FINAL MASTER PROJECT PROPOSAL

Title

Analysis of proteins by local probe microscopy

Supervisor(s)

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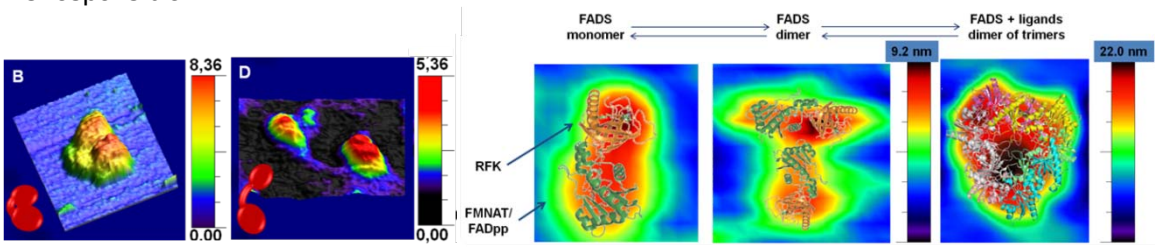
Summary of the project

The goal of the research line is the analysis of the mechanism of important proteins determining the association pattern, the quaternary organization and the intermolecular forces upon ligand binding -proteins, enzymes, DNA, metals, carbohydrates, inhibitors…- or catalysis. The study will be performed using atomic force microscopy (AFM), the only technique able to provide images of biological structures in a physiological environment with sub-nanometer resolution. Other variants of local probe microscopy, such as dip-pen nanolitography (DPN), may be used for the immobilization of proteins on surfaces; and also scanning tunneling microscopy (STM), capable of evaluating the conductive properties of electron-carrying proteins on a gold conductive surface. We currently have several projects under development with flavoenzymes, human glycosyltransferases, and mitochondrial respiratory complexes and supercomplexes.

The aim of the TFM is the analysis of the action mechanism of important proteins at the single molecule level. The work plan consists of three steps:
1- Optimization of protein immobilization on mica at the working conditions (buffer, pH, ligands) providing some movement that allows to study the conformational dynamics of the molecules. For STM, proteins could be attached to gold using DPN.
2- AFM imaging of the immobilized proteins preincubated with their ligands. STM measurements of flavoproteins to analyze topology and other properties.
3- Qualitative and quantitative analysis of the protein species detected. The conformational changes and association types of proteins when incubated with ligands or inhibitors will be observed.

The samples will be prepared in the biochemistry lab, and the measurements performed in the ambient AFMs of the ICTS Laboratory of Advanced Microscopies, at INA, of which A.I.G.Lostao is responsible.



Left: Conformational dynamics of single molecules of enzyme during the catalytic steps, which helped to solve the mechanism of action of human GalNAc-glycosyltransferase 2. Compact GalNAc-T2 bound to UDP-GalNAc (B), and extended structure bound to UDP-GalNAc/glycopeptide (D)[Nature comm. 6, 6937, 2015]. Right: Scheme representing the catalytic equilibrium between molecular FAD synthetase species. It was possible to distinguish between the riboflavin kinase (RFK) and the FMN adenylytransferase (FMNAT) modules at single enzyme molecules [BBA 1834, 665, 2013].