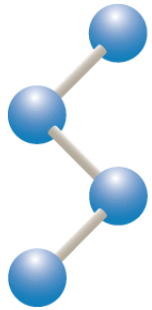


THE SCRIPPS RESEARCH INSTITUTE

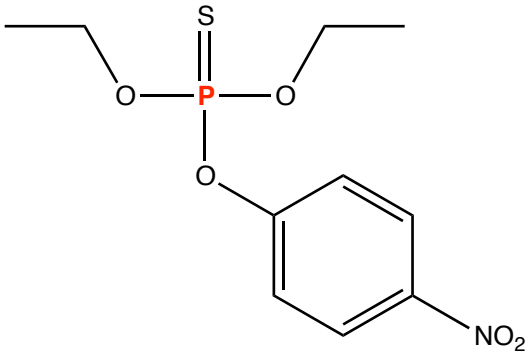
Computational design of catalytic triad-based organophosphate capture proteins

Chu Wang
The Cravatt Laboratory
The Scripps Research Institute, La Jolla
RosettaCON 2013

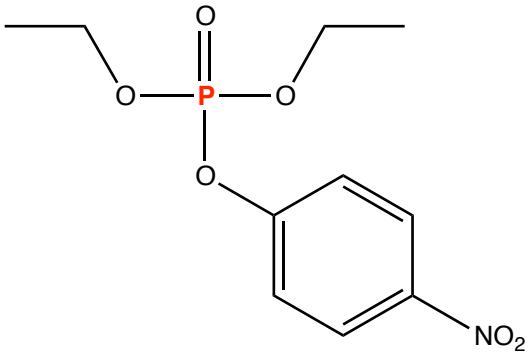
In Collaboration with Dr. Sridharan Rajagopalan, Baker Lab, UW

Organophosphates (OPs) are Highly Toxic

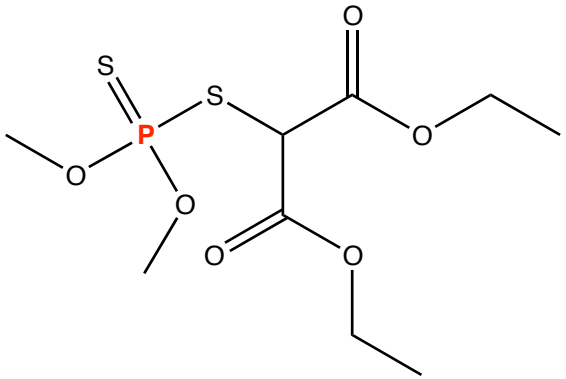
Pesticides



Parathion

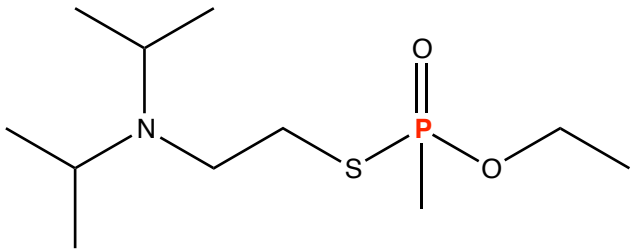


Paraoxon

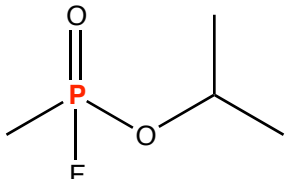


Malathion

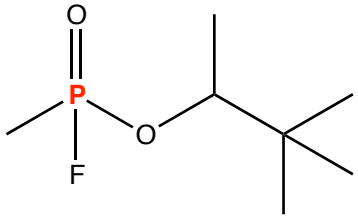
Nerve agents



VX

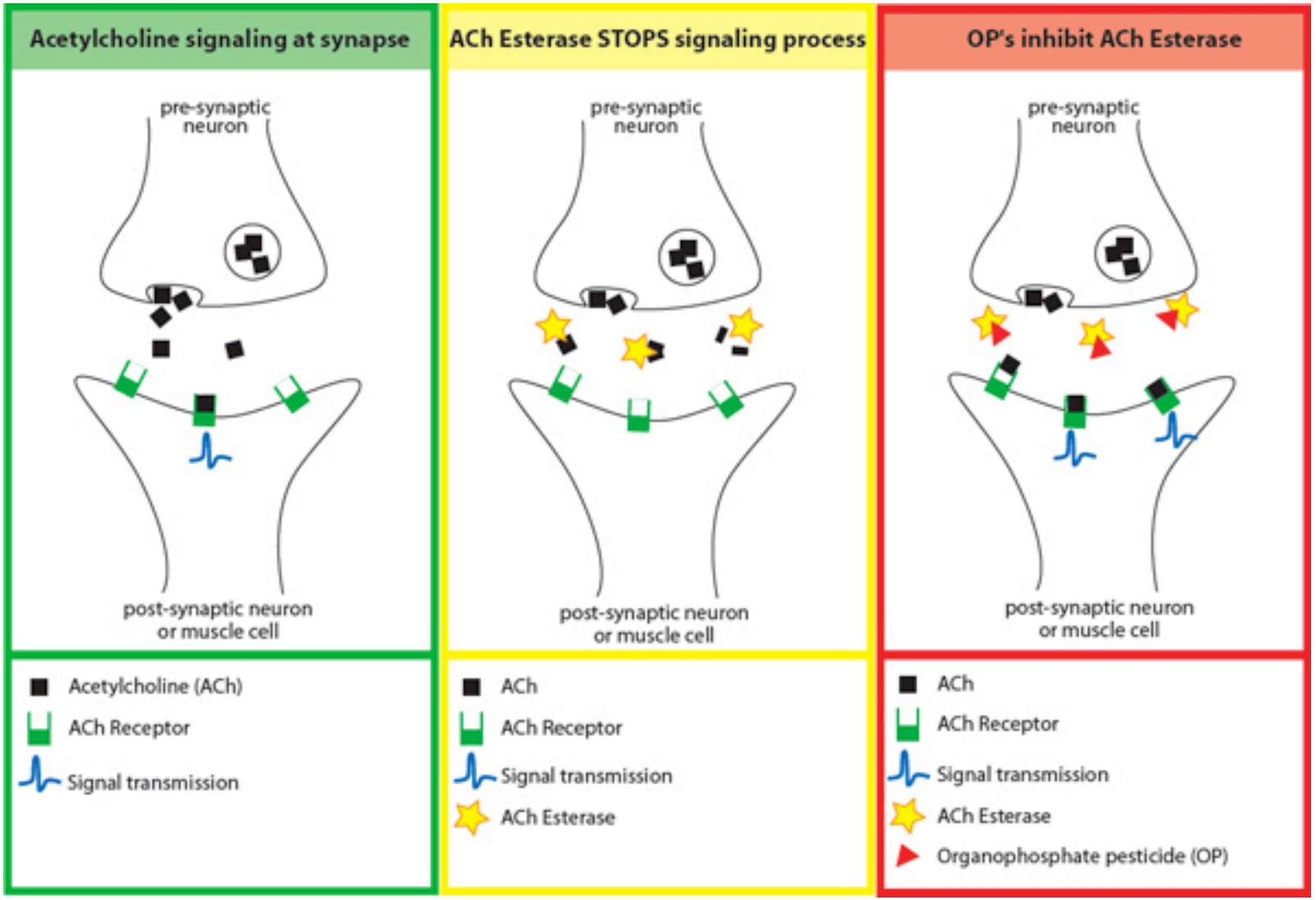


Sarin



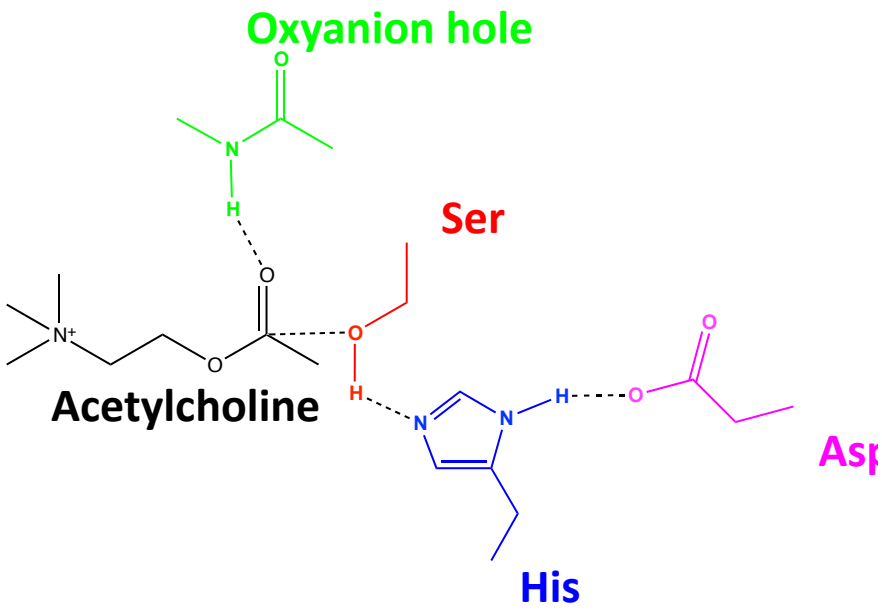
Soman

Molecular Target of OP Toxicity – Acetylcholine Esterase (AChE)

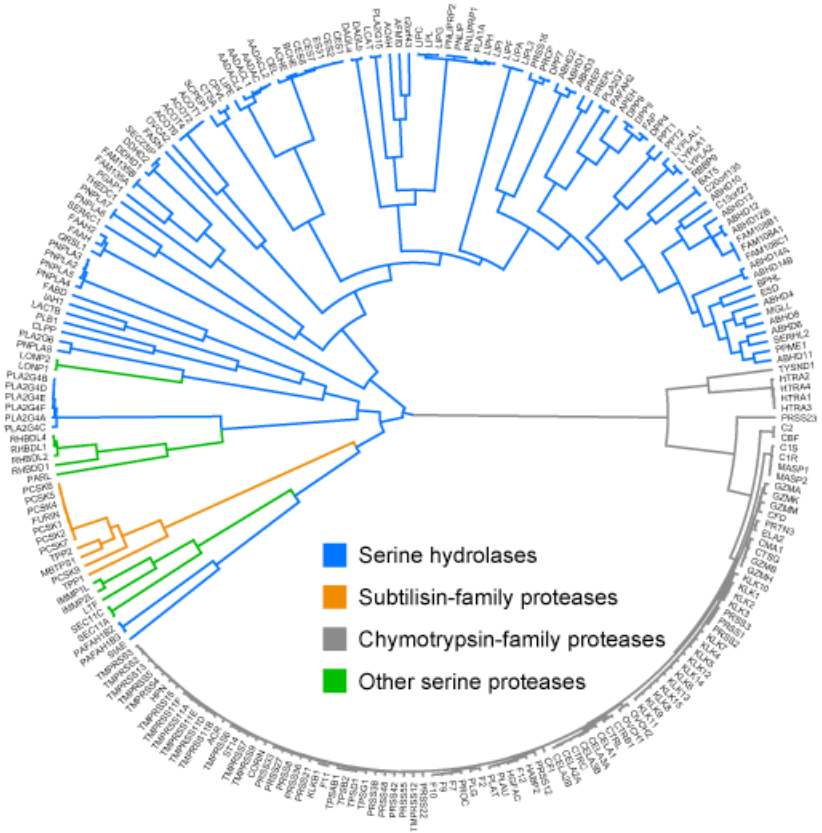


AChE -- an Essential Serine Hydrolase (SH) with a Conserved Catalytic Triad-based Active Site

Catalytic Triad

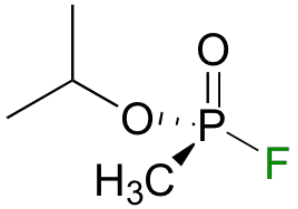


Serine Hydrolase Family Tree

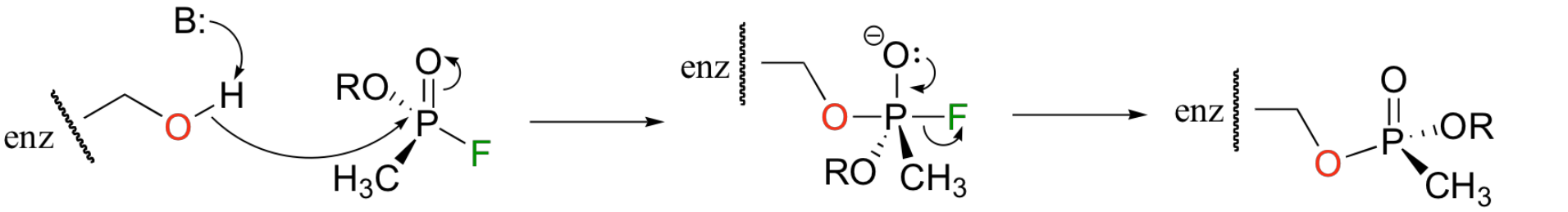


> 200 enzymes, ~ 1% of proteome
Proteases, esterase, lipase etc

Electrophilic OPs are Potent Inhibitors of SHs by Covalently Modifying the Serine Nucleophile



sarin



disabled acetylcholinesterase

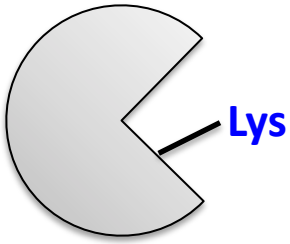
Bioscavengers to Prevent OP Poisoning

- Recombinant human butyrylcholinesterase (BChE) to sequester highly toxic OPs
  - 65kDa, large amounts required for stoichiometric inhibition.
  - 350 mg of human BCHE per 1 mg of cyclosarin.
  - Unwanted hydrolysis of endogenous esters, leading to imbalance of these metabolites in blood.

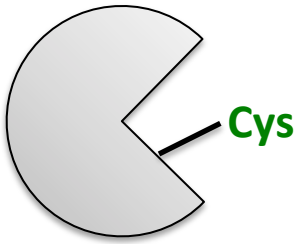
- *De Novo* Designs of proteins with activated serine nucleophiles as a new panel of OP scavenger agents
  - Smaller and diverse scaffolds
  - Specific for individual OP scavenging and detection
  - Controllable activity against endogenous substrates

- Towards design of *de novo* serine hydrolases.

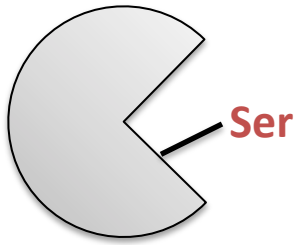
Active-Site Nucleophiles in Native Enzymes



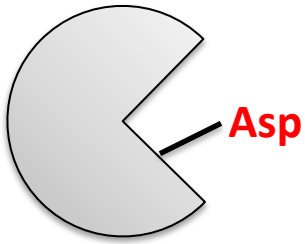
Aldolase



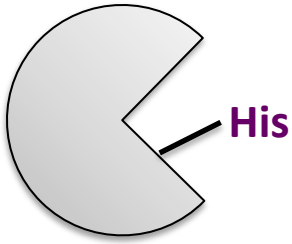
Cathespin



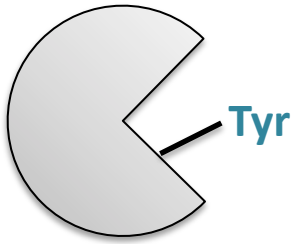
Trypsin



ATPase

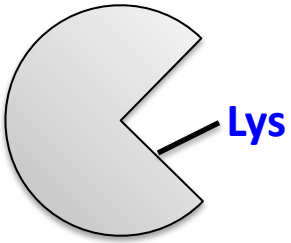


phosphokinase

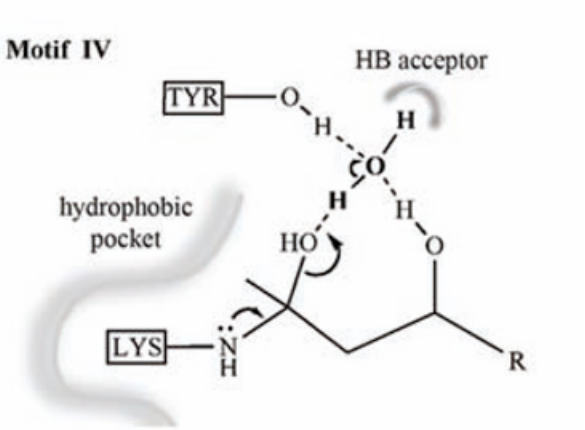


Topoisomerase

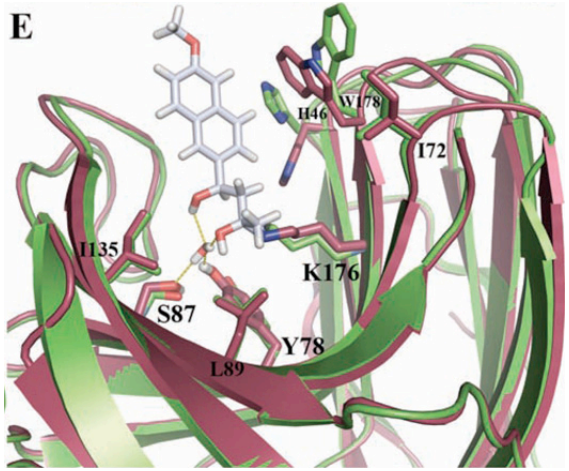
Active-Site Nucleophiles in Previous *De Novo* Designed Enzymes

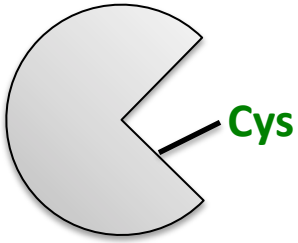


RA series
by Jiang et al.
Science, 2008

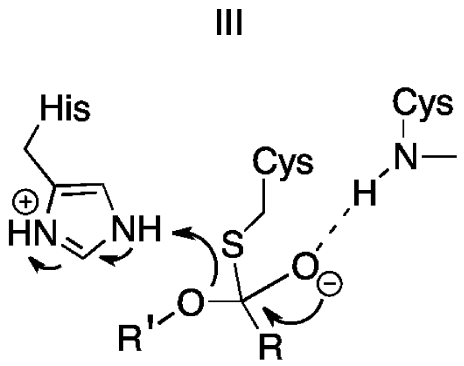


Buried Lysine

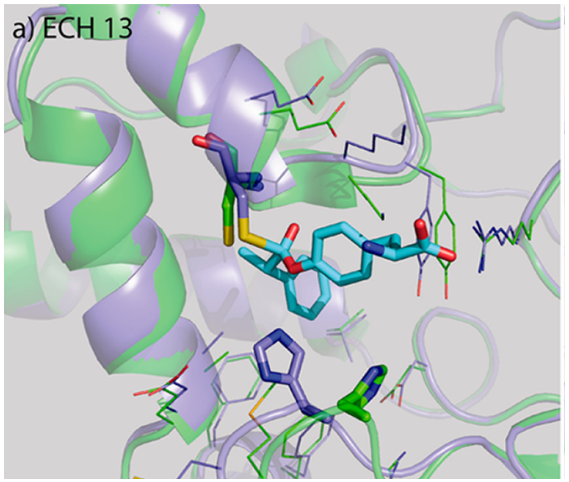




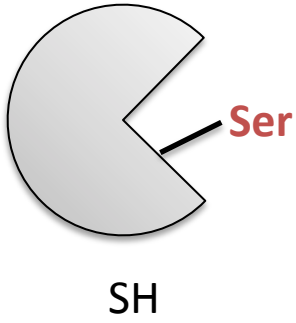
ECH series
by Richter et al.
JACS, 2012

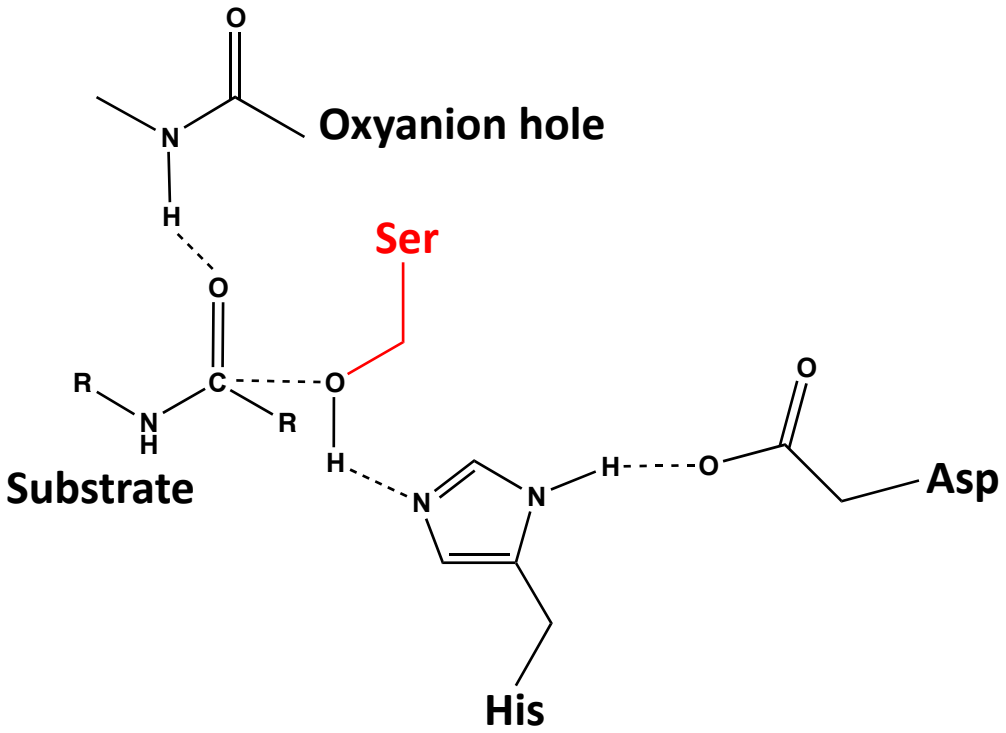


Cys-His Dyad



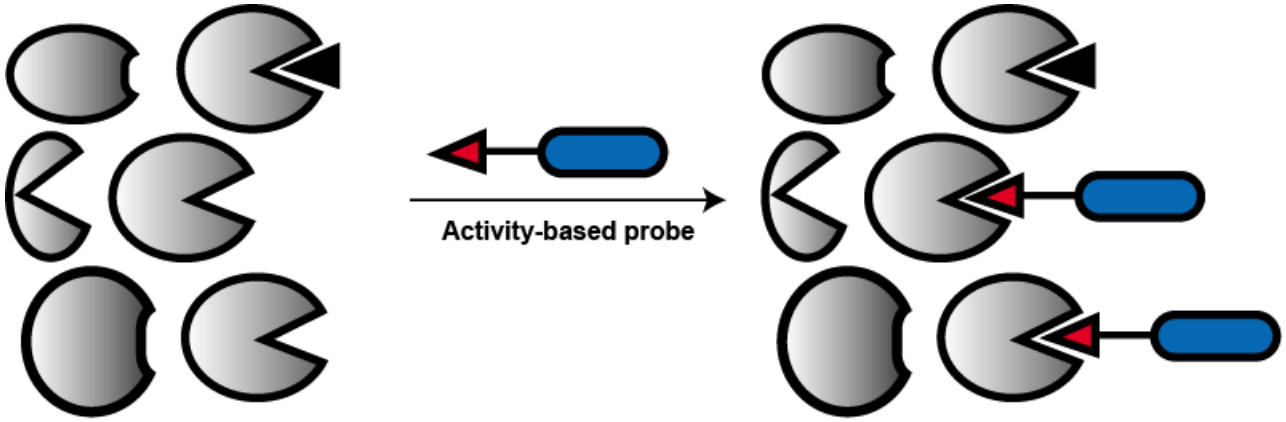
Challenges in Designing a Serine Nucleophile

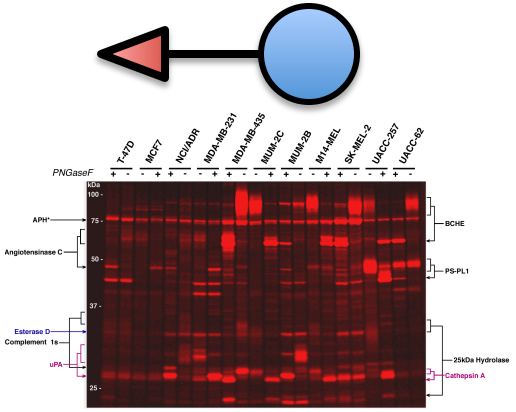




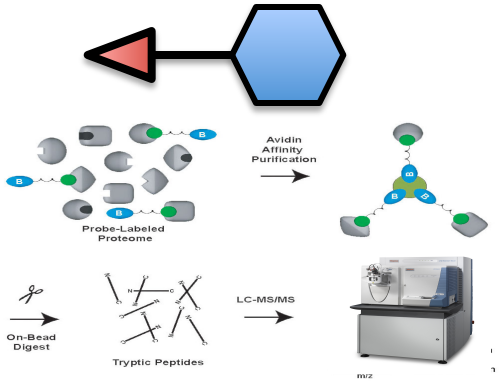
- Serine hydroxyl has a pKa of ~13 (vs. 8.00 for Cys and 10.5 for Lys)
- Requires precise designed interactions within the catalytic triad to activate
- How to screen and pick designs with activated serine nucleophile???

Activity-based Protein Profiling (ABPP)

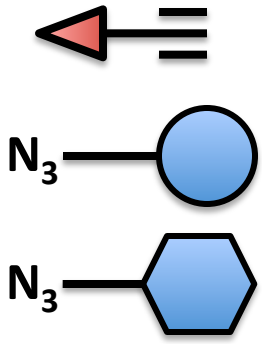




Gel-based ABPP



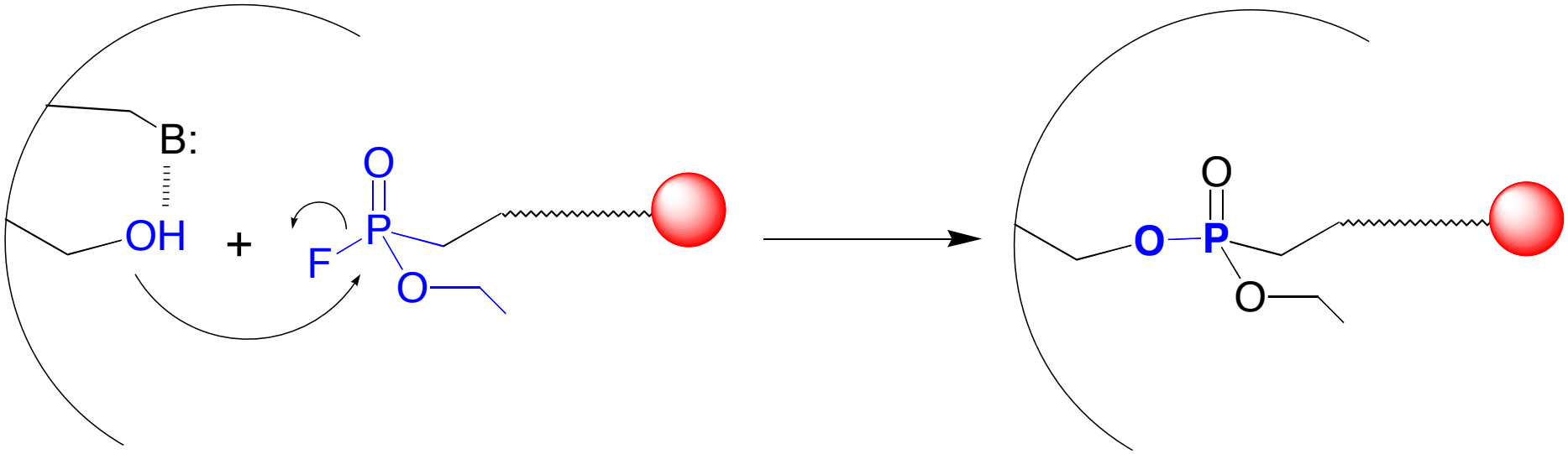
MS-based ABPP



"Click chemistry" -based ABPP

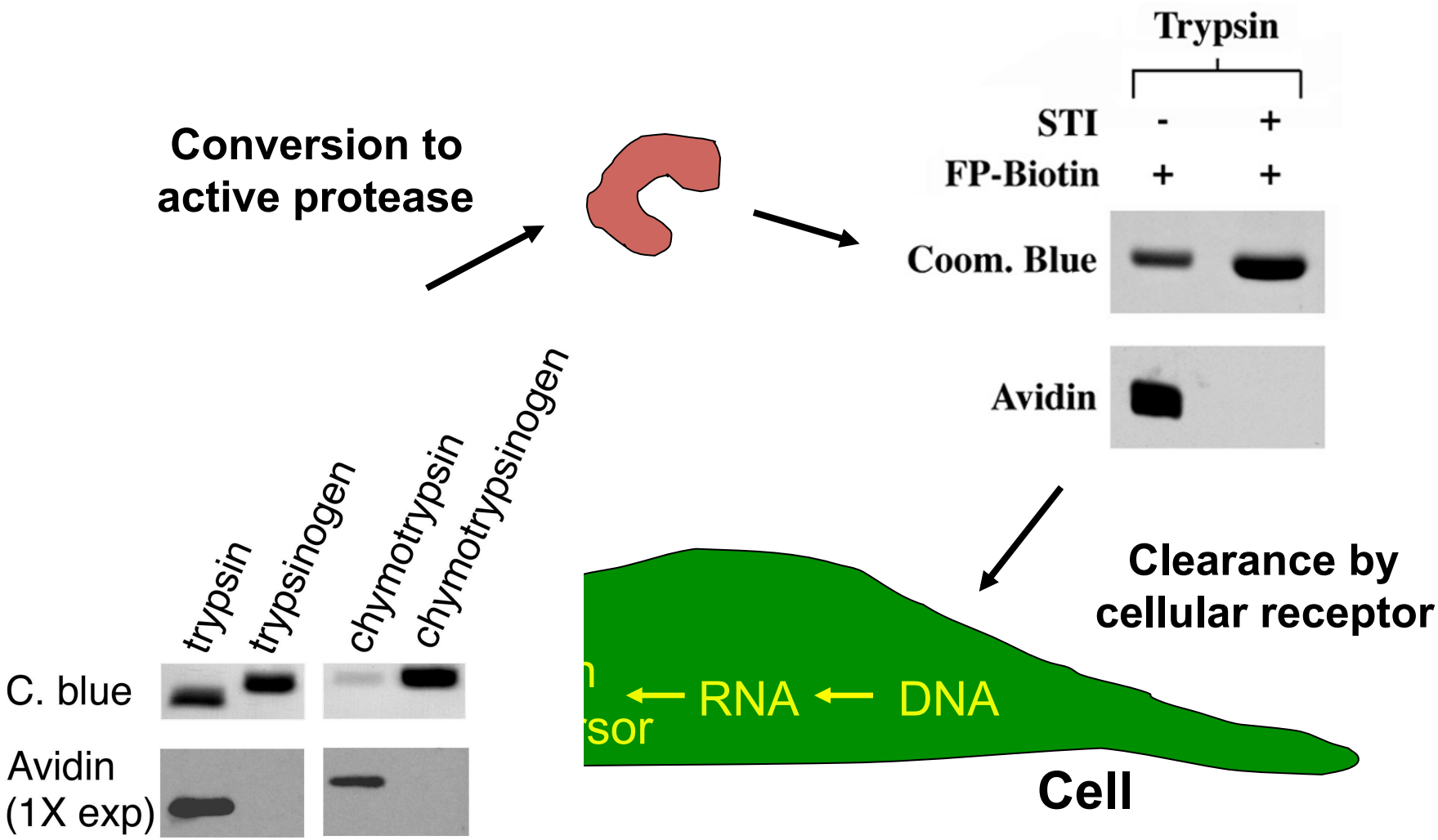
Liu Y et al. *Proc. Natl. Acad. Sci. USA.* 2000; Jessani et al, *Nature Method*, 2005.

OP-based Activity-based Probes for SHs

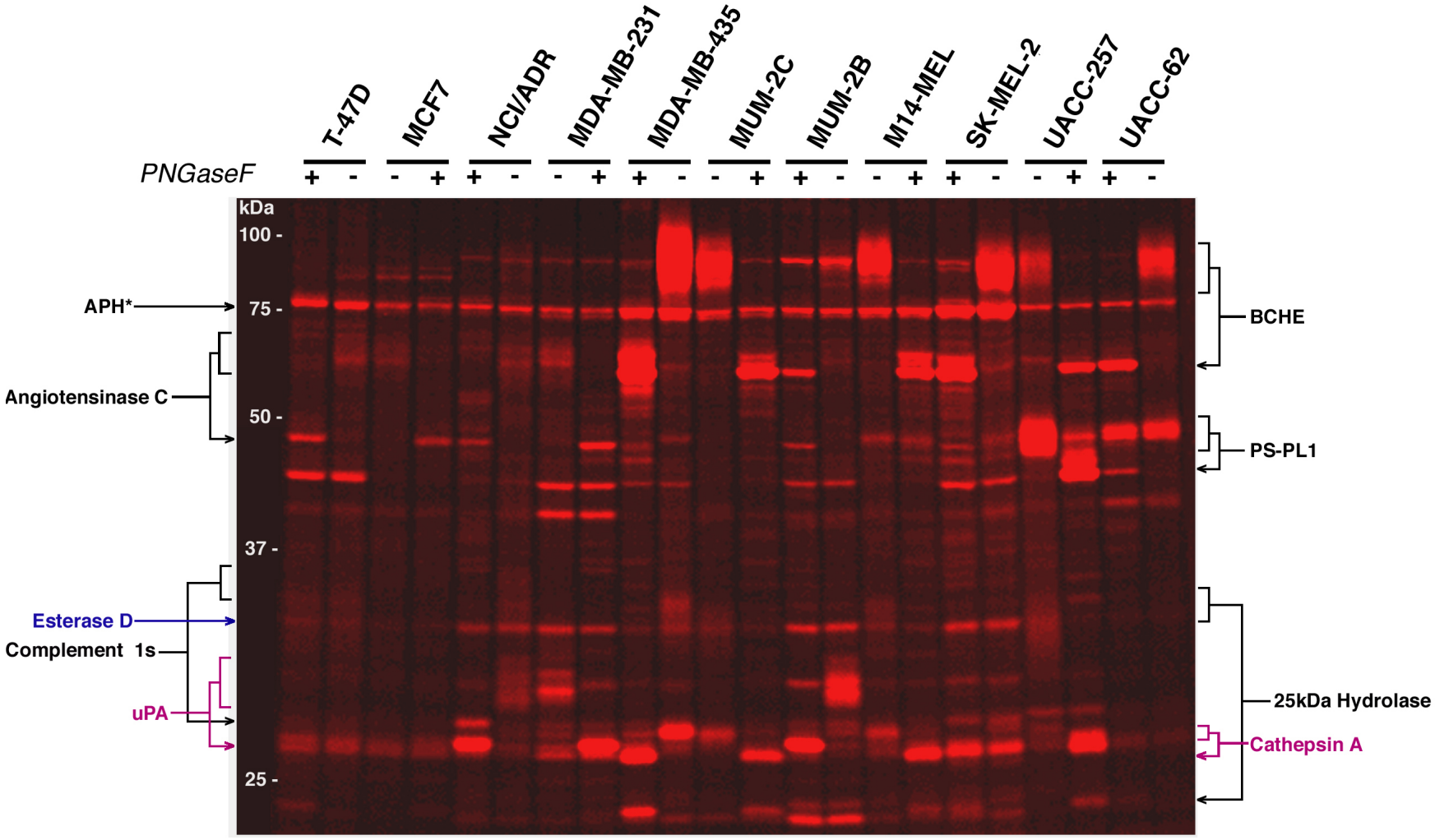


- **Fluorophore -- detection (in-gel fluoresence)**
- **Biotin – western blotting, enrichment**
- **Alkyne – "click chemistry" to conjugate with azide-functionalized fluorophore or biotin**

Activity-Based Protein Profiling (ABPP) of Serine Proteases

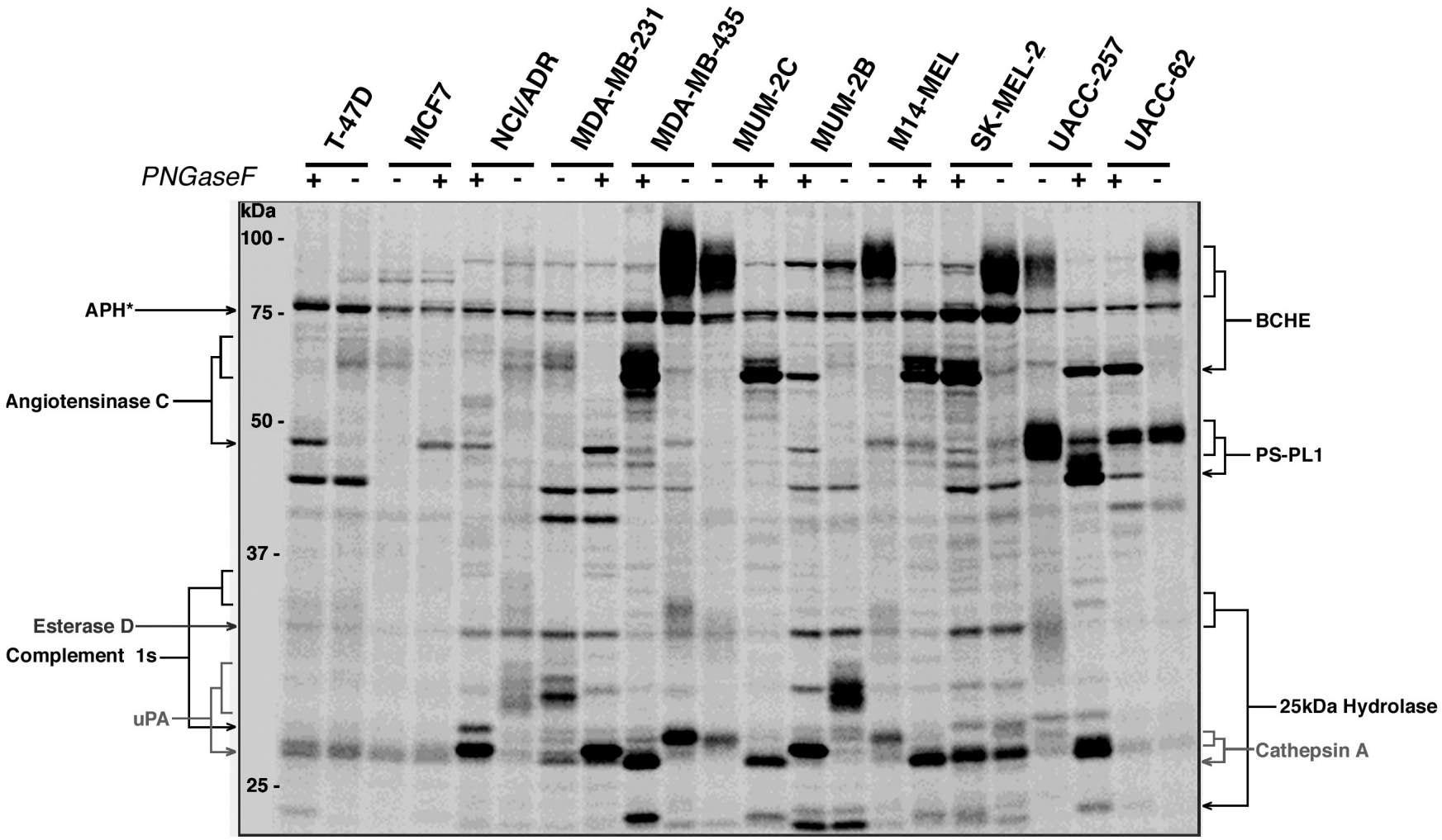


Profiling Proteome-wide SH Activities in Cancer



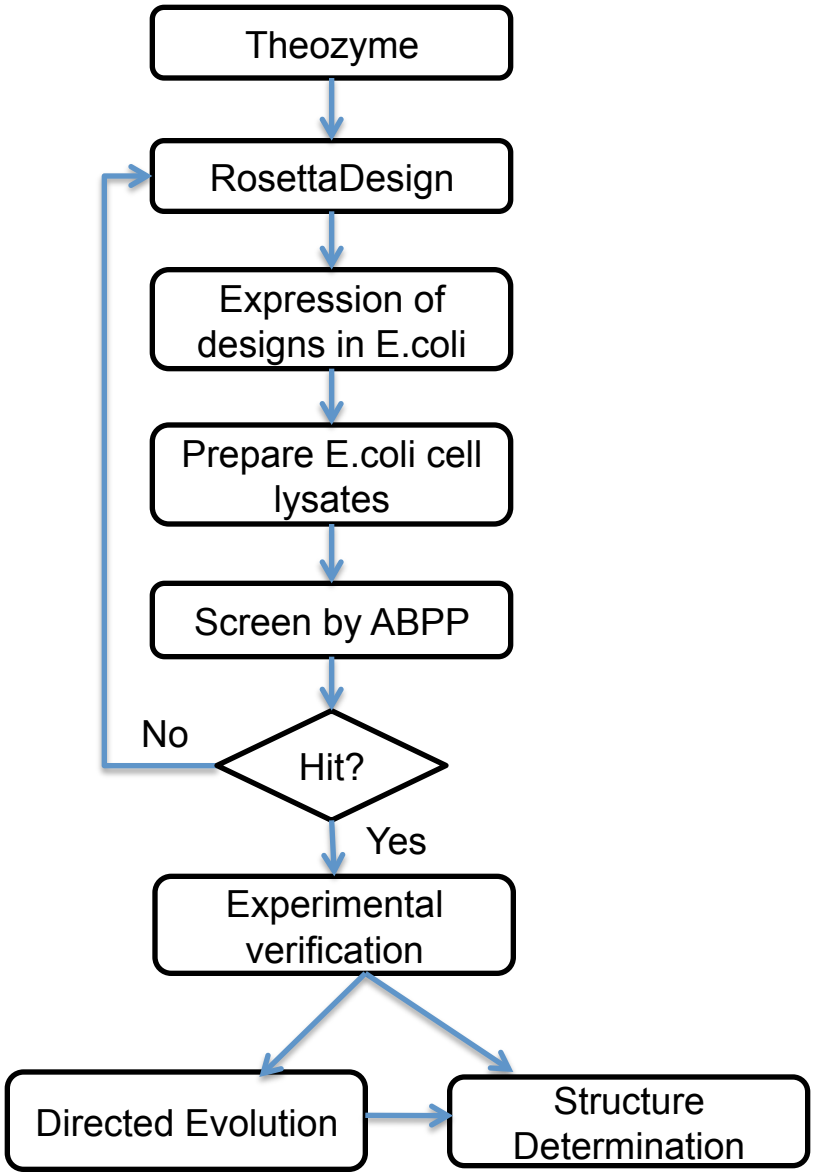
Secreted Proteomes of Cancer Cells

Profiling SH Activities in Cancer

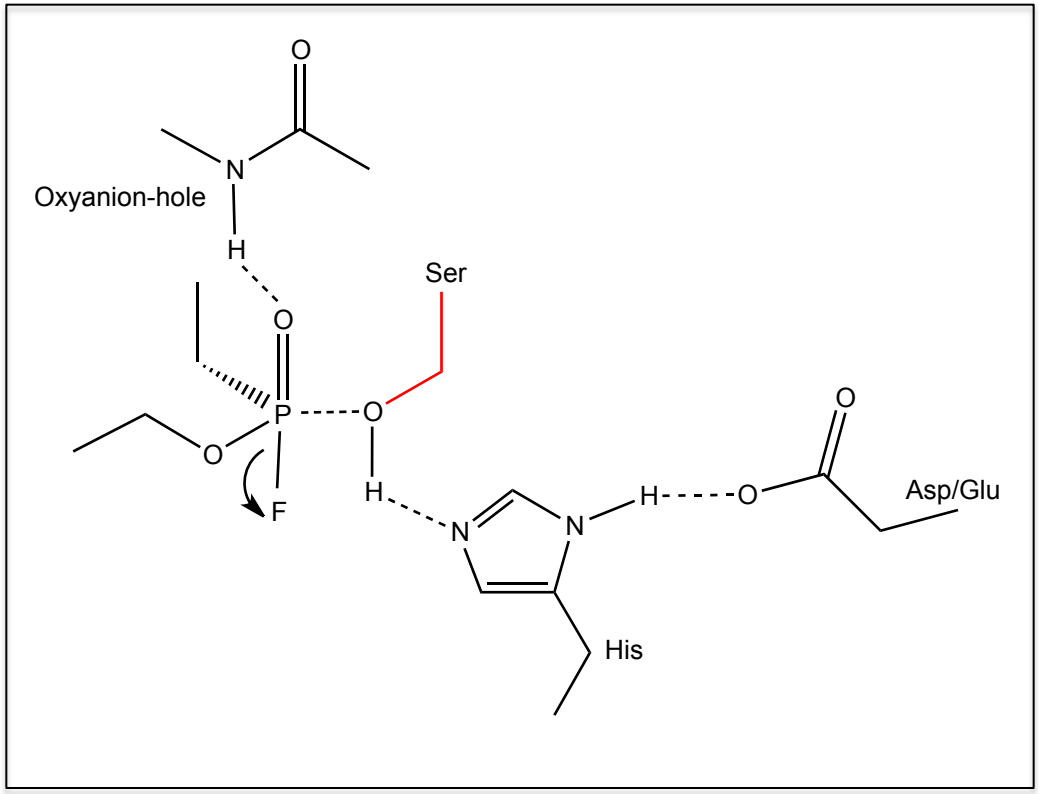


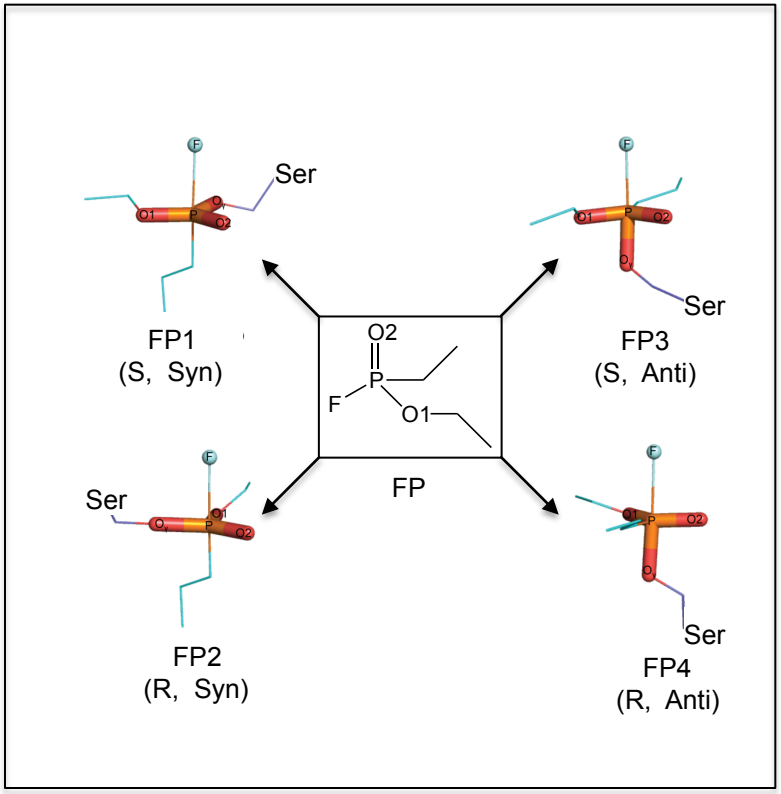
Secreted Proteomes of Cancer Cells

Overall Workflow



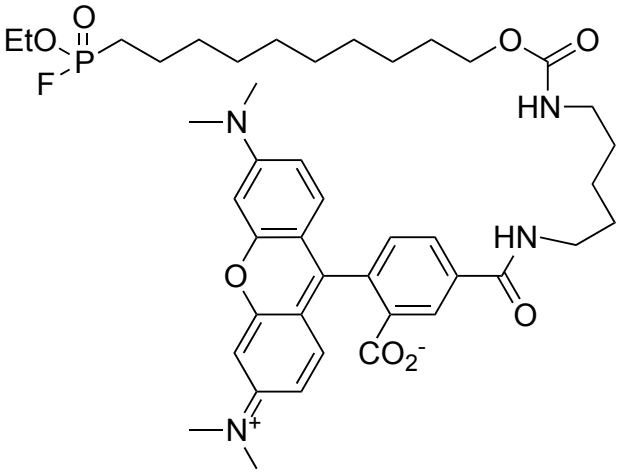
Theozyme & RosettaMatch & Design



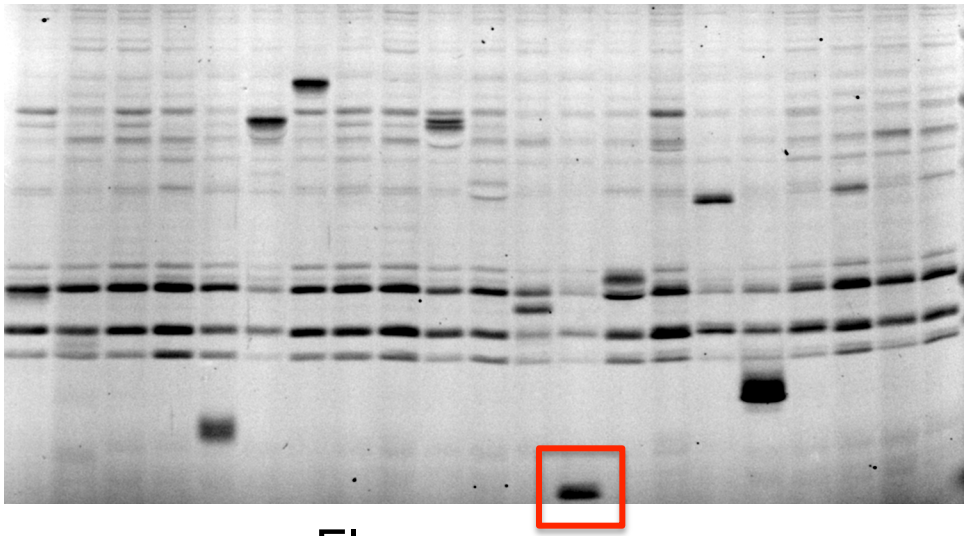


- Transition state of syn- or anti- attack of serine nucleophile in R or S isomers of OP
- Ensemble of Ligand conformers constructed by OpenEye's Omega software.
- RosettaMatch includes the Ser-His-Asp/Glu triad and an backbone NH as oxyanion hole

Screen By ABPP using OP-probes

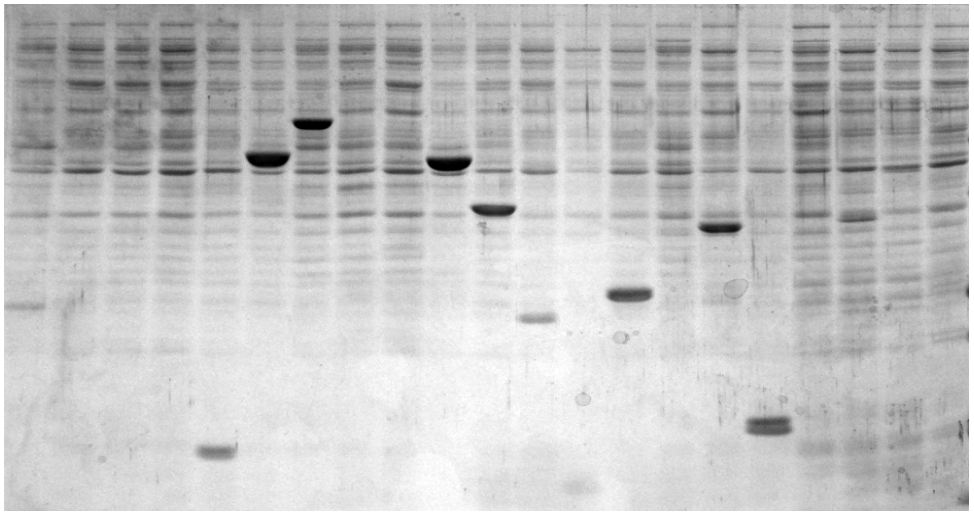


FP-rhodamine (FP-Rh)



Fluorescence

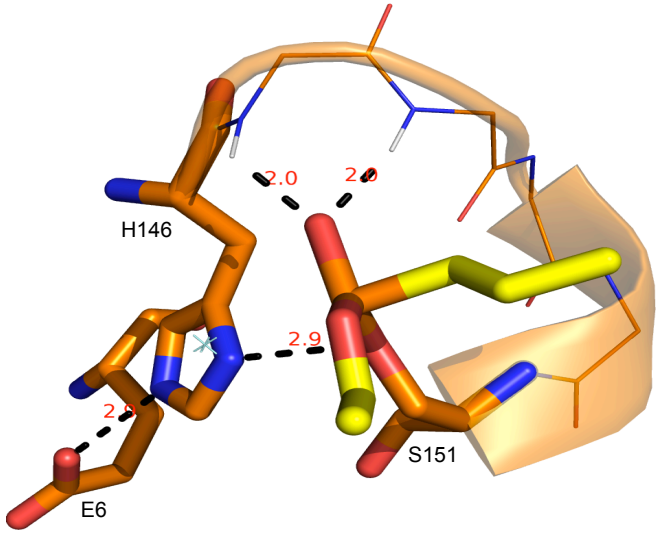
- 2mL of E.coli culture
- Lyse the pallet
- Prepare soluble lysate
- Label with FP-Rh for 1 hour
- Run SDS gel
- Scan fluorescence
- Stain with Coomassie blue
- Normalize fluorescence/abundance
- Fast screening (50 designs per day)



Coomassie Blue

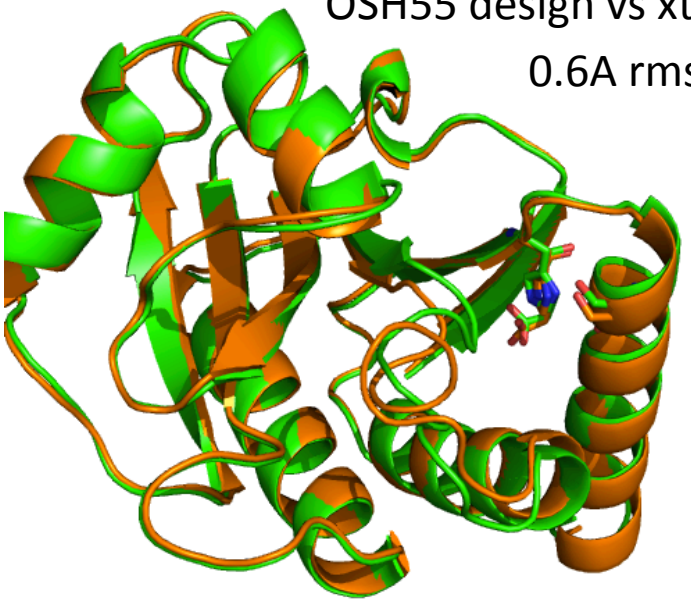
OSH55 Identified as a Potential Hit

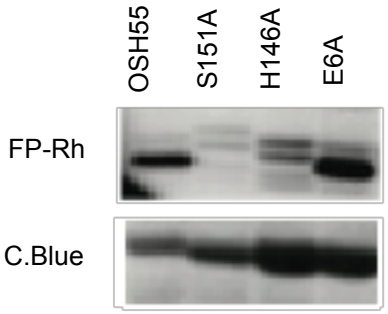
OSH55 design



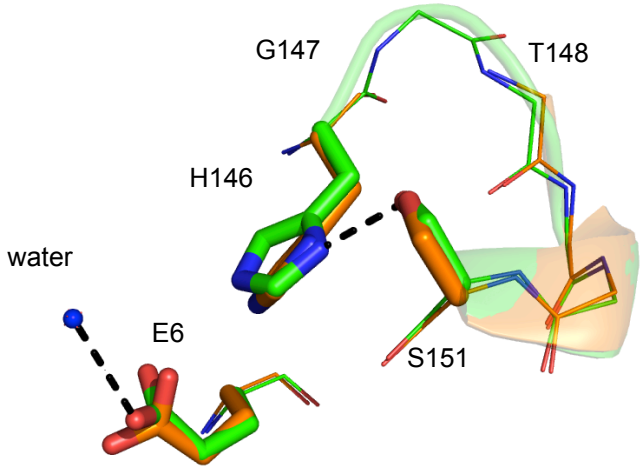
OSH55 design vs xtal

0.6A rmsd





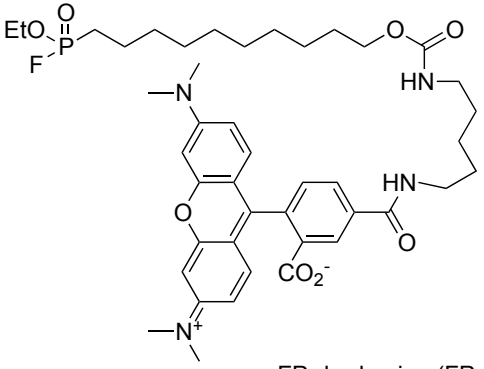
Ser and His knockouts abolish FP labeling



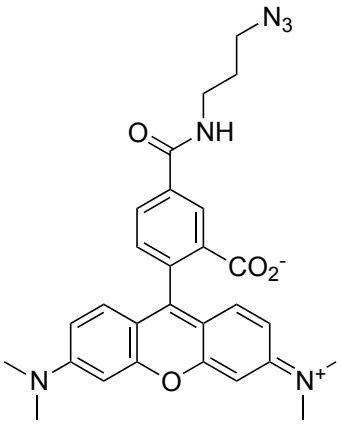
Glu6 is not positioned properly

Optimizing OSH55 Triad by RosettaDesign

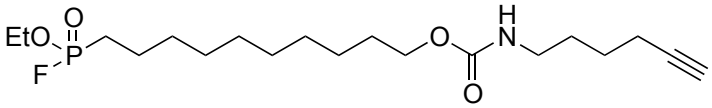
Designs	Mutations on wild-type
OSH55	No mutations (wild-type)
OSH55.4	E6H
OSH55.5	E6D
OSH55.6	E6A/L130K/G144E
OSH55.7	E6D/L159T
OSH55.8	E6A/A155H
OSH55.9	E6A/L130D/A155H
OSH55.10	E6A/A112S/G144D
OSH55.11	E6A / L126N/A155D
OSH55.12	E6A/ I133K/G144D
OSH55.13	T148R
OSH55.14	E6D/L159R
OSH55.15	E6D/L159T
OSH55.16	E6D/L126K
OSH55.17	E6D/I4R
OSH55.18	T149G
OSH55.19	I4N/L159S
OSH55.20	I4S/L130M/I133M



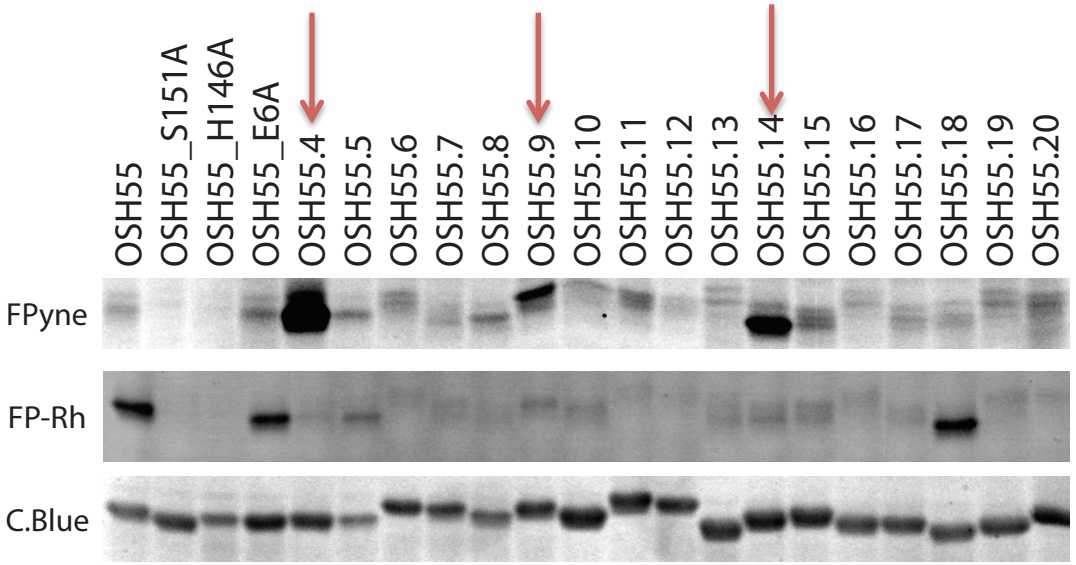
FP-rhodamine (FP-Rh)



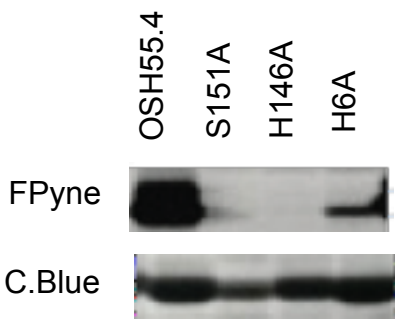
Azide-rhodamine

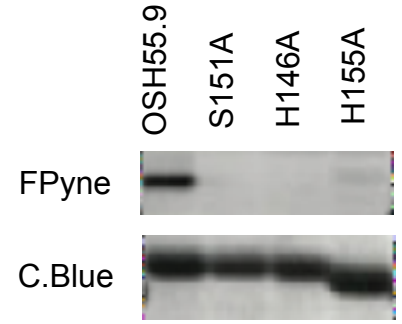


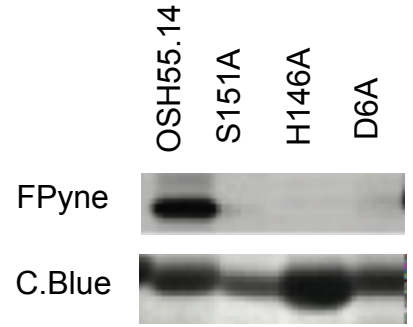
FP-alkyne (FPyne)

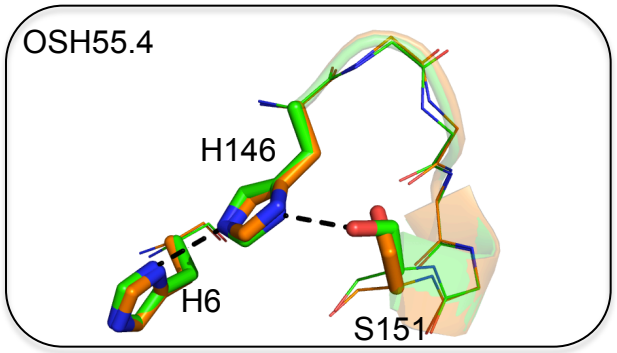


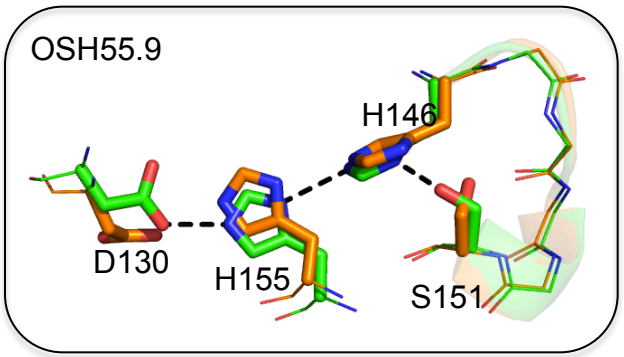
Three OSH55 Variants with Increased OP Reactivity and Accurate Triad Design

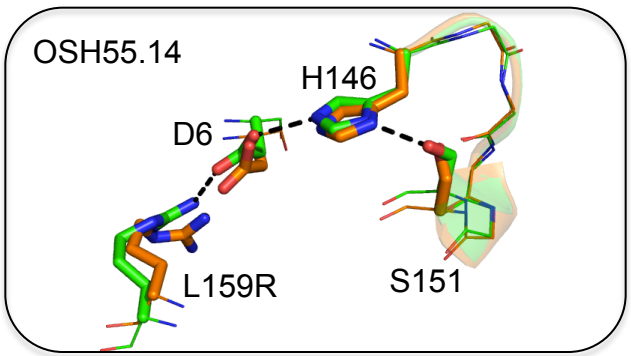




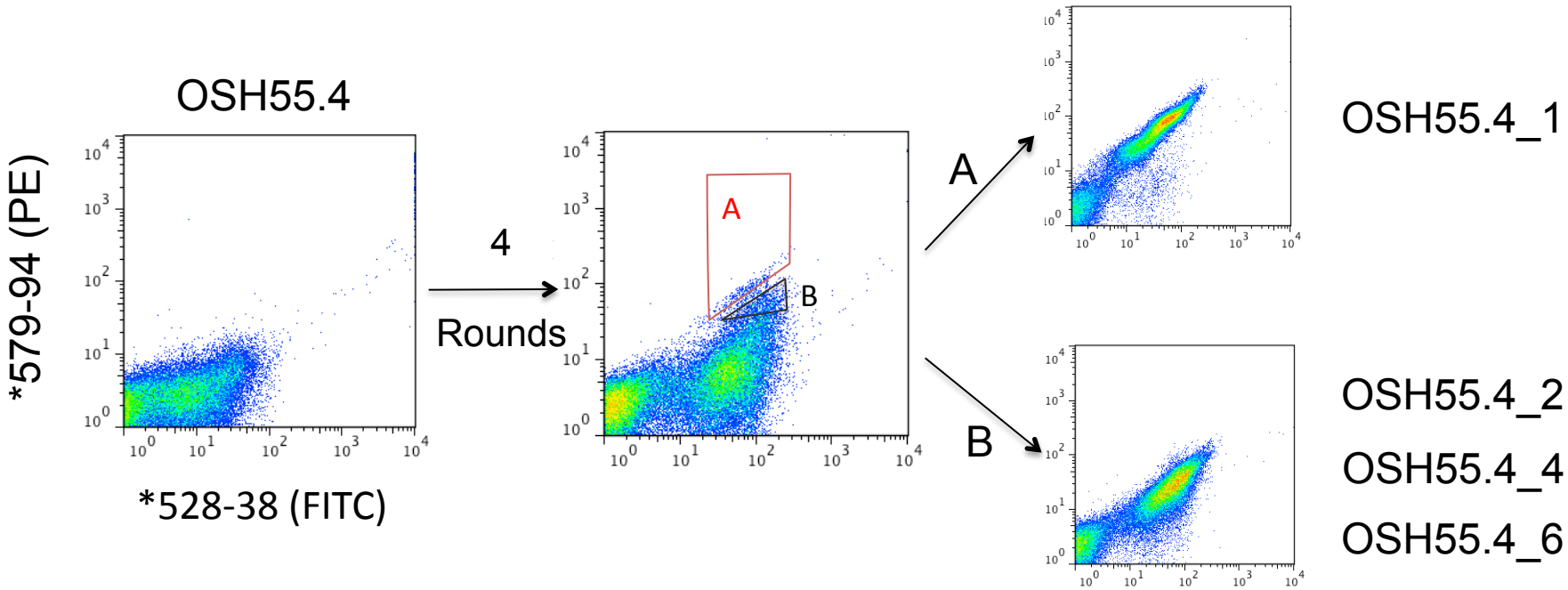


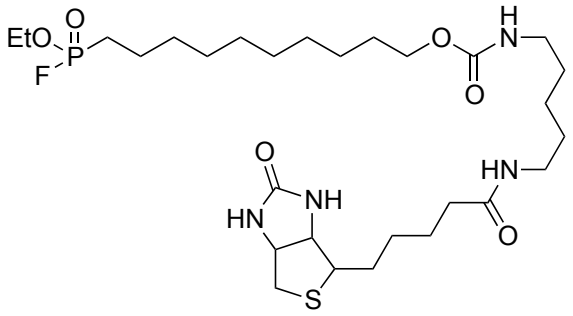






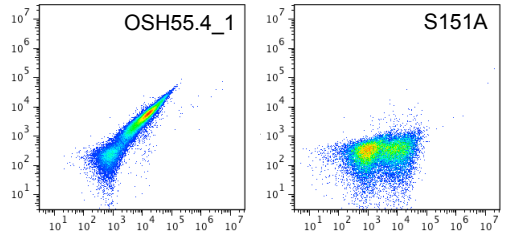
Improve OP Reactivity of OSH55.4 by Yeast Surface Display

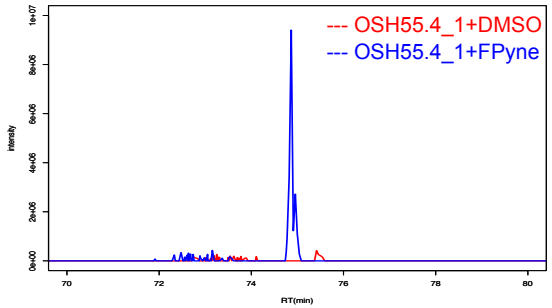




FP-biotin

Specific OP Adduction with S151 in the Triad





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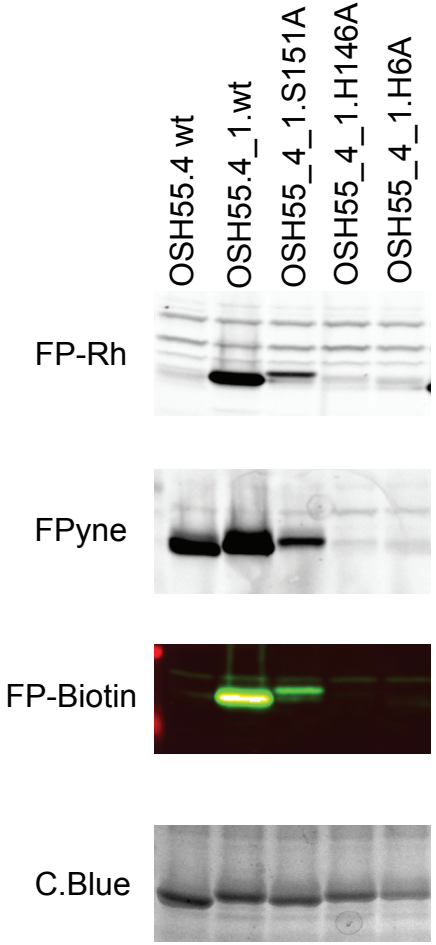
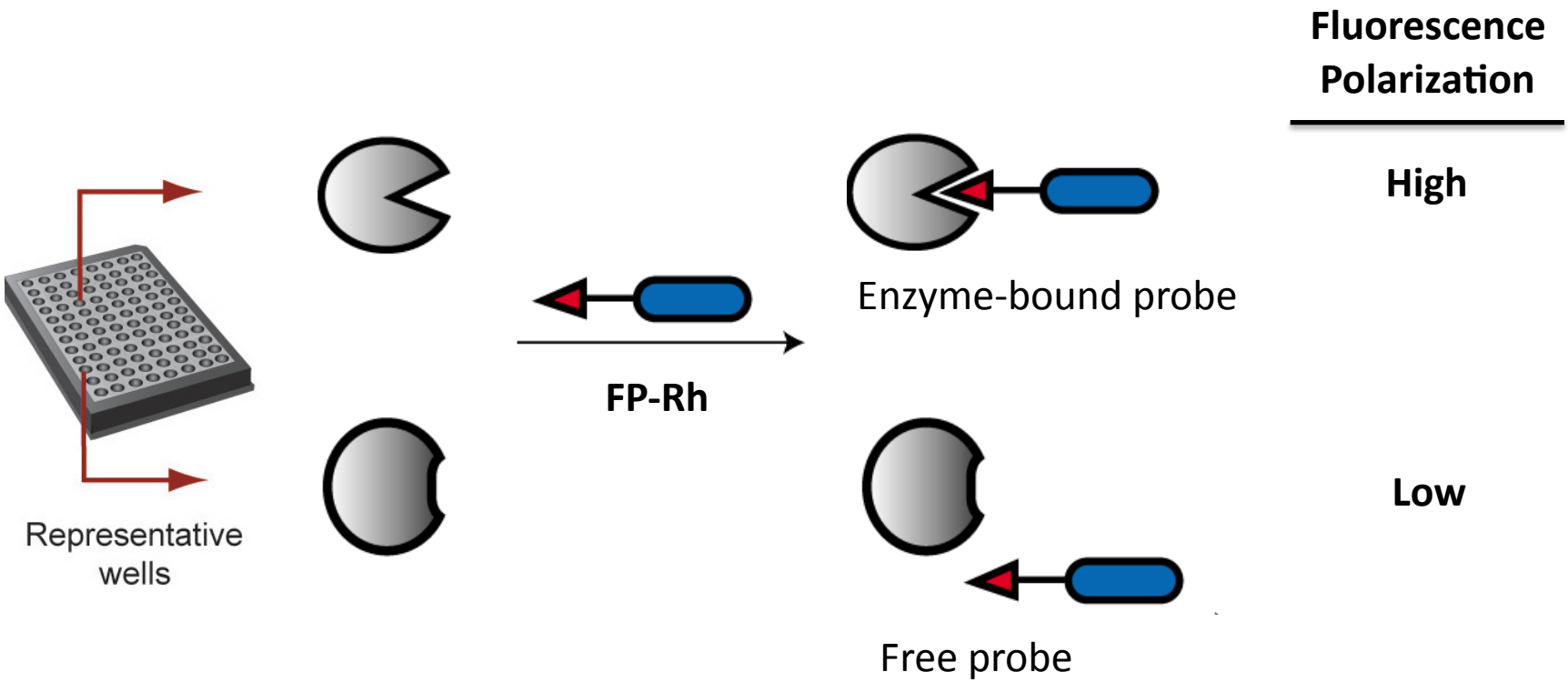
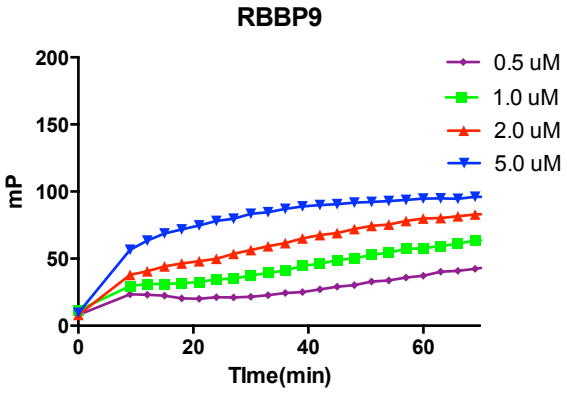
Charge 5+, m/z = 735.79010

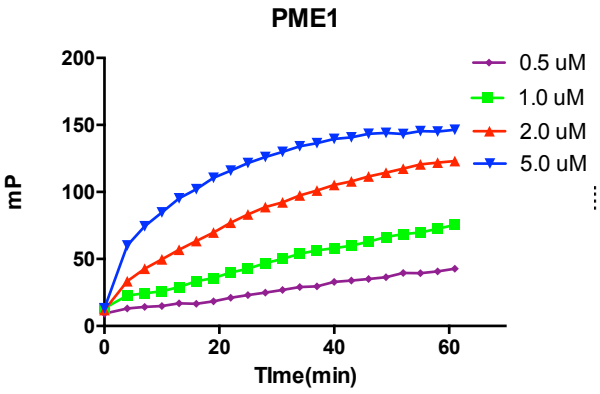


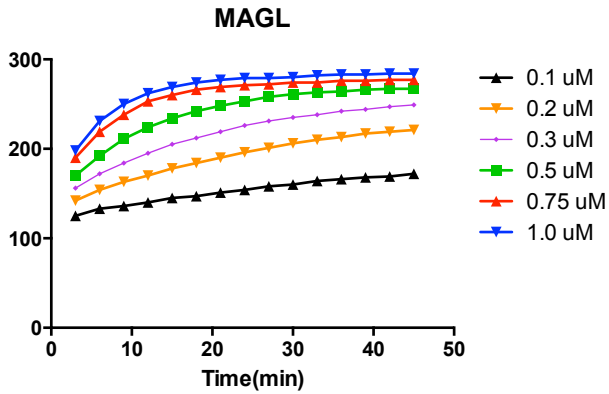
Measurement of Rate of OP Labeling by Fluorescence Polarization

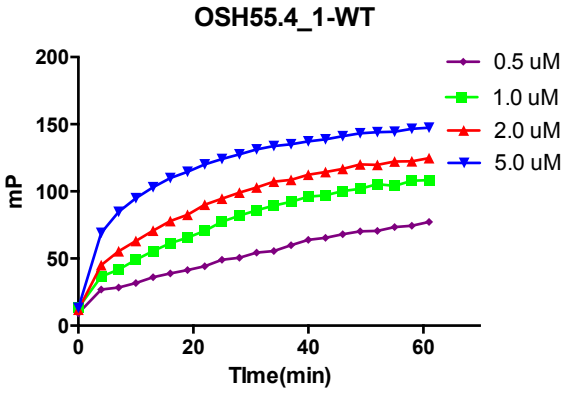


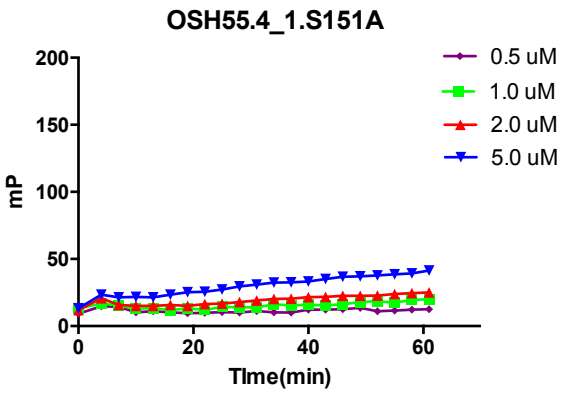
OSH55.4_1 Reacts with OP as FAST as Native Serine Hydrolases

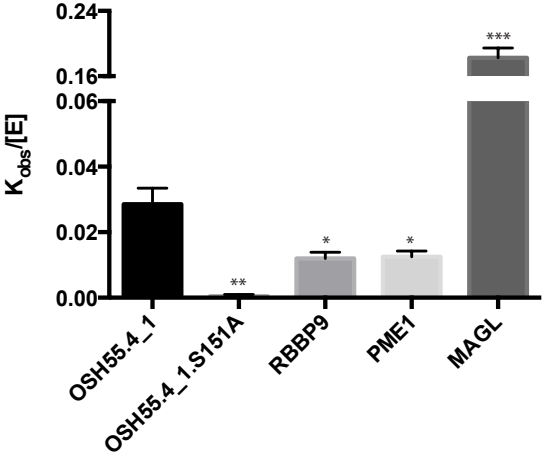






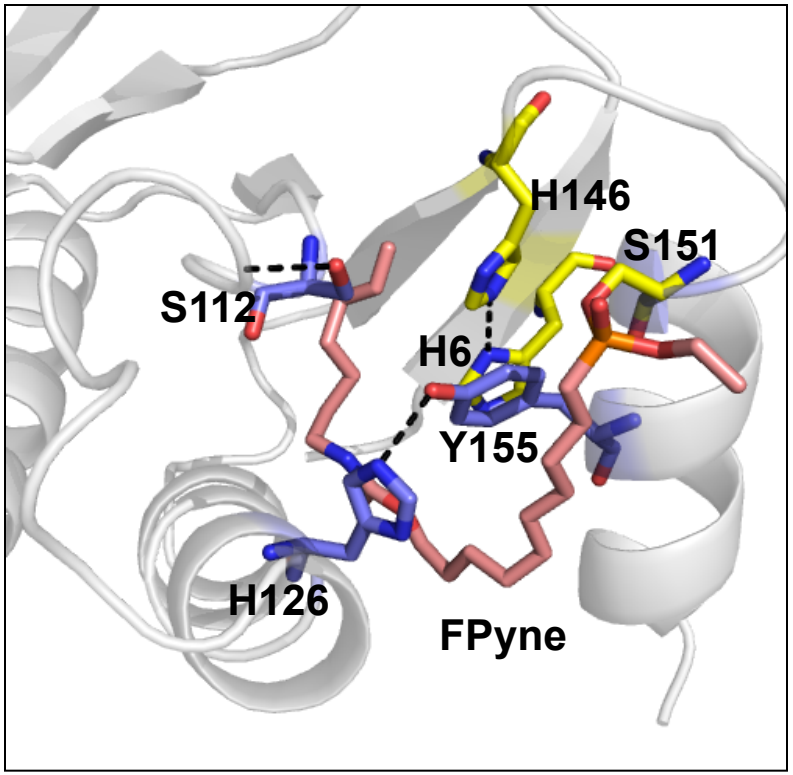




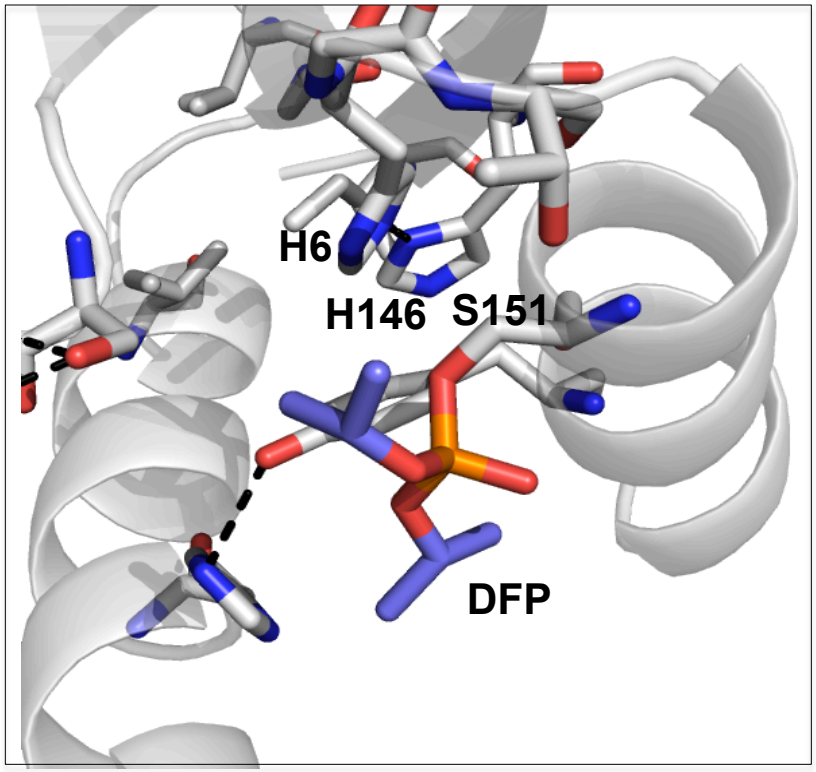


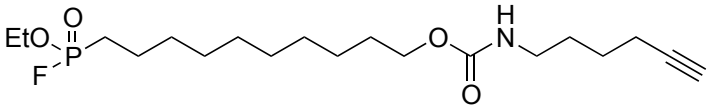
Structural Characterization of OSH55.4_1 Complexed with OPs

OSH55.4_1 with FPyne

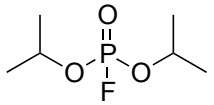


OSH55.4_1 with DFP





FPyne



DFP

Conclusion and Future Directions

- *De Novo* Design of proteins with authentic catalytic triads
- Xtal structures confirm very accurately designed interactions
- Serine nucleophile in the catalytic triad is activated
- The designed serine reacts with OP as fast as those in native SHs
- The designed protein binds to a toxic OP agent -- DFP
- ABPP can aid screening and testing functional protein designs
- Towards designs of enzymes with hydrolytic activity
- Towards designs of protein scavengers of toxic OP agents

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