Supplementary Information

Molecular Engineering of Organophosphate Hydrolysis Activity from a Weak Promiscuous Lactonase Template

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Table S1: Steady-state kinetic constants of *DrPLL*, selected mutants, and *PdPTE* for the hydrolysis of δ -nonanoic lactone at pH 8.3.

enzyme	δ -NL		
	k_{cat} [s^{-1}]	K_{M} [mM]	$k_{\text{cat}}/K_{\text{M}}$ [$\text{M}^{-1} \text{s}^{-1}$]
<i>DrPLL</i>	2.2×10^2	0.028	7.9×10^6
<i>DrPLL.1</i>	1.8×10^1	1.9	9.2×10^3
<i>DrPLL.7</i>	1.4×10^2	5.9	2.4×10^4
<i>DrPLL.8</i>	8.6×10^1	4.2	2.0×10^4
<i>DrPLL.9</i>	2.4×10^0	1.4	1.7×10^3
<i>DrPLL.10</i>	4.4×10^0	1.6	2.8×10^3
<i>PdPTE</i>	5.7×10^{-2}	0.84	6.8×10^1

Table S2: Kinetic parameters for the hydrolysis of individual enantiomers of **OP 1-5** catalyzed by *DrPLL* and *DrPLL.10*.

Substrate	$k_{\text{cat}}/K_{\text{M}}$ [$\text{M}^{-1} \text{s}^{-1}$]	wild-type <i>DrPLL</i>	<i>DrPLL.10</i>
OP 1	R _P	1.9×10^3	1.1×10^6
	S _P	1.0×10^0	1.3×10^3
OP 2	R _P	5.6×10^1	9.6×10^5
	S _P	3.0×10^{-1}	6.3×10^1
OP 3	R _P	3.0×10^1	1.1×10^6
	S _P	2.0×10^0	1.7×10^2
OP 4	R _P R _C + R _P S _C	6.2×10^0	1.3×10^4
	second	n.d.	2.5×10^0
	third	n.d.	3.4×10^{-1}
OP 5	R _P	7.1×10^0	8×10^4
	S _P	2.0×10^{-1}	1.7×10^1

n.d.: not determined. The enantiomeric preference for **OP 4** is $(R_P R_C + R_P S_C) > S_P R_C$ or $S_P S_C$. The rate constants for $k_{\text{cat}}/K_{\text{M}}$ of the second and third phases cannot be assigned to either $S_P R_C$ or $S_P S_C$.

Table S3: Ratios of k_{cat}/K_M , $(R_P)/(S_P)$, for the hydrolysis of chiral **OP 1-5** by *DrPLL* and *DrPLL.10*.

	R_P/S_P for 1	R_P/S_P for 2	R_P/S_P for 3	R_P/S_P for 4	R_P/S_P for 5
<i>DrPLL</i>	1.9×10^3	1.9×10^2	1.5×10^1	n.d.	3.6×10^1
<i>DrPLL.10</i>	8.5×10^2	1.5×10^4	6.5×10^3	5.2×10^3	4.7×10^3

n.d.: not determined. R_P/S_P for **OP 4** is equal to $k_{\text{cat}}/K_M (R_P R_C + R_P S_C) / k_{\text{cat}}/K_M$ for the second phase.

Table S4: Steady-state kinetic constants of *DrPLL*, engineered variants, and *PdPTE* for the hydrolysis of **OP 8** at pH 8.0.

enzyme	OP 8		
	k_{cat} [s ⁻¹]	K_{M} [mM]	$k_{\text{cat}}/K_{\text{M}}$ [M ⁻¹ s ⁻¹]
<i>DrPLL</i> ^a	n.d.	n.d.	n.d.
<i>DrPLL.1</i> ^b	7.3×10^{-3}	5.5	1.3×10^0
<i>DrPLL.7</i>	1.5×10^{-2}	7.7	1.9×10^0
<i>DrPLL.8</i>	6.5×10^{-3}	9.5	6.8×10^{-1}
<i>DrPLL.9</i>	6.7×10^{-3}	3.5	2.0×10^0
<i>DrPLL.10</i>	n.d.	n.d.	n.d.
<i>PdPTE</i>	1.0×10^0	0.8	1.2×10^3

n.d.: not detected. ^a: No activity was detected for *DrPLL* and *DrPLL.10* for the hydrolysis of **OP 8** up to 50 μM enzyme concentration.

Table S5: Data collection and refinement statistics for *DrPLL.8*, *DrPLL.9* and *DrPLL.10*.

	<i>DrPLL.8</i>	<i>DrPLL.9</i>	<i>DrPLL.10</i>
PDB ID	native, pH 8.0 4J5N	native, pH 8.5 4J2M	native, pH 6.5 4J35
Data collection			
beamline	SSRL BL 7-1	SLS PXII	SLS PXII
wavelength (Å)	0.9794	1.0	1.0
space group	P 31 21	P 31 21	P 31 21
unit cell dimensions	a=61.655Å b=61.655Å c=203.670Å $\alpha=\beta=90^\circ$, $\gamma=120^\circ$	a=61.78Å b=61.78Å c=203.71Å $\alpha=\beta=90^\circ$, $\gamma=120^\circ$	a=61.853Å b=61.853Å c=204.786Å $\alpha=\beta=90^\circ$, $\gamma=120^\circ$
resolution range (Å)	50.0-2.05 (2.09-2.05)	47.37-1.79 (1.88-1.79)	47.46-1.78 (1.88-1.78)
no. of observed reflections	540199	233864	231779
no. of unique reflections	29424	43548	44209
redundancy	18.4 (19.1)	5.4 (5.2)	5.3
mosaicity	0.351	0.249	0.110
I (%)	100 (100)	99.1 (97.7)	99.44
I/ σ I	36.5 (7.8)	13.60	9.83
R _{merge} (%) [†]	-	0.050 (0.492)	0.075 (0.505)
R _{sym} (%)	13.7 (55.8)	-	-
Refinement			
residues in molecule	T2-G323	T2-G323	T2-G323
R _{cryst} (%) [‡]	23.5	21.9	20.13
R _{free} (%) [‡]	26.1	25.05	22.61
no. of protein non-hydrogen atoms		2702	2592
no. of water molecules	209	273	173
average B factor (Å ²)	18.9	38	46.7
rmsd bond length (Å)	0.012	0.012	0.015
rmsd angles (deg)	1.60	1.337	1.477
Ramachandran plot (%)			
favored regions	92.5	97.20	98.12
allowed regions	7.5	2.18	1.88
disallowed regions	0.00	0.62	0.00

rmsd: root mean square deviation.

Values in parenthesis indicate statistics for the highest resolution shell.

[†]R_{merge} = $\frac{\sum_j \sum_i |I_j(hkl) - \langle I(hkl) \rangle|}{\sum_j \sum_i \langle I(hkl) \rangle}$, where I_j is the intensity measurement for reflection j and

$\langle I \rangle$ is the mean intensity over j reflections.

[‡]R_{cryst} or R_{free} = $\frac{\sum_i |F_o(hkl) - F_c(hkl)|}{\sum_i F_o(hkl)}$, where F_o and F_c are the observed and calculated structure factors, respectively. 5% of the reflections were excluded from the refinement and used to calculate R_{free}.

Figure S1: Structure based sequence alignment of PTE and selected PLL enzymes generated by 3D-coffee¹. Sequence alignment of *P. diminuta* PTE (*PdPTE*; gi|54778533), *D. radiodurans* Dr0930 (*DrPLL*; gi|15805954), *G. kaustophilus* *GKL* (gi|56420041), *S. solfataricus* *SsoPox* (gi|15899258), *R. erythropolis* *AhIA* (gi|229491665), and *M. tuberculosis* *PPH* (gi|15607371). Conserved residues are colored in shades of blue. Catalytically important residues for PTE and PLL enzymes are highlighted in orange. Catalytically important residues for PLL enzymes are highlighted in light green. The boxes highlight the residues corresponding to loops at the C-terminal ends of β -strands 7 and 8.

Figure S2: Structural superposition of *DrPLL* and *PdPTE*. *DrPLL* (PDB ID: 3FDK) and *PdPTE* (PDB ID: 1EZ2) are depicted in grey and green, respectively. (a) Parts of the backbone are shown, which illustrate major structural differences in the length and conformation of the $\beta\alpha$ -loops (predominately $\beta\alpha$ -loop 1, 3, 7 and 8). The over-all backbone conformation of *DrPLL* is very similar to that of *PdPTE* (backbone r.m.s.d = 1.3 Å). Residue Y97, which is conserved within the PLL family and located in $\beta\alpha$ -loop 3, aligns with W131 in *PdPTE*. (b) Excerpt of the active site of *DrPLL* with the docked tetrahedral intermediate that is formed upon the hydrolysis of δ -nonanoic lactone (depicted in dark grey). The lactone intermediate in *DrPLL* is positioned by polar interactions with Y97 (2.8 Å; Y97 aligns with W131 in PTE), R228 (5.3 Å; R228 aligns with H254 in *PdPTE*) and D264 (2.3 Å; D264 aligns with D301 in *PdPTE*). The side chain of the R228 residue is shifted by approximately 3 Å in the docked structure relative to the conformation observed in the crystal structure to accommodate the ligand (native conformation is not shown). (c) Excerpt of the active site of *PdPTE*, complexed with the substrate analogue di-isopropyl methyl phosphonate (DIMP); depicted in dark green. *PdPTE* exhibits only one hydrogen bond (3.2 Å) between N^{E1} of Trp-131 and the phosphoryl oxygen of DIMP. Together with D301 and D233, H254 is involved in proton shuttling in *PdPTE*. The equivalent residues in *DrPLL* to D301 and H254 are D264 and R228. The equivalent residue to D233 in *DrPLL* is G207 (not shown).

Figure S3: Time courses for the chemical (a) and enzymatic hydrolysis (b, c, d) of approximately 50 μM racemic **OP 3**. The stereoselectivity of *DrPLL* and *DrPLL.10* was assigned using the previously reported stereoselectivities of *PdPTE* and *PdPTE* variants².

(a) Time course of the chemical hydrolysis of racemic **OP 3** using 75 mM KOH. (b) Time courses for the enzymatic hydrolysis of racemic **OP 3** using 110 μM *DrPLL* (blue) and 4.9 nM *DrPLL.10* (red). *DrPLL.10* hydrolyzes the R_p and S_p enantiomers at significantly different rates. After 30 minutes one half of the initial substrate was hydrolyzed by *DrPLL.10*, and then 4.8 μM *DrPLL.10* was added to the reaction mixture to hydrolyze the remaining enantiomer. (c) The enzymatic hydrolysis of the R_p enantiomer of racemic **OP 3** was initiated with 4.9 nM *DrPLL.10*. After 27 minutes 0.65 nM *PdPTE*-G60A variant (dark cyan; preference for R_p , ratio R_p/S_p = 7600:1) was added and no further hydrolysis was observed. At 30 minutes 4.1 nM of the mutant *PdPTE*-H254G+H257W+L303T (dark purple; enantiomeric preference for S_p , ratio R_p/S_p = 1:120) was added to hydrolyze the remaining S_p enantiomer. (d) Enzymatic hydrolysis of racemic **OP 3** by 4.1 nM *PdPTE*-H254G+H257W+L303T (dark purple) and 0.65 nM *PdPTE*-G60A (dark cyan) as a function of time. At 23 and 32 minutes respectively, higher concentrations of enzyme (0.28 μM and 3 μM) were added for complete hydrolysis. Depending on the compound and enzyme variant used, one or two phases were observed and fit to a single exponential ($A(t) = y_0 + a(1 - e^{-k_1 t})$) or double exponential fit ($A(t) = y_0 + a(1 - e^{-k_1 t}) + b(1 - e^{-k_2 t})$).

Figure S4: Structural superpositions of *DrPLL.10* and *DrPLL* with focus on mutated positions. Wild-type *DrPLL* (PDB ID: 3FDK) and *DrPLL.10* (PDB ID: 4J35) are depicted in grey and blue, respectively. (a) Excerpts of structural superpositions of $\beta\alpha$ -loops 1, 2 and 3. The $\beta\alpha$ -loop 3, comprising the mutations Y97F and E101G, is shifted by 3 Å at positions A99-A103, due to the elimination of a hydrogen bond between D71 and the backbone nitrogen of A103 (dashed line). The mutations Y28L in $\beta\alpha$ -loop 1 and Y97F and E101G in $\beta\alpha$ -loop 3, caused an enlargement of the substrate binding pocket. (b) Excerpts of structural superpositions of $\beta\alpha$ -loops 5, 7 and 8 of *DrPLL* and *DrPLL.10*. The $\beta\alpha$ -loop 7, harboring the V235L mutation, is flexible. D179 in *DrPLL.10* ($\beta\alpha$ -loop 5) adopts an alternate conformation than E179 in wild-type *DrPLL*. The $\beta\alpha$ -loop 8, harboring the mutation P274L, is shifted by 1.7 Å.

Figure S5: Docking poses of DIMP and paraoxon in the crystal structure of *PdPTE*, obtained using the RosettaLigand program. *PdPTE* (PDB ID: 1E22) and the co-crystallized DIMP ligand are depicted in grey and dark grey, respectively. The docking poses are depicted in green and purple. (a) Active site and substrate binding pocket of *PdPTE* with docked DIMP ligand. One top-scoring docking pose of DIMP is superimposed with the native DIMP pose observed in the crystal structure. The two best docking poses of DIMP, superimposed with the native DIMP ligand, are shown in the inset in a different view. The docked DIMP ligands superimpose with an r.m.s.d of 0.5-0.8 Å with the native DIMP pose. (b) Active site and substrate binding pocket of *PdPTE* with docked **OP 7**. One top-scoring docking pose of paraoxon is superimposed with the native DIMP pose observed in the crystal structure. The phosphoryl oxygen of paraoxon is coordinated by the β -metal. The O-ethyl substituents at the phosphorus center of paraoxon fit the small (G60, S308, I106 and L303; I106 and L303 are not shown; overlined) and large (H254, H257, L271, M317; underlined) substrate binding pockets. The *p*-nitrophenyl moiety occupies the leaving group pocket (W131, F132, F306, Y309; boxed) of *PdPTE*. The two best docking poses of paraoxon, superimposed with the native DIMP ligand, are shown in the inset in a different view.

Figure S6: Docking poses of paraoxon in the crystal structure of *DrPLL.10*, obtained using the RosettaLigand program. The paraoxon docking poses are depicted in green and purple. The phosphoryl oxygen of paraoxon is hydrogen bonded to the R228 side chain (dashed lines) instead of coordinating the β -metal as observed in the modeled *PdPTE*-paraoxon complex (cf. **Figure S5b**). The *p*-nitrophenyl ring in the paraoxon docking pose is orientated towards $\beta\alpha$ -loops 1 and 3. The residue R228 in the docked structure is shifted compared to the conformation obtained by crystallization.

Figure S7: Structural superposition of the active sites of *DrPLL.10* and *PdPTE* with the top scoring docking poses for paraoxon. *DrPLL.10* and *PdPTE* are depicted in grey and green, respectively. One top scoring paraoxon docking pose is shown for each. The docking poses for paraoxon are identical with respect to the *p*-nitrophenol moiety, but differ in orientation of the O-ethyl substituents. The O-ethyl substituents of the docked paraoxon in *DrPLL.10* are shifted to avoid a steric clash with the F26 side chain. The residue R228 in the docked structure of *DrPLL.10* is shifted relative to the conformation obtained by crystallization (not shown).

Supplementary Methods

Library construction and site-directed mutagenesis to add and combine mutations in *drpll* gene variants.

The plasmid-encoded error-prone library was generated on the *DrPLL* wild-type template, applying the protocol essentially as described³. Briefly, the *drpll* gene was subjected to error-prone PCR in 3 independent reactions, which contained the template pTNA-*drpll*, GoTaq polymerase (Promega), unbalanced concentrations of nucleoside triphosphates (0.35 mM dATP, 0.4 mM dCTP, 0.2 mM dGTP, 1.35 mM dTTP), 0.75 mM MnCl₂, and 1 mM MgCl₂. The amplification products were gel-purified, and digested with *SphI* and *HindIII* (New England Biolabs), and the purified reaction products were ligated with equally digested pTNA plasmid. The ligation reaction was used to transform electrocompetent *E. coli* BW25113 $\Delta hisA\Delta hisF$ cells, plated and plasmids were prepared. For generating libraries of shuffled genes, selected *drpll* variants were applied to the Staggered Extension Process Process⁴. The assembly PCR reaction were further amplified by nested PCR, digested with *SphI* and *HindIII* (New England Biolabs) and recloned into pTNA vector for low constitutive expression. The *bona fide* activity enhancing exchanges were introduced and combined by site-directed mutagenesis, using overlap extension PCR⁵. The resulting modified *drpll* genes were amplified by PCR and cloned into the expression plasmid pET24a(+), using newly introduced restriction sites for *NdeI* and *XhoI*. Alternatively, targeted libraries were constructed using QuikChange Mutagenesis⁶. All constructs were entirely sequenced to exclude inadvertent mutations.

Heterologous expression of *PdPTE* and *DrPLL* variants, and purification of the recombinant protein.

PdPTE and *DrPLL* gene variants were expressed in *E. coli*. To this end, chemically competent *E. coli* BL21(DE3) Rosetta 2 and *E. coli* BL21(DE3) cells (Merck) were transformed with the pET30a(+)/pET24a(+)-*drpll* and pET20b(+)-*pdpte* constructs, and single colonies were used to inoculate 2 l LB and 8 l TB medium, respectively. The medium was supplemented with the corresponding antibiotics and 0.5 mM CoCl₂ and 1.0 mM CoCl₂, respectively, and cells were grown at 37 °C until OD₆₀₀ reached a value of 0.6. Following induction of gene expression with 0.5 mM IPTG and addition of extra 0.5 mM CoCl₂ for the

DrPLL expression cultures, the cell suspension was incubated overnight at 37 °C and 30 °C, respectively. Cells were harvested by centrifugation and the pellet was resuspended in 20 mM HEPES, pH 7.5, 100 µM CoCl₂ and 50 mM HEPES, pH 8.5, 100 µM CoCl₂, respectively. Cells were disrupted by sonication. The recombinant proteins were purified from the soluble fraction of the cell extract by protamine sulfate and ammonium sulfate precipitation and, either anion exchange chromatography or a combination of anion exchange chromatography and gel filtration chromatography. To this end, the soluble fraction of the cell extract was treated with 1% protamine sulfate. The supernatant was subjected to ammonium sulfate precipitation, using 45% and 60% ammonium sulfate for *DrPLL* and *PdPTE*, respectively. Pellets, containing the protein of interest, were resuspended in 15 ml of 20 mM HEPES, pH 7.5, 100 µM CoCl₂ or 50 mM HEPES, pH 8.5, 100 µM CoCl₂, respectively. Wild-type *DrPLL* and *DrPLL* variants were purified by Q Sepharose Fast Flow anion exchange chromatography (GE Healthcare). Wild-type *PdPTE* and *PdPTE* variants were purified by Superdex 200 or Superdex 75 size exclusion chromatography and DEAE Sepharose Fast Flow anion exchange chromatography (GE Healthcare). Combined pure protein fractions were dialyzed overnight against 5 l of 20 mM HEPES, pH 7.5, 100 µM CoCl₂ and 50 mM HEPES, pH 8.5, 100 µM CoCl₂, respectively.

Crystallization, data collection and processing, structure determination and refinement.

Crystals were obtained by sitting- and hanging drop vapor diffusion methods, performed at 22 °C and 20 °C, respectively. To this end, 1 µl (2 µl) of protein (approximately 12.8 - 13.5 mg/ml, supplemented with 1.0 mM CoCl₂ and inhibitor) and 1 µl (2 µl) of precipitating agent were mixed and placed on a siliconized coverslip, and equilibrated over 55 µl (500 µl) of the precipitating agent. Crystals of *DrPLL.8* were obtained in 10% (w/v) PEG-8000, 0.1 M imidazole pH 8.0, 0.2 M Ca(OAc)₂, 3% (v/v) di-isopropyl methyl phosphonate. Crystals of *DrPLL.9* were obtained in 0.01 M NiCl₂, 0.1 M Tris pH 8.5, 8% PEG MME 2000, 3% (v/v) di-isopropyl methyl phosphonate, 3% (v/v) MeOH. Crystals of *DrPLL.10* were obtained in 0.1 M MES pH 6.5, 1.3 M MgSO₄, 1% (v/v) diethyl 4-methylbenzylphosphonate. The Crystals were diamond shaped and purple in color. Crystals, transferred to ethylene glycol as cryo protectant, were flash frozen in liquid nitrogen, and data of single crystals were collected at the synchrotron beamline BL 7-1 (Stanford Synchrotron Radiation Lightsource) and PXII (Swiss Light Source) at 295 K and 100 K, recording single 0.1 or 0.5 degree images on a CCD

(ADSC QUANTUM 315r) or PILATUS detector, respectively. Data sets of *DrPLL.8* were indexed, integrated and scaled with the HKL2000 package⁷, and phases were determined by molecular replacement using Phaser version 2.1⁸. In case of *DrPLL.9* and *DrPLL.10*, data processing was done using XDS⁹, and the data quality assessment was performed using phenix.xtriage¹⁰. Molecular replacement was performed with MOLREP within the CCP4i suite¹¹. In all cases the structure of wild-type *DrPLL* (PDB ID 3FDK) was used as search model and initial refinement of the structures was performed using Refmac5¹² of the CCP4 suite¹³. For *DrPLL.8* difference electron density and omit maps were manually fit with the XtalView package¹⁴, and further refinement was done with CNS¹⁵. All structures were superimposed with Sequoia¹⁶. For *DrPLL.9* and *DrPLL.10*, the model was further improved in several refinement rounds using the automated restrained refinement within the program PHENIX¹⁰ and interactive modeling with Coot¹⁷. The final models were analyzed using the program MolProbity¹⁸. The data sets had a resolution of 1.78-2.05 Å, and cell dimensions were as follows: $a = 62 \text{ Å}$, $b = 62 \text{ Å}$, $c = 204 \text{ Å}$, $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$. The primitive trigonal space group was $P3_121$. The crystals contained one molecule in the asymmetric unit yielding a VM of approximately $3.24 \text{ Å}^3/\text{Da}$. Final R_{cryst} and R_{free} values for all structures were below 23.5% and 26.1%, respectively. The data collection and refinement statistics are summarized in Table S5.

Docking Modeling.

Docking calculations were performed with RosettaLigand as implemented in the Rosetta software suite version 3.3¹⁹. The program parameters applied were based on the parameters used earlier²⁰. RosettaLigand tries to find conformations of a receptor-ligand complex that minimizes the Rosetta energy score function, thereby employing a stochastic search algorithm. Many conformations are created, of which only the best scoring ones are used for further analysis.

Receptor preparation. Hydrogen atoms were added using Chimera²¹. A residue parameter file was created for the carboxylated Lysine KCX as described in the Rosetta documentation. The identity of the active site cobalt atoms for *DrPLL.10* was changed to zinc and distance constraints were calculated for the side chain atoms coordinating these metals in order to maintain the native coordination geometry. A penalty energy term was applied, if these

atoms deviated more than 0.2 Å from the measured distances. Rosetta's receptor minimization protocol was used to create ten minimized structures for each receptor protein. The structure with the lowest energy was used for docking calculations.

Ligand preparation. Ligand conformers were created with the software omega2, and charges added by molcharge²². Ligand parameter files were created as described in the Rosetta documentation. As starting position of the ligand DIMP in receptor PTE, a random conformation excluding the one the ligand assumes in the crystal structure was chosen to avoid biasing the docking algorithm towards the known binding mode. The starting positions of ethyl paraoxon were determined by roughly positioning an arbitrary ligand conformer in the binding pocket. For the *DrPLL.10*, this ligand conformer was superimposed at the phosphorus atom onto DIMP in the PTE structure 1EZ2, then superimposing this complex onto the *DrPLL.10* structure. For some docking runs, geometric constraints, derived from the PTE structure 1EZ2, were put on the phosphoryl oxygen and the phosphorus atoms of the ligand to enrich results containing the desired catalytic geometry. The distance between the phosphoryl oxygen and the interacting β -zinc ion was constrained to 2.5 ± 0.4 Å and the angle formed by zinc, phosphoryl oxygen and phosphorus atom was set to $140^\circ \pm 30^\circ$.

Docking calculations. For each receptor ligand pair, 5000 receptor-ligand conformations were created with the RosettaLigand application. For analysis, the top 5% by Rosetta's total energy score were ranked according to their predicted binding energies. The top 20 ranked binders were then used for analysis. This ranking scheme is suggested by the RosettaLigand documentation to search for suitable binders among low energy and therefore plausible complex structures.

Supplementary References

1. O'Sullivan, O., Suhre, K., Abergel, C., Higgins, D.G. & Notredame, C. 3DCoffee: combining protein sequences and structures within multiple sequence alignments. *Journal of molecular biology* **340**, 385-95 (2004).
2. Tsai, P.C. et al. Stereoselective hydrolysis of organophosphate nerve agents by the bacterial phosphotriesterase. *Biochemistry* **49**, 7978-87 (2010).
3. Claren, J., Malisi, C., Hocker, B. & Sterner, R. Establishing wild-type levels of catalytic activity on natural and artificial (beta alpha)8-barrel protein scaffolds. *Proc Natl Acad Sci U S A* **106**, 3704-9 (2009).
4. Zhao, H., Giver, L., Shao, Z., Affholter, J.A. & Arnold, F.H. Molecular evolution by staggered extension process (StEP) in vitro recombination. *Nat Biotechnol* **16**, 258-61 (1998).
5. Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K. & Pease, L.R. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* **77**, 51-9 (1989).
6. Wang, W. & Malcolm, B.A. Two-stage PCR protocol allowing introduction of multiple mutations, deletions and insertions using QuikChange Site-directed Mutagenesis. *Biotechniques* **26**, 680-682 (1999).
7. Otwinowski, Z. & Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. *Methods in Enzymology, Volume 276: Macromolecular Crystallography, part A*, 307-326 (1997).
8. McCoy, A.J. et al. Phaser crystallographic software. *J Appl Crystallogr* **40**, 658-674 (2007).
9. Kabsch, W. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J Appl Cryst* **26**, 795-800 (1993).
10. Adams, P.D. et al. PHENIX: building new software for automated crystallographic structure determination. *Acta Crystallogr D Biol Crystallogr* **58**, 1948-54 (2002).
11. Potterton, L. et al. Developments in the CCP4 molecular-graphics project. *Acta Crystallogr D Biol Crystallogr* **60**, 2288-94 (2004).
12. Murshudov, G.N., Vagin, A.A. & Dodson, E.J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D Biol Crystallogr* **53**, 240-55 (1997).
13. Collaborative Computational Project, N. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr D Biol Crystallogr* **50**, 760-3 (1994).
14. McRee, D.E. XtalView/Xfit--A versatile program for manipulating atomic coordinates and electron density. *J Struct Biol* **125**, 156-65 (1999).
15. Brunger, A.T. et al. Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr D Biol Crystallogr* **54**, 905-21 (1998).
16. Bruns, C.M., Hubatsch, I., Ridderstrom, M., Mannervik, B. & Tainer, J.A. Human glutathione transferase A4-4 crystal structures and mutagenesis reveal the basis of high catalytic efficiency with toxic lipid peroxidation products. *J Mol Biol* **288**, 427-39 (1999).
17. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* **60**, 2126-32 (2004).

18. Davis, I.W. et al. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res* **35**, W375-83 (2007).
19. Davis, I.W. & Baker, D. RosettaLigand docking with full ligand and receptor flexibility. *J Mol Biol* **385**, 381-92 (2009).
20. Khare, S.D. et al. Computational redesign of a mononuclear zinc metalloenzyme for organophosphate hydrolysis. *Nat Chem Biol* **8**, 294-300 (2012).
21. Pettersen, E.F. et al. UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-12 (2004).
22. Hawkins, P.C., Skillman, A.G., Warren, G.L., Ellingson, B.A. & Stahl, M.T. Conformer generation with OMEGA: algorithm and validation using high quality structures from the Protein Databank and Cambridge Structural Database. *J Chem Inf Model* **50**, 572-84 (2010).