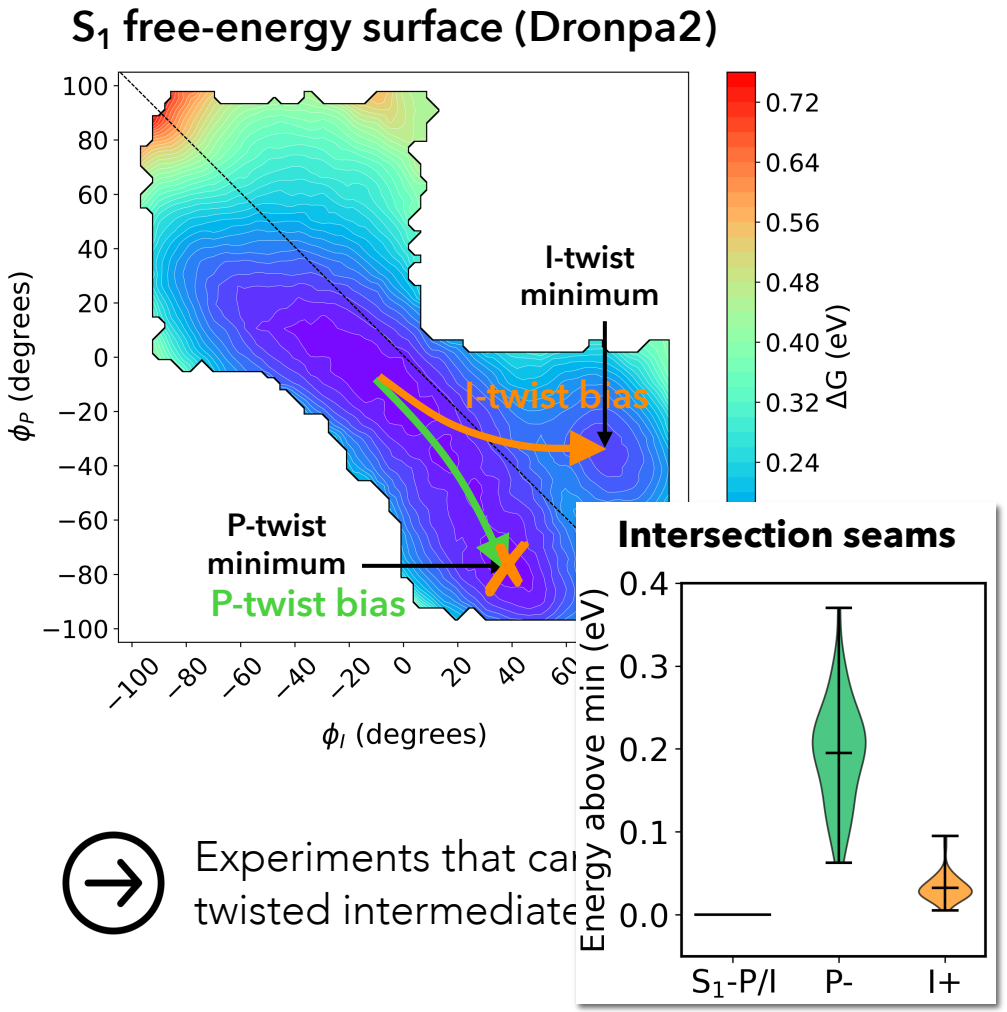
More space around the P-ring and the space is more symmetrically distributed

Jones, **NHL**, Martínez, *J. Am. Chem. Soc.* 2022, 144, 12732; Jones, **NHL**, Martínez, *Chem. Sci.* 2021, 12, 11347

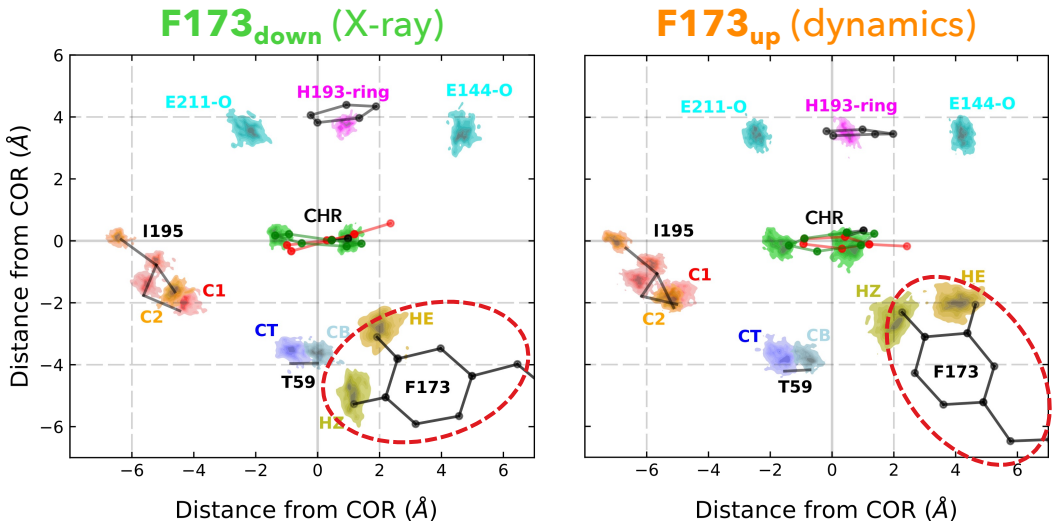
QM/MM(Amber ff14SB)-AIMS/α(0.64)-SA3-CASSCF(4,3)/6-31G*

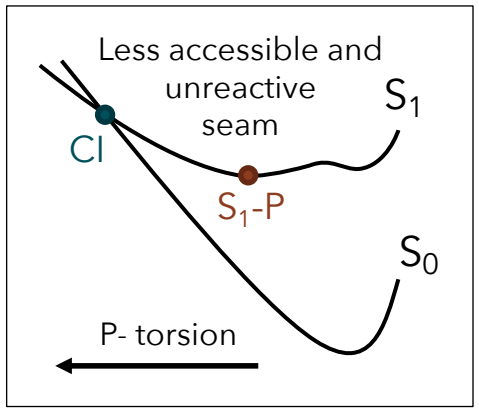
Structural biasing of excited-state pathways

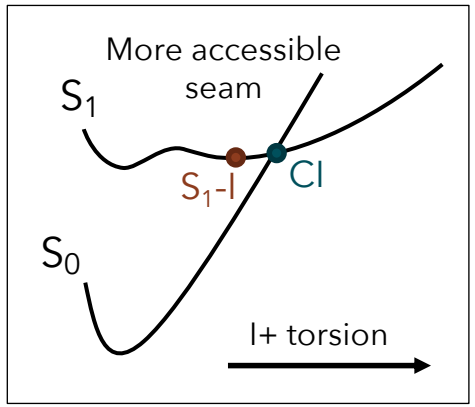




Experiments that can
twisted intermediate







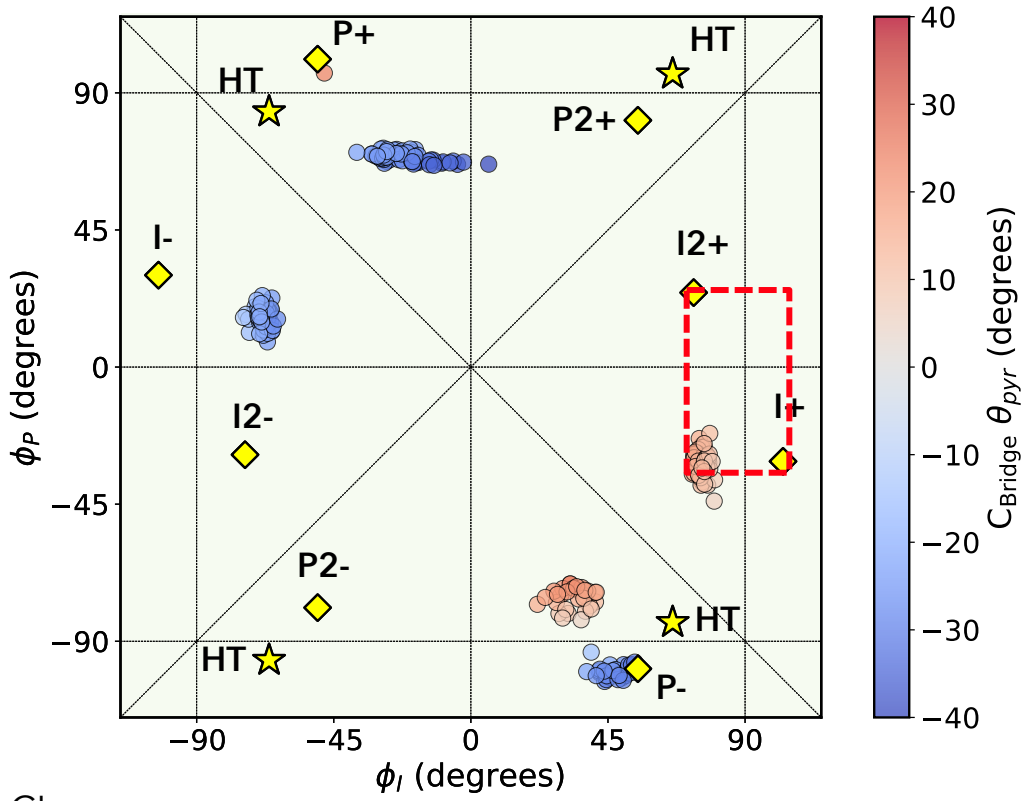
Twisted intermediates?
(unreactive seam)

Unreactive seam?

Couto, **NHL**, in preparation QM/MM(Amber ff14SB)-AIMS/α(0.64)-SA3-CASSCF(4,3)/6-31G*

How the protein affects the intersection seam

MECI maps: gas phase vs. Dronpa2

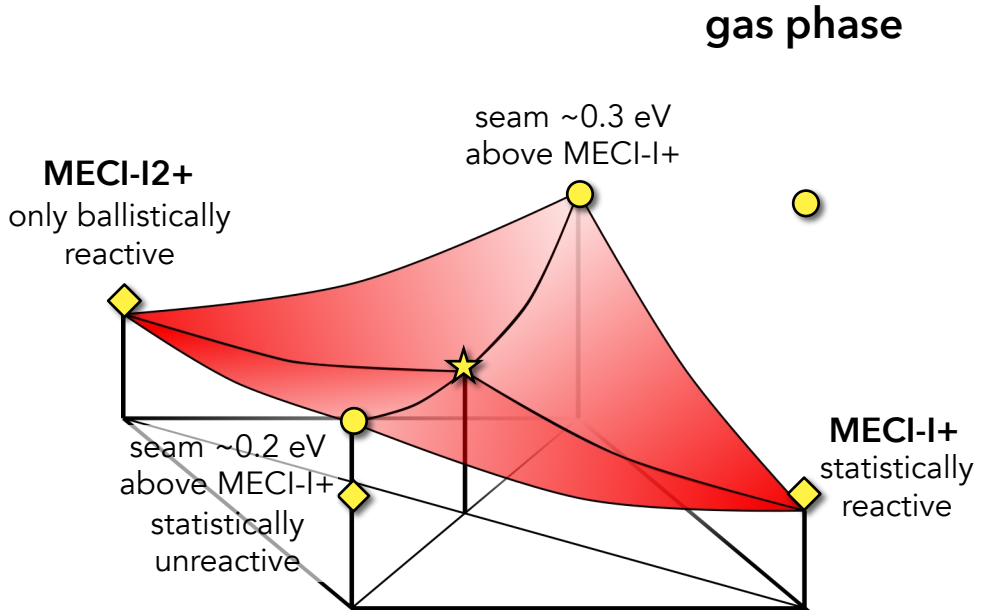


CI

MECI

seam TS

Schematic of I-twisted seam



Couto, **NHL**, In preparation

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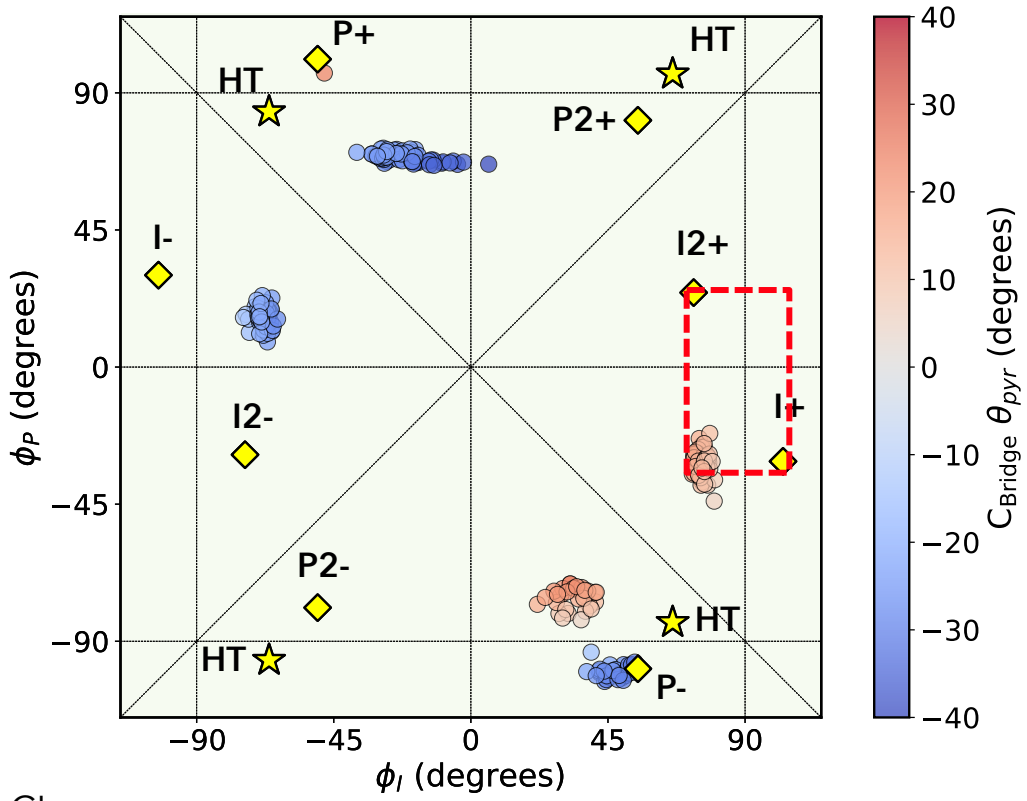

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MECI

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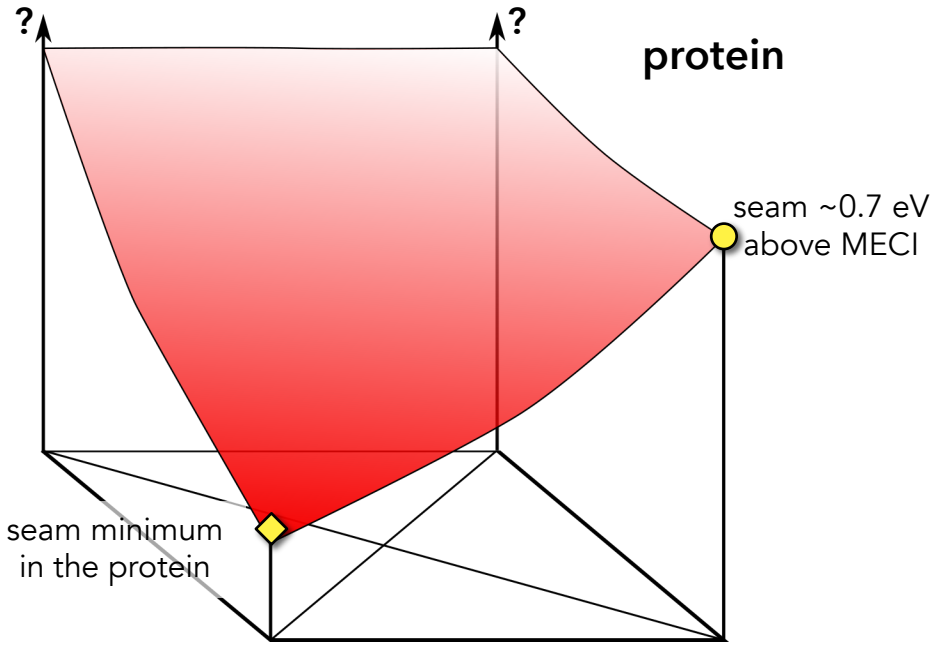
Schematic of I-twisted seam

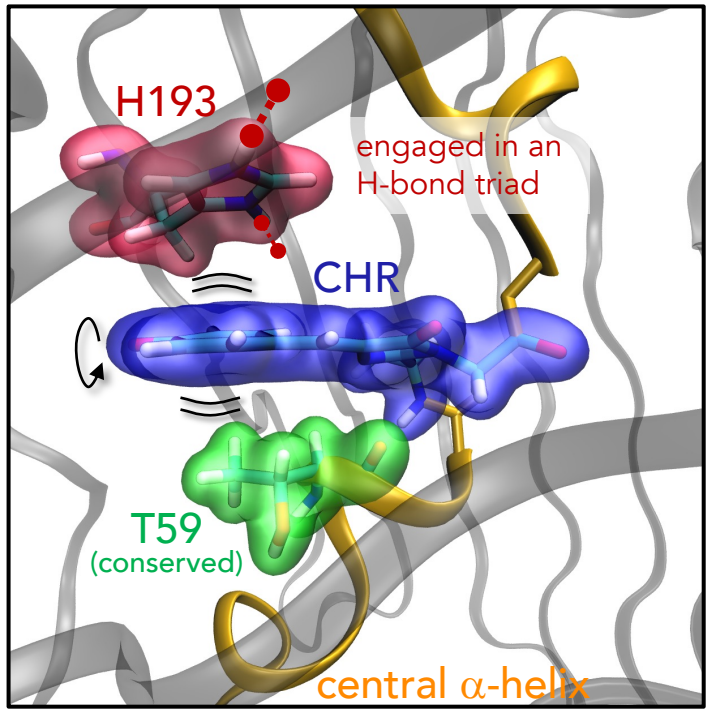


Unreactive part of I-twisted seam becomes minimum in the protein!

Couto, **NHL**, In preparation

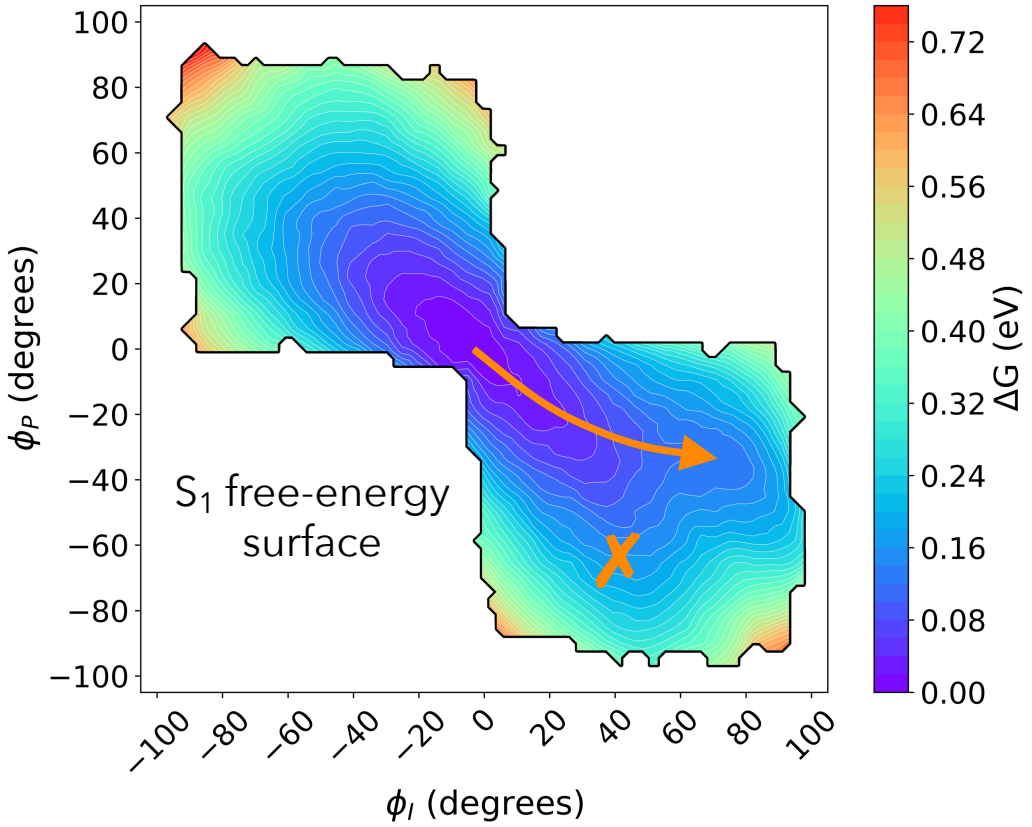
Photoisomerization bottlenecks

The jam in a sandwich cracker



fold hinders access to the
reactive part of I-twisted CI seam

2,3,5-F_3-Dronpa2



✓ Pathway selectivity

✗ Photoreactivity

Couto, **NHL**, In preparation

Where we are headed

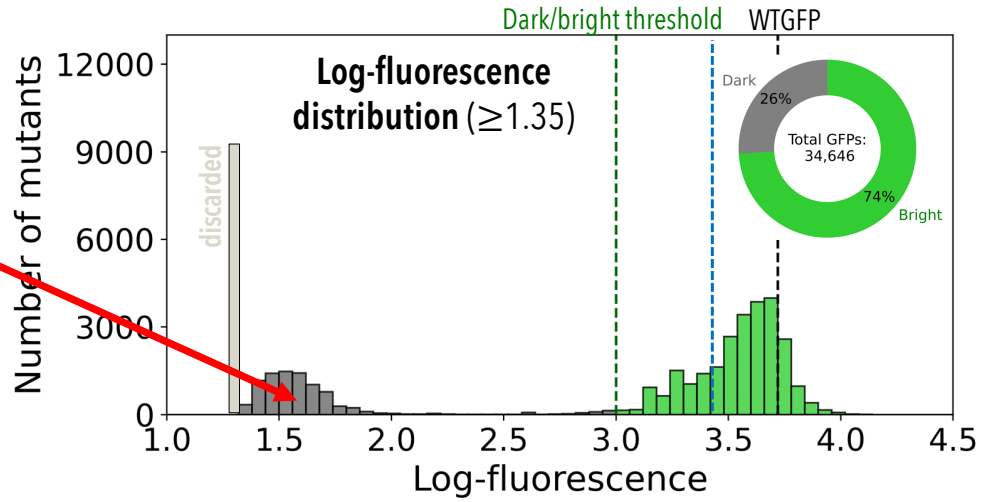
How far can we reshape the GFP fitness landscape toward photoisomerization?

Uncovering the "darkness"

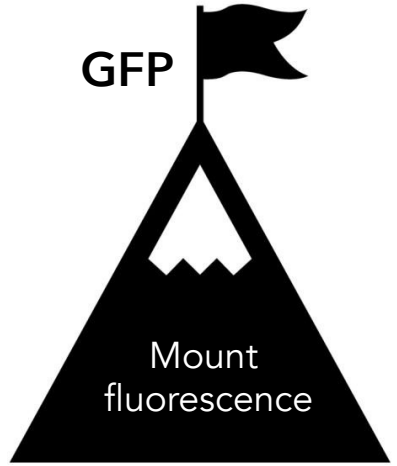
- Any efficient nonradiative decay involving photoisomerization?

OR is it "just"

- Protonated chromophore?
- Immature chromophore?
- Lack of protein folding?



GFP expt. data set: Sarkisyan *et al.*, *Nature,* 2016, 533, 397



How to escape the photofunctional optimum of the natural template…

Acknowledgments

KTH Royal Institute of Technology

Rafael Couto
Pratip Chakraborty
Martin van Horn
Athea Leiler Thomas

Collaborators (theory)

Chey M. Jones, Stanford
Todd J. Martínez, Stanford
Hayley Weir, Stanford
Keiran Thompson, Stanford
Alice Walker, Wayne State

Collaborators (experiment)

Jacob Kirsh, Stanford
Steven G. Boxer, Stanford
Michael Westberg, Aarhus

Thank you!

Two openings

- 2-year postdoctoral fellowship
- 4-year PhD position

Topic: theory for electronic spin-dependent nonadiabatic dynamics

nalist@kth.se

VILLUM FONDEN

Knut and Alice Wallenberg Foundation

Swedish Research Council

NAISS

HBDI⁻ dislikes hula-twist