



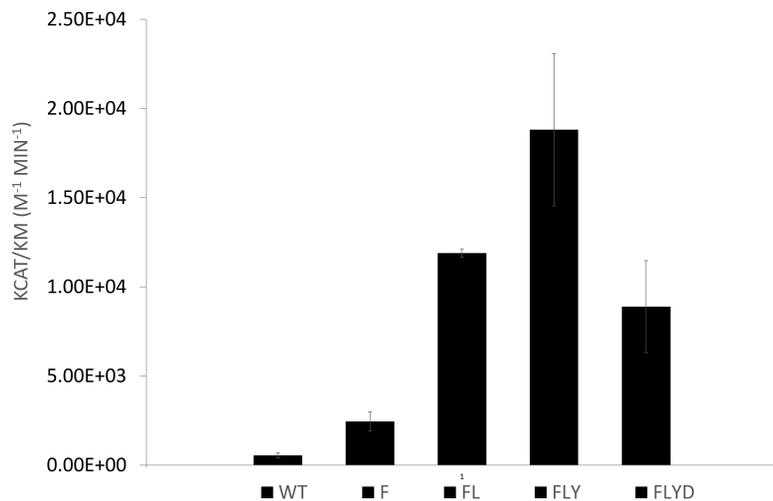
## Abstract

The enzyme organophosphorus acid anhydrolase (OPAA) has been shown to rapidly catalyze G-agent hydrolysis. There have been no previous reports of OPAA activity on VR (Russian VX, *O*-isobutyl S-[2-(diethylamino)ethyl] methylphosphonothioate) and wild-type OPAA has poor activity on VR. However, with a structurally aided protein-engineering approach, a 30-fold improvement in catalytic efficiency was realized via optimization of three amino acids within the small pocket of the substrate-binding site. X-ray structures provide potential structural rationales for their effect on the OPAA active site. A fourth mutation near the small pocket was found to relax the stereospecificity of the OPAA enzyme. Thus, it allows the altered enzyme to effectively process both VR enantiomers.

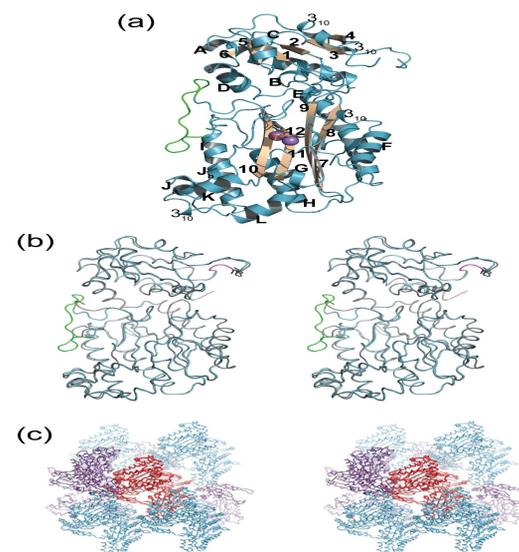
## OPAA Prime Mutants

**Table 1.** Genotypes and kinetic parameters of purified OPAA enzymes.

Enzyme	Genotype	$k_{cat}$ (min <sup>-1</sup> )	+/-	$K_m$ (μM)	+/-	$k_{cat}/K_m$ (M <sup>-1</sup> min <sup>-1</sup> )	+/-
WT	Wild-type	1.8	0.12	3280	551	548	128
F	Y212F	11	0.75	4482	680	2451	540
FL	Y212F/V342L	21	1.1	1767	258	11894	2349
FLY	Y212F/V342L/I215Y	38	2.7	1915	456	19642	6071
FLYD	Y212F/V342L/I215Y/H343D	18	1.3	2075	460	8890	258



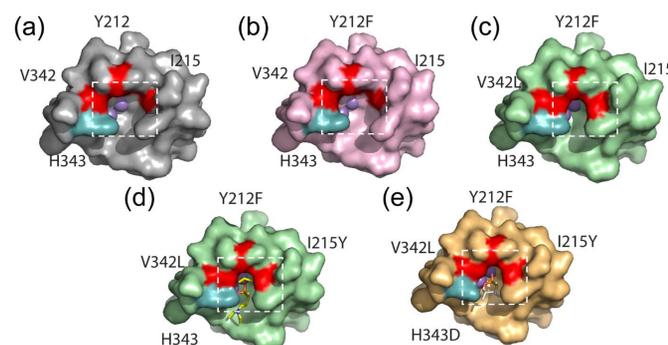
**Figure 1 Representation of KCAT/KM Values of 4 generations of OPAA Mutants on VR.**



**Figure 2. Tertiary and Quaternary structure of OPAA Y212F.**

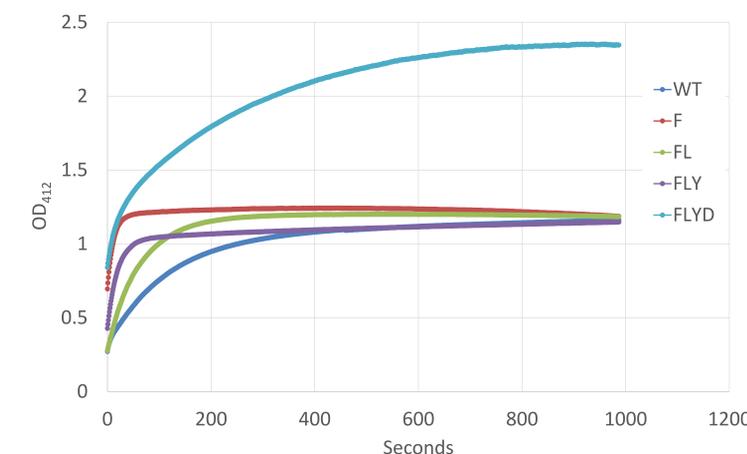
(a) Cartoon of Y212F bound with glycolate (GOA; orange) and interacting with Mn(II) (light purple). Helical regions in krypton, identified using letters, β-sheets in wheat, identified using numbers and the additional residues, which make the handle, are green. (b) Wall-eyed stereoview of Y212F wire diagram showing the extra residues making the handle (green) and the missing residues (magenta). (c) Wall-eyed stereoview of Y212F lattice (teal) overlaid with wild-type OPAA (purple; PDB entry 3L24); the monomer common to both lattices is rendered red.

## Surface Area of OPAA



(a) Surface area of the substrate-binding sites for the wild-type OPAA co-crystallized with mipafox (grey; PDB entry 3L7G), (b) Y212F with mipafox (light pink), and (c) FLY (light green), (d) same as (c) with VR P(+) enantiomer in active site, and (e) FLYD based on the FLY (light brown) active site with H343D and placement of VR P(-) in active site. The substrate-binding site in all panes is outlined in the white box and the amino acids 212, 342, and 215, are colored red with H343 dark teal. Mipafox in (a) and (b) removed for clarity of the small pocket space. Mn<sup>2+</sup> ions are light purple spheres.

## Stereochemistry Alteration



**Figure 3. Reaction profiles of wild-type, F, FL, FLY and FLYD OPAA Mutant Enzymes on 0.18 mM VR.**

## Conclusions

- Three successive mutations (Y212F, V342L, and I215Y) increased VR racemic catalytic efficiency more than 30-fold. A fourth mutation, H343D reduced the catalytic efficiency approximately 2-fold, but much more importantly, it greatly increased catalysis of the Sp or P(-) enantiomer, which binds acetylcholinesterase much more strongly than Rp or P(+).
- This structurally-aided modeling, informed by X-ray crystallography data, successfully predicted productive OPAA mutations with regard to activity and stereospecificity on VR.
- The FLYD mutant might be a good parent enzyme for further engineering of VR activity.