Rheological properties of normal and cancer cells measured by atomic force microscopy

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Measurements of single cell mechanics are crucial for understanding various cell behaviors such as migration and proliferation. It has been reported that mechanical properties of cancer cells differed from normal cells [1]. However, the viscoelastic (rheological) properties of the cells have not been fully understood. In this study, we measured rheological properties of a large number of human mammary epithelial cells (MCF-10A) and human mammary adenocarcinoma cells (MCF-7) by atomic force microscopy (AFM) [2], in which the force modulation mode was employed to measure the complex shear modulus, G^* , in a frequency range of 2-180 Hz [3] and the observed data was estimated using a power-law structural damping model [4]. As a result, the frequency dependence of G^* was well fitted to this model. The scale factor of modulus, G_0 , of MCF-10A cells was significantly larger than that of MCF-7 cells, which was consistent with the result observed on a different type of normal and cancer cells [5]. Other parameters regarding power-law rheology between the normal and cancer cells will be presented.

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1J1336 デンプン粒樹脂包埋切片の原子間力顕微鏡による力学特性の計測

Evaluation of mechanical properties of starch granules by using atomic force microscopy combined with resin embedded section

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The mechanical characterization of biological materials such as cell and organelle is important for understanding their structure and physiological function. In last decade, atomic force microscopy (AFM) has been used to investigate the mechanical properties, such as Young's modulus and hardness, of the biological material. However, the previous measurements have limitations, since the values of the mechanical properties are sensitive to the surface roughness of the specimen. Furthermore, the previous studies have been carried out for only the specimen surface. In order to exactly evaluate the properties, one should measure not only the surface but also inside of the specimen, since most biological specimens are nonuniform and their compositions are inhomogeneous.

At the previous meetings, we have reported AFM imaging combined with resin-embedded section method and structure of starch granules by using the method. Our previous methods can decrease surface roughness of the specimen and provides information about not only the surface but also inner of the specimen. In the present study, we investigated the mechanical properties of section of starch granules by using AFM combined with the resin-embedded section method. Based on the results, we will report the mechanical properties of the starch granules and relationship between the mechanical properties and the structure. This work is supported by Food Nanotechnology Project, MAFF.

1J1348 電子線励起作用による細胞膜上のナノパターン生成と生体分子操作にむけた応用

On Cell Membrane Nano Patterning Using Electron-Beam Induced Chemical Reaction Toward Single Bio Molecule Manipulation

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While previous studies have mainly applied an electron beam (EB) for imaging the molecular morphology of cellular subsystems, our submission now reports a new method of EB deposition and ablation in a non-vacuum liquid environment for a complete living cell; in the future, this research should lead toward nano-surgery of molecular mechanics on a cell membrane. We expect this first demonstration of in situ nano processing and cellular nano stimulation can be utilized for real time analysis of changes in membrane protein dynamics. Our nano processing on a living cell membrane using EB-induced deposition and ablation could directly manipulate the membrane physical dynamic function. Moreover, theoretically, the EB had less than a nanometer scale wave length, and the electron range in water was also nano scale. Therefore this method can provide new approaches for in situ molecular level dynamics manipulation on

membrane dynamics-related disorders, for example cancer metastasis, and repairing and regenerating neurons after injury; by combining it with single molecule imaging it can be used to understand how spatio-temporal dynamics affects their mechanisms.

1J1412 1分子タイムスタンプ FRET 時系列データから分子状態変化ダイナミクスを推定する変分ベイズ-隠れマルコフモデル解析

Variational Bayes-Hidden Markov Model for time-stamped single-molecule FRET time series data to analyze conformational state dynamics

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Single-pair fluorescence resonance energy transfer (spFRET) is a powerful tool to investigate conformational dynamics of biomolecules. Time series measurements on a single molecule enable us to trace dynamic behavior in realtime. Interpretation of its signal, however, is problematic because the signal to noise ratio is relatively low. We developed a novel method to analyze time series data of spFRET experiments, which reproduces the trajectory of transitions among the limited number of molecular states. The hidden Markov model (HMM) is employed to represent such situations and is extended to treat the time stamp signal, which records detection time of every single photon, leading to the ultimate time resolution by maximizing information. We introduced the variational Bayes (VB) to solve the HMM by optimizing the state transition trajectory and parameters. In addition, the VB has robustness in choosing the optimum model, i.e. the number of states. We conducted several sets of simulation to evaluate performance of our VB-HMM analysis. The time-stamp data is generated by Monte Carlo simulation and analyzed by VB-HMM methods. We compared results between the time-stamp and the photon-counting signals, and the donor-only and the two-color signals, and concluded that the time-stamped two-color FRET measurement followed by VB-HMM analysis was the best. We would like to introduce some examples of spFRET experiments analyzed by the VB-HMM, such as the spontaneous branch migration dynamics of the Holliday junction.

1J1424 ラマン分光計測による細胞分化ダイナミクスの計測

P77 A phase diagram of cell differentiation based on Raman spectroscopic measurements

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Raman spectroscopy of biological systems and single live cells permits the non-invasive sensing of information including molecular compositions, structures, and interactions about major components. The main interest of this presentation was to confirm whether the Raman technique was effective for distinguishing global dynamics of cell differentiation process, in which the conventional observations using biochemistry and fluorescence imaging provide invasive or specific information. Here, MCF-7, a breast cancer cell line, was used. MCF-7 is differentiated by heregulin into mammalian gland-like cells which produced lipid droplets in the cytoplasm, but the response is diverse in each single cell. Raman spectra of single live cells were obtained as a function of time of the heregulin treatment. A phase diagram was calculated based on the measured Raman spectra, utilizing the principal component analysis and the standardization of the distance. In the diagram, the multi-dimensional information of Raman spectra was projected on a 2D-plane of which the xy-axes were composed of the existing components of the cell and the newly produced components. The diagram indicated that the change in the concentrations of the existing chemical components at the cytoplasm was not monotonous and that the concentrations of the existing components were diversified before giving newly generated chemical species.

1J1436 Application of Micro Scoop Cantilever for Cell Biophysics

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Maintenance of intra- and extracellular mechanical environment is crucial for cell viability, tissue development and differentiation. To understand the relation between cellular mechanical properties and their responses, precise quantification both of the applied force as an input and the cellular response as an output is required. Single cell force spectrometry (SCFS) using atomic force microscope (AFM) helps us to clarify the relation in high spatiotemporal resolutions under physiological conditions, covering a wide range of force required for such study. To increase the throughput of SCFS experiments to