Structural biology created in 1950th has revealed in detail static three dimensional structures of many proteins using techniques represented by X-ray crystallography. However, protein molecules are dynamic in nature; the molecules fluctuate, undergo conformational changes, bind to and dissociate from the partner molecules and transverse a range of energy and chemical states. Therefore, we have had limitations in understanding how proteins function from their static structures. To make it possible to study the dynamic behavior of proteins, single-molecules biophysical techniques including single-molecule fluorescence microscopy and optical trap nanometry have been created and successfully used. However, the protein molecules themselves are invisible in the single-molecule observations even with super-resolution bypassing the diffraction limit. Thus, simultaneous assessment of structure and dynamics has long been infeasible, which is the main cause for the difficulty in understanding the functional mechanism of proteins.

To break this long-standing situation, high-speed atomic force microscopy (HS-AFM) has been developed (T. Ando et al., PNAS, 98, 12468, 2001; T. Ando et al., Prog. Surf. Sci. 83, 337, 2008). It enables direct visualization of dynamic structural changes and dynamic interactions occurring in individual proteins molecules at sub-100 ms temporal resolution. The revolutionary power of this new microscopy has recently been demonstrated in the studies of bacteriorhodopsin responding to light (M. Shibata et al., Nat. Nanotechnol. 5, 208, 2010), myosin V walking actin filaments (N. Kodera et al., Nature 468, 72, 2010), rotor-less F1-ATPase (T. Uchihashi et al., Science 333, 755, 2011), and others (see reviews: T. Ando et al., 42, 393, 2013; T. Ando et al., Chem. Rev., in press). The molecular movies with sub-molecular resolution have yielded significant findings, providing new insights into how the proteins function. The very recent progress in this microscopy has also been making it possible to observe dynamic molecular processes on live bacterial cells (H. Yamashita et al., J. Mol. Biol. 422, 300, 2012) and dynamic phenomena occurring in live eukaryotic cells (H. Watanabe et al., Rev. Sci. Instrum. 84, 053702, 2013). In this talk, I will review these studies and discuss future prospects of HS-AFM studies.

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