

Cryo-electron tomography of the apicomplexan invasion machinery in its native state reveals rigid body motion of the conoid and docked secretory machinery

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The apical complex is a conserved cytoskeletal structure that organizes the secretory and invasion machinery of all apicomplexan parasites, the causative agents of diseases such as malaria, toxoplasmosis, and cryptosporidiosis. Recent advances in cryo-electron tomography (cryoET) have allowed the high-resolution reconstruction of large complexes in their near-native states. Here, we use cryo-focused ion beam milling to generate a thin specimen of the coccidian apical complex, allowing us to visualize and compare its three-dimensional structure in the protruded and retracted states in situ by cryoET. Subtomogram averaging of the 8 nm repeats from the conoid fibers revealed the unusual nine-protofilament arrangement of tubulin and the tubulin-associated proteins that connect and likely rigidify the fibers. In spite of the open shape of the conoid fibers, neither the structure of the conoid spiral nor the tubulin fibers that comprise it are altered during cycles of protrusion and retraction. Thus the conoid moves as a rigid body, and is not compressible like a spring, as has been previously suggested. Instead, we found that the apical polar rings (APR), previously considered a rigid structure to which the conoid is docked, dilates during conoid protrusion. Furthermore, we identified previously undescribed actin-like filaments connecting the conoid to the APR during protrusion, consistent with a role of actin in driving conoid movements. In addition, our data capture the parasites in the act of secretion during conoid extension, allowing us to identify a network of interactions between the apical complex cytoskeleton and the parasite's secretory machinery.