Technology Opportunity, Ref. No. UA-20/008

Ultrahigh throughput directed evolution of biocatalysts

Obtaining enhanced variants of oxidoreductase enzymes is critically important for many industrial and biotechnological processes. An innovative new approach based on directed evolution is proposed.

Keywords Directed evolution, high-throughput screening, enzyme catalysis, oxidoreductases, cellular encapsulation, hydrogels

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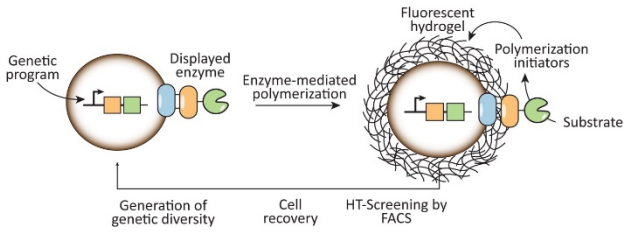
Reference Vanella at al. (2019), Biotechnol. Bioeng.: 116 (8): 1878-1886.

Background Obtaining enhanced variants of oxidoreductase enzymes is critically important for many industrial and biotechnological processes. Directed evolution is used to identify mutant enzymes that are stable under thermal, pH and solvent stress, or possessing enhanced catalytic turnover rates or substrate specificity. Genetic libraries of mutant enzymes are created and assayed using high-throughput screening methods. However, there exist **major challenges in large-scale enzyme screening**. The throughput of such campaigns is significantly limited by the requirement for reaction compartmentalization. This is typically achieved using robotic automation to pick individual colonies (genetic clones) for deep-well culture expansion and assay using soluble or solid substrates. Screening enzyme libraries using robotic automation is expensive, slow and achievable at a maximal rate of **~10,000 clones/week**.

Invention The invention is a new ultrahigh throughput screening method for identifying highly stable mutant enzymes. The method involves utilization of a chemical protocol that is completed within approximately 10 minutes. The chemical reaction results in localized formation of synthetic hydrogels on the surface of yeast cells displaying stable mutant enzymes. Cells displaying mutant enzymes with enhanced stability and catalytic turnover are encapsulated, while those cells displaying mutants that are less stable or possess lower catalytic turnover rates remain non-encapsulated. This means that the entire enzyme library can be tested in a single reaction vessel (i.e., one pot screening reaction), and subsequently sorted using flow cytometry at a rage of **~10^7 clones/hour**. Proof-of-concept studies using a model enzyme (FAD-dependent glucose oxidase) have been done, and several unique mutant enzymes with enhanced thermal stability and higher catalytic turnover rates have been identified. Several other candidate enzymes have been demonstrated to be compatible with the approach. Particularly interesting are FAD-dependent oxidoreductases.



Fields of Use One-pot large scale library screening to identify enzyme variants with improved properties in all fields of application.

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