Supporting Information

Improving *Pseudomonas fluorescens* esterase for hydrolysis of lactones

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Table S1. PCR primers for site-directed mutagenesis. Mutation site is in bold.

Target mutation	Primers
F158Y	Forward: 5' CTCAGTTCATCTCTGAC**TAC**AACGCTCCGTTCTACGG 3' Reverse: 5' CCGTAGAACGGAGCGTT**GTA**GTCAGAGATGAACTGAG 3'
F93W	Forward: 5' GAAGTTACCCTGGTTGGT**TGG**TCTATGGGTGGTGGTGAC 3' Reverse: 5' GTCACCACCACCCATAGA**CCA**ACCAACCAGGGTAACTTC 3'
L29P	Forward: 5' GTTCTCTCACGGTTGG**CCA**CTGGACGCTGACATGTG 3' Reverse: 5' CACATGTCAGCGTCCAG**TGG**CCAACCGTGAGAGAAC 3'
W28Q	Forward: 5' GTTCTGTTCTCTCACGGT**CAA**CTGCTGGACGCTGACATG 3' Reverse: 5' CATGTCAGCGTCCAGCAG**TTG**ACCGTGAGAGAACAGAAC 3
L30P	Forward: 5' GTTCTCTCACGGTTGGCTG**CCA**GACGCTGACATGTGGGAATAC 3' Reverse: 5' GTATTCCCACATGTCAGCGTC**TGG**CAGCCAACCGTGAGAGAAC 3'
I224P	Forward: 5' ACGGTGACGGTGACCAG**CCT**GTTCCGTTCGAAACCAC 3' Reverse: 5' GTGGTTTCGAACGGAAC**AGG**CTGGTCACCGTCACCGT 3'
I224A	Forward: 5' GGTGACGGTGACCAG**GCA**GTTCCGTTCGAAAC 3' Reverse: 5' GTTTCGAACGGAAC**TGC**CTGGTCACCGTCACC 3'
I224G	Forward: 5' GGTGACGGTGACCAG**GGA**GTTCCGTTCGAAAC 3' Reverse: 5' GTTTCGAACGGAAC**TCC**CTGGTCACCGTCACC 3'
F125V	Forward: 5' GTGCTGTTACCCCGCTG**GTA**GGTCAGAAACCGGACTAC 3' Reverse: 5' GTAGTCCGGTTTCTGACC**TAC**CAGCGGGGTAACAGCAC 3'
F158V	Forward: 5' CTCAGTTCATCTCTGAC**GTC**AACGCTCCGTTCTAC 3' Reverse: 5' GTAGAACGGAGCGTT**GAC**GTCAGAGATGAACTGAG 3'
F143V	Forward: 5' CGCTGGACGTTTTCGCTCGT**GTG**AAAACCGAACTGCTGAAAGA 3' Reverse: 5' TCTTTCAGCAGTTCGGTTTT**CAC**ACGAGCGAAAACGTCCAGCG 3'
F143W	Forward: 5' CTGGACGTTTTCGCTCGT**TGG**AAAACCGAACTGCTGAAAG 3' Reverse: 5' CTTTCAGCAGTTCGGTTTT**CCA**ACGAGCGAAAACGTCCAG 3'

Table S2. Kinetic constants for hydrolysis of lactones by wild-type PFE and selected variants.[a]

Enzyme	ε-Caprolactone				α-Angelicalactone				β-Butyrolactone			
	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}*s^{-1}$)	Fold change[b]	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}*s^{-1}$)	Fold change[b]	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}*s^{-1}$)	Fold change[b]
wt pfe	>30[c]	>600[c]	51	1.0	22±6	40±20	540	1.0	3.1±0.1	1.2±0.3	2,500	1.0
C194A	nd[d]	nd[d]	21	0.4	4.2±0.7	26±7	170	0.3	14±2	9±4	1,500	0.6
F93W	5±1	360±120	13	0.3	8±2	35±13	220	0.4	0.4±0.1	0.1±0.05	4,400	1.8
W28Q	17±4	480±190	36	0.7	5±3	23±18	240	0.4	1.4±0.5	4±3	340	0.1
L30P	nd[d]	nd[d]	2	0.04	6±5	80±90	68	0.1	0.3±0.0	1.0±0.5	270	0.1
F158Y/ C194A	nd[d]	nd[d]	28	0.5	16±3	15±5	1,100	2.0	2.5±0.3	6±2	380	0.2
F93W/F158Y	nd[d]	nd[d]	4	0.09	0.7±0.2	7±4	97	0.2	1.7±0.1	0.3±0.1	6,500	2.6
L29P/F93W	15±2	260±80	58	1.1	nd	nd	23	0.04	4.8±0.3	3.1±0.6	1,500	0.6

[a] Error limits are standard deviations for the best non-linear fit of the data to the Michaelis-Menten equation. [b] change in k_{cat}/K_m as compared to wild-type PFE. [c] The reaction rate increased linearly up to 600 mM substrate, so the minimum K_m value was 600 mM; the k_{cat}/K_m, was the slope of the line and the minimum k_{cat} was estimated from the product of k_{cat}/K_m and the minimum K_m. [d] nd = not determined because V_{max} could not be reached. To estimate k_{cat}/K_m, the increase in rate with increasing substrate concentration was fit to a line.

Table S3. Kinetic constants for hydrolysis of *p*NPAc by selected variants[a]

Enzyme	pNPAc			Fold change
	k_{cat}	K_m	k_{cat}/K_m	
	(s^{-1})	(mM)	($M^{-1}*s^{-1}$)	
wt pfe	44±0	1.7±0.2	26,000	1.0
F158Y	25±8	2±1	10,000	0.4
C194A	24±2	1.4±0.2	16,500	0.6
F93W	16±0	1.2±0.1	13,000	0.5
W28Q	18±2	0.7±0.2	24,400	0.9
F125V	19±0	1.1±0.1	16,500	0.6
I224A	47.6±0.0	1.3±0.1	36,500	1.4
F158V	26.3±0.0	0.04±0.01	640,000	24
F158Y/C194A	19.0±2.4	1.4±0.2	14,000	0.5
F93W/F158Y	16.2±0.0	3.0±0.4	4,600	0.2
F158VI224A	20.3±2.2	1.3±0.5	15,600	0.6
F125V/F158V	55.8±7.2	0.29±0.05	193,000	7.4
F143VF158V	57.8±5.8	0.29±0.06	202,000	7.8
F143WF158V	63.2±6.5	0.20±0.04	322,000	12.4
F125VF143VF158V	59.3±6.1	0.40±0.09	151,000	5.8
F143VF158VI224A	20.4±1.7	0.30±0.08	68,000	2.6

[a] Variants containing proline substitution showed very low activity and are not shown.

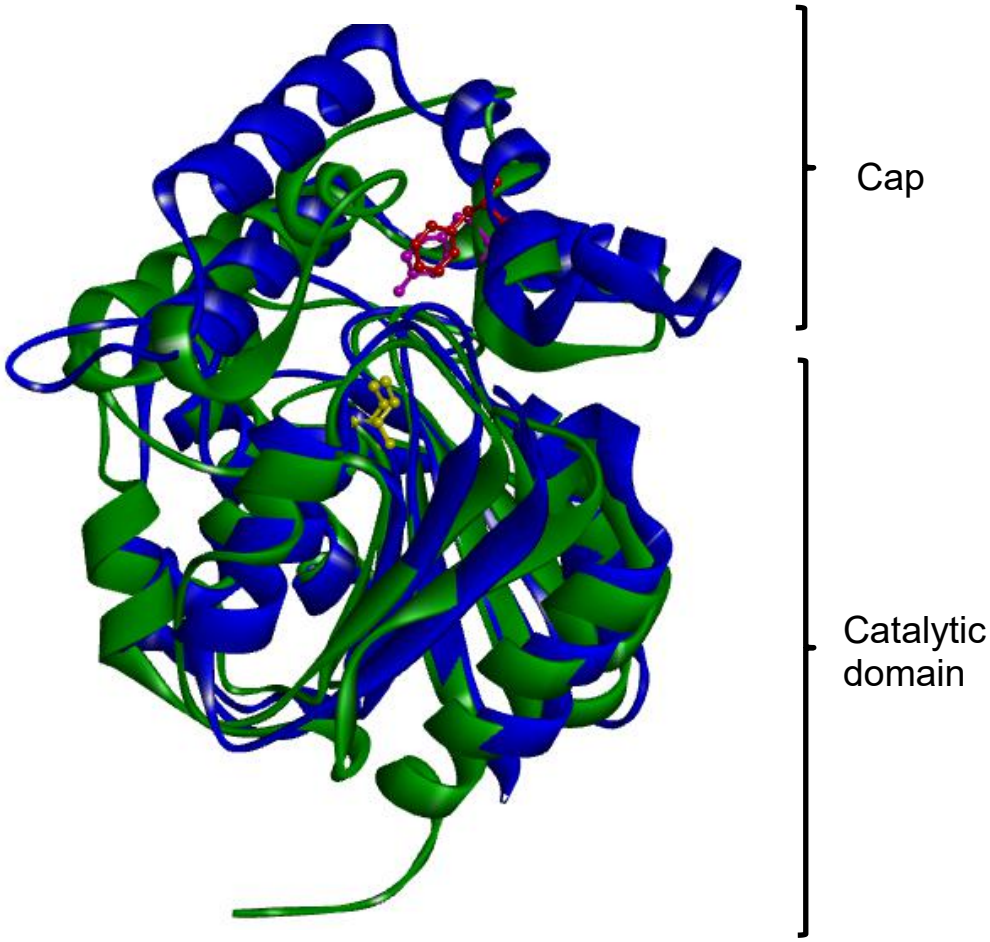

Figure S1. Overlay of ribbon diagrams of the structures of PFE (blue, pdb: 1va4) and OSL (green ribbon, pdb: 4g5x). Both adopt the α/β-hydrolase fold. and contain similarly positioned active-site serines (yellow ball and sticks), but differ in the leaving group region. PFE contains a phenylalanine (position 158, red ball and stick), while OSL contains a tyrosine (position 160, purple ball and stick). These two hydrolases share only 18% identical amino acids, but their main chain atoms overlap closely with root-mean-square deviation (RMSD) of 3.7 Å. The catalytic domains are more similar to each other (RMSD = 2.4 Å) than the cap domains (RMSD = 5.2 Å).

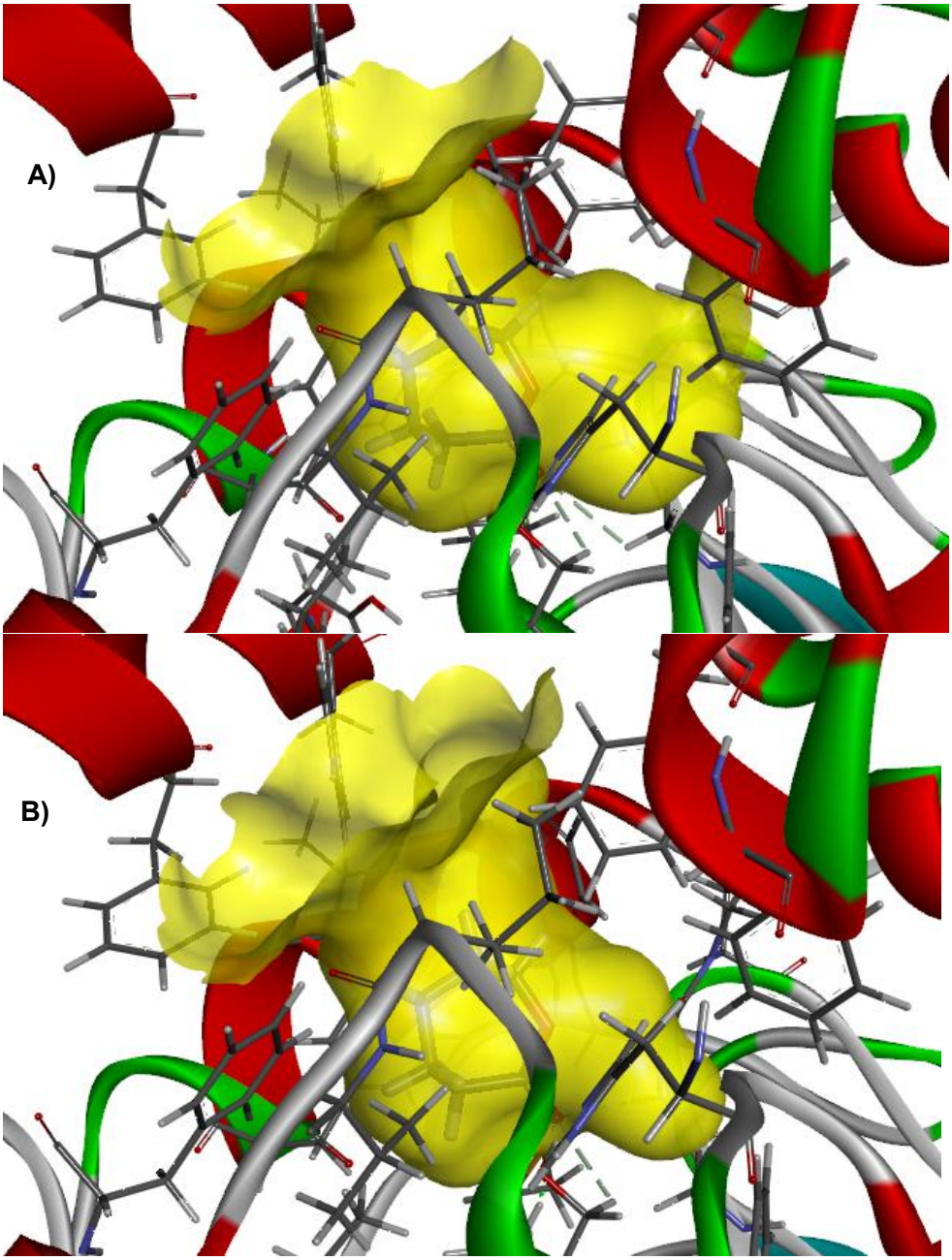

Figure S2. Substrate-binding pocket in wt PFE (A) and PFE-L29P (B). Yellow surface shows a smaller alcohol binding region in PFE L29P than in wt PFE. The substrate ε-caprolactone is docked in the binding pocket.

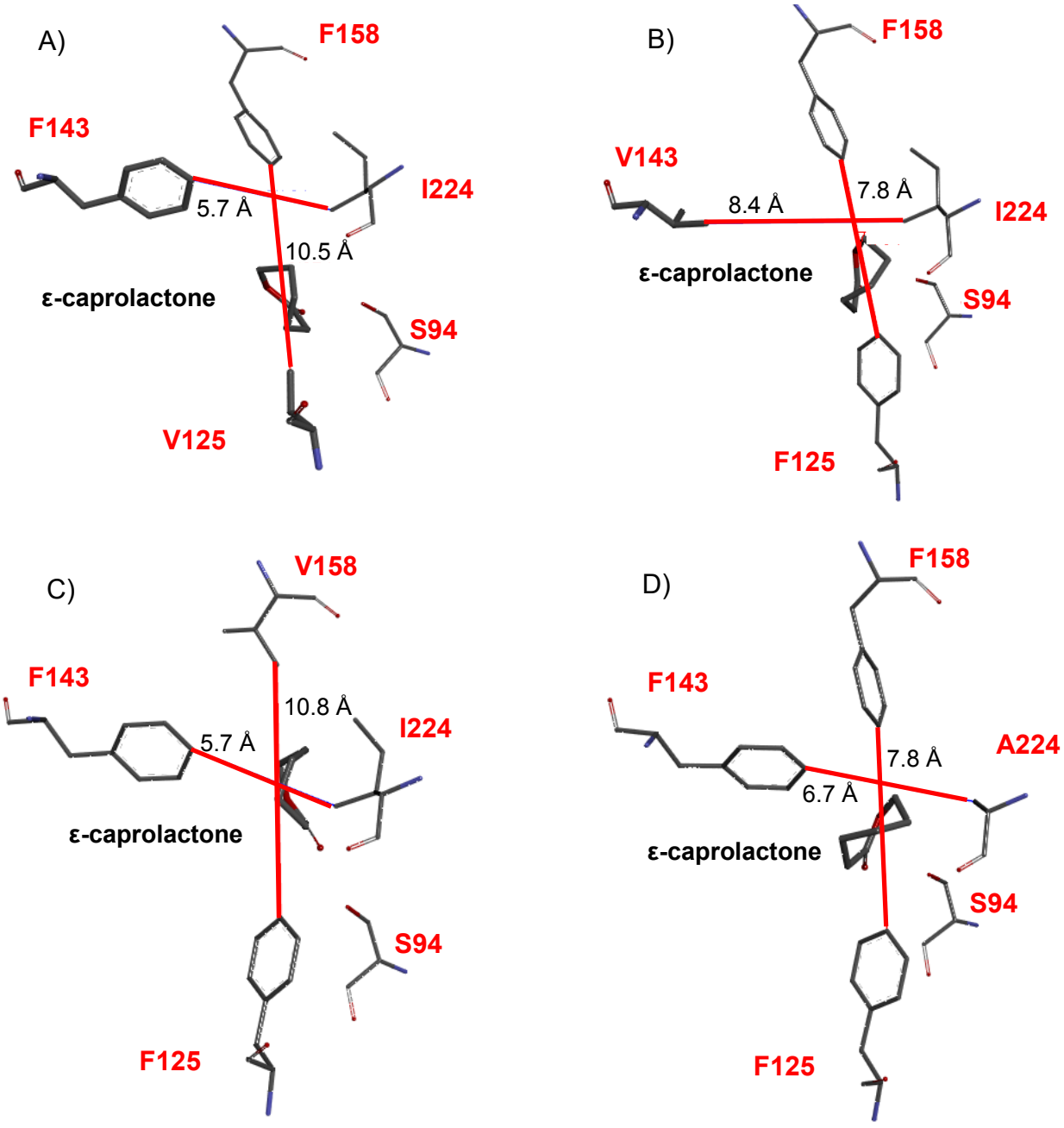

Figure S3. Estimated distances between residues around the top of substrate-binding pocket in selected variants. A)-F125V; B)-F143V; C)-F158V; D)-I224A. In wt PFE, the F143-I224 distance is 5.70 Å and the F125-F158 distance is 7.76 Å. The substrate ε-caprolactone docks in the binding pocket slightly differently in each case.

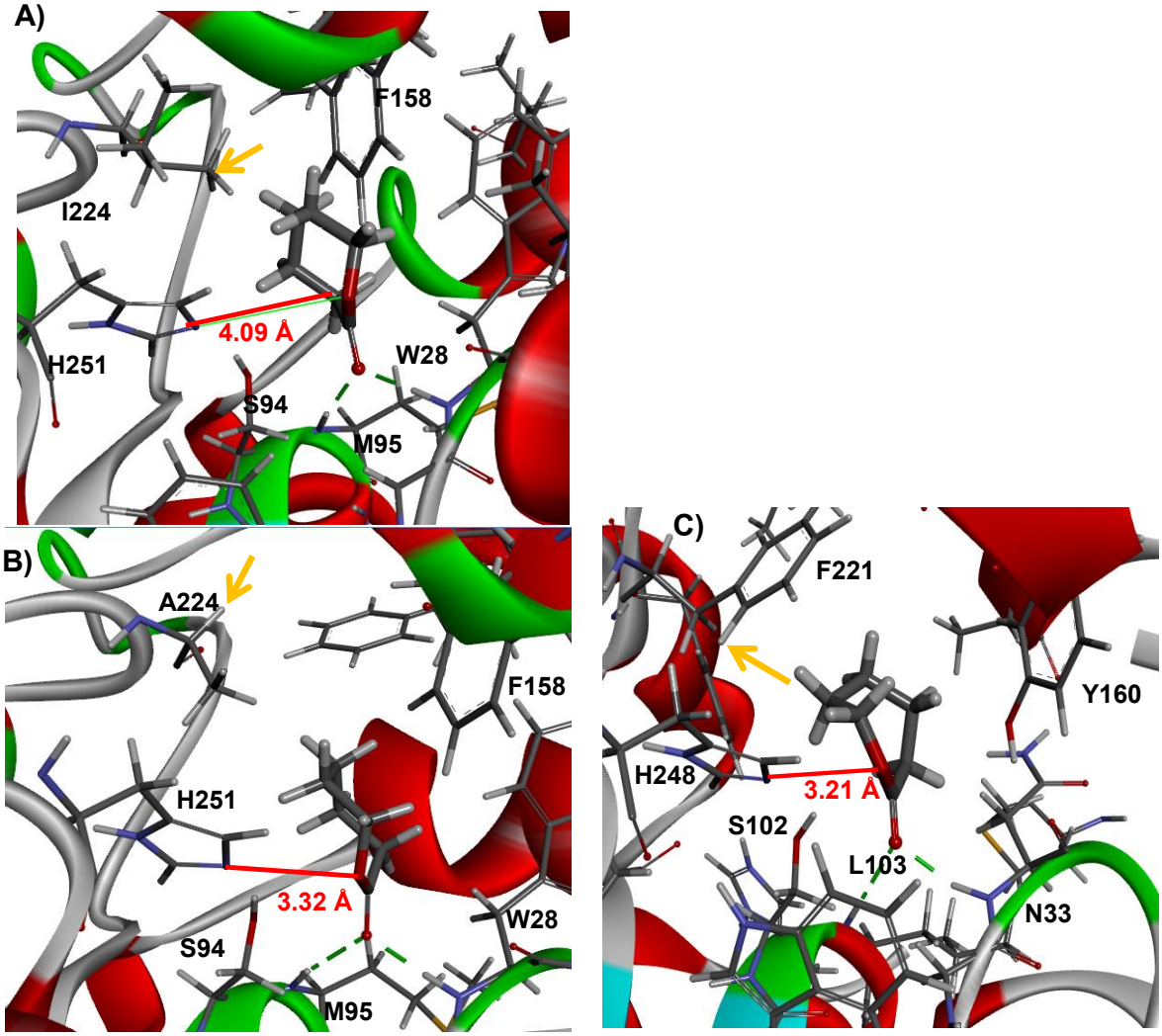

Figure S4. Docking of ε-caprolactone to the active site of wt PFE (A), PFE-I224A (B) and OSL (C). In all three proteins, the carbonyl oxygen of the lactone binds in the oxyanion hole. In wt PFE, the distance between Nε of H251 and O1 of ε-caprolactone 4.09 Å, which is too long for a hydrogen bond. In PFE-I224A, this distance shortens to 3.32 Å because residue A224 does not hinder the orientation of ε-caprolactone (orange arrow). In OSL, this distance is 3.21 Å. Residue F221 in OSL, which corresponds to I224 in wt PFE, also does not hinder the orientation of ε-caprolactone.

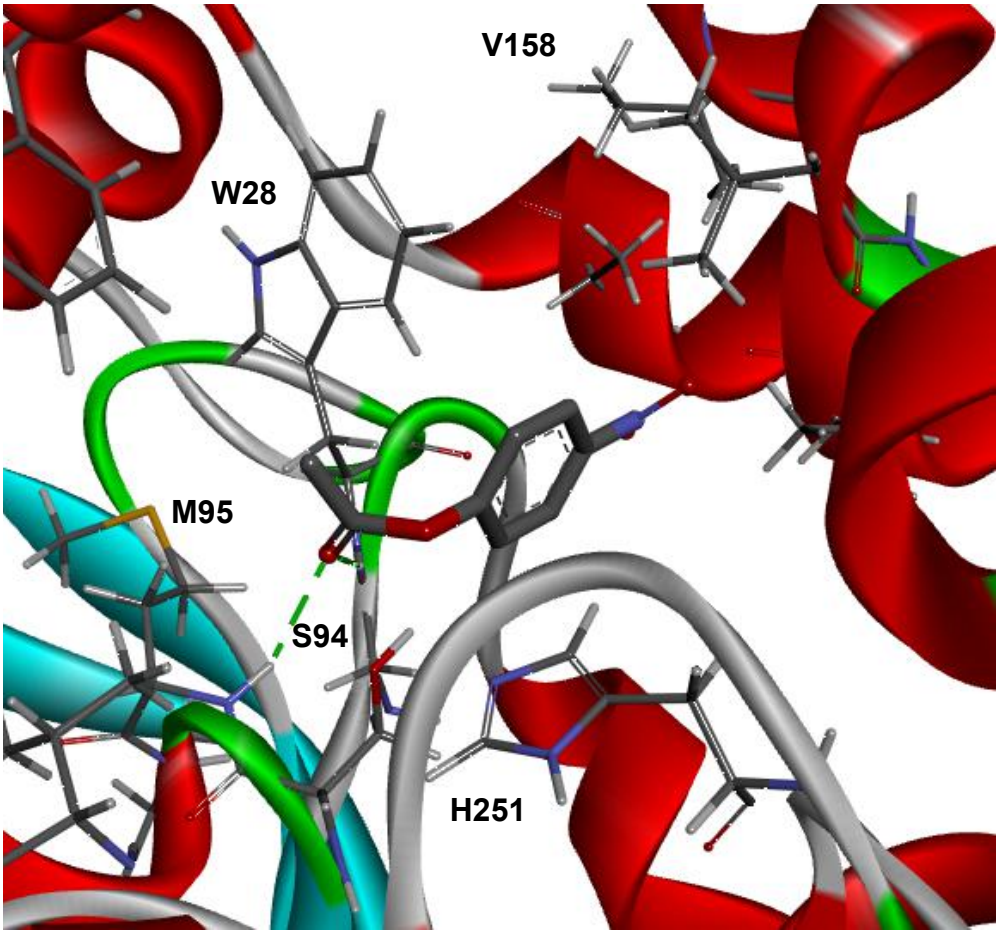

Figure S5. Docking of *p*NPAc to the active site of PFE-F158V. The carbonyl oxygen binds in the oxyanion hole. Attempted docking of *p*NPAc to the active site of wt PFE did not yield any bound poses because F158 hindered binding.