Directly observing protein molecules in dynamic action by high-speed atomic force microscopy

Toshio Ando
Nano Life Science Institute (WPI-NanoLSI), Kanazawa University
Kakuma-machi, Kanazawa 920-1192, Japan

All our vital activities depend on proteins. Therefore, deciphering how proteins function is the key to understanding life and disease. To this end, structural biology has solved the atomic structure of many proteins, but the acquirable information is largely limited to that of static snapshots. Single-molecule biophysics has recorded the dynamic action of proteins but only in an indirect way of observing optical markers attached to the molecules. To overcome the limitations of these major approaches, high-speed atomic force microscopy (HS-AFM) was initiated to be developed in 1993 [1] and finally established in 2008 [2]. HS-AFM enables direct visualization of protein molecules during their functional activity at sub-molecular spatial and ~100 ms temporal resolution, without disturbing their function [3]. In the last 15 years, the innovative power of this new approach has been continuously demonstrated by increasing studies across the world for a variety of proteins [3], from motor proteins [4,5] to ionic channels [6], and even to intrinsically disordered proteins [7]. The most remarkable feature revealed by these studies is that dynamic phenomena recorded in HS-AFM movies can quickly solve important biological questions, even those difficult to be addressed with other approaches. In this talk, I will briefly describe HS-AFM techniques including those under development for faster imaging (at 100 frames/s) and discuss molecular imaging studies with HS-AFM.

1. Ando T. et al. A High-speed atomic force microscope for studying biological macromolecules. *Proc. Natl. Acad. Sci. USA* 98, 12468−12472 (2001).
2. Ando T. et al. High-speed atomic force microscopy for nano-visualization of dynamic biomolecular processes. *Prog. Surf. Sci.* 83, 337−437 (2008).
3. Ando T. *High-speed atomic force microscopy in biology*. 309 pages, Springer Berlin, Heidelberg (2022).
4. Kodera N. et al. Video imaging of walking myosin V by high-speed atomic force microscopy. *Nature* 468, 72−76 (2010).
5. Uchihashi T. et al. High-speed atomic force microscopy reveals rotary catalysis of rotorless F_1-ATPase. *Science* 333, 755−758 (2011).
6. Lin Y.-C. et al. Force-induced conformational changes in PIEZO1. *Nature* 573, 230–234 (2019).
7. Kodera N. et al. Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy. *Nat. Nanotechnol*. 16, 181−189 (2021).