up to 50 nm. This capability has enabled several important studies on biological samples. Backscattered electron imaging has the ability to create morphological map from single cell to tissue scale. Combining the two techniques, we are able to obtain structural and isotopic information from the same sample, which can be useful to study complex biological problems at both single cell and tissue scale. (1) Single Cells: glutamine metabolism has been studied by correlative analysis, which makes it possible to measure the uptake of isotopically labelled molecules in specific organs in single cells (Fig 1a-1b). (2) Tissues: various mouse tissue samples have been imaged, which enables to trace 13C-labelled lipids in any structures throughout the tissues (Fig 1c-1e).

3020-Pos Board B712
A Time-Resolved CRYO-EM Study of Ribosome Subunit Association by Mixing-Spraying
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The emergence of a high-throughput data processing pipeline and efficient classification algorithms has revived the idea of time-resolved cryogenic electron microscopy (cryo-EM), i.e., capturing time-dependent structures in a biological specimen using cryo-EM. The blotting method now routinely used to prepare cryo-EM specimens does not allow to study a reaction in the second to minute range. Capturing faster reactions, in the sub-second range, has been a practical challenge, due to the required step of depositing the specimen on the grid. To address this challenge, Lu et al. (2009) have developed a novel method to prepare time-resolved cryo-EM specimens, by using a nano-fabricated mixing-spraying chip. The mixing-spraying chip allows a two-component reaction to proceed inside the chip for tens to hundreds of milliseconds (ms), which is then stopped by fast freezing. Here we used the improved mixing-spraying method to study ribosome subunit association, and particularly to capture the conformational changes of the ribosome in this reaction. Ribosome subunit association is a pivotal step in translation initiation. Previous ensemble kinetic studies suggested that the ribosome subunit association is a multi-step process, with the ribosomal inter-subunit bridges sequentially formed. Using time-resolved cryo-EM, we were able to capture the association reaction in a pre-equilibrium state by mixing the two ribosomal subunits and reacting for 140ms. However, the fraction population of the associated ribosomes in each conformation was stable from 140ms to 75min, indicating that the ribosome undergoes conformational changes faster than the 140ms time frame upon association of the subunits. Thus, we have demonstrated that the mixing-spraying method of time-resolved cryo-EM is able to visualize the states of macromolecules in a reaction within a sub-second time frame.

3021-Pos Board B713
Visualizing Biological Samples in Liquid Solution by Electron Microscopy
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Liquid water is essential to life on earth. The cell, the basic component of all living organisms, functions through the action of proteins in a liquid environment. Consequently, the ability to directly visualize protein structure at nanometer-resolution in a liquid environment would dramatically advance understanding of protein functions in living organisms, which can be useful to study complex biological problems at both single cell and tissue scale.

3022-Pos Board B714
Cryo-EM Studies of DRP1 Self-Assembly Provide Insights into the Mechanism of Mitochondrial Fission
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Dynamin-related protein 1 (Drp1) belongs to a family of large GTpase proteins that regulate membrane dynamics and morphology. Self-assembly of cytosolic Drp1 into larger oligomers on the surface of mitochondria is essential for enabling membrane fission. Preliminary studies show that in vitro reconstitutions of Drp1 emulate key features of the mitochondrial division machinery and provide a model system for evaluating conformational changes that drive membrane remodeling. To understand Drp1 self-assembly, cryo-electron microscopy (cryo-EM) will be used to determine the 3D structures of both the pre-assembly state and helical oligomers. More specifically, recombiant Drp1 forms stable tetramers in solution that are amenable to EM analysis. In the presence of non-hydrolyzable GTP analogs and/or synthetic liposomes, Drp1 further assembles into helical oligomers with varying diameters, suggesting ligand-induced conformational changes. Cryo-EM studies will determine the 3D structures of reconstituted Drp1 oligomers to reveal key intermolecular interactions and conformational changes that drive Drp1 self-assembly and mediate mitochondrial membrane fission.

3023-Pos Board B715
Cryo-Optical Imaging of Vitrified Bacterial and Human Cells by Scanning Transmission Electron Microscopy
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Cryo-transmission electron microscopy (CET) has emerged as a vital tool for structural biology studies of cells and viruses. Direct imaging of fully hydrated, vitrified material represents the state of the art for preservation of biological samples. Lacking heavy metal stains, CET relies on phase contrast typically obtained by defocusing the sample. The dependence on phase coherence, as well as cumulative radiation damage on frozen hydrated specimens, impose an inherent upper limit on sample thickness and usable tilt range. Even with energy filtration to remove the contribution of inelastic scattering, CET suffers from "missing wedge" effects and low signal-to-noise ratio. Scanning transmission electron microscopy (STEM) circumvents the need for phase contrast with the independent STEM detection in bright and dark field detectors provides sufficient contrast to show detailed cellular architecture similar to that provided by wide-field tomography. The important difference is that the sample remains dynamic with focus even at very high tilts up to 70°. This significantly improves the depth resolution in reconstructions. Sample thickness limitations are also relaxed. We demonstrate the cryo-STEM tomography (CSTET) method using unstained, vitrified bacteria and human epithelial cells.
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-bound 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 helically organized on LNT, as resolved by Cryo-electron microscopy at intermediate conditions were collected. A newly developed process of iterated rounds of 2D segmentation 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 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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3026-Pos Board B718
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 also 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 vitrified 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-serial 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 approach 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 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 (FVIII) 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 Ca2+ 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 on 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.