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Article

Unifying Mechanism for Thiol-Induced Photoswitching and Photostability of Cyanine Dyes

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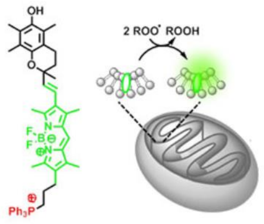
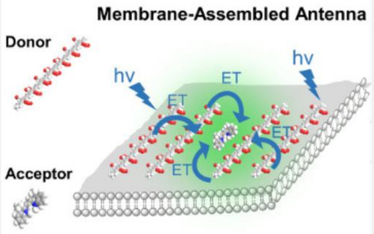
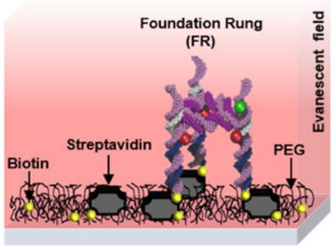
Research Description



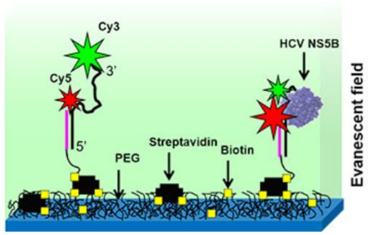
Imaging Redox Reactions With Newly Developed Fluorogenic Probes



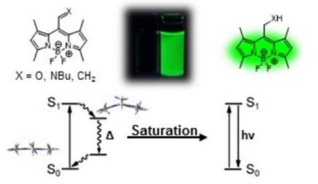
Exciton Transport in Lipid-Conjugated Polyelectrolyte Complexes



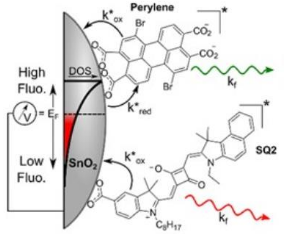
Synthesis and Single Molecule Visualization of Biomaterials



Single Molecule Biophysical Studies on Polymerase activity



Photophysical and Photochemical Properties of Fluorescent Dyes



Single Molecule Spectroelectrochemistry Studies

NIH NATIONAL CANCER INSTITUTE
Center for Cancer Research

Martin J. Schnermann, Ph.D.

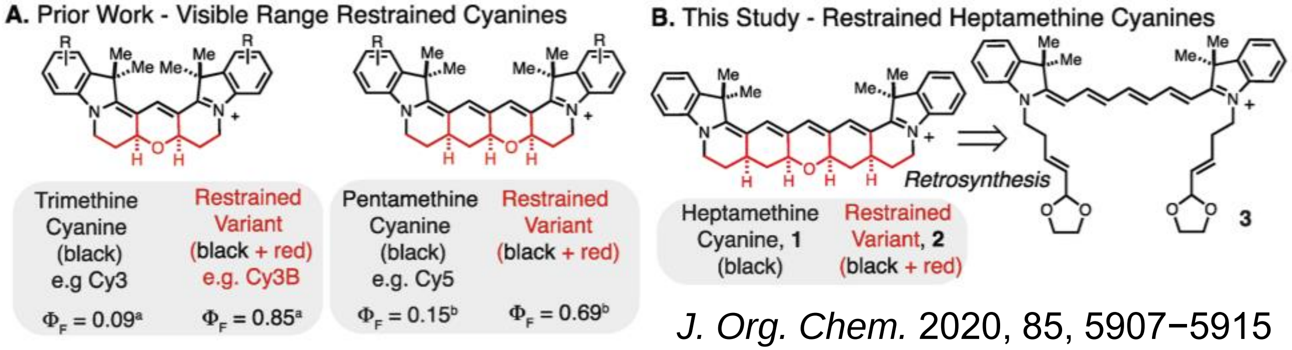
Senior Investigator
Chemical Biology Laboratory
Head, Organic Synthesis Section

The Schnermann lab uses the tools and concepts of modern organic chemistry to discover new molecules for cancer diagnosis and therapy. We focus on the development of new drug delivery and imaging methodologies. In the context of drug delivery, we develop innovative chemical strategies to deliver bioactive payloads with high precision. In the area of imaging, we create novel fluorescent molecules with improved properties for *in vivo* optical imaging and microscopy. While our studies are enabled by core expertise in organic synthesis and molecular design, trainees in the lab are engaged in highly interdisciplinary research using techniques that range from *in vitro* characterization to *in vivo* imaging. In addition to our studies, we embrace a highly collaborative approach with extensive interactions with both intramural and extramural investigators.

Link to additional information about Dr Schnermann's research.

Areas of Expertise

1) complex molecule synthesis, 2) synthetic methodology, 3) near-IR fluorescence, 4) natural product chemistry, 5) drug discovery, 6) drug delivery



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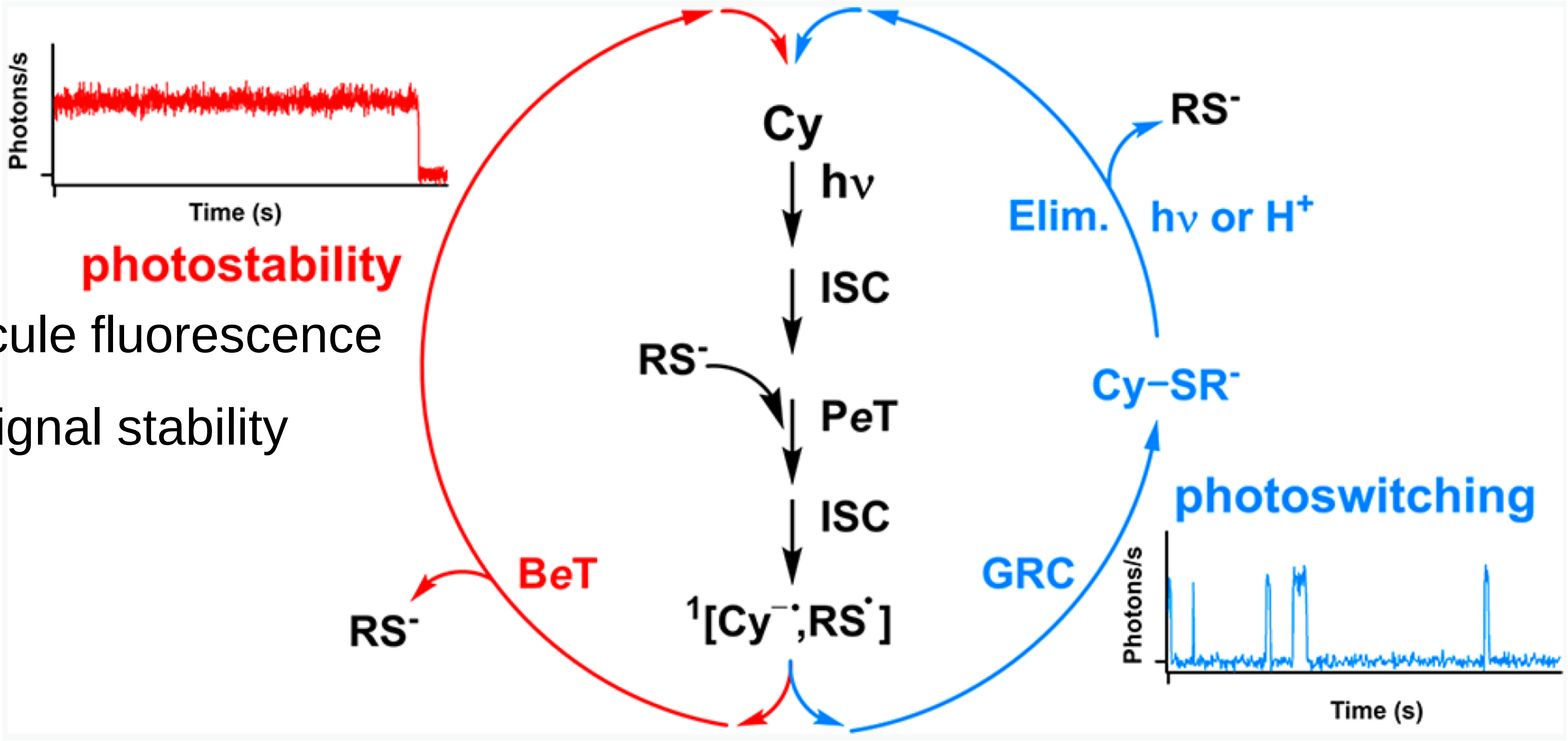


SMF: Single-molecule fluorescence

- ➢ Good intrinsic signal stability

Oxygen scavenger

β-mercaptoethanol (β-ME)

SMLM: super-resolution single-molecule localization microscopy

- ➢ Reversibly transition to dark transient states in photochemical reactions (photoswitching)
- ➢ Restricting the number of active fluorophores in the illumination field in a given imaging cycle to achieve super-resolution

SMF: Single-molecule fluorescence

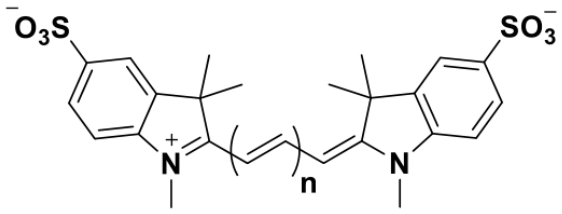
- ➢ Good intrinsic signal stability

- ➢ Account for enhanced brightness and extended survival time conveyed by β-ME to Cy3, Cy3B and Cy5B
- ➢ Formation of a non-emissive photoproduct for structurally related dyes Cy5, Cy5.5, and Cy7,as well as Alexa Fluor 647 and 750
- ➢ Provide a rationale for restoring the emissive state through a competing photochemical and thermal pathway

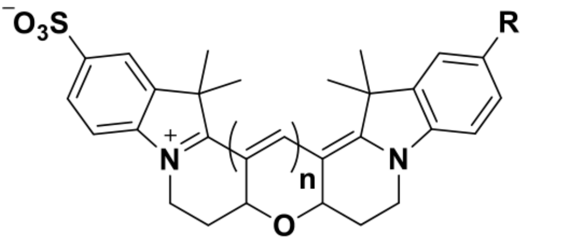
Molecular mechanism

SMLM: super-resolution single-molecule localization microscopy

- ➢ Reversibly transition to dark transient states in photochemical reactions (photoswitching)
- ➢ Restricting the number of active fluorophores in the illumination field in a given imaging cycle to achieve super-resolution



n=1 Cy3
n=2 Cy5
n=3 Cy7

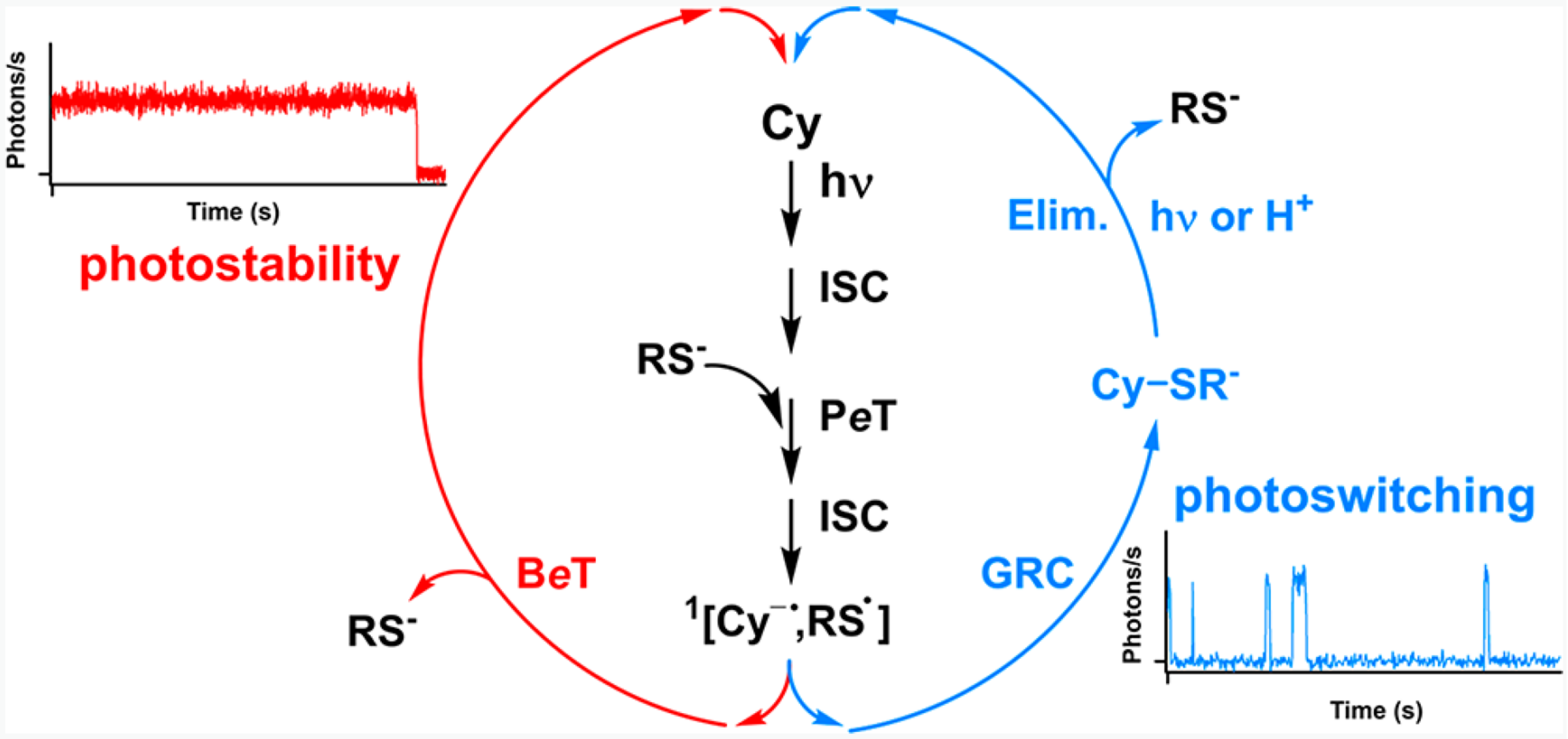


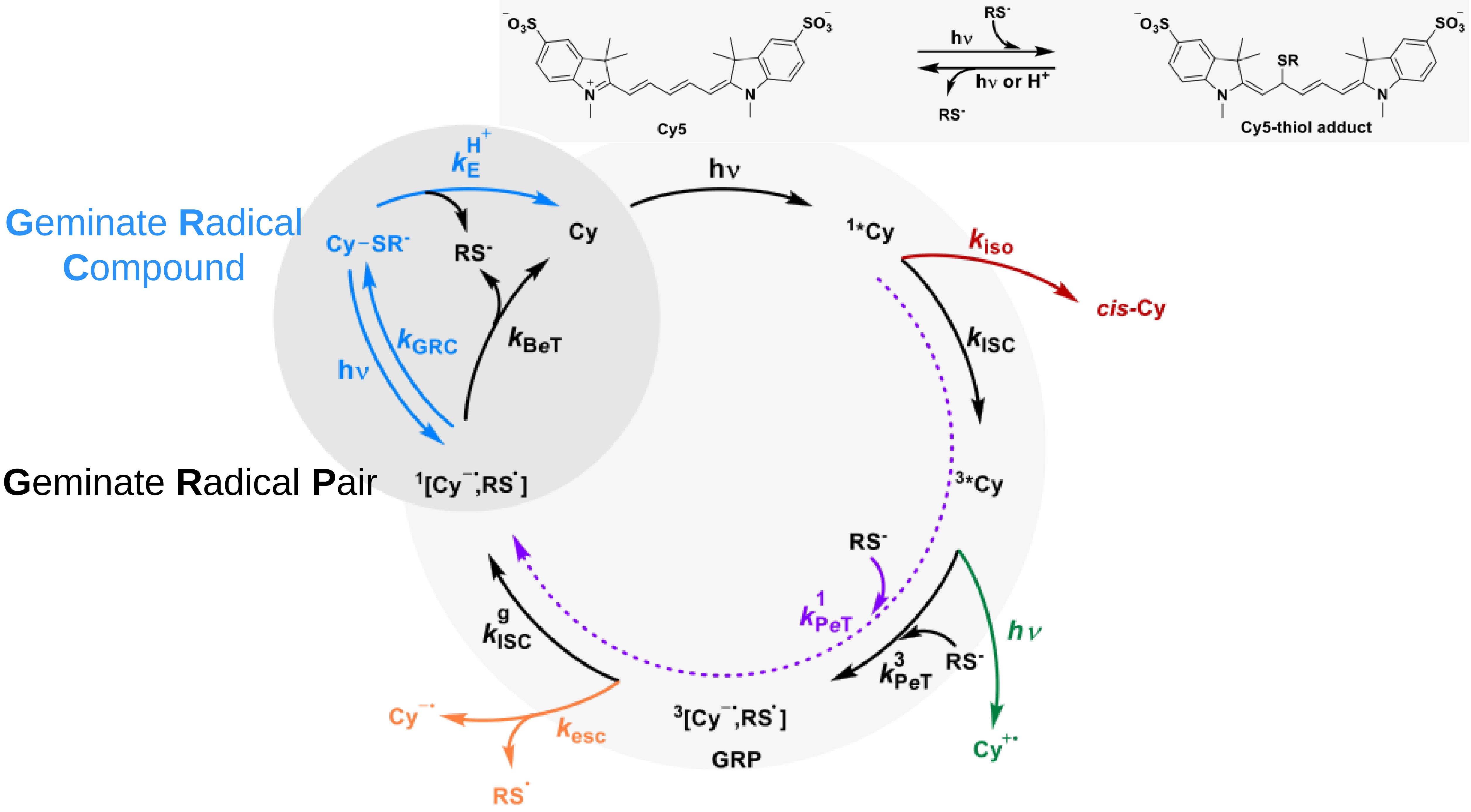
n=1, R $=CH_2CO_2^-$
Cy3B
n=2, R$=CO_2^-$
Cy5B

I. The photostabilizing role of aliphatic thiols such as β-ME and β-MEA results from quenching the triplet excited state of the fluorophore PeT. Different electron donor have also been reported as antifading agents

II. It was also proposed *via* heavy atom induction of the triplet excited state of Cy5 that photoswitching arises from the triplet excited state

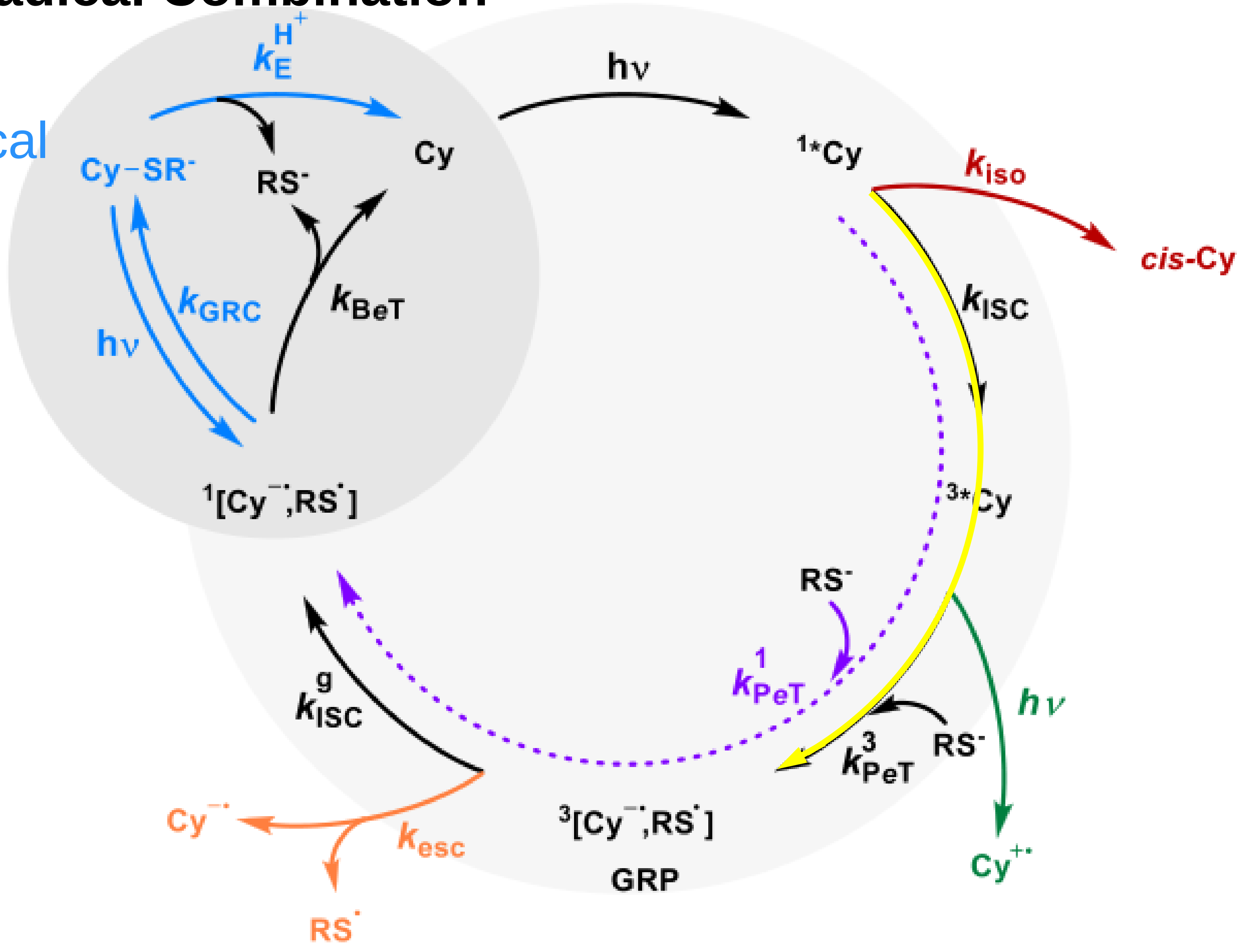
III. Quantitative back electron transfer (BeT) takes place when using β-ME



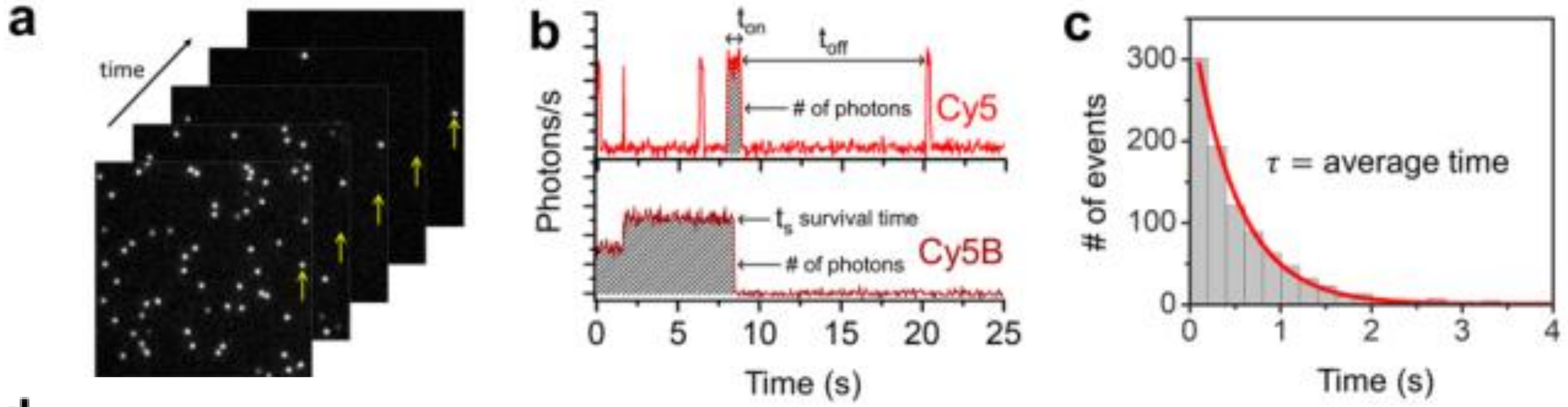

Cy5
Cy5-thiol adduct
hν
hν or H+
RS-
Geminate Radical Compound
Geminate Radical Pair
Cy−SR-
RS-
Cy
kE
H+
kGRC
kBeT
hν
1[Cy−•,RS•]
1*Cy
kiso
cis-Cy
kISC
3*Cy
1kPeT
3kPeT
hν
Cy+•
g kISC
kesc
Cy−•
RS•
3[Cy−•,RS•]
GRP

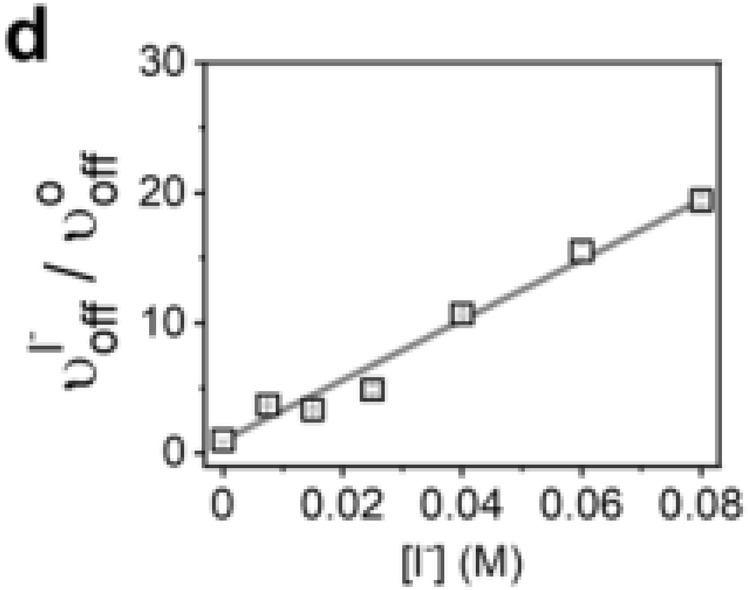
I. Dark State Genesis from the Triplet Manifold through Geminate Radical Combination

Geminate **R**adical **C**ompound



I. Dark State Genesis from the Triplet Manifold through Geminate Radical Combination



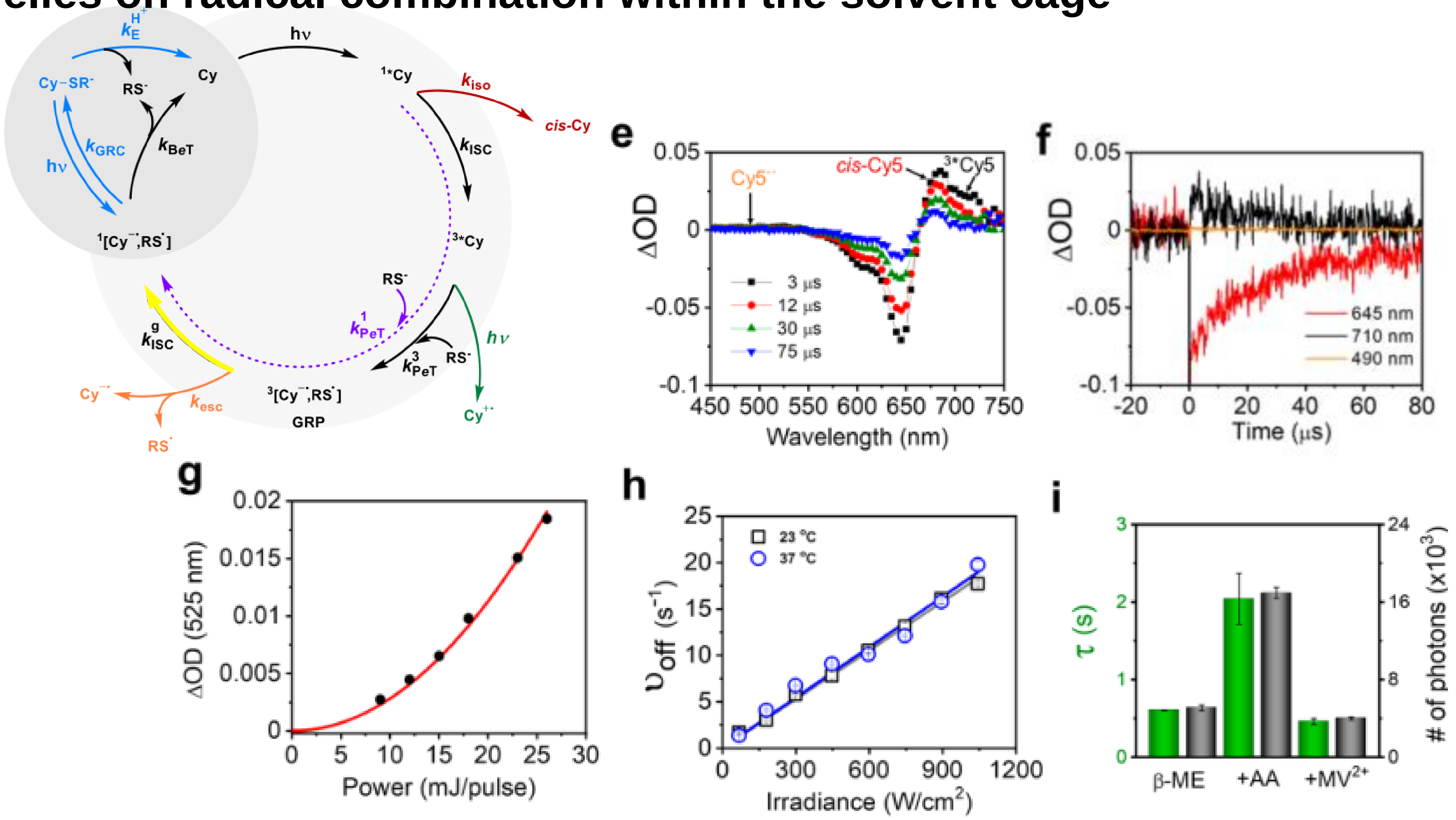


$$\frac{\tau_{on}^{o}}{\tau_{on}^{I-}} = \frac{\nu_{off}^{I^-}}{\nu_{off}^{o}} = 1 + \frac{k_{ISC}^{cat}}{k_{ISC}^{o}}[I^-]$$

Calculated: 231 ± 9 M^{-1}

Fluorescence correlation studies: 125 ± 65 M^{-1}

I. Formation of the Cy-thiol adduct from the triplet manifold relies on radical combination within the solvent cage



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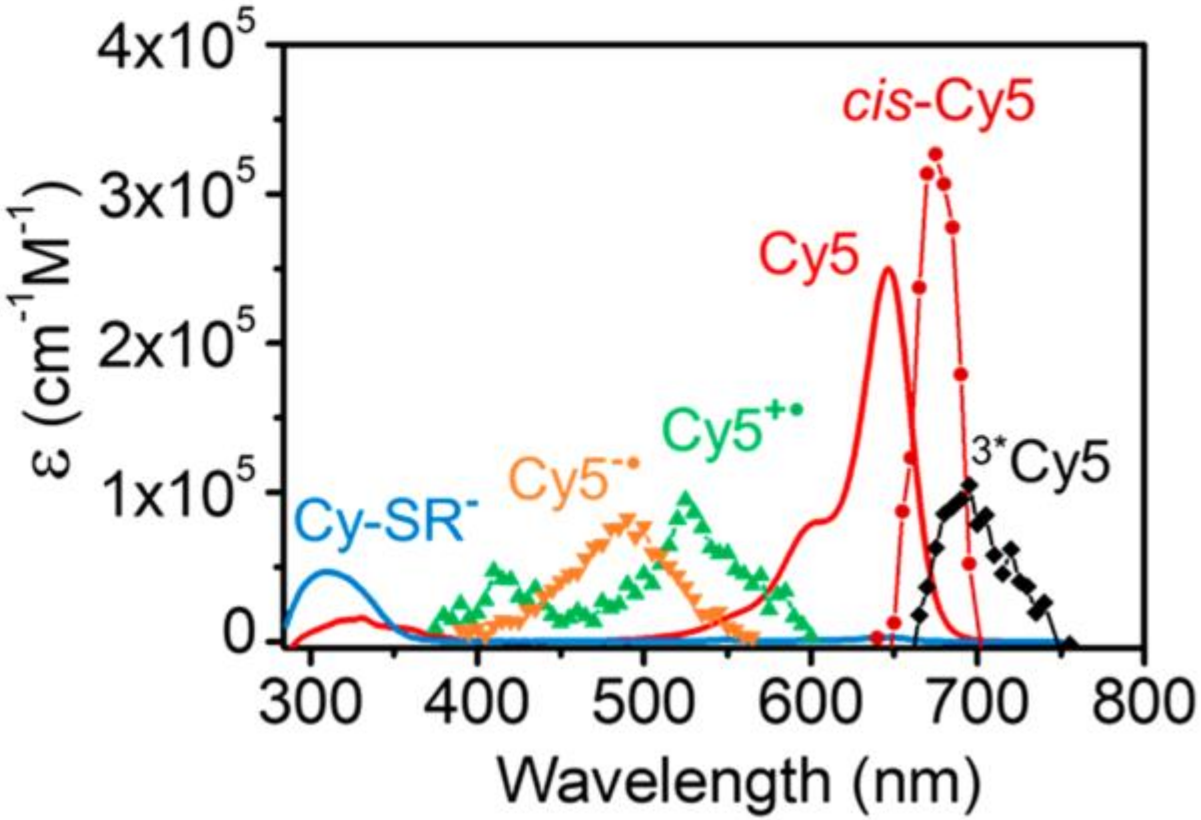


Table 1. Lifetimes and Spectral Properties for the Transient Species of Cy5 and Cy5B

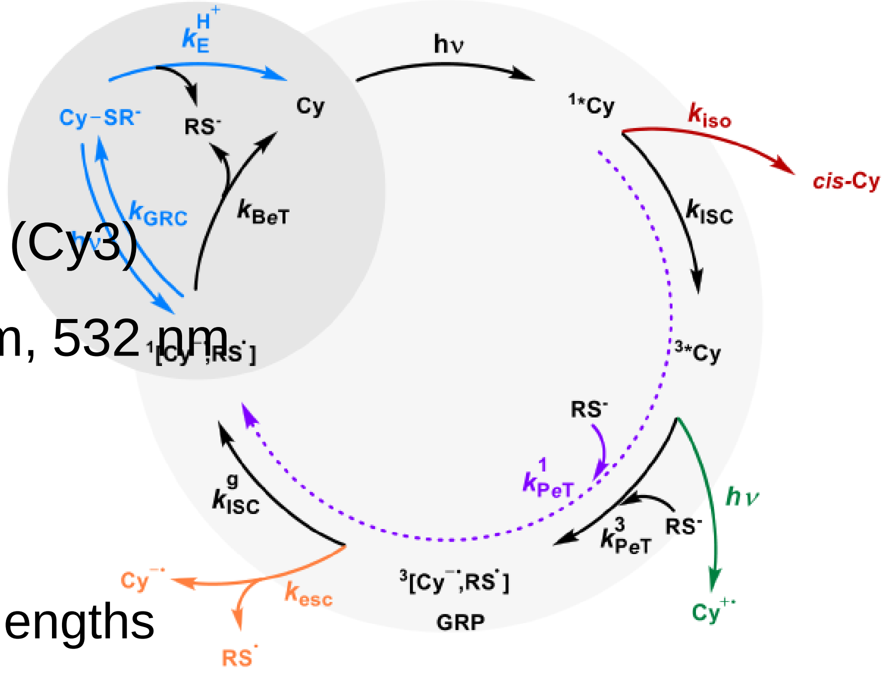
	τ (μs)		λ_{max} (nm)	ε (cm^{-1} M^{-1})
species	Cy5	Cy5B	Cy5	Cy5
cis-Cy	196 ± 5	n/a	675	326 000
^{3*}Cy	209 ± 20	172 ± 25	695	105 000
Cy5$^{+\bullet}$	440 ± 10	206 ± 6	525	95 000
Cy$^{-\bullet}$	853 ± 7	1042 ± 15	490	82 000
Cy-SR^{-}	>10^6	n/a	311	47 000

II. Photoinduced and Acid-Catalyzed Restoration of the Fluorescent State.

Uncaging of the thiolate moiety and restoration of the chromophore:

(i) Indirect illumination of a closely lying, orange-emitting fluorophore (Cy3)

(ii) Direct illumination at relatively short wavelengths, 337 nm, 405 nm, 532 nm.



Question:

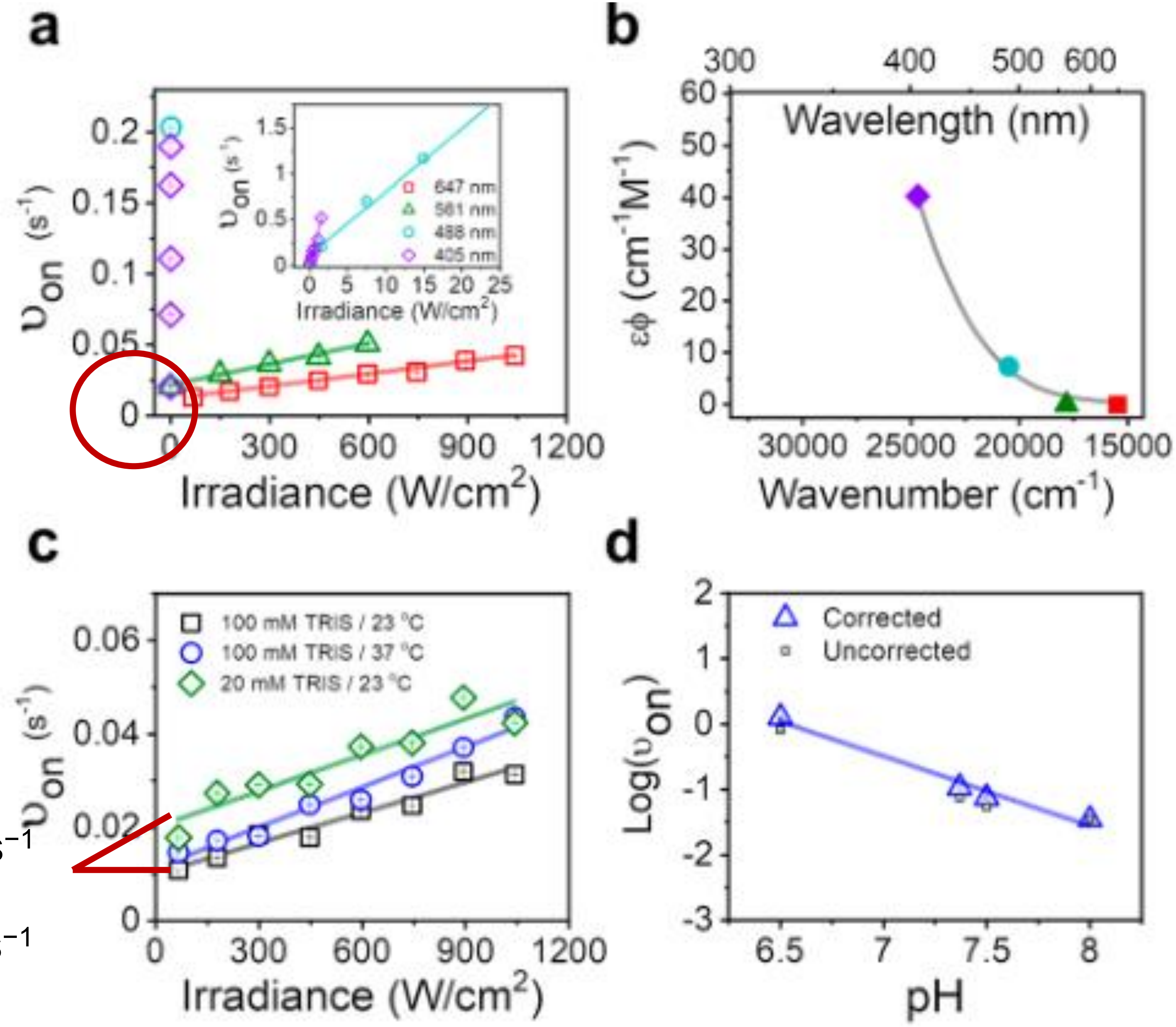
How the Cy5-thiol adduct may undergo photoinduced reactions at wavelengths where no absorption is detected

Speculation :

Broadening of the electronic transition of the 310 nm peaked Cy5-thiol adduct absorption

Solution :

Building the distribution of off times t_{off} (the times to Cy5 recovery) from single-molecule intensity−time trajectories



0.010 ± 0.001 s^{-1}

vs

0.011 ± 0.001 s^{-1}

III. Energy Landscape from DFT Calculations

The fates for all cyanines wasdictated by whether they may effectively undergo PeT with thiolates

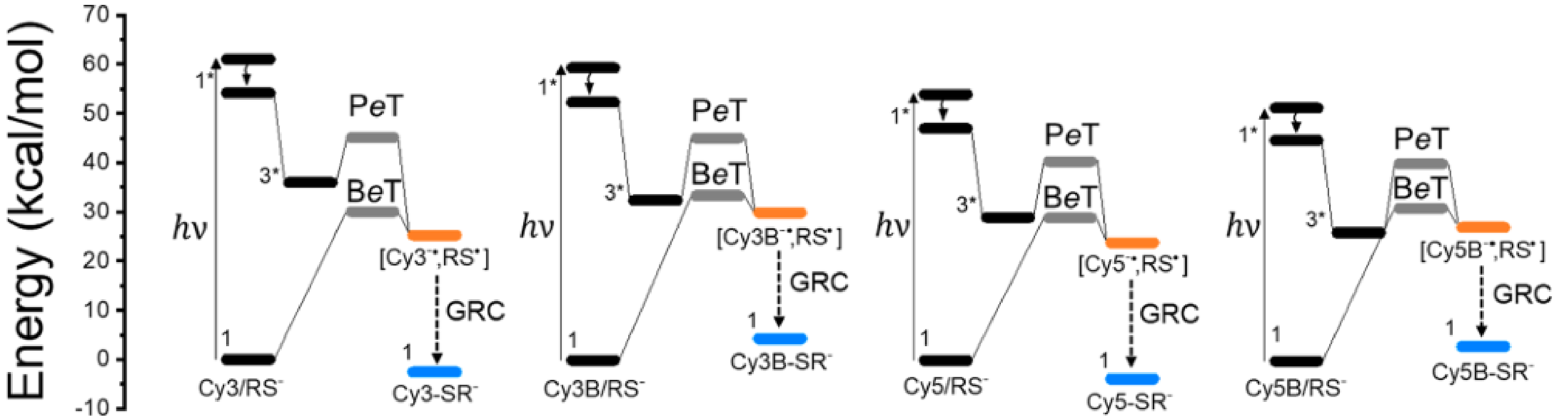
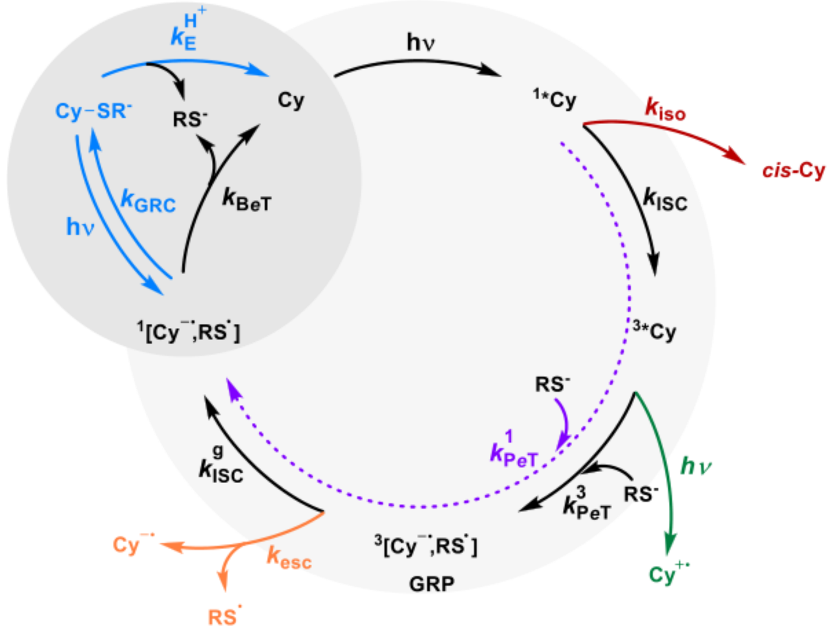


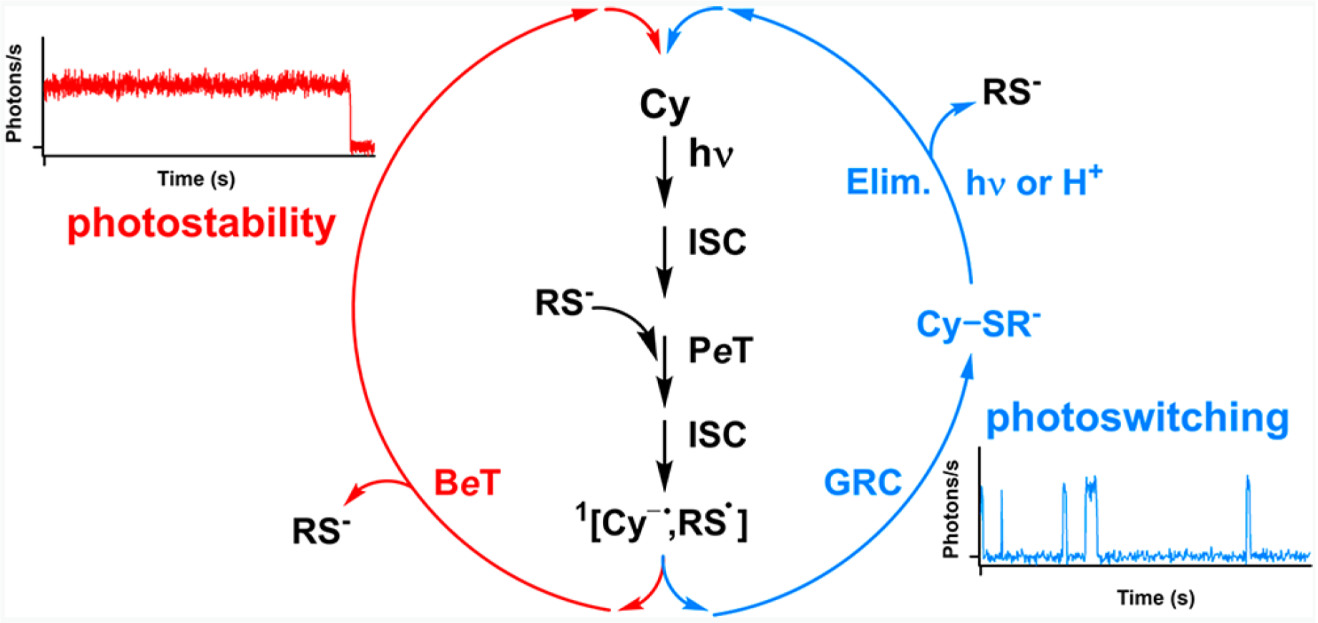
Figure 5. Energy landscape for Cy dye photoreactions. Energy of Cy/*β*-ME pairs and the computed transition states (gray) for the photoinduced electron transfer (PeT) and back electron transfer (BeT).

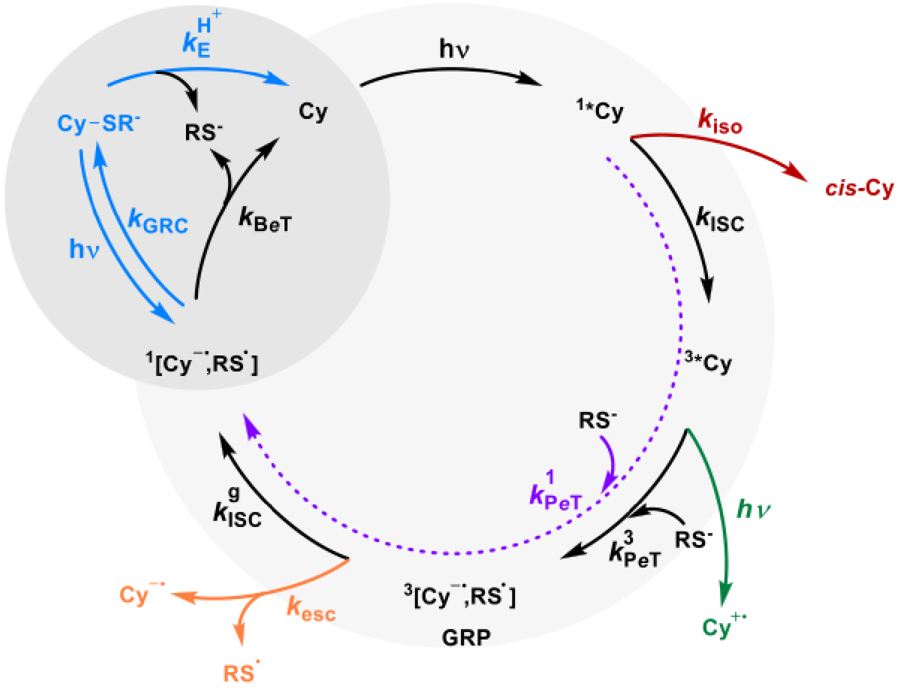
Table 2. Rate Constants for Photoinduced Electron Transfer to Cy Dyes by *β*-ME and Estimated Transition State Energies for Forward and Back Electron Transfer

dye/*β*-ME	k_{PeT} (s^{-1} M^{-1})	$\Delta E^{\ddagger}_{PeT}$ (kcal/mol)	$\Delta E^{\ddagger}_{BeT}$ (kcal/mol)
Cy3	$3.5 \pm 0.3 \times 10^{8}$	9.17	4.73
Cy3B	$1.6 \pm 0.1 \times 10^{7}$	12.60	3.50
Cy5	$9.6 \pm 0.5 \times 10^{7}$	11.39	5.01
Cy5B	$1.3 \pm 0.4 \times 10^{6}$	13.90	3.93



Conclusion





- Thiolates are required in all instances
- If extended survival times and stable signals, lower oxidation potentials, BeT, rather than GRC with the thiyl radical is desired
- If photoswitching toward super-resolution, a thiyl radical able to undergo GRC would be sought after SMLM is desired