Aix*Marseille université

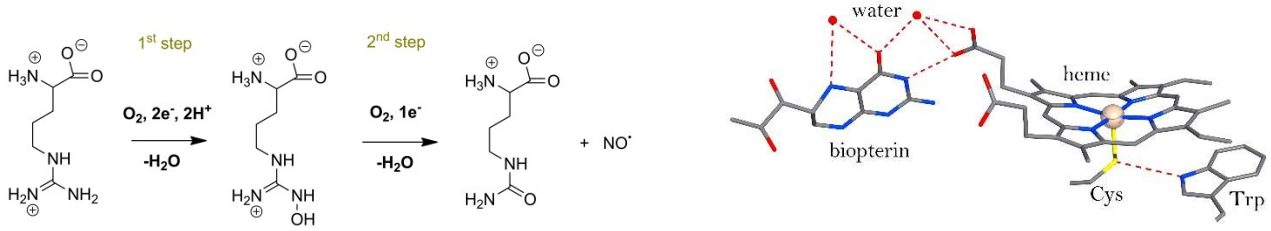
Doctoral school of Chemical Sciences

bip CNRS

PhD thesis title: **Biophysical studies of the NO-synthases family in diatoms**
Laboratory: Bioenergetics and Protein Engineering (BIP), Aix-Marseille University & CNRS (France)
Team: Biophysics of metalloproteins (Prof. B. Guigliarelli)
Supervisor: Dr. Pierre Dorlet, HDR (pdorlet@imm.cnrs.fr) http://bip.cnrs-mrs.fr/spip.php?rubrique134

Description of the PhD project:

Nitric oxide (NO•) is a ubiquitous biological messenger involved in numerous vital processes (regulation of the vascular tone, neuronal communication, …). In mammals it is synthesized by a family of enzymes the NO-synthases (NOS). Genome sequencing has led to the discovery of such enzymes in many different organisms throughout the tree of life, however their function in such cases remain unclear and poorly studied. The catalytic mechanism is also under debate to this day.[1-2] Only very recently, the first NOS from the plant kingdom was expressed and characterized by biophysical methods.[3] This has sparked a renewed interest in the role of these enzymes and of nitric oxide in photosynthetic organisms.



Left: The reaction catalyzed by NOS enzymes, producing nitric oxide in two oxidative steps from L-arginine. Right: active site of the oxygenase domain of NOS comprising the hemothiolate moiety associated with the tetrahydrobiopterin cofactor.

The aim of the project is to study the NOS family in diatoms, a very important group of phytoplankton. Two different enzymes chosen for their representative arrangement in different domains will be expressed and purified to be used in advanced biophysical studies of the cofactors and catalytic mechanism. In particular, multifrequency electron paramagnetic resonance (EPR) will be used to study the heme active site and its surroundings. Rapid kinetics approaches (rapid freeze quench, stopped flow) will help deciphering the catalytic mechanism. The interaction between the different domains of the enzyme will also be probed. A particular attention will be brought to the globin, a non-canonical heme domain of NOS present only in some species so far (cyanobacteria and diatoms): its influence on the electron transfer, catalysis and NO• chemistry will be under scrutiny.

The student will be hosted in the BIP lab in Marseille for a 3-year PhD contract from Aix-Marseille to begin in October 2019. The BIP lab has a strong expertise in spectroscopic and theoretical studies of metallo-enzymes. It hosts one of the major French EPR facilities of the national EPR network that includes continuous wave and pulsed EPR spectrometers operating at various frequencies (3 GHz, 9 GHz, 34 GHZ and 94 GHz) and equipped with multi-resonance capabilities.

Candidate profile:

The applicant must have a Master of Science in Chemistry or Physics with honors and some experience in spectroscopy. An interest in biological systems and biochemistry is also expected. Highly motivated, independent and dynamic, he/she should be able to work in a multidisciplinary team.
Please send applications including (i) a detailed CV, (ii) official transcripts of master and undergraduate studies, (iii) an application and motivation letter, and (iv) a recommendation letter of the Master's internship supervisor by e-mail at pdorlet@imm.cnrs.fr

Application deadline: 10th May 2019 – Interview in Marseille (doctoral school): **21st May 2019**.

References:

1. Brunel A, Santolini J, Dorlet P. (2012) *Biophysical Journal* 103, 109-117
2. Weisslocker-Schaetzel M, Lembrouk M, Santolini J, Dorlet P. (2017) *Biochemistry* 56, 748-756
3. Weisslocker-Schaetzel M, Andre F, Touazi N, Foresi N, Lembrouk M, Dorlet P, Frelet-Barrand A, Lamattina L, Santolini J. (2017) *Plant Sci* 265, 100-111