Electron Microscopy, Diffraction, and Scattering Techniques

3113-Pos Board B543
Cryo-Electron Tomography and Sub-Tomogram Averaging of Isolated Z-Discs from Honeybee Flight Muscle
Mara Rusu1, Dianne Taylor2, Kenneth Taylor2, John Trinick2
1School of Molecular and Cellular Biology, Leeds University, Leeds, United Kingdom, 2Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, USA.

The Z-disc is the mechanical linkage that transmits the active and passive forces developed by muscle sarcomeres. It is also the location of proteins involved in diverse other processes, such as stress sensing into signaling pathways controlling muscle growth and wasting. About 40 different proteins are now known to be in the Z-disc, some to which are transient and relocate to other destinations such as the nucleus. The main route of force transmission is through pairs of antiparallel α-actinin Z-bridges; these link the overlapped ends of thin filaments from adjacent sarcomeres. The gross structure of the Z-disc varies widely between different muscles and species. Thickness varies in proportion to the number of registers of Z-bridges. The lattice of thin filament ends is hexagonal in invertebrates, whereas in vertebrates it is tetragonal. However the structure of the Z-disc is known only in outline to ~7nm resolution and the detailed layout of its components is mostly unknown. Methods to isolate Z-discs date back 50 years but such preparations have not been subjected to modern electron microscopy methods, such as cryo-EM, tomography or image processing. An advantage of isolated discs is they are thin, which obviates the need for sectioning for microscopy, which is damaging. We have prepared Z-discs from honeybee flight muscle using high salt extraction and density gradient purification. Vitrified preparations were examined in the 300 kV Krios microscope at the MRC Laboratory of Molecular Biology, Cambridge, UK. Tilt series images were recorded to +/- 70° with a Falcon II direct electron detector. 3D reconstruction and sub-tomogram averaging used Protozo software.

3114-Pos Board B544
Label-Free Molecular Observations of Membrane-Associated Species using Backscattering Interferometry
Michael M. Baksh1, Ashley Lockwood1, Christopher Richards2, M.G. Finn1, David Heidary3
1Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA, 2Chemistry, University of Kentucky, Lexington, KY, USA, 3Chemistry, University of Kentucky, Lexington, KY, USA.

Membrane-associated proteins are integral components of cellular processes and disease pathogenesis. Quantitative observations of membrane protein interactions are extremely difficult; the membrane environment that is necessary to maintain appropriate structural and functional characteristics of such species interferes with or perturbs many analytical methods. In fact, typical assays to observe such interactions require the species of interest to be isolated and removed from the native membrane environment, usually with covalent modification. We will describe a label-free method to observe and evaluate membrane protein-ligand interactions in minimally-altered native membrane environments. This strategy is based on the use of backscattering interferometry (BSI) in which minute changes in the refractive index of the bulk solution caused by cognate ligand-receptor interactions are observed and quantified without the need for extrinsic molecular labeling. Combined with a method to present membrane proteins in an isotopi­scally-scattering matrix derived from the native cellular environment, Ashlwe are able to observe a variety of cognate ligand-receptor interactions over a large range of equilibrium binding affinities.
bearing nitrotriacetic acid substrits 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-targeted 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.

3116-Pos Board B546
Scanning Transmission Electron Tomography of Blood Platelets in Thick Sections
Jake D. Hoyne1, Gina N. Calco1, Bryan C. Kuo2, Maria A. Aronova1, Alioska A. Sousa1, Qiaping He1, Guofeng Zhang1, Irina D. Pokrovskaya1, Laura MacDonald1, Andrew A. Prince1, Brian Storrie2, Richard D. Leapman1.
1NIBIB, National Institutes of Health, Bethesda, MD, USA, 2Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.
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. The research was supported by the intramural program of the National Institute of Biomedical Imaging and Bioengineering, and the research in the Storrie lab was supported in part by NIH grant R01 HL119393.

3117-Pos Board B547
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
Atsuko H. Iwane1,2, Keisuke Ohta1,2,3
1Osaka University, Suita, Japan, 2QBiC, RIKEN, Suita, Japan, 3Kurume University, Kurume, Japan.
To better understand fundamental cellular properties, such as differentiation and division, we are developing whole 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 techniques 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. merolae). C. merolae is a primitive unicellular red algae whose cell division can be observed by 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.

3118-Pos Board B548
Regulation of Myosin VI Studied by Electron Microscopy
Dario Saczko-Brack1, Heike Ellrich1, Christine Werner1, Christopher Batters2, Claudia Veigel1.
1Department of Cellular Physiology, Ludwig-Maximilians-Universität München, Munich, Germany, 2Max-Planck-Institute for Structure and Dynamics of Matter, Hamburg, Germany.
Myosins are ATPase motors 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.

3119-Pos Board B549
The Steric Fine Structure of Maurer’s Cleft in “Unroofed” Plasmodium Falciparum-Infected Erythrocytes
Eri H. Hayakawa1, Fuyuki Tomakusai,2 Jiro Usukura1, Hiroyuki Matsuoka1, Takafumi Tsuboi1,3, Thomas E. Wellems2.
1Lab of Medical Zoology and Parasitology, Department of Infection and Immunity, Fuku Medical University, Shimotsuke, Japan, 2Department of Lipidomics, Grad school of Medicine, The University of Tokyo, Tokyo, Japan, 3Division of Integrated Project, EcoTopia Science Institute, Nagoya University, Nagoya, Japan, 2Malaria Research Unit, Cell-Free Science and Technology Research Center, Ehime University, Matsuyama, Japan, 3Lab of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases/NIH, Bethesda, MD, USA.
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.

3120-Pos Board B550
Towards Femtosecond Electron Diffraction of Proteins - Technical Challenges and Sample Preparation Strategies
Henrike M. Müller-Vermehrke1,2, Daniel Badali1, Oliver P. Ernst1, R.J. Dwayne Miller1,3.
1Chemistry, University of Toronto, Toronto, ON, Canada, 2Biochemistry, University of Toronto, Toronto, ON, Canada, 3Max-Planck-Institute for Structure and Dynamics of Matter, Hamburg, Germany.
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