Electronic Supplementary Information

Regioselective aromatic *O*-demethylation employing an artificial P450BM3 peroxygenase system

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Experimental Section

Materials

Guaiacol, 3-Methoxycatechol, *ortho*-Cresol, 2-Ethoxyphenol, 2-Methoxytoluene, Catechol, Anisole, 4-Methoxyphenol, Phenol, 2,6-Dimethoxyphenol and 3-Methoxycatechol were purchased from TCI. Veratrole, 2-Methoxyhydroquinone, 4-Methoxybenzene-1,3-diol, and 4-Methoxy-3-methylphenol were purchased from Bidepharm. All chemicals and solvents were used as purchased without further purification. Dual-functional small molecules including *N*-(*ω*-imidazol-1-yl hexanoyl)-*L*-phenylalanine (Im-C6-Phe) and *N*-(*ω*-imidazol-1-yl pentanoyl)-*L*-phenylalanine (Im-C5-Phe) were prepared according to the previous reported procedure by us.[1]

Expression and Purification of P450BM3

The P450 BM3 heme domain (BMP, residues 1–455) and a His-tag at the *N*-terminus was ligated with the vector pET-28a (+) digested with the same restriction enzymes. The pET-28a (+) vectors containing BMP and its variants were transformed into Escherichia coli BL 21(DE3) cells, and the cells were cultivated in LB medium containing 50 μg/ml kanamycin. The cultures were grown at 37 ºC with vigorous shaking (~200 rpm). When the OD600 of the cultures reached 0.8~1.0, the temperature was cooled to 30 ºC, and the expression was induced by the addition of IPTG (1 mM) and δ-aminolevulinic acid hydrochloride (0.5 mM). Following 16–20 h of expression, the cells were harvested by centrifugation and stored at -20 ºC. Purification was done by Ni-NTA metal-affinity chromatography. Cell pellets were resuspended in ice-cold buffer A (100 mM KPi, 100 mM NaCl, imidazole (20 mM), pH 7.4) and lysed by sonication. Cell debris was removed by centrifugation for 30 min at 20 000 g, and the crude cell extraction were applied to a 5 mL bed volume column pre-equilibrated with buffer A. Nonspecifically bound proteins were washed from the column with 5 column volumes of buffer A containing 30 mM imidazole. The bound protein was eluted with buffer B (100 mM KPi, 100 mM NaCl, imidazole (200 mM), pH 7.4). The purified protein solution was exchanged with buffer C (100 mM KPi, 100 mM NaCl, pH 7.4). BMP and its variants fractions were checked for purity by SDS-PAGE (Figure S1), concentrated by ultrafiltration and frozen in buffer C plus 50% glycerol at -20 ºC.

The formation of a ferrous CO complex was confirmed by UV-visible spectral change through the reduction of ferric heme of the wild type P450 and its mutants by addition of $Na_2S_2O_4$ in the presence of carbon monoxide (CO). The concentrations of P450s were measured from the CO difference spectra according to the reported method by using ε = 91 $mM^{-1}cm^{-1}$.[2]

Mutagenesis

All mutations were made by PCR based site-directed mutagenesis and verified by DNA sequencing. The primers used were as follows:

mutation	sequence (5'- 3')
F87A-F	***GCG***ACAAGCTGGACGCATGAAAAAAATTG
F87G-F	***GGT***ACAAGCTGGACGCATGAAAAAAATTGGAA
F87V-F	***GTG***ACAAGCTGGACGCATGAAAAAAATTGGAA
F87-R	TAACCCGTCTCCTGCAAAATCACGTACAA
T268A-F	***GCG***ACAAGTGGTCTTTTATCATTTGC
T268V-F	***GTG***ACAAGTGGTCTTTTATCATTTGC
T268I-F	***ATC***ACAAGTGGTCTTTTATCATTTGC
T268G-F	***GGT***ACAAGTGGTCTTTTATCATTTGCG
T268-R	TTCGTGTCCCGCAATTAAGAATG

General procedure for *O*-demethylation of aromatic ethers

P450BM3 (0.5 μM) was transferred to a glass sample bottle containing 0.1 M, pH 8.0 phosphate buffer, aromatic ether compounds (4 mM, dissolved in methanol) and dual-functional small molecule (DFSM) (500 μM, dissolved in pH 8.0 phosphate buffer). The reaction was initiated by the addition of H_2O_2 (30 mM, dissolved in pH 8.0 phosphate buffer). The reaction mixture was incubated in water bath at 25℃ for 30 min. The reaction was stopped by the addition of dilute HCl aqueous (1 M) and neutralized with an equal volume of KCl (1 M). The products were directly analyzed by HPLC (see below).

Product analysis by HPLC.

All measurements were performed two or more times and the control experiments were carried out in the absence of DFSM. A minimum of 6 calibration levels was used

with an r^2 coefficient of 0.996 or better for each analyte.

Analysis of samples was performed on an Agilent Technologies 1200 Series equipped with VWD. Each sample and standard were injected at a volume of 10 μL onto a Waters sunfire^{TM} C18 5.0 μm (4.6 mm × 150 mm column). The column temperature was maintained at 30 °C and the buffers used to separate the analytes of interest was 0.05% acetic acid in water (A)/acetonitrile (B).[3] The specific elution programs for the reactions of different substrates were as follows:

Anisole (1): The system was run with A-B (70:30) and under these conditions 4-Methoxyphenol eluted at 7.79 min, Phenol at 9.21 min, Guaiacol at 10.03 min and the substrate at 37.68 min. The flow rate was held constant at 0.6 mL min^{-1} resulting in a run time of 45 min. Calibration curve concentration for each reaction product and substrate varied between the ranges of 5 μM-400 μM and 0.05 mM-4 mM, respectively. 210 nm was used as the detection wavelength for analysis of the analytes of interest.

2-Methoxytoluene (2): The system was run with A-B (50:50) and under these conditions 4-Methoxy-3-methylphenol eluted at 6.55 min, O-Cresol at 7.69 min and the substrate at 23.32 min. The flow rate was held constant at 0.5 mL min^{-1} resulting in a run time of 30 min. Calibration curve concentration for each reaction product and substrate varied between the ranges of 5 μM-400 μM and 0.05 mM-4 mM, respectively. 210 nm was used as the detection wavelength for analysis of the analytes of interest.

Guaiacol (3): The system was run with A-B (83:17) and under these conditions 2-Methoxyhydroquinone eluted at 5.31 min, 4-Methoxybenzene-1,3-diol at 6.59 min, Catechol at 9.03 min, 3-Methoxycatechol at 9.86 min and the substrate at 23.94 min. The flow rate was held constant at 0.6 mL min^{-1} resulting in a run time of 30 min. Calibration curve concentration for each reaction product and substrate varied between the ranges of 5 μM–400 μM and 0.05 mM–4 mM, respectively. 210 nm was used as the detection wavelength for analysis of the analytes of interest.

2-Ethoxyphenol (4): The system was run with A-B (80:20) and under these conditions Catechol eluted at 6.93 min and the substrate at 29.33 min. The flow rate changed as follows: 0.6 mL min^{-1} at time t = 0 min; 1 mL min^{-1} at time t = 20 min; 1 mL min^{-1} at time t = 30 min; 0.6 mL min^{-1} at time t = 40 min. Calibration curve concentration for each reaction product and substrate varied between the ranges of 5 μM–400 μM and 0.05 mM–4 mM, respectively. 210 nm was used as the detection wavelength for analysis

of the analytes of interest.

1,2-Dimethoxybenzene (5): The system was run with A-B (70:30) and under these conditions Catechol eluted at 5.12 min, Guaiacol at 10.08 min and the substrate at 18.33 min. The flow rate was held constant at 0.6 mL min^{-1} resulting in a run time of 25 min. Calibration curve concentration for each reaction product and substrate varied between the ranges of 5 μM–400 μM and 0.05 mM–4 mM, respectively. 210 nm was used as the detection wavelength for analysis of the analytes of interest.

2,6-Dimethoxyphenol (6): The system was run with A-B (80:20) and under these conditions Pyrogallol eluted at 4.96 min, 3-Methoxycatechol at 9.63 min and the substrate at 21.25 min. The flow rate was held constant at 0.5 mL min^{-1} resulting in a run time of 30 min. Calibration curve concentration for each reaction product and substrate varied between the ranges of 5 μM-400 μM and 0.05 mM-4 mM, respectively. 210 nm was used as the detection wavelength for analysis of the analytes of interest.

1 N. Ma, Z. Chen, J. Chen, J. Chen, C. Wang, H. Zhou, L. Yao, O. Shoji, Y. Watanabe, Z. Cong, *Angew. Chem. Int. Ed.*, 2018, **57**, 7628.

2 T. Omura, R. Sato, *J. Biol. Chem.*, 1964, **239**, 2370.

3 S. J. B. Mallinson, M. M. Machovina, R. L. Silveira, M. Garcia-Borràs, N. Gallup, C. W. Johnson, M. D. Allen, M. S. Skaf, M. F. Crowley, E. L. Neidle, K. N. Houk, G. T. Beckham, J. L. DuBois and J. E. McGeehan, *Nat. Commun.*, 2018, **9**, 2487.

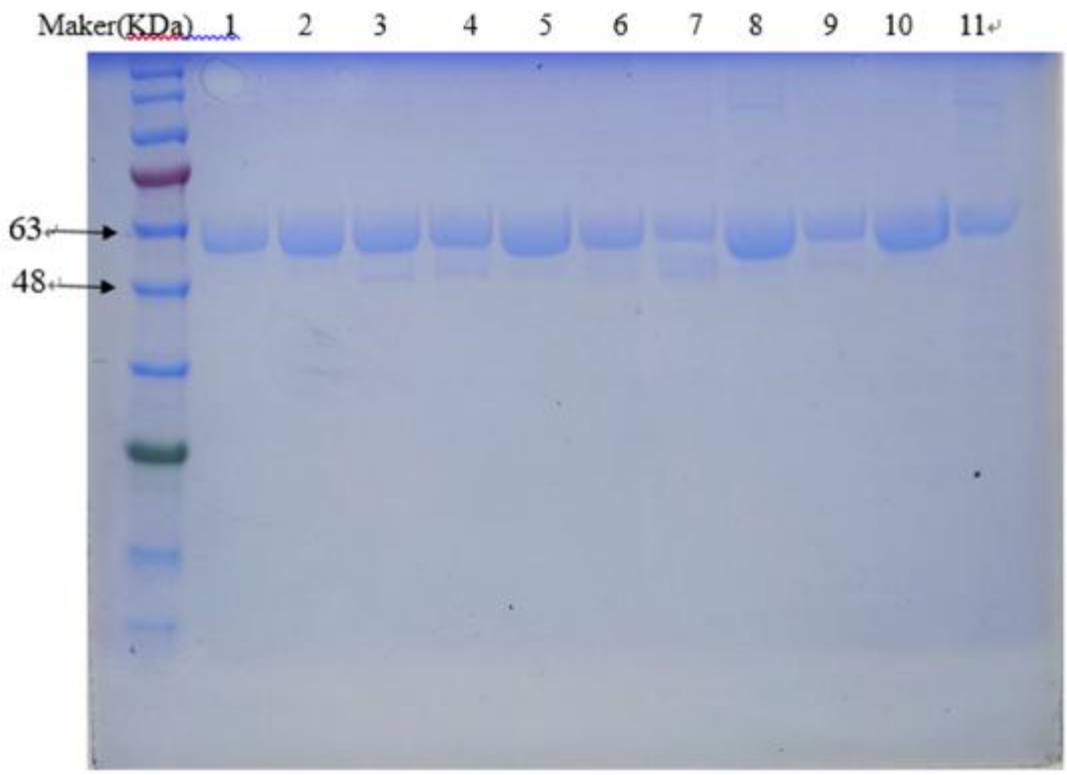
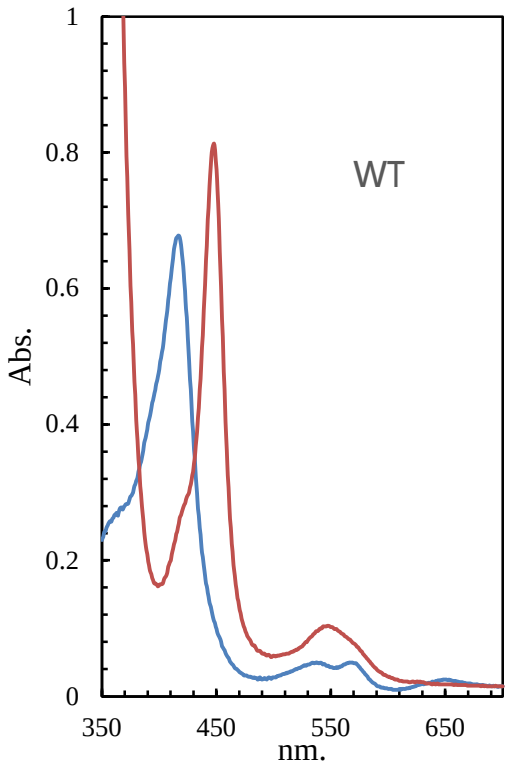


Figure S1 SDS-PAGE of P450BM3 and its mutants. Lane1-11: F87A, F87G, F87V, F87A/T268I, F87A/T268V, F87A/T268A, F87G/T268I, F87G/T268V, F87G/T268A, F87G/T268G, WT


WT
Abs.
nm.
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0.6
0.4
0.2
0
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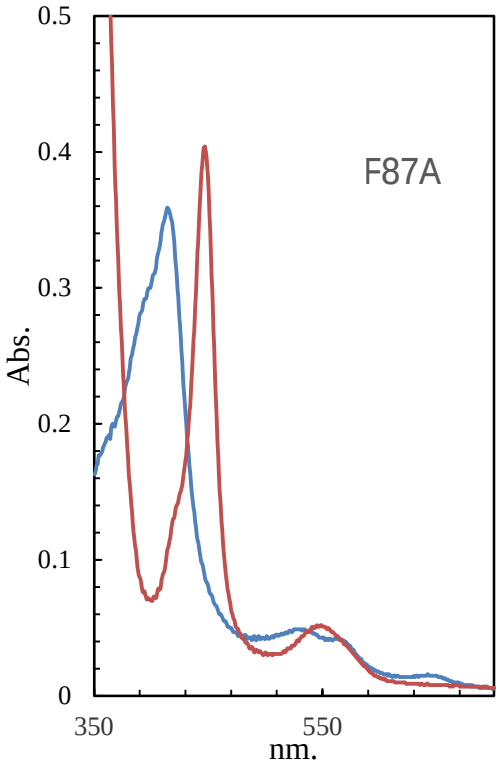
450
550
650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Abs.
nm.
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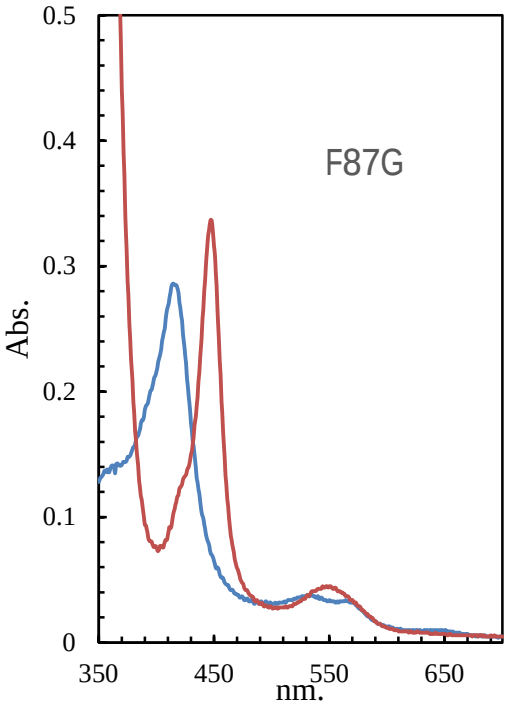
0.1
0
350
550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nm.
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0.2
0.1
0
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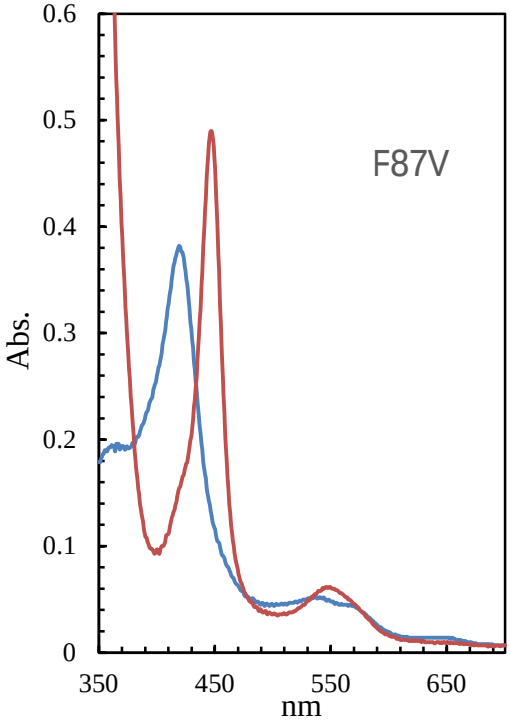
450
550
650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nm
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0.1
0
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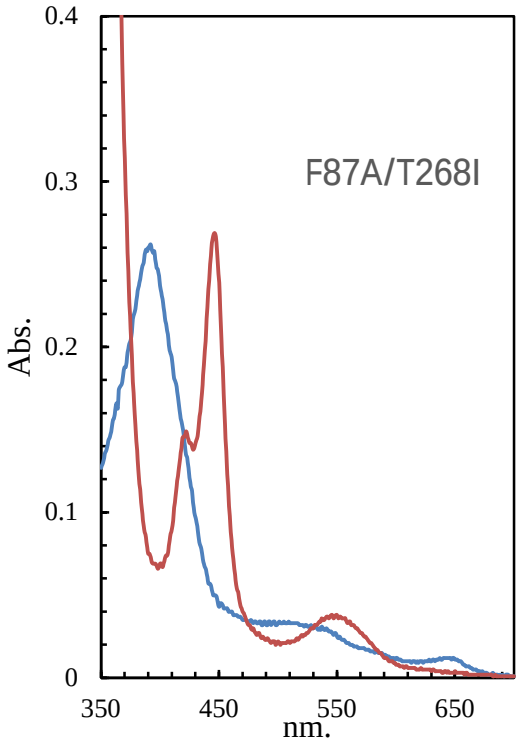
450
550
650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Abs.
nm.
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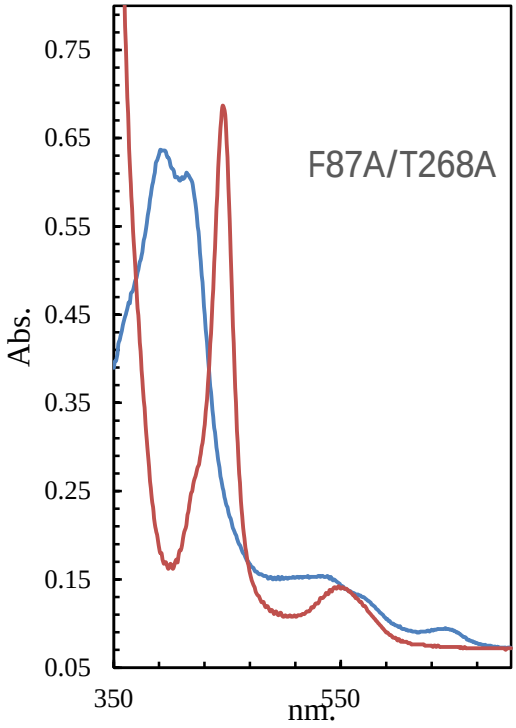
0.3
0.2
0.1
0
350
450
550
650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Abs.
nm.
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0.15
0.05
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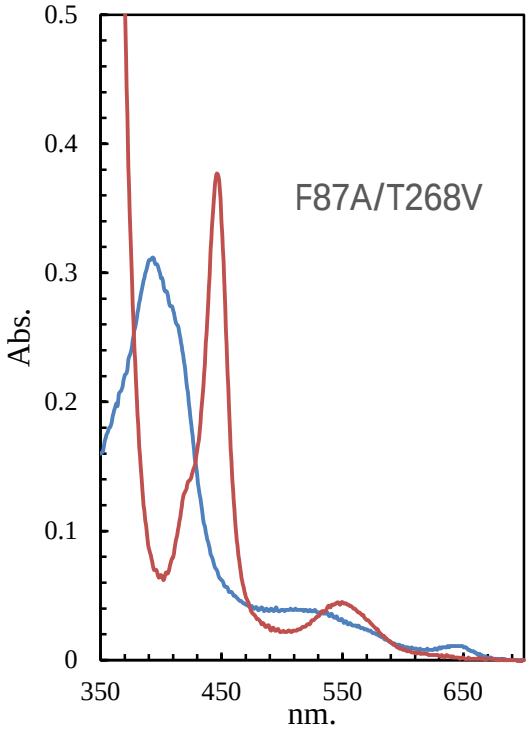
550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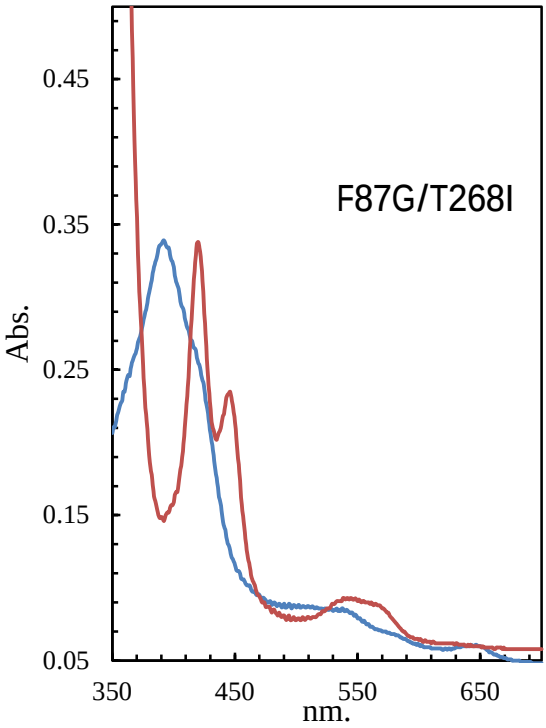
550
650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Abs.
nm.
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0.25
0.15
0.05
350
450
550
650

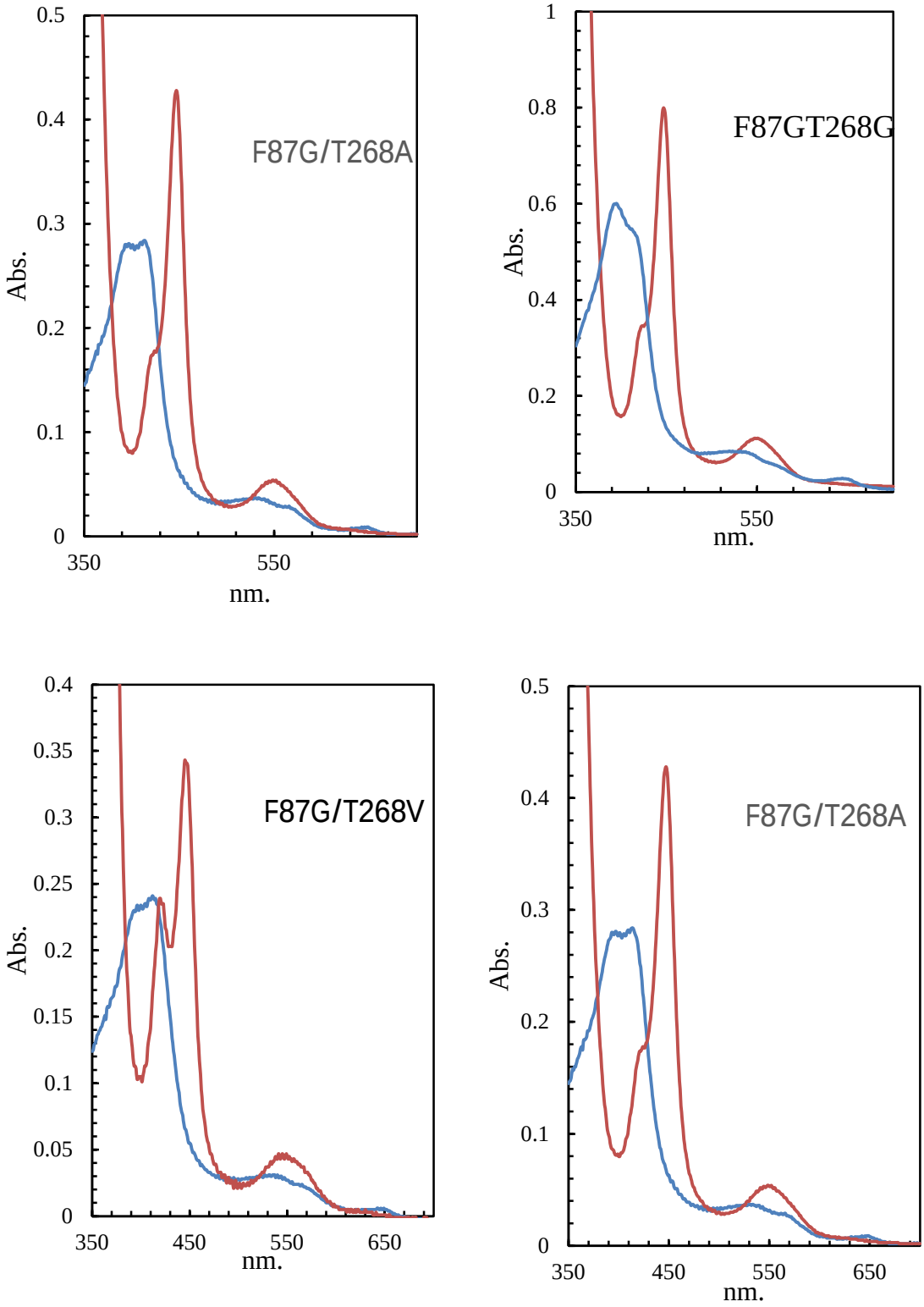
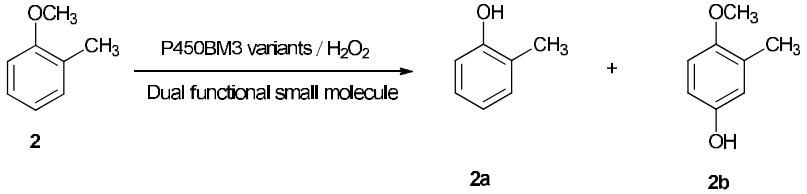


Figure S2. UV-visible spectral changes of the wild type P450BM3 and its mutants (blue line) upon addition of $Na_2S_2O_4$ (red line) for the formation of a ferrous CO complex through the reduction of ferric heme.

Table S1 Oxidation of 2-methoxytoluene(**2**) catalysed by P450BM3 peroxygenase system[ab]

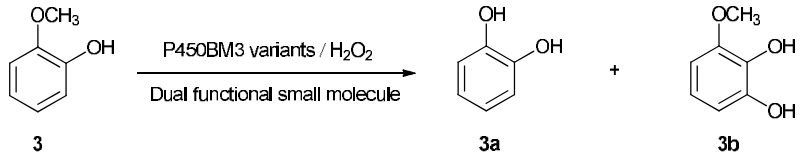


2 **2a** **2b**

Enzyme	DFSM	Total turnover numbers[c]		*o*-cresol (**2c**) selectivity/%
		2a	**2b**	
WT	Im-C6-Phe	1±0.1[e]	nd[d]	100
F87A	Im-C6-Phe	87±1.5	590±5.8	13
F87A	-	1±0.3	nd	100
F87G	Im-C6-Phe	161±0.4	47±0.6	77
F87G	Im-C5-Phe	11±0.2	nd	100
F87G	-	5±0.4	nd	100
F87V	Im-C6-Phe	2±0.2	nd	100
F87V	-	3±0.4	nd	100
F87A/T268I	Im-C6-Phe	356±12	25±1.4	93
F87A/T268I	Im-C5-Phe	73±4.6	nd	100
F87A/T268I	-	nd	nd	-
F87A/T268V	Im-C6-Phe	125±0.3	209±0.5	37
F87A/T268V	-	nd	nd	-
F87A/T268A	Im-C6-Phe	7±0.5	18±0.3	29
F87A/T268A	-	nd	nd	-
F87G/T268I	Im-C6-Phe	130±2.7	nd	100
F87G/T268I	-	nd	nd	-
F87G/T268V	Im-C6-Phe	349±11.2	71±2.6	83
F87G/T268V	Im-C5-Phe	12±0.3	nd	100
F87G/T268V	-	nd	nd	-
F87G/T268A	Im-C6-Phe	2±0.4	nd	100
F87G/T268A	-	nd	nd	-
F87G/T268G	Im-C6-Phe	nd	nd	-
F87G/T268G	-	1±0.1	nd	100

[a] Reaction conditions: P450BM3 (0.5 μM), 2-methoxytoluene (**2**) (4 mM), H_2O_2 (30 mM), DFSM (0.5 mM), in pH 8.0 phosphate buffer. [b] Hydroxylation of aromatic ring was observed by HPLC as a minor reaction product. [c] TON: Turnover numbers were estimated for 30-minutes reactions. [d] nd: not detected. [e] Average errors are representative of two or more independent measurements.

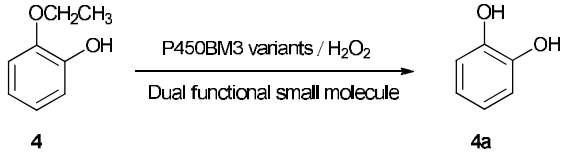
Table S2 Oxidation of guaiacol (**3**) catalysed by P450BM3 peroxygenase system[ab]



Enzyme	DFSM	Total turnover numbers[c]		Catechol (**3a**) Selectivity/%
		3a	**3b**	
WT	Im-C6-Phe	nd[d]	nd	-
F87A	Im-C6-Phe	539±1.2[e]	344±11.1	61
F87A	Im-C5-Phe	34±6.4	nd	100
F87A	-	nd	nd	-
F87G	Im-C6-Phe	495±8.6	236±11.3	68
F87G	Im-C5-Phe	nd	nd	
F87G	-	nd	nd	-
F87V	Im-C6-Phe	48±3.6	nd	100
F87V	-	nd	nd	-
F87A/T268I	Im-C6-Phe	96±1.0	16±0.1	85
F87A/T268I	-	nd	nd	-
F87A/T268V	Im-C6-Phe	42±0.7	22±1.7	65
F87A/T268V	-	nd	nd	-
F87A/T268A	Im-C6-Phe	96±6.1	nd	100
F87A/T268A	-	nd	nd	-
F87G/T268I	Im-C6-Phe	62±2.3	nd	100
F87G/T268I	-	nd	nd	-
F87G/T268V	Im-C6-Phe	205±2.1	43±2.0	83
F87G/T268V	Im-C5-Phe	nd	nd	
F87G/T268V	-	nd	nd	-
F87G/T268A	Im-C6-Phe	nd	nd	-
F87G/T268A	-	nd	nd	-
F87G/T268G	Im-C6-Phe	23±1.0	nd	100
F87G/T268G	-	nd	nd	-

[a] Reaction conditions: P450BM3 (0.5 μM), guaiacol (**3**) (4 mM), H_2O_2 (30 mM), DFSM (0.5 mM), in pH 8.0 phosphate buffer. [b] Hydroxylation of aromatic ring was observed by HPLC as a minor reaction product. [c] TON: Turnover numbers were estimated for 30-minutes reactions. [d] nd: not detected. [e] Average errors are representative of two or more independent measurements.

Table S3 Oxidation of 2-ethoxyphenol (**4**) catalysed by P450BM3 peroxygenase system[ab]

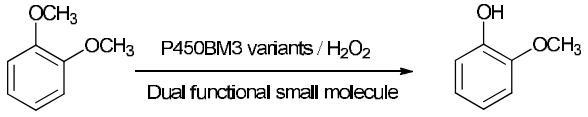


4 **4a**

Enzyme	DFSM	Total turnover numbers[c]
		5a
WT	Im-C6-Phe	nd[d]
F87A	Im-C6-Phe	69±2.9[e]
F87A	Im-C5-Phe	15±3.3
F87A	-	nd
F87G	Im-C6-Phe	35±0.8
F87G	-	nd
F87V	Im-C6-Phe	nd
F87V	-	nd
F87A/T268I	Im-C6-Phe	38±0.3
F87A/T268I	-	nd
F87A/T268V	Im-C6-Phe	4±0.6
F87A/T268V	-	nd
F87A/T268A	Im-C6-Phe	nd
F87A/T268A	-	nd
F87G/T268I	Im-C6-Phe	nd
F87G/T268I	Im-C5-Phe	nd
F87G/T268I	-	nd
F87G/T268V	Im-C6-Phe	65±1.0
F87G/T268V	Im-C5-Phe	nd
F87G/T268V	-	nd
F87G/T268A	Im-C6-Phe	5±0.8
F87G/T268A	-	nd
F87G/T268G	Im-C6-Phe	62±0.6
F87G/T268G	Im-C5-Phe	99±8.0
F87G/T268G	-	nd

[a] Reaction conditions: P450BM3 (0.5 μM), 2-ethoxyphenol (**4**) (4 mM), H_2O_2 (30 mM), DFSM (0.5 mM), in pH 8.0 phosphate buffer. [b] Hydroxylation of aromatic ring observed by HPLC as a minor reaction product wasn't calculated because of there is no standard product [c] TON: Turnover numbers were estimated for 30-minutes reactions. [d] nd: not detected. [e]Average errors are representative of two or more independent measurements.

Table S4 Oxidation of 1,2-dimethoxybenzene (**5**) catalysed by P450BM3 peroxygenase system[ab]

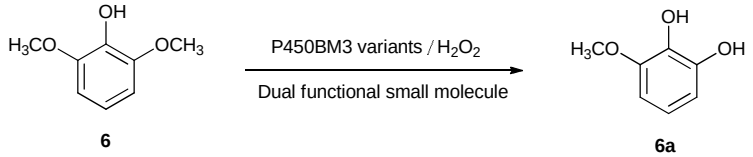


5 **5a**

Enzyme	DFSM	Total turnover numbers[c]
		4a
WT	Im-C6-Phe	nd[d]
F87A	Im-C6-Phe	53±1.5[e]
F87A	-	4±2.6
F87G	Im-C6-Phe	62±0.3
F87G	-	5±0.8
F87V	Im-C6-Phe	nd
F87V	-	9±0.5
F87A/T268I	Im-C6-Phe	287±3.0
F87A/T268I	Im-C5-Phe	49±3.3
F87A/T268I	-	nd
F87A/T268V	Im-C6-Phe	65±0.8
F87A/T268V	Im-C5-Phe	4±2.1
F87A/T268V	-	nd
F87A/T268A	Im-C6-Phe	18±1.1
F87A/T268A	-	2±0.8
F87G/T268I	Im-C6-Phe	83±1.7
F87G/T268I	Im-C5-Phe	nd
F87G/T268I	-	nd
F87G/T268V	Im-C6-Phe	6±1.1
F87G/T268V	-	nd
F87G/T268A	Im-C6-Phe	nd
F87G/T268A	-	nd
F87G/T268G	Im-C6-Phe	nd
F87G/T268G	-	nd

[a] Reaction conditions: P450BM3 (0.5 μM), 1,2-dimethoxybenzene (**5**) (4 mM), H_2O_2 (30 mM), DFSM (0.5 mM), in pH 8.0 phosphate buffer. [b] Hydroxylation of aromatic ring observed by HPLC as a minor reaction product wasn't calculated because of there is no standard product. [c]TON: Turnover numbers were estimated for 30-minutes reactions. [d] nd: not detected. [e]Average errors are representative of two or more independent measurements.

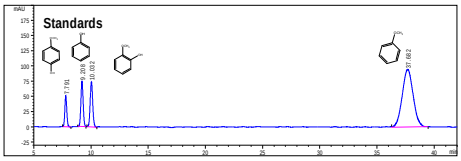
Table S5 Oxidation of 2,6-dimethoxyphenol (**6**) catalysed by P450BM3 peroxygenase system[ab]



6 **6a**

Enzyme	DFSM	Total turnover numbers[c]
		6a
WT	Im-C6-Phe	55±1.3[e]
F87A	Im-C6-Phe	23±0.2
F87A	-	46±1.8
F87G	Im-C6-Phe	106±1.9
F87G	Im-C5-Phe	43±1.2
F87G	-	29±0.9
F87V	Im-C6-Phe	nd[d]
F87V	-	35±2.1
F87A/T268I	Im-C6-Phe	68±0.4
F87A/T268I	-	49±0.8
F87A/T268V	Im-C6-Phe	76±3.7
F87A/T268V	-	61±2.0
F87A/T268A	Im-C6-Phe	60±0.2
F87A/T268A	-	55±0.8
F87G/T268I	Im-C6-Phe	82±2.0
F87G/T268I	Im-C5-Phe	65±1.5
F87G/T268I	-	37±7.5
F87G/T268V	Im-C6-Phe	165±0.9
F87G/T268V	Im-C5-Phe	45±0.1
F87G/T268V	-	55±1.3
F87G/T268A	Im-C6-Phe	38±0.1
F87G/T268A	-	34±1.4
F87G/T268G	Im-C6-Phe	78±1.2
F87G/T268G	-	43±2.1

[a] Reaction conditions: P450BM3 (0.5 μM), 2,6-dimethoxyphenol (**6**) (4 mM), H_2O_2 (30 mM), DFSM (0.5 mM), in pH 8.0 phosphate buffer. [b] Hydroxylation of aromatic ring observed by HPLC as a minor reaction product wasn't calculated because of there is no standard product [c] TON: Turnover numbers were estimated for 20-minutes reactions. [d] nd: not detected. [e]Average errors are representative of two or more independent measurements.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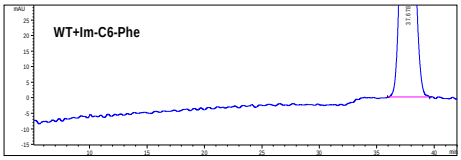
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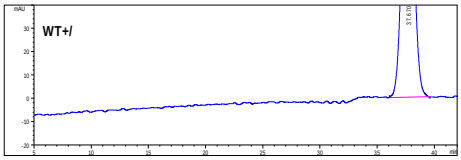
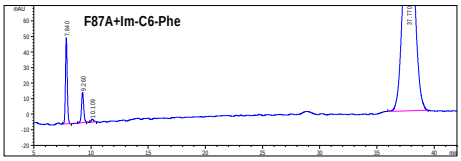

WT+/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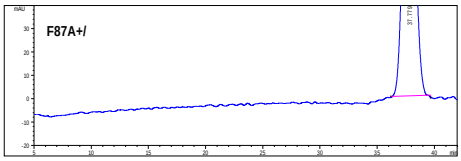
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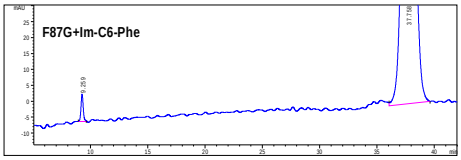
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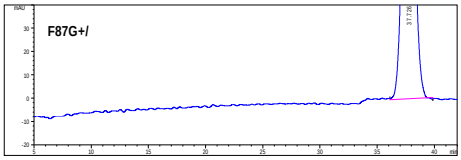
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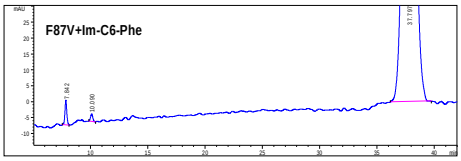
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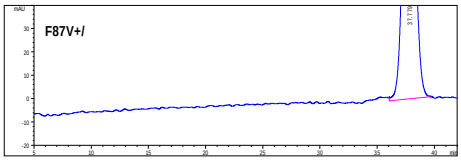
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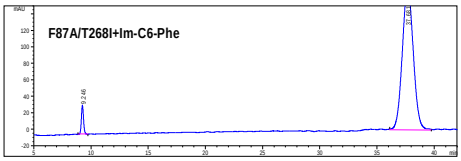
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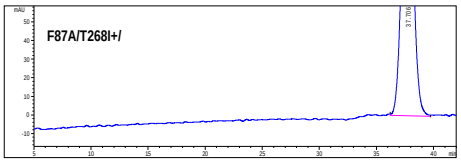
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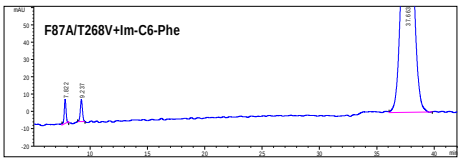
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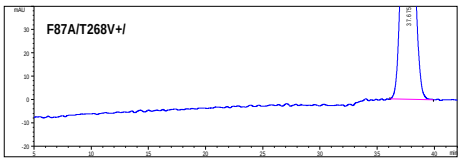
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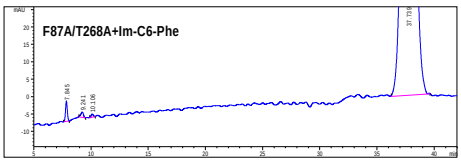
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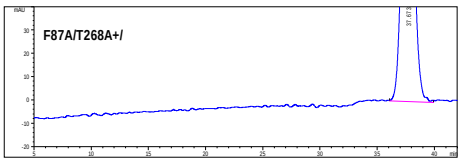
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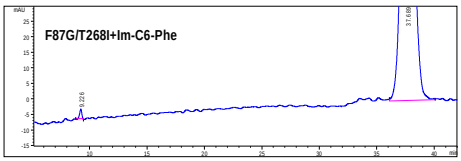
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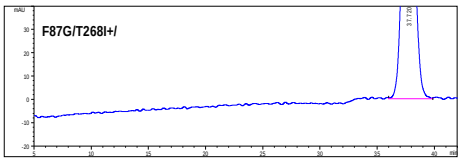
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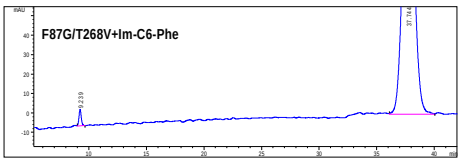
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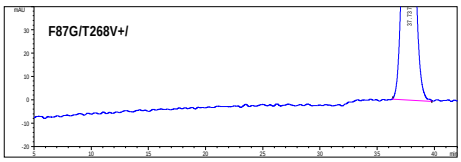
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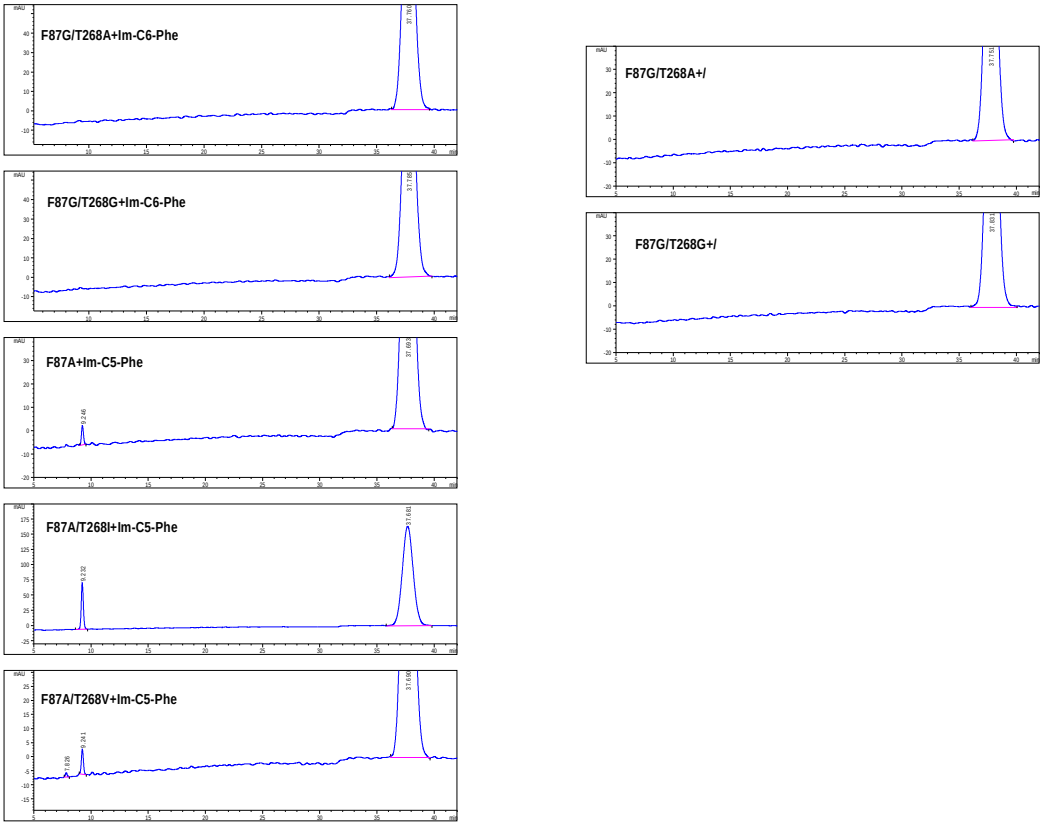
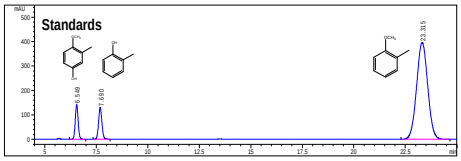
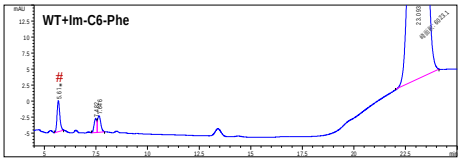
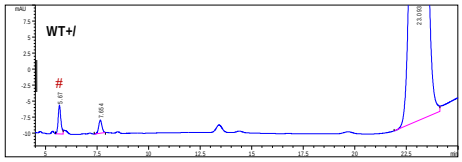


Figure S3 HPLC analyses for the oxidation of anisole (**1**) by P450BM3 and its mutants in the absence (right column) /presence (left column) of DFSM. *The top HPLC spectrum is for the mixed standard of 4-methoxyphenol (7.79 min), Phenol (9.21 min), guaiacol (10.03 min) and anisole (37.68 min).


Standards


WT+Im-C6-Phe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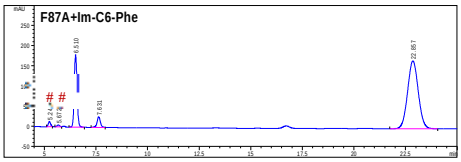
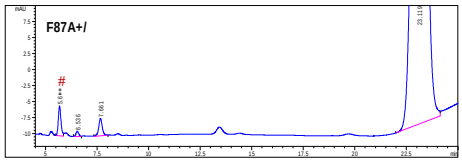

F87A+Im-C6-Phe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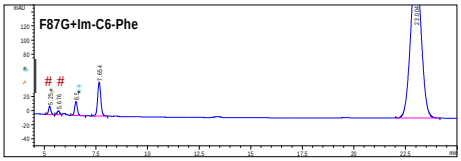
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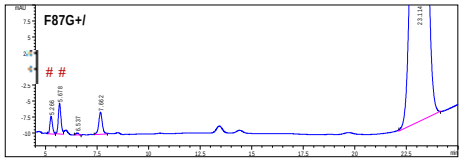
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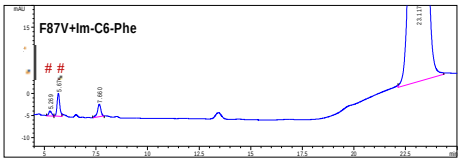
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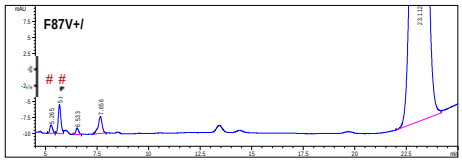
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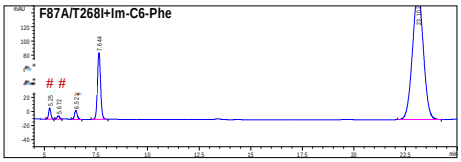
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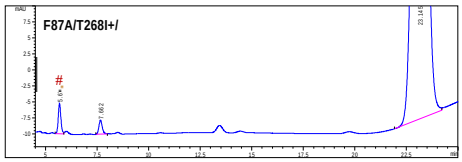
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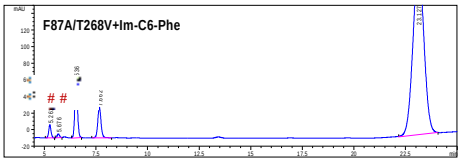
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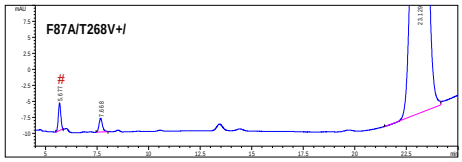
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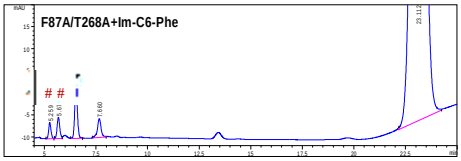
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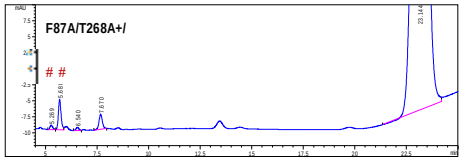
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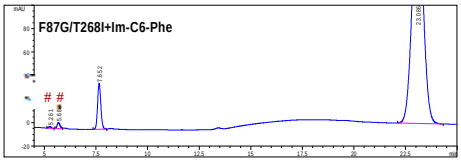
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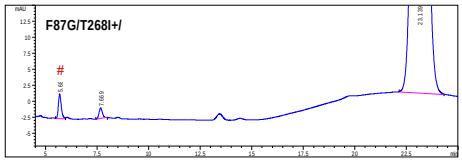
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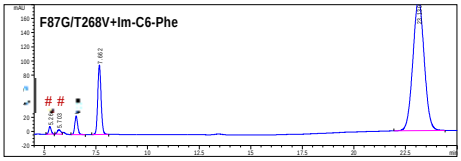
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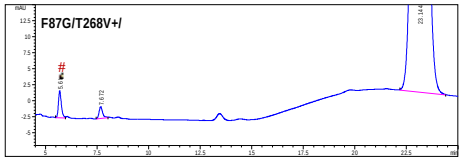
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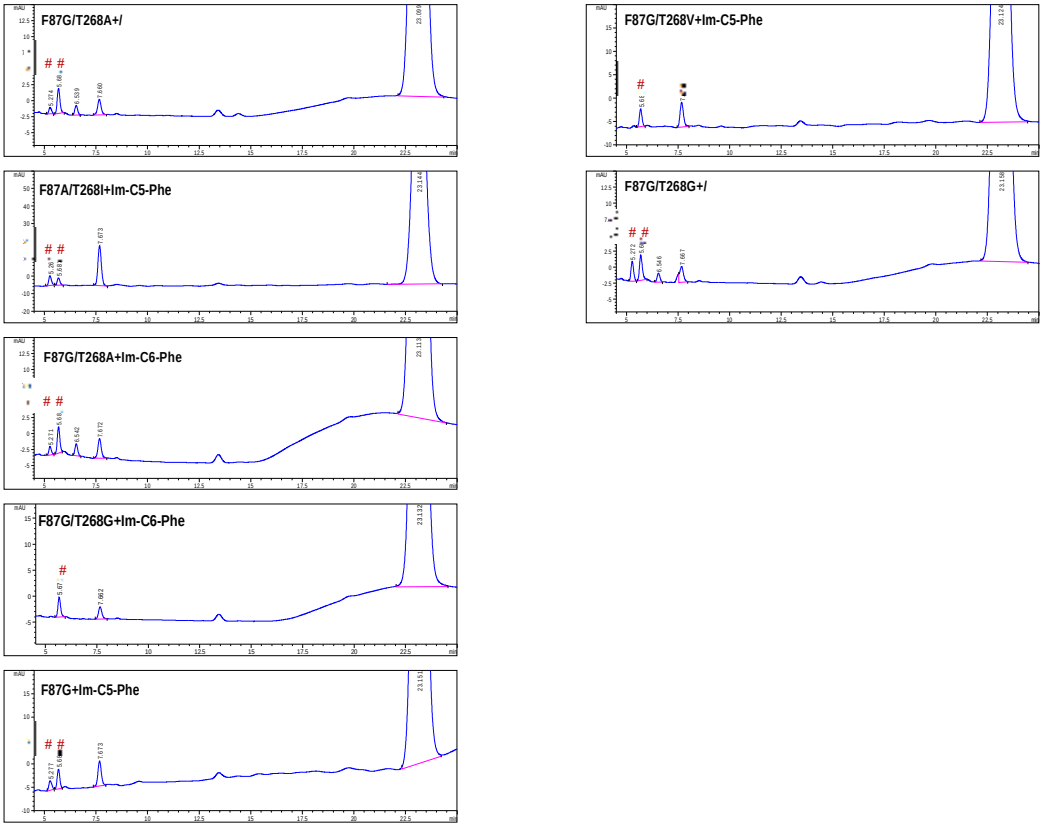
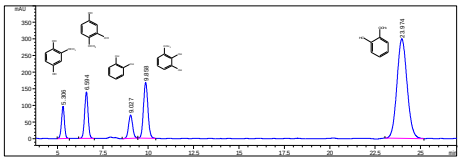
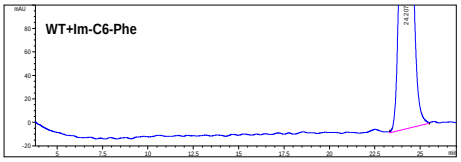
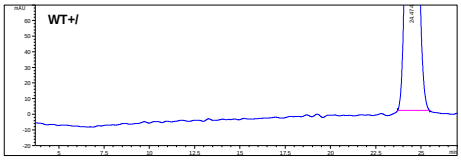


Figure S4 HPLC analyses for the oxidation of 2-Methoxytoluene (**2**) catalyzed by P450BM3 and its mutants in the absence (right column) /presence (left column) of DFSM. *The top HPLC spectrum is for the mixed standard of 4-Methoxy-3-methylphenol (6.55 min), O-cresol (7.69 min) and 2-methoxytoluene (23.32 min). ** The peaks labelled as "#" are from uncertain products.




WT+Im-C6-Phe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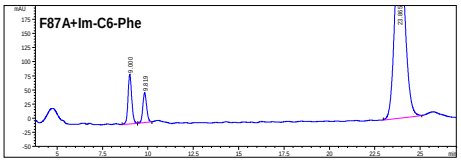
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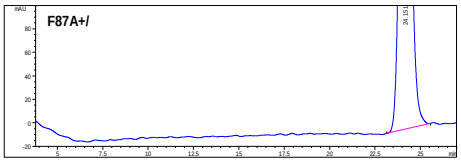
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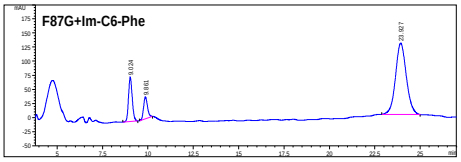
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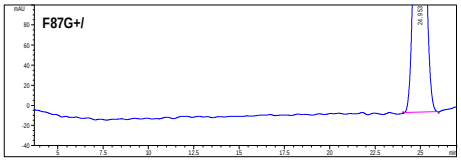
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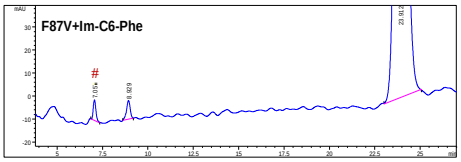
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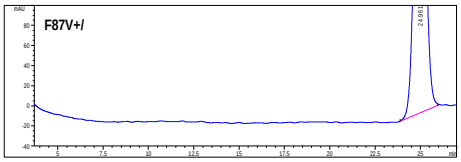
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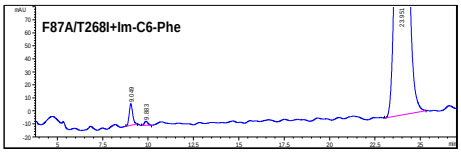
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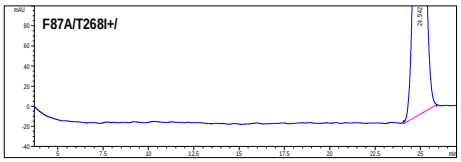
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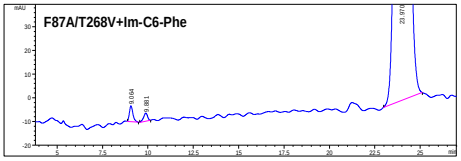
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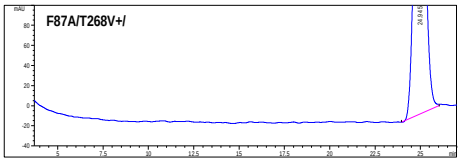
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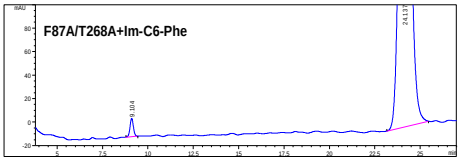
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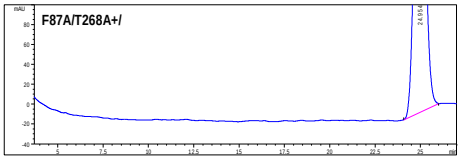
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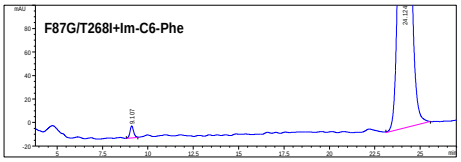
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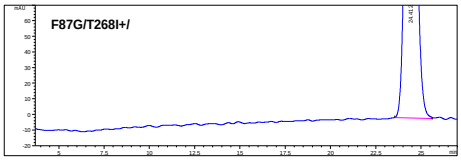
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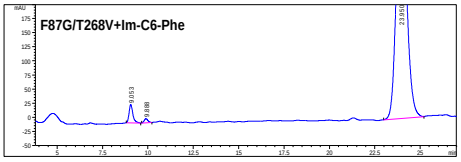
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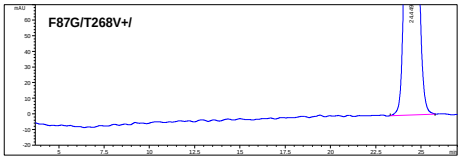
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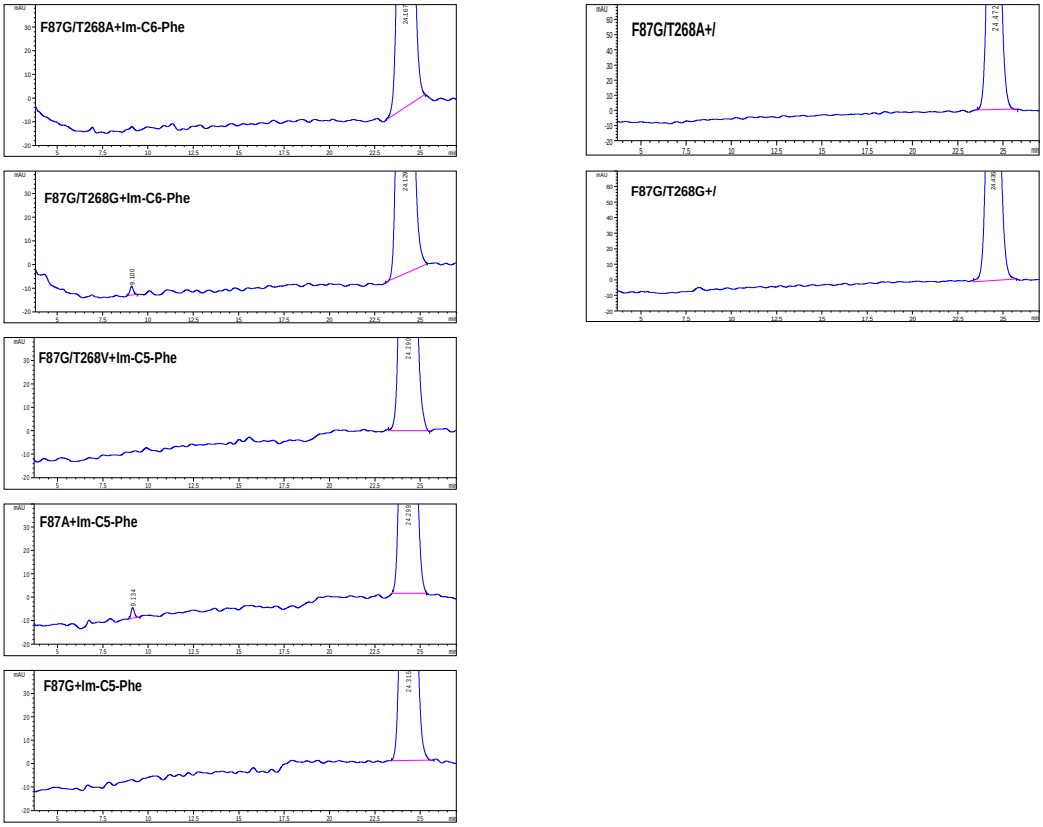
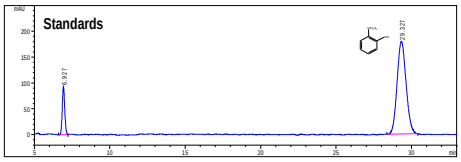
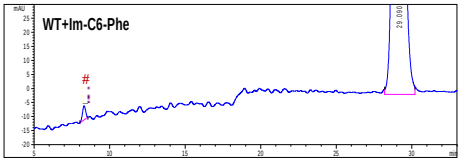
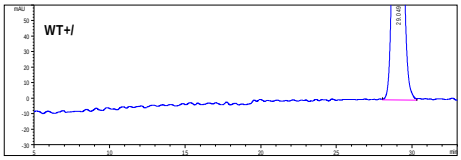


Figure S5 HPLC analyses for the oxidation of guaiacol (**3**) catalyzed by P450BM3 and its mutants in the absence (right column) /presence (left column) of DFSM. *The top HPLC spectrum is for the mixed standard of 2-methoxyhydroquinone (5.31 min), 4-methoxybenzene-1,3-diol (6.59 min), catechol (9.03 min), 3-methoxycatechol (9.86 min) and guaiacol (23.94 min). ** The peaks labelled as "#" are from uncertain products.


Standards


WT+Im-C6-Phe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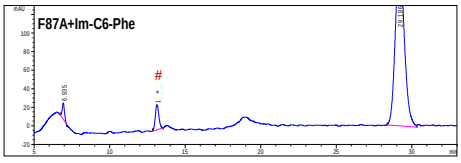
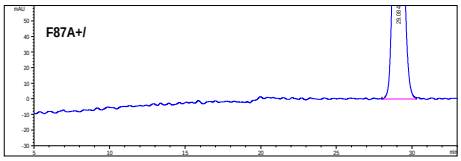

F87A+Im-C6-Phe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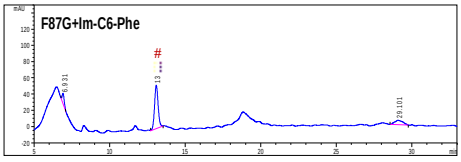
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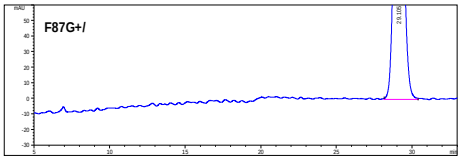
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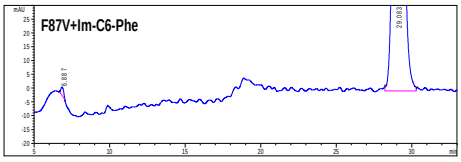
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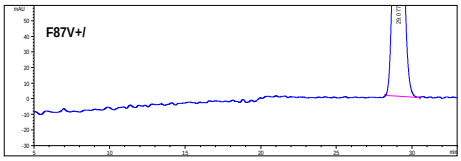
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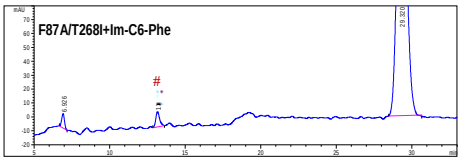
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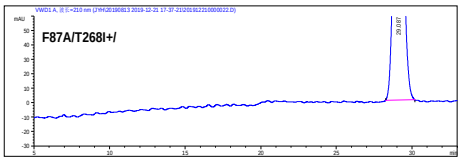
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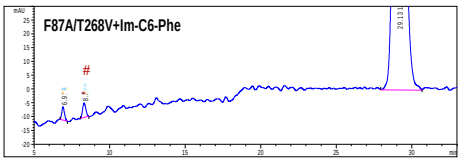
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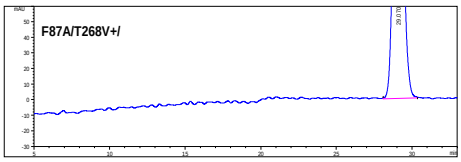
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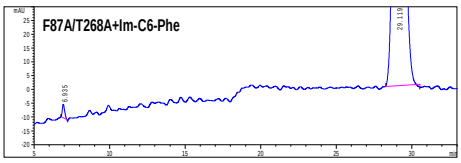
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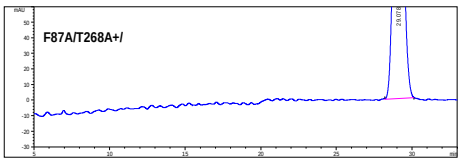
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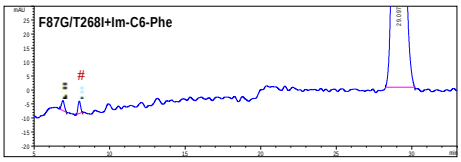
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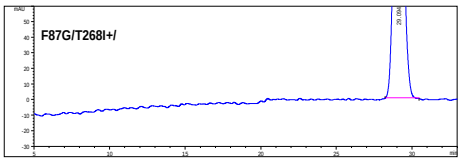
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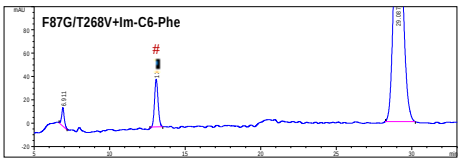
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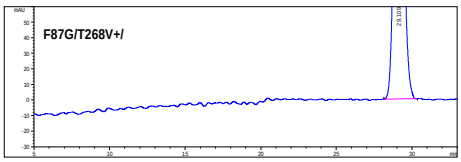
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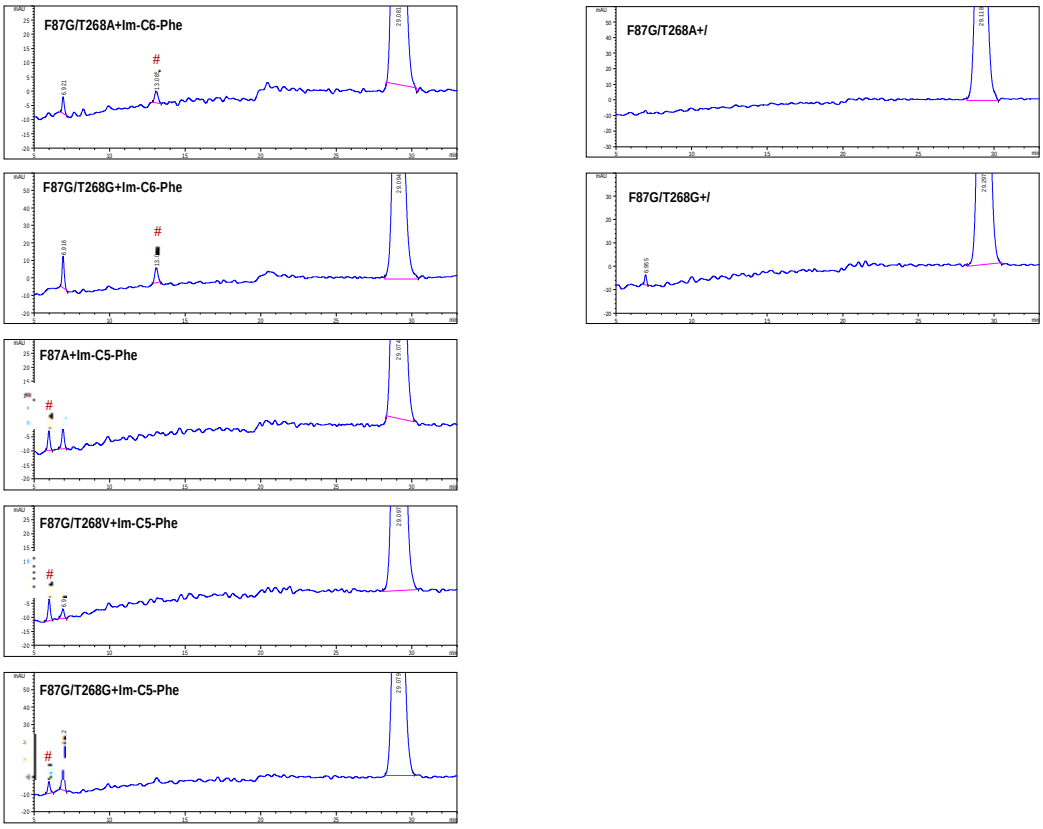
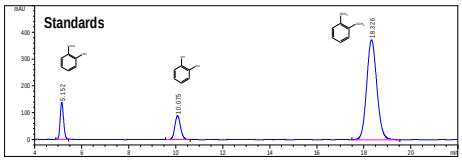
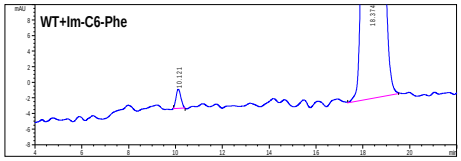
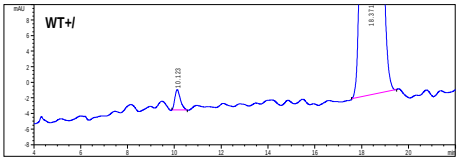
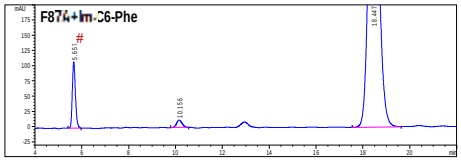


Figure S6 HPLC analyses for the oxidation of 2-ethoxyphenol (**4**) catalyzed by P450BM3 and its mutants in the absence (right column) or presence (left column) of DFSM. *The top HPLC spectrum is for the mixed standard of catechol (6.93 min) and 2-ethoxyphenol (29.33 min). ** The peaks labelled as "#" are from uncertain products.


Standards


WT+Im-C6-Phe


WT+/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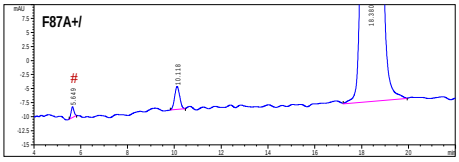
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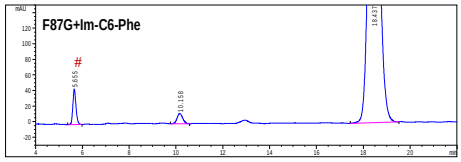
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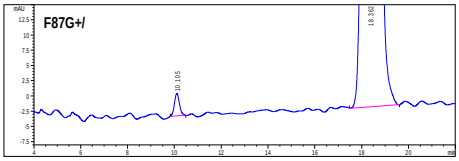
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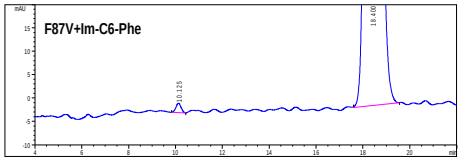
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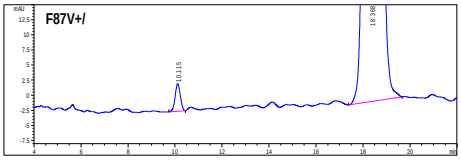
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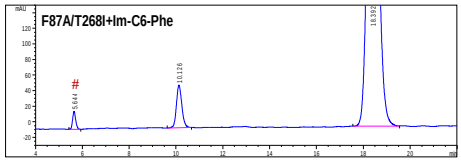
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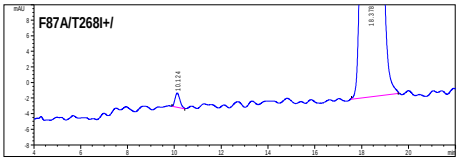
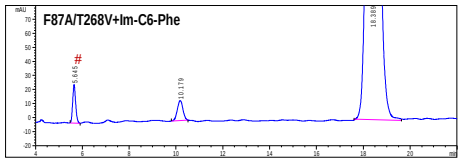

F87A/T268I+/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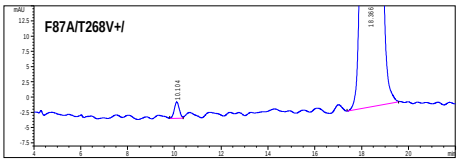
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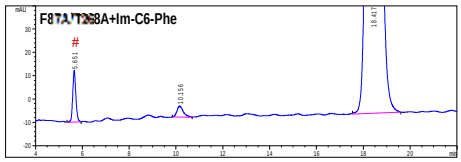
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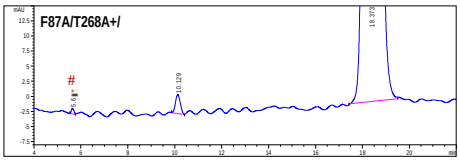
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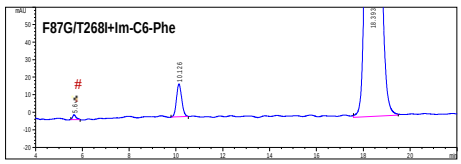
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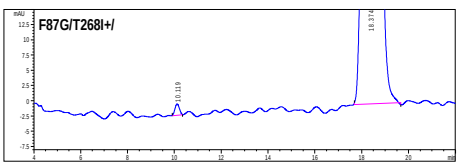
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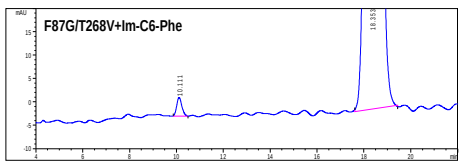
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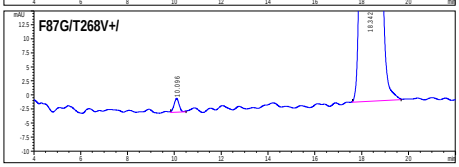
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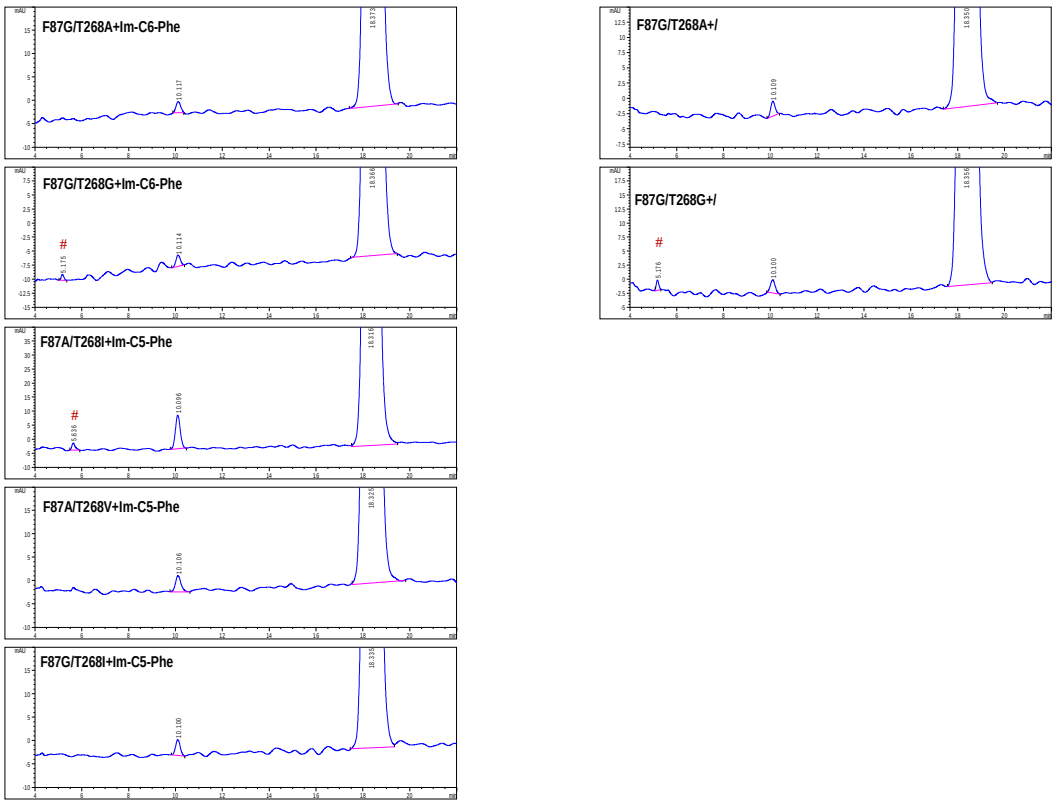
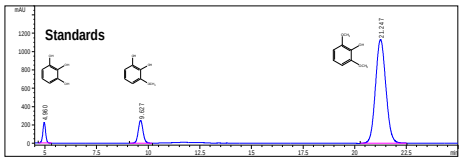
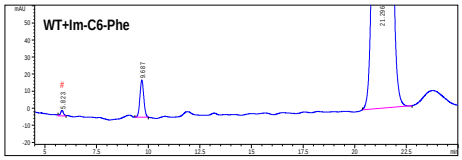
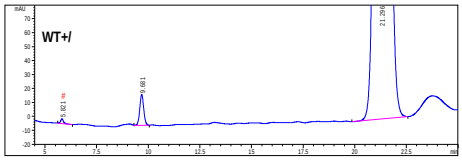
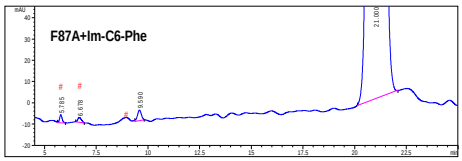


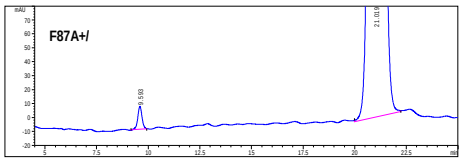
Figure S7 HPLC analyses for the oxidation of 1,2-Dimethoxybenzene (**5**) catalyzed by P450BM3 and its mutants in the absence (right column) /presence (left column) of DFSM. *The top HPLC spectrum is for the mixed standard of catechol (5.12 min), guaiacol (10.08 min) and 1,2-dimethoxybenzene (18.33 min). ** The peaks labelled as "#" are from uncertain products.

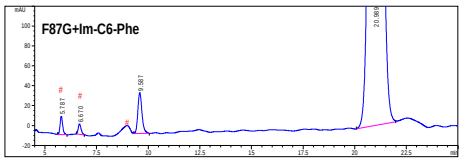

Standards


WT+Im-C6-Phe


WT+/


F87A+Im-C6-Phe


F87A+/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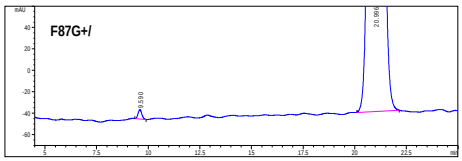
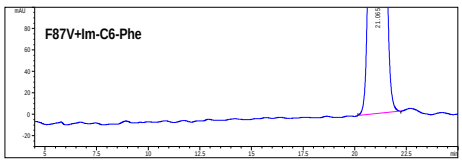

F87G+/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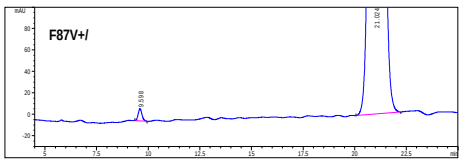
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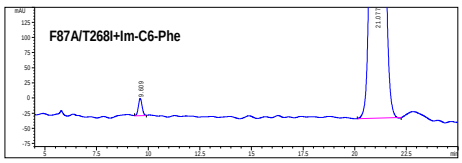
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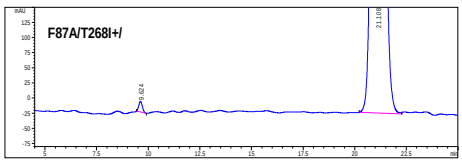
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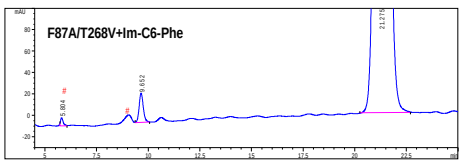
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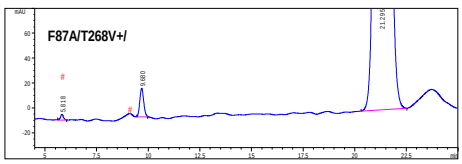
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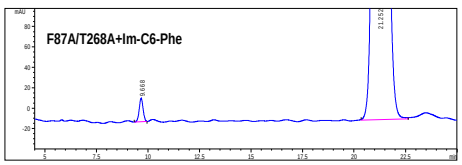
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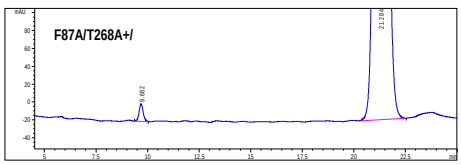
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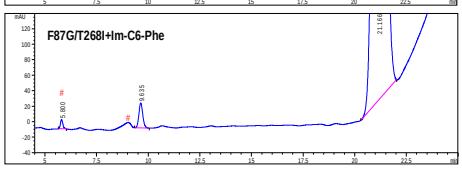
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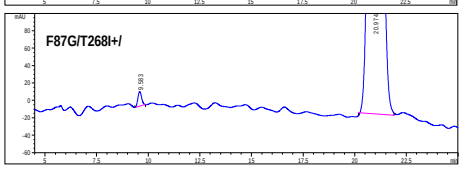
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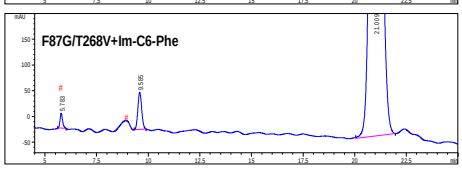
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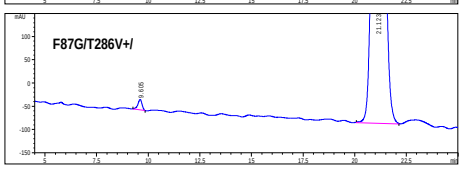
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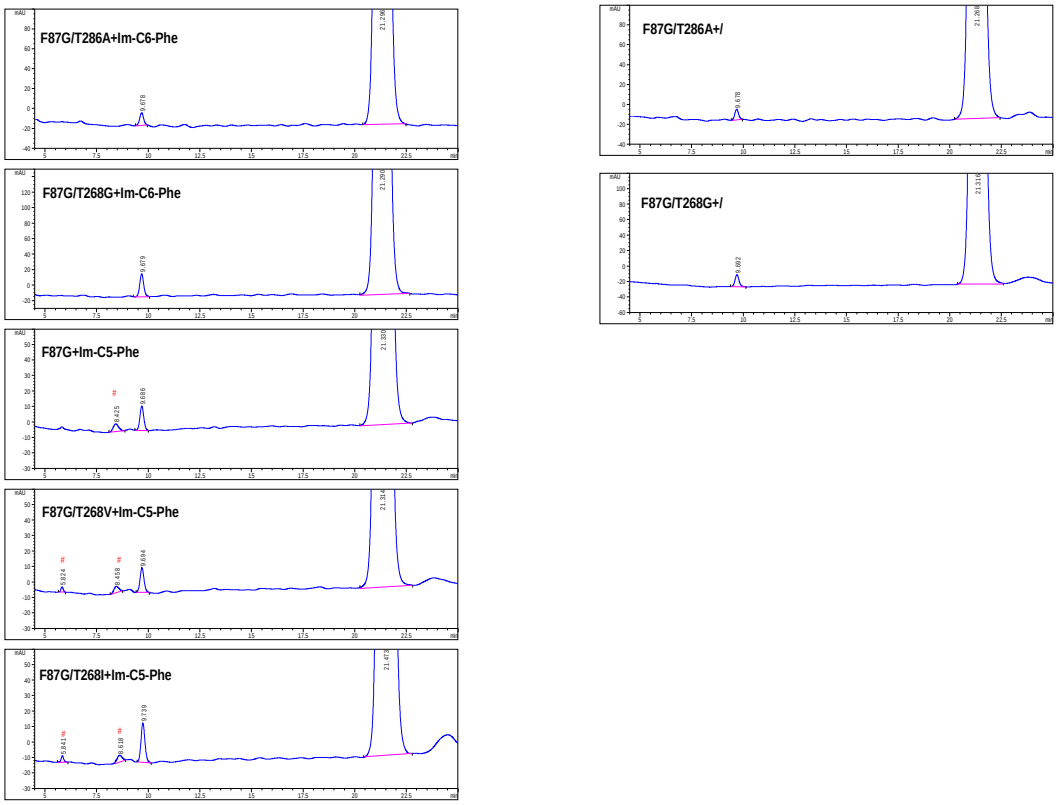

Figure S8 HPLC analyses for the oxidation of 2,6-Dimethoxyphenol (**6**) catalyzed by P450BM3 and its mutants in the absence (right column) or presence (left column) of DFSM. *The top HPLC spectrum is for the mixed standard of pyrogallol (4.96 min)，3-methoxycatechol (9.63 min) and 2,6-dimethoxyphenol (21.25 min). ** The peaks labelled as "#" are from uncertain products.