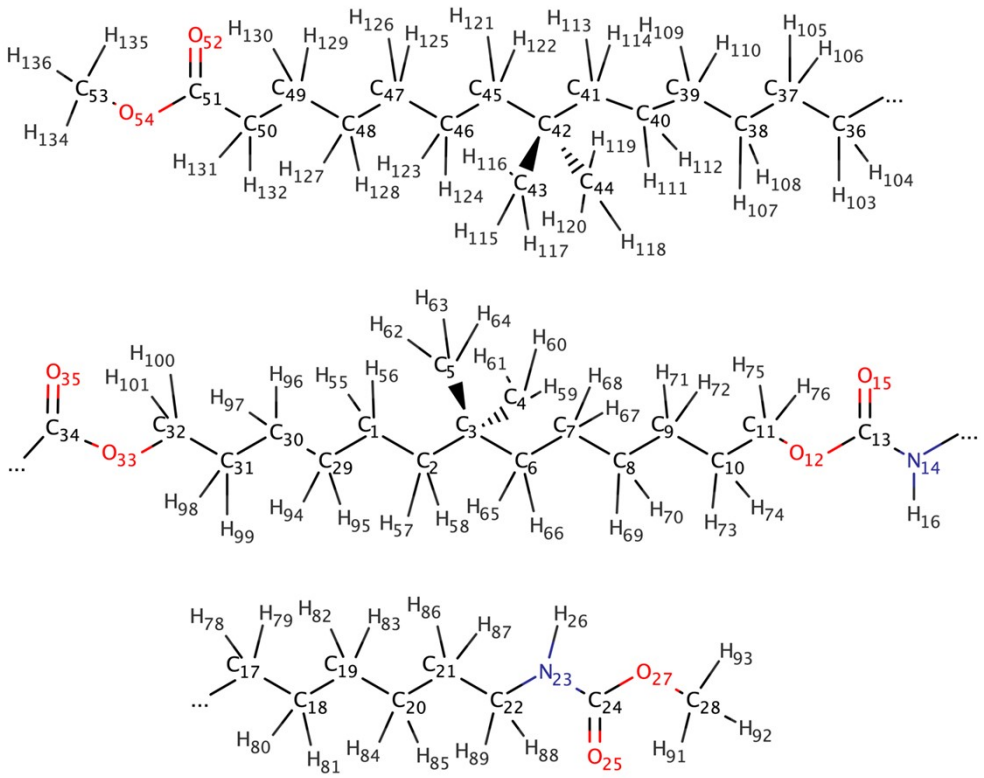
Supplementary Information

Computational study of the Mechanism of a polyurethane esterase A (PueA) from Pseudomonas chlororaphis

Katarzyna Świderek, Sergio Martí, Kemel Arafet and Vicent Moliner**

BioComp Group. Institute of Advanced Materials (INAM). Universitat Jaume I. 12071 Castellón. Spain.

Table S1. Atom types. charges (in a.u.) and parameters obtained after parametrization of Impranil obtained using structure molecule structure optimized at AM1 level of theory and GAFF force field.



Label	Type	Charge	Label	Type	Charge	Label	Type	Charge
C28	c3	0.1377	C7	c3	-0.0894	H108	hc	0.0432
H91	h1	0.0527	H67	hc	0.0537	C39	c3	-0.0824
H92	h1	0.0527	H68	hc	0.0537	H109	hc	0.0417
H93	h1	0.0527	C6	c3	-0.0664	H110	hc	0.0417
O27	os	-0.4479	H65	hc	0.0362	C40	c3	-0.0794
C24	c	0.7181	H66	hc	0.0362	H111	hc	0.0412
O25	o	-0.578	C3	c3	-0.048	H112	hc	0.0412
N23	n	-0.5119	C4	c3	-0.0931	C41	c3	-0.0704
H26	hn	0.3105	H59	hc	0.0352	H113	hc	0.0412
C22	c3	0.078	H60	hc	0.0352	H114	hc	0.0412
H88	h1	0.0702	H61	hc	0.0352	C42	c3	-0.05
H89	h1	0.0702	C5	c3	-0.0931	C43	c3	-0.0866
C21	c3	-0.1184	H62	hc	0.0352	H115	hc	0.0342
H86	hc	0.0557	H63	hc	0.0352	H116	hc	0.0342
H87	hc	0.0557	H64	hc	0.0352	H117	hc	0.0342
C20	c3	-0.0814	C2	c3	-0.0694	C44	c3	-0.0866
H84	hc	0.0427	H57	hc	0.0422	H118	hc	0.0342
H85	hc	0.0427	H58	hc	0.0422	H119	hc	0.0342
C19	c3	-0.0844	C1	c3	-0.0774	H120	hc	0.0342
H82	hc	0.0402	H55	hc	0.0397	C45	c3	-0.0714
H83	hc	0.0402	H56	hc	0.0397	H121	hc	0.0392

C18	c3	-0.1174	C29	c3	-0.0824	H122	hc	0.0392
H80	hc	0.0557	H94	hc	0.0472	C46	c3	-0.0814
H81	hc	0.0557	H95	hc	0.0472	H123	hc	0.0432
C17	c3	0.078	C30	c3	-0.0754	H124	hc	0.0432
H78	h1	0.0702	H96	hc	0.0367	C47	c3	-0.0784
H79	h1	0.0702	H97	hc	0.0367	H125	hc	0.0412
N14	n	-0.5099	C31	c3	-0.0854	H126	hc	0.0412
H16	hn	0.3085	H98	hc	0.0537	C48	c3	-0.0774
C13	c	0.7201	H99	hc	0.0537	H127	hc	0.0402
O15	o	-0.582	C32	c3	0.1384	H128	hc	0.0402
O12	os	-0.4499	H100	h1	0.0547	C49	c3	-0.0824
C11	c3	0.1514	H101	h1	0.0547	H129	hc	0.0592
H75	h1	0.0547	O33	os	-0.4439	H130	hc	0.0592
H76	h1	0.0547	C34	c	0.6291	C50	c3	-0.1274
C10	c3	-0.0844	O35	o	-0.543	H131	hc	0.0797
H73	hc	0.0532	C36	c3	-0.1264	H132	hc	0.0797
H74	hc	0.0532	H103	hc	0.0777	C51	c	0.6301
C9	c3	-0.0804	H104	hc	0.0777	O52	o	-0.546
H71	hc	0.0462	C37	c3	-0.0764	O54	os	-0.4429
H72	hc	0.0462	H105	hc	0.0552	C53	c3	0.1227
C8	c3	-0.0764	H106	hc	0.0552	H134	h1	0.0517
H69	hc	0.0347	C38	c3	-0.0794	H135	h1	0.0517
H70	hc	0.0347	H107	hc	0.0432	H136	h1	0.0517

MASS				**DIHE**				
c3	12.01	0.878		o-c-os-c3	1	2.7	180	-2
h1	1.008	0.135		o-c-os-c3	1	1.4	180	1
os	16	0.465		n-c-os-c3	2	5.4	180	2
c	12.01	0.616		h1-c3-os-c	3	1.15	0	3
o	16	0.434		os-c-n-hn	4	10	180	2
n	14.01	0.53		os-c-n-c3	4	10	180	2
hn	1.008	0.161		h1-c3-n-c	6	0	0	2
hc	1.008	0.135		c3-c3-n-c	1	0.5	180	-4
				c3-c3-n-c	1	0.15	180	-3
BOND				c3-c3-n-c	1	0	0	-2
c3-h1	330.6	1.097		c3-c3-n-c	1	0.53	0	1
c3-os	308.6	1.432		o-c-n-hn	1	2.5	180	-2
c-os	390.8	1.358		o-c-n-hn	1	2	0	1
c-o	637.7	1.218		o-c-n-c3	4	10	180	2
c-n	427.6	1.379		hc-c3-c3-n	9	1.4	0	3
hn-n	403.2	1.013		c3-c3-c3-n	9	1.4	0	3
c3-n	328.7	1.462		h1-c3-n-hn	6	0	0	2
c3-c3	300.9	1.538		c3-c3-n-hn	6	0	0	2
c3-hc	330.6	1.097		c3-c3-c3-hc	1	0.16	0	3
c-c3	313	1.524		c3-c3-c3-c3	1	0.18	0	-3
				c3-c3-c3-c3	1	0.25	180	-2
ANGLE				c3-c3-c3-c3	1	0.2	180	1

c-os-c3	63.3	115.98		h1-c3-c3-hc	9	1.4	0	3
h1-c3-h1	39.2	108.46		c3-c3-c3-h1	9	1.4	0	3
h1-c3-os	50.8	109.78		hc-c3-c3-hc	1	0.15	0	3
o-c-os	75.3	123.25		c3-c3-os-c	1	0.383	0	-3
n-c-os	75.3	109.22		c3-c3-os-c	1	0.8	180	1
c-n-hn	48.3	117.55		hc-c3-c3-os	1	0	0	-3
c-n-c3	63.4	120.69		hc-c3-c3-os	1	0.25	0	1
n-c-o	74.2	123.05		c3-c3-c3-os	9	1.4	0	3
h1-c3-n	49.8	108.88		c3-c-os-c3	1	2.7	180	-2
c3-c3-n	65.9	111.61		c3-c-os-c3	1	0	0	-1
c3-n-hn	45.8	117.68		c3-c-os-c3	1	1.15	0	3
c3-c3-hc	46.3	109.8		os-c-c3-hc	6	0	180	2
c3-c3-c3	62.9	111.51		os-c-c3-c3	6	0	180	2
c3-c3-h1	46.4	109.56		c-c3-c3-hc	9	1.4	0	3
hc-c3-hc	39.4	107.58		c-c3-c3-c3	9	1.4	0	3
c3-c3-os	68	107.97		o-c-c3-hc	1	0.8	0	-1
c3-c-os	68.9	110.72		o-c-c3-hc	1	0	0	-2
c-c3-hc	46.9	108.77		o-c-c3-hc	1	0.08	180	3
c-c3-c3	63.3	111.04		o-c-c3-c3	6	0	180	2
c3-c-o	67.4	123.2						
IMPROPER								
n-o-c-os	1.1	180	2					
c-c3-n-hn	1.1	180	2					
c3-o-c-os	1.1	180	2					
NONBON								
c3	1.908	0.1094						
h1	1.387	0.0157						
os	1.6837	0.17						
c	1.908	0.086						
o	1.6612	0.21						
n	1.824	0.17						
hn	0.6	0.0157						
hc	1.487	0.0157						

Table S2. List of the pKa of the titratable residues of PueA (aa) as derived from PROPKA3 semiempirical program. Protonation states were assigned at pH 7 according to the obtained

pKa values, except those marked with an asterisk: D* = deprotonated aspartate, H* = neutral histidine.

aa	propKa	aa	propKa	aa	propKa
D5	4.72	D563	4.58	Y252	13.07
D13	3.14	D576	4	Y292	13.71
D20	4.32	D578	2.78	Y318	17.08
D33	4.74	D589	3.26	Y331	13.04
D75	3.21	D597	3.47	Y388	16
D82	2.86	E38	3.56	Y404	10.42
D100	2.57	E77	3.8	Y425	11.87
D104	3.41	E112	4.97	Y436	13.46
D128	1.94	E122	4.35	Y487	11.72
D153	4.33	E134	4.93	Y559	12.22
D157*	7.05	E148	2.78	Y579	10.13
D161	6.26	E178	3.65	K7	8.83
D170	3.73	E222	4.31	K15	9.74
D186	3.75	E253	6.5	K78	11.52
D200	2.19	E327	4.11	K102	10.11
D218	4.57	E363	4.91	K113	11.39
D230	3.7	E390	5.35	K126	10.93
D245	3.46	E457	3.19	K169	10.57
D255	3.43	E537	3.86	K173	10.91
D262	3.54	E607	3.69	K182	12.66
D275	4.37	H30	6.57	K199	11.45
D279	3.64	H42	7.31	K229	10.68
D290	3.9	H85	6.09	K246	9.85
D320	3.34	H132*	7.43	K329	10.5
D332	3.96	H206	1.35	K336	10.17
D337	2.99	H274	5.16	K366	10.38
D347	2.54	H277*	7.13	K423	10.53
D357	3.02	H291	4.48	K456	11.46
D375	2.7	H313	6.97	K466	10.22
D378	5.26	H365*	9.89	K479	10.39
D387	6.16	H471	5.42	K501	10.4
D396	7.46	H503*	7.25	K564	10.98
D400*	8.23	H517	6.81	R106	15.47
D417	3.37	H550*	7.29	R141	10.44
D430	3.73	H582	5.71	R147	11.36
D439	3.87	H608	6.53	R223	12.48
D449	4.21	Y6	12.94	R259	12.02
D467	4.12	Y27	13.39	R324	12.53
D468	2.88	Y29	13.62	R350	13.19
D475	3.9	Y40	14.87	R360	11.99
D494	2.81	Y99	12.06	R392	14.49
D497	3.21	Y116	10.78	R399	12.63
D507	2.52	Y127	10.92	R438	11.66
D513	4.03	Y171	13.12	R448	12.46
D516	5.49	Y175	14.99	R492	12.29
D525	4.69	Y228	14.29	R555	12.36
D534	6.36	Y233	13.47	R580	13.59
D554	8.57	Y236	14.54		

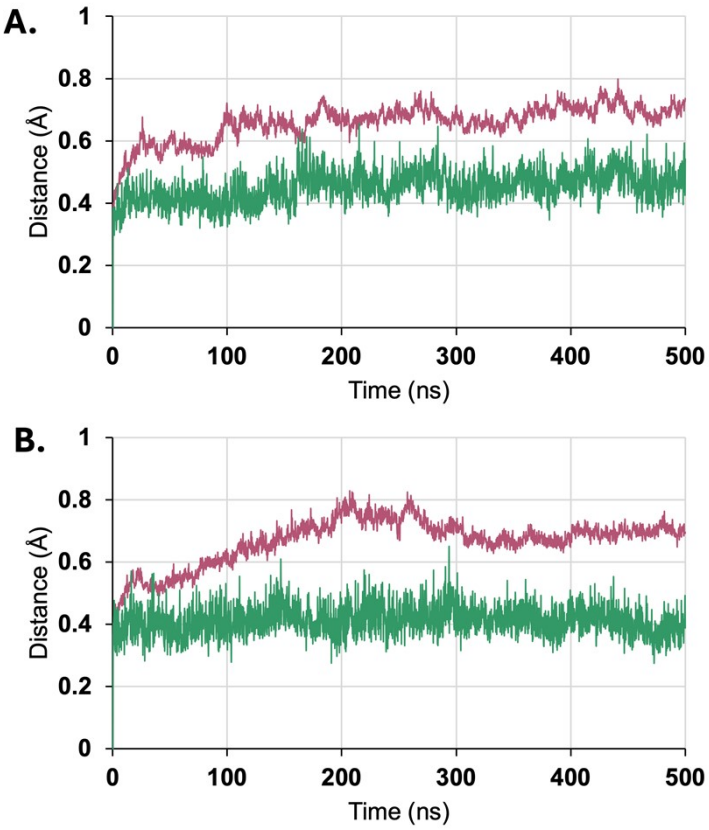


Figure S1. Time depending evolution of the RMSD along the 500 ns of unconstrained MD simulations of the reactant complex **RC1** (A) and **RC2** (B), computed with the position of Cα, C, O and N atoms of the protein backbone (pink line) and for heavy atoms of impranil substrate (green line).

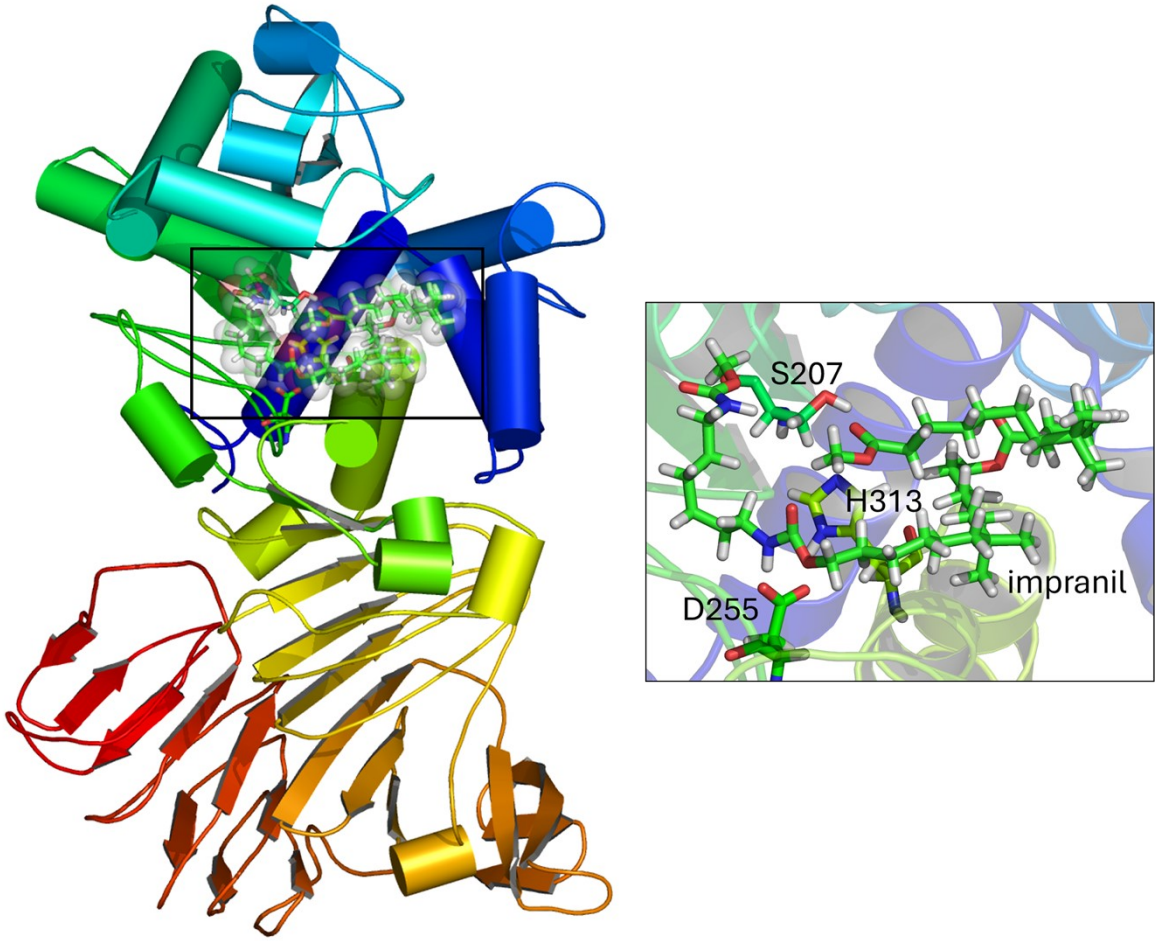


Figure S2. Structure of impranil when docked in the PueA enzyme, and detail of the active site, as the result of AutoDock vina.

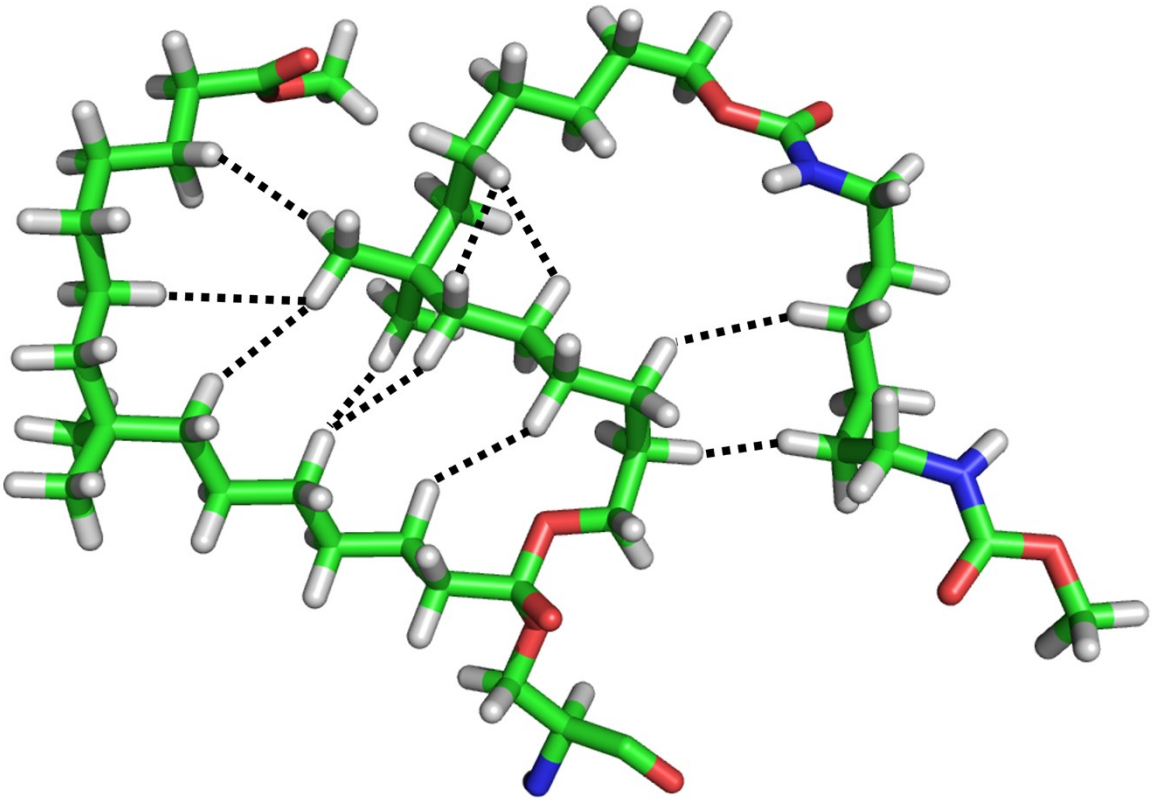


Figure S3. Detail of the conformation of the impranil when docked in the PueA enzyme. as the result of AutoDock Vina. Significantly short intramolecular interactions (CH···HC < 3 Å) are shown as dashed lines.

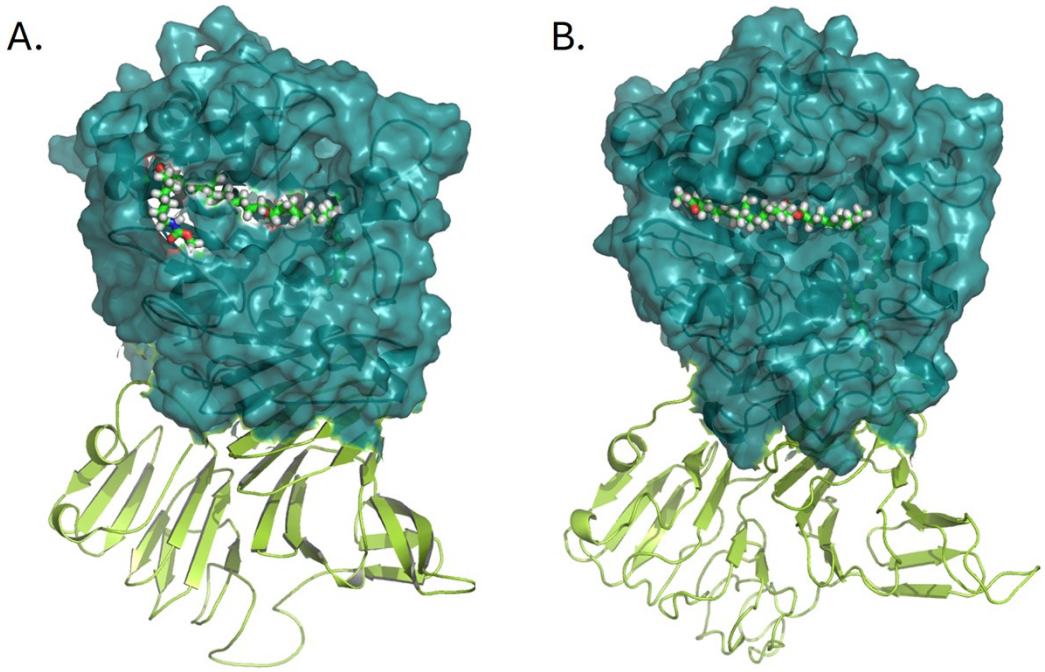


Figure S4. Representation of PueA with the extended conformation of impranil docked in the active site (in liquorice) with carbamate end-group exposed to the solvent. **RC1** (A) or inside the protein. **RC2** (B). Structures derived from 500 ns of unbiased MD simulation.

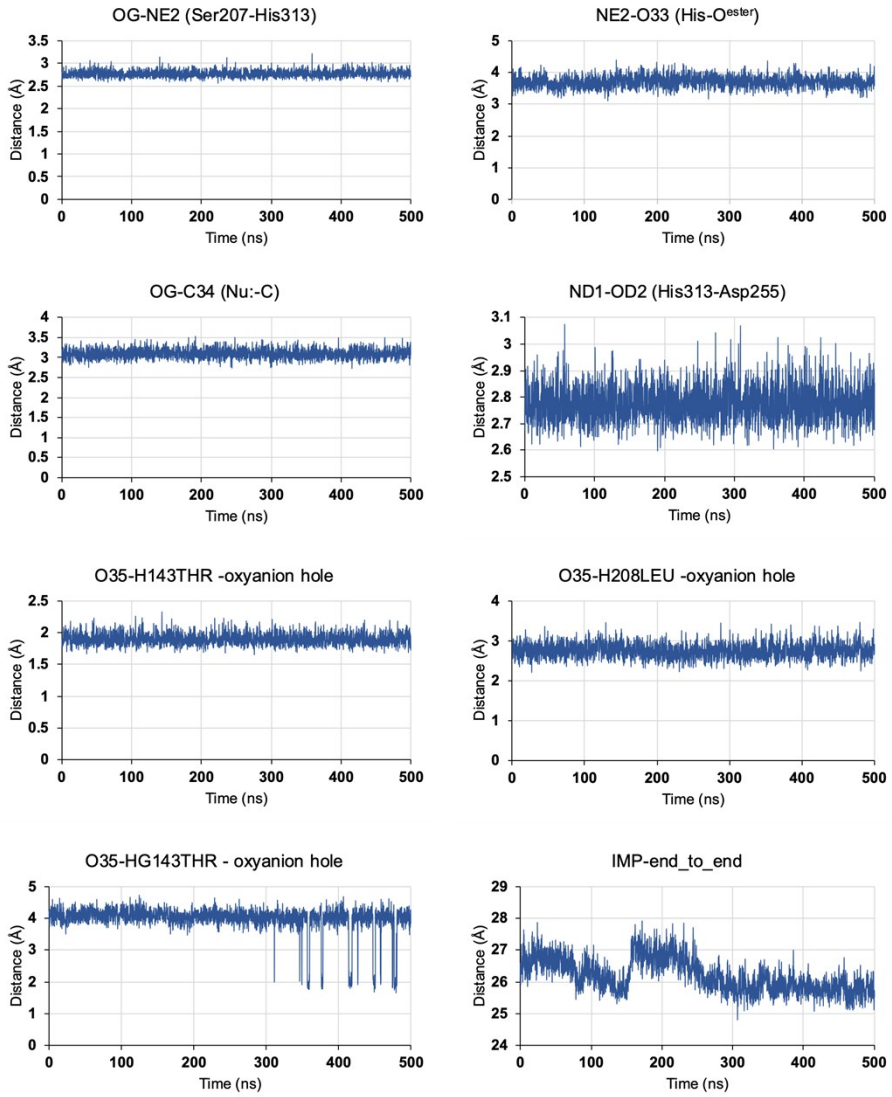


Figure S5. Time-dependent evolution of key distances along the 500 ns of free MD simulation of the non-covalent protein:ligand reactants complex with the extended conformation of impranil when the methyl carbamate end-group was exposed to the solvent, **RC1**.

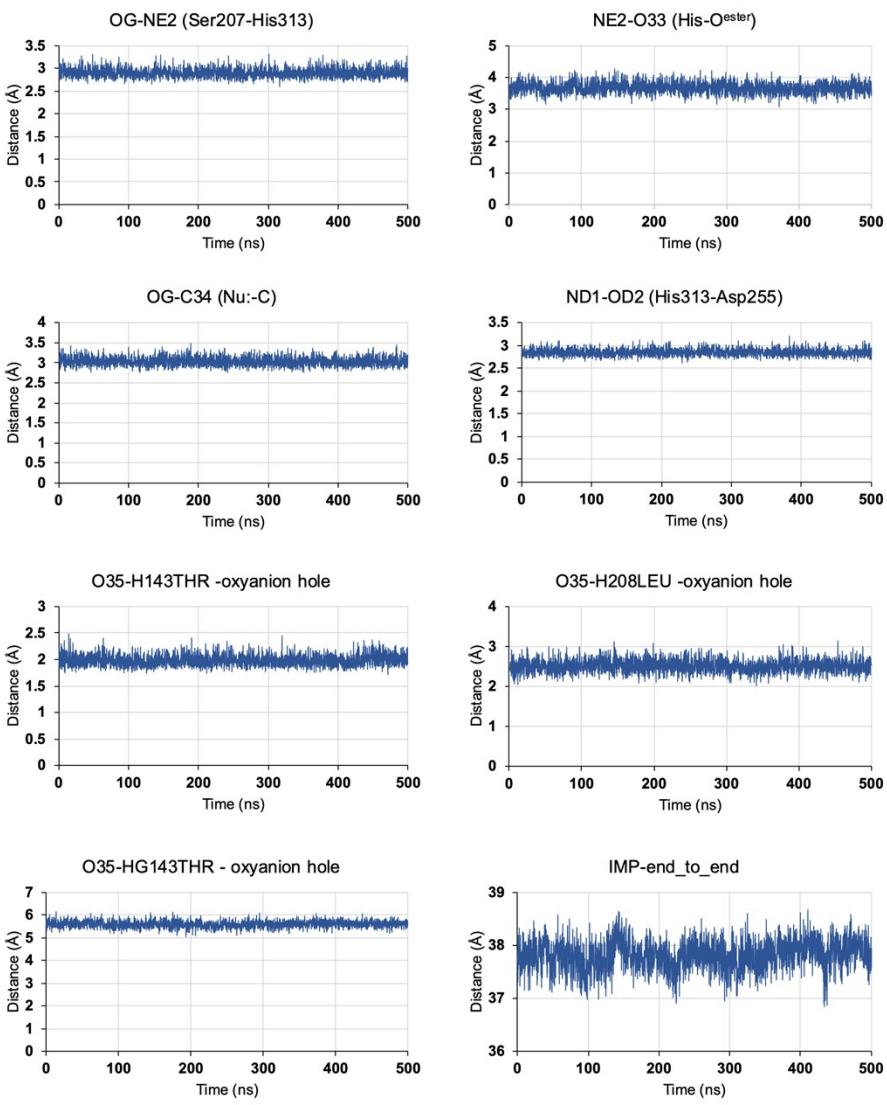


Figure S6. Time-dependent evolution of key distances along the 500 ns of free MD simulation of the non-covalent protein:ligand reactants complex with the extended conformation of impranil when the methyl carbamate end-group was exposed to the solvent, **RC2**.

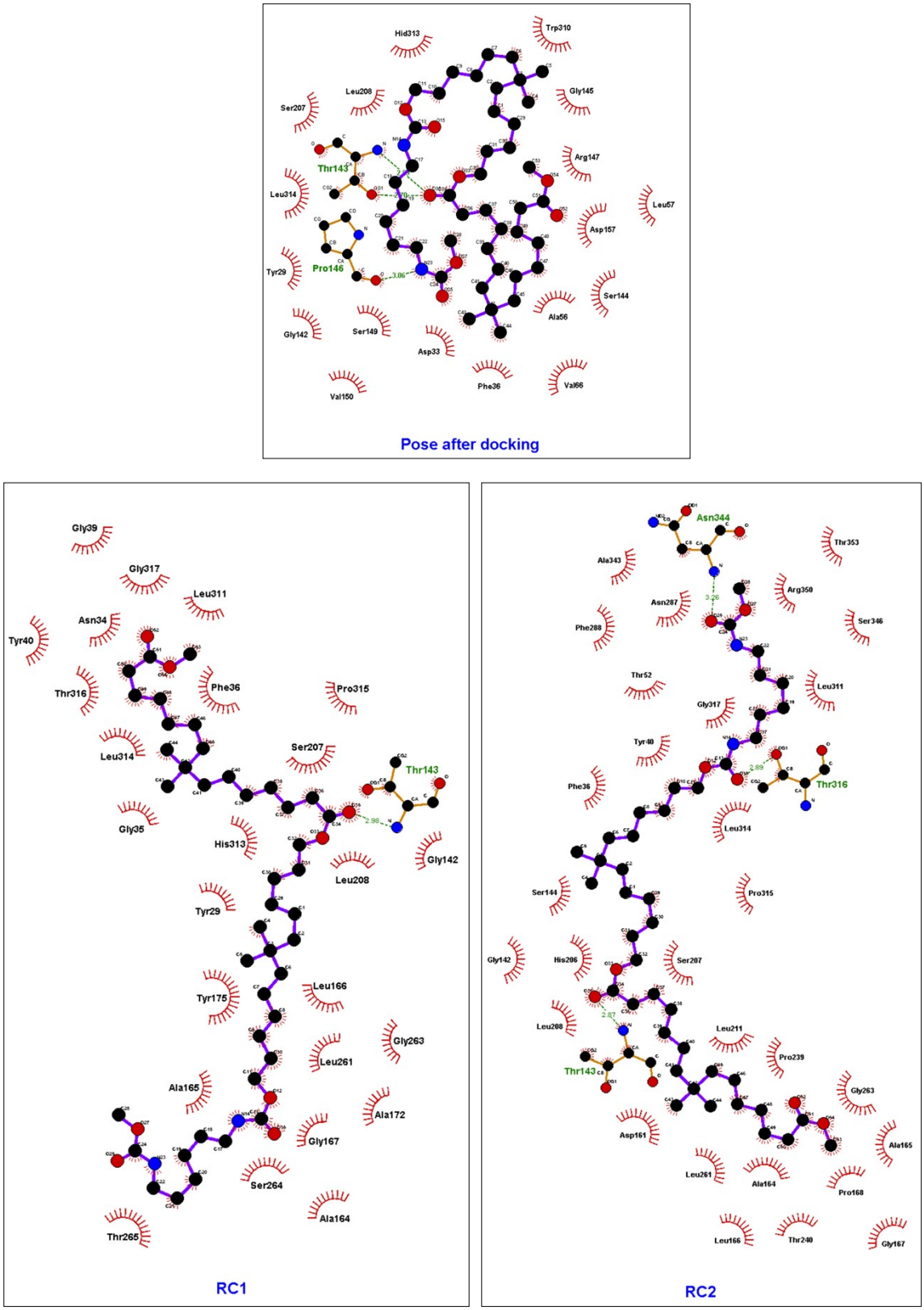


Figure S7. Diagram of hydrogen bonding interaction and hydrophobic contacts obtained for impranil pose generated after docking and in its RC1 and RC2 configurations. Schemes were generated using Ligplot+ ver. 2.2.8 program.

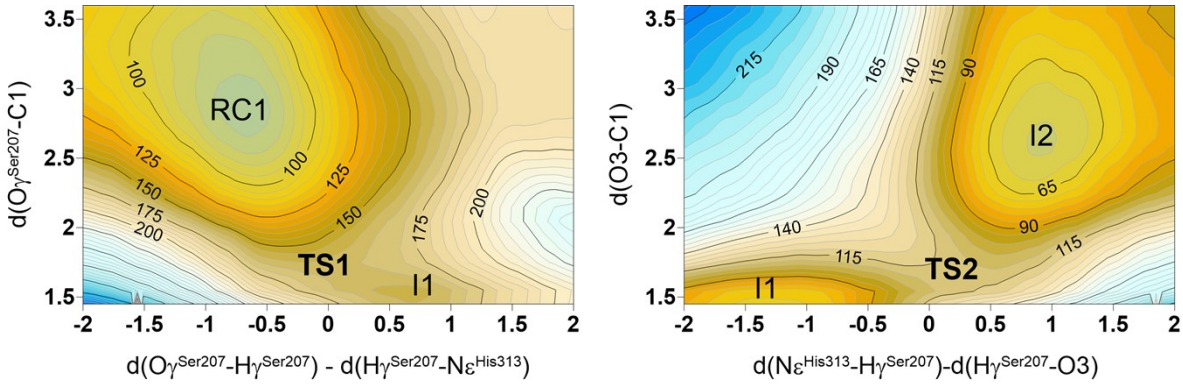


Figure S8. M06-2X:AM1/AMBER Free Energy Surfaces of acylation step starting from the **RC1**. Distances are given in Å and isoenergetic lines are in kcal·mol^{-1}.