

Title	EPR spectroscopy <i>in cellula</i>, developing new strategies
PI	Marco Bortolus
Research Group	EPR spectroscopy
Curriculum	Chemical Sciences
Location	DiSC (possibly Marseille)
Contact	web: https://www.chimica.unipd.it/eplab/
	email: marco.bortolus@unipd.it

Project description:

Electron paramagnetic resonance (EPR) spectroscopy, coupled to Site-Directed Spin Labeling, is well suited to study the dynamics-function relationship of recombinant proteins in solution and in the membrane, but is mainly applied *in vitro*. Moving towards *in cellula* experiments is highly interesting to map the dynamics of the chosen proteins in their native environment, and EPR would be ideally suited since the majority of the cellular components are not paramagnetic or are at too low a concentration to muddle the results. Unfortunately, commercial nitroxides, the spin labels of choice, can be easily reduced by the cellular environment, so an alternative must be found.

In this project, the PhD candidate will be investigating a series of non-commercial nitroxides to evaluate their efficacy for *in cellula* studies, and could synthesize new ones in collaboration with the University of Marseille, France. The project will focus on recombinant Calmodulin, a soluble, well-characterized protein which acts as a Ca²⁺ sensor and conformational switch; the protein is produced in collaboration with the Department of Biology at UniPD. The nitroxides, alone and labeling recombinant Calmodulin, will be studied at first in a solution of cellular extracts. The second step will be the optimization of the protocol to insert the protein inside viable cells.

This project is suited to those students who love biophysics, a multidisciplinary environment at the interface between chemistry and biology. It offers the possibility of working directly in a molecular biology laboratory and to spend a period abroad in Marseille, France.

**Publications:**

Doni, D.; Passerini, L.; Audran, G.; Marque, S.R.A.; Schulz, M.; Santos, J.; Costantini, P.; Bortolus, M.; Carbonera, D. Effects of Fe²⁺/Fe³⁺ Binding to Human Frataxin and Its D122Y Variant, as Revealed by Site-Directed Spin Labeling (SDSL) EPR Complemented by Fluorescence and Circular Dichroism Spectroscopies. *Int. J. Mol. Sci.* **2020**, *21*, 9619. <https://doi.org/10.3390/ijms21249619>