

Supporting information

Supplemental Fig. 1

Assessment of pre-aggregated tau solubility. Detergent (sarcosyl) was added to a sample of pre-aggregated tau to a final concentration of 1%. After 2 h of incubation, we examined the solution by AFM and found not only filaments but also granules, suggesting that both tau granules and filaments are detergent insoluble.

Supplemental Fig. 2

Thioflavin T (ThT) assay and AFM of tau samples incubated for several hours revealed that tau aggregation occurs in three phases. **(a)** Aggregation states were monitored by measuring ThT fluorescence intensity at the indicated time points. Solid line represents β -sheet formation of tau (tau aggregation) as a function of ThT fluorescence; dashed line represents β -sheet formation of BSA as a function of ThT fluorescence. Fluorescence is expressed in arbitrary units (a.u.; average \pm SD; n=3). **(b)** AFM images of granular tau oligomers and tau filaments. At the 6 h incubation time point, tau forms a structure having almost the same minor- and major-axis size. Fibrils begin to appear with longer incubation times. Magnified tau images are shown in the inset of each AFM micrograph. In each AFM image, we selected four different parts of the tau structure for in-depth analysis with NIH-image 1.62. The minor (diameter) and major (length) axes of these structures were then plotted in reference to major axis:minor axis slopes (lines). The slopes were calculated as follows: major axis=minor axis ($Y=X$) and major axis =2 minor axis ($2Y=X$). Structures having minor and major axis values lying between the slope lines were defined as tau granules, and those having minor and major axis values lying outside of the slope lines were defined as tau fibrils.

Supplemental Fig. 3

Immunoaffinity column chromatography purification of human brain aggregates. The immunoaffinity eluate was incubated in 1% sarcosyl (**a**), subjected to sucrose-gradient centrifugation, and fractionated into 6 fractions (**b–g**). Although no aggregates were found in fraction 1, we recovered granular aggregates from fraction 3 and short filaments from fraction 4.

Supplemental Fig. 4

(**a**) Continuous AFM with tapping mode revealed that *in vitro* generated tau filaments broke down into granular tau particles. (**b**) PHFs isolated from AD brains also broke down into granular tau oligomers. Arrows indicate breaking sites. Numbers represent the frequency of scans.

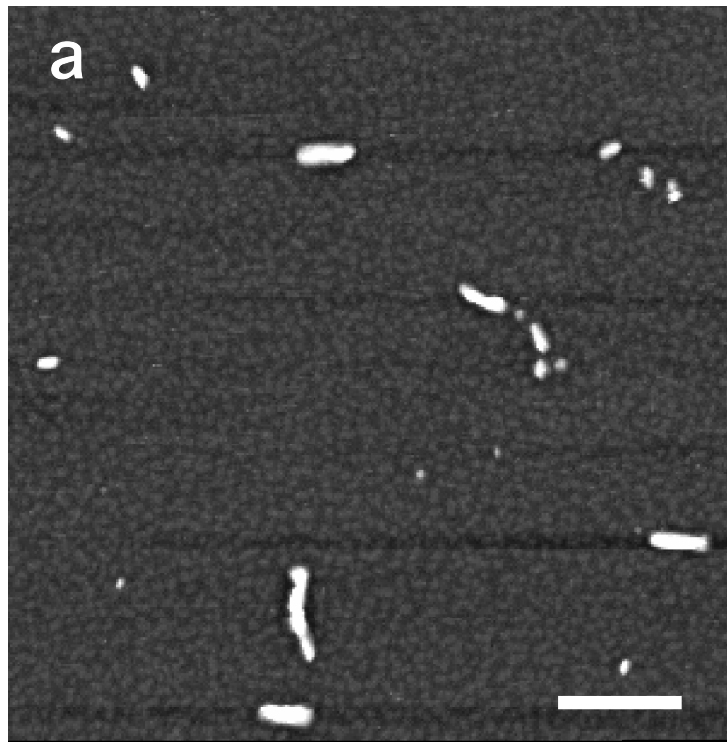
Supplemental methods

Purification of recombinant tau protein. Recombinant human tau (2N4R) in pRK172 vector was expressed in BL21 (DE3) *E. coli* cells. When cultures reached an absorbance of about 0.5–0.7 at 600 nm, cells were harvested by centrifugation at 3000G for 15 min. We obtained the most tau fragments from cultures with an absorbance that exceeded 0.7. After centrifugation, the pellets were resuspended in a buffer of 50 mM PIPES, 1 mM EGTA, 1 mM DTT (pH 6.4) containing protease inhibitors (H buffer) and sonicated. The homogenates were centrifuged at 27kG for 15 min and the supernatant was heated in boiling water for 10 min. After centrifugation at 27kG for 15 min, the supernatant was filtered with a 0.45-micrometer Millipore filter. The filtrate was loaded onto a phosphocellulose column (P11, Whatman) equilibrated with H buffer. After washing with 0.1 M NaCl in H buffer, tau protein was eluted with 0.3 M NaCl. The eluate was dialyzed against saturated ammonium sulfate, then precipitated by centrifugation at 50k rpm for 30 min (Beckman TLA 100.4). The pellets were dissolved in H buffer containing 0.5 M NaCl and boiled for 10 min. In this step, we avoided using β -mercaptoethanol because we found earlier that β -mercaptoethanol adducted tau and led to reduced

aggregation. After buffer exchange using a NAP-10 column (Amersham Biosciences), tau was further purified by reverse phase HPLC. After freeze-drying, tau was dissolved in water and stored as a stock solution at -30°C . Dissolving tau in water, but not in buffer, reduced disulfide bond formation. Tau protein concentration was determined using amino acid hydrolysis because calorimetric methods are sometimes inaccurate (*1*). From 8 liters of culture, we were able to obtain about 25–50 mg of tau protein.

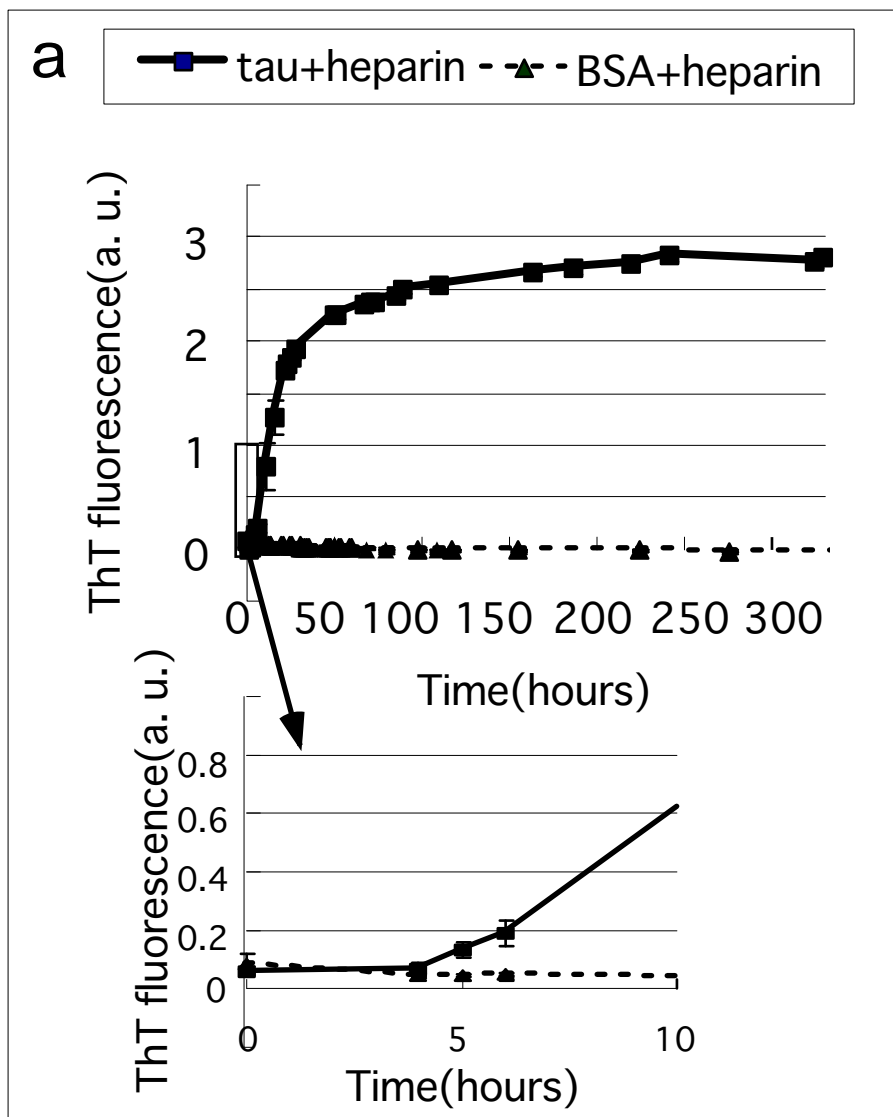
Reference

1. Gamblin, T. C., King, M. E., Kuret, J., Berry, R. W., and Binder, L. I. (2000) Oxidative regulation of fatty acid-induced tau polymerization. *Biochemistry* 39, 14203-10.

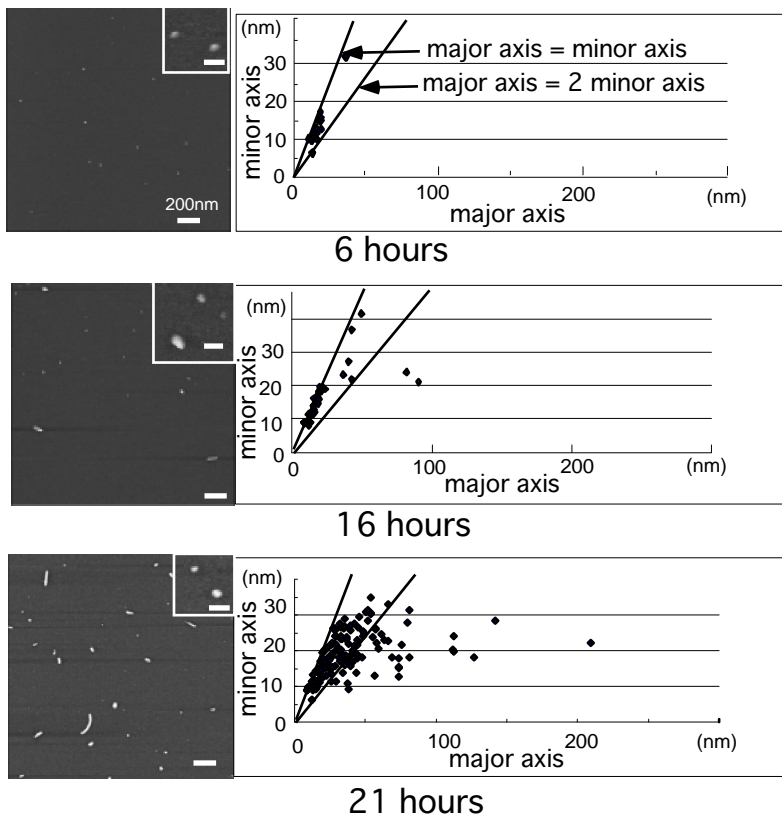


In detergent

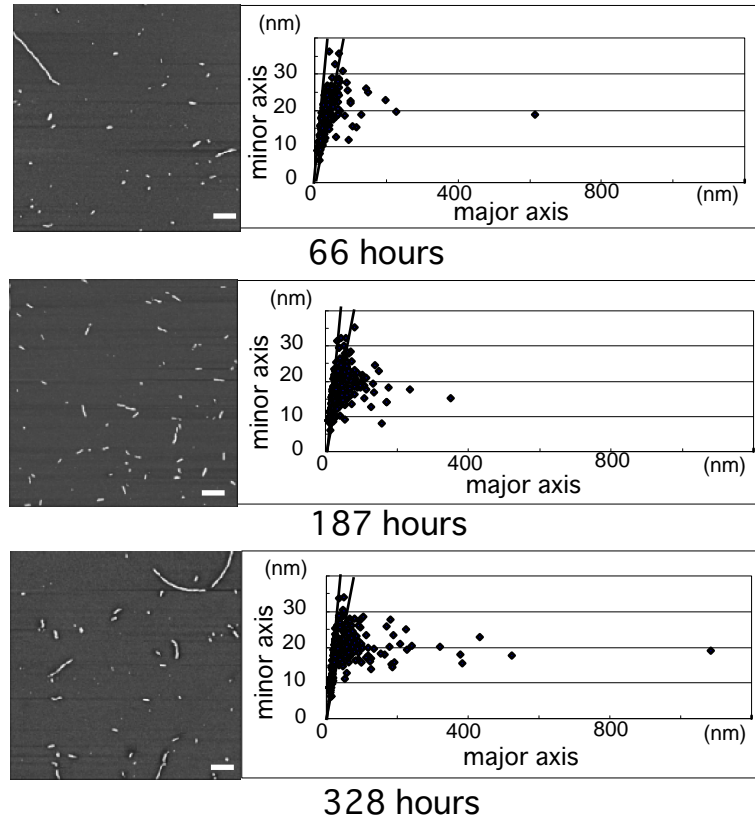
Supplemental figure 1



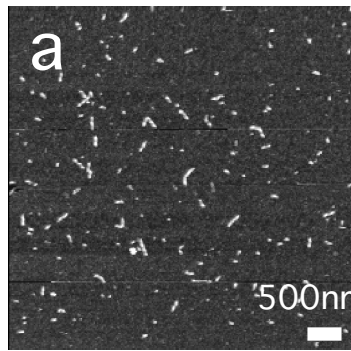
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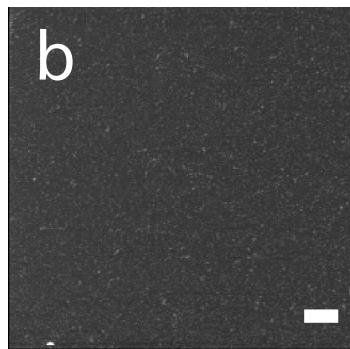
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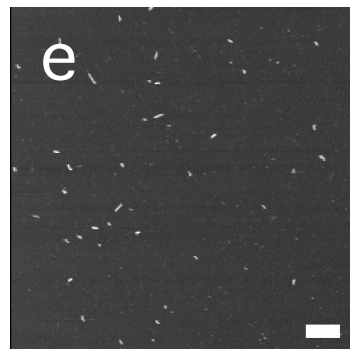
Supplemental figure 2



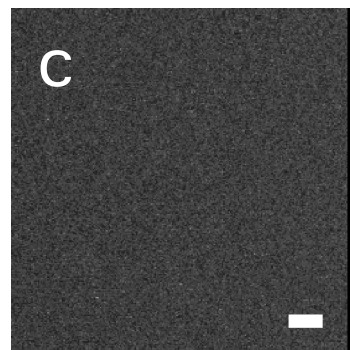
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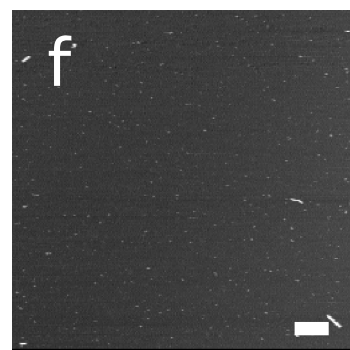
Fraction 1



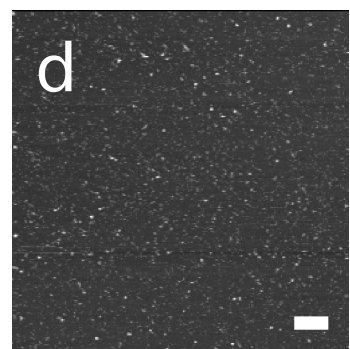
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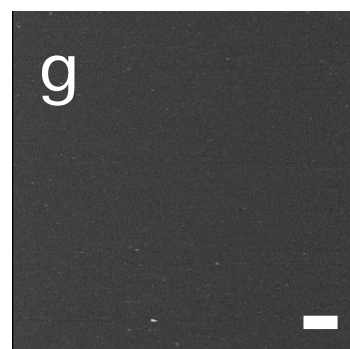
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Fraction 5

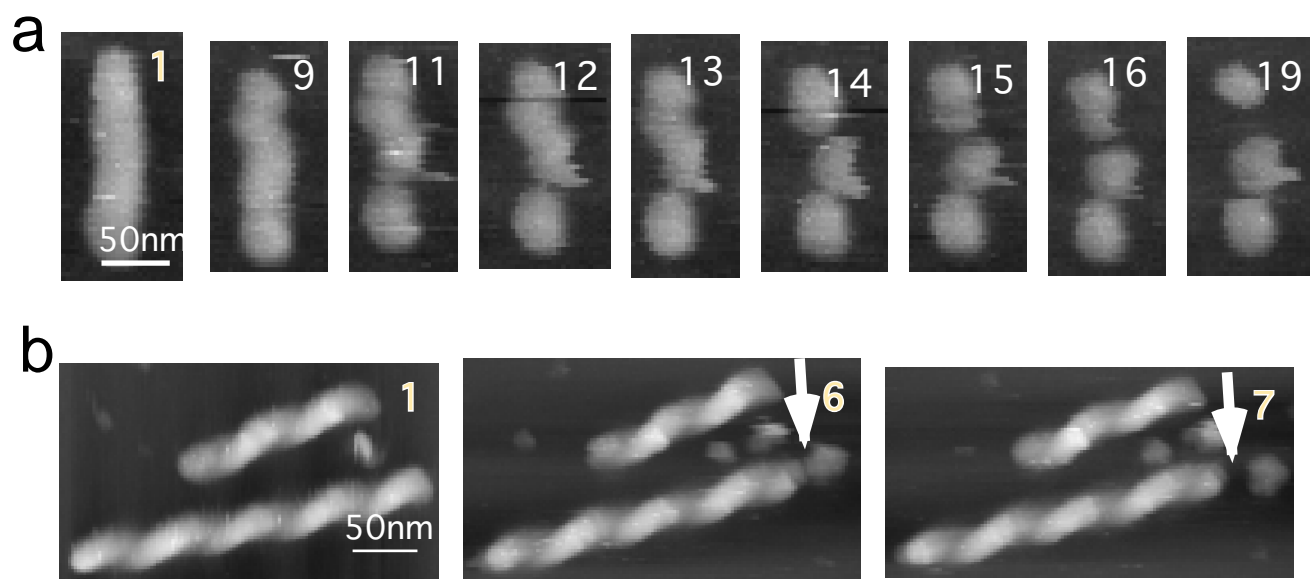


Fraction 3



Fraction 6

Supplemental figure 3



Supplemental figure 4