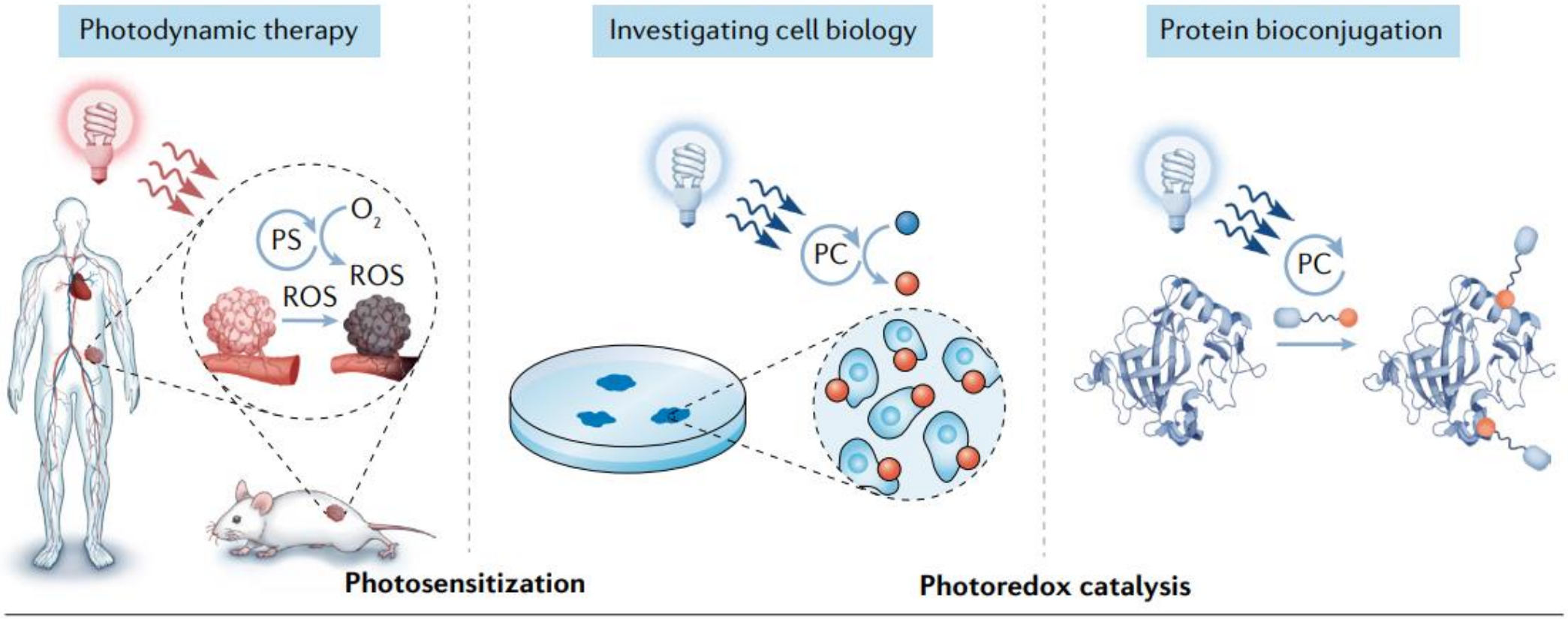
Development of proximity protein labeling

Hiroyasu Nakao

2021/10/07

Contents

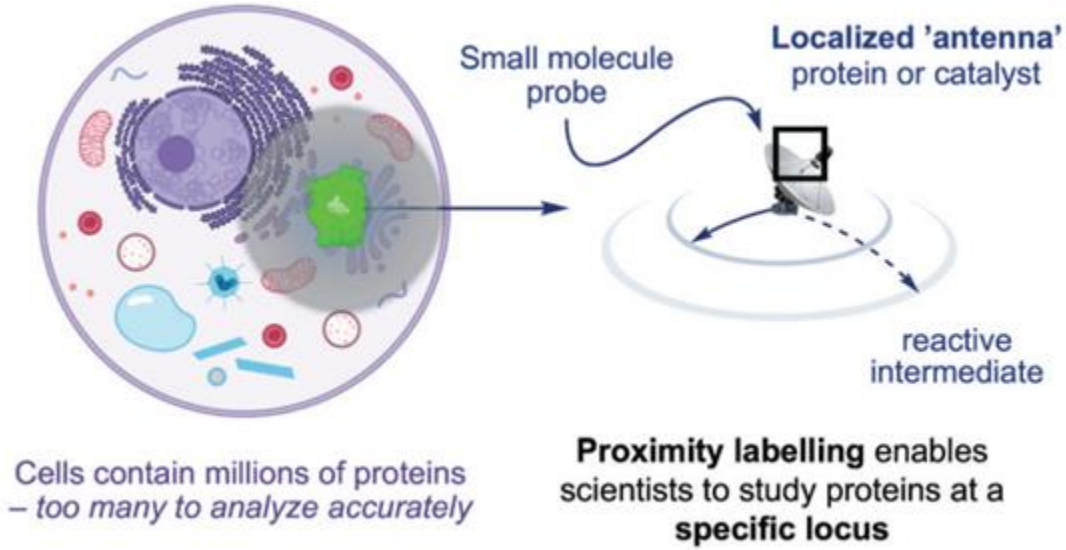
Introduction



Visible-light-driven catalysis has a tremendous impact on chemical synthesis, nanotechnology, energy and biological sectors.

O. Fedeyi, *et al. Nat. Rev. Chem*, **2021**, 5, 322.

Proximity protein labelling



✓ **Protein-protein interactions (PPIs), in which many proteins interact with other proteins, are important.**

✓ **Weak PPIs have until remained almost undetectable.**

✓ **Proximity protein labeling is one way to obtain information about PPIs.**

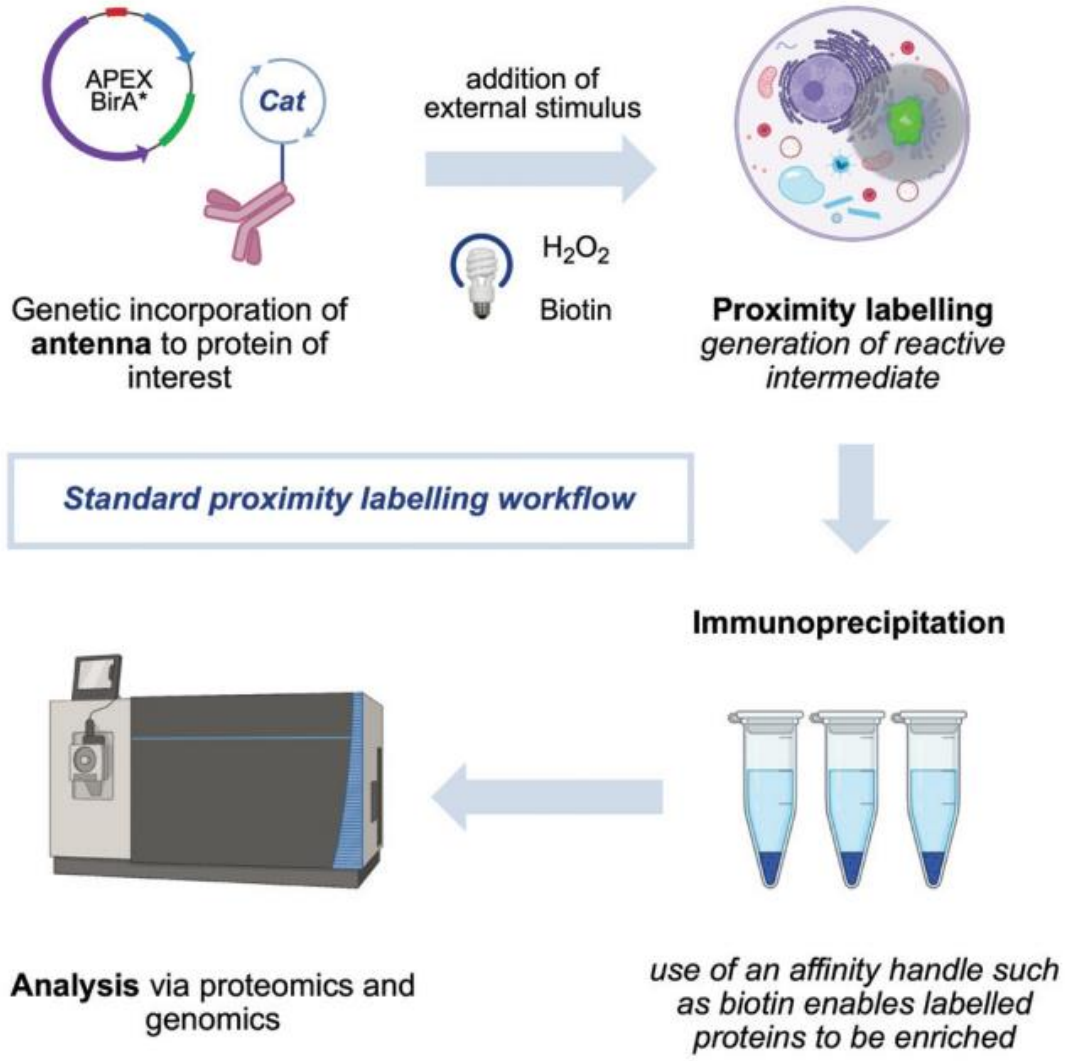
D. Macmillan, *et al*. *Chem. Soc. Rev*. **2021**, 50, 2911.

What is proximity protein labeling?

- ✓ **Proximity labelling is the localization of a small molecule or protein that can generate a reactive intermediate.**
- ✓ **Reactive intermediates cross-link with proteins.**
- ✓ **Extracted proteins can be analyzed by immunoblotting and chemoproteomic analysis**

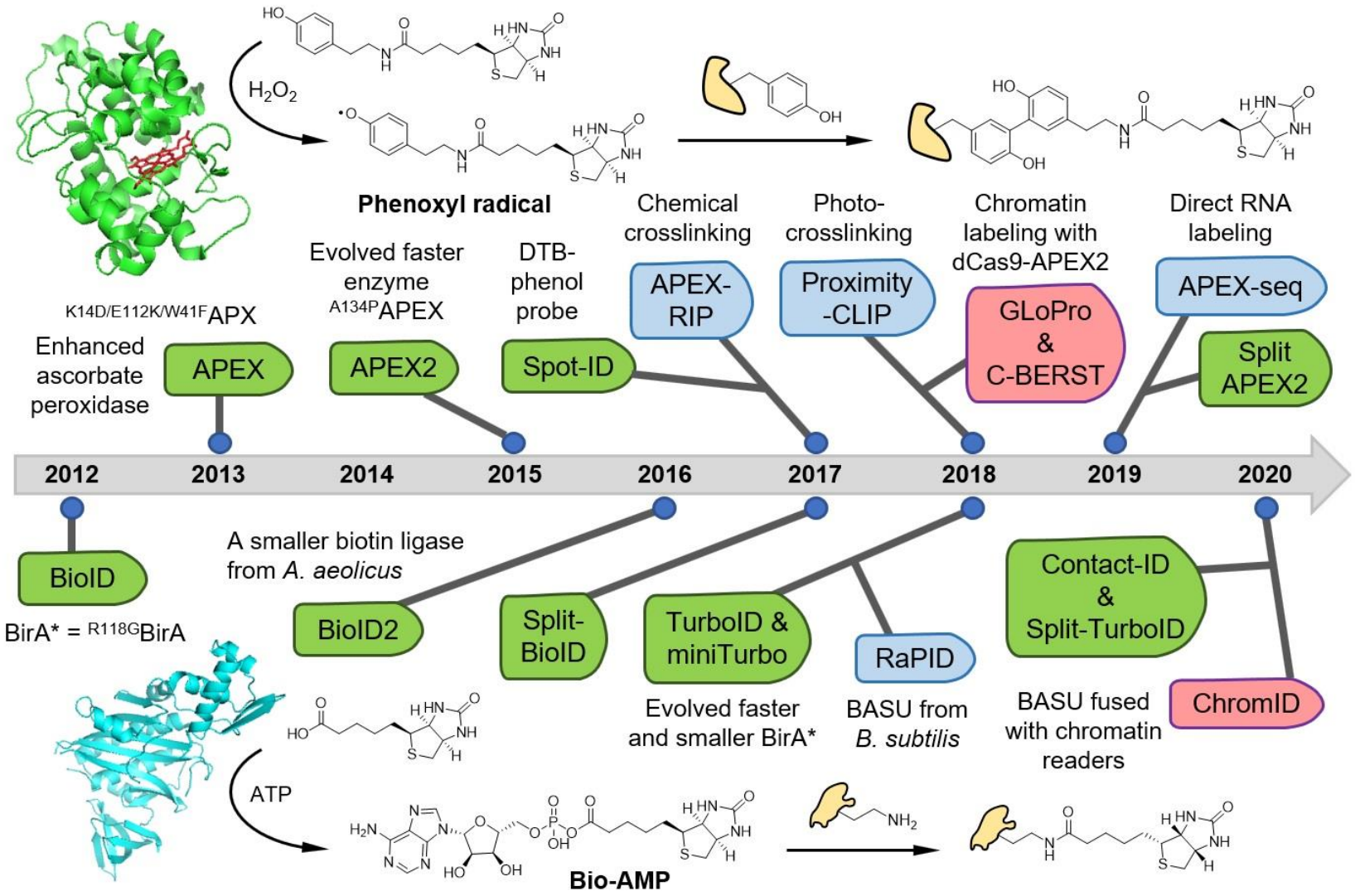
<u>Key issue</u>

- ✓ **labeling radius**
- ✓ **labeling time**



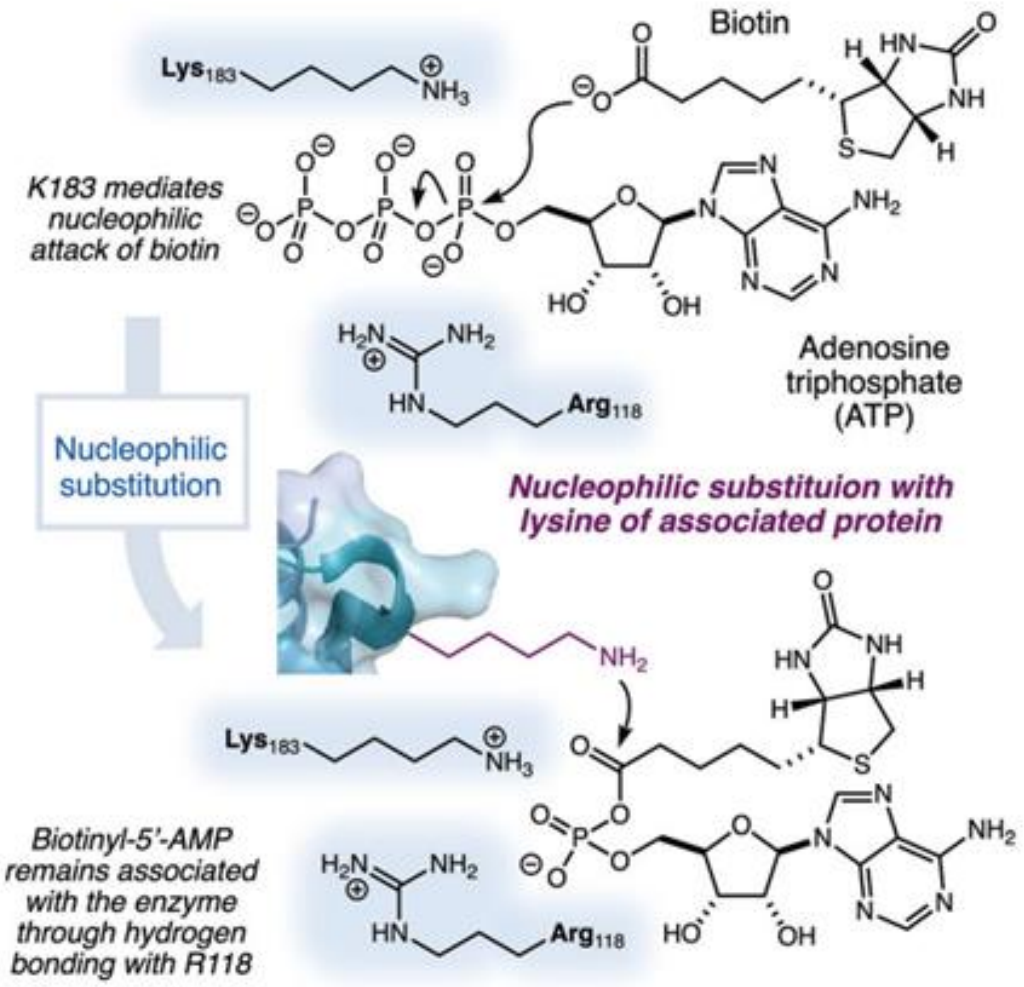
D. Macmillan, *et al*. *Chem. Soc. Rev*. **2021**, 50, 2911.

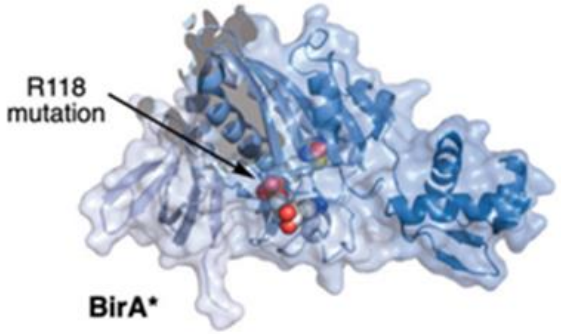
History



http://zoulab.org/Research_Proteomics.html

BioID





Biotinoyl-50AMP (BioAMP) is produced by the combination of biotin and ATP.

⇩

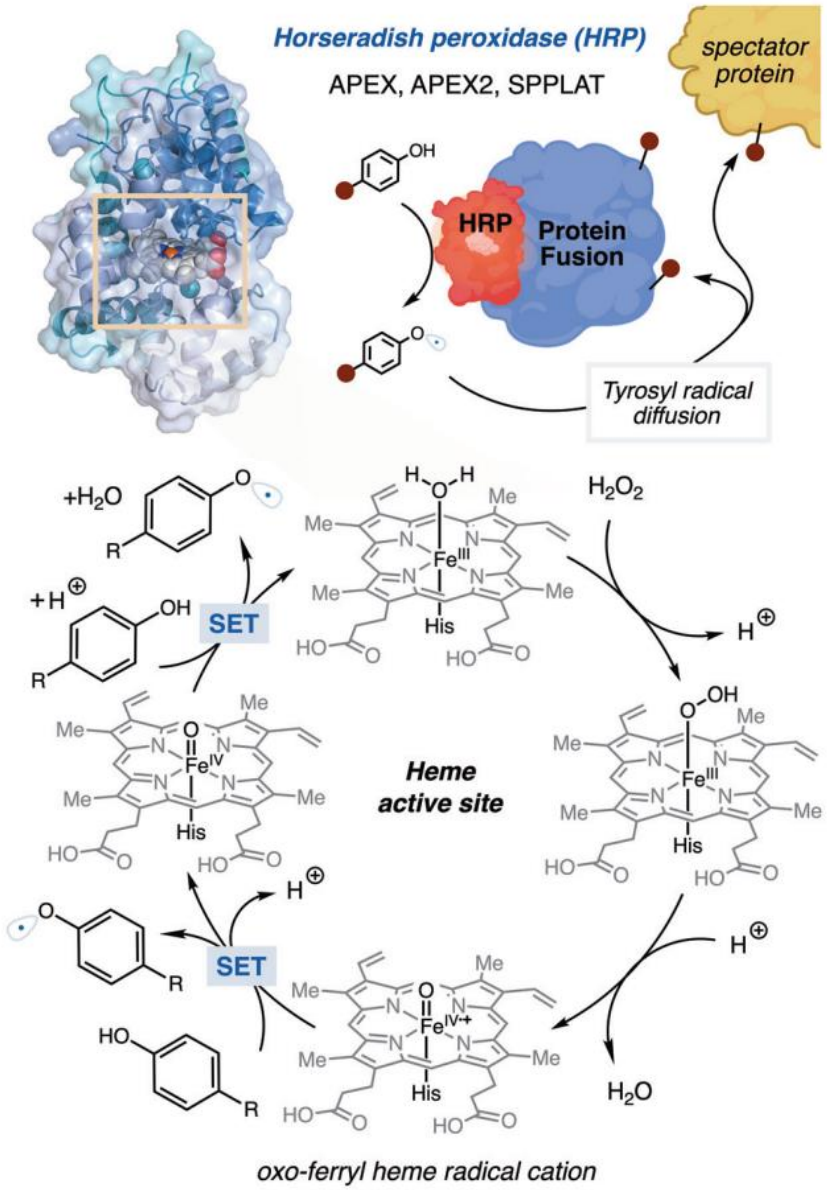
BioAMP is retained in the active site of BirA*.
BirA* : biotin ligase

⇩

When BioAMP is released from BirA, it reacts with surrounding lysine residues.

D. Macmillan, *et al*. *Chem. Soc. Rev*. **2021**, 50, 2911.

APEX



Iron(Ⅲ) is oxidized by hydrogen peroxide to become iron (IV).

⇩

Water and oxoferyl (FeIV) radical cations are generated via peroxo iron species.

⇩

This species is then reduced by phenol to neutral oxoferryl to produce phenoxyl radicals.

⇩

Phenoxy radicals crosslink with tyrosine and nucleic acids.

D. Macmillan, *et al*. *Chem. Soc. Rev*. **2021**, 50, 2911.

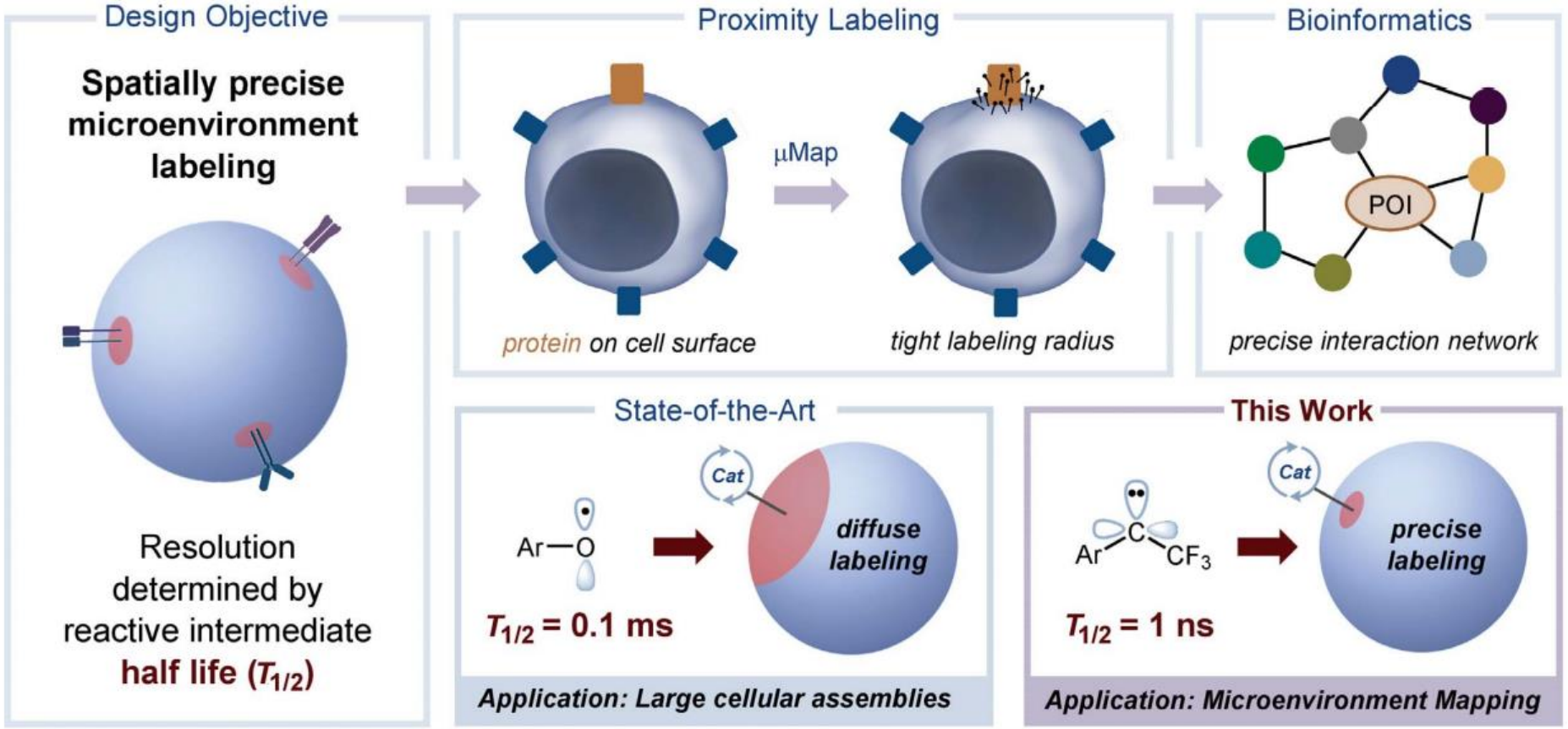
Protein proximity labeling

Method		Substrates	Labeling time	strength	limitations
APEX	APEX, APEX2	Biotin phenol (BP), H_2O_2	≦1 min	High temporal resolution High activity	H_2O_2 toxicity BP permeability
BioID	BioID2	Biotin, ATP	18 h	Non-toxic stable at higher temperatures	Poorly reactive Low temporal control Wide labeling radius
	AirID		3 h		
	TurboID		≦ 10 min	Highest activity	

cf. Takahashisan's proposal seminar

Contents

Microenvironment mapping



<u>catalyst</u>

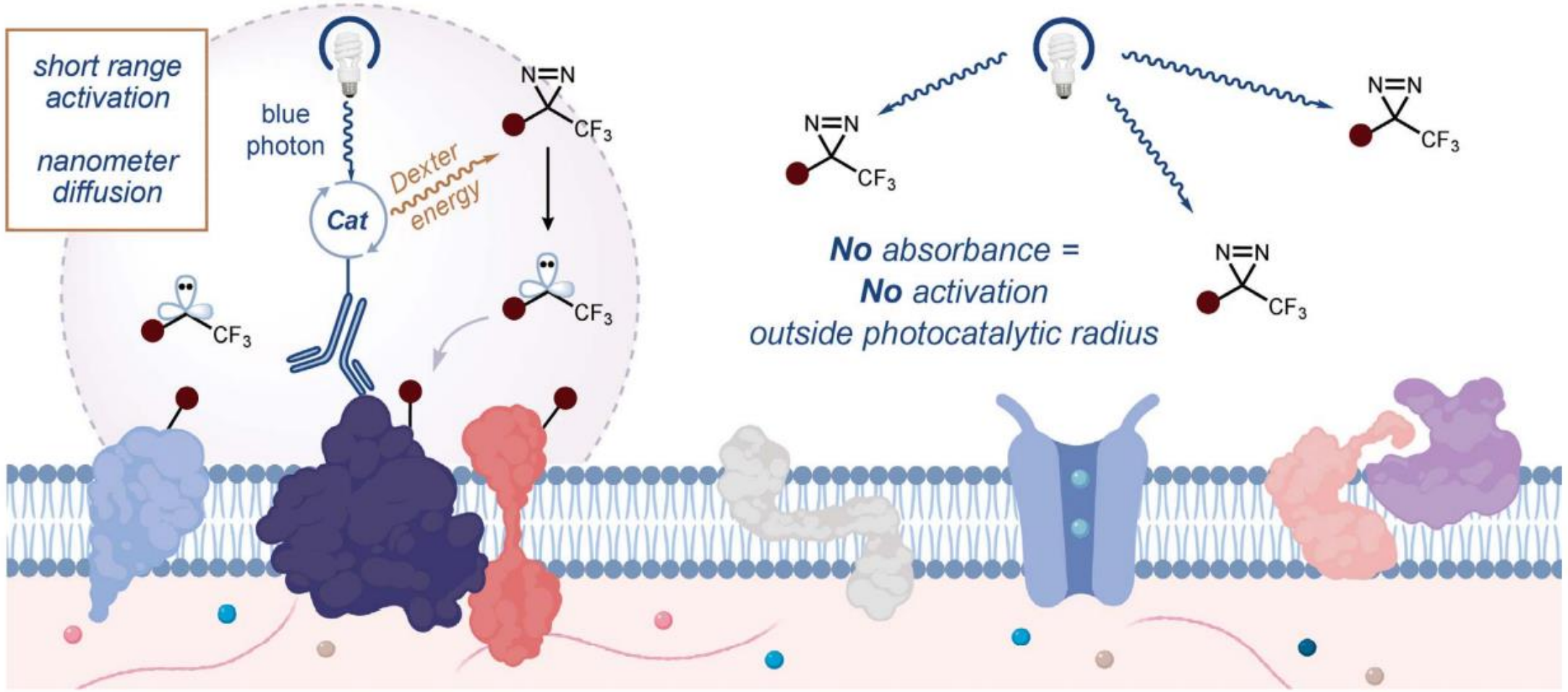
- ✓ **tolerant of aqueous conditions**
- ✓ **conjugate to antibodies, DNA, small ligand**
- ✓ **selectively activate chemical probes at a diffusion-limited rate**

<u>chemical probe</u>

- ✓ **activated within 1 nm of the catalyst radius**
- ✓ **not to undergo long-range diffusion after activation**

D. Macmillan *et al*. *Science*. **2020**, 367, 1091.

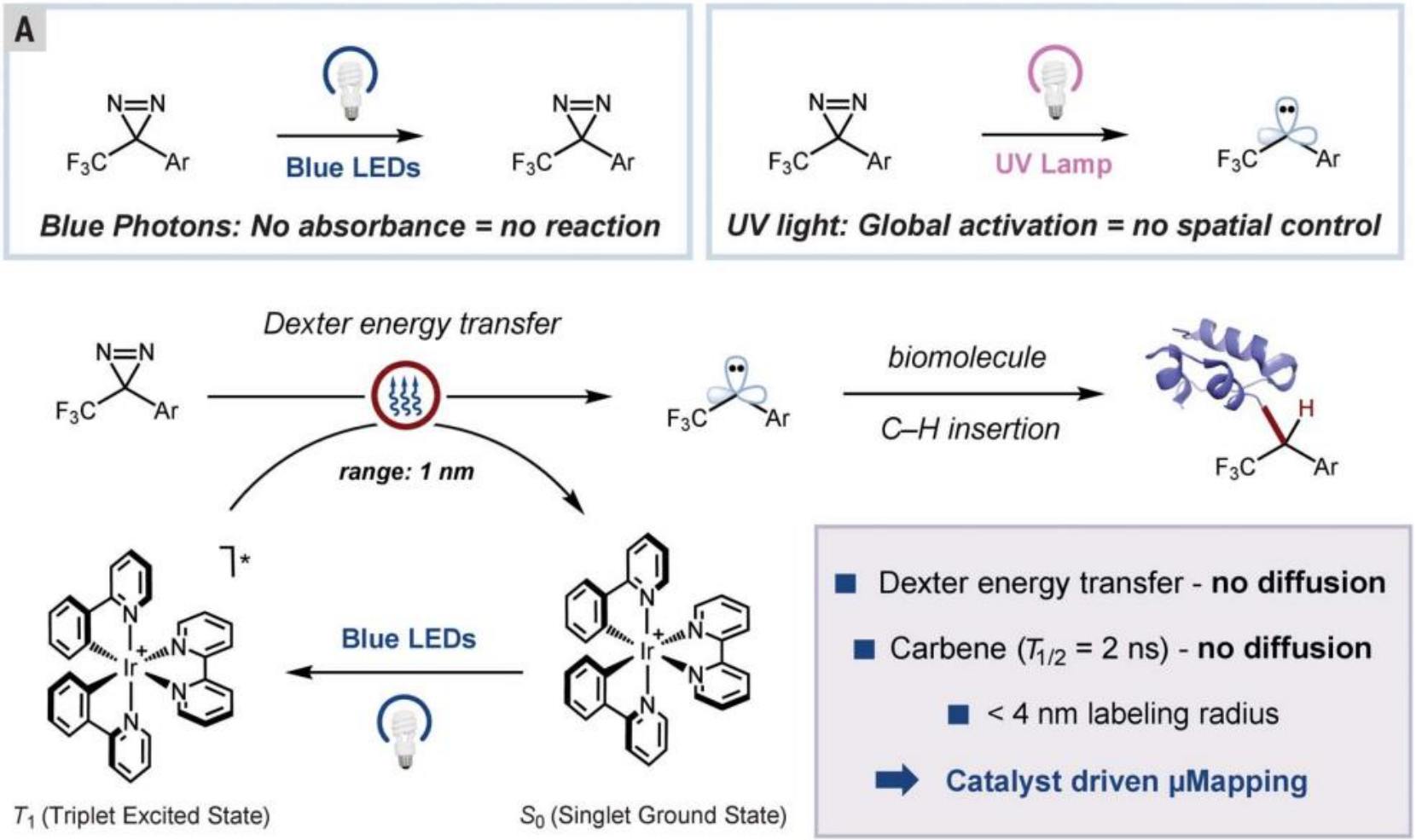
Carbene



Carbenes readily cross-link with C–H bonds found in all biomolecules [(k) = 3.1 × 10^9 s^{-1}]

D. Macmillan *et al*. *Science*. **2020**, 367, 1091.

Carbene



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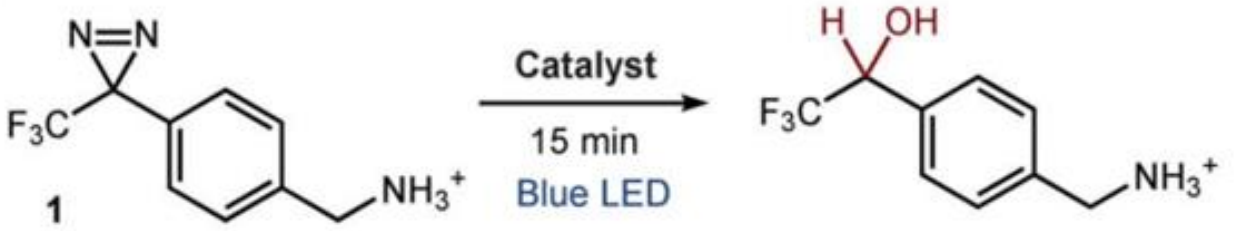
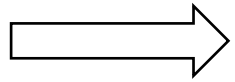
Photocatalyst

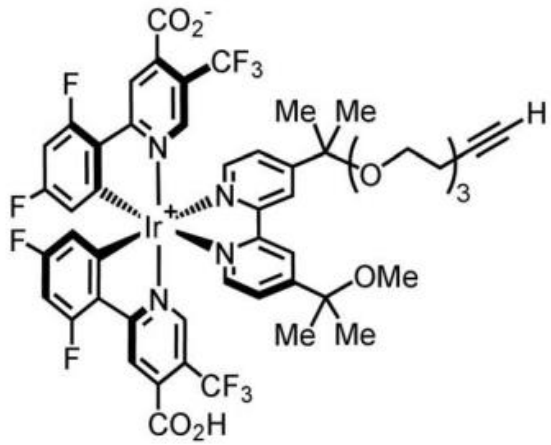
B



Catalyst	Conversion	E_T (kcal)	$E_{1/2}(ox)^*$	$E_{1/2}(red)$
$[Ir(dFCF_3ppy)_2(dtbbpy)]^+$ (2)	**100%**	**60.1**	**1.21**	**-1.37**
Blue LEDs only	0%	–	–	–
$Ir(ppy)_3$	0%	55.2	0.31	-2.20
$[Ru(bpz)_3](PF_6)_2$	0%	48.4	1.45	-0.80



Optimized Catalyst (3)



✓ **[Ir(dFCF3ppy)2(dtbbpy)]PF6(E_T = 60.1 kcal/mol) is the best photocatalyst.**

✓ **Iridium photocatalyst(3) aimed at biomolecular applications did not affect ability to remove nitrogen.**

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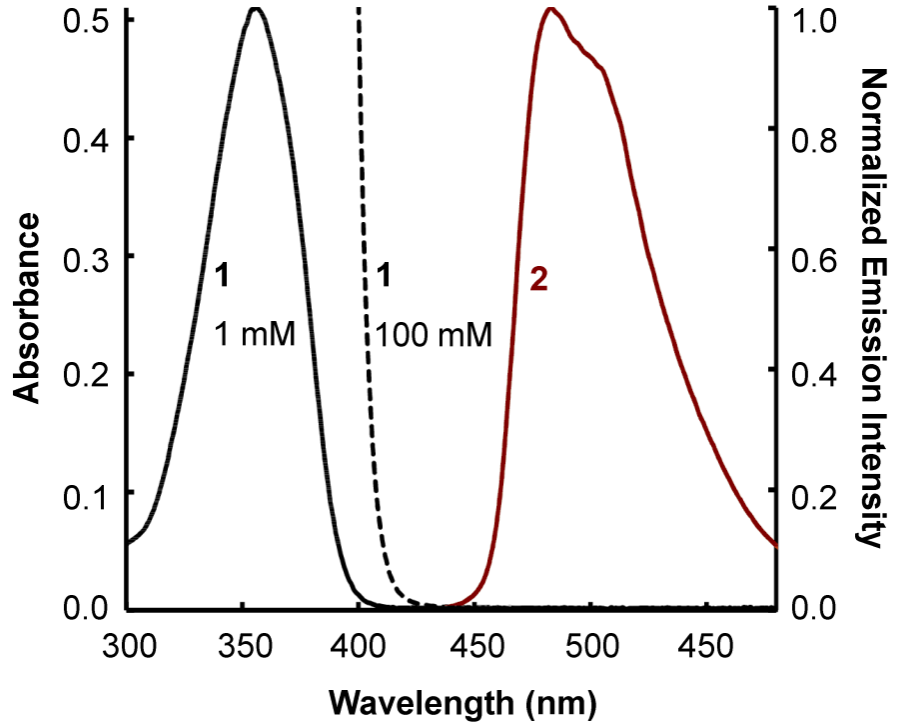
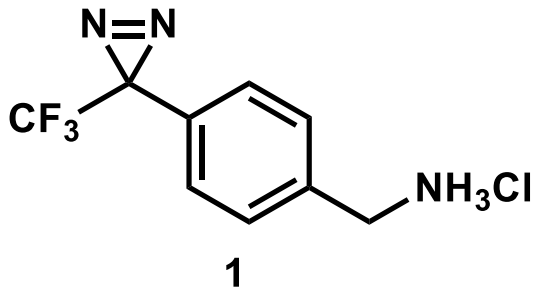
Dexter energy transfer vs Forster energy transfer





1

$[Ir(dFCF_3ppy)_2(dtbbpy)]PF_6$

2

✓ **No overlap was observed between the absorption spectrum of 1 and the emission spectrum of 2.**

✓ **It was suggested that it was a Dexter energy mechanism rather than a Forster energy transfer.**

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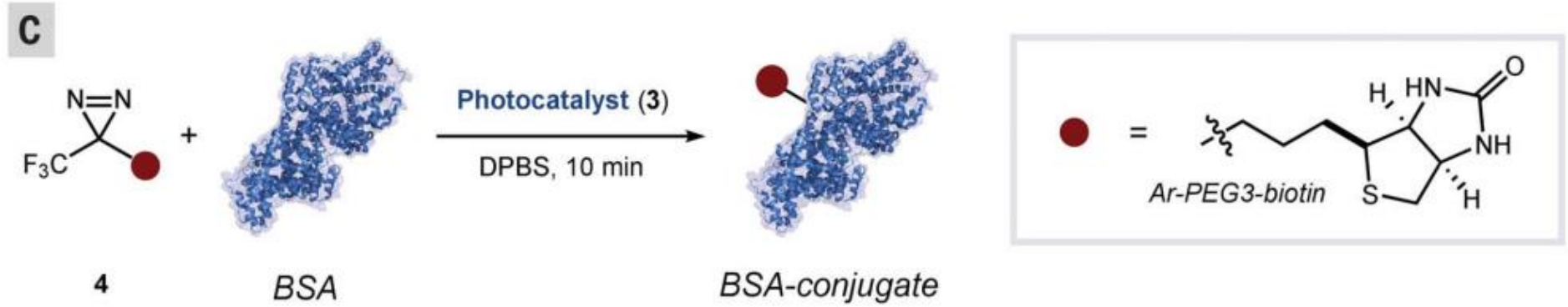
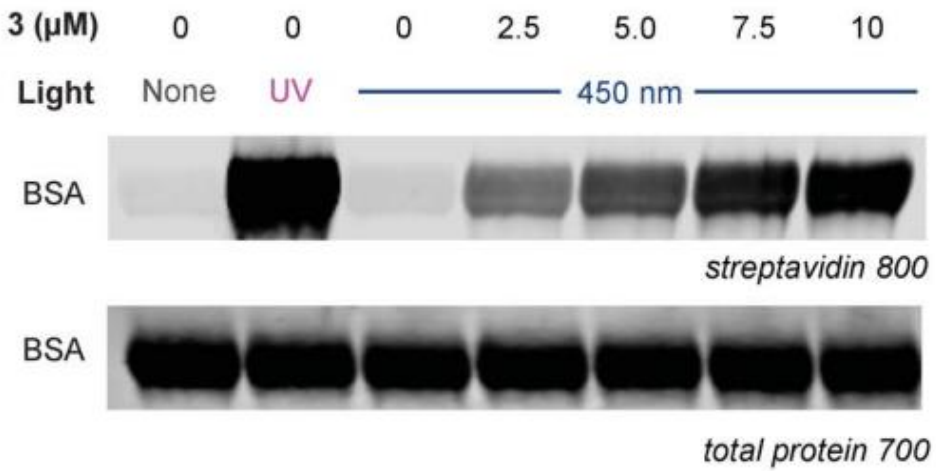
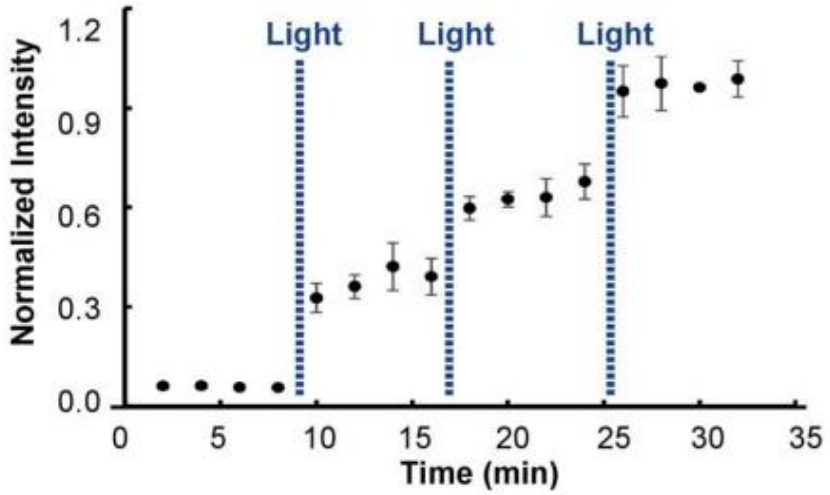
Protein labeling







✓ **No background reaction occurred.**

✓ **Turning the light source on or off affords fine temporal control over the labeling process**.

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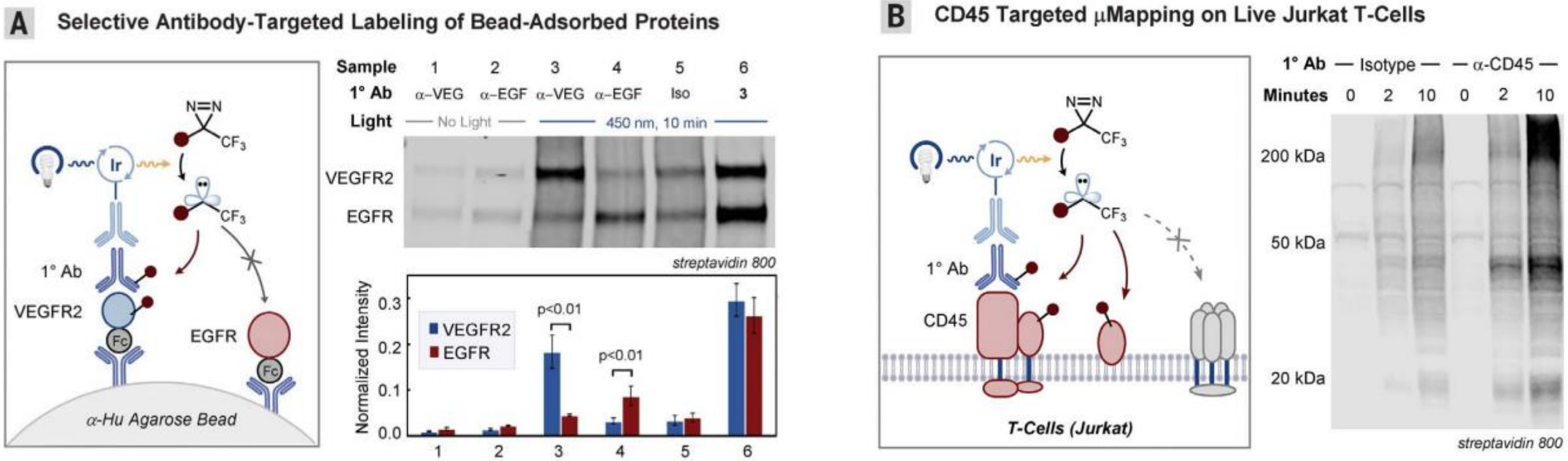
Selective proximity protein labeling



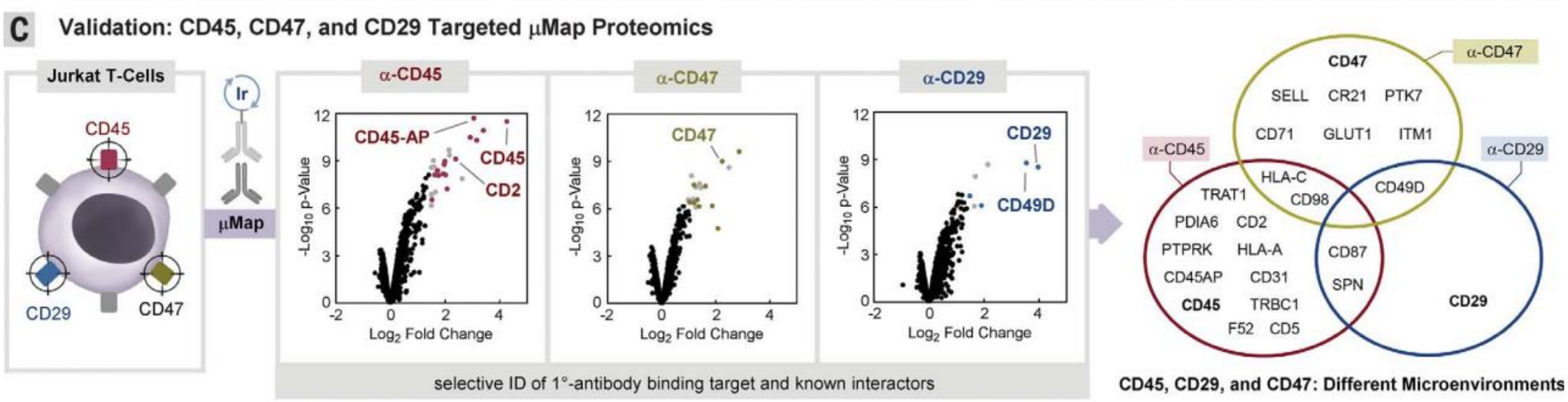
✓ **EGFR2/VEGFR2 was selectively labeled when irradiated with 450 nm light.**

✓ **When CD45-targeted μMap was performed on Jurkat cells, light- and time-dependent protein biotinylation was observed.**

D. Macmillan *et al*. *Science*. **2020**, 367, 1091.

µMAP proteomics

Could µMap differentiate between spatially separated microenvironments on the same cell membrane?



✓ The ability of µMap discriminate between unrelated microenvironments.

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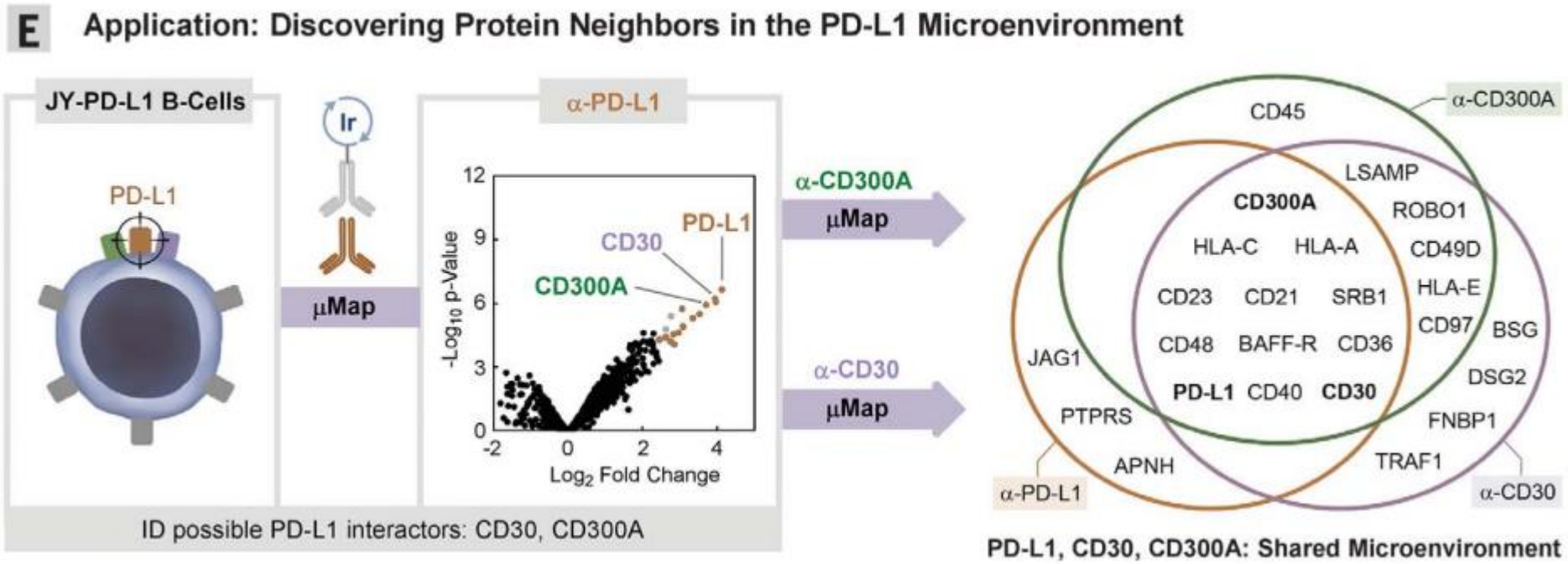
PD-L1

They investigate the proximal protein interactome of PD-L1 in B cells.



✓ **It was found that CD30 (a member of the tumor necrosis factor receptor family) and CD300A (an immunosuppressive receptor), may have a new interaction due to significant enrichment.**

✓ **Potential of mMapping provides new insights with respect to the microenvironments of checkpoint proteins.**

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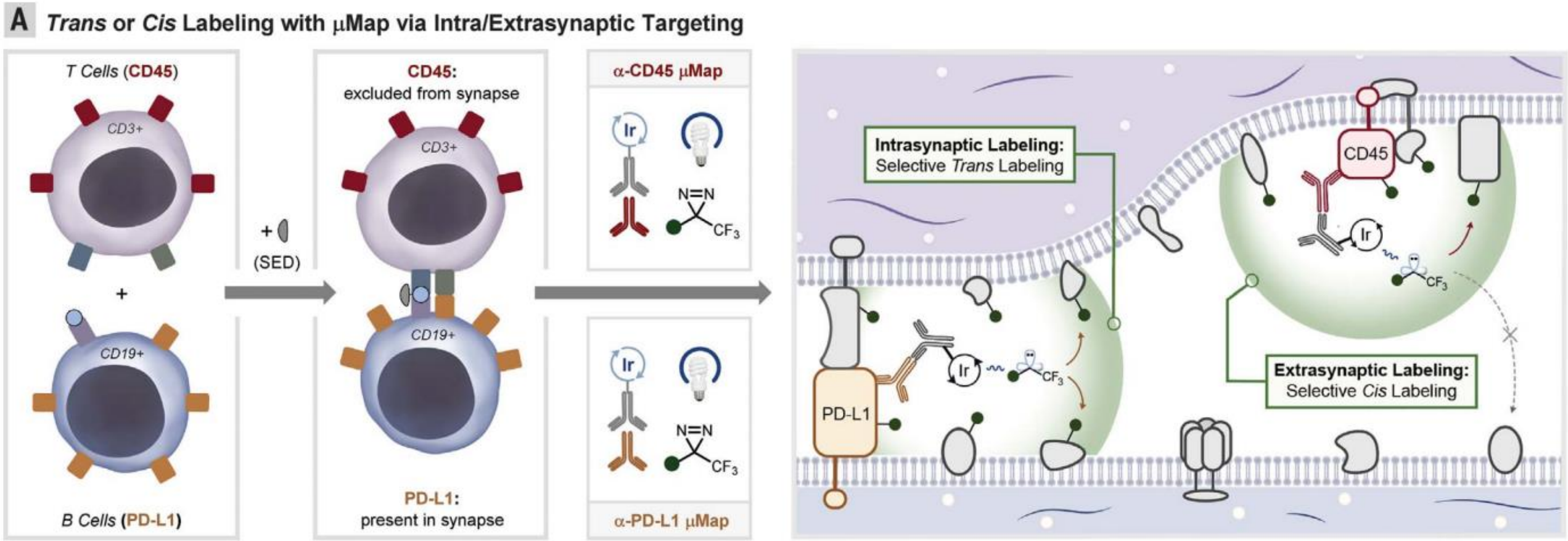
Intercellular communication



✓ **Understanding intercellular communication is important.**

✓ **Does biotinylation of the surface of APCs expressing PD-L1 (cis labeling) lead not only to biotinylation of T cells at adjacent synapses (trans labeling)?**

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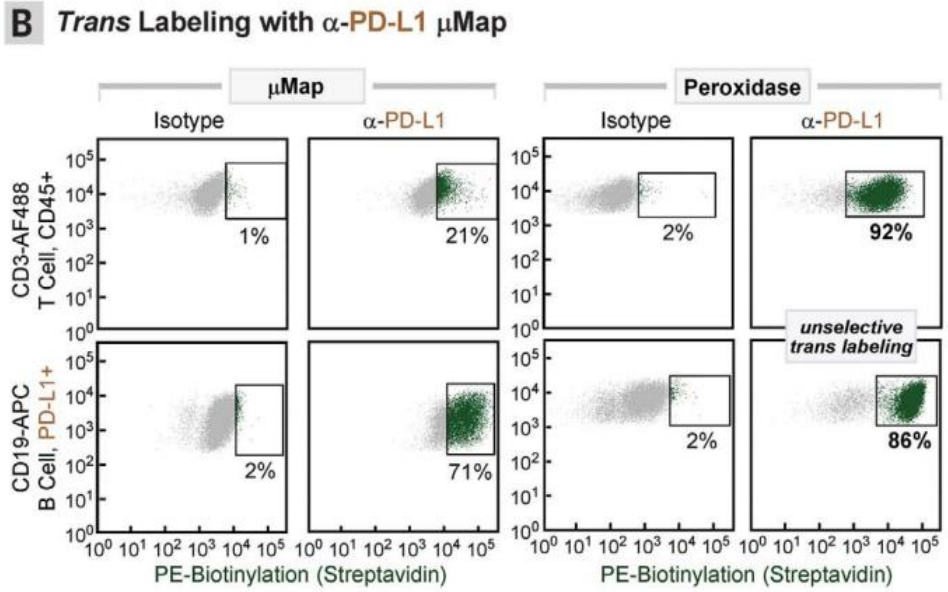
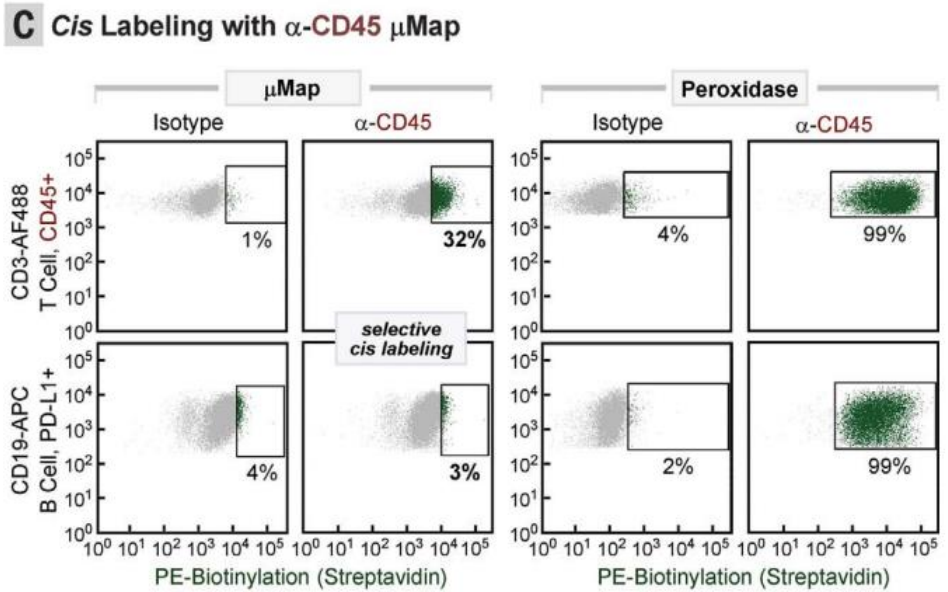
Intercellular communication

Trans labeling



PDL1–targeted μMap showed high selectivity for trans-labeling solely at the cis- and trans-cellular contact regions.

Cis labeling



CD45RO-targeted μMap led to selective cislabeling on the CD45RO-expressing Jurkat cells.

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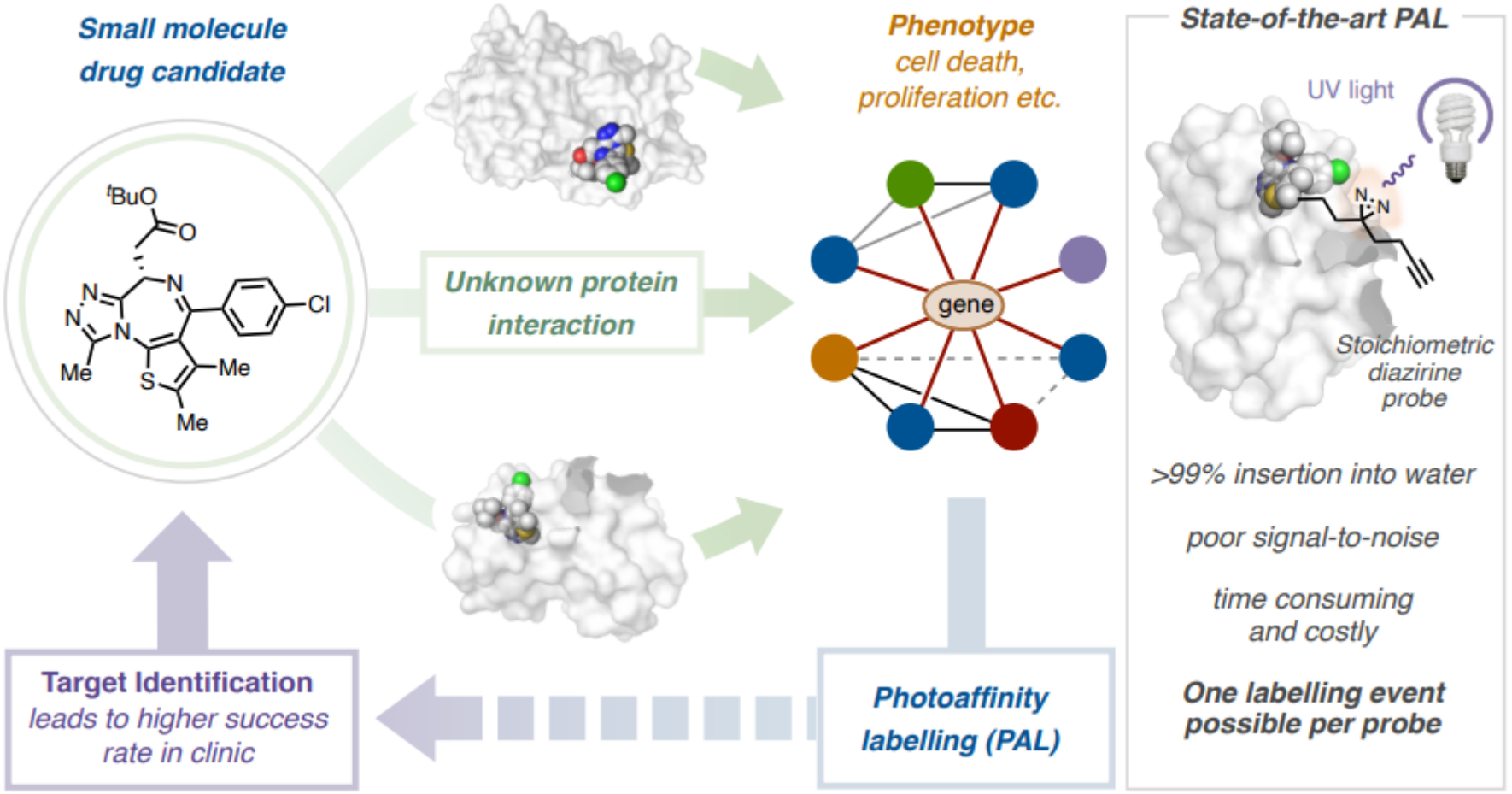
Target ID



✓ **Target ID remains a fundamental goal in drug discovery.**

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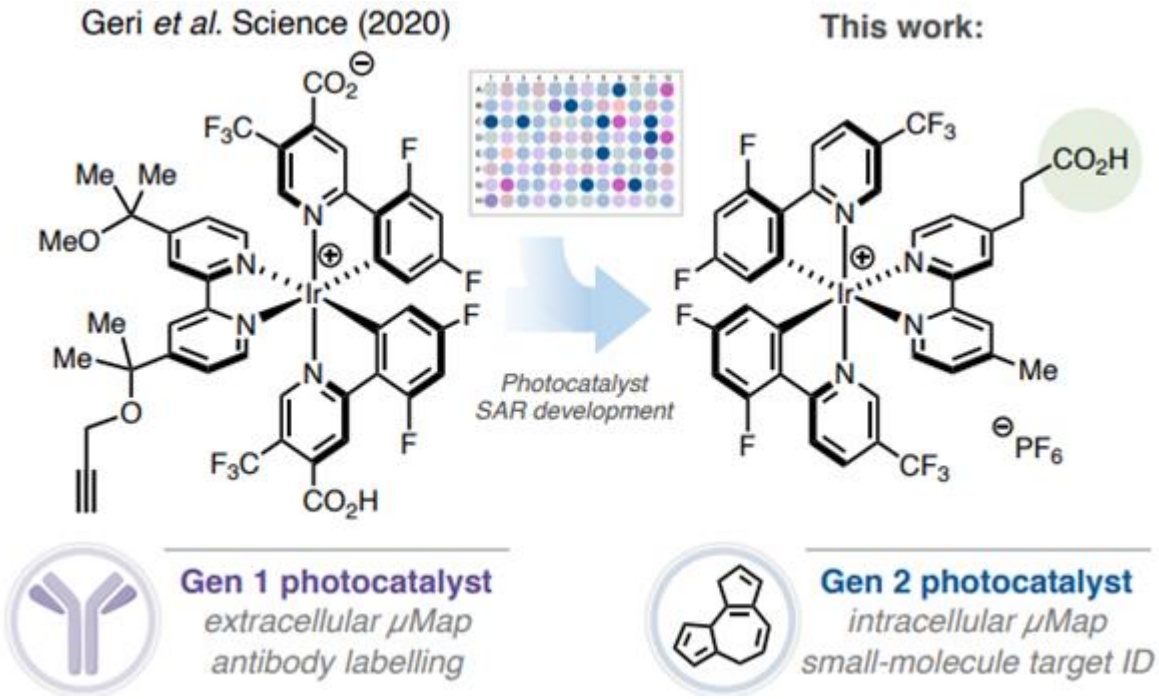
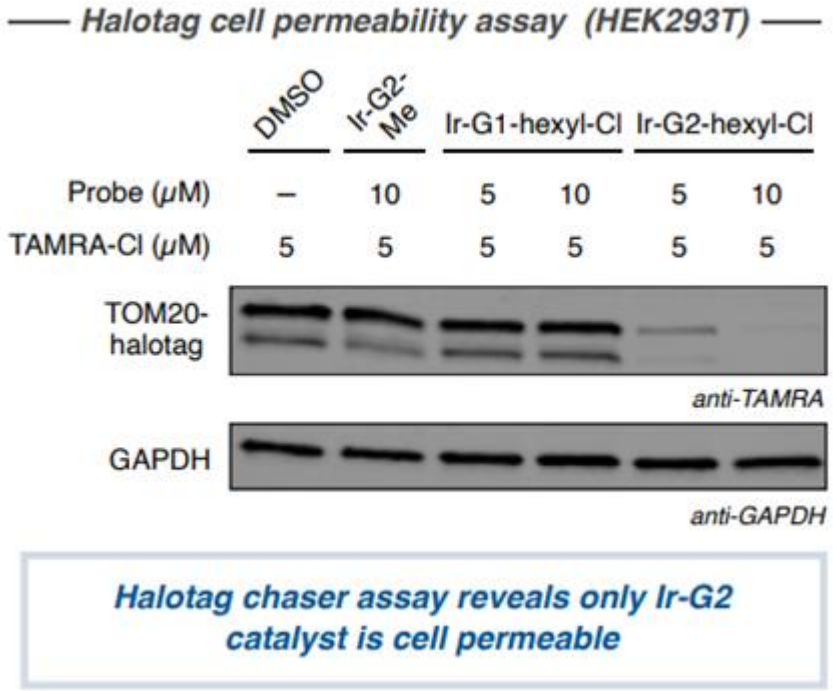
Design of photocatalyst





Problem

Cell permeable
Commercially available
Chemical manipulation
Retention of biological activity etc

By removing the carboxylic acid groups, the cationic photocatalyst (Gen 2) was rendered cell permeable

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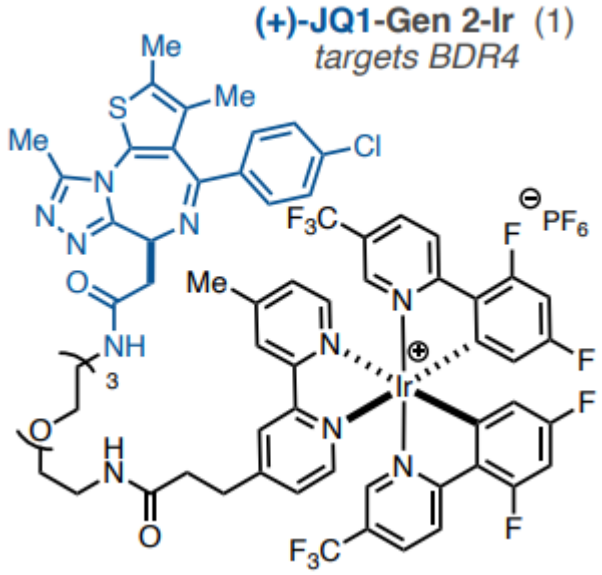
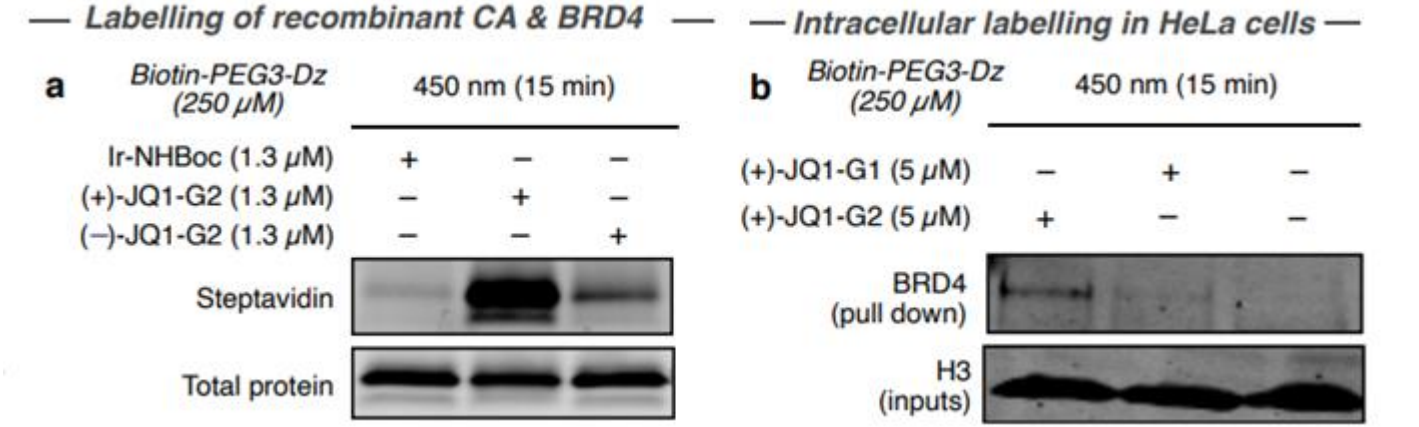
(+)-JQ128

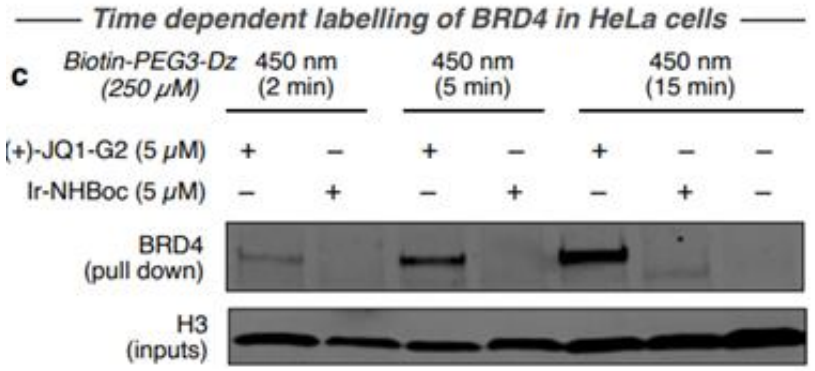


(+)-JQ-128 is a potent inhibitor of the BET family of bromodomain proteins (BRD2/3/4).



No labeling with inactive (-)-JQ1 enantiomer

No labeling with inactive (+)-JQ1-G1-Ir catalyst



Labeling increases with time due to signal amplification

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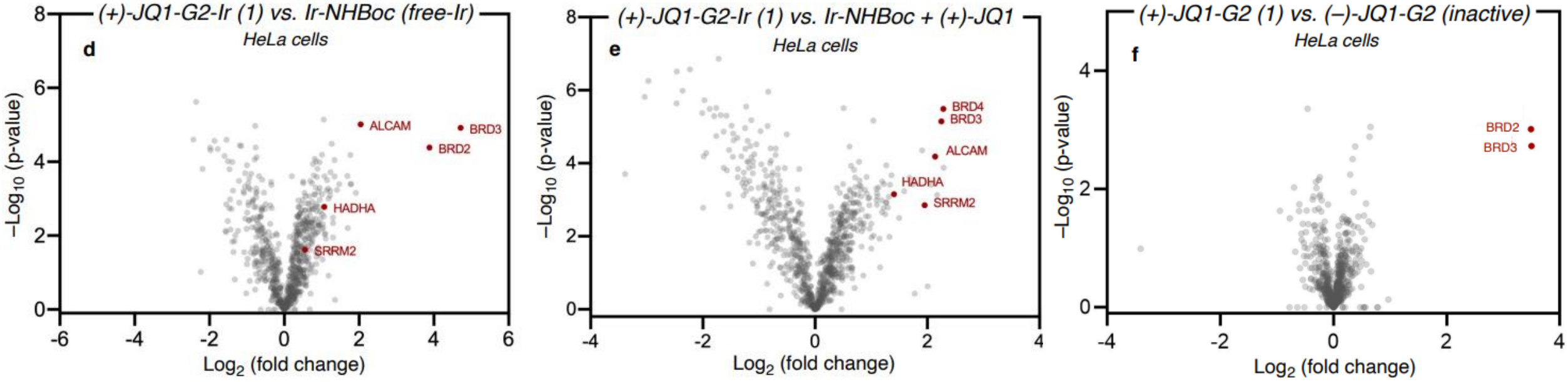
(+)-JQ128



- ✓ **Some BRD proteins are the most enriched.**
- ✓ **ALCAM (CD166) was also identified as being significantly enriched, but currently has no reported interaction with (+)-JQ1.**
- ✓ **CD166 was detected it was not enriched, indicating that binding may not be affected by the stereogenic center.**

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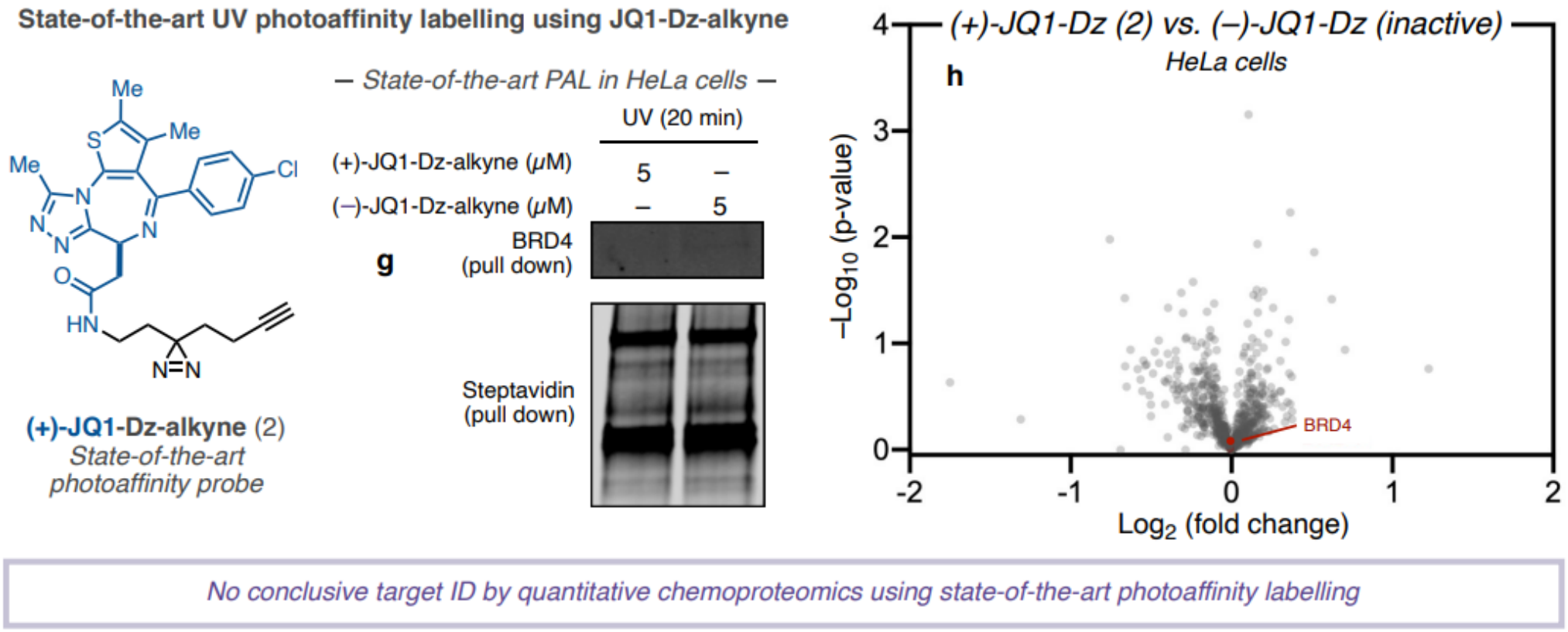
Classical photoaffinity labeling



No conclusive target ID by quantitative chemoproteomics using state-of-the-art photoaffinity labelling

Western blot and chemoproteomic analysis showed that the BRD protein was not enriched.

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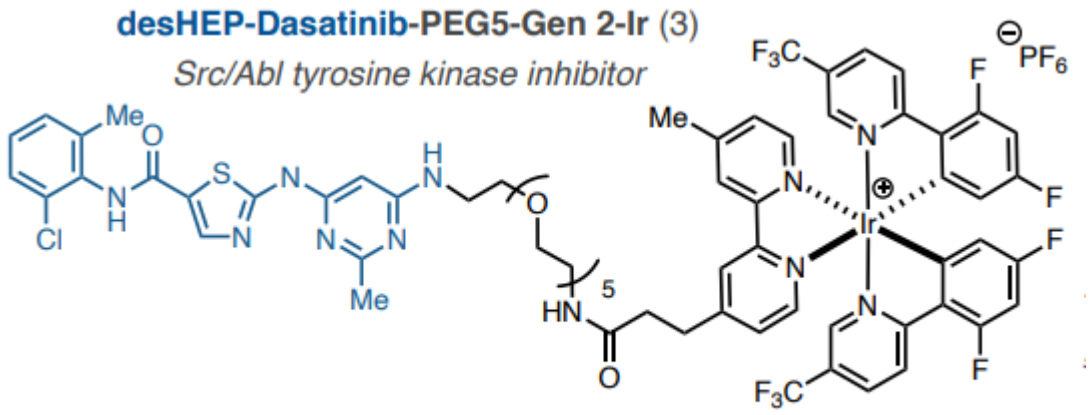
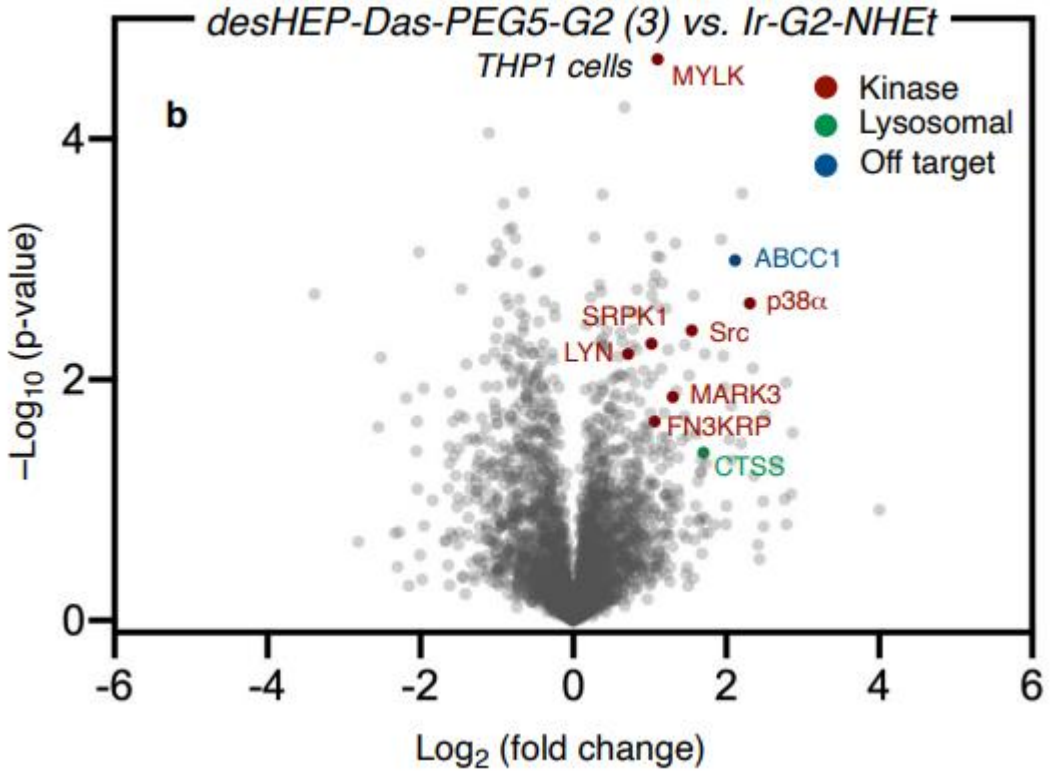
desHEP-Dasatinib



Dasatinib shows anti-cancer effects as a tyrosine kinase inhibitor



✓ **Interactions with several established kinases, including Src and Lyn, have been identified**.

✓ **They identified an off-target multidrug resistance transporter, ABCC1.**

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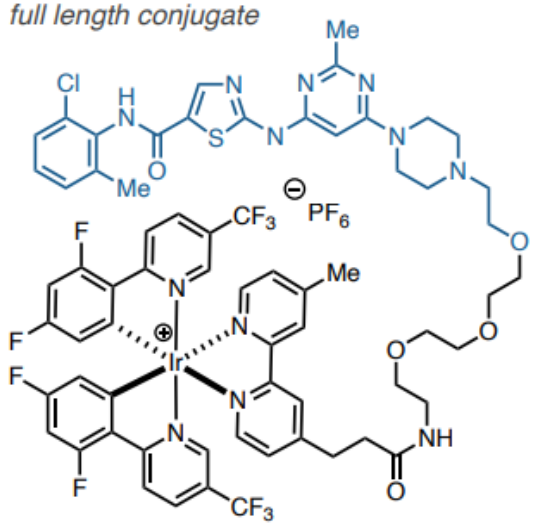
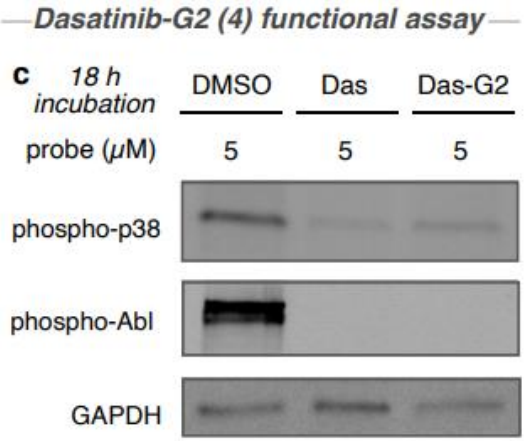
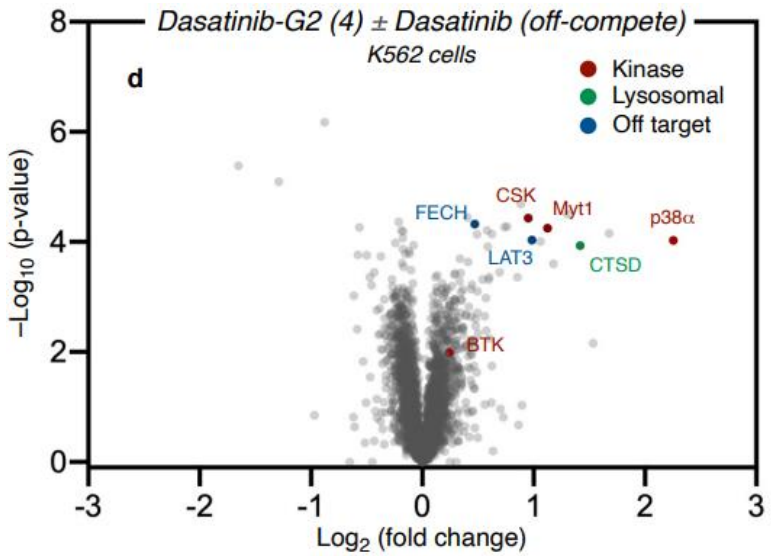
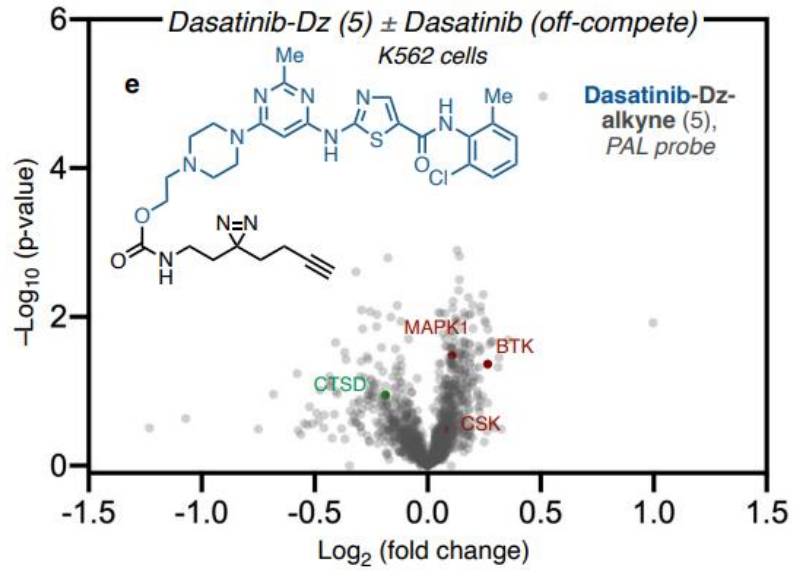
Dasatinib

Dasatinib-Gen 2-Ir (4)
full length conjugate









- ✓ **Iridium photocatalyst adapted to maintain biological function and cell permeability.**
- ✓ **Well-known kinases such as p38a, Myt1, and CSK kinases were extensively enriched.**
- ✓ **The most recent optical affinity labeling was that CSK, BTK and MAP were slightly enriched.**

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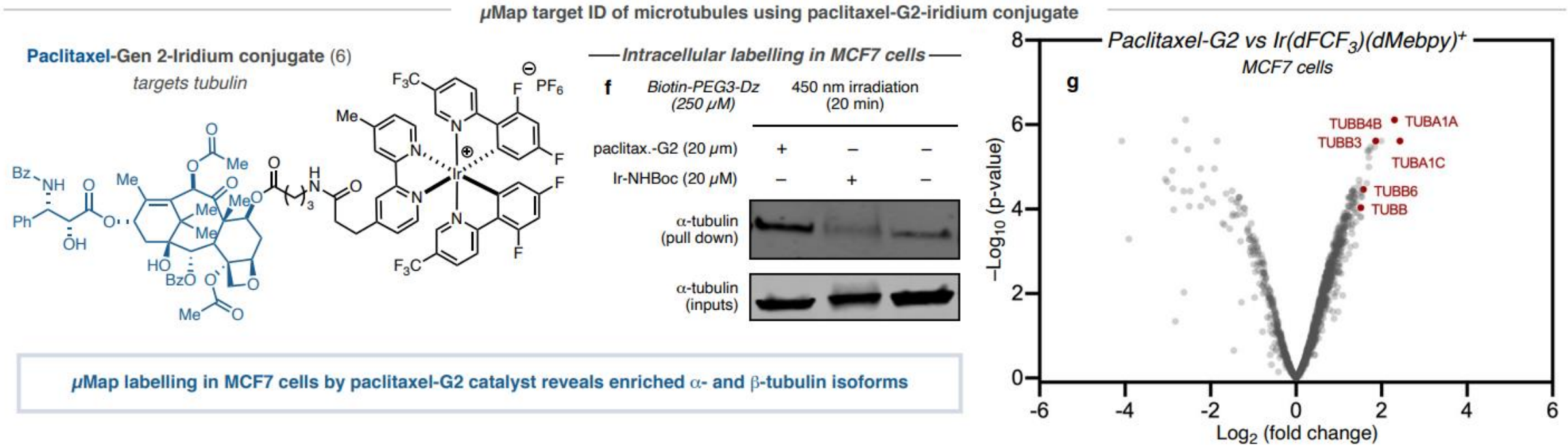
Paclitaxel



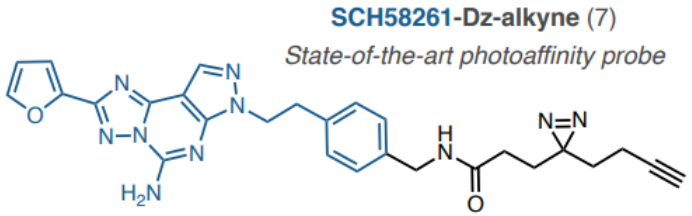
✓ **The anti-cancer mechanism of Paclitxel (Taxol) is not clear.**

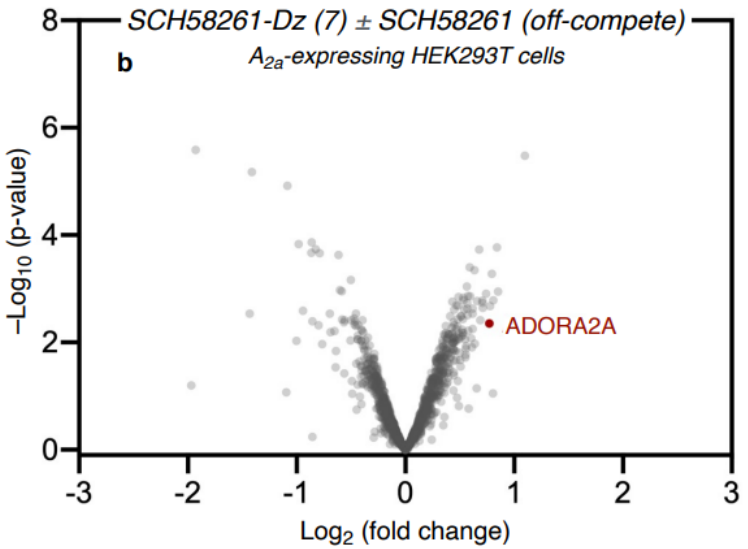
✓ **Western blot analysis using anti-a-tubulin clearly labeled the target protein compared to free iridium and DMSO.**

✓ **Tubulin isotypes aIa, bIII, bIVb, and aIc were found to be extensively labeled.**

D. Macmillan *et al. bioRxiv preprint.*

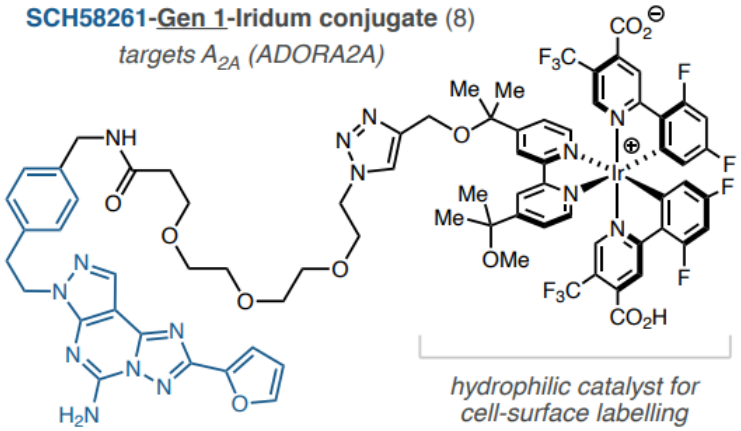
SCH58261

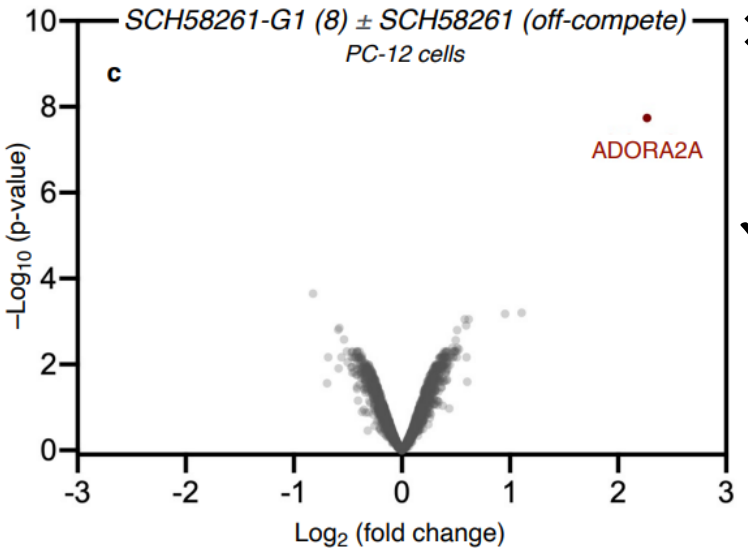




Validating the µMap Target ID Platform with Cell Membrane Proteins.

They chose the adenosine receptor A2a (ADORA2A) as a membrane target.





× PAL with SCH58261-Dz (7) showed low enrichment of ADORA2A.

✓ Photocatalyst labeling with SCH58261-G1(8) showed similarly high levels of ADORA2A enrichment.

D. Macmillan *et al. bioRxiv preprint.*

Summary

- ✓ A new mapping technology has been developed to replace APEX and BioID.
- ✓ µMap enables exploration of protein-protein interactions and intercellular communication.
- ✓ µMap targetID has allowed for the identification of multiple protein taegets across multiple drug classes and compartments.