

# Single Crystal Time-lapse Measurement Using Ultrasonic Acoustic Levitation

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**Abstract.** We are developing the acoustic levitation diffractometer, a new container-free diffractometer at the Swiss Light Source, Paul Scherrer Institut. By rotating single crystals in an acoustically levitated droplet and collecting the diffraction images by a fast-frame-rate X-ray image detector, the data collection can be completed within a few hundred milliseconds or shorter at room temperature. Here we report time-lapse measurements of ligand soaking using a single crystal. This was achieved by collecting a series of datasets after soaking ligand solution into single lysozyme crystals in a levitated droplet in the acoustic cavity of the diffractometer. Electron density maps of the lysozyme crystals obtained every 30 seconds after the ligand soaking showed meaningful conformational changes around the binding site of the ligand and the radiation damage for 300 seconds after the ligand soaking.

## INTRODUCTION

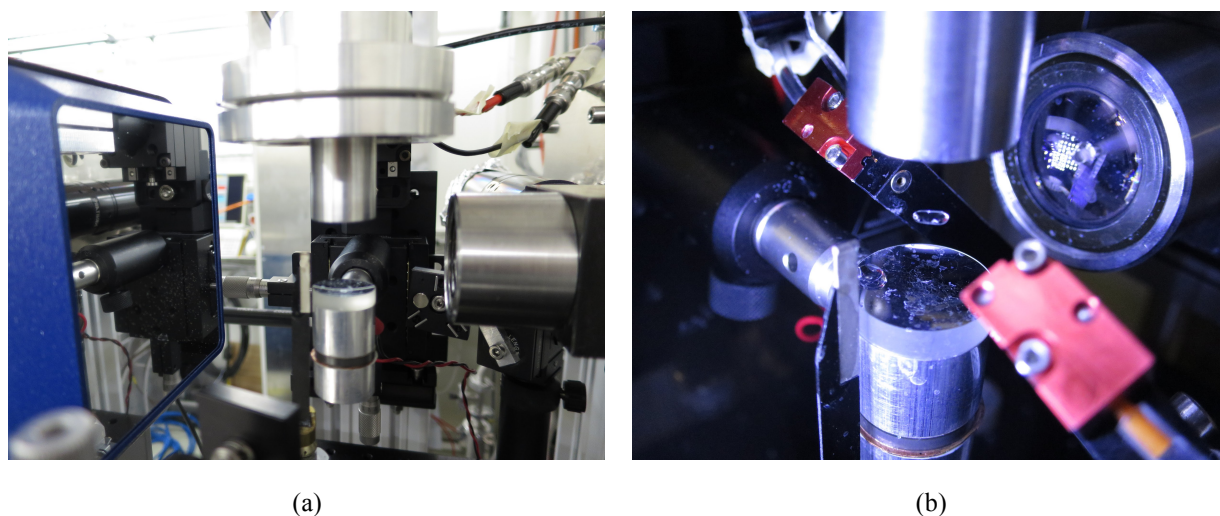
We are developing a new diffractometer, the acoustic levitation diffractometer (ALD), combining the ultrasonic levitation of samples-in-droplet in ambient air with a high-frame-rate X-ray image detector, at the Swiss Light Source (SLS), Paul Scherrer Institute. ALD allows for completing a dataset within short time at room temperature. Recently, we have demonstrated the successful acquisition of complete datasets with ALD using EIGER X 16M detector within a few seconds at the frame rate of 0.1 kHz [1]. With the recently installed ALD at SLS using EIGER X 1M detector with the frame rate up to 3 kHz, dataset acquisitions are completed within a few hundreds of milliseconds. Further advantages of ALD include the possibility to modify the crystal environment, such as pH, ligand concentration, temperature, humidity etc., while levitating the crystal in the cavity.

Time resolved studies have been reported at the synchrotron using different methods [2,3,4]. However, these experiments require a large number of crystals, and their observation timescale is limited at most in the order of nano- to micro-seconds. Also, the trigger type of those methods are not flexible because of the limitation of the observation time scale. We show in this work that, ALD allows for the study of slow reactions triggered by ligand soaking, over tens of seconds or even up to minutes, occurring in a single crystal, that will be easily extended for different triggering methods.

## EXPERIMENTAL SETUP

An ALD instrument operating at the ultrasound frequency of ~38 kHz combined with the EIGER X 1M detector was installed on X06SA beamline at the Swiss Light Source, Paul Scherrer Institut (**FIGURE 1**). For the ligand soaking experiment, we used two piezo driven droplet-on-demand dispensers with ALD. Droplets with a volume of 2-4  $\mu$ L containing a lysozyme crystal were loaded to the cavity of the levitator manually using a syringe.

Typical size of crystals was approximately 300  $\mu\text{m}$  in each dimension. The crystal rotation speed was adjusted to a few turns/sec. To increase the hit rate of the crystals by the X-ray beam, the experiments were normally conducted a few minutes after the loading when the droplet diameter was reduced to  $\sim 500\text{ }\mu\text{m}$  by evaporation. Subsequently, p-Toluenesulfonic acid solution at a concentration of 500 mM was soaked into the droplets for the initiation of the ligand binding by ejecting the solution by using the dispensers. We used two dispensers to eject two solution droplets from opposite sides of the levitating droplet to compensate the momentum of the ejected droplets. This way, we avoided the oscillation of the levitating droplet induced by the ejected droplets and minimized the stabilization time after the soaking to less than 1 s, that was otherwise more than 10 s without such compensation when the soaking was conducted using single dispenser. After dispensing the ligand solution, we collected datasets with 6000 diffraction images every 30 seconds at a frame rate of 3000 Hz at the X-ray photon energy of 10 keV with the beam size of 100  $\mu\text{m}$ -square in full-width at the half maximum at the detector distance of 60 mm. We collected 10 datasets in total from single lysozyme crystal. The diffraction images collected by ALD have unknown rotation axis and oscillation ranges. Therefore, we processed the images by the CrystFEL program suite [5] as a set of still images. Crystal structures were solved by the molecular replacement method with a model structure without water molecules and light metals, and subsequently refined with the CCP4 program suite.  $2mF_o - DF_c$  electron density maps for each dataset was rendered by COOT at 2.2  $\text{\AA}$  resolution limit. The resolution limit of the diffraction images was limited to this value because of the detector size and the distance of the detector to the crystals. Since a beam flux monitor was not installed for the present experiment, it was not possible to accurately calculate the dose for the datasets.



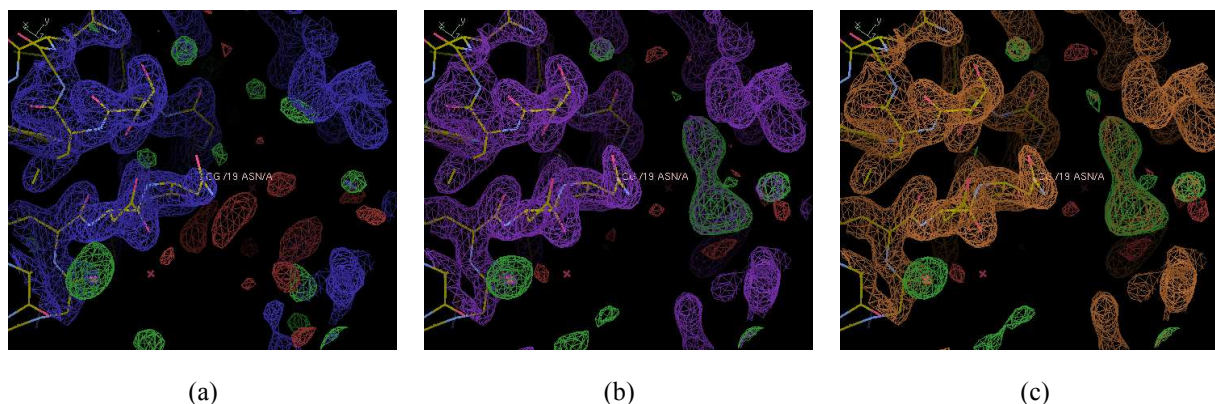
**FIGURE 1.** (a) Overview of the ultrasonic acoustic levitation diffractometer (ALD) with an EIGER X 1M detector on the X06SA beamline at the Swiss Light Source, Paul Scherrer Institut. (b) Piezo-driven dispensers for nanoliter liquid droplets (red) were placed at both sides of the acoustic levitator for the soaking. For illustrative purpose of the acoustic levitation, a large droplet (disk-shaped object in the middle with the volume larger than  $\sim 6\text{ }\mu\text{L}$ ) was levitated at the middle pressure node of the levitator acoustic cavity.

## RESULTS

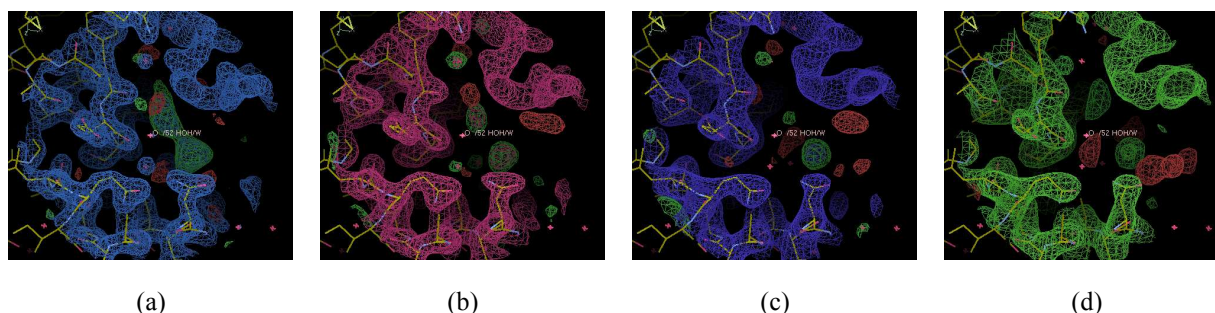
In **FIGURE 2** and **FIGURE 3**, we show the electron density maps obtained from 2 experiments with at different times after the ligand soaking was initiated by dispensing the solution. From the result shown in **FIGURE 2** (a) and (b), we found the ligand was soaked after 30 seconds. There was no difference to the electron density between 30 seconds (**FIGURE 2** (b)) and 120 seconds (**FIGURE 2** (b)) after the ligand soaking. Therefore, we conclude that the ligand compound was fully bound to the binding site in less than 30 seconds after the soaking. Interestingly, the electron density of the side chain (ASN 19) closest to the ligand compound was not clear before the soaking but became distinctly visible after 30 seconds of the ligand soaking. This suggests that the freely moving side chain was stabilized by the hydrogen bonds between the side chain and the ligand compound.

In the result shown in **FIGURE 3**, we observed how radiation damage became visible within the crystal structure in the electron density from the second crystal after 300 seconds. Radiation damage was clearly seen at the side

chains exposed to the protein surface in the electron density maps. With the increase of the measurement time, the electron density of the ligand (in green) gradually became weaker at the binding site. At 300 seconds, **FIGURE 3** (d), the radiation damage at the side chains was observed as the ambiguous density map.



**FIGURE 2.** Parts of 2Fo - Fc electron density maps of a lysozyme crystal (a) before ligand soaking, (b) after 30 seconds, and (c) after 120 seconds of ligand soaking. The sigma level of electron density of the ligand (in green) are approximately the same in (b) and (c). In (a) before the ligand soaking, the electron density of the side chain, ASN 19 (at the center of the figures) was not clear. In (b) after 30 second of ligand soaking, the ASN 19 density became distinctly visible, that is tentatively ascribed to the formation of hydrogen bonds between the side chain and the ligand.



**FIGURE 3.** Parts of 2Fo - Fc electron density maps of a lysozyme crystal (a) 30 s, (b) 120 s, (c) 210 s, and (d) 300 s after the ligand soaking. Electron density maps of the ligand in green gradually became weaker at the ligand binding site. In addition, radiation damage was visible at the side chains, that became ambiguous in comparison to those in the first map.

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