Supplementary Information for:

Supramolecular Filaments Containing a Fixed 41% Paclitaxel Loading

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S1 Molecule synthesis and characterization

S1.1 Chemicals

All amino acids were purchased from AAPPTEC (Louisville, KY). Rink Amide MBHA Resin was purchased from NovaBiochem (San Diego, CA). Paclitaxel was from Ava Chem Scientific (San Antonio, TX), 4-bromobutyric acid and thiourea were purchased from Sigma-Aldrich (St. Louis, MO). *N*,*N*'-diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP) were obtained from TCI, and all other reagents and solvents were sourced through VWR.

S1.2 AcCGVQIVYKK peptide synthesis

The peptide AcCGVQIVYKK was synthesized using AAPPTEC Focus XC synthesizer via standard Fmoc-solid phase technique. Fmoc groups were deprotected using 20% 4-methylpiperidine in DMF, and amino acid/HBTU/DIEA (4/3.98/6) was applied for coupling. The *N*-terminal amine was acetylated manually by reacting with 20% acetic anhydride in DMF. The finished peptide was cleaved from the resin with TFA/TIS/water (92.5:5:2.5) solution. The peptide was confirmed by MALDI-TOFMS m/z 1078.720 for [M+H], $C_{49}H_{83}N_{13}O_{12}S$, calcd. 1077.600.

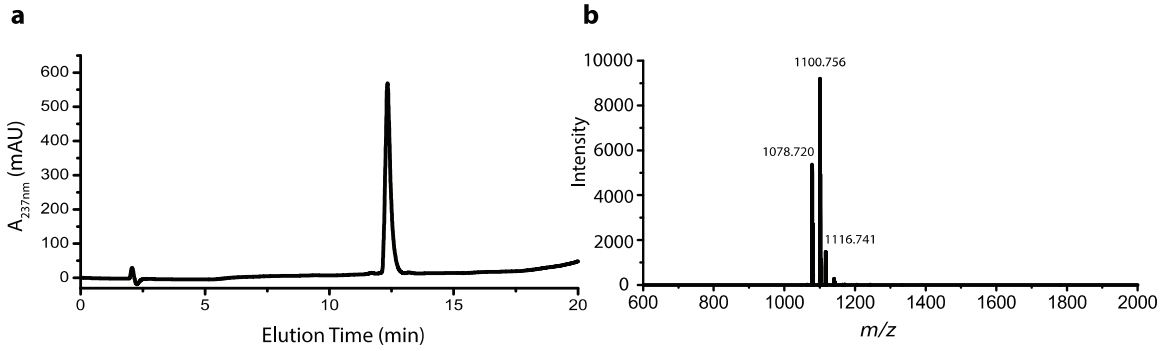

Figure S1 RP-HPLC trace (a) and MALDI-TOF MS (b) profile of the Tau peptide showing high purify and the expected molecular mass.

S1.3 4-(pyridin-2-yldisulfanyl) butyric acid synthesis

4-Bromobutyric acid (2g, 12mmol) and thiourea (0.96 g, 12.6 mmol) were dissolved in ethanol (50 mL) and refluxed at 90 °C for 4 h. After dropwise addition of a NaOH solution (4.8 g in 5:1 H_2O/ethanol), the mixture was refluxed for another 16 h and then cooled to room temperature. The white precipitate was collected and redissolved in water (40 mL). 4 M HCl was used to adjust the solution pH to 5, and the product was extracted into diethyl

ether. The organic phase was dried over anhydrous sodium sulfate to give 4-sulfanylbutyric acid as a colorless oil (310 mg, 15%), which was used in the next step without further purification. 4-sulfanylbutyric acid (105 mg, 0.87 mmol) and 2-aldrithiol (440 mg, 2.0 mmol, 2.3 eq) were dissolved in MeOH (1.3 mL) and stirred for 3 h. The solution was purified by RP-HPLC (5% to 95% of acetonitrile in water with 0.1% TFA over 45 min), combining product fractions and removing solvents to give 4-(pyridin-2-yldisulfanyl) butyric acid as an oil (118 mg, 59%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.56 (d, J=4.4 Hz, 1H), 7.84 (d, J=7.1 Hz, 1H), 7.78 (t, J=8.5 Hz, 1H), 7.26 (t, J=6.9 Hz, 1H), 2.88 (t, J=9.4 Hz, 2H), 2.51 (t, J=9.4 Hz, 2H), 2.04 (m, 2H) ppm; ESI MS m/z 230.0 for $[M+H]^+$, $C_9H_{11}N_{13}O_2S_2$, calcd.229.0.

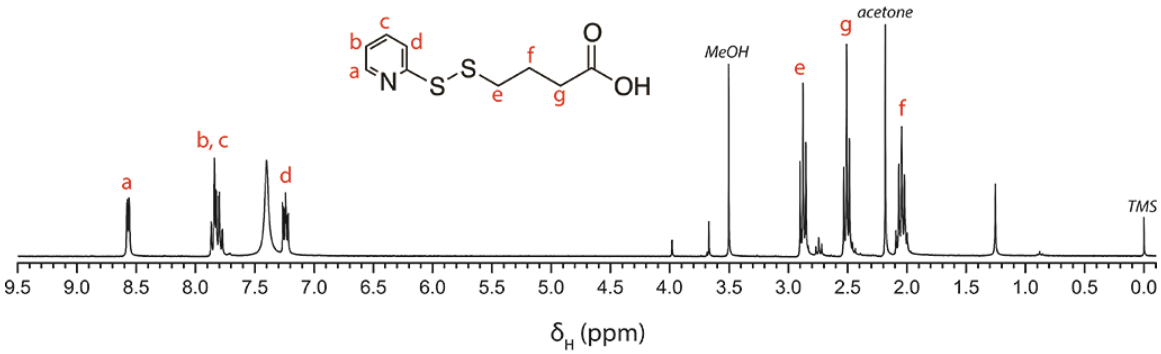

Figure S2 ^{1}H NMR spectrum of the synthesized 4-(pyridin-2-yldisulfanyl) butyric acid.

S1.4 Paclitaxel C2' ester synthesis

Paclitaxel C2' ester was synthesized using a previously published procedure.[1] Paclitaxel (186 mg, 0.22 mmol), 4-(pyridin-2-yldisulfanyl)butyric acid (100 mg, 0.44 mmol), DIC (68 µL, 0.44 mol), and DMAP(26.7 mg, 0.22 mmol) were added into an oven dried flask equipped with a stirrer bar, evacuated and refilled with nitrogen three times to remove air, then dissolved in anhydrous acetonitrile (12.7 mL). The reaction was allowed to stir in the dark at room temperature for 48 h. The solvents were removed *in vacuo* and the residue was dissolved in chloroform and purified by flash chromatography (3:2 EtOAc/ hexane), to give the product as a white solid (108 mg, 47%). ^{1}H NMR (400 MHz, CD_3OD):δ=8.43 (m, 1H), 8.15 (d, J=7.6 Hz, 2H), 7.76 (d, J=8.4 Hz, 2H), 7.64-7.38 (m, 13H), 7.09 (m, 1H), 6.93 (d,J=9.3 Hz, 1H), 6.31 (s, 1H), 6.25 (m, 1H), 5.98 (dd,J_1=8.8Hz,J_2=3.4 Hz, 1H), 5.70 (d,J=6.0 Hz, 1H), 5.52 (d,J=3.6 Hz, 1H), 4.99 (d, J=8.6 Hz, 1H), 4.48 (dd, J_1=9.1 Hz, J_2=6.0 Hz,1H), 4.33 (d, J=7.8 Hz, 1H), 4.21 (d, J=7.4 Hz, 1H), 3.83 (d, J=7.1 Hz, 1H), 2.79 (m, 2H), 2.60 (m, 2H), 2.45 (s, 3H), 2.38 (m, 1H), 2.23 (s, 3H), 2.05 (m, 1H), 1.93 (m, 4H), 1.69 (s, 3H), 1.24 (s, 3H), 1.15 (s, 3H) ppm; ESI MS: m/z 1065.2 for $[M+H]^+$, $C_9H_{11}N_{13}O_2S_2$, calcd.1064.3

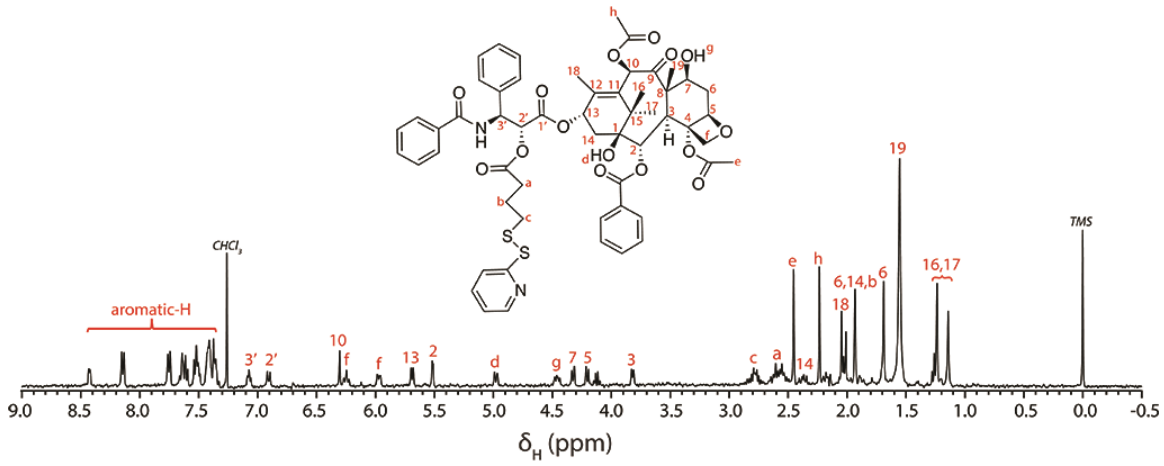

Figure S3 ^{1}H NMR spectrum of the synthesized Paclitaxel C2' ester.

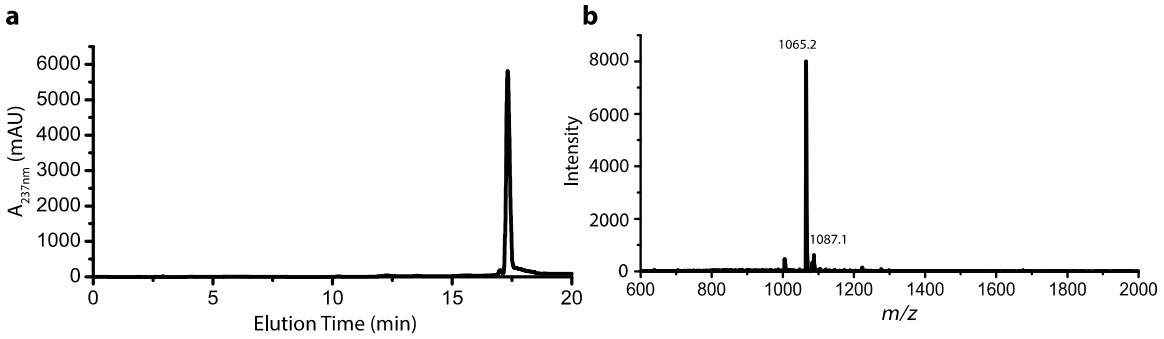

Figure S4 RP-HPLC trace (a) and ESI MS profile (b) of paclitaxel C2' ester showing high purify and the expected molecular mass.

S1.5 PTX-Tau synthesis

AcCGVQIVYKK (27.7 mg, 25.7 mmol) and paclitaxel C2' ester (54.7 mg, 51.4 mmol) were added to an oven dried flask equipped with a stirrer bar and evacuated and filled with nitrogen three times to remove the air. The reagents were then dissolved in anhydrous DMF (5 mL). The solution was allowed to stir for 16 h, before purification by RP-HPLC (30% to 95% acetonitrile in water with 0.1% TFA over 45 min). Product fractions were combined and lyophilized to give a PTX-Tau as a white powder. (31 mg, 60%). ESI MS: *m*/*z* 1016.7 for [M+2H], $C_9H_{11}N_{13}O_2S_2$, calcd.2031.9.

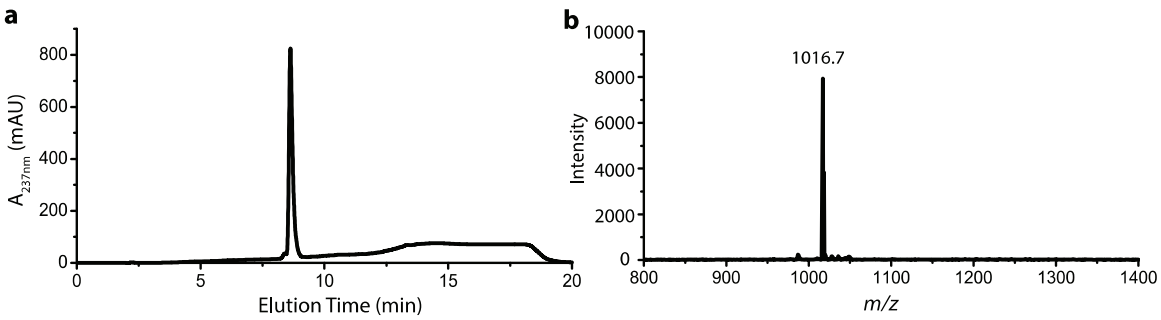

Figure S5 RP-HPLC trace (a) and ESI MS (b) profile of **PTX-Tau** showing high purify and the expected molecular mass.

S2 Self-assembly characterization

S2.1 Transmission Electron Microscopy

All the self-assembly experiments were performed at room temperature. **PTX-Tau** conjugate was directly dissolved into deionized water to make solutions of desired concentrations. Solutions were typically aged for 48 h before TEM imaging. TEM samples were prepared by adding ~10 μL solution onto a carbon-film-coated copper grid. After careful removal of the excess liquid using a piece of filter paper, a drop of 2% *w*/*v* uranyl acetate aqueous solution (~10 μL) was added onto the TEM grid as a negative staining agent. Again, excess solution was removed using a piece of paper to result in a thin film on the grid that was allowed to air dry before imaging. A Technai12 TWIN transmission electron microscope was used to image the prepared samples operating typically at 100 kV, with images recorded by a SIS Megaview III wide-angle CCD camera.

S2.2 Circular Dichroism (CD) Spectroscopy

CD spectra were recorded on a Jasco J-710 spectropolarimeter (JASCO, Easton, MD) using a 10 mm pathlength Spectrasil® quartz UV-Vis absorption cell (Starna Cells Inc., Atascadero, CA). Data was normalized with respect to sample concentration (5 μM), path length, and the number of residues from the expected β-sheet forming segment.

S2.3 Fluorescence Spectroscopy

1 mM Nile Red stock solution was prepared in acetone and diluted 100-fold into PTX-Tau solutions of various concentrations. The acetone was allowed to evaporate and the samples were equilibrated overnight.[2] Emission spectra for each sample were then recorded on a Fluorolog spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ) between 580 and 720

nm, with an excitation wavelength of 550 nm.

S3 AFM and cryo-TEM images of PTX-Tau filaments

The morphology of the observed supramolecular filaments formed by self-assembly of PTX-Tau conjugate was further confirmed using atomic force microscopy (AFM) and cryogenic transmission electron microscopy (cryo-TEM).

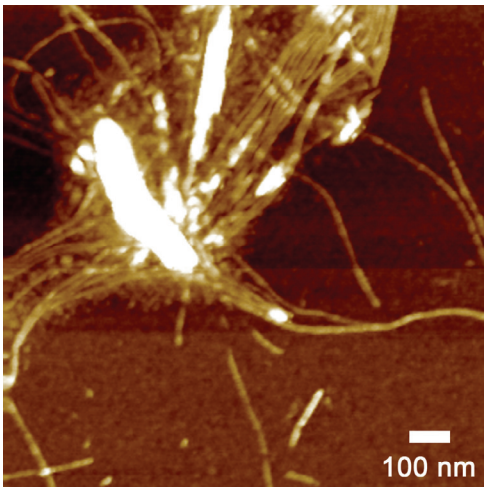

Figure S6 AFM image reveals dominant one-dimensional nanostructures formed by self-assembly of PTX-Tau drug amphiphile. The shape and dimension in AFM are consistent with those observed in conventional TEM.

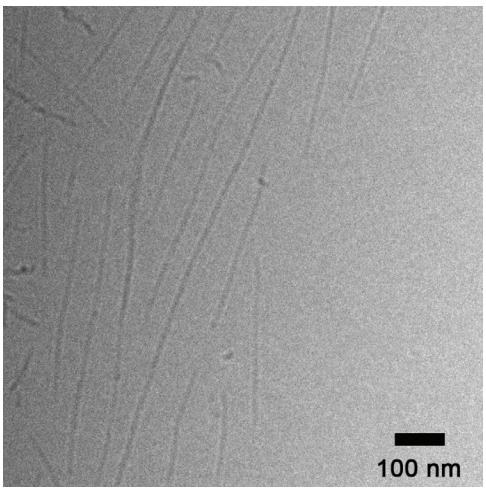

Figure S7 Cryo-TEM image reveals dominant one-dimensional nanostructures formed by self-assembly of PTX-Tau drug amphiphile. The shape and dimension in AFM are consistent with those observed in conventional TEM. This cryo-TEM image also reveals that the observed filamentous nanostructures are indeed formed in solution, not a result of drying effect during the sample preparation.

S4 Degradation study of PTX-Tau amphiphile

The release characteristics of the conjugate were studied at two concentrations, 5 and 100 μM. Briefly, a 20 mM glutathione stock solution was prepared in 20 mM PBS buffer (pH 7.4), while solutions of conjugate (10 and 200 μM) were prepared in water. Equal volumes of conjugate and GSH solution were then mixed to reach a final GSH concentration of 10 mM, and the mixture solution were incubated at 37°C for the desired periods of time (total 48 hours). Samples were taken at predetermined time points (0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48 h) and release of paclitaxel from conjugate was detected by HPLC. 2 ×PBS buffer was used to prepare the negative controls containing no GSH.

S5 Cytotoxicity study of PTX-Tau amphiphile

S5.1 Cell lines

MCF-7 cells were provided by Prof. Denis Wirtz's lab (Department of Chemical and Biomolecular Engineering, Johns Hopkins University). A549 and PC3-flu were provided by Prof. Justin Hanes and Prof. Martin G. Pomper of the Johns Hopkins Medical School, respectively. All cell lines were cultured according to providers' protocols. MCF-7 human breast cancer cells were grown in DMEM with 10% FBS and 1% antibiotics. A549 non-small cell lung cancer cells were grown in Advanced 1640 with 2mM L-glutamine, 10 mM HEPES and 1% FBS. PC3-flu prostate cancer cells were grown in RPMI 1640 with 10% FBS.

S5.2 Cell viability study of PTX-Tau amphiphile

The cytotoxicity of **PTX-Tau** was determined using a dose–response study. A 24-well plate was seeded with cell sat a density of 10^5 cells/well and incubated at 37 °C for 24 h. The cells were then treated with varying concentrations of paclitaxel or conjugate in cell medium and incubated for a further 48 h. Cell viability was determined by sulforhodamine-B (SRB, Sigma Aldrich) assay according to the manufacturer's protocol. The data was fitted using the Hill equation function within the Igor Pro program and used to obtain the IC_{50} values.

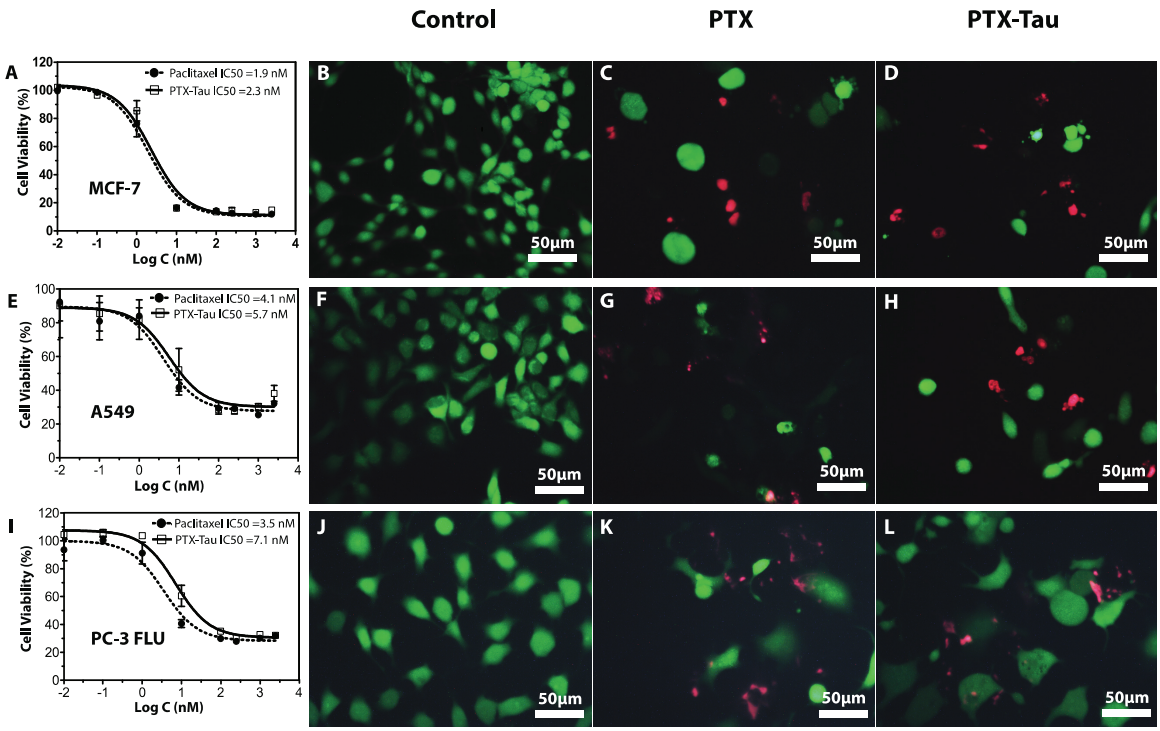

Figure S8 Cytotoxicity evaluation of **PTX-Tau** against MCF-7 breast cancer cells, A549 non-small cell lung cancer cells, and PC3-flu prostate cancer cells. Dose–response study of **PTX-Tau** and free PTX against MCF-7 (**A**), A549 (**E**), and PC3-flu (**I**). Cells were incubated with the PTX or **PTX-Tau** for 48 h and cell viability was determined by SRB assay. Data are given as mean ± s.d. (n = 3). Live/dead assay cell images of MCF-7 (**B**, **C, D**), A549 (**F, G, H**), and PC3-flu (**J, K, L**) cancer cells after 48 h incubation with DMSO (**B**, **F**, **J**), PTX (**C, G, K**) or **PTX-Tau** (**D, H, L**).

S5.3 Live/dead cell imaging

3×10^4 cells were plated onto a collagen-coatedsterile glass coverslip and incubated for 24 h. Cells were treated with 50 nM paclitaxel or **PTX-Tau**, using DMSO as a negative control. After incubating for 48 h, cells were washed with D-PBS buffer, stained with a solution containing 2 µM calcein AM and 4 µM EthD-1, and then imaged using fluorescence microscopy (JENCO, OR).

References

1. Dubikovskaya, E. A.; Thorne, S. H.; Pillow, T. H.; Contag, C. H.; Wender, P. A. *Proceedings of the National Academy of Sciences of the United States of America***2008**, *105*, 12128
2. Stuart, M. C. A.; van de Pas, J. C.; Engberts, J. *Journal of Physical Organic Chemistry***2005**, *18*, 929