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Towards deciphering Catalytic Mechanisms by Time-Resolved Serial Femtosecond Crystallography at XFELs

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Time-resolved serial femtosecond crystallography (TR-SFX) is a revolutionary scientific-technical breakthrough that makes use of the highly-intense, ultra-short X-ray pulses produced at X-ray Free Electron Lasers (XFELs) to study structural dynamics of biological macromolecules in "real time"by using nano/microcrystals under nearly "native" conditions. In our group, we are interested in applying this approach to study the reaction mechanisms of two proteins associated with fatal diseases. Human NQO1 protein, a flavoenzyme associated with cancer, Alzheimer´s and Parkinson´s disease and an attractive target for drug discovery is one. We have recently determined the first structure of NQO1 at LCLS (CA, USA) to 2.5Å resolution using the modulator droplet injector (MDI) developed by Alexandra Ros at Arizona State University (AZ, USA). A careful analysis of our structure has revealed that residues Tyr128 and Phe232, which have been described to play a key role in the function of the protein, show an unexpected flexibility within the crystals. This high-plasticity of NQO1 in the catalytic site provides us with the first structural evidence that the NQO1 functional cooperativity is driven by structural communication between the active sites through long-range propagation of cooperative effects across the NQO1 protein structure. Thus, understanding these functional aspects of NQO1 and its interaction with ligands (substrates and inhibitors) at the molecular level, will be critical to unravel NQO1' s role as an antioxidant and a potential target to treat common diseases by advancing in the design of new, more potent, and effective inhibitors that can be used in the clinic. To this end, TR-SFX experiments of the holo-NQO1 in the presence of the substrate NADH at various time delays are ongoing, with the ultimate goal of determining the high-resolution structures of the intermediates involved in the redox reaction mechanism of NQO1.
The second protein of interest to us is the penicillin binding protein 2a (PBP2a) of the methicillin-resistant Staphylococcus aureus (MRSA). PBP2a is used by MRSA as defense shield against beta-lactam antibiotics by an unique allosteric mechanism still not fully understood. We have recently obtained microcrystals of PBP2a and the initial SFX experiments will be conducted at the EuXFEL (Germany) in Fall 2022. For both proteins, NQO1 and PBP2a, it is clear how the setup of TR-SFX experiments is a crucial strategy for providing a new way to explore irreversible reactions and fast conformational changes, leading to generation of molecular movies of dynamic macromolecules in action. SFX at XFELs will represent a very turning point for drug design against cancer and for the design of more potent antibiotics.

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Primary author: GRIECO, Alice (Dept. Crystallography & Structural Biology. Institute of Physical Chemistry Rocasolano, Spanish National Research Council (CSIC), Madrid, Spain)

Co-authors: RUIZ-FRESNEDA, Miguel A. (Department of Microbiology, University of Granada, Granada,

Spain); PACHECO, Juan Luis (Department of Physical Chemistry, University of Granada, Granada, Spain); JERNIGAN, Rebecca (School of Molecular Sciences, Arizona State University, Tempe, AZ, USA;Center for Applied Structural Discovery, Biodesign Institute, Arizona State University, Tempe, AZ, USA); DOPPLER, Diandra (School of Molecular Sciences, Arizona State University, Tempe, AZ, USA; Center for Applied Structural Discovery, Biodesign Institute, Arizona State University, Tempe, AZ, USA); SONKER, Mukul (School of Molecular Sciences, Arizona State University, Tempe, AZ, USA; Center for Applied Structural Discovery, Biodesign Institute, Arizona State University, Tempe, AZ, USA); MOBASHERY, Shahriar (Department of Chemistry and Biochemistry, University of Notre Dame, IN, USA); CHANG, Mayland (Department of Chemistry and Biochemistry, University of Notre Dame, IN, USA); FROMME, Petra (School of Molecular Sciences, Arizona State University, Tempe, AZ, USA; Center for Applied Structural Discovery, Biodesign Institute, Arizona State University, Tempe, AZ, USA); HERMOSO, Juan A. (Dept. Crystallography & Structural Biology. Institute of Physical Chemistry Rocasolano, Spanish National Research Council (CSIC), Madrid, Spain); ROS, Alexandra (School of Molecular Sciences, Arizona State University, Tempe, AZ, USA; Center for Applied Structural Discovery, Biodesign Institute, Arizona State University, Tempe, AZ, USA); PEY, Angel L. (Department of Physical Chemistry, Unit of Excellence in Applied Chemistry to Biomedicine and Environment, and Institute of Biotechnology, University of Granada, Granada, Spain); MARTIN-GARCIA, Jose M. (Dept. Crystallography & Structural Biology. Institute of Physical Chemistry Rocasolano, Spanish National Research Council (CSIC), Madrid, Spain)

Presenter: GRIECO, Alice (Dept. Crystallography & Structural Biology. Institute of Physical Chemistry Rocasolano, Spanish National Research Council (CSIC), Madrid, Spain)

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