Supporting Information

Modification of Poly(ethylene glycol)-Capped Quantum Dots with Nickel Nitrilotriacetic Acid and Self-assembly with Histidine-Tagged Proteins

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Materials

CdSe-ZnS core-shell quantum dots (QDs) were synthesized as previously described.[1] The hydrophilic surface ligands (DHLA-PEG750-OMe and DHLA-PEG600-COOH) were synthesized as reported before.[2-5] All manipulations for synthesis of QDs were carried out under nitrogen (previously passed through a drying tower filled with anhydrous $CaSO_4$), unless otherwise stated. Air sensitive solids were handled in a MBraun Labmaster 130 glovebox (Stratham, NH). *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (sulfo-NHS) were purchased from Pierce Biotechnology (Rockford, IL) and used as received. *N,N*-bis(carboxymethyl)lysine was purchased from Fluka and used as received. Agarose (low EEO) was purchased from Fisher Scientific. All the other chemicals (including solvents) were purchased from Sigma Aldrich and Acros Organics.

Preparation of 5xHis-MBP and labeling with Texas Red dye

The *E. coli* derived maltose binding protein (MBP) expressing a C-terminal pentahistidine sequence (5xHis-MBP) was prepared using the procedures described.[6] 5xHis-MBP was labeled with Texas Red succinimidyl-ester dye (Life Technologies, Carlsbad, CA) as described in the same reference.[6] This reactive dye targets the primary amines on MBP of which there are more than 20. Previous mass spectral analysis of 5xHis-MBP labeled with succinimidyl-ester dyes following tryptic digestion has shown preferential labeling of lysine 141,143 and 145 which are surface located along with lysine 47 in the binding pocket.[6] However, the large number of available primary amines do not allow for site-specific labeling with this chemistry. MBP-Texas Red conjugates were purified from free dye with PD-10 desalting columns (GE Healthcare, Piscataway, NJ). UV-vis absorption analysis (MBP 69,000 $M^{-1}cm^{-1}$ at $\lambda_{280\ nm}$ and Texas Red 80,000 $M^{-1}cm^{-1}$ at $\lambda_{595\ nm}$, with ~18% Texas Red correction factor at $\lambda_{280\ nm}$) provided dye-to-protein (D/P) ratios of ~2.6.

Instrumentation

Electronic absorption spectra were recorded using an HP 8453 diode array spectrophotometer (Agilent technologies, Santa Clara, CA). Fluorescence spectra were collected using a Tecan Safire Dual Monochromator Multifunction Microtiter Plate Reader (Tecan, Research Triangle Park, NC). Fluorescence quantum yields were measured at room temperature using a Spex Fluorolog-3

spectrophotometer (Jobin Yvon Inc, Edison, NJ) equipped with a red-sensitive R2658 Hamamatsu PMT detector. The obtained fluorescence spectra were corrected using the spectral output of a calibrated light source supplied by the National Bureau of Standards. Rhodamine 6G in methanol (Φ_f = 0.93) was used as standard.

Cell Culture, Microinjection and Visualization of QD-Maltose Binding Protein (MBP) Assemblies

Adherent COS-1 cells (African green monkey cell line; ATCC, Manassas, VA) were cultured in complete growth medium (Dulbecco's Modified Eagle's Medium (DMEM), purchased from ATCC) supplemented with 1% (v/v) antibiotic/antimycotic (Sigma-Aldrich, St. Louis, MO) and 10% (v/v) heat inactivated fetal bovine serum (ATCC). Cells were cultured in T-25 flasks and incubated at 37ºC under 5% CO_2 atmosphere and a subculture was performed every 3-4 days as described previously.[7] For microinjection experiments, cells were trypsinized from T-25 flasks and seeded into the wells of coverslip bottom dishes (BD Biosciences, Bedford, MA) that were previously coated with 5 μg/mL fibronectin (Sigma-Aldrich). DHLA-PEG-NTA-capped QDs were injected directly into the cells as either QDs alone, QDs assembled with MBP-Texas Red conjugate or QDs mixed with transferrin conjugated to Texas Red. Injections were performed using an InjectMan NI2 micromanipulator equipped with a FemtoJet Programmable Microinjector delivery system (Eppendorf, Westbury, NY). This system allows the delivery of femtoliter aliquots of conjugate solution to individual cells. QD solutions used for delivery contained QDs at a concentration of ~3-5 μM in 0.5x PBS. After injection, the cells were imaged using an Olympus IX-70 microscope (Center Valley, PA). Cultures were excited using 488 nm light provided by a Xe lamp source combined with a 488 nm band pass filter (Chroma Technology, Rockingham, VT). The fluorescence signal was separated from the excitation signal using a 500 nm long-pass filter (Chroma Technology, Rockingham, VT) and collected on a DP71 color digital camera (Olympus, Center Valley, PA). Differential interference contrast (DIC) images of the cell cultures were also collected on the same Olympus IX-70 microscope using a bright field source. The images were then analyzed using DP Manager Software (Olympus, Center Valley, PA) and Image J (NIH, Bethesda, MD). After injections, the cells were incubated at 37ºC in Ringer's solution and were periodically taken out to ambient conditions for microscopy imaging.

Estimate of spectral overlap integral

The Förster separation distance corresponding to 50% energy-transfer efficiency, R_0, described in the text is expressed as a function of the medium refractive index, n_D, the donor quantum yield, Q_D, the orientation factor, κ_p^2, and the spectral overlap integral, I:

$$R_0 = \left(\frac{[9000 \times (\ln 10)] \times \kappa_p^{\,2}}{128 \pi^5 n_D^{\,4} N_A} Q_D I \right)^{1/6} \qquad (1)$$

I is a function of the overlap function between energy donor emission and acceptor absorption, $J(\lambda)$:[8]

$$I = \int J(\lambda)\, d\lambda = \int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda \,/\, \int F_D(\lambda) d\lambda, \qquad (2)$$

where $F_D(\lambda)$ is the fluorescence intensity of the donor (dimensionless), $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor ($M^{-1}cm^{-1}$). The calculated overlap integral for 550 nm emitting QD and Texas Red I = 3.14 x 10^{-13} $M^{-1}cm^3$, which is comparable with other QD-dye pairs we studied previously.[9]

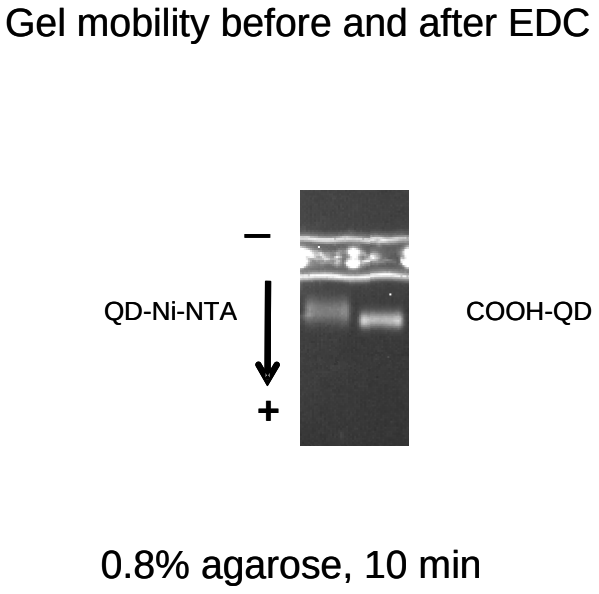

Figure S1: Gel image of QDs before and after coupling to Ni-NTA groups

References

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