SUPPORTING INFORMATION

Conformational Basis for Asymmetric Seeding Barrier in Filaments of Three- and Four-Repeat Tau

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This supplement contains:

Additional Experimental Procedures

5 Supplementary Figures

1 Supplementary Table

ADDITIONAL EXPERIMENTAL PROCEDURES

Preparation of Filaments of Fully Spin Labeled Tau. Double cysteine mutants of K18 and K19 were spin labeled as described above. After mixing 25 μM protein with heparin (at a protein: heparin molar ratio of 4:1) filaments were formed under agitation for 3 days at 25°C. Samples were pelleted at 100,000 × g for 30 minutes, washed with elution buffer, and transferred into borosilicate capillaries (0.6 mm inner diameter × 0.84 mm outer diameter).

CW EPR Measurements. Continuous wave EPR measurements of fibrils were recorded in a Bruker EMX spectrometer fitted with an ER 4119HS resonator. All spectra had a scan width of 150 G and an incident microwave power of 12 milliwatts. Spectra were normalized to the same number of spins using double integration. From all spectra, a minor background arising from 0.5% soluble tau was subtracted.

Negative Stain Electron Transmission Microscopy. Carbon coated copper grids (250-mesh) were placed for 40 s onto 10 μl drops of tau filaments (5 μM), then for 40 s on 10 μl drops of 2% uranyl acetate and subsequently air dried on filter paper. All images were taken with a Philips/FEI Tecnai-12 electron transmission microscope at 80 keV and equipped with a Gatan CCD camera.

SUPPLEMENTARY FIGURES

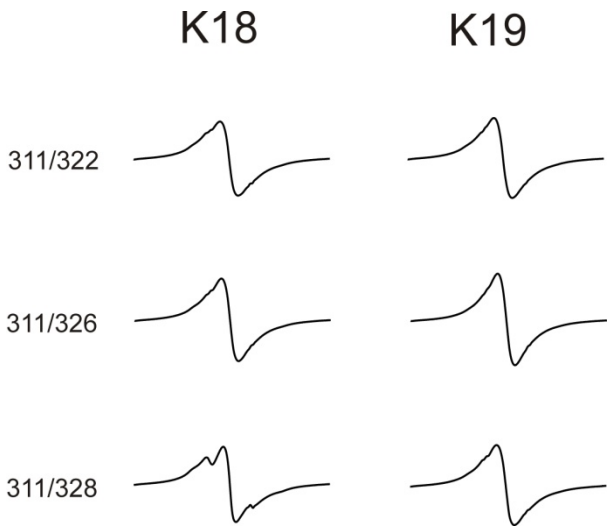

Figure S1. Continuous wave EPR spectra of K18 and K19 filaments reveal a stable core with parallel in-register arrangement of β-strands. The MTSL label was attached to cysteines at positions 311/322, 311/326, and 311/328 in R3.

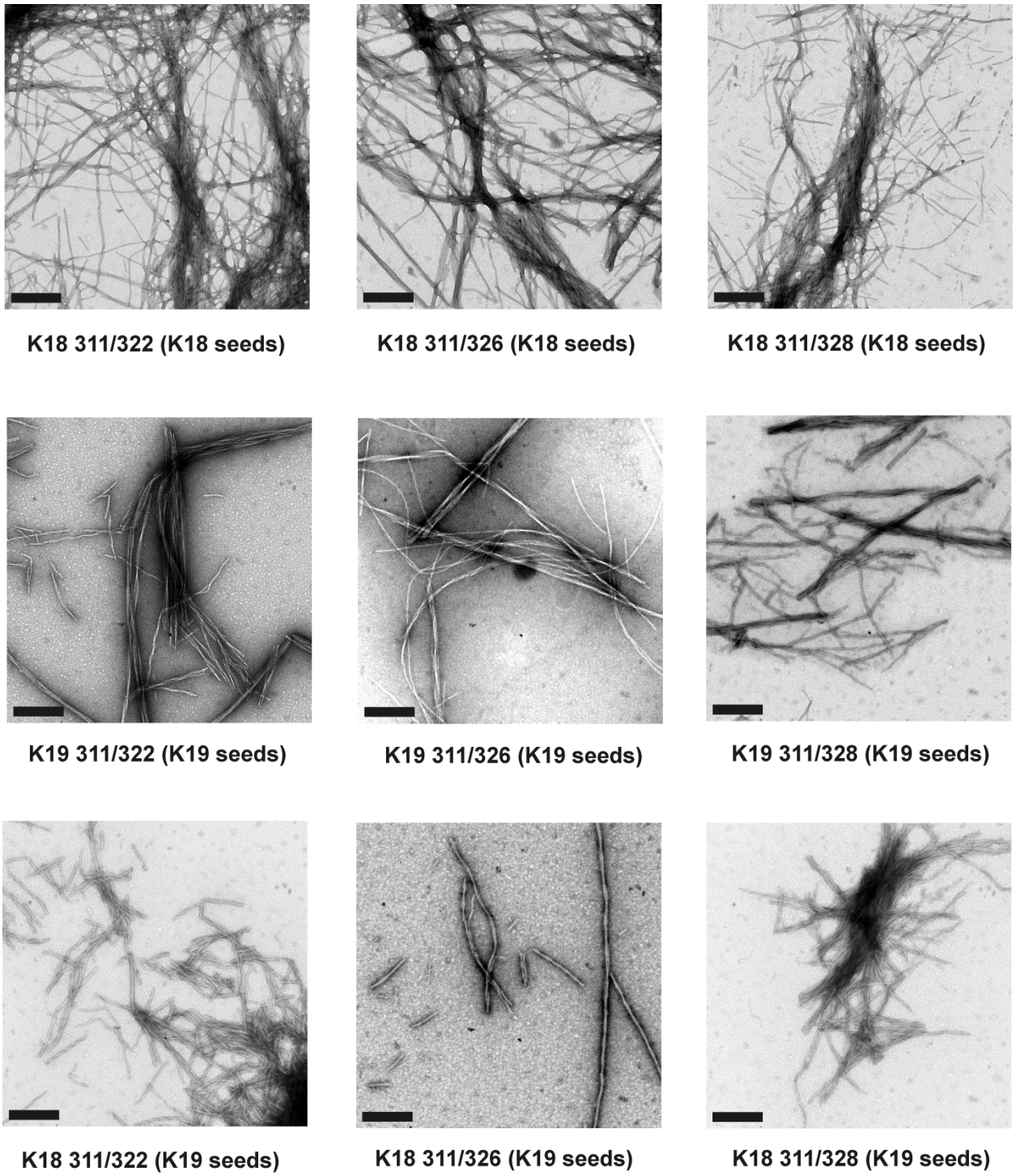

Figure S2. Electron micrographs of doubly spin labeled tau filaments. Filaments (1:50 molar ratio of labeled tau versus wild type tau) were negatively stained with uranyl acetate. Bar = 400 nm.

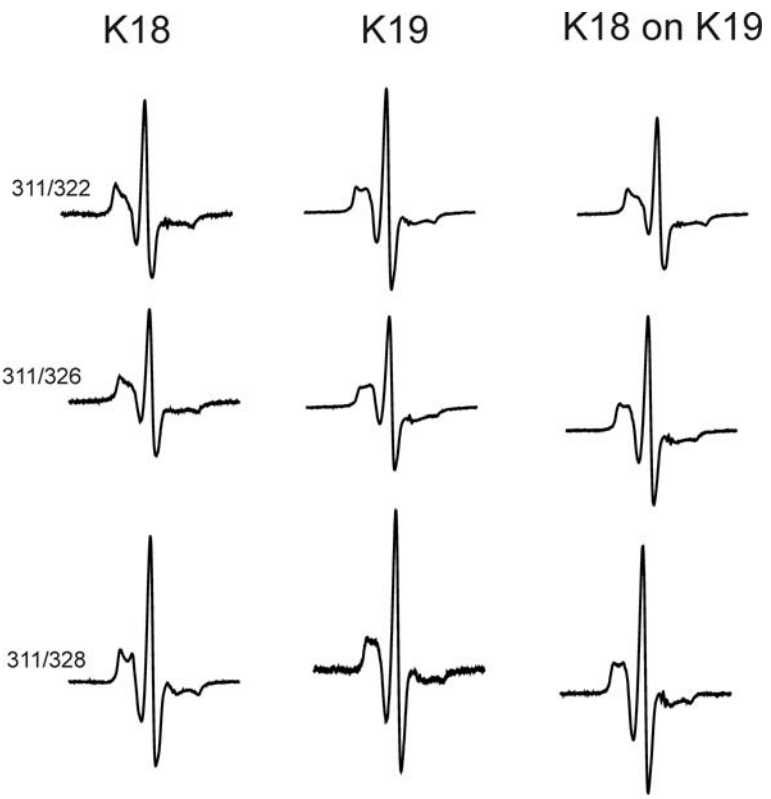

Figure S3. Continuous wave EPR spectra of spin diluted tau filaments (1:50 molar ratio of labeled tau versus wild type tau). The absence of spectral broadening indicates that labeled molecules are not preferentially stacked upon themselves.

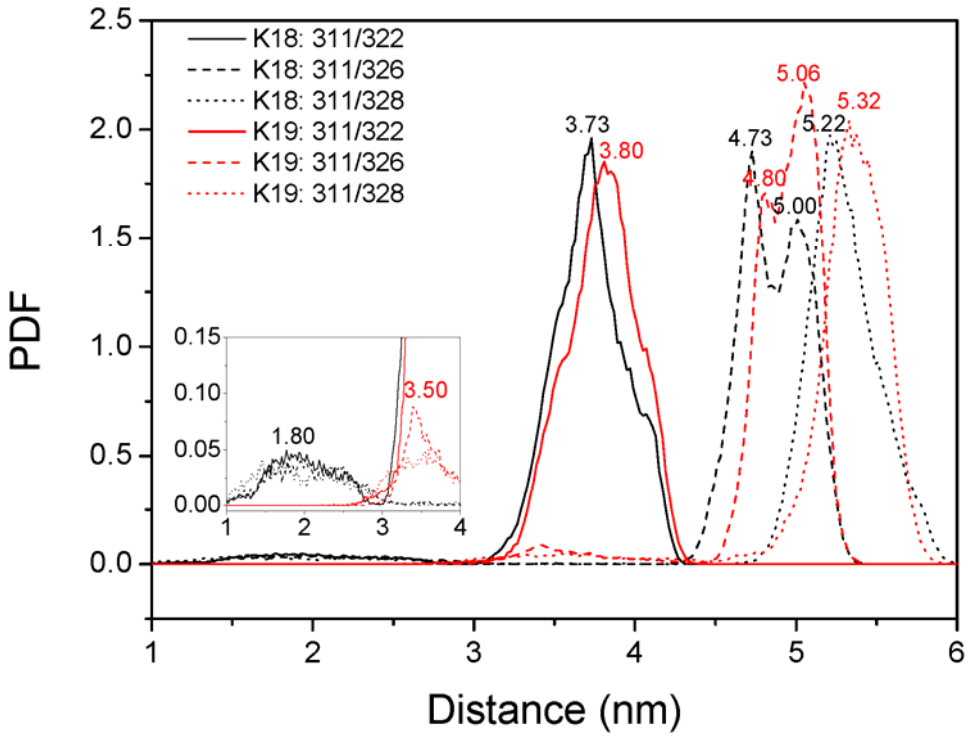

Figure S4. Calculated side chain distributions of K18 and K19 filaments in SL-shape. The distances in the theoretical models are between the terminal non-hydrogen atoms in the side-chains: NZ for K311, SG for C322, Cα for G326 and CD for I328. The distributions for 311/326 and 311/328 suggest that spin labels attached to these sites are separated by >5 nm and thus too long for characterization by DEER.

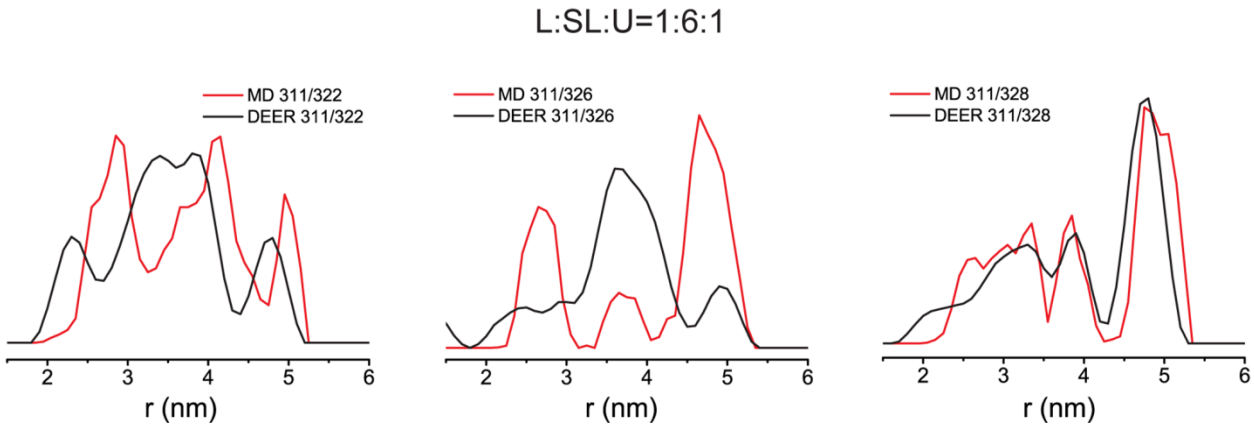

Figure S5. DEER distance distributions of 311/322, 311/326, and 311/328 for K18 are compared with rotamer distributions (MD simulations) with a mixture of L, SL, and U conformers at a 1:6:1 ratio. The fitting is optimized to have maximized overlap for 311/328. The less satisfactory fits for 311/322 and 311/326 indicate that the mutants have different preferences in the selection of seeds.

SUPPLEMENTARY TABLE

		L-shape	SL-shape	T-shape	6:3:1 mixture	1:6:1 mixture
MD-MC	311/322	0.203	0.200	0.446	0.171	0.149
	311/326	0.228	0.366	0.500	0.187	0.289
	311/328	0.248	0.294	0.340	0.169	0.111
MMM	311/322	0.261	0.185	0.419	-	-
	311/326	0.240	0.334	0.431	-	-
	311/328	0.293	0.307	0.359	-	-

Table S1. RMSD of the curve fitting using the MC/MD and MMM methods.