<u>Supporting Information</u>

Enabling Highly (*R*)-Enantioselective Epoxidation of Styrene by Engineering Unique Non-Natural P450 Peroxygenase

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Experimental Section

Materials

All chemical reagents were purchased from commercial sources (e.g. Aldrich, TCI, Fluka, and Alladin) and used without further purification until otherwise noticed. Styrene was freshly prepared by passing through a short column packed with aluminum oxide. Dual-functional small molecule (DFSM), *N*-(ω-imidazolyl)-hexanoyl-l-phenylalanine (Im-C6-Phe) was synthesized according to our previous report.[1]

Expression and Purification of Cytochrome P450BM3 Enzymes

The full-length of Cytochrome P450BM3 enzymes and its heme domain forms were respectively cultured and purified according to previous report.[1,2] Purified proteins were characterized by SDS pages (Figures S1). The formation of a ferrous CO complex was confirmed by UV/visible spectral change through the reduction of ferric heme of P450BM3 enzymes by addition of $Na_2S_2O_4$ in the presence of carbon monoxide (CO) (Figure S2). [3] The concentrations of P450BM3 and its variants were measured by Hemochrome binding assay.[4,5] A general procedure is shown as below.

A pyridine solution was made by combining pyridine (1.75 mL) and 1 M aqueous of NaOH (0.75 mL). The solution was mixed at room temperature then centrifuged for 30 s at 5000 rpm to remove excess aqueous base. To a cuvette containing 0.75 mL of protein solution in phosphate buffer (0.1 M, pH 8.0), 0.25 mL of the pyridine solution was added followed by 2 mg of sodium dithionite. A UV/vis spectrum was recorded immediately. Hemoprotein concentration was determined from the absorbance of the hemochrome complex using extinction coefficients of ε_{418} = 196 $mM^{/1}$ $cm^{/1}$. Absorbance was assigned as the difference between the peak max at 418 nm and the baseline at 420 nm as determined by extrapolating from two points on either side of the hemochrome peak (390 nm and 450 nm).

Mutagenesis and Recombination

All the mutations were made by PCR based site-directed mutagenesis and verified by DNA sequencing. The single mutants at the position of 87 were prepared according to previous report.[1] The F87 mutants were then used as parent templates to prepare the double mutants containing the positions of 87 and 268, respectively. The F87A/T268I, F87A/T268A mutant was used as parent template to prepare the triple mutants containing another position shown in Figure 3A. The quadruple mutants were respectively prepared by using the corresponding triple mutants as parent template. Beneficial mutations selected from the prepared mutations were recombined. All primers used were as follows.

The parent templates and primers used for the preparation of double mutants.

Parent template	primer	sequence
F87A	T268A-F	5'- GCGACAAGTGGTCTTTTATCATTTGC -3'
	T268V-F	5'- GTGACAAGTGGTCTTTTATCATTTGC -3'
	T268I-F	5'- ATCACAAGTGGTCTTTTATCATTTGC -3'
	T268L-F	5'- CTGACAAGTGGTCTTTTATCATTTGC -3'
	T268F-F	5'- TTCACAAGTGGTCTTTTATCATTTGC -3'
	T268W-F	5'- TGGACAAGTGGTCTTTTATCATTTGC -3'
	T268-R	5'- TTCGTGTCCCGCAATTAAGAATG -3'
F87G	T268V-F	5'- GTGACAAGTGGTCTTTTATCATTTGC -3'
	T268I-F	5'- ATCACAAGTGGTCTTTTATCATTTGC -3'
	T268-R	5'- TTCGTGTCCCGCAATTAAGAATG -3'
F87V	T268V-F	5'- GTGACAAGTGGTCTTTTATCATTTGC -3'
	T268I-F	5'- ATCACAAGTGGTCTTTTATCATTTGC -3'
	T268-R	5'- TTCGTGTCCCGCAATTAAGAATG -3'
F87I	T268V-F	5'- GTGACAAGTGGTCTTTTATCATTTGC -3'
	T268I-F	5'- ATCACAAGTGGTCTTTTATCATTTGC -3'
	T268-R	5'- TTCGTGTCCCGCAATTAAGAATG -3'
F87L	T268V-F	5'- GTGACAAGTGGTCTTTTATCATTTGC -3'
	T268I-F	5'- ATCACAAGTGGTCTTTTATCATTTGC -3'
	T268-R	5'- TTCGTGTCCCGCAATTAAGAATG -3'
BM3	T268V-F	5'- GTGACAAGTGGTCTTTTATCATTTGC -3'
	T268-R	5'- TTCGTGTCCCGCAATTAAGAATG -3'

The parent templates and primers used for the preparation of triple mutants.

primer	sequence
L75M-F	5'- CGATGAAATTTGTACGTGATTTTGCAGGAGAC -3'
L75F-F	5'- CGTTTAAATTTGTACGTGATTTTGCAGGAGAC -3'
L75Q-F	5'- CGCAGAAATTTGTACGTGATTTTGCAGGAGAC -3'
L75K-F	5'- CGAAAAAATTTGTACGTGATTTTGCAGGAGAC -3'
L75F-R	5'- CTTGACTTAAGTTTTTATCAAAGCGTGATTCATCG -3'
V78T-F	5'- CTTAAATTTACCCGTGATTTTGCAGGAGACG -3'
V78M-F	5'- CTTAAATTTATGCGTGATTTTGCAGGAGACG -3'
V78A-F	5'- CTTAAATTTGCACGTGATTTTGCAGGAGACG -3'
V78C-F	5'- CTTAAATTTTGCCGTGATTTTGCAGGAGACG -3'
V78F-F	5'- CTTAAATTTTTTCGTGATTTTGCAGGAGACG -3'
V78I-F	5'- CTTAAATTTATTCGTGATTTTGCAGGAGACG -3'
V78L-F	5'- CTTAAATTTCTGCGTGATTTTGCAGGAGACG -3'
V78S-F	5'- CTTAAATTTAGCCGTGATTTTGCAGGAGACG -3'
V78-R	5'- CGCTTGACTTAAGTTTTTATCAAAGCGTGAT -3'
A82-F	5'- GTTAGCGACAAGCTGGACGCATG -3'
A82G-R	5'- CCGTCTCCACCAAAATCACGTACAAATTTAAG -3'

A82V-R	5’- CCGTCTCCCACAAAATCACGTACAAATTTAAG -3’
A82I-R	5’- CCGTCTCCAATAAAATCACGTACAAATTTAAG -3’
A82L-R	5’- CCGTCTCCCAGAAAATCACGTACAAATTTAAG -3’
A82F-R	5’- CCGTCTCCAAAAAAATCACGTACAAATTTAAG -3’
A82M-R	5’- CCGTCTCCCATAAAATCACGTACAAATTTAAG -3’
A82S-R	5’- CCGTCTCCGCTAAAATCACGTACAAATTTAAG -3’
A82T-R	5’- CCGTCTCCGGTAAAATCACGTACAAATTTAAG -3’
A82E-R	5’- CCGTCTCCTTCAAAATCACGTACAAATTTAAG -3’
A82C-R	5’- CCGTCTCCGCAAAAATCACGTACAAATTTAAG -3’
A82D-R	5’- CCGTCTCCATCAAAATCACGTACAAATTTAAG -3’
A82N-R	5’- CCGTCTCCATTAAAATCACGTACAAATTTAAG -3’
L181F-F	5’- GTGCATTTGATGAAGCAATGAACAAGCTG -3’
L181Q-F	5’- GTGCACAGGATGAAGCAATGAACAAGCTG -3’
L181I-F	5’- GTGCAATTGATGAAGCAATGAACAAGCTG -3’
L181M-F	5’- GTGCAATGGATGAAGCAATGAACAAGCTG -3’
L181T-F	5’- GTGCAACCGATGAAGCAATGAACAAGCTG -3’
L181N-F	5’- GTGCAAATGATGAAGCAATGAACAAGCTG -3’
L181-R	5’- GGACCATACTTGTAATAAATGGATGAGGCT -3’
A184V-F	5’- CACTGGATGAAGTGATGAACAAGCTG -3’
A184I-F	5’- CACTGGATGAAATCATGAACAAGCTG -3’
A184L-F	5’- CACTGGATGAACTGATGAACAAGCTG -3’
A184M-F	5’- CACTGGATGAAATGATGAACAAGCTG -3’
A184F-F	5’- CACTGGATGAATTTATGAACAAGCTG -3’
A184T-F	5’- CACTGGATGAAACCATGAACAAGCTG -3’
A184Q-F	5’- CACTGGATGAACAGATGAACAAGCTG -3’
A184N-F	5’- CACTGGATGAAAATATGAACAAGCTG -3’
A184-R	5’- CACGGACCATACTTGTAATAAATGGATGAG -3’
R255S-F	5’- AGCTATCAAATTATTACATTCTTAATTGCGGG -3’
R255D-F	5’- GATTATCAAATTATTACATTCTTAATTGCGGG -3’
R255V-F	5’- GTGTATCAAATTATTACATTCTTAATTGCGGG -3’
R255L-F	5’- CTGTATCAAATTATTACATTCTTAATTGCGGG -3’
R255Q-F	5’- CAGTATCAAATTATTACATTCTTAATTGCGGG -3’
R255-R	5’- AATGTTCTCGTCATCAAGCGGC -3’
I263-F	5’- GGACACGAAATCACAAGTGGTCTTTTATC -3’
I263V-R	5’- CGCCACTAAGAATGTAATAATTTGATAGCG -3’
I263G-R	5’- CGCGCCTAAGAATGTAATAATTTGATAGCG -3’
A264-F	5’- GAAATCACAAGTGGTCTTTTATCATTTGCG -3’
A264C-R	5’- GTGTCCGCAAATTAAGAATGTAATAATTTGATAG -3’
A264S-R	5’- GTGTCCGCTAATTAAGAATGTAATAATTTGATAG -3’
A264T-R	5’- GTGTCCGGTAATTAAGAATGTAATAATTTGATAG -3’
E267-F	5’- ATCACAAGTGGTCTTTTATCATTTGC -3’
E267Q-R	5’- CTGGTGTCCCGCAATTAAGAATG -3’
E267L-R	5’- CAGGTGTCCCGCAATTAAGAATG -3’

A328V-F	5'- GTGCCTGCGTTTTCCCTATATGC -3'
A328S-F	5'- AGCCCTGCGTTTTCCCTATATGC -3'
A328-R	5'- AGTTGGCCATAAGCGCAGC -3'

The parent templates and primers used for the preparation of quadruple mutants.

Parent template	primer	sequence
F87A/T268I/V78A	A82-F	5'- GTTAGCGACAAGCTGGACGCATG -3'
	A82V-R	5'- CCGTCTCCCACAAAATCACGTACAAATTTAAG -3'
	L181Q-F	5'- GTGCACAGGATGAAGCAATGAACAAGCTG -3'
	L181M-F	5'- GTGCAATGGATGAAGCAATGAACAAGCTG -3'
	L181-R	5'- GGACCATACTTGTAATAAATGGATGAGGCT -3'
	A184L-F	5'- CACTGGATGAACTGATGAACAAGCTG -3'
	A184-R	5'- CACGGACCATACTTGTAATAAATGGATGAG -3'
F87A/T268I/A82V	L181Q-F	5'- GTGCACAGGATGAAGCAATGAACAAGCTG -3'
	L181M-F	5'- GTGCAATGGATGAAGCAATGAACAAGCTG -3'
	L181-R	5'- GGACCATACTTGTAATAAATGGATGAGGCT -3'
	A184L-F	5'- CACTGGATGAACTGATGAACAAGCTG -3'
	A184-R	5'- CACGGACCATACTTGTAATAAATGGATGAG -3'
F87A/T268I/A184L	L181Q-F	5'- GTGCACAGGATGAAGCAATGAACAAGCTG -3'
	L181M-F	5'- GTGCAATGGATGAAGCAATGAACAAGCTG -3'
	L181-R	5'- GGACCATACTTGTAATAAATGGATGAGGCT -3'
F87A/T268I/A184V	A82-F	5'- GTTAGCGACAAGCTGGACGCATG -3'
	A82 V-R	5'- CCGTCTCCCACAAAATCACGTACAAATTTAAG -3'
	A82M-R	5'- CCGTCTCCCATAAAATCACGTACAAATTTAAG -3'
	A82T-R	5'- CCGTCTCCGGTAAAATCACGTACAAATTTAAG -3'
F87A/T268I/A184I	A82-F	5'- GTTAGCGACAAGCTGGACGCATG -3'
	A82V-R	5'- CCGTCTCCCACAAAATCACGTACAAATTTAAG -3'
	A82M-R	5'- CCGTCTCCCATAAAATCACGTACAAATTTAAG -3'
	A82T-R	5'- CCGTCTCCGGTAAAATCACGTACAAATTTAAG -3'

General procedure for epoxidation of styrene catalyzed by full-length P450BM3

The full-length P450BM3 enzymes (0.5 μM) was transferred to a glass sample bottle containing 0.1 M, pH 8.0 phosphate buffer, styrene (4 mM, dissolved in methanol). The reaction was initiated by the addition of NADPH (2 mM, dissolved in pH 8.0 phosphate buffer). The reaction mixture was incubated in water bath at 25 ℃ for 30 min. The reaction mixture was neutralized and extracted with 1 mL of hexane (or ethyl acetate), and the organic phase was separated and dried with sodium sulphate anhydrous. The formation of styrene oxide and benzene acetaldehyde were identified according to the retention time of authentic samples by gas chromatography (GC). The catalytic turnover numbers (TON) of unreacted styrene and epoxide product were determined by using benzophenone as an internal standard according to the calibration curves prepared with the authentic samples (Figure S18). The optical purity of styrene oxide was determined with HPLC or GC.

General procedure for epoxidation of styrenes by H_2O_2-dependent P450BM3 (with styrene as an example)

The heme domains of P450BM3 variants (0.5 μM) were transferred to a glass sample bottle containing 0.1 M, pH 8.0 phosphate buffer, styrene (4 mM, dissolved in methanol), without or with Im-C6-Phe (2 mM, dissolved in pH 8.0 phosphate buffer). The reaction was initiated by the addition of H_2O_2 (80 mM, dissolved in pH 8.0 phosphate buffer). The reaction mixture was incubated in water bath at 25 ℃ or 4 ℃ for 30 min. The reaction mixture was neutralized and extracted with 1 mL of hexane (or ethyl acetate), and the organic phase was separated and dried with sodium sulphate anhydrous. The formation of epoxides and the corresponding acetaldehydes were identified according to the retention time of authentic samples by gas chromatography (GC). The catalytic turnover numbers (TON) of unreacted styrenes and the corresponding epoxide products were determined by using benzophenone as an internal standard according to the calibration curves prepared with the authentic samples (Figure S18). The optical purity of styrene oxide was determined with chiral HPLC or GC.

General procedure for Semi-preparative scale synthesis of (*R*)-styrene oxide by the DFSM-facilitated P450BM3 peroxygenase system.

2.5 μM heme domain of F87A/T268I/V78A/A82V (6 μM in the case of F87A/T268I/L181Q) was transferred to a glass flask containing 20 mL 0.1 M pH 8.0 phosphate buffer, 10 mM styrene (dissolved in 2% methanol) and 2 mM Im-C6-Phe. The reaction was initiated by the addition of 80 mM H_2O_2 and incubated at 0 °C for 30 min. The same amount of enzyme was added to the reaction mixture and incubated for another 30 min. 1mL reaction mixture was taken and extracted with 1 mL ethyl acetate, then the organic phase was separated and dried with anhydrous sodium sulphate. The concentration of unreacted styrene and the optical purity of styrene oxide formed were determined by gas chromatography (GC) and chiral GC, respectively.

The untreated reaction mixture was extracted with dichloromethane (10 mL x 3) for three times, the combined organic phase was dried with anhydrous sodium sulphate, filtered and concentrated. The crude product was purified by flash chromatography to give styrene oxide as colorless oil liquid (F87A/T268I/V78A/A82V: 13.0 mg, 54.2% yield; F87A/T268I/L181Q: 10.5 mg, 43.8% yield). ^{1}H NMR (600 MHz $CDCl_3$): δ 7.26~7.36 (m, 5H), 3.86-3.87 (t, J = 3.86, 1H), 3.14-3.16 (t, J = 3.15, 1H), 2.80-2.81 (dd, J = 2.81, 1H).

Instruments and Analytical Conditions

GC: The product analysis was performed on a Shimadzu GC-2010 plus gas chromatograph equipped with a DB-5 column (length: 30 m, internal diameter: 0.25 mm, film thickness: 1μm, Agilent, USA), a flame ionization detector, and an AOC/20i auto sampler system. The analytical conditions were as follows:

splitting ratio: 1/9, temperature program: injector 260 ºC, detector 300 ºC, 100 ºC oven for 1min, then 15 ºC/min gradient to 200 ºC, 60 ºC /min gradient to 280 ºC for 8 min (total 17 min).

Chiral GC: The chiral analysis was performed on a Shimadzu GC-2030 plus gas chromatograph equipped with a Astec CHIRALDEX G-TA column (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.12 μm, Germany, GER), a flame ionization detector, and an AOC/20i auto sampler system.

The analytical conditions of styrene epoxide, 2-fluorostyrene epoxide and 4-fluorostyrene epoxide were as follows:

splitting ratio: 1/9, temperature program: injector 200 ºC, detector 200 ºC, 80 ºC oven for 3 min, then 10 ºC/min gradient to 100 ºC for 5 min, 5 ºC /min gradient to 105 ºC for 7 min, 60 ºC/min gradient to 170 ºC for 3 min (total 19.08 min).

The analytical condition of 2-chlorostyrene epoxide was as follows:

splitting ratio: 1/9, temperature program: injector 200 ºC, detector 200 ºC, 80 ºC oven for 3 min, then 10 ºC/min gradient to 100 ºC for 5 min, 5 ºC /min gradient to 140 ºC for 2.5 min, 60 ºC/min gradient to 170 ºC for 2 min (total 20 min).

The analytical condition of 3-chlorostyrene epoxide was as follows:

splitting ratio: 1/9, temperature program: injector 200 ºC, detector 200 ºC, 80 ºC oven for 3 min, then 10 ºC/min gradient to 100 ºC for 5 min, 10 ºC /min gradient to 170 ºC for 10 min (total 24 min).

The analytical condition of 3-fluorostyrene epoxide was as follows:

splitting ratio: 1/9, temperature program: injector 200 ºC, detector 200 ºC, 80 ºC oven for 3 min, then 10 ºC/min gradient to 100 ºC for 5 min, 5 ºC /min gradient to 120 ºC for 2 min, 5 ºC /min gradient to 135 ºC, 60 ºC/min gradient to 170 ºC for 2 min (total 18.58 min).

Chiral HPLC: The chiral analysis was performed on a Hitachi plus HPLC chromatograph equipped with a Chiralpak AD-3 column (250 × 4.6mm, DAICEL), The analytical condition of 4-chlorostyrene was as follows:

splitting rate： 1.0 mL/min, mobile phase： hexane 100%, oven temperature: 25 ºC, UV detector:200 nm, Injection volume: 10 µL.

References

[1] Ma, N.; Chen, Z.; Chen, J.; Chen, J.; Wang, C.; Zhou, H.; Yao, L.; Shoji, O.; Watanabe, Y.; Cong, Z. Dual/functional small molecules for generating an efficient cytochrome P450BM3 peroxygenase. *Angew. Chem. Int. Ed.* **2018**, *57*, 7628/7633.

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[3] Omura, T.; Sato, R. The carbon monoxide/binding pigment of liver microsomes. I. evidence for its hemoprotein nature. *J. Biol. Chem.* **1964**, *239*, 2370/2378.

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[5] Chen, J.; Kong, F.; Ma, N.; Zhao, P.; Liu, C.; Wang, X.; Cong, Z. Peroxide-driven hydroxylation of small alkanes catalyzed by an artificial P450BM3 peroxygenase system. *ACS Catal.* **2019**, *9*, 7350-7355.

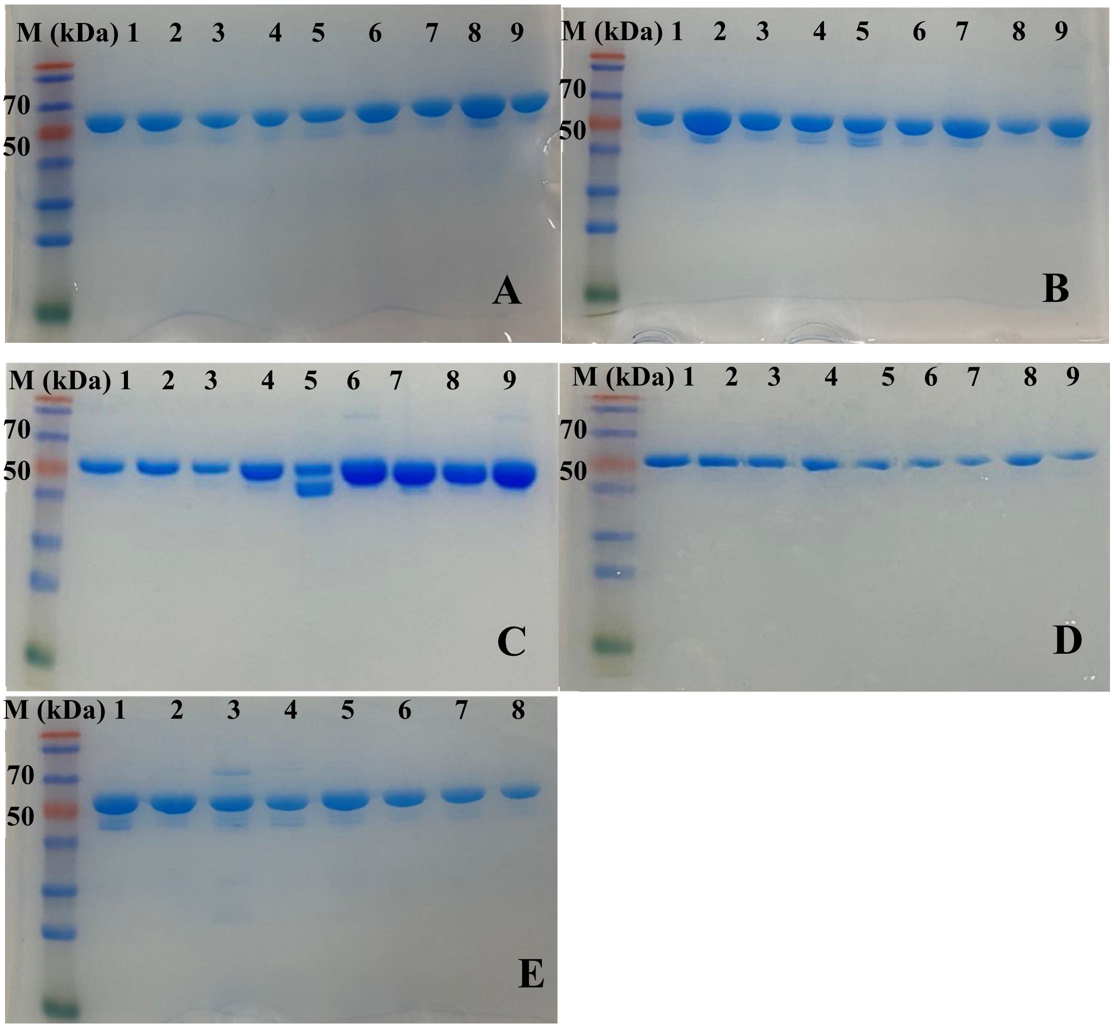
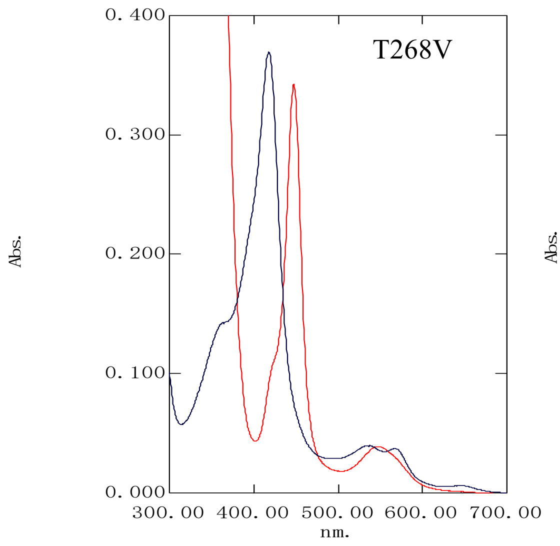


Figure S1 SDS Page of P450BM3 variants. A) Lane 1-9: F87A/T268I/L75M, F87A/T268I/L75Y, F87A/T268I/L75F, F87A/T268I/L75Q, F87A/T268I/L75K, F87A/T268I/L75T, F87A/T268I/V78T, F87A/T268I/V78M, F87A/T268I/V78A. B) Lane 1-9: F87A/T268I/V78C, F87A/T268I/V78F, F87A/T268I/V78I, F87A/T268I/V78L, F87A/T268I/V78S, F87A/T268I//L181F, F87A/T268I//L181Q, F87A/T268I/L181I, F87A/T268I/L181M. C) Lane 1-9: F87A/T268I/L181T, F87A/T268I/L181N, F87A/T268I/R255S, F87A/T268I/R255D, F87A/T268I/R255V, F87A/T268I/R255L, F87A/T268I/R255Q, F87A/T268I/I263V, F87A/T268I/I263Q. D) Lane 1-9: F87A/T268I/V78A/A82V, F87A/T268I/V78A/L181Q, F87A/T268I/V78A/L181M, F87A/T268I/V78A/A184L, F87A/T268I/A82V/L181Q, F87A/T268I/A82V/L181M, F87A/T268I/A82V/A184L, F87A/T268I/L181Q/A184L, F87A/T268I/L181M/A184L. E) Lane 1-8: F87A/T268I/A264C, F87A/T268I/A264S, F87A/T268I/A264T, F87A/T268I/E267Q, F87A/T268I/E267L, F87A/T268I/A328V, F87A/T268I/A328S, T268V. Lane M: molecular mass standards.


T268V
Abs.
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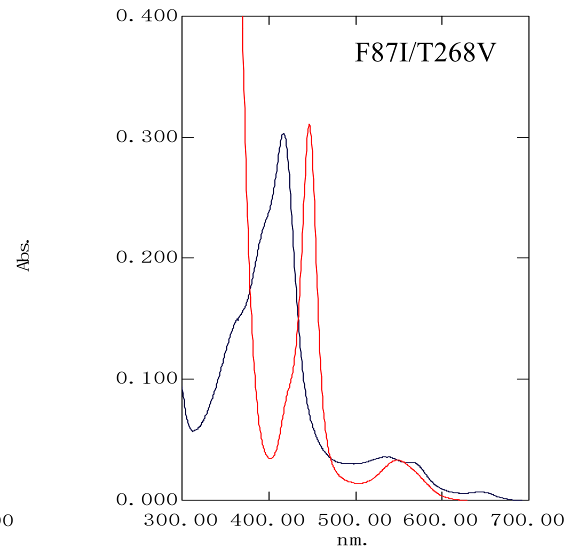
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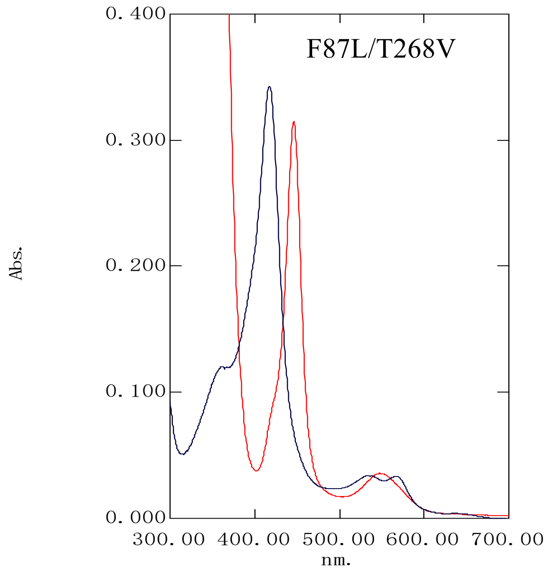
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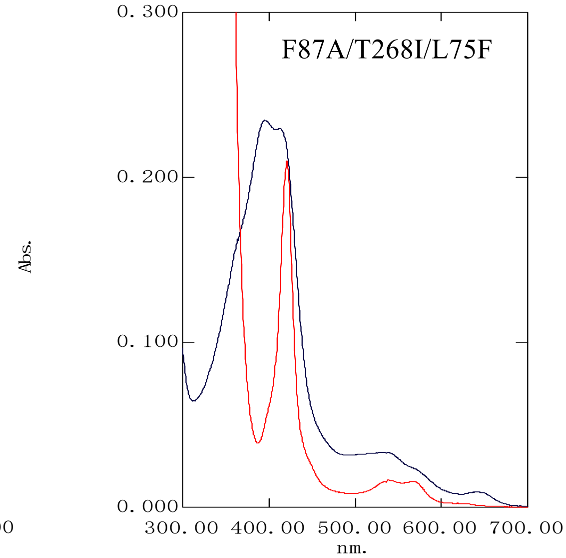
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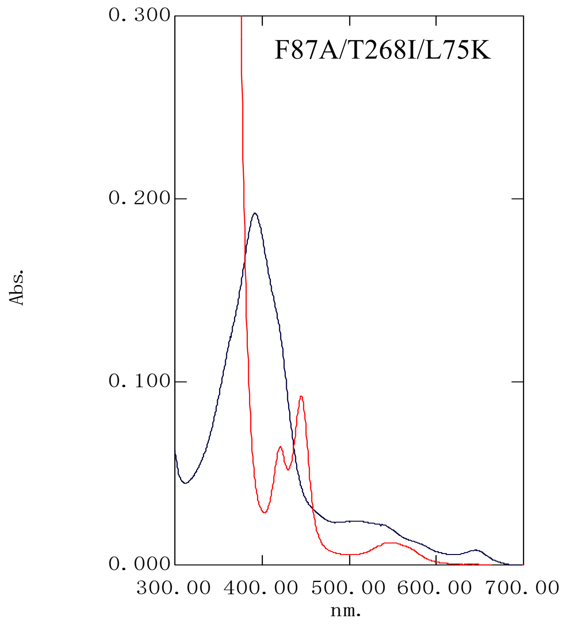
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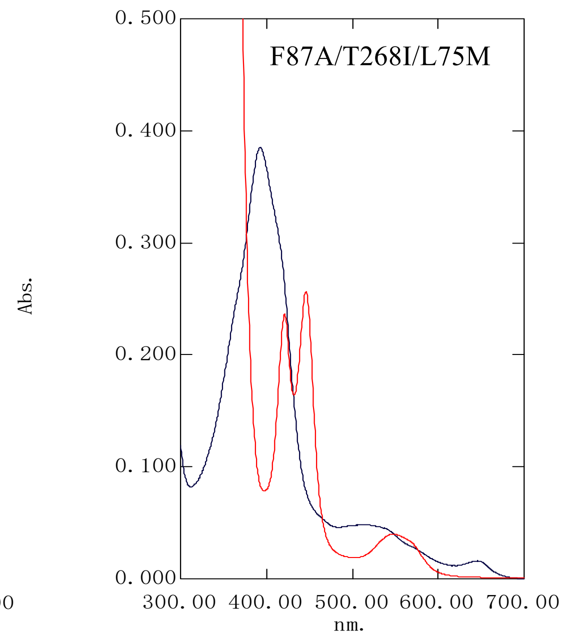
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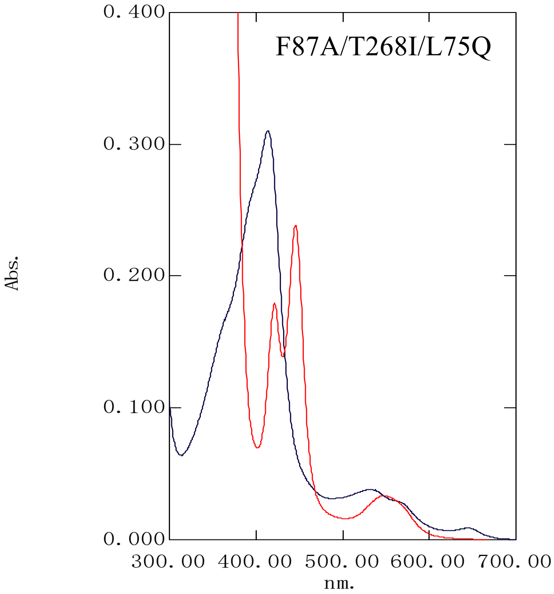
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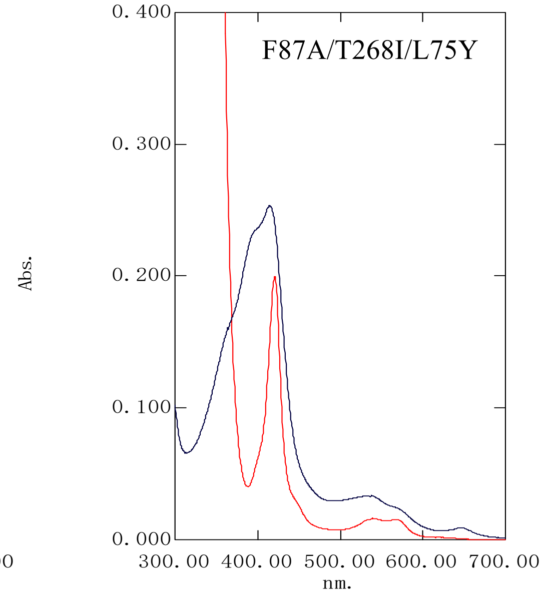
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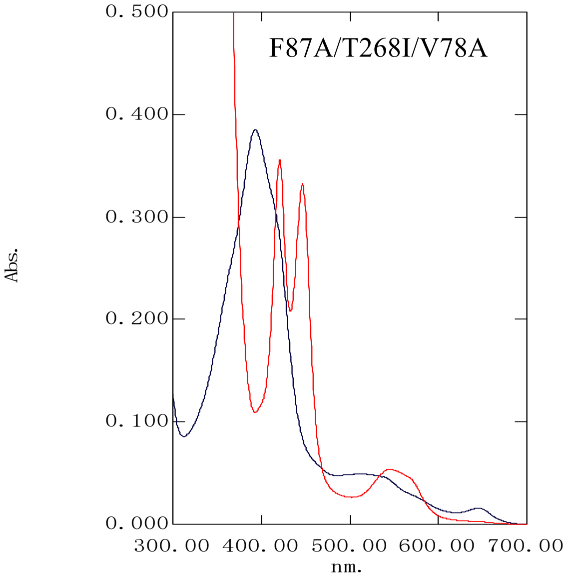
F87A/T268I/V78A
Abs.
0. 500
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0. 300
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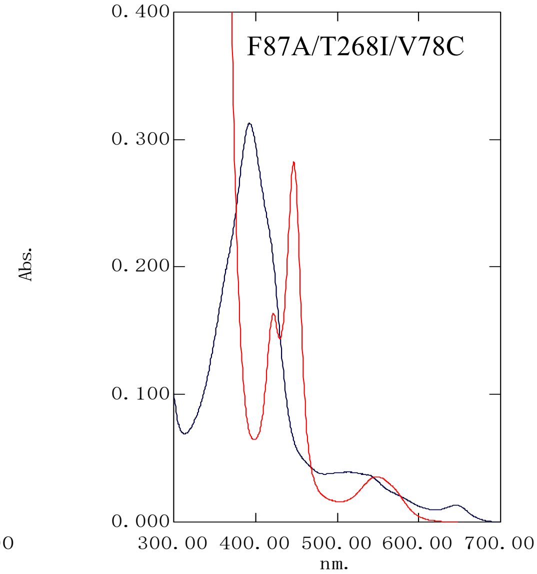
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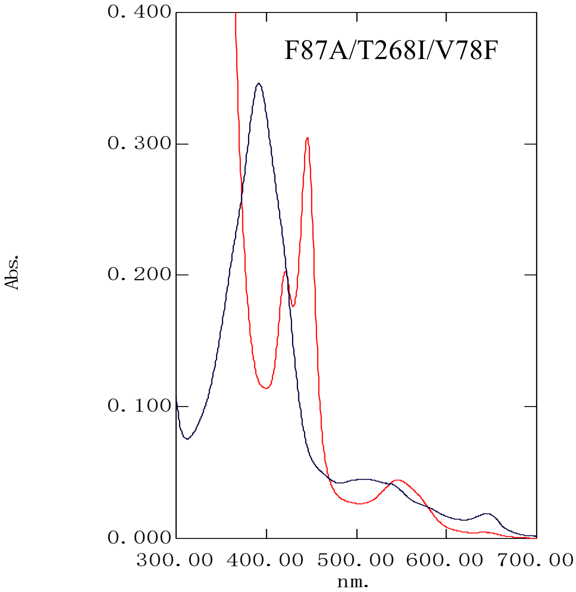
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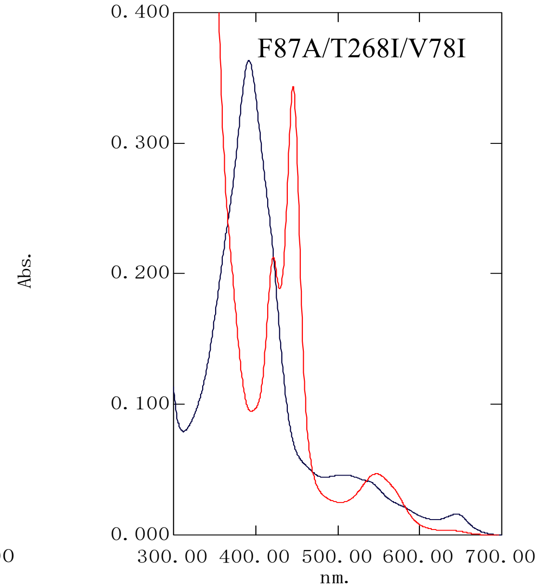
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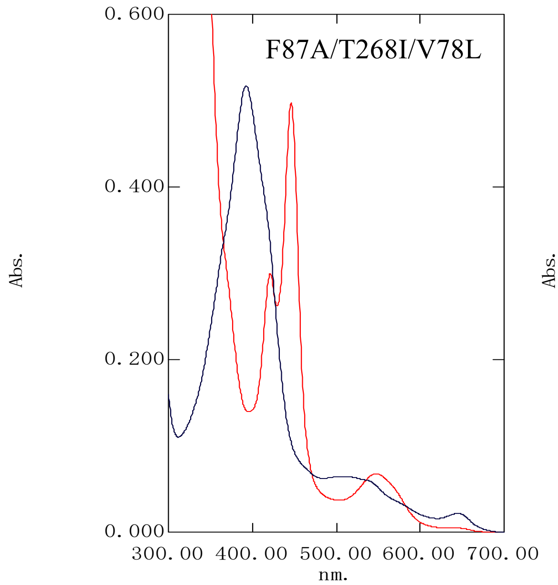
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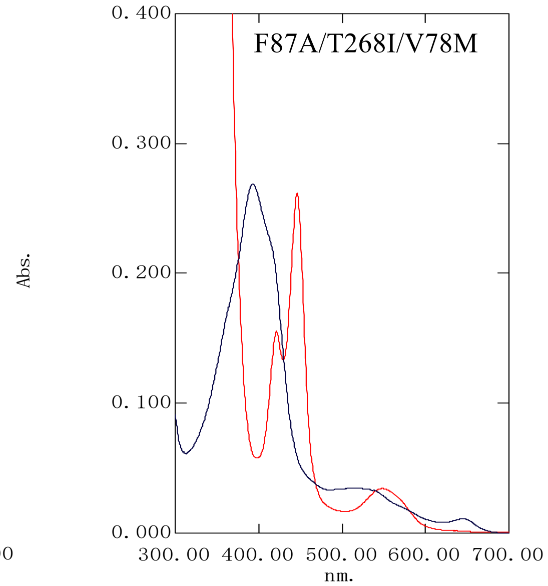
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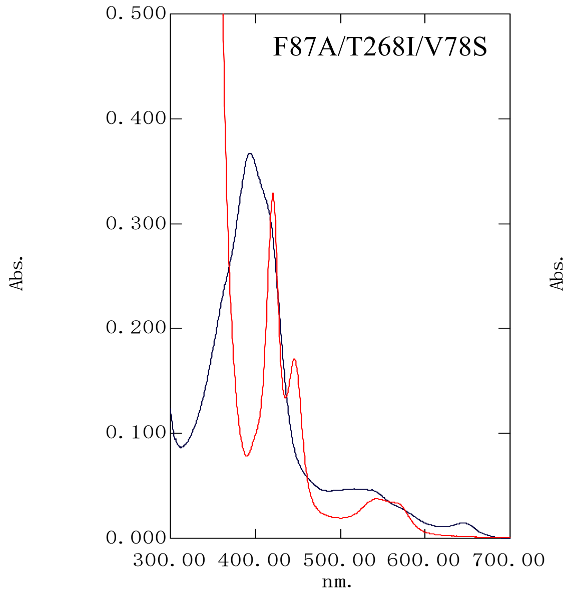
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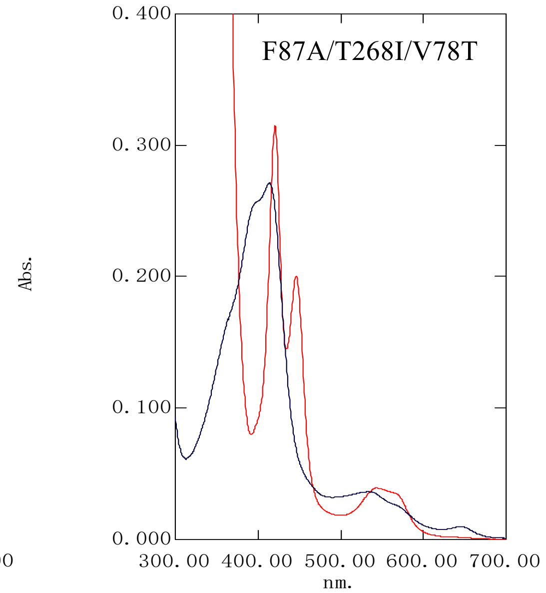
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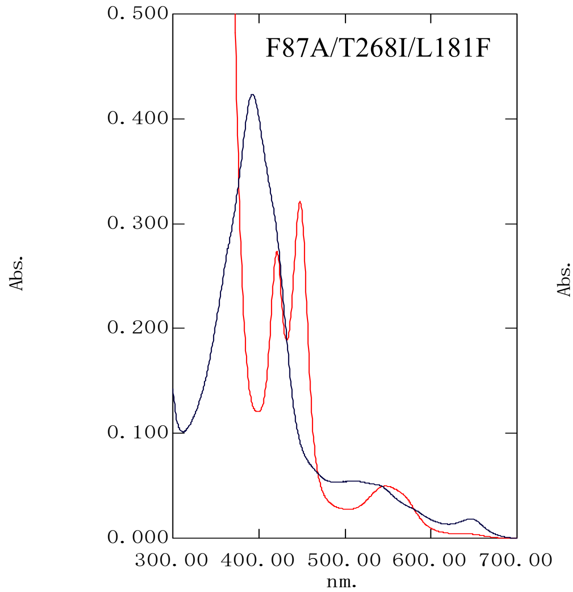
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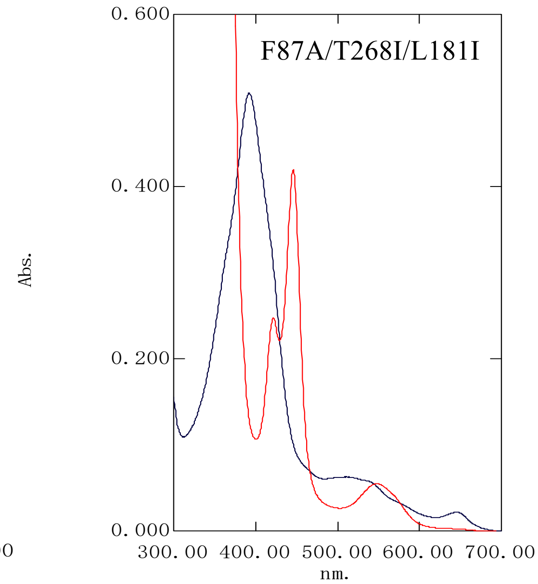
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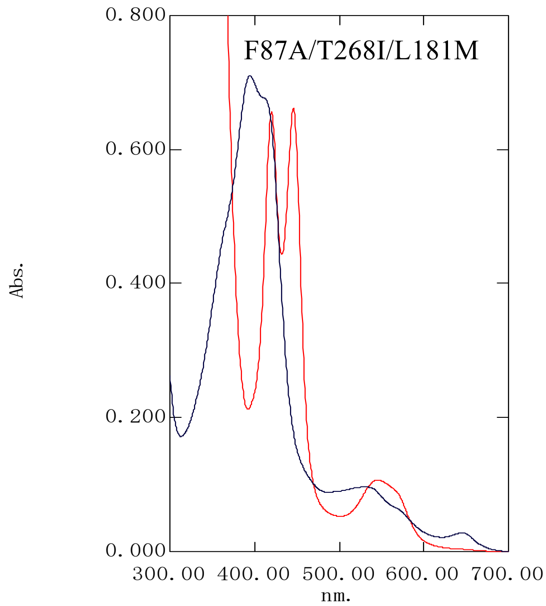
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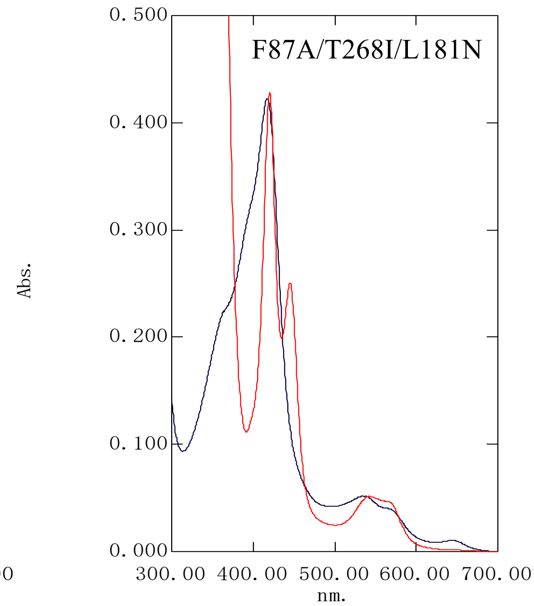
nm.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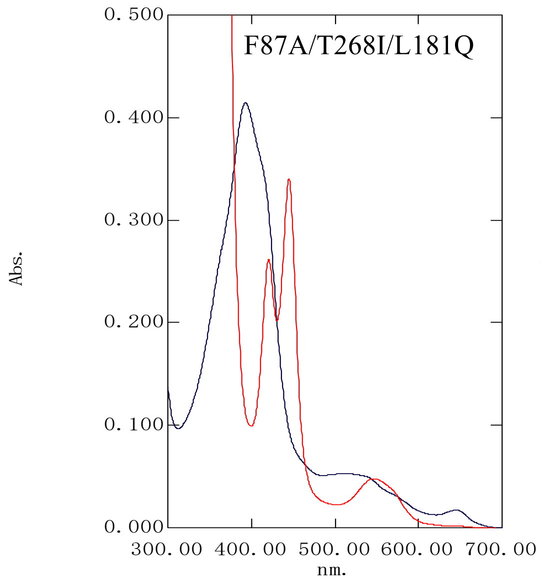
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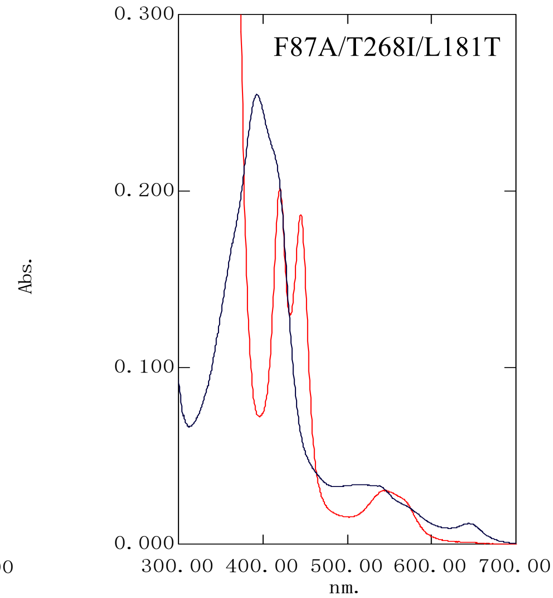
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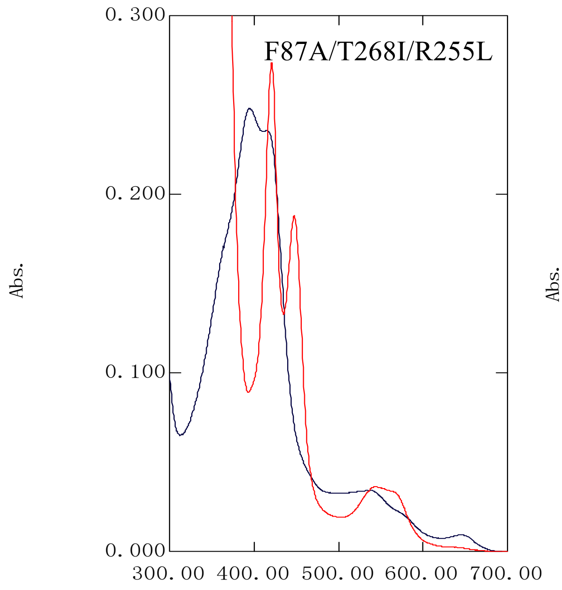
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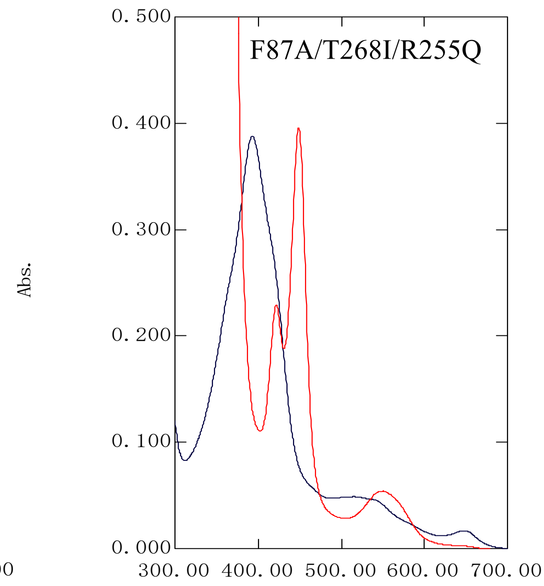
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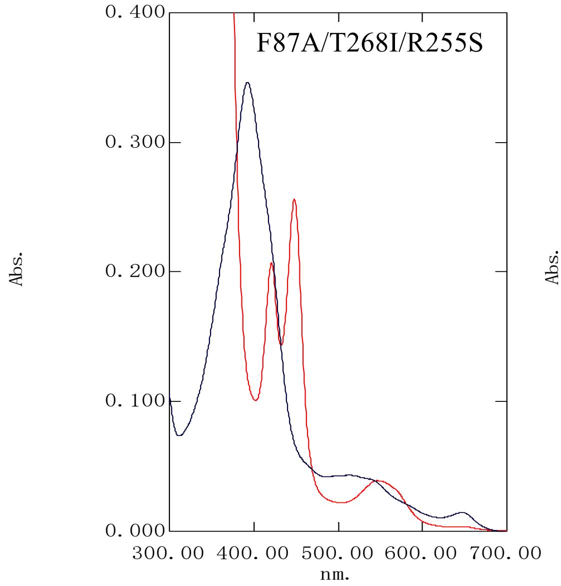
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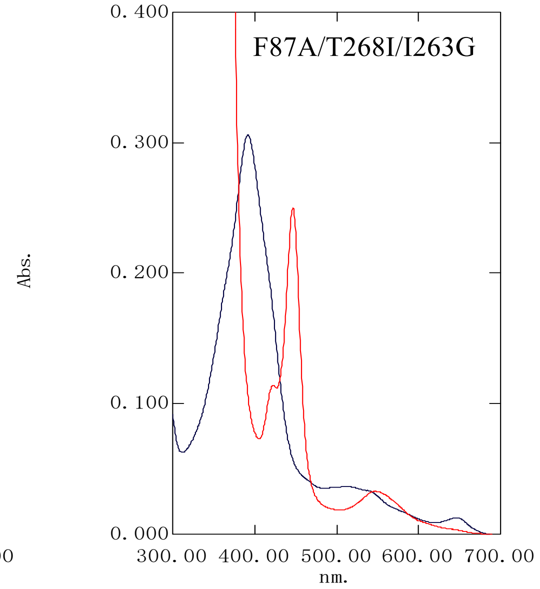
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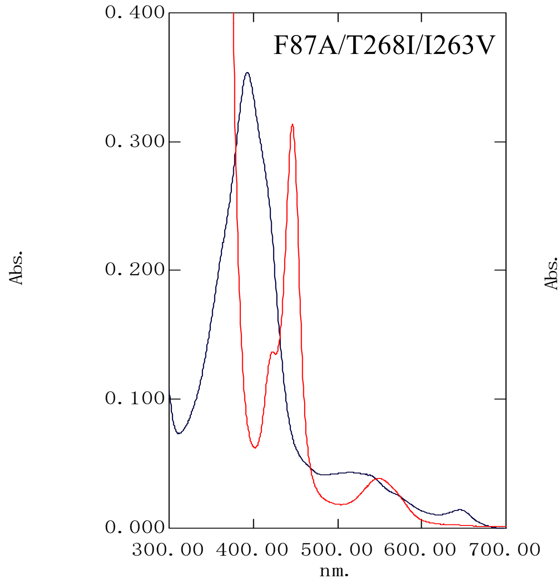
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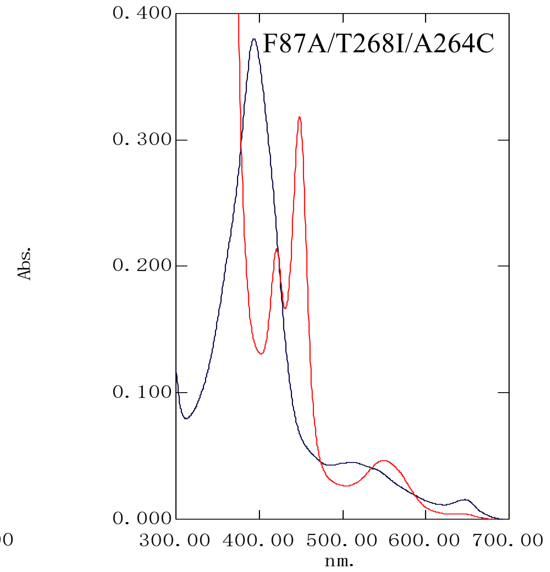
nm.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Abs.
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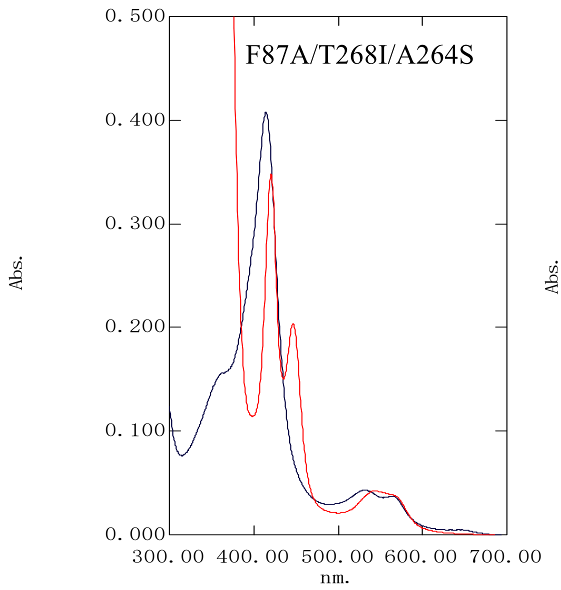
nm.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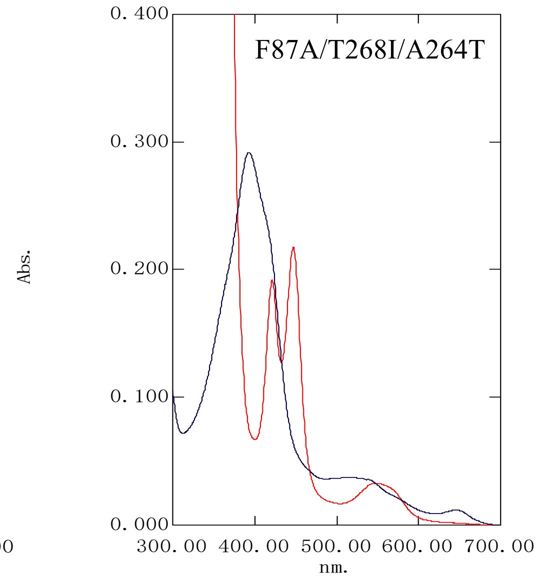
nm.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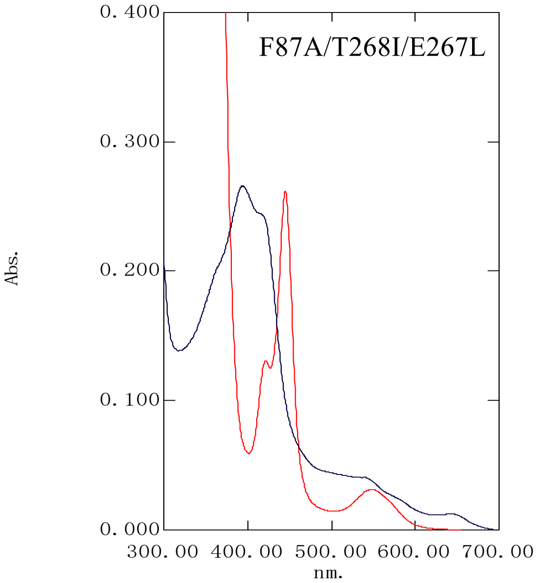
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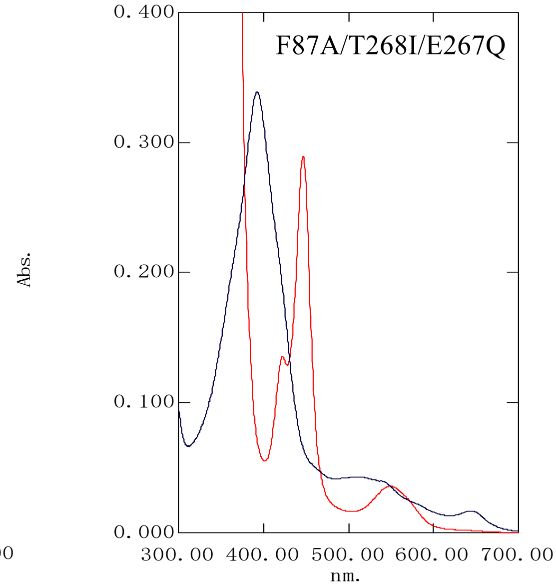
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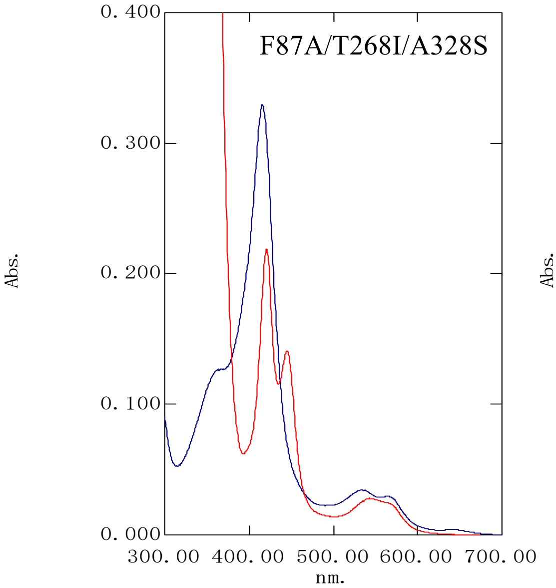
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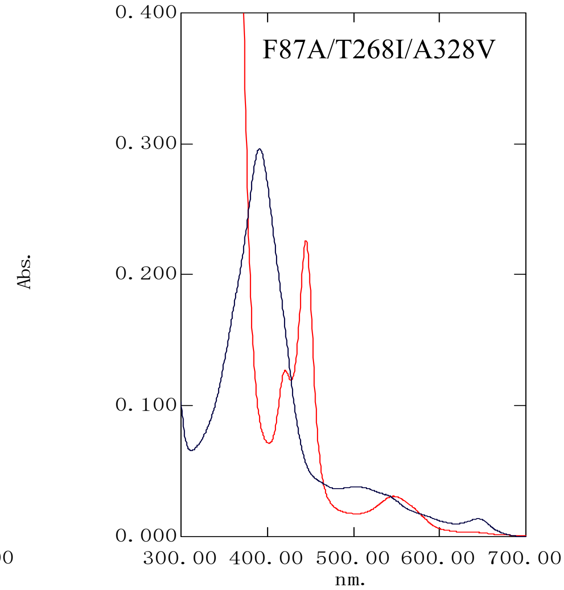
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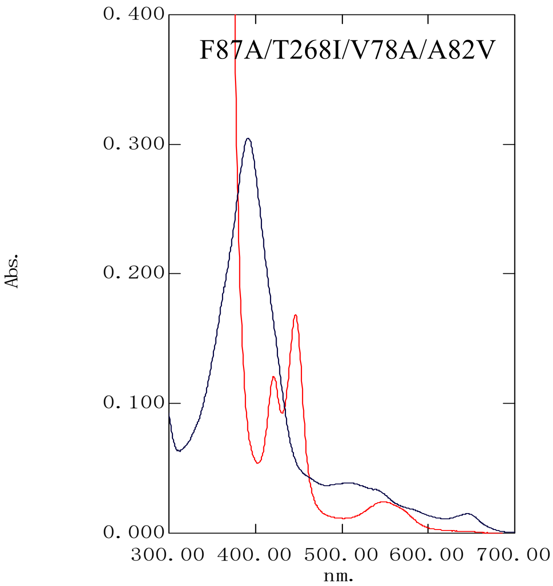
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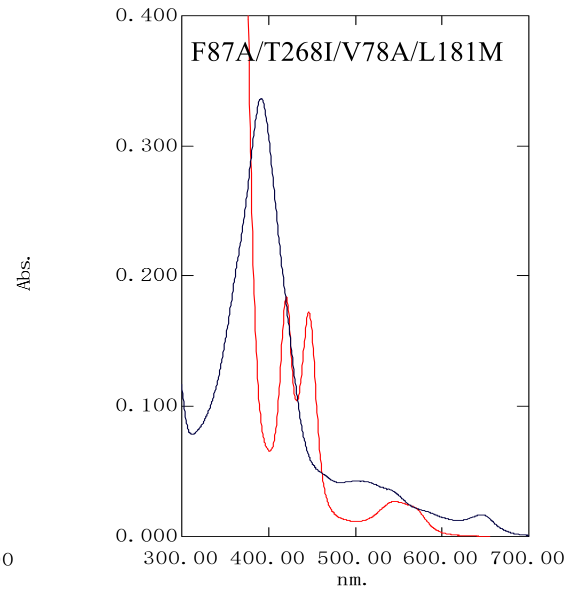
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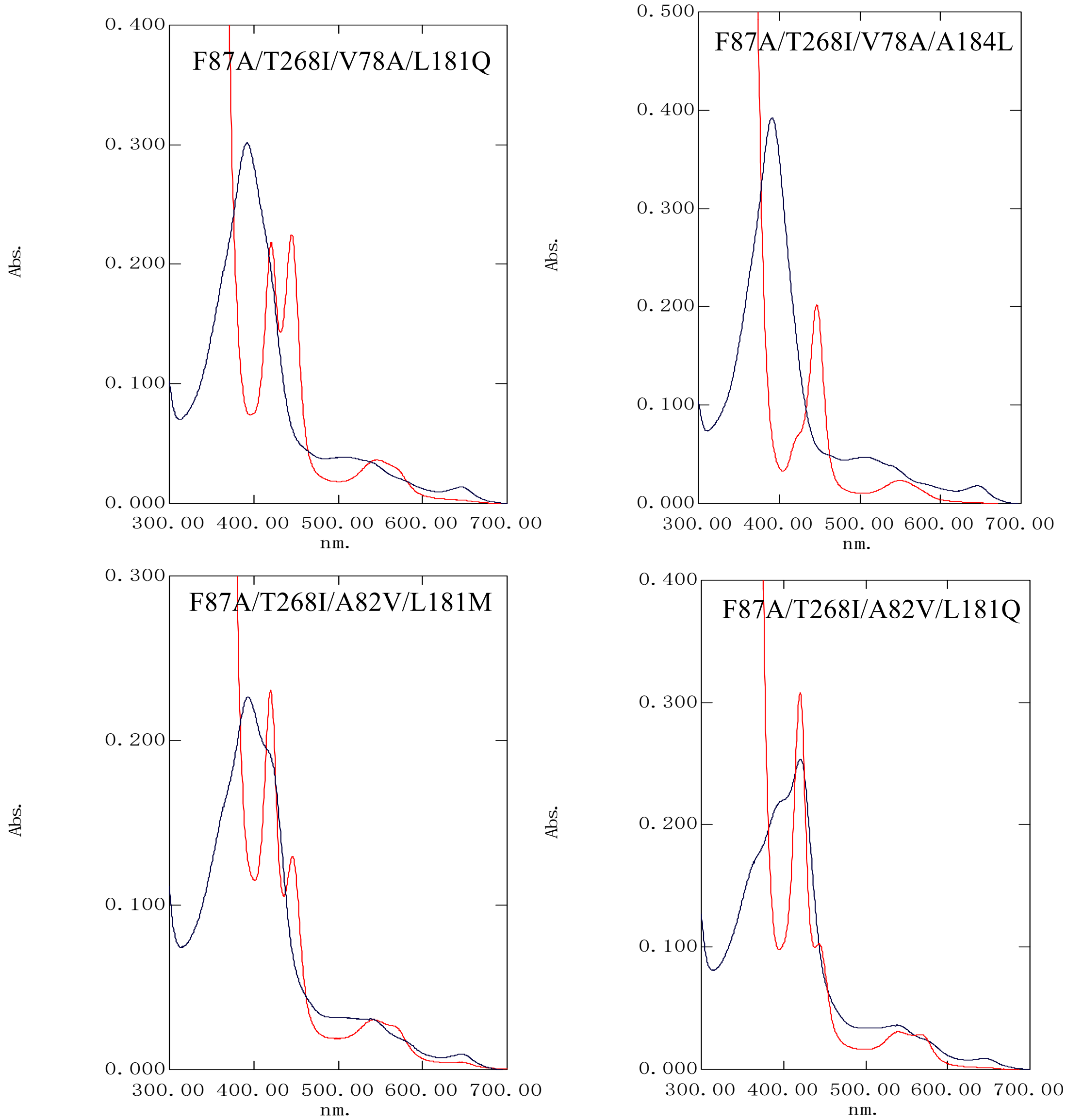
nm.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F87A/T268I/A82V/L181Q
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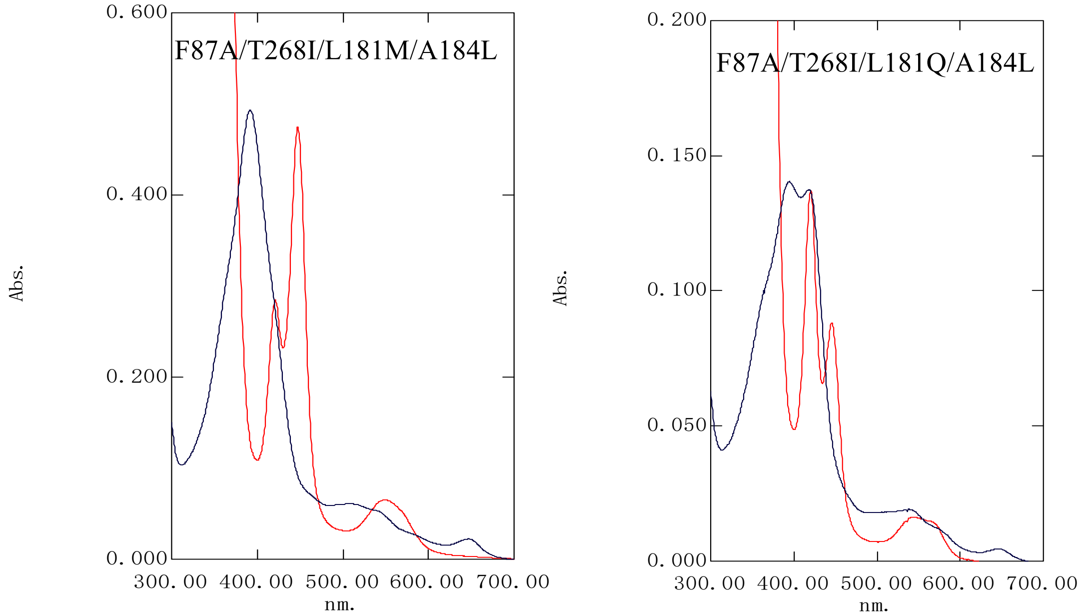
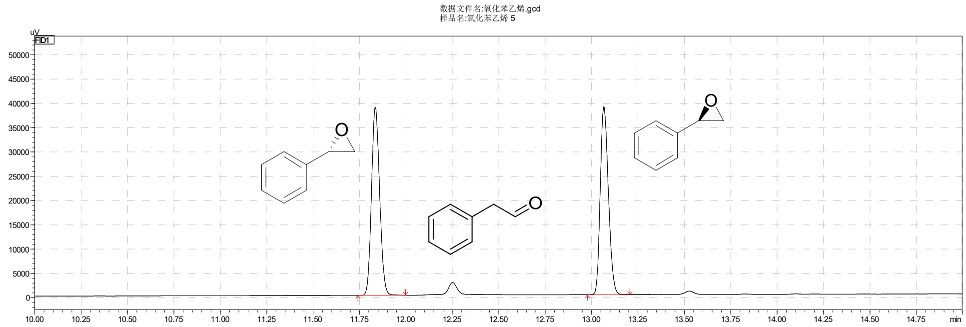
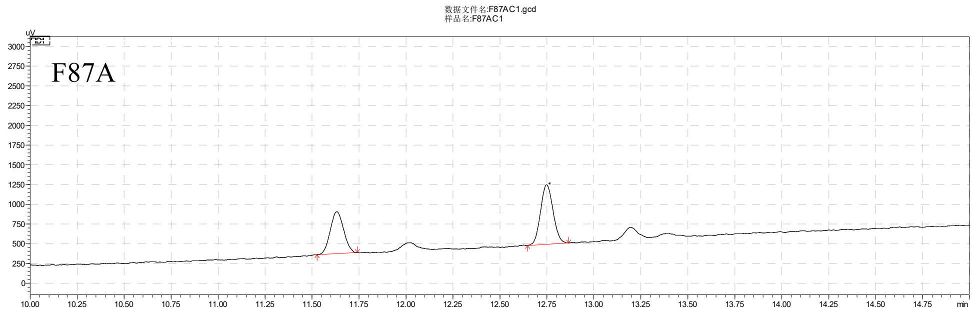


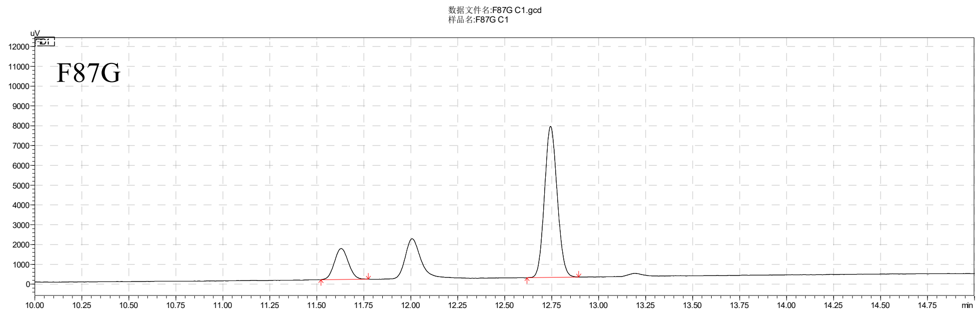
Figure S2 UV/visible spectral changes of P450BM3 variants (black line) upon addition of $Na_2S_2O_4$ (red line) for the formation of a ferrous CO complex through the reduction of ferric heme.



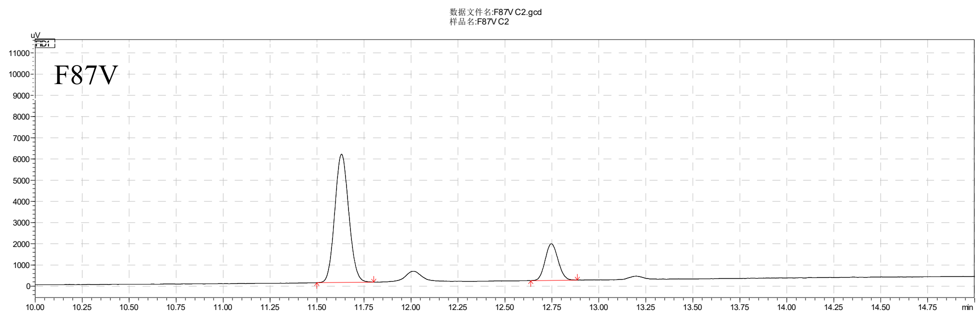
RT (min)	11.835 (*S*)	13.066 (*R*)
Area	117995	117726



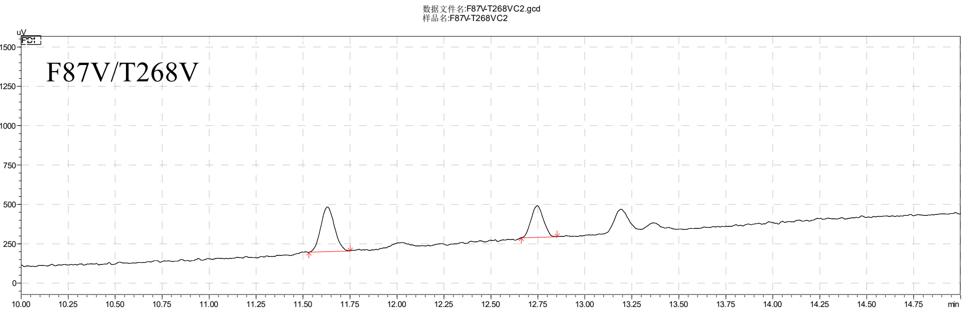
RT (min)	11.632 (*S*)	12.748 (*R*)
Area	2636	3505
ee % (*R*)	14.2	



RT (min)	11.630 (*S*)	12.744 (*R*)
Area	8094	36001
ee % (*R*)	63.3	

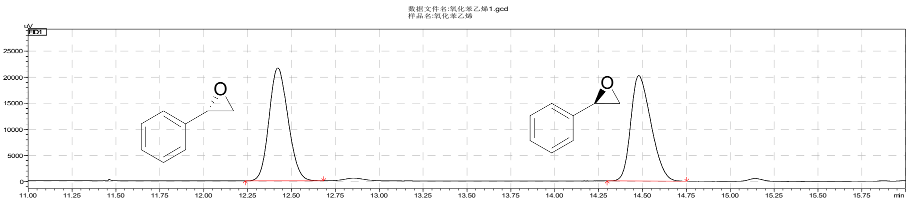


RT (min)	11.630 (*S*)	12.747 (*R*)
Area	31477	8281
ee % (*S*)	58.3	

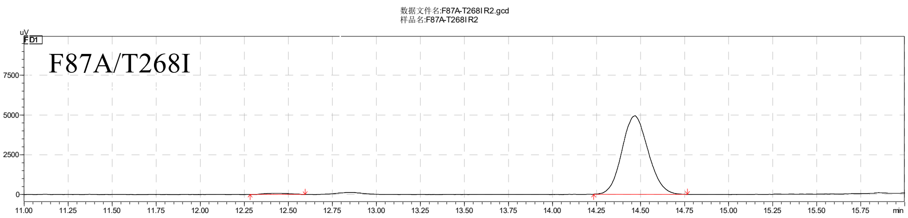


RT (min)	11.630 (*S*)	12.747 (*R*)
Area	1449	920
ee % (*S*)	22.3	

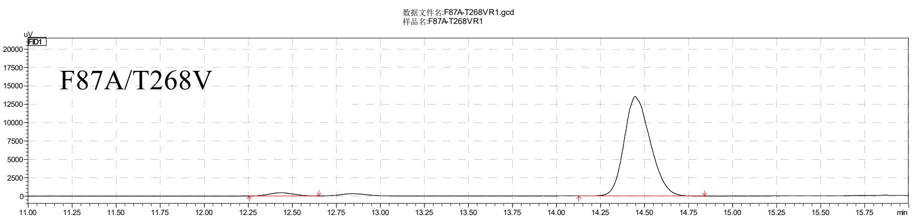
Figure S3 Typical chiral GC analyses for the epoxidation of styrene catalyzed by full-length P450BM3 mutants by addition of NADPH (2 mM) at 25 ℃. 1) the standard sample of styrene epoxide; 2) F87A; 3) F87G; 4) F87V; 5) F87V/T268V.



RT (min)	12.422 (*S*)	14.479 (*R*)
Area	163379	162083

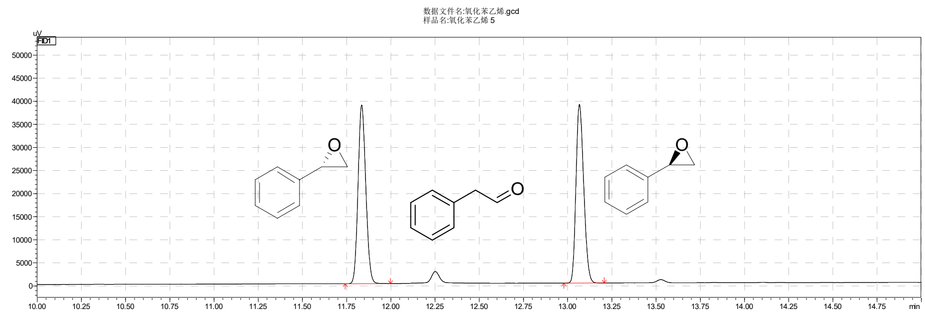


RT (min)	12.435 (*S*)	14.468 (*R*)
Area	705	51343
ee % (*R*)	97.3	

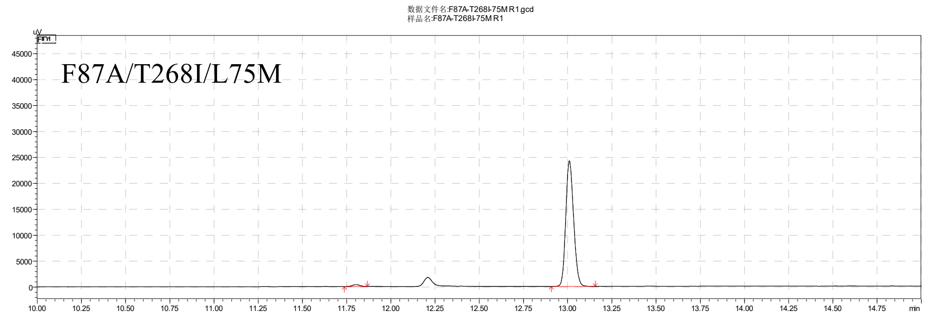


RT (min)	12.439 (*S*)	14.446 (*R*)
Area	4187	139324
ee % (*R*)	94.2	

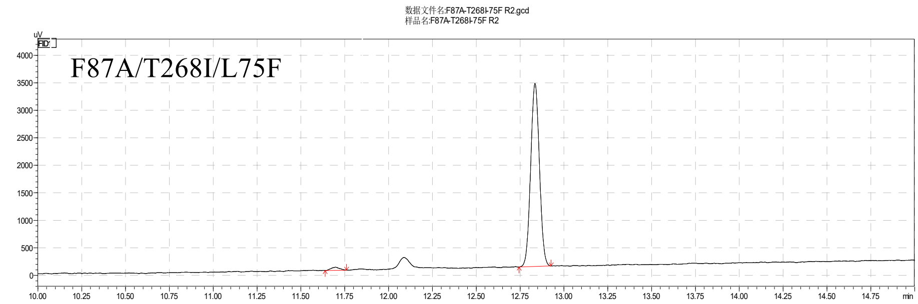
Figure S4 Typical chiral GC analyses for the epoxidation of styrene catalyzed by the double mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of styrene epoxide; 2) F87A/T268I; 3) F87A/T268V.



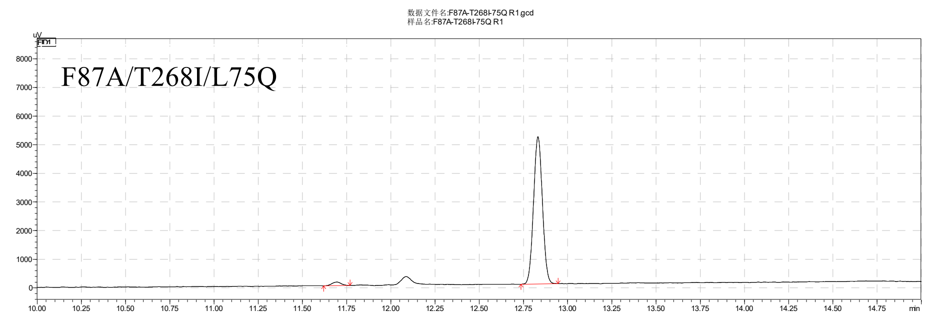
RT (min)	11.835 (*S*)	13.066 (*R*)
Area	117995	117726



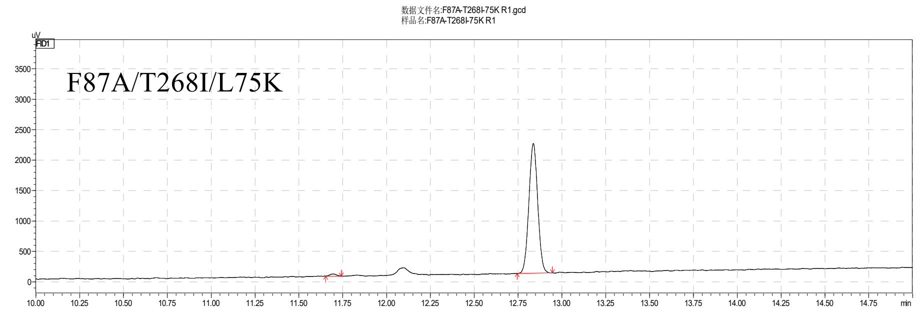
RT (min)	11.804 (*S*)	13.009 (*R*)
Area	1209	74632
ee % (*R*)	96.8	



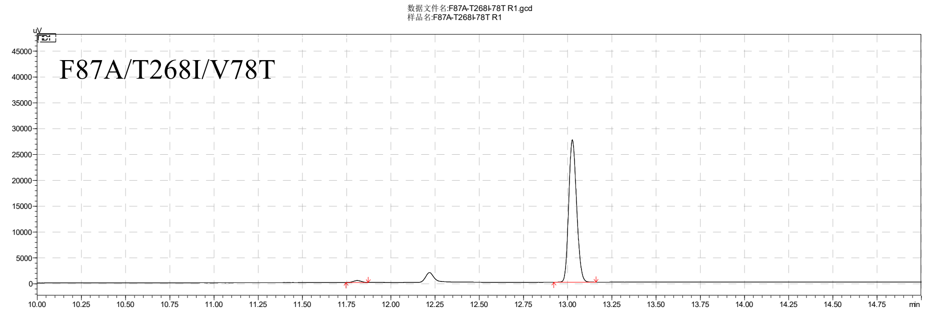
RT (min)	11.697 (*S*)	12.835 (*R*)
Area	196	11570
ee % (*R*)	96.7	



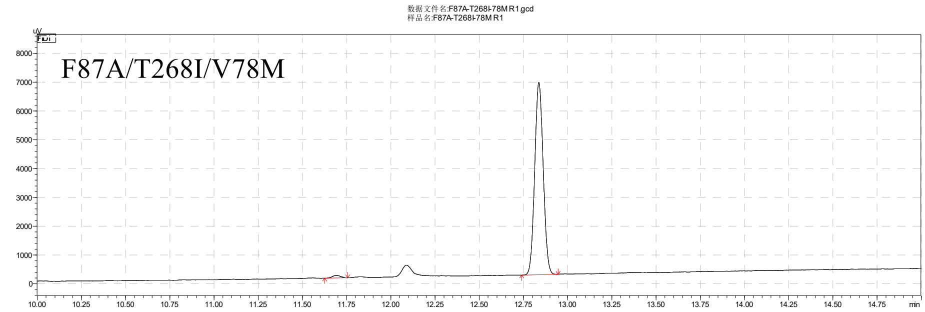
RT (min)	11.694 (*S*)	12.832 (*R*)
Area	457	18032
ee % (*R*)	95.1	



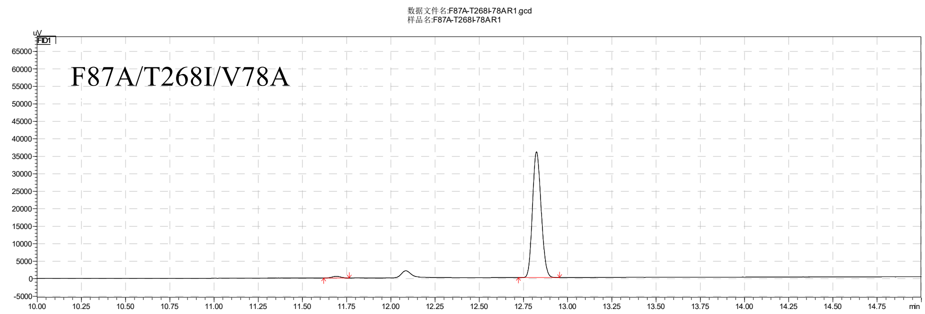
RT (min)	11.693 (*S*)	12.837 (*R*)
Area	94	7484
ee % (*R*)	97.5	



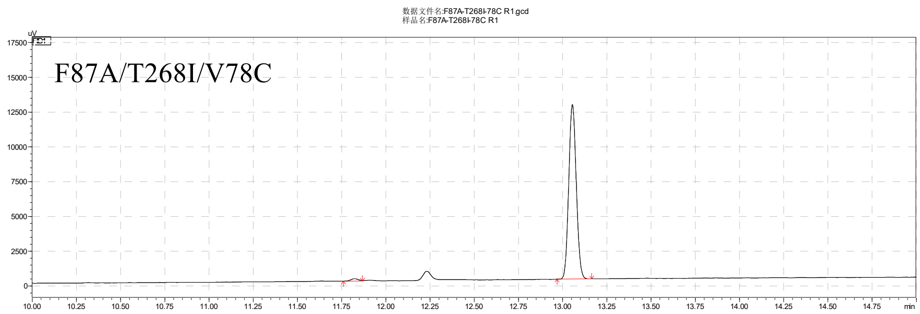
RT (min)	11.810 (*S*)	13.026 (*R*)
Area	1232	85492
ee % (*R*)	97.2	



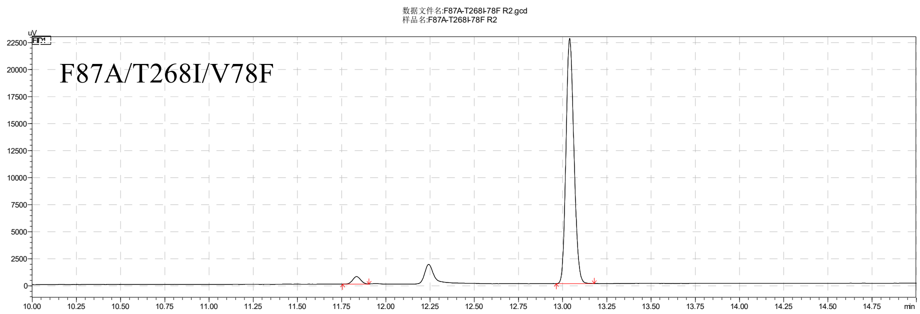
RT (min)	11.692 (*S*)	12.837 (*R*)
Area	284	22359
ee % (*R*)	97.5	



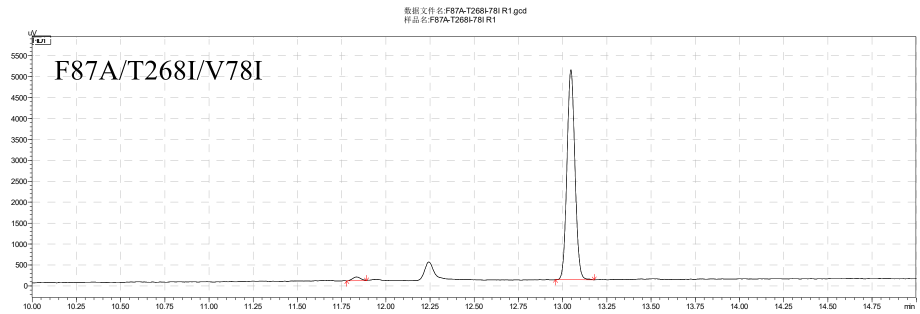
RT (min)	11.691 (*S*)	12.824 (*R*)
Area	1539	122768
ee % (*R*)	97.5	



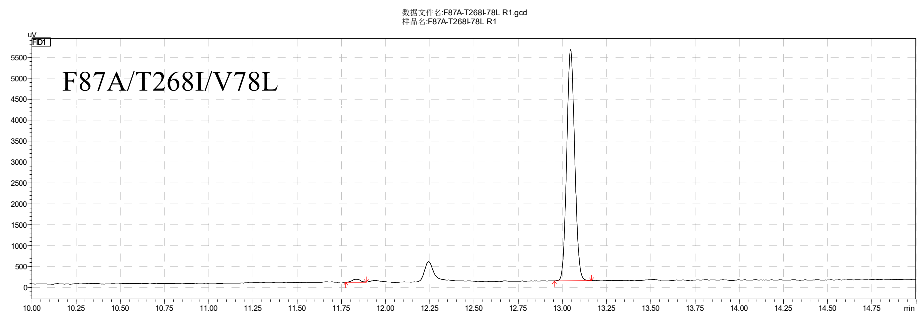
RT (min)	11.824 (*S*)	13.055 (*R*)
Area	522	37079
ee % (*R*)	97.2	



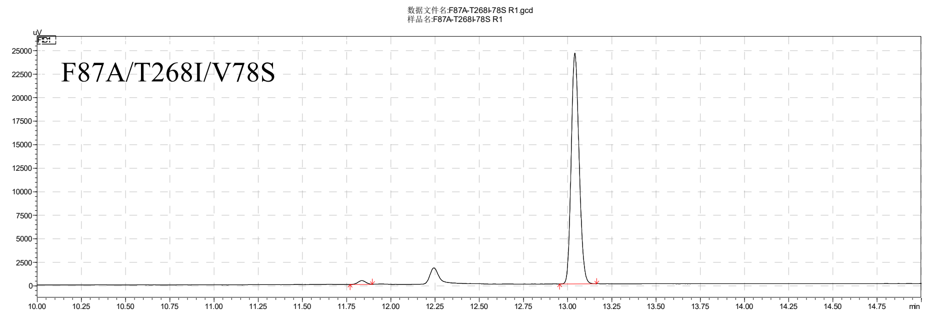
RT (min)	11.835 (*S*)	13.040 (*R*)
Area	2281	70820
ee % (*R*)	93.8	



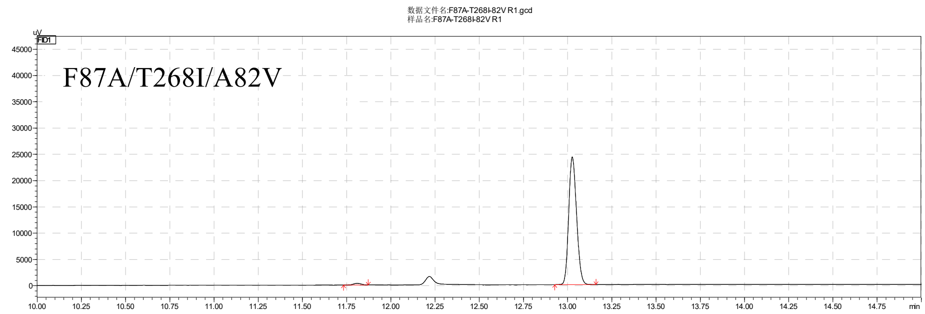
RT (min)	11.834 (*S*)	13.046 (*R*)
Area	294	15746
ee % (*R*)	96.3	



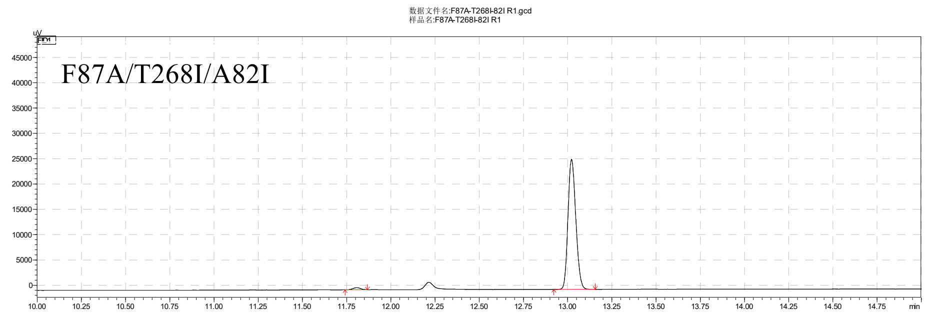
RT (min)	11.837 (*S*)	13.048 (*R*)
Area	266	19174
ee % (*R*)	97.3	



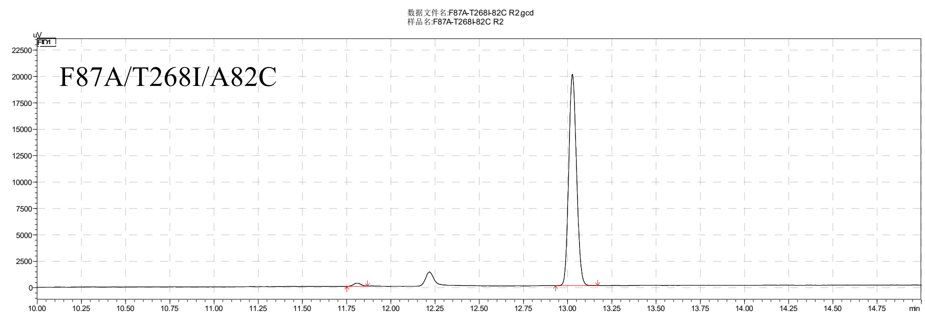
RT (min)	11.836 (*S*)	13.041 (*R*)
Area	1292	76365
ee % (*R*)	96.7	



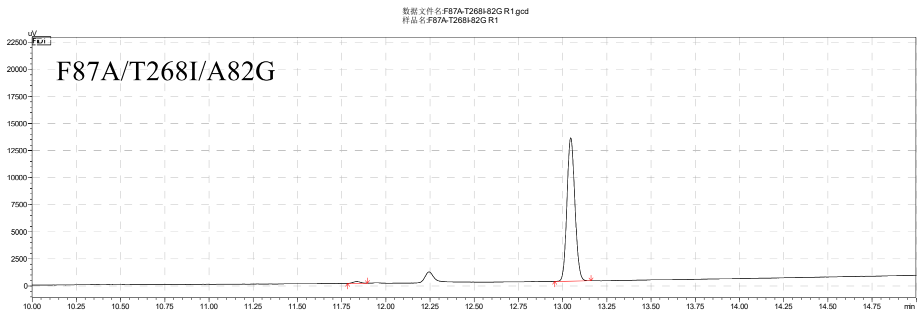
RT (min)	11.809 (*S*)	13.026 (*R*)
Area	1028	75223
ee % (*R*)	97.3	



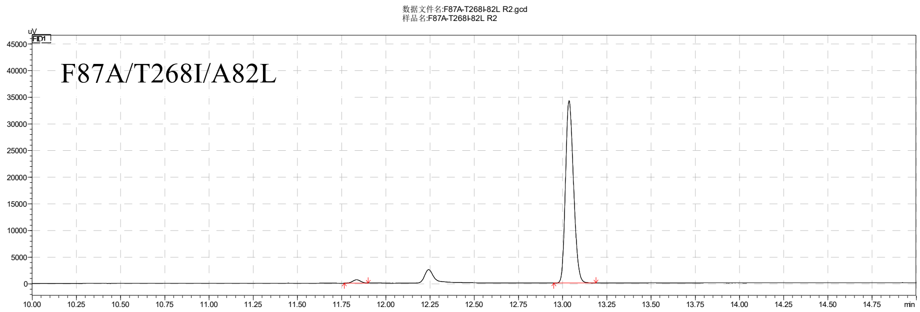
RT (min)	11.811 (*S*)	13.026 (*R*)
Area	1417	80564
ee % (*R*)	96.5	



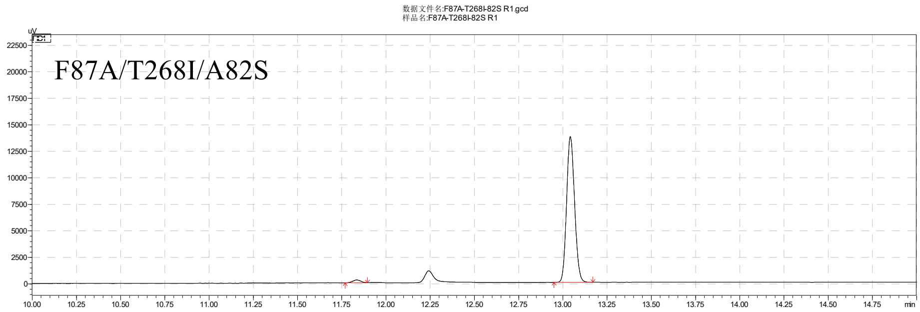
RT (min)	11.810 (*S*)	13.027 (*R*)
Area	939	61505
ee % (*R*)	97.0	



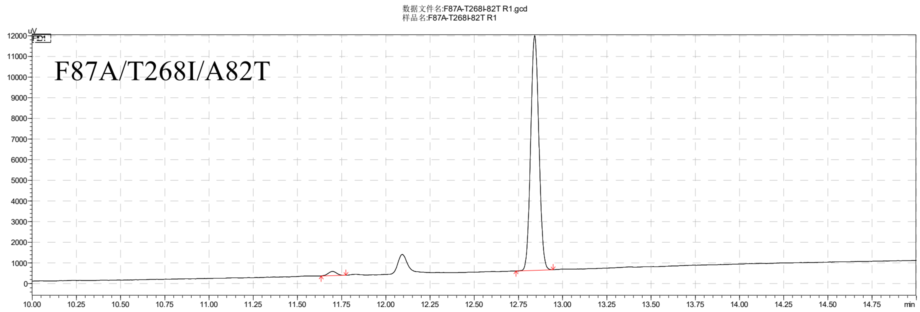
RT (min)	11.835 (*S*)	13.046 (*R*)
Area	534	41386
ee % (*R*)	97.4	



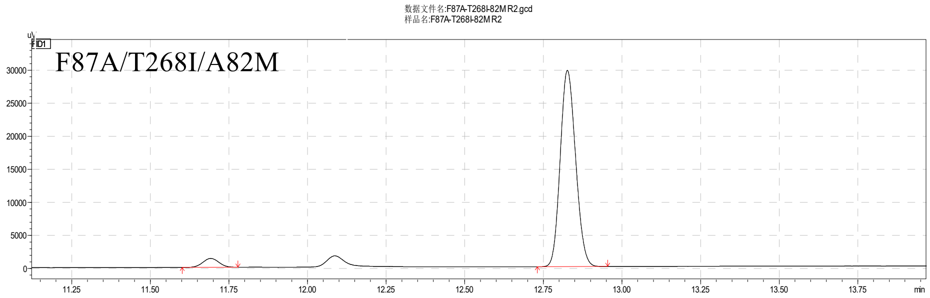
RT (min)	11.835 (*S*)	13.036 (*R*)
Area	2021	108406
ee % (*R*)	96.3	



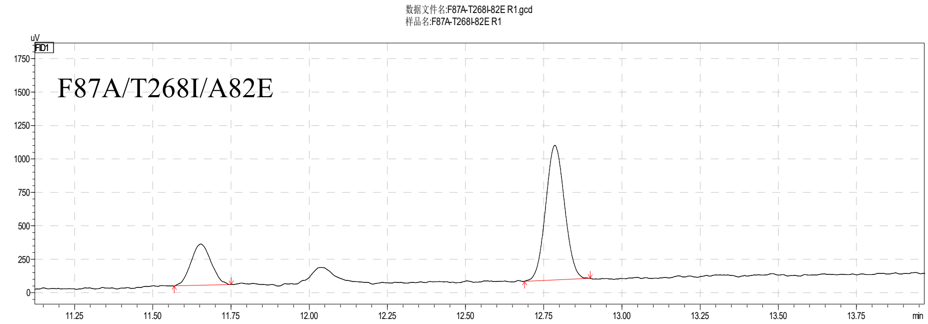
RT (min)	11.834 (*S*)	13.042 (*R*)
Area	923	43216
ee % (*R*)	95.8	



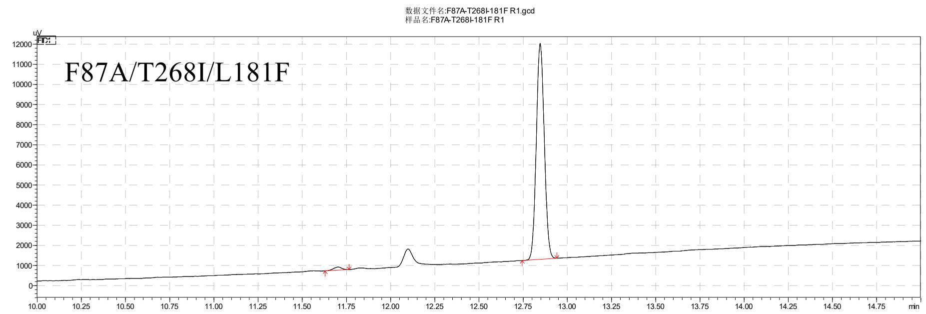
RT (min)	11.698 (*S*)	12.842 (*R*)
Area	693	36816
ee % (*R*)	96.3	



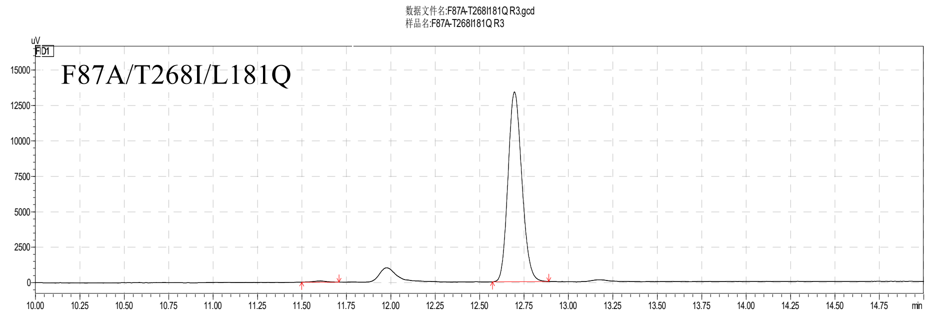
RT (min)	11.692 (*S*)	12.826 (*R*)
Area	4817	101016
ee % (*R*)	90.9	



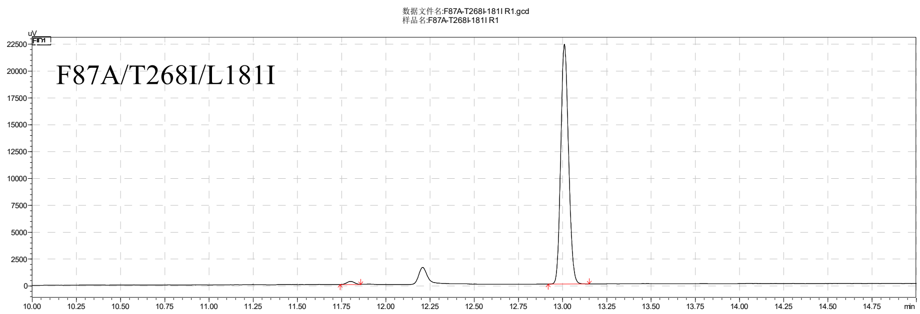
RT (min)	11.654 (*S*)	12.786 (*R*)
Area	1386	4231
ee % (*R*)	50.7	



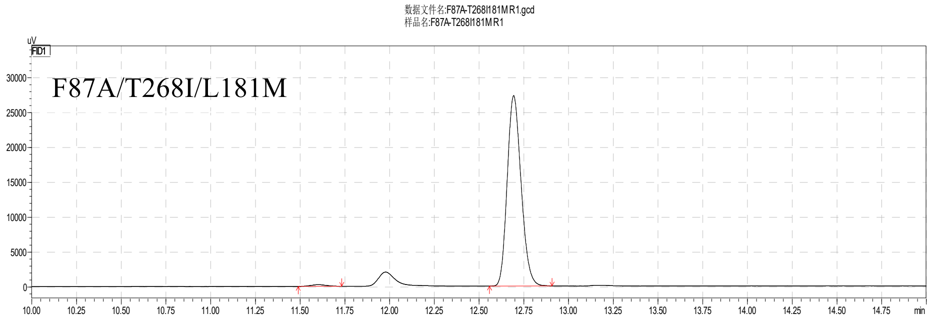
RT (min)	11.703 (*S*)	12.846 (*R*)
Area	509	33831
ee % (*R*)	97.0	



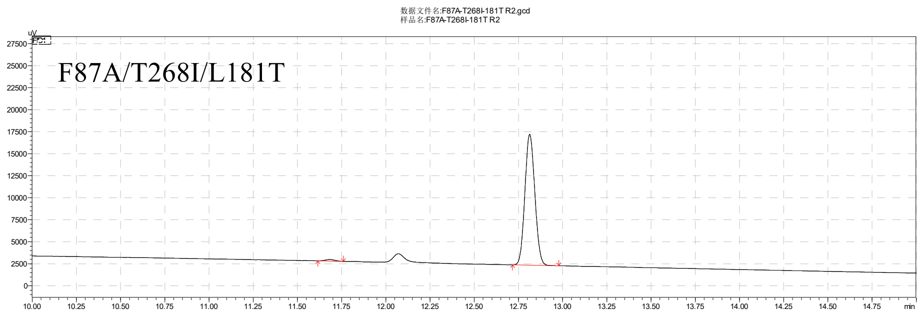
RT (min)	11.603 (*S*)	12.696 (*R*)
Area	448	72630
ee % (*R*)	98.8	



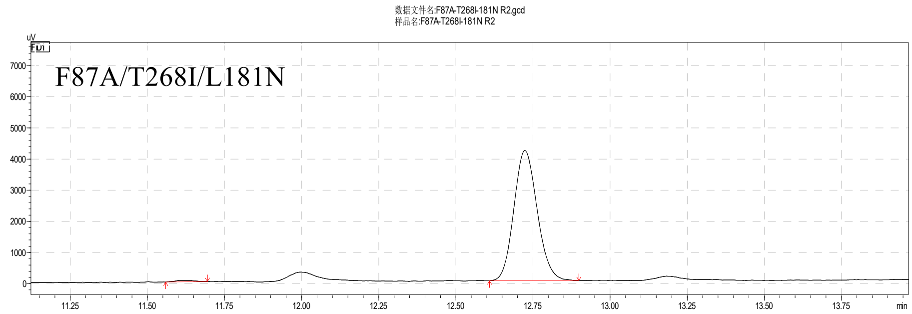
RT (min)	11.801 (*S*)	13.010 (*R*)
Area	941	68581
ee % (*R*)	97.3	



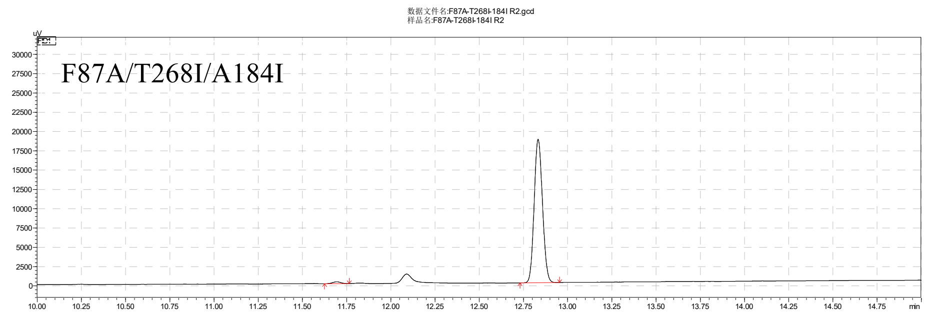
RT (min)	11.599 (*S*)	12.691 (*R*)
Area	1580	157174
ee % (*R*)	98.0	



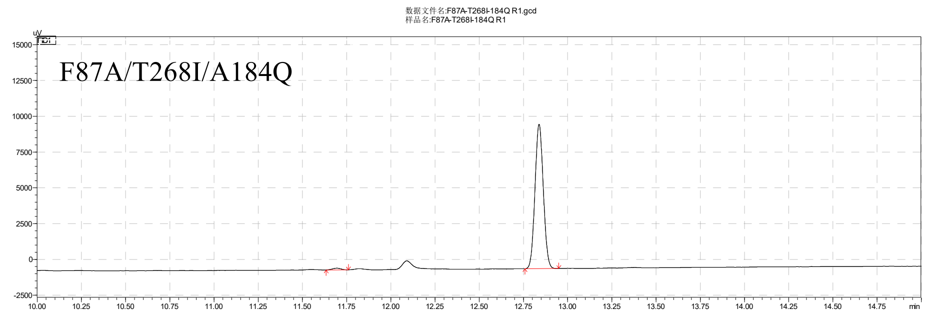
RT (min)	11.682 (*S*)	12.815 (*R*)
Area	689	58093
ee % (*R*)	97.7	



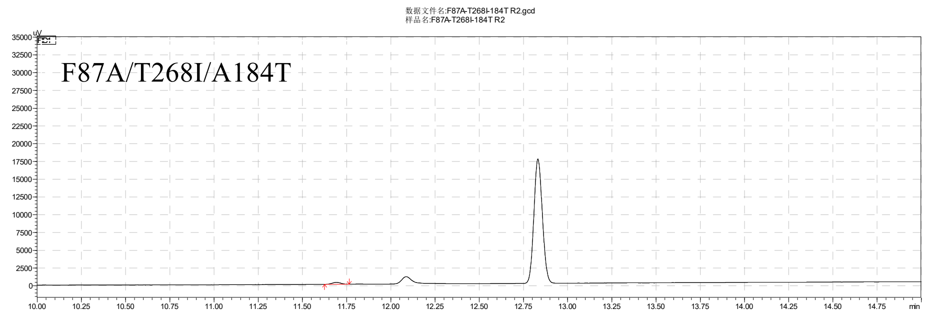
RT (min)	11.615 (*S*)	12.724 (*R*)
Area	205	21973
ee % (*R*)	98.2	



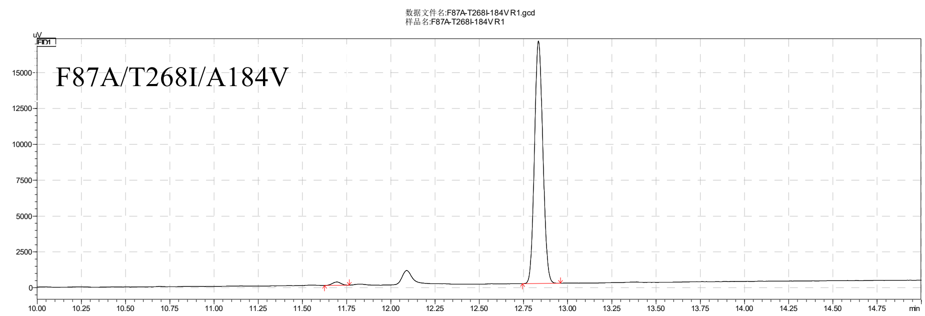
RT (min)	11.694 (*S*)	12.833 (*R*)
Area	790	61022
ee % (*R*)	97.4	



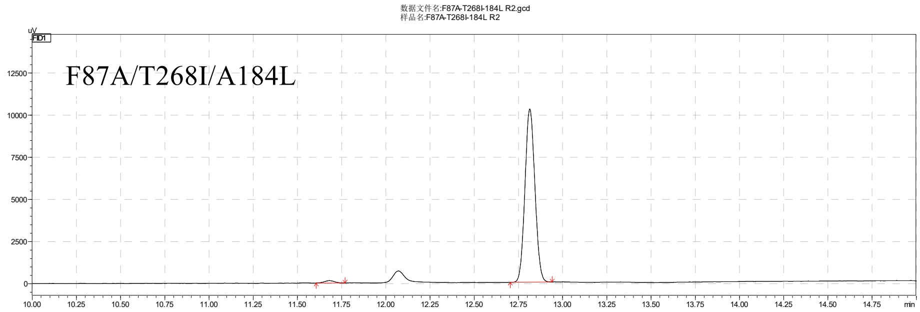
RT (min)	11.695 (*S*)	12.838 (*R*)
Area	417	33063
ee % (*R*)	97.5	



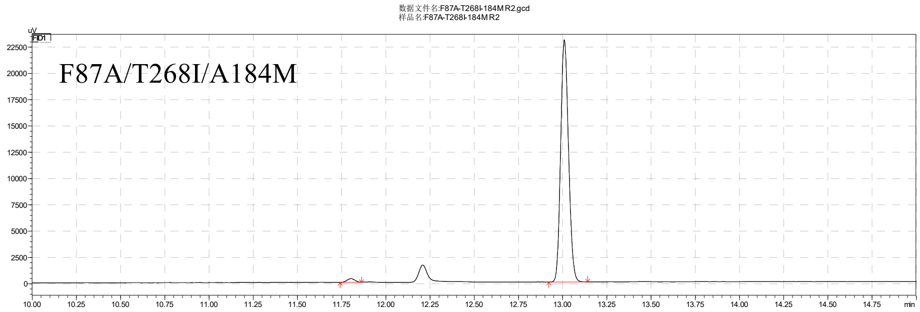
RT (min)	11.691 (*S*)	12.832 (*R*)
Area	875	58385
ee % (*R*)	97.1	



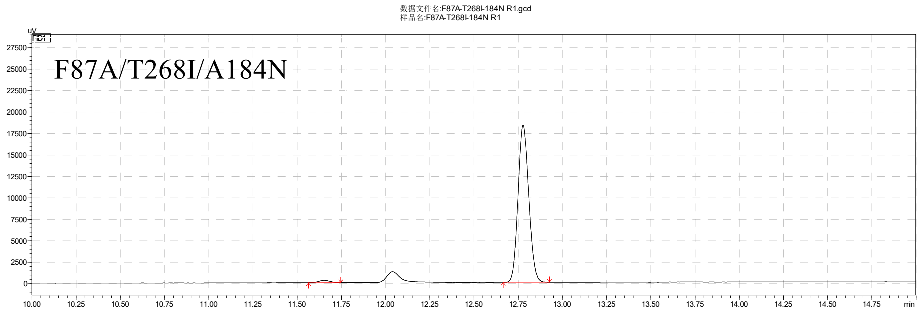
RT (min)	11.695 (*S*)	12.835 (*R*)
Area	781	55949
ee % (*R*)	97.3	



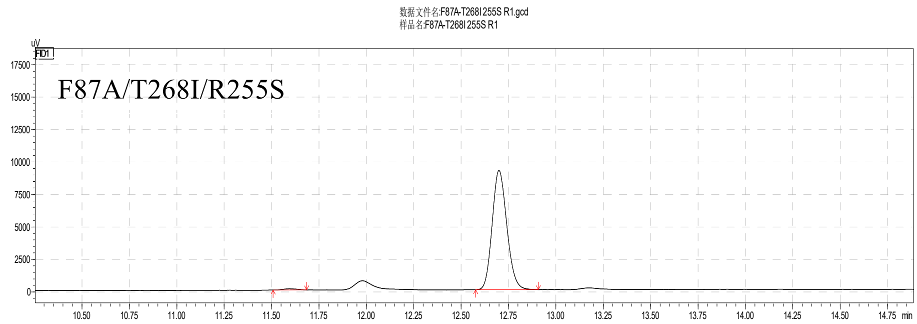
RT (min)	11.680 (*S*)	12.814 (*R*)
Area	546	40186
ee % (*R*)	97.3	



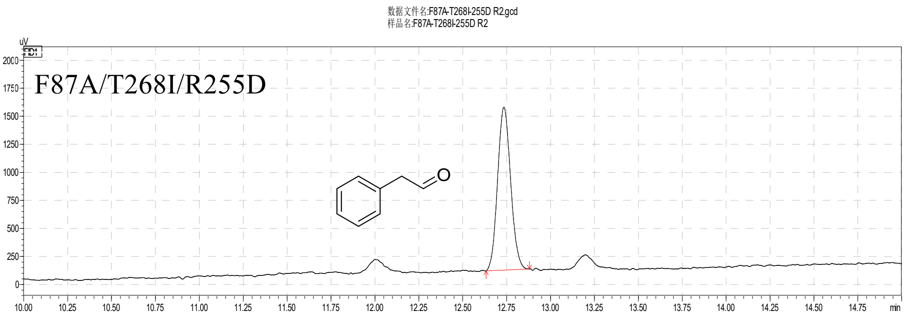
RT (min)	11.803 (*S*)	13.009 (*R*)
Area	1136	71497
ee % (*R*)	96.9	



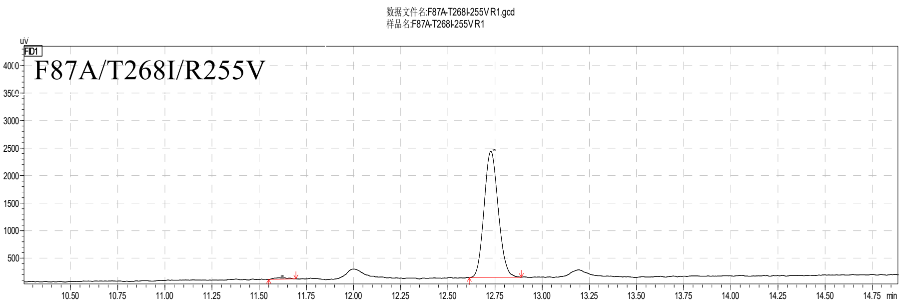
RT (min)	11.803 (*S*)	13.009 (*R*)
Area	1136	71497
ee % (*R*)	97.0	



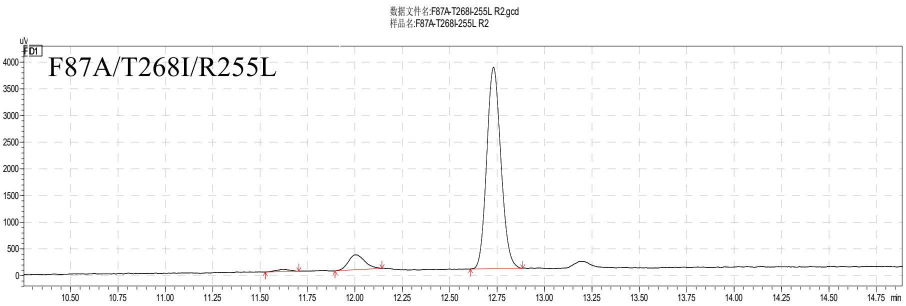
RT (min)	11.689 (*S*)	12.822 (*R*)
Area	384	35815
ee % (*R*)	98.1	



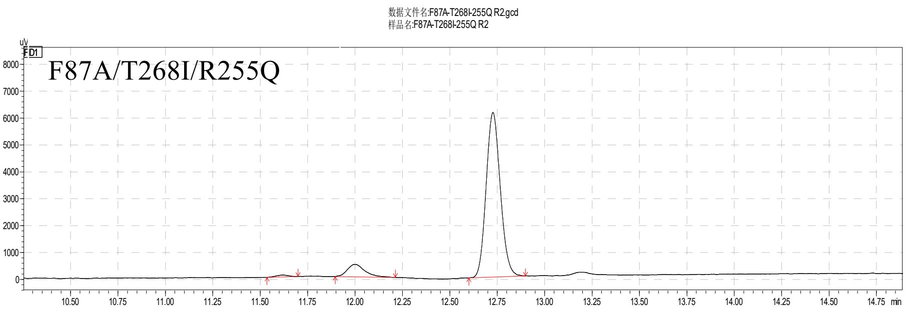
RT (min)		12.735 (*R*)
Area		7517
ee % (*R*)	>99%	



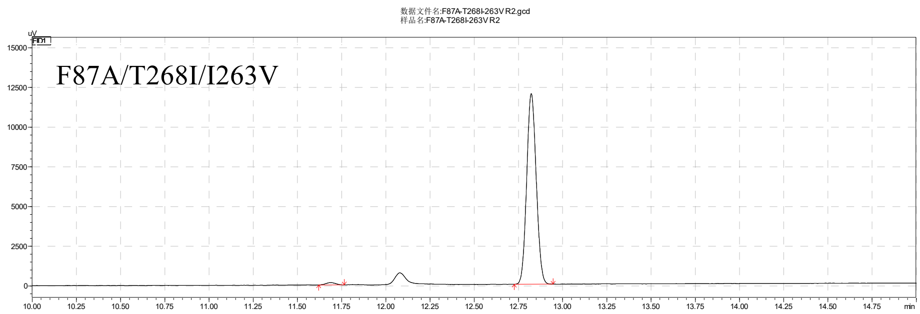
RT (min)	11.605 (*S*)	12.728 (*R*)
Area	118	11986
ee % (*R*)	98.1	



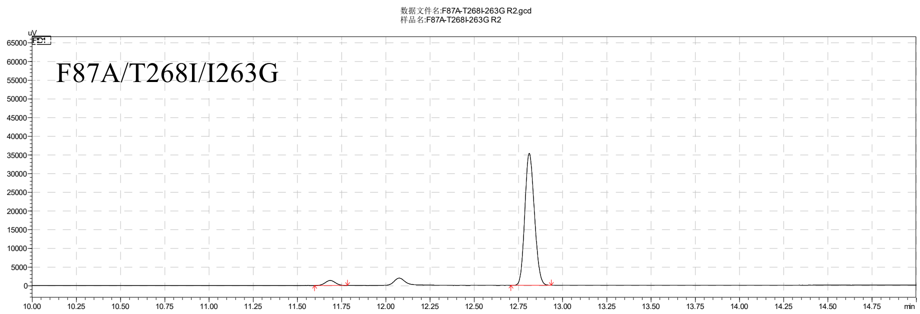
RT (min)	11.618 (*S*)	12.731 (*R*)
Area	204	19631
ee % (*R*)	98.0	



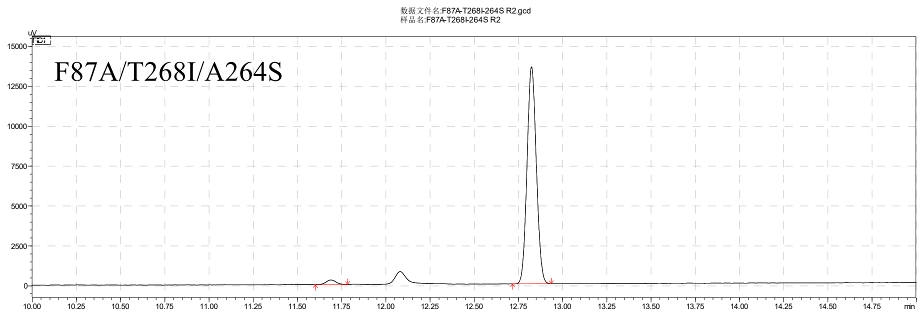
RT (min)	11.622 (*S*)	12.727 (*R*)
Area	292	31835
ee % (*R*)	98.2	



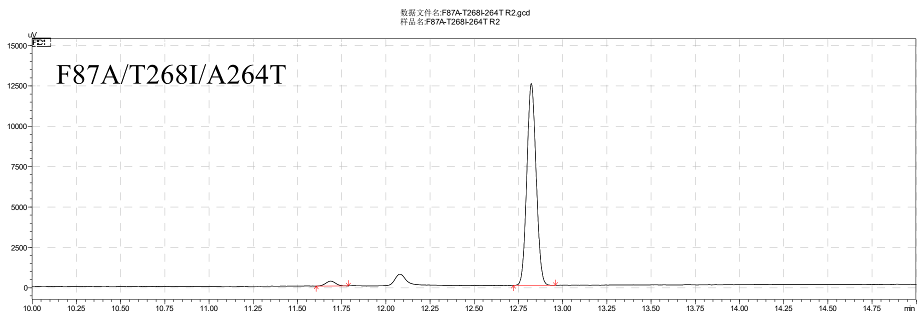
RT (min)	11.688 (*S*)	12.822 (*R*)
Area	557	45080
ee % (*R*)	97.6	



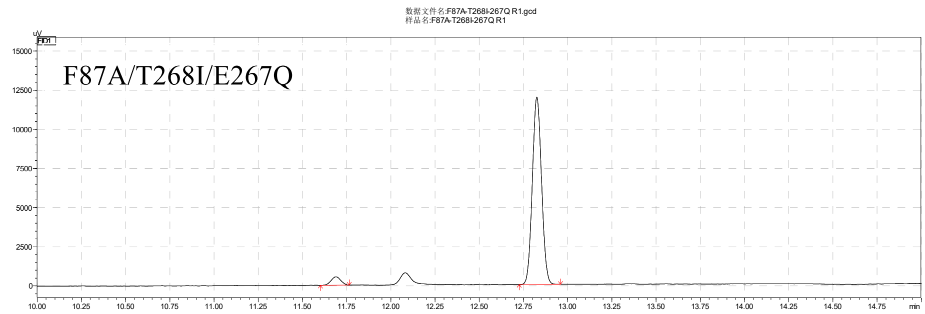
RT (min)	11.685 (*S*)	12.811 (*R*)
Area	5294	133343
ee % (*R*)	92.4	



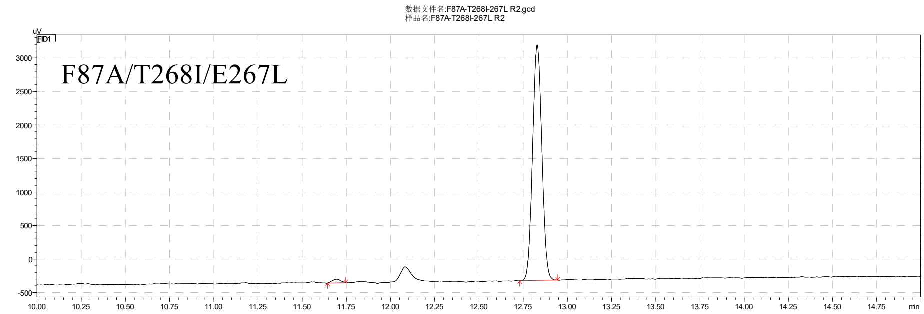
RT (min)	11.689 (*S*)	12.824 (*R*)
Area	1108	49783
ee % (*R*)	95.6	



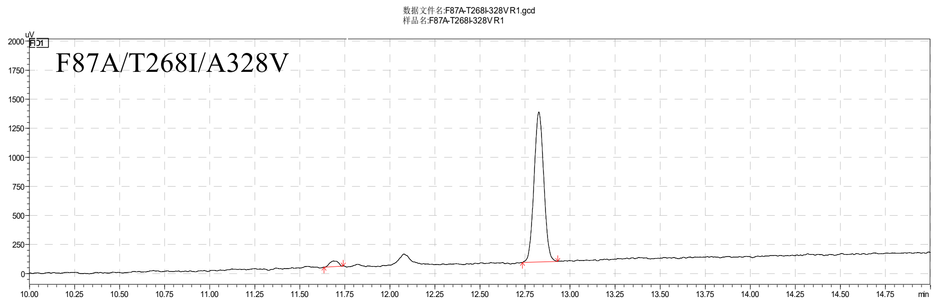
RT (min)	11.688 (*S*)	12.823 (*R*)
Area	1175	45905
ee % (*R*)	95.0	



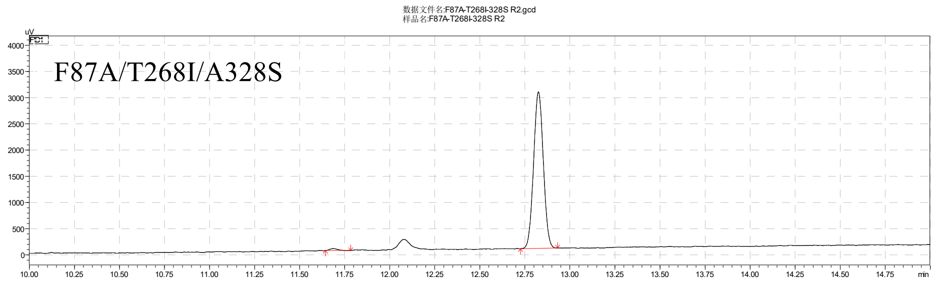
RT (min)	11.690 (*S*)	12.826 (*R*)
Area	2061	43419
ee % (*R*)	90.9	



RT (min)	11.696 (*S*)	12.829 (*R*)
Area	183	12864
ee % (*R*)	97.1	

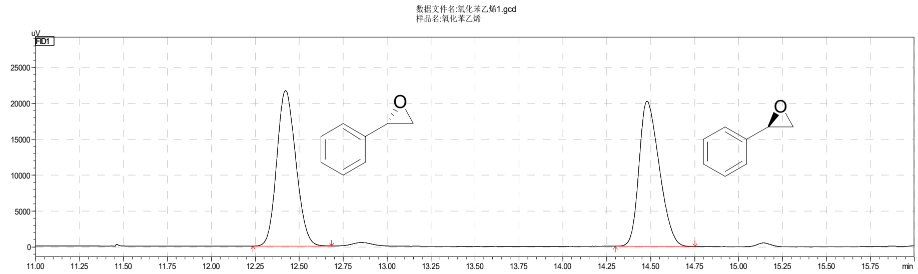


RT (min)	11.688 (*S*)	12.827 (*R*)
Area	158	4852
ee % (*R*)	93.7	

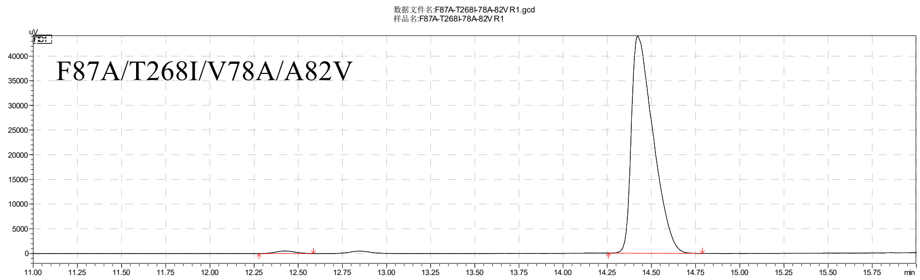


RT (min)	11.685 (*S*)	12.825 (*R*)
Area	112	11217
ee % (*R*)	98.0	

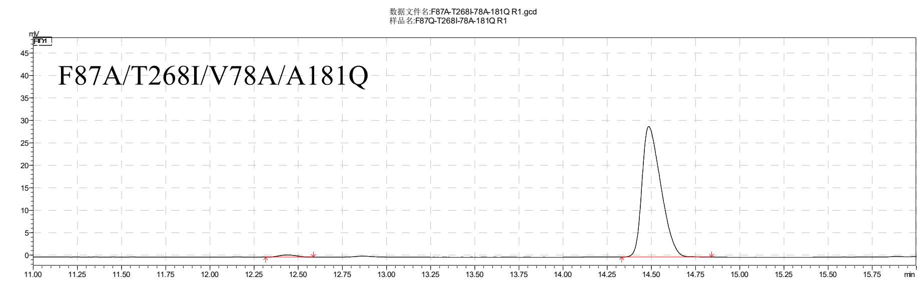
Figure S5 Typical chiral GC analyses for the epoxidation of styrene catalyzed by the triple mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 25 ℃ in the presence of Im-C6-Phe. 1) the standard sample of styrene epoxide; 2) F87A/T268I/75M; 3) F87A/T268I/L75F; 4) F87A/T268I/L75Q; 5) F87A/T268I/L75K; 6) F87A/T268I/V78T; 7) F87A/T268I/V78M; 8) F87A/T268I/V78A; 9) F87A/T268I/V78C; 10) F87A/T268I/V78F; 11) F87A/T268I/V78I; 12) F87A/T268I/V78L; 13) F87A/T268I/V78S; 14) F87A/T268I/A82V; 15) F87A/T268I/A82I; 16) F87A/T268I/A82C; 17) F87A/T268I/A82G; 18) F87A/T268I/A82L; 19) F87A/T268I/A82S; 20) F87A/T268I/A82T; 21) F87A/T268I/A82M; 22) F87A/T268I/A82E; 23) F87A/T268I/L181F; 24) F87A/T268I/L181Q; 25) F87A/T268I/L181I; 26) F87A/T268I/L181M; 27) F87A/T268I/L181T; 28) F87A/T268I/L181N;29) F87A/T268I/A184I;30) F87A/T268I/A184Q;31) F87A/T268I/A184T; 32) F87A/T268I/A184V; 33) F87A/T268I/A184L; 34) F87A/T268I/A184M; 35) F87A/T268I/A184N; 36) F87A/T268I/R255S; 37) F87A/T268I/R255D; 38) F87A/T268I/R255V; 39) F87A/T268I/R255L; 40) F87A/T268I/R255Q; 41) F87A/T268I/I263V; 42) F87A/T268I/I263G; 43) F87A/T268I/A264S; 44) F87A/T268I/A264T; 45) F87A/T268I/E267Q; 46) F87A/T268I/E267L; 47) F87A/T268I/A328V; 48) F87A/T268I/A328S.



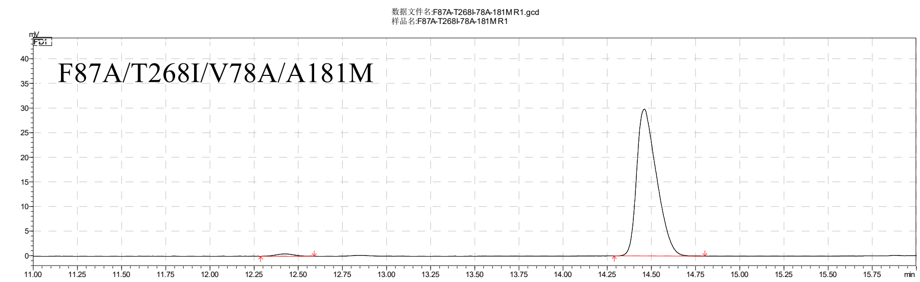
RT (min)	12.422 (*S*)	14.479 (*R*)
Area	163379	162083



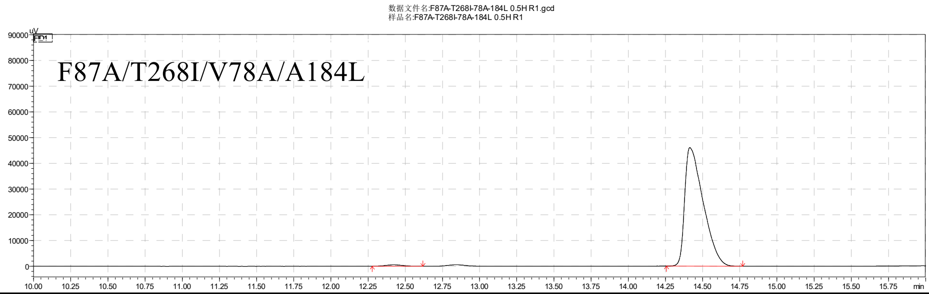
RT (min)	12.423 (*S*)	14.422 (*R*)
Area	3953	377400
ee % (*R*)	98.0	



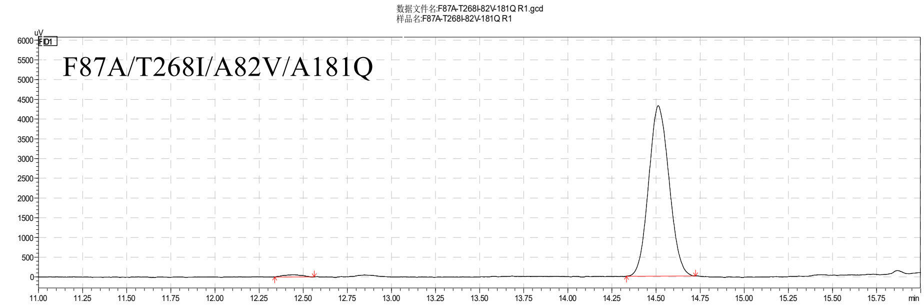
RT (min)	12.440 (*S*)	14.485 (*R*)
Area	3298	215644
ee % (*R*)	97.0	



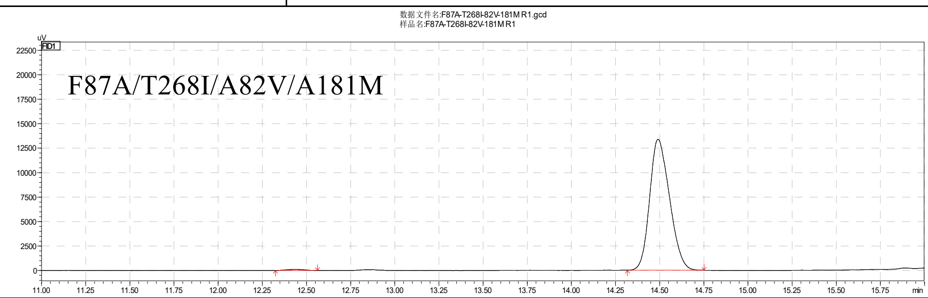
RT (min)	12.428 (*S*)	14.460 (*R*)
Area	3453	234069
ee % (*R*)	97.1	



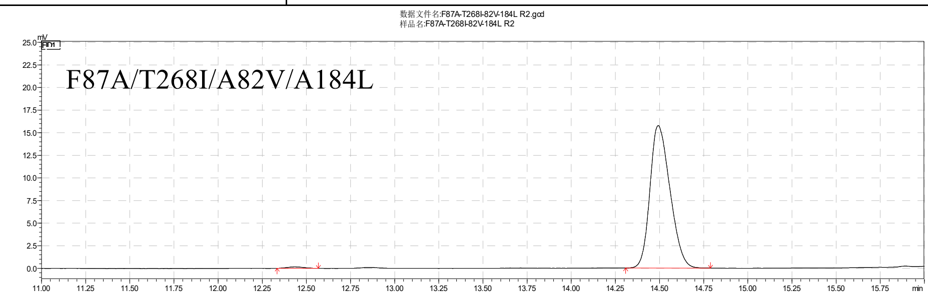
RT (min)	12.421 (*S*)	14.427 (*R*)
Area	4399	398008
ee % (*R*)	97.8	



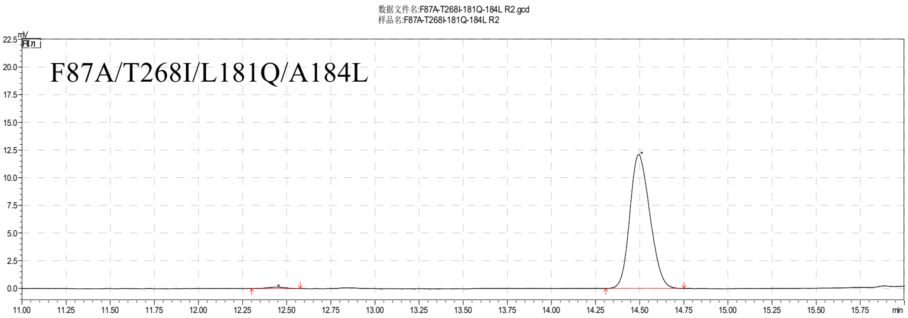
RT (min)	12.443 (*S*)	14.512 (*R*)
Area	385	34827
ee % (*R*)	97.8	



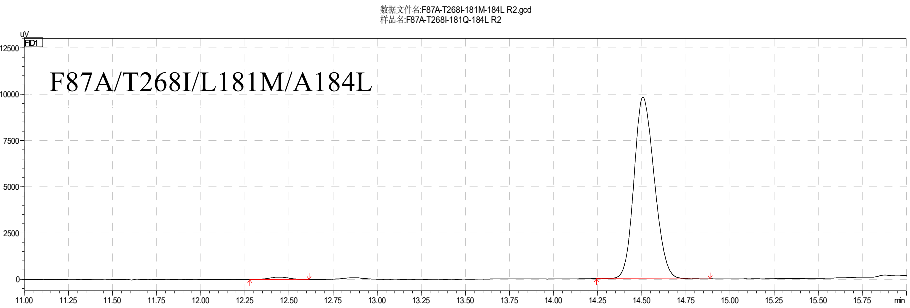
RT (min)	12.433 (*S*)	14.491 (*R*)
Area	802	104881
ee % (*R*)	98.5	



RT (min)	12.433 (*S*)	14.493 (*R*)
Area	1069	124477
ee % (*R*)	98.3	

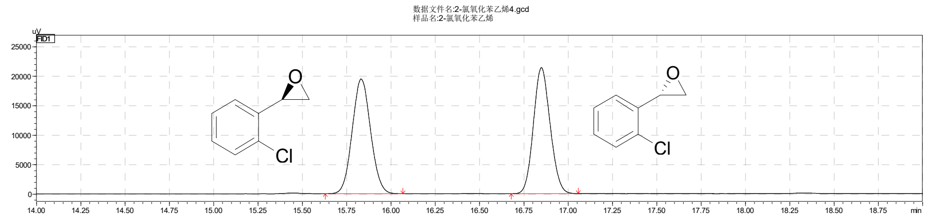


RT (min)	12.437 (*S*)	14.495 (*R*)
Area	895	95574
ee % (*R*)	98.1	

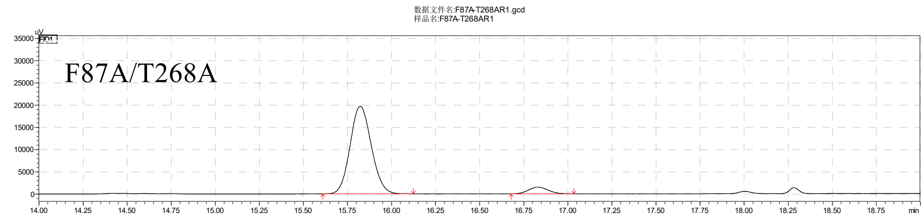


RT (min)	12.436 (*S*)	14.506 (*R*)
Area	1003	78631
ee % (*R*)	97.5	

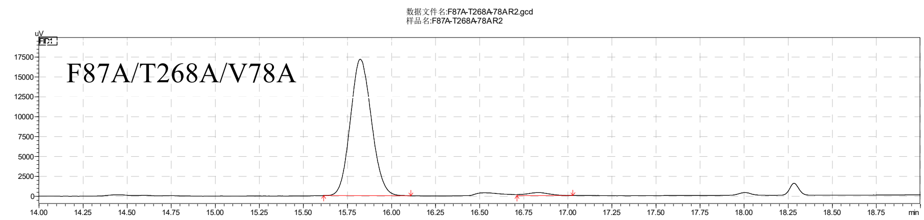
Figure S6 Typical chiral GC analyses for the epoxidation of styrene catalyzed by the quadruple mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of styrene epoxide; 2) F87A/T268I/V78A/A82V; 3) F87A/T268I/V78A/A181Q; 4) F87A/T268I/V78A/A181M; 5) F87A/T268I/V78A/A184L; 6) F87A/T268I/A82V/A181Q; 7) F87A/T268I/A82V/A181M; 8) F87A/T268I/A82V/A184L; 9) F87A/T268I/A181Q/A184L; 10) F87A/T268I/A181M/A184L.



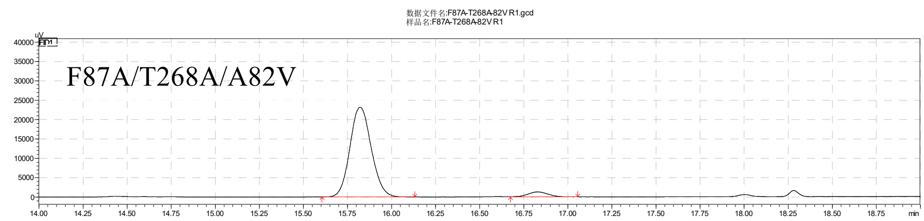
RT (min)	15.831 (*R*)	16.849 (*S*)
Area	140917	140444



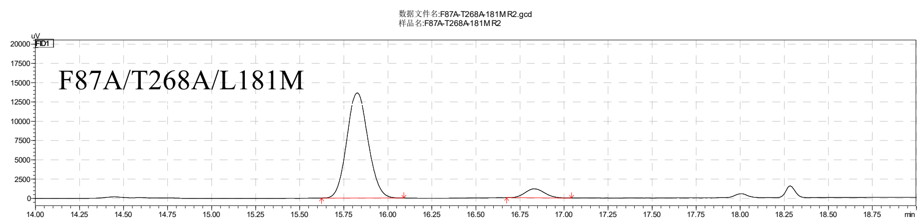
RT (min)	15.823 (*R*)	16.830 (*S*)
Area	164985	11641
ee % (*R*)	86.8	



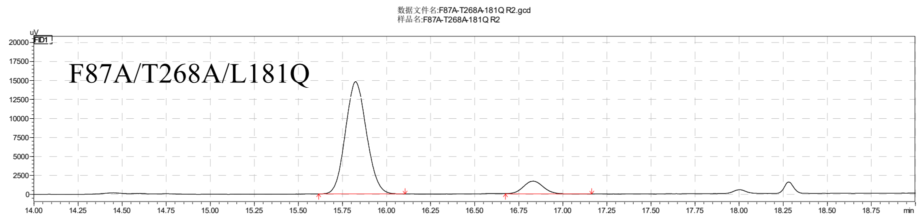
RT (min)	15.822 (*R*)	16.833 (*S*)
Area	143486	3429
ee % (*R*)	95.3	



RT (min)	15.822 (*R*)	16.829 (*S*)
Area	195003	10075
ee % (*R*)	90.2	

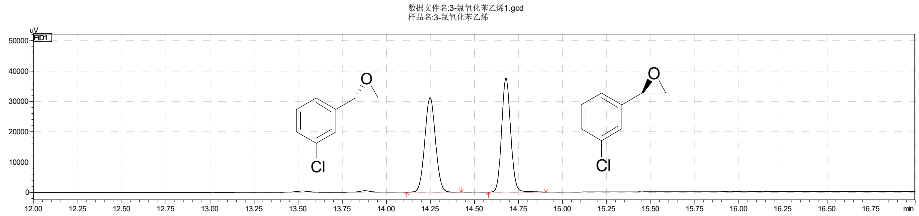


RT (min)	15.826 (*R*)	16.829 (*S*)
Area	114906	9138
ee % (*R*)	85.3	

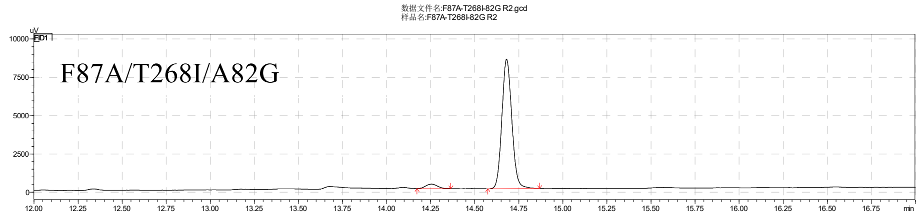


RT (min)	15.824 (*R*)	16.832 (*S*)
Area	123080	13452
ee % (*R*)	80.3	

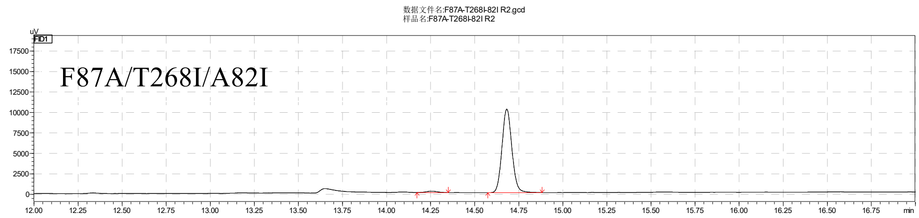
Figure S7 Typical chiral GC analyses for the epoxidation of 2-chlorostyrene catalyzed by the mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of 2-chlorostyrene epoxide; 2) F87A/T268A; 3) F87A/T268A/V78A; 4) F87A/T268A/A82V; 5) F87A/T268A/L181M; 6) F87A/T268A/L181Q.



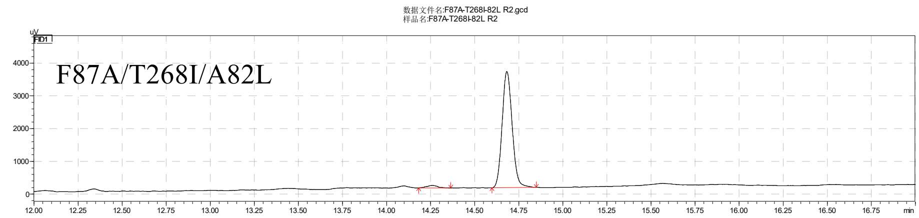
RT (min)	14.248 (*S*)	14.679 (*R*)
Area	131249	133575



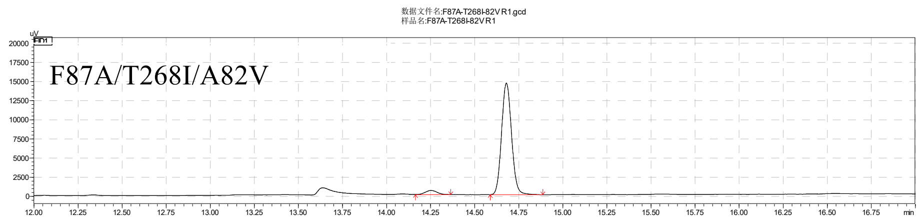
RT (min)	14.255 (*S*)	14.681 (*R*)
Area	1338	33228
ee % (*R*)	92.3	



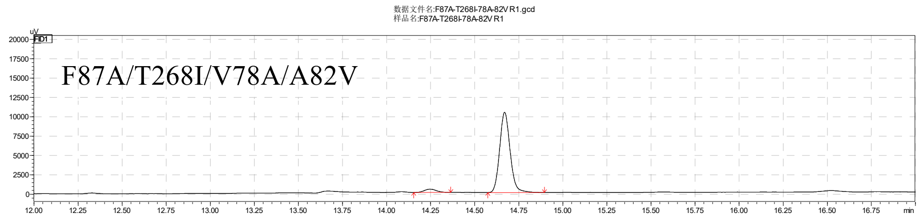
RT (min)	14.252 (*S*)	14.682 (*R*)
Area	759	39919
ee % (*R*)	96.3	



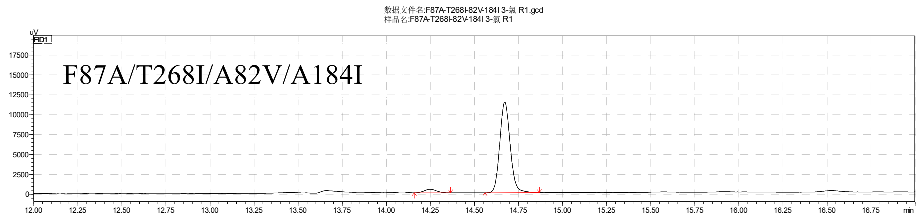
RT (min)	14.261 (*S*)	14.682 (*R*)
Area	322	14086
ee % (*R*)	95.5	



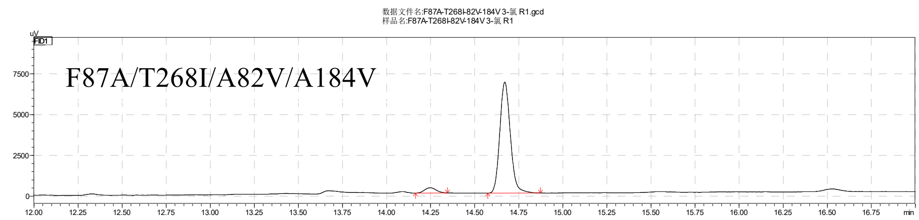
RT (min)	14.251 (*S*)	14.680 (*R*)
Area	2629	57574
ee % (*R*)	91.3	



RT (min)	14.247 (*S*)	14.670 (*R*)
Area	2037	42691
ee % (*R*)	90.9	

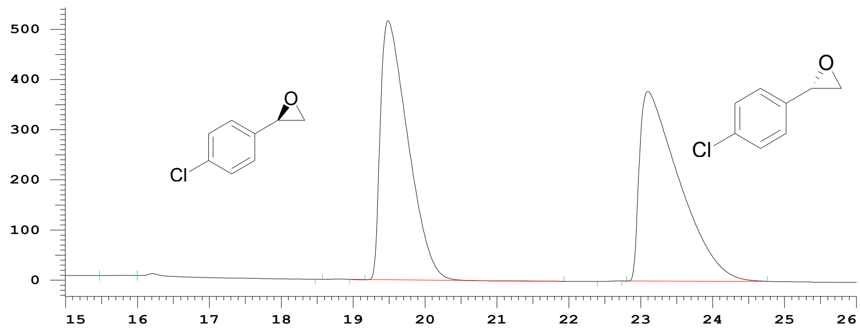


RT (min)	14.247 (*S*)	14.672 (*R*)
Area	2096	46741
ee % (*R*)	91.4	

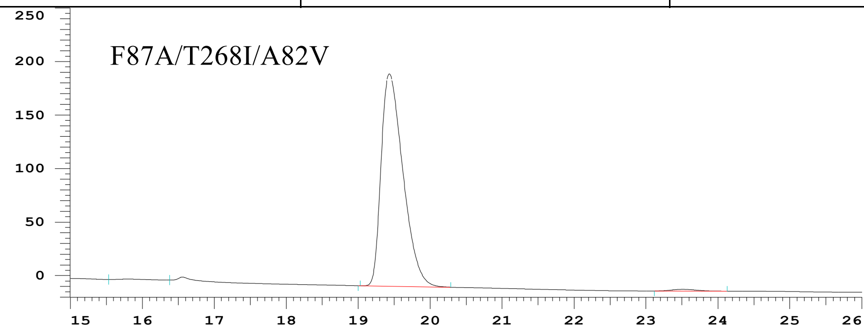


RT (min)	14.248 (*S*)	14.671 (*R*)
Area	1493	28370
ee % (*R*)	90.0	

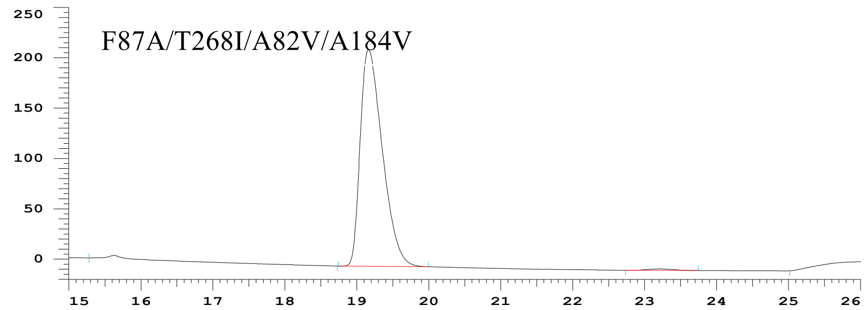
Figure S8 Typical chiral GC analyses for the epoxidation of 3-chlorostyrene catalyzed by the mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of 3-chlorostyrene epoxide; 2) F87A/T268I/A82G; 3) F87A/T268I/A82I; 4) F87A/T268I/A82L; 5) F87A/T268I/A82V; 6) F87A/T268I/V78A/A82V; 7) F87A/T268I/A82V/A184I; 8) F87A/T268I/A82V/A184V.



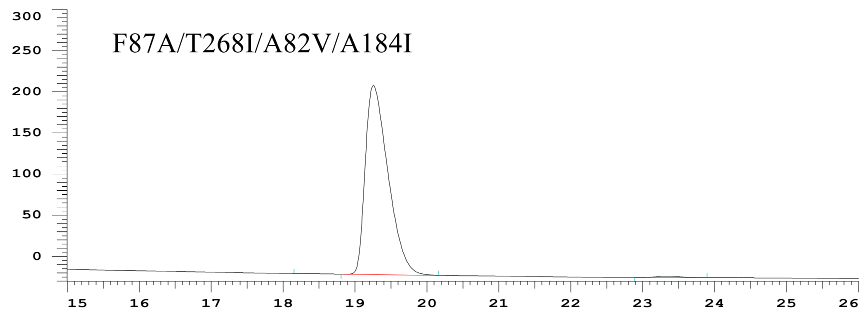
RT (min)	19.487 (*R*)	23.1 (*S*)
Area	13686771	14577173



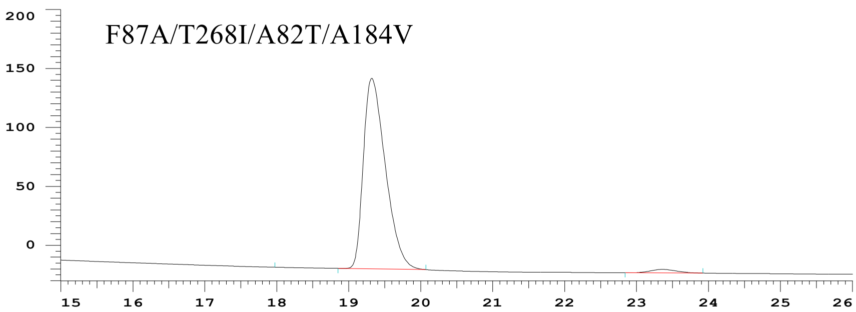
RT (min)	19.433 (*R*)	23.513 (*S*)
Area	4189595	37060
ee % (*R*)	98.3	



RT (min)	19.16 (*R*)	23.2 (*S*)
Area	4602696	36054
ee % (*R*)	98.4	

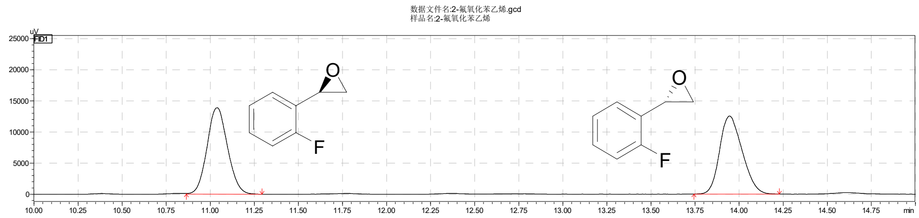


RT (min)	19.253 (*R*)	23.347 (*S*)
Area	5060257	39287
ee % (*R*)	98.5	

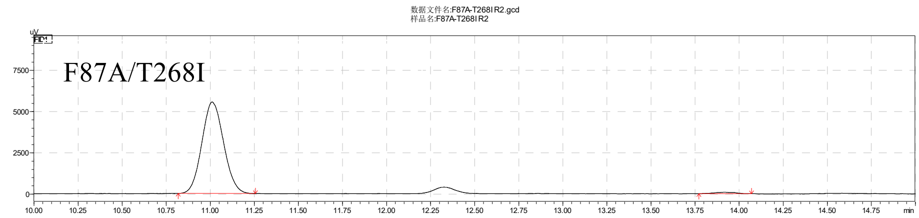


RT (min)	19.32 (*R*)	23.36 (*S*)
Area	3441219	75498
ee % (*R*)	95.7	

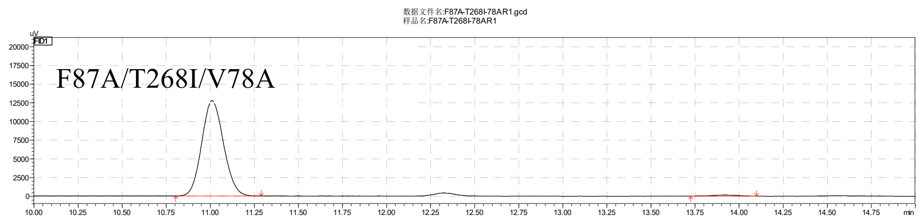
Figure S9 Typical chiral HPLC analyses for the epoxidation of 4-chlorostyrene catalyzed by the mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of 4-chlorostyrene epoxide; 2) F87A/T268I/A82V; 3) F87A/T268I/A82V/A184V; 4) F87A/T268I/A82V/A184I; 5) F87A/T268I/A82T/A184V.



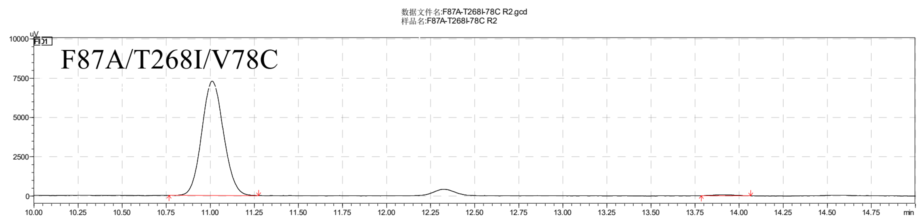
RT (min)	11.039 (*R*)	13.946 (*S*)
Area	112261	111902



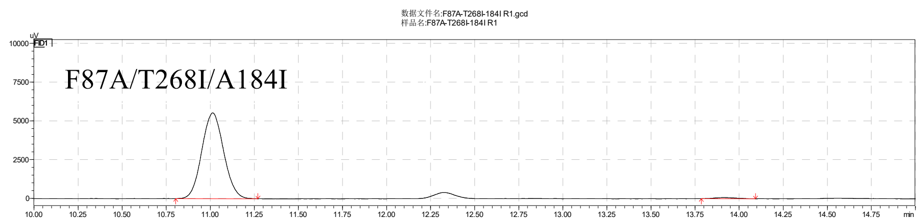
RT (min)	11.010 (*R*)	13.916 (*S*)
Area	47607	747
ee % (*R*)	96.9	



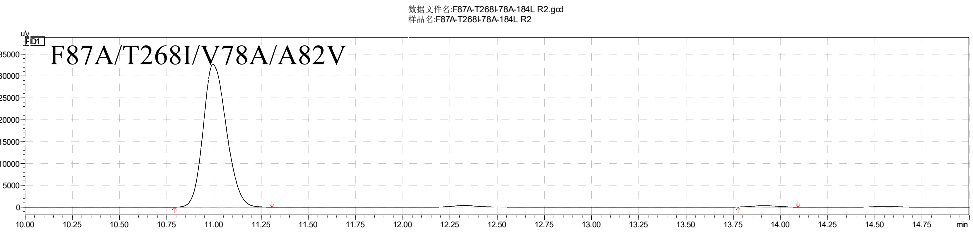
RT (min)	11.010 (*R*)	13.919 (*S*)
Area	109722	1387
ee % (*R*)	97.5	



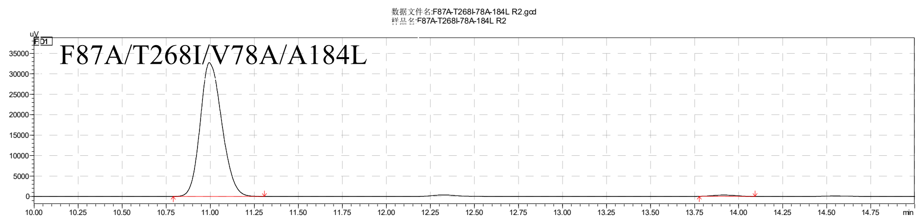
RT (min)	11.011 (*R*)	13.912 (*S*)
Area	62595	533
ee % (*R*)	98.3	



RT (min)	11.014 (*R*)	13.909 (*S*)
Area	47815	620
ee % (*R*)	97.4	

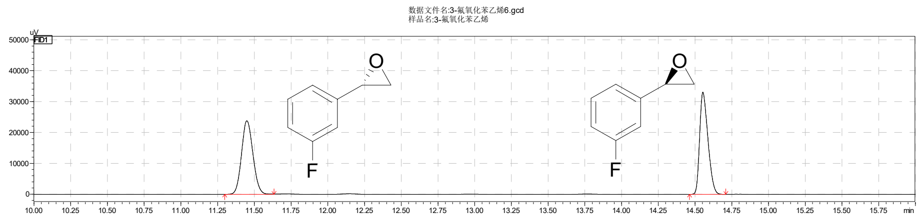


RT (min)	11.007 (*R*)	13.927 (*S*)
Area	184373	2175
ee % (*R*)	97.7	

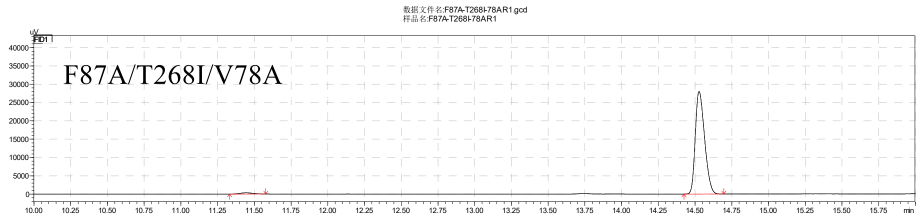


RT (min)	10.994 (*R*)	13.923 (*S*)
Area	279948	2796
ee % (*R*)	98.02	

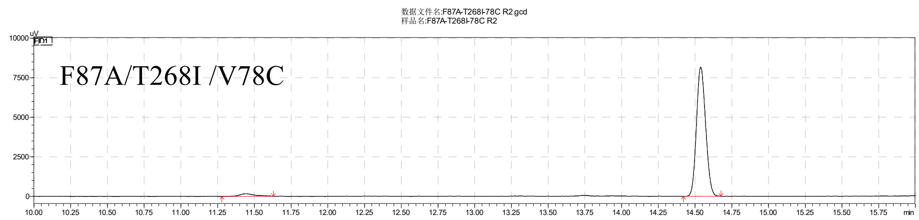
Figure S10 Typical chiral GC analyses for the epoxidation of 2-fluorostyrene catalyzed by the mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of 2-fluorostyrene epoxide; 2) F87A/T268I; 3) F87A/T268I/V78A; 4) F87A/T268I/V78C; 5) F87A/T268I/A184I; 6) F87A/T268I/V78A/A82V; 7) F87A/T268I/V78A/A184L.



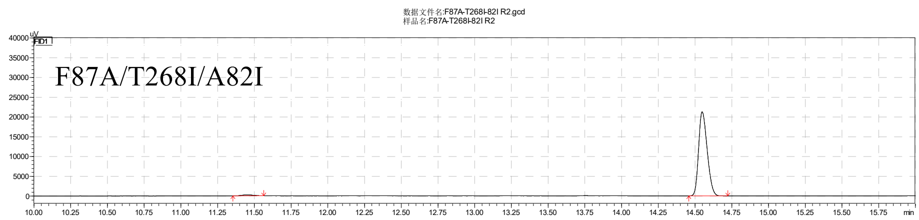
RT (min)	11.449 (*S*)	14.553 (*R*)
Area	128255	129815



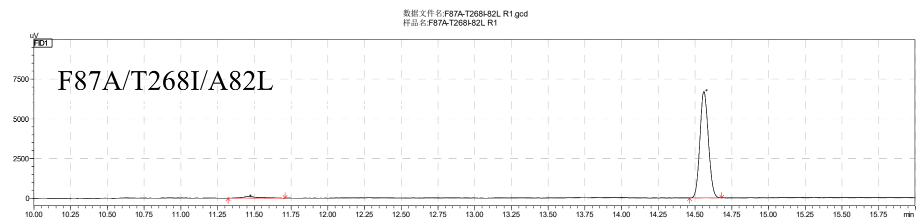
RT (min)	11.444 (*S*)	14.527 (*R*)
Area	2148	123088
ee % (*R*)	96.5	



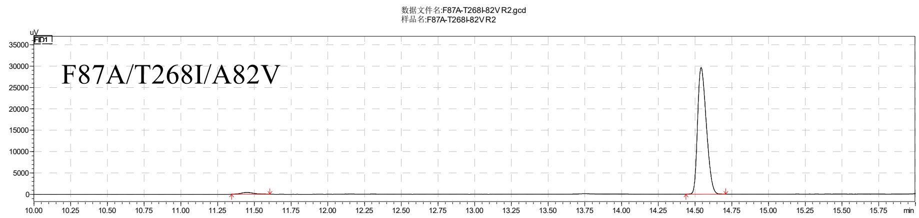
RT (min)	11.444 (*S*)	14.538 (*R*)
Area	1321	35581
ee % (*R*)	95.5	



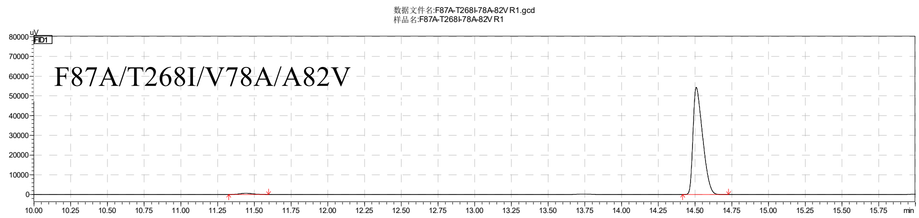
RT (min)	11.452 (*S*)	14.548 (*R*)
Area	1797	86400
ee % (*R*)	95.9	



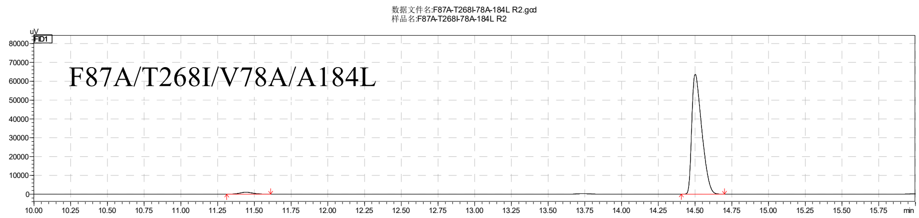
RT (min)	11.456 (*S*)	14.560 (*R*)
Area	789	26823
ee % (*R*)	94.2	



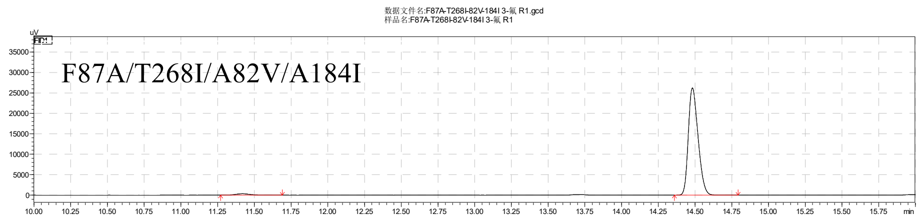
RT (min)	11.449 (*S*)	14.541 (*R*)
Area	2420	122935
ee % (*R*)	96.1	



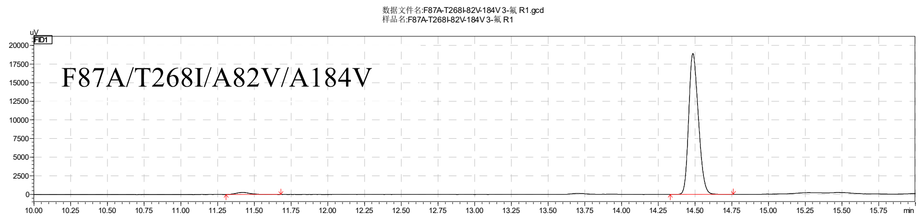
RT (min)	11.441 (*S*)	14.508 (*R*)
Area	3651	246550
ee % (*R*)	97.0	



RT (min)	11.442 (*S*)	14.501 (*R*)
Area	6267	301222
ee % (*R*)	95.9	

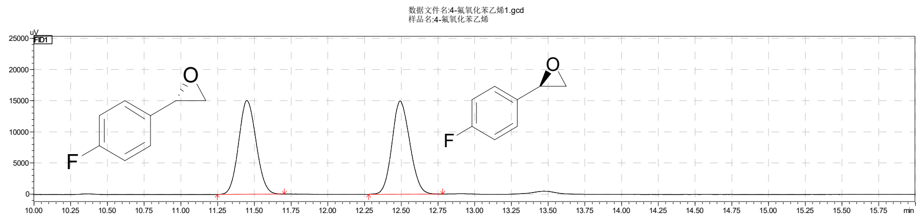


RT (min)	11.420 (*S*)	14.482 (*R*)
Area	2465	121186
ee % (*R*)	96.0	

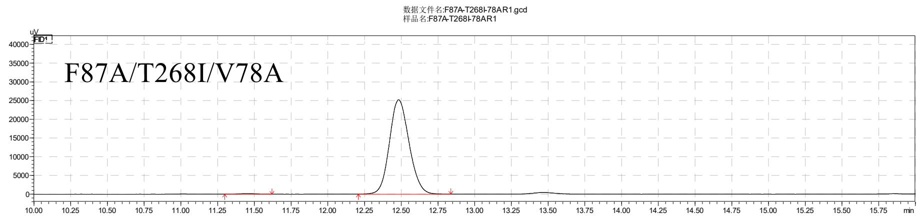


RT (min)	11.423	14.485
Area	1867 (*S*)	86641 (*R*)
ee % (*R*)	95.8	

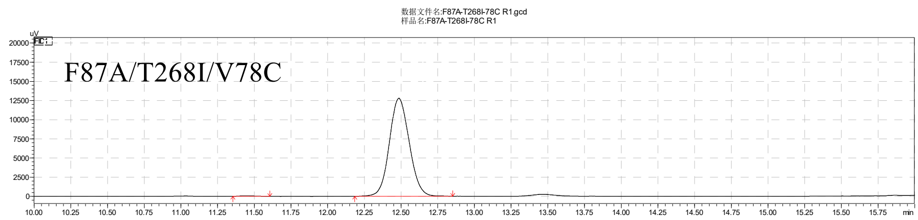
Figure S11 Typical chiral GC analyses for the epoxidation of 3-fluorostyrene catalyzed by the mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of 3-fluorostyrene epoxide; 2) F87A/T268I/V78A; 3) F87A/T268I/V78C; 4) F87A/T268I/A82I; 5) F87A/T268I/A82L; 6) F87A/T268I/A82V;7) F87A/T268I/V78A/A82V; 8) F87A/T268I/V78A/A184L; 9) F87A/T268I/A82V/A184I; 10) F87A/T268I/A82V/A184V.



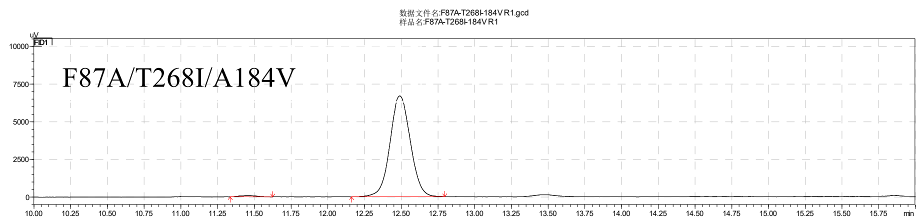
RT (min)	11.448 (*S*)	12.492 (*R*)
Area	125060	126331



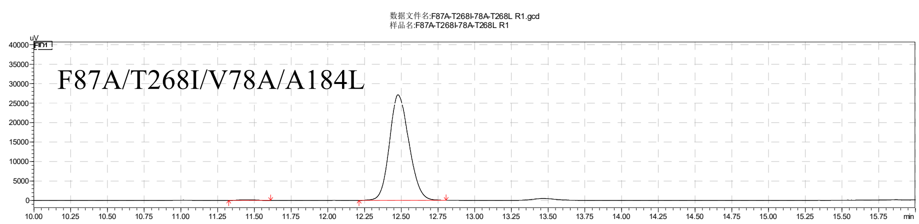
RT (min)	11.463 (*S*)	12.482 (*R*)
Area	1132	234908
ee % (*R*)	99.0	



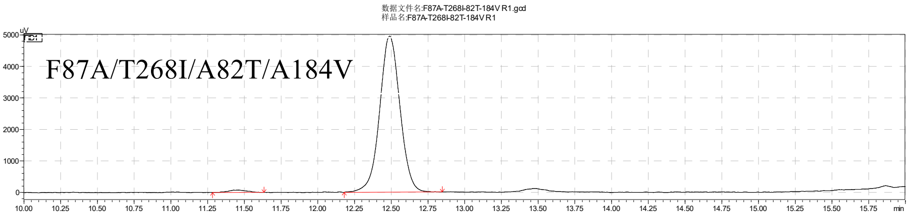
RT (min)	11.428 (*S*)	12.485 (*R*)
Area	412	120536
ee % (*R*)	99.3	



RT (min)	11.462 (*S*)	12.490 (*R*)
Area	688	64602
ee % (*R*)	97.9	



RT (min)	11.462 (*S*)	12.478 (*R*)
Area	976	250217
ee % (*R*)	99.2	



RT (min)	11.458 (*S*)	12.489 (*R*)
Area	671	47685
ee % (*R*)	97.2	

Figure S12 Typical chiral GC analyses for the epoxidation of 4-fluorostyrene catalyzed by the mutants of P450BM3 heme domain by addition of H_2O_2 (80 mM) at 4 ℃ in the presence of Im-C6-Phe. 1) the standard sample of 4-fluorostyrene epoxide; 2) F87A/T268I/V78A; 3) F87A/T268I/V78C; 4) F87A/T268I/A184V; 5) F87A/T268I/V78A/A184L; 6) F87A/T268I/A82T/A184V.

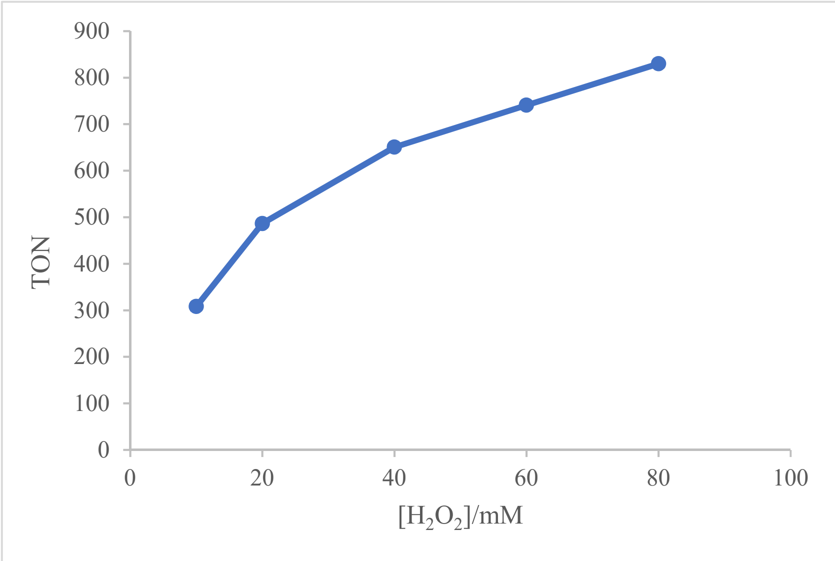


Figure S13 Optimizing the amount of H_2O_2 used in styrene epoxidation catalyzed by P450BM3 F87A/T268I/V78Cmutant (0.5 µM) in the presence of Im-C6-Phe (500 µM) at 25 °C.

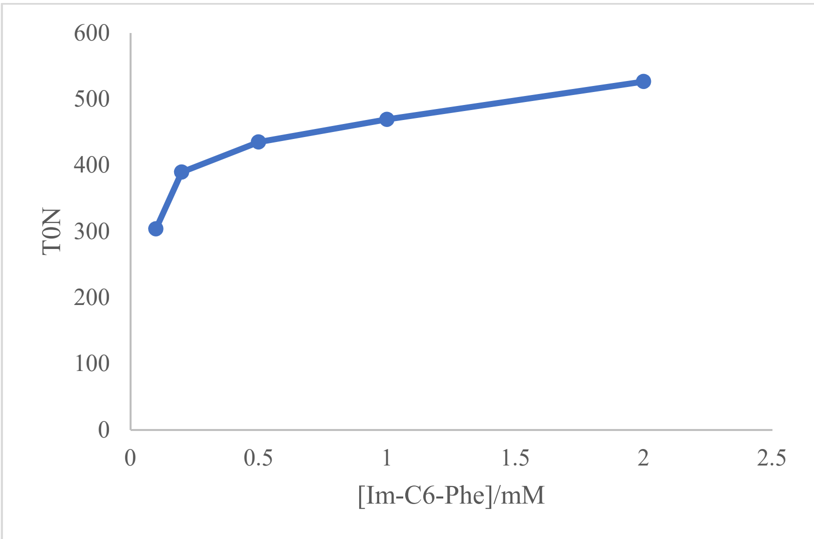
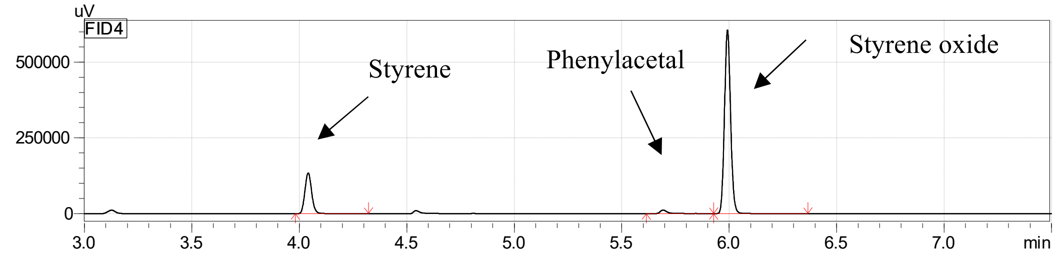


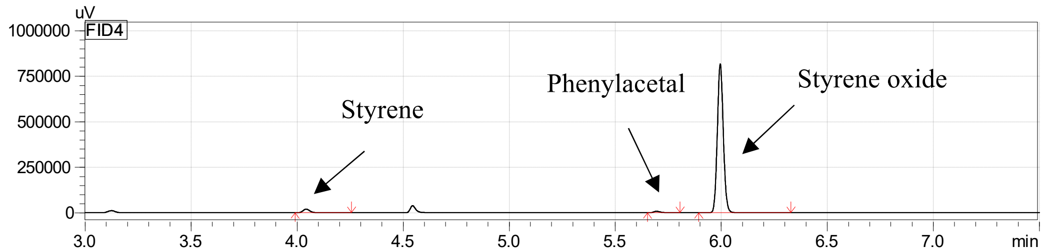
Figure S14 Optimizing the amount of Im-C6-Phe used in styrene epoxidation with H_2O_2 (20 mM) catalyzed by P450BM3_F87A/T268I/V78C mutant (0.5 µM) at 25 °C.

(A)



Enzyme	Styrene concentration / μM	Styrene oxide concentration / μM
F87A/T268I/L181Q	1592.6±53	5371.0±362

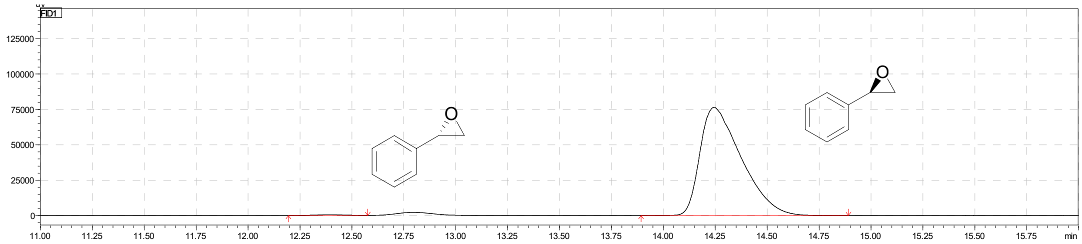
(B)



Enzyme	Styrene concentration / μM	Styrene oxide concentration / μM
F87A/T268I/V78A/A82V	341.6±85	6659.6±397

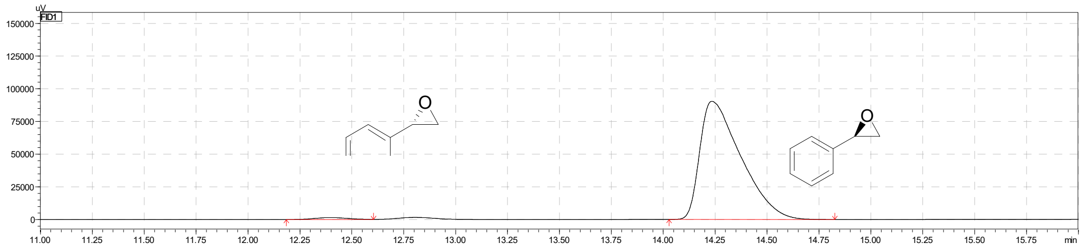
Figure S15 GC analyses for the unreacted styrene and formed styrene oxide in the semi-preparative scale epoxidation of styrene (10 mM) catalyzed by the mutants of F87A/T268I/L181Q (A) and F87A/T268I/V78A/A82V (B) upon addition of H_2O_2 (80 mM) in the presence of Im-C6-Phe (2 mM) in 0.1 M pH 8.0 phosphate buffer at 0 °C.

(A)



RT (min)	12.398 (*S*)	14.245 (*R*)
Area	6539	1057487
ee % (*R*)	98.8	

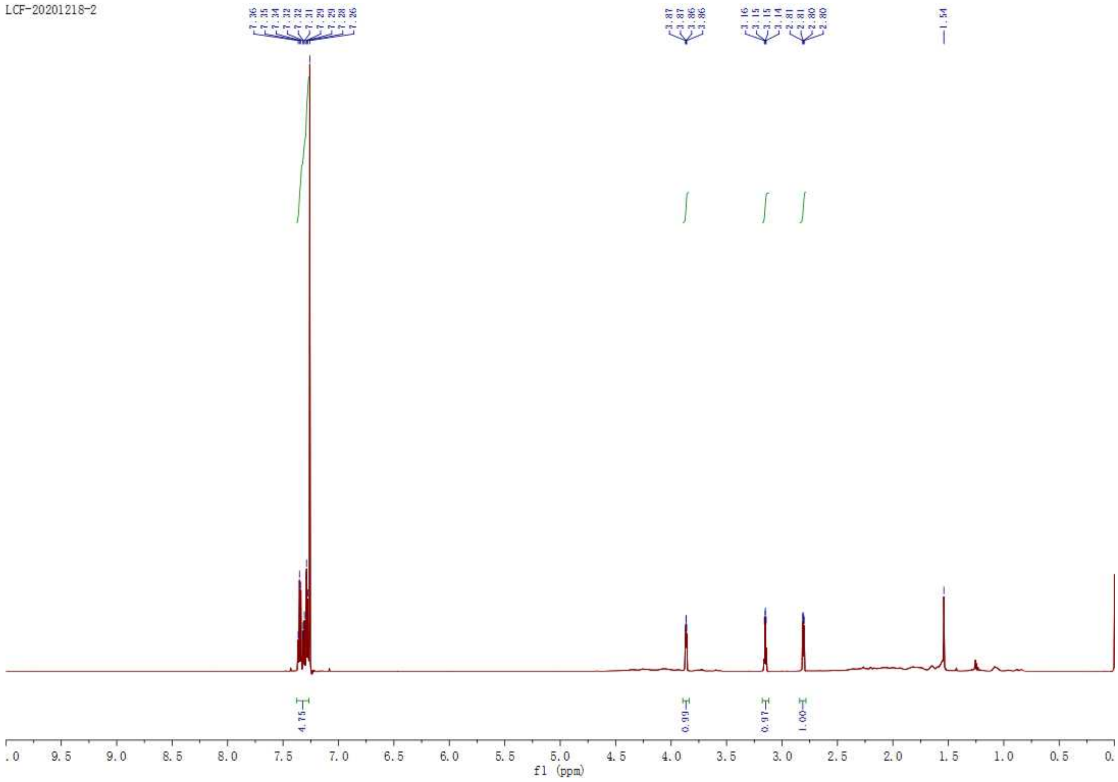
(B)



RT (min)	12.395 (*S*)	14.235 (*R*)
Area	16744	1241711
ee % (*R*)	97.4	

Figure S16 Chiral GC analyses for the semi-preparative scale epoxidation of styrene (10 mM) catalyzed by the mutants of F87A/T268I/L181Q (A) and F87A/T268I/V78A/A82V (B) upon addition of H_2O_2 (80 mM) in the presence of Im-C6-Phe (2 mM) in 0.1 M pH 8.0 phosphate buffer at 0 °C.

(A)



(B)

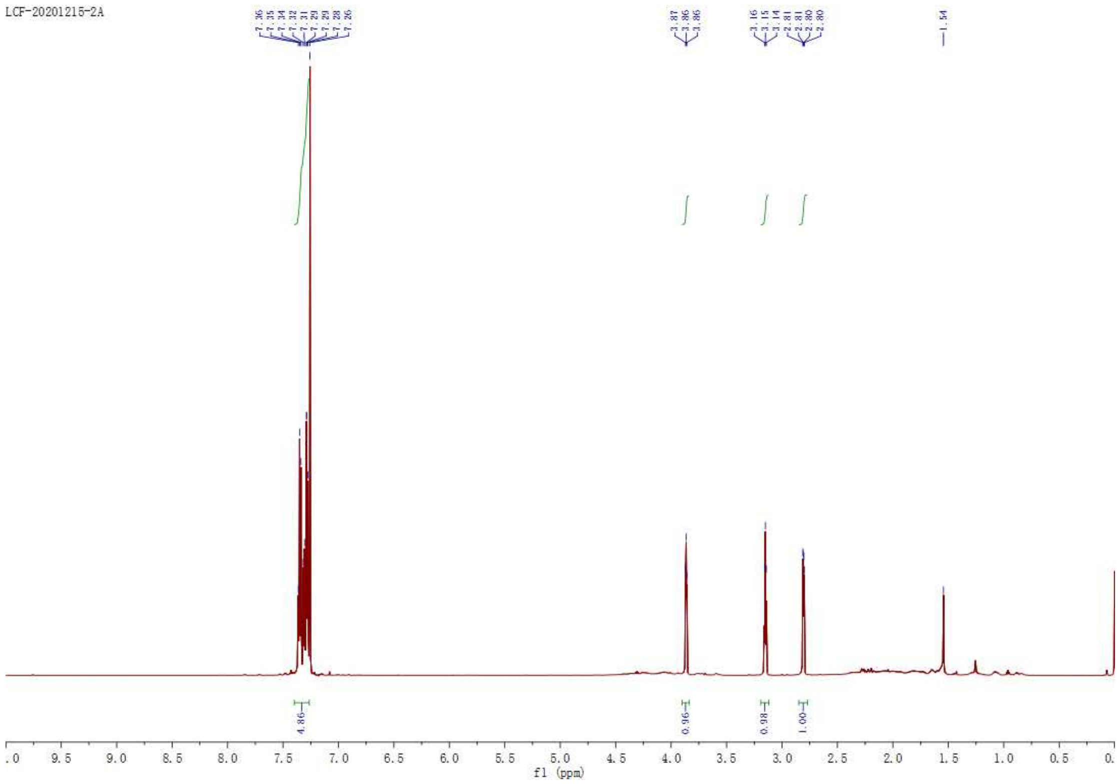
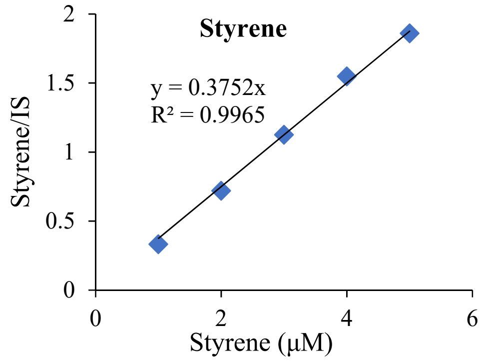
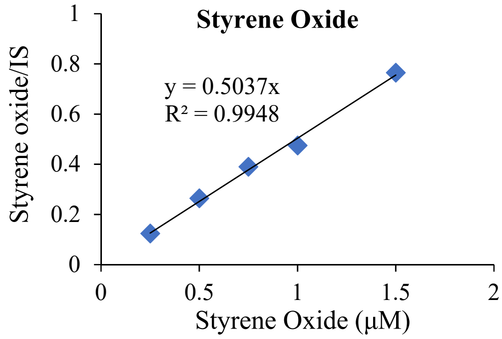
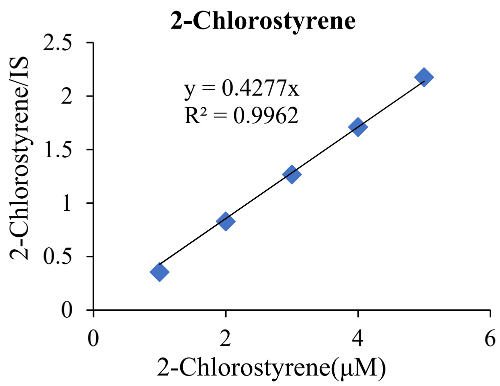
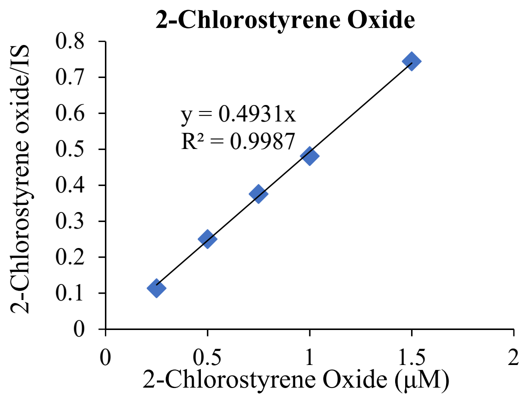


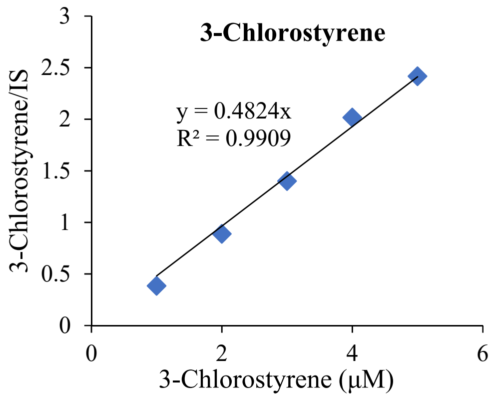
Figure S17 [1]H NMR spectra of styrene oxide isolated from the semi-preparative scale epoxidation of styrene (10 mM) catalyzed by the mutants of F87A/T268I/L181Q (A) and F87A/T268I/V78A/A82V (B) upon addition of H_2O_2 (80 mM) in the presence of Im-C6-Phe (2 mM) in 0.1 M pH 8.0 phosphate buffer at 0 °C.

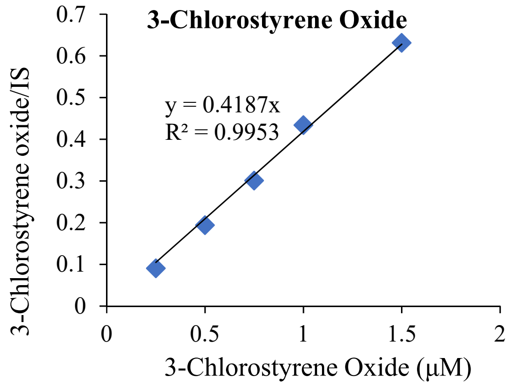

Styrene
y = 0.3752x
R² = 0.9965
Styrene/IS
Styrene (μM)

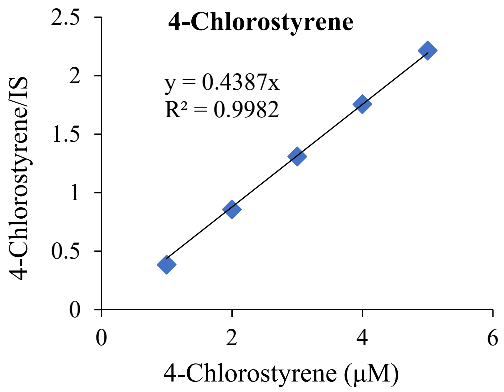

Styrene Oxide
y = 0.5037x
R² = 0.9948
Styrene oxide/IS
Styrene Oxide (μM)

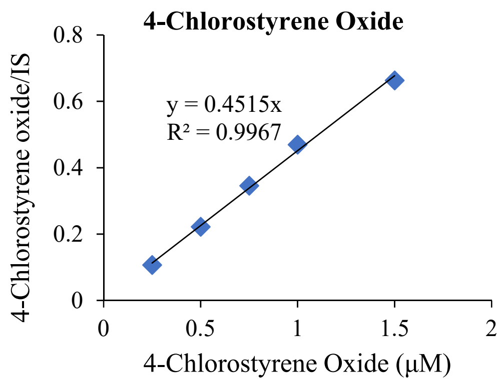

2-Chlorostyrene
y = 0.4277x
R² = 0.9962
2-Chlorostyrene/IS
2-Chlorostyrene(μM)


2-Chlorostyrene Oxide
y = 0.4931x
R² = 0.9987
2-Chlorostyrene oxide/IS
2-Chlorostyrene Oxide (μM)


3-Chlorostyrene
y = 0.4824x
R² = 0.9909
3-Chlorostyrene/IS
3-Chlorostyrene (μM)


3-Chlorostyrene Oxide
y = 0.4187x
R² = 0.9953
3-Chlorostyrene oxide/IS
3-Chlorostyrene Oxide (μM)


4-Chlorostyrene
y = 0.4387x
R² = 0.9982
4-Chlorostyrene/IS
4-Chlorostyrene (μM)


4-Chlorostyrene Oxide
y = 0.4515x
R² = 0.9967
4-Chlorostyrene oxide/IS
4-Chlorostyrene Oxide (μM)

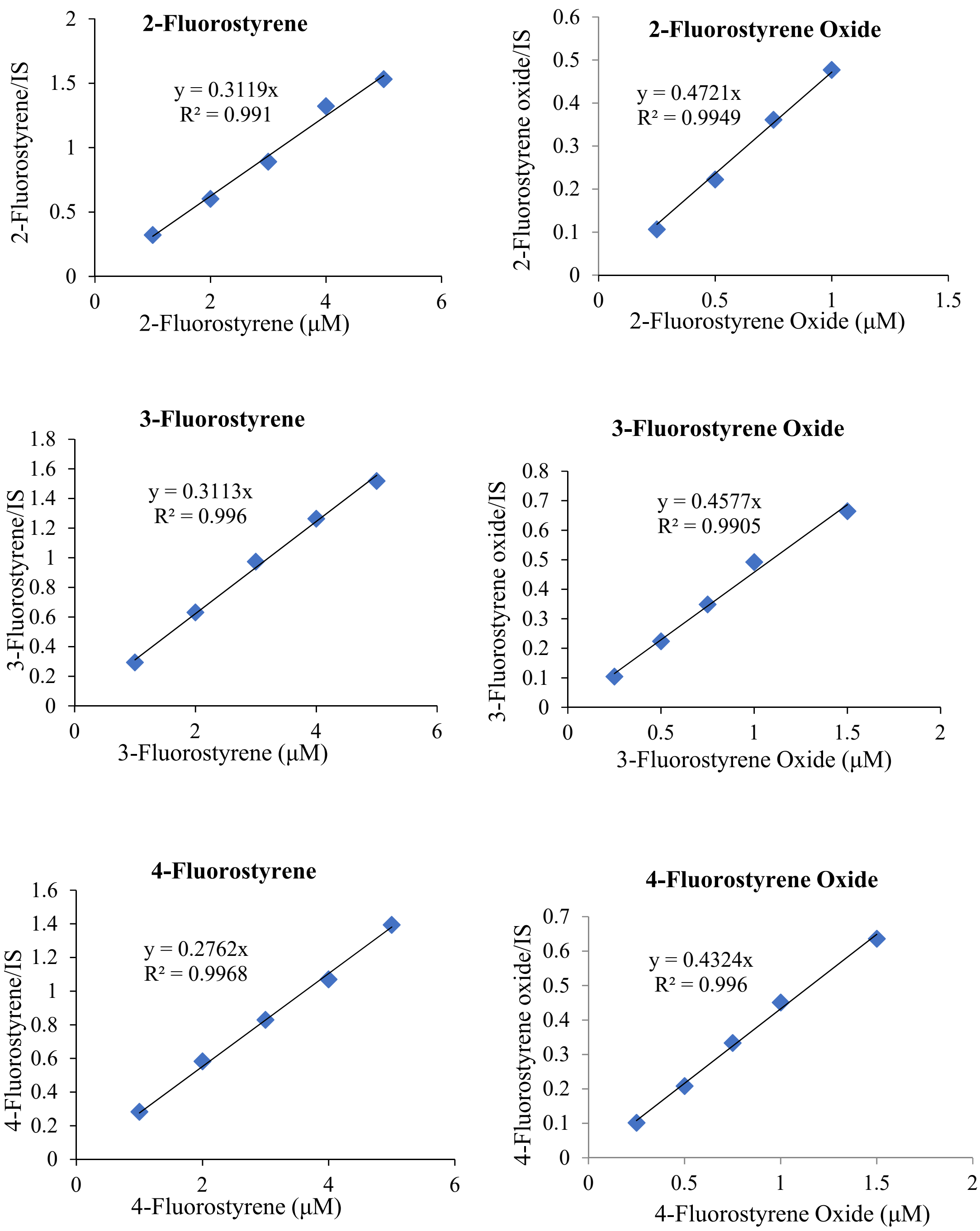


Figure S18 The calibration curves of styrene substrates and the corresponding epoxide products. IS: Internal standard.

Table S1 Screening of P450BM3 double mutants for the epoxidation of styrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268A	nd[d]	124±2
F87A/T268V	94	382±2
F87A/T268I	97	335±8
F87A/T268L	nd[d]	53±2
F87A/T268F	nd[d]	25±2
F87A/T268W	nd[d]	40±1
F87G/T268V	83	767±3
F87G/T268I	94	318±17
F87V/T268V	52	161±3
F87V/T268I	90	395±48
F87I/T268V	nd[d]	81±6
F87I/T268I	nd[d]	322±3
F87L/T268V	nd[d]	16±4
F87L/T268I	nd[d]	47±1

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (20 mM), Im-C6-Phe (0.5 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 25 °C. [b] All the control reactions did not show obvious activity of styrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S2 Screening of P450BM3 mutants for the epoxidation of styrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268I/L75M	97	1098±19
F87A/T268I/L75Y	nd[d]	59±1
F87A/T268I/L75F	97	80±1
F87A/T268I/L75Q	95	203±3
F87A/T268I/L75K	97	79±1
F87A/T268I/V78T	97	1006±19
F87A/T268I/V78M	97	296±4
F87A/T268I/V78A	97	1524±16
F87A/T268I/V78C	97	830±20
F87A/T268I/V78F	94	874±4
F87A/T268I/V78I	96	201±1
F87A/T268I/V78L	97	249±2
F87A/T268I/V78S	97	917±3
F87A/T268I/A82V	97	1027±22
F87A/T268I/A82I	97	1086±5
F87A/T268I/A82C	97	792±18
F87A/T268I/A82G	97	527±20
F87A/T268I/A82L	96	544±1
F87A/T268I/A82S	96	645±1
F87A/T268I/A82F	nd[d]	12±1
F87A/T268I/A82N	nd[d]	30±1
F87A/T268I/A82T	96	578±1
F87A/T268I/A82M	91	1472±2
F87A/T268I/A82E	51	nd[d]
F87A/T268I/L181F	97	481±2
F87A/T268I/L181Q	99	918±54
F87A/T268I/L181I	97	879±10
F87A/T268I/L181M	98	1576±82
F87A/T268I/L181T	98	879±52
F87A/T268I/L181N	98	355±1
F87A/T268I/A184F	nd[d]	nd[d]
F87A/T268I/A184I	97	635±1
F87A/T268I/A184Q	97	401±1
F87A/T268I/A184T	97	664±13
F87A/T268I/A184V	97	804±1
F87A/T268I/A184L	97	816±3
F87A/T268I/A184M	97	1015±3
F87A/T268I/A184N	97	nd[d]
F87A/T268I/R255S	98	298±1
F87A/T268I/R255D	>99%	120±1
F87A/T268I/R255V	98	164±1

F87A/T268I/R255L	98	292±5
F87A/T268I/R255Q	98	435±4
F87A/T268I/I263V	98	608±8
F87A/T268I/I263G	92	206±32
F87A/T268I/A264C	nd[d]	nd[d]
F87A/T268I/A264S	96	698±2
F87A/T268I/A264T	95	526±5
F87A/T268I/E267Q	91	478±18
F87A/T268I/E267L	97	148±3
F87A/T268I/A328V	92	47±1
F87A/T268I/A328S	98	150±3

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 25 °C. [b] All the control reactions did not show obvious activity of styrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S3 Screening of P450BM3 mutants for the epoxidation of styrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

F87A/T268I/V78A/A82V	98	4052±22
F87A/T268I/V78A/A181Q	97	2060±40
F87A/T268I/V78A/A181M	97	2051±36
F87A/T268I/V78A/A184L	98	4349±26
F87A/T268I/A82V/A181Q	98	236±1
F87A/T268I/A82V/A181M	99	699±9
F87A/T268I/A82V/A184L	98	1030±50
F87A/T268I/L181Q/A184L	98	815±22
F87A/T268I/L181M/A184L	97	538±17

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 4 °C. [b] All the control reactions did not show obvious activity of styrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S4 Screening of P450BM3 mutants for the epoxidation of 2-chlorostyrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268A	87	1724±7
F87A/T268I	50	153±1
F87A/T268V	51	514±1
F87A/T268A/V78A	95	1445±14
F87A/T268A/A82V	90	2023±24
F87A/T268A/L181M	85	1300±51
F87A/T268A/L181Q	80	1314±3

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 4 °C. [b] All the control reactions did not show obvious activity of 2-chlorostyrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S5 Screening of P450BM3 mutants for the epoxidation of 3-chlorostyrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268I/A82G	92	310±5
F87A/T268I/A82I	96	362±2
F87A/T268I/A82L	96	479±11
F87A/T268I/A82V	91	262±13
F87A/T268I/V78A/A82V	91	472±4
F87A/T268I/A82V/A184I	91	496±13
F87A/T268I/A82V/A184V	90	311±4

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 4 °C. [b] All the control reactions did not show obvious activity of 3-chlorostyrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S6 Screening of P450BM3 mutants for the epoxidation of 4-chlorostyrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268I/A82V	98	454±1
F87A/T268I/A82V/A184V	98	671±34
F87A/T268I/A82V/A184I	98	719±1
F87A/T268I/A82T/A184V	96	473±3

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 4 °C. [b] All the control reactions did not show obvious activity of 4-chlorostyrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S7 Screening of P450BM3 mutants for the epoxidation of 2-fluorostyrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268I	97	677±10
F87A/T268I/V78A	98	483±25
F87A/T268I/V78C	98	1215±11
F87A/T268I/A184I	97	488±23
F87A/T268I/V78A/A82V	98	2192±10
F87A/T268I/V78A/A184L	98	3480±216

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 4 °C. [b] All the control reactions did not show obvious activity of 2-fluorostyrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S8 Screening of P450BM3 mutants for the epoxidation of 3-fluorostyrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268I/V78A	97	1304±6
F87A/T268I/V78C	96	330±3
F87A/T268I/A82I	96	882±22
F87A/T268I/A82L	94	829±10
F87A/T268I/A82V	96	503±1
F87A/T268I/V78A/A82V	97	2876±130
F87A/T268I/V78A/A184L	96	3316±5
F87A/T268I/A82V/A184I	96	1215±42
F87A/T268I/A82V/A184V	96	887±8

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 4 °C. [b] All the control reactions did not show obvious activity of 3-fluorostyrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30/min reaction. [d] nd: not detected.

Table S9 Screening of P450BM3 mutants for the epoxidation of 4-fluorostyrene with H_2O_2 in the presence of Im-C6-Phe [a, b]

mutations	ee %	TON
F87A/T268I/V78A	99	2037±13
F87A/T268I/V78C	99	1312±11
F87A/T268I/A184V	98	680±13
F87A/T268I/V78A/A184L	99	2803±51
F87A/T268I/A82T/A184V	97	480±9

[a] Reaction conditions: P450BM3 (0.5 μM), H_2O_2 (80 mM), Im-C6-Phe (2 mM), styrene (4 mM) in pH 8.0 phosphate buffer at 4 °C. [b] All the control reactions did not show obvious activity of 4-fluorostyrene epoxidation in the absence of Im-C6-Phe. [c] TON: Turnover numbers were estimated over a 30 min reaction. [d] nd: not detected.