【Grant-in-Aid for Scientific Research (S)】

Science and Engineering (Interdisciplinary Science and Engineering)

Title of Project : Realization of nano-dynamics imaging of protein molecules in extremely soft membrane environments

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Research Area : Nano-Biosciences
Keyword : Single-molecule imaging, Nano-measurements

【Purpose and Background of the Research】

High-speed AFM (HS-AFM) that I developed enabled directly observing protein molecules in action. Following our pioneering imaging studies, various laboratories have been filming proteins in action using HS-AFM, enabling new discoveries that would be unable with conventional techniques. Nevertheless, HS-AFM imaging of membrane proteins working in extremely soft membranes, such as ion channels and translocons translocating polypeptides across membranes, has been infeasible. This is due to large deformation of the membranes caused by tip-contact, prohibiting high resolution imaging. Although this can be avoided by placing membrane fragments on a substrate, concentration gradients of ions and molecules across the membrane cannot be formed and polypeptides translocation across the membrane is hampered. In this study we will develop techniques that enable high resolution imaging of dynamic processes of proteins in soft membrane environments.

【Research Methods】

We will develop (i) a technique to suspend membrane protein-containing membranes at small areas, across which concentration gradients of ions and molecules can be formed, and (ii) high resolution scanning ion conductance microscopy (SICM), while further expanding our previous studies for high-speed and low-noise performances of SICM. SICM uses an electrolyte-filled glass pipette as a probe and relies on an ion current flowing between an electrode inside a pipette and another in an external bath solution (Fig. 1). The ion current passing through the small opening of the pipette is sensitive to the tip-sample surface separation. Hence, SICM can capture topographic images without tip-sample contact. To gain high resolution, the pipette tip has to be brought to the sample by a distance of the tip opening. However, the wall surrounding the opening is thick, and hence, an object right under the wall cannot be detected sensitively, leading to tip-sample contact. We will develop a technique to minimize the wall thickness and a technique to use carbon nanotubes as a probe for SICM. The innovative power of these techniques will be demonstrated by dynamic imaging of purified proteins and membrane proteins embedded in the outer and inner membranes of mitochondria.

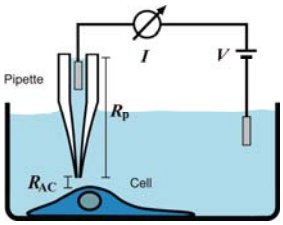

Fig.1 Schematic of SICM

【Expected Research Achievements and Scientific Significance】

SICM with high-speed, high-resolution and non-contact imaging capabilities will be materialized. This will make it possible to observe dynamic action of protein molecules working in extremely soft membranes. Moreover, imaging of suspended protein molecules will also become possible. Therefore, protein molecules in higher order structures, such as demembranated myofibrils, will be able to be observed. Future high-speed/high-resolution SICM has the potential to visualize the interior of live cells.

【Publications Relevant to the Project】

-T. Ando, T. Uchihashi, and S. Scheuring, Filming bio-molecular processes by high-speed atomic force microscopy (2014) *Chem. Rev.* **114**, 3120-3188.
-C.-C. Chen, Y. Zhou, and L. A. Baker, Scanning ion conductance microscopy (2012) *Annu. Rev. Anal. Chem.* **5**, 207-228.
-J. Geng *et al.*, Stochastic transport through carbon nanotubes in lipid bilayers and live cell membranes (2014) *Nature* **514**, 612-615.

【Term of Project】 FY2017-2021

【Budget Allocation】 126,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://biophys.w3.kanazawa-u.ac.jp/index.html