

**Time resolved study of globular and membrane protein crystals with 1000 Hz X-ray pulses.**

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This report is a preliminary brief review of what has been done during our first on site experiments on ID 29 ESRF.

Dates of experiments Feb 21-24, Feb 27 and March 1-3 in 2023 in total six days or 18 shifts.

We crystallized the following samples: photosystem I, NendoU (3 different mutants), BRD 3-1 as phycoyanin-C on site.

Tested fixed target provided by ESRF and LCP injection provided from our ASU team.

Co crystallized with and soaked ligands with NendoU. Soaked BRD 3-1 crystals with ligand.

All proteins gave diffraction quality crystals and could be tested with the different delivery methods.

**1.) Technical status of the beam line current**

Photon flux multiple of  $10^{15}$  photons/s

Chopped 1000 times per second

Each pulse currently 100 us (multiple of  $10^{11}$  photons)

Transmission can be reduced with foils

Camera 4 M Jungfrau

Taking 230 images per second

No laser excitation possible for pump probe experiments

**1a) Technical status of the beam line for time resolved experiments (not all of these are at the same time necessary)**

Photon flux multiple of  $10^{15}$  photons/s

Chopped 1000 times per second

Each pulse can be varied from 10- 100 us (multiple of  $10^{10}$  to  $10^{11}$  photons)

Transmission can be reduced with foils

Camera 4 M Jungfrau

Taking 1000 images per second

Laser excitation possible for pump probe experiments to measure synchronized eg

Dark-light alternating x-ray images.

## **2.) Diffraction results for fixed target**

### **Sample: NendoU**

For all NendoU mutants diffracting of crystals could be achieved.

Resolution was up to 3 Å in best case and preliminary hit rate 0.1-5 %.

Data have been collected with small targets each run was 80,000.

Several data sets have been obtained with and without ligand present.

Additional data have been obtained from co-crystallized NendoU mutants with ligand.

### **Sample Photosystem I**

In small fixed target frames no diffraction of photosystem I could be observed, in big fixed targeted frames

Photosystem I diffracted to 6-7 Å resolution with a low hit rate of 0.2 %. A physical stress of mounting seems to destroy the crystals, which has been proven by later adding a mesh as physical support in between the two mounting foils, here the resolution and hit rate had been improved.

### **Sample:C-Phycocyanin**

C-Phycocyanin worked good with fixed target a resolution of 3 Å could be achieved with a hit rate in between 3-6 %. With the high symmetry space group of H32 several datasets have been measured.

### **Sample BRD 3-1**

Crystals grown at ASU could be mounted on small fixed target frames and diffracted to 2 Å resolution with hit rates up to 10 %. Many datasets with and without pre soaked ligand have been obtained.

### **3.)LCP type jet provided by ASU**

To have a alternative method to deliver sample for diffraction experiments a liquid injection system developed by ASU was tested at ID 29. Here viscous media allow low flow rates of embedded crystals.

Here we tested two different media 8M PEG and Cellulose to achieve flow rates of below 2ul/min.

Stable jets could be achieved with both media. For each sample conditions of crystal flow and embedding have been tested.

### **Sample NendoU**

NendoU crystals could be embedded in 8M PEG as in Cellulose. Not only the embedded crystals looked by microscopy intact, diffraction was obtained to similar and sometime better resolution than the fixed target of the same mutant. A quantitative assessment has to be made in detail,

### **Sample Photosystem I**

Photosystem I crystals have been embedded in a wide range of concentration of 8 M PEG media. High concentration of media showed already damage via microscopy and even low concentration could not produce diffraction of crystals. With cellulose as embedding media a stability not only in the microscopic picture could be achieved. Here the problem is the embedding in the high viscous media. So far we could obtain diffraction up to 4 Å resolution with a hit rate of .3 %

### **Sample C-Phycocyanin**

Sample C-Phycocyanin could be embedded in 8M PEG. Diffraction and hit rate were to the same level as for fixed target. A quantitative assessment must be made in detail.

### **Sample BRD 3-1**

Crystals of BRD 3-1 have been embedded in 8 M PEG as in cellulose. Diffraction and hit rate were to the same high level as for fixed target. A quantitative assessment must be made in detail.

### **Summary**

This first series of beam times at ID 29 have been provided an excellent overview of the upcoming possibilities and challenges to make time resolved experiments with all of our samples.

We collected data sets for several mutants from NendoU with and without ligand which have not been published. For BRD 3-1 high resolution data (2Å) and better have been achieved as an new Apo protein

structure. C-Phycocyanin can be used for light dependent experiments Photosystem I has found a delivery medium which has to be optimized to achieve a diffraction of 3 A or better.

Overall, more than 150 TB data have been collected which are waiting for evaluation in detail with several publishable results.

(Jernigan et al., 2023; Zerio et al., 2023)