# Supplementary Information

**Production of trans-cinnamic and p-coumaric acids in engineered E. coli**

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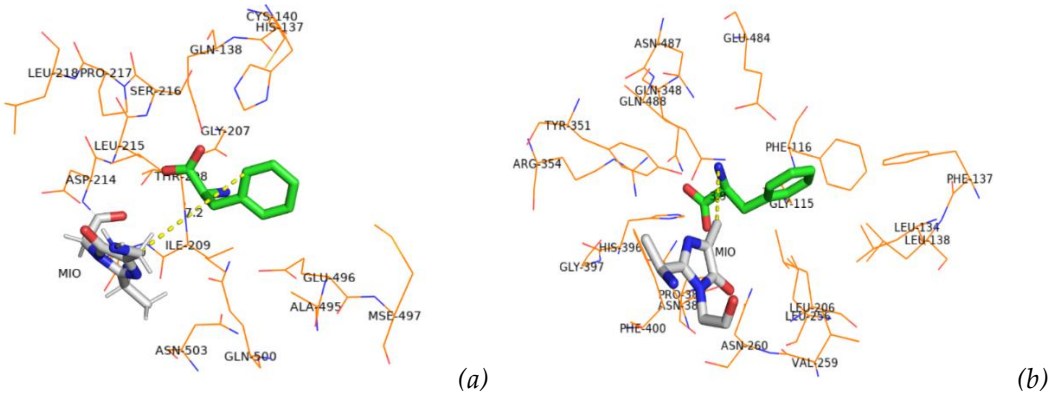
1. Molecular docking results for PAL and L-Phe

Based on the optimized reaction conditions, we aim to further increase the enzyme activity through molecular modifications to achieve higher yields of products. Two reaction mechanisms of PAL have been reported so far. In the molecular docking results, some results were found to be more consistent with the Friedel-Crafts's mechanism, while others were more compliance with the E1cb mechanism. According to this feature, targeted mutagenesis can be performed to improve the enzyme activity.

Figure S1. The molecular docking results of PAL and L-Phe




*(a)* *(b)*

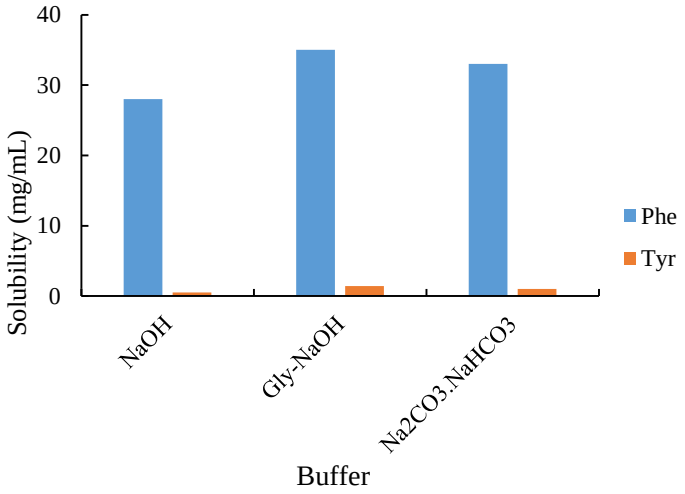
(a) PDB: 1T6J; (b) PDB: 1w27.

2. Solubilities of L-Phe and L-Tyr

To get a better solubility of L-Phe and L-Tyr, several different buffers were used and the results indicated that a basic pH is helpful to increase the solubility. Also, different buffer pairs may exhibit some differences for the solubility. Three different buffers were

finally used and the solubility results were obtained as the Figure S1 indicated below. The best performance was found in buffer Glycine-NaOH at pH 10, which provided a solubility of 33mg/mL and 1.4mg/mL for L-Phe and L-Tyr, respectively.

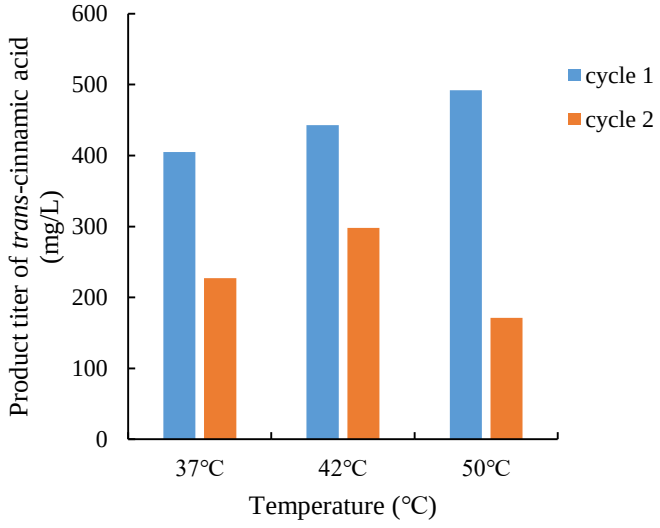
Figure S2. Solubilities of L-Phe and L-Tyr in NaOH, Gly-NaOH and $Na_2CO_3$.$NaHCO_3$ at pH 10.



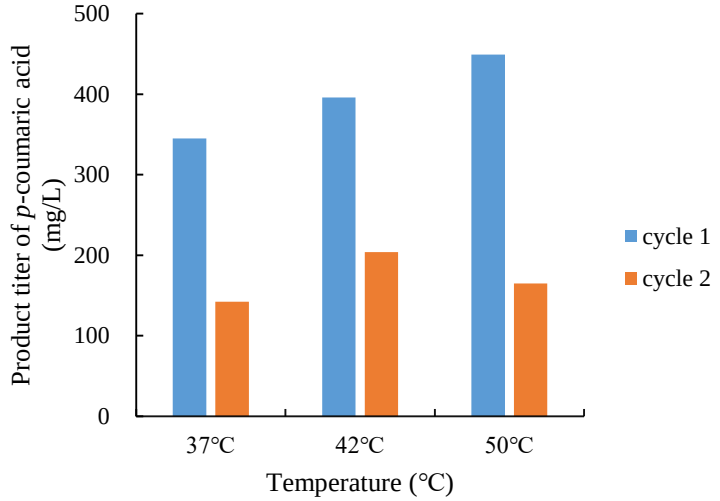

3. Temperatures for the second-round transformation

In the same reaction conditions, the second-round conversions were performed at 37 °C, 42 °C and 50 °C. The enzyme activity was evaluated by yields of products to select the appropriate reaction temperature. The results were shown in the following graph.

Figure S3. Results of two rounds transformation of trans-cinnamic acids and p-coumaric acid.




(a)



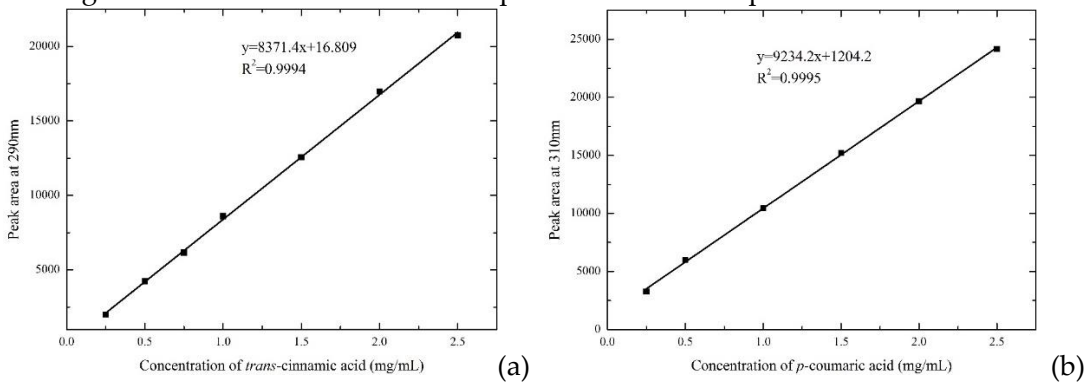

(b)

(a) Results of two rounds transformations of trans-cinnamic acid at 37°C, 42°C and 50 °C.
(b) Results of two rounds transformations of p-coumaric acid at 37°C, 42°C and 50 °C.

4. The standard curves for quantification of the trans-cinnamic acid and p-coumaric acid

To perform the standard curve for the calculation, the authentic samples of p-coumaric acid and trans-cinnamic acid were dissolved in ethanol to 2.5 mg/ml and then diluted to 2, 1.5, 1, 0.75, 0.5 and 0.25mg/ml, respectively. The peak areas of each concentration were detected by HPLC and plotted as standard curves to calculate the yields of products.

Figure S4. The standard curves for quantification of the product concentration.

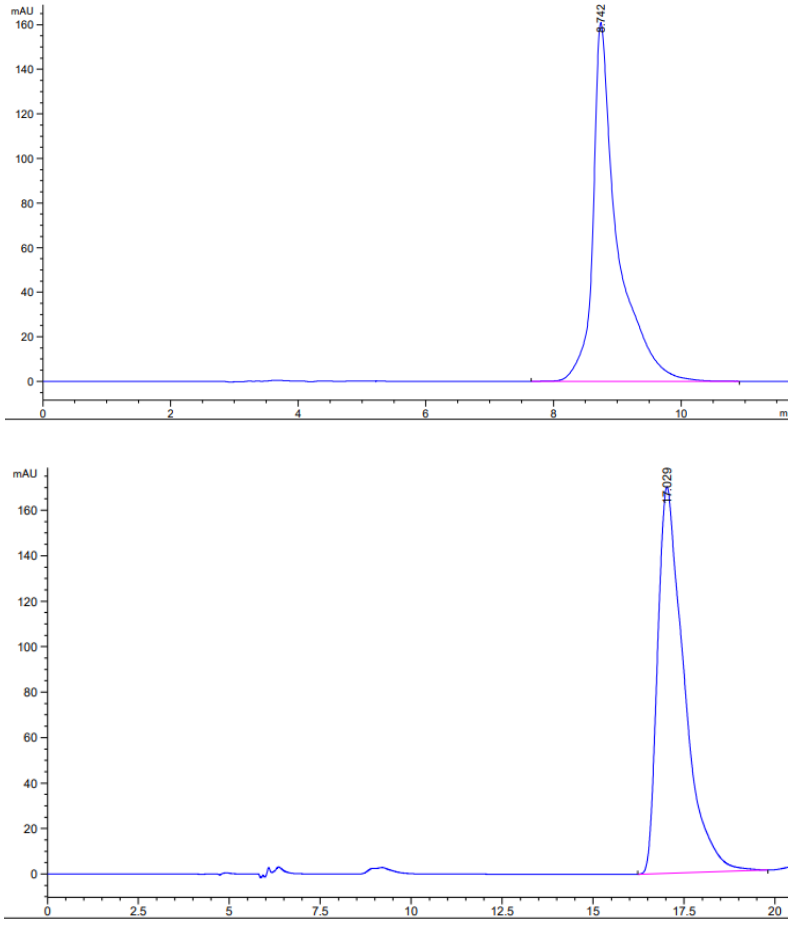



(a) trans-cinnamic acid, and (b) p-coumaric acid

For the isolation process, the reaction mixtures were centrifuged at 3500 rpm for 10 min, and supernatants were collected to analyze the product formation by HPLC. Meanwhile, the supernatants after centrifugation were adjusted to pH 4.2 by HCl and allowed to stand at room temperature for 30 mins, then the acidified supernatants were centrifuged again for separating the trans-cinnamic acid precipitate. Similarly, the supernatants after centrifugation were adjusted to pH 4.6 by $H_3PO_4$ to obtain p-coumaric acid and other steps were same as the isolation of trans-cinnamic acid.

5. The HPLC analysis of trans-cinnamic acid and p-coumaric acid.
   HPLC of trans-cinnamic acid was detected at 290 nm using a solution (50% of 1% acetic acid and 50% of acetonitrile) as mobile phase at flow rate of 0.7 mL/min. HPLC of p-coumaric acid was detected at 310 nm with the solution (60% of 0.1% formic acid and 40% of methyl alcohol) as mobile phase at flow rate of 0.6 mL/min.

Figure S5 The HPLC graph of trans-cinnamic acid and p-coumaric acid




The HPLC graph of trans-cinnamic acid(a) and p-coumaric acid (b).