Corner Engineering: A Directed Evolution Strategy for Enhancing Enzyme Resistance in Deep Eutectic Solvents

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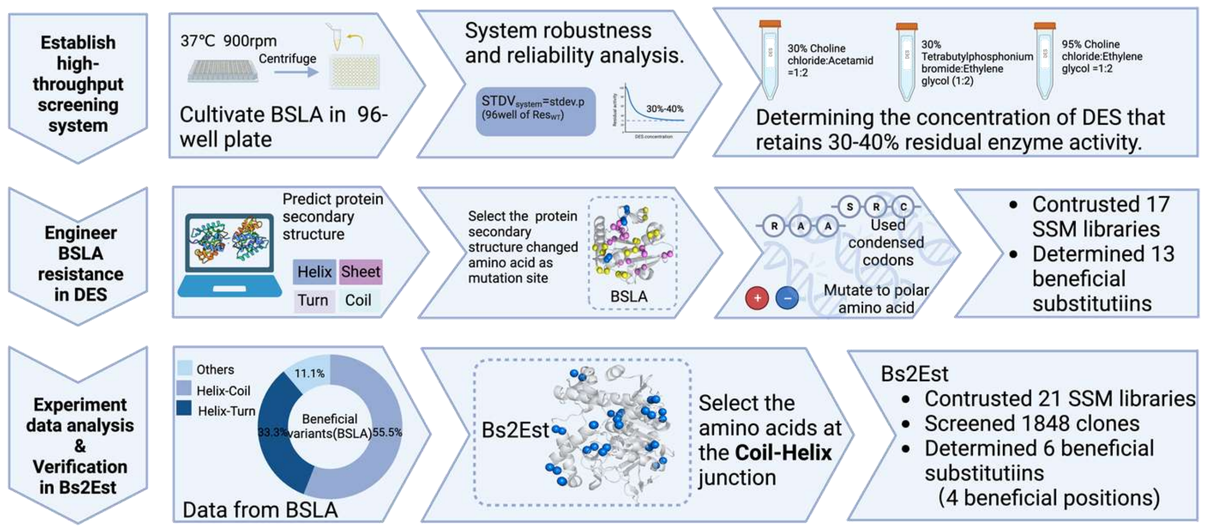
Abstract

- **As a green and cost-effective solvent, deep eutectic solvent (DES) shows promise in biocatalysis. However, enzyme activity in DESs is often inhibited, limiting their application. To address this, our project proposed a directed evolution strategy called Corner Engineering. Using *Bacillus subtilis* lipase A (BSLA) as a model, we thoroughly validated the effectiveness of the strategy and optimized it.**
- **The variant M137D/N138D displayed an impressive 3.0-fold increase in resistance compared to the WT in 95% (v/v) ChCl:ethylene glycol. To confirm the versatility and effectiveness of our optimized approach, we conducted validation experiments on *Bacillus subtilis* esterase (Bs2Est). In the 75% (v/v) ChCl:ethylene glycol, the resistance of these variants could reach up to 3.1-fold, thus affirming the broad applicability of our engineered enzyme strategy.**
- **The molecular investigations reveal that increased water molecules at substrate binding sites are the dominant determinant of elevated resistance, indicating a promising avenue for understanding enzyme-DES interactions.**

Introduction



Results

Establishment of the DES high throughput screening system

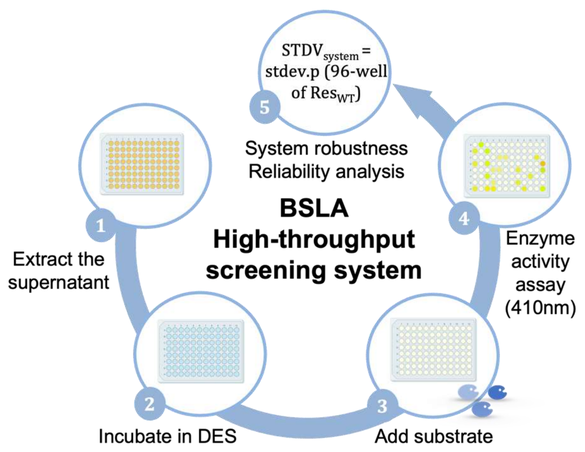


Figure 1.
Specific experimental steps to establish a high-throughput screening system

Directed evolution of BSLA

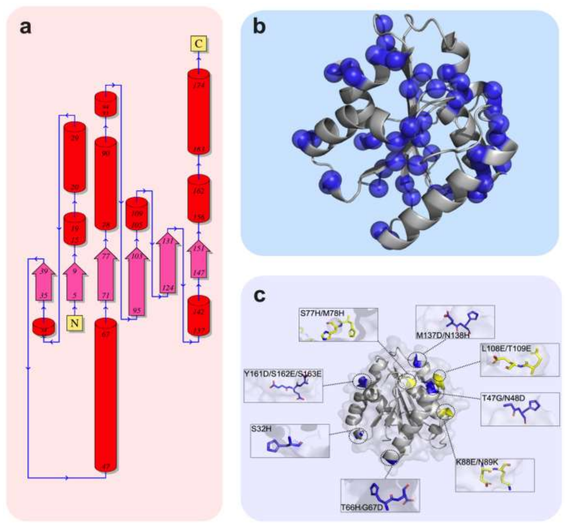
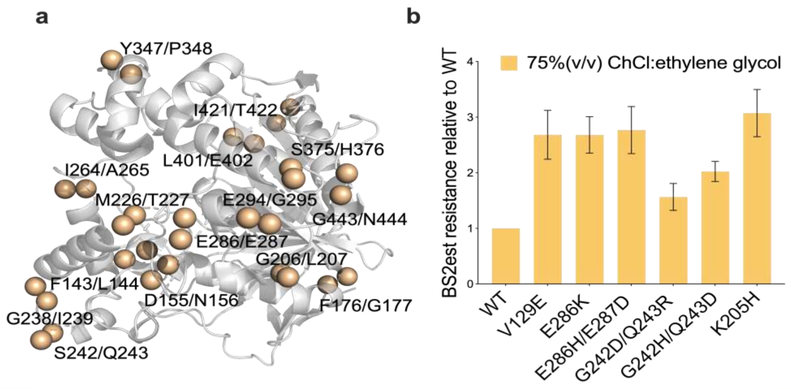


Figure 2.
With one more round rescreening, 84, 68, 64 beneficial variants were verified with improved DESs resistance (R_V> R_{WT} + 3σ) towards ChCl: ethylene glycol, ChCl: acetamide, and TBPB:ethylene glycol, respectively. Regarding the sequencing on the variants > 1.5-fold improvement towards at least two DESs, thirteen variants were identified and explored in all three DESs.

The transferability of Corner Engineering was confirmed by investigating Bs2Est



To further validate the effectiveness of this directed evolution strategy and improve the efficiency of directed evolution, Bs2Est were examined. The protein secondary structures of Bs2Est were obtained from EMBI-EBI. Taking various factors into consideration, the adjacent amino acids at the Coil-Helix junction were identified as mutation sites **(Figure 3a)**. Bs2Est libraries were screened in the presence of 75% (v/v) ChCl:ethylene glycol. After the initial screening and the second round of screening, six beneficial variants were identified, with K205H displaying approximately 3.1-fold improved resistance in ChCl:ethylene glycol **(Figure3b)**.

Investigation of DESs resistance profiles and kinetic characterization

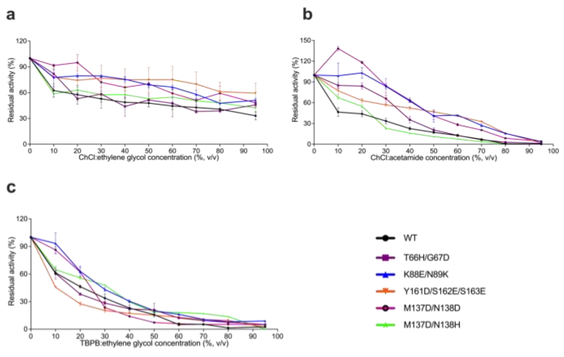


Figure 4.
DES resistance profiles of the BSLA. Residual activity of BSLA variants in different concentrations of
(a) ChCl: ethylene glycol
(b) ChCl: acetamide
(c) TBPB: ethylene glycol.

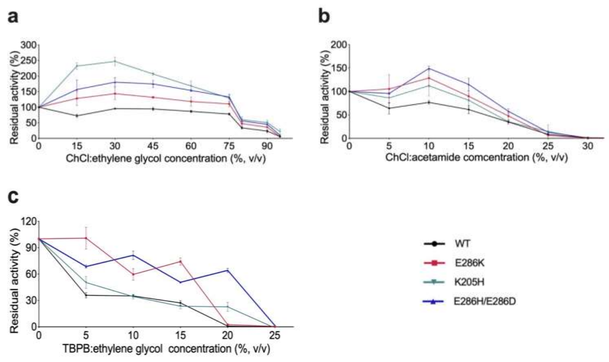


Figure 5.
Characterization of Bs2Est variants in DESs. Residual activity of BSLA variants in different concentrations of
(a) ChCl: acetamide
(b) ChCl: ethylene glycol
(c) TBPB: ethylene glycol.

Computational analysis revealed the molecular mechanism of improved DES resistance of variants

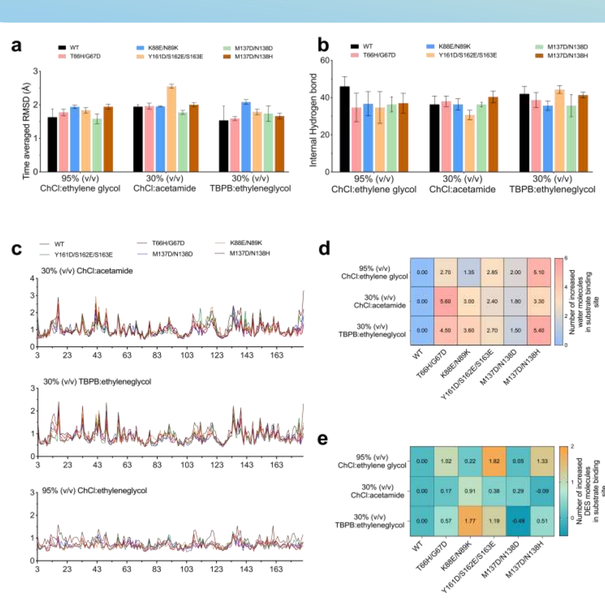


Figure 6.
Overall and local structural change of BSLA variants in cosolvents.

Discussion

This study, coupling corner engineering with obtained molecular insights, illuminates the enzyme-DES interaction patterns and fosters the rational design of more DES-resistant and thermostable enzymes. In summary, an effective directed evolution strategy was explored to better tailor the enzyme stability in DESs. By improving the resistance of enzymes, we can expand the application of enzymes in catalytic reactions, improve product purity and yield, and increase the flexibility and efficiency of industrial production. Corner Engineering can likely serve as a blueprint to reengineer other enzymes for efficient catalysis in DESs, thus further stimulating a broader application of biocatalysis in the emerging biobased economy.