

SUPPORTING METHODS

ATP determination

The samples for ATP determination were prepared by the method described previously.^[S2] The concentrations of ATP and ADP were detected by LC-20AT HPLC (Shimadzu, Japan) equipped with a C18 column (Agela Technologies, China) and a UV detector (254 nm). The mobile phase used was phosphate buffer consisting of 0.06 M K₂HPO₄ and 0.04 M KH₂PO₄ at pH 7.0 adjusted with 0.1 mol L⁻¹ KOH at a flow rate of 1 mL min⁻¹.

Samples for Intracellular Metabolites

20 mL of *E. coli* cells after 6 h cultivation was taken to prepare the intracellular metabolites according to the previous study.^[S1] Cell debris in methanol extracted solution was removed by centrifugation for 10 min at 13,000 rpm and -4 °C. The supernatant was used to measure the concentration of methionine and cystathionine by LC-MS.

LC-MS conditions

All measurements were performed on a Shimadzu LC-20AD HPLC system (Shimadzu, Japan) coupled with a QTRAP5500 mass spectrometer (AB SCIEX, USA). Data were acquired and evaluated via Analyst software (Analyst1.6.1, AB SCIEX, USA). The source was operated in ESI+ mode (CUR 20 psi, GS1 50 psi, GS2 50 psi, IS 5500 V, CAD MEDIUM, and TEMP 500°C, DP 40). The HPLC system is equipped with a ACQUITY UPLC BEH HILIC column (Waters, USA) (oven temperature 40 °C). The pump supplied a gradient with the following settings: 0 min, 100 % mobile phase B (0.1 % formic acid, 99.9 % H₂O), maintained for 3 min. Subsequently, the concentration of mobile phase A (2mmol L⁻¹ ammonium acetate) was increased to reach 40 % at 8 min, held constant for 1 min. Then the mobile phase B was increased to reach 100 % in 6 sec, held constant for 4min. And the flow rate was set to 0.3 mL min⁻¹.

SUPPORTING FIGURES

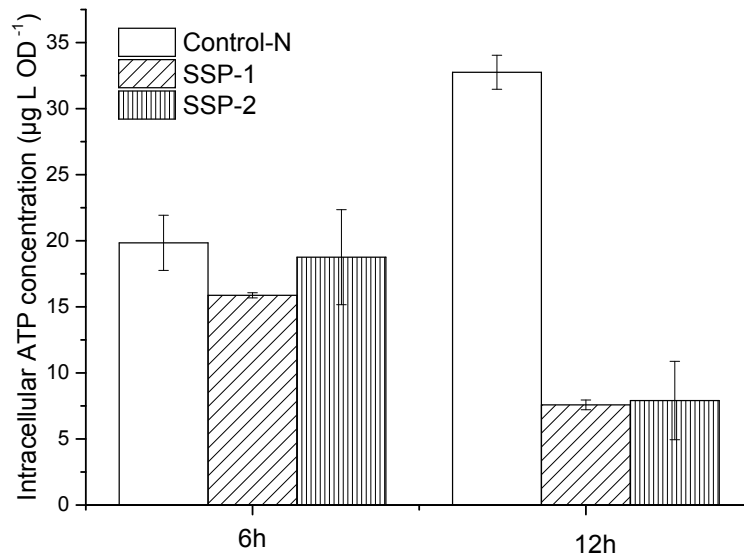


Fig.S1 Intracellular ATP concentration in the control and recombinant strains. Control-N: *E. coli* BL21 harboring pRSFDuet-1; SSP-1: *E. coli* BL21 harboring pRSFD-POS5Δ17; SSP-2: *E. coli* BL21 harboring pRSFD-*yffB-pntAB*

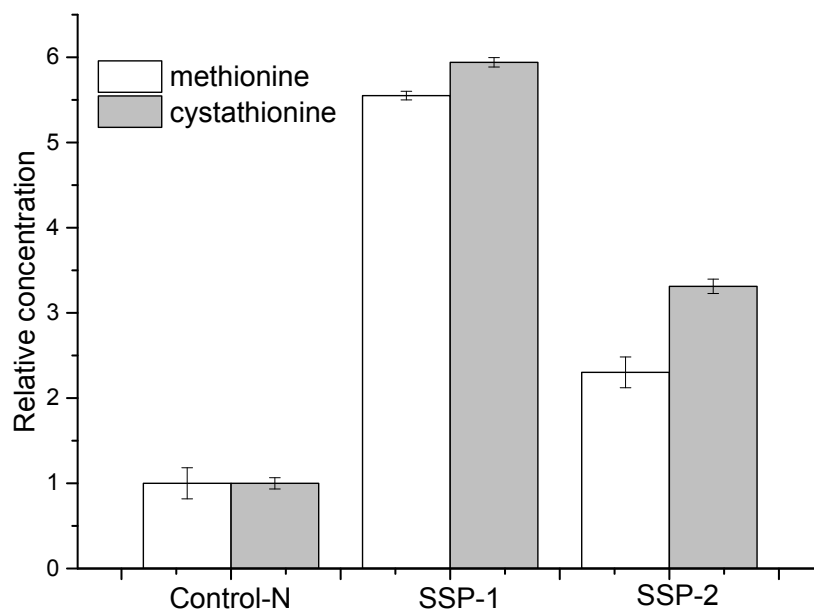


Fig.S2 Flask fermentation results of the relative concentration of methionine and cystathionine in the control and recombinant strains. Control-N: *E. coli* BL21 harboring pRSFDuet-1; SSP-1: *E. coli* BL21 harboring pRSFD-*POS5*Δ17; SSP-2: *E. coli* BL21 harboring pRSFD-*yffB*- *pntAB*. The HPLC-MS peak area of the methionine and cystathionine in the control strain was normalized to 1.0.

SUPPORTING REFERENCES

- S1. M. M. Wang, J. F. Sun, F. Y. Xue, F. Shang, Z. Wang and T. W. Tan, *Appl. Biochem. Biotechnol.*, 2012, **168**, 198-205.
- S2. A. V. Akhova, A. G. Tkachenko, *FEMS. Microbiol. Lett.*, 2014, **353**, 69-76.