

Table S1: Evaluation parameters of the generated LysB homology models.

No.	Name	No. of generated models by YASARA	YASARA Z-score ^a for hybrid model	Verify-3D ^b	ERRAT quality factor ^c	Prove ^d	Ramachandran (% residues in favored and allowed regions)	Ramachandran (% residues in the disallowed regions)	ProSA Z-score ^e
	D29	-----	-----	98.02%	97.95	0.9%	99%	1.0%	-7.62
1	Chy5	18	0.015	96.06%	98.37	3.6%	99.50%	0.5%	-7.74
2	SWU1	17	0.041	92.46%	93.44	2.3%	99.1%	0.9%	-7.57
3	Echild	2	-0.399	96.99%	98.03	3.2%	99.6%	0.4%	-8.18
4	RedRock	11	-0.575	85.54%	94.95	3.2%	99.6%	0.4%	-8.27
5	Goose	2	-0.439	97.67%	98	3.3%	98.6%	1.4%	-8.14
6	Twister	17	-0.293	99.61%	93.2	3.6%	99.1%	0.9%	-8.12
7	Sheen	16	-0.506	96.75%	94.95	2.5%	99.0%	1.0%	-7.54
8	Timshel	7	-0.590	86.46%	95.87	2.9%	99.3%	0.7%	-9.11
9	HelDan	46	-0.299	97.56%	95.79	3.1%	99.5%	0.5%	-7.26
10	BabyRay	70	-0.196	92.56%	88.03	5.0%	98.1%	1.9%	-7.21
11	BTCU-1	16	-0.501	99.19%	94.11	3.3%	99.1%	0.9%	-7.61
12	Anubis	14	-0.462	100.00%	95.88	2.5%	99.5%	0.5%	-8.23
13	Bxz2	21	-0.628	82.24%	100	2.3%	99.3%	0.7%	-7.01
14	Severus	21	-0.781	91.67%	93.98	3.3%	98.1%	1.9%	-8.45
15	Zakai	2	-0.896	83.40%	98.37	3.3%	98.6%	1.4%	-6.59
16	Wildcat	16	-0.934	91.19%	93.25	2.4%	99.5%	0.5%	-5.83
17	Ardmore	5	-1.369	87.65%	98.34	4.1%	98.6%	1.4%	-7.24

18	MS6	5	-1.369	88.45%	98.34	4.1%	98.6%	1.4%	-7.22
19	Hades	24	-1.645	80.18%	92.83	2.5%	98.9%	1.1%	-6.76
20	Saal	5	-1.611	86.06%	95.45	5.3%	98.6%	1.4%	-7.9
21	Pumpkin	6	-1.535	76.53%	97.76	4.9%	98.3%	1.7%	-5.49
22	Graduation	9	-1.199	83.58%	94.14	3.2%	99.1%	0.9%	-6.54
23	MrMagoo	16	-0.507	82.69%	92.85	2.8%	99.1%	0.9%	-6.86
24	<i>Dylan</i>	5	-1.235	94.55%	96.38	3.4%	98.6%	1.4%	-7.61
25	DS6A	14	-1.502	86.35%	95.03	3.7%	98.6%	1.4%	-6.48
26	Palestino	7	-2.245	81.37%	87.7	5.1%	99.6%	0.4%	-5.76
27	Obama12	46	-1.613	67.79%	97.48	3.3%	98.1%	1.9%	-5.61
28	Omega	39	-1.873	90.34%	98.93	2.9%	99.2%	0.8%	-8.31
29	Larva	79	-1.938	77.87%	91.32	2.7%	99.0%	1.0%	-5.9
30	Enkosi	89	-1.746	83.73%	97.11	3.4%	98.5%	1.5%	-6.69

^a Z-score describes how many standard deviations the model quality is away from the average high-resolution X-ray structure. Higher values are better, negative values indicate that the homology model looks worse than a high-resolution X-ray structure. The overall score thus captures the correctness of backbone- (Ramachandran plot) and side-chain dihedrals, as well as packing interactions as it is calculated from the equation: Overall Z-score = 0.145*Dihedrals + 0.390*Packing1D + 0.465*Packing3D

^b Verify_3D: Determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc.) and comparing the results to good structures. The model is regarded "Pass" if at least 80% of the amino acids have scored ≥ 0.2 in the 3D/1D profile.

^c ERRAT: Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolutions (2.5 to 3Å) the average overall quality factor is around 91%.

^d Prove: Calculates the volumes of atoms in macromolecules using an algorithm which treats the atoms like hard spheres and calculates a statistical Z-score deviation for the model from highly resolved (2.0 Å or better). It records "ERROR" if the %Buried outlier protein atoms exceed 5%.

^e ProSA Z-score: indicates the overall model quality in comparison to z-scores of all experimentally determined protein chains in current PDB. It can be used to check whether the z-score of the input structure is within the range of scores typically found for native proteins of similar size.

Table S2: LysB homologous proteins representatives of the seven groups, chain length and domain diversity.

No	LysB-	Uniprot ID	(%) Similarity to LysB- D29	Group	Total protein Length	Domains, protein families and residues	No. of domains
	D29	O64205	100	Standard	254	[PE-PPE, (PF08237); 51-125] [Cutinase, (PF01083); 41-169]	2
1	Chy5	R4JF81	99.2	1	254	[PE-PPE, (PF08237); 51-127] [Cutinase, (PF01083); 41-169]	2
2	SWU-1	I1V1E2	90.6			[PE-PPE, (PF08237); 51-125] [Cutinase, (PF01083); 41-169]	2
3	Echild	W0LJP4	76.6	2A	325	[PE-PPE, (PF08237); 109-191] [Cutinase, (PF01083); 109-238] [Flagellar hook-length control protein FliK (PF02120); 202-230]	3
4	RedRock	D3JZ76	74.5		325	[PE-PPE, (PF08237); 109-192] [Cutinase, (PF01083); 109-240]	2
5	Goose	K0G173	76.6	2B	324	[PE-PPE, (PF08237); 126-199] [Cutinase, (PF01083); 116-245]	2
6	Twister	H9NCI8	75.8		324	[PE-PPE, (PF08237); 126-198] [Cutinase, (PF01083); 116-249] [Uncharacterized protein family (UPF0565); 139-189]	3
7	Sheen	A0A0B5A441	70.2	2C	246	[PE-PPE, (PF08237); 51-119] [Cutinase, (PF01083); 69-162]	2
8	Timshel	G1DB29	70.2		325	[PE-PPE, (PF08237); 130-198] [Cutinase, (PF01083); 148-207]	2
9	HelDan	G1DA59	63	3	246	[PE-PPE, (PF08237); 50-122] [Cutinase, (PF01083); 41-123] [Phospholipase/Carboxylesterase (PF02230); 61-88]	3

10	BabyRay	A0A1D8EW72	63.1		244	[PE-PPE, (PF08237); 50-122] [Cutinase, (PF01083); 41-131] [Thioesterase domain (PF00975); 62-84]	3
11	BCTU-1	R9R488	63.0		246	[PE-PPE, (PF08237); 49-122] [Cutinase, (PF01083); 41-132] [Thioesterase domain (PF00975); 62-84]	3
12	Anubis	A0A097BYL0	62.9	4	321	[PE-PPE, (PF08237); 130-199] [Cutinase, (PF01083); 149-200]	2
13	Bxz2	Q857L1	63.3		321	[PE-PPE, (PF08237); 130-199] [Cutinase, (PF01083); 149-200]	2
14	Severus	R4JQ45	58.8		321	[PE-PPE, (PF08237); 122-199] [Cutinase, (PF01083); 149-268] [Protein of unknown function (PF05338); 283-311]	3
15	Zakai	A0A1D8EPB0	48.8	5A	342	[Protein of unknown function (PF05338); 80-94] [PE-PPE, (PF08237); 143-218] [Cutinase, (PF01083); 129-220]	3
16	Wildcat	Q19Y08	47.0	5B	321	[Putative peptidoglycan binding domain (PF01471); 17-71] [PE-PPE, (PF08237); 131-202] [Cutinase, (PF01083); 117-169]	3
17	Ardmore	D4N7H5	42.9	6A	332	[Putative peptidoglycan binding domain (PF01471); 15-58] [PE-PPE, (PF08237); 134-224] [Cutinase, (PF01083); 120-208]	3
18	MS6	Q9FZR9	42.9		333	[Putative peptidoglycan binding domain (PF01471); 15-58] [PE-PPE, (PF08237); 134-224] [Cutinase, (PF01083); 120-208]	3
19	Hades	A0A076YRQ3	42.9		332	[Statherin (PF03875); 14-43] [Putative peptidoglycan binding domain (PF01471); 8-59] [PE-PPE, (PF08237); 136-225] [Cutinase, (PF01083); 120-208]	4

20	Saal	W6AV51	41.7		333	[Putative peptidoglycan binding domain (PF01471); 38-58] [PE-PPE, (PF08237); 136-224] [Cutinase, (PF01083); 120-208]	3
21	Pumpkin	C9DCK3	41.7	6B	285	[PE-PPE, (PF08237); 52-110] [Cutinase, (PF01083); 49-177]	2
22	Graduation	V5R3P2	39.1	7A	322	[PE-PPE, (PF08237); 123-205] [Cutinase, (PF01083); 115-240]	2
23	MrMagoo	A0A1L6BYH7	38.6		325	[Putative peptidoglycan binding domain, (PF01471); 26-70] [PE-PPE, (PF08237); 118-207] [Cutinase, (PF01083); 110-188] [Lipase (class 3), (PF01764); 130-186] [Protein of unknown function, (PF11288); 147-194] [Bacterial virulence protein (VirJ), (PF06057); 133-167] [Molybdopterin oxidoreductase N-terminal, (PF18364); 286-315]	7
24	Dylan	S5ZH98	37.2		346	[PE-PPE, (PF08237); 135-232] [Cutinase, (PF01083); 128-225] [Phospholipase/Carboxylesterase, (PF02230); 169-194] [Protein of unknown function, (PF11288); 165-198]	4
25	DS6A	G8I4E1	37.4		345	[Protein of unknown function (PF11288); 68-117] [PE-PPE, (PF08237); 133-219] [Cutinase, (PF01083); 119-206]	3
26	Palestino	A0A1J0MAW0	36.4	7B	326	[PE-PPE, (PF08237); 91-187]	1
27	Obama12	W0LJG8	35.5		326	No conserved motif	0
28	Omega	Q854L1	31.3	7C	290	[Cutinase, (PF01083); 21-139]	1
29	Larva	G1FMT3	31.1		253	[Cutinase, (PF01083); 76-122]	1
30	Enkosi	A0A0M4R3S7	30.1		252	No conserved motif	0

Table S3: Types of active site conformations of LysB models and the best docking score upon docking of *p*-NP ligands.

No.	LysB-	Length of loop-5 ^a (aa)	<i>p</i> -NP ligands							Presence of GNP in the active site
			C4 <i>p</i> -NPB	C8 <i>p</i> -NPC	C10 <i>p</i> -NPD	C12 <i>p</i> -NPL	C14 <i>p</i> -NPM	C16 <i>p</i> -NPP	C18 <i>p</i> -NPS	
I. Tunnel conformation										
A. lysB-D29-like orientation										
	D29^b	14	Pose: 4 S: (-5.41)	Pose: 22 S: (-6.45)	Pose: 56 S: (-0.46)	Pose: 88 S: (-6.65)	OP	Pose: 136 S: (-7.56)	Pose: 163 S: (-8.07)	G
1	Chy5^b	14	Pose: 11 S: (-4.99)	Pose: 44 S: (-4.95)	Pose: 74 S: (-5.06)	NDP	Pose: 115 S: (-6.00)	Pose: 139 S: (-5.95)	NDP	G and P
B. Inverted lysB-D29 orientation										
1	SWU-1^b	13	Pose: 10 S: (-5.3)	Pose: 24 S: (-6.3)	Pose: 54 S: (-6.8)	Pose: 89 S: (-6.7)	Pose: 114 S: (-7.0)	Pose: 155 S: (-6.7)	Pose: 183 S: (-6.9)	GNP
2	Echild	13	NDP	NDP	NDP	NDP	Pose: 101 S: (-7.31)	OP	OP	-----
3	RedRock	13	NDP	NDP	NDP	NDP	NDP	Pose: 11 S: (-7.2)	NDP	GNP
4	Sheen	12	NDP	NDP	NDP	NDP	Pose: 87 S: (-5.76)	Pose: 105 S: (-6.23)	Pose: 138 S: (-6.12)	G and P
5	Timshel	12	NDP	NDP	Pose: 32 S: (-6.14)	Pose: 58 S: (-5.26)	Pose: 79 S: (-5.98)	NDP	Pose: 134 S: (-6.43)	G and P
6	BabyRay^b	9	NDP	NDP	NDP	NDP	NDP	NDP	NDP	-----
7	Ardmore	10	NDP	NDP	NDP	NDP	NDP	OP	NDP	G
8	Ms6	10	NDP	NDP	NDP	NDP	NDP	OP	NDP	G
9	Pumpkin^b	18	NDP	NDP	NDP	NDP	NDP	NDP	NDP	GNP

10	MrMagoo	14	Pose: 8 S:(-5.32)	NDP	NDP	NDP	OP	Pose: 174 S: (-4.29)	OP	-----
11	Dylan	10	NDP	NDP	NDP	NDP	NDP	NDP	NDP	GNP
12	Palestino^b	14	NDP	NDP	NDP	NDP	OP	NDP	OP	GNP
13	Obama12^b	19	Pose: 7 S:(-5.14)	Pose: 27 S:(-5.94)	OP	OP	OP	Pose: 160 S: (-6.56)	OP	GNP
14	Larva	15	NDP	NDP	NDP	NDP	NDP	NDP	NDP	G
15	Enkosi^b	16	OP	OP	OP	OP	OP	Pose: 147 S: (-6.6)	OP	-----
II. Deep Funnel conformation										
A. Very deep funnel										
1	Zakai	10	OP	OP	NDP	NDP	OP	NDP	NDP	G
2	Saal^b	10	Pose: 22 S:(-3.93)	Pose: 29 S:(-5.19)	OP	Pose: 97 S: (-6.5)	OP	OP	OP	GNP
3	Graduation	10	NDP	Pose: 41 S: (5.74)	NDP	NDP	Pose: 126 S: (5.94)	OP	NDP	-----
4	DS6A^b	11	OP	OP	OP	OP	OP	OP	OP	G
B. Less deep funnel										
1	Twister	9	OP	OP	OP	OP	OP	OP	OP	G and P
2	Wildcat	9	OP	OP	NDP	OP	OP	OP	OP	G and P
3	Hades^b	11	NDP	NDP	NDP	NDP	OP	OP	NDP	G
III. Superficial funnel conformation										
1	HelDan	7	NDP	NDP	NDP	NDP	NDP	NDP	NDP	G
2	BTCU-1	6	NDP	OP	NDP	NDP	NDP	NDP	NDP	G and P
3	Anubis^b	6	Pose: 9 S:(-4.39)	NDP	Pose: 61 S:(-5.46)	Pose: 104 S: (-4.76)	Pose: 118 S: (-5.5)	NDP	NDP	G and P
4	Bxz2	7	NDP	NDP	NDP	OP	OP	NDP	NDP	-----
5	Severus	7	NDP	NDP	OP	OP	NDP	NDP	NDP	G and P

IV. Long Shallow bowl conformation										
1	Omega ^b	16	NDP	NDP	NDP	NDP	NDP	NDP	NDP	G and P
V. Narrow buried cave conformation										
1	Goose ^b	9	NDP	NDP	OP	NDP	NDP	NDP	NDP	G and P

^alength of loop-5 according to the 3D structural alignment of each LysB 3D model to LysB-D29 crystal structure.

^b Representative members chosen for subsequent cloning, production and enzymatic assays.

Pose: ligand-protein conformation achieving H-bond between the catalytic Ser & ligand's ester bond (C=O).

S: (Docking score) a value calculated by MOE and indicate final energy for the ligand-substrate complex (the higher the negative value, the better the docking score).

OP: (Orientation pose) ligand-protein conformation where the catalytic Ser faces ester ligand's ester bond (C=O) with no H-bond formation.

NDP: (No Detected Pose) neither binding nor orientation pose.

Table S4: Quantitative measurements and structural alignments of LysB active sites to their counterparts of the α/β hydrolases.

No.	Item	Active site Conformation	size	No. of residues forming the active site	Volume of the active site (A ³)	Solvent Accessible Surface area (SASA) of the active site (A ²)	RMSD of alignment	Percentage of Sequence identity	TM-score
Std.	<i>Candida rugosa</i> lipase	Tunnel	149	38	903.3	455.9	0	0%	0.0526
Std.	LysB-D29		172	43	1838.5	1965.8	-----	-----	-----
1	LysB-Chy5		78	24	499.5	572.8	0.111	100%	0.7787
2	LysB-SWU-1		142	34	1176.5	1063.6	0	100%	0.9706
3	LysB-Echild-2		82	25	902.3	1346.5	0.286	44.44%	0.04
4	LysB-Redrock		26	14	103.3	93.1	0	0%	0.0022
5	LysB-Sheen		79	22	732.4	814.6	0.878	66.67%	0.0841
6	LysB-Timshel		93	25	602.1	592.7	0.257	80.0000%	0.04
7	LysB-Babyray		116	30	893.9	1177.1	1.706	68.7500%	0.1467
8	LysB-Ardmore		125	31	530.4	859.6	0.03	33.3300%	0.0645
9	LysB-Ms6		125	31	540.9	884.4	0.03	33.3300%	0.0645
10	LysB-Pumpkin		90	32	664.6	1137.2	0	0%	0.0624
11	LysB-Mrmagoo		139	37	848.4	1081.5	0.555	69.2300%	0.0645
12	LysB-Dylan		88	28	331.1	574.5	0.199	75.0000%	0.0614
13	LysB-Palestino		218	47	1863.1	2201.1	0.875	63.6400%	0.0293
14	LysB-Obama12		147	32	1072.1	1364.5	0.476	66.6700%	0.028
15	LysB-Larva	130	29	818	1299.2	0	0%	0.0697	
16	LysB-Enkosi	119	28	635.6	885.2	1.394	50.0000%	0.1235	
Std.	<i>Candida antartica</i> lipase B	Very deep funnel	123	34	838.5	809.2	-----	-----	-----
1	LysB-Zakai		74	21	459.5	366.6	0	0%	0.0707

2	LysB-Saal		186	43	957.8	1461.8	0.303	0%	0.0464
3	LysB-Graduation		77	22	239.5	389	0.755	0%	0.0455
4	LysB-DS6A		76	23	237.6	427.5	0.011	33%	N.D
Std.	<i>Pseudomonas cepacia</i> lipase	Less deep funnel	128	36	1235.9	947.2	-----	-----	-----
1	LysB-Twister		20	9	71.1	125.7	0	0%	N.D
2	LysB-Wildcat		74	24	437.9	559.6	0	0%	0.0417
3	LysB-Hades		61	19	329.1	437.8	0	0%	0.0802
Std.	Human pancreatic lipase	Superficial funnel	41	9	157.6	282.7	-----	-----	-----
Std.	<i>Fusarium solani</i> cutinase		67	23	421	910.2	-----	-----	-----
1	LysB-Heldan		60	17	304.8	638.9	0	0%	0.0588
2	LysB-BTCU-1		108	32	891.9	988.8	0	0%	0.0149
3	LysB-Anubis		113	30	797.3	728.4	0	0%	N.D
4	LysB-BXZ2		91	24	661	1029.7	0	0%	N.D
5	LysB-Severus	67	19	307.1	272.2	0	0%	N.D	
Std.	<i>Rhizomucor miehei</i> lipase	Long Shallow bowl	86	23	493.5	738.2	-----	-----	-----
1	LysB-Omega		92	28	814.8	819.4	0	0%	0.0536
Std.	Acetylcholine esterase	Narrow buried cave	151	38	867.6	766.5	-----	-----	-----
1	LysB-Goose		139	36	1342.1	1204	0.911	0.00%	0.0573

Std: Standard.

N.D: Not defined

Note: TM-score is a scoring function to assess the similarity of protein structures based on statistics [1, 2]:

0.0 < TM-score < 0.17, random structural similarity.

0.5 < TM-score < 1.00, in about the same fold.

Note: Alignment of the active site of LysB-D29 was done to *C. rugosa* lipase active site, while alignments of other tunnel-shaped LysB active sites were done to LysB-D29 active site and alignments of the other classes of LysB active sites were done to each corresponding of the class standard.

References

1. Zhang, Y.; Skolnick, J. Scoring function for automated assessment of protein structure template quality. *Proteins*. **2004**, *57*, 702-710.
2. Xu, J.; Zhang, Y. How significant is a protein structure similarity with TM-score= 0.5?. *Bioinformatics* **2010**, *26*, 889-895.

Table S5: Specific activities and kinetic parameters of LysB enzymes in comparison with relative cutinases and lipases.

Organism	Enzyme	Specific activity (U. mg ⁻¹) ^a			V _{max} (U. mg ⁻¹)			K _m (μM)			Reference
		<i>p</i> -NPB	<i>p</i> -NPL	<i>p</i> -NPP	<i>p</i> -NPB	<i>p</i> -NPL	<i>p</i> -NPP	<i>p</i> -NPB	<i>p</i> -NPL	<i>p</i> -NPP	
Mycobacteriophages											
D29	Endolysin B (LysB)	0.94±0.002	4.8±0.09	0.55±0.07	122.3	7.55	2.73	422.6	19.6	37.7	Present study - [1]
		1.61±0.031 ^b	1.4±0.016 ^b	0.07 ^b ±0.005							
		N.D	9.7±0.3	0.32±0.02	111.8	79.8	1.45	618.8	280.8	24.4	
		N.D	0.137±0.02	0.25±0.02	1.49	0.425	0.4	4172.12	1476.4	2833.32	
		N.D	0.018±0.004	0.0048±0.005	0.47	0.17	0.084	1268.5	3000	800	
		N.D	0.09±0.01	0.007±0.0009							
		N.D	0.02±0.001	0.002±0.003							
		N.D	0.01±0.001	0							
		2.7	0	N.D	49.8±1.1	N.D	N.D	211.2±4.8	N.D	N.D	
		0.35	0.21	N.D	1.7±0.4	N.D	N.D	107.7±27.0	N.D	N.D	
<i>Fusarium solani</i>	Cutinase I				1×10 ⁻⁶	0.008×10 ⁻⁶	N.D	3.5×10 ⁷	5.6×10 ⁷	N.D	[3]
	Cutinase II				1.2×10 ⁻⁶	0.002×10 ⁻⁶	N.D	7.5×10 ⁷	4.5×10 ⁷	N.D	[4]
								350±20 ^a	N.D	N.D	[5]
					0.638 ±0.024 ^a	N.D	N.D	0.121 ±0.009 ^a	N.D	N.D	[6]
								1.36±0.68	N.D	N.D	[7]
								1.26 ± 0.28	N.D	N.D	[8]
		Free cutinase				6.2 × 10 ⁻⁶	N.D	N.D			
	<i>Humicola insolens</i>	Cutinase						454±29	N.D	2246±347	[6]
							2.46±0.15	N.D	N.D	[10]	
367.6±7.6			N.D	N.D							
<i>Pseudomonas cepacia</i>	lipase				N.D	N.D	430±2	N.D	N.D	11×10 ³ ±0.8	[11]
					N.D	N.D	434±3	N.D	N.D	0.12×10 ³	[12]

										± 0.5	
		N.D	N.D	14200 ±1420							[13]
	Wild type	3.5 ×10 ³ ±0.016	N.D	7.9×10 ³ ±0.1							[14]
<i>Candida rugosa</i> lipase	free enzyme				N.D	N.D	1928.66	N.D	N.D	0.17	[15]
	LIP1	18±0.7	31±2.0	10±0.2							[16]
	LIP3	113±1.9	251±11	111±2.7							[17]
	LIP4	0.0029 ±0.0002	0.0044 ±0.0002	0.0061 ±0.0001							[18]
	lipase A	2.45	14.4	7.9							[19]
	lipase B	8.61	30.9	16.9							
	lipase A	350 ^b	0 ^b	0 ^b							
	lipase B	146 ^b	0 ^b	0 ^b							

^a One unit (U) of enzyme activity corresponds to the liberation of 1 µmol of *p*-nitrophenol per min under the assay conditions.

^b The assay was performed without addition of triton X-100 as a surfactant.

N.D: Not defined.

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