2P545

Imaging and direct structural analysis of biomolecules on the mammalian tissue surface using tandem mass spectrometry

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Mass spectrometry on 2D samples is known as imaging mass spectrometry, which enables us to visualize molecular distribution on a tissue surface. The technology has been developed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometers (MALDI-TOFMS). This technique can visualize biomolecules, however, it is difficult to identify the molecules precisely. As the molecular mass increased, the mass resolution is degraded. This phenomenon implies that signals in the heavier region contain multiple components. If we would like to determine the structure of molecules (e.g., amino acid sequences), a tandem mass spectrometry (MS^n) is essential. In the MS^n, ions in a specific *m/z* range are selected, dissociated, and the product ions analyzed sequentially. The structural information of interested molecules is reflected in the MS^n spectra as a peak pattern.For MS^n, we have to perform high ionization efficiency on the tissue surface. We had developed the methodology to overcome this problem. We can detect several biomolecules, for example, lipids, sugar chains, peptides, and proteins. In this poster, we will present the methodology and application results (imaging and structural analysis) directly from mouse brain section. In particular, we would like to give biological information to biological materials whose distribution was varied by physical stimulation.

2P546

Structural Analysis of Curly and Straight Human Hair Fibers by Scanning Microbeam SAXS

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It is well known that wool fibers, keratin fibers which bear strong similarities to human hair, contain two types of cortical cells, so-called orthocortex and paracortex, and the bilateral distribution of these is associated with the crimped shape of wool. It has been found by TEM observation and small angle X-ray scattering (SAXS) measurement that there are obvious differences in the geometrical arrangement of the intermediate filaments (IFs) in these cortical cells. For human hairs, the curvature of the fiber differs not only by ethnic group but also between individuals. There have been some reports on cortical cell types for human hairs but no information on their distributions and the relation to the curvature exists. The purpose of this study is to analyze the inhomogeneity in the IF arrangement in curly and straight human hairs.SAXS experiments were carried out at SPring-8 (Sayo, Japan) and Photon Factory (Tsukuba, Japan). The size of microbeam (less than 6 μm in diameter at the specimen) was small enough to analyze the outer and inner sides of the curl separately. Two-dimensional SAXS patterns from different transverse positions in the fiber were measured. The microstructure of Japanese curly and straight hairs, moderately curled Caucasian hairs, and strongly curled African-American hairs were investigated. As a result, a relationship between the lateral inhomogeneity of cortical cell distribution and macroscopic curl shape of human hair has been revealed.

2P547

Single cell based analysis on the polarity of *Escherichia coli* cells

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Rod-shaped bacteria *Escherichia coli* grow with the long axis of their body and reproduce two daughter cells by dividing in the middle. Each daughter has two poles, one is previously existed as a mother's pole (called as old pole) and the other is newly formed by division (called as new pole). Recent study revealed the cell that inherits the old pole exhibits a diminished growth rate. This result suggests there are some kinds of differences between old and new poles, in other words, *E.coli* cells have polarity and its polarity is thought to be inheritable from one generation to another.However, it is not well known that how the polarity is generated and retained, and what kind of significance the polarity has to cellular behavior. To approach these questions, we focused on the aspartate chemoreceptor Tar known to cluster at a cell pole. First we fabricated the thin micro order ditches on glass slide, and under the microscope, we cultured cells that express Tar-GFP in the micro ditches for over several generations by using on-chip single cell cultivation system. This system helped us to compare the mother with the daughter cell, or old with new pole easier than existing methods such as time-lapse observation of micro colonies based on agar pad method. Second we measured the effects of aspartate stimulus on a single cell in ditches. Asparatic acid is known to change the tumbling frequency and localization of Tar. So we examined the relation between the polarity and the temporal phenotypic changes brought on by aspartate stimulus.

2P548

Single-cell-based-observation of macrophage's phagocytic responses and the alteration of its morphology by activation

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Single-cell-based-observation of macrophage's phagocytic responses and the alteration of its morphology by activationHirofumi Shindoh1, Kazunori Matsumura1, Hiroshi Ishimoto2, Katsunori Yanagihara2, Shigeru Kohno2, Kenji Yasuda1.1The University of Tokyo, Tokyo, Japan, 2Nagasaki University, Nagasaki, JapanImmune system encompasses the various kinds of the cells that cooperatively respond to antigen be internalized, processed, and presented by the antigen-presenting cells(APCs); hence the study on the single cell responses of the APCs to well-characterized antigen stimulations is at first essential for understanding it. We, therefore, developed the method by which we can stimulate the APCs with the antigen in a site-and timing-specific manner and to observe their phagocytic responses at the single cell level.Alveolar macrophages were extracted from BALB/c mice, a model of the APC. The macrophages were observed by time-lapse. As an antigen, we used a cell wall of yeast, called zymozan. The site- and timing-specific stimulation by the zymozan was achieved by use of optical tweezers. The phagocyting and mobility responses were analyzed by the software programmed with LabVIEW.The analysis successfully revealed the existence of the lag time and the existence of two types of macrophages by distribution of phagocytic lag time. These types could be destinguished by area(S) and ratio(I) of circumference of macrophage to the circle of the same area of cell body. We defined one type activated macrophage, and the other type inactivated macrophage.

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