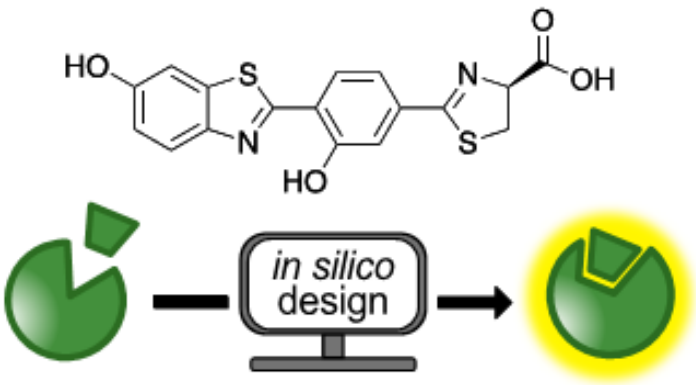
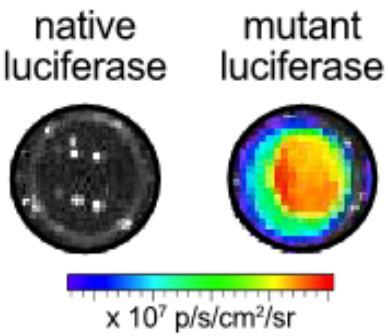
Multicomponent Bioluminescence Imaging with a π-Extended Luciferin





https://dx.doi.org/10.1021/jacs.0c01064

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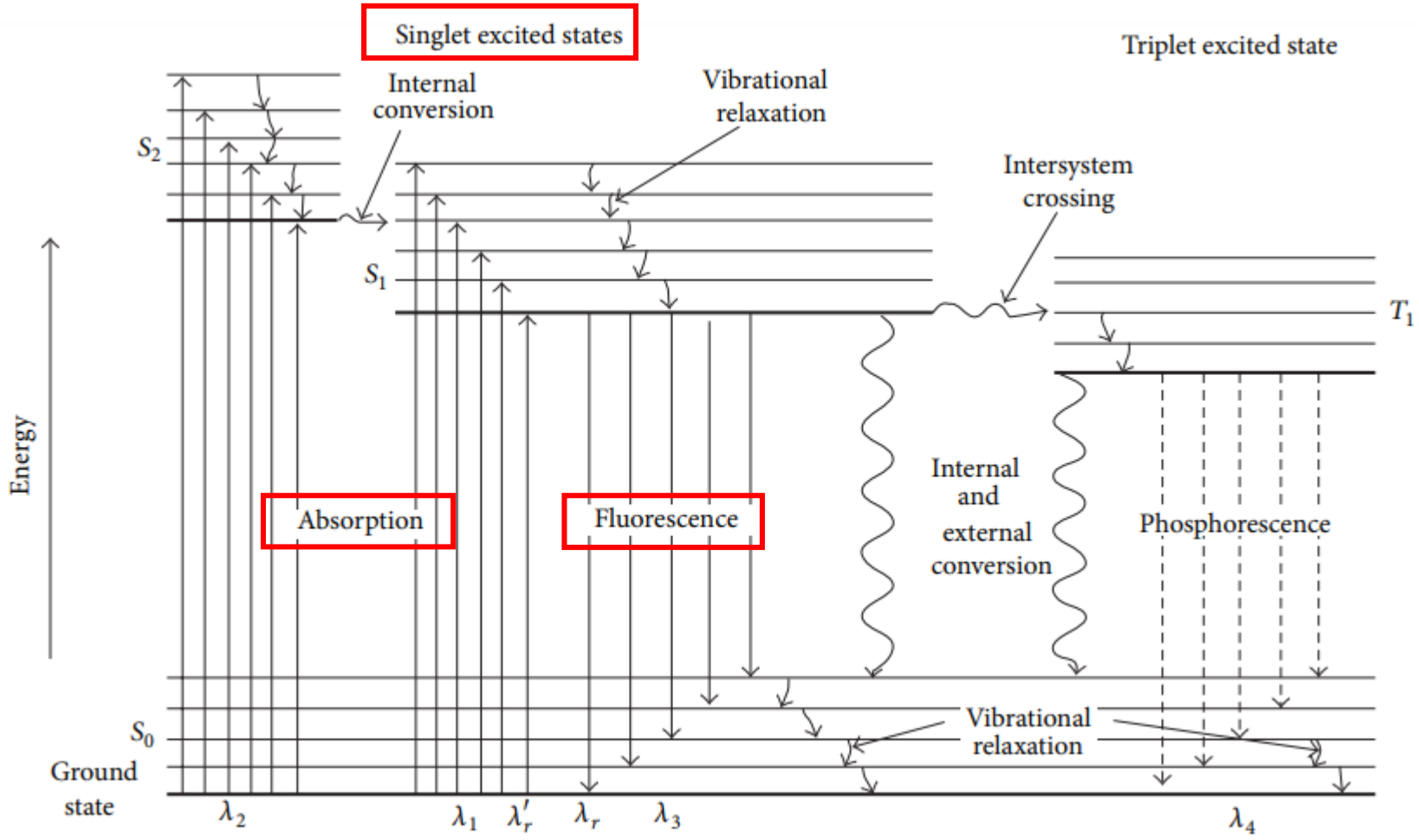
Supervisor: Prof. Zhaochao Xu

Date: Aug. 13th 2020

Outline

- *Background*
- *This work*
- *BRET & Triggerable Dioxetanes*
- *Summary*
- *Acknowledgement*

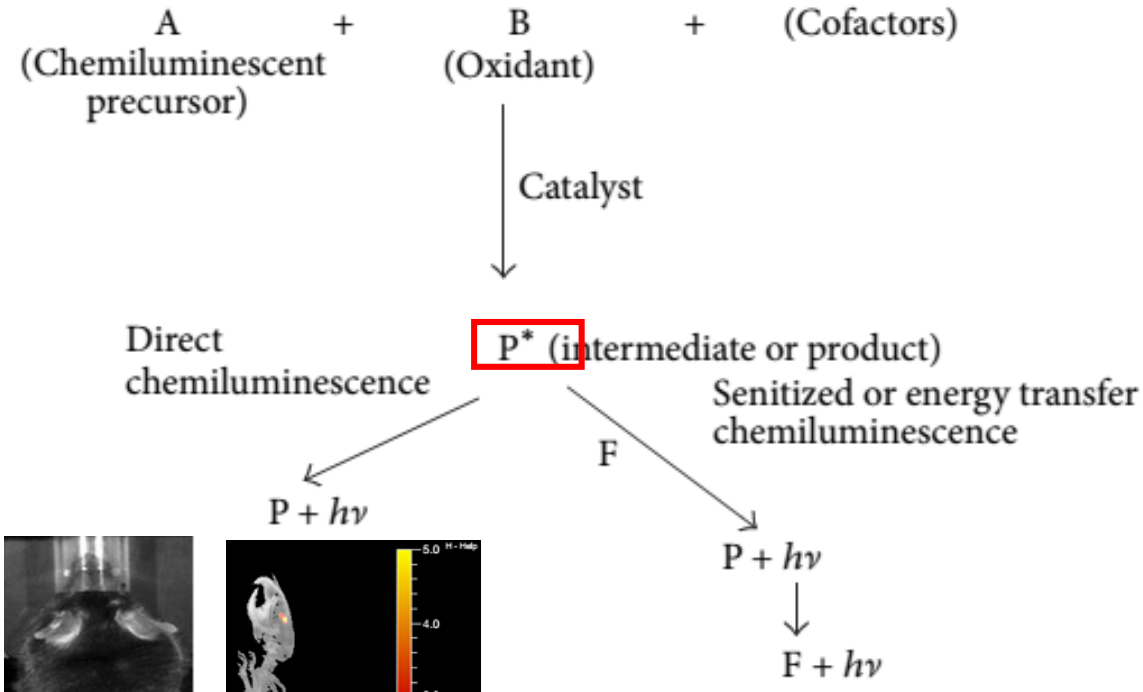
Nature of Chemiluminescence Reactions

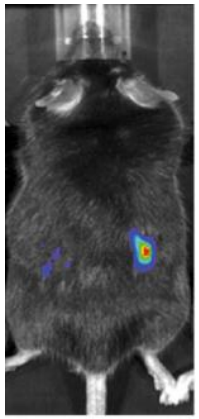


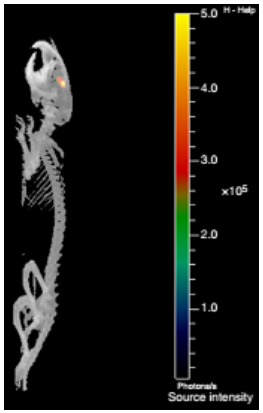
Photoluminescence – Jablonski diagram

ISRN Spectroscopy, 2013, 230858

Nature of Chemiluminescence Reactions







Basic Mechanism of Chemiluminescence

Chemiluminescence reaction yields one of the reaction products in an **electronic excited state** producing light on falling to the ground state.

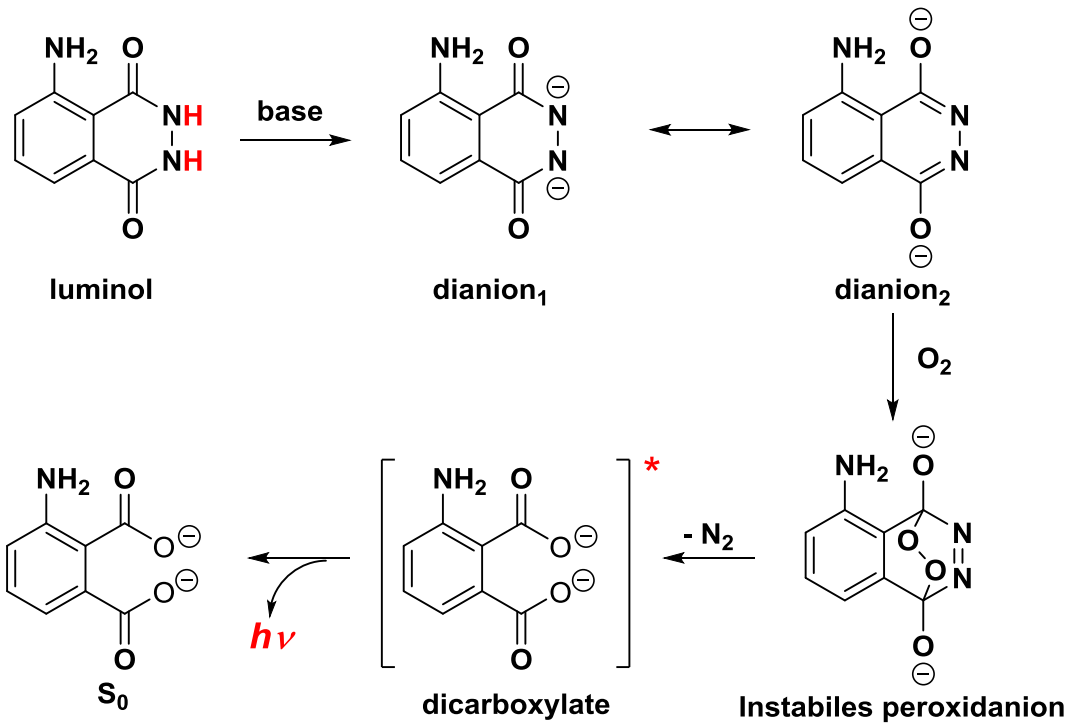
✓ $\Delta G = \Delta H - T\Delta S$

✓ Reaction pathway must be favorable to channel an electronic excited state.

✓ Photon emission must be a favorable deactivation process

ISRN Spectroscopy, 2013, 230858; Nat. commun. 2018, 9, 132

Nature of Chemiluminescence Reactions



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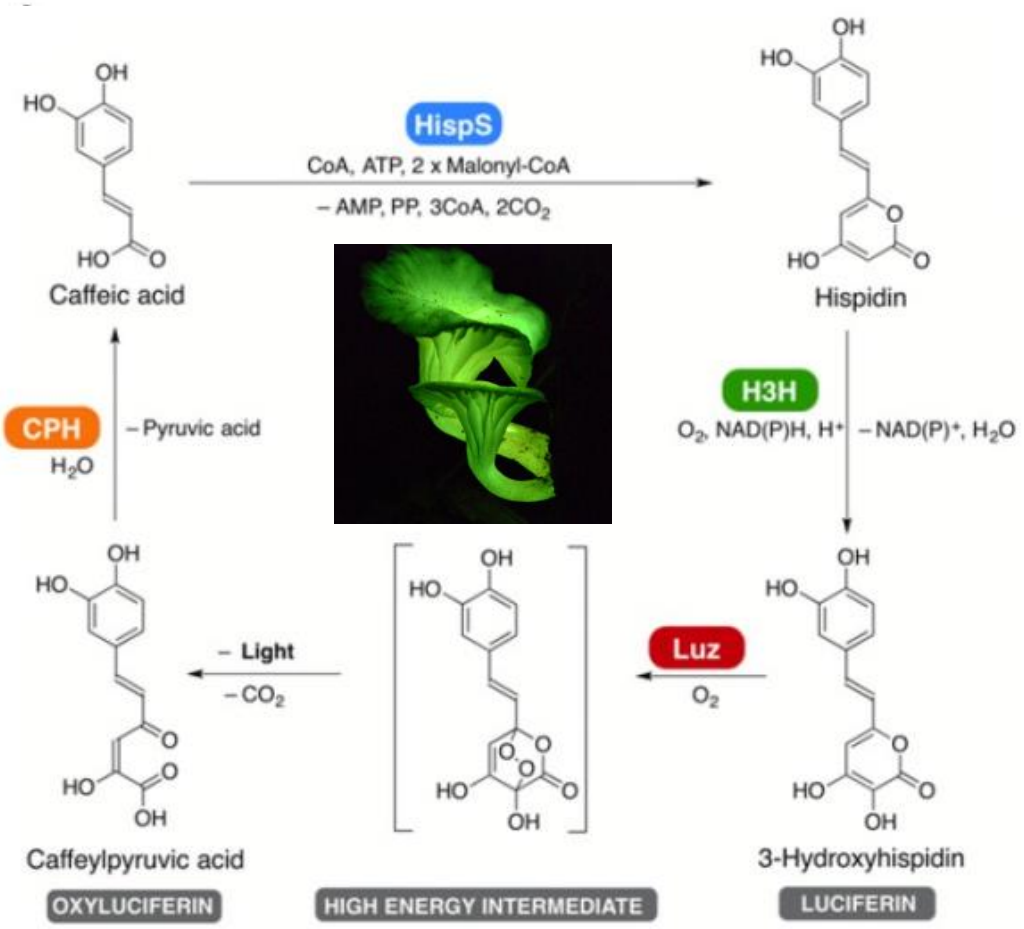
Nature of Chemiluminescence Reactions



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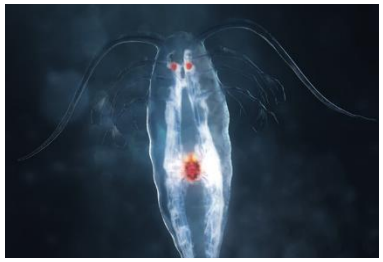
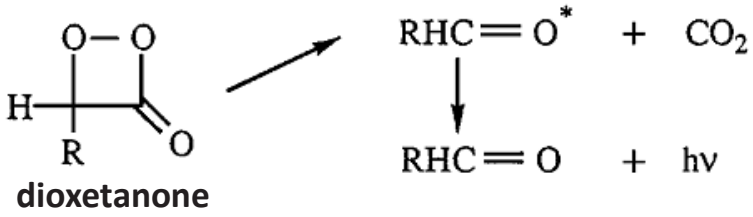
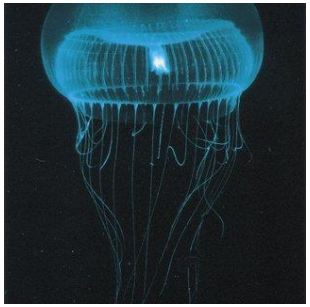
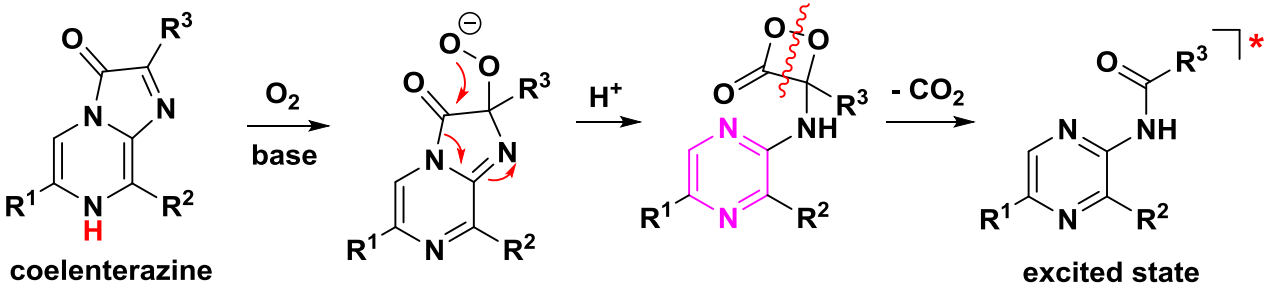
Bioluminescence Reactions

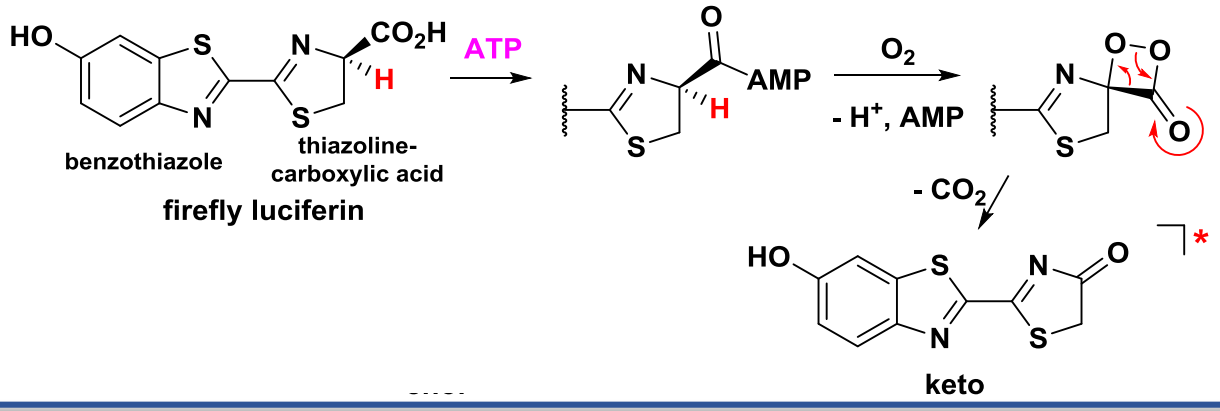


Bioluminescence Reactions



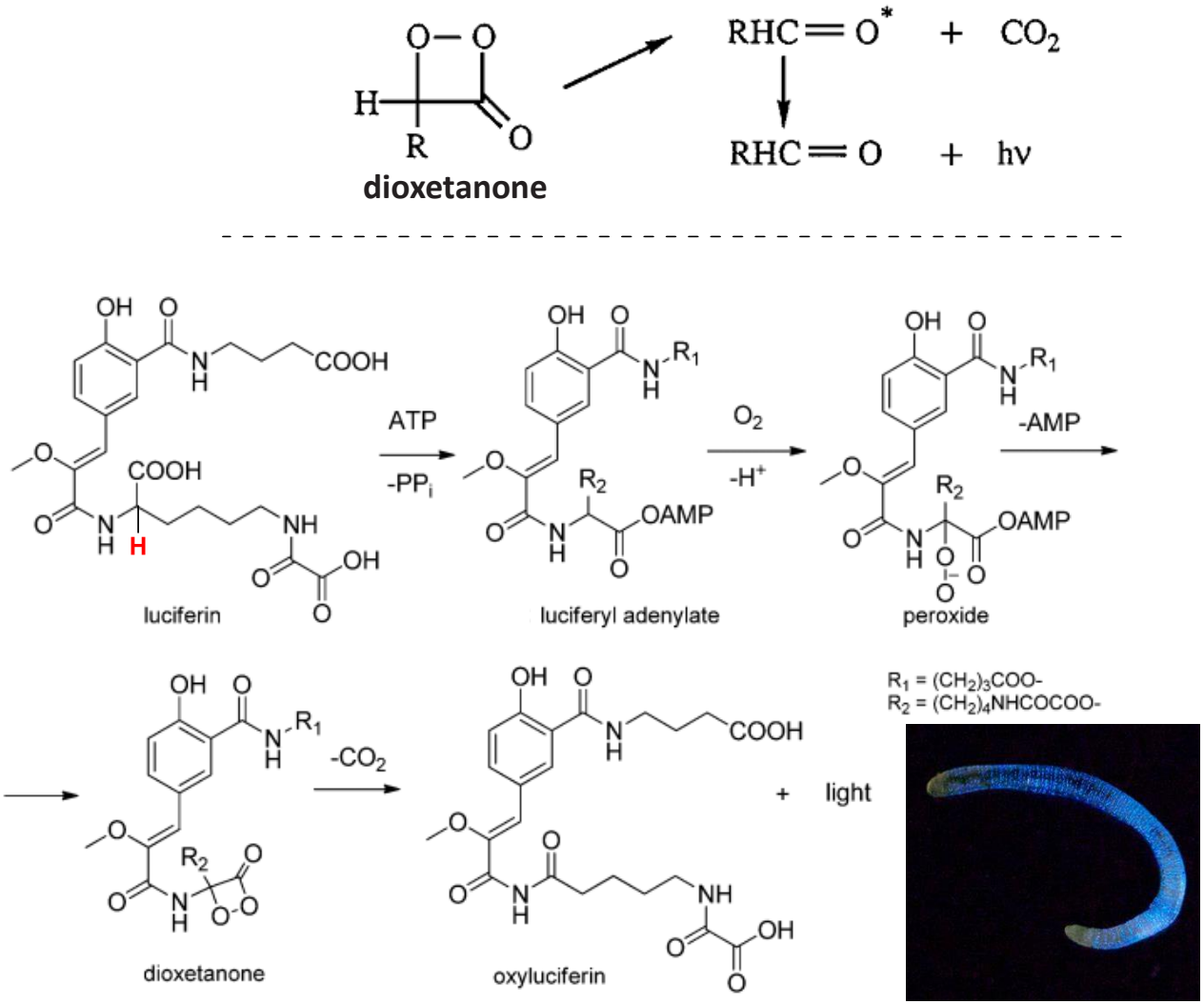






Cell. Mol. Life Sci. 2010, 67, 387; Chem. Soc. Rev., 2016, 45, 6048

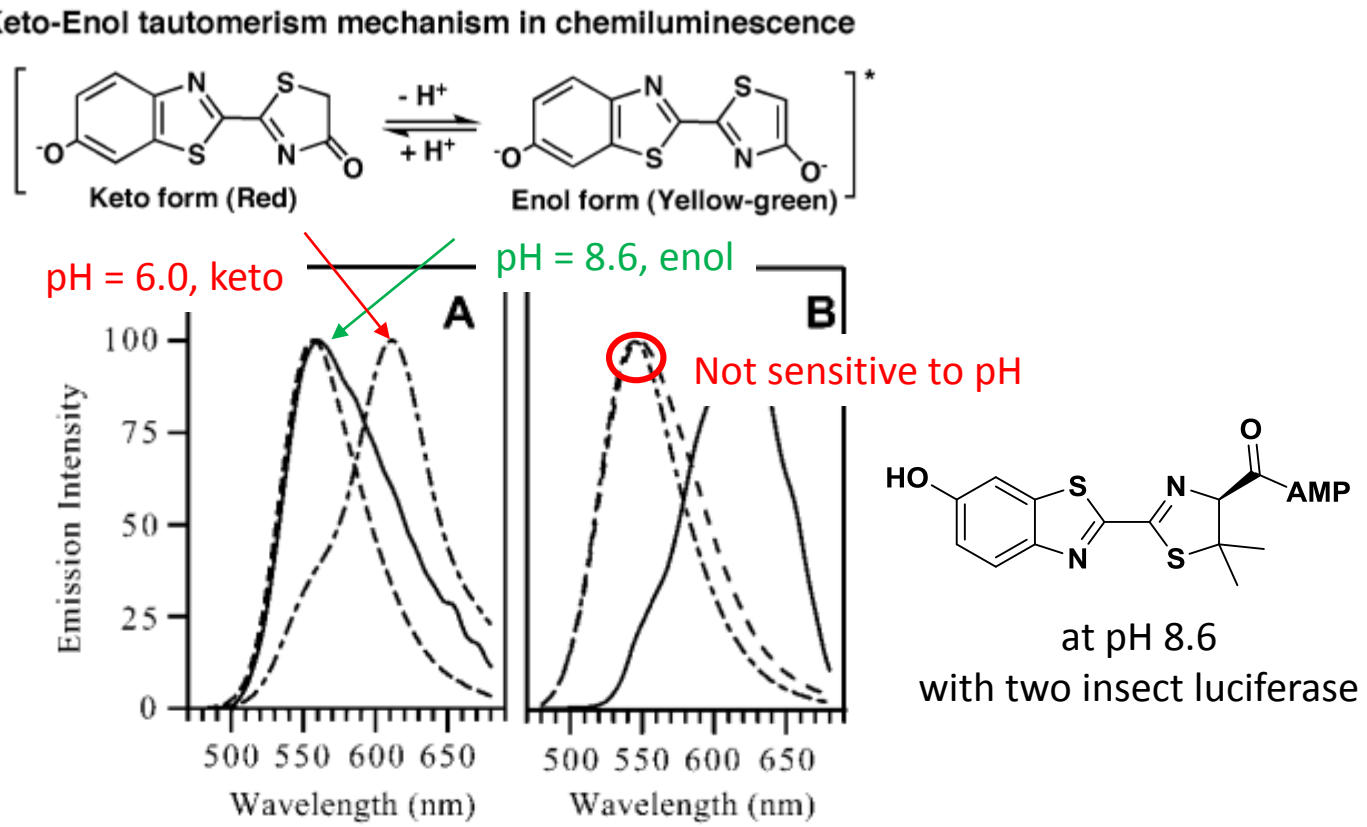
Bioluminescence Reactions



Cell. Mol. Life Sci. 2010, 67, 387; Chem. Soc. Rev., 2016, 45, 6048

Mechanisms for Light Emission

White



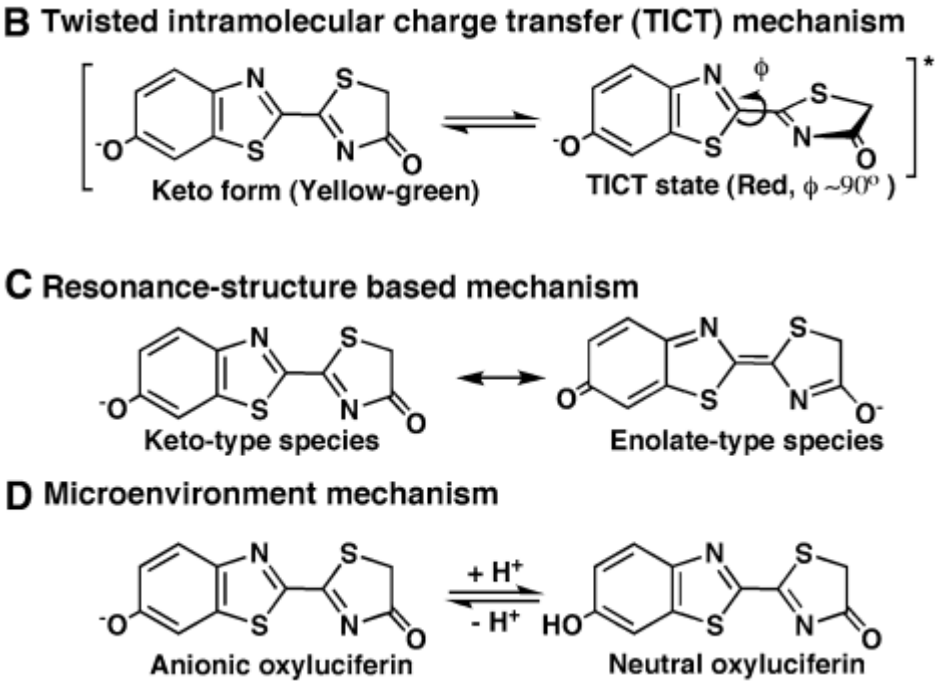
at pH 8.6
with two insect luciferase

Figure 1. Normalized bioluminescence emission spectra of Ppy (Panel A) and PplGR (Panel B) catalyzed reactions with LH_2 plus ATP-Mg at pH 8.6 (- - -) and pH 6.0 (— - —) and D-DiMeLH_2-AMP at pH 8.6 (—). Conditions used to obtain the spectra are in the Supporting Information.

Cell. Mol. Life Sci. 2010, 67, 387; JACS, 2002, 124, 2112

Mechanisms for Light Emission

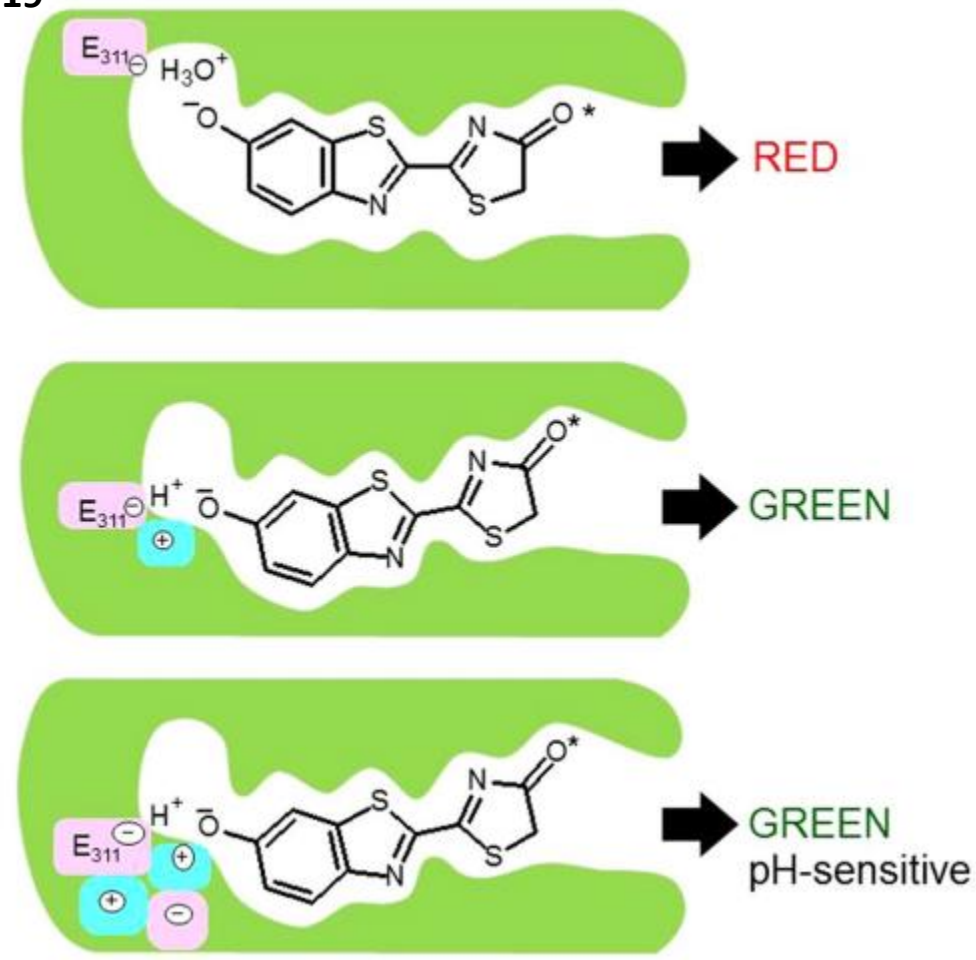
McCapra



Cell. Mol. Life Sci. 2010, 67, 387; JACS, 124, 2112

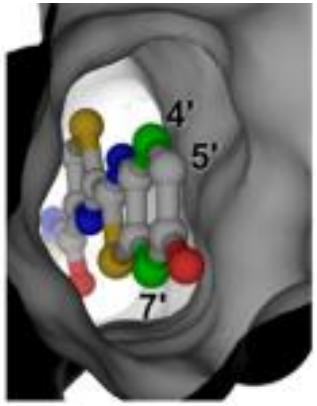
Mechanisms for Light Emission

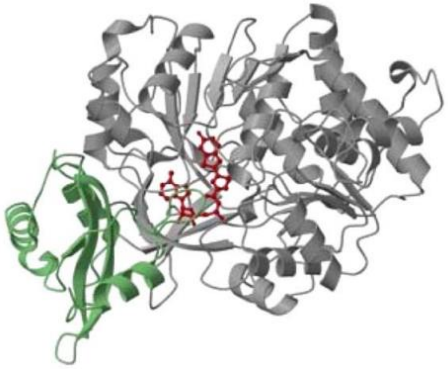
2019



(i) The **tight** accommodation of both the thiazole and benzothiazole parts of the luciferin inside the LBS to efficiently promote the adenylation and oxidative reactions, **increasing the light emission**

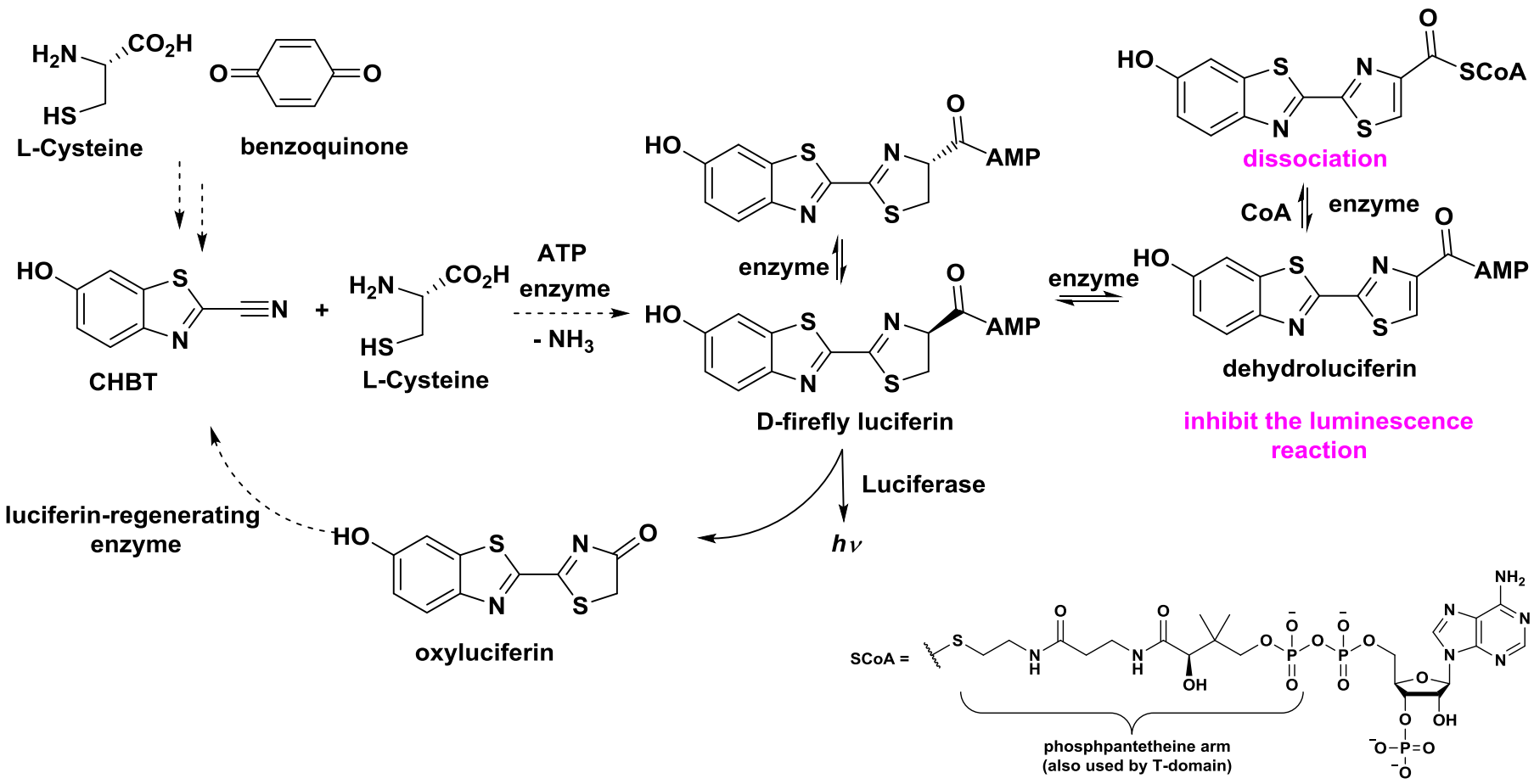
(ii) **Increasing of the cavity size** of the phenol binding pocket, relaxing and polarizing the environment around the phenolate group of excited oxyluciferin **producing red light**





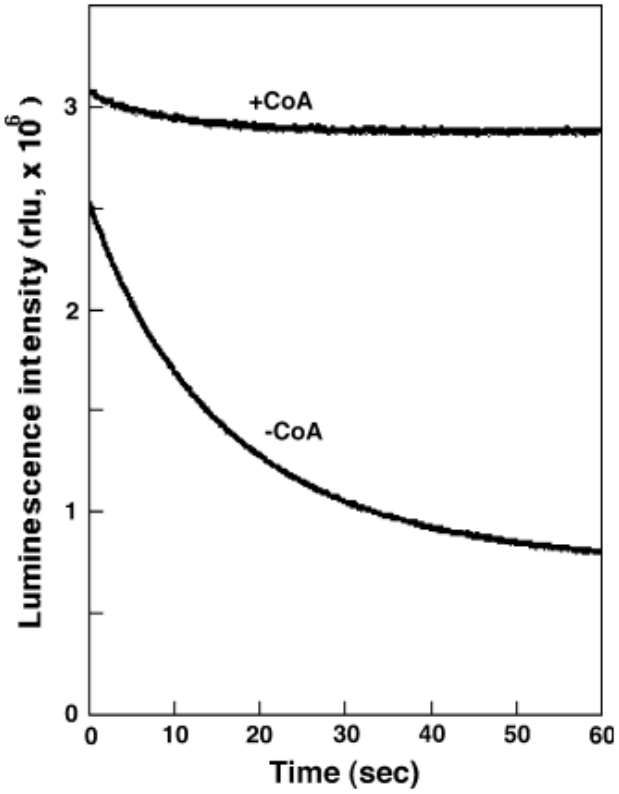
Scientific reports, 2019, 9,8998; Cell. Mol. Life Sci. 2010, 67, 387

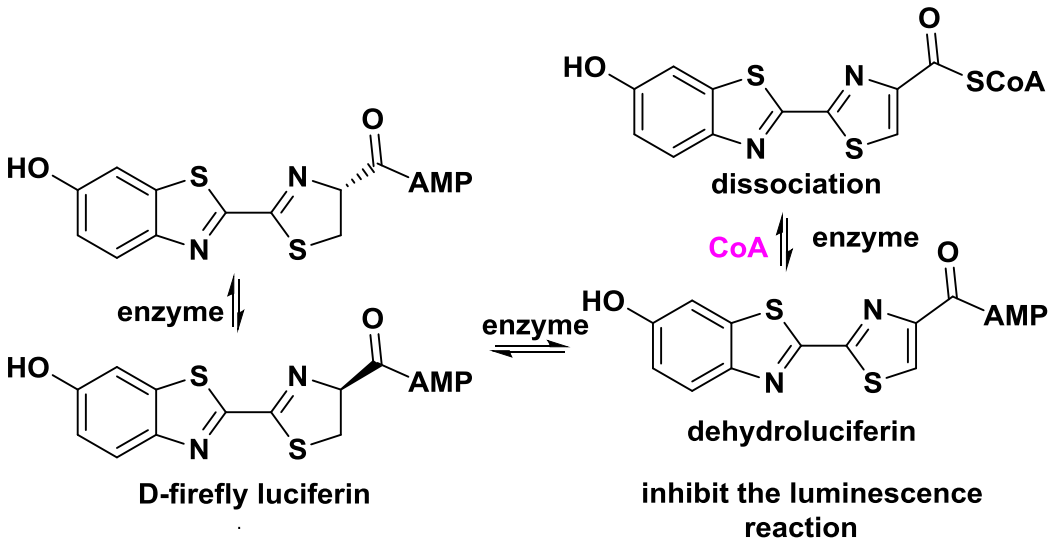
Biosynthesis, Recycling, Inhibition



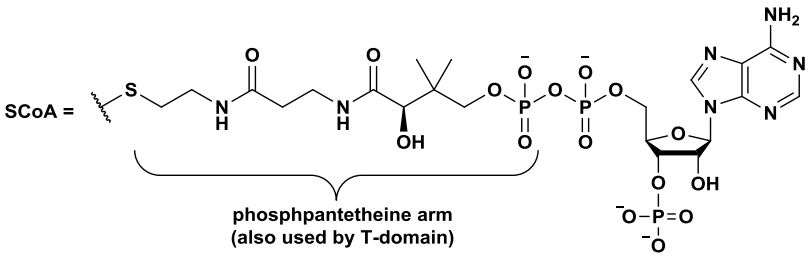
Cell. Mol. Life Sci. 2010, 67, 387; IUBMB Life, 2008, 61, 6

Biosynthesis, Recycling, Inhibition





The kinetics of the firefly luciferase reaction are effected by ATP, luciferin, O_2, CoA, temperature, pH, et al.



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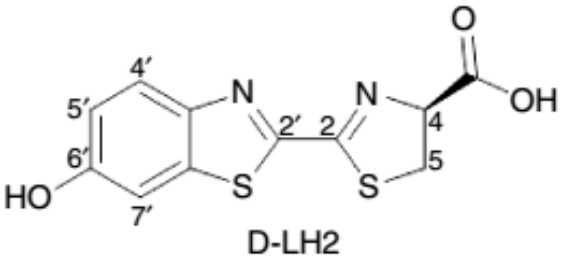
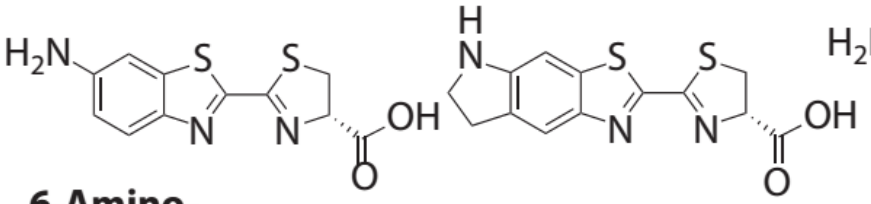
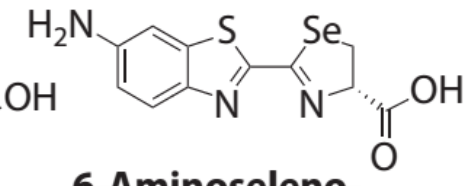
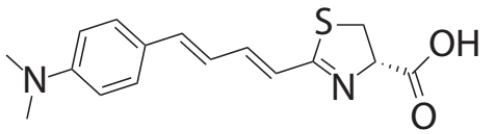
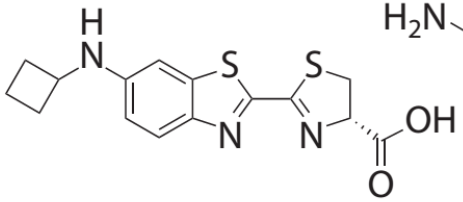
Representative D-luciferin Analogs

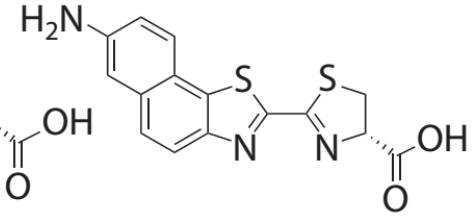


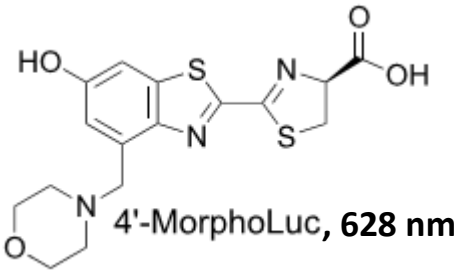












Tissue penetration is high at wavelengths greater than 620 nm.

1. sterically modified
2. electronically modified
3. heteroatom replacement

Disadvantages:
1. Emission intensity
2. Substrate specifity

Cell. Mol. Life Sci. 2010, 67, 387; Annu. Rev. Anal. Chem. 2019, 12, 9.1

Outline

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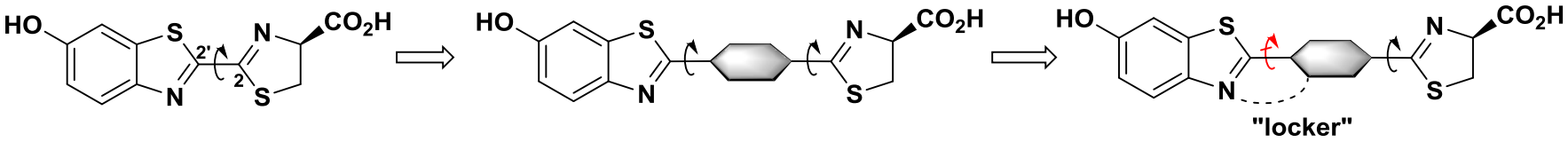
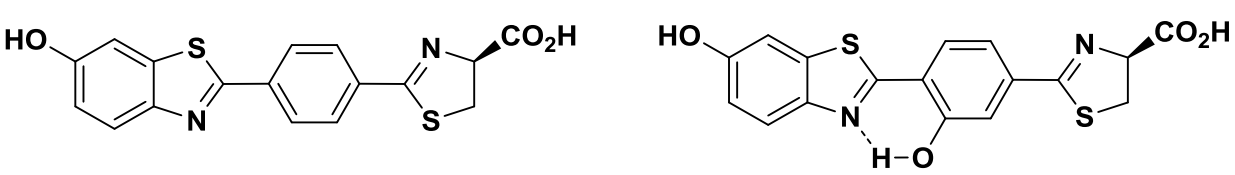
Design Logicality

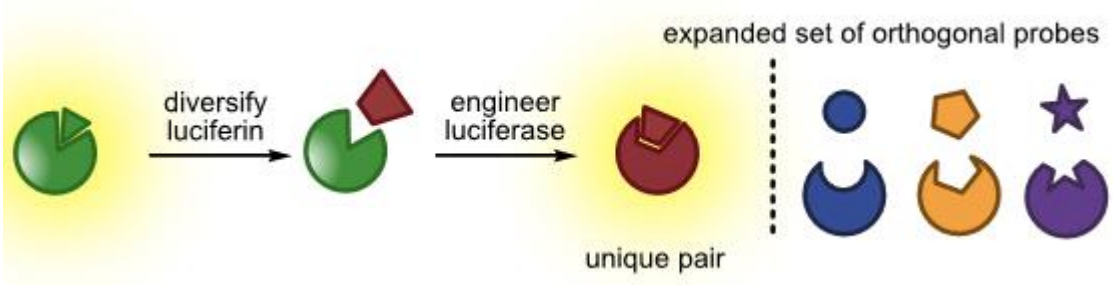


D-luciferin (Luc) rotationally labile partially restricted rotation

1. Substrate specificity: Only luciferases capable of enforcing a geometry should produce light, making them suitable candidates for orthogonal probe development.
2. Red shifted emission



Evolving an enzyme to control discrete chromophore conformation was a perceived challenge.



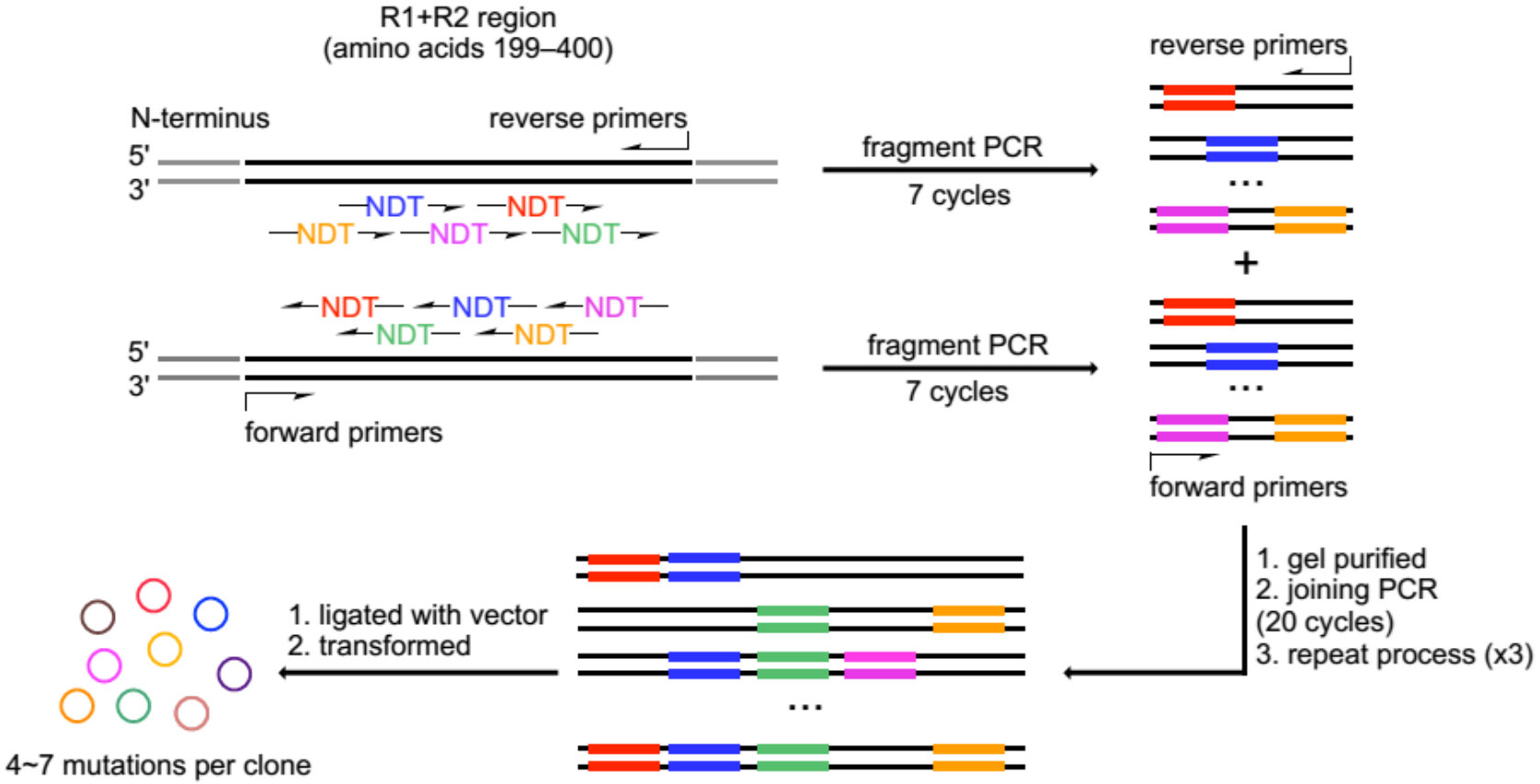
Parallel engineering to generate orthogonal luciferase-luciferin pairs

Evolutionary Luciferases via RosettaDesign

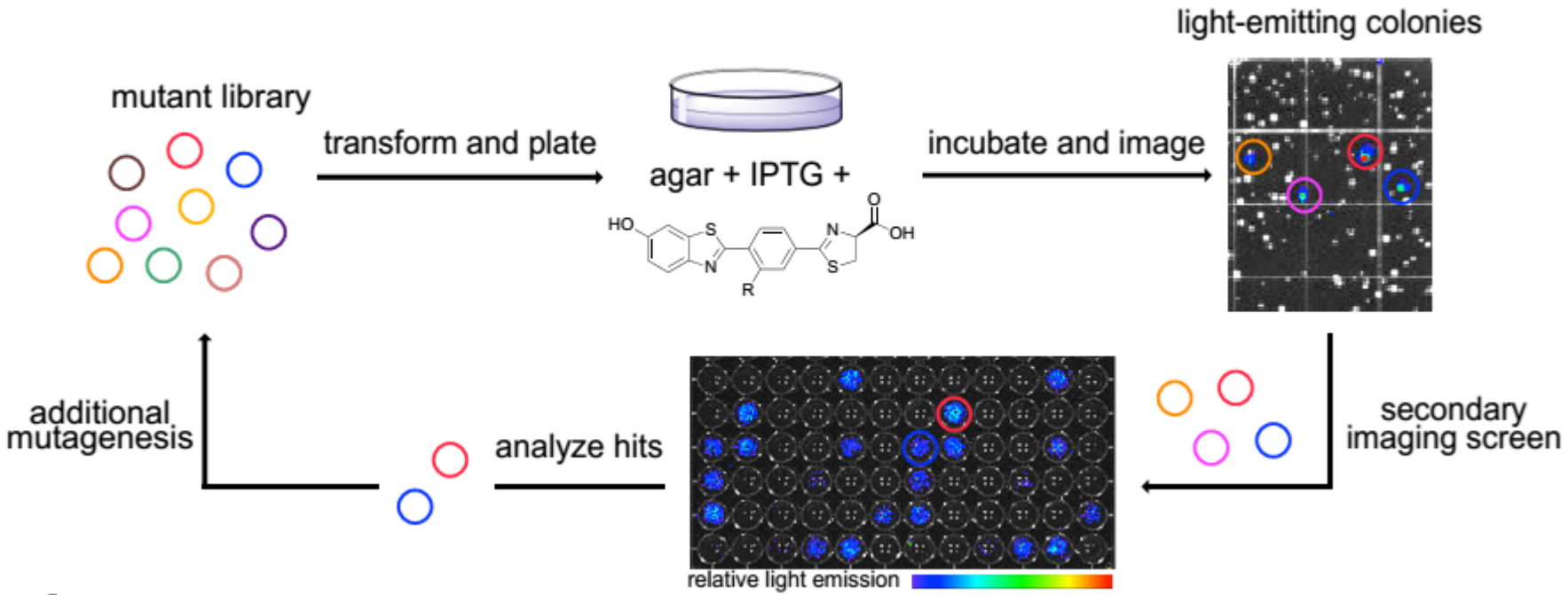
The modified luciferins were first docked into the luciferase enzyme

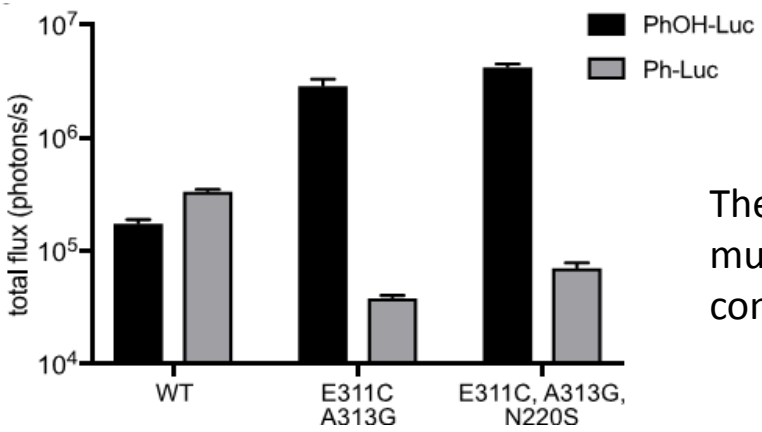
Optimize the residues surrounding the luciferins

Ranked by shape complementarity



Screening for Complementary Luciferases





The lack of hits for this analog suggested that it could take multiple mutations (>4) to remodel the luciferase active site to compensate for the substrate's flexibility.

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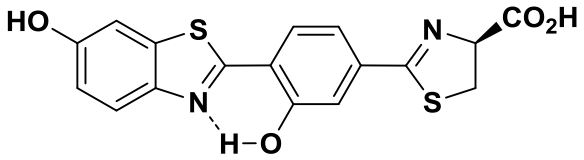
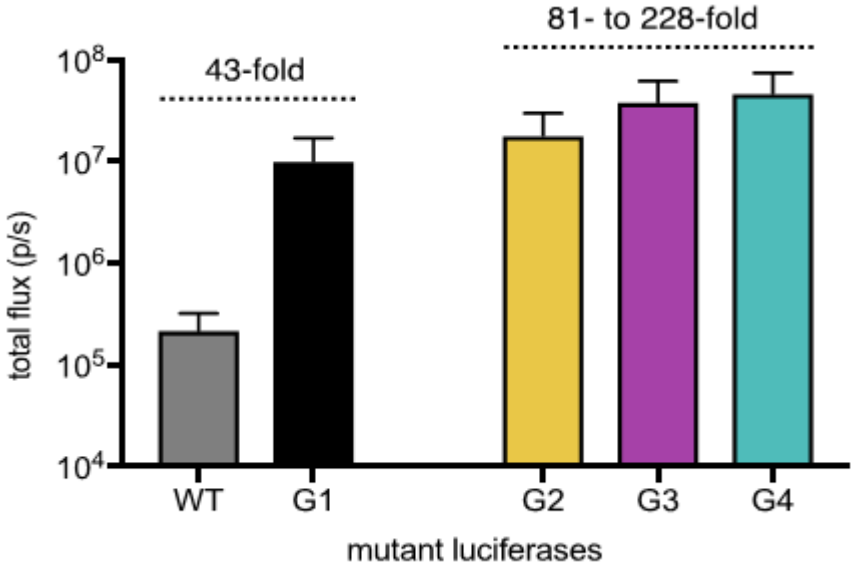
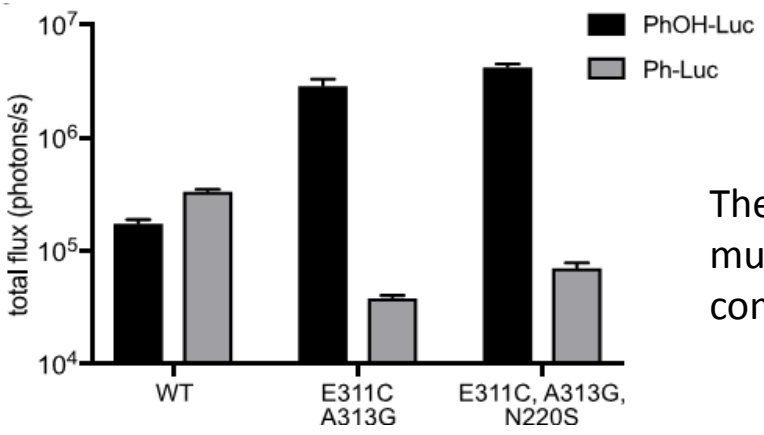


PhOH-Luc

Enzyme	218	220	249	311	313	314	316
WT	R	S	M	E	A	S	G
G1	-	-	-	C	G	-	-
G2	-	N	-	C	G	-	-
G3	K	N	L	C	G	-	-
G4	K	N	L	C	G	C	S





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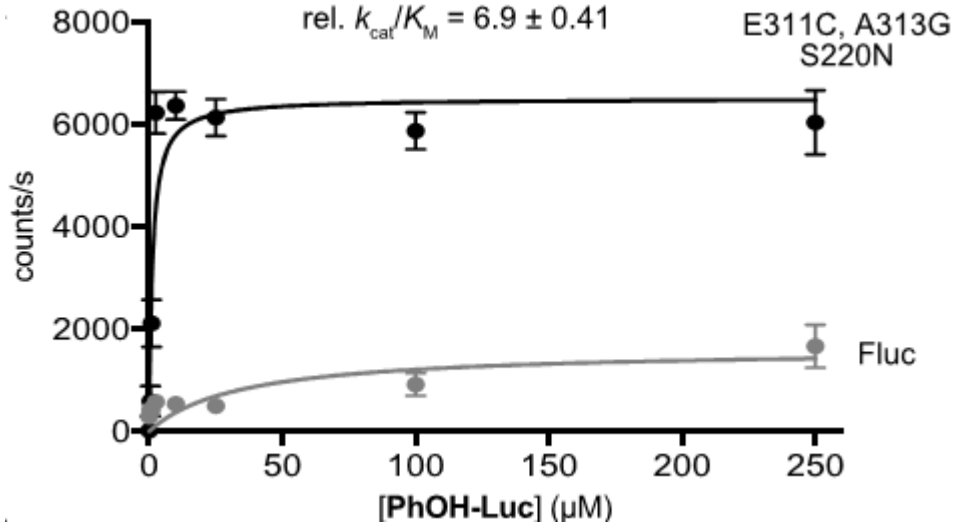
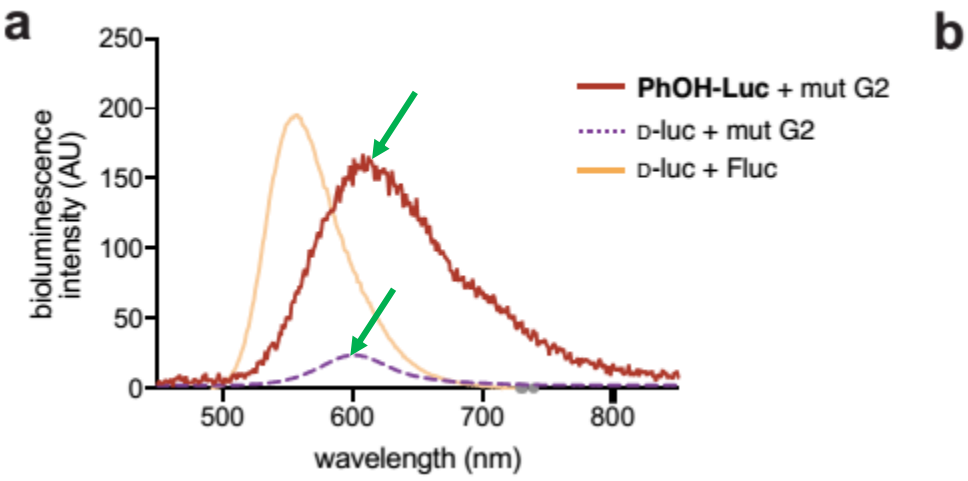
Bioluminescence Emission



~1.7–3.0% of Fluc/D-luc emission in lysate

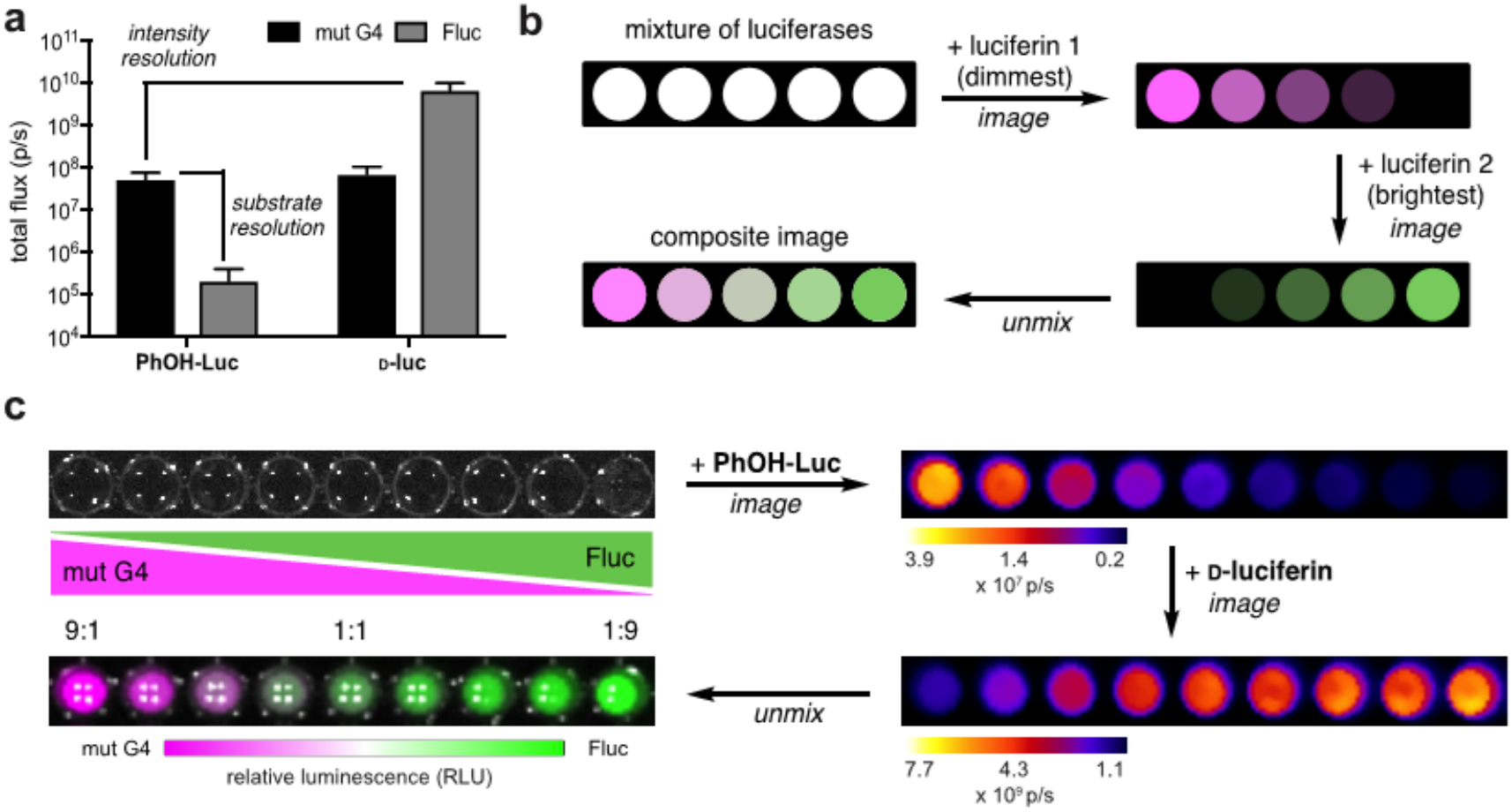
A similar red shift was observed when G2 was incubated with the native substrate, D-luc, suggesting that the change might not be substrate specific.



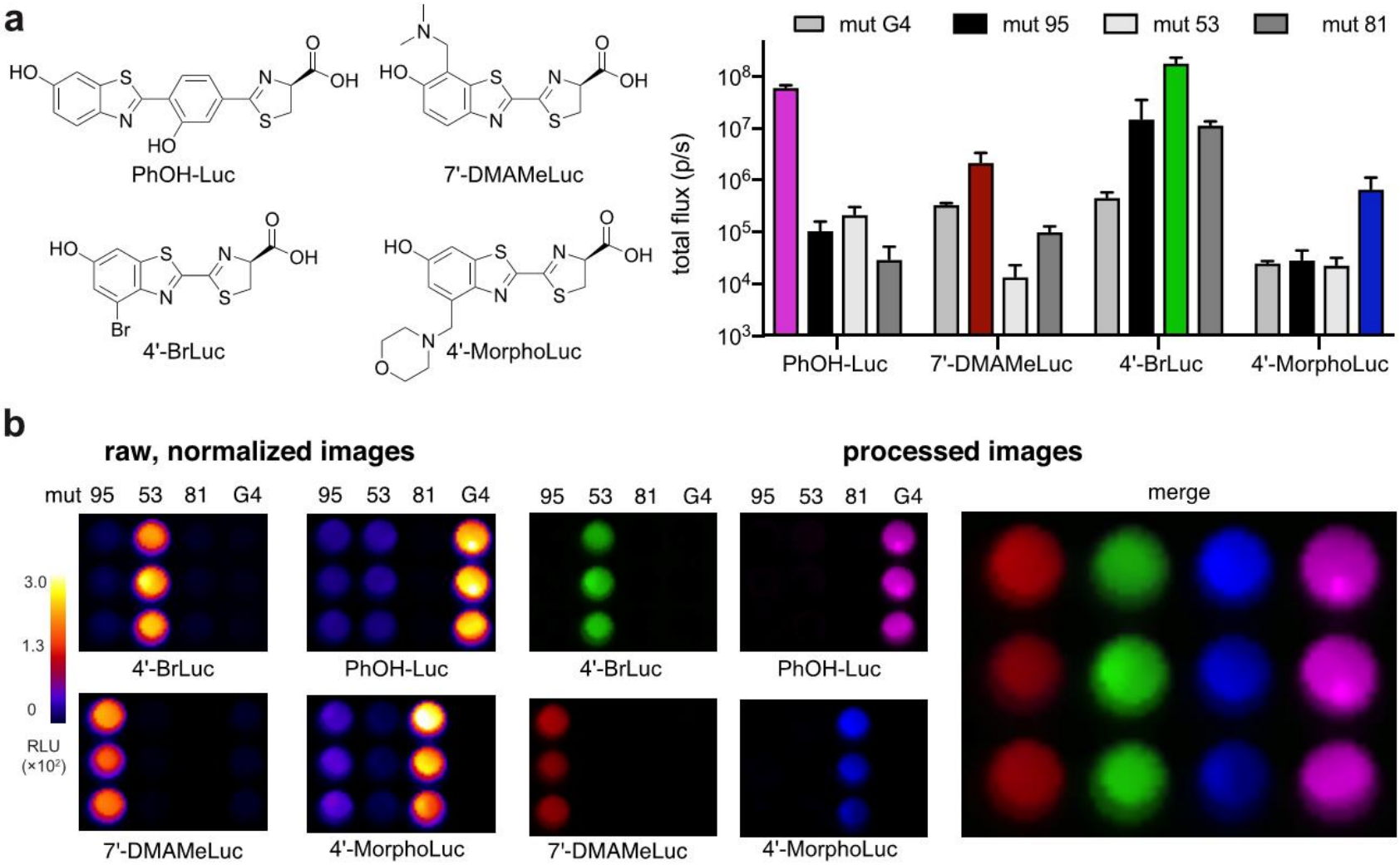
Luciferase	Luciferin	Emission maxima (λ_{em})
mut G2 (650 μg)	**PhOH-Luc** (250 μM)	608 nm
mut G2 (20 μg)	D-luciferin (100 μM)	603 nm
Fluc (6 μg)	D-luciferin (100 μM)	557 nm

Two-component Imaging

Firefly luciferin (Fluc) has been historically challenging: most synthetic analogs react with Fluc and most mutants react with D-luc.



Four-component Imaging



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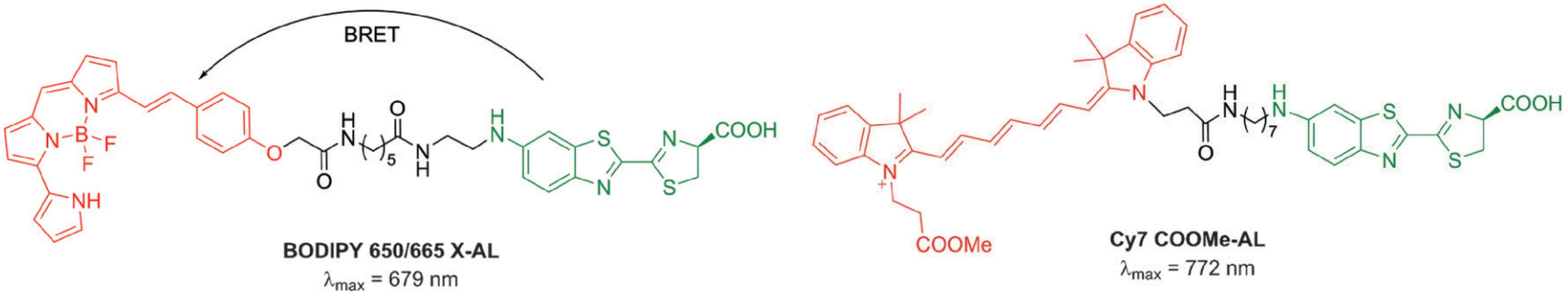
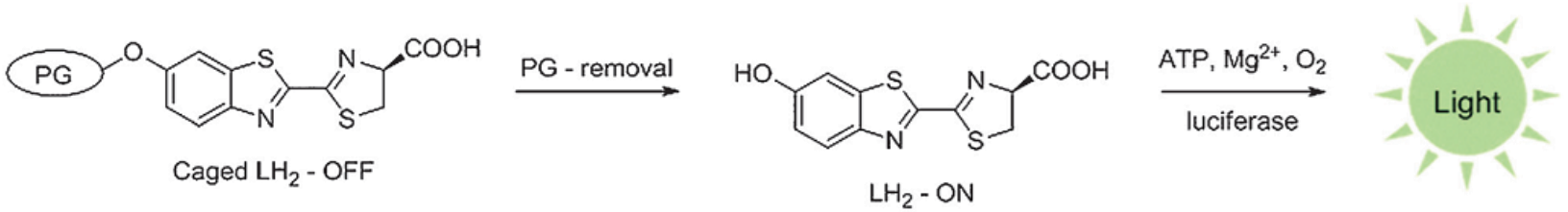
Bioluminescence Resonance Energy Transfer (BRET)



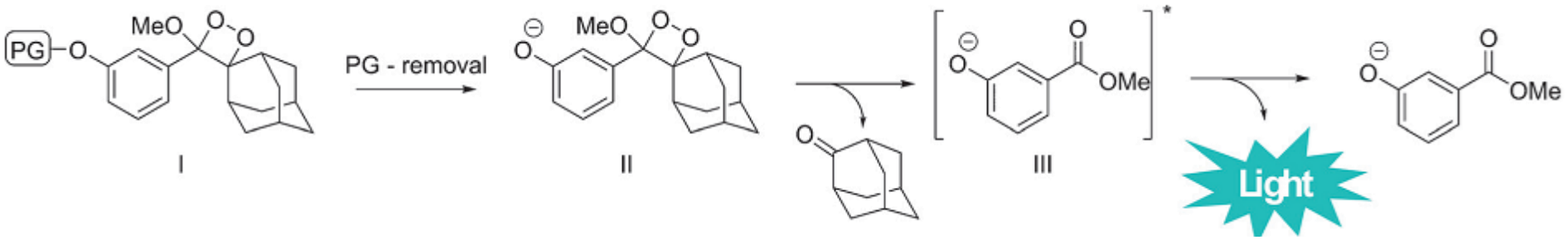
Luciferin emission is red-shifted by its conjugation to NIR-emitting dye (BRET)



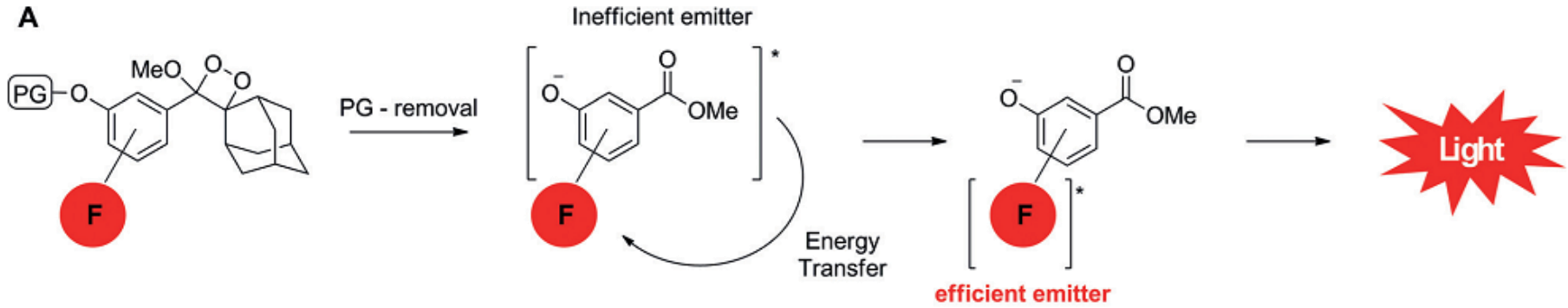
When the OH group of LH2 is caged, bioluminescence is off. The protecting group (PG) is removed by enzyme or analyte of interest, thus turning bioluminescence on.

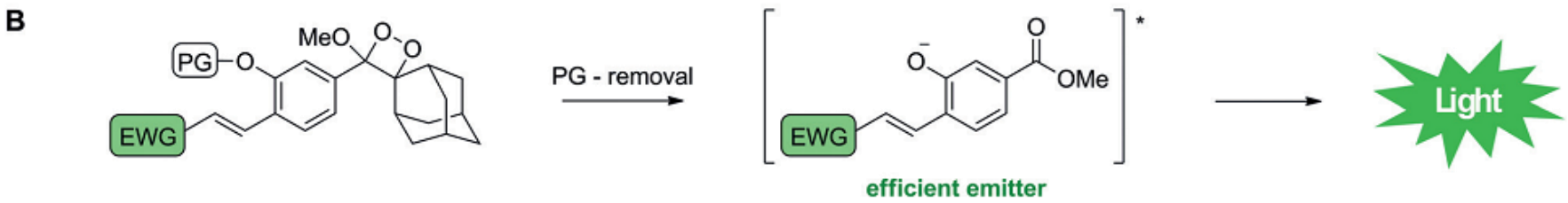
Angew. Chem. Int. Ed. 2017, 56, 16454

Triggerable Dioxetane



General structure and activation pathway of dioxetane-based probes (PG = protecting group).





Angew. Chem. Int. Ed. 2017, 56, 16454

Triggerable Dioxetane

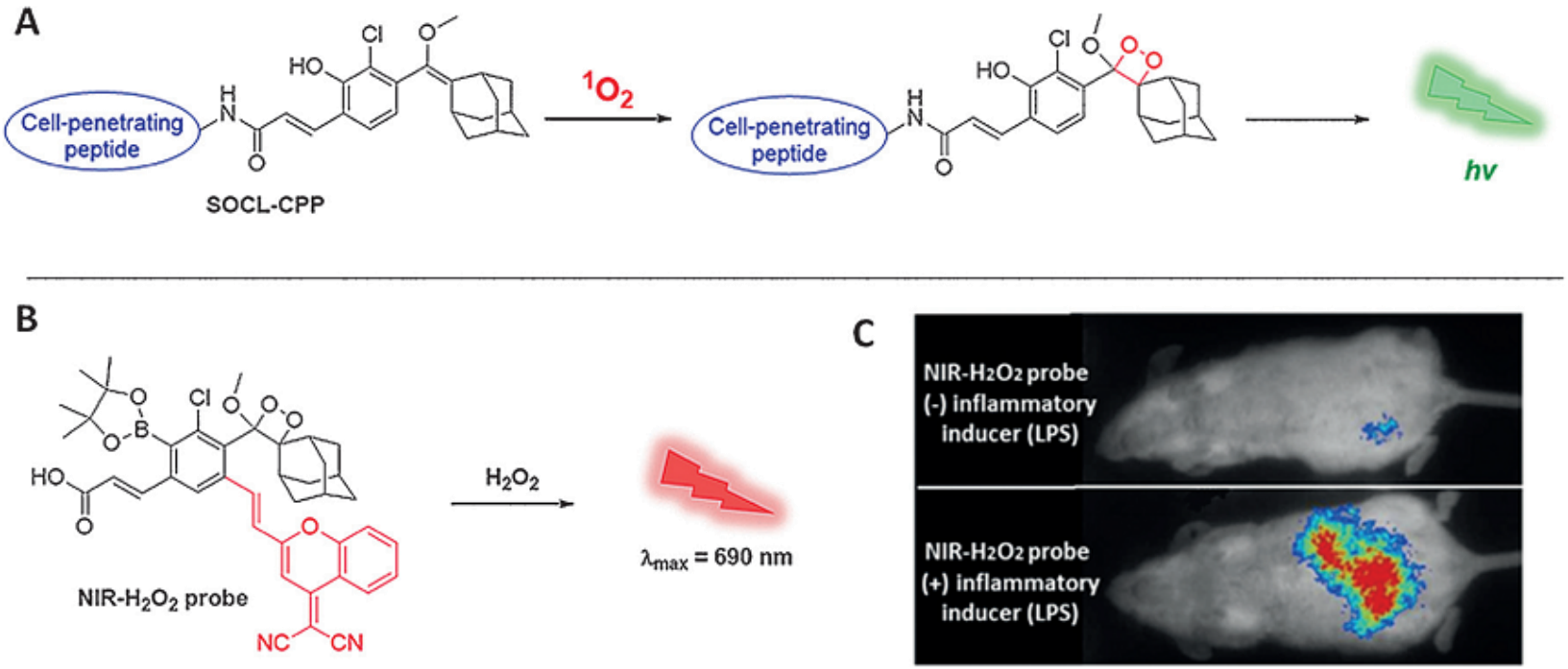


Figure 10. A) Structure of $^1O_2$ chemiluminescent probe **SOCL-CPP**, which emits green light upon reaction with $^1O_2$. B) Structure of NIR-emitting chemiluminescent probe with an extended π-electron system, which emits NIR light upon reaction with H_2O_2. C) In vivo imaging of endogenous H_2O_2 in the peritoneal cavity of mice during an LPS-induced inflammatory response, using the NIR-H_2O_2 probe.

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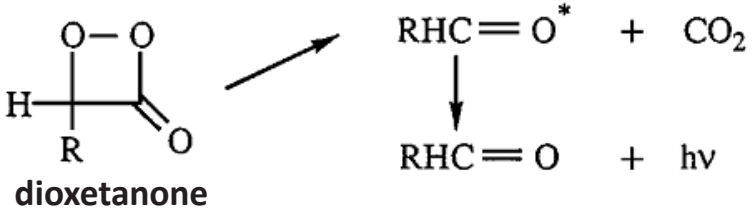
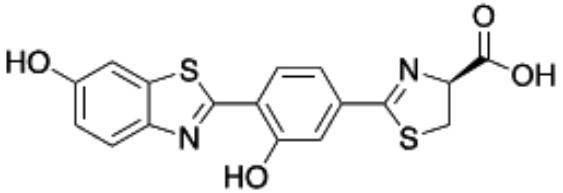
Outline

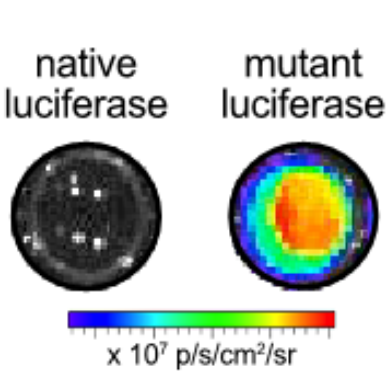
- ➢ *Background*
- ➢ *This work*
- ➢ *BRET & Triggerable Dioxetanes*
- ✓ *Summary*
- ✓ *Acknowledgement*

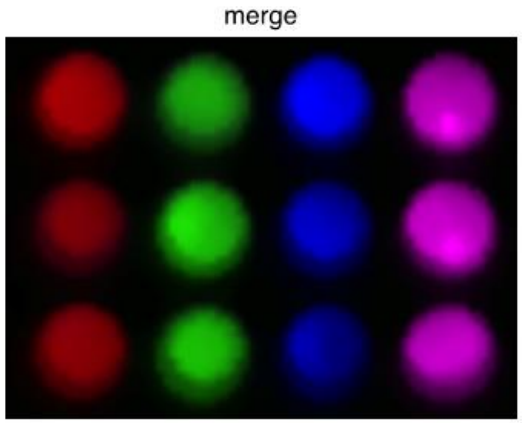
Summary

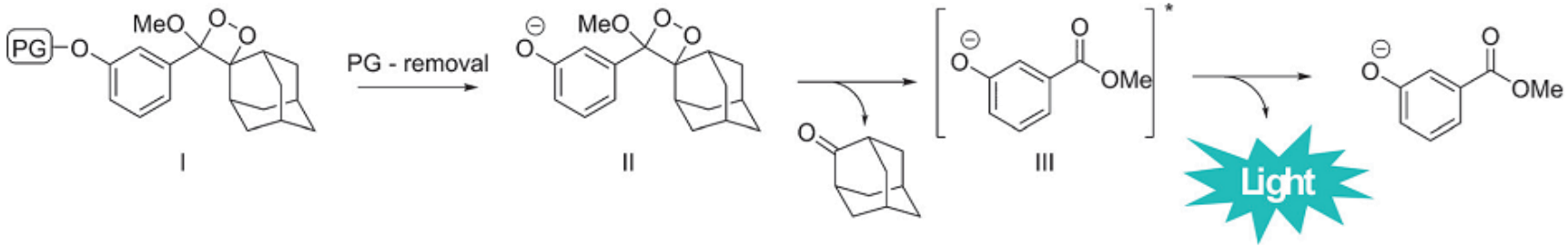


dioxetanone









Acknowledgement

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Thanks for Your Kind Attention!