ESRF	**Experiment title:** Conformational dynamics of KcsA channel protein in a single molecular level.	**Experiment number**: SC-2912
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Shifts: 17	**Local contact(s)**: **Laurent Guerin** **Michael Wulff**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Shigetoshi Oiki* University of Fukui
Hirofumi Shimizu* University of Fukui
Masayuki Iwamoto* University of Fukui
Takashi Konno* University of Fukui

Report:

The ion channel is a signal transduction molecule on the cell membrane. Upon stimuli, channel molecules undergo conformational changes that lead to opening and closing of the ion permeation pathway (gating). Ion flux through the open channel generates electrical signals, which propagates across the cell membrane, such as in neurons. We have elucidated underlying conformational change of the KcsA potassium channel during gating in a single-molecule level by a diffracted X-ray tracking (DXT) method (Shimizu, *et. al.* Cell 132: 67-78, 2008). In these studies, the conformational trajectories were traced under the equilibrium condition of low pH, and reversible opening and closing transitions were recorded. In the present study, to examine transient conformational changes upon pH jump, we established a new pH jump method.

The diffracted X-ray tracking method is a kind of dynamic Laue method, in which a channel protein bearing a gold nano-crystal is attached on a glass plate.

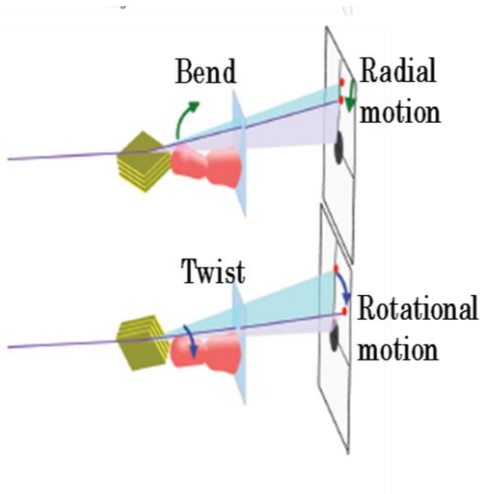

Fig. 1. Principles of the DXT method. KcsA was fixed on a glass plate and a gold nanocryatal was attached to the other side. When the channel bends, the diffraction spot from the gold nano-crystal moves along the radial direction, while the channel twists, the spot moves along the rotational direction.

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White X-rays supplied in the beamline ID09B was irradiated and diffraction spots were detected by image intensifier and high-speed camera. Last year, we set our own recording system (the image intensifier and the high-speed camera) in the beamline, and, with an aid of the beamline scientist, we manipulated spectrum of the X-ray beam for recording the diffraction spots with high signal-to-noise ratio. The background noise was significantly reduced and the recording rate as high as 5000 frames/sec was attained, which is more than hundreds times faster than the previous method. The high-speed tracking elucidated fine fluctuations of the conformations, and enabled confident tracking and retrieving coordinates of the diffraction spots on the image plane.

With these high resolution recording system, we built a pH-jump system. KcsA is a pH-dependent channel that exhibits reversible gating conformational changes at acidic pH, while it stays closed at neutral pH. Therefore, the channel should initiate opening transitions when the solution pH is changed from, for example, 7 to 4. Transient behavior of the opening should provide crucial information on the machanism of pH-sensing and initical processes of the conformational change.

The followings are basic results of the pH-jump experiment.

A pump-probe system

Solution pH was jumped from neutral to low by photolyzing a caged proton (1-(2-nitrophenyl)ethyl sulfate) with UV laser of 355 nm. The control experiments were performed to examine the degree and speed of the pH jump with collaboration of Dr. Antoni Royant (Cryobench of ESRF). To monitor changes in the solution pH, fluorescein was used (Fig. 2). pH changes in the solution upon irradiation of the caged compound were examined. Optimal duration of the laser pulse (2 mW, 335 nm) was determined to be 100 ms to evoke pH changes below 5.

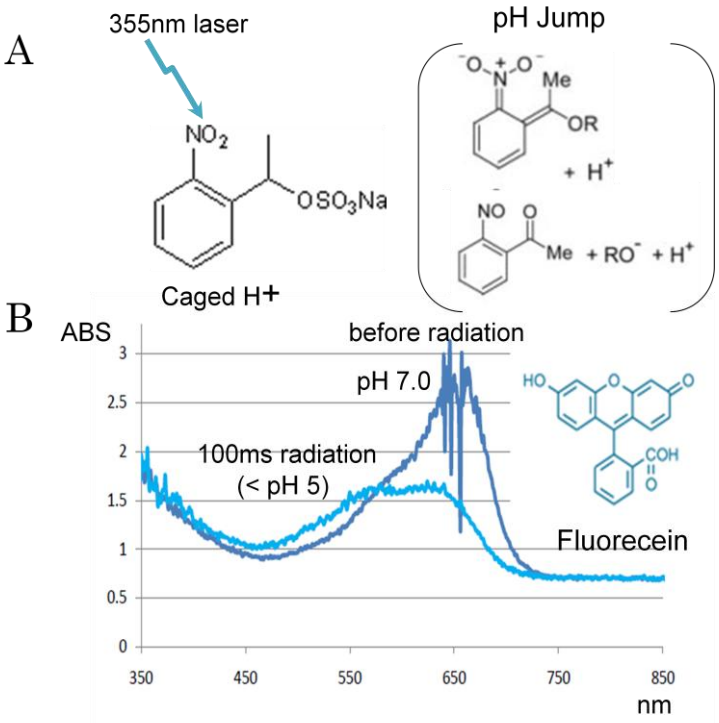

Fig. 2. pH changes by laser radiation of caged proton. **A.** Structure of caged proton (possible mechanism of proton release). **B.** The absorbance spectra before (blue) and after (light blue) 100 ms radiation of 2 mW laser (355 nm).

We constructed a pump-probe measurement system in the hatch of the ID09B with a help of beamline scientists (Fig.3). The laser was irradiated at 1s after the starting of the DXT measurement, thus the pH condition for the first 1s was pH 7.0, and then jumped to pH 5.0 for the last 3s.

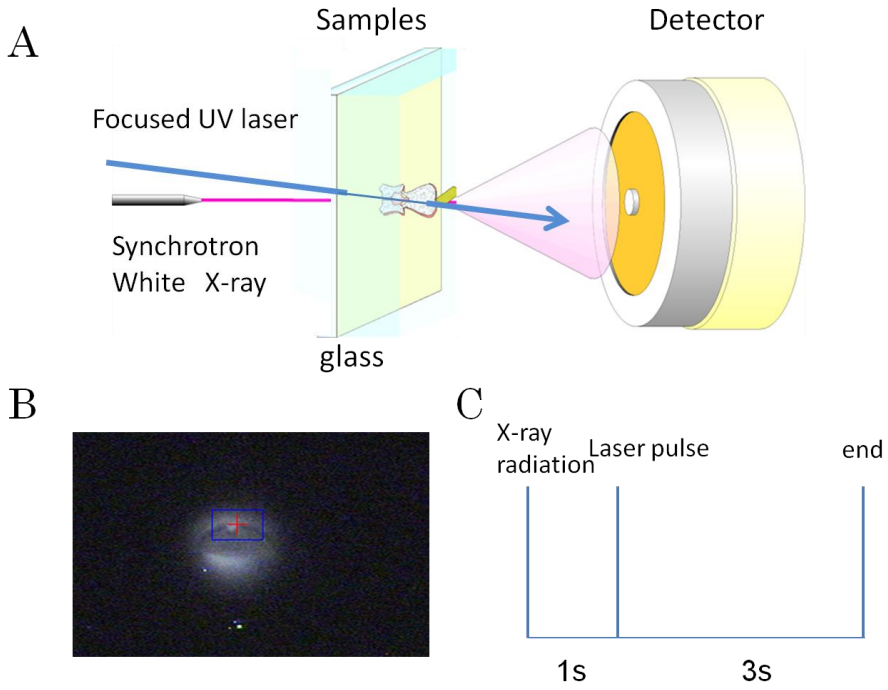

Fig. 3. pH jump experiments. **A.** Geometry of the pH jump experiment. The laser beam was aligned such that the beam was focused on the X-ray irradiation point. **B.** An image of the irradiated point on the sample surface. In the laser irradiated spot (white color), the focused spot of the X-ray (eye shaped mark) was overlapped. The size of the box (blue) is 50 μm. **C.** The trigger sequence of the pump-probe experiment. X-ray irradiation was started by the first trigger and continued for 4 sec. Laser was irradiated at 1s after the recording with 100 ms duration of pulse.

The channels exhibited small fluctuating motions for the first 1 s and then started large twisting motions. Tracing fine trajectories of twisting motion will provide crucial information of the initial step of the gating conformational changes. Our established method is promising that will reveal directionality of the twisting motion for the opening transition.

Automatic data collection system:

To perform the pH jump experiment efficiently, time sequence of the triggered events, such as opening and closing of the X-ray shutter, trigger for the camera, trigger & pulse duration of the laser, and transfer of the data, must be well organized. Also, several tens of data were collected from the sample on a glass plate by moving the irradiation spot on the sample, which should be motor-driven automatically. These sequences of time and positional control were automatically performed by a program written with an aid of the beamline scientists.

In parallel to these technical issues, Hirofumi Shimizu, a member of our team, has stayed as a visiting scientist for a year to accomplish the project efficiently and to acquaint the basic information of the beamline intimately. It is beneficial that he can learn sophisticated laser technology that the beamline sicentists are mostly involved.

In this beamtime we had two breakthroughs for measuring the conformational changes of potassium channels. Laser-triggered system elucidated early events of the pH-dependent conformational changes from closed state towards the open state. Automatical data retrieval system gave solutions for improving efficiency of data collections of single molecular measurement. Combining these two technical breakthroughs is promising that we will elucidate structural dynamics of channel protein for physiologically relevant function, as well as giving unprecedented data on the fundamental issue of protein dynamics in general.