6-month Experimental Report

BAG RESPONSIBLE: José Manuel Martín García

PROPOSAL TITLE: Towards 3D structure determination and dynamics of biological and non-biological samples using TR-SSX methods.

PROPOSAL REFERENCE: MX-2427

BAG PRINCIPAL INVESTIGATORS

- José Manuel Martin Garcia, CSIC - Instituto de Quimica Fisica Rocasolano Departamento de Cristalografia Serrano 119 ES - 28006 MADRID
- Carlos Martín-Gastaldo, Universidad de Valencia Instituto de Ciencia Molecular (ICMol) PO Box 22085 ES - 46071 VALENCIA

BAG PARTICIPANTS

- Alice Grieco (PhD student), CSIC - Instituto de Quimica Fisica Rocasolano Departamento de Cristalografia Serrano 119 ES - 28006 MADRID
- Jose Antonio Gavira, Instituo Andaluz Ciencias de la Tierra (IACT) Laboratorio Estudios Cristalograficos (LEC) Avenida de las Palmeras, 4 ES - GRANADA

SAMPLES TESTED

- Lysozyme. We will use lysozyme microcrystals soaked with bromophenol blue (BPB) for radiation damage experiments.
- MUV-15 (MOF from the University of Valencia 15). This will be a pump-probe TR-SSX in which microcrystals of MUV-15 will be illuminated by a laser and then probed by X-rays. Laser pulses will heat up the crystals allowing the solvent molecules of DMSO to come out of the sample. Solvent release shrinks the crystal lattice. So, we will gain insights into the structural dynamics of this flexible behavior (also known as “breathing” behavior) will provide new insights into the chemistry of MOFs allowing for the design and development of new type of MOFs with improved performances. For these experiments we will need a microfocus beam.
- NQO1 (NAP(P)H:quinone oxidoreductase 1). Our TR-SSX experiments will consist of mixing the substrate NADH with NQO1 microcrystals and follow the reaction progress. We will determine the crystal structure of structural intermediates at various time points that can enable to gain insights into the catalytic mechanism of this enzyme with the goal of advancing the design of more affective and potent inhibitors against NQO1. We will also work with the wild-type protein, which is a 30kDa protein.
- PBP2a (Penicillin Binding Protein 2a). Our TR-SSX experiments will consist of mixing crystals of PBP2a with the antibiotic oxacillin to follow the reaction progress. The main goal will be to determine the structure of reaction intermediates at various time points to gain insight into the antibiotic resistance mechanism of this enzyme. PBP2a microcrystals have been obtained and can be reproduced readily. Microcrystals have also been tested at ID30 beamline at ESRF and diffraction observed up to 2.5A resolution. For our TR-SSX experiments we will require a microfocus beam. For our experiments, only the wild-type protein will be explored. PBP2a is a 72 kDa protein.
- Flavodoxin (Fld). We intend to develop pump-probe TR-SSX experiments with this protein and follow the course of the reaction upon illumination. Fld is a small soluble electron-transfer flavoprotein of about 15 kDa that contains FMN as chromophore.
- Ferredoxin-NADP$^+$ reductase (FNR). We intend to develop TR-SSX experiments that will consist of mixing the substrate NADPH with FNR microcrystals and follow the reaction progress. We will determine the crystal structure of structural intermediates at various time points that can enable us to gain insights into the catalytic mechanism of this enzyme.
- Bi-11-b: This sample corresponds to a novel bismuth-based Metal-Organic Framework. The MOF is currently being investigated as heterogeneous catalyst for various multi-component reactions, involving the interaction between the metal atom and the catalytic substrates. However, this compound undergoes a phase transition when in presence of different organic solvents, and the atomic rearrangement of the MOF atoms is not determined yet, which in turn is expected to influence the material’s performance.

- CLG: This sample corresponds to a new Metal-Organic Framework synthesized following a recently developed synthetic methodology based on the use of heterometallic clusters as precursors. This synthetic methodology allows the incorporation of single-atom sites at specific positions in the MOF building units. The single crystal analysis is expected to demonstrate the formation of the proposed MOF structure, and confirm the suitability of this synthetic process for different kinds of organic linkers.

SUMMARY OF THE RESULTS OBTAINED

Experiment parameters:

- Photon energy: 11.56 keV
- Beam size: 4 x 2 um
- Flux: 10^{15} ph/sec
- Bandwidth: 1%
- Detector: JUNGFRAU 4M (detector distance was a bit closer, ~1.7 Å resolution)
- Pulse rep. rate: 925 Hz
- Pulse length: 90 us

1) Lysozyme: Lysozyme microcrystals (20 µm) grown in our labs at IQFR-CSIC were soaked with bromophenol blue (BPB) at pHs 6.5 and 7.5, incubated for a few days, and then transported to ESRF. Sample delivery was performed with the HVE acquired by ID29 team from Arizona State University (USA). Two high-viscous media were tested to deliver the microcrystals, the Super Lube grease and 20% hydroxy cellulose (HCE). Although we had some trouble getting a steady stream for the grease, overall, both media performed quite well with a very smooth stream. Several full data sets were collected at transmissions 10%, 20%, 40% and full beam with grease for the sample at pH 6.5. A few more data sets were collected at 10% and 20% transmissions with HCE for the sample at pH 7.5.

Data processing was done onsite by Shibom Basu. After 5K indexed frames, **we could see extra electron density that might belong to the BPB ligand**. We have recently resumed data processing remotely and we hope to have a full data set of lysozyme protein with BPB bound to it. Confirming the visibility of the ligand will enable us to develop radiation damage measurement in upcoming beamtimes.

2) MUV-15: MOF microcrystals were synthesized in the lab of the co-proposer, Carlos Martí-Gastaldo and delivered to the X-ray beam suspended in HCE and measurement made at 10%, 20% and 40% transmission. Poor diffraction was seen. So, we changed the setup to fixed target with the two mylar foil chips. We used the smaller chip, which uses just 3ul of sample. No good diffraction was seen from this sample in the end.

3) Fld and FNR: These two proteins were shipped to ESRF as frozen solution. Crystallization experiments were conducted onsite with no luck. Briefly, numerous experiments were tested unsuccessfully to reproduce the crystals obtained by our collaborators. Crystallization was done using free-interface diffusion (FID) and batch methods by varying the protein to precipitant ratio. In the case of FNR, “quasi” crystals were observed, which did not diffract. As for Fld, no precipitate of any kind was seen. A couple of batches with agitation experiments were also done for Fld in which phase separation was observed.

4) Bi-11-b and CLG MOFs: These two MOFs were resuspended in glycerol to prevent ethanol from evaporating while dispensing the sample onto the foil. Data sets collected using the mylar foil chips.

- Bi-11-b microcrystals (20-30 µm) were seen diffracting to the edge of the detector (~1.7Å). Data processing has recently been done remotely. A total of 4,241 frames have been indexed out of 629,000 into the space group C2/c with the unit cell dimensions: a= 31.83Å; b= 11.68Å; c= 27.38; α= 90°; β= 115.98°; γ= 90°. Data has also been integrated and merged. **Structure solution ongoing**.
- CLG crystals (1-5 µm) diffracted poorly. Data processing has recently been done remotely. A total of 970 frames have been indexed out of 834,000. Indexing gave us a very sharp peak distribution for the lengths of the unit cells, but too much error for the angles. Better crystals will be brought in our next beamtime.

5) NQO1: NQO1 was shipped to ESRF as frozen solution. Crystallization experiments were conducted onsite. Two crystal sizes were obtained, 50 µm and 20 µm. NQO1 microcrystals were mixed and incubated with NADH and dicoumarol. Crystals were delivered to the X-ray beam using the mylar foil chips. Diffraction was seen up to ~2.6 Å resolution. Full data sets were collected for:

- Unliganded NQO1 (apo-protein)
- NQO1 in complex with the substrate NADH
- NQO1 in complex with the inhibitor dicoumarol

Preliminary data processing was done onsite by Shibom Basu. From this, we have been able to identify the two ligands bound to the protein, which is a success. More detailed data processing and structure solution is currently ongoing.

6) PBP2a: PBP2a did not crystallize as normal. After several attempts in which higher protein concentrations (35 and 50 mg/ml) were tested, we could not manage to grow the crystals this time. It is worth noting that several crystallization tests have been carried out in our labs after the beamtime experiment and we have been able to produce the microcrystals, so that, we will bring this sample back in June experiment.

PUBLICATIONS

Two publications are expected to come out from the two experiments conducted so far.

BEAMLINE PERFORMANCE

The beamline performed as expected at all times in both experiments. We received fantastic support from all team members of ID29. We look forward to conducting our next experiments for which we hope to collect higher resolution data sets from MOF samples, as well as develop the first pump-probe and mixing time-resolved experiments with our samples.

NEW PROPOSED RESEARCHES AS CO-PROPOSERS

We have been experiencing an increment in the number of Spanish researchers interested in joining this BAG to conduct TR-SSX experiments on ID29. In fact, some of the samples tested in the second experiment were provided by our collaborators. So, we want to request the possibility to include the three researchers below as co-proposers in this BAG.

- Felipe Gándara, Instituto de Ciencias de Materiales (ICMM) Campus de Cantoblanco, C. Sor Juana Inés de la Cruz, 3, 28049 MADRID
- Marta Martínez Júlvez, Universidad de Zaragoza Mariano Esquillor, Edificio I+D Campus Río Ebro, 50018 ZARAGOZA
- Jose Antonio Gavira, Instituo Andaluz Ciencias de la Tierra (IACT) Laboratorio Estudios Cristalograficos (LEC) Avenida de las Palmeras, 4 ES - GRANADA