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Supporting Information for:

Distinct lipid membrane-mediated pathways of Tau assembly revealed by single-molecule analysis

Qiong-Qiong Yao[a,b,†], Jitao Wen[a,b,†], Sarah Perrett[a,b] and Si Wu[a,b,*]

[a] National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China.

[b] University of the Chinese Academy of Sciences, 19A Yuquan Road, Shijingshan District, Beijing 100049, China

[†] These authors contributed equally.

E-mail: wusi@ibp.ac.cn

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Methods

DLS experiments

DMPS vesicles were freshly prepared before each experiment. DMPS was diluted to 4 μM, 10 μM, 20 μM, 40 μM and 100 μM. A total volume of 20 μl sample was added to a cuvette and the particle sizes of lipid vesicles under different DMPS concentrations were detected by dynamic light scattering (DLS, Wyatt NanoStar 271-DPN). Three replicates were measured for each sample.

DiO assay

The dissolved DiO was incubated with DMPS vesicles at the molar ratio of 1:1000 for 20 min at room temperature. Wild-type Tau was mixed with 4 μM, 10 μM, 20 μM, 40 μM or 100 μM labeled DMPS vesicles at a final concentration of WT Tau of 0.2 μM. Samples were mixed thoroughly and measured by fluorescence spectrometer (Hitachi F-7000) with excitation at 485 nm and emission from 490 nm to 700 nm. The stained DMPS vesicles without addition of Tau were also detected as a control. For detection of the interactions between DMPS vesicles and Tau, 100 μM DiO-stained DMPS vesicles were incubated with 0.2 μM AF594-labeled Tau. The detection parameters were the same as above.

Binding affinity measurement

A concentration of 2 μM Tau was incubated with different concentrations of DMPS. The change of the intrinsic fluorescence of Tau induced by DMPS was measured using an FLS920 fluorimeter (Edinburgh Instruments, UK). The excitation wavelength was 278 nm and the emission of fluorescence was recorded from 290 nm to 400 nm. The fluorescence maximum at 307 nm was extracted and plotted against the DMPS concentration. The data were fitted to a one-site model similar to that applied in a previous study on K19-DMPS interaction[1].

Circular dichroism (CD) spectroscopy

Far-UV CD spectra of Tau in the absence and presence of DMPS were measured on a Chirascan plus spectrometer (Applied Photophysics, UK). A concentration of 2 μM Tau mixed with 40 μM, 100 μM, 200 μM, 400 μM or 1 mM DMPS was added to the cuvette (1 mm pathlength). The CD spectra were recorded from 190 nm to 260 nm with a scan rate of 30 nm min^{-1} and scan interval of 1 nm. Three repetitions were measured and averaged to obtain the final spectra.

Fluorescence correlation spectroscopy (FCS)

Fluorescence correlation spectroscopy (FCS) experiments were performed on the same instrument as used in smFRET measurements but with slight modifications. In FCS experiments, the fluorescence emission was filtered using an ET525/50 filter (Chroma) and a 50 μm pinhole (Thorlabs), and then split by a 50/50 splitter (Chroma) before being focused onto two avalanche photodiode detectors. The pseudo cross-correlation of the two channels, which aims to remove the effect of the afterpulse, was recorded using a correlation card (Flex02-01D, Correlator.com). The sample preparation using AF488-labeled Tau variants was the same as Scheme 1 described in Fig. 3. The mixture

of 20 μM unlabeled Tau dopant with 10% AF488 labeled Tau was incubated with different concentrations of DMPS and diluted 100-fold before FCS experiments. The collection of each FCS curve lasted 1 hour at Tau:DMPS ratios of 1:100, 1:200 and 1:500. At ratios of 1:20 and 1:50, correlation curves were recorded for 20 s and 60 cycles, due to the heterogeneity of the fluorescence intensity. In the time-dependent FCS experiment, the samples at Tau:DMPS=1:20 or 1:200 were incubated at 37 °C with shaking at 200 rpm. A volume of 1 μL sample was taken and diluted for FCS measurements. The FCS curve of AF488 free dye was also measured as a control to calibrate the focal volume between different experiments. The averaged autocorrelation curves ranging from 10 μs to 1 s were fit to the following functions to obtain the characteristic diffusional time of fluorescent species across the focal volume (τ_D):

$$G(\tau) = A\left(1 + \frac{\tau}{\tau_D}\right)^{-1} \quad (S1)$$

where A is the amplitude of the autocorrelation function.

Reference:

1. G. Künze, P. Barré, H. A. Scheidt, L. Thomas, D. Eliezer and D. Huster, *Biochim Biophys Acta*, 2012, **1818**, 2302-2313.

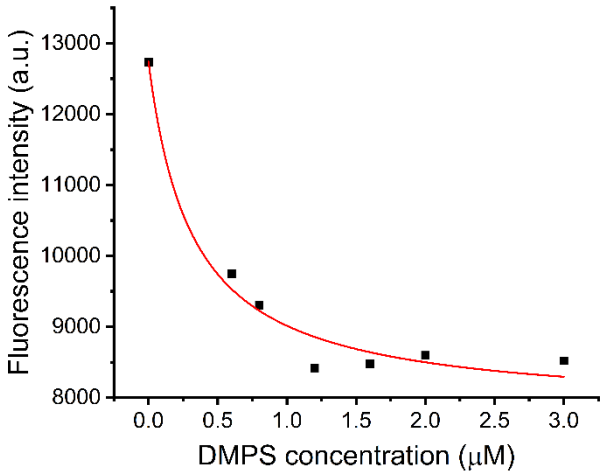

Fig. S1 Binding affinity between full-length Tau and DMPS measured by intrinsic fluorescence of Tau. A concentration of 2 μM Tau was titrated with a series concentration of DMPS. The K_d between Tau and DMPS was determined to be 0.32±0.12 μM by fitting the fluorescence intensity data to a one-site binding model (red line) as described previously[1].

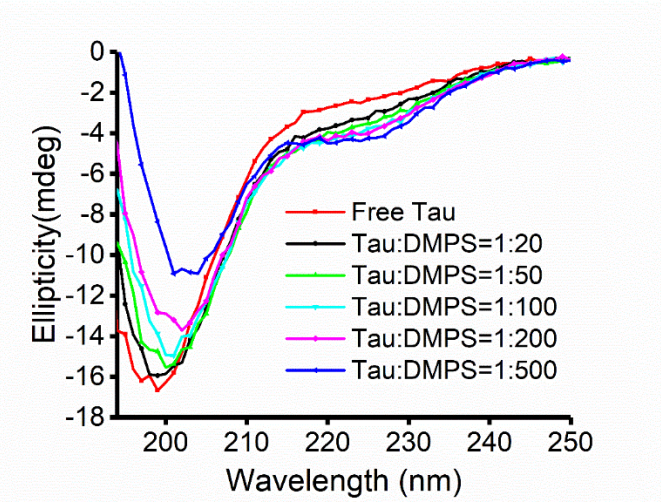

Fig. S2 CD spectra of Tau when interacting with DMPS. The CD spectra of 2 μM Tau (red curve) in the absence and presence of 40 μM DMPS (black curve), 100 μM DMPS (green curve), 200 μM DMPS (cyan curve), 400 μM DMPS (purple curve) and 1 mM DMPS (blue curve) are shown.

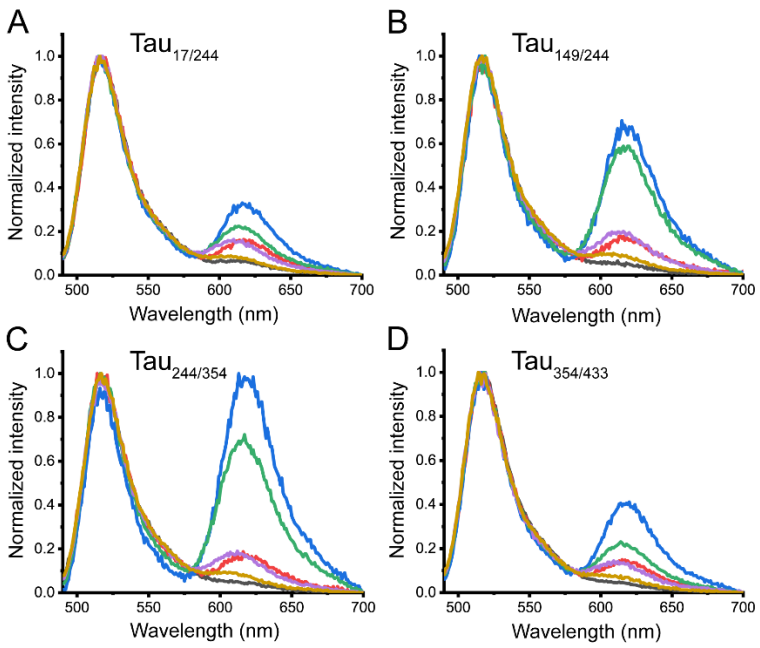

Fig. S3 Ensemble FRET measurements of inter-Tau interactions before and after incubation with different concentrations of DMPS vesicles. (A-D) The FRET spectra of an equimolar mixture of AF488-labeled and AF594-labeled $Tau_{17/244}$ (A), $Tau_{149/244}$ (B), $Tau_{244/354}$ (C) and $Tau_{354/433}$ (D) before (black curves) and after incubation with DMPS vesicles at Tau:DMPS ratios of 1:20 (red), 1:50 (blue), 1:100 (green), 1:200 (purple) and 1:500 (yellow). The concentration of Tau was 0.2 μM including 0.1 μM AF488-Tau and 0.1 μM AF594-Tau.

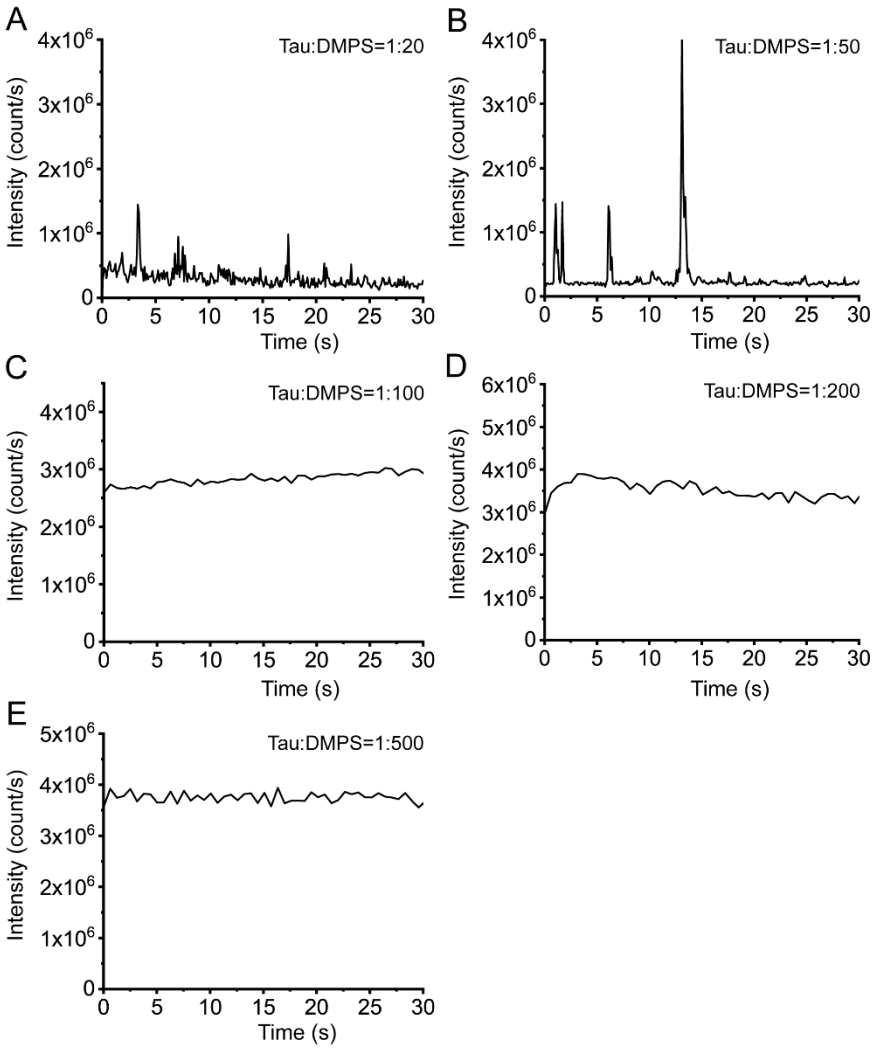

Fig. S4 Fluorescence intensity traces of AF488-Tau in the presence of different concentrations of DMPS vesicles. Examples of fluorescence traces of 0.2 μM Tau including 20 nM AF488-labeled Tau incubated with DMPS vesicles at Tau:DMPS ratios of 1:20 (A), 1:50 (B), 1:100 (C), 1:200 (D) and 1:500 (E). The traces of Tau:DMPS=1:20 and 1:50 show bursts significantly higher than the average intensity level.

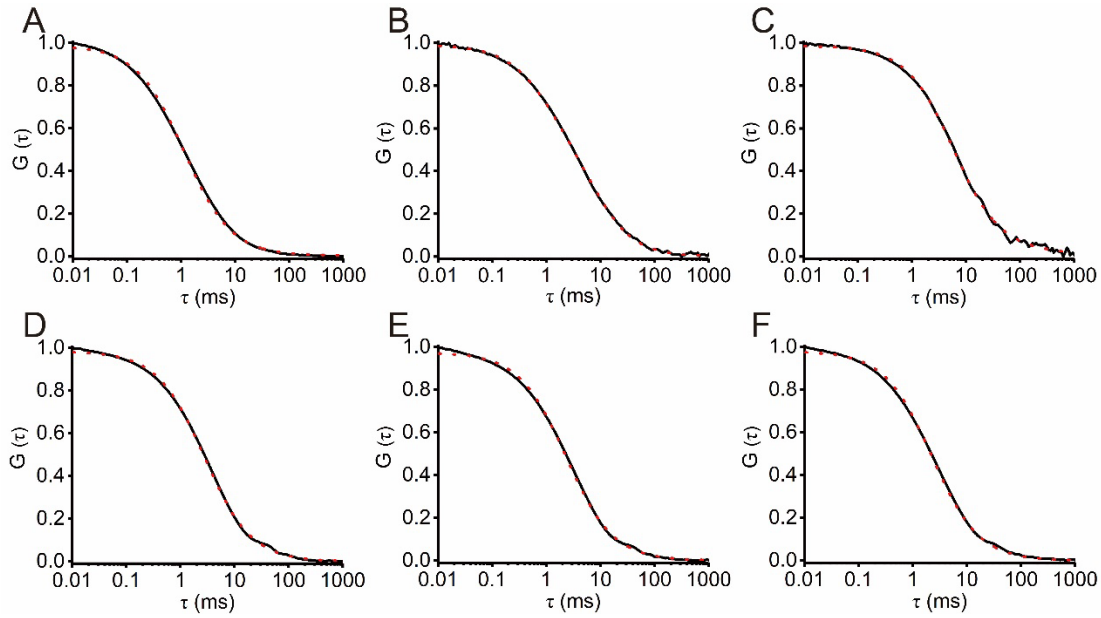

Fig. S5 FCS curves of AF488-Tau$_{244/354}$ incubated with different concentrations of DMPS vesicles. The FCS curves of the samples without DMPS (A), Tau:DMPS=1:20 (B), 1:50 (C), 1:100 (D), 1:200 (E) and 1:500 (F) were fitted to Eq. S1 (red dashed line) to obtained the characteristic diffusion time across the focal volume (listed in Table S3).

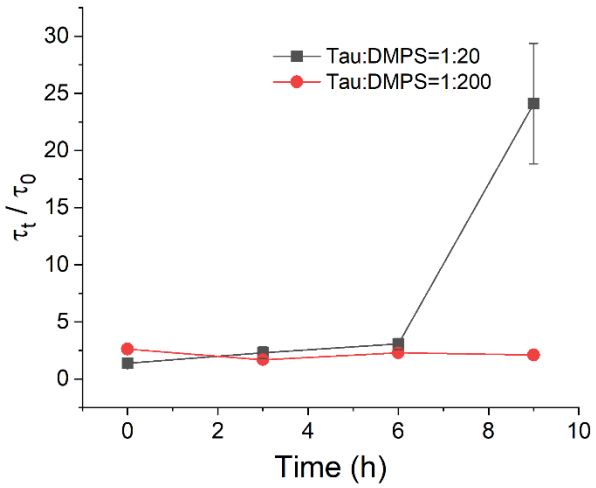

Fig. S6 The time-dependent changes of diffusional properties of AF488-labeled Tau measured by FCS. The samples of Tau:DMPS=1:20 (black squares) and Tau:DMPS=1:200 (red circles) incubated at 37 °C with shaking at 200 rpm were taken at different time points during the aggregation reaction. The ratios of the diffusional time of the Tau samples with DMPS (τ_t) to those of monomeric Tau without DMPS (τ_0) were plotted against time. The error bars are the standard errors for 40 repeated measurements.

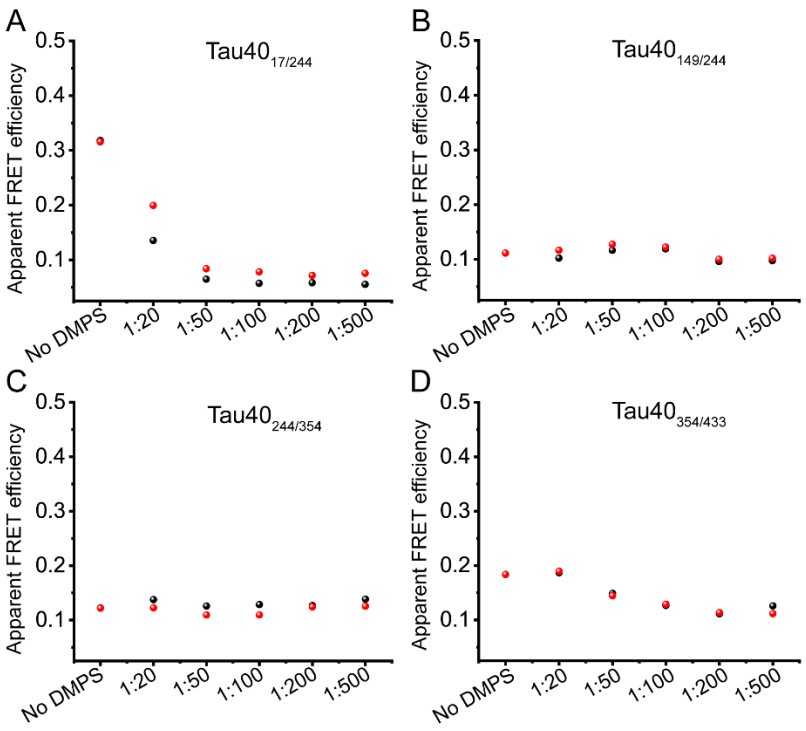

Fig. S7 Conformational changes of Tau after incubation for 40 h measured by ensemble FRET. The FRET spectra of 2 µM Tau protein containing 0.2 µM AF488/AF594-dual labeled Tau was measured before (black circles) and after incubation for 40 h (red circles) in the presence of different concentrations of DMPS vesicles. The apparent FRET efficiencies were calculated using $I_{acceptor}/(I_{donor}+I_{acceptor})$ extracted from the spectra without calibration of the contribution from donor-only labeled species, the direct excitation of acceptor and light leakage from donor to the acceptor detection range.

Table S1 DLS measurement of the diameter DMPS vesicles at different concentrations.

DMPS concentration	4 μM	10 μM	20 μM	40 μM	100 μM	142 μM
Diameter (nm)	43.0±2.5	41.1±0.1	42.1±0.6	40.9±2.0	37.4±0.3	39.4±0.3

Table S2 The distance changes of four regions in Tau protein after interaction with different concentrations of DMPS.

Tau constructs	$Tau_{17/244}$	$Tau_{149/244}$	$Tau_{244/354}$	$Tau_{354/433}$
Without DMPS	46.9±0.7	58.2±1.5	61.7±2.0	50.5±0.8
1:20 (Å)	81.1±3.0	64.9±2.6	67.3±6.4	63.7±4.7
1:50 (Å)	>100	64.3±2.5	68.8±7.2	64.3±4.9
1:100 (Å)	>100	63.7±2.4	69.5±7.7	70.3±8.3
1:200 (Å)	>100	73.1±5.2	76.5±13.9	73.1±5.2
1:500 (Å)	>100	74.1±5.7	>100	77.9±7.8

Table S3 The characteristic diffusion time of the complex formed by Tau and DMPS bilayer measured by FCS.

Tau variants	No DMPS ($\times10^{-3}$ s)	1:20 ($\times10^{-3}$ s)	1:50 ($\times10^{-3}$ s)	1:100 ($\times10^{-3}$ s)	1:200 ($\times10^{-3}$ s)	1:500 ($\times10^{-3}$ s)
$Tau_{17/244}$	1.3±0.1	3.4±0.2	4.5±0.7	3.3±0.2	3.2±0.1	3.0±0.1
$Tau_{149/244}$	1.4±0.1	3.4±0.1	4.6±0.3	3.3±0.1	3.2±0.2	3.2±0.1
$Tau_{244/354}$	1.4±0.2	2.9±0.1	4.6±0.4	3.0±0.1	2.9±0.2	2.5±0.2
$Tau_{354/433}$	1.3±0.1	3.2±0.2	4.4±0.1	3.2±0.1	2.8±0.1	2.8±0.1