Supporting Information

Dancing with oils – The interaction of lipases with different oil/water interfaces

Hugo A. L. Filipe[a*], Maëva C. F. Almeida[a], Rafaela R. Teixeira[a], Margarida I. M. Esteves[a], César A. Henriques[b] and Filipe E. Antunes[a]

[a] Coimbra Chemistry Centre, Dept. of Chemistry, University of Coimbra, Rua Larga, 3004-535, Coimbra, Portugal

[b] EcoXperience, HIESE, Quinta Vale do Espinhal, 3230-343, Penela, Portugal

* hlfilipe@uc.pt

Methods

Description, composition and construction of the model systems: A summary of the composition of the oil systems used in this work is shown in Table SI.

Table SI– Composition (triglyceride molecules) of the oil systems used in this work.

	Oil system							
	Olive	**Palm**	**Soy**	**Sunflower**	**Rapeseed**	**TOG/POPC**	**TPG/TOG**	**TPG/TOG/POPC**
TPG	-	250	75	25	25	-	350	300
TOG	500	250	115	150	325	400	150	100
TLG	-	-	310	325	150	-	-	-
POPC	-	-	-	-	-	100	-	100

A brief description of the protein addressed in this work is provided in the following text. TLL is one of the most famous enzymes in biotechnological applications.[1] The catalytic center is formed by the residues Ser146, Asp201, and His258. This is a noticeably thermostable enzyme, commercially available in both soluble and immobilized form. [2] The M37 lipase shows an extremely low activation energy and strong activity at low temperatures (25 °C). Its catalytic center is formed by the residues Ser174, Asp236, and His312.[3] RML is a relatively small extracellular fungal protein. It has three disulfide bridges (Cys29-Cys268, Cys40-Cys43, Cys235-Cys244) and the catalytic center has the residues Ser144, Asp203, and His257.[4] CALB is the lipase with the widest application in many industrial processes because of its high enantioselectivity, wide range of substrates, thermal

stability, and stability in organic solvents. Although it has never shown any significant interfacial activation, open and closed lid conformations and their protonation states were observed in the crystal structure of CALB at 0.91 Å resolution.[5] The catalytic center has the residues Ser105, Asp187, and His224.[5] BTL2 is a lipase that showed high stability at medium temperatures (50 °C), alkaline pH (9.0 – 11.0), and in organic solvents. The catalytic center is formed by the residues Ser114, Asp318, and His359.[6]. PPL (PLA2s) is a rather small protein that hydrolyzes phospholipids to produce free fatty acids and lysophospholipids.[7] The catalytic center is composed by the residues His48 and Asp99, including also other binding residues.[8] Phospholipases are ubiquitous lipid-degrading enzymes, therefore, the inclusion of these proteins may be considered when dealing with food fats containing some amount of phospholipids.

Data analysis from MD simulations: The several analyses of the interactions of the proteins with the O/W interface were done using different GROMACS analysis tools. Structural analysis including the calculations of Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) profiles were performed, using gmx rms and gmx rmsf tools, respectively. To study the interactions of the protein with the O/W interface, the time evolution of the distance of the protein to the center of mass (COM) of the oil phase was calculated using the gmx distance tool. The orientation of the protein at the O/W interface was also studied using the gmx rotmat tool, which was used to calculate the rotation matrix of the proteins regarding a reference configuration. For a better definition of the reference orientation, that is, the orientation when the protein is at the O/W interface, the first 500 ns of each simulation were discarded from the analysis. The density profiles were calculated with gmx density and the density maps with gmx densmap. The density maps were made by discarding the initial 500 ns of each simulation. The gmx select tool was also used to identify diverse protein-oil contacts. This analysis was used to identify two types of contacts. First, contacts between the residues of the protein that interacted with the O/W interface. Second, to identify contacts between specific triglyceride molecules that interacted with the protein binding site. The MD trajectories adopted by proteins during the simulations were visualized through Visual Molecular Dynamics (VMD).[9]

Results and discussion

Below are presented additional figures and tables with results as mentioned throughout the main text.

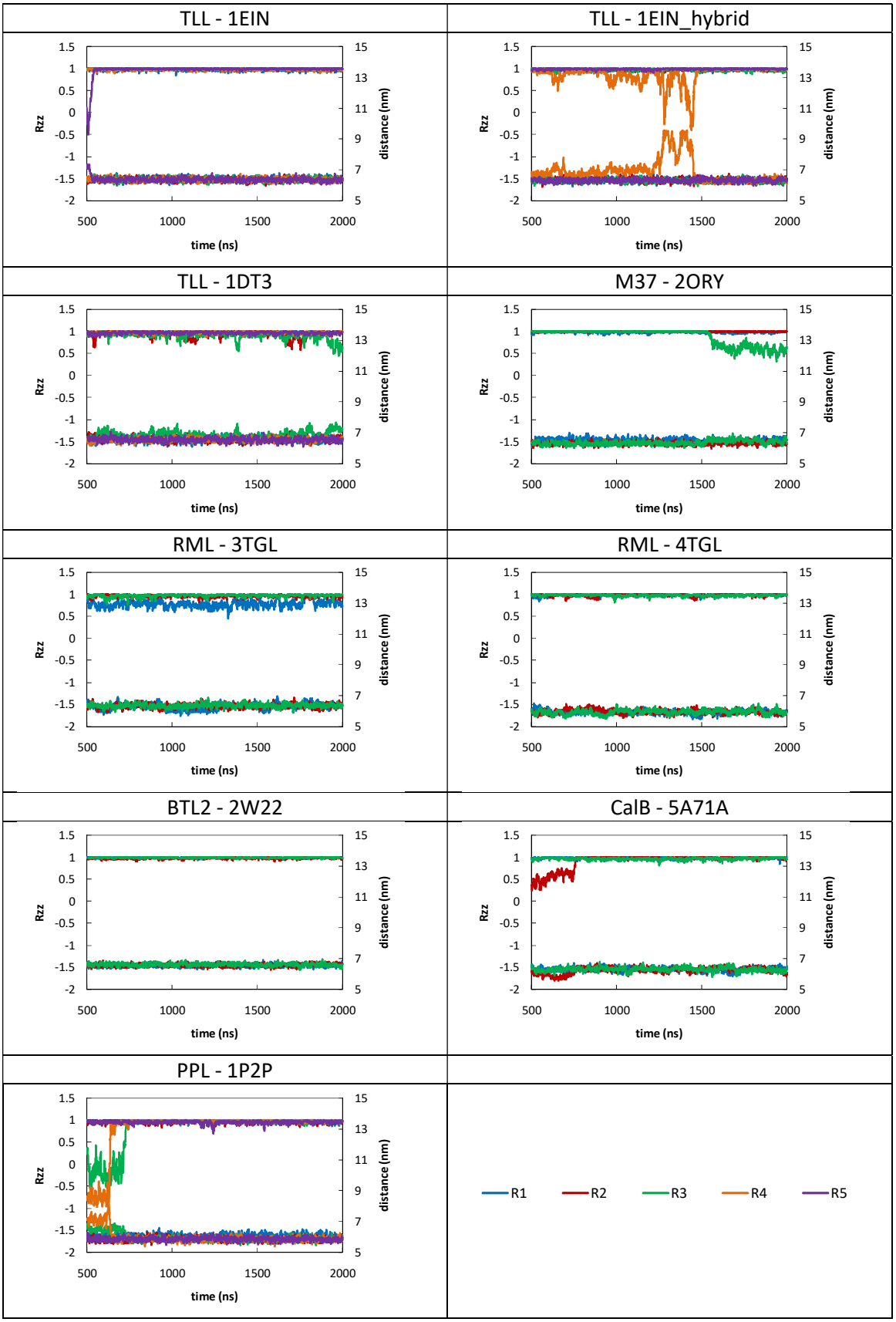

Figure S1 – Time evolution of the orientation (Rzz) and the distance of each protein to the center of mass (COM) of the oil phase in the TOG system. Rzz (left scale) and distance (right scale) values converge for the top and bottom of each panel, respectively. Data are shown for each simulation replicate.

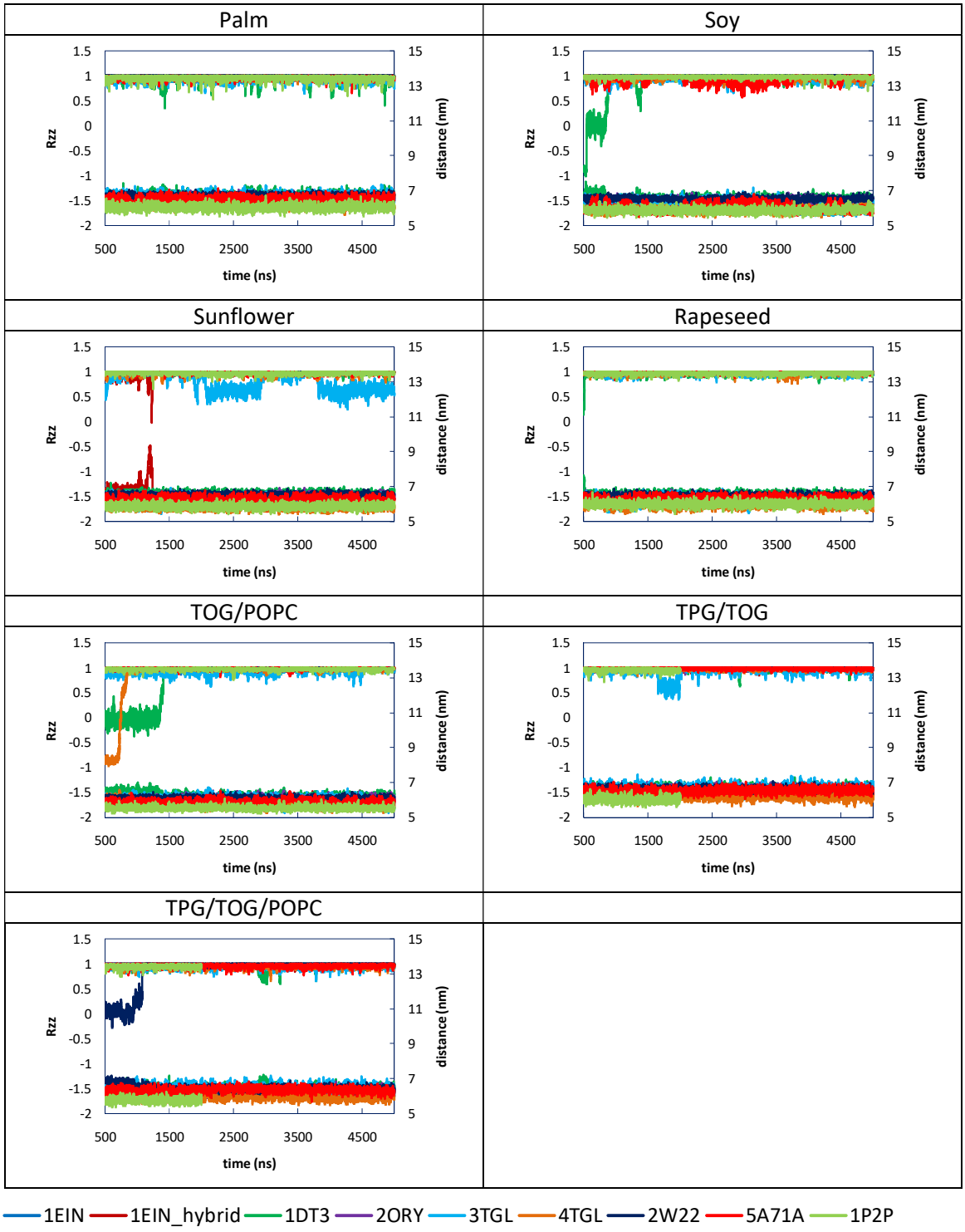

Figure S2 – Time evolution of the orientation (Rzz) and the distance of the proteins to the center of mass (COM) of each oil system. Rzz (left scale) and distance (right scale) values converge for the top and bottom of each panel, respectively. Data are shown for each protein.

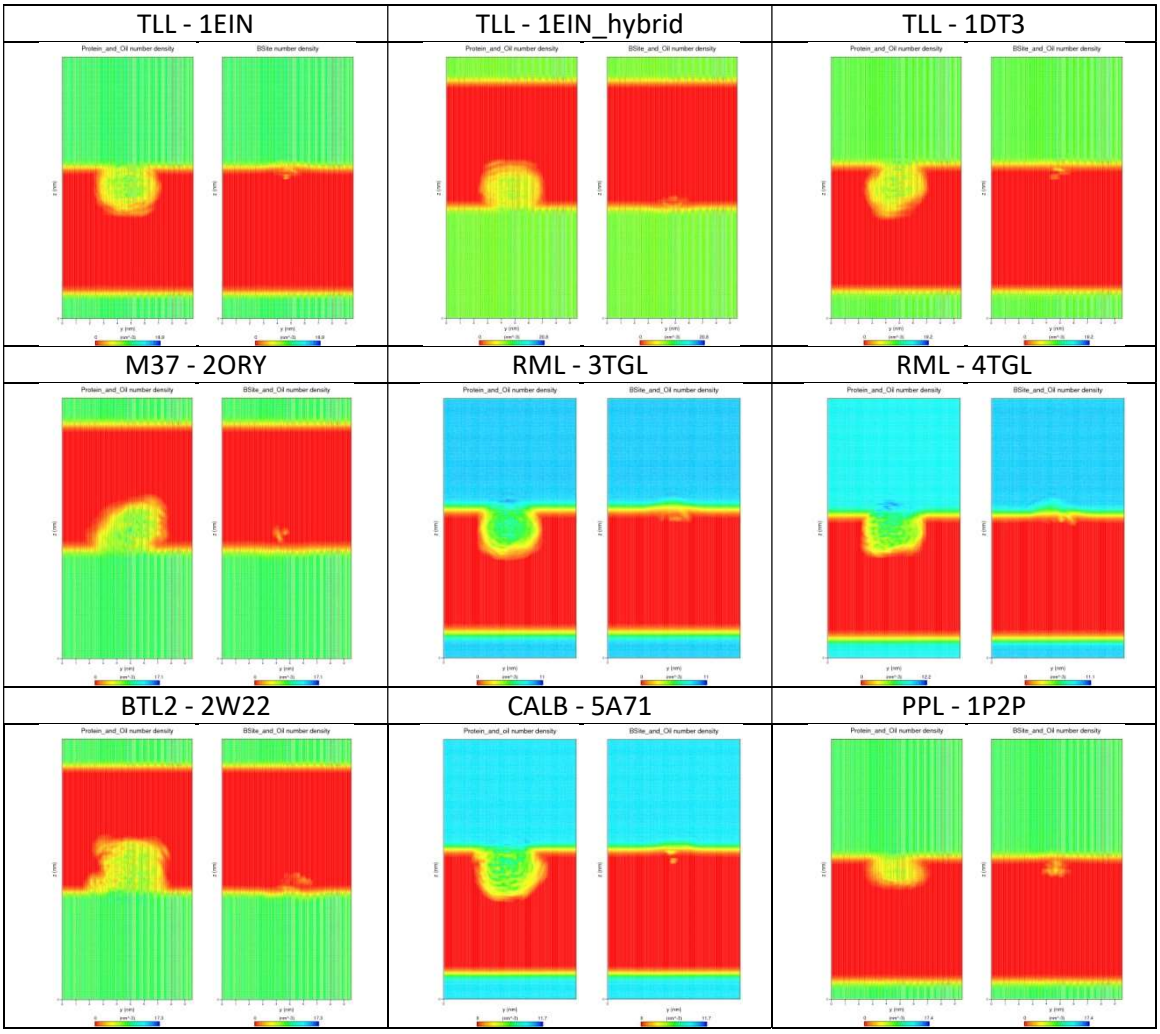

Figure S3 – Density maps for the simulations of the different proteins in the TOG system. Color code density bar increases from red to blue. Water region is shown as red. In all cases the protein is adsorbed at the O/W interface. For each protein, the density map for the whole protein is shown at left, while the representation of the protein binding site is shown at right.

Table SII – Quantitative analysis of the average number of contacts between the lipid molecules, in each oil mixture, and the binding site of each protein. The four proteins showing more interactions with the molecules of each oil are highlighted in yellow.

1EIN	1EIN hybrid	1DT3	2ORY	3TGL	4TGL	2W22	5A71	1P2P
TOG								
Palm								
Soy								
Sunflower								
Rapeseed								
TOG/POPC								
TPG/TOG								
TPG/TOG/POPC								

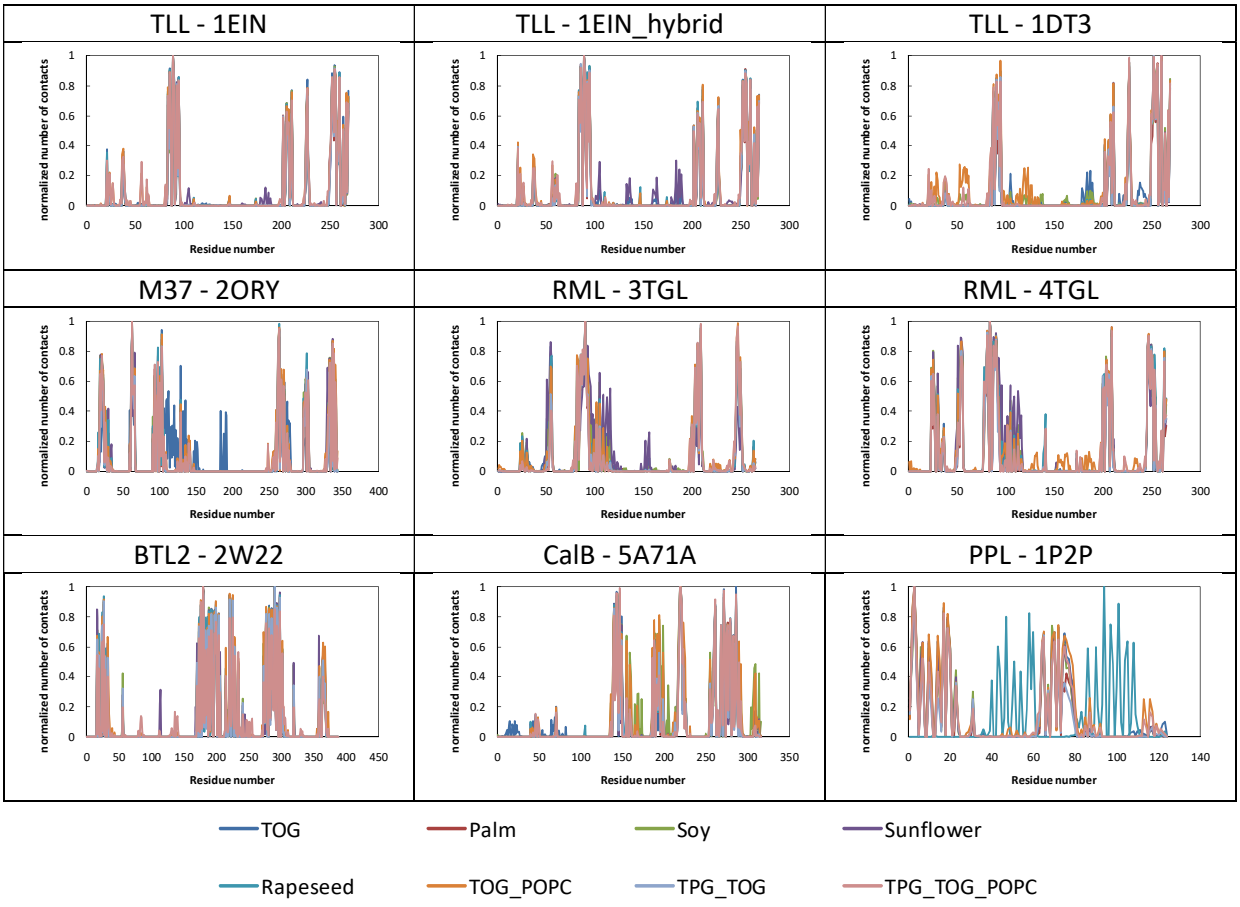

Figure S4 – Interaction of each protein with all oils. The most important regions of the primary sequence of each for the interaction with the oils, at the oil water interface are represented as the average normalized number of contacts obtained for all simulations of each protein in all oil systems.

Table SIII – Resume of the most important amino acid residues for the interaction of the different protein with the diverse oils.

Protein	Amino acid residues			
	Positive	**Negative**	**Polar**	**Non-polar**
TLL - 1EIN	ARG84	-	THR226 THR267	ILE86 TRP89 ILE90 LEU93 PHE95 ILE202 LEU206 PRO208 PHE211 LEU227 ILE252 PRO253 ILE255 PRO256 TRP260 LEU264 LEU269
TLL - 1EIN_hybrid	ARG84	ASP93	THR87 THR267	ILE86 TRP89 ILE90 TYR95 LEU206 PRO208 PHE211 LEU227 ILE252 PRO253 ILE255 PRO256 TRP260 LEU269
TLL - 1DT3	-	GLU87	ASN88 THR226	GLY91 LEU93 PHE95 PHE211 LEU227 ILE252 PRO253 ILE255 PRO256 TRP260 LEU269
M37 - 2ORY	SER25 HIS341 HIS343	GLU303	THR21 THR23 THR66 SER96 ASN103	GLY24 PRO64 PHE65 PHE68 PHE105 LEU264 LEU265 TYR266 ALA269 ALA273 ILE302 TYR304 VAL306 ALA335 ILE336 LEU339
RML - 3TGL	ARG86 HIS207	ASP91	ASN87 THR93 THR252	ILE59 TYR60 ALA90 LEU92 PHE94 VAL95 VAL97 PRO210 PHE213 PRO250 PHE251 VAL254
RML - 4TGL	ARG30 ARG86 HIS207 HIS257	-	SER83 SER84 THR93 THR252 SER253	TYR28 LEU58 ILE59 TYR60 ILE85 TRP88 ILE89 LEU92 PHE94 VAL95 ILE204 LEU208 PRO209 PRO210 PHE213 PRO250 PHE251 VAL254 LEU255 LEU267
BTL2 - 2W22	LYS190 HIS274	ASP366	THR18 THR178 THR201 THR279 CYS296 SER302	PHE17 TRP20 MET25 LEU26 PHE28 TYR30 VAL34 MET174 VAL175 PHE177 PHE181 PHE182 LEU184 VAL188 ALA191 VAL194 VAL198 PRO199 TYR200 VAL204 TYR205 PHE222 TYR225 PHE226 LEU229 LEU278 TYR283 PRO284 LEU286 ALA290 PHE291 VAL294 VAL295 PRO298 PHE299 TYR303
CalB - 5A71A	-	GLU188	-	VAL139 LEU140 PRO143 LEU144 ALA146 LEU147 VAL149 ILE189 VAL194 PRO218 LEU219 PHE220 VAL221 PRO260 LEU261 PRO268 VAL272 ALA275 ALA276 ALA279 ALA282 ALA283 VAL286
PPL - 1P2P	LYS10 HIS17	-	GLN4 SER7 THR70 SER72	LEU2 TRP3 LEU19 MET20 LEU64 VAL65 TYR69 TYR75

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