

Article

Improved Bioproduction of the Nylon 12 Monomer by Combining the Directed Evolution of P450 and Enhancing Heme Synthesis

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Contents:

Supporting Tables.....	S1
Table S1. Primers used for PCR and cloning.....	S2
Supporting Figures.....	S5
Figure S1. Replacement of the NADPH regeneration system.....	S5
Figure S2. The homologous modeling results of P450 reduction domain evaluated by MolProbity	S5
Figure S3. The homologous modeling results of P450 reduction domain evaluated by SAVES.....	S6
Figure S4. Gas chromatograms for C _{10:0} -C _{16:0} and their ω -hydroxylation products	S7
Figure S5. Electron ionization mass spectral fragmentations of trimethylsiloxyl (TMS) derivatives of C _{10:0} -C _{16:0} and their ω -hydroxylation products	S8
Figure S6. Assay of active P450	S9
Figure S7. Biotransformation of DDA to ω -AmDDA by P1-1 (E-M1-3/C-M2-2).....	S9
References.....	S10

Supporting Tables

Table S1. Primers used for PCR and cloning

Primer name	Primer sequence (5'-3') ^a	Description
CYPCPR-Gibson-F	TAATTTTGTTTAACTTTAACAGGAGGAACGGCATGC	To construct P450 mutants
	CGACTTTACCGCGTA	
CYPCPR-Gibson-R	TCCAGATCGCTGTAACCCATTATATATCTCCTTAGA	
	ATTCTTACCCAGCCACAC	
Petduet-Gibson-F	ATGGGTACAGCGATCTGGA	
Petduet-Gibson-R	AAAGTTAAACAAAATTATTTCTAGAGGGGAATTG	To construct His-Tagged P450
TP450-BsaI-GCCA-F	GGCTACGGTCTCAGCCATATGCCGACTTTACCGCGT	
	AC	
TP450-BsaI-TACC-R	GGCTACGGTCTCACCATGCCGTTCTCCTGTAAAG	
	T	
NHis-BsaI-F	GGCTACGGTCTCCATGGGCAGCAGCCATCATCATC	
	ATCATCACAGCAGCGGCTGGTGCCGCGCGGCAGC	
	CATGAGACCGTAGCC	
NHis-BsaI-R	GGCTACGGTCTCATGGCTGCCGCGCGGCACCAGGC	
	CGCTGCTGTGATGATGATGATGATGGCTGCTGCCA	
	TGGAGACCGTAGCC	
TP450-BsaI-ACTG-F	GGCTACGGTCTCCACTGAGAATTCTAAGGAGATAT	
	ATAATGGGT	
TP450-BsaI-AGGC-R	GGCTACGGTCTCACGGACCCAGCCCACACGTCTTTT	
	G	
CHis-BsaI-F	GGCTACGGTCTCCTCCGTCGACAAGCTTGCGGCCGC	
	ACTCGAGCACCACCACCACCACCACTGAGAGACCG	
	TAGCC	
CHis-BsaI-R	GGCTACGGTCTCTCAGTGGTGGTGGTGGTGGTCTC	
	GAGTGCGGCCGCAAGCTTGTGACGGAGGAGACCG	
	TAGCC	
hemB-1-F	CTTTAATAAGGAGATATACCATGACAGACTTAATCC	To construct plasmid pAC-hemBCDE
	AACGCCC	
hemB-1-R	TCTGCAGGCGCGCCGAGCTCTTAACGCAGAATCTTC	To construct plasmid pAC-hemB
	TTCTCAGCC	
hemB-2-F	GTATAAGAAGGAGATATACATATGACAGACTTAAT	
	CCAACGCCC	
hemB-2-R	GTTTCTTTACCAGACTCGAGTTAACGCAGAATCTTC	
	TTCTCAGCC	To construct plasmid pRS-hemB
hemB-3-R	ACCTGCAGGCGCGCCGAGCTCTTAACGCAGAATCT	
	TCTTCTCAGCC	To construct plasmid pAC-hemBCDE
hemC-1-F	AGAGCTCGGCGCGCCTGCAGATGTTAGACAATGTTT	
	TAAGAATTGCCACACG	
hemC-1-R	ACTCATATATTTTCTCCTTTTCATGCCGGGGCGTCTC	To construct plasmid pRS-hemBCD
	CGTTATA	
hemC-2-F	TATAAGAAGGAGATATACATATGTTAGACAATGTTT	
	TAAGAATTGCC	
hemC-2-R	TTTCTCCTTTTATAGATCTTCATGCCGGGGCGTCTC	
	CGTTA	

hemD-1-F	GCATGAAAGGAGGAGGAAAATATATGAGTATCCTTGTC ACCCGCC	To construct plasmid pAC- hemBCDE
hemD-1-R	CATTATGCGGCCGCAAGCTTTTATTGTAATGCCCGT AAAAGCGC	
hemD-2-F	AGATCTATAAAAAGGAGGAGGAAAATATATGAGTATCCT TGTCACCCGCC	To construct plasmid pRS- hemBCD
hemD-2-R	GTTTCTTTACCAGACTCGAGTTATTGTAATGCCCGTA AAAGCGC	
hemE-1-F	TATAAGAAGGAGATATACATATGACCGAACTTAAA AACGATCGTTATCT	To construct plasmid pAC- <i>hemE</i>
hemE-1-R	GTTTCTTTACCAGACTCGAGTTAGCGGTGATACTGT TCAGACAGT	
hemE-2-F	CTTTAATAAGGAGATATACCATGACCGAACTTAAA AACGATCGTTATCT	To construct plasmid pAC- hemEFGH
hemE-2-R	GAGCTCTTAGCGGTGATACTGTTTCAGACAGT	
hemF-1-F	CTTTAATAAGGAGATATACCATGAAACCCGACGCA CACCA	To construct plasmid pRS- <i>hemF</i>
hemF-1-R	CATTATGCGGCCGCAAGCTTTTACACCCAATCCCTG ACCTT	
hemF-2-F	AGTATCACCGCTAAGAGCTCATAAAAGGAGGAAA ATATATGAAACCCGACGCACACCA	To construct plasmid pAC- hemEFGH
hemF-2-R	TTTTATCTGCAGTTACACCCAATCCCTGACCTT	
hemG-1-F	TATAAGAAGGAGATATACATATGAAAACATTAATT CTTTTCTCAACAAGGG	To construct plasmid pRS- hemFGH
hemG-1-R	ACGCATATATTTTCCTCCTTTTATTTTCAGCGTCGGTT TGTCG	
hemG-2-F	ATTGGGTGTAAGTGCAGATAAAAGGAGGAAAATAT ATGAAAACATTAATTCTTTTCTC	To construct plasmid pAC- hemEFGH
hemG-2-R	CTTAAGCATTATGCGGCCGCTTATTTTCAGCGTCGGT TTGTCG	
hemH-1-F	AAATAAAAGGAGGAGGAAAATATATGCGTCAGACTAA AACCGGTATC	To construct plasmid pRS- hemFGH
hemH-1-R	GTTTCTTTACCAGACTCGAGTTAGCGATACGCGGCA ACAA	
hemH-2-F	TATAAGAAGGAGATATACATATGCGTCAGACTAAA ACCGGTATC	To construct plasmid pAC- <i>hemH</i>
pACYC-1-F	AAGCTTGCGGCCGCATAATG	
pACYC-1-R	GGTATATCTCCTTATTAAAGTTAAACAAAATTATTT CTACAGGG	To construct plasmids pAC- <i>hemE</i> and pRS- <i>hemF</i>
pACYC-2-F	CTCGAGTCTGGTAAAGAAACCGC	
pACYC-2-R	ATGTATATCTCCTTCTTATACTTAACTAATATACTAA GATGGGG	To construct plasmids pAC- <i>hemB</i> , pAC- <i>hemE</i> , pAC- <i>hemH</i> pRS- hemBCD and pRS- hemFGH
pACYC-3-F	GAGCTCGGCGCGCCTGCAGG	
pACYC-3-R	GGTATATCTCCTTATTAAAGT	To construct plasmids pRS- <i>hemB</i> and pRS- <i>hemF</i>

pACYC-4-F	GCGGCCGCATAATGCTTAAG	To construct plasmid pAC-hemEFGH
gdhA-EcoRI-RBS-F	NNNN <u>GAATTCT</u> AAGGAGATATATAATGGATCAGAC ATATTCTCTGGAG	To construct plasmid pETDuet-1- <i>rbs3-cyp153a-ncp^{G307A}-gdhA</i> (E-M1-3- <i>gdhA</i>)
gdhA-KpnI-R	NNNN <u>GGTACCTT</u> AAATCACACCCTGCGCCA	To construct plasmid pETDuet-1- <i>rbs3-cyp153a-ncp^{G307A}-icd</i> (E-M1-3- <i>icd</i>)
icd-EcoRI-RBS-F	NNNN <u>GAATTCT</u> AAGGAGATATATAATGGAAAGTA AAGTAGTTGTTCCGG	To construct plasmid pETDuet-1- <i>rbs3-cyp153a-ncp^{G307A}-fdh1</i> (E-M1-3- <i>fdh1</i>)
icd-KpnI-R	NNNN <u>GGTACCTT</u> ACATGTTTTCGATGATCGCG	To construct plasmid pCD- <i>pntAB</i>
fdh-EcoRI-RBS-F	NNNN <u>GAATTCT</u> AAGGAGATATATAATGGCCACCGT GCTGTGTG	To construct plasmid pCD- <i>sthA</i>
fdh-KpnI-R	NNNN <u>GGTACCTT</u> AGGTAAAGCGATAGCTCTGGG	
pntAB-NcoI-F	NNNN <u>CCATGGG</u> CCGAATTGGCATAACCAAGAGAAC G	
pntAB-SacI-R	NNNN <u>GAGCTCTT</u> ACAGAGCTTTCAGGATTGCAT	
sthA-NcoI-F	NNNN <u>CCATGGG</u> CCCACATTCTACGATTACGATGC CA	
sthA-SacI-R	NNNN <u>GAGCTCTT</u> AAAACAGGCGGTTTAAACCGTTT AACG	
fdh2-BglII-F	NNNN <u>AGATCTAT</u> GGCCAAAGTGCTGTGTGT	To construct plasmids pCD- <i>pntAB-fdh2</i> and pCD- <i>sthA-fdh2</i>
fdh2-BamHI-R	NNNN <u>GGATCC</u> CTTACACGGCTTTTTTAAATTTGGCTG	To construct plasmid C- <i>alkJ</i>
pCDFDuet-BamHI-F	NNNN <u>GGATCC</u> CTCGAGTCTGGTAAAGAAAC	
pCDFDuet-BglII-R	NNNN <u>AGATCT</u> GCATGTATATCTCCTTCTTA	
alkJ-F	ATGTACGACTATATAATCGTTGGTGCTGGA	
alkJ-R	TTACATGCAGACAGCTATCATGGC	
C-M2-2-F	TGATAGCTGTCTGCATGTAATCTGGTAAAGAAACCG CTGC	
C-M2-2-R	ACGATTATATAGTCGTACATCGGATCCTGGCTGTGG TGAT	
rbs2-F	GGCTAC <u>GGTCTCATTC</u> CTCAATAGCCTTGACTAAGG AGGTAAC <u>ATGGGGAGACCG</u> TAGCC	To construct plasmid C- <i>rbs2-alkJ</i>
rbs2-R	GGCTAC <u>GGTCTCC</u> CCATAGTTACCTCCTTAGTCAAG GCTATTGAGGAATGAGACCGTAGCC	
C-alkJ-BsaI-ATGG-F	GGCTAC <u>GGTCTCC</u> ATGGGCAGCAGCCATCACCATC ATCACCACAGCCAGGATCCGATGTACGACTA	
C-alkJ-BsaI-TTCC-R	GGCTAC <u>GGTCTCGG</u> GAATTGTTATCCGCTCACAATT CCCCTATAGTGAGTCGTATTAATTTCA	

^a Underline formatting indicates restriction sites.

Supporting Figures

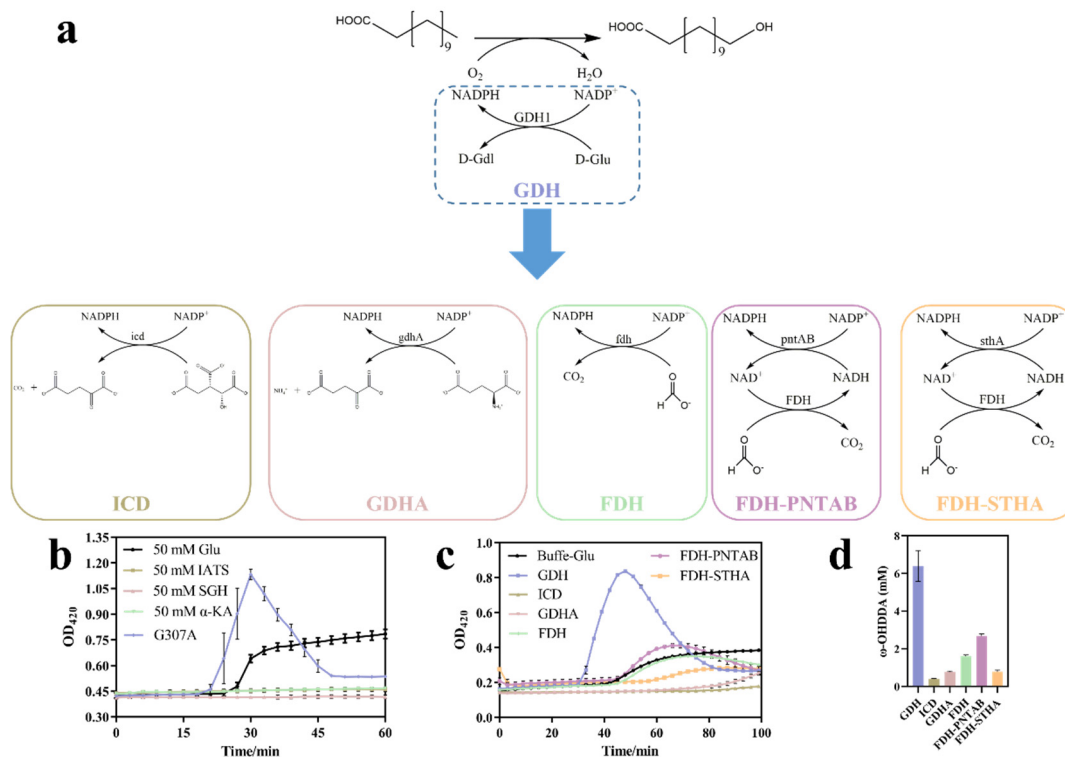


Figure S1. Replacement of the NADPH regeneration system. (a) Schematic of the NADPH regeneration systems tested. From left to right are ICD [1], GDHA [2], FDH [3], FDH-PNTAB [4,5] and FDH-STHA [4,5] systems. (b) The substrates and by-products (Glu, IATS, SGH and α -KA) required by NADPH regeneration system were detected by ABTS colorimetry. IATS, isocitric acid trisodium salt (substrate of ICD); SGH, sodium glutamate hydrate (substrate of GDHA); α -KA, 2-ketoglutaric acid (product of ICD); G307A, reaction solution after whole-cell biotransformation of M1. (c) ω -OHDDA production using different NADPH regeneration systems as detected by ABTS colorimetry. (d) ω -OHDDA production using different NADPH regeneration systems as detected by HPLC.

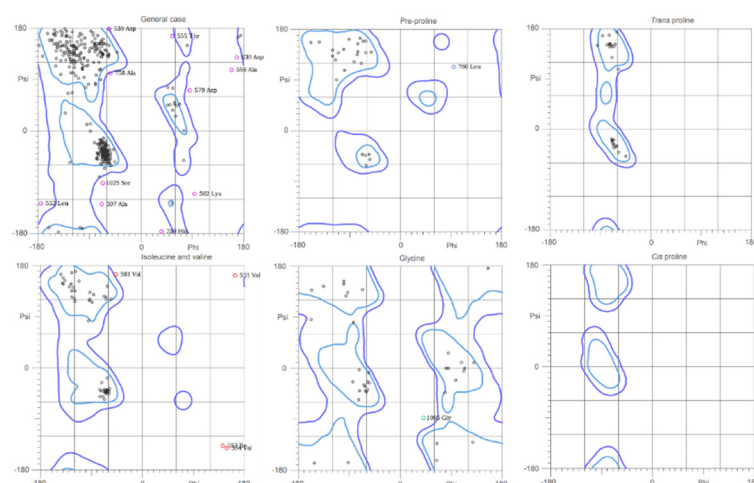


Figure S2. The homologous modeling results of P450 reduction domain evaluated by MolProbity [6].

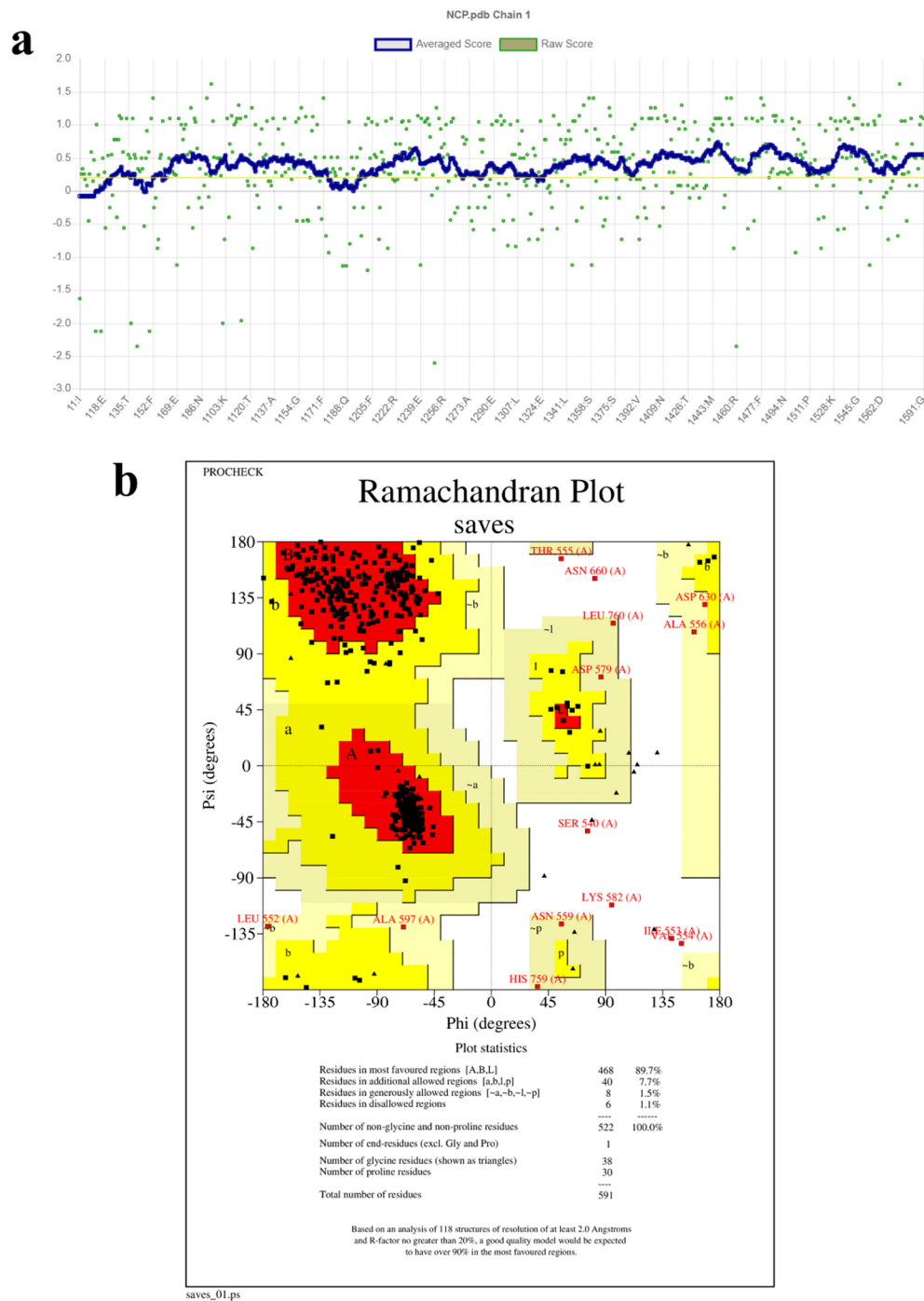


Figure S3. The homologous modeling results of P450 reduction domain evaluated by SAVES [7]. (a) VERIFY3D module evaluation results, which are used to test the three-dimensional structure compatibility of the protein model and at least 80% of the amino acids need to have scored ≥ 0.2 in the 3D/1D profile. (b) Ramachandran plot evaluation results.

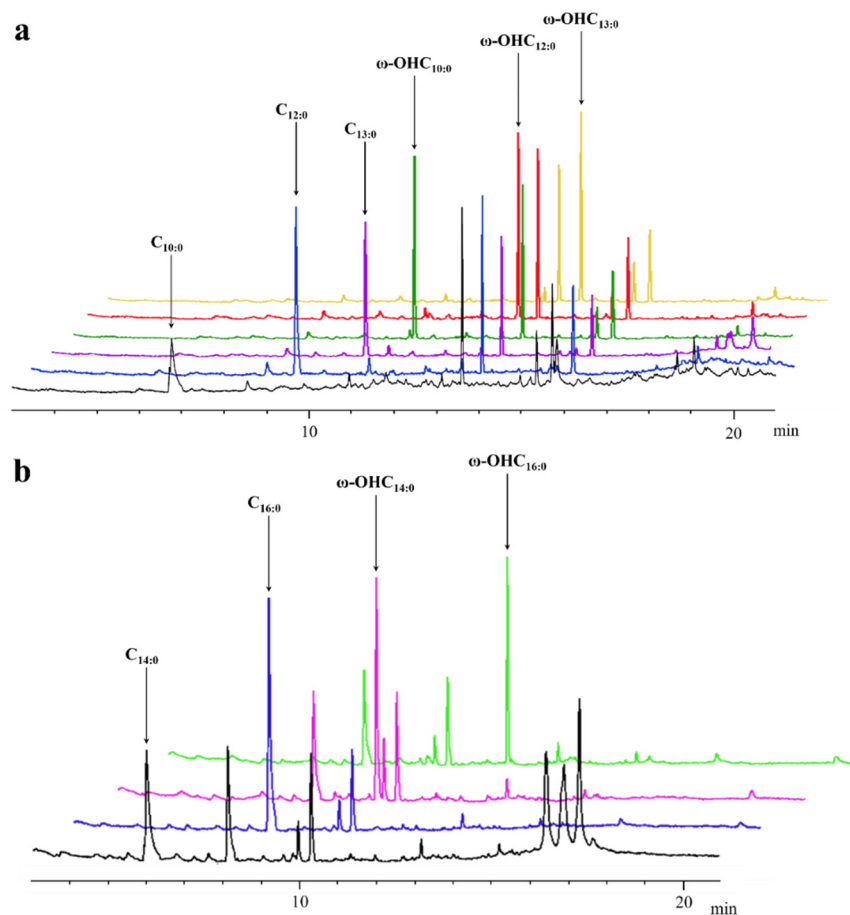


Figure S4. Gas chromatograms for $C_{10:0}$ - $C_{16:0}$ and their ω -hydroxylation products. Abbreviations: $C_{10:0}$, decanoic acid; $C_{12:0}$, lauric acid; $C_{13:0}$, tridecanoic acid; $C_{14:0}$, myristic acid; $C_{16:0}$, palmitic acid; ω -OHC $_{10:0}$, 10-hydroxydecanoic acid; ω -OHC $_{12:0}$, 12-hydroxylauric acid; ω -OHC $_{13:0}$, 13-hydroxytridecanoic acid; ω -OHC $_{14:0}$, 14-hydroxymyristic acid; ω -OHC $_{16:0}$, 16-hydroxypalmitic acid.

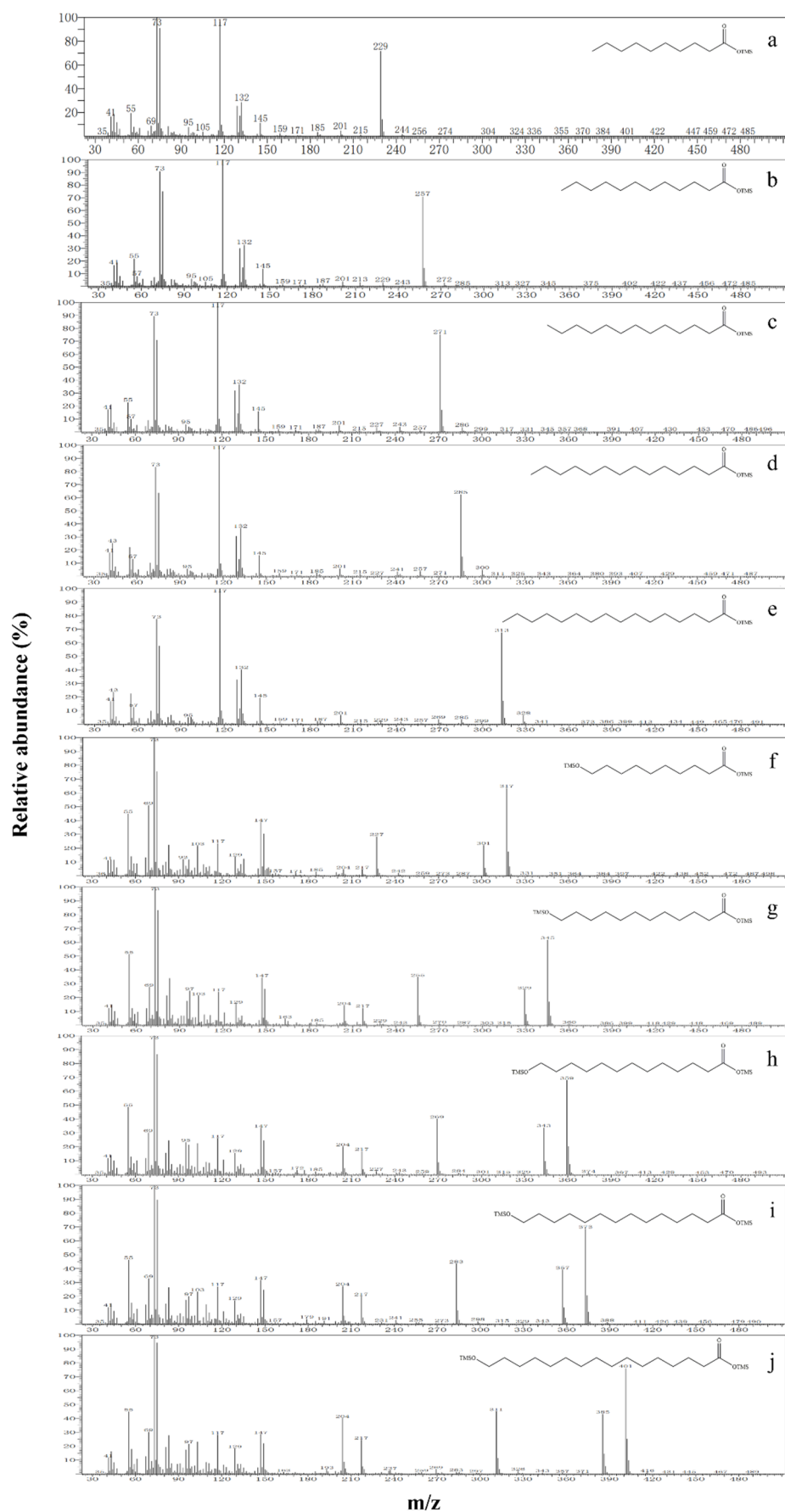


Figure S5. Electron ionization mass spectral fragmentations of trimethylsiloxy (TMS) derivatives of C_{10:0}-C_{16:0} and their ω -hydroxylation products. The compounds were identified as decanoic acid (a), lauric acid

(b), tridecanoic acid (c), myristic acid (d), palmitic acid (e), 10-hydroxydecanoic acid (f), 12-hydroxylauric acid (g), 13-hydroxytridecanoic acid (h), 14-hydroxymyristic acid (i), and 16-hydroxypalmitic acid (j). The common fragment ion at $m/z = 73$ occurs upon the loss of one TM ester group. Characteristic peaks of each fatty acids and ω -hydroxycarboxylic acid derivative are indicated in the corresponding mass spectra.

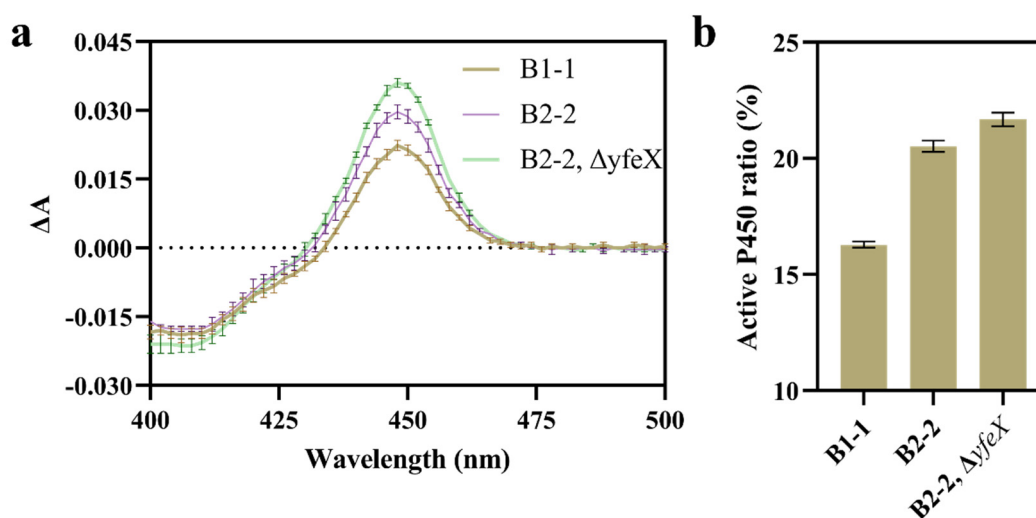


Figure S6. Assay of active P450. (a) CO spectra of P450. The absorbance at 490 nm was used as the "0" scale, i.e. $\Delta A = A_n - A_{490}$. (b) Content of active P450. The content of total P450 was determined by BCA kit [Sangon Biotech Ltd. (Songjiang District, Shanghai, China)].

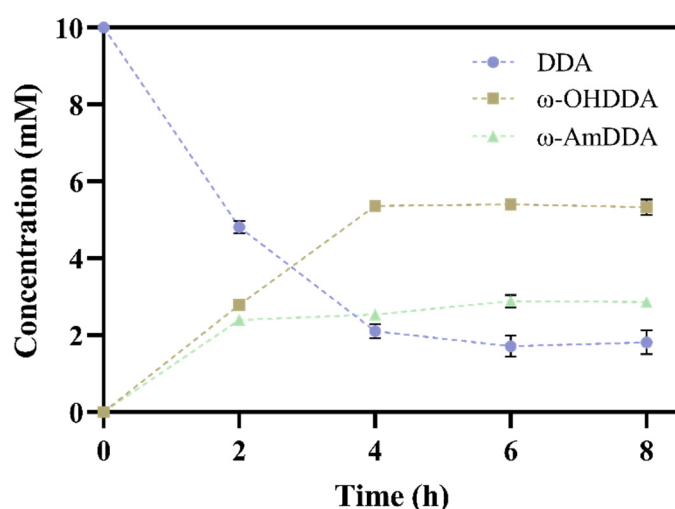


Figure S7. Biotransformation of DDA to ω -AmDDA by P1-1 (E-M1-3/C-M2-2) [8]. The reaction was performed with 50 g cww/L of resting cells in sodium phosphate buffer (100 mM, pH 8.0) containing 1% (w/v) D-glucose, $\text{NH}_3 \cdot \text{H}_2\text{O} / \text{NH}_4\text{Cl}$ (200 mM, $\text{NH}_3 \cdot \text{H}_2\text{O} : \text{NH}_4\text{Cl} = 1:10$) and 10.0 mM DDA (2% DMSO). Temperature was maintained at 30°C, agitation speed was maintained at 220 rpm, pH was maintained at 7.5-8.0 and the concentration of D-glucose was maintained at 0.5-1% (w/v) throughout the biotransformation process. All biotransformation reactions were performed in triplicate, and error bars represent standard deviations.

References

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