PNAS

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

Small molecule photocatalysis enables drug target identification via energy transfer

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Authors

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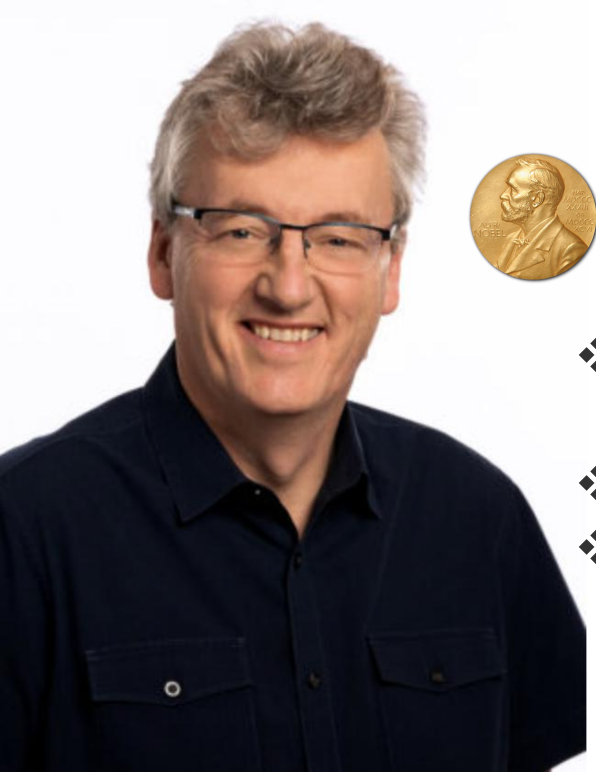

David MacMillan

Princeton University

- Nobel Prize in Chemistry (2021)
- Asymmetric organocatalysis
- Chemical biology

Aaron Trowbridge

Lecturer at university of Manchester

- Molecular Oxygen Editing
- Sustainable Photocatalysis

Background

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*During last decade, over **50% of drugs** in phase2 and phase3 clinical trials have failed……*

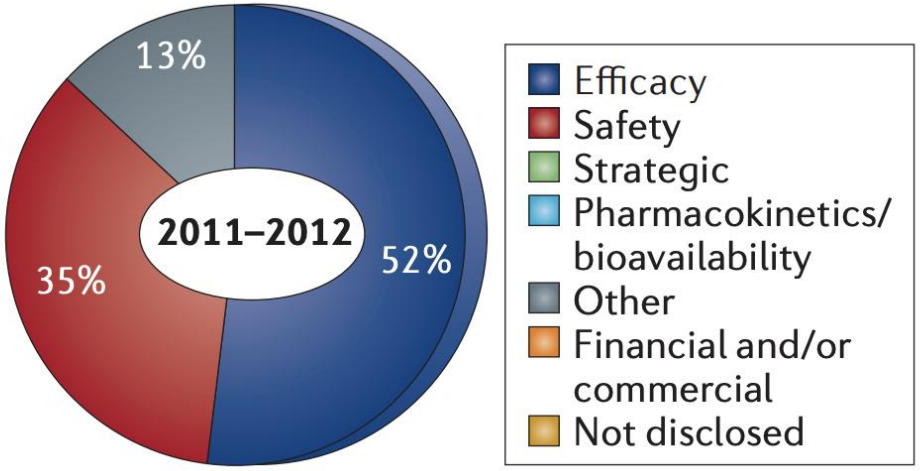

Fig1. Reasons for failures in Phase II and Phase III trials in 2011 and 2012

The lack of **Efficiency**

↓

Incomplete **target validation**

↓

The **Identification of biological target** and **understanding of interaction** at molecular level
(Target ID)

Arrowsmith, J., Miller, P. Phase II and Phase III attrition rates 2011–2012. Nat Rev Drug Discov 12, 569 (2013).

Background

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***Bioinformatics**, **Mass spectrometry**, **Chemical genetics** improved the understanding of cellular pathways…*

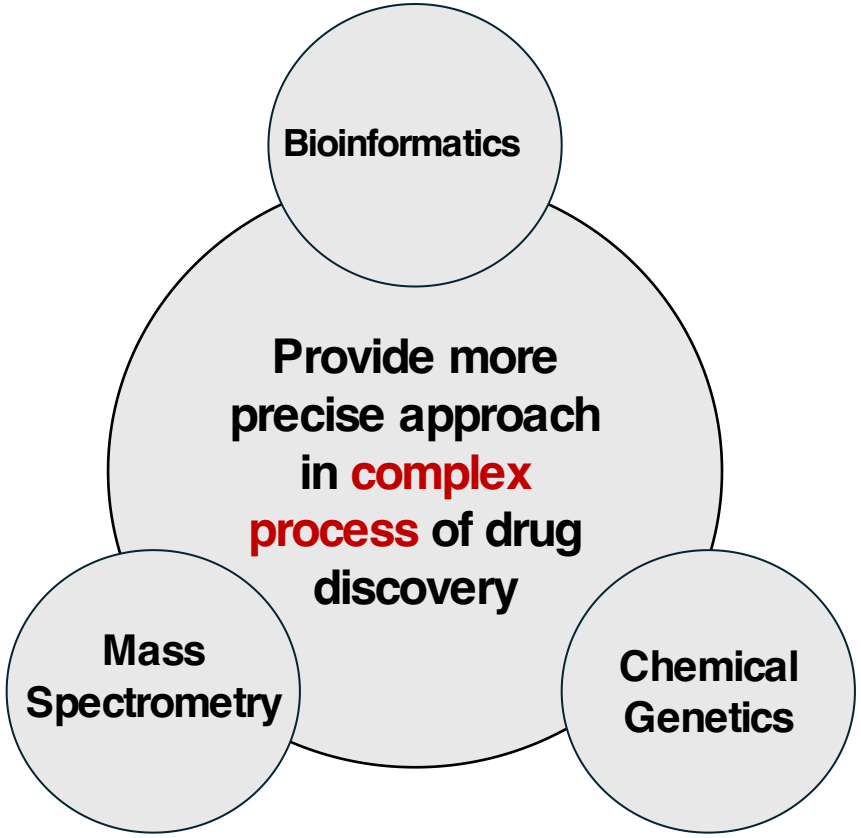

Fig1. Application of Different approach in drug discovery

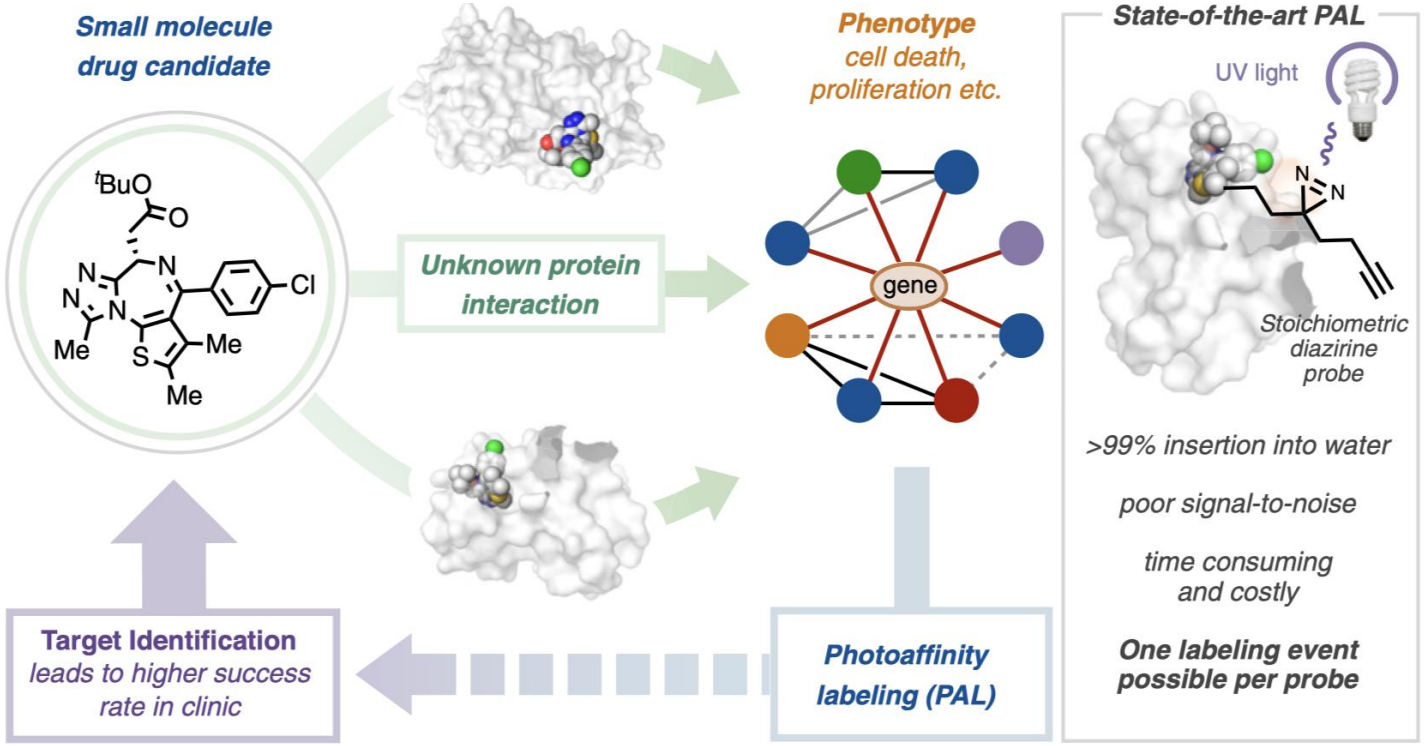

Fig2. Small molecule target ID in phenotypic screening-based drug discovery and its deficient

Improvements made by Photocatalytic

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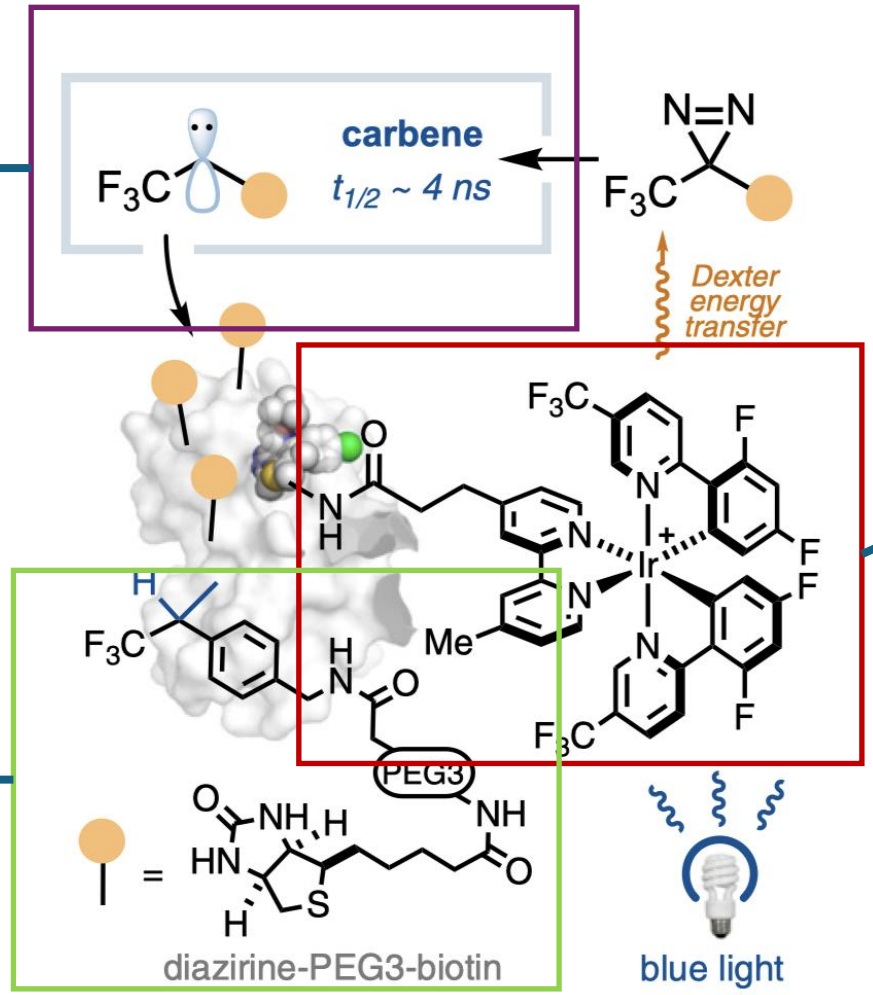
Carbene
- Rapidly form covalent bond with target proteins

Diazirine-biotin group
- Can be activated by Dexter energy transfer
- Labelling the target



Photocatalyst group
- Activated by blue light
- Further activate Diazirine to generate Carbene

- Increased specificity
- Signal Amplification
- Efficiency

Fig1. Visible light-mediated photocatalytic target ID

J. B. Geri et al., Microenvironment mapping via Dexter energy transfer on immune cells. Science 367, 1091–1097 (2020).

Designed Photocatalyst for Enhanced Cell Permeability and Intracellular Targeting

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- TAMRA–Cl: Fluorescent probes labeled with **TOM20–Hal**
- TOM20: A protein located on **the membrane of Mitochon**

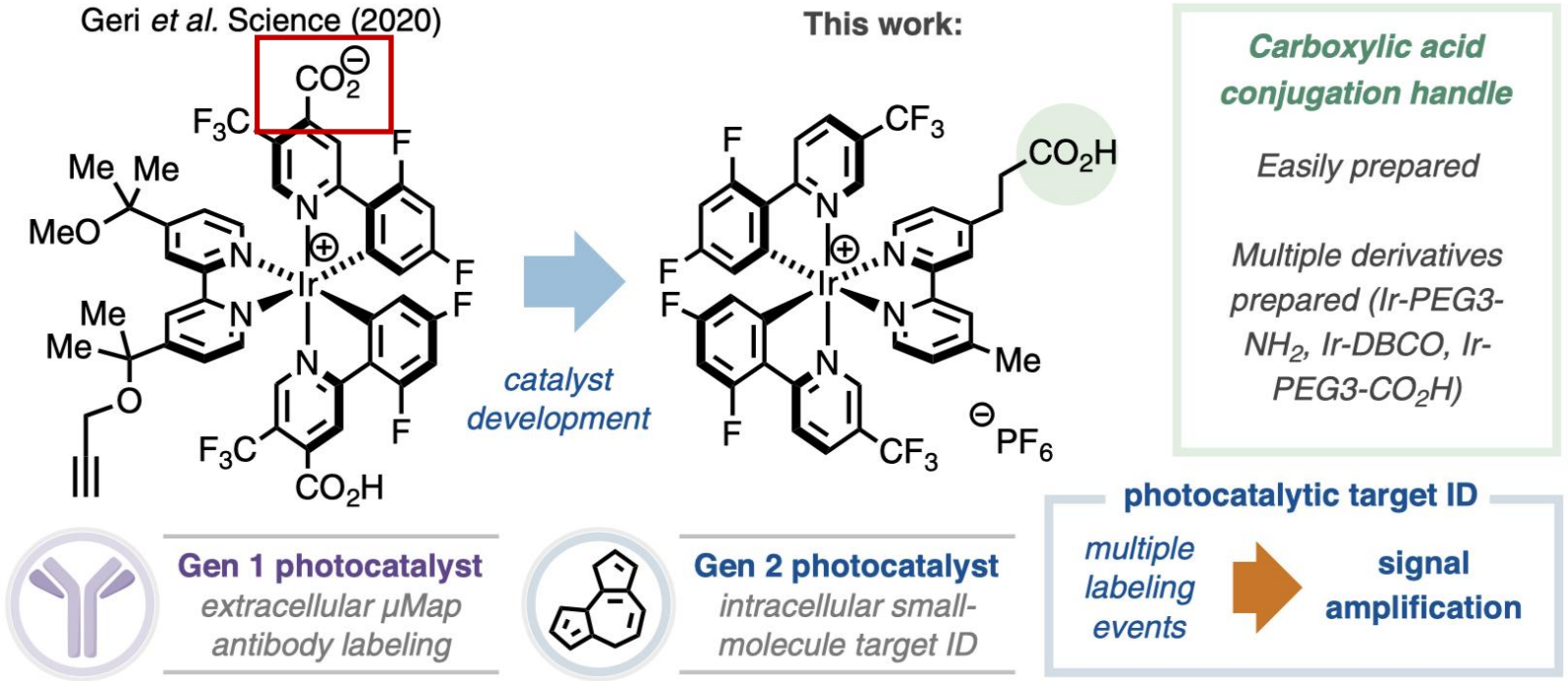

Fig1. intracellular photocatalyst suitable for small molecule target ID

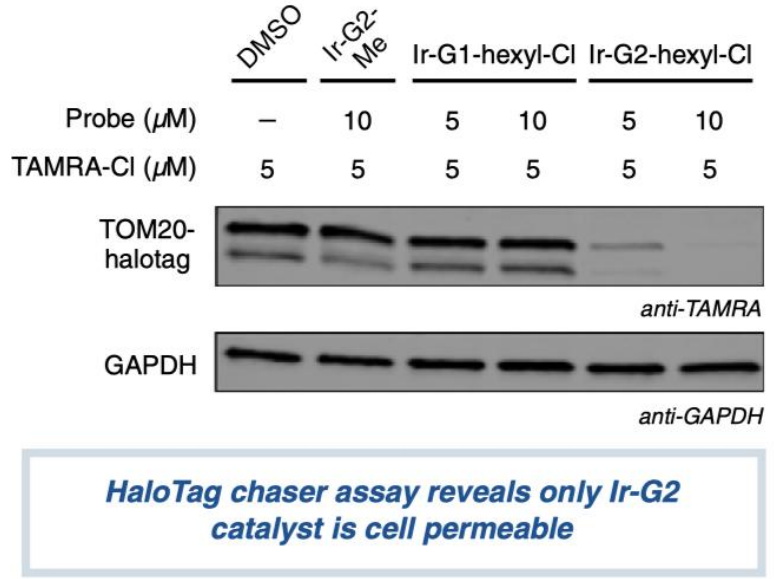

Fig2. Cell permeability of Ir-photocatalysts determined by HaloTag chaser assay

- **Changes of structure truly improve the permeability of Catalyst**

J. B. Geri et al., Microenvironment mapping via Dexter energy transfer on immune cells. Science 367, 1091–1097 (2020).

The photocatalyst truly increase the specificity of Target ID

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To further test whether this method can access any ***Ir-Drug conjugate***

Here, they employ an inhibitor of the BRD4, and a spectator protein CA

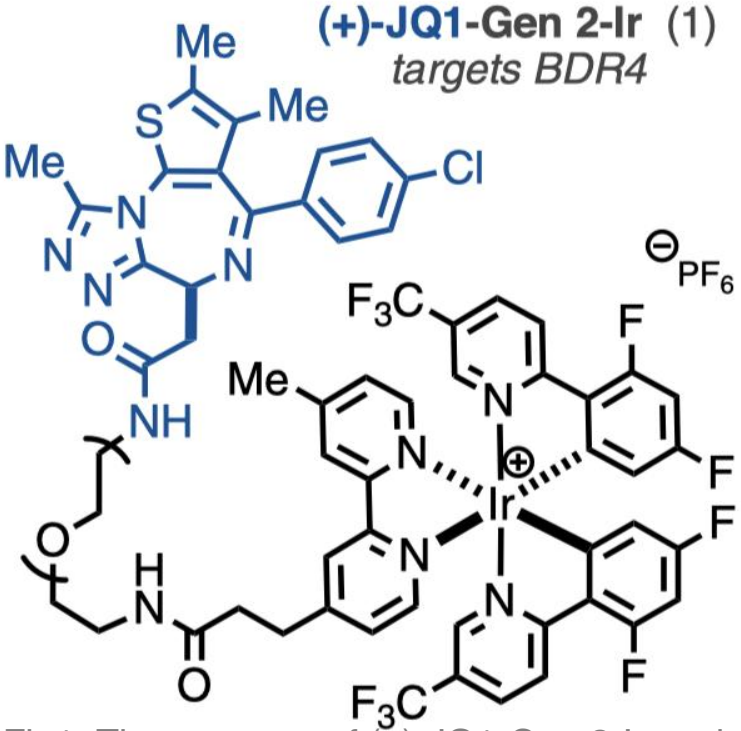

Fig1. The structure of (+)-JQ1-Gen 2-Ir conjugate

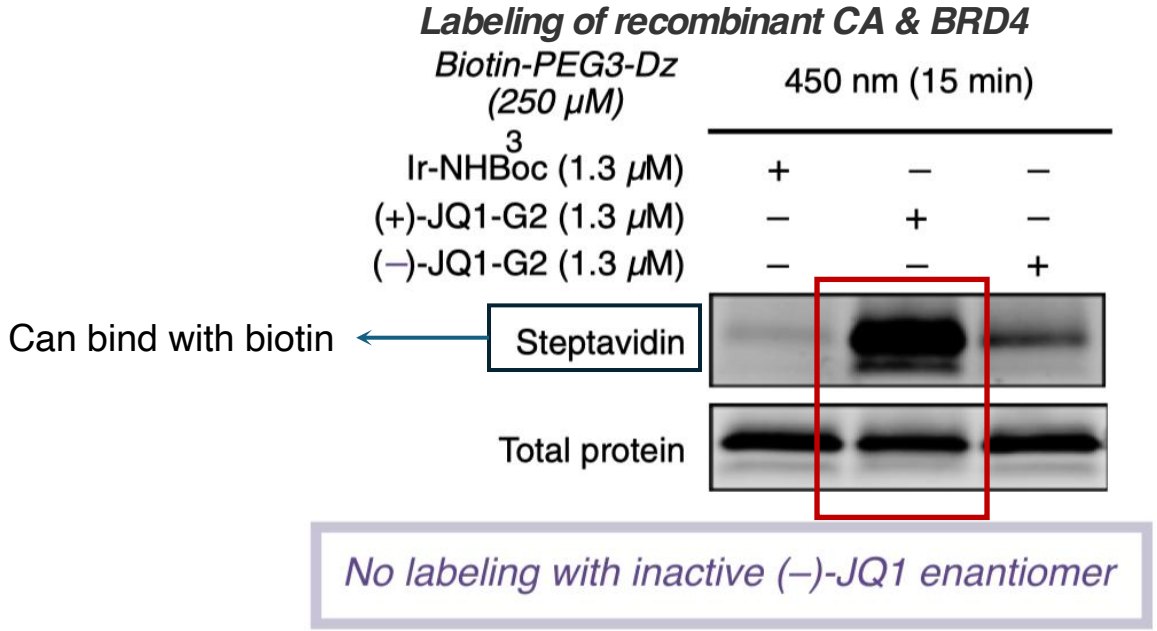

Fig2. Western blot assay result of labelling recombinant CA & BRD4

This Photocatalyst also works in the live cells in a time-dependent manner

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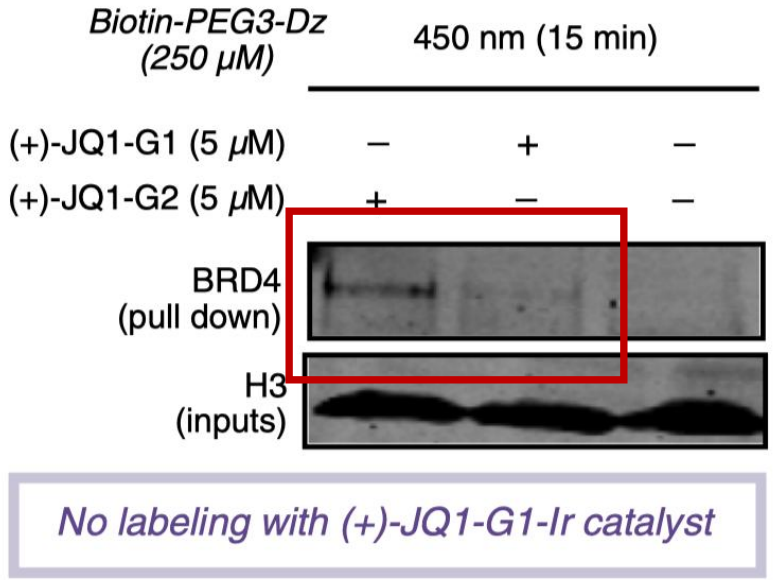

Fig1. Comparing permeability of G1- and G2-based (+)-JQ1 probes following irradiation in HeLa cells

- The **G2-based (+)-JQ1 probe** successfully identified the BRD4 protein

- The intensity of labeling was found to be linearly **related to irradiation time**

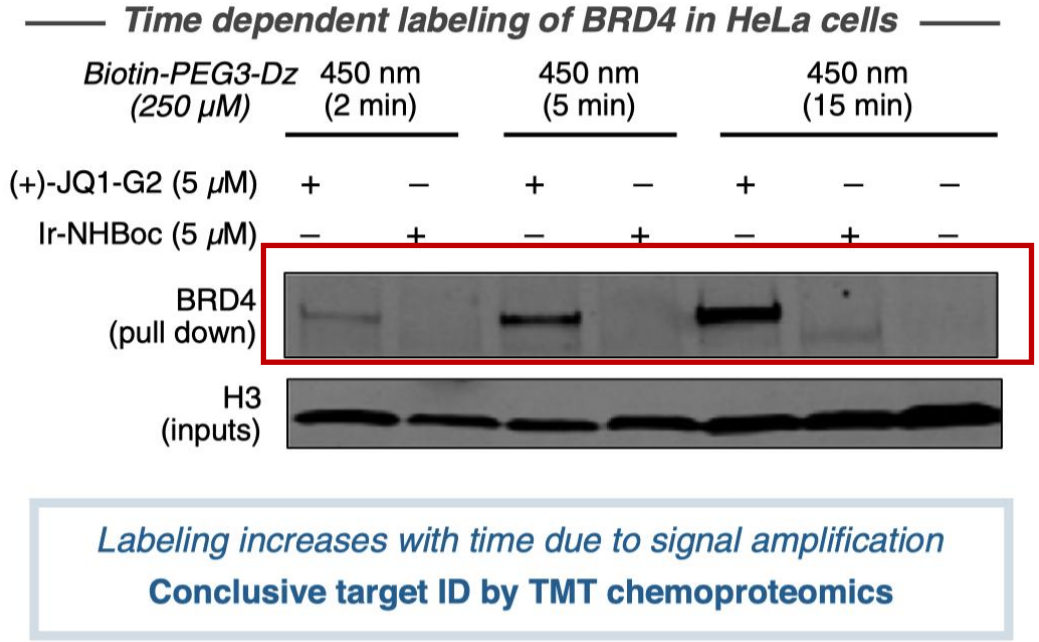

Fig2. BRD4 labeling increases over time (2-min, 5-min, and 15-min irradiation)

The Targeting of (+)-JQ1 is specific

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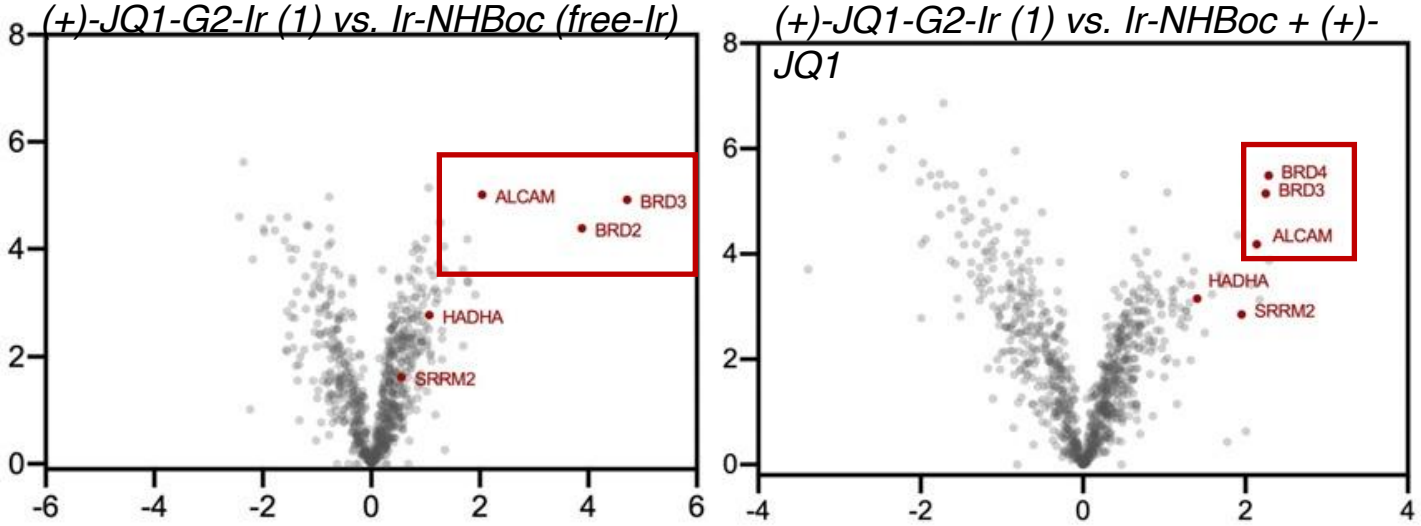

Fig1. Quantitative chemoproteomic analysis shows enrichment of several BRD proteins in HeLa cells labeled with (+)-JQ1-Gen 2

- BRD proteins are **highly enriched** due to the specifically binding of (+)–JQ1–Gen 2, and was **not affected by using Ir–NHBoc**
- The binding and labelling by (+)-JQ1 is indeed **specific**
- Comparing with (-)-JQ1-, only **BRD2 and BRD3** was enriched.
- This stereogenic center improves the **specificity of Targeting**

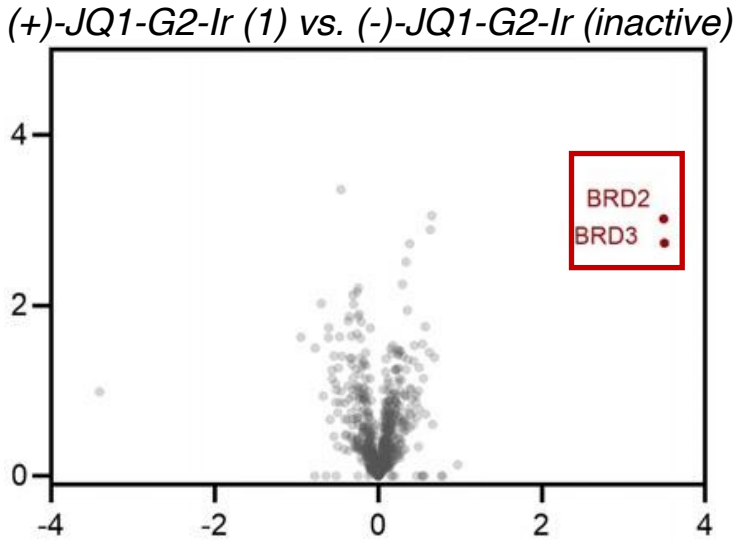

Fig2. Comparative analysis of the interactomes of (+)-JQ1-G2 enantiomers

Comparing with state-of-art PAL

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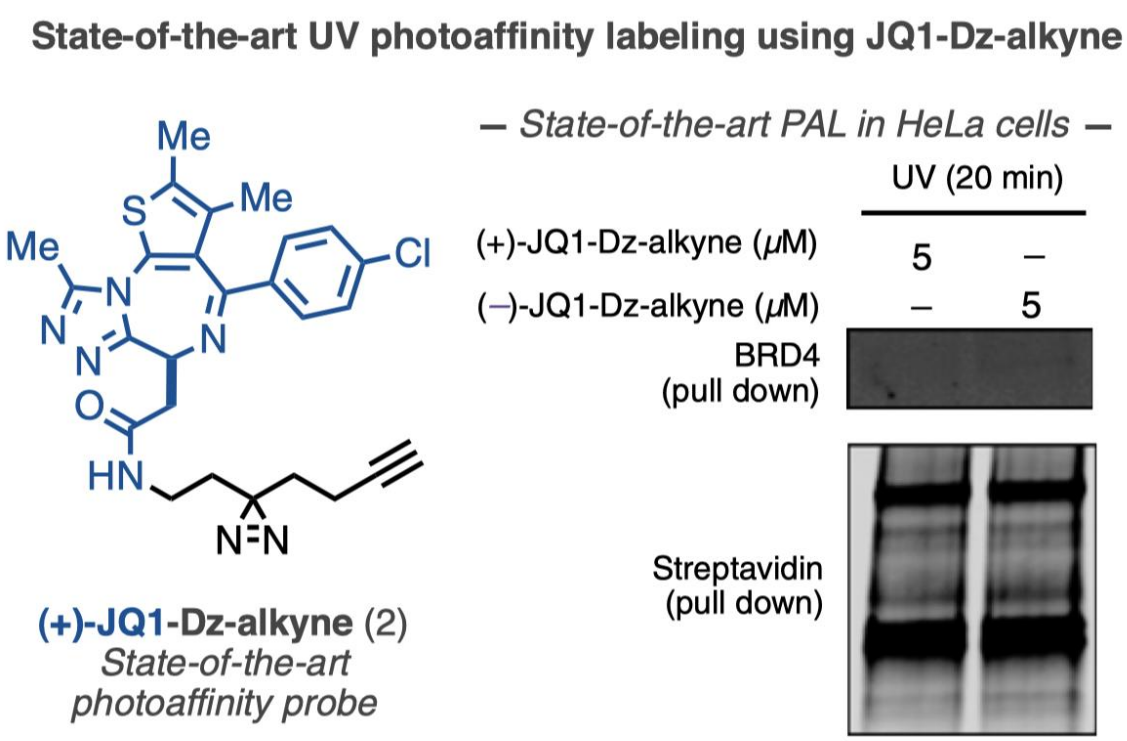

Fig1. State-of-the-art PAL employing active (+)-JQ1- and inactive (–)-JQ1-Dz-alkyne probes

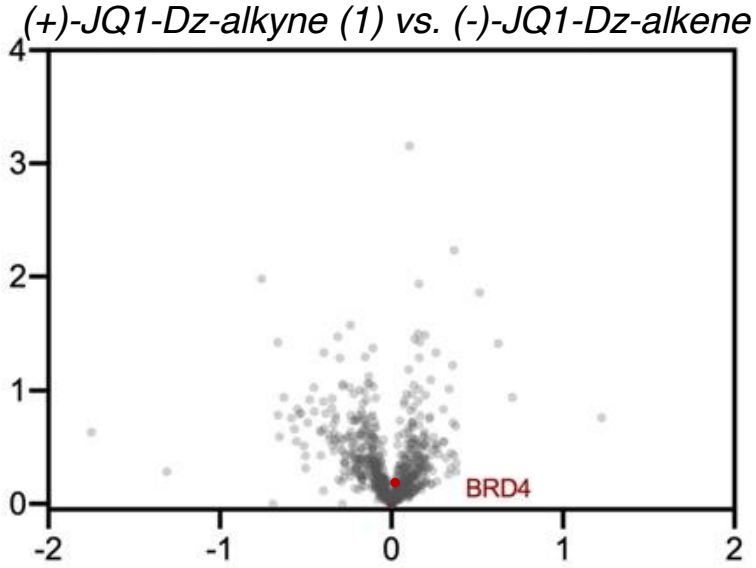

Fig2. TMT-based quantitative chemoproteomic analysis

- The UV-PAL **didn't lead** to selective enrichment of BRD4

Using the Photocatalyst to identify the target of Dasatinib(Das)

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- *Previous studies have demonstrated difficulties in **maintaining potency and cell permeability** using dasatinib-derived probes*

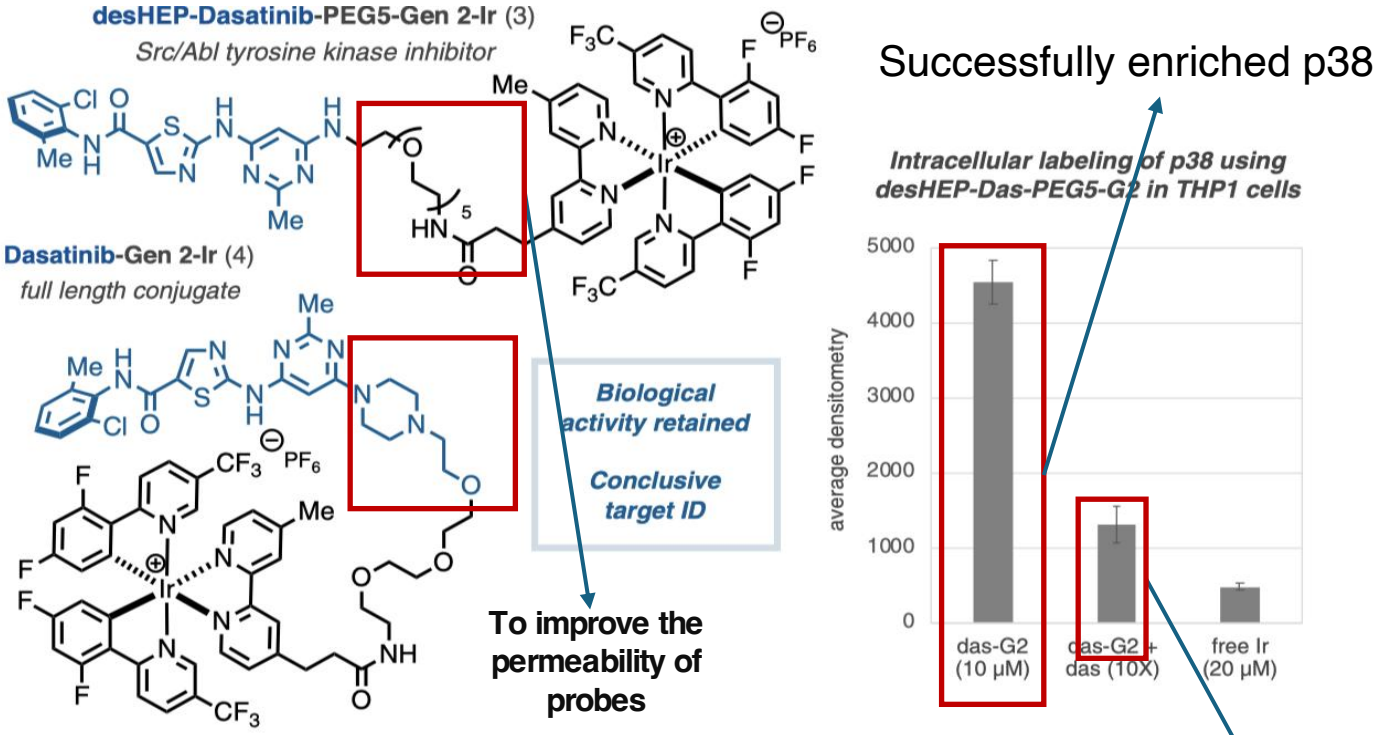

Fig1. The designed Probe and ntracellular labeling of p38 using desHEP-Das-PEG5-G2 in THP1 cells

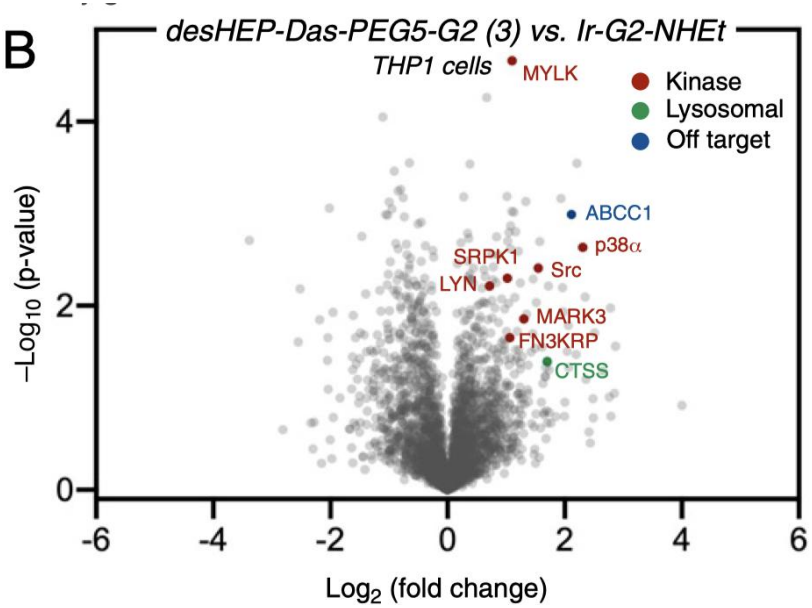

Fig2. Label-free proteomic analysis in THP1 cells

- The desHEP-probe **successfully identified** the target of Das
- Enrichment of several kinases (red), as well as lysosomal proteins (green)

Dasatinib-G2 probe can Identify the target protein as well

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The kinase binding to the probe or natural Das

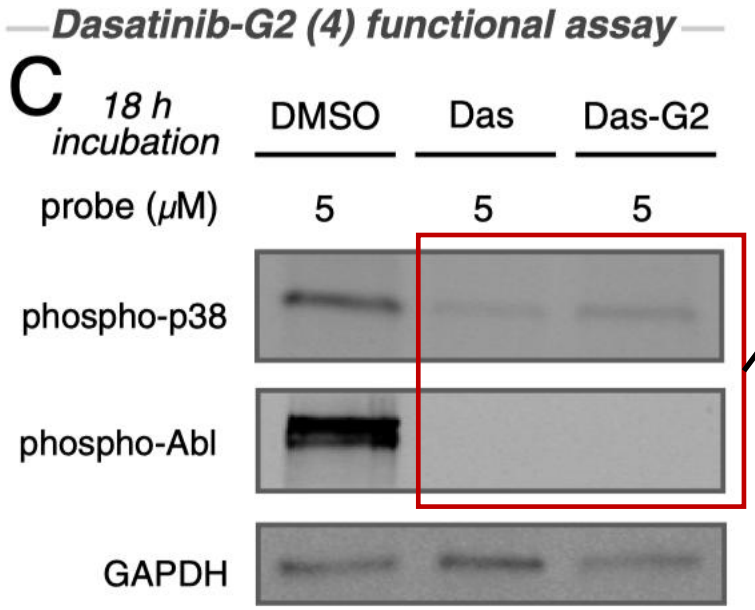

Fig1. Kinase activity assays

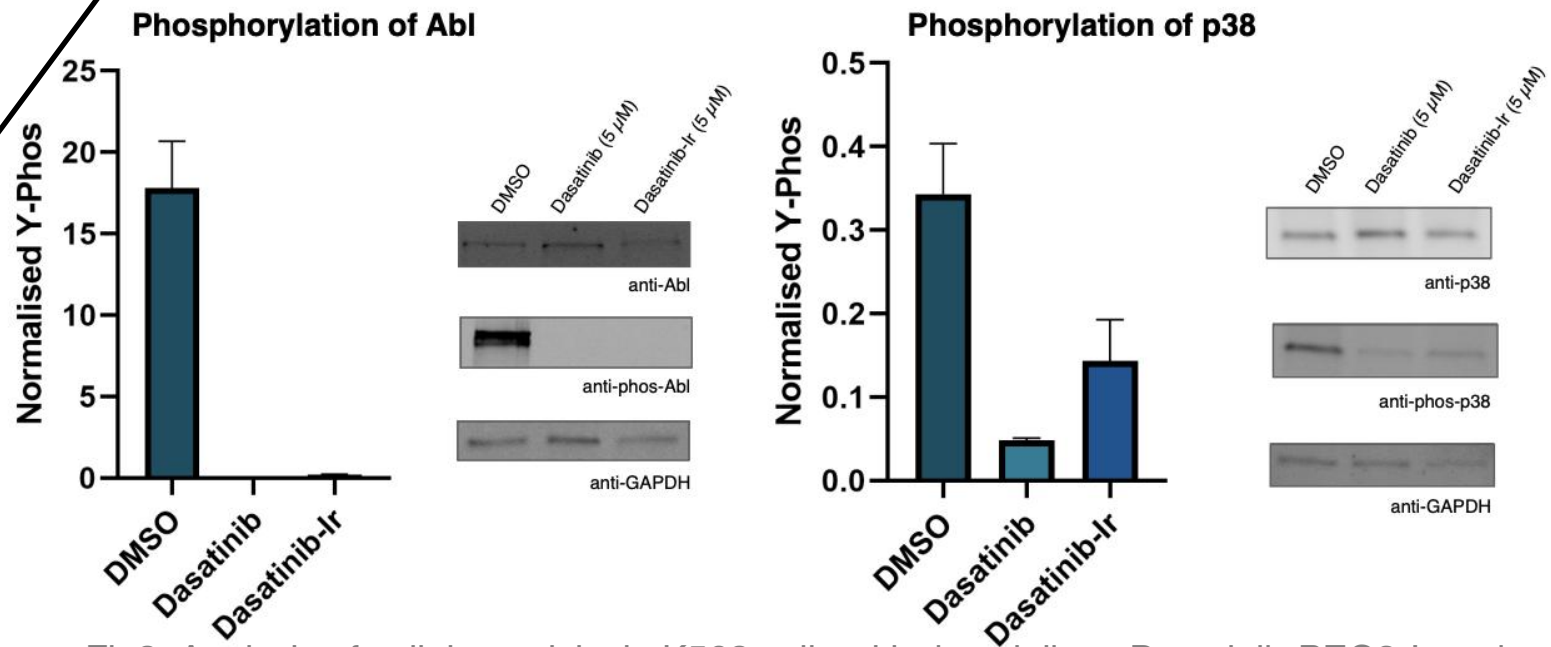

Fig2. Analysis of cellular activity in K562 cells with dasatinib vs Dasatinib-PEG3-Ir and DMSO controls.

Comparing with state-of-art PAL

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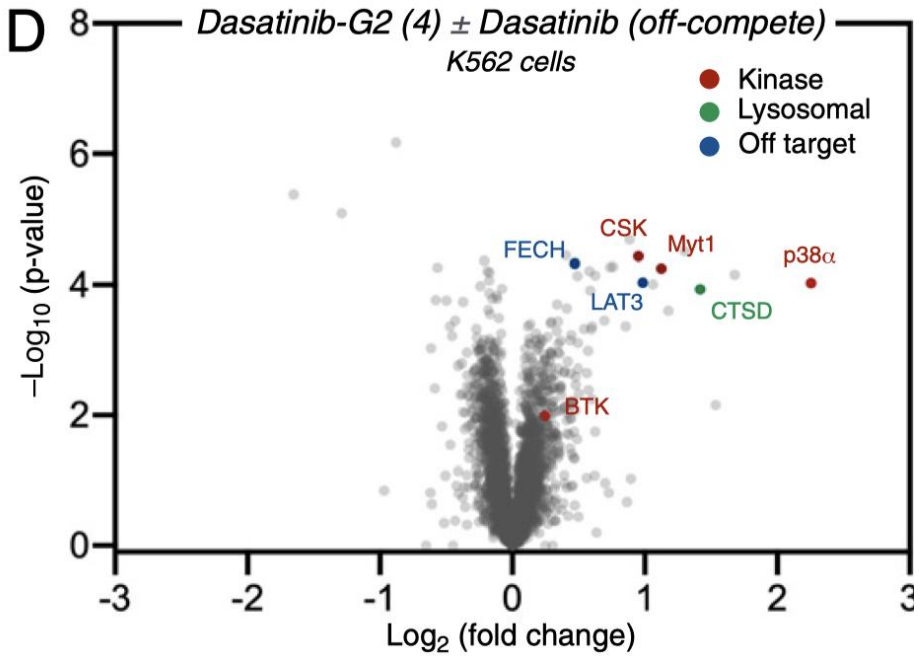

Fig1. TMT-based quantitative chemoproteomic analysis in K562 cells

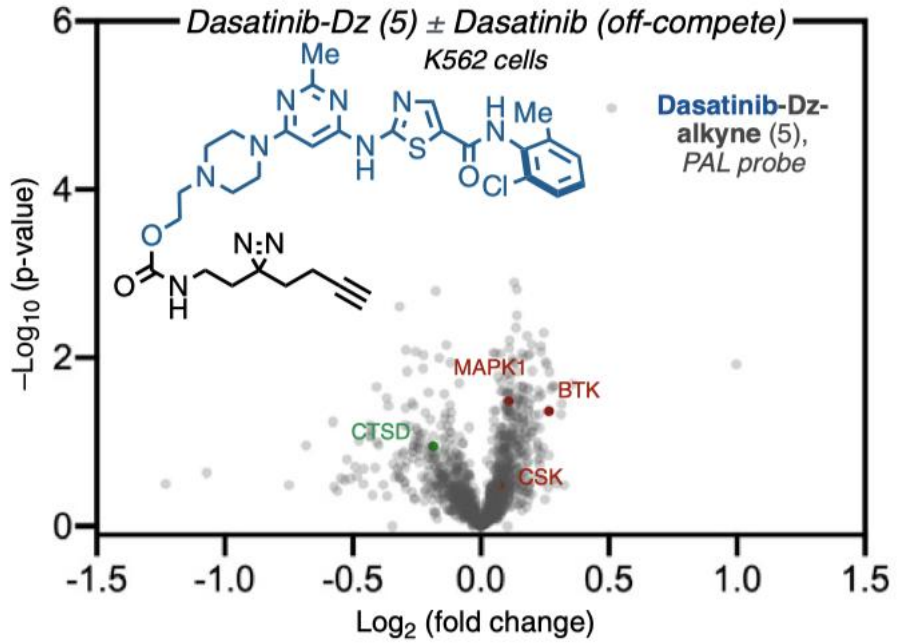

Fig2. TMT-based quantitative chemoproteomic analysis in K562 cells

- The Dasatinib-G2 identified extensive enrichment of **kinases p38, Myt1, and CSK**, confirming the **binding specificity**

- The Dasatinib-Dz only lead to **trace enrichment of certain kinases**

Identification of Paclitaxel targets

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- *Paclitaxel have been proposed to be binding to **microtubules**, leading to stabilization and mitotic arrest however, the full extent of its mechanism remains unclear*

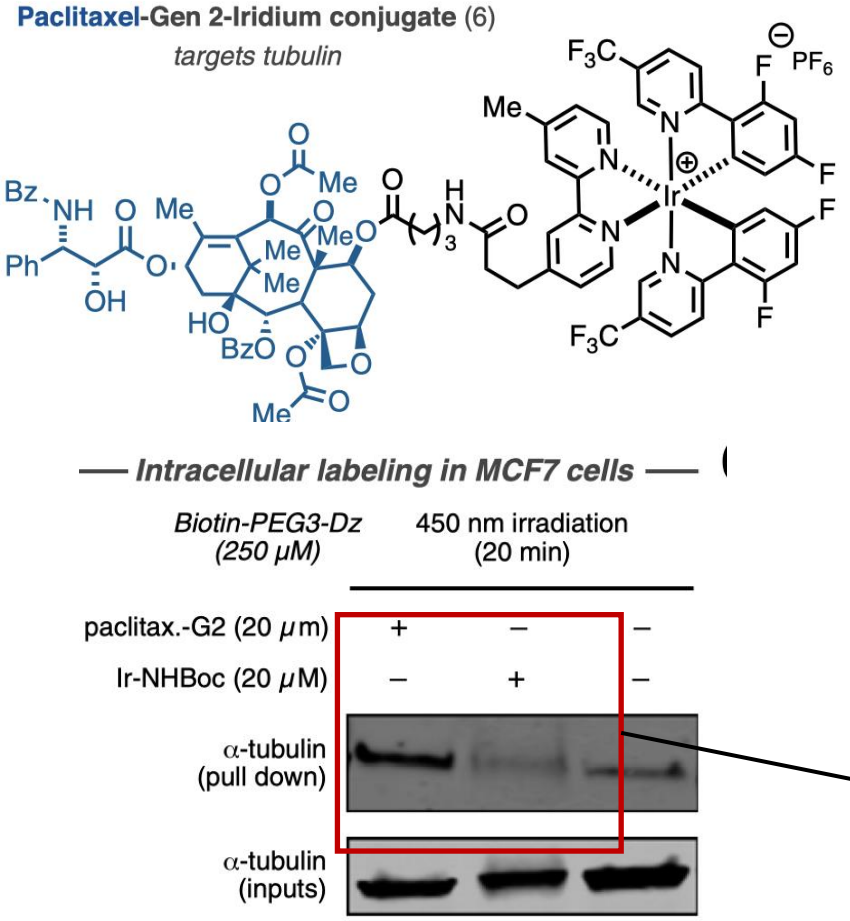

Fig1. Western blot analysis with anti-α-tubulin

- The **isoforms of tubulin** also enriched by photocatalyst

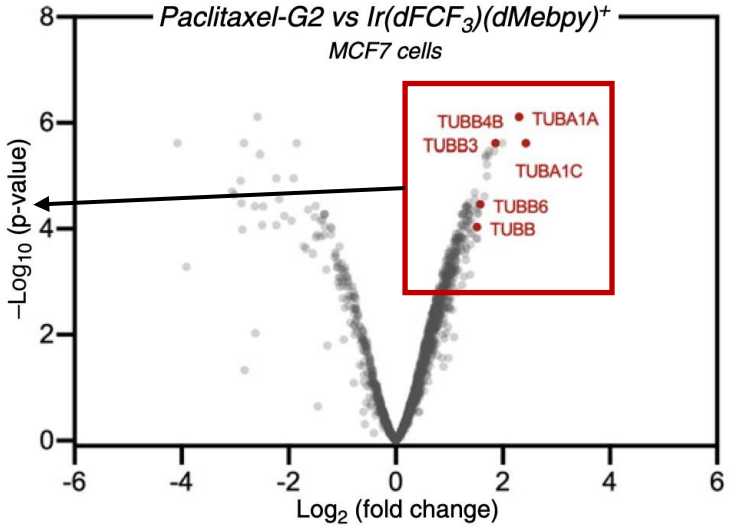

Fig2. TMT-based quantitative chemo-proteomic analysis in MCF7 cells

- **Successful labeling of tubulin** in breast cancer cells with Gen2-Iridium

Photocatalystic Target ID is an ideal platform for Targeting GPCRs

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After discussing the efficiency of photocatalytic target ID for Intracellular protein. We turned our attention to cell surface….

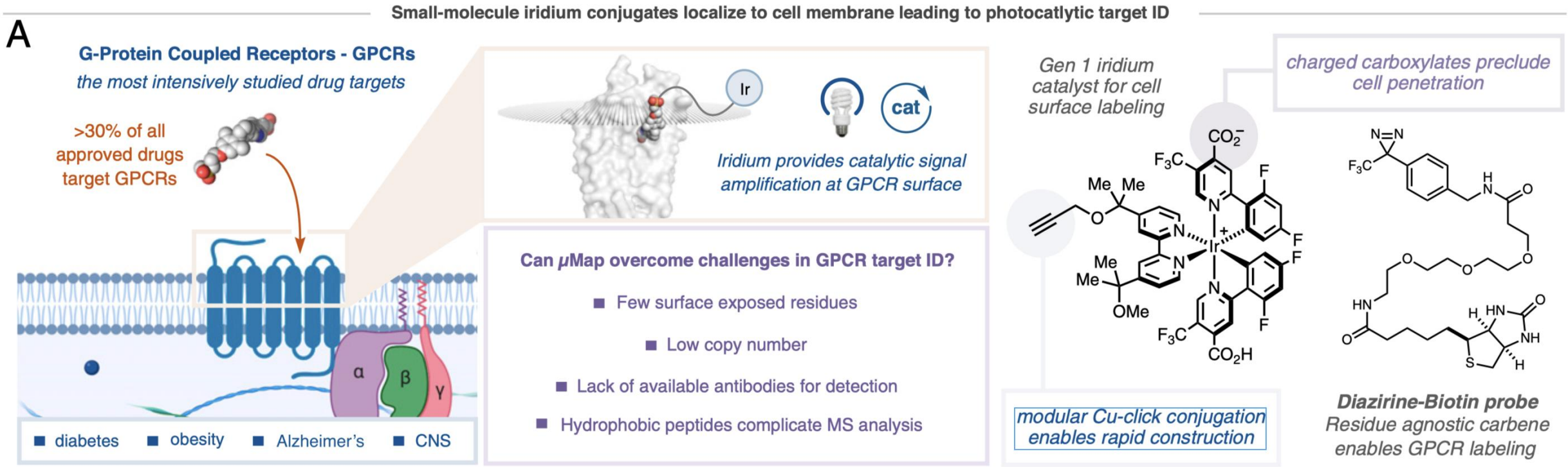

Fig1. An overview of the use of photocatalytic iridium conjugates for targeting G protein-coupled receptors (GPCRs)

Photocatalytic target identification (ID) versus classical UV-based PAL

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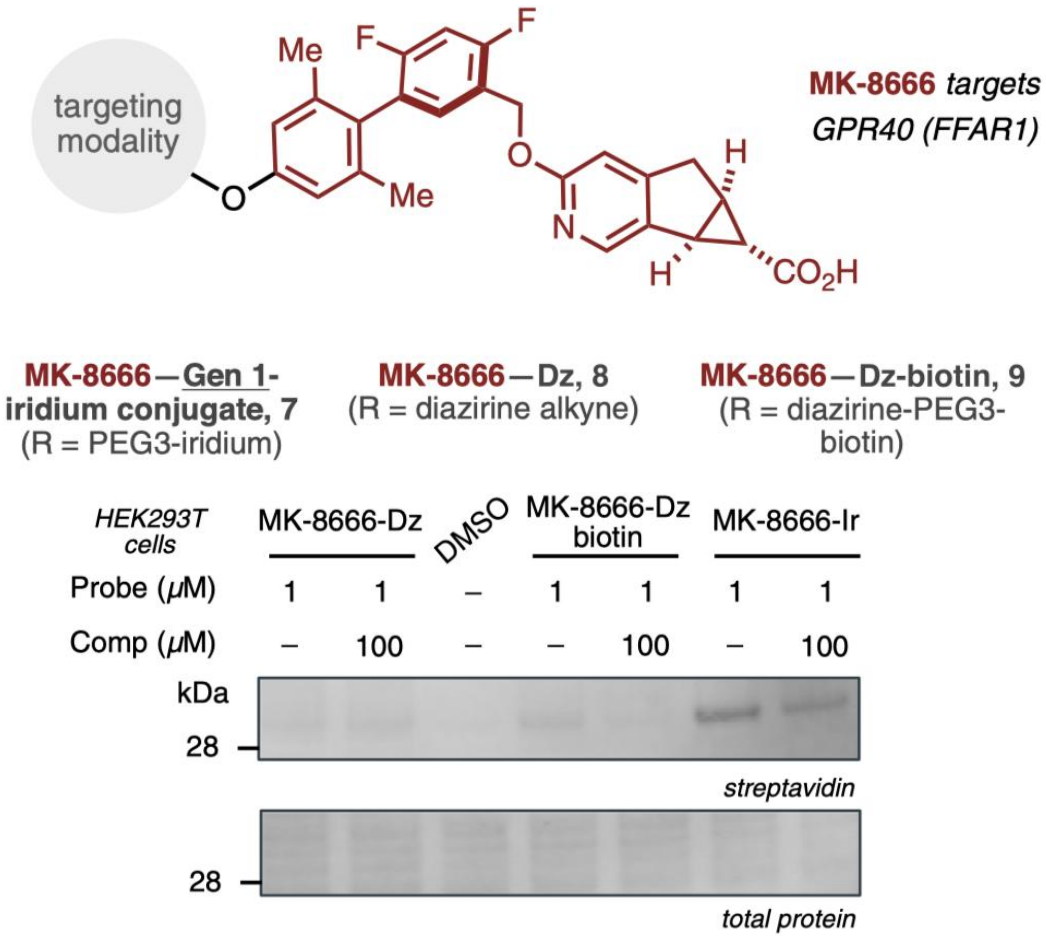


Fig1. Comparative analysis of labeling of GPR40

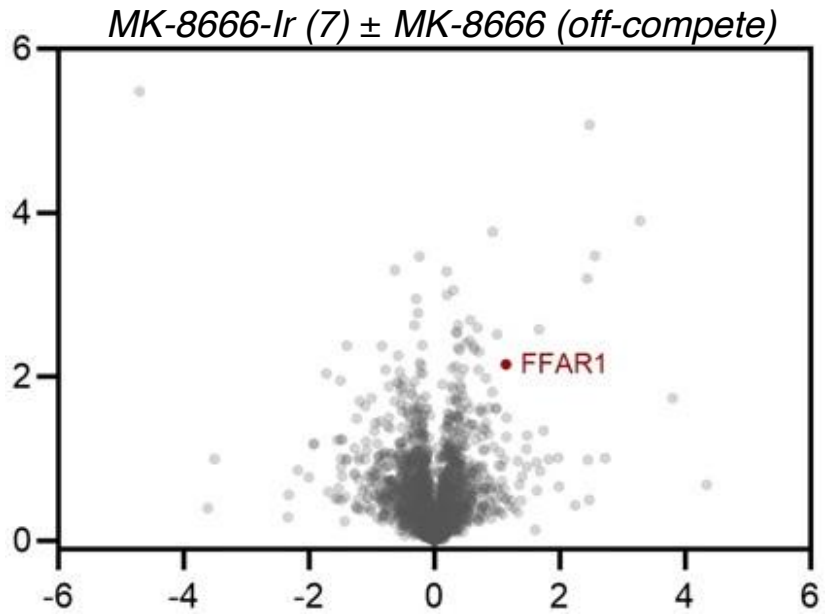
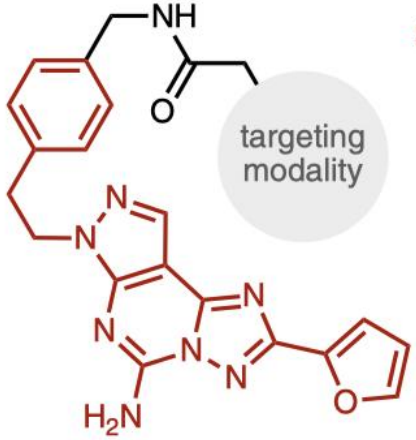


Fig2. TMT-based quantitative chemo-proteomic analysis in *GPR40-expressing HEK293T cells*

- Photocatalyst G1 **labelled more GPR40 protein** than Dz-biotin
- FFAR1 was **significantly** changed using MK-8666-Ir

Another example of membrane protein



SCH58261 *targets* A_{2A} *(ADORA2A)*

SCH58261—Gen 1-iridium conjugate, 10 (R = PEG3-iridium)

SCH58261—Dz, 11 (R = diazirine alkyne)

- Photocatalyst G1 **more significantly identified ADORA2A** in different cell types

SCH58261-Dz (11) ± SCH58261 (off-compete)

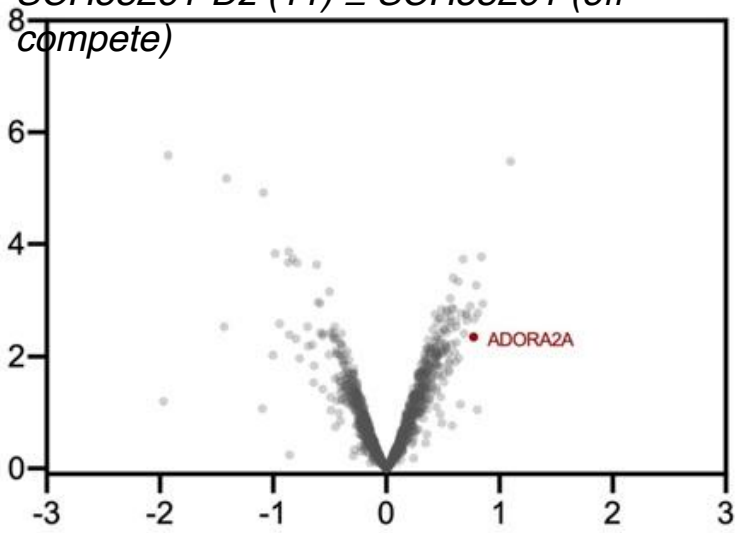


Fig1. TMT-based quantitative chemo-proteomic analysis in *A2a-expressing HEK293T cells*

SCH58261-G1 (10) ± SCH58261 (off-compete)

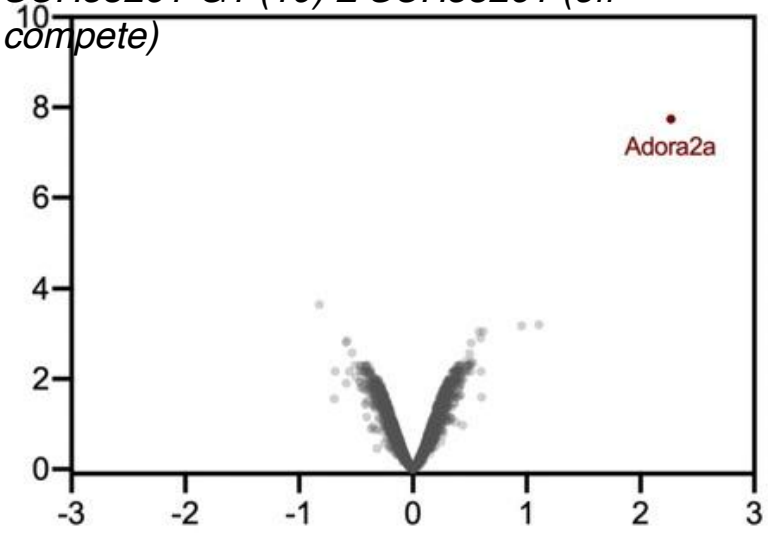


Fig2. TMT-based quantitative chemo-proteomic analysis in *PC-12 cells*

Pros and Cons

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Pros

- This research describe a **general platform for Target ID** of the intracellular proteins and **prove its reliability**
- The photocatalytic target ID has allowed for the identification of multiple protein targets and off-targets across **multiple drug classes** and **cellular compartments**
- The whole **process of validating the reliability** can also served as a reference for other similar researches
- This technology uses blue light, which ensure the **sample integrity**

Cons

- The author did not mention the limitation of this technology
- The specificity can be further improved
- There might be some side effect of this photocatalyst because of the metal ions

Questions

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- What are the difficulties for small molecule drug's target identification?

  Transient interaction

  Specificity

- What further research we can conduct with this new technology?