

## Supplementary materials

### Criteria for engineering cutinases: bioinformatics analysis of catalophores

Sara Fortuna<sup>1</sup>, Marco Cespugli<sup>1</sup>, Anamaria Todea<sup>1</sup>, Alessandro Pellis<sup>2</sup>, Lucia Gardossi<sup>1\*</sup>

<sup>1</sup>Department of Chemical and Pharmaceutical Sciences, University of Trieste, Via L. Giorgieri 1, Trieste, 34127, Italy

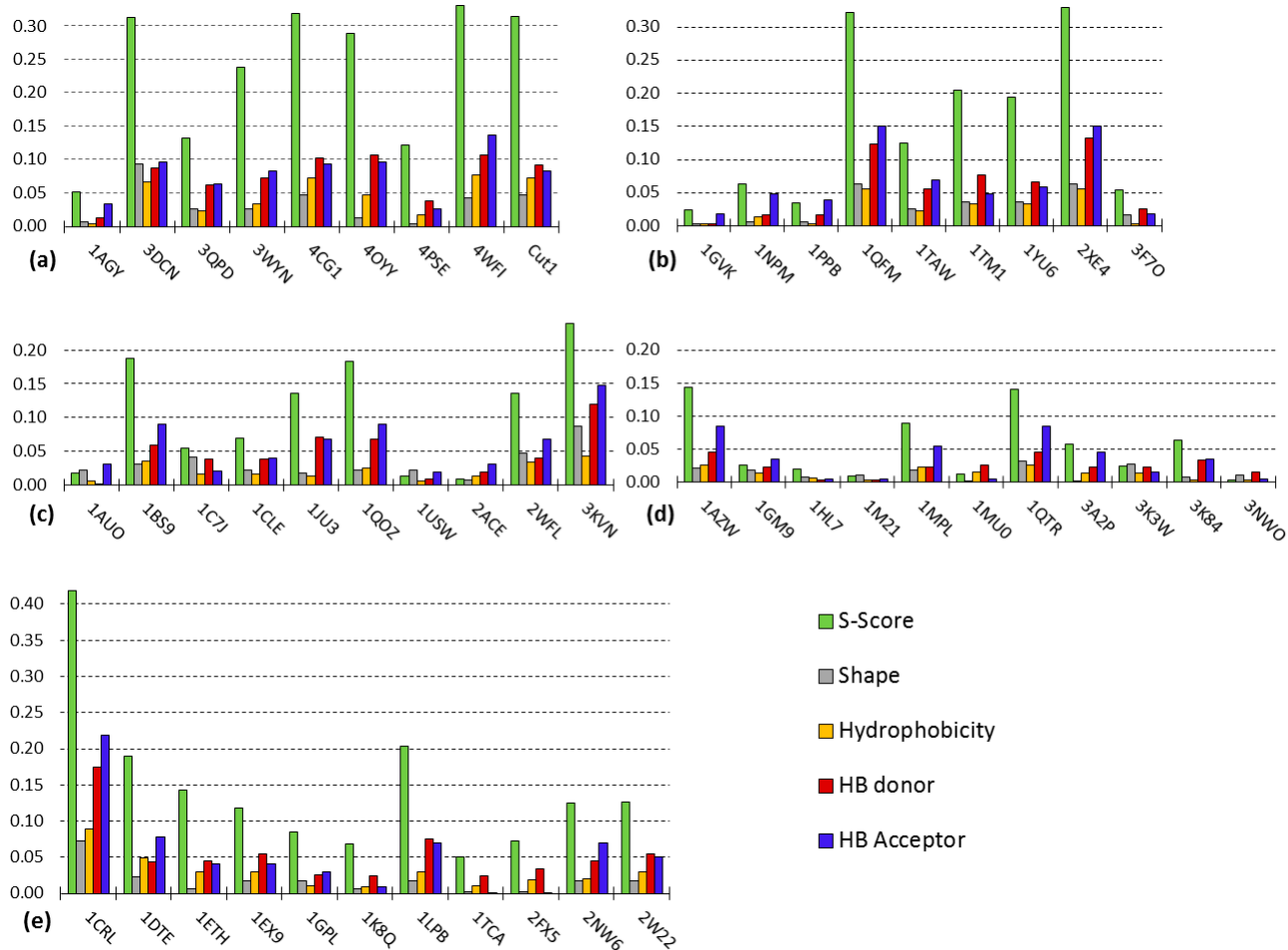
<sup>2</sup>University of Natural Resources and Life Sciences Vienna, Department of Agrobiotechnology, Institute of Environmental Biotechnology, Konrad Lorenz Strasse 20, 3430, Tulln an der Donau, Austria

\*Correspondence: [s.fortuna@units.it](mailto:s.fortuna@units.it) (S.F.); [gardossi@units.it](mailto:gardossi@units.it) (L.G.).

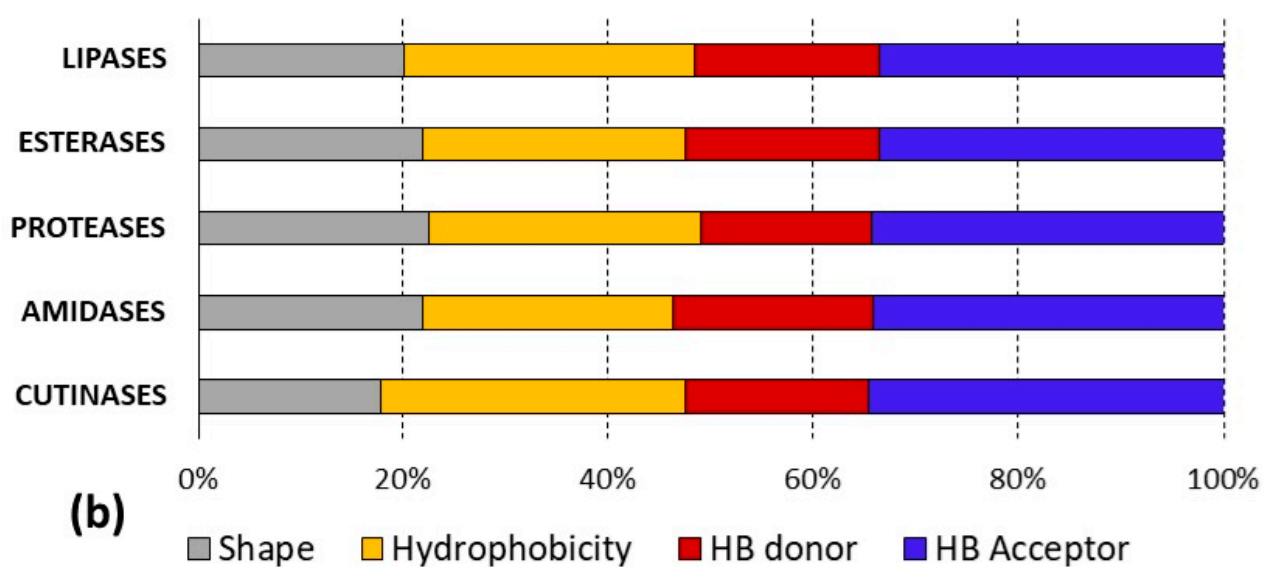
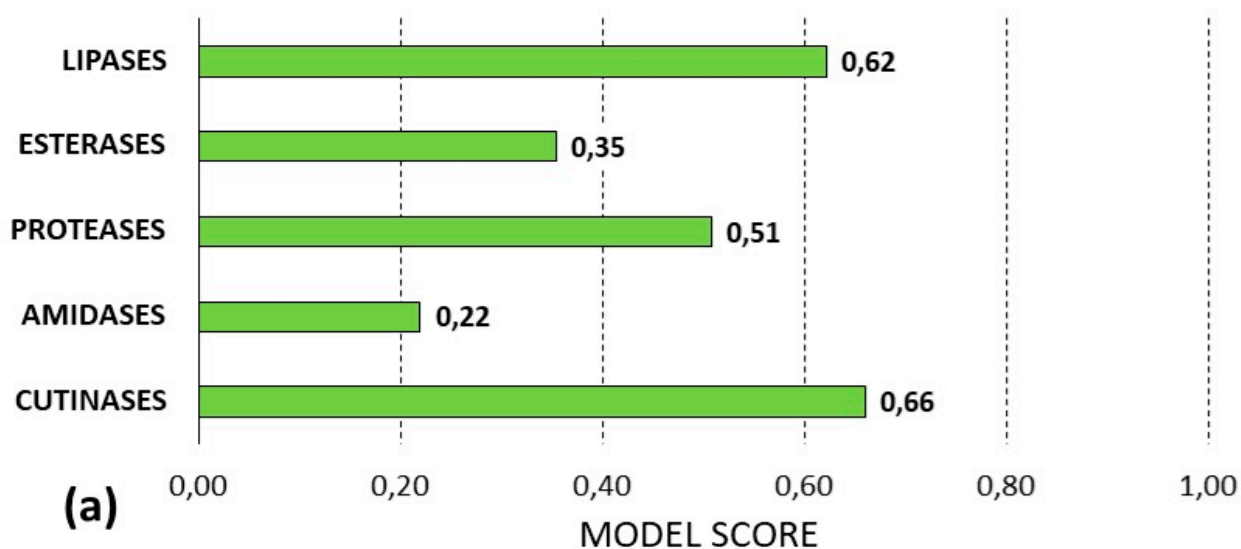
**Table S1:** PDB codes, sources and substrates of the serine-hydrolase used as dataset in the study.

Enzyme class	PDB code	Source	Substrate
Cutinases	1AGY	<i>Fusarium solani</i>	cutin, short esters, PET, PBS
	3DCN	<i>Glomerella cingulata</i>	cutin, triacylglycerol
	3QPD	<i>Aspergillus oryzae</i>	cutin, long chain esters
	3WYN	<i>Thermobifida alba</i>	cutin, PET, PBS, PLA
	4CG1	<i>Thermobifida fusca</i>	cutin, PET
	4OYY	<i>Humicola insolens</i>	cutin, PET, PBS, PLA, PBAT
	4PSE	<i>Trichoderma reesei</i>	cutin, triacylglycerol
	4WFI	<i>Saccharomonospora viridis</i>	short-chain triacylglycerol, PET
	Thc_Cut1	<i>Thermobifida cellulosilytica</i>	PET, PEF, PBAT
Lipases	1CRL	<i>Candida rugosa</i>	triacylglycerol
	1DTE	<i>Humicola lanuginosa</i>	triacylglycerol
	1ETH	<i>Sus scrofa</i>	triacylglycerol
	1EX9	<i>Pseudomonas aeruginosa</i>	triacylglycerol
	1GPL	<i>Cavia porcellus</i>	triacylglycerol
	1K8Q	<i>Canis lupus familiaris</i>	triacylglycerol
	1LPB	<i>Homo sapiens</i>	triacylglycerol
	1TCA	<i>Candida antarctica</i>	triacylglycerol
	2FX5	<i>Pseudomonas mendocina</i>	triacylglycerol
	2NW6	<i>Burkholderia cepacia</i>	triacylglycerol
	2W22	<i>Geobacillus thermocatenulatus</i>	triacylglycerol
Esterases	1AUO	<i>Pseudomonas fluorescens</i>	broad specificity

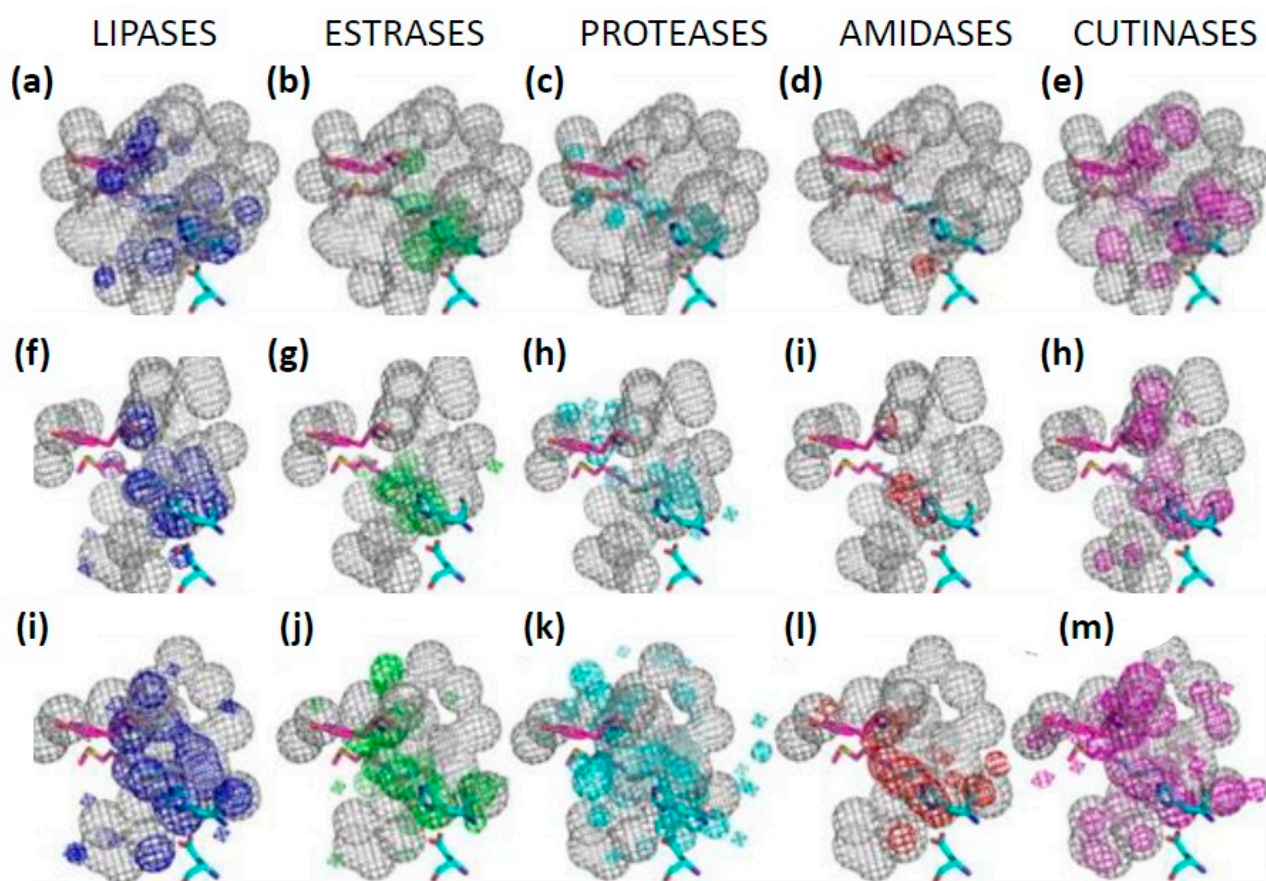
	1BS9	<i>Penicillium purpurogenum</i>	xylanes acetates
	1C7J	<i>Bacillus subtilis</i>	p-nitrobenzyl esters
	1CLE	<i>Candida cylindracea</i>	cholesterol esters
	1JU3	<i>Rhodococcus sp.</i>	cocaine
	1QOZ	<i>Tricoderma reesei</i>	xylanes acetates
	1USW	<i>Aspergillus niger</i>	feroloyl-polysaccharide
	2ACE	<i>Torpedo californica</i>	acetylcholine
	2H7C	<i>Homo sapiens</i>	CoA, palmitate and taurocholate
	2WFL	<i>Rauvolfia serpentine</i>	polyneuridine aldehyde
	3KVN	<i>Pseudomonas aeruginosa</i>	rhamnolipids
Proteases	1GVK	<i>Sus scrofa</i>	Ala- -Xaa
	1NPM	<i>Mus musculus</i>	Lys/Arg- -Xaa
	1PPB	<i>Homo sapiens</i>	Arg- -Gly fibrinogen
	1QFM	<i>Sus scrofa</i>	Pro- -Xaa (~30aa)
	1TAW	<i>Bos Taurus</i>	Lys/Arg- -Xaa
	1TM1	<i>Bacillus amyloliquefaciens</i>	uncharged P1
	1YU6	<i>Bacillus licheniformis</i>	uncharged P1
	2XE4	<i>Leshmania major</i>	oligopeptides
	3F7O	<i>Peacelomyces lilacinus</i>	peptides
Amidases	1AZW	<i>Xantomonas campestris</i>	NH-Pro- -Xaa
	1GM9	<i>Escherichia coli</i>	penicillin
	1HL7	<i>Microbacterium sp.</i>	$\gamma$ -lactam
	1M21	<i>Stenotrophomonas maltophilia</i>	C terminal amide
	1MPL	<i>Streptomyces sp.</i>	L-Lys-D-Ala- -D-Ala
	1MU0	<i>Thermoplasma acidophilum</i>	NH-Pro- -Xaa
	1QTR	<i>Serratia marcescens</i>	NH-Pro- -Xaa
	3A2P	<i>Arthrobacter sp.</i>	6-amino exanoate dimer
	3K3W	<i>Alcaligenes faecalis</i>	penicillin
	3K84	<i>Rattus norvegicus</i>	fatty acid amide
	3NWO	<i>Mycobacterium smegmatis</i>	NH-Pro- -Xaa



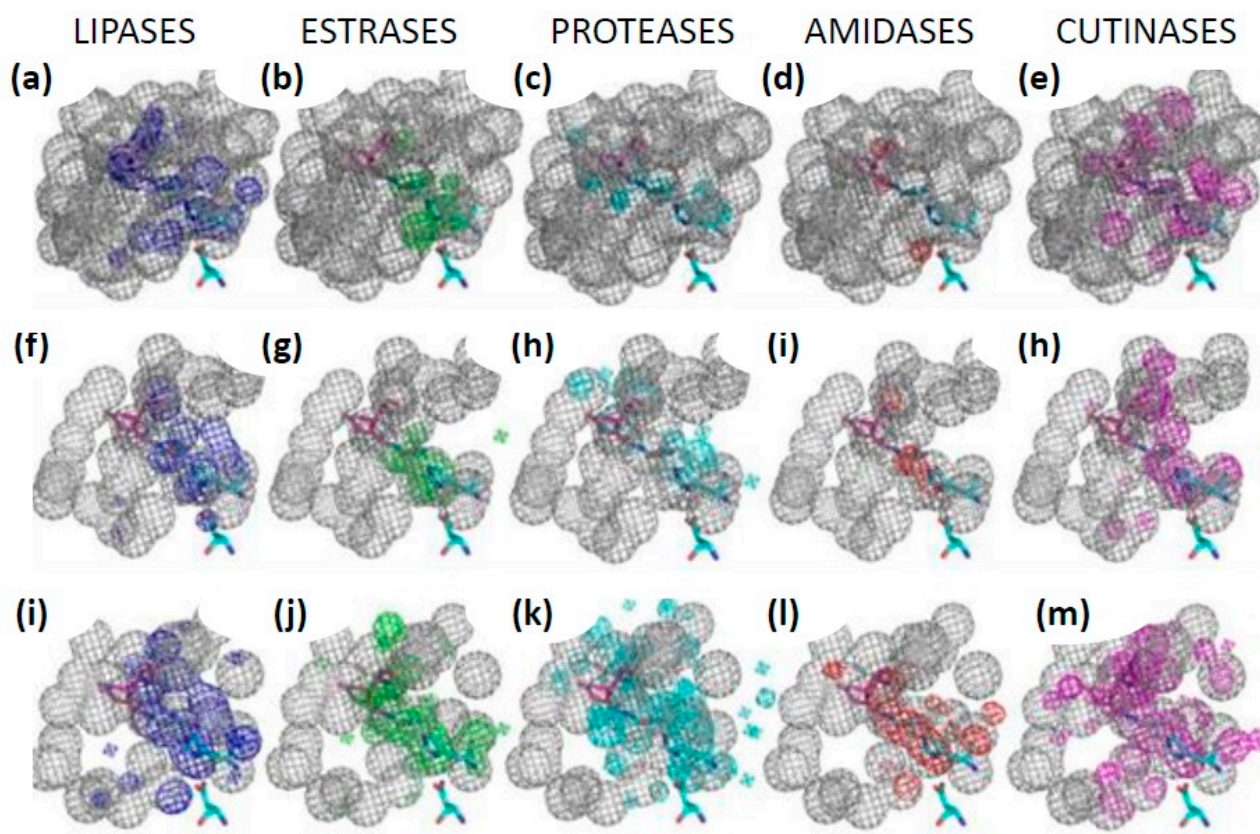
**Figure S1.** S-scores represented by deviation from the average value, low values mean that the structure is well fitted within the dataset. (a) cutinases, (b) proteases, (c) esterases, (d) amidases, (e) lipases.



**Figure S2.** Model scores calculated for the different sub-classes of hydrolases: (a) average score for each serine hydrolase sub-class, and (b) component contributions to the average score of each serine hydrolase sub-class. Color code: shape (gray), DRY probe (hydrophobicity, yellow), O probe (H-Bond donors, red), and N1 probe (H-Bond acceptors, blue).

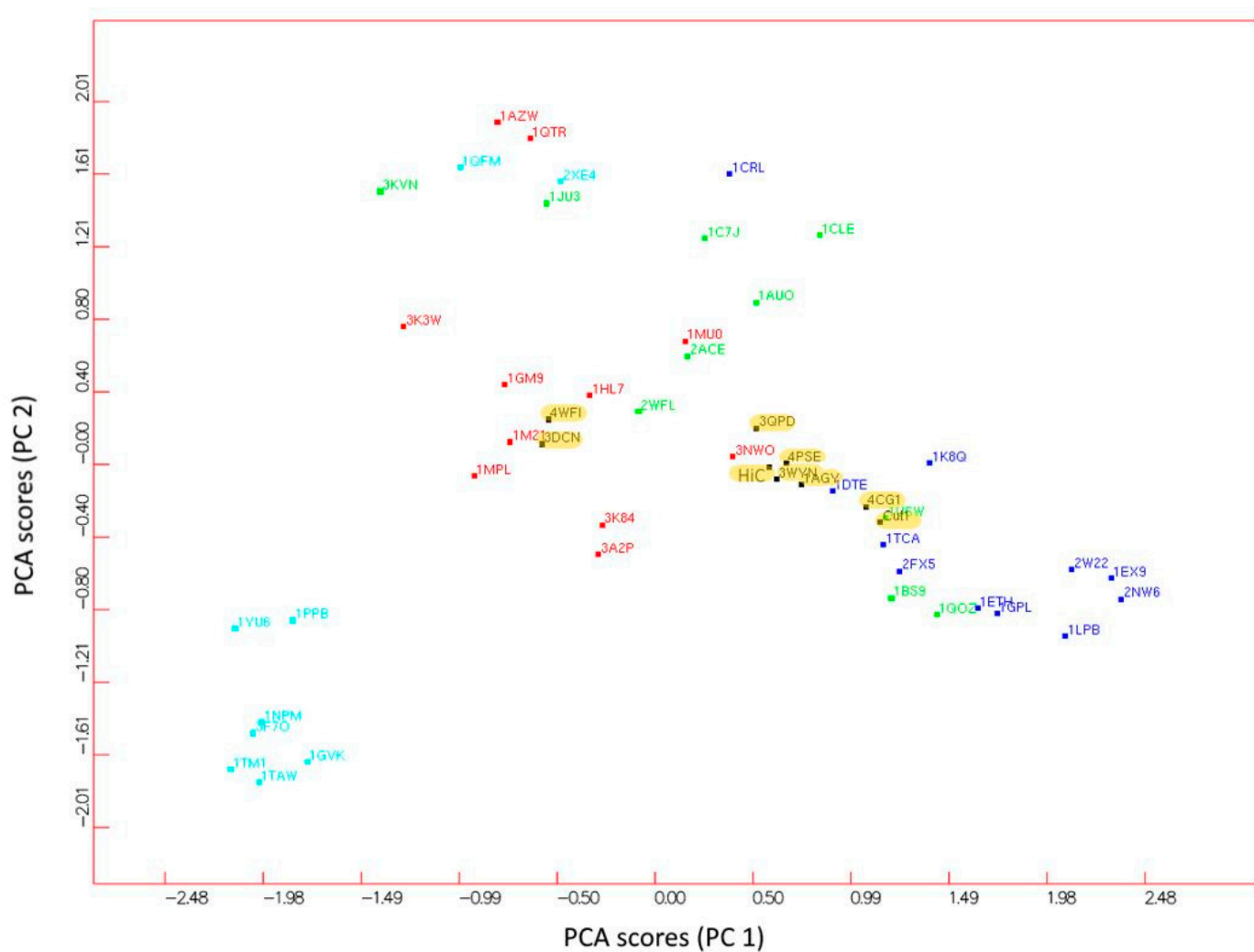


**Figure S3.** Superposition of the Thc\_cut1 MIFs (gray) on the calculated catalophores of each serine hydrolase sub-class. Mapping calculated from: (a-e) DRY probe (hydrophobicity), (f-h) O probe (H-Bond donors), and (i-m) N1 probe (H-Bond acceptors). For from left to right: lipases (blue), estrases (green), proteases (cyan), amidases (red), cutinases (magenta). The superimposition took as spatial reference the residues comprising the oxanion hole of the structure of Thc\_cut1 (Tyr61 and Met132, highlighted in magenta).

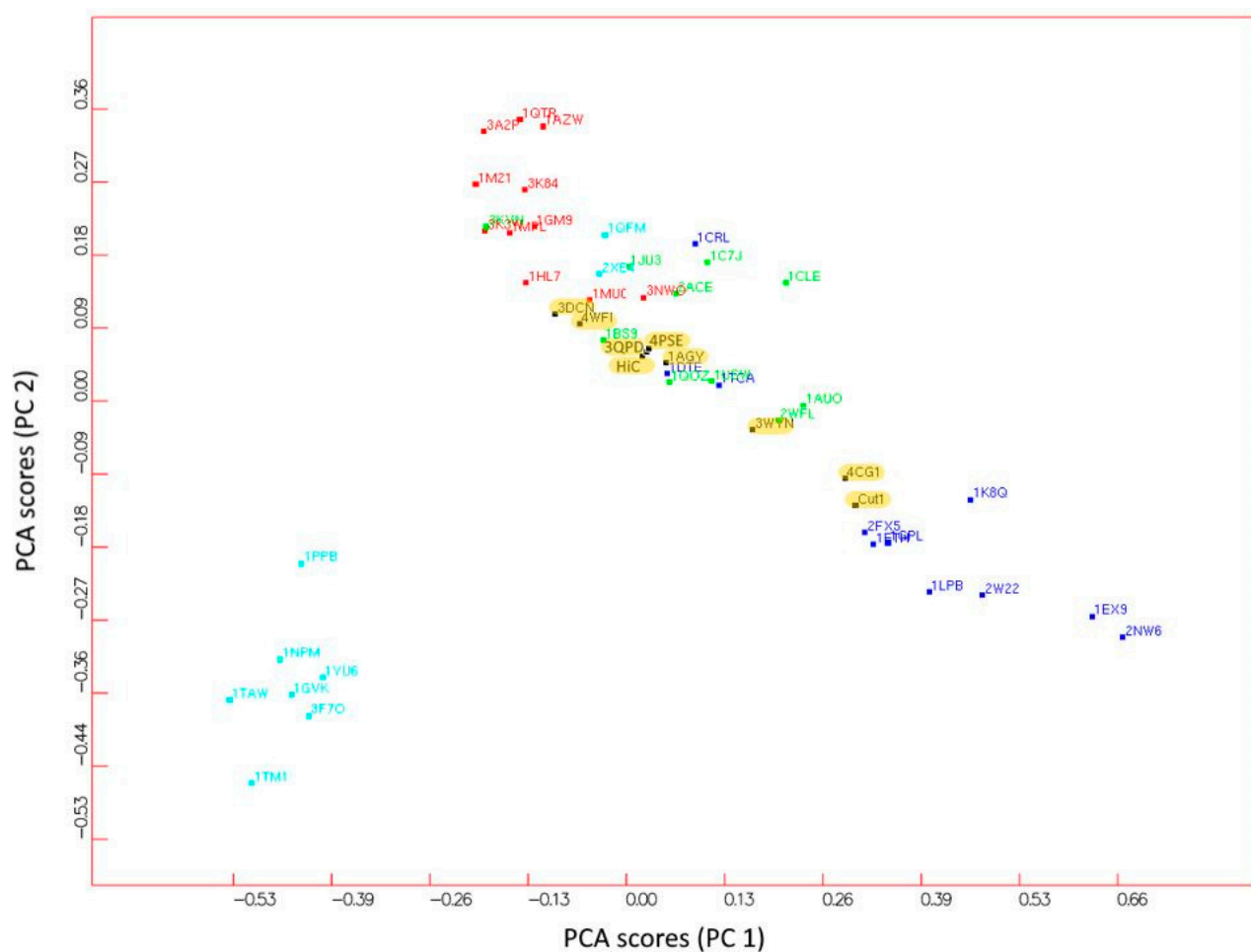


**Figure S4** Superposition of the CaLB MIFs (gray) on the calculated catalophores of each serine hydrolase subclass. Mapping calculated from: (a-e) DRY probe (hydrophobicity), (f-h) O probe (H-Bond donors), and (i-m) N1 probe (H-Bond acceptors). For from left to right: lipases (blue), estrases (green), proteases (cyan), amidases (red), cutinases (magenta). The superimposition took as spatial reference the residues comprising the oxyanion hole of the structure of CaLB (Thr40 and Gln106, highlighted in magenta).



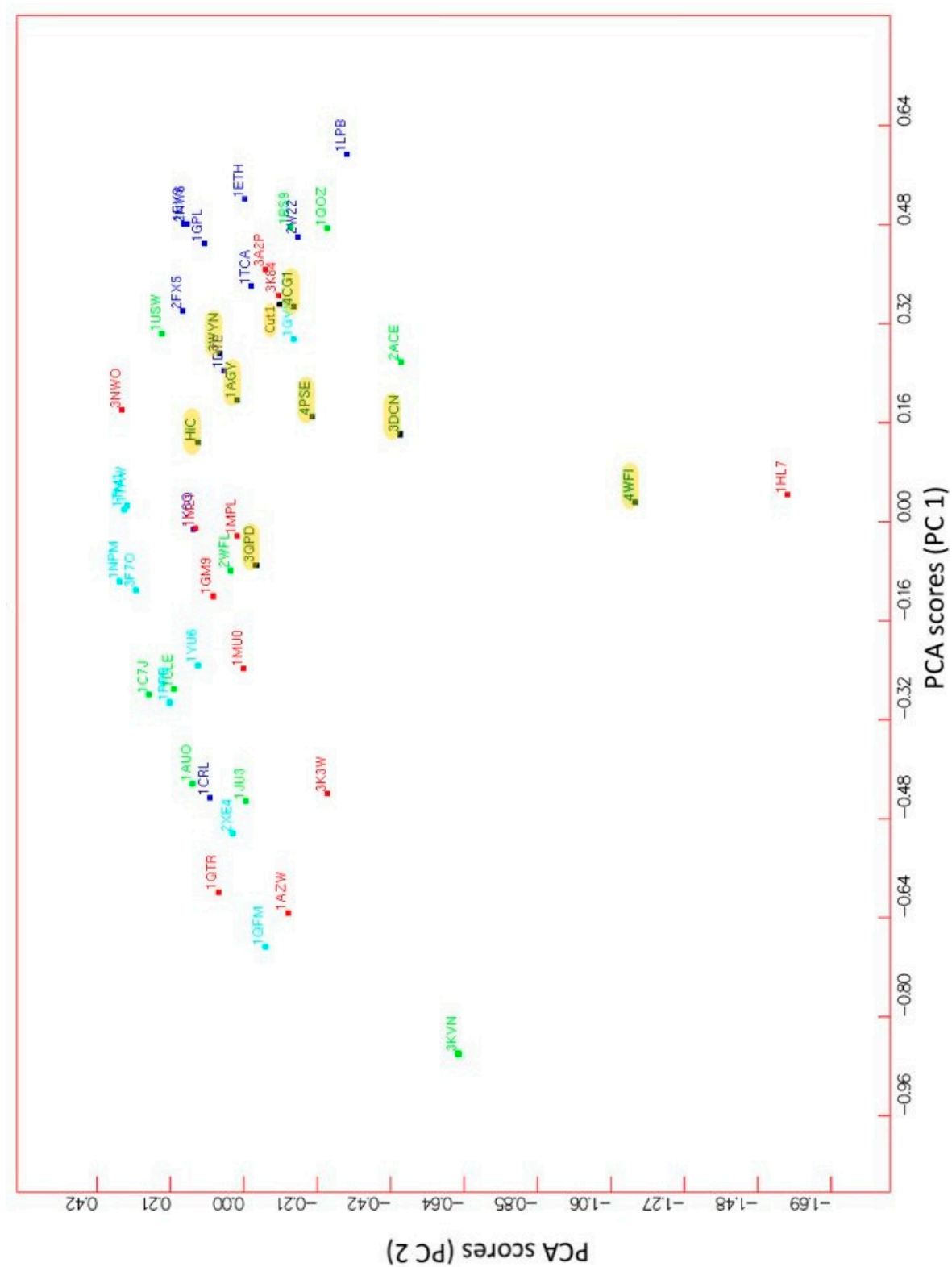


**Figure S5.** UPCA Model, Serine-hydrolases with cutinase projection: global score. Color code: lipases (blue), esterases (green), proteases (cyan), amidases (red), cutinases (yellow). Individual panels can be found as full page images in ESI.

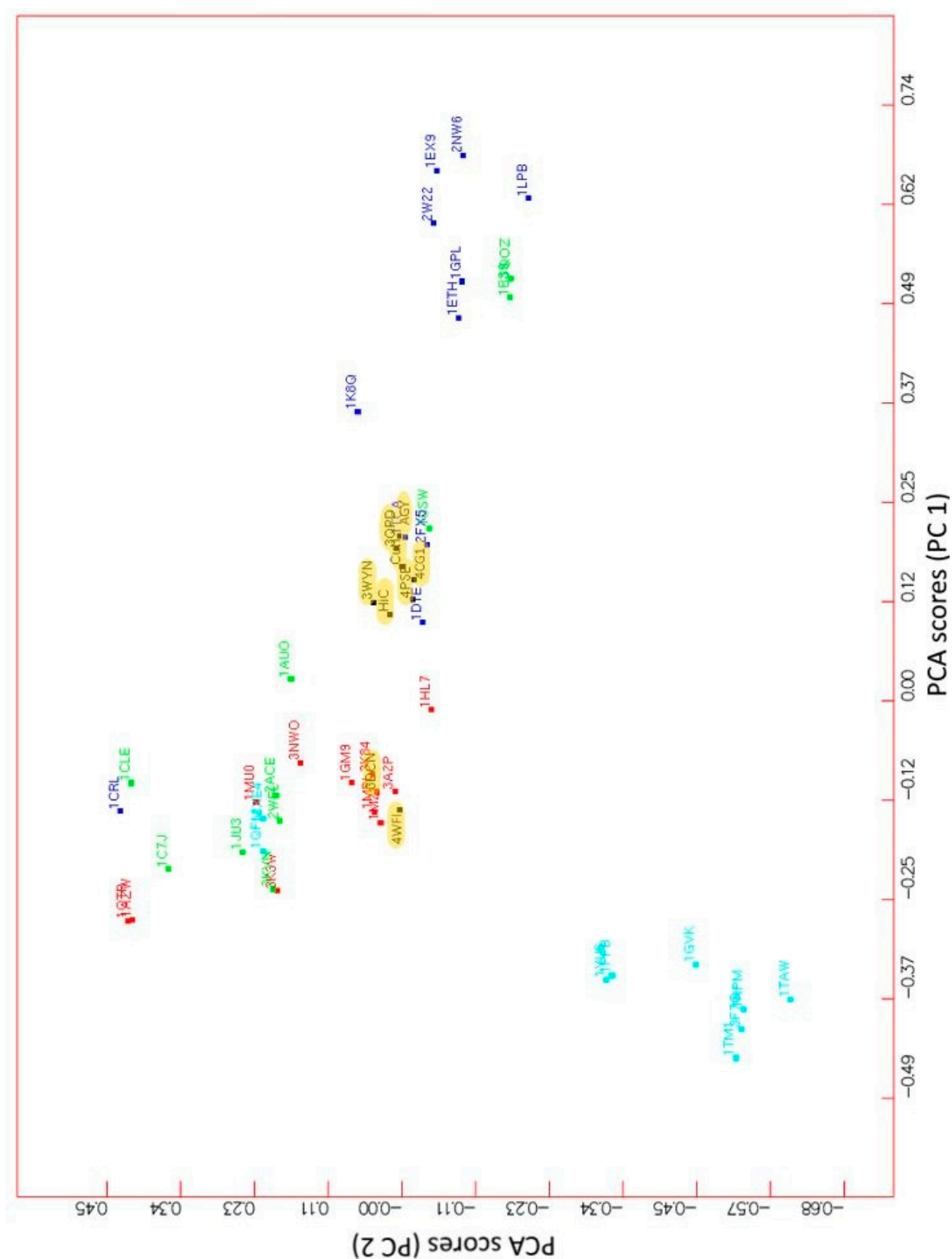


**Figure S6** UPCA Model, Serine-hydrolases with cutinase projection: O-probe score. Color code: lipases (blue), esterases (green), proteases (cyan), amidases (red), cutinases (yellow). Individual panels can be found as full page images in Figures S6-S9.

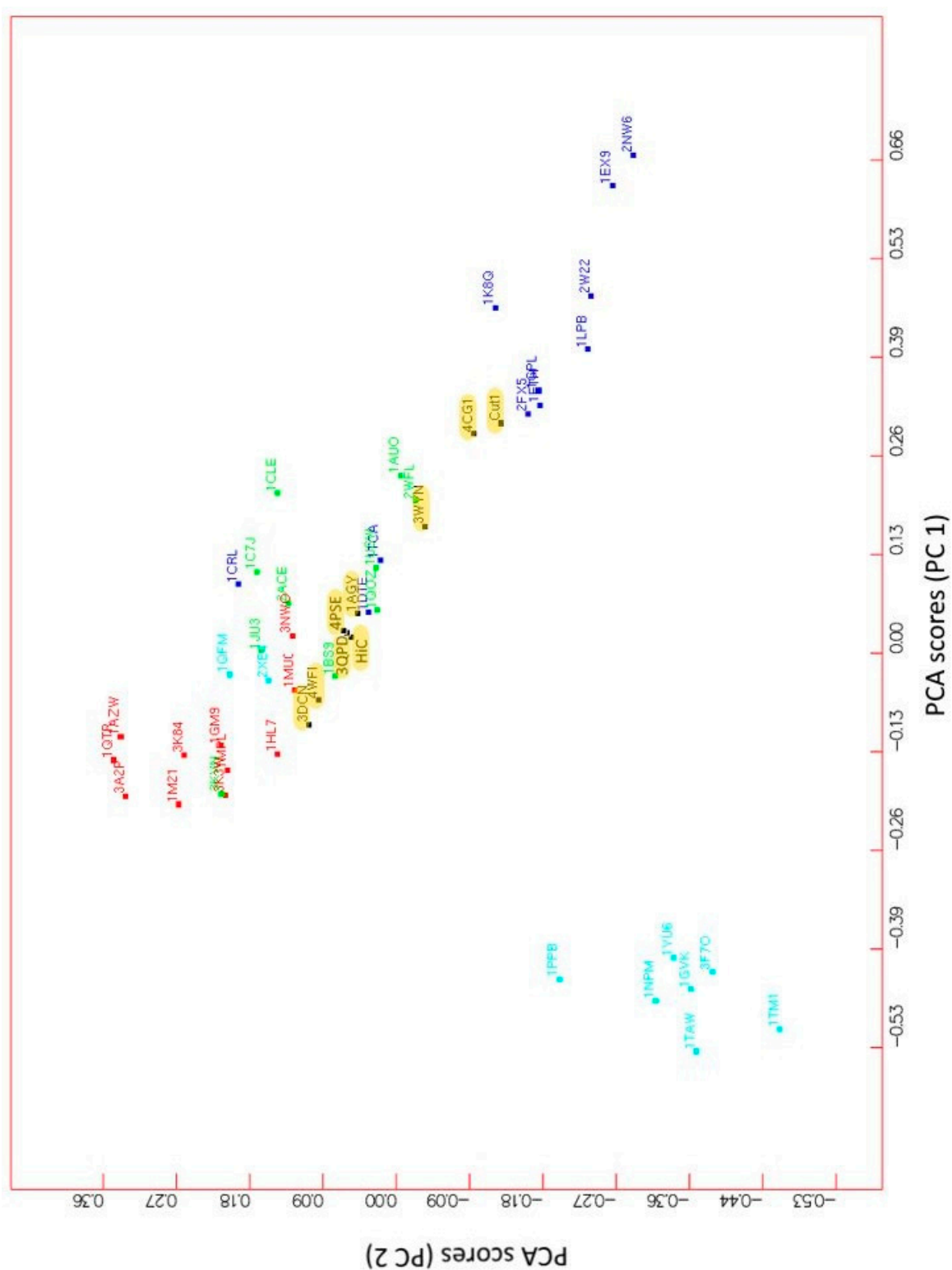




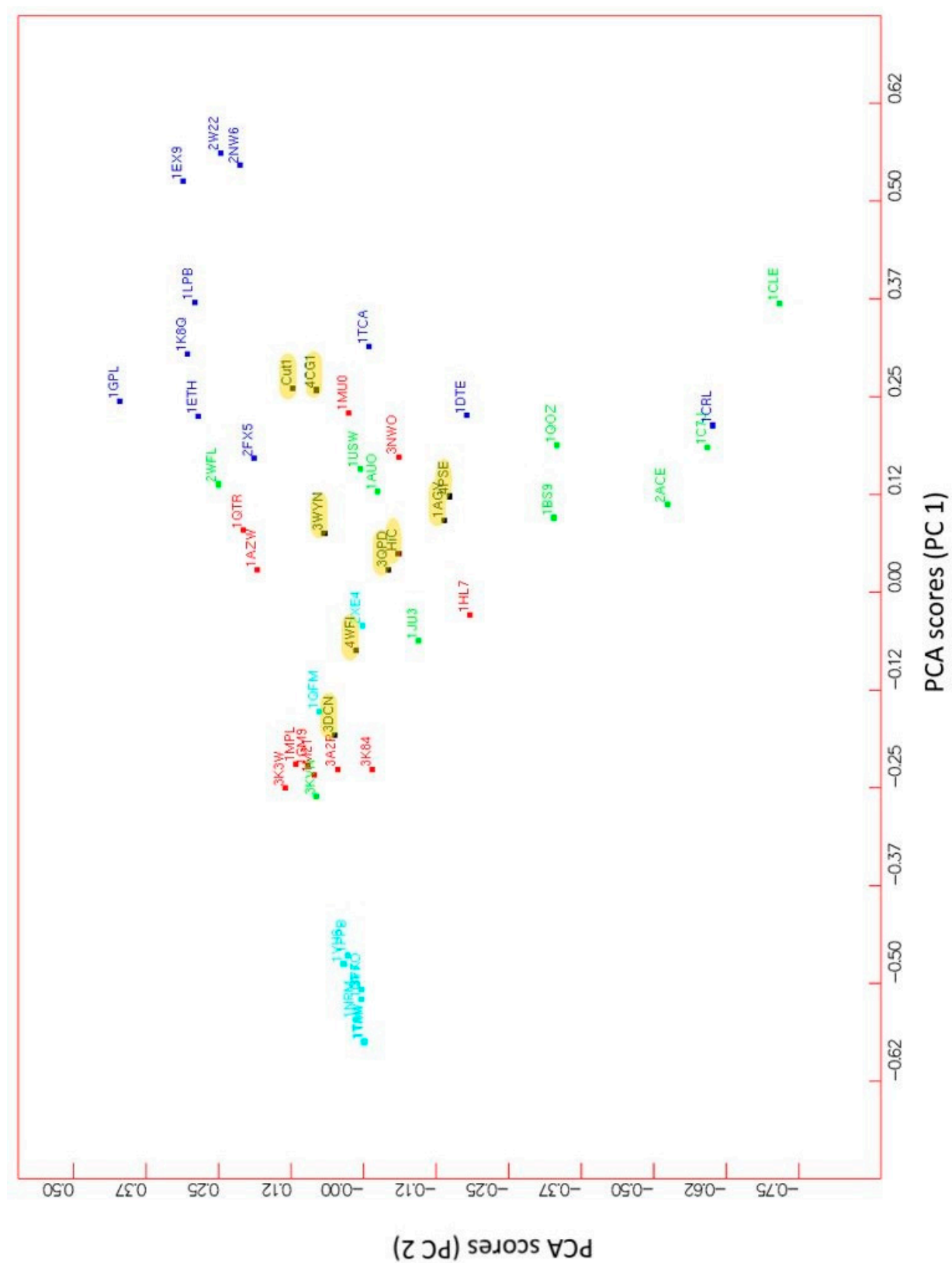
**Figure S7.** UPCA Model, Serine-hydrolases with cutinase projection: shape probe. Color code: lipases (blue), esterases (green), proteases (cyan), amidases (red), cutinases (yellow).



**Figure S8.** UPCA Model, Serine-hydrolases with cutinase projection: hydrophobicity probe. Color code: lipases (blue), esterases (green), proteases (cyan), amidases (red), cutinases (yellow).



**Figure S9.** UPCA Model, Serine-hydrolases with cutinase projection: H-bond acceptors probe. Color code: lipases (blue), esterases (green), proteases (cyan), amidases (red), cutinases (yellow).



**Figure S10.** UPCA Model, Serine-hydrolases with cutinase projection: H-bond donors probe. Color code: lipases (blue), esterases (green), proteases (cyan), amidases (red), cutinases (yellow).