Mapping of phase separation of supramolecular protein assemblies by live-cell holotomography microscopy

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Supramolecular protein assemblies (SMPAs) have demonstrated to be a great tool for studying the biophysical properties of human cells. Liquid-liquid phase separation is a driving mechanism in the assembly of various intracellular structures. An oligomerizing biomimetic system, known as Corelets, has helped to map phase separation within the cell subregions by means of protein condensation. Corelets are photosensitive phase-separating photoinduced oligomers of self-interacting proteins. Previous studies on protein condensation by localized oligomerization showed that sequestering protein ligands to slowly diffusing nucleation centers move the cell into different regions of the phase diagram, resulting in localized phase separation. The real-time oxidative-induced stress in human cells has also remained an enigma in cellular biology. A previous work on the redox state of the cell showed that supramolecular protein assemblies have a self-assembly interface sensitive to the exposure to a thiol-specific oxidizing reagent. We worked with a system of self-associating proteins built onto a ferritin core, genetically encoded for the expression of supramolecular protein assemblies based on a fusion construct between citrine, a yellow fluorescent protein variant and the heavy chain human ferritin. Here, we use a novel 3D label-free non-invasive fluorescent live-cell imaging method, which allows performing high-precision in-vivo holotomographic microscopy of subcellular structures, coupled with cryo-electronic tomography and image processing to evaluate real-space structure of solid and liquid localized phase separation and of SMPA oxidation within the cell nucleus; which allows us to use the refractive index as a method to map the emergence and prevalence of the Corelets in the cells. Our work has allowed the three-dimensional visualization of phase separating condensates in mammalian cells.

Keywords:

Protein condensation, label-free, refractive index

Reference:

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