Master Research Projects 2017

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Project: Evolutionary fitness of oxidoreductive enzymes

Areas: Protein Engineering/Molecular Biology/Biochemistry/Microbiology

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Directed laboratory evolution is a powerful protein engineering tool to tailor biocatalysts with improved features or new functions. By mimicking the principles of natural selection through iterative rounds of random mutagenesis and/or DNA recombination and screening, the time scale of evolution can be shortened to an experiment which can be conducted in the laboratory. Additionally laboratory evolution of proteins also contributes to understanding their functional properties and has the potential to test evolutionary theories and reproduce evolutionary scenarios.

McoA from the hyperthermophilic bacterium *Aquifex aeolicus* belongs to the multicopper oxidase family of enzymes. It is metalloxidase with a high efficiency for the oxidation of cuprous and ferrous ions, and notable thermoactivity (T_{opt} = 75°C) and thermostability (temperature values at the mid-point (T_m) of 110°C). Hyperthermostable enzymes are highly in demand for their robustness in biotechnological applications since thermostability is a major limiting factor preventing the industrial application of enzymes. For this reason we reported on a directed evolution approach that after four rounds of random mutagenesis followed by high-throughput screening changed the specificity of McoA from metals to aromatic organic substrates; the engineering approach led to the identification of a 2B3 variant featuring 100-fold higher efficiency than the McoA wild-type enzyme for the typical laccase substrate ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)).[1] Laccases are green catalysts that have found application in a large number of biotechnological applications in several industrial fields.

In this work we will track the evolutionary trajectory of McoA from a metalloxidase into a laccase and will recognize the dynamics and constraints of enzyme evolution. The kinetic, biochemical and biophysical characterization of evolutionary intermediates of the 2B3 variant will be performed. Additionally each of the mutations that accumulated over the evolution and their synergistic effect will be assessed through introduction of single and double substitutions in each intermediate; single mutants in wild-type background will also be characterized. The effects of mutations obtained in evolution will reveal mechanisms underlying epistasis (interaction between mutations) and how epistasis restricts evolutionary trajectories contributing to guide further the optimization of the enzyme. Finally, this work will advance our knowledge on the structural and functional determinants of substrate specificity and catalytic mechanisms within the multicopper oxidase family of enzymes.

1. Brissos, V., Ferreira, M., Grass, G., and Martins, L. O. (2015) Turning a hyperthermostable metallo-oxidase into a laccase by directed evolution, *ACS Catalysis 5*, 4932-4941.