Tau mediates microtubule bundle architectures mimicking fascicles of microtubules found in the axon initial segment

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Tau, an intrinsically disordered protein confined to neuronal axons, binds to and regulates microtubule dynamics. Although there have been observations of string-like microtubule fascicles in the axon initial segment (AIS) and hexagonal bundles in neurite-like processes in non-neuronal cells overexpressing Tau, cell-free reconstitutions have not replicated either geometry. Here we map out the energy landscape of Tau-mediated, GTP-dependent ‘active’ microtubule bundles at 37°C, as revealed by synchrotron SAXS and TEM. Widely spaced bundles (wall-to-wall distance $D_{w-w} \approx 25-41$ nm) with hexagonal and string-like symmetry are observed, the latter mimicking bundles found in the AIS. A second energy minimum ($D_{w-w} \approx 16-23$ nm) is revealed under osmotic pressure. The wide spacing results from a balance between repulsive forces, due to Tau’s projection domain (PD), and a stabilizing sum of transient sub-$k_B T$ cationic/anionic charge-charge attractions mediated by weakly penetrating opposing PDs. This landscape would be significantly affected by charge-altering modifications of Tau associated with neurodegeneration.

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Microtubules, a component of the eukaryotic cytoskeleton, are made up of 2β-tubulin heterodimers that dynamically assemble into hollow nanotubes composed of straight protofilaments. Microtubules are involved in a wide variety of cell functions (for example, intracellular trafficking, cell motility and chromosome segregation) through functionalization, in part, by microtubule-associated proteins (MAPs). One MAP in particular, Tau, is localized to neuronal axons and stabilizes microtubules upon binding (Fig. 1a), by partially suppressing microtubule dynamic instability (alternating periods of polymerization of tubulin into microtubules interrupted by catastrophe, or the rapid disassembly of microtubules following loss of the microtubule GTP cap). While Tau is developmentally regulated in neurons, Tau dysfunction in mature axons has been linked to many neurodegenerative ‘tauopathies’ (including Alzheimer’s, FTDP-17 (ref. 3), and, more recently, chronic traumatic encephalopathy in athletes suffering concussions). Tau consists of an amino-terminal tail (NTT) containing a projection domain (PD) and proline-rich region, followed by the C-terminal tail (CTT). Alternative splicing results in six wild-type (WT) isoforms, see Methods) are plotted in Fig. 2b–e, as a function of increasing $\Phi_{\text{tau}}$. The $D_{w-w}$ of microtubules stabilized active MT bundles (energy minimum at microtubule wall-to-wall distance $D_{w-w}$ of 25–41 nm) with hexagonal and string-like symmetry, the latter mimicking bundles found in the AIS. We propose a mechanism in which sub-3 $T$ cationic/ionic charge attractions by weakly penetrating Tau PDs on opposing microtubules stabilize active bundles. This mechanism is contingent on the overall microtubule length and thereby reconciles the lack of microtubule bundles in previous cell-free reconstitutions. With applied osmotic pressure, a second minimum ($D_{w-w}$ of 16–23 nm), indicative of antiparallel dipole–dipole interactions of interpenetrating Tau PDs, is revealed. Notably, we find that contrary to current dogma, the CTT alone is able to mediate relatively wide spacings in the absence of the NTT.

Results

SAXS reveals Tau-mediated hexagonally ordered MT bundles. SAXS is especially well suited to investigate Tau-directed higher-order assembly of microtubules (as seen at low resolution via differential interference contrast (DIC) microscopy; Fig. 1e,f), as solution scattering yields assembly structures in near-physiological conditions without tags/labels. Azimuthally averaged scattering of microtubules co-assembled with WT Tau isoforms ($\Phi_{\text{tau}}$ of 1/10) registers Bragg peak positions consistent with a two-dimensional (2D) hexagonal array ($q_{10}$, $q_{11} = 3^{1/2}q_{10}$, $q_{20} = 2q_{10}$, $q_{21} = 7^{1/2}q_{10}$, $q_{30} = 3q_{10}$, $q_{22} = 12^{1/2}q_{10}$) of microtubules with centre-to-centre distance $a_{1} = 4\pi/3^{1/2}q_{10}$ (Fig. 2a). Some peak positions are not apparent due to their proximity to the form factor minima (in particular, $q_{11}$ and $q_{22}$), necessitating line-shape analysis to separate scattering from individual microtubules (form factor, see Fig. 2a, bottom profile), the lattice of microtubules (structure factor) and background small-angle scattering. Following previous work, we modelled microtubules as hollow cylinders with ensemble-averaged inner radius $r_{m}$ (a fit parameter) and constant wall thickness $\delta$ (49 Å, in agreement with electron microscopy of microtubules). Each structure factor peak at reciprocal lattice vector $q_{hk} = q_{10}(h^{2} + k^{2} + hk)^{1/2}$ was represented as squared lorentzians (fit results as solid red lines in Fig. 2a, see Methods).

Even after $\approx 24$ h, there are no major changes in scattering (Supplementary Fig. 1), indicating that although the microtubules are dynamic (that is, hydrolyzing GTP), they have reached a steady state. The fit parameters, $r_{m}$ and $\delta$, calculated microtubule wall-to-wall distance $D_{w-w}$ ($\approx 37 ^{\circ}C$) is, mimicking the cytoskeletal environment of nerve cells with samples consuming the energy released by GTP hydrolysis). Synchrotron SAXS and plastic-embedded transmission electron microscopy (TEM) were used to obtain both angstrom-resolution ensemble-averaged structural information and nanometer-scale real-space local fine structure, respectively.

Our study reveals steady-state structures that are stable over time ($\geq 24$ h). Synchrotron SAXS studies under osmotic pressure allows us to map out the energy landscape of Tau-mediated, GTP-dependent active microtubule bundles at $37 ^{\circ}C$. In the absence of applied osmotic pressure, the microtubule reaction mixture exhibits Tau-induced phase separation into microtubule bundles (forming domains of high and low concentrations of microtubules in optical microscopy), unambiguously demonstrating the presence of an attractive component to Tau-mediated interactions between microtubules, as opposed to previous studies that had concluded that Tau acted as a purely repulsive spacer. SAXS and TEM reveal widely spaced bundles (energy minimum at microtubule wall-to-wall distance $D_{w-w}$ of 25–41 nm) with hexagonal and string-like symmetry, the latter mimicking bundles found in the AIS. We propose a mechanism in which sub-3 $T$ cationic/ionic charge attractions by weakly penetrating Tau PDs on opposing microtubules stabilize active bundles. This mechanism is contingent on the overall microtubule length and thereby reconciles the lack of microtubule bundles in previous cell-free reconstitutions. With applied osmotic pressure, a second minimum ($D_{w-w}$ of 16–23 nm), indicative of antiparallel dipole–dipole interactions of interpenetrating Tau PDs, is revealed. Notably, we find that contrary to current dogma, the CTT alone is able to mediate relatively wide spacings in the absence of the NTT.
three-repeat and four-repeat Tau isoforms, increasing PD length (increasing anionic block size, Fig. 1b) leads to increases in \(D_{ww}\) (for example, at \(\Phi_\text{Tau} = 1/10\) in Fig. 2d) \(D_{ww}\) increases from \(\approx 28–29\) nm (26–31 nm) to \(35–38\) nm (33–41 nm) in going from 3RS (4RS) to 3RL (4RL)). This behavior is consistent with observations in transfected cells\(^6\)–\(^8\), where Tau isoforms with longer PDs exhibit microtubule bundles with larger spacing. Notably, \(D_{ww}/R\) is nearly constant (\(\approx 8–11\), Fig. 2e). By plotting \(D_{ww}\) and \(D_{ww}/R\) for the six WT isoforms as a function of the overall charge of Tau (\(Q_{\text{Tau}}^\text{isoforms}\)), we see that \(D_{ww}\) decreases systematically as the overall charge of Tau increases. We note that the decrease in \(D_{ww}\) with increasing charge is accompanied by the simultaneous decrease in the size of the PD (that is, increases in Tau charge correspond to anionic inserts being removed). Thus, both the Tau PD size and electrostatics of Tau play a role in mediating these widely spaced bundles.

**Figure 1** | Tau-mediated microtubule assemblies and Tau charge distribution. (a) Cartoon showing Tau binding to a microtubule (red/blue) through its microtubule-binding region (yellow), with the projection domain (green/purple) and CTT (grey/teal) extending off the microtubule surface. (b) The average charge (dark blue for anionic character and dark grey for cationic character) of fully expressed 4RL Tau (top) as a function of primary sequence, with alternative splicing of exons 2 (red rectangle), 3 (orange) and 10 (blue) resulting in the five additional wild-type isoforms. Wild-type Tau consists of the amino-terminal tail (NTT), which includes the projection domain (PD, green/purple background) and proline-rich region (PRR, yellow), followed by the microtubule-binding region (MTBR, yellow) and carboxyl-terminal tail (CTT, grey/teal). Truncated Tau constructs were designed to understand the domain dependence of the CTT (3RS, 3C, missing CTT), anionic component of the projection domain (3RA(N-), missing anionic block of NTT) and the entire NTT (3RAN, missing NTT). (c) Prior electron microscopy revealed linear microtubule bundles in the axon initial segment (adapted from Peters et al. and reprinted by permission of Oxford University Press, USA). (d) Subsequent Tau cDNA transfection of SF9 cells revealed hexagonal arrays of microtubules in neurite-like processes (adapted from Chen et al. and reprinted by permission from Macmillan Publishers Ltd: Nature 360, 674–677, 1992).
interactions of comparable strength between Tau-coated microtubules at wide spacings.

**Truncated Tau shows Tau PD is unnecessary for microtubule bundles.** To further elucidate the nature of Tau-mediated microtubule–microtubule interactions, truncated Tau mutants (Fig. 1a, truncated Tau) were expressed/purified, and used for similar SAXS measurements (Fig. 3a) and subsequent parameter extraction from line-shape analysis (Fig. 3b–g): 3RS (truncation of the entire CTT), 3R (truncation of the anionic component of the NTT) and 3RN (truncation of the entire (2,1) 3RSNC (truncation of the entire NCT). No Tau

Figure 2 | SAXS and TEM show that Tau-assembled microtubules in active bundles recapitulate key in vivo features of microtubule spacing and linear bundles. (a) Azimuthally averaged SAXS data of WT Tau and microtubules registers Bragg peak positions consistent with hexagonal lattices for all six isoforms, as opposed to just microtubule form factor for no Tau (bottom profile). (b–e) Line-shape analysis of the SAXS data (resultant fits in red in a) yields the ensemble-averaged microtubule inner radius \( r_{in} \) (b), hexagonal lattice parameter \( a_H \) (c), wall-to-wall distance \( D_{w-w} \) (d) and \( D_{w-w}/R_G \) normalized by the calculated projection domain radius of gyration, \( R_G \) (e; see Methods). Parameters plotted are the result of line-shape analyses of two representative data measurements after 12 h to ensure equilibration, with samples made from independent tubulin purifications and Tau expressions/purifications. (f–g) The average \( D_{w-w} \) and \( D_{w-w}/R_G \) as a function of Tau net charge \( Q_{TAU} \) shows a monotonic decrease in \( D_{w-w} \) and a nearly constant \( D_{w-w}/R_G \) as a function of Tau net charge \( Q_{TAU} \) shows a monotonic decrease in \( D_{w-w} \) and a nearly constant \( D_{w-w}/R_G \). (3RM and 4RL, coincidentally, have the same charge by AA sequence and thus 4RL has been specially labelled.) (h) Electron microscopy of microtubules assembled with Tau (\( \Phi_{RM} = 1/20 \)) at low magnification show distinct bundled domains, demonstrating phase separation. (i) Domains of hexagonally ordered arrays of microtubules (identified in white outlines, \( \Phi_{RM} = 1/20 \)) with vacancies likely resulting from the suppressed (but still occurring) dynamic instability. (j) Linear bundles of microtubules (\( \Phi_{RM} = 1/20 \)), a result of extensive vacancy introduction and mimicking string-like microtubule bundles in the AIS. In (i) and (j), the staining process exaggerates the microtubule wall thickness. Scale bars, 1\( \mu m \) (h); 500 nm (i,j).
NTT). CTT elimination of 3RS Tau (3RSAC) has scattering associated with widely spaced bundles (Fig. 3a, top profile) and extracted parameters \(<f_{\text{in}}>\), \(a_1\) and \(D_{w-w}\) (Fig. 3b–d) similar to that of 3RS WT Tau (Fig. 2b–d), strongly indicating that the CTT is not critical to the WT mechanism of widely spaced bundles. Elimination of the anionic block from the PD (3RA(N-)) collapses the bundles (Fig. 3a, middle profile, Fig. 3d, \(D_{w-w} \approx 4-5\) nm), with a tight microtubule wall-to-wall spacing comparable to the radius of gyration of 3RA(N-) (\(R_G = 4.79\) nm, see Methods)\(^{20}\). This result indicates that the anionic block of the PD (a charged polymer containing overall anionic and cationic blocks) presents the dominant component of the repulsive barrier preventing neighbouring microtubules from getting closer.

Removal of the entire NTT of Tau (3RAN) results in a highly unexpected finding, where the SAXS data (Fig. 3a, bottom profile) give \(D_{w-w} \approx 22-24\) nm (Fig. 3d) compatible with widely spaced bundles, despite the nominal size (\(R_G = 4.0\) nm) of 3RAN. The relatively wide spacing seen upon elimination of the NTT (3RAN) indicates that the NTT is unnecessary for widely spaced bundling (under these conditions) and that, in its absence, the CTT (Fig. 1a) plays an equivalent role in determining the inter-microtubule interactions. TEM of microtubules assembled with 3RAN and 3RA(N-) (Fig. 3h,i) clearly shows microtubule bundles with relatively wide inter-microtubule spacing and in close contact, respectively, consistent with the SAXS data. For 3RAN and 3RSAC, \(D_{w-w}/R_G (\approx 8-11, \text{ Fig. 3e,g})\) is consistent with the WT ratio (Fig. 2e,g), suggesting
a similar mechanism of inter-microtubule interactions. However, 3RΔ(N-) presented markedly smaller Dw–w (E5nm) and Dw–w/RG (E2–4, Fig. 3e,g), suggesting a different inter-microtubule interaction regime.

Second energy minimum of bundles accessed via osmotic stress.

To understand the molecular mechanism of the Tau-mediated interactions between microtubules in active bundles, the force response behaviour of bundles was measured via SAXS of reaction mixtures under osmotic stress. We used high molecular-weight PEO-100k (size E40 nm, see Methods) to ensure that polymer depletant did not penetrate the inter-microtubule region in the widely spaced bundles, creating a polymer concentration exterior to microtubule bundles and thus exerting an osmotic pressure (P) on the bundle itself. By this method (Fig. 4a), we measured the microtubule wall-to-wall spacing Dw–w of bundles induced by 3RS and 3RL WT isoforms as a function of increasing P (Fig. 4b). The P–Dw–w curves for both WT isoforms exhibit an initial soft repulsion with Dw–w decreasing E3–4 nm up to P E40 Pa followed by a steep increase in slope (with Dw–w decreasing E2–3 nm for 40 Pa < P < E300–400 Pa) consistent with a highly repulsive barrier due to the PD with anionic blocks, resisting osmotic compression. Remarkably, above a critical pressure Pc (E300 and 400 Pa for 3RS and 3RL, respectively), there is an abrupt E5 nm decrease in Dw–w from E21.5 to E16.5 nm for 3RS and E27.5 to E22.5 nm for 3RL. This sudden jump is reflected in the SAXS data as a sudden large shift in peak position as P is increased to just above Pc (lines in Fig. 4a are a guide to the first peak below and above Pc).

Discussion

The osmotic pressure data and, more specifically, the abrupt transition above Pc are consistent with the onset of antiparallel dimerization between fully interpenetrating dipolar Tau PDs on opposing microtubule surfaces (Fig. 4c). Microtubule bundles at this intermediate spacing are in a second local energy minimum distinct from widely spaced bundles in the absence of PEO. Several findings support this model. First, reversibility measurements show this local minimum is stable, as bundles for
\[ P > P_0 \text{ do not relax to their previous spacings upon removal of PE0-100k but instead relax to the } D_{w-w} \text{ associated with } P_e. \] This implies the barrier between the second and widely spaced minima is greater than thermal energy \( k_B T \). Second, the wall-to-wall spacing observed for this local minimum for intermediate spacing bundles (\( D_{w-w} \approx 22.5 \text{ nm for 3RL} \)) is consistent with the recent work showing that PDs for microtubule-bound Tau are in an extended conformation (size \( \approx 23 \text{ nm for } -L \text{ isoforms} \)), twice that of the Tau PD physical diameter in solution\(^{21}. \)

Considering the minimum associated with widely spaced bundles, several findings with WT Tau isoforms point to the repulsive component emanating from the PD containing the anionic block: the steep repulsive barrier when PD chains are pushed together under osmotic pressure and the increase in \( D_{w-w} \) with increasing PD length (and increasing anionic block size, Fig. 2d). The observation of microtubule phase separation into widely spaced bundles (Figs 1f and 2h), upon addition of small amounts of WT Tau (as low as \( \Phi_{\text{Tau}} = 1/40 \)), demonstrates a Tau-mediated attractive component of the energy minimum, which overcomes the repulsive component, stabilizing microtubule bundles against dilution. To reconcile these wide spacings, we note that it was recently discovered\(^{20} \) that the effective size of Tau-stabilized microtubules (microtubule length \( \approx 7 \text{–} 11 \text{ µm} \)) is greater than thermal energy \( (4 + j)^{17}. \) Remarkably, this ion correlation mechanism predicts that \( D_{w-w} \approx \text{physical size} \) of the macromolecular counterion \( (z = 2(5/3)^{1/4} R_g) \), consistent with \( D_{w-w}/R_g \approx 2–4 \) for \( 3\text{RA(N-)} \) (Fig. 3e,g).

The proposed mode by which Tau bundles microtubules may have major implications for post-translational modifications of Tau associated with neurodegeneration, especially phosphorylation. In tauopathies, both cytosolic Tau and aggregated Tau in neurofibrillary tangles are hyperphosphorylated\(^{32,33} \). Our mechanism predicts that increased Tau phosphorylation would suppress Tau-mediated attractions and bundle formation. Although the identity of MAPs involved in fascicle formation in the AIS is not fully known, our results suggest that hyperphosphorylation of MAPs in the AIS (for example, either MAP Tau or other MAPs with similar polyampholytic structures in their PDs mediating attractions) would disrupt fascicles, and impair neuronal polarity crucial to healthy neurons.

This highly unusual interaction between widely spaced surfaces is made possible by the non-uniform charge distribution of the PD of Tau, where segments of the NTT (which shift the active zone to the mid-layer) are followed by shorter, cationic/anionic domains enabling attraction between Tau on opposing microtubule surfaces. We hope this discovery will spur analytical and computer modelling efforts, which take into account the specific sequence of Tau, to more quantitatively describe the Tau-mediated interactions and, in particular, the effect on Tau-mediated microtubule attractions in the presence of Tau phosphorylation and disease-related hyperphosphorylation. Furthermore, generalizable principles derived from this system could serve as inspiration for polymer-directed assembling materials.

**Methods**

**Purification of Tubulin and Tau.** Tubulin was purified from MAP-rich microtubules extracted from bovine brains. MAP-rich microtubules were obtained from crude brain extract by three polymerization/denaturalization cycles, after which tubulin was separated from MAPs with a phosphocellulose anionic exchange column. Tubulin was suspended in PEMS0 (50 mM Pipes (pH 6.8), 1 mM MgSO\(_4\) and 1 mM EGTA) with protein concentration between 7 and 12 mg ml\(^{-1}\), as measured by bovine serum albumin concentration standard. Solution was drop-frozen in liquid nitrogen and stored in a −70 °C freezer until use.

The concentration of each Tau stock was determined by SDS–polyacrylamide gel electrophoresis comparison with a Tau mass standard (originally measured via amino-acid analysis).

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Truncated Tau mutants were designed via the QuickChange Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) with appropriate introduction/deletion of start and stop codons: 3RSAC (truncation of the entire CTT, deleting residues 280–352 of 3RS), 3RAN (truncation of the anionic component of the NTT, deleting residues 2–117 of 3RL) and 3RAN (truncation of the entire NTT, deleting residues 2–255 of 3RL). Truncated Tau mutants were then expressed/purified, as above.

**Sample preparation.** After thawing frozen tubulin and Tau stocks, samples were prepared on ice, mixing tubulin, GTP and Tau such that final concentrations were 5 mg ml⁻¹, 2 mM and appropriate molar ratio of Tau to tubulin, respectively, in a final volume of 50 μl of PEM50 buffer. Samples were then polymerized in a 37°C for 40 min. If necessary, sample was brought to appropriate KCl concentration.

**Osmotic pressure samples.** A previous study measured the osmotic pressure (in Pa). P, of an aqueous solution of varying concentrations (c_mg ml⁻¹) and % w/v of poly(ethylene oxide) (Mw = 105,000 g mol⁻¹) at 35°C, which was taken as a reasonable approximation of the behaviour of PEO-100k at 37°C, absent further data. Data were fit to a second-order polynomial (following the mathematical form of a virial expansion) to determine a formula to relate an arbitrary PEO-100k concentration to a corresponding osmotic pressure (P, in Pa):

\[ P = 147.38vC^{0.1927} + 338.19wC^{1.0000} (\text{Pa}) \]

Alternatively, following a derivation in Rau et al., the osmotic pressure (P) on cylinders in a hexagonal lattice can be converted to a force per unit length between nearest cylinder pairs f as a function of the hexagonal lattice parameter a_H:

\[ f(a_H) = P_{osm} / a_H^{1.25} \text{ (N m}^{-1}) \]

PEO-100k was used as the osmotic depletant of choice compared with better-characterized depletants to parameters unique to our system: as stable inter-microtubule distances of up to 41 nm were observed, the distance of the depletion had to be smaller or equal than that distance to create a concentration differential inside/outside the microtubule bundle. Prior work measured the radius of gyration (R_g) of a function of PEO molecular weight (MW):

\[ R_g = 0.215M^{0.383} \text{ (nm)} \]

Thus, the effective depletion radius \( a \), \( a = 2R_g \pi^{-1/2} = 19.95 \text{ nm} \), or an effective depletion diameter, \( d = 40 \text{ nm} \), satisfies our experimental conditions that polymer not penetrate the space between microtubules in microtubule bundles.

**Small-angle X-ray scattering.** After polymerization, samples are loaded into 1.5-mm diameter quartz mark tubes (Hilgenberg GmbH, Malsfeld, Germany) at 9,500 rpm (176 μm diameter) with a Si(111) monochromator. Scattering data are taken with a 2D area detector (MarUSA, Evanston, Illinois) with a sample to detector distance of approximately 200 mm in the horizontal and 200 mm in the vertical and horizontal directions. To ensure reproducibility, scattering data were retaken for most samples at similar sample conditions using tubulin and Tau from different purifications and expressions/purifications, respectively.

**SAXS analysis.** Scattering data were azimuthally averaged and small-angle scattering was subsequently background subtracted by fitting the minima of scattering intensities to a polynomial equation. Data were then fit to the appropriate model using a custom MATLAB fitting routine using the Levenberg–Marquardt non-linear fitting routine. Microtubules were modelled as homogenous, hollow cylinders (with no expected scattering from Tau/PEO due to low electron density relative to water) with ensemble-averaged inner radius \( r_{in} \) (a fit parameter), wall thickness \( \delta \) (49 Å, an input parameter) and microtubule length L (20 μm, an input parameter for Tau-stabilized microtubules[36], averaged all orientations in q-space:

\[ |F(q)|^2 \propto \int \sin(qL/2) \sin(qL/2) (q_{r} + \delta) (-q_{r}L + \delta) - q_{r}L)I(q_{r},r_{in})^2 \]

where \( q_{r} \) and \( q_{r} \) are wavevectors perpendicular and parallel to the tubular axis, and \( I(q_{r},r_{in}) \) is the Bessel function of order 1. The structure-factor peaks (at reciprocal lattice vector for a hexagonal array, \( |g_{n}| = g_{n} + \delta^2 + h^2 \)) were modelled as squared lorentzians with peak amplitude \( A_{n} \) (a fit parameter) and peak width \( \sigma_{n} \) (a fit parameter, with \( \sigma_{n} \) corresponding to the average bundle width.

\[ S(q) = \sum (A_{n} / \sigma_{n}^2 + q_{r}^2 - \sigma_{n}^2)^2 \]

Fit of the intensities data, \( S(q) = |F(q)|^2 \) yielded the hexagonal lattice parameter \( a_{H} = 38 \text{ Å} \) and ensemble-averaged inner radius \( r_{in} \). \( \sigma_{n} \) was fit independently, while all other \( \sigma_{n} \) fit simultaneously, with \( \sigma_{n} \) approximately twice that of \( \sigma_{n} \).

**Plastic-embedded TEM sample preparation and TEM.** Samples for thin sections were centrifuged to a pellet at 9,500 g in 37°C for 30 min. Supernatant was removed and pellet fixed with 2% glutaraldehyde and 4% tannic acid overnight. The pellet was stained with 0.8% OsO₄ in PEM50 buffer for 1 h and subsequently rinsed four times with PEM50. Another stain of 1% uranyl acetate was applied for 1 h and rinsed with DI water.

Fixed and stained pellets were subsequently dehydrated with 25/50/75/100% solutions of acetone in DI water for 15 min apiece. Samples were embedded in resin, then embedded in spurr plastic and incubated overnight, with resin poured into flat embedding moulds and held at 65°C for 48 h and cooled overnight.

Plastic-embedded samples were then cut to ±50 nm slices with a microtome (Ted Pella, Redding, CA) and transferred to highly stable Formvar carbon-coated copper EM grids (Ted Pella, Redding, CA). Data were taken using the JEOL 1230 Transmission Electron Microscope.

**DIC Samples and DIC.** A SensiCam CCD camera (Cooke, Auburn Hills, MI) mounted on a Nikon Diaphot 300 with Xenon lamp (Sutter Instrument, Novato, CA) was used for optical microscopy measurements. Samples were centrifuged to a pellet at 9,500 g in 37°C and placed between two microscopic slides sealed with wax. Images were taken while slides were kept at 37°C by heat stage.

**Calculation of \( R_g \).** Previously, the radius of gyration (R_g) of WT Tau and truncated Tau domains in solution were found to scale as an unstructured protein with random-coil behaviour, with \( R_g = 0.1927M^{0.383} \) nm, which was subsequently used to calculate the \( R_c \) of the PD and truncated Tau used in our experiments.

**Data availability.** The authors declare that the data supporting the findings of this study are available within the article, and its Supplementary Information files, or from the authors on reasonable request.

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