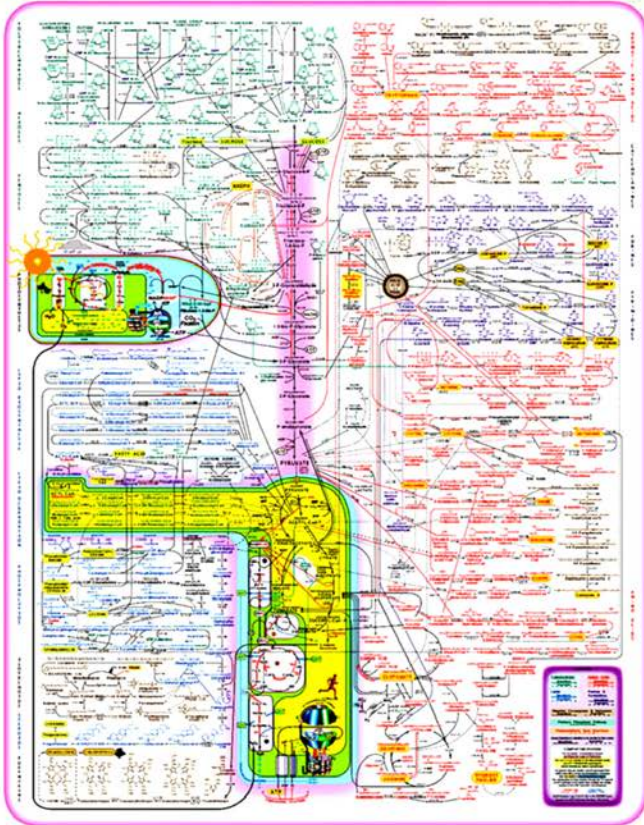


Activity-Based Proteomics – Protein and Ligand Discovery on a Global Scale

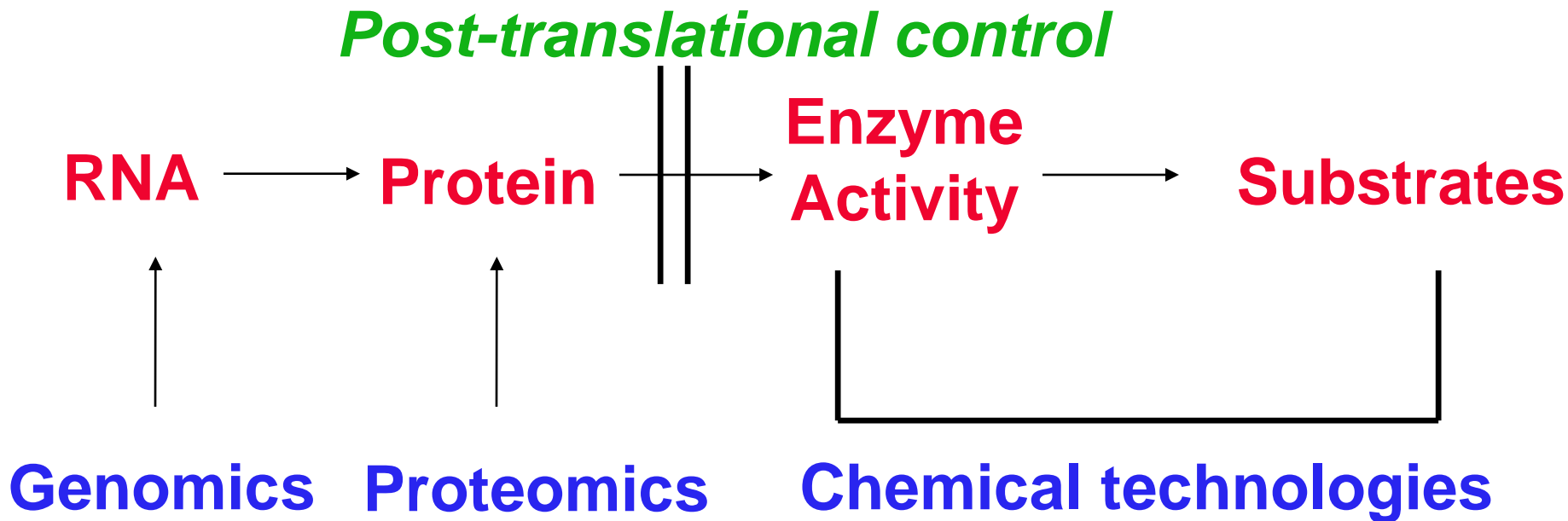
Benjamin F. Cravatt
Department of Molecular Medicine
The Skaggs Institute for Chemical Biology
The Scripps Research Institute

Current State of Understanding of Biochemical Pathways in Mammalian Cells



*Cell metabolism
(perception)*

Candidate Profiling Strategies for Mapping Biochemical Pathways



Overview

- **Activity-based Protein Profiling (ABPP) – Original Concepts and Technology**
- **Extending ABPP – Mapping the Ligandability of the Human Proteome**

Overview

- **Activity-based Protein Profiling (ABPP) – Original Concepts and Technology**
- Extending ABPP – Mapping the Ligandability of the Human Proteome

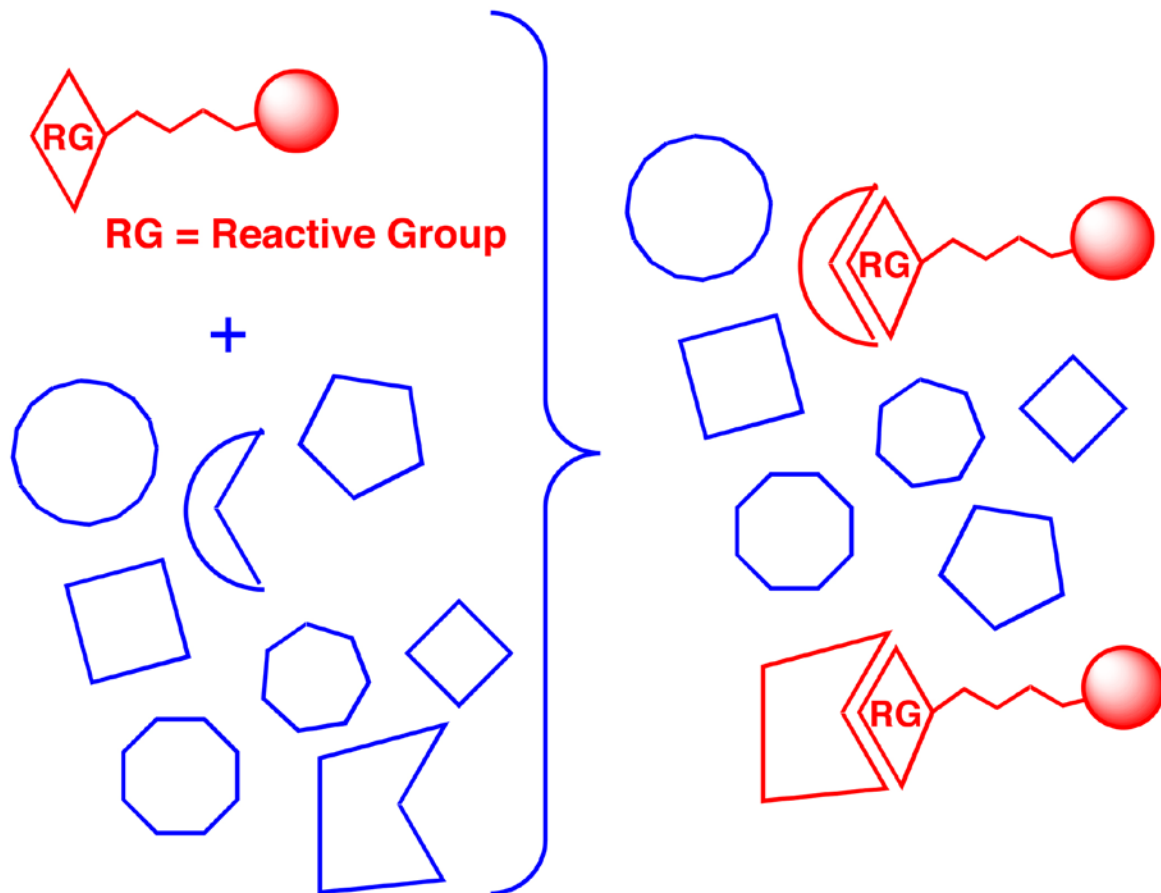
Chemical Probes for Activity-Based Profiling

• Activity-based probes should:

1) Bind and label **many enzymes** in proteomes

2) Labeling should be **activity-dependent**

3) Possess a **reporter tag** for detection/identification



Representative Enzyme Classes Addressed by Activity-Based Protein Profiling (ABPP)

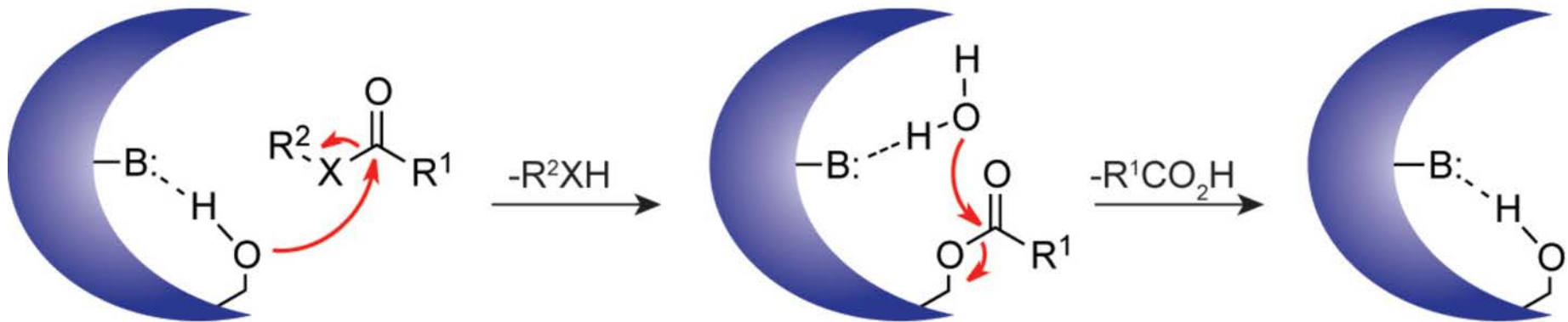
- **Serine hydrolases** (Cravatt et al)
- **Cysteine proteases** (Bogyo et al)
- **Histone deacetylases** (Cravatt et al)
- **Kinases, Phosphatases** (Activx, Taunton, Zhang)
- **Metalloproteases** (Cravatt et al, Yao et al)
- **Glycosidases** (Overkleeft, et al)
- **Cytochrome P450s** (Cravatt et al)

Representative Enzyme Classes Addressed by Activity-Based Protein Profiling (ABPP)

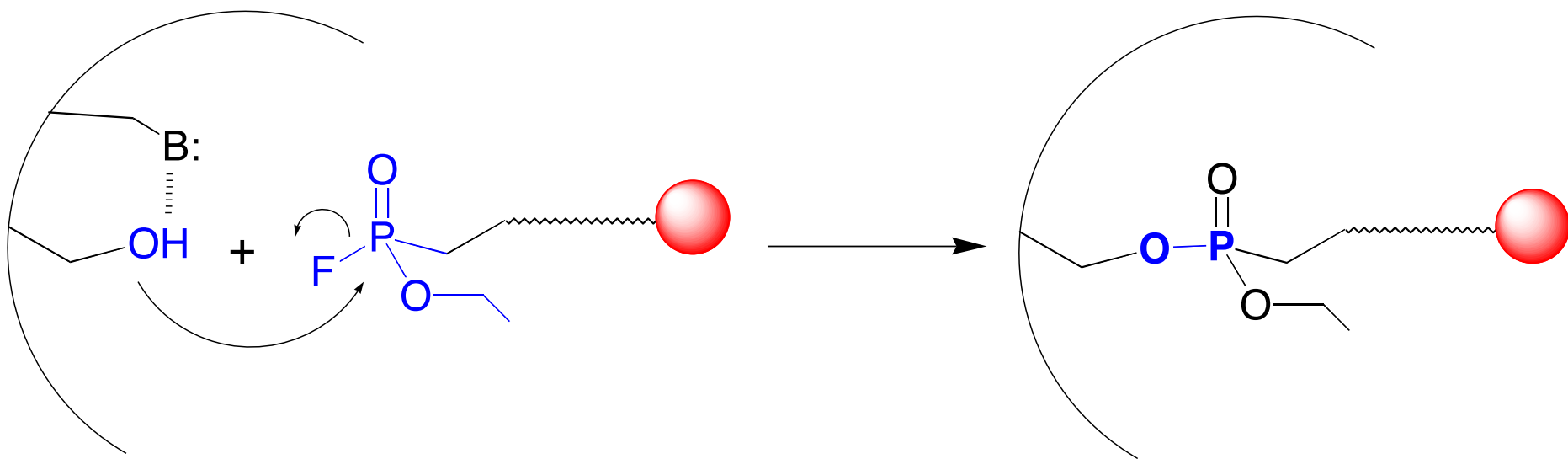
- **Serine hydrolases** (Cravatt et al)
- Cysteine proteases (Bogyo et al)
- Histone deacetylases (Cravatt et al)
- Kinases, Phosphatases (Activx, Taunton, Zhang)
- Metalloproteases (Cravatt et al, Yao et al)
- Aspartyl proteases (Li, et al)
- Cytochrome P450s (Cravatt et al)

Serine Hydrolases – A Large and Diverse Enzyme Class

- ~1-2% of all eukaryotic and prokaryotic proteomes
 - proteases, lipases, esterases, transacylases, amidases

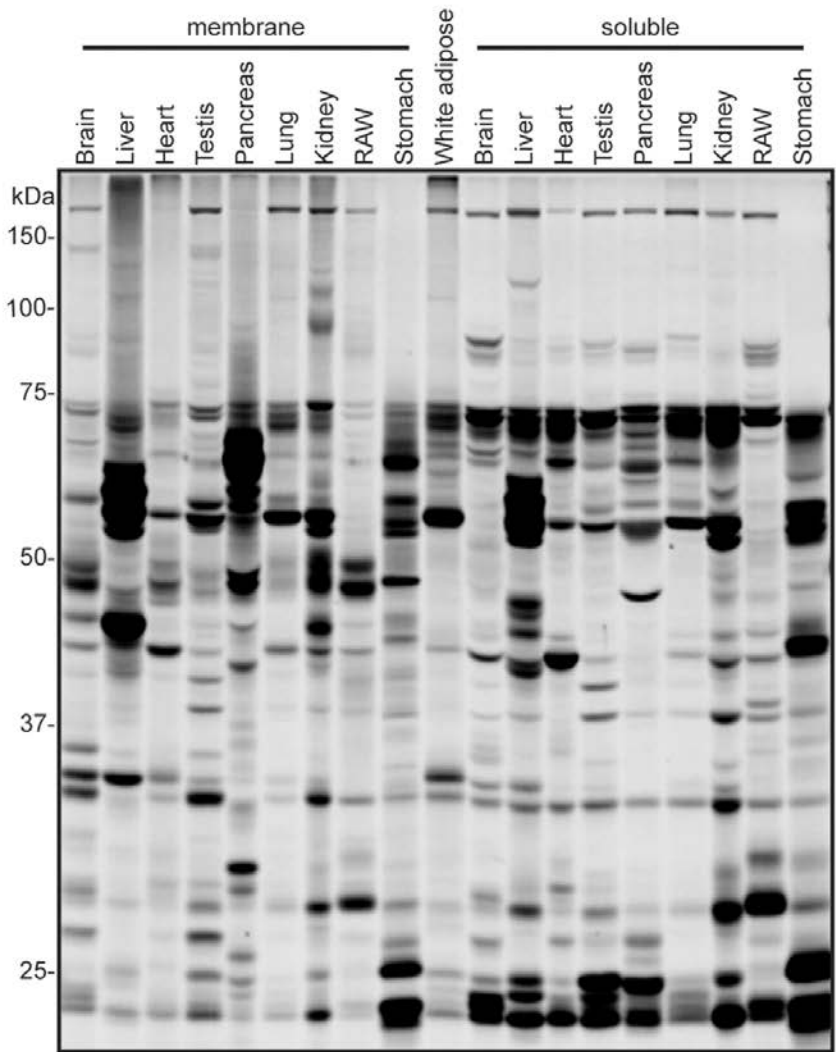
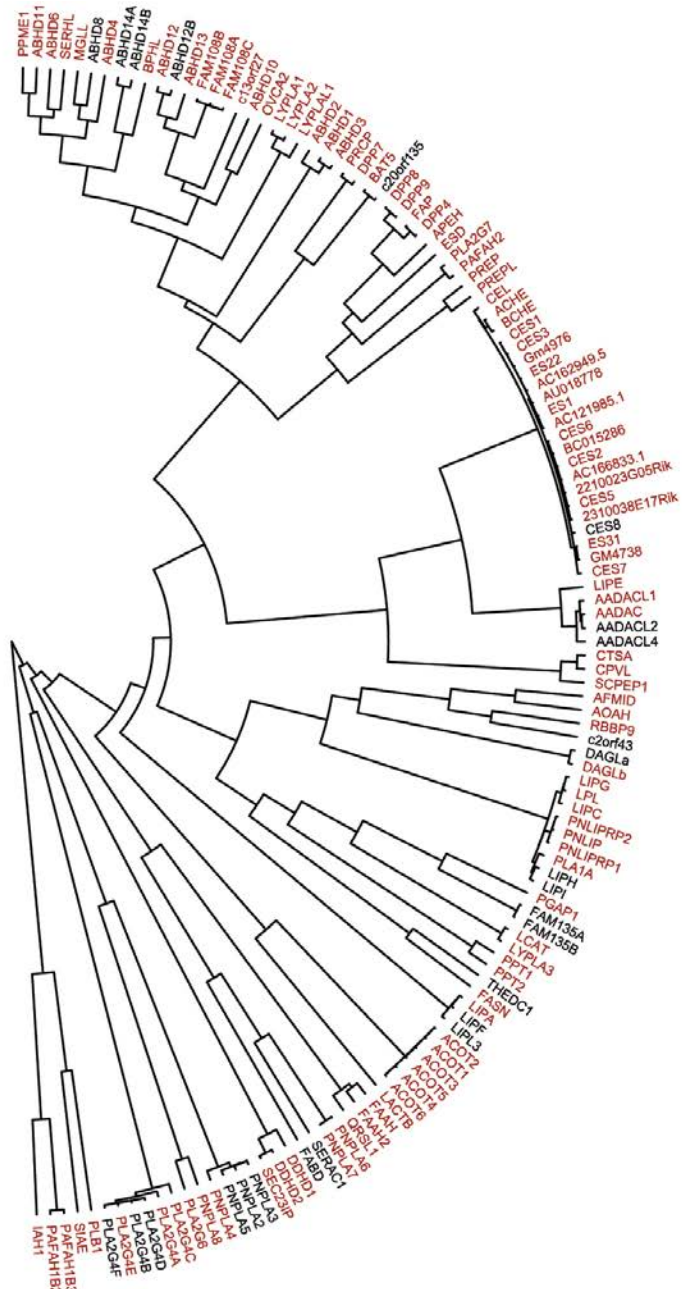


Fluorophosphonates as General Activity-Based Profiling Probes for Serine Hydrolases

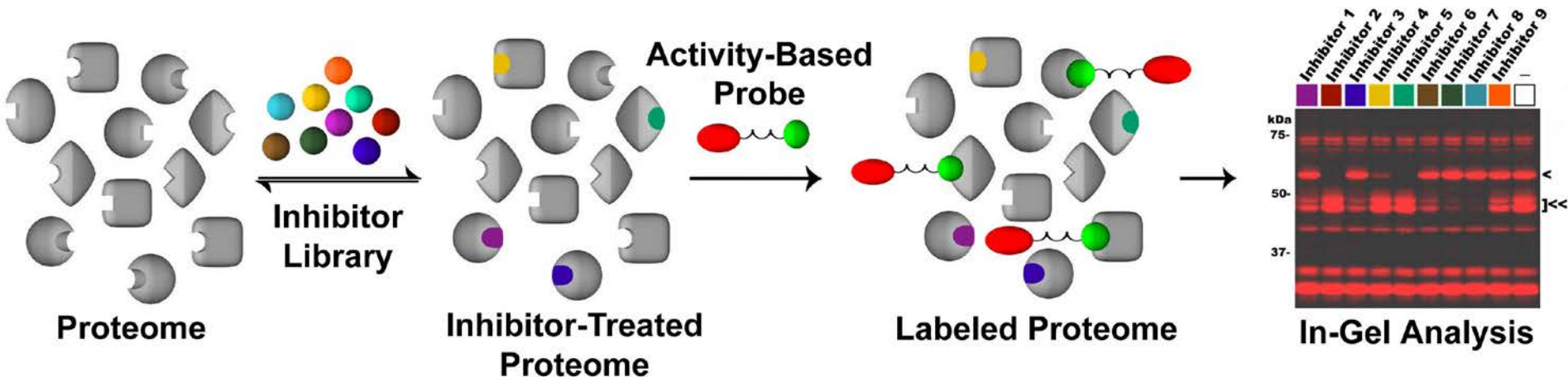


 - **Fluorophore** - detection (in-gel)
Biotin - enrichment

ABPP Coverage of Mammalian Serine Hydrolases



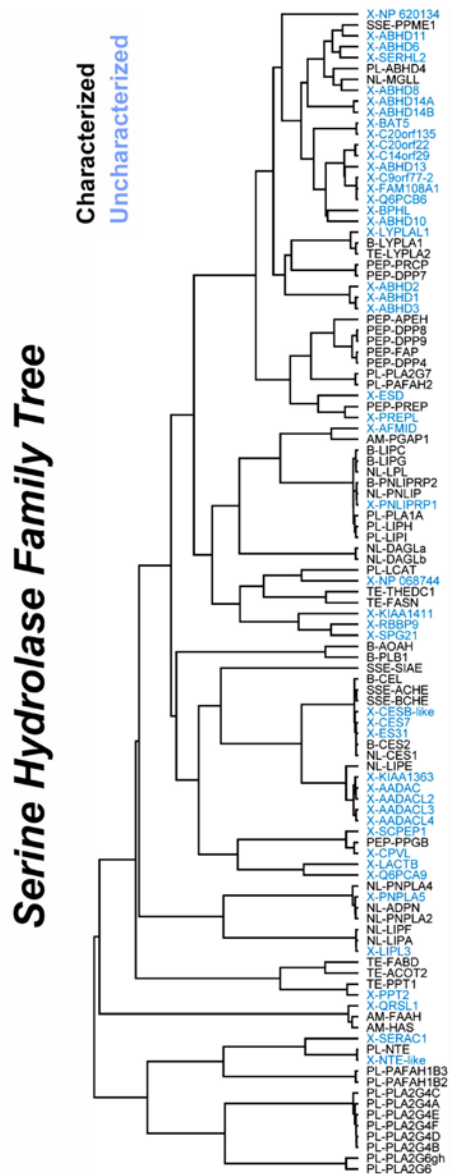
Inhibitor Discovery by Competitive Activity-Based Protein Profiling



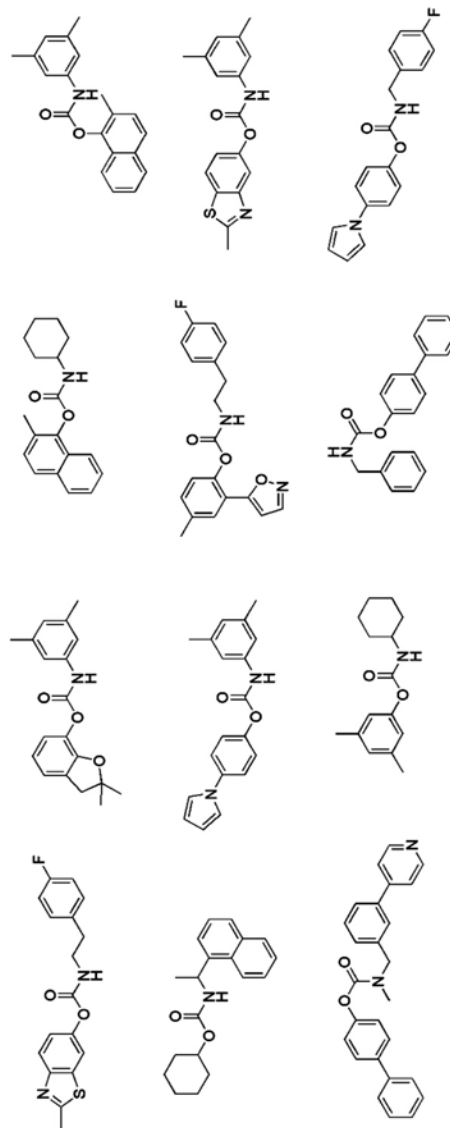
Advantages:

- No enzyme purification required
- No substrate assay required
- Evaluates both inhibitor *potency* AND *selectivity*

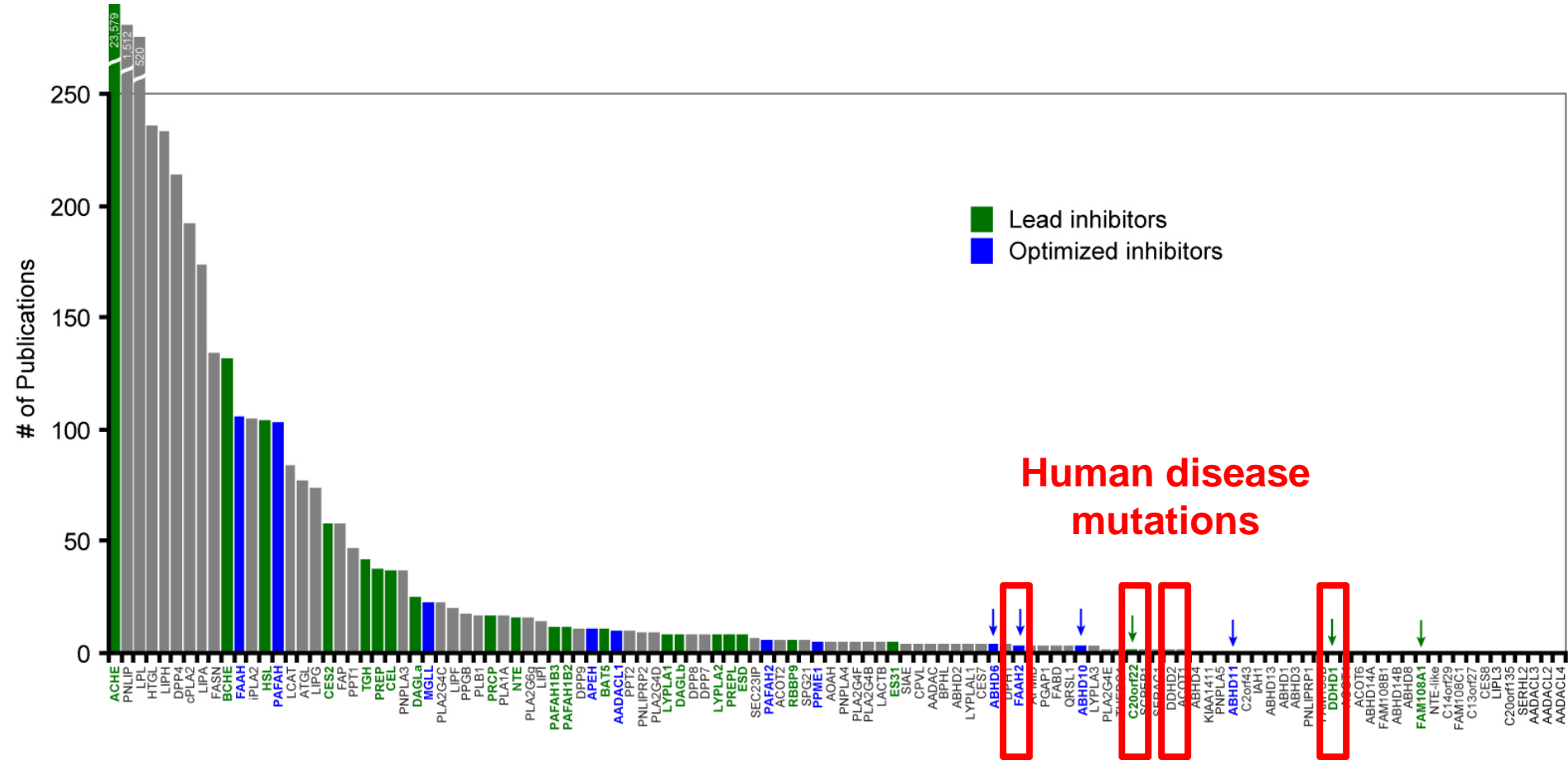
Systematic Discovery of Serine Hydrolase Inhibitors by Competitive ABPP



X



Toward a Complete Pharmacology For the Serine Hydrolase Superfamily



Integrating ABPP with Human Genetics to Map **Orphan Disease Mechanisms**



ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC

Jacqueline L. Blankman^{a,b}, Jonathan Z. Long^{a,b}, Sunia A. Trauger^c, Gary Siuzdak^c, and Benjamin F. Cravatt^{a,b,1}

^aThe Skaggs Institute for Chemical Biology and Departments of ^bChemical Physiology and ^cMolecular Biology, The Scripps Research Institute, La Jolla, CA 92037



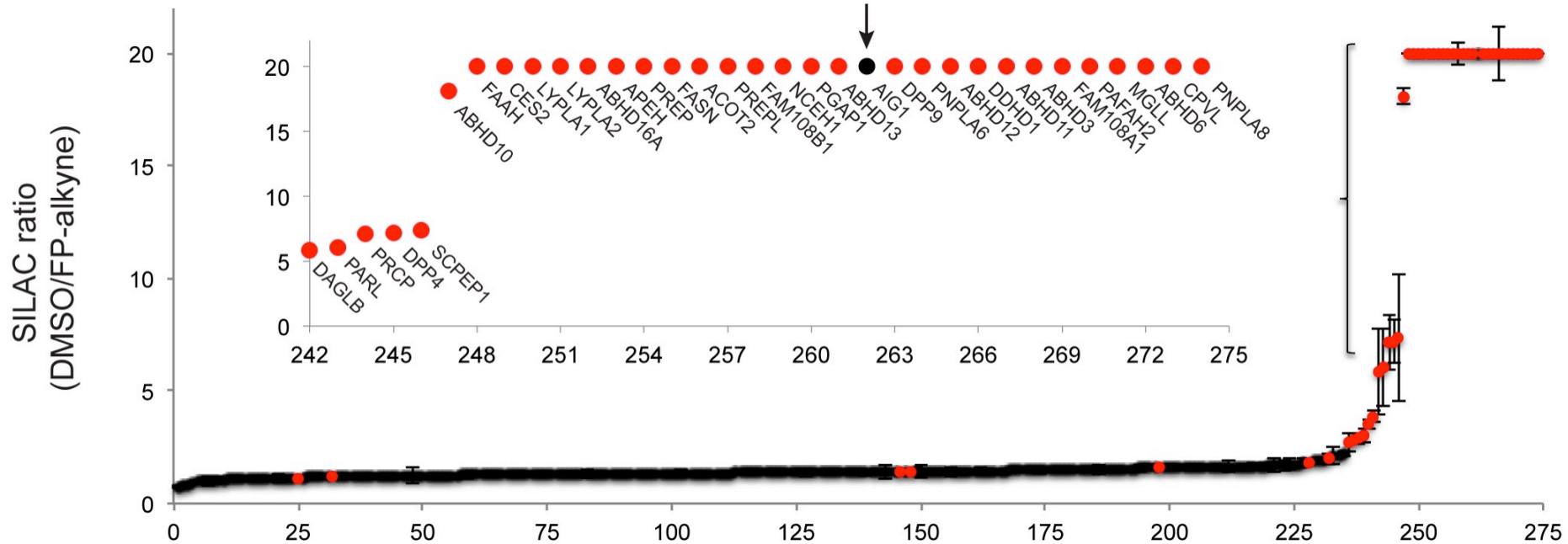
The hereditary spastic paraplegia-related enzyme DDHD2 is a principal brain triglyceride lipase

Jordon M. Inloes^{a,b,1}, Ku-Lung Hsu^{a,b,1}, Melissa M. Dix^{a,b}, Andreu Viader^{a,b}, Kim Masuda^{a,b}, Thais Takei^{a,b}, Malcolm R. Wood^c, and Benjamin F. Cravatt^{a,b,2}

^aThe Skaggs Institute for Chemical Biology and Departments of ^bChemical Physiology and ^cMolecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Excavating Cases of Convergent/Parallel Evolution of **Unprecedented Hydrolase Activities** by ABPP

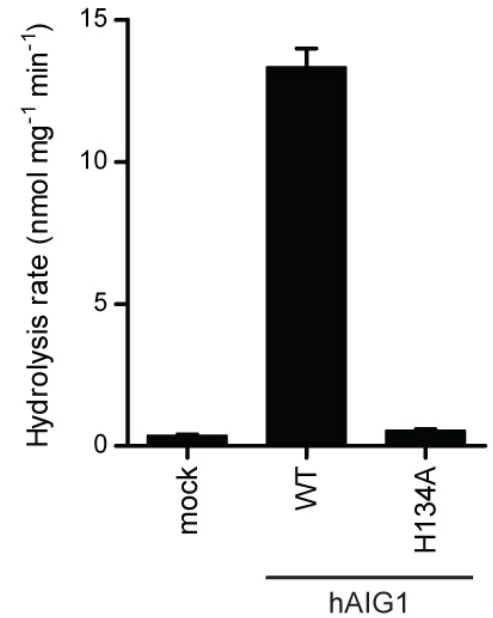
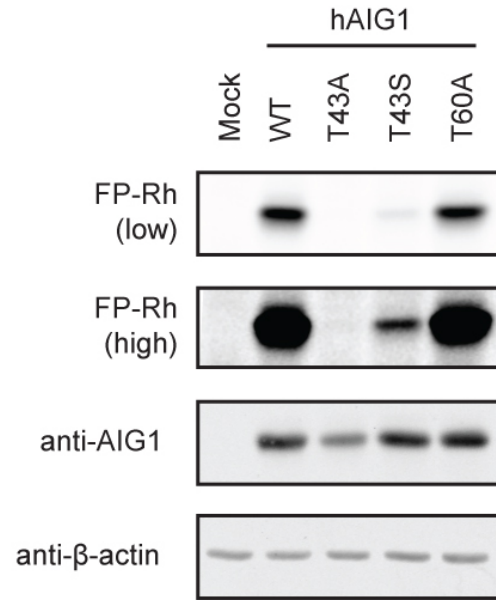
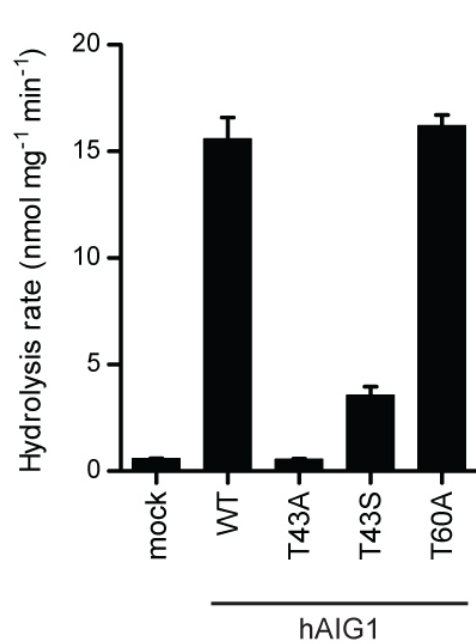
If FP reactivity marks hydrolase activity...



AIG1 (and ADTRP) are Multi-Pass Transmembrane Proteins of Poorly Characterized Function

<i>H. sapiens</i> AIG1	1	--MALVPCQ---VLRMAILLSYCSILCNKYKAIEMPSHOT-----YGGSWKFL	T	FIDLVIQAVFFGICVL
<i>M. musculus</i> AIG1		--MALVPCQ---VLRVAILLSYCSILCNKYKAIEMPSHOT-----YGGSWKFL	T	FIDLVIQAVFFGICVL
<i>D. rerio</i> AIG1	[51]	RKMALIPSQ---ILRVAILLSYFSILCNKYKAIDMPAHQT-----YGGSWKFL	T	FIDLVIQAVFFGVCVL
<i>A. gambiae</i> AGAP008626-PA	[14]	VYGALRTL---HFIVAVQFSY-GIYYDFTYVHFPPGMLRPGGEFGGKLYL	T	VWDAILQAVYFTICLL
<i>S. cerevisiae</i> YHR140W		MMSCLVPTRFTLTLNTACLLTSTWGFVRATSVVLPPLSK-----AGHKQFL	T	IISIIATIINNAVNIS
<i>H. sapiens</i> ADTRP		---MTKTST---CIYHFLVLSWYTFLNYYISQEGKDEVKPKILANGARWKYM	T	LLNLLLQTIIFYGVTCL
<i>D. rerio</i> LOC100537455	[10]	TASMAGVMK---TFCHIAAFSWYAFIIQCIYAKDVSDLPSGIFVYGGPWKYL	T	FLNLVLQMVFFGLASV
				* * *
<i>H. sapiens</i> AIG1	60	TDLSSLLTRGSGNQEQERQLKKLISLRDWMLAVLAFVGVFVAVFWIIYAYDREMIY---PKLLDNFIPG		
<i>M. musculus</i> AIG1		TDLSSLLTRGSGNQEQERQLRKLISLRDWTLAVLAFVGVFVAVFWTIYAYDREMIY---PRLLDNFIPG		
<i>D. rerio</i> AIG1		TDLSSLLTKGSASMEQERQLRKLIGLRDWMMAVLAFVGVFVVTMFWTLYLYDRDLIY---PRLLDNFIPQ		
<i>A. gambiae</i> AGAP008626-PA		NDFIGT-----NEVAPKRMPILIRKLDYMLAAFAFPVALNVGVTFWTLMAIDRELVF---PKALDAVFPG		
<i>S. cerevisiae</i> YHR140W		NYIIQR-----NNKMNLETKKKSDFISRHVTLPVSLVLESIVATVYWPLRRLFVNLIHMGVESTAKTPFPM		
<i>H. sapiens</i> ADTRP		DDVLKR-TKGG-----KDIKFLTAFRDLLFTTLAFPVSTFVFLAFWILFLYNRDLIY---PKVLDTVIPV		
<i>D. rerio</i> LOC100537455		ND-LQP-VGKG-----SKMSLLCLCKDLLFSVVFVPGMFVLLFWLIFAYDRQLVY---PASIDNFFPP		
				* * **
<i>H. sapiens</i> AIG1	128	WLNHGM H TTVLVPFILIEMRTSHHQYP---SRSSGLTAICTFSVGYILWVCWVHHVTGMWVYPF-LEHIG		
<i>M. musculus</i> AIG1		WLNHGM H TTVLVPFILIEMRTSHHQYP---SRSSGLAAICTFSVGYILWVCWIHHVTGMWVYPF-LEHIG		
<i>D. rerio</i> AIG1		WLNHGM H TTVLVPFIIIEMRTTHHRYP---SRPCGLLAVCAFAVGYYVWMCWVHSMTGWVYPL-LEHIG		
<i>A. gambiae</i> AGAP008626-PA		WLNHVM H TNIVIFMVIEICTSFRQYP---SRKAGLTGLGIFMASYLGWLHVVRHFGGIWVYPV-LDVLN		
<i>S. cerevisiae</i> YHR140W		TVDMAI H LYPILYLLADHYLSGSGTKFKLSNKHAWLIVTSLAFSYFQYLAFLIDAGQGQAYPYPLDVN		
<i>H. sapiens</i> ADTRP		WLNHAM H TFIFPITLAEVLRPHSYP---SKKTGLTLLAAASIAYISRILWLYFETGTWVYPV-FAKLS		
<i>D. rerio</i> LOC100537455		WLNHAM H TVVLPILFGEILLEPHVFP---KTKNGLAALGVVGLAYLGVVIWVYCTVGIWVYPL-LGLFS		

AIG1 (and ADTRP) are Founding Members of a New Class of **Transmembrane Thr-Hydrolases**



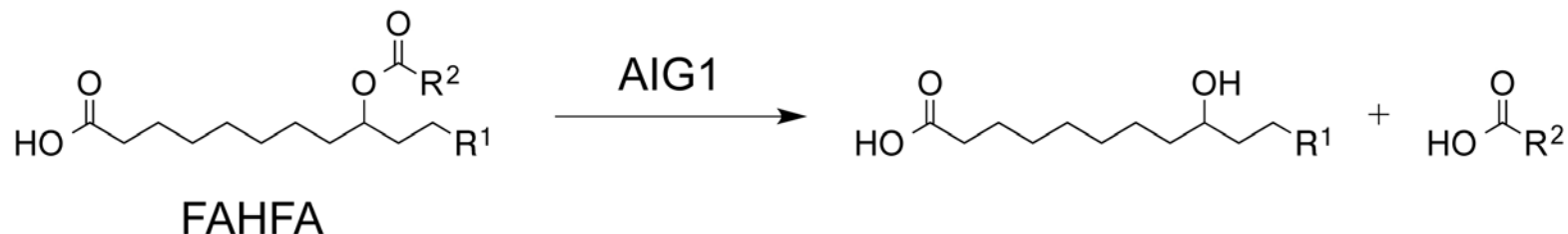
FAHFAs – A Class of Lipid Transmitters that Regulate Metabolic and Inflammatory Processes

Cell

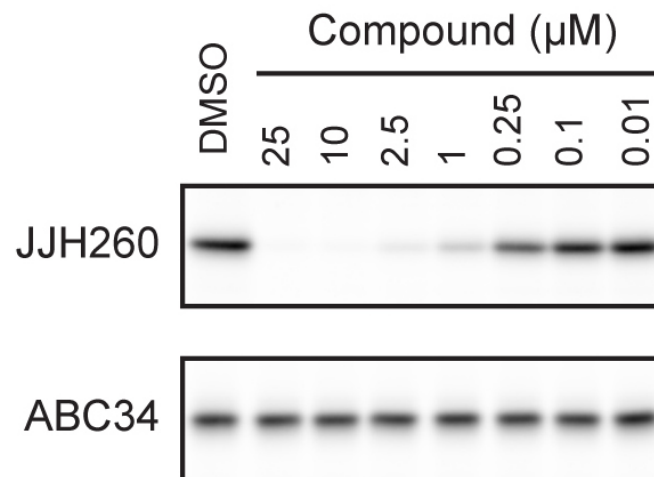
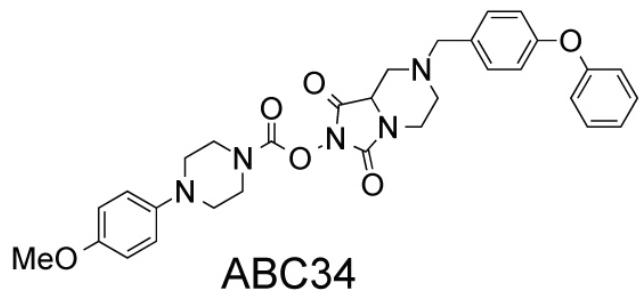
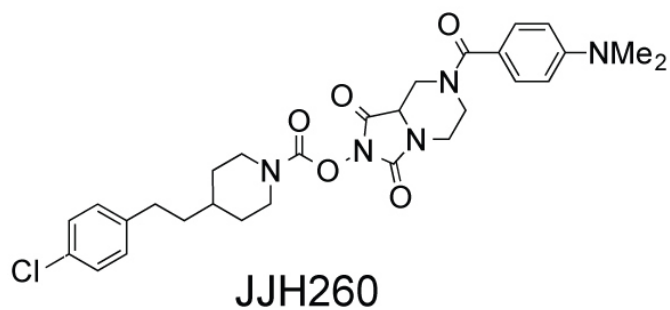
Discovery of a Class of Endogenous Mammalian Lipids with Anti-Diabetic and Anti-inflammatory Effects

Mark M. Yore,^{1,5} Ismail Syed,^{1,5} Pedro M. Moraes-Vieira,¹ Tejia Zhang,^{3,7} Mark A. Herman,¹ Edwin A. Homan,³ Rajesh T. Patel,² Jennifer Lee,¹ Shili Chen,^{3,7} Odile D. Peroni,¹ Abha S. Dhaneshwar,¹ Ann Hammarstedt,⁴ Ulf Smith,⁴ Timothy E. McGraw,² Alan Saghatelian,^{3,6,7,*} and Barbara B. Kahn^{1,6,*}

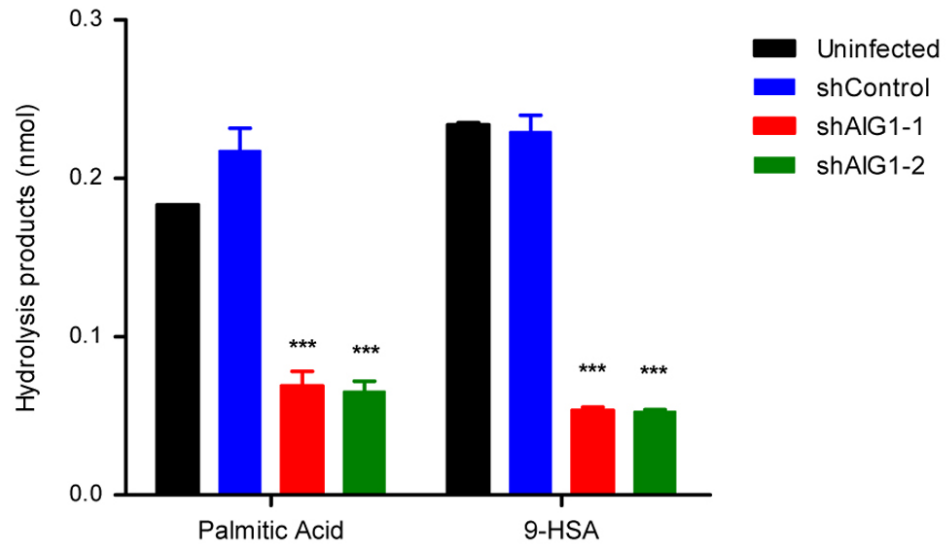
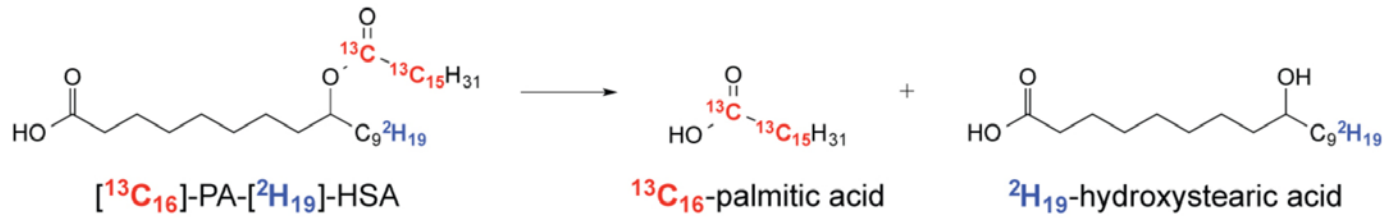
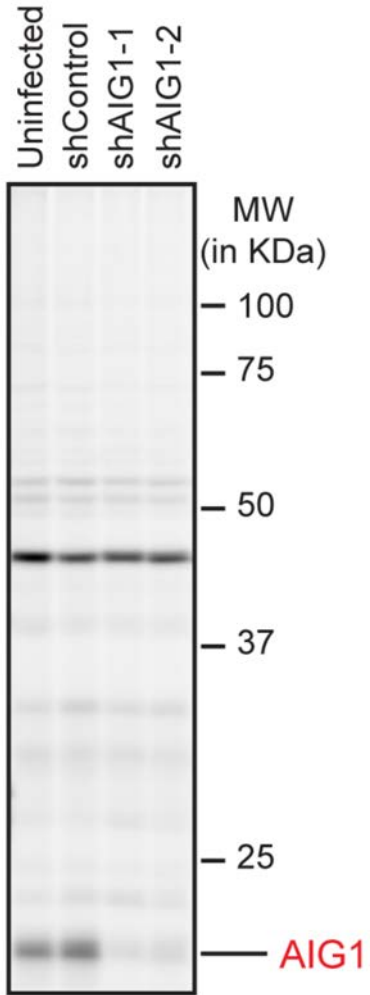
318 Cell 159, 318–332, October 9, 2014 ©2014 Elsevier Inc.



AIG1 Inhibitors Discovered by ABPP



AIG1 Regulates FAHFA Metabolism in Human Cells



Conclusions and Future Directions

- **AIG1 and ADTRP appear to represent a mechanistically unprecedented class of hydrolases**
 - **transmembrane Thr hydrolases**
- **Why such an unusual mechanism?**
 - **FAHFAs are unusual substrates**
- **Do AIG1 and ADTRP regulate FAHFAs in vivo?**
 - **potential relevance for treating metabolic disorders**

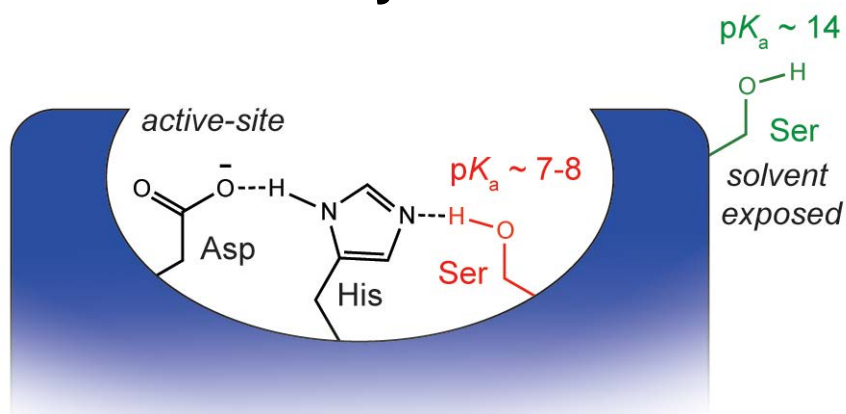
Chemical proteomics can assign functions to proteins that defy sequence- and structure-based predictions

Overview

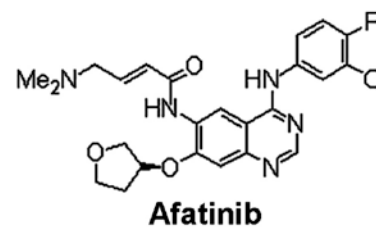
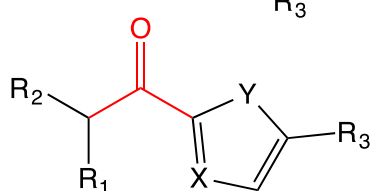
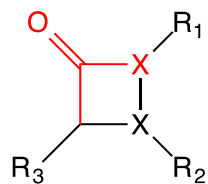
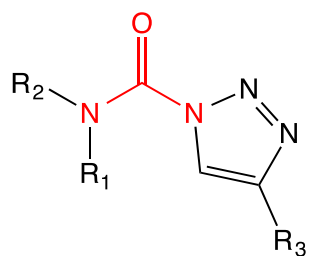
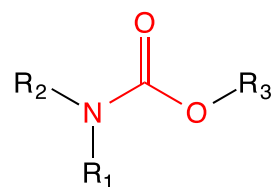
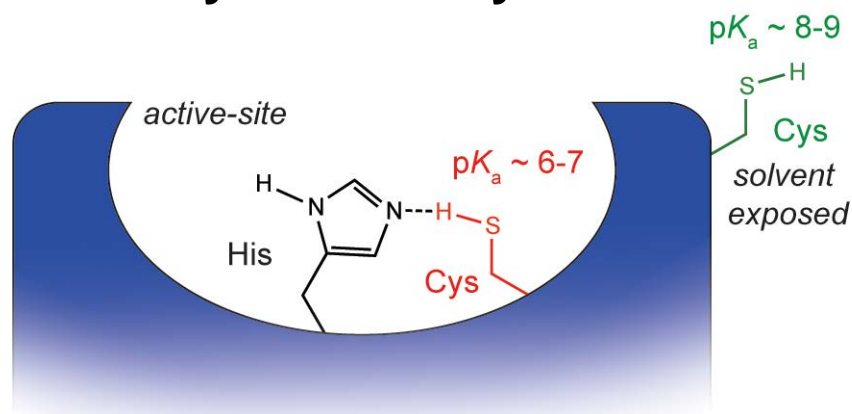
- Activity-based Protein Profiling (ABPP) – Original Concepts and Technology
- **Extending ABPP** – Mapping the Ligandability of the Human Proteome

Challenges and Opportunities for Design of Covalent Ligands that Target **Cysteine Residues**

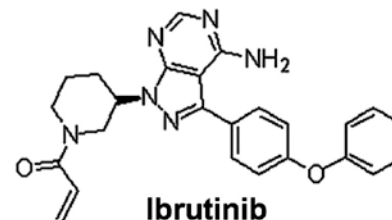
Serine hydrolase



Cysteine enzyme

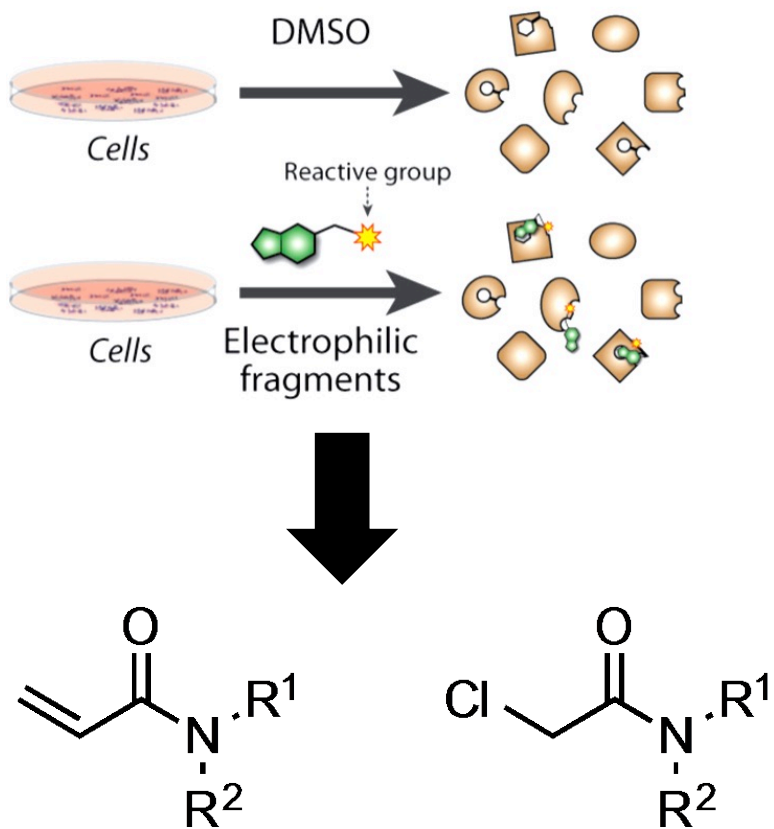


Afatinib

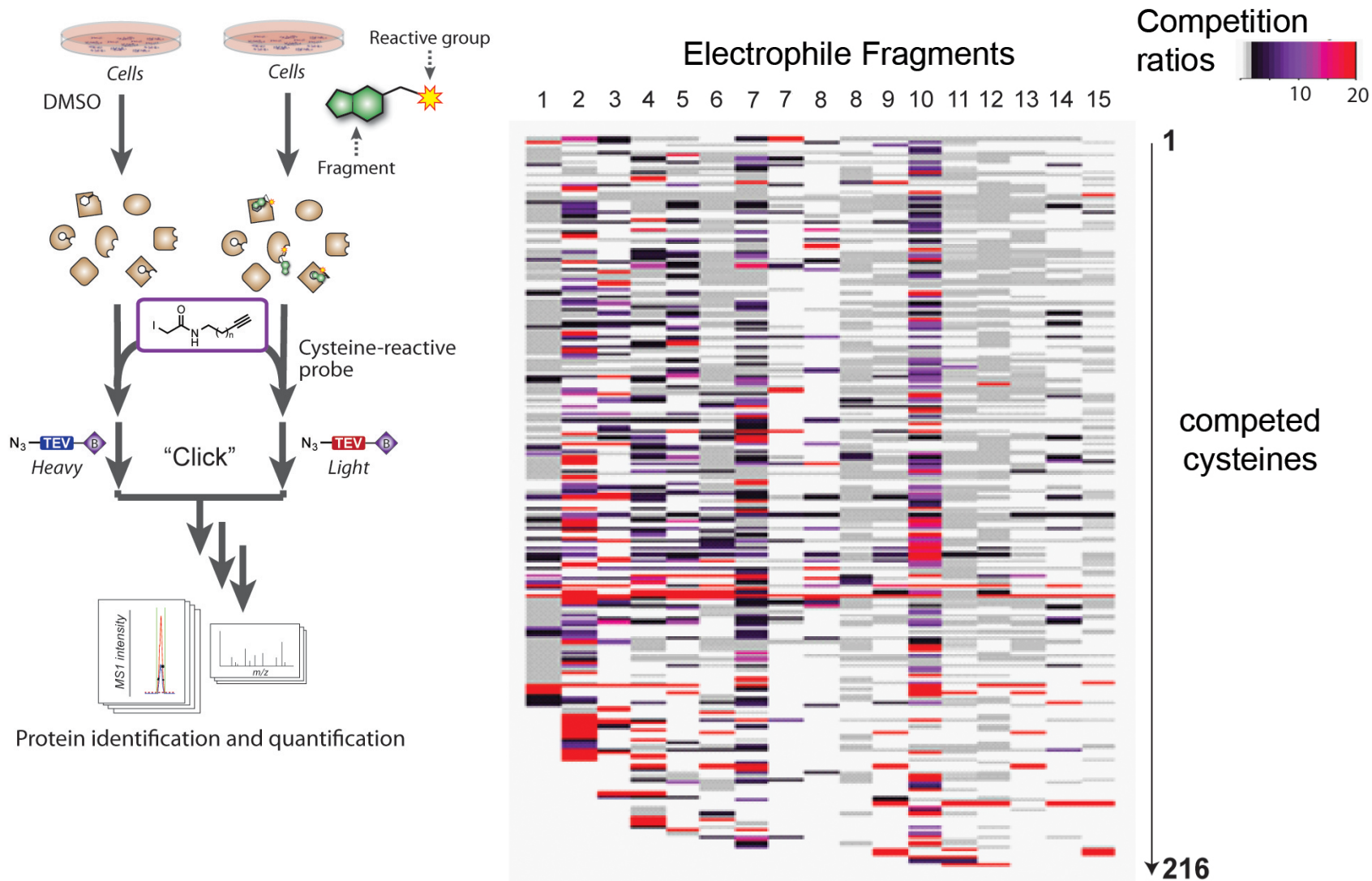


Ibrutinib

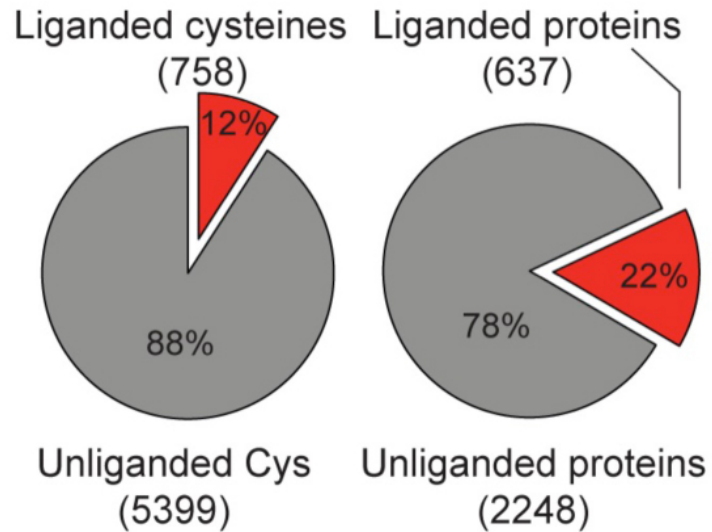
Proteome-Wide Covalent Ligand Discovery



Proteome-Wide Covalent Ligand Discovery



Proteome-Wide Covalent Ligand Discovery Accesses **New (Un)Druggable Space**



Initiator Caspases (CASP8 & CASP10) - Human Genetic Evidence for Key Roles in Immunology

letters to nature

.....

Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency

Hyung J. Chun^{*†}, Lixin Zheng^{*†}, Manzoor Ahmad^{*†}, Jin Wang^{*‡}, Christina K. Speirs^{*}, Richard M. Siegel^{*‡}, Janet K. Dale[§], Jennifer Puck^{||}, Joie Davis^{||}, Craig G. Hall[¶], Suzanne Skoda-Smith[¶], T. Prescott Atkinson[¶], Stephen E. Straus[§] & Michael J. Lenardo^{*}

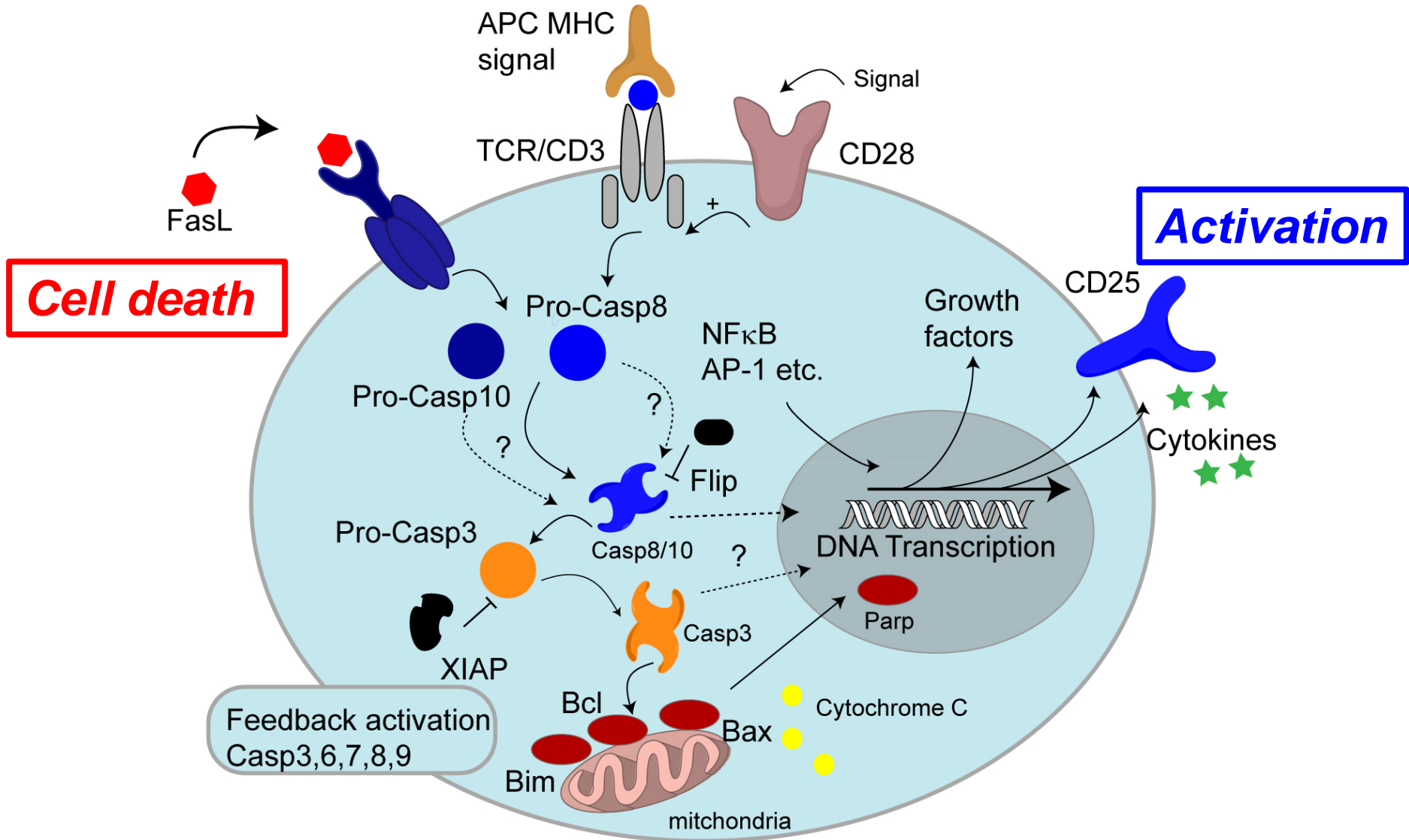
NATURE | VOL 419 | 26 SEPTEMBER 2002 | www.nature.com/nature

Cell, Vol. 98, 47–58, July 9, 1999, Copyright ©1999 by Cell Press

Inherited Human Caspase 10 Mutations Underlie Defective Lymphocyte and Dendritic Cell Apoptosis in Autoimmune Lymphoproliferative Syndrome Type II

Jin Wang,^{*} Lixin Zheng,^{*} Adrian Lobito,^{*} Francis Ka-Ming Chan,^{*} Janet Dale,[†] Michael Sneller,[‡] Xu Yao,^{||} Jennifer M. Puck,[§] Stephen E. Straus,[†] and Michael J. Lenardo^{*#}

Respective Roles of Caspase-8 and -10 in Human T Cell Biology



Initiator Caspases (CASP8 & CASP10) - Human Genetic Evidence for Key Roles in Immunology

letters to nature

.....
Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency

Hyung J. Chun^{*†}, Lixin Zheng^{*†}, Manzoor Ahmad^{*†}, Jin Wang^{*‡},
Christina K. Speirs^{*}, Richard M. Siegel^{*‡}, Janet K. Dale[§]
Jennifer Puck^{||}, Joie Davis^{||}, Craig G. Hall[¶], Suzanne Skoda-Smith[¶]
T. Prescott Atkinson[¶], Stephen E. Straus[§] & Michael J. Lenardo^{*}

PROBLEM: selective and drug-like inhibitors of caspases have proven difficult to generate

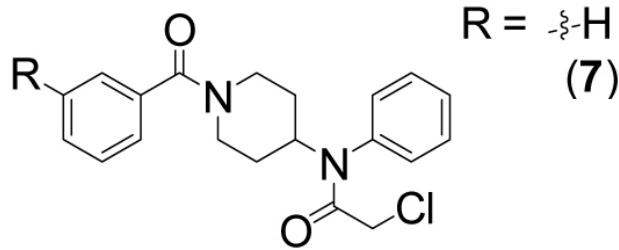
NATURE | VOL 419 | 26 SEPTEMBER 2002 | www.nature.com/nature

Cell, Vol. 98, 47–58, July 9, 1999, Copyright ©1999 by Cell Press

Inherited Human Caspase 10 Mutations Underlie Defective Lymphocyte and Dendritic Cell Apoptosis in Autoimmune Lymphoproliferative Syndrome Type II

Jin Wang,^{*} Lixin Zheng,^{*} Adrian Lobito,^{*}
Francis Ka-Ming Chan,^{*} Janet Dale,[†]
Michael Sneller,[‡] Xu Yao,^{||}
Jennifer M. Puck,[§] Stephen E. Straus,[†]
and Michael J. Lenardo^{*#}

Covalent Ligands that Target the **Pro (Inactive)** Forms of Caspases



CASP8 (C360)

Ramos



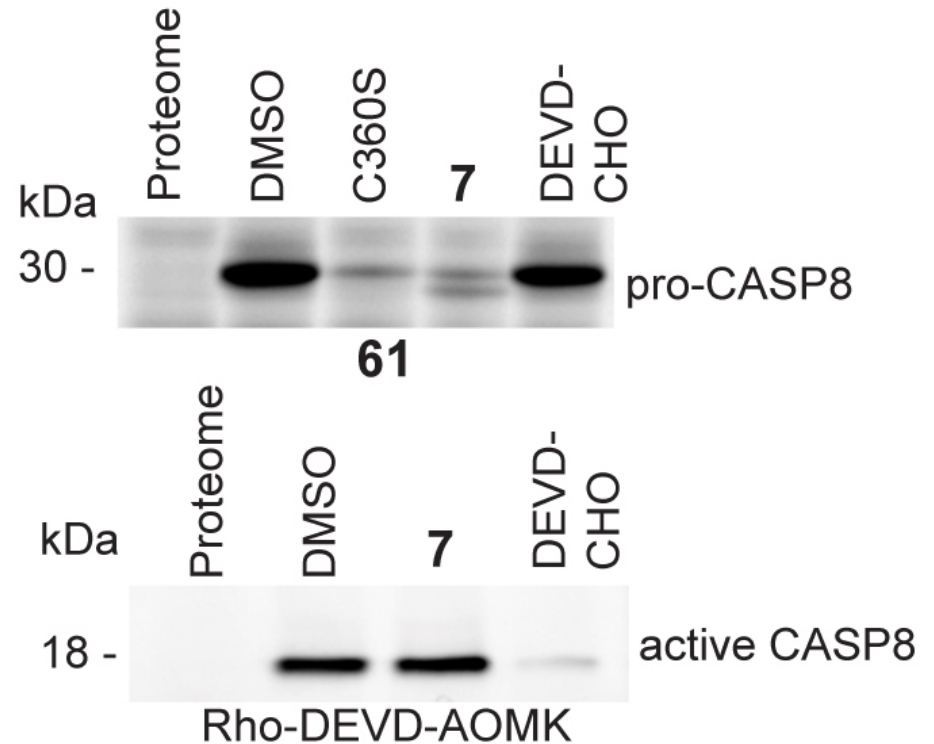
>20
7

Jurkat

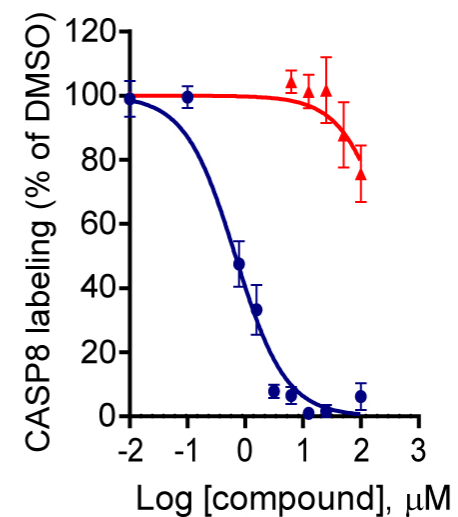
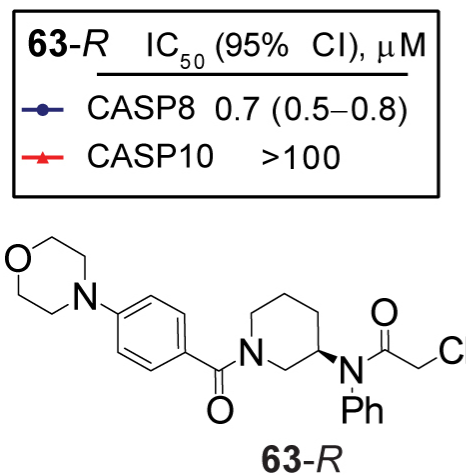
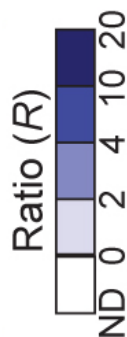
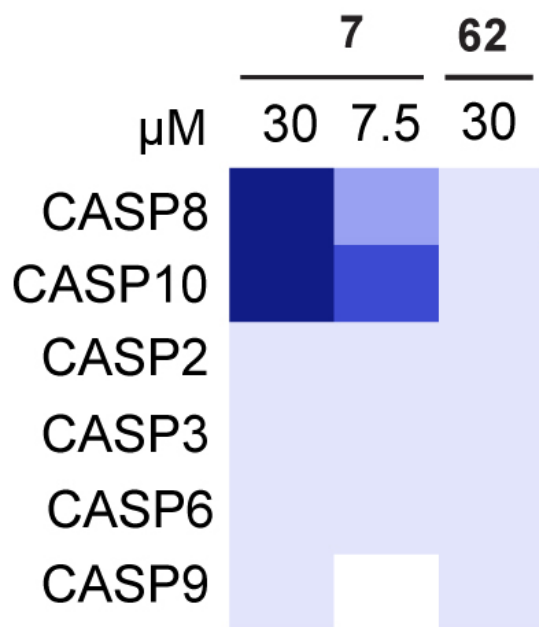


>20
7

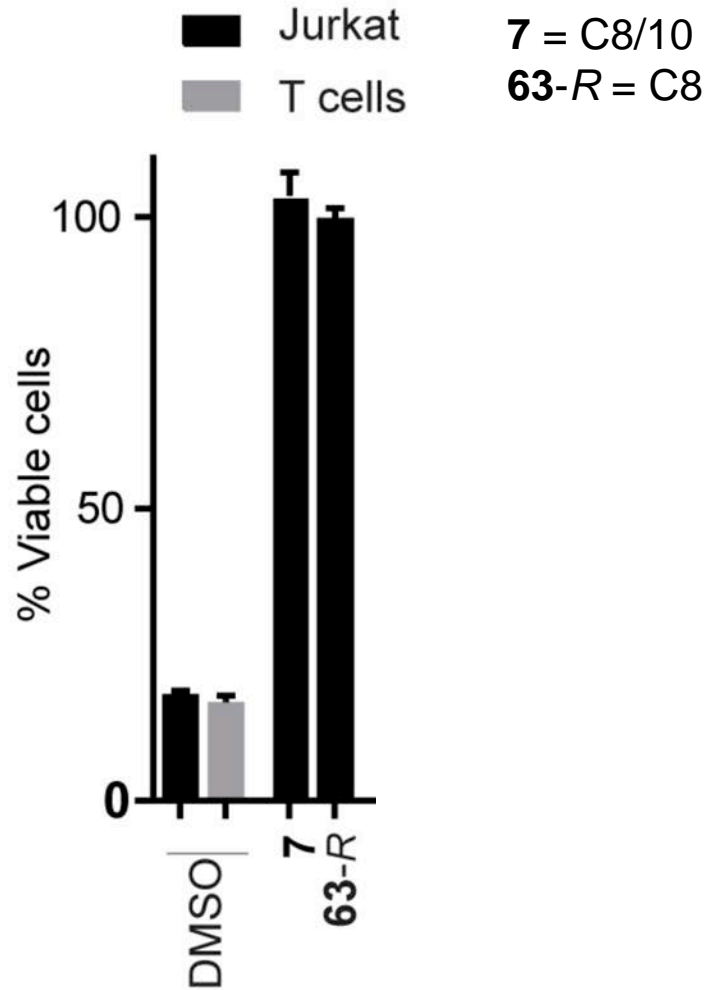
Ratio (R)
Fragment



Dual Pro-Caspase-8/10 and Selective Pro-Caspase-8 Ligands



FAS Ligand-Mediated Apoptosis in Human T Cells Requires **Both Caspase-8 and -10**



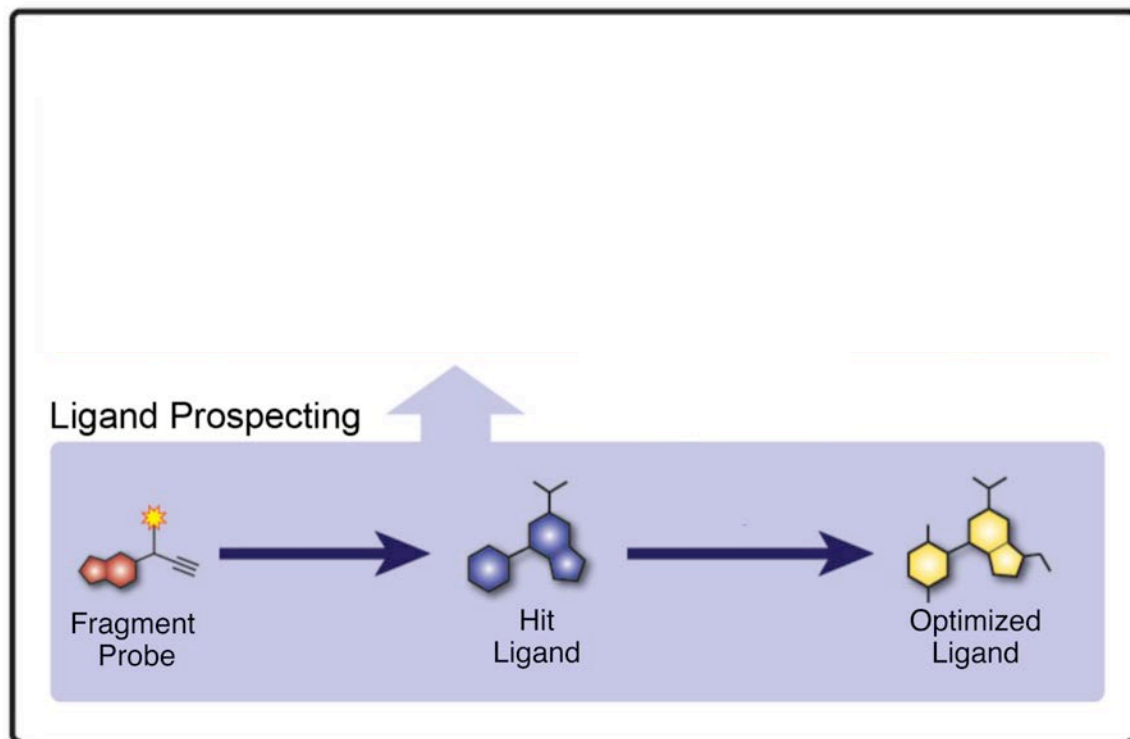
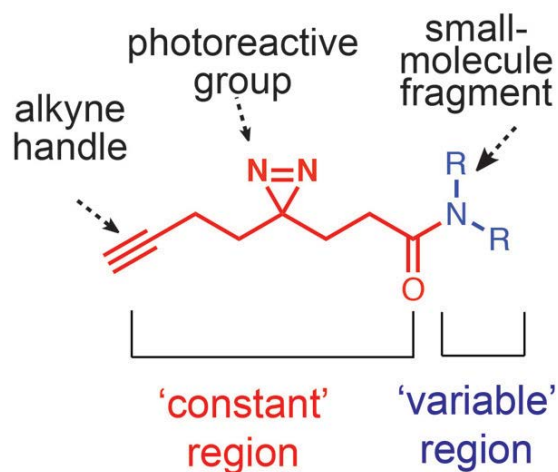
Conclusions and Future Directions

- Chemical proteomics reveals a **rich content of ligandable cysteines** in the human proteome
- Combining cysteine ligandability maps **with human genetics** identifies:
 - Novel way to drug initiator caspases important for human immunology
 - Sites of action for the immunosuppressive drug Tecfidera (dimethylfumarate) – Blewett *et al.* 2016
- Future Directions:
 - **Ligand optimization**
 - **Extension to other (non)-nucleophilic residues**

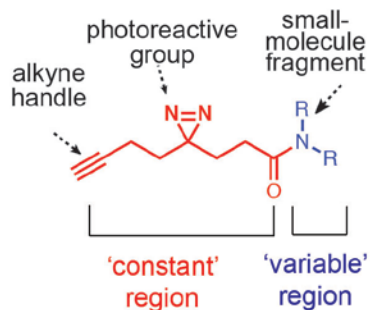
Conclusions and Future Directions

- Chemical proteomics reveals a rich content of ligandable cysteines in the human proteome
- Combining cysteine ligandability maps with human genetics identifies:
 - Novel way to drug initiator caspases important for human immunology
 - Sites of action for the immunosuppressive drug Tecfidera (dimethylfumarate) – Blewett *et al.* 2016
- Future Directions:
 - Ligand optimization
 - **Extension to other (non)-nucleophilic residues**

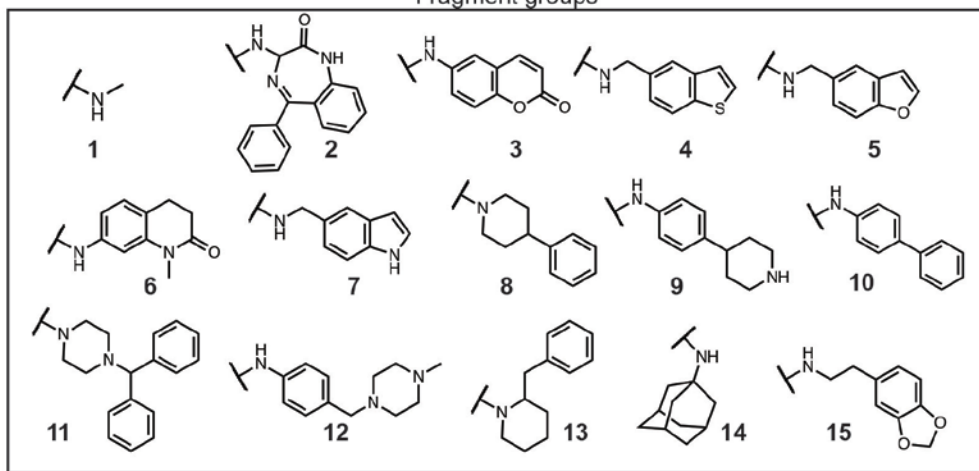
Proteome-Wide **Non-Covalent** Ligand Discovery with *Fully Functionalized Fragment Probes* (**Chris Parker**)



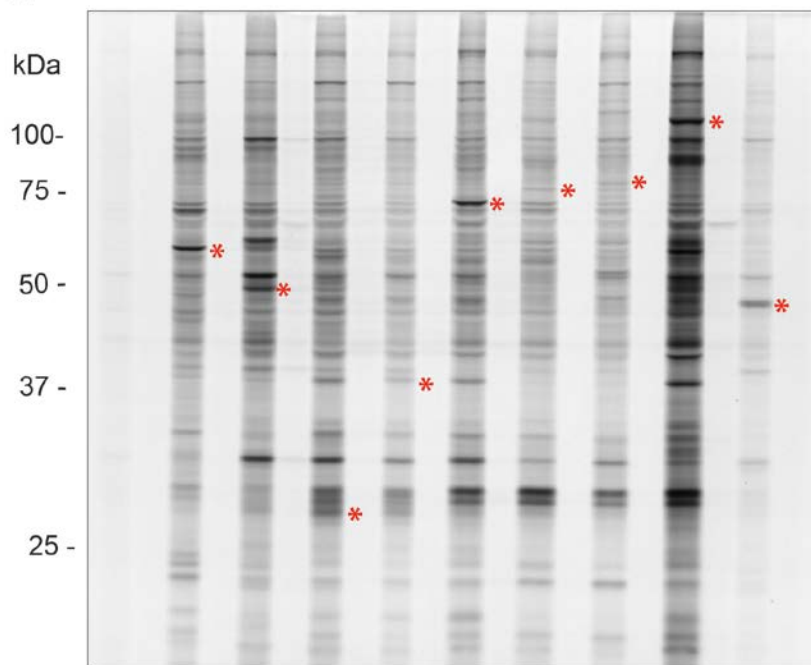
Proteome-Wide **Non-Covalent** Ligand Discovery with Fully Functionalized Fragment Probes



Fragment groups

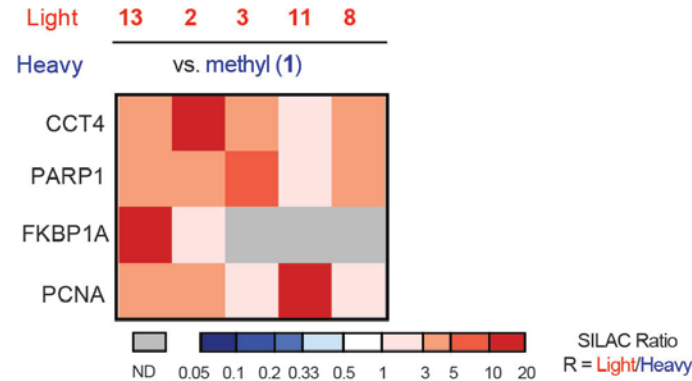
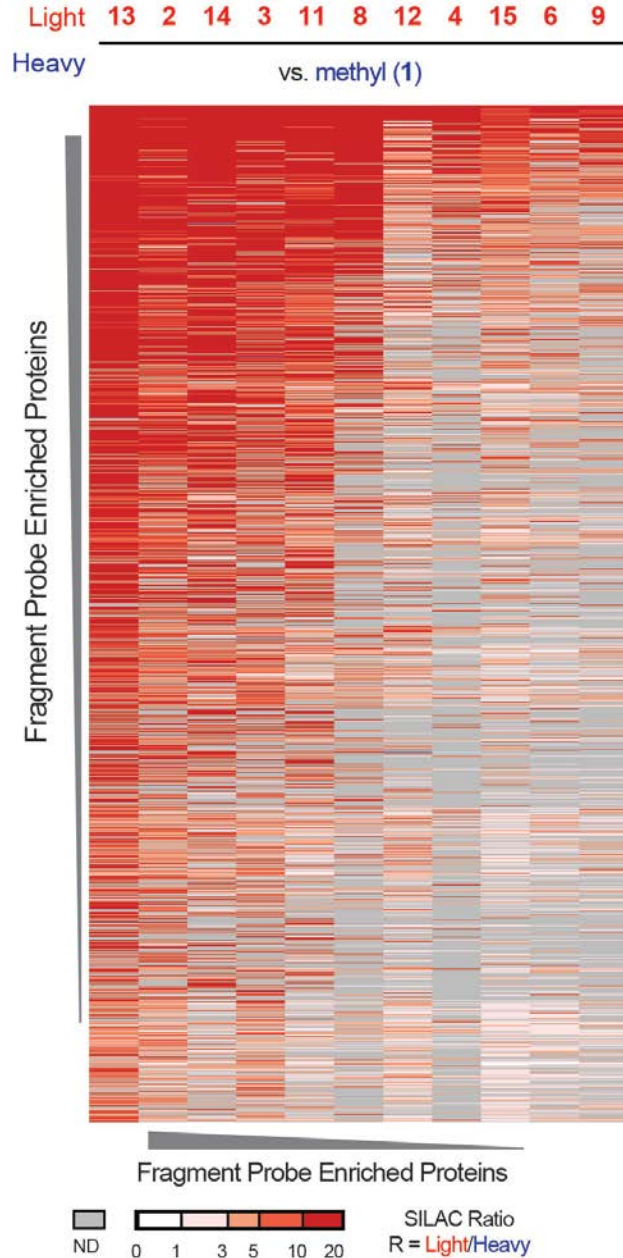


probe (20 μ M)	1	2	3	4	5	8	13	14	11	6		
UV	+	-	+	-	+	-	+	-	+	-	+	-



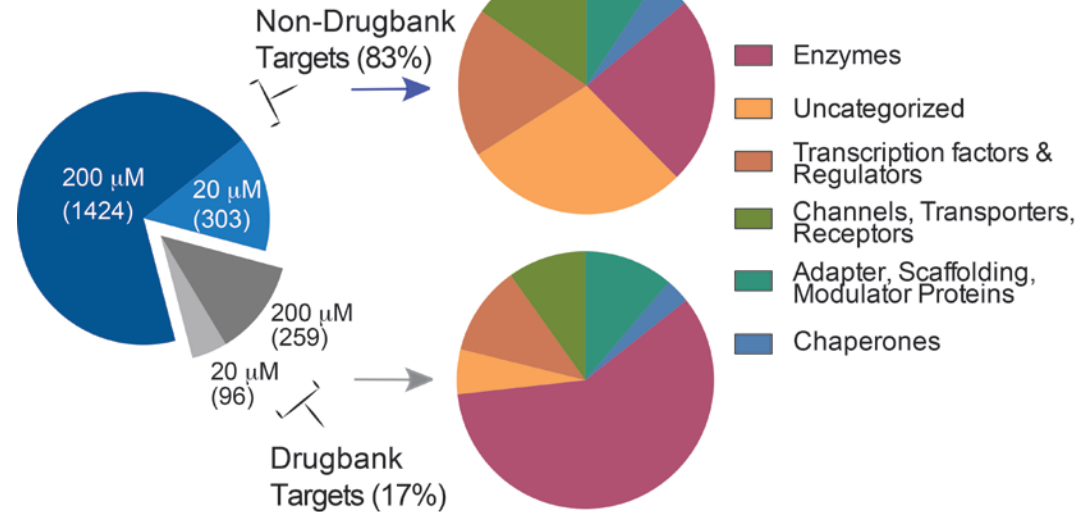
HEK293T (soluble)

Fragment-Based Ligand Discovery in Living Cells

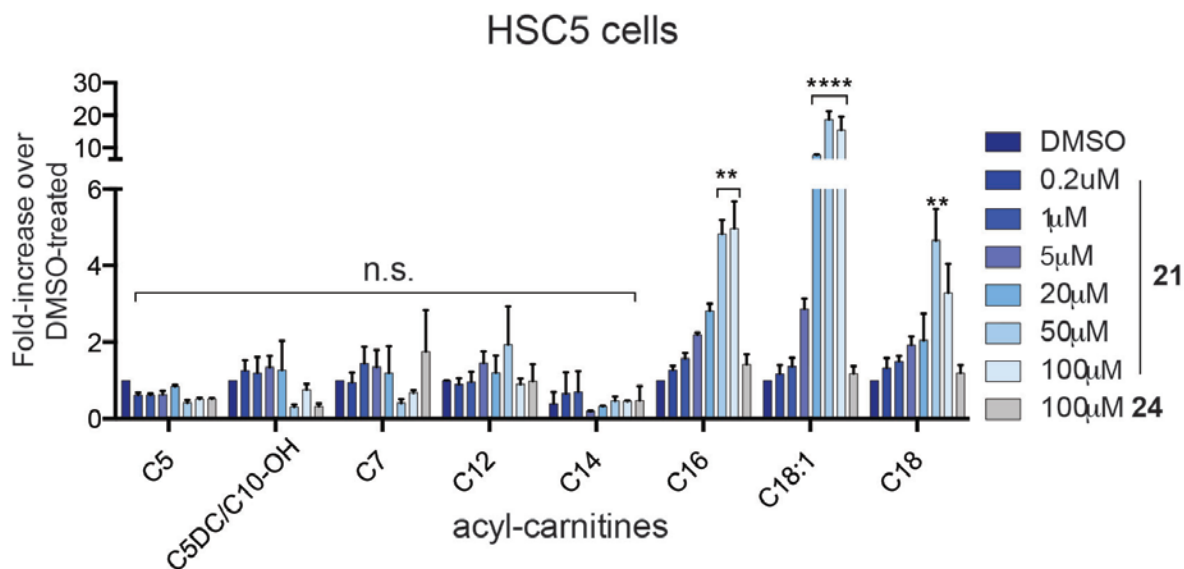
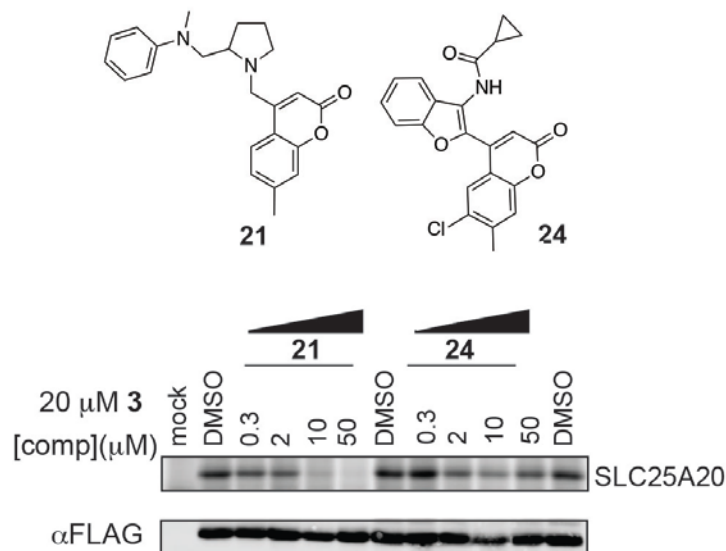
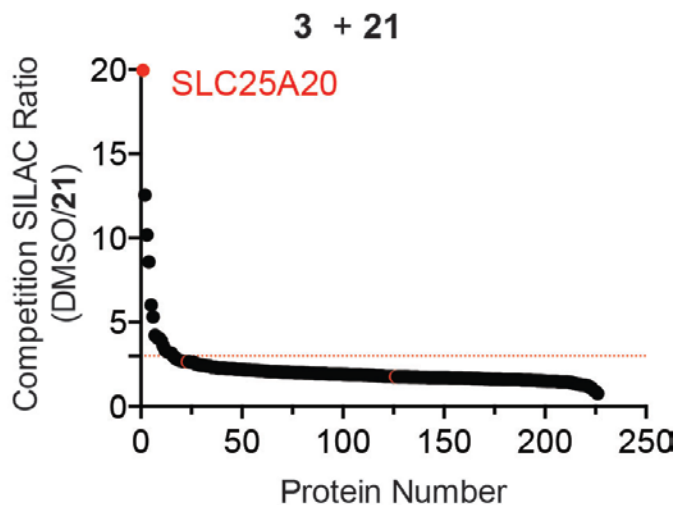


Fragment Probe Targets

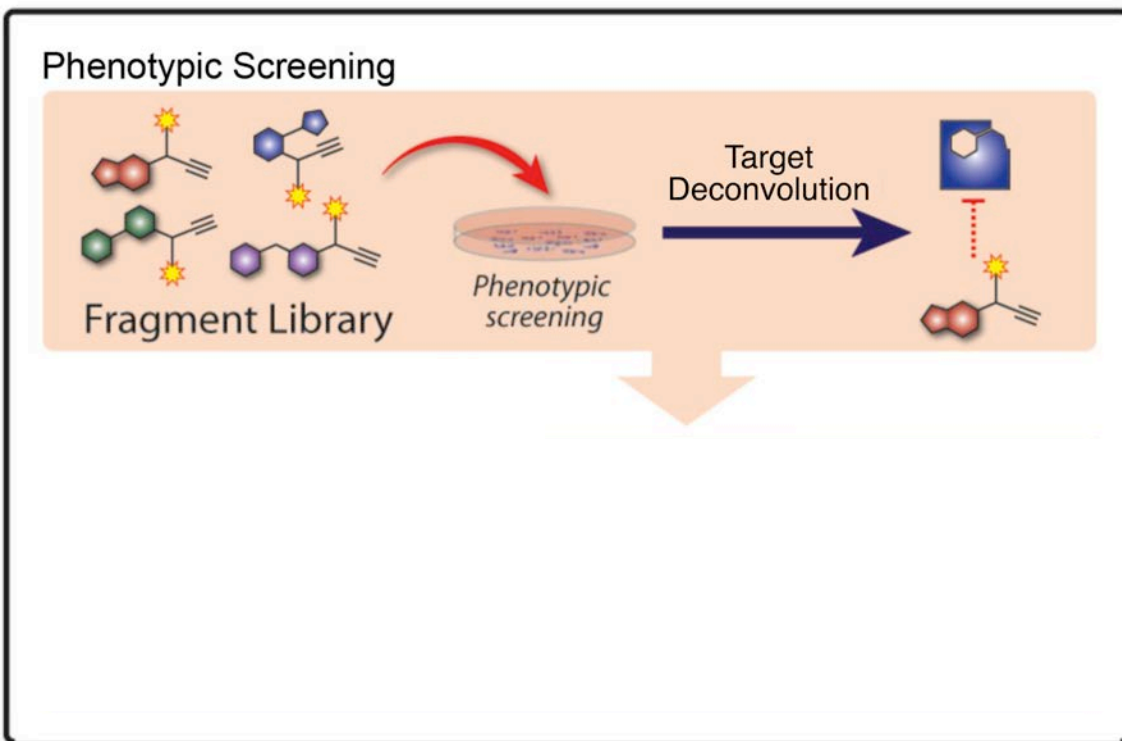
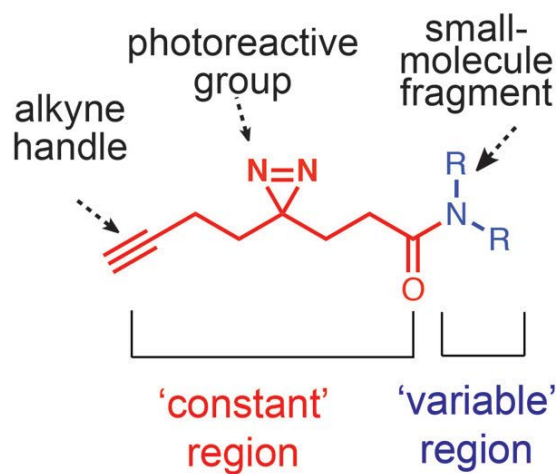
Protein Classification



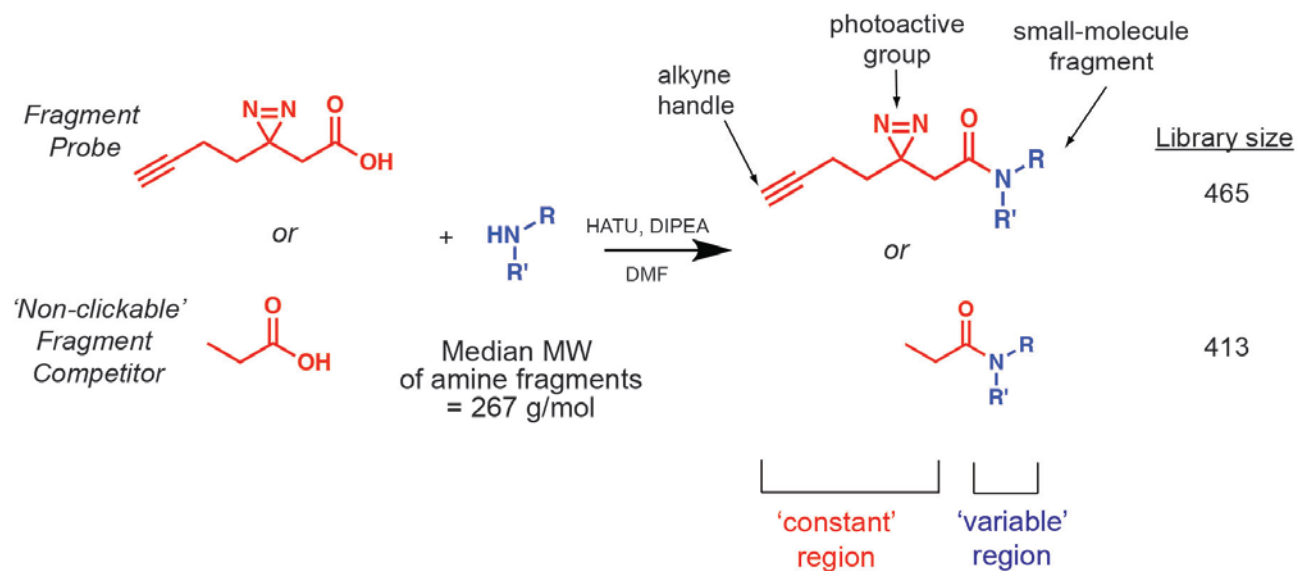
Discovery of an Inhibitor of the Mitochondrial Acylcarnitine Transporter SLC25A20



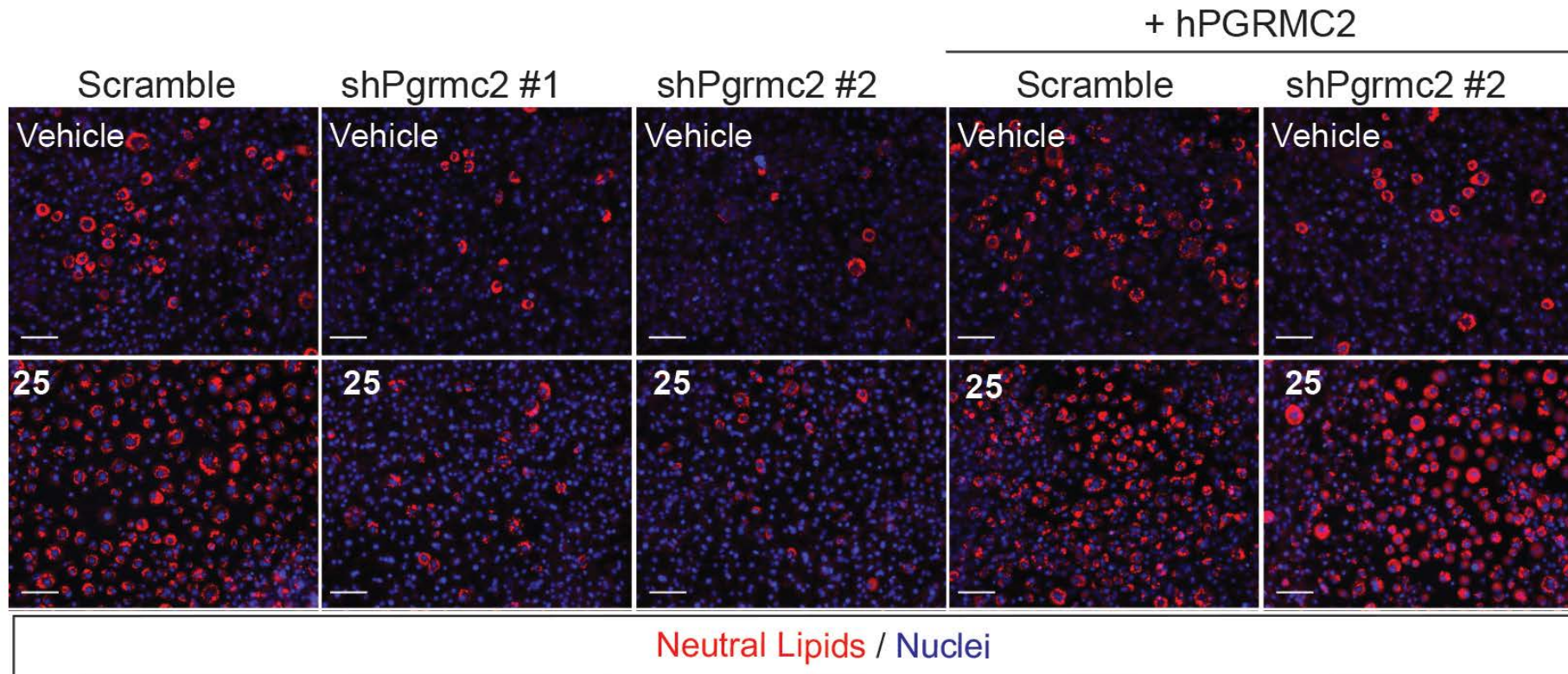
Proteome-Wide **Non-Covalent** Ligand Discovery with *Fully Functionalized Fragment Probes*



Phenotypic Screening w/ Fragment Library Identifies **Novel Ligand-Protein Pathway that Regulates Adipogenesis**



Phenotypic Screening w/ Fragment Library Identifies **Novel Ligand-Protein Pathway that Regulates Adipogenesis**



Conclusions and Future Directions

- Chemical proteomics enables **fragment-based ligand discovery** directly in living cells
- Applications include:
 - Discovery of first-in-class ligands for human proteins
 - Integrated phenotypic screening and target ID
- Future Directions:
 - **Ligand optimization for “undruggable” proteins**
 - **Improved site-of-labeling coverage**
 - **Additional phenotypic screens**

Acknowledgments

Cravatt lab members

- **Keriann Backus**
- Liron Bar-Peled
- Alice Chen
- Megan Blewett
- Armand Cognetta
- **Bruno Correia**
- Melissa Dix
- Stephan Hacker
- Jordon Inloes
- Taka Ichu
- Mike Lazaer
- Kenneth Lum
- Alice Chen
- Yujia Wang
- Daisuke Ogasawara
- **Chris Parker**
- **Will Parsons**
- Esther Kemper
- Kenji Sasaki
- Balyn Zaro
- Radu Suciu
- Katya Vinogradova

Collaborators

- D. Boger (TSRI)
- A. Galmozzi, E. Saez (TSRI)
- John Teijaro (TSRI)
- S. Forli, Art Olson (TSRI)
- M. Lawrence, C. Cavallaro, Johnson, G. Vite (BMS)
- M. Kolar, A. Saghatelian (Salk)
- M. van der Stelt (Leiden)

Funding Support

- NIH (NCI, NIDA, NIGMS)
- BMS
- American Cancer Society
- Pfizer, Abide, Vividion