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Improving the thermal stability of *trans*-epoxysuccinate hydrolase

Key words: *trans*-Epoxysuccinate hydrolase; *Pseudomonas koreana*; Molecular dynamics simulation; Thermal stability modification

Research Summary

This article mainly conducts thermostability modification of *trans*-epoxysuccinic acid hydrolase from the following aspects.

- **Prediction of important amino acid sites**
  - Molecular dynamics simulation screens out regions with high flexibility
  - Identify sites with significant impact on thermal stability based on B-factor
  - Simulation of saturation mutagenesis to screen for important mutants
- **Characterization of mutants**
  - Four mutants with improved enzyme activity
  - The thermal stability of the three mutants has been significantly improved
- **Improvement mechanism**
  - Salt bridges appear, and the intermolecular forces are enhanced
  - Molecular dynamics simulations show that the mutant is more stable than the wild type
  - Mutants exhibited varying degrees of reduced electrostatic potential energy and increased hydrophobicity in the vicinity of their mutation sites

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Innovation points

- **Screen important amino acid sites by combining molecular dynamics simulations (Fig. 1) with B-factor analysis (Table S2), and then simulate saturation mutagenesis at these sites to screen for critical mutants (Table 1).**

- **Three mutants with improved thermal stability of *trans*-epoxysuccinate hydrolase were obtained (Fig. 4).**

- **New salt bridges are formed in the interaction between the mutant and the substrate, further enhancing the intermolecular forces (Fig. 5); the mutant exhibits varying degrees of electrostatic potential reduction and hydrophobicity increase near the mutation sites (Fig. 8, Fig. 9).**

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