

Bioorthogonal Bond cleavage reaction

Literature seminar #3

2024/08/29

M2 Shuhei Terada

■ Introduction

- Bioorthogonal (ligation) reaction
- Bioorthogonal bond cleavage reaction

■ Main (state of the art)

- Gold-induced amide bond cleavage
- NIR-triggered photocatalytic decaging

■ Summary

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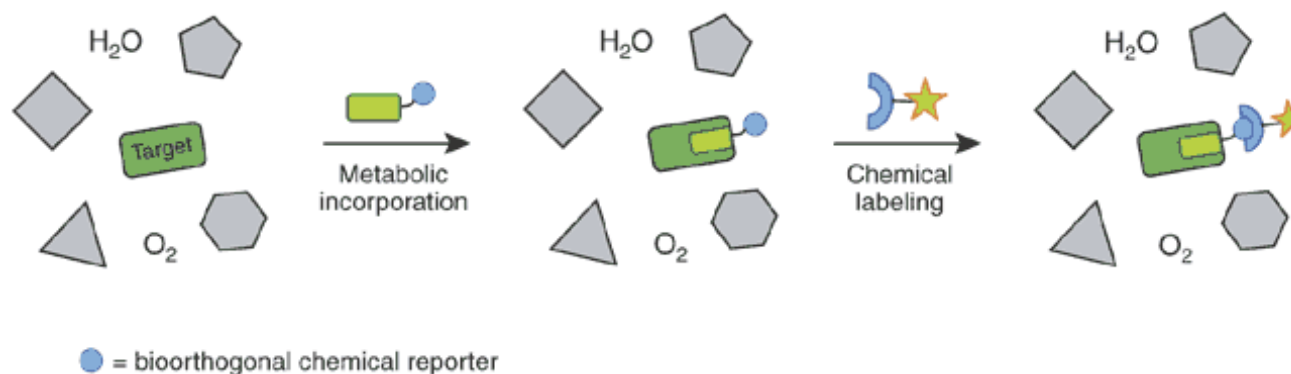


Carolyn Bertozzi
Stanford University

Requirement

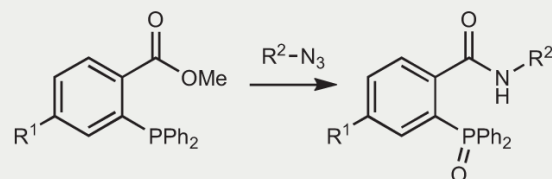
- Rapid reaction
- Proceed in physiological condition (water, ambient temperature, biocompatible pH)
- should be mutually and selectivity reactive
- neither interact nor interfere with the biological system

e.g. The bioorthogonal chemical reporter



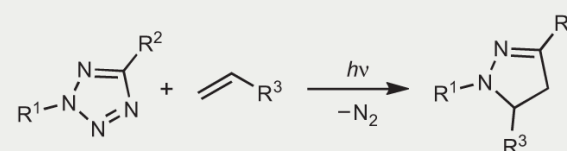
Staudinger ligation

In vivo formation of amide bonds



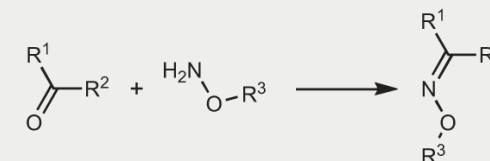
Photoinducible bioorthogonal chemistry

Spatio-temporal control with light



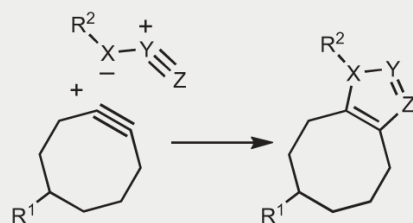
Oxime and hydrazone ligations

Enabled by ketone and aldehyde tags



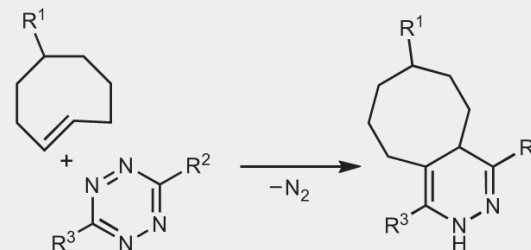
Strain-promoted [3 + 2] cycloaddition

Copper-free coupling with 1,3-dipoles



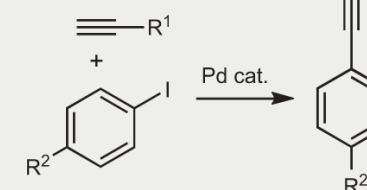
Tetrazine ligation

Exceptionally rapid kinetics



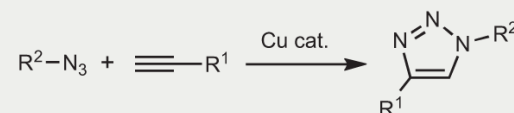
Palladium and ruthenium-catalysed couplings

Unique tags for biomolecule modification



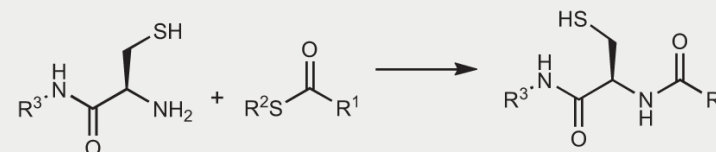
Copper-catalysed azide-alkyne cycloaddition

Minimal size, readily accessible, broadly useful



Native chemical ligation

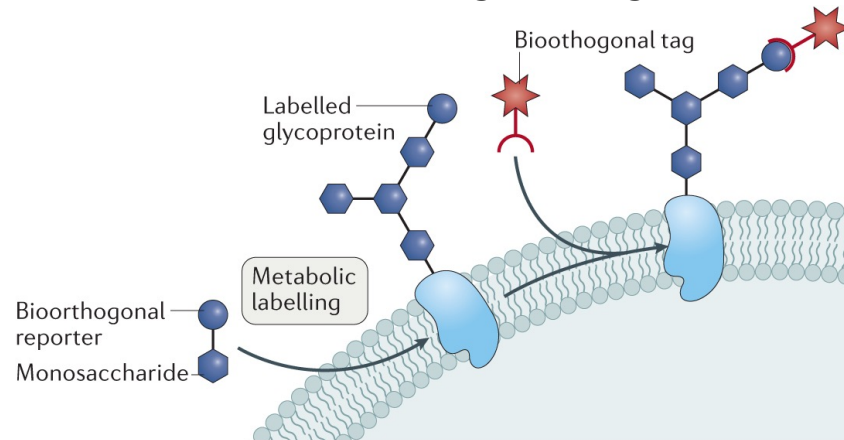
Protein synthesis from synthetic or expressed fragments



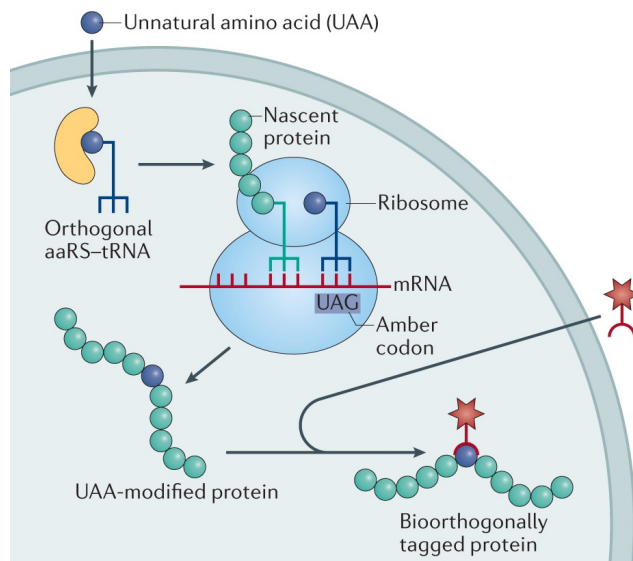
Bioorthogonal ligation reaction: application

Introduction

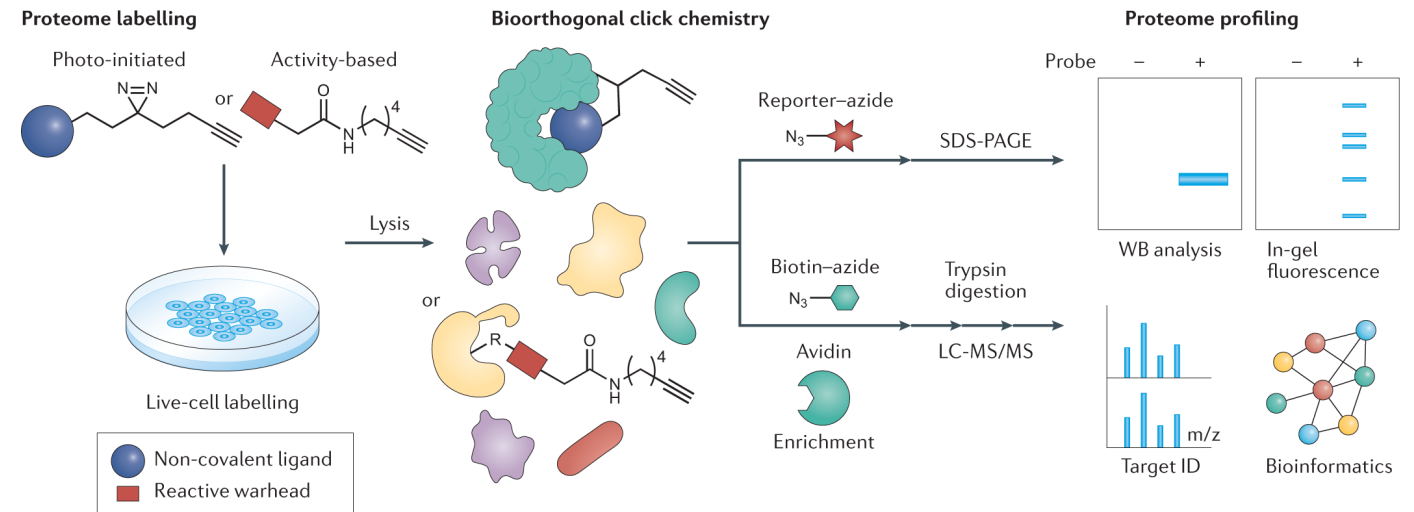
Metabolic engineering



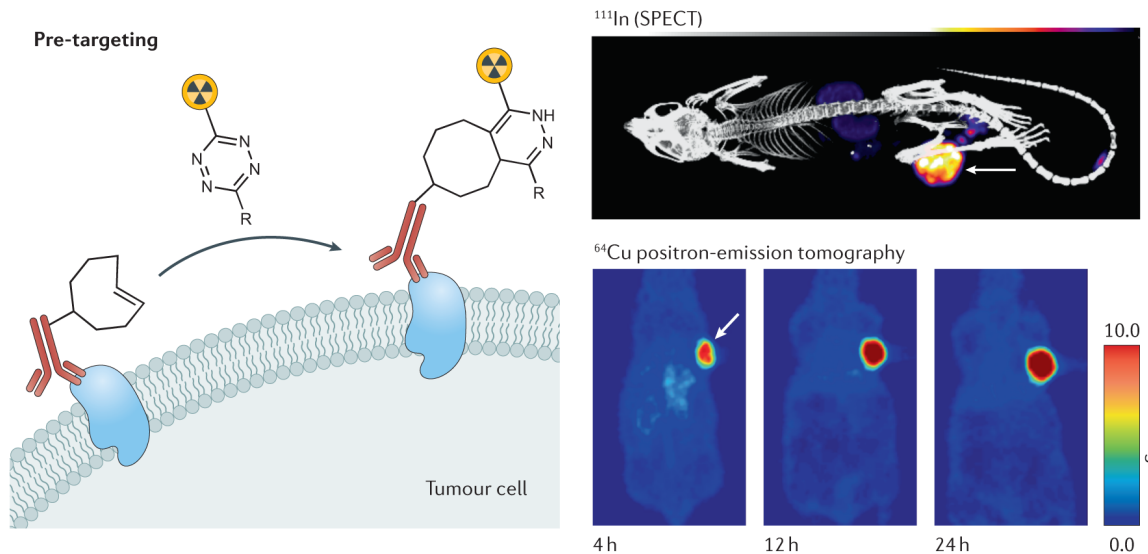
Genetic Code Expansion



Proteomics



Pre-targeting



■ Introduction

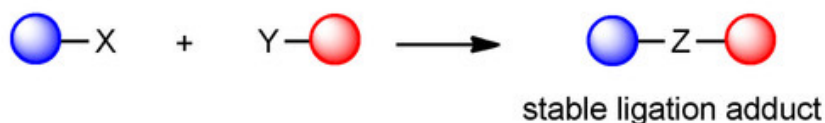
- Bioorthogonal (ligation) reaction
- Bioorthogonal bond cleavage reaction

■ Main (state of the art)

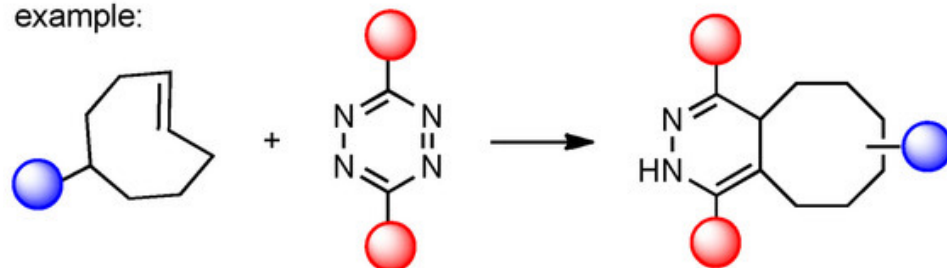
- Gold-induced amide bond cleavage
- NIR-triggered photocatalytic decaging

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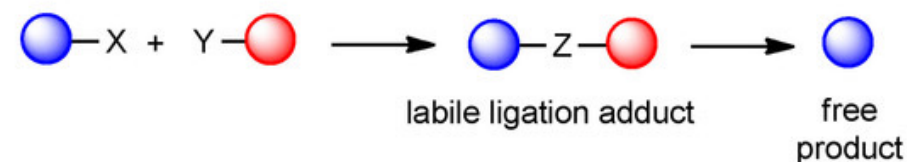
A) Ligation



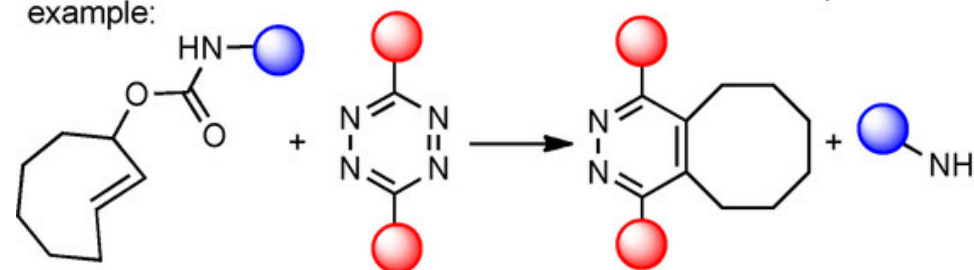
example:



B) Dissociation



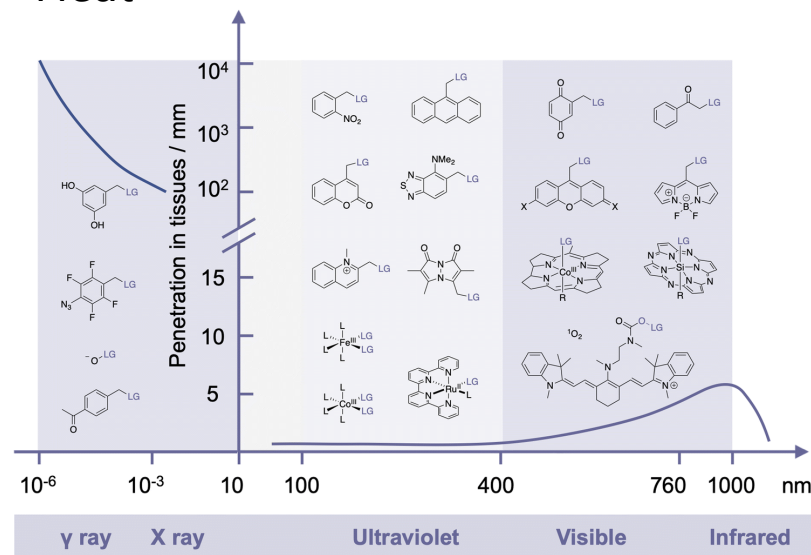
example:



- In terms of bio-orthogonality, it is less studied than the ligation reaction.
- Research has been conducted in terms of improving the generality of substrates and reaction efficiency.

Physical stimuli

- NIR
- Ionizing radiation
- Heat
- Electrical field
- Ultrasound etc.



Overview of photon tissue penetration trends

- ✓ Easy clinical application
- ✗ Tumor penetration (photon)
- ✗ Restrictions on chemical reactions that can be initiated

Chemical stimuli

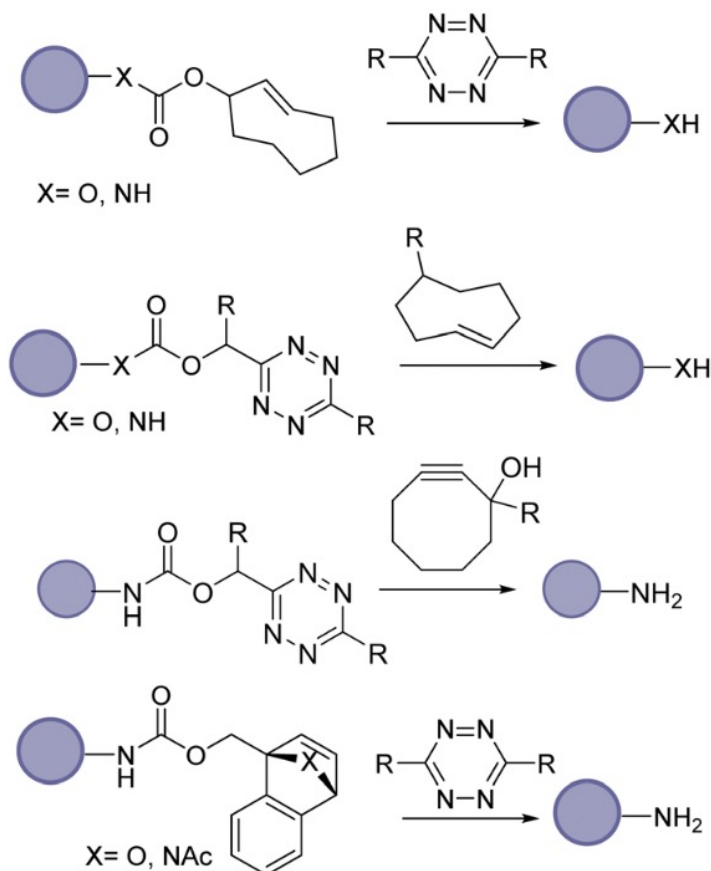
Small molecule

- Inverse electron demand Diels–Alder reaction
- Azide reduction reaction
- Desilylation reaction

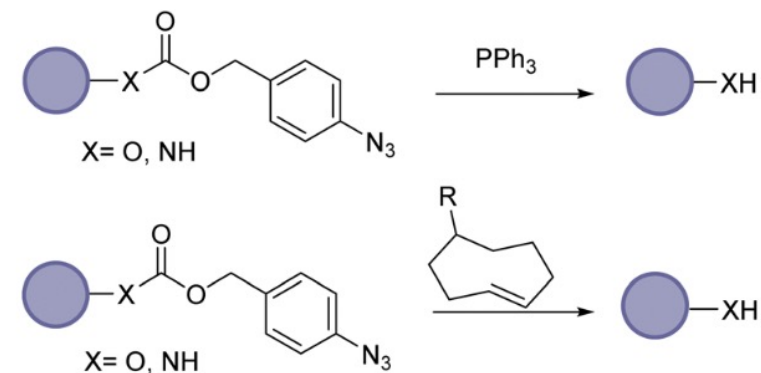
Transition Metal

- Deallylation
- Depropargylation
- Deallenylation
- Intramolecular Cyclisation

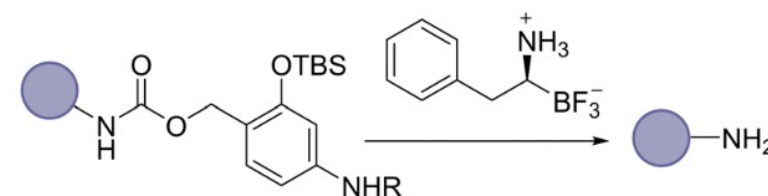
Inverse electron demand Diels–Alder reaction



Azide reduction reaction



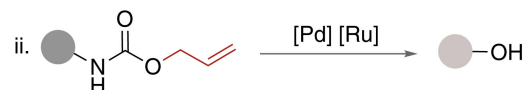
Desilylation reaction



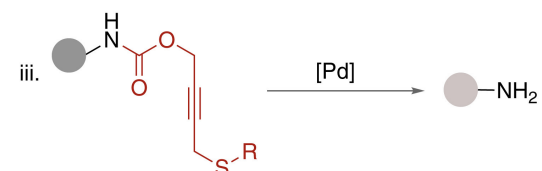
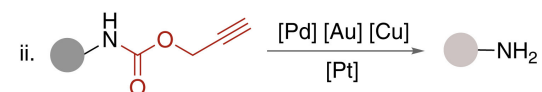
- ✓ high biocompatibility, fast reaction rate, and robustness
- ✗ Spatiotemporal activation

A Reaction:

(a) Deallylation



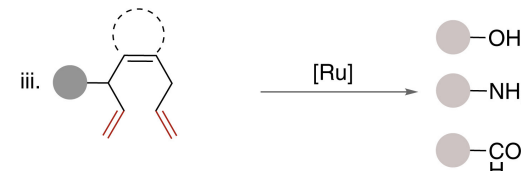
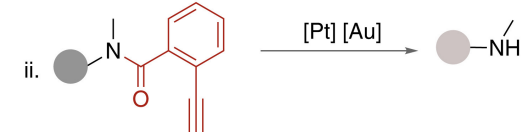
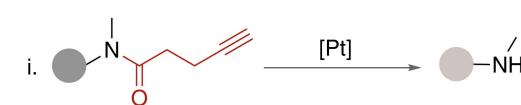
(b) Depropargylation



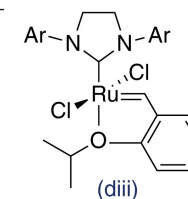
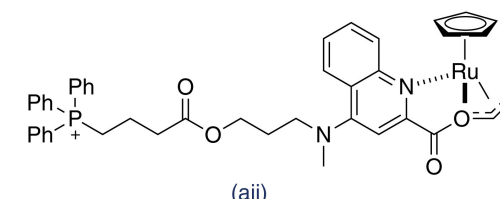
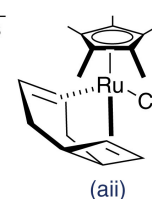
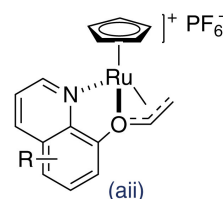
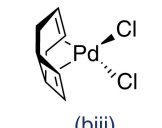
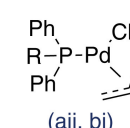
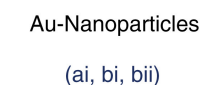
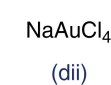
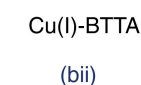
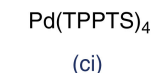
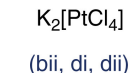
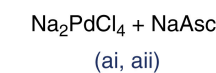
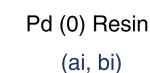
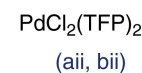
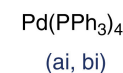
(c) Deallenylation



(d) Intramolecular Cyclisation



B Catalysts (reactions):

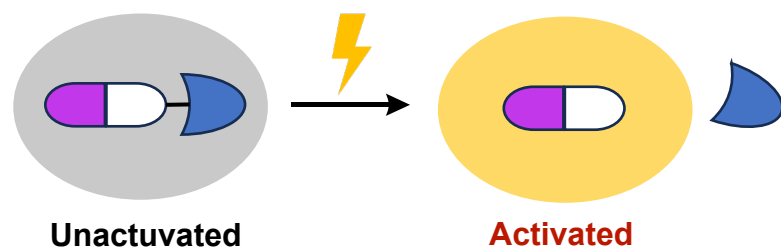


✓ Complement the substrate scope of other strategies.

✗ The variety of masking group is relatively limited. (Although metalloenzyme address this limitation)

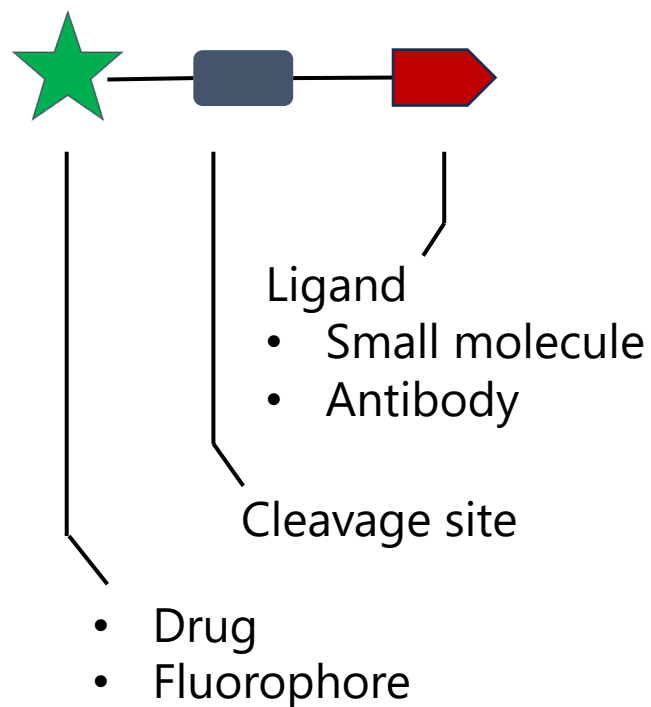
✗ Poor cell permeability, low catalytic activation in cell

Small molecule activation



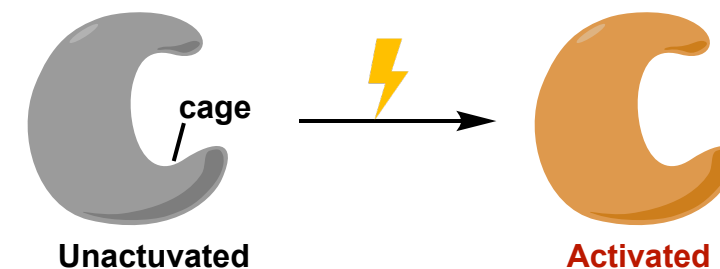
- Prodrug
- Chemical probe

Cleavage of bifunctional molecule



Protein activation

Caged AA is introduced by GCE



- Kinase
 - Fluorescent protein
 - nuclear localization sequence
 - Recombinase (Cre)
 - Gene editing enzyme (CRISPR/Cas9)
- etc.

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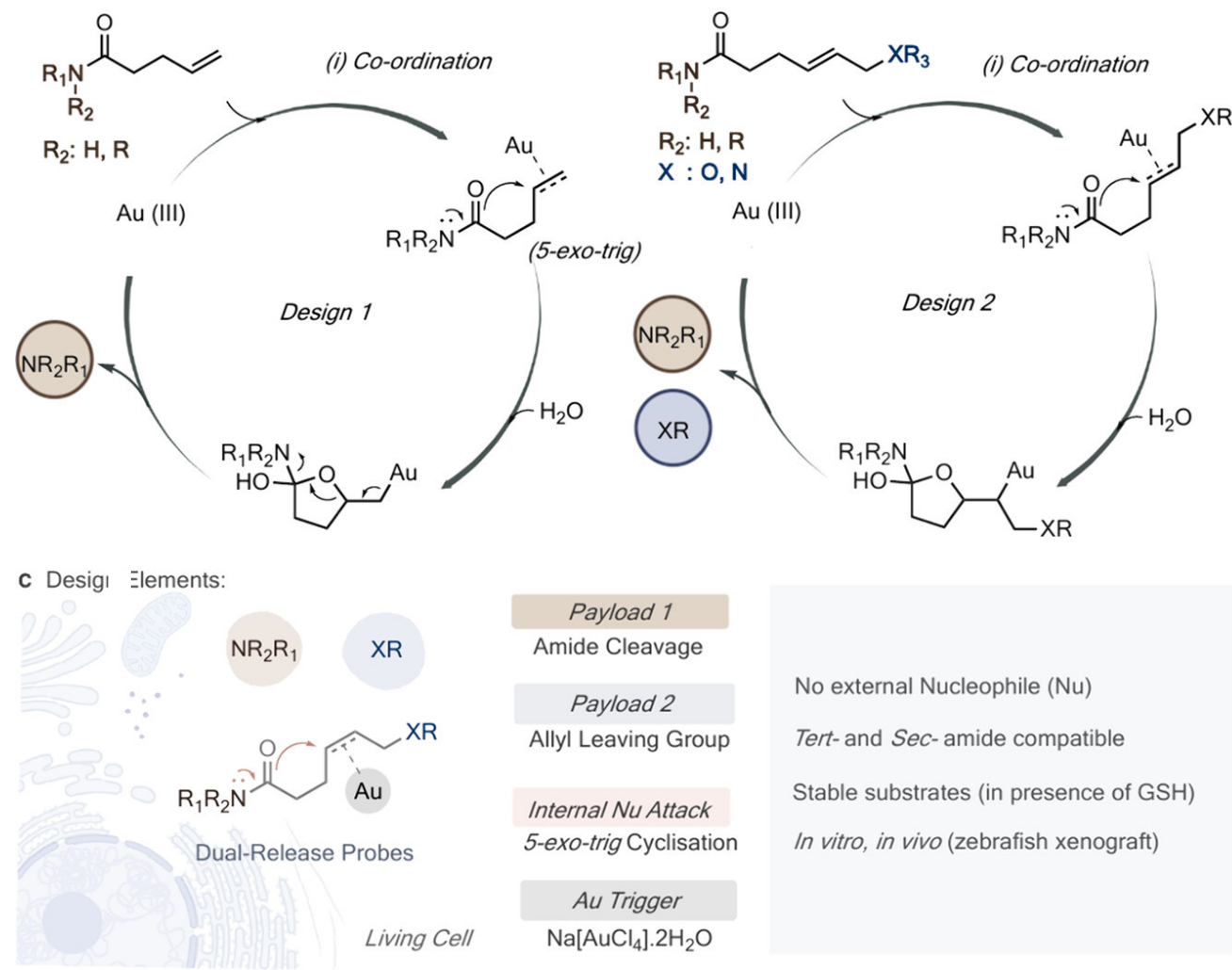
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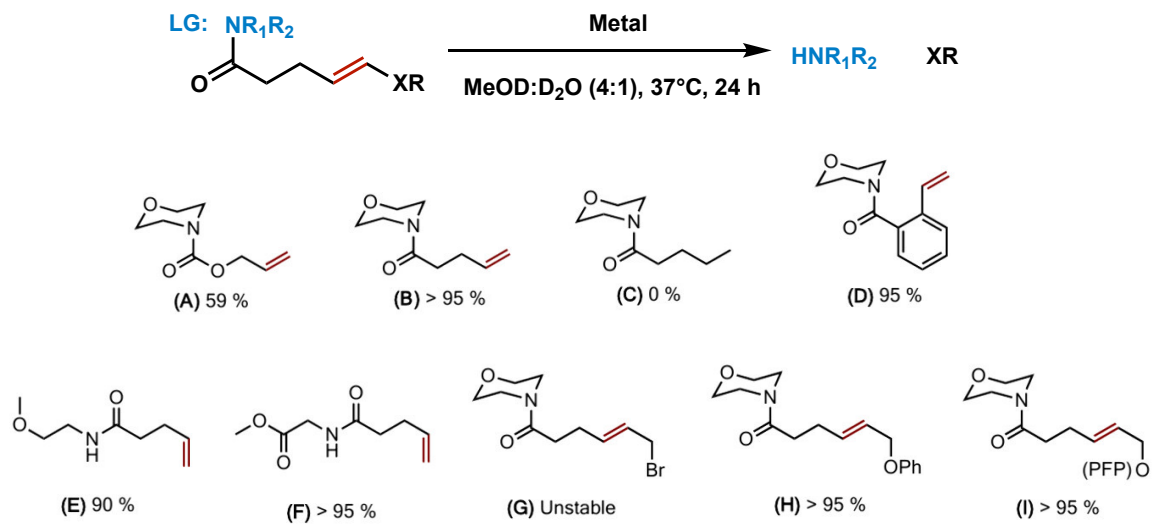
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Aim

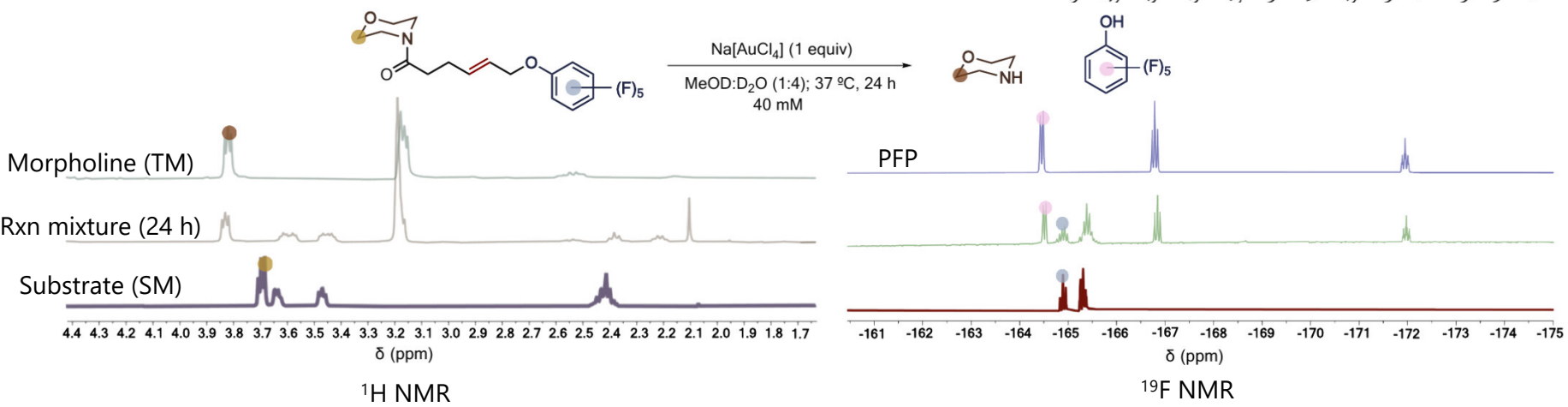
- Amide cleavage
- Dual-release



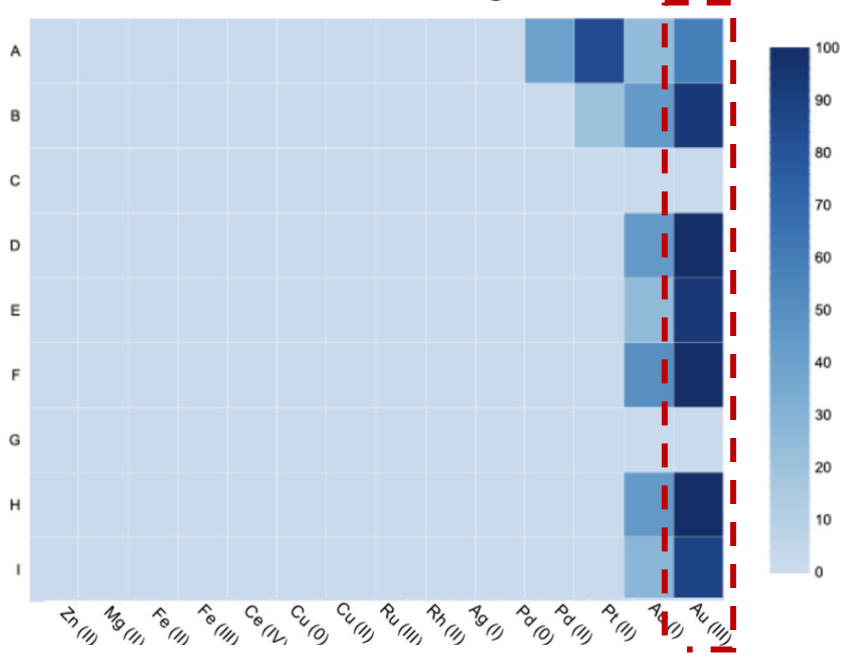


(Percentage: Efficiency of cleavage of amine)

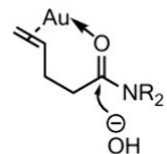
Dual release analysis of (I)



Screening

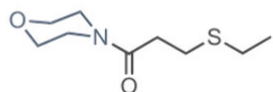


Mechanistic study

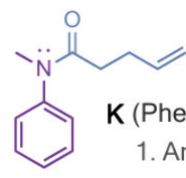
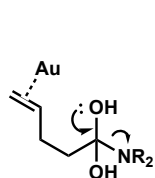


Possibility:

1. Alkene as directing group
2. Activates carbonyl for hydrolysis



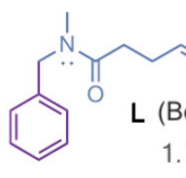
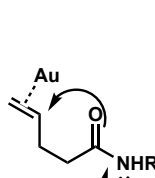
J *(no reaction) → Possibility 1 is eliminated



K (Phenyl Amide):

1. Amide LP in conjugation with ring
2. Aniline; good leaving group

*(trace)



L (Benzyl Amide):

1. No conjugation
2. Poorer leaving group

*($> 95\%$)

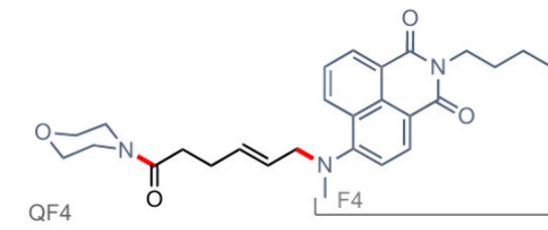
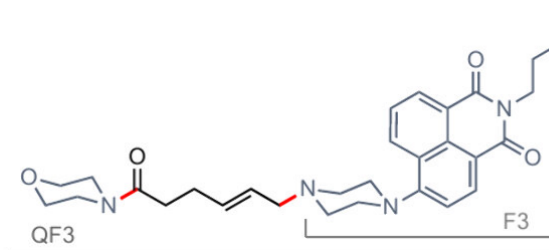
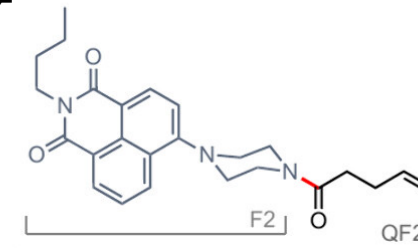
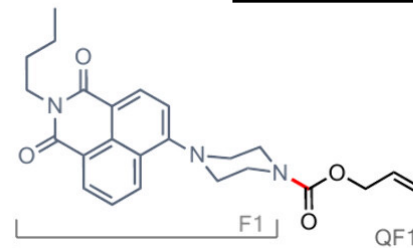
→ the carbonyl group should initially act as a Nu through its C-O resonance form

Reaction Conditions: Na[AuCl₄] (1 equiv.), MeOD: D₂O (1:4), 37 °C

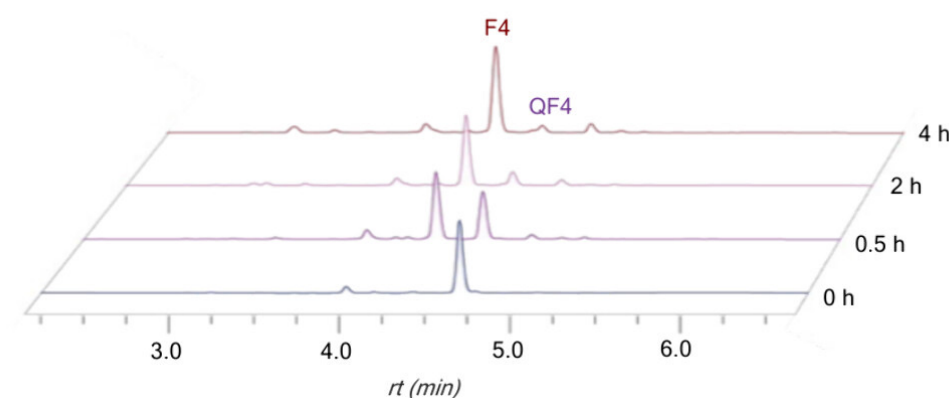
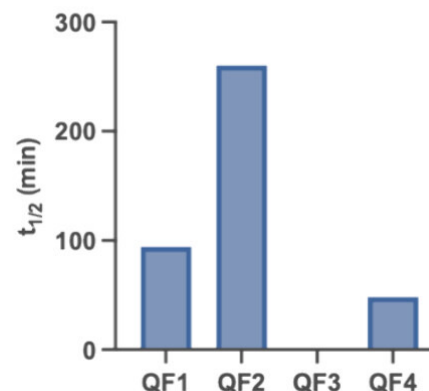
* Percentage conversion determined by ¹H NMR

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Kinetic study

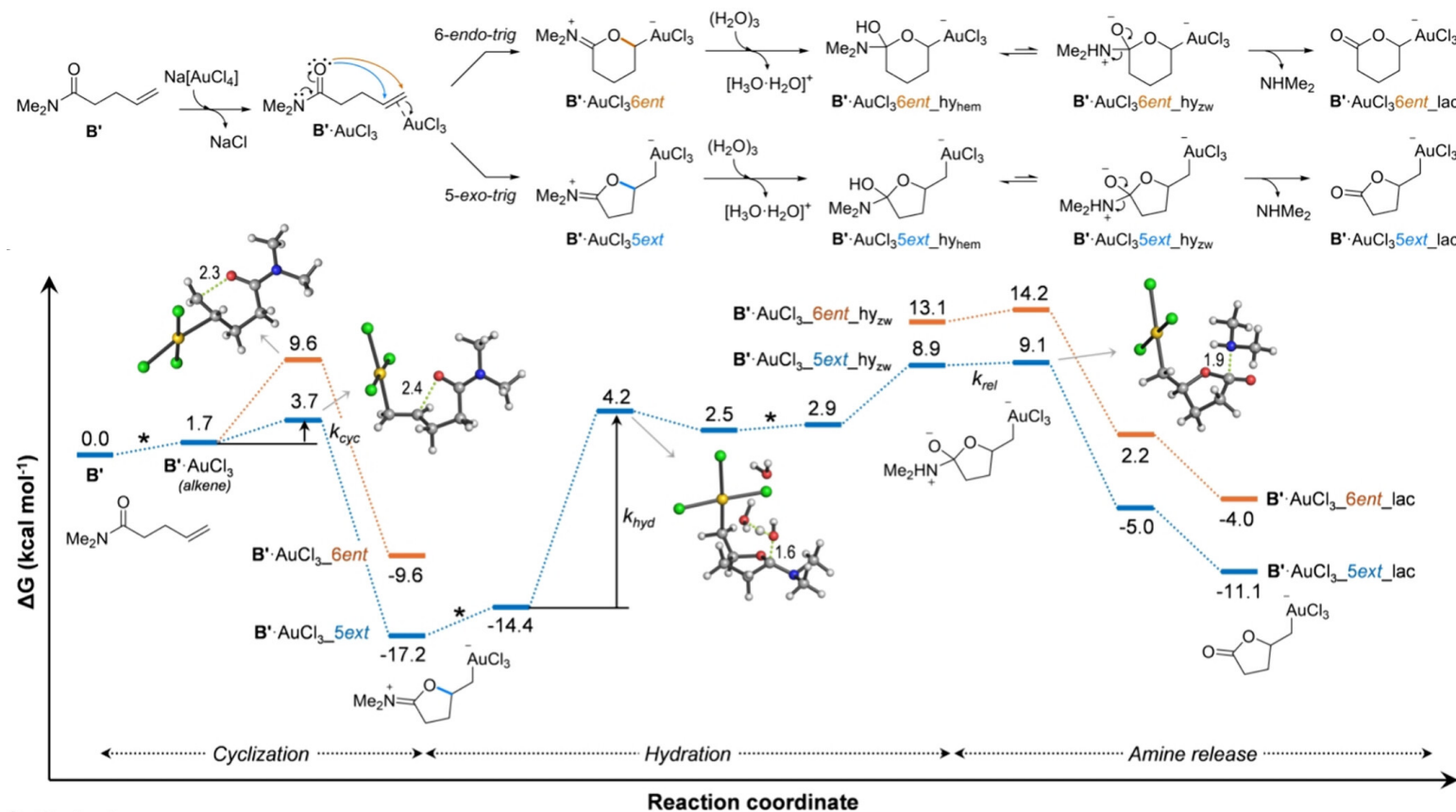


QF(x) [Quenched] + Na[AuCl₄] (10 - 50 equiv) $\xrightarrow[0.05 \text{ mM}]{\text{DMF : H}_2\text{O (1:1)}}$ F(x) [Fluorescent]

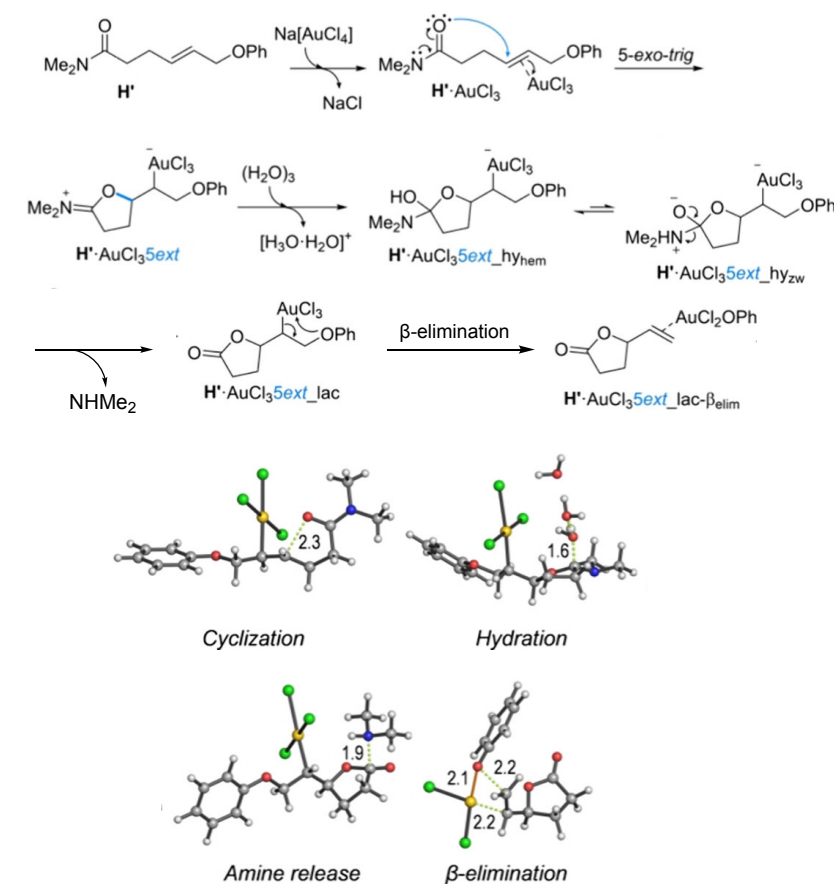


QF4 is ideal for in cellulo applications

Single-release

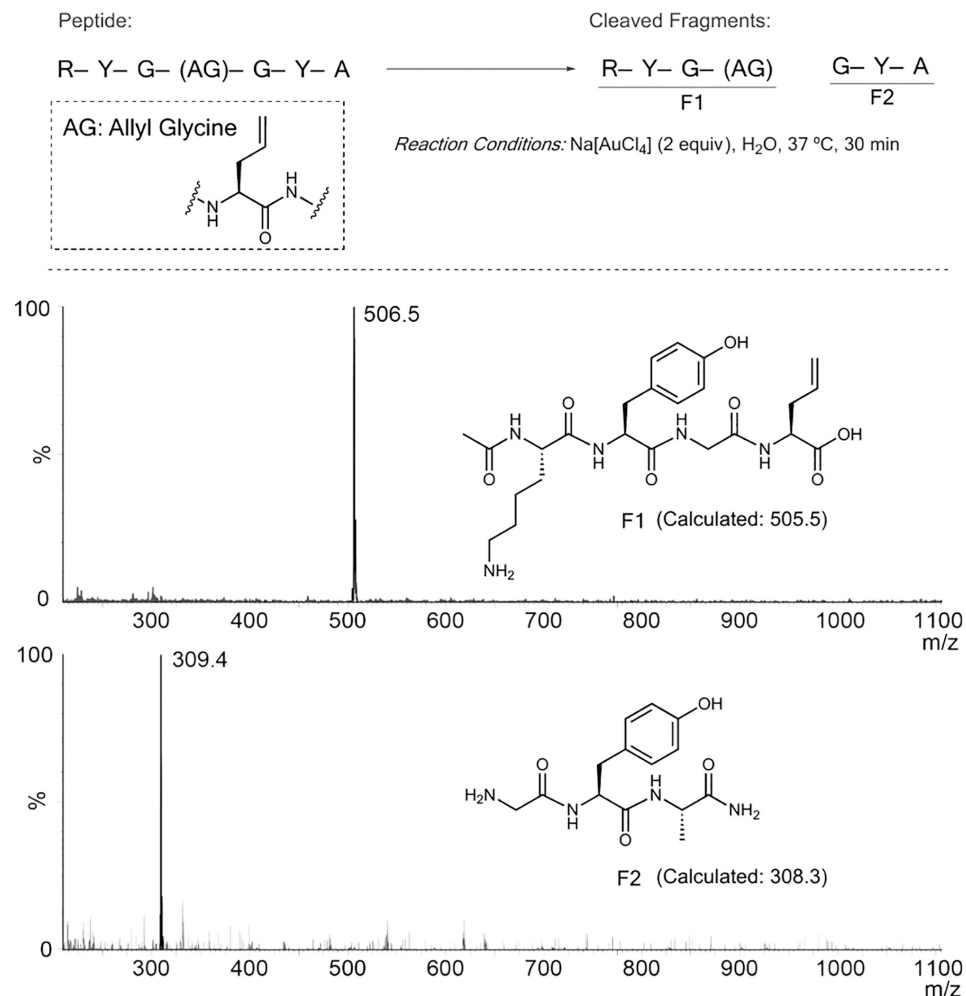


Dual-release



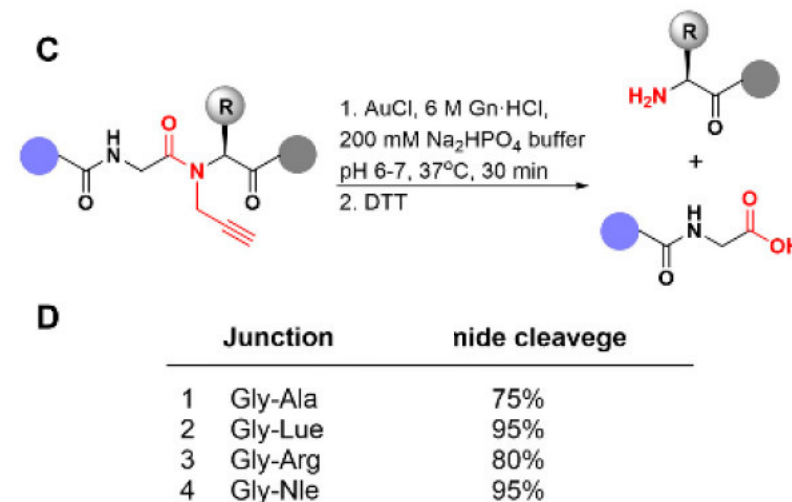
→ Quantum mechanical calculations also support the reaction mechanisms

Cleavage of model peptide



Previous report

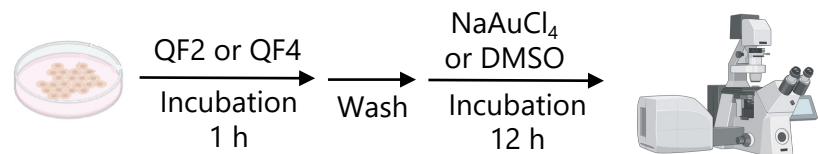
Au(I) mediated bond cleavage of N-propargylated peptide



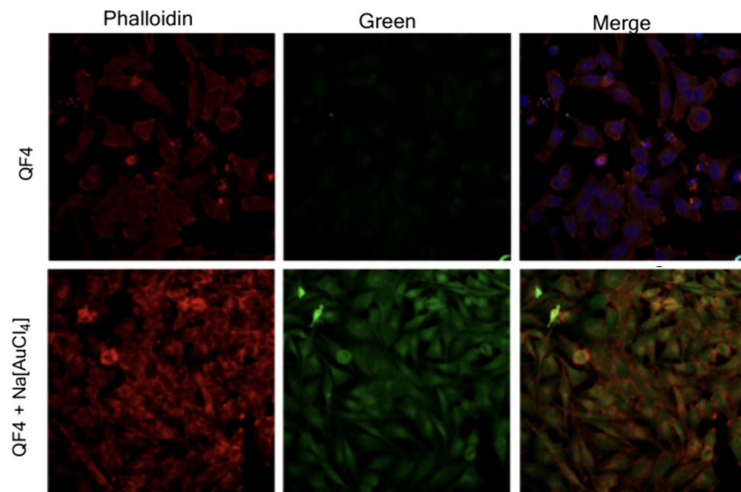
- **Bond-cleavage reaction proceeded on model peptide (PoC)**
- **Further investigation and optimization is needed for applications**

Au-Mediated Uncaging Reaction in Cells

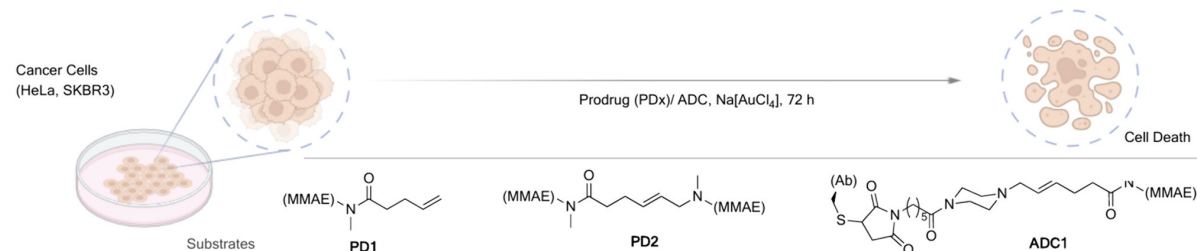
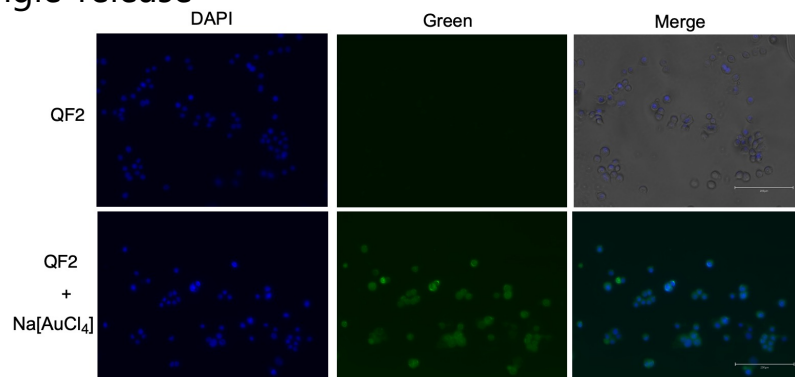
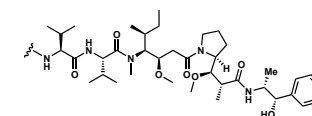
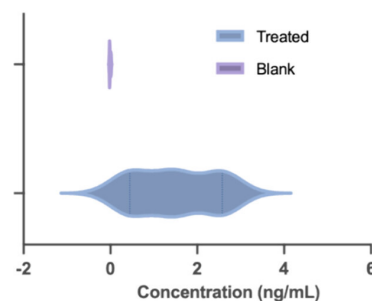
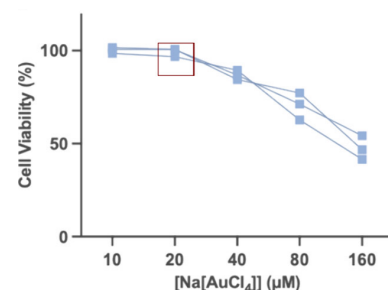
Main ①



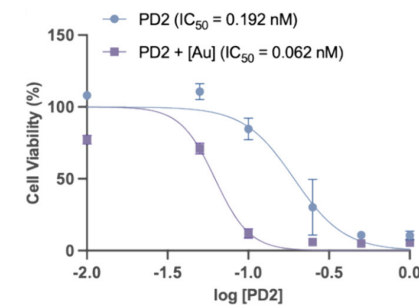
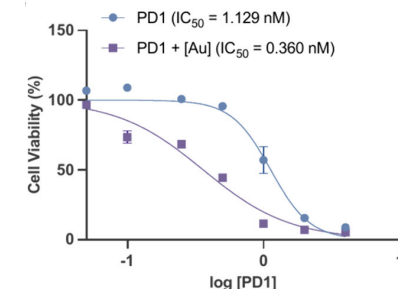
<Dual-release>



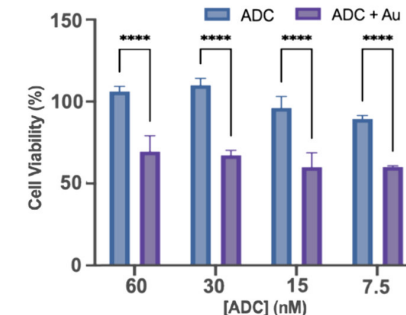
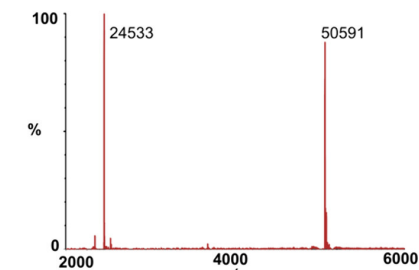
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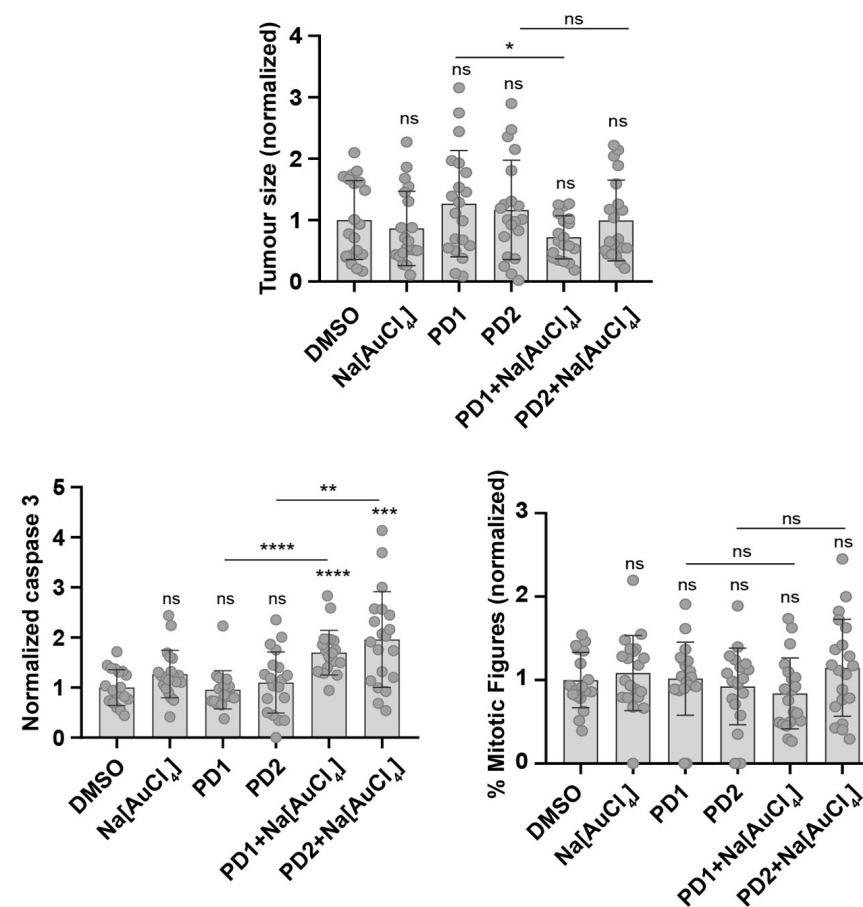
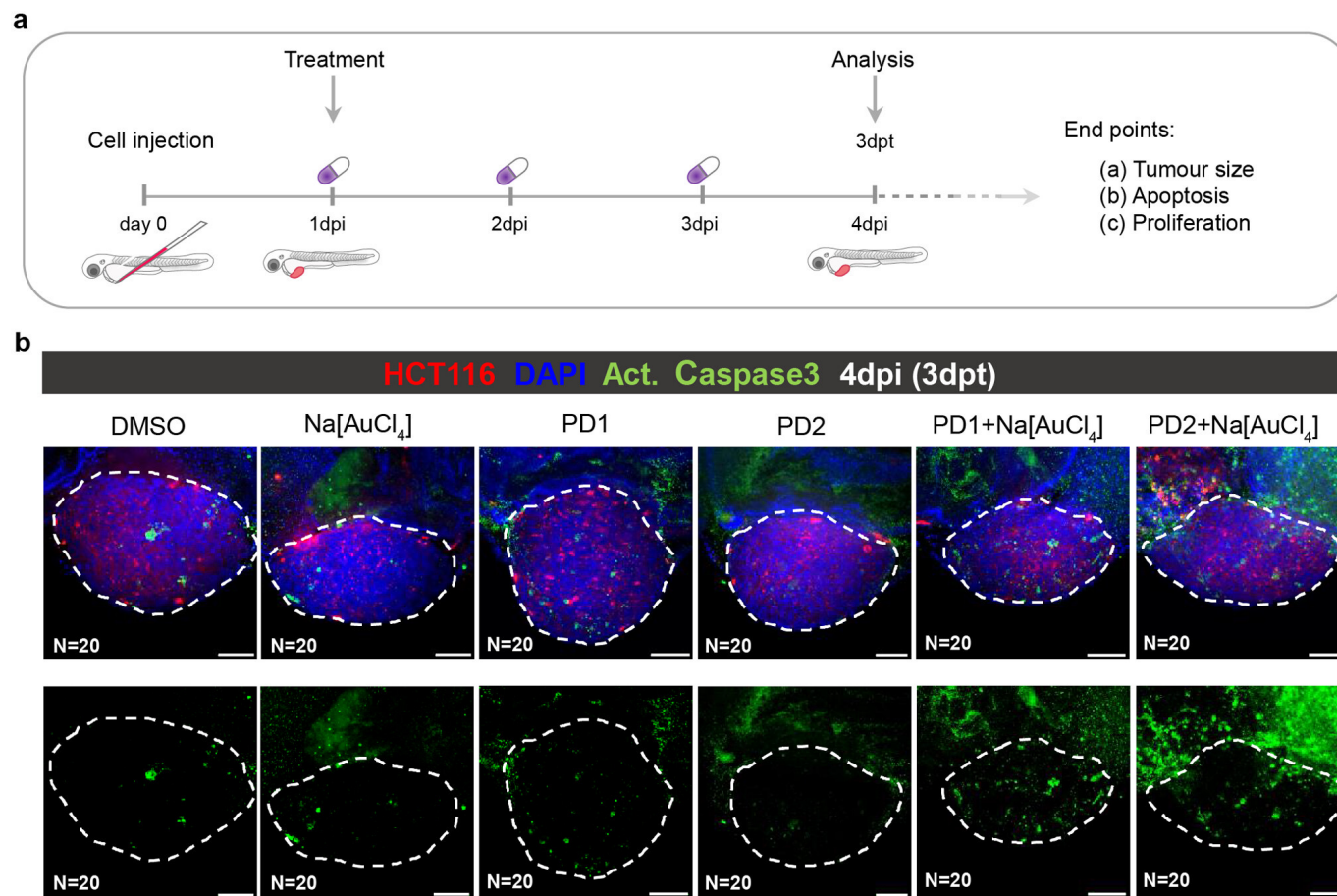
MMAE
antineoplastic agentProperties of NaAuCl₄

Effectiveness of prodrug



ADC





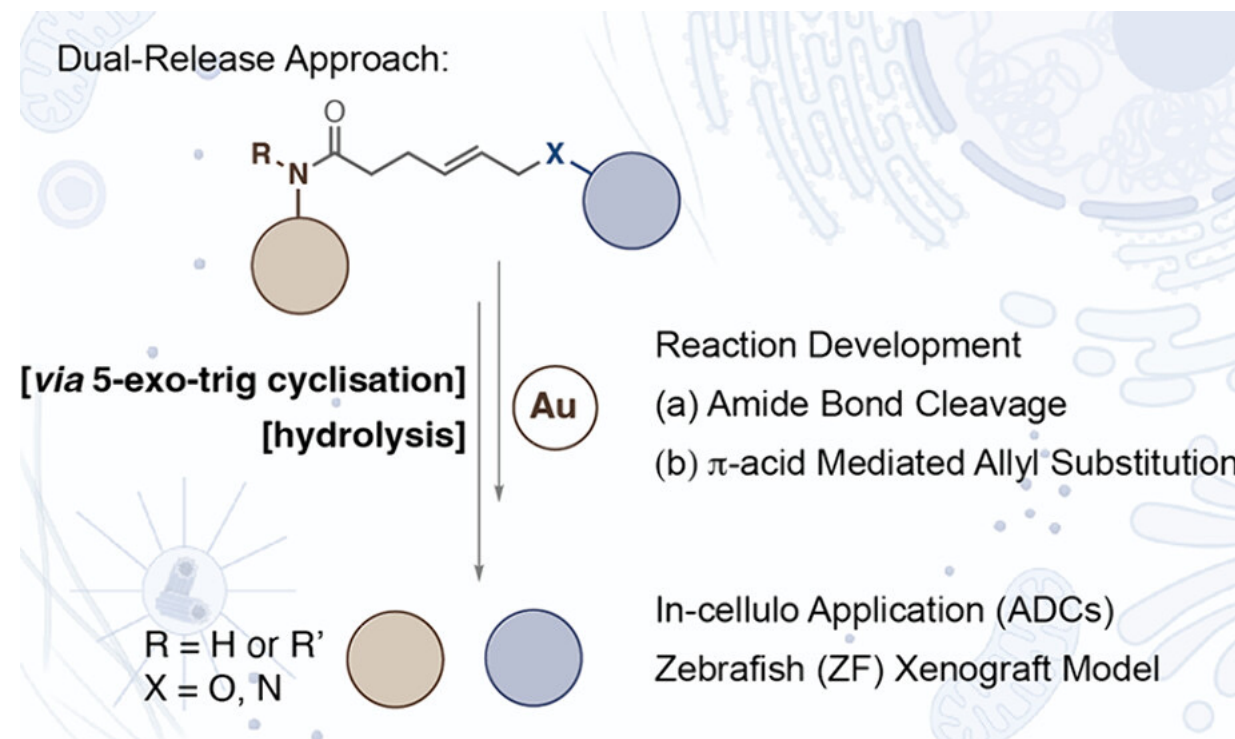
- Prodrug activation was accomplished in vivo

This time,

- Dual-release approach
- Amide bond cleavage
- Application in-cellulo (ADC)
in-vivo (zebrafish)

In future,

- Combining with nano particle
- Introducing of allyl glycine by Genetic code expansion



■ Introduction

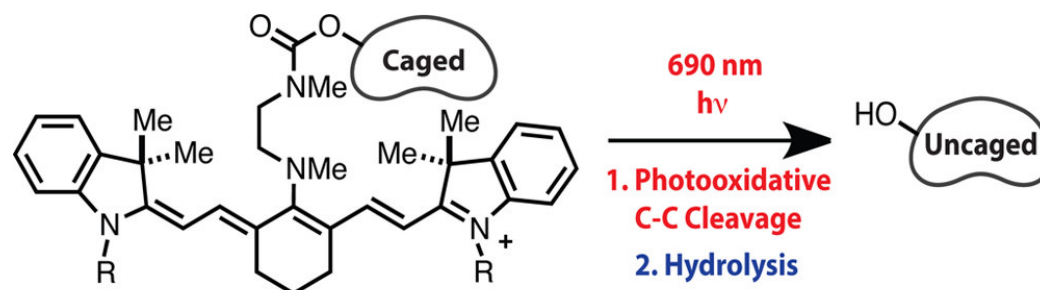
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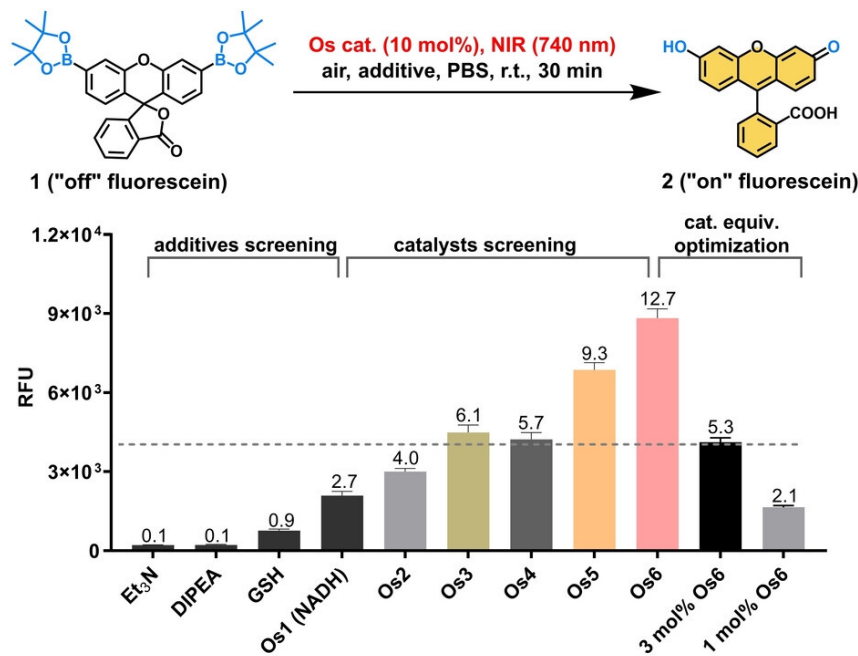
- Manipulation of biological processes in a remote-controlled, noninvasive manner
 - effective, but difficult (e.g. short wavelength light)
 - long wavelength light (NIR) is expected



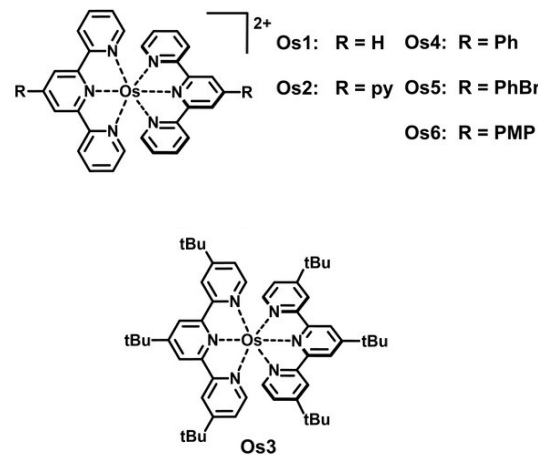
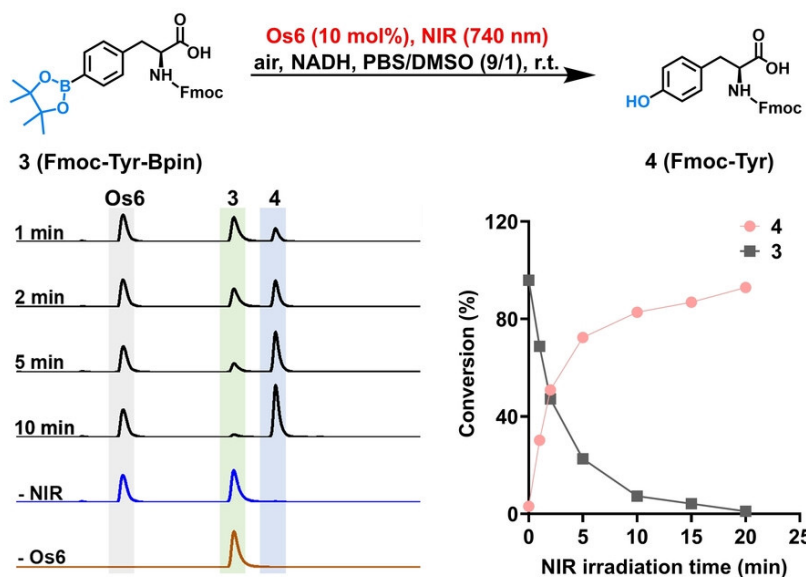
J. Am. Chem. Soc. **2014**, 136, 14153–14159

- Cyanine group
 - low energy conversion
- Metal might be good.

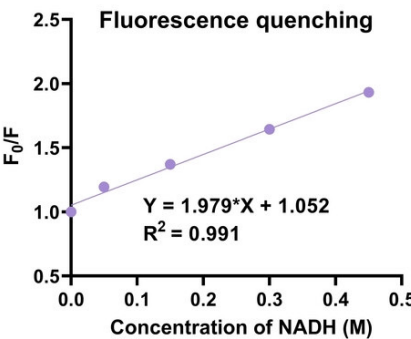
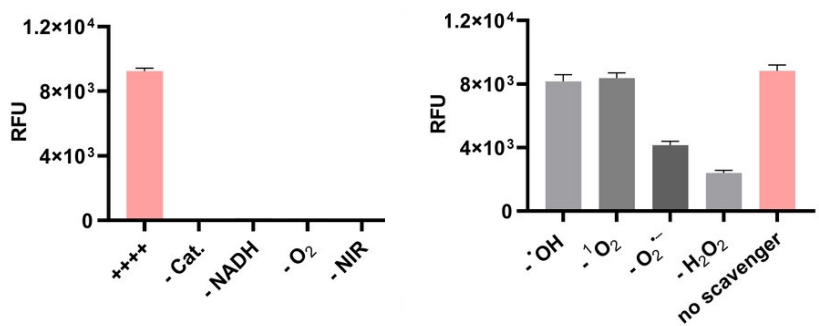
Reaction 1: fluorescein decaging



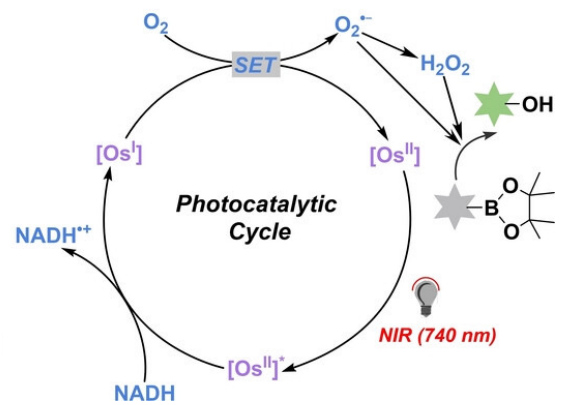
Reaction 2: Tyrosine decaging



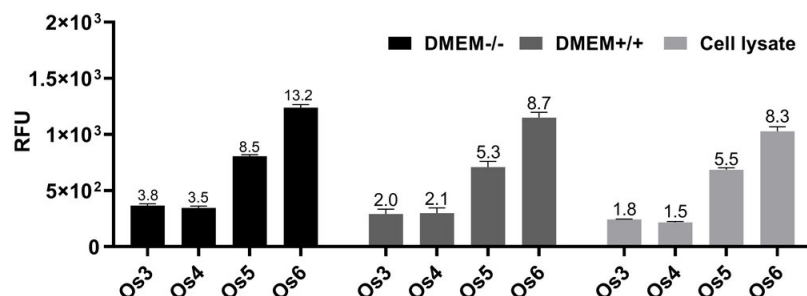
Investigation of reaction pathway



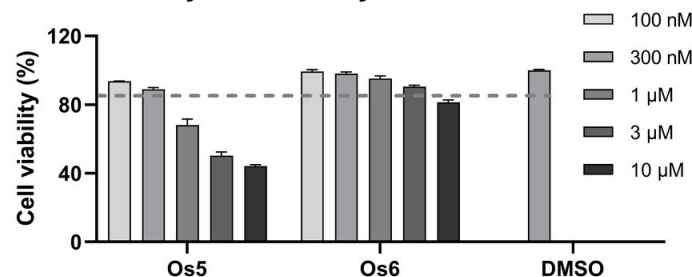
Proposed reaction mechanism



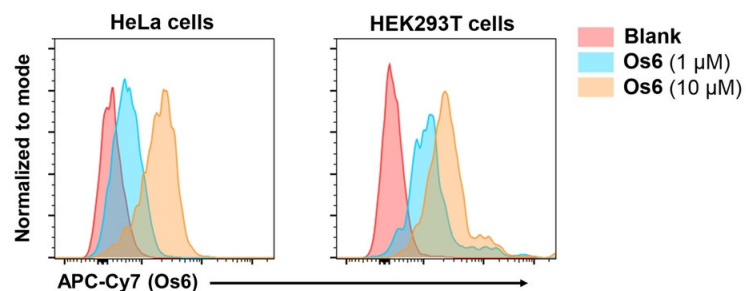
Catalyst optimization



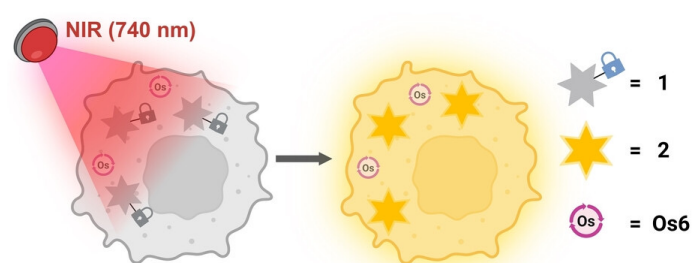
Photocytotoxicity evaluation



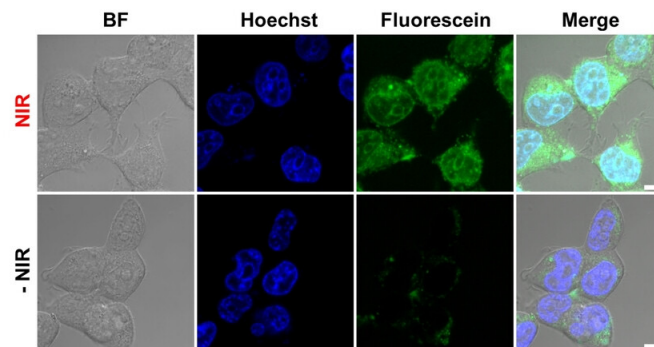
Evaluation of cellular uptake



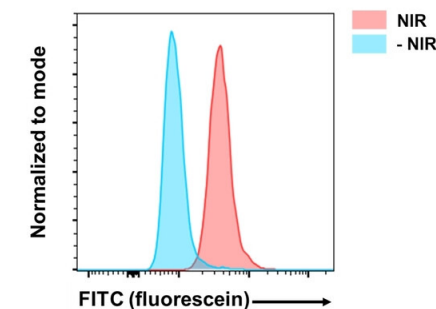
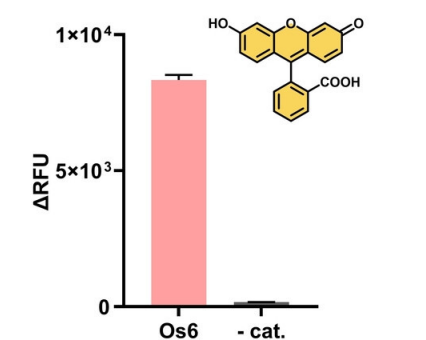
Schematic view of the activation of caged fluoresceine



Confocal microscopic imaging



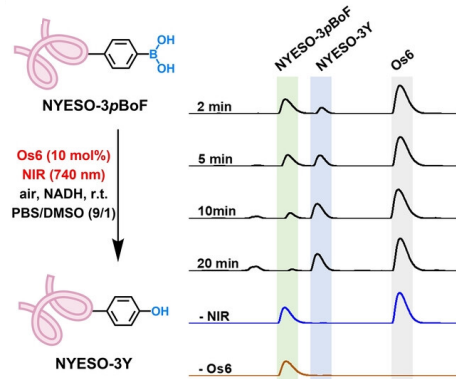
Statistical analysis of Fluorescence rescue



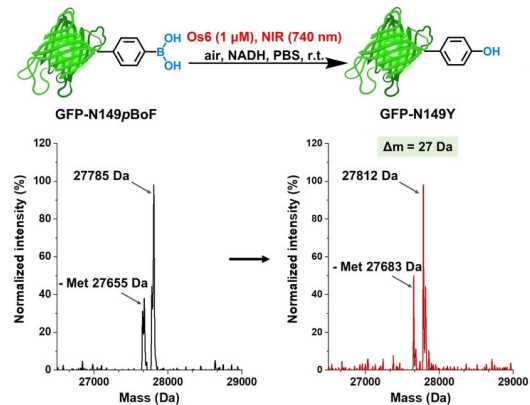
→ Decaging reaction proceeded in living cell

NIR-Triggered Protein Decaging via CAT-NIR System

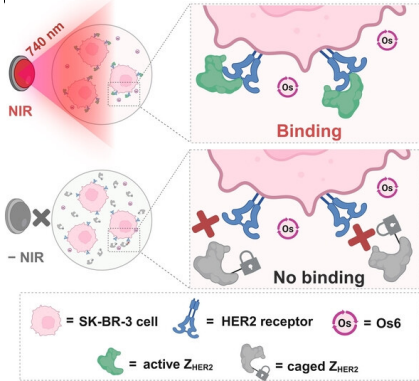
Decaging on model peptide



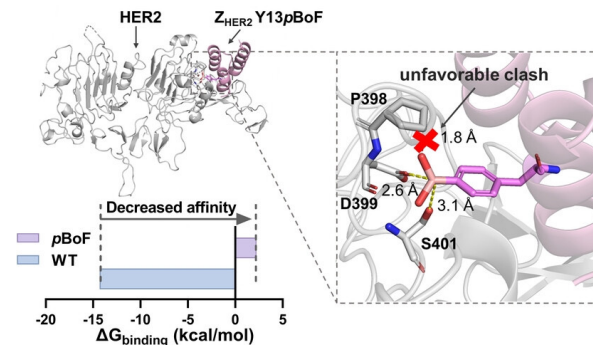
Decaging on model protein



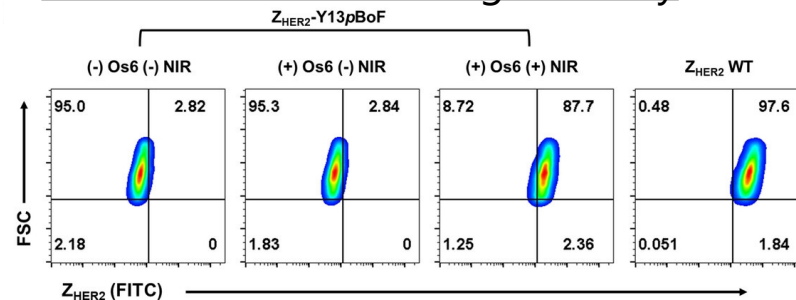
Schematic view of the activation of affibody



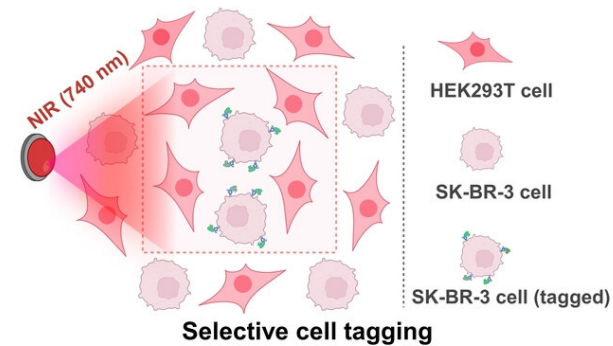
Structural analysis of Z_{HER2}-Y13pBoF



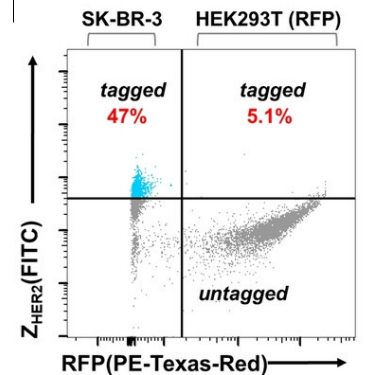
Validation of the binding recovery



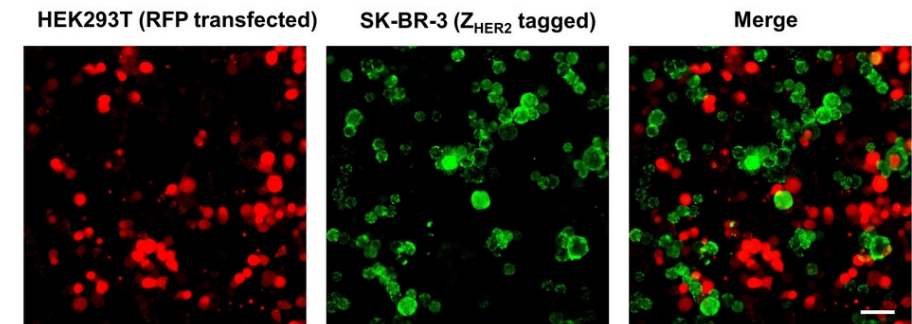
Schematic view of the selective cell tagging



Tagging percentage

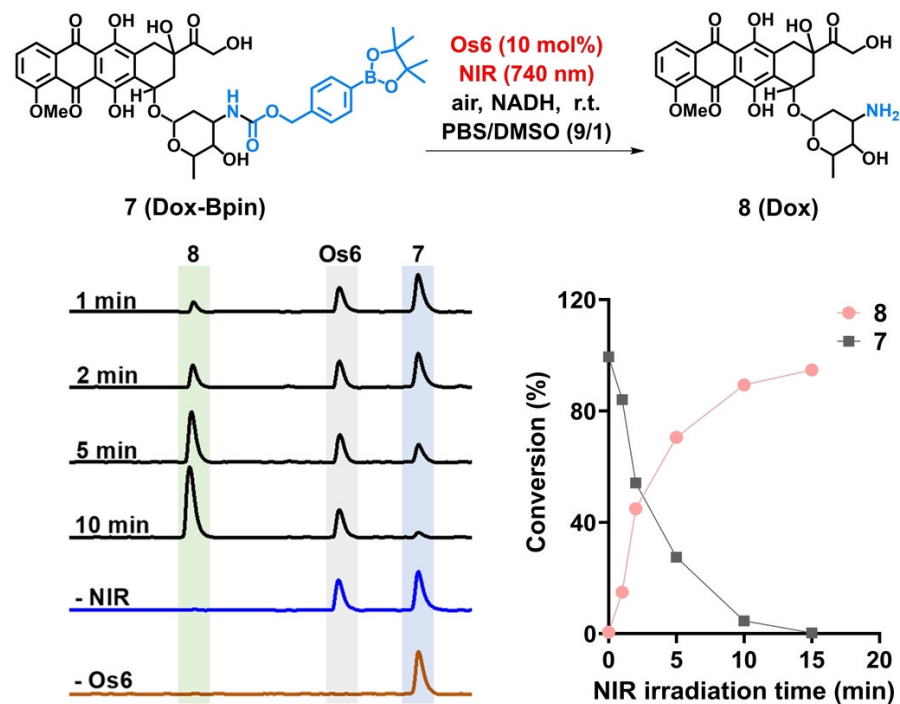


Confocal microscopic imaging

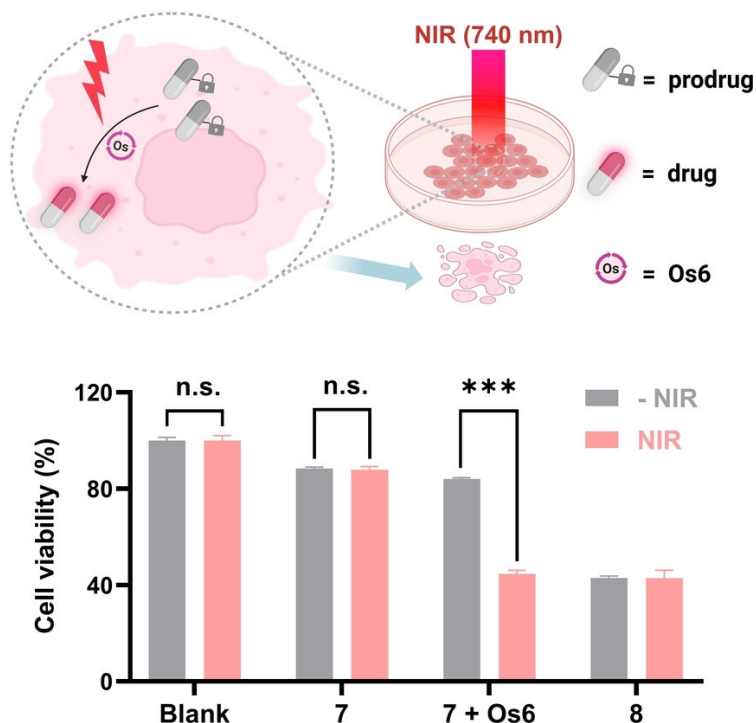


→ Protein decaging was succeeded

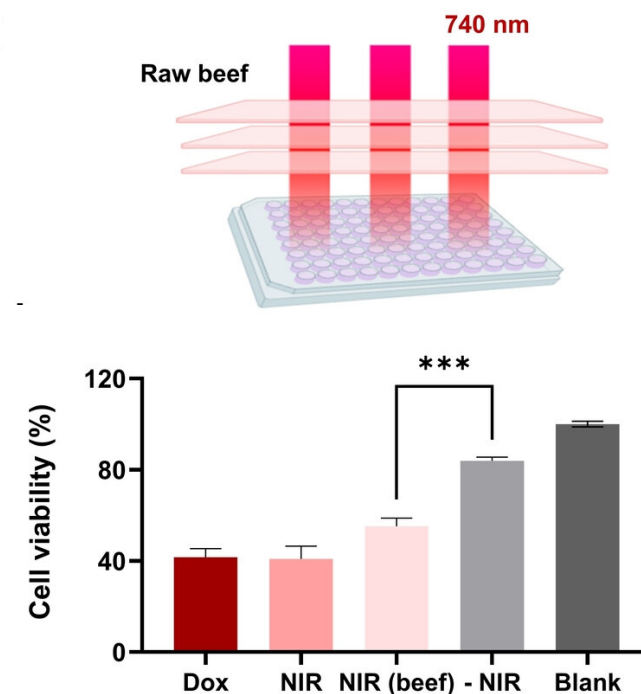
Kinetics of prodrug activation



Prodrug activation in living cell

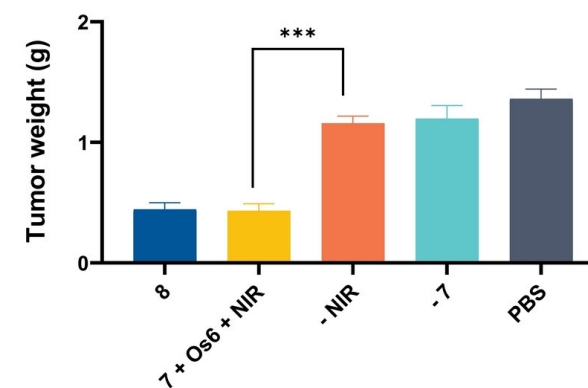
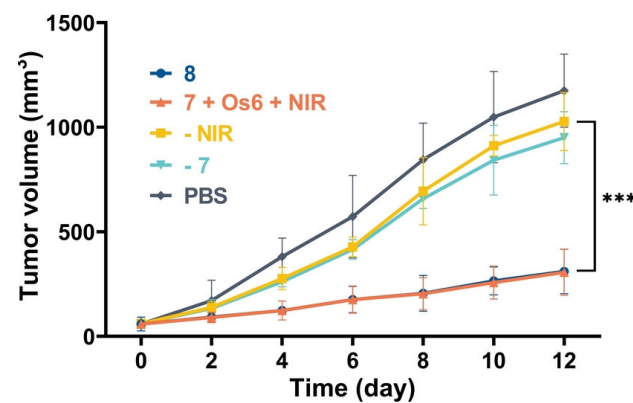
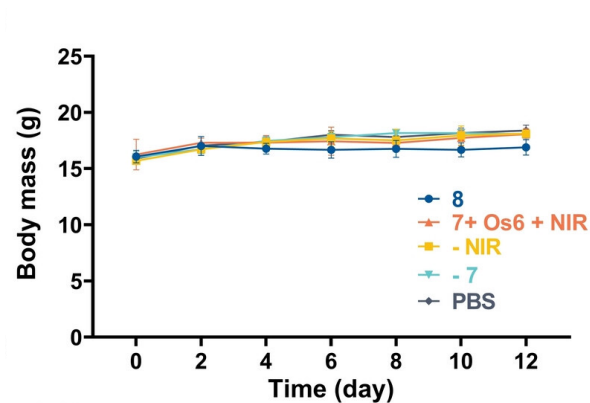
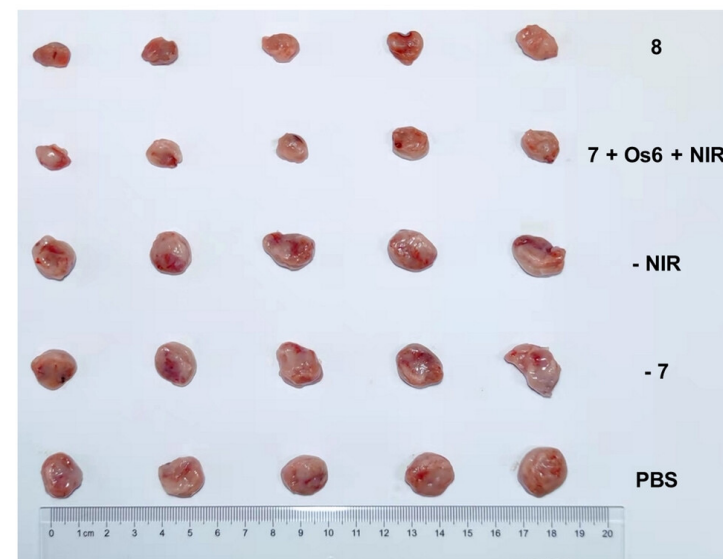
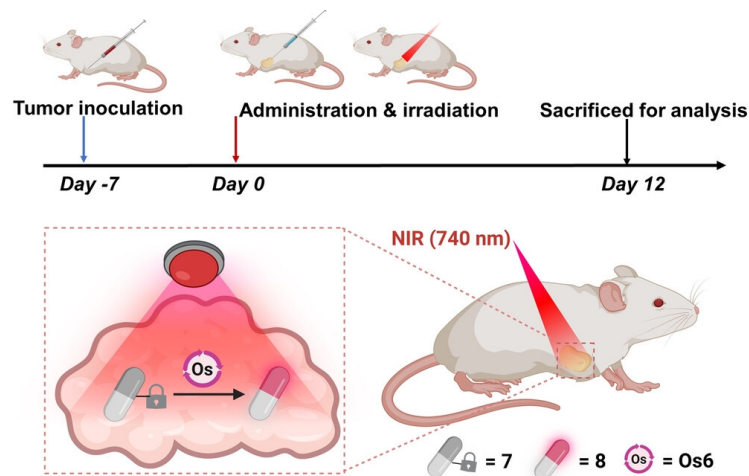


Evaluation of the tissue penetrating ability



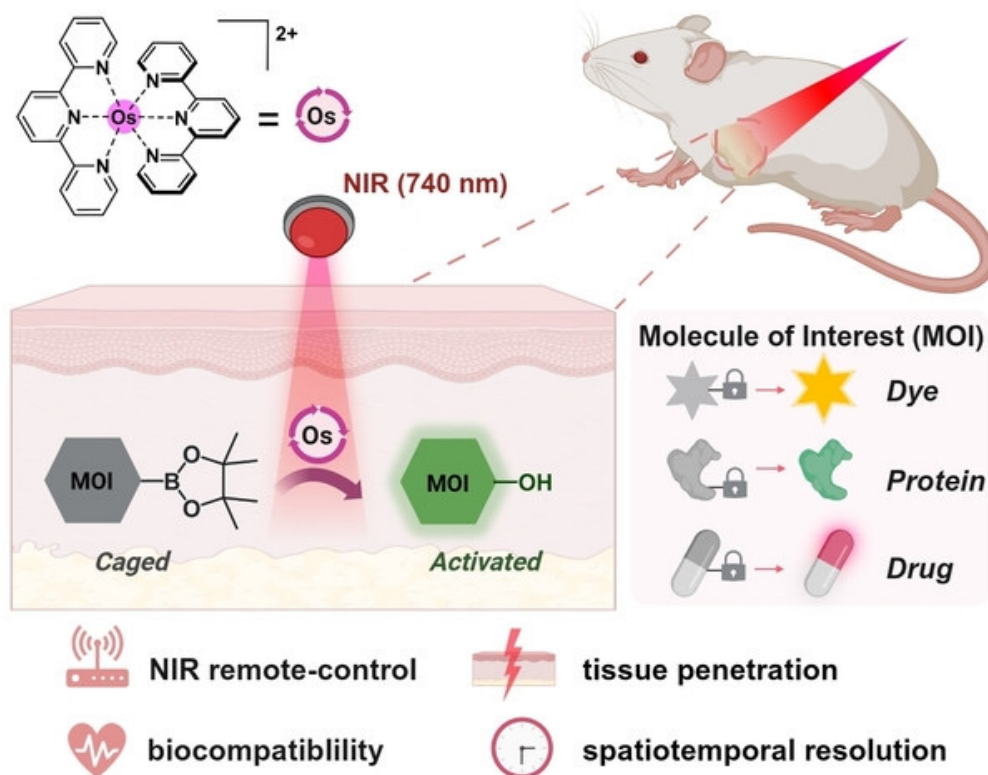
→ Prodrug activation is possible even in deep tissue

Schematic illustration of the remote-controlled prodrug activation in living mice



→ Effective prodrug activation was confirmed in the living mice was confirmed

CAT-NIR: Near Infrared Light Photocatalytic Decaging Chemistry



This time,

- developed a NIR-triggered photocatalytic decaging system
- The concept was proofed in vivo
- In-situ rescue of fluorophores, prodrugs, and biomolecules

In future,

- Protein gain-of-function study
- precise spatiotemporal resolution in manipulating biomolecules,

■ Introduction

- Bioorthogonal (ligation) reaction
- Bioorthogonal bond cleavage reaction

■ Main (state of the art)

- Gold-induced amide bond cleavage
- NIR-triggered photocatalytic decaging

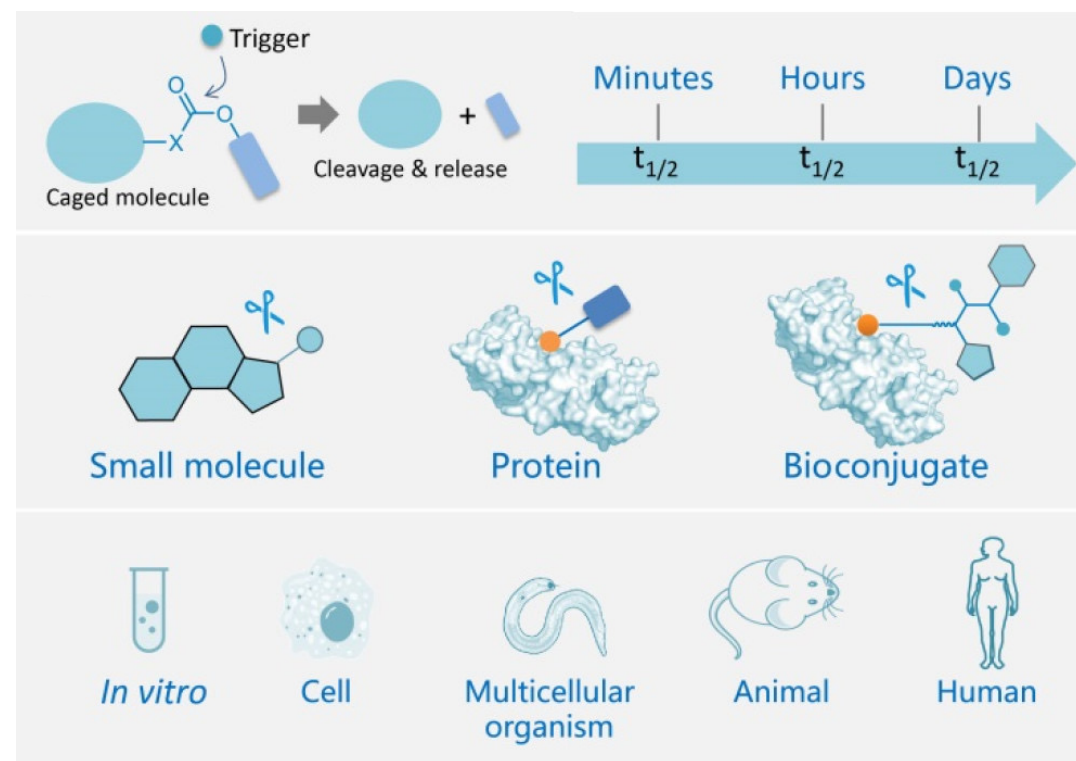
■ Summary

Summary

- Enables molecular functions to be regulated in living cells and animals
- Can be triggered by a variety of stimuli

Perspective

- Expanding reaction type and application
- Spatiotemporal control
- Clinical application



Thank you for your kind attention