Biological Applications of Chemiluminescence

B4 Hiroki Umeda

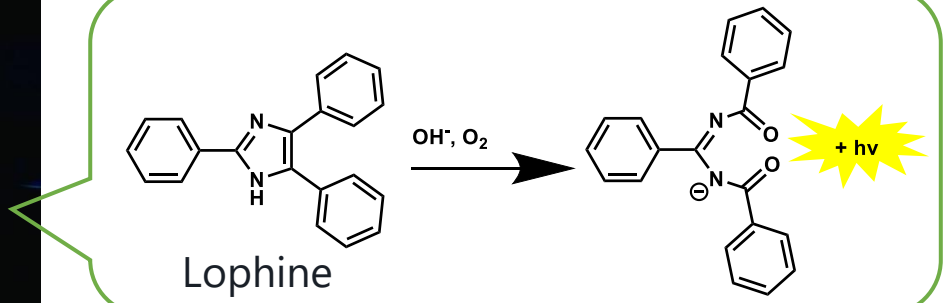
2021/12/15

Overview

[1]

BIOLUMINESCENCE (BL)

[2]

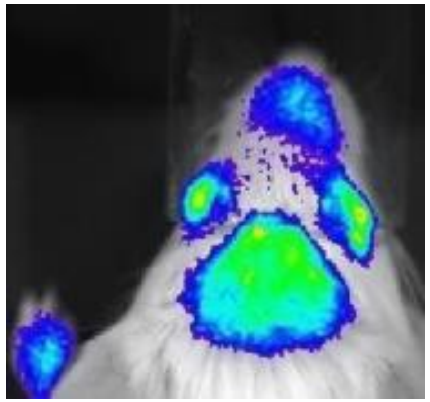


CHEMILUMINESCENCE (CL)

Biological Application

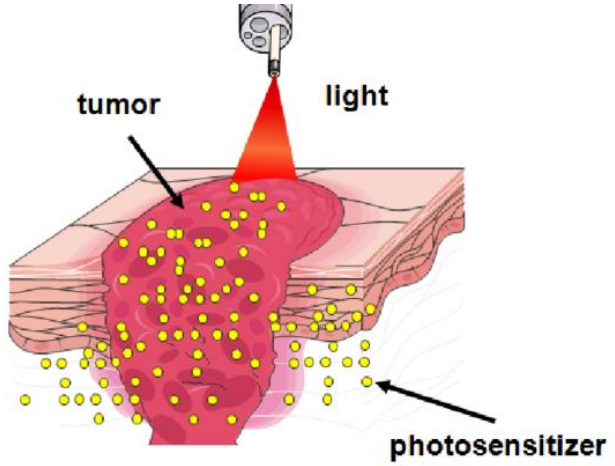
BIOIMAGING

[3]



PHOTODYNAMIC THERAPY

[4]



[1] https://itouyaryokan.com/blog/4247.html
[2] https://ja.eferrit.com/%E5%8C%96%E5%AD%A6%E7%99%BA%E5%85%89%EF%BC%9A%E5%AE%9A%E7%BE%A9%E3%81%A8%E4%BE%8B/
[3] https://www.nature.com/articles/s41467-020-17783-4#Sec21
[4] https://blog.rpmclasers.com/red-laser-diodes-for-photodynamic-therapy

Contents

- Basic Information on Chemiluminescence
- Application for Bioimaging
- Application for Photodynamic Therapy
- Mini-proposal
- Summary

Contents

- **Basic Information on Chemiluminescence**
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Chemiluminescence

Chemiluminescence (CL):

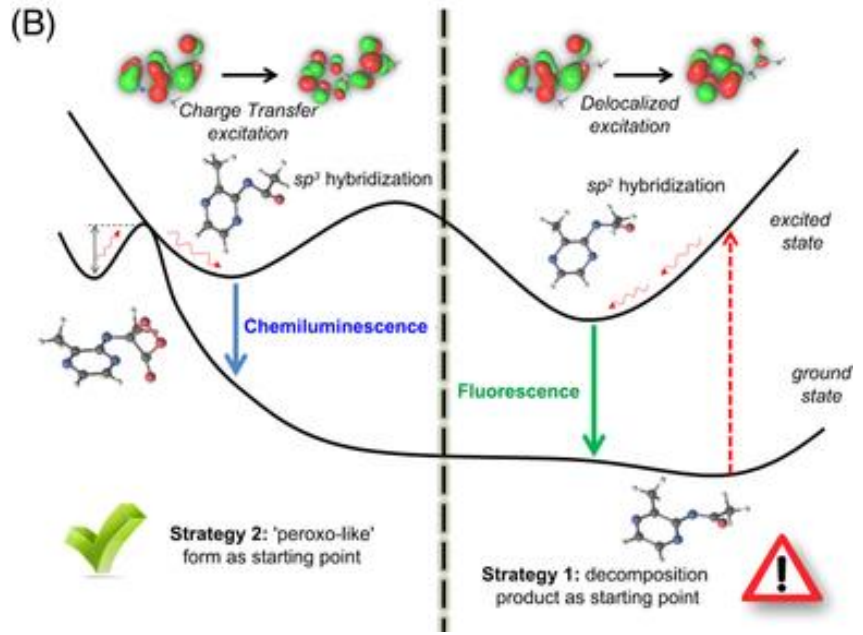
the emission of light as a result of chemical reaction

$$A\ (+B) \rightarrow C^* \rightarrow C + h\nu \quad \textbf{(eq.1)}$$

✓ **No excitation light required**

✕ **Poor CL efficiency**

$$\Phi_{CL} := \frac{moles\ of\ photons\ emitted}{moles\ of\ reactants} \quad \textbf{(eq.2)}$$

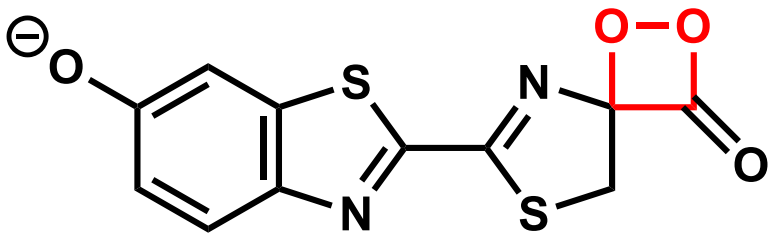


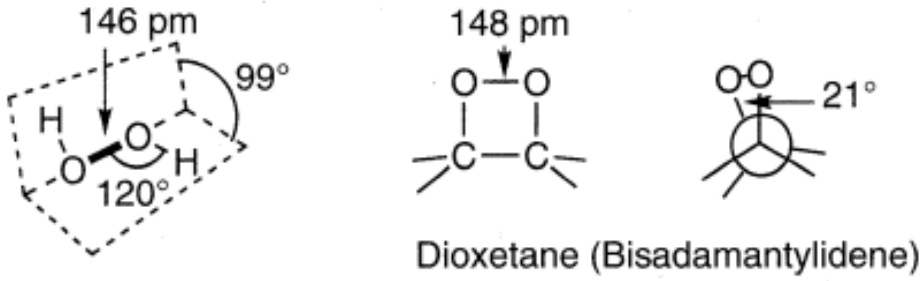
	Type	ΦCL
Firefly	BL	0.8
Sea firefly	BL	0.3
Luminol	CL	0.04
Ordinary compounds	CL	10^{-8}~10^{-3}

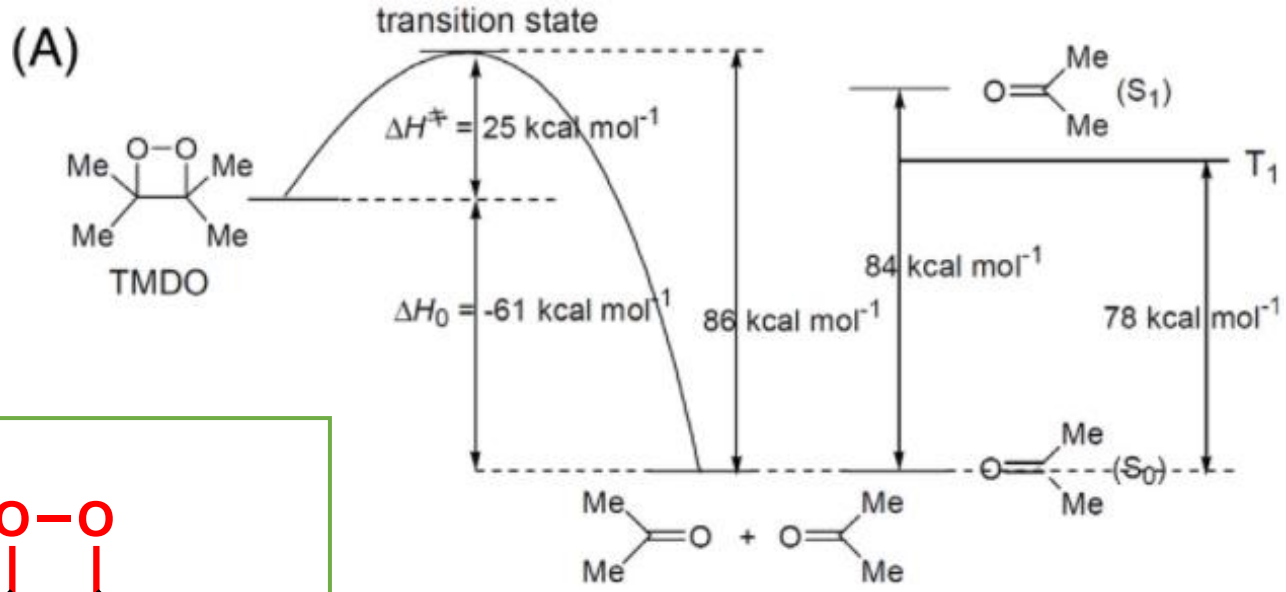
Motoyoshiya, J. 化学と教育. **2007**, 55(1), 32-35. / Xu, Fu-jian. *et al. Aggregate.* **2021**, xx, e140.

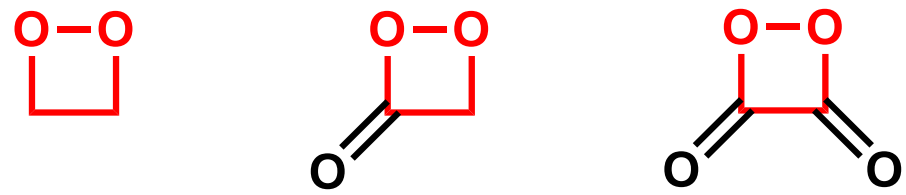
High-energy intermediate

Luminescence process of firefly luciferin → contribution of high-energy intermediate containing dioxetane moiety









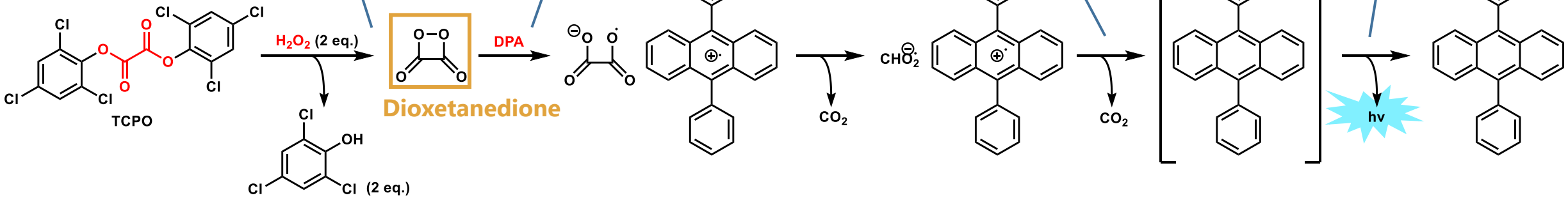
1,2-dioxetane 1,2-dioxetanone 1,2-dioxetandione

Representative Mechanism

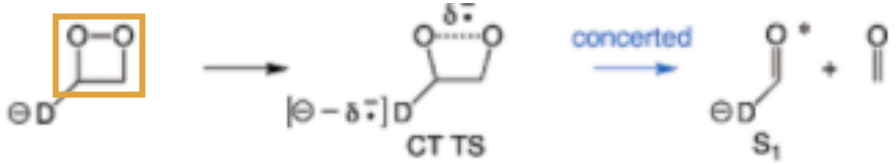

[1]

CIEEL (Chemically Initiated Electron Exchange Luminescence)

Formation of high-energy intermediate | **Decomposition (ET)** | **Chemiexcitation (BET)** | **Emission from the excited state**



CTIL
(charge-transfer-induced luminescence)



ET: electron transfer
BET: back electron transfer

Xu, Fu-jian. *et al. Aggregate.* **2021**, xx, e140.
[1] http://www.yamamotoningyou.net/omoty-04.htm

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Motivation

Drawbacks of near infrared fluorescence (NIRF) imaging:

- **Autofluorescence**
  Excitation light also excites other endogenous fluorophores.
- **Excitation Leakage**
  Excitation light close to the fluorescence wavelength can‘t be filtered out.
- **Stronger signals at shallow locations**
  Intense light at shallow locations results in a strong signal, including noise.
- **Short excitation wavelength**
  Due to Stokes shift, excitation light should be shorter than fluorescence.

→ **Low signal to noise ratio (SNR), Poor tissue penetration**

CL imaging (w/o the need for excitation light) can solve these problems !

Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

Challenges

Challenges of chemiluminescent (CL) imaging:

A) Narrow Substrate Scope

Only some substrates, such as **ROS**, can make high-energy intermediates.

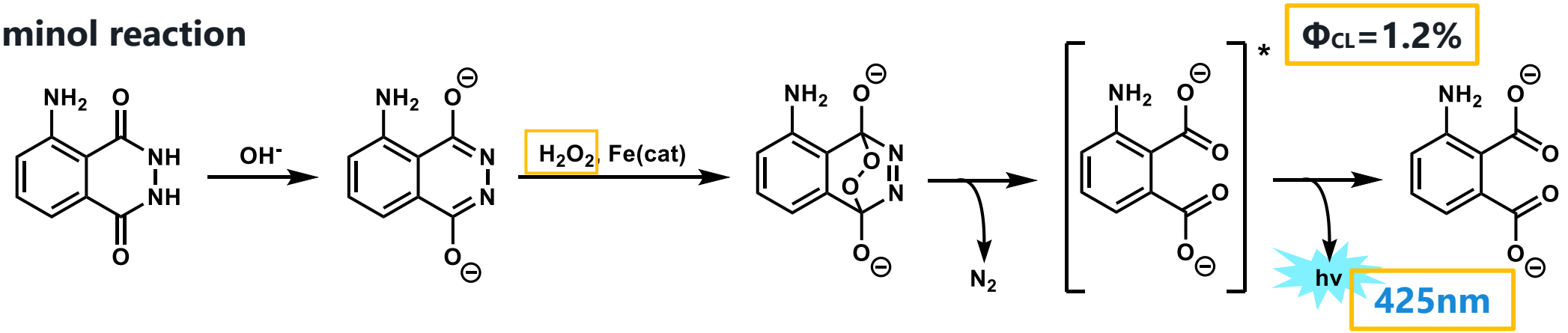
B) Weak CL brightness

Quantum yield of CL (Φ_{CL}) is low and difficult to detect.

C) Short wavelength

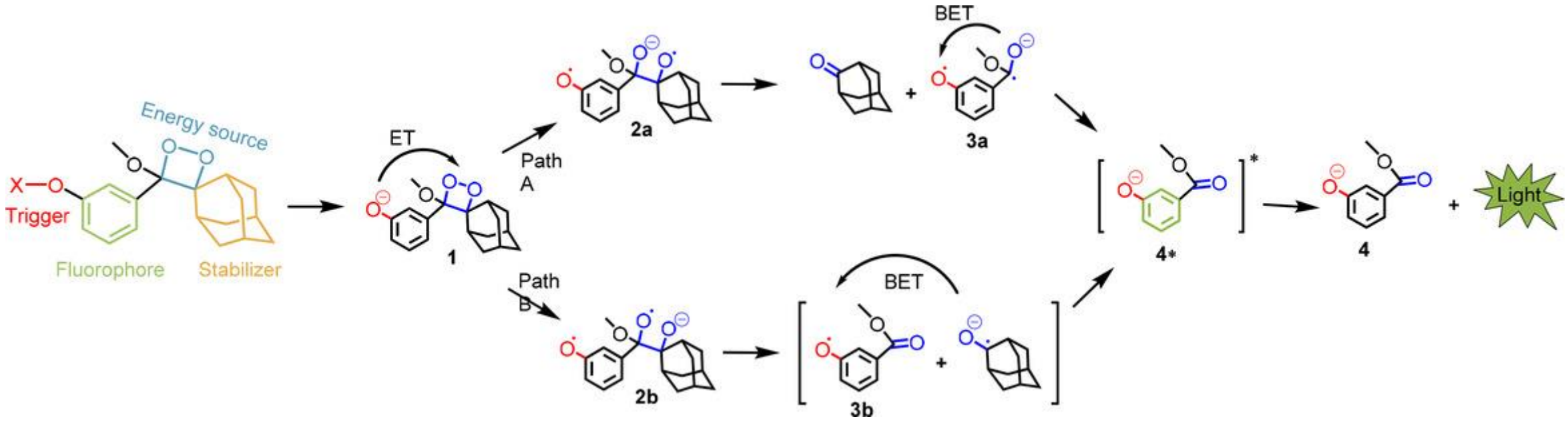
Short-wavelength light has poor tissue permeability.

Ex.) Luminol reaction



Magalhães, C. M. *et al. Chemphyschem.* **2016**, 17(15), 2286-2294. / Xu, Fu-jian. *et al. Aggregate.* **2021**, xx, e140.

Schaap's dioxetane

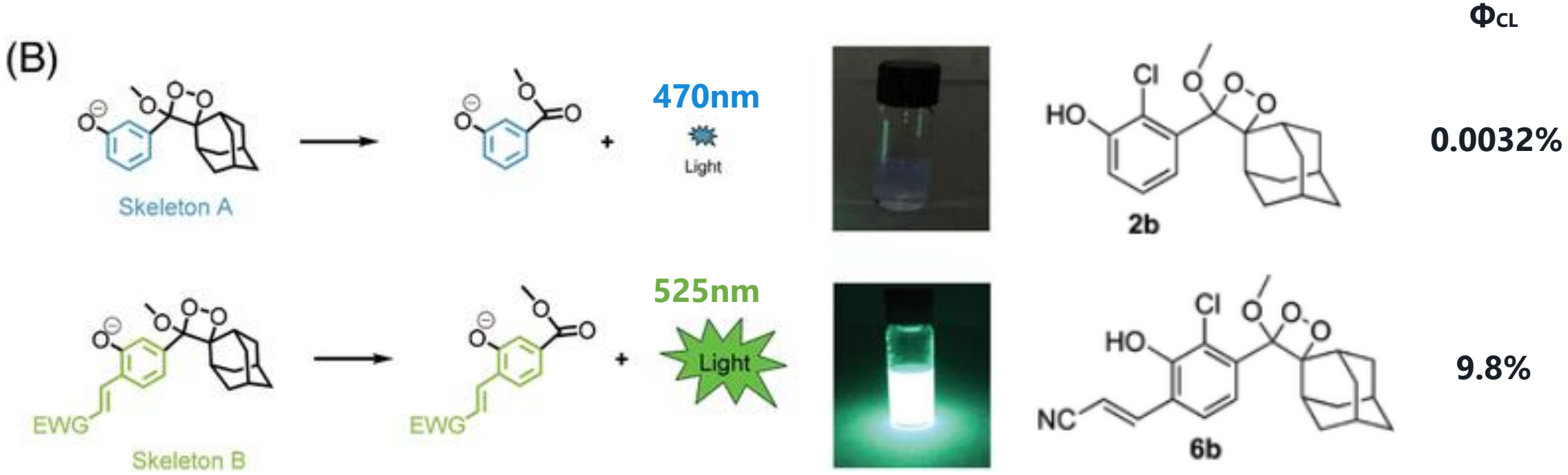


The energy source is in the molecule from the beginning.

→ It can be adapted to any substrate as long as the trigger group can be deprotected.

Schaap, A. P. *et al. Tetrahedron Lett.* **1987**, 28(9), 935-938.
Xu, Fu-jian. *et al. Aggregate.* **2021**, xx, e140.

Shabat's dioxetane



Φ_{CL}

0.0032%

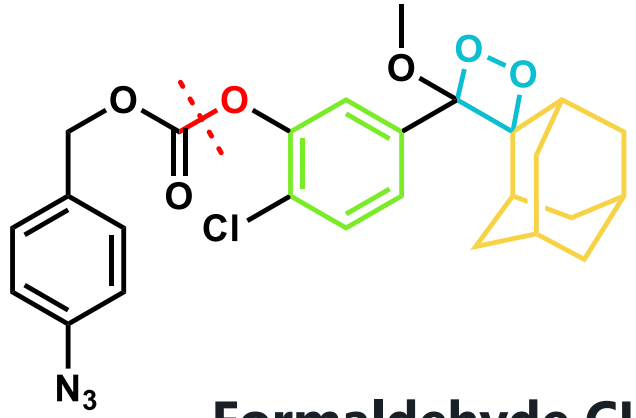
9.8%

Electron-withdrawing group (EWG) was introduced at the ortho position of the phenol in Schaap's dioxetanes (= skeleton A) → Redshift of wavelength and increase in quantum yield (Φ_{CL})

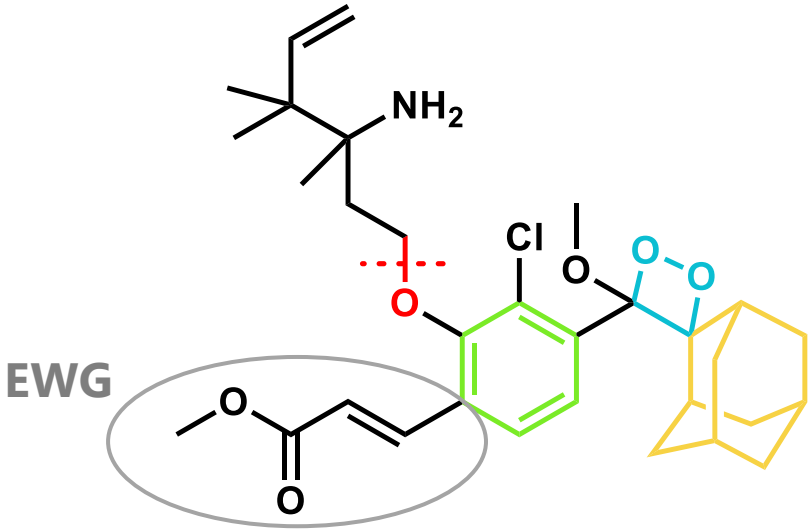
Shabat, D. *et al. ACS Cent. Sci.* **2017**, 3(4), 349-358.
Xu, Fu-jian. *et al. Aggregate.* **2021**, xx, e140.

Probe examples

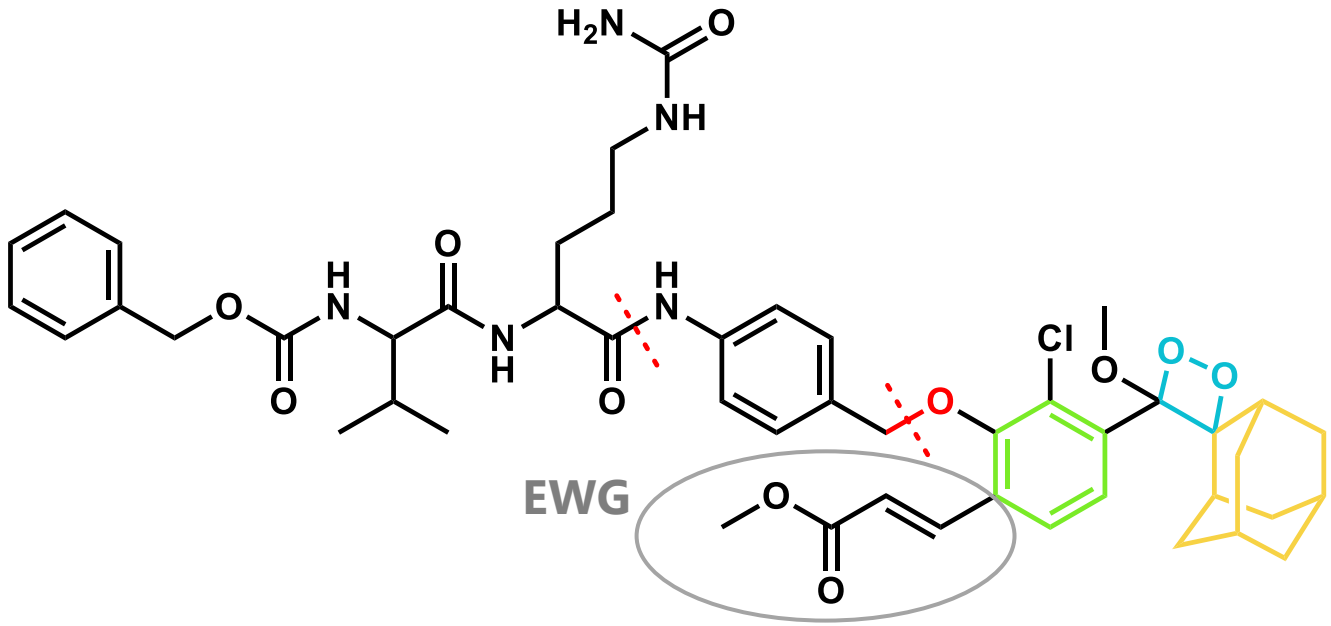
H_2S CL Probe



Formaldehyde CL Probe



Cathepsin B CL Probe



Applicable to *in vivo* imaging

Lippert, A. R. *et al. Chem. Sci.* **2015**, 6, 1979-1985.
Shabat, D. *et al. Angew. Chem. Int. Ed.* **2017**, 56, 15633–15638.
Shabat, D., Chang, C. J. *et al. Angew. Chem. Int. Ed.* **2018**, 57, 7508–7512.

New type probe

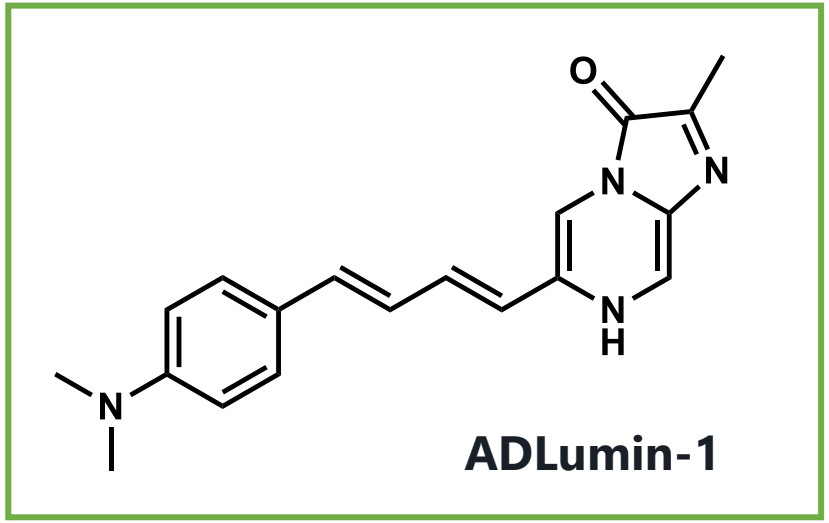
The limitation of Schaap's or Shabat's dioxetane:

Only applicable for substrates that can trigger phenol deprotection

New type probe:

Switching mechanism

(No need to deprotect phenol)



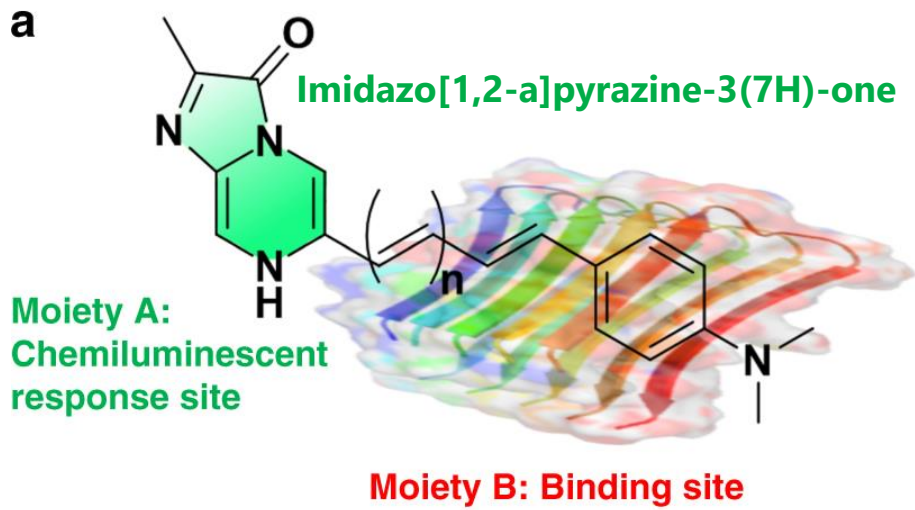
ADLumin-1

Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

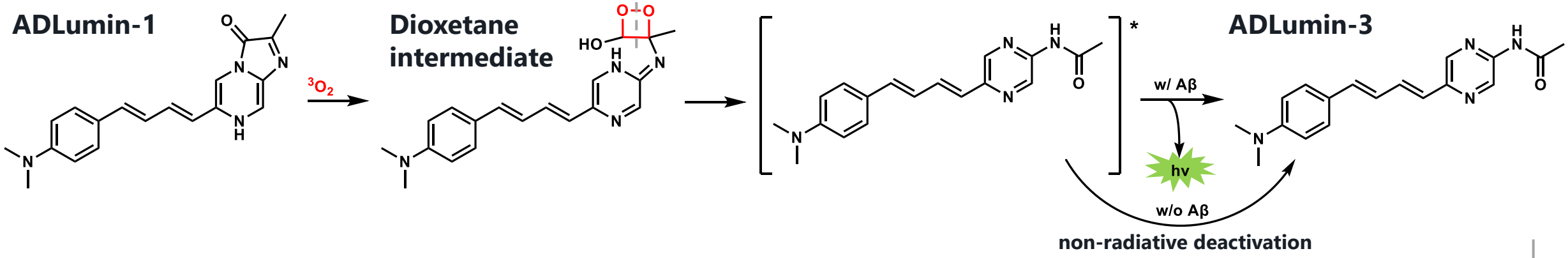
Mechanism

ADLumin-1:

- Probe for aggregated Amyloid-β (Aβ)
- Auto-oxidation is primary cause of CL
- ADLumin-3 release photons only when binding to Aβ



Tentative Mechanism:

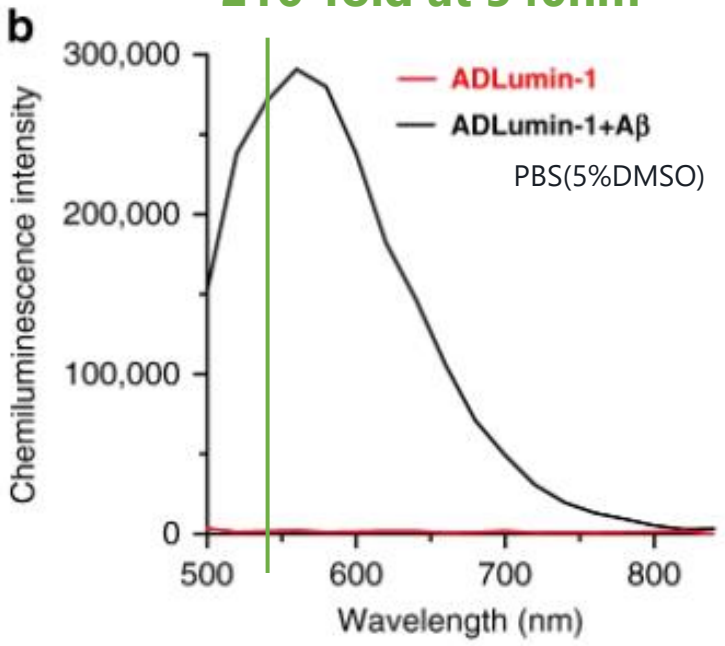


Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

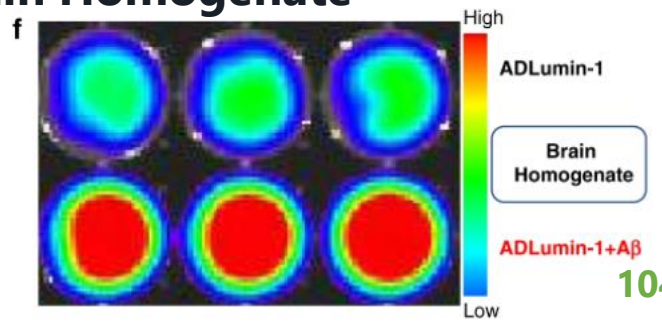
In vitro/vivo CL imaging

Pure solution

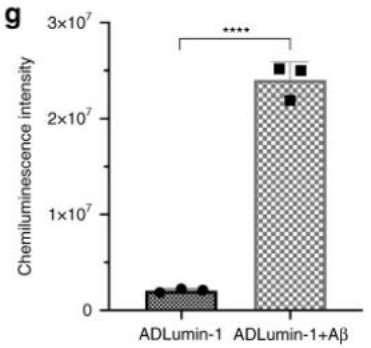
216-fold at 540nm



Brain Homogenate

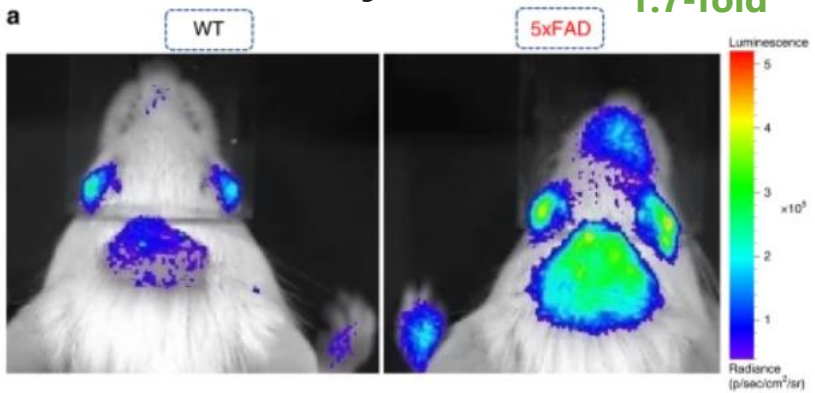


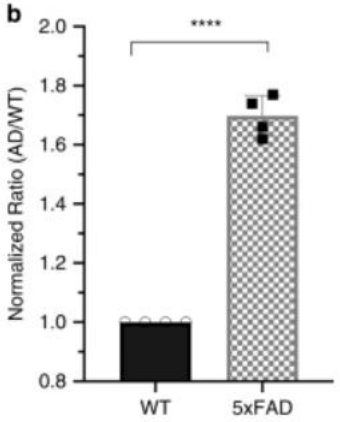
104-fold



***In vivo* brain (and eye)**

1.7-fold





✓ **Selective amplification for Aβ**

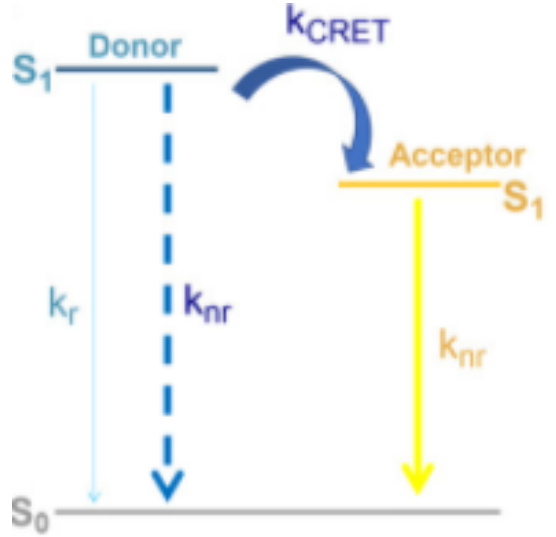
✓ **BBB permeability**

✕ **Wavelength of emission is short**

Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

CRET

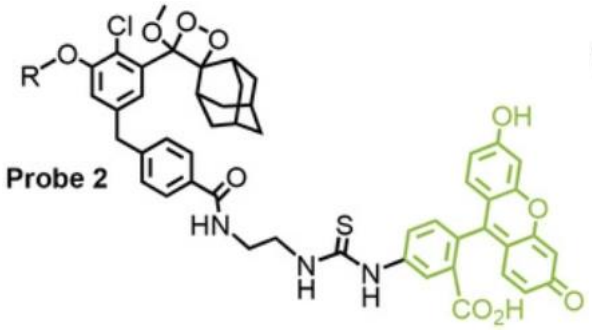
CRET(Chemiluminescence resonance energy transfer):
Nonradiative energy transfer process
from CL skeleton (Donor) to Fluorophore (Acceptor)
→ Longer wavelength, larger Φ_{CL}

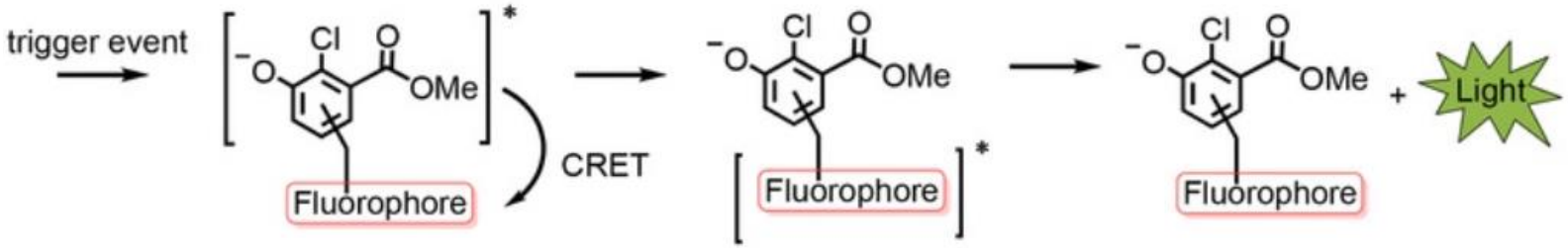


【Requirements】

- **Spectral Overlap** (between Em of D and Abs of A)
- **Proximity** (normally <10nm)

Ex.) Fluorescein tethered dioxetane





w/o Fluorophore: λ=470nm, Φ_{CL}=0.0033%
→ CRET Probe: λ=714nm, Φ_{CL}=0.38%

Xu, Fu-jian. *et al. Aggregate.* **2021**, xx, e140.

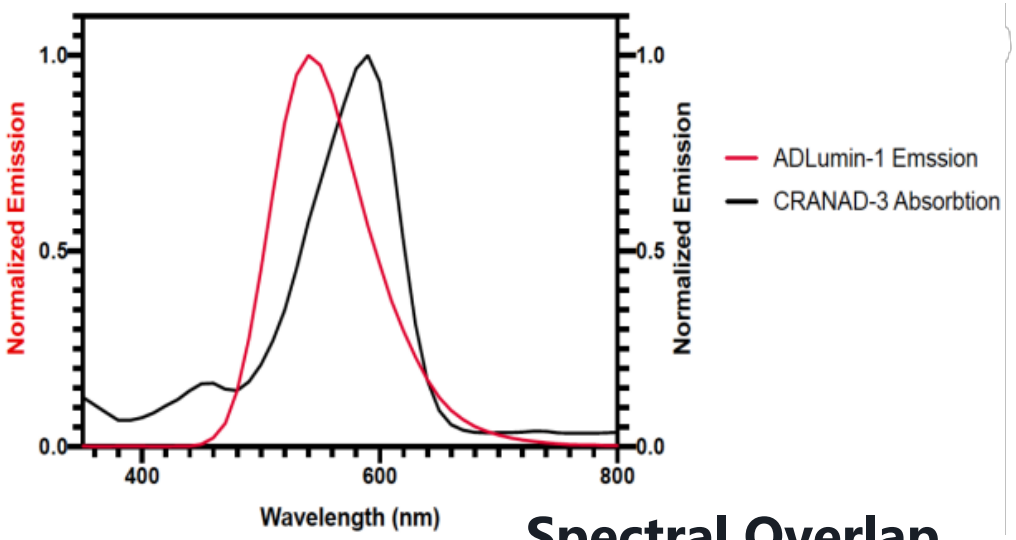
DAS-CRET

DAS-CRET(Dual-amplification of signal via CRET):

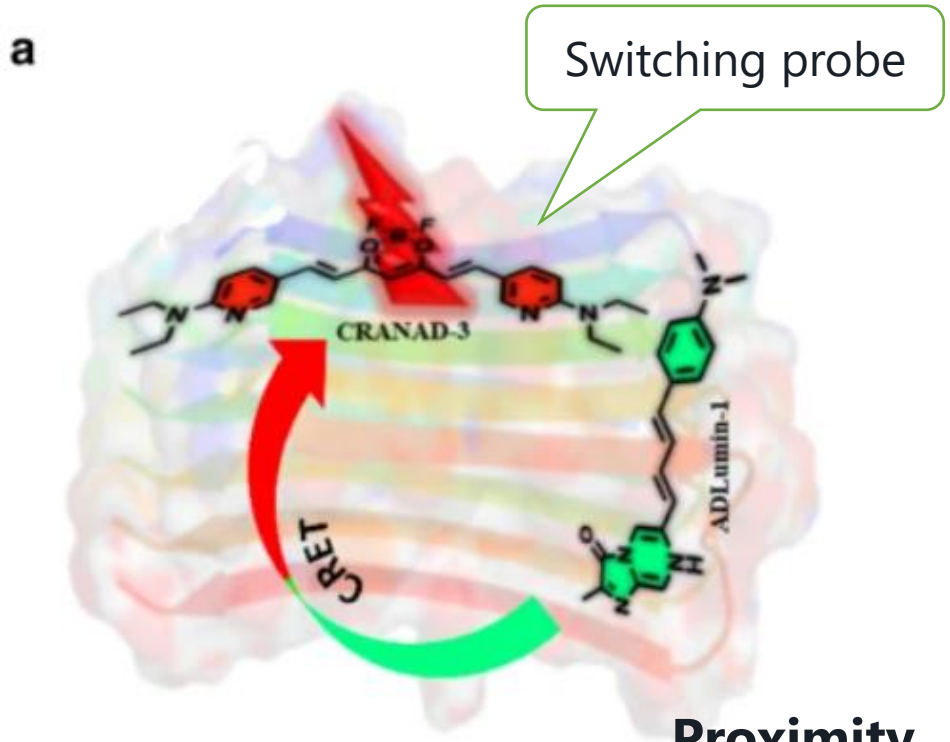
CRET by 2 molecules that amplify the signal upon Aβ binding

Donor：ADLumin-1（CL probe）

Accepter：CRANAD-3 (NIRF probe)



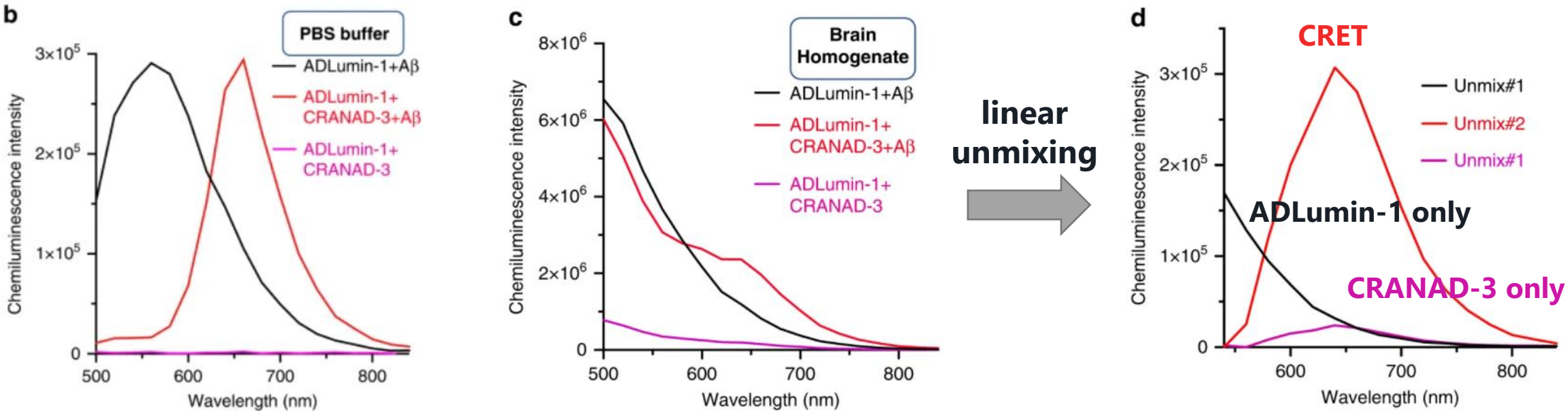
Spectral Overlap



Proximity

Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

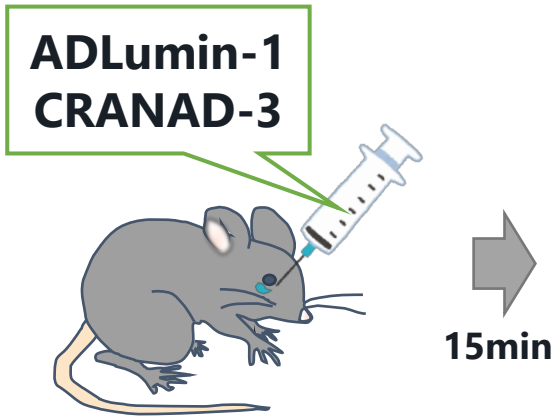
DAS-CRET *In vitro* Imaging



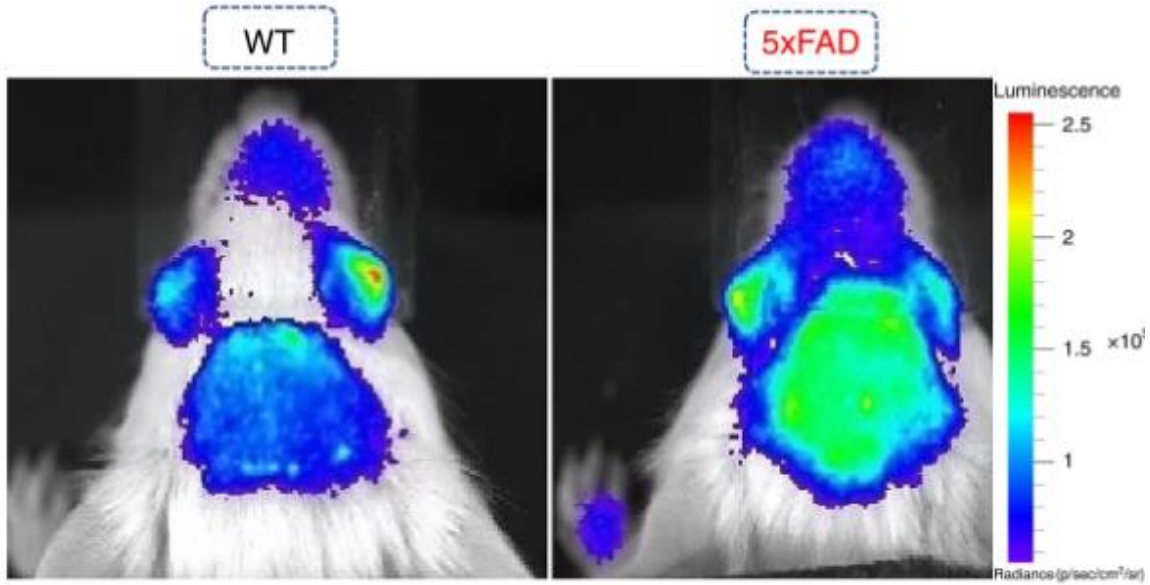
CRET is feasible in both pure solution and brain homogenate
(Longer wavelength (NIR) was achieved)

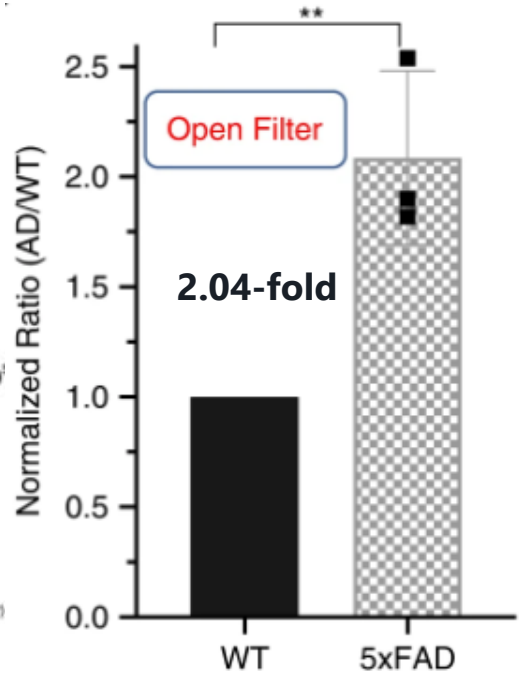
Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

DAS-CRET *In vivo* Imaging



15min





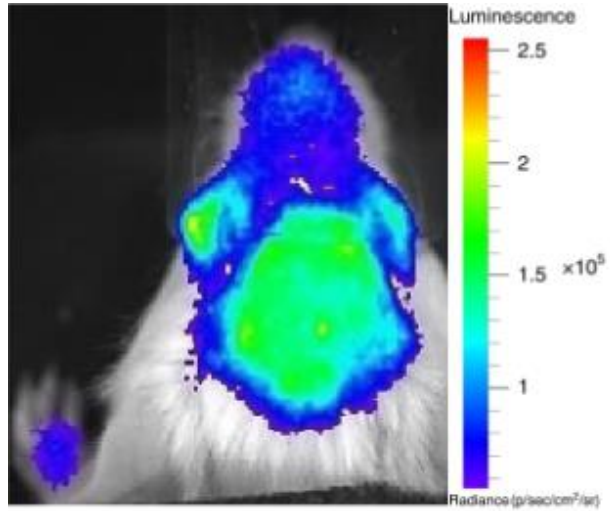
CRET *in vivo* Imaging is feasible in brain

- ✓ **Selective amplification for Aβ**
- ✓ **BBB permeability**
- \+ ✓ **Longer wavelength (NIR)**

Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

Perspective

- **New CL probe based on switching mechanism may broaden the target substrates of CL imaging.**
- **In particular, application for other aggregating-prone proteins should be easier.**
- **Monitoring of Aβ concentration by ocular imaging could be clinically useful.**



Ran, C. *et al. Nat. Commun.* **2020**, 11, 4052.

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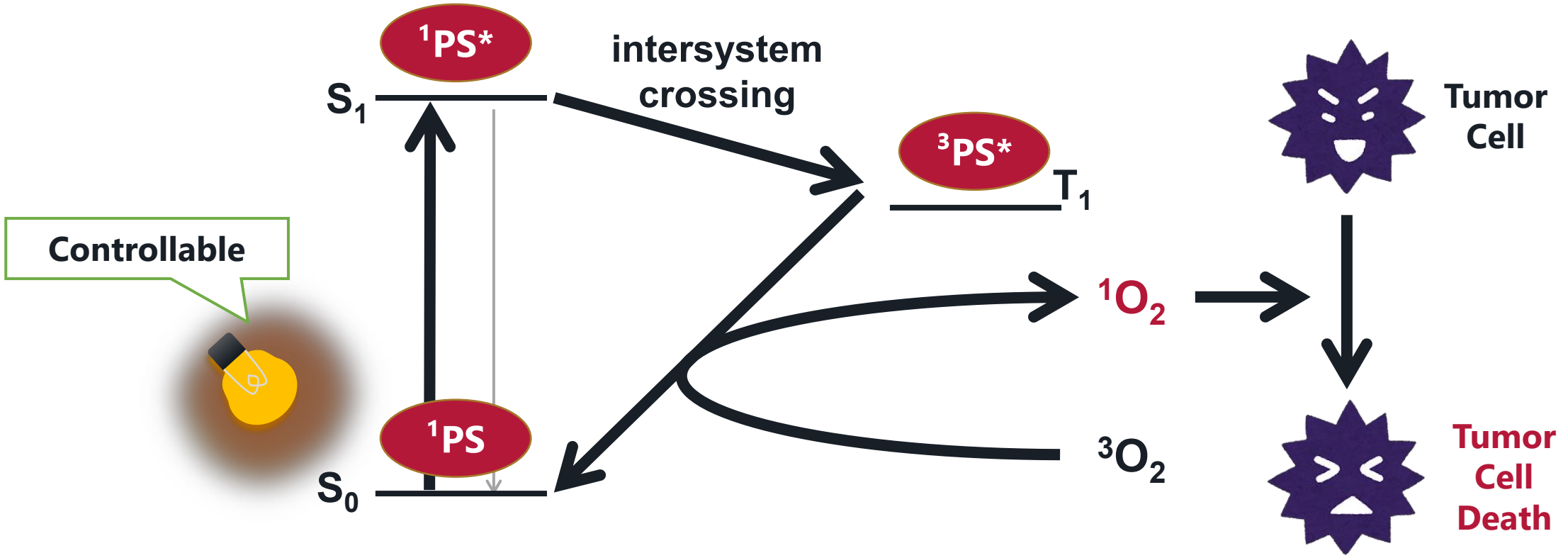
Photodynamic therapy (PDT)

Concept:

PS: Photosensitizer



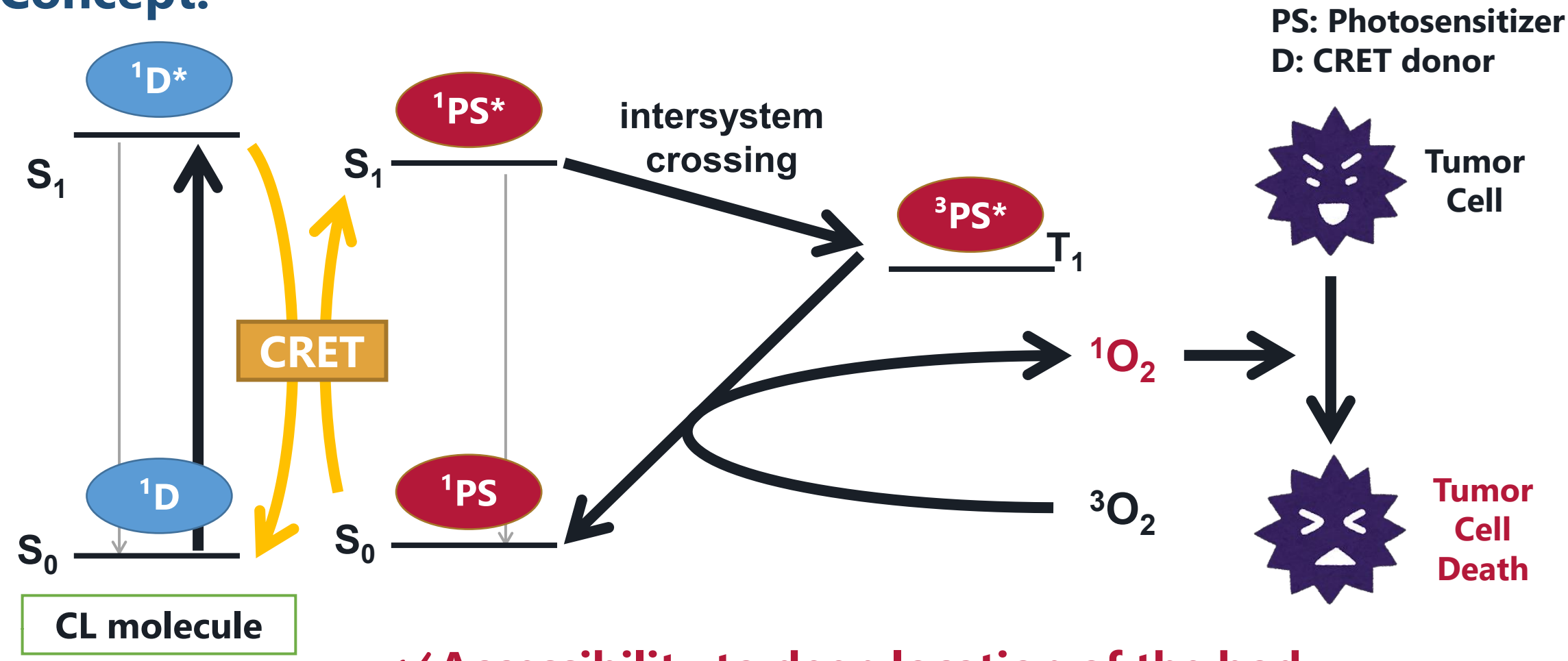
✓ **Fewer side effects due temporospatial regulation**

✕ **Only for localized and superficial tumor**

da Silva, L. P. *et al. ChemPhysChem.* **2016**, 17(15), 2286-2294.

CL initiated PDT

Concept:

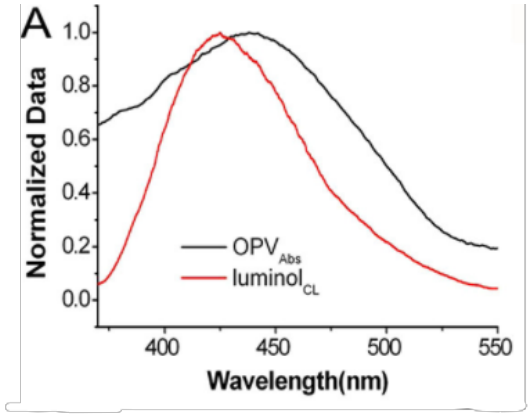


✓ **Accessibility to deep location of the body**

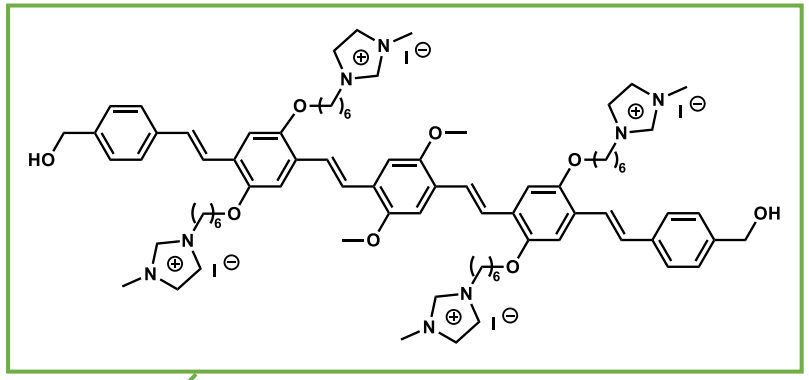
da Silva, L. P. *et al. ChemPhysChem.* **2016**, 17(15), 2286-2294.

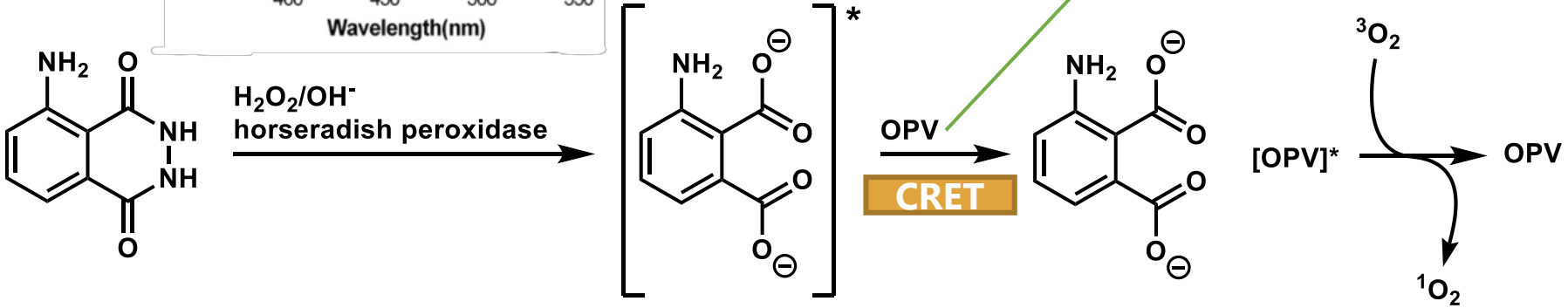
CL initiated PDT with Luminol

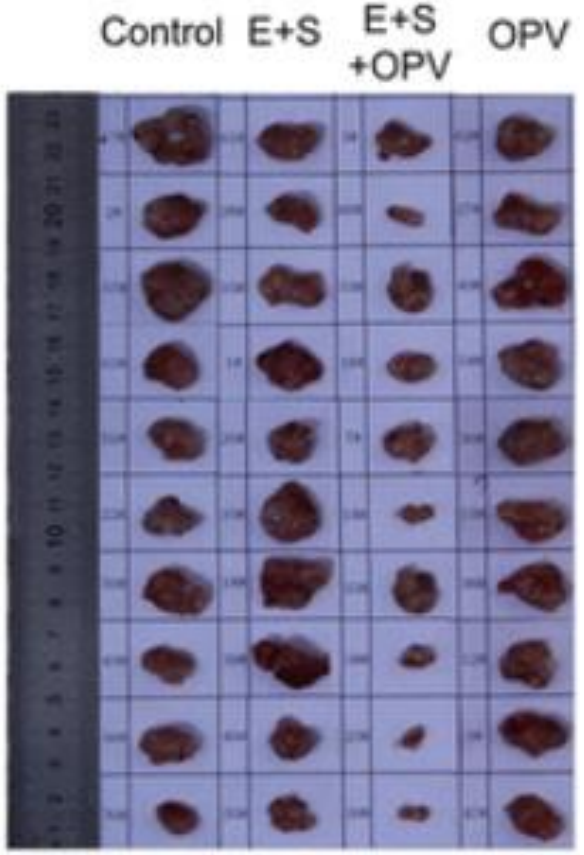
Strategies using luminol have been widely studied:



electrostatic interaction







HeLa cell tumor of nude mice (intratumoral injection)

✓ *in vivo* CL initiated PDT was achieved

✕ Concern about cytotoxicity to healthy cells

Intratumoral injection (not i.v.)

Wang, S. *et al. J. Am. Chem. Soc.* **2012**, 134(32), 13184-13187.

Unimolecular system for CL initiated PDT

Unimolecular system:

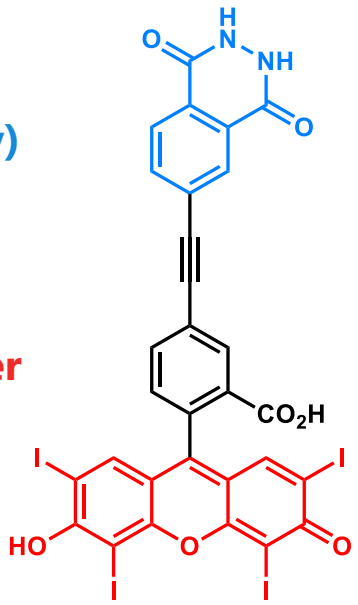
Low required concentration & Ease of delivery

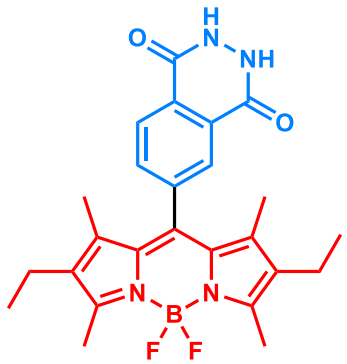
→ Smaller side-effect is expected.

Intramolecular CRET

Donor (CL body)

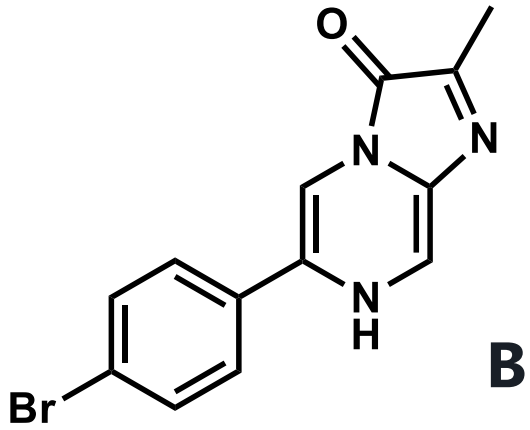
Accepter (PS)





Heavy atom free
→Low dark toxicity

Direct Excitation to Triplet



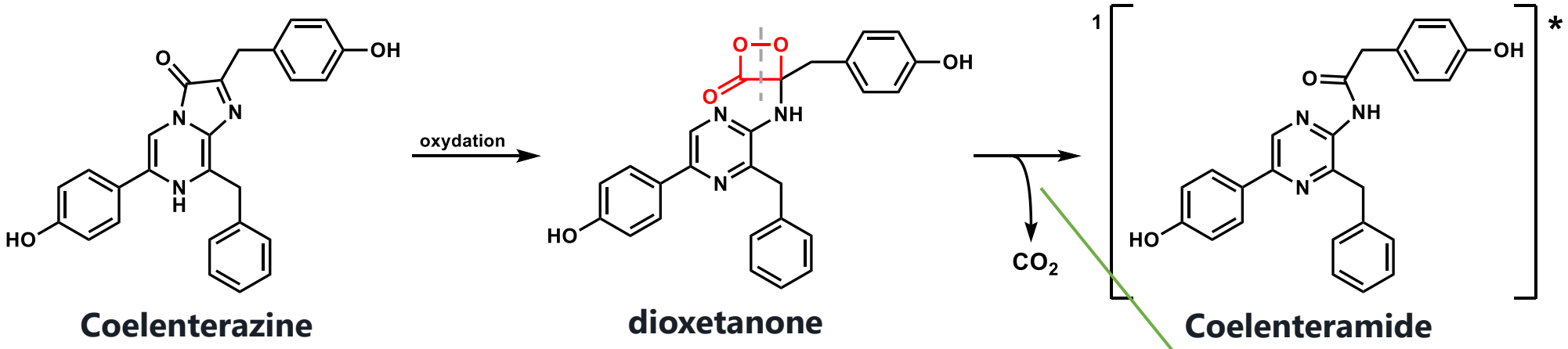
Br-Cla

Akkaya, E. U. *et al. ACS. Omega.* **2017**, 2(4),1367-1371.
Algi, F. *et al. ACS Appl. Bio Mater.* **2021**, 4(6), 5090-5098.
da Silva, L. P. *et al. Eur. J. Med. Chem.* **2019**, 183, 111683.

Molecular Design

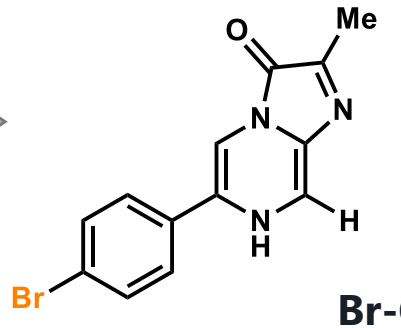
Mother Skeleton:

Coelenterazine containing Imidazo[1,2-a]pyrazine-3(7H)-one



Another path for T_1 by ISC (intersystem crossing)

Replace hydroxy group with bromine for heavy atom effect

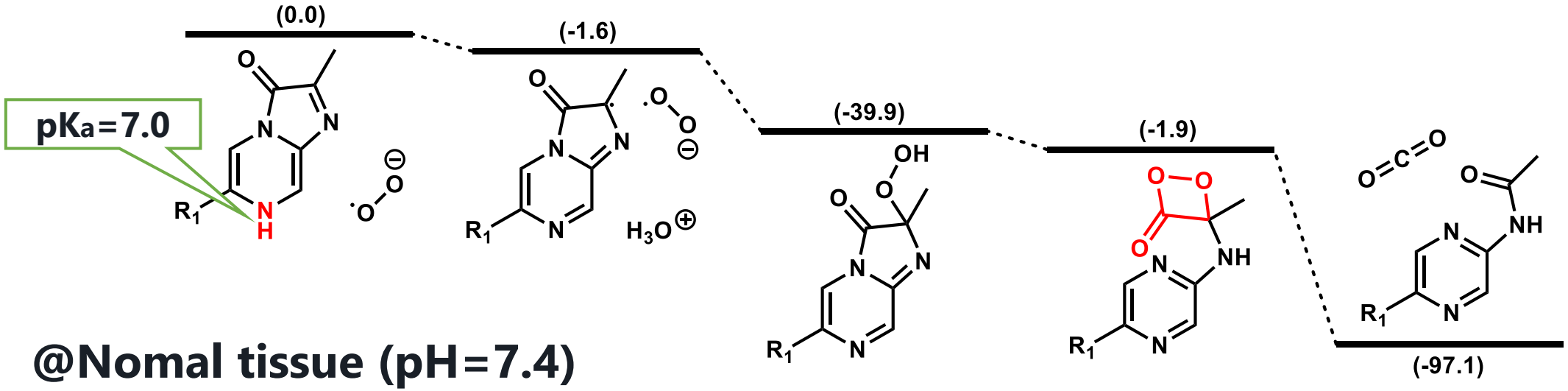


da Silva, L. P. *et al. J. Luminescence*. **2018**, 194, 139-145. / da Silva, L. P. *et al. Eur. J. Med. Chem.* **2019**, 183, 111683.

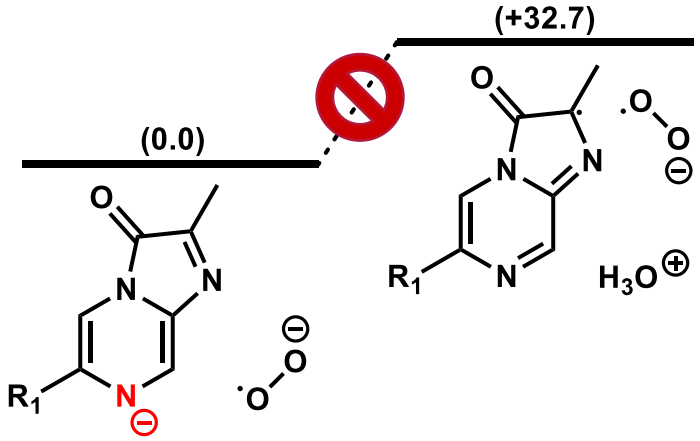
Reaction

Mechanism and energetics of the S_0 states (kcal/mol):

@Tumor tissue (pH=4.5~5.0)



@Nomal tissue (pH=7.4)



Dual tumor selectivity

- **Acidic pH**
- **overexpression of superoxide anion**

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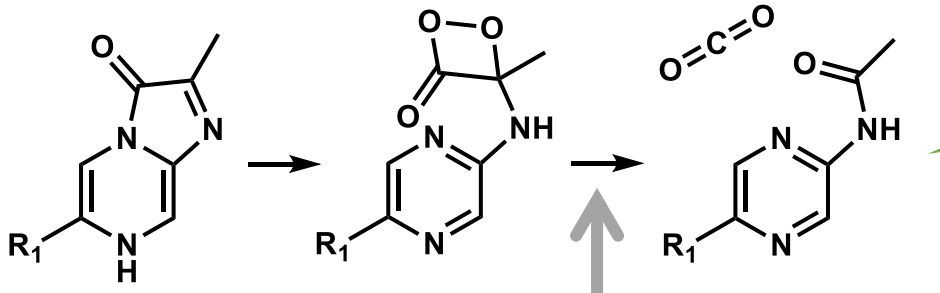
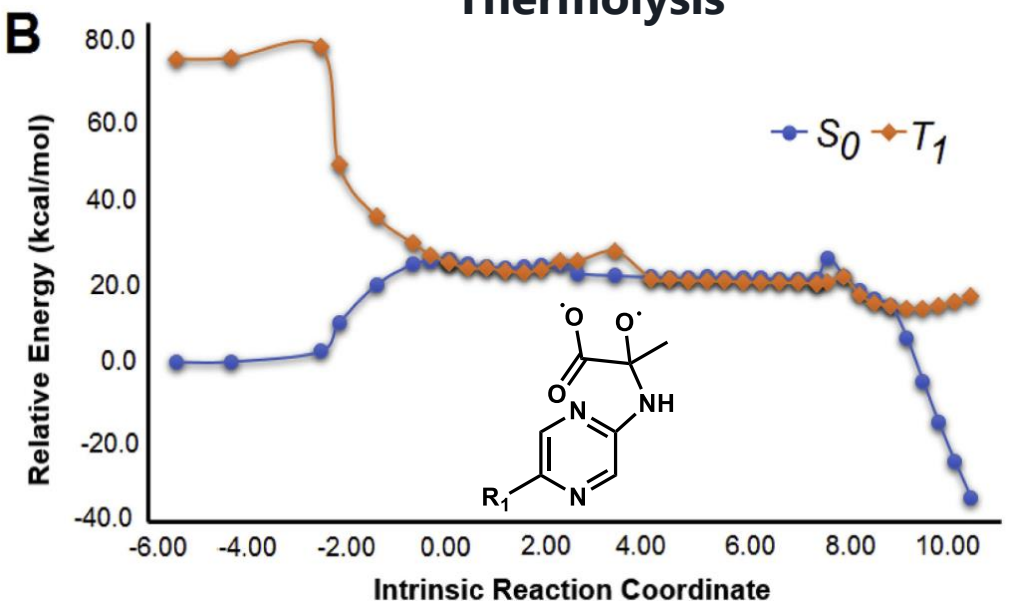
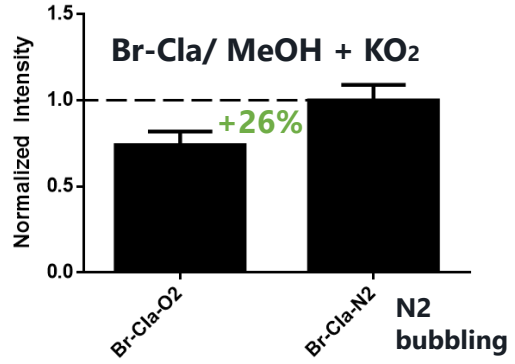
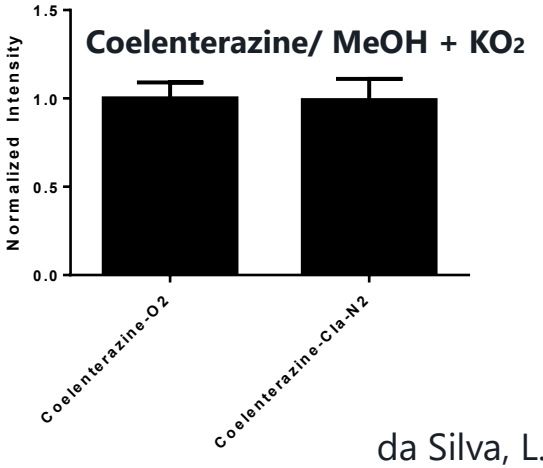
Confirmation of PS function



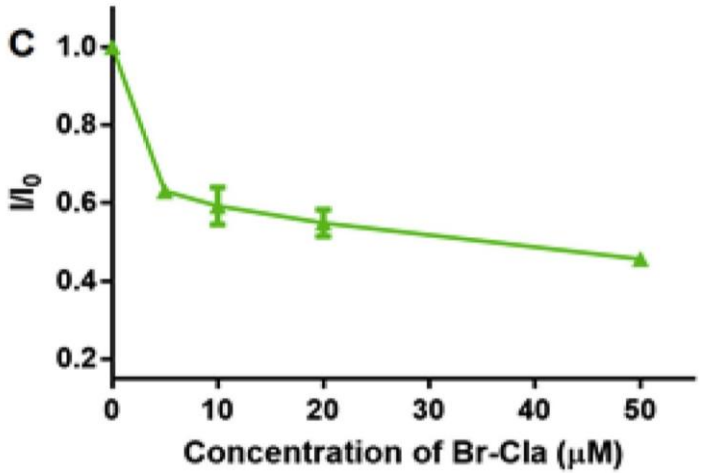
S_0-T_1 ΔE : 2.78eV > 0.98eV (=$^3O_2$-$^1O_2$ ΔE)







ABAD fluorescence



✓ ISC
✓ $^1O_2$ release

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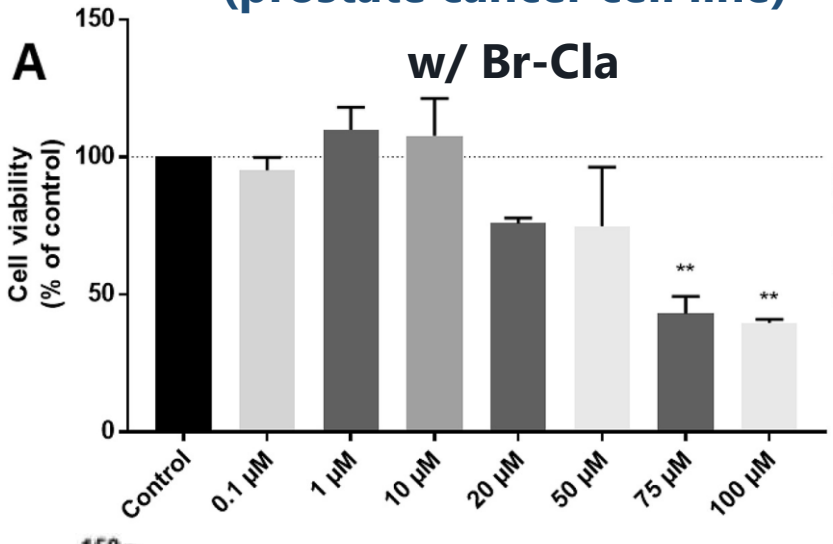
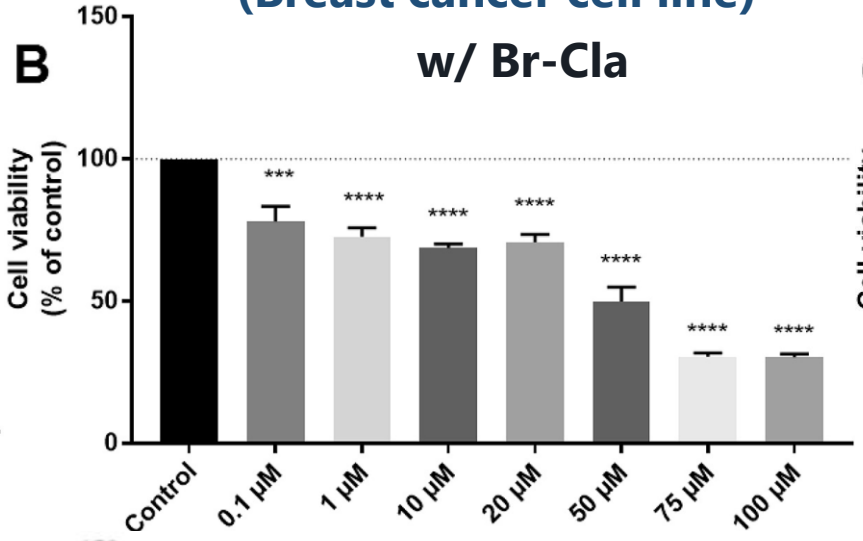
In vitro Cytotoxicity

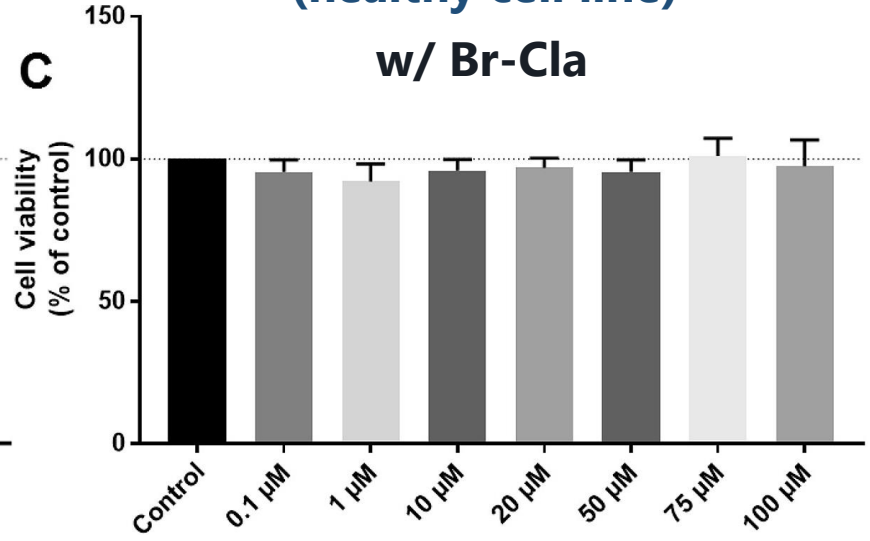
PC-3
(prostate cancer cell line)

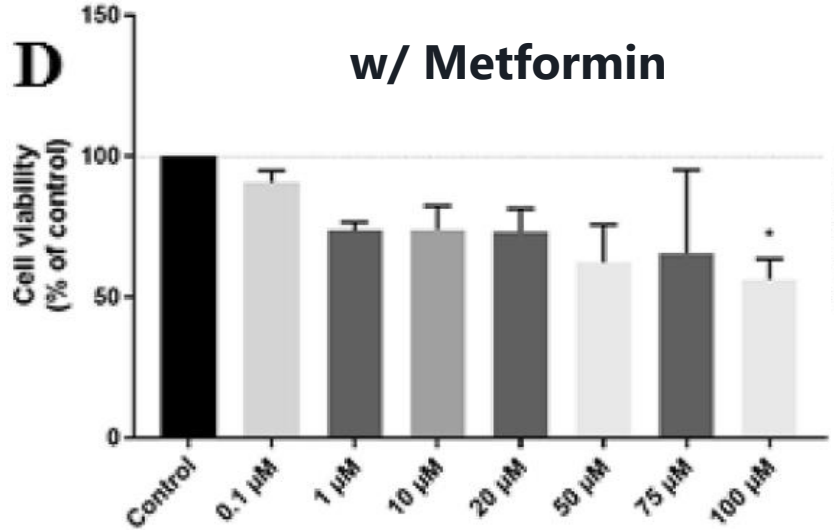


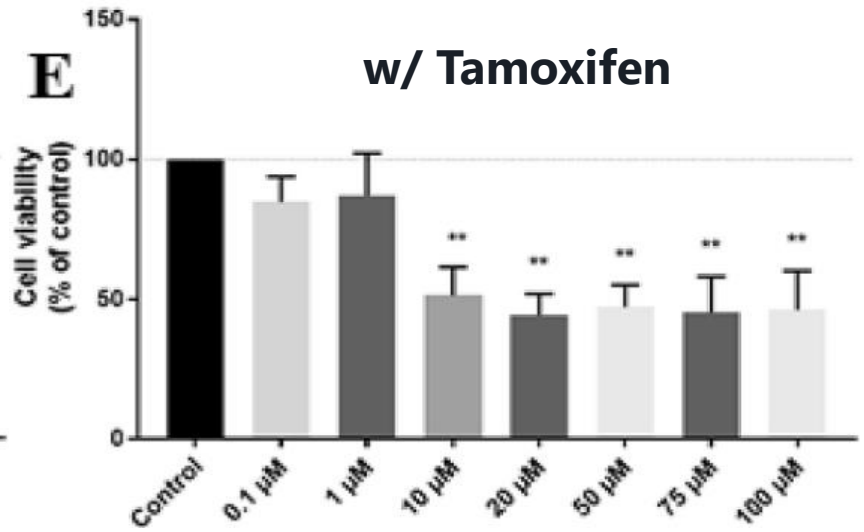
MCF-7
(Breast cancer cell line)



MCF-10A
(healthy cell line)







✓ **Tumor-selective cytotoxicity**

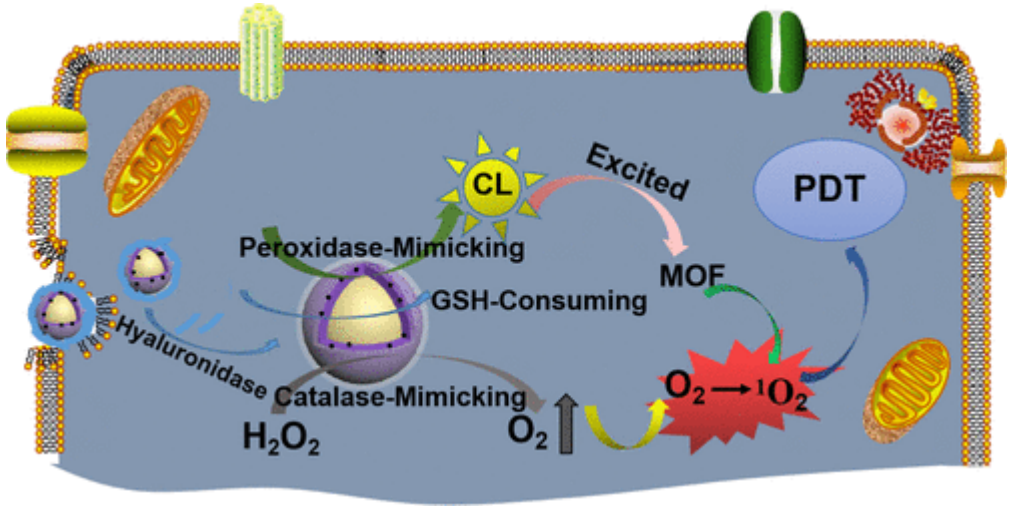
da Silva, L. P. *et al. Eur. J. Med. Chem.* **2019**, 183, 111683.

Perspective

- **There are many issues to be resolved.**
  Toxicity to normal tissue, inefficient CL, self-aggregation, hypoxia

- **Need an integrated approach to solve problems.**

- **It is expected that various approaches will be taken for practical use.**



Li, J. *et al. ACS. Appl. Bio. Mater.* **2021**, 4(4), 3490-3498.

Contents

- Basic Information on Chemiluminescence
- Application for Bioimaging
- Application for Photodynamic Therapy
- Mini-proposal
- **Summary**

Summary

- **The biological applications of CL were discussed from 2 aspects: imaging and PDT**

- **CRET is an important strategy in both.**

  Imaging: High Φ_{CL}, long wavelength

  PDT: Internal light source of PS

- **New diagnostic and therapeutic methods will be developed by CL imaging probes or CL initiated PDT**

Thank you for listening !