Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Fabrication of Bright and Small Size Semiconducting Polymer Nanoparticles for Cellular Labelling and Single Particle Tracking

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EXPERIMENTAL SECTION

Materials.

Poly(acrylic acid) (PAA, Mw~2,000), dodecylamine (DDA), 1-methyl-2-pyrrolidione (MPD, anhydrous, 99.5%) and 2-mercaptoethylamine hydrochloride (MEA, >98%) were purchased from Aladdin (Shanghai, China). Poly[(9,9-dioctylfl uorenyl-2,7-diyl)-alt-co-(1,4-benzo-(2,1',3)-thiadiazole)] (PFBT) was purchased from American Dye Source, Inc. (Quebec, Canada). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) were purchased from Thermo Fisher Scientific Inc. (Rockford, IL, USA). Qdots 565, streptavidin, biotinylated phycoerythrin and biotinylated monoclonal epithelial cell adhesion molecule (EpCAM) antibody were purchased from Life Technologies (Grand Island, NY, USA). Fluorescein, dicyclohexylcarbodiimide (DCC, 99%), anhydrous tetrahydrofuran (THF), methoxypolyethylene glycol amine (amine-PEG, MW: 750) and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Fabrication of amphiphilic multidentate polymer.

The amphiphilic multidentate polymer was synthesized according to the literature method by grafting dodecylamine and MEA onto the PAA backbone through carboxylic acid and amine linkage.[1,2] In a typical reaction for a nominal 40% grafting percentage of PAA (that is, 40% of the carboxylic acid groups are nominally modified with DDA portion), 0.2153 g of dried poly(acrylic acid) (PAA, 2.99 mmol based on -COOH group) was dissolved in 10 mL of 1-methyl-2-pyrrolidinone (MPD) in a 50 mL round-bottom flask at 60 °C for 24 h under constant stirring. In a separate reaction vessel, 0.3849 g of DCC was dissolved in 5 mL of MPD, added to the PAA solution, and allowed to react for

1 h at 60 °C under constant stirring for 30 min under N_2. Finally, 0.2258 g of DDA was dissolved in 5 mL of MPD, added to the PAA-DCC solution, and allowed to react for 24 h at 60 °C under constant stirring. The solution was then cooled to room temperature and filtered through filter paper to remove any unwanted dicyclohexylurea crystal byproducts. Afterward, 10 mL of 40 wt % NaOH solution was added to precipitate the polymer, and this was followed by centrifugation (4500 rpm, 5 min). The supernatant was discarded, and the pellet was washed with hot MPD (60 °C) and methanol to remove any impurities. The final solid was dried overnight and stored at room temperature (with yield of 86%). All other amphiphilic polymers used in the study were synthesized in a similar manner, but the molar ratio of grafted molecules to PAA was adjusted for the desired % modification. ^{1}H NMR spectra were recorded on a Varian Inova-400 NMR spectrometer operating at 400 MHz at room temperature, Figure S6. FT-IR spectra were collected using a Nicolet 730 FT-IR spectrometer, Figure S7.

SPNs fabrication and streptavidin conjugation.

The SPNs were fabricated by a modified co-precipitation method where an amphiphilic multidentate polymer (as synthesized above) was adopted as the phase transfer agent. In detail, 20 μL of 1 mg/mL PFBT polymer in THF was mixed well with 10 times of amphiphilic multidentate polymer in 1 mL THF. The mixture was then rapidly injected into 2 mL MilliQ water in a sonication bath (40 s). THF was removed by placing the vial on a magnetic hotplate with continuous nitrogen flow blowing above the bottleneck. After THF was completely removed, the SPNs solution was purified by filtration through a 0.2 μm filter to remove large particles. The excess amphiphilic polymers were separated from the SPNs by passing through a size exclusion column

(Sephacryl HR-300) or by washing the solution with Millipore centrifugal filter (cutoff range: 10 k).

The UV–vis and fluorescence spectroscopic characterizations were carried out on a UV-2450 ultraviolet–visible spectrophotometer (Shimazu Co., Japan) and a F-4500 FL spectrophotometer (Hitachi Co., Japan) respectively. The fluorescence QY of SPNs was measured by using fluorescein as a reference (with QY of 92%). The fluorescence lifetime of the SPNs was measured on a fluorescence lifetime spectrofluorometer (LaserStrobe^{TM}, Photon Technology International, USA) based on the time correlated single-photon counting technique. The hydrodynamic size and zeta-potential were determined by dynamic light scattering (DLS, Malvern Zetasizer NanoZS). Transmission electron microscopy (TEM) (JEM 1230, JEOL, Japan) was used to measure the size in a dried state.

The streptavidin was conjugated to the surface of SPNs with EDC/Sulfo-NHS. Firstly, the carboxyl groups on the surface of the SPNs were activated by EDC (100 ×) which can form an amine-reactive *O*-acylisourea intermediate. Since this intermediate is susceptible to hydrolysis, to improve the conjugation efficiency, we further added Sulfo-NHS (500 ×) to stabilize the intermediate by converting it to an amine-reactive Sulfo-NHS ester. The reaction solution which contains 120 μL of 1 mg/mL streptavidin was kept stirred for 3 h at room temperature until the crosslinking process was finished. To increase the stability and reduce the nonspecific interaction with other proteins, we added excessive amine-PEG (500×) to the reaction solution to deactivate the remained carboxyl groups and left the solution to react for another 3 h. Finally the resulting streptavidin-

SPNs bioconjugates were purified from free biomolecules by gel filtration with Sephacryl HR-300 gel media and stored at 4 °C prior to use.

Living cell labelling.

The breast cancer cell line MCF-7 was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured at 37 °C, 5% CO_2 in Eagles minimum essential medium supplemented with 10% Fetal Bovine Serum (FBS), 50 U/mL penicillin, and 50 μg/mL streptomycin. For cell membrane labeling, the live MCF-7 cells were cultured in a 35 mm Petri Dish until the density reach to 60-70% confluence. The cells were washed two times with 1×PBS (1% BSA) buffer prior to labeling. Typically, 2 μL of biotinylated primary anti-EpCAM antibody was incubated with 2.5 μL of streptavidin conjugated SPNs in 100 μL cell culture medium for 30 min at room temperature. The mixture was then added to the cell culture dish and co-incubated with the living MCF-7 cells for another 30 min at 4 °C. The excessive unbound SPNs were removed by washing the cell extensively with 1×PBS (1% BSA) buffer. For long time observation, the cell can be further fixed with 4% (V/V) paraformaldehyde solution. In the negative control and positive comparison experiments, the labeling procedures are the same except that no antibody was added and the streptavidin conjugated SPNs were replaced with streptavidin conjugated phycoerythrin respectively.

Cytotoxicity assay.

In order to test the cytotoxicity of SPNs, a MTT [3-(4,5-dimethylthiazlo-2-yl)-2,5-diphenyltetrazolium bromide] cell viability assay was performed to explore the effect of SPNs on MCF-7 cell proliferation. The MTT assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity.[3] Since for

most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs or nanoparticles on cell lines or primary patient cells. In brief, we added 200 μL of MCF-7 cells suspended in EMEM medium supplemented with 10% fetal bovine serum to each well of 96-well plate (with density of 4×10^3 cells/mL). The cells were incubated for 24 h and then the culture medium was replaced with 200 μL of EMEM containing SPNs with different concentrations. Then 20 μL of 5 g/L MTT solution was added to each well. The cells were further incubated for 4 h. After that, the culture medium containing MTT was removed, and 150 μL of DMSO was added to each well for approximately 5 min at room temperature. The absorbance of each well was measured at 490 nm using a microplate reader (Spectra Max i3, Molecular Devices Corp., USA).

Single particle imaging and tracking.

The single particle imaging experiments were performed on a Nikon Ti-U inverted epi-fluorescence microscope. To inhibit the vigorous thermal motion, the fluorescent objects were fixed on the 3-aminopropyltriethoxysilane (APTES) functionalized glass substrate through electrostatic interaction. Since the PFBT SPNs, Qdots 565, and phycoerythrin have strong absorption at around 488 nm, we adopted the 488 nm diode laser as the light source. To make sure the majority of the photons are collected by the camera, a long-pass filter (500 nm) was applied to separate the scattering light from the laser source. The photons from individual particles (or molecules) was then collected by a 100 × objective (NA 1.3) and recorded with an EMCCD (ultra897, Andor, UK). For the single particle tracking experiments, favorable amount of SPNs solution was added to the cell culture dish and then imaged with the setup described above. All of the data recorded

by the CCD camera were analyzed by the public image processing software, Image J (http://rsbweb.nih.gov/ij/).

SUPPORTING FIGURES

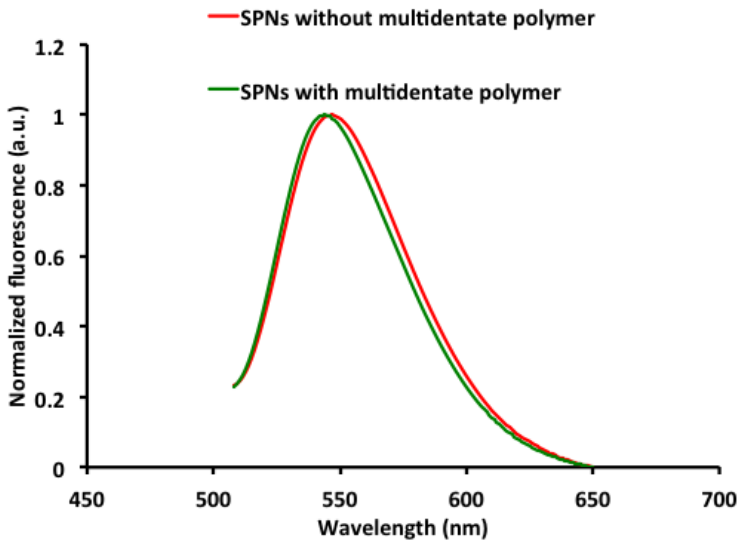

Figure S1. Fluorescence emission spectra of PFBT SPNs fabricated with (green) and without (red) multidentate polymer.

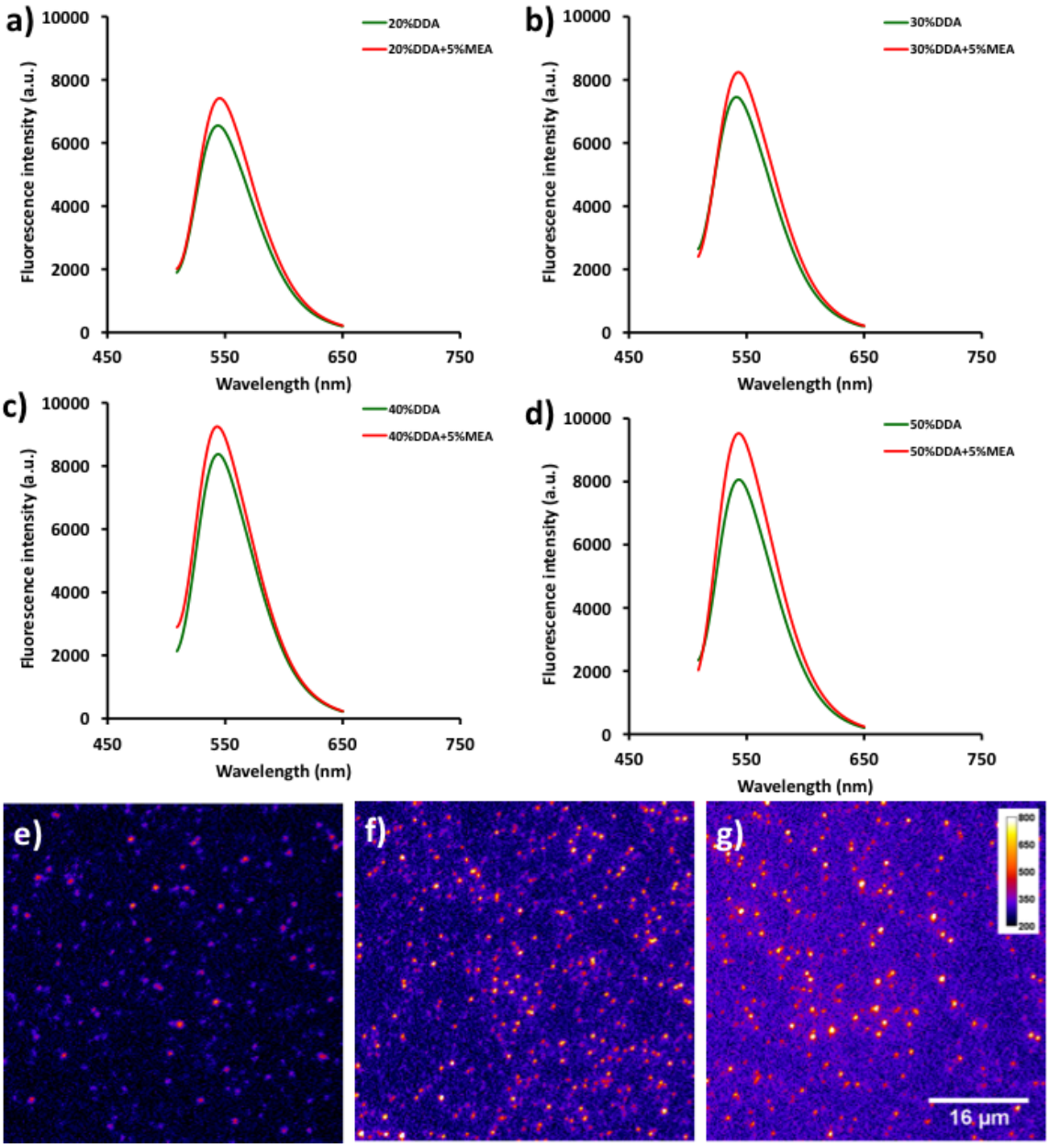

Figure S2. a)-d) fluorescence emission spectra of PFBT SPNs fabricated with different percent of DDA-PAA (green) and DDA-MEA-PAA (red) multidentate polymer. e)-f) fluorescence images of PFBT SPNs protected with 40% DDA in deionized water and 10

mM MEA solution respectively. g) the fluorescence image of SPNs protected with 40%DDA-5%MEA-PAA multidentate polymer.

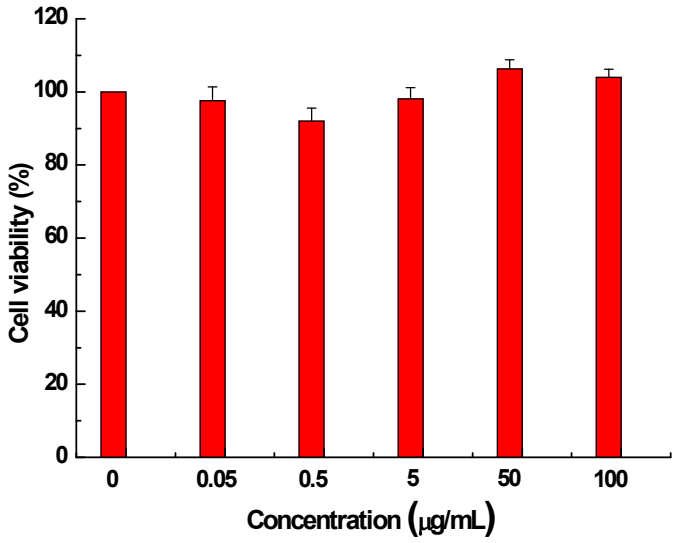

Figure S3. The cell viability assay of small size SPNs obtained from cultured MCF-7 cells using the MTT assay.

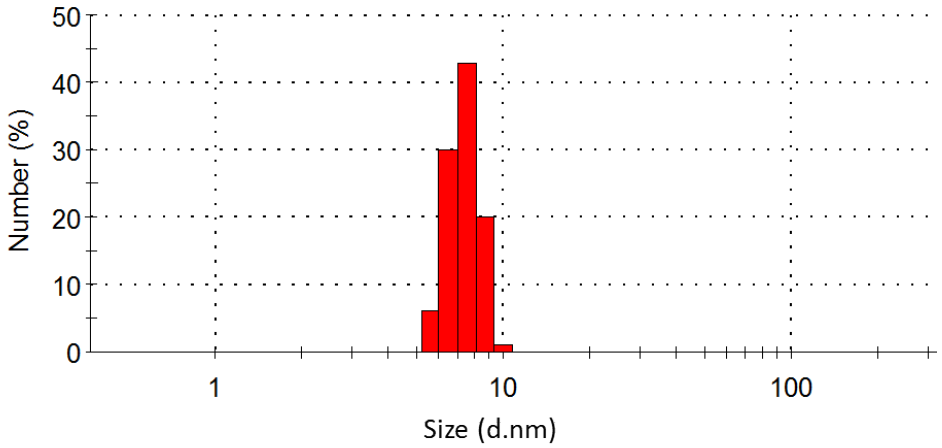

Figure S4. DLS measurement of SPNs after conjugated with streptavidin (7.3±1.0 nm).

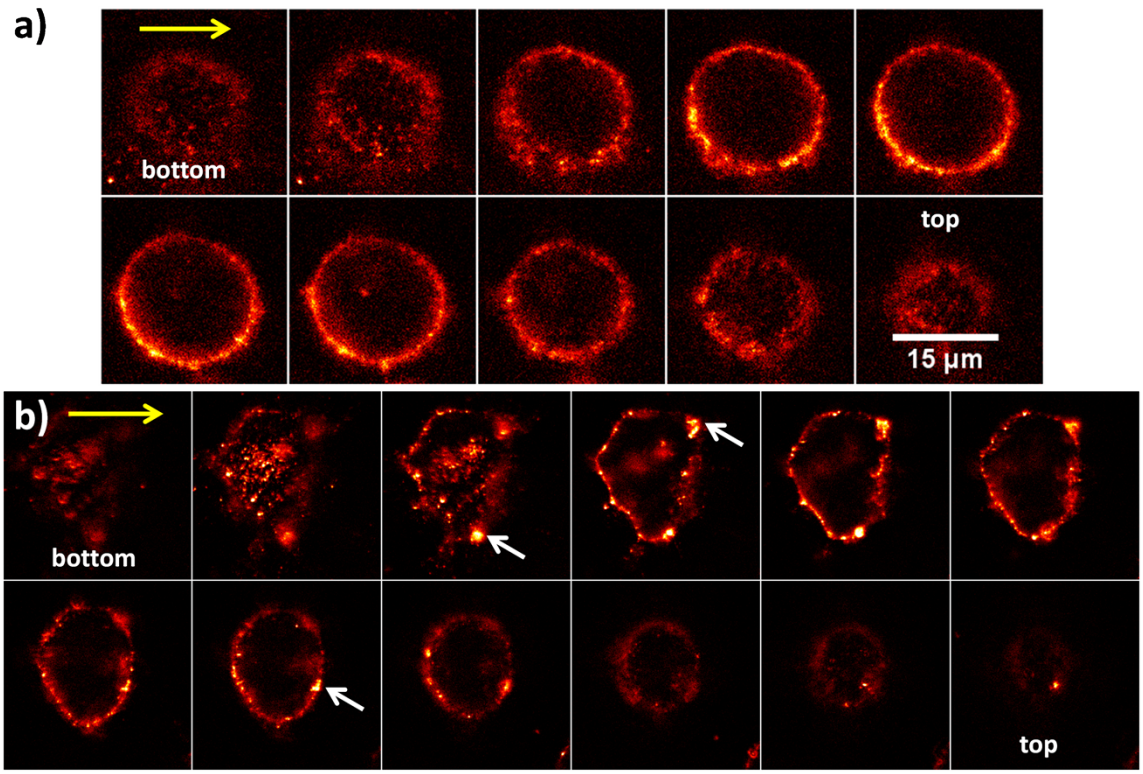

Figure S5. a) and b) are the 3D Z slicing fluorescence images of PFBT SPNs and phycoerytherin labeled MCF-7 cells respectively.

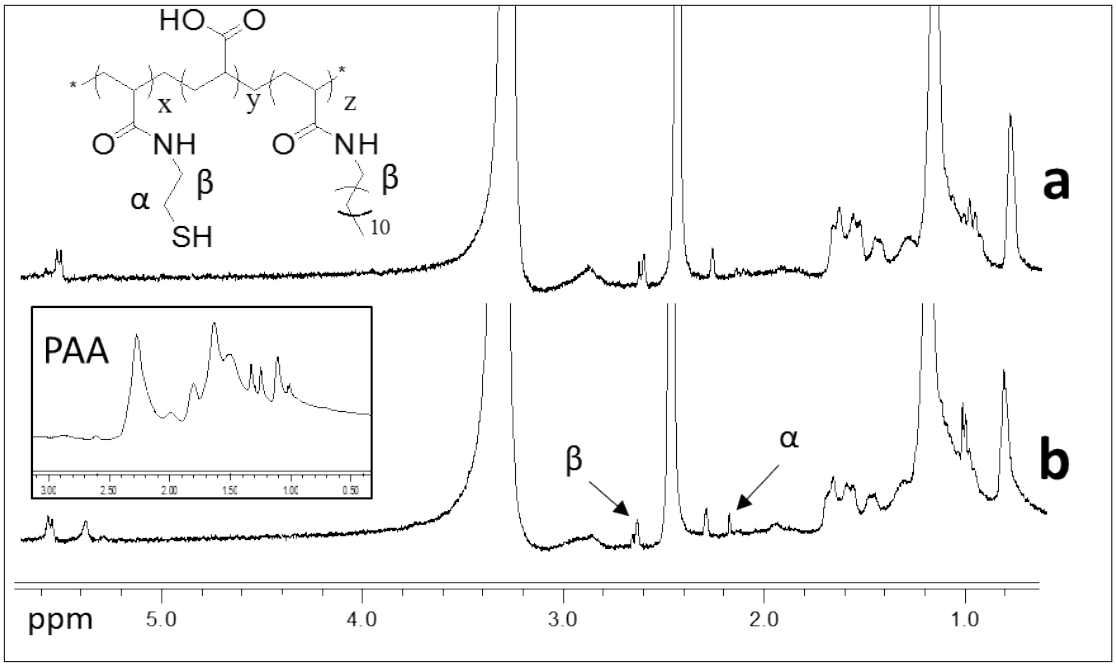

Figure S6. NMR spectra of 40%DDA-PAA (a) and 40%DDA-5%MEA-PAA (b) ligands measured in DMSO-d_6. Triplet peaks at 2.68 ppm (β) and 2.22 ppm (α) are ascribed to the protons in the MEA portion and the protons in the MEA and DDA portion, respectively. Inset shows the ^{1}HNMR spectrum of PAA and molecular structure of 40%DDA-5%MEA-PAA.

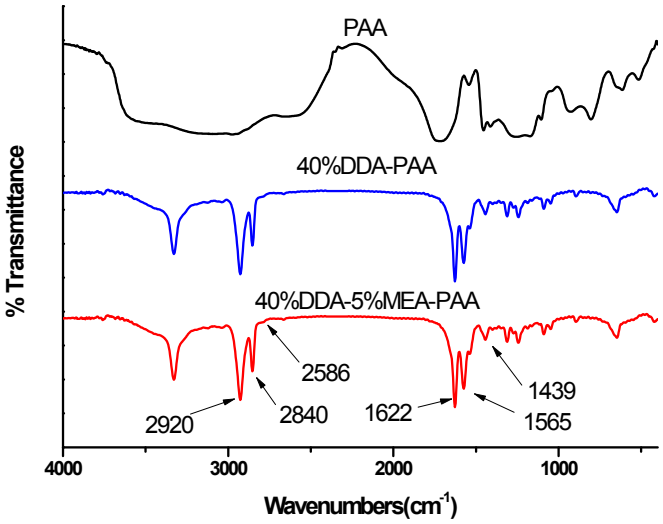

Figure S7. FT-IR spectra of PAA and multidentate polymers. In the spectra of 40%DDA-PAA and 40%DDA-5% MEA-PAA, strong peaks at 1565 and 1439 cm^{-1} are ν_{as} and ν_s of $–COO^-$, peeks at 2920 and 2840 cm^{-1} are the ν_{as} C-H and ν_s C-H of $–CH_2$ in the $–CH_2$ chain from PAA backbone, peak at 1622 cm^{-1} is the $\nu_{C=O}$ of –CONH- from the 40%DDA-5%MEA-PAA, peak at 2586 cm^{-1} is from SH-. These results indicate that we successfully obtained the desired ligands.

References:

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2. Anderson, R. E.; Chan, W. C. W. Systematic Investigation of Preparing Biocompatible, Single, and Small ZnS-Capped CdSe Quantum Dots with Amphiphilic Polymers. *ACS Nano* **2008**, *2*, 1341–1352.
3. van Meerloo J.; Kaspers G. J.; Cloos J. Cell sensitivity assays: the MTT assay. *Methods Mol. Biol.* 2011, 731, 237-245.