Tailoring In Situ Electron Microscopy Studies

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In situ transmission electron microscopy (TEM) in combination with high-resolution imaging, diffraction, and spectroscopy enables real-time observation of dynamic processes at the atomic scale and the correlation between site-specific atomic structure and properties. The in situ techniques include the application of external stimuli such as temperature, electric field, light, magnetic field, mechanical strain, temperature and reactive environments. Traditionally, these experiments rely on specialized TEM holders, making the integration of multiple excitations challenging. Advancements in micro-electromechanical systems (MEMS) have enabled the development of in situ TEM holders and chips giving access to experiments in both solid and liquid environments. We are tailoring the chip design for specific in situ studies to enable novel excitations with several experiments on one chip allowing better statistics and check of reproducibility, expanding the scope of in situ experiments. We also explore the use of 2-dimensional materials to design the properties of the TEM membranes supporting and enclosing the specimen. New ideas and concepts for is situ studies are explored using a TEM holder with a scanning tunneling microscope that enables piezo driven manipulation. By leveraging nanofabrication techniques, we develop MEMS chips that allow innovative, multi-modal in situ studies while allowing scalable wafer-level fabrication.

Structure Determination of Amyloid Fibrils by Cryo-EM

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A variety of mouse models are used in Alzheimer's disease (AD) research. However, little is known about the structural differences in aggregated Aβ between mouse models and humans or in vitro structures. These differences might help to understand why fibril-targeting drug candidates show efficacy when tested in mouse models but often fail to show the desired effect in clinical trials. We determined the structures of nine ex vivo Aβ fibrils from six different mouse models by cryogenic-electron microscopy (cryo-EM). We found that these fibril structures are different from the most dominant structures found in AD patients. A detailed assessment of the Aβ fibril structure is therefore important to the selection of appropriate mouse models for the preclinical development of novel plaque-targeting therapeutics and positron emission tomography imaging tracers in AD.

In addition to protein deposits, such as plaques in AD and Lewy bodies in α-synucleinopathies like Parkinson’s disease (PD), a high concentration of lipids is also found, suggesting a potential role for lipids in disease pathology. We present cryo-EM studies of Aβ40 and α-synuclein fibrils formed in the presence of liposomes. These structures provide detailed insights into fibril-lipid interactions and show that the fibrils can take up substantial amounts of lipids during formation, which results in lipid-decorated fibrils. This supports the notion that lipid extraction from cell membranes may be a mechanism contributing to fibril toxicity. Understanding these fibril-lipid interactions offers structural insights into disease-relevant processes in AD and PD.

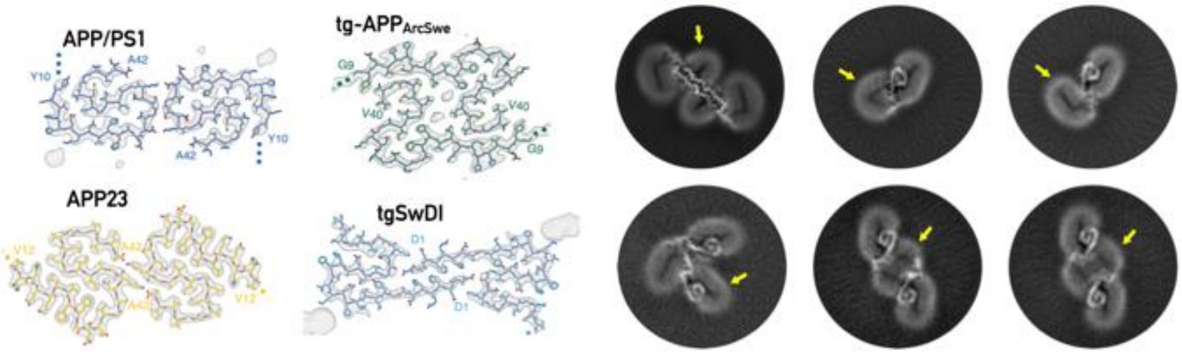

Fig. 1: Amyloid fibril structures of different Alzheimer mouse models (left) and amyloid fibrils decorated with lipids (right).

References:

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[2] B. Frieg*, M. Han*, et al. Nat Commun **15**(1):1297 (2024)
[3] M. Zielinski*, F.S. Peralta Reyes* et al. Nat Neurosci **26**:2073–2080 (2023)

WHAT IS THE BEST TEMPERATURE FOR ELECTRON CRYOMICROSCOPY OF BIOLOGICAL SPECIMENS?

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A decade on from the “resolution revolution” [1], electron cryomicroscopy (cryoEM) has become the dominant technique for determining the structure of biological macromolecular complexes. Electron diffraction measurements of protein crystals at liquid helium temperatures show reduced damage when compared to liquid nitrogen [2]. In cryoEM, there have been numerous attempts to reduce the effects of radiation damage by cooling the specimen beyond liquid-nitrogen temperatures, yet all failed to realize the potential improvement for single-particle structure determination. We recently found that this was due to the complex interaction of the electron beam with the specimen, including the reduced movement of radiolytic molecular fragments at low temperatures. Using this knowledge, we were able to eliminate the previous problems with imaging at liquid-helium temperatures and obtain structures where every micrograph was better than the equivalent using liquid-nitrogen cooling [3]. With this in mind, we have now set out to understand what the optimal temperature for structure determination might be, and in effect, how low is low enough. This will then enable a new generation of electron cryomicroscopes to be constructed that minimize the effects of radiation damage and allow smaller and more difficult structures to be determined.

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CONTRAST IN CRYO-TOMOGRAPHY BY 3.5 & 4D-STEM

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STEM cryo-tomography extends the range of *in situ* volume imaging from a few hundred nanometer to a micron or more. For such thick specimens, the weak phase object approximation is no longer satisfactory; contrast emerges from both coherent and incoherent scattering. Traditionally, symmetric disk or annular detectors were used for incoherent bright or dark field contrast. Phase contrast by STEM relies instead on off-axis detectors, typically either azimuthal segments (quadrant detectors) or pixelated cameras. Defocus in the illumination generates an image shift in real space, equivalent to parallax.

Using quadrant detectors, two parallax-filtered phase contrast methods prove useful for tomography of thick specimens[1]. One is simply a correction of the image shift prior to summing the individual signals: parallax bright field (pBF). The second is a variant of integrated differential phase contrast (iDPC): a gradient of the iDPC as a function of image shift, or equivalently defocus, producing a coherent amplitude image. To emphasize the parallax contribution, we call this method πDPC. Figure 1 shows a reconstructed virtual section from a FIB-sectioned multilayer of cells with intervening extracellular matrix filaments.

4D-STEM offers sophisticated analyses such as center of mass or ptychography. Iterative processing often fails to converge, however, for low-dose data. To this end we developed a direct method of ptychography based on shadow images that appear in the far-field diffraction pattern[2]. Keeping track of the probe overlap, a shadow montage reconstructs a 2D image with up-sampled resolution. Figure 2 shows a tomographic section of bacteriophage reconstructed from a tilt series of shadow montages. As the magnification depends on defocus, such reconstructions can be refocused to a range of depths, producing a multi-slice shadow volume. Combining these shadow volumes in tilt tomography separates between contradictory requirements for high lateral resolution vs depth of focus in a thick specimen.

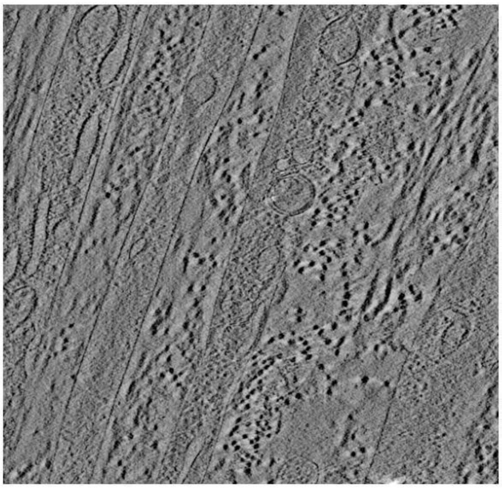

Fig. 1: πDPC tomogram of cell multi-layer cross-section. (dim. 4400x4400x750 nm)

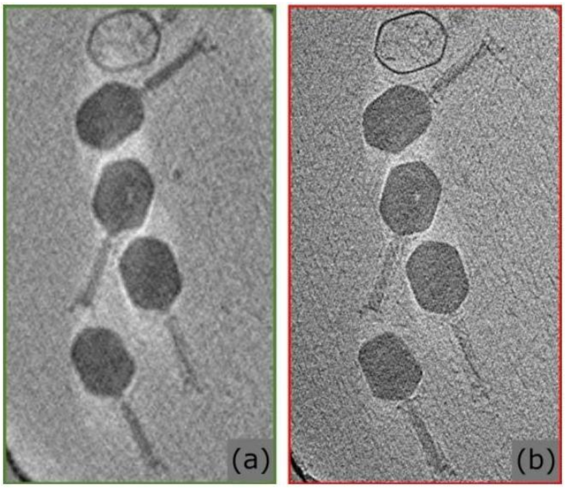

Fig. 2: T4 bacteriophage tomogram by πDPC (a) and up-sampled shadow montage (b).

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APPLICATION OF SPARSE SAMPLING TO THE MULTI-SCALE QUANTIFICATION OF DYNAMIC PROCESSES

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For most imaging and microanalysis experiments in high-resolution/in-situ/ultrafast (scanning) (transmission) electron microscopy ((S)(T)EM), the resolution and precision of the final result is determined by the interaction of the sample with the applied electron beam dose. Too much dose and the sample will either damage or the kinetics will be changed; not enough dose and there is insufficient spatial and/or temporal resolution to observe the phenomenon of interest. Recent results at the University of Liverpool (UoL) have shown that the optimal solution for dose control in any form of scanning/transmission electron microscopy is to form the image from discrete locations separated by as far as possible in space and time [1]. In scanned images/spectral maps this condition is satisfied by moving the beam over the area of the image using large jumps between the acquisition pixels, i.e. sparse sampling of the image. This form of imaging can be achieved with minor changes to the hardware and by using either a form of random walk scanning, a calibrated random scan or a mixture of conventional scanning and rapid beam blanking. The larger than standard jumps between pixel acquisition locations in this sparse sampling methodology creates problems with image interpretation, as the gaps between locations of acquisition are missing information (the image is under-sampled). Fortunately, we can use Inpainting to retrieve the missing information and form a full image. Figure 1 shows an example of a 4D STEM experiment, where the reduced number of pixels being acquired and the fast reconstruction of the image allows a live high angle annular dark field image to be formed. The live image allows the 4D acquisition to be optimized, and higher spatial resolution signals to be obtained during post-processing. Here I will discuss the methodology of Inpainting, with particular reference to the speed/efficiency of the reconstruction method and the potential for real-time video rate imaging across all forms of scanned images and spectral maps. In addition, the use of simulations to provide a starting point for image interpretation and to allow the microscope to adapt its own imaging conditions will be demonstrated. Finally, the integration of these de-noising/de-blurring and inpainting methods into ultrafast and low-dose TEM will also be discussed.

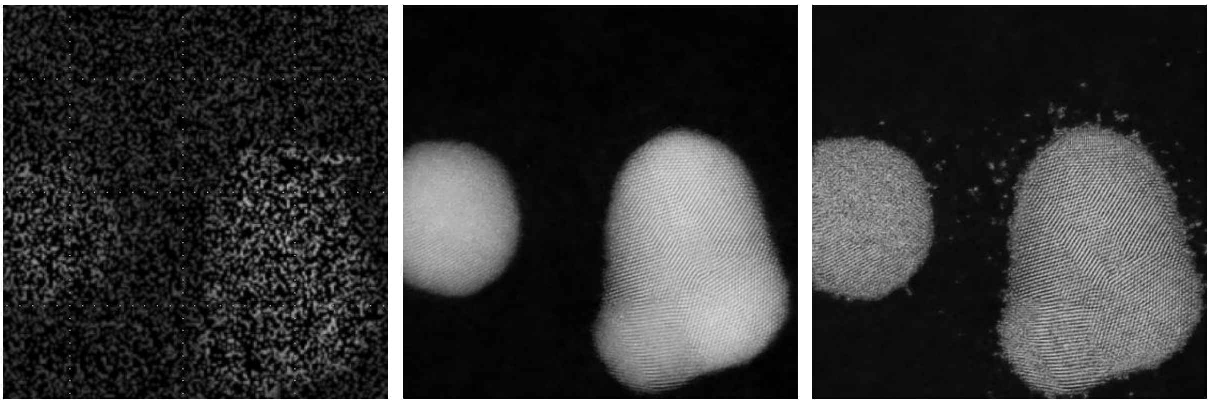

Fig 1: (a) Only 25% of the pixels are obtained in a 4D STEM image; (b) Inpainted fully sampled HAADF image of Au nanoparticles;(c) optimized post-acquisition reconstruction.

References:

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