Real time intramolecular motion of ligand-gated ion channel at the single molecule level

Most soluble proteins are composed of several subunits and the protein's function can be expressed by changing its intra-structure or its relative position in a multimeric protein. Moreover, 80% of drug targets are membrane proteins, which are composed of several subunits. Therefore, it is important to obtain information about such cooperative motions of each subunit in order to understand the mechanism of functional proteins. Because cooperative motions are thought be small (on the atomic scale) and fast (on the microseconds scale), a technique to acquire such dynamic information is desired in both academic and industry.

We have proposed a single molecule technique that utilizes short wavelength probes (X-rays) to monitor the internal motions of a single protein. We call it diffracted X-ray tracking (DXT), and it would be a powerful technique in biological sciences to detect atomic-scale dynamic motions of a protein on the single molecule level with several tens of microseconds time resolution. Figure 1 illustrates DXT. A protein is labeled with a nanocrystal, with a size of several tens of nanometers, and the motions of the nanocrystal coupled with the protein's motions are recorded as trajectories of the Laue spots from the nanocrystal. Professor Yuji C. Sasaki designed this technique in 1997 and presented it in 2000 at SPring-8 [1].

Here, using DXT with an improved time resolution at the high-flux beamline **BL40XU** [2], we performed real-time 100-μs-time resolution single molecule observations of nAChR from Torpedo, which is one of the most thoroughly studied members of the pentameric ligand-gated ion channel (pLGIC) family, and AChBP, a homologue to the extracellular ligand-binding domain of nAChR that is often used as a model for the ligand-binding domain of pLGICs. Based on an electron crystallographic structural study, it was hypothesized that the conformational changes associated with channel opening of nAChR would cause twisting and tilting motions in both the ligand-binding and channel domains. The DXT method can provide such information about the target proteins, as shown in Fig. 1(c). Thus, we applied it to investigate the gating mechanism of nAChR.

We immobilized a gold nanocrystal on the C-terminus of AChBP via a Met-tag (Fig. 1(a)) and on the extracellular side of nAChR via an antibody (Fig. 1(b)). Then we recorded the rotational motion of the gold nanocrystal on the target protein around two axes, tilting (θ) and twisting (χ), and compared the distributions under different experimental conditions, e.g., in the presence of an activator (acetylcholine: Ach), in the presence of a deactivator (α-bungarotoxin: αBtx),

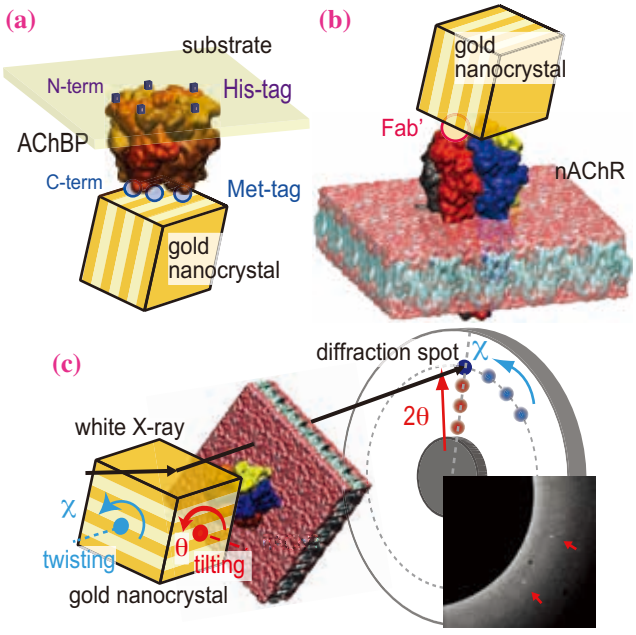

Fig. 1. DXT measurements of AChBP and nAChR. Schematic of the immobilization of gold nanocrystals on **(a)** AChBP and **(b)** nAChR. **(c)** Schematic of the tilting (θ) and twisting (χ) motions observed by DXT. [2]

and in the absence of any ligands. Figures 2(a) and 2(b) show the two-axis differential (ACh-αBtx) internal motion histograms of AChBP and nAChR, respectively, over a time interval of 100 μs. In Fig. 2(a), the ACh-positive area in the yellow-red color scale is distributed in the upper right, and the αBtx-positive area in the yellow-blue color scale is distributed in the lower left of the map. These results indicate that both the tilting and twisting motions of AChBP are facilitated in the presence of Ach, but inhibited in the presence of αBtx. AChBP is a homopentamer, and the ACh binding site is located at the boundary between subunits. The nanocrystal is labeled in the region sensitive to conformational changes upon ACh binding. Therefore, the enhanced nanocrystal motion, which is observed in the presence of ACh by DXT, reflects the conformational change of the ACh binding site of AChBP upon binding and unbinding of ACh. Figure 2(b) shows the differential (ACh-αBtx) internal motion histograms of nAChR, which are more complicated. The histogram can be divided into three areas as illustrated in right column of Fig. 2(b). Torpedo nAChR is a heteropentamer with two ACh binding sites. Additionally, prolonged exposure to ACh induces nAChR to adopt a desensitized state, indicating that nAChR can exist in multiple conformations. We hypothesize that the complex motion pattern observed by DXT is attributed to these heterogeneous characteristics of nAChR. Although we could not discern the motion pattern of the nAChR states in the current data set, ACh-induced conformational changes in the α-subunit of nAChR are clearly observed by our method.

The DXT technique can be used to monitor the dynamic behavior of a single molecule at all principal sites for intramolecular dynamics with an immobilizing motion tracer at the site. This technique is also expected to greatly contribute to the discovery of allosteric drugs without side effects because intramolecular dynamic information is essential to realize such drugs.

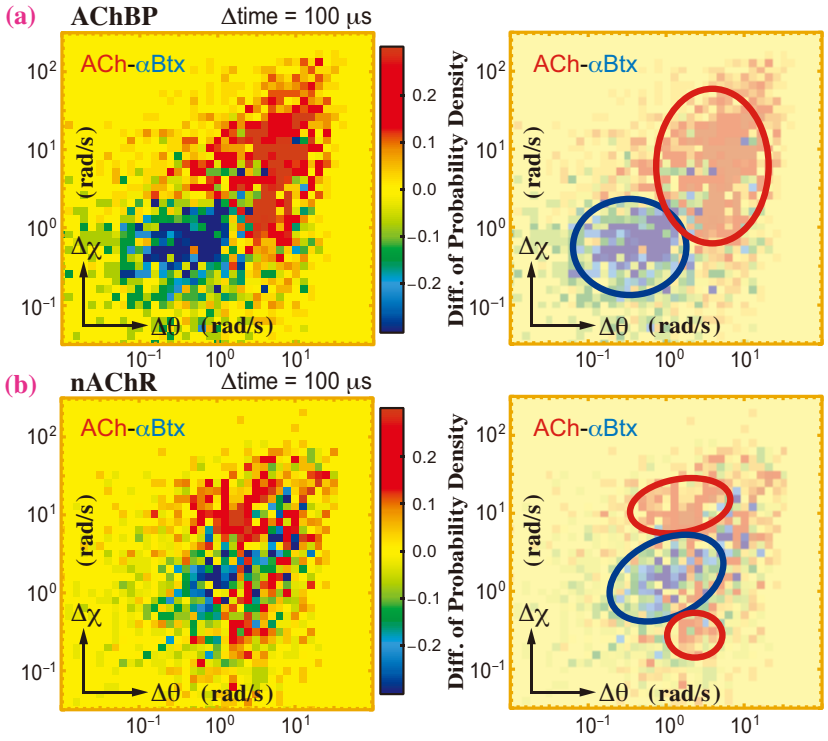

Fig. 2. Difference internal motion 2D histograms of AChBP and nAChR. [2]

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