

Project: Enzyme's fitness evolution

Areas: Biochemistry/Molecular Biology/ Microbiology

Supervisor: Lígia O. Martins **Co-supervisor:** Vânia Brissos

Over billions of years, evolution has created enzymes that enable biochemical reactions with remarkable catalytic power. However many interesting enzymes do not have enough robustness or efficiency for industrial or medical 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.

In this work we will track the evolutionary trajectory of the metaloxidase McoP for improved catalytic efficiency in the oxidation of lignin-related phenolic substrates and will design enzymatic systems for lignin depolymerisation. Lignin is a heterogeneous aromatic biopolymer that accounts for nearly 30% of the organic carbon on Earth. It is one of the few renewable sources for aromatic chemicals on which the chemical industry so heavily relies. Valorisation of lignin, currently considered a bio-waste, is increasingly recognized as being crucial to the economic viability of lignocellulose biorefineries, a promising alternative source of renewable chemicals, materials, energy and fuels for future sustainable development.

McoP from the hyperthermophilic *Archaea Pyrobaculum aerophilum* is a multicopper oxidase that catalyzes the oxidation of cuprous and ferrous ions as well as aromatic substrates at a lower efficiency. (1) McoP shows a notable thermostability (temperature values at the mid-point (T_m) of 110°C) and has therefore a high potential for biotechnological applications since stability is a major limiting factor in the industrial application of enzymes. Cutting-edge protein engineering methods for random and DNA shuffling will be used for improving McoP efficiency for phenolics' through evolutionary approaches.(2-4) Random libraries typically comprise several hundred to thousand variants and therefore require screening systems with sufficient throughput. Screening assays are readily available that can be run in robot-assisted microtiter plate-based formats. The kinetic, biochemical and biophysical characterization of evolutionary intermediates will be performed. The comparison of effects of individual mutations in different genetic backgrounds will allow the identification of epistatic, synergistic and antagonistic interactions between mutations clarifying the existence of restrictive evolutionary trajectories contributing to guide further the optimization of the enzyme. Finally, this work will allow the optimization of eco-friendly and sustainable new routes for lignin depolymerisation and production of bio-products and bio-materials .

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Contact:

Lígia O. Martins

Microbial and Enzyme Technology Lab

ITQB NOVA, Oeiras

Tel: 214469534,

E-mail: lmartins@itqb.unl.pt

<http://met.itqb.unl.pt/>