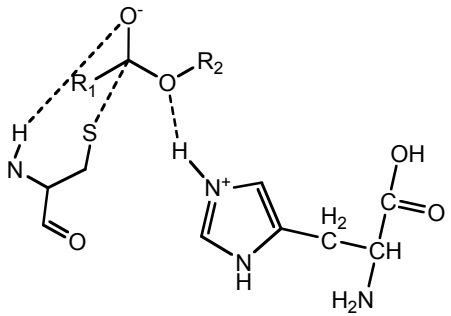
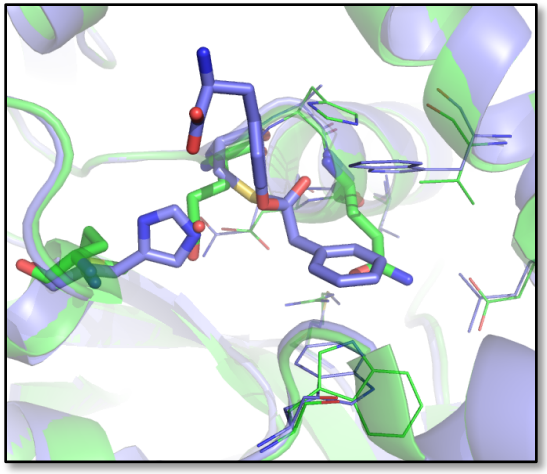
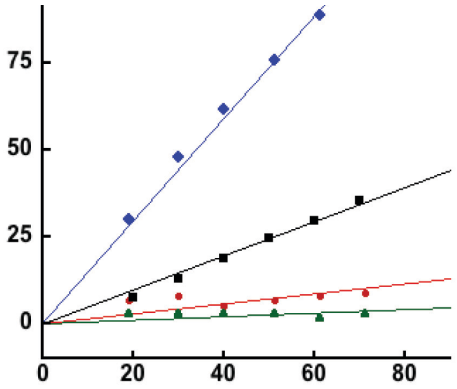
Computational *de-novo* design of esterase active sites







1. Design of ester hydrolases

2. Design of organophosphate binders
   (with Sridharan Rajagopalan)

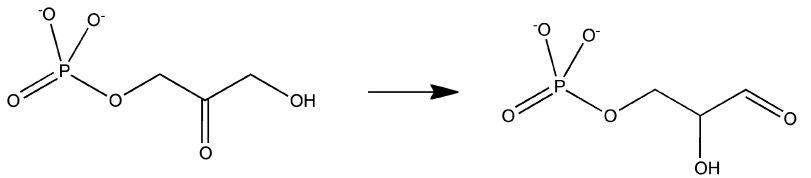
Florian Richter

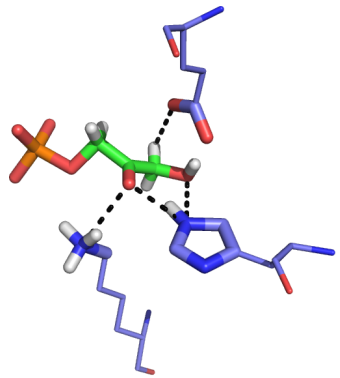
Baker Group

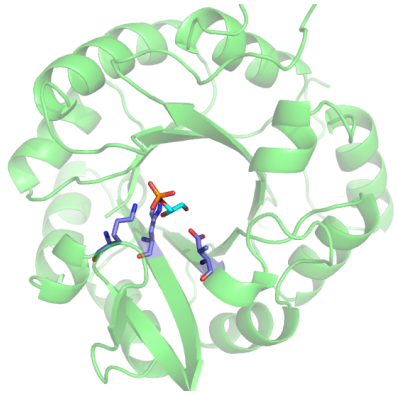
Intro to computational *de-novo* enzyme design

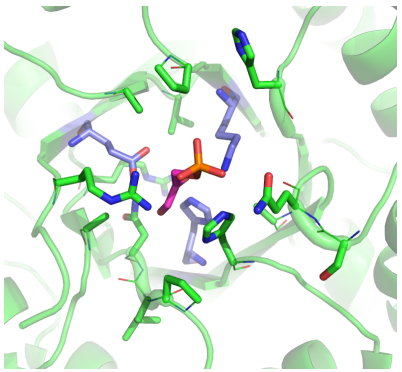
<u>Process divided into 4 steps</u>

1. Reaction -> Minimal active site ("Theozyme")
2. Place theozyme into a protein scaffold ("Matching")
3. Design sequence for the new active site
4. Experimental testing









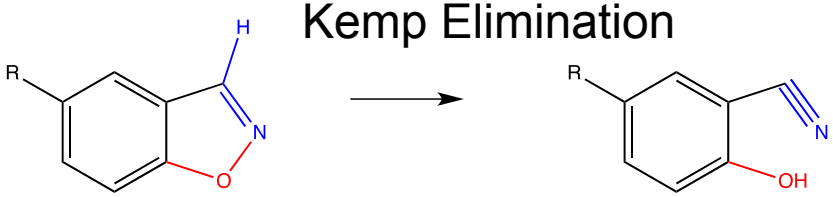
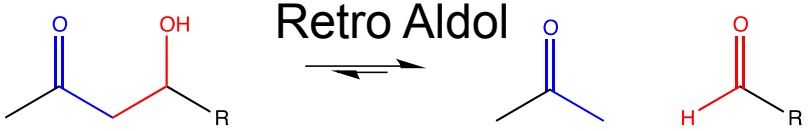
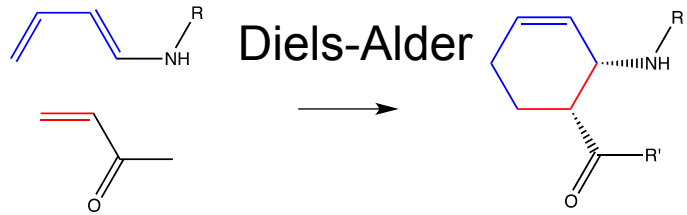
1

2

3

Computational Enzyme Design

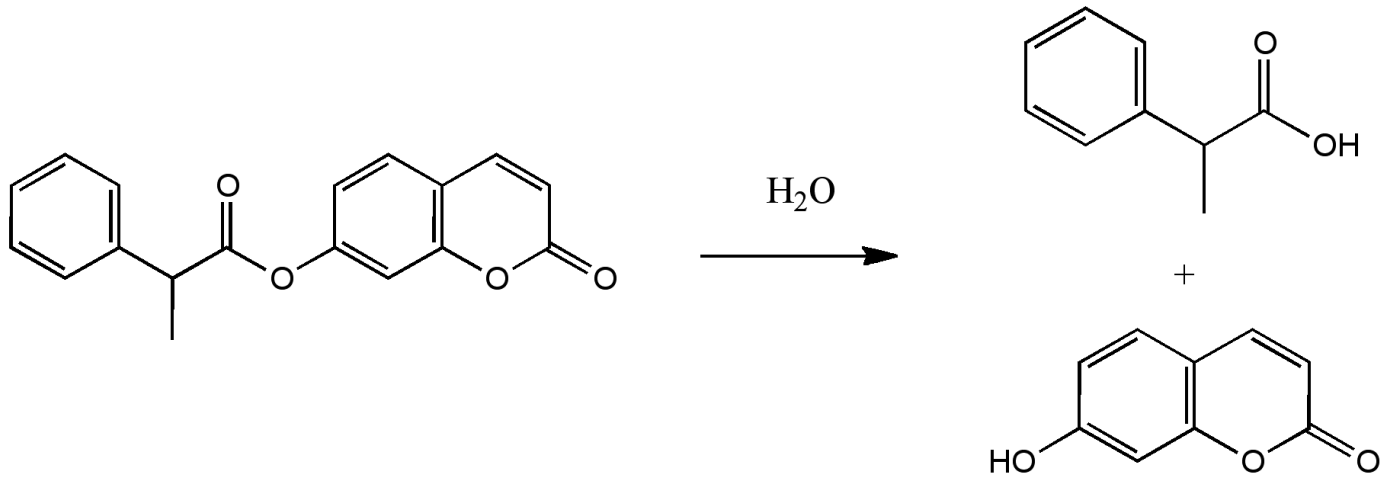
Previous achievements

Reaction	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M ($M^{-1}s^{-1}$)	k_{cat}/k_{uncat}	reference
 Kemp Elimination	0.29	1.8	163	$2*10^5$	*Nature* 453(2008), p190
 Retro Aldol	$5*10^{-5}$	0.62	0.11	$8*10^3$	*Science* 319(2008), p1387
 Diels-Alder	$3*10^{-5}$	3.5	0.008	n/a	*Science* 329(2010), p309

Natural enzymes: k_{cat}/K_M up to $10^8\ M^{-1}s^{-1}$, k_{cat}/k_{uncat} up to 10^{21}

Design of novel ester hydrolases

New Target reaction: ester hydrolysis



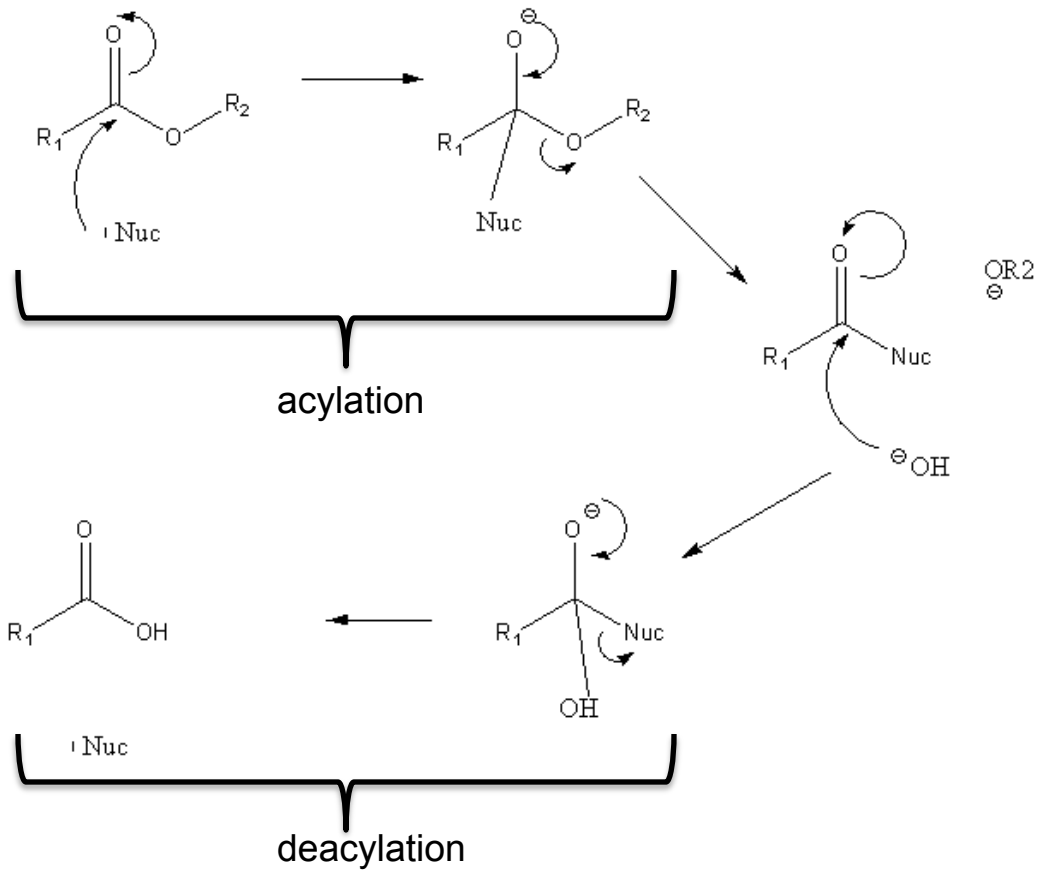
Why ester hydrolysis? ➔ Bechmark for computational enzyme design

- Computational design is a new technique with room for improvement
- Ester hydrolysis is one of the best studied reactions in (bio)chemistry
- Large amount of structural and biochemical data on natural esterases exists

➔How well can we recreate natural esterases using computational design?

Design of novel ester hydrolases

Reaction mechanism: an ester/amide gets split into an acid an an alcohol/amine



Key features:

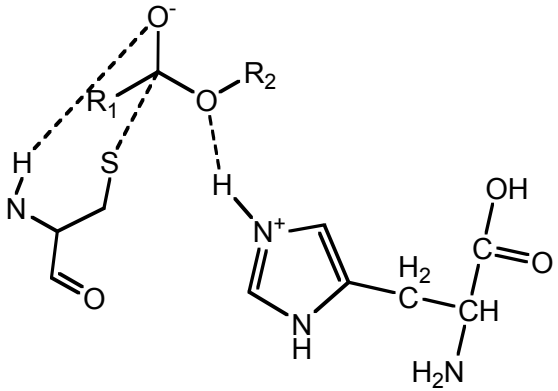
- Nucleophilic attack onto ester-carbon
- Tetrahedral intermediate
- Catalytic nucleophile binds covalently (‘acylenzyme intermediate’)
- Negative charge accumulation at ester-oxygen

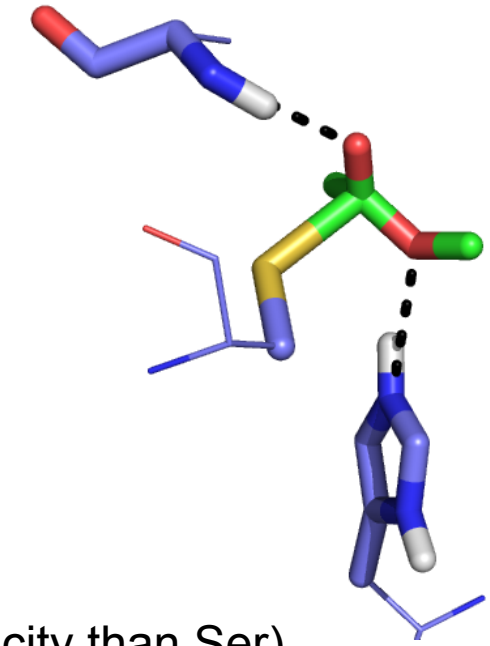
Design of novel ester hydrolases

Choice of theozyme

<u>Natural hydrolase active sites:</u>

- often use Ser or Cys as nucleophile
- Nucleophile activated by His or other protic residue
- Feature oxyanion-stabilizing elements (“oxyanion-hole”)





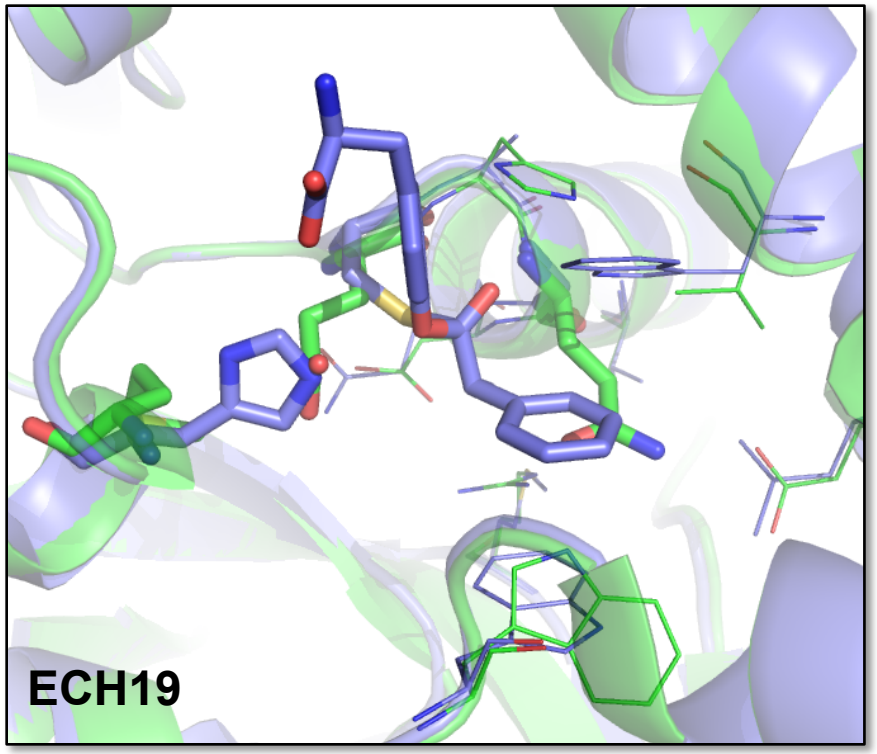
<u>Design theozyme</u>

- Cys as nucleophile (higher intrinsic nucleophilicity than Ser)
- His as proton shuttle (protonate leaving group, deprotonate water)
- Backbone-NH as oxyanion stabilizer

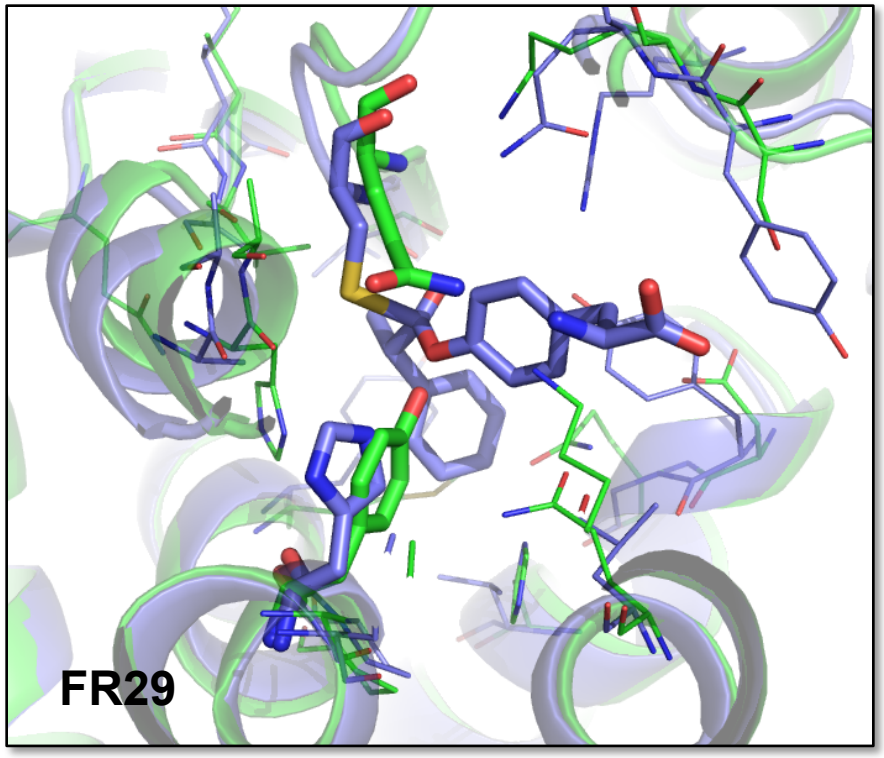
Design of novel ester hydrolases

•28 designs were tested, 4 had activity

(coloring: scaffold x-ray / design model)



Scaffold: galacturonide binding protein
11 Mutations
Catalytic site: E161C / M226H / Q163G



Scaffold: Trp – tRNA synthetase
20 Mutations
Catalytic site: Q9C / Y125H

Design of novel ester hydrolases

In-detail characterization of 4 active designs

1. Are they active for the right reason?
   - ➔ yes, catalytic residue knock-outs suggest activity is due to designed site

2. How active are they?
   - ➔ k_{cat}/K_M ~10^2 M^{-1} s^{-1}
   - ➔ < natural hydrolases, ≈ other computational *de-novo* designs
   - ➔ 2-phase kinetics observed (fast acylation / slow deacylation)

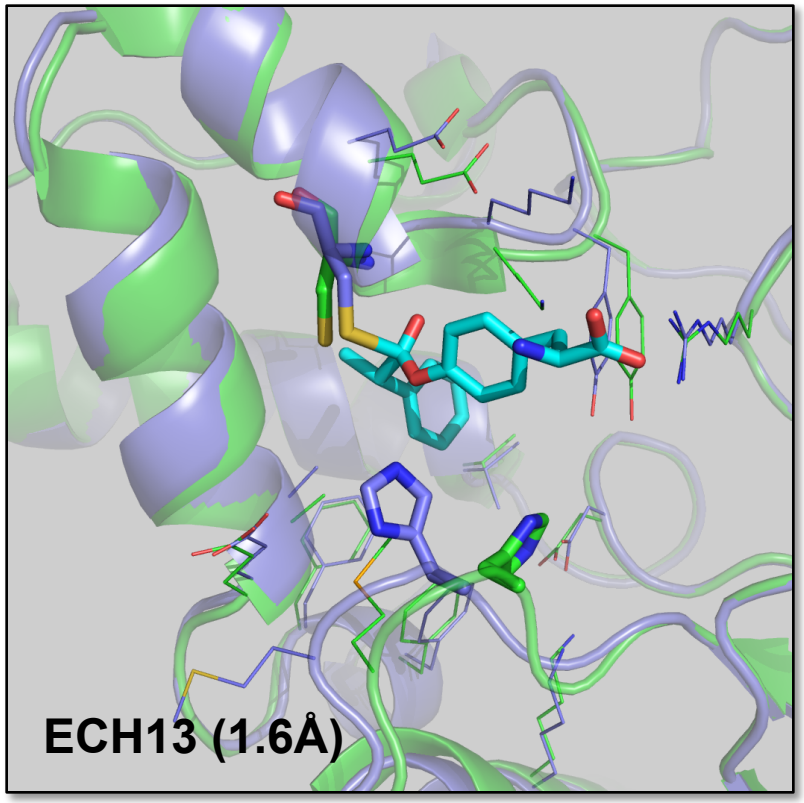
3. Does the catalytic mechanism work as designed?
   - ➔ covalent intermediate detected by Mass spectrometry
   - ➔ designs react with nucleophile-specific probe as good as natural cys hydrolases

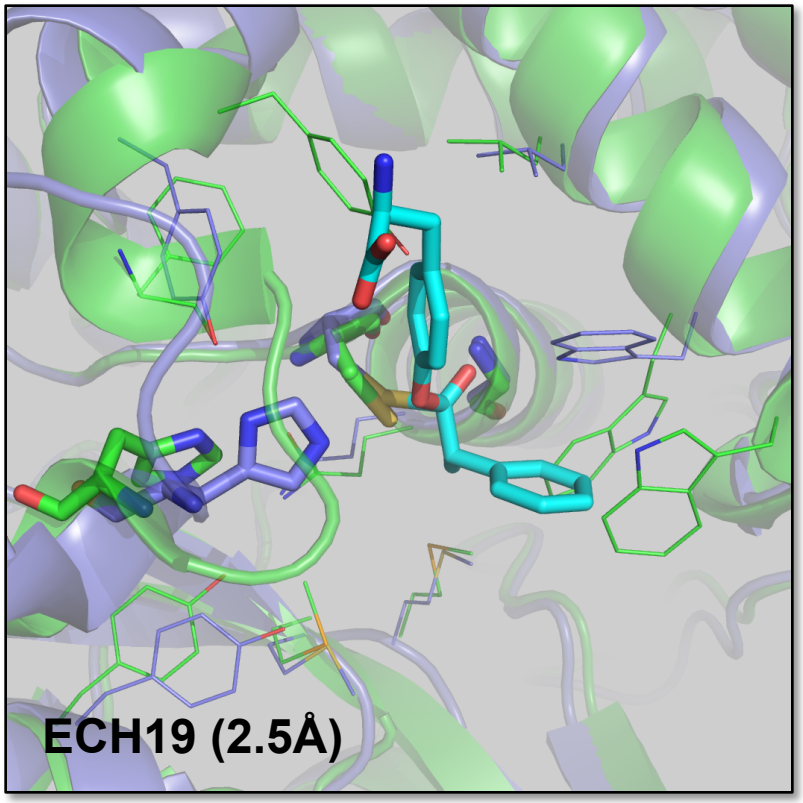
4. Does the structure look as designed?
   - ➔ X-ray structure elucidation
   - ➔ Molecular dynamics simulations

2.5. Does the structure look as designed?

Crystal structures of the 4 designs were determined

(coloring: design x-ray / design model)





- Overall shape of active site retained (Cα RMSD 0.97Å ECH13, 1.5Å ECH19)
- Cys in designed conformation
- His not in designed conformation, facilitated by small backbone shift
- Suggests reason for low overall activity and deacylation problems

In collaboration with A. Kuzin, L. Tong, et al, Northeast Structural Genomics Consortium (NESG)

Esterase design conclusions

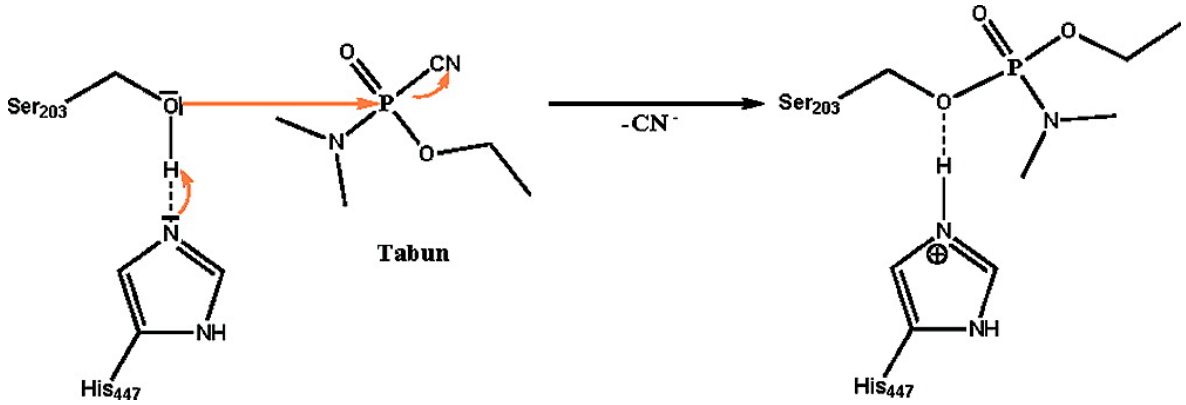
- Successfully designed esterase active site into 4 inert scaffolds
  - Scaffolds are structurally unrelated
  - ➔suggests we can design basic esterase catalytic machinery

- Crystal structures of designs and slow deacylation kinetics indicate that the biggest problem is the improperly positioned catalytic histidine
  - ➔attempts to improve the activity should thus focus on fixing the histidine position

- Designs are (relatively) bad catalysts but excellent nucleophiles
  - ➔Shows that nucleophilicity ≠ nucleophilic catalysis
  - ➔Suggest nucleophiles easier to design than catalysts

2. Design of organophosphate binders

- Organophosphates (OPs): Chemical warfare agents that inhibit esterases involved in synaptic transmission
- Act by covalently and irreversibly modifying the active site Serine catalytic nucleophile



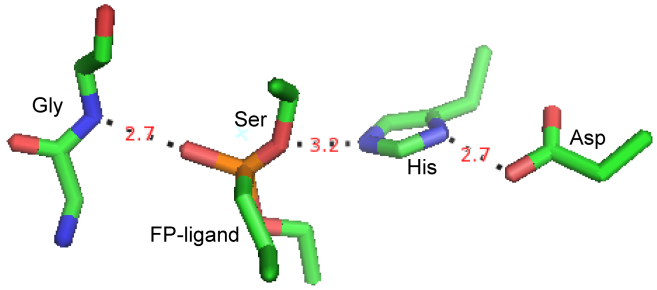
- A protein designed to react with OPs faster than native esterases could be used as a scavenger
- OP binding requires good nucleophile -> cys esterase results suggests design feasible
- OP transition state (TS) geometry different than ester hydrolysis TS geometry

➔Designs targeted towards OP-TS might have advantages vs. native esterases

(with Sridharan Rajagopalan)

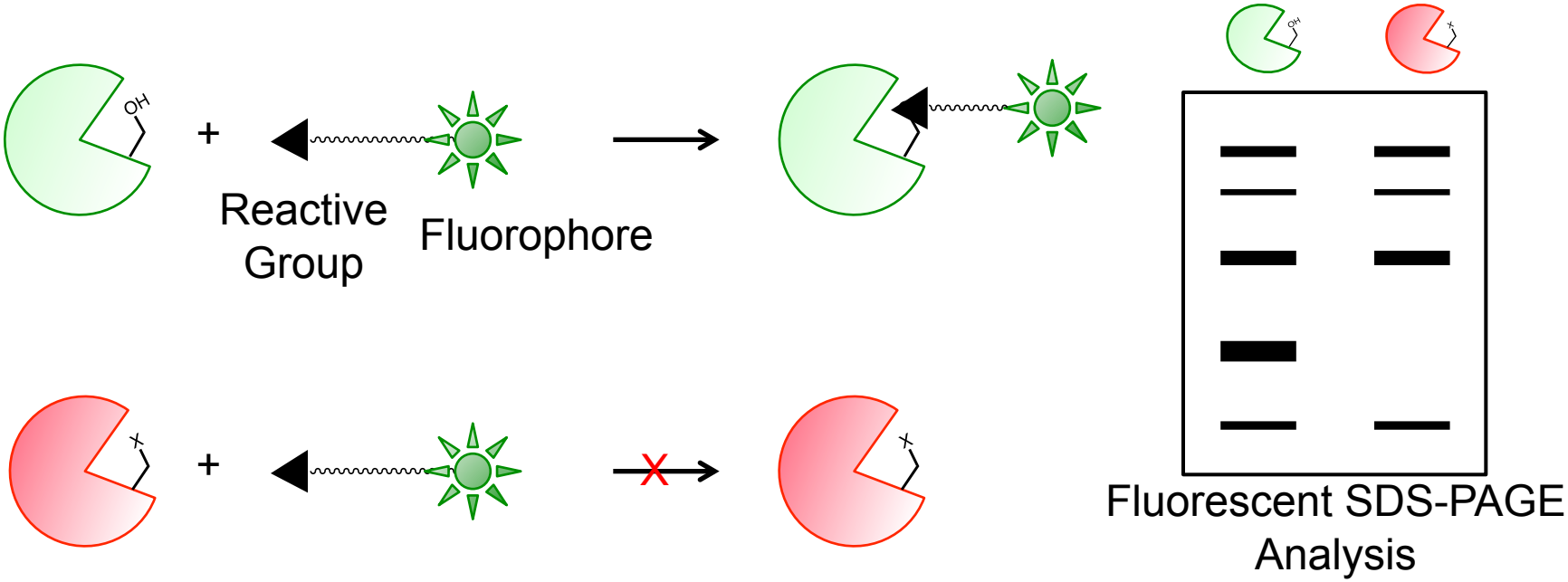
2. Design of organophosphate binders

•De novo enzyme design protocol was carried out for organophosphate binding



Theozyme used features a third residue (D/E/H) to ensure histidine is positioned properly

•Experimental setup: in gel screening with a fluorescently labeled OP probe



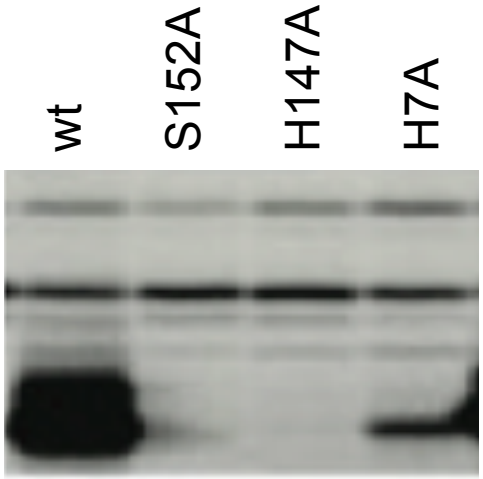
2. Design of organophosphate binders

- ~100 designs made, 4 active, OSH55 is most promising

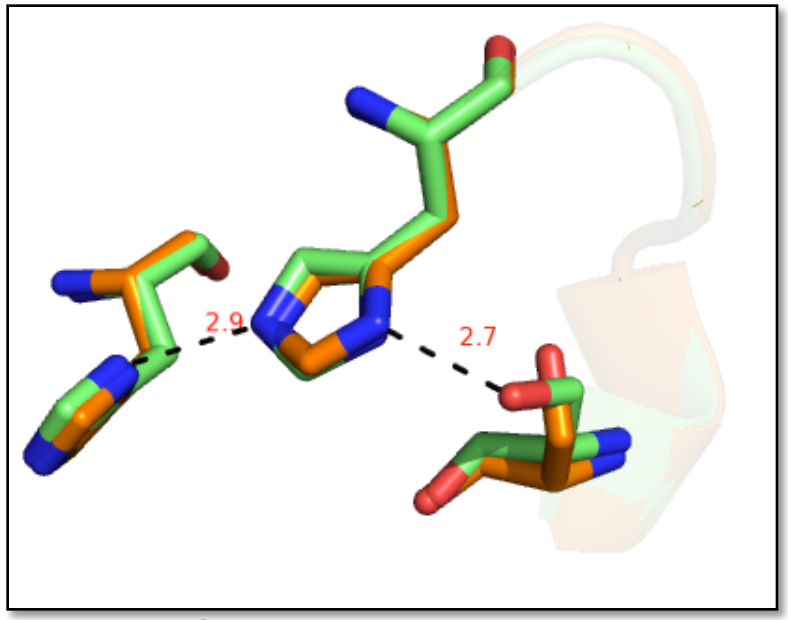
- Small (165AA), highly expressible, thermophilic scaffold

- Crystal structure confirms designed conformation
  ➔backing up histidine worked

- Knockouts confirm necessity of designed residues for OP binding



OP binding

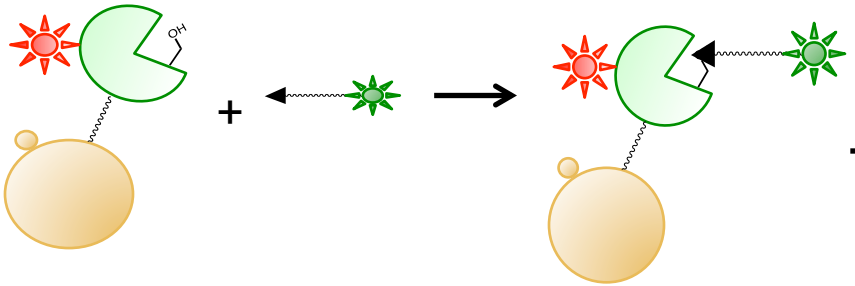


Crystal / Design

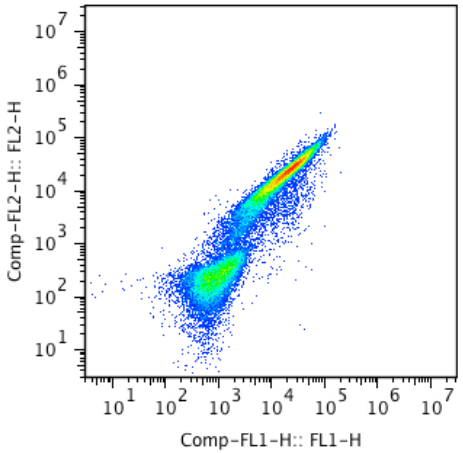
(with Sridharan Rajagopalan)

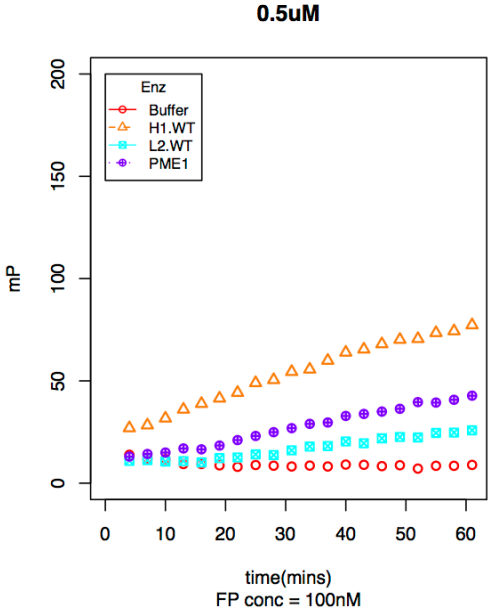
2. Design of organophosphate binders

- (Irreversible) OP binding easily accessible to high-throughput yeast display assay
- OSH55 library (6 binding site res randomized) was prepared and selected



FACS





- Binding was quantified for clones selected from library
- One clone found to react with OP faster than natural esterase!

(with Sridharan Rajagopalan)

De novo Enzyme Design

Conclusion

Can new enzymes be designed from scratch computationally?

•good: succesfully done for 5 very different reactions (deprotonation, C-C bond breaking, C-C bond forming, ester bond breaking, OP breakdown)

•less good: activity far below natural enzymes.

➔ several factors important for catalysis not modeled yet (differential stabilization, substrate access, dynamics, etc..

➔Long way to go till routine de-novo design of efficient catalysts

•However, if targets are picked wisely, useful molecules can be designed

Thanks

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•Zbigniew Pianowski

•Don Hilvert

Northeast Structural Genomics Consortium

•Alex Kuzin

•Liang Tong

Cravatt Lab @ Scripps

•Eranthie Weerapana

•Chu Wang

•Ben Cravatt

Questions?