LLPS DB Annotation

This is an example annotation document filled out with the information of the human tau protein. When annotating your protein(s), please delete the infromation in all fields, and fill out as many of them as possible with the details of the protein being annotated.

**Once you completed the annotation, please email it to: pancsa.rita@ttk.mta.hu**

Note: fill out a separate annotation document for all proteins separately.

**A) Common name(s) (as named in the article)**

Tau-F, Tau

**B) UniProt accession**

P10636-8

**C) Characterization of region(s) mediating LLPS**

Tau/MAP repeats

**D) UniProt region boundaries**

244-368

**E) LLPS region reference PMID**

PMID:28819146

**F) Binding partners of LLPS**

1) tRNA

2) heparin

3) tubulin

**G) Determinants of phase separation and droplet properties**

1) protein concentration of Tau

2) phosphorylation state

3) alternative splicing

4) salt concentration

5) temperature

**H) How is the condensate named by the authors?**

liquid droplets, complex coacervates

**I) Type of membraneless organelle**

cytoplasmic microtubule; condensed compartments of microtubule bundling

**J) Literature supporting the LLPS**

29472250 (research article), 28683104 (research article), 28819146 (research article), 28877466 (research article), 29734651 (research article), 30950394 (research article), 30068389 (research article), 31097543 (research article)

**K) Description of the LLPS system**

Non-centrosomal microtubule bundles play important roles in cellular organization and function. The concentration of tubulin into a condensed, liquid-like compartment composed of the unstructured neuronal protein tau is sufficient to nucleate microtubule bundles.Under conditions of molecular crowding, tau forms liquid-like drops. Tubulin partitions into these drops, where it nucleates and drives the formation of microtubule bundles. These bundles deform the drops and remain enclosed by diffusible tau molecules, exhibiting a liquid-like behavior. (PMID:28877466) Alternative splicing of Tau can regulate the formation of Tau-containing membrane-less compartments. Phosphorylation of Tau repeats promotes liquid–liquid phase separation at cellular protein conditions. Liquid droplets formed by the positively charged microtubule-binding domain of Tau undergo coacervation with negatively charged molecules to promote amyloid formation. LLPS promotes Tau fibrillization in the presence of heparin (polyaninon) (PMID:28819146). Tau complexes with RNA to form droplets. Uniquely, the pool of RNAs to which tau binds in living cells are tRNAs. The LLPS process is directly and sensitively tuned by salt concentration and temperature, implying it is modulated by both electrostatic interactions between the involved protein and nucleic acid constituents, as well as net changes in entropy. Despite the high protein concentration within the complex coacervate phase, tau is locally freely tumbling and capable of diffusing through the droplet interior. However, prolonged residency within the droplet state can result in the emergence of detectable β-sheet structures. Thus the droplet state can incubate tau and predispose the protein toward the formation of insoluble fibrils (PMID:28683104).

**L) Experimental techniques applied to prove/investigate LLPS**

To exclude the influence of intramolecular and intermolecular cross-linking through Tau’s two native cysteine residues, C291 and C322, turbidity measurements were performed in the presence of tris(2-carboxyethyl)phosphine (TCEP), mimicking the reducing environment inside neurons. Changes in solution turbidity can arise from liquid–liquid demixing/LLPS, but also from formation of other types of aggregates. To support the presence of a liquid phase separated state of the repeat domain of Tau, differential interference contrast (DIC) microscopy were performed. To demonstrate the presence of tau proteins in the liquid droplets, confocal microscopy of fluorescently labeled protein was used and at 37 °C, but not at 5 °C, fluorescent droplets were observed. To dissect the consequences of LLPS on the molecular properties of the repeat domain of Tau, NMR spectroscopy was used. The NMR data suggest that the protein stays largely disordered within liquid droplets, in agreement with the overall low content of regular secondary structure observed by CD spectroscopy. NMR measurements using attached paramagnetic nitroxide tag to the two native cysteines demonstrate that LLPS of Tau repeats results in a tight molecular mesh of amyloid-promoting elements. (PMID:28819146) Bright-field and fluorescence microscopy show that tau/tau-EGFP form drops in vitro in the presence of crowding agents. Fusion of tau droplets was visualized using dual-trap optical tweezers and Internal rearrangement of tau drops was monitored using fluorescence recovery after photo-bleaching (FRAP). (PMID:28877466) In vitro tau-RNA binding were detected using gel shift mobility assay and tau LLPS in the presence of RNA was investigated with light and confocal microscopy images of fluorescence-labeled proteins. Light microscopy images show that tau-RNA droplets form a complex coacervate phase. In vivo experiments show that tRNA transfection accumulates sarkosyl insoluble tau in human-induced pluripotent stem cell (hiPSC) derived neurons. (PMID:28683104)

**M) The experimental observations supporting the liquid material state of the condensates**

rheological traits (PMID:28819146, PMID:28877466), morphological traits (PMID:28819146, PMID:28877466, PMID:28683104), dynamic movement/reorganization of molecules within the droplet (PMID:28819146, PMID:28877466), temperature-dependence (PMID:28819146, PMID:28683104), reversibility of formation and dissolution (PMID:28683104)

**N) Type of RNA(s) required/used for the LLPS**

other type of RNA: tRNA

**O) PTMs that affect the formation or stability of LLPS**

262|S|phosphorylation|promotes|PMID:28819146|MARK2|Notes:none

324|S|phosphorylation|promotes|PMID:28819146|MARK2|Notes:none

356|S|phosphorylation|promotes|PMID:28819146|MARK2|Notes:none

148-395|K|hyperacetylation|weakens|PMID:29734651|p300|Notes:15 sites

**P) Disease mutations affecting LLPS**
K280del|dbSNP:rs1168968768|Frontotemporal dementias (FTD)|OMIM: 600274lPMID:29472250|Note: It causes tau oligomerization and aggregation.

P301L|dbSNP:rs63751273|Frontotemporal dementias (FTD)|OMIM: 600274lPMID:29472250|Note: It causes tau oligomerization and aggregation.

P301S|dbSNP:rs63751438|Frontotemporal dementias (FTD)|OMIM: 600274lPMID:29472250|Note: It causes tau oligomerization and aggregation.

A152T|dbSNP:rs143624519|Frontotemporal dementias (FTD)|OMIM: 600274lPMID:29472250|Note: It causes tau oligomerization and aggregation.

K274Q|None|Alzheimer disease (AD)|OMIM:104300|affects|PMID:31036717|Notes: It increases tau aggregation and enhances the cytotoxicity of tau oligomers.

**Q) Alternative splicing affecting LLPS**

Isoform P10636-5|weakened|PMID:28819146

**R) Molecular interaction types contributing to LLPS**

linear oligomerization/self-association (PMID:28683104); formation of amyloid-like/cross-beta/kinked/stacked beta-sheet structures (PMID:28819146); protein-RNA interaction (PMID:28683104)

**S) Determinants and mechanisms of LLPS formation**

Membrane cluster: N

Partner-dependent: N

RNA-dependent: N

PTM required for LLPS: N

Domain-motif interactions involved: N

Discrete oligomerization involved: N

**T) Functional class of membraneless organelle**

activation/nucleation/signal amplification/bioreactor

**U) Corresponding author contact**

Markus.Zweckstetter@dzne.de, stefan.diez@tu-dresden.de, hyman@mpi-cbg.de, kenneth.kosik@lifesci.ucsb.edu,songi@chem.ucsb.edu