Supporting Information

Improving and Inverting C_β-Stereoselectivity of Threonine Aldolase via Substrate-Binding-Guided Mutagenesis and a Stepwise Visual Screening

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Experimental Procedures

Materials

KOD Plus polymerase was purchased from Toyobo. *Dpn* I was obtained from NEB. Yeast extract and tryptone were purchased from Oxoid. DL-*threo*-phenylserine was obtained from Sigma-Aldrich, DL-*erythro*-phenylserine and DL-*threo*-phenylserine derivatives were synthesized as previously described.[1-3] Other reagents were purchased from Sigma-Aldrich, Alfa Aesar and Sinopharm Chemical.

Cloning and Expression of L-Threonine Aldolases

The reported L-TAs were selected as templates to identify new ones by using BLASTP search in NCBI. The codons of obtained putative sequences were optimized using the JCat algorithm.[4] The optimized genes were synthesized by Tianjin Institute of Industrial Biotechnology of Chinese Academy of Sciences and ligated into the pET-21a plasmid between the *Nde*I and *Xho*I restriction sites. Other genes were amplified by PCR using oligonucleotide primers with restriction site of *Nde* I and *Hind* III. All the genes contained six His-tag at C-terminal and the resultant plasmids were transformed into *E. coli* BL21(DE3). The source and nomenclature of the proteins were listed in Table S1.

Determination of Stereospecificity of L-TAs

The conversion and stereoselectivity of aldol condensation catalyzed by 18 soluble L-TAs were measured. The reactions were carried out in Tris-HCl buffer (100 mM, pH 7.5) with 1 M glycine, 100 mM benzaldehyde, 10 μM PLP and 10% DMSO (v/v) in a final volume of 1 mL. Negative control with *E. coli* BL21(DE3) was accomplished in the same fashion. The whole cell biotransformations were performed

at 25°C for 12 h. Chiral analysis was performed by HPLC after derivatization with *o*-phthaldialdehyde/*N*-acetyl-cysteine (OPA/NAC).[13]

Screening, Cloning and Expression of Phenylserine Dehydratase

Fifty two strains from our laboratory collection were each inoculated into 20 mL Luria-Bertani (LB) medium. The culture was shaken (200 rpm) at 30 or 37°C for 12 to 24 h. Cells were harvested by centrifugation (8000× *g*, 15 min), washed twice with 0.9 % NaCl and re-suspended in Tris–HCl buffer (100 mM, pH 7.5) with 10 mM DL-*threo*-phenylserine and DL-*erythro*-phenylserine. The mixture was shaken at 30 °C for 12 h, and dehydration activity was measured by 2,4-dinitrophenylhydrazine (DNPH) alkaline method[14] and HPLC.

Genomic DNA from *Paraburkholderia xenovorans* LB400 (DSM 17367) was prepared by using the TianGen Bacteria DNA Kit (TianGen Biotech). The gene encoding L-PxPSDH was amplified by PCR in a 50 μL reaction mixture, containing 50 ng genomic DNA, 1.0 mM $MgSO_4$, 0.2 mM dNTPs, 0.2 mM of each primer (5′-GGAATTCCATATGTCAACTGCCGCGCCGCAA-3′ and 5′-CCCAAGCTTTTATTGAGGAAACAGTGACTCGCTATGCCG-3′) and 1 U of KOD plus polymerase. The PCR product was digested with *Nde*I and *Xho*I and ligated into pET-28a. The recombinant plasmid was transformed into *E. coli* BL21 (DE3). L-PxPSDH was grown in LB medium containing 50 μg/mL kanamycin and expressed as previously described.

Purification of L-PxPSDH and L-PsTA

Cell pellets of L-PxPSDH and L-PsTA (800 mL culture) were re-suspended in 40 mL Tris-HCl buffer (100 mM, pH 7.5) containing 100 mM NaCl and disrupted by high-pressure homogenizer. The cell lysate was centrifuged at 12000 ×*g* for 30 min,

filtered through a 0.45-μm filter, and purified by Ni affinity chromatography with ÄKTA purifier at 4°C. The column was equilibrated with Tris-HCl buffer (100 mM, pH 7.5) containing 100 mM NaCl and 20 mM imidazole. The target protein was eluted with a linear gradient of Tris-HCl buffer (100 mM, pH 7.5) containing 100 mM NaCl and 500 mM imidazole. Enzyme fractions were analyzed by SDS-PAGE (Figure S2). The collected fractions were dialyzed and concentrated by ultrafiltration device (Amicon Ultra-15 30-kDa, Millipore) and stored in 10% glycerol at -80°C.

Determination of Stereospecificity of L-PxPSDH

The stereoselectivity of the enzymatic dehydration reaction was carried out as follows: 20 mM DL-*threo*-phenylserine or 20 mM DL-*erythro*-phenylserine, 0.5 mg/mL purified L-PxPSDH were added in Tris-HCl buffer (100 mM, pH 7.5). The mixture was shaken at 30°C for 12 h. The substrate profiling of L-PxPSDH was performed as follows: 0.5 mg/mL purified enzyme in Tris-HCl buffer (100 mM, pH 7.5) with 20 mM DL-phenylserine derivatives at 30°C for 4 h. Chiral analysis was performed by HPLC after derivatization with OPA/NAC (Table S2).

Detection of Activity and Stereoselectivity Using DNPH Assay

In the screening process two target compounds, benzaldehyde and phenylpyruvic acid, were tested by DNPH alkaline method to quantitatively determine carbonyl group.[15] 50 μL of benzaldehyde or phenylpyruvic acid test solution was mixed with an equal volume of 2 mM DNPH solution dissolved in 1 M HCl. After incubation for 30 min at room temperature (r.t.), 100 μL of 2 M NaOH (containing 5 g/L of Triton X-100 for benzaldehyde) was added. Absorbance was recorded at 470 nm and 440 nm for benzaldehyde and phenylpyruvic acid, respectively.

The aldol condensation reactions were performed with 10 mM benzaldehyde, 100 mM glycine, 10 μM PLP, 10% (v/v) DMSO and 0.5 mg/mL purified L-PsTA in Tris-HCl buffer (100 mM, pH 7.5) at 25°C for 12 h. The control experiments without enzyme were performed simultaneously. Benzaldehyde was measured by DNPH alkaline method after 25 μL of the mixture was diluted into 975 μL of Tris-HCl buffer. After boiling at 100°C for 15 min, the reaction solution was extracted with 1 mL dichloromethane to remove remaining benzaldehyde. 0.5 mg/mL purified L-PxPSDH was added to the aqueous phase. The mixture was incubated at 30°C for 4 h and the formed phenylpyruvate was detected by the DNPH alkaline method. Additionally, aldol condensation products were analyzed by HPLC after OPA/NAC derivatization.

Optimization of Colorimetric Method and Whole Cell Biotransformation

To simplify the steps of dilution and extraction, 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (purpald) was tested for the determination of benzaldehyde[16] and Fe^{3+} colorimetric method was used for the determination of phenylpyruvate.[17] The assays were performed in microtiter plate in the total volume of 200 μL.

Purpald method: 100 μL of benzaldehyde test solution was mixed with an equal volume of 80 mM purpald in 1 M NaOH. Absorbance was read at 540 nm after 30 min in the presence of air. The benzaldehyde testing solution with phenylserine and glycine were studied to check for possible interference for the above two methods.

Fe^{3+} colorimetric method: the Fe^{3+} chromogenic agent (100 mL) contained 0.05 g ferric chloride, 2 mL acetic acid and 60 mL DMSO. 40 μL of phenylpyruvate test solution in Tris-HCl buffer (100 mM, pH 7.5) was mixed with 160 μL of chromogenic agent. Absorbance was measured at 640 nm after incubation for 30 min at r.t. The phenylpyruvic acid testing solution with phenylserine, benzaldehyde, glycine and ammonium salt were studied to check for their possible interference.

To further simplify the screening process, we attempted to use whole cell biotransformation instead of purified enzyme. Eighteen soluble L-TAs were used for further testing. The pellet re-suspended in Tris-HCl buffer (100 mM, pH 7.5) with 100 mM glycine, 10 mM benzaldehyde, 10 μM PLP and 10% DMSO (v/v) in a final volume of 1 mL. The biotransformations were performed at 25°C for 12 h. After centrifugation, 100 μL of the mixture was withdrawn and measured by purpald method. The 900 μL remaining reaction solution was transferred to other tubes with the pellets of L-PxPSDH. The dehydration reaction was carried out at 30°C for 4 h. After centrifugation, reaction solution was detected by Fe^{3+} colorimetric method.

Enzyme-Substrate Docking

A homology model of L-PsTA was constructed based on the known crystal structure of L-PpTA from *P. putida* (PDB: 1V72) using Discovery Studio 4.1 (Accelrys). Co-factor PLP was copied from the template during the modeling. The model structure was further evaluated using Ramachandran plot. The receptor proteins were prepared by eliminating all bound water molecules, and hydrogen atoms were added to the enzyme molecule. The substrates molecules were optimized by applying the force field CHARMm. The docking were performed by CDOCKER with the default settings using phenylserine as substrate. Based on the interaction at the active site of enzymes, the related residues surrounding the binding pocket were selected.

The structural models of mutant D93H/E147D and D93N/E147D were also constructed based on the known crystal structure (PDB: 1V72) as same as wild-type enzyme L-PsTA. The substrate molecules were docked into wild-type enzyme and mutants by using flexible docking. The poses were analyzed and ranked according to the binding model of the PLP part and score functions.

Mutant Library Construction

The site-saturation mutagenesis of ten amino acid residues (S10, N12, Y35, H89, D93, H133, E147, R177, K207 and R321) lining the substrate-binding pocket was performed and then the sites extended to another eighteen amino acid residues (Q7, A9, D11, Y13, G15, A34, G36, V90, E91, T92, D131, P135, P137, A145, G149, A176, T206 and M323) (Table S3). The combinatorial active-site saturation libraries (S10/N12, A34/Y35, H89/D93, I132/H133, T146/E147, A176/R177, T206/K207, R321/M323) and combining saturation libraries (D93/E147, E147/R321 and R177/R321) were constructed (Table S4). Mutant libraries were constructed using overlap PCR and mega-primer PCR approach and the primers used are listed in supporting information. Single or combined sites used NNK degeneracy or NDT degeneracy, respectively.

50 μL reaction mixtures typically contained 50 ng plasmid, 1.0 mM $MgSO_4$, 0.2 mM dNTP, 5 μL polymerase buffer (10×), 0.2 mM of each primer and 1 U of KOD plus polymerase. The PCR was carried out under the following conditions: for mega-primer: 95°C 2 min, (95°C 30 sec, 58°C 30 sec, 58°C 30 sec) × 30 cycles, 68°C 5 min. For mega-PCR: 95°C 2 min, (95°C 30 sec, 58°C 30 sec, 68°C 7 min) × 24 cycles, 68°C 10 min. The PCR products were analyzed by agarose gel electrophoresis and digested with *Dpn* I restriction enzyme at 37°C for 4 h. The PCR products were purified and transformed into *E. coli* BL21(DE3).

Screening Procedures

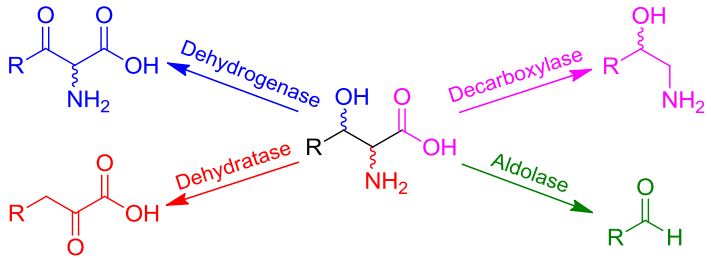
Colonies were picked up into 96 deep-well plates containing 500 μL LB medium with 100 μg/mL ampicillin and incubated at 37°C for 12 h with shaking. 20 μL overnight culture was diluted into 1 mL of fresh LB medium containing 100 μg/mL

ampicillin and incubated at 37°C until the OD_{600} reached 1.0. After induction by 0.2 mM IPTG at 30°C overnight, the cells were harvested by centrifugation (4000× *g*, 15 min). For storage at -80ºC, glycerol (final concentration of 10%) was added to each seed plate. The pellet re-suspended in Tris-HCl buffer (100 mM, pH 7.5) with 100 mM glycine, 10 mM aldehyde substrates and 10% DMSO (v/v) in a final volume of 600 μL. The biotransformations were performed at 25°C for 12 h. After centrifugation (4000× *g*, 15 min), 100 μL of the mixture was withdrawn and measured by purpald method. The 500 μL remaining reaction solution was transferred to new 96 deep-well plates with the pellets of L-PxPSDH and performed at 30°C for 4 h. After centrifugation (4000× *g*, 15 min), 40 μL of the mixture was withdrawn and detected by Fe^{3+} colorimetric method. The colonies, which showed higher activity or stereospecificity than the wild-type enzyme, were subjected to further study.

Activity and Stereoselectivity of Wild-Type and Mutant Enzymes

Wild-type and mutant enzymes were expressed and purified by following the procedures above (Figure S7). The activity and stereoselectivity of beneficial mutants were studied by using different aldehyde substrates and glycine. The general procedures was carried out as follows: aldehyde substrates (100 mM), glycine (1 M), PLP (10 μM), 10% DMSO (v/v) and purified mutants were mixed in Tris-HCl buffer (100 mM, pH 7.5) in a final volume of 1 mL, and the reaction was performed at 25°C for 12 h. The reaction mixture was diluted with 9 mL MeOH and stored at 4°C overnight. The resulting solution was subjected to HPLC analysis after derivatization with OPA/NAC to determine conversion an de value (Figure S8).

Supporting Scheme



Scheme S1. Enzymes involved in the transformations of β-hydroxy-α-amino acid

Supporting Tables

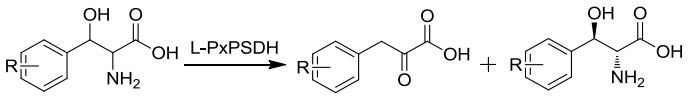
Table S1. Source of L-threonine aldolases

No.	Organism	Enzyme	GenBank Accession	Reference or source
1	*Aeromonas jandaei*	L-AjTA[a]	O07051.1	5
2	*Brevibacillus massiliensis*	L-BmTA[a]	WP_019119750	This study
3	*Burkholderia* sp.	L-BsTA[a]	WP_008921553	This study
4	*Caloramator australicus*	L-CaTA[a]	WP_008908414	This study
5	*Deinococcus deserti*	L-DdTA[a]	WP_012692929	This study
6	*Escherichia coli*	L-EcTA[b]	BAA20882	6
7	*Glutamicibacter arilaitensis*	L-GaTA[a]	WP_013349325	This study
8	*Geobacter sulfurreducens*	L-GsTA[a]	WP_010943783	7
9	*Hoeflea phototrophica*	L-HpTA[a]	WP_007198890	This study
10	*Pseudomonas aeruginosa*	L-PaTA[b]	AAG08798	8
11	*Pseudomonas putida*	L-PpTA[a]	BAD91544	9
12	*Pseudomonas* sp.	L-PsTA[a]	BAA24794	10
13	*Raoultella ornithinolytica*	L-RoTA[a]	WP_015584808	7
14	*Saccharomyces cerevisiae*	L-ScTA[a]	AAA72430	11
15	*Streptomyces griseus*	L-SgTA[a]	BAG23347	This study
16	*Shewanella loihica*	L-SlTA[b]	ABO23025	7
17	*Spirulina subsalsa*	L-SsTA[a]	WP_017307090	This study
18	*Thermoplasmatales archaeon*	L-TaTA[b]	WP_004556422	This study
19	*Trichodesmium erythraeum*	L-TeTA[a]	WP_011611295	This study
20	*Thermotoga maritime*	L-TmTA[a]	AAD36809	12

[a]Synthetic gene was was provided by the Tianjin Institute of Industrial Biotechnology of Chinese Academy of Sciences.

[b]Encoding gene cloned by PCR.

Table S2. Substrate profiling of L-PxPSDH[a]



Entry	X	Conv. (%)	ee (%)	Entry	X	Conv. (%)	ee (%)
1	*o*-F	50	>99	6	*p*-Cl	50	>99
2	*m*-F	38	61	7	*o*-Br	50	>99
3	*p*-F	50	>99	8	*m*-Br	37	59
4	*o*-Cl	50	>99	9	*p*-Br	50	>99
5	*m*-Cl	44	79				

[a]Reaction conditions: 0.5 mg/mL purified enzyme in Tris-HCl buffer (100 mM, pH 7.5) with 20 mM DL-*threo/erythro*-phenylserine derivatives at 30°C for 4 h.

Table S3. Primers used in site saturation mutagenesis

Name	Primer
Sec-1-NNK-R	ACGGTAAACAGAACCAACTTCGGTAGCCTGGGTGATG
Q7-NNK-F	ATGACCGACCAGTCTCAGNNKTTCGCTTCTGACAACTAC
A9-NNK-F	ACCAGTCTCAGCAGTTCNNKTCTGACAACTACTCTGG
S10-NNK-F	CAGTCTCAGCAGTTCGCTNNKGACAACTACTCTGGTATCTGCC
D11-NNK-F	CAGTTCGCTTCTNNKAACTACTCTGGTATCTGCCC
N12-NNK-F	GTCTCAGCAGTTCGCTTCTGACNNKTACTCTGGTATCTGCC
Y13-NNK-F	CAGTTCGCTTCTGACAACNNKTCTGGTATCTGCCCG
G15-NNK-F	TTCTGACAACTACTCTNNKATCTGCCCGGAAGCTTGG
A34-NNK-F	ACCACGGTCACGAACGTNNKTACGGTGACGACCAGTGGACCG
Y35-NNK-F	CCACGGTCACGAACGTGCTNNKGGTGACGACCAGTGGACCG
G36-NNK-F	TCACGAACGTGCTTACNNKGACGACCAGTGGACCGCT
Sec-2-NNK-R	GTAGTCGAAGTCTTCAGCCAGTTTACGGTTGAAGAACAG
H89-NNK-F	GAAACCGCTNNKGTTGAAACCGACGAATGCGGTGCTC
V90-NNK-F	TGCTCTGAAACCGCTCACNNKGAAACCGACGAATG
E91-NNK-F	TCTGAAACCGCTCACGTTNNKACCGACGAATGC
T92-NNK-F	GAAACCGCTCACGTTGAANNKGACGAATGCGGTGCT
D93-NNK-F	GCTCACGTTGAAACCNNKGAATGCGGTGCTCCG
D131-NNK-F	GTCAGNNKATCCACTACCCGAAACCGCGTGTTGTTAC
H133-NNK-F	CTGAAACGTCAGGACATCNNKTACCCGAAACCGCGTG
P135-NNK-F	GTCAGGACATCCACTACNNKAAACCGCGTGTTGTTACC
P137-NNK-F	GACATCCACTACCCGAAANNKCGTGTTGTTACCAT
A145-NNK-F	TGTTACCATCACCCAGNNKACCGAAGTTGGTTCTGTT
E147-NNK-F	CCATCACCCAGGCTACCNNKGTTGGTTCTGTTTAC
G149-NNK-F	CCAGGCTACCGAAGTTNNKTCTGTTTACCGTCCGGAC
Sec-3-NNK-R	GTCCCAAGAGCACATGAAACGAGCACCACCAGAACCGATGA
A176-NNK-F	ACCTGCACATGGACGGTNNKCGTTTCTCTAACGCTTGCGCTT
R177-NNK-F	ACCTGCACATGGACGGTGCTNNKTTCTCTAACGCTTGCGCTT
T206-NNK-F	GTTCTGTGCTTCGGTGGTNNKAAAAACGGTATGGCTGTTGGT
K207-NNK-F	GTTCTGTGCTTCGGTGGTACCNNKAACGGTATGGCTGTTGGT
Sec-4-NNK-F	TGCACATGGACGGTGCTCGTTTCTCTAACGCTTGCGCTT
R321-NNK-R	TCCCAAGAGCACATGAAMNNAGCACCACCAGAACCGAT
M323-NNK-R	TCCCAAGAGCAMNNGAAACGAGCACCACCAGAACCGAT

Table S4. Primers used in saturation mutagenesis

Name	Primer
S10/N12-F	AGTCTCAGCAGTTCGCTNDTGACNDTTACTCTGGTATCTGCCCG
S10/N12-R	ACCACCTTCAGAACGAGCGGTCAGCAGTTTAGAACCGTTAGAGAAG
A34/Y35-F	ACCACGGTCACGAACGTNDTNDTGGTGACGACCAGTGGACCGCT
A34/Y35-R	TCGGTAGCCTGGGTGATGGTAACAACACGCGGTTTCGGGTAGTG
H89/D93-F	TGCTCTGAAACCGCTNDTGTTGAAACCNDTGAATGCGGTGCT
H89/D93-R	CGAAAGCGCAAGCGTTAGAGAAACGAGCACCGTCCATG
I132/H133-F	CGTCAGGACNDTNDTTACCCGAAACCGCGTGTTGTTAC
I132/H133-R	CGAAAGCGCAAGCGTTAGAGAAACGAGCACCGTCCATG
T146/E147-F	CCATCACCCAGGCTNDTNDTGTTGGTTCTGTTTAC
T146/E147-R	CAACCGGGAACATCAGTTCAACACCCGGGATGTCAGCAACCAG
A176/R177-F	ACCTGCACATGGACGGTNDTNDTTTCTCTAACGCTTGCGCTT
A176/R177-R	CAACCGGGAACATCAGTTCAACACCCGGGATGTCAGCAACCAG
T206/K207-F	GTTCTGTGCTTCGGTGGTNDTNDTAACGGTATGGCTGTTGGTGA
T206/K207-R	GTCCCAAGAGCACATGAAACGAGCACCACCAGAACCGATGAAG
R321/M323-F	TGCACATGGACGGTGCTCGTTTCTCTAACGCTTGCGCTT
R321/M323-R	TCCCAAGAGCAAHNGAAAHNAGCACCACCAGAACCGAT
D93/E147-F	CTCACGTTGAAACCNDTGAATGCGGTGCTCCGGA
D93/E147-R	ACGGTAAACAGAACCAACAHNGGTAGCCTGGGTGATGGT
E147/R321 -F	CCATCACCCAGGCTACCNDTGTTGGTTCTGTTTAC
E147/R321-R	TCCCAAGAGCACATGAAAHNAGCACCACCAGAACCGAT
R177/R321-F	TGCACATGGACGGTGCTNDTTTCTCTAACGCTTGCGCTT
R177/R321- R	TCCCAAGAGCACATGAAAHNAGCACCACCAGAACCGAT

Supporting Figures

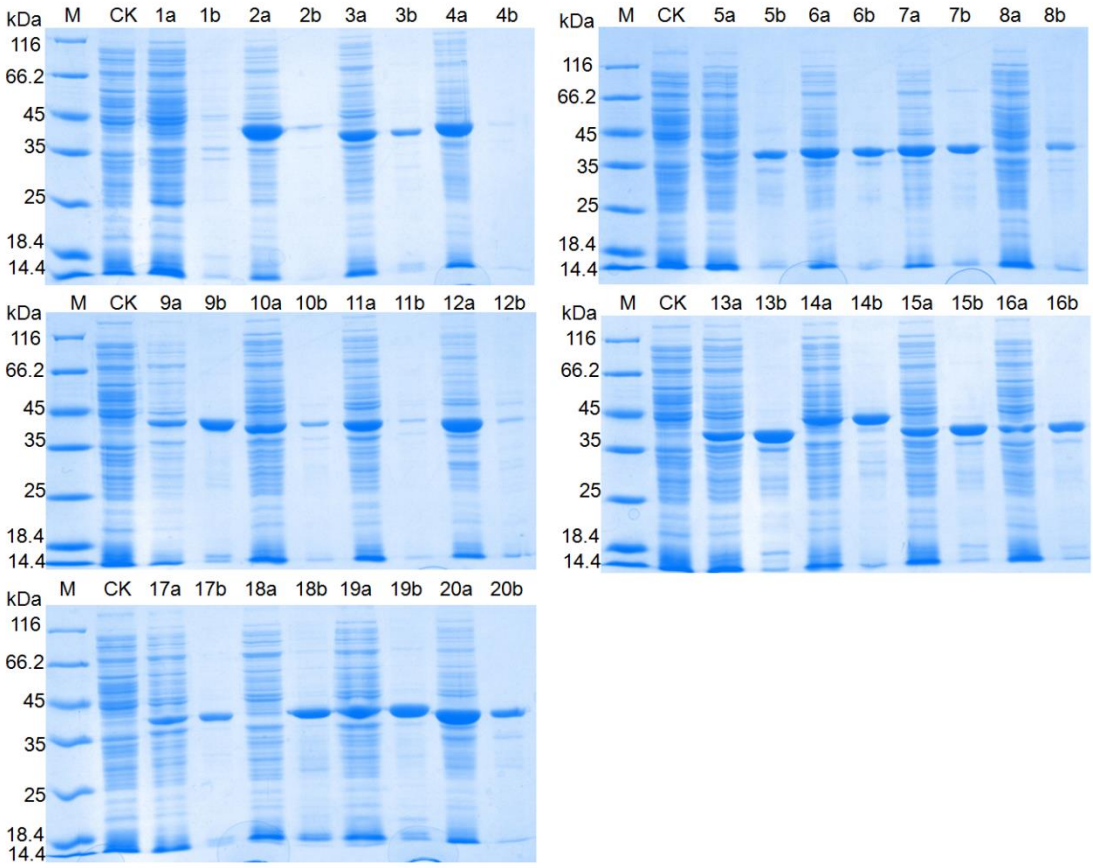


Figure S1. SDS-PAGE analysis of L-TAs. M: protein marker; CK: control check (non-induced whole cell extract); **a**: supernatant of the lysate; **b**: precipitate of the lysate. **1** L-AjTA, **2** L-BmTA, **3** L-BsTA, **4** L-CaTA, **5** L-DdTA, **6** L-EcTA, **7** L-GaTA, **8** L-GsTA, **9** L-HpTA, **10** L-PaTA, **11** L-PpTA, **12** L-PsTA, **13** L-RoTA, **14** L-ScTA, **15** L-SgTA, **16** L-SlTA, **17** L-SsTA, **18** L-TaTA, **19** L-TeTA, **20** L-TmTA.

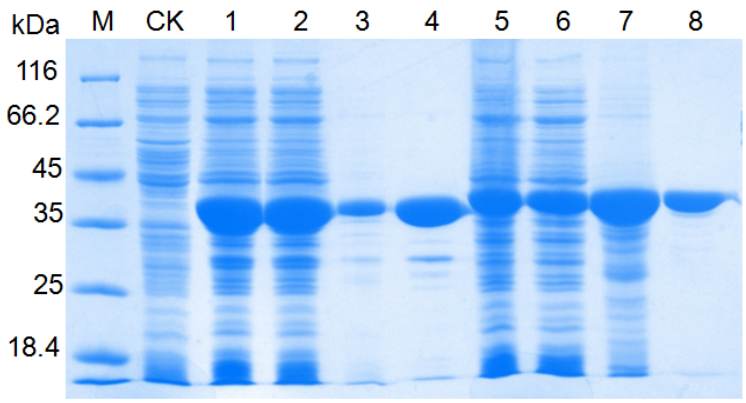


Figure S2. SDS-PAGE analysis of L-PsTA and L-PxPSDH. Lane M: protein marker; Lane CK: control check (non-induced whole cell extract); Lane 1-4, whole cell extract, supernatant, precipitate and purified enzyme of L-PsTA, respectively; Lane 5-8, whole cell extract, supernatant, precipitate and purified enzyme of PSDH, respectively.

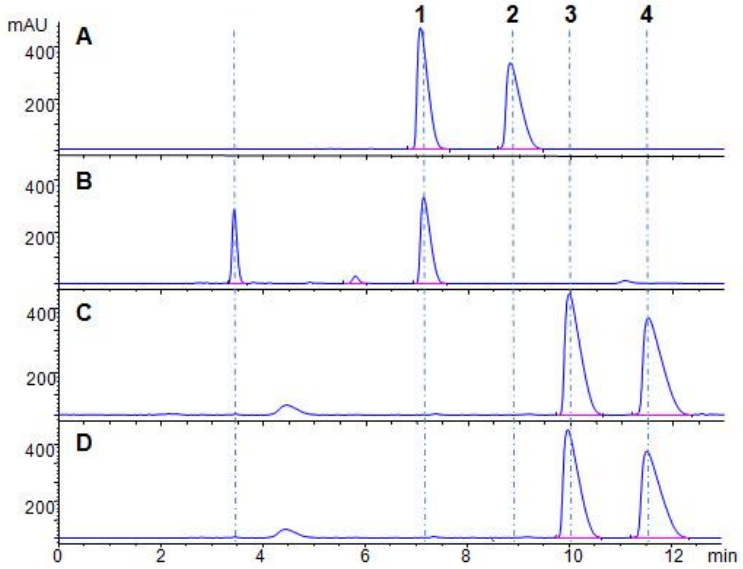


Figure S3. Determination of stereospecificity of PSDH by HPLC chromatography after derivatization with OPA/NAC. D-*threo*-phenylserine **1**, L-*threo*-phenylserine **2**, D-*erythro*-phenylserine **3**, L-*erythro*-phenylserine **4**. (A), (C) The control group of DL-*threo*-phenylserine or DL-*erythro*-phenylserine. (B), (D) The experimental group of DL-*threo*-phenylserine or DL-*erythro*-phenylserine with L-PxPSDH

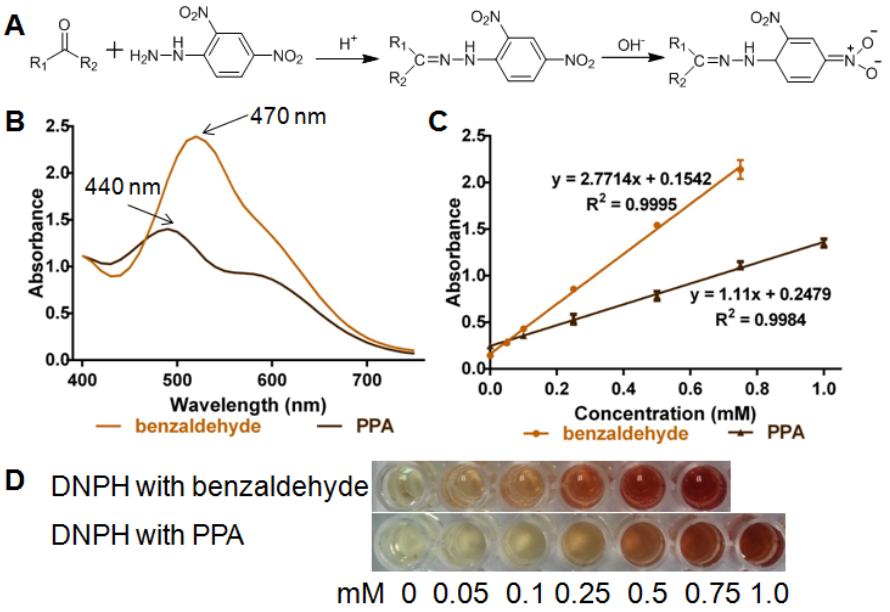


Figure S4. (A**)** Reactions of colorimetric assay with DNPH. **(**B**)** Multi-wavelength scanning. **(**C**)** Calibration curve. **(**D**)** Photos of products produced from substrates at different concentrations with derivatives.

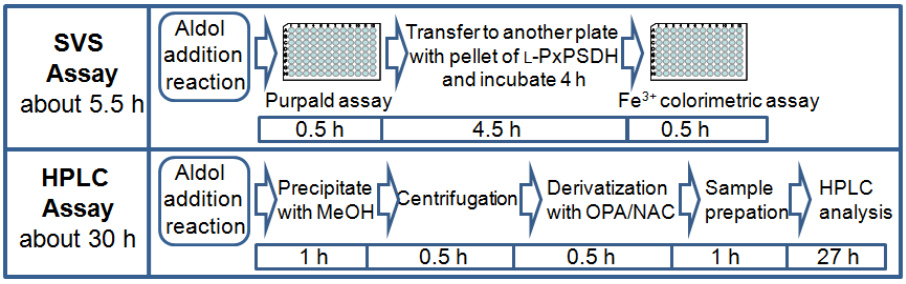


Figure S5. Comparative processing time between the SVS assay and HPLC assay

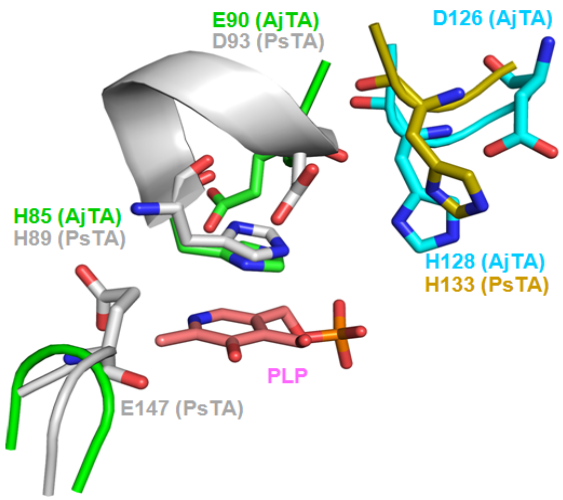


Figure S6. Superposition of the active sites of L-PsTA (amino acid residues from A monomer are shown as white; amino acid residues from B monomer are shown as yellow) and L-TA from *A. jandaei* (amino acid residues from A monomer are shown as green; amino acid residues from B monomer are shown as cyan).

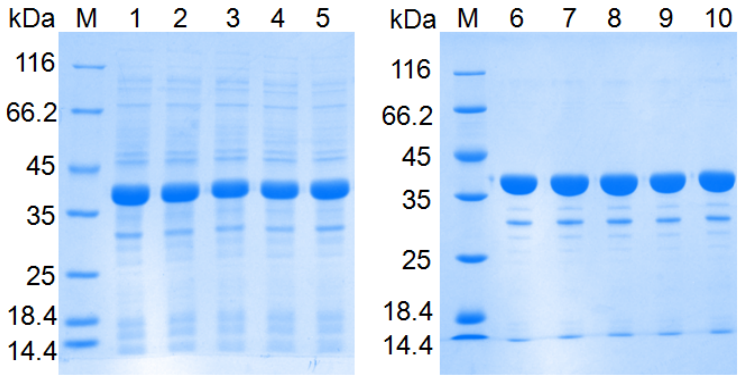


Figure S7. SDS-PAGE analysis of L-PsTA and mutants. M: protein marker; Lane 1-5, whole cell extract of WT L-PsTA, E147D, D93F/E147D, D93H/E147D and D93N/E147D, respectively. Lane 6-10, purified enzyme of WT L-PsTA, E147D, D93F/E147D, D93H/E147D and D93N/E147D, respectively.

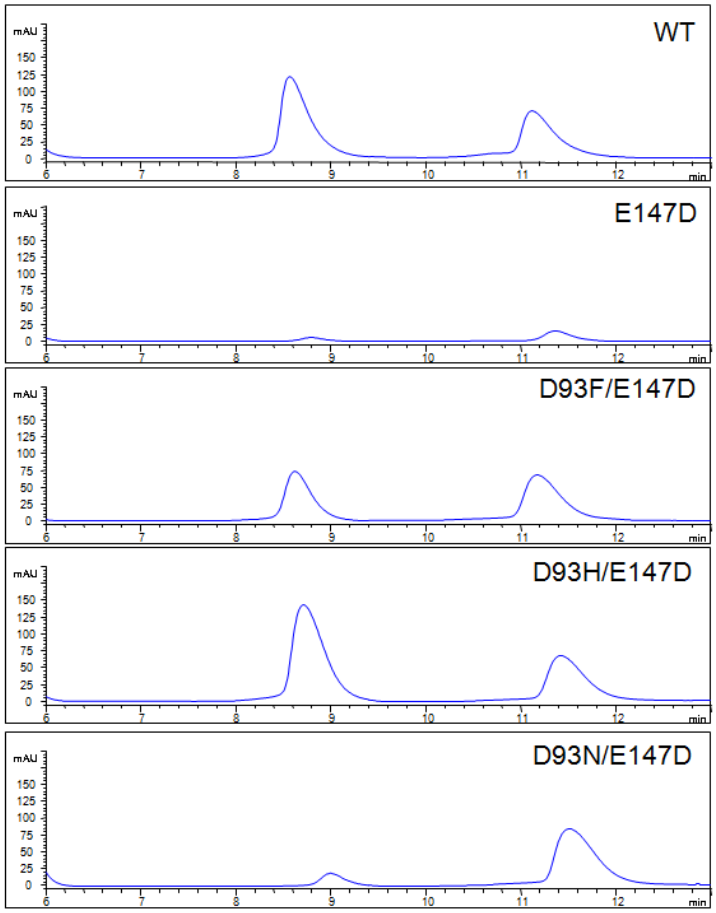


Figure S8. HPLC spectra of aldol condensation catalyzed by L-PsTA and mutants. L-Phenylserine ($t_{\text{L-}threo}$=8.7 min, $t_{\text{L-}erythro}$=8.7 min).

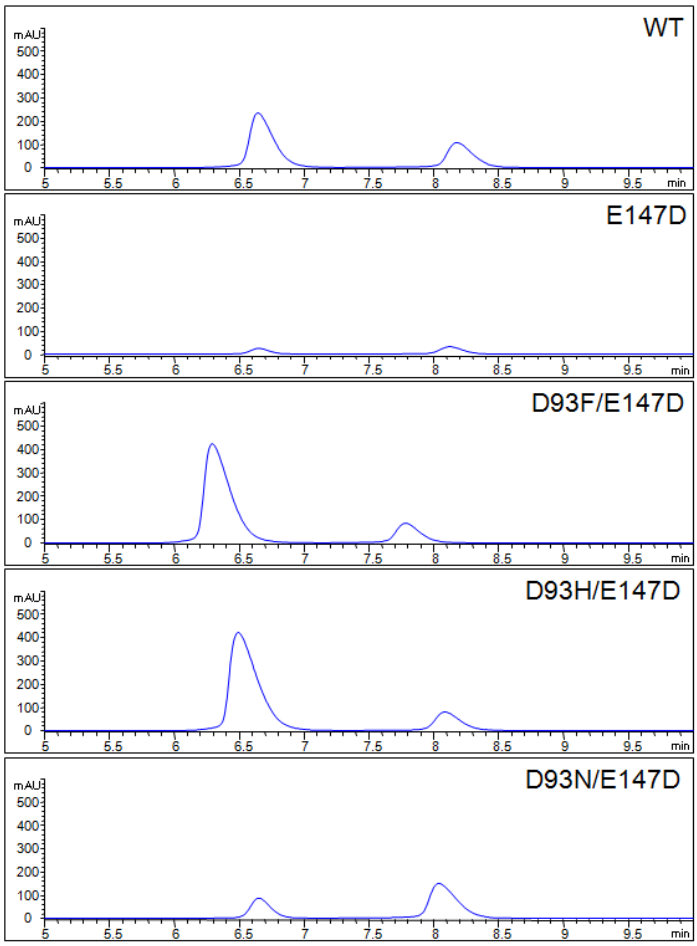


Figure S9. HPLC spectra of aldol condensation catalyzed by L-PsTA and mutants. L-*o*-Fluorophenylserine ($t_{\text{L-}threo}$=6.7 min, $t_{\text{L-}erythro}$=8.2 min).

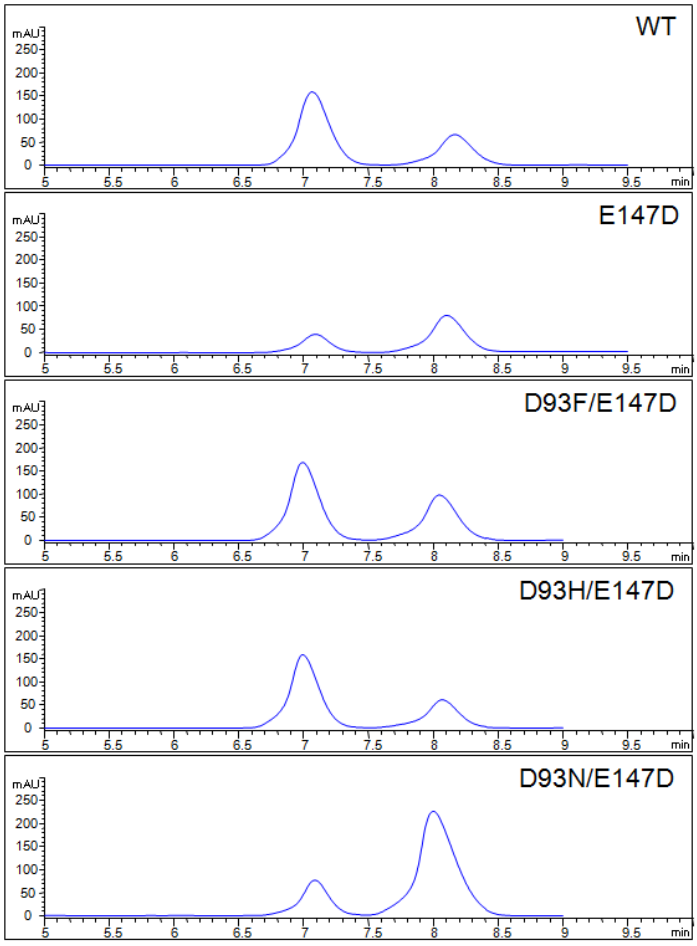


Figure S10. HPLC spectra of aldol condensation catalyzed by L-PsTA and mutants. L-*m*-Fluorophenylserine ($t_{L\text{-}threo}$=7.1 min, $t_{L\text{-}erythro}$=8.1 min).

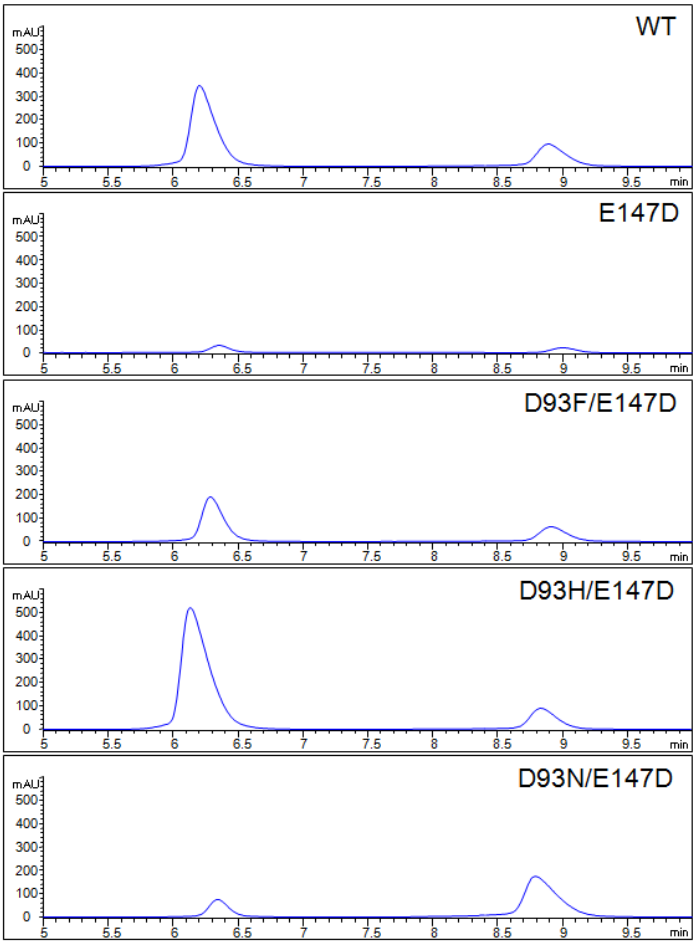


Figure S11. HPLC spectra of aldol condensation catalyzed by L-PsTA and mutants. L-*o*-Chlorophenylserine ($t_{L\text{-}threo}$=6.3 min, $t_{L\text{-}erythro}$=8.9 min).

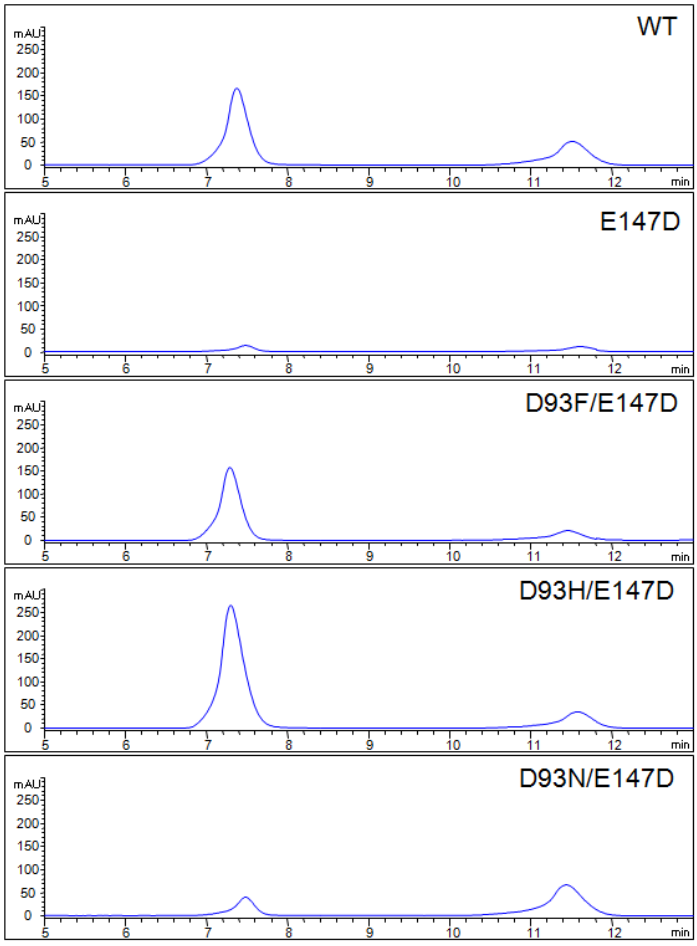


Figure S12. HPLC spectra of aldol condensation catalyzed by L-PsTA and mutants. L-*o*-Bromophenylserine ($t_{L\text{-}threo}$=7.4 min, $t_{L\text{-}erythro}$=11.5 min).

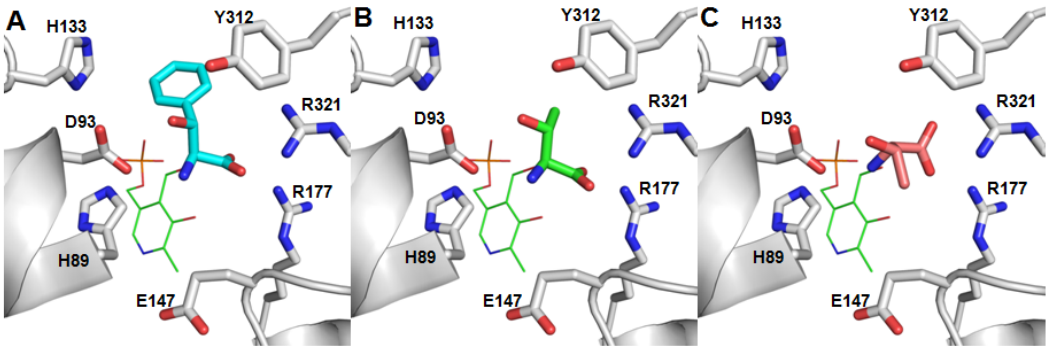


Figure S13. Catalytically active conformations of wild-type enzyme complexed with (A) L-*threo*-phenylserine (cyan), (B) L-threonine (green) and (C) L-*allo*-threonine (salmon). PLP is shown as green in lines.

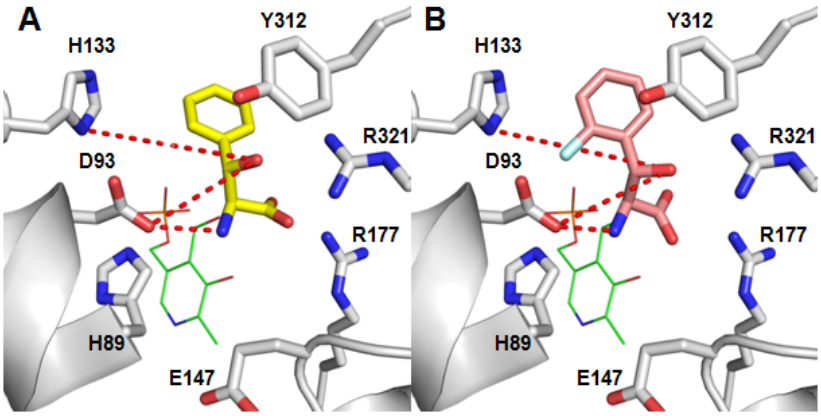


Figure S14. Flexible docking into the active sites of wild-type enzymes. (A) and (B) Catalytically active conformations of wild-type enzyme complexed with L-*erythro*-phenylserine (yellow) and L-*erythro*-*o*-fluorophenylserine (salmon), respectively. PLP is shown as green in lines.

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