

Driving tau into phase-separated liquid droplets

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Liquid–liquid phase separation of tau protein has been implicated in normal biological function as well as neurodegenerative diseases, including Alzheimer's. However, knowledge about these links is still scant, and the mechanisms driving tau into liquid droplets are poorly understood. A simplified *in vitro* system that uses unmodified human tau protein now suggests electrostatic interactions provide the basic instructions underlying liquid droplet formation.

The assembly of membraneless organelles through liquid–liquid phase separation (LLPS)² is a new paradigm for organizing biochemically relevant processes in the interior of cells (1, 2). LLPS results in droplets with elevated concentrations of proteins and nucleic acids. P-bodies, nucleoli, and stress granules are a few examples. Aberrant changes in droplet properties have been linked to various neurodegenerative diseases (3). Recently, it was discovered that tau, a protein found in the pathological filaments of Alzheimer's disease (AD), undergoes LLPS as well (4), a process that could be relevant to AD as tau droplets could convert into aggregates to establish the initial sites of filament assembly. Soluble tau is intrinsically disordered and possesses the multivalent characteristics one might expect from a protein that separates into different phases. However, the molecular mechanisms leading to tau droplet formation are poorly understood. Boyko *et al.* (5) now shed new light on the process in their demonstration that intermolecular electrostatic interactions between the negatively charged N-terminal region and the positively charged middle/C-terminal region of tau are the major forces driving the protein into droplets.

Initial work on tau liquid–liquid phase separation involved mixtures of tau and RNA (4), inspired by the observation that nucleic acids are often part of membraneless organelles. These experiments were recently expanded, indicating that tau can phase-separate with RNA under physiological conditions (6). Despite the tremendous insights gained from these studies, one caveat is that the majority of experiments were conducted with a construct of tau that lacked the negatively charged N-terminal region. Two other studies utilized tau protein expressed in

insect cells (7, 8). A special feature of tau purified from this system is that it is hyperphosphorylated, partially mimicking the phosphorylation status of tau in AD. Notably, tau protein isolated from this source was able to undergo liquid–liquid phase separation even in the absence of RNA. However, the physical properties of the tau droplets in the two studies were dramatically different with respect to their stability at elevated ionic strengths (7, 8), suggesting that variations in phosphorylation or other posttranslational modifications may have altered the interactions between tau molecules in the droplets.

The study by Boyko *et al.* (5) provides a fresh look at the interactions governing liquid–liquid phase separation of tau because it succeeds in forming phase-separated droplets of tau using recombinant, unmodified protein expressed in *Escherichia coli*. The authors used the largest of the six tau isoforms, tau441, also referred to as 2N4R or htau40. First, the authors demonstrated that physiologically relevant concentrations of tau (2–10 μM) and ionic strengths (150 mM NaCl) combined with a reagent that mimics intracellular crowding (PEG), led to spontaneous LLPS of tau. The molecules in the droplets were highly dynamic as judged by fluorescence recovery after photobleaching. Droplet size increased with protein concentration but decreased with ionic strength, pointing toward electrostatic interactions as the main drivers of phase separation. Markedly, 1,6-hexanediol, which inhibits formation of other LLPS systems and is thought to perturb hydrophobic interactions, had little effect on the formation and shape of liquid droplets, suggesting that under these conditions, hydrophobic interactions play only a negligible role. Importantly, this is in marked contrast to what had been observed for LLPS of tau isolated from insect cells (8). Additional experiments revealed that tau droplets dissolved with increasing temperature, a phenomenon associated with an upper critical solution temperature and suggestive of enthalpic contributions between protein segments. Surprisingly, these findings are opposite to what has been observed for tau/RNA droplets, which effectively dissolved with lowering temperature (6). Together, the observations suggest that the physical properties of tau droplets formed in these experiments are vastly different.

To pinpoint the interactions that lead to droplet formation of recombinant tau, Boyko *et al.* (5) next employed a set of well-designed constructs that lacked specific regions in the protein. Removal of the negatively charged N-terminal region (residues 1–117) or alternatively the positively charged C-terminal region (residues 118–441) completely abrogated droplet formation. Remarkably, when these two truncated proteins were added together, droplet formation was reestablished, indicating that interactions between the positively charged region of one

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² The abbreviations used are: LLPS, liquid–liquid phase separation; AD, Alzheimer's disease.

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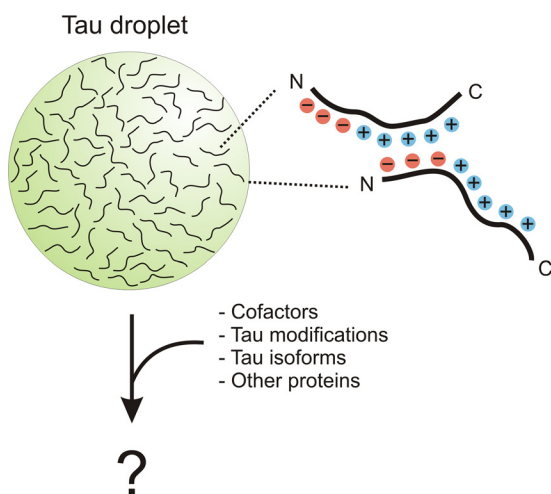


Figure 1. Model of liquid tau droplets and the effects of perturbations.

Droplets composed of tau441 are held together by intermolecular electrostatic interactions between the negatively charged N-terminal region and the positively charged middle/C-terminal region. It is expected that partitioning of different biomolecules into the preformed droplets could change the droplets' size, shape, and dynamics because of altered molecular interactions. The initial presence of these molecules, prior to liquid-liquid phase separation, could influence droplet formation itself (not shown). These effects can now be systematically studied, one class of molecules at a time.

protein and the negatively charged region of the other protein, drive LLPS. Interestingly, removal of the entire repeat region of tau (residues 244–369), which is involved in filament formation, reduced droplet formation but did not fully block it, suggesting that the positive charges in the proline-rich region of tau neighboring the repeats are sufficient for intermolecular interactions with the N terminus.

The beauty of the system established by Boyko *et al.* (5) is that it will now be possible to systematically interrogate the effects of biomolecular changes and other perturbations on droplet formation and/or evolution (Fig. 1). How do cofactors such as nucleic acids and polyphosphates alter the phase properties? What are the effects of specific tau modifications? Does the presence of multiple tau isoforms influence droplet behavior? Are there other proteins partitioning into the droplets? Can the system be used as a platform to identify compounds that affect tau LLPS, which could have potential therapeutic value? Ultimately, all these experiments will need to be verified in a biological setting that also captures the potential control mechanisms for LLPS of tau.

One big question that remains is what, if any, is the *in vivo* function of tau droplets? Are they reaction crucibles that induce the polymerization of tubulin (7)? Do they serve a role in

protein storage? And in the context of human health, can tau droplets initiate aggregation and thereby contribute to neurodegenerative diseases? There is some evidence in support of the latter (8), but much more work is needed. Pure droplets of unmodified tau are unlikely candidates for initiating aggregation because filament growth cannot be sustained in the absence of specific modifications (9) or cofactors (10). It is possible that posttranslational modifications and cofactors could facilitate structural changes in droplet-bound tau, leading to the formation of β -sheets (4, 8). This could explain some of the physicochemical differences between the tau droplets highlighted above. The system described by Boyko *et al.* (5) provides a valuable platform to further study and hopefully clarify these differences.

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