Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Blocking Tau Transmission by Biomimetic Graphene Nanoparticles

Runyao Zhu,[1] Kamlesh M. Makwana,[2] Youwen Zhang,[1] Benjamin H. Rajewski,[2] Juan R. Del Valle,[*,2] Yichun Wang[*,1]

[1] *Department of Chemical & Biomolecular Engineering, University of Notre Dame, Indiana 46556, United States.*

[2] *Department of Chemistry & Biochemistry, University of Notre Dame, Indiana 46556, United States.*

* Corresponding author: Yichun Wang (ywang65@nd.edu); Juan R. Del Valle (jdelvalle@nd.edu)

Supporting Figures

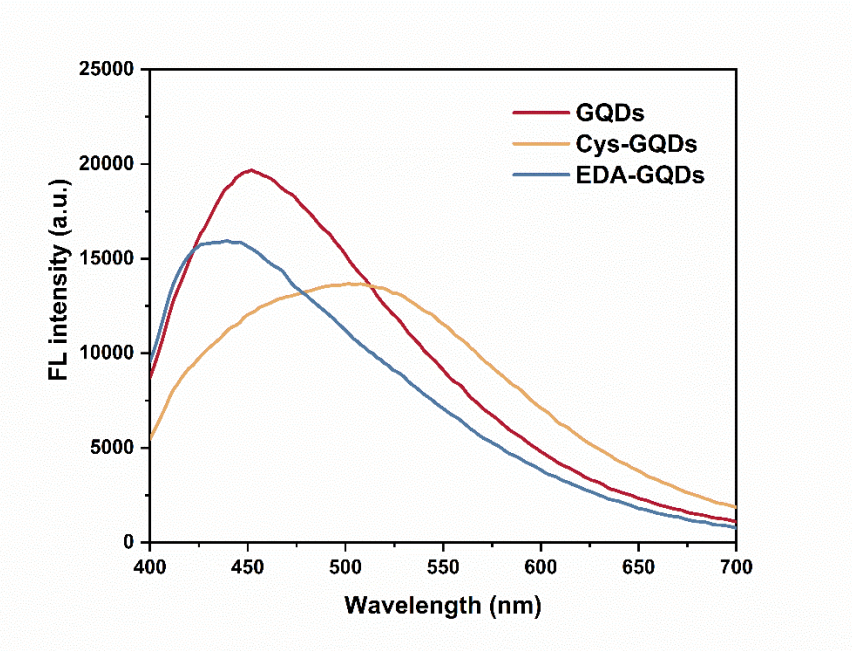

Fig. S1 Fluorescence spectra of graphene quantum dots (GQDs), cysteine (Cys)-GQDs, and ethylenediamine (EDA)-GQDs excited at 365 nm.

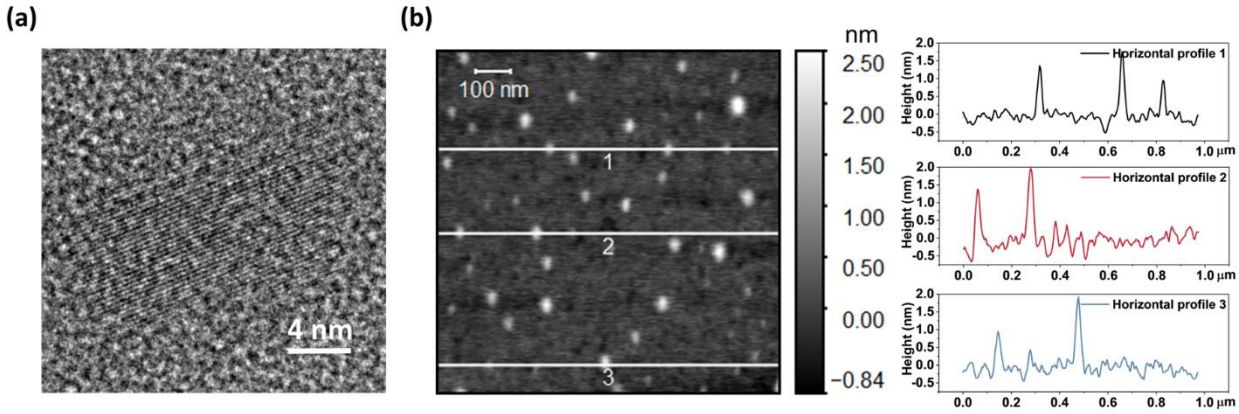

Fig. S2 (a) Transmission electron microscope (TEM) image of GQDs at high magnification (310 kx). It indicates high crystallinity of the GQDs, with a lattice parameter of 0.24 nm, lattice fringes of graphene. Scale bar: 4 nm. (b) Atomic force microscopy (AFM) image of GQDs and height profiles of GQDs along the horizontal lines 1, 2, and 3. The heights of GQDs are between 0.5 and 2 nm. Scale bar: 100 nm.

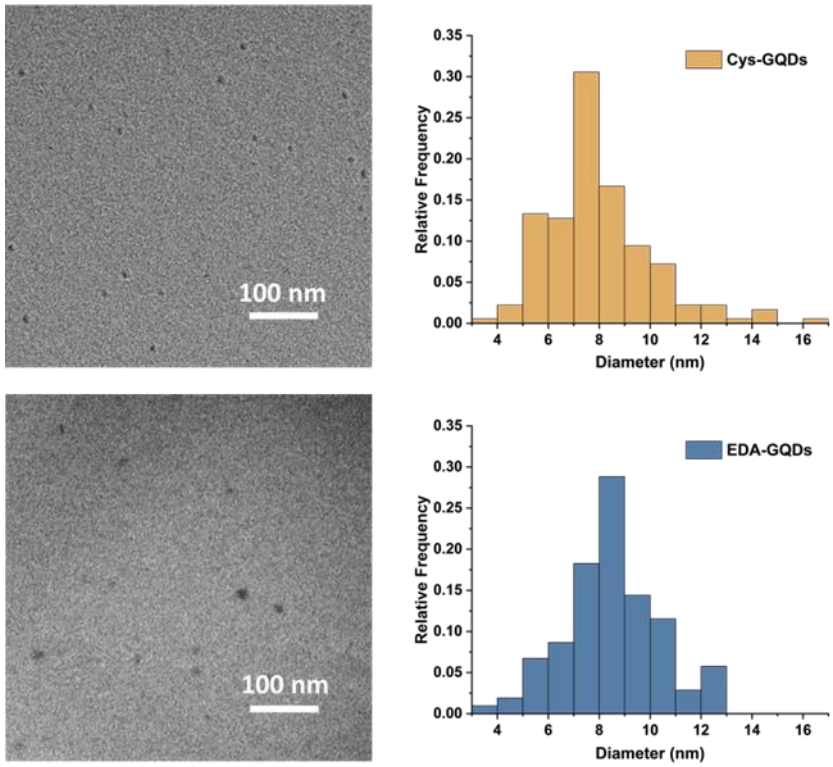

Fig. S3 TEM images and size distributions of Cys-GQDs and EDA-GQDs. The average diameters of Cys-GQDs and EDA-GQDs are 8.0 ± 2.0 nm and 8.5 ± 1.8 nm, respectively. (Scale bar: 100 nm).

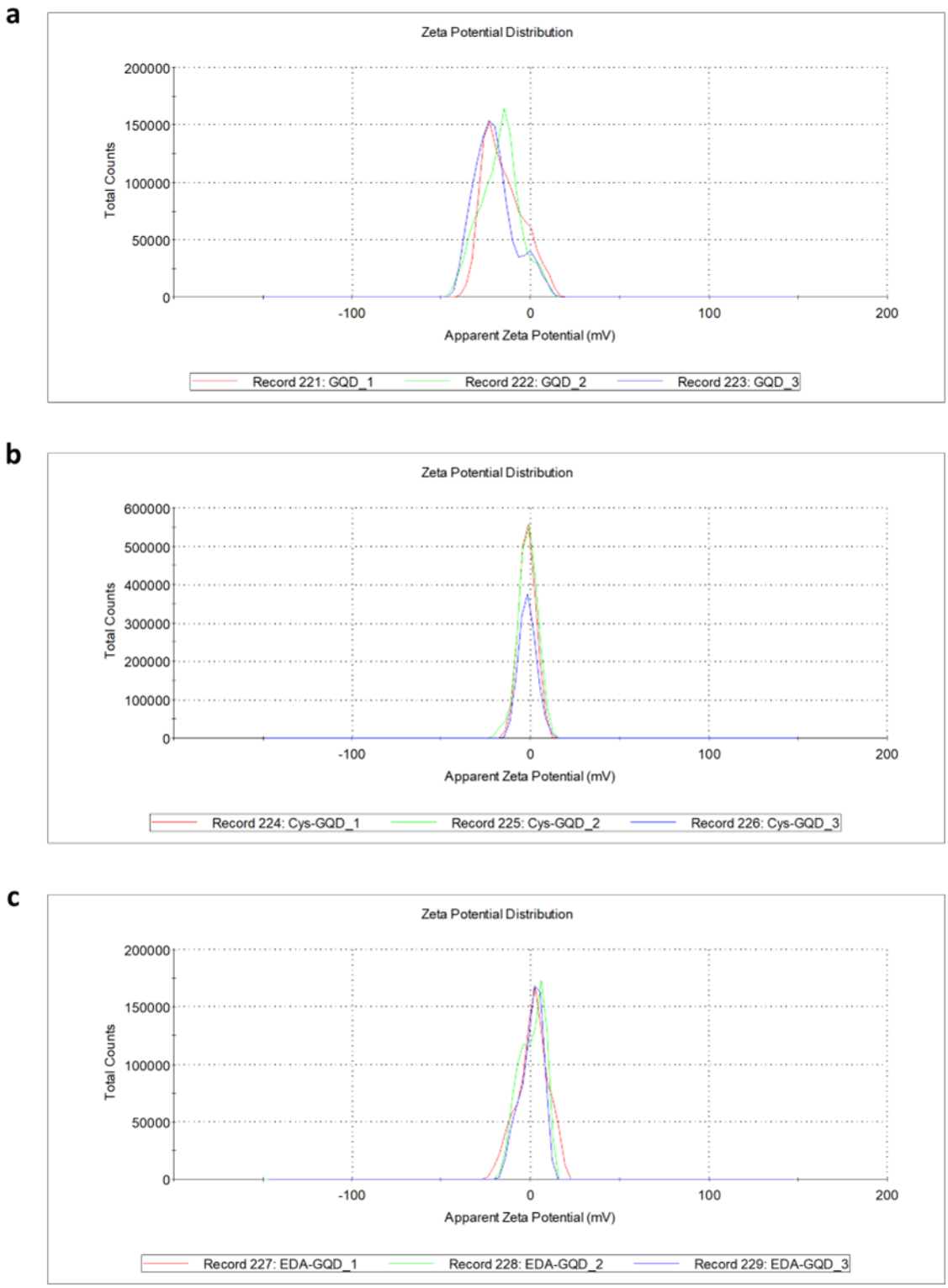

Fig. S4 Original data of zeta potentials of GQD, Cys-GQDs, and EDA-GQDs.

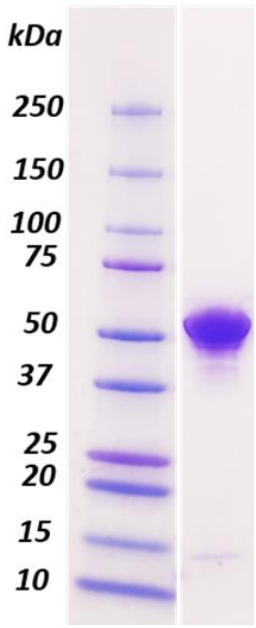

Fig. S5 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS/PAGE, Coomassie blue stain) of final purified tau_{P301L} protein.

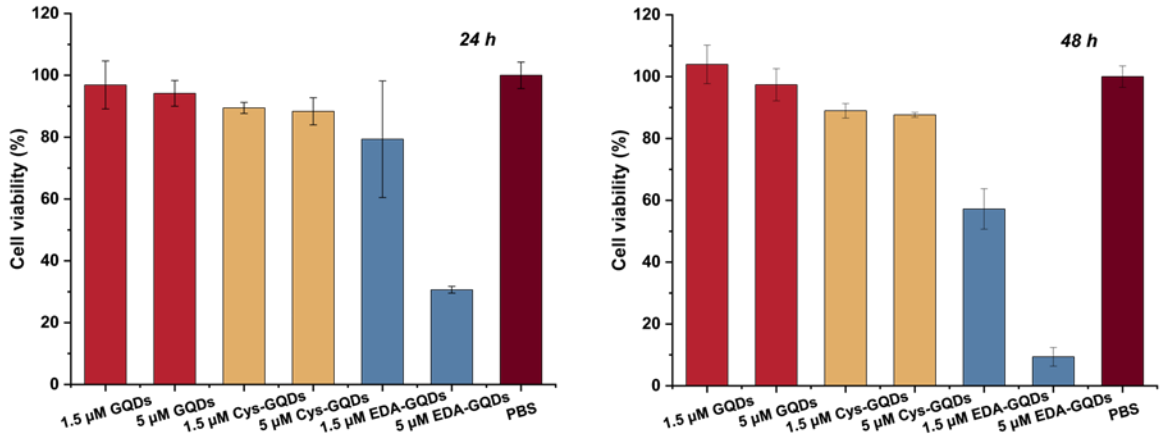

Fig. S6 The cell viability of SH-SY5Y human neuroblastoma cells after incubation with 1.5 μM and 5 μM of GQDs, Cys-GQDs, and EDA-GQDs for 24 and 48 h, tested by CCK-8 assay.

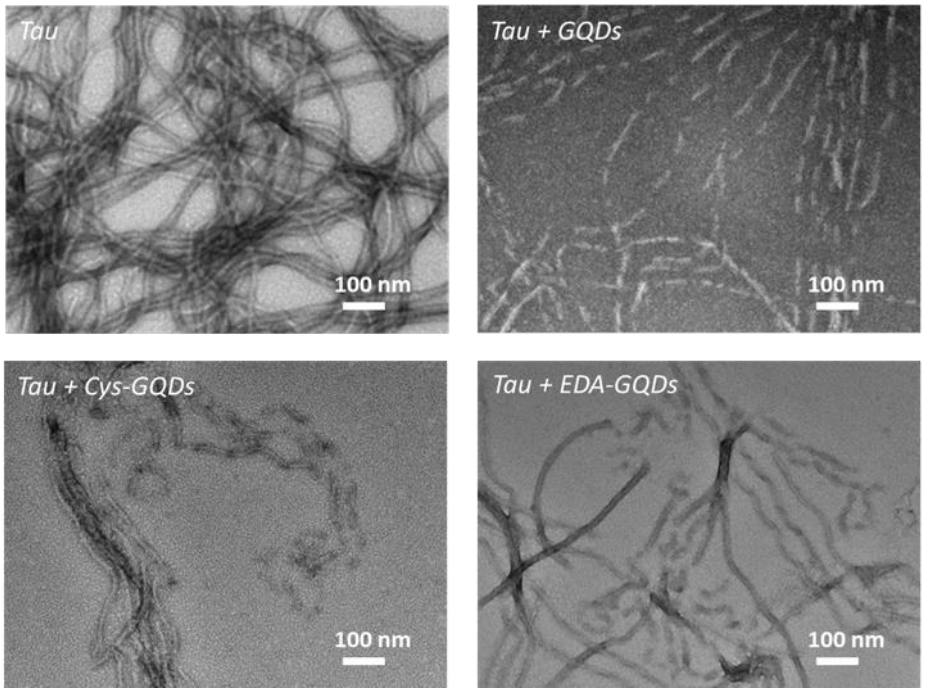

Fig. S7 TEM images of tau fibers (5 μM) incubated with/without 6.25 μM GQDs or functionalized GQDs for 2 days. (Scale bars: 100 nm)

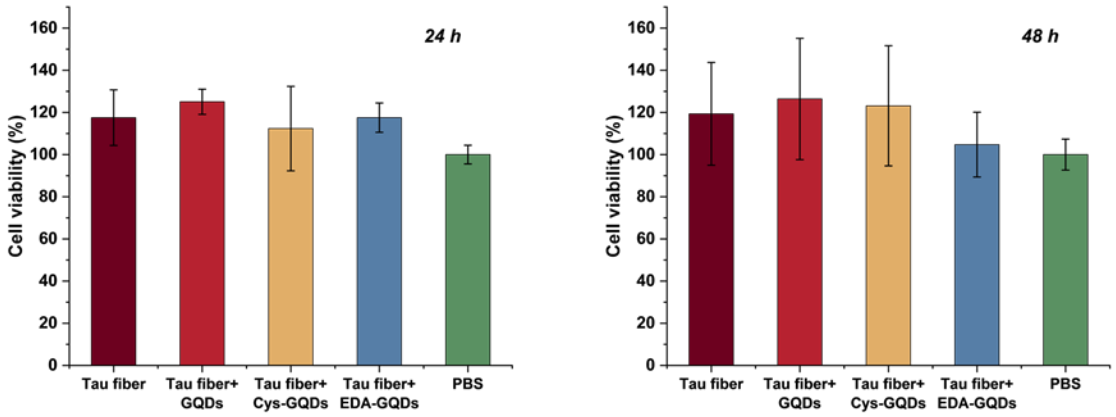

Fig. S8 10 μM of tau fibers and 12.5 μM of GQDs or functionalized GQDs were incubated for 7 days. The cell viability of SH-SY5Y human neuroblastoma cells after incubation with the mixture of 1 μM tau fibers and 1.25 μM GQDs or functionalized GQDs for 24 and 48 h, tested by CCK-8 assay.

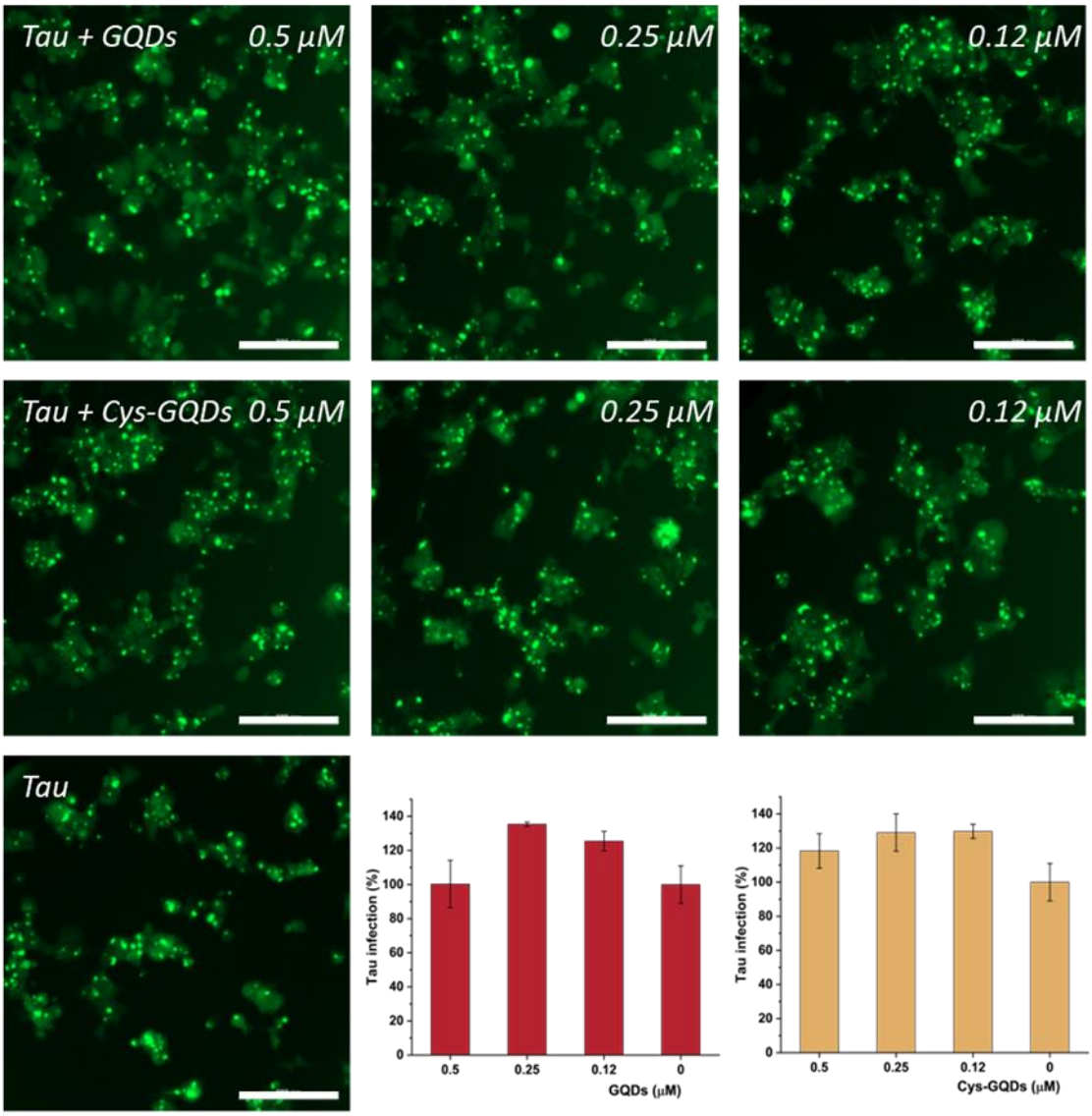

Fig. S9 Preformed tau fibrils (0.19 μM) were mixed with GQDs or Cys-GQDs and incubated for 0 h. Then, the mixture of tau fibrils and GQDs or Cys-GQDs was added to HEK293 cells stably expressing tau-RD (P301L/V337M)-YFP and incubated for 48 h. Representative images of cells were taken at 20× magnification under FITC channel (ex: 469 nm/em: 525 nm). The green puncta with high fluorescence represented the aggregation of tau in cells induced by exogenous tau fibers. Scale bar: 200 μm. Tau infection (%) in the bar graph shows the number of intracellular fluorescent puncta relative to control infection wells lacking the inhibitors.

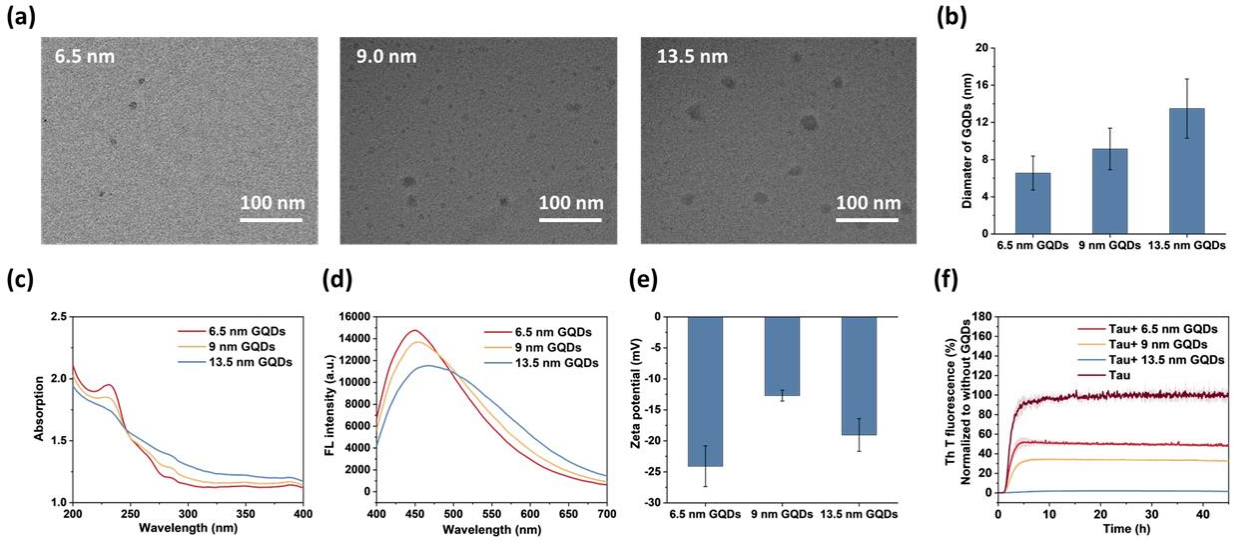

Fig. S10 The size effect of GQDs on the tau aggregation. (a) GQDs were separated using centrifuge filters with 3 KDa and 10 KDa molecular weight cutoffs. TEM images of GQDs with average diameters of 6.5, 9.0, and 13.5 nm. (Scale bar: 100 nm) (b) Size distribution of GQDs with average diameters of 6.5 nm, 9.0 nm, and 13.5 nm. (c) Absorption spectra, (d) fluorescence spectra (excited at 365 nm), and (e) zeta potentials of 1.25 μM GQDs with three different diameters. (f) Tau_{P301L} aggregation was inhibited with GQDs of different sizes with a concentration of 1.25 μM monitored by thioflavin T (ThT) fluorescence assay.

Supporting Tables

Table S1 The apparent elongation rate constants (k_{app}) and lag times (t_{lag}) of tau aggregation incubated with/without 6.25 μM of GQDs or functionalized GQDs.

	Tau aggregation (%)	k_{app} (h^{-1})	t_{lag} (h)
Tau	100 ± 9.27	0.76 ± 0.008	1.84 ± 0.011
Tau + GQDs	2.20 ± 0.23	0.70 ± 0.039	3.46 ± 0.063
Tau + Cys-GQDs	2.01 ± 0.082	0.38 ± 0.021	3.88 ± 0.096
Tau + EDA-GQDs	11.2 ± 0.10	0.95 ± 0.034	0.95 ± 0.030

Table S2 Average length and density of tau fibers were obtained from TEM images of tau protein incubated with/without GQDs or functionalized GQDs for 4 days. The length distribution is close to an exponential distribution, and thus its standard deviation is close to the mean value.

	Average length of tau fibers (nm)	Average density (fibers/μm^2)	Average length of tau fibers in the unit area (nm/μm^2)
Tau+ GQDs	277 ± 302	1.27 ± 0.48	371
Tau+ Cys-GQDs	333 ± 328	1.66 ± 0.98	506
Tau+ EDA-GQDs	571 ± 707	3.76 ± 0.84	2033
Tau	1623 ± 1255	4.53 ± 2.08	7352

Table S3 Amount of tau fibers per TEM mesh was obtained from TEM images of tau fibers incubated with/without GQDs or functionalized GQDs for 2 days.

	Amount of tau fibers per TEM mesh (μm^2)*
Tau fibers + GQDs	46.2 ± 1.3
Tau fibers + Cys-GQDs	7.6 ± 0.13
Tau fibers + EDA-GQDs	33.4 ± 0.3
Tau fibers	69.3 ± 0.9

$$*Amount\ of\ tau\ fibers\ per\ TEM\ mesh = \sum\left(\frac{Area\ of\ tau\ fibers\ in\ each\ mesh}{The\ number\ of\ mesh\ in\ one\ TEM\ grid\ (200)} \times \frac{The\ grey\ value\ of\ background - The\ mean\ grey\ value\ of\ tau\ fibers\ in\ each\ mesh}{The\ grey\ value\ of\ background}\right)$$