PhD thesis title: Probing the structural dynamics of the full-length cytochrome P450 reductase involved in the xenobiotic metabolism

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Context

Microsomal cytochrome P450s (CYPs) occupy a pivotal role in human drug (70% of commonly prescribed drugs), fatty acid and steroid metabolisms.[1,2] To perform their functions, CYPs require electrons, which are provided by a specific flavoprotein, the NADPH cytochrome P450 reductase (CPR). The electron transfer (ET) process involves a nicotinamide adenine dinucleotide phosphate (NADPH) co-factor, which provides a hydride to CPR. Electrons then transit via CPR's flavin cofactors, from flavin adenine dinucleotide (FAD) to flavin mononucleotide (FMN), reaching its final electron acceptor, the heme iron of CYPs.

The conformational dynamics of human CPR in solution, which involves transitions from a "locked/closed" to an "unlocked/open" state, is crucial for electron transfer. It has been studied through both spectroscopic and structural approaches, essentially on mutated or truncated proteins.[3–7] Still, molecular factors governing the conformational equilibrium are poorly understood for the full-length protein, including its specific interaction with CYPs impacting xenobiotic metabolism.

Description of the PhD project

Recently, we reported the study of soluble *Homo sapiens* CPR using Site-Directed Spin Labelling (SDSL) and Electron Paramagnetic Resonance (EPR).[8] We have successfully investigated the dynamics and conformational changes of the one electron reduced soluble form of human CPR by incorporating a non-canonical amino acid at selected strategic positions and subsequent specific nitroxide labelling. DEER experiments on semiquinone (sq)/nitroxide pairs on CPR, and molecular dynamics (MD) studies, have been conducted proving the existence of two main states in solution. The range of conformations of CPR may be crucial to the interaction and binding between CPR and CYPs, possibly via the selection of competent conformations depending on the redox state of CPR. The alterations in the conformational equilibrium of CPR induced by mutations on the flavin domains or by the binding of small molecules have been previously documented.[2,9] However, these studies did not concurrently track the redox state of flavins.

During the thesis, our innovative approach described above will be applied and developed to the full-length protein to study its native environment and its partnership with CYPs, by assessing the effect of membrane, the nature of lipids,[10–13] co-enzyme binding and redox chemistry in structural changes. It will involve the monitoring of CPR during the main flavine redox states implicated in ET and/or under varying ionic strengths. Complementary biophysical studies will be employed to fully study the protein, such as Cryo-EM (in collaboration). This will provide comprehensive insights into the dynamic interplay in CPR at various redox states, particularly those competent in ET to specific CYPs involved in the xenobiotic metabolism.

Candidate profile

The student will be hosted in the BIP lab in Marseille for a 3-year PhD contract from Aix-Marseille University to begin in October 2024. The BIP lab has a strong expertise in spectroscopic and theoretical studies of proteins. 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 and equipped with multi-resonance capabilities. The BIP lab is the only French lab working with the SDSL-EPR approach on biological systems.[8,14,15]

The candidate must have a Master of Science in Chemistry or Biochemistry. An interest in interdisciplinary projects is expected. Highly motivated, independent and dynamic, he/she should be able to work in a multidisciplinary team and have communication skills including for international collaborations. He/she will be involved in the biochemistry and the biophysics studies.

Please send applications by email before April, 20th 2024 including:
- a detailed CV
- an official transcript of master and undergraduate studies, and master exam grades
- a motivation letter for the project
- a recommendation letter of the Master's internship supervisor by e-mail at marlene.martinho@univ-amu.fr

Doctoral school interview in Marseille : May, 28th and 29th 2024

References

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