Supporting Information

for

Photodynamic micelles for amyloid β degradation and aggregation inhibition

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Materials and chemicals

Methoxy polyethylene glycol 5000 ($mPEG_{113}$-OH), copper bromide (CuBr, 99.99%), N,N,N',N",N"-Pentamethyldiethylenetriamine (PMDETA), 2-hydroxyethyl methacrylate (HEMA) and 2-(diisopropylamino) ethyl methacrylate (DPA), Tanshinone I (TAS), thioflavin T (ThT) and 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) were all purchased from Sigma-Aldrich (Shanghai, China). Photosensitizer chlorin e6 (Ce6) was purchased from Frontier Scientific Inc. (Logan, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), 4-dimethylaminopyridine (DMAP) and N-hydroxysuccinimide (NHS) were all ordered from TCI Chemical. Inc. (Shanghai, China). Amyloid-β fragment 1-40 (Aβ) was purchased from American peptide (CA, USA). Rabbit anti-oligomer (A11) polyclonal antibody was purchased from Thermo Fisher Scientific Inc. (California, USA). Alexa Fluor® 790 goat anti-rabbit antibody was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Ultracentrifugation tubes with molecular weight cut-off (MWCO) of 100 kDa or 3 kDa were obtained from Millipore (Shanghai, China). All chemicals are of analytical grade except those noted otherwise and used directly without further purification. Milli-Q water was used throughout the study.

Apparatus

The UV-Vis spectra were measured by a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). Fluorescence spectra were measured with a Cary Eclipse spectrofluorophotometer (Agilent Technologies, Palo Alto, CA,

USA). TEM images were collected with JEM-2100 microscopy (JEOL Ltd., Tokyo, Japan). CD spectra were obtained by J-815 CD spectrometer with a 1 mm path length standard cell at 25 °C (JASCO, Japan).

Polymer synthesis and Ce6 conjugation

$mPEG_{113}$-*b*-$P(DPA_{50}$-*r*-$HEMA_6)$ diblock copolymer bearing 6 pendant hydroxyl groups was synthesized by atomic transfer radical polymerization (ATRP) method, and it was then covalently labeled with Ce6 according to the procedure we reported previously.[1] The reaction product (abbreviated as PEG-*b*-PDPA-Ce6) was purified by dialyzing against dimethyl sulfoxide (DMSO) and water, respectively. Any trace Ce6 dye was removed by multiple ultrafiltration (Millipore, MWCO 100 kDa). The complete removal of free Ce6 from PEG-*b*-PDPA-Ce6 diblock copolymer was verified by high performance liquid chromatography (HPLC) examination using acetonitrile as the eluent. The Ce6 conjugation percentage was 6.6 wt% as determined by UV-Vis spectroscopic examination. Each polymer chain was conjugated with three Ce6 molecules accordingly.

Preparation of Ce6-conjugated micelles

Ce6-conjugated micelles were prepared by solvent evaporation method as described previously.[2] Briefly, 9.7 mg of PEG-*b*-PDPA and 0.3 mg of PEG-*b*-PDPA-Ce6 were dissolved in 200 μL of tetrahydrofuran (THF) in a glass vial. The solution was added into 1 mL water under ultrasonication. The resulting micelle solution was dialyzed against water to remove THF.

To prepare TAS-loaded Ce6 micelles, 9.7 mg of PEG-*b*-PDPA, 0.3 mg of PEG-*b*-

PDPA-Ce6 and certain amount of TAS (*e.g.*, 0.5 wt%, 2 wt% or 4 wt% of polymer weight) were dissolved in 200 μL of THF in a glass vial. Then the solution was added into 1 mL of DI water under constant sonication. The trace THF was removed by ultrafiltration (MWCO 3.0 kDa). The concentrated micelle solution was diluted into 0.5 mg/mL by Milli-Q water. The resulting micelle solution was dialyzed against water to obtain TAS-loaded Ce6 micelles.

Determination of TAS loading

To determine TAS encapsulation efficiency, the TAS-loaded Ce6 micelles were dissolved with THF. The absorbance of the solution was measured by UV-Vis spectrophotometer and TAS concentration was determined using standard curve method (Abs_{276nm}). The TAS encapsulation efficiency (the percentage of TAS encapsulated inside the micelles) was then calculated and included in Table S1.

To determine the TAS release efficiency, the TAS-loaded micelles (0.5 mg/mL) were incubated in 10 mM Tris-HCl buffer (pH 7.4) at 37^{o}C for 48 h, then free TAS was removed by ultrafiltration (MWCO 3.0 kDa). The concentrate was diluted with THF and determined by UV-Vis measurement. The TAS release efficiency (the percentage of TAS released from the micelles) was then calculated and included in Table S1.

ROS generation

Ce6 micelle-induced ROS generation was measured with fluorescence spectrophotometer using ABDA as a ROS indicator. Typically, Ce6 micelles solution were mixed with 70 μM of ABDA dye, then the solution was irradiated with a 655 nm

laser at photo density of 1.25 W/cm^2 for desired time intervals. The fluorescence intensity of ABDA was determined by a fluorescence spectrophotometer.

Photodegradation of Aβ protofibrils using Ce6 micelles

The Aβ (1-40) peptide power was dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) at a concentration 1 mg mL^{-1}. HFIP was removed under nitrogen to get a thin film. It was then stored at -80℃ until to use. For the preparation of fibrils, the peptides were dissolved in buffer (10 mM Tris–HCl, pH 7.4) and then incubated at 37^{o}C for 48 h.

Then Ce6 micelles were added to 50 μL 10 μM Aβ preformed protofibril solution. The mixture was vortexed to mix and then irradiated with a 655 nm laser for 60 min at room temperature. The degradation of Aβ protofibril was monitored using ThT fluorescence assay and TEM examination. Fluorescence measurements were carried out with excitation wavelength of 444 nm.

Degradation of Aβ protofibrils and inhibition of Aβ fibrillation using TAS-loaded Ce6 micelles.

To degrade Aβ protofibrils with TAS-loaded Ce6-micelles, 50 μL of 1.0 mg/mL TAS-loaded Ce6 micelles was added to 50 μL Aβ preformed protofibril solution (10 μM). The mixture was vortexed to mix and then incubated at 37^{o}C for 24 h, then irradiated with a 655 nm laser for 60 min at room temperature.

To investigate TAS-induced inhibition of Aβ fibrillation, 50 μL TAS-loaded Ce6 micelles (1 mg/mL) was added to 50 μL Aβ monomer solution (10 μM). The mixture was vortexed to mix and then incubated at 37 ^{o}C for 48 h. The ThT fluorescence and

the morphology of Aβ in the presence of these mixtures were monitored by fluorescence assay and TEM examination.

Dot blot assay

Proteins were dotted to a nitrocellulose membrane (GE Healthcare Life Science) and naturally dried. Then the nitrocellulose membrane was dyed by Ponceau S and blocked using 8% milk in 1× PBS at room temperature for 1 hour, followed by incubation with the primary antibody A11 at 4 °C overnight. Membranes were washed three times with PBST (0.1% Tween-20 in 1×PBS) buffer, incubated with Alexa Fluor®680 goat anti-mouse secondary antibody and visualized on a LiCor Odyssey imager.

Polyacrylamide gel electrophoresis and Coomassie brilliant blue staining

Electrophoresis buffer consisted of SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue (0.007%, wt/vol); 4.8 μL of buffer was added to 10 μL samples. Gels (10%) were run by applying 64 V for 2.5 h. The gel was stained with Coomassie brilliant blue overnight at 4 ^{o}C, then washed using eluent for several times until the band is clearly seen. Photo was taken on a film illuminator.

Cell toxicity assays

Phaeochromocytoma (PC12) cells were maintained in medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin antibiotics, and were grown in a 5% CO_2 atmosphere at 37 ^{o}C. For the MTT assay, cells were harvested from flasks and plated in 96-well polystyrene plates with approximately 7,000 cells per 100 μL of medium per well. Plates were incubated at 37 ^{o}C for 48 h to allow the

cells to attach, followed by introduction of Aβ protofibrils (10 μM), Aβ protofibrils (10 μM) with micelle (0.05 mg/mL) using laser, Aβ protofibrils (10 μM) with micelle (2% TAS, 0.05 mg/mL), Aβ protofibrils (10 μM) with micelle (2% TAS, 0.05 mg/mL) using laser and Aβ monomer (10 μM) with micelle (2% TAS, 0.05 mg/mL). After 48 h at 37^{o}C, the cells were treated with 20 μL MTT (5 mg/mL) for 4 h at 37^{o}C and then 100 μL DMSO was added to each well. Plates were shaken at room temperature for 10 min to dissolve the crystals before the absorbance at 490 nm was measured using a Tecan Safire5 microplate reader. Averages from three replicate wells were used for each sample and the control. Cell viability was calculated by dividing the absorbance of wells containing samples (corrected for background) by the absorbance of wells containing medium alone (corrected for background).

References

[1] H. Yu, Z. Xu, D. Wang, X. Chen, Z. Zhang, Q. Yin, and Y. Li, *Polym. Chem.* 2013, 4, 5052−5055.

[2] H. Yu, Y. Zou, Y. Wang, X. Huang, G. Huang, B. D. Sumer, D. A. Boothman, and J. Gao, *ACS Nano.* 2011, 5, 9246−9255.

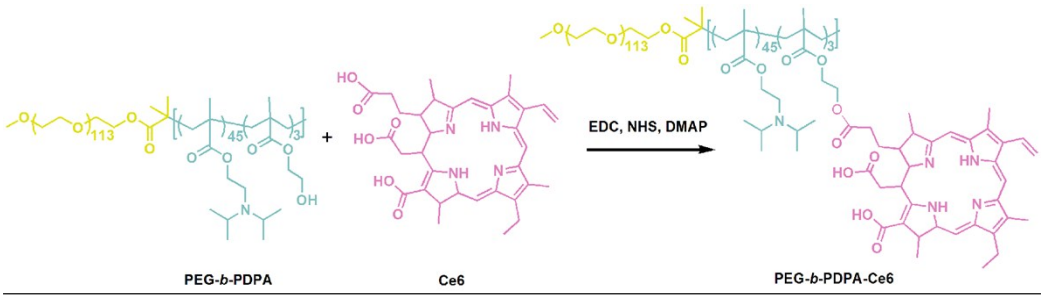

Fig. S1. Synthesis of Ce6-conjugated PEG-*b*-PDPA diblock copolymer.

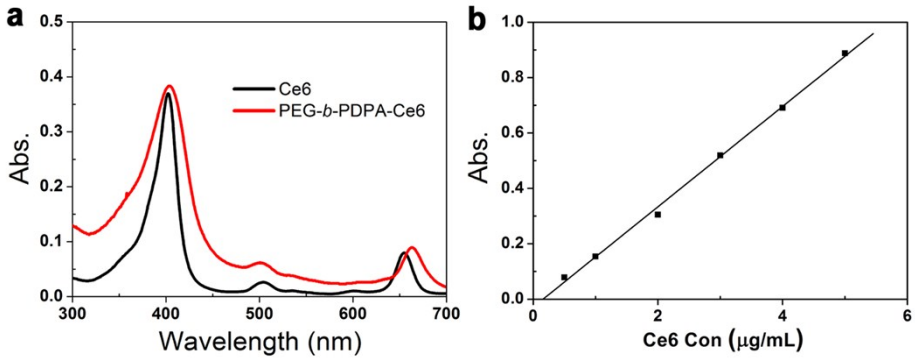

Fig. S2. (a) UV-Vis spectra of Ce6 and PEG-*b*-PDPA-Ce6; (b) Standard curve of Ce6 examined at Abs. 440 nm.

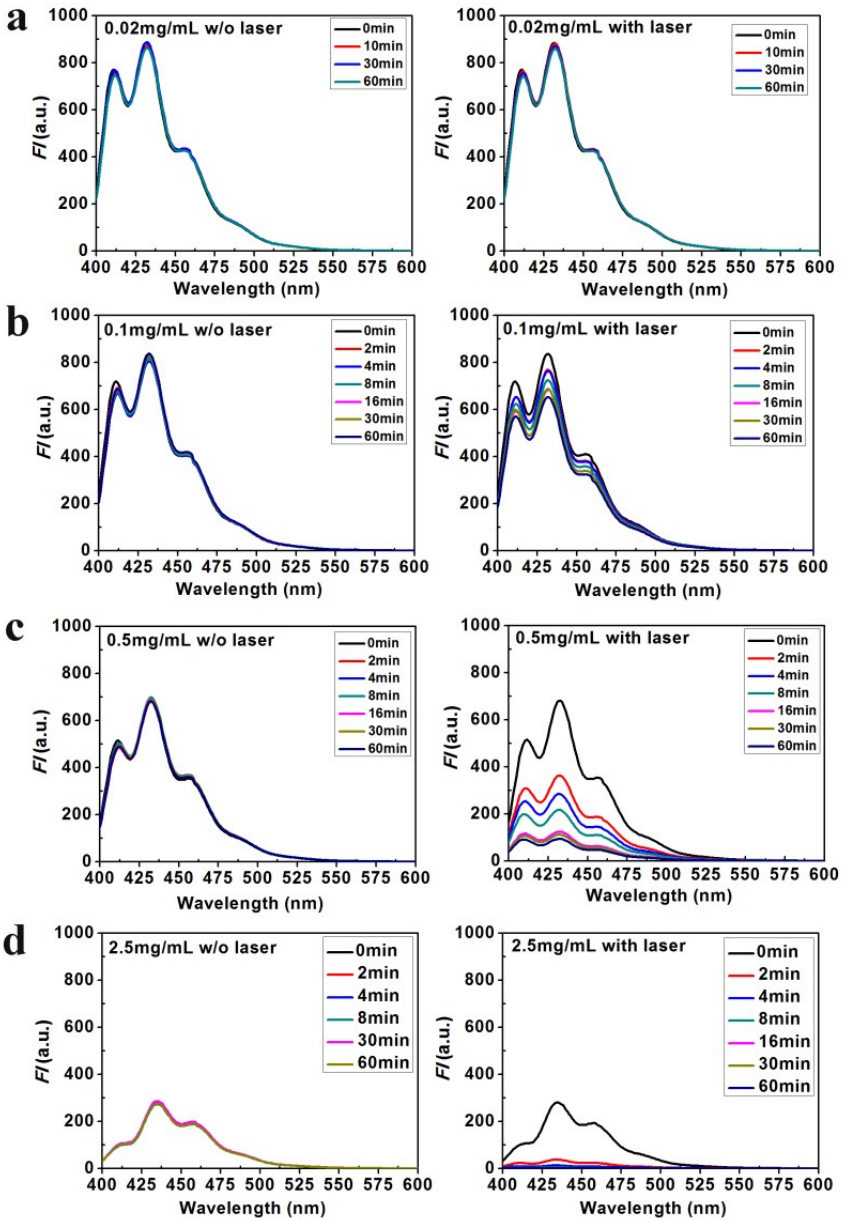

Fig. S3. Fluorescence spectra of ABDA in the presence of Ce6 micelles with or without laser irradiation for different time. Ce6 micelles concentration: (a) 0.02 mg/mL; (b) 0.1 mg/mL; (c) 0.5 mg/mL; (d) 2.5 mg/mL. [ABDA]=70 uM, Excitation wavelength: 380 nm.

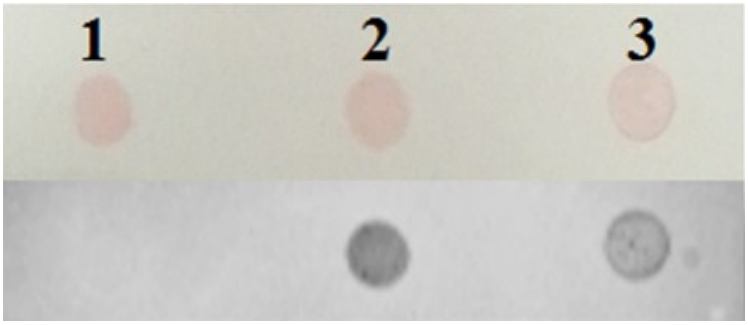

Fig. S4. Dot blot assay of Aβ samples. Top line: dyed by Ponceau S; Bottom line: incubated with the primary antibody A11 and Alexa Fluor®790 goat anti-mouse secondary antibody and visualized on a LiCor Odyssey imager. 1. Aβ monomer; 2 and 3. Aβ monomer incubated in 10 mM Tris-HCl buffer (pH = 7.4) at 37 ^{o}C for 24 h (2) and 48 h (3).

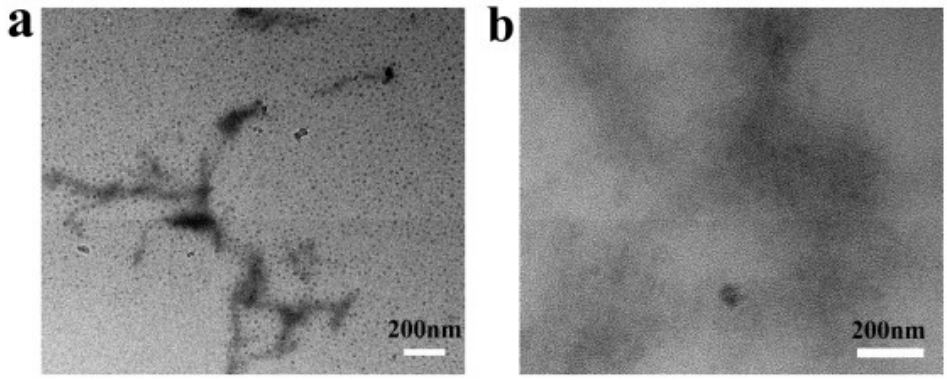

Fig. S5. (a). TEM image of Aβ oligomer obtained by incubating 10 μM Aβ monomer in 10 mM Tris-HCl buffer (pH = 7.4) at 37 ^{o}C for 24 h; (b). TEM image of Aβ oligomer treated with Ce6-micelles and irradiated with 655 nm NIR laser at photo density 1.25 W/cm^2 for 60 min at a micelle concentration of 0.5 mg/mL.

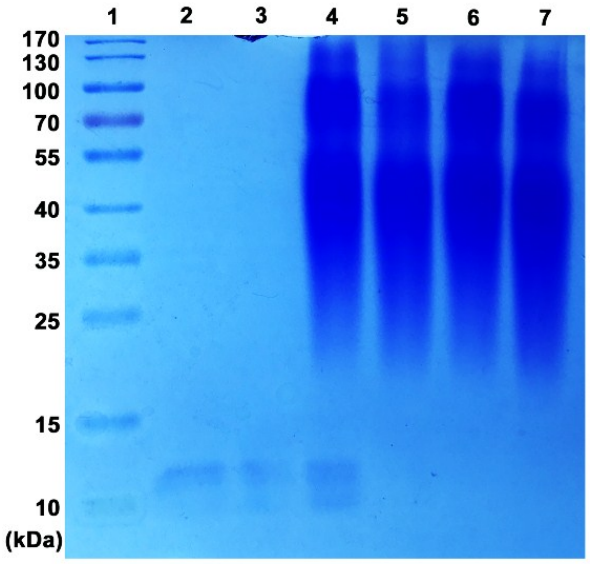

Fig. S6. Polyacrylamide gel electrophoresis and Coomassie brilliant blue staining. Aβ samples were obtained by incubating 20 μM Aβ monomer in 10 mM Tris-HCl buffer (pH = 7.4) at 37 ^{o}C for 24 h; Lane 1: marker; lane 2: Aβ; lane 3: Aβ + laser; lane 4: Aβ + micelle (0.5 mg/ml); lane 5: Aβ + micelle (0.5 mg/ml) + laser; lane 6: micelle (0.5 mg/ml); lane 7: micelle (0.5 mg/ml) + laser.

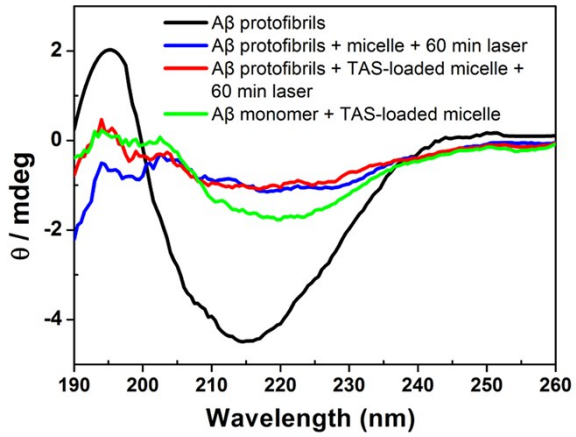

Fig. S7. CD examination of photodynamic micelle-induced Aβ degradation and aggregation inhibition.

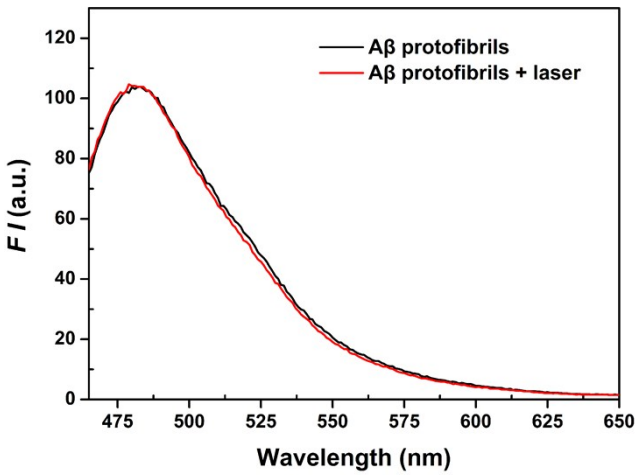

Fig. S8. Fluorescence spectra of ThT co-incubated with Aβ protofibrils with or without exposure to laser irradiation for 1 h.

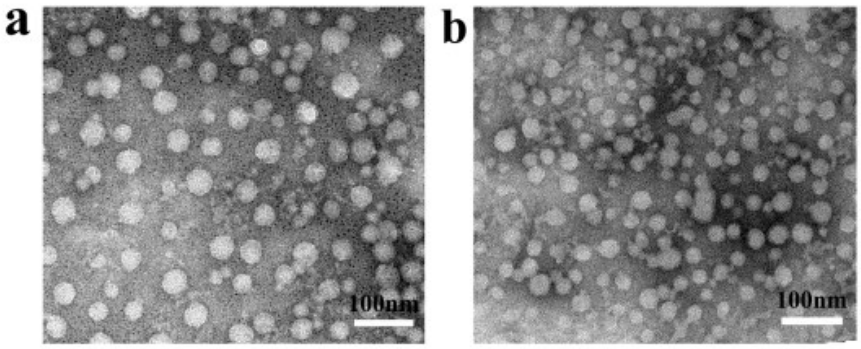

Fig. S9. TEM images of Ce6 micelles before (A) and after (B) exposure to laser for 1 h. Scale bar: 100 nm.

Table S1 The feeding ratio, encapsulation efficiency and release efficiency of TAS-loaded micelles.

Feeding ratio (wt%)	Encapsulation efficiency (%)	Release efficiency (%)
0.5	**45**	**30**
2.0	**36**	**37**
4.0	**27**	**43**