NW14A/2011G-608

Open-close dynamics of single protein molecule observed by diffracted X-ray tracking synchronized with pulse laser

Kouhei Ichiyanagi[1,2], Hiroshi Sekiguchi[2,3], Shunsuke Nozawa[4], Tokushi Sato[4], Ayana Tomita[4], Manabu Hoshino[4], Shin-ichi Adachi[4], Yuji C. Sasaki[1,2*],
[1]Graduate School of Frontier Sciences, the Univ. Tokyo, Kashiwa 277-8561, Japan
[2]JST-CREST Sasaski team, Kashiwa 277-8561, Japan
[3]JASRI SPring-8, Sayou-cho 679-5198, Japan
[4]Photon Factory, Tsukuba 305-0801, Japan

1 Introduction

Diffracted X-ray Tracking (DXT) measurement technique is a powerful tool to observe *in-situ* intermolecular motions of single protein. Understanding the rotational and tilting motions in channel and membrane protein molecules are a fundamental life scientific quest and basic research need in biological science. Sasaki et al., developed DXT technique using diffraction spot tracking from gold nanocrystals combined with the X-ray synchrotron source [1]. This technique can offer the milli-radian resolution of the rotational and tilting motion. We tracked θ and χ motions of the diffraction spot from gold nanocrystals attached on the cysteine group with the protein using the wide-energy band of X-ray (Fig.1). In reaction process between ATPase and protein, changing some rotation or tilt motions may occur inside protein with changing the conformation. In order to in-situ observing the intermolecular motion change under photo-dissociated ATPase reaction with the channel protein, we developed and constructed the DXT system synchronized with UV/VIS pulse laser at the NW14A beamline, the PF-AR. We demonstrated the open-close motion of group II chaperonine reacted with photo-dissociated ATPase.

2 Experiment

DXT measurement system was synchronized with 355 nm third harmonic generation of a nanosecond Q-switched Nd:YAG laser (Continuum, co. Inc., Powerlite 8000 plus, USA) for photo-dissociation of caged-ATP. The X-ray energy bandwidth and peak position were ΔE/E=15 % and 18 keV. The DXT movie can be measured by the CCD which is coupled with the image intensifier (C4880-10 and V7739P, Hamamatsu photonics, K.K, Japan). We took 90 diffraction images at the 36 ms/frames. The laser pulse was irradiated at the 31 frame.

3 Results and Discussion

A gold nanocrystal was attached on the top of chaperonin protein using Au-S bonding. The gold nanocrystal size was 20-40 nm. Fig.2 shows the mean square displacement of diffraction spot from gold nanocrystals attached on with the group II chaperonin before laser irradiation and after laser irradiation. The open-close motion is mainly associated with the c motion. In this work, the χ motion increased with producing the photo-dissociated ATPase after UV laser pulse irradiation.

We succeeded to observe the open-close dynamics in the group II chaperonin reacted with the ATPase.

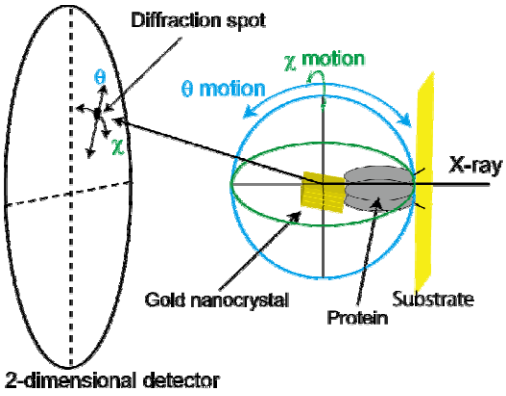

Fig. 1: The principle of diffraction X-ray tracking.

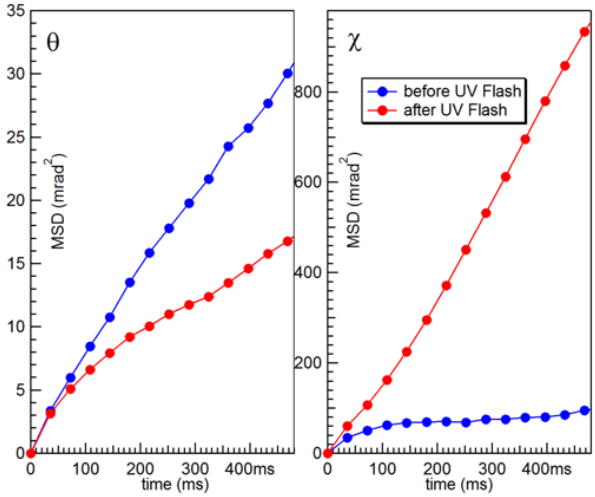

Fig. 2: Mean square displacement of θ and χ motions in the group II chaperonin before laser irradiation (blue line) and after laser irradiation (red line).

Acknowledgement

We thank the beamline staffs for supporting the DXT measurement.

References

[1] Y.C. Sasaki et al., Phys. Rev. Lett, **87**, 248102 (2001).

* ycsasaki@k.u-tokyo.ac.jp