

A. acidocaldarius	MNQCIPDRRTFSSLLAIKEGYLFIKNRVDQY	32
B. subtilis	MNEQIPHDKSIDNSITLLKEGYLFIKNRTERY	32
OleTJE	MATLKRDKGIDNTLKVLLKQGYLTNTQNRRL	31
S. agnetis	MAKQLPKDPGLDNTFKVLKEAYTVVPCRLKLF	32
S. delphini	MAKKLPKDTGLDNTLKIINEAYTVVPCRLKLF	32
S. intermedius	MAKKLPKDTGLDNTFKIINEAYTVVPCRLKLF	32
S. pseudintermedius	MAKKLPKDTGLDNTLKMINEAYTVVPCRLKLF	32
Sm46Δ29	MAKKLPKVGLDNTVDIIKGGYTVVPGKLEEF	32
Sm46 extended	MEVDSILVLRLLNLLKTGIQLEMNGGIKVAKKLPKVGLDNTVDIIKGGYTVVPGKLEEF	60
A. acidocaldarius	QSDIFEARLLLENVVCMHCAEAAKLFYNTLEFQCGALPKRVQKTLFGENAIQTLLDSTA	91
B. subtilis	NSDIFQARLLGNFICMTCAEAAKVFYCTDRFQRNALPKRVQKSLFGVNAIQGMDGSA	91
OleTJE	NTSVFQTKALGGKPFVVVTGKEGAEMFYNNVDVQREGMLPKRIVNTLFGKCAIHTVDGKK	91
S. agnetis	NSKAFQTTGGMKPIAVISCKEAAELFYNNNDVMQREKTLPKRVVNTLFGKCAIHTTKGV	92
S. delphini	GTKAFETRALGMKPFVVVISCKAAAEIFYDNDKISRKGTLPKRIVHTLFGKCAIHTTEKV	92
S. intermedius	GTKAFETRALGMKPFVVVISCKAAAKIFYDNDKISRKGTLPKRIVHTLFGKCAIHTTEKV	92
S. pseudintermedius	GTKAFETRALGMKPIVVISCKAAAEIFYDNDKISRKGTLPKRIVHTLFGKCAIHTTEKV	92
Sm46Δ29	DSKAFEVRLGGKKIAVMSCKEAAEIFYDNEKMEROGTLPKRIVNTLFGKCAIHTTAGKK	92
Sm46 extended	DSKAFEVRLGGKKIAVMSCKEAAEIFYDNEKMEROGTLPKRIVNTLFGKCAIHTTAGKK	120
A. acidocaldarius	HLHRKQLFLSLITPDQEKSLATATTQWRECAKVVENARVVLEEBAKRMICRIACQWIG	151
B. subtilis	HLHRKVLFLSLMTPPHOKRLAEIMTEEWKAAVTRWEKADEVVLFBBAKEILCRVACYWAG	151
OleTJE	HVDRKALFMSLMTTEGNINLYRELTRITWHANTORMESMDEVNIYRESIVLITKVGTWAG	151
S. agnetis	HVDRKALFMSLMTTEENLKYLRELTRNWFMHTEHMQNQEKNYIKESIYVLITKIGFRWAG	152
S. delphini	HVDRKALFMSLMTTEKNLKYLRELTRNYWFMHTERMQNKDEVNVYQFAGLILITKVGFWRWAG	152
S. intermedius	HVDRKALFMSLMTTEKNLKYLRELTRNYWFMHTERMQNMDDEVNVYQFAGLILITKVGFWRWAG	152
S. pseudintermedius	HVDRKALFMSLMTTEENLKYLRELTRNYWFMHTERMQNKDEVNVYQFAGLILITKVGFWRWAG	152
Sm46Δ29	HVDRKALFMSLMTDENLNLYRELTRNYWFMHTERMQSMDEVNVYQFAGLILITKVGFWRWAG	152
Sm46 extended	HVDRKALFMSLMTDENLNLYRELTRNYWFMHTERMQSMDEVNVYQFAGLILITKVGFWRWAG	180
A. acidocaldarius	VPLDESEVSKRADIFGAMVDAFGAVGPRHWKG.....RRARARAFBWLRCMTDETRICLR	206
B. subtilis	VPLKETEVKERADDFIDVDAFGAVGPRHWKG.....RRARERAFBWEIEMVEDARAGLL	206
OleTJE	VQAPPEDIERIATDMIDMSFRALG.GAFKG.YKASKBARRRFVDFWLEECIETTRKENI	209
S. agnetis	IHQTEEAQNAKMDMDIDMSFSGGLG.QTIGGGRKAKKARARVBOFLEKQIAIVGRWAG	211
S. delphini	LKQTDQAAQNAEDMNTMIDSEFSGLG.QSLKG.YREAKKARARVBOFLQDQIEAVRAGQQ	210
S. intermedius	LKQTDQAAQNAEDMNTMIDSEFSGLG.QSLKG.YRQAKKARARVBOFLQDQIEAVRAGQQ	210
S. pseudintermedius	LKQTDQAAQNAEDMNTMIDSEFSGLG.QSLKG.YREAKKARARVBOFLQDQIEAVRAGQQ	210
Sm46Δ29	IIQTPEEAQNAKMDMTMINSFVSLG.SAYKG.YKKAKKARKFVDFLEKQIIDVRKCKL	210
Sm46 extended	IIQTPEEAQNAKMDMTMINSFVSLG.SAYKG.YKKAKKARKFVDFLEKQIIDVRKCKL	238
A. acidocaldarius	SVDEHTPLHVVAFWRDVNGNLLDAQMVAIBLNLRLPIVAISTFITFSALALHHPHTWRD	266
B. subtilis	KTTSGTATHEMAFHTQEDGSQLDSRMAAIBLNLVRLPIVAISYFLVFSALALHHPKYKE	266
OleTJE	HPPECTALYEEAHWEDYLGNPMDSRTOAIDLMTFRPLIAINRFVSGLIHAMNPNITRE	269
S. agnetis	NAEPCTALYEEAHWEDYKGNPMDARLOAIDLNVVRPLAAVNRVFSYAVKAMTEYDQERI	271
S. delphini	YAEPCATLYEEAHWKDLNDQPMDFHLCAVDLNNIVRPLVAVNRVFSYGVKALIEFDQERK	270
S. intermedius	YAEPCATLYEEAHWKDLNNQPMDSHLCAVDLNNIVRPLVAVNRVFSYGVKALIEFDQERK	270
S. pseudintermedius	YAEPCATLYEEAHWKDLNDQPMDFHLCAVDLNNIVRPLVAVNRVFSYGVKALIEFDQERK	270
Sm46Δ29	HPEECTALYEEAHWEDLNDNPMDSHLCAVDLNNVVRPLAAINRFISYGVKVLIEFDQEKE	270
Sm46 extended	HPEECTALYEEAHWEDLNDNPMDSHLCAVDLNNVVRPLAAINRFISYGVKVLIEFDQEKE	298
A. acidocaldarius	RLKARNEADIE.MFVQEVRRYYPRAPFLGARVKKDFVWRGYEFKRGTLVLVDVYGTTHDA	325
B. subtilis	WLRSNGNSRERE.MFVQEVRRYYPRGPFLGALVKKDFVWNCEFKKGTSVLLDLYGTNHDP	325
OleTJE	KIKS..EPDYAYKFAQEVRRYYPFVFLPGKAKVDIDFCQVTPACVGLALDVGTHDE	327
S. agnetis	KLQVSDDPNYAYKFAQEVRRYIFPFVFLPGKLLKNIEFDGYRIKKGTFTLLDVGFTTHDP	331
S. delphini	KLQVTHDPNYAYKFAQEVRRYIFPFVFLPGRLKQTVEFDGFKLKKGTFTLLDIFGTTHDP	330
S. intermedius	KLQVTNDPNYAYKFAQEVRRYIFPFVFLPGRLTKTVEFDGFKIKKGTFTLLDIFGTTHDP	330
S. pseudintermedius	KLQVTNDPNYAYKFAQEVRRYIFPFVFLPGRLKKTVEFDGFKLKKGTFTLLDIFGTTHDP	330
Sm46Δ29	KLRLENNEDYAYKFAQEVRRYIFPFVFLPGRAAVDLEYDGYKIPAGMMTALDVGYGTTHDE	330
Sm46 extended	KLRLENNEDYAYKFAQEVRRYIFPFVFLPGRAAVDLEYDGYKIPAGMMTALDVGYGTTHDE	358
A. acidocaldarius	RLWDSFNEFRERFERMRKTVGFFDLIPQGGGDSHTGHRCEGEGATIEIMKASVDFIVNOID	385
B. subtilis	RLWDHDFRFRERFAEREENLFDMLPQGGGHAEGHRCBEGGITEVMKASLDLFIVHCHIE	385
OleTJE	SLWDDFNEFRERFETWDGSEFDLIPQGGGQYVTHRCAGEWITVIIMEETMKYFAEKIT	387
S. agnetis	ELFENPYQFNEDRENWDGSEFDLIPQGGGDFYTNHRCAGEWMTIIVMEETIKYFANKID	391
S. delphini	ELFENPYQFNEDRDNDWGSEFDLIPQGGGDFYTNHRCAGEWMTIIVMEETIQYFANKID	390
S. intermedius	ELFENPYQFNEDRENWDGSEFDLIPQGGGDFYTNHRCAGEWMTIIVMEETIQYFANKID	390
S. pseudintermedius	ELFENPYQFNEDRDNDWGSEFDLIPQGGGDFYTNHRCAGEWMTIIVMEETIQYFANKID	390
Sm46Δ29	DLWENPDQFNENRDNDWGSEFDLIPQGGGDFYTNHRCAGEWITVIIMEETMKYFANKIE	390
Sm46 extended	DLWENPDQFNENRDNDWGSEFDLIPQGGGDFYTNHRCAGEWITVIIMEETMKYFANKIE	418
A. acidocaldarius	FEVFAODLSYFLDVMFTLPKSGFVLTVHRKFIASPTIATPNGSEALPSE	435
B. subtilis	YDVPEOSLHYSLARMPSLPESGFMVGIRKRS.....	417
OleTJE	YDVPEODLEVLDINSIPGYVKSQFVTKNVREVVDR.....	422
S. agnetis	FEAFSODLSVKLDQFFGKVTSGTIIQNVRPRVQR.....	425
S. delphini	FEAFSODLSVKLDQFFGKVTSGTIIKSVPYRI.....	422
S. intermedius	FDASAFODLSVKLDQFFGKVTSGTIVIKNVYRI.....	422
S. pseudintermedius	FVVEAFODLSVKLSQFFGKVTSGTMIKNVYRI.....	422
Sm46Δ29	FDVFSODLSVKLDKLPGNVTSGTIISNVRPRVARK.....	425
Sm46 extended	FDVFSODLSVKLDKLPGNVTSGTIISNVRPRVARK.....	453

Figure S1. Protein sequence alignment of CYP-Aa162 from *A. acidocaldarius* (GenBank accession number: WP_008340313), P450_{BSβ} from *Bacillus subtilis* str. 168 (GenBank accession number: NP_388092), OleT_{JE} from *Jeotgalicoccus* sp. ATCC 8456 (GenBank accession number: ADW41779), CYP-Sm46 (labelled as Sm46 extended) from *Staphylococcus massiliensis* S46 (GenBank accession number: EKU50422), CYP-Sm46Δ29 (labelled as Sm46Δ29) from *S. massiliensis* (GenBank accession number: WP_039990689) and cytochrome P450 enzymes from other *Staphylococcus* species such as *S. agnetis* (GenBank accession number: KFE42911), *S. delphini* (GenBank accession number: WP_019165531), *S. intermedius* (GenBank accession number: WP_019167377) and *S. pseudintermedius* HKU10-03 (GenBank accession number: ADV05454).

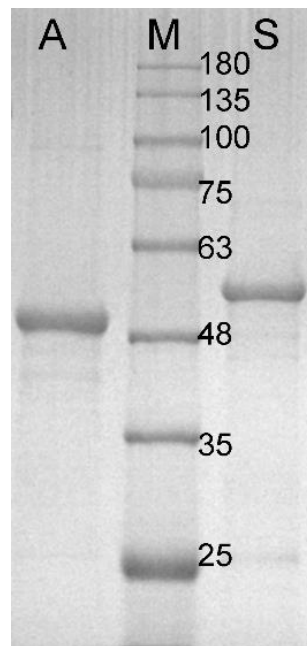


Figure S2. SDS-PAGE showing the purified His-tagged CYP-Aa162 (lane A) and CYP-Sm46 Δ 29 (lane S). Molecular sizes of the marker bands (lane M), from top to bottom, are 180, 135, 100, 75, 63, 48, 35 and 25 kDa respectively.

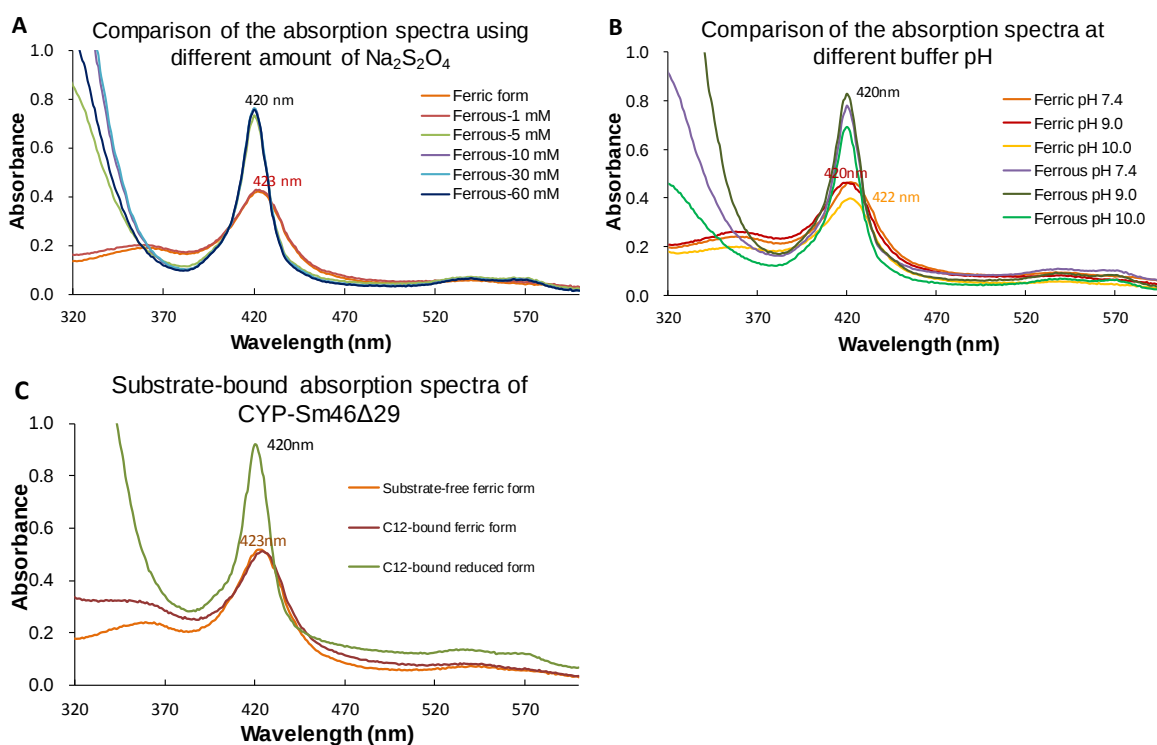


Figure S3. The UV-visible spectra of CYP-Sm46Δ29 (5 μM) under different conditions.

(A) The purified CYP-Sm46Δ29 was diluted in 50 mM Na_3PO_4 (pH 7.4) buffer containing 300 mM NaCl and 10% glycerol. Spectra are shown for the oxidized ferric form of the enzyme (orange line) and the ferrous-CO complex reduced by the indicated amount of $\text{Na}_2\text{S}_2\text{O}_4$. (B) The purified CYP-Sm46Δ29 was diluted in 50 mM Na_3PO_4 buffer containing 300 mM NaCl and 10% glycerol with different buffer pH as indicated. Then the absorption spectra were recorded respectively for the oxidized ferric form and the ferrous-CO adduct reduced by 10 mM $\text{Na}_2\text{S}_2\text{O}_4$. The protein precipitates at buffer pH lower than 7.0. (C) A molar excess (600 μM) of C_{12} lauric acid was pre-incubated with the enzyme at room temperature for 5 min before the absorption spectra were recorded. Binding of C_{12} FA did not seem to induce an apparent spin-state transition of the ferric heme. The Soret peak of the C_{12} -bound ferrous-CO adduct of the enzyme was still detected at 420 nm.

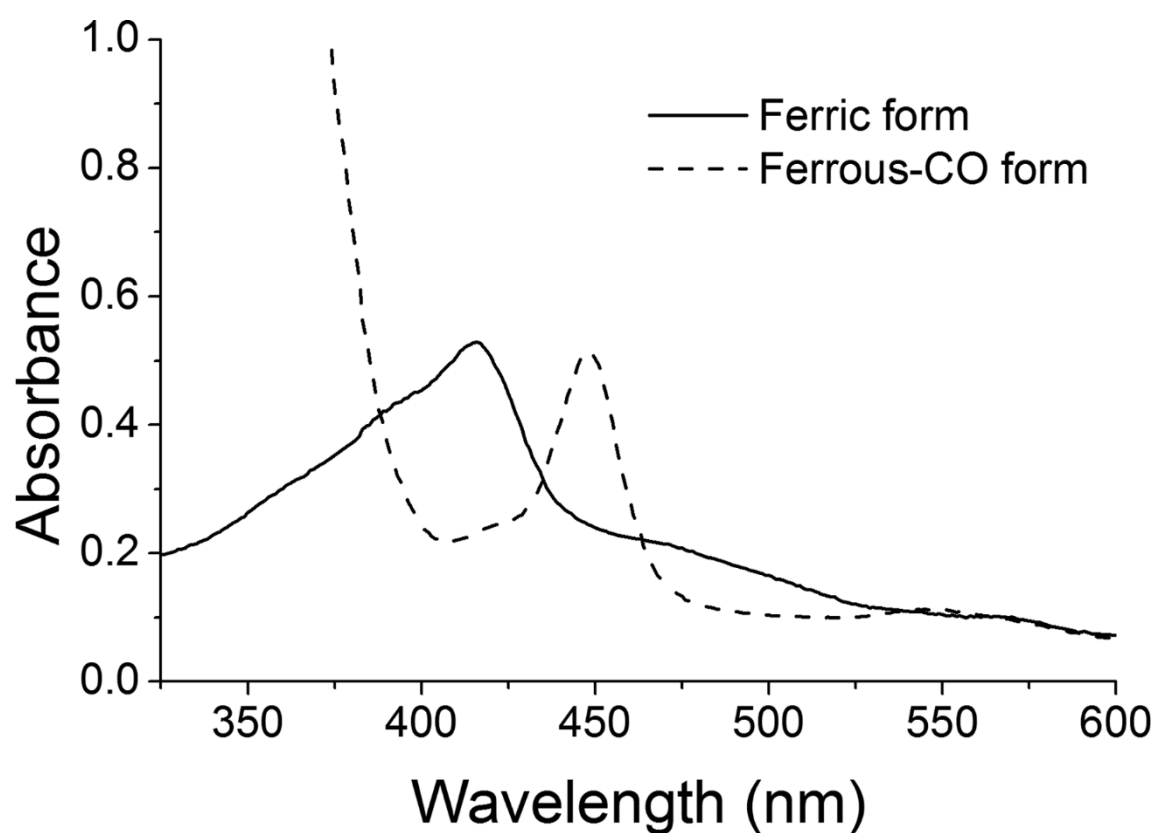


Figure S4. UV-visible spectra of the self-sufficient monooxygenase P450_{BM3}. The substrate-bound ferric form of P450_{BM3} (solid line) shows a Soret maximum at ~ 416 nm with undistinguishable β -band and a weaker α -band at 570 nm. The reduced ferrous-CO form of P450_{BM3} (dashed line) generated by the subsequent NADPH-initiated electron transfer features a shifted Soret peak to 448 nm.

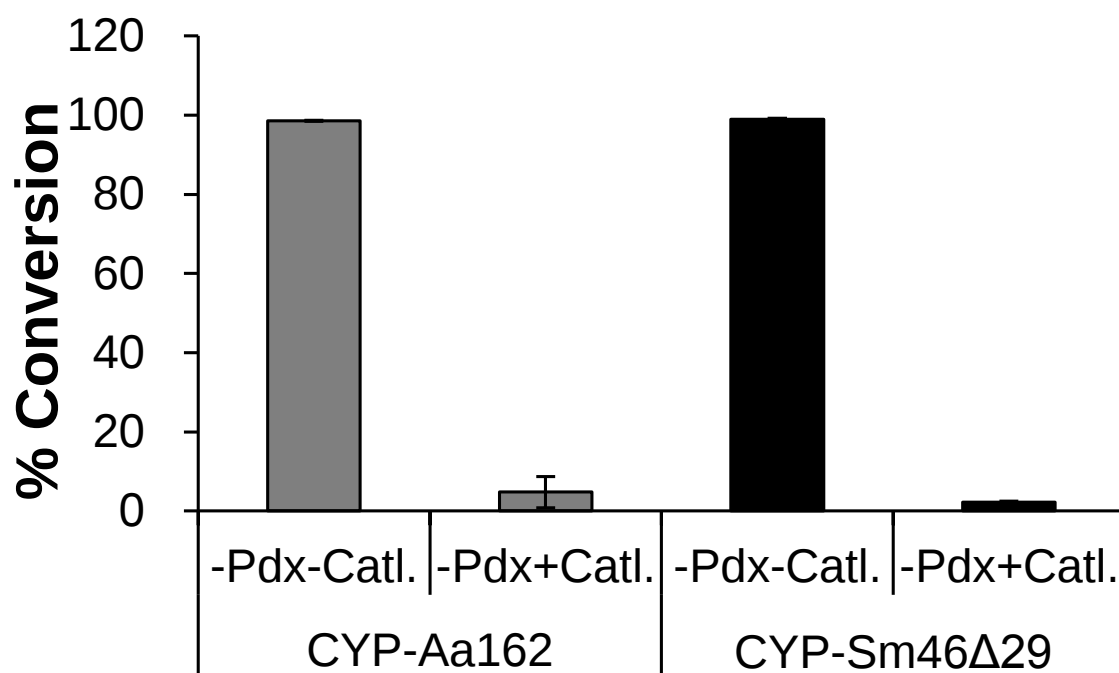


Figure S5. Effect of decoupling NADH oxidation and electron transfer on the catalytic conversion of lauric acid (LA) by CYP-Aa162 and CYP-Sm46Δ29. The reactions contained 0.2 mM LA, 2.0 μM CYP-Aa162 (or CYP-Sm46Δ29), 3.0 μM putidaredoxin reductase (PdR), 1 mM NADH in the absence and presence of 1200 U/ml catalase (Catl.). By subtracting putidaredoxin (Pdx) from the reaction system, the NADH oxidation was mandatorily decoupled from the Class I electron transfer chain to P450 enzymes. Any catalytic activity observed should be supported by the H₂O₂ generated from NADH oxidation and O₂ reduction. The percentage conversion of LA was determined by calculating the substrate consumption based on GC analysis. Results shown are mean ± SD of duplicated experiments.

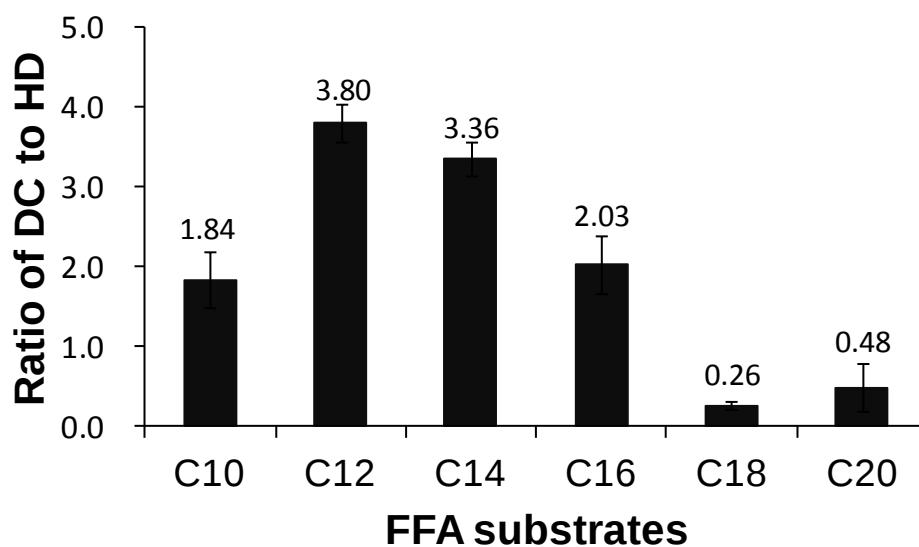


Figure S6. The ratios of free fatty acid (FFA) decarboxylation (DC) over hydroxylation (HD) by CYP-Sm46 Δ 29 against different FFA substrates. The decarboxylation activity was measured by detecting the 1-alkene yield using GC analytical method. The hydroxylation activity was estimated by subtracting the alkene production from the total substrate conversion. This indirect but more convenient method was validated with C₁₄ myristic acid substrate by direct measurement of the BSTFA/TMCS derivatized hydroxylation products. Results are shown as mean $\pm$ SD of duplicated experiments.

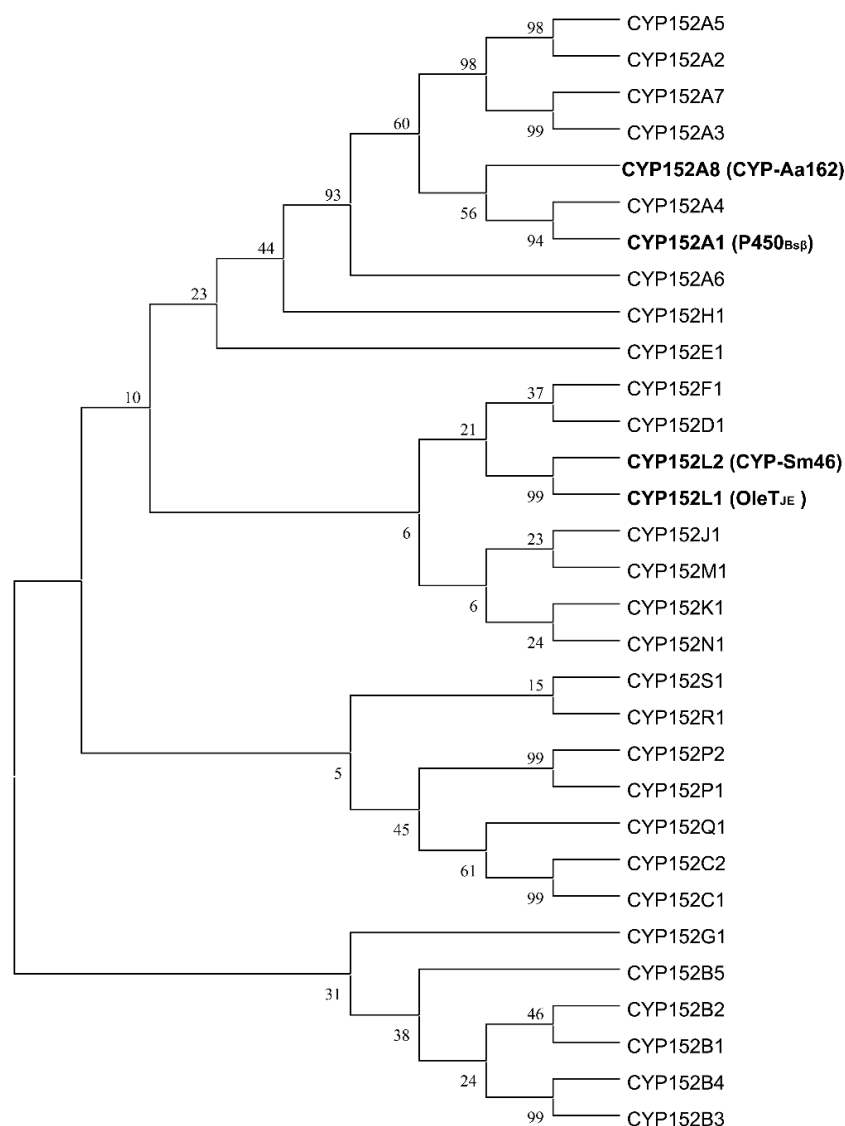


Figure S7. Phylogenetic tree for CYP-Aa162, CYP-Sm46 Δ 29 and other CYP152 family members. The sequences were aligned using Clustal W. The Neighbor-joining Tree was generated using MEGA 7.0 package. Bootstrap values shown next to the branches were computed from 1000 bootstrap tests. CYP-Sm46 is most closely related to the P450 fatty acid decarboxylase OleT_{JE} (CYP152L1), while CYP-Aa162 (CYP152A8) is much closer to the P450 fatty acid hydroxylase P450_{BSβ} (CYP152A1).

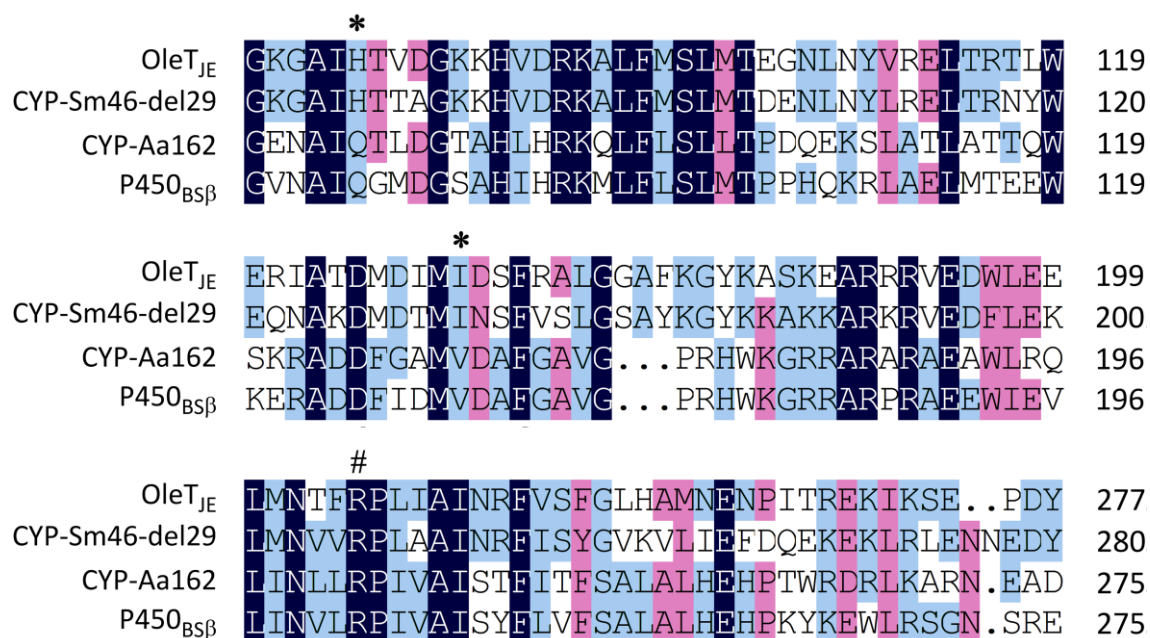


Figure S8. Protein sequence alignment of OleT_{JE}, CYP-Sm46Δ29, CYP-Aa162 and P450_{BSβ}. *: the only two residues that are distinct in the active sites of these four P450 peroxygenases, which are proposed to be important for product distribution; #: the key catalytic residue.

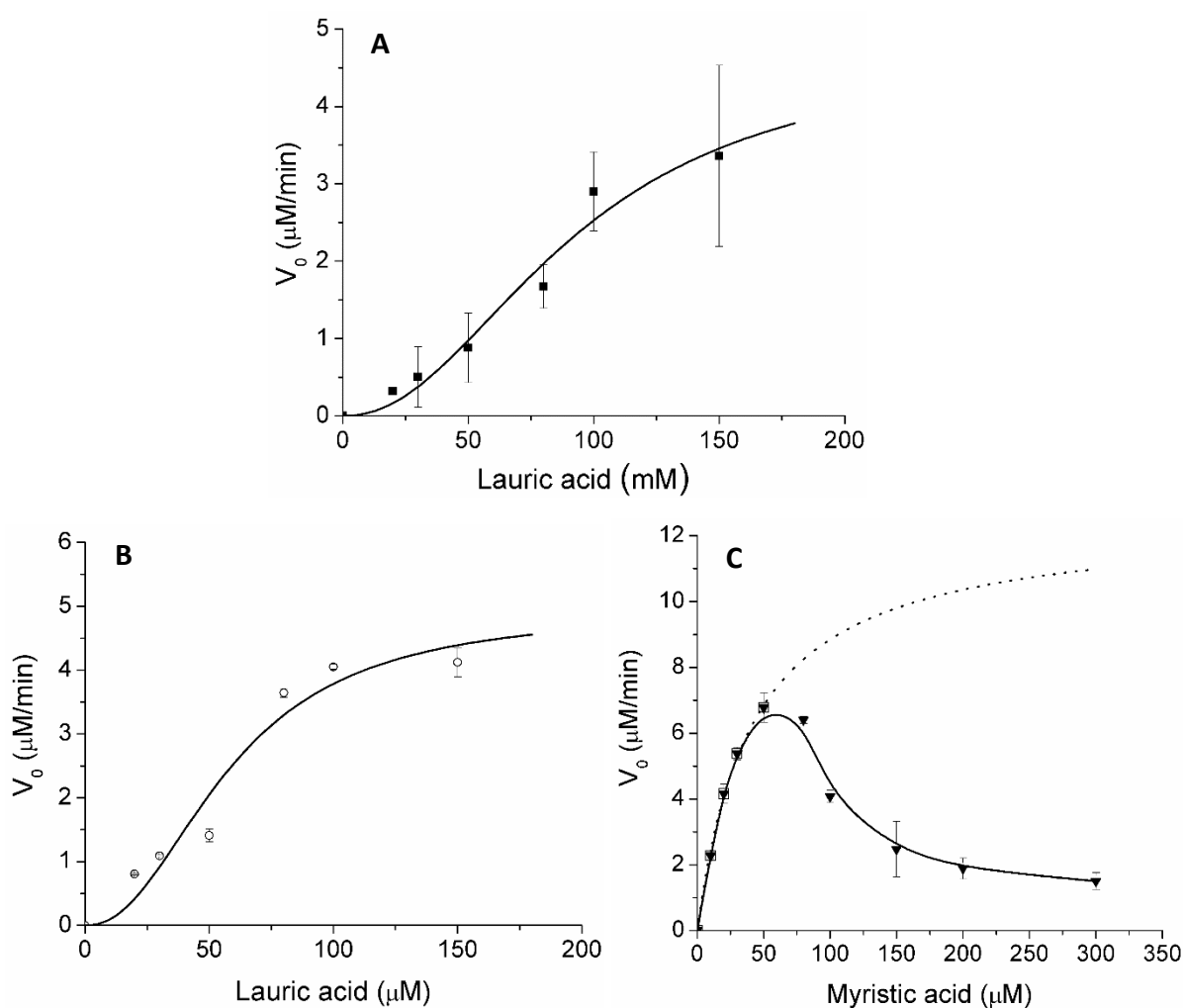


Figure S9. Kinetic curves of CYP-Aa162 and CYP-Sm46Δ29 against their optimal fatty acid substrates. (A) C₁₂ lauric acid substrate consumption rates by CYP-Aa162 were fitted to Hill equation; (B) 1-undecene formation rates by CYP-Sm46Δ29 were fitted to Hill equation; (C) Solid line: the plot of 1-tridecene formation rates by CYP-Sm46Δ29 as a function of increasing C₁₄ myristic acid concentrations, demonstrating substantial substrate inhibition. Dotted line: a hyperbolic curve fitted with Michaelis-Menten equation after truncating the inhibited rates at high C₁₄ substrate concentrations. The steady state kinetic parameters were calculated using OriginPro 8.0 and are summarized in Table 2.