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Takayuki Uchihashi, Simon Scheuring

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## Applications of high-speed atomic force microscopy to real-time visualization of dynamic biomolecular processes

Takayuki Uchihashi<sup>a</sup>, Simon Scheuring<sup>b,c</sup>

<sup>a</sup>Department of Physics, Kanazawa University, Kanazawa 920-1192, Japan.

<sup>b</sup>Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY 10065, USA.

<sup>c</sup>Department of Anesthesiology, Weill Cornell Medicine, New York, NY 10065, USA.

*Background:* Many biological processes in a living cell are consequences of sequential and hierarchical dynamic events of biological macromolecules such as molecular interactions and conformational changes. Hence, knowledge of structures, assembly and dynamics of proteins is the foundation for understanding how biological molecules work. Among several techniques to analyze dynamics of proteins, high-speed atomic force microscopy (HS-AFM) is unique to provide direct information about both structure and dynamics of single proteins at work.

*Scope of review:* The scope of this review is overviewing recent progresses of HS-AFM for studying dynamic processes of biomolecular systems. In the technical descriptions, key developments enabling fast and non-invasive imaging of biological samples are briefly mentioned. Then recent successful applications of HS-AFM are overviewed to showcase the power of HS-AFM in biological research.

*Major conclusions:* We discuss examples where HS-AFM movies captured important dynamic biological processes, including conformational dynamics of membrane proteins, processive movements of enzymes, assembly and disassembly processes of protein supramolecular structures, and dynamics in a two-dimensional protein crystal. These examples demonstrate the usability of HS-AFM to reveal biomolecular processes at high spatiotemporal (nanometer and subsecond) resolution.

*General Significance:* Real-time movies of unlabeled proteins at work captured by HS-AFM allowed us to directly gain insights into mechanisms of molecular actions. Together with further functional extensions, HS-AFM will enable researchers to investigate more complex biological systems involving multiple proteins and will become an indispensable technique for life science.

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