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Structures and functions of macromolecular assemblies by electron cryomicroscopy – the potential for biological sciences –

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Date: April 24th (WED), 2013 from 17:30

* Place has been changed.
Please be careful.

Place: Conference Room, Institute of Molecular Embryology BLDG 1F.

★発生医学研究所１階、カンファレンス室

High resolution structures of macromolecular assemblies and cellular architectures are essential for understanding the mechanisms of biological functions because they are all determined by the structures and dynamics of the molecules and cells at atomic level. Although X-ray crystallography and NMR are powerful tools for structural analysis of macromolecules, the difficulty in crystallization and the size limit of target molecules lay severe limitations on the former and the latter, respectively. Filamentous protein complexes are extremely difficult target because polymerization/depolymerization is dynamic and the length is not uniform. Electron cryomicroscopy (cryoEM) and image analysis is a powerful tool because there is no size limitation and only a very small amount of sample solution is required for data collection. The achievable resolution, however, had been limited by low contrast and signal to noise ratio (S/N) of cryoEM images due to low electron dose to avoid radiation damage. Therefore, tens of thousands of particle images have to be collected, classified, aligned and averaged for high-resolution 3D imaging. We have developed techniques to improve the S/N and contrast and to increase the speed of image collection by the use of an energy filter and CCD camera on an electron cryomicroscope with a field emission electron gun and gained nearly 5 times higher S/N and contrast by controlling ice thickness and also by raising the specimen temperature from 4 K to around 50 K. The undesirable charging is now gone, making almost all the collected images be used for analysis, while only a few % at 4 K (1). Image analysis with helical symmetry now allows the high-resolution structural analysis of filamentous protein assemblies to be completed within a week at resolution beyond 4 Å. I will discuss the potential of the method for future life sciences (2,3).

1. Fujii, T., Kato, T. & Namba, K. (2009) Specific arrangement of a-helical coiled coils in the core domain of the bacterial flagellar hook for the universal joint function. *Structure* **17**, 1485-1493.
2. Fujii, T., Iwane, A. H., Yanagida, T. & Namba, K. (2010) Direct visualization of secondary structures of F-actin by electron cryomicroscopy. *Nature* **467**, 724-729.
3. Gayathri, P., Fujii, T., Møller-Jensen, J., van den Ent, F., Namba, K., Löwe, J. A. (2012) A bipolar spindle of antiparallel ParM filaments drives bacterial plasmid segregation. *Science* **338**, 1334-1337.

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