Intitulé du Sujet de Thèse : Novel Gd^{3+}-tags and SDSL strategy for *in-cell* EPR spectroscopy

Laboratoire : Bioénergétique et Ingénierie des Protéines (BIP-UMR7281)

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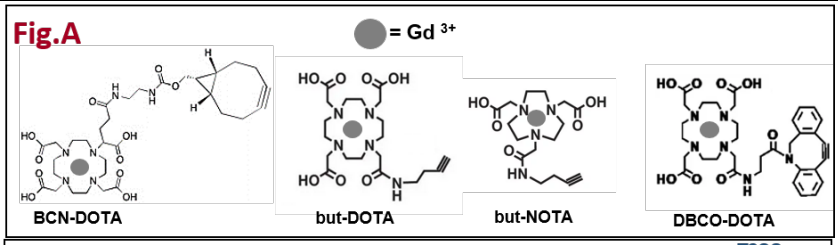
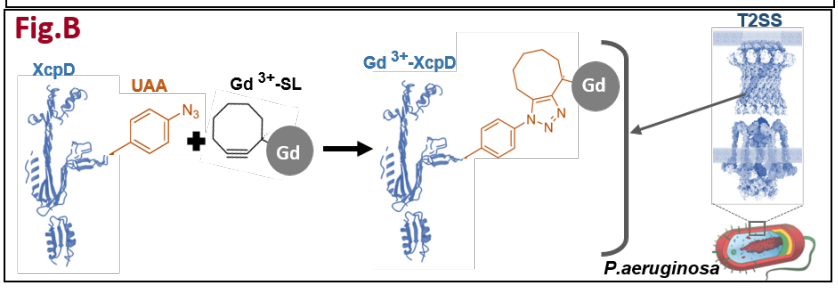
Contexte de l'étude

In-cell EPR spectroscopy is a ground-breaking biophysical method for bio-structural investigations on proteins inside cells[1]. In this context, proteins are usually modified *in vitro* introducing paramagnetic tags (or spin labels, SLs) onto sidechains of specific residues (i.e. Cys) using Site Directed Spin Labelling (SDSL) reactions. Once SLs are introduced, modified proteins are delivered into cells using different methods, such as electroporation or thermal treatments. Then, nanometric distances between SLs located in different regions can be measured with pulse EPR experiments for proteins in their native environments. In this way, conformational transitions and structural changes occurring in protein can be probed directly in the cellular space. However, the limited types of SDSL reactions and SLs available until now are hampering the application of *in-cell* EPR spectroscopy to a broad panel of biological systems.

This projects aim to develop an innovative intracellular SDSL strategy based on the use of unnatural amino acids (UAAs) and Gd^{3+}-based SLs[2] to extend the range of application of in-cell EPR spectroscopy in the field of structural biology. In details, this approach will allow to target the *P.aeruginosa* Type 2 Secretion System, a trans-membrane multi-protein nanomachine used by bacteria to secrete pathogenic agents in the environment[3]. The methodological developments brought by this project will be advantageous to acquire new insights about T2SS secretion mechanism at physiological conditions in its host-organism and to improve the state of the art of *in-cell* EPR spectroscopy at the same time.

Descriptif du projet





This PhD research project will be focused on the development of innovative intracellular SDSL reactions to enlarge the range of applications of *in-cell* EPR spectroscopy. Briefly, different types of UAAs will be initially incorporated onto a specific component of T2SS system using *CRISPR-cas* mutagenesis at the original chromosomal loci. These non-canonical residues have been chosen as spin labelling sites since selective SDSL techniques can be performed inside cell avoiding off-target reactions. For these reasons, the PhD student will initially screen a panel of Gd^{3+}-SLs (Fig.A) for different UAAs conjugations in order to select the optimal tag and labelling site based on the efficiency of UAAs mutagenesis, spin labelling yields, reaction rates and magnetic properties of the probe. Additional control experiments (fluorescence microscopy, mass spectrometry, circular dichroism, etc.) will also be performed to control the intracellular SDSL strategy. Once the spin labelling methodology optimized, the PhD student will perform pulse EPR measurements to measure inter-label distances in *P.aeruginosa* bacteria using various labelled variants of the protein of interest. In details, the main component of the T2SS outer membrane complex (XcpD secretin) will be targeted in this project (Fig.B). In this way, it will be possible to probe the secretion mechanism of T2SS nanomachine without perturbing its native environment and its physiological stoichiometry. The innovative intracellular SDSL strategy based on Gd^{3+}-SLs and UAAs will contribute to strength the state of the art of in-cell EPR spectroscopy, providing an alternative approach to carry out magnetic resonance experiments in living bacterial cells. The project will be supported by a funded-IM2B collaboration with *Dr. R.Voulhoux* (LCB-UMR7283), an expert of T2SS system and *CRISPR-cas* techniques in *P.aeruginosa*. The PhD project lies at the interface between biochemistry and biophysics, conferring to the PhD student a scientific background suitable for his/her future career in the field of scientific research. Candidates with background chemistry and magnetic resonance spectroscopies are particularly appropriate for this PhD project. The candidate should show also strong motivation and enthusiasm for experimental research activities.

Références Bibliographiques

[1] A. Bonucci et al. , ChemBioChem, 21 (2020) 451–460.

[2] Y. Yang et al., Phys. Chem. Chem. Phys., 19 (2017) 26944–26956.

[3] B. Barbat et al., Sci. Adv., 9 (2023) 40.