Supporting Information

Materials

All chemical reagents were acquired from commercial suppliers and used without further purification unless otherwise noted. Styrene was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan), and purified using alumina column chromatography to remove the polymerisation inhibitor immediately before the reaction. Dimethyl sulphoxide (DMSO) and methyl phenylacetate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Tris(hydroxymethyl)aminomethane (Tris), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), potassium chloride (KCl) and methanol were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Sodium dithionite ($Na_2S_2O_4$) was purchased from Sigma-Aldrich Co. (St. Louis, MO). Ethyl diazoacetate (EDA) was purchased from Toronto Research Chemicals, Inc. (North York, Canada). Ethyl trans-2-phenylcyclopropanecarboxylate was procured from PharmaBlock Sciences (Nanjing), Inc. (Nanjing, China). The decoy molecules used in this study were synthesised and characterised as reported previously.[1,2]

Experimental Procedures

P450BM3 mutant preparation

QuikChange site-directed mutagenesis[3] was employed using the pET-28a vector encoding the P450BM3 haem domain (1-455) as a template. To avoid insertion of tandem repeats of the primer sequence into the plasmid, we adopted the method described by Edelheit, Hanukoglu, & Hanukoglu, 2009.[4] The target plasmids were amplified in and extracted from the transformants (*Escherichia coli* DH5α), and confirmed to have the desired mutation by sequence analysis. Each expression vector encoding one of the two P450BM3 mutants was transformed into *E. coli* BL21(DE3) for protein expression.

Primer sequences used for site-directed mutagenesis

T268A:

Fwd: 5'-GCGGGACACGAA**GCA**ACAAGTGGTC-3'

Rev: 5'-GACCACTTGT**TGC**TTCGTGTCCCGC-3'

F87A:

Fwd: 5'-GCAGGAGACGGGTTA**GCT**ACAAGCTGGACGCATG-3'

Rev: 5'-CATGCGTCCAGCTTGT**AGC**TAACCCGTCTCCTGC-3'

Expression and purification of P450BM3 mutants

E. coli BL21(DE) expressing P450BM3 were suspended in 20 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM EDTA and then disrupted using an ultrasonicator at 4°C. After centrifugation, the supernatant was applied to a CELLUFINE A-500 anion-exchange column and washed with 20 mM Tris-HCl buffer (pH 7.4) containing 50 mM KCl. Tightly bound proteins including P450BM3 were eluted with 20 mM Tris-HCl buffer (pH 7.4) containing 200 mM KCl and desalted by spin centrifugation-dialysis using Amicon Ultra Centrifuge Filter Ultracel® (Millipore,Co.) with a MWCO of 30 kDa. The concentrated protein was further purified on an ÄKTAexplorer 100 equipped with a HiTrap 5 mL Capto DEAE column (GE Healthcare Life Sciences) in 20 mM Tris-HCl buffer (pH 7.4) over a KCl concentration gradient ranging from 100 mM to 250 mM. Eluted fraction containing P450BM3 were pooled, desalted, and concentrated with Amicon Filters, whereafter the protein was applied to a Sephacryl S-200 gel-filtration column equilibrated in 20 mM Tris-HCl buffer (pH 7.4) containing 100 mM KCl. P450BM3 fractions were collected, pooled, and concentrated, followed by estimation of the P450BM3 concentration by the pyridine haemochromogen assay.[5,6]

Biocatalytic Cyclopropanation

Reactions were carried out under argon and conducted according to a procedure reported in the literature.[7] The final concertation of all components of the reaction mixture were as follows: 1% DMSO, 3% methanol, 5 μM P450BM3, 200 μM decoy molecule, 10 mM styrene, 10 mM EDA, and 10 mM $Na_2S_2O_4$. 100 mM HEPES buffer was used to maintain pH at 8.0 during the reaction. After 14 hours, the reaction was quenched and the products were extracted into CH_2Cl_2. The internal standard (methyl phenylacetate) was also added at this point. The organic layer was analysed by GC-MS (Shimadzu GCMS-QP2010 SE) equipped with CycloSil-β column. The turnover numbers as well as diastereo- and enantioselectivities were estimated from calibration curves generated from the authentic samples. All biocatalytic reactions were performed at least in triplicate, and the unit of turnover number is expressed as (mol product) per (mol P450). The GC-MS conditions were as follows: column temperature: 115°C (50 min); 50°C min^{-1}; 220°C (10 min), injection temperature: 250°C, interface temperature: 250°C, carrier gas: helium, split ratio: 50. The retention times in GC-MS of the products and the internal standard were as follows: methyl phenylacetate (8.5 min), (1*S*,2*R*) isomer (32.1 min), (1*R*,2*S*) isomer (34.7 min), (1*R*,2*R*) isomer (42.0 min), and (1*S*,2*S*) isomer (43.2 min).

Computational Experiments

Molecular dynamics simulation and docking simulation

The structures of the P450BM3 T268A and F87A/T268A mutant were obtained from the protein data bank and their PDB IDs are 1YQO and 3DGI, respectively. The missing loop in the 3DGI

chain A structure was modelled with Modeller 9.21[8,9] prior to the following simulations. Protein protonation states were predicted using the H++ sever.[10,11] Quantum chemical calculations for parametrisation of non-amino acid molecules, required for molecular dynamics simulations, were performed with Gaussian 09 rev. E.01.[12] The force field parameters and atomic partial charges of the haem carbene intermediate were generated with MCPB.py[13] and VFFDT[14] at B3LYP/6-31(d) level. In accordance with the literature, the spin state of the intermediate is set as an open-shell singlet.[15] As for the parameters of R-Ibu-Phe, R.E.D.-III tools[16] was employed to derive RESP-A1 charges.[17] The docking simulation of R-Ibu-Phe into the F87A/T268A mutant was carried out with rDock.[18]

Each structure was solvated in a box with a 10 Å buffer of TIP3 water molecules[19] and neutralised by addition of Na^+ ions using the AMBER *leap* module.[20] AMBER14 and GAFF2 force fields[21,22] were applied to protein and non-protein solutes, respectively. The resulting topology files were converted into Gromacs format with the tool ACPYPE.[23] Molecular dynamics simulations were performed as follows using Gromacs.[24,25] The system energy was minimised with the steepest descent method followed by the conjugate gradient method. The system was then heated to 300 K in a 200 ps NVT MD simulation, in which harmonic position restraints of 1000 kJ mol^{-1} $Å^{-2}$ force constant were applied on all the atoms of the solutes except for the hydrogen atoms. The restraints were gradually relaxed to 10 kJ mol^{-1} $Å^{-2}$ in successive NPT simulations, whereby the system was equilibrated to 300 K and 1.01325 bar. Finally, a 200 ns production run was conducted. To maintain the temperature and pressure, Berendsen thermo- and barostats[26] were employed, and for the production MD, the Parrinello-Rahman method[27,28] was used for pressure coupling. The particle mesh Ewald method[29,30] was used for long-range electrostatics and the cut-off value for Lennard-Jones and electrostatic interactions was set to 9 Å. P-LINCS algorithm[31] was applied to constrain the bonds involving hydrogen. A 2 fs time step was used for the simulations. Each resulting 200 ns MD simulation trajectory was converted into AMBER format, and then analysed with the cpptraj module.[32] The most "average-like" structure of the last 50 ns of the 200 ns simulation was extracted from the trajectory, based on the value of the root mean square deviation of all Cαs. The docking simulation of styrene was conducted on AutoDock Vina.[33] The initial structure of styrene was downloaded from the PubChemQC server.[34]

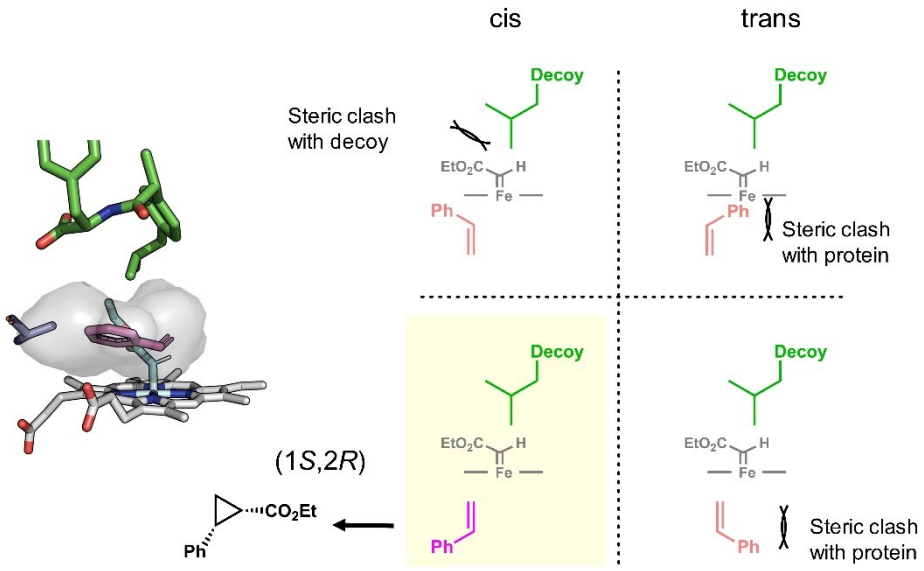

Fig. S1 Proposed geometries of the reaction complex to allow cyclopropanation of styrene catalysed by the F87A/T268A mutant of P450BM3 in complex with R-Ibu-Phe. The least amount of steric repulsion is possible when styrene is in a conformation to yield the (1*S*,2*R*) isomer.

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