

## Thursday 2 July 2020 1.00 pm



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## Studying Phase Separation In Cellulo using Cryo-Electron Tomography: Endocytosis & Ribosome Biogenesis

Liquid-liquid phase separation (LLPS) is emerging as a new concept of cellular organization without the need for membranes to separate and control biochemical processes. As such, LLPS compartments are notoriously hard – if not impossible – to purify without endangering their structural integrity. We therefore use in situ cryo-electron tomography, which consists of cryo-focused ion beam (FIB) milling in conjunction with cryo-electron tomography (cryo-ET), and a quantitative analysis of cellular constituents using visual proteomics, to study sites of LLPS in cellulo. Some of these can be rare, or confined to specific locations within a cell. They therefore require 3D-targeted FIB milling to not be missed.

We here describe two examples of cellular liquid-liquid phase separation to which we apply these advanced techniques: the nucleolus, which has a central role in ribosome biogenesis, and the newly discovered END compartment, which serves as a buffer for proteins involved in endocytosis, when its normal function is impaired. In both cases, in situ cryo-ET reveals molecular details of the underlying cellular processes and helps to put them into context with the overall cellular organization and biochemical analysis. Therefore, in situ cryo-ET can serve as a powerful tool for structural biology of LLPS compartments, bridging the gap between fluorescence microscopy and single particle cryo-EM.

Host: Prof. Dr. Patrick Cramer



Zoom access data will be mailed before the seminar