measurement system that records the extracellular field potentials and the contractile motions in lined-up cardiomyocyte network simultaneously. Cardiomyocytes were isolated from embryonic mouse heart by collagenase treatment and seeded in rectangular microchambers fabricated on the multi electrode array (MEA) chip for field potential recordings. To detect contractility of cardiomyocytes, the cells were labeled with the fluorescent polystyrene beads and the motions of beads were treated with image-based analysis. Hence, electrophysiological properties (interspike intervals and field potential durations) and mechanophysiological properties (displacements and velocities of beads) were obtained simultaneously. In the presence of verapamil, typical L-type calcium channel blocker, beating rate decreased accompanying with shortening of field potential duration (FPD) and its fluctuation increase, and contractility was weakened significantly with its fluctuation increase. The results indicate that the fluctuation of force generation should also become a biomarker of cardiotoxicity risk more correctly in drug development, also might be used for the clarification of mechanisms.

3PS024 白色 X 線源を用いた X 線 1 分子計測法の開発

Development of Diffracted X-ray Tracking measurement system using various wide-band energy X-ray sources

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Diffracted X-ray tracking (DXT) enable to measure milliradian resolved rotating and tilting motion of single protein molecules using high-brilliant and wide-band energy X-ray source. The tilting (θ) and rotating (χ) angle resolution and temporal resolution in this measurement are 0.9 mrad, 6 mrad and from 5 μ s/frame to 36 ms/frame corresponding to the photon flux. Tracking the diffraction spot from gold nanocrystals attached functional group with protein was developed for revealing the detailed fluctuation and functionalized motions in protein reaction processes at the beamline BL28B2, and BL40XU at the SPring-8 and NW14A at the PF-AR, KEK. (1) We installed the X-ray toroidal mirror at the beamline BL28B2 because the wide-angle diffraction spot tracking of gold nanocrystal markers in the measurement range $\Delta\theta = 7.4^\circ$ can be observed in this beamline. (2) The undulator beamline BL40XU (the measurement range, $\Delta\theta = 4.2^\circ$) allows high speed DXT in microsecond time region using the CMOS CCD camera. (3) New DXT measurement system which was synchronized with pulse laser system (Nd: YAG laser, pulse-width 10 ns, 10 Hz, UV-Vis) has succeeded to observe the open-close motion of membrane protein in reaction process at the beamline NW14A (measurement range, $\Delta\theta = 2.1^\circ$). Thus, the biological science applications of DXT can high profit from observation of the in-vivo functionalized motions of single protein molecules. Here, we will present the development of DXT measurement system and the future plan.

3PS025 CT 撮影用投影型 X 線顕微鏡の開発

Development of Projection X-ray Microscope for Micro-Tomography

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The projection X-ray microscope makes use of a very small X-ray light source excited by the focused electron beam on a thin metal target. If an object is placed just below the metal target, the expanding X-rays enlarge the shadow of the object with infinite focal depth. We have developed the microscope by modifying a scanning electron microscope (SEM) and achieved a resolution about 0.1 μ m. It is advantageous to investigate an opaque sample with better resolution than an optical microscope. And also has additional benefit to investigate three-dimensional structure of the thick specimen which can not studied by an electron microscopy. Taking these advantages, we have applied projection X-ray microscope for three-dimensional (3-D) structure analysis by means of cone-beam computed tomography (CT). To get higher resolution and better contrast, the choice of the metal target is important. The size of X-ray source determines the resolution, thus the electron beam should be focused as small as possible by condenser lens of SEM. And it should have a good balance to get enough strength of X-ray. The electron beam irradiated at the metal target is scattered in the metal and the scattered area is smaller in heavy metal. To get better contrast the wave length of X-ray should be longer utmost it penetrate the sample. In this case light metal target works better because of longer wavelength of K α characteristic X-rays. By accounting these conditions we have optimized the metal target for biological specimens.

3PS026 XFEL を用いた 3 次元分子イメージングのための雑音下における 2 次元回折像の類似度自動同定

Automatic similarity identification of 2D diffraction patterns with noisy background for 3D coherent x-ray diffractive imaging

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We proposed a method for classifying and assembling two-dimensional diffraction patterns to reconstruct the three-dimensional diffraction intensity functions (A. Tokuhisa, et. al., Acta Crystallographica, A68, 366 (2012)). In the classification process, similarity of each pair of patterns is judged based on the correlation of corresponding pixels along the concentric circles. When two diffraction patterns are similar to each other, a straight line with high correlations appears from the origin to a certain high scattering-angle region. Theoretically, the integrated value of the correlation value along this line is proportional to the extent of the similarity and can be used as threshold for the classification. This correlation line disappears with increasing scattering-angle due to the quantum noise and the structural irregularity. In our estimation, diffraction images of 10^6 orders will be required to construct the 3D structure with 3 Å resolution for a molecule with a radius of 200 Å. In this case, the necessary number of pattern comparisons is $\sim 10^{10}$ order. To achieve such a huge number of comparisons, we have to develop an automatic system. In this meeting, we will report how to deal with such a huge amount of data and our current status of the system.

3PS027 植物細胞の飛行時間型二次イオン質量分析法と多変量スペクトル分離法による評価

Evaluation of plant cells using time-of-flight secondary ion mass spectrometry and multivariate curve resolution

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Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is one of the most sensitive surface analysis methods providing submicron-scale chemical mapping. It is, however, often difficult to interpret TOF-SIMS spectra of complex samples such as tissues and cells. In this study, multivariate curve resolution (MCR) was employed in order to extract less complex TOF-SIMS spectra from original TOF-SIMS data of plant tissue. As a result, components related to intracellular tissues and cell wall tissues, respectively, were extracted from both samples. By comparing the component of the main sample to that of the reference sample, peaks specific to the main sample were indicated.

3PS028 Shannon エントロピーの可視化による質量顕微鏡データの情報解析 Information analysis by visualizing Shannon entropy using Imaging Mass Spectrometric data

Noritaka Masaki, **Mitsutoshi Setou** (Hamamatsu University School of Medicine, Department of Cell Biology and Anatomy)

Microscopic Imaging Mass Spectrometry (MIMS), which we have developed cooperatively with Shimadzu Corporation and others, enables us to analyze spatial distribution of enormous molecules in the resolution of single cell level. MIMS has demonstrated its successful performance by revealing key molecules and their distribution in various research fields, e.g., medical development, agriculture, and material analysis. On the other hand, ageing and some inflammation are difficult to understand using a few key molecules. Principal component analysis and machine learning are also inadequate to elucidate such phenomena overlaying high-dimensional space. In this study, we try to figure out biological state in the framework of overall behavior of molecules, citing the famous argument by Erwin Schrödinger who first utilized entropy for biology to understand living organisms as system that autonomously maintaining their order against disruption.

In our previous presentation in this biophysical society, we visualized Shannon entropy in mouse brain to quantify *in situ* information held in molecular composition. Entropy image reveals characteristic distribution irrespective to anatomical representation. To confirm and discuss such spatial variation in information entropy, we propose a new data processing that takes advantage of

spatial distribution by binning mass spectrum. This bundle of mass spectrum corresponds to maximum likelihood estimation and entropy image calculated from bundled spectrum shows more distinct pattern than normal entropy image.

3PS029 シャペロン GroEL フットボール型複合体を介したタンパク質フォールディングの直接観察

Direct observation of protein folding mediated by the football-shaped GroEL-GroES complex

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The chaperonin GroEL is an essential molecular chaperone that mediates protein folding together with its cofactor GroES in *Escherichia coli*. It has been commonly believed that GroES binds alternatively to each ring of GroEL, which is composed of two identical rings stacked back to back, during the functional cycle. In other words, an asymmetric GroEL-GroES complex (the bullet-shaped complex) is formed throughout the cycle, whereas a symmetric GroEL-(GroES)₂ complex (the football-shaped complex) is not formed. We have recently shown that the football-shaped complex coexists with the bullet-shaped complex during the functional cycle. However, how protein folding proceeds in the football-shaped complex remains poorly understood. Here, we used green fluorescent protein (GFP) as a substrate to visualize the folding in the football-shaped complex by single-molecule fluorescence techniques. First, we directly counted the number of refolded GFP molecules in the football-shaped complex, and showed that GFP can fold in both rings of the complex. Next, we performed the real-time imaging of GFP folding in the football-shaped complex. Remarkably, the folding was a sequential two-step reaction, and the kinetics was in excellent agreement with that in the bullet-shaped complex. These results demonstrate that the same reactions take place in both cavities of the football-shaped complex in the same manner to facilitate protein folding. These findings shed new light on the functional cycle including the football-shaped complex.

3PS030 等方性と偏光変調性の 2 つの全反射型蛍光顕微鏡を組み合わせる Combining of isotropic TIRFM and polarization-modulation TIRFM

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Two different types of total internal reflection fluorescence microscopy, TIRFM, have been independently developed in our research group. The first one is an isotropic TIRFM, *iTIRFM* (Adachi *et al.*, *Cell* 2007), in which the hollow-cone light generated by a diffractive diffuser is parallelized and focused to the back focal plane of the objective. Single fluorophores at the sample plane are almost uniformly excited even when their orientation are randomly distributed, because the incident light comes from all directions and so the resultant evanescent field contains all (*X*, *Y* and *Z*) polarization-components. The second one is a polarization-modulation TIRFM, *pmTIRFM* (Nishizaka *et al.*, *Nat. Struct. Mol. Biol.*, 2004; Masaie *et al.*, *Nat. Struct. Mol. Biol.*, 2008), which enables us to detect orientation of single fluorophores in *XY*-plane by analyzing a phase of the oscillation of their intensity.

We here combined these two techniques on the same Nikon microscope equipped with a commercial 'perfect-focus system.' Two different illuminations are switchable in a second by translating two filter cubes, which are located at the different points on the optical axis, with electric motors. This experimental setup allows to characterize single fluorophores quantitatively in terms of intensity, orientation and anisotropy, which uncover the change in local environment of multiple catalytic sites in a single protein molecule, such as F₁-ATPase, by directly visualizing repetitive binding and hydrolysis of 2'-Cy3-EDA-ATP.

3PS031 炎症時におけるヒト単球サイトカイン分泌のライブセルイメージング Live-cell secretion imaging assay of the inflammatory cytokine from human monocytes

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Inflammatory cytokines play a key role in the initiation of inflammation. However, their secretion dynamics especially at the single cell resolution still

remain unclear. For example, IL-1 β is a leaderless protein, lacking the signal peptide that typically directs secreted proteins through the classical endoplasmic reticulum-Golgi secretory pathway. Therefore the mechanisms of cellular release into the extracellular environment remain the subject of much investigation. To overcome this, we developed live-cell secretion imaging assay by total internal reflection fluorescence illumination with micro-fabricated well array chip. Here we tried to monitor the release dynamics of LPS activated human monocyte before and after additional ATP stimulation. Forty wells were observed every 1 minute for 4 hours. While little signals of IL-1 β could be observed before ATP stimulation, the fraction of the IL-1 β secreting cells largely increased after ATP stimulation. Moreover they showed strong IL-1 β signal and increased in their membrane permeability indicated with SYTOX dead cell staining reagent. This change seems to represent a "Terminal release", which is usually associated with cell death called "pyroptosis". This work thus succeeded in imaging of IL-1 β secretion from living cells at a high temporal resolution, which will enable us to investigate acute-phase inflammatory reactions of monocytes at the single-cell level for the first time.

3PS032 大腸菌異物排出タンパク質複合体コンポーネントの細胞内動態解析 Intracellular dynamics of the xenobiotic efflux proteins in *Escherichia coli*

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The rising occurrence of multidrug-resistant bacteria is a serious worldwide issue. One of the major causes of the multidrug resistance is the elevated expression of an RND-type xenobiotic efflux transporter complex, which consists of three components, the inner membrane transporter, the membrane fusion protein, and the outer membrane channel. Among the five inner membrane transporters of *Escherichia coli*, only AcrB, which forms a complex with the membrane fusion protein AcrA and the outer membrane channel TolC, is constitutively expressed. Interestingly, the other inducible transporters are also coupled to TolC. This raises an interesting question: does a newly synthesized inner membrane transporter replace the pre-existing transporter in complex with TolC or form a ternary complex *de novo*? In this study, we aim at visualization of the construction processes of the efflux protein complex. First, we constructed a strain expressing a chromosome-encoded fusion protein AcrB-GFP. We observed with total internal reflection fluorescence microscopy (TIRFM), most fluorescent foci of AcrB-GFP did not show lateral movement, whereas they were moving incessantly in the absence of TolC. Second, a residue in the extramembrane loop of TolC was replaced by Cys, and the resulting mutant was stained with the thiol-reactive fluorescent reagent to demonstrate co-localization of AcrB-GFP with labeled TolC. These results suggest that AcrB is fixed when it forms a complex with TolC, which penetrate through the peptidoglycan layer.

3PS033 1 分子蛍光イメージングを用いた転写活性化による Arp4 の動態変化の定量解析

Quantitative analysis of changes in molecular dynamics of Arp4 upon transcriptional activation using single-molecule fluorescence imaging

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Actin-related protein 4 (Arp4) is a component of a chromatin-remodeling complex. Chromatin-remodeling is thought to occur with direct binding of Arp4 to histones, indicating that Arp4 is involved in transcription control. Consequently, molecular dynamics of Arp4 is expected to change in correlation with transcriptional activity. However, the details of relationship between molecular dynamics of Arp4 and transcriptional activity remain unclear. In this study, we performed quantification of molecular dynamics of Arp4 both before and after stimulation with the phorbol ester PMA, as a transcriptional activator. We carried out single-molecule fluorescence imaging of the nucleus of CHO cells expressing TagRFP-T fusion proteins of Arp4 at a low level. The residence time of individual Arp4 molecules, the time from appearance to disappearance of the fluorescence spot, was measured in the images. We found that a residence time after stimulation tends to become shorter than before. It means that transcriptional activation causes dissociation of Arp4-containing chromatin-remodeling complexes from the target sites. In yeast, the number of the