Photoredox Trp-Selective Modification.

Literature Seminar

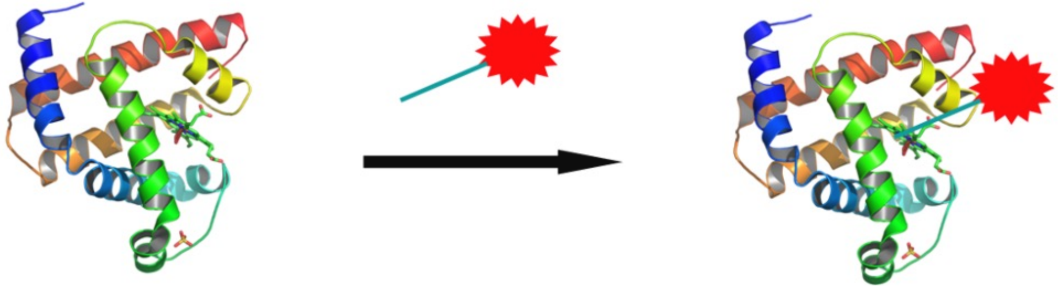
M2 Ryo Kuroda

2023/11/9

Contents

Contents

Importance of Protein Bioconjugation



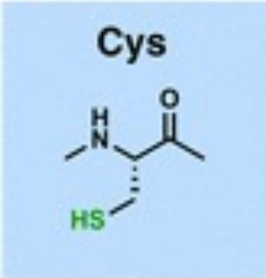
Biomolecules
(Peptides/Proteins)

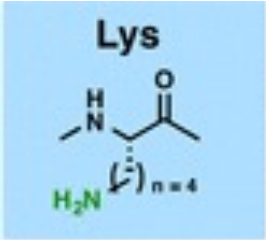
Supernatural Functions

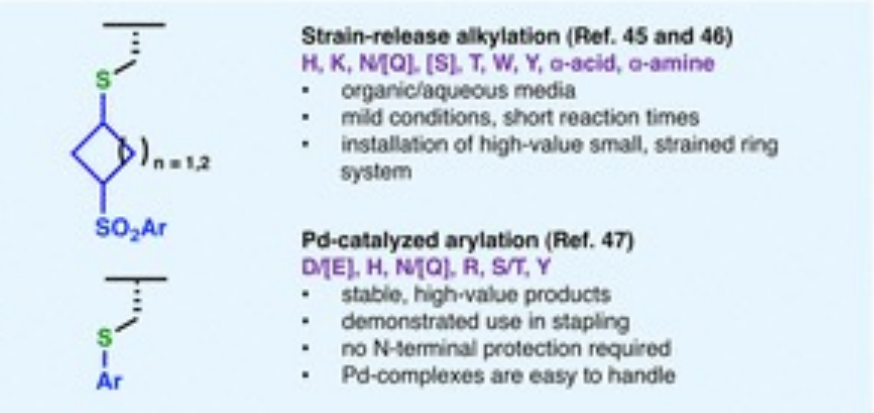
Therapeutics
Diagnostics
Biomaterials

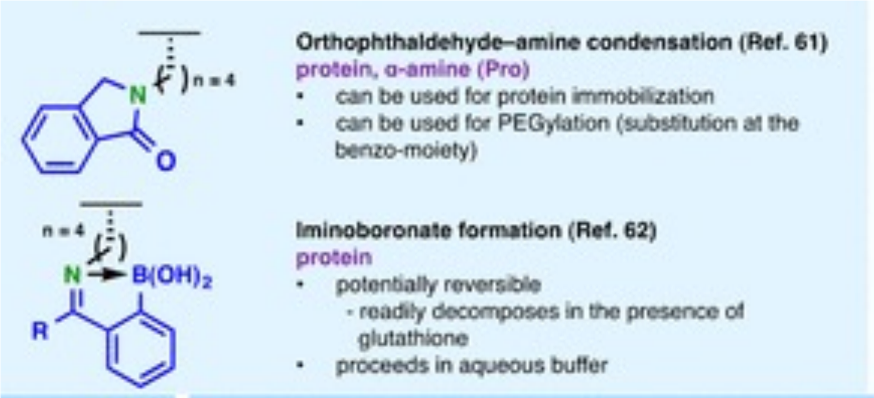
Chiang, CW. *et al. Commun Chem.* **2020**, *3, 171.*

Problem of Traditional Modifications







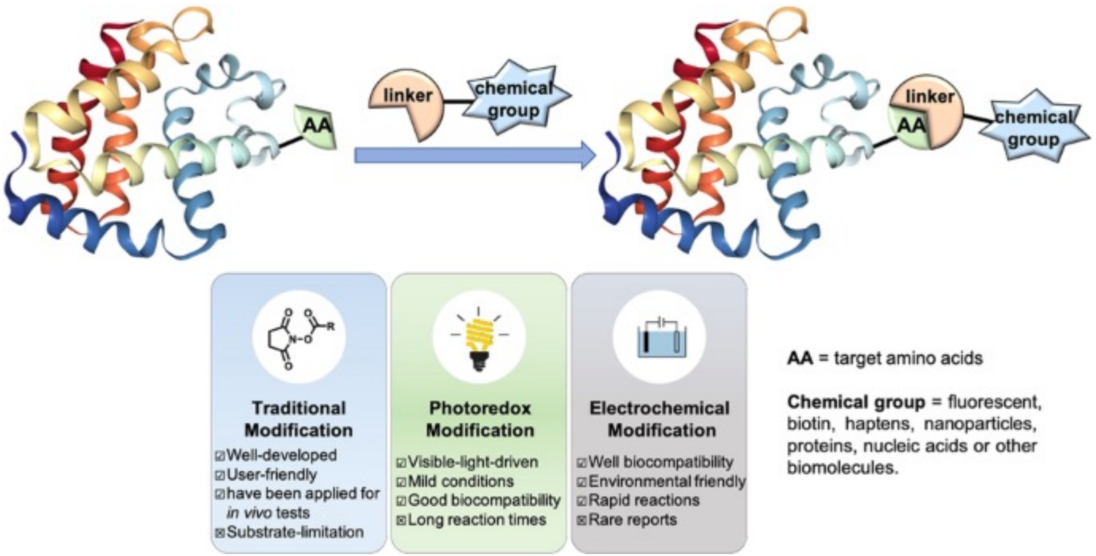


Problem

- Side reactions of Cys and Lys units,
- Limited site- and chemo-selectivity
- Limited functional group tolerance

Chiang, CW. *et al. Commun Chem.* **2020**, *3, 171.*

Advantages of SET

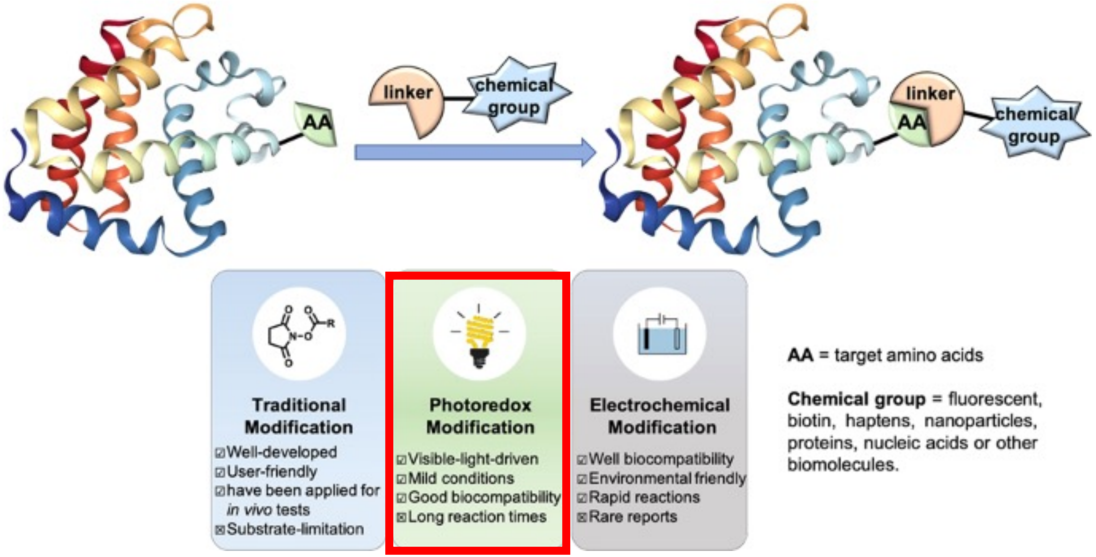


Chiang, CW. *et al. Commun Chem.* **2020**, *3, 171.*

SET

- Less side reactions.
- Mild reaction conditions
- High site-selectivity
- High functional group tolerance

Advantages of Photoredox Bioconjugation



Chiang, CW. *et al. Commun Chem.* **2020**, *3, 171.*

- ✓ **Photoredox bioconjugation**
  - Mild and biocompatible conditions
  - The kinetics of the reaction could be easily controlled.
- ✓ **Visible-light-induced photocatalytic methods**
  - The structures of bioactive molecules are preserved.
  - Quite rare

Advantages of Trp

Advantages of Tyr and Trp

- ✓ C(sp^2)–H functionalization of aromatic compounds has been extensively developed
- ✓ Reactivity can be activated by SET

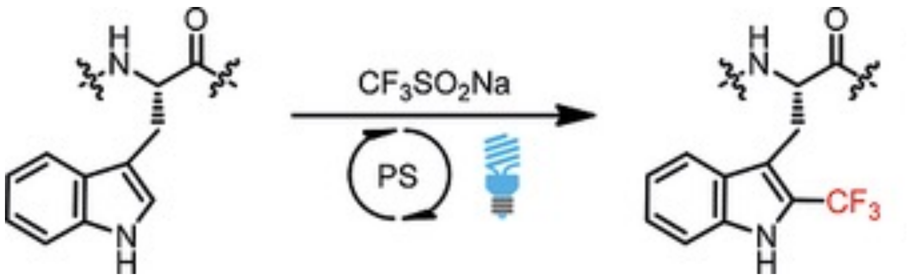
Advantages of Trp

- ✓ The rarest of the amino acids
- ✓ Widely and evenly dispersed in the proteome
- ✓ The most electron-rich π-system of amino acids
  - Electrostatically driven non-covalent
  - H-bonding to neighboring functionality *via* indolic N–H bond.

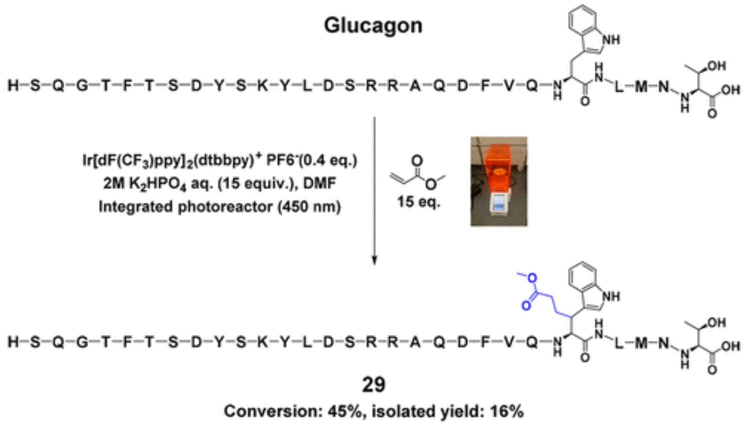
- ✓ Enriched at centers of biochemical significance
- ✓ Maintain protein structural integrity through non-covalent interactions.

Chiang, CW. *et al. Commun Chem.* **2020**, *3, 171.*

Problem of Traditional Photoredox Trp Modifications



Chiang, CW. *et al. Eur. J. Org. Chem.* **2019**, *46, 7596–7605.*



Shi, ZC. *et al. J. Am. Chem. Soc.* **2018**, *140, 6797–6800.*

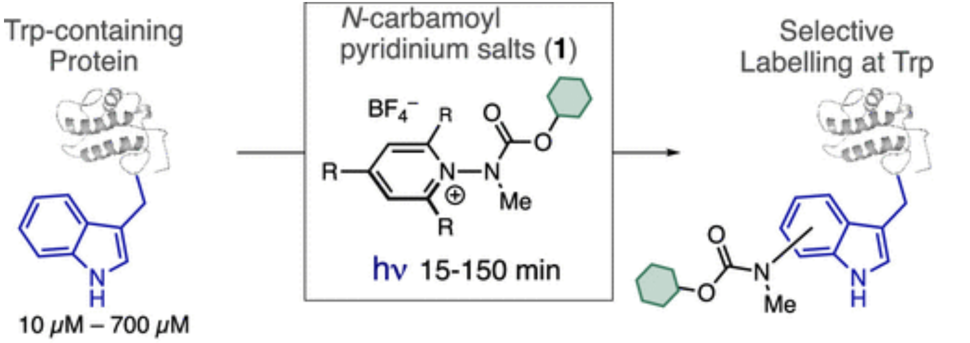
Problem

- ✓ Depends on Trp-selective electrophile generation
  - ● Compete with other biological nucleophiles
- ✓ **Limited biocompatibilities**
  - ✓ Few examples of the use as probes *in situ*

Contents

Trp-Selective Modification via PET

Selective Modification of Tryptophan Residues in Peptides and Proteins Using a Biomimetic Electron Transfer Process



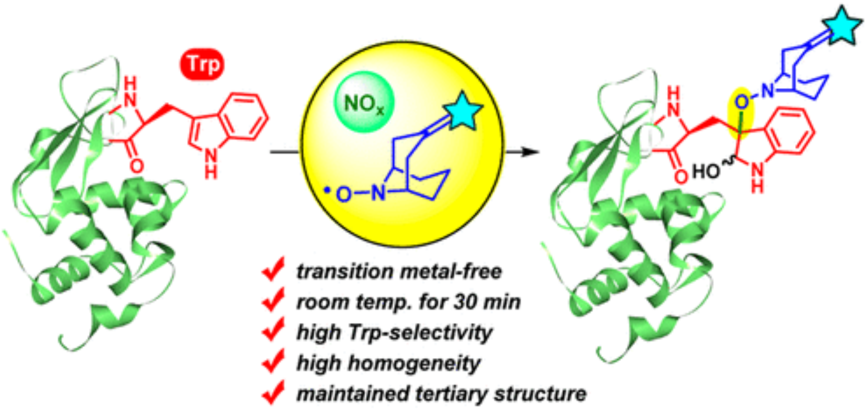
■ Putative mechanism: Photo-induced electron transfer ■ No catalysts or cosolvents

■ Wavelength dependent activation by directly accessing [Trp]* or [1]*

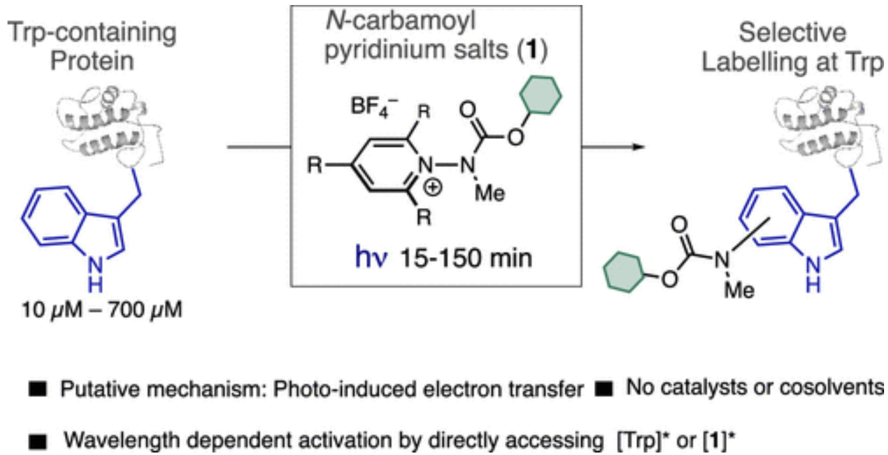
Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

- ✓ PET between Trp and the pyridinium salt
- ✓ Trp-selectivity
- ✓ Pure aqueous conditions
- ✓ *N*-carbamoylpyridinium salt

Problem Presentation



Kanai, M. *et al. J. Am. Chem. Soc.* **2016**, *138, 10798– 10801,*

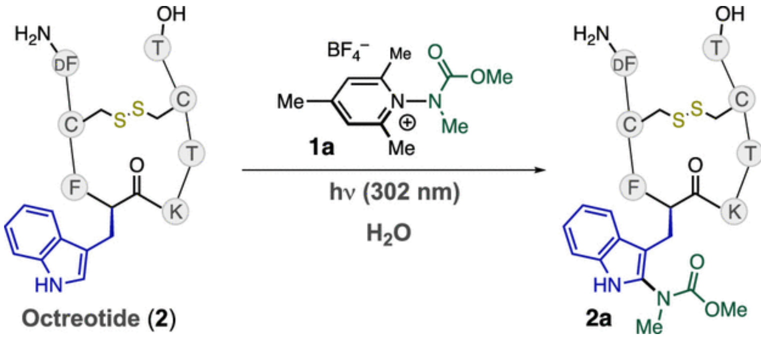


Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

Trp-selective electrophiles
→Compete with other biological nucleophiles

This research :
Trp's inherent photolability

Selected Optimization



Entry	[2] μM	[1] mM	Buffer[a]	Additive	*t* (min)	Conversion of 2[b]	%2a (mono/di)[b]
1	100	10	NH_4OAc		30	>95%	34% (>20:1)
2	100	7	NH_4OAc		30	>95%	27% (>20:1)
3	100	7	NH_4OAc	1 mM GSH	30	>95%	95% (>20:1)
4	10	7	NH_4OAc	1 mM GSH	30	>95%	95% (>20:1)
5	100	7	Na_2HPO_4	1 mM GSH	45	>95%	<95% (3:1)
6	100	7	NaOAc	1 mM GSH	30	>95%	95% (>20:1)
7[c]	100	7	NH_4OAc	1 mM GSH	30	0%	0%

Best

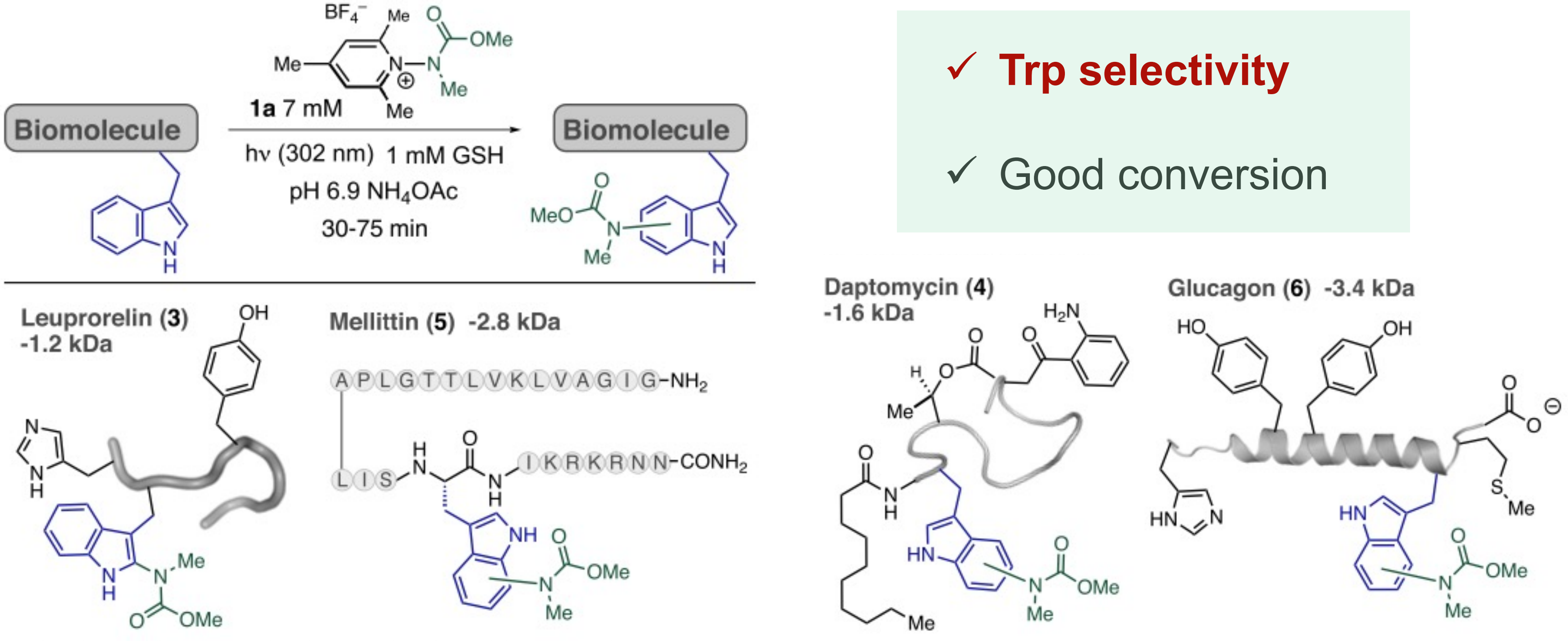
- ✓ GSH acts as a reactive oxygen species scavenger
- ✓ **Trp selectivity**
- ✓ High conversion

1a

- ✓ Water solubility
- ✓ Stability
  - Methylated positions inhibits nucleophilic addition

Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

Substrate Scope



100 μM: **>95% conversion**[a,b]
label:degradation ratio: 14:1
500 μM: 92% conversion[a,c]
73% isolated yield of 3a[d]

70 μM: **85% conversion**[a]
label:oxidation ratio: > 20:1

10 μM: **95% conversion**[a]
mono:di labelling ratio: 9:1

10 μM: **86% conversion**[a,e,f]
label:oxidation ratio: 13:1

- ✓ **Trp selectivity**
- ✓ Good conversion

Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

Mechanistic Studies

(A) Temporal control experiments with 2 and 1a

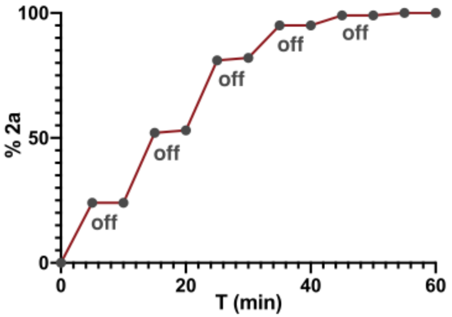
(B) Additive and light-perturbation experiments

- ✓ Spin-trap TEMPO and NaI inhibited the reaction
  - Quencher of Trp fluorescence
- ✓ A large excess of prenyl alcohol has no effect
  - It trap free N-centered radical

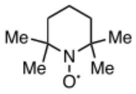
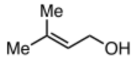
(D) Mechanistic and NMR experiments

- ✓ R,S-Trp (>95% C-4 deuteration (302 nm), 75% C-4 deuteration (311 nm))
- ✓ 2a、3a (Modification site : indole C2)

A

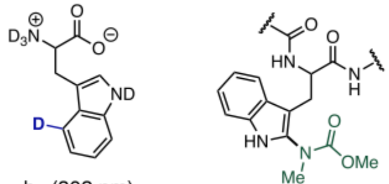


B

Additive	% conversion[a]	% 2a[a]
none	>95%	>95%
	34%	<10%
NaI (7 mM)	<5%	<5%
	>95%	>95%

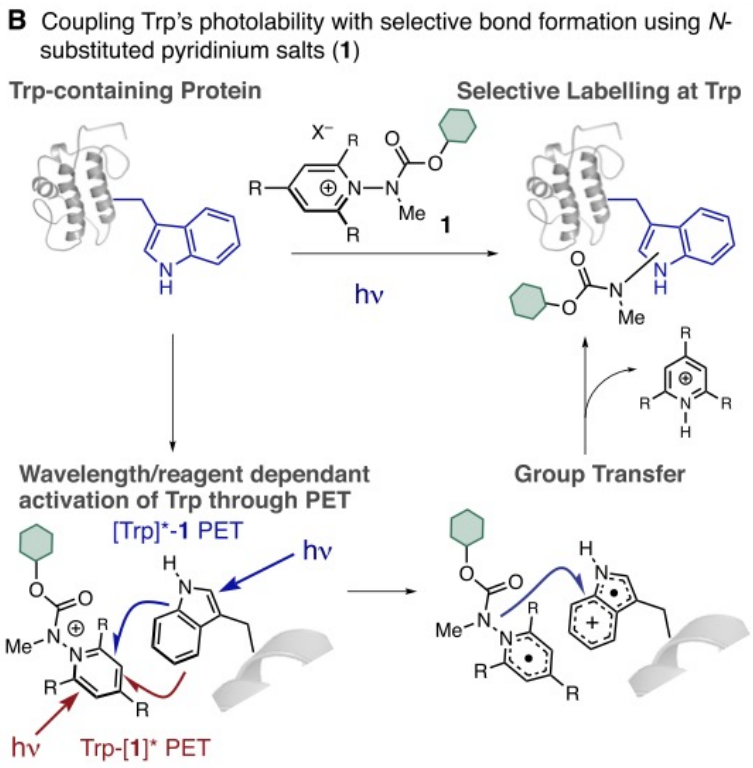
hν condition	% conversion[a]	% 2a[a]
365 nm[c]	0%	0%
305 nm longpass filter:		
30 min	35%	35%
120 min	>95%	>95%

D NMR Characterisation:



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

Mechanistic Studies



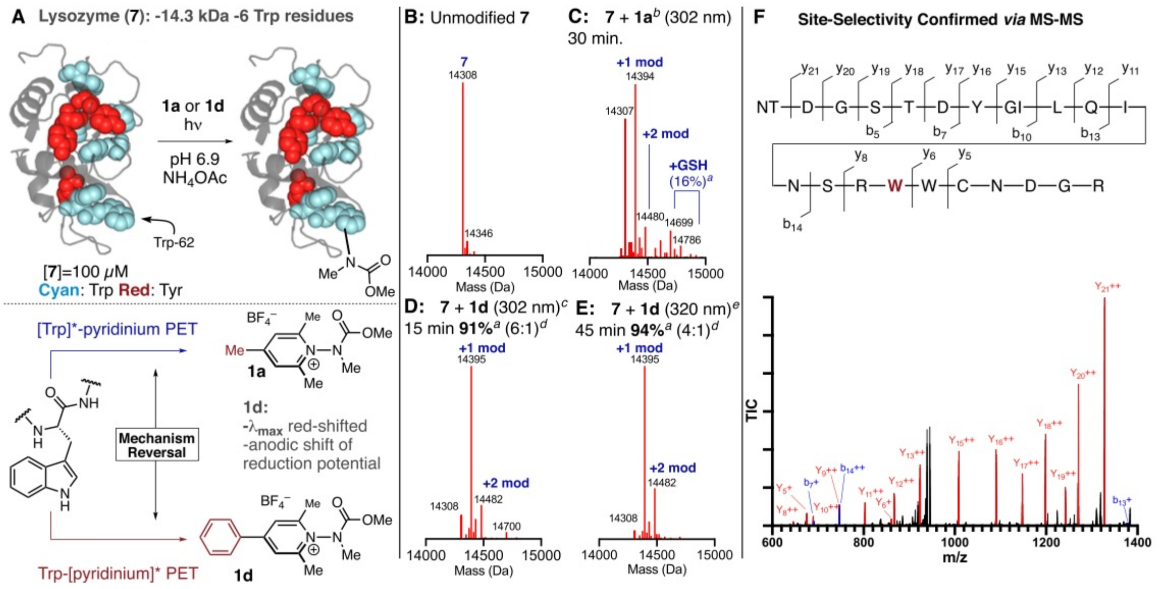
Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142*, 9112–9118.

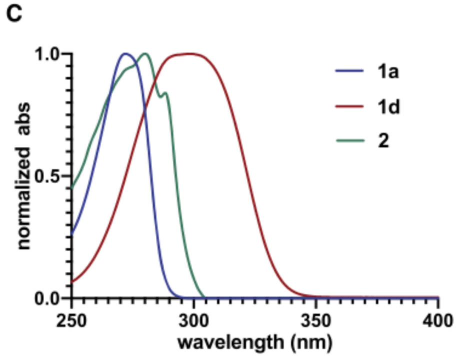
- ✓ Quantum yield of the labeling of 2 is consistent with that of Trp-photionization
  →Electron transfers from [Trp]*
- ✓ **PET advances the reaction**

Possible mechanism

1. [Trp]* activates 1 as a single electron reductant
2. The labile N-N bond of 1 undergoes homolytic cleavage
3. Trp radical and a reactive N-centered radical generates, recombines and modifies Trp selectively.

Modification of lysozyme





Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

Problem of 1a

- ✓ Cys-glutathionylation
  - Reduction of proximal disulfide by [Trp]*
  - Thiyl radical exchange

1d

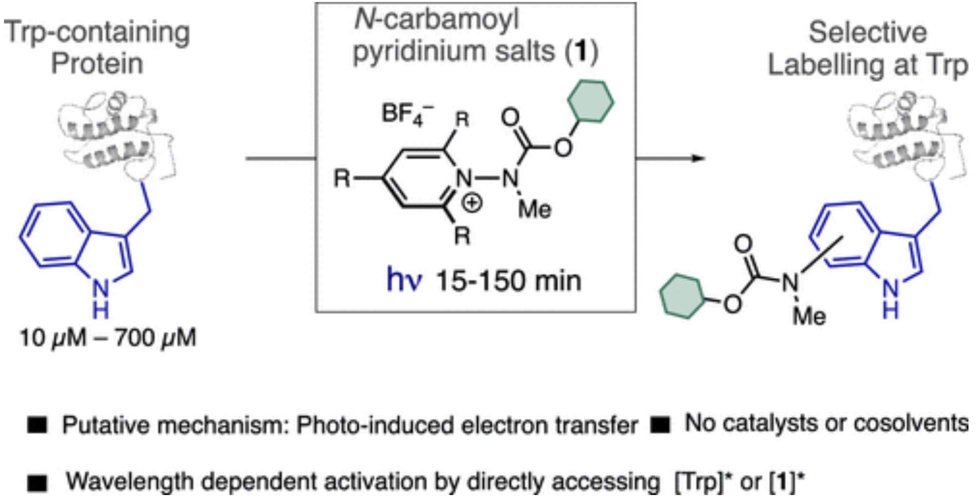
- ✓ Trp→[1]* is the main pathway
- ✓ Minimize degradation due to intraprotein PET and avoid side reactions
- ✓ (C) Irreversible reduction potential shifted anodic compared to 1a

Modification of 1d

- ✓ **No glutathionylation**
- ✓ **Trp-62 selectivity**

Short Summary

Selective Modification of Tryptophan Residues in Peptides and Proteins Using a Biomimetic Electron Transfer Process



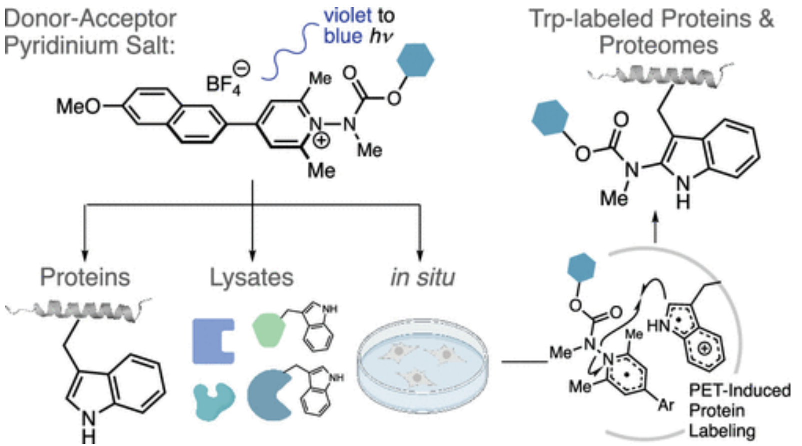
Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

- ✓ PET between Trp and the pyridinium salt
- ✓ Site selectivity for Trp and tolerant to other amino-acid
- ✓ Pure aqueous conditions
- ✓ *N*-carbamoylpyridinium salt

Contents

Trp-Modification via PET Using Visible Light

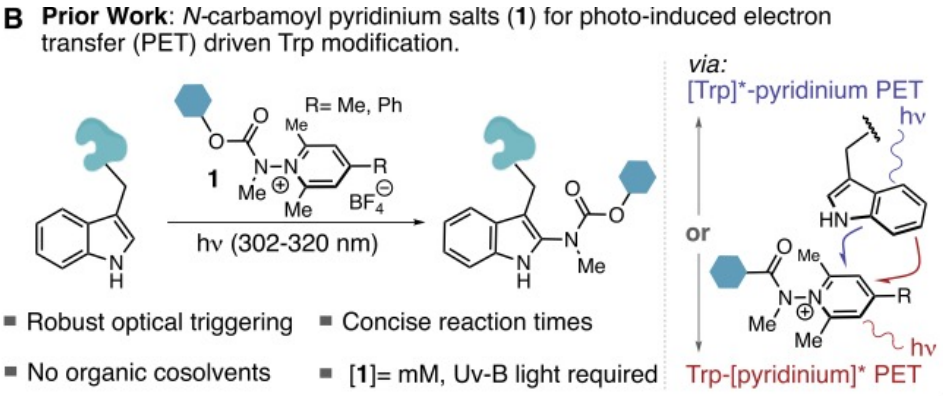
Donor–Acceptor Pyridinium Salts for Photo-Induced Electron-Transfer-Driven Modification of Tryptophan in Peptides, Proteins, and Proteomes Using Visible Light



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2022**, *144, 14, 6227–6236*

- ✓ Trp modification through PET
- ✓ *N*-carbamoyl pyridinium salts have donor–acceptor relationship
- ✓ Surface exposed Trp-selectivity of peptides and proteins.
- ✓ Enrichment from live cell

Problem Presentation



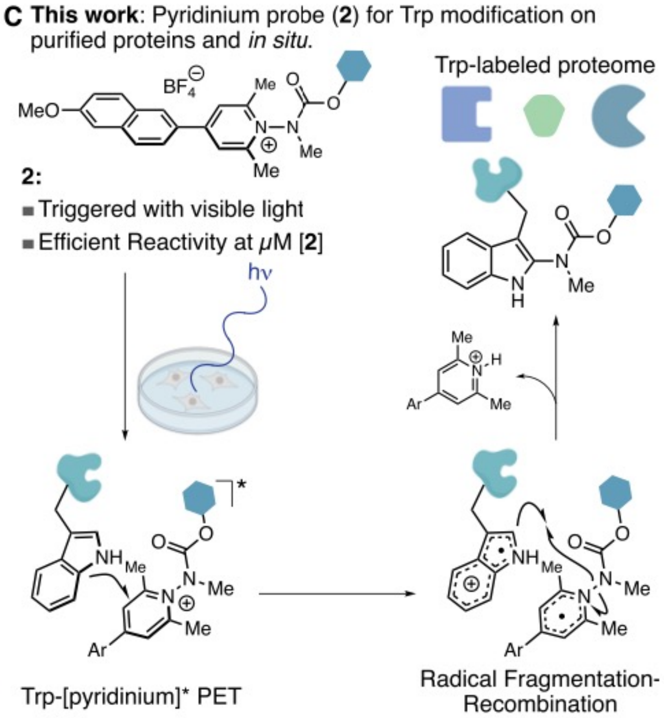
Taylor, M. T. *et al. J. Am. Chem. Soc.* **2020**, *142, 9112–9118.*

N-carbamoylpyridinium salt

Disadvantages

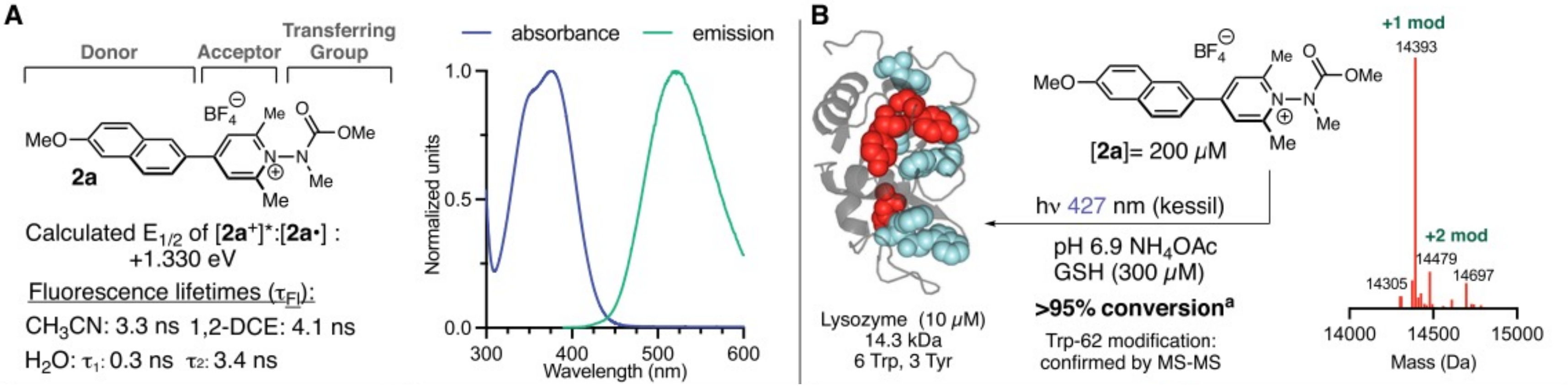
✓ UV-B light induced photodegradation of labile proteins and unintended cellular stress

Proteomic Profiling

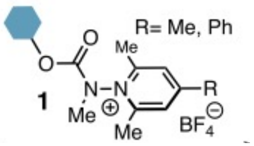


Taylor, M. T. *et al. J. Am. Chem. Soc.* **2022**, *144, 14, 6227–6236*

Catalyst design



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2022**, *144, 14, 6227–6236*



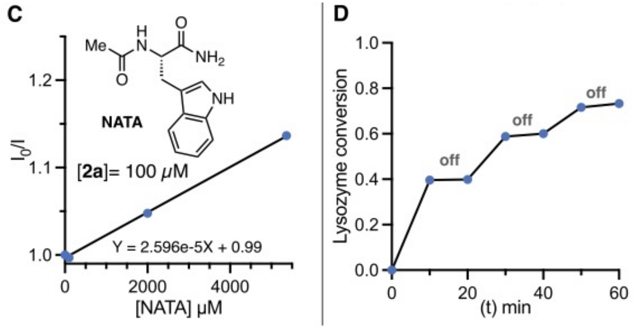
Pyridinium scaffold 2a

- ✓ Low photo-oxidation potential of 2
  - Donor-acceptor relationship
- ✓ **Photoexcitation of 2a with visible spectrum**
  - Absorbance of 2a > 450 nm

(B) Labeling of lysozyme

- ✓ Performance: 2a >> 1
- ✓ Visible light

Study of photophysical properties



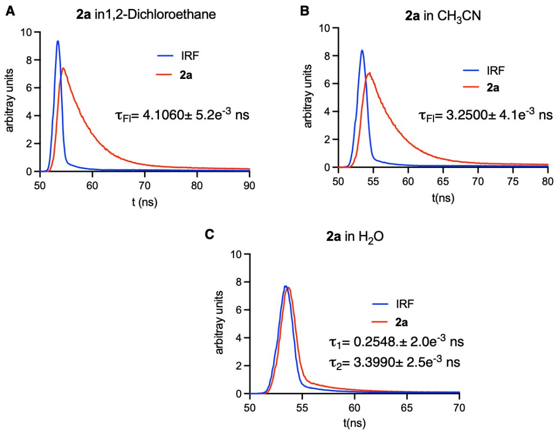


Figure S15. Fluorescence lifetimes of **2a** and **2b**.

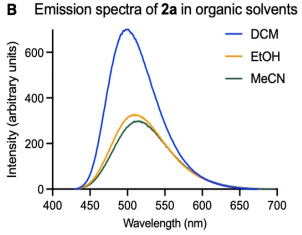


Figure S14. Absorption and emission spectra

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(C) Stern–Volmer plot of fluorescence quenching of 2a with NATA

(D) Temporal control experiments

(S15) Fluorescence lifetimes of 2a

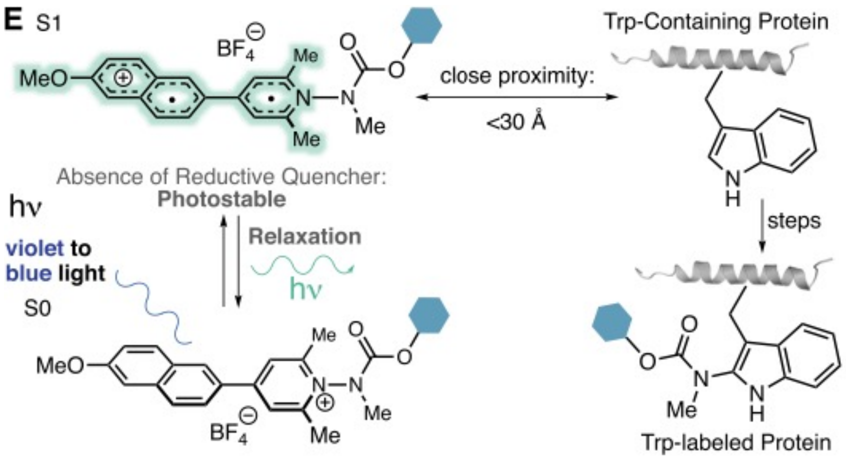
✓ Lifetime decreases as solvent polarity increases

(S14) Emission spectra of 2a

✓ Fluorescence intensity decreases as solvent polarity increases

1. Excited state of 2a is suppressed in aqueous systems.
2. 2a* cannot defuse widely

Study of photophysical properties



(E) Mechanistic considerations

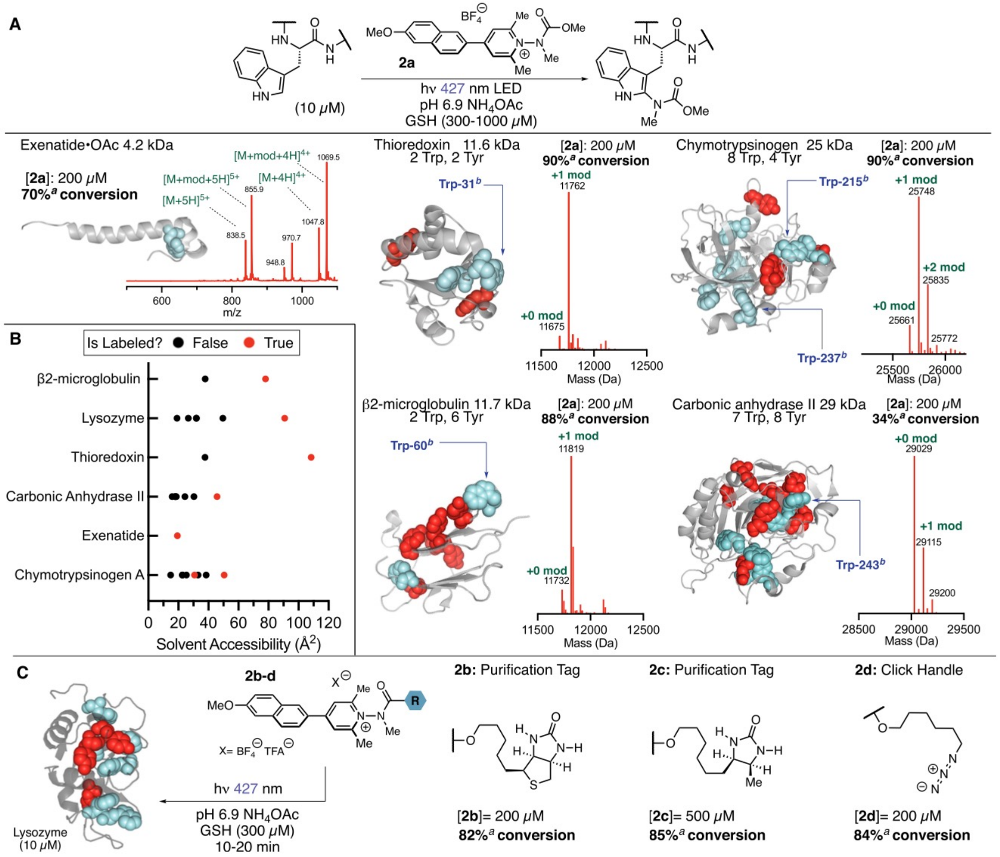
- ✓ Excitation from S0 to S1 is supported by naphthyl → pyridinium charge transfer
- ✓ The redox potential of 2a is low

Reason for Trp selectivity

1. Short-lived excited state
2. Protein-2a pre-complexation via hydrophobic effects
3. Kinetic preference for Trp

Taylor, M. T. *et al. J. Am. Chem. Soc.* **2022**, *144, 14, 6227–6236*

Substrate scope and Transferring Group Scope



(A) Substrate scope

- ✓ **Trp-selectivity**
- ✓ Clean reaction profiles
- ✓ No photodegradation

(B) Solvent accessibilities

- ✓ **Selectivity for the most solvent-accessible residue**

(3C) Transferring group scope

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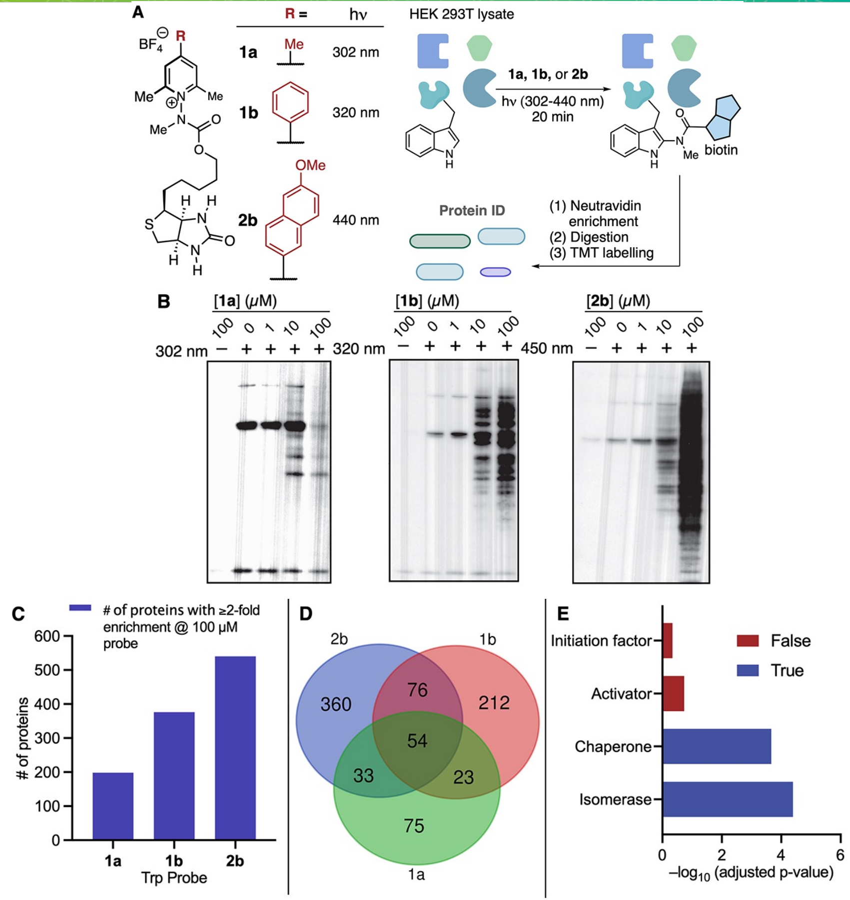
Evaluation of Trp probe designs



(B) Post-enrichment elution profile

(C) Number of proteins identified at 100 μM

(D) Overlap of proteome coverage by each probe

✓ 2b had the most unique enriched proteins

(E) Classes of proteins showing significant enrichment with 2b

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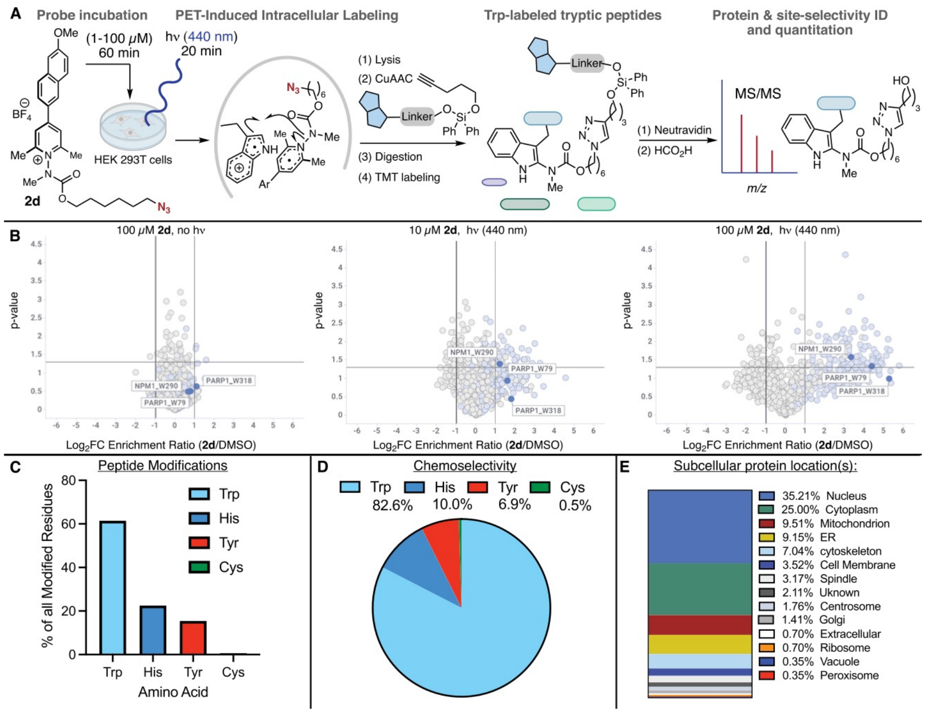
Peptide-level enrichment of Trp in cells



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2022**, *144, 14, 6227–6236*

(B) Volcano plots showing enrichment of the tryptophan-ome

- ✓ Trp-selectivity in situ
- ✓ Ability to enrich the tryptophan-ome directly from cell culture.

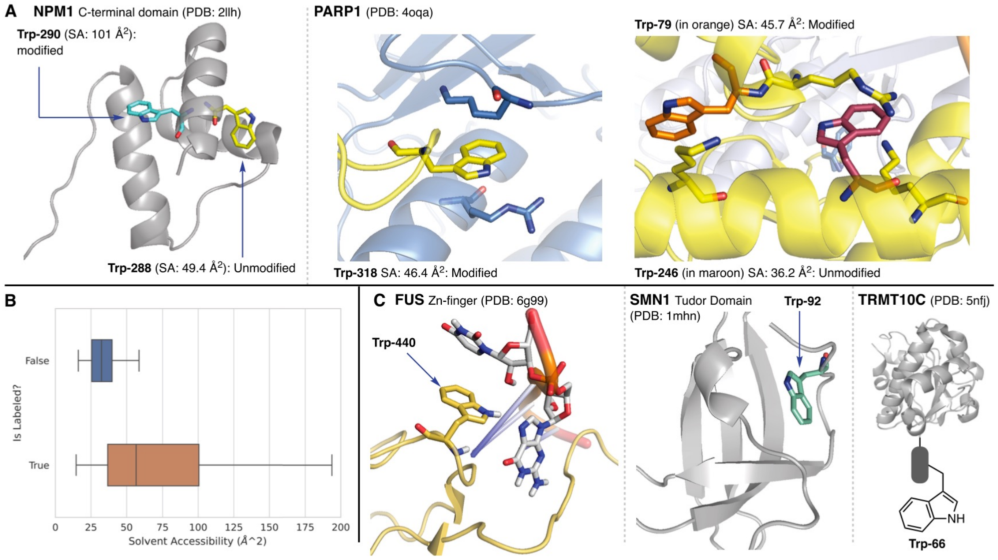
(C) Residue modifications by percentage

(D) Chemo-selectivity based on amino acid relative frequency

(E) Subcellular localization of Trp-modified proteins

- ✓ 2d readily crosses the plasma membrane
- ✓ the net positive charge of 2d doesn`t restrict Trp modification to mitochondrial proteins.

Peptide-level enrichment of Trp in cells



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2022**, *144, 14, 6227–6236*

(A) Modification of functional Trp

- ➢ Trp290 of NPM1 (genomic homeostasis)
- ➢ Trp79 and Trp318 in PARP1 (DNA damage detection and repair)

(B) Comparison of solvent accessibility in modified and unmodified Trp in situ

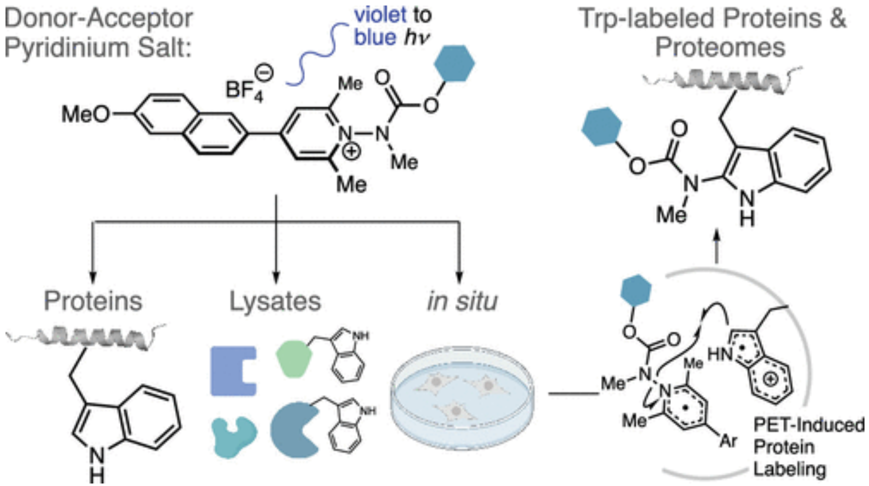
✓ **Selectivity of Trp with enhanced surface exposure**

(C) Modified Trp on proteins associated with disease

- ➢ Trp440 in FUS (neurodegenerative diseases)
- ➢ Trp92 in the Tudor domain of SMN1 (spinal muscular atrophy)
- ➢ Trp66 in the mitochondrial protein TRM10C

Short Summary

Donor–Acceptor Pyridinium Salts for Photo-Induced Electron-Transfer-Driven Modification of Tryptophan in Peptides, Proteins, and Proteomes Using Visible Light



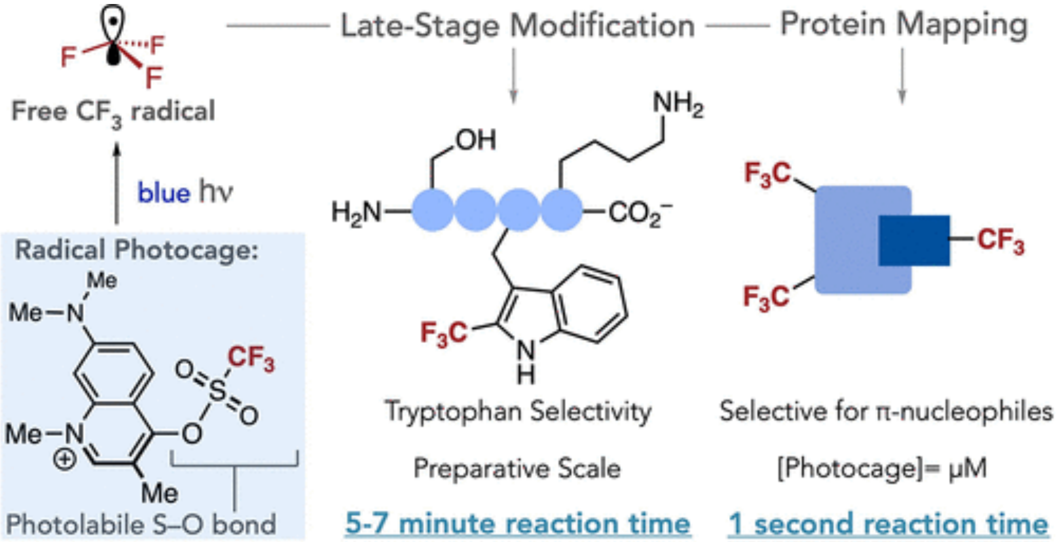
Taylor, M. T. *et al. J. Am. Chem. Soc.* **2022**, *144, 14, 6227–6236*

- ✓ Donor–acceptor pyridinium salt scaffold enables PET-driven Trp modification
- ✓ Good conversions and selectivity
- ✓ The carbamate transferring group can install functional handles to proteins.
- ✓ Enrichment of the tryptophan-ome from both lysates and live cell

Contents

Trifluoromethylation Using Radical Photocges

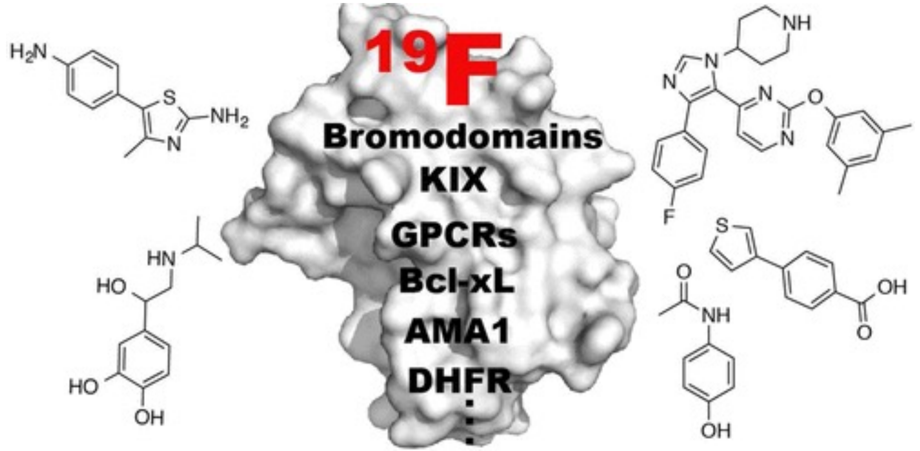
Rapid Biomolecular Trifluoromethylation Using Cationic Aromatic Sulfonate Esters as Visible-Light-Triggered Radical Photocages



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2023**, *145, 42, 22878–22884*

- ✓ Quinolinium sulfonate ester achieves decaging
- ✓ Protein and protein-interaction mapping
- ✓ Scalable peptide trifluoromethylation

Importance of Incorporation of CF3



Incorporation of CF3 into proteins

✓ Precision alteration of physiochemical properties with minimal steric change
- Hydrophobicity
- Small size
- Strong electron-withdrawing

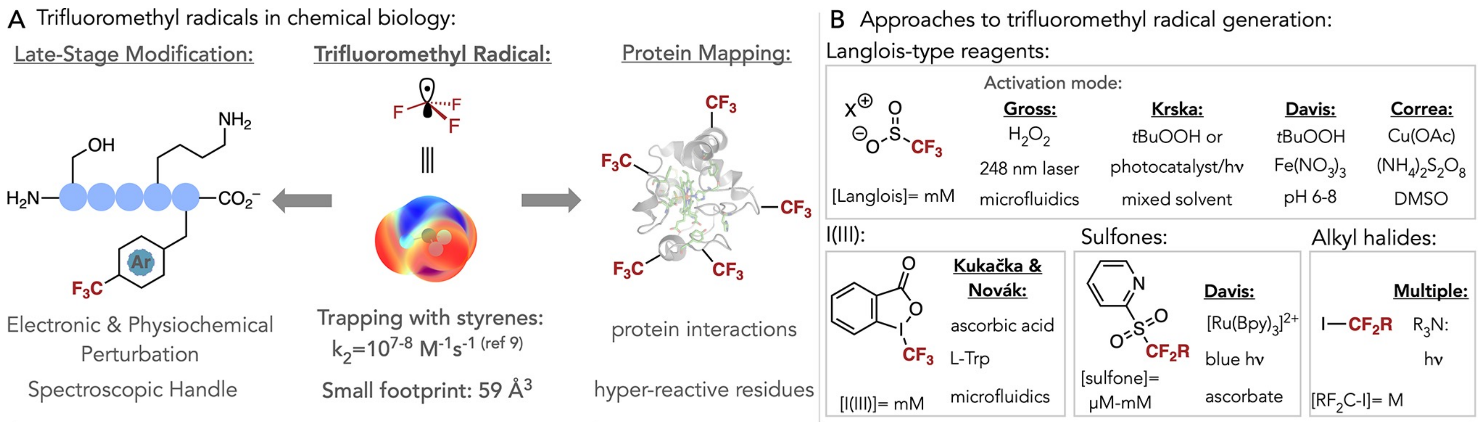
✓ ^{19}F-labeled amino acids accelerate drug design

^{19}F-NMR
- High sensitivity
- Extreme responsiveness to the local environment
- Broad chemical shift range

Problem
Few approaches compatible with biomolecular structures

W. C. K Pomerantz. *et al. J. Med. Chem*, **2016**, *59*, *5158–5171.*

Background research



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2023**, *145, 42, 22878–22884*

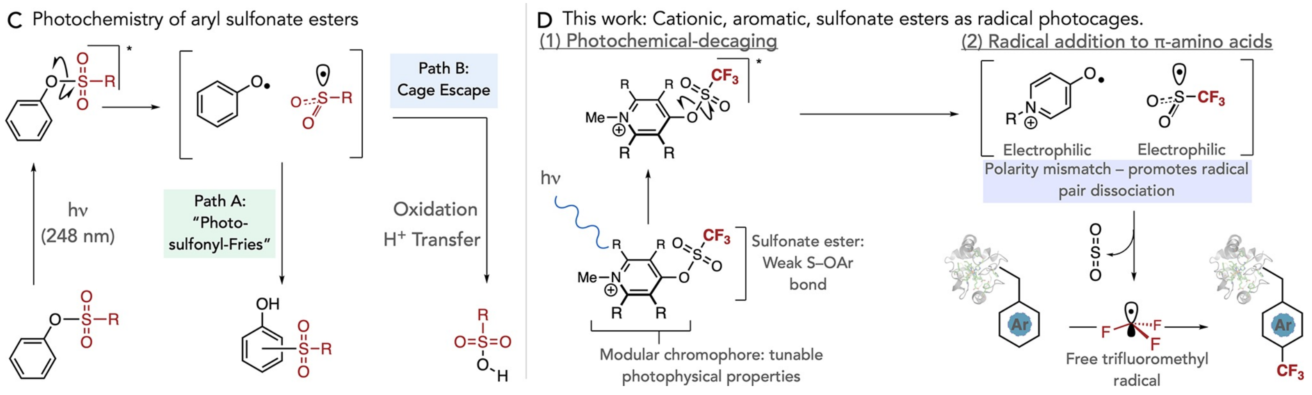
(B) Example of trifluoromethylation of biomolecules by radicals

- ✓ Langlois reagent
- ✓ Sulfonylpyridine photocatalyst
- ✓ Hypervalent iodine reagents
- ✓ Perfluoroalkyl iodide

(A) Advantages of $CF_3\cdot$

1. Electrophilicity
2. Small

This research



Taylor, M. T. *et al. J. Am. Chem. Soc.* **2023**, *145, 42, 22878–22884*

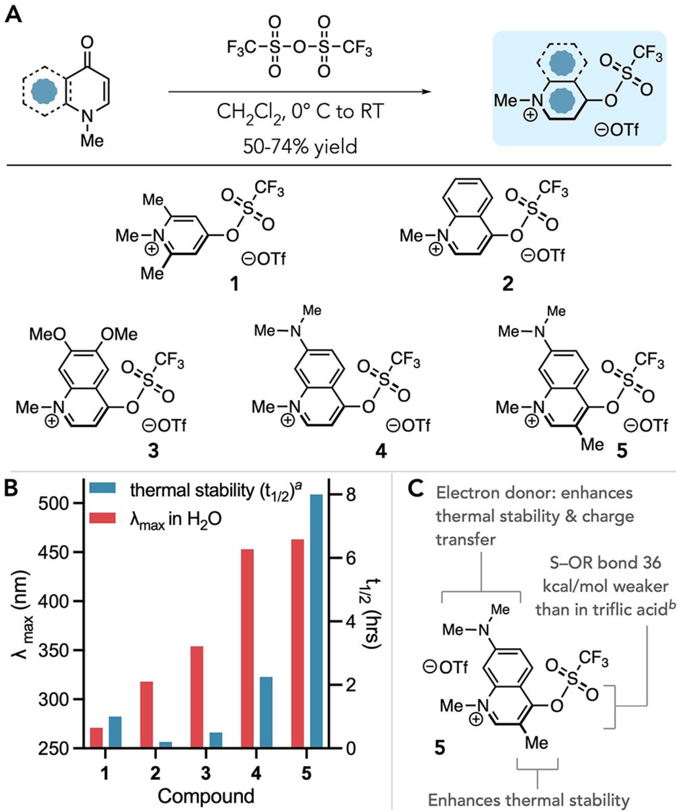
(C) Photochemistry of aromatic, sulfonate esters

✓ They have a weak S-OAr bond that cleaves homolytically upon photoexcitation to generate sulfonyl and phenoxy radicals.
→ They can be used as photocages

(D) Proposed mechanism

1. Cationic, aromatic chromophore further weakens the photolabile S-OAr bond and promotes the dissociation of radical pairs by photolysis.
2. This liberates free CF_3 radicals.
3. Then, this radical captures peptides and proteins.

Selected Optimization



(2A) Synthesis of photocages 1–5

(2B) Evaluation of thermal stability and absorption properties

➢ **5**

- **Significantly improved half-life**
- **Absorbance shifted into the visible spectrum**

(2C) Structural properties of 5

- S-OAr bond is weak enough to be cleaved by photoexcitation

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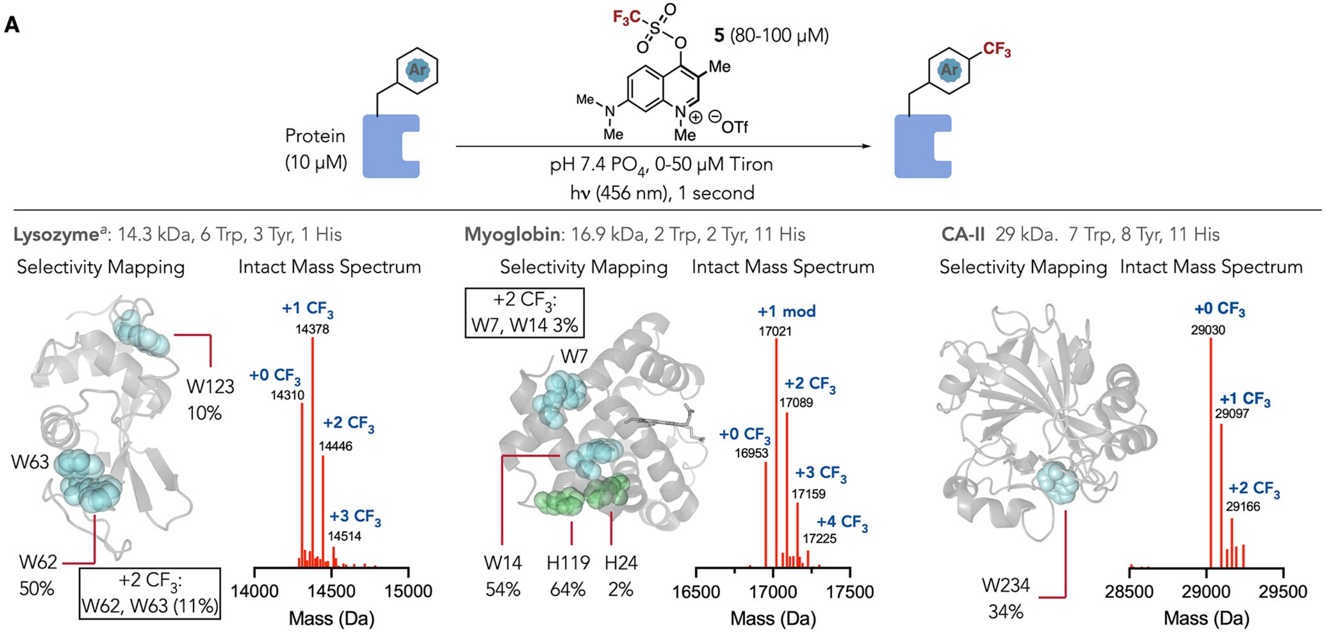
Protein footprinting



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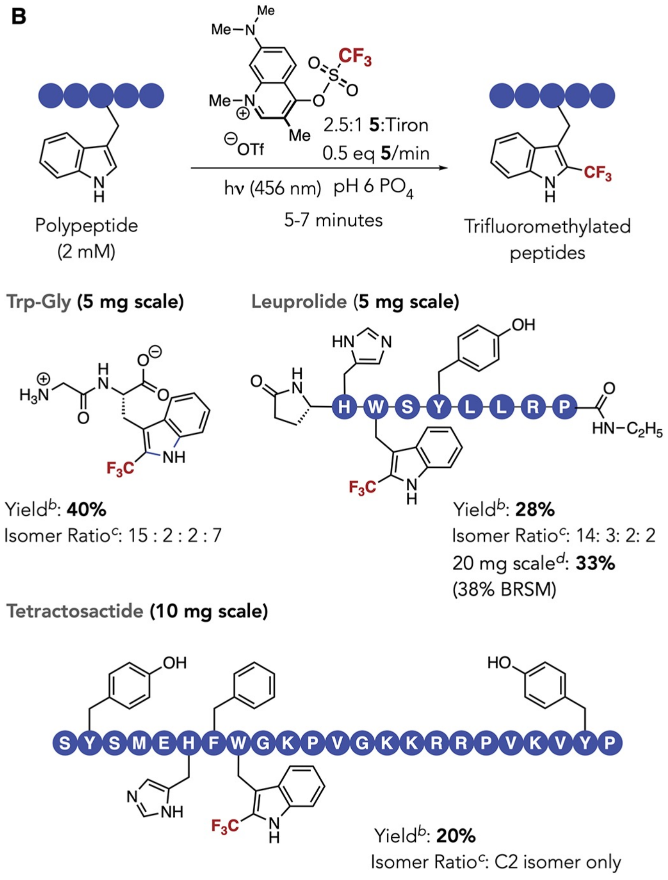
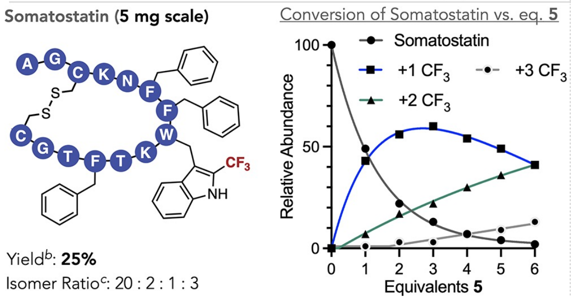
✓ **Chemoselectivity toward nucleophilic aromatic residues**

Preparative scale synthesis





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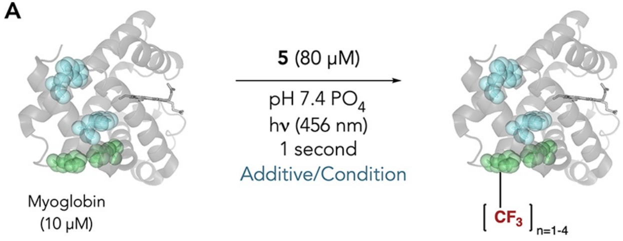
(B) Preparative scale synthesis

- **Somatostatin**
  - +1 CF3 reached maximum after addition of 3 eq of 5
  - Total isolation yield : 25%
  - Regioisomer (main : C2, minor : C4, C7)
- **Gly-Trp, leuprolide, Tetracosactide**
  - 20-40%, The main modification site: Trp

✓ **Applicable to larger scales**

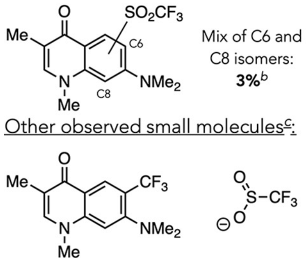
Additive and condition studies

A



Entry	Additive/Condition	% conversion[a]
1	none	**82%**
2	no hν	**0%**
3	10 µM **5**	**22%**
Additive addition *before* irradiation:		
4	TEMPO (500 µM)	**0%**
Additive addition *after* 1 second of irradiation:		
5	TEMPO (500 µM)	**78%**

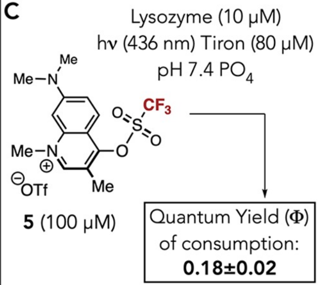
B Recombination products:



Mix of C6 and C8 isomers: **3%**[b]

Other observed small molecules[c]:

C



(A, entry 3) A significant conversion was obtained with 1s-irradiation.
(A, entry 4) TEMPO inhibited modification.
(A, entry 5) Addition of TEMPO after 1 s-irradiation didn`t reduce conversion. The reaction time is 1 s.

(B) Byproducts included a radical-pair recombination product.

(C) Quantification of light

(D) 5 decaged to label myoglobin at biological pH.

D

i. Additive/pertubation prior to irradiation

Entry	**[5]**	**Time hν**	**Additive/perturbation**	**[Additive]**	**Conversion (%)**
1	80 µM	1 s	---	0 µM	82
2	80 µM	1 s	TEMPO	500 µM	0
3	80 µM	1 s	vial shielded from hν	0 µM	0
4	10 µM	1 s	---	0 µM	22
5	80 µM	1 s	pH 5 PO_4	0 µM	71
6	80 µM	1 s	pH 6 PO_4	0 µM	70
7	80 µM	1 s	pH 8 PO_4	0 µM	71

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Possible mechanism

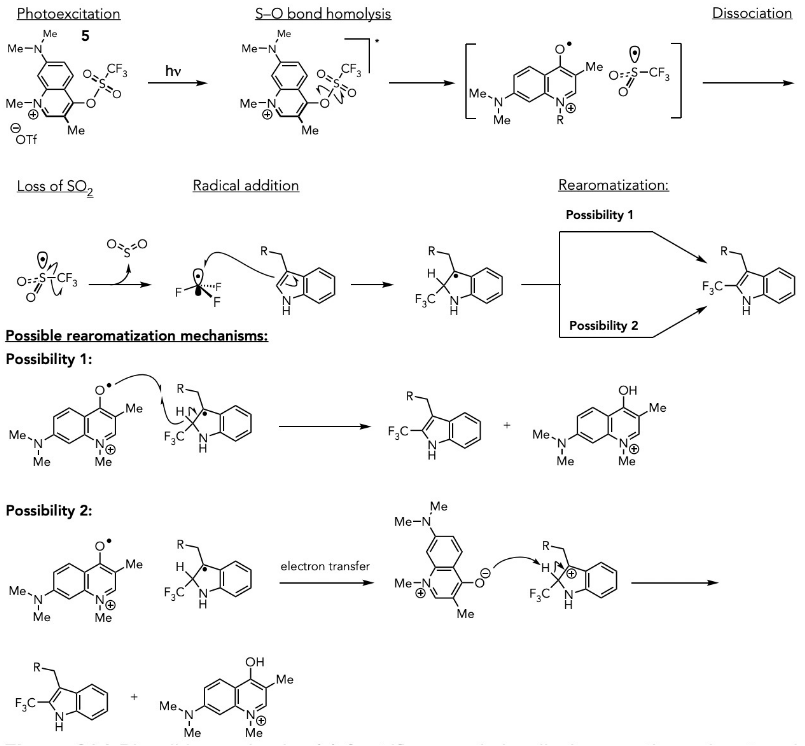
Possible mechanism

1. Optically induced photolysis of labile S-OAr bonds forming quinoloxy-sulfonyl radical pairs.
2. Thermal extrusion of SO2 and radical alkylation of π-nucleophiles with free trifluoromethyl radicals.

Evidence supporting a radical mechanism

(1) Regioisomers of trifluoromethylated Trp-conjugates
(2) TEMPO inhibited modification.
(3) Byproducts included a radical-pair recombination product.

Plausible Mechanism for photo-induced trifluoromethylation with 5:



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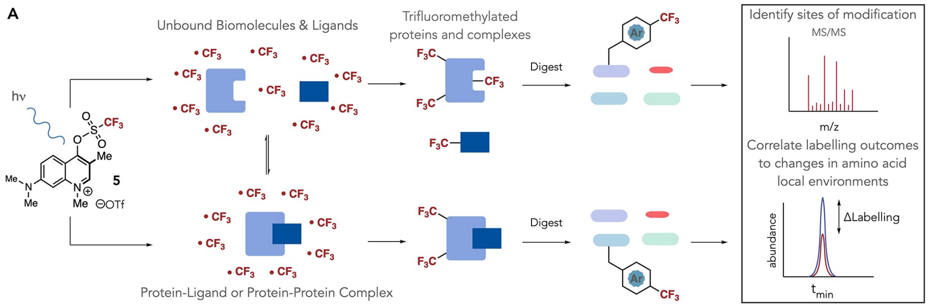
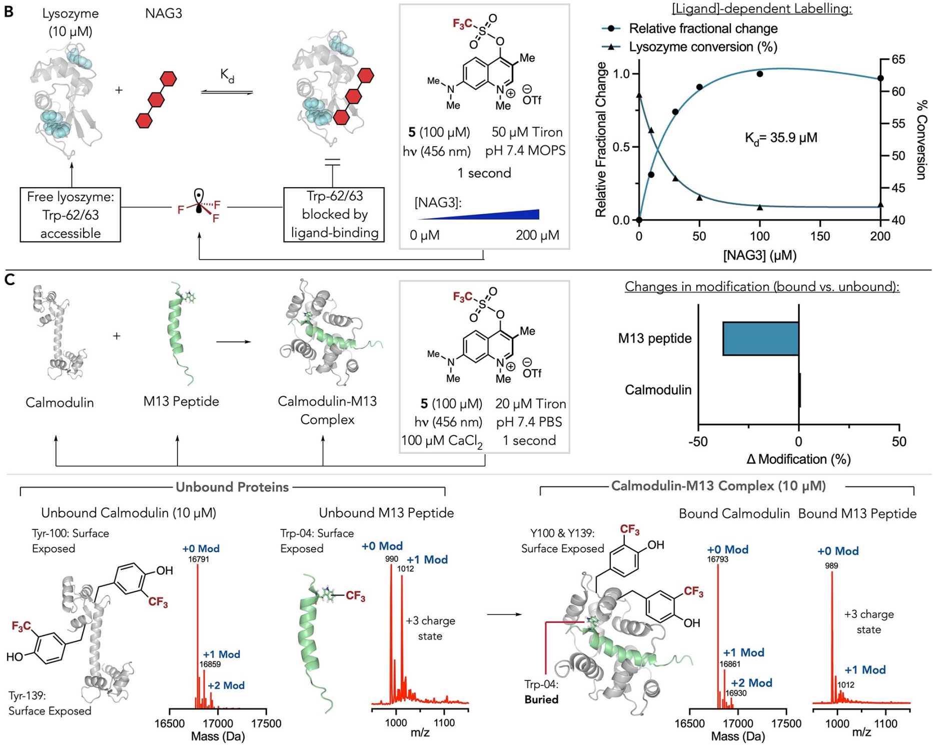
Mapping of protein interactions





(B) Mapping of the interaction between lysozyme and NAG3

- The binding affinity was consistent with previously reported values .

(C) Mapping of the calmodulin–M13 complex

✓ : Calmodulin–M13 complex hides M13-W4

- **Unbound Proteins**
  - Strong labeling with only M13-W4
- **Calmodulin–M13 complex**
  - M13-W4 labeling was dramatically reduced.

✓ Protein mapping using 5 allows investigation of environmental changes.

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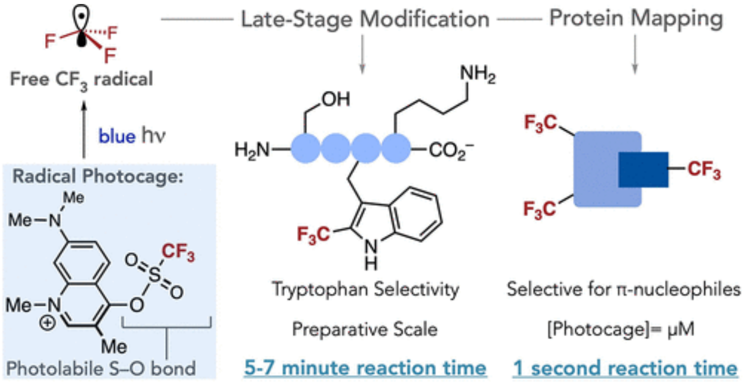
Short summary

Rapid Biomolecular Trifluoromethylation Using Cationic Aromatic Sulfonate Esters as Visible-Light-Triggered Radical Photocages



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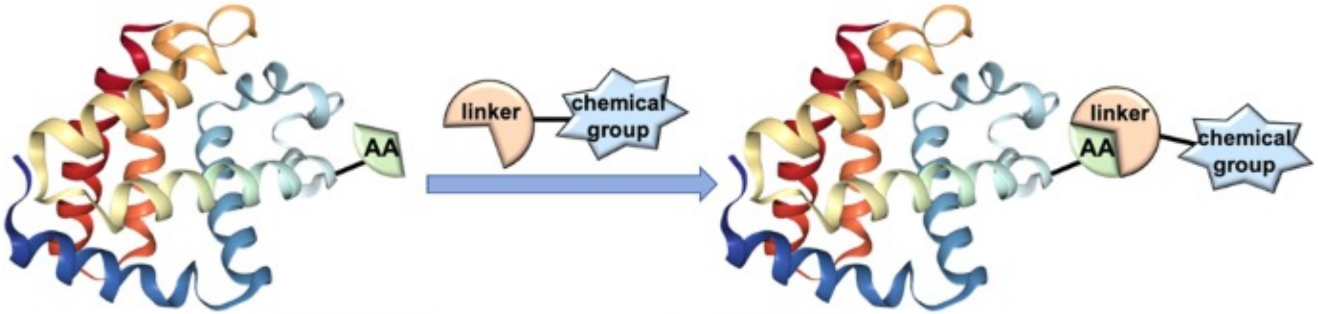
- ✓ Scaffold 5 enables ultrarapid protein labeling via a photolysis of S–O bond followed by liberation of a trifluoromethyl radical.
- ✓ Protein and protein-interaction mapping
- ✓ Preparative scale trifluoromethylation of peptides.

Contents

Summary



Photoredox Trp modification

- ✓ Mild and biocompatible conditions.
- ✓ Trp-selectivity
- ✓ Enrichment of the tryptophan-ome from both lysates and live cell
- ✓ Protein and protein-interaction mapping