Research Reports in Molecular Biology
E-Science Information

Research Reports in Molecular Biology

Volume 3, Issue 1
Editorial

Balancing Stability and Activity: Innovations in Enzyme Engineering for Industrial Use

Nima Ghahremani Nezhad[1,2*]

[1]Enzyme and Microbial Research Center, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[*]**Corresponding author: Nima Ghahremani Nezhad,** Enzyme and Microbial Research Center, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

E-mail: GS52916@upm.edu.my

Received: January 06, 2025; **Accepted:** January 20, 2025; **Published:** January 27, 2025

Editorial

Enzymes serve as essential catalysts in industrial processes, enabling a vast range of chemical reactions with exceptional precision and efficiency. These biocatalysts are indispensable in sectors like food processing, textiles, pharmaceuticals, and biofuel production, offering sustainable and environmentally friendly solutions [1]. Despite their utility, one major limitation hinders their widespread application: their structural instability under high-temperature conditions. Thermostability is critical for industrial enzymes, as elevated temperatures can speed up reaction rates and minimize contamination risks. However, attempts to improve thermostability often lead to reduced catalytic efficiency, creating a long-standing trade-off that poses significant challenges to researchers and industry professionals. Addressing this issue demands innovative strategies that simultaneously enhance both the stability and activity of enzymes [2].

This discussion highlights advanced strategies for achieving simultaneous improvements in thermostability and activity of industrial enzymes. It focuses on the interplay between protein engineering methodologies, structural biology insights, and computational modeling. By synthesizing the latest developments, the aim is to provide a comprehensive understanding of how these tools are transforming enzyme design and paving the way for their expanded industrial use.

A major challenge in enzyme engineering lies in the intricate relationship between protein structure and function. Enzymes derive their catalytic efficiency from a delicate balance between flexibility and rigidity. While rigidity often contributes to stability, excessive rigidity can hinder substrate binding and reduce catalytic efficiency. Approaches to enhance thermostability, such as introducing disulfide bonds, hydrophobic interactions, or proline substitutions, may inadvertently restrict the dynamic movements necessary for efficient catalysis. For instance, mutations aimed at stabilizing regions far from the active site can alter the geometry of the active site itself, compromising functionality. Similarly, modifying residues near the active site to improve substrate interaction can destabilize the overall enzyme structure, underscoring the importance of a nuanced, integrated approach [3]. Directed evolution has proven to be a transformative technique for enzyme optimization, mimicking natural selection to create variants with enhanced properties. This approach involves subjecting enzyme libraries to iterative cycles of random mutagenesis and screening, enabling the identification of improved variants. Tools like error-prone PCR and DNA shuffling have been instrumental in generating diverse enzyme libraries [4]. Recent advancements in high-throughput screening have further streamlined this process, allowing the exploration of large sequence spaces with greater efficiency. Rational design, on the other hand, employs detailed structural information, often derived from crystallography or molecular dynamics simulations, to introduce targeted mutations. This method relies on understanding the interplay between enzyme structure and function, enabling precise modifications. Semi-rational approaches, such as consensus-guided mutagenesis and site-saturation mutagenesis, blend rational design with the exploratory power of directed evolution, making them particularly effective in overcoming the trade-offs between stability and activity. Integrative strategies that combine these methods have demonstrated remarkable success in improving both properties simultaneously. Computational tools, including molecular docking and machine learning algorithms, are increasingly being employed to predict mutation outcomes, further enhancing the efficiency of the engineering process [5]. The hydrophobic core is crucial for maintaining protein stability, and targeted modifications in this region can significantly enhance thermostability. For example, substituting small hydrophobic residues with larger ones can fill internal cavities, reducing flexibility and increasing structural robustness [6]. However, such changes must be carefully designed to avoid disrupting the enzyme’s active site. Similarly, optimizing electrostatic interactions, such as salt bridges and hydrogen bonds, can reinforce structural integrity. Computational algorithms like PoPMuSiC and FoldX are widely used to predict stabilizing mutations that enhance electrostatic networks, particularly around active or substrate-binding sites. Another effective strategy involves truncating disordered regions at the N- or C-terminus. These regions, while contributing to enzyme flexibility, can also cause instability under thermal stress. Truncation reduces the entropy of the unfolded state, resulting in a more compact and stable [2,3]. Advancements in computational biology have revolutionized the field of enzyme engineering, offering powerful tools for predicting and analyzing the effects of structural modifications. Molecular dynamics simulations provide detailed insights into protein flexibility and stability, allowing researchers to identify hotspots for potential mutations. Machine learning models, trained on extensive datasets of enzyme structures and activities, can accurately predict the effects of specific mutations, reducing reliance on trial-and-error approaches. These computational tools not only expedite the engineering process but also enable more targeted interventions, resulting in enzymes with tailored properties [7].

The applications of engineered enzymes span numerous industries, including biofuels, food production, pharmaceuticals, and environmental remediation. For example, thermostable amylases and cellulases are essential for biomass conversion in biofuel production, while lipases with enhanced activity are critical for producing biodegradable plastics and pharmaceuticals [8-10]. As industries increasingly demand sustainable and efficient processes, the role of engineered enzymes is expected to grow significantly. Looking ahead, the integration of synthetic biology with enzyme engineering offers exciting possibilities.

By designing synthetic pathways and incorporating non-natural amino acids, researchers can create enzymes with entirely novel functionalities. Additionally, adaptive enzymes capable of responding to environmental changes could revolutionize their use in dynamic industrial settings.

Achieving simultaneous thermostability and activity improvements in industrial enzymes remains a complex yet rewarding challenge. Advances in protein engineering, supported by computational tools and integrative approaches, have greatly enhanced our capacity to design enzymes that meet specific industrial requirements. By addressing the inherent trade-offs between stability and activity, researchers are unlocking the full potential of biocatalysts, enabling more sustainable, efficient, and cost-effective industrial processes. As we continue to explore the molecular intricacies of enzyme functionality, fostering interdisciplinary collaborations among structural biology, computational modeling, and industrial biotechnology will be crucial. Such efforts will accelerate the development of next-generation enzymes, driving innovation in industries reliant on these remarkable catalysts. With continued scientific and technological advancements, the future of enzyme engineering holds immense potential to transform industrial landscapes and address global challenges.

References

1. Zhang Y, He S, Simpson KB (2018) Enzymes in Food Bioprocessing - Novel Food Enzymes, Applications, and Related Techniques. Curr Opin Food Sci 19: 30-35.
2. Nezhad NG, Abd Rahman RNZ, Normi YM, Oslan SN, Shariff FM, et al. (2022) Thermostability Engineering of Industrial Enzymes Through Structure Modification. Appl Microbiol Biotechnol 106: 4845-4866.
3. Nezhad NG, Abd Rahman RNZ, Normi YM, Oslan SN, Shariff FM, et al. (2023) Recent Advances in Simultaneous Thermostability-Activity Improvement of Industrial Enzymes Through Structure Modification. Int J Biol Macromol 232: 123440.
4. Xing H, Wang P, Yan X, Yang Y, Li X, et al (2023) Thermostability Enhancement of Escherichia Coli Phytase by Error-Prone Polymerase Chain Reaction (Eppcr) and Site-Directed Mutagenesis. Front Bioeng Biotechnol 11: 1-10.
5. Albayati SH, Nezhad NG, Taki AG, Rahman RNZRA (2024) Efficient and Easible Biocatalysts: Strategies for Enzyme Improvement: A review. Int J Biol Macromol 276: 133978.
6. Nguyen C, Young JT, Slade GG, Oliveira RJ, McCully ME, (2019) A Dynamic Hydrophobic Core and Surface Salt Bridges Thermostabilize a Designed Three-Helix Bundle. Biophys J 116(4): 621-632.
7. Zhang J, Humaidi AJ, Al-Dujaili A, Duan Y, Alzubaidi L et al., (2021) Review of Deep Learning: Concepts, CNN Architectures, Challenges, Applications, Future Directions. Journal of Big Data 8(1).
8. Wang C, Huang R, He B, Du Q (2012) Improving the thermostability of alpha-amylase by combinatorial coevolving-site saturation mutagenesis. BMC Bioinformatics 13: 263.
9. Buhari SB, Nezhad NG, Normi YM, Shariff FM (2024) Insight on Recently Discovered PET Polyester - Degrading Enzymes Thermostability and Activity Analyses. 3 Biotech 14: 31.
10. Patel AK, Singhania RR, Sim SJ, Pandey A (2019) Thermostable Cellulases: Current Status and Perspectives. Bioresour Technol 279: 385-392.