Cryo-EM single particle analysis meets cryo-ET at the sperm tail

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The advent of cryogenic-electron microscopy (cryo-EM) as a key tool for structural biologists has brought forth a whole range of applications to gain structural information at high-resolution. Cryo-EM single-particle analysis (SPA) approaches typically entail overexpression and purification of the target protein. Larger and more complex molecular assemblies often require extensive optimisation of expression, purification and reconstitution procedures. Additionally, prior knowledge of the composition of the structure of interest is required. In-situ approaches employing cryo-focused ion beam (FIB) milling and cryo-electron tomography (cryo-ET) have proven incredibly useful in exploring protein structures within cells, in some cases even at high-resolution. Such in-situ strategies do not require purification of the target protein or protein complex yet are often still limited in throughput and achievable resolution. During my talk, I will show how we expand the range of samples attainable for SPA towards more native samples, specifically towards complex macromolecular assemblies that cannot be reconstituted, and show how SPA is used as a discovery tool for de-novo protein identification.