Multi-Functional Conjugates to Prepare Nucleolar-Targeting CdS Quantum Dots

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Supporting Information

Experimental Section

Materials

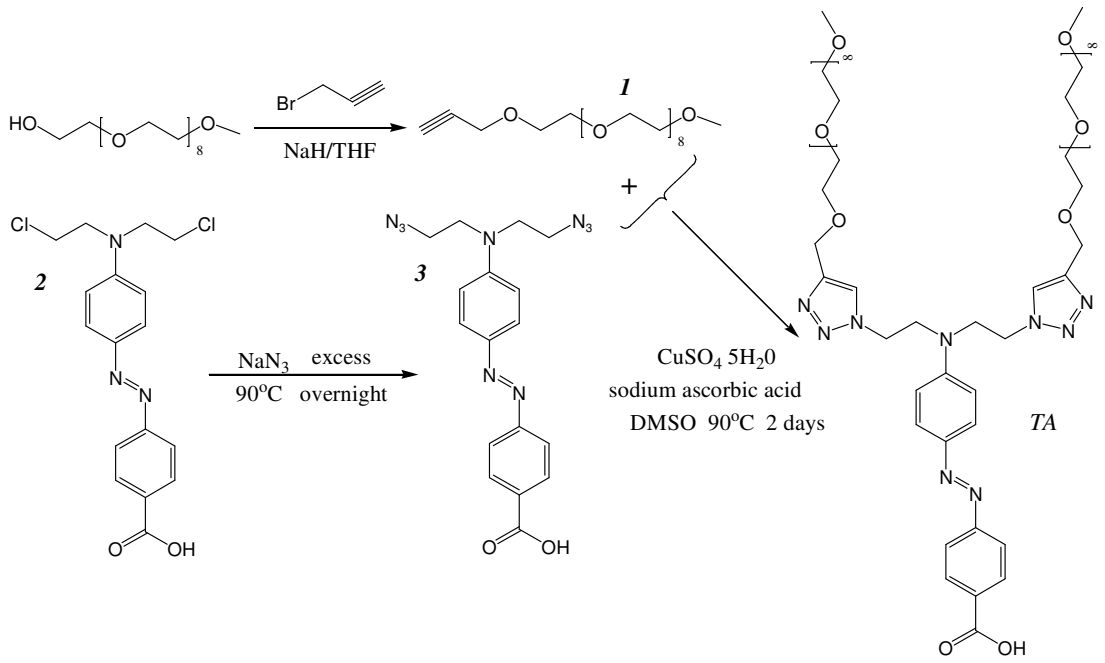
Monomethoxy-polyethylene glycol 350 (Alfa Aesar, Britain) was dried by boiling in toluene. 3-Bromo-propyne (80%, stored in toluene solution, Sinopharm Chemical) was passed through neutral alumina column before use. Calf thymus DNA (ct-DNA, Sigma D1501) and Tris-HCl buffer (5 mM Tris-HCl, 50 mM NaCl, pH = 7.4) were handled with standard molecular biology techniques. A solution of ct-DNA gave ratios of UV absorbance at 260 and 280 nm of about 1.9:1, indicating that the DNA

was sufficiently free of protein. The DNA per nucleotide was determined with UV-vis. absorbance spectrum by assuming $\varepsilon_{260nm} = 6600\ M^{-1}\ cm^{-1}$.

Methods

^{1}H NMR (300 MHz) spectra were recorded on a Bruker 300 NMR instrument, using tetramethylsilane (TMS) as an internal reference. UV-vis. absorption spectra were measured on a Shimadzu UV-2401PC spectrometer. Fluorescence spectra were measured on a Shimadzu RF-5301PC fluorescence spectrophotometer. In the photo-isomerization experiments of azobenzene, UV irradiation was carried out with a UV lamp (5 W) with emission at 302 nm, and exposure by visible light was performed using a Philips day light bulb (> 400 nm, 23 W). Raman spectroscopy with the different laser excitation lines (325 nm and 514.5 nm) was performed on RAMALOG 6 (SPEX, USA) at room temperature. X-ray photoelectron spectroscopy (XPS) was performed with a Thermo-VG Scientific ESCALAB 250 XPS spectrometer with a Mg K_α X-ray source (1253.6 eV). X-ray diffraction (XRD) was carried out on silicon (100) wafer on a MAC Science M18X X-ray diffractometer using Cu K_α line (λ = 0.154056 nm). Transmission electron microscopy (TEM) of samples was carried out in on a JEOL-2010 microscope operated at 200kV. Cellular staining was evaluated under an epifluorescence microscope with UV, blue and green excitation sources (IX-71-F22FL/PH; Olympus, Tokyo, Japan) and photographed with a digital camera (DP70-Set2; Olympus).

Synthesis of the TA ligand (bis-[2-(4-PEG-[1,2,3]triazol-1-yl)-ethyl]-amino -azobenzoic acid). The synthesis of ***TA*** was generally illustrated in Scheme S1. 4-{4-[Bis-(2-chloro-ethyl)-amino]-phenylazo}-benzoic acid (BAA, 2) and 4-{4-[Bis(2-azidoethyl)-amino]-phenylazo}-benzoic acid (3) were synthesized in our previous work.[S1]

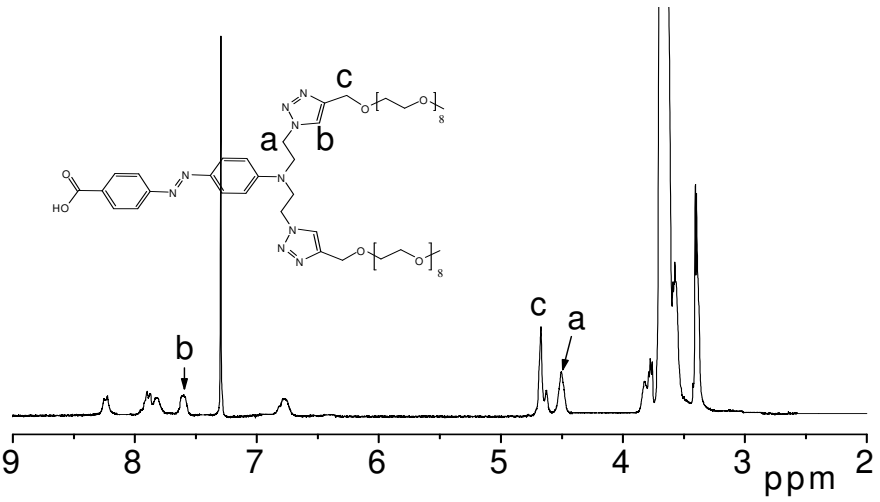


Scheme S1. Synthesis of the ligand ***TA***.

*Synthesis of alkyne PEG (**1**).* To a solution of monomethoxy-polyethylene glycol 350 (3.50 g, 10 mmol) in dried THF (50 ml) was added NaH (70% w/w in mineral oil, 0.51 g, 11 mmol) at 0 ^{o}C with frequent venting. After stirring of 30 min, propargyl bromide (80% in toluene, 1.31 g, 11 mmol) was added slowly, and the mixture was stirred at 0 ^{o}C for 1h and then refluxed overnight. The suspensions were filtered, and then the filtrates were dried by rotary evaporation under reduced pressure at 60 ^{o}C to remove volatiles. The crude product was dissolved in 50 ml water and extracted with

dichloromethane for 3 times. The solution was dried to give the purified product ***1*** (3.26 g, 86.1%). ^{1}H NMR (300 MHz, δ ppm, $CDCl_3$): 2.42 (s, 1H, -C≡C*H*), 3.38 (s, 3H, -CH_3), 4.20 (s, 2H, -C≡C-CH_2-), 3.64 (t, 32H, -CH_2-CH_2-).

*Synthesis of bis-[2-(4-PEG-[1,2,3]triazol-1-yl)-ethyl]-amino-azobenzoic acid (**TA**).* A round bottom flask was charged with 4-{4-[bis(2-azidoethyl)-amino]-phenylazo}-benzoic acid (0.453 g, 1.2 mmol), product ***1*** (1.03 g, 2.64 mmol), DMSO (10 ml), followed by sodium ascorbic acid (0.104 g, 0.56 mmol) and $CuSO_4 \cdot 5H_20$ (0.066 g, 0.28 mmol). The mixture was stirred at room temperature for 2 days. The reaction was filtered to remove salts, and the filtrates were dried under vacuum. The crude product was passed through a silica gel column by being eluted first with tetrahydrofuran/dichloromethane (1:1) to remove excess ***1***, and then eluted with methanol to give the purified product ***TA*** (0.89 g, 64.1%). The ^{1}H NMR (300 MHz) of ***TA*** was illustrated in the following:



*Synthesis of CdS QDs in aqueous solution of **TA***. In a typical experiment, a round bottom flask was charged with ***TA*** (0.1 g, 0.087 mmol), de-ionized water (33 ml) and cadmium chloride (0.0016 g, 0.0087 mmol). The solutions were stirred at room temperature for 3 h and then sulfureted hydrogen gas was slowly bubbled into the mixture for another 30 min. The mixture was stirred at room temperature for 24 hours. The mixture was extracted by chloroform (10 ml) for three times. The chloroform phases were collected and dried by rotary evaporation. The obtained viscous liquid was dissolved in de-ionized water to form a solution of 1 mg/ml.

With constant concentrations of cadmium chloride and varying *TA* concentrations, QDs with different *TA*/Cd(II) ratios were obtained.

*Stability of **TA**-capped CdS QDs.* The stability of QDs at different ionic strengths and pH values was estimated by photo-fluorescence analysis.

CdS QD aqueous solution of 200 μL (1 mg/mL) were added to volumetric flasks filled with 25 mL KCl aqueous solutions with different concentrations (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 M, respectively). The solutions were stored at room temperature for 1 day before photo-fluorescence analysis.

CdS QD aqueous solution of 200 μL were added to volumetric flasks filled with 25 mL Tris-HCl/NaOH buffers with different pHs (1.0, 3.0, 5.0, 7.0, 9.0, 11.0 and 13.0, respectively). The solutions were stored at room temperature for 1 day before photo-fluorescence analysis.

*Cell culture and labeling with **TA**-capped CdS QDs.* Human lung carcinoma (A549), human cervical cancer cell HeLa and human hepatocellular carcinoma (HEP G2) cells were separately cultured (at 37 ^{o}C, 5% CO_2) in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% bovine calf serum (BCS) and 1% penicillin.

After 48 h, when cells had attached to the surface and spread well, they were washed with phosphate buffered saline (PBS), and then fixed with methanol/acetone (1/1, v/v) mixture for 20 min. Fixed cells were washed with PBS for three times. For quantum dots staining, cells were incubated with aqueous solution of ***TA***-capped QDs (0.1 mg/ml) for 30 min, and then were washed with PBS for three times.

TEM observation of QD-stained HeLa cells. QD-stained fixed HeLa cells were collected in a centrifugal tube, and were centrifugated. After the top solution layer had been removed, PBS was added, and the cells were ultrasonic dispersed again. The procedure of ultrasonic dispersion-centrifugation was repeated for three times. The HeLa cells were dehydrated with acetone, and then were embedded in epoxy resin. Ultrathin sections (60 nm) were examined in the TEM.

Ethidium bromide exclusion assay. Ethidium bromide (1.0 μmol/L) and ct-DNA (10.0 μmol/L) were mixed in Tris-HCl buffer (pH = 7.4). After 10 min incubation at room temperature, various amounts of ligand ***TA*** were added to the DNA-ethidium bromide mixture and then incubated for 30 min. Excitation (λ_{ex}) and emission (λ_{em}) wavelengths were 302 and 586 nm, respectively. The fluorescence of the DNA

solutions in Tris buffer with ethidium bromide was set to 100%, measured against a background of ethidium bromide without DNA.

As a control, ethidium bromide exclusion assay was also carried out for the compound (2)/ct-DNA according to the same procedure.

Measurement of quantum yield (QY). The quantum yield of all compounds is determined according to equation (1)[S2]

$$\Phi = \left(\frac{F_{sample}}{F_{ref}}\right)\left(\frac{A_{ref}}{A_{sample}}\right)\Phi_{ref}\left(\frac{n^2_{sample}}{n^2_{ref}}\right) \qquad (1)$$

where F_{sample} and F_{ref} are the measured fluorescence (area under the fluorescence spectra) of the sample and the reference respectively, A is the absorbance at the same excitation wavelength, Φ_{ref} is the quantum yield of the reference, and n is the refractive index. Rhodamine B is used as a reference (Φ = 0.88) in these measurements.

When measuring the QY of CdS QDs, the fluorescence was monitored at 520 nm with excitation of 390 nm; whereas fluorescence at 650 nm was monitored for QY measurement of band C (surface complexes) with the excitation at 550 nm.

Cyclic Voltammetry Measurements. A potential versus current profile for the aqueous 1,2,3-triazole/$CdCl_2$ (1.25 mM) solutions were obtained at a sweep rate of 1 mV/s at room temperature in the potential range of 2 and −2 V versus the Ag/AgCl (saturated KCl) in 0.1 M KNO_3 (aq) at 25 ^oC. The electrodes for cyclic voltammetry were Pt imbedded in glass. Electrochemical experiments were performed with a CHI660D Cyclic Voltammetry with a three-electrode configuration.

Conductometric titration. A solution of 1,2,3-triazole in anhydrous ethanol (40 mM) was slowly added from a titrator to 60 mL of cadmium acetate/ethanol solution (1.66 mM) at 25 ^{o}C. The conductance of the mixture was monitored by DDS-11 conductivity meter (Shanghai, China). Titration of 1,2,3-triazole (10 mM), and cadmium acetate (1.66 mM) to 60 mL anhydrous ethanol were also carried out as control tests, respectively.

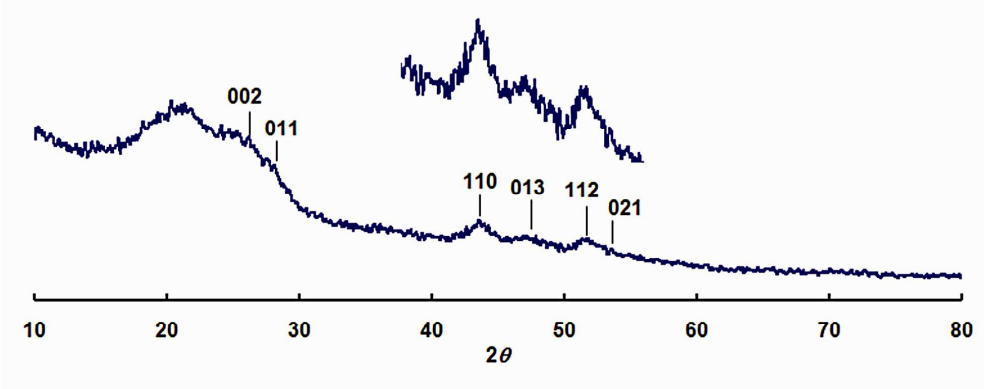


Figure S1. XRD of ***TA***-capped CdS QDs. The strong peak at about 20 degree arose from the scattering of amorphous polymers.

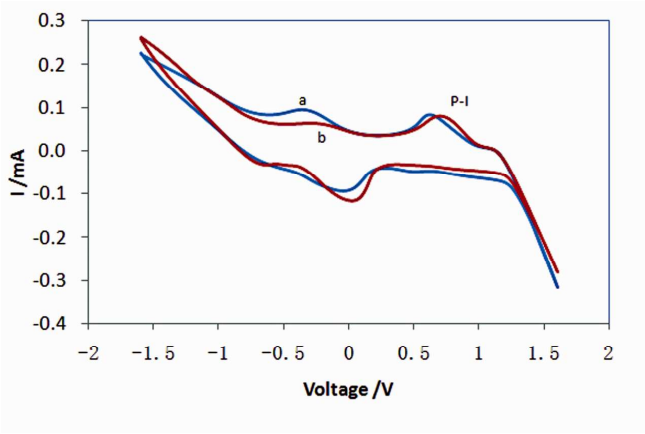


Figure S2. Cyclic voltammogram of (a) 1.25 mM $CdCl_2$ in aqueous 0.1 M KNO_3, and (b) 1.25 mM $CdCl_2$ in aqueous 0.1 M KNO_3/1,2,3-triazole solution with molar ratios [1,2,3-triazole]/[Cd(II)] of 2.

References

(S1) Shen, X.; Liu, H.; Li, Y.; Liu, S. *Macromolecules* **2008**, 41, 2421-2425.

(S2) (a) Cho, D.; Mattice, W. L.; Porter, L. J.; Hemingway, R. W. *Polymer* **1989**, 30, 1955-1958. (b) Williams, A. T. R.; Winfield, S. A.; Miller, J. N. *Analyst* **1983**, 108, 1067-1071.