Electronic Supplementary Material (ESI) for Biomaterials Science

Supporting Information

N-acetyl-L-cysteine capped quantum dots offer neuronal cell protection by inhibiting beta (1-40) amyloid fibrillation

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Experimental

Synthesis and characterization of NAC-capped CdTe QDs

All chemicals were purchased from Sigma unless otherwise mentioned. Mono-dispersed CdTe-core QDs were synthesized according to the scheme reported previously with minor modifications.[S1] Briefly, 0.2 mmol of tellurium powder and 0.5 mmol of $NaBH_4$ were loaded in a 25 mL two-necked flask with N_2 flow. Then 5.0 mL of distilled water was added. The reaction mixture was heated at 80 ˚C for 30 min under N_2 flow to get a deep red clear solution. (Stored at room temperature under the protection of N_2 for further use) Then, 0.2 mmol of $CdCl_2$ solution and 0.34 mmol of *N*-Acetyl-L-cysteine (NAC) solution were mixed to make a 40 mL solution, and pH of the solution was adjusted to 12.0 by adding 1.0 M NaOH solution with stirring. The solution was transferred into a three-necked flask with N_2 flow. Under stirring, freshly prepared NaHTe solution (0.4 mmol) was added to the Cd precursor solution at room temperature. Then the reaction mixture was heated to reflux (100 ˚C) for 5 min and removed from heat afterward. The solution was purified by centrifugation and dried under vacuum; the dried CdTe QDs powder was kept in the dark for future use. The UV-visible absorption and fluorescence spectra were obtained by Cary 300 UV-visible spectrophotometer (Varian, Inc., USA) and Perkin Elmer LS-50B fluorescence spectrometer (Buckinghamshire, U.K.) respectively. The concentration of the synthetic NAC-QDs solution was calculated as reported.[S2]

Sample preparation

Beta (1-40) amyloid was purchased from Invitrogen and used without further purification. Stock $A\beta_{1\text{-}40}$ solution was prepared by dissolving in 400 µL 0.02 % ammonium solution and stored at –20 ˚C until usage. The fibril seeds solution was prepared as: 4 µL stock solution was diluted to 40 µL with phosphate buffer (pH7.4). After sonication for 10 s, the reaction mixture was incubated in a water bath at 37 ˚C for 24 hours. Prior to each experiment, sample of the fibril seeds was sonicated for 5 s thrice.

Aβ monomer ($A\beta_{1\text{-}40}M$) solution was prepared by diluting stock Aβ monomer solution to 50 µM with buffer. Mixture of Aβ monomer and fibril seeds ($A\beta_{1\text{-}40}MS$) solution was prepared by diluting stock Aβ monomer solution and fibril seeds solution to 50 µM and 1.8 µM respectively with buffer. The seeding fibril solution ($A\beta_{1\text{-}40}F$) contained same composition as the ($A\beta_{1\text{-}40}MS$) solution, except that it was incubated at 37 ˚C for 1 hour before performing MTT/ROS assay.

Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium (Gibco) containing 10 % fetal bovine serum (FBS) (Gibco) in a 5 % CO_2 humidified environment at 37 ˚C.

Inhibition effect of NAC-QDs on $A\beta_{1\text{-}40}$ fibrillation

10 nM of NAC-QDs solution was mixed with 50 µM of the mixture of $A\beta_{1\text{-}40}$ monomer and fibril seeds in 0.1 M phosphate buffer, the resultant mixture was incubated at 37 ˚C for 1 hour. The resulted $A\beta_{1\text{-}40}$ fibril was imaged by TIRFM-EMCCD system. Length of 100 fibrils was analyzed by the free-domain software *ImageJ*. The distribution of fibril length was displayed by a histogram with the software *OriginPro 8*.

Cell viability assay

Cells were plated at a density of 1 x 10^4 cells per well on 96-well plates in 200 µL medium. After 24 hr, the culture medium was replaced by fresh medium containing 0.2 % serum. In the QDs cytotoxicity experiment, 40 µL quantum dots solution was added into each well with a final concentration of 10^{-6} M to 10^{-9} M. In MTT assay, 40 µL quantum dots solutions in a final concentration 0.2 x 10^{-8} M with (1) $A\beta_{1-40}M$ solution, (2) $A\beta_{1-40}F$ solution and (3) $A\beta_{1-40}MS$ solution (final concentration of $A\beta_{1-40}$ was 10 µM) were added into each well respectively. Control experiment of the MTT assay was performed in the absence of QDs solution addition. The plates were incubated at 37 ˚C for 2, 6 and 24 hours, respectively. The medium of each well was replaced by 200 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) (5mg/mL) solution and further incubated for 3 hours at 37 ˚C. The MTT solution was aspirated off and then 100 µL of DMSO (Sigma-Aldrich) was added to each well to dissolve the formazan crystals. The plates were agitated on a plate shaker for 15 minutes and the absorbance was measured at 540 nm with the reference set to 690 nm using absorbance microplate reader (BioTek, ELx800^{TM}).

Assay of intracellular ROS levels

The oxygen stress in living cell model was evaluated by a dichlorofluorescein (DCF) assay. After plating and treatment, the culture medium of each well was replaced by 200 µL of 2',7'-dichlorofluorescin diacetate (DCFH-DA) (100 µM) solution and incubated for 30 minutes in 5 % CO_2 dark environment at 37 ˚C. Then the DCFH-DA solution was removed and the cells were washed with PBS twice. Then all wells were filled with 200 µL culture medium containing 0.2 % serum. In the ROS assay, 40 µL quantum dots solutions of a final concentration 0.2 x 10^{-8} M with (1) $A\beta_{1-40}M$ solution, (2) $A\beta_{1-40}F$ solution and (3) $A\beta_{1-40}MS$ solution (final concentration of $A\beta_{1-40}$ was 10 µM) were added into each wells respectively. Control experiment of the MTT assay was performed in the absence of QDs solution. The plates were incubated at 37 ˚C for 2, 6 and 24 hours, respectively. After incubation, the microplate reader (BioTek, ELx800^{TM}) was used to measure the fluorescence intensity of the samples with excitation at 485 nm and the emission at 530 nm

Localization of Congo Red-labeled fibrils in SH-SY5Y cells

To investigate the distribution of fibrils in cell environment, SH-SY5Y cells were incubated with $A\beta_{1-40}F$ solution in a final concentration of 10 µM. 20 µM of Congo red solution was then added right before imaging. The cells were visualized by Confocal Laser Scanning Microscope (Olympus Fluoview FV1000).

Statistical analysis

All results were determined in three independent trials and presented as means ± SEM. All data was analyzed by One-Way ANOVA with PASW *Statistics 18* software. Significant was set as $p < 0.05$.

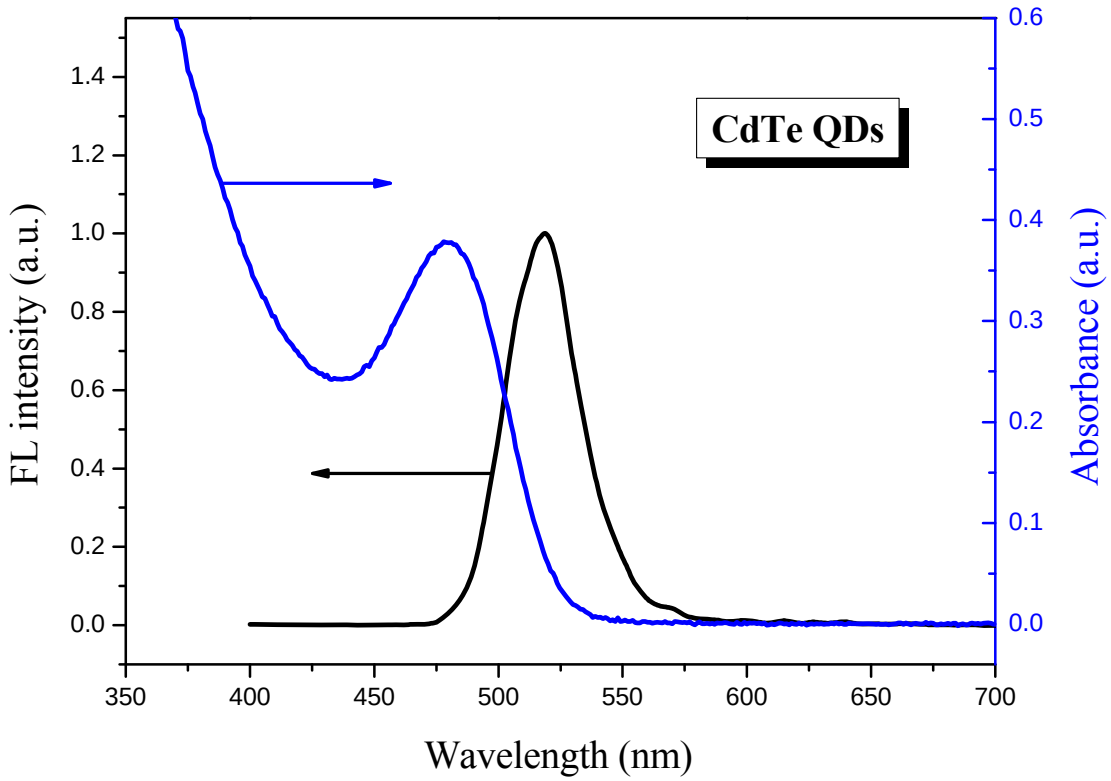

Figure S1. Emission spectra and corresponding UV-vis spectra of NAC-CdTe QDs.

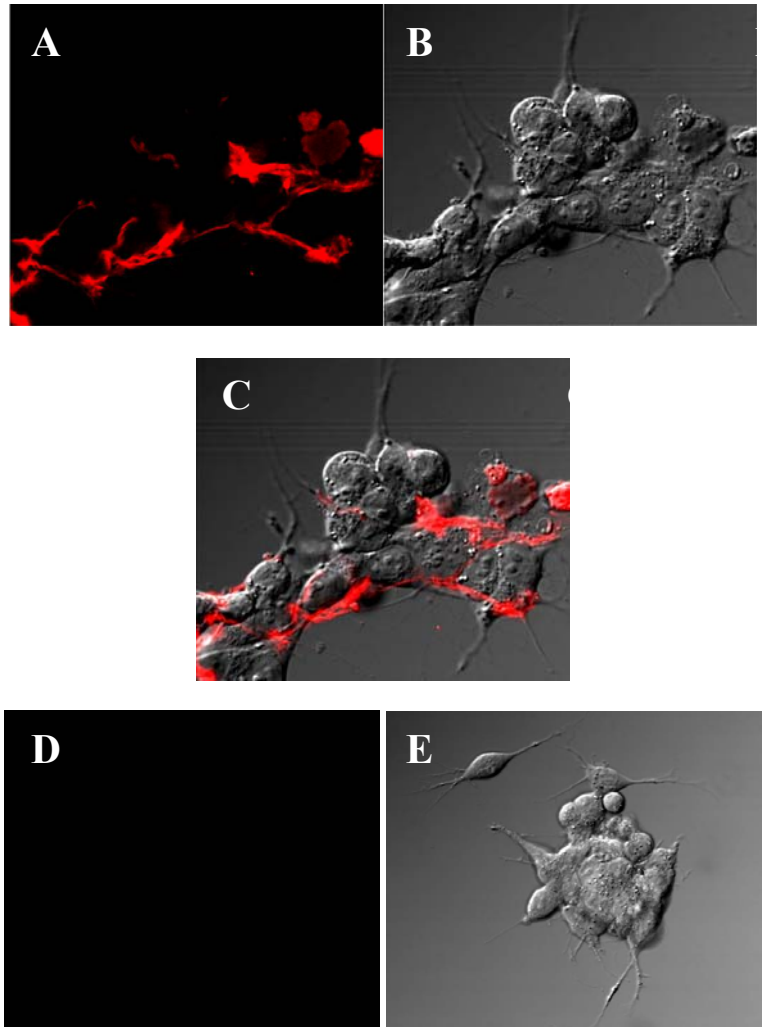

Figure S2. Localization of $A\beta_{1\text{-}40}F$ in SH-SY5Y cell culture was captured and shown in the images. Congo red was used to visualize the location of $A\beta_{1\text{-}40}F$ in cells. After incubating with $A\beta_{1\text{-}40}F$ solution for 24 hours, Congo red was added and fluorescence images were captured. $A\beta_{1\text{-}40}F$ located and accumulated outside the cell membrane. (A) Fluorescence images of SH-SY5Y cells and (B) DIC images of SH-SY5Y cells were captured. (C) Merging of the Fluorescence and DIC image of SH-SY5Y cells was shown and indicated the localization of the $A\beta_{1\text{-}40}F$ in cells. (D) and (E) shown the images captured from SH-SY5Y cells without any treatment and regarded as the control in the present experiment. (D) Fluorescence image of SH-SY5Y cells and (E) DIC images of SH-SY5Y cells were captured.

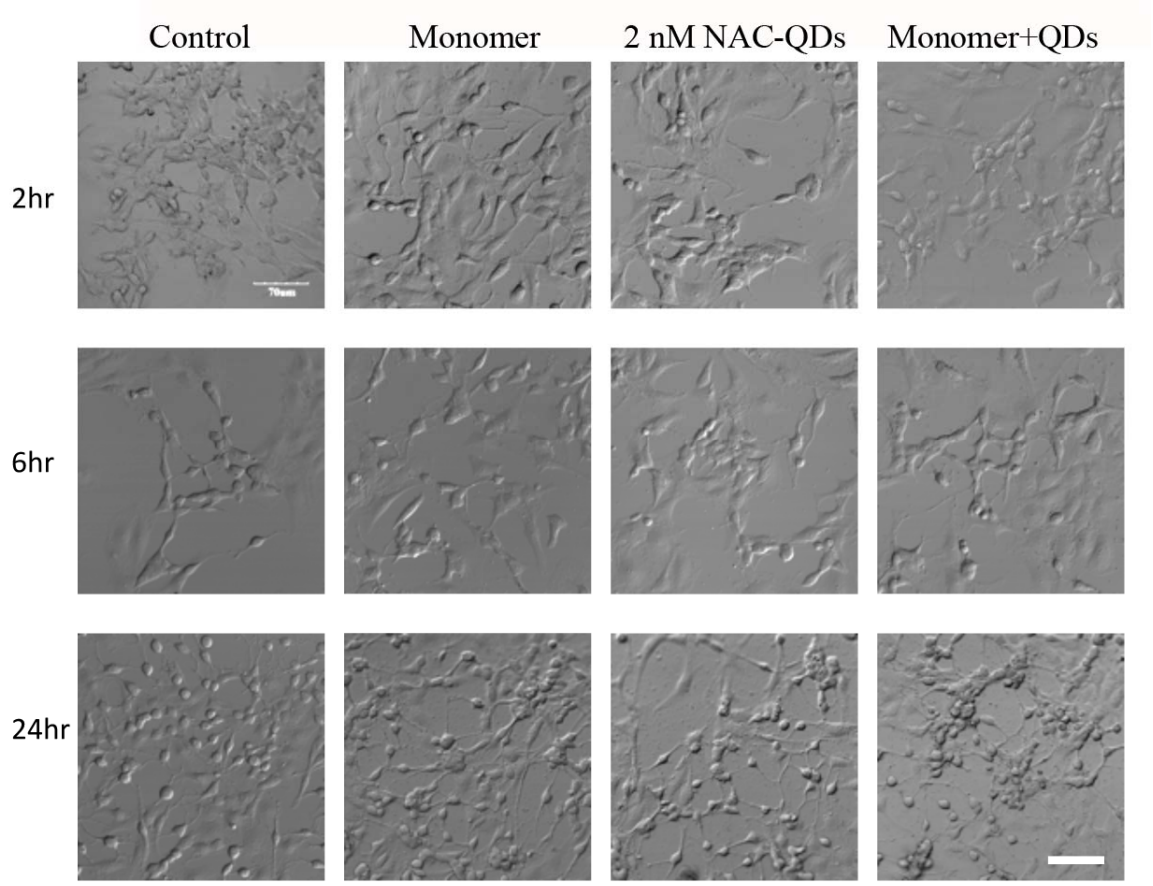

Figure S3. Differential Interference Contrast (DIC) images of SH-SY5Y cells incubated in the presence of 10 μM $A\beta_{1\text{-}40}$ monomer, 2 nM NAC-QDs, and $A\beta_{1\text{-}40}$ monomer with NAC-QDs for 2 hour, 6 hour and 24 hours. No obvious difference in morphology of the neural cells was observed in the addition of $A\beta_{1\text{-}40}$ or quantum dots.

References

S1: Zou, L.; Gu, Z. Y.; Zhang, N.; Zhang, Y. L.; Fang, Z.; Zhu, W. H.; Zhong, X. H., *J. Mater. Chem.* **2008,** 18, 2807-2815.

S2. Yu, W. W.; Qu, L. H.; Guo, W. Z.; Peng, X. G., *Chem. Mat.* **2003,** 15, 2854-2860.