ESRF	**Experiment title:** **Tracing conformational dynamics of channel protein during active gating.**	**Experiment number**: SC-2684
Beamline: ID09B	**Date of experiment**: from: June 12, 2009 to: June 16, 2009	**Date of report**: Aug. 31, 2009
Shifts: 12	**Local contact(s)**: **Laurent Guerin** Michael Wulff	*Received at ESRF:*

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Report:

Ion channel is a membrane protein that transduces various types of stimuli into opening and closing of ion permeation pathway (gating) for electrical signal transduction. For ion channels electrophysiological methods enabled detecting dynamic behavior of channel function in a single molecular level, in which gating is recorded as discrete jumps between binary levels of current amplitudes. To examine underlying conformational changes during gating, we have established a method to measure dynamics of channel protein in a single molecular level using diffracted X-ray tracking (DXT) method (Shimizu et al., 2008). DXT method is a kind of dynamic Laue using white X-rays. We succeeded to detect twisting motion of the KcsA potassium channel molecule around the symmetrical axis of the molecule during gating.

The purpose of this project is to improve the previous DXT method and to gain more quantitative information for the conformational dynamics of channel proteins in a single molecular level.

In the previous study we have encountered an inherent problem of beam characteristics having narrow bandwidth. In earlier studies for other types of proteins, the diffraction spots could be traced throughout the recording period of 1 s, since the range of the conformational changes were limited. On the other hand, the KcsA channel exhibited unexpectedly large conformational changes upon gating and the diffraction spots run out of the recording range in the 2 θ angle. This limited range of detection area failed to capture whole trajectories of conformational changes. To circumvent this problem we explored possibilities for use of

white X-rays with broad spectrum available in the beamline of ID09B in ESRF. Our first trial in this beamtime enabled great advances in the recording quality and gave promising data.

The experimental setup

KcsA channel protein is a membrane protein and stable in solubilized condition, exhibiting normal gating behavior at acidic pH. To detect fine conformational changes of channel molecular, rather than Brownian motion in the aqueous solution, the channel was fixed on the glass surface through a binding site in the extracellular loop. A gold nanocrystal was attached at another end of the channel molecule. This sample was packed in a water filled enclosure and white-X-rays were irradiated normal to the glass surface. Our own image intensifier and high-speed camera were brought and set in the beamline. The diffraction spots were recorded using the above camera system. At acidic pH channels keep active gating and the trajectories of the diffraction spots were traced in the rate of 5000 Hz for 1 s.

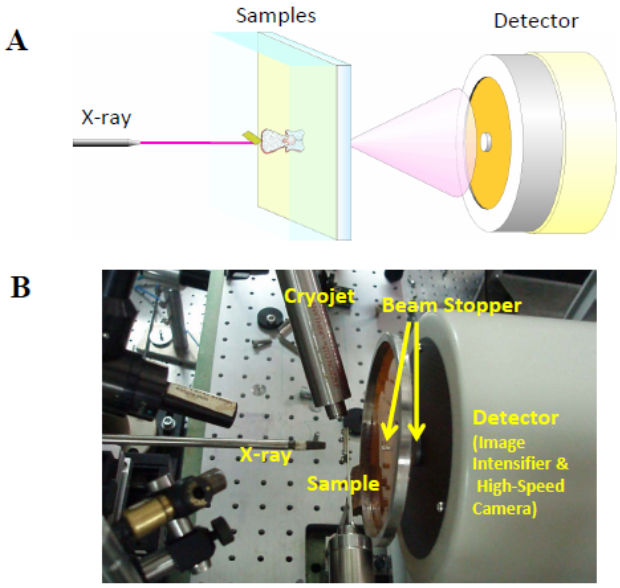

Fig. 1. The experimental set in the beamline ID09B. KcsA channel was attached on the glass surface and a thin water layer (7 um) was overlaid. The image intensifier was placed 40 mm from the sample. The detection area of the image intensifier was 20 cm in diameter. The high-speed camera is x pixels with 16 bit resolution.

In this beamtime we examined the effect of spectrum on the trajectories of diffraction spots. The spectrum of the white X-rays could be manupulated freely by combinations of parameters. The beamline scientists suggested several options. These were great benefits of using the beamline ID09B. By changing the gap and slits for the undulator (U17 or U20) and inserting an aluminum plate with different thickness, the shape of the spectrum was modified significantly (Fig. 2 A-E). Finally, we found the appropriate spectrum (Fig. 2E) for the DXT mehtod. The spectrum became wide, covering from 15 keV to 30 keV and amplitudes of the flux was readily tunable through

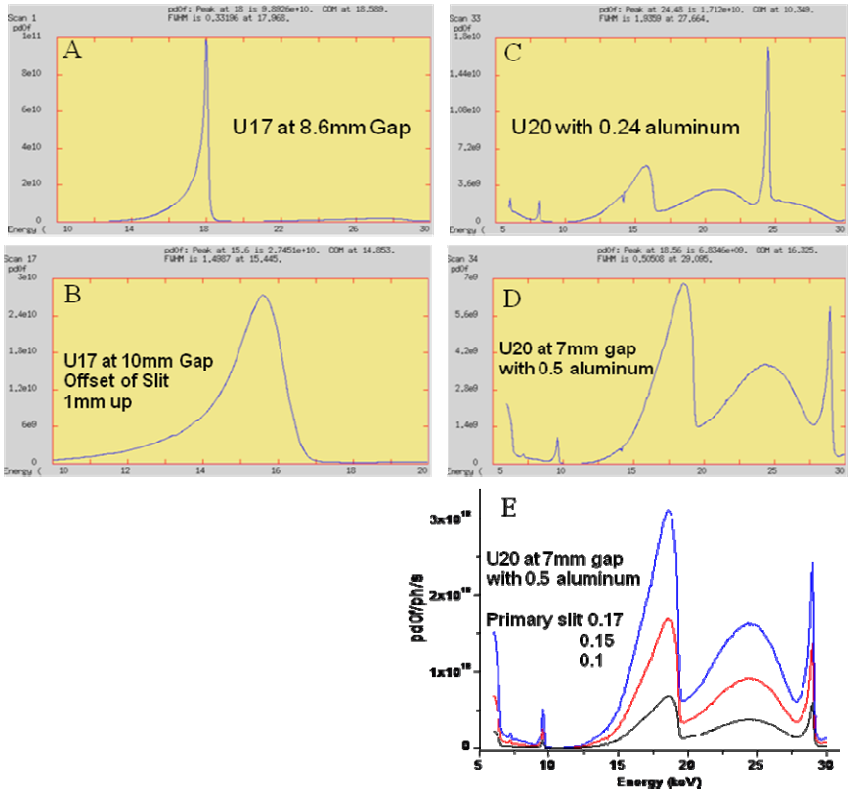

Fig. 2. The spectrum of the beam using the undulator U20. The shape and amplitude distribution were controlled by changing the gap and the inserted aluminum plate with different thickness.

adjusting the primary slit. This spectrum is nice since scatter from the water layer is minimized by attenuating flux at around 15 keV.

The flux was enough for detecting diffraction spots from single gold nanocrystals. Representative data are shown in Fig. 3. In our experiments

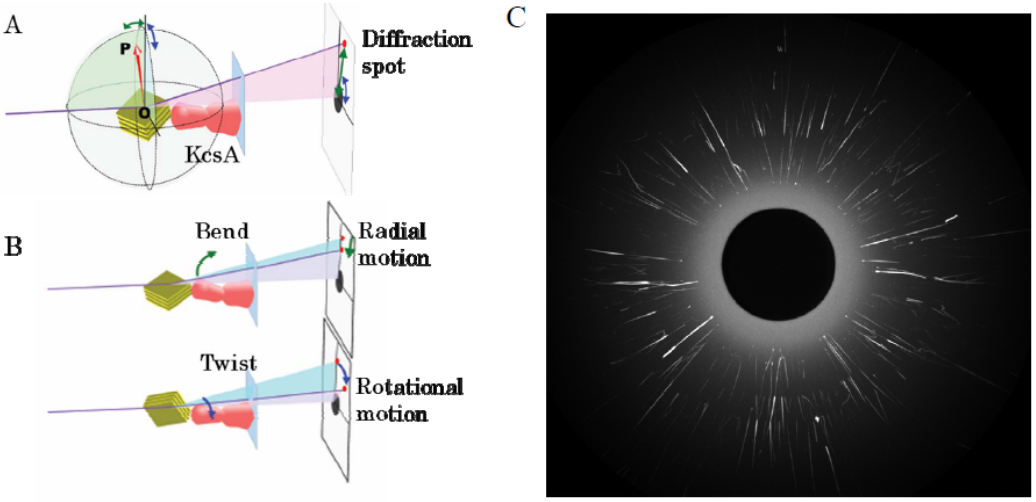

Fig. 3. A. Geometry of the sample, beam and image plane. B. Orientation of the KcsA channel on the glass plate and the direction of the incident beam. C. A superimposed image of diffraction spots. Each spot represents motions of singel molecule.

the channel molecules are oriented in upright position on the glass surface and the white X-rays are irradiated in parallel to the longitudinal axis of the channel. Therefore, if channel molecule tilts or bend along the axis, the diffraction spots move on the image plane in 2 θ direction. On the other hand, if channel molecule rotates around the axis, the spots move circumferentially.

To examine the detection range in 2 θ angle under modified beam spectra, measurements were performed at relatively high temperature of 25°C, where the bend motion of KcsA channel is enhanced. Fig. 3C is a representative record. The diffraction spots moved across a wide range of the 2 θ angle, which have never been attained before. We found in these superimposed trajectories that a continuous trajectory exhibited changes in its brightness along the 2 θ angle. To further examine this feature, a representative trajectory is shown in Fig. 4. The brightness is not uniform and changes significantly. This trend prevails for all the trajectories. The brightness pattern reminded us the shape of the spectrum of X-rays (Fig. 2). This additional information has never been expected and will be served for more accurate

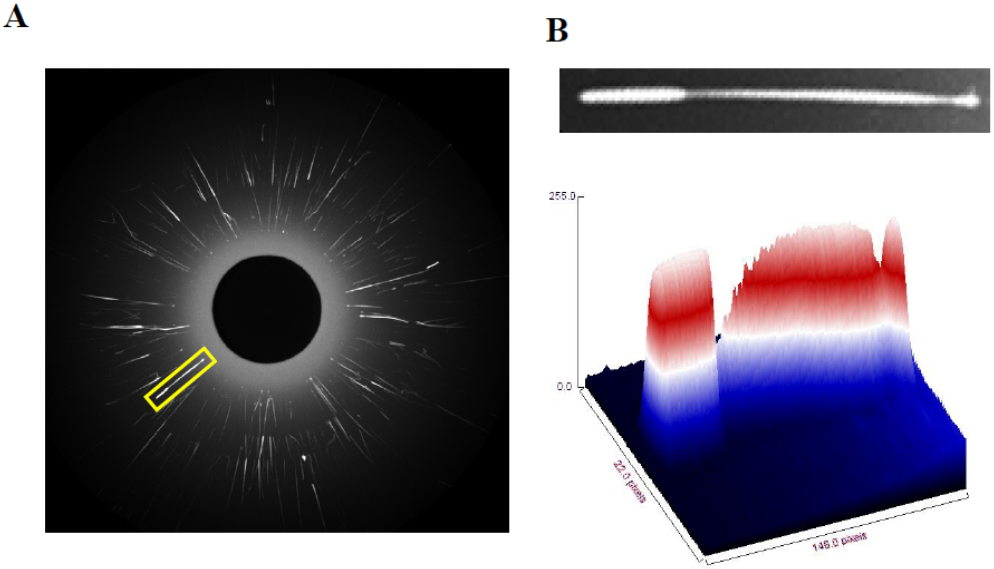

Fig. 4. A. Trajectories of diffraction spots for 1 s (5000 images were overlaid). B. A trajectory with brightness. A trajectory (yellow box in A) exhibited unon-uniform brightness along the trajectory (upper trace), which is represented as 3-D plot with the vertical axis indicating brightness (lower panel). The profile of the brightness reflects those of the energy for irradiated X-rays (Fig. 2E).

quantitative analysis for the conformational dynamics of channel proteins.

Using this spectrum, experiments were performed in a condition that the channel exhibits twisting motion (not shown). Circumferential motion of the diffraction spots could be traced even with concomitant large motions along the 2 θ angle, indicating that our strategy using broad white X-rays are valid and promising.

In the first trial of beamtime in the beamline of ID09B, we obtained promising data. Constructive aid by the beam scientists facilitated the experiments significantly. Developing our method would provide unprecedented data for confomational dynamics of channel proteins. Those trajectories will be used for building the energy landscape of channel conformation.