Supportive Information

Gold Nanoparticle-Quantum Dot-Polystyrene Microspheres as Fluorescence Resonance Energy Transfer (FRET) Probes for Bioassays

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Experimental Section

Materials and methods

All chemicals were used as received without further purification. Technical grade (90%) trioctylphosphine oxide (TOPO), technical grade (90%) trioctylphosphine (TOP), cadmium oxide (99.99+ %), selenium powder (-100 mesh, 99.5+ %), lauric acid (98+ %), technical grade (90%) hexadecylamine (HDA), diethyl zinc ($Zn(Et)_2$) solution 1.0 M in heptane, hexamethyldisilathiane ($(TMS)_2S$), 16-mercaptohexadecanoic acid (MHDA, 90%), methanol anhydrous (99.8%), chloroform (99.5+ %), DL-Dithiothreitol (DTT) were purchased from Sigma-Aldrich. Custom polyhistidine peptides were purchased from CPC Scientific. Polystyrene microspheres were purchased from Miscospheres-nanospheres. Citrate-stabilized 5 nm gold nanoparticles were purchased from Ted Pella. Rhodamine B was purchased from Alfa Aesar. Succinimidyl esters Alexa 488 and Rhodamine X were purchased from Invitrogen.

Synthesis of TOPO-Capped CdSe/ZnS Luminescent Quantum Dots - TOPO-capped CdSe/ZnS quantum dots were prepared following a method described elsewhere[1]. Briefly, 12.7 mg of cadmium oxide and 200-250 mg of lauric acid were mixed under nitrogen atmosphere. The mixture was heated to ~200 °C to fully dissolve the cadmium oxide (clear color). Then, 2.0 g of TOPO and 2.0 g HDA were added to the solution under constant stirring. Temperature was raised to 250°C before being cooled down slowly to around 200°C. 80 mg selenium powder was then dissolved in 2.0 mL solution of TOP before being rapidly injected into the solution under vigorous stirring. The

temperature was increased to 280°C before being cooled down upon the desired color of quantum dots. For shell coating, a 2.0 mL TOP solution containing 250 μL $(TMS)_2S$ and 1 ml $Zn(Et)_2$ was drop-wise injected into the solution. The reaction mixture was kept at 200°C for one hour before cooling to room temperature and washed three times with methanol, centrifuged at 4,000 rpm for 10 minutes each, and re-dissolved in chloroform and stored in the dark.

Water-Soluble MHDA-Capped and His-tag-Capped Quantum Dots - 50.0 mg of 16-mercaptohexadecanoic acid (MHDA) was heated to 80°C until melted. Then 5.0 mL of TOPO-capped QDs at ~1.0 μM were added to the above solution and stirred overnight at 70°C. The solution was cooled down to room temperature. Then, 5 mL of 0.2 M tetramethylammonium hydroxide pentahydrate (pH ~10) were gradually added to the mixture over 30 minutes under continuous stirring. A two-phase solution resulted after two hours. The top aqueous layer, 16-MHDA coated QDs, was collected, centrifuged, and washed with deionized water. Spin dialysis was performed with 100,000 Da cut off molecular weight centrifuge tube. MHDA-capped QDs then were incubated with 10-20 fold excess of synthetic His-tag peptide H_2N-K_2-H_6-E_2-COOH at room temperature under constant stirring for 1-2 hours. The histidine binding to the surface of QDs occurs through Zn coordination[2]. The final product, QD-His, was washed with deionized water three times by spin dialysis using a 100,000Da cutoff molecular weight centrifuge tube. QD-MHDA is not very stable in water or buffer for long term, and could further cause problem with coupling chemistry. Coating with PolyHis peptide increases the solubility and stability of QDs in aqueous solutions.

Synthesis of Amino-Functionalized Gold Nanoparticles via Cystamine - 2.0 mL of $5.0x10^{13}$ particles/ml citrate-stabilized gold nanoparticles (AuNPs) averaging 5 nm in diameter were incubated with 50.0 mg cystamine in 1.0 mL water for 2 hours at room temperature under continuous mixing. The product was then washed by spin dialysis using a 30,000Da cut-off molecular weight centrifuge tube three times with deionized water.

Cystamine and cysteamine were previously, largely used because the thiol groups bind to the gold surface leaving the free amino groups available for further modification chemistry[3,4]. The most favorable attachment situation of cystamine dithiols on the surface of gold involves a cleavage of disulfide bond[3.] Previous spectroscopic data suggest that dithiols binds preferably through a single thiol end to gold surfaces [5].

Conjugation of QD-His to Carboxylated-Polystyrenes using EDC Coupling - EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) coupling is common condensation chemistry between carboxyl groups and amino groups to produce stable amide bond. EDC is often used in combination with N-hydroxysuccinimide (NHS) or sulfo-NHS to increase coupling efficiency. 0.5 ml of 50 mg/mL carboxyl-modified polystyrene particles ranging from 6-10 μm in diameter in deionized water were incubated with 1.0 mg *N*-Hydroxysulfosuccinimide sodium salt, 2 drops of *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide and 1.0 mL DI water for 15 minutes at room temperature. The mixture was centrifuged and washed three times with deionized water. The EDC activated particles were then resuspended in 0.5 mL DI water and

incubated with approximately 50 to 100-fold in molar excess to number of carboxylic groups on PS surface, for 2 hours at room temperature. This strategy was to ensure that PS surface is completely saturated with QDs and there were no available free carboxyl sites on PS surface after the conjugation, thus enabling QDs for further conjugations. The product, QD-PS, was centrifuged and washed with deionized water three times and then stored in dark conditions at 4 ^{o}C.

Conjugation of Molecular Fluorophores to Aminated-Polystyrenes – 0.5 mL of 6-10 µm polystyrene-NH_2 in water was incubated with excess amount (50 to 100-fold in molar excess) of amine-reactive succinimidyl esters Alexa 488 and Rhodamine X for at least 2 hours at room temperature. The product, dye-labeled PS was centrifuged and washed with DI water several times before being stored at 4 ^{o}C in the dark.

Conjugation of Cystamine-Au to QD-Polystyrenes – A QD-PS solution was activated with the EDC/NHS as described above. It was then incubated with 50-100 fold excess of cystamine-Au for 2 hours at room temperature. The high concentration of AuNPs induces therefore complete fluorescence quenching of QDs. The product was centrifuged and washed three times with deionized water before analysis.

TEM Imaging- Transmission electron microscopy was performed on a Jeol 2010 LaB6 and attached EDAX Genesis analyzer. Sample preparation involved placing QDs or gold NPs samples on carbon-coated copper grids and air-dried the grids. Particle size measurements were carried out on raw TEM images.

Fluorescence Spectroscopy and Fluorescence Imaging Measurements - Emission spectra were measured using a spectrofluorometer (PTI International, Model QM-1) equipped with a 75-W continuous Xe arc lamp as a light source, and by using an inverted fluorescence microscope (Olympus IX70) equipped with a 250 mm Acton spectrograph and a high performance 16 bit resolution, back illuminated CCD camera (Roper Scientific). Luminescence images were obtained using a digital luminescence imaging microscopy system. The system consisted of an inverted fluorescence microscope (Olympus IX51) equipped with a 100 W Hg lamp as a light source. The fluorescence images were collected via a 40X microscope objective with NA = 0.9. A filter cube containing a 450 ±10 nm band-pass excitation filter, a 505 nm dichroic mirror, and a 515-nm long pass emission filter was used to ensure spectral imaging purity. A high performance 16 bit resolution, back illuminated CCD camera of Roper Scientific was used for digital imaging.

Luminescence Properties of CdSe/ZnS QD when coated with TOPO, MHDA and His Peptides – As mentioned in this paper, we have carried out a ligand exchange reaction, to exchange the hydrophobic TOPO ligands with MHDA ligands in order to provide the CdSe/ZnS QD with aqueous solubility. The MHDA ligands were then bound to peptide molecules that contain multiple histidine residues through Zn mediated chelation. This increased the stability of the QD in aqueous solution and their chemical accessibility. Figure S1 shows the luminescence properties of 1µM solutions of CdSe/ZnS QD when coated with TOPO, MHDA and histidine peptides. Figure S1(a) shows that the luminescence intensity of the QD slightly decreases when the TOPO ligands are exchanged with MHDA ligands. However, the luminescence intensity

partially recovers when the MHDA ligands are coated with the histidine peptide molecules. It was reported previously that His-tag binding to the thiol ligand-coated QD increases their emission quantum yield [6]. More importantly figure S1(b) shows that the normalized spectra of the QD are almost identical. This indicates that their photophysical properties of the QD are not significantly affected by these ligand reactions.

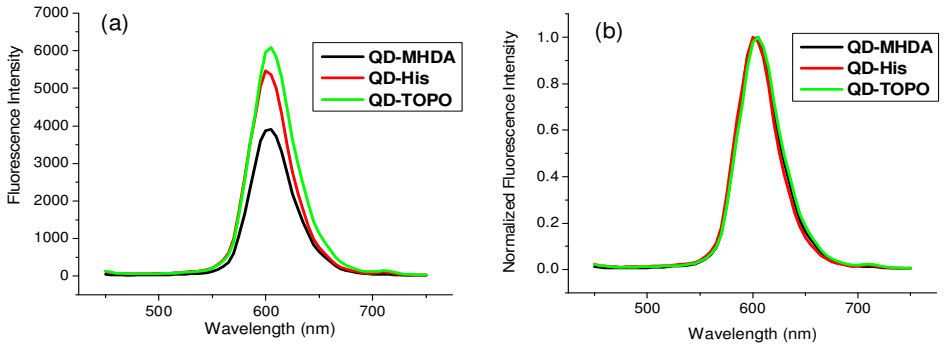

Figure S1 – Luminescent properties of QD-TOPO, QD-MHDA, and QD-His. a) Emission spectra and (b) normalized emission spectra of QD-TOPO (green), QD-MHDA (black) and QD-His(red) (λ_{ex}=450 nm)

Quantum yield (QY) values of QD-TOPO, QD-MHDA and QD-His were further determined using Rhodamine B with 590nm emission wavelength as a standard. All samples were excited at 550 nm and the emission intensity was measured at 590nm. The QY values for QD-TOPO, QD-MHDA and QD-His were found to be 0.56, 0.37 and 0.46 respectively. These results are in agreement with previous studies in which the QY of peptide coated water soluble QD was found to be lower than the QY of QD-TOPO[7].

To calculate the absolute quantum yield we used the following formula[8,9]:

$$QY_{QD} = QY_{st} (G_{QD}/G_{st}) (\rho_{QD}^2/\rho_{st}^2) \qquad [1]$$

QY_{QD} is the emission quantum yield of the QD sample. QY_{st} is the emission quantum yield of a standard sample. In our measurements, Rhodamine B dissolved in ethanol was used as standard with an emission quantum yield of 0.70. G_{QD} and G_{st} were the gradients of the QD and Rhodamine B solutions. The gradients were the slopes of linear plots describing the fluorescence intensity versus absorbance at increasing concentrations of QD and Rhodamine B. ρ_{QD} and ρ_{st} are the refractive indices of the solvents used to dissolve QD and Rhodamine B. The refractive indices were 1.4460 for QD-TOPO in chloroform, 1.333 for QD-MHDA and QD-His in water and 1.361 for Rhodamine B in ethanol.

TEM characterization for size and distribution of AuNPs and QDs

TEM images of AuNPs and QD-TOPO show that both types of nanoparticles average 5nm in diameter with uniform size distribution within 10% variation. The EDS data shows characteristic peaks of Cd, Se, Zn and S for CdSe/ZnS and Au for AuNPs.

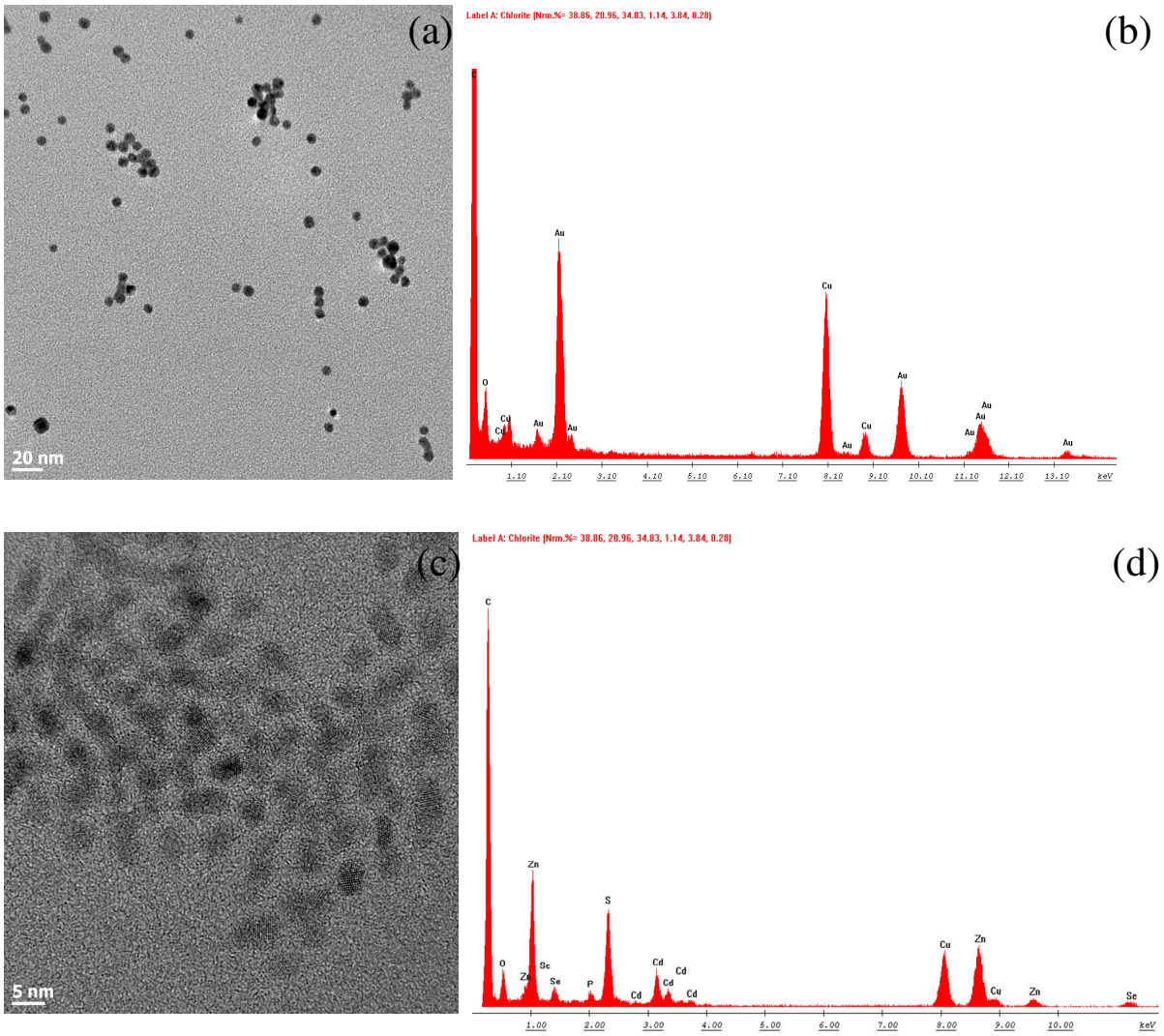

Figure S2 – TEM image (a) and EDS analysis (b) of AuNPs shows the presence of characteristic peaks for Au. TEM image (c) and EDS analysis (d) of QD-TOPO showing uniform size distribution and characteristic peaks for Cd, Zn, Se and S.

DTT effect on QDs photoluminescence

As a control experiment, we investigated whether DTT affects the photoluminescence of QD-PS and whether it interferes with our data interpretation. We determined the fluorescence signal of QD-PS when incubated with 5 mg/ml DTT for 1 hour and in the absence of DTT. DTT quenching of QD luminescence was about 5%.

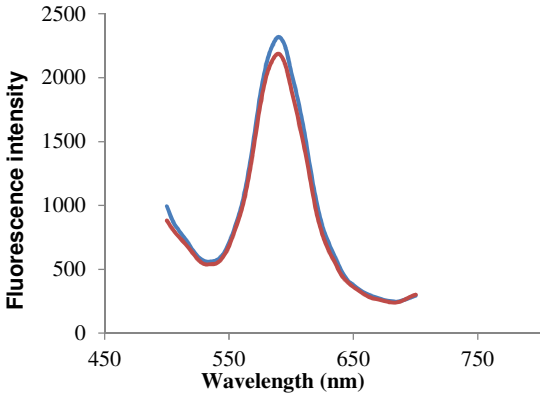

Figure S3 – Luminescence of QD-PS in the absence of DTT (blue line) and following incubation with 5 mg/ml DTT for 1 hour (red line).

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