CYP153A71 from *Alcanivorax dieselolei*: oxidation beyond monoterminal hydroxylation of *n*-alkanes

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Supporting Information

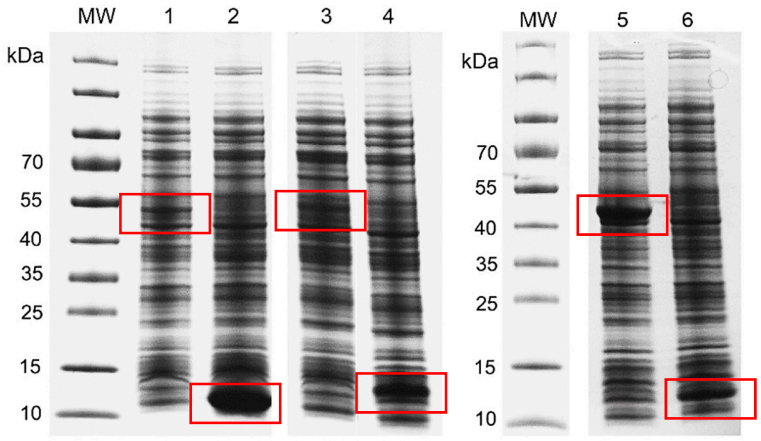


Figure S1. SDS-PAGE analysis of redox partner proteins of CYP153A6 and CYP153A13. *E. coli* CFE of lane 1: FdR-A6 (pET-22b(+)), lane 2: Fdx-A6 (pET-22b(+)), lane 3: FdR-A6 (pET-28b(+)), lane 4: Fdx-A6 (pET-28b(+)), lane 5: FdR-A13 (pETDuet-1), lane 6: Fdx-A13 (pETDuet-1). MW: PageRuler Prestained protein ladder.

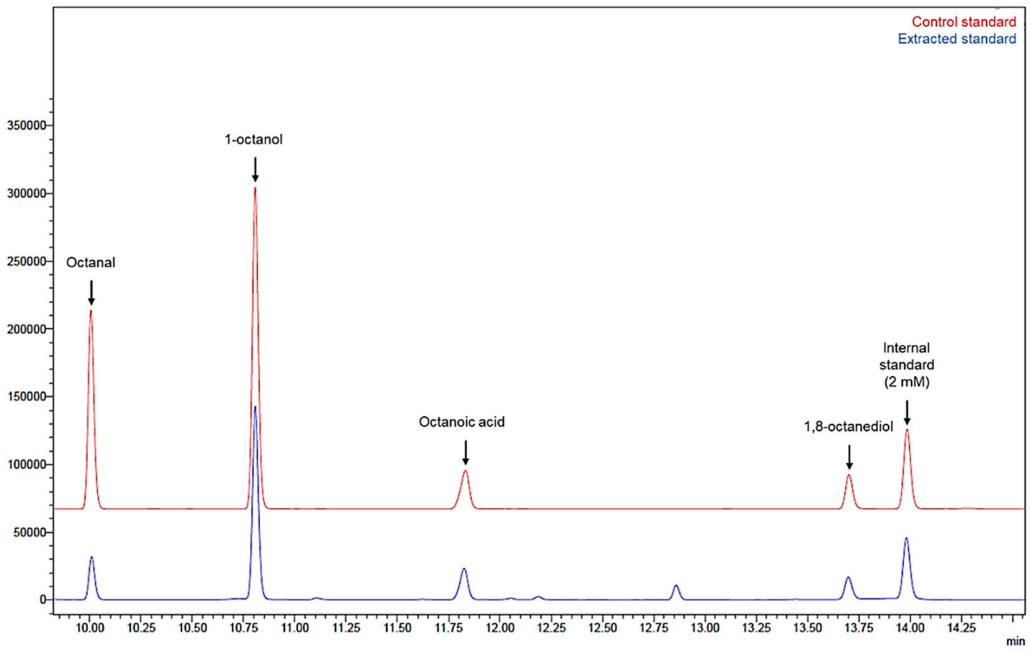


Figure S2. Comparative GC-FID chromatograms showing the extraction efficiency of hydroxylated products obtained from *n*-octane. For the control standard, products were directly prepared to final concentration in ethyl acetate containing internal standard. For the extracted standard, products were made up to final concentration in 1 mL reaction buffer [200 mM Tris (pH 8) containing 100 mM glucose and glycerol] in a 40 mL amber vial and extracted using ethyl acetate containing internal standard (equivalent to biotransformation extractions). Expected final product concentrations for both chromatograms: 1-octanol (7.5 mM), octanal (7.5 mM), octanoic acid (1.5 mM), 1,8-octanediol (1.5 mM) and internal standard (2 mM).

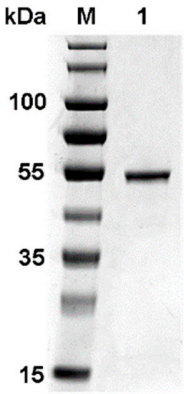


Figure S3. SDS-PAGE analysis of purified CYP153A71. M: PageRuler Prestained Protein Ladder, 1: purified CYP153A71 (53.8 kDa).

Table S1. Data collection and refinement statistics for CYP153A71.

Data collection	
X-ray source	Beamline i03, Diamond Light Source
Wavelength (Å)	0.9763
Resolution (Å)	46.11 – 1.95 (2.00 – 1.95)
Space group	$P2_1$
Unit cell parameters	
a/b/c (Å)	57.66 / 76.82 / 107.34
$\alpha/\beta/\gamma$ (°)	90.00 / 90.53 / 90.00
Unique reflections	66934 (4466)
Completeness	97.8 (97.0)
R_{merge}	0.129 (1.365)
Average $I/\sigma(I)$	7.2 (1.2)
Multiplicity	3.4 (3.5)
CC (1/2)	0.995 (0.519)
Refinement	
R_{work}/R_{free}	0.2290 / 0.2707
Molecules in ASU	2
Average B factor (Å) :	
Protein	43.25
Ligand	28.33
Solvent	40.91
RMSD:	
Bond length (Å)	0.0040
Bond angle (°)	1.2564
Ramachandran distribution (%)	
(favoured/allowed/outlier)	94.91 / 4.48 / 0.61
Molprobity score	1.6

(Values for highest resolution shell indicated in brackets)

Table S2. Targeted genes from *A. dieselolei* B-5 and *A. borkumensis* SK2.

Gene	Accession No	Primer sequences[1]	Annealing Temp	Cloning
CYP153A71	ACQ99381	5′-CAT ATG TCA ACC AAG TCA GGC ACG ATG-3′ 5′-GTC GAC TCA ATT TTT TGC GGT CAA CTT GAC-3′	55°C	*Nde*I *Sal*I
FdR-A71	AFT70351	5′-CAT ATG ACA ACA CTG TTG CAA AAG AAG TAT-3′ 5′-GTC GAC TTA TGC CAA CGA TTT CAA TTC-3′	55°C	*Nde*I *Sal*I
Fdx-A71	ACZ62813	5′-CAT ATG GGT AAG ATT ACG TTC ATT GAG CAT-3′ 5′-CTC GAG CTA CAT CTG AAA TTC AGG CAA-3′	55°C	*Nde*I *Xho*I
CYP153A13	CAL15649.1	5′-CAT ATG TCA ACG AGT TCA AGT ACA AGT AAT GAC-3′ 5′-AAG CTT TAT TTT TTA GCC GTC AAC TTA ACC ATC-3′	57°C	*Nde*I *Hind*III
FdR-A13	CAL15651.1	5′-CAT ATG GAA AAC GAA AAA CAA GAT GCC ACT G-3′ 5′-CCT AGG TCA GAT CAA AGA CTT TAA TTC AAC ATC C-3′	58°C	*Nde*I *Avr*II[2]
Fdx-A13	CAL15648.1	5′-CAT ATG GGA AAA ATC ACC TTT ATT GAG AAT G-3′ 5′-CCT AGG TTA TTT TTG CCT GGA TGT CAT TAC TTG-3′	58°C	*Nde*I *Avr*II

[1] Underlined sequences indicate introduced restriction sites for directional cloning. PCRs were initiated by a denaturation step (95°C, 2 min) followed by 40 cycles of annealing (10 sec) and elongation (70°C, 1 min). A final elongation step (70°C, 4 min) was included to ensure completion of all amplicons.

[2] Cloned to pET28 digested with *Nde*I/*Nhe*I to create compatible sticky ends.

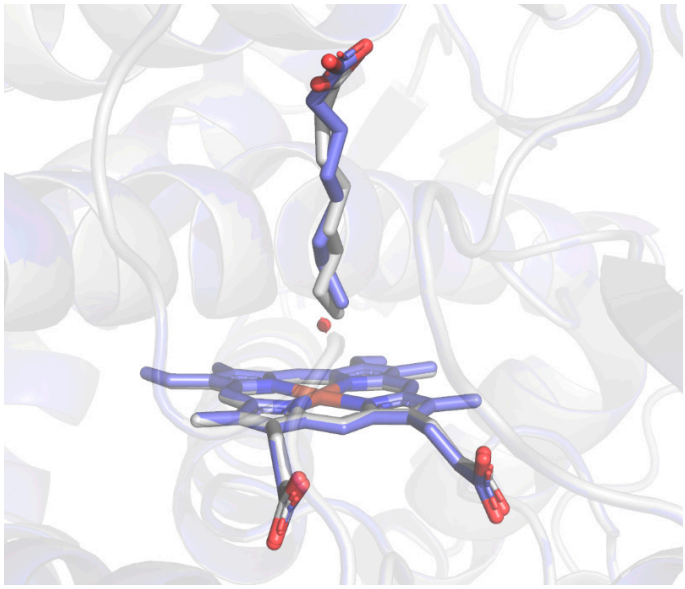
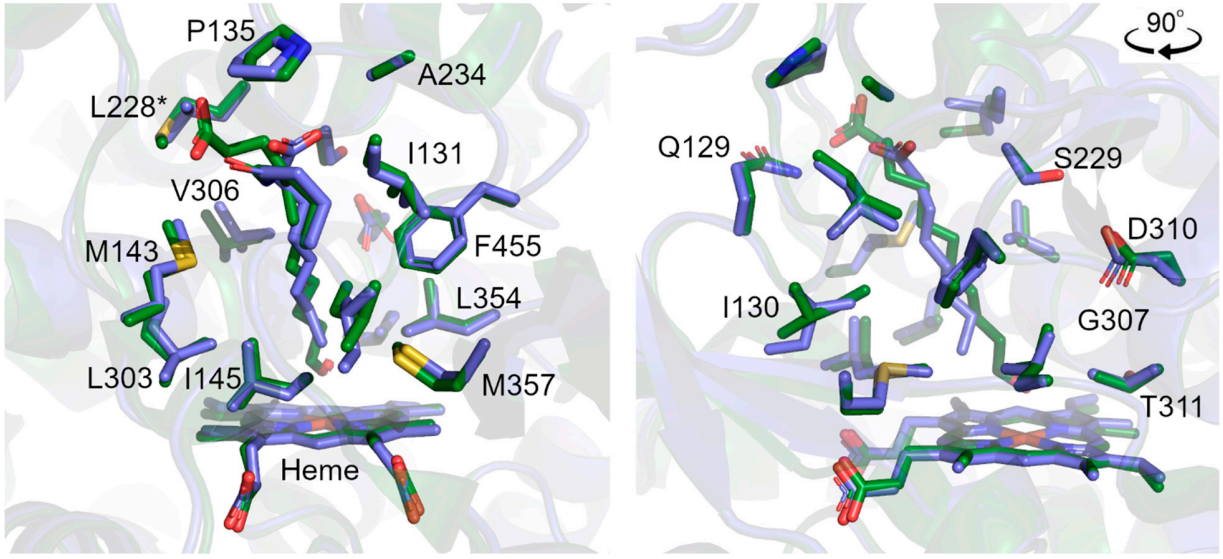


Figure S4. Overlay of CYP153A71 chain A (blue) and chain B (gray) showing the two different orientations of octanoic acid.



(**a**) (**b**)

Figure S5. (a) Overlay of CYP153A71 chain A (blue) and CYP153A33 (green, 5FYG) showing the amino acids lining the active sites. Numbering is that of CYP153A71 with I130 adopting a different conformation from that observed in CYP153A33. *L228 from the F-helix is a methionine in CYP153A33. **(b)** View rotated 90°.

Table S3. Percentage amino acid sequence identity between different CYP153A.

	CYP153A16	CYP153A33	CYP153A13	CYP153A71	CYP153A7	CYP153A6	CYP153A34
CYP153A16		65.7	65.4	65.4	50	48.7	49.7
CYP153A33	65.7		81.3	81.9	47.1	48.2	48.2
CYP153A13	65.4	81.3		83.8	47.9	47.1	47.5
CYP153A71	65.4	81.9	83.8		48.6	49	49.7
CYP153A7	50	47.1	47.9	48.6		65.6	64.7
CYP153A6	48.7	48.2	47.1	49	65.6		76.2
CYP153A34	49.7	48.2	47.5	49.7	64.7	76.2	