Master Research Projects 2017

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Project: Methyl-NMR for studying substrate binding to a hyperthermophilic metalloxidase
Areas: Biochemistry/Enzymology/Biotechnology
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McoA from *Aquifex aeolicus* shows a remarkable efficiency for the oxidation of cuprous and ferrous ions and a notable thermoactivity (T_{opt} = 75°C) and thermostability (temperature values at the mid-point (T_m) of 110°C). Thermostability is a major limiting factor preventing the industrial application of enzymes and therefore hyperthermostable enzymes are highly in demand for their robustness in biotechnological applications. For this reason we have recently reported on a directed evolution approach that led to an engineered mutant showing a 100-fold higher efficiency for the typical aromatic laccase substrate ABTS [1]. In the X-ray structures of McoA wild type and mutant enzymes, the methionine-rich (Met-rich) segment, a striking feature of McoA, that occludes the substrate binding site could not be solved (unpublished results). This impaired significantly the understanding of McoA functional properties since this segment is thought to play a critical role in the catalytic specificity of the enzyme.

In this proposal, NMR spectroscopy will be used to study the interaction of the Met-rich region of the hyperthermophilic McoA enzyme with copper and other substrates. NMR spectroscopy is a powerful tool for studying biomolecular interactions, in part due to isotopic labelling schemes, which allow the specific incorporation of NMR-active nuclei into biomolecules. In this sense, isotopic labelling will be employed to selectively ^{13}C-label the methyl positions of all Met-residues (12) on McoA, with the ultimate goal of defining spectroscopic probes of protein structure and binding, particularly, at a region that is yet structurally and mechanistically poorly understood.

The expected results will give insight the molecular features of the substrate binding site of *A. aeolicus* McoA, close to the T1 copper centre, and will advance our knowledge on the structural and functional determinants of substrate specificity and catalytic mechanisms in 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.