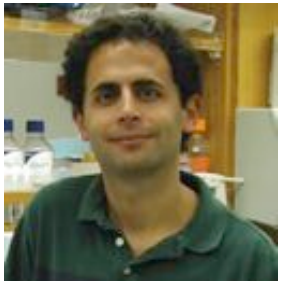
The Career of Benjamin F. Cravatt

Group Meeting: November 3rd, 2010

Benjamin D. Horning



- For a complete listing of Dr. Cravatt's publications, including pdf links, please visit http://www.scripps.edu/chemphys/cravatt/refs.html

The Career of Benjamin F. Cravatt

Biographical notes

▪ Education

B.A. (History) and B.B. (Biological Sciences): Stanford University (1992); Research with John H. Griffin

Ph.D.: The Scripps Research Institutre (TSRI); Dale Boger, Richard Lerner, and Norton Gilula (d. 2000)

- Crossing Extreme Mechanistic Barriers by Antibody Catalysis - Syn Elimination to a Cis Olefin. *J. Am. Chem. Soc.* **1994**, *116*, 6013-6014
- Chemical characterization of a family of brain lipids that induce sleep. *Science* **1995**, *268*, 1506-1509
- Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **1981**, *384*, 83-87

▪ Career

- Graduated from TSRI late 1996, began his independent career there January 1997 (no post-doc).
- Matthew Patricelli, his first graduate student, started working with him as he was finishing up his Ph.D. thesis.
- Promoted to Associate Professor (with tenure) in 2001, Full Professor in 2004 (TSRI)
- Currently Norton B. Gilula Chair in Chemical Biology, Chair of Chemical Physiology, TSRI

▪ Companies

Founder, ActivX Biosciences, Inc. No longer on Board of Directors, still on Scientific Advisory Board.

▪ Awards

- Searle Scholar Award (1998-2001)
- Eli Lilly Award in Biological Chemistry (2004)
- Cope Scholar Award (2005)
- Irving Sigal Young Investigator Award (2007)
- Tetrahedron Young Investigator Award in Bioogranic and Medicinal Chemistry (2008)

▪ Publications:

>230 publications (h-index = 66)

Cited >12,000 times, >55 average citations per article*

*According to a Web of Science search on 10-30-2010

The Career of Benjamin F. Cravatt

Ex-group members

- Alan Saghatelian (post-doc)
  Currently Professor, Harvard University

- Erin Carlson (post-doc)
  Currently Assistant Professor,
  Indiana University

- Stephan A. Sieber (post-doc)
  Currently Chair of Organic Chemistry II,
  Technical University of Munich

- Eranthie Weerapana (post-doc)
  Currently Assistant Professor, Boston College

- Several former group members working at ActivX Biotechnology, Merck, Pfizer

http://www.scripps.edu/chemphys/cravatt/alumni.html

The Career of Benjamin F. Cravatt

Frequent collaborators

- Eric Sorensen
  Arthur Allan Patchett Professor in
  Organic Chemistry, Princeton University

- Dale Boger
  Richard and Alice Cramer Professor of Chemistry
  The Scripps Research Institute

- Aron H. Lichtman
  Professor
  Department of Pharmacology and Toxicology
  Medical College of Virginia Campus
  Virginia Commonwealth University

- Richard Lerner,
  President, The Scripps Research Institute

http://www.scripps.edu/chemphys/cravatt/refs.html

The Beginning

Cats

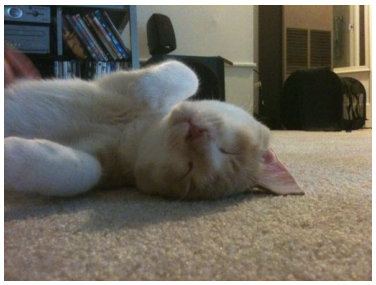
- Cerebrospinal fluid from sleep-deprived cats was analyzed, and the structure of *cis*-9,10-octadecenoamide (oleamide) was elucidated(via combination of MS, GC, TLC, IR, NMR, and ozonolysis)

- Oleamide was shown to induce sleep in rats, with significant enhancement as compared to *trans* isomer, other olefin isomers, and shorter or longer alkyl chains

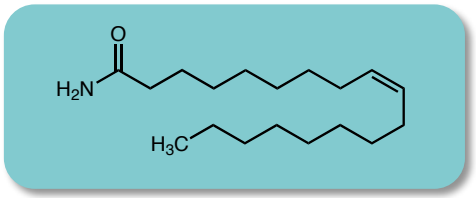
- Oleamide is a member of a family of recently discovered neuromodulatory fatty-acid amides

- Regulation was proposed to occur via hydrolysis to inactive oleic acid, and such enzymatic activity was found in rat brain membrane fractions

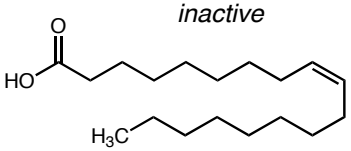
- Sleep deprivation



rat brain membrane fraction



Oleic acid
inactive

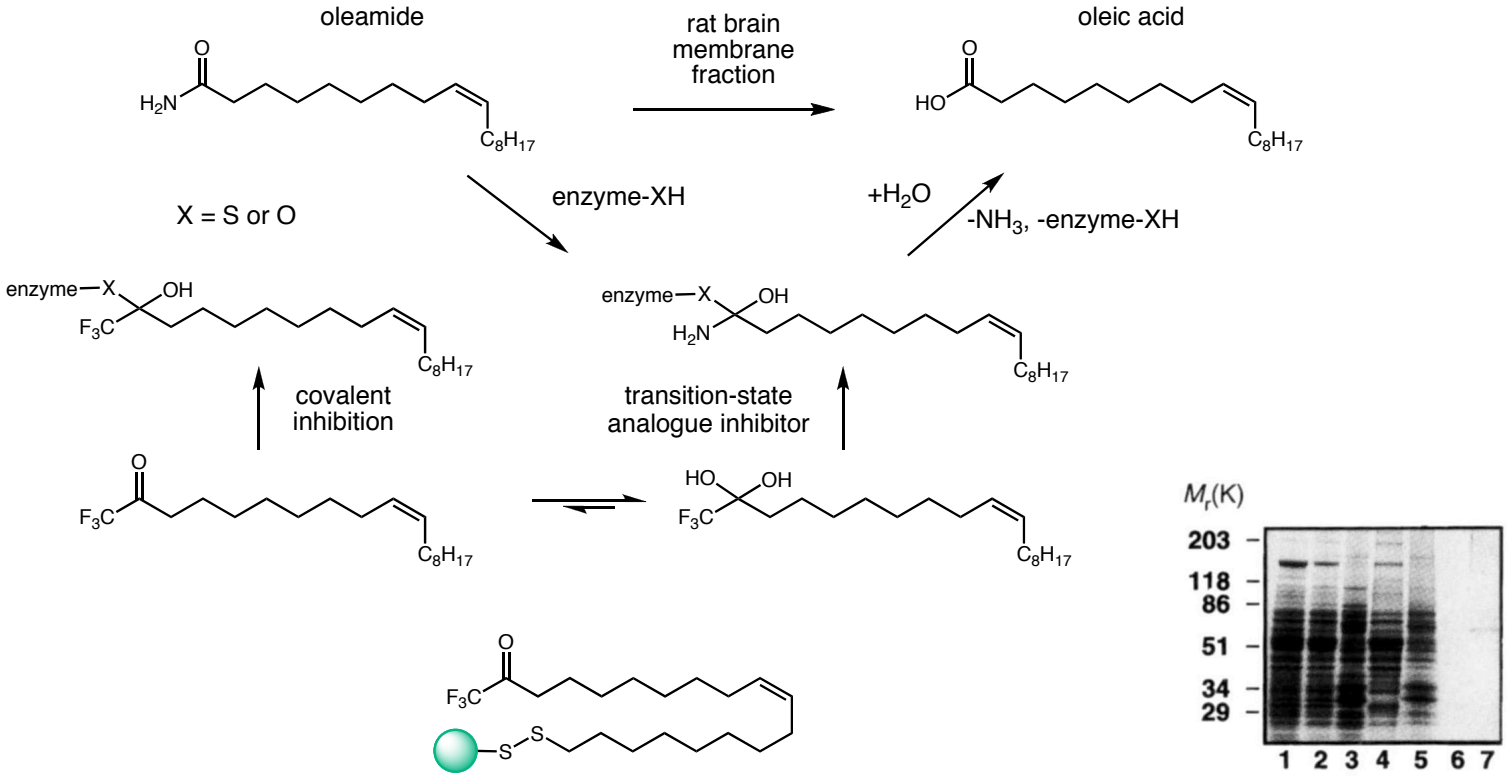


Cravatt *et al*. *Science* **1995**, *268,* 1506-1509

Discovery of FAAH

Purification via affinity chromatography

- Membrane-bound proteins, such as that which hydrolyzes oleamide, are difficult to isolate/characterize
- Trifluoromethyl ketone derivates of oleamide were shown to be potent inhibitors of oleamide hydrolase activity

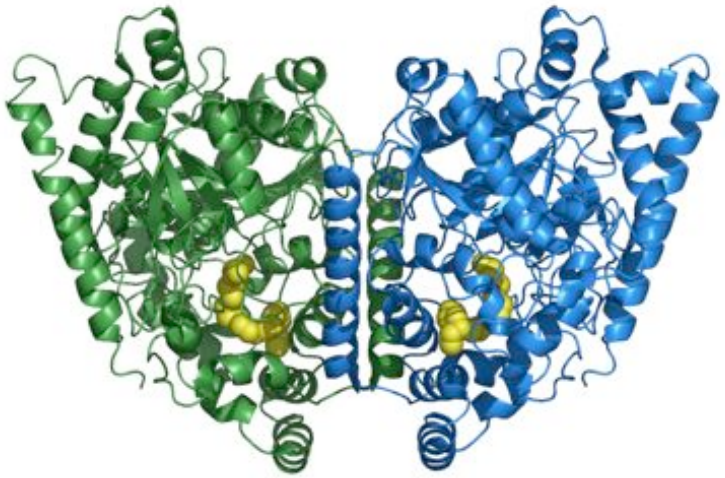


- A trifluoromethyl ketone-based column was synthesized and used to purify the enzyme, which was named fatty-acid amide hydrolase, or FAAH

Cravatt *et al. Nature* **1996**, *384,* 83-87 Oleamide hydrolase activity inhibitors: Patterson *et al. J. Am. Chem. Soc.* **1996**, *118*, 5938-5945

Discovery of FAAH

A non-conventional serine protease, and modulator of fatty-acid amide levels

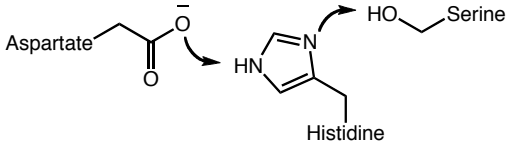


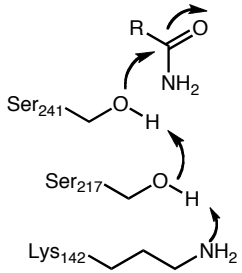
- The human version of FAAH shares 82% identity with rat FAAH, 84% identity with mouse FAAH
- All three FAAHs have similar substrate specificity, apparent molecular size, and inhibitor sensitivity (trifluoromethyl ketones)
- FAAH is the main enzyme that controls levels of neuromodulatory fatty-acid amides

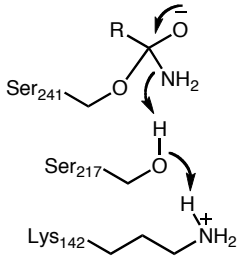
Cravatt *et al. Proc. Natl. Acad. Sci.* **1997**, *94,* 2238-2242 crystal structure of FAAH: Cravatt *et al. Science* **2002**, *298*, 1793-1796

Discovery of FAAH

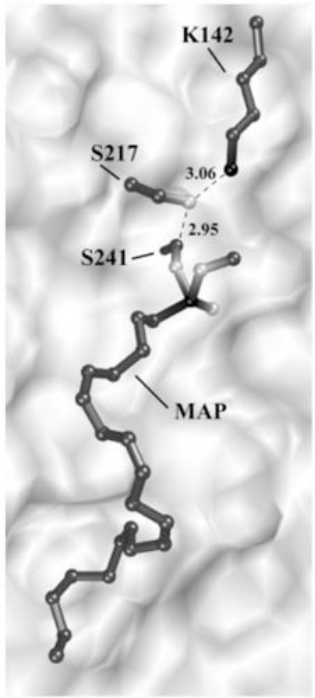
Serine proteases







- Serine alkoxide proceeds to esterify substrate, leading to hydrolysis
- Serine alkoxide is hydrolytic agent, but activation occurs via a distinct mechanism



- Amide and ester hydrolysis occur at the same rate; this is believed to be due to protonation of the leaving group to facilitate its departure
- Point mutants were made to understand catalytic activity, oleoyl methyl ester versus oleamide hydrolysis rates were used to determine selectivity

Enzyme	Ser241	Ser217	Lys142	k_{cat}	ester/amide
FAAH	+	+	+	14.4±0.5	0.38
S241A	-	+	+	undetectable	-
K142A	+	+	-	$3.4\pm0.5 \times 10^{-4}$	320
S217A	+	-	+	$4.3\pm0.5 \times 10^{-3}$	0.21

Cravatt *et al. J. Biol. Chem.* **2003**, *278,* 37393-37399

Discovery of FAAH

Other fuctions of FAAH

- Oleamide and Anandamide are endocannabinoid fatty-acid amides modulated by FAAH
- FAAH (-/-) mice show THC-like response to anandamide
- FAAH inhibition can lead to therapeutically useful responses, including helping fight pain, relieving anxiety, and managing substance dependencies (shown to affect alcohol and THC dependency)

Cravatt *et al*. *Biol. Reprod.* **2009**, *80*, 235-242: Genetic loss of Faah compromises male fertility in mice

Cravatt *et al*. *Neuropsychopharmacology* **2007**, *32*, 1570-1582: Role of endocannabinoids in alcohol consumption and intoxication: Studies of mice lacking fatty acid amide hydrolase

Cravatt *et al*. *J. Neurobiol.* **2004**, *61*, 149-160: The endogenous cannabinoid system and its role in nociceptive behavior

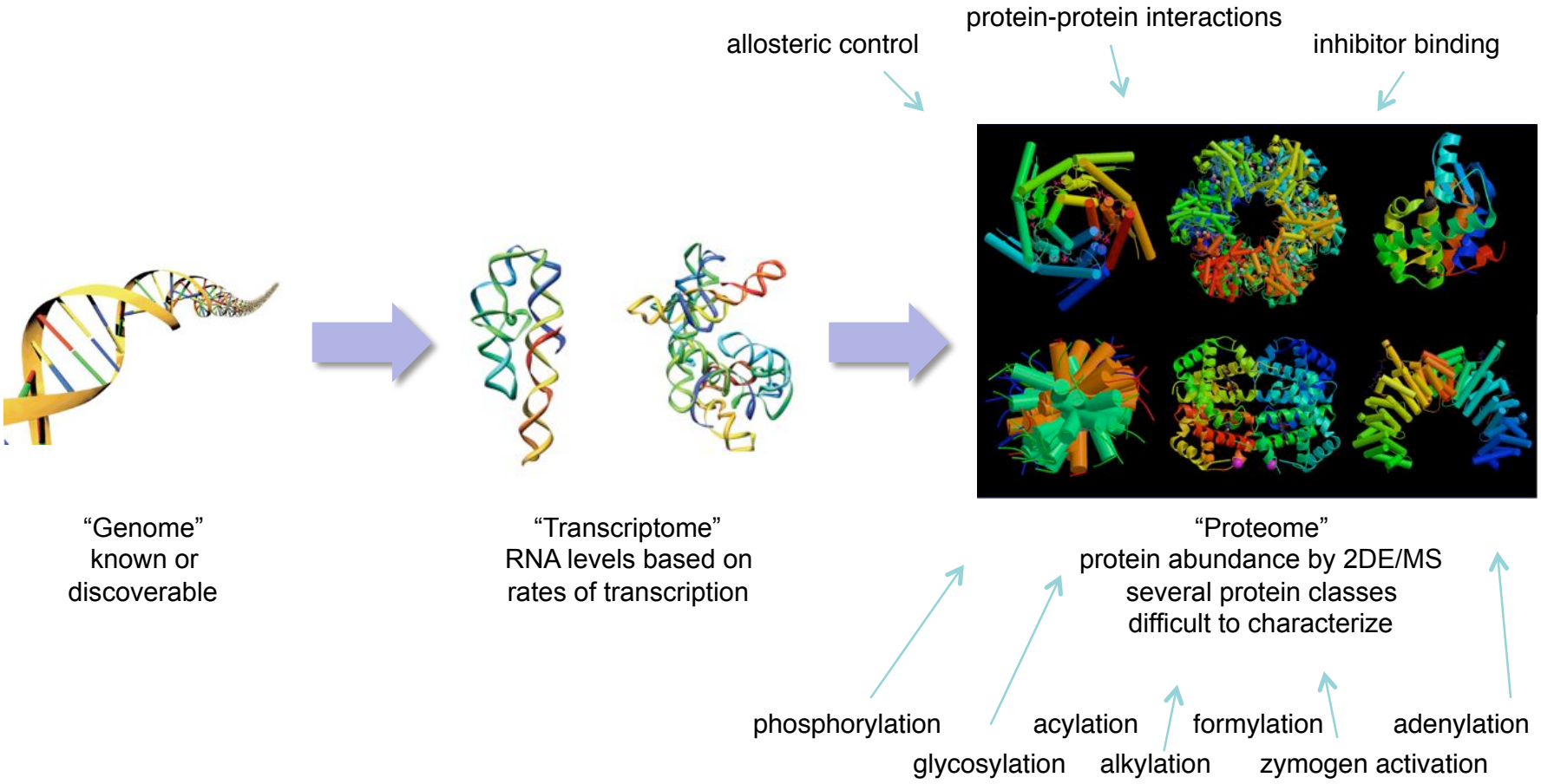
Cravatt *et al*. *Curr. Opin. Chem. Biol.* **2003**, *7*, 469-475: Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system

Cravatt *et al*. *Annu. Rev. Biochem.* **2005**, *74*, 411-432: Structure and function of fatty acid amide hydrolase

Activity-Based Protein Profiling

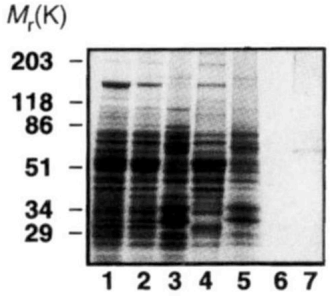
The need for new proteomics technologies

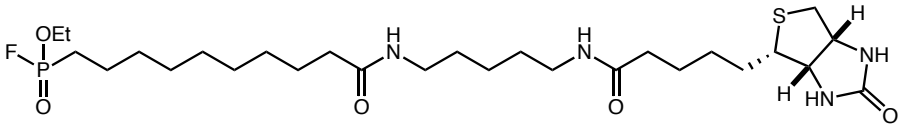
- Proteomic research attempts to elucidate the function of the myriad of products encoded by the genome
- Previous research has involved monitoring transcription rates and protein abundancies
- Monitoring expression levels of proteins does not account for post-translational modifications that modulate protein activity



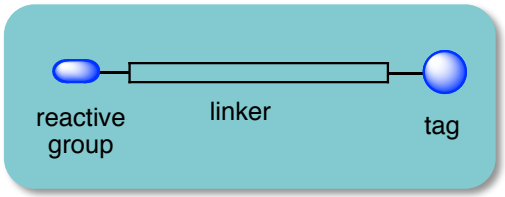
Activity-Based Protein Profiling

Entry via Serine Hydrolases



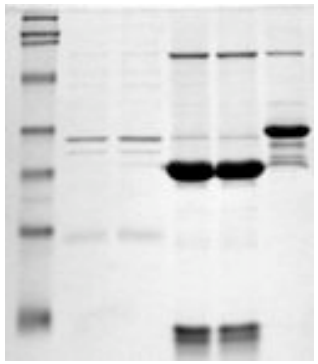


fluorophosphate FP-biotin biotin



- FAAH was a very minor constituent of the rat brain cell lysate

- Avidin will bind selectively to any biotinylated substrate

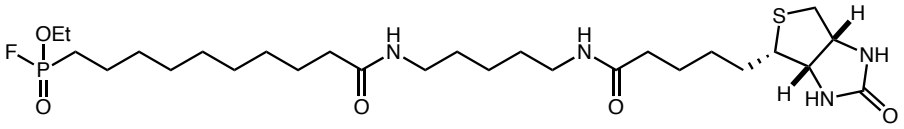


- Addition of H_2O_2 causes staining via horseradish peroxidase conjugated to Avidin

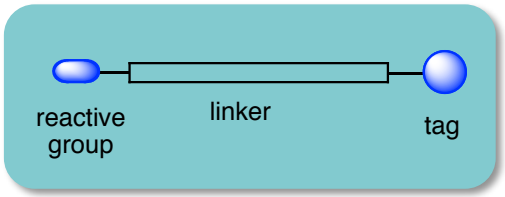
Cravatt *et al. Proc. Natl. Acad. Sci.* **1999**, *96,* 14694-14699

Activity-Based Protein Profiling

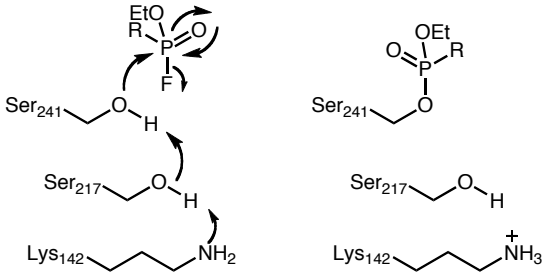
Entry via Serine Hydrolases



fluorophosphate FP-biotin biotin



- Phosphate ester formation is irreversible and selective for nucleophilic active site residues
- Once the protein is covalently linked to biotin, it can be easily visualized utilizing Avidin (biotin binding protein)
- Oxophilicity of fluorophosphate ensures orthogonality to other nucleophilic residues (cysteine)

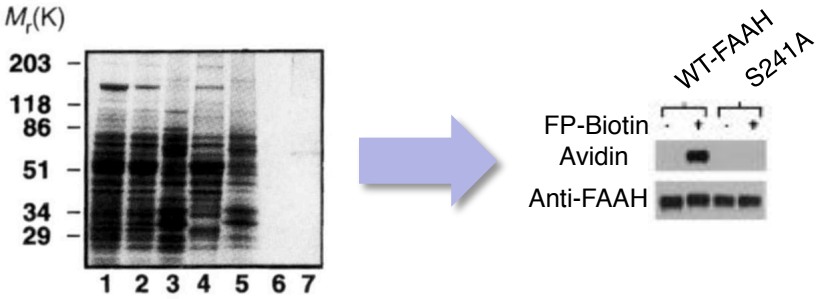


Cravatt *et al. Proc. Natl. Acad. Sci.* **1999**, *96,* 14694-14699

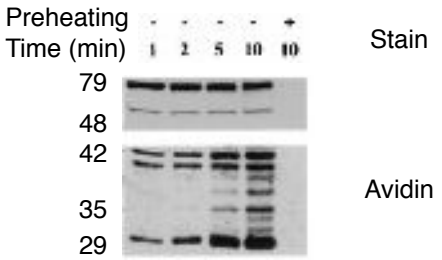
Activity-Based Protein Profiling

FP-Biotin enables activity-based analysis

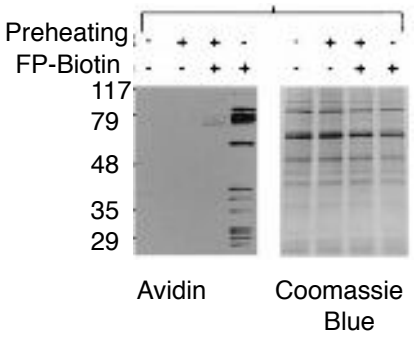
- Treatment of cell lysate (rat testis) with FP-Biotin, separation via SDS-PAGE and visualization with Avidin is performed
- Activity-dependent binding was shown via comparing heated (denatured) enzyme to properly folded
- Prolonged exposure reveals lower abundance/activity proteins, but still does not tag inactive ones
- The presence of an active site inhibitor prevents tagging, consistent with active site-specific tagging

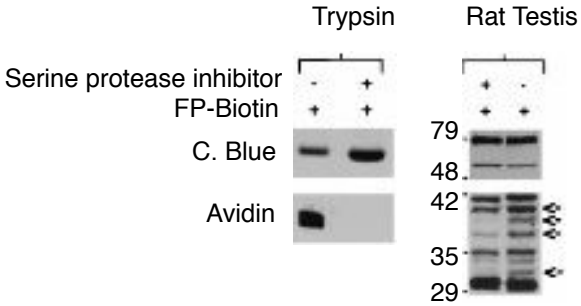


- FP-Biotin binds FAAH in an activity-dependent manner



- Only properly folded (active) enzymes are labeled





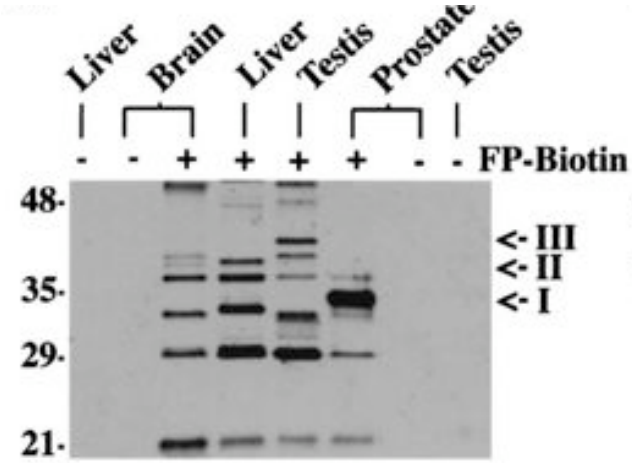
- Inhibited enzymes are not labeled

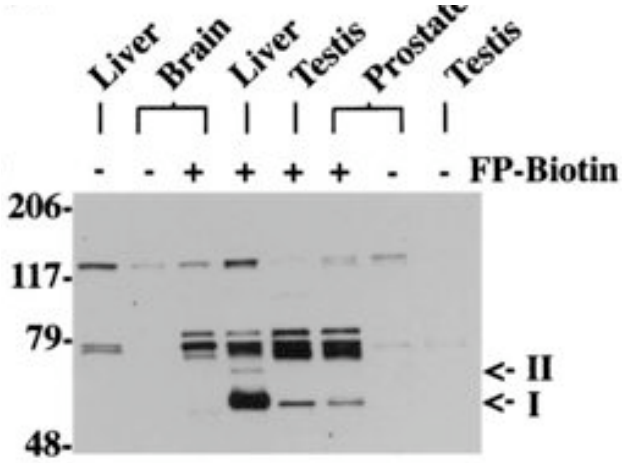
Cravatt *et al. Proc. Natl. Acad. Sci.* **1999**, *96,* 14694-14699

Activity-Based Protein Profiling

FP-Biotin enables activity-based analysis

- Activity levels of serine hydrolase enzymes in different rat tissues can be monitored simultaneously
- Activity-dependent binding was shown via comparing heated (denatured) enzyme to properly folded
- Prolonged exposure reveals lower abundance/activity proteins, but still does not tag inactive ones
- Proteins are believed to all belong to the serine hydrolase class



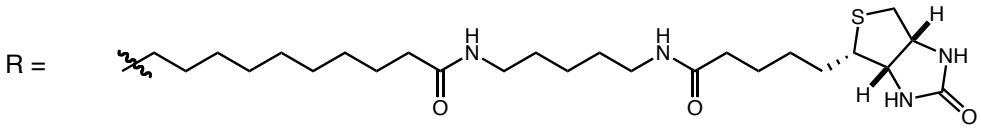


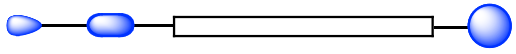
Cravatt *et al. Proc. Natl. Acad. Sci.* **1999**, *96,* 14694-14699

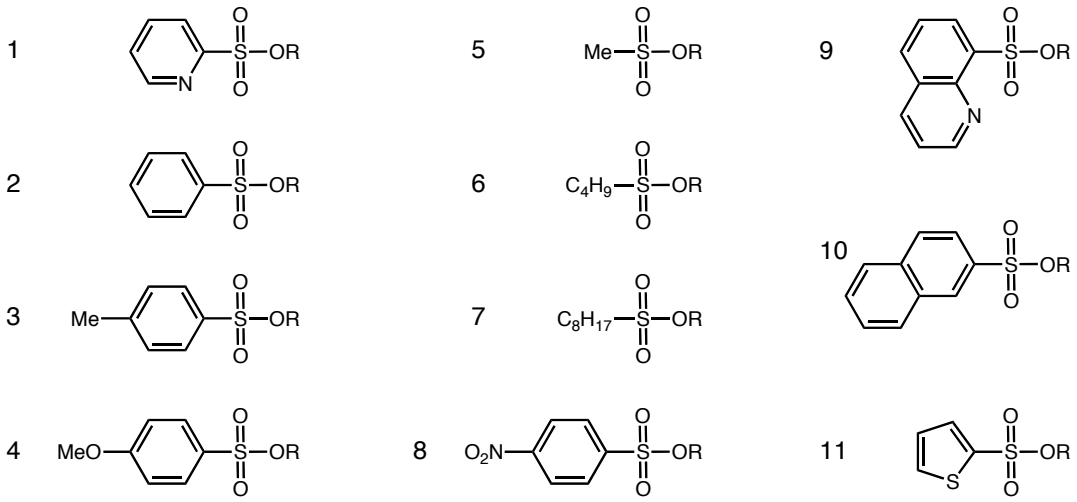
Activity-Based Protein Profiling

Second-generation ABPs (activity-based probes)

- Previous work was based on known covalent inhibitor of serine proteases
- For enzyme families which are poorly characterized, no inhibitors may be know
- Non-directed activity-based probes could allow screening of novel enzymes, and help characterize recently-discovered ones
- To accomplish this, a library of probes was constructed and tested





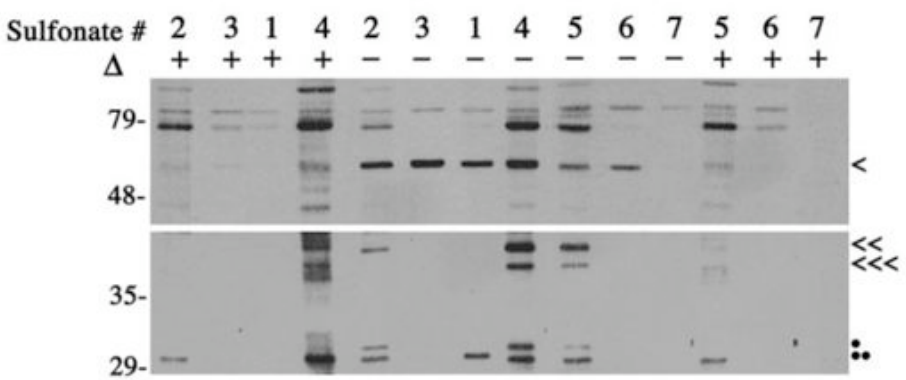


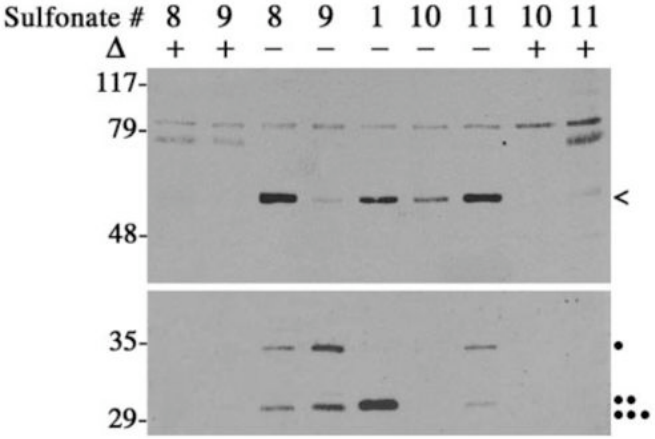
Adam, G.C.; Cravatt, B. F.; Sorensen, E. J. *Chem. Biol.* **2001**, *8*, 81-95

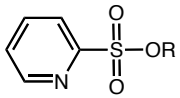
Activity-Based Protein Profiling

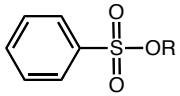
Second-generation ABPs (activity-based probes)

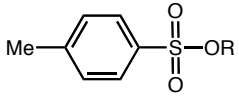
- Synthesized probes were tested against rat testis

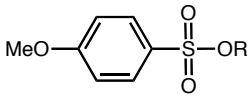


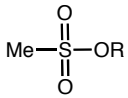


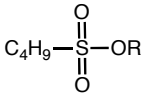
1 

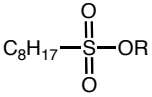
2 

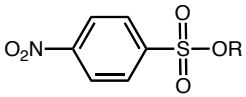
3 

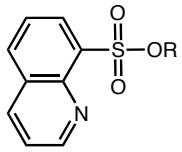
4 

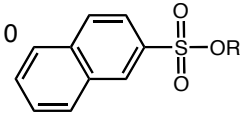
5 

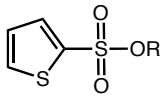
6 

7 

8 

9 

10 

11 

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Activity-Based Protein Profiling

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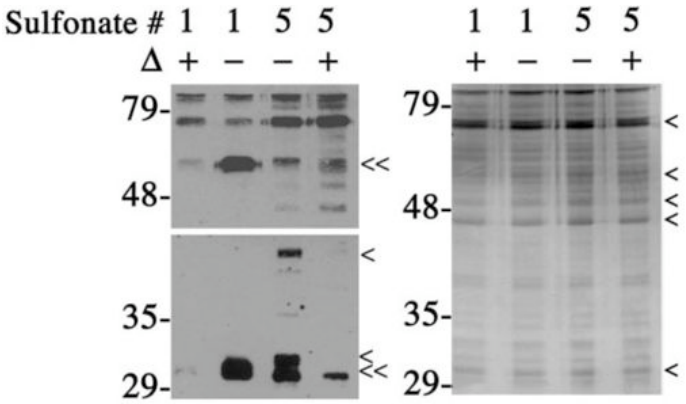
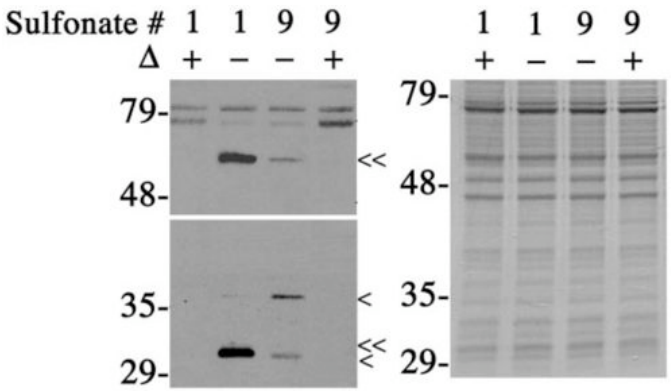
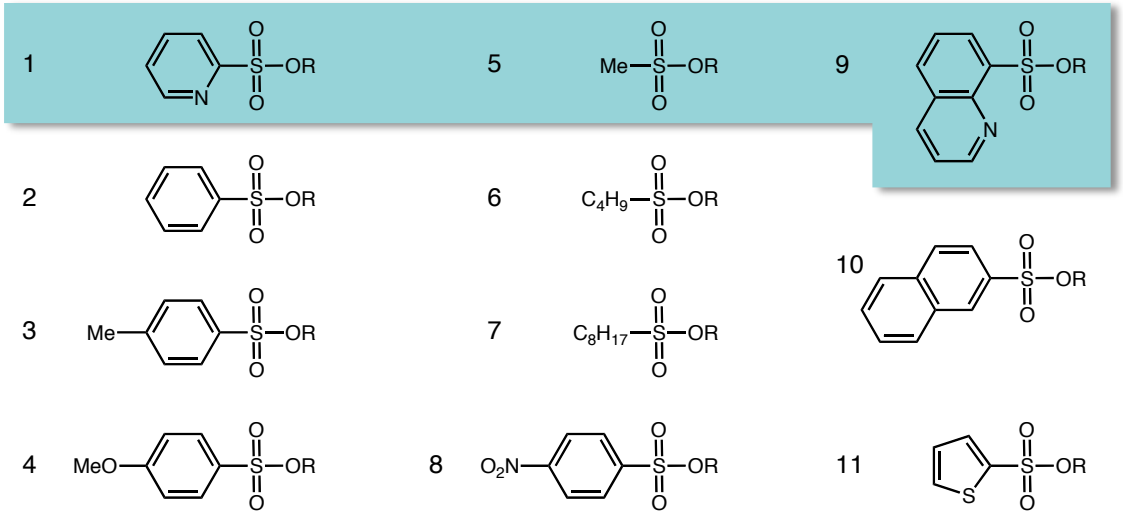
- Synthesized probes were tested against rat testis



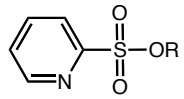




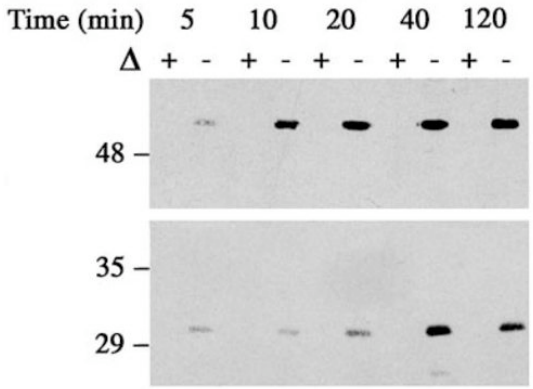
Adam, G.C.; Cravatt, B. F.; Sorensen, E. J. *Chem. Biol.* **2001**, *8*, 81-95

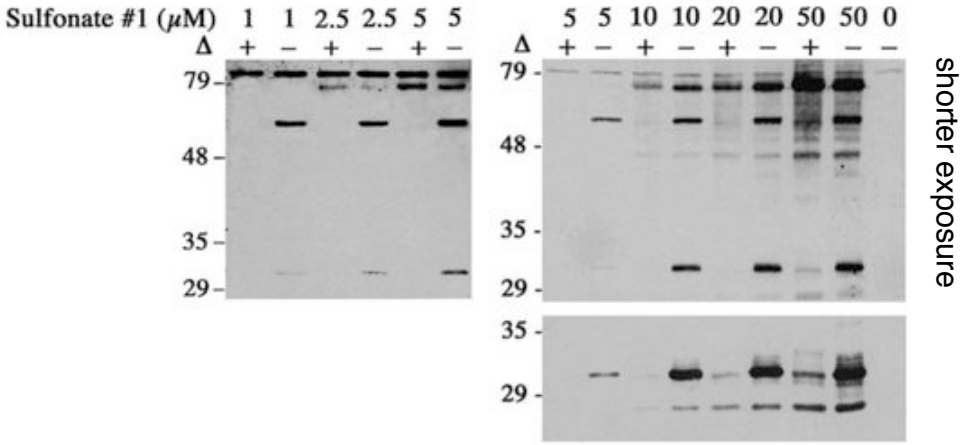
Activity-Based Protein Profiling

Second-generation ABPs (activity-based probes); factors that affect tagging

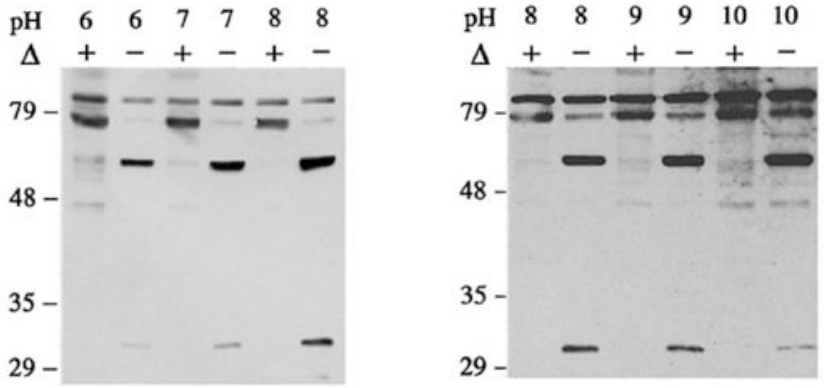


- Time and probe concentration

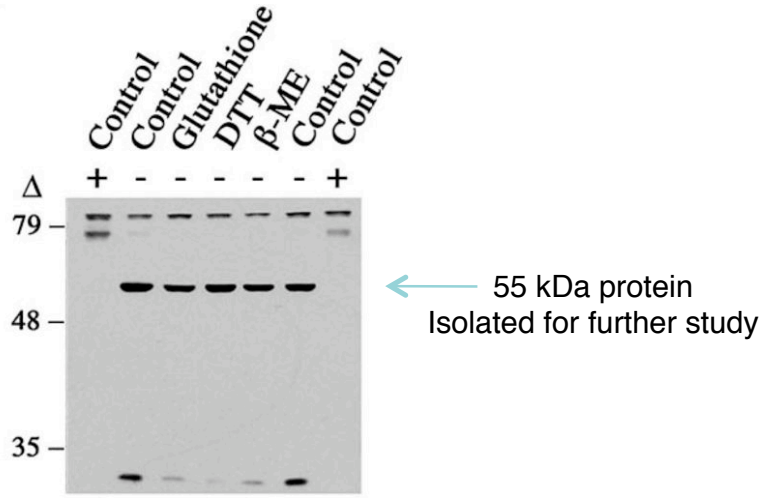




- pH-dependence



- Exogenous nucleophile (thiol) tolerance

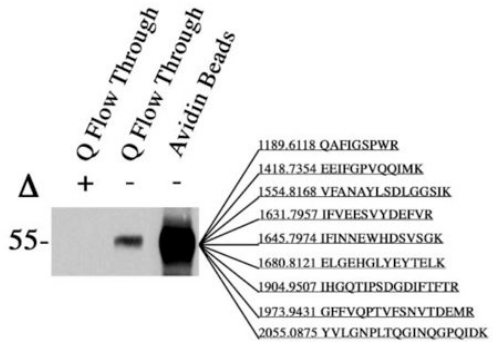


Adam, G.C.; Cravatt, B. F.; Sorensen, E. J. *Chem. Biol.* **2001**, *8*, 81-95

Activity-Based Protein Profiling

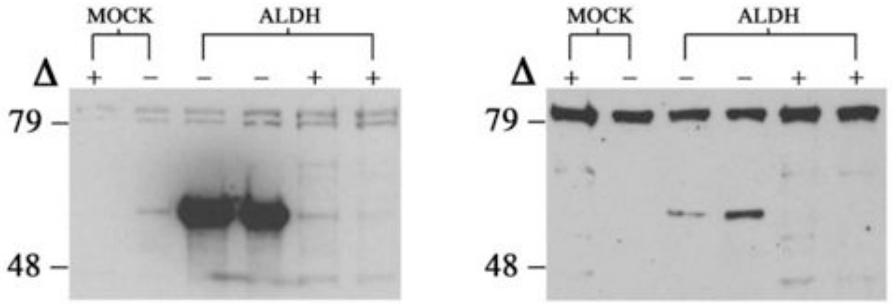
Identification of protein targets

- Rat liver was shown to have the highest expression of a 55 kDa protein, and it was isolated from this source
- Initial purification was achieved by anion-exchange chromatography (Q-Sepharose), and then using Avidin Beads



- After isolation, the protein was digested with trypsin and the fragments analyzed via MS (MALDI-TOF), identifying the protein as cytosolic 2 class I aldehyde dehydrogenase (cALDH-I)

- The cALDH-I DNA was transfected into mamallian cells and expressed

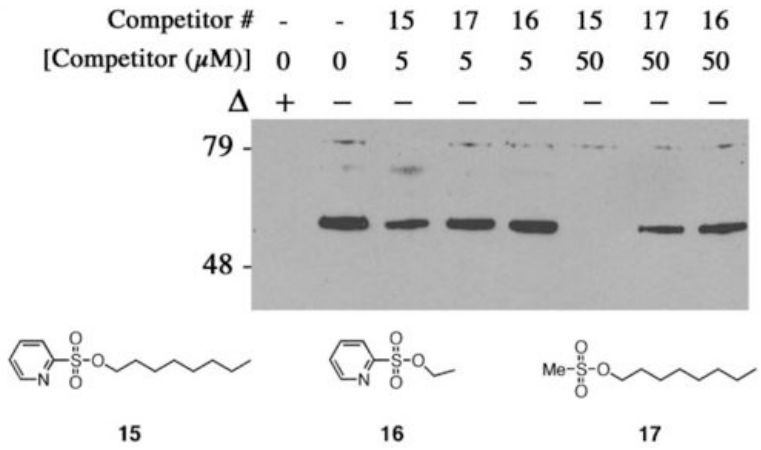


Adam, G.C.; Cravatt, B. F.; Sorensen, E. J. *Chem. Biol.* **2001**, *8*, 81-95

Activity-Based Protein Profiling

Discovery of inhibitors using ABPP

- Inhibitors for cALDH-I were next screened as competitors for the activity-based probe 1



Principles of ABPP thus far

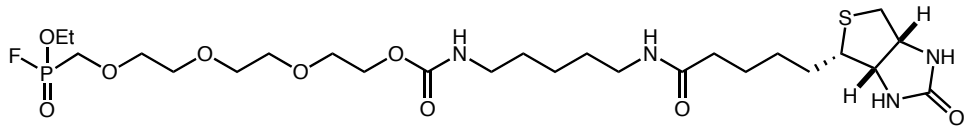
- Probes bind selectively to enzymes with intact active sites
- Denatured enzymes and active site mutants do not react (side chain residues are not reactive)
- Probes can be used to determine availability of active site
- Inhibitor-bound proteins are unreactive (only shown for covalent inhibitors)

Adam, G.C.; Cravatt, B. F.; Sorensen, E. J. *Chem. Biol.* **2001**, *8*, 81-95

Activity-Based Protein Profiling

Advancements in probe design

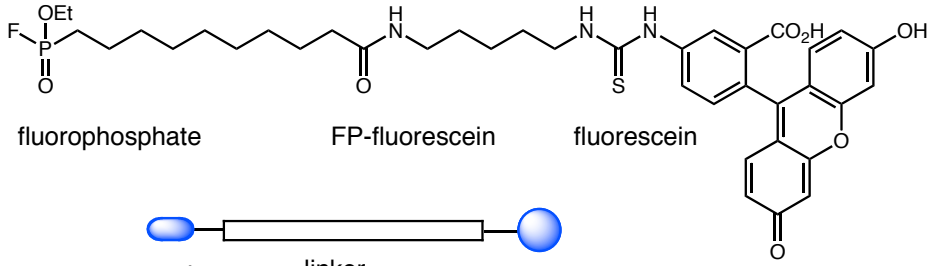
- A PEG-linked ABP shows a complementary tagging profile to standard FP-biotin



fluorophosphate FP-PEG-biotin biotin

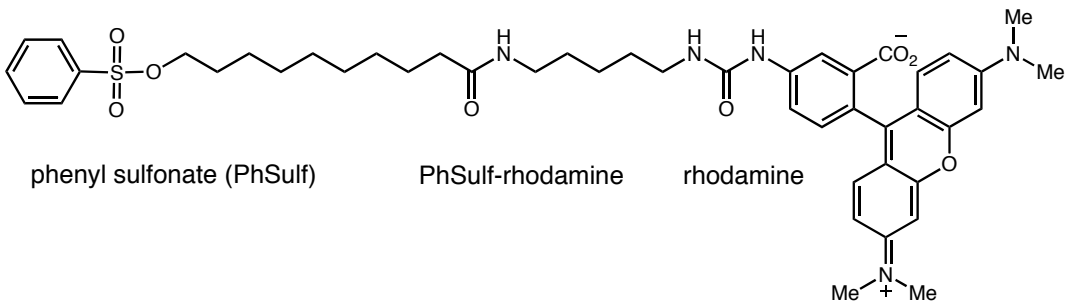
Kidd, D.; Liu, Y.; Cravatt, B. F. *Biochemistry.* **2001**, *40*, 4005-4015

- The biotin tag can be replaced by fluorescent handles such as rhodamine and fluorescein

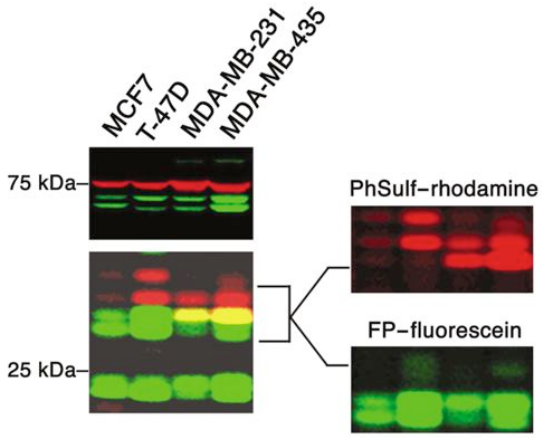


fluorophosphate FP-fluorescein fluorescein

reactive group linker tag



phenyl sulfonate (PhSulf) PhSulf-rhodamine rhodamine



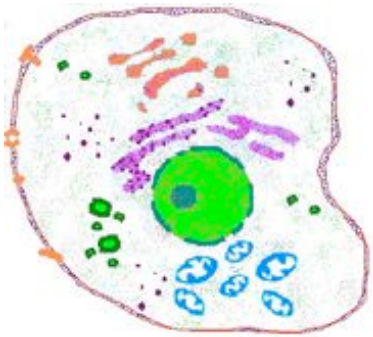
- Multiplexing ABPs

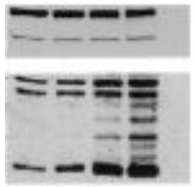
Adam, G.C.; Sorensen, E. J.; Cravatt, B. F. *Nature Biotechnology* **2002**, *20*, 805-809

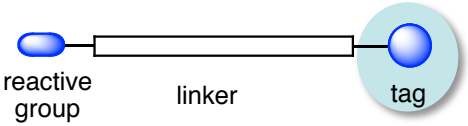
Activity-Based Protein Profiling

In vivo ABPP

- Standard APBB is performed on cell lysates







- Biotin or fluorescent tag precludes *in vivo* applications

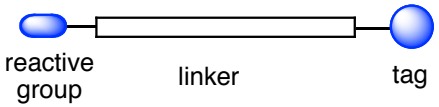
- Does lysing the cell affect the state of the proteome? Can ABPP be applied *in vivo*?

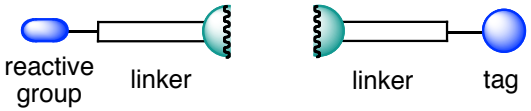
Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686-4687

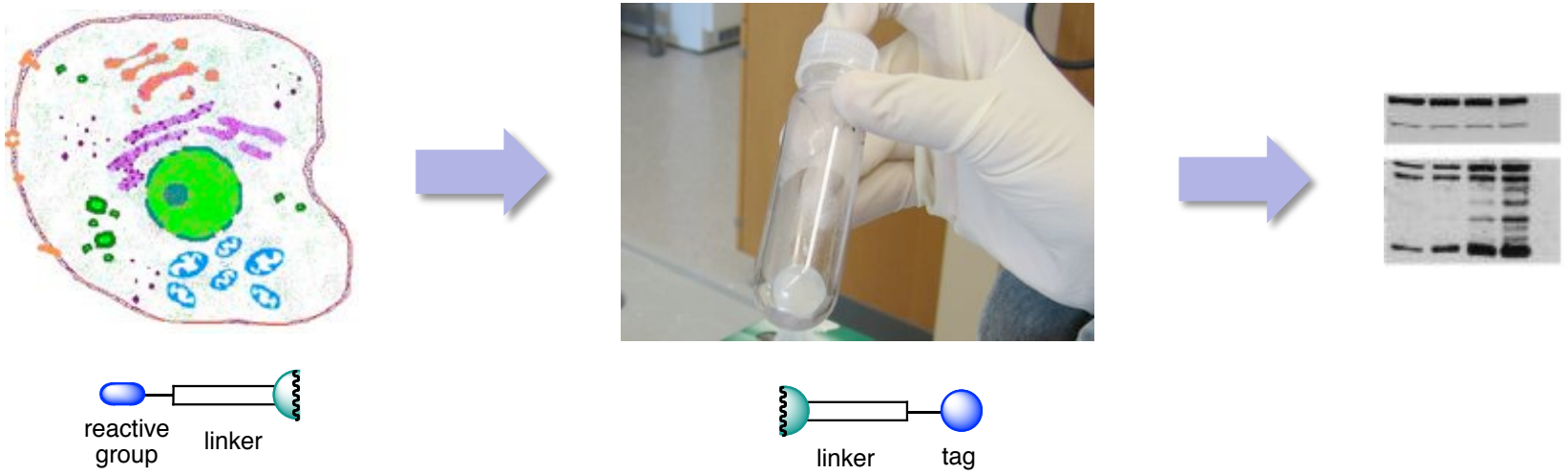
Activity-Based Protein Profiling

In vivo ABPP

- Separation of the reactive group and the tag would allow *in vivo* protein functionalization and *in vitro* tagging





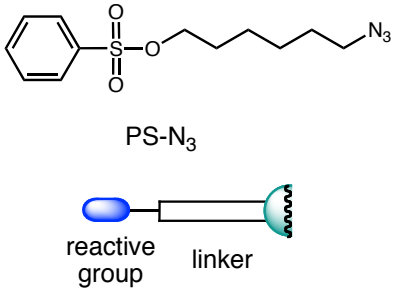


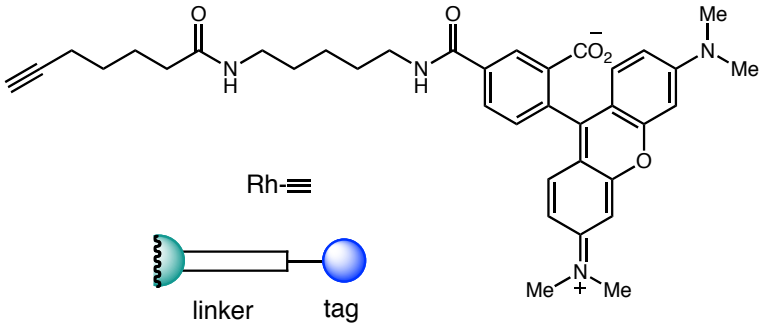
Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686-4687

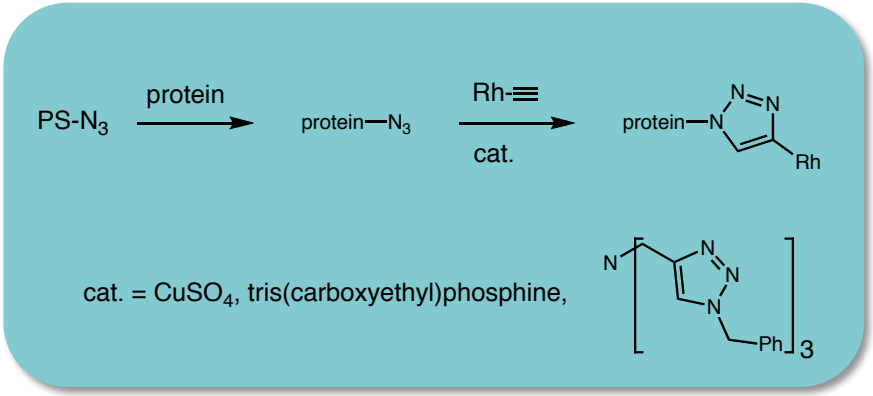
Activity-Based Protein Profiling

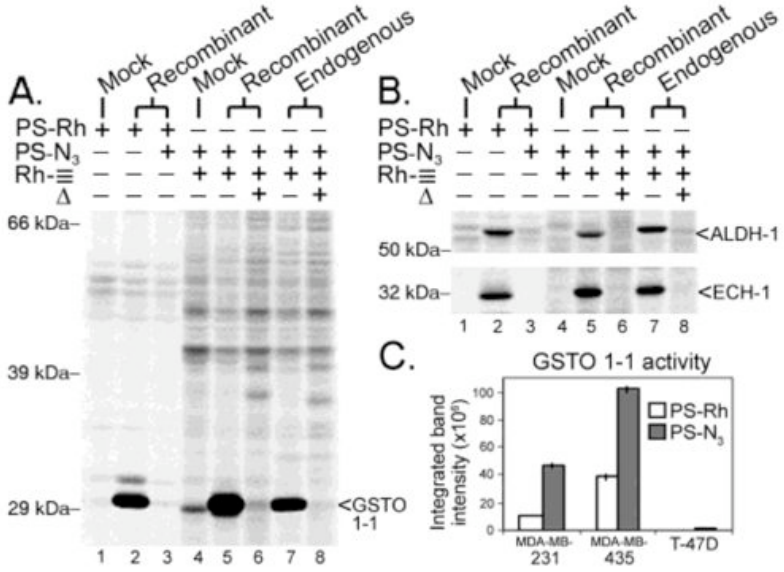
In vivo ABPP using click chemistry

- Alkylation of protein can be performed separately from Huisgen cyclization, but still allows detection







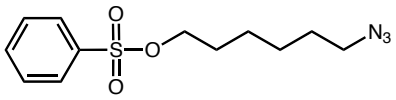


Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686-4687

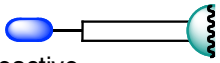
Activity-Based Protein Profiling

In vivo ABPP using click chemistry

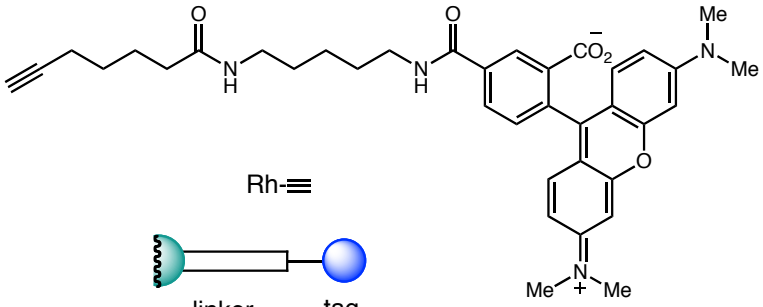
- ABPP was shown to be possible even in eukaryotes



PS-N_3

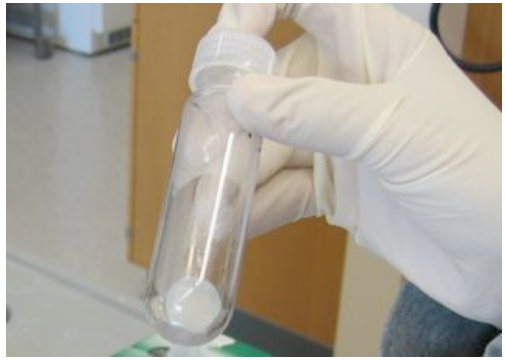


reactive group linker

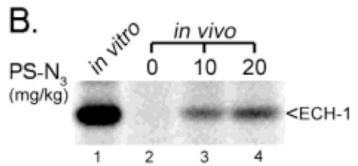


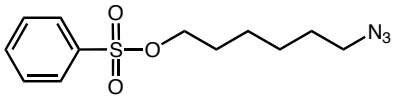
Rh-≡

linker tag

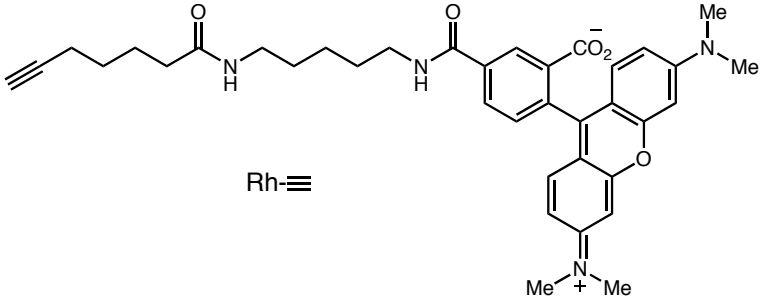


"Cu"





PS-N_3



Rh-≡

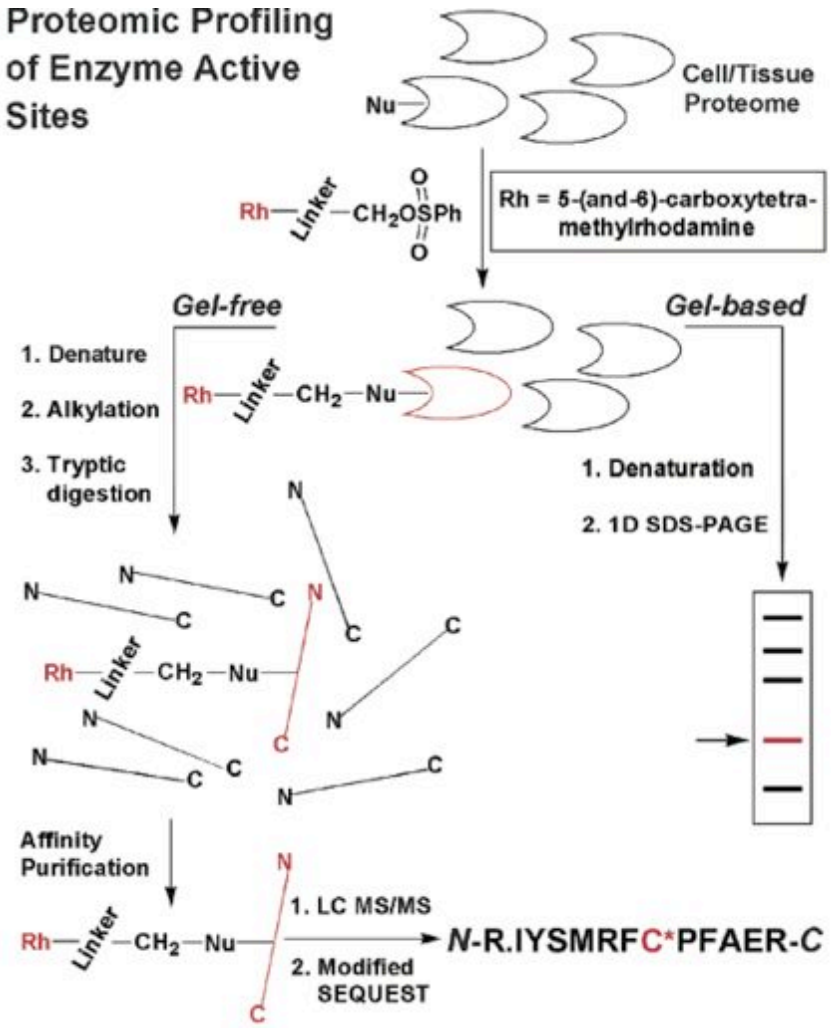
- Injected directly into live mouse

Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686-4687

Activity-Based Protein Profiling

Gel-free ABPP and probe labeling site determination

- To expand the coverage of ABPP, non-directed probes are combined with LC-MS/MS analysis



- Known enzyme targets of PS-Rh were used, including:

  Glutathione S-transferase omega (GST)
  Aldehyde dehydrogenase-1 (ALDH1)
  Enoyl CoA hydratase-1 (ECH1)
  Dimeric dihyrodiol dehydrogenase (DDH)
  3β-hydroxysteroid dehydrogenase/isomerase-1 (3HSD1)

- MS/MS analysis allows determination of active site sequence and probe labeling location

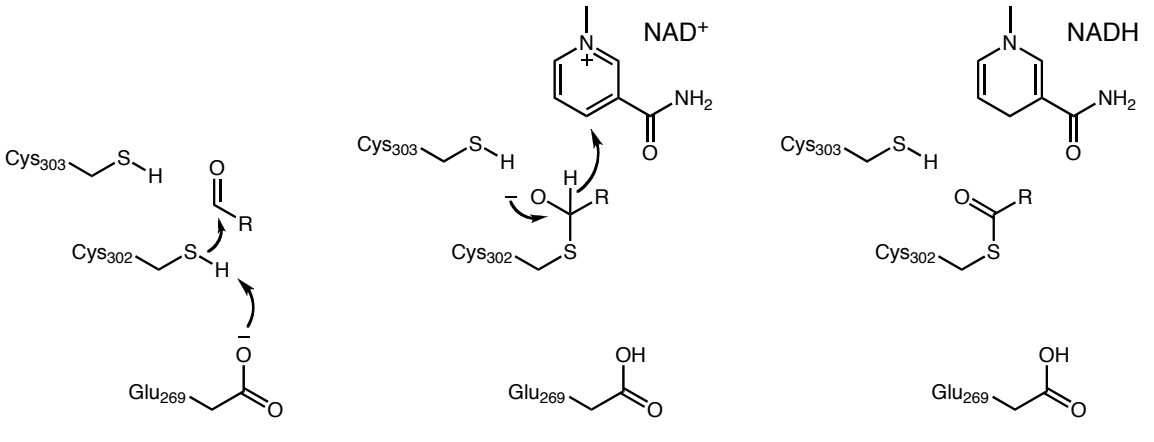
- ALDH1 showed labeling at a non-nucleophilic active-site residue

Cravatt, B. F. *et al. J. Am. Chem. Soc.* **2004**, *126*, 1363-1368

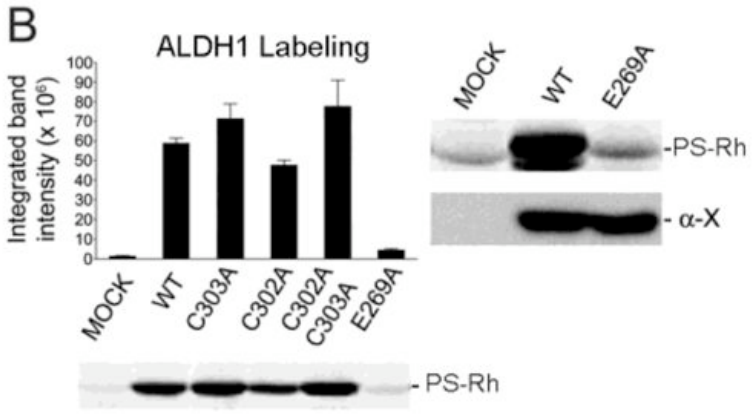
Activity-Based Protein Profiling

Gel-free ABPP and probe labeling site determination

- Point mutations of ALDH1 were made to confirm labeling specificity



- Labeling occurs selectively on E269

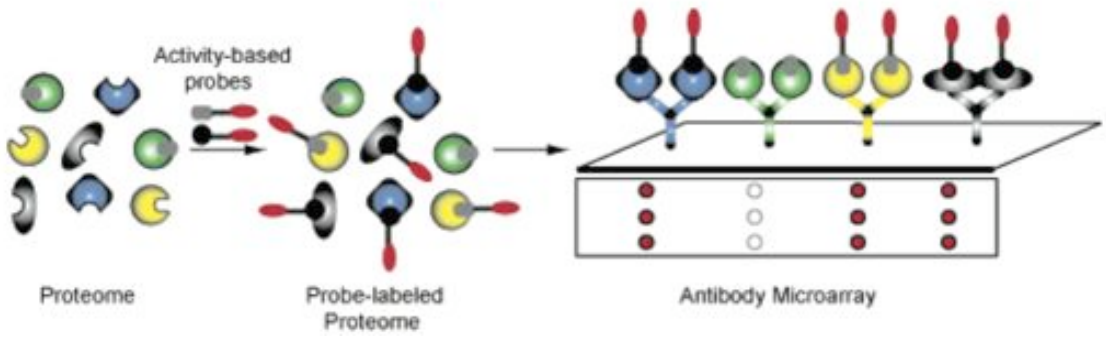


Cravatt, B. F. *et al. J. Am. Chem. Soc.* **2004**, *126*, 1363-1368

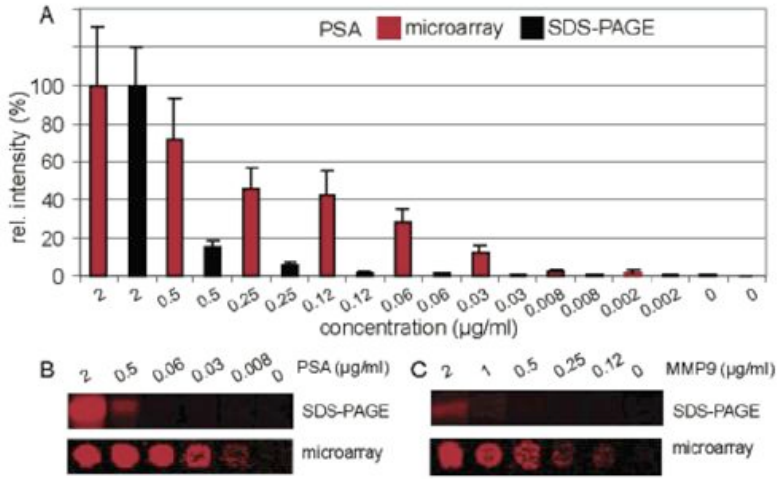
Activity-Based Protein Profiling

Microarray platforms allow more sensitive probing

- For known enzymes with established antibodies, microarrays can be used to monitor activity levels



- This technique is more sensitive than previous gel-based approaches



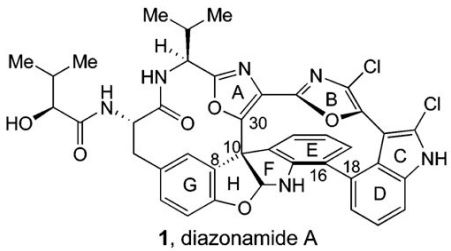
Cravatt, B. F. *et al. J. Am. Chem. Soc.* **2004**, *126*, 15640-15641

Applications of ABPP

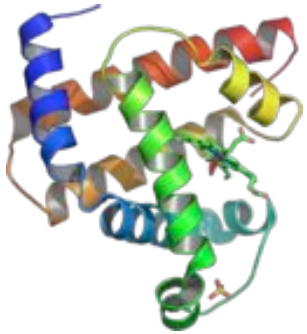
Towards reversible inhibitors of enzymes in complex proteomes

- "Standard" enzyme inhibitor assays screen lead compounds against a single protein at a time

- Protein expression and purification, as well as development of a specific substrate assay are required

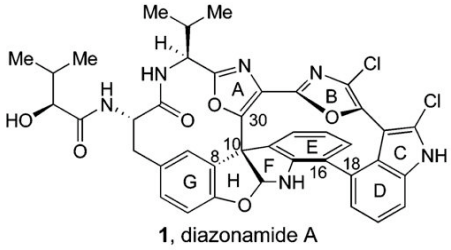


1, diazonamide A

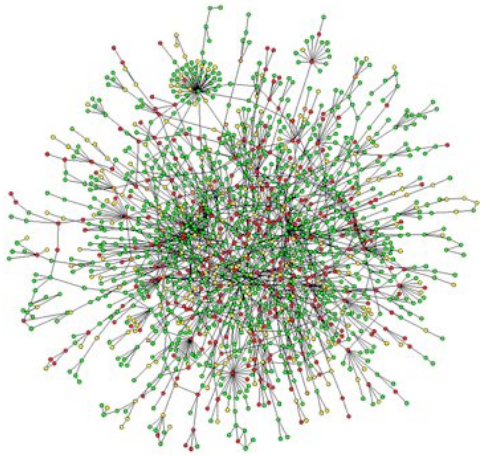


- Potency one target at a time
- Selectivity only after multiple runs

- The ability to screen compounds again a variety of proteins simultaneously has many advantages



1, diazonamide A



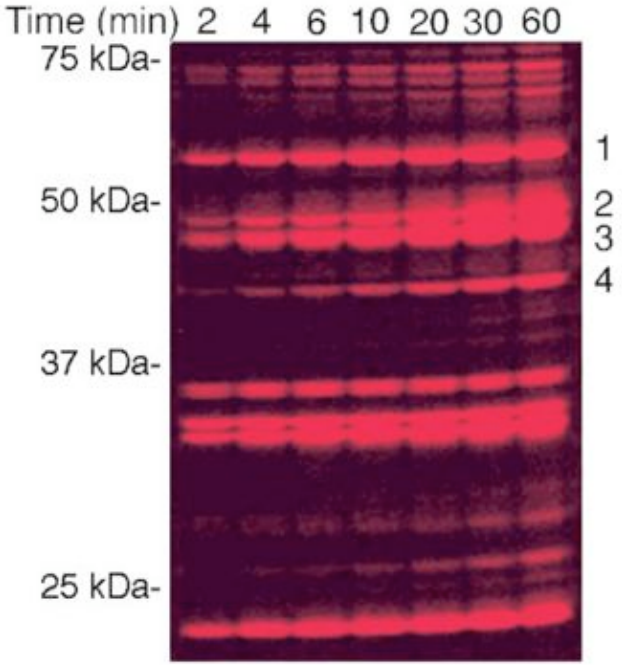
- Potency *and* selectivity of many targets with one assay

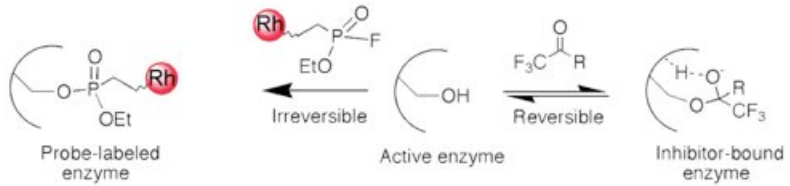
Leung, D.; Hardouin, C.; Boger, D. L.; Cravatt, B. F. *Nature Biotechnology* **2003**, *21*, 687-691

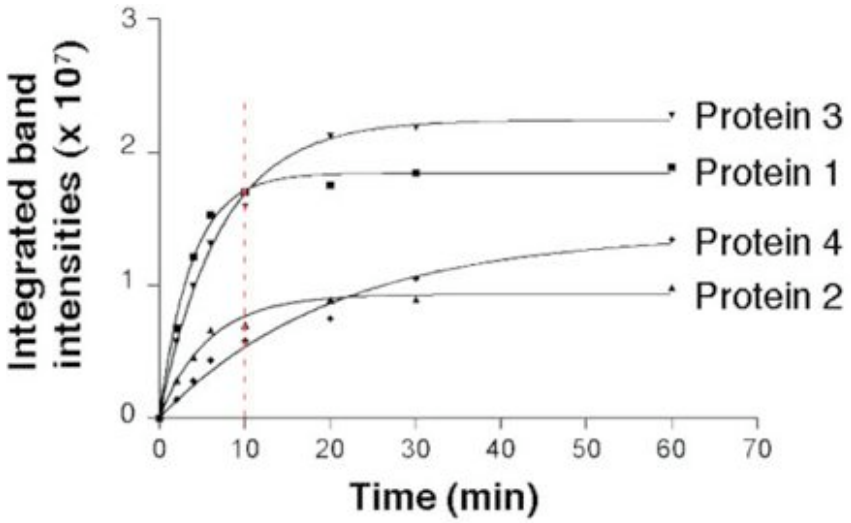
Applications of ABPP

Towards reversible inhibitors of enzymes in complex proteomes

- Previous ABPP work involved testing selectivity of *irreversible* inhibitors; reversible inhibitors require monitoring changes in probe labeling (probe labeling must be incomplete)







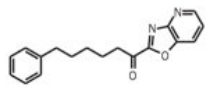
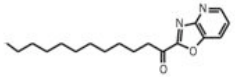
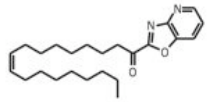
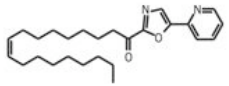
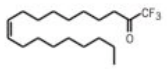
- An assay time of 10 minutes was deemed optimal

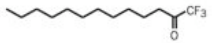
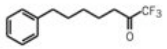
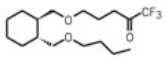
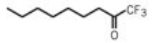
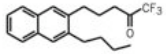
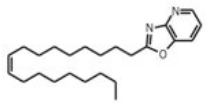
Leung, D.; Hardouin, C.; Boger, D. L.; Cravatt, B. F. *Nature Biotechnology* **2003**, *21*, 687-691

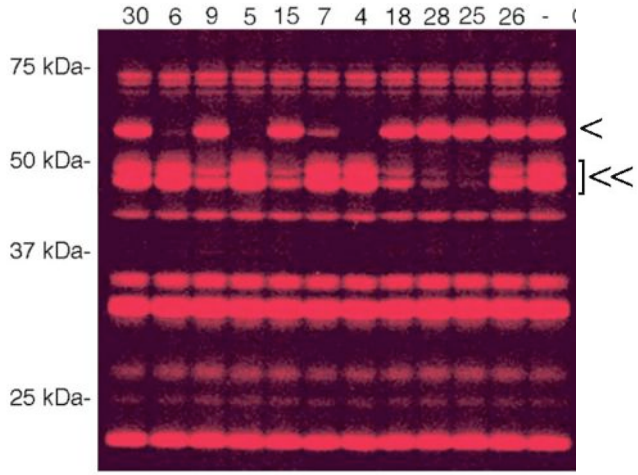
Applications of ABPP

Towards reversible inhibitors of enzymes in complex proteomes

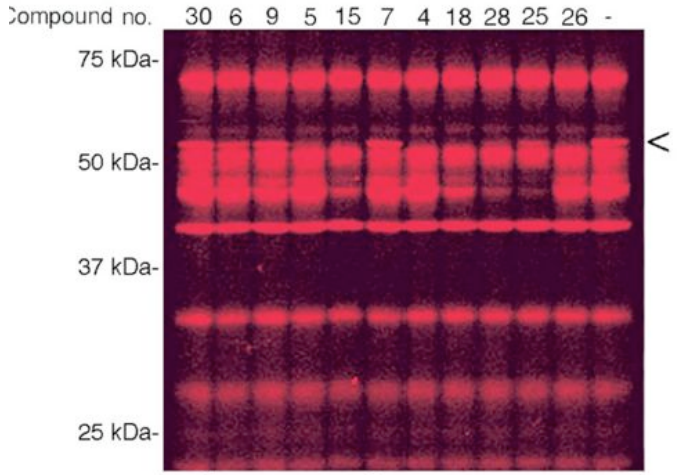
- A longstanding collaboration with the Boger laboratory has yielded many FAAH inhibitors

		60-kDa brain enzyme (FAAH)	45- and 50-kDa brain enzyme (KIAA1363)	60-kDa heart enzyme (TGH)
4		1.1 (0.72–1.7)	>100,000	1.4 (1.1–1.9)
5		0.81 (0.56–1.1)	50,000 (21,000–120,000)	83 (69–100)
6		44 (31–62)	62,000 (23,000–160,000)	1,400 (880–2,400)
7		150 (84–280)	>100,000	>100,000
9		4,500 (3,100–6,500)	1,100 (730–1,500)	4,800 (1,900–12,000)

		60-kDa brain enzyme (FAAH)	45- and 50-kDa brain enzyme (KIAA1363)	60-kDa heart enzyme (TGH)
15		11,000 (6,200–21,000)	470 (320–690)	61 (47–79)
18		5,300 (3,300–8,300)	770 (570–1,100)	0.47 (0.10–2.2)
25		15,000 (8,100–28,000)	52 (38–71)	320 (230–450)
26		30,000 (19,000–48,000)	1,500 (1,300–1,700)	1.8 (1.0–3.1)
28		37,000 (16,000–84,000)	110 (86–150)	160 (86–310)
30		>100,000	>100,000	>100,000



Brain membrane proteome



Heart membrane proteome

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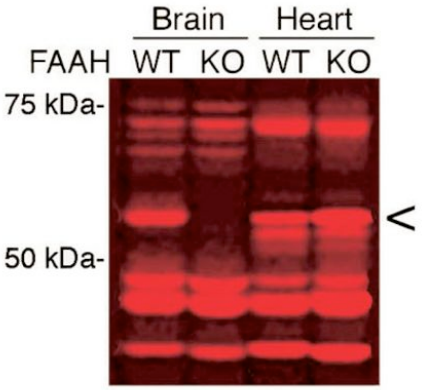
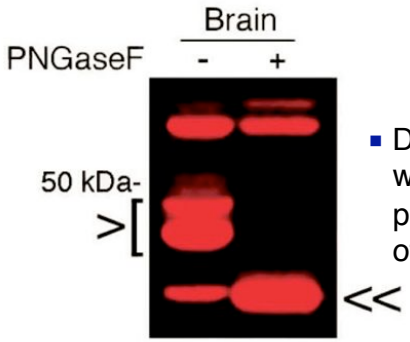
Applications of ABPP

Towards reversible inhibitors of enzymes in complex proteomes

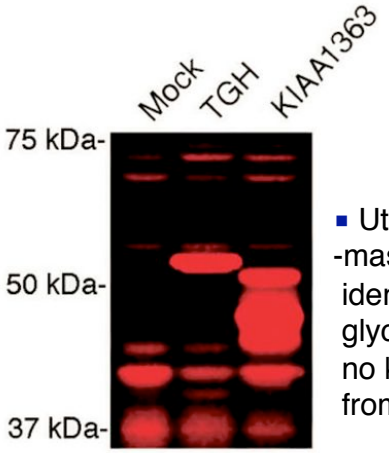
- A longstanding collaboration with the Boger laboratory has yielded many FAAH inhibitors



- FAAH knockout mice were used to show that the 50 kDa proteins in brain and heart tissue were distinct enzymes



- Deglycosylation of the 45 and 50 kDa proteins with similar probe affinities yielded a single 40 kDa protein, indicating different glycosylation states of one enzyme



- Utilization of FP-biotin and avidin chromatography -mass spectrometry, the 60 kDa enzyme was identified as triacylglycerol hydrolase (TGH), and the glycosylated protein as KIAA1363, an enzyme with no known substrates. Expression of these proteins from cDNA confirmed their identities

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Applications of ABPP

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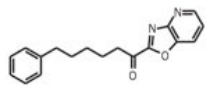
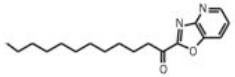
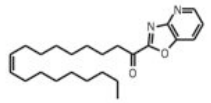
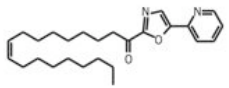
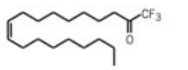
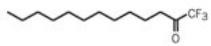
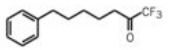
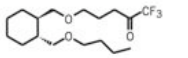
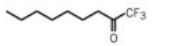
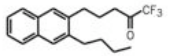
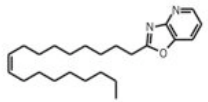
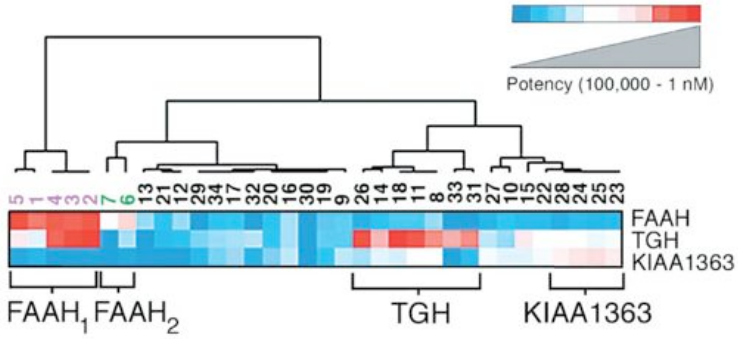
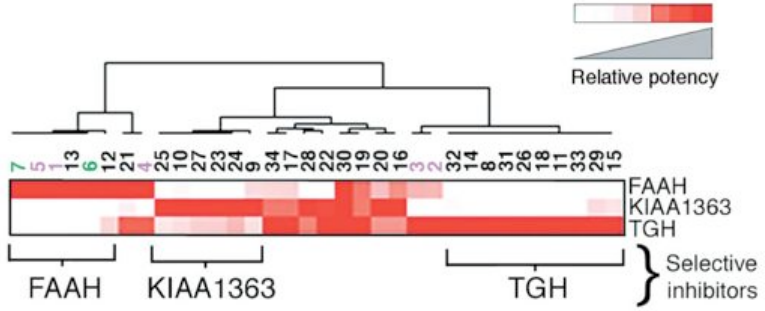
		60-kDa brain enzyme (FAAH)	45- and 50-kDa brain enzyme (KIAA1363)	60-kDa heart enzyme (TGH)
4		1.1 (0.72–1.7)	>100,000	1.4 (1.1–1.9)
5		0.81 (0.56–1.1)	50,000 (21,000–120,000)	83 (69–100)
6		44 (31–62)	62,000 (23,000–160,000)	1,400 (880–2,400)
7		150 (84–280)	>100,000	>100,000
9		4,500 (3,100–6,500)	1,100 (730–1,500)	4,800 (1,900–12,000)
15		11,000 (6,200–21,000)	470 (320–690)	61 (47–79)
18		5,300 (3,300–8,300)	770 (570–1,100)	0.47 (0.10–2.2)
25		15,000 (8,100–28,000)	52 (38–71)	320 (230–450)
26		30,000 (19,000–48,000)	1,500 (1,300–1,700)	1.8 (1.0–3.1)
28		37,000 (16,000–84,000)	110 (86–150)	160 (86–310)
30		>100,000	>100,000	>100,000





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