Intitulé du Sujet de Thèse : Advanced Methodological Strategies for Investigating Protein Structural Dynamics in Cellular Environments Using EPR Spectroscopy

Laboratoire : Bioénergétique et Ingénierie des Protéines

Equipe : « Flexibilité structurale des biomolécules en milieux complexes »

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Descriptif du projet

Studying protein structural dynamics and protein-protein interactions (PPIs) in living cells is critical to improve our understanding of how the intracellular medium tunes biological functions of proteins but also represents one of the current greatest challenges in structural biology.[1]
During the last decade, the investigation of bio-macromolecules in the cellular environment has attracted increasing interest and involved different techniques. Among magnetic resonances spectroscopies suitable for this approach, Electron Paramagnetic Resonance spectroscopy coupled to Site-Directed Spin Labeling (SDSL-EPR) has emerged as a promising tool to study protein local dynamics and conformational ensembles. Since 2010 and the first in-cell EPR experiment, remarkable progresses have been made in the development of suitable methodologies for the in-cell EPR approach.[1, 2, 3] However, despite these advances, performing EPR in living cells is still challenging and strongly limited by: *i)* a low signal-to-noise ratio affecting distance measurements between spin labels by Pulsed Dipoler spectroscopy (PDS-EPR);[3] *ii)* the lack of appropriate methods enabling to keep cell viable during EPR experiments and preventing time-resolved EPR investigations.
The aim of this PhD project is to overcome these obstacles and bring SDSL-EPR beyond its actual limits and towards EPR vision at the molecular level inside cells. Using new sensitivity-enhanced nitroxide labels for PDS-EPR spectroscopy and a bioreactor, we will be able study protein structure in native environments – inside living cells – to reveal how both healthy and disease-relevant cellular environments influence protein structure. In the near future, the developed tools will be applied to the study of the chaperone protein HtpG dynamics under different conditions (stressed, healthy or damaged cells) and would allow unique molecular-level studies of disease-related protein alterations.

Références Bibliographiques

[1] A. Pierro, A. Bonucci, A. Magalon, V. Belle, and E. Mileo, *Chemical Reviews* **2024**, 124, 9873.

[2] A. Pierro, K. C. Tamburrini, H. Leguenno, G. Gerbaud, E. Etienne, B. Guigliarelli, V. Belle, B. Zambelli and E. Mileo. *iScience*, **2023**, 26, 107855.

[3] A. Pierro, A Bonucci, D. Normanno, M. Ansaldi, E. Pilet, O. Ouari, B. Guigliarelli, E. Etienne, G. Gerbaud, A. Magalon, V. Belle, E. Mileo*. *Chem.- Eur. J.*, **2022**, 28, e202202249.