



Selection of DNA-encoded chemical libraries against endogenous membrane proteins 60% on live cells

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Yi Man Eva Fung^{ID 1}, Xiaomeng Li^{ID 1}, Cen Huang², Yan Cao^{ID 3}✉, Yizhou Li^{ID 2,4}✉ and Xiaoyu Li^{ID 1,5}✉

Reporter: Jin Li
Date: 2020-12-24

About the authors



Xiaoyu Li

Resume:

1997年获北京大学有机化学学士学位;

2002年获美国芝加哥大学化学博士学位;

2002年至2004年在美国哈佛大学化学与化学生物学系从事博士后研究，进行DNA模板控制和药物筛选等方面的研究；

之后于生物技术公司 Ensemble Discovery 担任Principle Scientist，从事药物研发工作；

之后于2007年至2009年于美国 Broad Institute 化学生物学平台任研究员，从事活性小分子的靶点识别以及蛋白组质谱的研究工作；

2009年-2015年在北京大学化学与分子工程学院任特聘研究员；

于2015年10月加入香港大学化学系，从事生物有机化学，化学生物学和药物化学方面的研究。

李笑宇：为原创药快速筛选造一个钥匙库

Research Field

Chemical biology and bioorganic chemistry,

Drug screening and discovery,

Target identification of bioactive molecules,

Nucleic acid chemistry,

Development of molecular probes and functional devices for Chemical Biology research

About the authors



Yizhou Li

简历：

2019.03-至今 重庆大学药学院•创新药物研究中心，教授（博士生导师）

2017.09-2019.02 重庆大学药学院•创新药物研究中心，重庆大学“百人计划”特聘研究员（博士生导师）

2014.09-2017.08 瑞士苏黎世联邦理工大学（ETH），药学博士后，基于DNA编码化合物库的药物研发，合作导师：Prof. Dario Neri

2009.09-2014.07 北京大学,有机化学专业，理学博士，DNA模板控制的化学生物学研究，导师：李笑宇研究员

2005.09-2009.07北京大学，化学专业，理学学士，过渡金属催化的惰性化学键转化，导师：施章杰教授

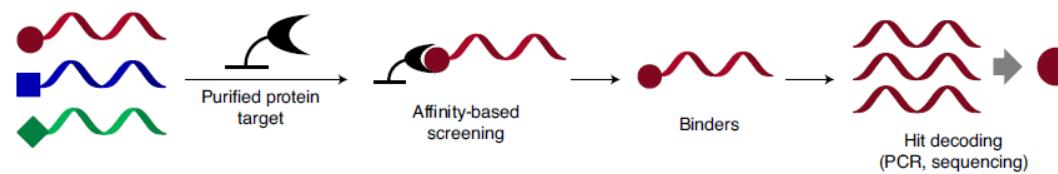
研究领域：

- 以核酸编码化合物库（DNA-encoded library）筛选技术为手段的新药先导化合物研究；
- 以核酸作为工具的化学生物学研究；
- 对生物大分子的选择性化学修饰；
- 活性小分子探针设计、合成及靶点识别

About the DEL(DNA-encoded chemical library)

DNA编码化合物库(DNA Encoded Compound Library, 简称DEL)合成与筛选的概念最早由美国Scripps研究院的Sydney Breener(2002年诺贝尔生理与医学奖获得者)和Richard Lerner(时任Scripps研究所所长)于1992年提出。

DEL技术是**组合化学**和**分子生物学**的完美结晶，并在高通量测序技术的迅速发展下得到了巨大的推动，使得先导化合物的筛选变得前所未有的快捷和高效。

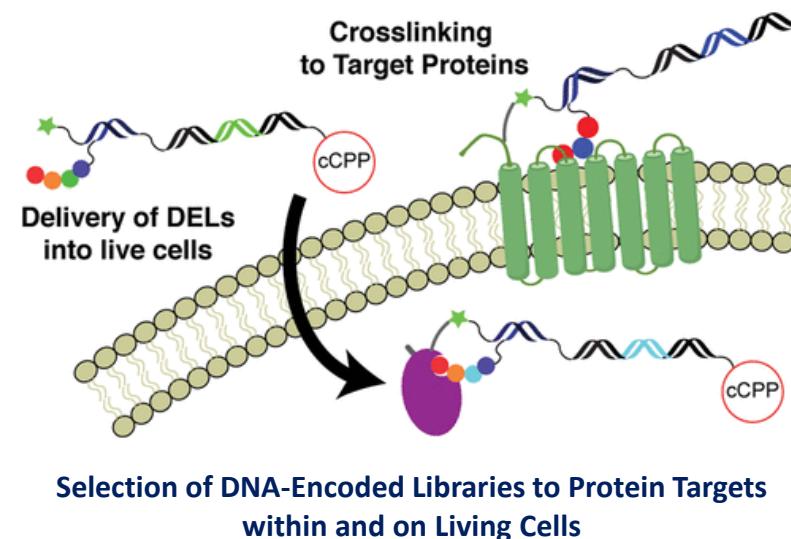
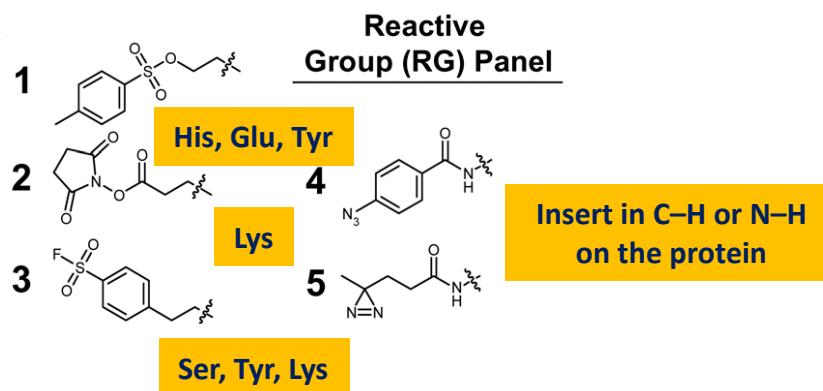
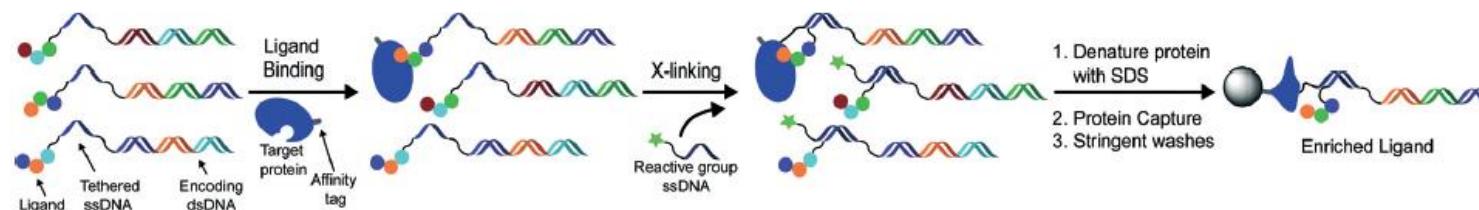


“bind-wash-elute”

DEls are typically selected against **purified** and **immobilized** proteins

Method to label proteins with DELs

Crosslinking of DNA-linked ligands to target proteins for enrichment from DNA-encoded libraries



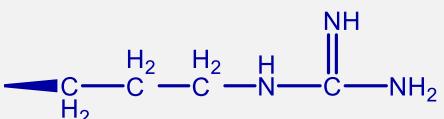
AMINO ACIDS

Ala Arg Asn Asp Cys

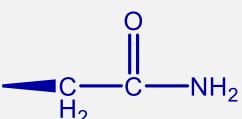
Ala
Glu
Leu
Ser



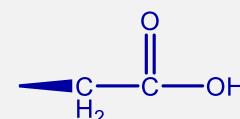
Arg
Gln
Lys
Thr



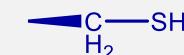
Asn
Gly
Met
Trp



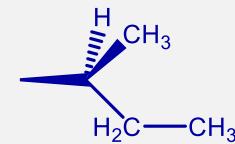
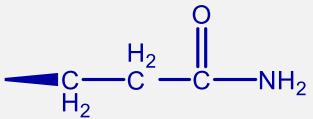
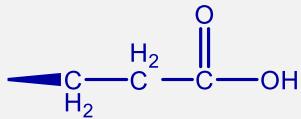
Asp
His
Phe
Tyr



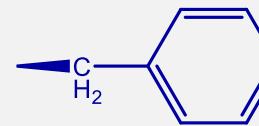
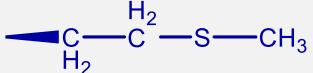
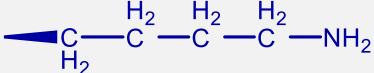
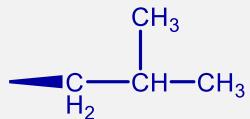
Cys
Ile
Pro
Val



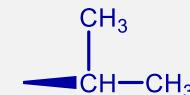
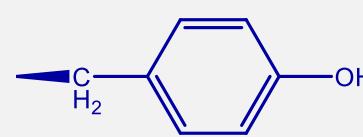
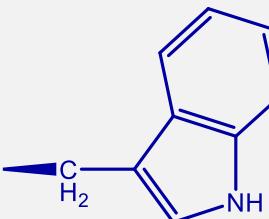
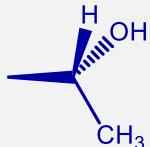
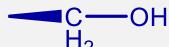
Glu
Gln
Gly
His
Ile



Leu
Lys
Met
Phe
Pro

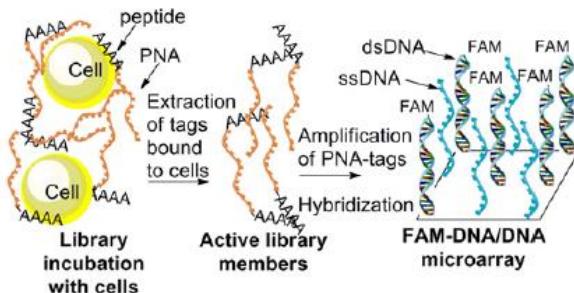


Ser
Thr
Trp
Tyr
Val

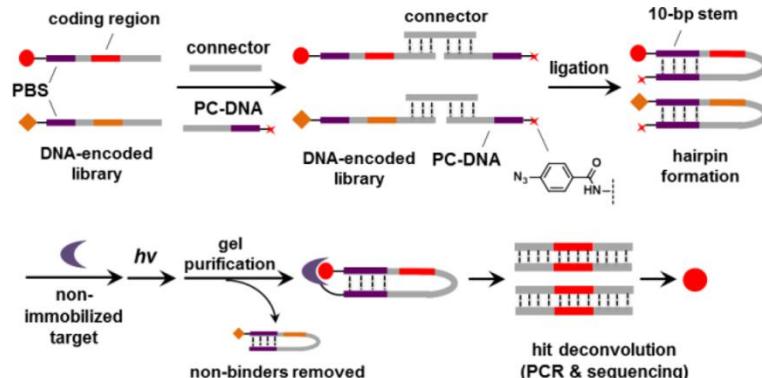


Method to label proteins with DELs

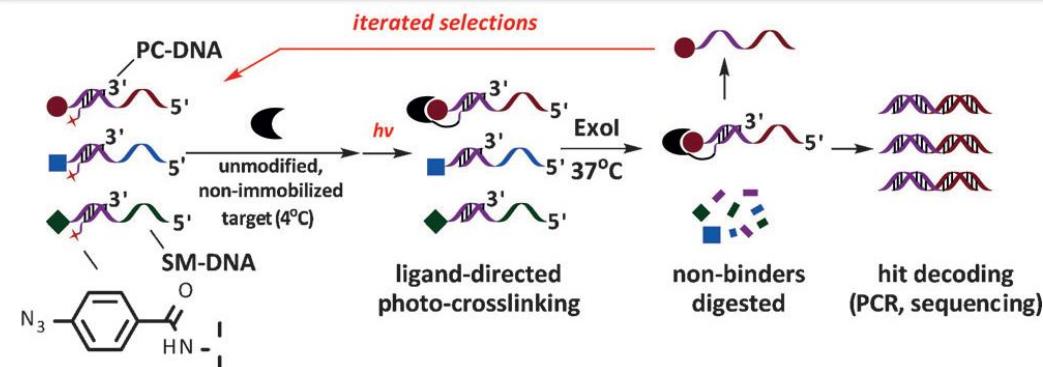
Decoding a PNA Encoded Peptide Library by PCR: The Discovery of New Cell Surface Receptor Ligands



ligate-cross-link-purify

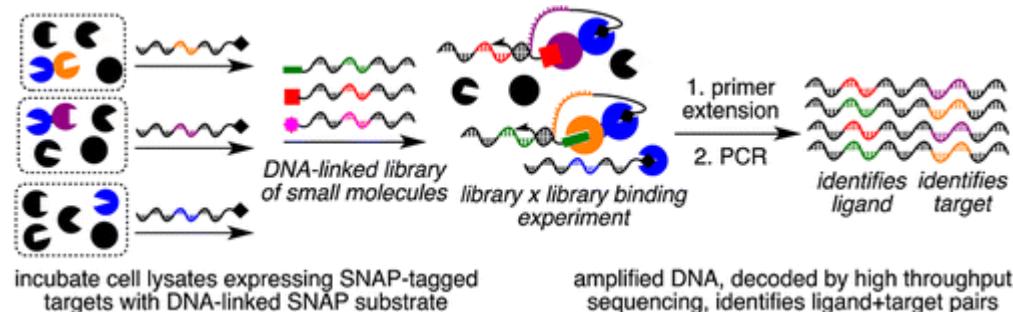


Selecting a DNA-Encoded Chemical Library against Non-immobilized Proteins Using a “Ligate–Cross-Link–Purify” Strategy



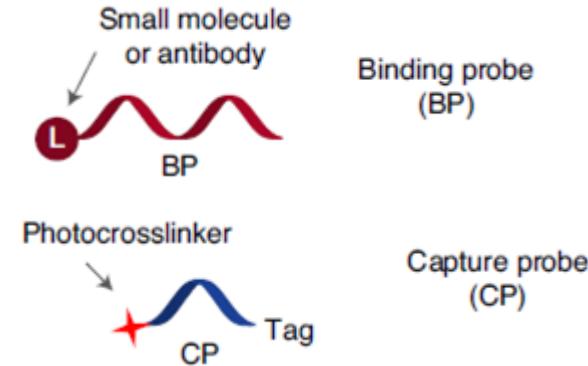
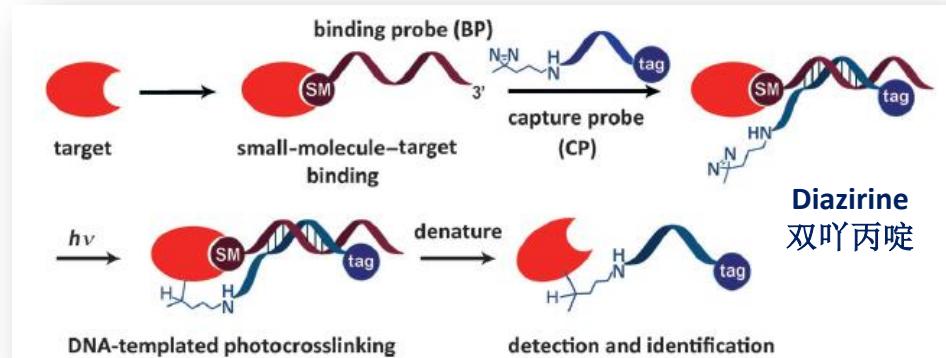
Selection of DNA-Encoded Small Molecule Libraries Against Unmodified and Non-Immobilized Protein Targets

interaction determination using unpurified proteins (IDUP)

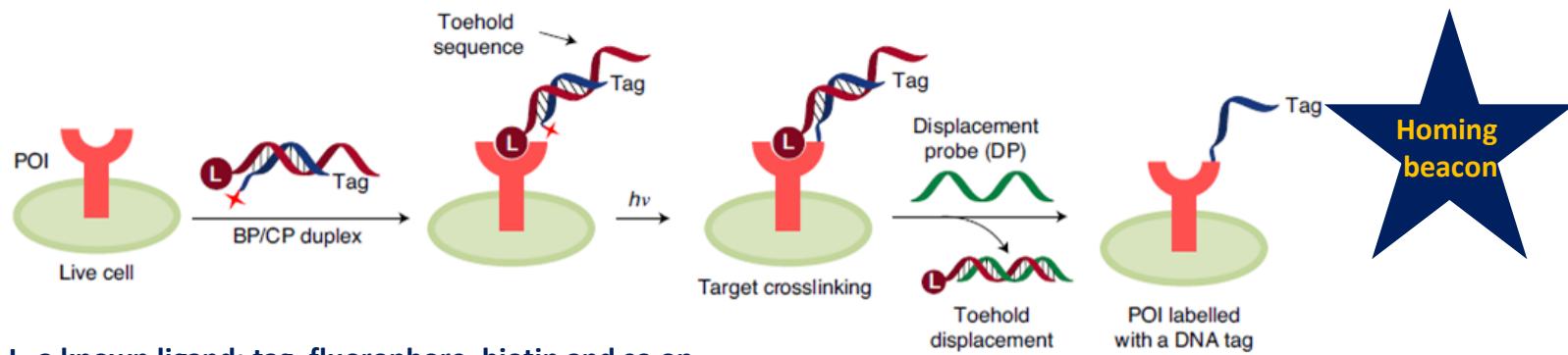


Identification of Ligand–Target Pairs from Combined Libraries of Small Molecules and Unpurified Protein Targets in Cell Lysates

Method to label membrane proteins with a DNA tag

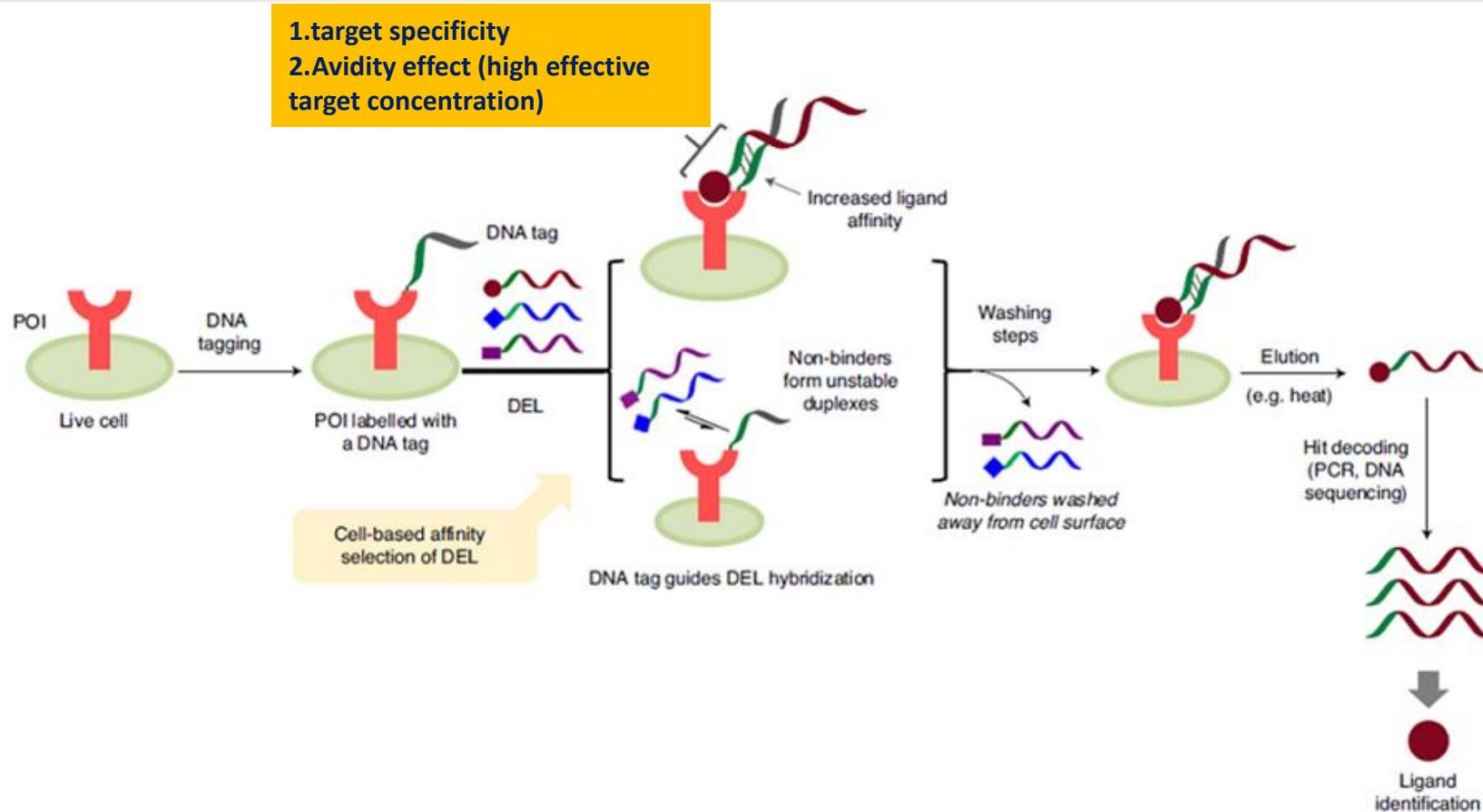


“DNA-programmed photoaffinity labeling (DPAL)”

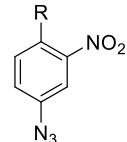
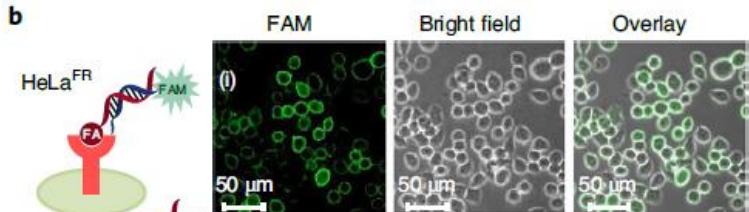
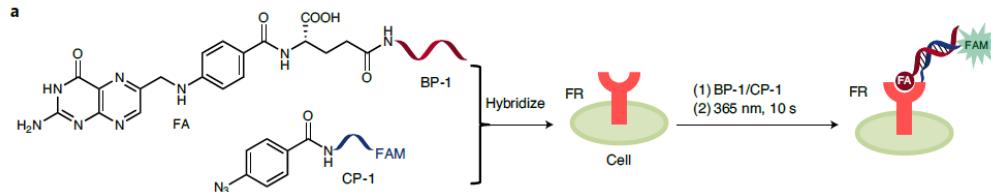


L, a known ligand; tag, fluorophore, biotin and so on

The proposed cell-based selection method

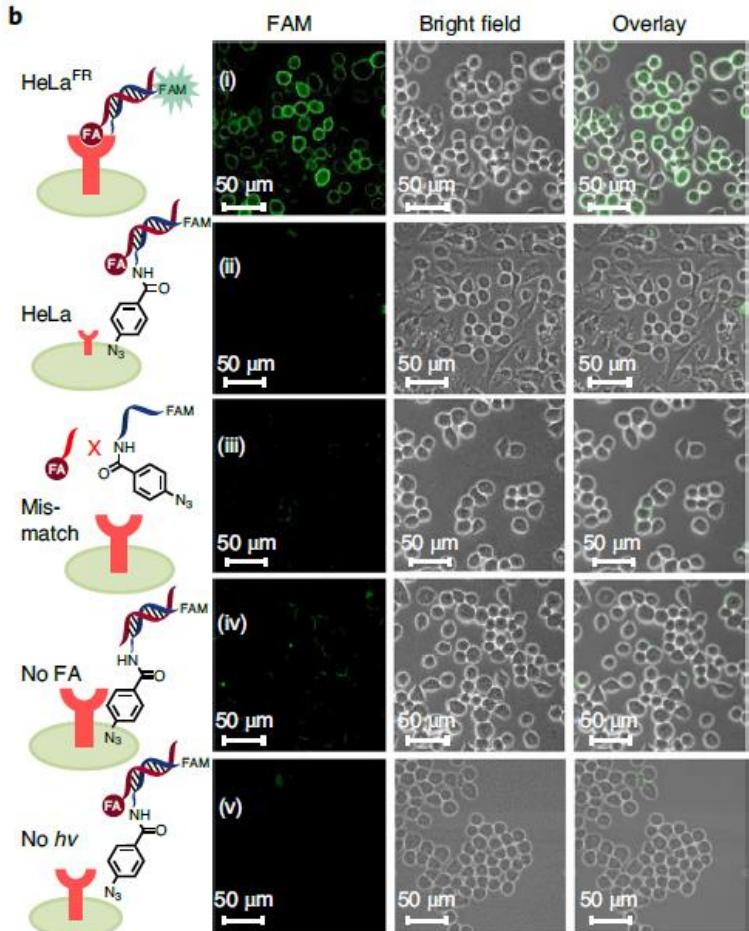
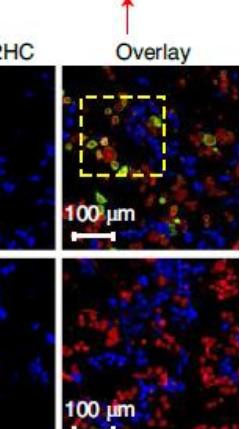
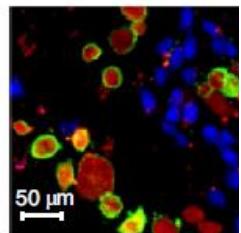


Membrane proteins on live cells can be specifically labelled with DNA tags using DPAL



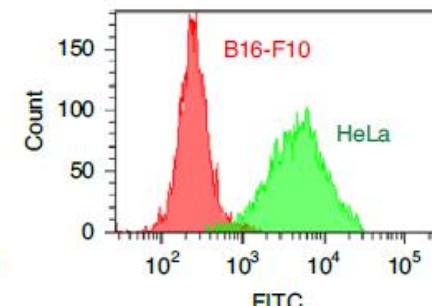
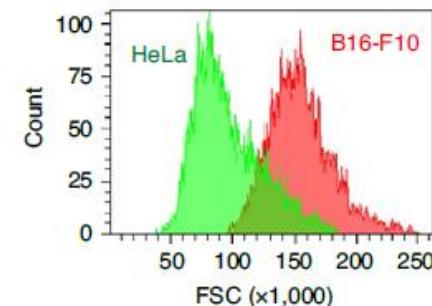
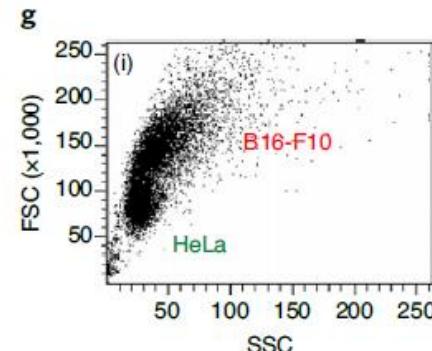
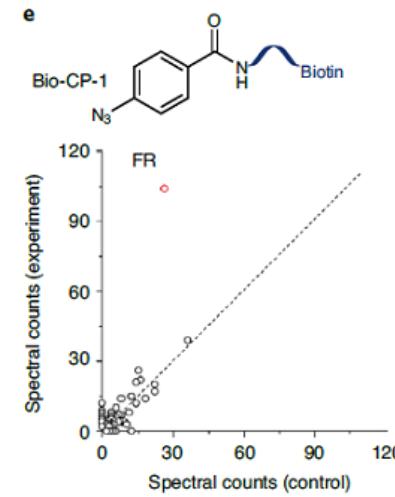
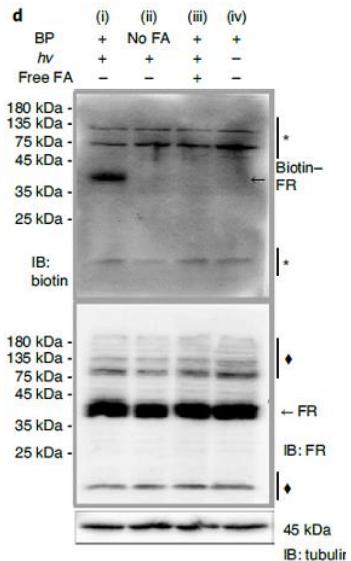
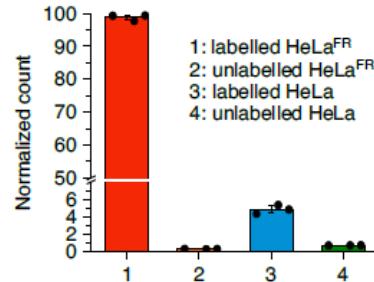
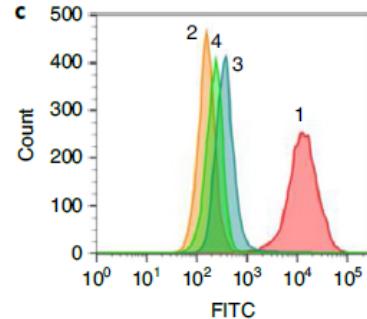
Nature, 1969, 224, 511

Upon irradiation with UV light at wavelengths below 310 nm, simple aryl-azides extrude molecular nitrogen to form short-lived (1 ns) singlet nitrenes which rearrange to form dehydroazepines; the latter react rapidly with amines to form robust adducts.

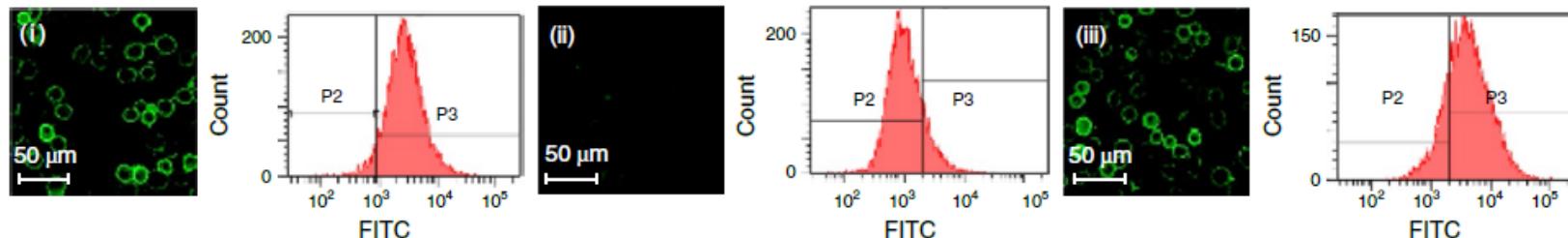
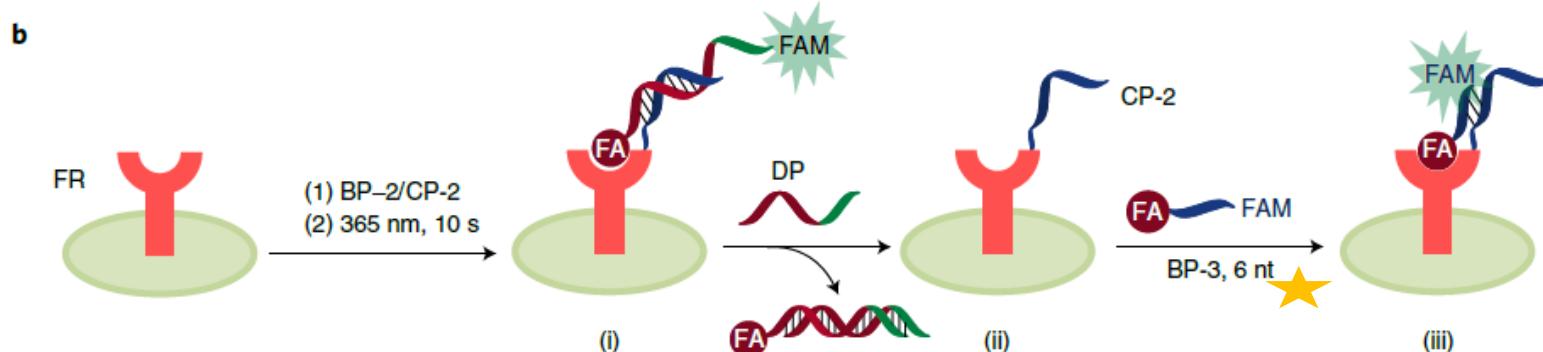
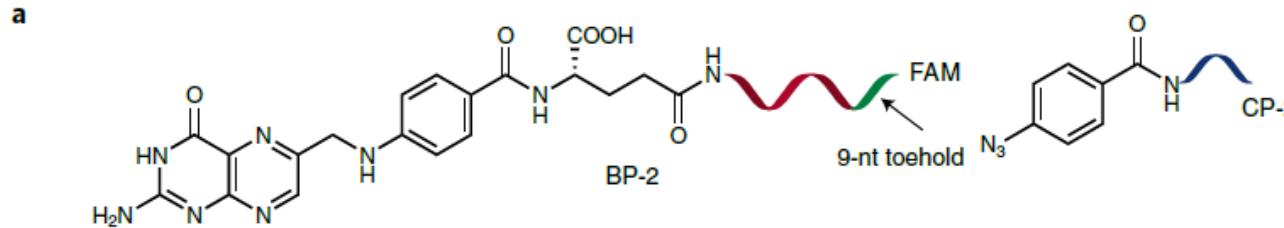


Integrin
CA-12

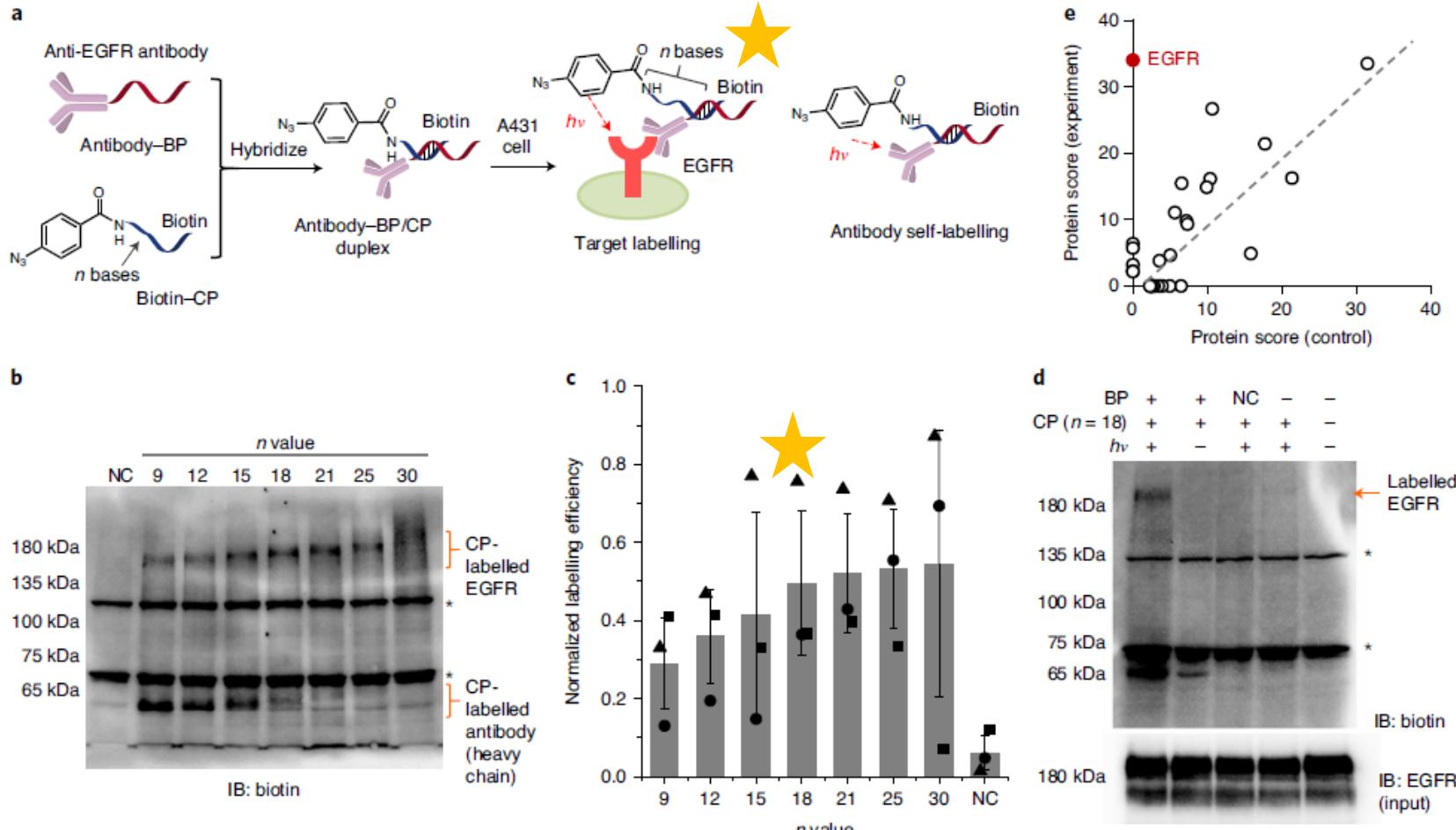
Membrane proteins on live cells can be specifically labelled with DNA tags using DPAL



BP removal using toehold displacement after the labelling of FR



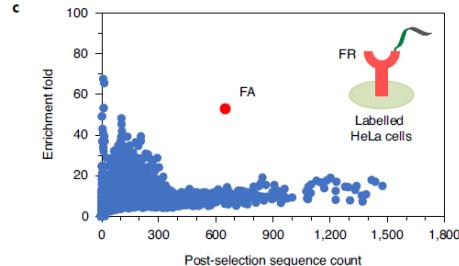
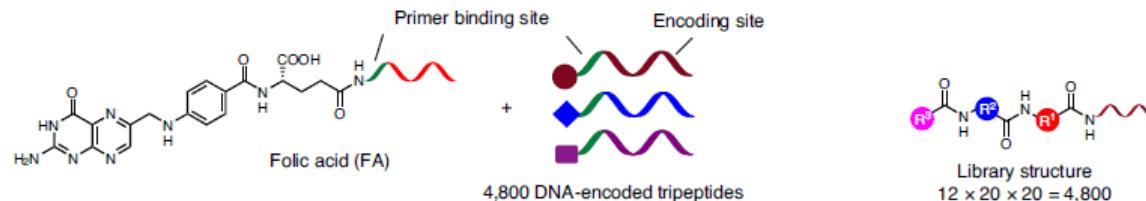
Cell-based labelling of EGFR with antibody-guided probes (~56% efficiency)



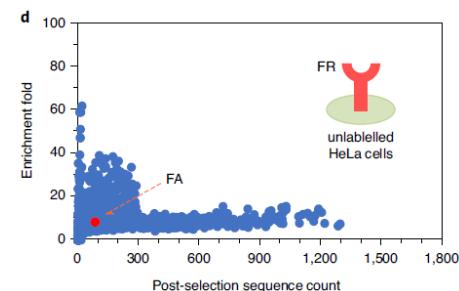
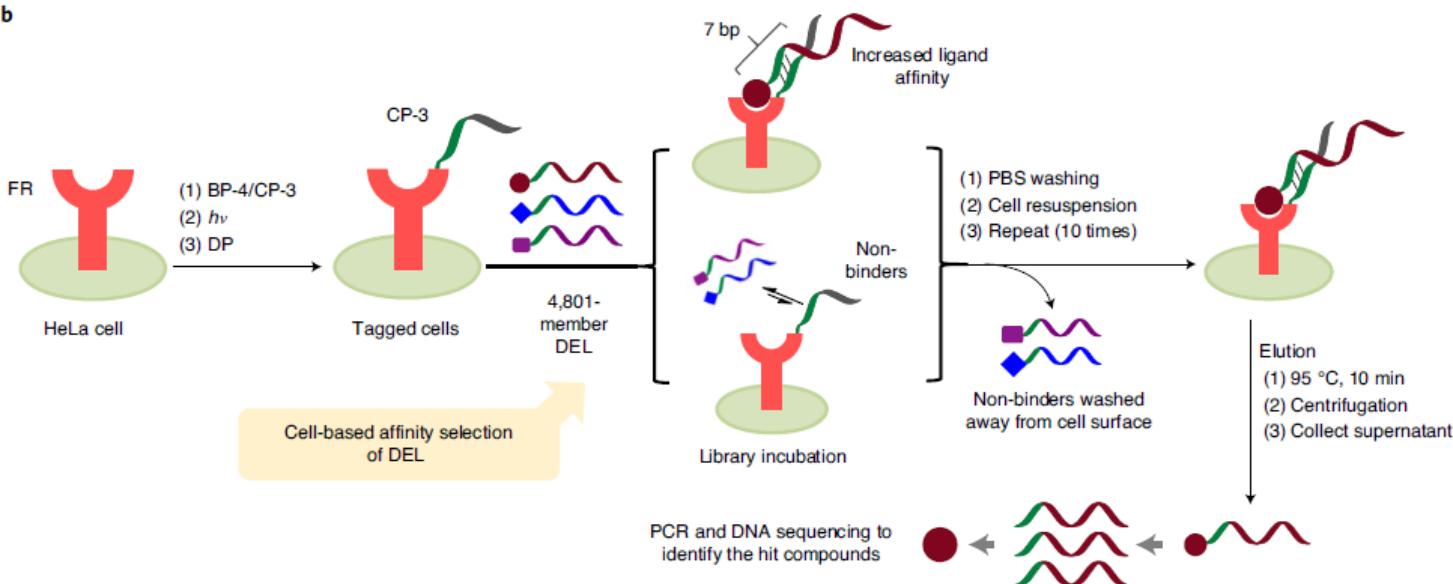
Selection of DELs against membrane proteins on live cells

DEL selection against DNA-tagged FR on HeLa cells

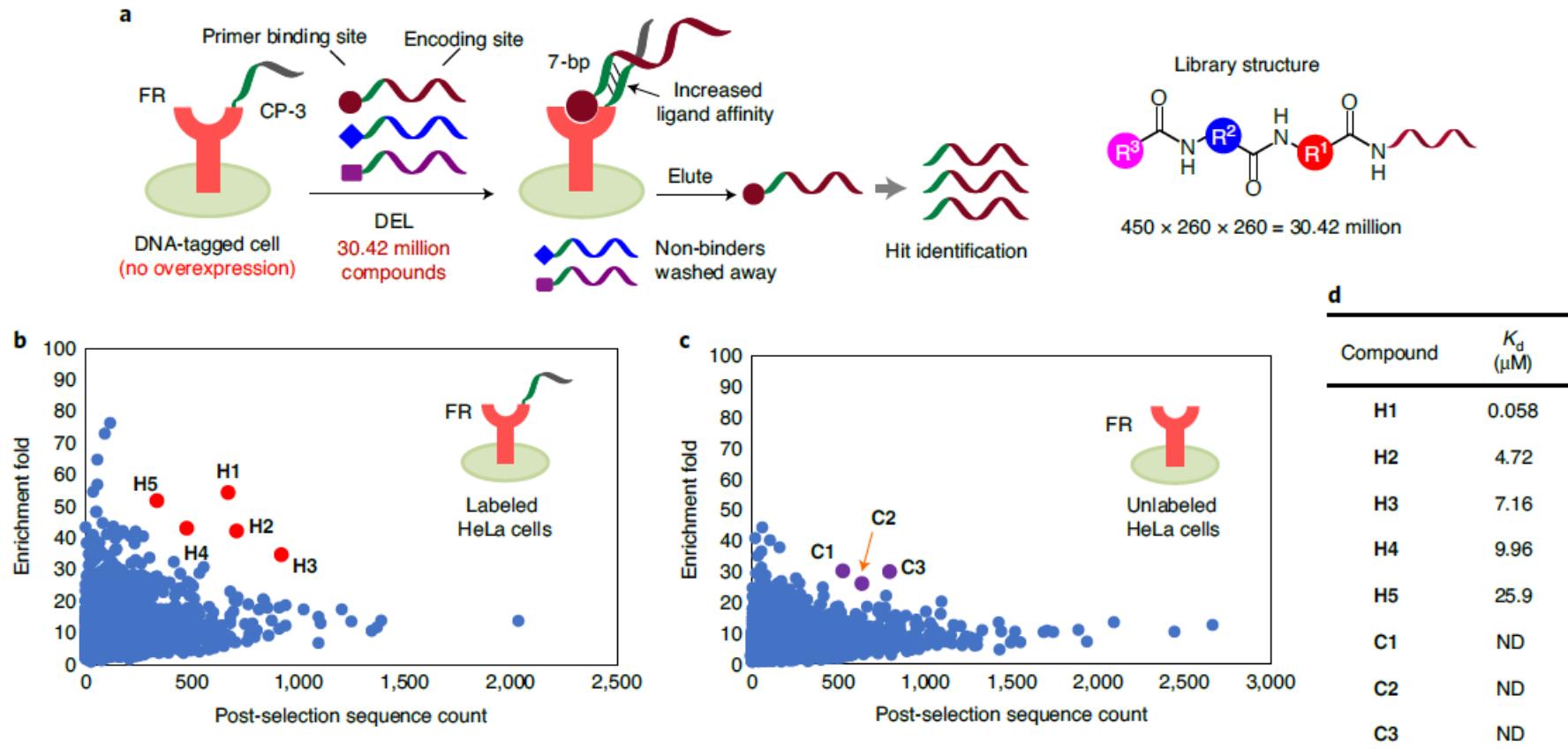
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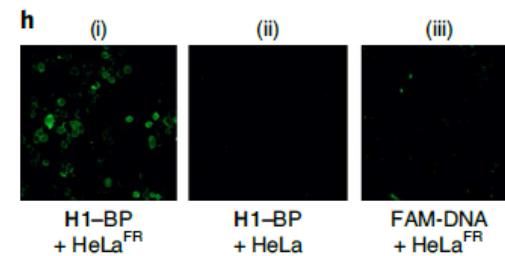
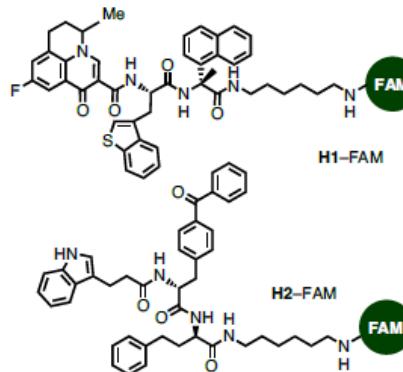
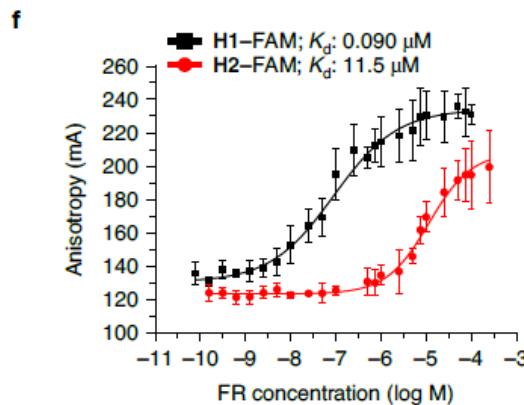
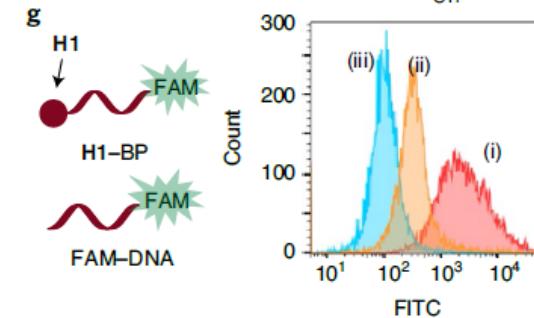
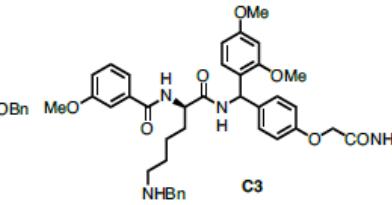
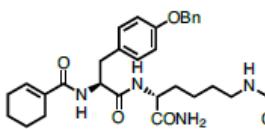
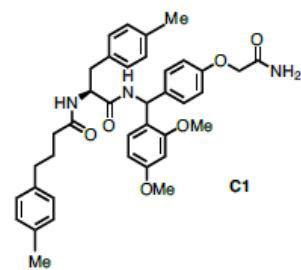
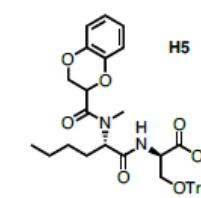
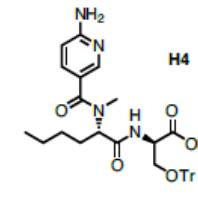
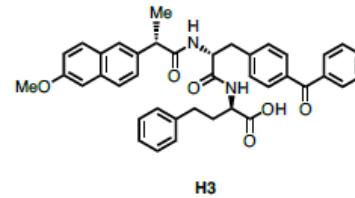
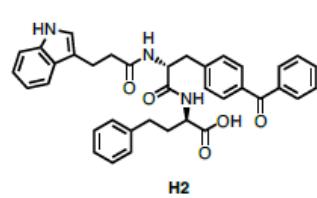
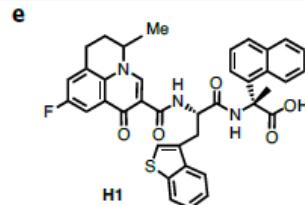
b



Selection of a 30.42-million DEL against FR on live cells

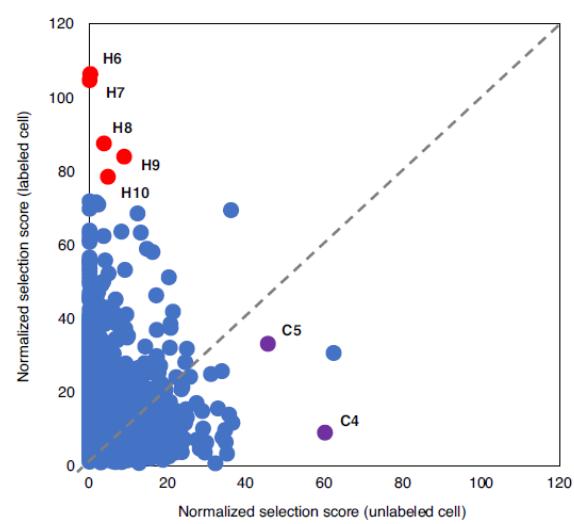
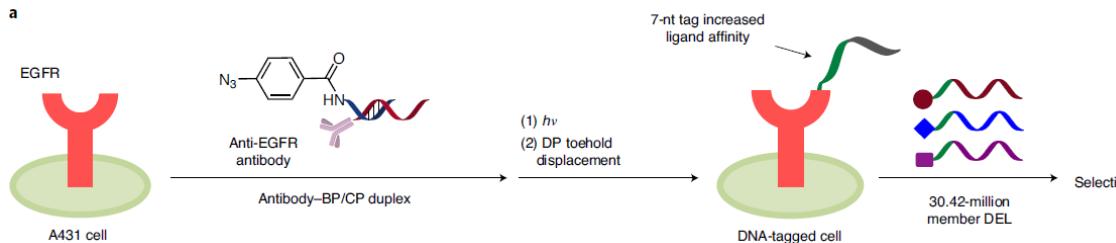


Selection of a 30.42-million DEL against FR on live cells

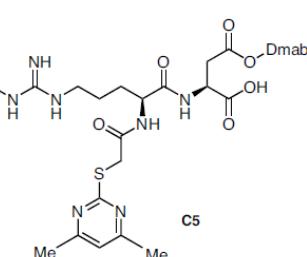
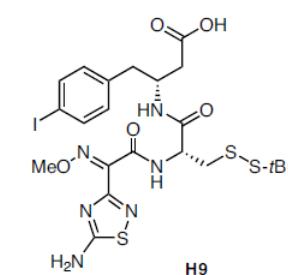
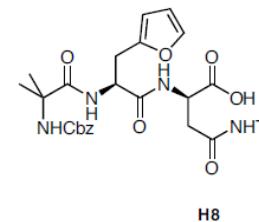
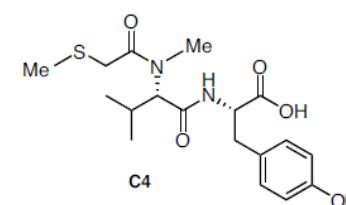
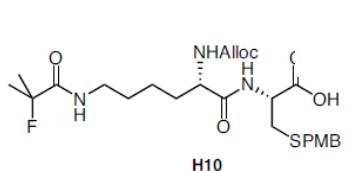


Selection of a 30.42-million DEL against DNA-tagged EGFR on live cells

17



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



Thanks for your attention!