SUPPLEMENTAL MATERIAL

TAXOL-STABILIZED MICROTUBULES PROMOTE THE FORMATION OF FILAMENTS FROM UNMODIFIED FULL-LENGTH TAU *IN VITRO*.

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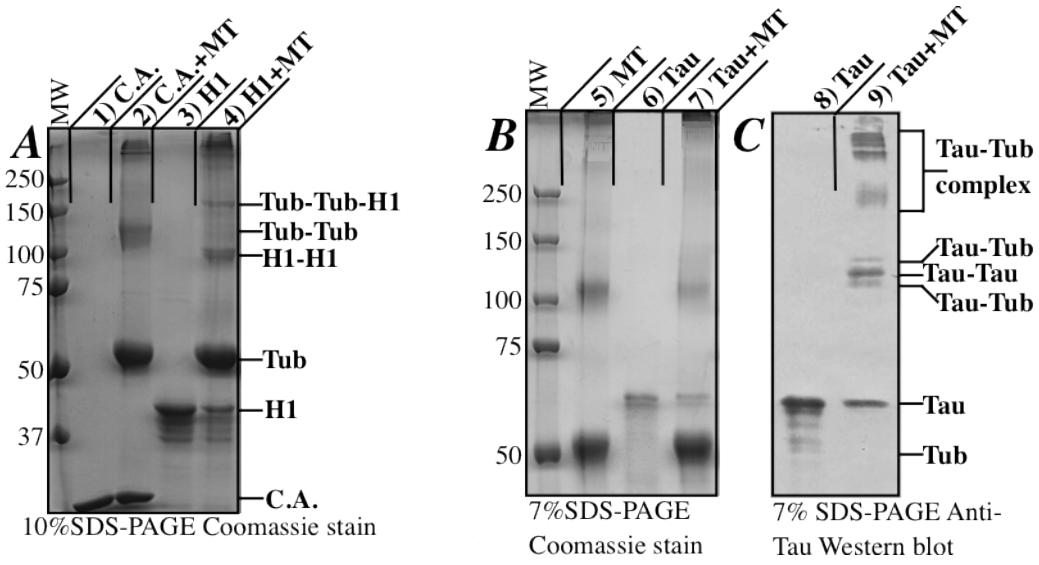
Figure S1:

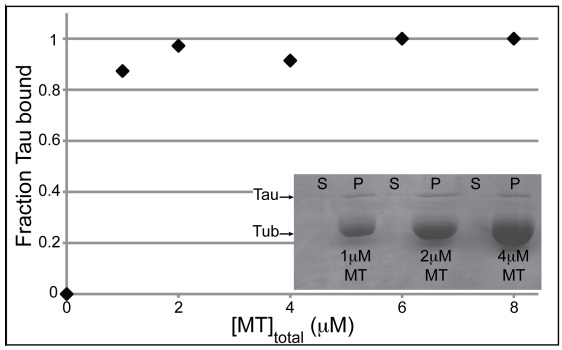


Supplemental Figure 1: Cross-linked products of Tau and MT incubations confirm that Tau–Tau interactions are promoted by MTs. (A) 10% SDS-PAGE Coommassie stain showing cross-linked products of positive and negative controls (CLIP-170 construct, H1, and carbonic anhydrase, C.A., respectively) when incubated with MTs. H1 cross-links with tubulin; C.A. does not cross-link with tubulin. **(B)** 7% SDS-PAGE Coommassie stain of the cross-linked products of MTs alone, Tau alone, and Tau incubated with MTs (4μM each). Tau alone runs anomalously high at ~65 kDa. MTs alone cross-link to form higher MW bands at ~105 kDa. Tau does not crosslink with itself. Tau incubated with MTs forms additional higher MW bands that are hard to visualize with Coommassie stain given the background caused by cross-linked tubulin dimers. **(C)** Cross-linked products of Tau alone and Tau with MTs (same samples as in *B*) that are probed with Tau monoclonal mouse antibody. The observation that three bands appear at approximately 130 kDa (interpreted as one Tau-Tau band and two Tau-tubulin bands) is consistent with previous observations (Makrides *et al.*, 2003) that MTs induce Tau-Tau interactions. Band identities are deduced from all possible additive weights between Tau and tubulin: bottom band (115-120kDa)= 1Tau + 1 tubulin (50 or 55kDa), middle band (130kDa)= 2 Tau, and top band (146-147kDa)= either 1 tubulin (55kDa) + 2 Tau or 2 tubulin (50kDa each) +

1 Tau. Lanes are as follows: 1) C.A. alone; 2) C.A. + MTs; 3) H1 alone; 4) H1 + MTs; 5) MTs alone; 6) Tau alone; 7) Tau + MTs; 8) Tau alone; 9) Tau + MTs.

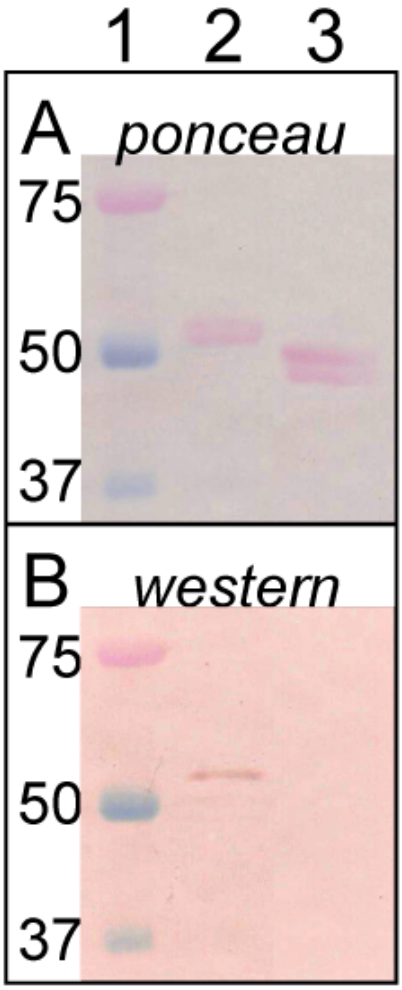
Methods for Protein Cross-linking Experiments: Proteins were incubated in PEM buffer supplemented with 10μM Taxol at 37^{o}C for 30mins. Cross-linking agents NHS (*N*-Hydroxylsuccinimide) and EDC (ethyl(dimethylaminopropyl) carbodiimide) were added to the reaction mixtures to final concentrations of 5mM and 2mM respectively. The reaction was further incubated at room temperature for 30 mins then subsequently quenched with SDS buffer. Samples were boiled, run on a 7% SDS-PAGE gel, and western blot transferred. Tau was probed with mouse monoclonal antibody purchased from Sigma (CAT T5530).

Figure S2:



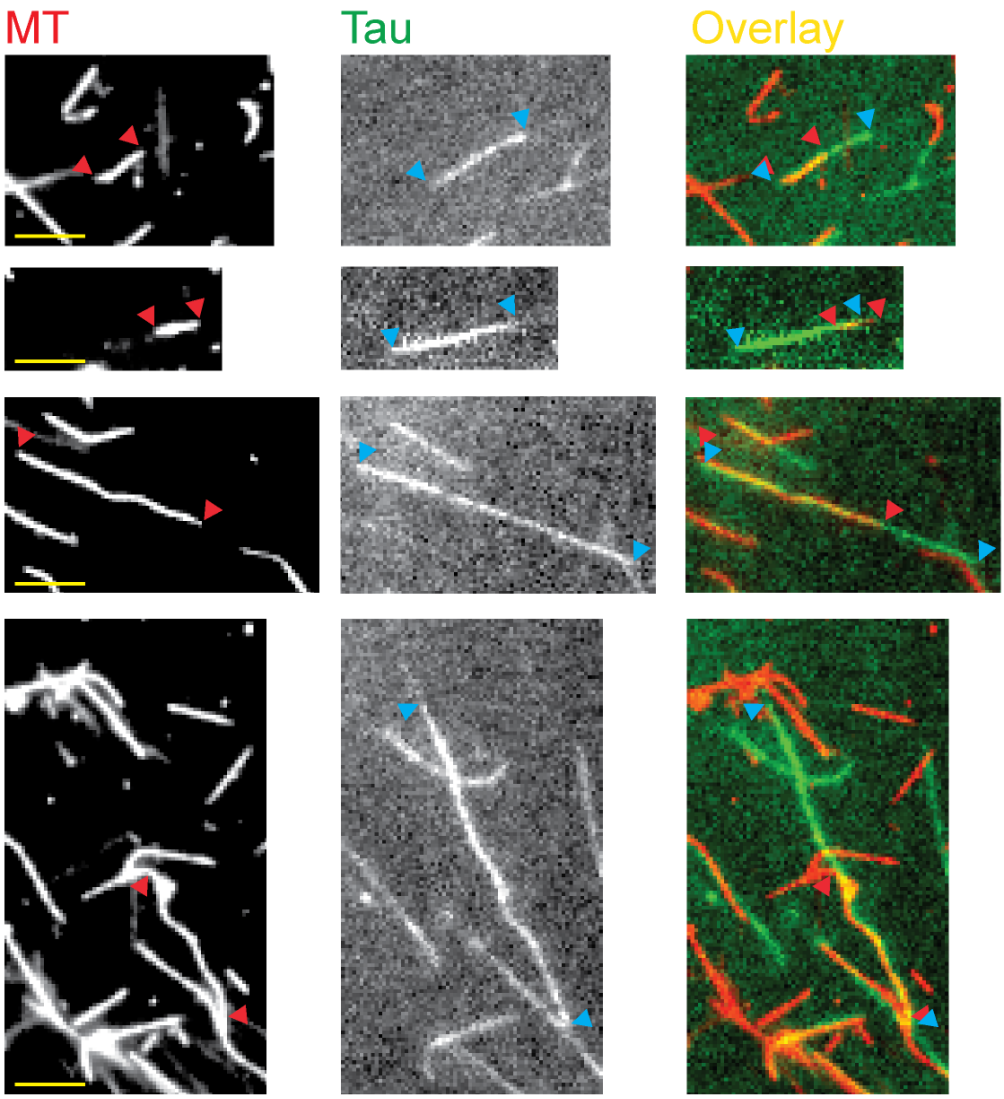
Supplemental Figure 2: The apparent affinity of Tau for MTs is time-dependent. To further test the hypothesis that the variability of the data in Figure 1D is due to variations in the extent of Tau polymerization, we repeated the experiments of Figure 1D, but this time incubated for one hour instead of 15mins. The data show that the Tau protein completely sediments out of solution when incubated for an hour in the presence of a range of different concentrations of MTs. This observation is in agreement with the data in Figure 6 showing that Tau forms filaments to completion after a 1hr incubation with MTs, and also the data in Figure 4 showing that the Tau filaments cosediment with MTs. The insert shows a gel segment to help visualize that the Tau band appears in the pellet fraction, P, with the tubulin (S is the supernatant fraction). Although measuring the apparent affinity of Tau for MTs using this approach appears to be a time-dependent process (more Tau appears in the pellet fraction after longer incubation times), these data agree with the observations in Figure 6 that the apparent time-dependence is the result of Tau filaments forming and sedimenting out of solution and that halting the filament formation process at different times before it reaches completion results in variable amounts of Tau that cosediment with MTs.

Figure S3:



Supplemental Figure 3: Complete digestion of MTs with subtilisin is confirmed by western blot analysis. Subtilisin cleaves the C-terminal tails of tubulin. After a short digestion time, subtilisin first removes the β-tubulin tail. After a longer digestion period, the subtilisin is able to cleave off both the α- and β-tubulin tails to completion according to previously established protocol (Zhu *et al.*, 2009). *Lane 1)* Molecular weight marker; weights are shown to the left of the bands. *Lane 2)* Normal, untreated Taxol-stabilized MTs. We can see a double band appearance from the separation of α- and β-tubulin as it ran through the SDS-PAGE gel. *Lane 3)* Subtilisin-treated MTs incubated long enough to remove both C-terminal tubulin tails. Note: the weights of the bands shift down a little, indicating the loss/removal of the tubulin tails. All proteins used are ~2μM. **(A)** The ponceau stain of the nitrocellulose membrane prior to antibody probing. **(B)** Western blot using the colorimetric NBT/BCIP detection system and probed with mouse monoclonal 1A2 anti-α-tubulin antibody, which targets the α-tubulin C-terminal tail. In lane 2, we see the antibody detects the normal MTs. In lane 3, we see that the antibody did not detect its epitope, showing that the MTs are completely digested by subtilisin and are lacking the C-terminal α-tubulin tail.

Figure S4:



Supplemental Figure 4: Fluorescence microscopy observations provide evidence that Tau filaments can polymerize off of MT ends. While imaging the experiments shown in Figure 2, there were a number of events in which the Tau filaments appeared to be fibrillizing off of the MT ends. They are depicted here with the MTs in the left panels, the Tau images in the center panels, and the overlayed images in the right panels. The MTs are shown in red, the Tau in green, and the overlay in yellow. Red and blue arrowheads have been placed on the images to aid in the visualization of the filament endpoints. The red arrowheads point at the ends of the MTs; the blue arrowheads point at the ends of the Tau filaments. In the indicated cases, the Tau decorates the length of the MT and continues as a filament beyond the length of the MT. Scale bars = 10μm.