Supplementary Information for

Reshaping the Active Pocket of Promiscuous Lactonases for Degrading Bulky Organophosphate Flame Retardants

Yuanyuan Zhang,[†,a] Xiaoyan Zhang,[†,a] Huan Cheng,[†,a] Mario Roque Huanca Nina,[a] Jun Ge[*,b] and Yunpeng Bai[*,a]

[a]State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, 200237.

[b]Key Lab for Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China.

*Email: junge@mail.tsinghua.edu.cn, ybai@ecust.edu.cn

[†] These authors contribute equally.

Contents

Materials and methods

Chemicals, Reagents, and Genes. Triphenyl was phosphate purchased from Bailingwei Chemical Technology Co. (Shanghai, China). Methyl parathion was purchased from Shanghai Pesticide Research Center. Phenol and p-nitrophenol were purchased from Saen Chemical Technology Co. (Shanghai, China). T4 DNA ligase, PrimeSTAR HS DNA polymerase, recombinant Taq DNA polymerase and restriction enzymes were purchased from TaKaRa (Dalian, China). Dpn *I* was purchased from New England Biolabs. The DmpR gene, promoter Po, and *sf*GFP gene were synthesized by Generay Biotech Co. (Shanghai, China). pSB1C3 plasmid was purchased in Wuhan Miaoling Biotech Co. The expression host *E. coli* strain BL21(DE3), *E. coli* DH5α and expression vector pET-28a (+) were preserved in our laboratory.

Design and construction of whole-cell biosensors. The DmpR gene from *Pseudomonas* sp. CF600 with its cognate promoter Po, and *sf*GFP gene were synthesized by Shanghai Generay Biotech Co. First, the DmpR gene was placed between the constitutive promoter BBa_J23114, the ribosome binding site BBa_B0034, and the terminator BBa_B0014 to form the sensing module. The Po promoter, the ribosome binding site BBa_B0034, the *sf*GFP gene, and the terminator BBa_B0015 were fused to form the reporter module. Then, the sensing and reporter modules were first assembled in the BioBrick standard vector pSB1C3 plasmid (pSB1C3-DSF, Wuhan Miaoling Biotech, China) and then transformed into *E. coli* DH5α, yielding the phenol-responsive biosensor *E. coli* DH5α/pSB1C3-DSF. Finally, seamless cloning method was used to subclone the whole segment of pSB1C3-DSF which was inserted

into the *pooph*-containing plasmid pET28a-*Po*OPH at the restriction sites of *Bgl* II and *Sgr*A I, and transformed into *E. coli* BL21 (DE3) cells to yield the whole-cell biosensor *E. coli* BL21/pET28a-OPH-DSF.

Characterization of whole-cell biosensors. The recombinant *E. coli* cells (*E. coli* BL21/pET28a-OPH-DSF) were inoculated into 4 mL LB media with 50 μg mL^{-1} kanamycin, and shaken at 37ºC, 180 rpm for 12 h. Then, the culture was diluted (1:100 v/v) in fresh medium and cultured until the OD_{600} reached 0.6 when the effector (phenol, p-nitrophenol and methyl parathion) was added to induce protein expression. The culture was grown at 30ºC for different period before the cells were harvested by centrifugation (12,000×g, 6 min). For fluorescence analysis, the harvested cells were washed with PBS buffer (100 mM, pH 7.0) twice, resuspended in PBS buffer with OD_{600} 0.2~0.8 and 200 μL sample was added in 96-well microtiter plate. The fluorescence (λ_{EX} = 488 nm and λ_{Em} = 510 nm) was monitored every 2 h using a microplate spectrophotometer (SpectraMax M2, Molecular Devices, USA). All the experiments were repeated 3 times. For fluorescence microscopy analysis, the resuspended culture (OD_{600} 0.2) was added with 50% glycerol and 10 μL sample was dropped on the glass slide for fluorescence detection (600× magnification) using an inverted fluorescence microscope (Nikon, Japan). The photos were processed with NIS-Viewer.

High-throughput Screening of model libraries. The cell culture (*Po*OPH_{M2} and *Po*OPH_{WT}) was induced with methyl parathion (100 μM) overnight at 30ºC and mixed with different ratios (1:1,1:10,1:100,1:1000 and 1:10000) to form different binary

libraries. The negative control (without cells) and the positive control (*Po*OPH$_{M2}$ and *Po*OPH$_{WT}$) were also prepared for analysis. 100 μL pool samples were diluted with 1 mL PBS buffer (sterile before sorting), and washed twice before analysis and screening using FACS Jazz (BD Biosciences) with excitation wavelength at 488 nm (PTM3: FITC channel) and emission at 530/30 nm. System pressure was adjusted for analysis at the events (5000 events/s). Sorting threshold was determined by analyzing 100,000 cells, and the sorting ratio was set 1% for Lib-A (1:100), 0.1% for Lib-B (1:1000) and Lib-C (1:10000). Lib-C was sorted twice before collection. The sorting data was analyzed by the software flowJo.

The sorted sample was collected with the fresh LB medium (50 μg mL^{-1} kanamycin). The sorted cells were recovered by shaking at 180 rpm, 37℃ for 1h, and diluted for spreading at a LB solid medium, cultured at 37℃ overnight and picked up with sterile toothpicks to inoculate in a 200 μL LB media containing 50 μg mL^{-1} kanamycin in 96-well plates. The cultures were grown overnight at 37ºC and 220 rpm, and then the broth was transferred to another plates containing 600 μL LB media and 50 μg mL^{-1} kanamycin. The plates were incubated at 37ºC and 220 rpm for 3 h, and protein expression was induced with the addition of 0.2 mM IPTG and 0.1 mM TPHP at 16ºC 20 h. The activity of crude enzymes in cell extract was determined by measuring the hydrolysis of methyl parathion at 405 nm absorption. The positive *Po*OPH$_{M2}$ cells were further confirmed by DNA sequencing.

Directed evolution of the promiscuous *Po*OPH$_{M2}$ for degrading TPHP. The site-directed mutagenesis method was employed on residues around the substrate-binding

pocket of *Po*OPH within 4Å～6Å. The variants were generated using the QuikChange site-directed mutagenesis protocol. pET28-*Po*OPH$_{M2}$ was used as the initial template. The degenerate primers (Table S1) were used as the mutant primers, and the high-fidelity Primer STAR was used for fragment amplification. Following digestion of the template by *Dpn* I, the PCR products were transformed into *E. coli* DH5α. After culturing for 16 h, the bacteria on plates were scraped off and the mixed plasmids were extracted. The mutated genes in mixed plasmids were amplified by PCR and digested by restriction endonucleases of *Bam*H I and *Hin*d Ⅲ, ligated with recombinant plasmid pET28-DSF, and transformed into *E. coli* BL21 for subsequent screening.

For screening the mutant library of *Po*OPH, the mutated clones were picked with sterile toothpicks to inoculated in 200 μL LB media containing 50 μg mL^{-1} kanamycin in 96-well plates. The cultures were grown overnight at 37ºC and 220 rpm, and then the broth was transferred to another plates containing 600 μL LB media and 50 μg mL^{-1} kanamycin. The plates were incubated at 37ºC and 220 rpm for 3 h, and protein expression was induced with the addition of 0.05 mM IPTG and 0.1 mM TPHP at 30ºC for another 24 h. Then, the cells in each well were harvested, washed and resuspended in PBS buffer in the second 96 well-plate, and fluorescence were measured using a Synergy LX multi-mode microplate reader (BioTek, U.S.). The clones with higher fluorescence per OD_{600} were selected for further validation.

Protein Purification. The selected variants were inoculated into LB medium with 50 μg mL^{-1} kanamycin. When the OD_{600} reached 0.8, 0.4 mM IPTG was added to induce the protein expression at 16ºC for 24 h. The cells were harvested by

centrifugation and resuspended in Buffer A which contained 50 mM Tris-HCl (pH 8.0), 500 mM NaCl and 20 mM imidazole before disrupted by ultrasonication. After removing the cell debris by centrifugation at 12,000 rpm for 0.5 h, the supernatant was filtered on a 0.45 μm ultrafiltration membrane and loaded onto a Ni^{2+}-NTA affinity column (with Buffer A pre-equilibrated). The fractions were collected by gradient elution with 20 mM Tris-HCl (pH 8.0), 500 mM NaCl and increasing concentration (20-500 mM) of imidazole. Fractions containing the purified target protein verified by SDS-PAGE were combined and concentrated via Ultrafiltration tube (10 kDa, Millipore), and the imidazole was washed out with Buffer C (50 mM Tris-HCl, pH 8.0 and 150 mM NaCl), then the harvested protein was stored at 4ºC.

Activity assays and kinetic analysis. The TPHP hydrolysis activity of different *Po*OPH mutants was assayed by monitoring the reaction rates using HPLC. The reaction solution of 500 μL contained 425 μL Tris-HCl (50 mM, pH 9.0), 25 μL TPHP (100 mM, methanol dissolved), and 50 μL purified enzyme (diluted to 1 mg/mL), which was shaken at 1000 rpm and 30ºC. After different time periods, the hydrolysis reaction was stopped by the addition of 50 μL H_2SO_4 (10%, v/v), and extracted twice with 1.5 eq volumes of ethyl acetate. After volatilization, the sample was re-dissolved in 200 μL methanol for HPLC analysis. Kinetic parameters were obtained by measuring the reaction rates with substrate concentrations from 0.1 to 5 mM, using 1 mL solution containing 10 μL purified enzyme (diluted to 2 mg/mL) and fitting data to the Michaelis-Menten equation. The optimum temperature and pH were obtained by measuring enzyme activities at temperatures ranging from 20 to 70ºC and in different

buffers, respectively. Four different buffers were sodium citrate buffer (50 mM, pH 4.0-6.0), phosphate buffer (50 mM, pH 6.0-8.0), Tris-HCl buffer (50 mM, pH 7.0-9.0) and glycine-NaOH buffer (50 mM, pH 8.0-11.0). All the enzyme assays were performed in triplicates and spontaneous hydrolysis rate was subtracted. HPLC analysis conditions: aqueous C18 column; mobile phase: methanol: water = 90:10; flow rate: 0.6 mL min^{-1}; detection wavelength: 270 nm; injection volume: 10 μL.

Thermostability Analysis. The T_{50}[15] values were determined for an activity-based thermostability analysis. Purified *Po*OPH$_{WT}$ and variants (1 mg/mL) were incubated in Thermal Cycler T100 (BioRed) at temperatures ranging from 40 to 85ºC for 15 min and chilled on ice for 5 min. The residual activities of heat-treated enzymes against TPHP were determined by HPLC. As prescribed above, the reaction system contained 425 μL Tris-HCl (50 mM, pH 9.0), 25 μL of TPHP (100 mM, methanol dissolved), and 50 μL purified enzyme (diluted to 1 mg/mL), the relative activity data of a variant at different temperatures was obtained and fitting data to the Boltzmann equation.

Molecular Docking. The structures of *Po*OPH mutants were modeled based on the crystal structure of its parent enzyme *Po*OPH$_{M2}$ (PDB: 4O98, 2.25Å) using SWISS-MODEL web server (http://www.swissmodel.expasy.org/). The substrate molecule TPHP was minimized in energy with Chem3D, and the output was in PDB format. The center coordinates were calculated using VMD, and the *Po*OPH catalytic triads His144, His294, Asp143, His139, His141, and Asp247, His226, were centered, and the box size was 15 Å. The substrate molecules are docked into the protein structure and a suitable

conformation with the lower binding energy binding mode was analyzed in Pymol.

Degradation of TPHP. The degradation of TPHP were catalyzed by different *Po*OPH mutants and the products were assayed using HPLC. Typically, the 200 μL reaction solution contained 50 mM Tris-HCl (pH 9.0), 10 mM TPHP and 2 mg/mL purified enzymes, which was shaken at 1000 rpm and 30ºC. After different time periods, the hydrolysis reactions were stopped by adding 50 μL H_2SO_4 (10%, v/v), and extracted twice with 1.5 eq volumes of ethyl acetate. After volatilization, the samples were re-dissolved in 200 μL methanol for HPLC analysis.

Figures and Tables

Table S1.

Primers used in this study

Cloning primers	
sfGFP- *Xba* Ⅰ-FP	5'-GCTCTAGAATGGTGAGCAAGGGCGAGGAGCT -3'
sfGFP-*Hind* ⅢI-RP	5'-CCCAAGCTTTTACTTGTACAGCTCGTCCAT -3'
J23114-B0034-FP	5'- CCAGGCATCAAATAAAACGAAAGGC -3'
J23114-B0034-RP	5'- GCTCTGGAAGTGGATCAGGTTGGT -3'
pUC19-De-open-FP	5'- ATGCCGATCAAGTACAAGCCTGAAA -3'
pUC19-De-open-RP	5' - CAGTGTGACTCTAGTAGAGAGCGTTC -3'
pSB1C3- *Xbal*- FP	5'- CTAGTCTAGAATAAGCATTTGCTCAAGCGGCCTT -3'
pSB1C3-*Eco*R I-RP	5'- CCGGAATTCCTAGCCTTCGATGCCGATTTTC -3'
DmpR-sfGFP-insert-FP	5'- TAAGCATTTGCTCAAGCGGCCT -3'
DmpR-sfGFP-insert-RP	5'- AAATAATAAAAAAGCCGGATTAATAATCTGG -3'
pET28a-open-FP	5'- ATCCGGCTTTTTTATTATTTCACCGGCGCCACAGGTGC-3'
pET28a-open-RP	5'- GCCGCTTGAGCAAATGCTTAAGATCTCGATCCCGCGAA-3'
Mutagenesis primers	
*Po*OPH-Y54X-FP	5'- GTATGACGGC**NNK**ATCGACGGGCCTGCCAGCCT -3'
*Po*OPH-Y54X-RP	5' - GCCCGTCGAT**MNN**GCCGTCATACAGAGCGGTGA -3'
*Po*OPH-V55X-FP	5'- TGACGGCTAC**NNK**GACCTGCCTGCCAGCCTGCT -3'
*Po*OPH- V55X -RP	5' - CAGGCAGGTC**MNN**GTAGCCGTCATACAGAGCGG -3'
*Po*OPH- V55I -FP	5'- TGACGGCTAC**ATC**GACCTGCCTGCCAGCCTGCT -3'
*Po*OPH- V55I -RP	5'- CAGGCAGGTC**GAT**GTAGCCGTCATACAGAGCGG -3'
*Po*OPH- D56X -FP	5'- CGGCTACATC**NNK**GGGCCTGCCAGCCTGCTCAA -3'
*Po*OPH- D56X -RP	5'- TGGCAGGCCC**MNN**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56G -FP	5'- CGGCTACATC**GGC**GGGCCTGCCAGCCTGCTCAA -3'
*Po*OPH- D56G -RP	5' - TGGCAGGCCC**GCC**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56A -FP	5'- CGGCTACATC**GCA**GGGCCTGCCAGCCTGCTCAA -3'
*Po*OPH- D56A -RP	5'- TGGCAGGCCC**TGC**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56V -FP	5'- CGGCTACATC**GTG**GGGCCTGCCAGCCTGCTCAA -3'
*Po*OPH- D56V -RP	5'- TGGCAGGCCC**CAC**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56L -FP	5'- CGGCTACATC**CTC**GGGCCTGCCAGCCTGCTCAA -3'
*Po*OPH- D56L -RP	5'- TGGCAGGCCC**GAG**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56I -FP	5'- CGGCTACATC**ATC**GGGCCTGCCAGCCTGCTCAA -3'
*Po*OPH- D56I -RP	5'- TGGCAGGCCC**GAT**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56F -FP	5'- CGGCTACATC**TTC**GGGCCTGCCAGCCTGCTCAA -3'
*Po*OPH- D56F -RP	5'- TGGCAGGCCC**GAA**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56P -FP	5'- CGGCTACATC**CCT**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56P -RP	5'- TGGCAGGCCC**AGG**GATGTAGCCGTCATACAGAG -3'
*Po*OPH- D56S -FP	5'- CGGCTACATC**TCG**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56S -RP	5'- TGGCAGGCCC**CGA**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56T -FP	5'- CGGCTACATC**ACG**GGGCCTGCCAGCCTGCTCAA-3'

*Po*OPH- D56T -RP	5'- TGGCAGGCCC**CGT**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56H -FP	5'- CGGCTACATC**CAC**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56H -RP	5'- TGGCAGGCCC**GTG**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56W -FP	5'- CGGCTACATC**TGG**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56W -RP	5'- TGGCAGGCCC**CCA**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56C -FP	5'- CGGCTACATC**TGC**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56C -RP	5'- TGGCAGGCCC**GCA**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56E -FP	5'- CGGCTACATC**GAA**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56E -RP	5'- TGGCAGGCCC**TTC**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56Y -FP	5'- CGGCTACATC**TAC**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56Y -RP	5'- TGGCAGGCCC**GTA**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56M -FP	5'- CGGCTACATC**ATG**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56M -RP	5'- TGGCAGGCCC**CAT**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56N -FP	5'- CGGCTACATC**AAC**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56N -RP	5'- TGGCAGGCCC**GTT**GATGTAGCCGTCATACAGAG-3'
*Po*OPH- D56Q -FP	5'- CGGCTACATC**CAA**GGGCCTGCCAGCCTGCTCAA-3'
*Po*OPH- D56Q -RP	5'- TGGCAGGCCC**TTG**GATGTAGCCGTCATACAGAG-3'
*Po*OPH-L57X-FP	5'- CTACGTCGAC**NNK**CCTGCCAGCCTGCTCAAGGG -3'
*Po*OPH-L57X-RP	5'- GGCTGGCAGG**MNN**GTCGACGTAGCCGTCATACA -3'
*Po*OPH-L57G-FP	5'- CTACGTCGAC**GGC**CCTGCCAGCCTGCTCAAGGG -3'
*Po*OPH- L57G -RP	5'- GGCTGGCAGG**GCC**GTCGACGTAGCCGTCATACA -3'
*Po*OPH-A88X-FP	5'- CGTGCAGACT**NNK**GTCAACGCCTACCTGATCAA -3'
*Po*OPH-A88X-RP	5'- AGGCGTTGAC**MNN**AGTCTGCACGCCTTTCTCCG -3'
*Po*OPH-V89X-FP	5'- GCAGACTGCG**NNK**AACGCCTACCTGATCAACAC -3'
*Po*OPH-V89X-RP	5'- GGTAGGCGTT**MNN**CGCAGTCTGCACGCCTTTCT -3'
*Po*OPH-N90X-FP	5'- GACTGCGGTC**NNK**GCCTACCTGATCAACACTG -3'
*Po*OPH-N90X-RP	5'- CAGGTAGGC**MNN**GACCGCAGTCTGCACGCCTT -3'
*Po*OPH-A107X-FP	5'- CGATACCGGC**NNK**GCCCAGTGCTTCGGCCCGAC -3'
*Po*OPH- A107X -RP	5'- AGCACTGGGC**MNN**GCCGGTATCGATCAGCACCA -3'
*Po*OPH-F111X-FP	5'- CGCCCAGTGC**NNK**GGCCCGACTCTCGGCGTGGT -3'
*Po*OPH-F111X-RP	5'- GAGTCGGGCC**MNN**GCACTGGGCGGCGCCGGTAT -3'
*Po*OPH-T114X-FP	5'- CTTCGGCCCG**NNK**CTCGGCGTGGTGCAGACCAA -3'
*Po*OPH-T114X-RP	5'- CCACGCCGAG**MNN**CGGGCCGAAGCACTGGGCGG -3'
*Po*OPH-L115X-FP	5'- CGGCCCGACT**NNK**GGCGTGGTGCAGACCAACCT -3'
*Po*OPH-L115X-RP	5'- GCACCACGCC**MNN**AGTCGGGCCGAAGCACTGGG -3'
*Po*OPH-L140X-FP	5'- GCTCACCCAC**NNK**CACCCAGACCATGCCTGCGG -3'
*Po*OPH-L140X-RP	5'- GGTCTGGGTG**MNN**GTGGGTGAGCAGCACGGTAT -3'
*Po*OPH-P142X-FP	5'- CCACCTGCAC**NNK**GACCATGCCTGCGGCCTGGT -3'
*Po*OPH-P142X-RP	5'- AGGCATGGTC**MNN**GTGCAGGTGGGTGAGCAGCA -3'
*Po*OPH- A145X -FP	5'- CCCAGACCAT**NNK**TGCGGCCTGGTCAACGCCGA -3'
*Po*OPH-A145X-RP	5'- CCAGGCCGCA**MNN**ATGGTCTGGGTGCAGGTGGG -3'
*Po*OPH-A145S-FP	5'- CCCAGACCAT**TCG**TGCGGCCTGGTCAACGCCGA -3'
*Po*OPH-A145S-RP	5'- CCAGGCCGCA**CGA**ATGGTCTGGGTGCAGGTGGG -3'
*Po*OPH-M188X-FP	5'- CATGCAGGGC**NNK**TTCAAGATGGCGCGACAGGC -3'

*Po*OPH-M188X-RP	5'- CCATCTTGAA**MNN**GCCCTGCATGCCTTCGGGGG -3'
*Po*OPH- M188F -FP	5'- CATGCAGGGC**TTC**TTCAAGATGGCGCGACAGGC -3'
*Po*OPH-M188F-RP	5'- CCATCTTGAA**GAA**GCCCTGCATGCCTTCGGGGG -3'
*Po*OPH-M188W-FP	5'- CATGCAGGGC**TGG**TTCAAGATGGCGCGACAGGC -3'
*Po*OPH-M188W-RP	5'- CCATCTTGAA**CCA**GCCCTGCATGCCTTCGGGGG -3'
*Po*OPH-M188A-FP	5'- CATGCAGGGC**GCA**TTCAAGATGGCGCGACAGGC -3'
*Po*OPH-M188A-RP	5'- CCATCTTGAA**TGC**GCCCTGCATGCCTTCGGGGG -3'
*Po*OPH-M191X-FP	5'- CTTTTTCAAG**NNK**GCGCGACAGGCAGTCGCACC -3'
*Po*OPH-M191X-RP	5'- CCTGTCGCGC**MNN**CTTGAAAAAGCCCTGCATGC -3'
*Po*OPH-M191T-FP	5'- CTTTTTCAAG**ACC**GCGCGACAGGCAGTCGCACC -3'
*Po*OPH-M191T-RP	5'- CCTGTCGCGC**GGT**CTTGAAAAAGCCCTGCATGC -3'
*Po*OPH-I248X-FP	5'- ATGGGGCGAC**NNK**CTGATTAACCACGCCGTGCA -3'
*Po*OPH-I248X-RP	5'- GGTTAATCAG**MNN**GTCGCCCCATACCAGCAGGC -3'
*Po*OPH-I250X-FP	5'- CGACATTCTG**NNK**AACCACGCCGTGCAGTTCG -3'
*Po*OPH-I250X-RP	5'- GGCGTGGTT**MNN**CAGAATGTCGCCCCATACCA -3'
*Po*OPH-I250H-FP	5'- CGACATTCTG**CAC**AACCACGCCGTGCAGTTCG -3'
*Po*OPH-I250H-RP	5'- GGCGTGGTT**GTG**CAGAATGTCGCCCCATACCA -3'
*Po*OPH- I250A -FP	5'- CGACATTCTG**GCA**AACCACGCCGTGCAGTTCG -3'
*Po*OPH-I250A-RP	5'- GGCGTGGTT**TGC**CAGAATGTCGCCCCATACCA -3'
*Po*OPH-I250G-FP	5'- CGACATTCTG**GGC**AACCACGCCGTGCAGTTCG -3'
*Po*OPH-I250G-RP	5'- GGCGTGGTT**GCC**CAGAATGTCGCCCCATACCA -3'
*Po*OPH-V262X-FP	5'- GCCTGAAGTG**NNK**TGGGAGTTCGATGTCGACAG -3'
*Po*OPH-V262X-RP	5'- CGAACTCCCA**MNN**CACTTCAGGCTTGGCGAACT -3'
*Po*OPH-W263X-FP	5'- TGAAGTGGTC**NNK**GAGTTCGATGTCGACAGCGA -3'
*Po*OPH-W263X-RP	5'- CATCGAACTC**MNN**GACCACTTCAGGCTTGGCGA -3'
*Po*OPH-W263I-FP	5'- TGAAGTGGTC**ATT**GAGTTCGATGTCGACAGCGA -3'
*Po*OPH-W263I-RP	5'- CATCGAACTC**AAT**GACCACTTCAGGCTTGGCGA -3'
*Po*OPH- W263S -FP	5'- TGAAGTGGTC**TCG**GAGTTCGATGTCGACAGCGA -3'
*Po*OPH-W263S-RP	5'- CATCGAACTC**CGA**GACCACTTCAGGCTTGGCGA -3'
*Po*OPH-W263G-FP	5'- TGAAGTGGTC**GGC**GAGTTCGATGTCGACAGCGA -3'
*Po*OPH-W263G-RP	5'- CATCGAACTC**GCC**GACCACTTCAGGCTTGGCGA -3'
*Po*OPH-F265X-FP	5'- GGTCTGGGAG**NNK**GATGTCGACAGCGACCAGGC -3'
*Po*OPH-F265X-RP	5'- TGTCGACATC**MNN**CTCCCAGACCACTTCAGGCT -3'
*Po*OPH-A293X-FP	5'- GGTCGCTGGT**NNK**CACCTGCCCTTCCCCGGCCT -3'
*Po*OPH-A293X-RP	5'- AGGGCAGGTG**MNN**ACCAGCGACCCACAGCTTGT -3'

Figure S1

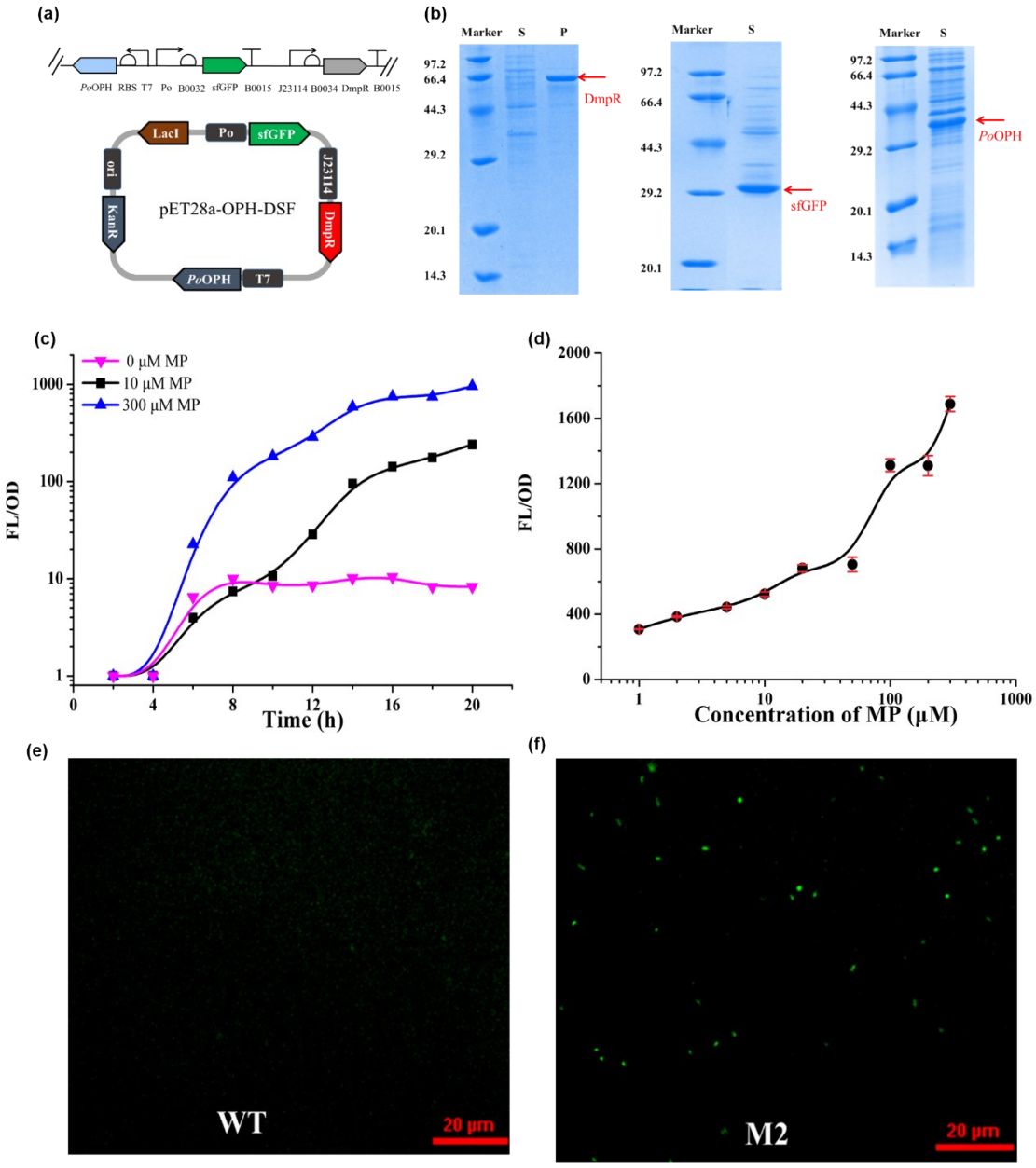

Figure S1 Construction of the whole-cell biosensor *E. coli* BL21/pET28a-OPH-DSF. (**a**) The building blocks of the genetic circuit and the plasmid used for protein expression. (**b**) SDS-PAGE analysis of DmpR, sfGFP and *Po*OPH. S: the supernatant of the cell lysate. P: the precipitation of the cell lysate. (**c**) Time-course detection of MP by biosensor; (**d**) The dose–response curve for detecting MP from 0 to 300 μM for 20 h at 30 °C, 180 rpm; (**e**) Confocal fluorescence images of the wild-type *Po*OPH$_{WT}$ and (**f**) *Po*OPH$_{M2}$ in presence of MP.

Figure S2

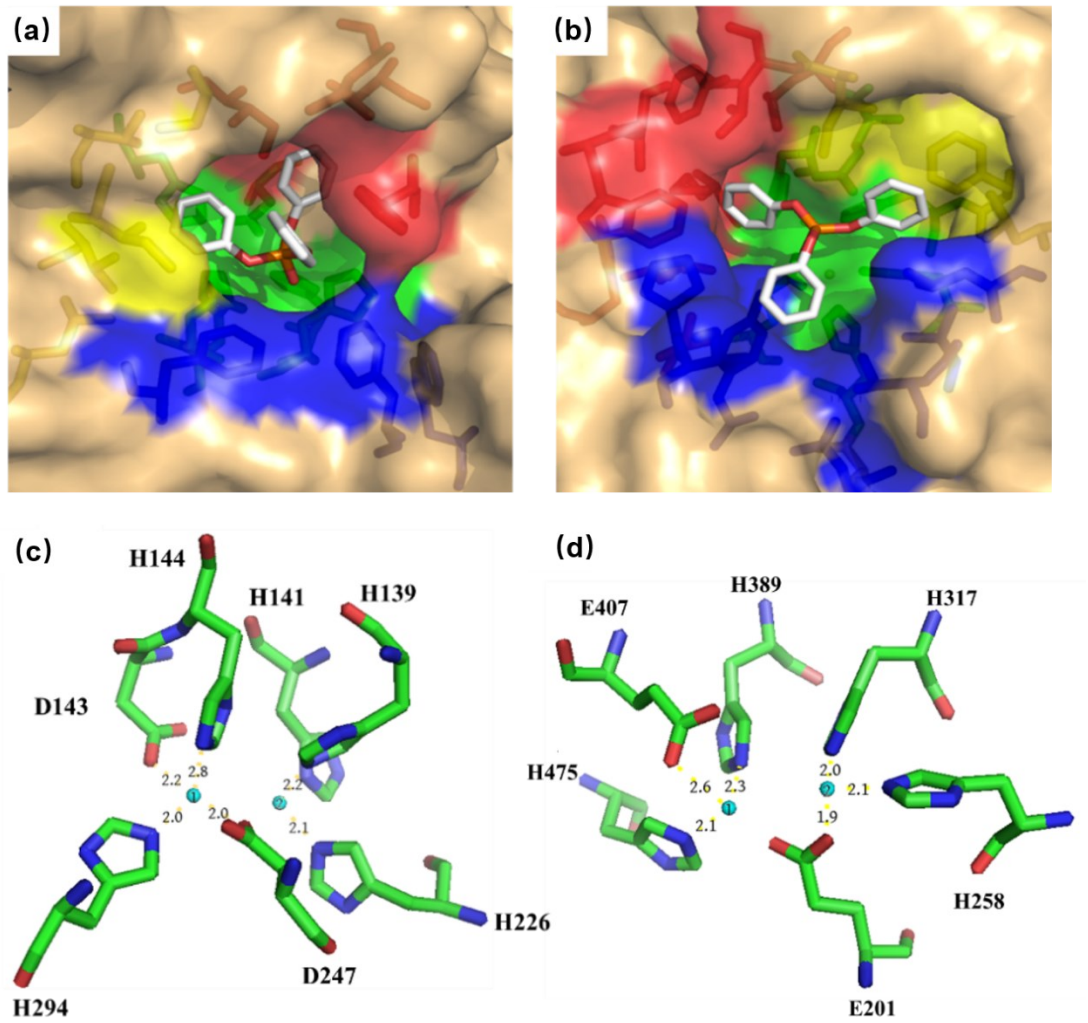

Figure S2 Model of TPHP docked in the active site of (**a**) *Po*OPH$_{M2}$ and (**b**) *Sb*-PTE. In the figure, the green part indicates the catalytic center, the blue part indicates the leaving group binding site, and the red and yellow indicate two side chain group binding sites, respectively. Superposition of metal coordinating residues in (**c**) *Po*OPH$_{M2}$ and (**d**) *Sb*-PTE, two mental ions are shown in cyans spheres, zinc ions for *Po*OPH$_{M2}$ and manganese ions for *Sb*-PTE.

Figure S3

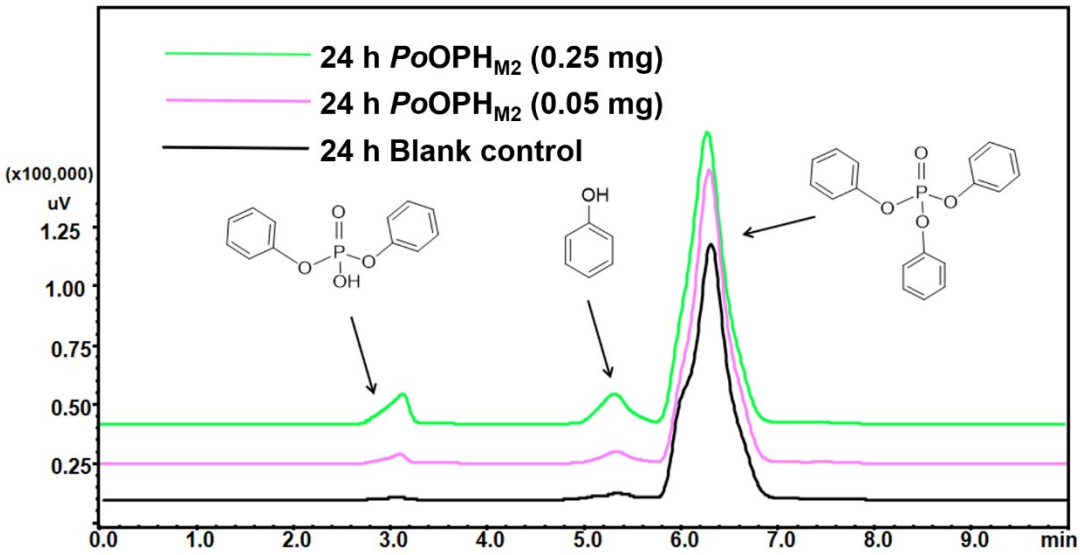

Figure S3 The HPLC analysis for the reaction mixture with purified *Po*OPH$_{M2}$. The reaction system was 500 μL, and the concentration of TPHP was 5 mM. The amount of pure enzyme added was 0.05 and 0.25 mg, respectively, and the blank control was a reaction solution without enzyme. The reaction was terminated after 24 h of reaction at 30°C, and the liquid phase was performed after extraction.

Figure S4

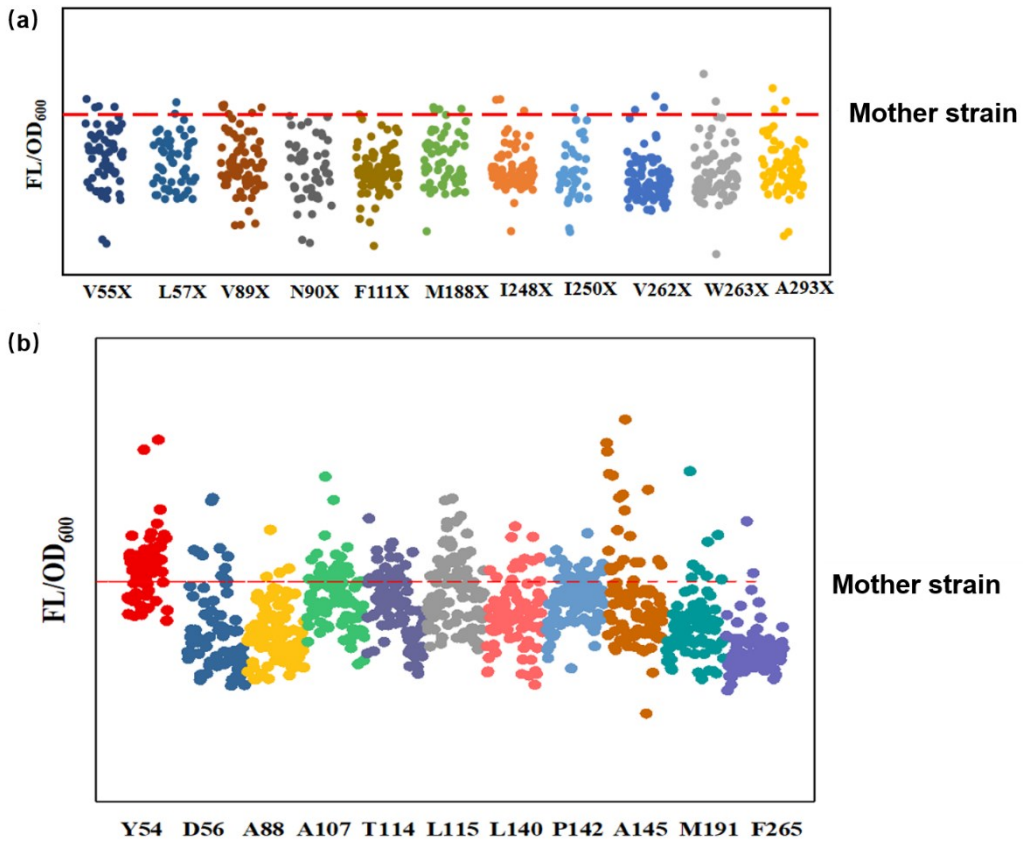

Figure S4 (**a**) Fluorescence screening of 11 saturated mutagenesis libraries within 4Å of TPHP binding pocket; (**b**) (A) Fluorescence screening of 11 saturated mutagenesis libraries within 6Å of TPHP binding pocket.

Figure S5

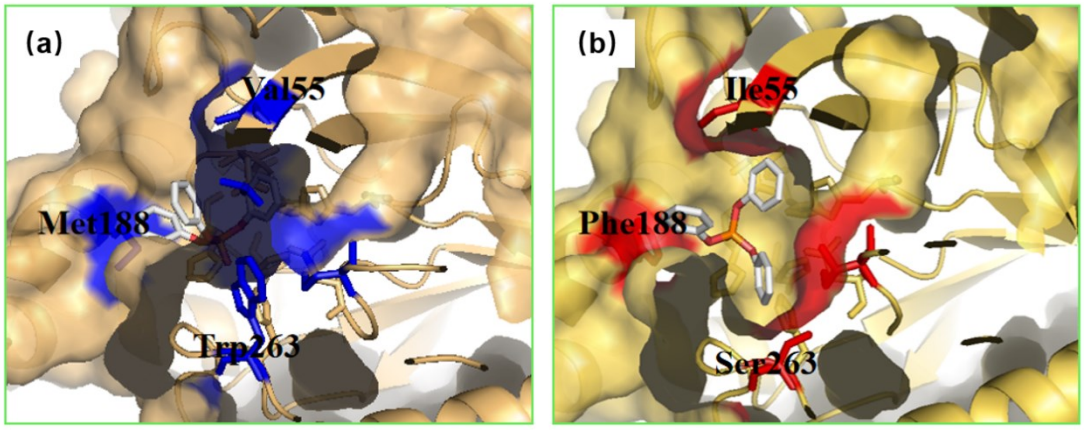

Figure S5 Illustration of the product leaving channel of *Po*OPH$_{M2}$ (**a**) and *Po*OPH$_{V4}$ (**b**), where Trp263 was mutated to Ser263.

Figure S6

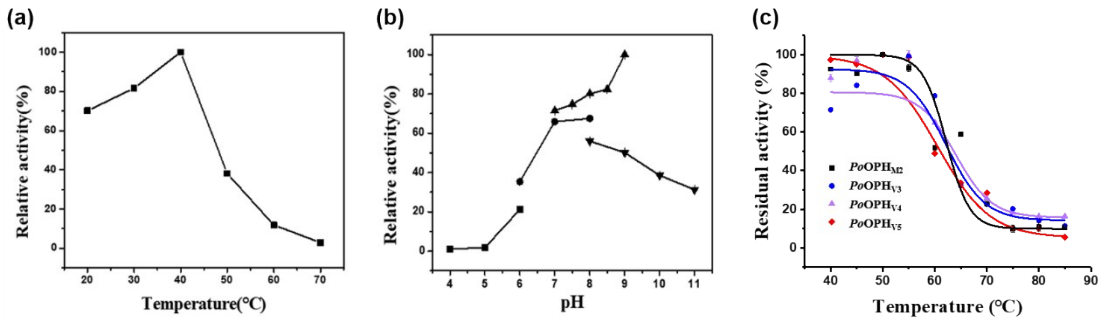

Figure S6 Effects of temperature (**a**) and pH (**b**) on the activity of *Po*OPH$_{V5}$. (■) Sodium citrate buffer (50 mM, pH 4.0-6.0), (●) Phosphate buffer (50 mM, pH 6.0-8.0), (▲) Tris-HCl buffer (50 mM, pH 7.0-9.0) and (▼) Glycine-NaOH buffer (50 mM, pH 8.0-11.0). (**c**) Thermostability characterization of *Po*OPH$_{M2}$ and its mutants. *Po*OPH$_{M2}$ (blank squares), *Po*OPH$_{V3}$(blue circles), *Po*OPH$_{V4}$ (purple triangles) and *Po*OPH$_{V5}$ (red diamonds). All enzymes were diluted to the concentration of 1 mg/mL, incubated at different temperatures (40-85ºC) for 15 min and cooled for 5 min. The residual activity towards TPHP was determined by HPLC.