Affinity Probe and Quantitative Chemical Proteomics

LS 2020/05/30 Koichi Kamiya

Contents

1. **Introduction**
   Affinity probe and chemical proteomics

2. **Approaches for “undruggable” proteins**

3. **Main paper**

   Wang, Y.; Dix, M. M.; Bianco, G.; Remberg, J. R.; Lee, H. Y.; Kalocsay, M.; Gygi, S. P.; Vite, G.; Lawrence, R. M.; Parker, C. G.; Cravatt, B. F.
   ***Nat. Chem*. 2019, *11*, 1113.**

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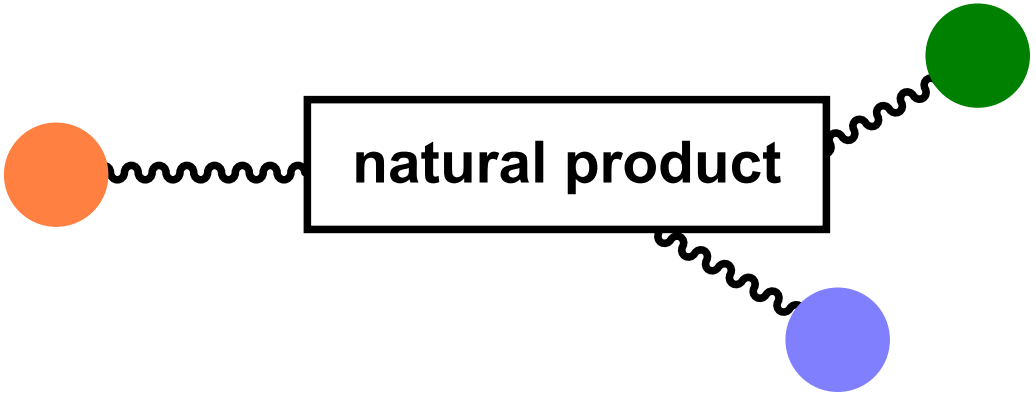
Affinity Probe

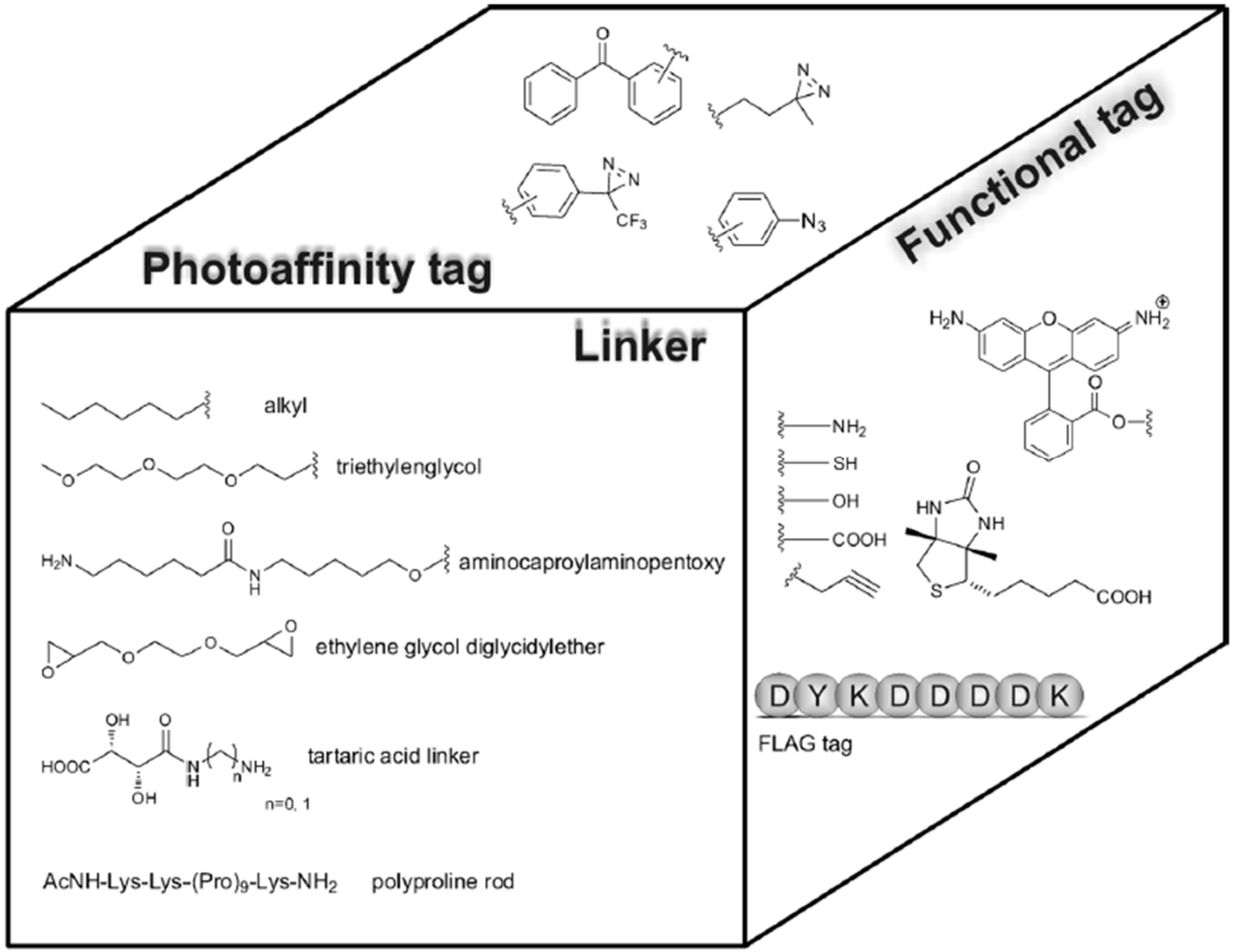


: Tag

: linker

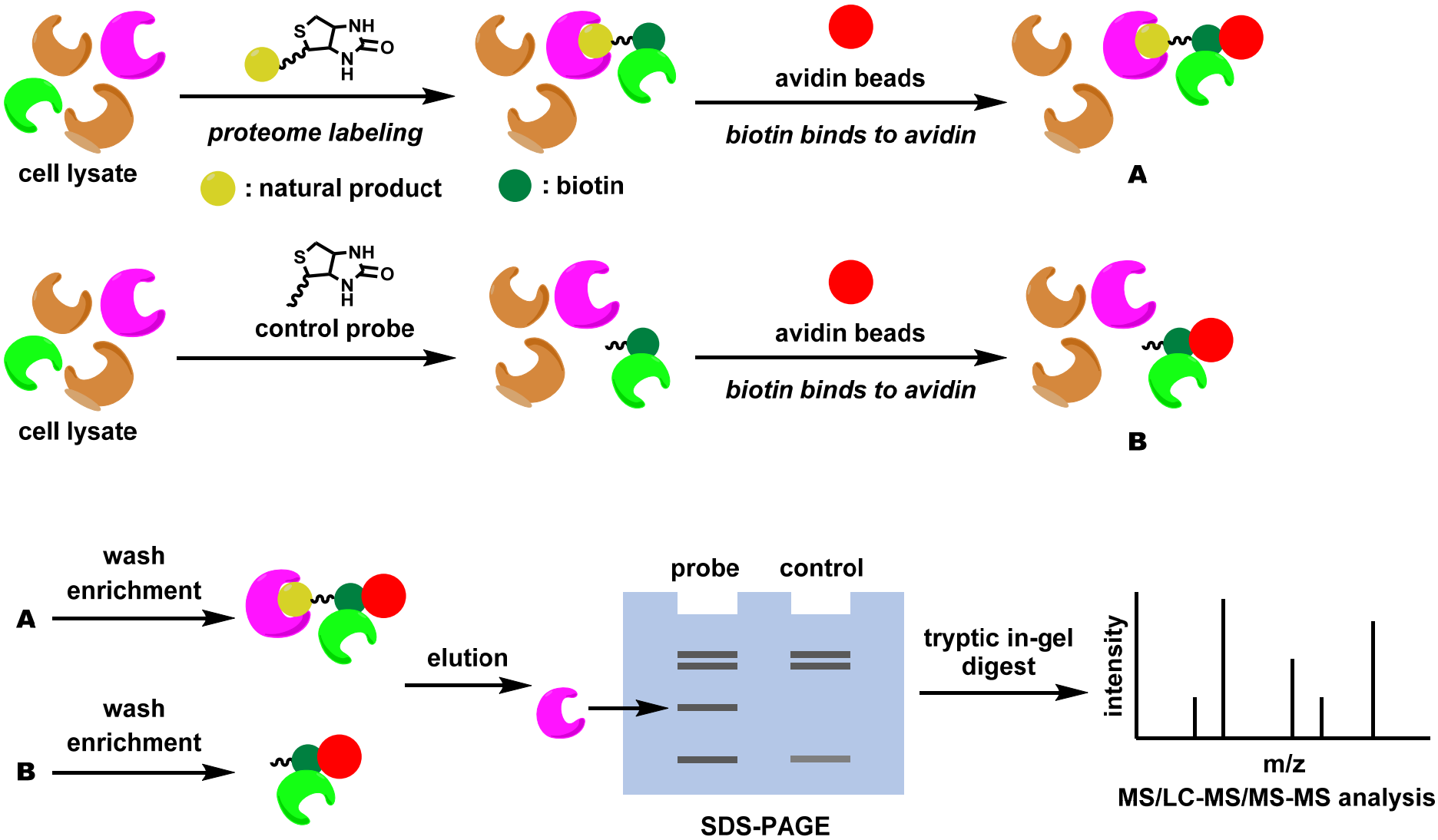
- Chemically modified natural product
- Powerful tool for target identification of natural product

Tag and Linker

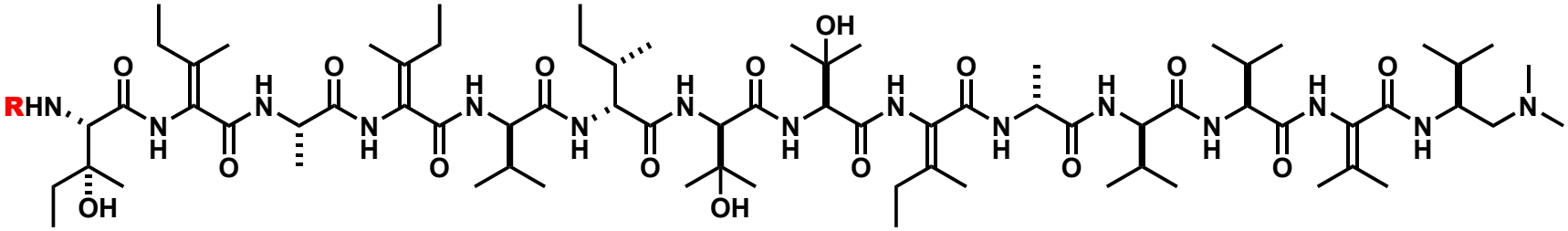


Ziegler, S.; Pries, V.; Hedberg, C.; Waldmann, H. *Angew. Chem. Int. Ed.* **2013**, *52*, 2744.

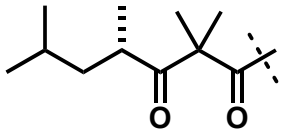
Workflow for Target Identification

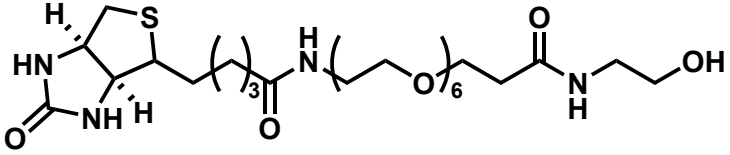


Target Identification of Yaku'amide B

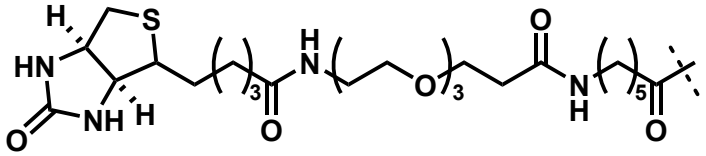


yaku'amide B (**1b**) : **R** =

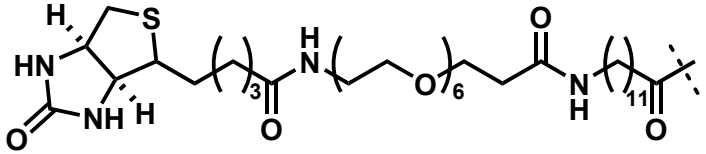




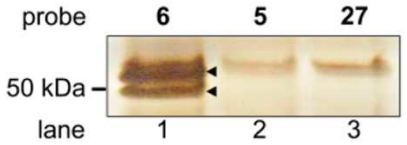
probe **5** : **R** =

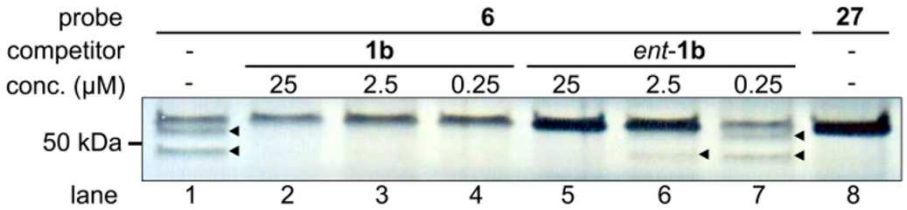


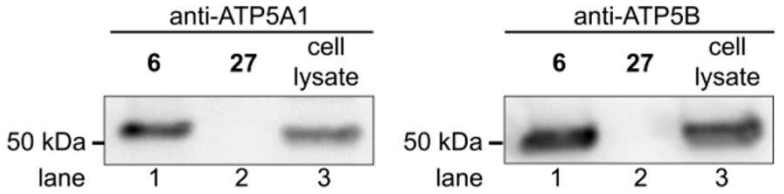
probe **6** : **R** =



control probe **27**



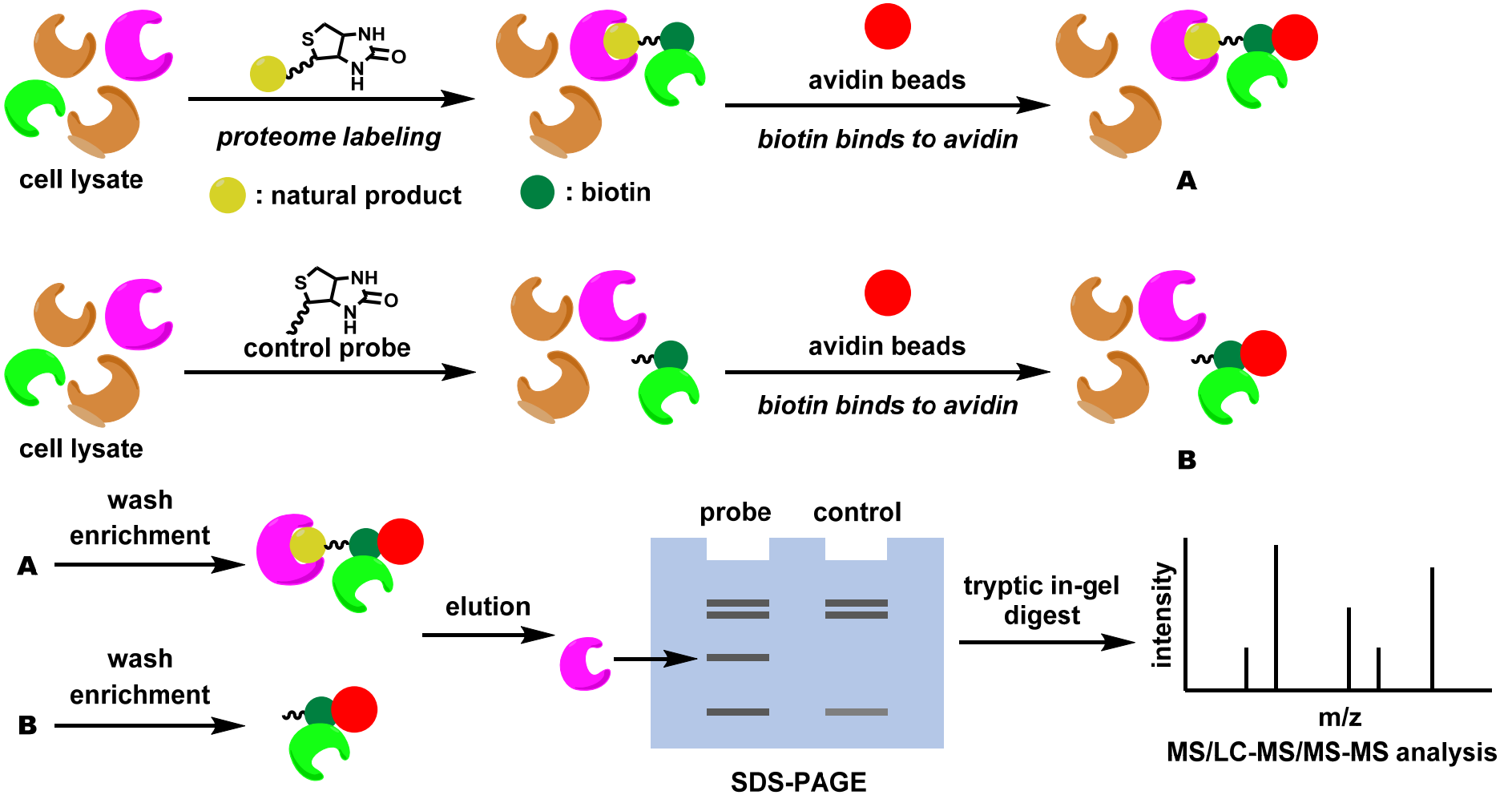




- α,β-subunits of mitochondrial F_oF_1-ATP synthase were found to be target proteins of yaku'amide B

Kitamura, K.; Itoh, H.; Sakurai, K.; Dan, S.; Inoue, M. *J. Am. Chem. Soc.* **2018**, *140*, 12189.

Limitation

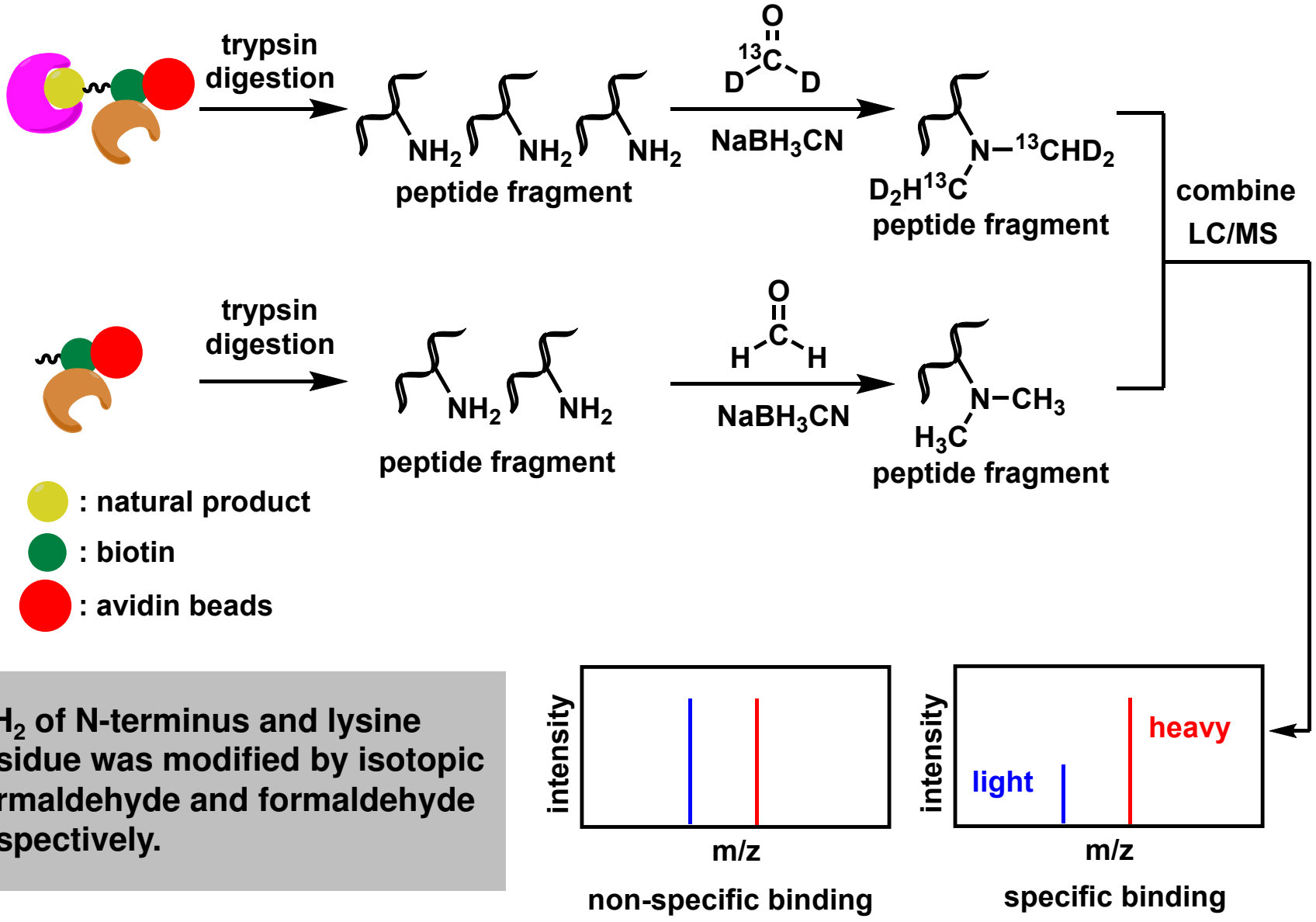


- Low abundant proteins are difficult to determine
- Comparisons between proteins identified with active and inactive probes are sometimes difficult.

→

- Quantitative chemical proteomics are used

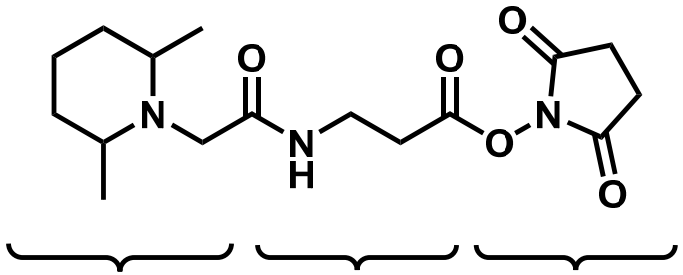
Reductive Dimethylation (ReDiMe)



- NH_2 of N-terminus and lysine residue was modified by isotopic formaldehyde and formaldehyde respectively.

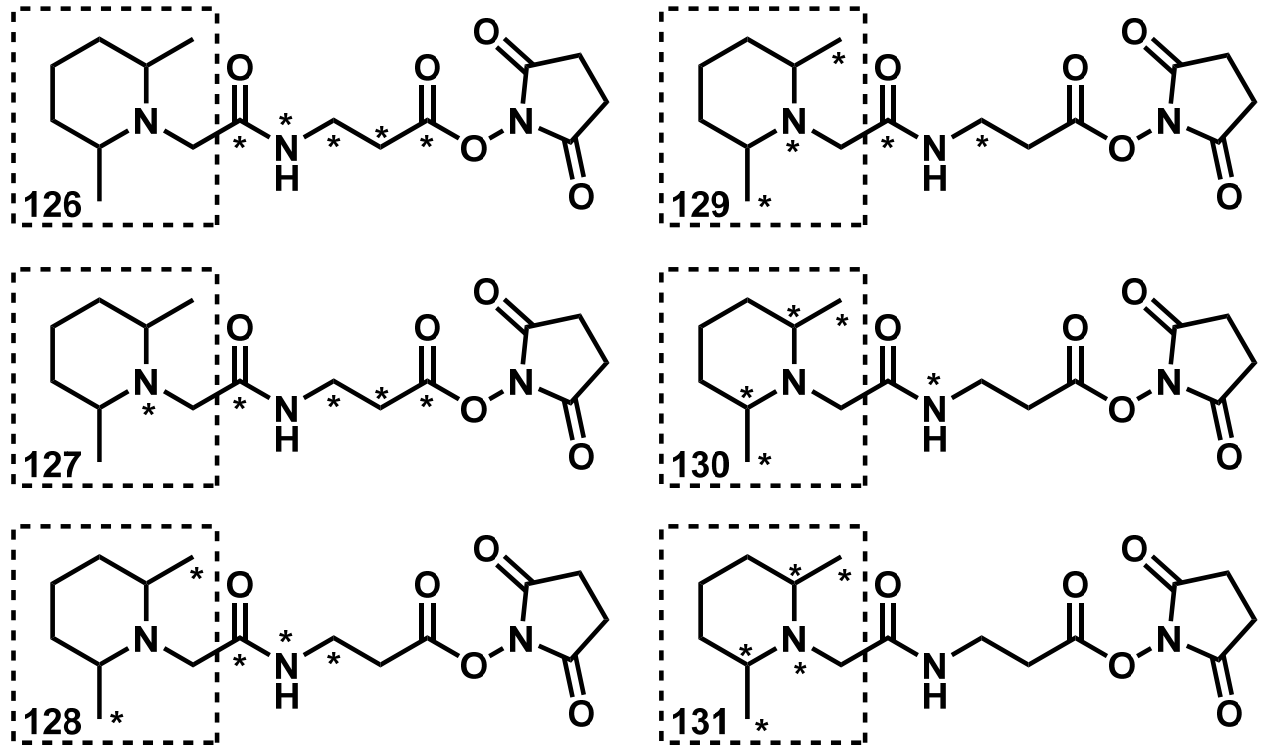
Boersema, P. J.; Rajimakers, R.; Lemeer, S.; Mohammed, S.; Heck, A. J. R. *Nat. Protoc.* **2009**, *4*, 484.[9]

Tandem Mass Tag (TMT) (1)



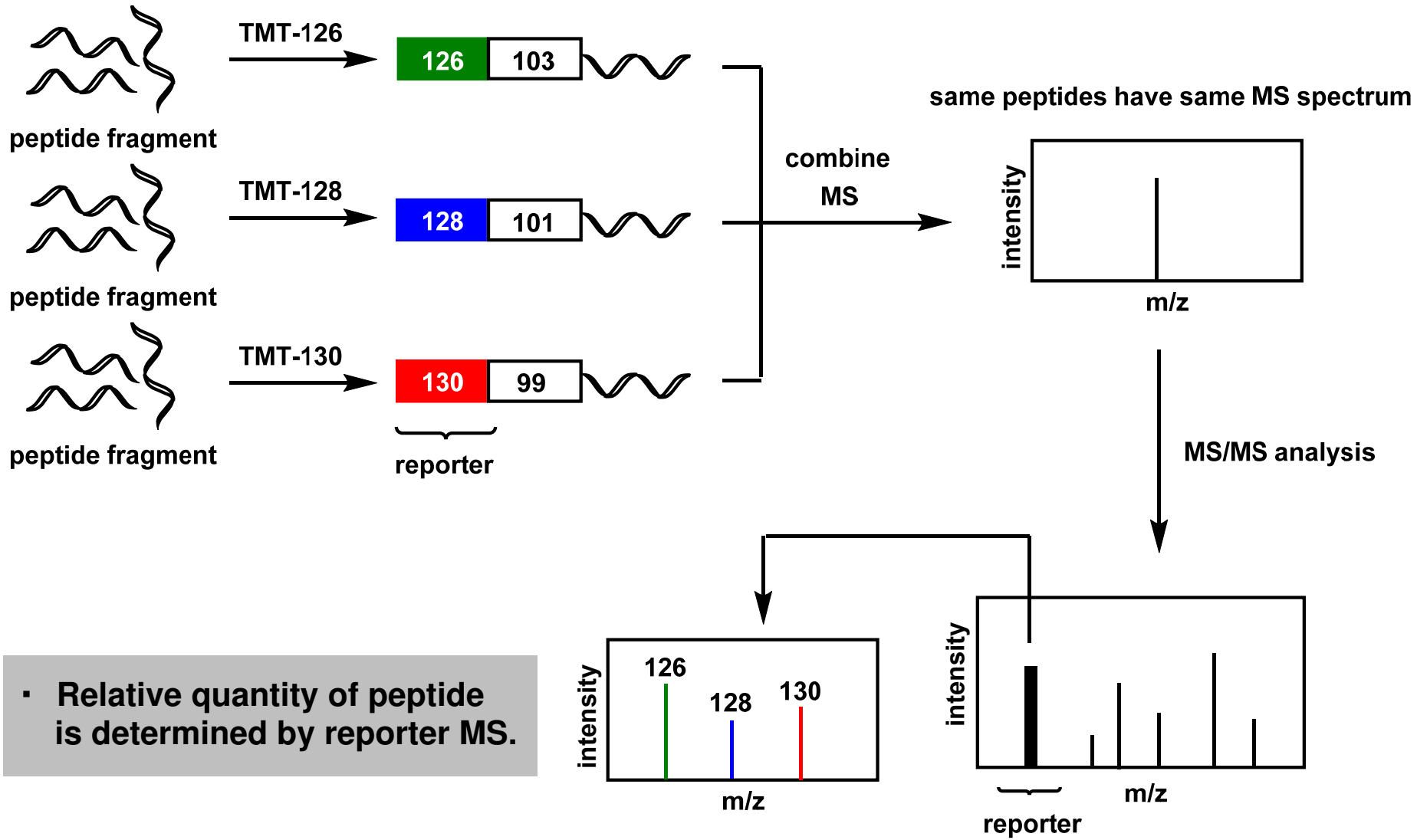
Mass Reporter | Mass Normalizer | NH_2 Reactive group

- NH_2 of N-terminus and lysine residue was modified by isotopic TMT tags.



Dayon, L.; Hainard, A.; Licker, V.; Turck, N.; Kuhn, K.; Hochstrasser, D. F.; Burkard, P. R.; Sanchez, J. *Anal. Chem.* **2008**, *80*, 1895.

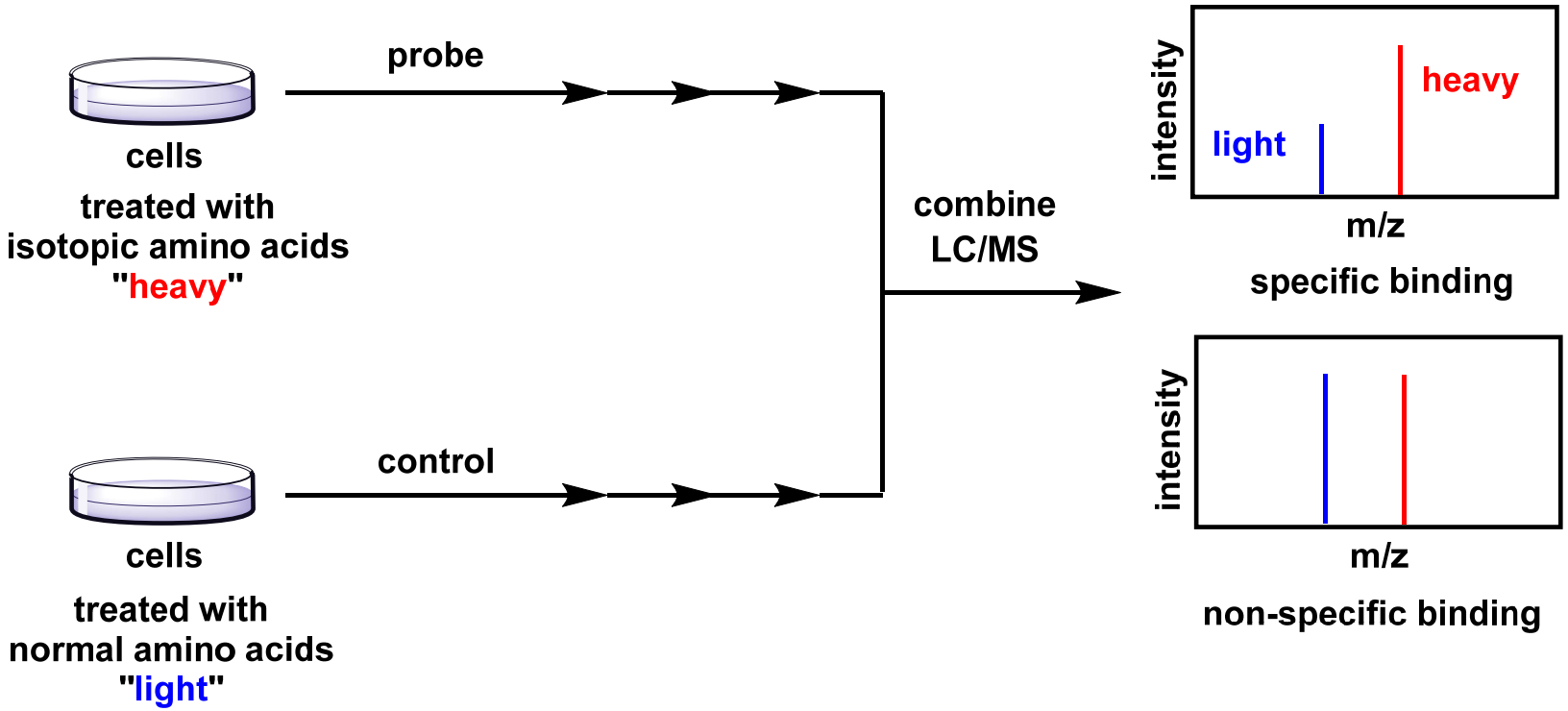
Tandem Mass Tag (TMT) (2)



- **Relative quantity of peptide is determined by reporter MS.**

Dayon, L.; Hainard, A.; Licker, V.; Turck, N.; Kuhn, K.; Hochstrasser, D. F.; Burkard, P. R.; Sanchez, J. *Anal. Chem.* **2008**, *80*, 1895.

Stable Isotope Labeling using Amino Acids in Cell Culture (SILAC)



- Cells were pre-treated with isotopic amino acids and normal amino acids respectively.
- Thus, cells metabolically take in each amino acid to furnish isotopic proteins and normal proteins respectively.

Ong, S. E.; Blagoev, B.; Kratchmarova, I.; Kristensen, D. B.; Steen, H.; Pandey, A.; Mann, M. *Mol. Cell. Proteomics.* **2002**, *1*, 376.

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Prof. Benjamin F. Cravatt

1992: B.S. Biological Sciences, Stanford Univ.
B.A. History, Stanford Univ.

1996: Ph.D. Macromolecular and Cellular Structure and Chemistry, The Scripps Research Institute (Prof. Dale Boger, Prof. Richard Lerner)

1996-2001: Assistant Professor, The Scripps Research Institute

2001-2004: Associate Professor, The Scripps Research Institute

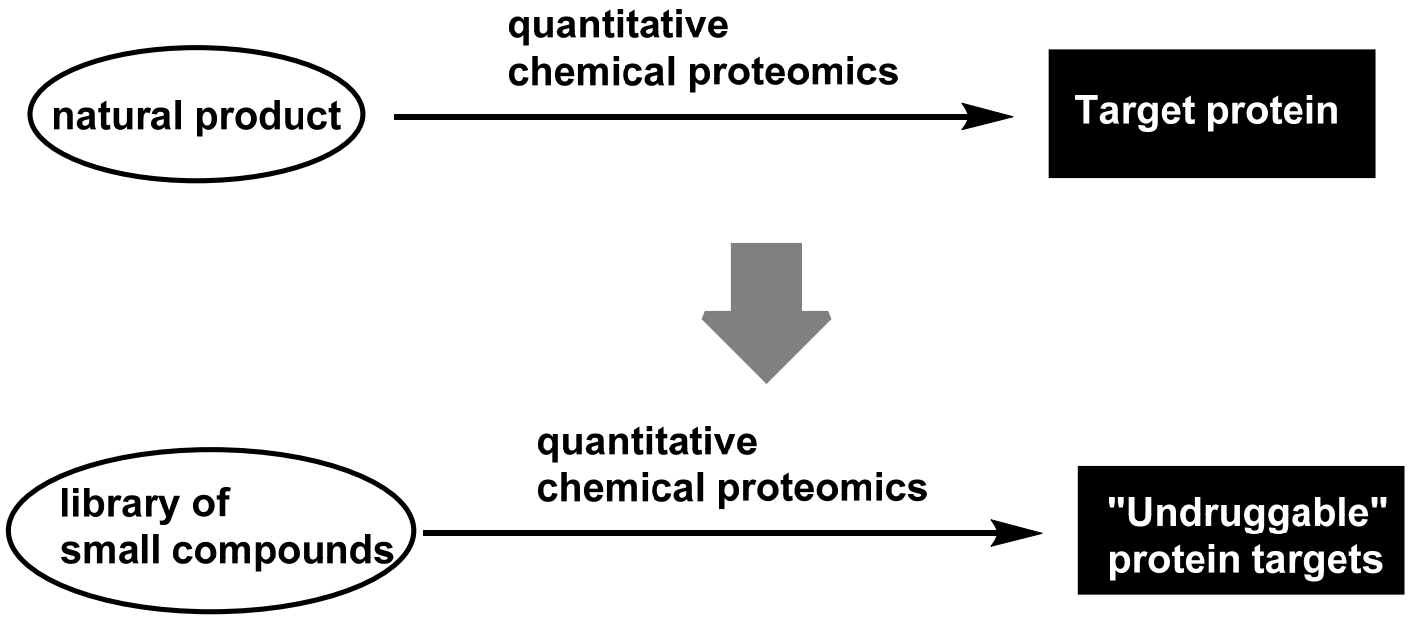
2004-: Professor, The Scripps Research Institute

Address to “Undruggable” Proteins

Table 1 | **Comparison of the druggable genomes of selected eukaryotes**

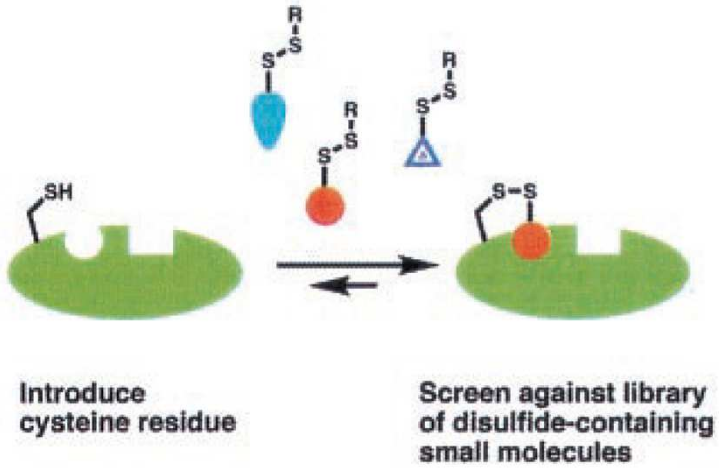
	Homo sapiens	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Saccharomyces cerevisiae*
Total number of predicted genes[8,9,16]	~30,000	13,601	18,424	6,241
Number of proteins in proteome*	21,688	13,849	17,946	6,127
Number of estimated druggable targets	3,051	1,714	2,267	508
Percentage that are predicted druggable targets	~10–14%	12%	12%	8%

Three hundred and seventy-six targets identified to bind rule-of-five-compliant drugs have had InterPro domains assigned. The prevalence of these InterPro domains in various genomes has then been determined. Twenty-three more bacterial and viral drug targets for which InterPro assignments could not be made have not been included in any of our analyses. *Data taken from InterPro, 29 October 2001.

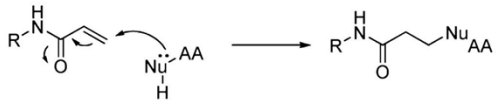
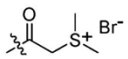
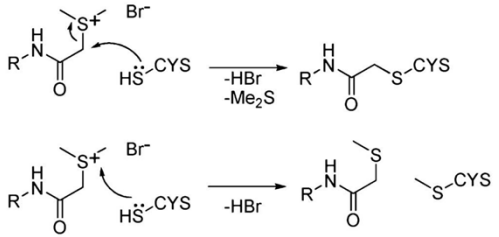
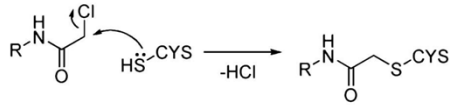
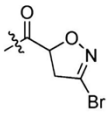
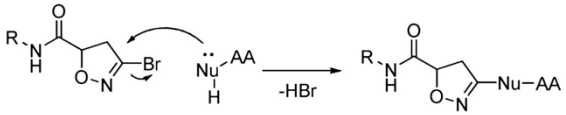
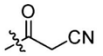
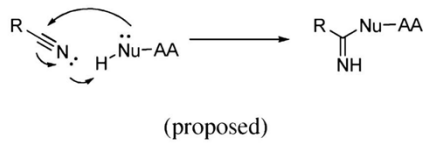
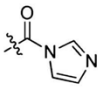
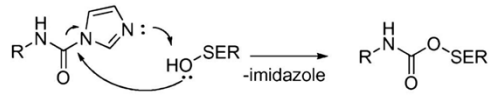


Hopkins, A. L.; Groom, C. R. *Nat. Rev. Drug Discov.* **2002**, *1*, 727.

Covalent bond Formation by Cysteine Residue

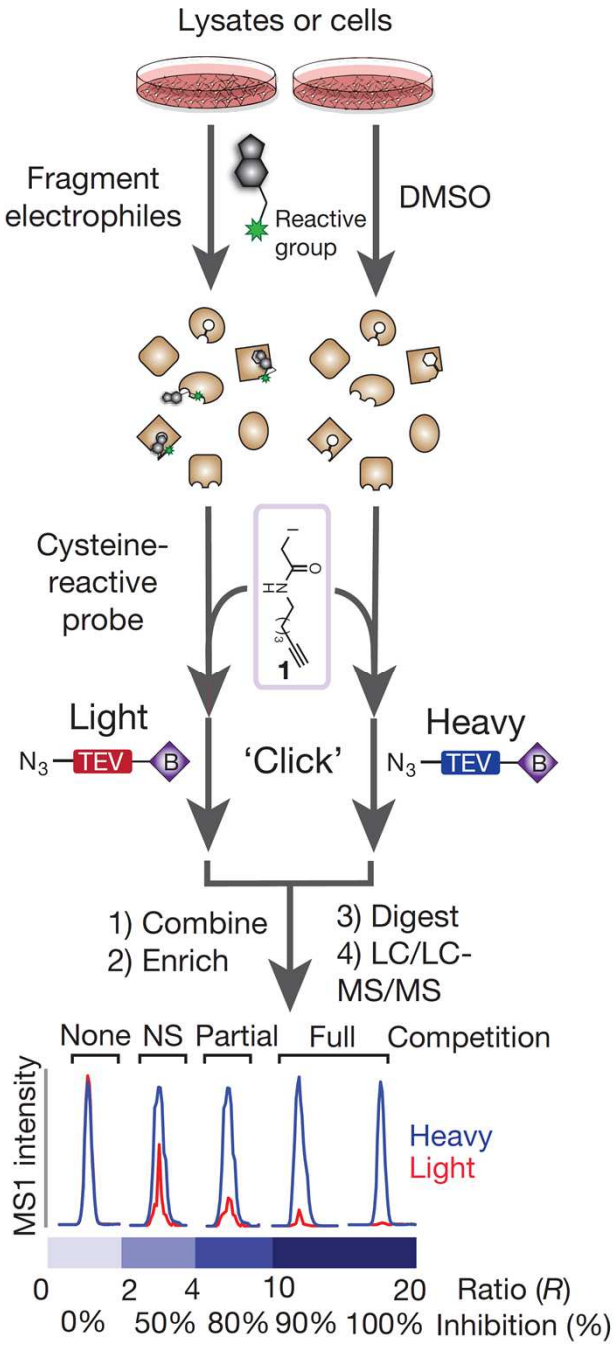


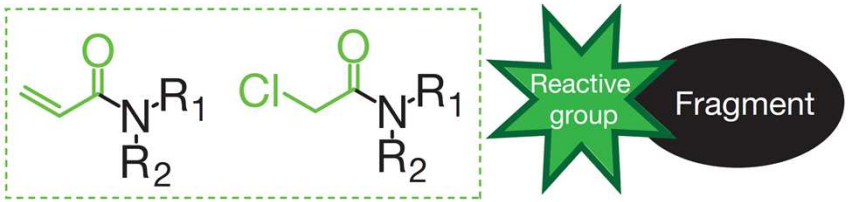
- **Improve biological efficiency**
- **Side effect can be minimized**
- **Can access regions of protein that are difficult to target through binding affinity alone**

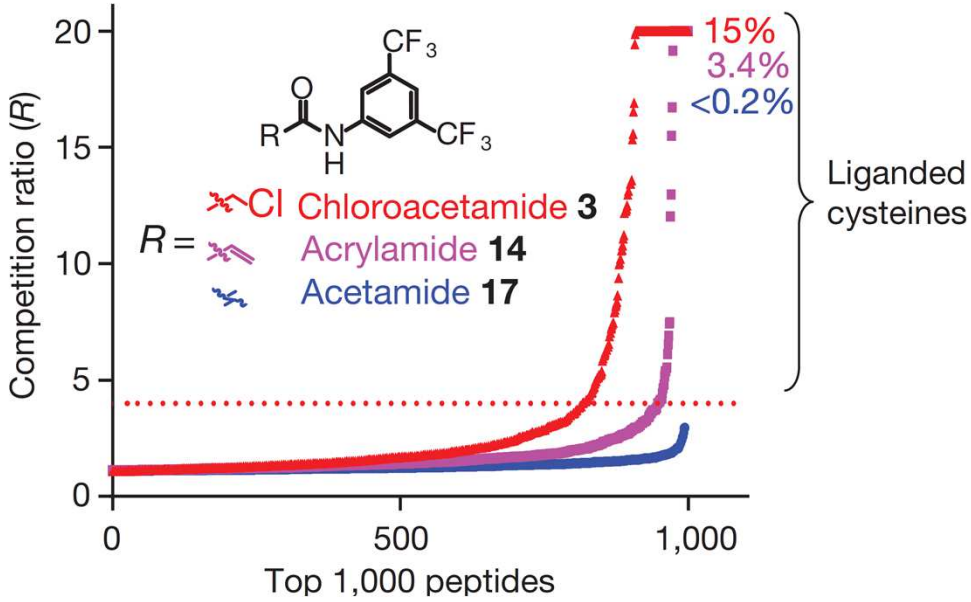
#	Electrophile	Targeted nucleophiles	Example	Binding mode
1		-	-	-
2		Cysteine, Threonine	Afatinib[43] Syringolin A[14]	
3		Cysteine	Transglutaminase inhibitors[12, 44]	
4		Cysteine	Metazachlor[11]	
5		Cysteine	Bromoacivicin[16, 45, 46]	
6		Serine Cysteine	NEP-inhibitors[47] Balicatib[48]	 (proposed)
7		Serine	L-norvaline methylester azolide[18]	 (proposed)

1) Erlanson, D. A.; Braisted, A. C.; Raphael, D. R.; Randal, M.; Stroud, R. M.; Gordon, E. M.; Wells, J. A. *Proc. Natl Acad. Sci. USA.* **2000**, *97*, 9367.
2) Jöst, C.; Nitsche, C.; Scholz, T.; Roux, L.; Klein, C. D. *J. Med. Chem.* **2014**, *57*, 7590.

Proteome-Wide Covalent Ligand Discovery

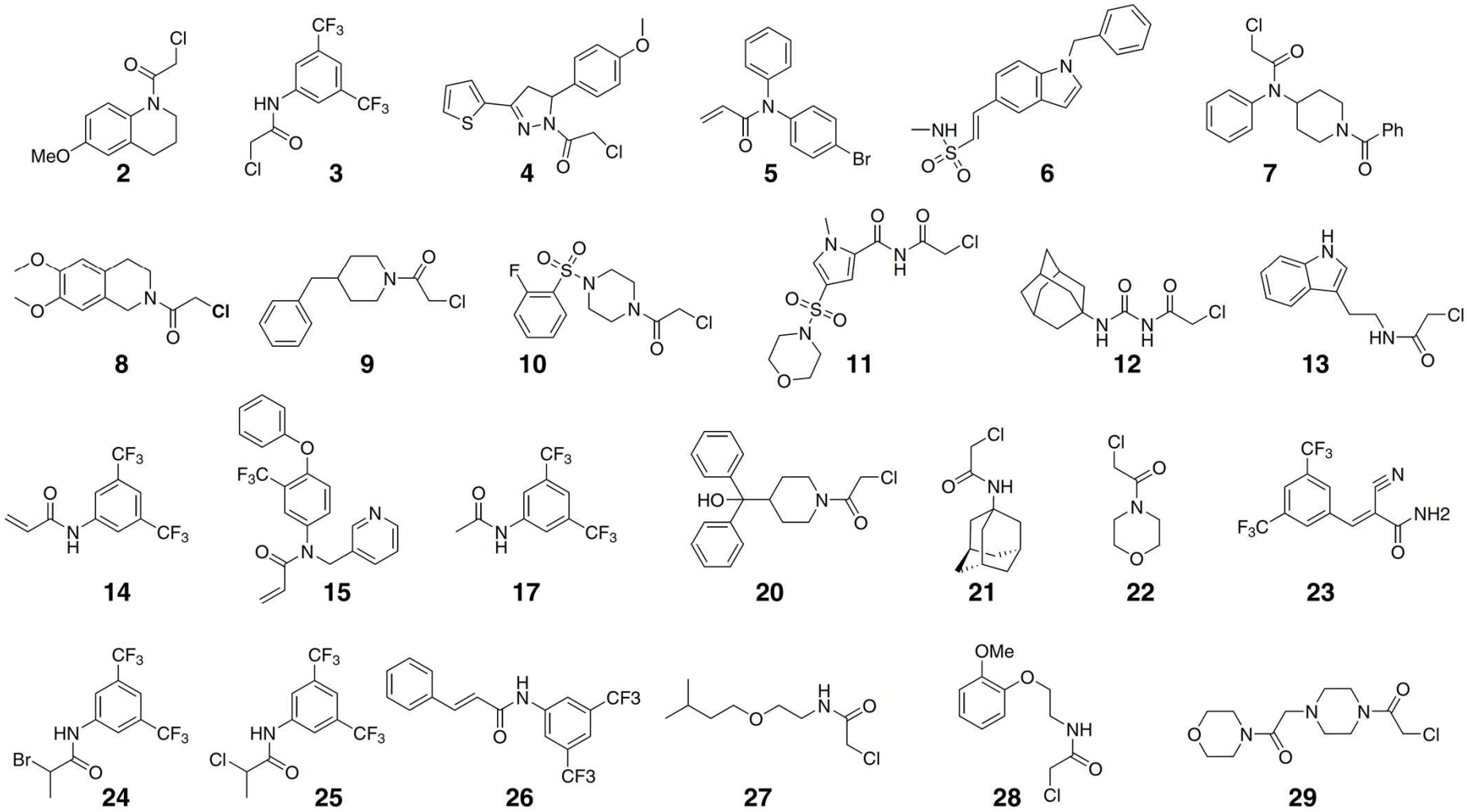






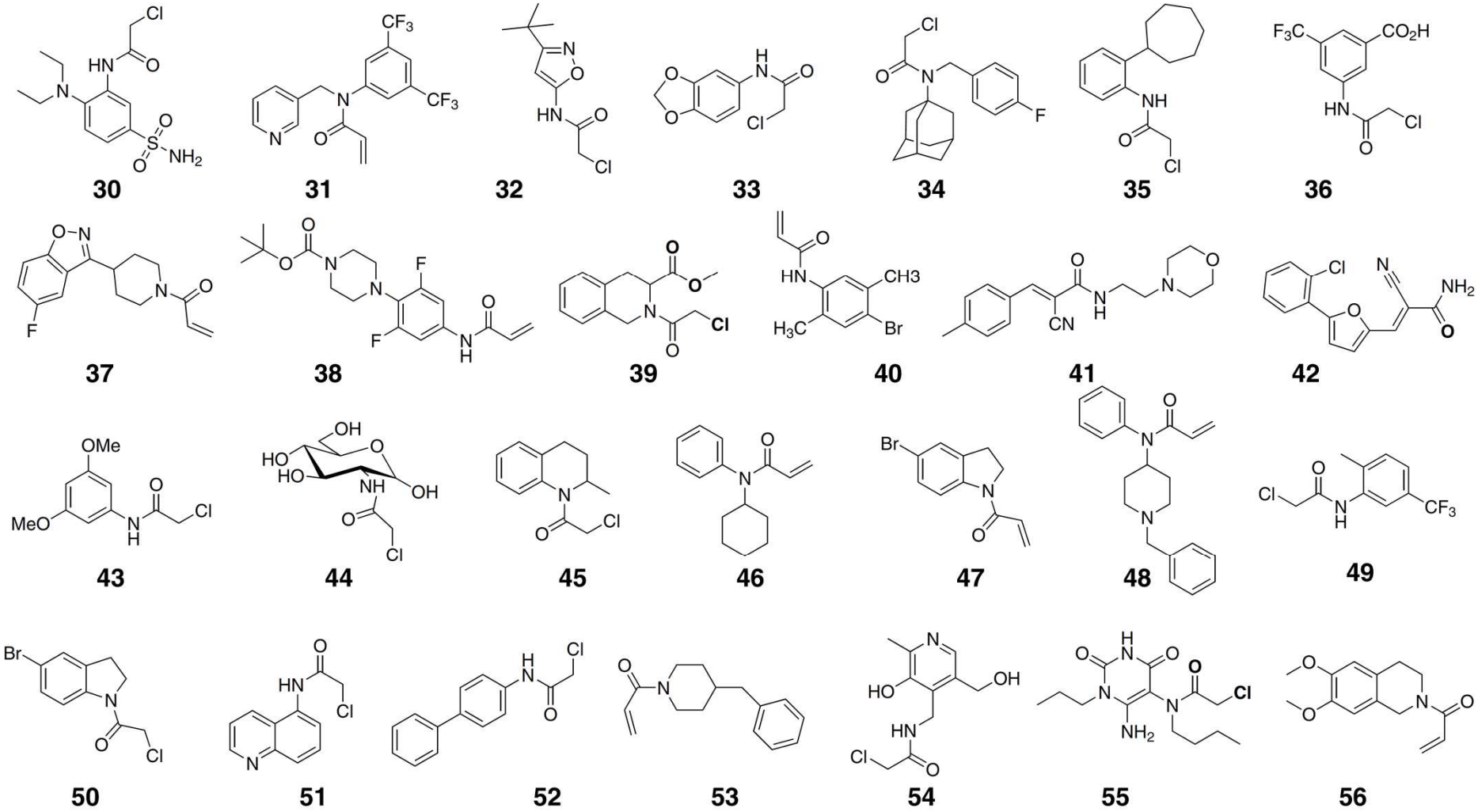
Backus, K. M.; Correia, B. E.; Lum, K. M.; Forli, S.; Horning, B. D.; González-Páez, G. E.; Chatterjee, S.; Lanning, B. R.; Teijaro, J. R.; Olson, A. J.; Wolan, D. W.; Cravatt, B. F. *Nature* **2016**, *534*, 570.

Electrophilic Fragment Library (1)



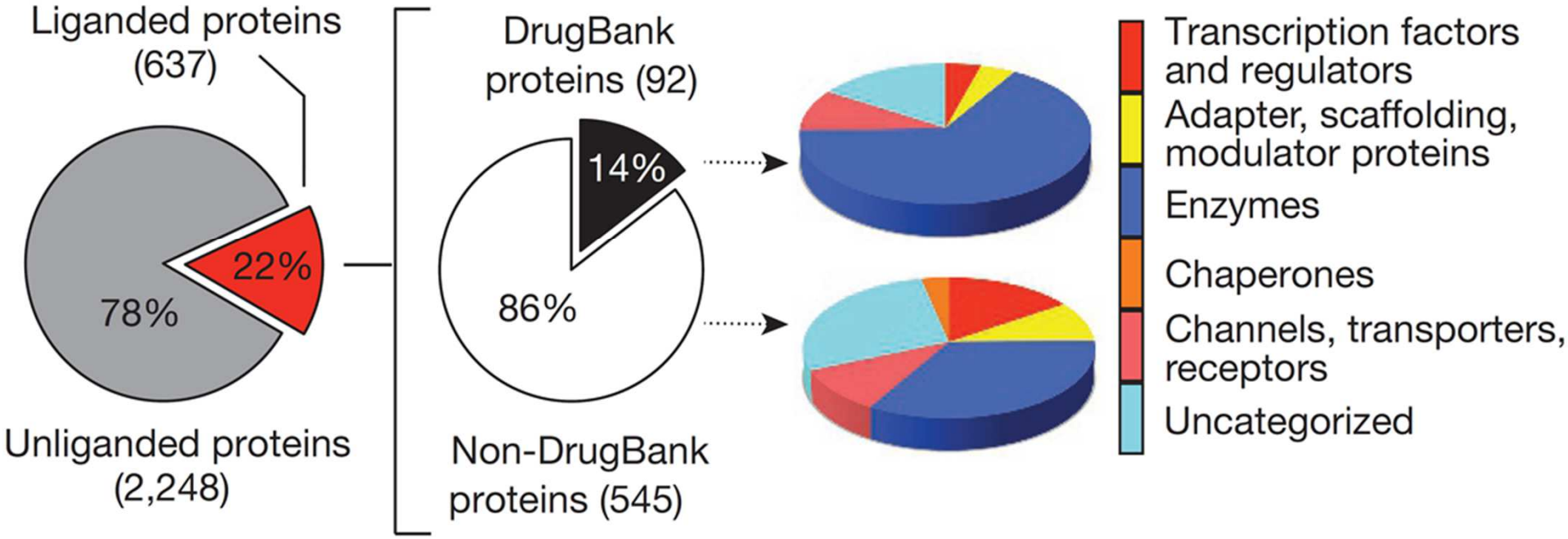
Backus, K. M.; Correia, B. E.; Lum, K. M.; Forli, S.; Horning, B. D.; González-Páez, G. E.; Chatterjee, S.; Lanning, B. R.; Teijaro, J. R.; Olson, A. J.; Wolan, D. W.; Cravatt, B. F. *Nature* **2016**, *534*, 570.

Electrophilic Fragment Library (2)



Backus, K. M.; Correia, B. E.; Lum, K. M.; Forli, S.; Horning, B. D.; González-Páez, G. E.; Chatterjee, S.; Lanning, B. R.; Teijaro, J. R.; Olson, A. J.; Wolan, D. W.; Cravatt, B. F. *Nature* **2016**, *534*, 570.

Proteins Liganded by Fragment Electrophiles



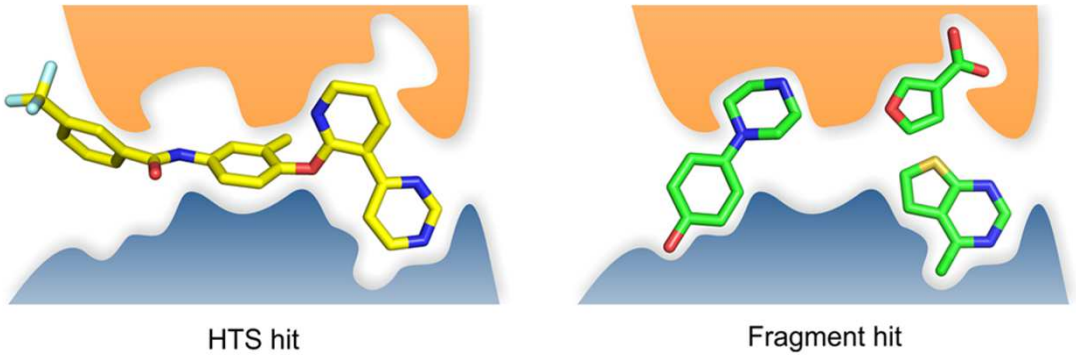
- **Non-DrugBank proteins were successfully detected as new proteins targets by covalent interaction.**

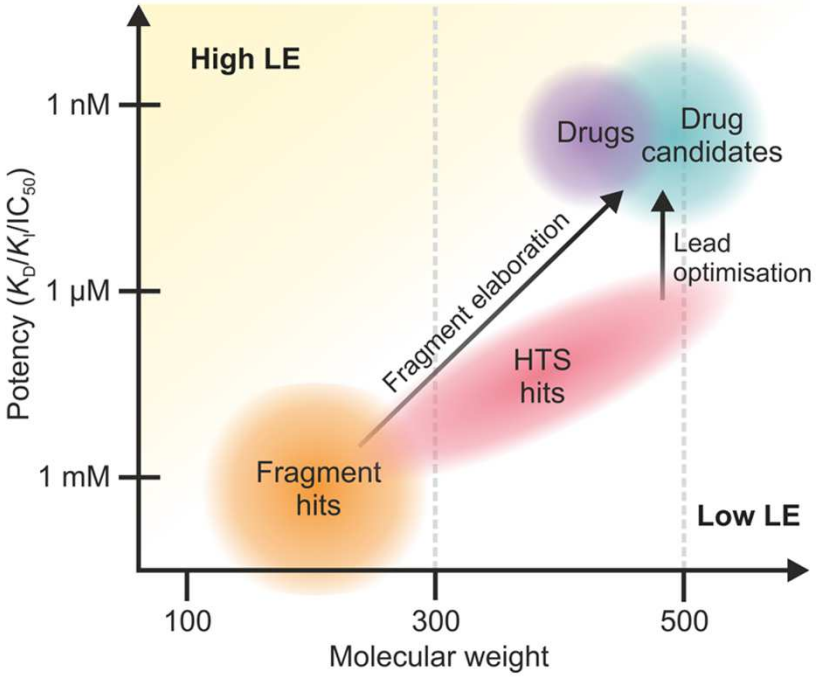
Next question

- **Non-covarent fragment-protein interaction can be profiled in the same way?**

Backus, K. M.; Correia, B. E.; Lum, K. M.; Forli, S.; Horning, B. D.; González-Páez, G. E.; Chatterjee, S.; Lanning, B. R.; Teijaro, J. R.; Olson, A. J.; Wolan, D. W.; Cravatt, B. F. *Nature* **2016**, *534*, 570.

Fragment-Based Ligand and Drug Discovery (FBLD)

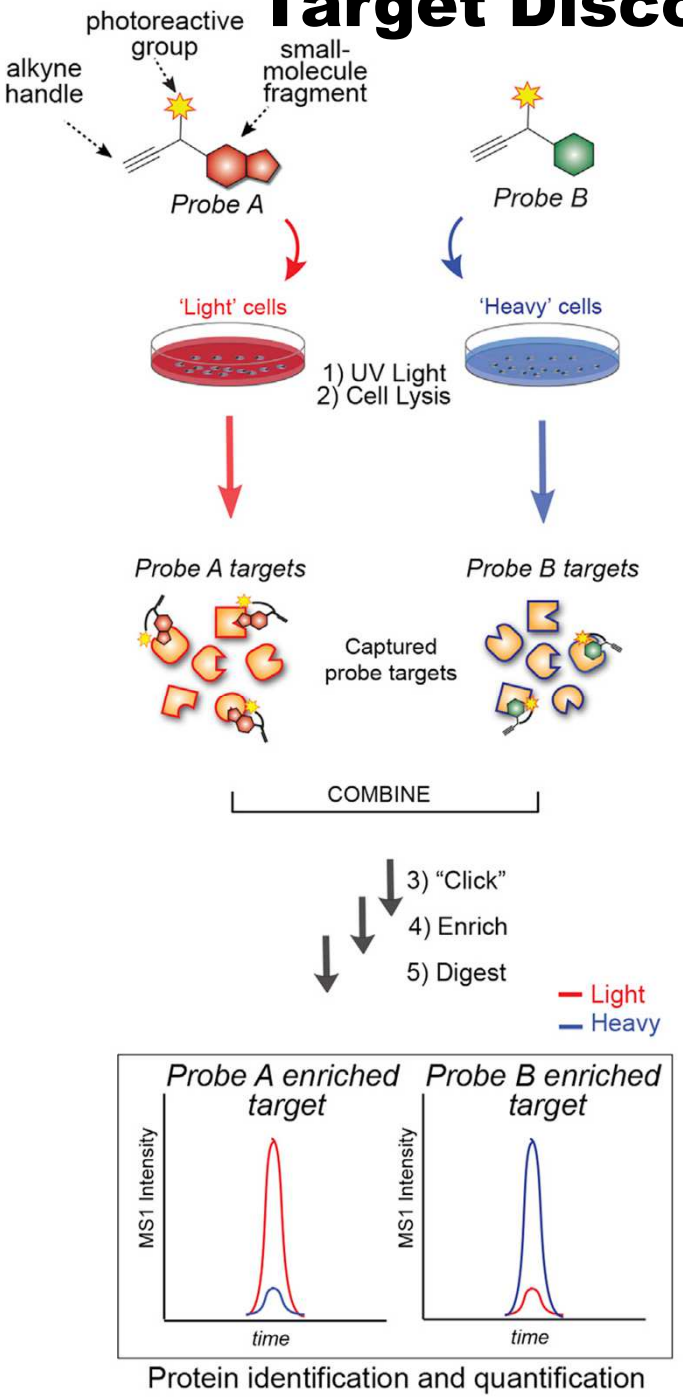


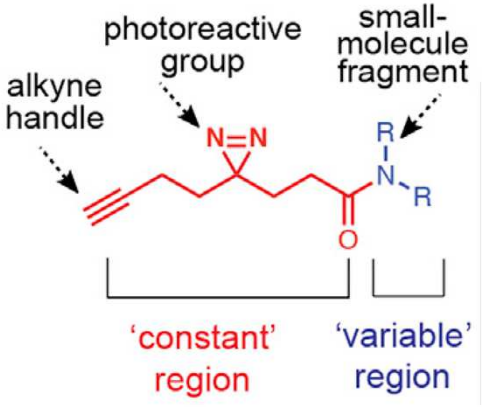


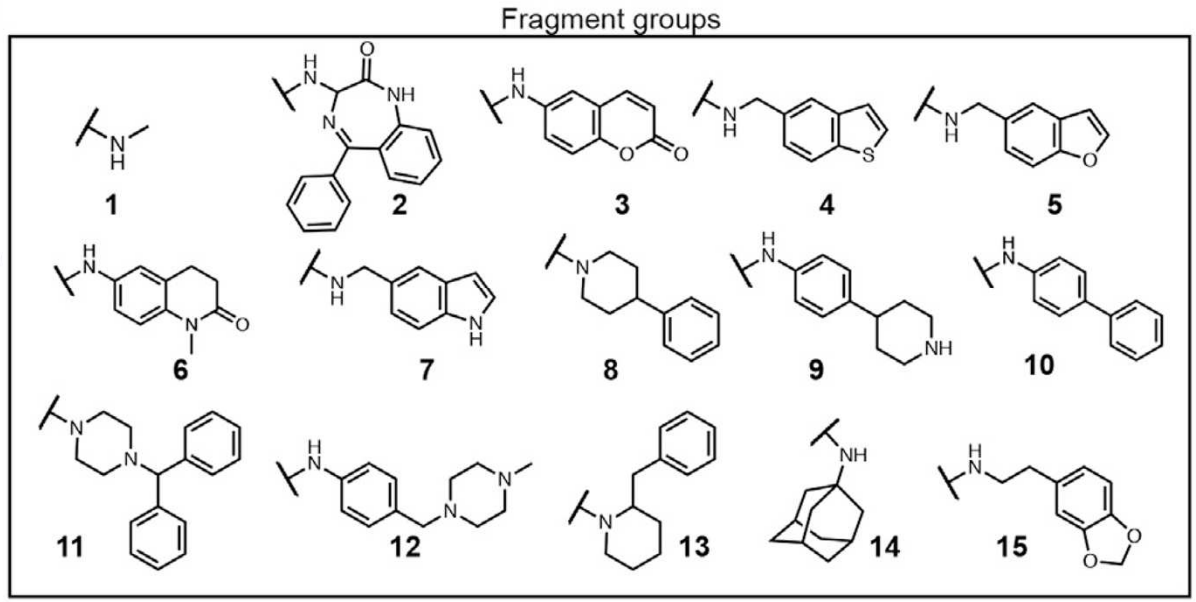
- **It is constructed by low-molecular weight compounds (<300 Da).**
- **Structurally small hit compounds can be efficiently optimized into more potent ligands.**
- **Can access to proteins that have proven difficult to target using high-throughput screening of complex compounds.**

Scott, D. E.; Coyne, A. G.; Hudson, S. A.; Abell, C. *Biochemistry* **2012**, *51*, 4990.

Target Discovery by Non-covalent Ligands

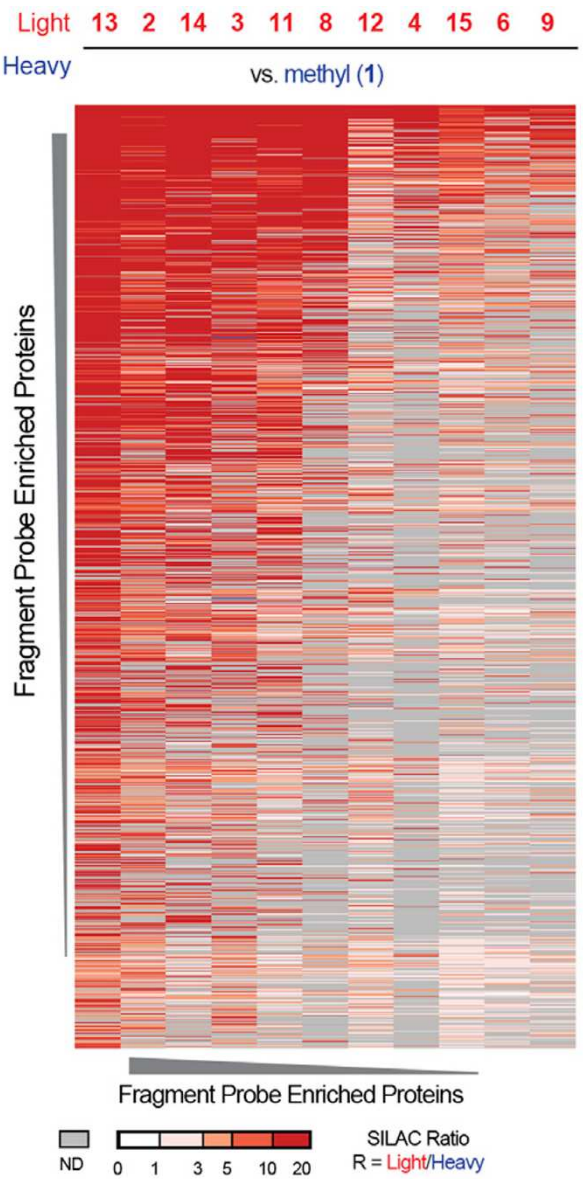


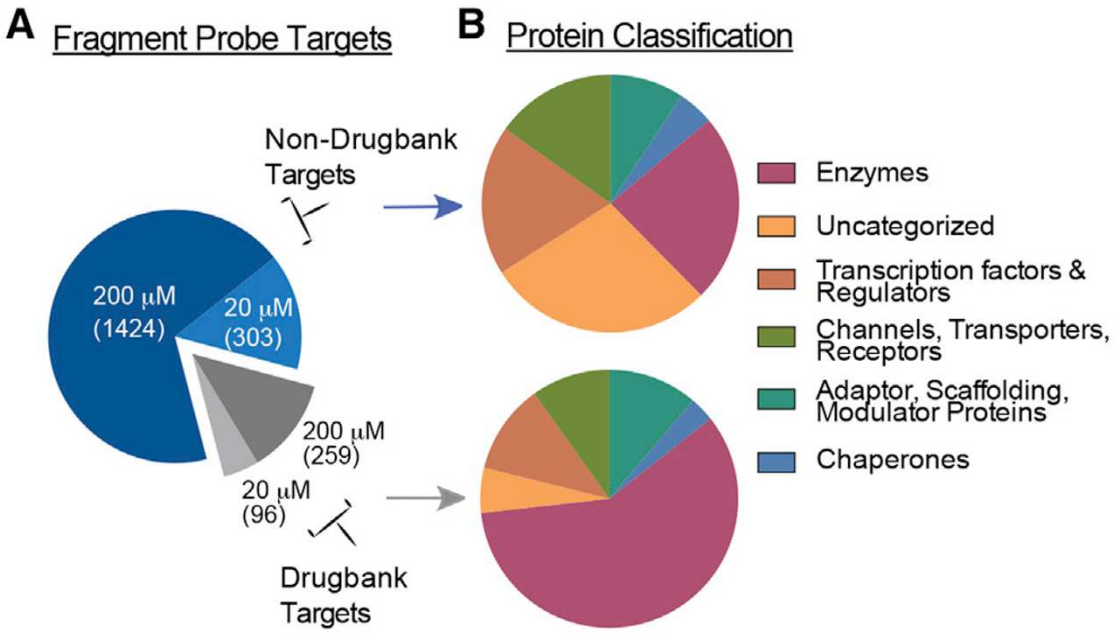




Parker, C. G.; Galmozzi, A.; Wang, Y.; Correia, B. E.; Sasaki, K.; Joslyn, C. M.; Kim, A. S.; Cavallaro, C. L.; Lawrence, R. M.; Johnson, S. R.; Narvaiza, I.; Saez, E.; Cravatt, B. F. *Cell* **2017**, *168*, 527.

Proteins Liganded by Non-covalent Ligand





- **Non-DrugBank proteins were successfully detected as new proteins targets by non-covalent interaction.**

limitations

- **The probes showed complicated profiles in their proteomic interaction.**
- **lack of detailed stractural information for SAR study.**

enantiomeric probes were used. (physicochemically matched, differ only in stereochemistry)

Parker, C. G.; Galmozzi, A.; Wang, Y.; Correia, B. E.; Sasaki, K.; Joslyn, C. M.; Kim, A. S.; Cavallaro, C. L.; Lawrence, R. M.; Johnson, S. R.; Narvaiza, I.; Saez, E.; Cravatt, B. F. *Cell* **2017**, *168*, 527.

Contents

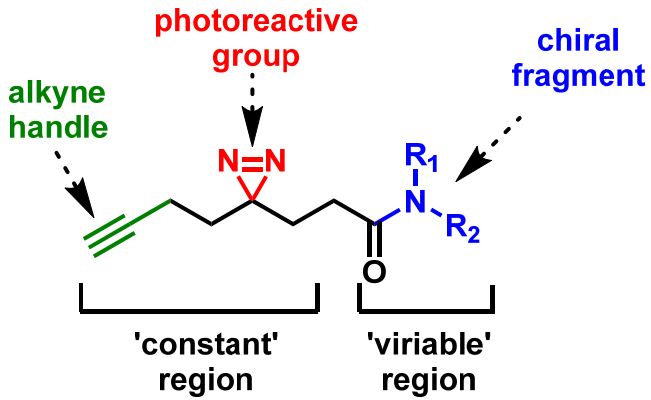
1. Introduction
   Affinity probe and chemical proteomics

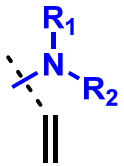
2. Approaches for “undruggable” proteins

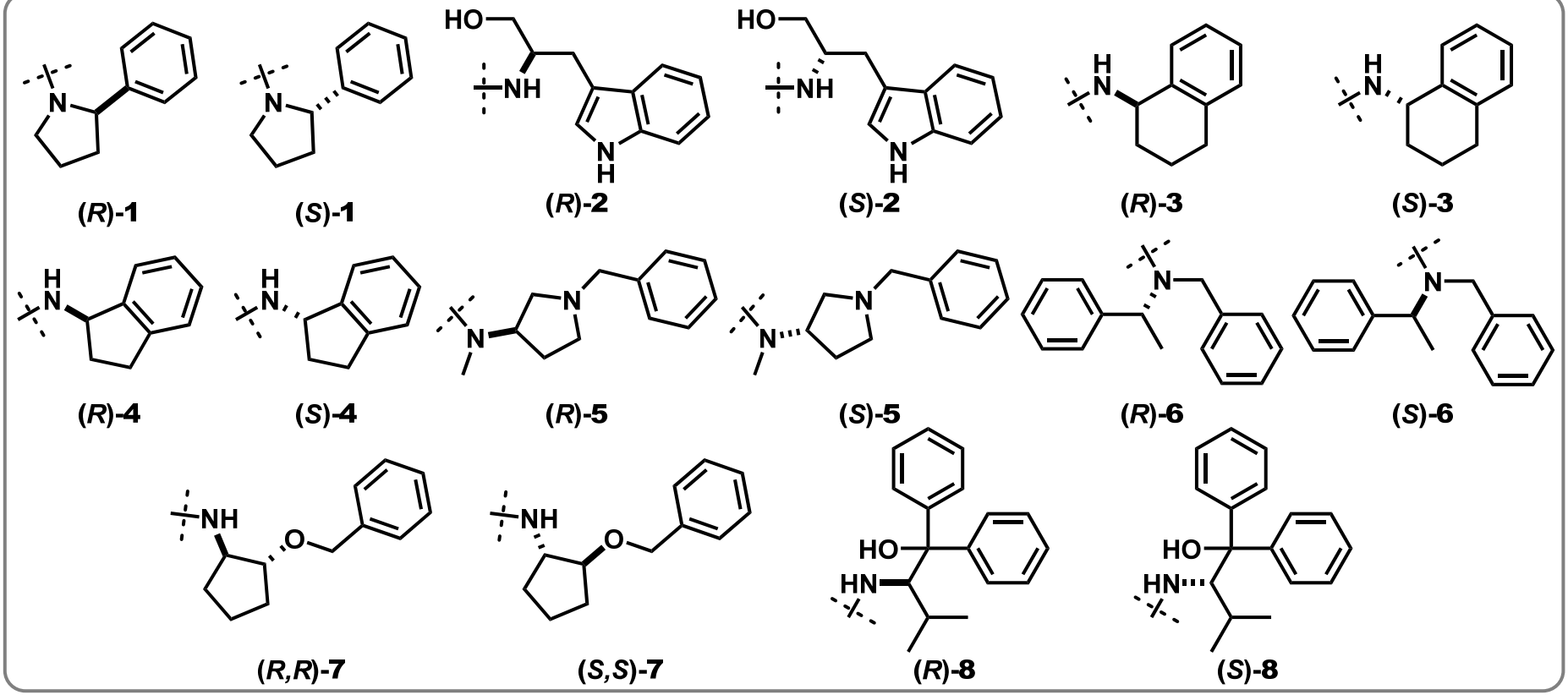
3. **Main paper**

   Wang, Y.; Dix, M. M.; Bianco, G.; Remberg, J. R.; Lee, H. Y.; Kalocsay, M.; Gygi, S. P.; Vite, G.; Lawrence, R. M.; Parker, C. G.; Cravatt, B. F.
   ***Nat. Chem*. 2019**, *11*, 1113.

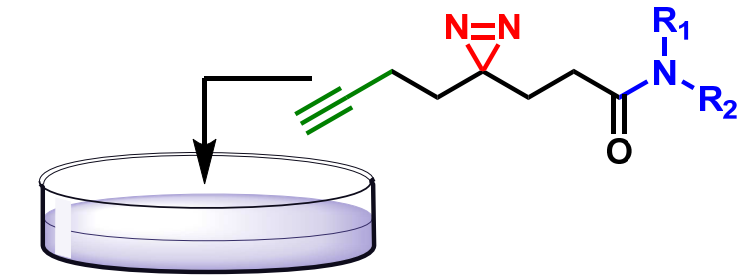
Design of Enatiomeric Probe Pairs







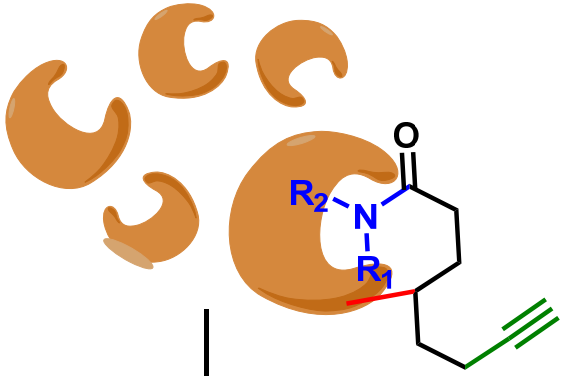
Gel-Based Profiling



HEK293T cells

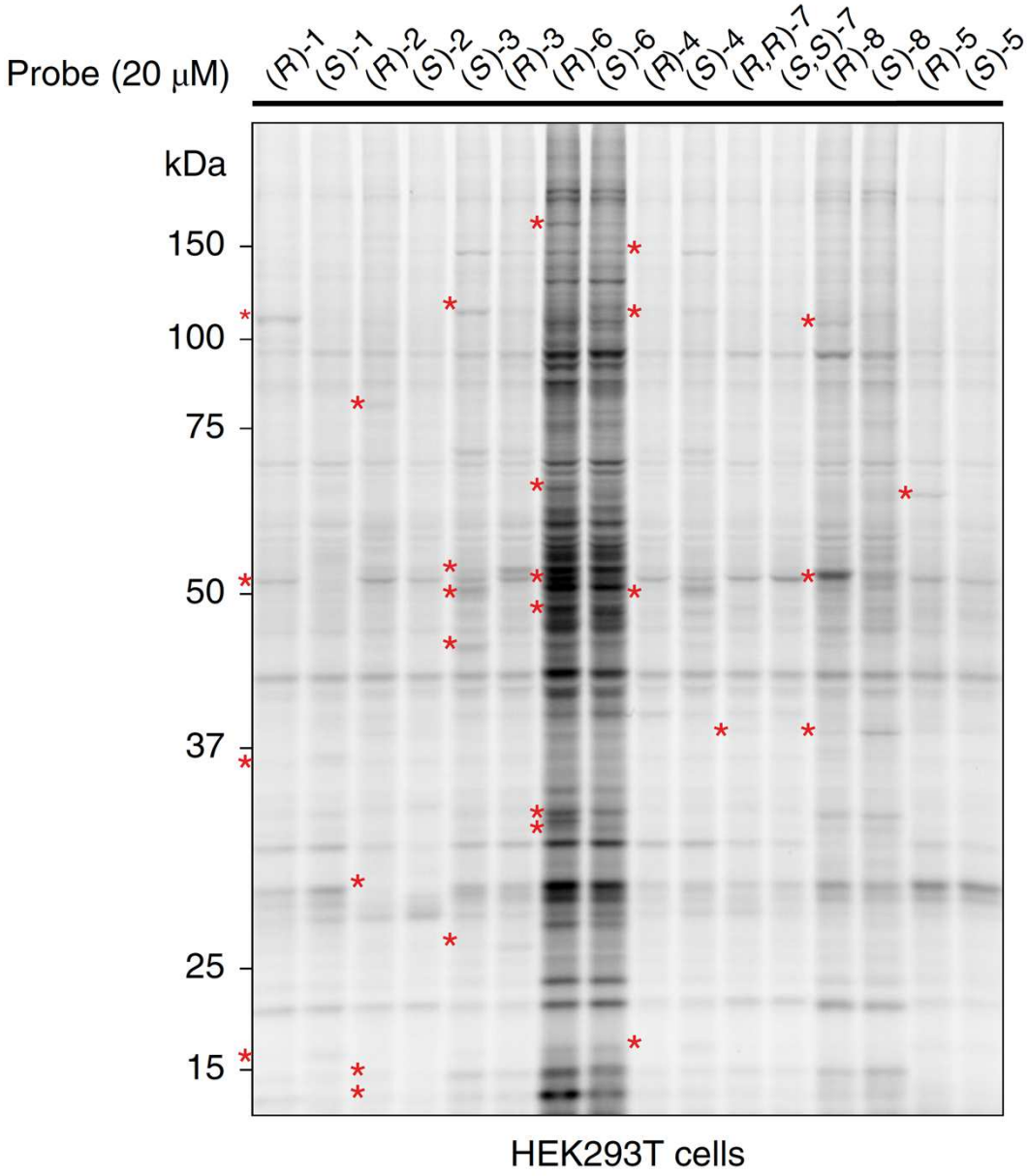
(1) UV light

(2) Cell lysis



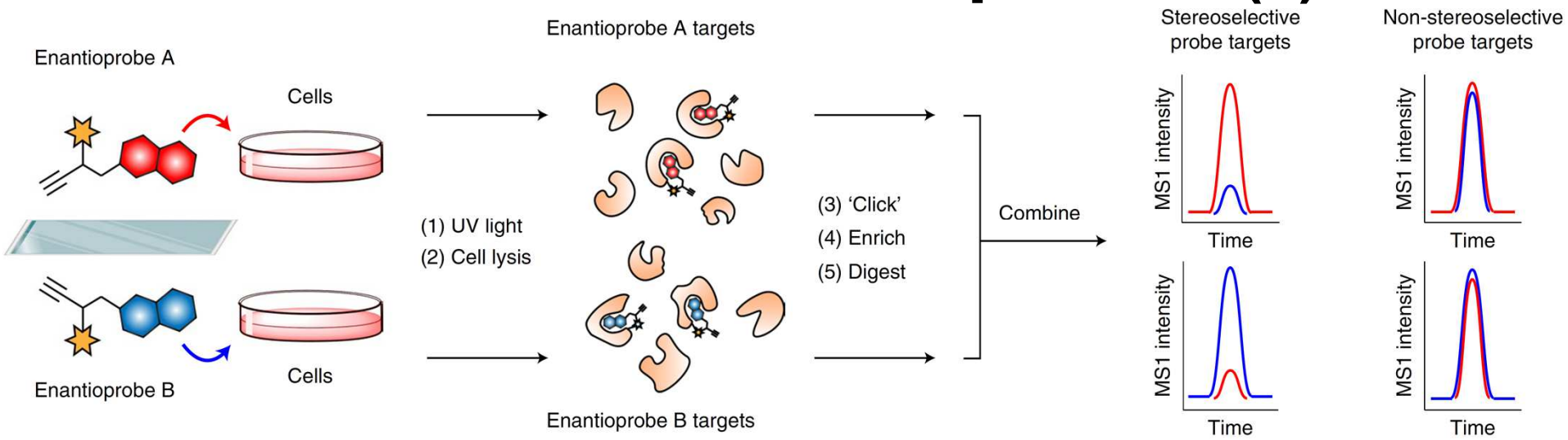
(3) 'click' reaction with azide-rhoidamine tag

electrophoresis

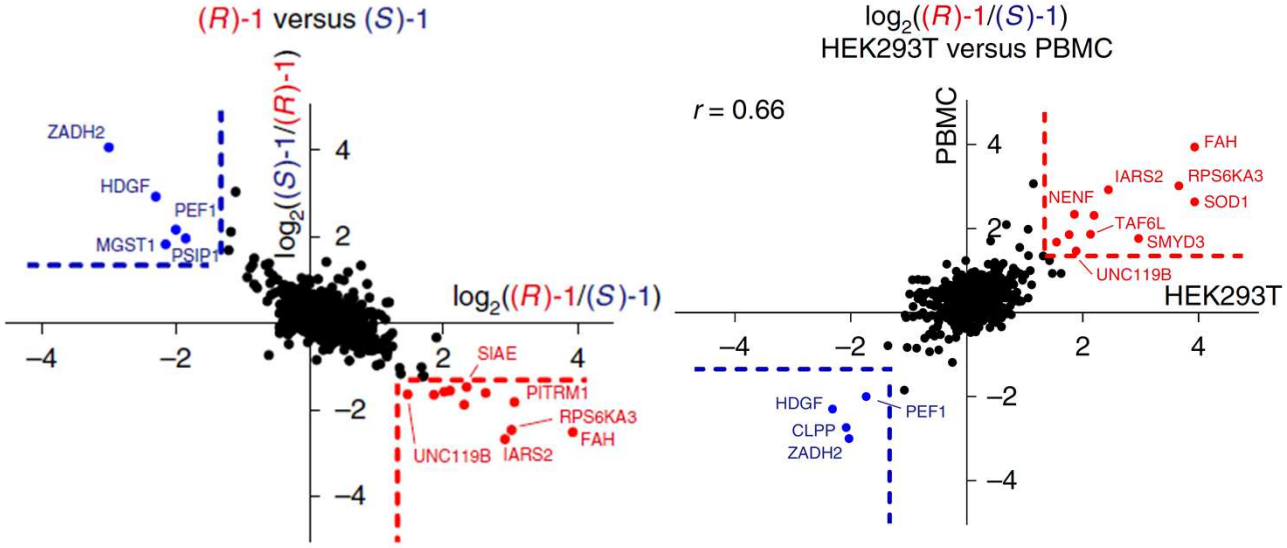


*representative stereoselective enantioprobe-protein interaction

Chemical Proteomics Experiment (1)



Method : ReDiMe for PBMCs and SILAC for HEK293T cells

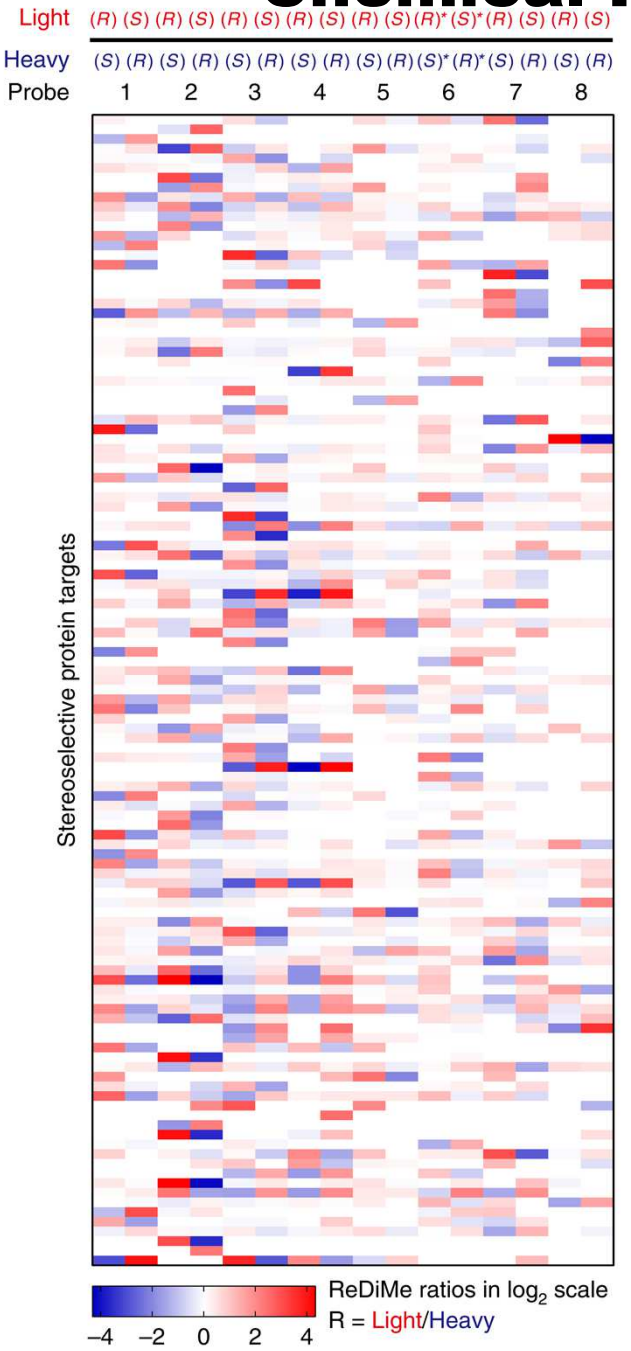


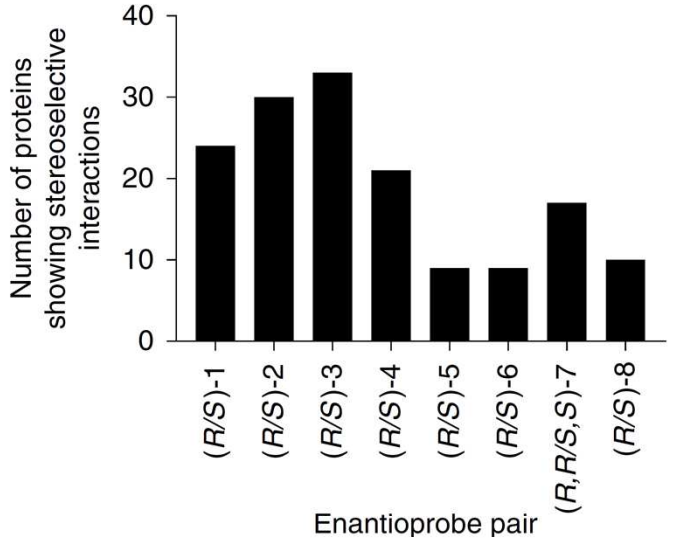
- **The *R* and *S* enantiomers have equivalent potential to preferentially enrich proteins**

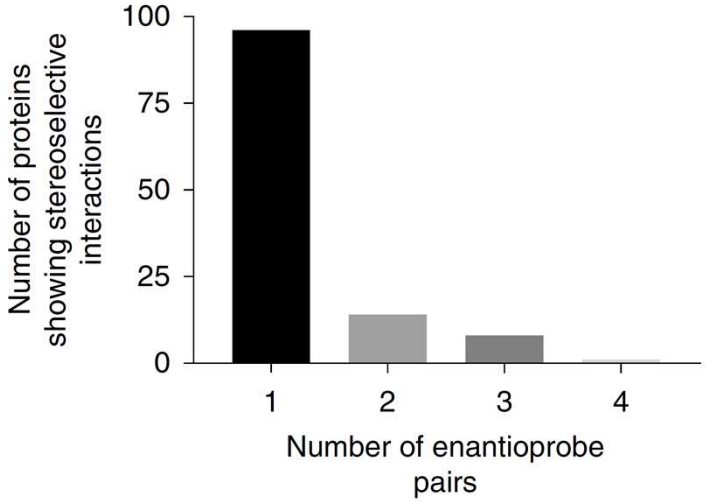
- **Both cells showed consistent profile**

An average value of >2.5-fold considered as 'stereoselective'

Chemical Proteomics Experiment (2)

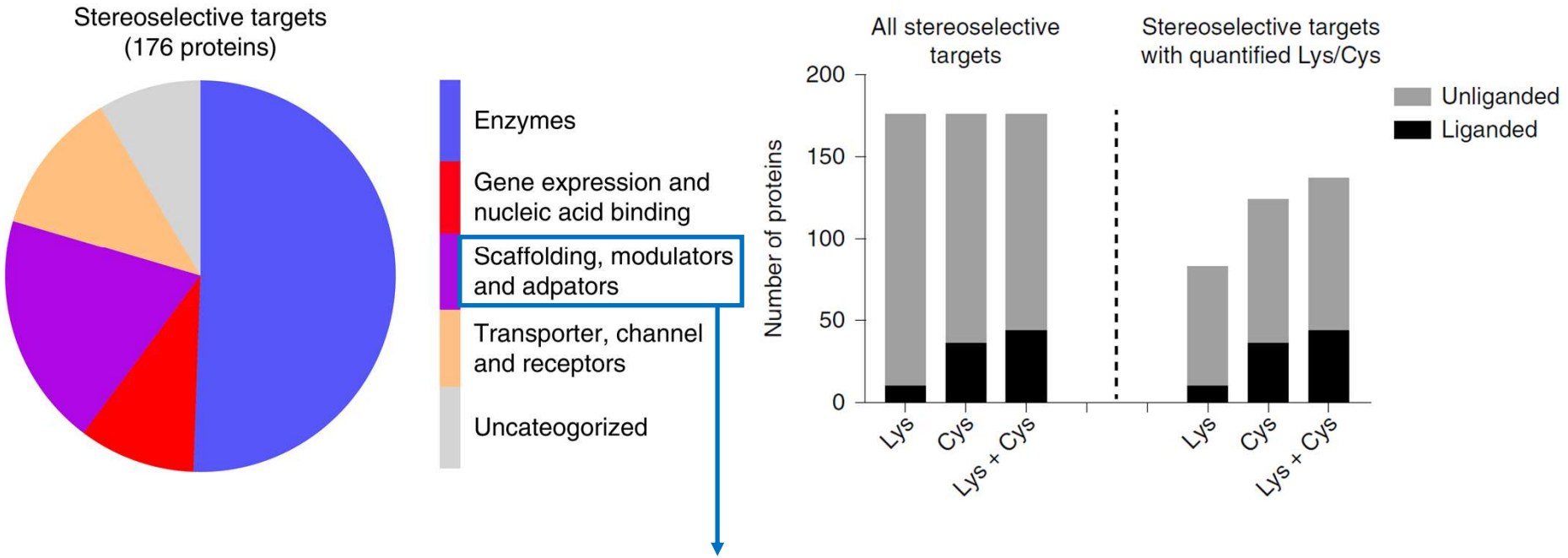






・The majority of proteins shows stereoselective interaction with only one of the enantioprobe pairs.

Targeted Protein by Enantioprobes

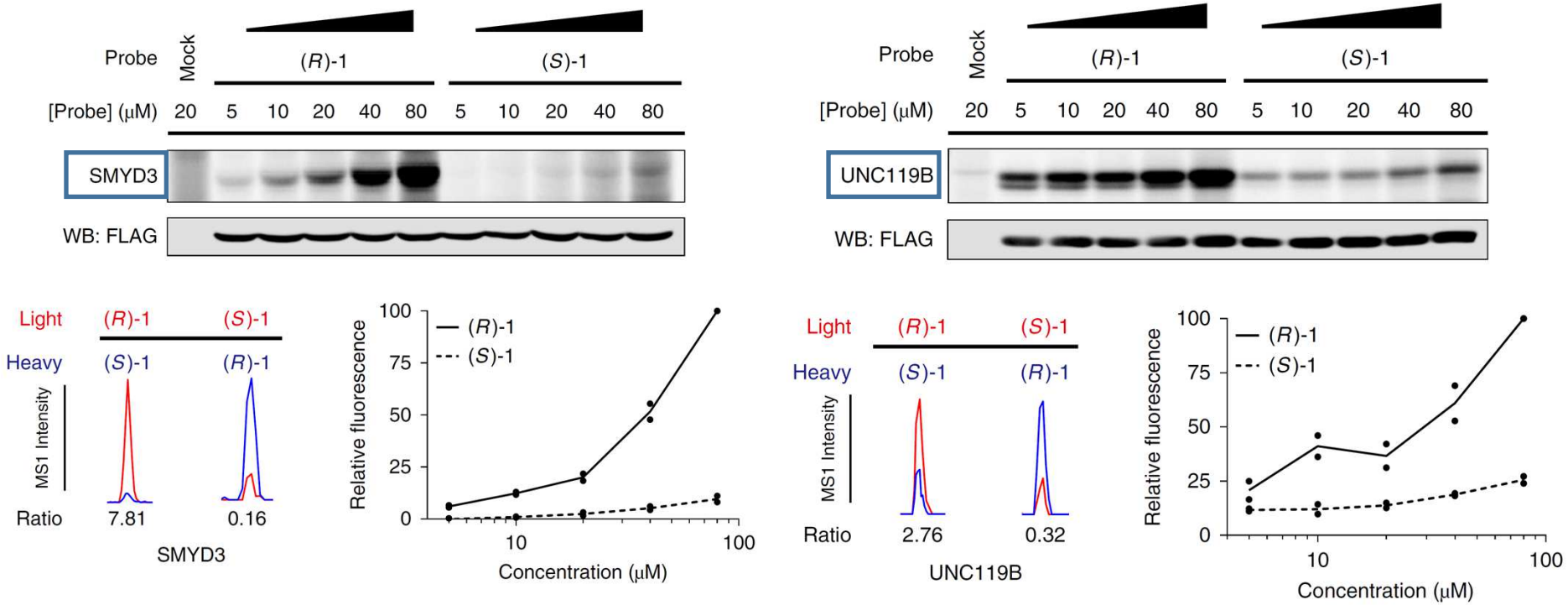


historically considered challenging to target with small molecules

There are limited overlap of enantioprobe targets with proteins that were targeted by covalent fragments

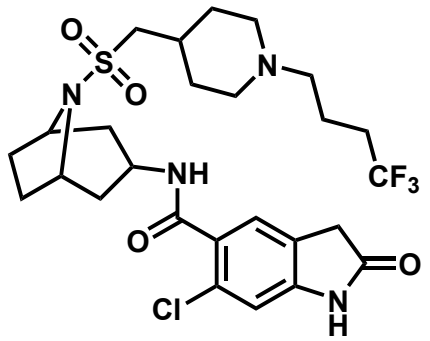
Covalent and non-covalent fragments target different proteins

Interaction with Recombinantly Expressed Protein

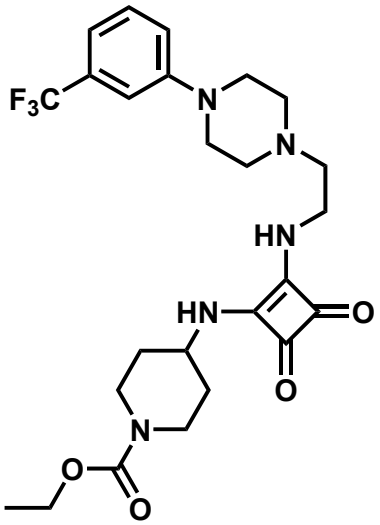


Enantioprobe (*R*)-1 stereoselectively binds to SMYD3 and UNC119B

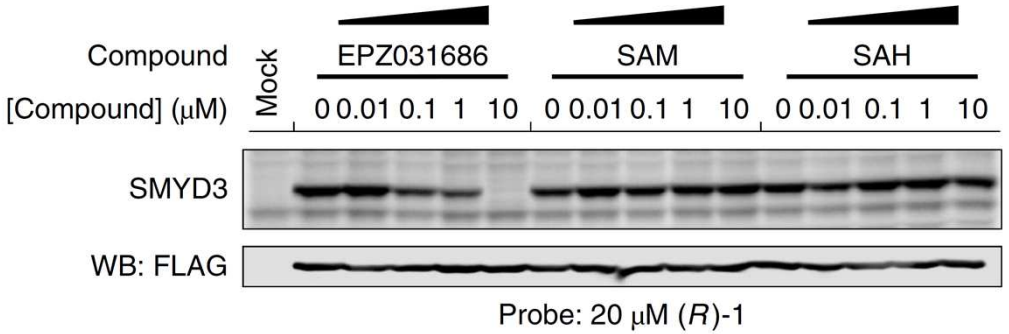
Competitive Experiment by Known Inhibitor

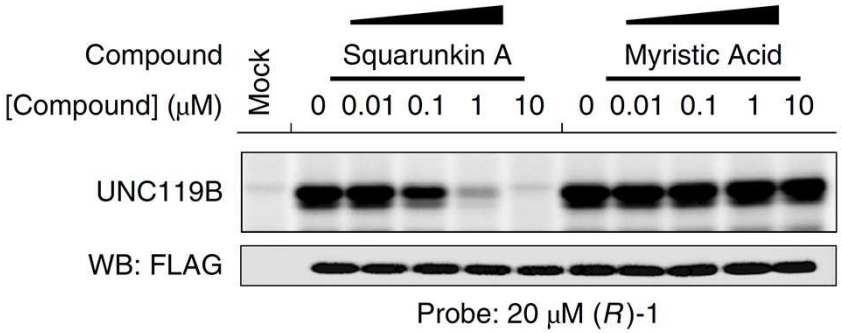


EPZ031686: inhibitor of SMYD3



squarunkin A: inhibitor of UNC119B



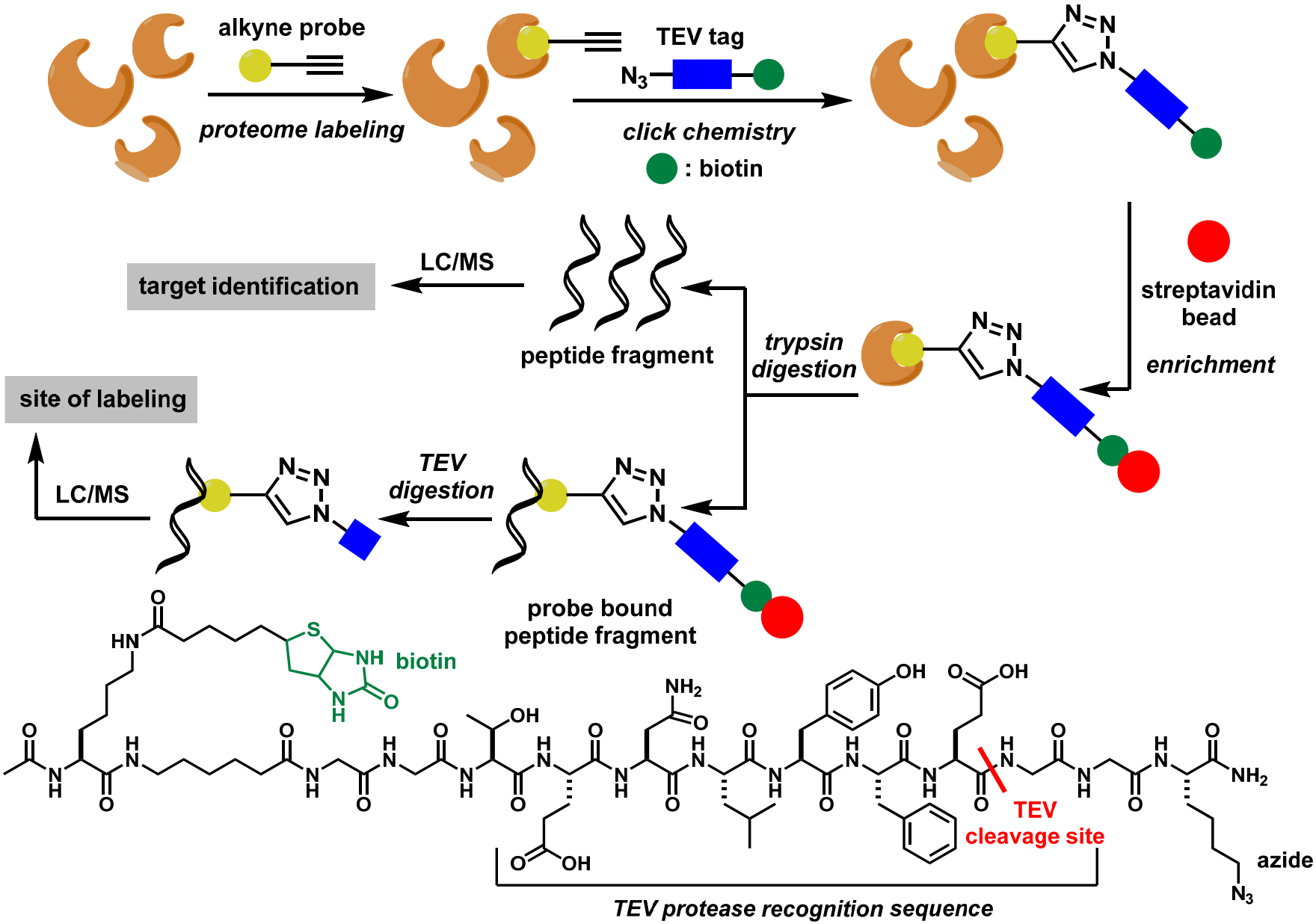


Protein targets were blocked by increase concentrations of inhibitors (EPZ031686, squarunkin A)

Probe (*R*)-1 may bind to the proteins at the same site of inhibitors

Binding sites of probe (*R*)-1 to the proteins were next determined.

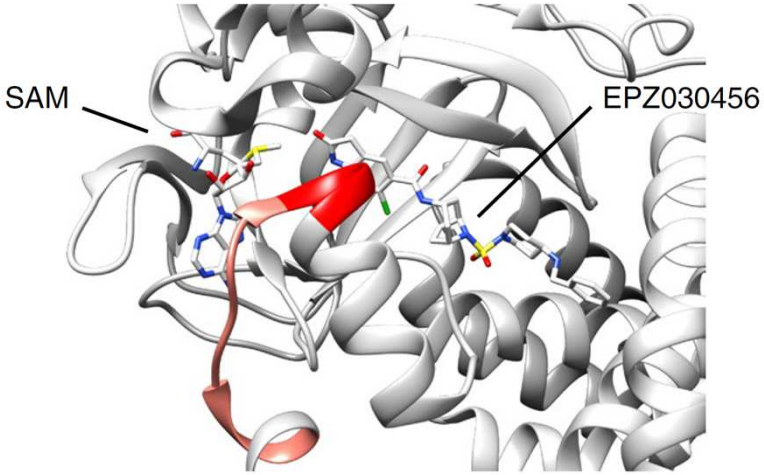
Method for Determinination of Binding Site



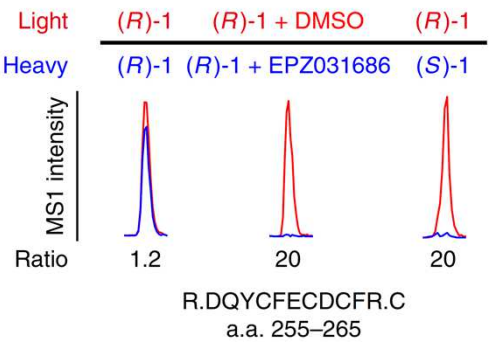
Weerapana, E.; Speers, A. E.; Cravatt, B. F. *Nat. Protoc.* **2007**, *2*, 1414.

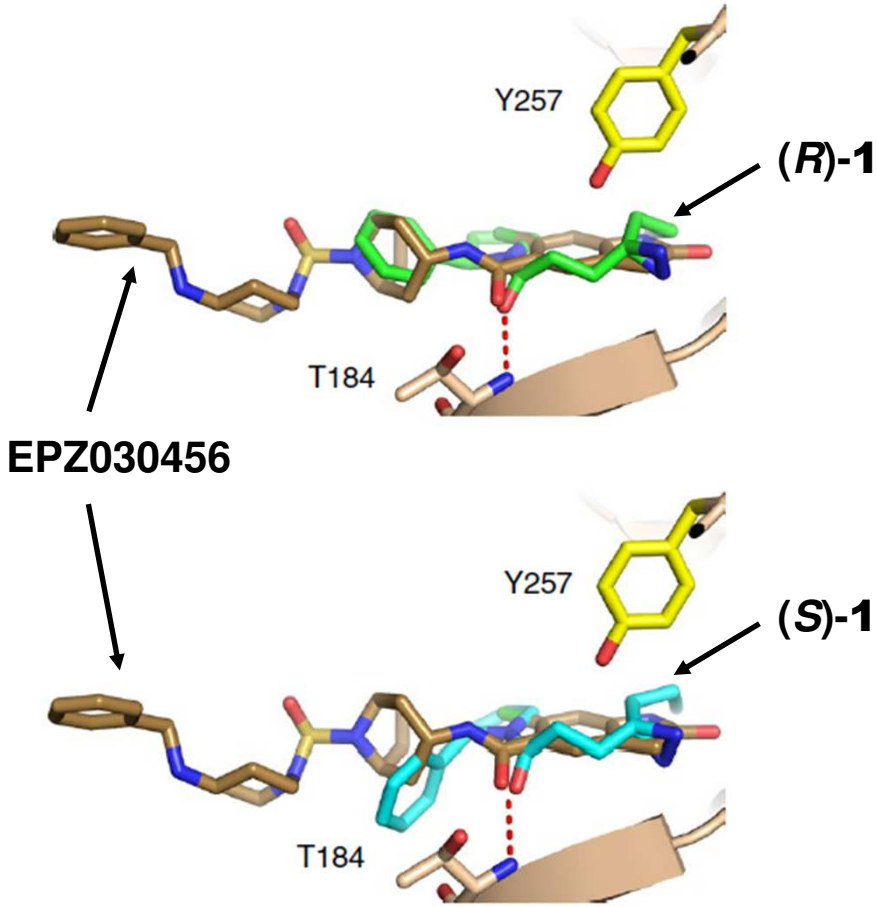
Structural Study

SMYD3



PDB : 5CCM

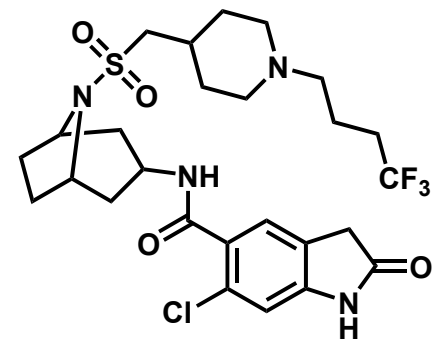




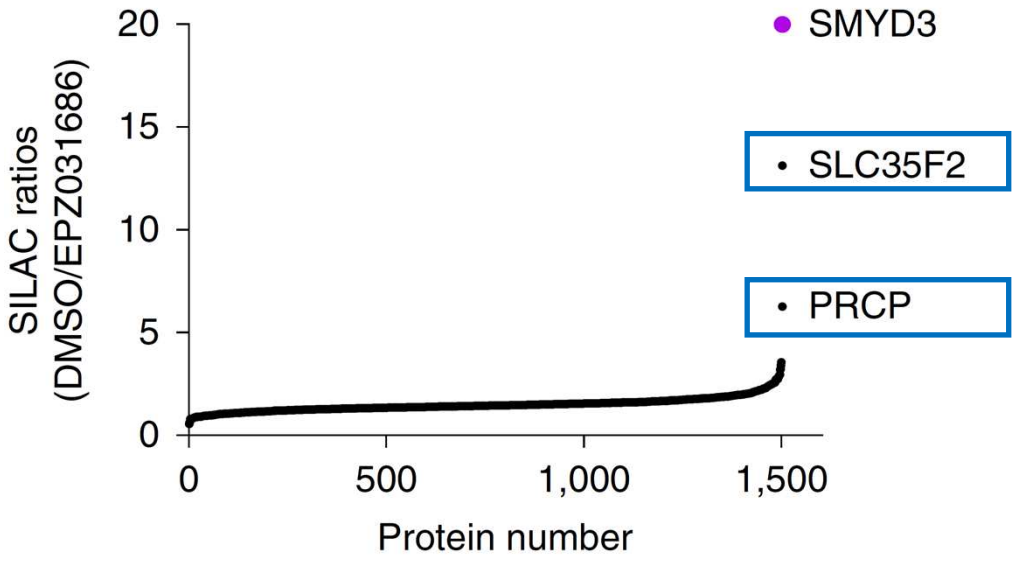
(*R*)-1 was found to bind at residue 255-267 (light red).

- **Docking simulation reveal that (*R*)-1 overlapped with EPZ030456.**
- **On the other hand, phenyl ring of (*S*)-1 went towards threonine residue.**
- **This affected the difference of affinity of each probe.**

Other Blocked Proteins by Known Inhibitor



EPZ031686: inhibitor of SMYD3

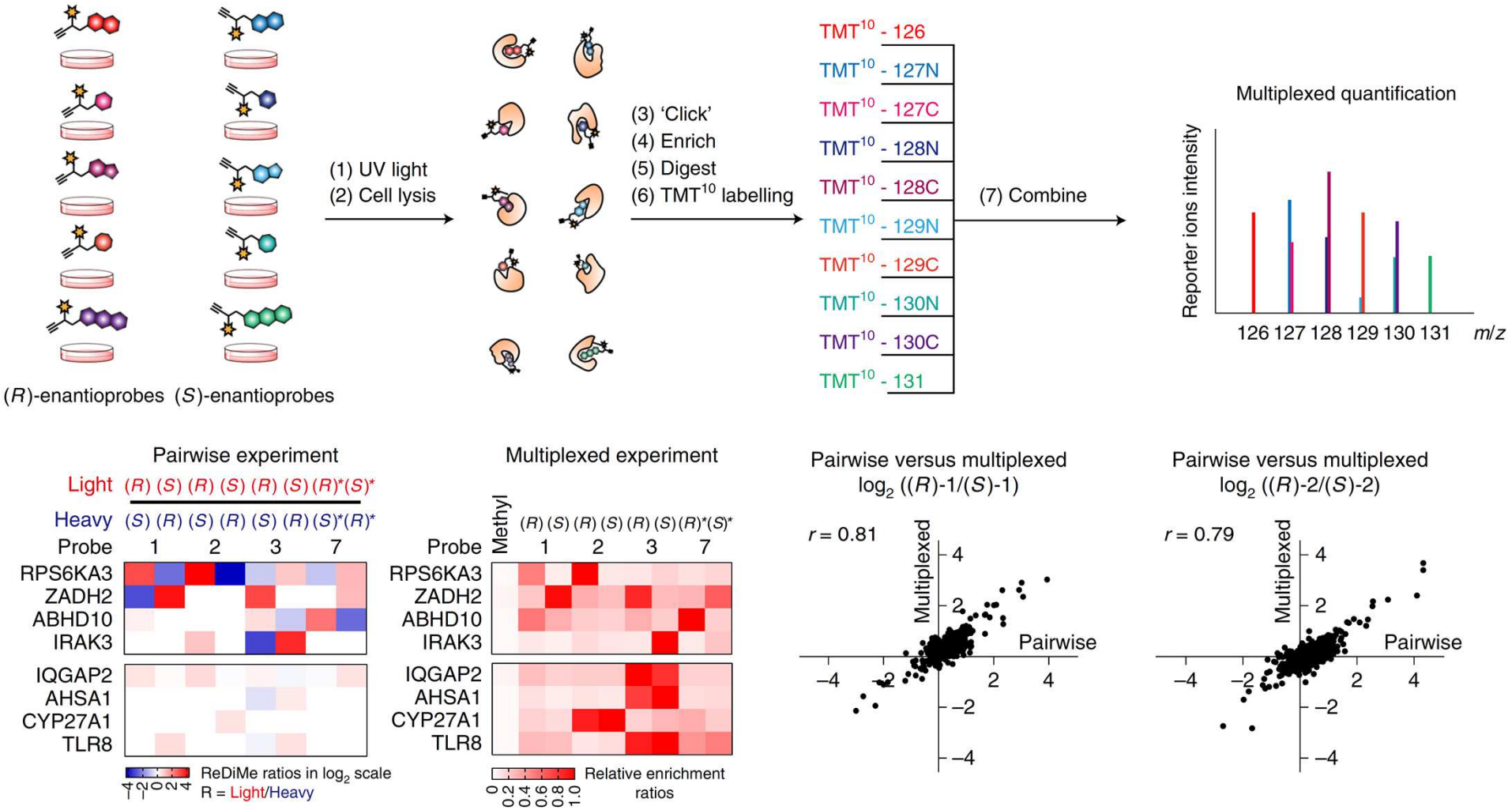


(*R*)-**1** vs EPZ031686

- **Probe-protein interactions of SLC35F2 and PRCP were also blocked by EPZ031686.**
- **SLC35F2 and PRCP did not show evidence of stereoselective interactions with enantioprobes, which suggest that these proteins may specifically bind enantioprobes, with no stereochemical preference.**

Alternative method was needed to address these types of interaction.

Multiplex Analysis of Enantioprobe-Protein Interaction



- **The multiplexed method could find both proteins which have stereoselective and chemoselective interaction with enantioprobe.**

Summary

- **Quantitative chemical proteomics analysis using ‘enantioprobes’ discovered stereoselective probe-protein interaction.**

- **TMT-based multiplexed analysis can be used for discovery of stereoselective interaction without loss of sensitivity and accuracy.**

- **These multiplexing experiments provide additional SAR information by identifying proteins that interact in a chemoselective, rather than stereoselective, manner with the enantioprobes.**