



	Experiment title: X-ray crystallography of temperature induced conformational changes in cardiac Myosin	Experiment number: LS-2806
Beamline: ID09	Date of experiment: from: 3.12.2018 to: 9.12.2018	Date of report:
Shifts: 18	Local contact(s): Matteo Levantino	<i>Received at ESRF:</i>

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Report:

The aim of the proposal was to study the temperature-induced conformational changes in cardiac myosin. On the way towards this ultimate goal, we conducted initial IR-laser induced temperature jump (T-jump) pink beam serial crystallography experiments at ID09.

The first aim of the experiment was to install the Roadrunner goniometer for fast fixed-target scanning at beamline ID09. In addition to the goniometer, we also installed an on-axis viewing system with a fully motorized positioning system. This allowed coupling of the IR-laser into the in-line viewing system through a fiber. This setup is supposed to facilitate easy adjustment of laser to X-ray position as the laser spot can be adjusted within a certain range through the on-axis microscope. Additionally we installed our own detector, a Jungfrau 1M. All this was achieved in very close collaboration with our local contact Matteo Levantino, with great efforts from his side.

After reaching spatial and temporal overlap of X-ray and IR-laser pulses, we used hen egg-white lysozyme as first test case. Lysozyme is a simple and robust system that is readily available and yields reproducible crystals. Crystals were grown in batch and then deposited on micro-patterned silicon chips. Excess liquid was removed by blotting and crystals were maintained in a humidity-controlled helium atmosphere during data collection.

During the data collection we observed unusually large Bragg reflections on the detector. We assigned these to high mosaicity of the crystals. However, after further in depth analysis after the beamtime it turned out that the bias voltage of the detector was set too low. Unfortunately, this prevented us to collect data of the highest quality.

Nevertheless, we attempted to analyze the pink beam serial crystallography data with our newly developed pipeline within CrystFEL^{1,2}.

We used an 5 ns IR-laser pulse (1490 nm, 280 μ J, 30 μ m FWHM) to induce a T-jump of approximately 30K and probed with a 5 μ s X-ray pulse with delays of 5, 10, 15, 30, 100, 200, 1000 μ s delay. Despite the trouble with the detector, we were able to index and integrate between 2000 and 9000 pattern per time point. With this data we were able to solve the structures which refined to acceptable R values (see Tab. 1).

No major changes could be detected in unit cells of crystals or in the solved, pumped structures compared to the ground state. However, more careful analysis of global as well as local temperature factors indicated indeed higher B factors for pumped structures (see Tab.1). Already at the first time point of 5 μ s, a clear increase in the Wilson B as well as the atomic displacement factor is detectable.

Table 1: Analysis of refined lysozyme structures from T-jump pump-probe experiment

laser delay [μ s]	res limit [\AA]	Rwork	Rfree	WilsonB [\AA^2]	 [\AA^2]
off	1.7	17.44	21.81	15.8	25.5
5	1.8	16.81	21.02	17.43	27.7
10	1.7	18.38	22.47	14.38	27.03
15	1.9	19.18	25.4	19.53	29.2
30	1.9	19.71	26.58	19.07	30.79
100	1.8	17.63	22.76	17.49	29.59
200	1.7	17.43	21.98	16.44	27.11
1000	1.8	18.01	21.32	17.8	28.65

With this experiment we have shown that we can indeed achieve thermal excitement of crystals through an IR-laser pulse with crystals presented to the X-ray beam by a fixed-target silicon chip. This work is the foundation for more elaborate crystal systems such as the cardiac myosin as suggested in the proposal. Especially with the EBS upgrade we expect a tighter X-ray focus, enabling us to use smaller crystals. In addition, the energy spectrum from the undulator is expected to be smaller and more symmetric which should improve data processing.

Altogether we are excited for the continuation of this proposal. We feel well prepared for the proposed experiments.

References:

1. Gevorkov, Y. *et al.* pinkIndexer – a universal indexer for pink-beam X-ray and electron diffraction snapshots. *Acta Crystallogr. Sect. Found. Adv.* **76**, 121–131 (2020).
2. Tolstikova, A. *et al.* 1 kHz fixed-target serial crystallography using a multilayer monochromator and an integrating pixel detector. *IUCrJ* **6**, 927–937 (2019).