Photocages activated by visible light
- meso-Methyl BODIPY photocages and their improvements -

Literature seminar #3
M2 Shinpei Takamaru
2024/08/01 (Thu)

Contents

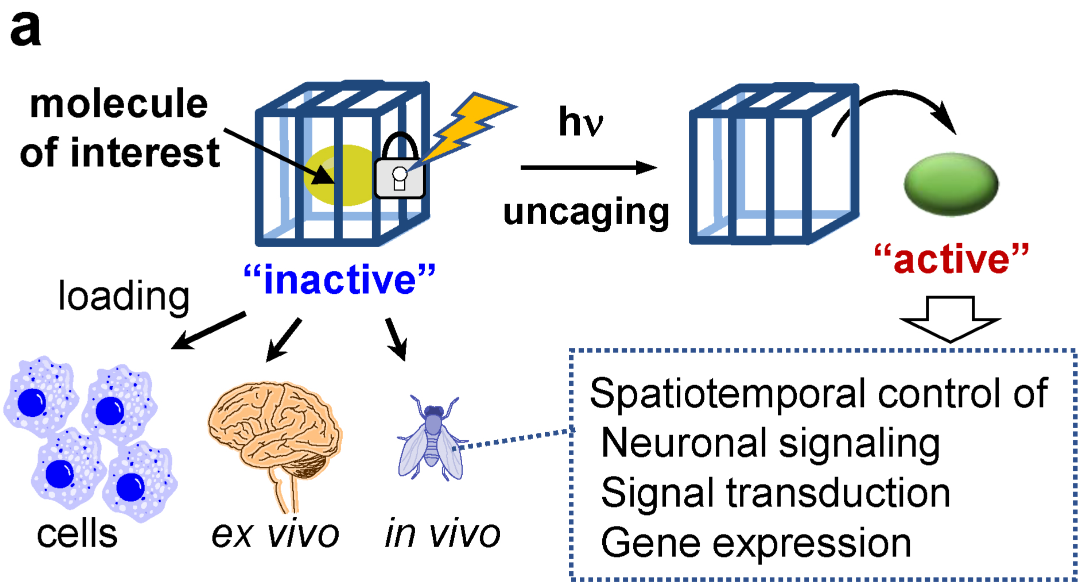
What is photocaged compound?

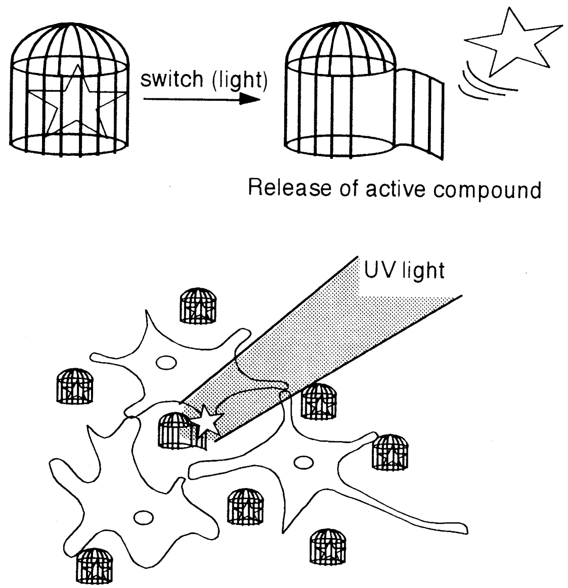
Photocaged compounds

・temporarily **mask biological activity** through photoremovable protecting groups

・target substances are released upon exposure to **specific wavelengths of light**

・**high spatial and temporal resolution (Spatiotemporal control)**





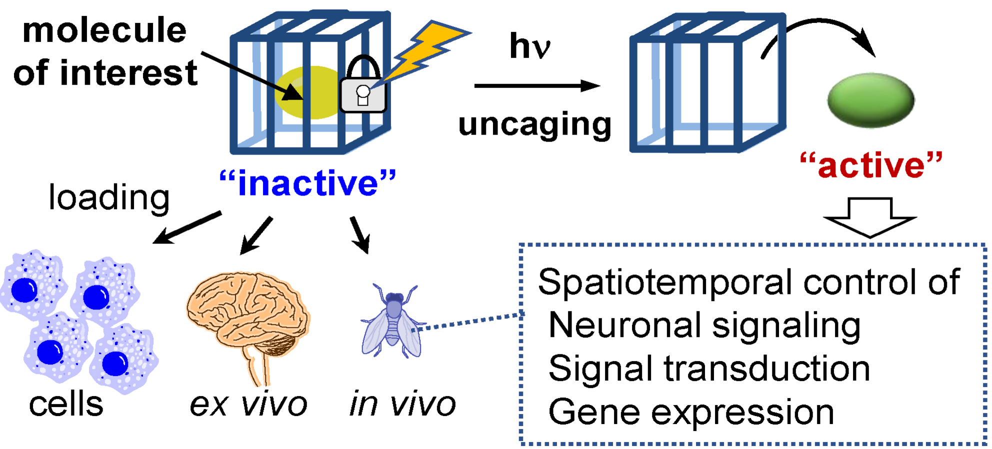
Furuta, T. *YAKUGAKU ZASSHI* **2022**, *142*, 495-502.
Tatsu, Y., *et al*. *Comparative Physiology and Biochemistry* **1998**, *15*(2), 141-147.
Chen, X., *et al. Smart Molecules* **2023**, *1*(1), e20220003.

What is photocaged compound?

Advantageous features of using light to promote controlled release

1. **clean and inexpensive energy**
2. **more selective** than thermally activated reaction
3. high controllability (varying the intensity and wavelength)
4. long wavelength light is both less damaging to cells and more penetrable into cells

a



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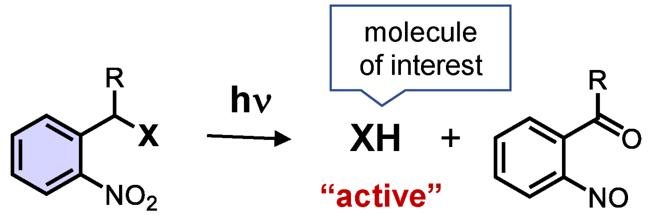

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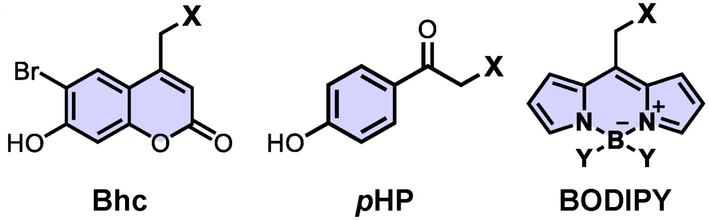
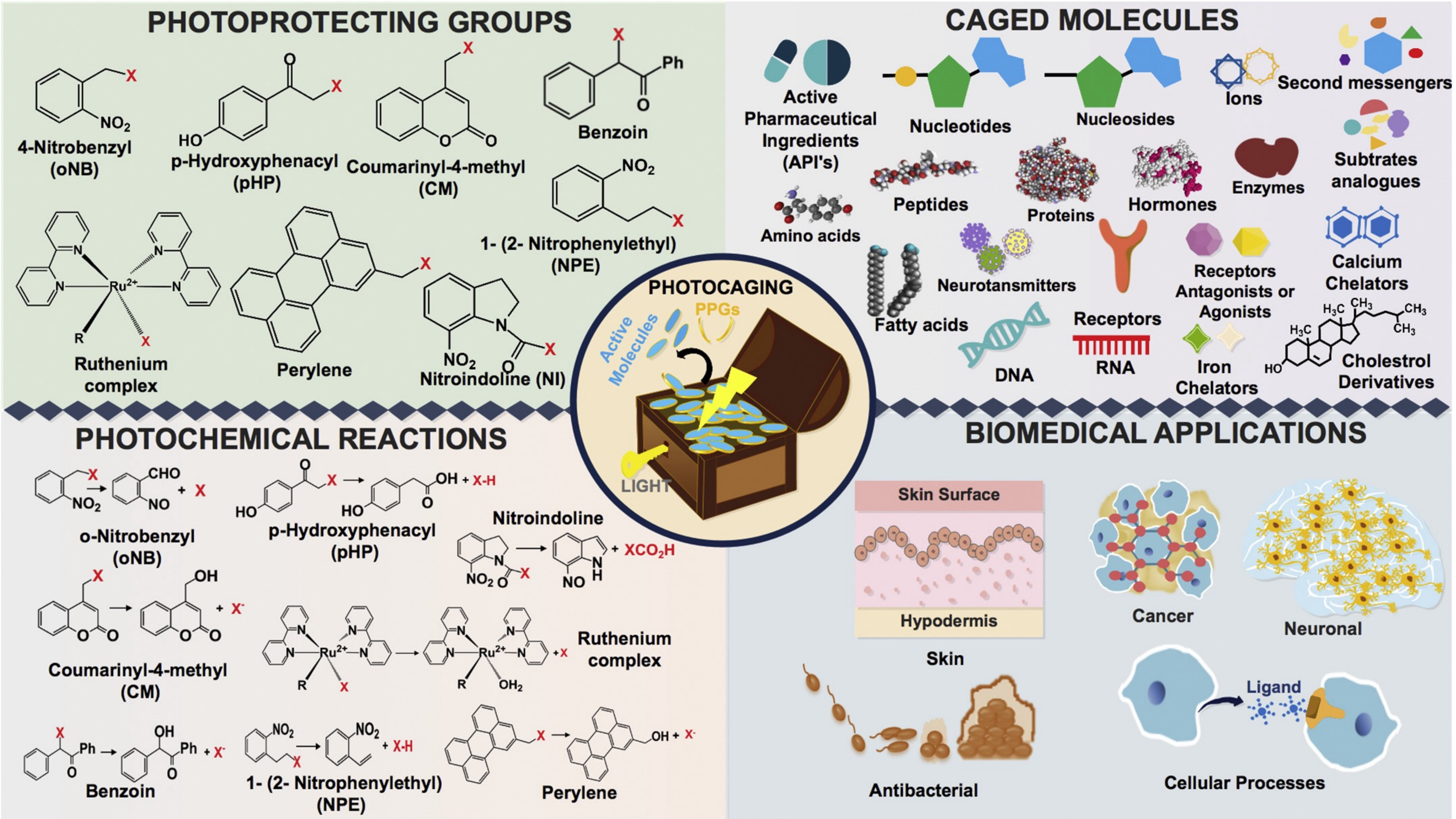


Furuta, T. *YAKUGAKU ZASSHI* **2022**, *142*, 495-502.
Chen, X., *et al. Smart Molecules* **2023**, *1*(1), e20220003.

Wide applications in various fields



Reis, R. L., *et al. Journal of Controlled Release* **2019**, *298*, 154-176.
Chen, X., *et al. Smart Molecules* **2023**, *1*(1), e20220003.

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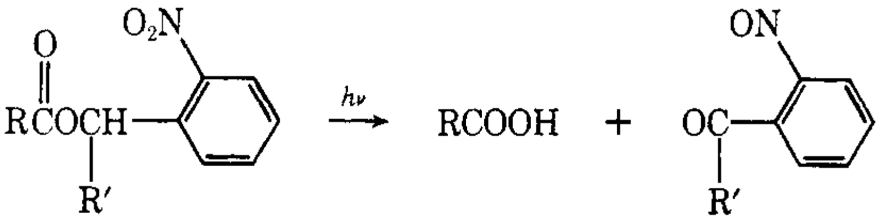
Discovery of *o*-nitrobenzyl derivatives as "photocages"

Discovery of photo-sensitive reaction (*o*-nitrobenzyl based compound)


Derek Harold Richard Barton

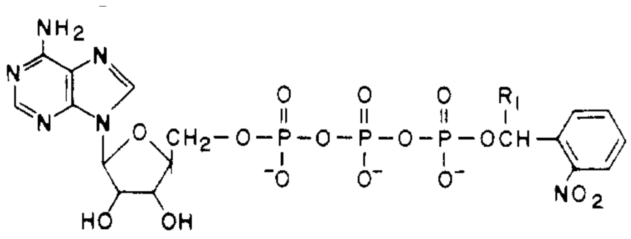

Robert Burns Woodward

Scheme III

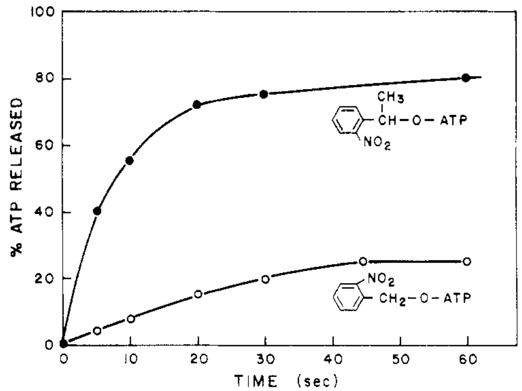


P. Schofield, *et al. Tetrahedron Lett.* **1962**, *16*, 697-699.
Woodward, R. B., *et al. J. Am. Chem. Soc.* **1970**, *92*, 6333-6335.
Barton, D. H. R., *et al. Tetrahedron Lett.* **1962**, *23*, 1055-1057.
https://www.nobelprize.org/prizes/chemistry/1969/barton/biographical/
https://www.chem-station.com/chemist-db/archives/2007/09/-robert-burs-woodward.php

Photocaged compound (caged-ATP)


"CAGED-ATP"

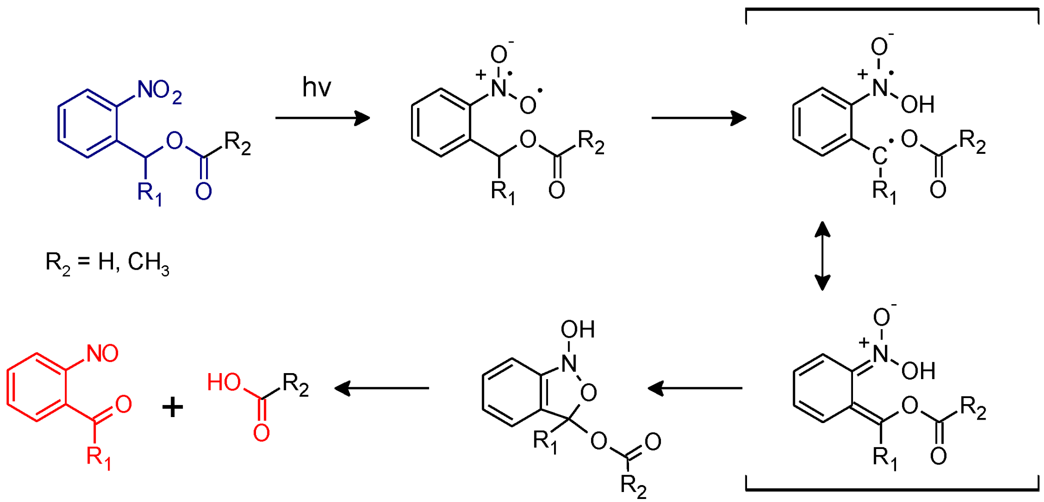

Joseph Frederick Hoffman



Hoffman, J. F., *et al. Biochemistry* **1978**, *17*, 1929.
https://medicine.yale.edu/news-article/in-memoriam-joseph-frederick-hoffman-phd/

Example of utilizing *o*-nitrobenzyl photocages

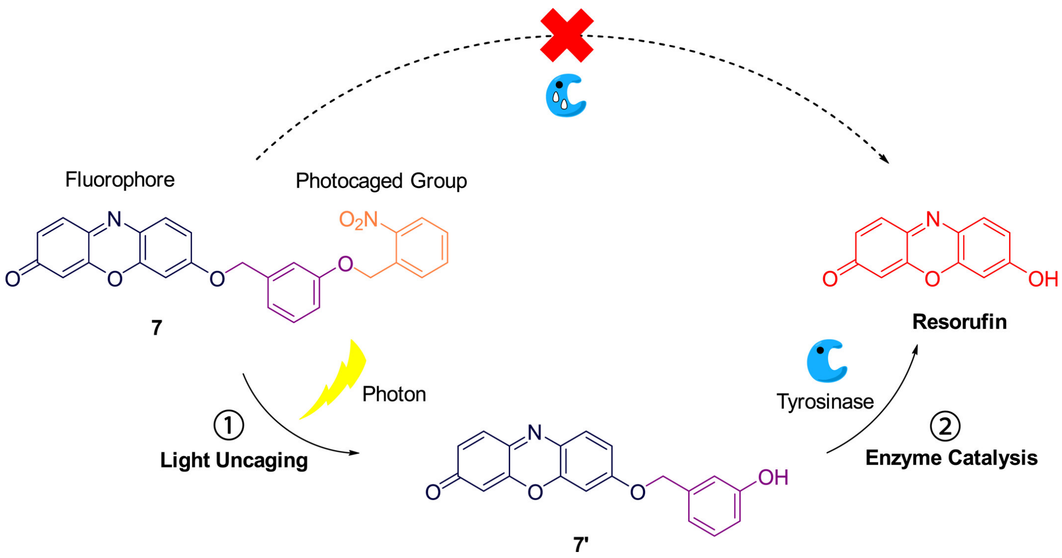
Proposed mechanism



- O-radical produced by UV irradiation withdraws hydrogen at the benzyl position.

Sangermano, M., *et al. Materials* **2020**, *13*(12), 2777.
https://www.chem-station.com/blog/2012/04/post-374.html

One of the examples utilizing *ortho*-nitrobenzyl photocage

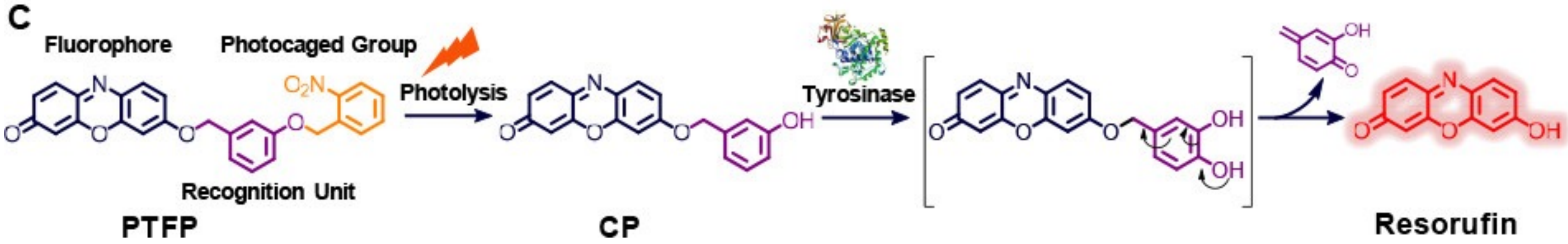


- This photocage is inactive to tyrosinase (TYR) because the *o*-nitrobenzyl causes steric hindrance.

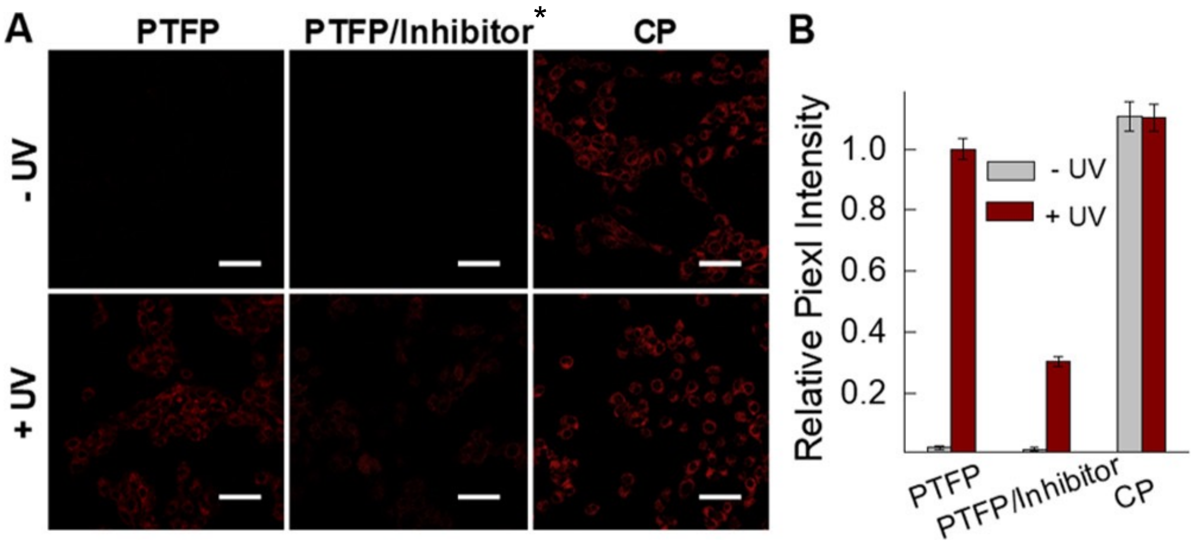
Yang, R. *et al. Anal. Chem.* **2020**, *92*(10), 7194-7199.
Chen, X., *et al. Smart Molecules* **2023**, *1*(1), e20220003.

Example of utilizing *o*-nitrobenzyl photocages

One of the examples utilizing *ortho*-nitrobenzyl photocage



(Control probe)



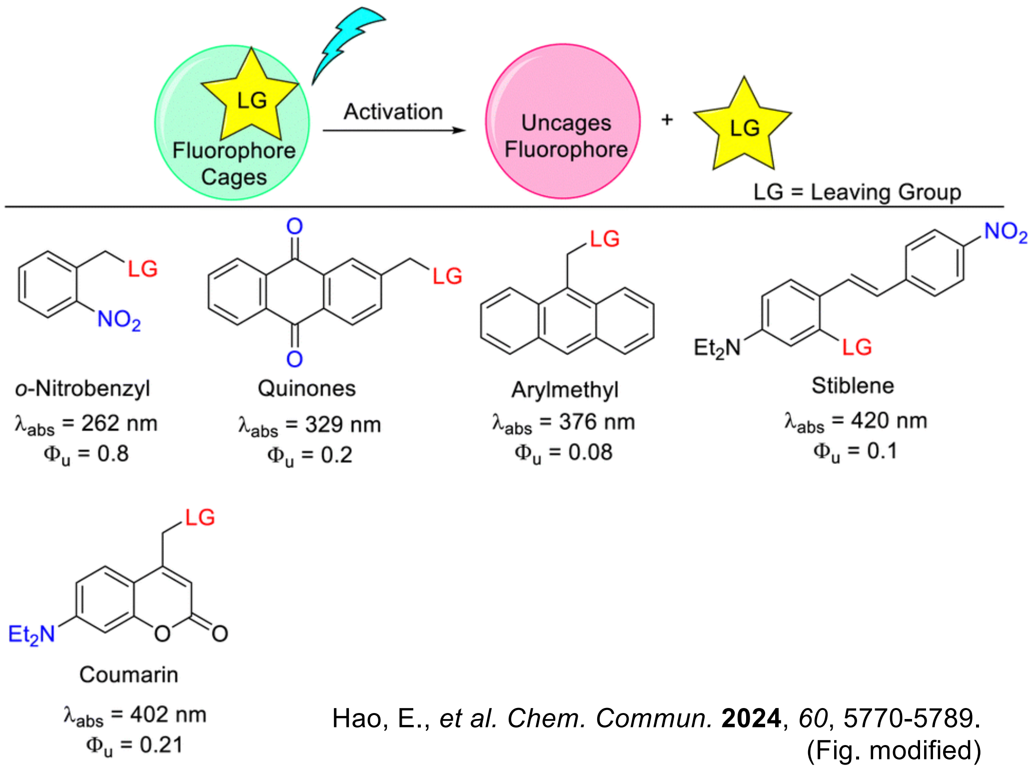
*: Tyrosinase inhibitor (kojic acid)

- **PTFP**-treated cells: brightly fluoresced only under irradiation
- **CP**-treated cells: exhibited bright fluorescence both condition

→ photochemically activated for visualizing intracellular endogenous TYR activity with artificial control

Yang, R. *et al. Anal. Chem.* **2020**, *92*(10), 7194-7199. (Fig. modified)
Chen, X., *et al. Smart Molecules* **2023**, *1*(1), e20220003.

Many photosensitive groups have been developed



Hao, E., *et al. Chem. Commun.* **2024**, *60*, 5770-5789.
(Fig. modified)

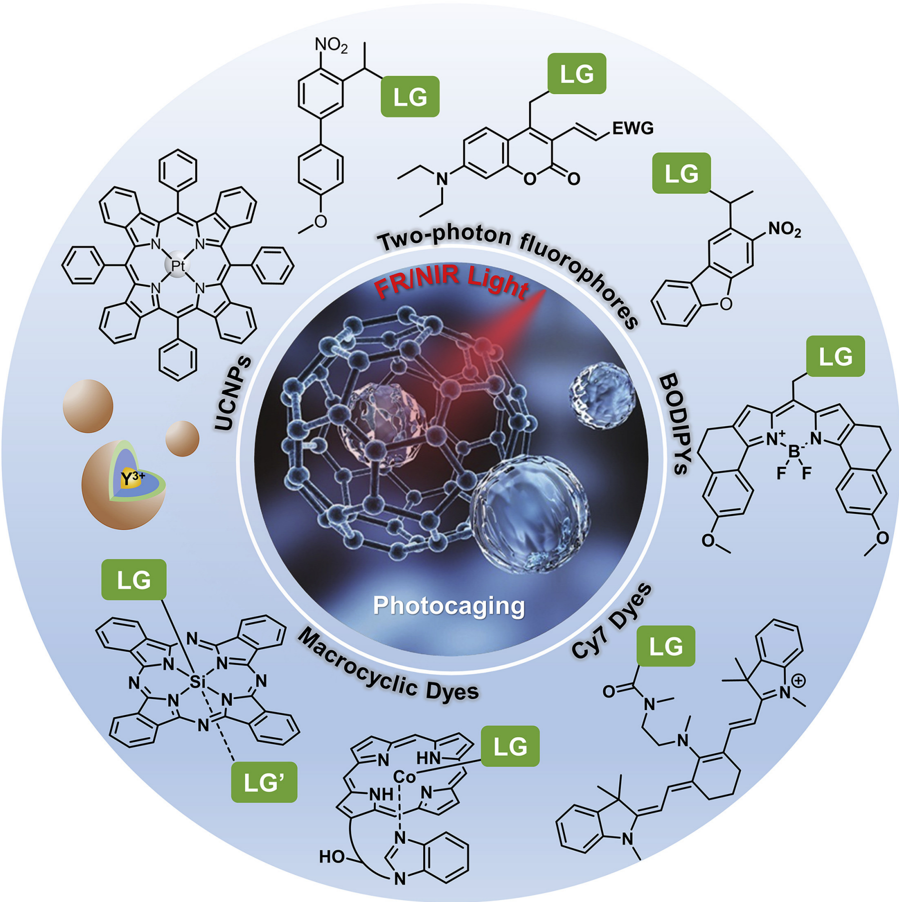
<u>Limitations of these photocages</u>

- sensitive to ultraviolet (UV) and blue light below 450 nm wavelength

1. Inherent **phototoxicity (e.g. DNA damage)**
2. Limited tissue permeability
   → restrict their **biological applications**
3. **Background noise interference** in living systems under short-wavelength light excitation

→ photocages activated by longer wavelength are needed

Many photosensitive groups have been developed

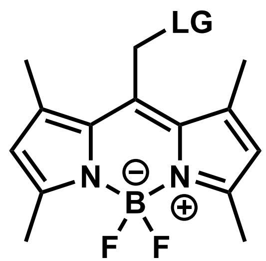


Development of visible / NIR light activated cages

- BODIPYs
- Cy7 Dyes
- Phthalocyanine
- Cyanocobalamin

etc.

→ focused on **BODIPY photocages**
(Today's seminar)



Kim, J. S., *et al. Chem* **2023**, *9*(1), 29-64.

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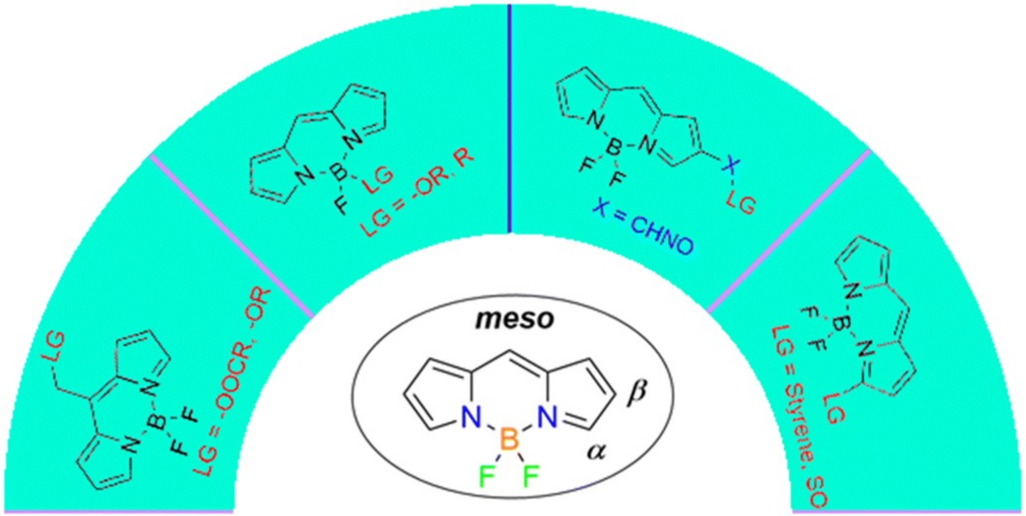
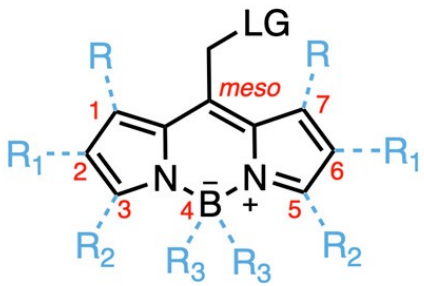
Various types of BODIPY photocages



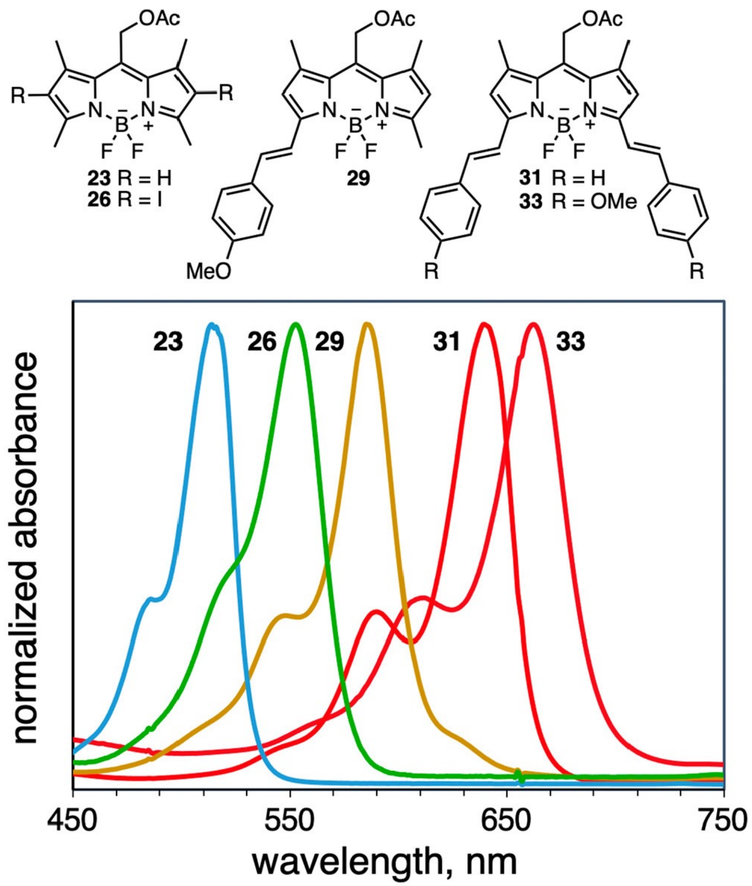
Hao, E., *et al. Chem. Commun.* **2024**, *60*, 5770-5789.

meso-Methyl BODIPY photocages



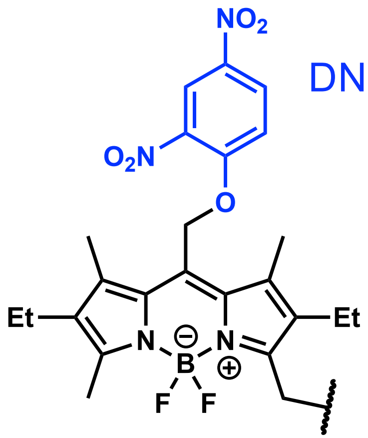
Winter, A. H., *et al. J. Am. Chem. Soc.* **2023**, *145*(32), 17497-17514.

Absorptions of BODIPY PPGs (Green ~ Red light)



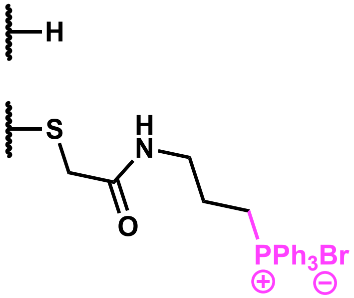
Winter, A. H., *et al. J. Am. Chem. Soc.* **2023**, *145*(32), 17497-17514.

Example of meso-Methyl BODIPY photocages

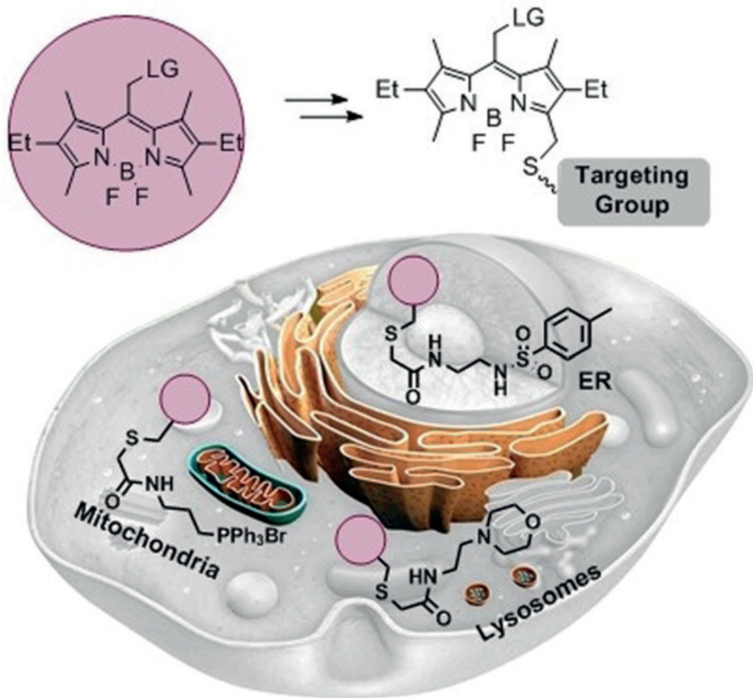


DNP = uncoupler of mitochondrial oxidative phosphorylation
→ decrease mitochondrial membrane potential ($\Delta\psi_m$)

14
16



Mitochondria targeting

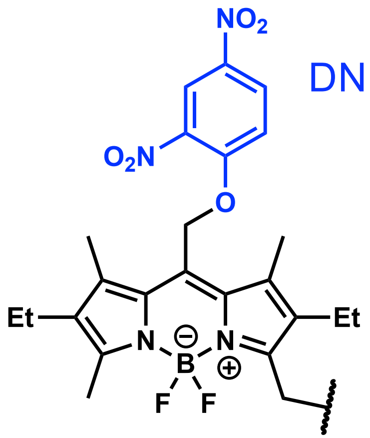


- sub-cellular organelles are dispersed in cells
- selective light-irradiation of a complete organelle is impractical

→ Organelle-specific photocages

Weinstain, R., *et al. Angew. Chem., Int. Ed.* **2019**, *58*, 4659-4663.

Example of meso-Methyl BODIPY photocages



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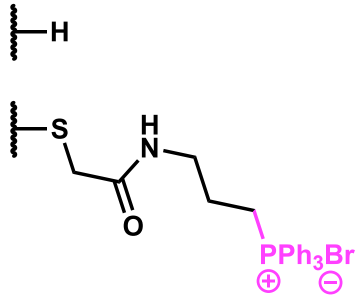
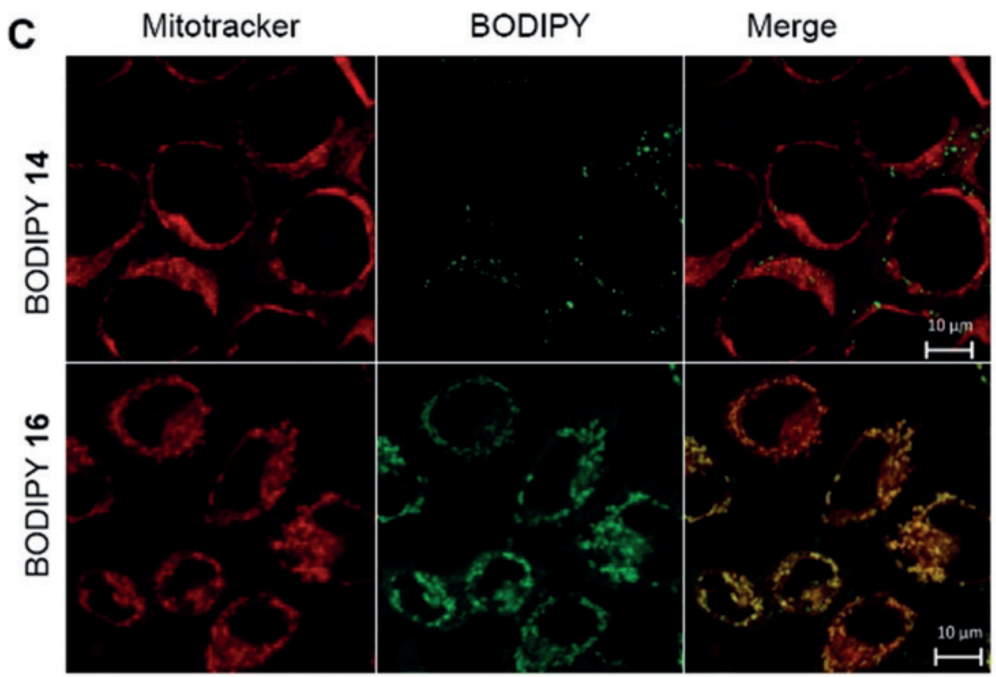


Mitochondria targeting



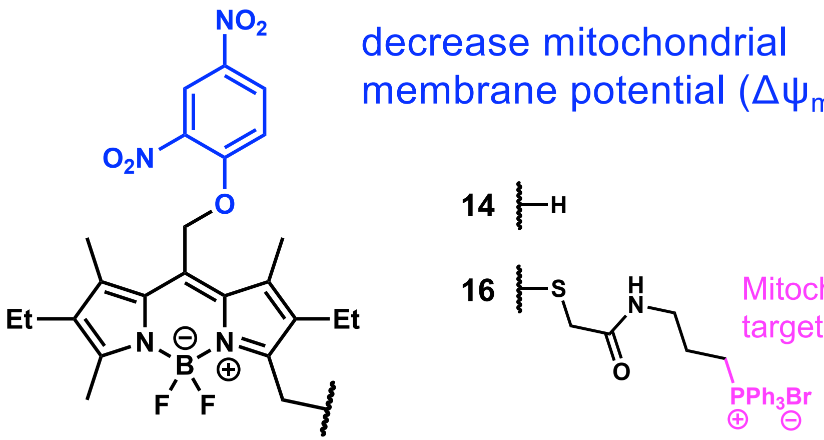
- sub-cellular organelles are dispersed in cells
- selective light-irradiation of a complete organelle is impractical

→ Organelle-specific photocages

Targeting compounds are co-localized with Mitotracker.

Weinstain, R., *et al. Angew. Chem., Int. Ed.* **2019**, *58*, 4659-4663.

Example of meso-Methyl BODIPY photocages

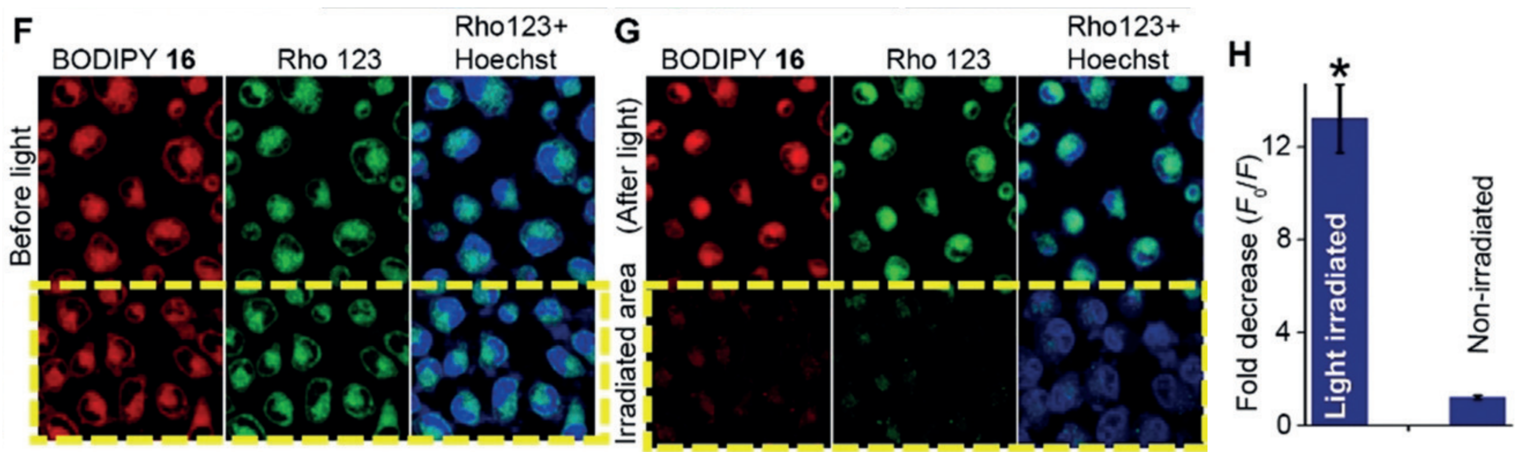


decrease mitochondrial membrane potential ($\Delta\psi_m$)

Rhodamine 123 (Rho123) :
(intact cells) accumulates in mitochondria

(reduction in $\Delta\psi_m$)
redistribution of the dye to the cytoplasm
→ a decrease in fluorescence signal

Photo-release → decrease signal of Rho123



Rho123 signal decreased by light-irradiation
→ **photo-release was achieved!**

← averaged fluorescence intensities of cells within **the irradiated area versus those outside of it**

Weinstain, R., *et al. Angew. Chem., Int. Ed.* **2019**, *58*, 4659-4663.

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Improvement points of BODIPY photocages

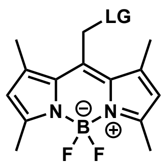
Improvement points of BODIPY photocages

- **Low photoreaction quantum yield** (ϕ_r) even though large extinction coefficients (ε_{irr}) at the λ_{max}

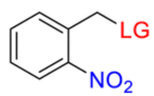
→ need to improve quantum yield (ϕ_r)

Representative value

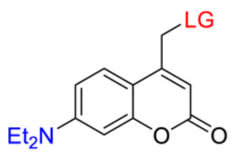
	BODIPY	*o*-nitrobenzyl	coumarin
ε_{irr} [$M^{-1}cm^{-1}$]	40,000~ 150,000	< 20,000	< 40,000
ϕ_r	< 0.01	0.5 ~ 0.7*	~ 0.2*



BODIPY



o-Nitrobenzyl



Coumarin

ε_{irr} : extinction coefficients at the λ_{max}

→ Value of how much of the light applied is absorbed

ϕ_r : photoreaction quantum yield / uncaging quantum yield

→ The percentage of photons used for uncaging when a single photon is absorbed by a compound

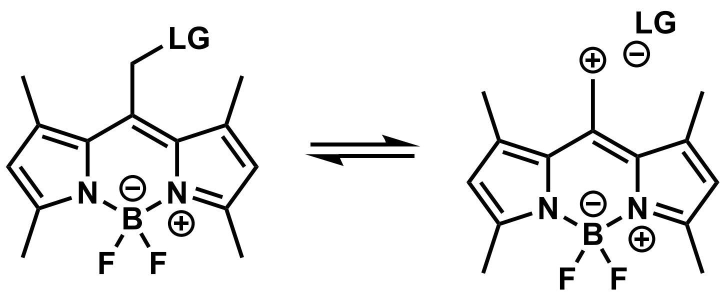
Winter, A. H., *et al. J. Am. Chem. Soc.* **2023**, *145*(32), 17497-17514.
*: Hao, E., *et al. Chem. Commun.* **2024**, *60*, 5770-5789.
(Fig. modified)

Several strategies to improve quantum yield

Improvement points of BODIPY photocages

- **Low photoreaction quantum yield** (ϕ_r) even though large extinction coefficients (ε_{irr}) at the λ_{max}

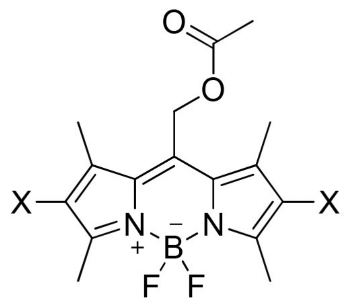
→ need to improve quantum yield (ϕ_r)



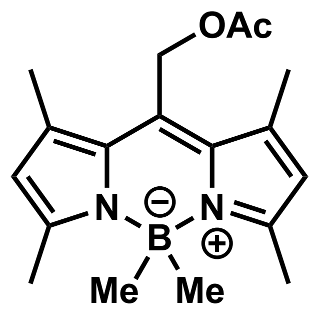
< Strategies to improve quantum yield >

1. Stabilization of carbocation intermediate
2. Heavy atom effect
3. Boron methylation

etc.



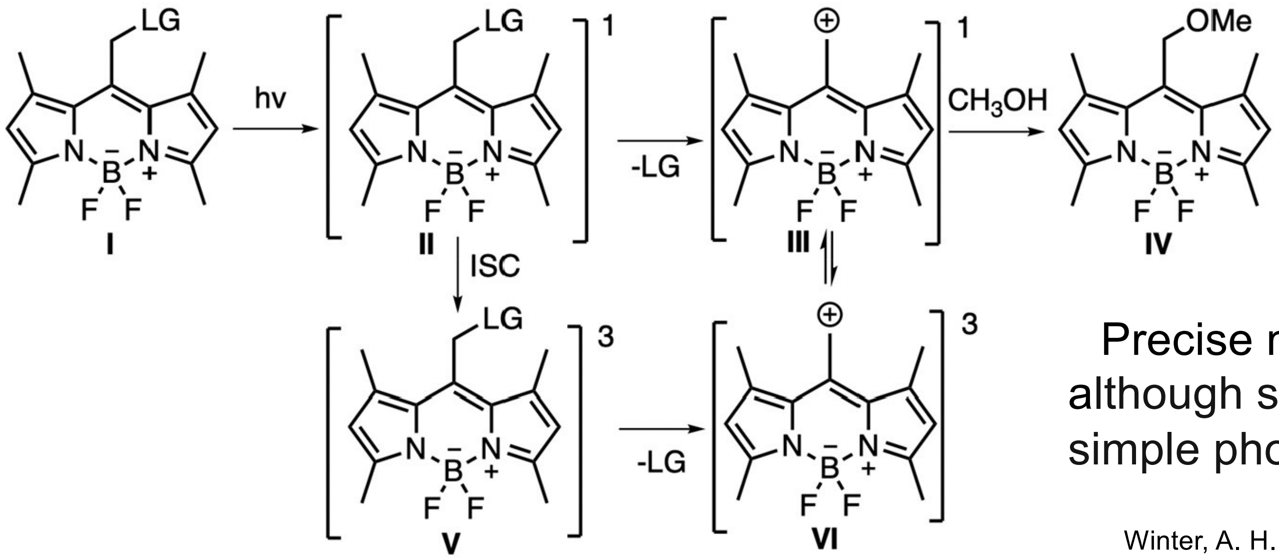
2: X = H
2-Cl: X = Cl
2-Br: X = Br
2-I: X = I



Feringa, B. L., *et al. J. Am. Chem. Soc.* **2022**, *144*(27), 12421-12430.
Weinstain, R., Winter, A. H., Klan, P., *et al. J. Am. Chem. Soc.* **2017**, *139*(42), 15168-15175.
Winter, A. H., *et al. J. Org. Chem.* **2024**, *89*(10), 6740-6748.

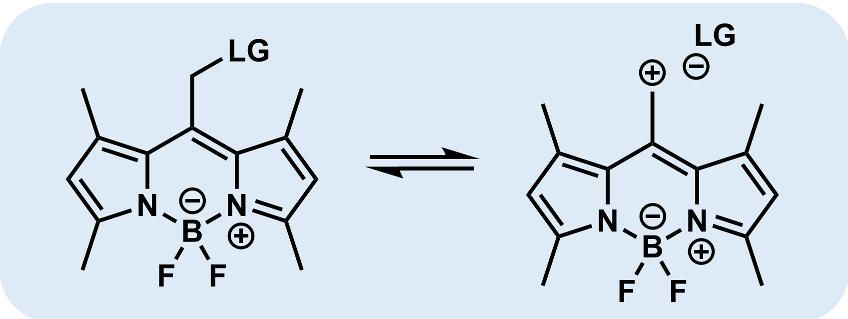
1. Proposed mechanism of photo-release

<u>Proposed mechanism of photo-release (photo-S_N1 mechanism)</u>



Precise mechanism remains unknown, although several observations suggest a simple photo-S_N1 type mechanism.

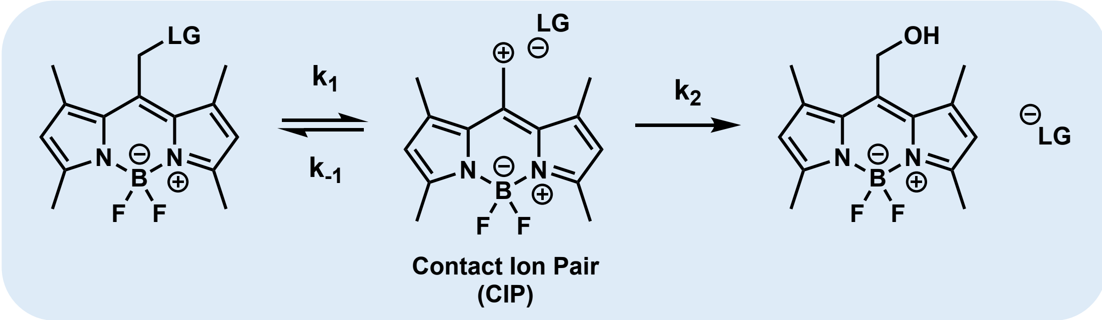
Winter, A. H., *et al. J. Am. Chem. Soc.* **2023**, *145*(32), 17497-17514.

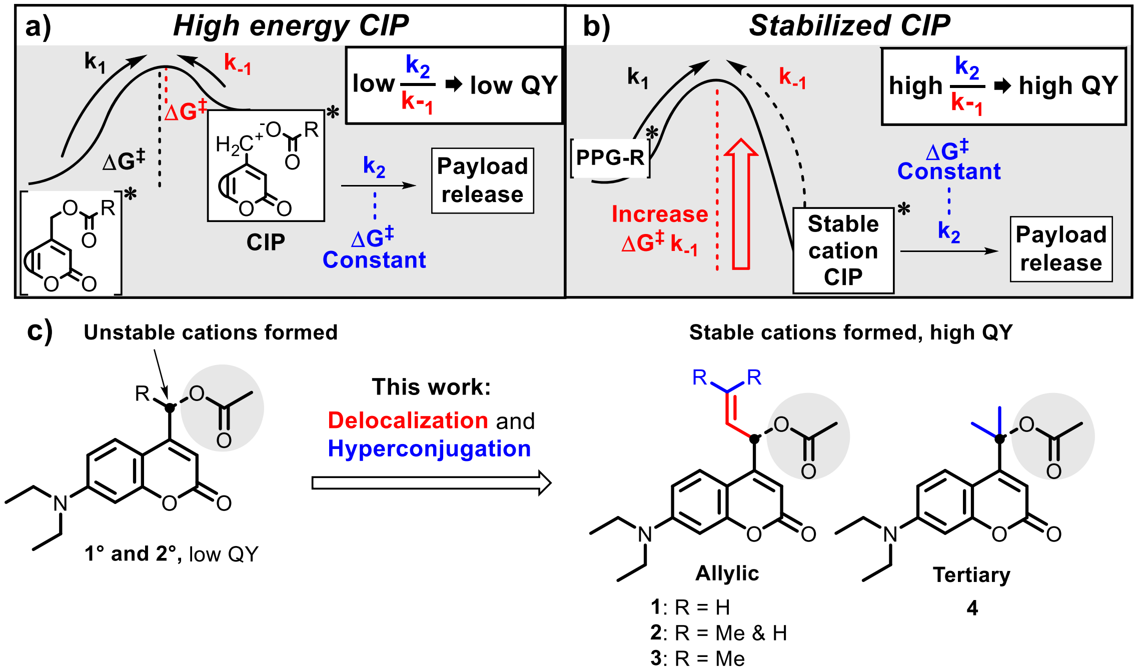


✗ unstable cation intermediate
→ ion pair recombination
→ lower the quantum yields

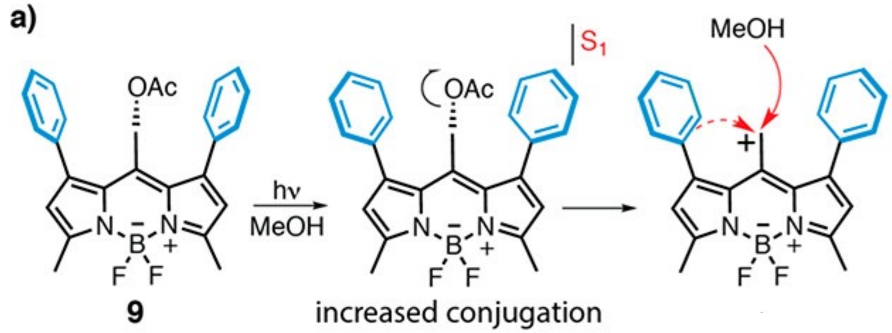
Feringa, B. L., *et al. J. Am. Chem. Soc.* **2022**, *144*(27), 12421-12430.
Winter, A. H., *et al. J. Am. Chem. Soc.* **2020**, *142*(36), 15505-15512.

1. Stabilization of carbocation intermediate



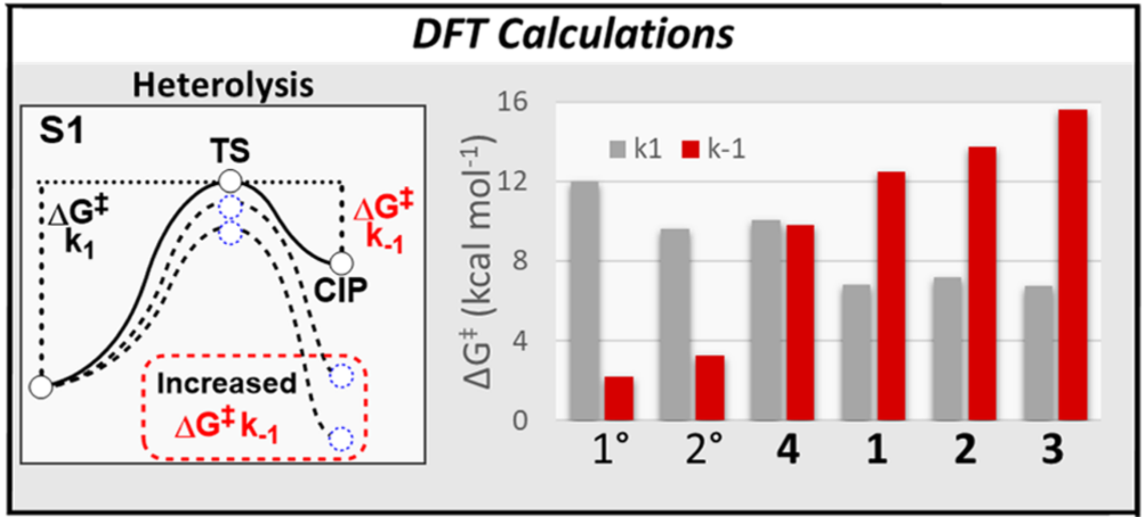


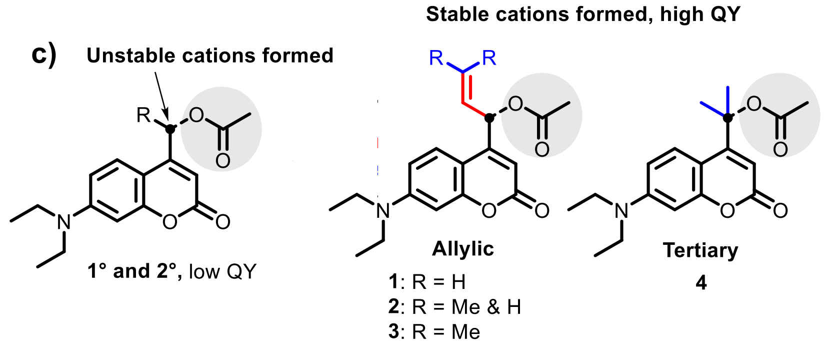
Feringa, B. L., *et al. J. Am. Chem. Soc.* **2022**, *144*(27), 12421-12430.

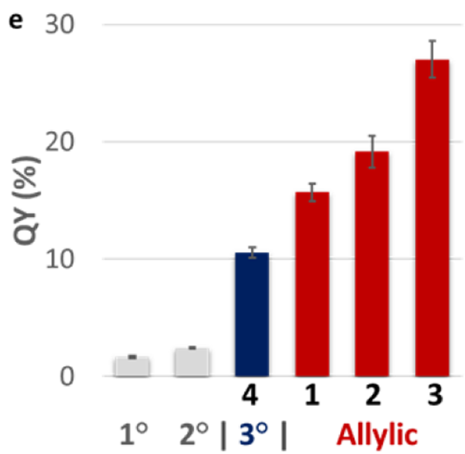


Winter, A. H., *et al. J. Am. Chem. Soc.* **2020**, *142*(36), 15505-15512.

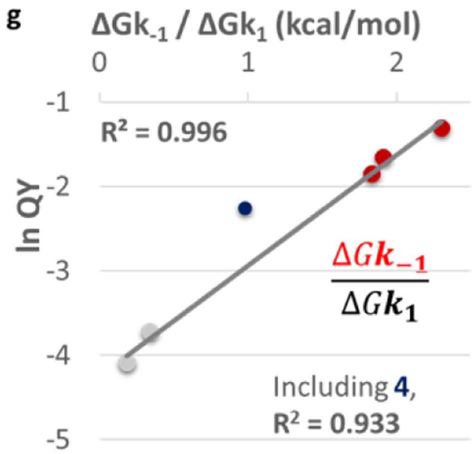
1(1). Cation stabilization results in high QY (ϕ_r)







QY: Quantum yield (ϕ_r)

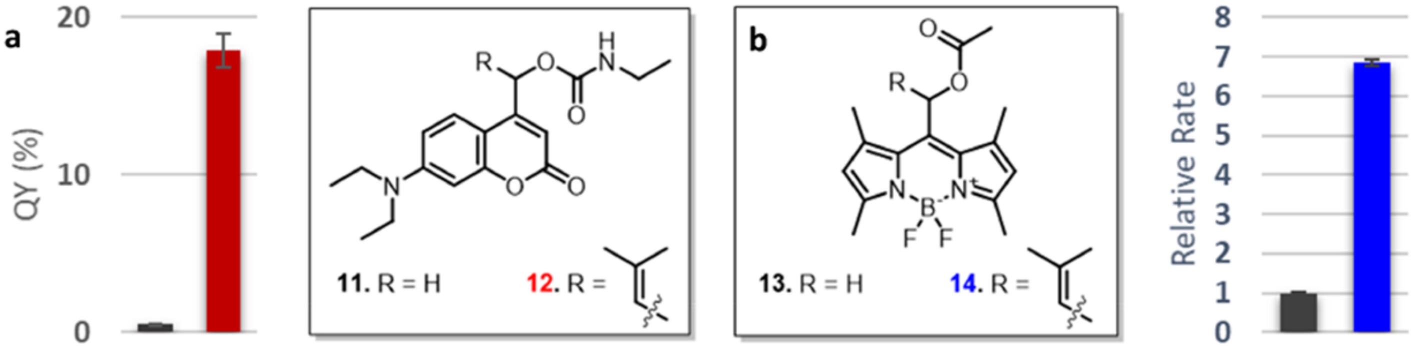


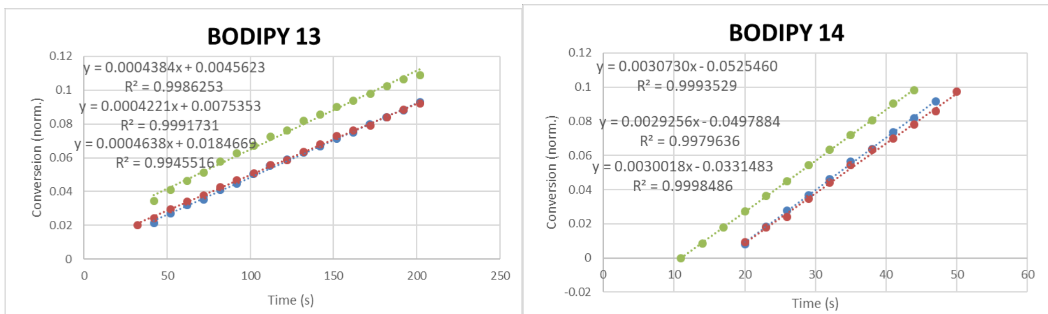
By using DFT calculation,
delocalization or hyperconjugation works well.

→ allylic coumarin **3** showing a QY (ϕ_r) as high as 27%, a **16-fold increase** over its primary coumarin model
→ relative barrier height ratio of k_{-1} over k_1 showed a strong correlation with QY (ϕ_r)

Feringa, B. L., *et al. J. Am. Chem. Soc.* **2022**, *144*(27), 12421-12430.

1(1). Apply the strategy to BODIPY photocage





	BODIPY 13	BODIPY 14
rate of deprotection [s^{-1}]	4.41×10^{-4}	3.00×10^{-3}
S.D.	1.72×10^{-5}	6.02×10^{-5}

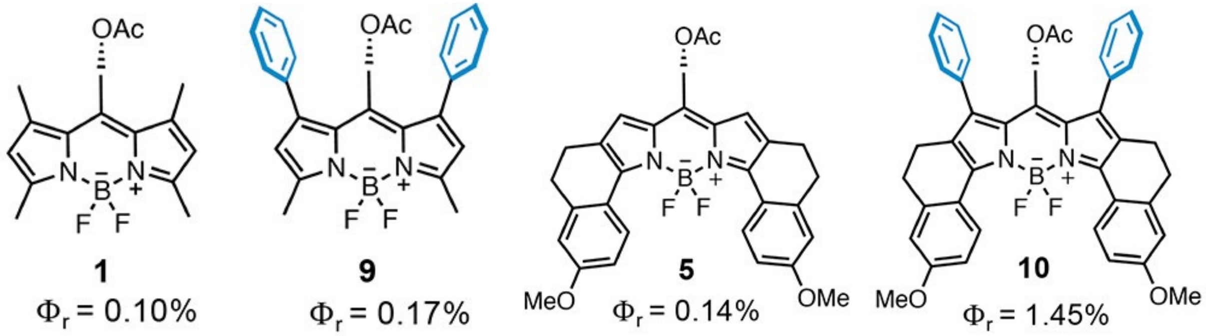
*: three replicates

Apply to BODIPY photocages:
QY (ϕ_r) of allylic-BODIPY **14** showed a 7-fold increase over **13**

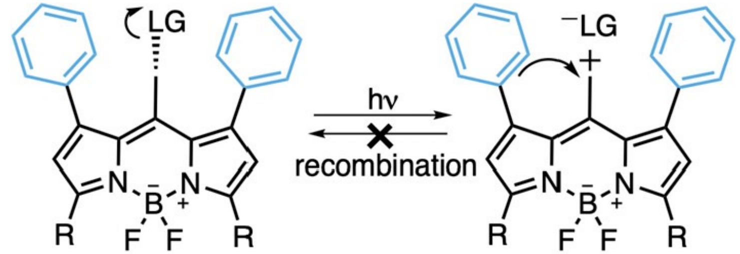
Rate of deprotection of Allylic-BODIPY **14** was 7-fold improvement.

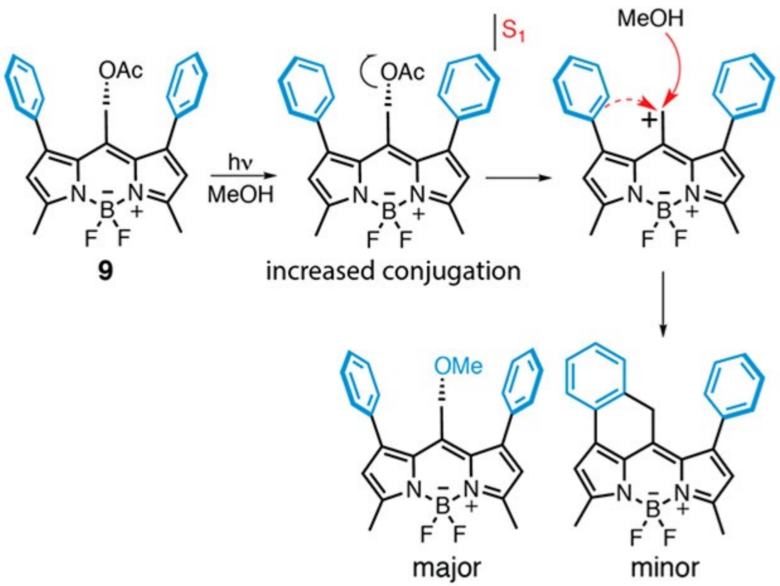
Feringa, B. L., *et al. J. Am. Chem. Soc.* **2022**, *144*(27), 12421-12430.

1(2). 1,7-diaryl substitutions



Concept





Strategy for stabilizing carbocation intermediate by 1,7-diaryl substitutions

→ lead to an increase in the quantum yield of photorelease

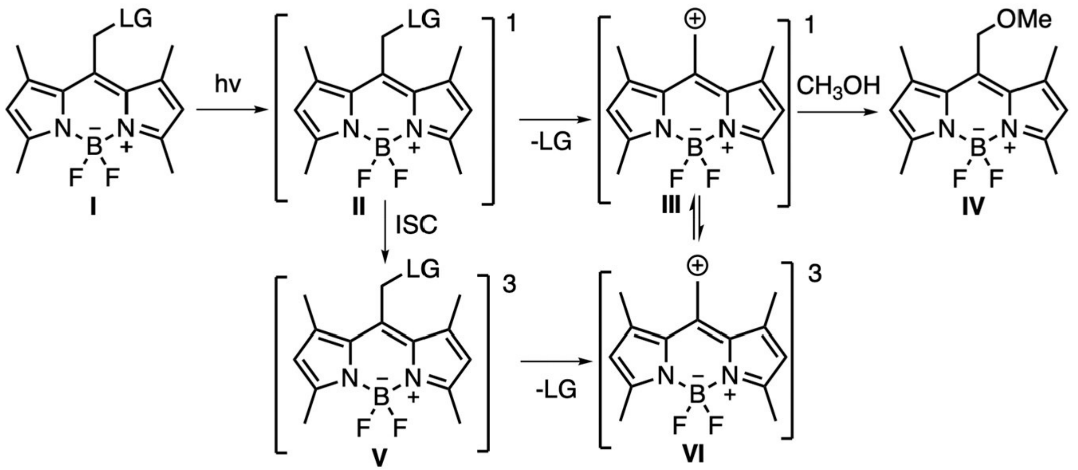
the trapping product : **minor** product
the typical solvent trapping adduct: **major** product

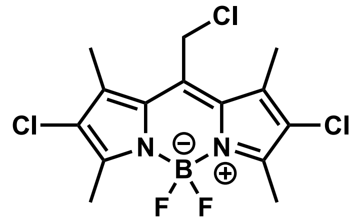
→ not only by trapping cation but also involving excited state?

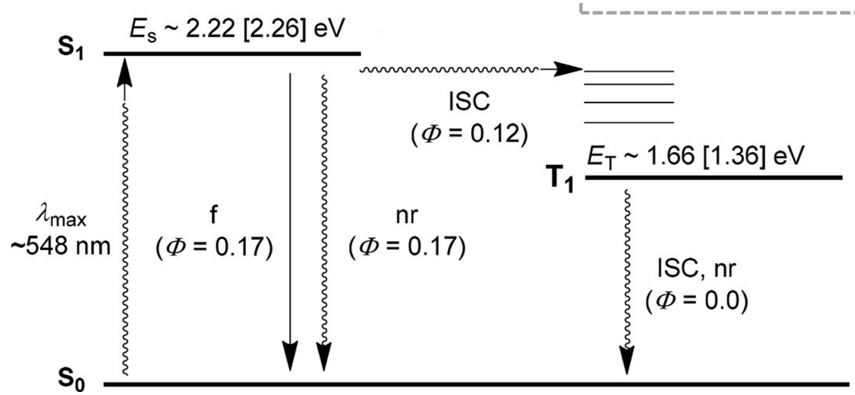
Winter, A. H., *et al. J. Am. Chem. Soc.* **2020**, *142*(36), 15505-15512. (Fig. modified)
Winter, A. H., *et al. J. Am. Chem. Soc.* **2023**, *145*(32), 17497-17514.

2. Heavy atom effect (2,6-dihalogen substitution)

Proposed mechanism of photo-release







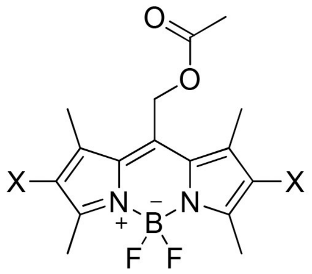
Both singlet-excited state and triplet-excited state are involved in photo-release

Generally, triplet-excited state has longer lifetime than singlet-excited state (forbidden transition)

→ **Promote intersystem crossing (ISC)** by using **heavy atom effect**

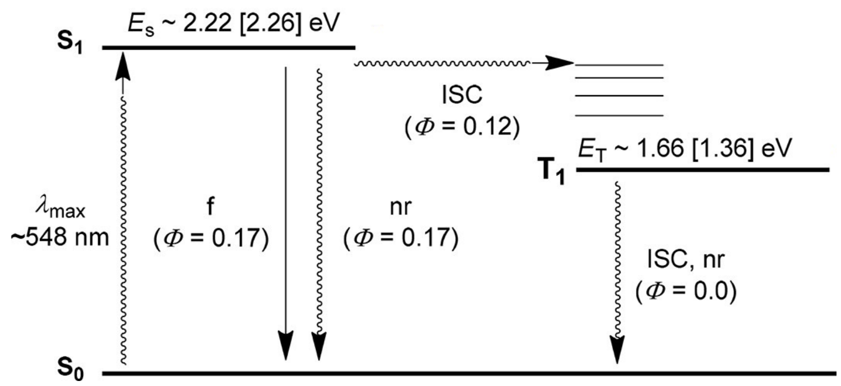
Winter, A. H., *et al. J. Am. Chem. Soc.* **2023**, *145*(32), 17497-17514.
Weinstain, R., Winter, A. H., Klan, P., *et al. J. Am. Chem. Soc.* **2017**, *139*(42), 15168-15175. (Fig. modified)

2. Heavy atom effect (2,6-dihalogen substitution)



2: X = H
2-Cl: X = Cl
2-Br: X = Br
2-I: X = I

compound	Φ_{ISC} (%)	Φ_r (%)
2	< 0.05	0.14
2-Cl	22	0.29
2-Br	54	0.70
2-I	84	0.99

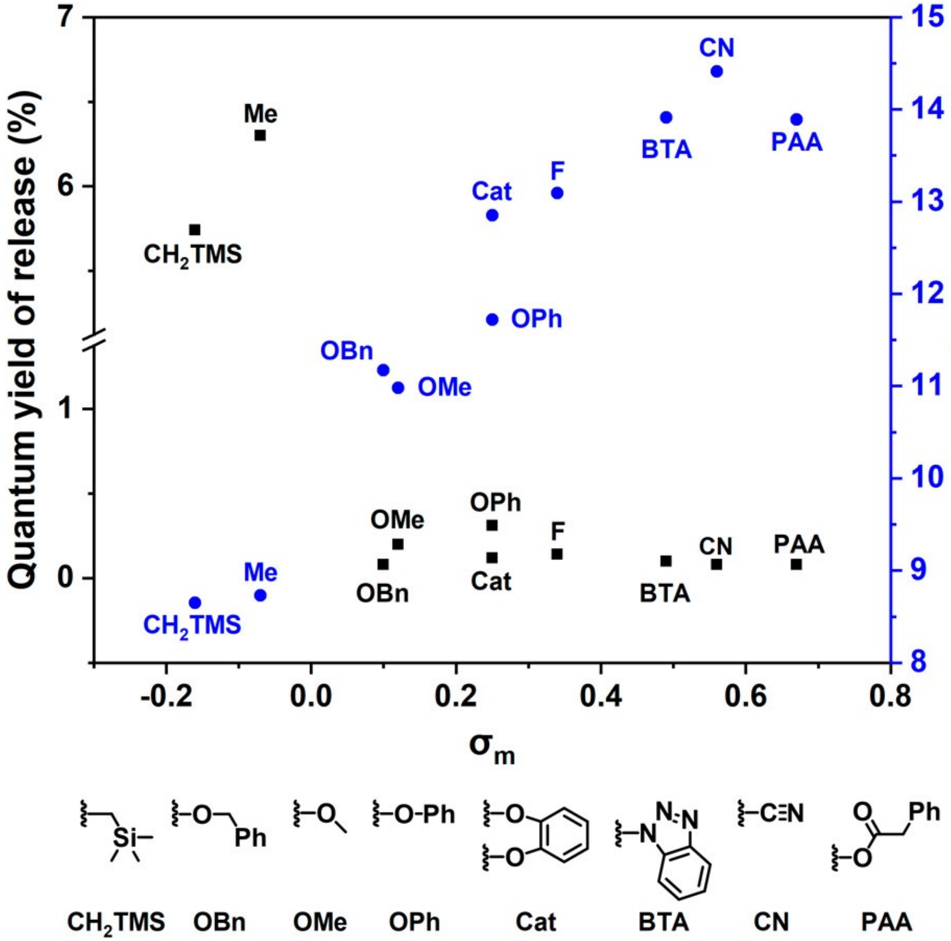


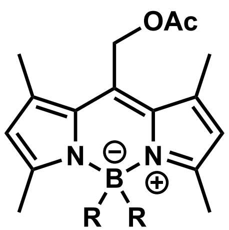
2,6-dihalogen analogues exhibited an efficient ISC in the order of H < Cl < Br < I substitution

By introducing heavy atom, promote ISC and increase photo-release quantum yield

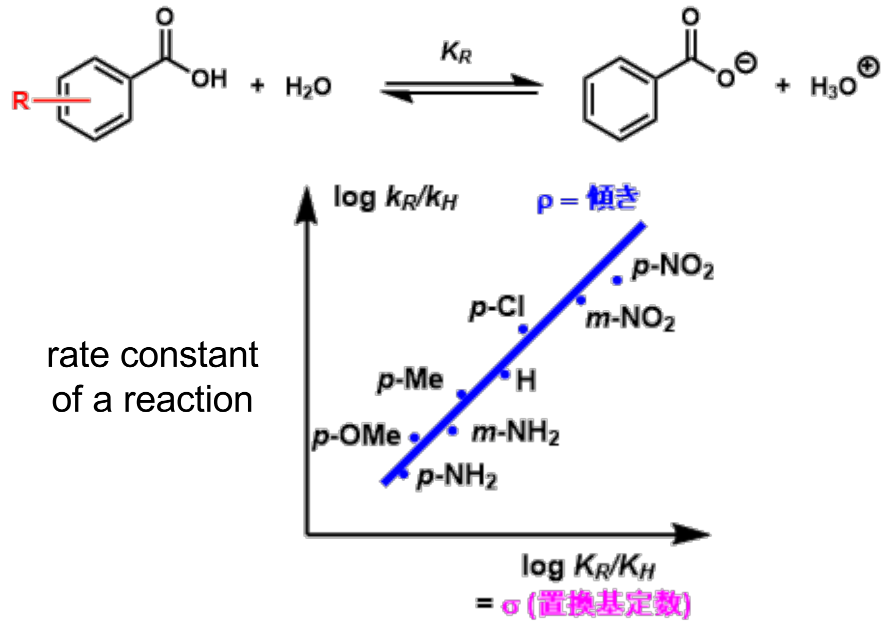
Winter, A. H., *et al. J. Am. Chem. Soc.* **2023**, *145*(32), 17497-17514.
Weinstain, R., Winter, A. H., Klan, P., *et al. J. Am. Chem. Soc.* **2017**, *139*(42), 15168-15175. (Fig. modified)

3. Boron methylation





substituent constant (σ)



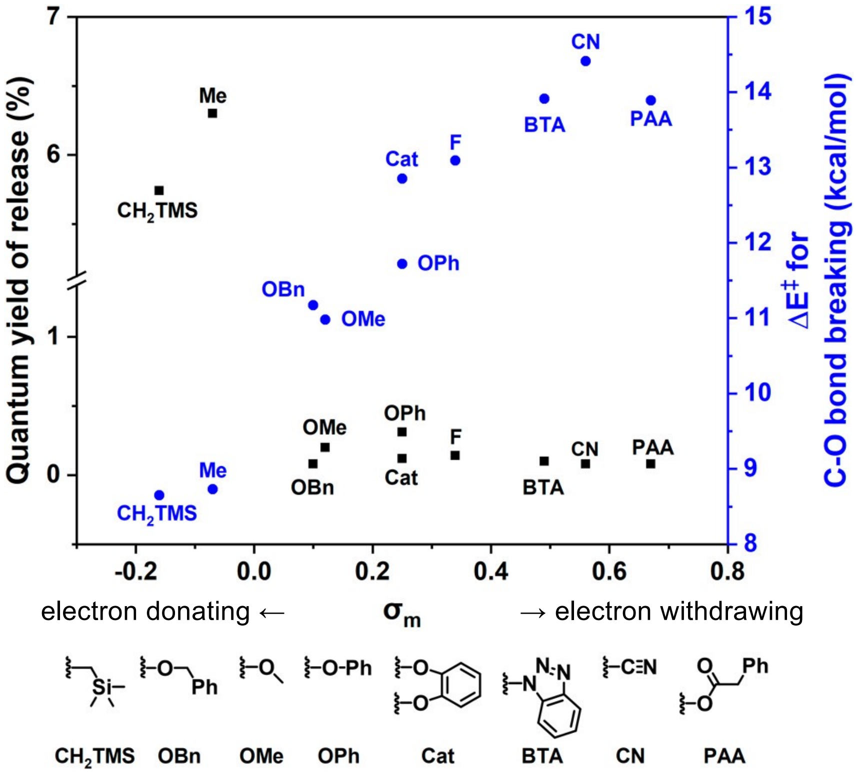
rate constant of a reaction

← electron donating → electron withdrawing

https://www.chem-station.com/blog/2002/04/Hammett_equation.html

Winter, A. H., *et al. J. Org. Chem.* **2024**, *89*(10), 6740-6748. (Fig. modified)

3. Boron methylation



compound (R)	ε [×10^4 $M^{-1}cm^{-1}$]	Φ_r (%)
Me	6.1	6.30*
CH_2TMS	7.6	5.74
F	7.1	0.14*
OBn	7.2	0.08
OMe	4.0	0.20
OPh	9.2	0.31
Cat	5.9	0.12
BTA	5.5	0.10
CN	4.6	0.08
PAA	10.0	0.08

*: Values reported previously

No correlation between the experimental Φ_r and σ_m
→ donating substituents **lower barrier** for photo-release (mechanism remains unknown)

Boron methylation exhibited a Φ_r of 6.3%, **a 41-fold increment** of Φr compared to the parent BODIPY 1-F

Winter, A. H., *et al. J. Org. Chem.* **2024**, *89*(10), 6740-6748. (Fig. modified)
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  2. Discovery of photocages and an example of *ortho*-nitrobenzyl photocages
- Main
  1. meso-Methyl BODIPY photocages
  2. Several strategies for improving photo-release quantum yield
- **Summary**

Summary

Photocaged compounds are in the spotlight
→ **Spatiotemporal control**

Discovery and development of ***o*-Nitrobenzyl photocages**

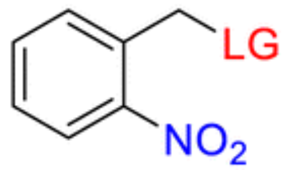
< several limitations >

- Inherent **phototoxicity**
- Limited tissue permeability

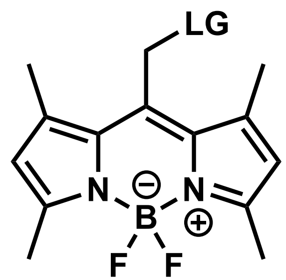
BODIPY and other photocages (activated by visible-light)
→ high ε but **low φ (quantum yield)**

several strategy to improve **quantum yield**
cation stabilization, heavy atom effect, B-methylation

→ will become a powerful tool with a broad range of applications



o-Nitrobenzyl



BODIPY

Thank you

for your kind attention!