

Supplementary data

Table S1. Catalytic activity for the WT and mutants using ABTS as substrate.

CotA-laccase	Catalytic activity (U/L)
WT	55.12
E186A	171.38
E186R	210.74
E186K	22.41
F205Y	7.33
R208G	196.38
K315N	111.96
G322S	15.90
A376I	101.72
L385W	329.62
I417G	30.80
P454S	9.74
D500G	62.56

Table S2. Primers used in this study.

Gene	Primer	Sequence (5' to 3') ¹
<i>E186A</i>	<i>E186A-F</i>	TTGAACTTGCCGAAGGGAG <u>C</u> ATATGAAATC
	<i>E186A-R</i>	<u>G</u> CTCCCTTCGGCAAGTTCAATGAACGCTCT
<i>E186R</i>	<i>E186R-F</i>	TTGAACTTGCCGAAGGGAC <u>G</u> ATATGAAATC
	<i>E186R-R</i>	<u>C</u> GTCCTTCGGCAAGTTCAATGAACGCTCT
<i>E186K</i>	<i>E186K-F</i>	ATTGAACTTGCCGAAGGGAA <u>A</u> ATATGAAAT
	<i>E186K-R</i>	<u>T</u> TCCCTTCGGCAAGTTCAATGAACGCTCTT
<i>F205Y</i>	<i>F205Y-F</i>	CATGAAGATGGTTCATTG <u>T</u> ATTATCCGCGG
	<i>F205Y-R</i>	<u>T</u> ACAATGAACCATCTTCATGAAATGATTTA
<i>R208G</i>	<i>R208G-F</i>	TGGTTCATTGTTTTATCCG <u>G</u> GGCAGCCTGA
	<i>R208G-R</i>	<u>C</u> CGGATAAAACAATGAACCATCTTCATGAA
<i>K315N</i>	<i>K315N-F</i>	GAAAAACCGTGACACTGAA <u>C</u> AACCGGATCG
	<i>K315N-R</i>	<u>G</u> TTCACTGTCACGGTTTTTCTTCTGCTCG
<i>G322S</i>	<i>G322S-F</i>	AACCGGATCGGCTGCGG <u>C</u> TACAAGACGCA
	<i>G322S-R</i>	<u>G</u> AGCCGCAGCCGATCCGGTTTTTCAGTGTC
<i>A376I</i>	<i>A376I-F</i>	AATCTGTCATTGGGCGCGA <u>T</u> CGTTGACCAA
	<i>A376I-R</i>	<u>A</u> TCGCGCCCAATGACAGATTCTGAGGGCA
<i>L385W</i>	<i>L385W-F</i>	CAATATGGAAGACCTGTT <u>T</u> GGCTTTTAAAC
	<i>L385W-R</i>	<u>C</u> AAACAGGTCTTCCATATTGGTCAACGGCC
<i>I417G</i>	<i>I417G-F</i>	ATCAATGCCGGAAGGGCG <u>G</u> CCATCCGATC
	<i>I417G-R</i>	<u>C</u> CCGCCCTTCCGGCATTGATGATCGACCAG
<i>P454S</i>	<i>P454S-F</i>	TTTTACCGGTCCGGCAGTT <u>T</u> CTCCGGCACC
	<i>P454S-R</i>	<u>A</u> AACTGCCGGACCGGTAAAACAAGTTCTC
<i>D500G</i>	<i>D500G-F</i>	CTTGAGCACGAAGATTACG <u>G</u> TATGATGCGC
	<i>D500G-R</i>	<u>C</u> CGTAATCTTCGTGCTCAAGGATGTGGCAG

¹ The underlined sequences indicate mutant sites and cloning sites.

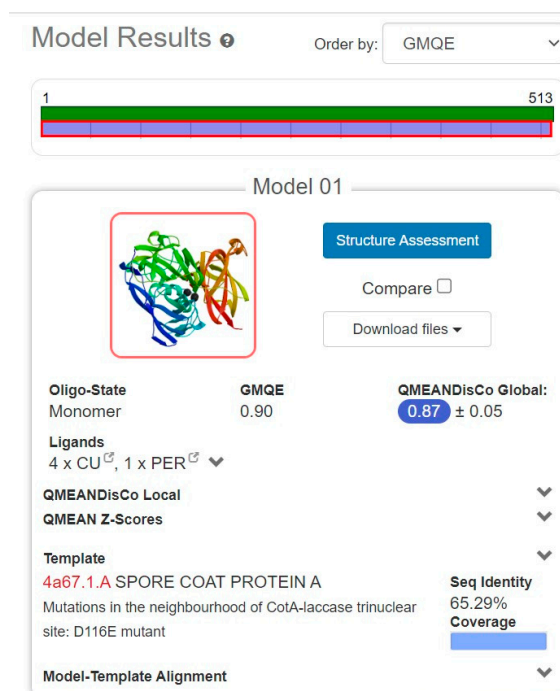


Figure S1. Homologous modeling of CotA-laccase from *Bacillus licheniformis* ANSB821.

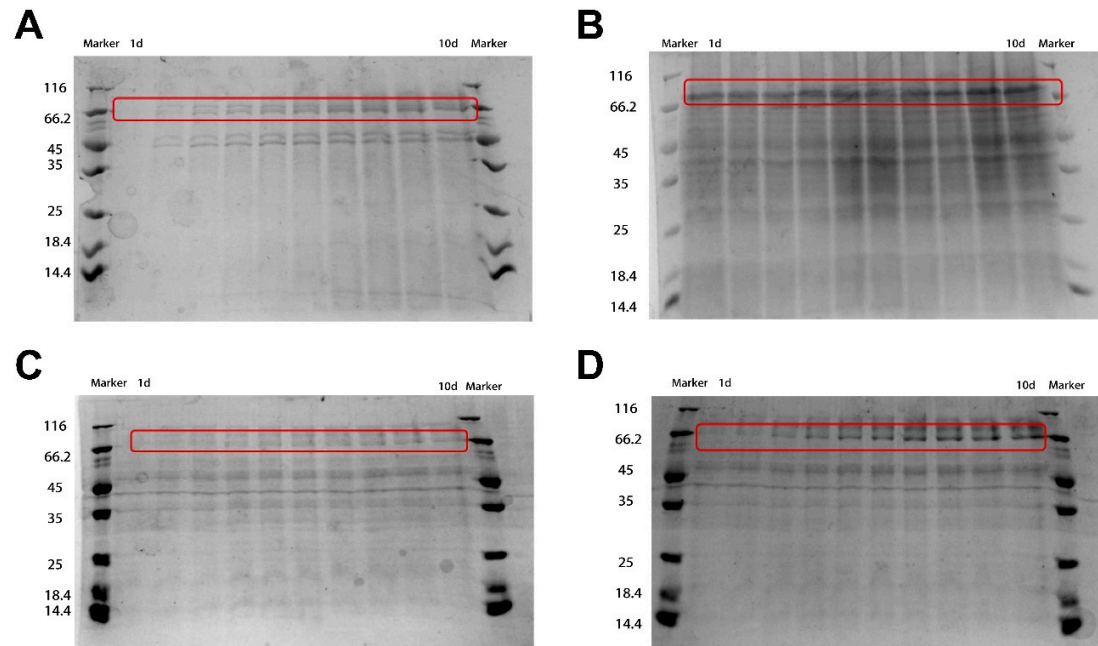


Figure S2. SDS-PAGE of the fermentation supernatant of GS115-pPICZαA-WT (A), GS115-pPICZαA-N-WT (B), GS115-pPICZαA-N-E186A (C), and GS115-pPICZαA-N-E186R (D) cultured for 1-10 d.