

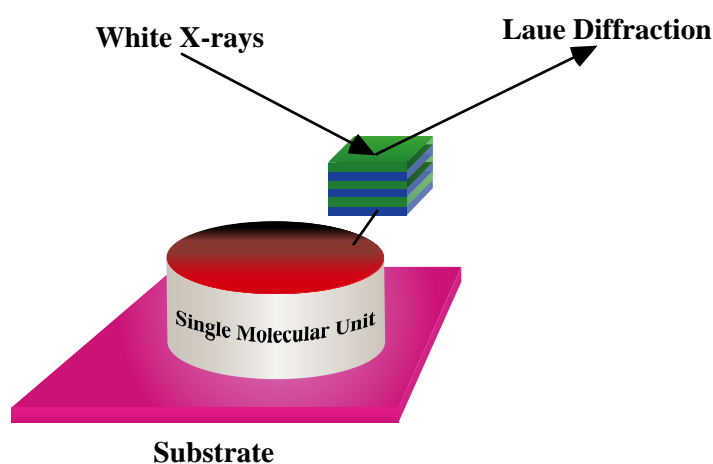
## Dynamical Observations of Individual Protein Molecules with X-rays

In order to understand how protein molecules operate in bio-systems such as membrane or cells, some X-ray techniques were developed to give the time-and-space averaged structural information about protein molecules. Recently, the dynamic behavior of single bio-molecules were directly visualized in real time under an optical microscope [1,2]. These single-molecule techniques have been providing positional information at an accuracy of about  $\lambda/100$ , far below the optical diffraction limit ( $\sim\lambda/2$ ). In this work, we demonstrated the direct observations of the rotating motion of an individual single nanocrystal, which is bound to individual bio-molecules, using time-resolved Laue diffraction. We achieved time-resolved X-ray ( $\lambda_{X\text{-ray}} \sim 0.1$  nm) observations of picometer-scale ( $\lambda_{X\text{-ray}}/100$ ) slow Brownian motion in individual bio-molecules in various aqueous solutions.

Most X-ray experiments are based on the average of several observations of many molecules and the behavior of each molecule cannot be determined. In this report, we proposed a new

X-ray methodology for direct observations of the behavior of single molecular units in real time and in real space. The new system (Fig. 1), which we call Diffracted X-ray Tracking (DXT), monitors the Brownian motions of a single molecular unit by observations of X-ray diffracted spots from a nanocrystal, tightly bound to an individual single molecular unit in bio-systems [3,4]. DXT does not determine any translational movements, but only orientational movement. Here, in order to detect intra-molecular Brownian motions in individual single biological molecules on a picometer scale, we utilized individual diffraction spots from a nanocrystal, which was tightly bound to the DNA molecules under observation (Fig. 2).

We used the white X-ray mode (Laue mode) of the RIKEN Structural Biology II beamline **BL44B2** to record Laue diffraction spots from Au nanocrystals. The photon flux at the sample position was estimated to be about  $10^{15}$  photon/sec/mm<sup>2</sup> in the energy range of 7 - 30 kV. The X-ray beam's focal size was 0.2 mm (horizontal)  $\times$  0.2 mm (vertical). A diffraction spot was monitored with an X-ray image intensifier (Hamamatsu Photonics, V5445P) and a CCD camera (Hamamatsu Photonics, C4880-82) with  $656 \times 494$  pixels. The average exposure time



*Fig. 1. Schematic drawing of the detection system for single molecular tracking with X-rays (not to scale). Diffracted X-ray Tracking (DXT) monitors the motion of a single nanocrystal with the guidance of a diffraction spot from the individual nanocrystal itself, which is labeled with the individual single molecular unit.*

was 1 msec.

Figure 3 shows movements of diffraction angles  $\theta$  from a single nanocrystal coupled to a single DNA molecule (18-mer) at 4  $\text{\AA}$ . The observed spots randomly move along the direction of  $\theta$ . From analyzed data, the observed displacement of  $\theta$  is assigned as directed Brownian motion [5].

In the future, the biggest challenge will be to observe individual and rare biological processes in living cells. DXT can be used to monitor not translational motions but orientational ones on picometer scales. DXT can be expected to observe the structural changes accompanying the activation of ion channels in living cells. Such changes are known as tilting or small orientational motions of the helix in channel pores. DXT can also be expected to monitor the dynamics of ion channels through ionic flux measurements by the patch-clamp technique.

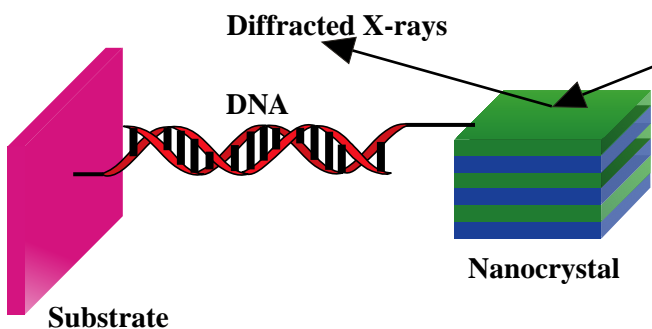


Fig. 2. Schematic drawing of the X-ray single-molecular detection system for individual DNA molecules in aqueous solutions (not to scale). The diameter of the nanocrystal and the DNA molecule are about 15 nm and 2.5 - 3 nm, respectively.

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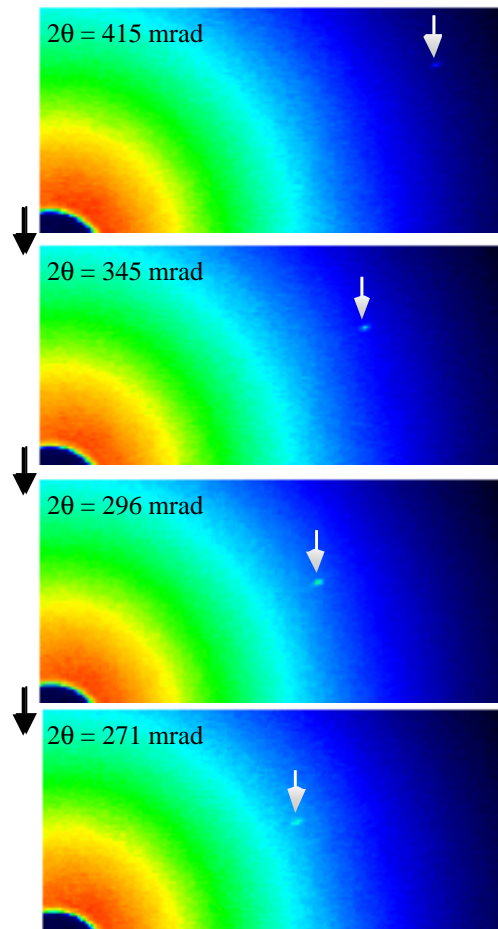


Fig. 3. Examples of the diffracted spots from the single nanocrystal in aqueous solutions appeared as brightly shining dots (white-blue). Frames are spaced at 180-ms intervals. The exposure time was 1 s.

### References

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