Supplementary Material

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Type  Name     Alignment                                                  Position
      LCC      ------------------------------------------------SNPYQRGPNPT  11
I     Cut190   -------------------------------------MRGSHHHHHHGSNPYERGPDPT  22
      TfCut2   ------------------------------------------------ANPYERGPNPT  11
IIb   PETase   --MNFPRASRLMQAAVLGGLMAVSA-------------------AATAQTNPYARGPNPT  39
      PET12    MPPDCVLPRRLAAAALLASATLVPL-------------------SAAAQTNPYQRGPDPT  41
IIa   PET5     --MN---KSILKKLSFGTSVLLVSMNALSWTPSPTPNPDPIPDPTPCQDDCDFTRGPNPT  55
      PE-H     MPFNK--KSVLA--LCGAGALLFSMSALANNPAP-------TDPGDSGGGSAYQRGPDPS  49

      LCC      RSALT-ADGPFSVATYTVSRLSVSGFGGGVIYYPTGTS-LTFGGIAMSPGYTADASSLAW  69
I     Cut190   EDSIEAIRGPFSVATERVSSF-ASGFGGGTIYYPRETDEGTFGAVAVAPGFTASQGSMSW  81
      TfCut2   DALLEARSGPFSVSEENVSRLSASGFGGGTIYYPREN--NTYGAVAISPGYTGTEASIAW  69
IIb   PETase   AASLEASAGPFTVRSFTVS-R-PSGYGAGTVYYPTNAG-GTVGAIAIVPGYTARQSSIKW  96
      PET12    TRDLEDSRGPFRYASTNVR-S-PSGYGAGTIYYPTDVS-GSVGAVAVVPGYLARQSSIRW  98
IIa   PET5     PSSLEASTGPYSVATRSVASS-VSGFGGGTLHYPTNTT-GTMGAIAVVPGFLLQESSIDF  113
      PE-H     VSFLEADRGQYSVRSSRVSSL-VSGFGGGTIYYPTGTT-GTMGAVVVIPGFVSAESSIDW  107

      LCC      LGRRLASHGFVVLVINTNSRFDYPDSRASQLSAALNYLRT---SSPSAVRARLDANRLAV  126
I     Cut190   YGERVASQGFIVFTIDTNTRLDQPGQRGRQLLAALDYLVE---RSDRKVRERLDPNRLAV  138
      TfCut2   LGERIASHGFVVITIDTITTLDQPDSRAEQLNAALNHMIN---RASSTVRSRIDSSRLAV  126
IIb   PETase   WGPRLASHGFVVITIDTNSTLDQPSSRSSQQMAALRQVASLNGTSSSPIYGKVDTARMGV  156
      PET12    WGPRLASHGFVVITLDTRSTSDQPASRSAQQMAALRQVVALSETRSSPIYGKVDPNRLAV  158
IIa   PET5     WGPKLASHGFVVITISANSGFDQPASRATQLGRALDYVINQSNGSNSPISGMVDTTRLGV  173
      PE-H     WGPKLASYGFVVMTIDTNTGFDQPPSRARQINNALDYLVSQNSRSSSPVRGMIDTNRLGV  167

      LCC      AGHSMGGGGTLRIAEQNPSLKAAVPLTPWHTDKT-FN-TSVPVLIVGAEADTVAPVSQHA  184
I     Cut190   MGHSMGGGGSLEATVMRPSLKASIPLTPWNLDKT-WGQVQVPTFIIGAELDTIAPVRTHA  197
      TfCut2   MGHSMGGGGSLRLASQRPDLKAAIPLTPWHLNKN-WSSVTVPTLIIGADLDTIAPVATHA  185
IIb   PETase   MGWSMGGGGSLISAANNPSLKAAAPQAPWDSSTN-FSSVTVPTLIFACENDSIAPVNSSA  215
      PET12    MGWSMGGGGTLISARDNPSLKAAVPFAPWHNTAN-FSGVQVPTLVIACENDTVAPISRHA  217
IIa   PET5     VGWSMGGGGALQLAS-GDRLSAAIPIAPWNQGGNRFDQIETPTLVIACENDVVASVNSHA  232
      PE-H     IGWSMGGGGTLRVAS-EGRIKAAIPLAPWDTTSYYASRSQAPTLIFACESDVIAPVLQHA  226

      LCC      IPFYQNLPSTTPKVYVELDNASHFAPN---SNNAAISVYTISWMKLWVDNDTRYRQFLCN  241
I     Cut190   KPFYESLPSSLPKAYMELDGATHFAPN---IPNTTIAKYVISWLKRFVDEDTRYSQFLCP  254
      TfCut2   KPFYNSLPSSISKAYLELDGATHFAPN---IPNKIIGKYSVAWLKRFVDNDTRYTQFLCP  242
IIb   PETase   LPIYDSMSR-NAKQFLEINGGSHSCANSGNSNQALIGKKGVAWMKRFMDNDTRYSTFACE  274
      PET12    SSFYNSFSSSLAKAYLEINNGSHTCANTGNSNQALIGKYGVAWIKRFVDNDTRYSPFLCG  277
IIa   PET5     SPFYNRIPSTTDKAYLEINGGSHFCANDGGSIGGLLGKYGVSWMKRFIDNDLRYDAFLCG  292
      PE-H     SPFYNSLPSSIDKAFVEINGGSHYCGNGGSIYNDVLSRFGVSWMKLHLDEDSRYKQFLCG  286

      LCC      VNDPALS---DFRTNNRHCQ----------------------                   258
I     Cut190   NPTDR-----AIEEYRSTCPYKLN------------------                   273
      TfCut2   GPRDGLF--GEVEEYRSTCPFYPNSSSVDKLAAALEHHHHHH                   282
IIb   PETase   NPNSTRVSDFRTA----NCSLE-------------HHHHHH-                   298
      PET12    APHQADLRSSRLSEYRESCPY---------------------                   298
IIa   PET5     PDHAANR---SVSEYRDTCNY---------------------                   310
      PE-H     PNHTSDS---QISDYRGNCPYL-------------EHHHHHH                   312
```

Figure S1 Multiple sequence alignment of PE-H with amino acid sequences of different cutinases and PET hydrolases using the program Clustal Omega. The enzymes were assigned to different types of polyester hydrolases. Amino acid residues of the catalytic triad are marked by a red triangle, disulfide forming cysteine residues are highlighted in orange and connected by an orange line. Amino acids of the extended loop region, specific for type II PET hydrolases, are framed in red. Abbreviations are: leaf-branch compost metagenome cutinase (LCC); *Saccharomonospora viridis* cutinase (Cut190); *Thermobifida fusca* cutinase (TfCut2); *Ideonella sakaiensis* PET hydrolase (PETase); *Polyangium brachysporum* PET hydrolase (PET12); *Oleispira antarctica* PET hydrolase (PET5).

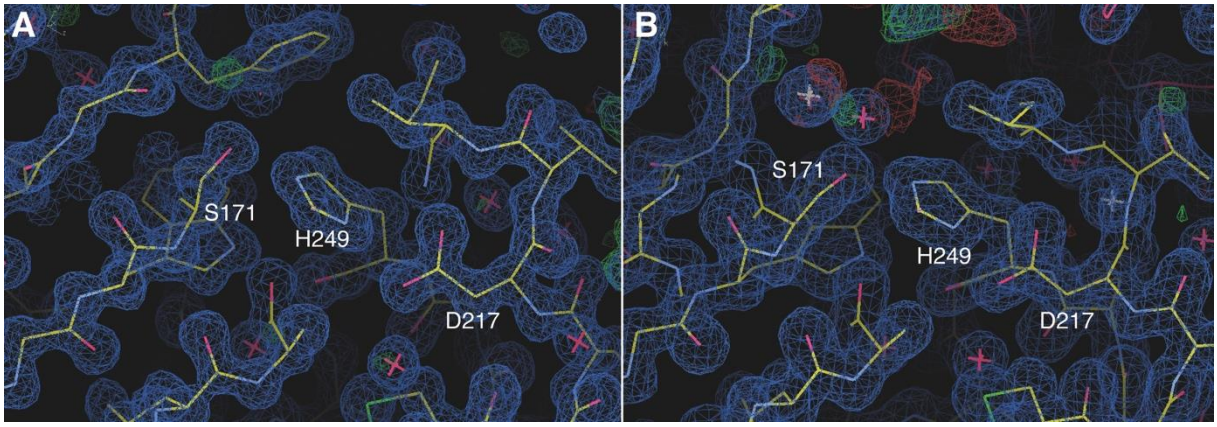

Figure S2 Electron densities around the active site of **(A)** PE-H WT (PDB code 6SBN) and **(B)** PE-H Y250S (PDB code 6SCD) with residues of the catalytic triad highlighted with labels. 2FoFc contoured at 1 σ.

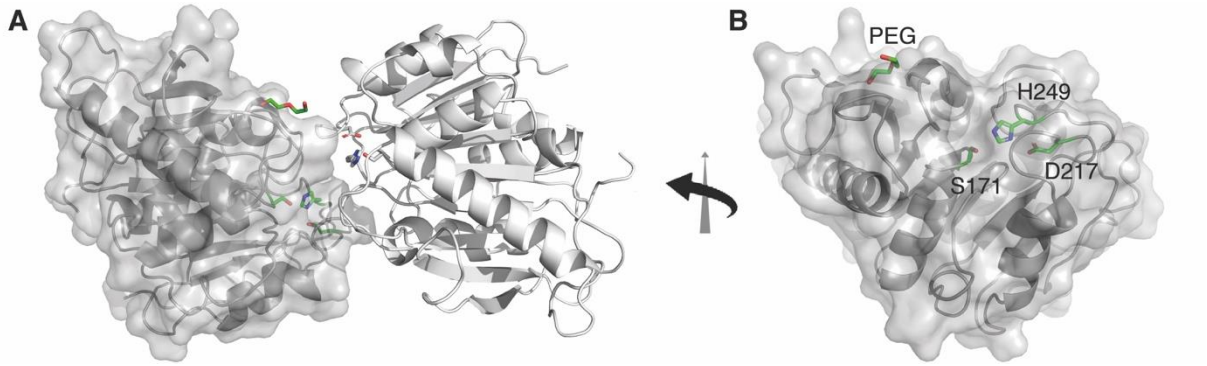

Figure S3 (**A**) Asymmetric unit containing two molecules of PE-H Y250S (PDB code 6SCD) depicted as cartoon showing the monomer with bound PEG molecule also in surface representation. In (**B**) the left molecule is rotated counter-clockwise with the residues of the catalytic triad as well as the PEG molecule shown as green sticks.

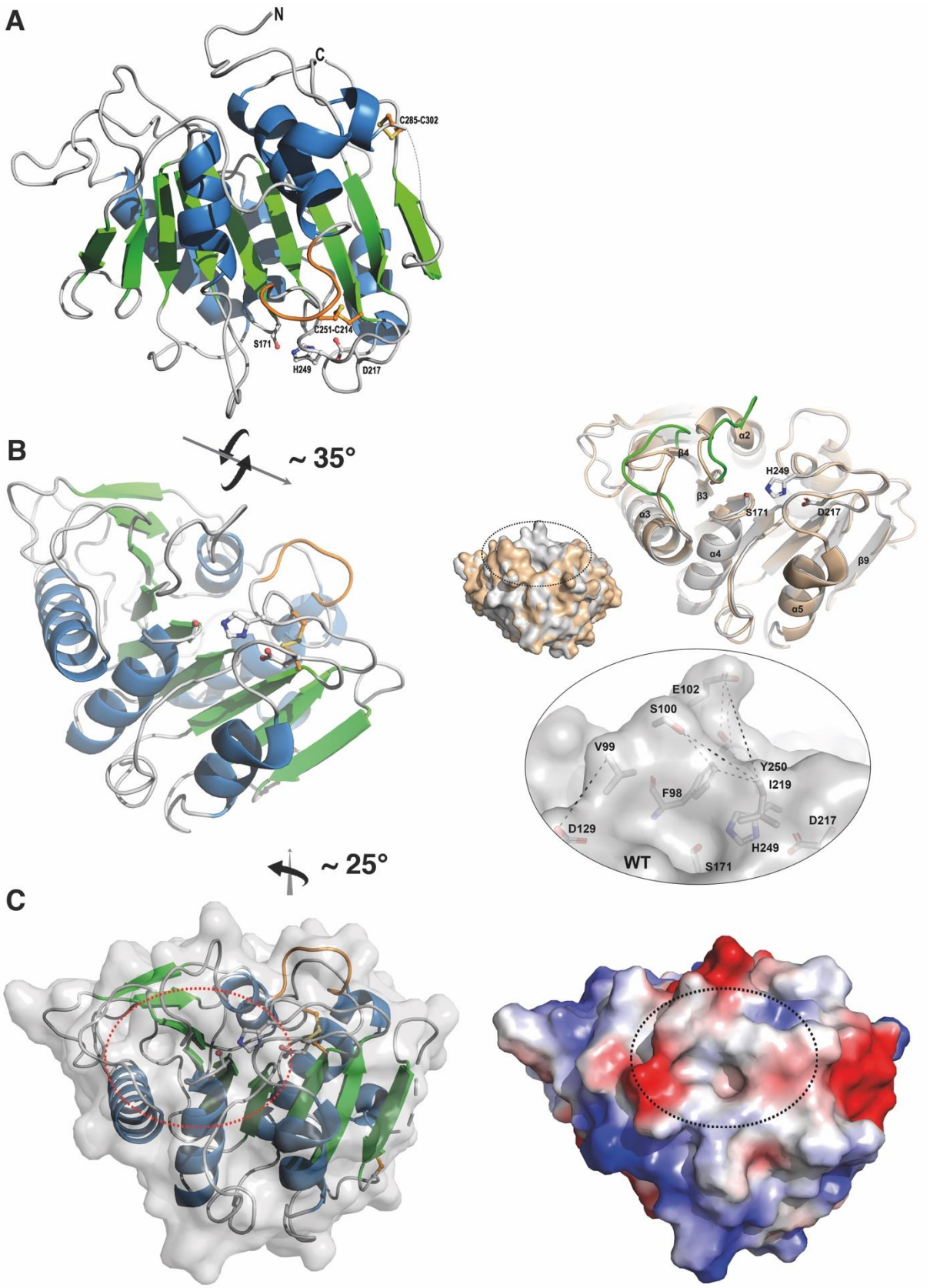
A
N
C
C285-C302
C251-C214
S171
H249
D217
B
~ 35°
α2
β4
H249
β3
S171
D217
α3
α4
β9
α5
E102
S100
V99
Y250
I219
F98
D129
D217
H249
WT
S171
~ 25°
C

Figure S4 (**A**) Orientation of PE-H as reference to Figure 4. (**B**) PE-H is rotated for around 35° from bottom to top to zoom in the active site (as in Figure 5). In (**C**) PE-H is additionally rotated counter-clockwise for around 25° for a better view on the active site cleft (as in Figure S5).

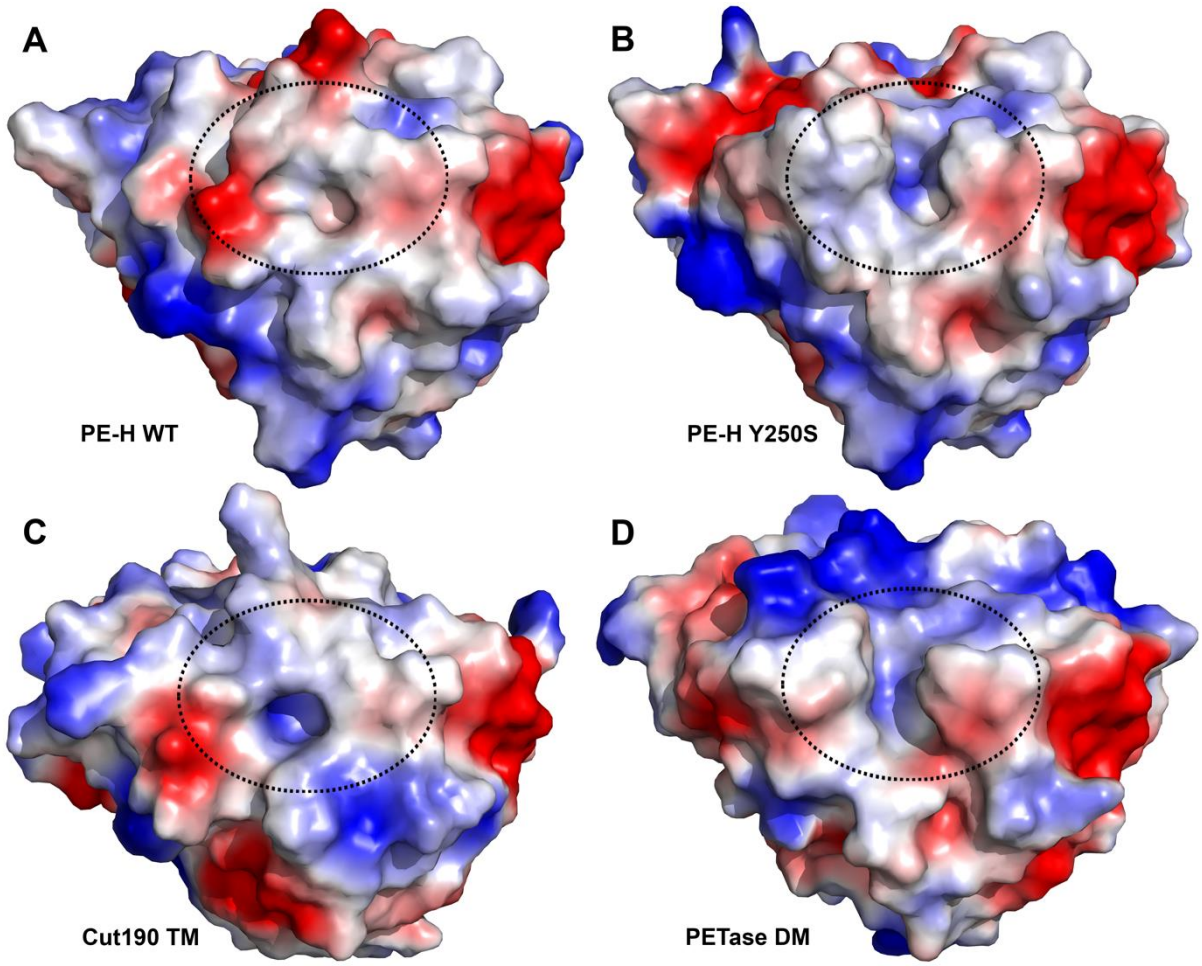

Figure S5 Surface representation of PE-H variants and two structural homologues. **(A)** shows wild type PE-H (PDB code 6SBN), **(B)** PE-H Y250S (PDB code 6SCD), **(C)** Cut190 triple mutant (TM) from *S. viridis* (PDB code 5ZRR) and **(D)** PETase double mutant (DM) from *I. sakaiensis* (PDB code 5XH3). Color code of the electrostatic surface is blue for positive, red for negative charge. All molecules are shown in identical orientation with the active site cleft positioned in the middle and marked by a dashed line.

Table S1 Amino acid composition of PETase (6EQE) and PE-H (6SBN) at selected positions as marked in the main text (i to iii).

	active site			(i)	(ii)	(iii)					
PETase	S160	D206	H237	S238	W159	S242	G243	N244	S245	N246	Q247
PE-H	S171	D217	H249	Y250	W170	G254	G255	S256	I257	Y258	N259

Table S2 Thermal melting points (Tm) of PE-H (WT) and different variants determined by nano differential scanning fluorimetry. The top row shows the respective single amino acid substitutions. Mutations G254S, S256N, I257S, Y258N, and N259Q were combined (ext.) to resemble the amino acid combination of the extended loop region of PETase.

	WT	G254S	S256N	I257S	Y258N	N259Q	ext.	Y250S
Tm [°C]	50.8	39.7	49.5	48.5	43.8	45.2	39.4	49.8

Table S3 Data collection and refinement statistics of PE-H

	PE-H	**PE-H Y250S**
Beamline	ID29, ESRF, Grenoble	P13, DESY, Hamburg
Crystal parameters		
Space group	C 2 2 21	I 21 21 21
Unit cell parameters: a, b, c (Å) α, β, γ (°)	 68.81, 80.01, 88.91 90, 90, 90	 94.53, 98.27, 121.23 90, 90, 90
Data collection and processing		
Wavelength	0.9762	0.9505
Resolution (Å)	50.00 – 1.09 (1.15 – 1.09)	77.34 – 1.35 (1.42-1.35)
Total reflections	1293506 (198125)	494296 (78069)
Unique reflections	101774 (16134)	122029 (19634)
Multiplicity	12.7 (12.3)	4.1 (4.0)
Completeness (%)	99.5 (98.4)	99.11 (99.26)
I/σ(I)	16.20 (4.70)	14.98 (2.52)
Wilson B-factor ($Å^2$)	10.4	16.7
R-merge	0.076 (0.429)	0.043 (0.439)
R-meas	0.080 (0.448)	0.050 (0.505)
CC 1/2	0.999 (0.970)	0.998 (0.849)
Refinement statistics		
Reflections used in refinement	99673	116011
Reflections used for R-free	2057	5715
R work (%)	10.69	11.3
R free (%)	13.70	15.2
Number of non-hydrogen atoms macromolecules ligands solvent	2401 2028 5 368	4590 4048 49 493
RMS (bonds) RMS (angles)	0.027 2.206	0.026 2.430
Ramachandran plot: favoured (%) Allowed (%) outliers (%)	 96.5 2.7 0.8	 97.3 2.5 0.2
Average B-factor ($Å^2$)	14.0	22.0
Model content		
Monomers ASU	1	2
Protein residues	263 (38-285; 292-306)	532 (40-305)
Ligand	1 ACT, 1 NA	1 SO4, 3 PO4, 1 ACT, 1 PEG, 2 GOL, 3 CL, 3 NA
Waters	368	495
PDB code	6SBN	6SCD

(Statistics for the highest resolution shell are shown in parentheses)

Table S4: Structure based alignment of wild type PE-H and PE-H Y250S against pdb entries. Top 10 of the most similar structures are given with their corresponding alignment quality (Q-score), root mean square deviation (RMSD), number of aligned residues (N_{algn}), their sequence identity (Seq-%), their PDB number (Target PDB), and description of the PDB entry (Protein).

Wild type PE-H (pdb-code: 6SBN)						
##	**Q-score**	**RMSD**	**N_{algn}**	**Seq-%**	**Target PDB**	**Protein**
1	0.76	1.18	246	51	5zrr:A	**cutinase** Cut190 S176A/S226P/R228S mutant (*Saccharomonospora viridis*)
2	0.75	1.20	245	51	5xg0:B	*1* **PET hydrolase** *(Ideonella sakaiensis)*
3	0.75	1.18	244	51	6ane:A	*2* **PET hydrolase** *(Ideonella sakaiensis)*
4	0.75	1.24	245	51	4cg2:A	***3*** **PET hydrolase** *(Thermobifida fusca)*
5	0.75	1.25	245	51	4cg1:A	***4*** **PET hydrolase** *(Thermobifida fusca)*
6	0.74	1.22	240	53	5luj:A	***5*** **cutinase 2** *(Thermobifida cellulosilytica)*
7	0.74	1.25	244	52	5lul:A	*6* **cutinase 2** R19S/R29N/A30V mutant *(Thermobifida cellulosilytica)*
8	0.74	1.32	246	52	5luk:A	*7* **cutinase 2** R29N/A30V mutant *(Thermobifida cellulosilytica)*
9	0.73	1.28	245	51	5lui:A	**cutinase 1** (*Thermobifida cellulosilytica*)
10	0.73	1.25	242	52	5zoa:A	***8*** **cutinase** *(Thermobifida fusca)*
PE-H Y_{250}S chain A (pdb-code 6SCD)						
1	0.84	1.17	259	51	5xh3:A	**9** **PET hydrolase** R103G/S131A mutant (HEMT complex) *(Ideonella sakaiensis)*
2	0.84	1.17	259	51	5xfz:A	**10** **PET hydrolase** R103G/S131A mutant *(Ideonella sakaiensis)*
3	0.83	1.11	257	51	6qgc:A	**11** **PETase** *(Ideonella sakaiensis)*
4	0.83	1.14	257	51	5xfy:A	**12** **PET hydrolase** S131A mutant *(Ideonella sakaiensis)*
5	0.83	1.16	257	51	5xh2:A	**13** **PET hydrolase** R103G/S131A mutant (pNP complex) *(Ideonella sakaiensis)*
6	0.83	1.10	256	52	6eqg:C	**14** **PET hydrolase** *(Ideonella sakaiensis)*
7	0.83	1.08	253	52	4wfk:A	**15** **cutinase** Cut190 S226P mutant (Ca(2+)-bound state) *(Saccharomonospora viridis)*
8	0.83	1.08	253	52	4wfj:A	**16** **cutinase** Cut190 S226P mutant (Ca(2+)-bound state) *(Saccharomonospora viridis)*
9	0.83	1.08	255	52	6eqg:B	**17** **PET hydrolase** *(Ideonella sakaiensis)*
10	0.83	1.07	255	52	6eqh:A	**18** **PET hydrolase** *(Ideonella sakaiensis)*