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3D Cryo-Electron Tomography Studies on Microtubule – MAP Interactions in vitro and in situ

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Microtubules are highly dynamic cytoskeletal structures that are involved in various cellular functions, thereby interacting with a diverse group of proteins. These proteins or protein complexes are typically grouped into molecular motors (kinesins, dyneins) and non-motor MAPs (Tau, TPX, EB1 etc.). Many of these proteins, including the dimeric microtubule building block αβ-tubulin itself have been extensively studied in vitro and their structure has been solved to near atomic detail. Many kinesin motor head domains and some MAPs have been analyzed in complex with microtubules by 3D cryo-electron microscopy (cryo-EM). Most of the cryo-EM studies were performed in vitro and with so-called 3D averaging procedures such as helical reconstruction, assuming a particular (e.g. helical) symmetry in the complexes. However, the recent rise of cryo-electron tomography (cryo-ET), promoted by new microscopy hardware such as large sensitive detectors, but also by new developments in tomographic reconstruction software opened new avenues for 3D studies on large intracellular organelles and macromolecular assemblies. Also, cryo-sectioning performed on vitrified samples without the need for substitution and embedding prior to sectioning is an emerging technology that opens completely new possibilities in observing cellular structures at physiological conditions. Here an outlook is presented into the possibilities as well as the current limitations of cryo-ET and vitrified sectioning for high-resolution structural analysis in cell biology. New in vitro and in situ labeling methods are now being developed to mark protein complexes by high-density labels such as little metal clusters, which are either large enough for direct visualization, or detected by difference mapping. The parallel assignment of multiple labels may be achieved by electron energy-loss spectroscopy. This will allow labeling approaches for electron microscopy that are similar to the simultaneous use of different colors in fluorescence light microcopy.

One of the most challenging aspects when leaving the simple environment of an in vitro approach and venturing into the complex environment of an intact cell is the assignment of density boundaries between complexes as well as the unambiguous identification of structural elements within a complex. To this end we present our 3D study on the ventral disk of *Giardia*. *Giardia intestinalis* is a unicellular, flagellated parasite of mammals, infecting ~1 billion people worldwide. Giardiasis is caused by ingesting water contaminated with cysts, which then excyst into trophozoites in the duodenum of the host. The trophozoite has a highly specialized microtubule (MT) cytoskeleton—4 pairs of structurally distinct flagella, and the ventral disc (VD). Trophozoites attach and detach from intestinal epithelia by a coordinated action of the cytoskeleton within the VD. Our large-area tomography data shows the VD consists of an array of ~45 parallel MTs that forms a counter-clockwise spiral ~8.5µm in diameter. Each VD MT has a micro-ribbon (MR) that extends ~200nm from the dorsal side of the MT into the cytoplasm and occurs along the entire length of the MT (3-7um). MRs connect laterally to each other via bridging elements. We have isolated the cytoskeletons of trophozoites and prepared them for cryo-electron tomography. We used our volume-averaging program, PEET, to average segments of 16nm long axial repeats (2 αβ-tubulin dimers) along VD MTs. Averages were calculated either from individual tomograms, or included all 6 tomograms (7400 repeats). A large protein complex, called the side-arm is found every 8nm (1 αβ-tubulin dimer) along the length of VD MTs. The side-arms span 5 protofilaments and have various linkers among them and to their neighboring side-arms, such that all are connected together along the length of the MT. The MRs are made of 3 parallel sheets with repeating structures along their length. There is a linker-bridge between the MR and a protofilament of the MT every 16nm. Individual tomograms have averages that are distinct from each other, with the greatest variety occurring in the side-arms. Our data suggests that an estimated 25,000side-arms are working in concert with each other and the MRs to induce coordinated conformational changes within the VD driving attachment and detachment.

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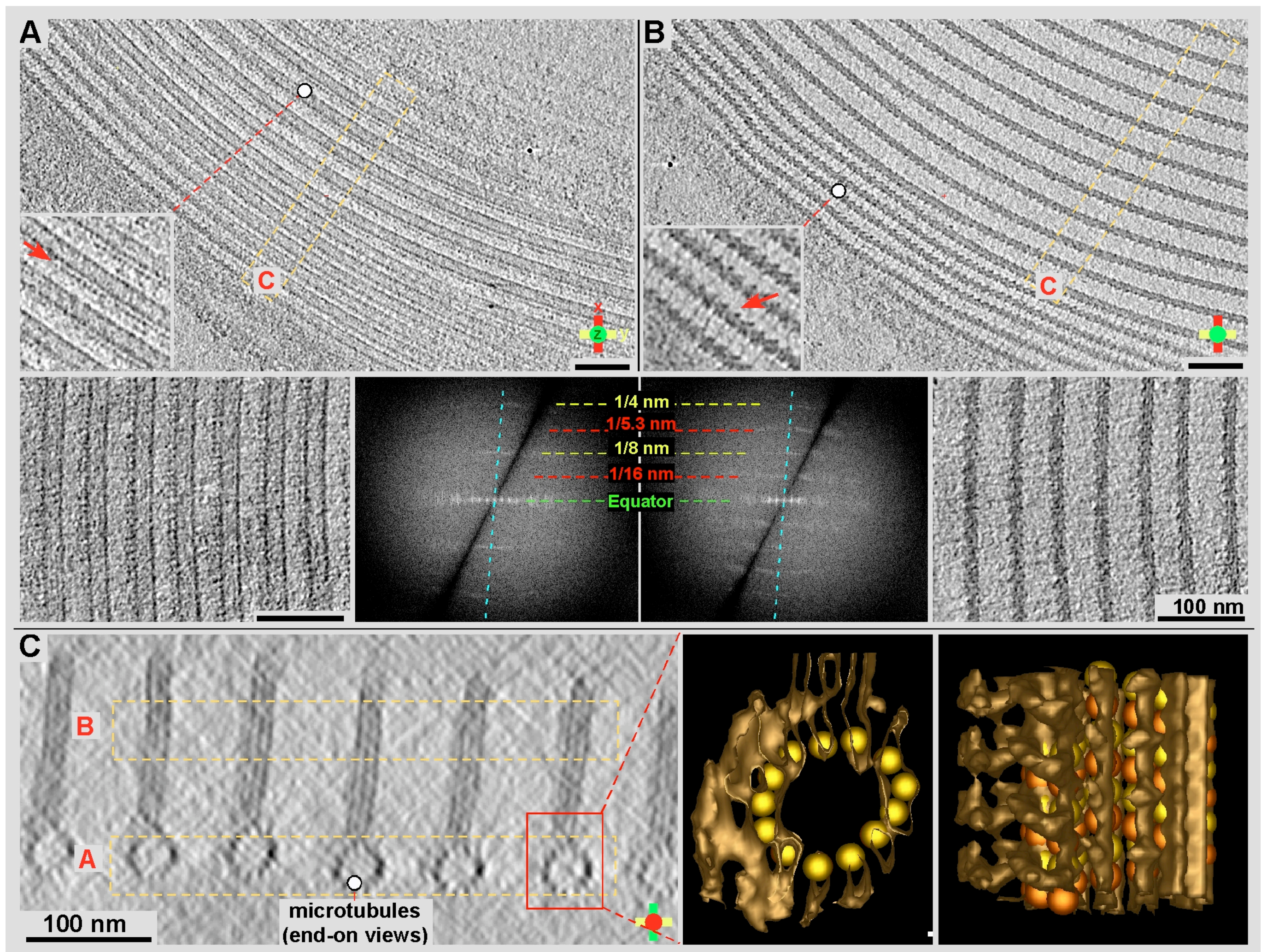

Figure 3: Cryo-electron tomography on isolated and vitrified ventral discs revealed the microtubule and microribbon architecture of this unique structure. **A** and **B** show different tomographic slices in top-view (projection perpendicular to the disc plane) of the disc. **C** shows a side view of the disc and an averaged 3-D reconstruction end-on and from the side. Frames A and B in **C** correspond to the top-view slices in **A** and **B** respectively. **A:** a plane within the microtubule layer reveals a strong decoration along tubes towards the cell-periphery side of the disc (see arrow in upper inset) while the face on the cell-interior side of the disc is essentially lacking any protein density. Diffraction of the selected area in lower inset reveals an 8-nm layerline pattern typical for the 8-nm ab -tubulin repeat in microtubules. **B:** slice through the microribbons reveals cross-bridges between them that appear to be responsible for narrowing the lateral distance between the microribbons near the outer edge of the ventral disc, suggesting a conformation change of the ventral disc could be responsible for *Giardia* attachment to intestinal microvilli. Diffraction of the selected area in lower inset reveals a 16-nm layerline pattern indicating a repetitive pattern consistent with the length of two 8-nm $\alpha\beta$-tubulin repeats in microtubules **C:** Side views show clearly the three sheets that form the microribbons, and their connection to the microtubules that is slightly offset to cell-periphery side of the microtubule in **A**. Due to the missing wedge of data in these tomograms the resolution is lower along the projection axis (z-axis) and therefore the cross-bridges visible in B are not resolved in **C**.

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