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

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Department of Molecular Medicine
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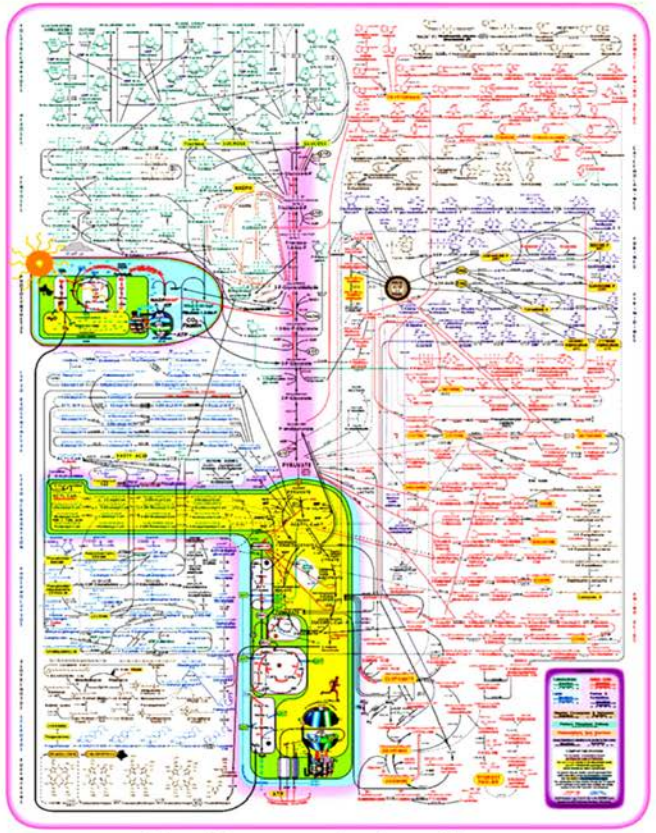
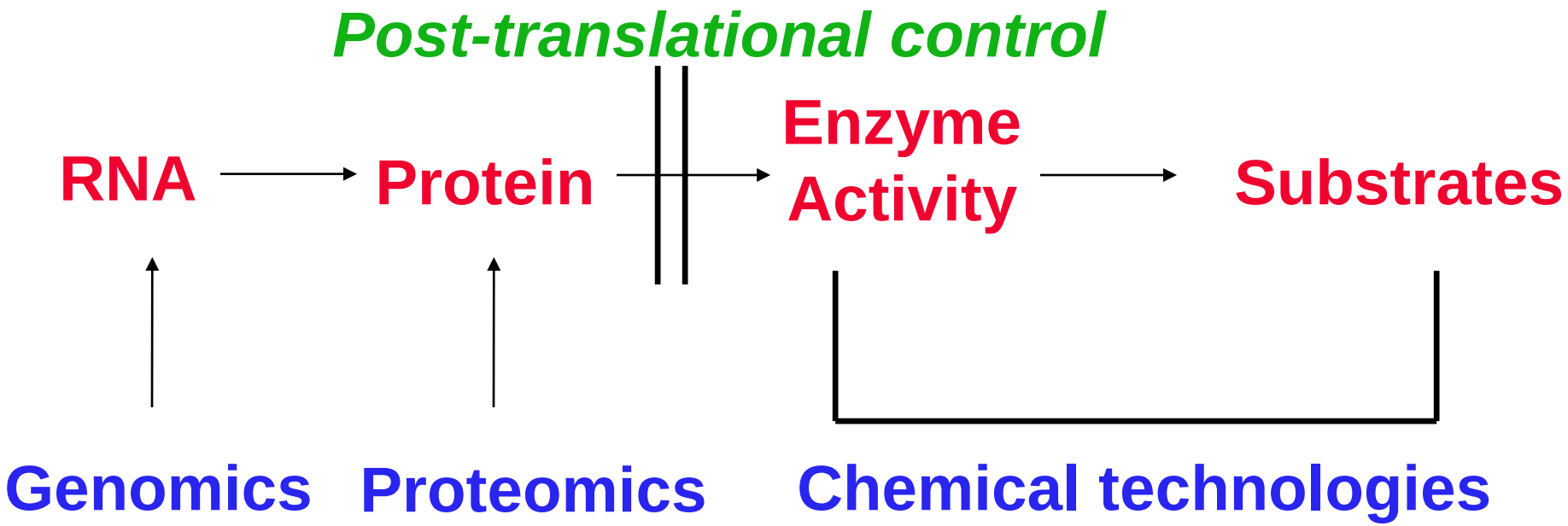
The Scripps Research Institute

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**

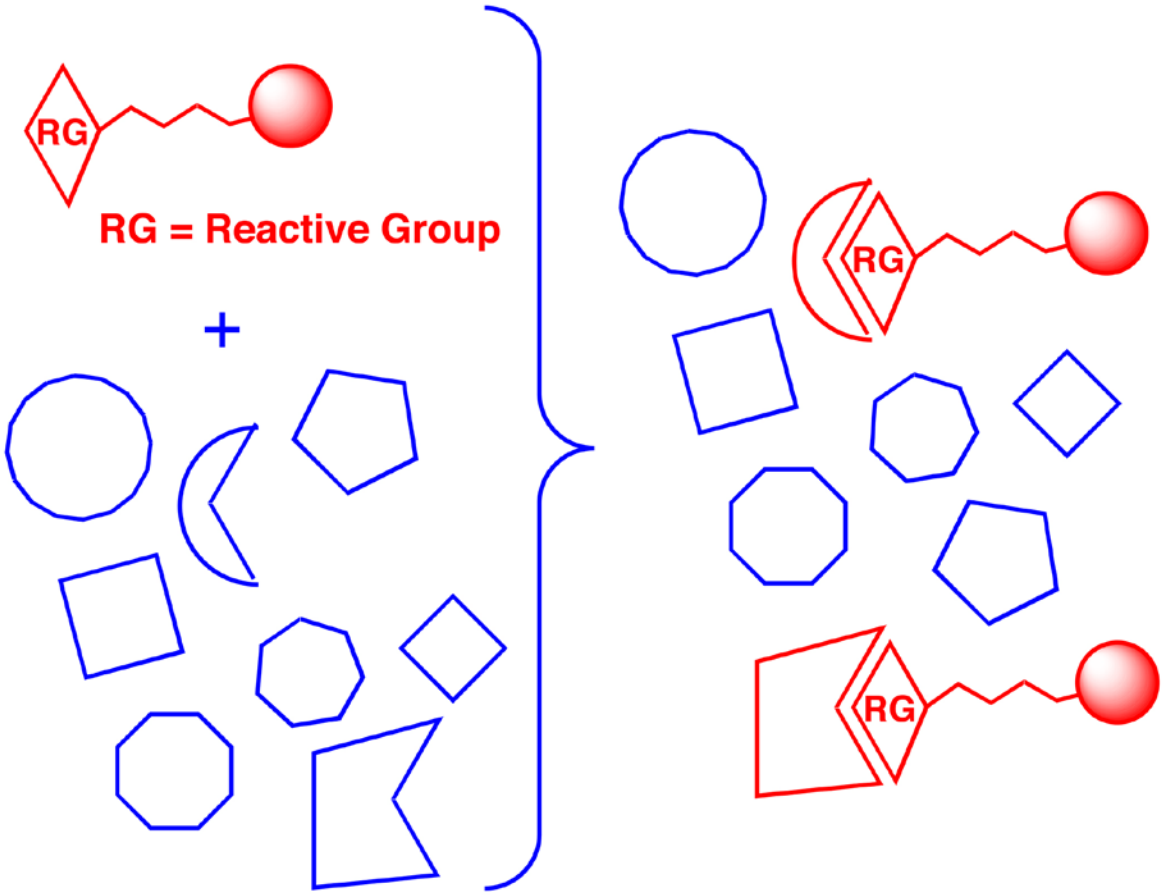
Chemical Probes for Activity-Based Profiling

- **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)

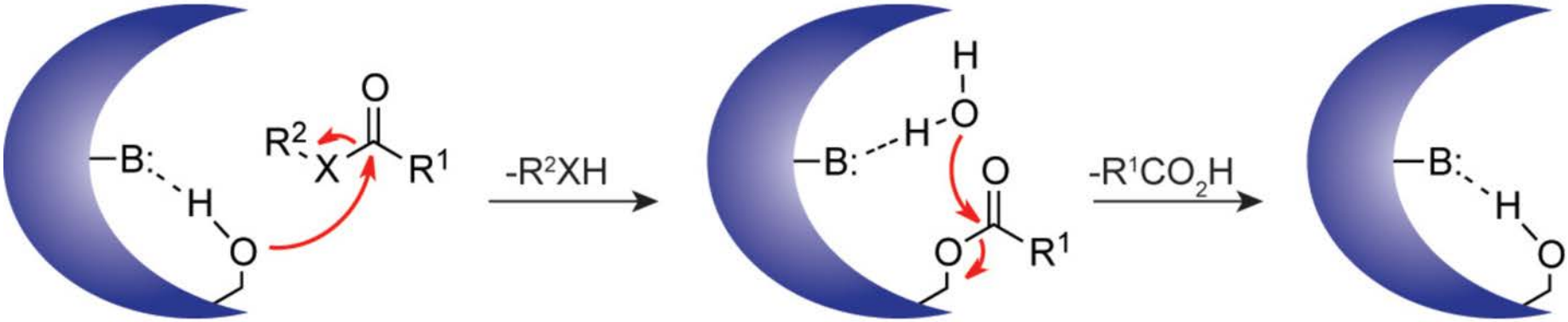
- Aspartyl proteases (Li, et al)

- Cytochrome P450s (Cravatt et al)

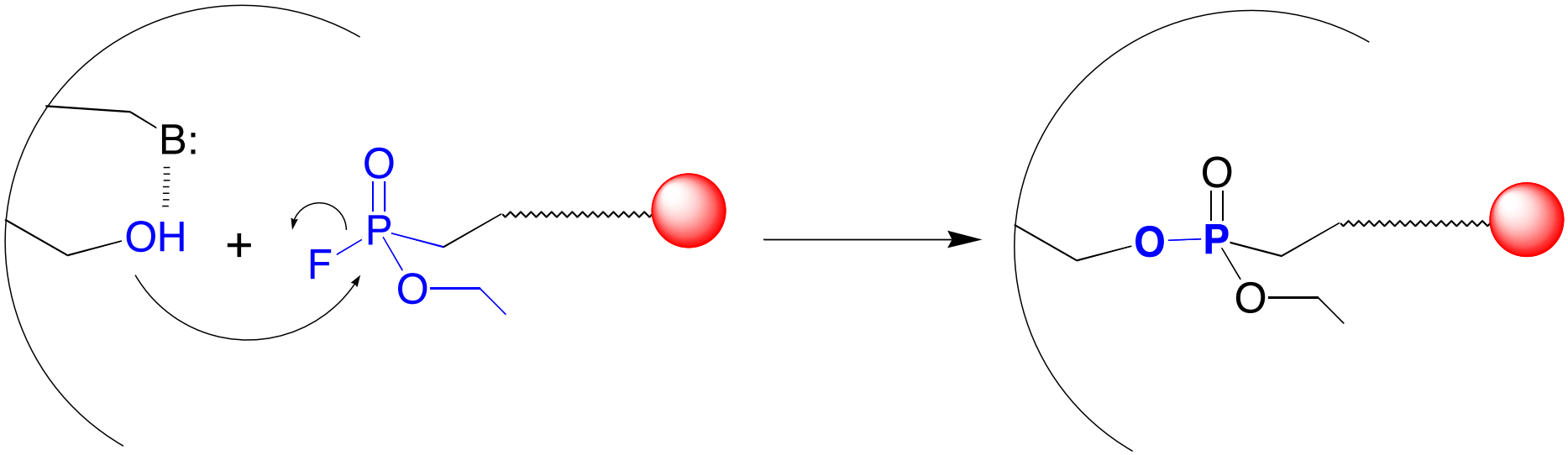
Serine Hydrolases – A Large and Diverse Enzyme Class

- **~1-2% of all eukaryotic and prokaryotic proteomes**

  - proteases, lipases, esterases, transacylases, amidases

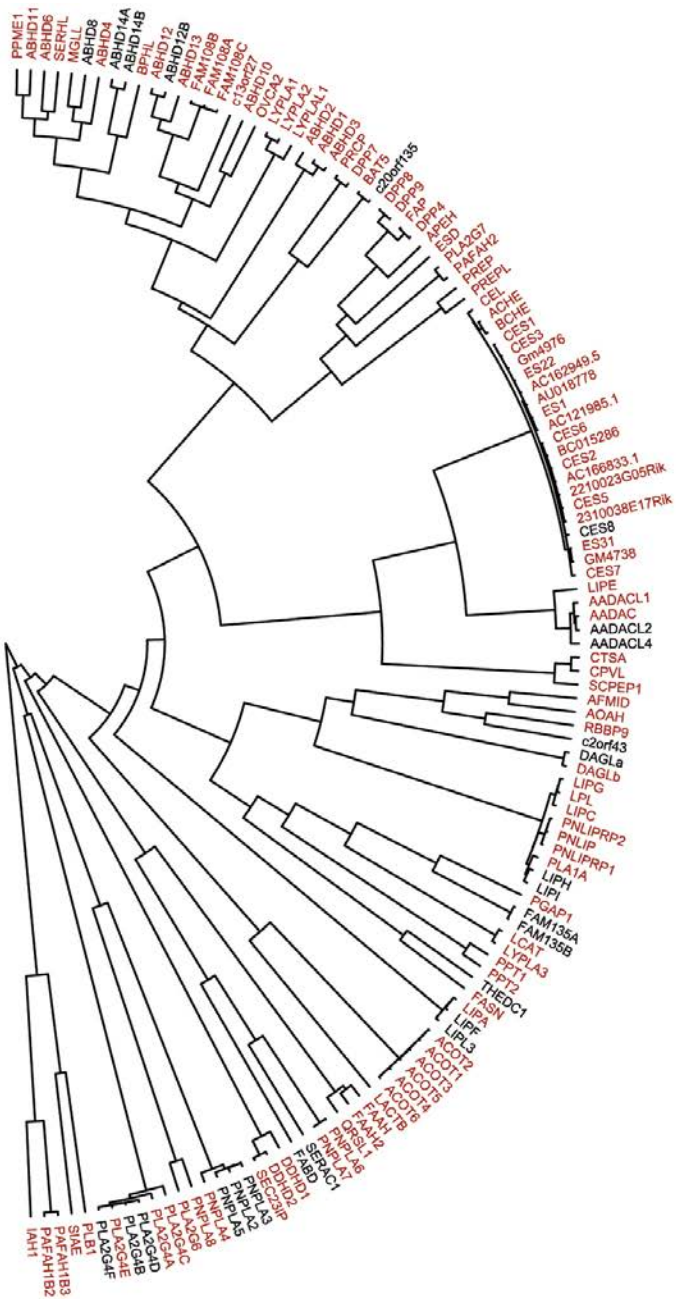


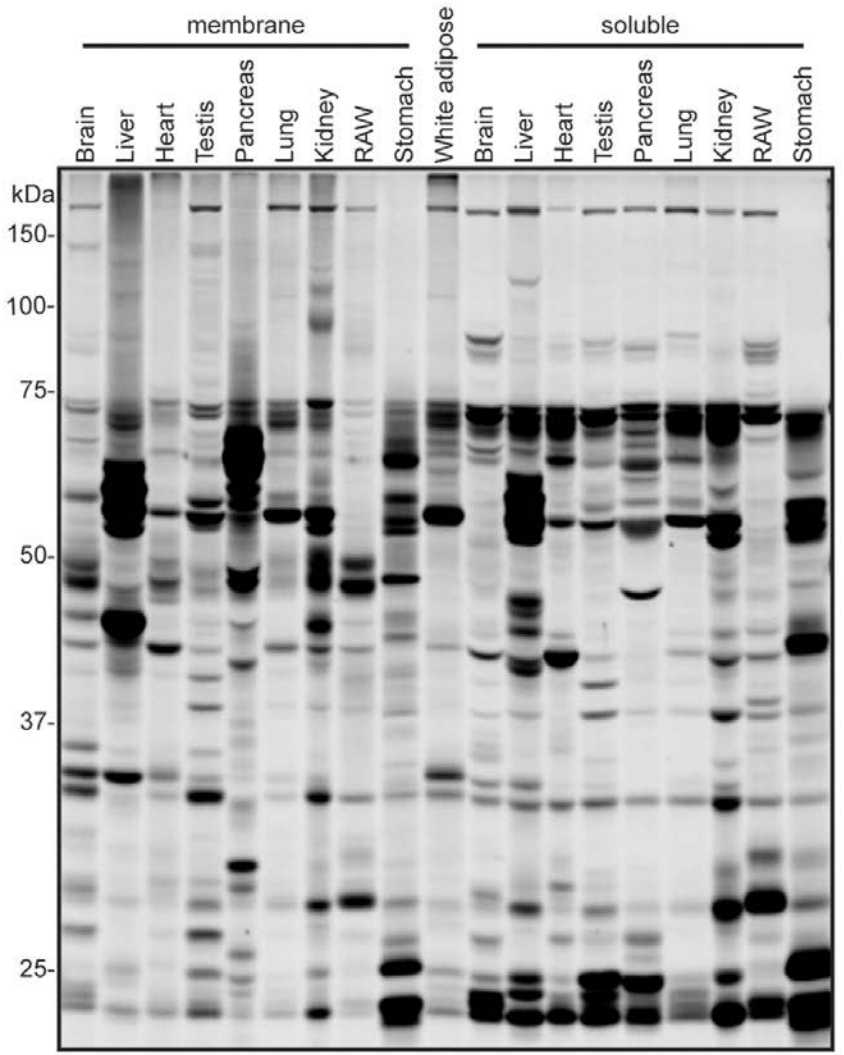
Fluorophosphonates as General Activity-Based Profiling Probes for Serine Hydrolases



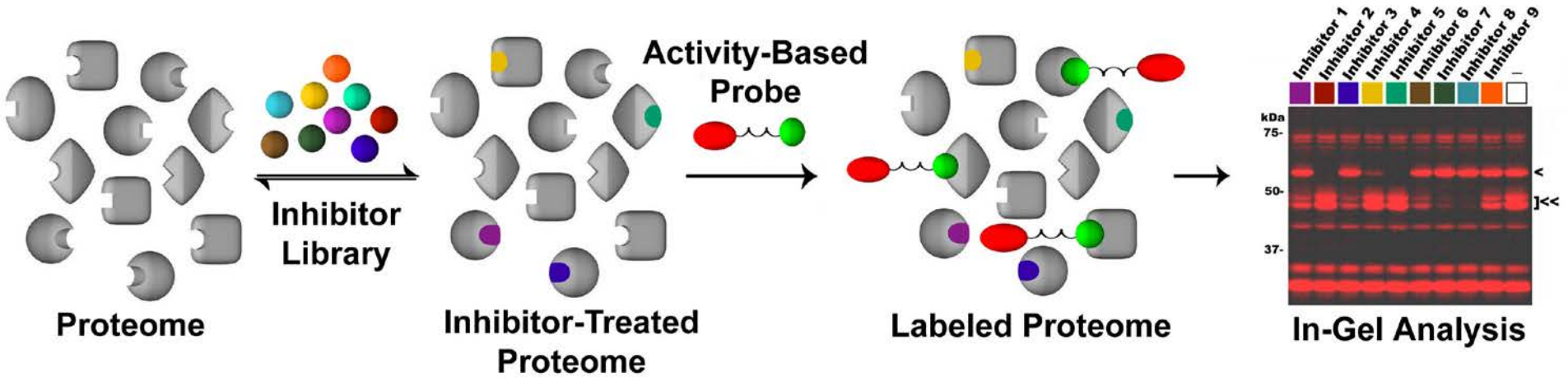
- Fluorophore - detection (in-gel)
Biotin - enrichment

ABPP Coverage of Mammalian Serine Hydrolases





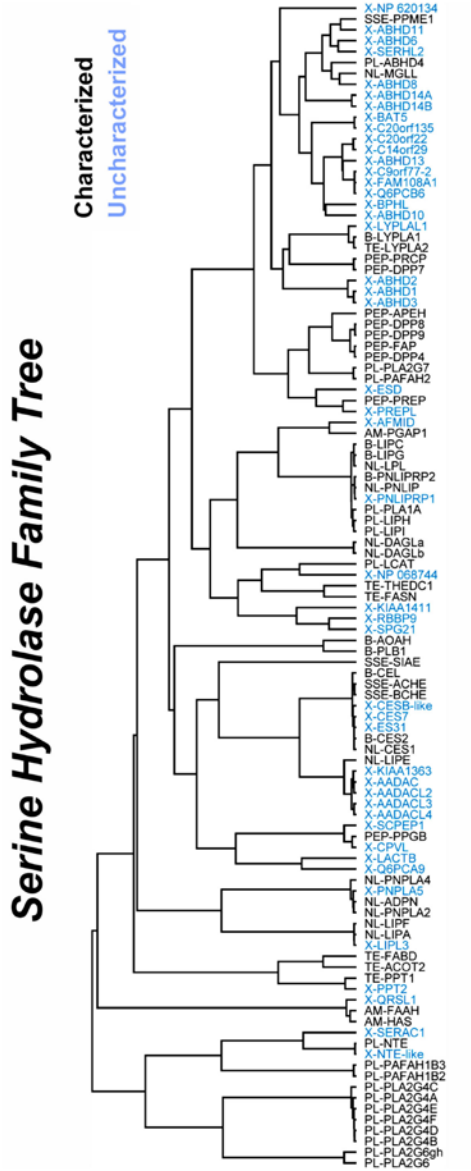
Inhibitor Discovery by Competitive Activity-Based Protein Profiling



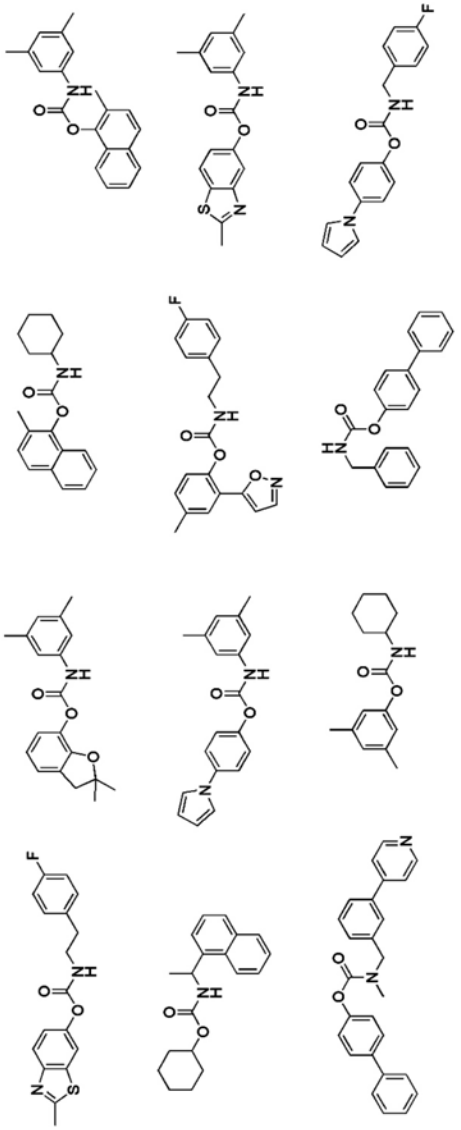
Advantages:

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

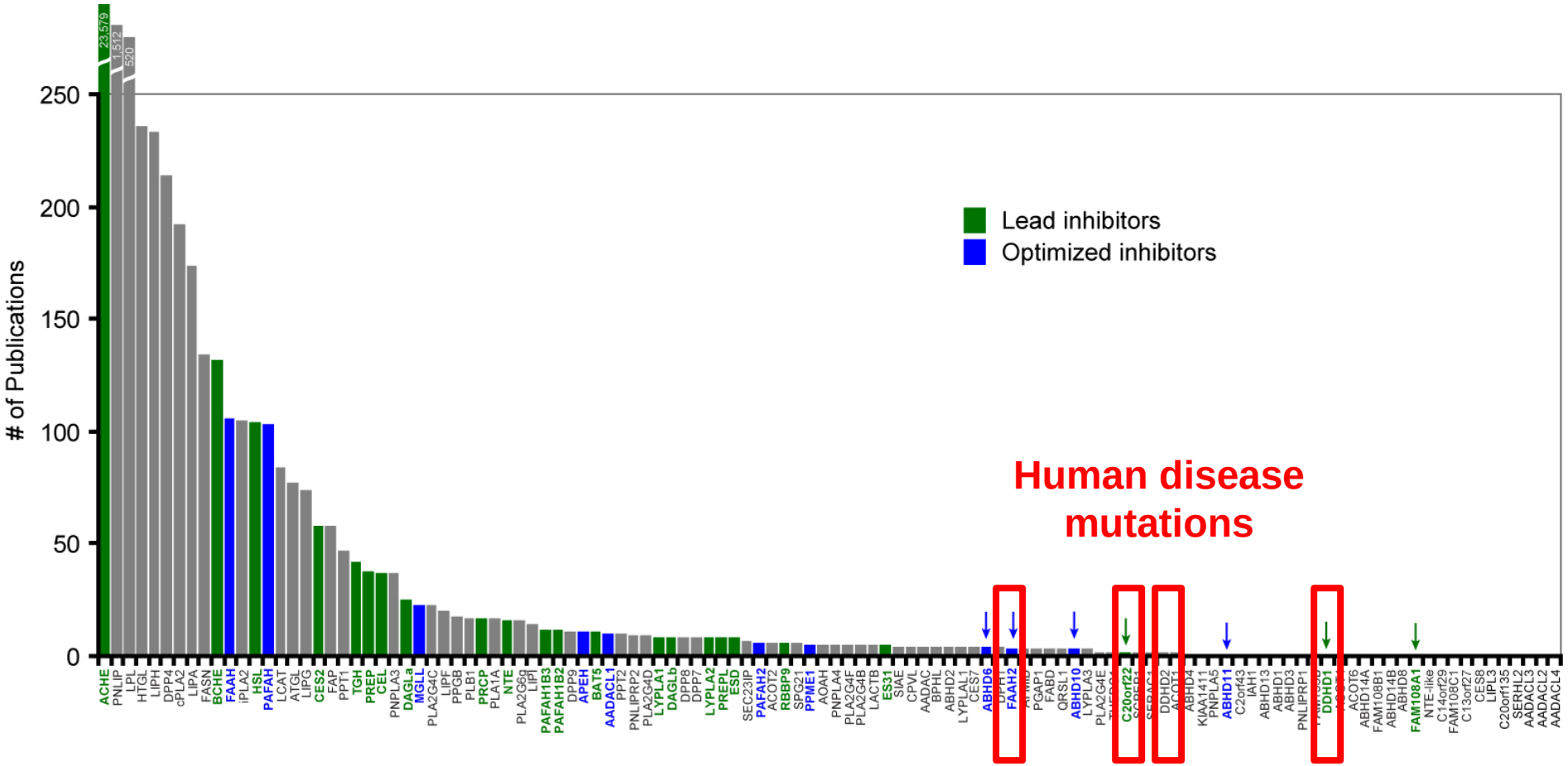
Systematic Discovery of Serine Hydrolase Inhibitors by Competitive ABPP



X



Toward a Complete Pharmacology For the Serine Hydrolase Superfamily



Integrating ABPP with Human Genetics to Map Orphan Disease Mechanisms

PNAS

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]

[a]The Skaggs Institute for Chemical Biology and Departments of [b]Chemical Physiology and [c]Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

PNAS

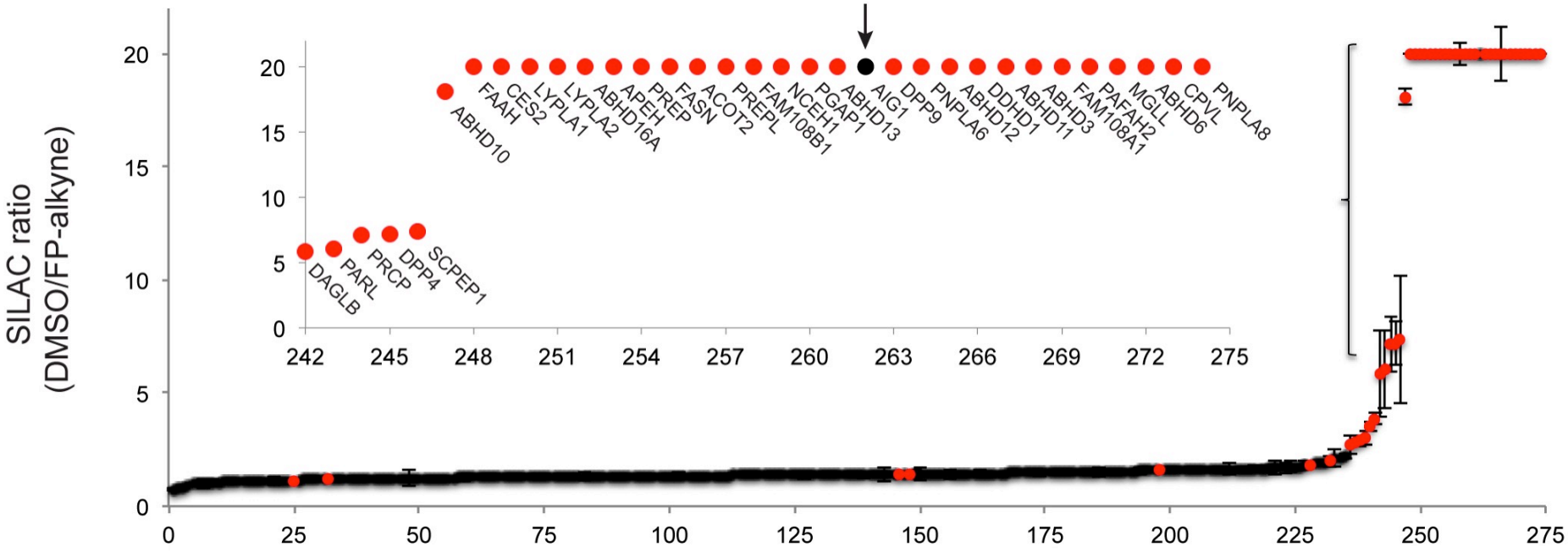
The hereditary spastic paraplegia-related enzyme DDHD2 is a principal brain triglyceride lipase

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]

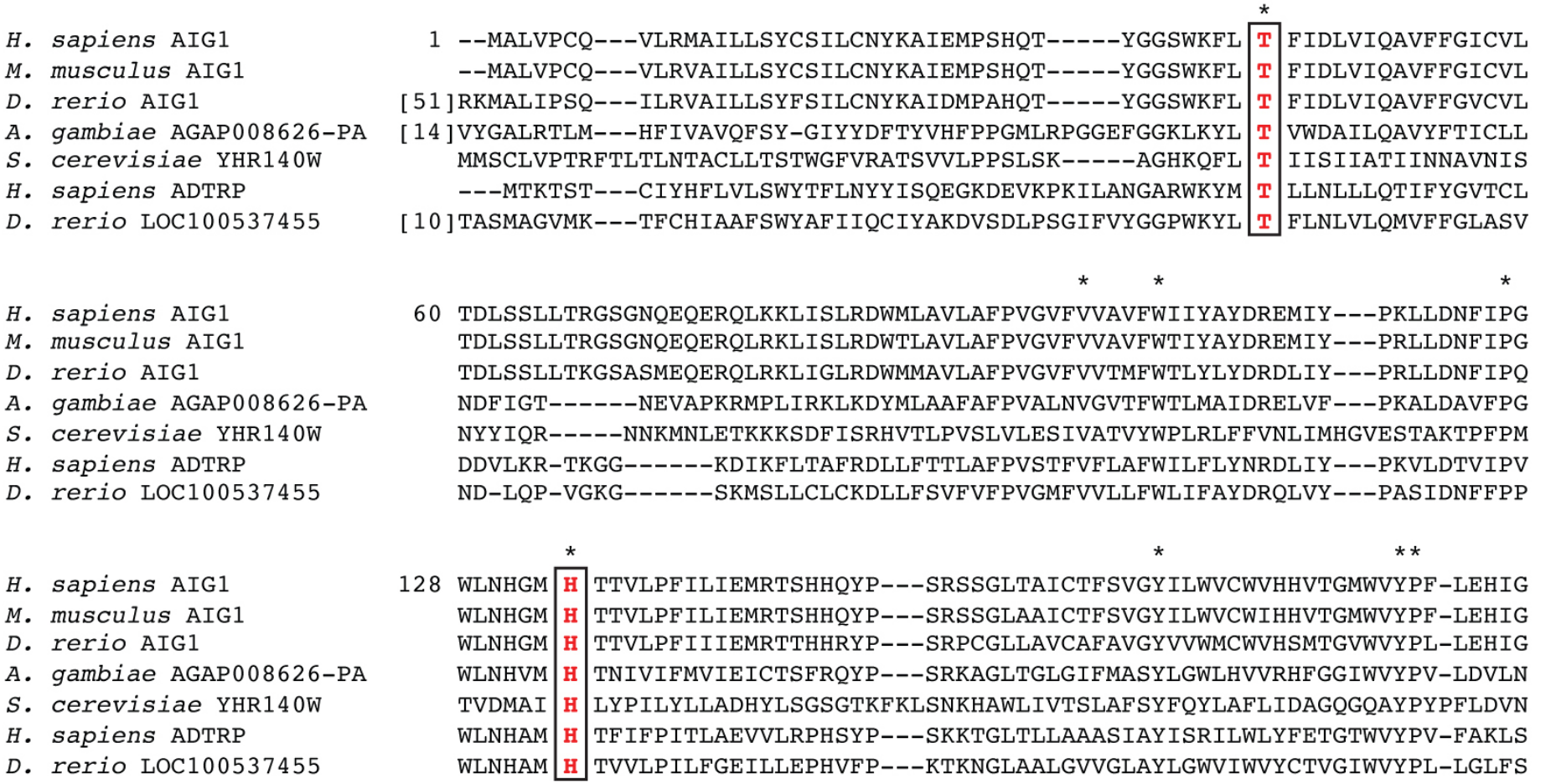
[a]The Skaggs Institute for Chemical Biology and Departments of [b]Chemical Physiology and [c]Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Excavating Cases of Convergent/Parallel Evolution of Unpredecented Hydrolase Activities by ABPP

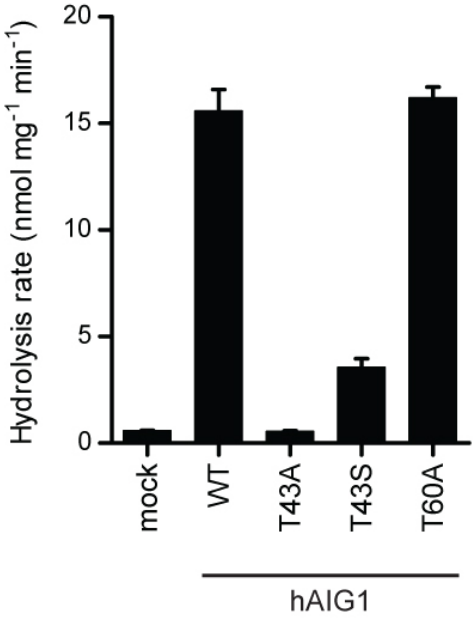
If FP reactivity marks hydrolase activity…

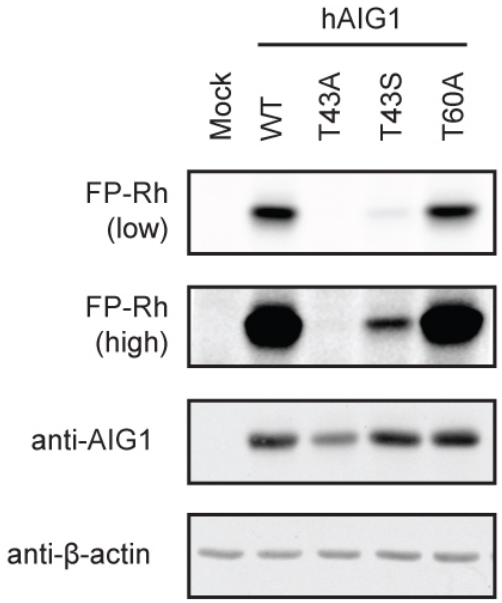


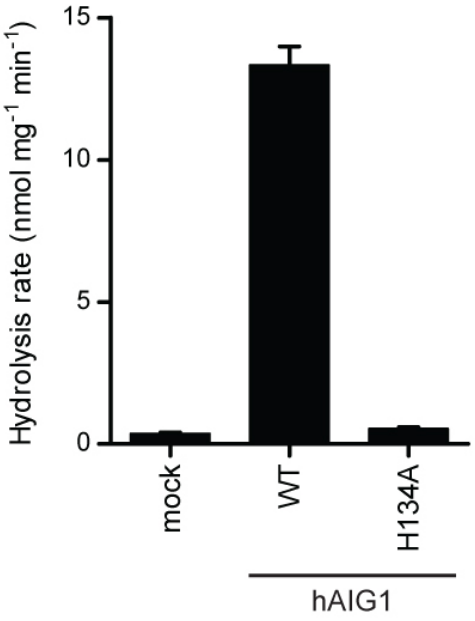
AIG1 (and ADTRP) are Multi-Pass Transmembrane Proteins of Poorly Characterized Function



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







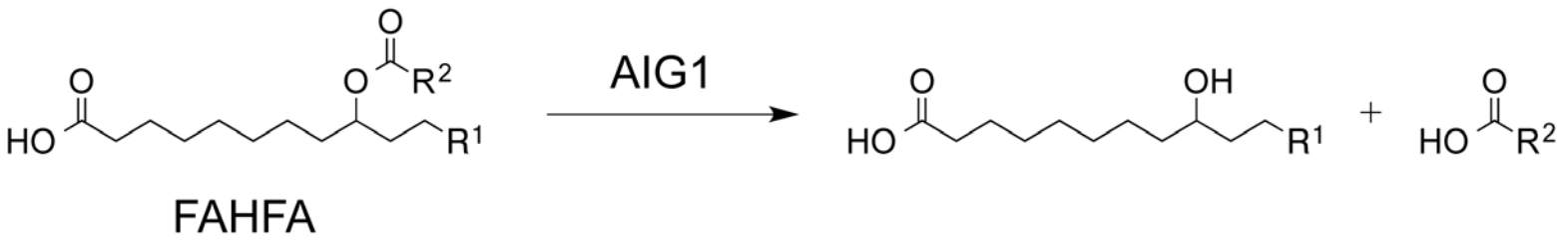
FAHFAs – A Class of Lipid Transmitters that Regulate Metabolic and Inflammatory Processes

Cell

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

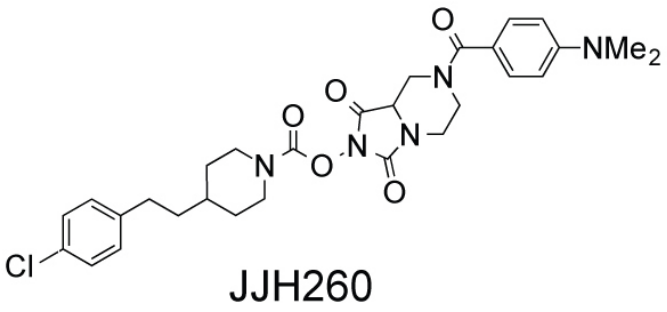
Mark M. Yore,[1,5] Ismail Syed,[1,5] Pedro M. Moraes-Vieira,[1] Tejia Zhang,[3,7] Mark A. Herman,[1] Edwin A. Homan,[3] Rajesh T. Patel,[2] Jennifer Lee,[1] Shili Chen,[3,7] Odile D. Peroni,[1] Abha S. Dhaneshwar,[1] Ann Hammarstedt,[4] Ulf Smith,[4] Timothy E. McGraw,[2] Alan Saghatelian,[3,6,7,*] and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

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

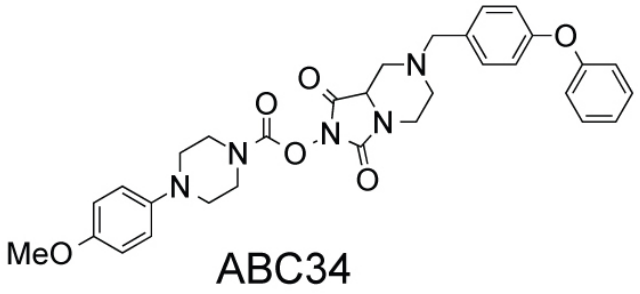


FAHFA

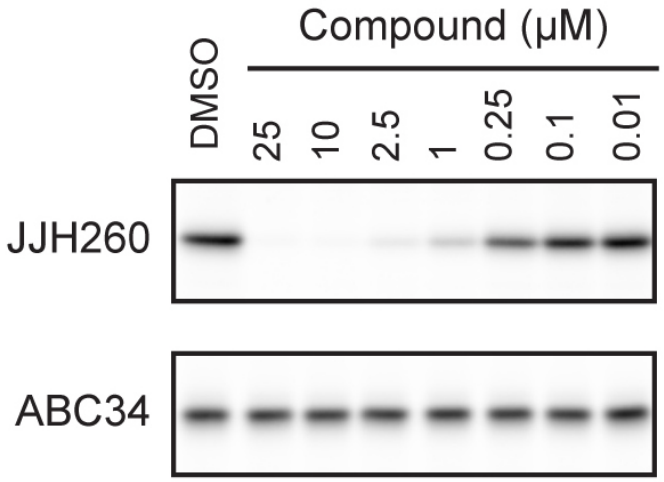
AIG1 Inhibitors Discovered by ABPP



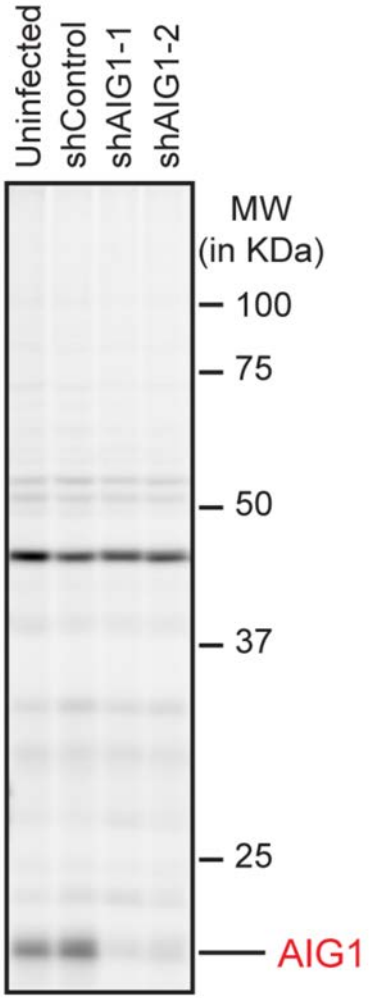
JJH260

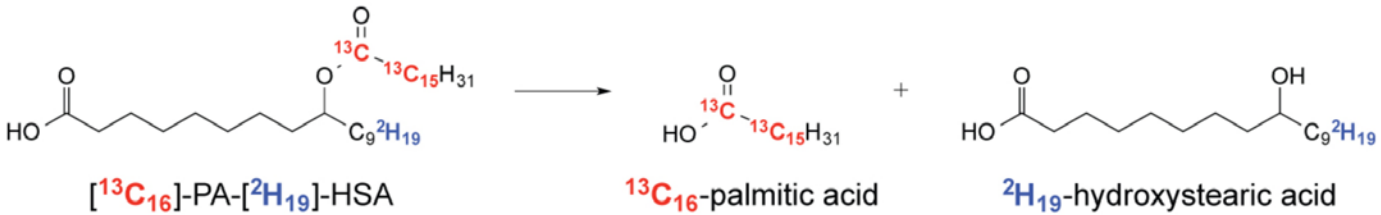


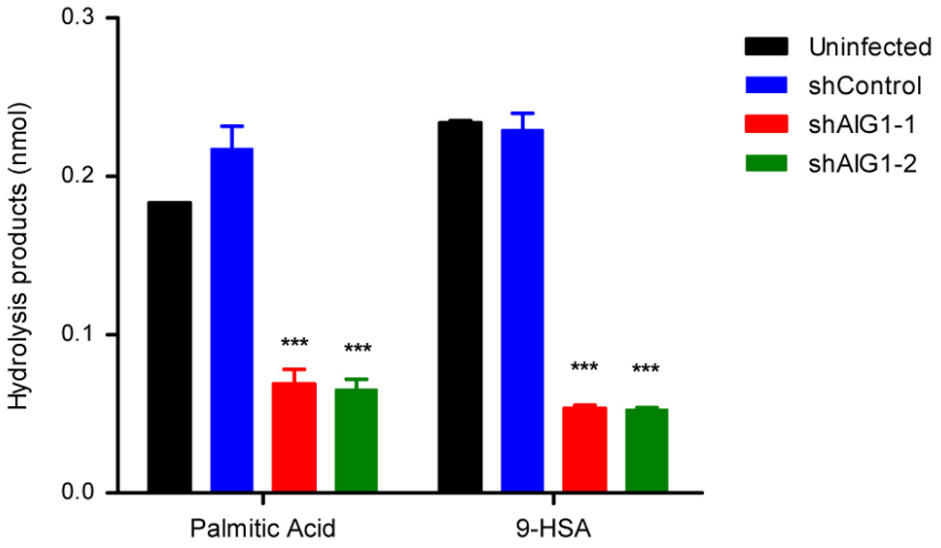
ABC34



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

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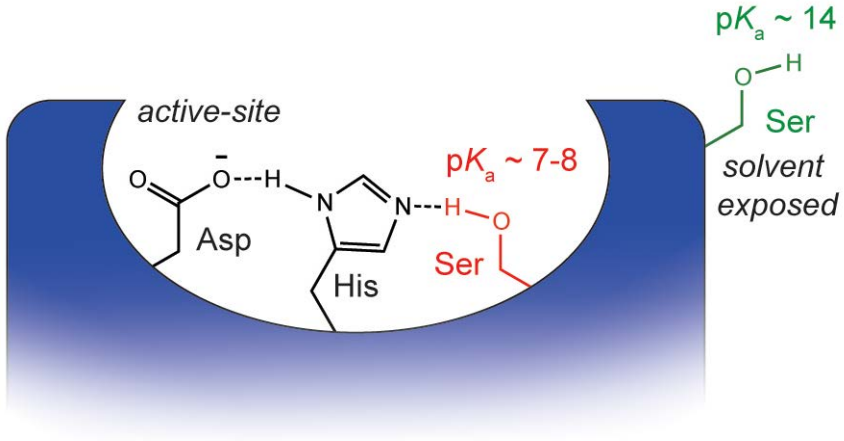
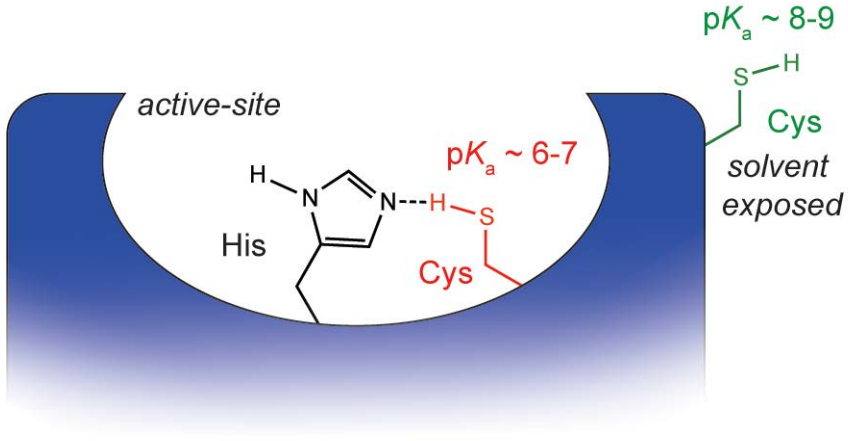
- **Extending ABPP – Mapping the Ligandability of the Human Proteome**

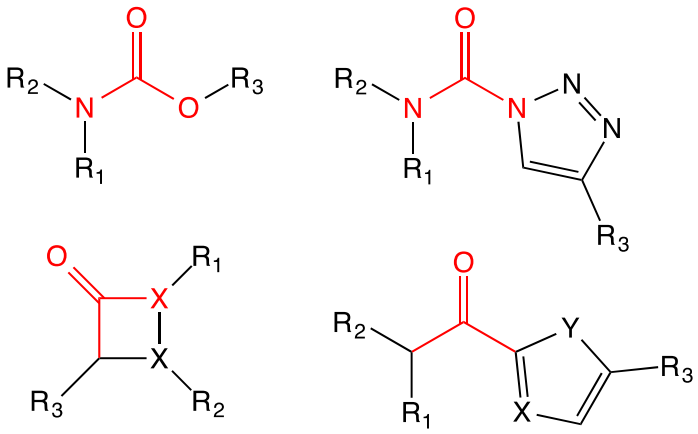
Challenges and Opportunities for Design of Covalent Ligands that Target Cysteine Residues

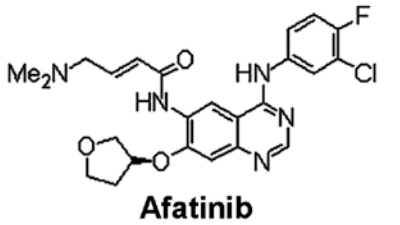
Serine hydrolase

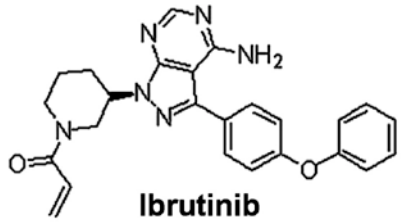


Cysteine enzyme

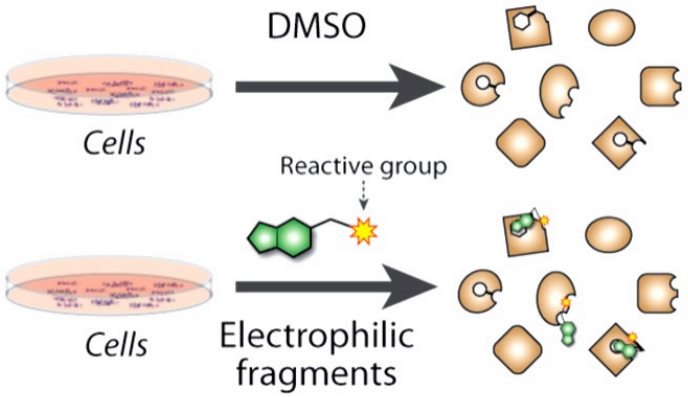


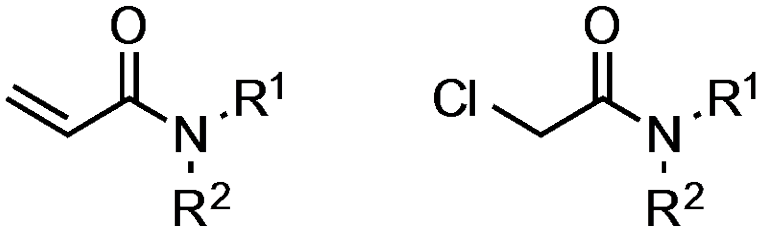






Proteome-Wide Covalent Ligand Discovery





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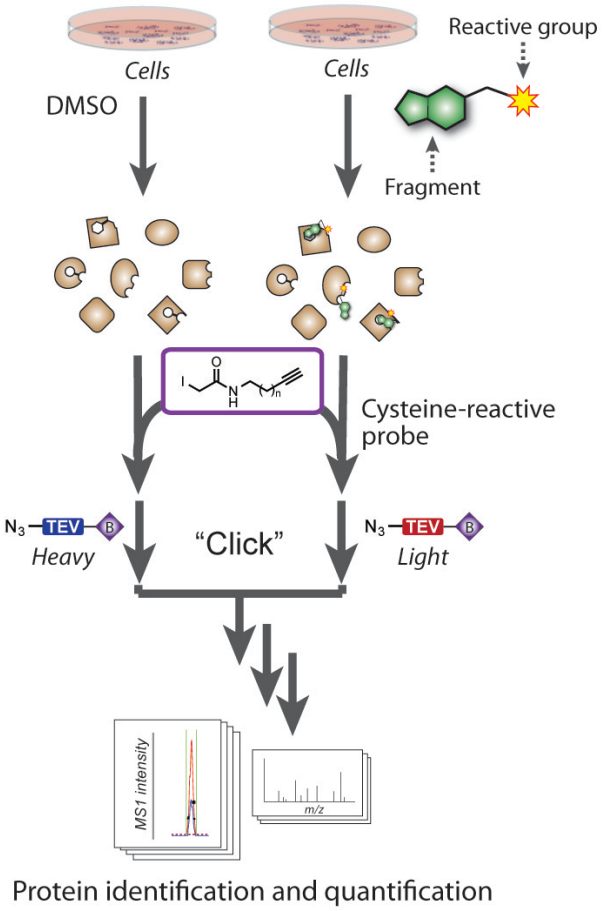
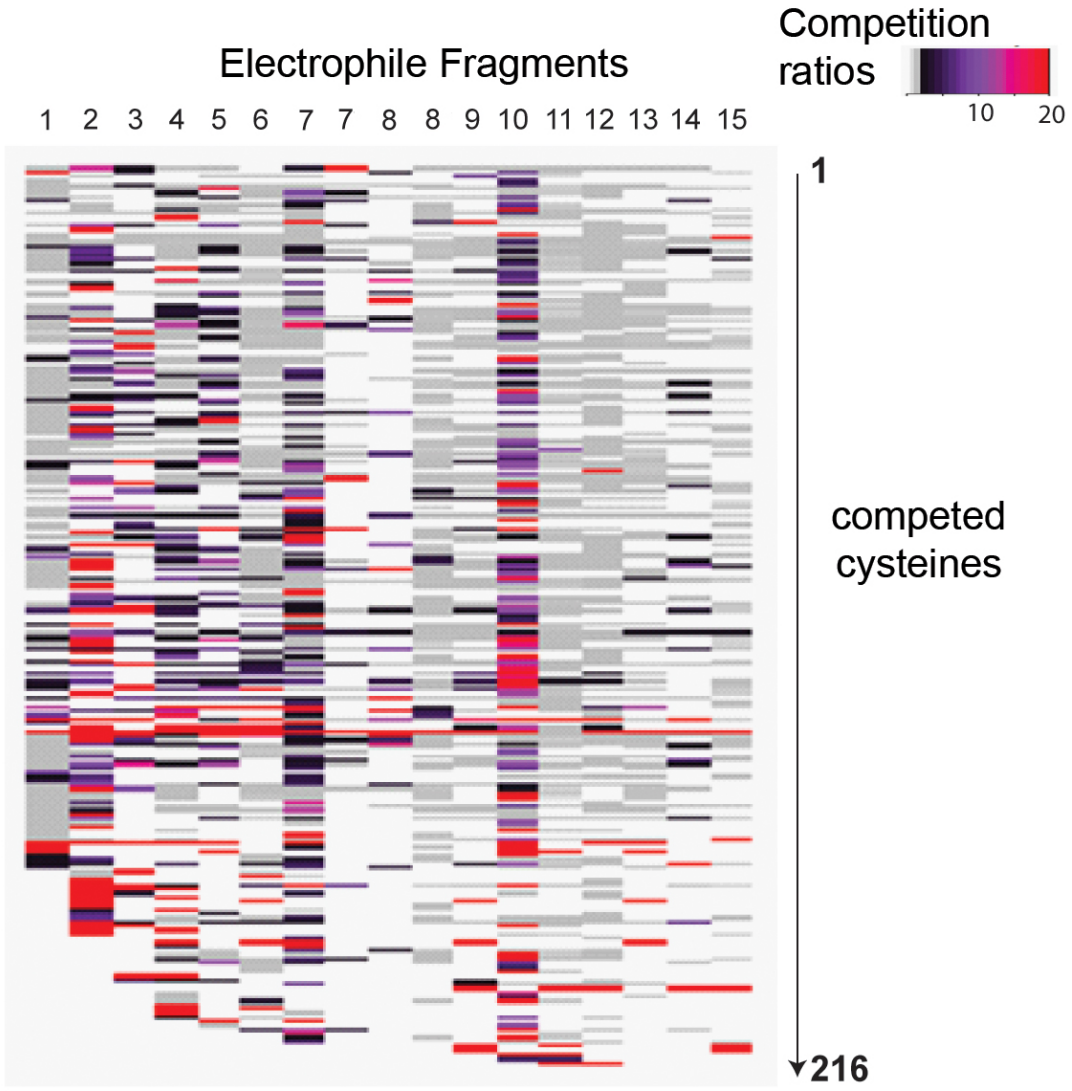
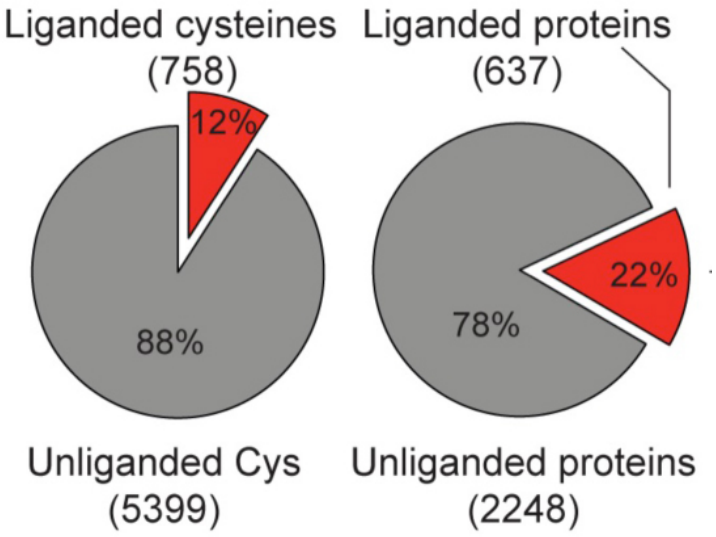




Proteome-Wide Covalent Ligand Discovery Accesses New (Un)Druggable Space



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

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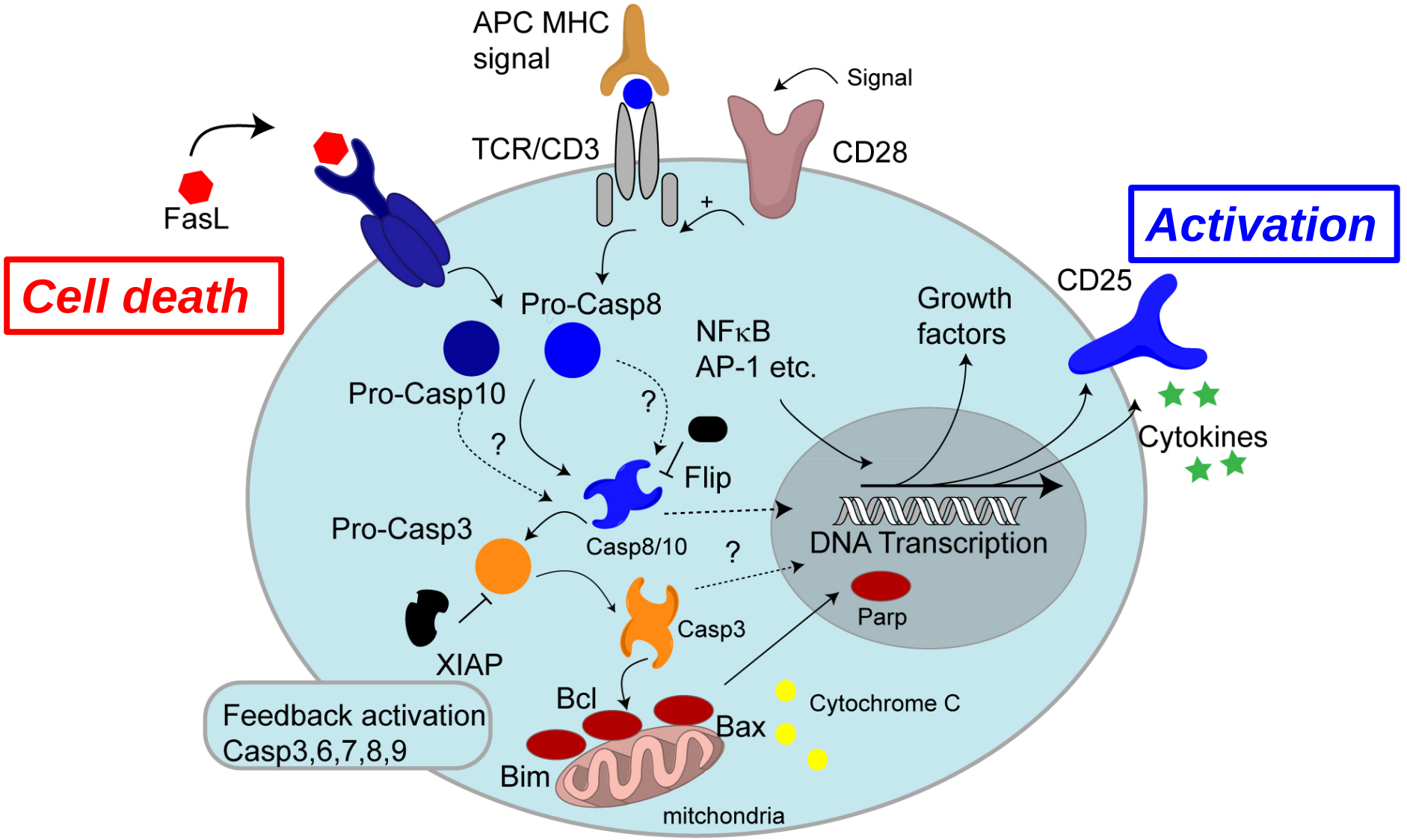
Inherited Human Caspase 10 Mutations Underlie Defective Lymphocyte and Dendritic Cell Apoptosis in Autoimmune Lymphoproliferative Syndrome Type II

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



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

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

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

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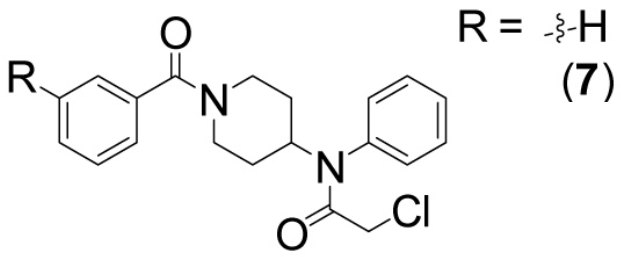
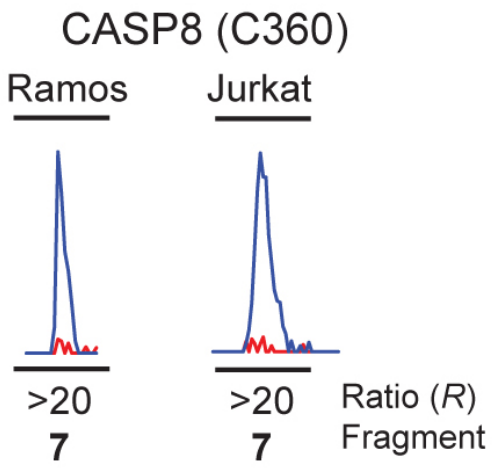
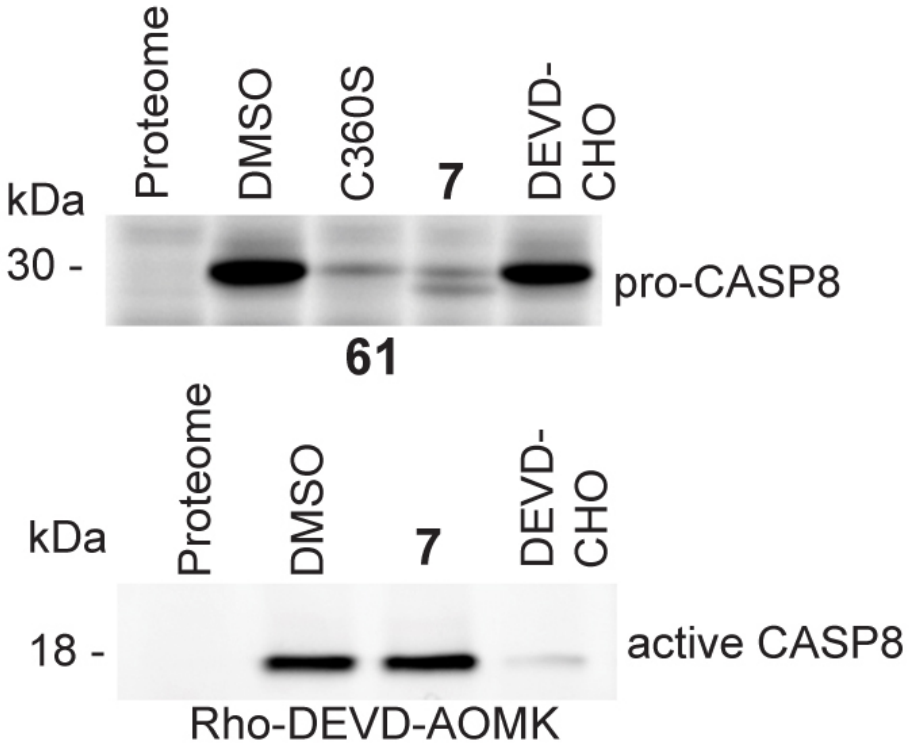
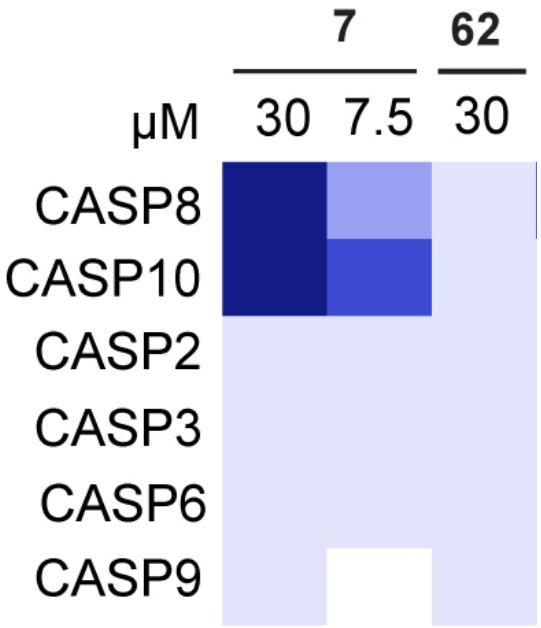
Covalent Ligands that Target the Pro (Inactive) Forms of Caspases

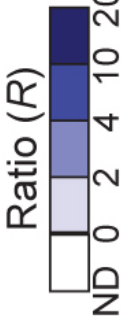






Dual Pro-Caspase-8/10 and Selective Pro- Caspase-8 Ligands

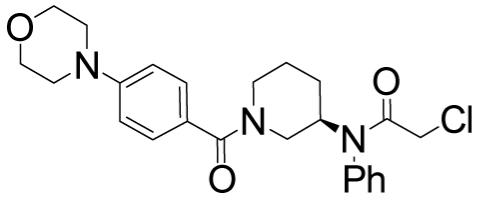




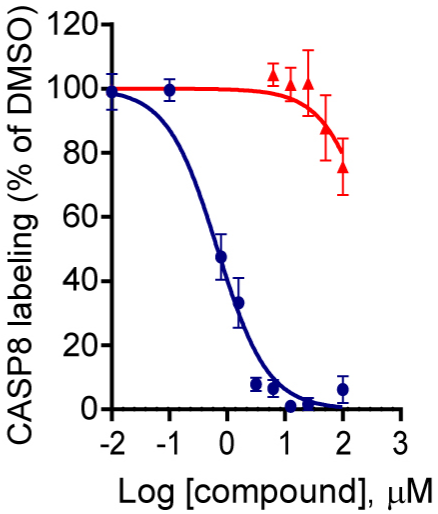
63-*R* IC_{50} (95% CI), μM

CASP8 0.7 (0.5–0.8)

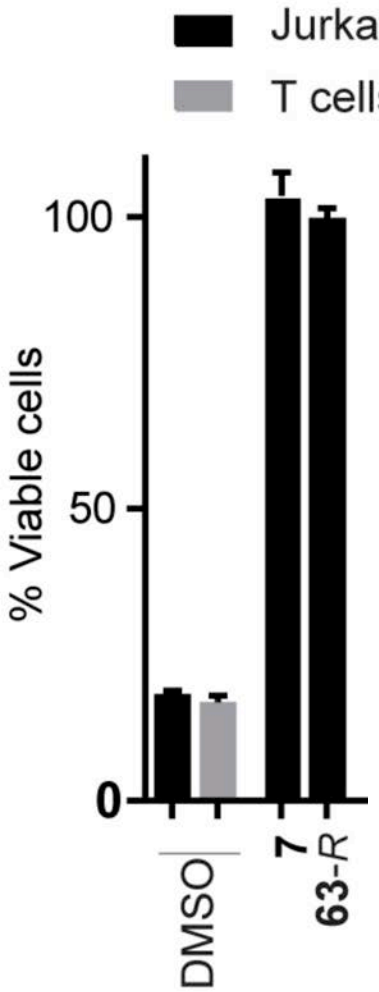
CASP10 >100



63-*R*



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



7 = C8/10
63-*R* = C8

Conclusions and Future Directions

- **Chemical proteomics reveals a rich content of ligandable cysteines in the human proteome**

- **Combining cysteine ligandablity 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

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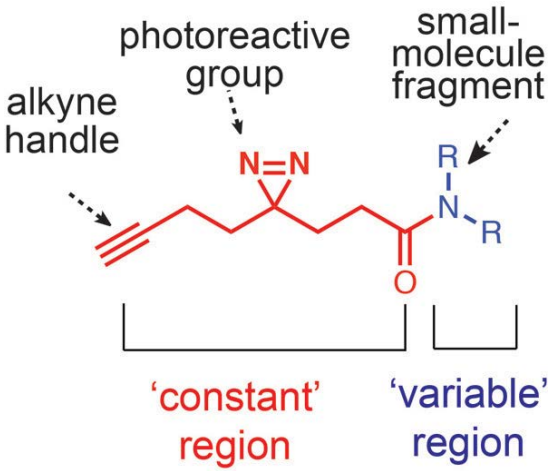
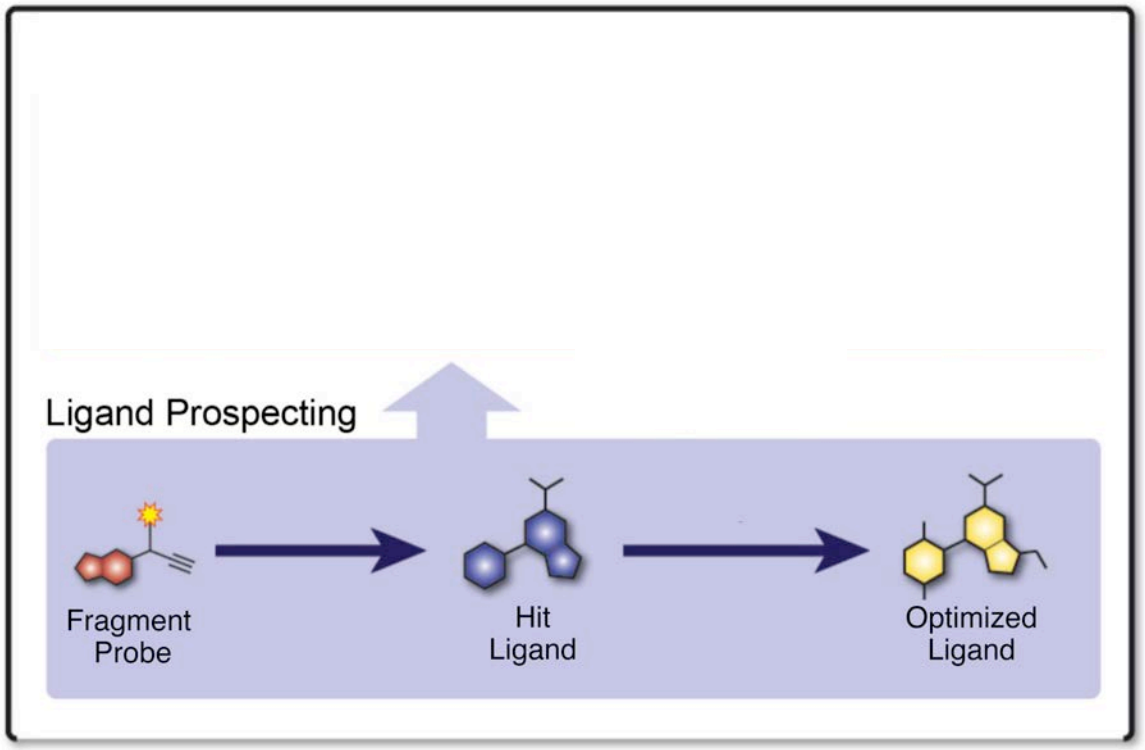
  – Ligand optimization

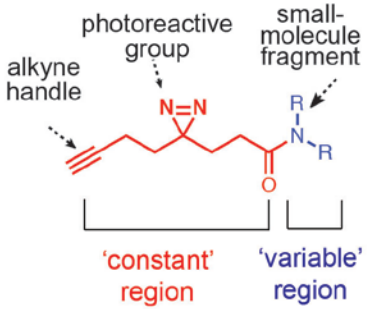
  – **Extension to other (non)-nucleophilic residues**

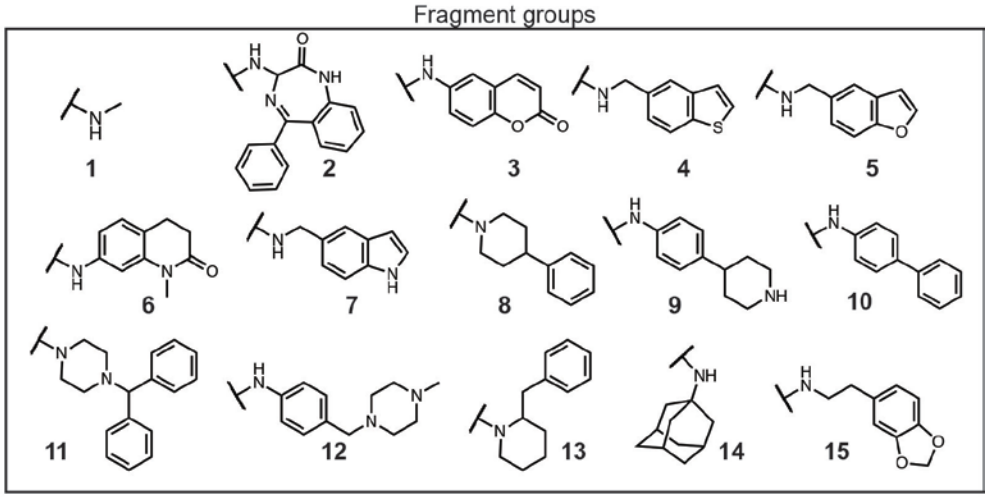
Proteome-Wide Non-Covalent Ligand Discovery with *Fully Functionalized Fragment* Probes (Chris Parker)

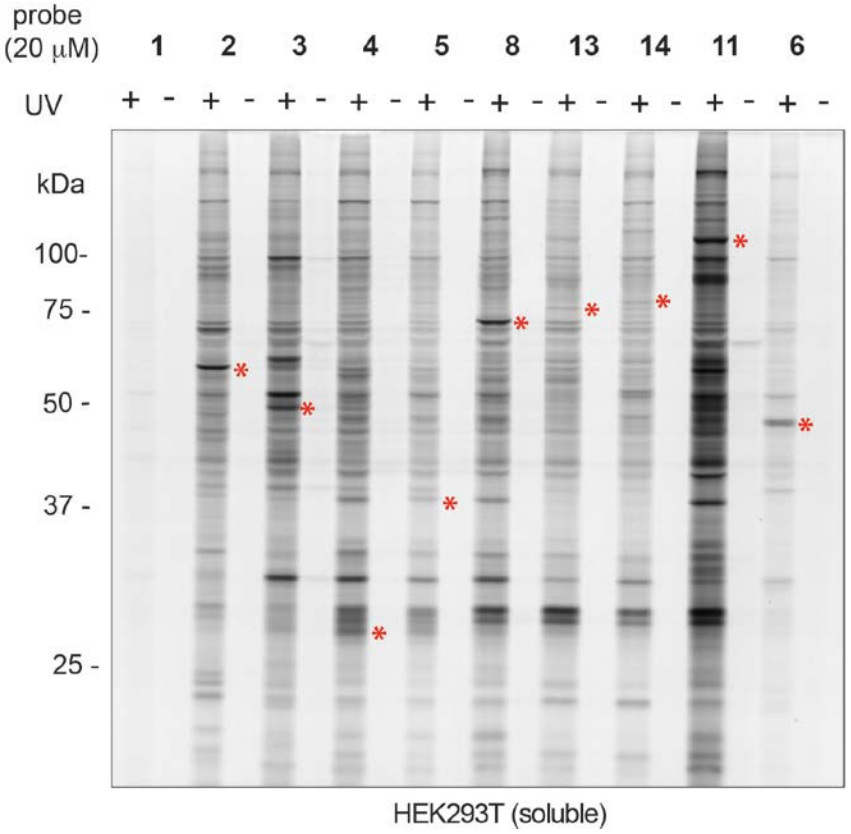




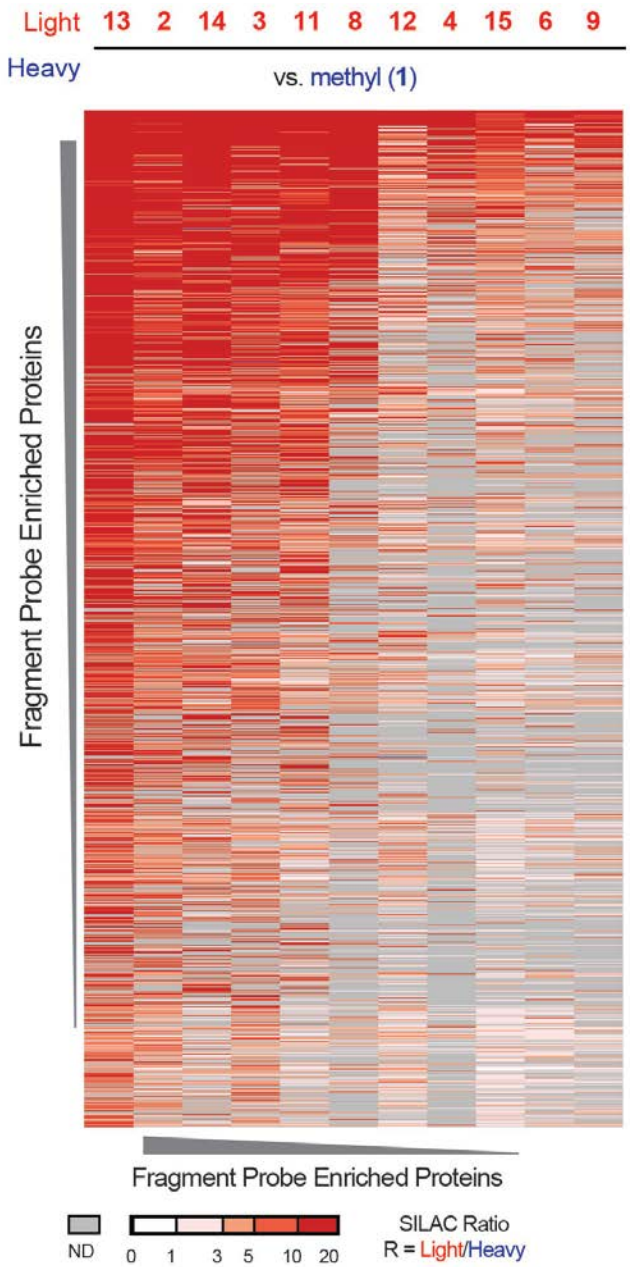
Proteome-Wide Non-Covalent Ligand Discovery with *Fully Functionalized Fragment* Probes

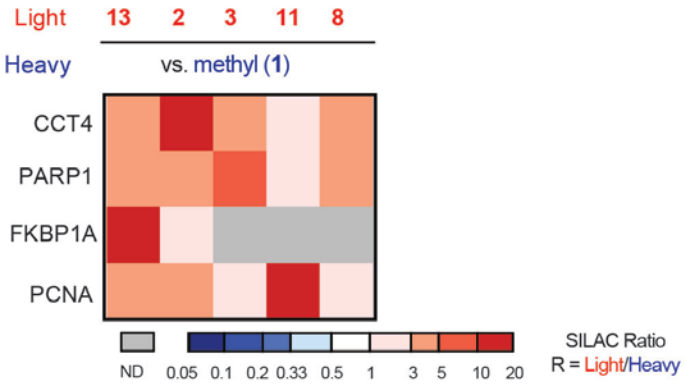


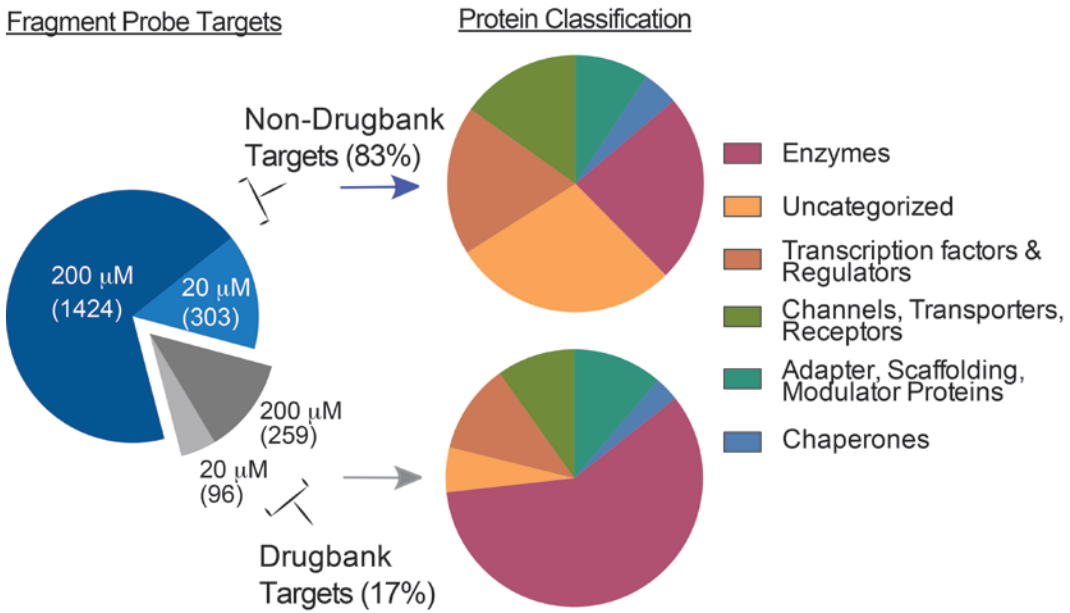




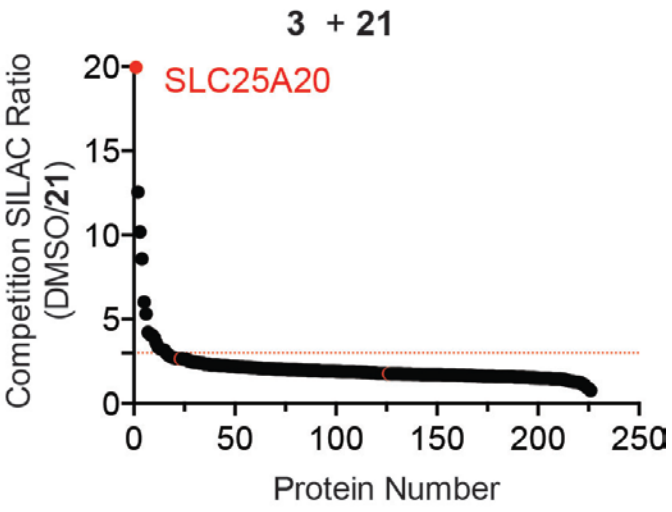
Fragment-Based Ligand Discovery in Living Cells

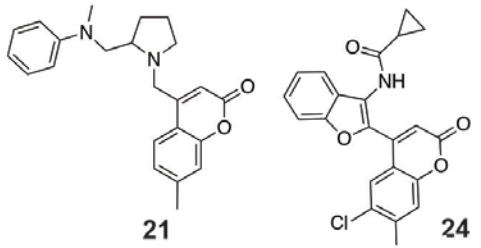


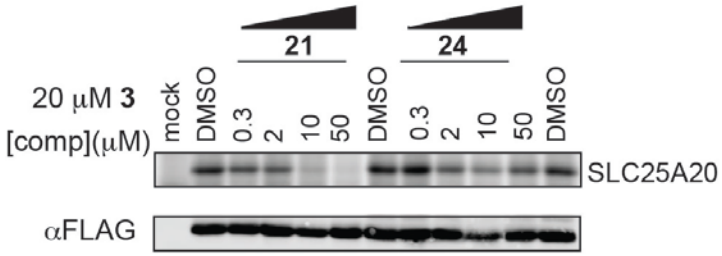


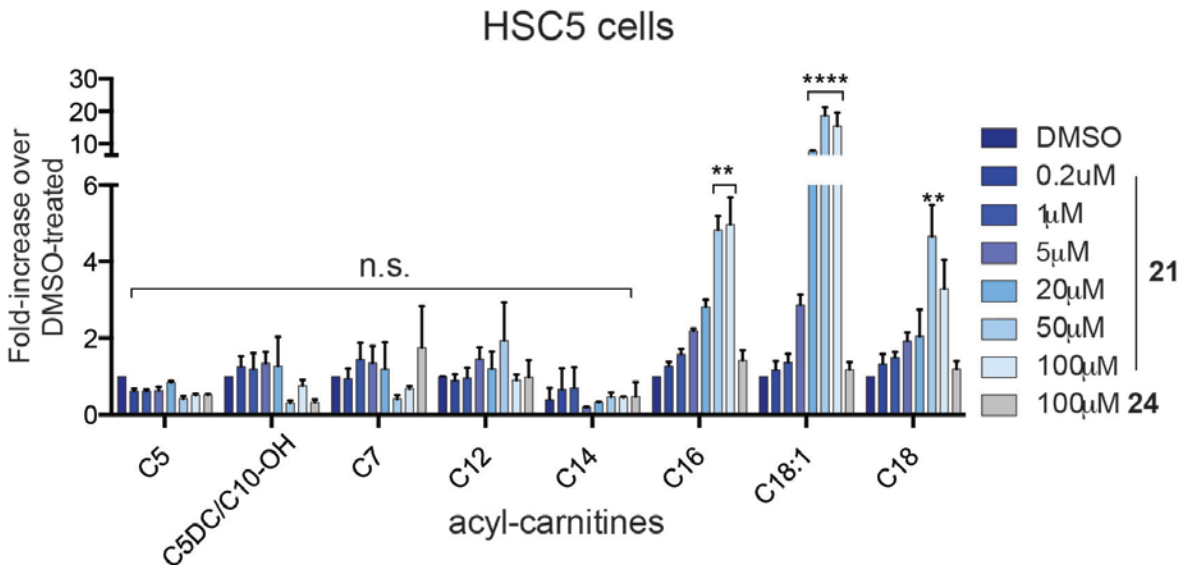


Discovery of an Inhibitor of the Mitochondrial Acylcarnitine Transporter SLC25A20

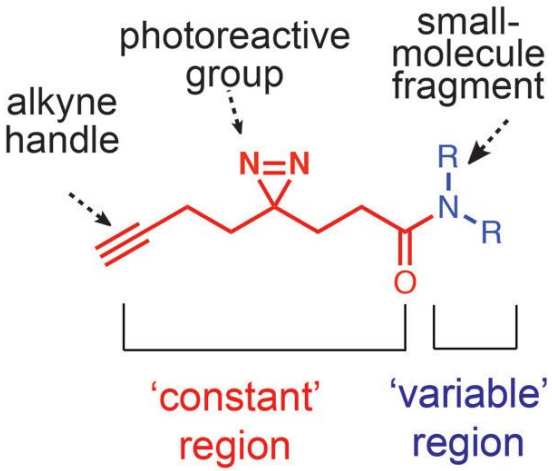


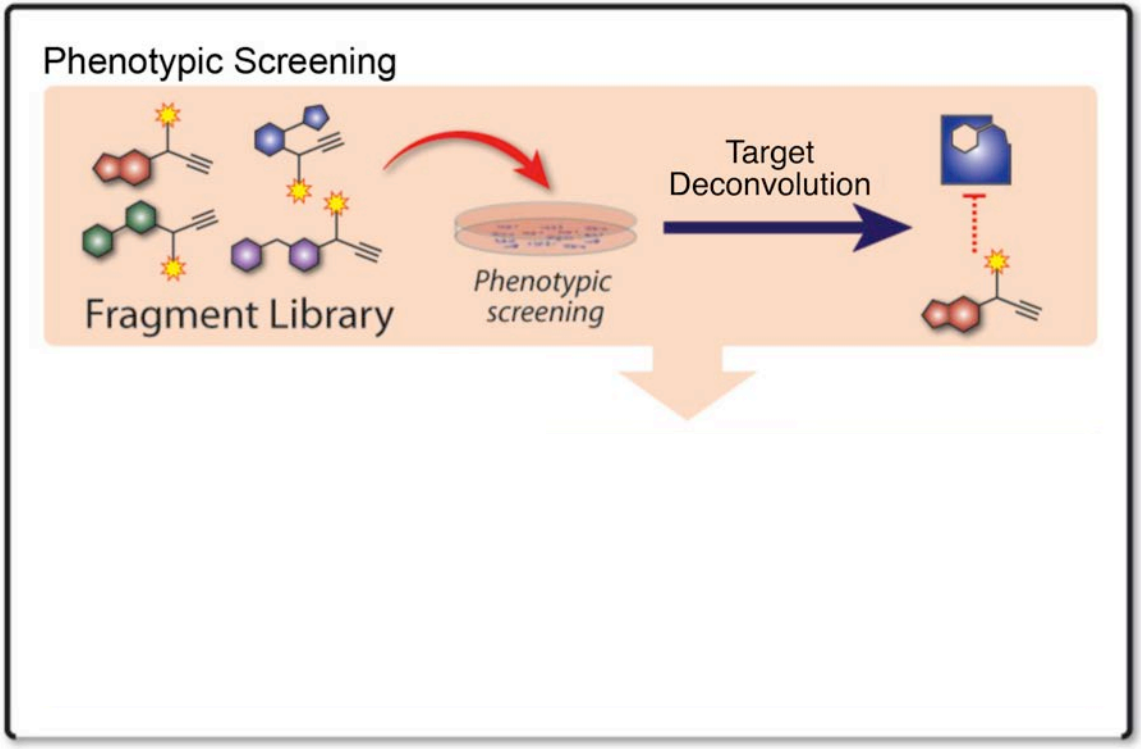




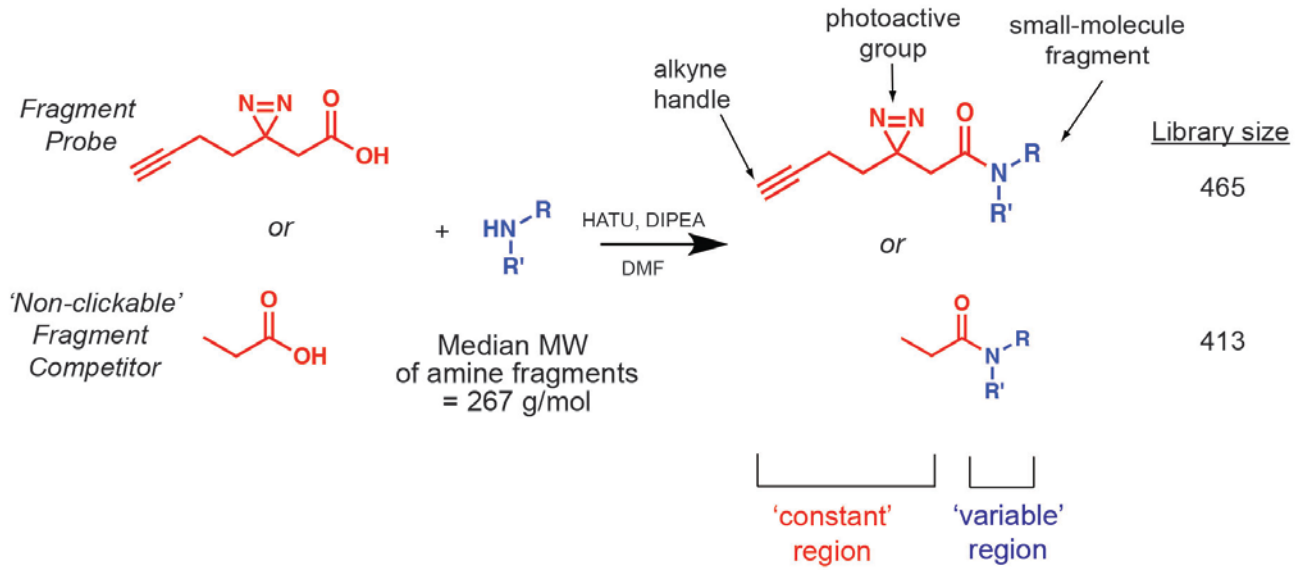


Proteome-Wide Non-Covalent Ligand Discovery with *Fully Functionalized Fragment* Probes

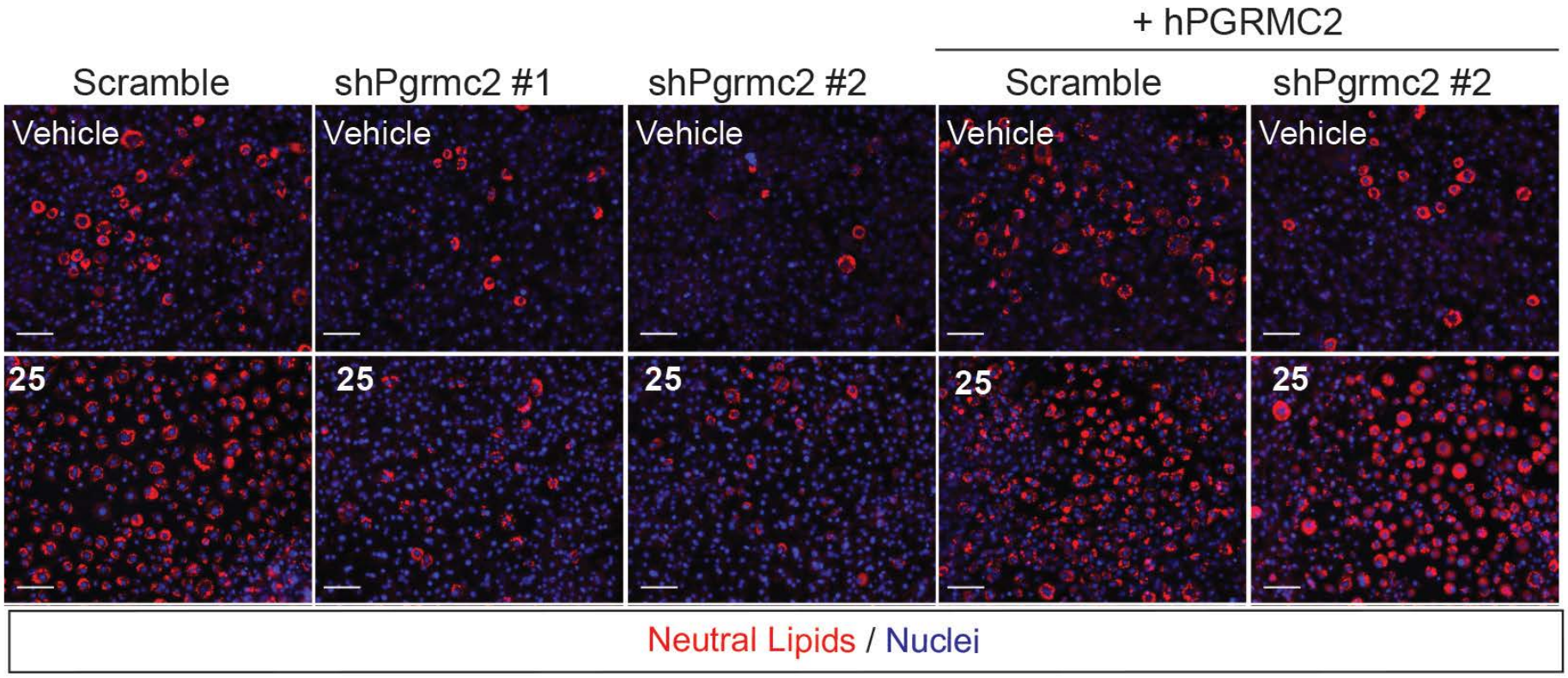




Phenotypic Screening w/ Fragment Library Identifies Novel Ligand-Protein Pathway that Regulates Adipogenesis



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

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