Supplementary information

Methods and Materials

Protein expression and purification. An N-terminal truncated form of 2N4R tau, tau187, containing the microtubule binding domain (residues 255-441 from the longest human tau isoform 2N4R with a 6× His-tag at the N-terminus) was used for in vitro studies. Mutated variants of tau187 were prepared using site-directed mutagenesis: tau187P301L contains P301L mutation; tau187C291SP301L contains C291S and P301L mutations; tau187C291SC322S contains C291S and C322S mutations; tau187G272CS285C contains C291S, C322S, G272C and S285C mutations. Except otherwise stated, the experiments reported in this manuscript were carried out on tau187C291SP301L, which is referred to as tau throughout the text.

The expression and purification of tau187 have been previously reported (1–3). E. coli BL21 (DE3) cells were transfected with constructed DNA variants and stored as frozen glycerol stock at −80 °C. Cells from glycerol stock were grown in 10 mL luria broth (LB, Sigma Aldrich, L3022) overnight and then used to inoculate 1 L of fresh LB. Growth of cells were performed at 37 °C, 200 rpm with addition of 10 μg/mL kanamycin (Fisher Scientific, BP906) until optical density at λ = 600nm reached 0.6–0.8. Expression was induced by incubation with 1 mM isopropyl-ß-D-thiogalactoside (Fisher Bioreagents, BP175510) for 2–3 h. Cells were harvested with centrifugation at 4500 g for 20 min. Harvested cells were resuspended in lysis buffer (Tris-HCl, pH = 7.4, 100 mM NaCl, 0.5 mM DTT, 0.1 mM EDTA) added with 1 Pierce protease inhibitor tablet (Thermo Scientific, A32965), 1 mM PMSF, 2 mg/mL lysozyme, 20 μg/mL DNase (Sigma, DN25) and 10 mM MgCl₂ (10 mM), and incubated on ice for 30 min. Samples were then frozen and thawed for 3 times using liquid nitrogen, then centrifuged at 10,000 rpm for 10 min to remove cell debris. 1 mM PMSF was added again and samples were heated at 65 °C for 12 min and cooled on ice for 20 min. Cooled samples were then centrifuged at 10,000 rpm for 10 min to remove the precipitant. The resulting supernatant was incubated overnight with Ni-NTA resins pre-equilibrated in buffer A (20 mM sodium phosphate, pH = 7.0, 500 mM NaCl, 10 mM imidazole, 100 μM EDTA). The resins were loaded to a column and washed with 20 mL of buffer A, 25 mL buffer B (20 mM sodium phosphate, pH = 7.0, 1 M NaCl, 20 mM imidazole, 0.5 mM DTT, 100 μM EDTA). Purified protein was eluted with 15 mL of buffer C (20 mM sodium phosphate, pH = 7.0, 0.5 mM DTT, 100 mM NaCl, 300 mM imidazole). Eluents were analyzed by SDS-PAGE to collect the pure fractions. Proteins were then buffer exchanged into DTT-free working buffer (20 mM ammonium acetate, pH 7.0) and immediately frozen and kept at −80 °C until usage.

Preparation and digestion of tau fibrils. In vitro experiments were carried out in working buffer (20 mM ammonium acetate, pH 7.0) for RNA fibrils and (20 mM ammonium acetate pH 7.0, 100 mM NaCl, 1.5 mM CaCl₂) for heparin fibrils. Heparin-induced tau fibrils (heparin fibrils) were prepared by incubating 20 μM tau, 5 μM heparin (Galen laboratory supplies, HEP001) and 20 μM Thioflavin T at 37 °C. RNA-induced tau fibrils (RNA fibrils) were prepared by mixing 20 μM of tau with 300 μg/ml RNA (polyU, Sigma, P9528) and incubated at room temperature in the presence of 10 μM Thioflavin T. Immediately after mixing, samples were pipetted onto a micro plate (Corning, 3844) and fluorescence intensity was monitored in a Bio-Tek Synergy 2 microplate reader (excitation 440/30, emission 485/20, number of flash 16). Data were plotted using home-made R package gen5helper (https://www.github.com/yanxianucsb/gen5helper).

When ThT fluorescence reached maximum, samples were collected to be tau fibrils before digestion (referred to as ‘Before’ in figures). Digestions of heparin were carried out by incubating heparin fibrils with Bacteroides heparinase I (heparinase, New England Biolabs Inc. P0735S) and incubated at 30 °C for 8
hours. Digestions of RNA were carried out by incubating RNA fibrils with RNase A (RNase, Thermo Scientific, AB-0548) at room temperature for 8 hours. Different concentration of heparinase/RNase were used (in all figures, 1× heparinase: 1 Units of enzyme per 1 μg of heparin; 1× RNase: 2.5 μg/mL). The sample denoted as “Control” in Fig. 1, “Non-digested” in Fig. 2 and “Non-digested fibril” in Fig. 3A followed the identical incubation times and temperatures than the digested samples but received buffer instead of enzymes.

Pelleted fibrils (used in Fig. 2B RNA fibrils, Fig. 3A and Fig. S4) were prepared from tau fibrils before digestion, by centrifugation at 53,000 rpm (TLA-100.3 rotor) for 1h and resolubilizing in working buffer.

Quantification and characterization of digested heparin. Tau fibrils were prepared with Spin-labeled heparin (heparin-SL) by incubating 4:1 molar ratio of tau:heparin-SL overnight at 37 °C, with a final concentration of 80 μM tau and 20 μM heparin-SL. After the sample reached its maximum ThT fluorescence, 2 μL of heparinase was added to 20 μL of sample, and 2 μL of buffer was added to a control sample. The samples were incubated at 30 °C for 12-16 h. Cw-EPR spectra were recorded, and the samples were then filtered through a 0.22 μm MilliporeMilllex-GV filter. Cw-EPR spectra were recorded of the filtrates and referred to as “1× heparinase” for the digested sample, and “Non-digested” for the undigested sample in Fig. 2B. The double integral of cw-EPR spectra were used to quantify the number of spin labels in the samples and therefore the concentration of heparin. The values were divided by the double integral before filtration in order to obtain the percentage of heparin that passed the filter reported in Fig. 2B.

To determine the mobility of the tethered spins, the width of the central peak at half its maximum value (CPWHM) was measured. Values before and after digestion, and before and after filtration are compared to soluble heparin in Fig. S7. The CPWHM provides a proxy for the mobility of the spin label through its relationship to the rotational correlation time(4, 5). Each experiment was repeated 3 times independently. Additionally, after digestion, 20 μL of sample were centrifuged for 20 minutes at 14,000×g. The resultant pellet was resuspended in buffer, and the cw-EPR spectrum was recorded and denoted as “SL-heparin-fibrils-digested-pellet” in Fig. S7.

Quantification of digested RNA.

To remove low-molecular-weight RNA, 5 mg/mL RNA was first dialyzed (Pur-A-Lyzer 12,000 Da, Sigma Aldrich) against a 1000-fold excess of deionized water for 16 h. The concentration of RNA inside the dialysis membrane (high-molecular-weight RNA, referred to as HMW RNA) was then calculated by UV absorbance at 260 nm (A260, the extinction coefficient is 0.025 (μg/mL)−1 cm−1) using a Shimadzu UV-1601 spectrophotometer (Shimadzu Inc.). The absorbance at 280 nm (A280) was recorded and the A260/A280 ratio was used to assess RNA purity. A260/A280 ratios of 1.9 or above were found, indicating a reliable RNA purity.

RNA fibrils were then prepared with 60 μM tau and 900 μg/mL HMW RNA, followed by pellet preparation described above and resolubilized in the same volume of working buffer. Digested RNA fibrils were subjected to 1× RNase treatment, and non-digested RNA fibrils treated by an equivalent volume of working buffer. 60 μL of both digested and non-digested RNA fibrils were then dialyzed in 800 μL of working buffer for at least 24 h, followed by the measurement of A260 and A280 described above to determine RNA concentration and purity of the solution outside of the membrane.

Two controls were used where digested and non-digested HMW RNA alone (without tau) was dialyzed under the same conditions of the above RNA fibril samples. RNA concentrations outside of the membrane were determined by the same above method in Fig. 2B. Each sample was repeated 3 times independently.
Re-aggregation from digested RNA fibrils. 300 μL RNA fibrils were prepared by mixing 20 μM of tau with 300 μg/ml RNA and incubated at room temperature. ThT fluorescence was monitored in the presence of 10 μM ThT. When ThT fluorescence reached its maximum, samples were collected and ultracentrifuged at 53,000 rpm (rotor TLA-100.3) for 1 h at 4 °C. Pellet was washed and resuspended in 300 μL working buffer. The digestions of RNA were carried out by incubating RNA fibrils with 1× RNase at room temperature for 8 h. To separate and purify monomer in the digested RNA fibrils, each sample was first filtered through 0.2 μm Nalgene syringe filter (#720-1320, Thermo Scientific) and the size exclusion chromatography was conducted by injecting 250 μL sample onto ENrich SEC 70 10 x 300 Column (GE Healthcare Life Sciences) connected to a BioRad NGC Quest 10 FPLC system, and 0.5 mL fractions were collected with a BioFrac fraction collector (BioRad). Prior to sample injection, the column was washed by 30 mL degassed Milli-Q water at a flow rate of 1 mL/min and equilibrated with 50 mL degassed working buffer at 1 mL/min. Samples were eluted from the column with 30 mL working buffer at 0.75 mL/min after sample injection. Fractions of each elution peaks were collected right after elution. Elution peak assignment was done by comparing with the elution profile of purified tau187 monomers. Fractions corresponding to monomer were concentrated using Amicon Ultra-4 centrifugal filters (Fisher Scientific, #UFC801024) at 10 kDa cutoff and were further analyzed by SDS-PAGE to confirm that these fractions were monomeric tau. The concentration of tau monomer was determined by UV-Vis absorbance at 274 nm. Re-aggregation was performed by incubating 20 μM monomer from digested RNA fibrils, 5 μM heparin, and 20 μM ThT at 37 °C, and compared with a control sample containing 20 μM tau monomer freshly purified, 5 μM heparin and 20 μM ThT, while monitoring ThT fluorescence. The entire reaggregation experiment was repeated in 3 times independently. For each repeat, overlapped in Fig. 3A, the maximum ThT of the control sample was used as a normalization value for both the control and the re-aggregation curve.

BN-PAGE. For quantification of tau monomers, tau fibrils before and after enzyme digestions were prepared as described above with either 100 μM tau and 25 μM heparin or 20 μM tau and 300 μg/mL RNA. Sample were loaded onto a BN-PAGE using 3~12% Bis-Tris gradient gels (Novex), following the instructions of the manufacturer. For digested and non-digested heparin fibrils, 24 μg equivalent monomers were loaded. For digested and non-digested RNA fibrils, 4 μg were loaded.

Western blot. Samples of sarkosyl soluble, wash, and sarkosyl insoluble fractions from rTg4510 extraction were run on an SDS-PAGE gel (Bio-Rad), transferred to a nitrocellulose membrane, blocked in 5% BSA/TBST, and blotted with primary antibody PHF-1 (Peter Davies) 1:1000 in 5% BSA/TBST overnight at 4°C. The next day membranes were washed 3× with TBST and incubated with secondary antibody (Invitrogen, A21057) for 1h at RT. Membranes were washed 3× and developed on a LI-COR Odyssey scanner (LI-COR Biosciences).

TEM. 10 μL of recombinant tau fibril samples were applied to copper grid (Electron Microscopy Science, FCF-300-Cu) cleaned with plasma for 20 s. Samples were stained with 10 μL 1.5 w/v % uranyl acetate and were analyzed using a FEI Tecnai G2 TEM microscope (FEI) at room temperature. For mouse-derived fibrils (Fig. S15), tau fibrils extracted from rTg4510 mice cortex (0.5 μg) were adsorbed on 200-mesh formvar-coated copper grids, washed, and stained with a 2% uranyl acetate solution. Grids were then imaged with a JEOL JEM-1230 (JEOL USA, Inc) at the indicated magnifications.

Fibril dimension quantification. TEM images were acquired as described in Methods and Materials at 5 μm defocus. Fibril dimensions were measured manually using ImageJ, by an independent observer who had no knowledge of the sample identity. The maximum width of each fibril was measured along
every distinguishable period. 10 images of 3 independent grids were measured for each digested fibril, and non-digested fibril sample. A total of 390 measurements were taken for the digested fibrils, and 338 measurements were taken for undigested fibrils.

**Spin labeling of tau and heparin.** Protein was spin-labelled using MTSL (1-Acetoxy-2,2,5,5-tetramethyl-δ-3-pyrroline-3-methyl) Methanethiosulfonate) purchased from Toronto Research Chemicals. Prior to labelling, samples were treated with 5 mM DTT, which was removed using a PD-10 desalting column. Then, 10× to 15× molar excess MTSL to free cysteine was incubated with the protein at room temperature overnight. Excess MTSL was removed using a PD-10 desalting column. Labelling efficiency, defined as the molar ratio of tethered spin labels over the cysteines, was measured to be 50-60% for double-cysteine mutants (Tau187G272CS285CC291SC322S) and about 90% for single-cysteine mutant (Tau187C291SP301L).

Heparin sodium salt was spin-labeled with 4-amino-TEMPO through derivatization of the carboxyl group of the uronic acid residues of heparin. 0.5 mmol heparin was mixed with 0.9 mmol 4-amino-TEMPO, 0.1 mmol ethylenediamine, and 0.5 mmol N-hydroxysuccinimide on ice. 0.7 mmol EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) was added and stirred on ice for 30 min. The solution was stirred for 7 h at room temperature, frozen at -20 °C, then lyophilized. Lyophilized samples were dissolved in 45 mL ethanol, centrifuged, and the supernatant was decanted. The pellet was washed with ethanol twice more and dried via vacuum, then dissolved in H2O and stored in freezer. Heparin sodium salt has a reported average molecular weight of 12-15 kDa. The resulting heparin was determined to contain an average of 1.2±0.2 spin labels per heparin molecule via cw-EPR double integrals.

**Continuous wave Electron Paramagnetic Resonance (cw-EPR).** Cw-EPR experiments were carried out on Bruker EMX X-band spectrometer with dielectric cavity (ER4123D). 4 μL of sample was loaded into a capillary tube (0.6 mm i.d., 0.8 mm o.d.) and sealed at one end with critoseal. Samples were irradiated with 2 mW incident microwave power at 9.8 GHz using a 1 G modulation amplitude and a sweep width of 150 G or 200 G.

Spin counting was obtained by calculating the doubled integral of the acquired derivative spectra, which is proportional to the number of spin in the cavity. A 2nd polynomial fitted baseline was subtracted to the first integral before applying the second integration.

**EPR simulation.** The cw-EPR spectra of tau-SL recorded were subjected to single-, double- or triple-component EPR simulations. The simulation were performed using MultiComponent developed by Christian Altenbach (University of California, Los Angeles) as previously reported (3). For all EPR spectra fitting, the magnetic tensors A and g were used as constraints. For tau-SL the tensor A and g were set as previously reported (3).

The single-component fit was obtained from tau-SL without any cofactor present. The parameters of fitted single-component was used as a mobile/fast- component in the double- and triple-component fitting. For double-component fitting of tau-SL, the immobile/slow-component was set to be identical to the mobile/fast-component except the tilt angles of the diffusion tensor βD = 36°, the scaling coefficient of orienting potential (c20), and the rotational diffusion constant (R). The fitting parameters are the scaling (population) of two components (p1, p2), c20 and R of the immobile/slow-component. For triple-component fitting of tau-SL, the spin exchange component was set to be identical to the immobile/slow-component except including Heisenberg exchange frequency ωss = 140 MHz. The fitting parameters are the population of three components (p1, p2, p3), c20 and R of the immobile/slow/exchange-components. The parameters
were chosen to minimize the number of fitting parameters. For clarity, rotational diffusion constant \( R \) was transformed into rotational correlation time \( \tau_R \) by \( \tau_R = 1/(6R) \). Fitted results for tau-SL were noted in the manuscript.

**Double Electron Electron Resonance (DEER).** Tau187G272CS285CC291SC322S was expressed and spin labeled as tau-SL, which contains two spin labels in order to probe distances between residues 272 and 285. Tau187C291SC322S (tau-cysless) was expressed as cysteine-less to avoid disulfide bonding. The protein stocks were concentrated, and buffer exchanged against \( \text{D}_2\text{O} \)-based buffer (100 mM NaCl, 20 mM ammonium acetate, 1.5 mM \( \text{CaCl}_2 \)) using Amicon centrifugal concentrators (10 kDa cutoff). A 1:10 molar ratio tau-SL:tau-cysless sample of 57 \( \mu \text{M} \) tau-SL and 570 \( \mu \text{M} \) tau-cysless was incubated with 157 \( \mu \text{M} \) heparin at room temperature for 24 h to prepare heparin fibrils (denoted as “before digestion” in Fig. 3B). Heparin fibrils were then incubated with 120 U of heparinase at 30 °C overnight to prepare digested heparin fibrils (denoted as “0.4× Heparinase” in Fig. 4B). 35 \( \mu \text{L} \) samples were mixed with 15 \( \mu \text{L} \) \( \text{D}_8 \)-glycerol (30% volume) before transferring to a quartz tube (2 mm i.d.) and frozen using liquid nitrogen. Seeded-fibrils sample (Fig. 4B) was prepared as follow: A 1:10 molar ratio tau-SL:tau-cysless sample of 15 \( \mu \text{M} \) tau-SL and 150 \( \mu \text{M} \) tau-cysless was incubated at RT with 825 \( \mu \text{g/ml polyU} \) and 1% (protein mass) mouse brain-extracted tau fibrils. After 12h, the sample was pelleted and the fibrils solubilized in 35 \( \mu \text{L} \) buffer and 15 \( \mu \text{L} \) \( \text{D}_8 \)-glycerol before transferring to a quartz tube (2 mm i.d.) and freezing using liquid nitrogen.

Four-pulse DEER experiments were carried out at 85 K using the Q-band Bruker E580 Elexsys pulse EPR spectrometer operating at ~34 GHz and equipped with a 300 W TWT amplifier. The following DEER pulse sequence was used: \( \pi_{\text{obs}}/2 - \tau_1 - \pi_{\text{obs}} - (t - \pi_{\text{pump}}) - (\tau_2 - t) - \pi_{\text{obs}} - \tau_2 - \text{echo} \). Rectangular observe pulses were used with lengths set to \( \pi_{\text{obs}}/2 = 10-12 \text{ ns} \) and \( \pi_{\text{obs}} = 20-24 \text{ ns} \). A chirp \( \pi_{\text{pump}} \) pulse was applied with a length of 20-24 ns and a frequency width of 133 MHz. The observe frequency was 150 MHz higher than the center of the pump frequency range. \( \tau_1 \) was 180 ns and \( \tau_2 \) was set between 1.8 ms and 2.4 ms. The DEER experiment was accumulated for ~12 h.

The DEER signal was first baseline-corrected and followed by reconstruction of the distance distribution. For baseline correction, natural log of the DEER signal was taken and then linear baseline was obtained using the linear polynomial function. The DEER background signal is an exponential decay and it’s logarithm provides a linear region from which the baseline can be easily obtained. A new Picard-Selected Segment-Optimized Singular Value Decomposition (or PICASSO) method (6) was applied to obtain distance distributions from the DEER signal, which is an ill-posed problem. The method finds the optimal distribution value at each distance or distance range by determining the different singular value cut-offs associated with the optimal solution at each distance using the Picard condition (6). The Picard condition informs when the solution diverges, allowing the selection of singular value cut-off before the divergence point. The method ensures optimal convergence at all distance ranges, preventing a premature or unstable solution as well as any spurious peaks. It also avoids negative values in distributions as they originate from premature and unstable solution. A singular value cut-off of 0.5-0.7 was used throughout for all datasets. Fig. S17 overlaps the experimental DEER signal with the signal calculated from the obtained distance distributions, for the 4 data sets presented in Fig. 3B and 4B. To calculate uncertainty in the distance distribution, an error analysis is conducted. The minimum and maximum values are calculated for singular value contributions where distance distributions are converged to the accurate solution and remain converged. The minimum and maximum values provide the uncertainty in the optimal distribution.

**Immunocytochemistry.** Cells were fixed with 4% paraformaldehyde for 15 min at room temperature followed by three washes with PBS. Cells were permeabilized with 0.25% Triton X-100 in PBS and blocked for 1 hr in blocking buffer at room temperature (1% BSA, 300 mM Glycine, 0.1% Gelatin, 4% Donkey Serum in TBST). After blocking, cells were incubated with primary antibody PHF-1 (Peter Davies) diluted
1:1000 in blocking buffer overnight at 4 °C. The day after cells were washed three times (5 min each) with 0.05% Tween-20 in PBS. Secondary antibody (Life Technologies, A21422 1:1000) was incubated for 1 h at RT, washed three times with 0.05% Tween-20 in PBS and imaged with an Olympus IX71 Microscope.

**Mouse Fibril Extraction.** Cortex or hippocampal tissue from rTg4510 mice were homogenized in high salt buffer (10 mM Tris-HCl pH 7.9, 0.8 M NaCl, 1 mM EDTA, 2 mM DTT, 0.1% sarkosyl, 10% sucrose, complete protease inhibitor cocktail (Roche), phosphatase inhibitor cocktail (Thermo Scientific), and 0.1 mM PMSF). Tissue was homogenized and clarified (10,000 × g, 10 min) in three sequential rounds to obtain a pooled supernatant fraction. This fraction was 0.45 μm filtered and additional sarkosyl (1% final volume) added to the sample incubated at 4°C with rotation. The following day the sample was ultracentrifuged at 100,000 × g for 2 h at 4 °C. Pellet was washed with PBS, resuspended in PBS, passed through a 27 G needle and ultracentrifuged again at 100,000 × g for 1 h at 4°C. The final pellet was washed, resuspended in PBS and protein concentration was determined via BCA assay (Thermo Scientific). The final product was characterized in Fig. S15.

**Cellular Seeding assay.** H4 neuroglioma cells stably expressing mCerulean-K18 (P301L/V337M) or mCerulean-tau187 (P301L/V337M) were cultured in DMEM supplemented with 10% FBS, 100 μg/ml penicillin/streptomycin. Cultures were maintained in a humidified atmosphere of 5% CO₂ at 37 °C. For seeding experiments, cells were plated in a 96-well plate and the following day various tau species were transfected (50 ng/μL final) using Lipofectamine 2000 (Thermo Fisher). The seeds indicated as “sonicated” were sonicated 30sec with the micro tip of a Qsonica sonicator. For quantification of the assay (Fig. S9 and 3C), the number of cells containing one or several puncta were counted and divided by the total number of cells in the well. Each well on a cultured plate provide one point. Quantification results originate from two independent cultures.

**In vitro seeding assay.** In the first generation, 20 μM tau187C291S in (100 mM NaCl, 20 mM ammonium acetate) was seeded with 5% mass of either mouse-extracted fibrils or heparin-induced fibrils. The heparin-induced fibrils were sonicated in a bath sonicator for 20 min before uses. 20 μM thioflavin T was present in the buffer. “+cofactor” denotes the presence of 120 μg/ml polyU RNA while “-cofactor” demotes no RNA added. After seed addition, samples were placed in a 384-well microplate (Corning 3844) and ThT fluorescence was measured in a plate reader (Biotek Synergy 2) at 37 °C with shaking. After 24 h, the samples were used as seeds for the second generation. 10% mass of the end product of the first generation was added, after 20 min sonication in a bath sonicator, to fresh tau187C291S with or without cofactor. First generation with/without cofactor was used to seed second generation with/without cofactor, respectively.

**Supplementary discussions**

**DEER data processing.** For the DEER signals processed, the singular value cut-off for optimal solution ranged between 0.5-0.7, which results in highly resolved distance distributions. Both narrow and broad
distributions can be easily be distinguished in this cut-off range. The use of a similar singular value cut-off range also allows the reliable comparison of distance distributions without any parameter bias. This is of particular importance for the comparison made in Fig. 4B where mouse-seeded fibrils are found to show more distinct and narrow populations than heparin fibrils. The uncertainty analysis reveals that the distributions are stable and different peaks are reliable.

The evolution times for the DEER signals were similar for all the 4 samples, i.e. around 1.7 µs, to effectively compare the different distance distributions. When longer evolution times were available, the distributions were calculated for several evolution times (1.7, 2 and 2.4 µs) and compared in Fig. S18a, b and c. Truncation of the time-domain data is expected to primarily impact the certainty at longer distances, however, as shown in Fig. S18, the distance distribution is quite similar. The good agreement between the different distributions shows that 1.7 µs is enough to obtain accurate distribution. A comparison of the time-domain data for heparin induced aggregation and seeded aggregation are shown in Fig. S19.

**Heparinase does not digest a unique population of fibrils.** We measured and compared the width of the fibrils by TEM of both non-digested and digested heparin fibrils (see Fig. S5). Only a single population of fibers can be identified, under our resolution, in both cases, as shown by a reasonable Gaussian fit, suggesting that heparinase treatment does not preferentially digest a unique population of fibril morphology. The average measured fibril width was found similar before (15.3 ± 3.9 nm) and after (14.9 ± 2.8 nm) digestion, revealing no detectable differences in the average fibril width between the digested and non-digested fibril population (p=0.08). The average width measured agrees well with reported values for paired helical filaments found in patients with Alzheimer’s disease(7). The measured distribution was found narrower after digestion, which might be due to an artifact from the measurement. Further in-depth high resolution studies will be needed to fully characterize the relation between fibril structure and cofactor digestion.
Supplementary figures

Fig. S1. Assembly of tau fibrils with cofactors. **A.** Schematic representation of the tau construct used in this work (2N4R 255-441) that include the four repeat domains (R1-R4) as well as the C terminal region; **B.** Representative TEM image of heparin-induced tau fibrils and **C.** RNA-induced tau fibrils.
Fig. S2. ThT fluorescence trace of heparin-/RNA-induced tau fibrillation followed by cofactor enzymatic digestion. A. Heparin-induced samples were incubated at 37°C until heparinase treatment where temperature was decreased to, and stabilized at, 30°C. B. RNA-induced fibrillation and RNase treatment were performed at room temperature. In the control samples buffer was added instead of enzymes.
Fig. S3. ThT fluorescence of pre-digested heparin/RNA inducing tau fibrillization. Heparin-/RNA-inducing tau fibrillization were performed with pre-digested heparin/RNA at the same experimental conditions as described in Methods and Materials. Pre-digested heparin/RNA was prepared respectively using heparin pre-incubated with 1× heparinase overnight at 30°C or RNA pre-incubated with 200× RNase overnight to ensure full digestion.
**Fig. S4. Digestion of pelleted fibrils.** The same experiments as in Fig. 1A were carried out, on fibrils that were first pelleted at 53,000 rpm (TLA-100.3) for 1h and resuspended in working buffer. Results are overall similar than without centrifugation (Fig. 1A), although a pelleted RNA fibril sample seems to be more susceptible to digestion (~90%) than non-pelleted fibril sample (70-80%).
Fig. S5. Dimension quantification of fibrils before and after digestion. TEM images show heparin fibrils A. before and B. after 1× heparinase treatment; RNA fibrils C. before and D. after 1× RNase treatment. Histograms with gaussian fits of the measured heparin fibril widths showed two similar populations of fibrils E. before and F. after 1× heparinase treatment.
Fig. S6. ThT fluorescence of spin labeled (SL) heparin inducing tau fibrillation. ThT fluorescence of heparin-SL and heparin mixed with tau at tau:heparin molar ratio of 4:1. Absolute concentrations were 80 $\mu$M and 20 $\mu$M for tau incubated with heparin-SL, respectively, and 20 $\mu$M and 5 $\mu$M for tau incubated with heparin, respectively.
Fig. S7. cw-EPR lineshape analysis of heparin-SL. Changes in cw-EPR lineshape show the evolution of heparin mobility with respect to aggregation and digestion. (inset) The central peak width half-maximum (CPWHM) of digested and undigested fibrils, before and after filtration shows the relative mobility of heparin. Soluble heparin is the most mobile species (dark blue), and upon fibrillation, the heparin becomes less mobile as indicated by a broader peak (green). Digestion increase the average mobility indicated by a narrower peak (red), but not to the same point than free heparin, suggesting that a portion of bound heparin is released upon digestion. When this digested sample is pelleted to select only fibril (blue), the peak become even broader showing that the remaining fibrils after digestion contain bound heparin.
Fig. S8. Cellular seeding assay of tau fibrils. H4 neuroglioma cells stably expressing mCerulean-K18 with aggregation-prone mutations (P301L/V337M) were treated with different types of seeds. Each picture exemplifies the images used to quantified aggregation in Fig. S9 and 3C. Scale bar is 50 μm.
Fig. S9. Quantification of \textit{in vivo} seeding. Cells expressing A. mCerulean-K18 (P301L/V337M) or B. mCerulean-tau187 (P301L/V337M) were exposed to different types of seeds. The number of cells containing one or more fluorescent puncta over the total number of cells is reported in the Y axis. No difference was visible between digested and non-digested fibrils, while all recombinant seeds needed sonication to be seeding active.
Fig. S10. ThT fluorescence of in vitro seeding experiments. Time resolved ThT curves of the seeded tau under the same conditions as in Fig. 4A. The control represents seed with cofactor and without monomeric tau. Digested fibrils have a lower capacity to seed aggregation than undisturbed fibril seeds.
Figure S11. Controls of *in vitro* seeding assays. ThT fluorescence of 20 µM tau187C291S incubated with and without cofactor (polyU RNA at 120 µg/ml) showed that fibrils were not seeded in the absence of seeds. As a reference, mouse-fibrils seeds were added in the same condition (+seeds +cofactor).
Fig. S12. Full aggregation curves of seeding experiments presented in Fig. 4A.
Fig. S13. Enzyme digestion of heparin-/RNA-induced tau fibrils trigger a loss of ThT signal for full-length tau as well as for different tau187 mutants. Tau constructs: full-length 2N4R (“2N4R”), fragment 255-441 (“tau187”) with P301L (“tau187P301L”) or with C291S and C322S (“tau187C291SC322S”). Spin labeled tau used in cw-EPR measurement and EPR simulation (“tau187C291SP301L-SL”) was also tested. Standard deviation is shown for n ≥ 2.
Fig. S14. ThT fluorescence of \textit{in vitro} seeding with mouse-extracted seeds. Tau was incubated with 1\% mass mouse extracted seed in the absence of additional cofactors. The aggregation was followed by ThT fluorescence in the absence (control) and presence of a cocktail of enzymes (2.5 μg/ml RNase A, 10 U/μL RNase T1, 500 μg/ml DNase, 0.5 U/μL heparinase I). This experiment shows that the postulated cofactor present in the mouse-fibril seeds (see discussion section in the manuscript) and responsible for efficient seeding is not sensitive to any of these enzymes.
Fig. S15. Characterization of mouse brain-extracted fibrils. **A.** TEM image of tau fibrils extracted from rTg4510 mouse cortex. Scale bar represents 500 nm. **B.** Western blot analysis of mouse fibrils with PHF-1 antibody (recognizes p396/404). The first lane is the sarkosyl soluble fraction “S”, second lane is the wash fraction “W”, and the final lane is the sarkosyl insoluble pellet “F”.
Fig. S16. ICC characterization of in-vivo seeding assay. H4 neuroglioma cells stably expressing mCerulean-tau187 (P301L/V337M) were treated with (A) or without (B) tau fibrils purified from mouse brain. Cells were then fixed and stained for PHF-1 (p396/404) reactivity (C & D). Puncta observed in mouse fibril samples were PHF-1 positive, while soluble, diffuse tau in the cytoplasm was PHF-1 negative (merge E & F).
Fig. S17. DEER signal and fitting. A. before heparin aggregation, B. heparin induced aggregation, C. digested, and D. seed induced aggregation. The reconstructed DEER signal (fit) is shown in red from the distance distribution and the experimental DEER signal is shown in black. The “2+1” artifact is observable in (A) and (C) at the end of the DEER trace and was not cut from analysis.
Fig. S18. Comparison of distance distributions calculated with various dipolar evolution times. The distance distributions are shown for (a) before aggregation, (b) heparin induced aggregation, and (c) seeded aggregation. The dipolar evolution times are compared to verify that the dipolar evolution time has only minor impact on the distance distribution.
Fig. S19. Comparison of DEER signal for heparin induced tau aggregation and seeded tau aggregation (distance distribution shown in Fig. 4B).
Table S1. Fitting results for Cw-EPR spectra of tau-SL before and after digestion. $c_20$, scaling coefficient for orienting potential, $p_1$, $p_2$, $p_3$, percentage of mobile, immobile and exchange components, respectively. $t_{R,1}$, rotational correlation time of mobile component, fitted using spectra of solution tau-SL samples and used as constraint for fibril and digested fibril samples, $t_{R,2}/t_{R,3}$, rotational correlation time of immobile and exchange components.

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<th>$p_1$</th>
<th>$p_2$</th>
<th>$p_3$</th>
<th>$t_{R,1}$, ps</th>
<th>$t_{R,2}/t_{R,3}$, ns</th>
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Supplementary references


