SUPPORTING INFORMATION FOR:

Nitrilotriacetic acid-derivatized quantum dots for simple purification and site-selective fluorescent labelling of active proteins in a single step

Manish Gupta, Anne Caniard, Angeles Touceda-Varela, Dominic Campopiano and Juan C. Mareque-Rivas*

School of Chemistry, University of Edinburgh,

Joseph Black Building, King's Buildings,

West Mains Road, Edinburgh, EH9 3JJ, UK

Tel:44-(0)131-650-4761

Fax:44-(0)131-650-4743

Contents:

Materials and Methods
QD Synthesis
Expression and Purification of His_6-SjGST and untagged GST
Protein Binding Studies
GST Activity Assay

Fig. S1 TEM and HRTEM images of the hydrophobic ((a) and (b)) and hydrophilic ((c) and (d)) CdSe-ZnS core-shell nanocrystals.

Fig. S2 X-ray photoelectron spectra highlighting the (A) N 1s and (B) Ni 2p transitions of the DHLA-Ni(NTA) coated CdSe-ZnS core-shell QDs.

Materials and Methods. All chemicals were obtained from commercial sources and used as received. Cadmium oxide (CdO, 99.5%), tri-*n*-octylphosphine oxide (TOPO, 99%), tri-*n*-butylphosphine (TBP, 97%), hexadecylamine (HDA, 98%), diethylzinc ($ZnEt_2$,1M in hexane), hexametyldisilathiane [$(TMS)_2S$ 98%], nickel (II) chloride, *N*-hydroxysuccinimide (NHS, 98%) and *N*-*N*-bis(carboxymethyl)-L-lysine hydrate were purchased from Sigma Aldrich. 1-Ethyl-3-[3'-(dimethylamino)propyl]carbodiimide was purchased from Ademtech. Stearic acid (≥98.5) was purchased from Fluka. Selenium powder (Se, 99.999%) was obtained from Alfa Aesar.

Fluorescence studies were carried out with an Edinburgh Instruments FS900 fluorimeter. Excitation was at 350 nm with bandwidths of 2 nm for excitation (unpolarised) and emission (unpolarised). Temperature was maintained at 25 °C.

X-ray photoelectron spectra were obtained with a VG Scientific Sigma Probe (UK) XPS system. The Al Kα anode X-ray source (hυ = 1486.6 eV) was operated at 200W and the take-off angle for photoelectrons was 37º. Samples were mounted with a spring clip. In a typical experiment, a few survey scans in the -10 to 1100 eV kinetic energy range were collected at a resolution of 1 eV. Then, detailed scans of 20-60 eV over a single feature were collected at a resolution of 0.2 eV. During the measurements the pressure was 10^{-9}-10^{-10} Torr.

High-resolution transmission electron microscopy (HRTEM) studies were conducted on a JEOL JEM-2011 electron microscope operating at 200 kV. The samples were prepared by depositing a drop of a solution of nanocrystals in pyridine onto a copper specimen grid coated with a holey carbon film and allowing it to dry.

Enzyme concentrations were determined by Bradford assay on a UNICAM UV-vis spectrometer with bovine serum albumin as a standard.[1] The QD concentration was determined by the method of Peng et al.[2] Enzyme activity assays were conducted on a CARY 300 SCAN UV-vis spectrometer.

QD Synthesis. Tri-*n*-butylphosphine selenide (TBP-Se, 1M) was prepared in a N_2-filled glove box by shaking 0.08 g of selenium powder in 1 mL of TBP. A ZnS stock solution was prepared in a N_2-filled glove box by reacting $(TMS)_2S$ (0.5 ml, 2.5 mmoles) with $ZnEt_2$ (3.5 ml, 3.5 mM in hexane) in TOP (6 ml).

TOPO-coated CdSe-ZnS QDs. The TOPO-coated core-shell CdSe-ZnS QDs were synthesised and purified according to previously reported procedures.[3] In a typical synthesis, CdO (0.013 g, 0.1mmol) and stearic acid (0.254 g, 0.89 mmol) were loaded into a three-neck flask and heated to 225 °C under N_2 flow and stirring. Once the

mixture was completely dissolved, it was allowed to cool to room temperature. Then, TOPO (3 g) and HDA (1 g) were added and the mixture was heated to 225 °C under N_2 flow and vigorous stirring. At this temperature, 1 mL of freshly prepared TPB-Se solution (1 M) was quickly injected into the flask. Following injection the temperature was adjusted to ~200 °C to promote nanocrystal growth. On reaching the desired nanoparticle size, as determined by UV/vis and fluorescence spectroscopy,[2] the temperature is lowered to ca. 100 °C to stop further growth. Then, the solution is heated to 210 °C. At this temperature, 0.5 ml of the ZnS stock solution was slowly injected in. The temperature of the mixture was set to 100 °C and stirred for 2 hr. After cooling to room temperature, the nanocrystals were dispersed in chloroform and precipitated by addition of methanol. After centrifugation the supernatant liquid phase was removed. This procedure was repeated at least three times. The precipitates were combined and air-dried at room temperature. The results reported in this study are for nanocrystals which had the first absorption band at 594 nm, and a maximum emission peak at 610 nm with excitation at 350 nm, but the same results were obtained with QDs of different sizes and emission maxima.

DHLA-coated CdSe-ZnS QDs. Dihydrolipoic acid (DHLA) was prepared according to the method given by Uyeda *et al.*[4] Thiotic acid (1.22 g) was dissolved in an aqueous solution of $NaHCO_3$ (25 ml, 0.25 M) and the resultant solution was cooled to 0 °C. $NaBH_4$ (0.9 g) was added and the temperature was maintained at ca. 5 °C with continuous stirring for about 2 hr in nitrogen. The reaction mixture was acidified with HCl (3.3 M) to pH 1. The product was extracted with toluene (~30 ml) dried by adding $MgSO_4$. The final product was isolated as colourless oil.

Then, the water-soluble DHLA-coated CdSe/ZnS QDs were prepared according to the method given by Mattousi *et al.*[5] In a typical reaction, TOPO-coated CdSe-ZnS QDs (50 mg) and DHLA (50 mg) were mixed in DMF (15 ml). The solution was heated to 60-70 °C and stirred overnight. Then, potassium-*tert*-butoxide (100 mg) was slowly added to deprotonate the terminal lipoic acid groups. The nanoparticles were separated by centrifugation and the supernatant liquid was discarded. The resulting quantum dots were dried under vacuum. The QDs were further purified by redissolving them in water and passing this solution through a 100K microsep filter.

NTA-coated CdSe/ZnS QDs. *N-N*-bis(carboxymethyl)-L-lysine hydrate (NTA) (5 mg, 0.0132 mmoles) was dissolved in 3 ml of HEPES (20 mM, pH 7.5). Activation of

carboxylate groups of the DHLA-coated QDs was performed by mixing the QDs (9 mg) in 1 ml of EDC (3 mM in HEPES) and 1ml of NHS (3 mM in HEPES) with stirring for 30 min. Then, 1ml of NTA (4.4 mM) was added and the solution was stirred for 24 hrs. The resulting NTA-modified nanocrystals were purified using a Nanosep 100k omega membrane filter (low protein-binding, modified polyethersulfone on polyethylene substrate). The Ni-NTA coated nanocrystals were prepared in the same way using the preformed complex, which was prepared by mixing of *N,N*-bis(carboxymethyl)-l-lysine hydrate (NTA) (5 mg, 0.0132 mmoles) and $NiCl_2$ (5 mg, 0.021 mmoles) in 3 ml of HEPES (20 mM) with stirring for 1 hr using. Alternatively, the NTA-coated nanocrystals (18 μM) were dissolved in water and reacted with $NiCl_2$ (200 μM) for 1 hr. The excess nickel was removed by passing the solution through a 100K microsep filter.

Expression and Purification of His_6-SjGST and untagged GST

Overexpression of *Schistosoma japonicum* His_6-GST and untagged GST were achieved as we described previously[6] with the following modifications. Fresh LB broth medium (3 L) were inoculated with 100 mL of seed culture and induced with isopropyl thio-β-D-galactoside (IPTG) (0.1 mM final concentration) when OD_{600} reached 0.6. The cell debris was removed by centrifugation at 15000 g for 1 h at 4 °C. Purification of *Schistosoma japonicum* His_6-GST was achieved as described previously.[6]

Purification of untagged GST. The cell lysate was applied to a GSTPrep FF 16/10 column (20 mL, GE Healthcare Life science) pre-equilibrated with 1x PBS binding buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4). The column was then washed with 5 column volumes of binding buffer (100 mL) before bound material was eluted with an elution buffer (10 mM glutathione, 50 mM Tris/HCl, pH 8.0) over 5 column volumes (100 mL). Fractions were analysed by SDS-PAGE, and those containing GST were pooled and dialyzed overnight against 4 L of a 20 mM potassium phosphate buffer (pH 7.0) at 4 °C. Liquid chromatography mass spectrometry (LCMS) analysis of the pure enzyme gave the molecular mass of the SjGST as 28059 (± 0.9 Da), in good agreement with the predicted value of 28057 Da for the monomer. The final yield of untagged GST using this method was ~ 25 mg per L of bacterial culture.

Protein Binding Studies

Protein binding studies were carried out by incubating 100 μl of the corresponding QD (1.5×10^{-5} M) with 75 μl of His-GST and Untagged-GST (1.09 mg/ml) for 2 hr at room temperature. The enzyme-QDs complexes were separated from the unbound enzyme molecules by passing this solution through a Nanosep 300k Omega membrane filter (low protein-binding, modified polyethersulfone on polyethylene substrate). The retenate containing the QD-bound enzyme was treated with 175 μl of a PBS solution of imidazole (0.5 M) to release the enzyme. Retenate and filtrate fractions were analysed by SDS-PAGE. Incubation of QDs with cell lysates was carried out at 4 ^{o}C.

GST Activity Assay

The GST activity was determined spectrophotometrically by measuring the change in absorbance at 340 nm (ε = 9600 M^{-1} cm^{-1}).[7] The solution for the blank assay was composed of potassium phosphate buffer (850 μl, 0.1 M, pH 6.5), 1-chloro-2, 4-dinitrobenzene (50μl, 40 mM) and GSH (100 μl, 80 mM). The solution for sample assay was composed of potassium phosphate buffer (800 μl, 0.1 M, pH 6.5), 1-chloro-2,4-dinitrobenzene (50 μl, 40 mM), GSH (100 μl ,80 mM) and GST (His-GST 50 μl, 2.7 mg/ml; Untagged-GST 50 μL, 1.09 mg/ml free and immobilized on QD-NTA-Ni^{+2} (9 μM)). The absorbance was measured at 340 nm for five minutes (T = 25 °C).

References:

1. M. Bradford, *Anal. Biochem*. 1976, **72**, 248.

2. W. W. Yu, L. Qu, W. Guo and X. Peng, *Chem. Mater*., 2003, **15**, 2854.

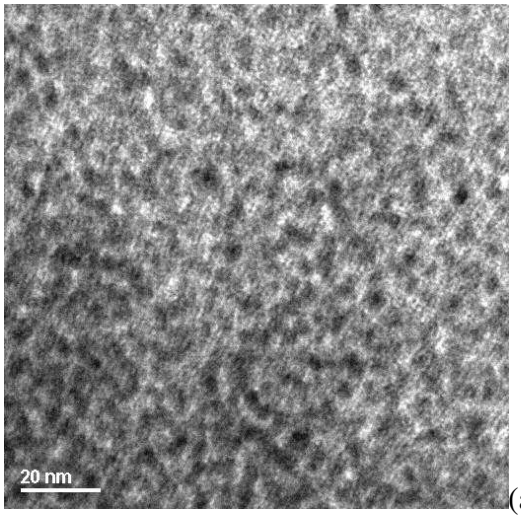
3. (a) L. Qu and X. Peng, *J. Am. Chem. Soc*., 2002, **124**, 2049; (b) T. Jin, F. Fujii, E. Yamada, Y. Nodasaka and M. Kinjo, *J. Am. Chem. Soc*., 2006, **128**, 9288.

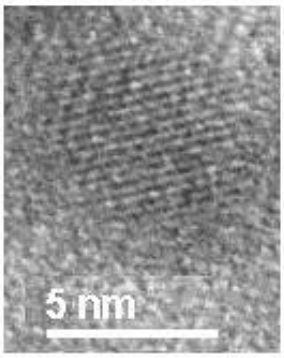
4. H. T. Uyeda, I. L. Medintz, J. K. Jaiswal, S. M. Simon and H. Mattoussi, *J. Am. Chem. Soc.*, 2005, **127**, 3870.

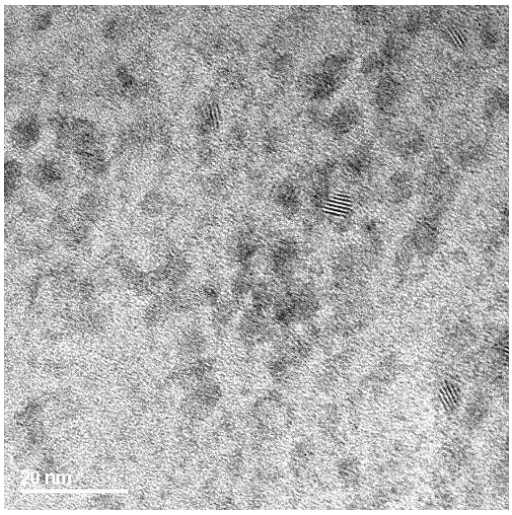
5. H. Mattoussi, J. M. Mauro, E. R. Goldman, G. P. Anderson, V. C. Sundar, F. V. Mikulec and M. G. Bawendi, *J. Am. Chem. Soc*., 2000, **122**, 12142.

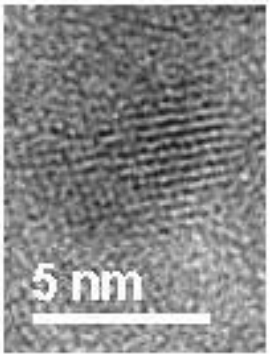
6. B. Shi, R. Stevenson, D. J. Campopiano and M. F. Greaney, *J. Am. Chem. Soc*., 2006, **128**, 8459.

7. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. Biol. Chem*., 1974, **249**, 7130.



(a)


(b)


(c)


(d)

Fig. S1 TEM and HRTEM images of the hydrophobic ((a) and (b)) and hydrophilic ((c) and (d)) CdSe-ZnS core-shell nanocrystals.

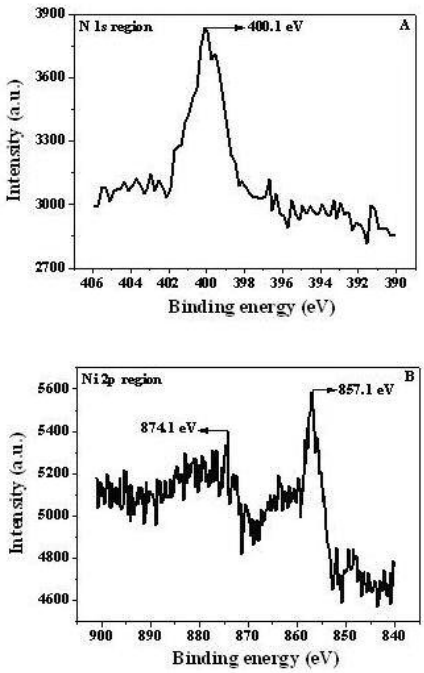


Fig. S2 X-ray photoelectron spectra highlighting the (A) N 1s and (B) Ni 2p transitions of the DHLA-Ni(NTA) coated CdSe-ZnS core-shell QDs.