EnPlex: a high-throughput method for family-wide profiling of enzyme activity

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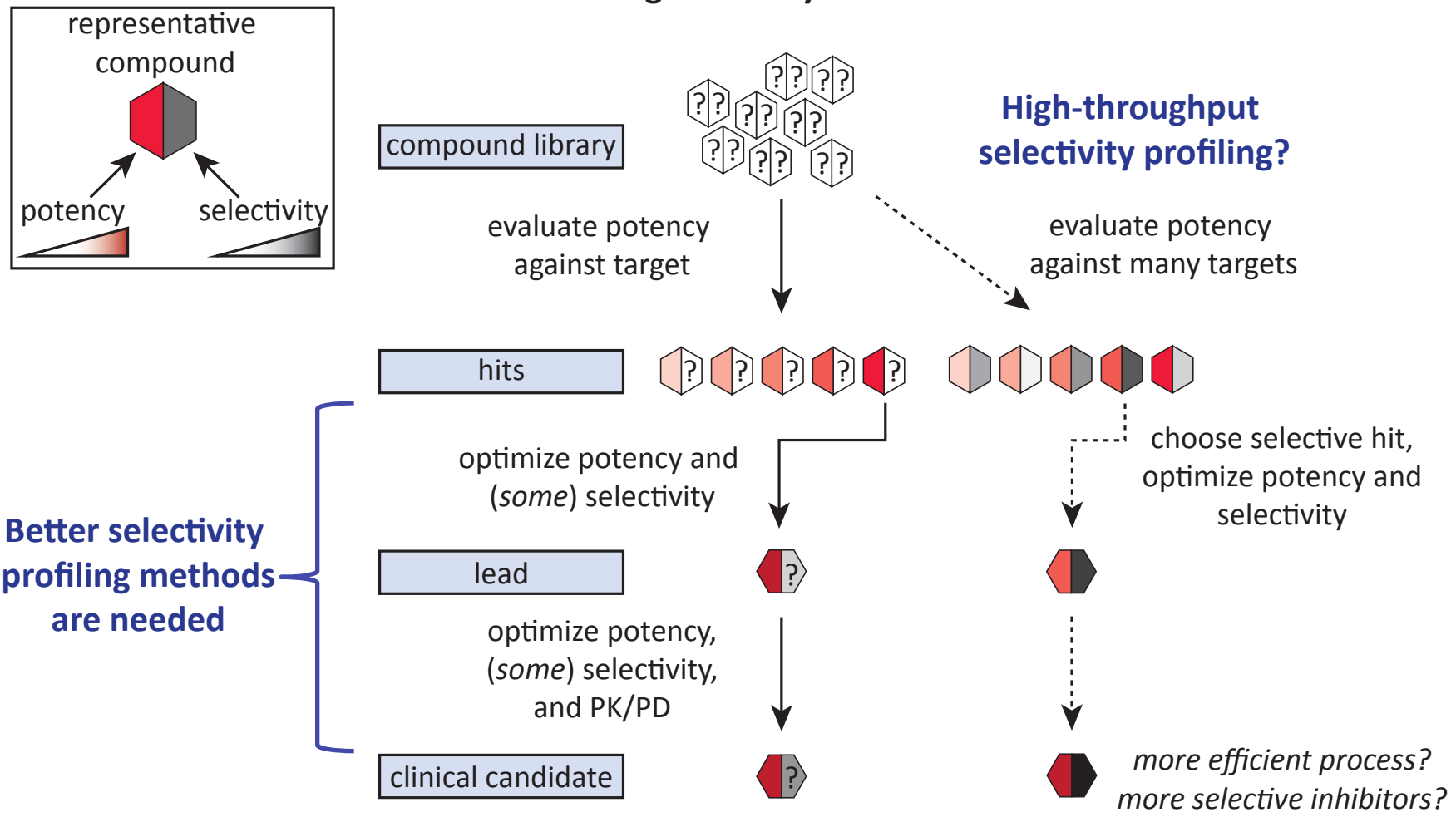
November 5, 2014

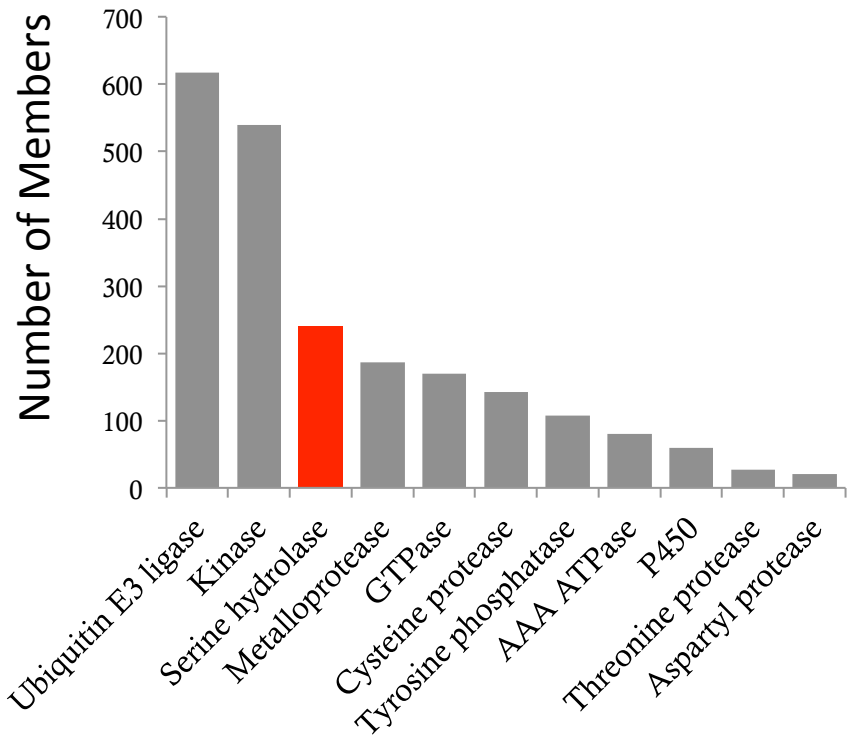
BROAD INSTITUTE

Potency typically drives drug discovery, selectivity follows

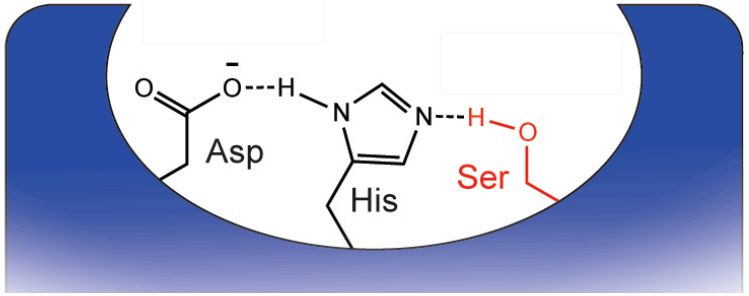


Selectivity determination is difficult due to the number and similarity of serine hydrolases

Major Human Enzyme Families



Serine hydrolases



- Involved in clotting, neural signaling, inflammation, cancer
- Currently 8 enzymes are targeted by 14 drugs
- Majority are uncharacterized

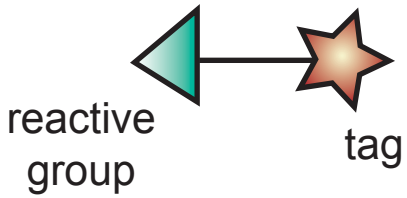
The ideal solution? A high-throughput, multiplexed enzyme activity assay

1. **Selectivity profiling** of drugs and chemical probes

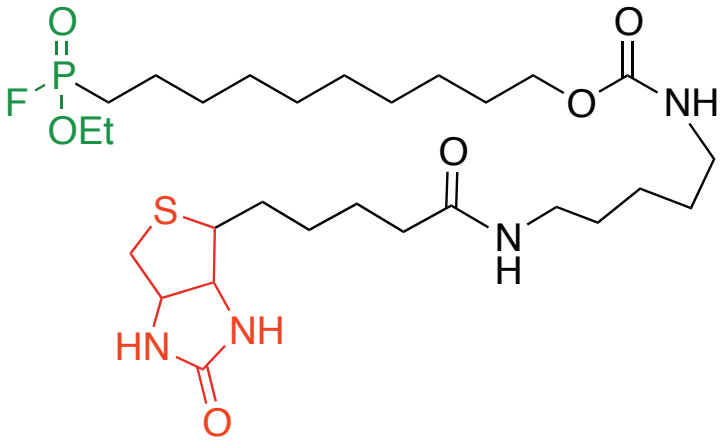
2. **Inhibitor discovery** based on both **potency** and **selectivity**

Activity-based protein profiling (ABPP): a general method to assay enzyme activity

activity-based probe



fluorophosphonate (FP)-biotin

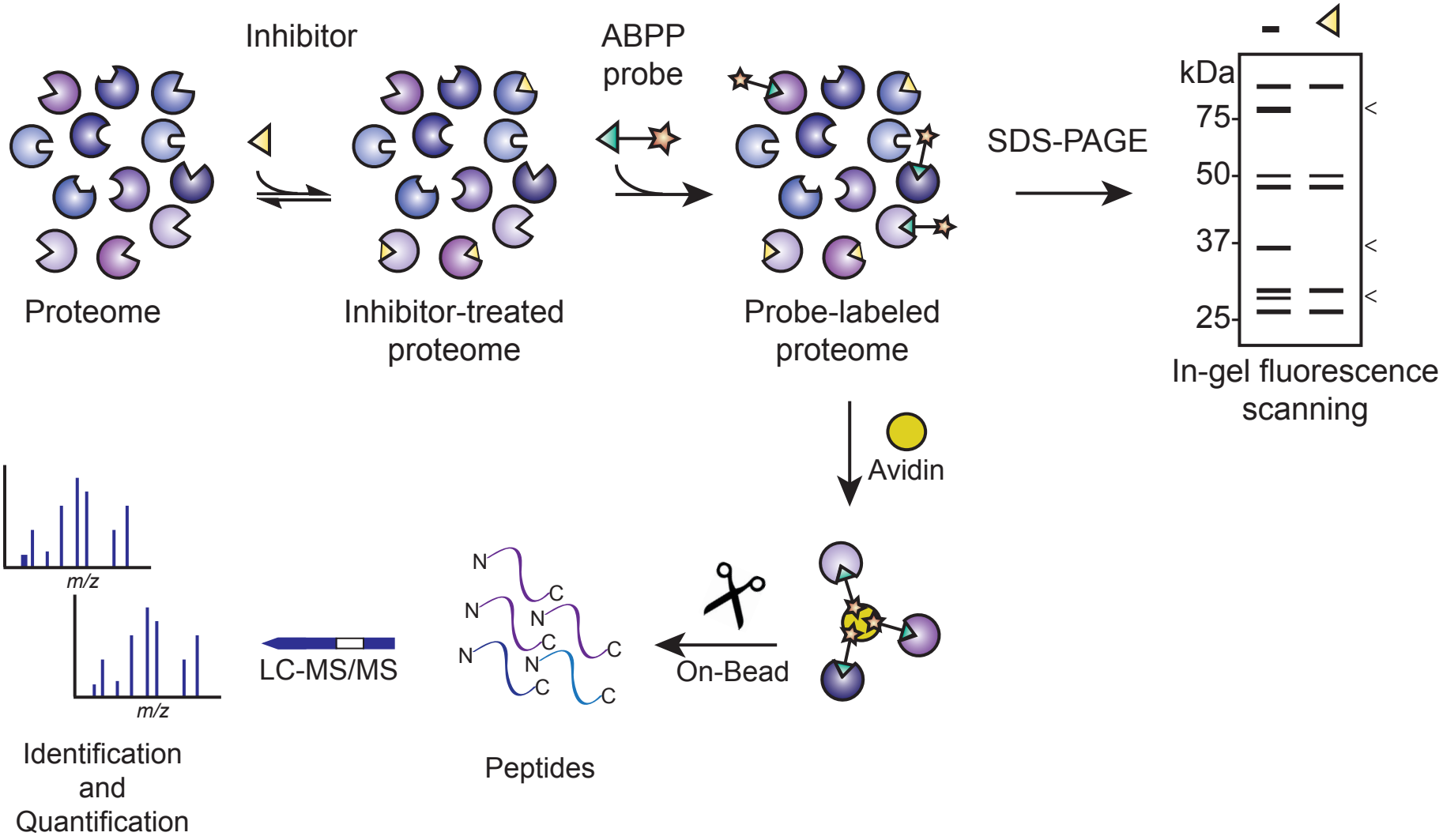


active serine hydrolase

probe-labeled serine hydrolase

Evans & Cravatt, Chem Rev, 2006

Competitive ABPP can be used for inhibitor discovery



Evans & Cravatt, *Chem Rev*, 2006

The advantages and limitations of ABPP for inhibitor discovery

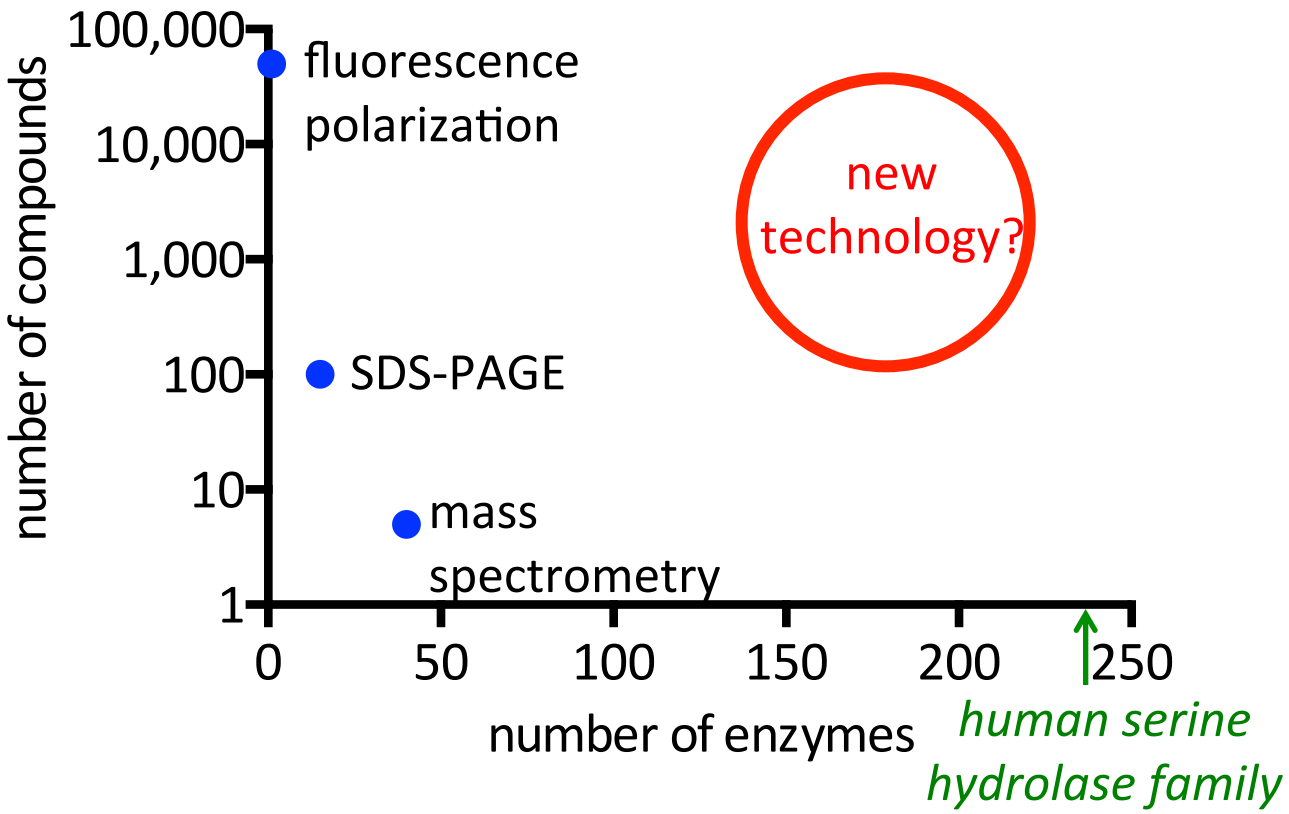
Advantages

- Does not require a specific substrate assay
- Can simultaneously evaluate multiple enzymes

Limitations

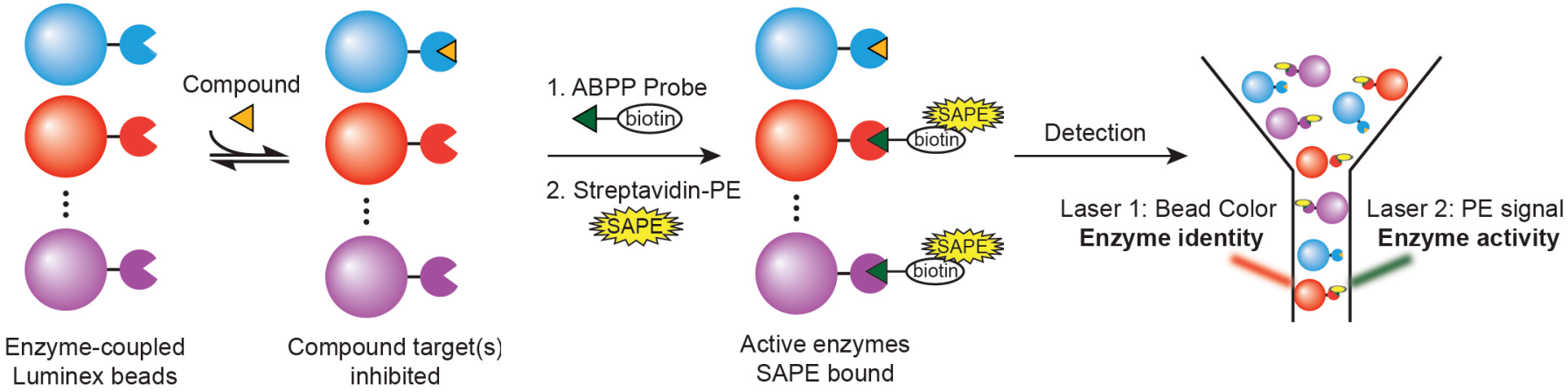
- Typically assays only a small number of enzymes
- Laborious detection methods severely limit throughput

Comparing ABPP screening platforms (for serine hydrolases)



Is a possible to simultaneously screen many enzymes in high-throughput?

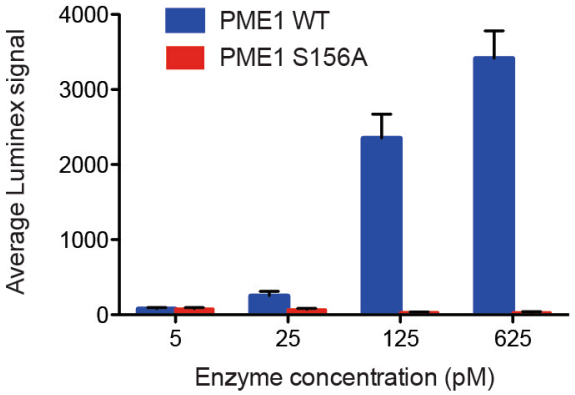
EnPlex: A multiplexed, bead-based platform for enzyme inhibitor screening



Bachovchin et al. *Nat Chem Biol*, 2014

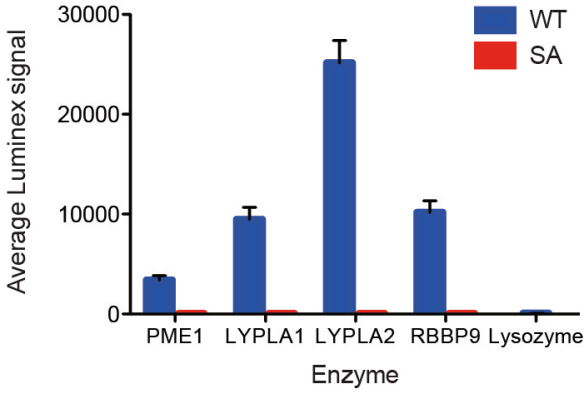
EnPlex is sensitive and robust assay for inhibitor discovery

Extraordinary sensitivity:



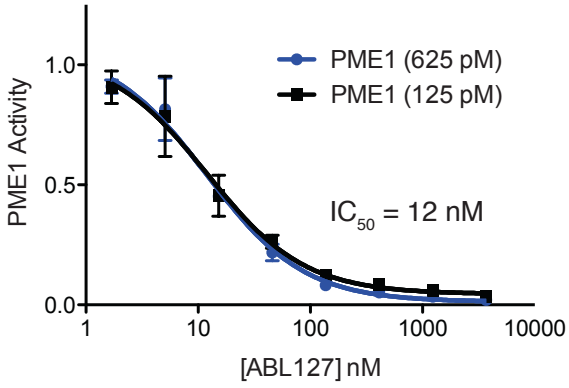
Requires **8,000-fold less protein** than fluopol-ABPP

Compatibility with many enzymes:



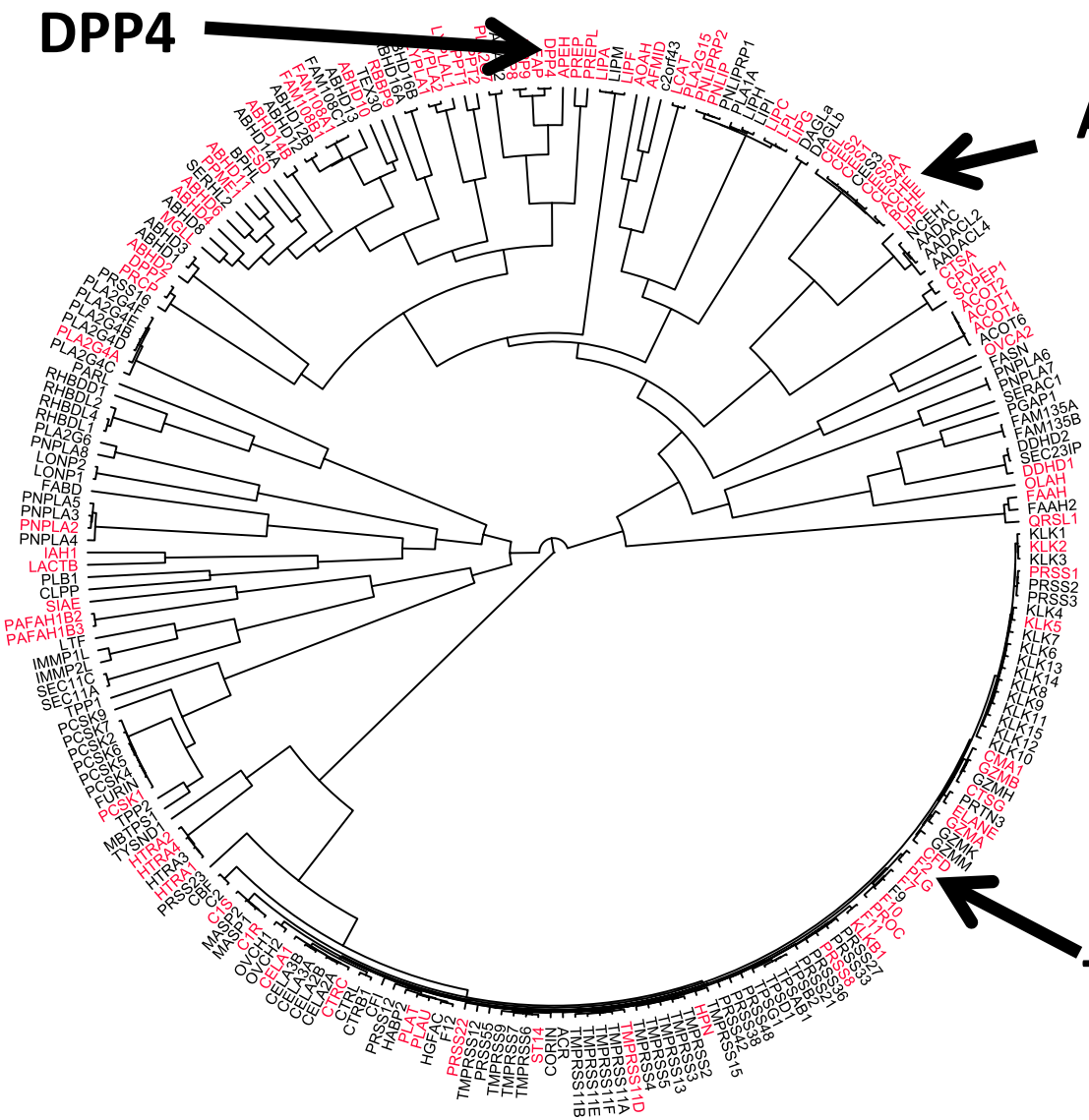
Catalytically dead enzymes give no signal

Works in a competitive format:



Accurate IC_{50} determination

Assembly of a diverse 97 human serine hydrolase panel

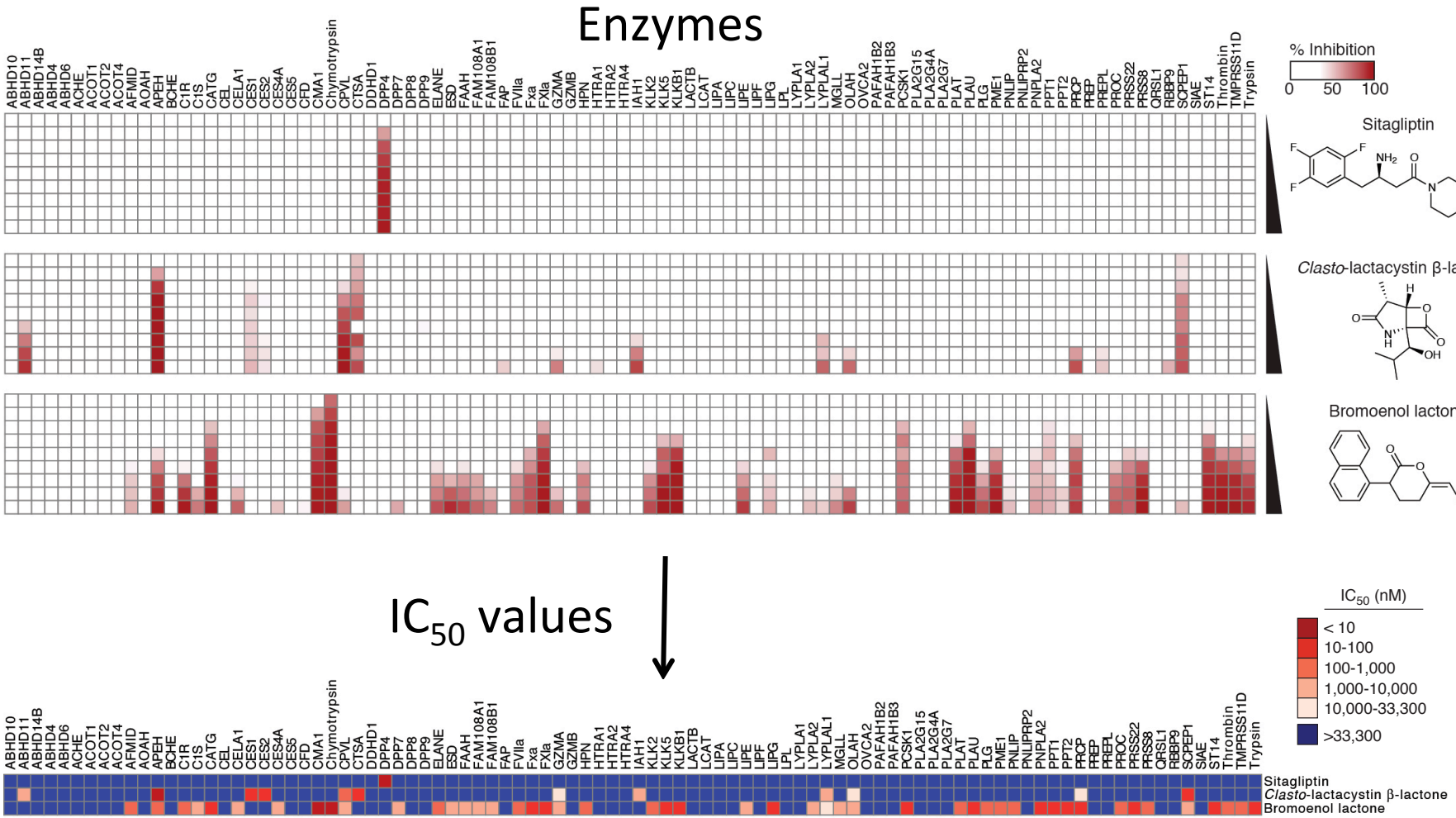


Uses of a high-throughput, multiplexed enzyme activity assay

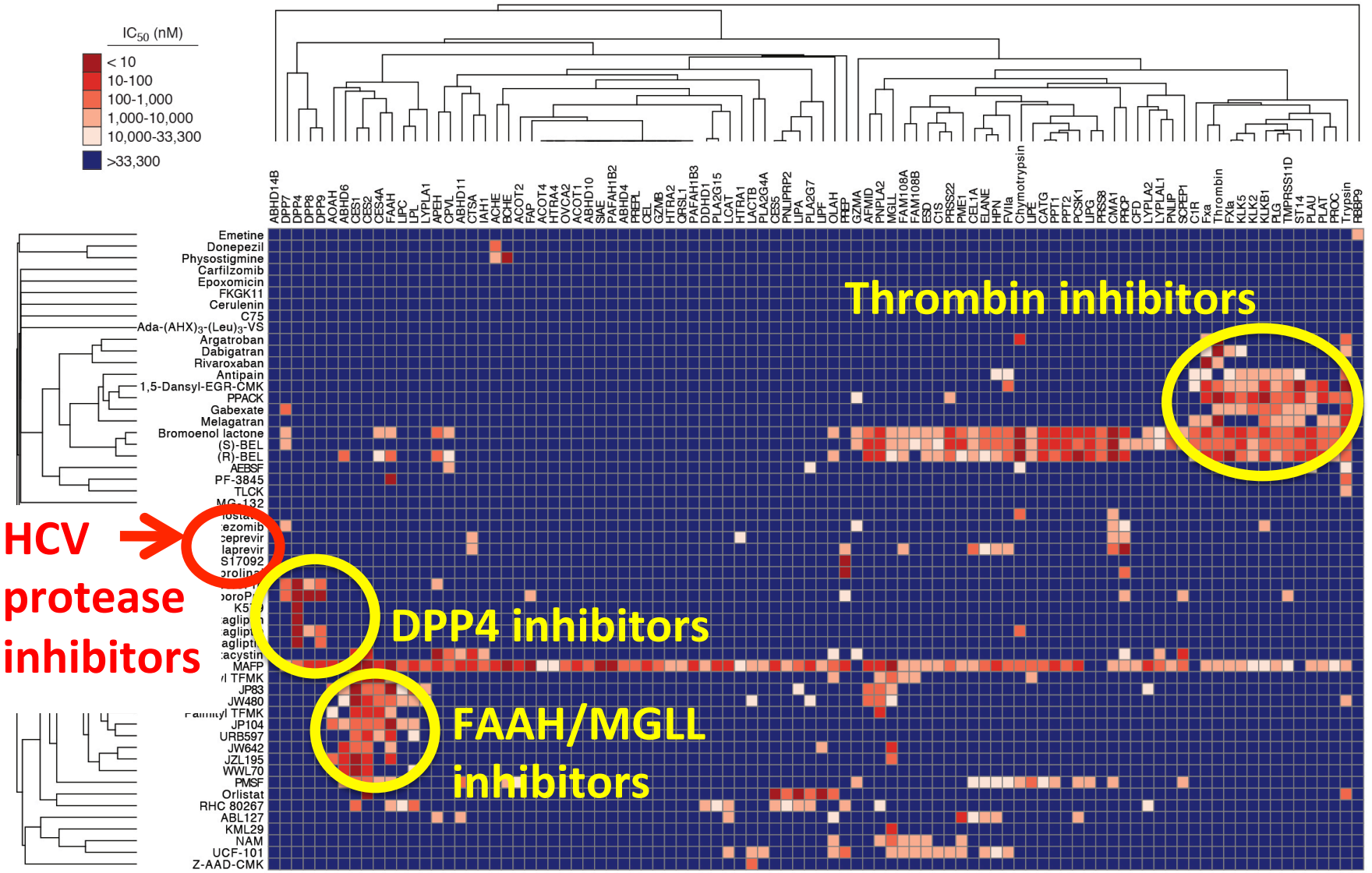
1. **Selectivity profiling** of drugs and chemical probes

2. **Inhibitor discovery** based on both **potency** and **selectivity**

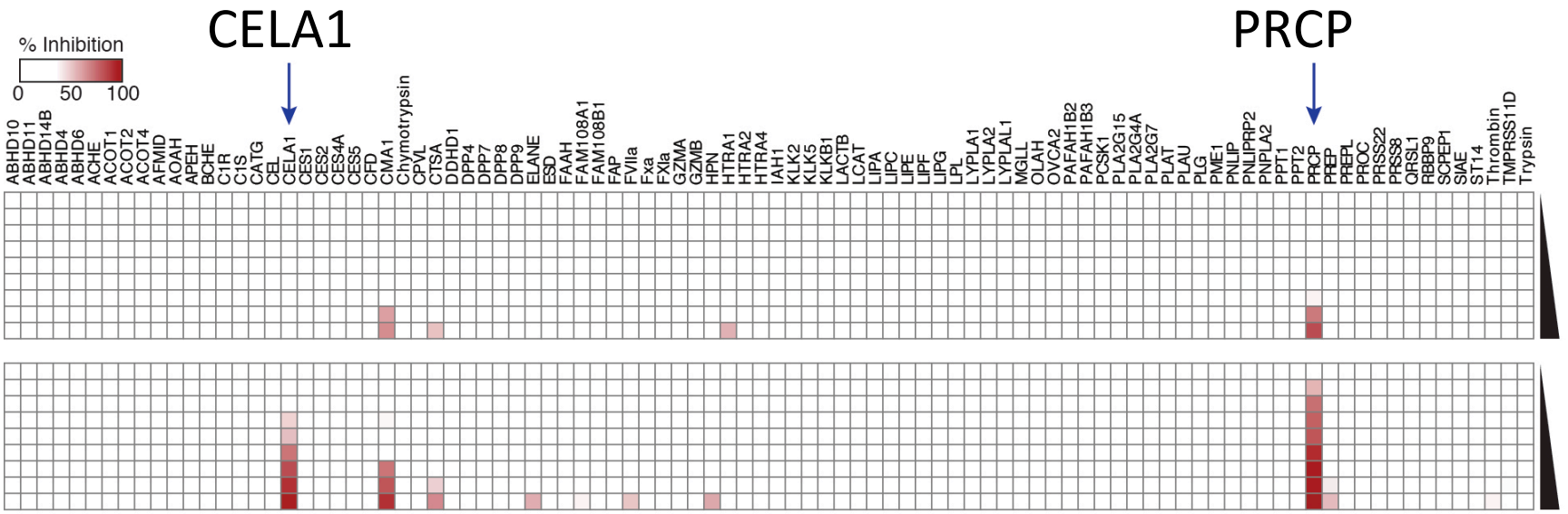
Representative EnPlex data of known inhibitors

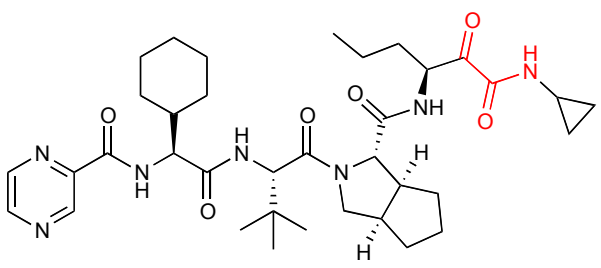


First family-wide selectivity profiling of commonly used inhibitors

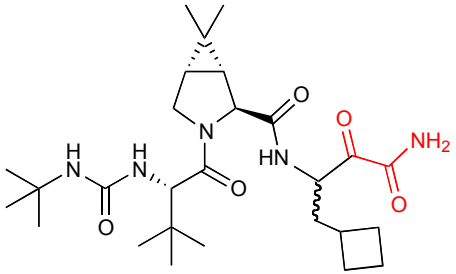


CELA1 and PRCP are previously unknown off-targets of telaprevir



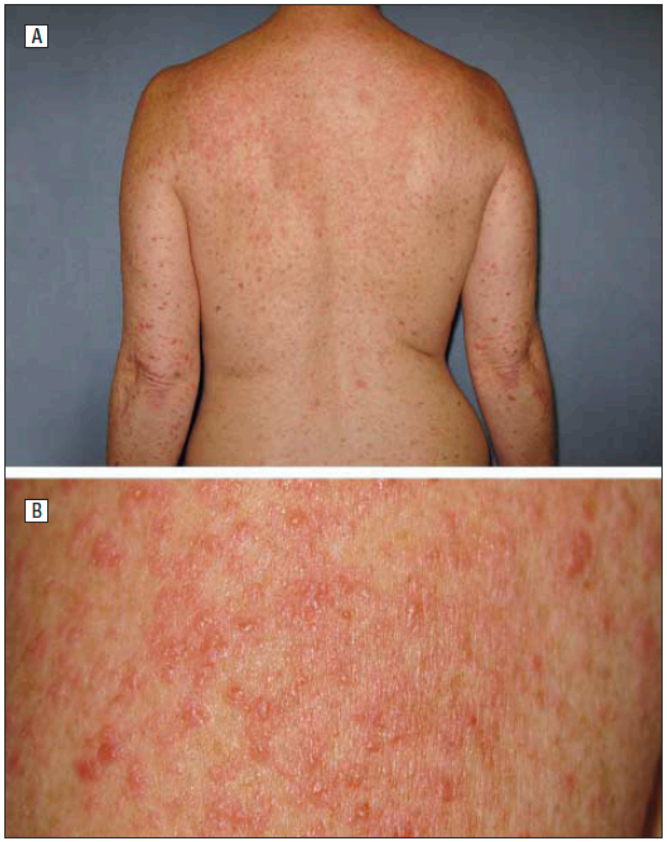


Telaprevir
(Incivek)



Boceprevir
(Victrelis)

Telaprevir is associated with a severe rash that is not currently understood



Roujeau et al. JAMA Dermatol, 2013

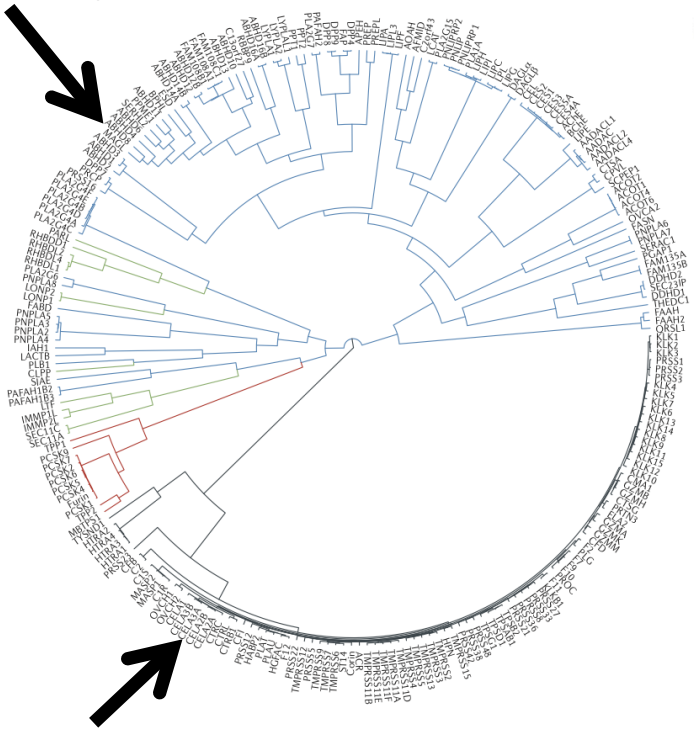
- Telaprevir received a Black Box Warning from the FDA in December 2012
- PRCP and CELA1 possible mediators?

  -CELA1 is only expressed in skin, function is poorly understood

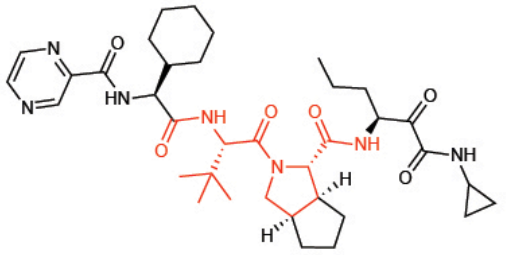
  -PRCP globally expressed, regulates several bioactive peptides

Telaprevir-PRCP interaction underscores the value of family-wide profiling

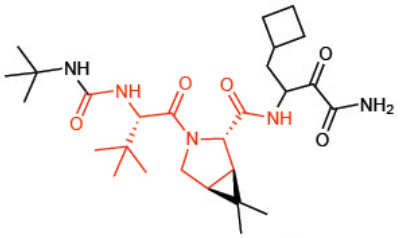
PRCP: α/β hydrolase fold,
cleaves after proline



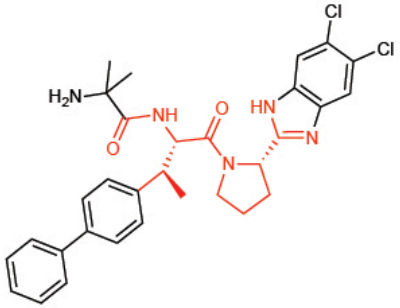
NS3 protease and CELAs:
chymotrypsin-like folds,
cleave after hydrophobic residues



Telaprevir
(Vertex)

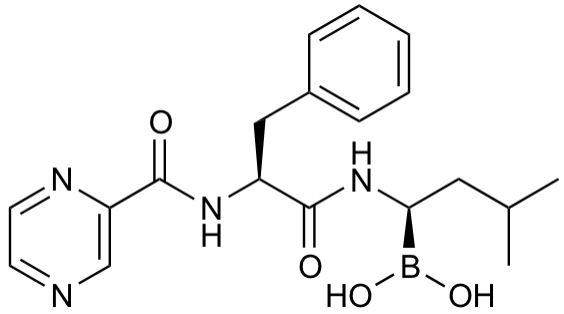


Boceprevir
(Merck)

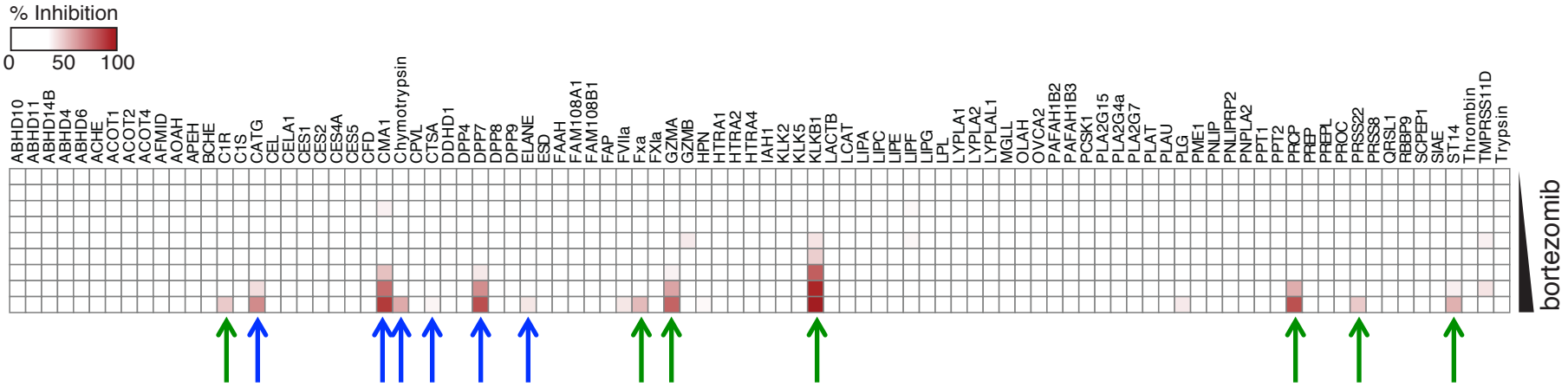


PRCP inhibitor "8o"
(Merck)

Bortezomib highlights comprehensiveness of EnPlex compared to existing methods



Bortezomib, first-in-class proteasome inhibitor approved for multiple myeloma



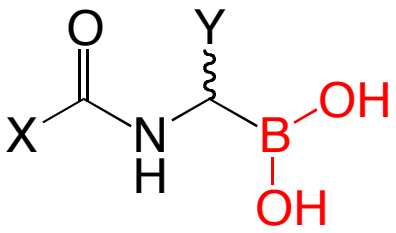
- Previous studies discovered 6 serine protease targets
- EnPlex found those 6, plus 7 additional targets

Uses of a high-throughput, multiplexed enzyme activity assay

1. **Selectivity profiling** of drugs and chemical probes

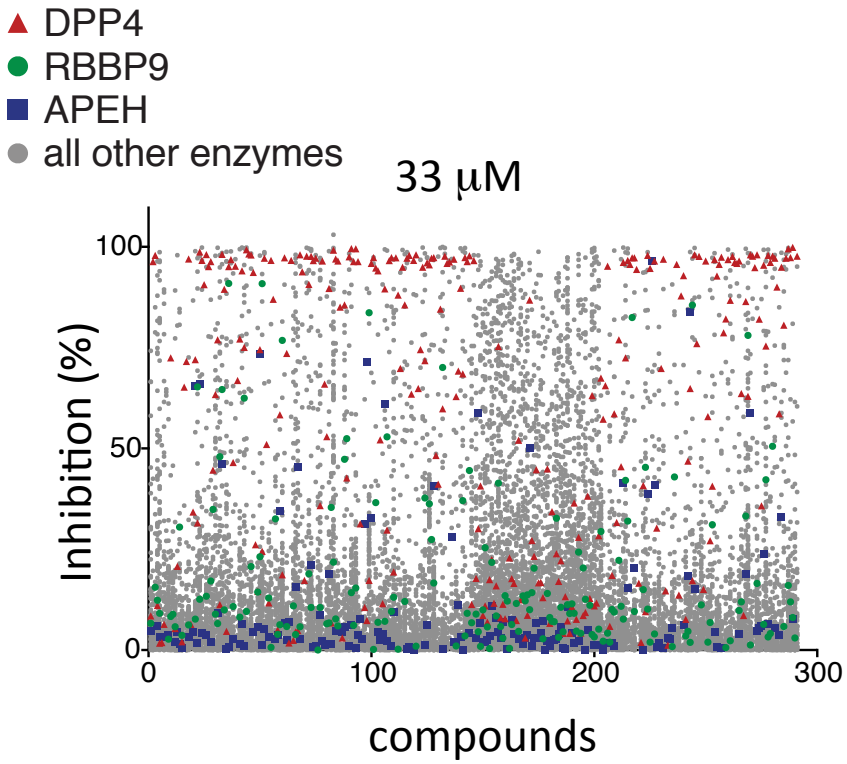
2. **Inhibitor discovery** based on both **potency** and **selectivity**

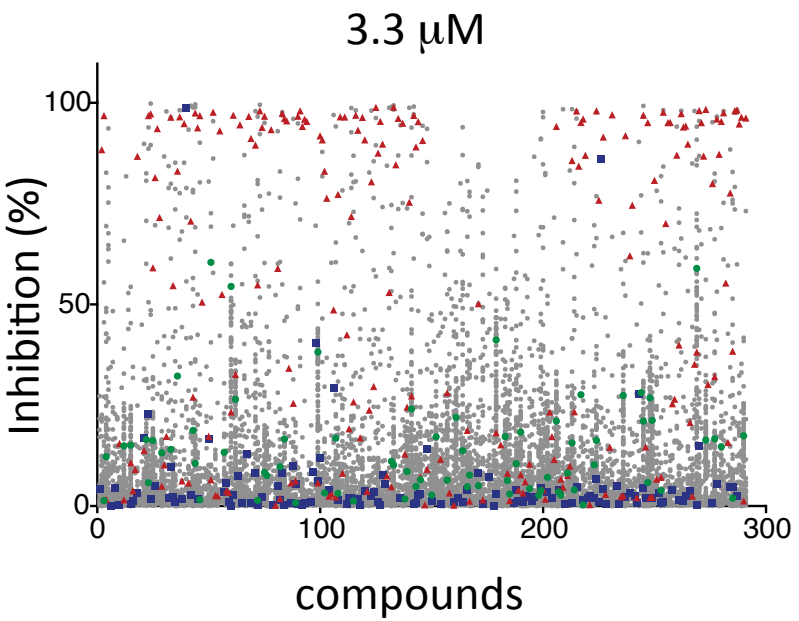
Inhibitor discovery from a boronic acid library



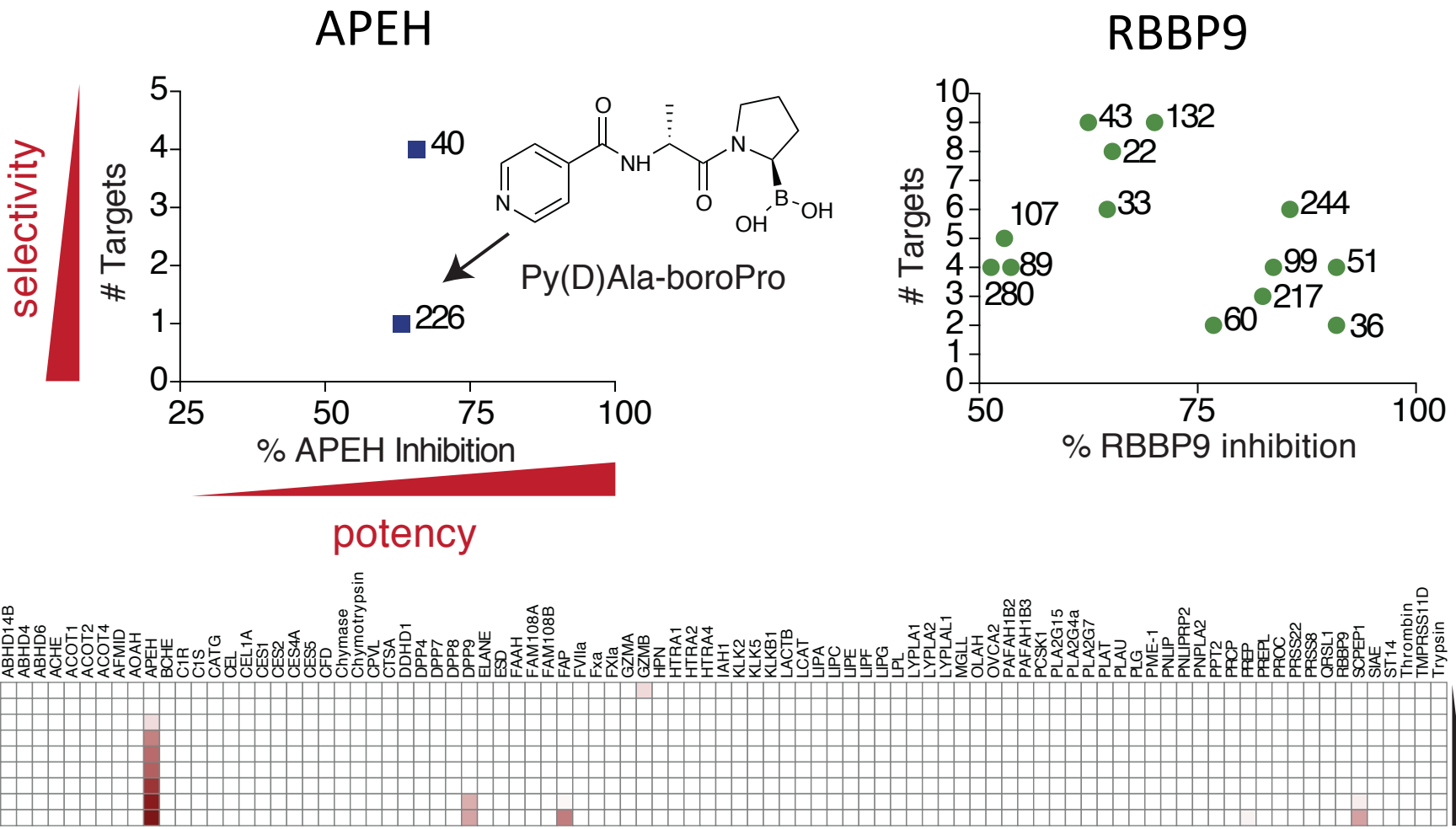
~600 boronic acids

Boronic acids drive **potency**, but achieving **selectivity** is challenging

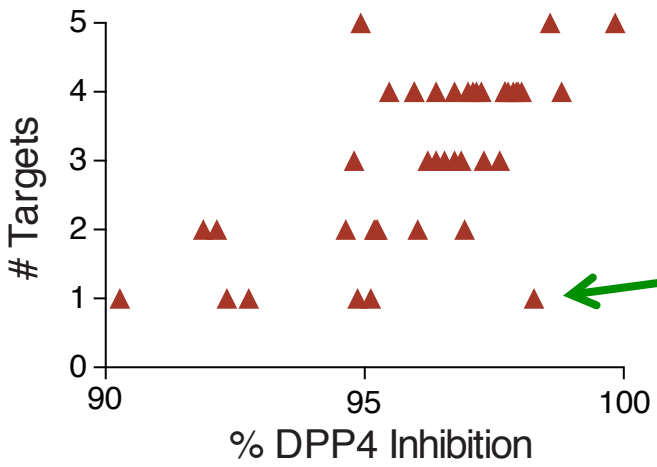


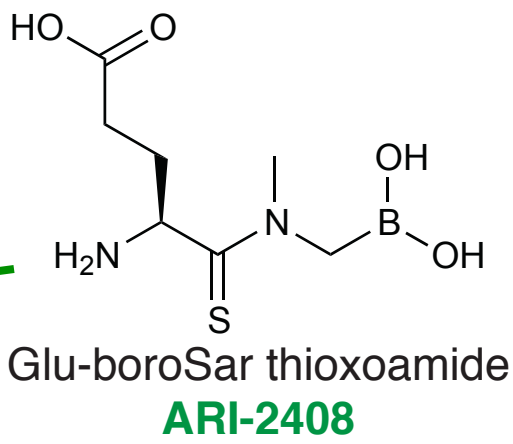


Examples of lead inhibitors: APEH and RBBP9



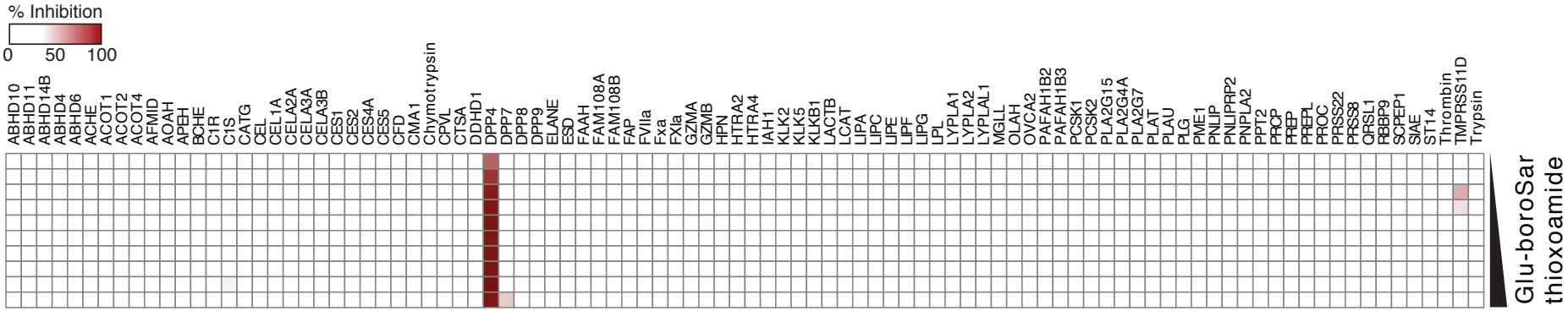
Glu-boroSar thioxamide (ARI-2408): A remarkably selective DPP4 inhibitor





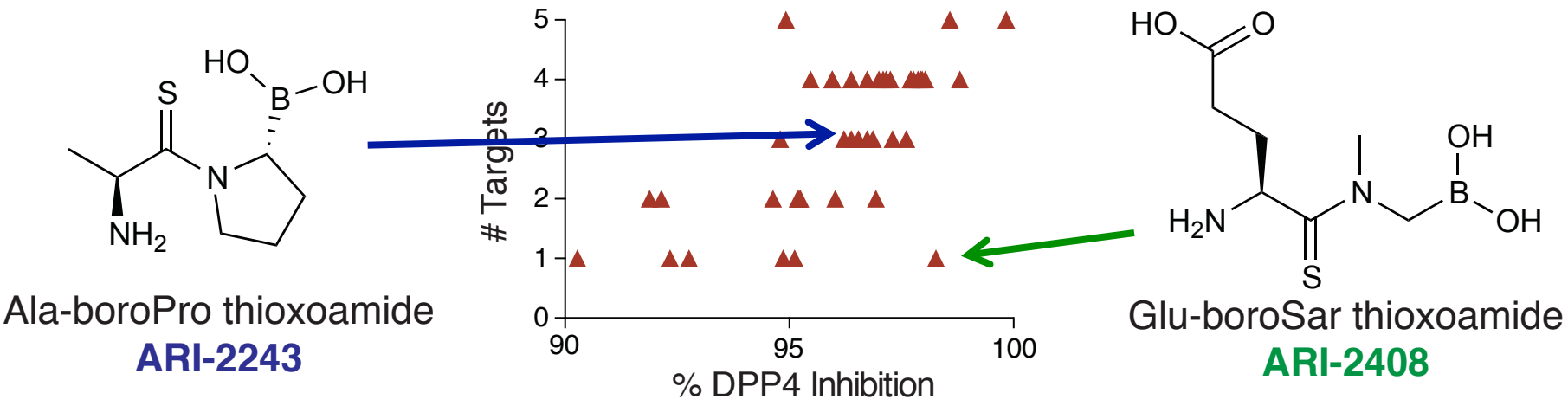
Glu-boroSar thioxoamide

ARI-2408

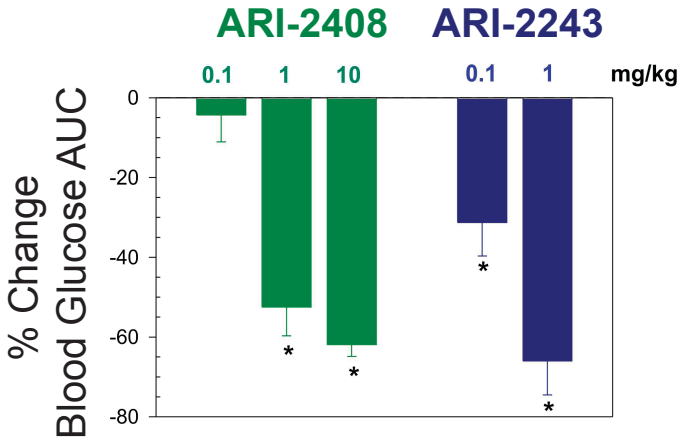


Demonstrates boronic acids can be selective inhibitors

ARI-2408 *vs.* ARI-2243: Similar in efficacy, but dramatically different in safety



Similar efficacy in OGTT:



ARI-2408 safe, ARI-2243 toxic:

Animals with adverse events (e.g., skin lesions, edema, or death)			
Dose (mg/kg)	ARI-2408	ARI-2243	Sitagliptin
0.3	-	5/12	
1.0	-	11/12	-
3.0	-	9/12	-
100.0	0/4	-	0/6
300.0	0/6	-	-

Summary of the EnPlex technology and its applications

EnPlex: a multiplexed, high-throughput enzyme activity assay that can profile **100s of enzymes** against **1000s of compounds**

Applications:

- Aid lead selection and optimization in drug discovery (DPP4, APEH, RBBP9)
- Profile existing drugs for selectivity (sitagliptin, bortezomib)
- Discover off-target interactions mediating toxicity (telaprevir)
- Repurpose drugs and probes
- Mechanistic insights into enigmatic biology

Acknowledgements

Todd Golub

Golub Lab:
Luke Koblan
Jason Barnett
Jadwiga Grabarek
David Peck
Channing Yu
John Davis

NIH/NIAID:
Robert Munford

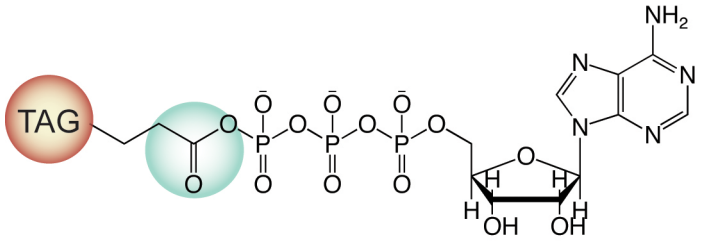
Benjamin Cravatt

Cravatt Lab:
Katsunori Tsuboi
Shasha Ji
Jon Long

Tufts Medical School:
Jack Lai
Wengen Wu
Sarah Poplawski
William Bachovchin
David Sanford

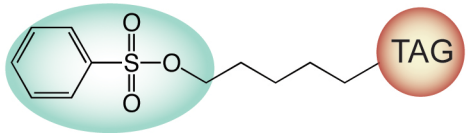
Current Activity-Based Probe Repertoire

- **Directed approaches**
  - **Cysteine proteases (Bogyo et al)**
  - **Metalloproteases (Cravatt et al, Yao et al)**
  - **Kinases, Phosphatases (Activx, Taunton, Zhang)**
  - **Histone deacetylases (Cravatt et al)**
  - **Cytochrome P450s (Cravatt et al)**
  - **Protein Arginine Deiminases (Thompson et al)**
  - **Protein deubiquitinases (Kessler et al)**



BHAcATP probe (Kinases)

- **Non-directed approaches (Cravatt et al)**
  - **Alcohol/aldehyde dehydrogenases**
  - **Enoyl hydratases**
  - **Epoxide hydrolases**
  - **Glutathione S-transferases**
  - **Oxidoreductases**
  - **Transglutaminases**



Phenyl sulfonate probe (activated cysteines; GSTO1)