bearing nitrilotriacetic acid substituents at the end of a poly(ethylene glycol)-grafted surface that promotes specific capture of protein targets for single particle reconstruction analysis. The utilization of these grids for specific adsorption of the targeted protein onto the grid surface results in well-controlled surface concentration enhancements and a days-to-minutes reduction in time required for the preparation of a purified sample for cryoEM analysis from an E. coli expression system. The selective and reversible capture of his-tag T7 bacteriophage and GroEL from crude lysates, as well as purified nanodisc-solubilized his-malFGK2, on these NTA-modified grids with an exceptionally low level of adsorption by non-target proteins has been observed. Our data illustrates the utility of these grids for selective capture from complex mixtures, detergent-solubilized membrane protein isolates, and expression systems yielding low copy numbers of the desired target in a manner that is well-suited for single particle reconstruction analysis.

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Scanning Transmission Electron Tomography of Blood Platelets in Thick Sections

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Electron tomography in the scanning transmission electron microscope (STEM) can be performed on sections of stained plastic-embedded tissues or cells of 1 to 2 micrometer thickness without effects of chromatic aberration because there are no imaging lenses after the specimen. By using a small STEM probe convergence angle of 1-2 mrad the geometrical broadening of the probe is restricted, which enables a spatial resolution of a few nanometers. Furthermore, by using an axial bright-field detector instead of the standard high-angle annular dark-field detector, image blurring due to multiple elastic scattering can be reduced in the lower part of the specimen. Here, we have applied STEM tomography to elucidate the 3D ultrastructure of human blood platelets, which are small anucleate blood cells that aggregate to seal leaks at sites of vascular injury and are important in the pathology of atherosclerosis and other diseases. Of particular interest are the morphological changes that occur in alpha-granules, which contain important proteins released when platelets are activated. Axial bright-field STEM electron tomographic tilt series were acquired at an accelerating voltage of 300 kV from 1.5-micrometer thick sections of platelets that had been prepared by rapid freezing and freeze-substitution; and the tomograms were reconstructed from dual-axis tilt series. The tomographic reconstructions revealed changes in ultrastructure that occurred on platelet activation including release of alpha granules through channels connecting to the plasma membrane.
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Three-Dimensional Microstructural Visualization of Mitosis using Focused Ion Beam-Scanning Electron Microscope (FIB-SEM) and 3Mv Ultra-High Voltage Electron Microscope (UHVEM) Tomography with Nanoscale Resolution at Whole Cell Level

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To better understand fundamental cellular properties, such as differentiation and division, we are developing whole single cell 3D-structure analysis technologies based on innovative electron microscopy. These new techniques are designed to reveal the dynamics and structure of intracellular material such as organelles and supramolecular proteins. Our main technologies include (1) Cryo-Tomography using Scanning-TEM and (2) FIB (Focused Ion Beam)-SEM and 3D-reconstruction. FIB-SEM is normally used to visualize metals and ceramics. We have modified it for the 3D reconstruction of an entire cell at a nanoscale resolution that lies between those of electron microscopy tomography and X-ray tomography.
Last annual meeting, we described how FIB-SEM could visualize the basic 3D architecture of *Cyanidioschyzon merolae* (*C. marolae*). *C. marolae* is a primitive unicellular red algae whose cell division can be observed my manipulating the light/dark cycle. By synchronizing cells to a 6-h light/18-h dark cycle, we obtained $>$ 75% S/M-phase cells at 89 hrs after synchronous culture start. Using these cells and FIB-SEM, we observed unique architectures of whole *C. merolae* cells during the mitotic cycle and successfully made 3D-models of individual double-membrane organelles such as the nucleus, chloroplast and mitochondria, and of single-membrane organelles such as the ER, lysosome and peroxisome using ImageJ and Amira 3D software. Using UHVEM tomography, we also observed the 3D-structure of phycobilisomes, which are essential supramolecular complexes on the surface of the thylakoid membrane in chloroplasts. Although many reports have provided structural models, we offer the first 3D-structural model of the membrane surface from specimens that were not purified using specific detergents.

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Regulation of Myosin VI Studied by Electron Microscopy

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Myosins are ATPase motor proteins that are activated by and traffic along actin filaments. This large protein family is divided into many classes with different functional properties and specializations for various roles, including membrane anchorage, longer range transport of cargo vesicles or cell signaling.
Myosin class VI is unique due to its reversed directionality along actin filaments, moving towards the pointed end, in contrast to almost all other classes, which move towards the barbed end of F-actin. Whilst the directionality is well studied, other characteristics such as activation, cargo and lipid binding or dimerization are not fully understood. Using size exclusion chromatography, titration studies and gliding filament assays we investigated myosin VI back-folding, cargo binding and mechanical activity.
Furthermore, we applied electron microscopy and single particle image processing to determine the structural properties of myosin VI in different ionic and nucleotide conditions. Two dimensional class averages based on various alignment and classification methods were made that allow for a detailed structural analysis including a comparison with crystal structures.

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The Steric Fine Structure of Maurer's Cleft in "Unroofed" Plasmodium Falciparum-Infected Erythrocytes

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Plasmodium falciparum produces additional membrane systems, Maurer's cleft (MC) and tubulovesicular network (TVN), in the host erythrocytes. The parasites use these membrane compartments to transport proteins to the surface of erythrocytes. Previous studies reported the structure of MCs by transmission electron microscopy (TEM) using ultra-thin layer specimen and suggested physical connections between MCs and erythrocyte membrane via an extension of MC membrane. However, fine structures of MC including filamentous extensions smaller than the thickness of diamond knives were likely missing in the TEM images. To obtain intact structural information of MCs, we used unroofing/rip-off technique for both normal- and parasitized-erythrocytes and successfully captured accurate oval/global shape of MCs with elongated-fine filamentous extensions (diameters $<$10 nm). We also treated parasitized erythrocyte with aluminum tetrafluoride, which are known to inhibit intracellular vesicle transport, to clarify if the oval/global structures are MCs. In the presence of aluminum tetrafluoride, the vesicle was no longer observed in parasitized erythrocytes. This result was in agreement with the previous study (Trelka DP, et al., Mol Biochem Parasitol, 2000), demonstrating the oval/global structures are MCs which extends filaments to host erythrocyte membrane. Our EM images demonstrated that MCs in P. falciparum-infected erythrocyte involve fine filaments reaching erythrocyte membrane which may provide a direct transport pathway for their proteins to the surface of erythrocytes.

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Towards Femtosecond Electron Diffraction of Proteins - Technical Challenges and Sample Preparation Strategies

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To study protein dynamics in real-time with atomic resolution is one of the dream experiments in biophysics. Up to now experimental tools with full