Supporting Information

Tau interacts with the C-terminal region of α-synuclein, promoting formation of toxic aggregates with distinct molecular conformations

Anvesh K. R. Dasari[1], Rakez Kayed[2], Sungsool Wi[3], and Kwang Hun Lim[1,*]

[1]Department of Chemistry, East Carolina University, Grenville, NC 27858, USA

[2]Departments of Neurology, Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX, 77555, USA.

[3]Center of Interdisciplinary Magnetic Resonance (CIMAR), National High Magnetic Field Laboratory (NHMFL), 1800 East, Paul Dirac Dr., Tallahassee, FL 32310, USA.

Table S1. Relative content of secondary structure of α-synuclein at different incubation times.

Incubation time (days)	Helix (%)	β-strand (%)	Turn (%)	Disordered (%)
0	5	3	9	83
2	11	13	15	61
3	16	13	17	55
5	11	23	19	45

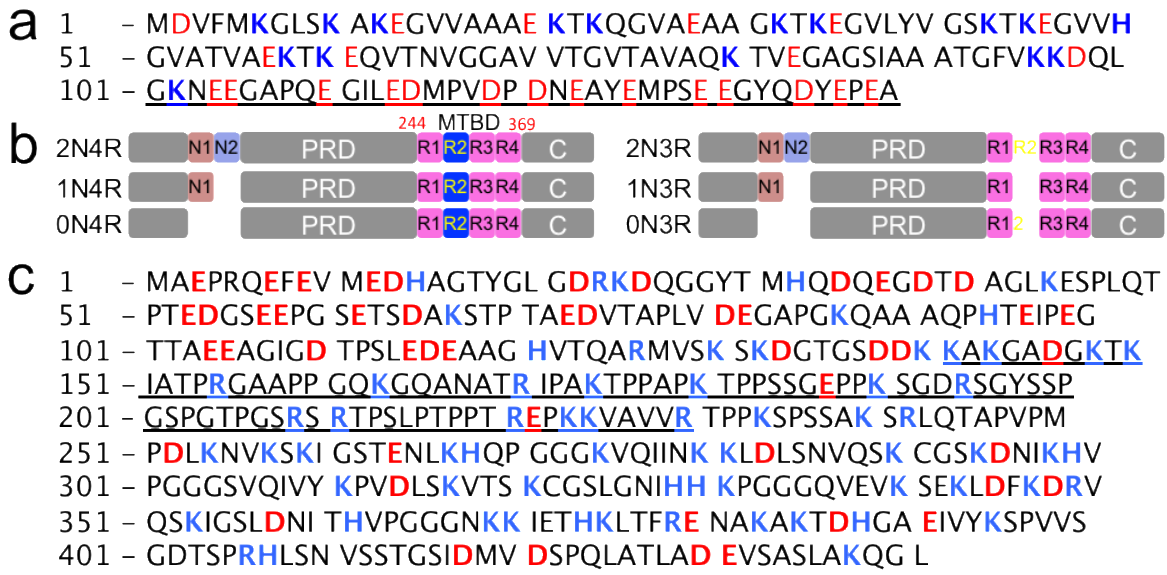

Figure S1. (a) Amino acid sequence of α-synuclein with negatively (red) and positively (blue) charged amino acids, respectively, at the neutral pH. (b) An alternative splicing of the tau gene leads to six isoforms of the tau protein. The six isoforms consist of the N-terminal region with 0, 1, 2 inserts, prolin-rich domain (PRD), microtubule binding domain with three or four repeats, and C-terminal region (C). (c) Amino acid sequence of full-length (2N4R) tau. The theoretical pI of the underlined regions is 3.4 and 11.4 for α-synuclein (a) and tau (c), respectively.

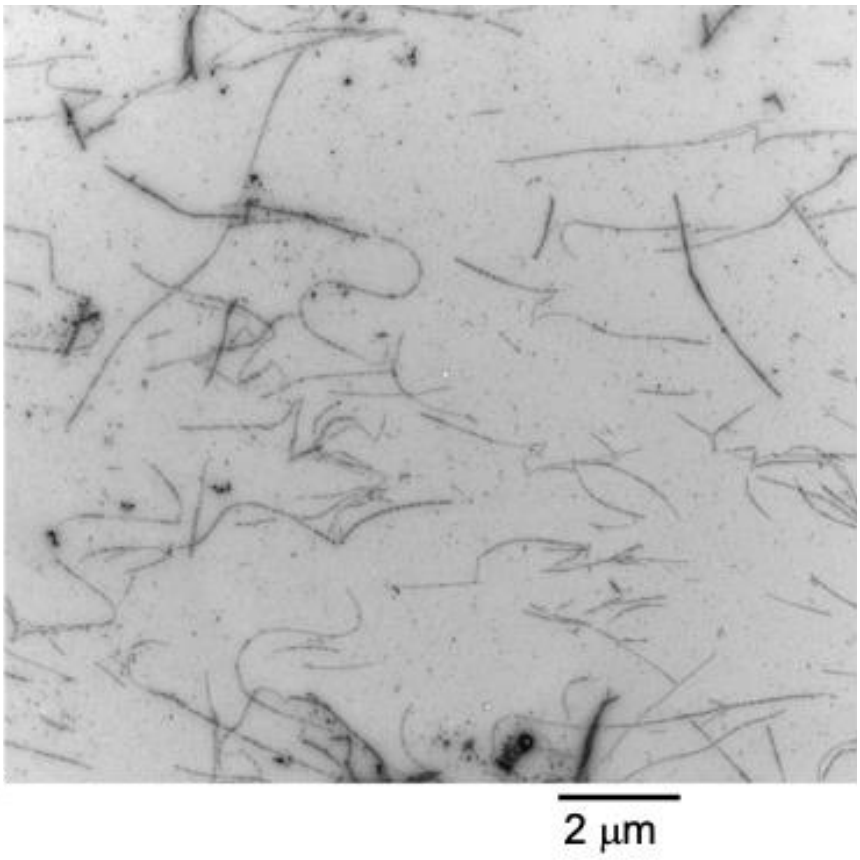

Figure S2. TEM image of a mixture of α-synuclein (70 μM) and tau (20 μM) incubated for 1 day at 37 °C in 10 mM phosphate buffer (pH 7.4).

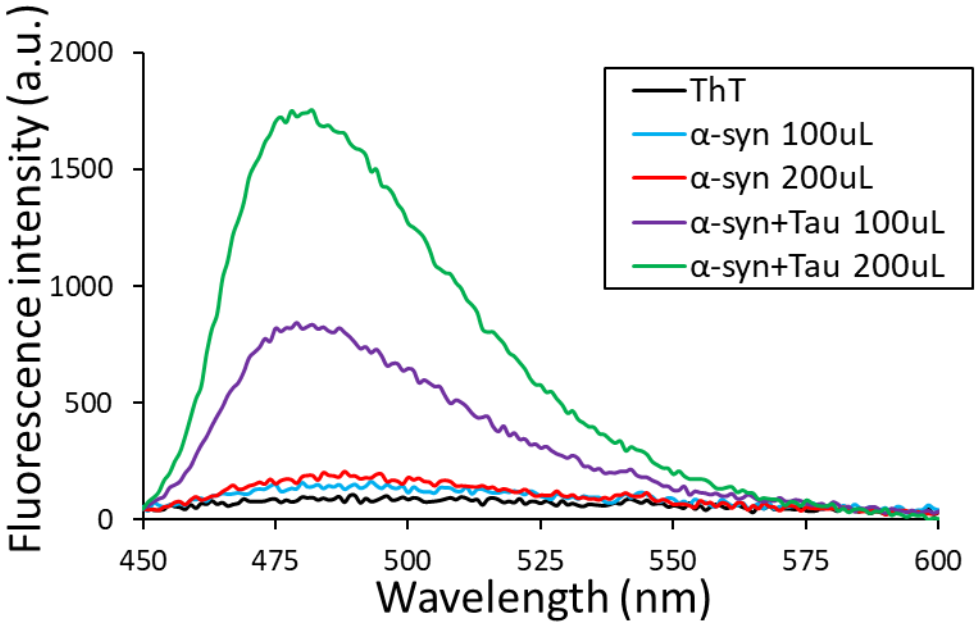

Figure S3. Thioflavin T (ThT) fluorescence emission spectra of the pure α-synuclein (70 μM) and a mixture of α-synuclein (70 μM) and tau (20 μM) incubated for 1 day at 37 °C. For the fluorescence measurements, 50 μL of I mM ThT in PBS buffer (pH 7.4) was mixed with the protein solutions, and the emission spectra were recorded with an excitation at 440 nm.

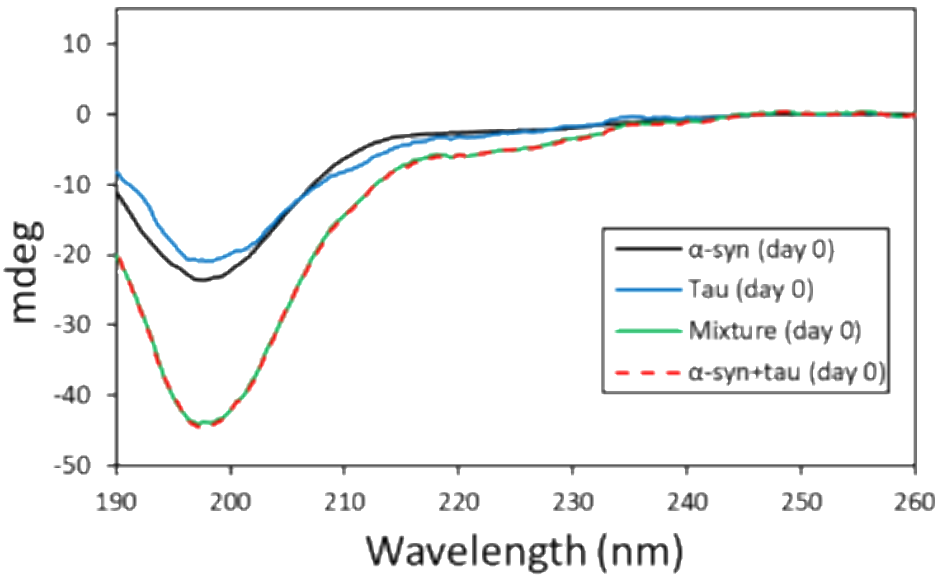

Figure S4. CD spectra of the pure α-synuclein (10 μM, black), pure tau (3 μM, blue), mixture of the two proteins (green), and sum of the two pure proteins (dotted red).

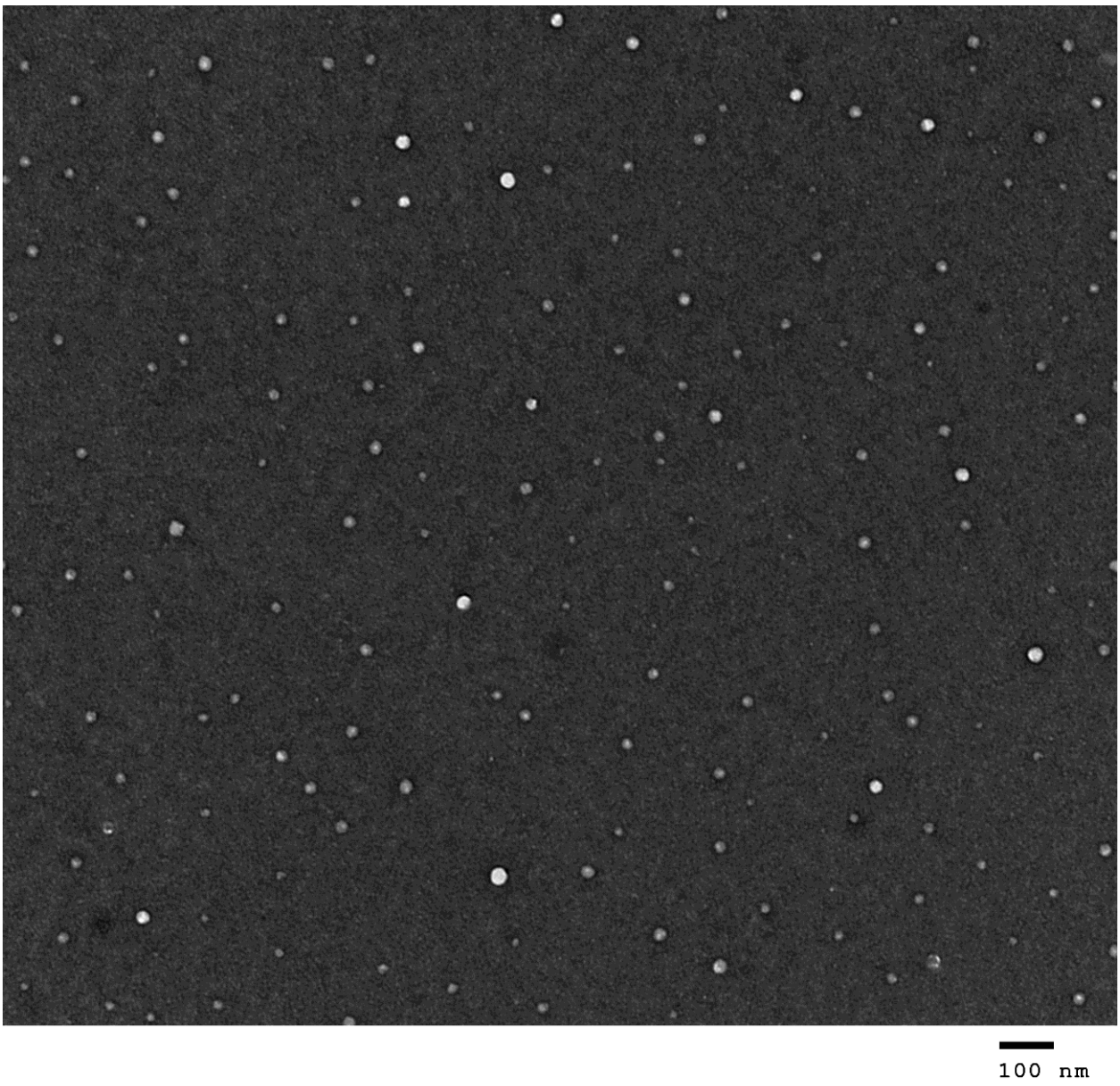

Figure S5. TEM image of protein aggregates formed by co-incubation of α-synuclein and tau. The mixture of α-synuclein (10 μM) and tau (3 μM) was incubated at 37 °C for two days under constant agitation at 250 rpm.

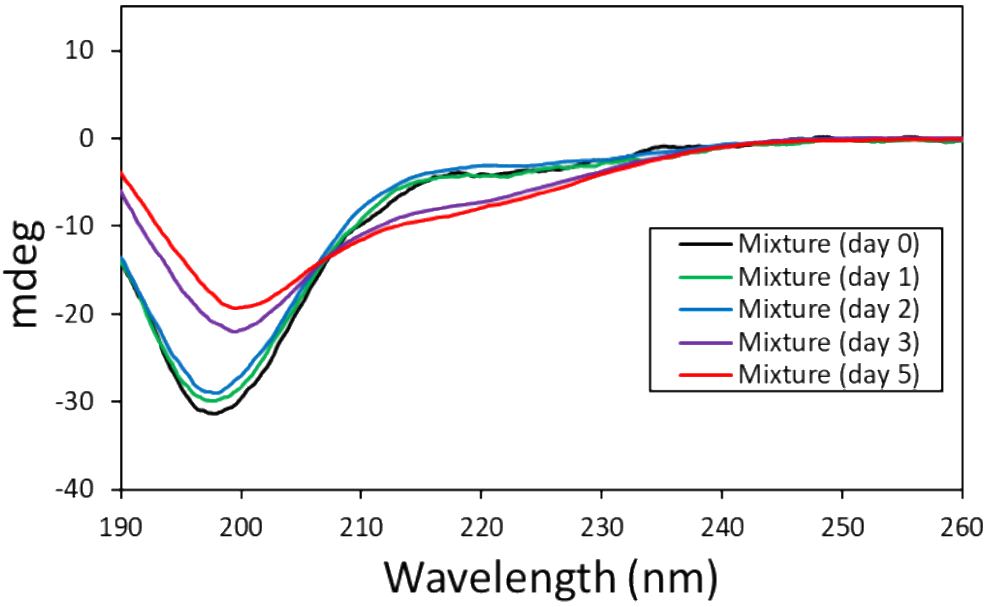

Figure S6. CD spectra of the mixture of α-synuclein (10 μM) and tau (1 μM) acquired at different incubation times.

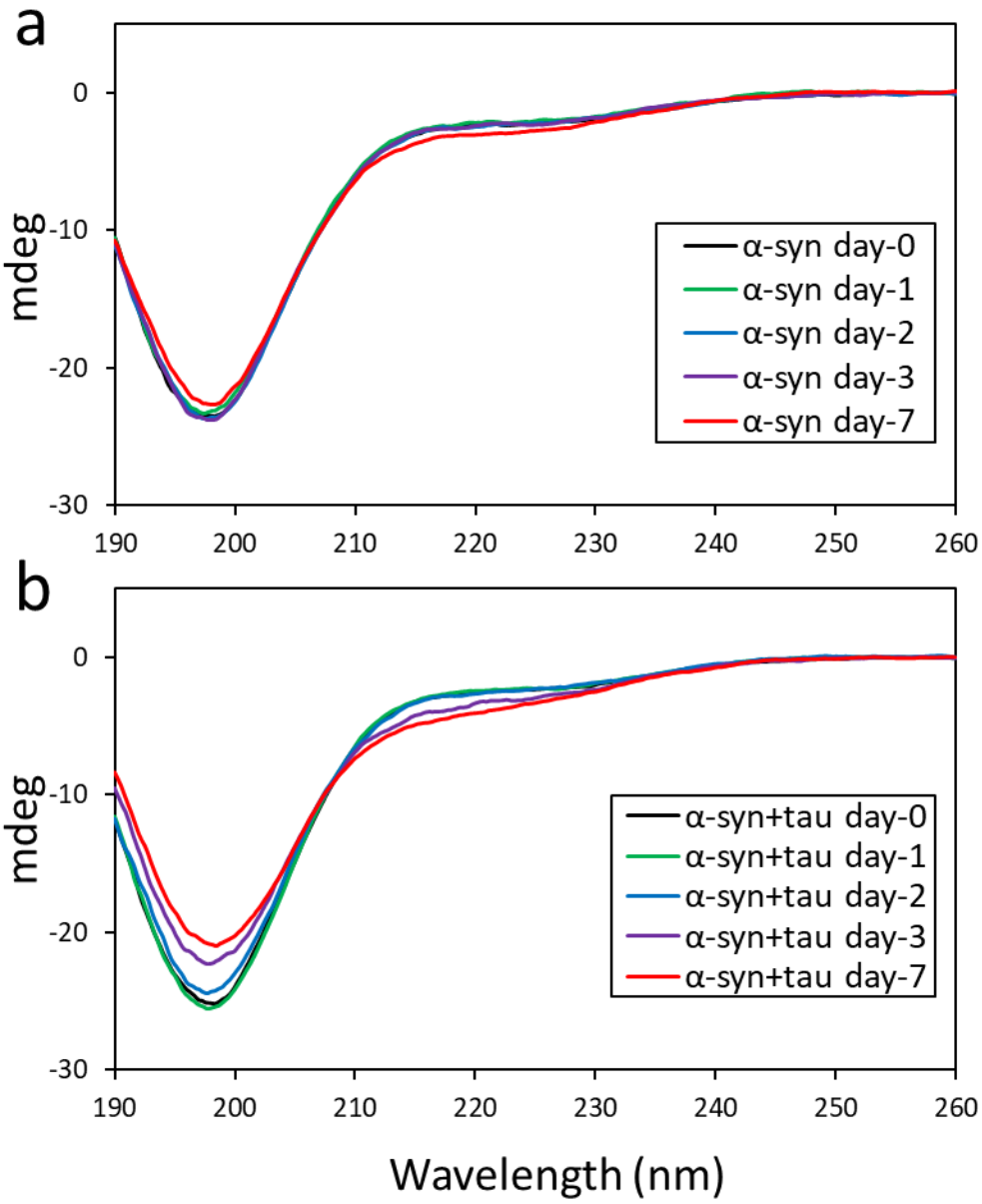

Figure S7. CD spectra of the pure α-synuclein (70 μM) (a) and the mixture of α-synuclein (70 μM) and tau (0.7 μM) (b) acquired at different incubation times.

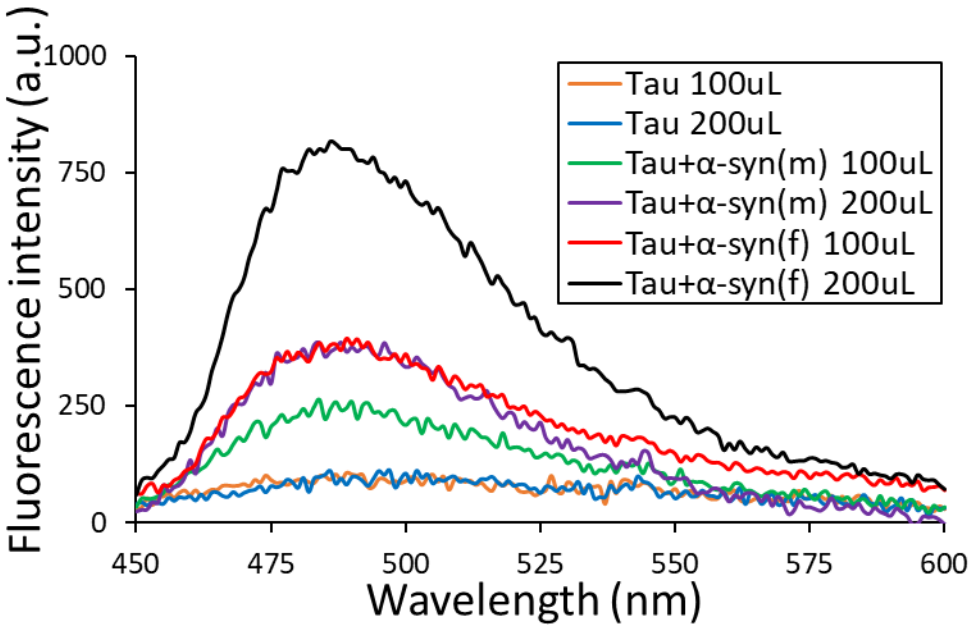

Figure S8. Thioflavin T (ThT) fluorescence emission spectra of the pure tau (10 μM), a mixture of tau (10 μM) and α-synuclein monomers (1 μM), and a mixture of tau (10 μM) and α-synuclein filaments (1 μM, monomer concentration). For the fluorescence measurements, the protein samples were incubated for 3 days at 37 °C.

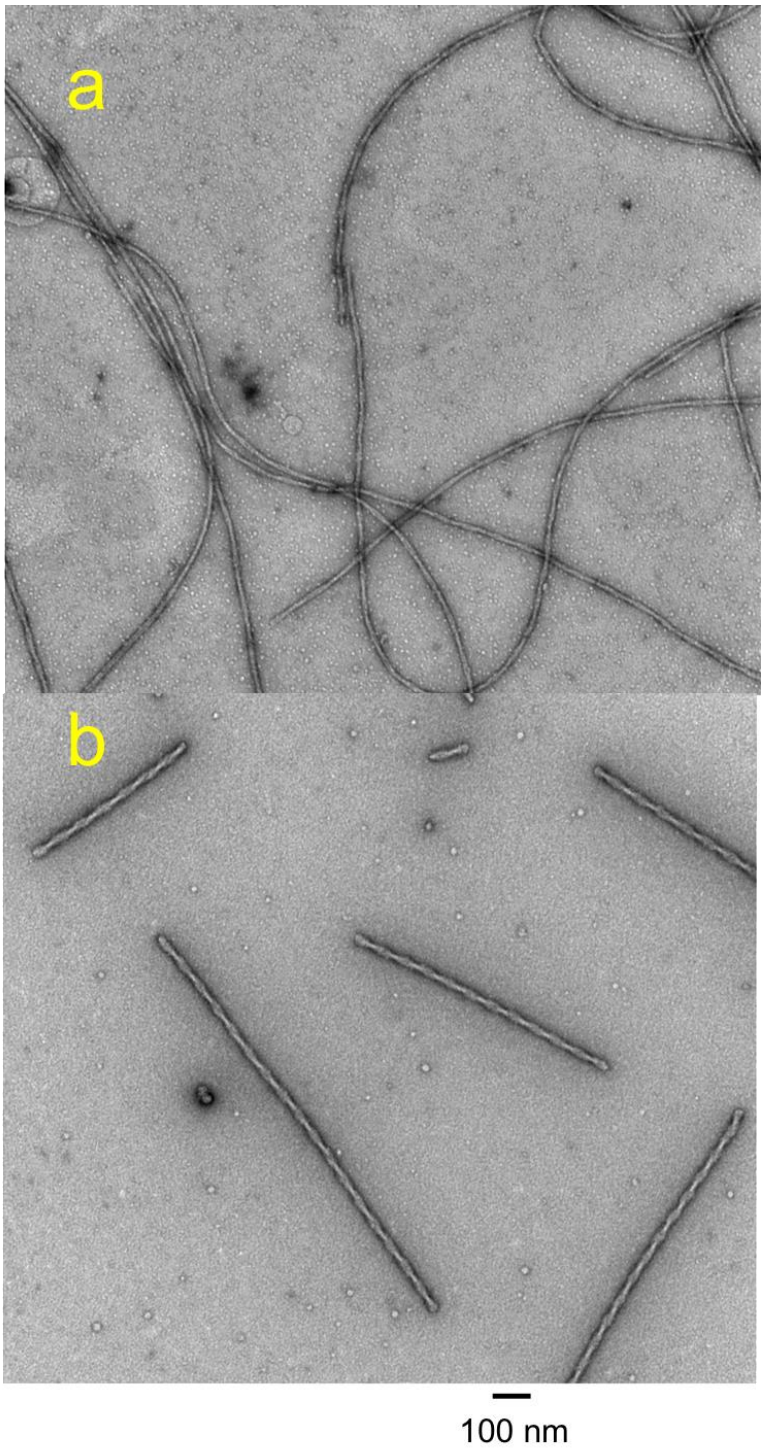

Figure S9. TEM images of the pure α-synuclein (70 μM) (a) and mixture of α-synuclein (70 μM) and tau (20 μM) (b). The pure α-synuclein and mixture of the proteins were incubated at 37 °C for one week and one day, respectively.

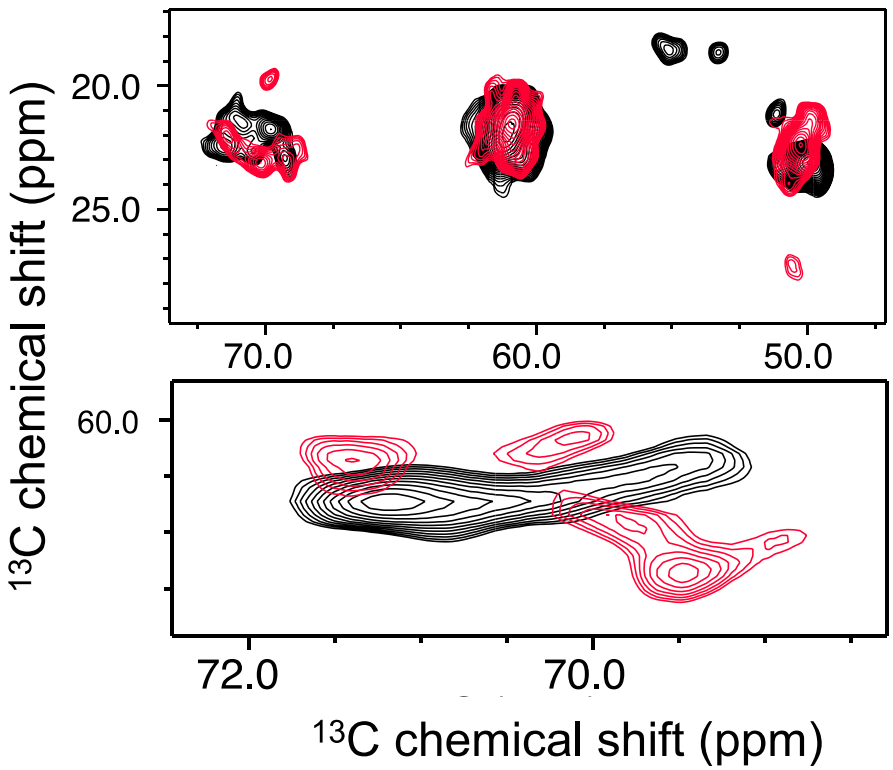

Figure S10. 2D DARR solid-state NMR spectra for the sidechain regions in the two α-synuclein filaments formed in the absence (black) and presence of tau (red).

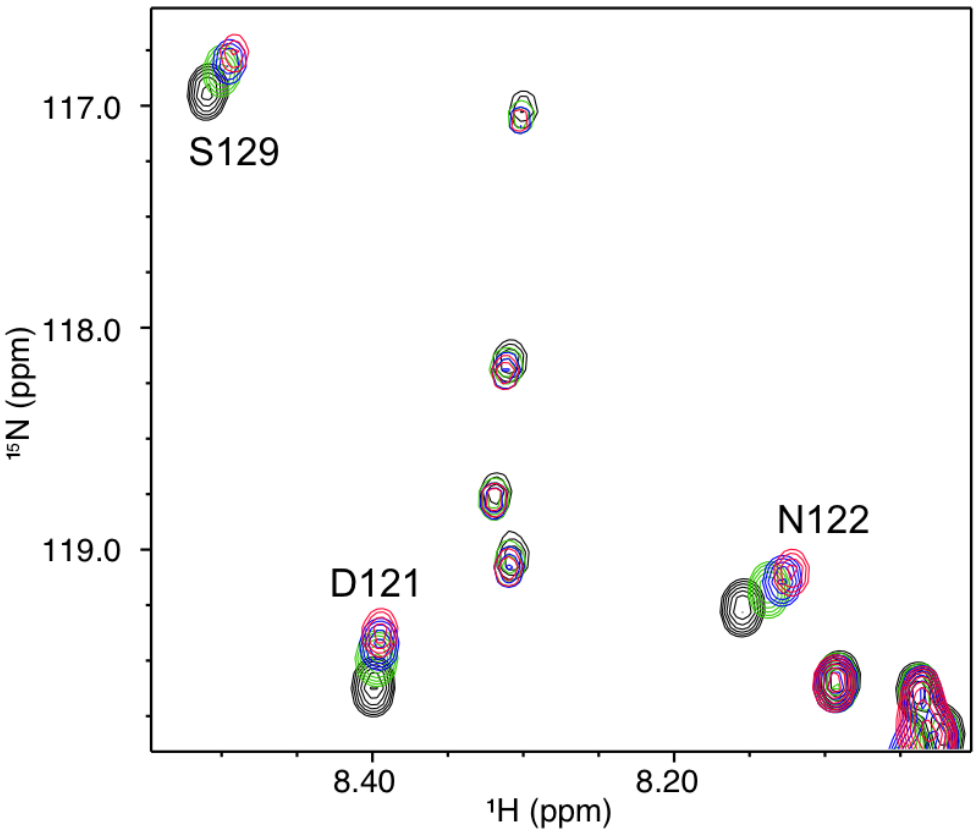

Figure S11. Overlaid $^{1}H/^{15}N$ HSQC NMR spectra of α-synuclein (70 μM) in the presence of tau (35 μM (green), 70 μM (blue), and 140 μM (red). The NMR cross-peaks from the residues in the C-terminal region gradually decrease in intensity at higher tau concentrations. Dissociation constant (K_d) was calculated based on the chemical shift changes of the C-terminal regions using the software NMRviewJ. The week affinity (K_d of 8.1 μM ± 2.0 μM) falls in the K_d values (pM – μM) of the interactions between intrinsically disordered proteins.

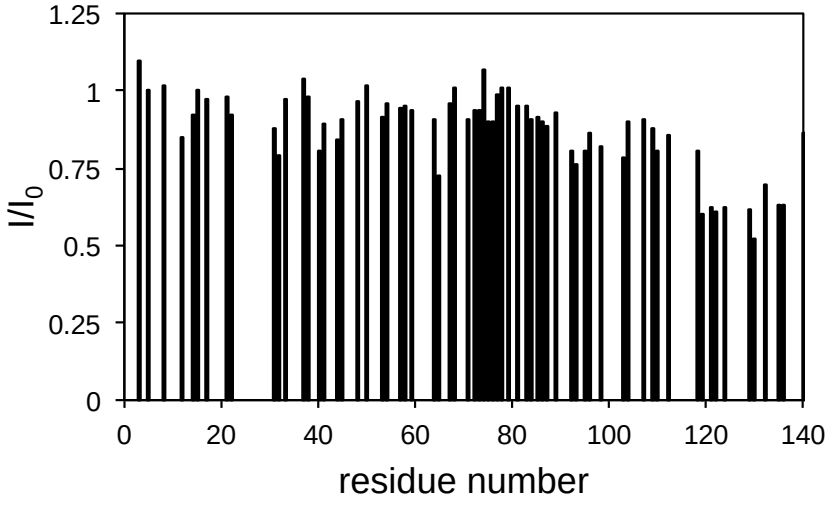

Figure S12. Changes in the NMR signal intensity of α-synuclein (70 μM) in the presence of tau (140 μM). I_0 and I are the cross-peak intensity in the 2D HSQC NMR spectra of the pure α-synuclein and mixtures of the two proteins, respectively.

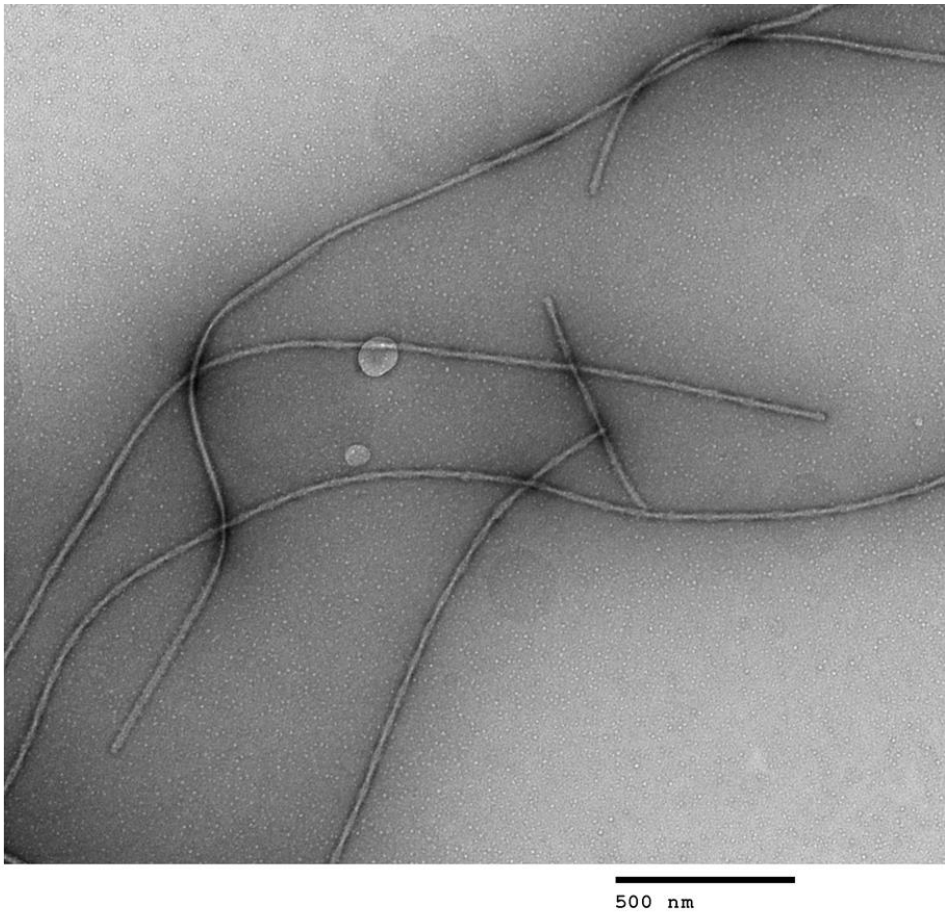

Figure S13. TEM image of a mixture of α-synuclein (70 μM) and tau (7 μM) incubated for 2 days at 37 °C in 10 mM phosphate buffer (pH 7.4).