non-reactive protein is effective to reduce the nonspecific interaction (J. Wakayama et al., Anal. Biochem., in press). In the present study, we investigated the influence of temperature on the energy landscape of interaction of antibody and antigen using the methods. We measured the rupture force between anti-beta-lactoglobulin antibody and beta-lactoglobulin at various temperatures and pulling velocities by AFM. We will discuss the temperature effect on the energy landscape of the antibody-antigen interaction. This work is supported by Food Nanotechnology Project, MAFF.

3P-307 4CCDを用いた4色単分子同時蛍光計測システムの開発

Simultaneous Four-Color Imaging of Single Fluorescent Molecules Using Dichroic Mirrors and Four Charge-Coupled Devices

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We developed a four-color total-internal-reflection fluorescence microscopy (TIRFM) system using dichroic mirrors and four charged-coupled devices (CCDs) to simultaneously detect four spectrally distinct fluorophores at the single-molecule (SM) level with high time resolution. In this system, four species of fluorophores, Alexa Fluor 488, Cy3, Cy5, and Cy5.5, were immobilized on a fused silica substrate and irradiated simultaneously by two excitation beams from an Ar ion laser (488 and 514.5 nm) and diode laser (642 nm) through their total internal reflection on the substrate surface. Then, images of fluorescence emitted from the fluorophores were collected simultaneously with the four CCDs at a rate of 10 Hz. Signal to noise ratio (S/N) here is defined as the ratio of the SM fluorescence intensity before photobleaching to the background noise after photobleaching in the time course of the SM fluorescence intensity. The average S/Ns derived from hundreds of fluorophores turned out to be above ten for all four species. We also assessed the accuracy of fluorophore classification based on the ratios between the SM fluorescence intensities obtained by the four CCDs simultaneously. We found the error rates of fluorophore classification to be less than 4% for all the species.

3P-308 原子間力顕微鏡・蛍光相関分光法を用いた細胞膜表面の局所領域測定

Local measurement of cell surfaces with atomic force microscopy and fluorescence correlation spectroscopy

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Biological molecules of cell surface such as lipids, cholesterol, and membrane proteins interact with each other and the underlying structures. To understand the mechanism of the interactions, we need to clear the relation between the dynamics and the mechanics of cell surfaces. In order to investigate the relationship, we developed a combined system of atomic force microscopy (AFM) and fluorescence correlation spectroscopy (FCS), and we attempted to measure simultaneously both the diffusion coefficient of molecules and the nanomechanics of living cell surfaces in local regions. The AFM/FCS system was composed of an AFM (Asylum Research, USA) mounted on an inverted optical microscope (TE2000, Nikon) with a photon counting system used a photomultiplier. In the preliminary experiments, we modified the membrane of HeLa cells with phospholipids with fluorescence probes such as quantum dots, and we measured the diffusion coefficients of probes in local regions by FCS. Simultaneously, we could apply a loading force and/or measure mechanical properties of cell surfaces in the local regions by AFM. Therefore we consider that our new method of AFM/FCS system is contributed to clear the relation between dynamics and the mechanics of cell surfaces. The performance of the system and the results regarding the relationship between the dynamics and mechanics of cell surfaces will be presented in detail.

3P-309 AFMと平面パッチクランプ法を組み合わせた新規分子計測法の開発

Development of new molecular measurement method of patch-clamping combined with AFM

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In 1976, patch-clamping was developed by Neher and Sakmann, and pioneering work on single-ion channels started with electrophysiology experiments that could measure the flow of ions through single nanopores in cell membranes. More recently, as development of Atomic Force Microscopy (AFM), it have opened up an even wider range of new research area for single biological molecule, and which allow us to perform operation and physical measurement of biological molecule such as viscosity, stress, and force curving of the acceptor interaction at single molecule. However, single molecule measurement method that consists of AFM and patch-clamping, has not been developed. In order to develop a new single molecule measurement method that can measure the interaction with the single channel protein and other molecule from both sides of electrophysiological and physical measurements, we made up planner patch-clamping for AFM

3P-310 ポルフィリン錯体からなる分子ベアリングの1分子計測実験

Single molecule experiments of molecular bearing of porphyrin complexes

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Recent single molecular detection techniques based on optical microscopy made direct visualization of molecular motor such as a working F1-ATPase. Although the bottom-up fabrication of nanoscopic motor continues to attract great attention, it appears to be a challenge to visualize molecular rotation of such supramolecular assembly at molecular resolution in real space. We report herein that scanning tunneling microscopy (STM) study of hetero double-decker porphyrin derivative (DD) and hetero triple-decker porphyrin derivative (TD) deposited on Au (111) surface by means of "pulsed injection method". The DD molecules are known to form highly ordered adlayers on highly oriented pyrolytic graphite surface. We have found that both DD and TD, deposited by pulse injection method, form highly ordered adlayers on Au(111), indicating the strong molecular recognition between the alkyl chains of lower porphyrin units. We also successfully observed rotation of topmost porphyrin of both DD and TD. Importantly, high-resolution images exhibited that compound DD is arranged in 1:1 mixture of M and P chirality on the surface. Furthermore we have found that compound DD lying on the chasm of the assemblies alters its chirality from M to P upon applying electric field through the 22deg rotation of the upper porphyrin ring. To the best of our knowledge, there have been no reports of STM where such movement of "molecular bearing" (not rotation of entire molecular unit over the surface) has been detected in this way.

3P-311 原子間力顕微鏡を用いた多数細胞粘弾性の精密測定

Viscoelastic Properties of Many Living Cells Investigated by Atomic Force Microscopy

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The rheology of living cells plays a key role in determining cell function. The force-modulation mode with the atomic force microscopy (AFM) is a useful method for measuring the complex shear modulus, $G^*(\omega)$, quantitatively in a wide frequency region^[1-4]. However, in the previous AFM studies, the statistics of rheological properties has not been fully investigated. Thus, in the present study, we used a microarray technique^[5] to measure a large number of cells by AFM and calculated $G^*(\omega)$ of each cell. Moreover, we investigated the correlation between $G^*(\omega)$ and cytoskeleton. In the experiments, mouse fibroblast cells (NIH3T3) were arranged on the wells of microarray, and the force modulation experiments were examined in each cell ($n > 100$) with a colloidal silica probe of cantilever. The number distribution of the $G^*(\omega)$ could be estimated in the frequency range from 0.1 Hz to 200 Hz. As a result, we found that the number distribution of $G^*(\omega)$ (the real part of $G^*(\omega)$) and $G''(\omega)$ (the imaginary part of $G^*(\omega)$) exhibited a log-normal distribution. Additionally, the relationship between $G^*(\omega)$ and cytoskeleton will be presented in detail. This work was supported by industrial Technology Research Grant Program in 2006 from NEDO of Japan.

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3P-312 AFM力学測定の実用化への応用

Applications of AFM Force Measurement for Life-science Field

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The force measurement mode of AFM (atomic force microscopy) allows us to study the interaction force between bio-molecular pairs at the single molecular level and the mechanical property of biomaterials, and also to manipulate biomolecules in the cell. To conduct this technique more precisely and effectively for biological studies, we should address issues described below.

1. Quantification of the lateral force applied on cantilever during manipulation experiments
2. Quantification of the immobilized molecules on the tip of cantilever during measurement of interaction force between biomolecules
3. Reduction of non-specific interaction between tip of cantilever and substrate surface
4. Shortening of measurement and analysis for statistic analysis of rupture force between biomolecules.

In this presentation, we will review our strategies to overcome problems described above and our applications of force measurement of AFM.

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3P-313 回転自由度を制限したレーザートラップ法

Laser trap nanometry with non-spherical shaped probe

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Laser trap nanometry is a powerful tool to reveal the physiological character of various biomolecules. In this method, a spherical bead with biomolecule captured