**Gold Nanocrystals as 3D High-precision Motion Tracker**

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*in vivo* observations have greatly progressed due to the remarkable development of fluorescence single-molecule detection techniques using visible lights. These single-molecular techniques have provided positional information at an accuracy of about wavelength/100, far below the optical diffraction limit (wavelength/2). In 1998, we achieved time-resolved x-ray (wavelength~0.1nm) observations of 3-dimentional (3D) picometer-scale (wavelength/100) Brownian motions in individual DNA molecules1. We proposed a method to observe intramolecular motions by labeling gold nanocrystals with individual single protein molecule and observing the motions of diffracted X-ray spots from labeled individual gold nanocrystals. This DXT (=Diffracted X-ray Tracking) can trace all rotational 3D motions within single protein molecule using white X-rays. The cysteine and methionine site in the protein molecules can have a covalent bond to the surface of gold nanocrystals. Therefore, we succeeded time-resolved (to nano-seconds from micro-seconds) x-ray observations of dynamical Brownian motions of individual single channel in aqueous solutions through the labeled gold nanocrystals for the first time in the world2. Until now, we are trying to observe Brownian motions of actin-myosin interactions, denatured proteins3,4, functional protein membranes2,5 (bacteriorhodopsin, AChBP, AChR, and KvAP), antigen- antibody interactions6,7, peptide/MHC complex for T cell activation8, and monitoring super-weak force (pN).

Additionally, we successfully observed the nano-scale dynamics of supersaturated protein (lysozyme) solutions with time-resolved X-ray observations9. We demonstrated that supersaturated protein solutions have femto newton-scale force fields. This observed force field by manipulated nanocrystal is originated from asymmetric Brownian motions, we call as nano-flow field.

As described above, normal DXT must use white x-rays. Therefore, when using monochromatic X-rays, it is impossible to track all motions of diffraction spots. However, we detected a clear blinking in diffracted X-ray intensity. Now, we call Diffracted X-ray Blinking (DXB). The observed X-ray blinking intensity from the labeled and moving gold nanocrystals correlated with the velocity of the diffraction spots by autocorrelation function (ACF). Recently, we developed this technique to observe the molecular dynamics using laboratory X-ray source; Rigaku FR-D (Cu anode, 50kV, 60mA) and a high sensitive detector; PILATUS-100K. We try to distinguish molecular dynamics of AChBP between with or without toxin using new our laboratory analytical instrumentation. We have recently succeeded in being able to measure *in vivo* single molecular observations in living cells with DXB even with a laboratory x-ray source for the first time in the world.

**References**


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