reconstruction quality, CS reconstructions were compared with weighted back-projections of two classes of phantom: (1) derived from experimental data, and (2) derived from a generative model that approximates the structure of membrane-bound organelles. We are investigating the application of the CS approach for decreasing acquisition times and electron dose, and for improving the reconstruction quality of electron tomograms recorded from micrometer-thick specimens of neuronal tissues in the scanning transmission electron microscope.

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3025-Pos Board B717

Helical Organization of Coagulation Factor VIII on Lipid Nanotubes

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Human factor VIII (FVIII) is a multidomain-plasma glycoprotein critical for blood coagulation which when activated (FVIIIa) functions as a co-factor to the serine protease factor IXa (FIXa) within the membrane-bound tenase complex. We have developed single bilayer lipid nanotubes (LNT) resembling by dimensions and lipid composition the activated platelet pseudopodia holding the tenase complex *in vivo*. We have successfully organized helically recombinant FVIII on LNT and calculate the membrane-bound structure at resolution sufficient to resolve its membrane-bund domain organization.

In this work, we present the macromolecular organization of human FVIII helically organized on LNT, as resolved by Cryo-electron microscopy at intermediate resolution (10 - 15 Å) which we compare to the porcine FVIII membrane-bound structure from helical assemblies on LNT at the same experimental conditions. We compare these structures to the FVIII structures when organized in 2D and 3D crystals and when attached to lipid nanodisks (ND), to define the conformational space and flexibility of this important for blood coagulation macromolecule.

Our goal is to identify the FVIII protein-protein and protein-lipid interfaces critical for the tenase complex assembly and function.

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Answering Real Biological Questions by Combining Cryo-TEM, XRD and NMR

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A new frontier exists in unraveling interactive biological and biochemical processes and pathways at the macromolecular level. Of critical importance is the three-dimensional visualization of protein complexes and molecular machines in their native functional state. Three techniques play a major role in orchestrating this.

Nuclear magnetic resonance (NMR) has the capability to study specific protein domains or fragments and their role in protein folding and dynamics and in ligand binding. X-Ray crystallography (XRD) provides high-resolution but more static 3D structures of apo and liganded proteins, mainly in a monomeric or dimeric state after crystallization. To unravel more physiologically relevant situations, it is essential to visualize multimeric complexes in their tertiary and quaternary state and their interaction with other complexes. By performing cryo-transmission electron microscopy (cryo-TEM) applications like single particle analysis or tomography, this can be achieved. Cryo-TEM provides complementary information to NMR and XRD that can be crucial for understanding the structure of protein complexes that when combined can help answer real biological as well as medically relevant questions.

Recent developments in cryo-TEM have brought the three major structural biology technologies closer together. When combined with molecular dynamics simulations, a continuum has been reached on all important aspects with regards to resolution and macromolecular scales which allows for full deployment of the combination of these technologies.

Here, we will illustrate the historical context of these technologies with respect to one another and show how recent developments have reached the critical requirements needed to fully unleash the power of structural biology for not just answering fundamental questions, but actually contributing to curing diseases and improving health. We will discuss the future of structural biology based on results from recent publications and expected developments over the next several years.

3027-Pos Board B719

Zernike Phase-Contrast Electron Tomography of Microtubule-Related Complexes in Axonemes

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In cryo-electron microscopy of vitreously frozen biological specimens, phase-plate imaging can greatly increase the signal-to-noise ratio of the micrographs. When routinely used, this imaging technique may be the method of choice for cryo-electron tomography, in which each image in the tilt-series data set suffers from a high noise background due to the low-electron-dose exposure. Utilizing the Zernike phase-contrast imaging technique, we have carried out an electron tomographic study of the axonemal microtubule doublets and their associated radial spokes. The reconstructed tomogram displays a strong contrast. The quality of the resultant density maps, obtained by sub-volume averaging of a single tomogram in this study, is comparable with that from the averaging of many cryo-tomograms without using the phase-plate.

3028-Pos Board B720

Flexible Fabs in the Refinement of Complexes by Single-Particle Transmission Electron Microscopy

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An emerging strategy for solving medium-resolution (5-10 Å) structures entails complexing the protein of interest with monoclonal antibodies and solving the complex by single particle transmission electron microscopy (TEM.) The addition of Fabs to target molecules is an attractive strategy because it increases the mass of the complex (improving its resolvability by single-particle TEM), adds a fiducial marker that aids the interpretation of the resulting volume maps, and potentially elucidates an immunological site of interest. We note a possible limitation to this approach owing to the localization of the Fabs in the resulting complex. Alignment parameters that optimize the Fab constant domains' convergence may come at the expense of the visualization of the core particle of interest. We evaluate the implications of this conjecture with respect to both experimental and synthetic data.

3029-Pos Board B721

Resolving the Structural Basis of Factor VIII Activation

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Activated Factor VIII (FVIIIa) is the essential co-factor for the serine protease Factor IXa (FIXa) in the membrane-bound Tenase complex. In the blood coagulation process, the interaction between FVIIIa and FIXa in presence of Ca^{2+} and phospholipids amplifies the proteolytic activity of Factor IXa more than 100.000 times. The Tenase complex catalyzes the activation of Factor X and initiates a cascade of multi-protein complex formation which amplifies the Thrombin production and secures the formation of a blood clot.

Here we report for the first time the 3D-structure of the membrane-bound FVIIIa form, which is the most physiologically relevant in relation to the delicate equilibrium of hemostasis and thrombosis.

The structure of the membrane bound light chain (domains A3-C1-C2) of human FVIII helically organized onto lipid bilayer nanotubes (LNT) clarified that the domains adopt a different organization than in the crystal structure which represents the molecule in solution. These differences of the membrane-bound FVIII structure have to be confirmed for the full length FVIII protein and the active form. To achieve this, high resolution Cryo-EM data of porcine FVIII and FVIIIa helically organized on LNT at closest to physiological conditions were collected. A newly developed process of iterated rounds of 2D refinement was applied to select only highly organized homogenous helical segments for an effective iterative helical real-space reconstruction (IHRSR). The calculated porcine FVIII and FVIIIa membrane-bound structures at sub-nanometer resolution were combined with flexible docking to define the changes in the FVIIIa interfaces important for its function.

Understanding the structural differences and similarities between the membrane-bound activated FVIII and the non-activated FVIII will make an indispensable contribution to the field of coagulation in determining unique targets for the design of specific therapeutic drugs against Hemophilia A and thrombosis.