Electronic Supplementary Information (ESI)

Engineering Arginine Cross-Linked Mercaptoundecanoic acid CdSe/CdS/ZnS Quantum Dots for Two-Photon Imaging of Live Cancer Cells

Ken-Tye Yong,*[a] GaiXia Xu[b], and Indrajit Roy[c]

[a]School of Electrical and Electronic Engineering, Nanyang Technological University, 639798 Singapore. *E-mail: ktyong@ntu.edu.sg
[b]Institute of Optoelectronics, Key Laboratory of Optoelectronics Devices and Systems of Ministry of Education/Guangdong Province, Shenzhen University, Shenzhen 518060, P.R. China
[c]Department of Chemistry, University of Delhi, Delhi-110 007, India.

A. Synthesis of Quantum Dots (QDs)

CdSe/CdS/ZnS QDs were prepared by growing a CdS/ZnS graded shell on CdSe core as described in our previous studies. Briefly, 4 mmol cadmium oxide, 15 mL oleic acid, and 3 g TOPO were loaded into a 100 ml three-necked flask. Next, the reaction mixture was slowly heated under an argon atmosphere to 250ºC. After 10 to 15 minutes of heating, a clear homogeneous solution was obtained. The reaction mixture was maintained at 250ºC for another 5 minutes, then 1.5 ml of 1 M TOP-Se was rapidly injected. The reaction was stopped after 1-2 minutes by removing the heating mantle. The QDs were separated from the surfactants solution by addition of ethanol and centrifugation. The reddish QD precipitate could be readily redispersed in various organic solvents (hexane, toluene, and chloroform). CdSe QD solution was prepared in advance by dissolving ~0.2-0.23 g of CdSe QDs in ~5 mL of toluene. Separately, 2 mmol of cadmium oxide, 4 mmol of zinc acetate, and 5 g of TOPO were dissolved in 10 mL of oleic acid. The reaction mixture was heated to 170^{o}C for ~30 minutes under an argon flow, after which the CdSe QD solution was injected slowly under stirring into the hot reaction mixture. The reaction mixture was held at 170ºC, with a needle outlet that allowed the toluene to evaporate. After ~10 minutes of heating, the needle was removed, and then the reaction temperature was raised to 200^{o}C. Upon reaching the temperature, 2 mL of TOP-S was added drop wise into the reaction mixture. The reaction mixture was then held at ~200^{o}C for 10 to 15 minutes, after which an aliquot was removed via syringe and was injected into a large volume of toluene at room temperature, thereby quenching any further growth of the QDs. The QDs were separated from the toluene solution by addition of ethanol and centrifugation.

B. Preparation of Arginine cross-linked MUA coated QDs

Three mmol mercaptoundecanoic acid was dissolved in ~15 mL of chloroform under vigorous stirring. After stirring for 10 to 15 minutes, 2.5 mL of concentrated (~30 mg/mL) CdSe/CdS/ZnS QD solution was added into this mixture. Approximately one minute later, to this vigorously stirring solution, 2 mL of ammonium hydroxide was added. This solution was stirred overnight at room temperature. The QDs were separated from the surfactant solution by addition of ethanol and centrifugation. The QD precipitate was re-dissolved in 15 mL DMSO for further arginine cross-linking process. The arginine cross-linked MUA QDs were obtained by mixing DMSO quantum dots solution with both N,N′-Dicyclohexylcarbodiimide DCC (~30 mmol) and arginine (~15 mmol) under vigorous stirring for 2.5 hours. After 2.5 hours, the arginine coated QDs were precipitated from the solution by addition of ethanol and centrifugation. The precipitate was

redispersed in 10 mL HPLC water with pH adjusted to 10 and the solution was sonicated for 10 to 15 minutes. After that, the solution was further filtered using a syringe filter with a nominal pore diameter of 0.45 μm. The arginine coated QDs have relatively good colloidal stability and no precipitation was observed after several weeks. This stock solution was kept in the refrigerator at 4^{o}C for further use. These arginine coated QDs are stable over a wide range of pH values (~7 - 11).

C. Conjugation of Arginine-coated QDs with transferrin

150 μL Arginine coated QD stock solution was mixed with 50 μL of 0.5 mg/mL 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride solution and gently mixed for 2 to 3 minutes. Next, 200 μL of 2 mg/mL transferrin was added into this mixture and incubated at room temperature for 2 to 3 hours to allow the protein to covalently bond to the Arginine coated QDs. The QD-transferrin bioconjugates were further purified by centrifugation.

D. Cell staining studies

For in vitro imaging with arginine coated QDs, the human cancer cell-line was cultured in Dulbecco minimum essential media (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin, and 1% amphotericin B. The day before treatment, cells were seeded in 35 mm culture dishes at a confluency of 70-80 %. On the treatment day, the cells in serum-supplemented media were treated with the Tf-conjugated QDs for two hours at 37^{o}C. After two hours, the cells were washed thrice with PBS and directly imaged using confocal microscope. Two-photon microscopy images were obtained using confocal microscope with two-photon laser excitation which is the modelocked Ti/Sapphire laser pulses of <200 fs at 884nm using Mira 900.

E. Characterization methods

The emission spectra were collected using a Fluorolog-3 Spectrofluorimeter (Jobin Yvon; fluorescence spectra). All the samples were dispersed in hexane and loaded into a quartz cell for measurements. Transmission Electron Microscopy (TEM) images were obtained using a JEOL model JEM-100CX microscope at an acceleration voltage of 80 kV. The specimens were prepared by drop-coating the sample dispersion onto an amorphous carbon coated 300 mesh copper grid, which was placed on filter paper to absorb excess solvent.

F. Dynamic light scattering (DLS)

Dynamic light scattering(ZetaPlus Plus90, Brookhaven) was used to estimate the hydrodynamic diameter of the arginine coated QDs.

G. Cytotoxicity of Arginine coated QDs

Briefly, human pancreatic cancer (Panc-1) cells were trypsinized and resuspended in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were seeded at a density of 0.2-1 million cells/well in a 24-wellplate at 37^{o}C and 5% CO_2 for 24 hours and washed with PBS. Desired concentrations of arginine coated QDs were added to each well with 4 duplicates for each concentration. After 48 hours of incubation, the supernatant was removed, and the cells were washed with PBS buffer. To evaluate cell viability, 100 μL of MTS solution was added to each well and incubated the mixture at 37^{o}C for 4 h. After incubation, each well was treated with 100 μL of DMSO for 5

minutes. The optical absorbance was measured at 570 nm on a plate reader. In the MTS assay, the absorbance of formazan at 570 nm is directly proportional to the number of live cells.

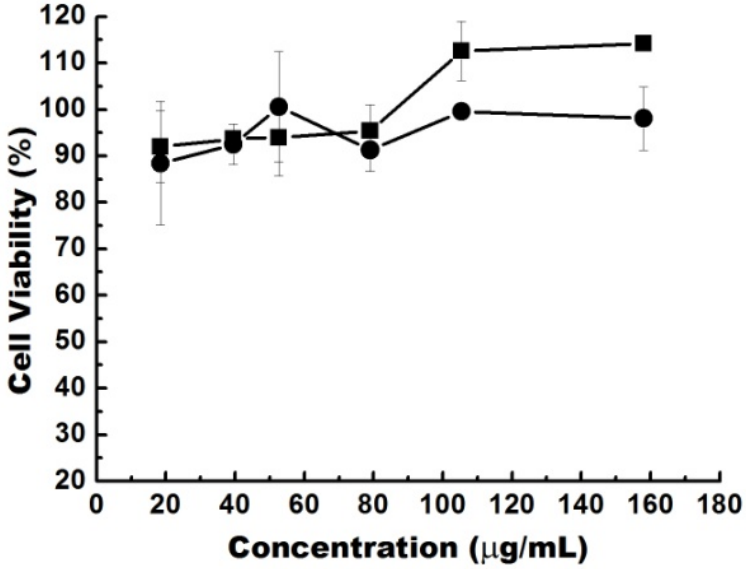

Figure S1. Cytotoxicity studies of Panc-1 cells treated with functionalized ACM-QDs. MTS assays illustrating cell viability upon exposing the cells with different concentration of ACM-QD formulation for 24 (square) and 48 hours (circle).