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**Time-resolved observations of liquid-liquid phase separation at the nanoscale using *in situ* liquid
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.

[1] Y. Shin and C.P. Brangwynne, Liquid phase condensation in cell physiology and disease, *Science*, **357**, 1253 (2017).

[2] H. Cai, B. Gabryelczyk, M.S.S. Manimekela, G. Gruber, S. Salentinig, A. Miserez, Self-coacervation of modular squid beak proteins – a comparative study, *Soft Matter*, **13**, 7740 (2017).