

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project Year 2024-2025

Laboratory/Institute: IBS Team: ELMA and GSY Royant **Director:** W. Weissenhorn **Head of the team:** B. Franzetti & A.

Name and status of the scientist in charge of the project: Dr Dominique MADERN and Dr Sylvain Engilberge
HDR: yes (DM)
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Program of the Master's degree in Biology:

□ Structural Biology of Pathogens

<u>Title of the project</u>: Functional protein crystallography on malate dehydrogenase using a nano injector

<u>Objectives (up to 3 lines)</u>: We will develop an innovative method to study the dynamics of enzymes during catalysis. This method combines X-ray crystallography at room temperature on single crystal and *in crystallo* optical spectroscopy with nanodroplets injection. This approach will enable the recording of molecular movies depicting enzymatic activites and allow for atomic-scale visualization of substrates binding and processing.

<u>Abstract (up to 10 lines):</u> Recent advances in artificial intelligence have significantly influenced the field of structural biology. It is now possible to obtain excellent static structural models predicted solely from protein sequences. However, predicting protein dynamics, such as short atomic displacements, reorganization, multiple conformations, and catalysis, remains challenging. Room temperature crystallography offers the possibility to decipher enzyme function. The challenge lies in initiating the reaction and evaluating the kinetics of the reaction in the crystalline state. We are developing a new methodology called functional crystallography, which combines X-ray diffraction and *in crystallo* optical spectroscopy with a piezo nanodispenser that can dispense droplets of 2 to 70 nl onto a single crystal. The top-hat beam of the BM07 beamline will allow us to record low-dose room temperature datasets on single crystals to monitor the dynamic properties of non-allosteric and allosteric enzymes involved in metabolism. We will follow the conformational changes associated with the direct injection of substrates and activators in a time-resolved manner.

Methods (up to 3 lines): Protein purification, Enzymology, Crystallization, X-ray diffraction.

<u>Up to 3 relevant publications of the team :</u> Robin AY, Brochier-Armanet C, Bertrand Q, Barette C, Girard E, Madern D. Deciphering Evolutionary Trajectories of Lactate Dehydrogenases Provides New Insights into Allostery. Mol Biol Evol. 2023 Oct 4;40(10):msad223.

Bertrand Q, Coquille S, Iorio A, Sterpone F, Madern D. Biochemical, structural and dynamical characterizations of the lactate dehydrogenase from Selenomonas ruminantium provide information about an intermediate evolutionary step prior to complete allosteric regulation acquisition in the super family of lactate and malate dehydrogenases. J Struct Biol. 2023 Dec;215(4):108039. doi: 10.1016/j.jsb.2023.

Aumonier S. Engilberge S, Caramello N, von Setten D, Gotthard G, Leonard GA, Mueller-DieckmannC, Royant A. Slow protein dynamics probed by time-resolved oscilation crystallography at room temperature. IUCrJ. 2002, Sept 28, 9(Pt 6):756-767.

<u>Requested domains of expertise (up to 5 keywords)</u>: Biochemistry, enzymology, crystallography, crystallization.