Time-resolved observations of liquid-liquid phase separation at the nanoscale using in situ transmission electron microscopy

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Liquid-liquid phase separation (LLPS) of proteins into concentrated microdroplets (also called coacervation) is a phenomenon that is increasingly recognized to occur in many biological processes, both inside and outside the cell [1]. While it has been established that LLPS can be described as a spinodal decomposition leading to demixing of an initially homogenous protein solution, little is known about the assembly pathways by which soluble proteins aggregate into dense microdroplets. Using a recently developed technique enabling the observation of matter suspended in liquid by transmission electron microscopy (TEM), we observed how a model intrinsically disordered protein (IDP) phase-separates in liquid environment. The model protein used in these experiments is Histidine-rich Beak Proteins 2 (HBP-2), a protein present in the beak of squids that can be recombinantly obtained [2]. Our observations reveal for the first time dynamic mechanisms by which soluble proteins self-organize into condensed microdroplets, with nano-scale and milli-second space and time resolution, respectively. With this method, the nucleation and initial growth steps of the LLPS could be captured, opening the door for a deeper understanding of other biomacromolecular complexes exhibiting LLPS ability [3].

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