Electronic supplementary information

Control of Stereoselectivity of Benzylic Hydroxylation Catalysed by Wild-Type Cytochrome P450BM3 Using Decoy Molecules

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Experimental Section

Materials

Cytochrome P450BM3 was prepared according to methods described previously.[1] The concentration of the enzyme was determined by CO difference spectra.[2]

Escherichia coli cells expressing P450BM3 suspended in 20 mM Tris-HCl (pH 7.4) were disrupted using an ultrasonicator at 4ºC. After removing cell debris by centrifugation, the supernatant was applied to a CELLUFINE A-500 anion-exchange column (JNC). Weakly bound impurities were removed with 20 mM Tris-HCl containing 50 mM KCl (pH 7.4) and tightly bound proteins including P450BM3 were eluted with Tris-buffer containing 250 mM KCl. Vividly red P450BM3 fractions were pooled and desalted by spin-centrifugation dialysis using an Amicon® Ultra Centrifuge Filter Ultracel® (Millipore,Co.) with a MWCO of 30 kDa, followed by further purification using a DEAE 650S anion-exchange column (TOSOH). P450BM3 was eluted with Tris-HCl buffer over a KCl concentration gradient ranging from 0 to 120 mM. Eluted fractions were pooled and concentrated before applying to a Sephacryl S-300 gel-filtration column (GE Healthcare), equilibrated with 20 mM Tris buffer and 100 mM KCl (pH 7.4) and the P450BM3 fraction was collected.

All chemical reagents were purchased from commercial sources and used without further purification. Ethylbenzene, propylbenzene, (*S*)-(+)-1-indanol, and cyclohexanepentanoic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO). 1-phenylethyl alcohol, (*R*)-(+)-1-phenylethyl alcohol, 2-ethylphenol, 1-phenyl-1-propanol, 4-propylphenol, (*R*)-(+)-1-phenyl-propanol, indan, 1-hydroxyindan, 2-hydroxyindan, 5-hydroxyindan, 1,2,3,4-tetrahydronaphthalene, 1,2,3,4-tetrahydro-1-naphthol, (*S*)-(+)-1,2,3,4-tetrahydro-1-naphthol and BSTFA-TMCS (99:1) were obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). The following chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan): acetophenone, phenylmethyl acetate.

Measurement

Ultraviolet-visible spectra were recorded on a Shimadzu UV-2600 PC spectrophotometer. Gas

chromatography (GC) and GC-MS analysis were performed with Shimadzu GC-2014 ATF and Shimadzu GCMS-QP2010 SE both equipped with a cyclosil-β column (Agilent Technologies, Inc., 30m × 0.25 mm). 1H NMR spectra were measured by A-400 spectrometer and ECA600 Delta (JEOL). ^{1}H NMR chemical shifts were reported versus tetramethylsilane (TMS) and referenced to residual solvent peaks (DMSO-d_6). ESI-TOF-MS spectra were measured by micrOTOF II (BRUKER ANALYTIC).

Decoy molecules

The synthesis and characterisation of PFC9-Trp, PFC9-Phe, PFC9-Met were reported previously. [1] Other decoy molecules were synthesised according to published procedures.[3] C9-Phe, C9-Trp, Ph-C5-Phe, 5CHVA-Trp, 5CHVA-Phe, (R/S)-Ibuprofen-Phe, Z-Pro-Phe, Z-Pro-Met and Z-Gly-Phe were synthesised by the same method as described below.

General procedure for preparation of *N*-acyl amino acids (Ph-C5-Phe as an example):

To a mixture of 5-phenylpentanoic acid (356 mg, 2.0 mmol), L-phenylalanine methylester hydrochloride (430 mg, 2.0 mmol), 1-hydroxybenzotriazole monohydrate (HOBt•H2O, 338 mg, 2.5 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC•HCl, 489 mg, 2.5 mmol) in dry DMF (5 mL) was added N,N'-diisopropylethylamine (0.65 g, 5 mmol) and stirred at room temperature for 13 h. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with sat. $NaHCO_3$ aq. and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by column chromatography (SiO_2, hexane / EtOAc = 7 / 3).

The solution of Ph-C5-Phe-Me in 1M LiOH aq. / THF (4 / 1, 10 mL) was stirred at 60^{o}C for 3 h and then cooled to ambient temperature. 1 M HCl and EtOAc were added to the mixture and the aqueous phase was extracted (30 mL × 2) with EtOAc. The organic phases where combined, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude solid was recrystallised in hexane / EtOAc to give the product Ph-C5-Phe (544 mg, 84%).

Nonanoyl-L-Phenylalanine (C9-Phe):

^{1}H-NMR (DMSO-D_6) δ: 12.66 (1H, s), 8.11 (1H, d, *J* = 8.3 Hz), 7.28-7.17 (5H, m), 4.42 (1H, td, *J* = 9.0, 4.2 Hz), 3.05 (1H, dd, *J* = 13.7, 4.4 Hz), 2.82 (1H, dd, *J* = 13.7, 10.2 Hz), 2.02 (2H, t, *J* = 7.3 Hz), 1.37 (2H, quin, *J* = 7.3 Hz), 1.29-1.10 (10H, m), 0.86 (3H, t, *J* = 6.8 Hz). ESI-MS: *m/z* 328.19 ($[M+Na]^+$), 350.17 ($[M-H+2Na]^+$), 633.40 ($[2M+Na]^+$), 655.38 ($[2M-H+2Na]^+$), 938.60 ($[3M+Na]^+$), 960.58 ($[3M-H+2Na]^+$).

Nonanoyl-L-Tryptophan (C9-Trp):

^{1}H-NMR (DMSO-D_6) δ: 12.55 (1H, s), 10.82 (1H, s), 8.03 (1H, d, J = 8.3 Hz), 7.52 (1H, d, J = 7.8 Hz), 7.32 (1H, d, J = 8.3 Hz), 7.12 (1H, d, J = 2.0 Hz), 7.05 (1H, t, J = 7.3 Hz), 6.97 (1H, t, J = 7.3 Hz), 4.46 (1H, td, J = 8.3, 4.9 Hz), 3.15 (1H, dd, J = 14.6, 4.9 Hz), 2.98 (1H, dd, J = 14.6, 8.8 Hz), 2.05 (2H, t, *J* = 7.1 Hz), 1.40 (2H, quin, *J* = 7.2 Hz), 1.32-1.08 (10H, m), 0.85 (3H, t, J = 6.8 Hz). ESI-MS: *m/z* 367.21 ([M+Na]$^+$), 711.43 ([2M+Na]$^+$), 1055.64 ([3M+Na]$^+$)

(5-phenylpentanoyl)*-L-*phenylalanine (Ph-C5-Phe):

^{1}H NMR (DMSO-d_6, 600MHz at 80°C) δ: 12.30 (1H, brs), 7.78 (1H, d, *J* = 6.6 Hz), 7.26-7.13 (10H, m), 4.48 (1H, td, *J* = 9.0, 4.8 Hz), 3.06 (1H, dd, *J* = 14.4, 5.4 Hz), 2.87 (1H, dd, *J* = 14.4, 9.6 Hz), 2.52 (1H, t, *J* = 7.2 Hz), 2.09 (2H, t, *J* = 7.2 Hz), 1.47 (4H, m). ESI-MS: *m/z* 348.16 ([M+Na]$^+$) 370.14 ([M-H+2Na]$^+$) 673.33 ([2M+Na]$^+$) 695.32 ([2M-H+2Na]$^+$) 324.16 ([M-H]$^-$) 739.54 ([2M-H]$^-$) 649.32 ([2M-H]$^-$)

(5-cyclohexylpentanoyl)*-L-*tryptophan (5CHVA-Trp):

^{1}H NMR (DMSO-d_6, 400MHz) δ: 12.55 (1H, brs), 10.81 (1H, s), 8.03 (1H, d, *J* = 7.6 Hz), 7.53 (1H, d, *J* = 7.6 Hz), 7.32 (1H, d, *J* = 8.0 Hz), 7.12 (1H, d, *J* = 2.0 Hz), 7.05 (1H, t, *J* = 7.4 Hz), 6.75 (1H, t, *J* = 7.2 Hz), 4.46 (1H, td, *J* = 8.4, 5.2 Hz), 3.15 (1H, dd, *J* = 14.4, 5.2 Hz), 2.98 (1H, dd, *J* = 14.8, 8.8 Hz), 2.05 (2H, m, *J* = 7.6, 1.6 Hz), 1.62 (5H, m, *J* = 9.6 Hz), 1.38 (2H, m, *J* = 7.2 Hz), 1.21-1.05 (8H, m), 0.79 (2H, q, *J* = 7.2 Hz). :MS *m/z* 371.24 ([M+H]$^+$) 393.22 ([M+Na]$^+$) 741.47 ([2M+H]$^+$) 763.45 ([2M+Na]$^+$) 1133.68 ([3M+Na]$^+$) 1503.91 ([3M+Na]$^+$) 369.21 ([M-H]$^-$) 739.44 ([2M-H]$^-$) 1109.67 ([3M-H]$^-$) 1479.90 ([4M-H]$^-$)

(5-cyclohexylpentanoyl)*-L-*phenylalanine (5CHVA-Phe):

^{1}H NMR (DMSO-d_6, 400MHz) δ: 12.78 (1H, s), 8.22 (1H, d, *J* = 8.4 Hz), 7.39-7.29 (5H, m), 4.52 (1H, td, *J* = 13.2, 4.8 Hz), 3.16 (1H, dd, *J* = 13.6, 4.4 Hz), 2.94 (1H, dd, *J* = 14.0, 10.0 Hz), 2.14 (2H, t, *J* = 7.6 Hz), 1.74 (5H, m), 1.46 (2H, q, *J* = 6.8 Hz), 1.34-1.20 (8H, m), 0.91 (2H, q, *J* = 11.0 Hz). ESI-MS: *m/z* 354.22 ([M+Na]$^+$) 685.44 ([2M+Na]$^+$) 1016.66 ([3M+Na]$^+$) 330.21 ([M-H]$^-$) 661.44([2M-H]$^-$)

***(2-(4-isobutylphenyl)propanoyl)*-L-*phenylalanine (R-Ibu-L-Phe)*:**

^{1}H-NMR ($CDCl_3$) δ: 7.82 (1H, br s), 7.17-7.08 (7H, m), 6.82 (2H, d, *J* = 7.3 Hz), 5.76 (1H, d, *J* = 7.3 Hz), 4.83 (1H, q, *J* = 6.2 Hz), 3.51 (1H, q, *J* = 7.0 Hz), 3.08 (1H, dd, *J* = 13.9, 5.1 Hz), 2.99 (1H, dd, *J* = 13.9, 6.1 Hz), 2.48 (2H, d, *J* = 7.3 Hz), 1.91-1.84 (1H, m), 1.46 (3H, d, *J* = 6.8 Hz), 0.92 (6H, d, *J* = 6.8 Hz). ESI-MS: *m/z* 376.18 ([M+Na]$^+$), 729.38 ([2M+Na]$^+$), 1082.58 ([3M+Na]$^+$), 1104.57 ([3M-H+2Na]$^+$)

***(2-(4-isobutylphenyl)propanoyl)*-L-*phenylalanine (S-Ibu-L-Phe)*:**

^{1}H-NMR ($CDCl_3$) δ: 8.06 (1H, br s), 7.22-7.20 (3H, m), 7.10-7.07 (4H, m), 6.96-6.94 (2H, m), 5.76 (1H, d, *J* = 6.8 Hz), 4.74 (1H, q, *J* = 6.3 Hz), 3.54 (1H, q, *J* = 7.2 Hz), 3.15 (1H, dd, *J* = 13.8, 5.2 Hz), 3.00 (1H, dd, *J* = 14.0, 6.7 Hz), 2.46 (2H, d, *J* = 7.3 Hz), 1.91-1.80 (1H, m), 1.49 (3H, d, *J* = 7.3 Hz), 0.90 (6H, d, *J* = 6.6 Hz). ESI-MS: *m/z* 376.19 ($[M+Na]^+$), 729.40 ($[2M+Na]^+$), 1082.60 ($[3M+Na]^+$), 1104.58 ($[3M-H+2Na]^+$)

((benzyloxy)carbonyl)*-L-*prolyl*-L-*methionine (Z-Pro-Met):

^{1}H NMR (DMSO-d_6, 400MHz) δ: 12.67 (1H, s), 8.25 (1H, dd, *J* = 16.0, 8.0 Hz), 7.37-7.28 (5H, m), 5.09-4.94 (2H, m), 4.28 (1H, dd, *J* = 8.4, 3.6 Hz), 4.23 (1H, d, *J* = 8.8 Hz), 3.49-3.39 (2H, m), 2.40-2.33 (2H, m), 2.16-2.14 (1H, m), 2.04 (1H, s), 1.96 (3H, s), 1.84-1.80 (4H, m). ESI-MS *m/z* 403.14 ($[M+Na]^+$) 783.29 ($[2M+Na]^+$) 1163.44 ($[3M+Na]^+$) 379.13 ($[M-H]^-$) 759.29 ($[2M-H]^-$)

(Benzyloxy)carbonyl)*-L-*prolyl*-L-*phenylalanine (Z-Pro-Phe):

^{1}H-NMR (DMSO-D_6) δ: 12.70 (1H, s), 8.17 (1H, dd, *J* = 39.0, 7.8 Hz), 7.38-7.18 (10H, m), 5.10-4.84 (2H, m), 4.50-4.39 (1H, m), 4.22 (1H, d, *J* = 8.3 Hz), 3.45-3.35 (2H, m), 3.07-2.86 (2H, m), 2.09-1.99 (1H, m), 1.77-1.68 (3H, m). ESI-MS: *m/z* 419.16 ($[M+Na]^+$), 441.14 ($[M-H+2Na]^+$), 859.30 ($[2M-2H+3Na]^+$)

(Benzyloxy)carbonyl)glycyl*-L-*phenylalanine (Z-Gly-Phe):

^{1}H-NMR (DMSO-D_6) δ: 12.78 (1H, s), 8.12 (1H, d, *J* = 7.8 Hz), 7.42-7.20 (11H, m), 5.02 (2H, s), 4.44 (1H, td, *J* = 8.1, 5.2 Hz), 3.61 (2H, qd, *J* = 16.7, 6.4 Hz), 3.04 (1H, dd, *J* = 13.9, 5.1 Hz), 2.89 (1H, dd, *J* = 13.4, 9.0 Hz). ESI-MS: *m/z* 379.12 ($[M+Na]^+$), 735.26 ($[2M+Na]^+$)

Hydroxylation of ethylbenzene

The oxidation of ethylbenzene was carried out in 20 mM Tris-HCl (pH 7.4) buffer containing 100 mM KCl at 25°C for 5 min in the presence of 0.5 μM P450BM3, 10 mM ethylbenzene, 5 mM NADPH and 100μM decoy molecule. Ethybenzene and decoy molecules were dissolved in DMSO and added to the reaction mixture. The final volume of the reaction mixture was 1 mL containing 1.5% (v/v) DMSO. NADPH consumption was monitored by the absorption at 340 nm by UV-vis spectra. After 5 min reaction, dichloromethane was added to the reaction mixture to quench the reaction, and the products were extracted into the organic layer. Phenylmethyl acetate was also added to reaction mixture as an internal standard. Reaction extract was analysed by GC and GC-MS.

The conditions for GC were as follows: column temperature: 110°C (30 min); 10°C min^{-1}; 220°C (15 min), injection temperature: 250°C, interface temperature: 250°C, detector temperature: 250°C, carrier gas: helium. The retention times of ethylbenzene, products and the internal standard were as follows: ethylbenzene (2.1 min), phenylmethyl acetate (11.1 min), (*R*)-1-phenylethanol (12.7 min), (*S*)-1-phenylethanol (13.6 min), 2-ethylphenol (23.2 min) and 4-ethylphenol (27.0 min). GC-MS condition: column temperature: 110°C (30 min); 20°C min^{-1}; 220°C (15 min), injection temperature: 250°C, interface temperature: 200°C, ion source temperature: 200°C, carrier gas: helium. The retention times of products and the internal standard were as follows: phenylmethyl acetate (11.7 min), (*R*)-1-phenylethanol (13.6 min), (*S*)-1-phenylethanol (14.6 min), 2-ethylphenol (24.7 min) and 4-ethylphenol (28.2 min). Reactions using each decoy molecule were performed at least three times. The unit of turnover rate is expressed as (nmol product) per min per (nmol P450).

Hydroxylation of propylbenzene, indane, and tetralin

The oxidation of propylbenzene, indane, and tetralin were conducted in the same manner as ethylbenzene, but the products obtained from these substrates were derivatised to silylated alcohols by BSTFA-TMCS (99:1). The products of propylbenzene and indane oxidation were analysed by GC-MS, and those of tetralin oxidation were analysed by GC. The conditions for GC or GC-MS analysis and retention times of substrates, products and the internal standard were as follows:

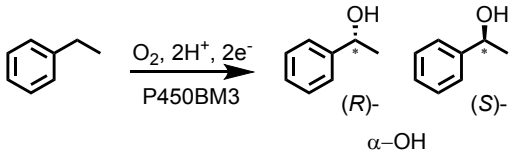
Propylbenzene: GC-MS condition: column temperature: 90°C (30 min); 20°C min^{-1}; 220°C (10 min), injection temperature: 250°C, interface temperature: 200°C, ion source temperature: 200°C, carrier gas: helium. Retention times: propylbenzene (5.3 min), (*S*)-1-phenylpropanol-BSTFA derivative (15.0 min), (*R*)-1-phenylpropanol-BSTFA derivative (15.5 min), phenylmethyl acetate (29.5 min), 4-propylphenol-BSTFA derivative (33.5 min).

Indane: GC-MS condition: column temperature: 110°C (30 min); 20°C min^{-1}; 220°C (15 min), injection temperature: 250°C, interface temperature: 200°C, ion source temperature: 200°C, carrier gas: helium. Retention times: indane (4.7 min), phenylmethyl acetate (11.9 min), (*R*)-1-indanol-BSTFA derivative (18.7 min), (*S*)-1-indanol-BSTFA derivative (19.7 min), 4-indanol-BSTFA derivative (23.4 min), 1-indanone (28.7 min), and 5-indanol-BSTFA derivative (30.7 min). The peak of 1-indanone and 4-indanol-BSTFA derivative was assigned by GC-MS fragmentation.

Tetralin: GC condition: column temperature: 110°C (40 min); 30°C min^{-1}; 220°C (10 min), injection temperature: 250°C, interface temperature: 250°C, detector temperature: 250°C, carrier gas: helium. Retention times: tetralin (9.5 min), phenylmethyl acetate (11.1 min), (*R*)-1-tetralol-BSTFA derivative (32.0 min) and (*S*)-1-tetralol-BSTFA derivative (34.3 min). No further products were

detected by GC-MS analysis.

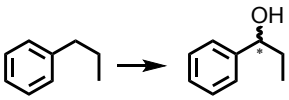
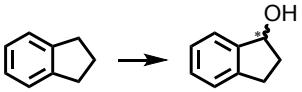
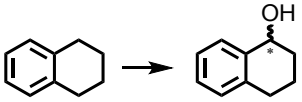
Table S1. Hydroxylation of ethylbenzene catalysed by WT P450BM3 in the presence of decoy molecules.[a]



Decoy molecule	Turnover rate [/min/P450]	α-OH [%]	ee [%] (*R*/*S*)	Coupling efficiency[b] [%]	Further Products
None	147 ± 11	83	45 (*R*)	23	2-ethylphenol (17%)
PFC8	296 ± 13	90	57 (*R*)	35	2-ethylphenol (10%)
PFC9	337 ± 9	90	61 (*R*)	37	2-ethylphenol (10%)
PFC10	324 ± 23	91	68 (*R*)	34	2-ethylphenol (9%)
PFC9-Trp	292 ± 16	87	45 (*R*)	29	2-ethylphenol (13%), 4-ethylphenol (trace)
PFC9-Phe	373 ± 46	89	52 (*R*)	31	2-ethylphenol (11%), 4-ethylphenol (trace)
PFC9-Met	297 ± 3	85	39 (*R*)	35	2-ethylphenol (15%), 4-ethylphenol (trace)
C9-Trp	285 ± 6	84	40 (*R*)	55	2-ethylphenol (16%), 4-ethylphenol (trace)
C9-Phe	277 ± 24	86	48 (*R*)	54	2-ethylphenol (14%), 4-ethylphenol (trace)
Ph-C5-Phe	276 ± 11	91	68 (*R*)	66	2-ethylphenol (9%), 4-ethylphenol (trace)
5CHVA-Trp	408 ± 13	92	80 (*R*)	56	2-ethylphenol (8%)
5CHVA-Phe	447 ± 13	94	85 (*R*)	47	2-ethylphenol (6%)
(*R*)-Ibu-Phe	321 ± 5	77	7 (*S*)	35	2-ethylphenol (23%), 4-ethylphenol (trace)
(*S*)-Ibu-Phe	386 ± 17	78	14 (*R*)	38	2-ethylphenol (22%), 4-ethylphenol (trace)
Z-Gly-Phe	211 ± 9	73	18 (*R*)	52	2-ethylphenol (27%), 4-ethylphenol (trace)
Z-Pro-Phe	324 ± 9	72	4 (*S*)	43	2-ethylphenol (28%), 4-ethylphenol (trace)
Z-Pro-Met	239 ± 6	76	31 (*R*)	38	2-ethylphenol (24%), 4-ethylphenol (trace)

[a] Reaction conditions: 10 mM ethylbenzene, 5 mM NADPH, 100 µM decoy molecule, 100 mM KCl and 0.5 µM P450BM3 in 20 mM Tris-HCl buffer (pH 7.4) at 25°C for 5 min. [b] ([Products]/[NADPH consumption]) × 100.

Table S2. Hydroxylation of propylbenzene, indane, and tetralin.catalysed by WT P450BM3 in the presence of decoy molecules.[a]

Reaction scheme	Decoy molecule	Turnover rate [/min/P450]	α-OH [%]	ee. [%] (*R*/*S*)	Coupling efficiency[b] [%]	Further products
Propylbenzene 	None	165 ± 8	>99	89 (*R*)	32	4-propylphenol (trace)
	PFC9-Phe	305 ± 12	>99	94 (*R*)	32	4-propylphenol (trace)
	5CHVA-Phe	479 ± 15	>99	95 (*R*)	66	4-propylphenol (trace)
	(*R*)-Ibu-Phe	308 ± 13	96	86 (*R*)	51	4-propylphenol (4%)
	Z-Pro-Phe	175 ± 13	97	81 (*R*)	42	4-propylphenol (3%)
Indane 	None	36 ± 2	>99	16 (*S*)	8	indanone (trace)
	PFC9-Phe	216 ± 8	96	6 (*R*)	24	4-indanol (4%), 5-indanol (trace), indanone (trace)
	5CHVA-Phe	435 ± 27	97	53 (*R*)	44	4-indanol (3%), 5-indanol (trace), indanone (trace)
	(*R*)-Ibu-Phe	314 ± 5	90	45 (*S*)	39	4-indanol (7%), 5-indanol (3%), indanone (trace)
	Z-Pro-Phe	302 ± 10	90	56 (*S*)	43	4-indanol (7%), 5-indanol (3%), indanone (trace)
Tetralin 	None	20 ± 1	>99	55 (*S*)	7	-
	PFC9-Phe	212 ± 16	>99	69 (*S*)	17	-
	5CHVA-Phe	320 ± 31	>99	13 (*S*)	49	-
	(*R*)-Ibu-Phe	332 ± 40	>99	89 (*S*)	66	-
	Z-Pro-Phe	260 ± 15	>99	96 (*S*)	82	-

[a] Reaction conditions: 10 mM substrate, 5 mM NADPH, 100 µM decoy molecule, 100 mM KCl and 0.5 µM P450BM3 in 20 mM Tris-HCl buffer (pH 7.4) at 25°C for 5 min. [b] ([Products]/[NADPH consumption]) × 100.

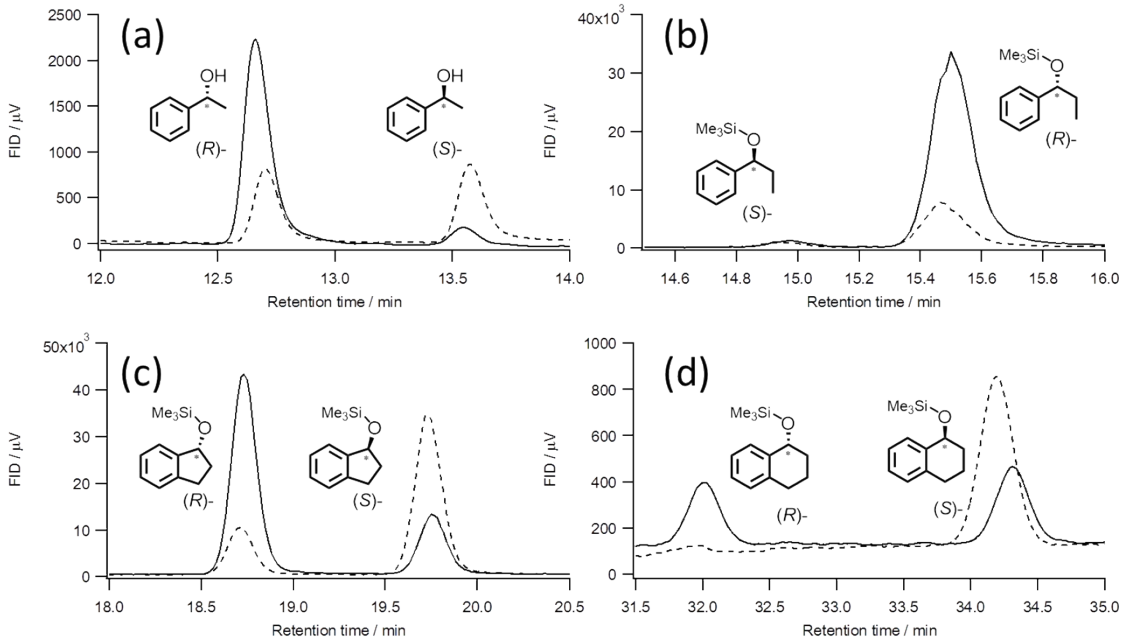


Fig. S1 Chromatograms of products in benzylic hydroxylation reactions by P450BM3 with 5CHVA-Phe (solid line) and Z-Pro-Phe (dashed line). (a): Ethylbenzene hydroxylation, (b): propylbenzene hydroxylation, (c): indane hydroxylation, (d): tetralin hydroxylation.

Crystallisation of P450BM3 with 5CHVA-L-Trp, Data Collection, and Refinement.

Buffer containing purified P450BM3 was exchanged with 50 mM Tris-HCl (pH 7.4) containing 200 μM of 5-cyclohexylvaleroyl-L-tryptophan and 1.0% (v/v) dimethyl sulfoxide and concentrated to 30 mg/mL by centrifugation using Amicon Ultra filter units (Millipore,Co.). An aliquot of the concentrated P450BM3 solution (1 μM) was mixed with 1 μM of a reservoir solution composed of 50 mM Tris-HCl (pH 8.5), 260 mM MgCl, and 17% (w/v) PEG8000. Cocrystals were grown by sitting-drop vapour diffusion method at 20 °C. Crystals were harvested and flash-cooled in liquid nitrogen. X-ray diffraction data sets were collected at SPring-8 (Hyogo, Japan) on the beamline BL26B2 equipped with a MAR225 CCD detector at 1.0 Å wavelength and 100 K. The program HKL2000[4] was used for integration of diffraction intensities and scaling. The structure was solved by molecular replacement with MolRep.[5] The structure of P450BM3 with *N*-perfluorononanoyl-L-tryptophan (3WSP) was used as a search model. Model building and refinement were performed using COOT[6] and REFMAC5.[7] The 5-cyclohexylvaleroyl-L-tryptophan model was generated employing a Dundee PRODRG server[8] and used in the refinement with COOT and REFMAC5. The final refinement statistics are summarised in Table S3.

Table S3 Data collection and refinement statistics

PDB code	5XHJ
Data collection	
Wavelength (Å)	1.000
Space group	$P2_1$
Cell dimensions	
a, b, c (Å)	58.653, 146.779, 63.642
α, β, γ (°)	90.000, 97.251, 90.000
Resolution (Å)	50.00-2.00 (2.00-2.07)
No. of total observed reflections	245648
No. of unique reflections	69112
R_{merge} [a, b] (%)	3.3 (14.7)
Completeness [a] (%)	96.7 (95.9)
$I/\sigma(I)$ [a]	35.2 (7.4)
Redundancy [a]	3.6 (3.5)
Refinement statistics	
Resolution range (Å)	20.00-2.00
No. of monomer/asymmetric unit	2
R_{work}/R_{free} [c, d] (%)	17.8/20.6
RMSD bond length [e] (Å)	0.008
RMSD bond angles [e] (º)	1.3159
No. of atoms	8040
Average *B*-factor (Å^2)	30

[a] The values in parentheses are for the highest resolution shell.

[b] $R_{merge} = \Sigma_{hkl}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the average intensity of the *i* observations.

[c] $R_{work} = \Sigma_{hkl}|F_{obs}(hkl) - F_{calc}(hkl)|\Sigma_{hkl}F_{obs}(hkl)$, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively.

[d] R_{free} was calculated with 5% of the reflections that were not included in the refinement.

[e] r. m. s. d. = root mean square deviation

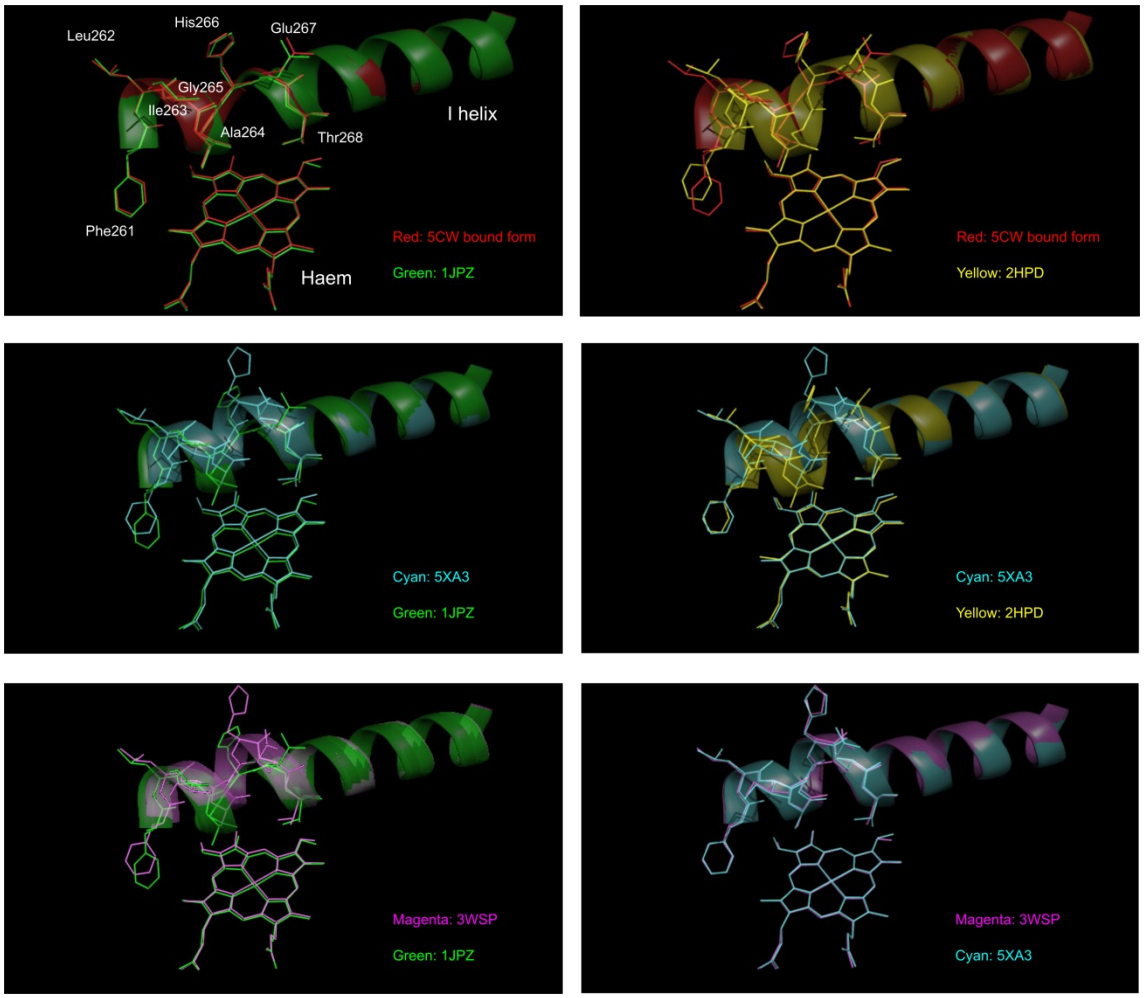


Fig. S2 Superimposed structures of the I helix and residues contained in the I helix (Phe261, Leu262, Ile263, Ala264, Gly265, His266, Glu267 and Thr268).

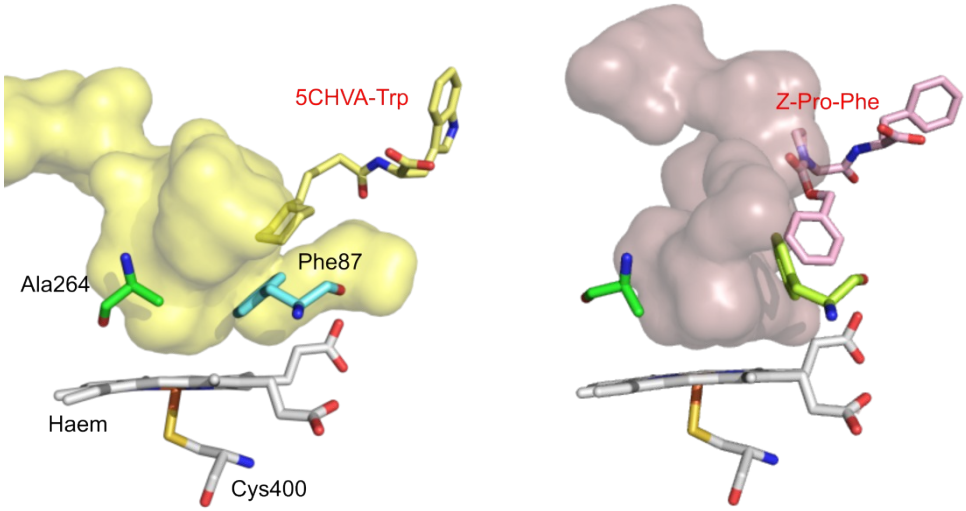


Fig. S3 Cavities of the active site depicted by surface model. (Left) 5CHVA-Trp- and (right) Z-Pro-Phe bound P450BM3

Docking simulation

The docking simulations were performed by AutoDockFR and AutoGridFR.[9] To prepare the active species compound I, receptor structures were edited by Maestro (Schrödinger Release 2016-2: Maestro, version 10.6, Schrödinger, LLC, New York, NY, 2016).[10] Indane model structure was obtained from Pubchem database.[11] Structures of receptor and ligand for simulations were prepared by AutoDock Tools.[12] Phe87, Leu262, Ile263, His266, Glu267 and Thr268, located around haem, were set as flexible residues. Partial charge of compound I species and Cys400 were derived from previous research.[13] The docking pockets were generated by AutoGridFR and pockets at the haem distal side were chosen for each simulation. After each simulation, results were visualised and hydrogen atoms of indane were added by Pymol.[14]

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