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SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

Selection of DNA-encoded chemical libraries against endogenous membrane proteins on live cells

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- Chemical biology
- Nucleic acid chemistry
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- DNA-encoded Chemical Library (DEL) in drug discovery
- Chemical biology study of nucleic acids
- Design, synthesis of bioactive small molecules and target identification

Background

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- *Membrane proteins are situated in the hydrophobic lipid bilayer of the cell membrane, which makes in vitro biochemical techniques, such as **protein expression and purification**, extremely difficult*

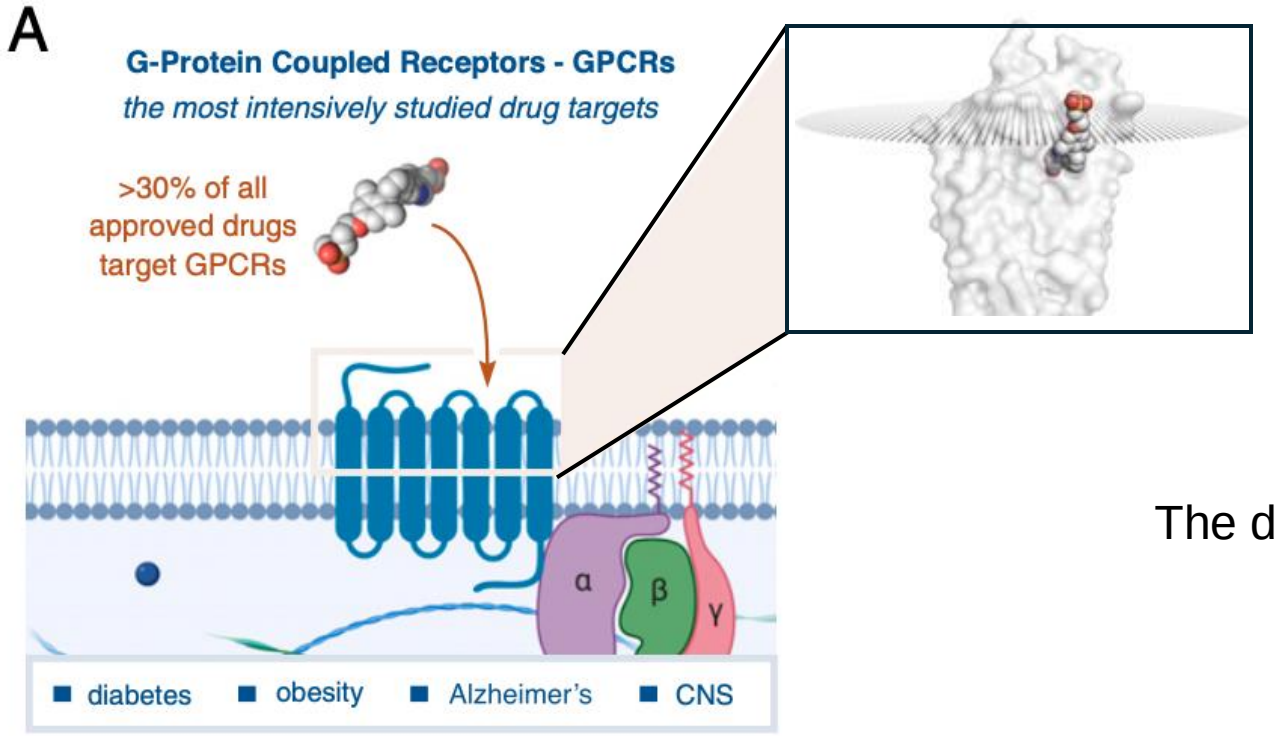

Fig1. Schematic overviews of GPCRs

"Outside their native contexts, membrane proteins may lose important biological features"

The development of **ligand discovery methods** compatible with natural cellular conditions

Trowbridge, Aaron et al. "Small molecule photocatalysis enables drug target identification via energy transfer.", PNAS

Background

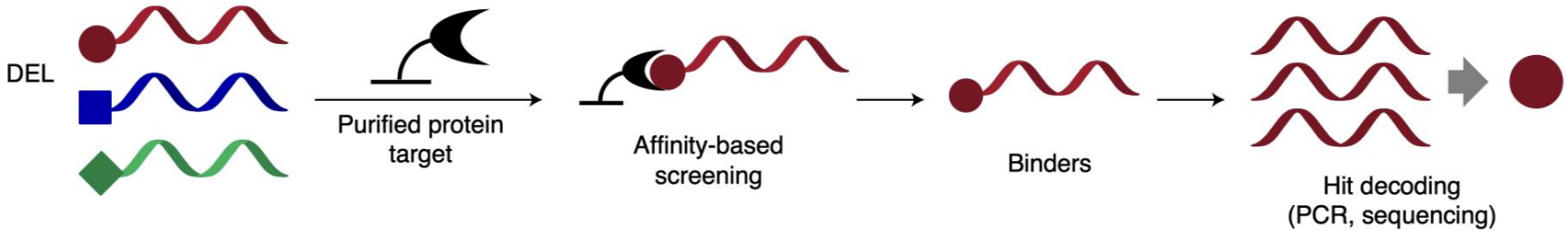

Fig1. Process of DEL screening

❖ *"Target-specific DEL selection against* ***endogenous membrane proteins on live cells*** *has yet to be achieved."*

Major difficulties:

- ❖ The cell surface contains many **other proteins and biomolecules**
- ❖ Difficulties in identifying the **ligands** with a **dissociation constant (*Kd*) higher** than or close to the target concentration

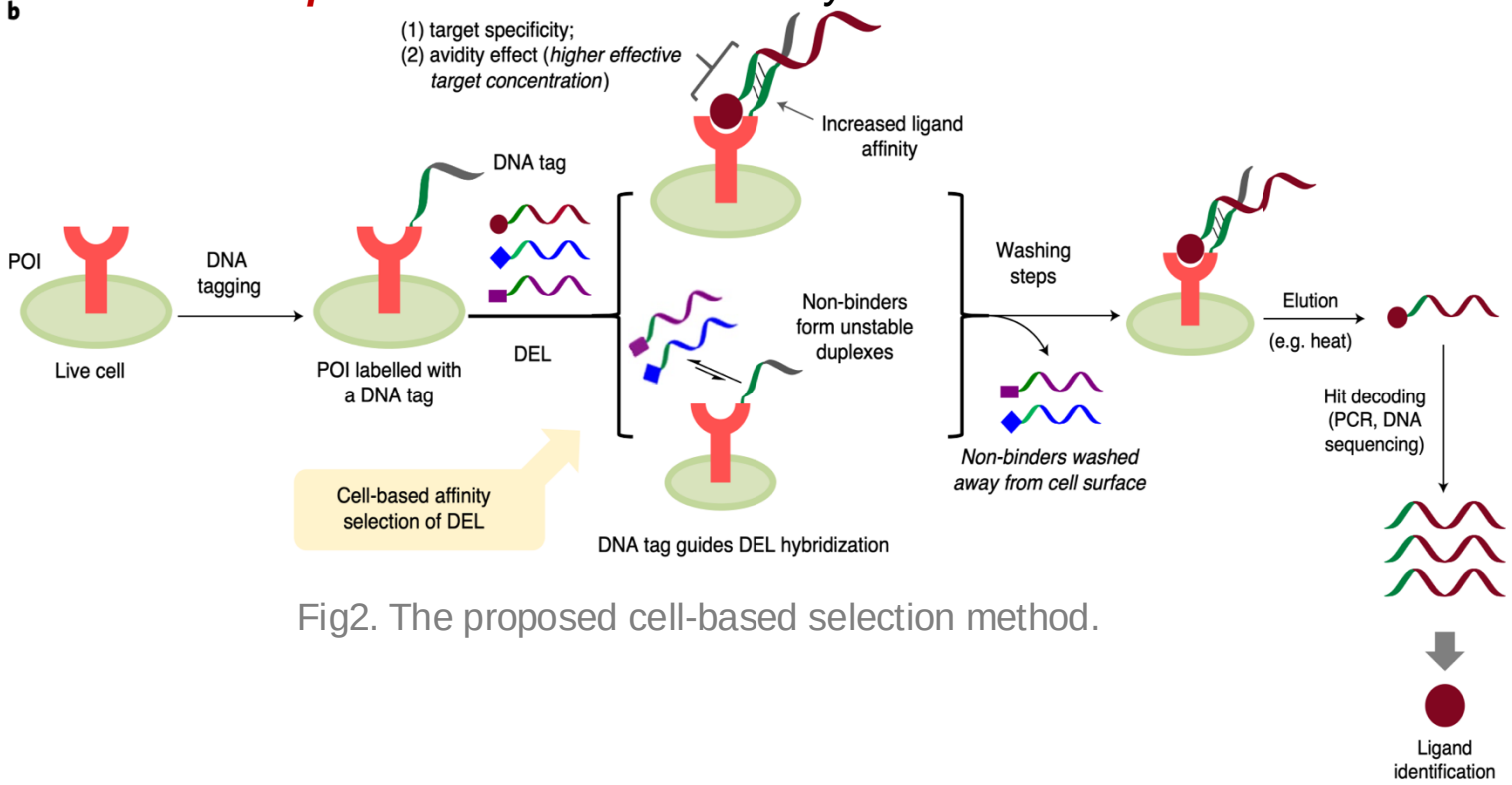

Fig2. The proposed cell-based selection method.

Method to label membrane proteins with a DNA tag

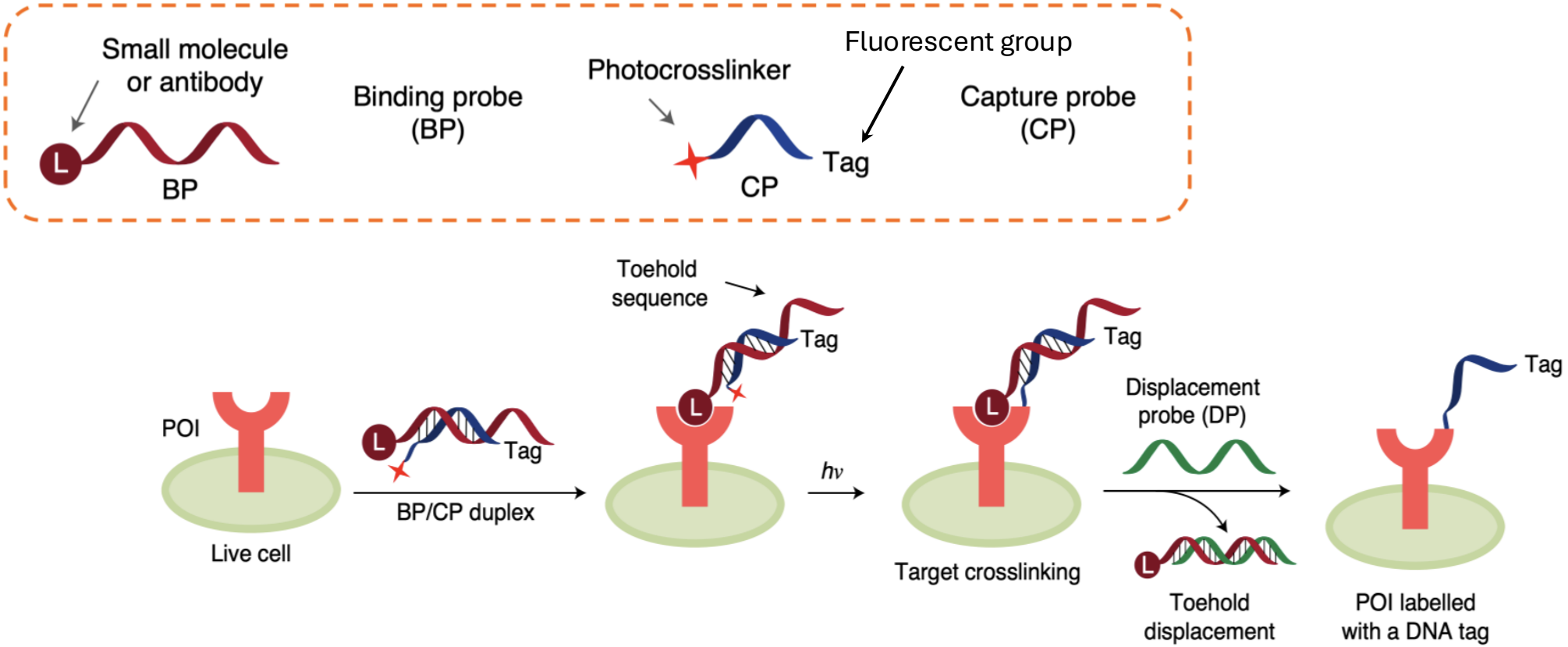

Fig1. Schematic overviews of how to label the membrane proteins with DNA tag

Validating the specificity of FR labelling

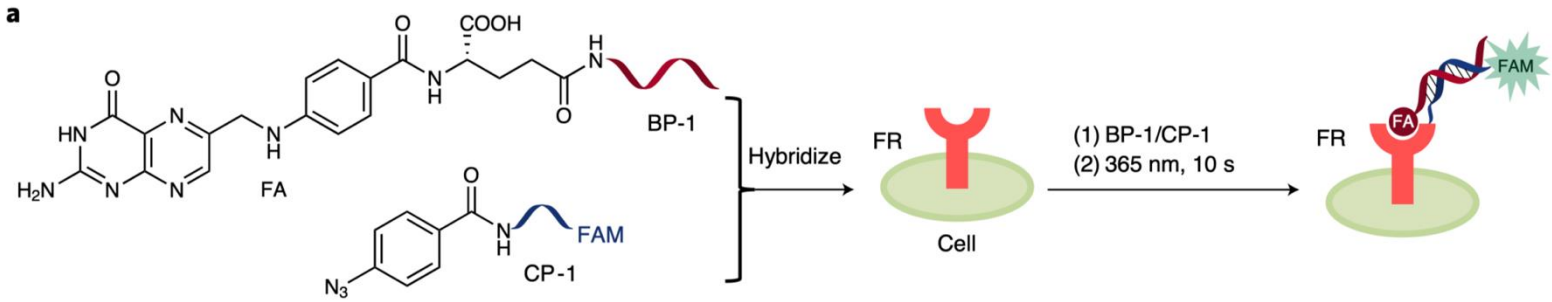

Fig1. Schematic Cells were labelled with BP-1/CP-1

- Fluorescence mostly **surrounded the cell membrane**
- The labelling was **specific** and dependent on **DNA-mediated photo-crosslinking**

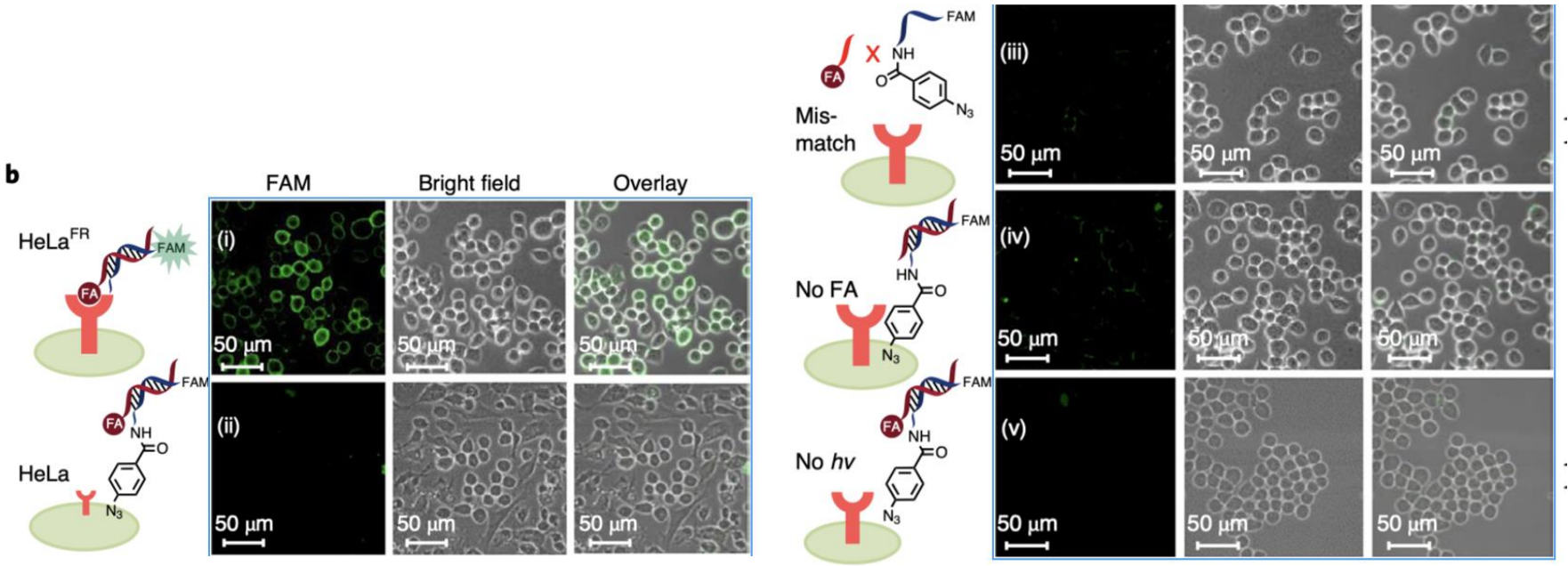

Fig2. Confocal images of the labelled cells: HeLa

Validating the specificity of FR labelling

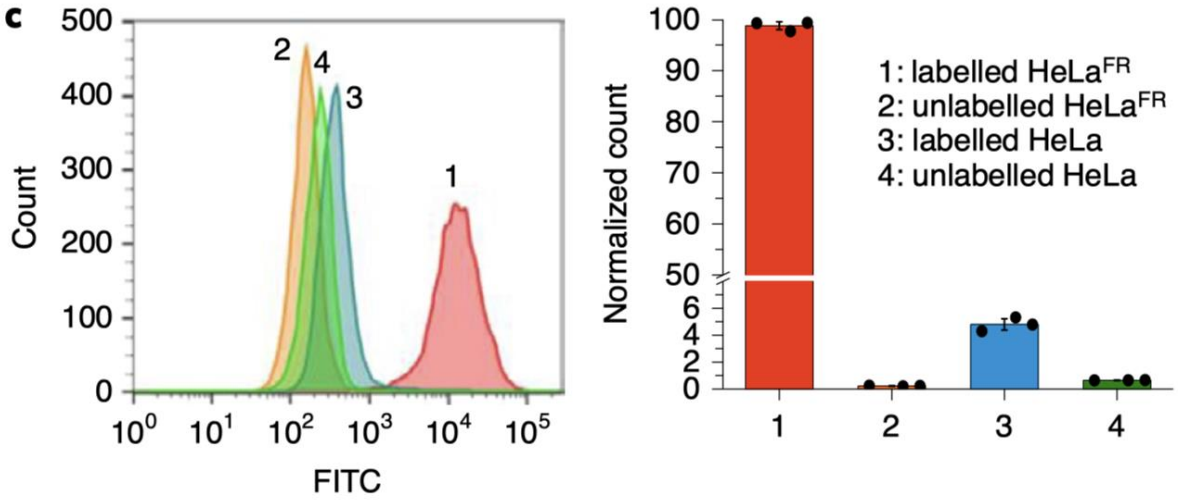

Fig1. Flow cytometry analysis of the labelled or unlabelled cells

- FR was **specifically labelled**
- Mass spectrometry (MS) analysis confirmed the **labelling specificity**

- HeLa^{FR} cells were more **efficiently labelled**

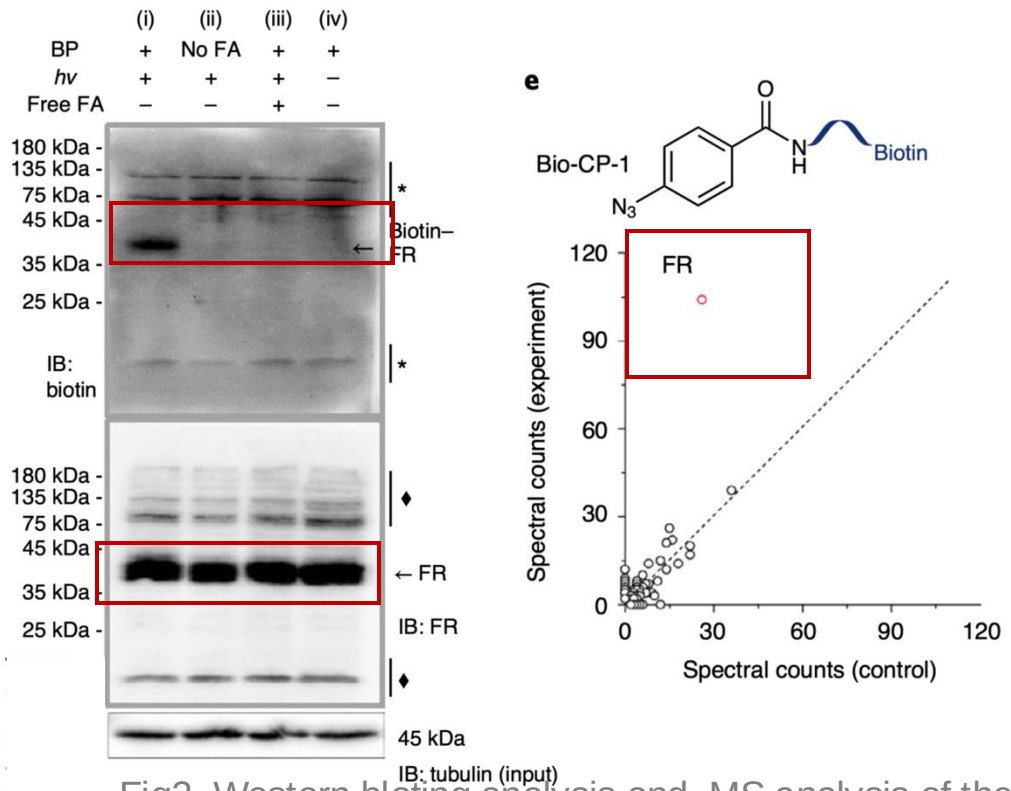

Fig2. Western bloting analysis and MS analysis of the affinity-purified proteins

The BP-1/CP-1 tend to label cell with a higher expression of POI

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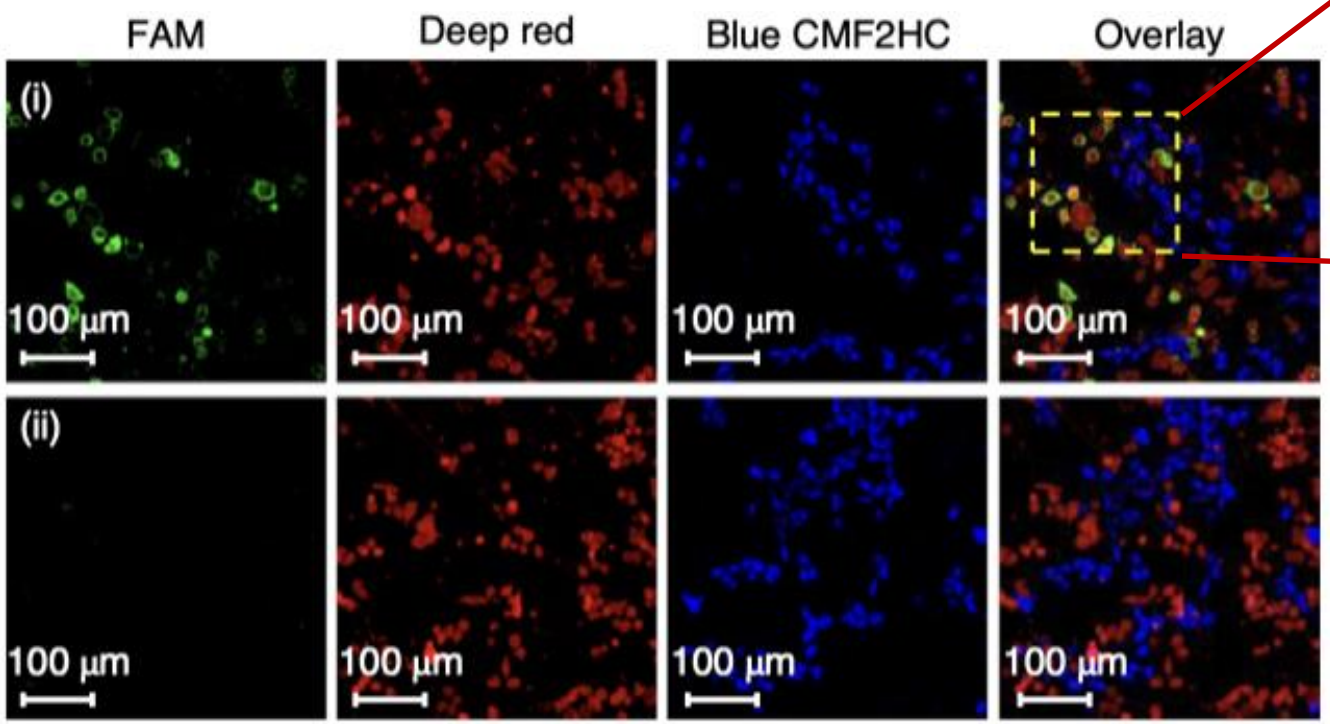
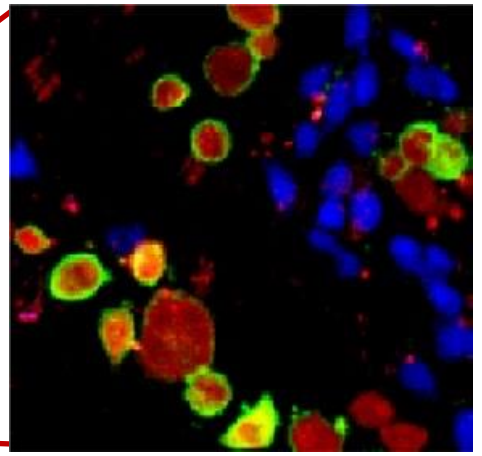


Fig1. HeLaFRand B16-F10 cells were stained with CellTracker Deep Red and Blue CMF2HC dyes



B16-F10 *Have a low FR expression*

- Nearly all the labelling occurred on **HeLa^{FR} cells (red)**
- **B16-F10 cells (blue)** were barely labelled.

The BP-1/CP-1 tend to label cell with a higher expression of POI

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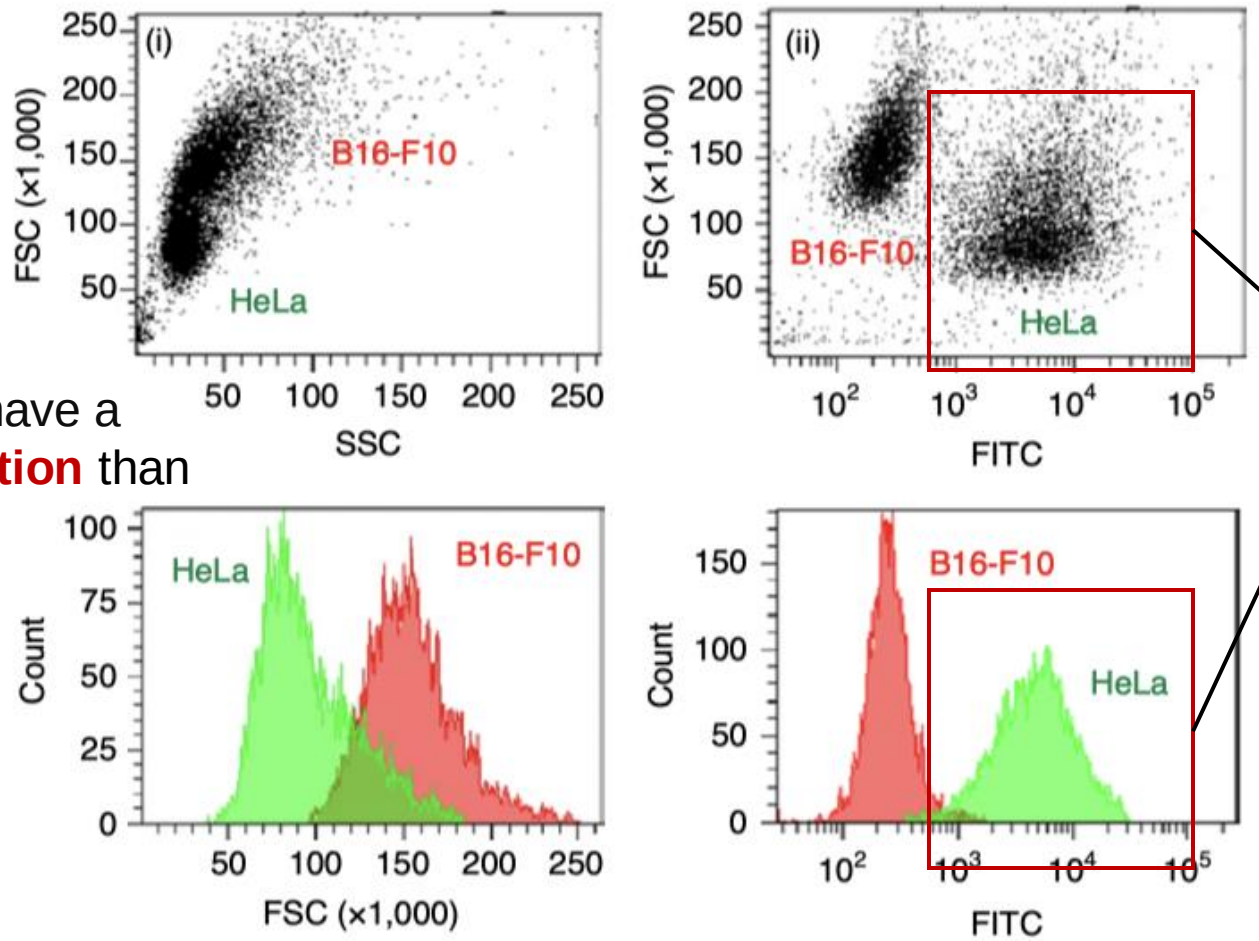
- HeLa cells (green) have a **lower FSC distribution** than B16-F10 cells



- B16-F10 cells show **low FITC fluorescence** while HeLa cells exhibit **high FITC fluorescence**

Fig1. Analysed with flow cytometry by cell size and granularity (i) and by fluorescence (ii)

BP removal using toehold displacement after the labelling of FR

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- Make this fragment can be **displaced by toehold**
- And use FAM to visualize **whether this tag is changed**

- Small molecule ligand

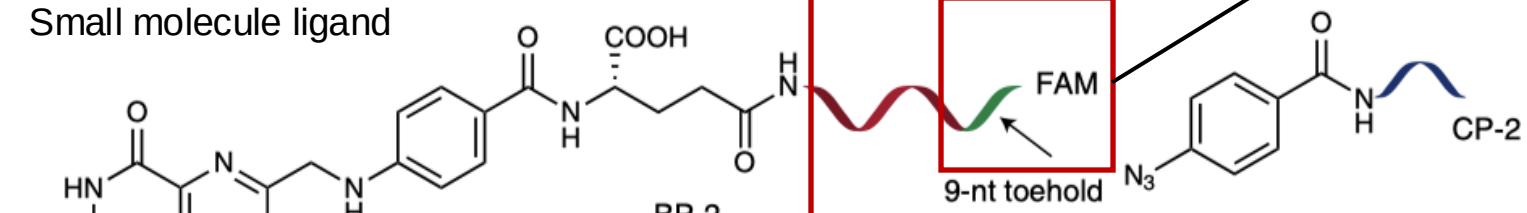

Fig1. The structures of BP-2 and CP-2

To validate

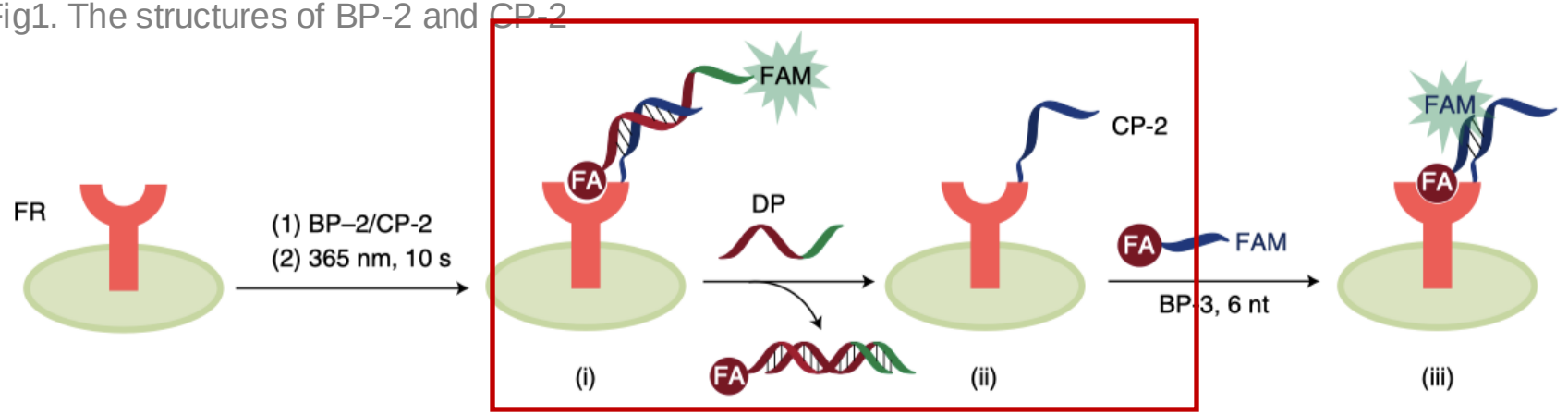

Fig2. Schematic Overview of BP removal

The tag could hybridize with DNA-conjugated small molecules

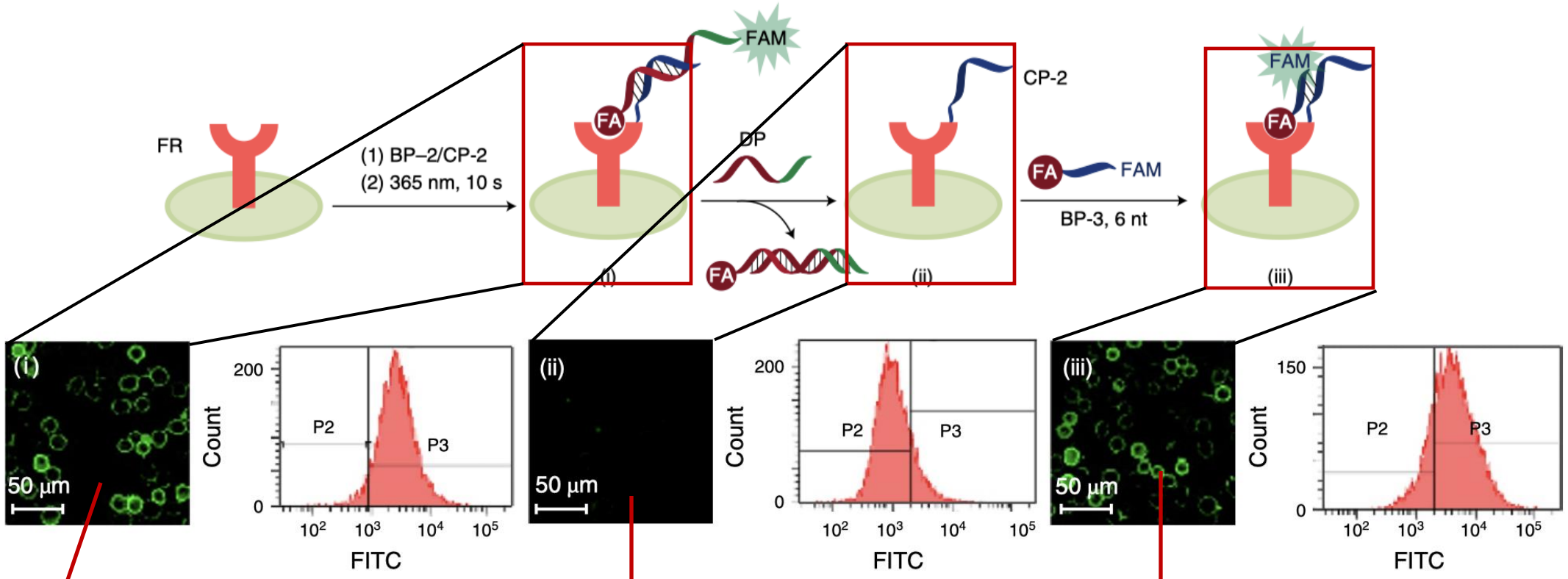

Fig1. Micrograph images of the BP removal process

Strong fluorescence was observed after labelling

DP addition greatly **reduced the signal**

BP-3 **restored** the cell fluorescence

❖ Small-molecule ligands can direct the labelling of membrane proteins with a DNA tag on live cells

Using antibody to target the POI

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"Many membrane proteins do not have a known small-molecule ligand"

- **CP may crosslink to the antibody** may reduce the labelling efficiency.

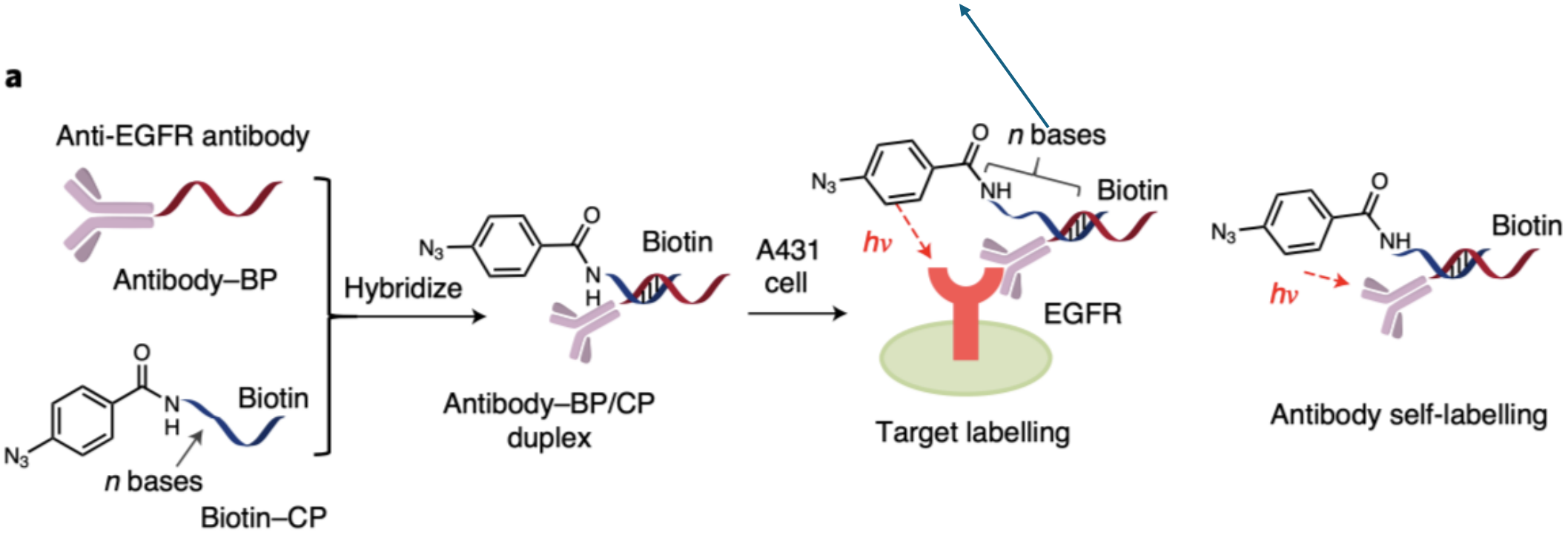

Fig1. Schematic Overview of using Antibody-BP to target the POI

The level of self-labelling could be lower by increasing the length of n-bases

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❖ CPs with ***n* = 18, 21 and 25** showed efficiently labelling with relatively low levels of self-labelling

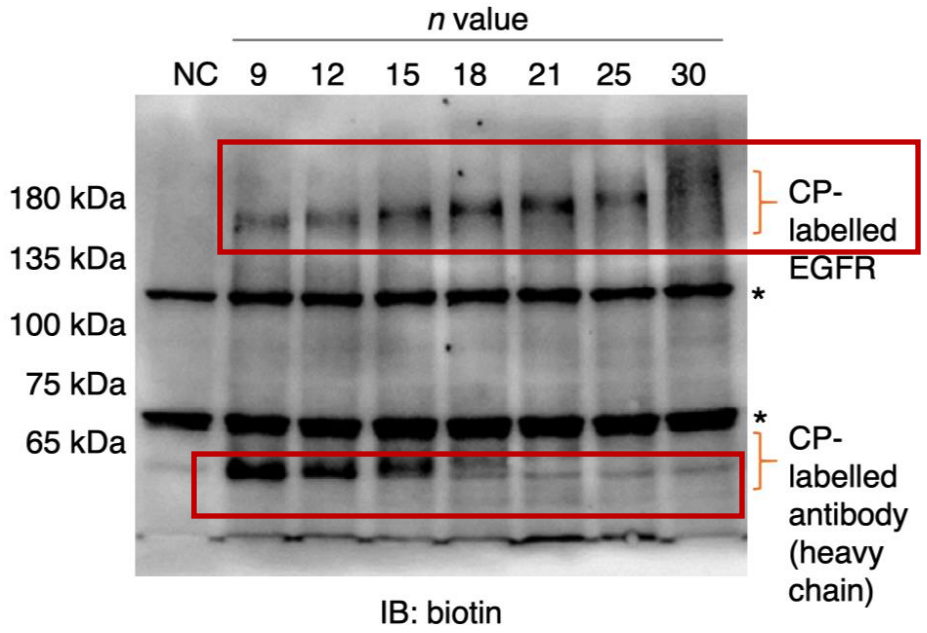

Fig1. Western blotting of labelling of EGFR with antibody-guided probes

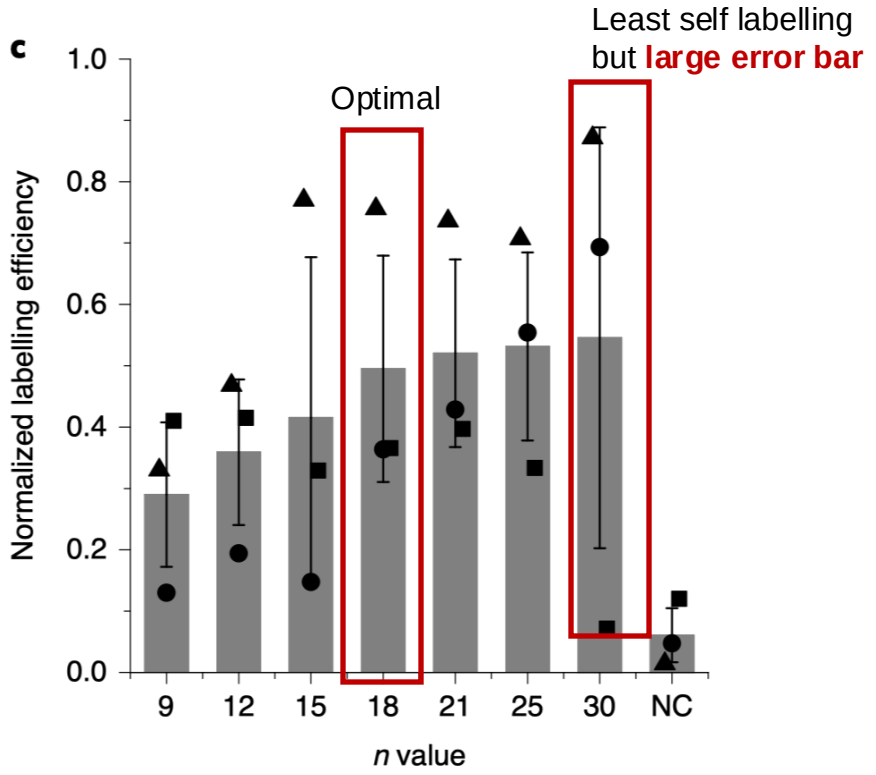

Fig2. Column graph summarizing the labelling results

Validation of the specificity of using antibody labelling

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- Several negative control experiments shows **no or little labelling**

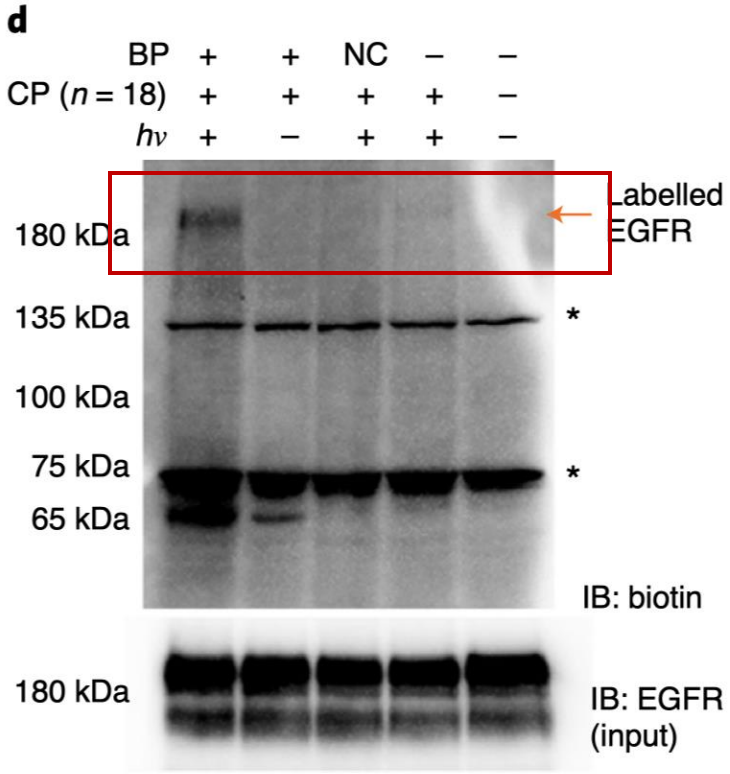

Fig1. The labelling experiment with antibody–BP/CP (n = 18)

- MS characterization confirmed the **labelling specificity**

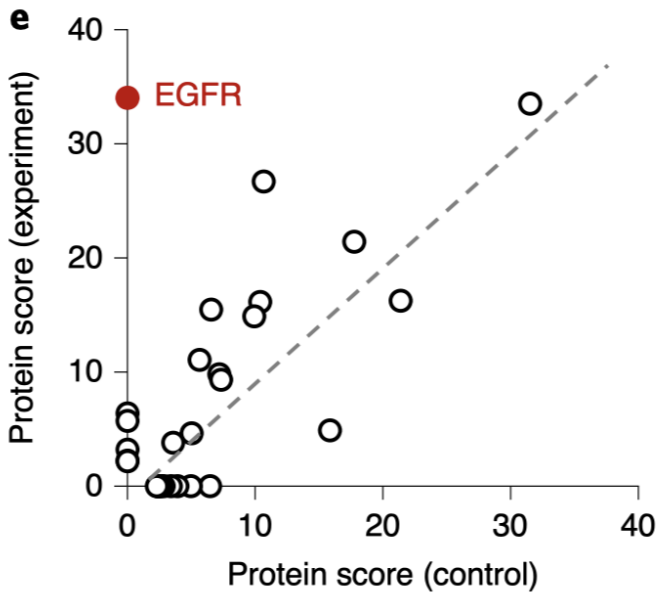

Fig2. MS analysis of the affinity-purified proteins

Selection of DELs against membrane proteins on live cells

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- Prepare **a 4,800-member tripeptide DEL** and spiked in an FA–DNA as a positive control

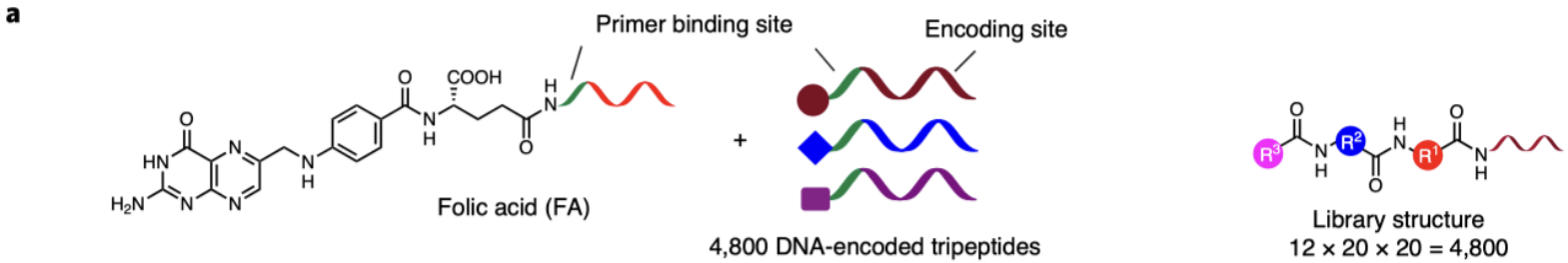

Fig1. The composition of the 4,801-member DEL

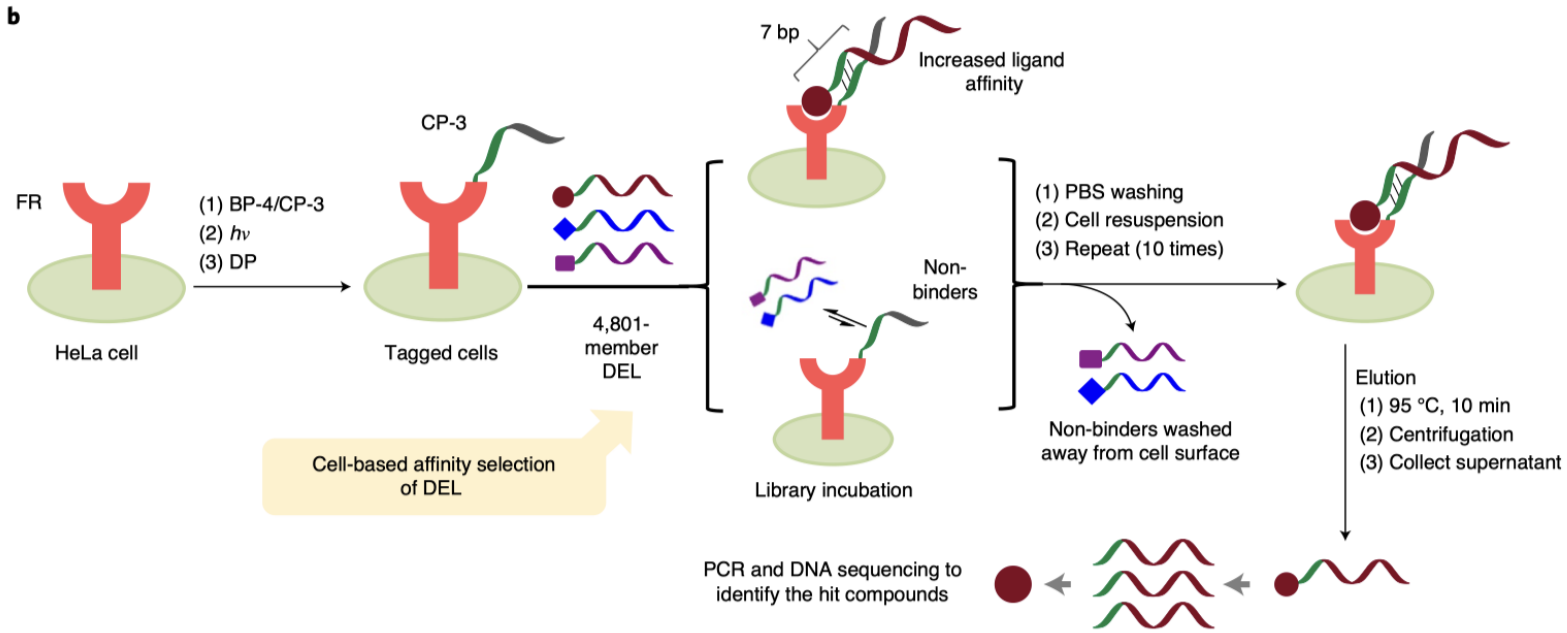

Fig2. Regular HeLa cells were labelled with BP-4/CP-3 and incubated with the library

- HeLa cells were labelled with an **FA-conjugated BP/CP probe pair** (BP-4/CP-3

FA-FR system validated the reliability of This DEL selection

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- With the labelled cells, **FA was strongly enriched**, whereas **FA was not enriched with the unlabelled cells**

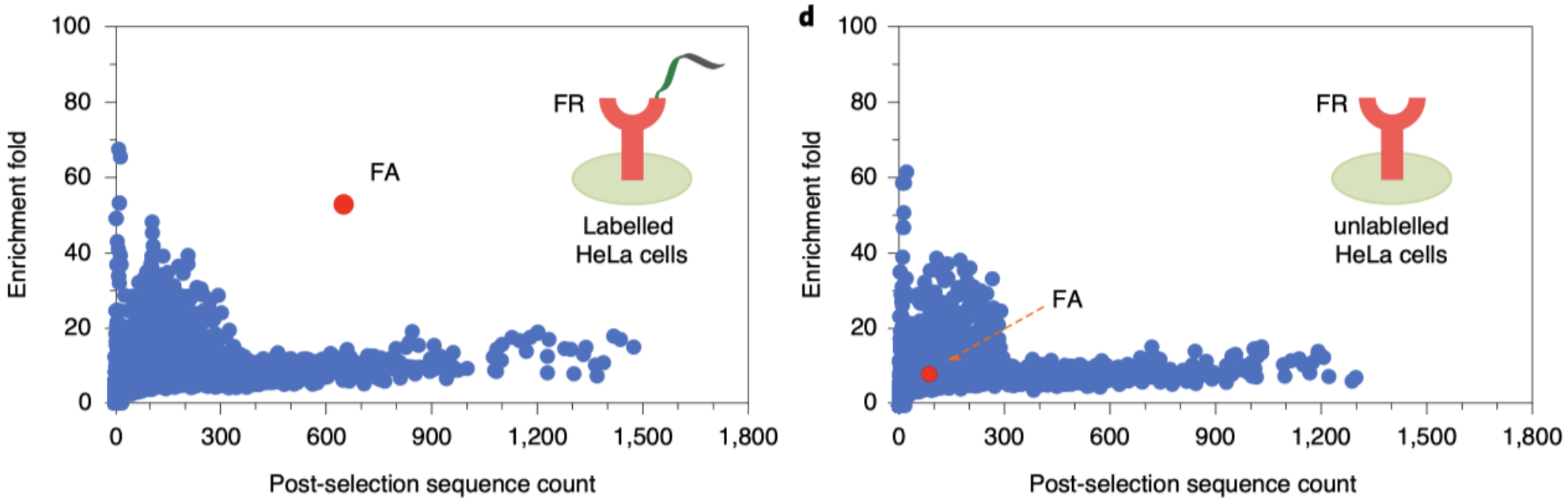

Fig1. Scatter plots of the selection results for the labelled (left) and unlabelled (right)

Validating the selection with a larger Library

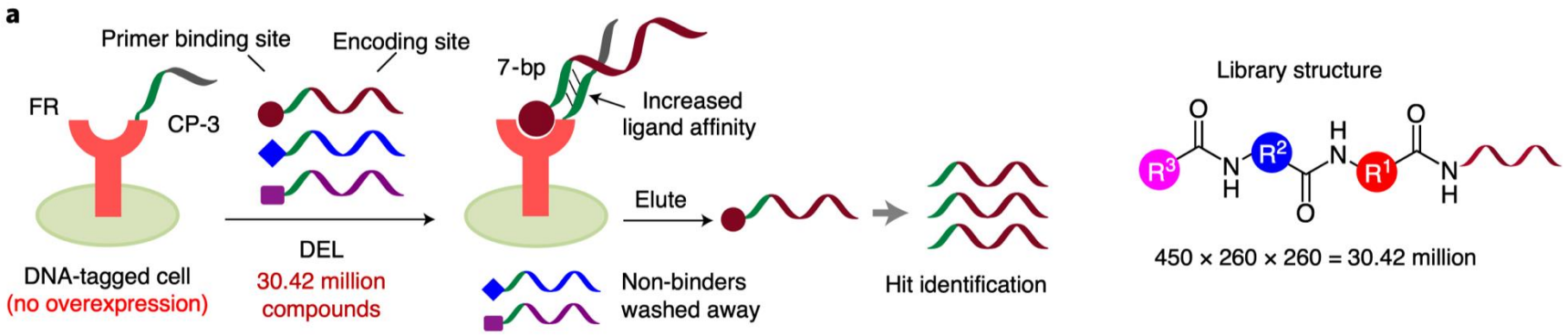

Fig1. The selection scheme and library structure

- The selection with the tagged cells identified **several distinctly enriched compounds**
- In the untagged cell, the selection also identified some **different compounds**

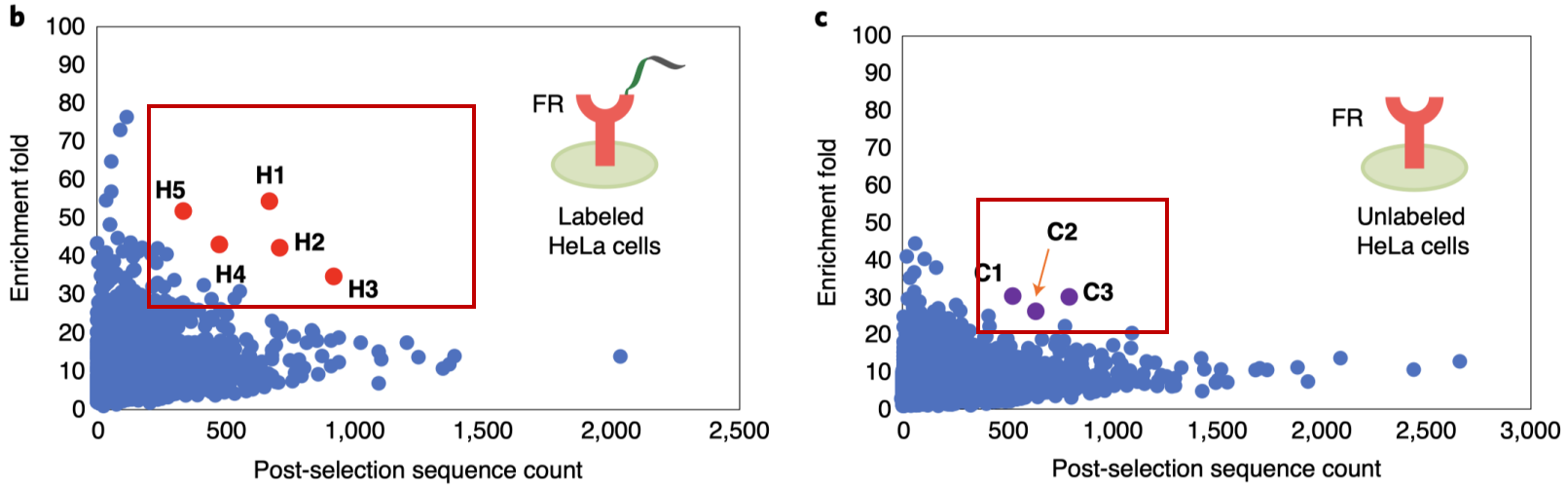

Fig2. Scatter plots of the labelled (left) and unlabelled (right) cells

Synthesize different to assay binding-affinities with SPR

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- H2–H5 showed **low micromolar affinities**, whereas H1 had a relatively **high binding affinity**
- SPR analysis showed C1–C3 were **not FR binders**

Table1. SPR analysis of the 'off-DNA' hit compounds

Compound	K_d (μM)
H1	0.058
H2	4.72
H3	7.16
H4	9.96
H5	25.9
C1	ND
C2	ND
C3	ND

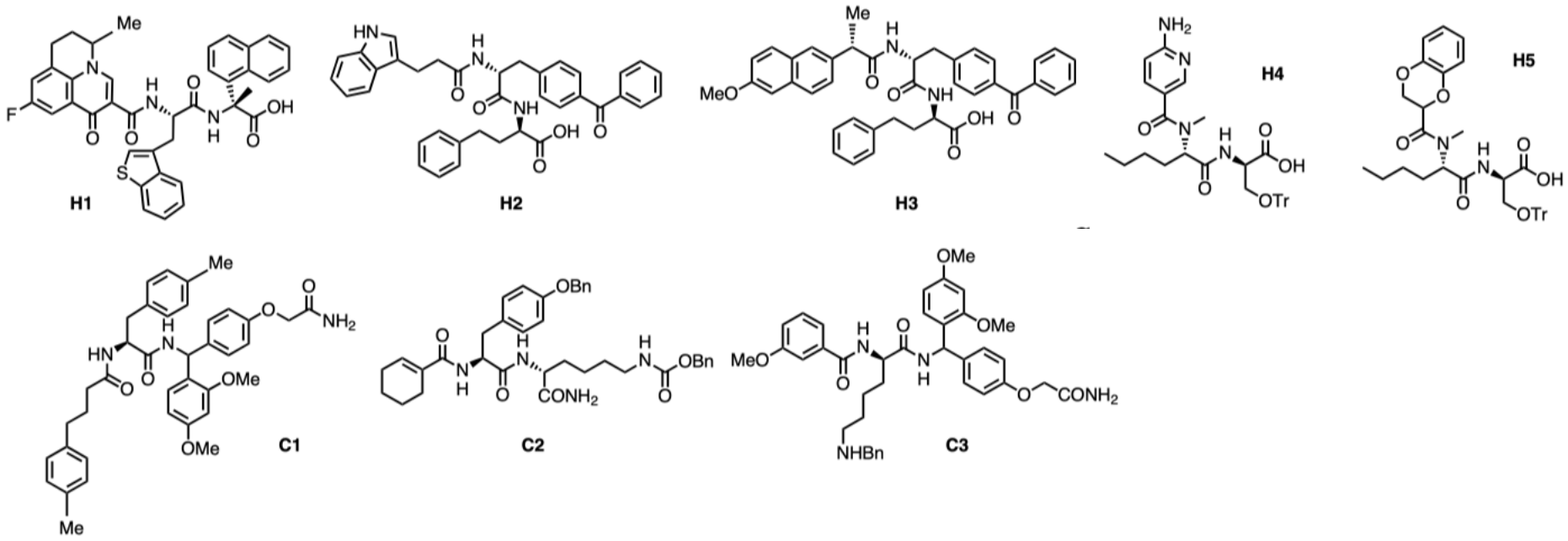

Fig1. Structures of the hit compounds

Test the affinity of the strongest binder H1

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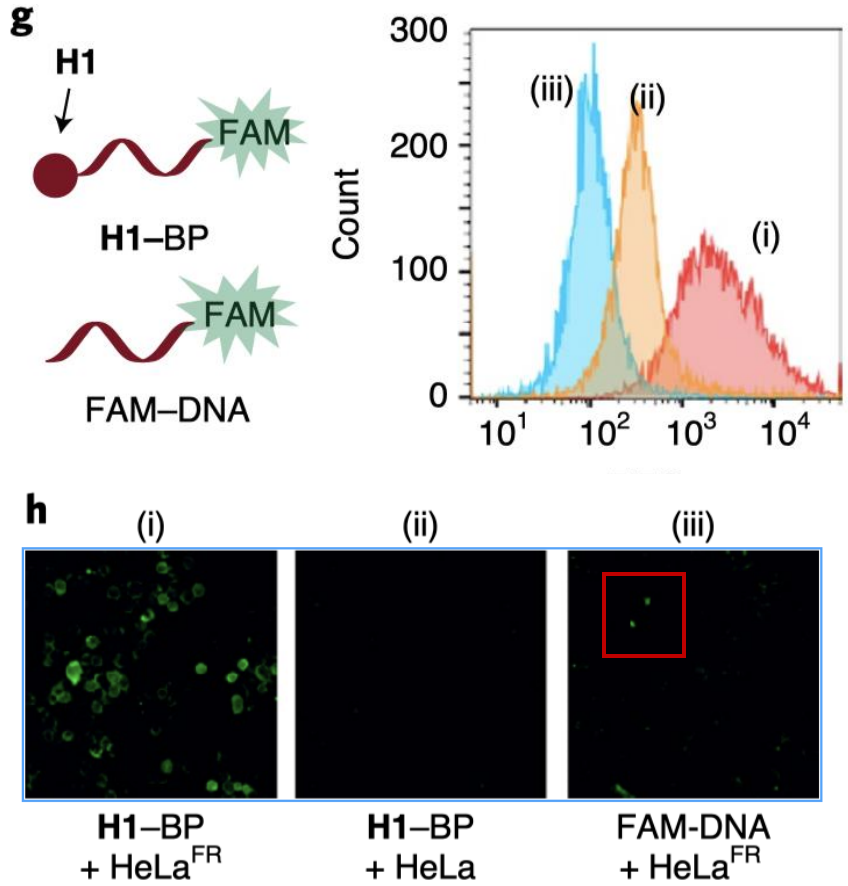


Fig1. Flow cytometry (upper panel) and fluorescent imaging (lower panel) of cells stained with H1–BP/HeLa

- HeLa^{FR} cells **could be stained with H1–BP** (i), but not with FAM–DNA without H1 (iii)
- HeLa cells with a low FR expression also **yielded a low fluorescence (ii)**

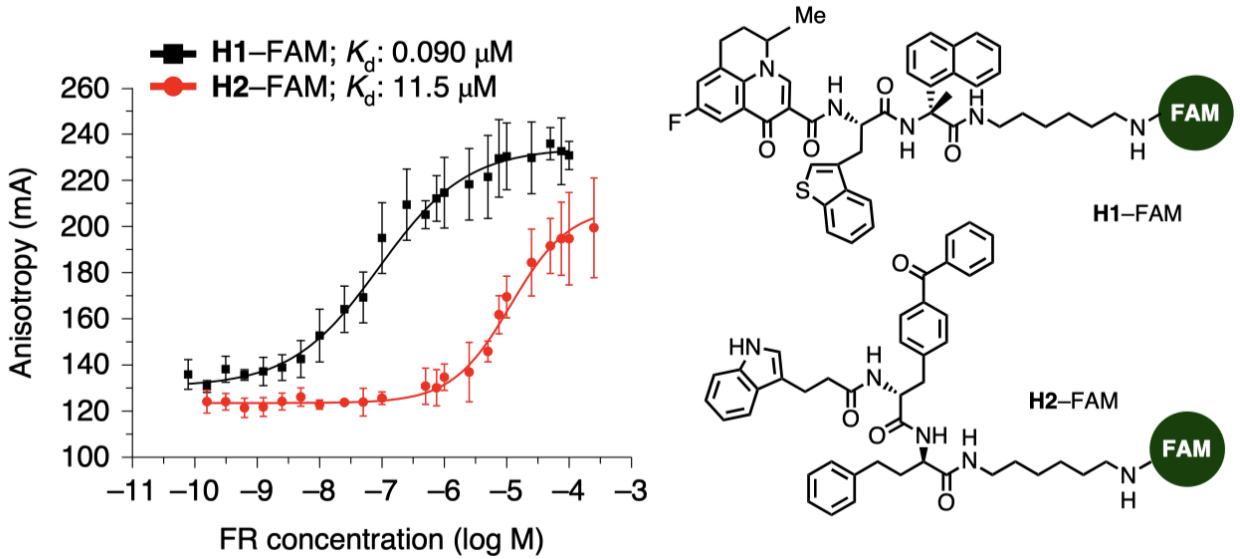


Fig2. Fluorescence polarization analysis of H1–FAM and H2–FAM

Whether antibody-based probe could be subjected to DEL selection

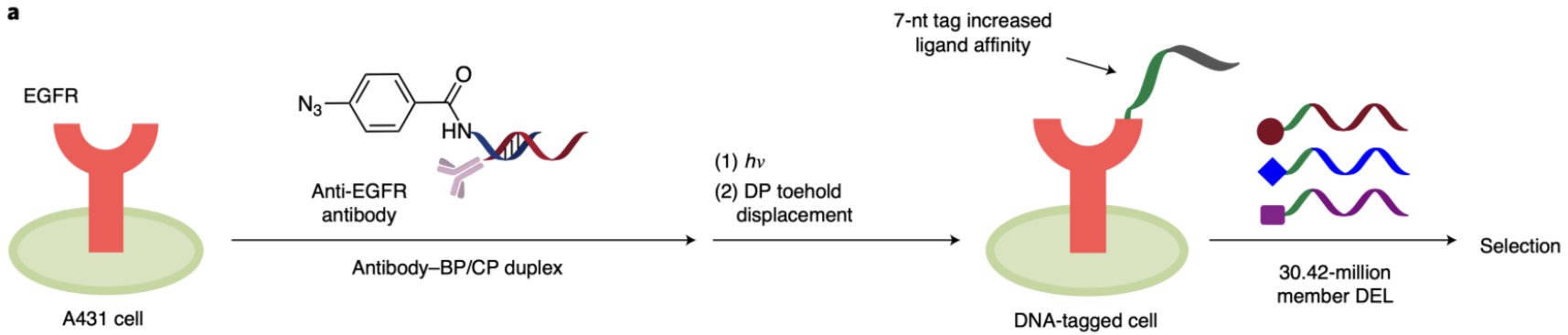
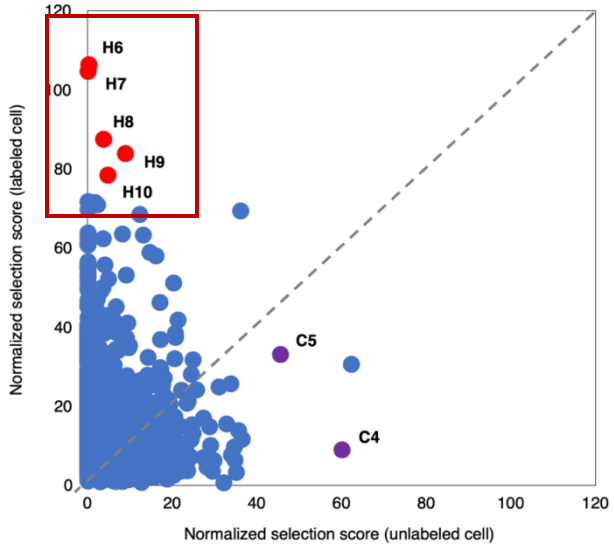


Fig1. EGFR on A431 cells was labelled with an anti-EGFR-antibody–BP/CP

- The compounds specifically enriched with the tagged cells could be easily identified



Compound	K_d (μM)
H6	12.0
H7	4.2
H8	35.8
H9	18.0
H10	67.5
C4	>200
C5	>200

Fig1. The selection results and its SPR analysis

Conclusion

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Pros

- Allow the DEL selection on live cells
- Could identify weak binders without high protein concentrations
- Both small molecules and antibodies can be used to guide the labelling

Cons

- Dependency on Known Ligands
- Restricted Target Region
- Non-Specific Antibody Conjugation
- Limited to Extracellular Domains

Questions

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What challenges does this DEL-based method solve for ligand discovery in membrane proteins, and how does it compare to traditional approaches?

What are the implications of this method for drug discovery targeting difficult-to-drug proteins?