Supporting information

Engineering of Amine Dehydrogenase for Asymmetric Reductive Amination of Ketone by Evolving *Rhodococcus* Phenylalanine Dehydrogenase

Li Juan Ye [†], *Hui Hung Toh*[†], *Yi Yang*[†], *Joseph P. Adams*[‡], *Radka Snajdrova*[‡] *and Zhi Li**[,†]

[†]Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117585 (Singapore).

[‡] Medicines Research Centre, GlaxoSmithKline R&D Ltd, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY (UK)

E-mail: chelz@nus.edu.sg; Fax: (+65)-6779-1936; Phone: (+65)-6516-8416

Contents

1. Chemicals

Following chemicals were obtained from suppliers and used without further purification: phenylacetone (≥ 99%, Acros), 4-phenyl-2-butanone (≥ 98%, Sigma-Aldrich), 1-methyl-3-phenylpropylamine (≥ 98%, Sigma-aldrich), *S*-1-methyl-3-phenylpropylamine (≥ 98%, Sigma-aldrich), phenylpyruvic acid (≥ 98%, Sigma-Aldrich), L-phenylalanine (≥ 98%, Sigma-Aldrich), fluorophenylacetone (≥ 98%, Alfa Aesar), iodonitrotetrazolium chloride (INT) (≥ 95%, Alfa Aesar), phenazine ethosulfate (PES) (≥ 90%, Sigma-aldrich), 1-hydroxy-3-phenylpropan-2-one (≥ 95%, Aurora Fine Chemicals), Isopropyl β -Dthiogalactopyranoside (IPTG) (≥ 99%, Sigma-Aldrich), and NAD^+ (≥ 96.5%, Calbiochem), NADH (≥ 96.5%, Calbiochem).

Amphetamine as substrate for deamination activity screening was synthesized according to the reported method [S1]. Ammonium hydroxide solution (28.0-30.0% NH_3 basis), NH_4Cl (≥99.5%), K_2HPO_4 (≥98%), KH_2PO_4 (≥98%), imidazole (≥98%), 1.0 M perchloric acid solution, dimethylamine (≥98%) were purchased from Sigma-Aldrich.

2. Strains and biochemicals

E. coli T7, Q5 High-Fidelity DNA Polymerase, *Dpn* I were purchased from New England Biolabs (NEB). Plasmid pET28a was obtained from Novagen. T4 DNA quick ligase kit and deoxynucleotide (dNTP) DNA loading dye (DNA ladders) were purchased from Thermo Scientific. Oligonucleotides were synthesized by AIT biotech, Singapore. LB Broth, bacto agar, tryptone, and yeast extract were purchased from Biomed Diagnostics. Plasmid extraction kit and B-PER reagent were obtained from Thermo Scientific. Glucose dehydrogenase was bought from *Pseudomonas* sp. was bought from Sigma-Aldrich.

3. Analytic method

HPLC analysis for determination of the concentrations of substrate phenylacetone 1 or 4-phenyl-2-butanone 3 and product amphetamine 2 or 1-methyl-3-phenylpropylamine 4

The concentrations of the substrates and products were determined by using a Shimadzu prominence HPLC system with UV detection at 210 nm. Elute: 80% TFA (pH3.0): 20% acetonitrile. Flow rate: 0.5 mL/min. Retention times: 13.3 min for **1** and 4.4 min for **2**; 26.4 min for **3** and 6.2 min for **4** with an Agilent Poroshell 120 SB-Aq C18 column (100 × 4.6 mm, 2.7 μm); 39.0 min for **3** and 7.0 min for **4** with an Agilent Poroshell 120 SB-Aq C18 column (150 × 4.6 mm, 2.7 μm).

Chiral HPLC analysis for *ee* determination of amination product amphetamine 2

The *ee* value of the amine products **2** was determined by using a Shimadzu prominence HPLC system (reverse phase) with a Crownpak CR (+) column (250 × 4.6 mm) (Daicel Co., Japan) and UV detection at 210 nm. Elute: 70% $HClO_4$ (pH1.8): 30% MeOH. Flow rate: 0.6 mL/min. Retention times: 19.4 min for **1**, 11.3 min for (*S*)-**2**, and 13.3 min for (*R*)-**2**.

Chiral HPLC analysis for *ee* determination of amination product 1-methyl-3-phenylpropylamine 4

The *ee* value of the amine products **4** was determined by using a Shimadzu prominence HPLC system (normal phase) with an OD-H column (250 × 4.6 mm) (Daicel Co., Japan) and UV detection at 254 nm. Elute: 90% DEA (0.1%) in hexane: 10% *i*- propanol. Flow rate: 0.6 mL/min. Retention times: 22.8 min for **3**, 13.5 min for (*S*)-**4** and 17.6 min for (*R*)-**4**.

4. Cloning and expression of phenylalanine dehydrogenase from *Rhodococcus* sp. M4 in *E. coli*

The synthetic gene with optimized codon usage of phenylalanine dehydrogenase (pheDH) from *Rhodococcus*. sp. M4 was obtained from Genscript (USA). To clone the gene into plasmid pET28a, two primers were designed: forward: CGCCATATGAGTATCGATTCGGC; reverse: CCGCTCGAGGGCAGTTGCAGTTG. pheDH gene was amplified by PCR using MJ Research PTC-200 thermal cycler with the following program: 95 °C for 5 min, (95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min) × 30 cycles, 72 °C for 10 min. PCR reaction tube contained 1 μL each of forward and reverse primer (2.5 μM), 5 μL Standard Taq Buffer, 1 μL dNTP Solution Mix, 1 μL (50 ng) template plasmid (pUC57_pheDH), 1 μL Taq DNA Polymerase, and 40μL ultra-pure H_2O. The amplified PCR fragments were purified with PCR purification Kit (Qiagen). The purified gene samples as well as vector pET28a sample were then digested with *Nde* I and *Xho* I. The digestion mixture was incubated for 2 h at 37 °C and purified again with PCR purification Kit (Qiagen). The purified DNA concentration of each sample was measured at 280 nm by Nanodrop. The ligation was conducted using Quick ligation kit (NEB) [1 μL pET28a vector (50 ng), 3.5 μL insert DNA fragment (200 ng), 0.5 μL Quick T4 DNA Ligase, and 5 μL of Quick Ligation Buffer]. The ligation solution was incubated in room temperature for 30 min and then transformed into chemical competent DH5α cells, which were then plated on LB agar plates with 50 μg/mL kanamycin. The constructed plasmids were confirmed by DNA sequencing. Finally, the purified plasmids were transformed into chemical competent *E. coli* T7 cells and plated on LB agar plates containing 50 μg/mL kanamycin to give *E.coli* (pheDH).

E.coli (pheDH) was grown in TB medium. After induction with 0.5 mM IPTG at 2 h, cells were grown at 22 °C overnight and then harvested. The expression was checked by SDS-PAGE) (Fig. S1) of the cell-free extract prepared by B-PER cell lysing reagent (Thermo Scientific) and the amination activity of the pheDH toward natural substrate phenylpyruvic acid was checked spectrophotometrically by following UV absorbance at 340 nm of the reaction mixture containing 2 mM NAD^+, 5 mM phenylalanine, and 1 g protein/L of cell-free extract of *E.coli* (pheDH) in 0.1 M glycine buffer at pH 9.6 and 30 °C.

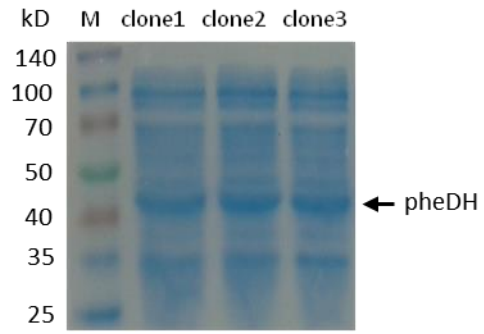

Fig. S1 SDS-PAGE of cell-free extract of *E. coli* expressing pheDH.

5. Identification of suitable amino acid residues for the evolution of pheDH from *Rhodococcus* sp. M4 as new amine dehydrogenase

The first round mutagenesis: Lys66 and Asn262 have direct interaction with the carboxylate group of natural substrate. Therefore we started enzyme engineering by simultaneous randomization of the residues Lys 66 and Asn262 in the catalytic site for accepting ketone substrates.

The second round mutagenesis: 20 residues within 6 Å from the natural substrate phenylalanine in the active site of pheDH (PDB ID: 1C1D) were selected for single site saturation mutagenesis (Fig. S2 and Table S1). Lys78 and Asp118 are excluded because they are essential for the catalysis based on catalytic mechanism.

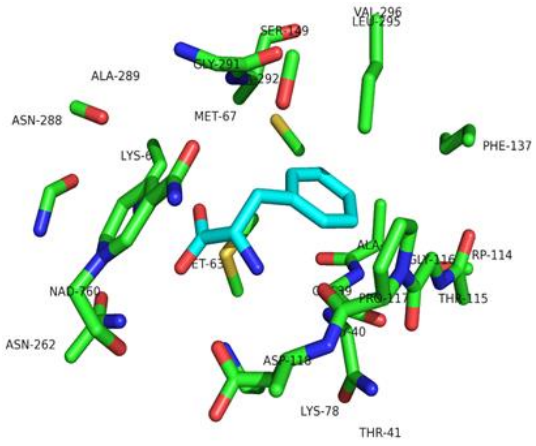

Fig. S2 Active site of *Rhodococcus* pheDH (PDB ID: 1C1D) bound with phenylalanine

Table S1. 20 amino acid residues of 1C1D within 6 Å from phenylalanine bound to *Rhodococcus* pheDH

No.	Residue	No.	Residue
1	A38	11	F137
2	G39	12	S149

3	G40	13	T153
4	T41	14	N 263
5	M63	15	N 288
6	M68	16	A 289
7	W114	17	G 291
8	T115	18	A 292
9	G116	19	L 295
10	P117	20	V 296

6. Double site saturation mutagenesis of *Rhodococcus* pheDH and high-throughput screening of positive mutants

Principle of high-throughput screening assay

The first round double-site saturation mutagenesis library was screened by using a formazan-based colorimetric assay [S2]. The principle is shown in Fig. S3. Positive mutants can deaminate 1-methyl-3-phenylpropylamine **4** and produce NADH which creates deep red formazan from PES and INT.

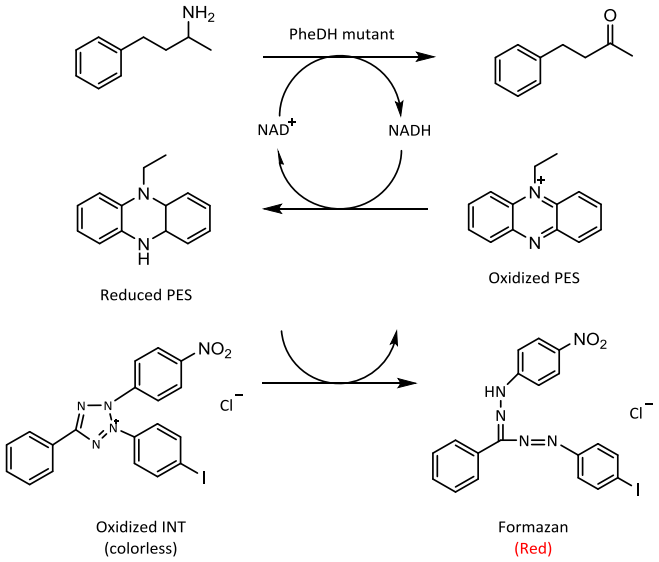

Fig. S3 Reaction scheme of high-throughput screening method based on formazan colorimetric assay

Library building

Plasmid pET28a containing the gene of the pheDH was extracted from *E. coli* (pheDH) and used as a template for saturation mutagenesis. Double-site saturation mutagenesis of Lys 66 and Asn262 was carried out with Strategene QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. The mutant library at selected sites was generated using PCR with primers (Table S2) containing NNK codons covering all 20 possible amino acids.

The PCR product was first digested by using *Dpn* I and then transformed into 50 μL XL10-Gold Ultracompetent Cells (Stratagene) according to the manufacturer's instructions. The cells were incubated in a shaker at 37°C and 250 rpm for 1 h. Colonies containing plasmid were selected on LB agar plates containing 50 μg/mL kanamycin.

Table S2. PCR primer set for the double-site saturation mutagenesis

Site	Primers
K66	gcgatgactttg**nnk**atggcagtctccaat
N262	gcaggggccgca**nnk**aatgtgattgc

Screening procedure

The colonies were picked manually, inoculated into 600 μL TB medium in each well of 96-well plates, and grown at 37 °C for 6-8 h. 100 μL culture was inoculated into 900 μL TB medium containing 50 μg/mL kanamycin and 0.5 mM IPTG. After expression at 22°C for 12 h, the plates were centrifuged at 4000 *g* for 15 min, and the obtained cell pellets were lysed by adding B-PER reagent. The cell-free extract was split in 20 μL aliquots into 2 rows of a 96-well microtiter plate: a reaction and a background row. Buffer containing 0.5 mM NAD^+, 5 μg/mL PES, 0.3 mg/mL INT and 0.1 M glycine buffer at pH 9.6 was added to the background row. The reaction buffer containing 10 mM amines **2** or **4** and other components of the background buffer was added to the reaction row. Plates were allowed to incubate for 30 min at room temperature, and the UV absorbance was determined at 495 nm. ΔA495 ($A_{495\ reaction} - A_{495\ background}$) was used to calculate the deamination activity.

7. Single site saturation mutagenesis of *Rhodococcus* pheDH and high-throughput screening of positive mutants

Library building

Second round single site saturation mutagenesis was made at each of the 20 selected amino acid residues, respectively. The mutant library was built by using the method reported by Reetz [S3], with primers containing NNK codons as shown in Table S3.

The mutant library at each selected sites was generated using PCR with the primers containing NNK codons. Each PCR reaction tube contained 2 μL each of 20 forward and reverse degenerate primer of a particular target site, 10 μL HF Buffer, 2 μL dNTP Solution Mix, 0.1 μL (10 ng) template plasmid pET28-pheDH_K66Q/N262C, 0.5 μL Q5 High-Fidelity DNA Polymerase, and 36.6 μL ultra-pure H_2O. PCR amplification was carried out on MJ Research Thermal Cycler using the following thermal cycling protocol: 98 °C for 3 min, (98 °C for 10 s, 58 °C for 30 s, 72 °C for 4 min) × 30 cycles, 72 °C for 10 min.

PCR products were subjected to DNA gel electrophoresis and the location of DNA in gel was detected using gel imaging system. The band containing the product was excised from the gel, and purified using

QIAquick Gel Extraction Kit (Qiagen). The purified DNA was digested with methylated restrictive enzyme *Dpn* I at 37°C for 2 h, then ligated with 1U T4 DNA ligase in a total volume of 20 µL at 22 °C for 2 h. The ligated DNA was transformed into 50 µL competent *E. coli* T7 (NEB). The cells were incubated in a shaker at 37 °C and 250 rpm for 1 h. Colonies containing the gene were selected on LB agar plates containing 50 µg/mL kanamycin.

Table S3. PCR primer set for the single-site saturation mutagenesis

Site	Primers
A38f	ccggcg**nnk**gccggtacccgtg
A38r	gccgagttgcgtactatctaaacggataacaaagtggg
G39f	ccggcggcc**nnk**ggtacccgtg
G39r	gccgagttgcgtactatctaaacggataacaaagtggg
G40f	gcggccggc**nnk**acccgtgcgg
G40r	cgggccgagttgcgtactatctaaacggataacaaagtggg
T41f	gccggcggt**nnk**cgtgcggcac
T41r	cgccgggccgagttgcgtactatctaaacggataacaaagtg
M63f	gctggcgcg**nnk**actttgaaaatgg
M63r	caacttacccgcatcggtcagtgc
M68f	gcgatgactttgaaa**nnk**gcagtctccaat
M68r	gccagccaacttacccgcatcggt
W114f	ctgagtggtaactat**nnk**acaggtccggat
W114r	cttatcaatgttttccgcatgaatacgaagaatacg
T115f	ggtaactattgg**nnk**ggtccggatgttaatacg
T115r	actcagcttatcaatgttttccgcatgaatacgaagaatacg
G116f	ctgagtggtaactattggaca**nnk**ccggatgttaatacgaattc
G116r	cttatcaatgttttccgcatgaatacgaagaatacgcg
P117f	gacaggt**nnk**gatgttaatacgaattcag
P117r	caatagttaccactcagcttatcaatgttttc
F137f	cggaatttgtt**nnk**gggcgcagcctggagc
F137r	tcgtatcgttcagtgtatccatatctgctg
S149f	ggagcaggctcc**nnk**gcattcacaacc
S149r	gccacgctccaggctgcgc
T153f	gcattcaca**nnk**gctgtgggcgtgggc
T153r	actggagcctgctccgccacgctccag
N 263f	gccgcaaat**nnk**gtgattgccgacgaagcagc
N 263r	ccctgccacaactgaacagtcaagcgtgcg

N 288f	cccgactttgtggcg**nnk**gccggaggtgctattc
N 288r	cgcgtacaagatcccccgagcgtg
A 289f	gactttgtggcgaat**nnk**ggaggtgctattcacctg
A 289r	gggcgcgtacaagatcccccgagcgtg
G 291f	gcgaatgccgga**nnk**gctattcacctggtg
G 291r	cacaaagtcgggcgcgtacaagatccc
A 292f	gcgaatgccggaggt**nnk**attcacctggtg
A 292r	cacaaagtcgggcgcgtacaagatccc
L 295f	ggtgctattcac**nnk**gtgggccgtg
L 295r	tccggcattcgccacaaagtcgg
V 296f	gctattcacctg**nnk**ggccgtgttcac
V 296r	acctccggcattcgccacaaagtcgg

Screening procedure

The cell growth procedure and cell free extract preparation are the same as described in section 6. The assay for the second round library screening was also based on the deamination of **2** or **4**, but with UV detection of the formation of NADH. 20 μL cell-free extract were added to each well of a 96-well plate containing deamination buffer of 0.5 mM NAD^+, 10 mM amine **2** or **4**, and 0.1 M glycine buffer at pH 9.6. Plates were incubate for 30 min, followed by the detection of UV absorbance at 340 nm.

8. Specific activity of positive mutants for the asymmetric amination of ketone 1 and 3

Cell growth and cell-free extract preparation

E. coli cells expressing the positive pheDH mutants were grown at 37 ℃ and 250 rpm in TB medium containing 50 μg/mL kanamycin for 2 h. Enzyme expressions were induced by addition of IPTG to a final concentration of 0.5 mM, and the cells were then grown at 22 ℃ for 12 h. Cells were harvested by centrifugation at 12, 000 *g* for 5 min, and the cell pellets were lysed with B-PER reagent 4 mL/g wet cell at room temperature for 15 min. After centrifugation at 12,000 *g* for 10 min, the supernatant was taken as cell-free extract for amination reaction.

Amination of ketone 1 and 3

Amination reactions were performed in NH_4Cl/NH_4OH buffer (0.5 M, pH 9.6) containing 10 mM **1** or **3**, 1 mM NADH, and cell-free extract containing mutant enzyme 1 mg protein /mL. The reactions were carried out at 30℃ and 250 rpm. The cell-free extracts of mutant enzyme were added to initiate the reactions. Samples were taken at 30 min for HPLC analysis to determine the product concentration and calculate the specific activity.

9. Molecular modelling of pheDH and its mutants and substrate binding poses

Molecular dynamics (MD) simulations and substrate dockings were performed to explore the structure basis of the experimentally observed biocatalytic behaviour of the wild type and mutated enzymes. The X-ray crystal structure of pheDH from *Rhodococcus* sp. M4 (PDB ID: 1C1D) was used as a template for molecular modelling, where one homo-dimer subunit was chosen for post-processing. The structure models of the double and triple mutants (K66Q/N262 & K66Q/N262/S149G) were generated via ACCELRYS Discovery Studio [S4]. MD simulations of wild type and enzyme models (inclusive of NADH) were executed with GROMACS 4.5 [**S5**] by using GROMOS 53a6 force field, a recording interval of 0.2 ps and a SPC water solvent system with 150 mM NaCl. Particle Mesh Ewald (PME) method was applied for long-range electrostatic interactions, and van der Waals interactions were restricted to 10 Å, with all bond lengths constrained via LINCS algorithm [S6]. The systems were energy minimized with steepest descent algorithm and relaxed at NVT ensemble and NPT ensemble using a V-rescale thermostat and Parrinello-Rahman barostat to equilibrate at 300 K and 1 atm. NPT coupled production runs of 5 ns were conducted, and recorded enzyme conformers were clustered using Gromos algorithm [S7] with least-squares-fitted RMSD cutoff at 0.16 nm. The main cluster centroids were docked with phenyl pyruvate and substrates **1** and **3**, by using Autodock VINA [S8, S9]. Docked results were evaluated via the criteria of binding energy scores with non-fitted RMSD cutoff at 1 Å.

Fig. S4 shows the docked posture of the natural substrate phenyl pyruvate in the wild type enzyme pheDH from *Rhodococcus* sp. M4. Substrate carbonyl-O located at ~3 Å from Lys78, and the carboxyl moiety oriented near the hydrophilic residue Asn262.

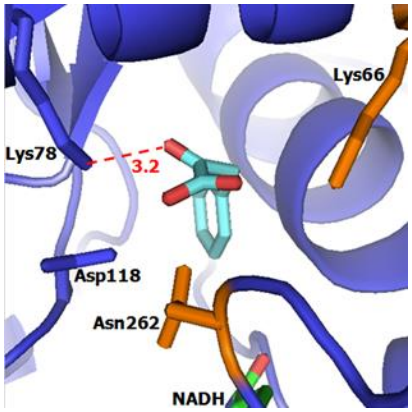

Fig. S4 Enzyme-substrate binding pose of phenyl pyruvate in pheDH from *Rhodococcus* sp. M4. Distances (in angstrom) are denoted by dashed lines.

10. Sequence alignment between TM_pheDH and the reported pheDH double mutant from *Bacillus badius*

In our research, pheDH was from *Rhodococcus* sp. M4, while the reported evolution of pheDH was from *Bacillus badius* [S10]. The amino acid sequences of the two enzymes share 32% identity and 65%

similarity (Fig. S5). Two catalytic residue K78 and D118 (according to pheDH_*Rhodococcus* number [S11], in square) are conserved, and two residues (K66, N262, underlined) which have interaction with carboxylate group are also conserved.

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CLUSTAL 2.1 multiple sequence alignment


1C1D_Rhodococcus      ---MSIDSALN--------WDGEMTVTRFDKMTGAHFVIRLDSTQLGPAAGGTRAAQYSQ 49
pheDH_Bacillus        MSLVEKTSIIKDFTLFEKMSEHEQVVFCNDPATGLRAIIAIHDTTLGPALGGCRMQPYNS 60
                         :.  * ::         : * .*   *  ** : :* :..* **** ** *   *..

1C1D_Rhodococcus      LADALTDAGKLAGAMTLKMAVSNLPMGGGKSVIALPAPRHSIDPSTWARILRIHAENIDK 109
pheDH_Bacillus        VEEALEDALRLSKGMTYKCAASDVDFGGGKAVIIG-DPQKDKSP----ELFRAFGQFVDS 115
                      : :** ** :*: .** * *.*:: :****:**    *::. .*    .::* ..: :*.

1C1D_Rhodococcus      LSGNYWTGPDVNTNSADMDTLNDTTEFVFGRSLERGGAGSSAFTTAVGVFEAMKAT-VAH 168
pheDH_Bacillus        LGGRFYTGTDMGTNMEDFIHAMKETNCIVGVPEAYGGGGDSSIPTAMGVLYGIKATNKML 175
                      *.*.::**.*:.**  *:    . *: :.* .   **.*.*::.**:**: .:***

1C1D_Rhodococcus      RGLGSLDGLTVLVQGLGAVGGSLASLAAEAGAQLLVADT------DTERVAHAVALGHTA 222
pheDH_Bacillus        FGKDDLGGVTYAIQGLGKVGYKVAEGLLEEGAHLFVTDINEQTLEAIQEKAKTTSGSVTV 235
                       * ..*.*:*  :**** ** .:*.   * **:*:*:*         :. *::.: . *.

1C1D_Rhodococcus      VALEDVLSTPCDVFAPCAMGGVITTEVARTLDCSVVAGAANNVIADEAASDILHARGILY 282
pheDH_Bacillus        VASDEIYSQEADVFVPCAFGGVVNDETMKQFKVKAIAGSANNQLLTEDHGRHLADKGILY 295
                      ** ::: *  .***.***:***:. *. : :. ..:**:*** :  *  .  *  :****

1C1D_Rhodococcus      APDFVANAGGAIHLVGREVLGWSESVVHERAVAIGDTLNQVFEISDNDGVTPDEAARTLA 342
pheDH_Bacillus        APDYIVNSGGLIQVAD-ELYEVNKERVLAKTKHIYDAILEVYQQAELDQITTMEAANRMC 354
                      ***::.*:** *::.. *:   .:. *  ::  * *:: :*:: :: * :*. ***. :.

1C1D_Rhodococcus      GR----RAREASTTTATA-------- 356
pheDH_Bacillus        EQRMAARGRRNSFFTSSVKPKWDIRN 380
                       :    *.*. *  *::.
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Fig. S5 The amino acid sequence alignment of the pheDH from *Rhodococcus* sp. M4 with the pheDH from *Bacillus badius* by ClustalW2. In blue square are the conservative catalytic residues. Red underlined are the two residues which have hydrogen bond with the carboxyl group of the natural substrate phenylalanine.

11. Asymmetric amination of ketone 1 and 3 with TM_pheDH to (*R*)-2 and (*R*)-4, respectively

Cell growth procedure for *E.coli* (TM_pheDH) and cell-free extract preparation are the same as described in section 8.

Reactions

Reaction was performed in 10 mL NH_4Cl/NH_4OH buffer (pH 9.6, 0.5 M), 1 mM ketone **1** or **3**, 1 mM NADH, and the cell-free extract of *E. coli* (TM_pheDH) (1mg protein/mL) at 30 °C for 30 min. The reaction was stopped by adding the equal volume of acetonitrile, followed by centrifugation. The supernatant was taken for HPLC analysis. The chromatograms are shown in Fig S6a-d.

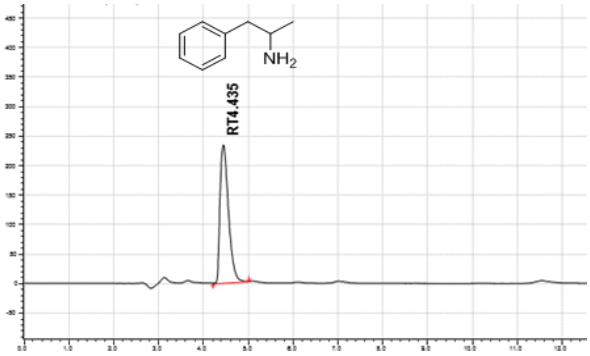

Fig. S6a HPLC chromatogram of amphetamine **2** standard

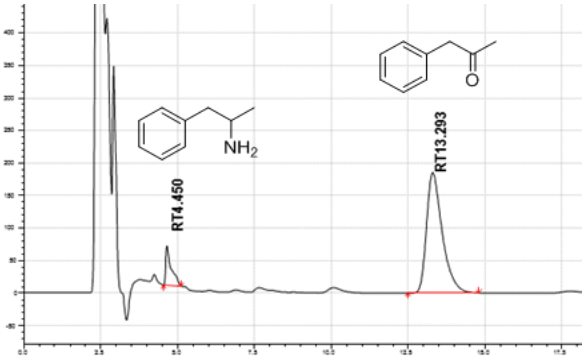

Fig. S6b HPLC chromatogram of the biotransformation mixture from TM_pheDH-catalyzed amination of **1** at 30min

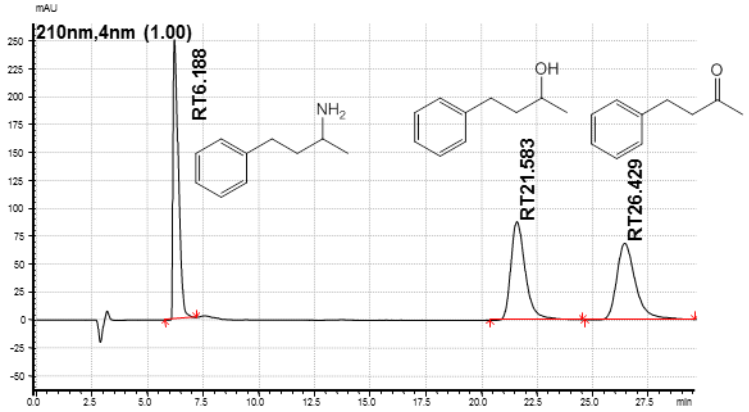

Fig. S6c HPLC chromatogram of 4-phenyl-2-butanone **3** and 1-methyl-3-phenylpropylamine **4** standards

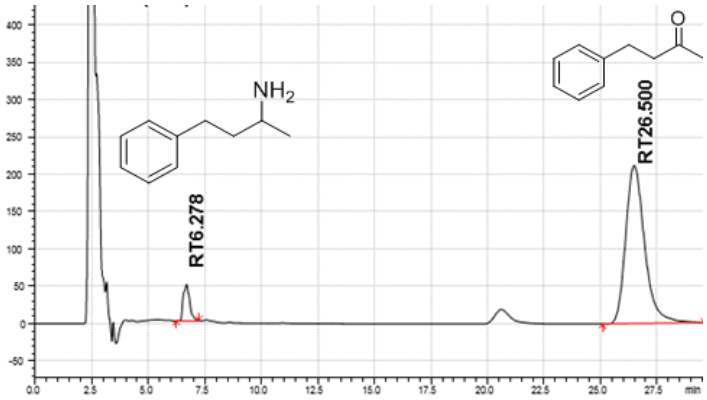

Fig. S6d HPLC chromatogram of the biotransformation mixturefrom TM_pheDH-catalyzed amination of 4-phenyl-2-butanone **3** at 30 min

To determine the enantioselectivity, samples were taken at 30 min, extracted with chloroform, dried, re-dissolved in mobile phase (70% $HClO_4$ (pH 1.8): 30% MeOH for **2** or 90% DEA (0.1%) in hexane: 10% *i*-propanol for **4**), and analyzed by chiral HPLC. The chromatograms were shown in Fig. S7 and S8.

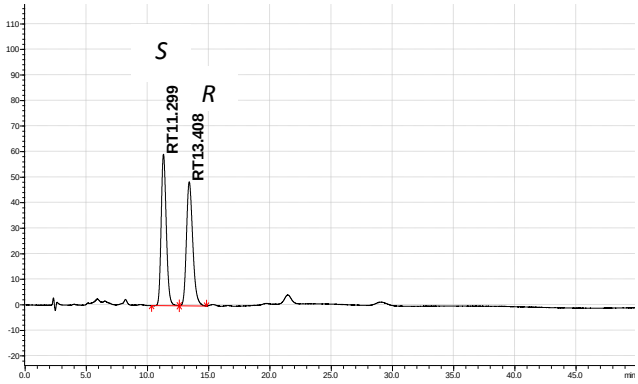

Fig. S7a Chiral HPLC chromatogram of racemic amphetamine **2** standard.

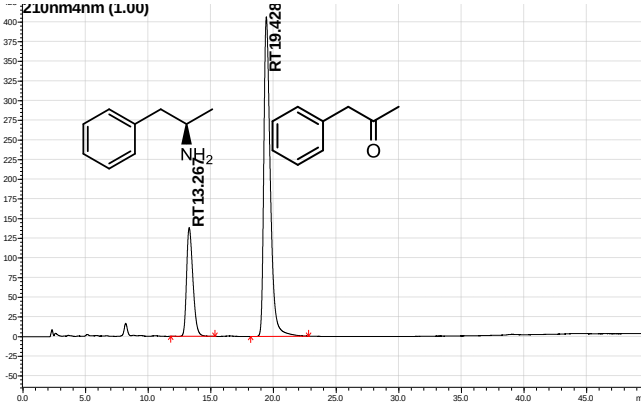

Fig. S7b Chiral HPLC chromatogram of the biotransformation mixture of TM_pheDH-catalyzed amination of phenylacetone **1** to amphetamine **2** at 30 min

The peaks could be clearly assigned to (*R*)- or (*S*)-**2** in Fig. S7a by comparison the retention times with the reported ones with exact the same column and mobile phase [S12]. The reaction product of TM_pheDH shows only an (*R*)-amphetamine **2** peak in Fig. S7b, indicating an *ee* of > 98%.

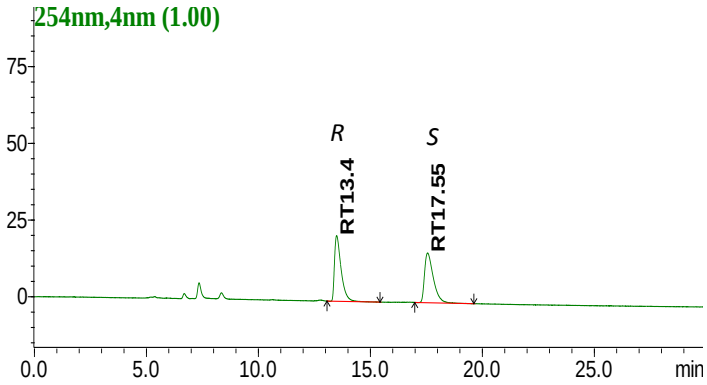

Fig. S8a Chiral HPLC chromatogram of racemic 1-methyl-3-phenylpropylamine **4** standard

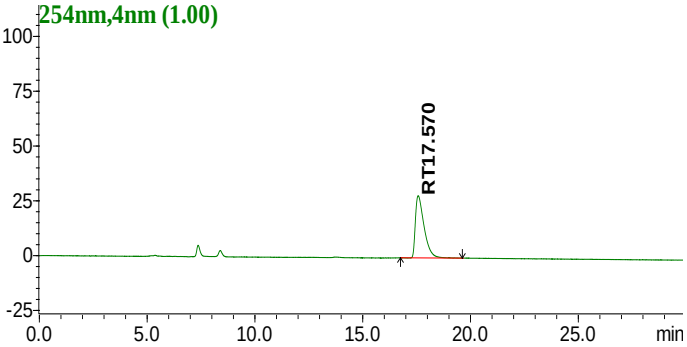

Fig. S8b Chiral HPLC chromatogram of (*S*)-1-methyl-3-phenylpropylamine **4** standard

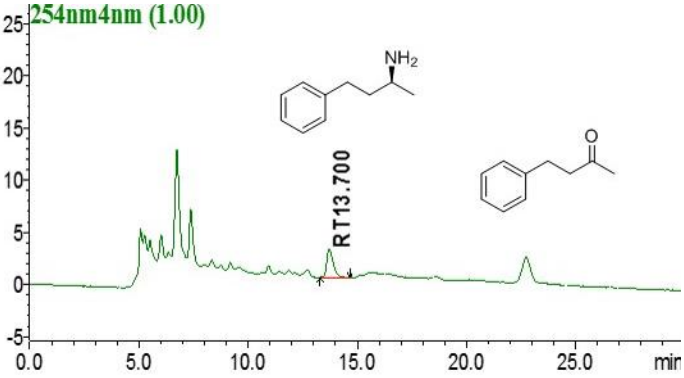

Fig. S8c Chiral HPLC chromatogram of the biotransformation mixture of TM_pheDH-catalyzed amination of 4-phenyl-2-butanone **3** to 1-methyl-3-phenylpropylamine **4** at 30 min

In comparison with the peak of (*S*)-1-methyl-3-phenylpropylamine **4** (Fig. S8b), the product peak in Fig. S8c can be easily assigned as (*R*)-**4**. No peak of (*S*)-**4** in Fig. S8c indicated the *ee* of (*R*)-**4** is > 98%.

12. Purification of his-tagged TM_pheDH

TM_pheDH was engineered in pET28 vector in *E.coli* T7 with N-terminal His-tag. After 12 h of cell growth with the enzyme expression with IPTG, cells (500 mL liquid culture) were harvested by centrifugation at 3220 *g* and 4 °C for 15 min. The cell pellets were then re-suspended in 170 mL 10 mM potassium phosphate buffer (pH 8.0) containing 0.3 M NaCl, 10 mM imidazole to an OD_{600} of 30. Cells were broken by passing through cell disruptor at 21 psi for 2 times, followed by centrifugation (15000 *g*, 4 °C, and 60 min).

The his-tagged TM_pheDH were purified by using ÄKTA purifier (GE Healthcare) system at 4 °C with UV at 280 nm to monitor protein concentration. The cell-free extracts were loaded onto a Ni-NTA column, and non-his-tagged proteins were washed out by using 50 mM potassium phosphate buffer (pH 8.0) containing 0.3 M NaCl and 50 mM imidazole. The his-tagged protein was then eluted by using 50 mM potassium phosphate buffer (pH 8.0) containing 0.3 M NaCl and 500 mM imidazole. The fractions containing his-tagged TM_pheDH were concentrated using Millipore Amicon Ultra-4 centrifugal filter device (10 kDa) at 3220 *g* and 4°C, washed three times with 50 mM potassium phosphate buffer (pH 8.0), and concentrated again. Figure S9 demonstrated the purity of the obtained his-tagged TM_pheDH.

Protein concentration of the purified enzyme was determined with the Bradford assay and bovine serum albumin as standard. Roughly 108 mg of pure enzyme was obtained from 170 mL cell free extract with 18 mg/mL protein.

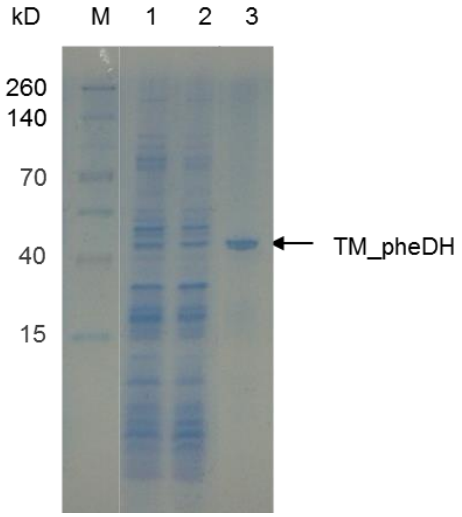

Fig. S9 SDS-PAGE of purified N-his-tagged TM_pheDH expressed from *E. coli*. M, molecular weight marker; Lane 1, Cell-free extract of *E. coli* (pheDH); Lane 2, Cell-free extract of *E. coli* (TM_pheDH); Lane 3, Purified TM_pheDH.

13. Determination of kinetic data for the asymmetric amination of ketone 1 and 3 with purified his-tagged TM_pheDH, respectively

Purified his-tagged TM_pheDH was mixed with 1 mL NH_4Cl/NH_4OH buffer (pH 9.6, 0.5 M) containing 0.25-10 mM ketone **1** or **3**, 2 mM NADH, and 1 μM enzyme. Reactions were performed at 30℃ and stopped at 10 min by adding the equal volume of acetonitrile. After centrifugation, the supernatant was taken for HPLC analysis to determine the product concentration. The kinetic data (K_m, V_{max} k_{cat} and k_{cat} /K_m) were calculated accordingly from the Lineweaver-Burk plot (Fig. S10).

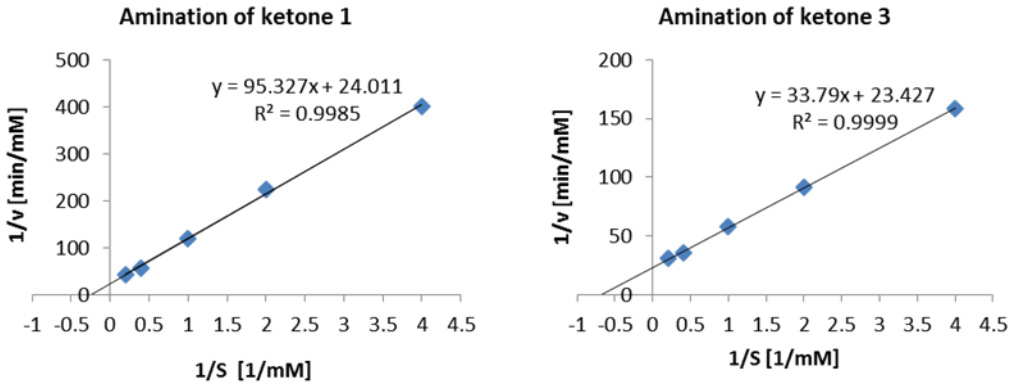

Fig. S10 Lineweaver-Burk curves of TM_pheDH-catalyzed amination of ketone phenylaceton **1** and 4-phenyl-2-butanone **3**

14. Asymmetric amination of 4-phenyl-2-butanone 3 to (*R*)-4 with purified TM_pheDH, coupled with GDH for cofactor regeneration

1mL NH_4OH/NH_4Cl buffer (pH9.6, 0.5M) containing 15mM substrate phenylacetone **1**, 0.005mM NAD^+, 4 mg protein /mL TM_PheDH, and 40 U/mL GDH from *Pseudomonas* was shaken at 250 rpm and 30 °C. Samples were taken at different time intervals, and diluted 2 fold with 5% TFA. After centrifuge at 12, 000 *g* for 1 min, the supernatant was further diluted 5 fold with 80% TFA (pH 3.0) and 20% ACN, and the samples were used for HPLC analysis. The reaction was stopped at 60 h.

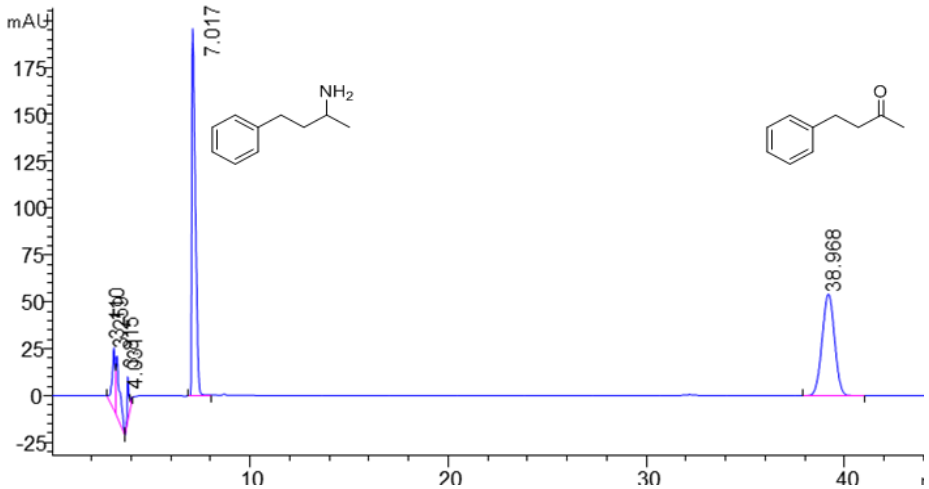

Fig. S11a HPLC chromatogram of 4-phenyl-2-butanone **3** and 1-methyl-3-phenylpropylamine **4** standards

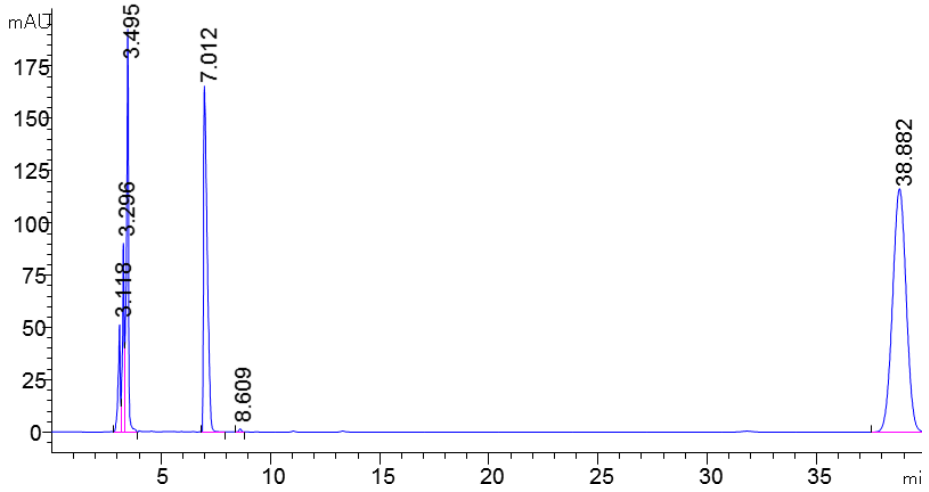

Fig. S11b HPLC chromatogram of the biotransformation mixture from TM_pheDH-catalyzed amination of 4-phenyl-2-butanone **3** to (*R*)-**4** coupled with GDH-catalyzed cofactor regeneration at 24 h

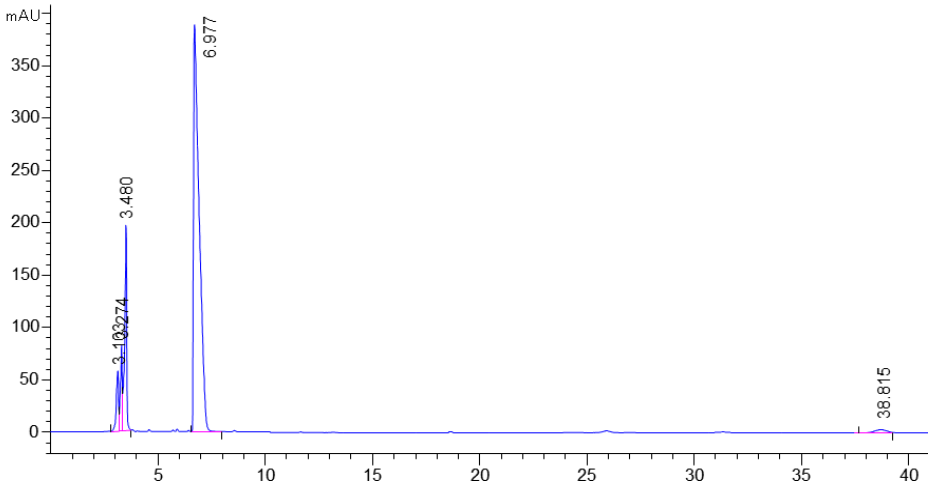

Fig. S11c HPLC chromatogram of the biotransformation mixture from TM_pheDH-catalyzed amination of 4-phenyl-2-butanone **3** to (*R*)-**4** coupled with GDH-catalyzed cofactor regeneration at 60h

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