Master 2 Research Project
Coupling between NMR and EPR spectroscopies

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General context and objectives

The magnetic resonance platforms (Nuclear Magnetic Resonance (NMR) and Electronic Paramagnetic Resonance (EPR)) applied to structural and dynamic biology, located on the CNRS Joseph Aiguier campus, aim to study biological objects at the molecular level. The EPR platform is also part of the RENARD national network - Research Infrastructure CNRS 3443. EPR is a method of choice for paramagnetic species and cannot detect diamagnetic entities which are the main objects of study in NMR. The physics behind these two methods is the same. However, although the complementarity between these two spectroscopies is no longer to be demonstrated, very few studies have really exploited it and the two worlds remain mostly separated with "NMR labs" and "EPR labs". Given the strong scientific skills of these platforms and the associated teams, as well as the subjects of study which are often identical, the challenge is to establish a stronger association between these two techniques. The objective of this Master project is to achieve the attribution of certain hyperfine couplings of a copper protein by a concerted approach using NMR and EPR without using specific isotopic labelling. The complementarity of skills between the two platforms will also be exploited to set up innovative methodologies in NMR and EPR for the rapid acquisition of spectra. In NMR, pulse calibration with variable radio frequencies for selective excitation and inversion of proton amides will shorten the inter-scan delay, and thus shorten the acquisition time. In addition, the implementation of the non-uniform acquisition mode for multidimensional spectra will also reduce their acquisition time. In EPR, we will test and optimize sequences not yet used routinely on the platform for the measurement of hyperfine couplings. These technical improvements will then benefit other users of the platforms.

Master project

CopI is a protein recently discovered in the bacterium *Rubrivivax gelatinosus* [1]. It is involved in the bacterial resistance to copper. Its structure and mechanism of action are not known at this time. We are working on the spectroscopic characterization of this protein and have been able to show that it coordinates at least two Cu (II) ions [2]: a square planar site located in the N-terminal portion of the protein and a green copper-type site. CopI is thus the first single-domain cupredoxin comprising two distinct copper sites. At the BIP laboratory, we produce this protein in *E. coli*, it is extremely soluble and overexpressed in large quantities. We can also produce it with uniform isotopic labeling for structural NMR purposes. Preliminary data show that the protein NMR resonances are narrow, making it a good model for a coordinated approach to NMR and EPR. The NMR study of the diamagnetic protein is in progress as well as the construction of a structural model. The Cu (II) sites have also been characterized by EPR [2] and more detailed studies are in progress. The objective of the internship will be to use a combined approach of NMR and EPR to fully characterize the copper coordination sites without resorting to specific isotopic labeling.
To achieve this, the student will record experiments of attribution for the NMR resonances of the side chains of the protein in its paramagnetic state for comparison with that of the diamagnetic state in order to assign the

nuclei of the CopI protein responsible for the hyperfine couplings with paramagnetic centers, previously measured in EPR (see below). These couplings are composed of a Fermi contact term and a dipolar coupling which cause a displacement of the NMR resonances and induce a faster relaxation ("paramagnetic relaxation enhancement", PRE). The student will set up recently developed NMR experiments which shorten their acquisition time while maintaining equal sensitivity [3,4]. The non-uniform mode of acquisition of the second and third dimensions of three-dimensional NMR spectra will also be tested and exploited by the student [5]. These features being the prerequisite for any molecular analysis by NMR, the development of these experiments which reduce the acquisition time of the spectra from several days to a few hours will allow other users of the platform to reduce their time spent on the spectrometer, and to free up machine time for other uses. The feasibility of these methods for pulse EPR will also be investigated.
The measurement of hyperfine couplings can be done directly by pulse EPR. For this, the student will rely on usual sequences (ESEEM, HYSCORE) to set up and optimize existing sequences and methods but not routinely used on the platform (DEFENSE, DONUT, pulse shaping, …). These new coupling measurement strategies (in particular the development of acquisition scripts) will then be accessible to other users of the platform.
In addition, the expertise in NMR and EPR that the student will acquire during the implementation of these new methods will be of added value for its integration into spectroscopic platforms. Indeed, many spectroscopists are specialized in one or the other spectroscopy, and a profile having skills in both will be highly rewarding. The interdisciplinarity of the subject located at the chemistry-biology-physics interface will be an additional asset in the student's training.

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[3] A.M.E. Cassaignau, H.M.M. Launay, M.-E. Karyadi, X. Wang, C.A. Waudby, A. Deckert, A.L. Robertson, J. Christodoulou, L.D. Cabrita, Nat Protoc 11 (2016) 1492–1507.
[4] P. Schanda, H. Van Melckebeke, B. Brutscher, J. Am. Chem. Soc. 128 (2006) 9042–9043.
[5] C. a Waudby, J. Christodoulou, Journal of Magnetic Resonance 219 (2012) 46–52.

Expected profile for the candidate

The student will have a solid background in physical chemistry, and at least a basic understanding of EPR and NMR spectroscopies. Knowledge in biology/biochemistry and/or bioinorganic chemistry will be appreciated.

The Master training position will start in early 2021 at the BIP lab in Marseille.
Applications (CV, letter of motivation, …) should be sent before the end of November 2020 to one of the supervisors.