Supporting Information

Highly selective synthesis of D-amino acids from readily available L-amino acids by one-pot biocatalytic stereoinverting cascade

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Effects of biocatalyst form and reaction conditions on PmLAAD catalyzing oxidative deamination of L-Phe

L-Phe (50 mM) was added into 2 mL Tris-HCl buffer (50 mM, pH 7.5), and the recombinant *E. coli* BL21(DE3)/pET-28a(+)-PmLAAD wet cells (100 mg/mL), the soluble fraction from recombinant *E. coli* BL21(DE3)/pET-28a(+)-PmLAAD wet cells (100 mg/mL) lysate, and the insoluble fraction from recombinant *E. coli* BL21(DE3)/pET-28a(+)-PmLAAD wet cells (100 mg/mL) lysate were used as the biocatalyst, respectively. Then the reactions were carried out at 220 rpm and 37 °C for 30 min. To investigate the effect of reaction temperature on PmLAAD whole cells catalyzing oxidative deamination of L-Phe, L-Phe (50 mM) and PmLAAD whole cells (100 mg/mL) were added into 2 mL Tris-HCl buffer (50 mM, pH 7.5), and the reaction was carried out at 220 rpm and different temperatures (30, 37, 45, 50, 55, and 60 °C) for 30 min. To investigate the effect of initial pH value on PmLAAD whole cells catalyzing oxidative deamination of L-Phe, L-Phe (50 mM) and PmLAAD whole cells (100 mg/mL) were added into 2 mL Tris-HCl buffer (50 mM, pH 7-10) or Na_2CO_3-$NaHCO_3$ buffer (50 mM, pH 8.5-10), and the reaction was carried out at 220 rpm and 45 °C for 30 min. To investigate the effect of biocatalyst concentration on PmLAAD whole cells catalyzing oxidative deamination of L-Phe, L-Phe (50 mM) was added into 2 mL Tris-HCl buffer (50 mM, pH 9.0) with the concentration of PmLAAD whole cells varying from 10 to 200 mg/mL, and the reaction was carried out at 220 rpm and 45 °C for 30 min. The PPA generated in the reaction process was measured by using ferric chloride solution and the amount of L-Phe was determined by HPLC after FDAA derivation.

Effects of pH value and temperature on the activity of BsFDH

The activity assaying system for BsFDH consisted of 50 mM sodium formate, 2 mM $NADP^+$, and 50 mM Tris-HCl buffer (pH 7.5) at a final volume of 200 μL. After incubation, reactions were performed in 96-well plates using a Spectramax M2e microplate reader (Molecular Devices, USA) and initiated by the addition of a 20 μL enzyme solution. The OD_{340} was monitored at 30 °C using a molar extinction coefficient of 6.22 $mM^{-1} \cdot cm^{-1}$. The influences of pH on the activity of BsFDH were measured at various pH by using 50 mM Tris-HCl (pH 7.5-10) at 30 °C. The influences of temperature on the activity of BsFDH were measured at various temperature (30-45 °C) in 50 mM Tris-HCl (pH 9.0).

Supplementary Tables

Table S1. Effect of the PmLAAD biocatalyst form on the oxidative deamination of L-Phe

Biocatalyst form	Yield, %
Whole cells	91.7
Soluble fraction[a]	56.4
Insoluble fraction[b]	6.1
Purified protein	0

[a] 100 mg/mL PmLAAD wet cells were suspended in Tris-HCl buffer (50 mM, pH 7.5) and lysed by sonication, then the cell lysate was centrifuged at 12,000 × g for 20 min. The supernatant was used as the soluble fraction.

[b] 100 mg/mL PmLAAD wet cells were suspended in Tris-HCl buffer (50 mM, pH 7.5) and lysed by sonication, then the cell lysate was centrifuged at 12,000 × g for 20 min. The precipitate was resuspended in equal volume Tris-HCl buffer (50 mM, pH 7.5), which was used as the insoluble fraction.

Supplementary Figures

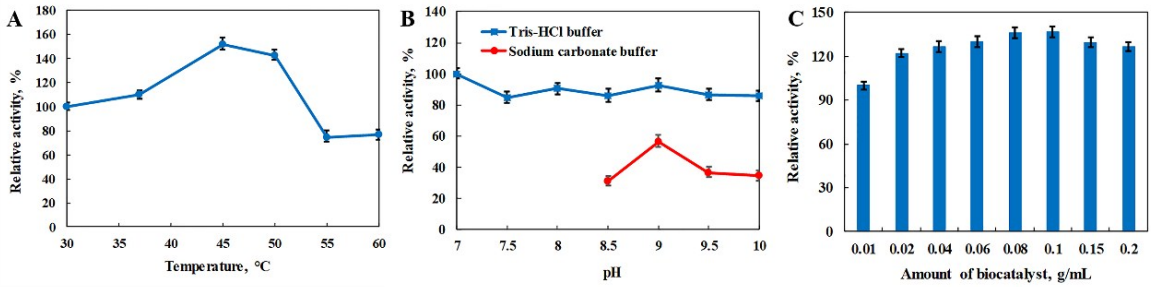


Figure S1. Effects of reaction conditions on PmLAAD whole-cell catalyzed oxidative deamination. (A) Effect of temperature on PmLAAD whole-cell catalyzed oxidative deamination. Reaction mixture in 2 mL Tris-HCl buffer (50 mM, pH 7.5) was comprised of 100 mg/mL PmLAAD wet cells and 50 mM L-Phe, and the reactions were carried out at 200 rpm for 30 min. (B) Effect of pH value on PmLAAD whole-cell catalyzed oxidative deamination. Reaction mixture in 2 mL Tris-HCl buffer (pH 7-10, 50 mM) or Na_2CO_3-$NaHCO_3$ buffer (pH 8.5-10, 50 mM) was comprised of 100 mg/mL PmLAAD wet cells and 50 mM L-Phe, and the reactions were carried out at 200 rpm and 45 °C for 30 min. (C) Effect of PmLAAD whole-cell concentration on the oxidative deamination. Reaction mixture in 2 mL Tris-HCl buffer (50 mM, pH 9.0) was comprised of 10-200 mg/mL PmLAAD wet cells and 50 mM L-Phe, and the reactions were carried out at 220 rpm and 45 °C for 30 min. The values were averaged from triplicate measurements and the standard deviations are indicated as error bars.

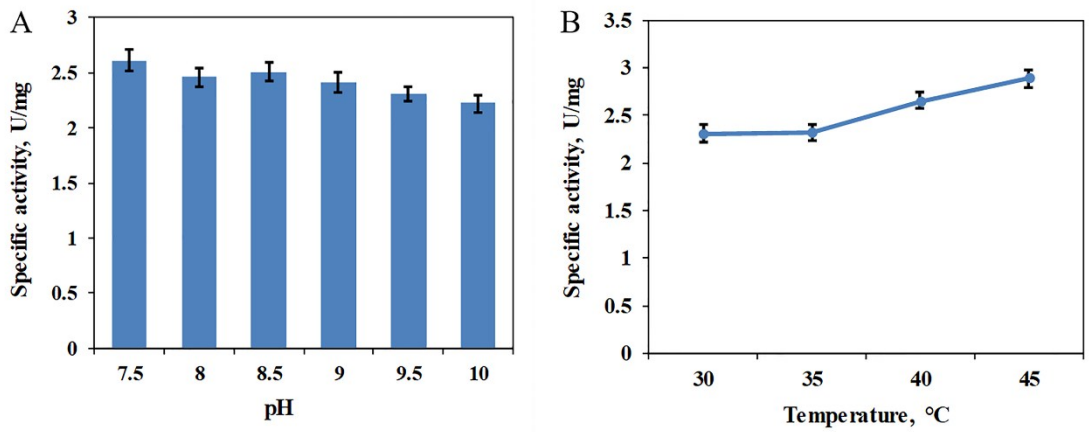


Figure S2. Effects of pH and temperature on the activity of BsFDH. (A): Effect of pH value on the specific activity of BsFDH. The reaction buffer was 50 mM Tris-HCl, pH 7.5-10. (B): Effect of temperature on the specific activity of BsFDH. The reaction temperature was 30-45 ℃, and the reaction buffer was Tris-HCl (50 mM, pH 9.0). The values were averaged from triplicate measurements and the standard deviations are indicated as error bars.

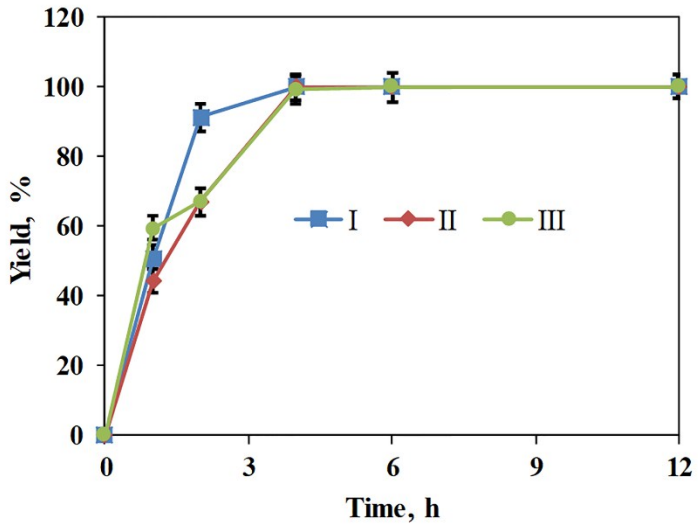


Figure S3. Time course for synthesis of D-Phe from PPA catalyzed by StDAPDH/H227V–BsFDH coupling system. (I) The reaction system contained 10 mM PPA, 20 mM NADPH, 30 mM NH_4Cl, and 4 mg/mL StDAPDH/H227V in Tris-HCl buffer (50 mM, pH 9.0); (II) The reaction system contained 10 mM PPA, 1 mM NADPH, 30 mM NH_4Cl, 20 mM soudim formate, 4 mg/mL StDAPDH/H227V, and 0.35 mg/mL BsFDH in Tris-HCl buffer (50 mM, pH 9.0); (III) The reaction system contained 10 mM PPA, 1 mM $NADP^+$, 30 mM NH_4Cl, 20 mM soudim formate, 4 mg/mL StDAPDH/H227V, and 0.35 mg/mL BsFDH in Tris-HCl buffer (50 mM, pH 9.0). All the reactions were carried out at 45 °C and 220 rpm. The values were averaged from triplicate measurements and the standard deviations are indicated as error bars.

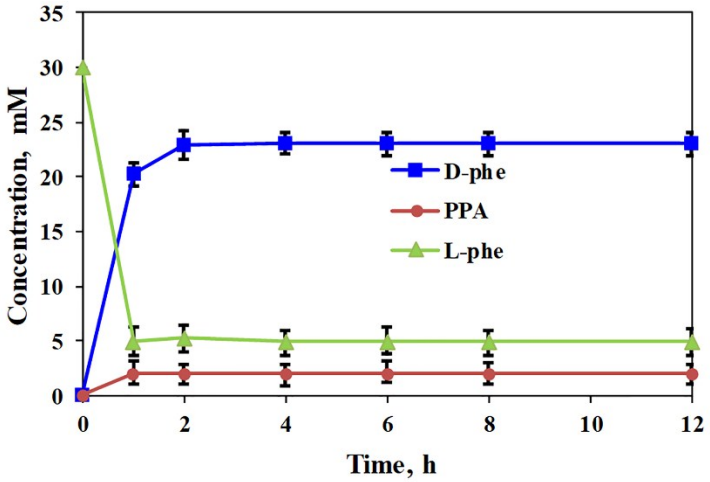
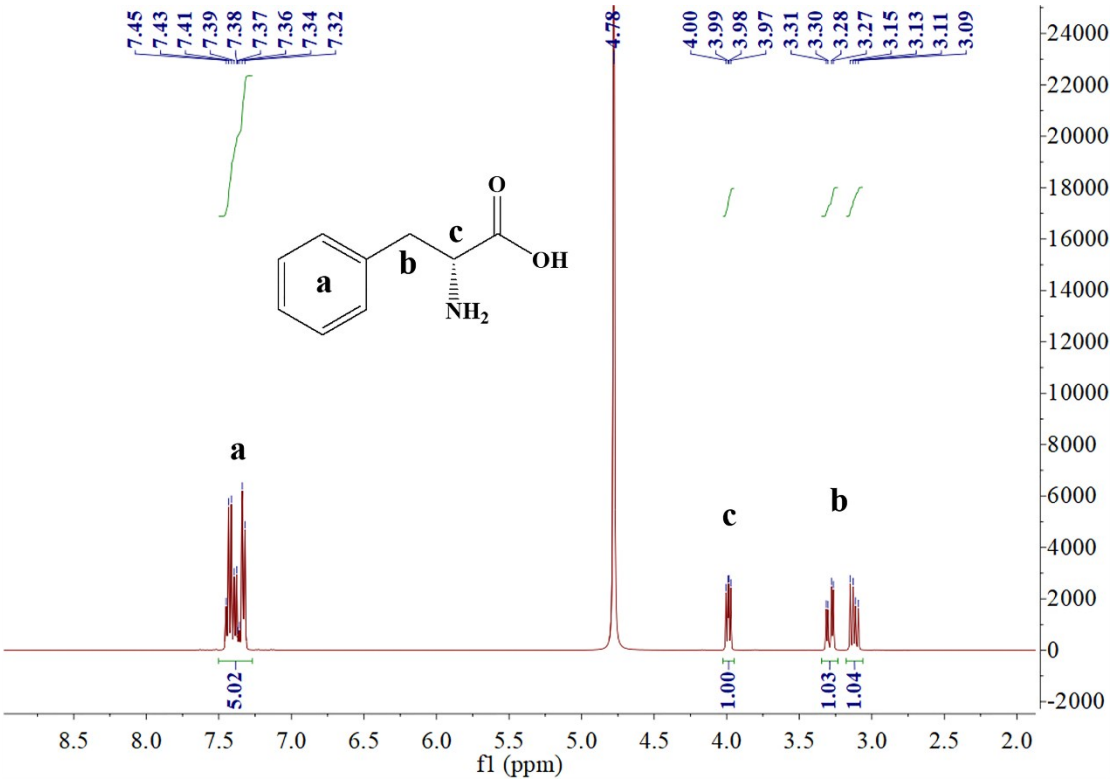


Figure S4. Time course for synthesis of D-Phe from L-Phe catalyzed by PmLAAD–StDAPDH/H227V–BsFDH cascade system. The reaction mixture contained 30 mM L-Phe, 90 mM NH_4Cl, 60 mM sodium formate, 3 mM $NADP^+$, 100 mg/mL PmLAAD whole-cell biocatalyst, 4 mg/mL StDAPDH/H227V, 0.35 mg/mL BsFDH, and Tris-HCl buffer (50 mM, pH 9.0). The reactions were carried out at 45 °C and 220 rpm. The values were averaged from triplicate measurements and the standard deviations are indicated as error bars.



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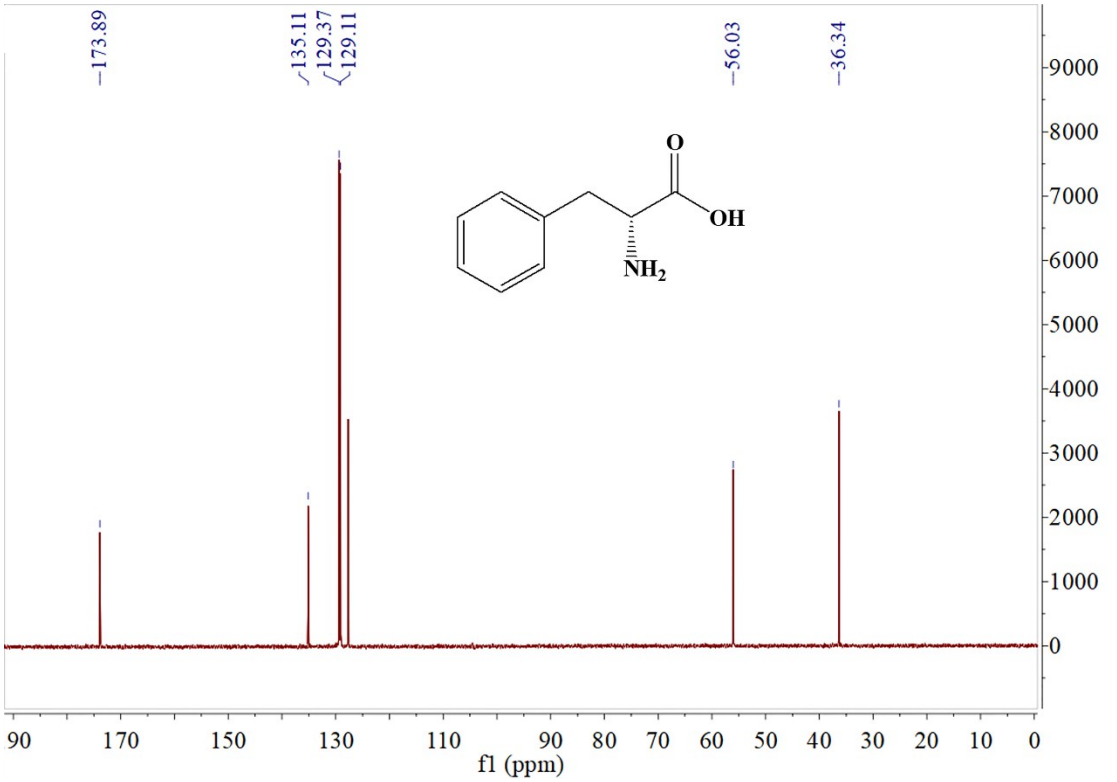
117 **Figure S5.** [1]H-NMR spectra of synthesized D-Phe by PmLAAD-StDAPDH/H227V-
118 BsFDH cascade system.



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120 **Figure S6.** [13]C-NMR spectra of synthesized D-Phe by PmLAAD-StDAPDH/H227V-
121 BsFDH cascade system.

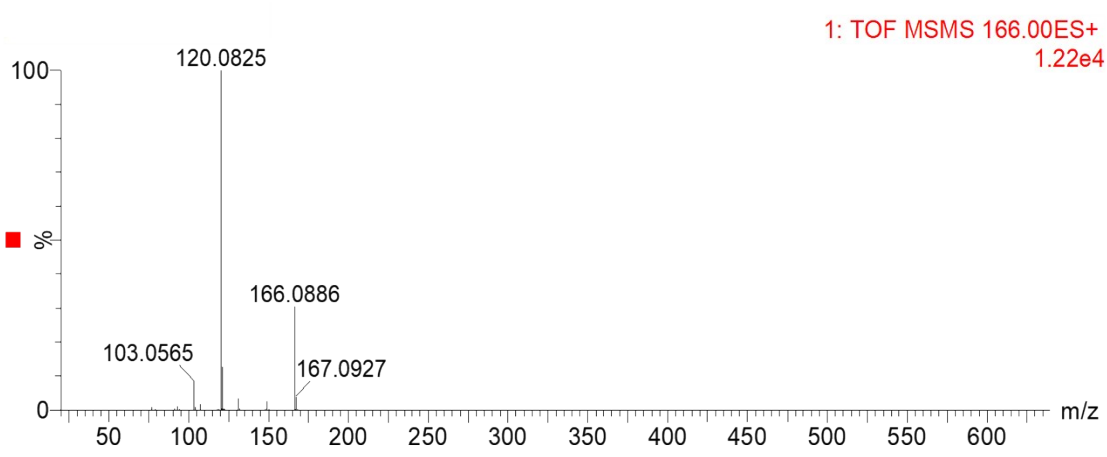


Figure S7. MS spectra of synthesized D-Phe by PmLAAD-StDAPDH/H227V-BsFDH cascade system.

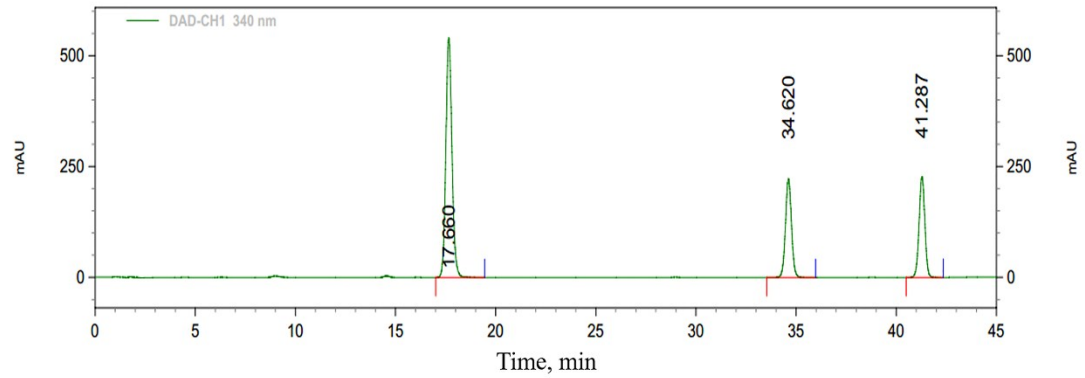


Figure S8. HPLC analysis of L-Phe and D-Phe. Retention time of L-Phe is 34.620 min; retention time of D-Phe is 41.287 min.

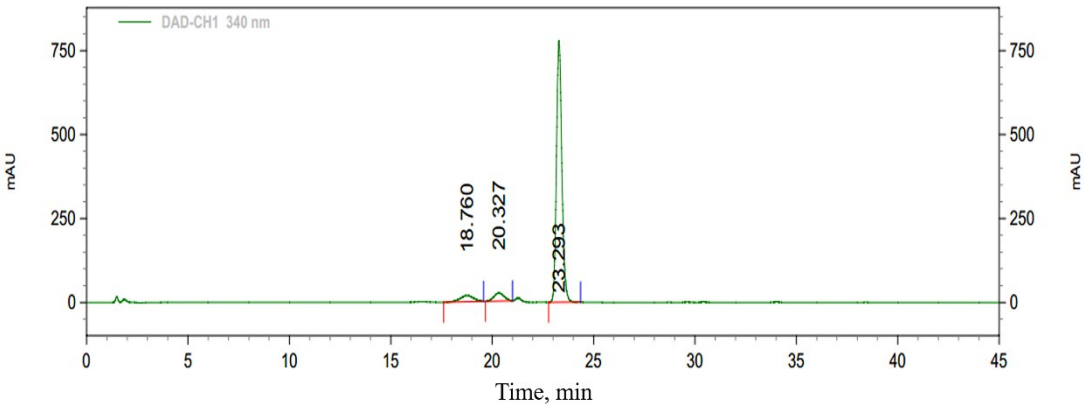


Figure S9. HPLC analysis of L-glutamic acid and D-glutamic acid. Retention time of L-glutamic acid is 18.760 min; retention time of D-glutamic acid is 20.327 min.

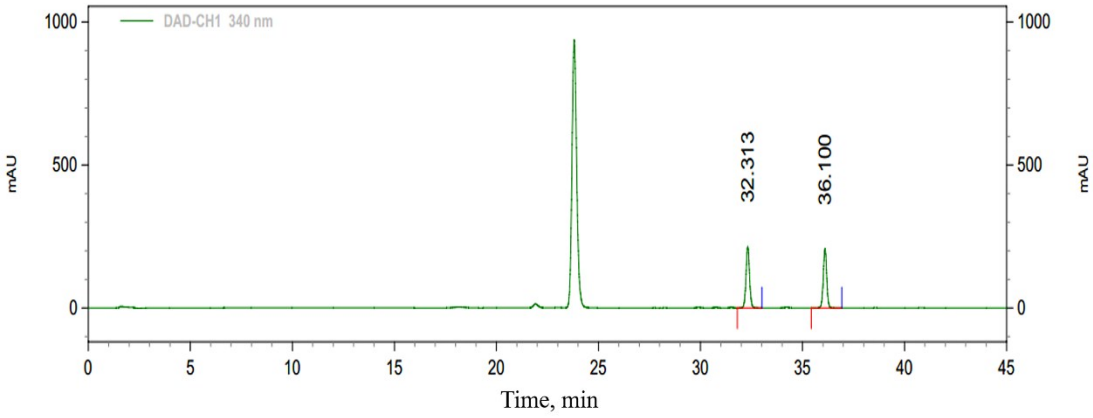


Figure S10. HPLC analysis of L-leucine and D-leucine. Retention time of L-leucine is 32.313 min; retention time of D-leucine is 36.100 min.

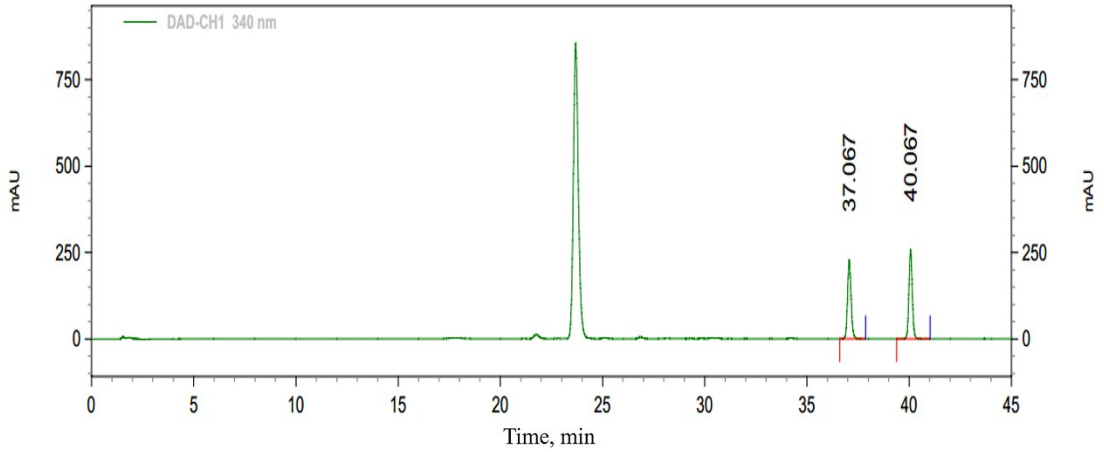


Figure S11. HPLC analysis of L-tyrosine and D-tyrosine. Retention time of L-tyrosine is 37.067 min; retention time of D-tyrosine is 40.067 min.

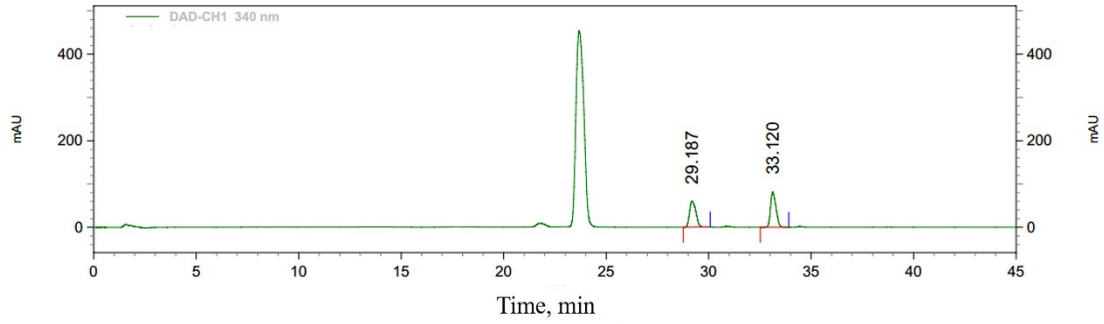


Figure S12. HPLC analysis of L-norvaline and D-norvaline. Retention time of L-norvaline is 29.153 min; retention time of D-norvaline is 33.120 min.

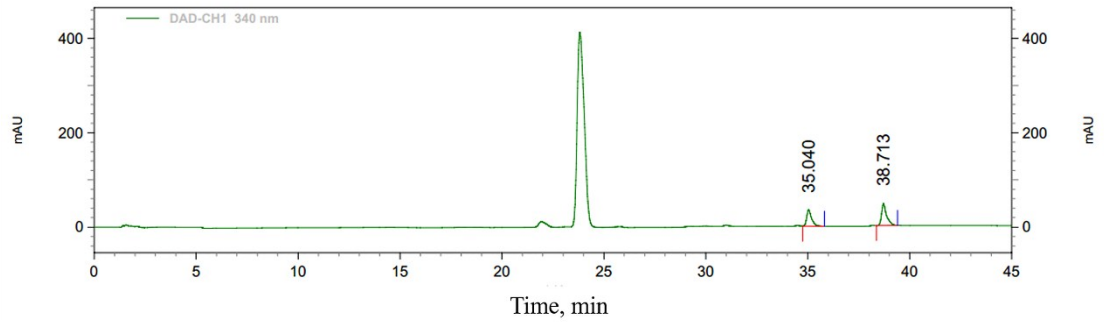
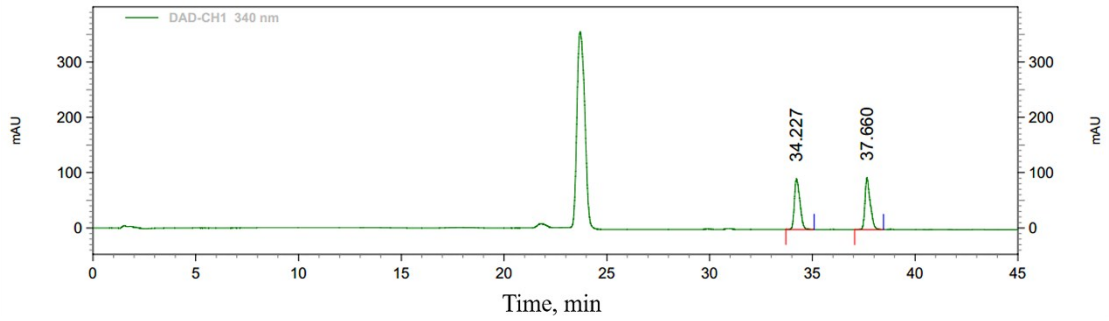


Figure S13. HPLC analysis of L-homophenyalanine and D-homophenyalanine. Retention time of L-homophenyalanine is 35.04 min; retention time of D-homophenyalanine is 38.713 min.



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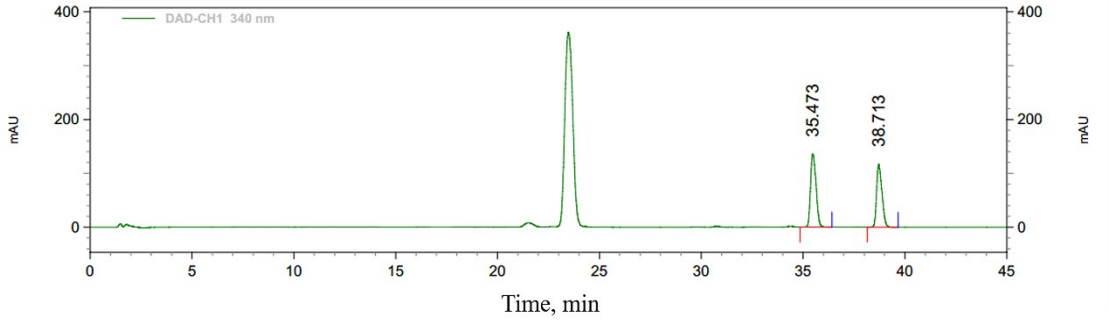
149 **Figure S14.** HPLC analysis of 2-chloro-L-phenylalanine and 2-chloro-D-
150 phenylalanine. Retention time of 2-chloro-L-phenylalanine is 37.66 min; retention
151 time of 2-chloro-D-phenylalanine is 34.227 min.



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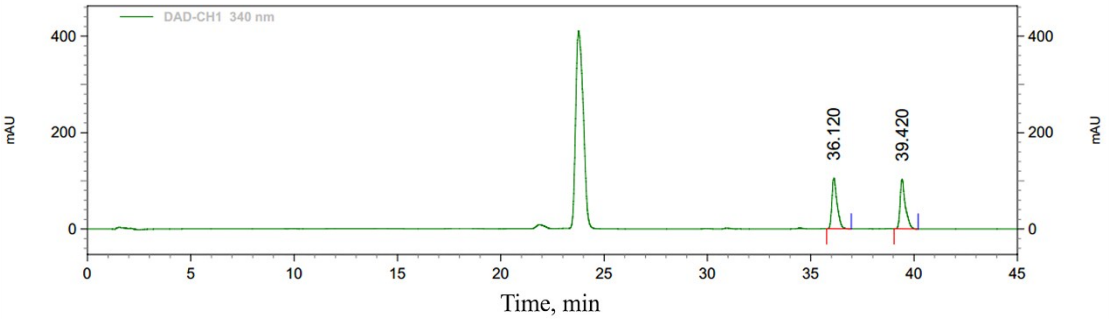
153 **Figure S15.** HPLC analysis of 3-chloro-L-phenylalanine and 3-chloro-D-
154 phenylalanine. Retention time of 3-chloro-L-phenylalanine is 35.473 min; retention
155 time of 3-chloro-D-phenylalanine is 38.713 min.



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157 **Figure S16.** HPLC analysis of 4-chloro-L-phenylalanine and 4-chloro-D-
158 phenylalanine. Retention time of 4-chloro-L-phenylalanine is 36.12 min; retention
159 time of 4-chloro-D-phenylalanine is 39.42 min.

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