Università di Pisa

MoLECoLab
PISA

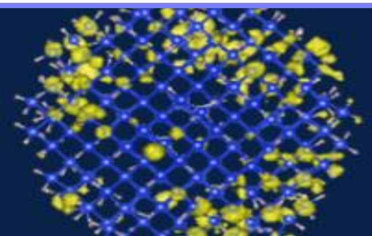
What controls excitation energy transfer in xanthophyll-binding microbial rhodopsins?

Structural and spectroscopic insights from multiscale modeling

Laura Pedraza-González

laura.pedraza@unipi.it

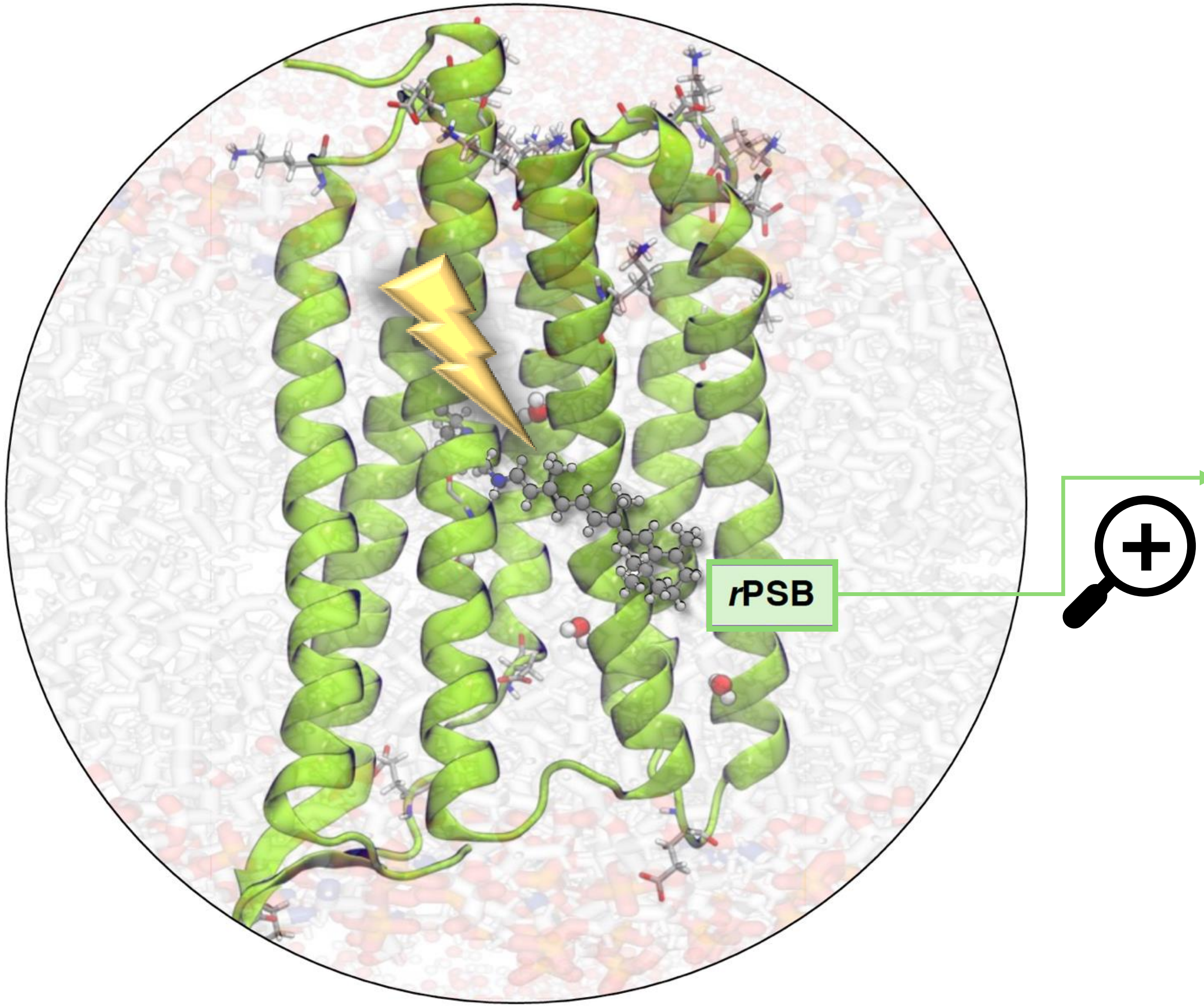
25/02/2026



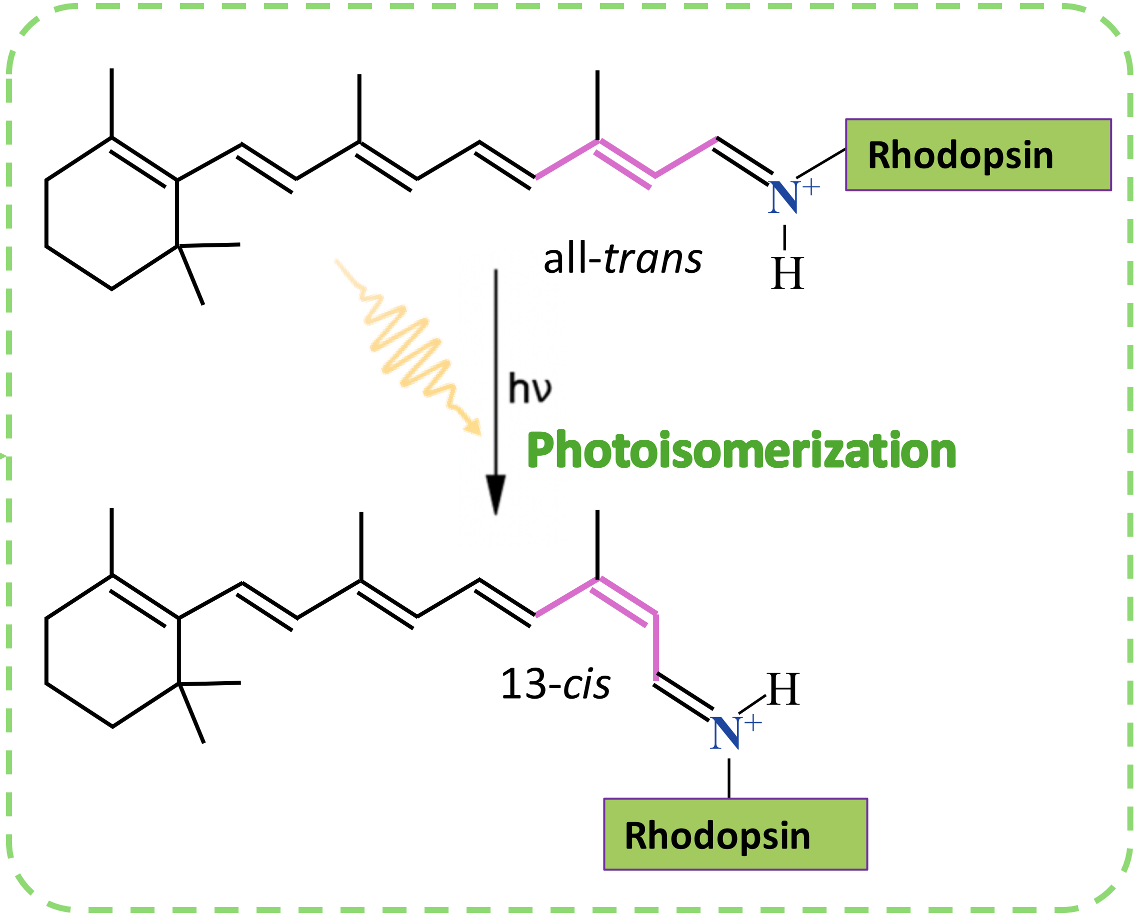
Virtual International Seminar on Theoretical Advancements (VISTA)

Rhodopsins: Nature's molecular translators of light

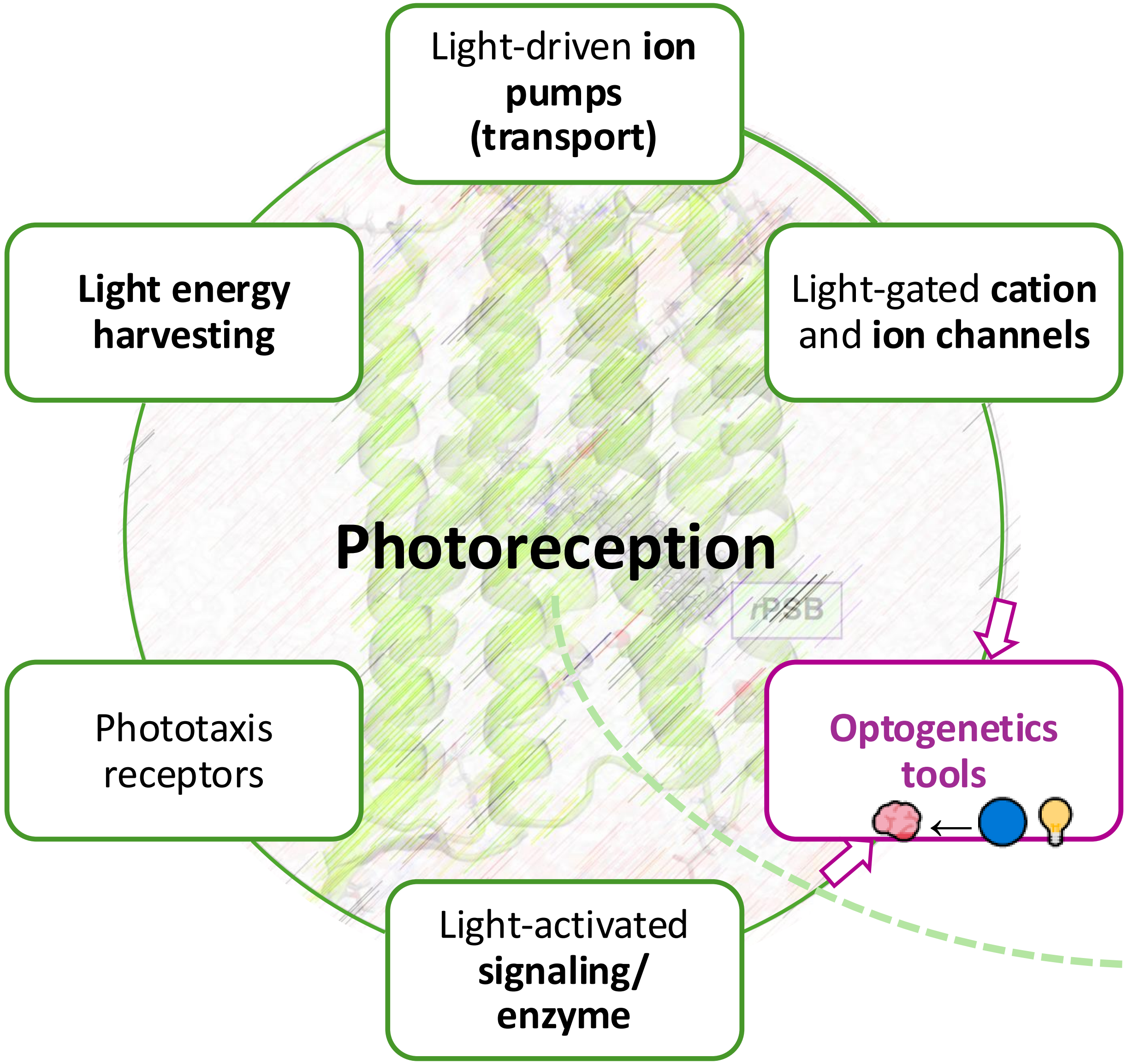
The photoreceptor:
7 α-helix transmembrane



The photoswitch:
Retinal protonated Schiff-base **(rPSB)**



Rhodopsins: Diversity and biological functions



Light-driven **ion pumps (transport)**

Light energy harvesting

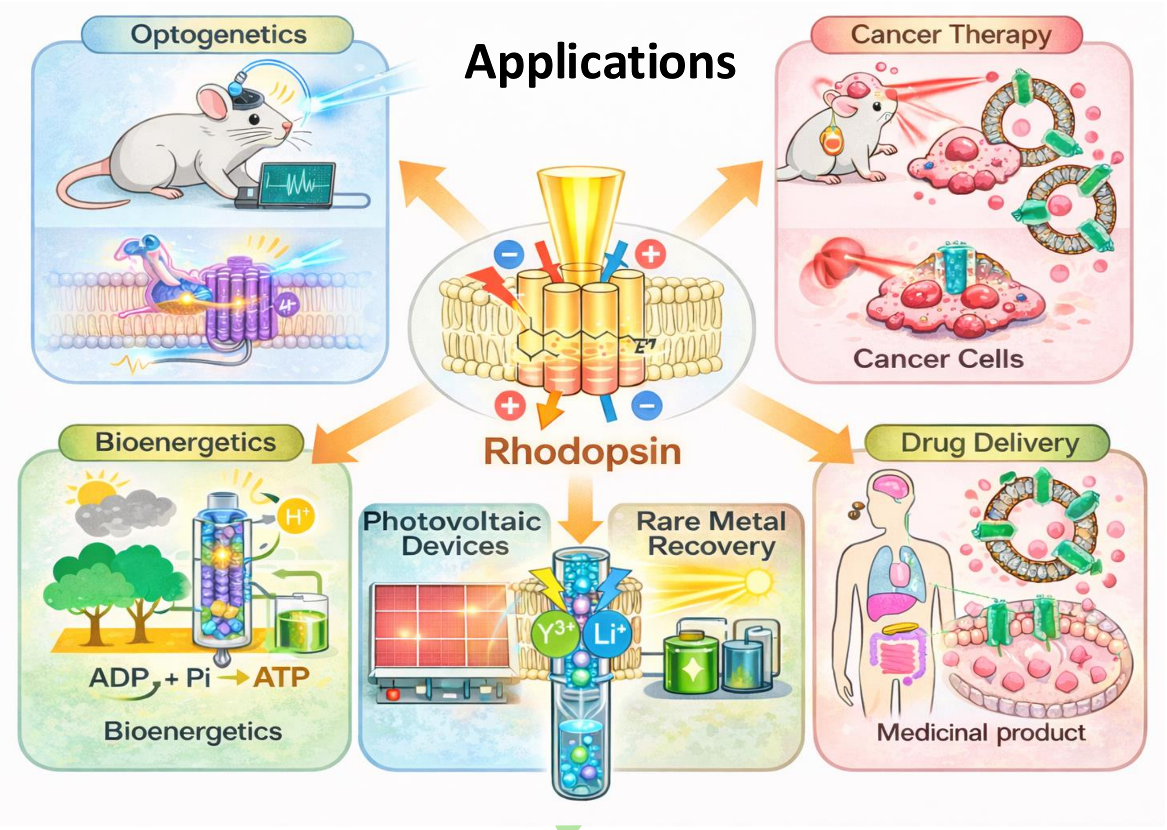
Light-gated **cation** and **ion channels**

Photoreception

Phototaxis receptors

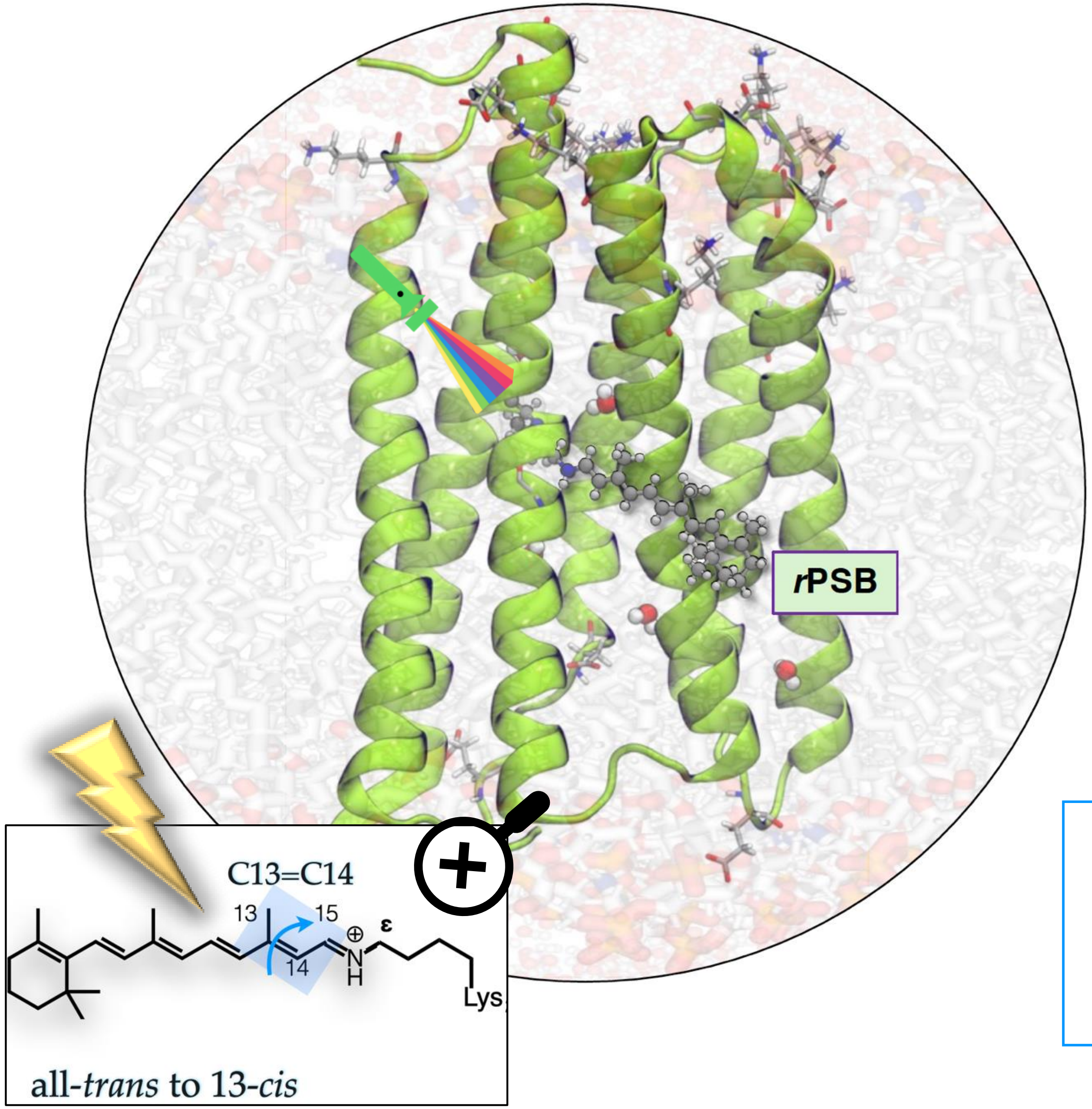
Optogenetics tools

Light-activated **signaling/ enzyme**

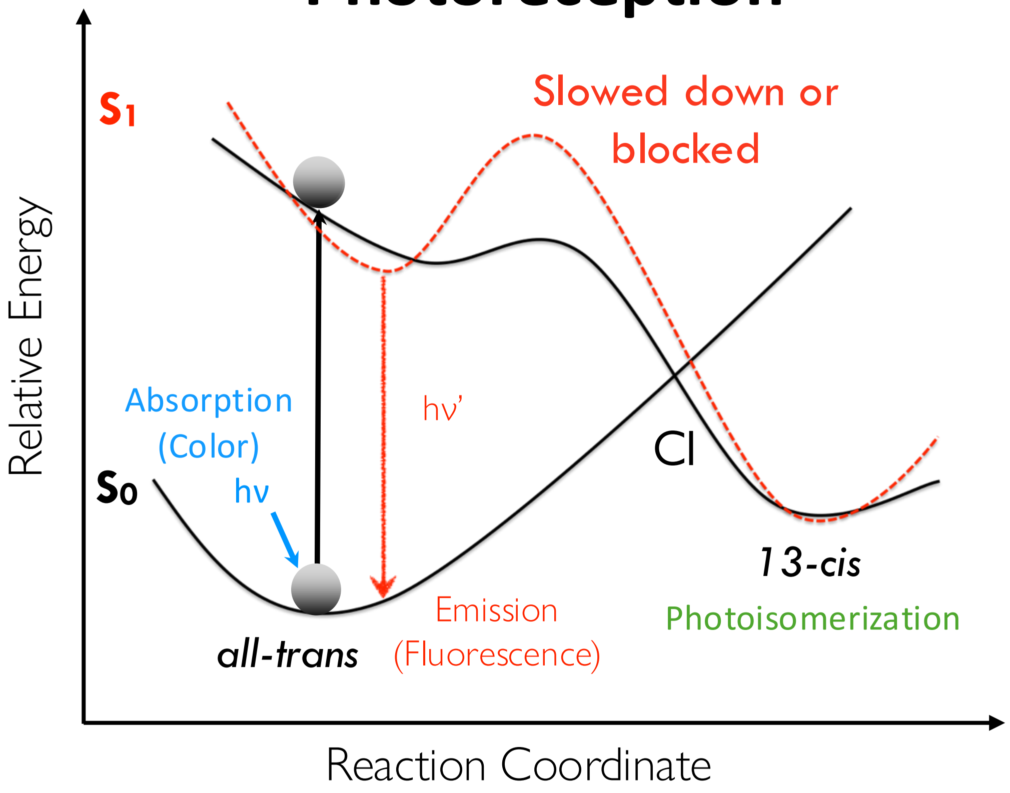


Ernst, O., et al. *Chem. Rev.* 2014, 114, 1, 126–163
K. Keiichi, A. Shibukawa, and Y. Sudo. Biochemistry 2019, 59(3), 218-229
Zhao, H., et al. *Adv. Photonics* 2025, 7(5), 054001-054001

How rhodopsins (retinal) respond to light



Photoreception



Absorption wavelength diversification **(Color)**

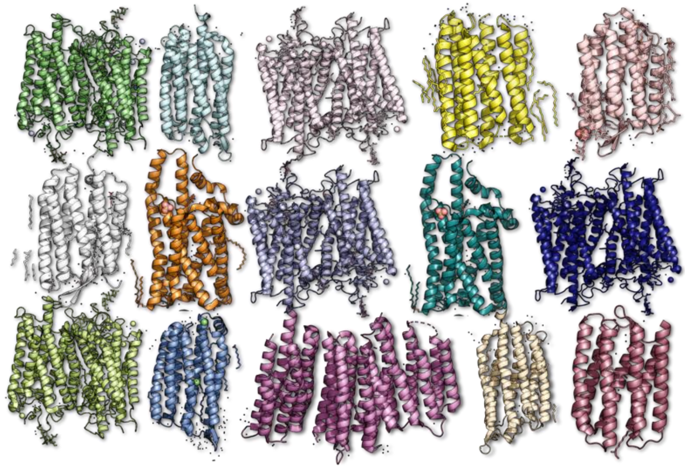
Emission wavelength diversification **(Fluorescence)**

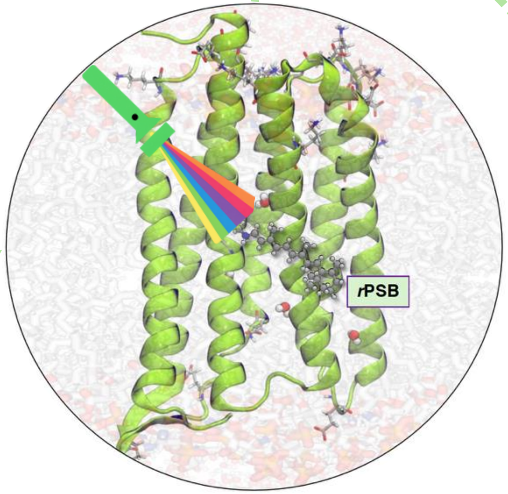
Photo-isomerization **(Quantum Yield)**

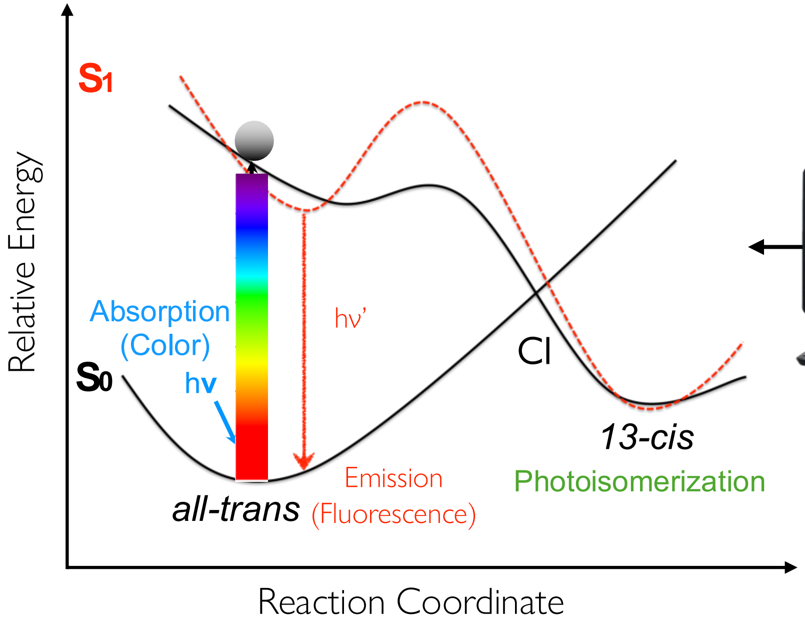
L. Pedraza-González; L. Barneschi; D. Padula; L. De Vico; M. Olivucci. *Top Curr Chem,* **2022**, *380(2),* 21

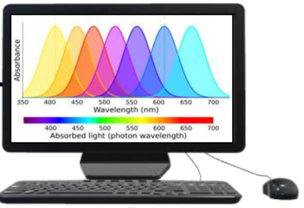
How nature fine-tunes rhodopsin properties

Properties of **retinal** chromophore modulated by the **protein sequence**





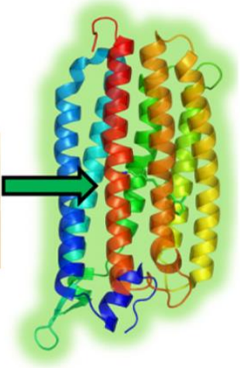




Prof. Massimo Olivucci

LCPP

Automatic Rhodopsin Modeling protocol (ARM)



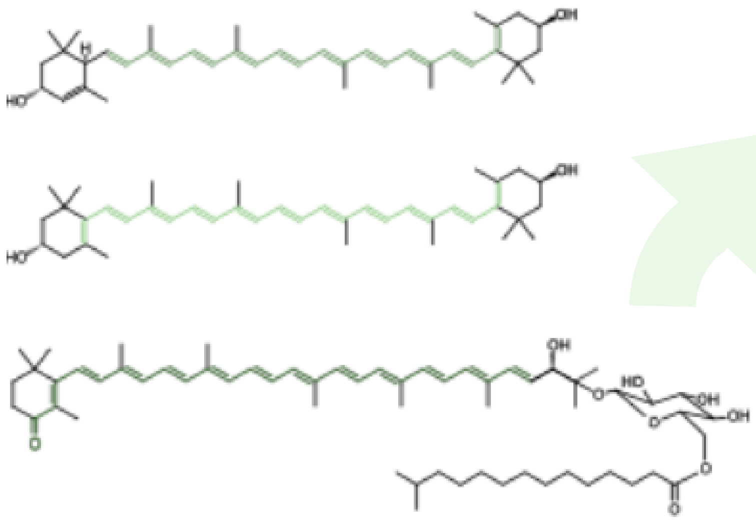
L. Pedraza-González; L. De Vico; M.d.C. Marín; F. Fanelli; M. Olivucci. *J. Chem. Theory Comput.* **2019**, 15 (5), 2847–2862

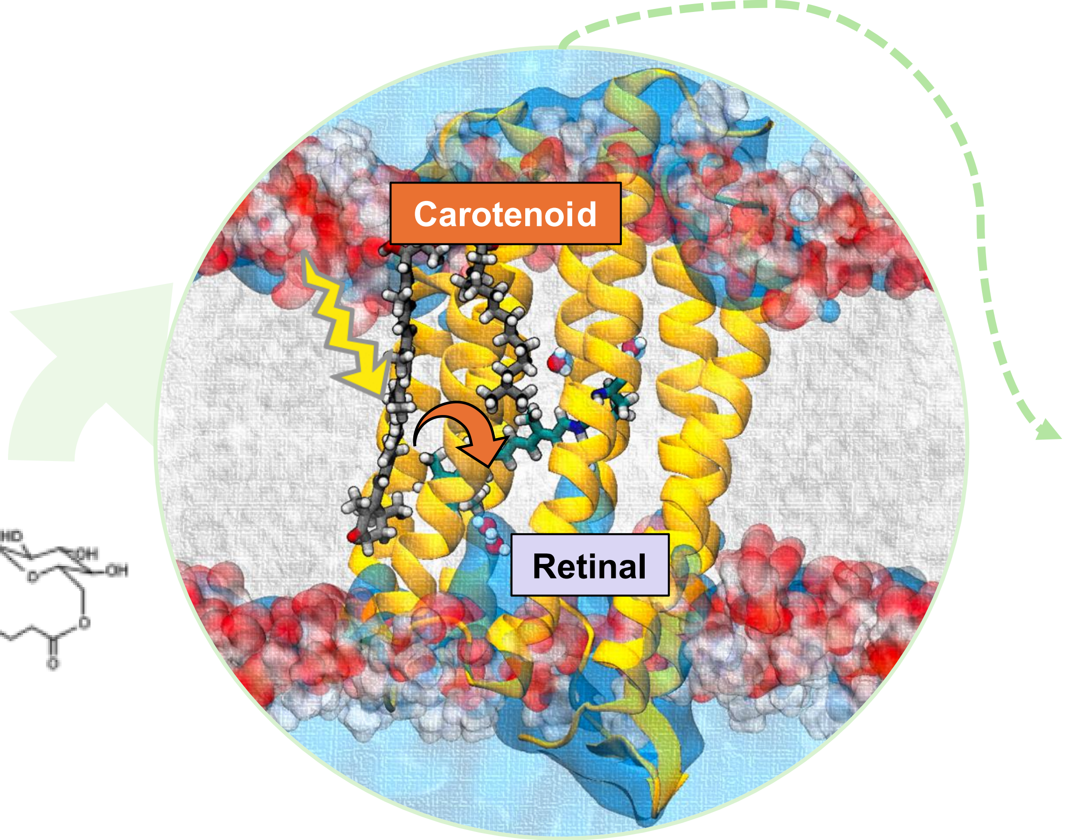
L. Pedraza-González; L. Barneschi; D. Padula; L. De Vico; M. Olivucci. *Top Curr Chem,* **2022**, *380(2),* 21

L. Pedraza-González; L Barneschi; M. Marszałek; D. Padula; L. De Vico; M. Olivucci. *J. Chem. Theory Comput*. **2023**, 19 (1), 293-310

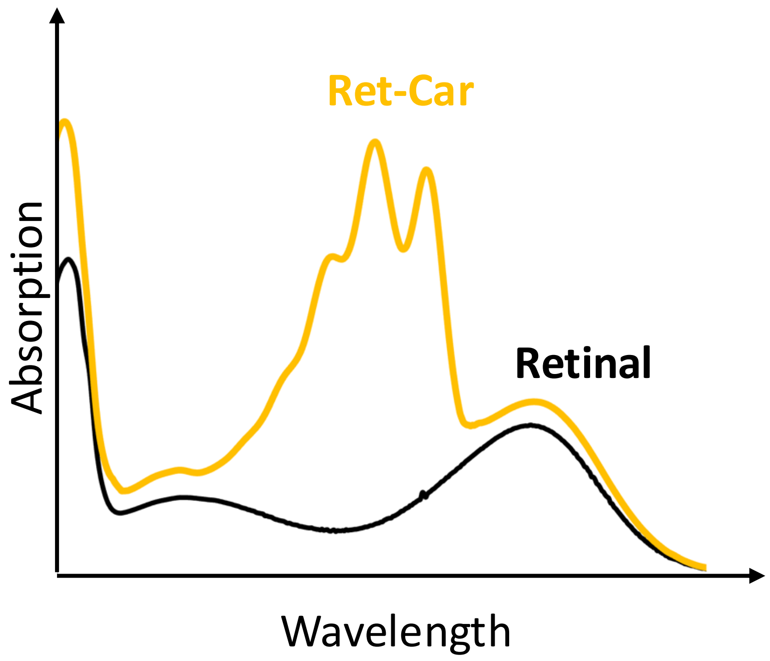
Carotenoid Antenna Rhodopsins

Light harvesting via **carotenoid → retinal** energy transfer



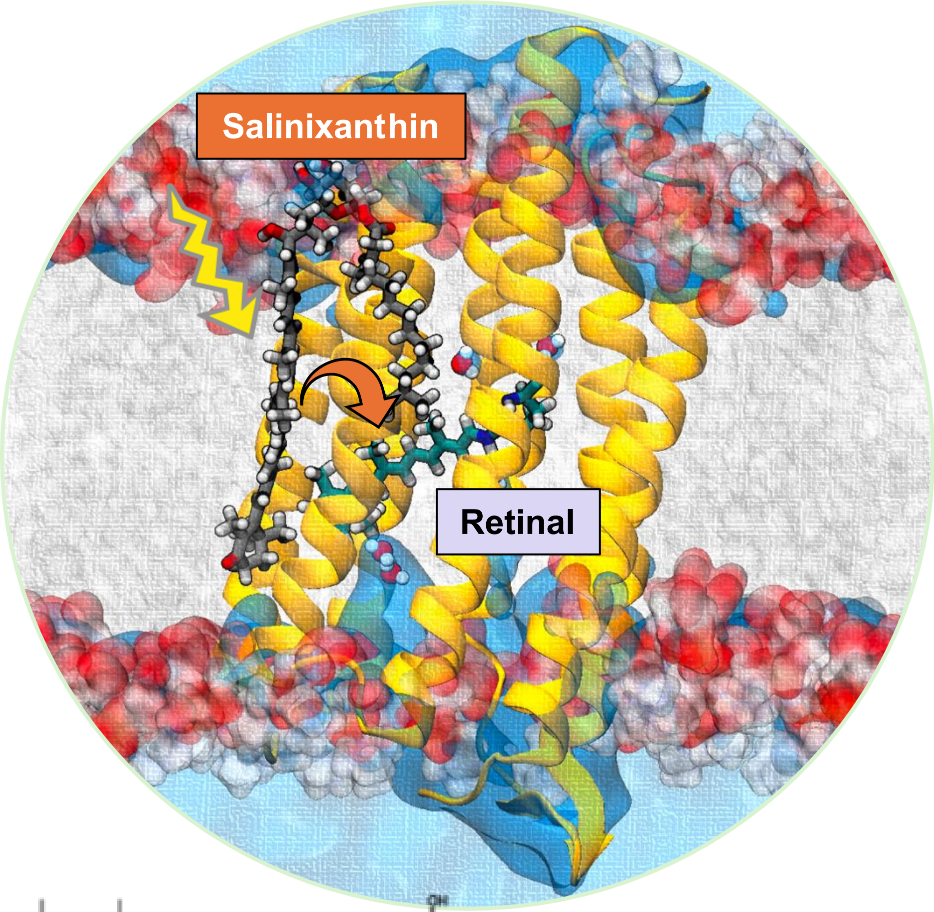


Expanded absorption window



Carotenoids extend the spectral range and transfer excitation to retinal in < 100 fs

*Sr*XR: The first Carotenoid-Assisted Rhodopsin (early 2000s)



Discovery

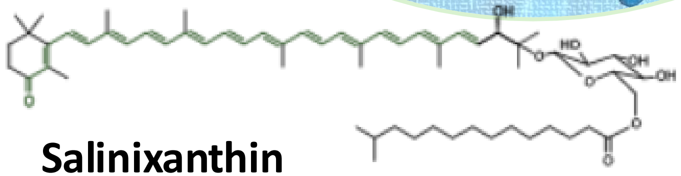
- Identified in halophilic bacterium *Salinibacter ruber*
- Adaptation to high-salinity, high-irradiance environments

Specificity

- Binds 4-keto xanthophyll salinixanthin
- Other carotenoids do not support EET

Photophysics

- Ultrafast Car → Ret EET
- Efficiency ≈ 40%

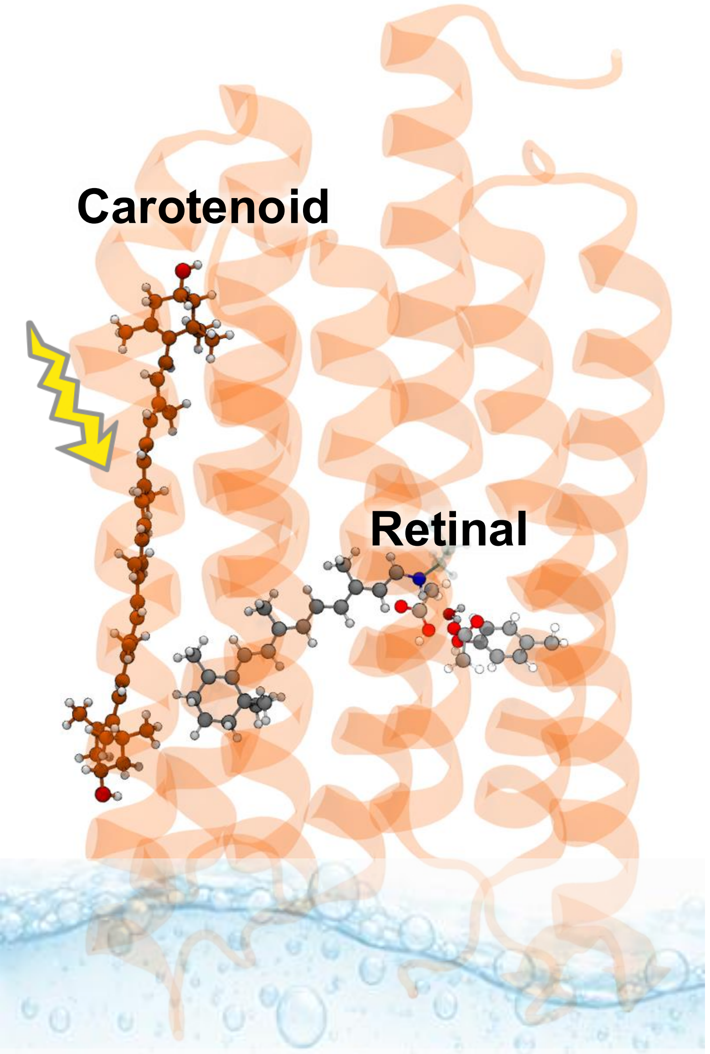


Salinixanthin

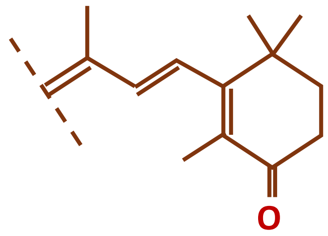
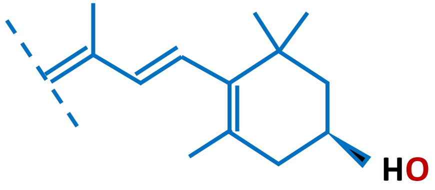
Conclusion at the time: The 4-keto group is essential for EET

S. P. Balashov; E. S. Imasheva; V. A. Boichenko; J. Antón; J. M. Wang; J. K. Lanyi. Science **2005**, 309 (5743), 2061–2064.

Kin4B8: Broad binding, selective energy transfer (2023)



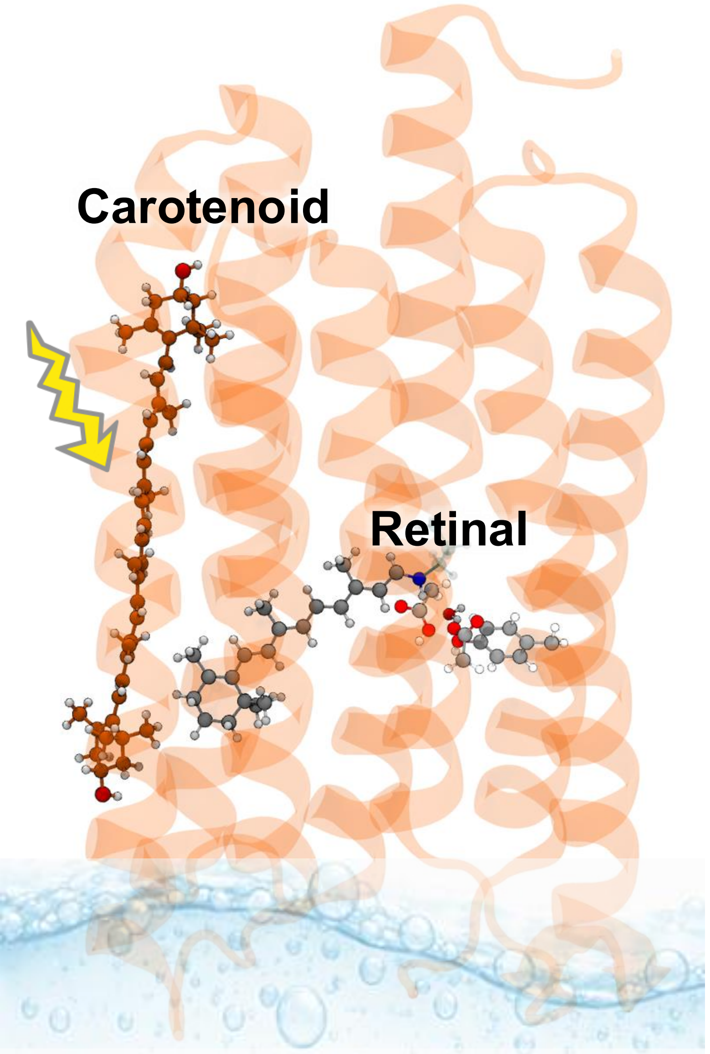
Light-driven proton pump
from a freshwater bacterium

	Binding	**Energy transfer**
4-Keto		- No detectable EET - No contribution to light harvesting
3-Hydroxylated		- Ultrafast EET (<100 fs, ~56%) - Extends spectral coverage

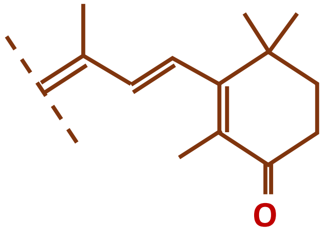
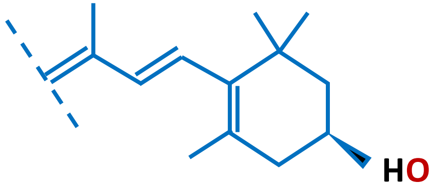
Conclusion: 4-keto group is not required for EET

Chazan, A. et al. *Nature*. **2023**, 615, 535–540
I. Das; et al. JACS Au **2025**, *5* (7), 3070–3081

Kin4B8: Broad binding, selective energy transfer (2023)



Light-driven proton pump
from a freshwater bacterium

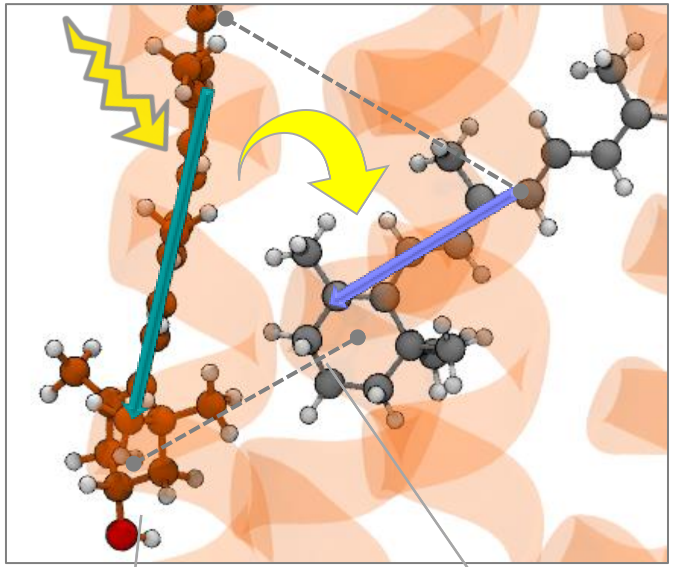
	Binding	Energy transfer
4-Keto		- No detectable EET - No contribution to light harvesting
3-Hydroxylated		- Ultrafast EET (<100 fs, ~56%) - Extends spectral coverage

What structural, spectroscopic and electronic factors enable EET only in hydroxylated xanthophylls (Kin4B8)?

G. Salvadori, P. Saraceno, A. Santomieri, C. John and L. Pedraza-González, *Chem. Sci.*, **2025**, 16, 18423–18437
G. Salvadori, P. Saraceno, C. John, L. Cupellini and L. Pedraza-González, *ChemRxiv*, **2026**. DOI: 10.26434/chemrxiv.15000075/v1

Mechanism governing Carotenoid → Retinal EET?

$$\text{Car } S_2 \xrightarrow{\text{EET}} \text{Ret } S_1$$



Donor:
Carotenoid

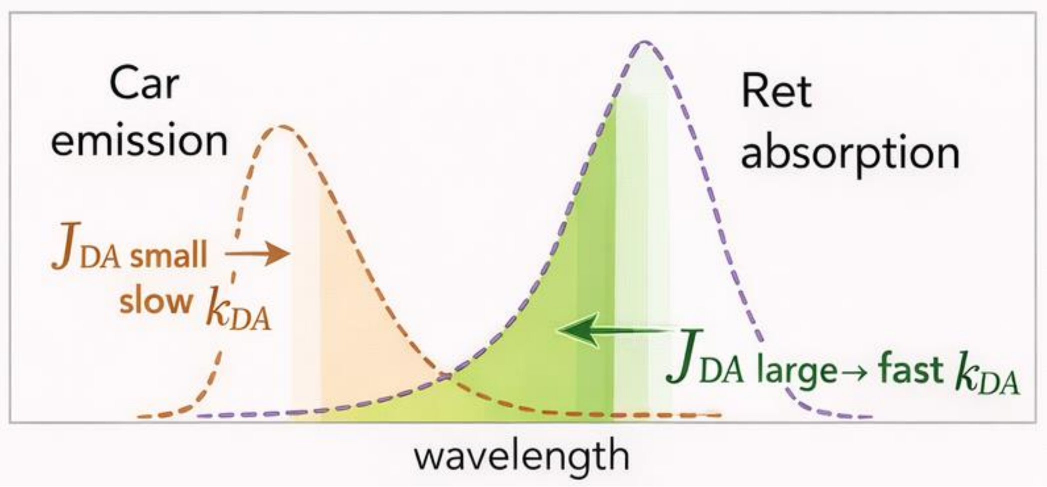
Acceptor: Retinal

Förster Resonance Energy Transfer (FRET)

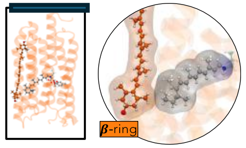
$$k_{DA} \propto |V_{DA}|^2 \times J_{DA}$$

Electronic coupling

Spectral Overlap



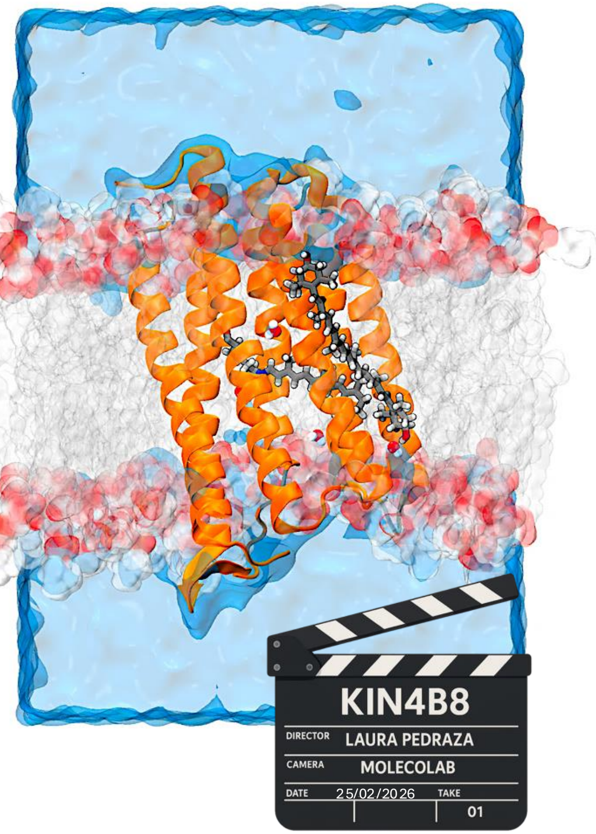
Quantifying V_{DA} and J_{DA} requires an atomistic description of the system



Our solution:

Multiscale computational modeling

Structural sampling: Capturing protein dynamics



Kin4B8-ZEA

Cryo-Em [8I2Z]

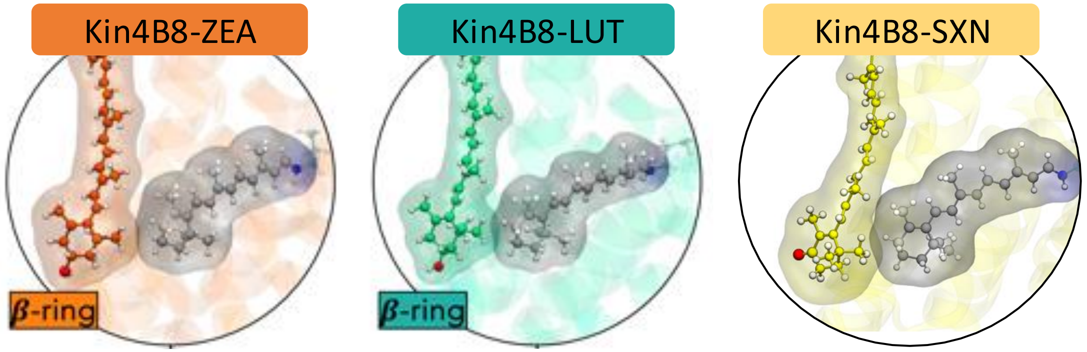
-LUT **-SXN**

Molecular Docking

Molecular Dynamics (MD)

Conformational sampling (1 μs)

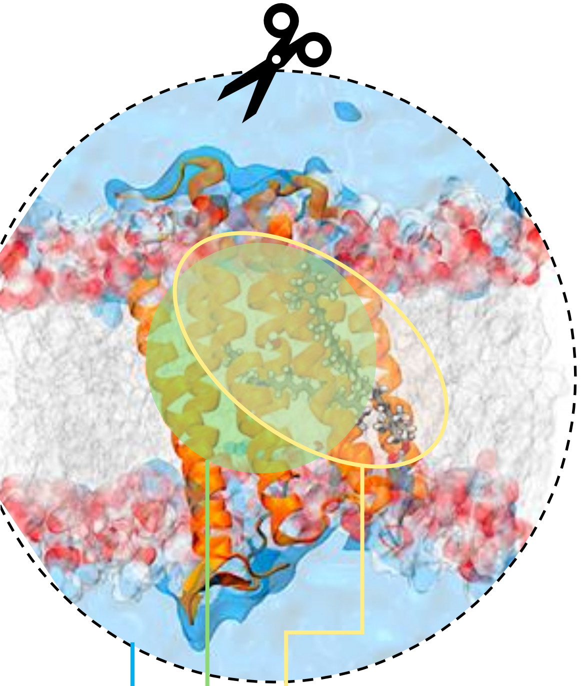
Static structures are not enough: Dynamic ensembles enable computation of spectra and EET rates



G. Salvadori, P. Saraceno, A. Santomieri, C. John and L. Pedraza-González, *Chem. Sci.*, **2025**, 16, 18423–18437

G. Salvadori, P. Saraceno, C. John, L. Cupellini and L. Pedraza-González, *ChemRxiv,* **2026**. DOI: 10.26434/chemrxiv.15000075/v1

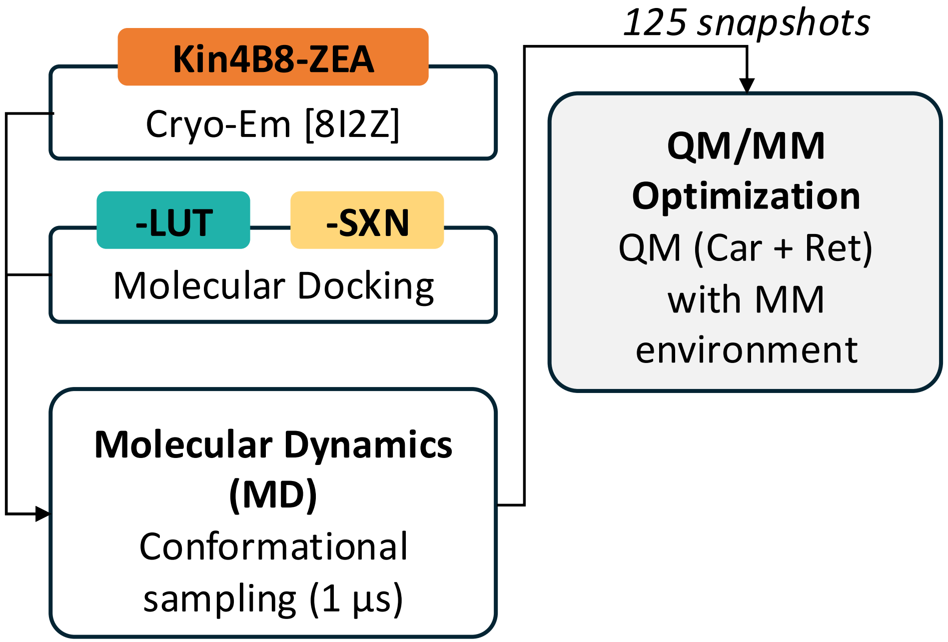
Electronic structure: Chromophore(s) excited-states in Kin4B8



QM:
Car + Ret

MM mobile:
4 Å from Ret

MM fixed:
30 Å from Ret



Kin4B8-ZEA
Cryo-Em [8I2Z]

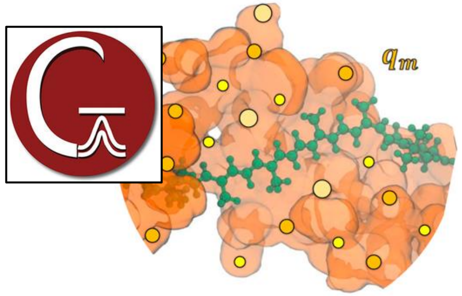
-LUT **-SXN**
Molecular Docking

Molecular Dynamics (MD)
Conformational sampling (1 µs)

125 snapshots

QM/MM Optimization
QM (Car + Ret) with MM environment

OPT@S$_0$ DFT CAM-B3LYP/6-31G(d)//AMBER

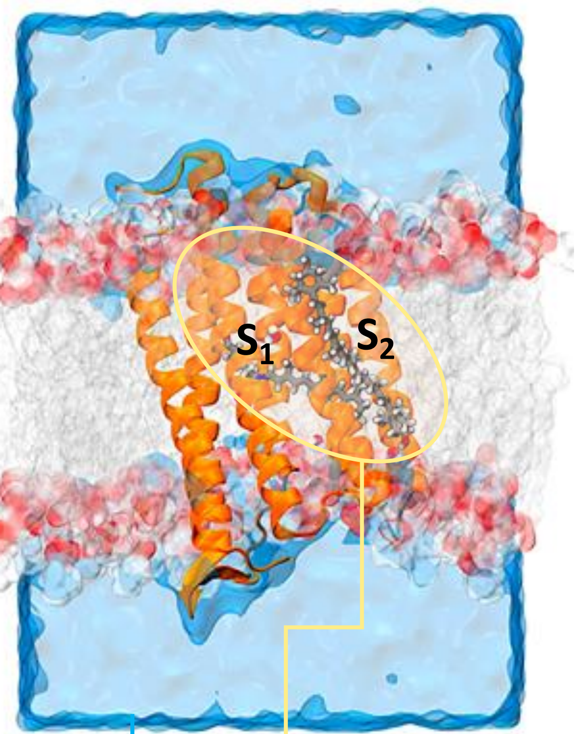


Electrostatic Embedding

Electrostatic interactions between QM & MM subsystems

G. Salvadori, P. Saraceno, A. Santomieri, C. John and L. Pedraza-González, *Chem. Sci.*, **2025**, 16, 18423–18437

Electronic structure: Chromophore(s) excited-states in Kin4B8



QM:
Car or Ret

MM:
Car or Ret and rest of the Kin4B8 system

Kin4B8-ZEA
Cryo-Em [8I2Z]

-LUT **-SXN**
Molecular Docking

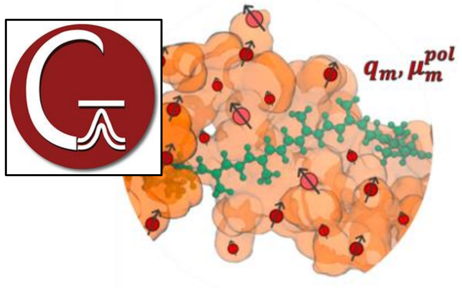
Molecular Dynamics (MD)
Conformational sampling (1 μs)

125 snapshots

QM/MM Optimization
QM (Car + Ret) with MM environment

QM/MMPol
Site energies (Ret S_1, Car S_2)

TD-DFT CAM-B3LYP/6-31+G(d)//MMPol



Polarizable Embedding
(induce dipole approximation)

Electrostatic + mutual polarization between QM & MM subsystems

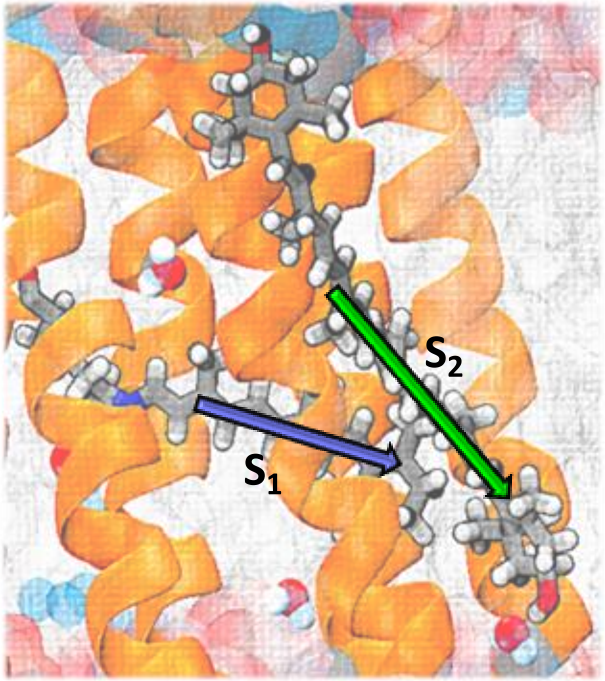

Prof. Benedetta Mennucci

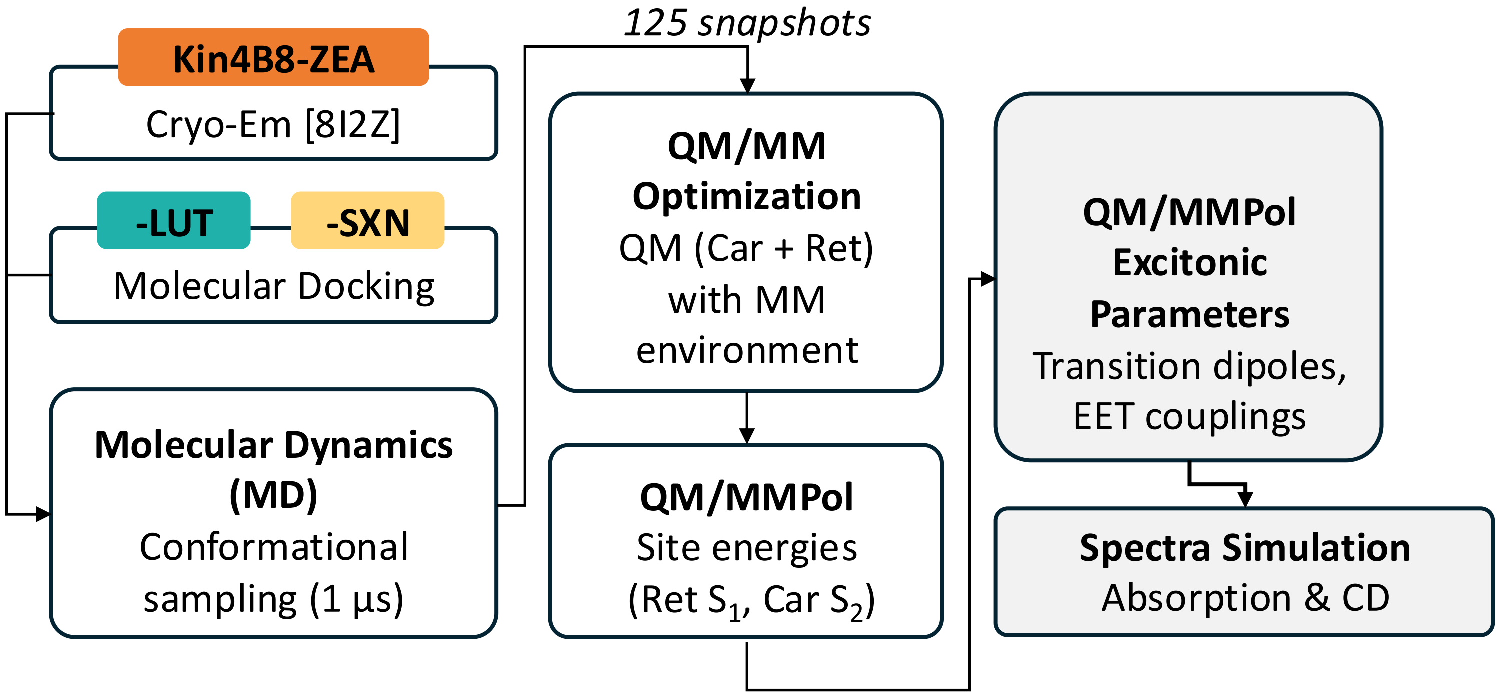

Prof. Filippo Lipparini

Nottoli, M.; Bondanza, M.; Mazzeo, P.; Cupellini, L.; Curutchet, C.; Loco, D.; Lagardère, L.; Piquemal, J.-P.; Mennucci, B.; Lipparini, F, *WIREs Comput. Mol. Sci*. **2023**, 13, e1674.

G. Salvadori, P. Saraceno, A. Santomieri, C. John and L. Pedraza-González, *Chem. Sci.*, **2025**, 16, 18423–18437

Excitonic interactions: From couplings to spectra





Kin4B8-ZEA
Cryo-Em [8I2Z]

-LUT **-SXN**
Molecular Docking

Molecular Dynamics (MD)
Conformational sampling (1 μs)

125 snapshots

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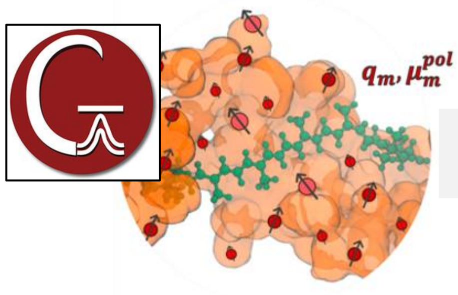
QM (Car + Ret) with MM environment

QM/MMPol
Site energies
(Ret S_1, Car S_2)

QM/MMPol Excitonic Parameters
Transition dipoles, EET couplings

Spectra Simulation
Absorption & CD



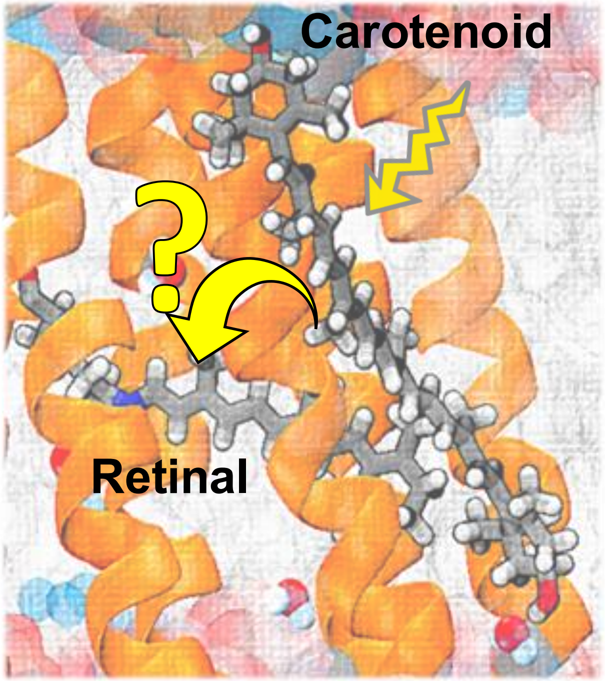
Polarizable Embedding
(induce dipole approximation)

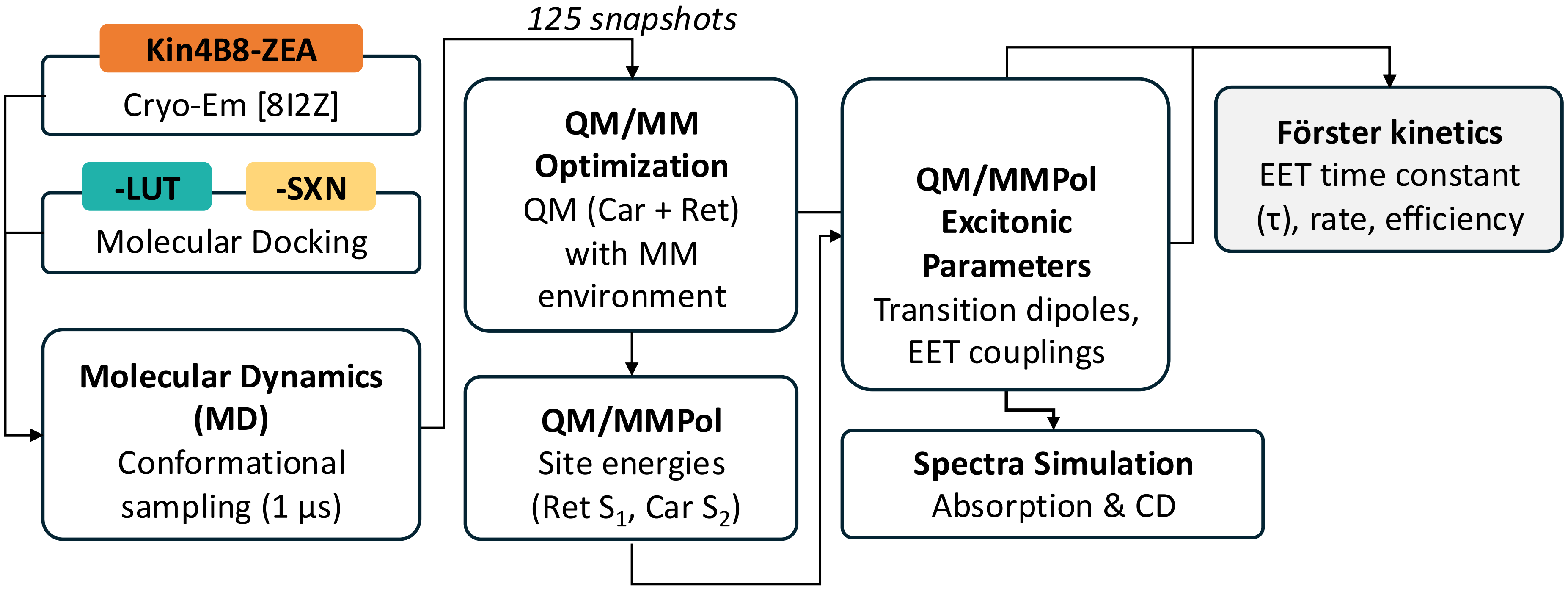
$$\hat{\mathcal{H}}_{\text{exc}} = \sum_i \underbrace{\mathcal{E}_i}_{\text{Site energies}} |i\rangle \langle i| + \sum_{i\neq j} \underbrace{V_{ij}}_{\text{Electronic coupling}} |i\rangle \langle j|$$

https://github.com/Molecolab-Pisa/pyQME/tree/main/pyQME

G. Salvadori, P. Saraceno, A. Santomieri, C. John and L. Pedraza-González, *Chem. Sci.*, **2025**, 16, 18423–18437

Multiscale integration: From structure to EET efficiency





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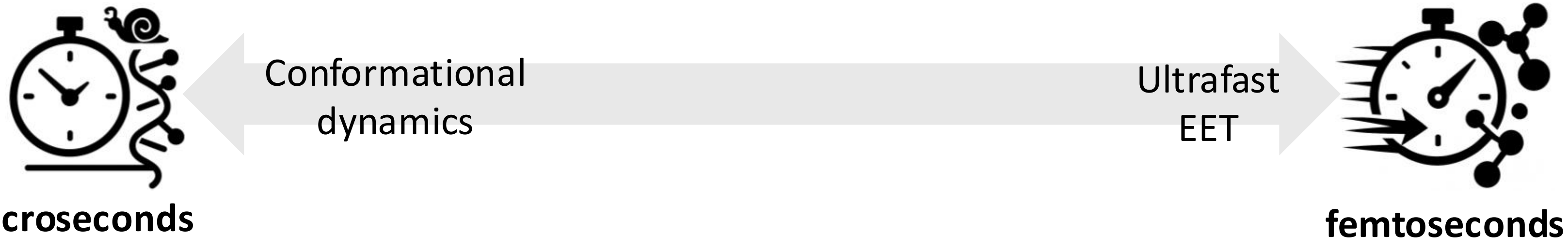
Site energies (Ret S_1, Car S_2)

QM/MMPol Excitonic Parameters
Transition dipoles, EET couplings

Spectra Simulation
Absorption & CD

Förster kinetics
EET time constant (τ), rate, efficiency



Conformational dynamics

Ultrafast EET

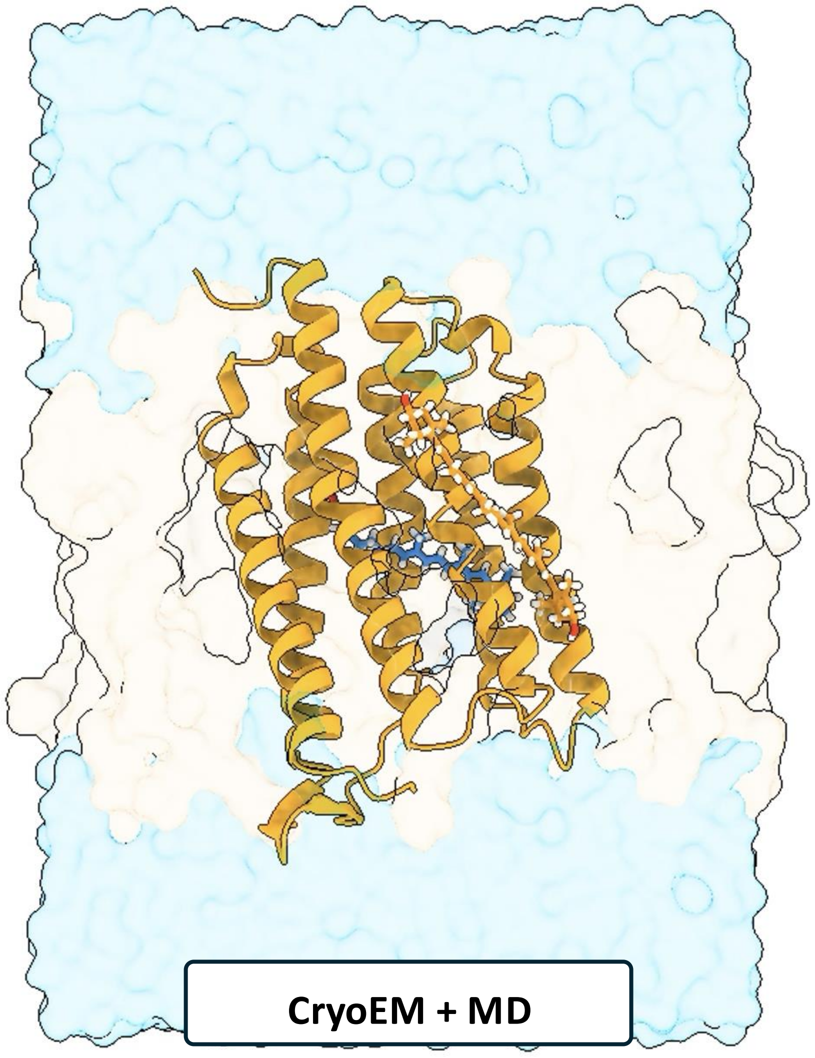
microseconds

femtoseconds

G. Salvadori, P. Saraceno, A. Santomieri, C. John and L. Pedraza-González, *Chem. Sci.*, **2025**, 16, 18423–18437

Conserved Carotenoid binding across Xanthophyll classes

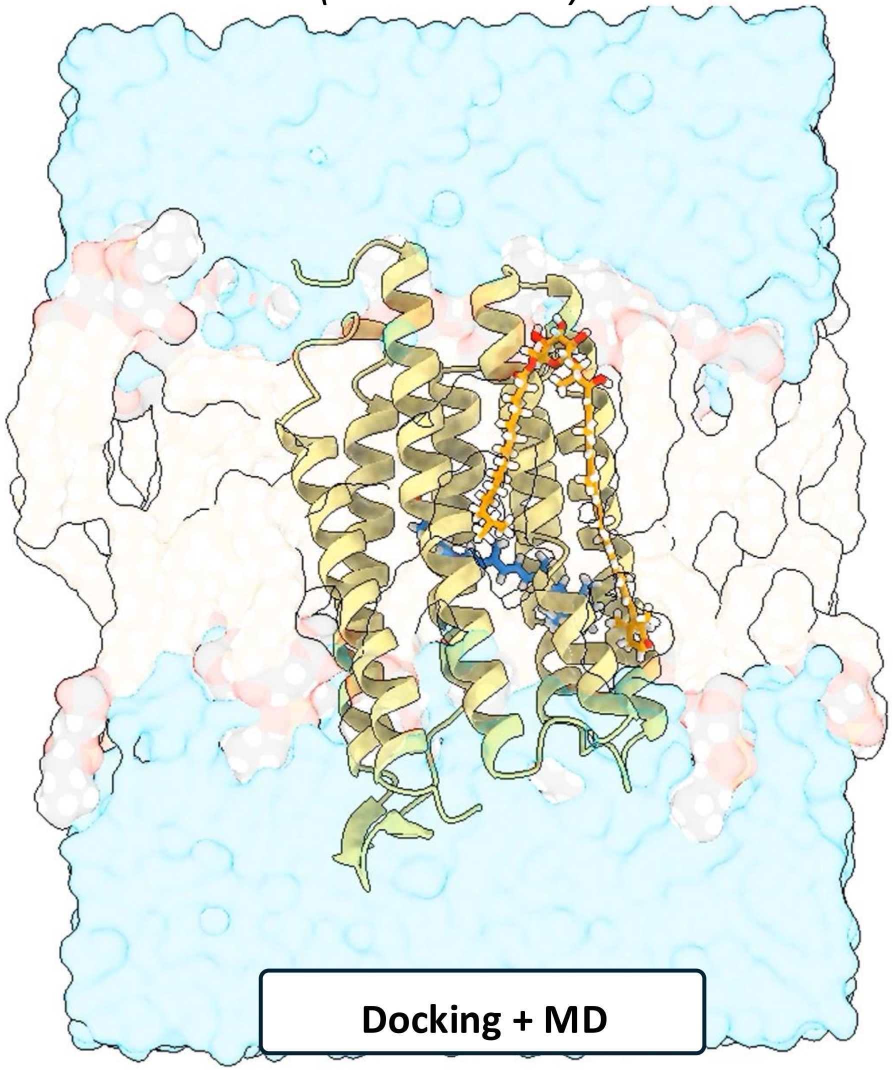
3-Hydroxylated Xanthophylls
(Zeaxanthin)



CryoEM + MD

Dr. Giacomo Salvadori

4-Keto Xanthophylls
(Salinixanthin)



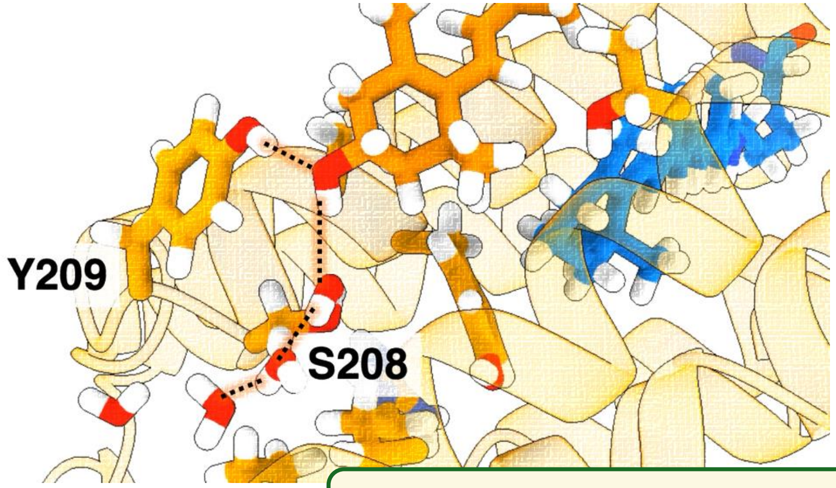
Docking + MD

L. Pedraza-González and coworkers, *Chem. Sci.*, 2025, **16**, 18423–18437

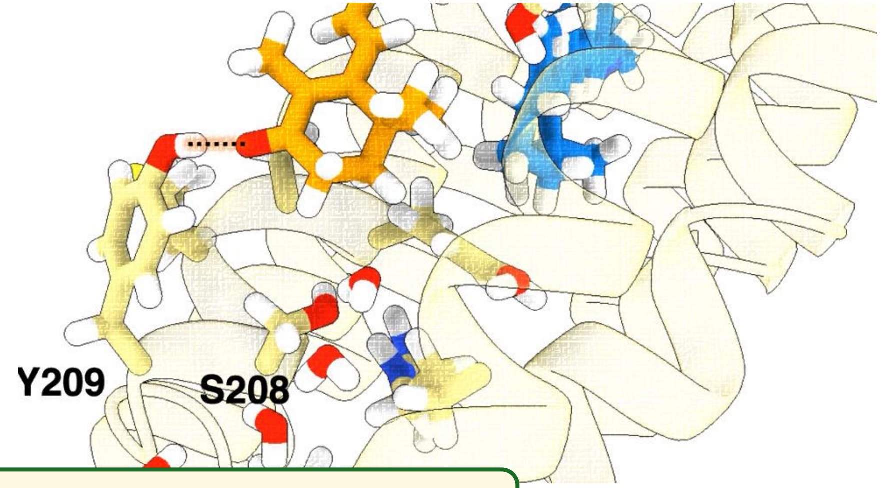
L. Pedraza-González and coworkers, *ChemRxiv,* 2026. DOI:10.26434/chemrxiv.15000075/v1

Conserved Carotenoid binding across Xanthophyll classes

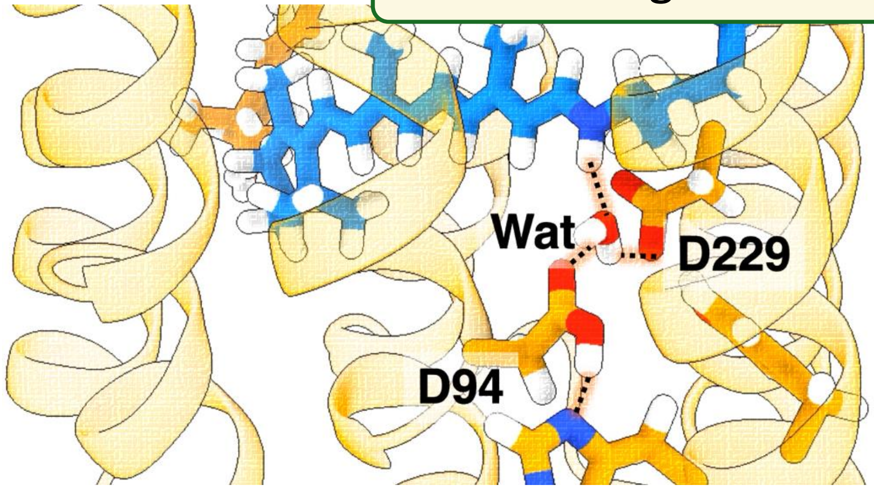
3-Hydroxylated Xanthophylls
(Zeaxanthin, Lutein)

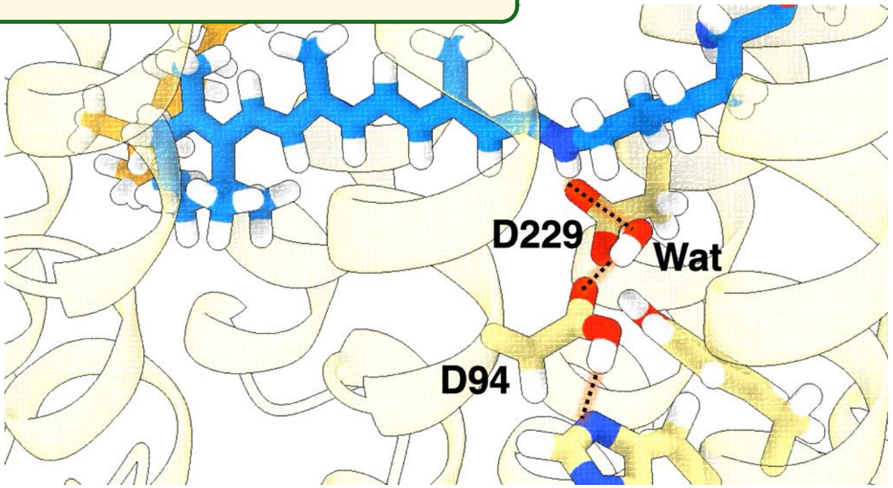


4-Keto Xanthophylls
(Salinixanthin)



Binding is similar… let’s validate our atomistic models!



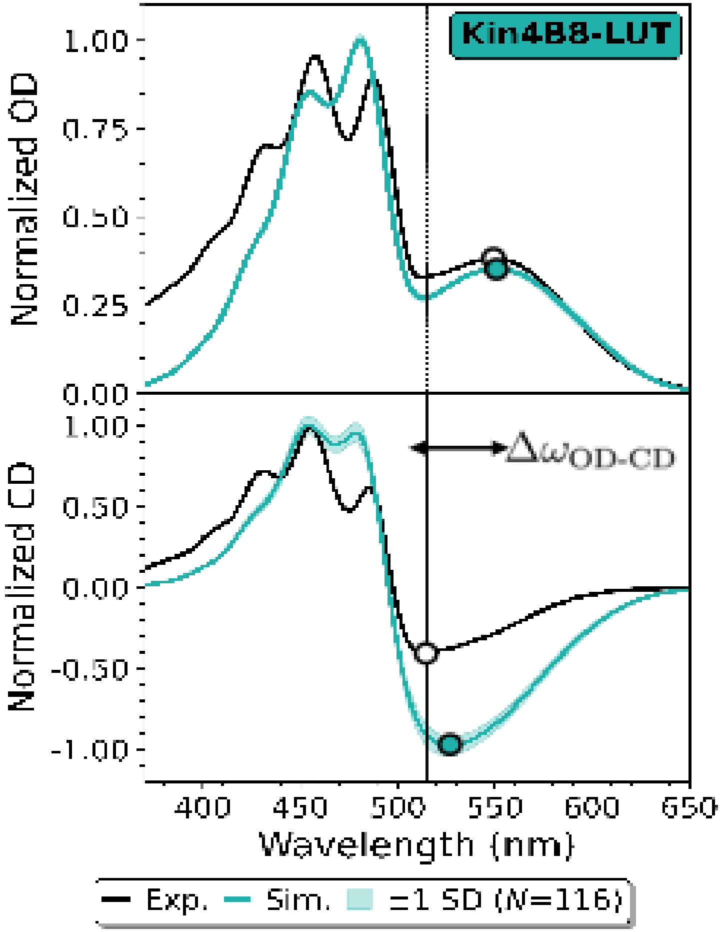


L. Pedraza-González and coworkers, *Chem. Sci.*, 2025, **16**, 18423–18437

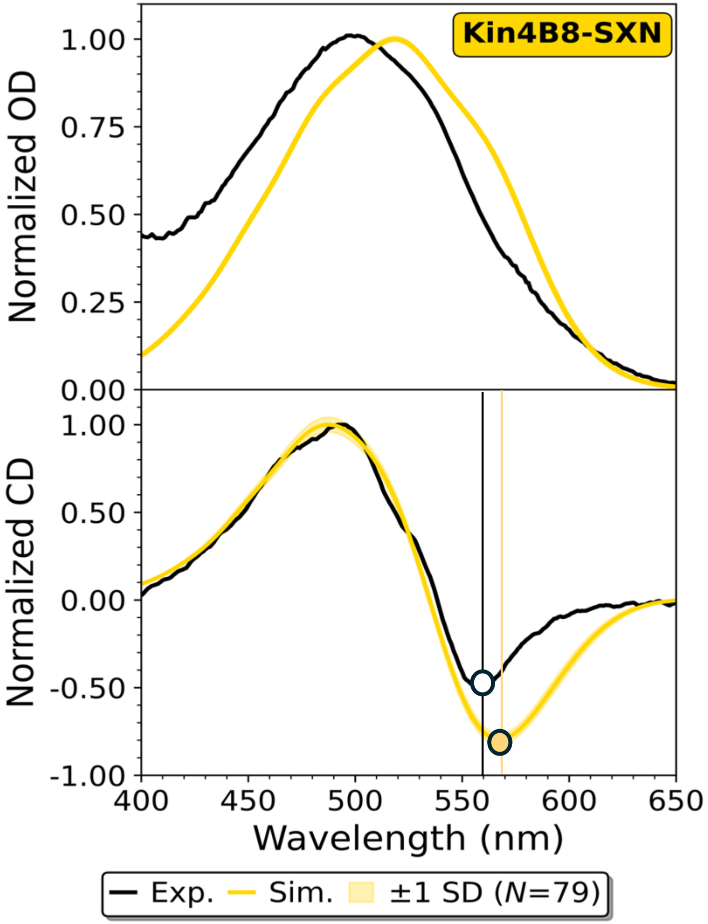
L. Pedraza-González and coworkers, *ChemRxiv,* 2026. DOI:10.26434/chemrxiv.15000075/v1

Distinct optical signatures for 3-OH and 4-Keto Xanthophylls

3-Hydroxylated Xanthophylls



4-Keto Xanthophylls



Piermarco Saraceno

Dr. Chris John

Experimental spectra already distinguish xanthophyll classes

What is the origin of biphasic CD shape?

Why Kin4B8-SXN has a broader absorption?

🟩 Clear vibronic shape

✓ Reduced vibronic shape

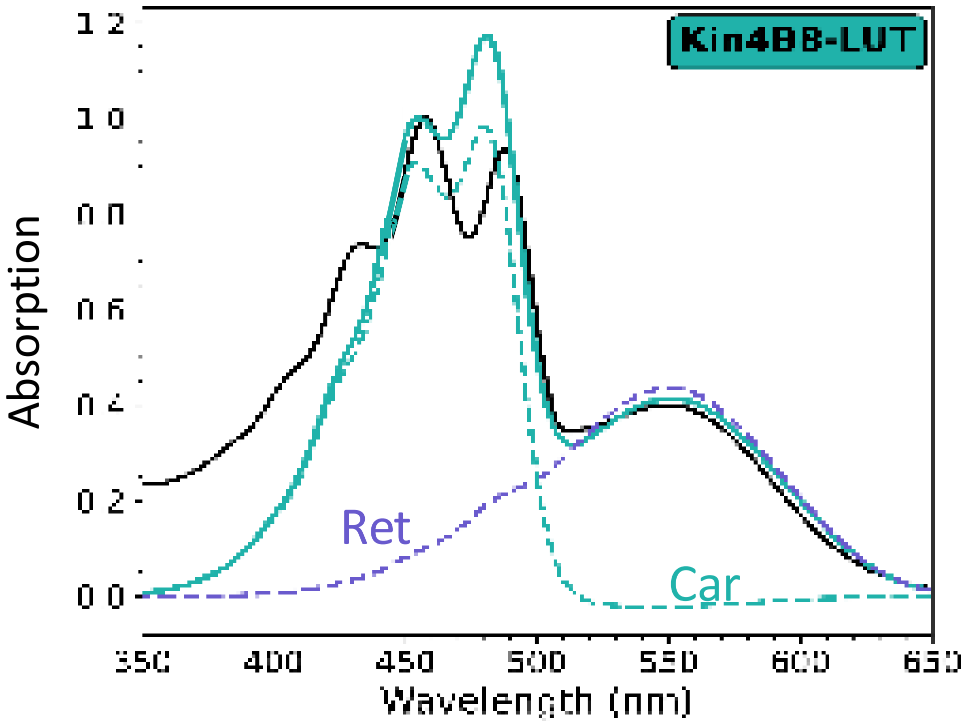
🟩✓ Strong, well-defined biphasic CD is conserved in all systems

Exp: Chazan, A. et al. *Nature.* **2023**, 615, 535–540

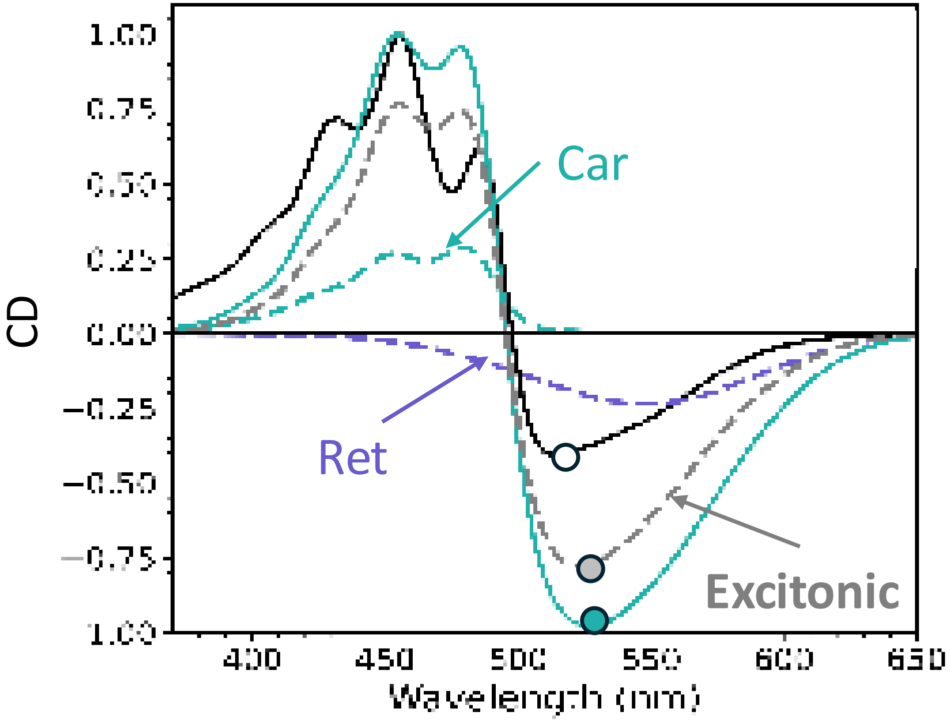
Sim: L. Pedraza-González and coworkers, *Chem. Sci.*, 2025, **16**, 18423–18437; L. Pedraza-González and coworkers, *ChemRxiv*, 2026. DOI:10.26434/chemrxiv.15000075/v1

Exciton origin of the biphasic CD

Additive behavior



Non additive behavior



Absorption = largely additive

$\mathbf{CD_{total} = CD_{intrinsic} + CD_{excitonic}}$

Piermarco Saraceno

Excitonic contribution dominates CD lineshape

Biphasic CD directly refects strong inter-chromophore coupling

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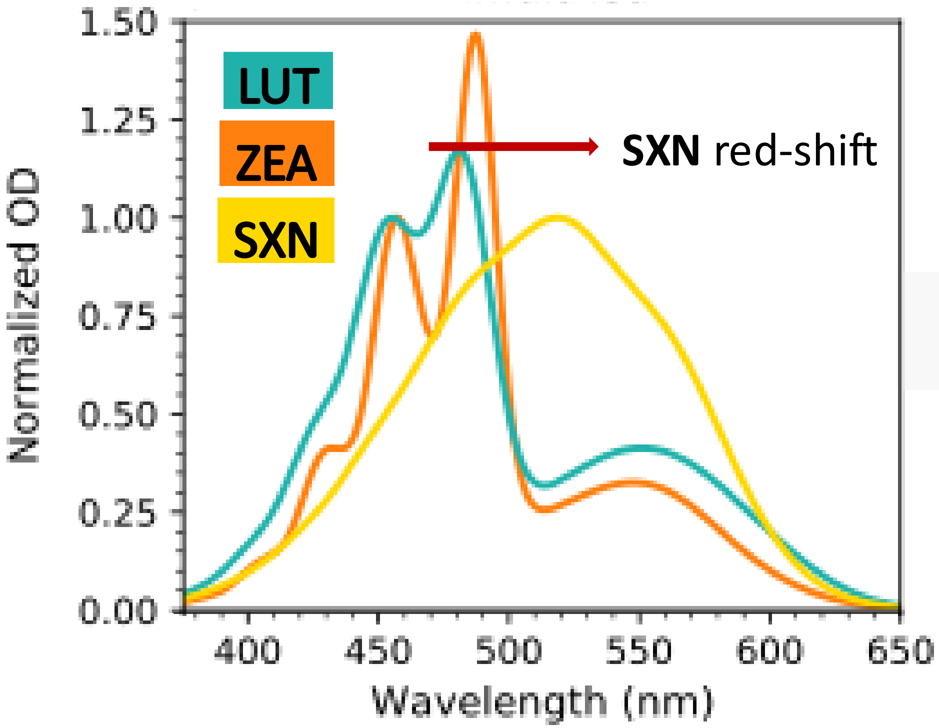
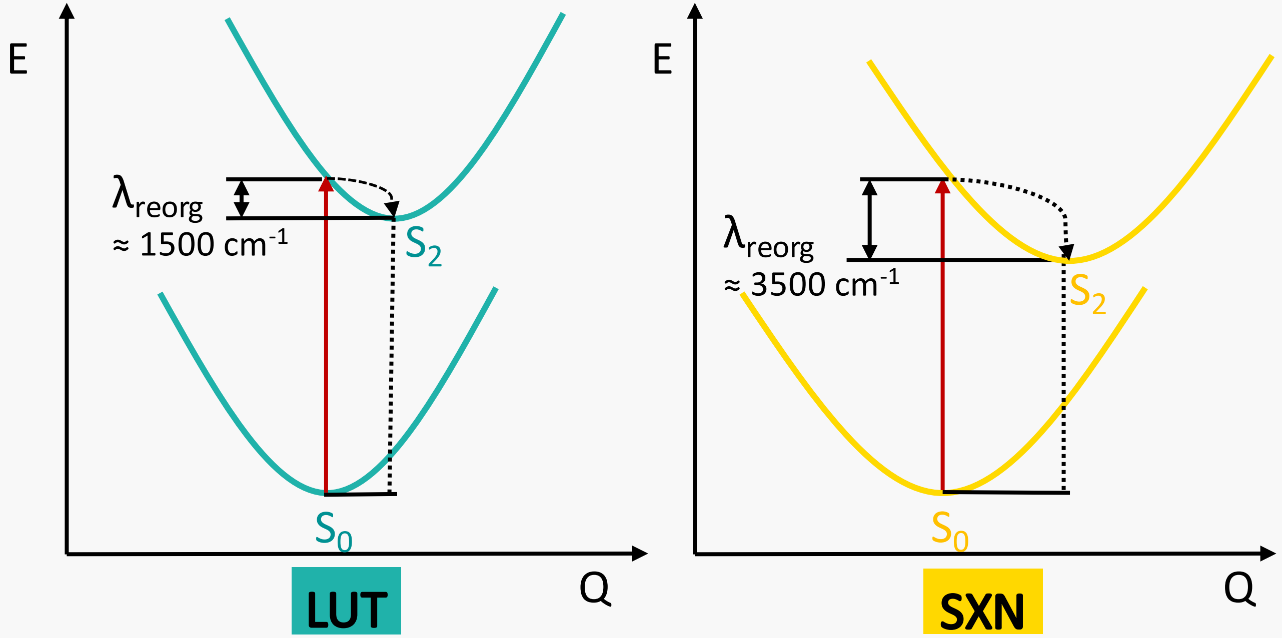
Absorption lineshape is set by Car vibronic coupling

Absorption spectra Kin4B8-Car



Reorganization energy (λ_{reorg})

Excitation energies fluctuate due to the nuclear motion



SXN broader / less resolved vibronic progression → reorganization energy **λ(SXN) ≈ 2xλ(LUT/ZEA)**

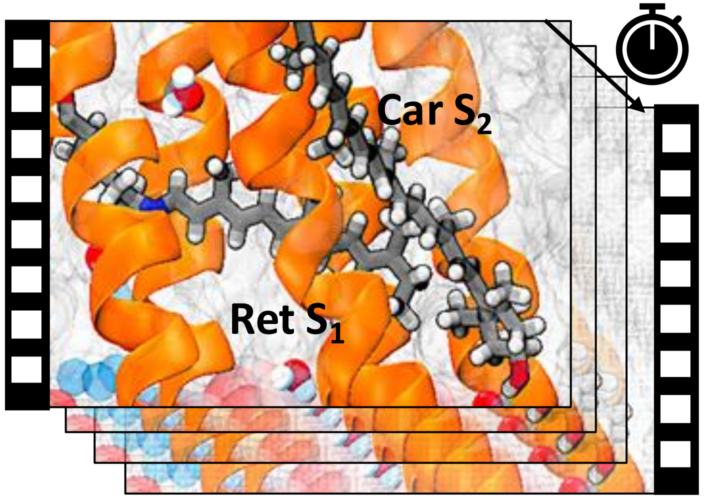
Larger λ → broader/shifted vibronic envelope

Dr. Chris John

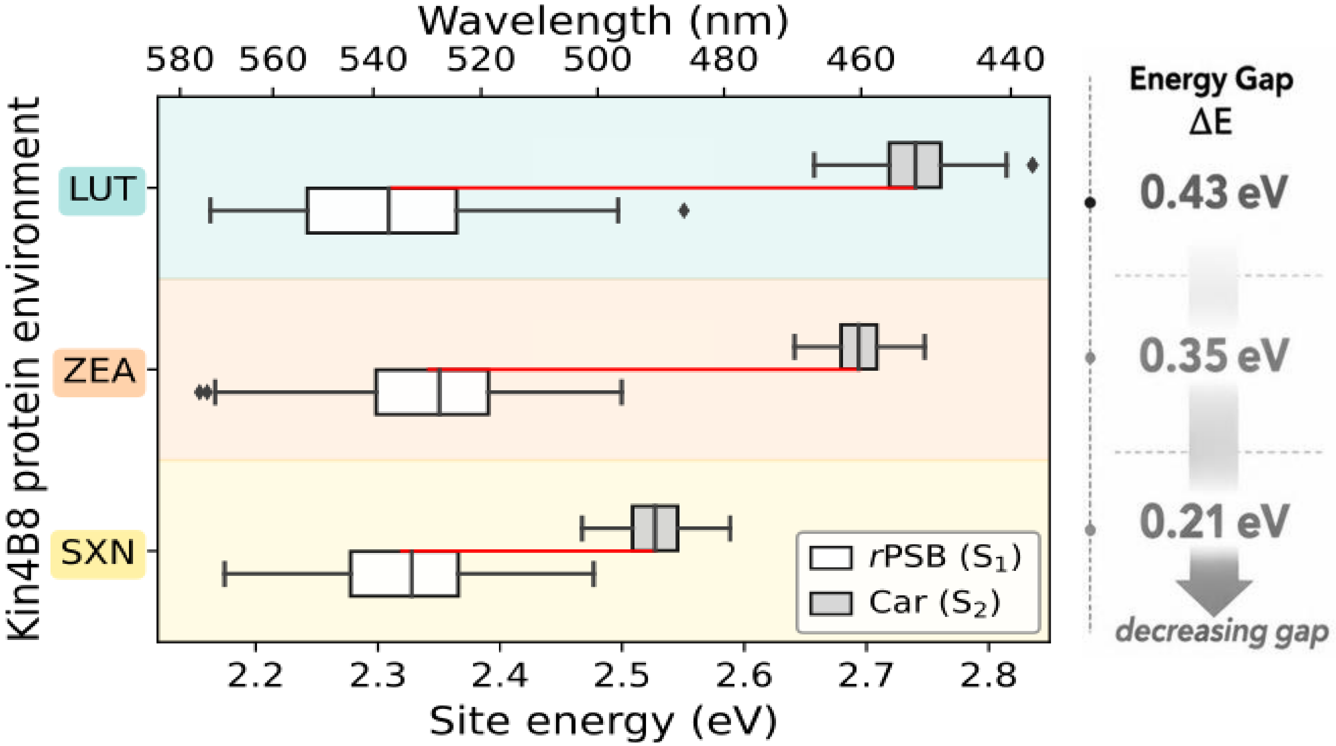
L. Pedraza-González and coworkers, *Chem. Sci.*, 2025, **16**, 18423–18437

L. Pedraza-González and coworkers, *ChemRxiv,* 2026. DOI:10.26434/chemrxiv.15000075/v1

SXN S_2 red-shift reduces Car(S_2)-Ret(S_1) energy gap



QM/MMPol Sites **Car S_2, Ret S_1**



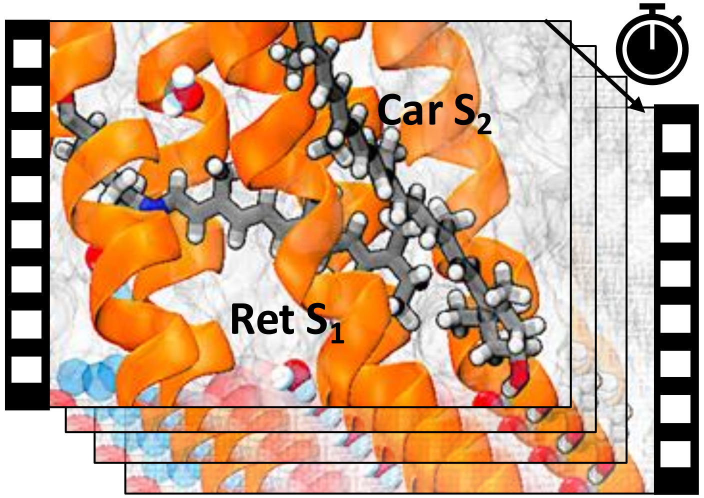
Energy gap: Kin4B8-**LUT** ≈ **-ZEA** >> **-SXN**

Different energy gap between 3-OH and 4-keto

L. Pedraza-González and coworkers, *Chem. Sci.*, 2025, **16**, 18423–18437; L. Pedraza-González and coworkers, *ChemRxiv,* 2026. DOI:10.26434/chemrxiv.15000075/v1

Electronic Couplings are comparable across complexes

EET rate: $k_{DA} \propto |V_{DA}|^2 \times J_{DA}$

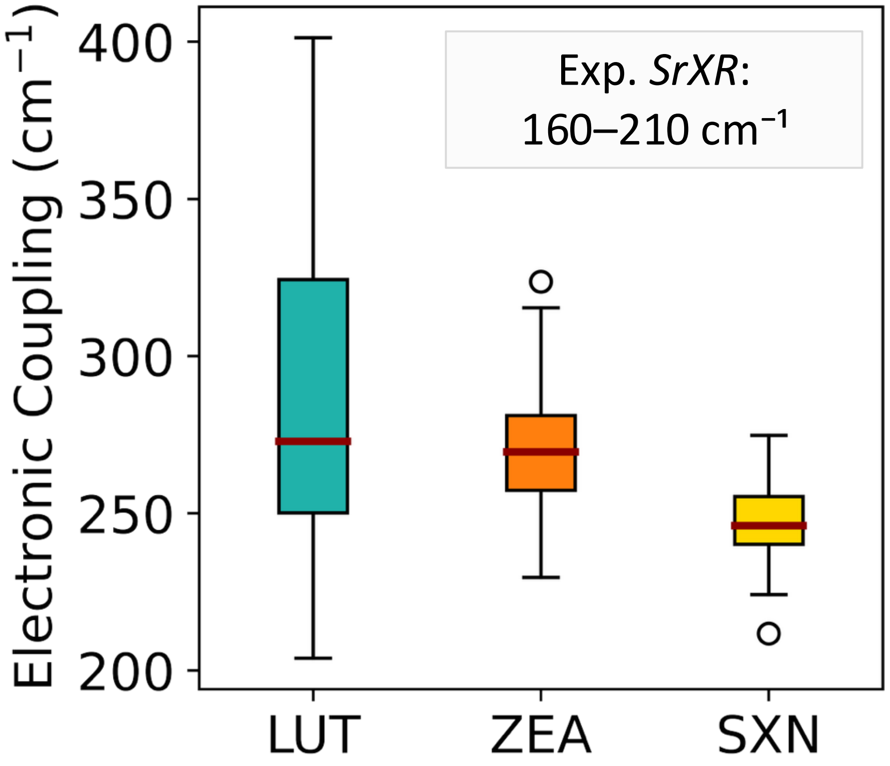


QM/MMPol Couplings **Car S_2 → Ret S_1**

(Direct integration of transition densities)

$$V^{MMPol}_{r\text{PSB-SXN}} = -\sum_{l} \int \mathrm{d}\mathbf{r}'\, \rho^{T*}_{\text{SXN}}(\mathbf{r}') \cdot \mu_l^{MMPol}(\rho^{T}_{r\text{PSB}})$$

Curutchet, Mennucci, *Chem. Rev.* (2017), 117, 294



Strong coupling in all Kin4B8 complexes

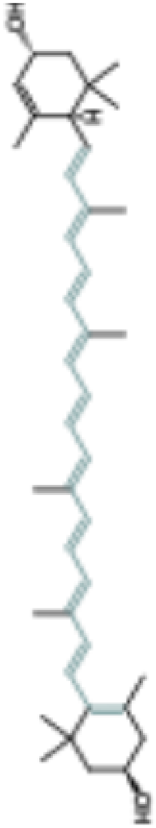
If coupling is comparable, the origin of selectivity must lie in resonance — i.e., spectral overlap

L. Pedraza-González and coworkers, *Chem. Sci.*, 2025, **16**, 18423–18437; L. Pedraza-González and coworkers, *ChemRxiv,* 2026. DOI:10.26434/chemrxiv.15000075/v1

Spectral overlap determines EET selectivity

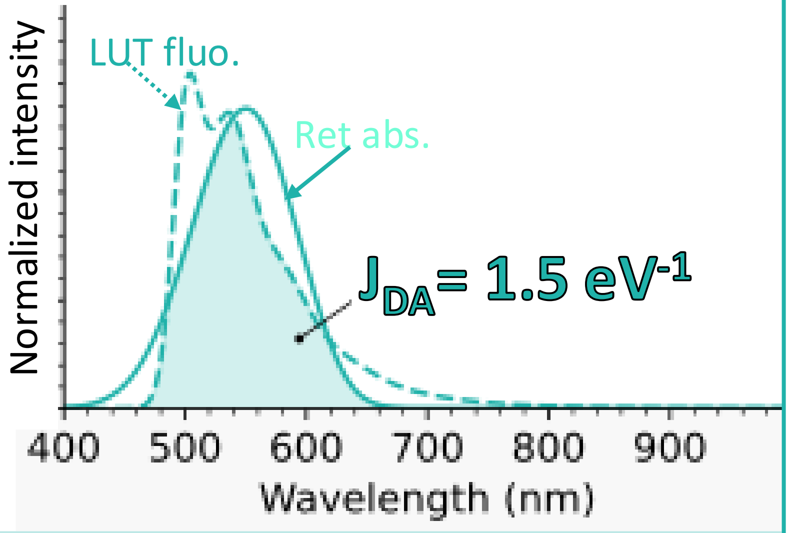
EET rate: $k_{DA} \propto |V_{DA}|^2 \times J_{DA}$

✓ Binding: conserved ✓ Coupling V_{DA}: strong & comparable



Kin4B8-LUT (3-OH)

Efficient antenna

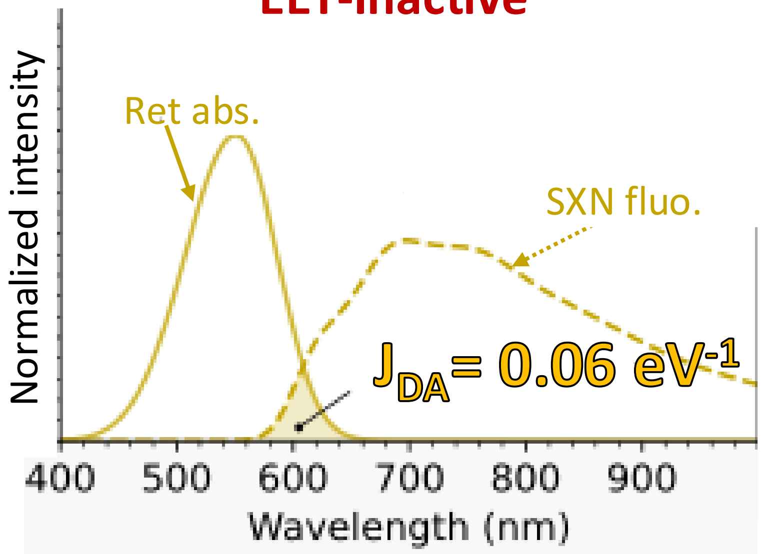


$|V_{DA}|$ = 283 cm^{-1}

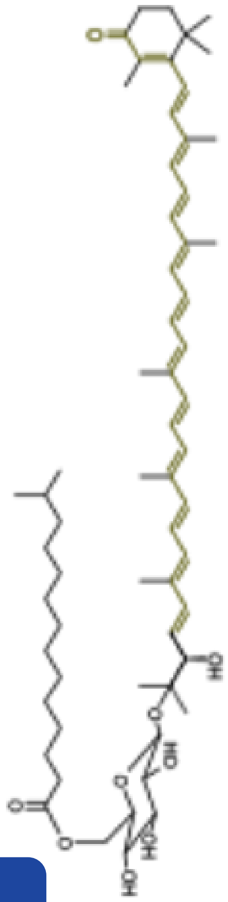
VS

Kin4B8-SXN (4-keto)

EET-Inactive



$|V_{DA}|$ = 248 cm^{-1}



Similar coupling + very different spectral overlap → elective EET

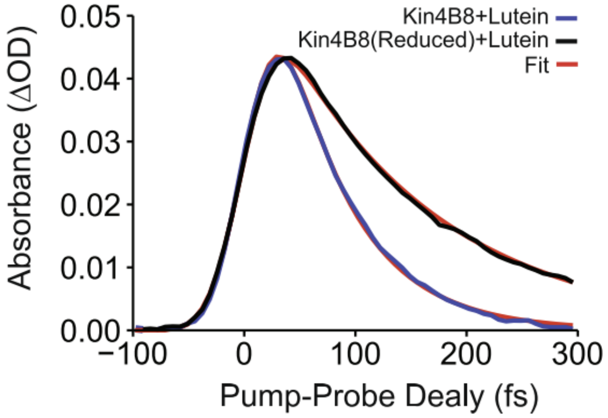
L. Pedraza-González and coworkers, *ChemRxiv*, 2026. DOI:10.26434/chemrxiv.15000075/v1

Experimental input and Kinetic model for EET

Experimental input

Transient absorption (LUT) defines

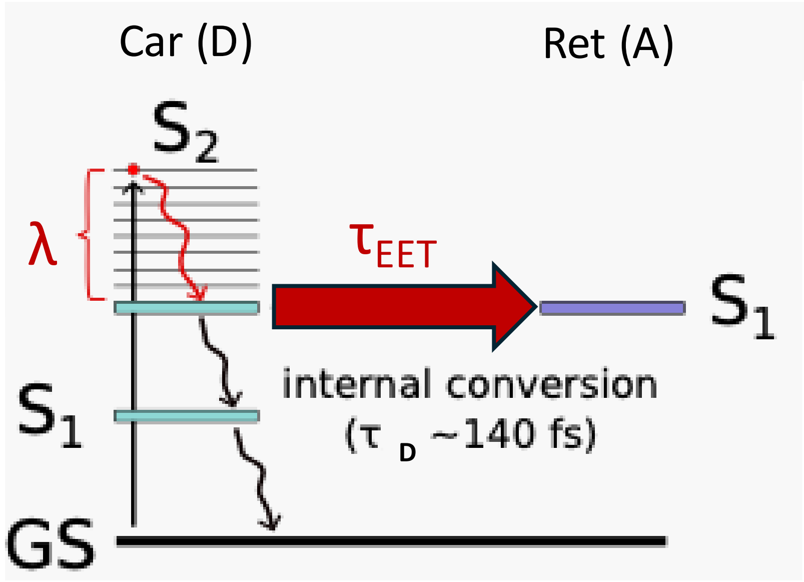
τ_D = 140 fs
(Car S_2 lifetime)



Experimental benchmark
(not model input):

Φ = 57%
τ_{EET}(exp) ≈ 105 fs

Kinetic scheme



Kinetic model

Input: τ_D fixed from experiment (140 fs)

No explicit Car $S_2 \rightarrow S_1 \rightarrow S_0$ channel

Single pathway: Car(S_2) → Ret(S_1)

QM/MMPol → excitonic + vibronic param.

Förster rate (output) → τ_{EET} **,** Φ

$$\Phi = \frac{k_{\mathrm{EET}}}{k_{\mathrm{EET}} + k_{\mathrm{IC}}} = \frac{\tau_D}{\tau_{\mathrm{EET}} + \tau_D}$$

Origin of **EET rate** differences:

- Energy gap
- Electronic coupling
- Spectral overlap

Exp: Chazan, A. et al. *Nature*. **2023**, 615, 535–540
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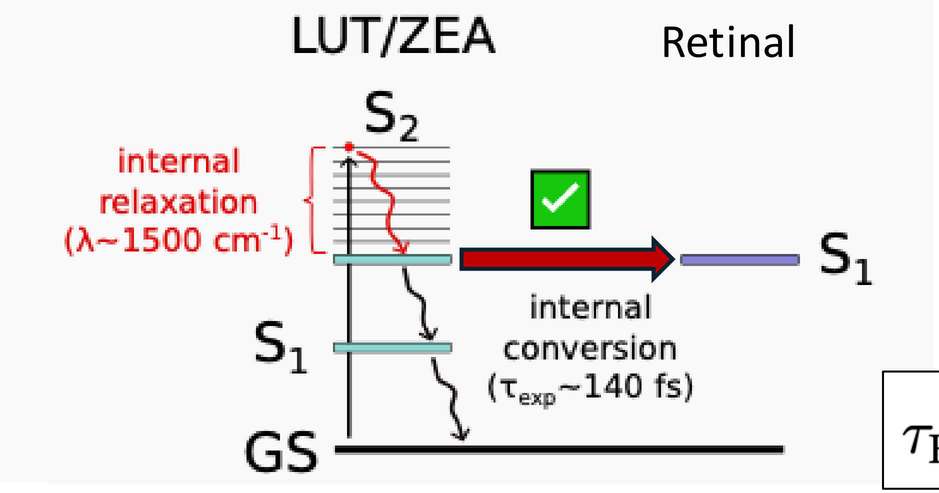
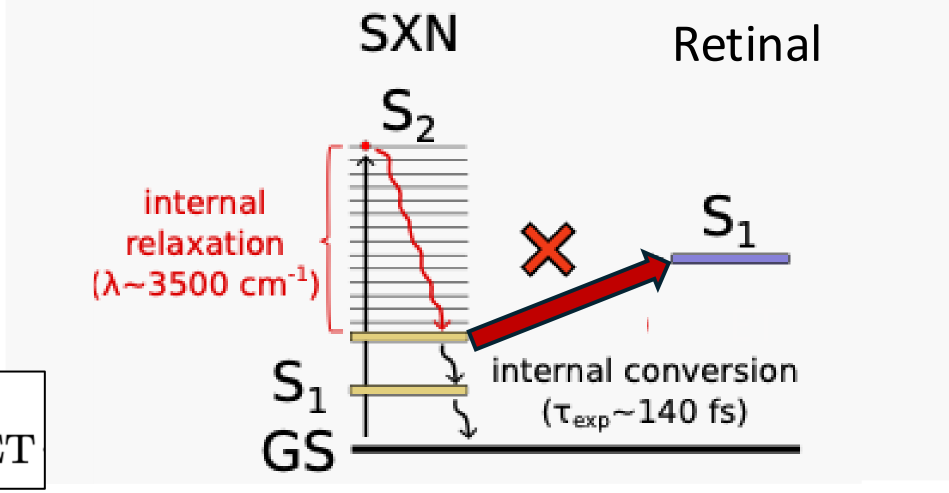
From femtosecond to picosecond EET in Kin4B8-Car

3-Hydroxylated: **ultrafast EET (fs)**

4-Keto: **slow EET (ps)**

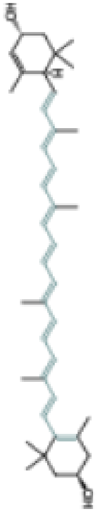


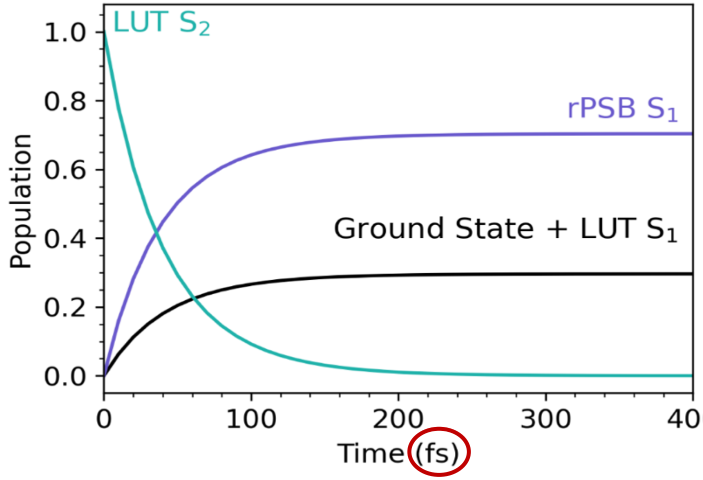


$$\tau_{\text{EET}} = 1/k_{\text{EET}}$$

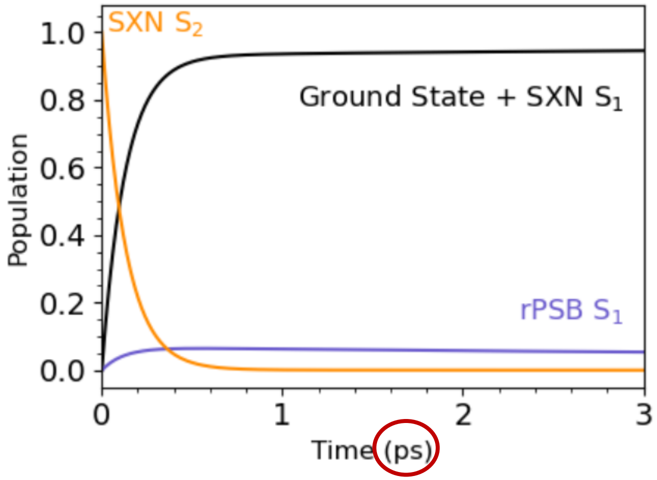
τ_{EET} = ~ 60-70 fs, Φ= 70%

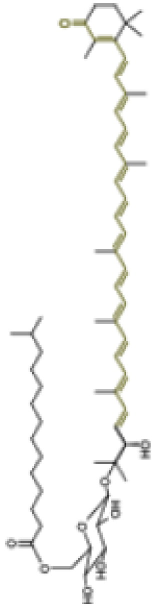
τ_{EET} = ~ 9 ps, Φ= 7%





EET 100× slower





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Sim: L. Pedraza-González and coworkers, *Chem. Sci.*, 2025, **16**, 18423–18437; L. Pedraza-González and coworkers, *ChemRxiv,* 2026. DOI:10.26434/chemrxiv.15000075/v1