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Cryo EM Study of Protein Complexes Bound to Streptavidin Crystal Layers
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Conventional cryo-EM specimens use either continuous or holey, glow-discharge treated carbon films to prepare samples. As a result, particles are either immobilized by binding to an unnatural (i.e. carbon-film) surface, or they collide multiple times (due to rapid diffusion) with the freshly blotted air-water interface. In either case there is a risk that considerable structural heterogeneity may occur, or even that particles may undergo severe denaturation. Binding biotinylated particles to streptavidin monolayer crystals is a more structure-friendly way to immobilize particles. We have developed a protocol for preparing biotinylated protein complexes and tethering them to the surface of streptavidin monolayer-crystals. Three different multi-protein complexes have been used to demonstrate the generality of this method. The biotinylated protein complexes bind to the streptavidin crystal layer with high affinity, whereas the unbiotinylated control samples do not do so. In addition, the same technique can be used to produce a uniform distribution of Euler angles. The thermosomes, known to adopt a preferred orientation with other methods of cryo-EM specimen preparation, have been used as a test sample. Indeed, the results confirm that a uniform distribution of Euler angles is obtained for the biotinylated thermosome. We thus conclude that biotinylation, together with binding to 2-D crystals of streptavidin, is a better method to prepare protein complexes for cryo-EM study.

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Three Dimensional Imaging of Maurer's Clefts in "Unroofed" *Plasmodium Falciparum*-Infected Erythrocytes by Transmission Electron Microscopy
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Plasmodium falciparum human malaria parasites establish unique membrane systems in the cytoplasm of their host erythrocytes. These systems include Maurer's Clefts (MCs), which are evident as small membranous structures in host erythrocyte periphery and are thought to support the trafficking of *P. falciparum* proteins to the host cell surface. Three-dimensional structures of MCs and their tethering to the host erythrocyte membrane have been reported from reconstructions of transmission electron microscopy (TEM) and fluorescence images (Hanssen *et al.* Mol. Microbiol. 2008 Feb;67(4): 703–18.). To examine the structure of MCs and their proximal interactions in host erythrocytes, we have used an unroofing/rip-off method to expose the intracellular contents of parasitized erythrocytes and collect structural information by TEM. MCs in the unroofed TEM images range in dimension from 200 nm to 700 nm and have easily-resolved tethers that are 170 nm to 450 nm in length. These tethers are present in networks that connect the MCs to the skeleton of the host erythrocyte membrane.

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Measuring Membrane Potentials with Cryo-EM?
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In materials science it is well known that electrostatic potentials produce phase-shifts of electron waves which are detectable in phase-contrast electron microscopy. The question arises, are membrane potentials preserved during rapid freezing and cryo-EM imaging of liposomes? Imaged with 200 keV electrons, a 200 mV membrane potential in a 30 nm liposome is expected to produce a phase shift of about 50 mrad, resulting in approx. 10% change in image intensity. (The maximum phase shift due to the liposome membrane alone is about 200 mrad.) Calculations showed that phase-plate imaging would be necessary to detect the signal. We used a JEOL JEM2200FS microscope equipped with a Zernike phase plate having a cut-on frequency of approximately 1/100 nm. Liposomes with or without added valinomycin were adhered to a carbon film and then washed a buffer to create a 350:1 K+ gradient, either inwardly or outwardly directed. In liposomes in the 20-30 nm size range we observed a contrast difference relative to the no-valinomycin controls as expected from positive and negative membrane potentials ~100 mV in size. Thus the diffusion potential set up by a K+ gradient appears to remain throughout the freezing and imaging process.

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Molecular Architecture of OPA1, the Dynamin-Related GTPase Involved in Mitochondrial Fusion
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Mitochondria are dynamic organelles that constantly undergo fusion and fission events. Four dynamin-related GTPases have been shown to facilitate mitochondrial dynamics. One of them, OPA1, mediates mitochondrial inner membrane fusion. Mutations in OPA1 have been linked to severe disease phenotypes characterized by progressive degeneration of the retinal ganglion cells, thus leading to blindness. OPA1 is a nuclear encoded protein that is inserted into the inner mitochondrial membrane through an N terminal transmembrane domain. There it undergoes proteolytic processing yielding a long, membrane-bound (OPA1) and a short, diffusible form (OPA1s). Fusion of the inner mitochondrial membrane requires both forms of OPA1, and the ability of OPA1 to bind and hydrolyze GTP. In previous structural studies, we have shown that recombinant OPA1s binds to lipid preparations with a composition similar to that of the inner mitochondrial membrane (Ban et al., 2010). Our findings indicated that the interaction of OPA1s with suitable membranes stimulates oligomerization and self-assembly. Using cryo-electron microscopy, we have visualized and analyzed the topological arrangement of OPA1 on these tubules and showed that the protein wraps around the perimeter of these tubes in a helical fashion. Interestingly, the protein formed a two-dimensional lattice on the lipid bilayer indicating that specific intermolecular interactions are at play. We are now investigating the molecular architecture of such OPA1-lipid assemblies using biochemical and high-resolution electron microscopy (EM) methods. Here, we report on strategies employed to generate substrates suitable for EM analysis and on our current insights into the properties and architecture of OPA1 assemblies.
Reference:
Ban, T., Heymann, J. A., Song, Z., Hinshaw, J. E., Chan, D. C.: OPA1 disease alleles causing dominant optic atrophy have defects in cardiolipin-stimulated GTP hydrolysis and membrane tubulation. Hum. Mol. Genet. 2010, 19: 2113-22.

Molecular Dynamics I

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Development of a Force Field Topology Database for Detergents for Molecular Dynamics Simulations with the Amber Force Fields
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Membrane proteins (MPs) are amphiphilic in nature. As such, to be manipulated and studied in aqueous solution and to remain biologically active, MPs need to be transferred in a suitable membrane-mimicking environment such as detergent solutions. Unfortunately, there is no systematic way of identifying the ideal detergent for any given MP, as empirical criterions are often used in this undertaking. Thus, a better knowledge of the interactions between MPs and detergents at the molecular level could improve this situation. Following this approach, our work has focused on the molecular dynamics (MD) simulations of MPs in detergent solutions. In contrast to others FFs (such as CHARMM or GROMOS), AMBER does not provide specific parameter/FF library set for detergents. Consequently, studying MPs by MD with the AMBER FF is not straightforward. In this context, we have developed using R.E.D. Server a complex FF Topology DataBase (FFTopDB) (i.e. RESP partial charges embedded in a large set of FF libraries) for an ensemble of ionic and non-ionic detergents compatible with AMBER FF (available at http://q4md-forcefieldtools.org/). Key points of the procedure are the definition of elementary "building blocks" with well-identified conformations as well as the derivation of reproducible RESP charge values. The FFTopDB contains more than 75 molecular fragments, which can be used to construct ~70 different detergents. The approach has been validated by performing MD simulations on dodecylphosphocholine (DPC) micelles with the AMBER99SB parameters and compared to results obtained from CHARMM36 and GROMOS53A6 simulations. We find that this combination of parameters reproduces accurately the micelles structural and