Supporting information

A Light-controlled Biocatalytic System for Precise Regulation of Enzymatic Decarboxylation

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Experimental Procedures

1. General information

All chemicals and regents, including antibiotics, were of analytical grade and were purchased from J&K Chemical Ltd. (Beijing, China) and Sigma-Aldrich (Shanghai, China). Phanta® Max SuperFidelity DNA polymerase was purchased from Vazyme Biotech Co. Ltd. (Nanjing, China). DpnI and dNTPs were obtained from Fermentas Co. Ltd. (Shenzhen, China). All oligonucleotides were synthesized in high-performance liquid chromatography (HPLC)-purity by Sangon Biotec Corporation (Shanghai China).

2. Genes, plasmids and microorganisms

The gene of a fatty acid photodecarboxylase was codon-optimized and synthesized by Tsingke company (Hangzhou, China) and inserted into the expression vector pET-28b. The genetic circuit of OptoFAP was constructed on the plasmid pACYCduet-1. Site-directed mutagenesis (SDM) or site-saturated mutagenesis (SSM) was preformed using QuikChange Kit with Phanta Max Super-Fidelity DNA Polymerase (Vazyme, Nanjing, China), and the primers were listed in **Table S1**. The PCR reaction mixture (50 μL) contained: 20 μL ddH_2O, 25 μL Phanta Max buffer, 1 μL dNTP, 1 μL forward primers, 1 μL reverse primer and 1 μL DNA Polymerase. The PCR program was operated as below: 95 °C for 5 min (1 cycle); 90 °C for 30 s, 60-70 °C for 30 s, 72 °C for 10 min (30 cycles); and following by 1 cycle of 72 °C for 10 min. The PCR products were analyzed by agarose gel electrophoresis and digested at 37 °C for 15 min (1 μL DpnI and 5 μL cut-smart buffer with 50 μL PCR products). 10 μL DpnI-digested PCR products were transformed into *E. coli* BL21(DE3) competent cell. The transformation mixture was incubated with 600 μL of LB medium at 37 °C with shaking of 200 rpm/min for 1h, and then spread on LB-agar medium containing 50 μg/mL kanamycin and 50 μg/mL ampicillin. A single colony from a plate was incubated in 5 mL of M9 medium (with 50 μg/mL kanamycin and 50 μg/mL ampicillin) at 37 °C for 8 h, and the plasmid was extracted by Plasmid Mitprep Kits and was confirmed by DNA sequencing (TsingKe Inc.). Positive strains verified by sequencing were stored at -80 °C freezer.

Table S1. Primers used for site-directed mutagenesis

Primer	Nucleotide sequence (5'-3')
G402Y-F	CCCGGACtatGTTAGCACCTACGTTCGTT
G402Y-R	GTAGGTGCTAACataGTCCGGGTCCAGCG

The sequence of genetic circuit of OptoFAP (Yellow highlight indicates P_{lacIQ} promoter; green highlight indicates RBS; pink highlight indicates the gene of YF1-FixJ; red highlight indicates P_{FixK2} promoter; brown highlight indicates the represor cI-LVA; grey highlight indicates T_{B0015} terminator; dark green highlight indicates P_R promoter; cyan highlight indicates *lacI_ssrA*-(AAV); dark yellow highlight indicates T_{T7} terminator; violet highlight indicates P_{T7} promoter; blueish green highlight indicates *CvFAP*.

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3. Gene expression and protein production

The recombinant *E. coli* cells were inoculated into 10 mL M9 minimal medium containing 50 μg/mL kanamycin and grown at 37 °C for 12 h under light. Then, 2 mL of cell culture was transferred into 100 mL of fresh M9 minimal medium containing 50 μg/mL kanamycin and incubated for another 2-3 h. The gene of *Cv*FAP expression was induced by 0.1 mM IPTG or darkness when the OD_{600} at 0.6. To obtain the purified *Cv*FAPs, cells were harvested, after 18 h of incubation at 20 °C, by centrifugation at 8,000 rpm and 4 °C for 10 min. The cells were resuspended in 100 mM phosphate buffer (pH 8.0). The cells were sonicated and then centrifuged at 12,000 rpm for 10 min at 4 °C, which yielded a clear supernatant as crude enzyme solution was stored at -80 °C for further experiments. The purification process of *Cv*FAP was performed as previously reported[1]. The precipitate was resuspended in buffer A (50 mM phosphate buffer pH 7.0 containing 500 mM NaCl). The cells were disrupted by ultrasonication for 10 min (1 sec on, 1 sec off), and the suspension was centrifuged at 12,000 rpm for 10 min to yield a clear lysate. The N-terminal His_6-tagged *Cv*FAP was purified using IMAC on a Nickel-NTA column (Bio-Rad, CA) by eluting with buffer B (buffer A containing 500 mM imidazole).

4. SDS-PAGE determination of *Cv*FAP protein

32 μL of the enzyme solution and 8 μL protein dye were added to 1.5 mL EP tube and bathed in boiling water for 10 min. After confirming that the electrophoresis tank does not leak, 7-8 μL sample supernatant was added into the perforation of the prefabricated adhesive. The electrode buffer was poured into the electrophoresis tank until it is flush with the notch, and then electrophoresis was run at 100 V for 40 min until the protein sample reaches the red line below. The gel was carefully removed, stained and eluted, and observed under an ULTRAVIOLET lamp with a white background.

5. Product detection methods

Method1 for detection of amino acids: High performance liquid chromatography (HPLC) equipped with U3000 with C18 column (Unitary C 18, 5 μm, 100 A, 4.6 mm × 250 mm) was carried out for detection of the concentration and *ee* of amino acids that was derivatized with *o*-phthalaldehyde (OPA) and N-acetyl-L-cysteine (NAC)[2]. The fluorescence wavelength was set as λ_{Ex} = 340 nm and λ_{Em} = 450 nm. The column temperature and the flow rate of mobile phase were set as 30 °C and 1.0 mL/min, respectively. Mobile phase (pH=3.7) was 50 mM ammonium acetate combined 10% (v/v) methanol.

Method 2 for detection of hydroxyl acids[3]: The 200 μL of reaction solution were added to a 1.5 mL centrifuge tube and were acidify by adding 30 μL HCl (6 M) to stop the reaction. And then 1000 μL analytical pure ethyl acetate were added in the mixture. Each tube was turned up and down gently 50 times to mix. After centrifuging at 8000 rpm for 2 min, 400 μL supernatant were removed into a new centrifuge tube. In addition, 400 μL ethyl acetate, 30 μL methanol and 15 μL diazomethane were added for derivatization. Finally, 5 μL 100 Mm N-octanol was added for gas phase analysis as internal standard. The peak area ratio was X-axis, and the mole ratio was Y-axis. The gas phase procedure was performed at 120 °C for 15 minutes at the initial temperature, and then at 20 °C/min to 200 °C for 3 min (flow rate: 0.4 mL/min; shunt ratio: 30: 1; column: BGB-174).

6. Molecular docking and in silico analysis

To prepare the ligand structure, ChemBioDraw was used to create the ligand molecule and the energy minimization. Before docking, the receptor protein (PDB: 5NCC) was added hydrogens first[4], and then saved as PDBQT format. Then, by setting a number of grid points, the spacing and the center position of the Grid Box, the proper search space was fixed which covers the entire substrate pocket of the receptor protein. The

molecular dockings were run by Autodock Vina yielding the location of the ligand structure and the corresponding receptor-ligand affinity[5]. 20 consecutive runs were performed and the highest ranked score from each run was used to calculate the average score of each flexible ligand configuration. The optimal configurations of resulted substrate-enzyme complexes were visualized by PyMOL software.

7. Scale-up of chemical production in a 5 L bioreactor

To validate our circuits for L-PPT production on a gram scale density conditions and larger culture volumes, we grew a 5-ml overnight culture of *E. coli* harboring OptoFAP circuit in M9 media under blue light at 37 °C. We then set up a BioFlo120 system with a 5 L bioreactor (Baoxin Biotech, Shanghai, China) and added 2 L of sterile media (M9 + 5% glucose + kanamycin + ampicillin). The reactor was set to 37 °C, pH 7.5 (maintained using a base feed of glycerin or ammonia, and a minimum dissolved oxygen percentage of 20 (maintained by adjusting the agitation rate between 200 and 800 r.p.m. and by injecting air at a flow rate of 1 VVM. Blue LED panels were placed in a rectangular formation ~20 cm from the reactor such that the light illuminated ~75% of the bulk surface area at an intensity of 100–150 μmol m^{-2} s^{-1}. The *E. coli* cells were grown for ~4 h under blue light until reaching an OD_{600} of 0.6. The enzyme production was triggered by turning off lights and wrapping bioreactor with aluminum foil and black cloth. Meanwhile, 14.4 g *rac*-PPL was added into the bioreactor as the substrate for the one-pot system. Thereafter, every half an hour, 1 ml samples was taken out form the system for HPLC analysis as described above.

Results and Discussion

8. Testing of *E. coli* containing OptoFAP circuit under different conditions

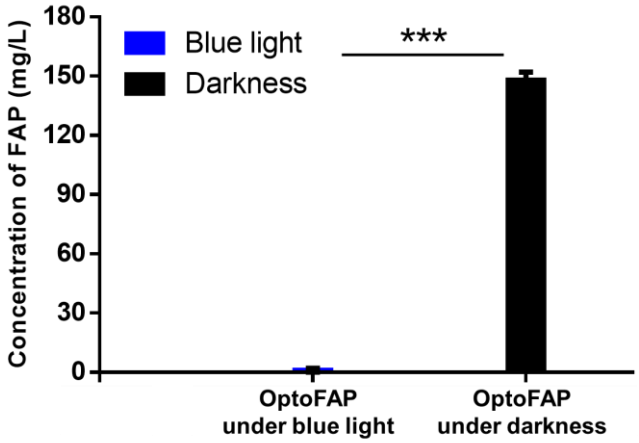

Figure S1. *Cv*FAP expression levels under different conditions. *Cv*FAP expressed from OptoFAP circuit under continuous blue light or continuous darkness after OD600 reached 0.6. *** $P < 0.001$. Statistics are derived using a two-sided t-test. Data are representative of n = 5 independent experiments.

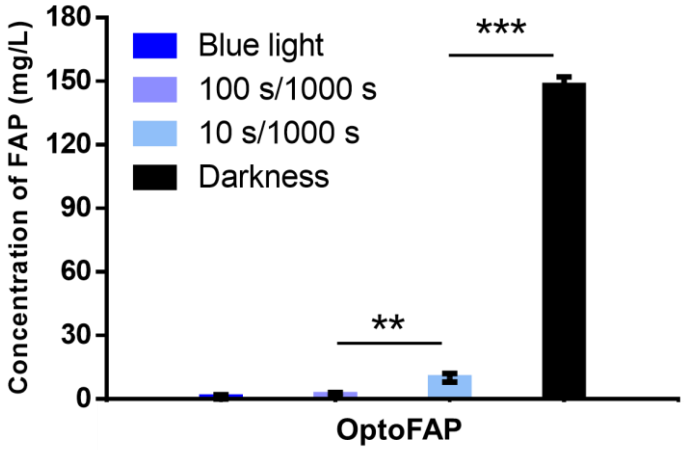

Figure S2. *Cv*FAP expression levels under different duty cycles of light or darkness. From left to right: full light, 100 s on/1,000 s, 10 s on/1,000 s and full darkness. ** $P < 0.01$, *** $P < 0.001$. Statistics are derived using a two-sided t-test. Data are representative of n = 5 independent experiments.

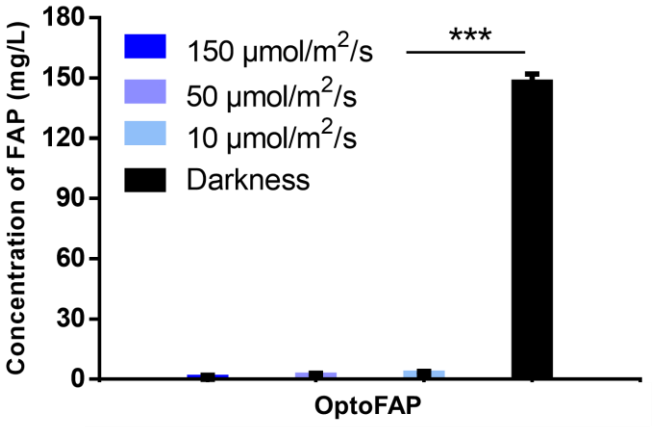

Figure S3. Tunability of OptoLAC circuits with light intensity. From left to right: 150 µmol/m^2/s, 50 µmol/m^2/s, 10 µmol/m^2/s and full darkness. *** P < 0.001. Statistics are derived using a two-sided t-test. Data are representative of n = 5 independent experiments.

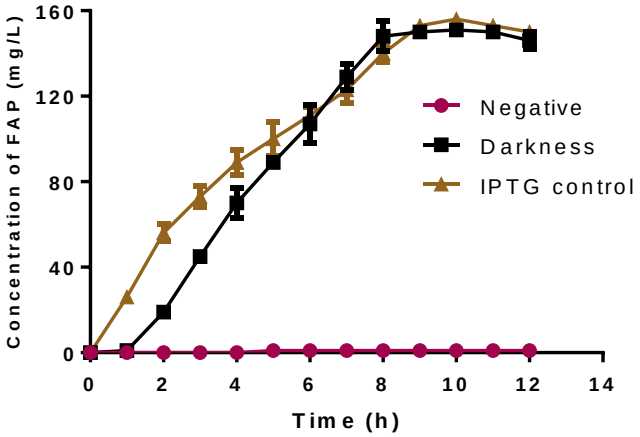

Figure S4. Time course of *Cv*FAP expression from OptoFAP circuit under darkness or IPTG. *Cv*FAP expressed from OptoFAP circuit under darkness or 0.1 mM IPTG after OD_{600} reached 0.6. Negative control: lacking of the *Cv*FAP FAP Data are representative of n = 5 independent experiments.

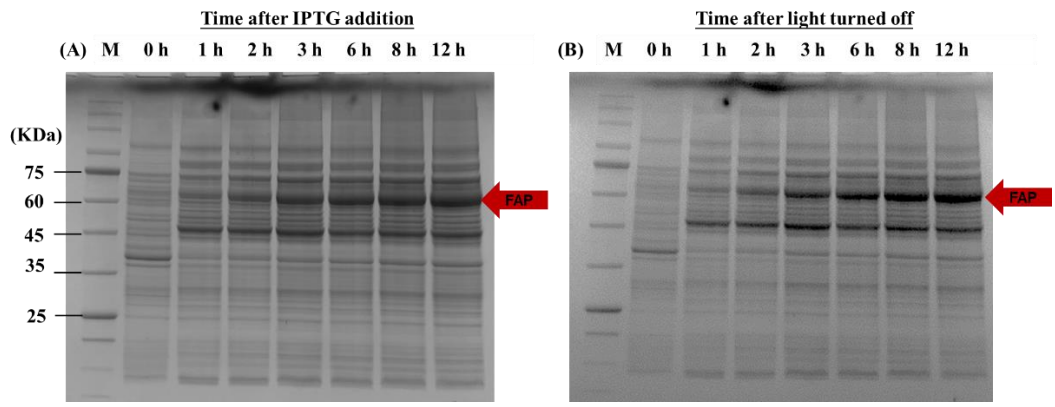

Figure S5. Time course of *Cv*FAP production in SDS-PAGE. (A) CvFAP production by adding 0.1 mM IPTG after OD600 reached 0.6; (B) CvFAP production under darkness after OD600 reached 0.6.

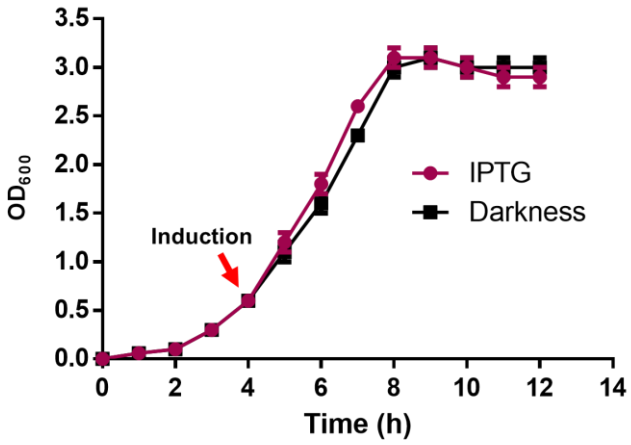

Figure S6. The growth curve of *E. coli* BL21(DE3). The stains were grown up under light until OD_{600} reached 0.6. The red curve presents the growth curve that 0.1 mM IPTG induced for protein production at 4 h, while the black curve presents the growth curve that produced by darkness induction.

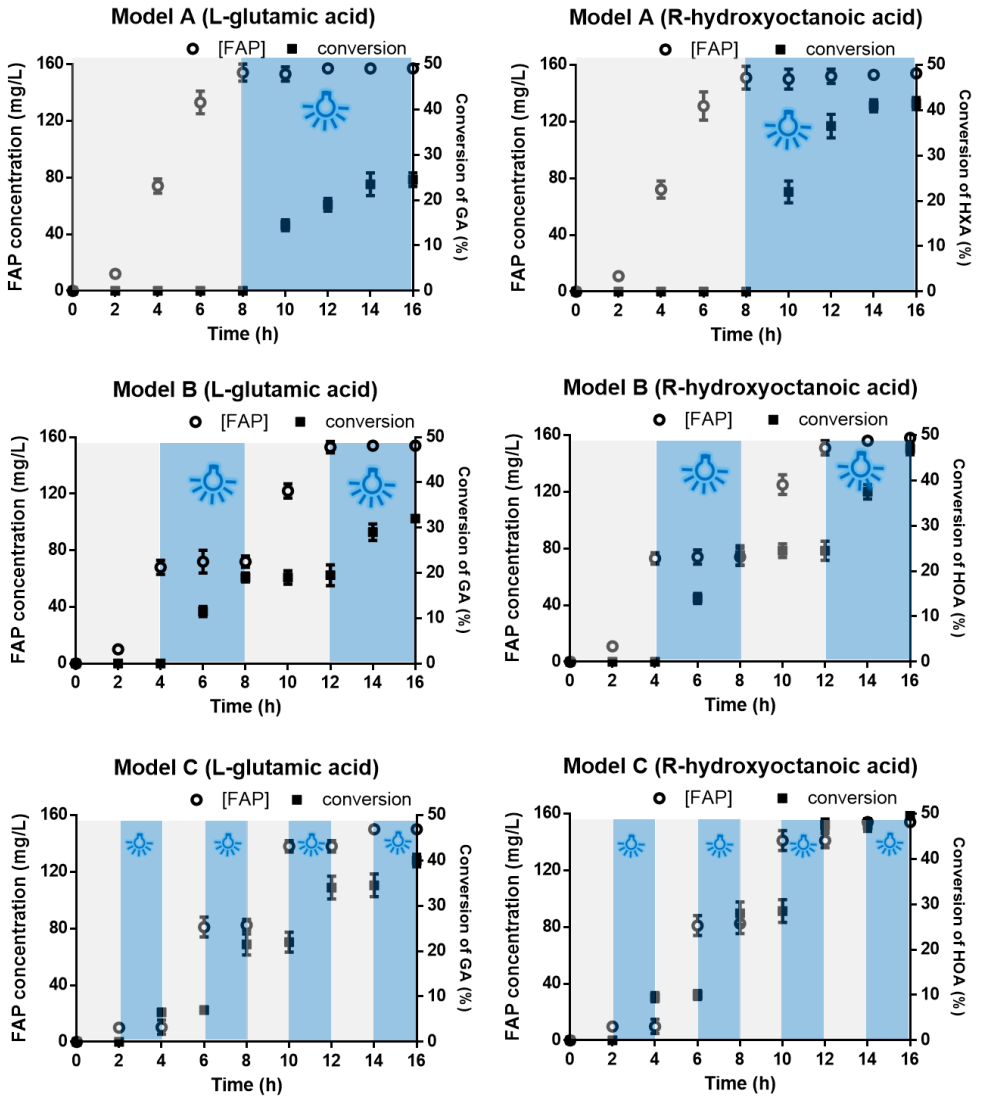

Figure S7. Time course curves of FAP, hydroxy acid and amino acid in the developed biocatalytic system OptoFAP. The concentrations of enzyme and conversions v.s. time were shown in three lines: illumination in Model A (the first row), illumination in Model B (the second row), illumination in Model C (the third row).

Table S2. The efficiency comparison of classical stepwise system and developed one-pot system

System	The procedure	Gene expression	Conversion of the tested substrates	The *e.e.* values of the products	The total time from gene expression to products isolation
Stepwise system	Gene expression-biocatalyst isolation-biotransformation in separate pots	chemical induction/ irreversible	25%-42%	90-99%	>24 h (8 h for gene expression and 16 h for biotransformation and the time for cell isolation)
One-pot system	Gene expression and biotransformation in one pot and controlled by illumination	Reversibly controlled by light on or off	46%-49%	92-99%	16 h (gene expression and biotransformation in one pot)

9. The designed bioreactor illumination setup

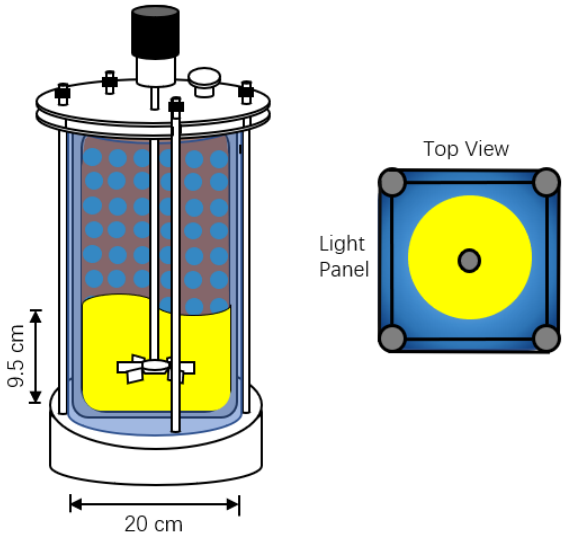

Figure S8. Bioreactor illumination setup. The side- and top-view of a light-controlled one-pot system in a 5-L bioreactor (with 2 L medium), with four light panels arranged rectangularly ~20 cm from the glass vessel wall such that the light intensity at the vessel surface is between 100-150 μmol/m^2/s.

10. The NMR data of the compounds used in this study

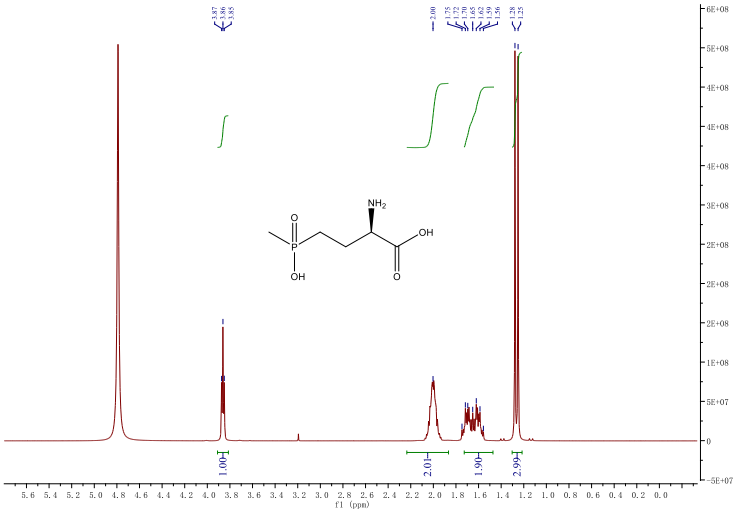

Figure S9. ^{1}H NMR spectrum of produced L-PPT. ^{1}H NMR (500 MHz, D_2O): δ 3.86 (t, *J* = 6.0 Hz, 1H; CH), 2.00 (s, 2H; CH_2), 1.80 – 1.50 (m, 2H; CH_2), 1.26 (d, *J* = 13.8 Hz, 3H; CH_3).

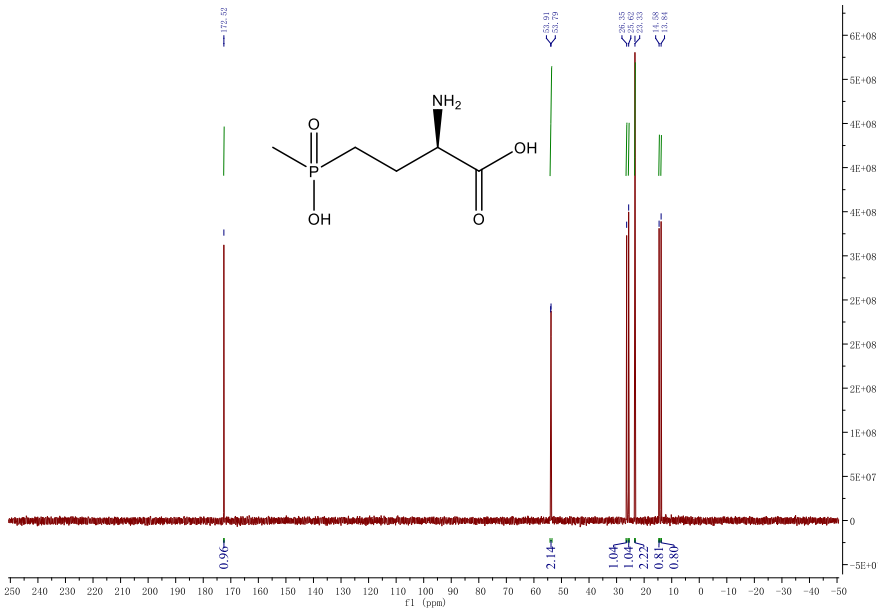

Figure S10. ^{13}C NMR spectrum of produced L-PPT. ^{13}C NMR (126 MHz, D_2O): δ 172.52 (s; C), 53.85 (d, *J* = 15.6 Hz; CH), 26.35 (s; CH_2), 23.33 (s; CH_3).

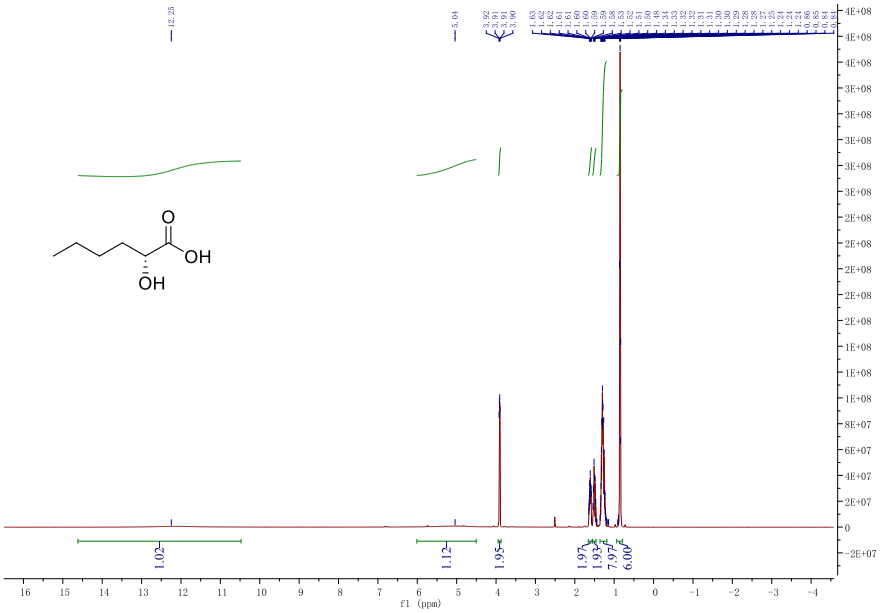

Figure S11. ^{1}H NMR of (R)-2-hydroxyhexanoic acid (500 MHz, DMSO): δ 12.25 (s, 1H; OH), 5.04 (s, 1H; OH), 3.91 (dd, *J* = 7.8, 4.5 Hz, 2H; CH), 1.66 – 1.56 (m, 2H; CH_2), 1.55 – 1.46 (m, 2H; CH_2), 1.36 – 1.19 (m, 8H; CH_2), 0.94 – 0.79 (m, 6H;CH_3).

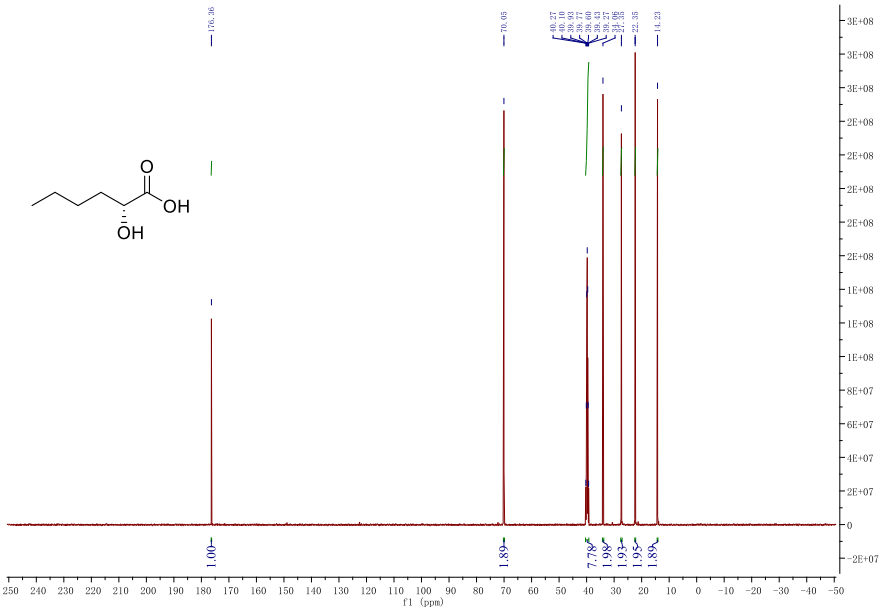

Figure S12. ^{13}C NMR of (R)-2-hydroxyhexanoic acid (126 MHz, DMSO): δ 176.36(s; C), 70.05(s; CH), 34.06(s; CH_2), 27.35(s; CH_2), 22.35(s; CH_2), 14.23(s; CH_3).

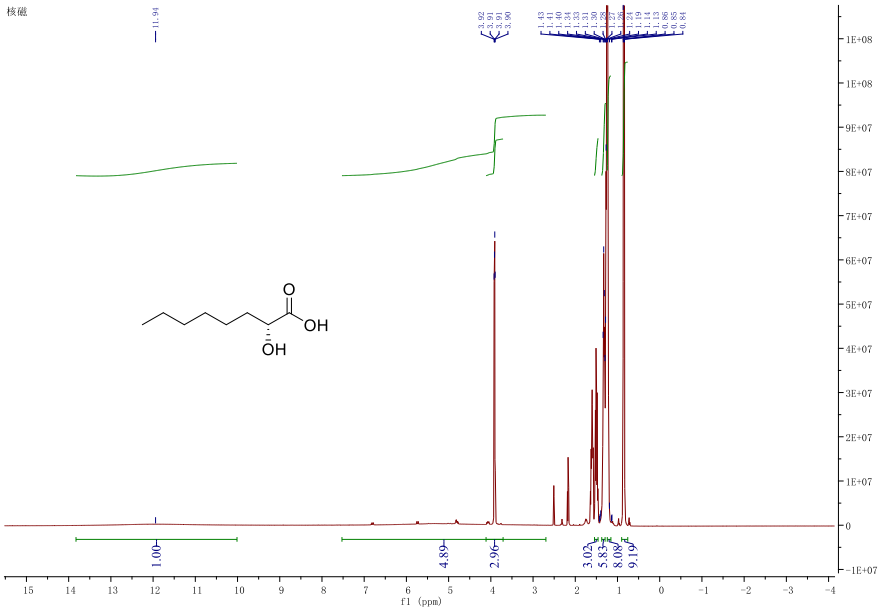

Figure S13. ^{1}H NMR of (R)-2-hydroxyoctanoic acid (500 MHz, DMSO): δ 11.94 (s, 1H; OH), 3.91 (dd, *J* = 7.8, 4.5 Hz, 8H; CH_2), 1.50 (td, *J* = 14.6, 7.5 Hz, 3H; CH), 1.32 (dd, *J* = 14.4, 7.5 Hz, 6H; CH_2), 1.21 (d, *J* = 24.5 Hz, 8H; CH_2), 0.85 (t, *J* = 6.9 Hz, 9H; CH_3).

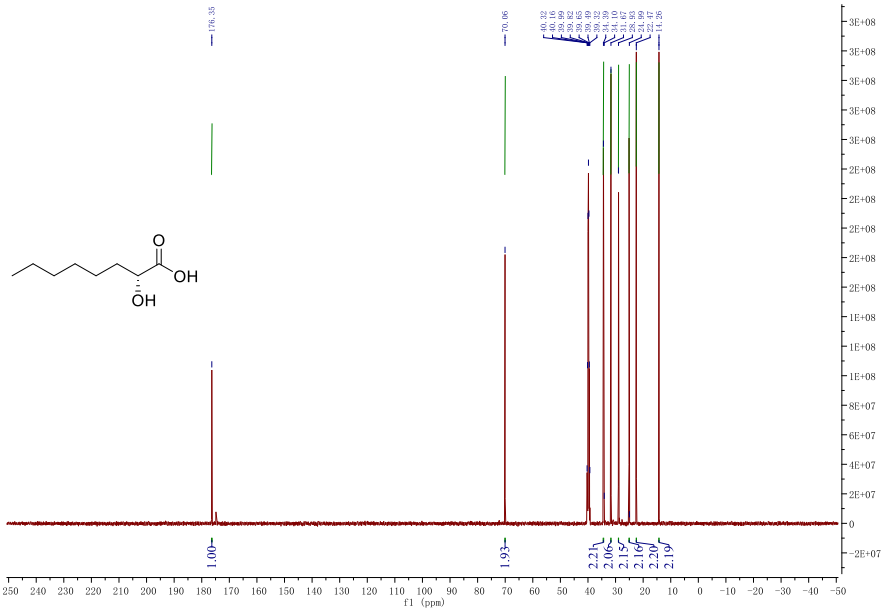

Figure S14. ^{13}C NMR of (R)-2-hydroxyoctanoic acid (126 MHz, DMSO): δ 176.35(s; C), 70.06(s; CH), 34.39(s; CH_2), 31.67(s; CH_2), 28.93(s; CH_2), 24.99(s; CH_2), 22.47(s; CH_2), 14.26(s; CH_3).

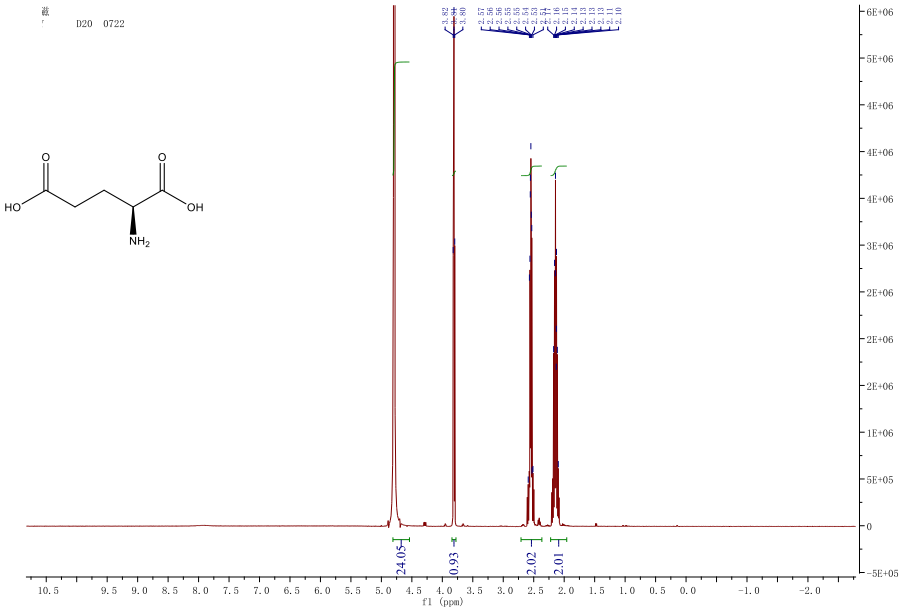

Figure S15. ^{1}H NMR of L-glutamic acid (500 MHz, D_2O): δ 4.79 (s, 2H;CH_2), 3.81 (t, *J* = 6.4 Hz, 1H; CH), 2.71 – 2.37 (m, 2H; CH_2), 2.22 – 1.96 (m, 2H; CH_2).

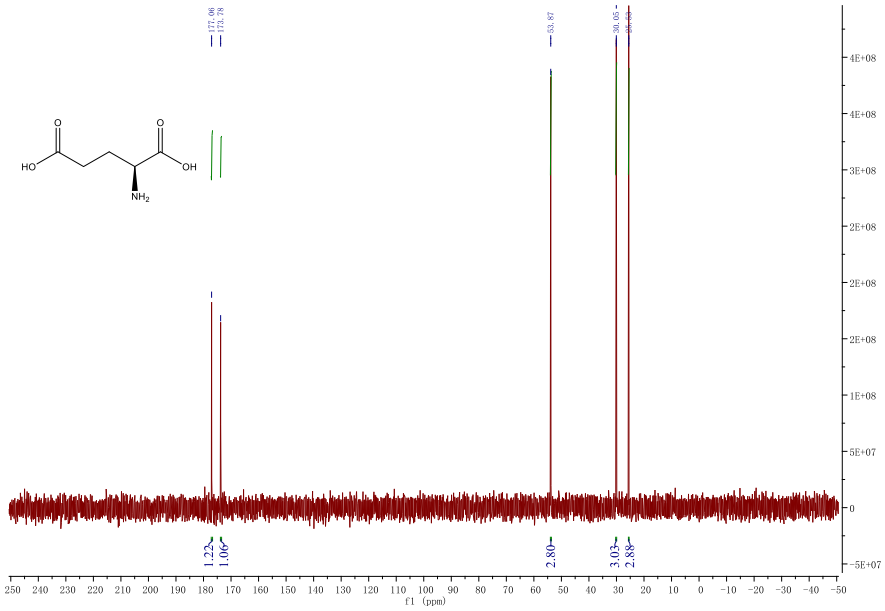

Figure S16. 13C NMR of L-glutamic acid (126 MHz, D_2O): δ 177.06 (s; C), 173.78 (s; C), 53.87 (s; CH), 30.05 (s; CH_2), 25.53 (s; CH_2)

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