Microtubules gate tau condensation to spatially regulate microtubule functions

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Tau is an abundant microtubule-associated protein in neurons. Tau aggregation into insoluble fibrils is a hallmark of Alzheimer’s disease and other types of dementia¹, yet the physiological state of tau molecules within cells remains unclear. Using single-molecule imaging, we directly observe that the microtubule lattice regulates reversible tau self-association, leading to localized, dynamic condensation of tau molecules on the microtubule surface. Tau condensates form selectively permissible barriers, spatially regulating the activity of microtubule-severing enzymes and the movement of molecular motors through their boundaries. We propose that reversible self-association of tau molecules, gated by the microtubule lattice, is an important mechanism of the biological functions of tau, and that oligomerization of tau is a common property shared between the physiological and disease-associated forms of the molecule.

Microtubules (MTs) are dynamic, polarized filaments that are critical for a wide variety of cellular functions. Highly polarized cells, such as neurons, rely on the MT cytoskeleton to develop and maintain a specialized cellular architecture. Neurons devote substantial resources towards the regulation of diverse MT-based processes, such as polarized active transport by molecular motors, spatial control of MT dynamics and post-translational modifications of tubulin³. Given the central importance of MTs, it is not surprising that defects in MT-based pathways often lead to neurodevelopmental or neurodegenerative diseases in humans.

In Alzheimer’s disease, the intrinsically disordered MT-associated protein (MAP), tau (MAPT), forms insoluble neurofibrillary tangles through aberrant self-association, a process that is strongly correlated with neuronal death¹. Although tau self-association drives the formation of neurofibrillar tangles in disease, a non-pathological role for the self-association of tau molecules in cells is less clear. Biochemical studies have suggested that tubulin, or MTs, might mediate tau oligomerization⁴. Furthermore, multivalent interactions between tau molecules can drive liquid–liquid phase separation (LLPS) of tau in solution, promoting aggregation⁵. Single-molecule studies showed that tau molecules can exist in either static or diffusive populations when bound to MTs, and that tau binds heterogeneously along MTs⁶–¹⁰. Although these studies reveal a diverse range of molecular behaviours, how these behaviours relate to the physiological roles of tau in the cell is unknown.

MT-bound tau has been proposed to regulate the movement of motor proteins. Tau inhibits the anterograde movement of kinesin-1¹¹–¹³, but its effect on the retrograde movement of cytoplasmic dynein is less clear. High concentrations of tau were reported to reduce the attachment rate of dynein to MTs¹⁴, whereas an unactivated, putative dynein–dynein co-complex tended to reverse direction after encountering tau on the MT surface¹. Tau also regulates the activity of MT-severing enzymes, partially blocking the MT-severing activity identified in Xenopus egg extracts¹⁵. Overexpression of tau protects against katanin- or spastin-induced MT severing in cells¹⁶–¹⁸, whereas loss of tau in diseased neurons indirectly leads to aberrant spastin activity¹⁹. Dysfunction of MT-based transport or MT turnover could conceivably explain the neurotoxicity observed in tauopathies, but a cogent molecular mechanism for how tau affects such activities is lacking.

We set out to explore the molecular basis of tau’s complex behaviours using in vitro reconstitution with purified components. We directly observed the binding of bacterially expressed green fluorescent protein (GFP)-tagged full-length (2N4R) human tau (Supplementary Fig. 1a) to taxol-stabilized MTs in vitro. Tau molecules initially bound diffusely along the entire MT lattice, followed by the nucleation and expansion of denser regions of molecules that we term ‘condensates’ owing to the localized increase in protein density (Fig. 1a,b, Supplementary Video 1). Tau condensates expanded along the MT with asynchronous, highly variable growth rates from one or both ends of the condensate, and merged with nearby condensates (Fig. 1a,b, Supplementary Fig. 1b). At a fixed total concentration of tau, condensate intensity quickly reached a steady state (Fig. 1c). Nucleation of new condensates and further condensate expansion were rarely observed after 10 min, suggesting that the condensation process reached equilibrium in our assay conditions.

Varying the total tau concentration revealed an increase in both condensate frequency and the density of diffusively bound tau surrounding the condensates (Fig. 1d,e). Tau condensates appeared when the total tau concentration was at least 0.25 nM, whereas only diffuse tau binding was observed at lower concentrations, revealing a critical concentration for condensation under our conditions. Pixel intensity distributions of tau along MTs segmented into two distinct populations (dim and bright), which provided a quantitative signature of the condensation process (Supplementary Fig. 1c). Increasing the tau concentration progressively occluded our ability to distinguish condensates from their surroundings as overall total fluorescence increased on the MT (Fig. 1e,f). We also observed a concentration-dependent increase in frequency, percentage total coverage of the MT lattice and size of the condensates (Fig. 1f, Supplementary Fig. 1d). Accounting for the increase in diffuse tau signal surrounding the condensate, we observed a concentration-independent density of tau within condensates, suggesting that the
Fig. 1 | MTs gate the spatial condensation of tau on the lattice. a, Time-lapse images of tau condensate nucleation and expansion. The pink arrows indicate nucleation sites; n = 4 chambers. b, Kymograph of a (left). The pink arrows indicate nucleation sites. Right, kymograph of condensate dissolution; n = 2 chambers. c, Quantification of tau intensity within condensates over time. Data are mean ± s.d. n = 36 condensates (3 chambers). The calculated time constant for intensity saturation (τ) is shown. d, Images of increasing tau concentrations (equal brightness and contrast); n = 3 chambers.

e, Concentration curve of condensate intensity normalized to total tau intensity. Data are mean ± 95% confidence interval (CI); n = 4, 52, 144, 233, 275 and 329 condensates (from left to right; 3 chambers each). f, Quantification of different parameters of tau concentration titrations. Data are mean ± 95% CI; n = 77, 100, 104, 111, 100 and 90 MTs (from left to right; 3 chambers each). g, Concentration curve of condensate intensity normalized to increases in tau intensity surrounding condensates. Data are mean ± 95% CI; n = 52, 233, 275 and 329 condensates (from left to right; 3 chambers each). h, Tau accumulation on highly curved regions of MTs is indicated by magenta arrows; n = 3 chambers. i, Tau binding to taxol-stabilized and GMP-CPP-stabilized MTs (top). Bottom, tau channel. Right, quantification of tau intensity in taxol-stabilized versus GMP-CPP-stabilized MTs. Note total intensity (total) versus intensity outside of condensates (lattice). Data are mean ± s.d.; n = 90 (4 chambers), 98 (3 chambers) and 97 (4 chambers) (from left to right; 3 chambers each. j, Tau condensates (green, indicated by the magenta arrows) on native GDP MT lattice (blue), stabilized at both ends with GMP-CPP caps (red); n = 3 preparations of MTs. k, Tau (green) binding to subtilisin-treated (red) and untreated MTs (blue; top images). Bottom images, tau channel. Quantification of tau intensity at 0.5 nM (top) and 20 nM (bottom). Note use of x8 neutral-density (ND) filter for 20 nM tau. Data are mean ± s.d. n = 107 (3 chambers) and 38 (3 chambers) (for left and right, respectively) continuous MT segments for 0.5 nM tau; n = 116 (4 chambers) and 62 (4 chambers) (for left and right, respectively) continuous MT segments for 20 nM tau. For i and k, statistical analysis was performed using two-sided Student’s t-tests. For a, b (horizontal), d, h and i, scale bars, 2 μm; j and k, scale bars, 5 μm; b (vertical), scale bars, 2 min (left), 10 min (right). See Supplementary Fig. 1, Supplementary Video 1 and Supplementary Table 1 for source data.
tau molecules occupy the total available MT surface within condensate boundaries (Fig. 1g). Finally, condensates slowly dissolved after removal of soluble tau from the solution (Fig. 1b, Supplementary Fig. 1b), revealing that tau condensation is reversible.

Similar to observations in live cells, we observed that tau invariably accumulated at regions of high MT curvature (Fig. 1h), revealing that the molecule intrinsically recognizes tubulin lattice conformations. Although tau bound diffusely to MTs assembled with either taxol or the non-hydrolyzable GTP analogue guanosine-\(5'-[\alpha,\beta]-\text{methylene}\)triphosphate (GMP-CPP), condensates only formed on taxol-stabilized, or native guanosine diphosphate (GDP) MT lattices (Fig. 1i–j, Supplementary Fig. 1c), revealing that condensation is gated by the nucleotide state of the MT lattice. This observation probably explains the previously reported approximately sevenfold differences in the binding affinity of tau for GMP-CPP versus GDP MTs. High-resolution cryogenic electron microscopy imaging of GMP-CPP MTs compared with GDP MTs revealed an expansion of around 2.2 Å in the inter-tubulin dimer distance of GMP-CPP MTs. We therefore hypothesize that the spacing between tubulin dimers regulates the ability of tau to undergo condensation on the lattice.

The unstructured C-terminal tails of tubulin have been reported to affect tau binding to MTs. In these experiments, tau was labelled on its two endogenous cysteines, which have recently been suggested to make direct contact with tubulin, possibly a confounding factor in the interpretation of these data. In our assays, removal of the tail domains of tubulin by subtilisin digestion led to uniform tau binding to the digested MTs; by contrast, undigested MTs showed clear tau condensation (Fig. 1k, Supplementary Fig. 1c). Thus, tubulin tails are required for tau condensation. Surprisingly, intensity analysis revealed that more tau bound to subtilisin-digested MTs compared with wild-type MTs, in apparent contrast to previous results. However, a fortyfold increase in the total tau concentration revealed a greater tau intensity on undigested MTs compared with digested MTs (Fig. 1k, Supplementary Fig. 1c). At this concentration, tau condensates are not easily discernible on wild-type MTs owing to the large amounts of total tau on the lattice. We propose that tubulin tails hinder the ability of single tau molecules to directly contact the surface of the tubulin dimer, but tau condensation—mediated in part by tubulin tails—facilitates direct contact with the tubulin dimer surface, resulting in a higher overall density of tau on the MT lattice.

We hypothesized that tau self-association underlies condensation, and set out to test this idea. Fluorescence recovery after photobleaching (FRAP) experiments revealed that tau condensates recovered approximately twofold more slowly than diffusively bound tau outside of condensate boundaries (Fig. 2a,b), demonstrating that bulk turnover of tau molecules is slower within condensates. We directly observed single SNAP-TMR-labelled tau molecules (Supplementary Fig. 1e) as they encountered tau condensates composed of GFP-labelled tau (Fig. 2c, Supplementary Video 2). Outside of condensates, most SNAP-TMR–tau molecules rapidly diffused along the MT lattice with an average dwell time of 6.2 s (Fig. 2d). Single tau molecules diffused to condensate boundaries, after which their behaviour altered markedly, transitioning from highly mobile to statically bound (Fig. 2c). Consistent with this, the measured diffusion coefficient was substantially reduced within condensates, from 4.6 × 10^{-2} μm^2 s^{-1} outside of condensate boundaries (\(n = 191\) molecules) to 4 × 10^{-4} μm^2 s^{-1} within condensates (\(n = 108\) molecules). Therefore, tau condensation substantially reduces the molecular dynamics of tau molecules on the MT surface. Within condensates, dwell times for single tau molecules increased sixfold, indicating that cooperative interactions between tau molecules underlie the cohesiveness of the condensate (Fig. 2c,d). In further support of the dynamic nature of tau condensates (Fig. 1b,c), we observed single tau molecules transitioning into and out of the condensate boundary, switching behaviour repeatedly between immobility and rapid diffusion (Fig. 2c). We conclude that interactions between tau molecules, gated by the MT lattice (Fig. 1), strongly reduce the molecular dynamics of tau on the MT surface, providing a possible molecular mechanism for tau condensation.

Tau condensates share qualities of reversible LLPS that were previously observed for tau in solution. Both processes are reversible, concentration dependent, result in distinct phases composed of low and high concentrations of tau, and are relatively insensitive to ionic strength. Furthermore, condensates merge as they encounter each other, similar to phase-separated droplets of tau after LLPS. To explore whether tau condensates share similar material properties to phase-separated tau, we exposed condensates to 1,6-hexanediol (1,6-HD), an aliphatic alcohol that is hypothesized to disrupt weak-hydrophobic interactions between proteins and has been shown to dissolve tau LLPS droplets. Tau condensates were rapidly dissolved by 1,6-HD without affecting the overall diffusive tau binding to either taxol-stabilized or GMP-CPP-stabilized MTs (Fig. 2e). Phase-separated tau and MT-bound tau condensates therefore share a similar level of sensitivity to 1,6-HD, and we speculate that weak hydrophobic interactions underlie both processes. However, in contrast to phase-separated tau in solution, tau condensates on MTs did not rapidly harden into gel-like structures, remaining susceptible to rapid 1,6-HD-mediated dissolution even after 5 h of incubation on MTs.

We used 1,6-HD to further examine the role of the MT lattice in tau condensation. Surprisingly, tau condensates formed, dissolved after addition of 1,6-HD and re-formed after removal of 1,6-HD largely at the same locations on the MT lattice (Fig. 2f). We frequently observed the formation of a new condensate, or the lack of condensate reformation after the removal of 1,6-HD (Fig. 2f, Supplementary Video 3). This observation indicates that local regions of the MT lattice could act as nucleation hotspots for tau condensation, although we cannot rule out that a small nucleus of tau oligomers could remain bound to these regions during 1,6-HD exposure. However, tau condensation was sporadic at certain hotspots, suggesting that a lingering tau nucleus is not strictly required for condensation (Fig. 2f). These hotspots may represent areas of local lattice distortion, similar to the highly curved regions that invariably accumulate tau (Fig. 1h). Our results show that tau condensation is reversible and probably driven by the same types of molecular interactions between tau molecules that lead to LLPS of tau in solution. The slower kinetics of FRAP recovery and longer dwell times within condensates differ from the typical properties of an LLPS system in solution, which we surmise may be due to scaffolded interactions of tau molecules with the MT.

We next sought evidence that tau condensation can occur in vivo. We stained mouse hippocampal neurons with two different pan-tau antibodies at various days after plating, and observed developmentally dependent tau localization to puncta along MTs, which strikingly resembled tau condensates in vitro (Fig. 2g, Supplementary Fig. 2). These puncta were not as apparent in neurons after 3 days in vitro (DIV3), even after axon specification by DIV4, as marked by unphosphorylated-specific tau-1 antibody staining (Supplementary Fig. 2a). However, by DIV7, tau puncta were abundant in all neurons (Supplementary Fig. 2). Similar staining has been previously reported, and some studies have suggested that tau puncta represent distinct hotspots because they are resistant to Triton X-100 extraction. However, in vitro, tau condensates were similarly resistant to Triton X-100 (Fig. 2h), raising questions about this interpretation. Overexpression of tau results in regions of higher tau density along neurite processes, which may be similar to the tau foci observed here. Thus, tau localization in neurons is diverse, and puncta of tau that resemble tau condensates form inside of neurons in a developmentally regulated manner.
**Fig. 2 | Tau condensation reduces molecular dynamics of tau molecules on the MT lattice.**

**a,** Image and kymograph of FRAP of a tau condensate. The arrow indicates a bleaching event and dashed yellow lines indicate the photobleached region; *n* = 4 chambers. Scale bars, 5 μm (horizontal), 20 s (vertical).

**b,** Image, GFP intensity plot and kymograph of a MT containing GFP-tau condensates and single molecules of SNAP-TMR–tau. The magenta arrows indicate a static tau molecule within a condensate. The blue arrow indicates a diffusive molecule outside of a condensate. The red arrows indicate a tau molecule entering a condensate and the green arrow indicates a tau molecule leaving a condensate; *n* = 4 chambers. Scale bars, 2 μm (horizontal), 10 s (vertical).

**c,** Quantification of FRAP recovery from both inside and outside (lattice) condensate boundaries. Data are mean ± s.d. *n* = 24 regions for both condensates and lattice; *n* = 24 segments (4 chambers).

**d,** Cumulative frequency plot and calculated time constants (τ) of SNAP-TMR–tau dwell times inside (τC, condensate) and outside (τL, lattice) GFP-tau condensates; *n* = 451 molecules (3 chambers) and 167 molecules (4 chambers) for lattice and condensates, respectively.

**e,** Images of tau (green) bound to taxol-stabilized (blue) or GMP-CPP-stabilized (red) MTs before and after addition of 1,6-HD. Tau concentration was kept constant during buffer exchange (left). Right, plot of tau intensity outside of condensates (lattice) or total GFP intensity in the presence of 1,6-HD. Data are mean ± s.d. *n* = 71 (3 chambers), 80 (3 chambers) and 103 (3 chambers) (from left to right) continuous MT segments. Statistical analysis was performed using two-sided Student’s t-tests. Scale bar, 5 μm.

**f,** Kymograph of alternating washes of 1 nM tau with or without 1,6-HD. Alternating buffer exchange scheme is shown at the top, tau concentration was kept constant during buffer exchange. Magenta arrows indicate condensate nucleation. The blue arrow indicates failure to reform after 1,6-HD washout. The yellow arrow indicates new nucleation; *n* = 2 chambers. Scale bars, 5 μm (vertical), 5 min (horizontal).

**g,** Images of immunostained DIV7 mouse hippocampal neurons using the GTX49353 (Genetex) pan-tau antibodies; *n* = 4 preparations of neurons. Scale bars, 25 μm (left), 10 μm (right).

**h,** Image of in vitro tau condensates in the presence of 1% Triton X-100. *n* = 2 chambers. Scale bar, 5 μm. See Supplementary Fig. 2, Supplementary Videos 2 and 3, and Supplementary Table 1 for source data.
Subsequently, we set out to map the regions of tau that are responsible for self-association within condensates. In neurons, six tau isoforms exist that differ in the number of projection domain inserts (N) and in the number of MT binding repeats (R). All of the isoforms contain an MT-binding domain (MTBD) flanked by a proline-rich region and pseudo-repeat region (Fig. 3a). Alternative splicing in the MTBD or projection domain did not grossly perturb tau condensation (Fig. 3a,b, Supplementary Fig. 1a). We assayed for tau oligomerization by first forming condensates with full-length mScarlet-tagged tau (2N4R), followed by introduction of equimolar amounts of various GFP-tagged tau isoforms (2N4R, 2N3R and 0N3R). We compared the enrichment of GFP-tau isoform signal within the boundaries of full-length mScarlet–tau condensates with the GFP signal outside of mScarlet–tau condensates. We observed a twofold to threefold enrichment of GFP–2N4R, GFP–2N3R and GFP–0N3R isoforms within mScarlet–tau condensates, indicating that alternative splicing does not grossly affect the ability of a tau molecule to incorporate into 2N4R tau condensates (Fig. 3c).

We truncated different domains of tau and found that, in contrast to published data on tau LLPS, the MTBD alone was weakly excluded from condensates, whereas the isolated projection domain exhibited only diffuse binding (Fig. 3a,c, Supplementary Figs. 1a and 3c). The isolated C terminus of tau segregated into condensates, albeit more weakly than full-length tau (1.2-fold versus 2.9-fold), as did a ‘bonsai’ construct that consisted of a fusion between the N and C terminus but lacked the MTBD (1.3-fold; Fig. 3a,c, Supplementary Fig. 3c). These data indicate that the C terminus of tau licenses other portions of the molecule into condensates, albeit more weakly than full-length tau (1.2-fold versus 2.9-fold), as did a ‘bonsai’ construct that consisted of a fusion between the N and C terminus but lacked the MTBD (1.3-fold; Fig. 3a,c, Supplementary Fig. 3c). These data indicate that the C terminus of tau licenses other portions of the molecule into condensates. Consistently, in cases in which the MTBD was excluded from condensates, the addition of the flanking N-terminal proline-rich and C-terminal pseudo-repeat domains (mini-tau) restored segregation into condensates to levels similar to those seen for full-length tau (Fig. 3c).

Fig. 3 | The C-terminal pseudo-repeat region of tau licenses the rest of the molecule into tau condensates. a, Schematic of natural tau isoforms and the constructs used. Orange boxes indicate alternatively spliced N-terminal inserts. Blue boxes indicate the proline-rich domain. Green boxes indicate MT binding repeats. Yellow boxes indicate the pseudo-repeat domain. Constructs labelled in green text form condensates on their own and constructs labelled in black text do not. b, Images of tau condensates formed from different alternatively spliced or artificially truncated tau constructs; n = 3 chambers each. Scale bar, 2 μm. c, Quantification of the fold enrichment of various tau constructs into 2N4R tau condensates compared with the MT lattice surrounding the condensate. Inset: magnified image for clarity of MTBD, projection domain (Proj), C-terminal fragment (C-term) and tau bonsai constructs. Statistical analyses for significance for enrichment (black) or exclusion (red) of a given GFP-construct within mScarlet–2N4R tau condensates is indicated on the right (Supplementary Fig. 3). Data are mean ± 95% CI; n = 211, 143, 158, 189, 208, 149, 296 and 239 GFP–2N4R condensates (from top to bottom; 3–6 chambers for each construct). d, Quantification of the fold enrichment of mini-tau and C-terminal deletion constructs into 2N4R tau condensates. Data for mini-tau reproduced from c for comparison. Data are mean ± 95% CI; n = 239, 122 and 75 GFP–2N4R condensates (from left to right; 3–4 chambers). For c and d, statistical analysis was performed using two-sided Student’s t-tests. See Supplementary Fig. 3 and Supplementary Table 1 for source data.
Fig. 4 | Tau condensates form selectively permeable barriers that regulate distinct MT functions. a, Kymograph of processive DDB, tau intensity is indicated on the left (top). Red lines indicate the maximum intensity position (condensates). Bottom, event distribution for DDB. White indicates proportion that detach during pause; n = 336 events (7 chambers). b, Plot of tau intensity with a kymograph of diffusive DDB or p150Glued molecules (top). Bottom, event distribution for p150Glued; n = 240 (3 chambers). c, Model of tau (R2x4, Protein Data Bank: 6CVN; orange) and the dynein MTBD (DYNC1H1, Protein Data Bank: 3J1T; yellow) highlighting no steric clash between the two. MT plus (+) and minus (−) ends are labeled. d, Distribution of DDB behaviours at ON3R or mini-tau condensates. DDB behaviour at 2N4R condensates were reproduced from a; n = 336 events (7 chambers), 226 events (3 chambers) and 399 events (5 chambers) for 2N4R, ON3R and mini-tau, respectively. e, Peak tau intensity within condensates for each DDB behaviour. Data are mean ± s.d. n = 26, 178, 108 and 13 events for each behaviour (from left to right; 7 chambers). NS, not significant. f, Tau intensity and kymographs of DDH or DDF behaviour (top). Bottom, distribution of DDH and DDF behaviours at condensates. DDB behaviour was reproduced from a; n = 336 events (7 chambers), 268 events (8 chambers) and 697 events (4 chambers) for DDB, DDH and DDF, respectively. g, Tau intensity and kymographs of DDB–L and DDB behaviour (left). Right, passing and pausing distribution for DDB–L. Distribution of DDB behaviour was reproduced from a. The green arrows indicate the DDB–L complex and the magenta arrows indicate DDB complexes; n = 336 events (7 chambers) and 237 events (10 chambers) for DDB and DDB–L, respectively. For a, f and g, see also Supplementary Fig. 4b. h, Images of mScarlet-tau condensates with GFP–spastin before and after 5 min incubation (left). Right, kymographs from each channel. Magenta arrows indicate regions of spastin-mediated MT destruction. Bottom right, normalized MT intensity after spastin severing inside and outside condensates. Data are mean ± s.d. n = 228 and 181 continuous MT segments inside and outside condensates (4 chambers), respectively. For d–h, statistical analysis was performed using two-sided Student’s t-tests. ****P < 0.0001. For a, b, f, g, scale bars, 2 μm (vertical), 15 s (horizontal); h, scale bars, 1μm (horizontal), 2 min (vertical). See Supplementary Fig. 4, Supplementary Videos 4 and 5, and Supplementary Table 1 for source data.
requires interactions with multiple amino acids located within this region (Fig. 3d, Supplementary Fig. 3d). Removal of the entire pseudo-repeat domain (mini-tauΔ28) largely abolished incorporation into 2N4R tau condensates (Fig. 3d, Supplementary Fig. 3d). Our data suggest that potential hydrophobic interactions within the pseudo-repeat region are important for the intermolecular interactions that underlie tau condensation. Interestingly, this region was previously shown to enhance the MT affinity of tau, and disease-associated mutations within this domain affect the dynamicity of tau on MTs in living cells.

Previous studies have suggested that tau can regulate the movement of molecular motors, including the cytoplasmic dynein–dynactin complex\(^\text{1,24}\). However, this reported dynein–dynactin behaviour\(^\text{25}\) is not broadly consistent with the strongly processive movement of activated dynein–dynactin–cargo-adapter complexes discovered subsequently\(^\text{6}\). To reconcile these findings, we investigated the effects of tau condensates on the motility of activated dynein–dynactin–cargo-adapter complexes. We found the majority of processive dynein–dynactin–BicD2N (DDB)\(^\text{31}\) complexes passed through condensates (84%), often displaying considerable pausing at the condensate boundary (49%; Fig. 4a, Supplementary Fig. 4a,b, Supplementary Video 4). A small population of motor complexes (~3%) exhibited unidirectional movement before switching to a bidirectional state at tau condensates (Supplementary Fig. 4b,c). Similarly, DDB motors that displayed only diffusive behaviour on the MT\(^\text{1,2}\) reversed direction at tau condensates (Fig. 4b, Supplementary Fig. 4b). This behaviour was very similar to a purified p150\(\text{CDM}\) construct, a component of the dynactin complex that diffuses along MTs through interactions with the tubulin-tail domains\(^\text{2,3}\) (Fig. 4b). We conclude that tau condensates are permissive to processive dynein movement but not to dynactin-mediated diffusion. Thus, in contrast to the plus-end-directed motor kinesin\(^\text{1,11,12}\), dynein is physically capable of traversing tau condensates. The behavioural difference between these motors can be explained by recent cryo-electron microscopy structures of tau on the MT\(^\text{22}\). Although the kinesin motor domain has a large steric clash with MT-bound tau, we found that the much smaller dynein MT binding domain does not (Fig. 4c, Supplementary Fig. 4d–f). Combined with recent work showing how orthogonal MAP proteins facilitate kinesin-1 transport\(^\text{4}\), our results provide a comprehensive view for how tau may directly modulate the bidirectional movement of intracellular cargos driven by dynein and kinesin-1.

We sought to determine the domains of tau that contribute to the notable pausing of processive dynein complexes (Fig. 4a). Condensates formed from the shortest natural tau isoform (ON3R) or the mini-tau construct, enabled progressively greater numbers of motor complexes to pass unimpeded (47% and 63%, respectively; Fig. 4d, Supplementary Fig. 4b). We also found that motor behaviour was not correlated with peak pixel intensity within tau condensates (Fig. 4e), indicating that the distribution of motor behaviours does not depend on any differences in tau density within condensates.

Recent data have defined adapter-dependent variations in the number of dynein dimers linked to the dynactin scaffold\(^\text{11,12,24}\). BicD2N predominantly recruits only one dynein dimer, whereas the adapter Hook3 largely recruits two dynein dimers per dynactin. Dynein–dynactin–Hook3 (DDH) complexes passed through tau condensates without pausing at significantly higher rates (55%) compared with DDB (35%), indicating that pairs of dynein dimers are better able to navigate tau condensates unimpeded (Fig. 4f, Supplementary Fig. 4b). Dynein–dynactin–Rab11-FIP3 (DFD) showed significantly increased rates of passing through tau condensates without pausing (68%), indicating that Rab11-FIP3 probably recruits a pair of dynein dimers similar to Hook3 (Fig. 4f, Supplementary Fig. 4b). Another dynein regulator, LIS1, directly impinges on the mechanochemistry of dynein and allosterically controls DDB velocity\(^\text{5,6}\). Analysis of DDB complexes that contained bound LIS1 (DDB–L) revealed that these complexes were also significantly better at crossing tau condensates unimpeded (59% versus 35%; Fig. 4g, Supplementary Fig. 4b). These results reveal that allosteric control of dynein motor activity alters the motor’s ability to traverse condensates, and suggest that tau condensation could modulate the velocity and run lengths of retrograde traffic in a cargo-dependent manner in vivo. In this context, we note that changes in dynein run lengths result in neurodegenerative and neurodevelopmental phenotypes\(^\text{25,26}\).

The molecular mechanism for tau-mediated inhibition of MT severing is unknown. We found that a truncated, active form of the MT severing enzyme spastin\(^\text{19}\) was largely excluded from tau condensates (Fig. 4h). As a result, tau condensates protected the underlying MT lattice from spastin-mediated severing, whereas the lattice surrounding the condensate was destroyed (Fig. 4h, Supplementary Video 5). Complementary results presented in the companion paper by Siahaan et al.\(^\text{25}\) reveal that this principle extends to the other major MT severing enzyme, katanin. As a consequence, tau condensates affect both molecular motor movement and MT severing by acting as selectively permissible barriers for MT effector proteins.

Phosphorylation plays a key role in tau biology and disease. The recombinant tau protein used in this study was produced in bacteria and therefore contains no post-translational phosphorylation. We observe only minor differences in MT-binding affinity and condensation kinetics between our study and that of Siahaan et al.\(^\text{25}\), which utilized baculovirus-expressed partially phosphorylated tau\(^\text{34}\). Tau phosphorylation is therefore not required for condensation but may tune the kinetics of condensate formation and growth by modulating the affinity of tau for the MT, and potentially the tau interactions that drive condensation.

In summary, our study has uncovered a regulated form of reversible tau oligomerization that functionally compartmentalizes the MT lattice. We propose that tau condensation is a physiological form of tau self-association, which is gated and scaffolded by the MT lattice, that can be harnessed by cells to spatially direct diverse MT-based molecular pathways. Furthermore, our results demonstrate that oligomerization is not exclusive to the pathological form of tau. As tau condensation is sensitive to total tau concentration, we speculate that the loss of tau monomer to alternative self-association pathways, such as the fibrillization observed in tauopathies, will negatively impact the condensate formation, dynamics and functions of tau in neurons.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41556-019-0375-5.

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Author contributions
R.J.M., R.T. and K.M.O.-M. conceived the project. R.T., A.J.L. and T.T. produced reagents. R.T. performed all in vitro experiments. J.H. and S.S. provided hippocampal cells. R.J.M., R.T. and K.M.O.-M. conceived the project. R.T., A.J.L. and T.T. produced reagents. R.T. performed all in vitro experiments. J.H. and S.S. provided hippocampal neuron cultures, D.W.N. created molecular models and M.V. performed data analysis.

Competing interests
The authors declare no competing interests.

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Methods

MT assembly. Porcine brain tubulin was isolated using the high-molarity PIPES procedure and then labelled with biotin NHS ester, Dylight-405 NHS ester or Alexa647 NHS ester as described previously (http://mitoshin.hms.harvard.edu/ files/mitoshinlab/comparing_tubulin_labels_staining_tubulinstaining.pdf). Pig brains were obtained from a local abattoir and used within 4 h after death. MTs were prepared by incubation of 100 μM tubulin with 1 mM GTP for 10 min at 37 °C, followed by dilution into a final concentration of 20 μM tubulin for an additional 20 min. GMP-CP MTs were prepared similarly but with 1 mM GMP-CP instead of GTP without taxol. MTs were pelleted at 80,000 r.p.m. over a 25% sucrose cushion in a TLA-100 rotor and the pellet was resuspended in 50 μl BRB80 containing 10 μM taxol. For removal of tubulin C-terminal tail, MTs were further treated with subtilisin for 1 h at 37 °C as described previously11. The reaction was terminated by 1 mM PMSF and pelleted at 80,000 r.p.m. Concentration of subtilisin used and digestion were assayed by Coomassie staining and recombinant p150Glued binding. For GMP-CP capped MTs, 100 μM of Alexa647-labelled and biotin-labelled tubulin was added to 0.5 mM GMP-CP in BRB80 (80 mM potassium PIPES pH 6.8, 1 mM MgCl₂, and 1 mM EGTA) at 37 °C for 30 min. Then, 10 mM GTP was added along with 40 μM Alexa455-labelled tubulin for an additional 30 min. Finally, 20 μM Alexa647-labelled and biotin-labelled tubulin was added along with 1 mM GMP-CP for at least 30 min. Capped MTs were kept at 37 °C until use. For GMP-CP-capped MT experiments, n is defined as the number of preparations of MTs used to derive images.

Protein constructs. All of the human tau and spastin constructs were cloned into pET28A cassette for expression. The constructs contain an N-terminal 6×His-tag and tandem Strep-Tags connected by a G-S linker. The sequence is as follows: MGSSHHHHHHSSGLVPRGSWMHPQFEKGS SGSSGSASWHPQFEKGS. This cassette is then followed by the fluorophore (sFluor, mScarlet or SNAP) followed by a precision protease cleavage site. Human tau cDNA was purchased from Transcombs (BC1050260). Fully active, truncated spastin (ΔK28) was cloned into pET-28a-Strep-Tags (sFluor). Full-length human tau was purchased from Addgene (16316). Amino acid boundaries for tau constructs are as described in Fig. 3a. In brief, the projection domain inserts were from E45 to T102. The proline-rich domain encompassed S198–L243, and the MTBD was defined as Q244–E372. The second repeat (exon 10) removed in 3R tau constructs spans K274–G304. The repeat region consists of T373–V399.

Protein purification. Tau and spastin were expressed in BL21 (DE3) cells (Agilent). The cells were grown at 36 °C until an optical density of 600 nm (OD600) of 0.6, then induced with 0.4 mM isopropyl-β-D-thiogalactoside overnight at 18 °C. Cells were resuspended in buffer X and lysed using an Emulsiflex C-3 (Avestin). Proteins were affinity purified using Strep XT beads (IBA). Tau constructs were further purified by anion exchange using a HiTrap Q HP column in Protein Buffer pH 7.5 (50 mM Tris-HCl pH 7.5, 2 mM MgCl₂, 1 mM EGTA and 10% glycerol) with a salt gradient from 100 mM to 400 mM. Full-length tau constructs were further purified by size-exclusion chromatography using a Superose 6 10/300 column in protein buffer pH 8.8. All mini-tau-based constructs were resuspended for only 4 h and were purified similarly to all of the other tau constructs. For mini-tau constructs and spastin, we performed cation exchange using a HiTrap SP HP column with the same conditions as for the other tau constructs. Dynactin–cargo-adapter complexes were purified from rat brain lysate as described previously31. In brief, all of the SNAP-tagged adapter protein constructs were purified by affinity as above and further purified by size-exclusion chromatography using a Superose 6 10/300 column in 60 mM HEPES pH 7.4, 50 μM potassium acetate, 2 mM MgCl₂, 1 mM EGTA and 10% glycerol. Dynactin–cargo-adapter complexes were labelled using a 4:1 ratio of dye:SNAPf-tagged protein at 2 μM SNAPf-TMR, SNAP, Alexa647 or SNAP-Alexa488 dye (NEB) during the isolation procedure and were frozen in small amounts and stored at −80 °C. The protein concentration was assessed using a Nanodrop One (ThermoFisher). Protein concentrations are given as the total amount of fluorophore (monomer) in the assay chamber. All of the buffers and chemicals used were obtained from Sigma-Aldrich.

TIRF microscopy. All total internal reflection fluorescence (TIRF) microscopy experiments were performed on a custom-built through-the-objective TIRF microscope (Technical Instruments) based on a Nikon Ti-E stand, motorized ASI microscope (Technical Instruments) and a Nikon Ti2 stand, LU-n4 four laser unit, EMCCD camera (iXon Ultra 897) and a high-speed filter wheel (Finger Lakes Instruments). All imaging was performed using a ×100 1.45 NA objective (Nikon) and the ×1.5 tube lens setting on the Ti-E. Experiments were conducted at room temperature. The microscope was controlled using Micro-Manager software. For imaging of tau binding at 20 nM per time point, a 8 neutral-density filter was used to reduce total signal intensity. Of note, some tau molecules were found to localize outside of MTs. Tracks from such molecules tended to show a diffusion coefficient in the order of 1 × 10⁻⁶ cm²/s. This can be taken as an estimate of background drift and vibration of the optical stage and is sufficiently low that it can be hard to computationally correct. Combine this with drift, which is lower than the diffusion width, therefore, 3.94 × 10⁻⁶ cm²/s may be a slight overestimate but not a major departure from the true value.

Single-molecule diffusion measurements. Tracks of molecules were assessed for co-localization with condensates by manual inspection. On rare occasions, tracks were found to briefly co-localize with the condensates but were mostly motile outside such regions. These latter tracks were not considered for analysis of diffusion within the condensates. A total of 108 tracks were therefore identified and their mean squared displacement (MSD) curves were then calculated and averaged. The diffusion coefficient, which was estimated from the averaged curve, was computed (<?x^2> = 2Δx²) and found to be 3.94 × 10⁻⁶ μm²/s. α is the mean distance from starting point, D is the diffusion coefficient, and t is time. Of note, some tau molecules were found to localize outside of MTs. Tracks from such molecules tended to show a diffusion coefficient in the order of 1 × 10⁻⁶ cm²/s. This can be taken as an estimate of background drift and vibration of the optical stage and is sufficiently low that it can be hard to computationally correct. Combine this with drift, which is lower than the diffusion width, therefore, 3.94 × 10⁻⁶ μm²/s may be a slight overestimate but not a major departure from the true value.
carefully dissected and the meninges were removed. Dissociation was achieved by a combination of enzymatic digestion using papain and pipetting homogenization (Worthington Biochemical Corporation). Neurons were resuspended in neuron growth medium (Neurobasal medium containing 2% B-27 supplement, 2% GlutaMAX solution, glucose, and penicillin and streptomycin). A total of approximately 10^5 neurons were plated in poly-d-lysine-coated coverslips. Growth medium was changed every 2 d. The morning a vaginal plug was observed was considered E0.5. All animals were used with approval from the University of California Davis Institutional Animal Care and Use Committees. The use of animals complied with all of the relevant ethical regulations regarding animal research.

At the desired DIV, the neurons were fixed in 4% PFA for 20 min and permeabilized using 0.3% Triton X-100 for 5 min. Neurons were blocked with 5% BSA and incubated with primary antibodies at a 1:500 dilution (anti-tau, Genetex, 49353 or Thermo Scientific, 13-6400; anti-beta tubulin, Abcam, ab6046), then incubated with fluorescently labelled secondary antibodies at a 1:1,000 dilution (anti-chicken, A11039; anti-mouse, A28175 and A28180; and anti-rabbit, A27039) and mounted with Vectashield.

Coverslips were imaged using a Leica TCS SPE-II RYBV with automated DM68 with a Leica laser launch (25 mW, 405 nm; 10 mW, 488 nm; 20 mW, 561 nm; 18 mW, 635 nm). All imaging was performed using a HC PL APO CS2 x63 1.40 NA objective (Leica). Experiments were conducted at room temperature. The microscope was controlled using Leica LAS X software and analysed with ImageJ. For qualitative images, n is defined as the number of neuronal preparations used.

For quantification of tau puncta, the brightest plane for a given z stack was selected for analysis. The non-neuronal area was filtered by its low intensity, although bright spots outside of neuronal area were also occasionally masked by hand. The loci of tau enhancement in all cases were identified by fitting local intensity to Gaussian or quadratic profiles. Good fits were identified by imposing a limit on squared residuals and cut-offs on fitted peak widths in x and y (both to avoid fitting single pixel specs and large broad peaks, such as cell bodies). All cut-offs were held the same for all data being analysed. The density of enhanced tau loci was quantified as the number of loci divided by the number of pixels that correspond to neuronal fluorescence.

Data analysis for condensate enrichment. mScarlet–2N4R tau condensates were used as fiducials for condensate boundaries. Background-subtracted mean intensities were obtained for a line scan along the MT. Each straight and uninterrupted (no MT overlaps) stretch of MT was counted as a single data point. Data points from two different protein preparations of mScarlet–2N4R tau condensates were pooled. Fold enrichment was calculated by dividing each data point for condensate intensity by the average value of associated lattice intensity.

Dynemin–dynactin–adapter behaviour assays. Dynemin–dynactin–adapter movement was visualized manually using kymographs. The behaviours at condensates were characterized in the following manner. A loss of signal at a condensate was binned as ‘detach’. Continuing through the condensate boundary without a change in velocity was binned as ‘pass’. A ‘pause’ was defined as a diffraction-limited stop in processivity for longer than 3 frames (1.5 μs). These molecules were then binned as pause–pass and pause–detach following the same behaviours as above. ‘Bidirectional’ was binned as molecules that exhibit diffraction-limited movement in a single direction for further than 1 μm, then reversed direction after encountering a condensate.

Peak tau intensity for a condensate was derived by first averaging the intensities per pixel over time for a condensate throughout the entire video. In the event of substantial stage drift, the intensities at the time of the events were used. The ‘Find Peaks’ plugin using default conditions for ImageJ was then used to determine the peak intensity. Background intensity of a non-MT region nearby was then subtracted from this value to determine background-subtracted peak intensity.

Single-molecule intensity and photobleaching analysis. The Fiji plugin ComDet was used to analyse the intensities of TMR-labelled SNAP–2N4R-tau molecules bound to MTs. The estimated particle size was set to 3 px and the intensity threshold was set to 3 s.d. Photobleaching analysis was performed on TMR-labelled SNAP–2N4R-tau molecules that were non-specifically bound to the observation chamber to avoid the confounding factors of tau diffusion and dissociation from MTs. Kymographs were generated by drawing a line across these molecules and the intensity of the molecules was then plotted against time. Photobleaching steps were then assessed manually by visual inspection of the intensity plots.

Quantification of spastin severing. Linear regions of MT lattice inside and outside condensates were identified, and the MT intensity at the end of acquisition (~10 min) was normalized to initial MT intensity from the first frame. MT regions that detached from the coverslip during the severing reaction were excluded from analysis.

Intensity distribution analysis. Pixel intensities were obtained from continuous uninterrupted regions along the MT as shown in Fig. 3. The pixel intensities were normalized to the average pixel intensity of all regions in the same field of view to normalize for changes in imaging conditions (such as laser intensity and TIRF angle). All of the normalized values were aggregated and graphed on a relative frequency plot.

Statistics and reproducibility. All data were collected from at least two independent trials with at least three independent experimental chambers per trial. All of the repeated independent experiments showed similar results. Unless otherwise stated, all data were analysed manually using ImageJ (FIJI). Graphs were created using GraphPad Prism v7.0a and v8.0.1 and statistical analyses were also performed using this software. Major points on graphs represent data means and the error bars represent variation or associated estimates of uncertainty. For comparison of DDB behaviours at condensates, the data were collected into a contingency table with four assay conditions and five observable outcomes. For data in Supplementary Fig. 4b, the aggregate analysis of the observed outcome frequencies for the entire table was performed using the Pearson’s χ² test and showed significance at very high levels (P < 0.0001). However, some outcome counts were low (below five) so the analysis was performed again using Fisher’s exact test and again significance was extremely high (P < 0.0001). DDB outcomes were compared pairwise with other assay conditions (using data for all outcomes and for just pass and pause–pass pair of outcomes) and significant differences (P < 0.0001) were seen for all comparisons using both tests. Where appropriate, two-sided statistical tests were performed.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Source data for all statistical analyses can be found on Supplementary Table 1. All other data that support the findings of this study are available from the corresponding authors on reasonable request.

Code availability
The custom analysis code used in this study is available from the corresponding authors on reasonable request.
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## Software and code

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**Data collection**

- Micromanager v.1.4, Nikon Elements AR v.4, Leica Application Suite X v.3.1

**Data analysis**

- Graphpad PRISM v.7 and v.8, Matlab R2017B, Nikon Elements AR v.4, ImageJ v.2, 1.52a, Leica Application Suite X v.3.1

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Life sciences study design

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Sample size

No statistical methods were used to determine sample size. Sample size was sufficient to determine robust statistical differences by Student’s t-test, Fisher’s exact test, and Pearson’s chi-squared test where relevant.

Data exclusions

No data was excluded.

Replication

All data was collected from at least two independent trials with at least three independent experimental chambers per trial. All findings were successfully replicated in all trials.

Randomization

Randomization was not used. Experimental conditions were maintained between experimental groups to control for covariates.

Blinding

Blinding was not possible as experimental set-up, and data-analysis were performed by the same group of authors.

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| n/a | Involved in the study |
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|     | Eukaryotic cell lines |
|     | Palaeontology        |
|     | Animals and other organisms |
|     | Human research participants |
|     | Clinical data        |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
|     | ChIP-seq              |
|     | Flow cytometry        |
|     | MRI-based neuroimaging |

### Antibodies

Antibodies used

Antibody - Supplier #CatalogueNumber: Tau – Genetex #49353, or Thermo Scientific #13-6400, Beta-Tubulin – Abcam #ab6046 (T46), Anti-chicken – Thermo Scientific #A11039, Anti-mouse – Thermo Scientific #A28175, Thermo Scientific #A28180, Anti-rabbit - Thermo Scientific #A27039. Primary antibodies used at 1:500 dilutions. Secondary Antibodies used at 1:1000

Validation

Full validation from supplier website. Genetex #49353 - IHC WB IF, rodent Cultured Neurons, rodent brain lysate. Thermo Scientific #13-6400 - WB IHC ICC IF, Rodent and human brain sections, rodent cultured neurons. Abcam #ab6046 - IHC WB ICC/IF ELISA IP, Mouse Rat Chicken Human Xenopus Levis Zebrafish Chinese hamster, samples include purified protein, cultured neurons, and brain lysates, over 536 citations available on supplier website.

### Animals and other organisms

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| Laboratory animals | Embryonic mouse - CD1 from Charles River, E16.5-17.5, Undetermined sex (pooled neurons). Mother mouse - CD1 from Charles River, 2 months to 1 year, Female. Porcine brains were obtained from adult pigs of mixed sex from a commercial slaughterhouse. |
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