Progress in field-free magnetic imaging on Thermo Scientific transmission electron microscopes

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Field-free magnetic imaging using scanning transmission electron microscopy (STEM) enables nanometer- to ångström-scale resolution [1], but routine measurements is often limited by diffraction contrast from bend contours. We report field-free magnetic imaging on Thermo Scientific transmission electron microscopes (TEMs) using a frame-based precession workflow implemented via AutoScript TEM, where programmable control of electron-beam tilt and rotation suppresses diffraction-related effects and enhances magnetic contrast. The approach is demonstrated on representative magnetic materials, including $Nd_2Fe_{14}B$, B20-FeGe, and Fe_3O_4 thin films. Cryogenic measurements and momentum-resolved EELS are also explored under field-free conditions. These results highlight how AutoScript TEM enables automated acquisition strategies, supporting scalable, multimodal characterization workflows for emerging magnetic materials.

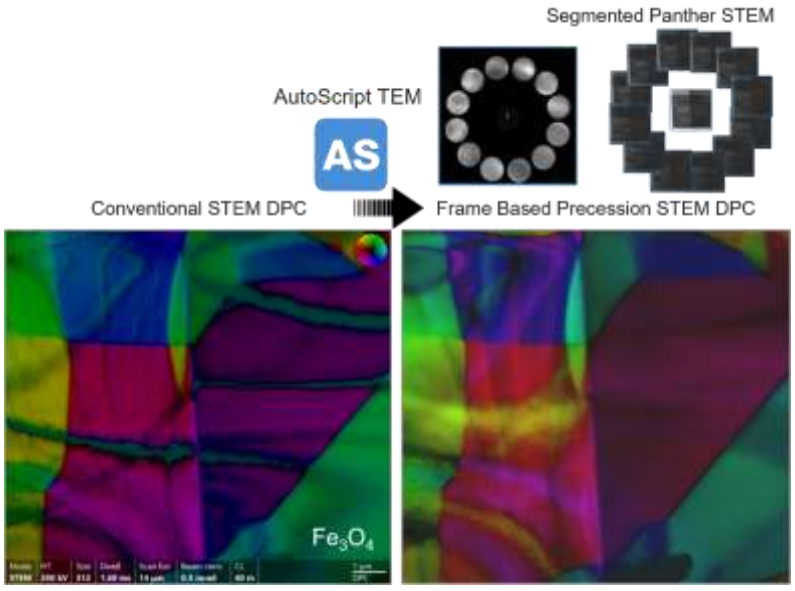

Fig. 1: Field-free imaging of an Fe_3O_4 thin film. (left) Conventional STEM-DPC with diffraction contrast. (right) Frame-based precession STEM-DPC suppressing diffraction contrast.

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Development of a C_c/C_s Corrector Based on Hexapole and Quadrupole Fields

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Spherical and chromatic aberrations are fundamental and inevitable limitations of the lenses used in transmission electron microscopes. Although the spherical aberration correctors have been widely used for high-resolution imaging, chromatic aberration correctors remain limited in number, despite their importance for various applications such as imaging of thick specimens, observation using electron guns with a wide energy spread or a low accelerating voltage, and imaging using objective lenses with large chromatic aberration coefficients. In this study, we have developed a chromatic and spherical aberration corrector based on quadrupole and hexapole fields.

In the developed corrector, thick hexapole fields generate negative spherical aberration as in conventional hexapole-type spherical aberration correctors. To produce negative chromatic aberration, superimposed electric and magnetic quadrupole fields are introduced between the two hexapole fields. The quadrupole quadruplets also function as a transfer doublet for the hexapoles. By using this configuration shown in Figure 1, chromatic and geometric aberrations can be corrected simultaneously. The corrector has been installed in the imaging system of a transmission electron microscope.

Measurements of the residual aberrations indicate that the first-order chromatic aberration was corrected, and the second-order chromatic astigmatism became dominant. The correction of geometric aberrations allows an aberration-free angle of more than 30 mrad. The effect of chromatic aberration correction has been demonstrated by intentionally inducing energy oscillations. Images of gold particles on an amorphous film indicate that clear Thon rings can be obtained under C_c corrected condition even with an energy oscillation of 100 eV as shown in Figure 2. This aberration corrector is expected to significantly improve image quality in cases where chromatic aberration is the dominant limiting factor [1].

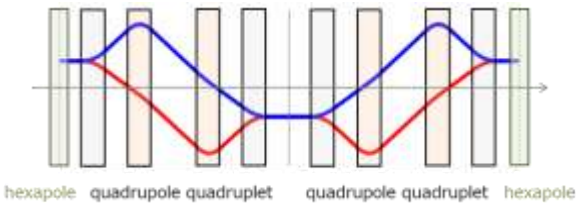

Fig. 1: Optical configuration of the developed C_c/C_s corrector

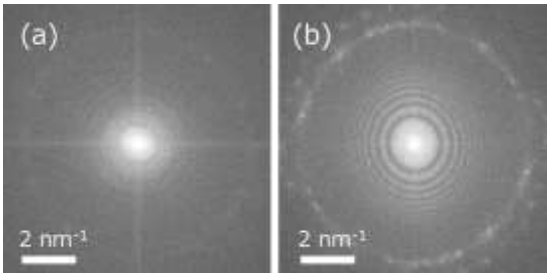

Fig. 2: Fourier transforms of TEM images with an energy oscillation of 100 eV under (a) uncorrected and (b) C_c/C_s corrected conditions.

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Exploring electron dose fractionation in TEM for material science applications using the latest generation of fast hybrid pixel detectors

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Electron dose fractionation is an established technique in the life-science TEM community, recording individual electron hits at the detector plane is maximizing the amount of useful information that can be extracted with a limited number of electrons. [1] The latest generation of direct electron counting hybrid pixel detectors achieve framerates above 100kHz [2], extend the dose fractionation principle into dose rates closer to the realm of material science TEM. Currents between 1-10 pA can be used for electrons homogenously distributed within a frame as in conventional TEM imaging. Under these conditions, interpretable TEM images can be acquired in less than a second, while retaining information about individual electron hits in individual sub-frames. This acquisition mode allows for sensitive evaluation of sample changes induced by the electron beam or external stimuli. Dose fractionation can further enhance detector DQE [3] and allow for precise drift correction.
In the present work, the detector setup and data processing are guided by a simulated detector response according to Allpix[2] model. [4] Benefits and drawbacks of dose rate fractionation for real-life material science samples are explored; in both imaging and diffraction modes with particular focus on electron damage process for beam sensitive samples and dynamic (in-situ triggered) processes.

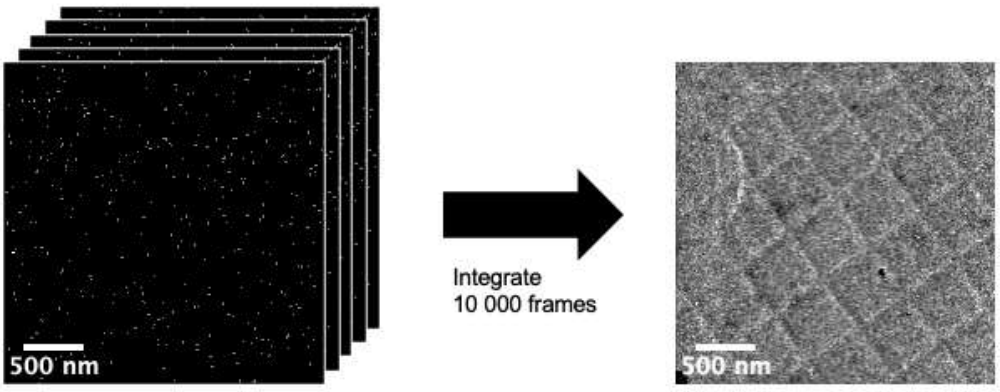

Figure 1. Illustrates the principle of dose fractionation with a fast detector. 10 000 images resolving individual electron events been acquired over the course of 1s with DECTRIS ARINA CZT detector. Dose rate: 130 e/px/s

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ATOMIC-RESOLUTION COUNTED EELS ON NON-ABERRATION-CORRECTED MICROSCOPES USING GIF CONTINUUM AND ADVANCED eaSI WORKFLOWS

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While aberration correction has greatly improved the spatial resolution of the STEM, the reality of beam-specimen interactions in a typical EELS experiment often means the resolution is limited by the SNR of the data and not by the probe size. Recent advances in direct electron detection and high-speed spectrum imaging workflows maximize the SNR of the collected data enabling atomic-resolution EELS even on non-aberration-corrected TEM/STEM instruments, overcoming traditional limits imposed by drift, sample instability, and detector readout noise. Modern counting cameras such as the K3 and the Stela hybrid pixel detector deliver single-electron sensitivity, negligible read noise, sharp point-spread functions, and extremely high frame rates, allowing dose-efficient spectrum imaging at all accelerating voltages.

Combined with the GIF Continuum platform and eaSI (efficient and synchronized spectrum imaging) technology, these detectors facilitate fully synchronized EELS, EDS, and imaging acquisition while providing automated metadata correlation and live drift correction. Continuous drift correction applied between successive passes removes drift artifacts that would otherwise be present in long, single-scan acquisitions, enabling large-area or atomic-resolution spectrum imaging even during long or dynamic in-situ experiments.

Dose-fractionated EELS acquisition, enabled by high-speed counting detectors, is particularly powerful for both robust and beam-sensitive materials. Due to the low readout noise and high sensitivity of the counting detectors, individual sparsely populated spectra can be sum together to improve the SNR. Instead of long single-pass dwell times, many sparse, low-dose passes are acquired and selectively summed post-collection. This permits removal of compromised passes (beam damage, contamination, drift), and preserves high-fidelity ELNES information by rejecting damaged passes and retaining pristine chemical state information.

These capabilities extend naturally to cryo-EELS. Using Gatan's new liquid-nitrogen cryo holder, atomic resolution can be maintained despite the typically high drift rates at low temperature. Ultra-short pixel times (110–339 µs) minimize beam dose, and increase the frame rate of spectrum image acquisition, helping minimize sample instabilities due to thermal drift. Furthermore, direct detection enables high-quality mapping up to 3000 eV energy range at probe currents as low as 20 pA on an uncorrected 200 kV system.

Together, the combination of counted EELS, dose fractionation, continuous drift correction, and eaSI multimodal acquisition redefines what is achievable on non-aberration-corrected instruments. These workflows enable reliable atomic-resolution EELS on a broad range of materials—including highly beam-sensitive systems—supporting advanced in-situ, cryogenic, and multimodal STEM experiments.

SENSITIVITY AND ROBUSTNESS OF QUANTITATIVE PHASE ANALYSIS AND STRAIN MEASUREMENTS BY PRECESSION-ASSISTED 4D-STEM

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The introduction of fast pixelated detectors with a large dynamic range has revived the advanced methods of electron diffraction for characterization of a broad range of materials and phenomena by analytical scanning transmission electron microscopy [1,2]. Electron diffraction maps (4D-STEM datasets) can now be acquired efficiently from large regions of interest in just several minutes. The quality of acquired diffraction patterns can be substantially improved by beam precession [3], which reduces the deleterious effects of dynamic scattering in the data while increasing the number of detected spots in acquired diffraction patterns. Techniques based on template matching, such as phase-orientation mapping, then become more accurate and robust, especially when several phases are present in the sample. Beam precession also results in more homogeneous intensities of individual diffraction spots which facilitates more accurate determination of their position to the sub-pixel level, making the analytical diffraction methods based on “center-of-mass” measurements, such as strain analysis or differential phase contrast, more accurate and precise.

The power of analytical 4D-STEM measurements enhanced by beam precession with precise microscope alignments and fast data acquisition speeds [4], will be demonstrated on the challenging examples of phase analysis of pleiomorphic materials, such as annealed HZO thin films [5], and strain measurements in advanced Si:SiGe multi-layers and technology nodes.

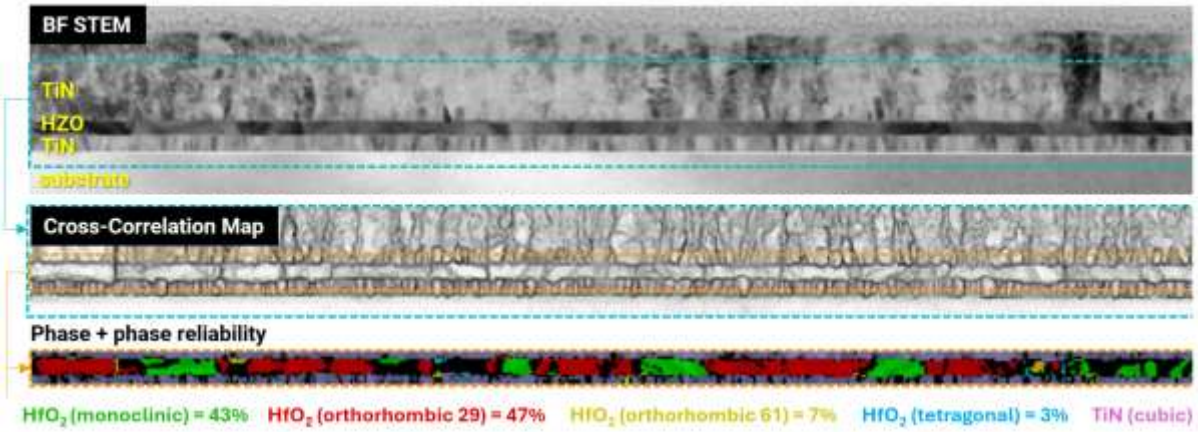

Fig. 1: Example of quantitative phase analysis of grains in an annealed HZO film by 4D-STEM enhanced by precise beam precession [5]. Multiple possible HZO phases were considered and evaluated, including the monoclinic (green) and the orthorhombic (red).

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IN SITU STRUCTURAL ORGANIZATION OF THE AUTOPHAGY CARGO RECEPTOR P62 STUDIED WITH A CORRELATIVE LIGHT AND ELECTRON MICROSCOPY PIPELINE

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The autophagy cargo receptor p62/SQSTM1 acts as a central hub in autophagy; it bridges the poly-ubiquitinated tag of cargo to several autophagy regulators and the autophagosomal membrane. p62 forms helical filaments *in vitro* and phase-separates in the cytosol to form p62-cargo biological condensates [1,2]. We aimed to understand the molecular mechanism behind the formation of these large intracellular structures by studying both the *in vitro* structure of p62 as well as the *in situ* structural organization using cellular cryo electron tomography. To characterize these protein condensates inside the context of the cell, we have used a correlative light, energy-dispersive X-ray and cryo-electron microscopy workflow [3,4]. Besides liquid liquid phase separated p62 bodies, spherical cellular structures of approximately 500nm in diameter were found; all containing a homogeneous, feature-less core and a very dense coat of varying thickness. Our results show that these structures are likely lipophagy intermediates; a lipid droplet core is surrounded by a shell of up to 18 nm thick composed of p62, and highly concentrated in calcium, phosphorus and magnesium. The structure is surrounded by an ER-derived membrane with calcium transporters in close proximity. We hypothesize that the calcium present here could trigger autophagy initiation on the surface of the lipid droplets after p62 has recognized them as cargo. This novel application of using energy-dispersive X-ray spectroscopy to characterize these frozen hydrated cellular lamellae was instrumental in elucidating this biological process, and we think it could be of wider interest to the community to expand more correlative light and electron microscopy workflows to incorporate elemental mapping.

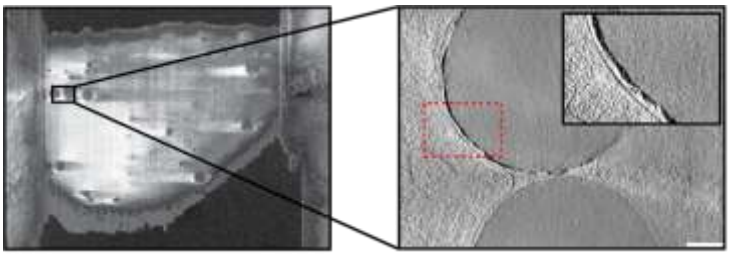

Fig. 1. Left panel; SEM image of a 150 nm cryo lamella of a human cell expressing mCherry-p62 and a 72 hour ATG5 knockdown. Right panel; Slice through a cryo electron tomogram acquired on the lamella, showing a p62(+) structure with an electron dense region

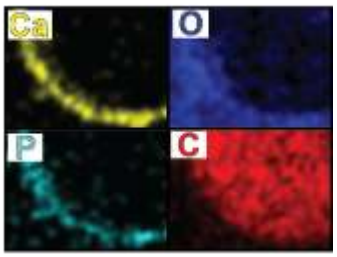

Fig. 2. EDX spectral maps recorded on the zoomed in section of the lamella displayed in figure 1.

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Cryo-electron tomography of p62-mediated aggrephagy in cells

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Selective autophagy pathways rely on cargo receptors such as p62 to recognize ubiquitinated substrates and direct them toward autophagosomes for degradation [1]. Aggrephagy, the selective autophagy of protein aggregates, is particularly relevant in the context of neurodegenerative diseases. Several neurodegeneration-associated proteins, including α-synuclein and mutant huntingtin (mHTT), are prone to aggregation and have been identified as substrates of autophagy [2]. The aggregation of these proteins is believed to play a key role in the pathogenesis of their respective disorders, Parkinson's disease (PD) and Huntington's disease (HD) [3]. Although cryo-electron microscopy (cryo-EM) has provided high-resolution structures of α-synuclein and huntingtin fibrils [4,5], knowledge of how these proteins are organized as aggregates in cells and engage the autophagy machinery remains relatively limited. We opted to study the *in situ* ultrastructure of these α-synuclein and huntingtin aggregates using cryo-electron tomography (cryo-ET). This technique enables three-dimensional, *in situ* visualization of macromolecular assemblies within intact cells. Furthermore, combining correlative light and electron microscopy (CLEM) with cryo-ET allows targeted investigation of p62-positive protein aggregates, providing insight into the structural basis of aggrephagy at the cellular level and the effect of p62's overexpression on the ultrastructure of the aggregates.

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SUPER-RESOLVED INTEGRATED CRYO-FLUORESCENCE IMAGING ON LAMELLA FOR PRECISION CRYO-ET

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Cryo focused ion beam (cryo-FIB) milling expands the range of biological samples accessible to cryo-electron tomography (cryo-ET). METEOR 2.0 is Delmic's integrated cryo-fluorescence microscope (cryo-FM), utilizing high-NA objectives and LED imaging for high-sensitivity, non-destructive fluorescence imaging throughout the FIB milling process. We investigated the resolving power of METEOR 2.0 imaging in combination with a super-resolving technique, enhanced Super-Resolution Radial Fluctuations (eSRRF) [2]. While super-resolution has been demonstrated on stand-alone cryo-FMs [3-4], this is the first demonstration of super-resolution in an integrated cryo-FM.

We used METEOR 2.0 to acquire fluorescence timelapses of DNA-origami nanorulers with precisely defined fluorophore separations under cryogenic conditions. eSRRF post-processing yielded a 2-fold improvement in lateral resolution. As a proof-of-concept, we applied the METEOR 2.0 - eSRRF workflow to co-localizing fluorescent puncta in lamellae of mRFP-GFP-LC3 HeLa cells. METEOR 2.0 imaging revealed co-localizing fluorescent puncta clearly in polished lamellae, and eSRRF processing sharpened structures and improved peak separation, validating colocalization. The super-resolved fluorescence images correlated closely with distinct organelles visible when imaged in TEM. Together, this work demonstrates that the METEOR 2.0 and eSRRF workflow overcomes the diffraction barrier and improves effective resolution. This setup enables precise, fluorescence-guided cryo-ET acquisition, and strengthens target validation of features below the diffraction limit.

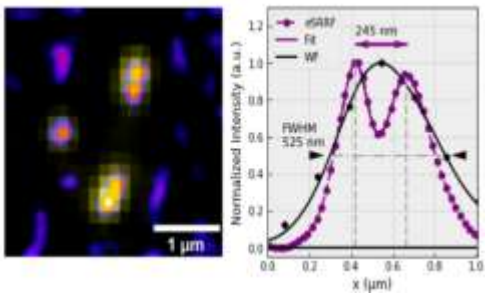

Fig. 1: Widefield (yellow) and eSRRF (heatmap) imaging of fluorescent nanorulers (left). The intensity profile (right) of 1 nanoruler shows eSRRF peak separation and resolution enhancement.

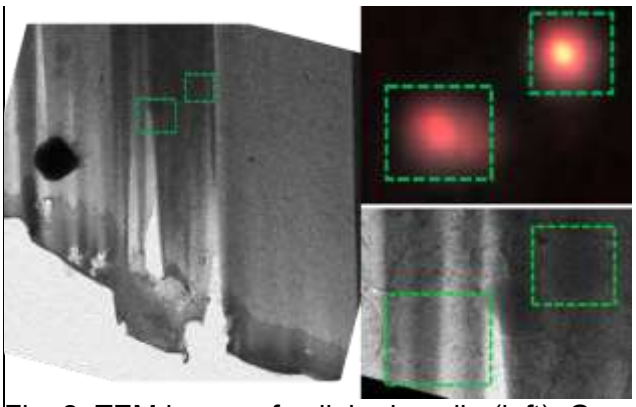

Fig. 2: TEM image of cellular lamella (left). Green boxes highlight organelles identified in both eSRRF-processed fluorescent images (top right) and TEM imaging (bottom right).

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Need for Speed: Staggered Cryo-Plasma FIB Milling Enables High-Throughput Cryo-ET

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Cryo-focused ion beam (cryo-FIB) milling combined with cryo-electron tomography (cryo-ET) enables structural analysis of macromolecules directly within their native cellular environment [1]. However, its application to thick specimens such as tissues and organoids remains limited by slow, labor-intensive lamella preparation using conventional gallium-based FIB systems. Plasma FIB (PFIB) microscopes promise dramatically increased throughput through higher beam currents, yet the consequences of high-current milling for biological sample integrity are largely unknown.

We systematically explored current-dependent damage induced by high-current xenon PFIB milling in cryo-ET workflows. Using high-pressure frozen *S. cerevisiae* and serial lift-out [2] strategies, we generate damage gradients with currents up to 2,500 nA and assess structural preservation from the specimen scale down to the molecular level via subtomogram averaging. Across thousands of tomograms, we evaluate how increased milling currents influence lamella quality and macromolecular integrity.

In addition, we provide a high-throughput staggered milling strategy to leverage high plasma currents for rapid, large-volume preparation, validated on tissue samples (Figures 1 and 2). This framework balances throughput and structural preservation in PFIB-based cryo-ET. It opens the door to systematic, high-resolution structural studies of medically relevant tissues and complex biological systems.

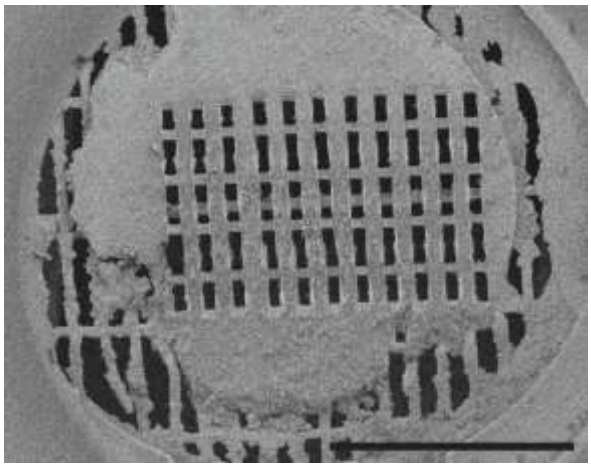

Fig. 1: Example of staggered milling, illustrating the preparation of 44 lamellae on a 70 µm thick specimen. Scale bar 1 mm.

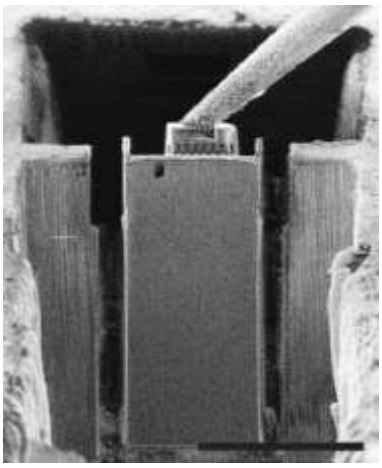

Fig. 2: Whole-depth lift-out from a 100 µm thick specimen generated with the staggered milling strategy. Scale bar 50 µm.

References:
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Cryo-STEM: center-of-mass methods applied to biological specimens

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Scanning transmission electron microscopy (STEM) is a well-established method for the characterization of materials. However, it is not a widely used method for imaging biological samples in cryogenic conditions. Biological specimens are dose-sensitive, often have poor contrast against ice background and can therefore be challenging to visualize. Recently, we showed that integrated Differential Phase Contrast (iDPC)-STEM can be applied on vitrified biological specimens and near atomic resolution can be determined (1). In STEM the resolution is dependent on the convergence semi-angle (CSA) of the focused beam. Using the iDPC-STEM approach, the maximum contrast of the image can be achieved in focus. In this work, tobacco mosaic virus (TMV), 70S ribosomes, apoferritin and hemoglobin have been used as specimen at 4.0 mrad. In case of TMV near-atomic resolution results are demonstrated, in both types of data collection, using a 4-quadrant segmented and a pixelated detector. However, these challenges serve as a springboard for further research on the method in structural biology. Addressing the theoretical and experimental limitations can lead to methodological improvements and an expanded applicability of single-particle analysis to complex biological systems.

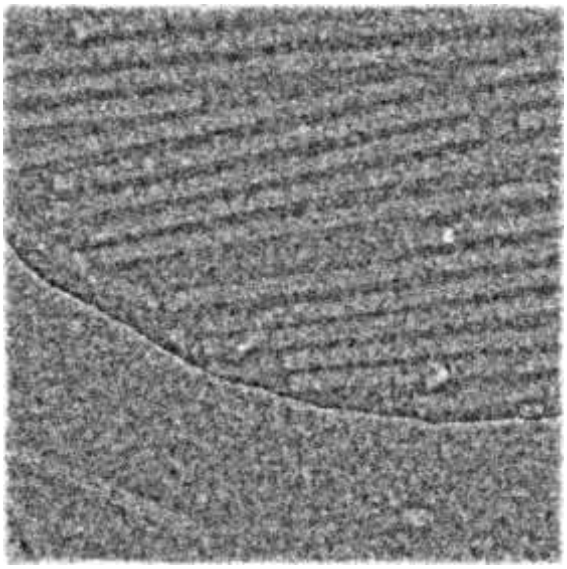

Fig. 1: TMV embedded in vitreous ice.

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FUNCTIONAL INVESTIGATION OF THE ESCRT-III HOMOLOGUE VIPP1 USING *IN SITU* CRYOGENIC ELECTRON TOMOGRAPHY

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The endosomal sorting complex required for transport machinery (ESCRT-0 to -IV) is one of few cellular systems employed to remodel membranes away from the cytoplasm. Central to this process are assemblies of ESCRT-III proteins, which increasingly constrict the membrane until fission is achieved[1,2]. The ESCRT-III homologue Vesicle-Inducing Protein in Plastids 1 (Vipp1) shows membrane remodeling activity *in vitro* and is critical for thylakoid membrane maintenance and biogenesis in both chloroplasts and cyanobacteria. While recent structural and functional studies revealed that Vipp1 belongs to the ESCRT-III superfamily, the cellular context of Vipp1 remains poorly understood[2,3]. In this work, we use the cyanobacterium *Synechocystis* PCC6803 as a model for photosynthetic organisms to investigate Vipp1 function *in situ*. High-intensity light stress triggers Vipp1 localization as punctae close to the thylakoid membrane. Cryogenic electron tomography reveals that these punctae coincide with the formation of small, stress-induced vesicles located towards thylakoid membrane convergence zones. These vesicles are uniform in size and consistently situated between plasma and thylakoid membrane. Based on preliminary findings, we aim to decipher the role of Vipp1 in thylakoid membrane biogenesis and maintenance under stress conditions.

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Structural characterization of a novel bacterial ESCRT-III protein in E. coli

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The structures and functions of endosomal sorting complexes required for transport (ESCRT)-III proteins in bacteria, such as PspA and Vipp1, have been intensively studied in recent years[1,2]. In *E. coli*, an additional potential PspA-homolog, YjfJ, has been recently identified while a biochemical, structural and functional characterization is entirely lacking. Here we show that YjfJ from *Escherichia coli* is a bona fide member of bacterial ESCRT-III proteins. Using cryo-EM, we solved eight structures of YjfJ helical filaments to a resolution of 3.0 to 4.0 Å as well as two structures of YjfJ in presence of membranes at 4.4 and 6.0 Å. In these structures YjfJ monomers adopt the typical ESCRT-III fold as well as extensive plasticity typical for bacterial ESCRT-III proteins. Our data also revealed that apo state YjfJ polymers are very similar to apo state PspA polymers, whereas they change to Vipp1-like polymers upon lipid reconstitution. Preliminary functional data suggest that YjfJ and PspA may exhibit complementary functions in *E. coli*, where YjfJ may play a critical role in maintaining the membrane fluidity under cold (stress) conditions. The characterization of YjfJ will help to understand the evolution of ESCRT-III proteins in bacteria as well as membrane remodeling in *E. coli*.

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STRUCTURAL ORGANIZATION OF p62 FILAMENTS

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Autophagy is a conserved cellular process essential for maintaining intracellular homeostasis by degrading and recycling cytoplasmic material such as aggregated proteins, damaged organelles, and pathogens [1]. In selective autophagy, cargo receptors like p62/SQSTM1 (p62) recognize poly-ubiquitinated cargo and interact with Atg8 family proteins via a LC3-interacting region (LIR) motif, facilitating targeted degradation [2]. p62 is a multi-domain protein with an N-terminal Phox1 and Bem1p (PB1) domain, an intrinsically disordered region (IDR) containing the LIR motif, and a C-terminal ubiquitin-associated (UBA) domain. The PB1 domain enables p62 polymerization into filaments, which is critical for autophagy, while the UBA domain captures ubiquitinated cargo [3, 4].

To gain structural insights into p62 assembly, we determined the cryo-electron microscopy (cryo-EM) structure of full-length p62. The structure revealed a double-helical PB1-domain scaffold, with a partially disordered C-terminal region residing within the lumen and major groove of the filament. Binding studies complemented by single-molecule fluorescence demonstrated that LC3b and poly-ubiquitin modulate p62 filament dynamics, inducing either disassembly or filament bundling, respectively. Notably, our structural analysis suggested that the LIR motif is positioned toward the filament's exterior, facilitating molecular access to LC3b and supporting its role in autophagic cargo recruitment.

A multimeric AI-predicted model of p62 using AlphaFold3 expanded by helical symmetry is consistent with the well-resolved cryo-EM density. The model further suggested that the UBA domain aligns with the major groove near the PB1 scaffold, while the LIR motif remains exposed, enabling efficient LC3b binding.

Extending these findings in the cell, cryo-electron tomography (cryo-ET) of ATG5-deficient cells revealed p62 oligomers coating lipid droplets. These assemblies were associated with calcium-rich layers, as confirmed by compositional spectroscopy, suggesting a role for ion dynamics in early lipophagy. Together with proteomic identification of ER calcium channels, our results support a model in which p62 polymers not only recognize ubiquitinated lipid droplet cargo but may also coordinate calcium-dependent events that help recruit the autophagy machinery.

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CROSS-SEEDING OF TAU AND α-SYNUCLEIN IN THE ORIGIN OF THE SYNUCLEINOPATHIES

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Amyloid aggregation of specific proteins is strongly associated with distinct neurodegenerative disorders. The deposition of α-synuclein (α-syn) amyloid fibrils in the brain defines a group of progressive diseases known as synucleinopathies, whereas tau aggregation is a hallmark of tauopathies. Recent advances in cryo-electron microscopy (cryo-EM) have uncovered numerous polymorphs of both α-syn and tau aggregates, enabling correlations between patient-derived α-syn polymorphs and specific synucleinopathies, as well as linking diverse tau polymorphs to various tauopathies. Nonetheless, significant structural differences exist between brain-derived amyloid aggregates and those formed *in-vitro*, underscoring the challenge of reproducing physiological aggregation pathways in the laboratory.

Currently, *in-vitro* protocols have been established that induce the aggregation of recombinant tau fragments, recapitulating the structure of certain patient-derived tau polymorphs. Building on this, we have successfully developed conditions for the *in-vitro* aggregation of full-length recombinant tau that closely mimics patient-derived tau fibrils. This advance is particularly significant, as recent studies highlight the critical role of tau's terminal regions in amyloid elongation, allowing us to use these lab-generated polymorphs for spreading and propagation studies.

Regarding α-syn aggregation mechanisms, we hypothesize that the cross-seeding of α-syn onto tau aggregates, which are more prone to aggregation than α-syn, could be a critical initial event in the formation and propagation of α-syn amyloid fibrils. Our laboratory has explored the cross-seeding potential of various tau polymorphs, aggregated *in-vitro* from full-length recombinant tau and tau fragments, in the presence of recombinant wild-type α-syn monomers and in HEK cells overexpressing wild-type α-syn. Using cryo-EM, we aim to pinpoint the structural determinants of tau that mediate cross-seeding and to evaluate the pathological significance of these cross-aggregates.

THE EFFECT OF MET35 OXIDATION ON THE FORMATION AND STRUCTURE OF AMYLOID-β(1-42) FIBRILS

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Amyloid-β (Aβ) is associated with Alzheimer's disease (AD) pathology. In AD, Aβ(1–40) and Aβ(1–42) undergo a transition from disordered monomers to oligomers and protofibrils rich in β-sheets, and finally to mature amyloid fibrils. The only methionine residue in Aβ, Met35, is located in the hydrophobic C-terminal region and is susceptible to oxidation. Its oxidation has been linked to oxidative stress. Indeed, 10-35% oxidized Met35 has been detected in association with Aβ fibrils isolated from the brains of deceased AD patients [1]. Previous studies at low resolution have shown that in vitro conversion of Met35 to methionine sulfoxide (Met35ox) induces measurable changes in Aβ structure and morphology [2]. Met35ox has been proposed to play a role in β-amyloidosis by modulating aggregation pathways and potentially limiting the formation of protofibrils, which are key intermediates in fibril assembly [3, 4]. To gain structural and kinetic information at the atomic level, we prepared ^{13}C,^{15}N-labeled Aβ Met35 wild-type (wt-Aβ) and ^{13}C,^{15}N-Met35ox-Aβ by oxidizing lyophilized wt-Aβ with air over several months. Aggregation kinetic measurements followed by solution heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectroscopy and Thioflavin T (ThT) fluorescence assays demonstrate that Met35 oxidation of Aβ alters its fibrillization profiles by drastically slowing Aβ(1–42) assembly and shifting the lag and growth phases of aggregation. Met35 oxidation modestly impairs amyloid fibril growth. However, atomic force microscopy (AFM) and transmission electron microscopy (EM) reveal that Met35ox-Aβ still can form amyloid fibrils and amorphous aggregates after ~9 months of incubation in an aqueous solution of 30% acetonitrile at pH 2 [Fig. 1]. Under these conditions, wild-type (wt) Aβ forms long, straight LS-shaped amyloid fibrils, the structures of which have been solved at near-atomic resolution by cryo-electron microscopy (cryo-EM) and solid-state NMR [5]. Cryo-EM is used for high-resolution structural analysis to resolve the atomic architectures of Met35ox-Aβ fibrils and to assess their structural differences compared to wt-Aβ fibrils grown under identical conditions. These results will help to clarify the role of oxidative methionine modifications, particularly the formation of Met35ox-Aβ due to oxidative stress, and its consequences for fibril formation in Alzheimer's disease (AD).

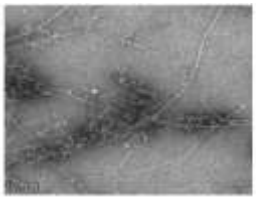

Fig. 1: EM image of Met35ox stained with 2% Uranyl acetate

Fig. 2: EM image of Met35 control stained with 2% Uranyl acetate

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Polymorphism in *Ex-Vivo* & *In-Vitro* Aβ42 Fibrils

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Amyloid-β (Aβ) fibrils derived from Alzheimer's disease brain tissue exhibit well-defined, disease-relevant folds that can now be resolved to near-atomic resolution by cryo-electron microscopy. Here, we report a subtle yet significant deviation in the layer-assembly pattern of previously described type-I *ex vivo* fibrils [1] (Fig. 1). *In vitro* assembled Aβ fibrils consistently display pronounced structural polymorphism and, critically, have yet to reproduce the dominant type 1 architecture observed in brain tissue. As an illustrative example, the first solved Aβ42 fibril structure, which shows the LS Fold [2] (Fig. 2), demonstrates this divergence. These limitations extend to standard mouse models, which raises critical questions about the faithfulness of current experimental systems in modeling disease-relevant Aβ folds for translational drug development. In this work, we discuss the persistent challenges in replicating clinically relevant Aβ fibril conformations *in vitro*.

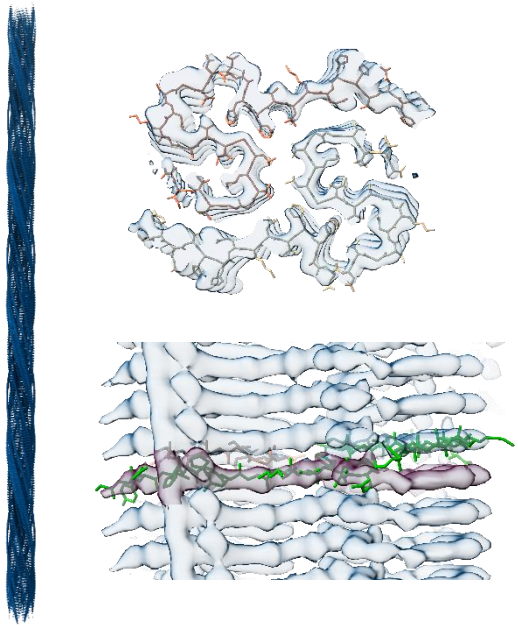

Fig. 1: Ex-vivo type 1 fibril fold with new layer shift

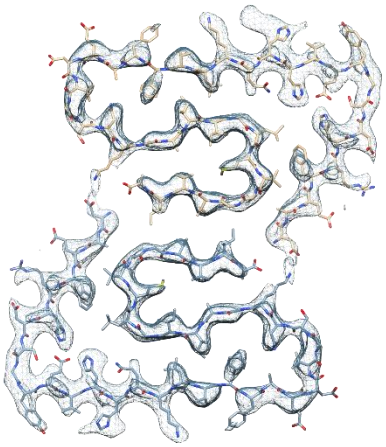

Fig. 2: LS in-vitro fold

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Therapeutic Insulin Glargine Forms Amyloid Fibrils distinct from Human Insulin Fibrils: A Cryo-EM Study

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Insulin is a critical hormone for blood glucose regulation and diabetes treatment. However, insulin is prone to forming amyloid fibrils, a process that reduces its effectiveness and poses challenges for storage and stability, particularly in warmer climates and when cold chains cannot be guaranteed. Therefore, we employed cryo-electron microscopy to investigate insulin amyloid fibril formation at the molecular level and provide a structural foundation for developing strategies to prevent aggregation of therapeutic insulin. In this study, we determined at near-atomic resolution a previously unknown trimeric fibril structure of recombinant human insulin, as well as the dimeric structures of two insulin glargine fibril polymorphs, that formed at elevated temperature in its therapeutic formulation. Of note, insulin glargine is one of the most widely used insulin analogues in clinical practice. Importantly, the structure and protofilament folds of both dimeric insulin glargine fibril polymorphs differ significantly from those of recombinant human insulin fibrils. This demonstrates that minor engineered sequence modifications in insulin analogues can produce fundamentally different fibril structures. Therefore, structural information from fibrils of recombinant human insulin is not readily transferable to its therapeutic analogues. Our findings provide valuable molecular insights to address the challenge of therapeutic insulin aggregation and have direct implications for the engineering of next-generation insulin analogues. Specifically, these insights can guide the optimization of sequence modifications, formulation, and storage conditions to improve drug stability and efficacy.

A Novel Eukaryotic Ribosome Factor Enables Translation Restart Following Cellular Dormancy

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Dormancy is a survival strategy employed by all domains of life to withstand prolonged nutrient deprivation and environmental stress, marked by a global shutdown of protein synthesis. However, the molecular mechanisms driving ribosome inactivation and reactivation during and after dormancy in eukaryotes remain poorly understood. Here, using high-throughput *in situ* cryo-electron tomography, we obtain a 3.3 Å map (up to 2.4 Å locally) of the hibernating ribosomes in which we identify SNOR, a novel 12 kDa SBDS-like ribosome-associated factor in *Schizosaccharomyces pombe*. We find that this factor is upregulated and associates with ribosomes during induced dormancy triggered by glucose depletion. SNOR contributes to protein synthesis repression by binding the ribosome to probe the peptidyl transferase center (PTC), block tRNA-binding sites, and cap the polypeptide exit tunnel (PET). Importantly, we show that SNOR is essential for the restart of protein synthesis upon glucose reintroduction and exit from dormancy. SNOR is evolutionarily conserved and specifically upregulated in response to glucose stress in fungi. These findings reveal a previously unrecognized ribosome-associated factor that links glucose stress and cellular dormancy to surveillance of protein synthesis and highlight the power of *in situ* structural biology to uncover stress-responsive regulators of translation.

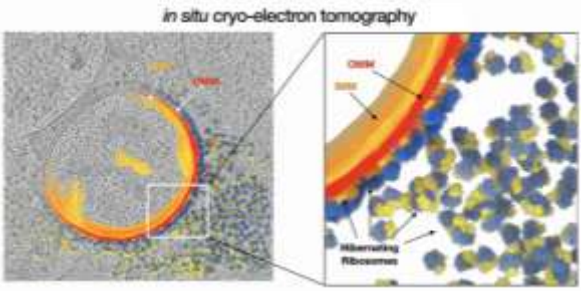

Fig. 1: Hibernating ribosomes by *in situ* cryo-ET. Slice through a tomogram of a mitochondrion decorated with ribosomes from a *S. pombe* cell grown for 7 days in EMM containing 0.5% (w/v) glucose. Segmentation highlights the mitochondrion and surrounding ribosomes. Inset shows a close-up of ribosomes at the mitochondrial surface; the inner mitochondrial membrane (IMM) is shown in orange, the outer mitochondrial membrane (OMM) in red, and ribosomal SSU and LSU in yellow and blue, respectively.

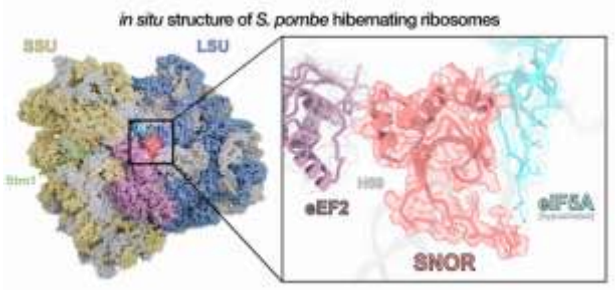

Fig. 2: *in situ* cryo-ET reconstruction of the hibernating ribosome. Map shown as a semi-transparent surface with the fitted atomic model rendered as a cartoon. Ribosomal proteins of the SSU and LSU are colored yellow and blue, respectively. Stm1 is shown in green, eEF2 in purple, eIF5A in cyan, and SNOR in red. (D) Inset shows a close-up of SNOR interface at the peptidyl transferase center (PTC). The post-translated hypusinated residue of eIF5a is also observed interacting with the C-terminal tail of SNOR.

Structural basis for the semiquinone stability and superoxide production in the quinone reduction site of the cytochrome bc_1

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The complex III (cytochrome bc_1) is considered one of the main sources of reactive oxygen species (ROS) in mitochondria. Although low-level ROS generation is important to homeostasis and cell signaling, elevated levels may have deleterious effects, including cellular damage. It is well established that inhibition of the ubiquinone-reduction (Q_i) site of the cytochrome bc_1 by antimycin leads to an elevated generation of superoxide at the ubiquinol-oxidation (Q_o) site. However, in the case of the Q_i site, the possibility of superoxide production was left out of consideration. The general paradigm states that the Q_i semiquinone intermediate (SQ_i) is stabilized within the site by a tight network of hydrogen bonds, which makes any reactions with molecular oxygen unlikely.

Herein, we challenge this paradigm. We have used *R. capsulatus* cytochrome bc_1, a common and convenient model for mitochondrial complex III, to investigate potential for ROS generation at the Q_i site. We show that in the cytochrome bc_1, superoxide can also be formed in the Q_i site. Moreover, we show that this deleterious reaction can be enhanced by I49P mutation, a bacterial equivalent of the mitochondrial disease-related mutation S35P.

To further investigate the effect of the mutation, we determined detailed cryo-EM structures of wild-type and I49P cytochrome bc_1 with ubiquinone species bound in the Q_i site. In the I49P, we revealed weakening of the hydrogen bonding network between D252 and the ubiquinone. We identify this change in hydrogen bonding as the factor responsible for the SQ_i destabilization that results in enhanced ROS production.

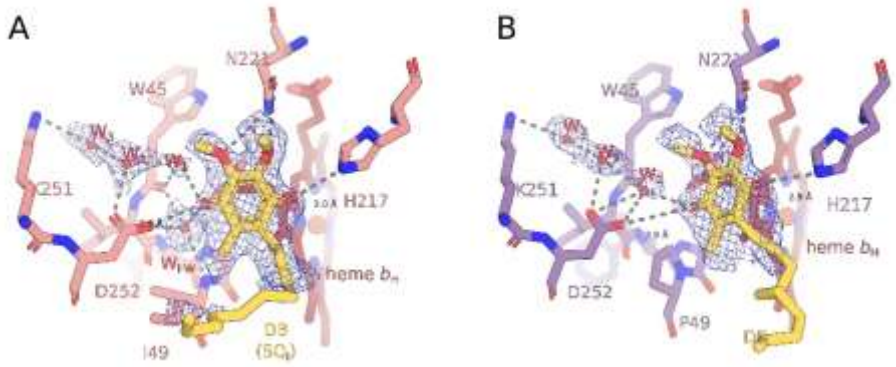

Fig. 1: Structural changes in cytochrome bc_1 active site (Q_i) deriving superoxide production: native form WT (A) and I49P mutant (B).

ON AN UNUSUAL AHARONOV-BOHM EFFECT WITHOUT ENCLOSING A MAGNETIC FLUX

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Recently, it was claimed that the static magnetic vector potential of a toroidal solenoid acts as a lens on an electron wave passing through the field-free torus opening [1]. Arrangements were shown for a converging lens and a diverging lens, and it was claimed that the spherical aberration of an electron-optical system can be corrected with a toroidal solenoid. The hypothetical lens effect was claimed to be caused by the invalidity of Stokes' theorem in a field-free, simply-connected region *R* enclosing the electron wave (Fig. 1), which produces an unusual Aharonov-Bohm effect [2] without enclosing the magnetic flux through the solenoid *S*.

In [3] it was shown, however, that the key results in [1] are incorrect due to a number of basic calculation errors, and that there is no theoretical evidence for the unusual Aharonov-Bohm effect. In response to [3], a particular loop integral of a magnetic vector potential was presented in [4], which was claimed to not vanish although the loop lies in a field-free, simply-connected region. This constitutes a counterexample to Stokes' theorem, and would again support the theory of the unusual Aharonov-Bohm effect.

Yet, the conjecture about the non-vanishing loop integral in [4] is wrong, and the counterexample is therefore invalid. This is shown in [5] using only the field-free condition $\boldsymbol{B}_S = \mathrm{rot}\,\boldsymbol{A}_S = 0$ and the basic rules for parameter integrals, and without using Stokes' theorem. Therefore, Stokes' theorem is not invalidated, leaving no room for the unusual Aharonov-Bohm effect postulated in [1].

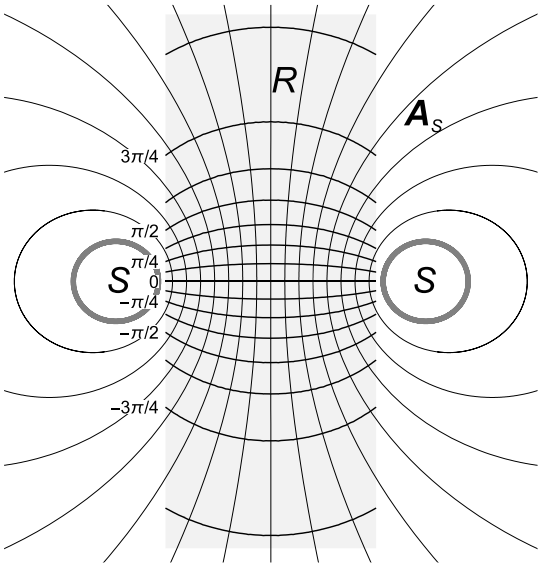

Fig. 1: Cross-section through a cylindrical region R in the magnetic vector potential $\boldsymbol{A}_S$ of a toroidal solenoid S with a poloidal surface current density (thick circles). The labeled contours denote surfaces of constant phase $2\pi q/h \int \boldsymbol{A}_S \cdot d\boldsymbol{r} = 2\pi qf/h$ relative to the horizontal midplane, with the electron charge q, the Planck constant h, path coordinates $\boldsymbol{r}$, and a magnetic flux of h/q in S.

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ULTRASTABLE CRYOGENIC SCANNING ELECTRON MICROSCOPY ENABLED BY A CRYOGEN-FREE JOULE-THOMSON MICROCOOLER

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Preparation and characterization of soft matter and biological samples in their native state is essential for revealing their underlying structural and biological mechanisms. Cryogenic scanning electron microscopy (SEM) and focused ion beam (FIB) instruments have been widely adopted for these applications. However, traditional cryogenic stages, using liquid nitrogen cooled copper braids or cold nitrogen gas, often suffer from long cooling path and large cooling mass, thus longer cool-down time, persisting thermal drift and higher than base cryogenic temperature, as well as mechanical vibration and restricted stage movement. In order to overcome most of these barriers, we introduced a compact, cryogen-free microcooler that leverages Joule-Thomson (JT) effect [1] for cryo-SEM/FIB applications. Followed by overpressure nitrogen gas expansion through a miniaturized MEMS-based throttling device, this system provides on-demand, localized cooling with high thermal gradient and minimal cooling mass. This compact architecture eliminates the need for bulky cryogen storage, significantly reduces cooling loss, in turn enhancing operational stability and flexibility.

To evaluate its stability, we installed such a JT microcooler device on a Zeiss GeminiSEM 460 (Fig. 1) and conducted high-resolution image analysis to quantify thermal drift as well as the mechanical drift across a broad frequency spectrum (up to 5000 Hz). These metrics serve as a benchmark, allowing for a direct comparison among different cooling solutions. The results demonstrate that our JT microcooler solution enables ultrastable, cryogenic temperature and minimum vibration, while having potential for seamless sample transfer within a compact vacuum footprint, offering a versatile solution for cryogenic correlative microscopy.

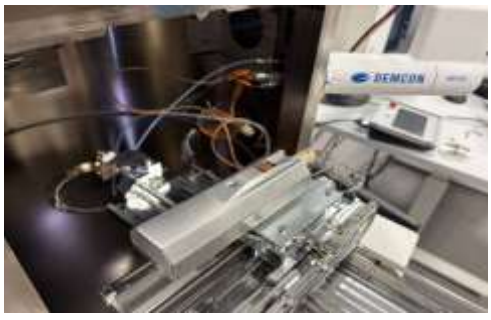

Fig.1: JT microcooler device on a Zeiss GeminiSEM 460

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Time-Resolved Nanoscale Thermal Transport Measurements in STEM

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Measurements of thermal transport at the nanometer scale remain scarce despite their importance for thermal management in semiconductor devices or decoherence in quantum materials. We present a platform for time-resolved nanoscale thermal transport measurements in a Nion HERMES scanning transmission electron microscope (STEM) combining laser excitation with vibrational electron energy-loss spectroscopy (EELS) thermometry [1].
A fiber-coupled laser is integrated via a modified aperture mechanism at an objective lens port, introducing optical excitation without placing optical components inside the polepiece gap. This preserves large tilt angles and compatibility with diverse (in-situ) holders. Pulsed laser excitation is synchronized with an externally gated Dectris ELA direct detector, enabling 50 ns temporal resolution while maintaining sub-10 meV energy resolution and relatively high flux.
Local temperatures are determined from the ratio of energy-loss and energy-gain phonon intensities using the principle of detailed balance. Time-dependent temperature evolution is analyzed with a forward-time central-space heat diffusion model including radiative losses, allowing simultaneous extraction of thermal conductivity and heat capacity. Using amorphous carbon thin films as a model system, we obtain a thermal conductivity of 1.24 W m^{-1} K^{-1} and a heat capacity of 821 J kg^{-1} K^{-1} [1], consistent with literature values.
Moreover, we have also performed the first time-resolved phonon dispersion measurements under non-equilibrium conditions. Fig. 1 shows the setup of the laser-excitation experiment and Fig. 2 phonon dispersions of graphite for two different time windows. The dispersion during heating (1807 K) exhibits stronger gain than the dispersion after cooling for 40 µs (1433 K).
This framework enables quantitative, time-resolved studies of nanoscale heat transport without complex microfabrication and is compatible with in-situ and tomographic experiments.

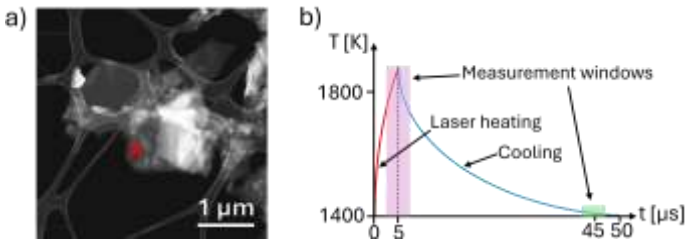

Fig. 1: Setup of time-resolved phonon dispersion measurements of graphite. a) Overview of the flake with marked measurement position. b) Sketch of temperature over time with measurement windows.

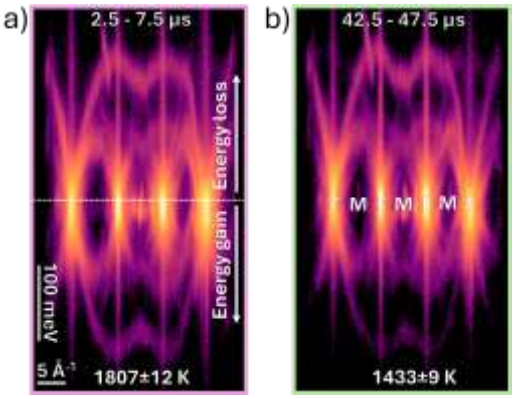

Fig. 2: Time-resolved phonon dispersion of graphite. a) Phonon dispersion for purple time window from Fig 1 b. b) Result for green window.

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A Strategy for Characterizing Domain Walls Motion on Racetrack Memory From 2D to 3D of Iron Nano-printed by Focused Electron Beam Induced Deposition

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Spintronic device development relies on precise control of magnetic domain wall (DW) dynamics in complex nanostructures. Three-dimensional racetrack memory (RTM) offers a promising high-density and energy-efficient approach [1]; however, deterministic DW motion under current-induced operation in 3D architectures remains challenging due to structural failure under current-induced operation. This RIANA-funding [2] project provides a strategy that establishes a solid foundation to study the current-induced DW motion in complex 3D RTM systems while avoiding structural failure associated with FEBID-only fabrication.
Focused electron beam-induced deposition (FEBID) using an FEI Helios Nanolab 660 FIB-SEM at UoG, UK, is used to fabricate Fe-based RTM on MEMS-based TEM DENS-chips through the developed growth methodology of 3D Fe-FEBID in the framework of F3ast [3], as shown in Fig. 1(a). Magnetic imaging of the nanostructures is performed using off-axis holography [4], as shown in Fig. 1(b). To prevent structural failure of the 3D RTM-based chips and to have magnetic imaging from 2D to 3D as shown in Fig. 1(c), a helium ion microscopy (HIM) subtractive process is used to selectively refine FEBID-fabricated structures at the Ion Beam Centre (IBC), HZDR, Dresden, Germany [5]. 3D micromagnetic finite-element simulations using the Tetmag software package are performed to model time-dependent magnetization dynamics in curved **S**-shaped and cornered **V**-shaped ferromagnetic nanostructures as shown in Fig. (2), providing guidance for structural optimization that facilitates controlled DW motion in 3D RTM different geometries [6].

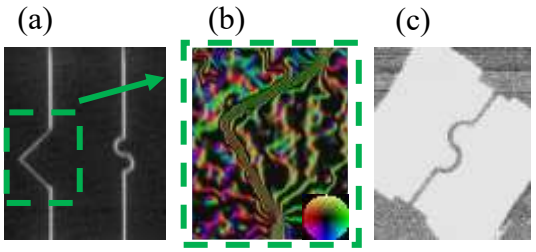

Fig.1: RTM tracks: (a) V- and S-shaped on membrane TEM chips, (b) magnetic induction of a 2D V-shaped, (c) 3D S-shaped formed by HIM milling on the membrane.

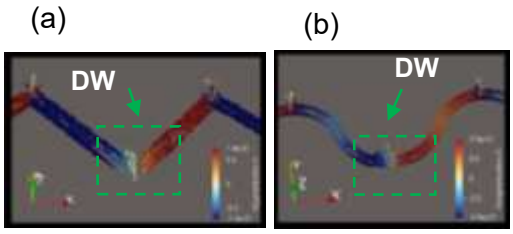

Fig. 2: Tetmag Micromagnetic simulated models of 3D RTM revealing vortex-type DWs (a) cornered-**V**-like structure, (b) curved-**S**-like structure

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MAXIMIZING PHOTOCATALYTIC EFFICIENCY OF CARBON NITRIDES BY TAILORING THEIR LOCAL STRUCTURE

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Poly heptazine imides (PHIs) are 2D ionic carbon nitrides that are recognized as emerging earth-abundant photocatalysts. Their direct application in industry is hampered by several factors, including high recombination rates, deficient charge transport and the lack of absorption above 460 nm. In this work we explore several approaches for fine-tuning the nanostructure of PHIs in order to improve interlayer charge transfer in these compounds and thus their photocatalytic performance: (1) controlled modification of the local crystal structure though the substitution of various cations (M = H^+, Na^+, K^+, Mg^{2+}) in the channels of the organic framework; (2) creating a heterojunction between PHI and other carbon nitrides, e.g. poly(triazine imide) (PTI). Low-dose HR-TEM and ADF-STEM, energy filtered electron radial distribution function analysis obtained from RED data, VEELS and EXELFS analysis enabled us to obtain a detailed understanding of the local structure, coordination around different atoms, chemistry and electronic properties of modified PHIs and to correlate them with photocatalytic activity.

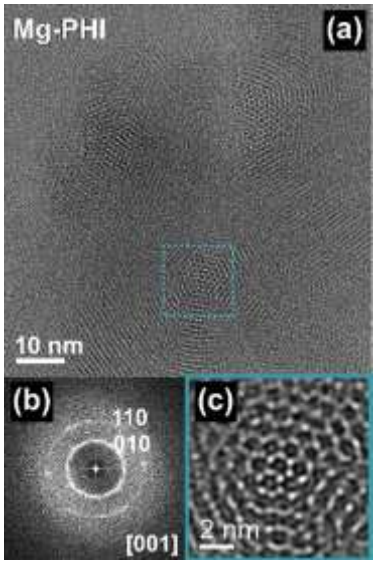

Fig. 1: (a) “flower-like structures” with rotation of layers by a random angle (usually 2-8°); (b) FFT pattern from (a). enlargement from (a).

For M-PHIs with various cations (M = H^+, Na^+, K^+, Mg^{2+}) we found that both the short-range order (due to the sizes and charges of the solvated metal cations) and the medium-range order (due to the formation of twist domains between carbon nitride layers, Figure 1) influence the photocatalytic H_2 evolution rate. Na-PHI displays the best performance among other PHIs with metal cations in oxidation state +1, as the size of the solvated Na^+ cation and the rotational defects enable cations to occupy positions in the channels equidistantly from N in polyheptazine layers. The combination of twist defects and the even higher charge of Mg^{2+} promotes a faster interlayer charge transfer and a more efficient polarization of the heptazine backbone in Mg-PHI, leading to an unprecedented quantum efficiency of 7.14% [1].

Site defects and the structural alignment of two phases at the interface are the main reasons of enhanced photocatalytic O_2 reduction activity of the PTI/K-PHI heterojunction compared to pure constituent phases. The analysis of SE- and ADF-STEM images, ELNES, EXELF, and of the general thermochemical behavior of precursors enabled us to conclude that PHI crystallites grow on PTI layers formed at a gas-liquid interface in the salt melt following the $[001]_{PTI}/[001]_{K\text{-}PHI}$ orientation. This crystallographic alignment promotes the charge transfer from PTI to PHI creating an electron-reach interface [2].

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Magnetic interactions in van der Waals heterostructures

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Magnetic van der Waals (vdW) materials are promising for memory and logic applications because their properties are highly tunable and they integrate readily into heterostructures that exploit proximity and interlayer effects. However, depth-resolved imaging of interlayer coupling is challenging in plan-view, where contrast is integrated through the sample. Here we use cross-sectional Fe_3GeTe_2 (FGT)/graphite/FGT heterostructures to probe coupling between vertically stacked FGT layers across separations of 0-110 nm. We use Lorentz TEM and off-axis electron holography to visualize the domain structure (Fig. 1(a, b)) and map the magnetic field inside and outside the vdW heterostructures (Fig. 1(c, d)), resolving how the interaction evolves with separation distance. We find that domain alignment between FGT flakes is set by their separation, and that a weak interaction persists even at a separation of 110 nm. These measurements provide design guidance for vdW heterostructure devices that rely on controlled interlayer interactions between magnetic textures.

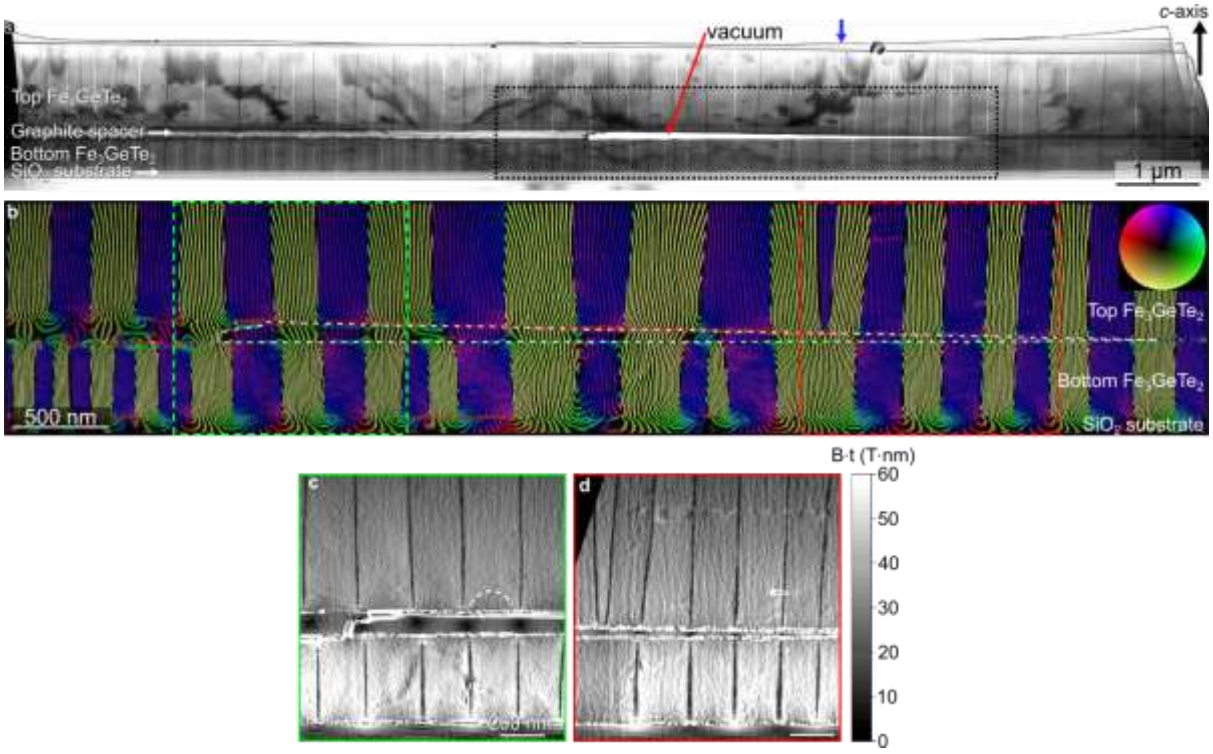

Fig. 1: (a) Lorentz TEM image obtained at 95 K with ~0.3 mm defocus after zero-field cooling. The blue arrow indicates the position of the first defect in the domain wall alignment between the top and bottom FGT layer. (b) Magnetic contour map acquired at 95 K by off-axis electron holography. The contour spacing is 2π/7 rad. (c, d) In-plane projected magnetic induction calculated from the regions indicated by the green and red rectangles in (b). The striped white arc highlights a typical surface region with reduced magnetic induction.

Enhancing Temporal Resolution in Ultrafast Transmission Electron Microscopy Using a V-Band Cavity

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Ultrafast transmission electron microscopy (UTEM) has revolutionized our ability to probe materials at femtosecond time- and nanometer length scales. To illustrate, time-resolved bright-field UTEM can capture the propagation of strain-waves across nanostructures [1], and photon-induced nearfield electron microscopy (PINEM) can yield optical phase-resolved dynamic movies [2]. Despite its capabilities, a major hurdle in UTEM remains the degradation of electron pulse properties due to Coulomb repulsion and dispersive propagation [3]. To counteract this, large-scale particle accelerators and ultrafast electron diffraction (UED) instruments utilize microwave cavities to achieve temporal compression [4, 5]. In UTEM, however, these cavities have only been used to chop the continuous beam of a conventional electron source [6], not for shaping and compressing the phase space of a pulsed electron source.

In this contribution, we demonstrate the use of a V-Band microwave cavity to manipulate the longitudinal phase space of electron pulses produced by femtosecond photoemission in our UTEM [7]. Operating in the TE_{102} resonant mode with a simple box geometry, this cavity facilitates both longitudinal control and transversal streaking. Additionally, the cavity is built into a custom TEM sample holder equipped with a built-in rectangular waveguide to ensure efficient microwave delivery.

To characterize the cavity, we perform microwave reflection and transmission measurements and map the internal cavity fields by spectroscopic imaging. By generating the driving microwave signal directly from the femtosecond laser with an ultrafast photodiode, we achieve intrinsic synchronization [8]. Following this, we assess the residual jitter between the microwave field and the laser using event-based, energy- and time-resolved electron detection.

Incorporating cavity-based phase space control will overcome the space-charge limitations of electron pulses in the UTEM, thereby allowing operation at higher flux and temporal resolution. Moreover, leveraging the microwave cavity to shape the phase space of the electron probe creates unique opportunities for novel temporal and spectroscopic studies.

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Megaelectron-Volt Ultrafast Electron Microscope the Future of Electron Imaging

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Aberration correction electron optics and cold field-emission electron source made the transmission electron microscope (TEM) a popular tool to image atomic and nano-scale objects. Cryogenic electron microscopy (Cryo-EM) revolutionized the bio-structure science, and recently it is explored to investigate radiation-sensitive battery and energy materials. But non-physiological environments, sample damage and electron beam-induced sample movements greatly limit the science impact of both TEM and Cryo-EM. To address those challenges, we propose to develop ultrafast electron microscope based on megaelectron electron beams (MeV-UEM). The development of high-brightness electron sources made it possible to explore megaelectronvolt electrons for Ultrafast Electron diffraction and Microscope (MeV-UED/UEM) [1-2]. MeV-UED [3-4] had produced broad and transformative impact on ultrafast science, such as the first 2-D materials ultrafast structure dynamics [5], light-induced transient states [6,7], molecular movies of canonical interception & ring-opening [8-9], and the first hydrogen bond structure dynamics in liquid water [11]. The proposed MeV-UEM is capable of single-shot imaging with atomic spatial resolution (0.3 nm) and sub-nanosecond temporal resolution. With sub-ns pulse duration, the proposed MeV-UEM will eliminate the imaging blurring by the beam induced sample movement and fast backbone protein movement. Using MeV electrons will lead to much less sample damage, open the possibility of imaging thick biological samples in its native environments. We will present the plan of employing accelerator technologies, such as superconducting photocathode RF gun, novel electron optics and high field magnet to realize MeV-UEM.

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LIGHT-ENHANCED ELECTRON MICROSCOPY: ATOMIC-SCALE LABELLING OF CHEMICAL FUNCTIONALITY

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Transmission electron microscopy (TEM) allows atomic-scale resolution imaging across a variety of disciplines. In biology and medicine, cryo-EM facilitates reconstruction of the structure of complex proteins and organelles[1]. In chemistry, environmental TEM (ETEM) enables the investigation of heterogeneous materials under reactive gas environments and elevated temperatures, providing insight into catalytic transformations[2,3].

Despite their success in determining **structure** and **composition**, TEM-based techniques remain largely unable to correlate these fundamental properties with **function** at the atomic scale. This limitation constitutes a major barrier to further progress, as functional information, *i.e.*, how specific sites participate in a process, cannot be inferred from structure alone. In catalysis, understanding function is critical for the rational design of catalytic nanoparticles. In biology, locating where certain proteins are expressed inside a cell would accelerate drug discovery.

We develop a new generation of electron microscopy modalities: correlating structure and composition with function at the atomic scale. The concept relies on tunable **light injection** into frontier cryo- and environmental-TEM platforms as a new route to create the desired **functional contrast** (Fig. 1). Light excitation induces changes in the **projected electrostatic potential landscape**, arising from light-responsive electronic or dipolar states, localized at distinct atomic sites with specificity that depends on the light wavelength and can thus be tuned during experiment to distinguish between sites of different functionality. The light-activated changes to the potential landscape are then imprinted onto the probing electron wavefront and retrieved using phase-sensitive methods such as electron holography and ptychography.

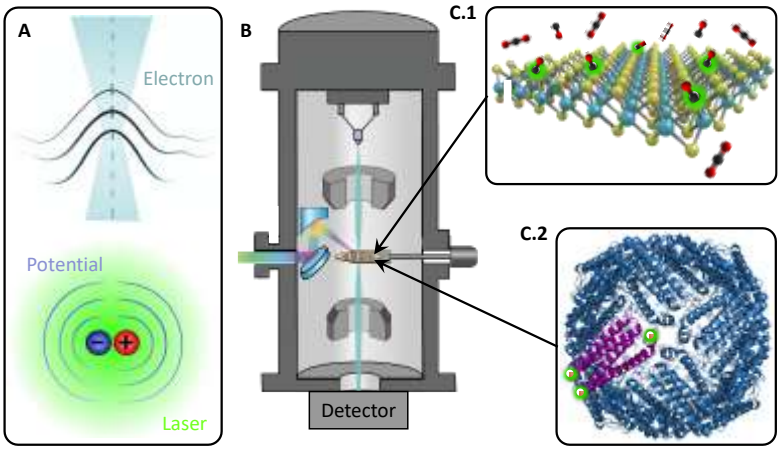

Fig. 1 | Light-enhanced electron microscopy for labeling of chemical functionality. (**A**) Light irradiation on a specimen (green) generates carrier redistribution, changing the local potential landscape (bottom), which is imprinted on the electron probe wavefront (top). (**B**) A specialized light injection module (called "Stork") is being developed and installed in a cryo-EM and an environmental TEM (ETEM). (**C**) Proposed proof-of-concept experiments in catalysis (C.1), and biomolecular labeling (C.2), each using a dedicated marker that reacts selectively to excitation by light of a particular wavelength.

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The role of DFCP1 in replication organelle membrane biogenesis in SARS-CoV-2

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Double-membrane vesicles (DMVs) are dedicated replication organelles responsible for the RNA synthesis of many positive-strand viruses like SARS-CoV-2. While viral proteins nsp3, nsp4, and nsp6 are essential for DMV formation, the involvement of host factors required for this process remains poorly understood.
Here, we identify DFCP1, an autophagy-associated protein involved in omegasome constriction, as a key host regulator of DMV biogenesis [1]. Confocal microscopy studies of overexpressed DFCP1 in VeroE6 cells showed that DFCP1 localized adjacent to lipid droplets, influencing their size and abundance, an effect further elevated during DMV biogenesis. In situ cryo-electron tomography of cells overexpressing DFCP1 revealed extensive ER membrane remodeling, including small vesicle shedding into the cytosol. Using a minimal DMV-inducing system based on viral nsp3–4 and nsp6, we observed that DFCP1 overexpression increases DMV size, while the ATPase-deficient mutant K193A leads to severe DMV deformation. Cryo-ET further captured multiple intermediate states of DMV biogenesis, showing that DMVs emerge from tethered ER membranes. These observations support a mechanistic model in which DFCP1 coordinates lipid droplet dynamics and ATPase-driven ER remodeling to promote proper DMV formation.
Together, our findings establish DFCP1 as a dual regulator of DMV biogenesis, linking lipid droplet homeostasis with membrane remodeling during viral replication. This work advances our understanding of virus–host interactions, connects autophagic membrane remodeling to viral replication organelles, and identifies DFCP1 as a potential antiviral target.

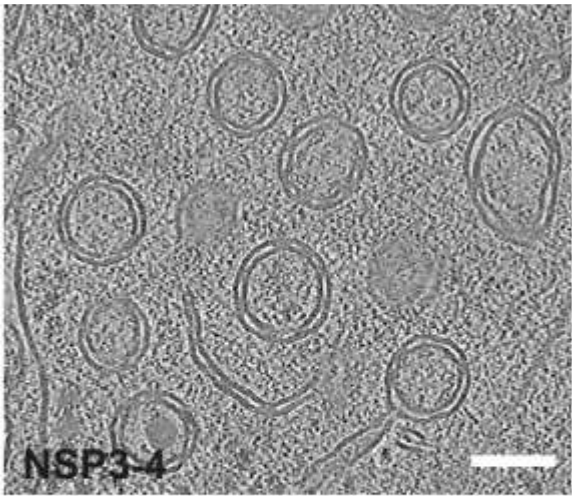

Fig. 1: DMV cluster induced by nsp3 and nsp4

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CRYO-ELECTRON TOMOGRAPHY SAMPLE PREPARATION AT THE ERNST RUSKA-CENTRE JÜLICH

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The Cryo-Electron Microscopy (cryo-EM) facility at the Ernst Ruska Centre (ER-C), Forschungszentrum Jülich, Germany, is a premier national user facility offering global access to state-of-the-art cryo-EM infrastructure. Our instrumentation suite includes advanced transmission electron microscopes (Thermo Fisher Krios G4, Arctica G2, Talos120) and scanning electron microscopes (Aquilos 2 with Delmics METEOR in-column fluorescence module and CERES ice shield and Arctis cryo-plasma-FIB with iFLM), supporting a wide range of applications such as negative staining, single-particle cryo-EM, cryo-electron tomography, life science STEM, and cryo-correlative light and electron microscopy (cryo-CLEM).
Complementing our microscopic capabilities, the facility hosts comprehensive sample preparation equipment including the Vitrobot Mark IV+, Leica EM GP2, CryoSol VitroJet, and Wohlwend Compact 3 high-pressure freezer. We provide end-to-end workflow access—from sample preparation to image acquisition—with minimal supervision for experienced users and robust training or full service for newcomers. Since 2022, over 100 external projects have been supported globally. Looking ahead, the ERC2.0 initiative, funded by BMBF, will expand our infrastructure with additional high-end instrumentation, further strengthen CLEM applications. This poster presents ongoing projects utilizing the Aquilos 2 system and Arctis cryo-plasma-FIB, along with initial results establishing Wohlwend Compact 3 high-pressure freezer workflows.

Cryo-EM Instrumentation at the Ernst Ruska-Centre: Enhanced Platforms for Cryo-STEM and -CLEM Workflows

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The cryo-electron microscopy (cryo-EM) facility at the Ernst Ruska-Centre (ER-C), Forschungszentrum Jülich, Germany, is a leading national user facility providing global access to cutting-edge cryo-EM infrastructure. The facility's instrumentation supports a broad range of structural biology methods, including single-particle analysis, tomography, cryo-scanning transmission electron microscopy (cryo-STEM), and cryo-correlative light and electron microscopy (cryo-CLEM).

Cryo-focused ion beam (FIB) systems include an Arctis plasma FIB and a modified Aquilos 2, equipped with Meteor and Ceres systems (Delmic), enabling efficient in situ fluorescence imaging. TEM resources include a Talos 120 and Talos Arctica for screening and conventional imaging, as well as a STEM-optimized Krios G4 with Arina and Panther detectors for high-contrast, low-dose cryo-(S)TEM. Since September 2025, the installation phase of a helium-cooled, double aberration-corrected Krios (BioTool) with combined STEM/TEM capabilities has started, designed to explore and push the boundaries of resolution, contrast, and dose dependencies in cryo-(S)TEM workflows.

Comprehensive sample preparation tools – Vitrobot MK4+, Leica EM GP2, VitroJet and Wohlwend HPF – supports the entire pipeline. Since 2022 over 100 external projects have been hosted, engaging both experienced structural biologists and early-stage trainees.

Read more: https://er-c.org/index.php/facilities-2/life-science/

Fig. 1: BioTool microscope

Fig. 2: User training on Arctica

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Instruct-ERIC: Get access to advanced structural biology services

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