Supporting Information

A plug-and-play chemobiocatalytic route for one-pot controllable synthesis of biobased C4 chemicals from furfural

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Materials

Furfural (99%), EY (80%), RB (95%), HFO (98%), β-Ala (99%) and IRA-900 were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). FCA (98%), L- and D-Asp (99%) were from Aladdin Biochemical Co., Ltd. (Shanghai, China). MA (98%) and FA (99%) were from Sangon Biotech Co., Ltd. (Shanghai, China). DVB was from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). TEMPO (98%) was from Sigma-Aldrich (USA). All other chemicals used in this study were of analytical grade. Laccase from *Trametes* sp. that contains approximately 18 mg protein per g crude enzyme is a gift from Amano Enzyme Inc. (Japan). The recombinant strain *E. coli*_CtVDH2_NOX and the plasmids p43E_EcAspA and pET24a_CgPanD were recently constructed by us.[1-3] Their gene sequences were available in supporting information.

One-pot photobiocatalytic synthesis of HFO

HFO synthesis in a concurrent mode: 4 mL of phosphate buffer (0.2 M, pH 7) containing 50-150 mM furfural, 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells and 2 mol% photocatalyst (EY / RB) was irradiated under 30 W green LEDs (530-540 nm) at 25 ℃ for 9 h. Aliquots were withdrawn at specified time intervals from the reaction mixtures, and then diluted with the corresponding mobile phase prior to HPLC analysis. All the experiments were conducted at least in duplicate, and all the data were the averages of experimental results. The yields were determined by HPLC, based on the corresponding calibration curves.

HFO synthesis in a stepwise mode: 4 mL of phosphate buffer (0.2 M, pH 7) containing 100-200 mM furfural and 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃ for 4-9 h (tuning pH to 7 every 2 h). After the reaction, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 30% acetone and 2 mol% photocatalyst. The reaction mixtures were irradiated under 30 W green LEDs (530-540 nm) at 25 °C for 18-36 h. Aliquots were withdrawn at specified time intervals from the reaction mixtures, and then diluted with the corresponding mobile phase for HPLC analysis. All the experiments were conducted at least in duplicate. The yields were determined by HPLC, based on the corresponding calibration curves.

One-pot, three-step synthesis of MA

Typically, 4 mL of phosphate buffer (0.2 M, pH 7) containing 100 mM furfural and 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃ (tuning pH to 7 every 2 h). After 4 h, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 30% acetone and 2 mol% photocatalyst. The reaction mixtures were irradiated under 30 W green LEDs (530-540 nm) at 25 °C for 18 h. Then, the pH of the mixtures was tuned to 5.0, followed by adding 5 mg/mL laccase and 20-100 mol% TEMPO; the reaction mixtures were incubated at 150 r/min and 30 ℃ for 12-120 h. Aliquots were withdrawn at specified time intervals from the reaction mixtures, and then diluted with the corresponding mobile phase for HPLC analysis. All the experiments were conducted at least in duplicate. The yields were determined by HPLC, based on the corresponding calibration curves.

One-pot synthesis of FSA and FA

Typically, 4 mL of phosphate buffer (0.2 M, pH 7) containing 50-100 mM furfural and 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃ for 2-4 (tuning pH to 7 every 2 h). Then, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 2-10 mol% EY@DVB. The reaction mixtures were irradiated under 30 W green LEDs (530-540 nm) at 25 °C and 200 r/min for 24-48 h.

For FA synthesis, the pH of the above mixtures was tuned to 5.0, followed by adding 5 mg/mL laccase and 40-100 mol% TEMPO; the reaction mixtures were incubated at 150 r/min and 30 ℃ for 6-24 h. Aliquots were withdrawn at specified time intervals from the reaction mixtures, and then diluted with the corresponding mobile phase for HPLC analysis. All the experiments were conducted at least in duplicate. The yields were determined by HPLC, based on the corresponding calibration curves.

One-pot synthesis of HBEA, and HBA

HBEA synthesis: 4 mL of phosphate buffer (0.2 M, pH 7) containing 50 mM furfural and 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃ for 2. Then, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 5 mol% EY@DVB. The reaction mixtures were irradiated under 30 W green LEDs (530-540 nm) at 25 °C and 200 r/min for 24 h. Finally, 1.2 equivalent of $NaBH_4$ was added to the reaction mixtures, followed by incubation at 150 r/min and 30 ℃. After 1 h, almost all FSA was converted to HBEA, based on NMR analysis.

HBA synthesis: 4 mL of phosphate buffer (0.2 M, pH 7) containing 50 mM furfural and 50

mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃ for 2. Then, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 5 mol% EY@DVB. The reaction mixtures were irradiated under 30 W green LEDs (530-540 nm) at 25 °C and 200 r/min for 24 h. Finally, 1 mL methanol, 3 mM CuCl and 2.5 equivalent of $NaBH_4$ was added to the reaction mixtures, followed by incubation at 150 r/min and 30 ℃. After 0.5 h, almost all FSA was converted to HBA, based on HPLC analysis. The yields were determined by HPLC, based on the corresponding calibration curves.

One-pot, four-step synthesis of biobased L-Asp

Generally, 4 mL of phosphate buffer (0.2 M, pH 7) containing 50 mM furfural and 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃. After 2 h, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 5 mol% EY@DVB. The reaction mixtures were irradiated under 30 W green LEDs (530-540 nm) at 200 r/min and 25 °C. After 24 h, tuning pH to 5 was conducted, followed by adding 5 mg/mL of laccase and 80 mol% of TEMPO; then the reaction mixtures were incubated for 12 h at 150 r/min and 30 ℃. Then, approximately 350 mM aqueous ammonia was added to tune pH to 8.5, followed by adding 50 mg/mL (wet cell weight) *E. coli*_EcAspA cells and 1 mM $MgCl_2$; the mixtures were incubated at 150 r/min, and 30 ℃ for 2 h. Aliquots were withdrawn at specified time intervals from the reaction mixtures, and then diluted with the corresponding mobile phase prior to HPLC analysis. All the experiments were conducted at least in duplicate, and all the data were the averages of experimental results. The yields were determined by HPLC, based on the corresponding calibration curves.

One-pot synthesis of β-Ala

Generally, 4 mL of phosphate buffer (0.2 M, pH 7) containing 50 mM furfural and 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃. After 2 h, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 5 mol% EY@DVB. The reaction mixtures were irradiated under 30 W green LEDs (530-540 nm) at 200 r/min and 25 °C. After 24 h, tuning pH to 5 was conducted, followed by adding 5 mg/mL of laccase and 80 mol% of TEMPO; then the reaction mixtures were incubated for 12 h at 150 r/min and 30 ℃. Then, approximately 200 mM aqueous ammonia was added to tune pH to 7, followed by adding 50 mg/mL (wet cell weight) *E. coli*_EcAspA cells, 50 mg/mL *E. coli*_CgPanD and 1 mM $MgCl_2$; the mixtures were incubated at 150 r/min, and 30 ℃ for 12 h. Aliquots were withdrawn at specified time intervals from the reaction mixtures, and then diluted with the corresponding mobile phase for HPLC analysis. All the experiments were conducted at least in duplicate. The yields were determined by HPLC, based on the corresponding calibration curves.

Scale-up synthesis of L-Asp

Phosphate buffer (0.2 M, pH 7, 50 mL) containing 100 mM furfural and 50 mg/mL (wet cell weight) *E. coli*_CtVDH2_NOX cells was incubated at 150 r/min and 30 ℃. After 4 h, the cells were removed by centrifugation (8000 r/min, 4 ℃, 10 min), followed by adding 5 mol% EY@DVB. The reaction mixture was irradiated under 30 W green LEDs (530-540 nm) at 200

r/min and 25 °C. After 60 h, tuning pH to 5 was conducted, followed by adding 5 mg/mL of laccase and 80 mol% of TEMPO; then the reaction mixture was incubated for 24 h at 150 r/min and 30 ℃. Then, approximately 350 mM aqueous ammonia was added to tune pH to 8.5, followed by adding 50 mg/mL (wet cell weight) *E. coli*_EcAspA cells and 1 mM $MgCl_2$; the mixture was incubated at 150 r/min, and 30 ℃ for 4 h. The crude product of 233 mg (an isolated yield of 36%) was isolated from the reaction mixture through isoelectronic point precipitation (pH 2.8) by adding H_2SO_4.

Derivatization of amino acids

0.1 mM phenyl isothiocyanate (PITC) solution: 1 mL of PITC (99%; Aladdin, Shanghai, China) was added to 83 mL of chromatographically pure acetonitrile, mixed, and stored under 4 °C and dark conditions.

1 mM triethylamine (TEA) solution: 14 mL of chromatographically pure TEA was added to 86 mL of chromatographically pure acetonitrile, mixed, and stored under 4 °C and dark conditions.

Derivatization steps: 0.25 mL of PITC solution and 0.25 mL TEA solution were added to 0.5 mL of the reaction mixtures, and settled for 1 h under dark condition. Upon the reaction, 0.7 mL hexane was added and mixed; after phase separation, the aqueous phase was subjected to HPLC analysis.

HPLC analysis

The reaction mixtures were analyzed on a Zorbax Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA) by using a reversed phase HPLC equipped with a Waters 996 photodiode array detector (Waters, USA). The column temperature was 35 °C. For quantitative analysis of furfural, FCA, HFO and FSA, the mixture of acetonitrile/0.4% $(NH_4)_2SO_4$ solution with pH 3.5 (10:90, v/v) was used as the mobile phase at the flow rate of 0.6 mL/min. The retention times of furfural (maximum absorption wavelength: 278 nm), FCA (246 nm), HFO (210 nm) and FSA (219 nm) are 12.10, 5.91, 4.82 and 4.16 min, respectively. The mixture of acetonitrile/0.4% $(NH_4)_2SO_4$ solution with pH 3.5 (5:95, v/v) at a flow rate of 0.6 mL/min was used as the mobile phase for quantifying FA (210 nm), while the mixture of methanol/0.02% phosphoric acid solution (5:95, v/v) at the same flow rate was used for quantifying MA (210 nm). The retention times of FA and MA are 4.75 and 8.28 min, respectively. Prior to analyzing amino acids, the derivatization was conducted using PITC under basic conditions (see **Derivatization of amino acids** for details). The mixture of acetonitrile/0.1 M sodium acetate solution (7:93, v/v) at a flow rate of 0.6 mL/min was used for quantitatively analyzing L-Asp and β-Ala at 240 nm. The retention time of L-Asp and β-Ala are 5.51 and 18.53 min, respectively. For determining the configuration of aspartic acid (no requirement of derivatization), the reaction mixtures were analyzed on a Chirex 3126 column (Phenomenex, USA) by using HPLC (Agilent, USA) at 254 nm, with 0.2 mL/min of the mixture of 2 mM CuSO4 solution/isopropanol (9:1, v/v) as the mobile phase. The retention times of L- and D-aspartic acid were 27.90 and 30.12 min, respectively.

HPLC analysis for Scheme 2B

The reaction mixtures were analyzed on a Zorbax Eclipse XDB-C18 column (4.6 mm × 250

mm, 5 μm, Agilent, USA) by using a reversed phase HPLC equipped with a Waters 2489 UV/Visible detector (Waters, USA). The column temperature was 35 ºC. The mixture of acetonitrile/0.4% $(NH_4)_2SO_4$ solution with pH 3.5 (5:95, v/v) was used as the mobile phase at the flow rate of 0.6 mL/min. The derivatization using PITC was carried out prior to L-Asp analysis.

Construction and cultivation of recombinant *E. coli*_ EcAspA strain

The primer (P1: 5'-GTGGACAGCAAATGGGTCGCGGATCCATGTCAAACAACATTCGTATCG AAGA-3'; P2: 5'-TGGTGGTGGTGGTGGTGCTCGAGTGCGGCCGCAAGCTTTTACTGTTCGCTTT CATCAGT-3') was designed on the basis of the gene sequence of the plasmid p43E_EcAspA and synthesized by Sangon Biotech (Shanghai, China). The gene EcaspA was amplified with the plasmid p43E_EcAspA as template. Then, the PCR product were double-digested using the restriction enzymes BamH I and Hind III; then, the resulting product was linked with the expression vector pET28a that was digested with the same restriction endonucleases. The resulting recombinant plasmid pET28a_EcaspA was verified by restriction analysis and nucleotide sequencing.

The recombinant plasmid was transformed into *E. coli* BL21(DE3) and pre-cultivated in 30 mL Luria-Bertani (LB) medium containing 30 mg/L kanamycin at 37 °C and 180 r/min overnight. Then, 100 mL LB medium containing 30 mg/L kanamycin was inoculated with 1 mL of an overnight culture. Cells were grown at 37 °C and 180 r/min. When the optical density at 600 nm (OD_{600}) reached 0.6-0.8, IPTG was added for inducing protein expression at the final concentration of 0.2 mM, followed by incubation at 24 °C and 180 r/min for 20 h. The cells were harvested by centrifugation (8000 r/min, 5 min, 4 °C) and washed twice with 0.85% NaCl solution.

Cultivation of *E. coli*_CtVDH2_NOX and *E. coli*_CgPanD

Cultivation of *E. coli*_CtVDH2_NOX was conducted according to a recent method.[3] Briefly, recombinant *E. coli*_CtVDH2_NOX cells were pre-cultivated overnight in 30 mL of LB medium containing 100 mg/L ampicillin at 37 °C and 180 r/min. Then, 100 mL LB medium containing 100 mg/L ampicillin was inoculated with 1 mL of the overnight culture, followed by cultivation at 37 °C and 180 r/min. When the optical density at 600 nm (OD_{600}) reached 0.6-0.8, IPTG (as inducer) was added to the medium at a final concentration of 0.1 mM, followed by induction at 20 °C and 160 r/min for 20 h. The cells were harvested by centrifugation (8000 r/min, 5 min, 4 °C) and washed twice with 0.85% NaCl solution.

Cultivation conditions of *E. coli*_CgPanD was similar to those of *E. coli*_ EcAspA, with the exception of 0.8 mM of IPTG and the induction temperature of 37 °C.

The gene sequences of EcAspA and CgPanD were showed as below.

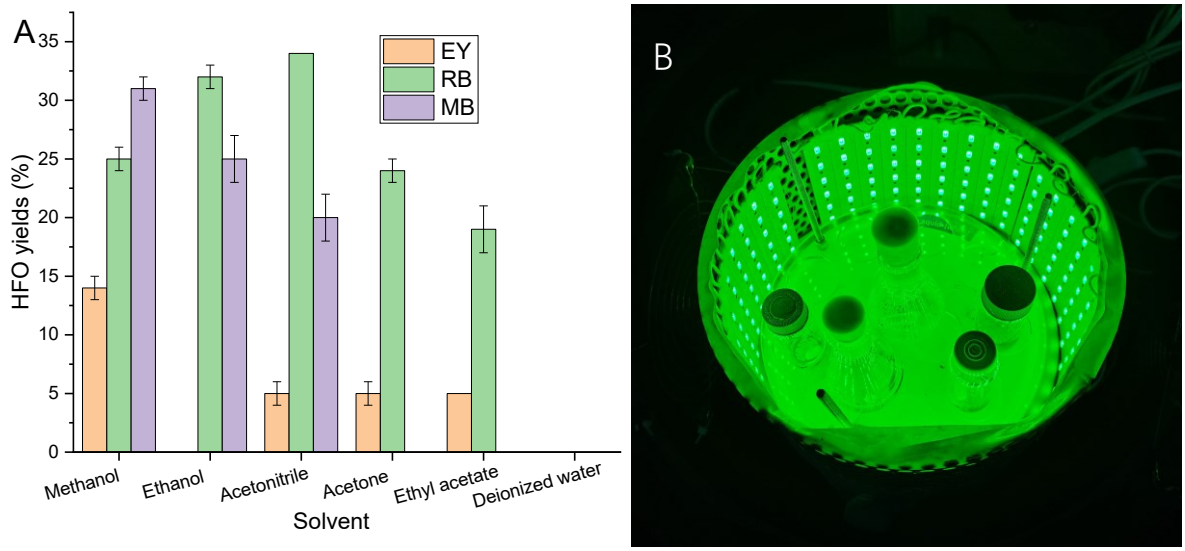


Figure S1. Effect of solvents on photocatalytic synthesis of HFO (A) and the homemade photoreactor (B). Reaction conditions: 10 mM furfural, 2 mol% photocatalyst, 5 mL solvent, 30 W green LEDs (530-540 nm), 25 °C, 6 h. Ethanol used herein refers to 95% ethanol; EY, EY; RB, Rose Bengal; MB, methylene blue.

Table S1. Effect of solvents on photocatalytic synthesis of HFO

Entry	Photocatalyst	Solvent	HFO yield (%)
1	RB	Methanol	96
2	RB	Ethanol [a]	96
3	RB	Acetonitrile	97
4	RB	Acetone	96
5	RB	Ethyl acetate	97
6	RB	N,N-Dimethylformamide	98
7	RB	Tetrahydrofuran	95
8	RB	2-Methyltetrahydrofuran	95
9	RB	γ-Valerolactone	87
10	RB	H_2O [b]	26
11	EY	Methanol	94
12	EY	Ethyl acetate	90
13	EY	Dimethyl carbonate	91
14	EY	γ-Valerolactone	90
15	EY	H_2O [b]	23

Reaction conditions: 10 mM FCA, 2 mol% photocatalyst**,** 5 mL solvent, 30 W green LEDs (530-540 nm), 25 °C, 6 h. [a] 95% ethanol. [b] Deionized water

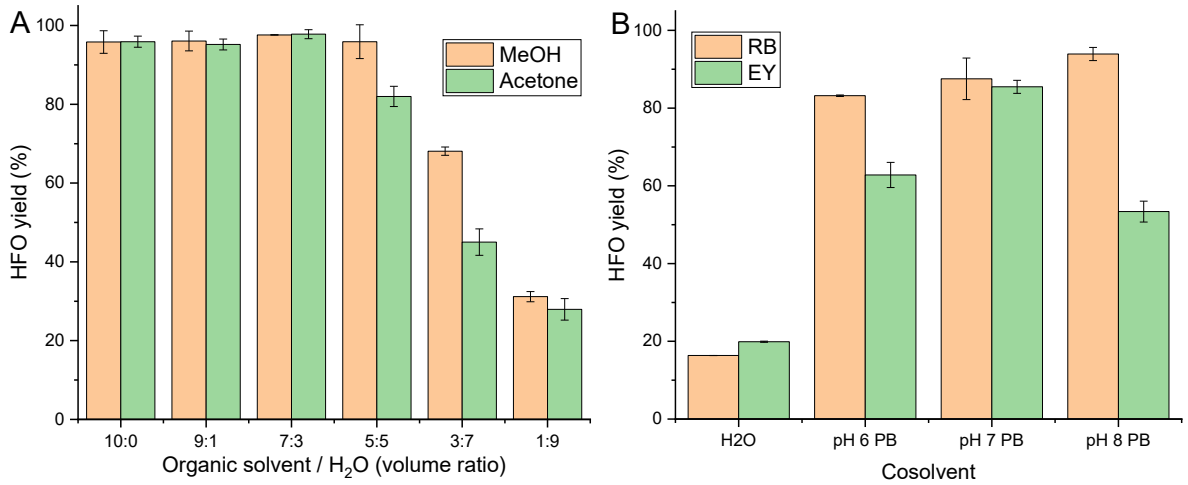


Figure S2. Photocatalytic synthesis of HFO from FCA in organic solvent aqueous solutions: (A) the effect of the volume ratio of organic solvent to deionized H_2O; (B) comparison of the effect of deionized H_2O and phosphate buffer. Reaction conditions for Figure S2A: 10 mM FCA, 2 mol% RB, 5 mL the mixture of organic solvent and water, 30 W green LEDs (530-540 nm), 25 °C, 6 h; reaction conditions for Figure S2B: 50 mM FCA, 2 mol% RB / EY, 4 mL aqueous solution containing 30% acetone, 30 W green LEDs (530-540 nm), 25 °C, 9 h. PB: 200 mM phosphate buffer.

Table S2. Immobilization of EY on different polymers

Polymer	EY (mg)	Amount of EY immobilized per gram of resin (mg/g)
IRA-900	300	295
NKA-II	300	102
DVB	300	299

Conditions: 300 mg EY, 1 g polymer (IRA-900, Amberlite® IRA-900 anion exchange resin; NKA-II, NKA-II macroporous adsorption resin; DVB, Styrene DVB D392 weakly basic anion resin based on a styrene-divinylbenzene copolymer), 40 mL deionized water, 30 ℃, 100 r/min, 10 h.

Table S3. The photosensitizer leakage from polymer-supported EY

Polymer-supported EY	Loss of EY (%)
EY@IRA-900	1
EY@NKA-II	2
EY@DVB	3

Conditions: The concentration of EY present in solution was determined after 10 mL of phosphate buffer (0.2 M, pH 7) containing 10 mg polymer-supported EY was incubated at 30 ℃, 100 r/min for 10 h.

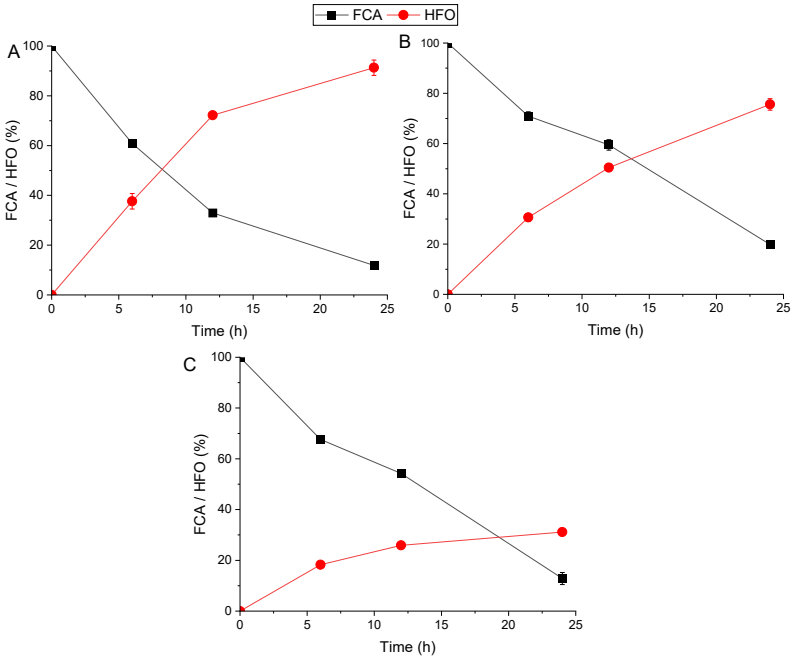


Figure S3. Photocatalytic conversion of FCA to HFO by EY@NKA-II (A), EY@IRA-900 (B), and EY@DVB (C). Reaction conditions: 50 mM FCA, 2 mol% immobilized EY, 4 mL phosphate buffer (0.2 M, pH 7), 25 °C, 200 r/min, 30 W 530 nm-540 nm Green LEDs

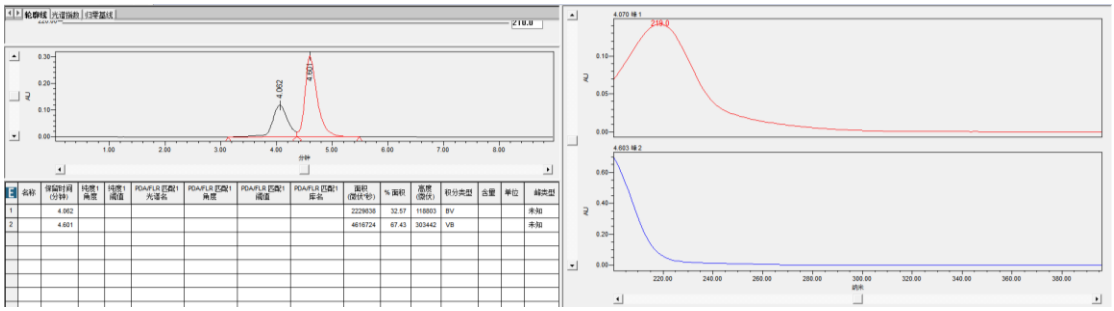


Figure S4. HPLC analysis of the unknown chemical. The peaks of the unknown chemical and HFO appear at 4.06 and 4.60 min, respectively.

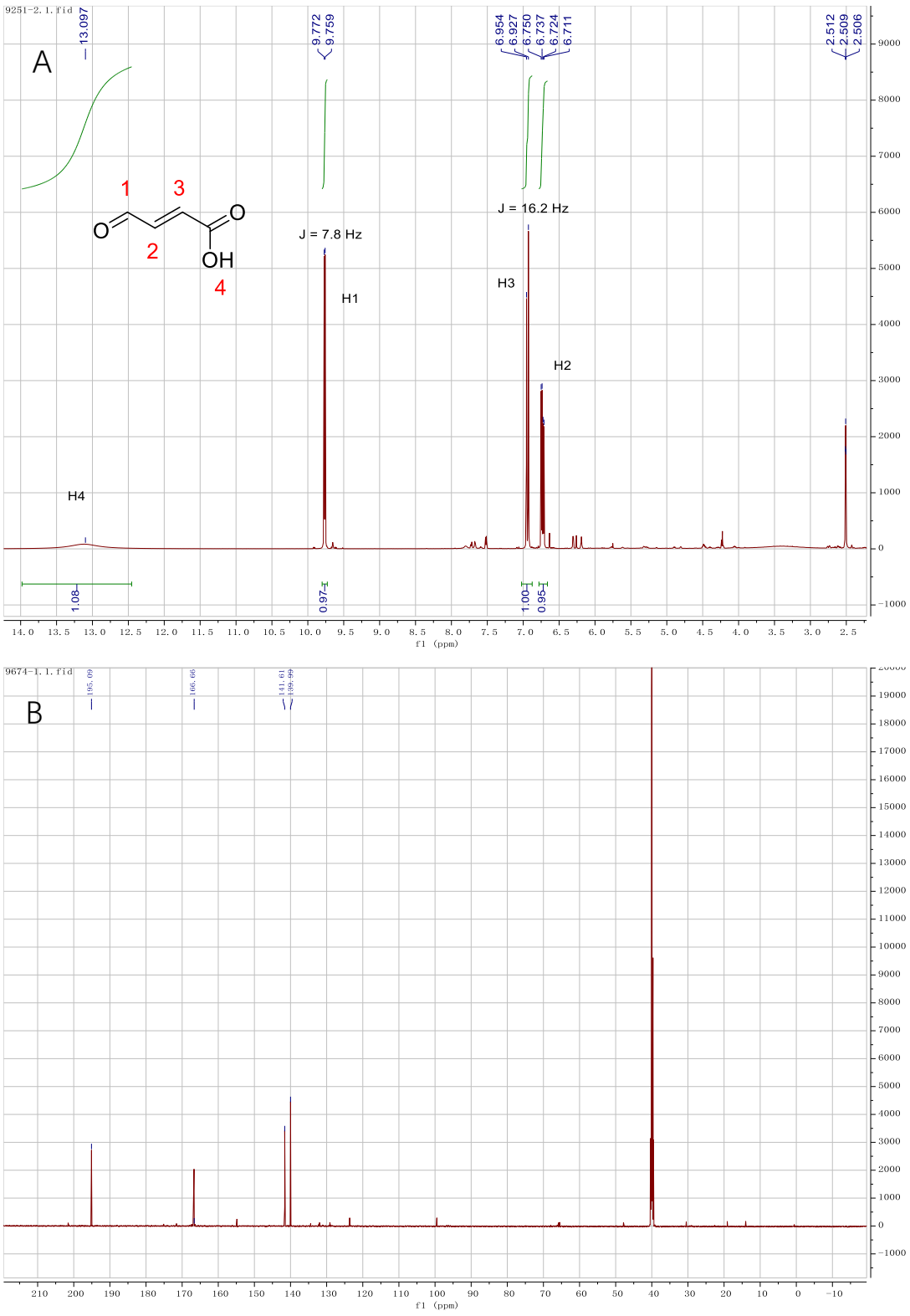


Figure S5. ^{1}H (A) and ^{13}C NMR (B) spectra of FSA (in d_6-DMSO, 600 MHz)

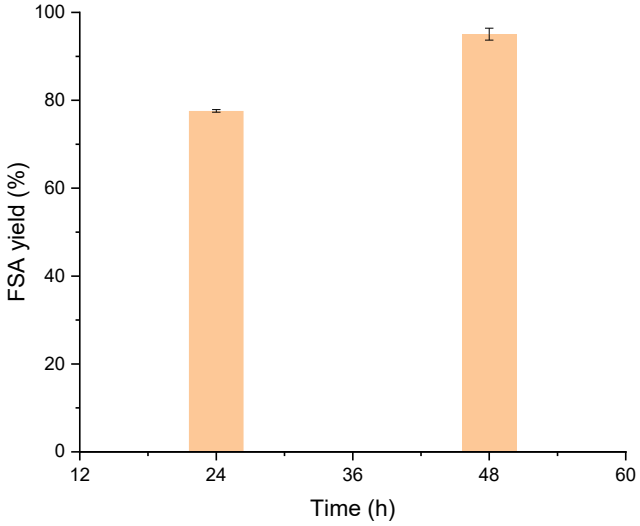


Figure S6. Synthesis of FSA from HFO by DVB. Reaction conditions: 10 mM HFO, 1.8 mg DVB (corresponding to the amount of polymer present in 2 mol% EY@DVB), 4 mL phosphate buffer (0.2 M, pH 7), 30 ℃, 150 r/min.

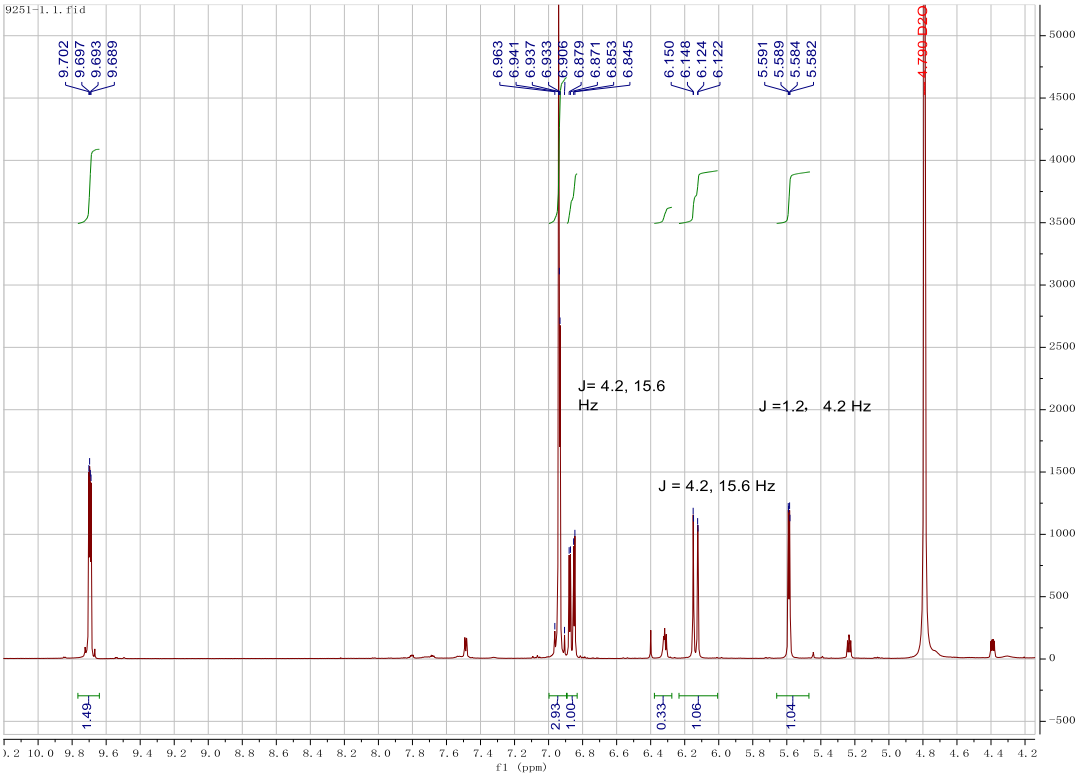


Figure S7. ^{1}H NMR spectrum of FSA (in D2O, 600 MHz)

The chemical shift of the proton present in the formyl group of FSA was changed from 9.69 ppm to 5.58 ppm upon hydration. Based on the ratio of the integration of the peak at 5.58 ppm to the sum of the peak integrations at 5.58 and 9.69, the hydration level is around 41%.

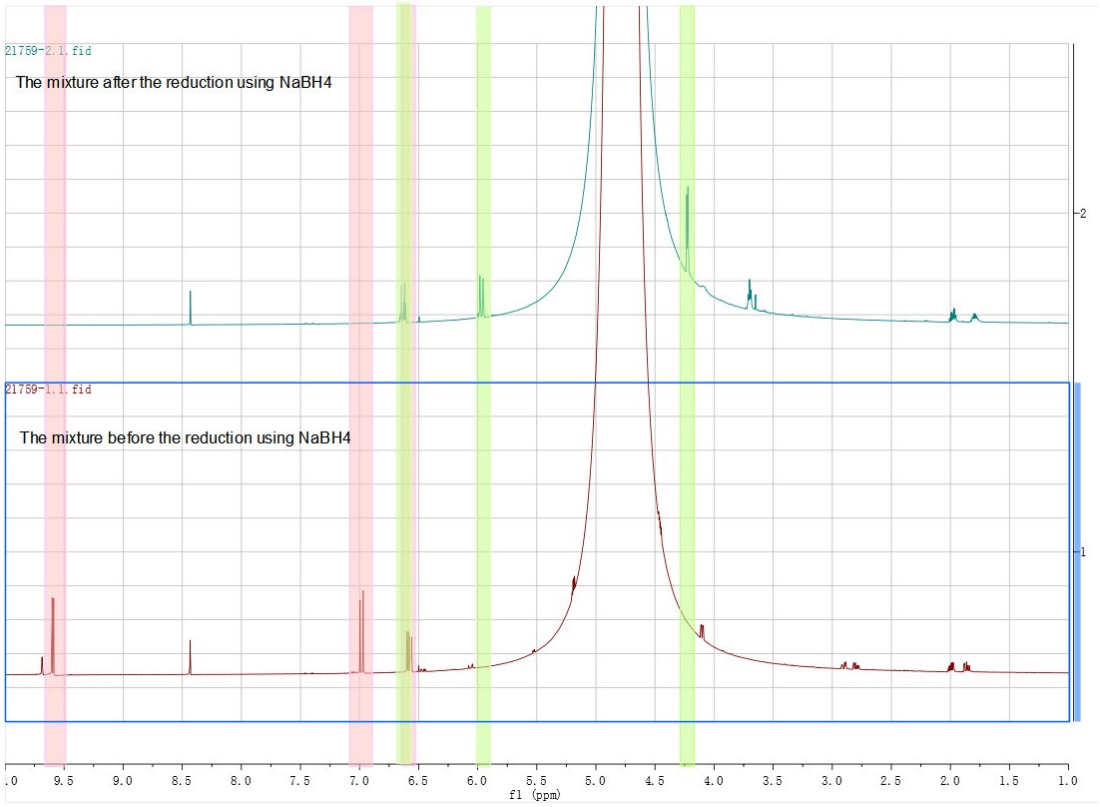


Figure S8. Comparison of the composition change of the reaction mixture before and after reduction using $NaBH_4$. Reaction conditions: 50 mM furfural, 50 mg/mL *E. coli*_CtVDH2_NOX cells, 4 mL phosphate buffer (0.2 M, pH 7), 150 r/min, 30 ℃, 2 h; removing cells, then adding 5 mol% EY@DVB, 30 W green LEDs (530-540 nm), 25 °C, 200 r/min, 24 h; adding 1.2 eq. $NaBH_4$, 150 r/min, 30 ℃, 1 h; after 0.48 mL of sample was withdrawn from the reaction mixture, followed by adding 0.12 mL of D_2O, ^1H NMR spectra were recorded.

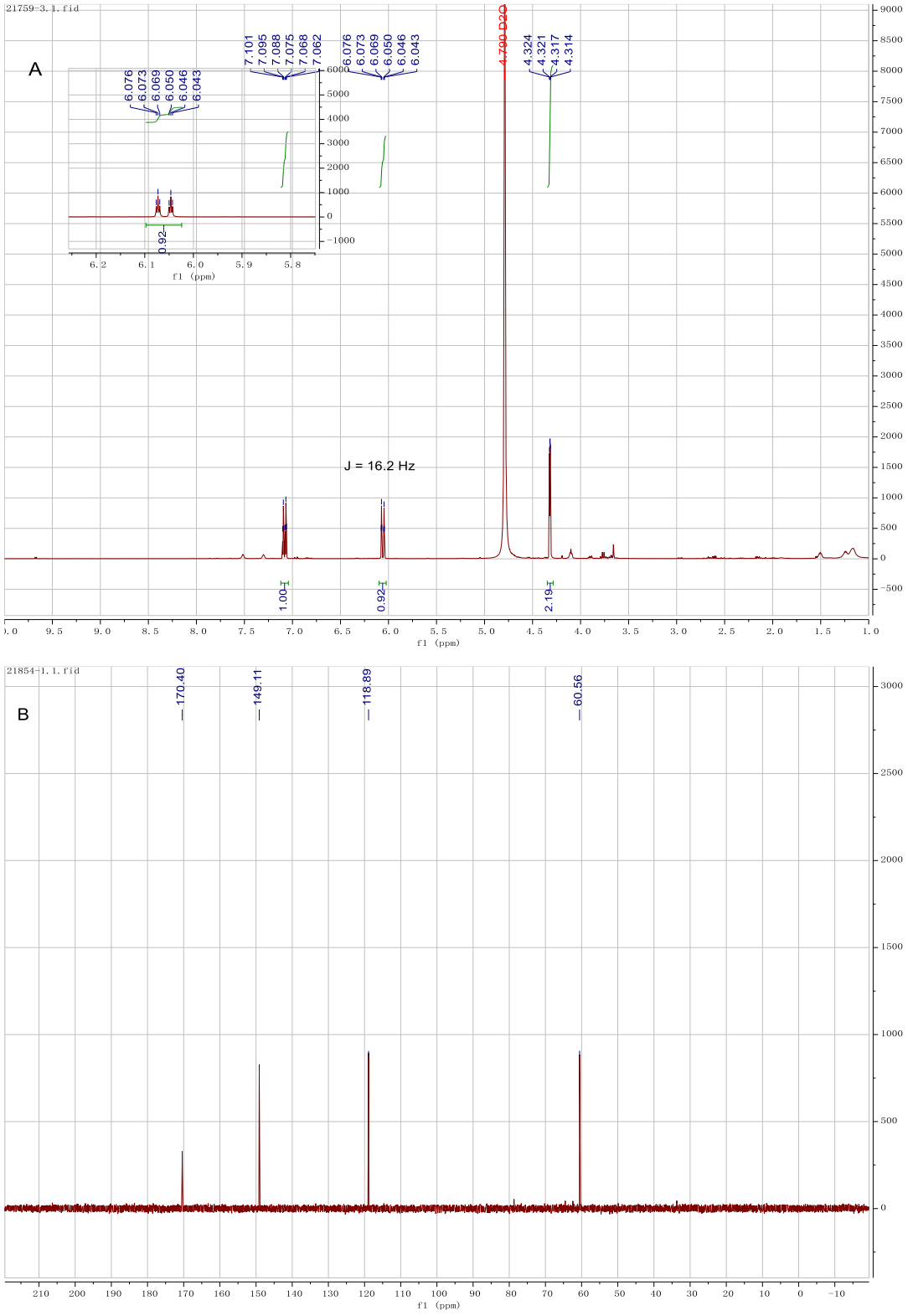


Figure S9. ^{1}H (A) and ^{13}C (B) NMR spectra of HBA (in D_2O, 600 mM)

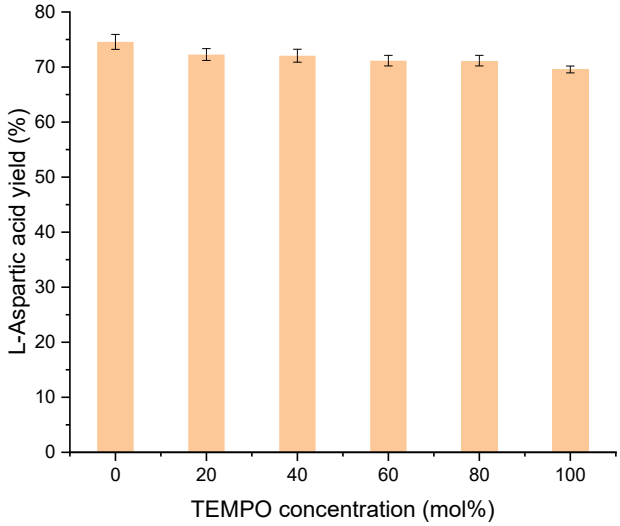


Figure S10. Effect of the TEMPO concentrations on the synthesis of L-Asp by *E. coli*_EcAspA. Reaction conditions: 50 mM FA, 50 mg/mL *E.coli*_EcAspA cells, 0-100 mol% TEMPO, 4 mL phosphate buffer (0.2 M, pH 7), 1 mM $MgCl_2$, 350 mM NH_3 (after adding NH3, pH of the reaction mixture was changed to around 8.5), 150 r/min, 30 °C, 2 h.

Table S4. One-pot, four-step synthesis of amino acids

Entry	TEMPO (mol%)	Biocatalyst [a]	Time (h)	Product	Yield (%)
1	40	*E. coli*_EcAspA	52 (2+24+24+2)	L-Asp	64
2	60	*E. coli*_EcAspA	40 (2+24+12+2)	L-Asp	62
3	80	*E. coli*_EcAspA	40 (2+24+12+2)	L-Asp	63
4	100	*E. coli*_EcAspA	34 (2+24+6+2)	L-Asp	57
5	80	*E. coli*_EcAspA and *E. coli*_CgPanD	50 (2+24+12+12)	β-Ala	75 (2) [a]

Reaction conditions: 50 mM furfural, 50 mg/mL *E. coli*_CtVDH2_NOX cells, 4 mL phosphate buffer (0.2 M, pH 7), 150 r/min, 30 ℃, 2 h; removing cells, then adding 5 mol% EY@DVB, 30 W green LEDs (530-540 nm), 25 °C, 200 r/min, 24 h; tuning pH to 5, then adding 5 mg/mL laccase and 40-100 mol% TEMPO, 150 r/min, 30 ℃, 6-24 h; adding approximately 350 mM aqueous ammonia to tune pH to 8.5, followed by adding 50 mg/mL *E. coli*_EcAspA cells and 1 mM $MgCl_2$, 150 r/min, 30 ℃, 2 h; for β-alanine synthesis, tuning pH to 7 by adding approximately 200 mM aqueous ammonia, followed by adding 50 mg/mL *E. coli*_EcAspA, 50 mg/mL *E. coli*_CgPanD and 1 mM $MgCl_2$, 150 r/min, 30 ℃, 12 h . [a] the value in parenthesis represents the yield of L-aspartic acid.

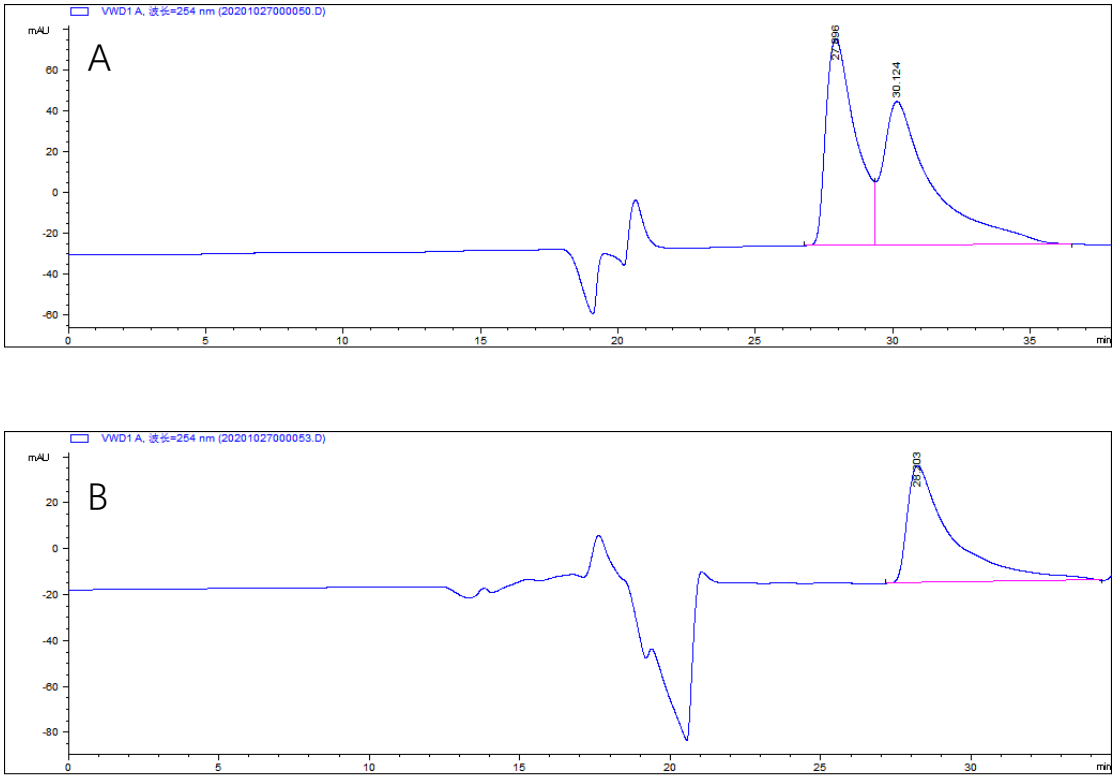


Figure S11. Chiral analysis of the product configuration: (A) racemic aspartic acid; (B) the product produced in the cascade reaction. Retention times of L- and D-Asp were 27.9 and 30.1 min, respectively.

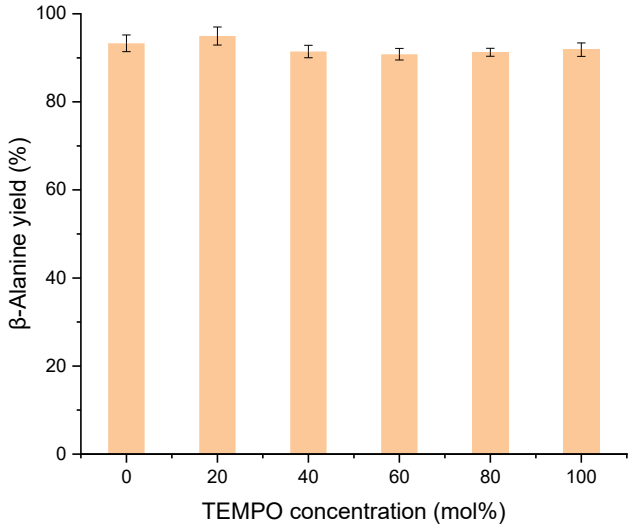
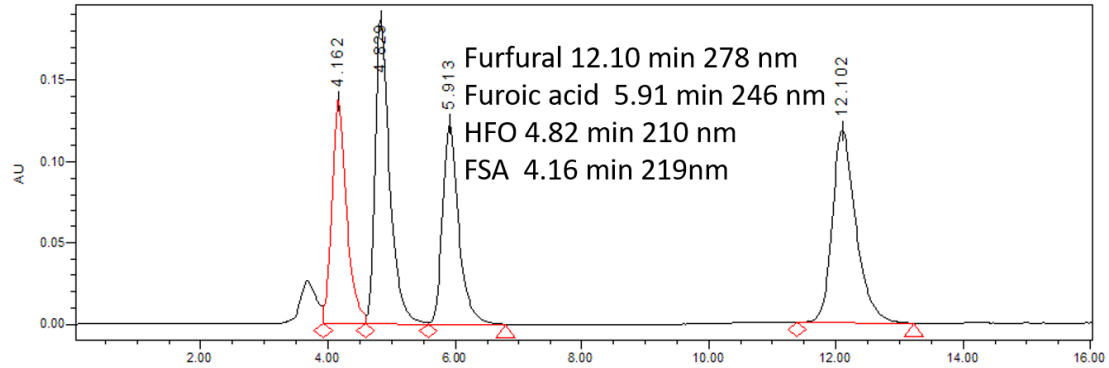


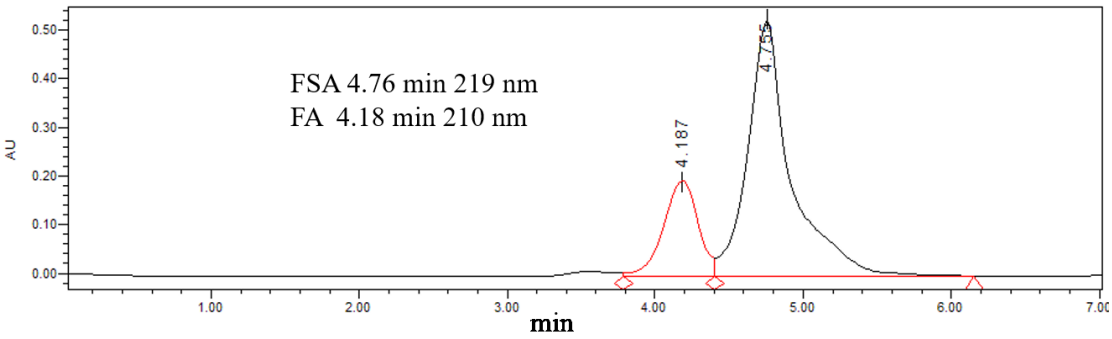
Figure S12. Effect of the TEMPO concentrations on the synthesis of β-Ala by *E. coli*_CgPanD. Reaction conditions: 50 mM L-Asp, 50 mg/mL *E.coli*_CgPanD cells, 0-100 mol% TEMPO, 4 mL phosphate buffer (0.2 M, pH 7), 150 r/min, 30 °C, 8 h.

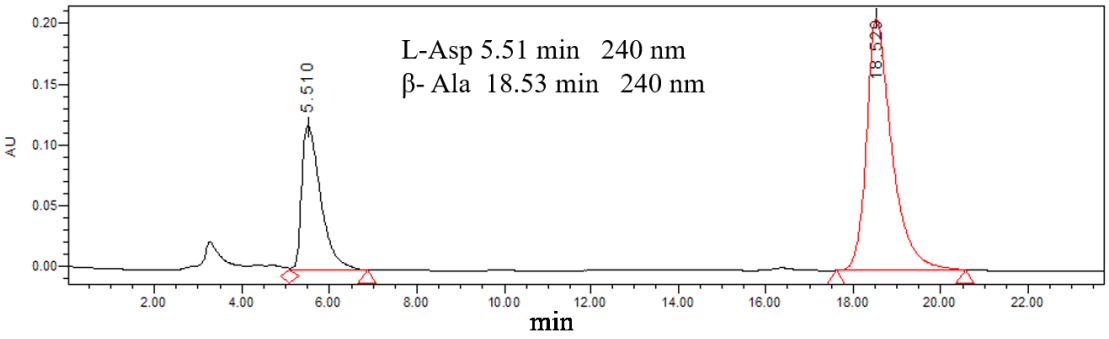
Table S5. Estimation of waste generated in the scale-up synthesis of L-Asp (without consideration of the product purification)

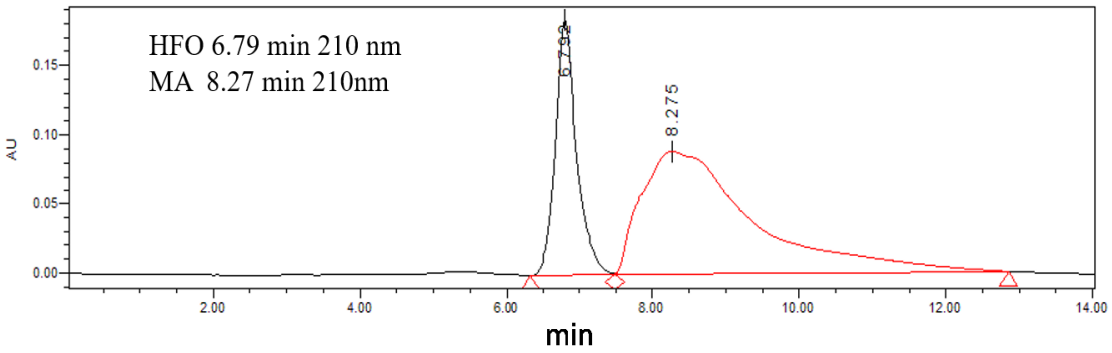
Component	E factor contribution (kg waste per kg product)
Water	156.6
Buffer salt	4.1
EY@DVB	2.3
Cells	3.9
Laccase	0.8
TEMPO	2.0
NH_3	0.7
Sum	170.4

HPLC chromatograms

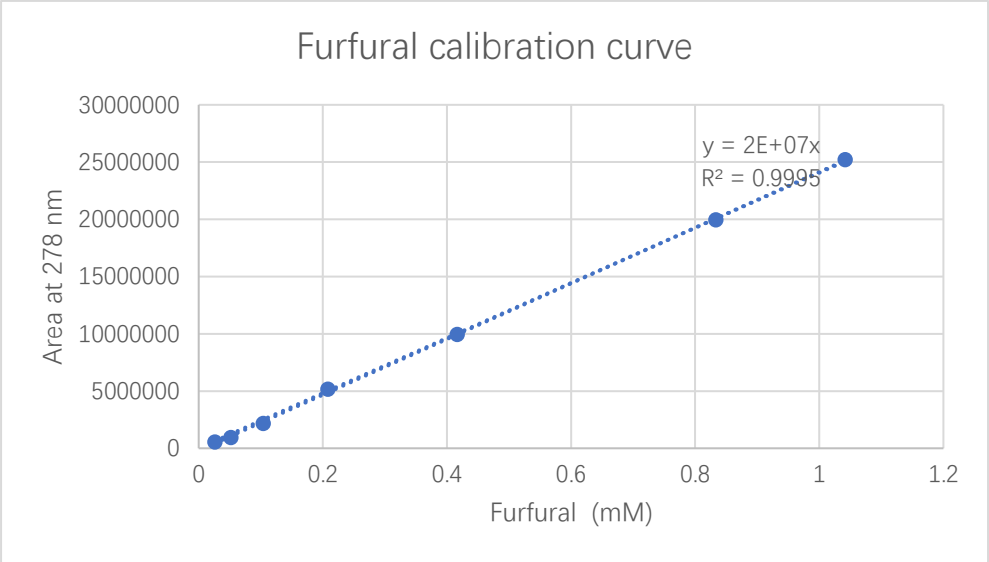


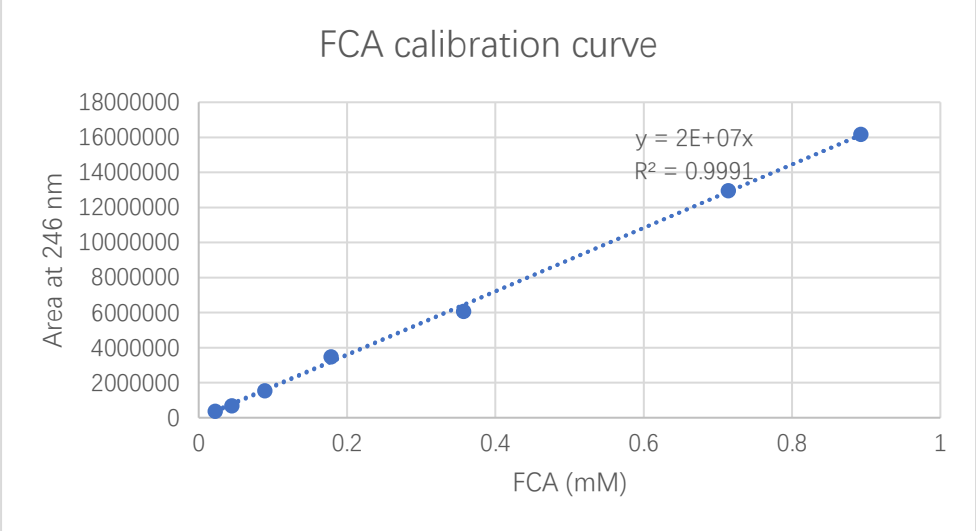


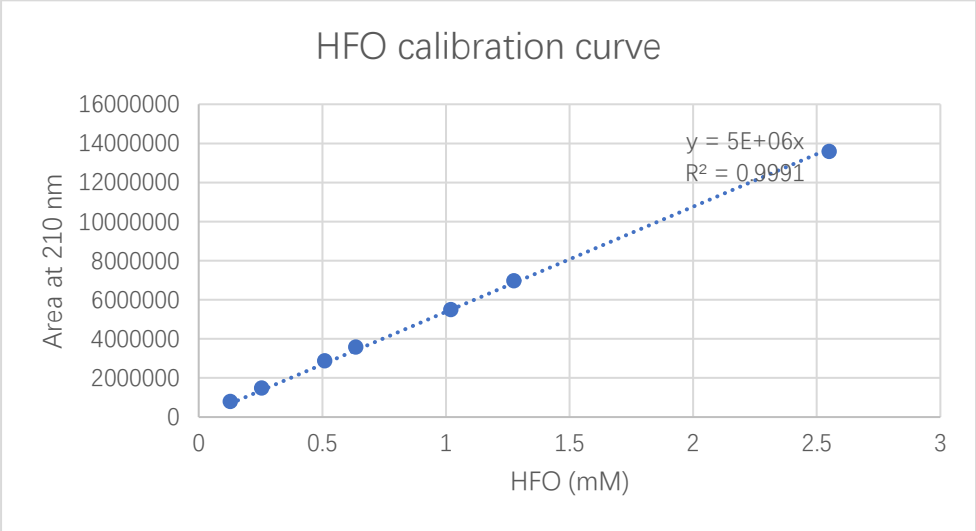


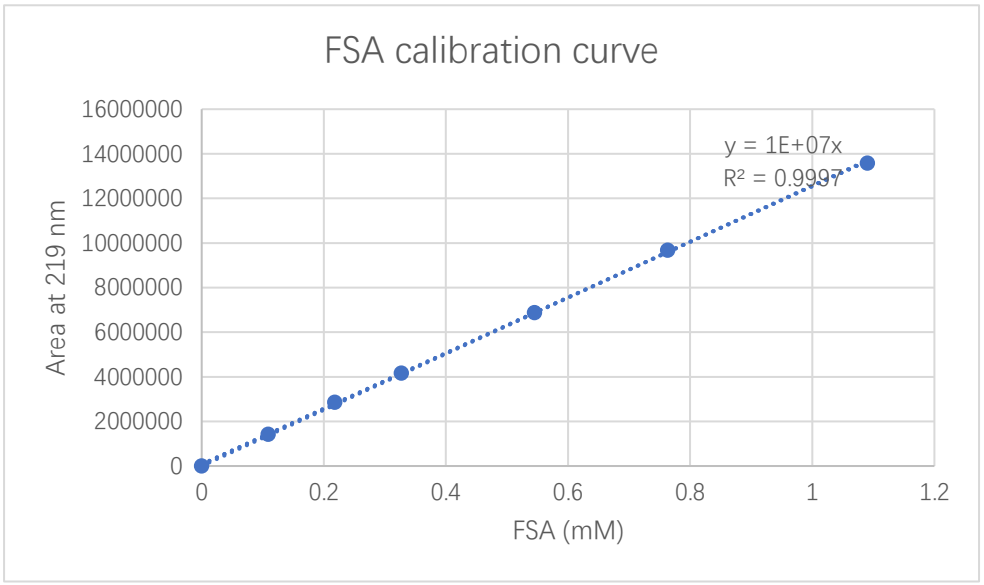


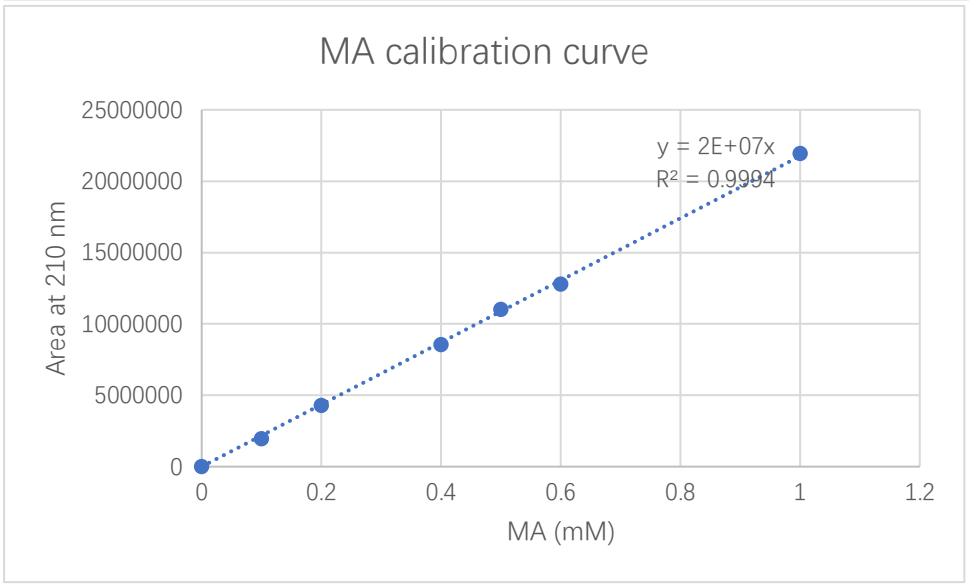
Calibration curves

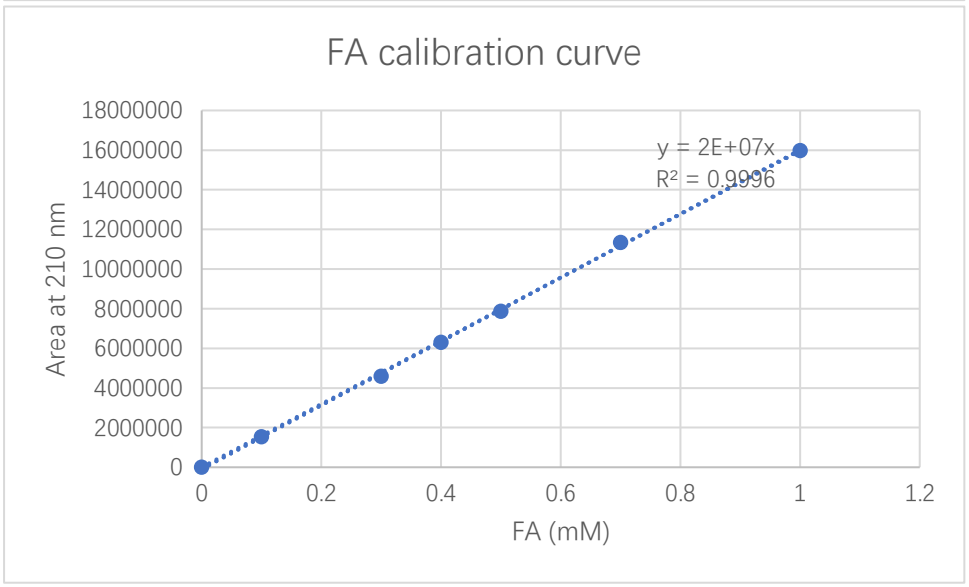


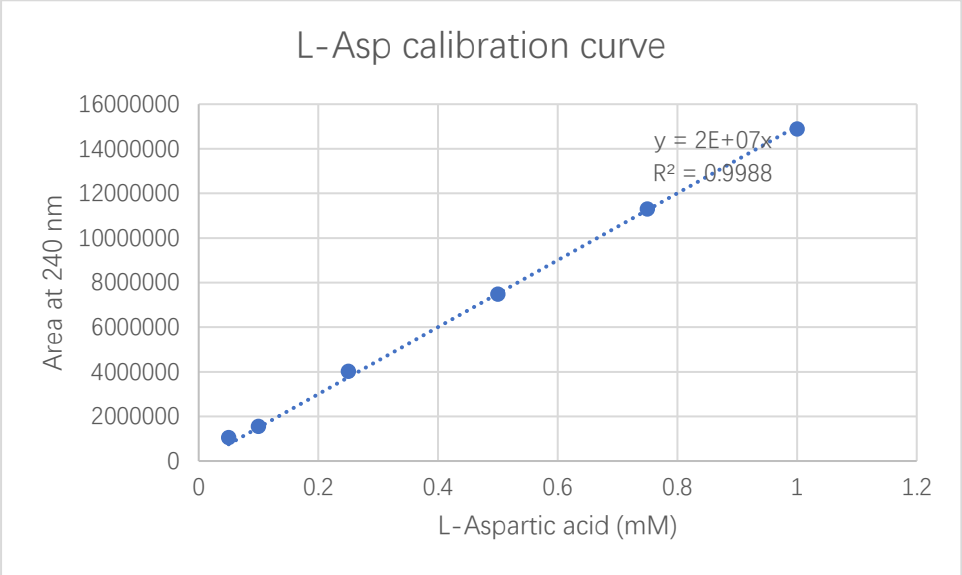


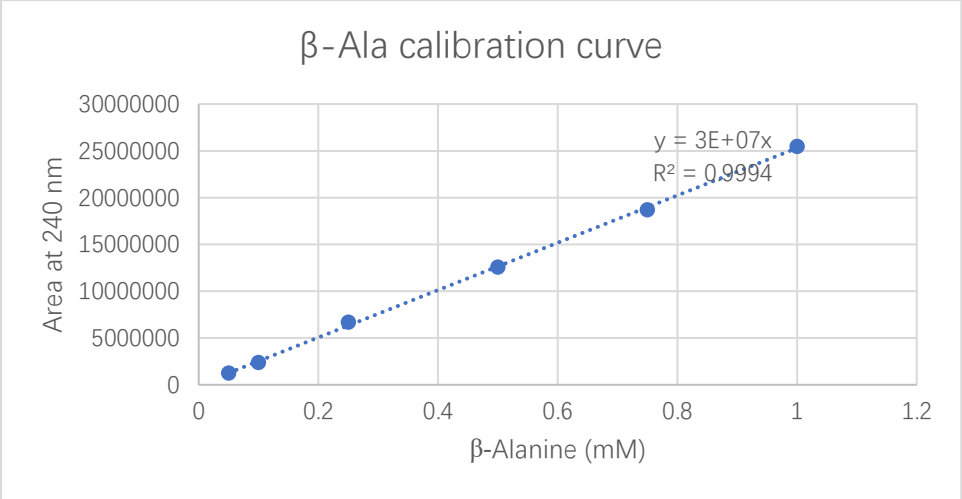


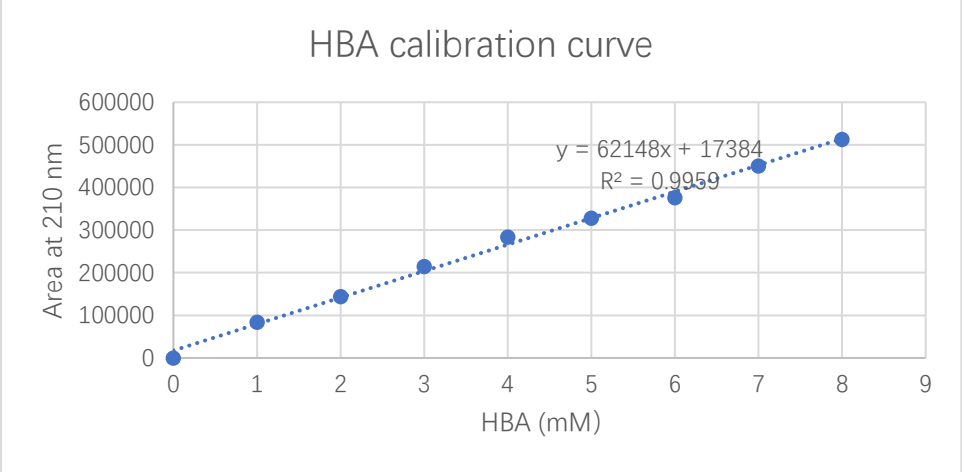

FSA calibration curve
y = 1E+07x
R² = 0.9997
Area at 219 nm
FSA (mM)
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
0.2
0.4
0.6
0.8
1
1.2


MA calibration curve
y = 2E+07x
R² = 0.9994
Area at 210 nm
MA (mM)
25000000
20000000
15000000
10000000
5000000
0
0
0.2
0.4
0.6
0.8
1
1.2


FA calibration curve
y = 2E+07x
R² = 0.9996
Area at 210 nm
FA (mM)
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
0.2
0.4
0.6
0.8
1
1.2


L-Asp calibration curve
y = 2E+07x
R² = 0.9988
Area at 240 nm
L-Aspartic acid (mM)
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
0.2
0.4
0.6
0.8
1
1.2


β-Ala calibration curve
y = 3E+07x
R² = 0.9994
Area at 240 nm
β-Alanine (mM)
30000000
25000000
20000000
15000000
10000000
5000000
0
0
0.2
0.4
0.6
0.8
1
1.2


HBA calibration curve
y = 62148x + 17384
R² = 0.9959
Area at 210 nm
HBA (mM)
600000
500000
400000
300000
200000
100000
0
0
1
2
3
4
5
6
7
8
9

EcAspA gene sequence

ATGTCAAACAACATTCGTATCGAAGAAGATCTGTTGGGTACCAGGGAAGTTCCAGCTGATGCCT
ACTATGGTGTTCACACTCTGAGAGCGATTGTAAACTTCTATATCAGCAACAACAAAATCAGTGAT
ATTCCTGAATTTGTTCGCGGTATGGaAATGGTTAAAAAAGCCGCAGCTATGGCAAACAAAGAGC
TGCAAACCATTCCTAAAAGTGTAGCGAATGCCATCATTGCCGCATGTGATGAAGTCCTGAACAA
CGGAAAATGCATGGATCAGTTCCCGGTAGACGTCTACCAGGGCGGCGCAGGTACTTCCGTAAA
CATGAACACCAACGAAGTGCTGGCCAATATCGGTCTGGAACTGATGGGTCACCAAAAAGGTGA
ATATCAGTACCTGAACCCGAACGACCATGTTAACAAATGTCAGTCCACTAACGACGCCTACCCG
ACCGGTTTCCGTATCGCAGTTTACTCTTCCCTGATTAAGCTGGTAGATGCGATTAACCAACTGCG
TGAAGGCTTTGAACGTAAAGCTGTCGAATTCCAGGACATCCTGAAAATGGGTCGTACCCAGCTG
CAGGACGCAGTACCGATGACCCTCGGTCAGGAATTCCGCGCTTTCAGCATCCTGCTGAAAGAA
GAAGTGAAAAACATCCAACGTACCGCTGAACTGCTGCTGGAAGTTAACCTTGGTGCAACAGCA
ATCGGTACTGGTCTGAACACGCCGAAAGAGTACTCTCCGCTGGCAGTGAAAAAACTGGCTGAA
GTTACTGGCTTCCCATGCGTACCGGCTGAAGACCTGATCGAAGCGACCTCTGACTGCGGCGCTT
ATGTTATGGTTCACGGCGCGCTGAAACGCCTGGCTGTGAAGATGTCCAAAATCTGTAACGACCT
GCGCTTGCTCTCTTCAGGCCCACGTGCCGGCCTGAACGAGATCAACCTGCCGGAACTGCAGGC
GGGCTCTTCCATCATGCCAGCTAAAGTAAACCCGGTTGTTCCGGAAGTGGTTAACCAGGTATGC
TTCAAAGTCATCGGTAACGACACCACTGTTACCATGGCAGCAGAAGCAGGTCAGCTGCAGTTG
AACGTTATGGAGCCGGTCATTGGCCAGGCCATGTTCGAATCCGTTCACATTCTGACCAACGCTT
GCTACAACCTGCTGGAAAAATGCATTAACGGCATCACTGCTAACAAAGAAGTGTGCGAAGGTT
ACGTTTACAACTCTATCGGTATCGTTACTTACCTGAACCCGTTCATCGGTCACCACAACGGTGAC
ATCGTGGGTAAAATCTGTGCCGAAACCGGTAAGAGTGTACGTGAAGTCGTTCTGGAACGCGGT
CTGTTGACTGAAGCGGAACTTGACGATATTTTCTCCGTACAGAATCTGATGCACCCGGCTTACAA
AGCAAAACGCTATACTGATGAAAGCGAACAGTAA

CgPanD gene sequence

ATGCTGCGCACCATCCTCGGAAGTAAGATTCACCGAGCCACTGTCACTCAAGCTGATCTAGATT
ATGTTGGCTCTGTAACCATCGACGCCGACCTGGTTCACGCCGCCGGATTGATCGAAGGCGAAA
AAGTTGCCATCGTAGACATCACCAACGGCGCTCGTCTGGAAACTTATGTCATTGTGGGCGACGC
CGGAACGGGCAATATTTGCATCAATGGTGCCGCTGCACACCTTATTAATCCTGGCGATCTTGTG
ATCATCATGAGCTACCTTCAGGCAACTGATGCGGAAGCCAAGGCGTATGAGCCAAAGATTGTG
CACGTGGACGCCGACAACCGCATCGTTGCGCTCGGCAACGATCTTGCGGAAGCACTACCTGGA
TCCGGGCTTTTGACGTCGAGAAGCATTTAG

References

1. Z. Liu, L. Yu, L. Zhou and Z. Zhou, *Appl. Environ. Microbiol.*, 2019, **85**, e01327-01319.
2. W. Cui, Z. Shi, Y. Fang, L. Zhou, N. Ding and Z. Zhou, *Biotechnol. Lett.*, 2014, **36**, 121-126.
3. X.-Y. Zhang, X. Wang, N.-W. Li, Z.-W. Guo, M.-H. Zong and N. Li, *ChemCatChem*, 2020, **12**, 3257–3264.