

Cell-free protein synthesis: A versatile platform for biocatalyst discovery

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Cell-free protein synthesis (CFPS) offers a powerful alternative to *in vivo* expression systems by enabling rapid, flexible, and high-throughput protein production without the constraints of cellular viability. Using CFPS systems based on *Escherichia coli* extracts, we have successfully synthesized various enzymes across five classes, demonstrating CFPS as a robust tool for biocatalyst screening and optimization. Key applications include the evaluation of enzyme homologs and variants, expressibility of genes from diverse organisms, and synthesis under non-natural conditions including more complex proteins like membrane proteins. The openness of CFPS systems allows for precise control of reaction environments, enabling them to be used as a flexible platform for the development of biocatalysts.