Passaging Human Tauopathy Patient Samples in Cells Generates Heterogeneous Fibrils with a Subpopulation Adopting Disease Folds

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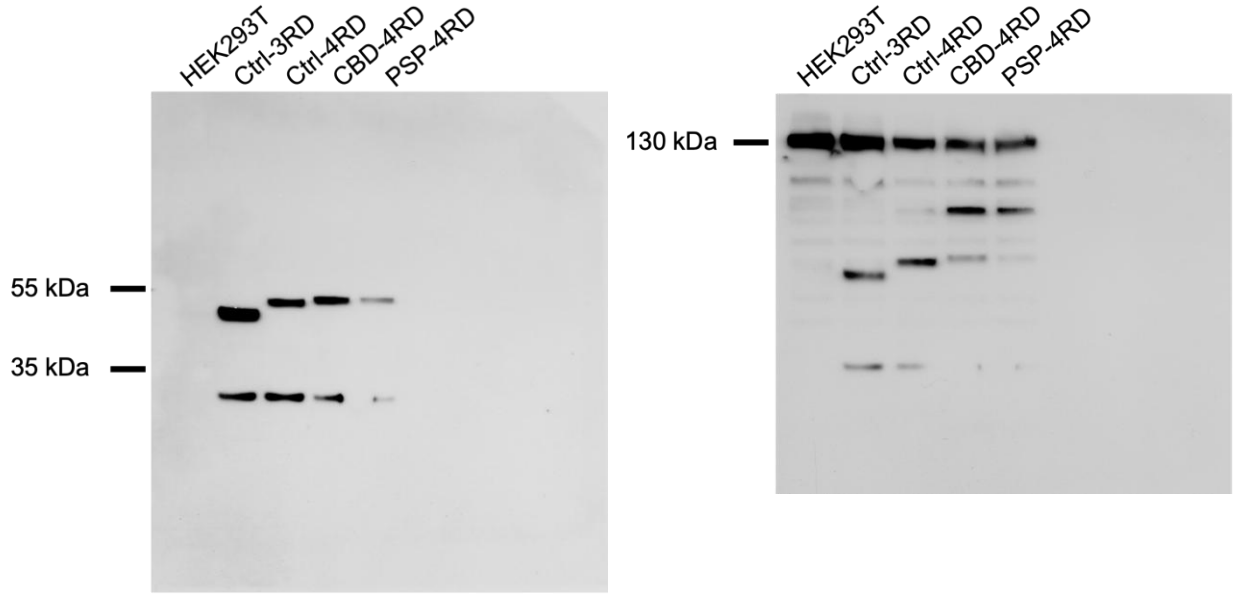

Figure S1. Uncropped blots of lysates collected in RIPA buffer from naïve HEK293T cells, Tau3RD*VM-YFP cells, Tau4RD*LM-YFP cells, and Tau4RD*LM-YFP cells stably infected with CBD or PSP tau prions shown in Fig. 2c. Left, membrane probed for the presence of the tau-YFP fusion protein using the GFP primary antibody. Right, membrane probed for vinculin as a loading control.

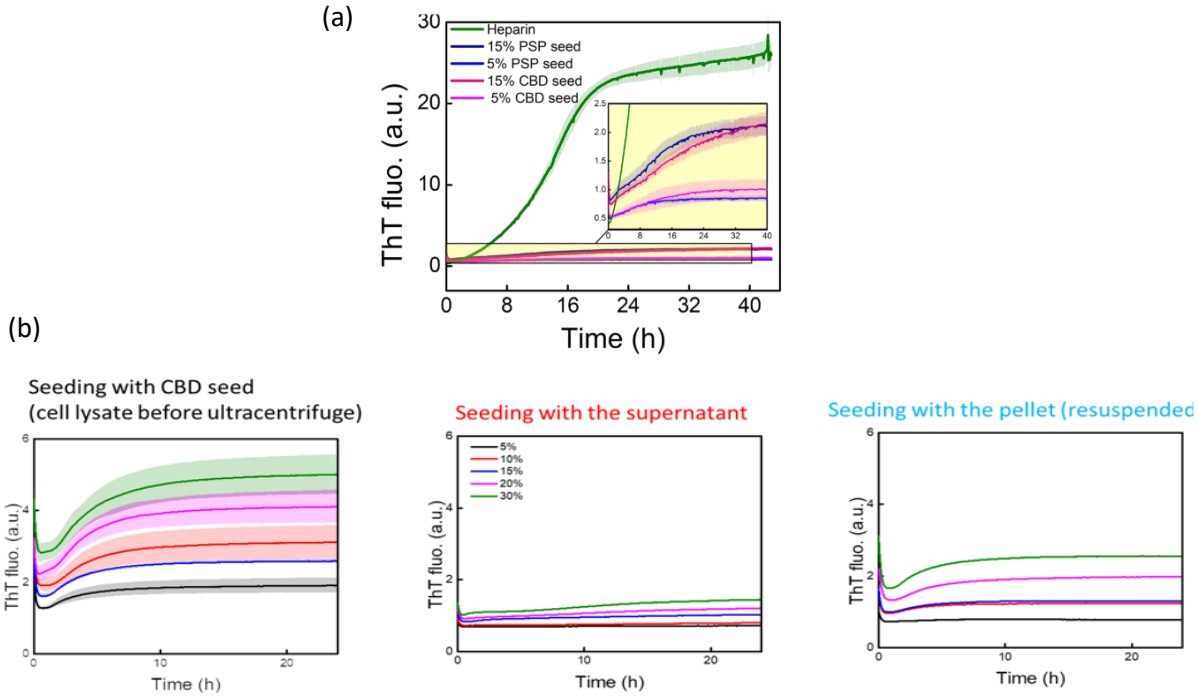

Figure S2. (a) ThT fluorescence of tau fibrillization induced by heparin (green), or cell-passaged seeds (lysates) from CBD (pink) or PSP (blue) patient samples for 0N4R construct. The lysate-seeded aggregation generated a relatively low maximum ThT; an enlarged curve is shown in the inset. 0N4R (25 μM) was mixed with stoichiometric amounts of heparin (8.25 μM, 17% by mass) or cell-passaged lysates [either 5% (lighter-color lines) or 15% by mass (darker-colored lines)] and was aggregated in the presence of ThT at 37°C. (b) ThT fluorescence of recombinant Tau187 fibrillization induced by cell-passaged CBD seed. Tau187 fibrillization in the presence of ThT at 37°C using CBD-seeded lysate (left) without treatment (middle) supernatant following ultracentrifugation, and (right) resuspended pellet following ultracentrifugation.

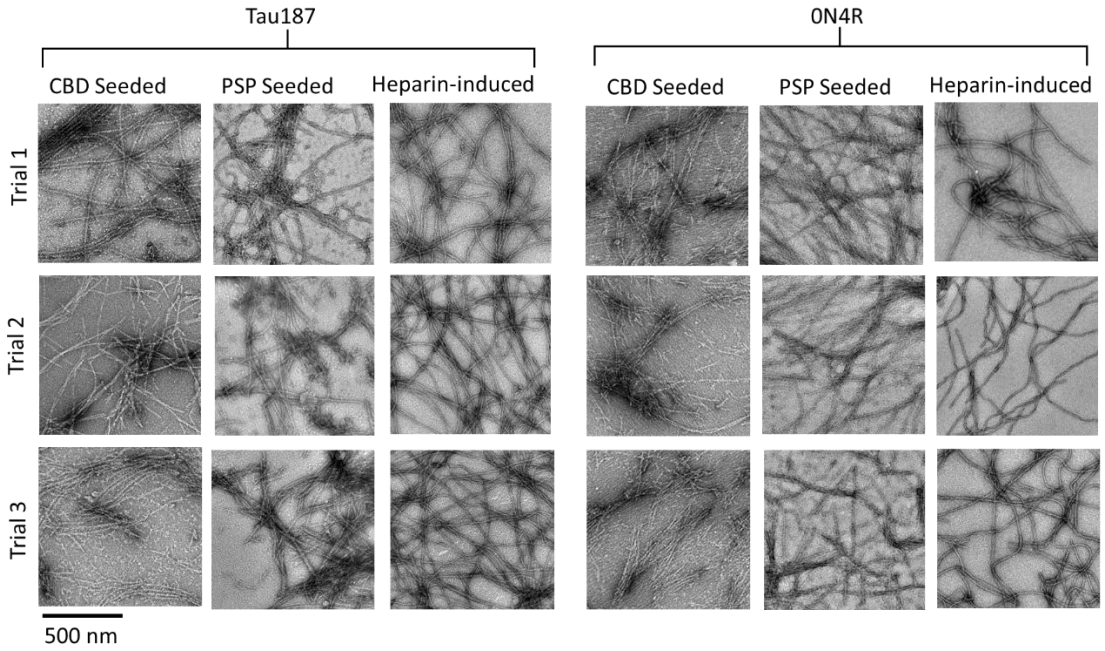

Figure S3. Negative-stain TEM of seeded fibrils. Triplicates (Trials 1-3) of negative-stain TEM images are shown for Tau187 (left) and 0N4R (right) seeded fibrils. Fibrillization was induced by CBD lysate (left), PSP lysate (middle), or heparin (right).

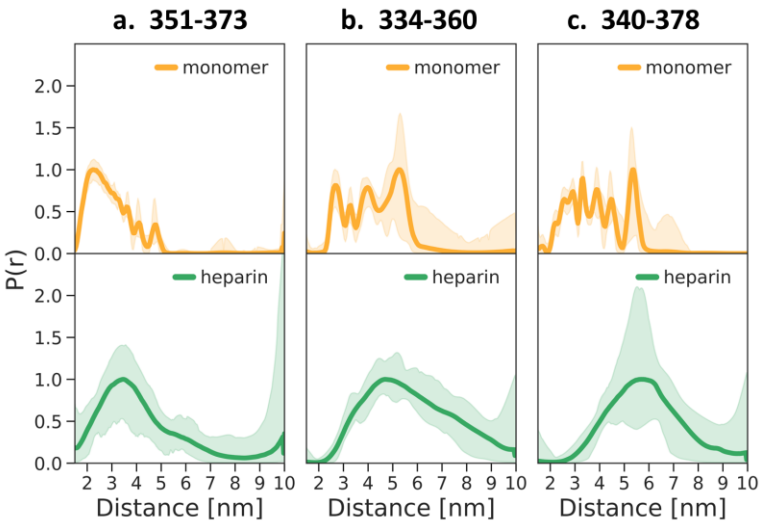

Figure S4. P(r) comparison between monomer and heparin induced fibrils. Measurements from residues (a) 351-373, (b) 334-360, and (c) 340-378. Technical replicates of P(r) measurements for residues 351 & 373 are in **Fig. S5**, residues 334 & 360 are in **Fig. S6**, and residues 340 & 378 are in **Fig. S7**.

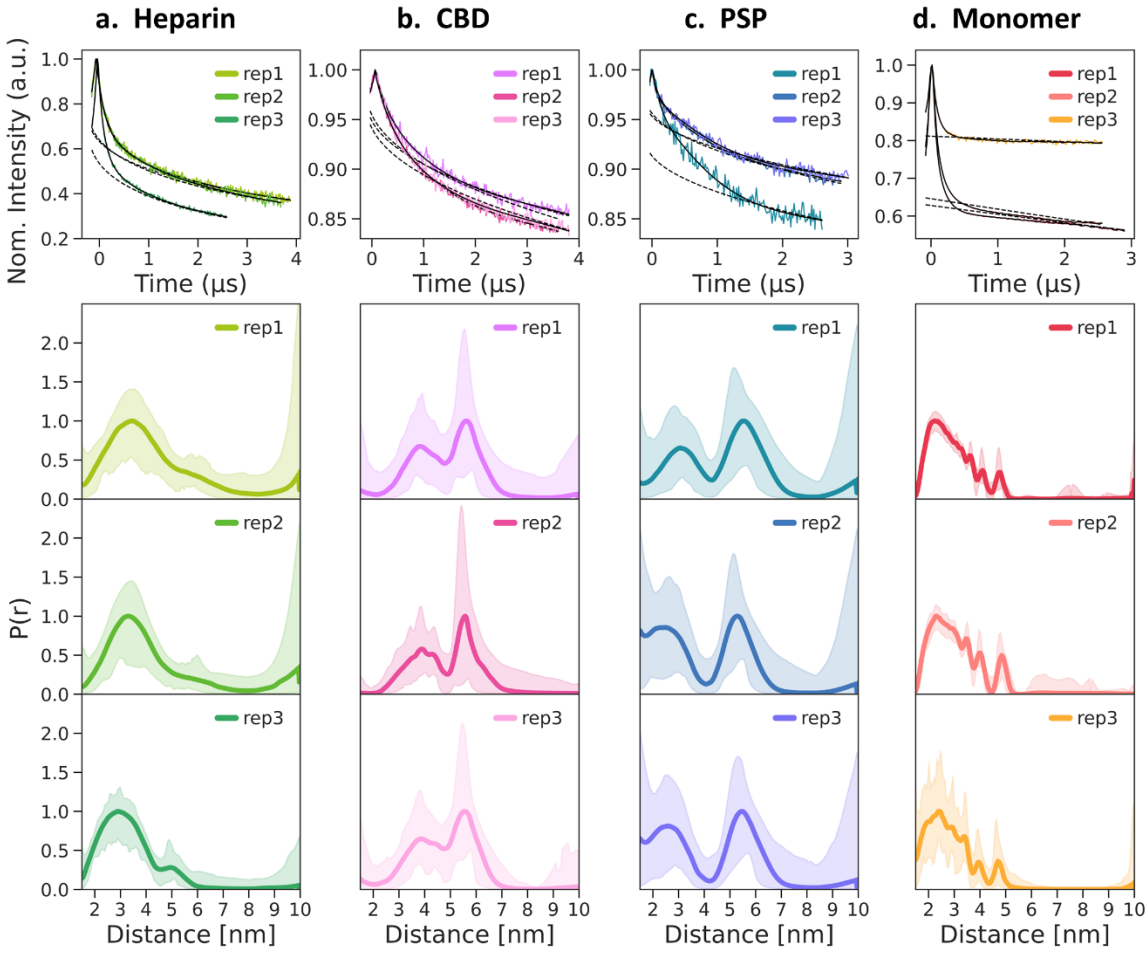

Figure S5. Technical replicates of P(r) measurements for residues 351 & 373. Independent triplicates (reps 1-3) of the intra-molecular DEER distance distribution, P(r), measured for residues 351 & 373 are shown for fibrils induced by (a) heparin, (b) cell-passaged CBD tau, (c) cell-passaged PSP tau, and (d) monomer. From top to bottom, figures show time-domain data with its fit and background fit, and distance distributions P(r).

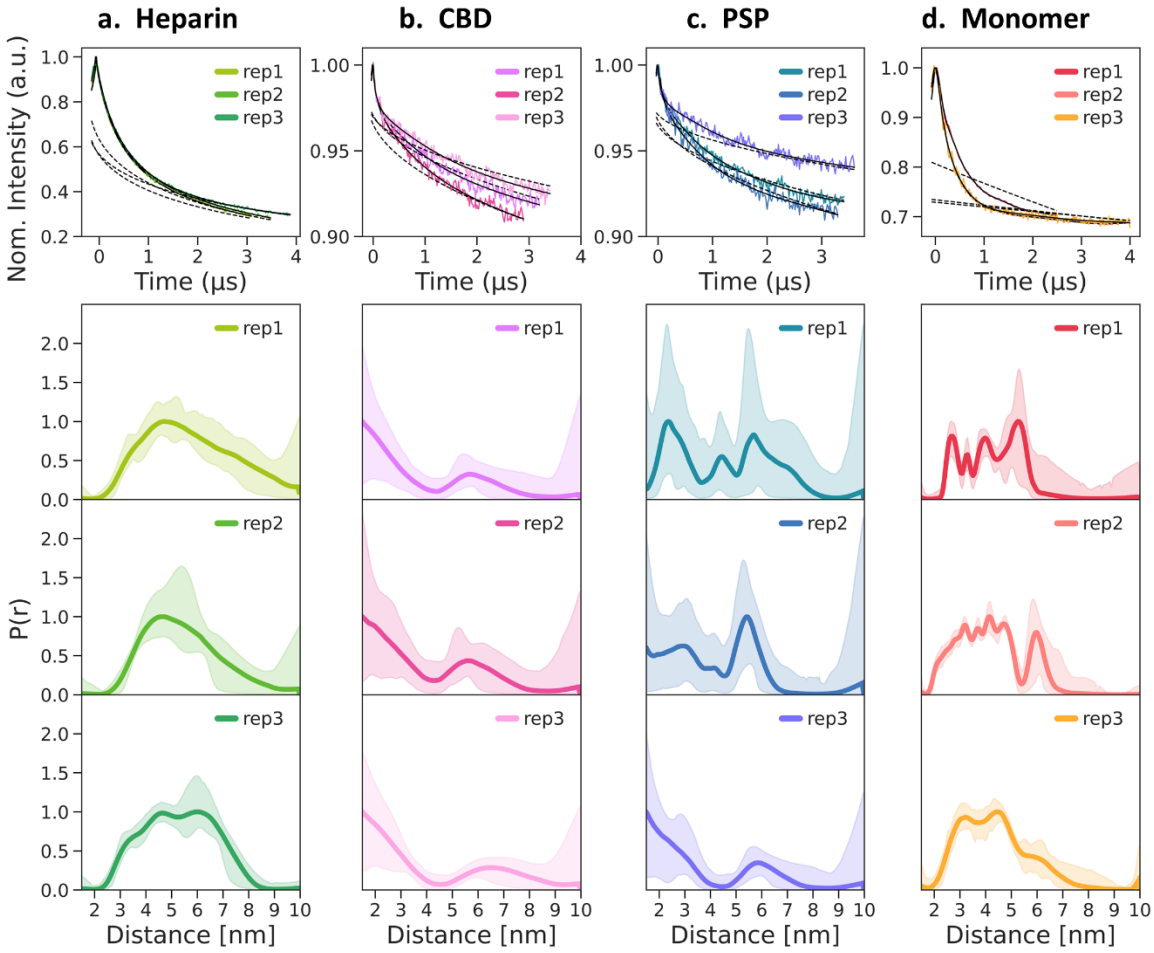

Figure S6. Technical replicates of P(r) measurements for residues 334 & 360. Independent triplicates (reps 1-3) of the intra-molecular DEER distance distribution, P(r), measured for residues 334 & 360 are shown for fibrils induced by (a) heparin, (b) cell-passaged CBD tau, (c) cell-passaged PSP tau, and (d) monomer. From top to bottom, figures show time-domain data with its fit and background fit, and distance distributions P(r).

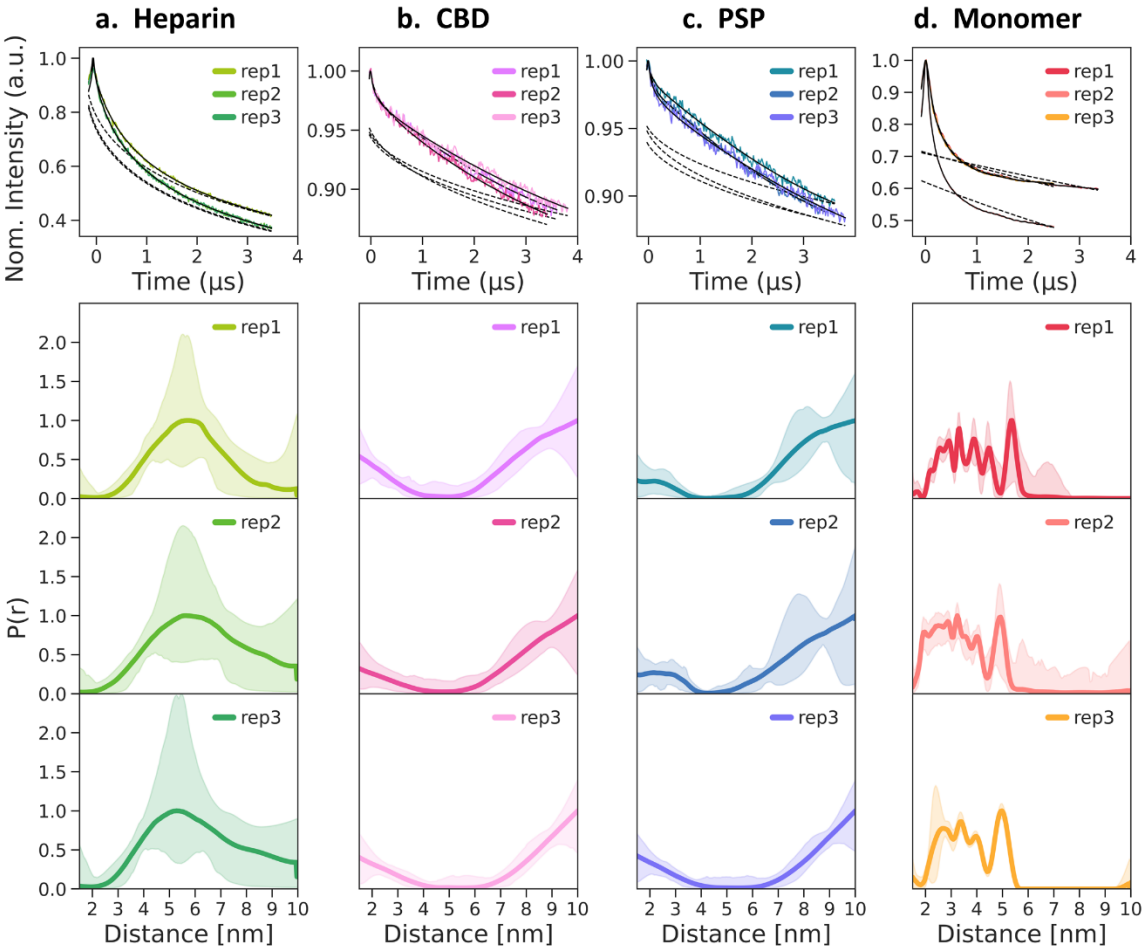

Figure S7. Technical replicates of P(r) measurements for residues 340 & 378. Independent triplicates (reps 1-3) of the intra-molecular DEER distance distribution, P(r), measured for residues 340 & 378 are shown for fibrils induced by (a) heparin, (b) cell-passaged CBD tau, (c) cell-passaged PSP tau, and (d) monomer. From top to bottom, figures show time-domain data with its fit and background fit, and distance distributions P(r).

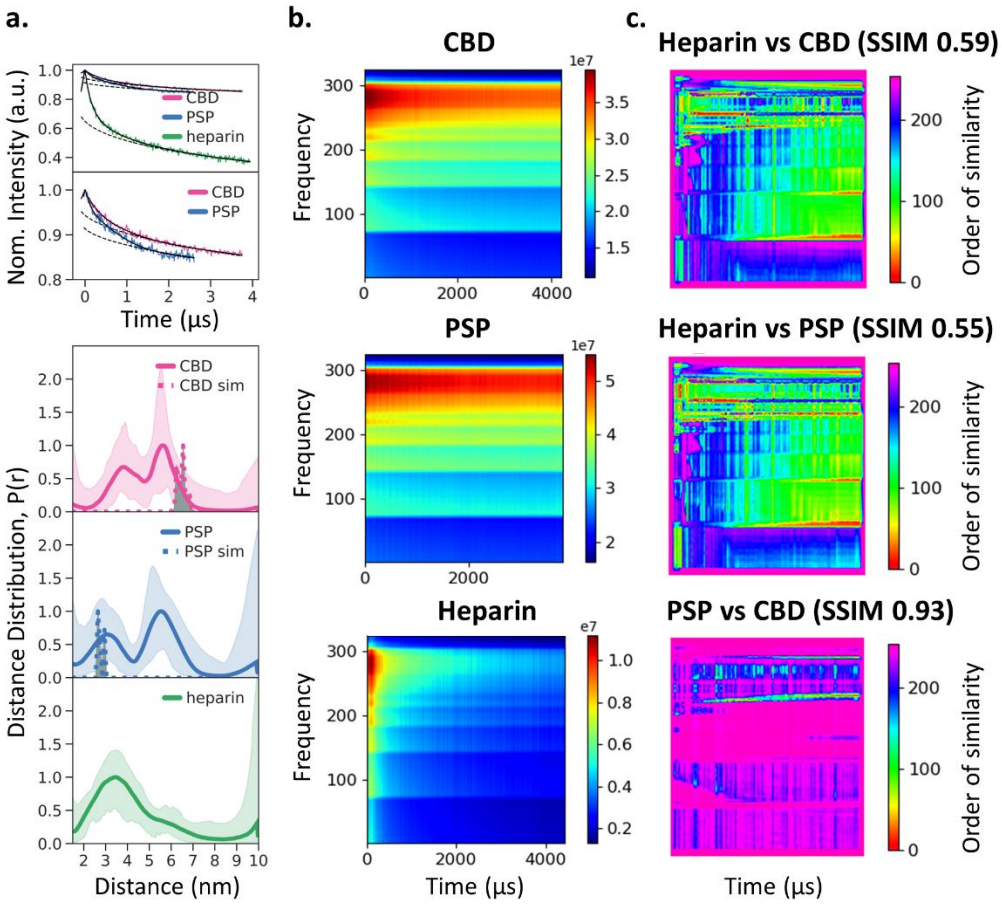

Figure S8. Pattern analysis of P(r) measurements for residues 351 & 373. (a) DEER time domain data (top) and P(r) (bottom) of Tau187 labeled at sites 351 & 373 are shown for cell-passaged CBD (pink), cell-passaged PSP (blue), and heparin seeded fibrils (green). (b) Spectrograms from CWT calculations of DEER time domain data are shown for cell-passaged CBD seeded fibrils (top), cell-passaged PSP seeded fibrils (middle), and heparin-induced fibrils (bottom). (c) Similarity gradient plots comparing spectrograms of heparin vs cell-passaged CBD fibrils (top), heparin vs cell-passaged PSP middle (middle), and cell-passaged PSP vs CBD seeded fibrils (bottom). The similarity gradient plot is shown as frequency vs time (top to bottom: high to low frequency bands) with the magnitude of similarity displayed in different colors, where red presents the least similarity and pink represents high similarity.

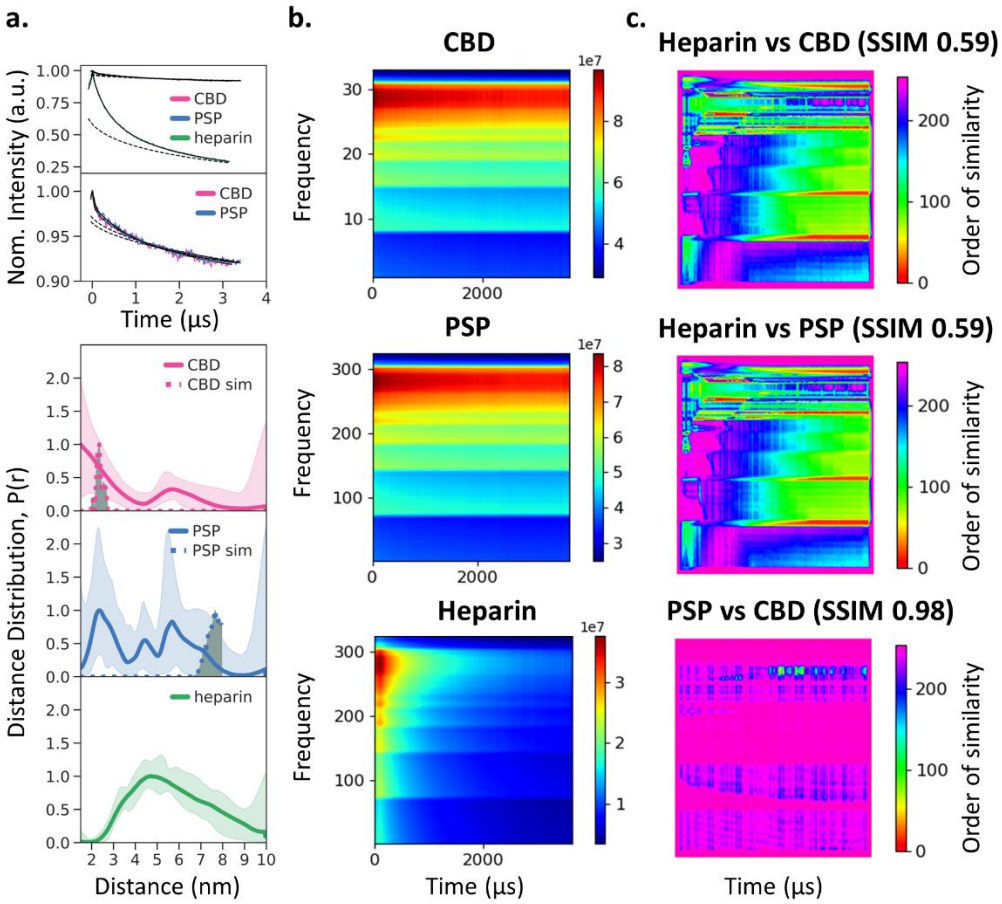

Figure S9. Pattern analysis of P(r) measurements for residues 334 & 360. (a) DEER time domain data (top) and P(r) (bottom) of tau187 labeled at sites 334 & 360 are shown for cell-passaged CBD (pink), cell-passaged PSP (blue), and heparin seeded fibrils (green). (b) Spectrograms from CWT calculations of DEER time domain data are shown for cell-passaged CBD seeded fibrils (top), cell-passaged PSP seeded fibrils (middle), and heparin-induced fibrils (bottom). (c) Similarity gradient plot comparing spectrograms of heparin vs cell-passaged CBD fibrils (top), heparin vs cell-passaged PSP middle (middle), and cell-passaged PSP vs CBD seeded fibrils (bottom). The similarity gradient plot is shown as frequency vs time (top to bottom: high to low frequency bands) with the magnitude of similarity displayed in different colors, where red presents the least similarity and pink represents high similarity.

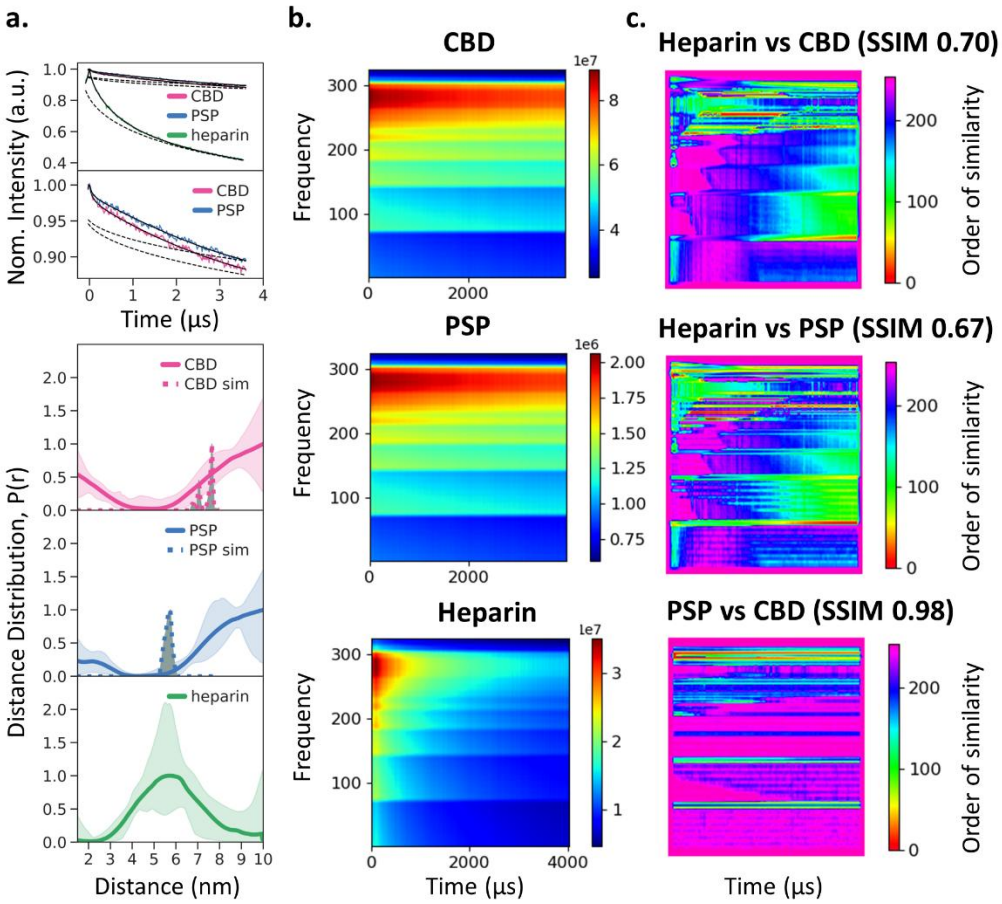

Figure S10. Pattern analysis of P(r) measurements for residues 340 & 378. (a) DEER time domain data (top) and P(r) (bottom) of tau187 labeled at sites 340 & 378 are shown for cell-passaged CBD (pink), cell-passaged PSP (blue), and heparin seeded fibrils (green). (b) Spectrograms from CWT calculations of DEER time domain data are shown for cell-passaged CBD seeded fibrils (top), cell-passaged PSP seeded fibrils (middle), and heparin-induced fibrils (bottom). (c) Similarity gradient plot comparing spectrograms of heparin vs cell-passaged CBD fibrils (top), heparin vs cell-passaged PSP middle (middle), and cell-passaged PSP vs CBD seeded fibrils (bottom). The similarity gradient plot is shown as frequency vs time (top to bottom: high to low frequency bands) with the magnitude of similarity displayed in different colors, where red presents the least similarity and pink represents high similarity.

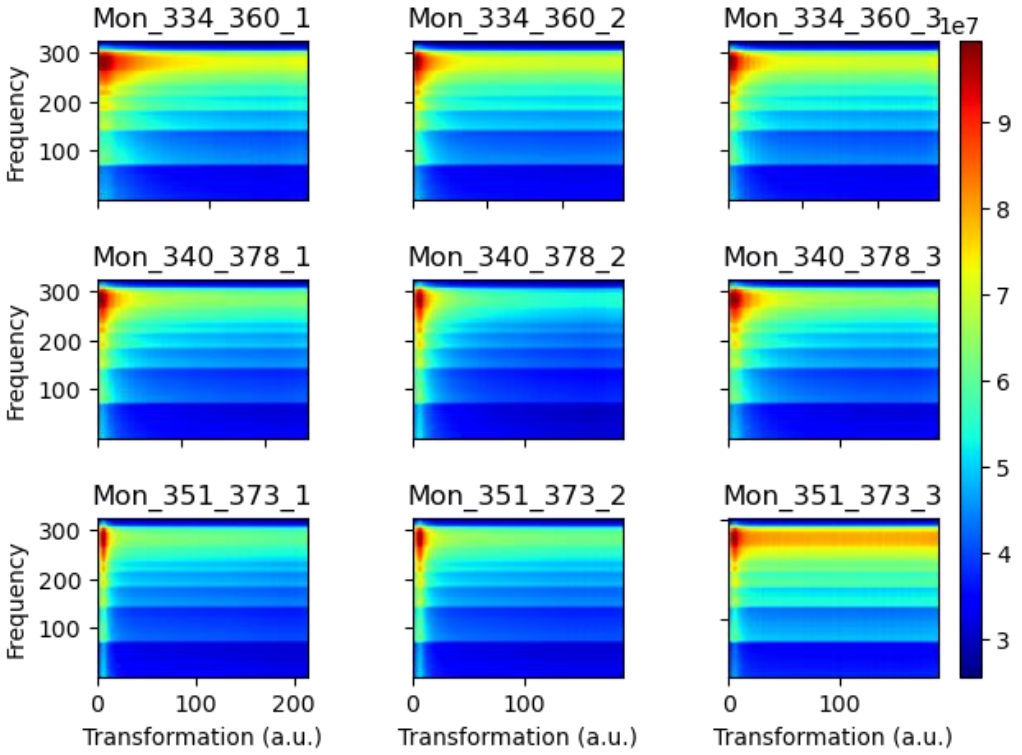

Figure S11. Spectrograms from CWT calculations of monomers. The spectrograms from CWT of independent triplicates (reps 1-3) of the DEER time-domain data of tau monomer labeled at residues 334 & 360 (top), 340 & 378 (middle), and 351 & 373 (bottom).

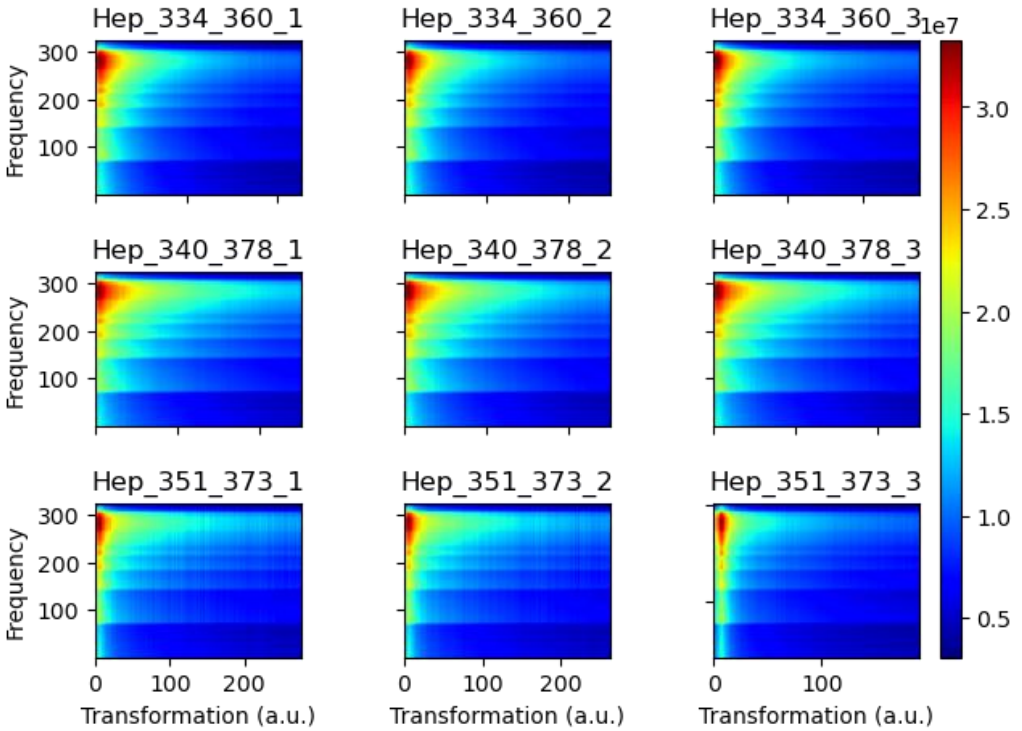

Figure S12. Spectrograms from CWT calculations of heparin induced fibrils. The spectrograms from CWT of independent triplicates (reps 1-3) of the DEER time-domain data of heparin-induced fibrils labeled at residues 334 & 360 (top), 340 & 378 (middle), and 351 & 373 (bottom).

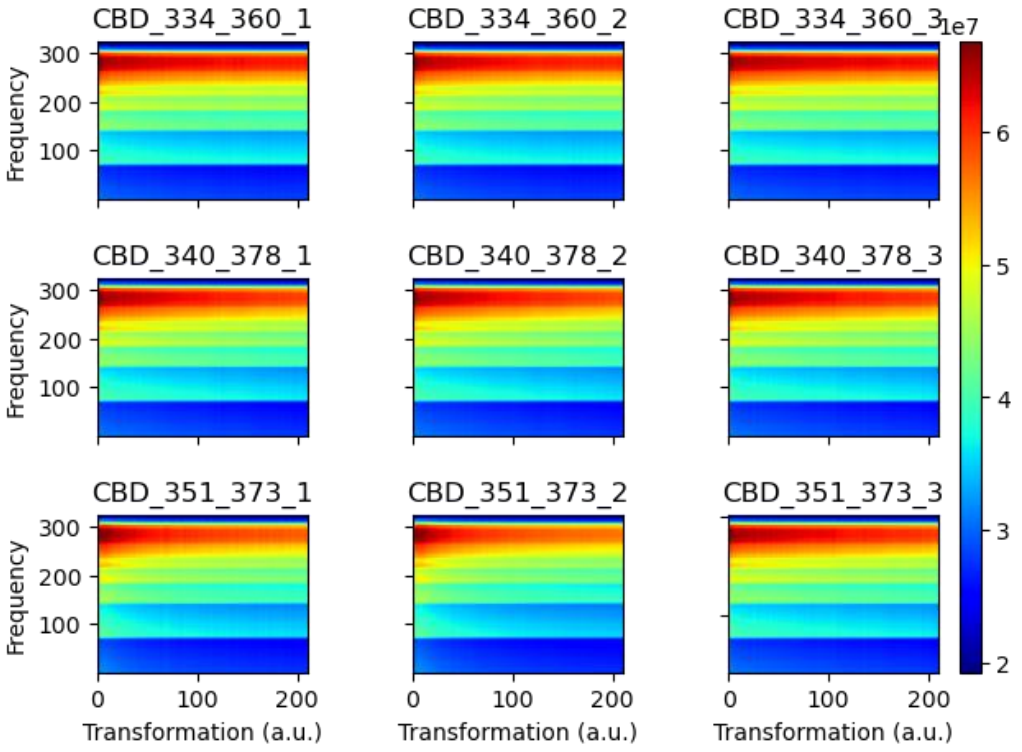

Figure S13. Spectrograms from CWT calculations of cell-passaged CBD seeded fibrils. The spectrograms from CWT of independent triplicates (reps 1-3) of the DEER time-domain data of cell-passaged CBD seeded fibrils labeled at residues 334 & 360 (top), 340 & 378 (middle), and 351 & 373 (bottom).

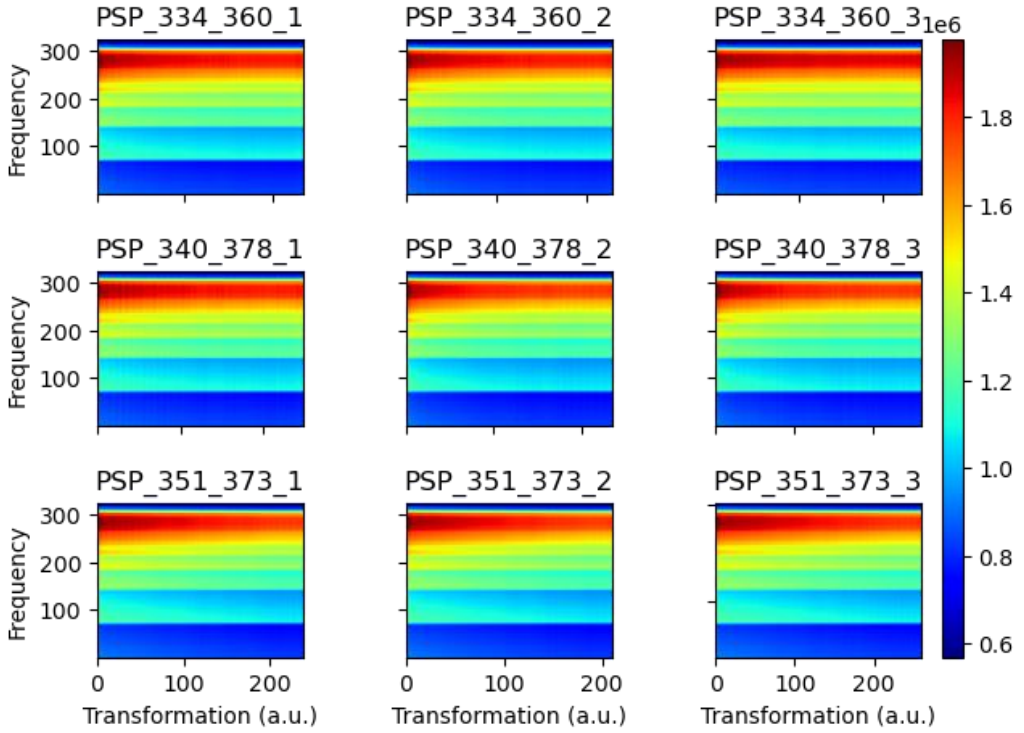

Figure S14. Spectrograms from CWT calculations of cell-passaged PSP seeded fibrils. The spectrograms from CWT of independent triplicates (reps 1-3) of the DEER time-domain data of cell-passaged PSP seeded fibrils labeled at residues 334 & 360 (top), 340 & 378 (middle), and 351 & 373 (bottom).

Residues	Sample type	Replicate 1	Replicate 2	Replicate 3	Avg SSIM	STD SSIM
334-360	Monomer	0.837	0.853	0.841	0.844	0.007
	Heparin	1.000	0.963	0.963	0.975	0.018
	CBD	0.718	0.724	0.712	0.718	0.005
	PSP	0.727	0.729	0.714	0.723	0.006
340-378	Monomer	0.875	0.913	0.873	0.887	0.018
	Heparin	0.919	0.931	0.927	0.926	0.005
	CBD	0.722	0.724	0.718	0.722	0.002
	PSP	0.722	0.730	0.719	0.724	0.005
351-373	Monomer	0.862	0.866	0.788	0.839	0.036
	Heparin	0.885	0.900	0.921	0.902	0.015
	CBD	0.735	0.749	0.747	0.744	0.006
	PSP	0.720	0.733	0.730	0.728	0.005

Table S1. SSIM index of each spectrogram comparison to one set control spectrogram. Spectrograms for all DEER data were compared to that of the replicate one of heparin-induced fibril labeled at residues 334-360 (highlighted in yellow). The average and standard deviation of the replicates from each condition are displayed.