Chemo-proteomics approaches for in-depth profiling of drug-target interactions in living cells

April 2, 2025

Bruno Correia

Protein environment dramatically changes the chemical properties of amino-acids

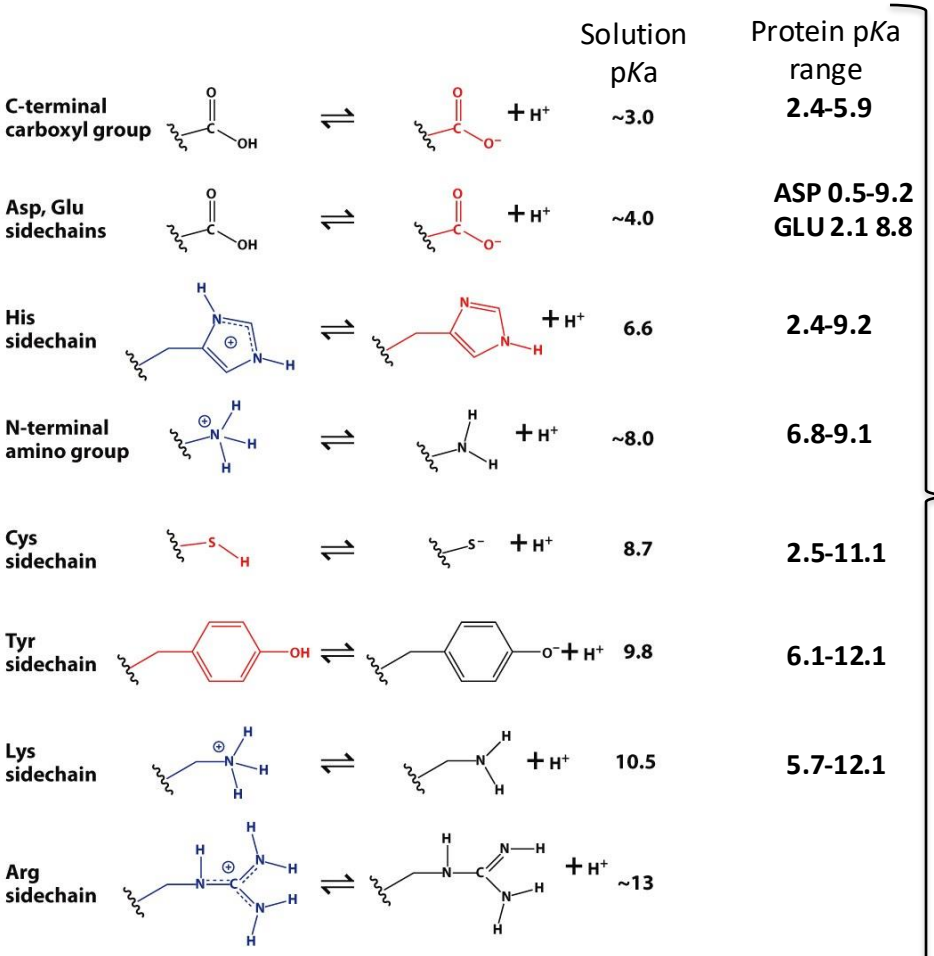


Figure 10.11 The Molecules of Life (© Garland Science 2013)

Basic or **acidic** forms prevalent at pH= 7

Protein environment

↓

Side-chain p*K*as

↓

Protonation states of ionizable groups

↓

Protein Structure

-Stability

-Solubility

Protein Function

-Enzimatic activities

↓

Protein Reactivity is directly related to the side-chain protonation state

↓

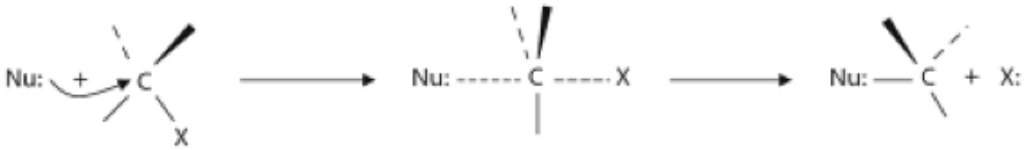
Example: Deprotonated Cys and Ser residues are very reactive nucleophiles and often catalytic residues in enzymes

↓

Key message: Intrinsic protein reactivities provide "handles" for chemical biologists to mine protein's biochemical and cellular functions

Protein Reactivity and Nucleophiles

Nucleophilic Substitution Reaction (S_N2)



Nucleophile(Nu) attacks an electron deficient center displacing a good leaving group(X)

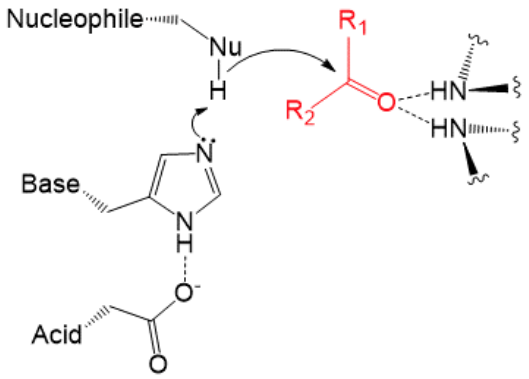
Some "guideline" rules for nucleophilicity:

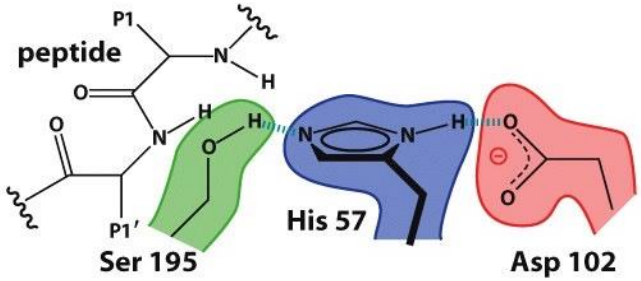
-NH_2^->RO^->OH^->ArO^->RNH_2>NH_3>H2O

-S^-(e.g. cys) > O^-(e.g. ser)

Examples of catalysis:
Catalytic Triads in Serine Proteases

pKa ↑12





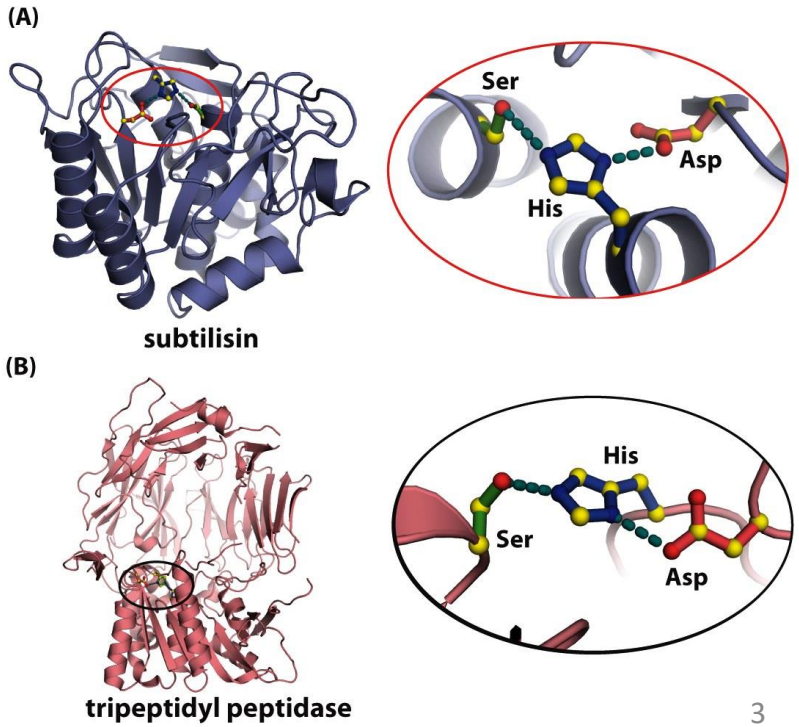
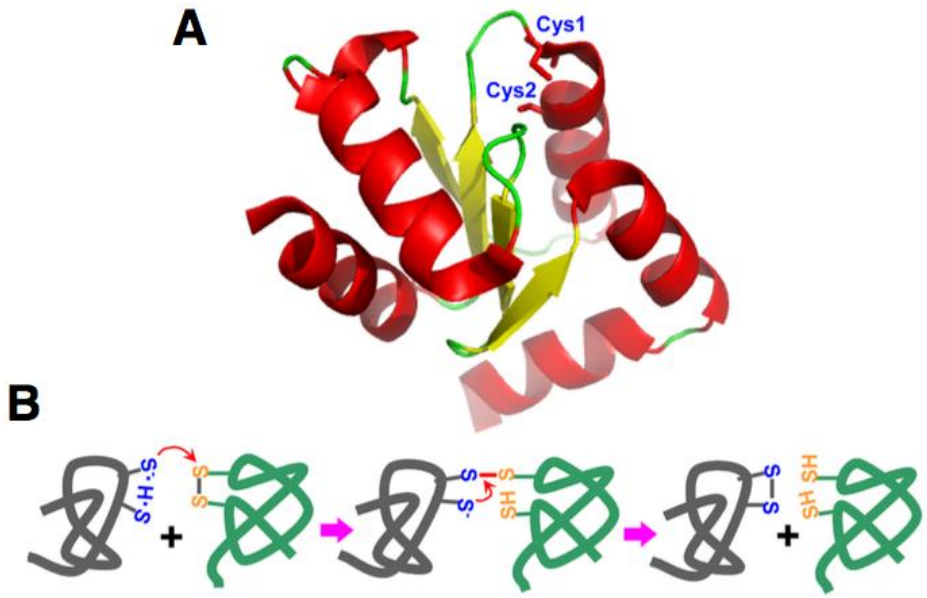


Figure 16.41 The Molecules of Life (© Garland Science 2013)

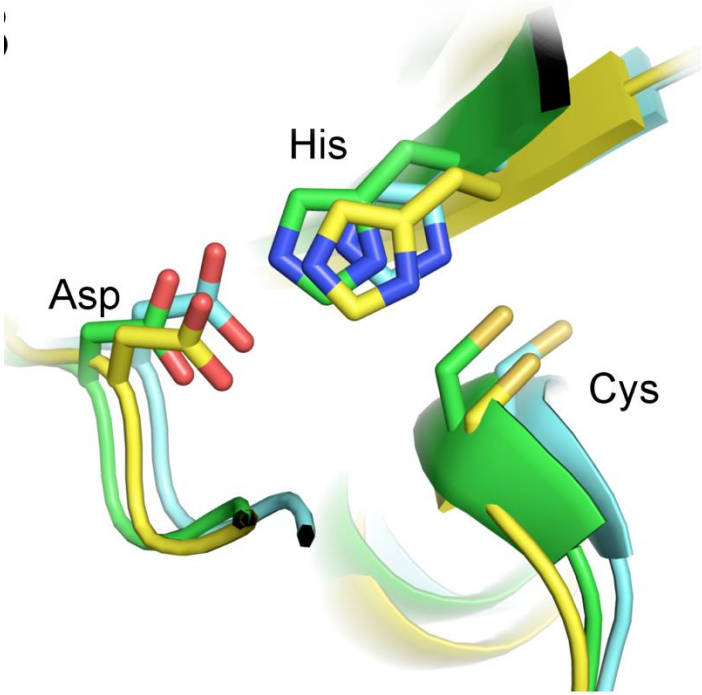
Protein Reactivity and Nucleophiles

Cysteine-based catalysis

Oxyreductases



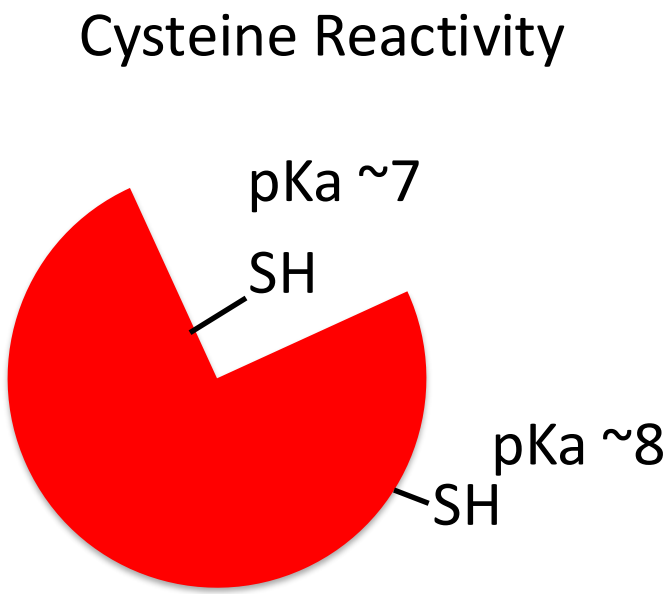
Transglutaminase



-2 cysteine residues coordinate catalysis

-A more canonical catalytic triad with a nucleophilic cysteine

Formulating the problem in a chemical biology perspective

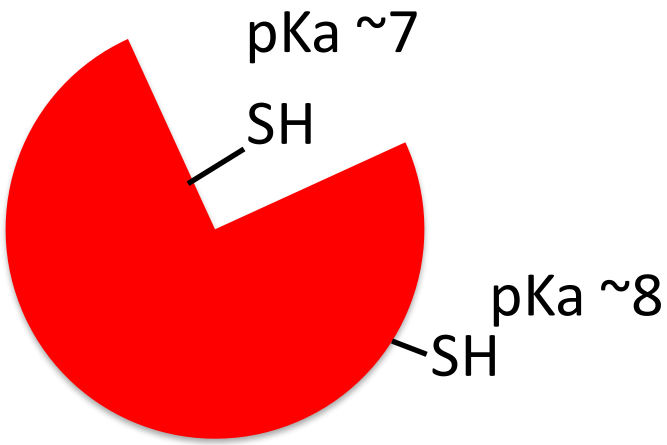


-Unlike “functional” and “non-functional” serines, cysteines are more reactive/nucleophilic independently of their chemical environment

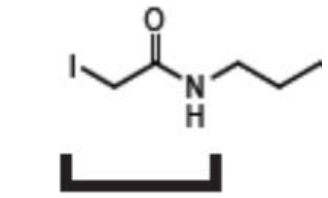
-Which problem does this poses ?

Formulating the problem in a chemical biology perspective

Cysteine Reactivity

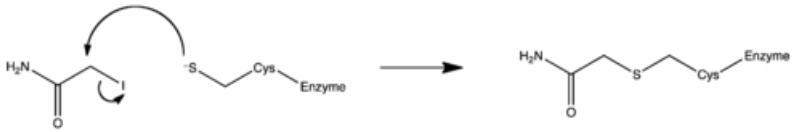


Cysteine labeling probe



Iodoacetamide

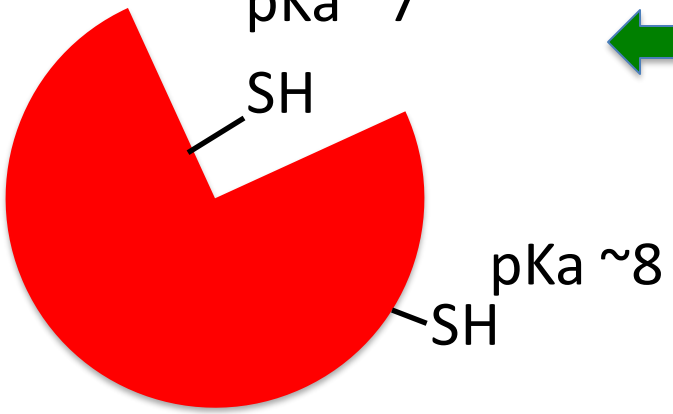
Cysteine labeling mechanism



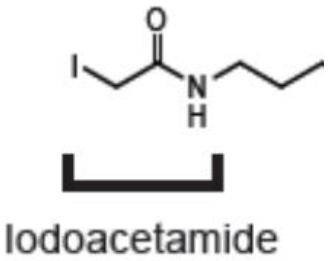
-Iodoacetamide will label cysteines that are accessible and not involved in dissulfide bonds

Formulating the problem in a chemical biology perspective

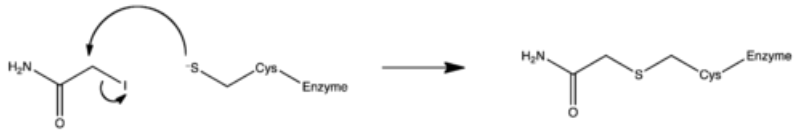
Cysteine Reactivity



Cysteine labeling probe



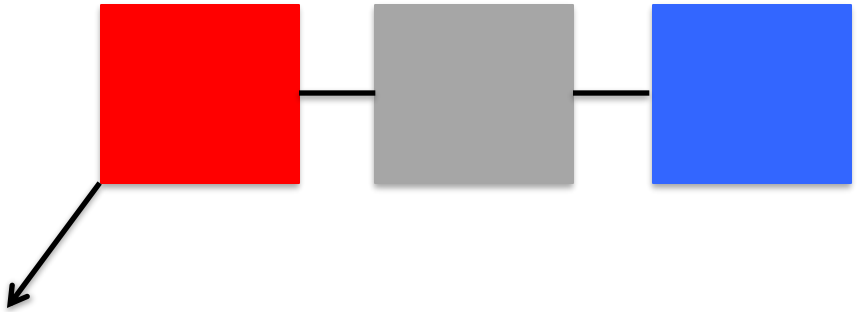
Cysteine labeling mechanism



-Iodoacetamide will label cysteines that area accessible and not involved in dissulfide bonds

Chemical Probes

-Basic building blocks

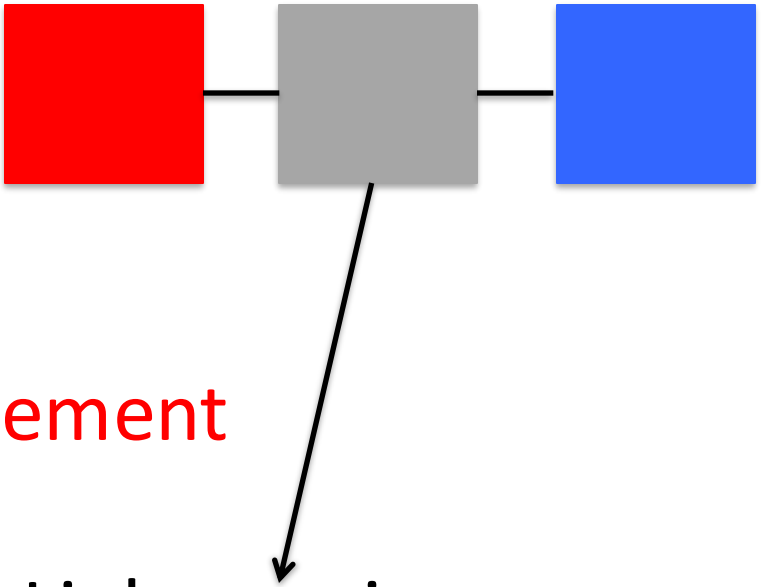


-Specificity element

+ Chemical Reactivity
+ Binding component
+ Or both

Chemical Probes

-Basic building blocks



-Specificity element

-Linker region

+ Non functional (just for solubility)

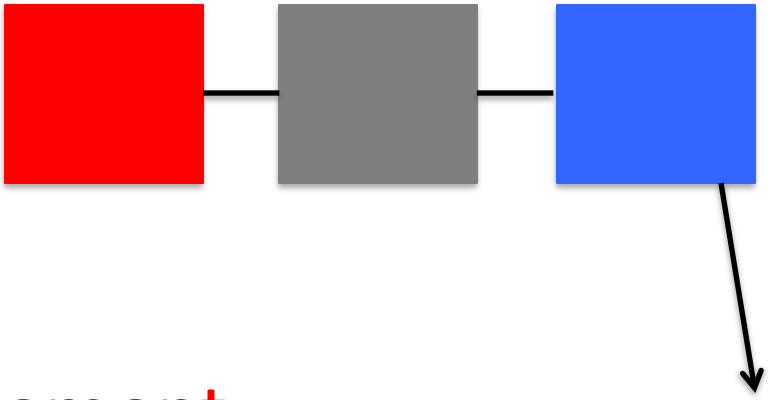
+ Functional

-encode cleavage site

-isotopic tag for MS

Chemical Probes

-Basic building blocks



-Specificity element

-Linker region

-Reporter element

+Directly attached

(biotin, dye)

+Latent

(azide, alkyne)

Chemical Probes

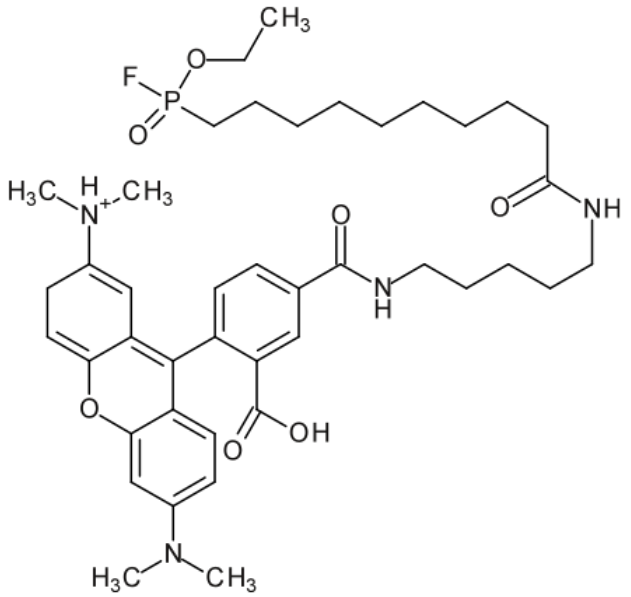
-Basic building blocks

-Specificity element

-Linker region

-Reporter element

An example:



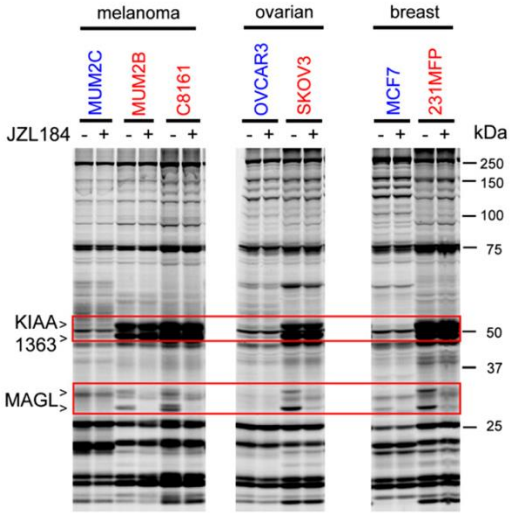
Where is what ?

Chemical Probes

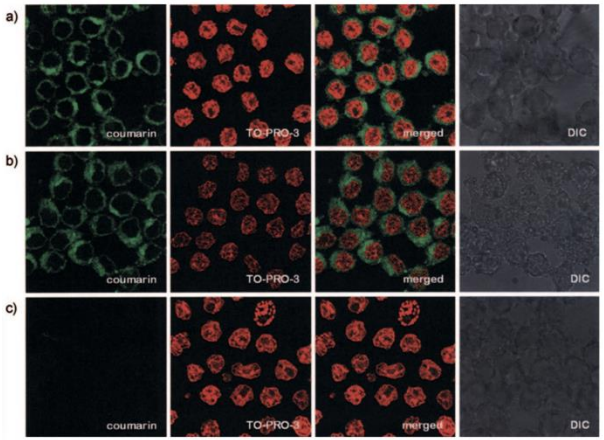
-Basic building blocks

-Reporter element

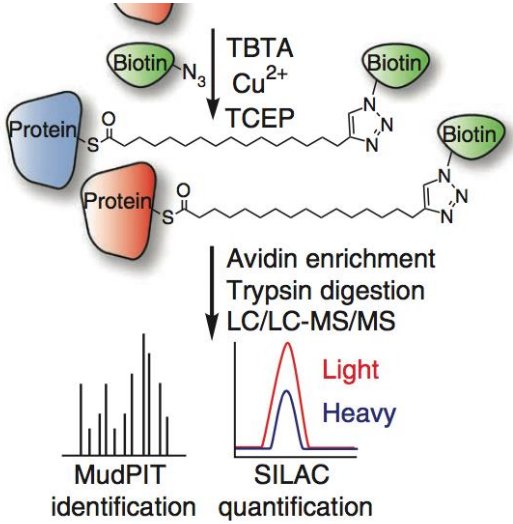
Fluorescence Gel



Microscopy



MS analysis

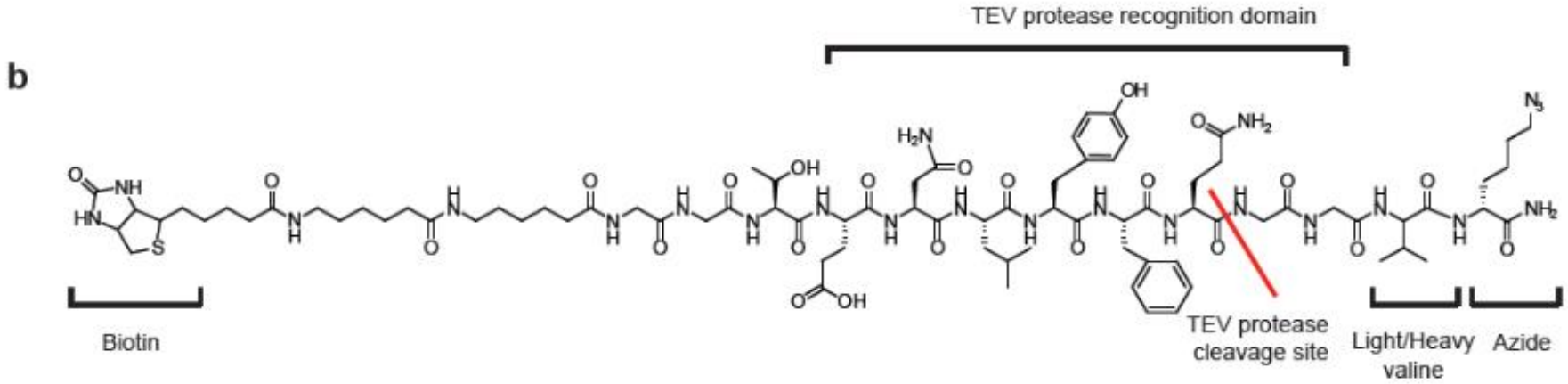


Chemical Probes

-Basic building blocks

-Linker region

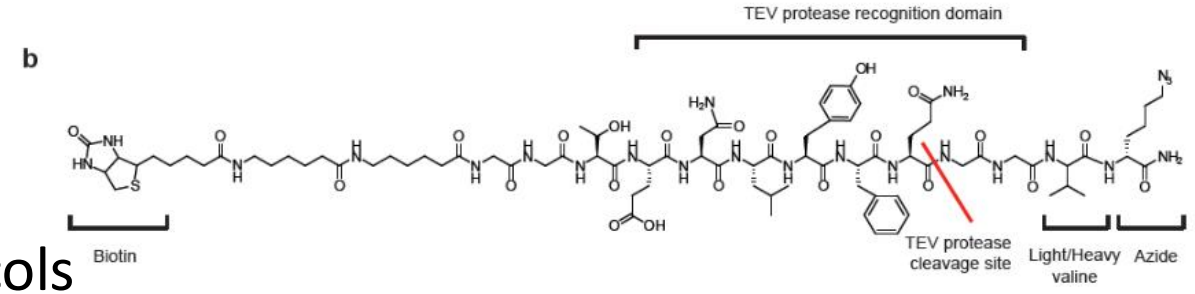
(with additional functionality)



Chemical Probes

-Basic building blocks

-Linker region



-Catch and release protocols

-Catch with Biotin

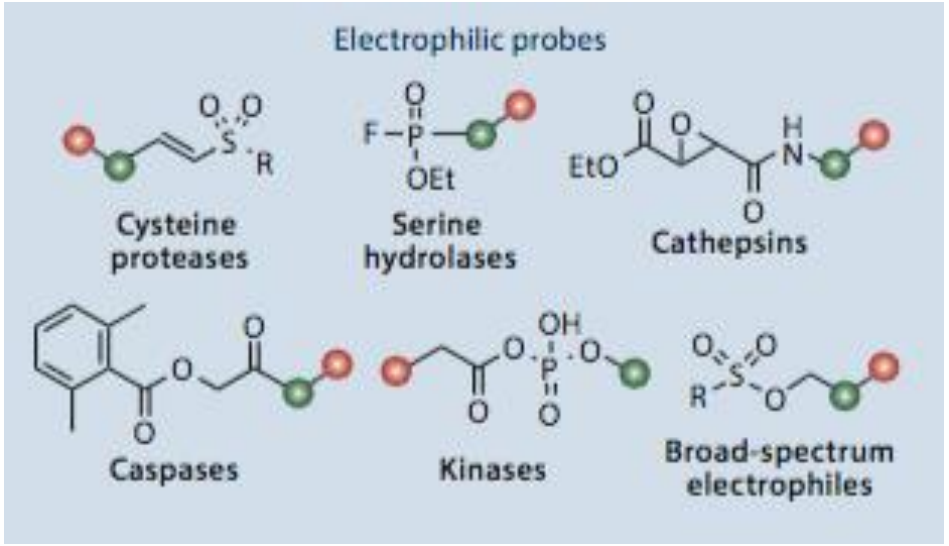
-Release with TEV protease

Chemical Probes

-Basic building blocks

-Specificity element

+ Chemical Reactivity



Take-Home Messages

-Bio-orthogonal chemistries are widely used in probe development

-They can be compatible with living cells or other processed biological samples (cell lysates, tissues, etc)

-Often used together with unnatural aminoacids and chemical probes

-Chemical probes have three basic elements: specificity, linker, reporter

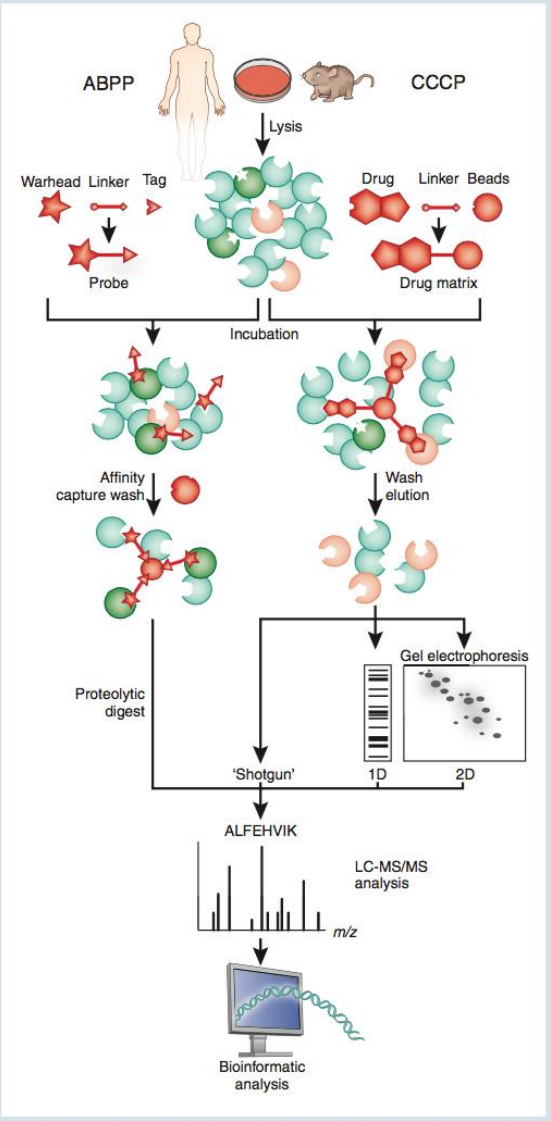
Typical strategies for target identification/deconvolution

Biological sample

Small Molecule

Biochemical processing

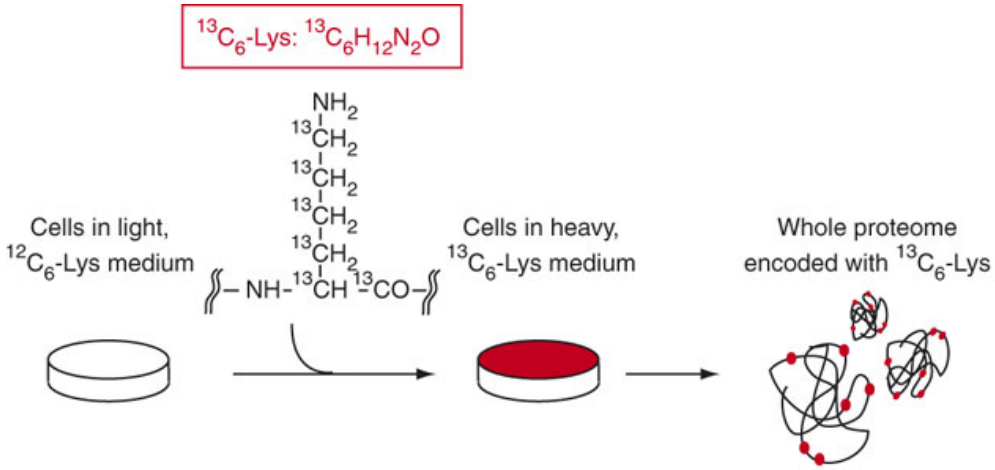
Protein Identification



Proteomics approaches

Mass Spectrometry – Quantitative approaches

-The most used strategy is called Stable Isotope Labeling by Amino acids in Cell culture (SILAC)



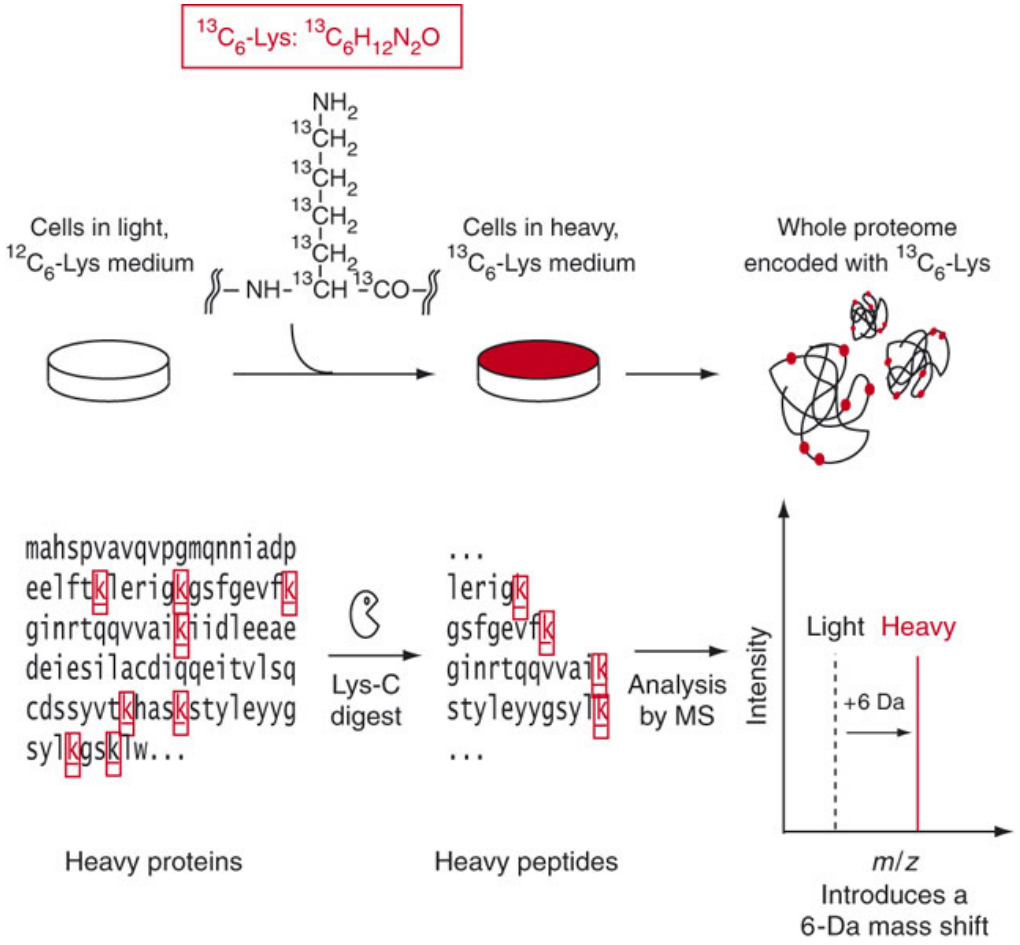
-Heavy Isotopes of arginines and lysines are typically used

-Cells have to be passaged a number of times for full incorporation.

-But there are other possibilities

Mass Spectrometry – Quantitative approaches

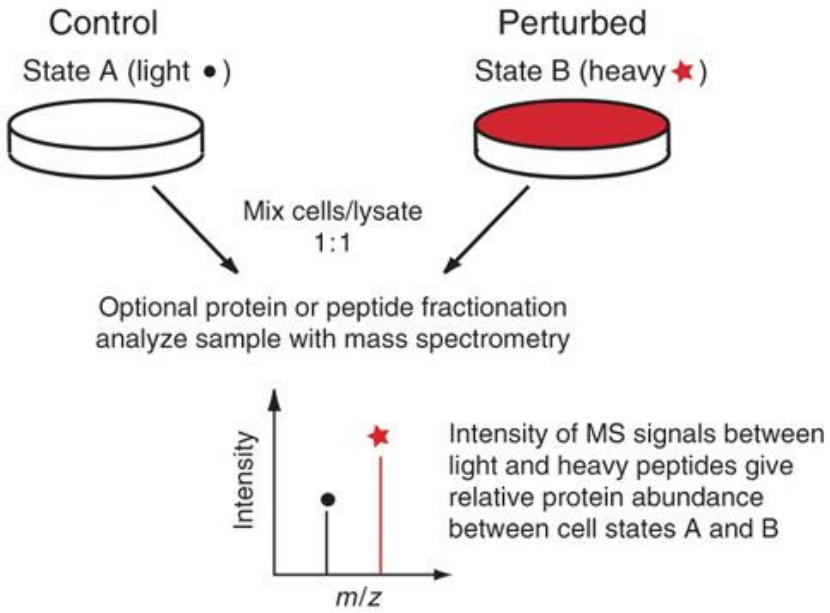
-The most used strategy is called Stable Isotope Labeling by Amino acids in Cell culture (SILAC)



-Peptides containing heavy amino acids have a distinct an identifiable mass signature

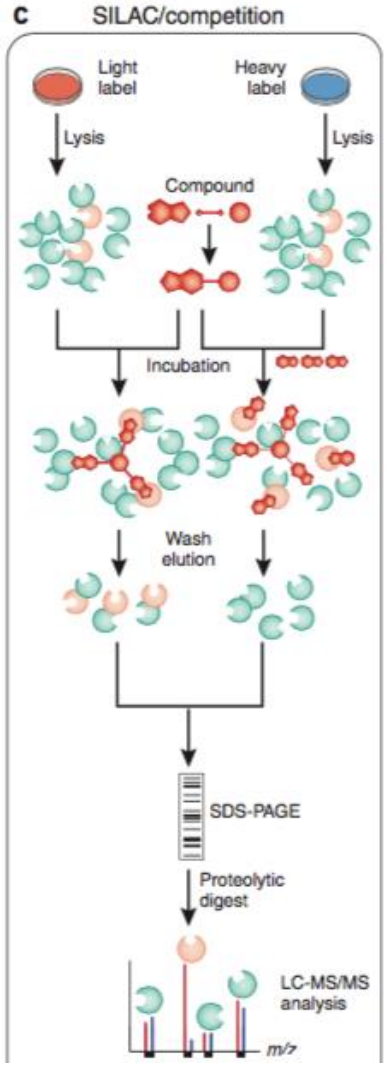
Mass Spectrometry – Quantitative approaches

-Typical SILAC experiment always include a reference state and a perturbed state



- The perturbations can be diverse.
- Specially amenable to treatment with chemical compounds for quantitative analysis of downstream effects.

Quantitative Chemoproteomics



-SILAC cells lysed an incubated with compounds

-small molecule immobilize in matrix

-Perturbed condition incubated with competitor

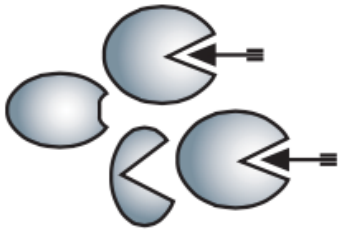
-Eluted proteins are analyzed by LC-MS/MS

-How do we distinguish the true targets ?

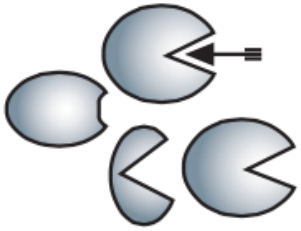
Quantitative reactivity profiling predicts functional cysteines in proteomes

Eranthie Weerapana[1,2]*, Chu Wang[1,2]*, Gabriel M. Simon[1,2], Florian Richter[3,4], Sagar Khare[3,5], Myles B. D. Dillon[2], Daniel A. Bachovchin[1,2], Kerri Mowen[2], David Baker[3,4,5] & Benjamin F. Cravatt[1,2]

Proteomes
(cell lysates)



Proteome A



Proteome B

-Initially the proteomes are handled separately

Quantitative reactivity profiling predicts functional cysteines in proteomes

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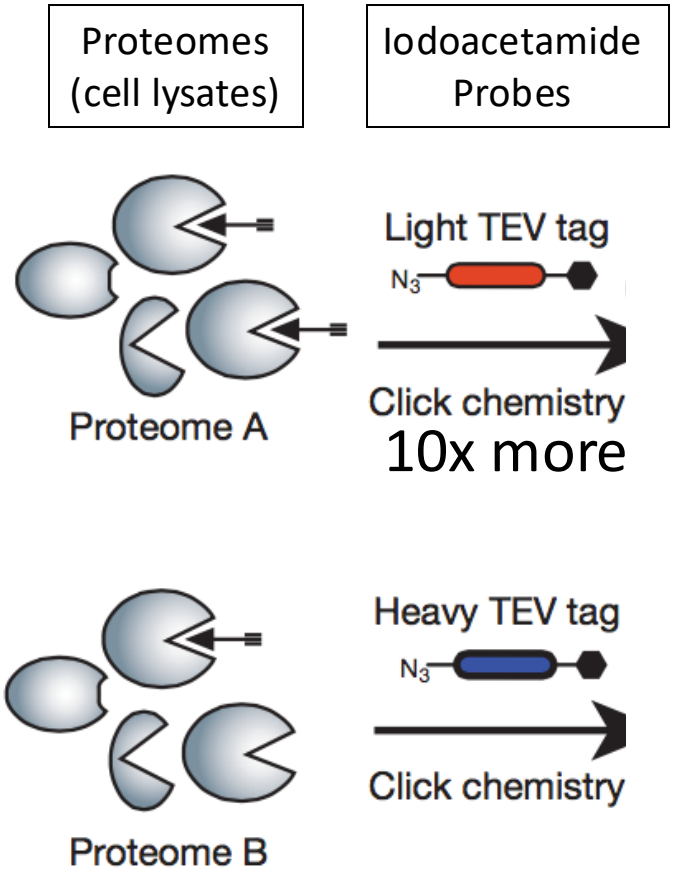

-Proteome are incubated with different probe concentrations

Quantitative reactivity profiling predicts functional cysteines in proteomes

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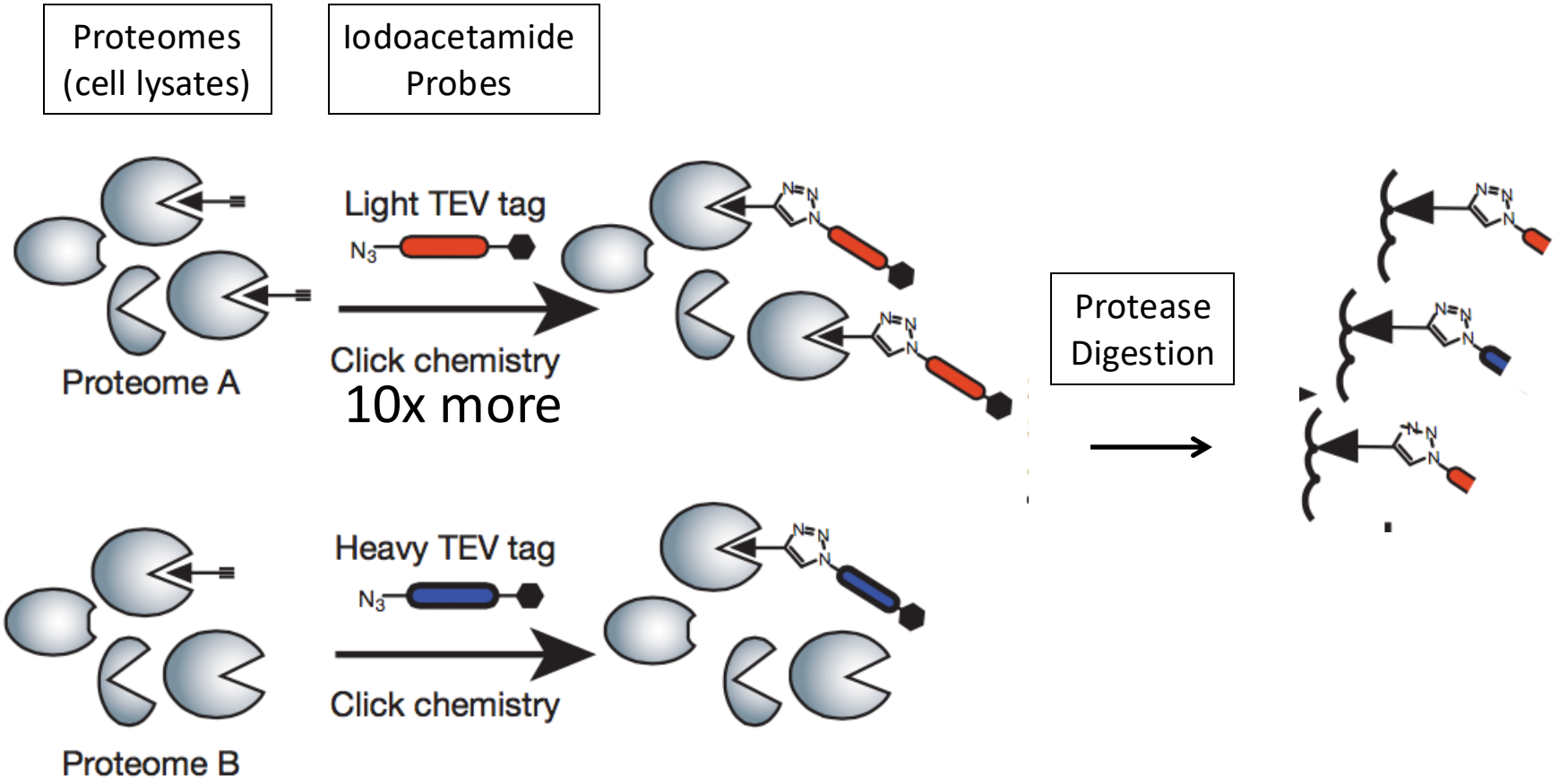

-In the lower concentration proteome proteins with more reactive cysteines will be preferentially labeled

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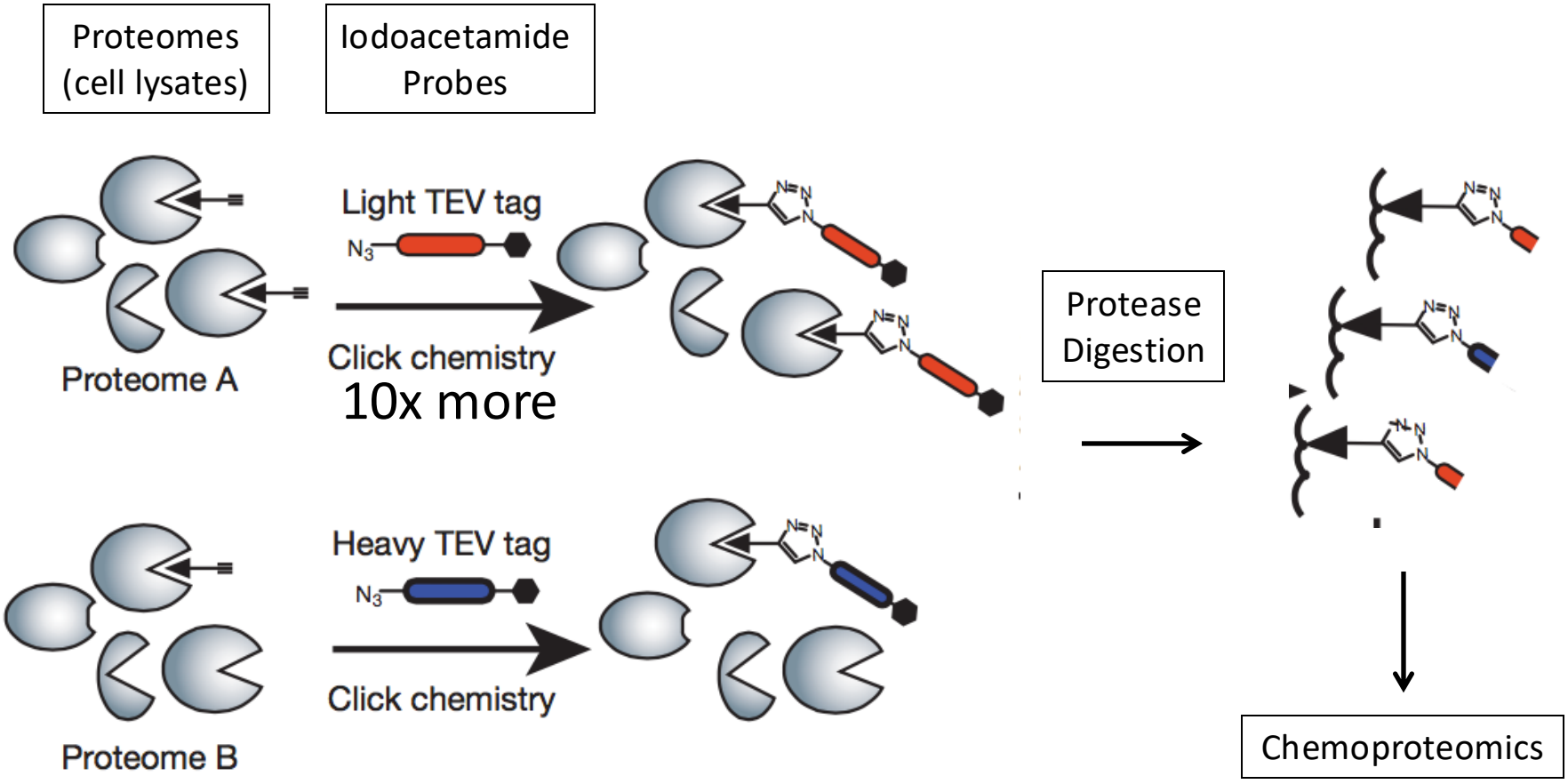

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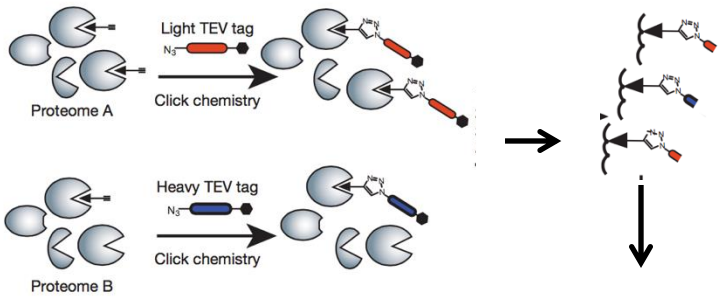
Biochemical processing stage



Digested Petides from labeled proteins

Chemoproteomics

Data collection and processing

LC-MS/MS

-Peptide sequence
-Residue labeled
-Proteins Identity
-Labeling Ratios

Quantitative reactivity profiling predicts functional cysteines in proteomes

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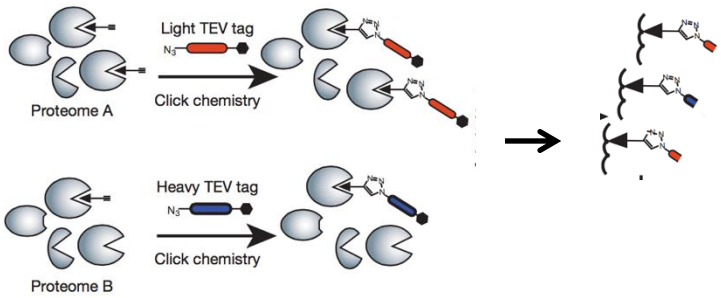


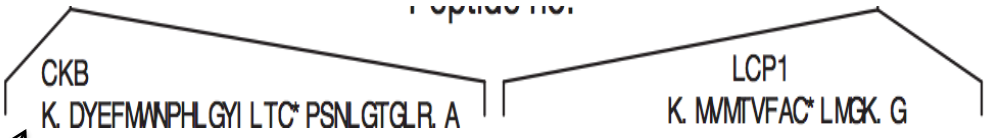
Data collection and processing

↓

LC-MS/MS

↓

-**Peptide sequence**
-**Labeled Residue**
-**Proteins Identity**
-Labeling Ratios



Quantitative reactivity profiling predicts functional cysteines in proteomes

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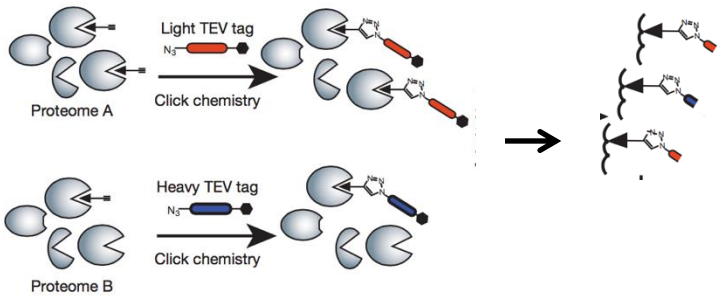
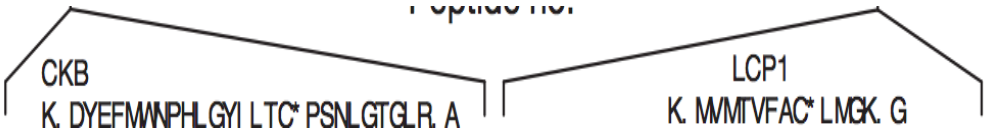
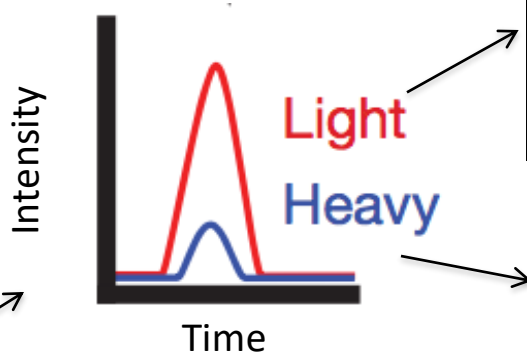


Data collection and processing

LC-MS/MS

-Peptide sequence
-Labeled Residue
-Proteins Identity
-**Labeling Ratios**





Tags or proteins with abundant isotopes(e.g. C12, N14, H1 …)

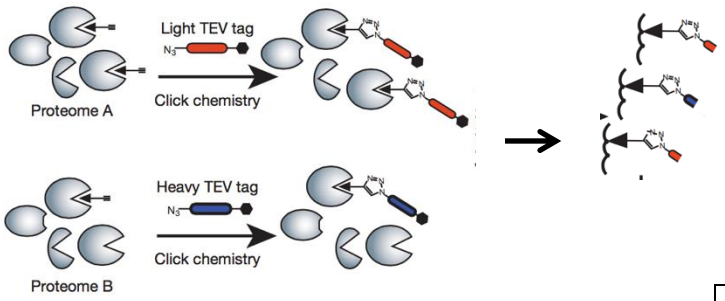
Tags or proteins with less abundant isotopes(e.g. C13, N15, H2 …)

Ratio between light and heavy peptides serves as a measurement of cysteine reactivity

Quantitative reactivity profiling predicts functional cysteines in proteomes

Eranthie Weerapana[1,2]*, Chu Wang[1,2]*, Gabriel M. Simon[1,2], Florian Richter[3,4], Sagar Khare[3,5], Myles B. D. Dillon[2], Daniel A. Bachovchin[1,2], Kerri Mowen[2], David Baker[3,4,5] & Benjamin F. Cravatt[1,2]

Biochemical processing stage



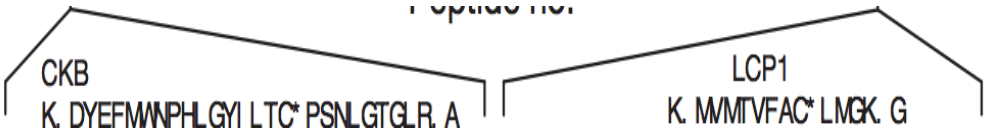
Data collection and processing

↓

LC-MS/MS

↓

-Peptide sequence
-Labeled Residue
-Proteins Identity
-**Labeling Ratios**



Question: Which ones are the most reactive cysteines ?
High or low ratio ?

Quantitative reactivity profiling predicts functional cysteines in proteomes

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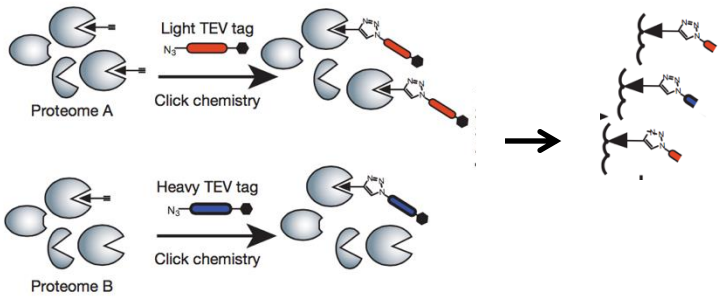

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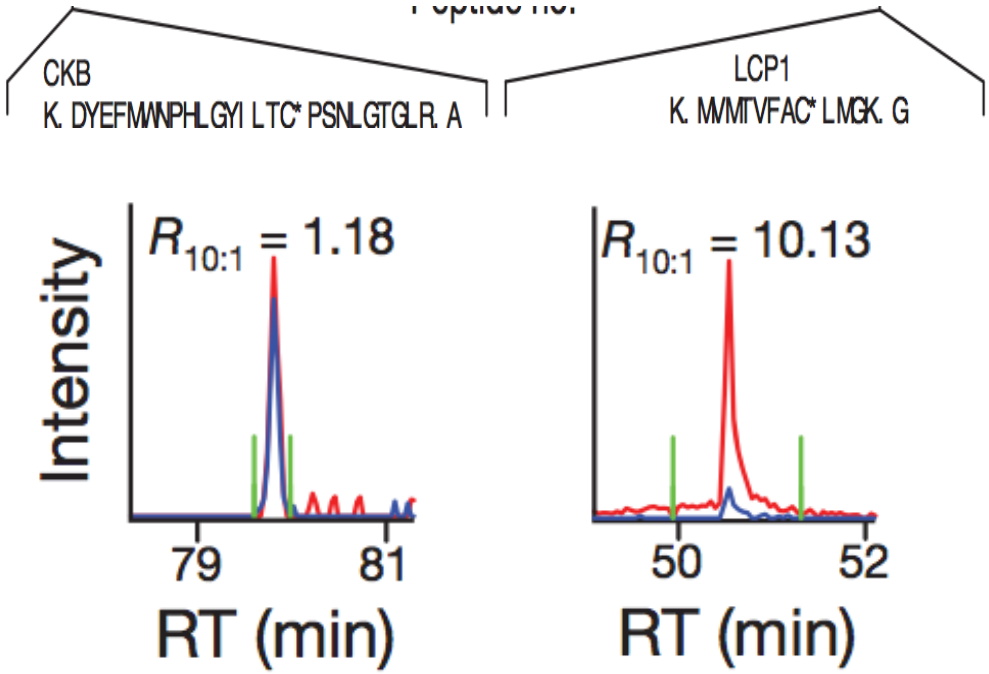

Lower the ratio – higher the reactivity

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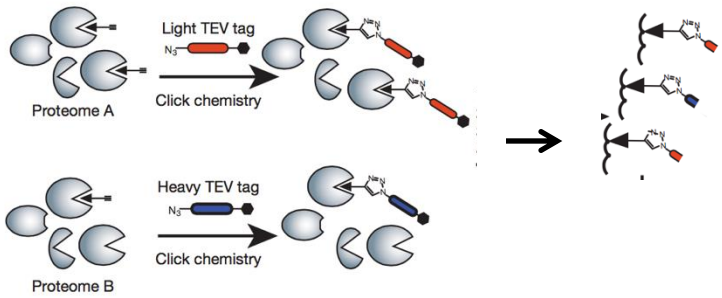

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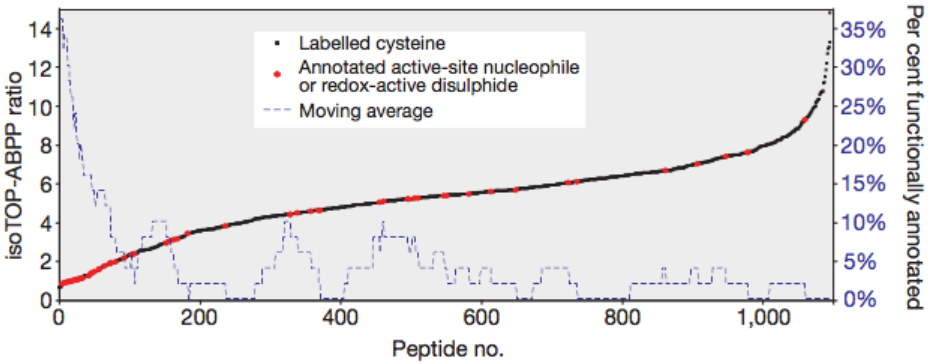
Biological interpretation



Cysteines can be sorted by reactivity

Quantitative reactivity profiling predicts functional cysteines in proteomes

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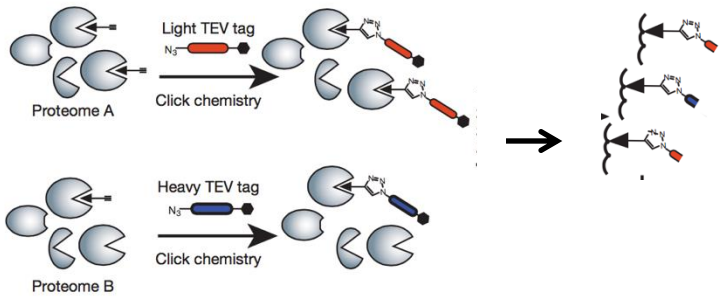

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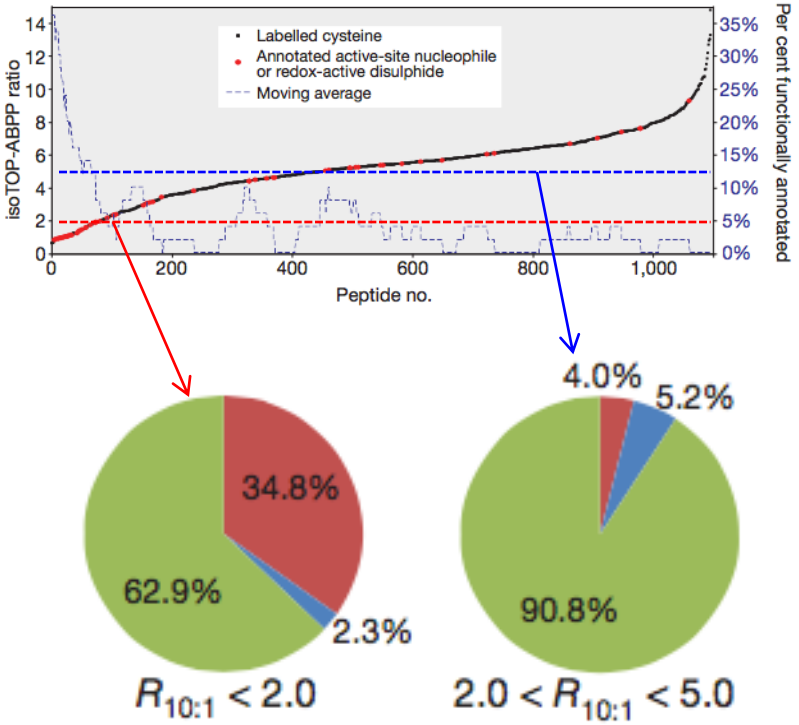
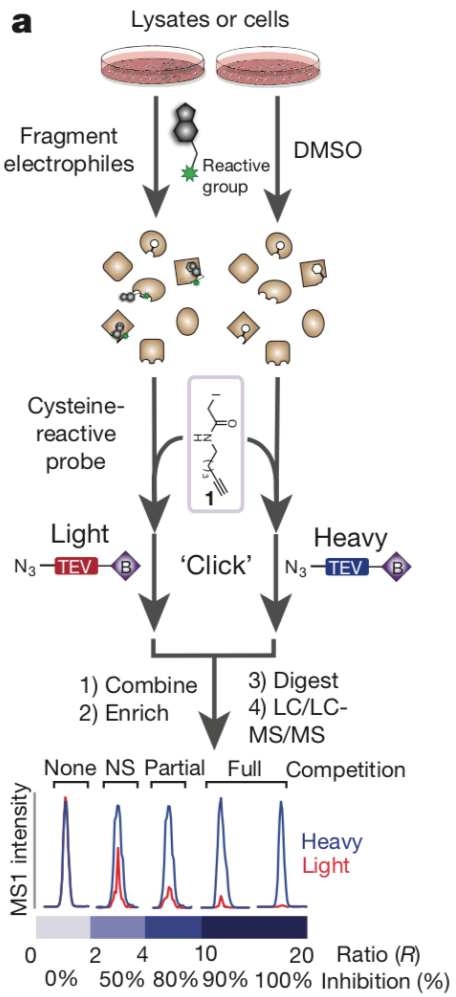


Highly reactive cysteines (low ratio) are enriched in functionally annotated residues (34.8% Vs 4.%)

Proteome-wide covalent ligand discovery in native biological systems

Keriann M. Backus[1]*, Bruno E. Correia[1]*, Kenneth M. Lum[1], Stefano Forli[2], Benjamin D. Horning[1], Gonzalo E. González-Páez[3], Sandip Chatterjee[3], Bryan R. Lanning[1], John R. Teijaro[4], Arthur J. Olson[2], Dennis W. Wolan[3] & Benjamin F. Cravatt[1]



What is the critical point that enables the measurement of inhibition ?

Proteome-wide covalent ligand discovery in native biological systems

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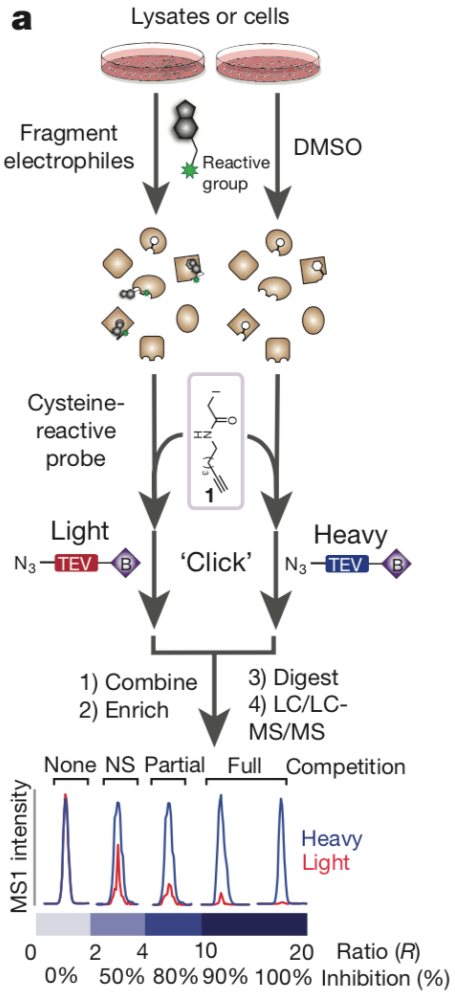

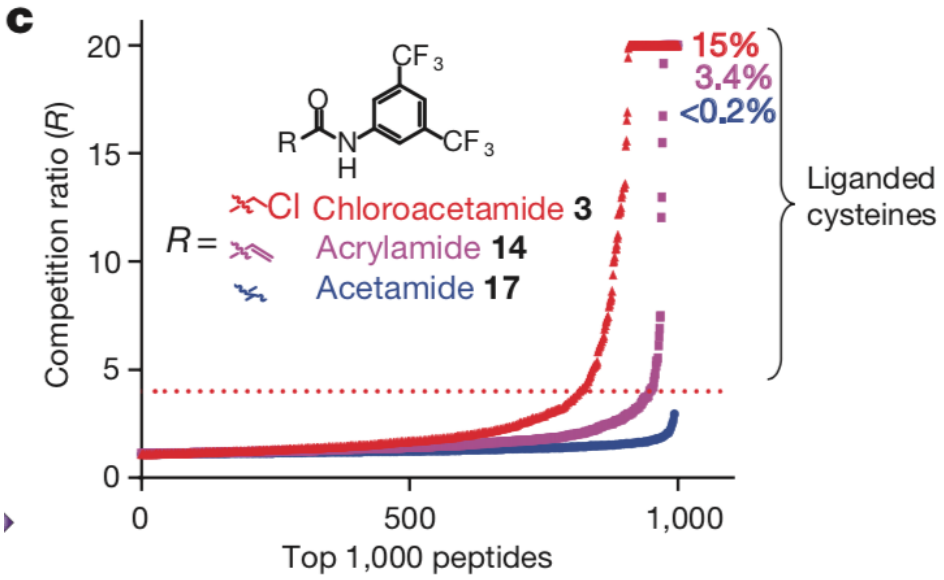

Which intrinsic property of the compounds changes ?

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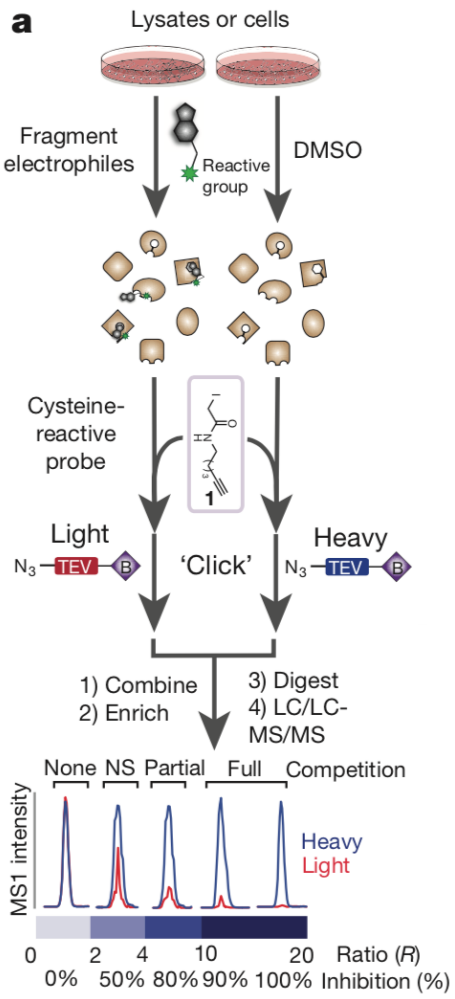

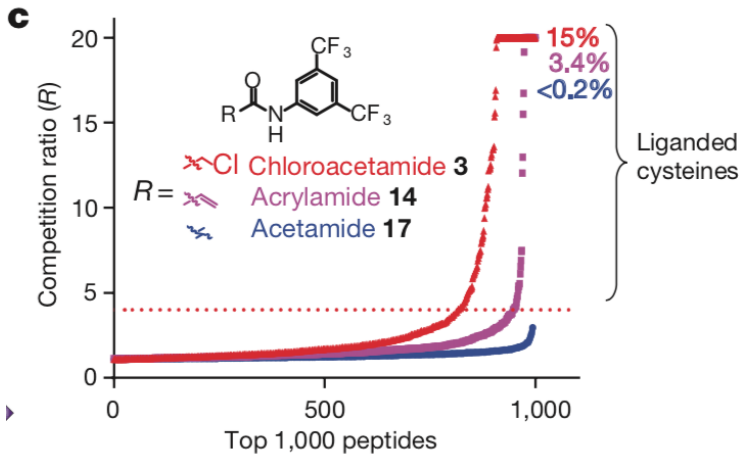

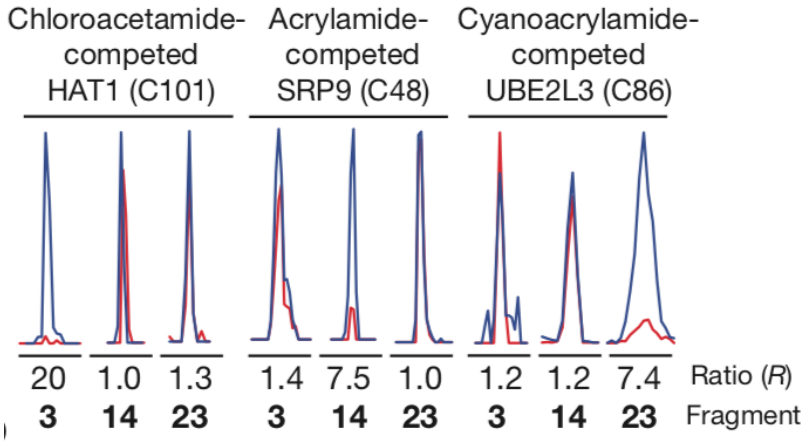

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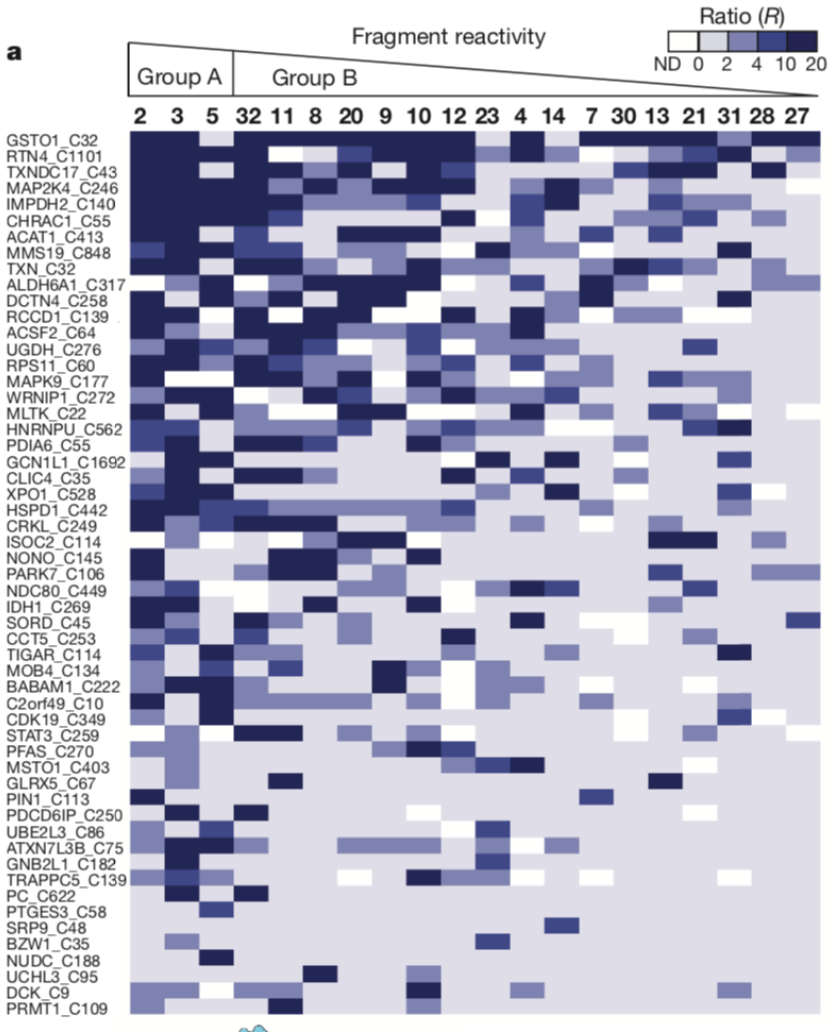
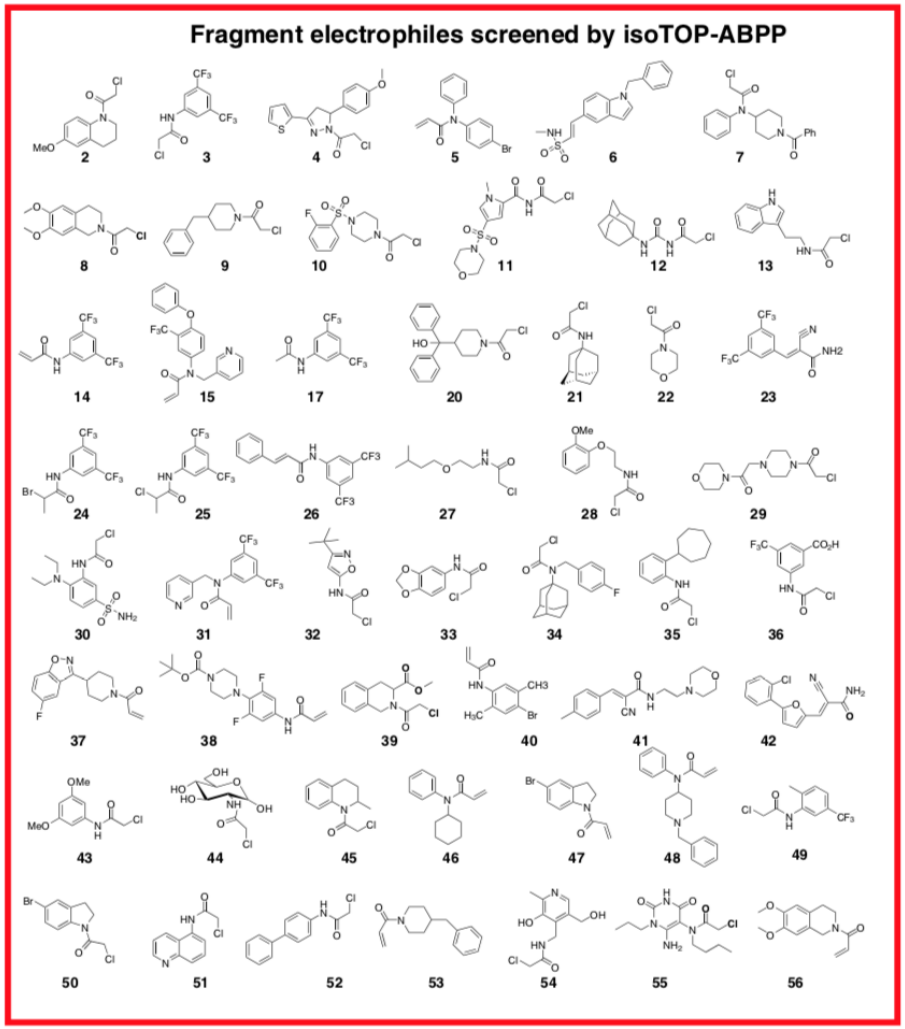
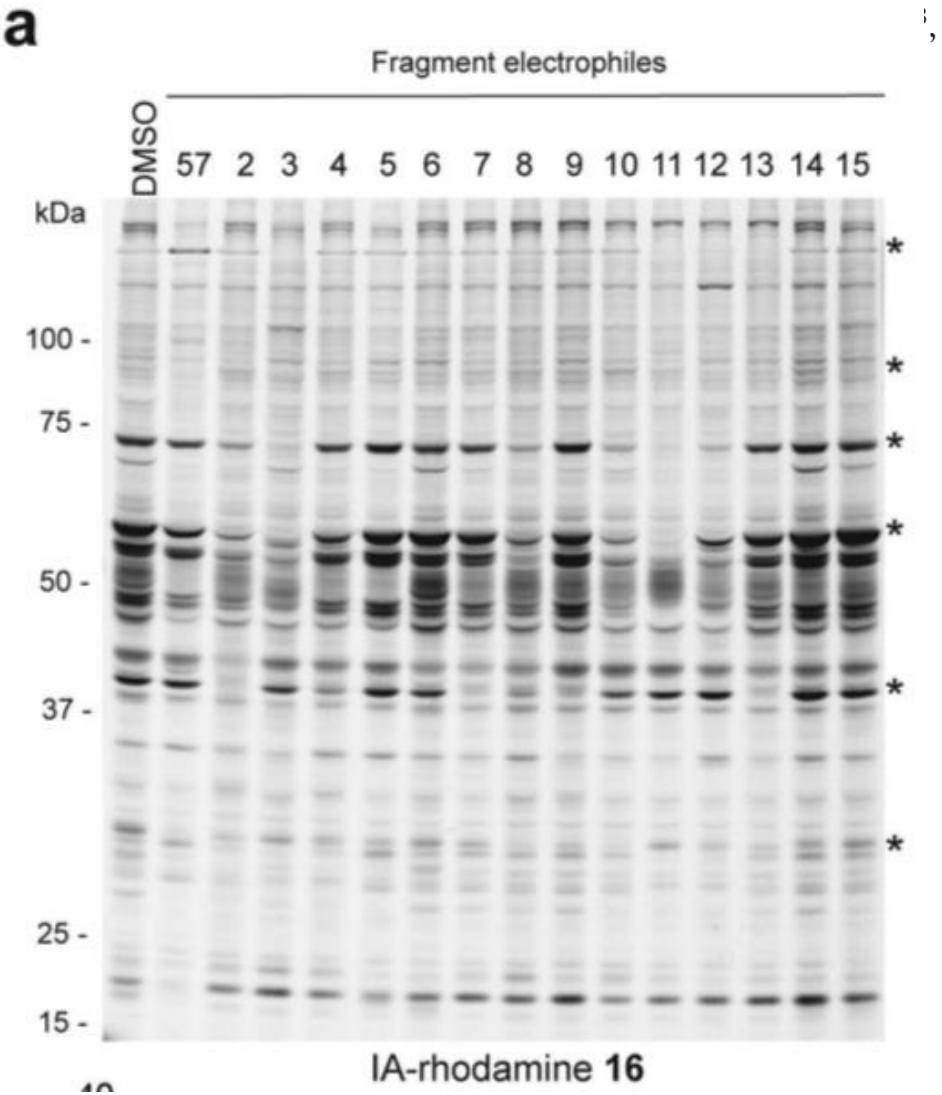


Which striking features are present on this ligandability map ?

Proteome-wide covalent ligand discovery in native biological systems





What do we see in the gel ?

Chemical Probes

-Basic building blocks

-Specificity element

+ Binding specificity

IMPORTANT: Generally we always need a covalent bond between protein target and the probe – **merely binding groups don't have that functionality**

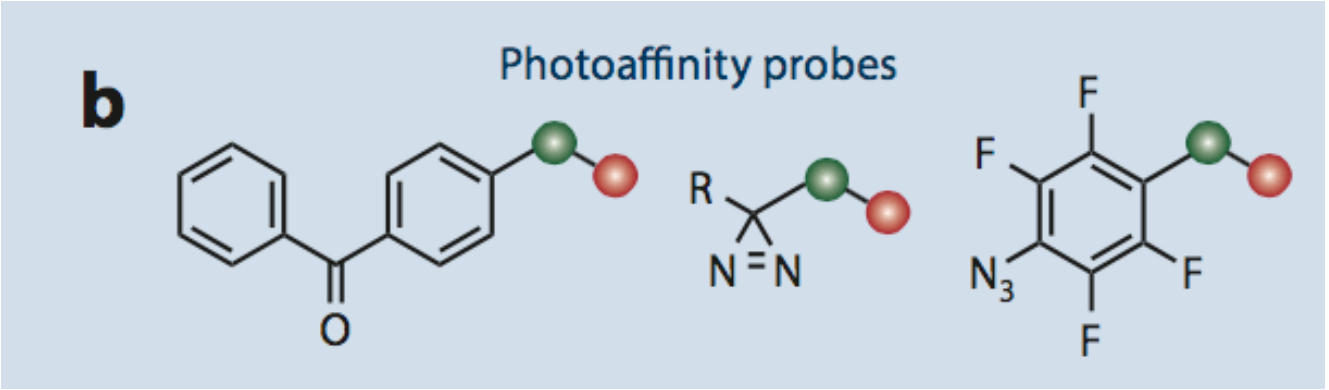
SOLUTION: Photocrosslinkable group can be added to the probe

Chemical Probes

-Basic building blocks

-Specificity element

SOLUTION: Photocrosslinkable group can be added to the probe



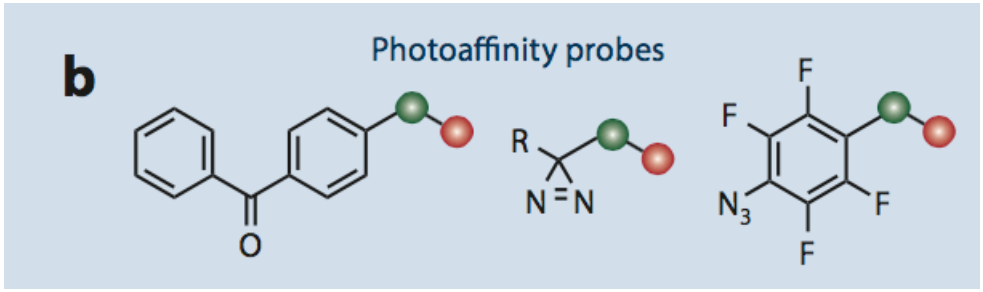
To forge a covalent bond with the target these groups have to be irradiated with UV light.

Chemical Probes

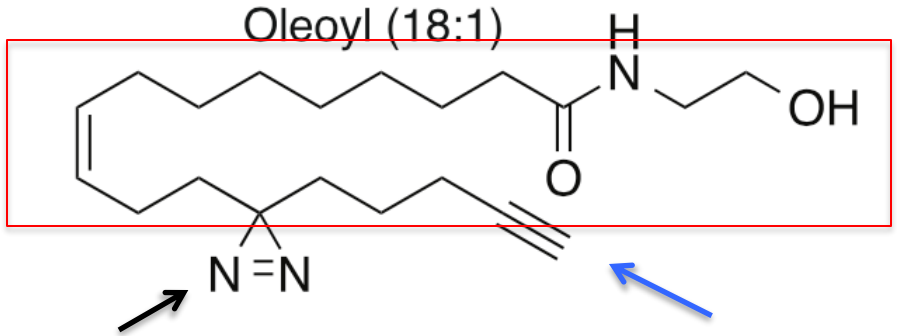
-Basic building blocks

-Specificity element

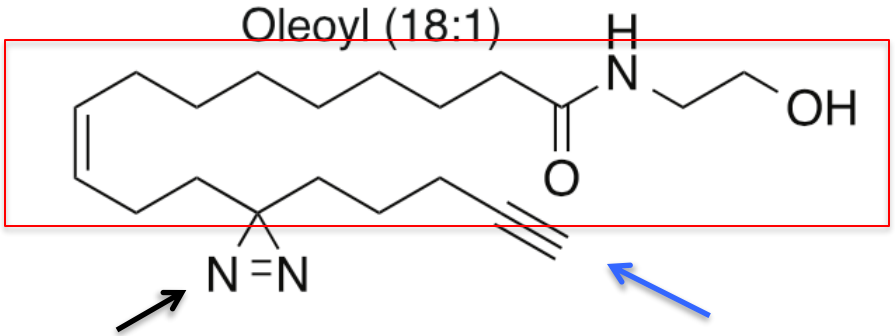
SOLUTION: Photoaffinity group can be added to the probe



Lipid Probe
(anandamide)



Chemical Probes



One can map the interactions between lipids and proteins.

Cell

Resource

A Global Map of Lipid-Binding Proteins and Their Ligandability in Cells

Micah J. Niphakis,[1,2,*] Kenneth M. Lum,[1,2] Armand B. Cognetta III,[1] Bruno E. Correia,[1] Taka-Aki Ichu,[1] Jose Olucha,[1] Steven J. Brown,[1] Soumajit Kundu,[1] Fabiana Piscitelli,[1] Hugh Rosen,[1] and Benjamin F. Cravatt[1,*]

[1]The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

[2]Co-first author

*Correspondence: mniphak@gmail.com (M.J.N.), cravatt@scripps.edu (B.F.C.)

http://dx.doi.org/10.1016/j.cell.2015.05.045

Quantitative Chemoproteomics with Activity based probes

What if could do this in live cells ?

Cell

Resource

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*Correspondence: mniphak@gmail.com (M.J.N.), cravatt@scripps.edu (B.F.C.)

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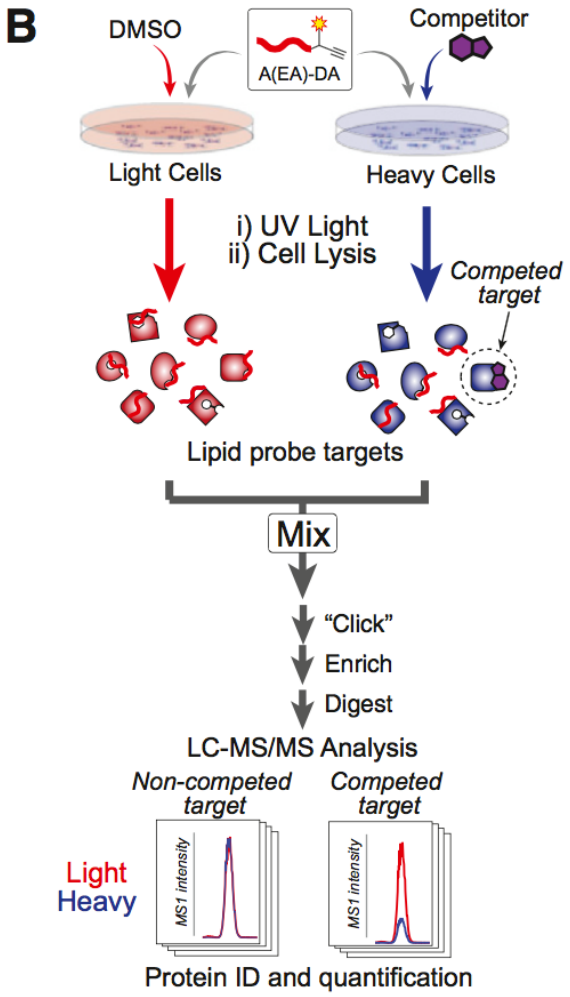
WE CAN !

How ?

Bio-orthogonal Chemistry

Quantitative Chemoproteomics with Activity based probes

What if could do this in live cells ?



Quantitative Chemoproteomics with Activity based probes

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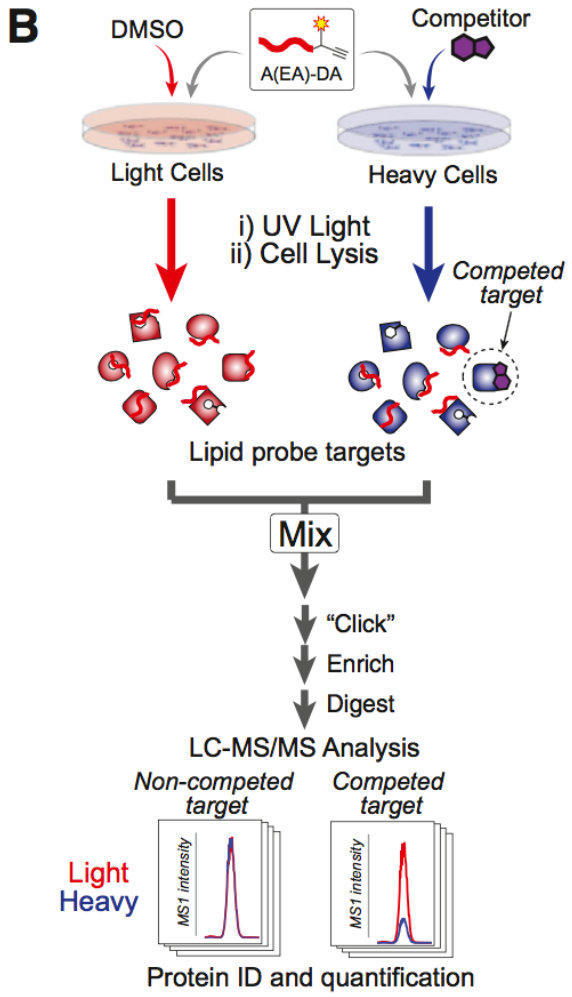
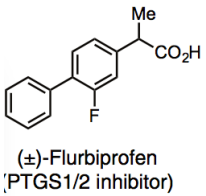
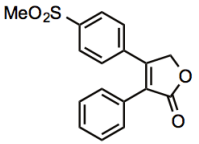


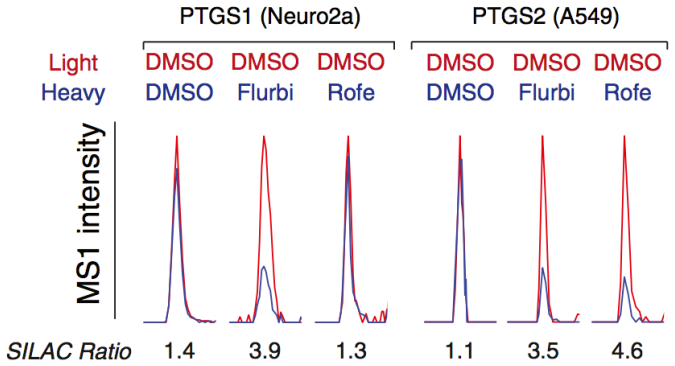


(±)-Flurbiprofen
(PTGS1/2 inhibitor)



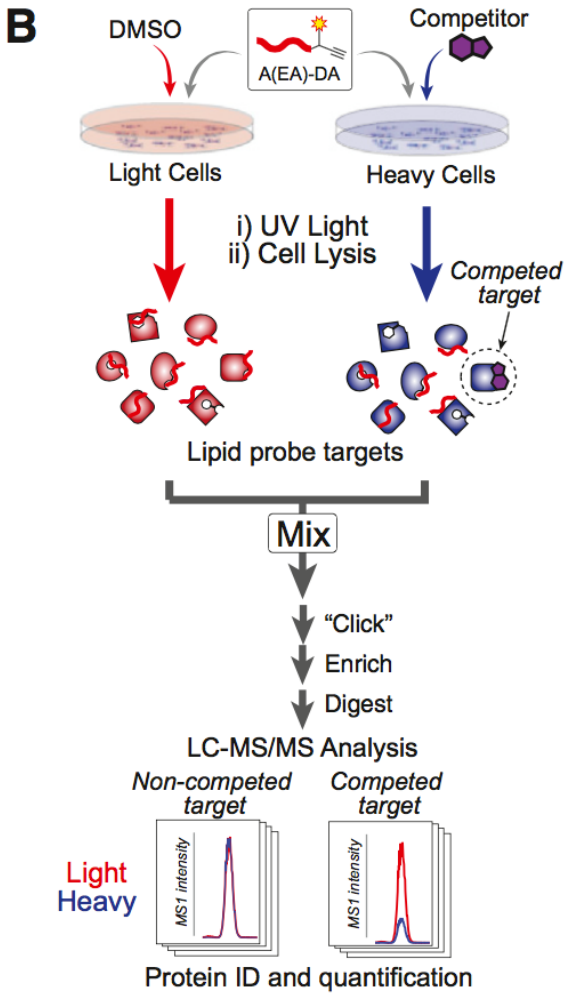
Rofecoxib
(PTGS2 inhibititor)

SILAC Ratios



Quantitative Chemoproteomics with Activity based probes

What if could do this in live cells ?



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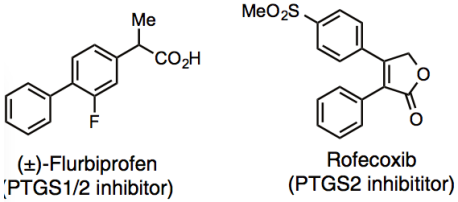

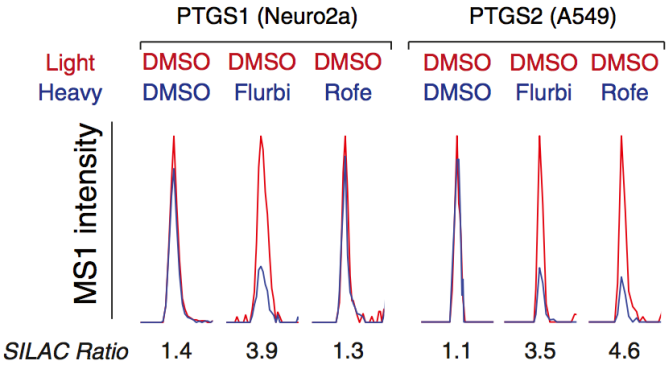

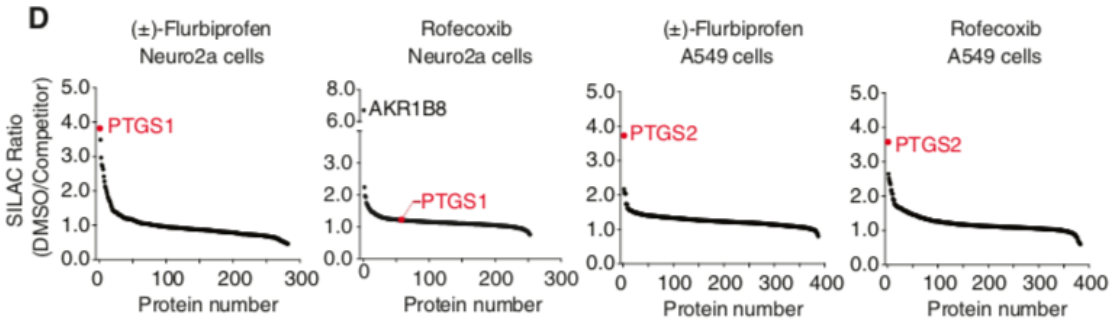

Are the drugs specific ?

Quantitative Chemoproteomics with Activity based probes

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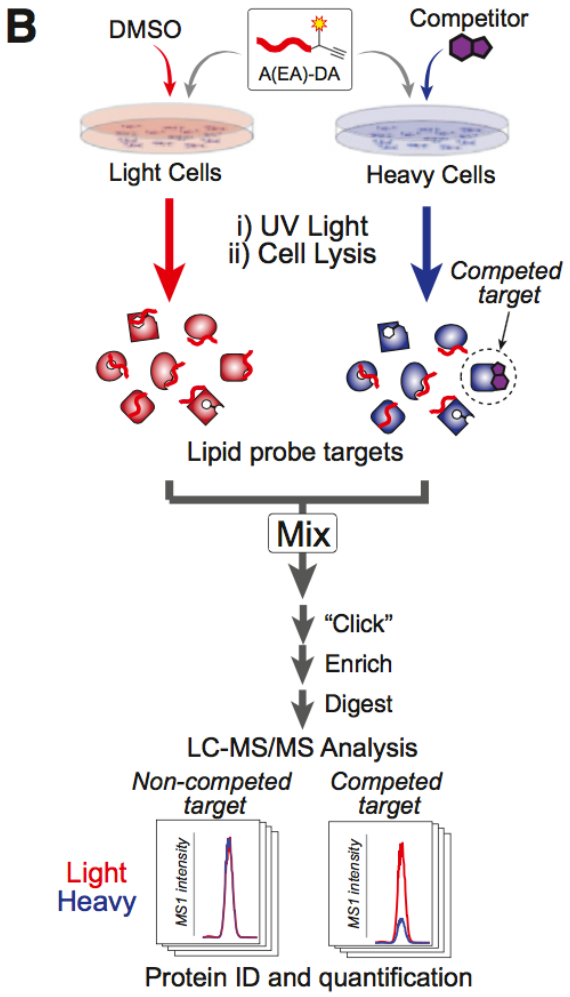
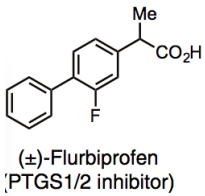


SILAC Ratios



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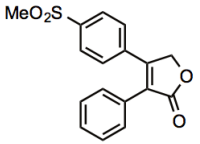

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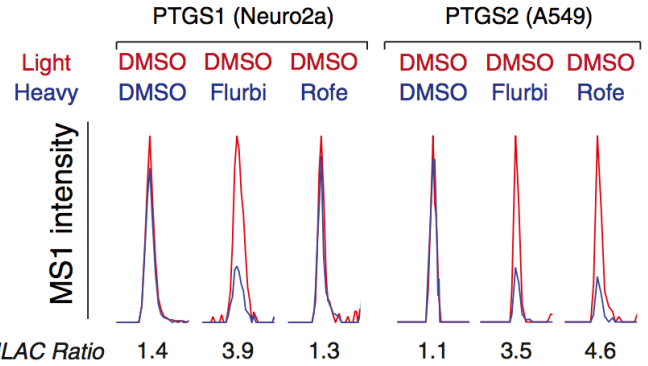
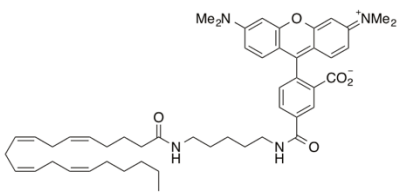
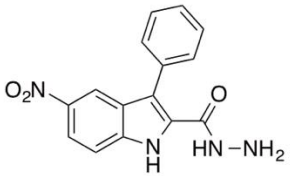


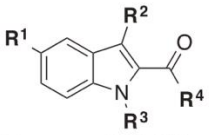
Fluorescent probe



Inhibitors

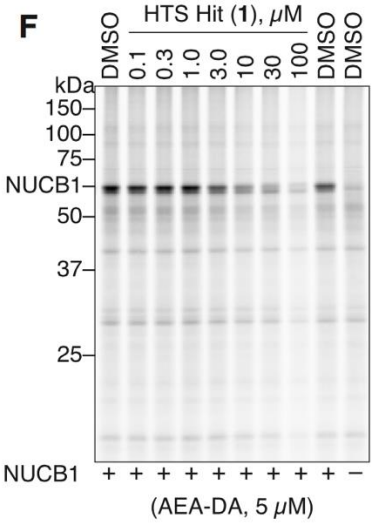


HTS Hit (**1**)



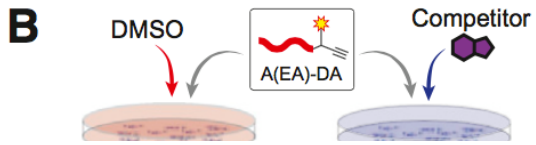
Compounds **2–22**

Gel based



Quantitative Chemoproteomics with Activity based probes

What if could do this in live cells ?



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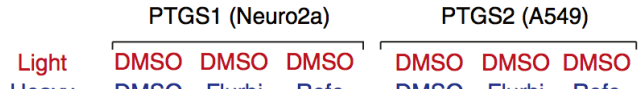
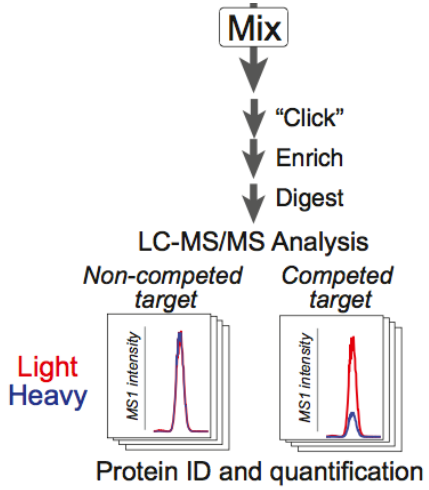
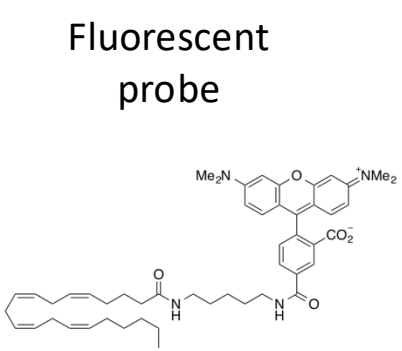
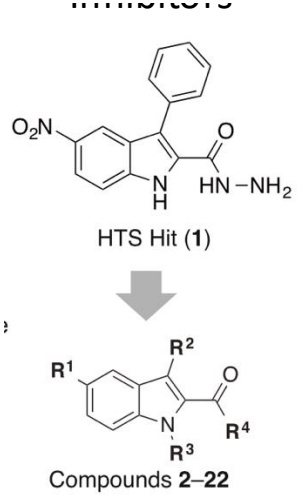


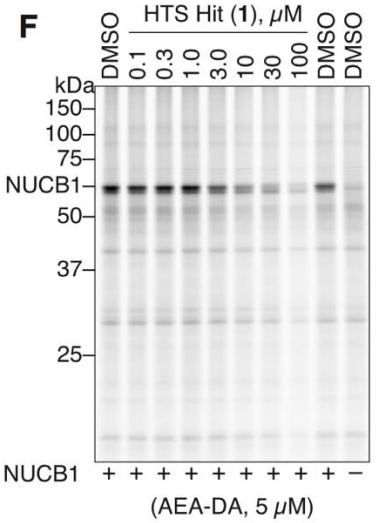
This is all good and great !

But what is the main limitation of probe-based approaches?









Take-Home Messages

-A key challenge in drug discovery is to understand the mode of action of small-molecule candidates.

-Understanding the targets of a small molecules is essential in drug discovery.

-Chemoproteomics allows for a broad characterization of the targets of small molecules.

-Chemoproteomics together with bioorthogonal chemistry approaches can characterize the target landscape in living systems.

-Powerful (quantitative) proteomics approaches are becoming ubiquitous in biological research.(And most likely you will have to use them at some point)