Aix*Marseille université

Ecole Doctorale des Sciences Chimiques ED250

Contrats Doctoraux **2019**

**Title :** **Multi-Copper Enzymes as logic systems**

**Laboratory** : ISM2, team BiosCiences

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**Context:** Biocomputing is attracting more and more attention.[1] Current research is driven by potential applications in biotechnology and medicine. In this field, one of the objectives is the integration of several functional units in a molecular structure giving rise to multiple responses following stimuli of various chemical or physical natures, thus allowing different logical operations to be performed within the same multifunctional molecule. Among the objects studied, enzymes are efficient and specific molecular machines. Each enzyme is a logic system whose effectors are substrates, products, inhibitors, pH, redox potential or even light.

In redox metalloenzymes, a unidirectional ET occurs if a rate limiting step depends on the oxidation state of a center (diode like). In addition, in response to environmental conditions, the catalytic properties of the enzymes change making the output signal conditional (transistor like).

**Description of the project :** Multi-Copper Oxidases (MCOs) are particularly robust biomolecules that couple the oxidation of substrates to the reduction of $O_2$ to $H_2O$ via successive intramolecular electron (ET) transfers (Fig. 1, top) thus behaving like a capacitor in which up to $4e^-$ and $4H^+$ are stored transiently.[2] **We are seeking for a motivated candidate with a training in physico-chemistry or bio-physics to study the response of a typical MCO (laccase) to different stimuli.**

The thesis work will comprise (i) a characterization of both native and surfaces–modified (e.g. photo-sensitizers) laccases and (ii) a thourough spectrocopic, magnetic, kinetic characterization of the enzymes under different stimuli (substrates, electrodes, light , etc.).

The effect of the orientation of probes located on the surface with respect to the two different copper centers (surface mono-nuclear, buried tri-nuclear aggregate, Fig. 1, bottom) will be studied. Intramolecular ETs, the distribution of $e^-$ on the 4 coppers and the evolution of the reaction (intermediates, reduction of $O_2$ to 2 or $4e^-$ or inverse reactions) will be studied by spectroscopic measurements (UV/VIS, EPR), stopped-flow and electrochemistry combined with advanced kinetic processing and modeling.

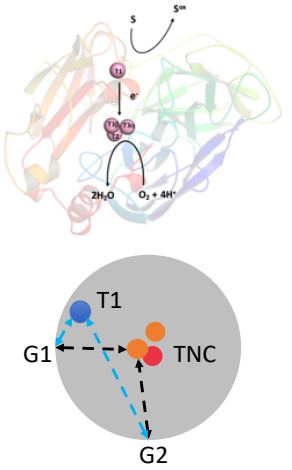


**Figure 1:** Top: structure of a MCO. $Cu^{II}$ centres are depicted as pink spheres. Bottom: location of $Cu^{II}$ ions relative to surface grafting points.

**Hosting laboratory:** BiosCiences is multidisciplinary (biochemistry, chemistry, physico-chemitry). The candidate will be able to rely on the team's know-how in: i) the production of laccase variants on the $gL^{-1}$ scale;[3] ii) the precise grafting of the surface of the variants with photo-sensitizers; [4] iii) promoting the reduction of $O_2$ by the enzyme under irradiation; [5] iv) the precise orientation of variants on the surface of materials (eg gold, carbon nanotubes, magnetic nanoparticles, $TiO_2$).[6]

**References:**

1 – E. Katz, V. Privman, *Chem. Soc. Rev.*, **2010**, *39*, 1835. This review has received 398 citations so far.
2 – E. I. Solomon et al., *Chem. Rev.*, **2014**, *114*, 3659.
3 – Y. Mekmouche, et al., *J. Biosci. Bioeng*. **2014**, *117*, 25.
4 – V. Robert, et al., *Chem Plus Chem*, **2017**, 82, 607.
5 – a) A. J. Simaan, et al., *Chem. Eur. J.* **2011**, 17, 11743; b) T. Lazarides, et al., *J. Am. Chem. Soc.*, **2013**, *135*, 3095.
6 - N. Lalaoui, et al. *ACS Catalysis* **2016**, *6*, 1894