From In Situ Chromatin and HIV-1 Nuclear Transport to Atomic-Resolution Pol II Dynamics

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The cell nucleus is a crowded, chromatin-rich environment that shapes both gene expression and the life cycle of nuclear-replicating viruses. In this talk, I will present an integrated cryoEM framework that bridges in situ cryo-electron tomography (cryoET) of the nuclear landscape with in vitro atomic-resolution structures and dynamics of RNA polymerase II (Pol II).

I will first describe in situ cryoET studies of chromatin organization and nucleosome heterogeneity in intact nuclei, providing native-context views of chromatin-dense regions and nuclear ultrastructure [1,2]. I will then focus on HIV-1 nuclear entry and intranuclear transport within this dense chromatin environment, using correlative cryoET to localize and structurally characterize viral assemblies and transport intermediates relative to nuclear landmarks and chromatin architecture [3,4]. Complementing these in situ measurements, I will present atomic-resolution cryo-EM reconstructions of Pol II complexes that resolve ordered water molecules and define their functional roles in catalysis, while capturing conformational states and dynamics throughout the nucleotide addition cycle [unpublished]. Finally, I will discuss recent cryoET method development enabling high-fidelity analysis of thick and heterogeneous nuclear specimens, including cc-correction, improved tilt-series alignment, and advanced CTF estimation and modeling strategies.

Together, these advances connect nuclear-scale organization and viral trafficking to molecular-scale transcription machinery and provide a quantitative framework for interpreting nuclear processes across length scales.

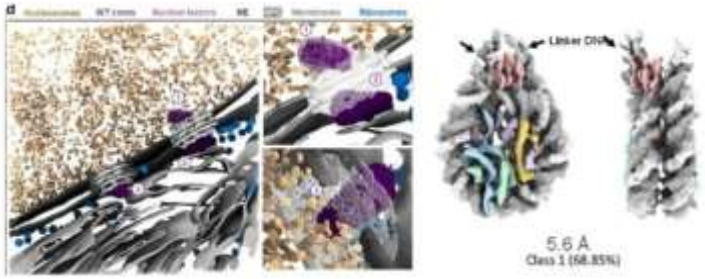

Figure 1: Nuclear import of HIV-1 cores and structures of nucleosomes in situ by correlative cryoET.

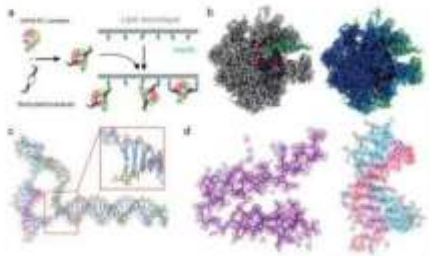

Figure 2: Sub-2 Å cryoEM structure of RNA polymerase II elongation complex resolved using mspSA affinity grids [5]

References:
[1] Hou et al., Nat Commun 14(1):6324 (2023)
[2] Hou et al., BioRxiv DOI:10.64898/2025.12.12.693995 (2025)
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Unlocking the secrets of the cytoskeleton and heart muscle

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Sarcomeres are force-generating and load-bearing devices of muscles. A precise molecular picture of how sarcomeres are built underpins understanding their role in health and diseases. We determined the molecular architecture of native skeletal and cardiac sarcomeres and structures of sarcomeric proteins using cryo-focused-ion-beam milling (cryo-FIB) and electron cryo-tomography (cryo-ET). Our in situ structures of the native thin (actin) and thick (myosin) filaments, including nebulin, titin and myosin binding protein C (MyBP-C), show for the first time how these critical proteins interact and regulate the filaments during muscle contraction. Unlike muscle, where the filaments of actin and myosin are stably arranged in sarcomeres, cells move and change their shape by controlling the appearance, growth, and shrinkage of actin filaments. However, the underlying structural mechanisms have remained elusive. To this end, we determined high-resolution structures of actin filaments alone and in complex with different actin-binding proteins revealing the molecular details of ATP hydrolysis, phosphate release, and the interactions governing filament assembly and disassembly. Taken together, our cryo-EM studies provide deep insights into muscle structure, actin filament polymerization and depolymerization and the interaction between actin and myosin and their associated proteins, which form the basis of muscle contraction and regulation.

Pushing the limits of detection in STEM EELS: magnon spectroscopy in an electron microscope

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Nearly a decade since first demonstration, vibrational electron-energy-loss spectroscopy has pushed the capabilities of analytical in a scanning transmission electron microscope (STEM) [1]. Phonon eigen modes can now be detected at atomic resolution [2], along with their dispersion in momentum space [3], and related to local atomic structure and chemistry. Magnons are quasiparticles representing the collective excitation of spins in magnetic materials. They, along with hybrid magnon-phonon quasiparticles (magnon polarons), are the basis for the operation of new spin wave transfer logic devices. They occupy the same energy loss windows as phonon modes, suggesting that STEM-EELS may offer the ability to detect them at the nanoscale. Here, we show that bulk THz magnons can be excited and detected at the nanoscale using high-energy-resolution STEM EELS [4] with the help of hybrid-pixel electron detectors. Momentum-resolved (ω-q) vibrational EELS measurements on antiferromagnetic material systems reveal the unambiguous dispersion behaviour of the magnon signal (Fig. 1) in NiO. The experimental findings are shown to be in excellent agreement with theoretical momentum-resolved magnon EELS dispersion curves (Fig. 1), calculated using theoretical methodologies to electron inelastic scattering of magnons and phonon-magnon coupling in an electron microscope [5]. Finally, we explore the limits of spatial resolution, by performing atomically resolve measurements and discuss of the contrast formation in atomically resolved magnon maps.

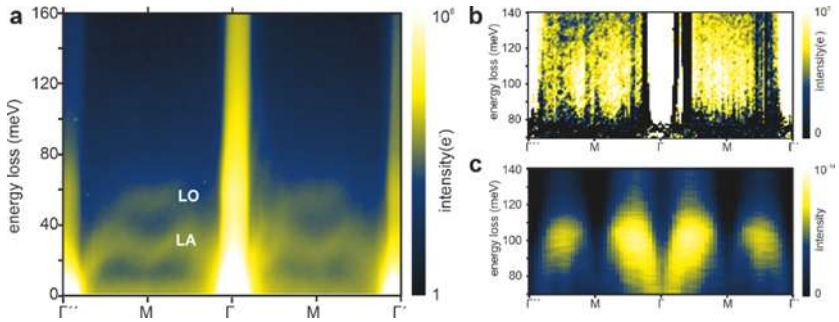

Figure 1. a. ω-q EELS map along the Γ → M q-path of NiO, showing the dispersion of the NiO LA / LO phonon branches. b Experimental background-subtracted and c calculated ω-q EELS maps showing the dispersion of the magnon bands.

References:

[1] O.L. Krivanek et al, Nature 514, 209, (2014).
[2] F.S. Hage et al, Science 367, 1124-1127, (2020).
[3] F.S. Hage et al, Science Advances 4, eaar7495, (2018).
[4] D.M. Kepaptsoglou et al, Nature 644, pages83–88 (2025)
[5] J.Á. Castellanos-Reyes et al, Phys. Rev. Let. 134, 036402 (2025).

Frontiers in Short Range Order - Measurement and Phenomenological Implications

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This lecture will discuss recent work at Sydney on the measurement of short range order (SRO) and its phenomenological implications in materials science. Our scattering-based approach to determine pair distribution functions will be discussed, emphasising the significance of accurate background subtraction and elliptical distortion. The interoperability of our approach between different diffraction intensity profiles will be explained. Our non-scattering approach using atom probe tomography (APT) will be presented, and our sensitivity analysis that describes clear regimes where the measurement of species-specific SRO is viable using APT will be summarised. Finally, our reason for seeking to quantify these aspects of the solid solution will be discussed since the phenomenology of clustering-type SRO enables the tuning of mechanical and functional properties of materials. Examples will be presented across aluminium alloys, metallic glasses and high entropy alloys, some of which have translated into industrial materials engineering practice.