## WORKING PLAN

# Turning an Hyperthermophilic Metallo-oxidase into a Laccase by Directed Evolution

**Área:** Biotechnology/Enzyme Engineering

**Laboratory:** Microbial and Enzyme Technology

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**SUMMARY**

This proposal focuses on research involving bacterial multicopper oxidases as a tool for biotechnology. These are copper-containing enzymes that oxidize a wide range of substrates including polyphenols and are considered useful biocatalysts for diverse biotechnological applications (Pereira et al. 2009). Nowadays, enzymes have found many applications in the industrial, analytical, environmental and biomedical sectors. However, catalysts which are stable to high temperatures, can function in diverse solvents, tolerate wider ranges of pH, catalyse reactions not encountered in nature, are still scarce. Directed evolution has developed quickly to become a method of choice for protein engineers in order to generate enzymes having desired properties. Multicopper oxidase from *Aquifex* (McoA) is a remarkable hyperthermostable copper-activated metallo-oxidase with low efficiency towards aromatics oxidation (Fernandes et al. 2007, 2009). The goal is to change the substrate specificity of McoA from metals to bulky aromatic (poly) phenols and amines, through directed evolution (Brissos et al. 2009).

Research strategies will cover: a) Construction of libraries of McoA mutants by combinatorial techniques, b) High-throughput robotic screening towards target substrates, c) Expression, production and purification of the best mutants and d) Biochemical and stability characterisation of mutants.

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Fernandes, AF, Soares, CM, Pereira, MM, Huber, R, Grass, G, Martins, LO. 2007. A robust metallo-oxidase from the hyperthermophilic bacterium *Aquifex aeolicus*. FEBS J. 274: 2683-94

Fernandes, AF, Martins, LO, Melo, EP. 2009. The hyperthermophilic nature of the metallo-oxidase from *Aquifex aeolicus*. Biochimica et Biophysica Acta. 1794: 75-83

Pereira, L, Coelho, AV, Viegas, CA, Correia dos Santos, MM, Robalo, MP, Martins, LO. 2009. Enzymatic biotransformation of the azo dye sudan orange G with bacterial CotA-laccase. J Biotechnology. 139: 68-77.