

Evolving enzymes for biorefinery applications

Proposers

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Introduction

Biocatalysis is considered a key component for the development of a sustainable bio-economy in Europe and the use of enzymes as biocatalysts has been constantly growing in the chemical, food, textile, pulp and paper and pharmaceutical industries. Enzymes are sustainable, selective and efficient, and offer a variety of benefits such as cleaner reactions with lower energy requirements. Over billions of years, evolution has created enzymes that enable biochemical reactions with remarkable catalytic power. However most native enzymes do not have enough robustness or efficiency for biotechnological applications. Directed evolution (DE) is a powerful engineering tool where the time scale of evolution can be shortened to an experiment conducted in the laboratory. DE mimics the principles of natural selection, through iterative rounds of random mutagenesis/recombination and screening of mutants. The application of an ultrahigh-throughput screening platform using droplet-based microfluidic devices can provide revolutionary improvements required to significantly advance both the scale and speed of screening at lower costs.

Partner 1

The MET lab at ITQB NOVA applies cutting-edge protein engineering techniques for improvement of enzymes' performance and robustness and approach long-standing questions on enzyme function and evolution.

Partner 2

The Thin-Film MEMS and BioMEMS group at INESC MN has extensive experience developing and characterizing thin-film silicon MEMS resonators as well as PDMS-based microfluidics.

Project outline/goal

Our aim is to construct a tool-box of robust peroxidases with improved efficiency and stability for chemical transformation of diverse phenolic-lignin related compounds. Evolutionary approaches such as error-prone PCR and DNA shuffling will be used for changing the enzyme's specificity. Libraries of variants will be screened using robotic screenings and chip-in-the-lab application. The in-depth biochemical and biophysical characterization of hits and evolutionary intermediates, will allow assessing the potential application of enzymes as well as identifying structural and functional determinants, to track evolutionary trajectories and recognize the dynamics and constraints of enzyme evolution. In parallel, this project will explore how microfluidic systems (most likely, but not exclusively, droplet-based) can be used to increase the

speed and decrease the costs both of specific steps and also the overall workflow of the evolutionary approaches described above.

Student profile *Profile sought: preference, but not limited, to students with a background in Biological Engineering, Biochemistry, Biomedical Engineering or Engineering Physics, with an interest in exploring complex advanced microsystems for practical applications.*