Substantially improving the enantioconvergence of PvEH1, a \textit{Phaseolus vulgaris} epoxide hydrolase, towards \textit{m}-chlorostyrene oxide by laboratory evolution

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\textbf{Abstract}

\textbf{Background:} Epoxide hydrolase can regioselectively catalyze the oxirane ring-opening hydrolysis of rac-epoxides producing the corresponding chiral diols. In our laboratory, a gene named \textit{pveh1} encoding an EH from \textit{Phaseolus vulgaris} was cloned. Although the directed modification of \textit{PvEH1} was carried out, the mutant \textit{PvEH1Y3} showed a limited degree of enantioconvergence towards racemic (\textit{rac}-) \textit{m}-chlorostyrene oxide (\textit{mCSO}).

\textbf{Results:} \textit{PvEH1} and \textit{PvEH1Y3} were combinatorially subjected to laboratory evolution to further enhance the enantioconvergence of \textit{PvEH1Y3} towards \textit{rac}-\textit{mCSO}. Firstly, the substrate-binding pocket of \textit{PvEH1} was identified using a CAVER 3.0 software, and divided into three zones. After all residues in zones 1 and 3 were subjected to leucine scanning, two \textit{E. coli} transformants, \textit{E. coli/pveh1Y149L} and \textit{E. coli/pveh1P184L}, were selected, by which \textit{rac-mCSO} was transformed into (\textit{R})-\textit{m}-chlorophenyl-1,2-ethanediol (\textit{mCPED}) having 55.1\% and 27.2\% ee\textsubscript{p}. Secondly, two saturation mutagenesis libraries, \textit{E. coli/pveh1Y149X} and \textit{E. coli/pveh1P184X} (X: any one of 20 residues) were created at sites Y149 and P184 of \textit{PvEH1}. Among all transformants, both \textit{E. coli/pveh1Y149L} (65.8\% \alpha\textsubscript{S} and 55.1\% ee\textsubscript{p}) and \textit{E. coli/pveh1P184W} (66.6\% \alpha\textsubscript{S} and 59.8\% ee\textsubscript{p}) possessed the highest enantioconvergences. Finally, the combinatorial mutagenesis was conducted by replacements of both Y149L and P184W in \textit{PvEH1Y3}, constructing \textit{E. coli/pveh1Y3Z2}, whose \alpha\textsubscript{S} reached 97.5\%, higher than that (75.3\%) of \textit{E. coli/pveh1Y3}. In addition, the enantioconvergent hydrolysis of 20 mM \textit{rac-mCSO} was performed by \textit{E. coli/pveh1Y3Z2}, giving (\textit{R})-\textit{mCPED} with 95.2\% ee\textsubscript{p} and 97.2\% yield.

\textbf{Conclusions:} In summary, the enantioconvergence of \textit{PvEH1Y3Z2} was successfully improved by laboratory evolution, which was based on the study of substrate-binding pocket by leucine scanning. Our present work introduced an effective strategy for the directed modification of enantioconvergence of \textit{PvEH1}.

\textbf{Keywords:} Epoxide hydrolase, Enantioconvergence, \textit{m}-Chlorostyrene oxide, \textit{m}-Chlorophenyl-1,2-ethanediol, Laboratory evolution, Substrate-binding pocket

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Background
Optically pure epoxides and vicinal diols, the highly value-added and versatile building blocks, were widely applied in pharmaceutical, fine chemical and agrochemical industries [1–3]. For example, (R)-pCPED was used for the synthesis of (R)-Eliprodil, a neuroprotective agent for the treatment of ischemic stroke, while (R)-mCPED for β3-adrenergic receptor agonists, such as SR 58611 and AI-9677 [4]. With the ever-increasing environmental consciousness, the biocatalysis mediated by whole resting cells or enzymes was considered to be an alternative to chemocatalysis that required expensive chiral ligands and hazardous metals, such as Jacobsen's asymmetric ring-opening hydrolysis and epoxidation [5, 6].

Epoxide hydrolases (EHs; 3.3.2.-), existing widely in nature, can enantioselectively and/or regioselectively catalyze the oxirane ring-opening hydrolysis of rac-epoxides, retaining epoxide enantiomers and/or producing the corresponding chiral diols. Based on the catalytic mechanisms of given EH-substrate pairs, the asymmetric hydrolysis of rac-epoxides was divided into two pathways: kinetic resolution and enantioconvergent hydrolysis [7]. The former can retain single epoxide enantiomers with an intrinsic limitation of 50% maximum yield, while the latter can produce optically pure vicinal diols with 100% theoretically yield [8].

The mono-enzymatic catalysis was an ideal bioprocess for preparing chiral diols via convergent hydrolysis of epoxides, but few naturally existing EHs had high and opposite regioselectivities towards (R)- and (S)-epoxides [9]. To completely and quickly hydrolyze rac-epoxide, EH applied in enantioconvergent hydrolysis also have to possess a low enantioselectivity (i.e., enantiomeric ratio, E). In view of the merits of mono-enzymatic catalysis and the shortage of highly enantioconvergent EHs, it is necessary to excavate novel EHs or to modify specific local configurations of the existing EHs by protein engineering [10]. Among the different gene mutagenesis techniques, saturation mutagenesis (SM) at sites lining the enzyme’s binding pocket has emerged as a particularly viable approach to improve selectivity [11, 12]. For example, through five rounds of iterative saturation mutagenesis of nine residues at sites lining in the substrate-binding pocket (SBP) of Aspergillus niger M200 EH (AnEH), its best mutant, named H:12-A1, was selected, whose regioselectivity coefficients (αS values) towards (S)-SO and -pCSO were higher than those of AnEH, and by which rac-SO and -pCSO were transformed into (R)-PED and -pCPED, respectively, with over 70% enantiomeric excess (eeP) [13]. Several other research groups also reported the laboratory evolution of residues located in the SBP of EHs [14, 15].

Previously, to improve the enantioconvergence of PvEH1 towards styrene epoxides, its laboratory evolution was carried out based on the computer-aided design. Among all tested mutants of PvEH1, PvEH1L105I/M160A/M175I (renamed PvEH1Y32), was selected (Additional file 1: Table S1). Its enantioconvergence towards rac-mCSO increased to 69.7% from 1.0% eeP of PvEH1 [10]. In our present work, the SBP of PvEH1, identified and analyzed using a CAVER 3.0 software (http://www.caver.cz/), was divided into three zones. To substantially improve the enantioconvergence of PvEH1, the zones 1 and 3 were subjected, in which all residues were subjected to leucine scanning [16], namely, substituting target residues with Leu, to identify the sites where Leu mutants had best EH enantioconvergence. Two saturation mutagenesis libraries, E. coli/pveh1Y149X and /pveh1P184X, were constructed and screened to select the best substituted residues at their respective sites. Then, the combinatorial mutagenesis of PvEH1Y3 was conducted by replacing its Y149 and P184 with the selected best substituted residues, thereby creating one five-site mutant of PvEH1, named PvEH1Y32/Y149L/P184W or PvEH1Y32. Lastly, the enantioconvergent hydrolysis of rac-mCSO was carried out using resting cells of E. coli/pveh1Y32.

Materials and methods
Strains, plasmids and chemicals
E. coli BL21(DE3) and pET-28a (+) (Novagen, Madison, WI) were used for the construction of recombinant plasmid and expression of pveh1 or its variant, while PrimeSTAR HS DNA polymerase and DpnI endonuclease (TaKaRa, Dalian, China) were used for the leucine scanning and site-saturation mutagenesis of pveh1 as well as the combinatorial mutagenesis of pveh1Y32, pET-28a-pveh1 and /pveh1Y3 and their corresponding E. coli/ pveh1 and /pveh1Y3 were constructed and stored in our lab. Rac-mCSO, (R)- and (S)-mCPED were purchased from Energy (Shanghai, China). All other chemicals were of analytical purity.

Homology modeling of PvEH1 and identification of its SBP
Using the known crystal structure of a Vigna radiata EH at 2.0 Å resolution (VrEH1, PDB: 5XMD), sharing 87.4% identity with PvEH1, as the template, the three-dimensional (3-D) structure of PvEH1 was modeled using the MODELLER 9.11 program (http://salilab.org/modeller/ (Additional file 1: Figure S2), and subjected to molecular mechanics optimization by CHARMM27 force field in the GROMACS 4.5 package (http://www.gromacs.org/) (Additional file 1: Figure S3). The 3-D structure with the best geometry quality, which was validated by Structure Assessment in SWISS-MODEL (https://swissmodel .expasy.org/assess), was obtained, and visualized by a
PyMOL software (http://pymol.org/) (Additional file 1: Figures S4 and S5). The SBP of a modeled PvEH1’s 3-D structure was identified and analyzed using a CAVER 3.0 software, and artificially divided into three zones 1–3 (Fig. 1).

**Leucine scanning and site-saturation mutagenesis of PvEH1**

The zones 1 and 3 of PvEH1’s SBP were subjected, in which all residues were subjected to leucine scanning. The variants of pveh1 were designed by substituting the target residues-encoding codons with Leu-encoding codon, synthesized by Genewiz (Suzhou, China), and transformed into E. coli BL21, respectively, thereby constructing the corresponding E. coli transformants, such as E. coli/pveh1 Y149L and /pveh1 P184L. Through screening, the specific residue sites where Leu mutants catalyzed the enantioconvergent hydrolysis of rac-mCSO with the highest ee:p values of (R)-mCPED were identified for the further studies.

Based on the results of leucine scanning, the saturation mutagenesis of a Y149- or P184-encoding codon in pveh1 was carried out using a one-step whole-plasmid PCR method [17]. The primers of saturation mutagenesis were designed as reported previously [18], and synthesized by Sangon (Shanghai, China) as listed in Additional file 1: Table S2. Using pET-28a-pveh1 as a template, the mutagenesis PCR was performed by PrimeSTAR HS DNA polymerase using a pair of primers, Y149X-F/Y149X-R or P184X-F/P184X-R, as following conditions: a denaturation at 95 °C for 4 min, 30 cycles of at 98 °C for 10 s, 55 °C for 15 s and 72 °C for 6 min, and an extra elongation at 72 °C for 10 min. The target PCR products, pET-28a-pveh1 Y149X or -pveh1 P184X (X: any one of 20 residues), were digested by DpnI at 37 °C for 6 h to decompose the methylated template DNA, and transformed into E. coli BL21(DE3), respectively, thereby constructing the mutagenesis library, E. coli/pveh1 Y149X or /pveh1 P184X. Using both the ee:p of (R)-mCPED and c of rac-mCSO as indexes, E. coli/pveh1 Y149X and /pveh1 P184X were screened, respectively. The best E. coli transformant of each library at residue site Y149 or P184 of PvEH1 was selected, expressing the EH mutant with the highest enantioconvergence towards rac-mCSO.

**Combinatorial site-directed mutagenesis of PvEH1Y3**

The combinatorial site-directed mutagenesis was designed by residue replacements of Y149L and P184W in PvEH1 Y3, and also carried out by one-step whole-plasmid PCR. The PCR primers were designed according to the nucleotide sequence of pveh1 Y3 and codons encoding the selected mutation residues, and synthesized by Sangon (Additional file 1: Table S3). Using pET-28a-pveh1 Y3 as the template, the first round of PCR was conducted using a pair of primers, Y149L-F/Y149L-R, under the same PCR conditions as described above. Thereafter, using the first-round PCR product as the template, the second round of PCR was carried out using another pair of primers, P184W-F/P184W-R. The target PCR product, pET-28a-pveh1 Y3/Y149L/P184W or -pveh1 Y3Z2, was digested by DpnI, and transformed into E. coli BL21(DE3), thereby constructing one E. coli transformant, named E. coli/pveh1 Y3Z2, harboring a five-site variant of pveh1 or a two-site one of pveh1 Y3.

**Analytic methods of HPLC and GC chromatographies**

The activity of PvEH1 or its mutant as well as the conversion ratio (c) of rac-mCSO defined as the ratio of its depleted concentration to initial one was assayed by high-performance liquid chromatography (HPLC), using an e2695 apparatus with an XBridge BEH C18 column (Waters, Milford, MA). The mobile phase of methanol/H$_2$O (7:3, v/v) was used at 0.8 mL/min, and continuously...
monitored using a Waters 2489 UV–Vis detector at 220 nm. The generated diols (R)- and (S)-mCPED were analyzed by HPLC with a Chiralcel OD-H column (Daicel, Osaka, Japan). The n-hexane/isopropanol (9:1, v/v) was used as a mobile phase. Because (R)- and (S)-mCSO can not be separated by OD-H, they were assayed by chiral gas chromatography (GC), using a GC-2010 system (Shimadzu, Tokyo, Japan) with a CP-Chirasil-DEX CB column (Agilent, Santa Clara, CA) and a flame ionization detector. The injector and detector temperatures were 220 °C, while the column temperature was programmed from 110 to 190 °C at 10 °C/min. The nitrogen gas carrier was used at 3.0 mL/min.

EH expression of E. coli transformant and EH activity assay
E. coli transformant harboring pveh1 or its variant, such as E. coli/pveh1 or /pveh1V322, was inoculated into LB medium supplemented with 100 μg/mL kanamycin, and cultured at 37 °C overnight as the seed culture. Then, the same fresh medium was inoculated with 2% (v/v) seed culture, and grown until OD_{600} reached 0.6–0.8. The expression of PvEH1 or its mutant was induced by 0.5 mM IPTG at 20 °C for 10 h. The induced transformant cells were collected, and resuspended in 100 mM Na_2HPO_4–NaH_2PO_4 buffer (pH 7.0) to 100 mg wet cells/mL. E. coli BL21(DE3) transformed with pET-28a(+), named E. coli/pET-28a, was used as the negative control.

The hydrolytic conditions for the PvEH1 or its mutant activity assay were as follows: 475 μL cell suspension of E. coli transformant, suitably diluted with 100 mM phosphate buffer (pH 7.0), was mixed with 25 μL 200 mM rac-mCSO, incubated at 25 °C for 10 min, and terminated by adding 2 mL methanol. The sample was assayed by HPLC with a C18 column. One EH activity unit (U) was defined as the amount of whole wet cells catalyzing the hydrolysis of 1 μmol rac-mCSO per minute under the given assay conditions.

EH enantioconvergence assay
EH enantioconvergence assay was carried out as follows: 1.8 mL suitably diluted cell suspension was mixed with 200 μL 200 mM rac-mCSO and incubated at 25 °C. Aliquots of 100 μL sample were periodically drawn out, and extracted with 900 μL ethyl acetate, and then assayed by HPLC to calculate the c of rac-mCSO and ee_{p} of (R)-mCPED.

Results and discussion
3-D structural analysis of PvEH1 and its SBP identification
Most EHs belonged to α/β hydrolase superfamily, which consisted of a core domain, an α/β domain and a lid domain (Fig. 2a) [20]. Like 3-D structures of other EHs, that of PvEH1 also contained a core domain, a β-sheet packed between two layers of 9 α-helices and a lid domain. Its active center was made up of two polarizing tyrosine residues: Y150 and Y234 and catalytic triad D101–H299–D264 (Fig. 2b). To avoid mutants losing catalytic activities, these five sites cannot be replaced by other residues. After being homologically modeled and optimized, 3-D structure of PvEH1 was analyzed to identify the SBP of PvEH1. The SBP was located in the core domain and the lid domain mainly (Fig. 1a). It was reported that SBP can be divided according to the zone where the substrate binding with the enzyme [16]. Herein, as shown in Fig. 1b, the SBP of PvEH1 was divided into three zones subjectively: zone 1 (residues A120-V126, M129, V135-G142 and Y149), zone 2 (residues D101, Y150, Y234, D264 and H299) and zone 3 (residues M160-T162, A171-M175 and R180-L186). Five residue sites in active center which displayed important roles in the catalytic reaction were in zone 2. Compared with zone 3, zone 1 involved in more residues (17 vs. 15). Similar with the SBP of LEH [15], that of PvEH1 just looked like a dumbbell, which contained two big cavities and a tunnel connecting them.

Leucine scanning of the SBP
Amino acid substitutions at sites lining the SBP of EH may evolve its stereoselectivity [20]. As lacking the basic understanding of the prerequisites for regioselectivity in nucleophilic attack, each residue in zone 1 and zone 3
was replaced by leucine respectively to construct *E. coli* transformant expressing single-site mutant, except that the residue was leucine originally, for finding out the key sites influencing the enantioconvergence. The $e_{en}$ values and activities of all the mutants towards rac-**m**CSO were measured respectively. The results were outlined in Additional file 1: Table S4. Among all the mutants, $Pv$EH1$^{Y149L}$ displayed a marked increase in enantioconvergence, and $e_{en}$ reached to 55.1%. On other side, residue replacement like M129L in zone 1 reversed the configuration of the main diol product partly (Fig. 3). The similar phenomenon was also observed in the reshaping of LEH SBP, in that case, a one-site mutant LEH$^{L114F}$ favored the formation of ($R,R$)-diol, while LEH the formation of ($S,S$)-diol [15]. Compared with residue replacements in zone 1, those in zone 3 had smaller effects on the catalytic activities (Fig. 3). For example, different from mutant $Pv$EH1$^{Y149L}$, $Pv$EH1$^{P184L}$ had an increased enantioconvergence and its activity was not decreased. By the way, mutant $Pv$EH1$^{M129L}$ displayed the highest catalytic activity, while mutant $Pv$EH1$^{Y149L}$, the lowest except $Pv$EH1$^{P137L}$ (no activity). There was an increase in the enantioconvergence of mutant $Pv$EH1$^{Y149L}$, but under the comparison with other studies which have been reported, it is still not ideal enough [21, 22].

Saturation mutagenesis of Y149 or P184 in *PvEH1*

To figure out which residues at sites 149 and 184 are most beneficial for improving the enantioconvergence of *PvEH1*, saturation mutagenesis libraries *E. coli/pveh1$^{Y149X}$* and *E. coli/pveh1$^{P184X}$* were constructed and screened. According to the “22c-trick” method reported by Kille et al. [18] a special mixture of three primers was employed to create a degeneracy of 22 unique codons coding for the 20 amino acids. To ensure a full coverage of potential mutants, well above a theoretical coverage of > 95%, all libraries were oversampled at least threefold. That is, 66 *E. coli* transformants from each

![3-D structure of *PvEH1* (a) and its active center (b). The active center of was *PvEH1* made up by two polarizing tyrosine residues: Y150 and Y234 and catalytic triad D101-H299-D264](image)

![Bar chart: The enantioconvergence and relative activity part mutants from leucine scanning of zone 1 (contain $Pv$EH1$^{Y149L}$, $Pv$EH1$^{M129L}$ and $Pv$EH1$^{V126L}$) and zone 3 (contain $Pv$EH1$^{P184L}$, $Pv$EH1$^{T162L}$ and $Pv$EH1$^{M160L}$), where catalytic activity of $Pv$EH1 was used as a control](image)
library were needed. All transformants were screened by HPLC to select the highest mutant in ee_p value, then confirmed by DNA sequencing. As a result, in saturation mutagenesis library E. coli/pveh1P184X, four E. coli transformants with an over 35% in ee_p value were obtained, that is, E. coli/pveh1P184E, E. coli/pveh1P184D, E. coli/pveh1P184M and E. coli/pveh1P184W, whose P184-encoding codon (CCT) was verified to be mutated to E-, D-, M- and W-encoding codons (GAG, GAT, ATG and TGG). As shown in Fig. 4, E. coli/pveh1P184E and E. coli/pveh1P184D could not transform 10 mM rac-mCSO completely, while E. coli/pveh1P184M and E. coli/pveh1P184W could. E. coli/pveh1P184W displayed the highest enantioconvergence in saturation mutagenesis library E. coli/pveh1Y149X. The ee_p of (R)-mCPED catalyzed by E. coli/pveh1P184W was 59.8%, which was nearly 58-fold higher than that by PVEH1. Unfortunately, most E. coli transformants in saturation mutagenesis library E. coli/pveh1Y149X displayed no catalytic activity towards mCSO except E. coli/pveh1Y149L. Taking into consideration of studies on active areas in VrEH2 and StEH1 [21, 23], it is likely that amino acid sites around site 149 (including 149) may display important roles in the catalytic activity. On the other hand, the exchanges (Y149L and P184W) were crucial for the regioselectivity regulation [13].

The regioselectivity coefficients (β_R and α_S) were applied to quantitatively evaluate the preference attacking on Cβ of (R)-epoxide which could afford the diol of unchanged (R)-configuration, and on Cα of (S)-epoxide which could lead to the (R)-diol by inversion of configuration. It was necessary to determine regioselectivity coefficients which were helpful to understand the mechanism of enantioconvergence [8]. The α_S values of mutants PVEH1_Y149L and PVEH1_P184W increased from 10.3% to 65.8% and 66.6%, which straight contributed to the high enantioconvergence. But they were still lower than that of PVEH1_Y3 (Table 1).

**Residue replacements of both Y149L and P184 W in PVEH1_Y3**

According to the research of Ye et al. [10] mutant PVEH1_Y3 displayed a limited enantioconvergence towards mCSO (ee_p = 69.7%). But it still did not meet the requirement of production of (R)-mCPED. For further enhancing the enantioconvergence, we took the advantage of a cooperative mutational effect from replacements Y149L and P184W [24]. Mutant PVEH1_Y3Z2 (containing residues replacements: L105I, M160A, M175I, Y149L and P184W) was engineered, using PVEH1_Y3 as the template. The enantioconvergence of mutant PVEH1_Y3Z2 was 94-fold higher than that of PVEH1 (ee_p 1.0%). Compared regioselectivity coefficients of PVEH1_Y3Z2 with those of PVEH1, it was observed that almost all the improvement in enantioconvergence was contributed by the increase of α_S and there was no obviously change in the β_R. The α_S of PVEH1_Y3Z2 (97.5%) was higher than that of StEH1 (97%) and mbEH A (79%) [4, 25]. Combining with the research by Kotik et al. [13] we surmised that with the beneficial amino acid exchanging, the position of nucleophilic attack from PVEH1_Y3Z2 is switched from Cβ to Cα of the oxirane ring of (S)-mCSO. On the other hand, nucleophilic attack of the oxirane ring of (R)-mCSO remained largely unaltered in the PVEH1_Y3Z2, which creates an enantioconvergence towards rac-mCSO.

**Enantioconvergent hydrolysis of rac-mCSO by E. coli/pveh1Y3Z2**

To confirm the details in the improvement in enantioconvergence of PVEH1_Y3Z2, the ee_p, c, (R)- and (S)-mCPED concentrations in the enantioconvergent hydrolysis of rac-mCSO catalyzed by E. coli/pveh1Y3Z2 were monitored by chiral HPLC. With 20 mM rac-mCSO and 100 mg/mL E. coli/pveh1Y3Z2 adding into

| EH          | β_R/% | α_S/% | ee_p/% |
|-------------|-------|-------|--------|
| PVEH1       | 94.1  | 10.3  | 1.0±0.1|
| PVEH1_Y149L | 89.2  | 65.8  | 55.1±1.7|
| PVEH1_P184W | 93.2  | 66.6  | 59.8±1.7|
| PVEH1_Y3    | 94.4  | 75.3  | 69.7±3.3|
| PVEH1_Y3Z2  | 97.9  | 97.5  | 96.1±2.9|

Table 1 Results of measurements of regioselectivity coefficients
and 97.2%, respectively, and the chiral HPLC spectra for enantioconvergence was achieved in this work (ee_p from 1.0 to 95.2% vs. from 3 to 70.5%).

Conclusions
In this work, the enantioconvergence towards mCSO was conferred on PvEH1 by a laboratory evolution. Firstly, after identified and analyzed using a CAVER 3.0 software, the SBP of PvEH1 was studied by leucine scanning. The result shows that PvEH1Y149L and PvEH1P184L have a marked increase in enantioconvergence, which means that sites 149 and 184 played important roles in the enantioconvergent hydrolysis of rac-mCSO. Secondly, to confirm the best residue at each site, saturation mutagenesis libraries E. coli/pveh1Y149X and E. coli/pveh1P184X were constructed and screened. Mutants PvEH1Y149L and PvEH1P184W have the highest enantioconvergence in each saturation mutagenesis library. Thirdly, mutant PvEH1Y3Z2 containing five residue replacements was constructed using PvEH1Y3Z2 as the template. The enantioconvergence of PvEH1Y3Z2 was 94-fold higher than that of PvEH1. The analysis of regioselectivity coefficients indicated that the position of nucleophilic attack from mutant PvEH1Y3Z2 switched from Cβ to Cα of the oxirane ring of (S)-mCSO, which led to the improvement in enantioconvergence. At last, enantioconvergent hydrolysis by E. coli/pveh1Y3Z2 was monitored. The result showed that the ee_p and yield of (R)-mCPED were 95.2% and 97.2%, when the rac-mCSO was transformed into mCPED completely (c = 99.5%).

Supplementary information

Table 2 Chart of different EHs with high enantioconvergence

| EH     | Substrate | ee_p/| Product | References |
|--------|-----------|------|---------|------------|
| pveh1Y3Z2 | mCSO      | 95.4 | (R)-mCPED | This work  |
| SteH1  | mCSO      | 91   | (R)-mCPED | [4]        |
| VEH2M028N | pNSO    | 98   | (R)-pNPED | [21]       |
| Kau2   | mCSO      | 92   | (R)-mCPED | [22]       |
| Kau2 F:13-B11 | pCSO   | 97   | (R)-pCPED | [26]       |
| pveh3G170E/F187I | pCSO  | 92.8 | (R)-pCPED | [27]       |

Table 2 Chart of different EHs with high enantioconvergence

A volume of 5.0 mL, hydrolysis of rac-mCSO was carried out by resting cells at 25 °C, until c reached over 99%. With a large amount of (R)-mCPED produced, few (S)-mCPED was formed, which decreased ee_p value. Among the hydrolysis, the ee_p was all over 93%. The rac-mCSO was transformed into mCPED within 6 h completely (c = 99.5%). According to the Fig. 5, at the end of the hydrolysis, the ee_p and yield were 95.2% and 97.2%, respectively, and the chiral HPLC spectra of production was seen in Additional file 1: Figure S1b. By now, among all the reported EHs which the author has ever known, Pveh1Y3Z2 owned the highest enantioconvergent hydrolysis of rac-mCSO by whole cells which expressed Pveh1Y3Z2. The enantioconvergent hydrolysis of mCSO was carried out at 25 °C, 2 mL volume containing 20 mM rac-mCSO, 100 mg/mL E. coli/pveh1Y3Z2 wet cells until the conversion reached over 99%. The reaction course of aliquots of sample were drawn out periodically, extracted with ethyl acetate, and analyzed by chiral HPLC and GC.

Additional file 1: Figure S1. Chiral HPLC spectra for enantioconvergent hydrolysis of rac-mCSO by Pveh1Y3Z2 including analysis of rac-mCSO and rac-mCPED (a), and analysis of (R)-mCPED (b). Figure S2. The comparison of the homology model (red) with the template model (green). Figure S3. The change in RMSD values of the whole model. The RMSD values of the whole model tends to 0.18 nm. Figure S4. The Ramachandran plots of the model. The Ramachandran favored residue sites was 96.07%, which means that the most distribution of residues is good and the model could be believed. Figure S5. The local quality estimate of every residue. Compared with the template model, the identity of amino acids lining the substrate-binding pocket was 93.94%. The local similarity value of most residue in the substrate-binding pocket was 93.94%. The local similarity value of most residue in the substrate-binding pocket was 0.8, which means the residue was highly similar to the template model. Table S1. Enantioconvergences and activities of Pveh1 and its three-site mutant towards rac-mCSO. Table S2. PCR primers used for site-saturation saturation mutagenesis of pveh1. Table S3. PCR primers used for combinatorial site-directed mutagenesis of pveh1. Table S4. Catalytic characteristics of representative mutants from leucine scanning mutagenesis.
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Authors’ contributions
XZ and CL designed this study. XZ performed most experiments. All the authors analyzed the data. XZ and MW mainly wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated and/or analysed during this study are included in this article.

Ethics approval and consent to participate
Not applicable.

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Not applicable.

Competing interests
The authors declare that they have no competing interests.

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