3P007 大気圧電子顕微鏡 (ASEM) によるタンパク質微結晶と細胞内複合体の液中観察

Direct electron microscopy of protein crystals and Mycoplasma cells in solution using the Atmospheric SEM

Tatsuhiko Ebihara¹, Masaaki Kawata¹, Hidetoshi Nishiyama², Miki Senda³, Mari Sato¹, Mitsuo Suga², Toshiya Senda³, **Chikara Sato¹** (¹AIST, ²JEOL, ³KEK)

The new Atmospheric Scanning Electron Microscope (ASEM) observes samples in solution under an open atmosphere. It is, at the same time, a Correlative Light-Electron Microscope (CLEM), in which the optical microscope (OM) and SEM quasi-simultaneously observes samples. In the system, an inverted SEM observes the wet sample from beneath an open dish while an OM observes it from above. The film of ASEM dish can be coated variously like glass, which allows various cells to be cultured including neurons.

Immuno-ASEM successfully visualized molecular complexes in neuron primary culture and STIM1 in T cells in solution [1]. The ASEM not only visualized cells, but protein micro-crystals [2].

[1] J Struct Biol 180, (2012) 259-270. [2] Int. J. Mol. Sci. 13, (2012) 10553-10567.

3P008 EM Navigator と Yorodumi による 3 次元電子顕微鏡構造データの利用

Using 3D electron microscopy data by EM Navigator and Yorodumi

Hirofumi Suzuki^{1,2}, Haruki Nakamura^{1,2} (¹IPR, Osaka univ., ²PDBj)

More than 2300 structure data analyzed by 3D electron microscopy (3DEM) are deposited in EM Data Bank (EMDB) and Protein Data Bank (PDB). Compared to representative structure data, it is more difficult to utilize the 3DEM data. The structures are large and complex, and EMD data are in 3D map format, not atomic models. To make it easy even for 3DEM non-specialist, we have been developing and managing two web sites. EM Navigator (<http://pdbj.org/emnavi/>) is a 3DEM data explorer for EMD and 3DEM data in PDB. Yorodumi (<http://pdbj.org/yorodumi/>) is a 3D structure viewer especially for complex data in both databanks. In the presentation, we will describe the recent progress of these services.

3P009 電子顕微鏡の傾斜ペアを利用した構造の異なるタンパク質単粒子画像の分類

Separating single particle images of protein in the different conformations using tilt pair transmission electron microscopy

Yutaka Ueno, Kazunori Kawasaki, Shouhei Mine (*AIST Health Research Institute*)

In single particle analysis of protein and biological macromolecules, tilt pair images have been utilized to build the first structural models. With negatively stained specimens, particle images are sometimes stochastically distorted, although it could also happen in the native environment. We tried to segment single particle images in the different conformation using tilt pair observation. Based on a simple flattening model, a tilt angle correction was examined to yield correct volume reconstruction for the target molecule. The segmentation was considered as a typical graph-cut problem and images from different conformations were segmented. Our preliminary results in application for a structural study of glucosaminidase purified from archaea will be discussed.

3P010 NMR タンパク質立体構造決定のための新規構造最適化法の開発

Development of a new refinement method for NMR protein structure determination

Manato Shimazaki¹, Teppei Ikeya¹, Masaki Mishima¹, Yutaka Ito¹, Peter Guentert^{1,2} (¹Grad. Sch. Scii., Tokyo Metropolitan Univ, ²Inst. Biophys. Chem., Goethe Univ Frankfurt)

NMR protein structure calculation adopts severe simplifications of the nonbonded interactions to rapidly obtain global structures. It is thus necessary to perform the subsequent refinement of the structures with a physical forcefield. While various refinement approaches are proposed using MD simulations, there are a few examples to optimize the NOESY assignment and calibration of the distances from NOE peak intensities based on the refined conformations. Recently, we implemented into the program CYANA a new structure refinement method that recursively improves the structures and NOE assignments, and addresses the calibration based on Bayesian inference. Here, we show the results applying it to several test data and its feasibility for the protein structure refinement.

3P011 Structural analysis of antimicrobial peptide CP1 with LPS by NMR

Mihwa Baek¹, Masakatsu Kamiya^{1,2}, Taichi Nakazumi¹, Satoshi Tomisawa¹, Yasuhiro Kumaki³, Takashi Kikukawa^{1,2}, Makoto Demura^{1,2}, Keiichi Kawano², Tomoyasu Aizawa^{1,2} (¹Grad. Sch. Life Sci., Hokkaido Univ., ²Fac. Adv. Life Sci., Hokkaido Univ., ³Grad. Sch. Sci., Hokkaido Univ.)

Cecropin P1 (CP1) is 31 amino acids peptide and exhibits antimicrobial activity against gram-negative bacteria. In this study, we investigated the interaction with LPS and CP1₁₋₂₉ because AMPs that target Gram-negative bacteria bind to the negatively charged LPS. We have applied tr-NOESY to determine the high-resolution structure of CP1₁₋₂₉ in LPS. NOE peaks were observed at the C-terminal region in LPS, but, were not detected in aqueous solution. CD measurement was also carried out using CP1 and analogues of CP1 (CP1₁₋₂₉ and CP1₁₋₂₀, the lacks of C-terminal amino acids). In LPS, α -helical content of CP1₁₋₂₀ is lower than CP1 WT or CP1₁₋₂₉'s one. We suggest that CP1₁₋₂₉ accepts α -helical structure with LPS at C-terminal region.

3P012 高圧力下で見られるべん毛繊維の動的多型性 Dynamic polymorphism of bacterial flagellar filaments at high pressure

Masayoshi Nishiyama¹, Yoshiyuki Sowa² (¹Kyoto University, ²Hosei University)

Escherichia coli cells smoothly swim in solution by rotating their flagellar filaments, each of which is composed of a single protein, flagellin. In response to mechanical torque and solvent conditions, the supercoiled structure is dynamically changed from a left-handed form (normal) to the others including right-handed forms, and vice versa. Here, we show that application of pressure can change the supercoiled structure of flagellar filaments from normal to coiled or curly I form. The application of pressure is thought to enhance the structural fluctuation and/or association of water molecules with the exposed regions of flagellin molecules, and results in switching the helical shape from normal to coiled or curly I form.