BIOTRANS
LA ROCHELLE 2023

N°850 / OC / PC
TOPIC(s) : Enzyme discovery and engineering / Artifical intelligence / computational methods

A microfluidics-enabled workflow for rapid large-scale fitness data generation informs imine reductase engineering

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PURPOSE OF THE ABSTRACT
Directed evolution is the method of choice for optimizing biocatalysts but is often time consuming and its success is unpredictable. To meet the fast pace of product development in industry the turnaround time of engineering enzymes needs to become shorter. Directed evolution can be especially slow and challenging when fitness peaks are rare or poorly accessible in sequence space. Here we show that the availability of large-scale sequence–function data in enzymes relevant to biocatalysis can guide and thereby accelerate enzyme engineering. As a model system we chose an imine reductase (IRED) from S. roseum (SrIRED) catalysing reductive amination. To rapidly assess the fitness of >10000 IRED variants, we developed a cheap and versatile absorbance-activated droplet sorting-based ultra-high throughput screening setup. Coupled to a novel deep sequencing protocol combining next generation- and long-read-sequencing, this workflow allows the generation of large-scale fitness data using unbiased mutagenesis by error-prone PCR and the resolution of epistasis in less than 2 weeks. Using this novel workflow hot spots for engineering substrate specificity in the active site and at the periphery of the enzyme were identified. We used higher-order mutation data to score the combinability of individual improving mutations and rationally engineered highly active variants (10-fold improved kcat/KM) displaying positive epistasis showing that our workflow can give rapid access to highly improving variants. Due to its versatility and broad applicability, we envision that this workflow will become a valuable part of the protein engineer's toolkit for rapid enzyme engineering.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

BIBLIOGRAPHY