Table S1 Strains and plasmids used in this study

Strain or plasmid	Relevant genotype/description [a]	Source [b]
Escherichia coli DH5α	F^- Φ80d/lacZ⊿M15, ⊿(lacZYA-argF) U169, recA1, endA1, hsdR17 (rK^-, mK^+), phoA, supE44, λ^-, thi-1, gyrA96, relA1 (CICIM B0006)	CICIM-CU
Bacillus licheniformis CICIM B6902		
45A0	Wild type (CICIM B6902)	CICIM-CU
45A1	Recombinant strain harboring pMA5, Kan^r	This work
AbTH	Recombinant strain harboring pMA5-*AbTH*, Kan^r	This work
SeTH	Recombinant strain harboring pMA5- *SeTH*, Kan^r	This work
BcTH	Recombinant strain harboring pMA5- *BcTH*, Kan^r	This work
EsTH	Recombinant strain harboring pMA5- *EsTH*, Kan^r	This work
SrTH	Recombinant strain harboring pMA5- *SrTH*, Kan^r	This work
TbTH	Recombinant strain harboring pMA5- *TbTH*, Kan^r	This work
TcTH	Recombinant strain harboring pMA5- *TcTH*, Kan^r	This work
CsSPR	Recombinant strain harboring pMA5- *CsSPR*, Kan^r	This work
CtSPR	Recombinant strain harboring pMA5- *CtSPR*, Kan^r	This work
PdSPR	Recombinant strain harboring pMA5- *PdSPR*, Kan^r	This work
RsSPR	Recombinant strain harboring pMA5- *RsSPR*, Kan^r	This work
VsSPR	Recombinant strain harboring pMA5- *VsSPR*, Kan^r	This work
XpSPR	Recombinant strain harboring pMA5- *XpSPR*, Kan^r	This work
Escherichia coli Bl21 (DE3)		Laboratory stock
EcDHPR	Recombinant strain harboring pET28a-EcDHPR, Kan^r	This work
Plasmids		
pMA5	*E. coli-Bacillus* shuttle vector, Amp^r in *E. coli*, Kan^r in *B. subtilis*	Laboratory stock
pMA5-*AbTH*	pMA5 carrying the *AbTH* gene, Amp^r, Kan^r	This work
pMA5- *SeTH*	pMA5 carrying the *SeTH* gene, Amp^r, Kan^r	This work
pMA5- *BcTH*	pMA5 carrying the *BcTH* gene, Amp^r, Kan^r	This work
pMA5- *EsTH*	pMA5 carrying the *EsTH* gene, Amp^r, Kan^r	This work
pMA5- *SrTH*	pMA5 carrying the *SrTH* gene, Amp^r, Kan^r	This work
pMA5- *TbTH*	pMA5 carrying the *TbTH* gene, Amp^r, Kan^r	This work
pMA5- *TcTH*	pMA5 carrying the *TcTH* gene, Amp^r, Kan^r	This work
pMA5- *CsSPR*	pMA5 carrying the *CsSPR* gene, Amp^r, Kan^r	This work
pMA5- *CtSPR*	pMA5 carrying the *CtSPR* gene, Amp^r, Kan^r	This work
pMA5- *PdSPR*	pMA5 carrying the *PdSPR* gene, Amp^r, Kan^r	This work
pMA5- *RsSPR*	pMA5 carrying the *RsSPR* gene, Amp^r, Kan^r	This work
pMA5- *VsSPR*	pMA5 carrying the *VsSPR* gene, Amp^r, Kan^r	This work
pMA5- *XpSPR*	pMA5 carrying the *XpSPR* gene, Amp^r, Kan^r	This work
pET28a-EcDHPR	pET28a carrying the EcDHPR gene, Amp^r	This work

[a] Kan^r, kanamycin resistance; Amp^r, ampicillin resistance. For recombinant mutants, 30 μg/mL kanamycin or 100 μg/mL ampicillin were used when necessary.

[b] CICIM-CU, Culture and Information Center of Industrial Microorganisms of China Universities.

Table S2 Primers used in this study

Primers	Sequences of primers (5′ to 3′) [a]	Restriction sites
AbTH-F/R	GGAATTCCATATGATGAACGACGAATACGAGCAGGAG/CCGGATCCTTAA	*Nde* Ⅰ/*Bam*H Ⅰ

	AGTCTTGTCAGAAAGCGGTTCAGTTC	
SeTH-F/R	GGAATTCCATATGATGGACACGGATACGATGATCAGAC/CCGGATCCTTAT GAACAAGGTGATGCCATTGCTCC	*Nde* Ⅰ/*Bam*H Ⅰ
BcTH-F/R	GGAATTCCATATGATGTTCGAAGAAGGCCAACTTTACG/CCGGATCCTTAT GCTGCTTGTGCTGCTAATCTTC	*Nde* Ⅰ/*Bam*H Ⅰ
EsTH-F/R	GGAATTCCATATGATGTTCGAAGAAGCGCAGTATTTTG/CCGGATCCTTAA TCAACTGCTGCTGCTCTAAGTCC	*Nde* Ⅰ/*Bam*H Ⅰ
SrTH-F/R	GGAATTCCATATGATGTTCGAAGAAGCGCAGTATTTTG/CCGGATCCTTAT CCTTGTCTTGCGTCTGCAATAAGTC	*Nde* Ⅰ/*Bam*H Ⅰ
TbTH -F/R	GGAATTCCATATGATGTTCGAAGAAGCGCAGTATTTTG/CCGGATCCTTAT CTTCCGTCTGCTTCTCTAACAAGTC	*Nde* Ⅰ/*Bam*H Ⅰ
TcTH-F/R	GGAATTCCATATGATGATGGAAGAAGCGCAGTACTTTG/CCGGATCCTTAT GATCCTGCTGATGCTGCTAATGC	*Nde* Ⅰ/*Bam*H Ⅰ
CsSPR-F/R	GGAATTCCATATGATGAGAAGATCAGCACTGATCACGG/CCGGATCCTTA AAGTGCTCCTGCTGCCGGAA	*Nde* Ⅰ/*Bam*H Ⅰ
CtSPR-F/R	GGAATTCCATATGATGAAACATATCCTGCTGATCACGG/CCGGATCCTTAC AGATCGCCTGCTGTCGG	*Nde* Ⅰ/*Bam*H Ⅰ
PdSPR-F/R	GGAATTCCATATGATGCAGAAAATCCTTATCACGGGC/CCGGATCCTTAAA GCTGGCTGTAGTTCGGGTTG	*Nde* Ⅰ/*Bam*H Ⅰ
RsSPR-F/R	GGAATTCCATATGATGACAAGAACACTGGCAGCAC/CCGGATCCTTATGC GATGTTTCTCAGATCGTCCAG	*Nde* Ⅰ/*Bam*H Ⅰ
VsSPR-F/R	GGAATTCCATATGATGAACCAGAAGACGTTCATGGTC/CCGGATCCTTACG GGTAGTTCTTTTCGACAAGCG	*Nde* Ⅰ/*Bam*H Ⅰ
XpSPR-F/R	GGAATTCCATATGATGAAGATCCTGGTCACGGCG/CCGGATCCTTAGTACT TCTCGATGAACTTGAACTCGTTC	*Nde* Ⅰ/*Bam*H Ⅰ
EcDHPR-F/R	CGGAATTCTAATGGATATCATTTCTGTCGCCTTAAAGCG/CCCAAGCTTTT AGTGGTGGTGGTGGTGGTGCACTTCGGTTAAGGTGATGTTTTGCGG	*Eco*R Ⅰ/*Hin*dⅢ

[a] The restriction sites is underlined.

Table S3 Gene accession numbers of synthesized nucleotide sequences

Gene	Gene accession number	Species source
AbTH	MT835273	*Actinobacteria bacterium*
SeTH	MT835274	*Saccharothrix espanaensis*
BcTH	MT835275	*Beutenbergia cavernae*
FsTH	MT835276	*Frankia sp. EAN1pec*
SrTH	MT835277	*Streptosporangium roseum*
TbTH	MT835278	*Thermobispora bispora*
TcTH	MT835279	*Thermomonospora curvata*
CsSPR	MT835280	*Candidatus Synechococcus spongiarum*
CtSPR	MT835281	Chlorobaculum thiosulfatiphilum
PdSPR	MT835282	*Photobacterium damselae*
RsSPR	MT835283	*Ralstonia solanacearum*
VsSPR	MT835284	*Vibrio sp.* C7
XpSPR	MT835285	*Xenorhabdus poinarii*

Table S4 Enzyme activities and protein concentrations during purification process

	Protein concentration (mg/L)	Enzyme activity (nmol/min/mL)	Total enzyme activity (nmol/min)	Specific enzyme activity (nmol/min /mg)	Protein recovery (%)	Purification folds
Crude enzyme	254	4.58	915.47	18.16	100	1
Concentration by freeze drying	2492	42.15	800.87	16.91	87.48	0.93
Ion exchange chromatography	44.29	6.41	493.82	144.80	53.24	7.97

Table S5 *K*i constant comparison between crude enzyme and purified enzyme

	Crude enzyme SrTH	Purified protein SrTH
Total protein content	1.25 g/L	44.29 mg/L
The ratio of target protein	2.7 %	21.8 %
Target protein content	34.06 mg/L	9.66 mg/L
*K*i of target protein	113 ± 18 μM	98 ± 16 μM

Fig. S1 Ramachndran plot analysis for SrTH protein model.

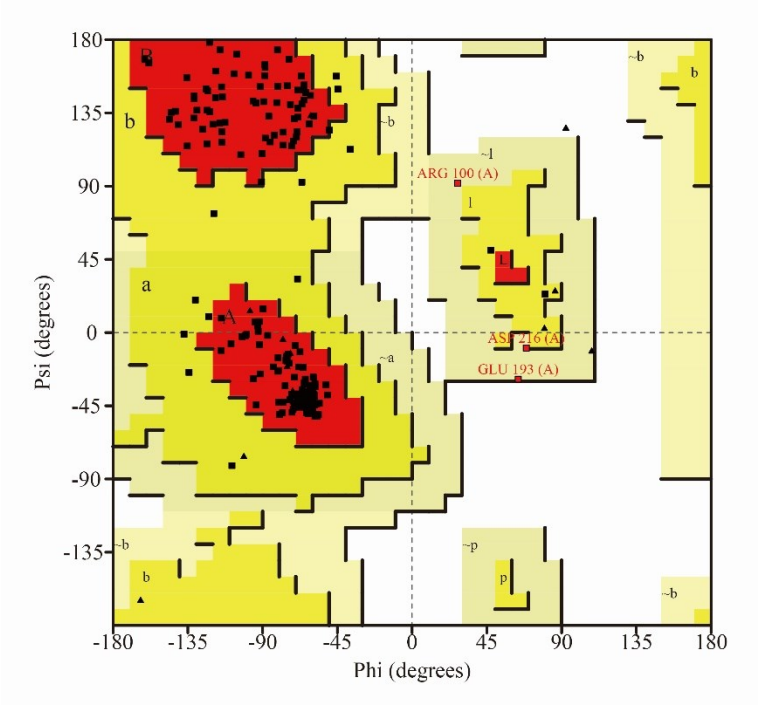

Fig. S1 Ramachndran plot analysis for SrTH protein model. The phi-psi torsion angle of the protein is shown in the Ramachandran plot which depicts that 93.6% of the amino acid residues are in the core region (red), 5.0% residues in allowed (yellow) and 1.4% in generously allowed region.

Fig. S2 Oxidation process analysis of ascorbic acid and L-Dopa under natural condition.

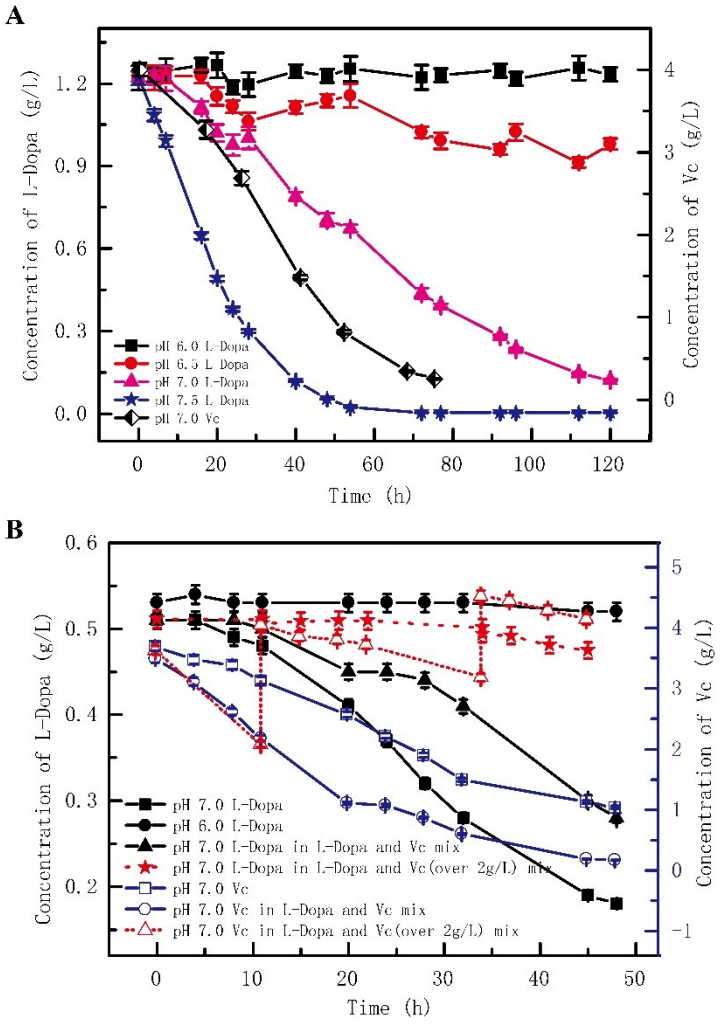

Fig. S2 Oxidation process analysis of ascorbic acid and L-Dopa under natural condition. The samples in 50 mmol/L sodium phosphate buffer were shaked at 37℃, 180 rpm and analyzed by HPLC. A: The oxidation rate of L-Dopa at different pH. The oxidation rate of L-Dopa and ascorbic acid will increase with the pH of the solution increases.B The effect of ascorbic acid on the oxidation of L-Dopa. Ascorbic acid can alleviate the oxidation of L-Dopa but cannot completely prevent it. When the mass ratio of ascorbic acid to L-Dopa is over 4:1, 90% dopa will not be oxidized at pH 7.0.

Fig. S3 Inhibition analysis during the crude enzyme reaction of TcTH by 7,8-dihydrobiopterin

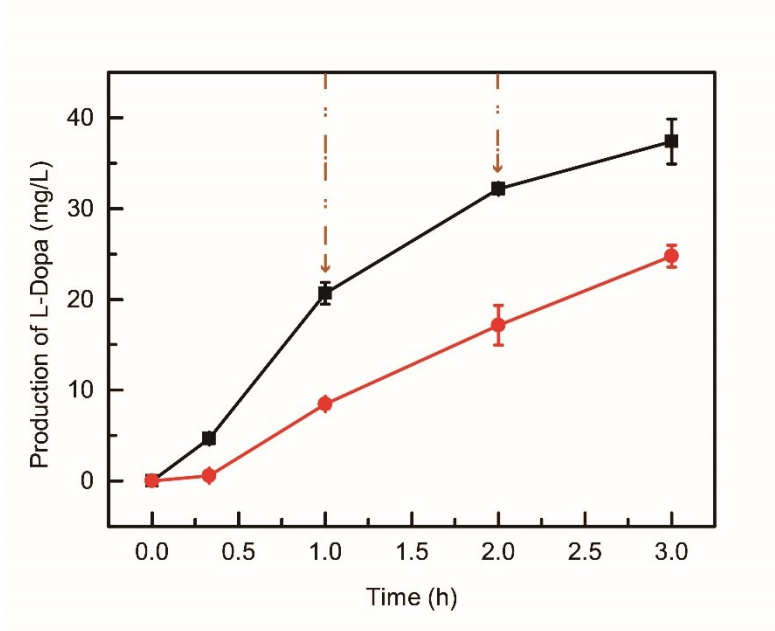

Fig. S3 Inhibition analysis during the crude enzyme reaction of TcTH by 7,8-dihydrobiopterin. 1 mmol/L BH2 was added into the reaction (●) with adding equal volume of ddH_2O as control (■). In the reaction, we supplemented with 1.5 g/L tyrosine, 5 g/L ascorbic acid, 100 μmol/L Fe^{2+} and 1 g/L crude enzyme powder containing TcTH. 1 g/L crude enzyme powder containing TcTH and 0.25 mmol/L BH4 were supplemented in the solution per 1 hour.

Fig. S4 The regeneration pathway of BH4 in eukaryotes

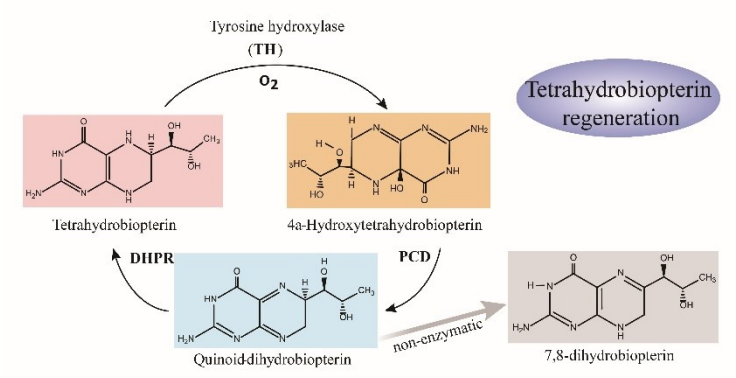

Fig. S4 The regeneration pathway of BH4 in eukaryotes.

Fig. S5 High performance liquid chromatography peaks during BH4 oxidation analysis under natural condition.

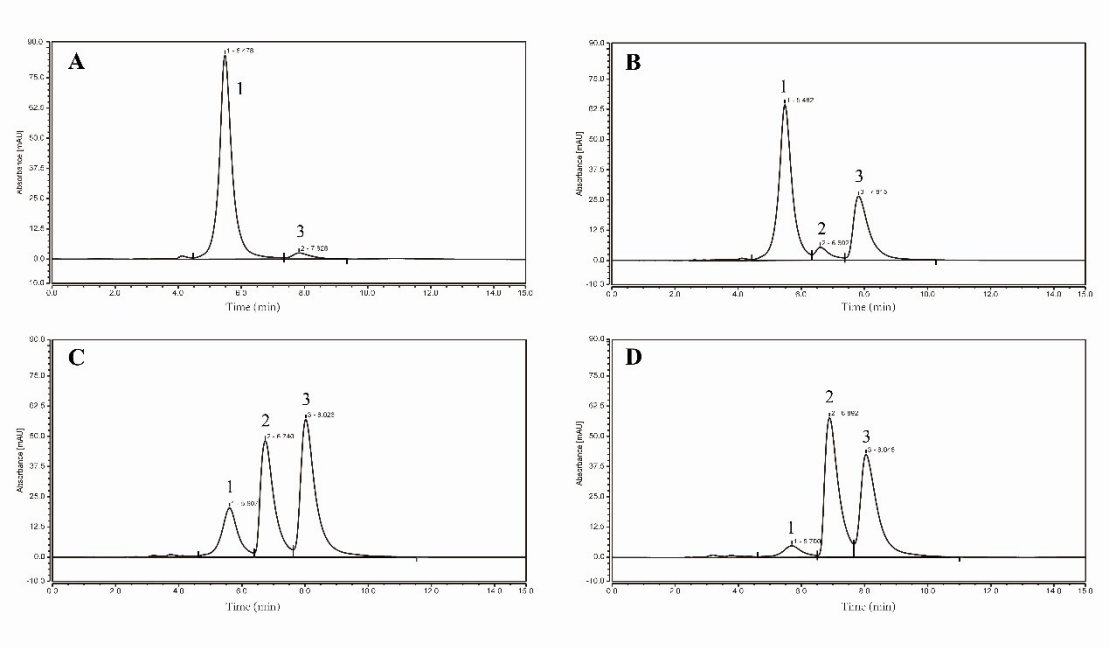

Fig. S5 High performance liquid chromatography peaks during BH4 oxidation analysis under natural condition. PeaK 1 BH4；PeaK 2 BH2；PeaK 3 q-BH2. A: 0 h; B: 3 h; C: 15 h; D: 18 h.

Fig. S6 Product analysis during BH4 oxidation analysis under natural condition.

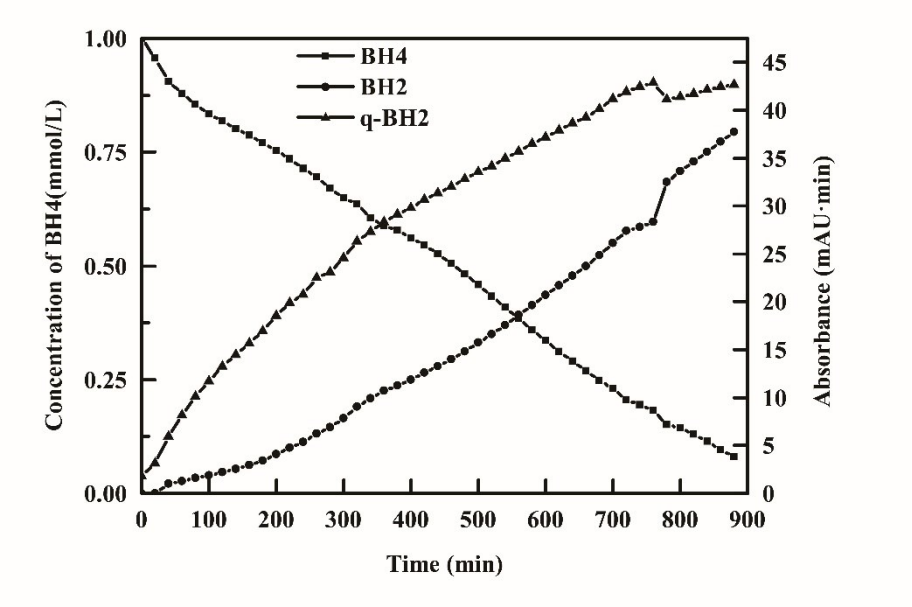

Fig. S6 Product analysis during BH4 oxidation analysis under natural condition.

Fig. S7 Preparation of BH2

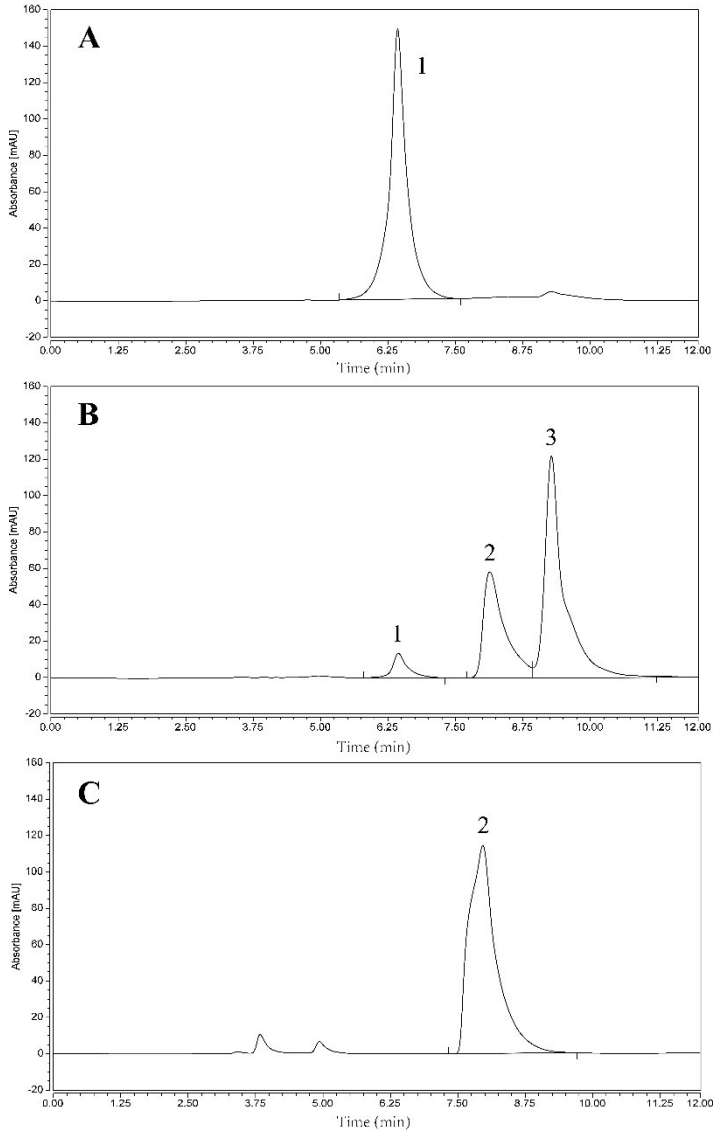

Fig. S7 Preparation of BH2. 1 mmol/L BH4 were shaked at 37℃, 250 rpm and analyzed by HPLC with a Waters XSelect®HSS T3 C18 column (250*4.6 mm). PeaK 1 BH4；PeaK 2 BH2；PeaK 3 q-BH2. A: 0 h; B: 24 h; C: 60 h.

Fig. S8 Preparation of q-BH2

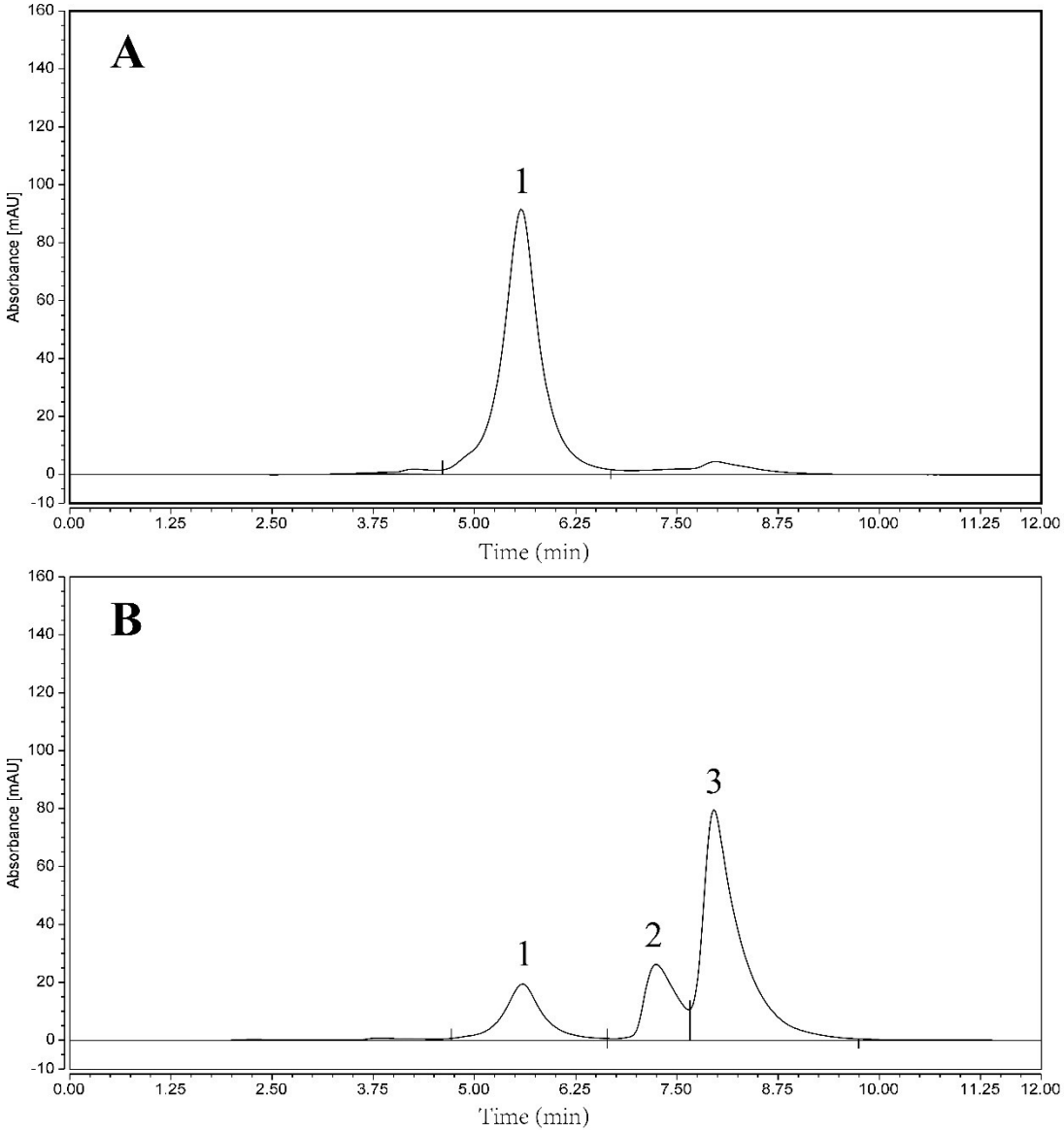

Fig. S8 Preparation of q-BH2. 1 mmol/L BH4 and 1 mmol/L 2,6-dichloroindophenol were shaked at 37℃, 250 rpm and analyzed by HPLC with a Waters XSelect®HSS T3 C18 column (250*4.6 mm). PeaK 1 BH4；PeaK 2 BH2；PeaK 3 q-BH2. A: 0 h; B: 1.33 h.

Fig.S9 Expression of SrTH in *E. coli* (DE3) using pET28a

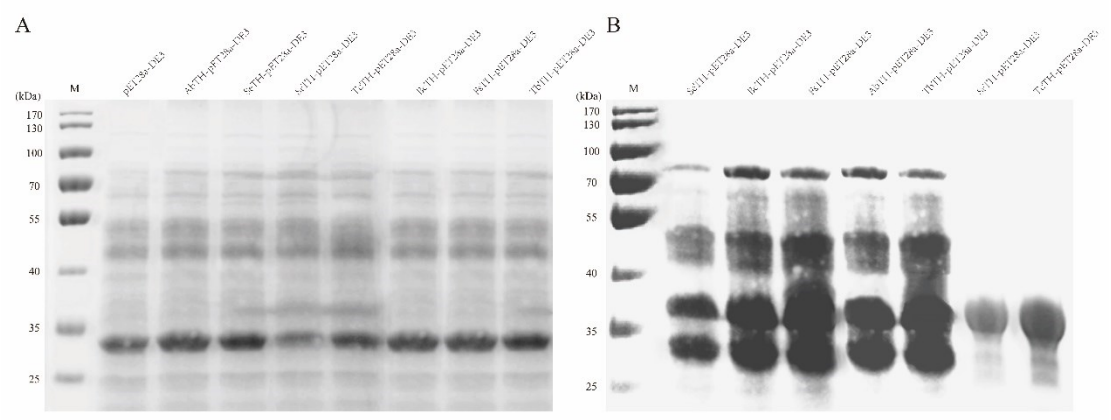

Fig. S9 Expression of SrTH in *E. coli* (DE3). The SrTH was expressed in *E. coli* (DE3) cells using pET28a and analysed using SDS-PAGE. Induction with 0.1 mmol/L IPTG was carried out at 25 °C. (A) Crude enzyme supernatant of 7 tyrosine hydroxylases using SDS-PAGE: Lane 1, marker; lane 2, Control; lane 3, AbTH; lane 4, SeTH; lane 5, SrTH; lane 6, TcTH; lane 7, BcTH; lane 8, FsTH and lane 9, TbTH; (B) Inclusion body of 7 tyrosine hydroxylases using SDS-PAGE: Lane 1, marker; lane 2, SeTH; lane 3, BcTH; lane 4, FsTH; lane 5, AbTH; lane 6, TbTH; lane 7, SrTH; lane 8, TcTH.

Fig. S10 Expression and purification of SrTH

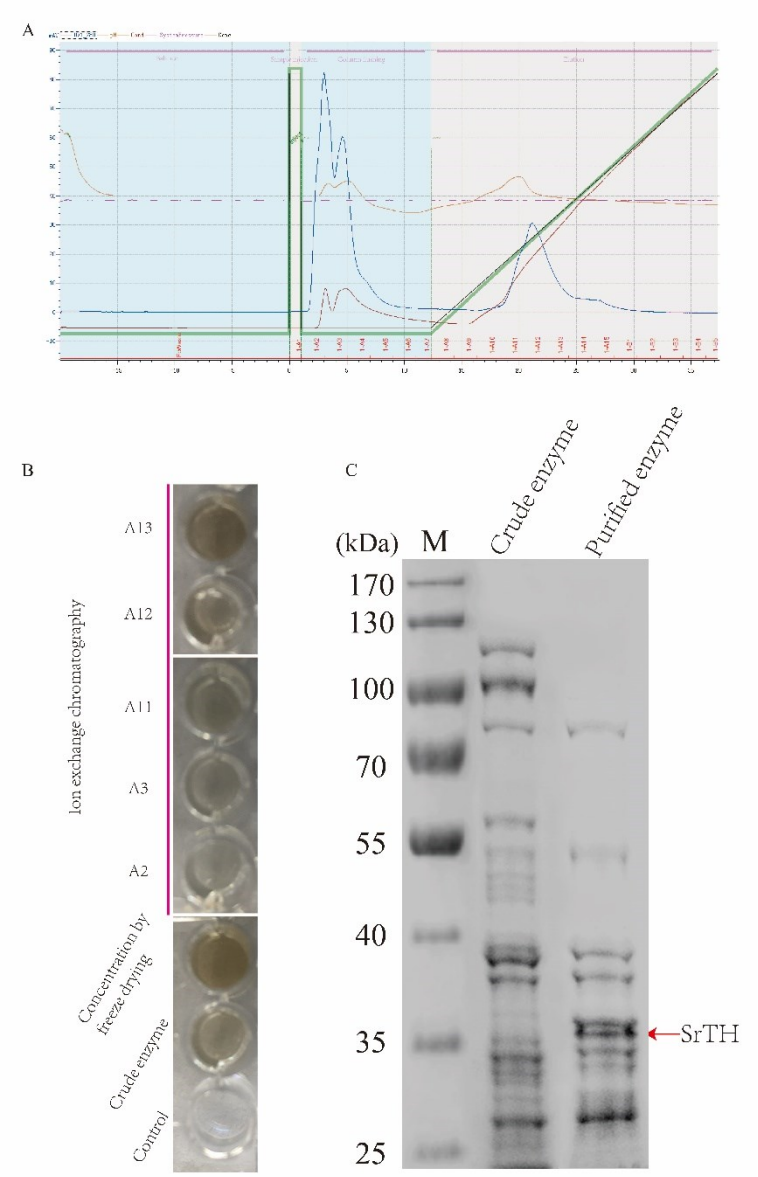

Fig. S10 Expression and purification of SrTH. The SrTH was expressed in *B. licheniformis* cells, purified by ion exchange chromatography (DEAE-cellulose column) and analysed using SDS-PAGE. (A) The process of Protein purification; (B) Enzyme activity detection of all the fractions; (C) Protein analysis using SDS-PAGE: Lane 1, marker; lane 2, crude enzyme; lane 3, the purified protein SrTH.

Fig. S11 The optimal catalytic temperature and pH of SrTH

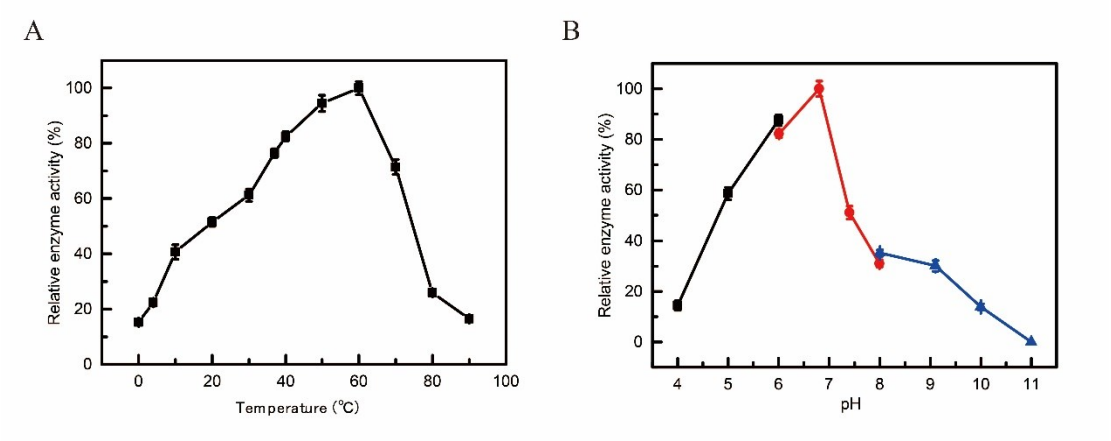

Fig. S11 The optimal catalytic temperature and pH of SrTH. (A) The optimal catalytic temperature of SrTH; (B) The optimal catalytic pH of SrTH: (■) The pH was regulated by 100 mmol/L sodium acetate buffer; (●) The pH was regulated by 100 mmol/L sodium phosphate buffer; (▲) The pH was regulated by 100 mmol/L sodium carbonate buffer.

Fig.S12 Fluorescence emission spectra of BH4-SrTH system

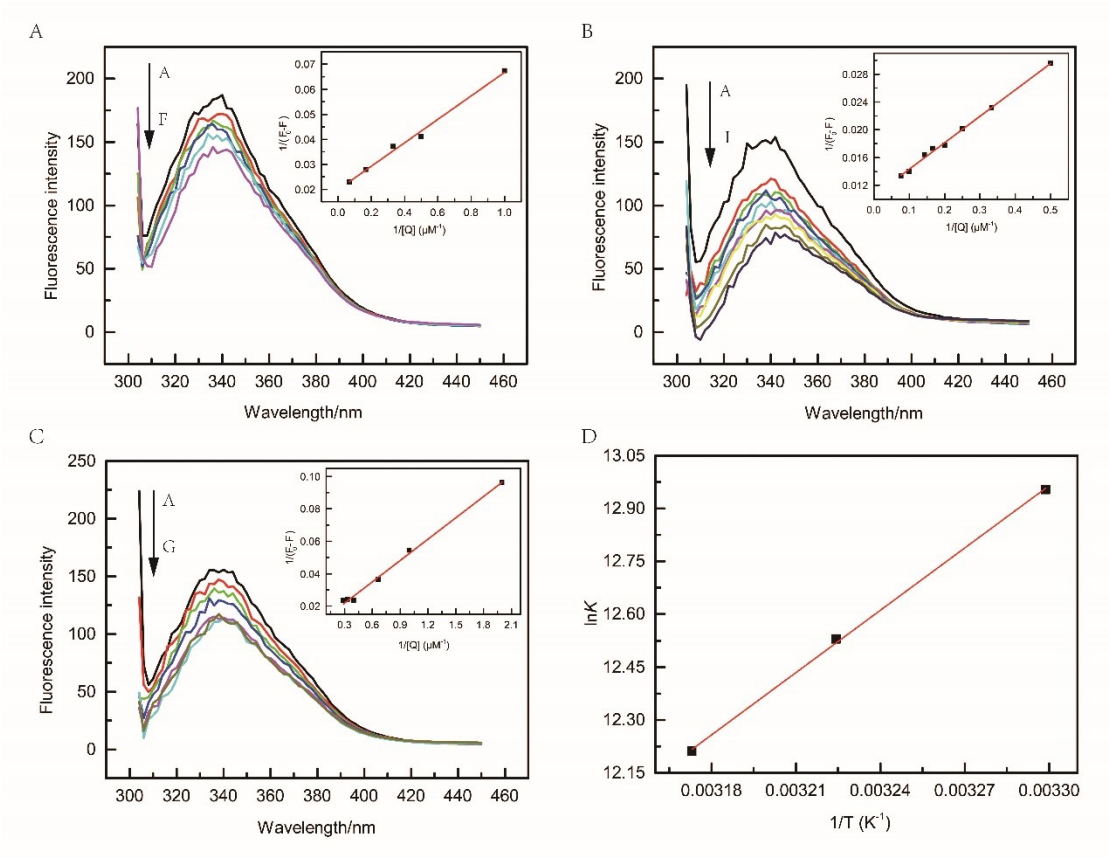

Fig. S12 Fluorescence emission spectra of BH4-SrTH system in 20 mM sodium phosphate buffer (pH 7.0) at different temperatures, λ_{ex}=265 nm. (A) 303 K, A: SrTH (44 mg/L), B-F: 1, 2, 3, 6 and 15 μmol/L, respectively. (B) 310 K, A: SrTH (44 mg/L), B-I: 2, 3, 4, 5, 6, 7, 10 and 13 μmol/L, respectively. (C) 315 K, A: SrTH (44 mg/L), B-G: 0.5, 1, 1.5, 2.5, 3.0 and 3.5 μmol/L, respectively. (D) Van't Hoff plot, pH=7.0, c(SrTH)=44 mg/L. Inset: Plot of $1/(F_0-F)$ against 1/[Q] for BH4-SrTH system at three temperatures (303, 310, and 315 K).

Fig.S13 Fluorescence emission spectra of BH2-SrTH system

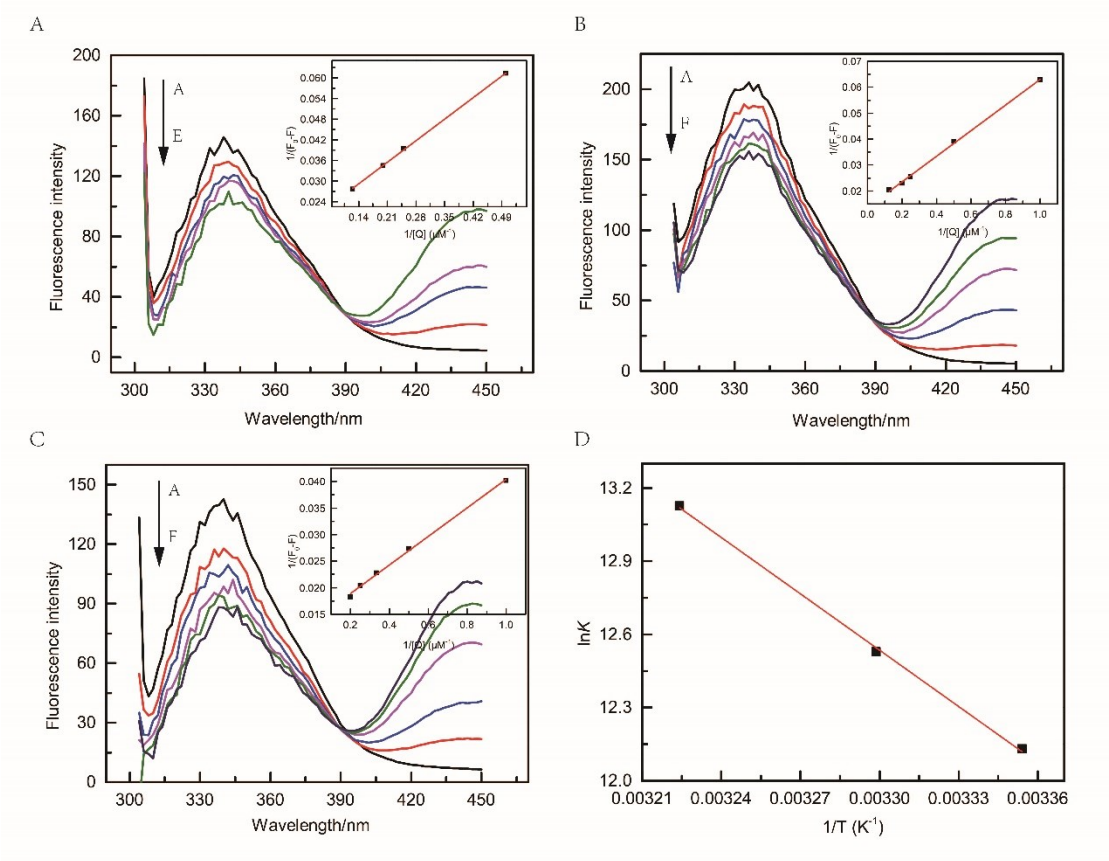

Fig. S13 Fluorescence emission spectra of BH2-SrTH system in 20 mM sodium phosphate buffer (pH 7.0) at different temperatures, λ_{ex}=265 nm. (A) 298 K, A: SrTH (44 mg/L), B-E: 2, 4, 5 and 8 μmol/L, respectively. (B) 303 K, A: SrTH (44 mg/L), B-F: 1, 2, 4, 5 and 8 μmol/L, respectively. (C) 310 K, A: SrTH (44 mg/L), B-F: 1, 2, 3, 4 and 5 μmol/L, respectively. (D) Van't Hoff plot, pH=7.0, c(SrTH)=44 mg/L. Inset: Plot of $1/(F_0-F)$ against 1/[Q] for SrTH-BH2 system at three temperatures (298, 303, and 310 K).

Fig.S14 Fluorescence emission spectra of BH2-SrTH system with adding 10 μmol/L BH4

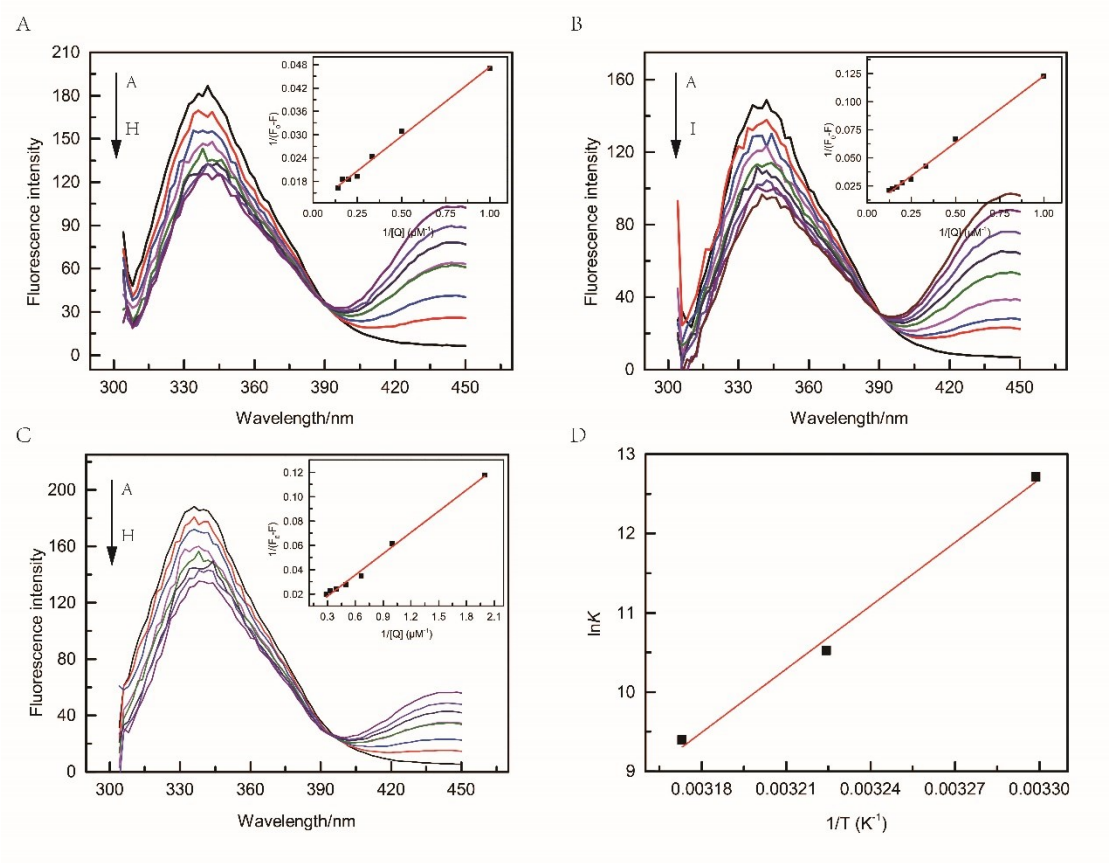

Fig. S14 Fluorescence emission spectra of BH2-SrTH system with adding 10 μmol/L BH4 in 20 mM sodium phosphate buffer (pH 7.0) at different temperatures, λ_{ex}=265 nm. (A) 303 K, A: SrTH (44 mg/L), B-H: 1, 2, 3, 4, 5, 6 and 7 μmol/L, respectively. (B) 310 K, A: SrTH (44 mg/L), B-I: 1, 2, 3, 4, 5, 6, 7 and 8 μmol/L, respectively. (C) 315 K, A: SrTH (44 mg/L), B-H: 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 μmol/L, respectively. (D) Van't Hoff plot, pH=7.0, c(SrTH)=44 mg/L. Inset: Plot of $1/(F_0-F)$ against 1/[Q] for BH2-SrTH system with adding 10 μmol/L BH4 at three temperatures (303, 310, and 315 K).

Fig.S15 Fluorescence emission spectra of PH2-SrTH system

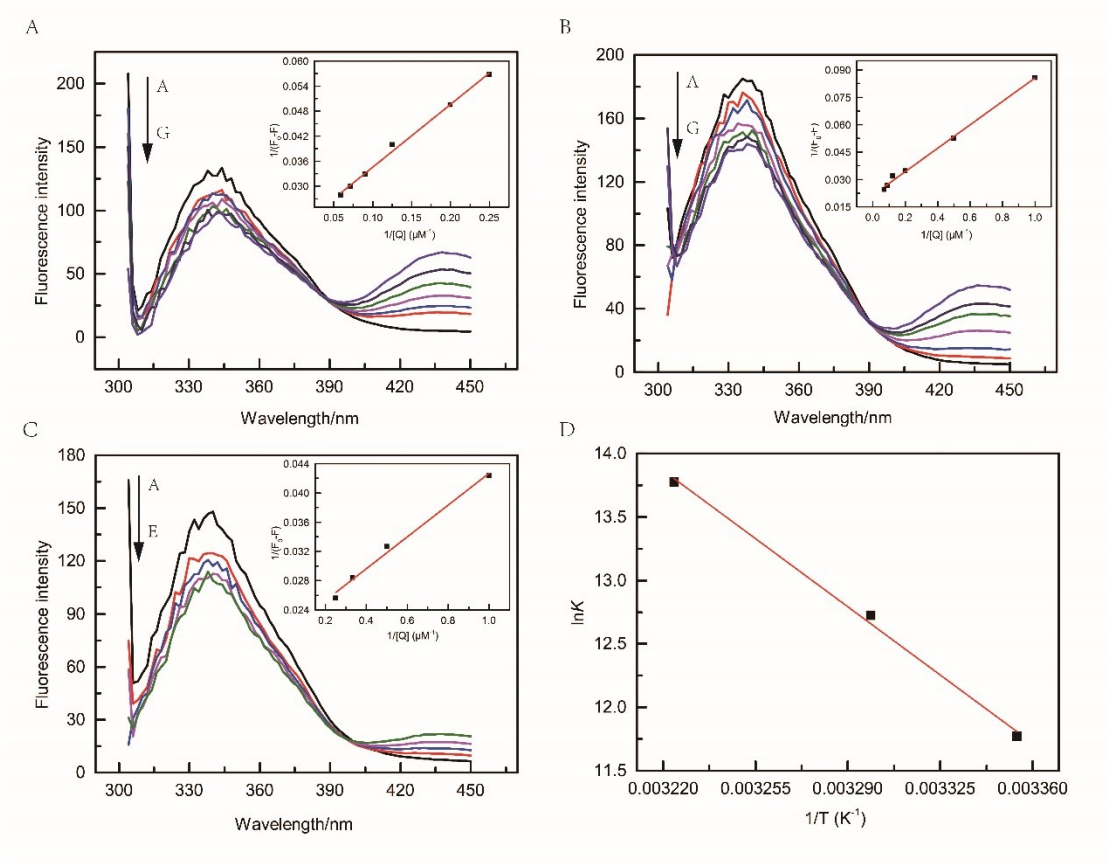

Fig. S15 Fluorescence emission spectra of PH2-SrTH system in 20 mM sodium phosphate buffer (pH 7.0) at different temperatures, λ_{ex}=265 nm. (A) 298 K, A: SrTH (44 mg/L), B-G: 4, 5, 8, 11, 14 and 17 μmol/L, respectively. (B) 303 K, A: SrTH (44 mg/L), B-G: 1, 2, 5, 8, 11 and 14 μmol/L, respectively. (C) 310 K, A: SrTH (44 mg/L), B-E: 1, 2, 3 and 4 μmol/L, respectively. (D) Van't Hoff plot, pH=7.0, c(SrTH)=44 mg/L. Inset: Plot of $1/(F_0-F)$ against 1/[Q] for SrTH-PH2 system at three temperatures (298, 303, and 310 K).

Fig. S16 BH4 regeneration by EcDHPR

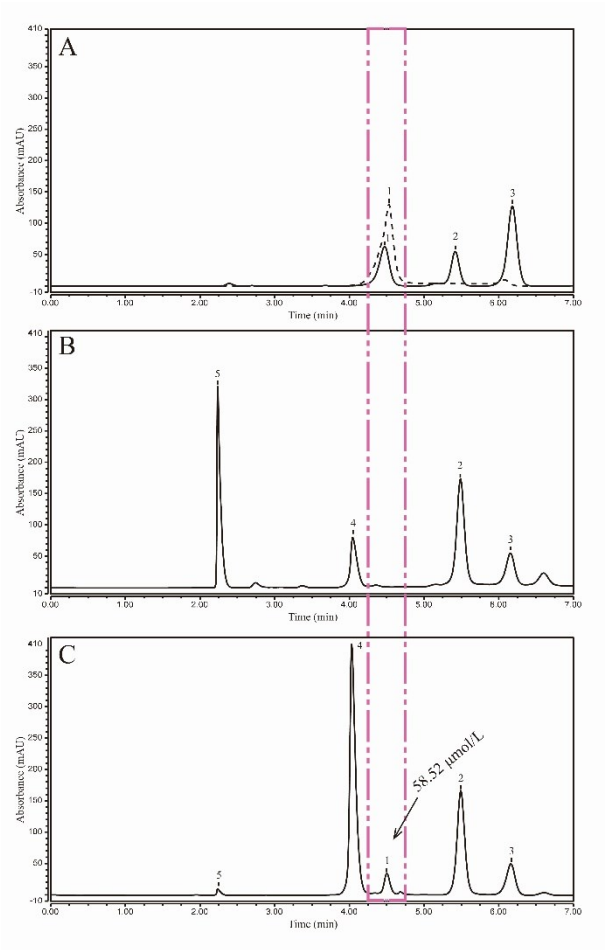

Fig. S16 BH4 regeneration by EcDHPR. The synthesis of BH4 using 0.1g/L pure EcDHPR, 0.5 mmol/L q-BH2 and 1 mmo/L NADH was at 25 °C for 1h. In the enzyme reaction, 1mmo/L NADH was almost completely converted into NAD (+) and the content of BH4 produced by EcDHPR was 58.52 μmol/L in the solution at 1h. A: BH4 standard (dash line) and product analysis after treated by 2,6-dichloroindophenol (solid line); B: control with adding corresponding volume of HEPES-NaOH (pH7.4) buffer; C: sample with adding 0.1g/L pure EcDHPR. PeaK 1 BH4；PeaK 2 BH2；PeaK 3 q-BH2; PeaK 4 NAD (+);PeaK 5 NADH. The samples were analyzed by HPLC with a Spursil 5 μm C18 column (250*4.6 mm).

Fig. S17 Functional identification of TH/SPR dual-enzyme synthesis systems by combined whole-cell catalyst

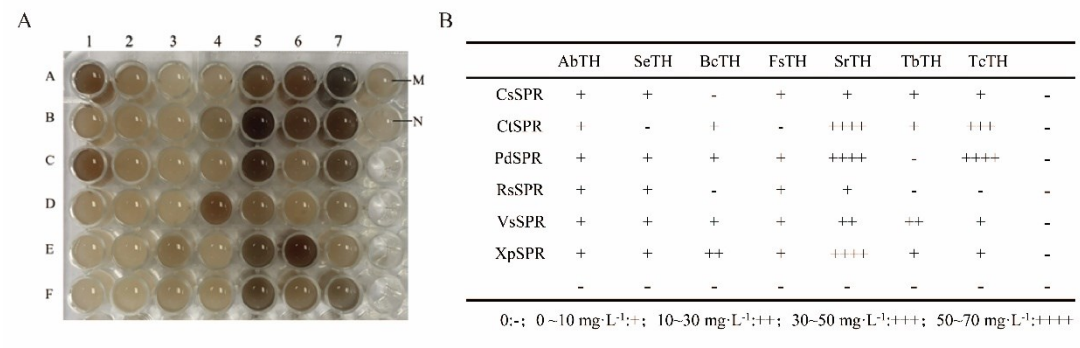

Fig. S17 Functional identification of TH/SPR dual-enzyme synthesis systems by combined whole-cell catalyst. The two kinds of cells expressing TH or SPR were mixed by OD_{600} 10: OD_{600} 10 in two-by-two combination with 10 mmol/L sodium phosphate buffer (pH 7.0). In the process, 2 g/L L-Tyrosine was added and the reaction was at 37 °C, 250 rpm for 24 h-36 h with 30 mL in the 250 mL flasks. After combined whole-cell catalyst by different groups, the color change was recorded and L-Dopa in the supernatant was detected by HPLC. (A) Color of solution; 1-7: AbTH, SeTH, BcTH, FsTH, SrTH, TbTH and TcTH; M: *B. licheniformis* with pMA5 only as control 1; N: *B. licheniformis* with SrTH only as control 2; A-F: CsSPR, CtSPR, PdSPR, RsSPR, VsSPR and XpSPR. (B) Classification according to L-Dopa production.