Direct observation of three-dimensional ferritin crystallization with molecular resolution

Houben, L.[1], Weissman, H.[1], Wolf, S.G.[1] and Rybtchinski, B.[1]

[1] Weizmann Institute of Science, Israel

The formation of crystalline order in ferritin aggregates too small for diffraction studies was investigated using a novel approach that combines three-dimensional tomographic reconstruction with order parameter analysis. This work was driven by the need for a fundamental understanding of crystal growth of weakly interacting proteins on a molecular level. At present, only tentative ideas exist about interactions in particular in solution and during crystallization. Offering simplicity and controllability the condensation of ferritin molecules from solution was chosen to get insight into the nucleation and crystallization mechanism that lead to ordered crystals.

The approach (Fig. 1) involved cryo-fixation of aging stages during ferritin condensation from solution and subsequent three-dimensional tomography in the STEM. Tomograms were analysed to yield the three-dimensional ferritin molecule coordinates for the real-space refinement of the structure of ferritin agglomerates. Site-specific order on the molecular level within the ferritin agglomerates was refined quantitatively by order parameter analysis based on a data-base correlation technique.

Our studies elucidate a mechanism of ferritin crystallization, revealing a critical role of initial protein aggregation and gradual ordering in ferritin aggregates. Gradual ordering occurs in parallel with densification in the inner parts of the aggregates. The involvement of amorphous aggregates and ordering occurring via desolvation is in agreement with two-step nucleation theory.

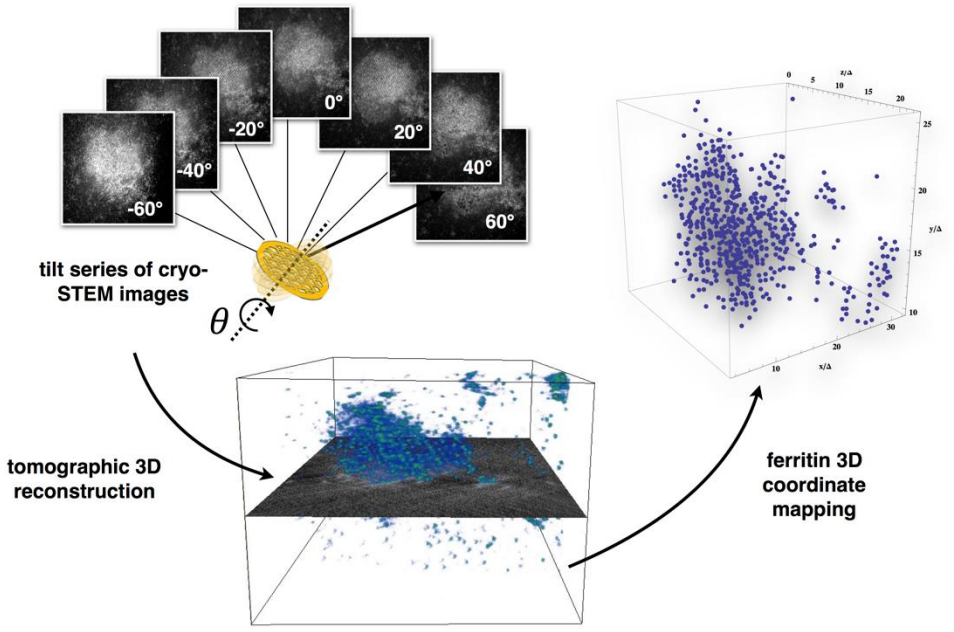

Fig. 1: Direct visualization and quantification of three-dimensional ferritin crystallization with molecular resolution. Snapshots of aging stages of the crystallisation are preserved in a cryo-sample for STEM tomography. Volume reconstruction and peak refinement yields centre-of-mass coordinates for each ferritin molecule with Fe-core in all three dimensions for quantitative order analysis.