Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information

Low-toxic Mn-doped ZnSe@ZnS Quantum Dots Conjugated with Nano-hydroxyapatite for Cell Imaging

Ronghui Zhou,[1] Mei Li,[2] Shanling Wang,[2] Peng Wu,[2] Lan Wu,[2]* Xiandeng Hou[1, 2]

[1]College of Chemistry, [2]Analytical & Testing Center, Sichuan University, Chengdu, 610064, China

*Corresponding authors, E-mails: wulan@scu.edu.cn;

Experimental Section

1. Chemical Reagents.

All reagents used were of at least analytical grade. Zinc stearate ($ZnSt_2$, 99.99%), stearic acid （HSt，98%），selenium （Se，≥99.999%），manganese chloride ($MnCl_2$, 99.99%), tetramethylammonium hydroxide pentahydrate (TMAH), oleic acid (OA, AR), octadecylamine（ODA，≥97%）, dimethyl sulfoxide (DMSO)，genistein， amiloride，chlorpromazine and ethanol absolute (AR) were brought from Aladin, Shanghai, China. 1-Octadecene (ODE, 90%), and oleylamine (OAm, 70%), *o*-phosphoethanolamine (PEA) and 3-mercaptopropionic acid (MPA) were purchased from Sigma-Aldrich. $ZnAc_2.7H_2O$ and Tris were obtained by Sinopharm Chemical Reagent Co., Ltd, Shanghai,China. $Ca(NO_3)_2$, NaF, $Na_3PO_4.12H_2O$, $Na_2HPO_4.12H_2O$, NaH_2PO_4, and Na_2S were supplied by Chengdu Kelong Chemical Reagent Company, China. Ultrapure water (18.25 MΩ·cm) produced with a purification water machine (PCWJ-10, Pure Technology Co. Ltd, Chengdu, China) was used throughout this work.

2. Characterization

The UV-Vis and fluorescence emission spectra were obtained with a UV-1700 UV/Vis spectrophotometer (Shimadzu, Japan) and an F-7000 spectro-fluorometer (Hitachi, Japan); TEM and EDX were performed with a Tecnai G2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., USA); X-ray diffraction (XRD) spectra were carried out using an X'Pert Pro MPD X-ray diffractometer (Philips, Netherlands) with Cu Kα (λ=1.5406 A°); X-ray photoelectron spectroscopy (XPS, PHI5000 Versa Probe, ULVAC-PHI) was used to show the valent state of the product; Fourier transform infrared spectra (FTIR) were collected using a Nicolet IS10 FTIR spectrometer (Thermo Inc.,America); The concentration of Zn^{2+} was measured with an inductively coupled plasma atomic emission spectrometer (SPECTRO ARCOS, Germany).

3. Synthesis of ZnSe:Mn@ZnS QDs

Mn-doped ZnSe QDs was synthesized via the "nucleation-doping" approach developed by Peng *et al.*[1-3] $MnSt_2$ was first prepared via the precipitation reaction between $MnCl_2$ and stearic acid (SA). The manganese stock solution was prepared by dissolving $MnSt_2$ (0.025 g) in 2.5 g ODE and heated to 100 °C after being degassed under argon flow. The zinc precursor solutions were prepared by dissolving $ZnSt_2$ (0.36 g) and SA (0.04 g) in 2.4 g ODE.

Typically, 0.0474 g selenium powder and 0.1 g oleylamine in 9 g ODE were loaded in 100 mL four-neck flask and degassed at 100 °C for 30 min by bubbling with argon. Then the temperature was further raised to 280 °C and kept at this temperature until selenium powder was completely dissolved. At this temperature, 2 mL $MnSt_2$ solution was injected into the reaction system. The reaction mixture was allowed to cool to 260 °C and reacted for 2 min to form MnSe nanoclusters. Subsequently, 1 mL heated Zn stock solution was added to the solution together with 200 µL oleylamine to activate the zinc carboxylate . After stirring at 250 °C for 15 min, the second Zn stock solution (1 mL) with 200 µL oleylamine was added and the temperature cooled down to 240 °C, the reaction mixture stayed at the temperature for another 15 min. Finally, the reaction was cooled to room temperature, and the nanocrystals were purified using acetone/$CHCl_3$ extraction for 3 times. After purification, 40 mg the as-synthesized Mn-doped ZnSe QDs was dissolved in 2 mL chloroform and treated with 1 mL MPA (neutralized with NaOH). The mixture was shaken for 30 min with sonication. The chloroform solution gradually became turbid as the long hydrocarbon chains of the original ligands were replaced by the carboxyl groups of MPA. The MPA-coated QDs precipitate was isolated by centrifugation and decantation, and the excess MPA was further removed by washing the precipitate three times with chloroform.

For the growth of ZnS shell[4], the above MPA-capped Mn doped ZnSe nanocrystals was dissolved in water and deaerated with argon bubbling for 30 min. Then 1mL of 0.24 mM Na_2S solution and 1 mL of 0.24 mM $Zn(CH_3COO)_2$ solution were dropped alternately at 50 ºC. Reaction temperature was maintained at 70 ºC for 40 min. The final solution of MPA-capped ZnSe:Mn@ZnS core/shell nanocrystals was purified with ethanol and stored in distilled water with a concentration of 5 mg/mL.

4.The procedure for the determination of the fluorescence quantum yields

The PL QY of ZnSe:Mn@ZnS QDs was obtained by using the comparative method of Williams et al.[5] Rhodamine 6G was used as the standard for the quantum yield correction, which has an overlapping emission peak with ZnSe:Mn@ZnS QDs and can be excited at the same wavelength. The Rhodamine 6G (Q_R= 0.95) was dissolved in absolute ethanol (refractive index (n_R) of 1.360) and the ZnSe:Mn@ZnS QDs was dissolved in distilled water (n_X= 1.333). The absorbances of QDs and Rhodamine 6G solution were adjusted with identical absorbance (0.05) at 350 nm, and then a simple ratio of the integrated fluorescence intensities of the two solutions (recorded under identical conditions) will yield the ratio of the quantum yield values (Q_X) according to the following equation:

$$Q_X = Q_R \cdot \frac{A_R}{A_X} \cdot \frac{F_X}{F_R} \cdot \left(\frac{n_X}{n_R}\right)^2$$

where Q_R is the quantum yield of the standard, F is the corrected emission intensity and n is the average refractive index of the solution.

5. Synthesis of monodispersed nano-FAp

The oleic acid-capped monodisperse nano-FAp nanoparticles was synthesized based on a hydrothermal method. [6-8] Firstly, 0.5 g octadecylamine and 4 ml oleic acid were dissolved into 16 mL ethanol under agitation. Then, $Ca(NO_3)_2$ (0.5 g in 7.5 mL distilled water) and NaF (0.17 g in 2 mL distilled water) were added into the above solution in order. After stirring for 5 minutes, an aqueous solution of $Na_3PO_4.12H_2O$

(0.5 g in 7.5 ml distilled water) was dropped into above solution and the mixture was agitated for about 10 min. Finally, the mixture was transferred into a 40 mL autoclave, which was then treated at temperature of 100 ºC for about 10 hours. When the autoclave was cool down to room temperature, the products could be collected at the bottom of the vessel, and then washed with ethanol, distilled water and a small amount of cyclohexane.

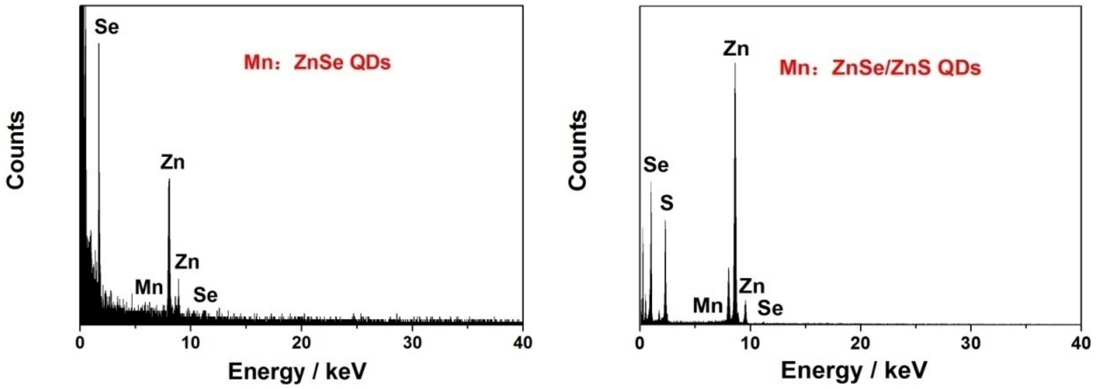

Figure S1. EDX results of Mn:ZnSe QDs and Mn:ZnSe@ZnS QDs

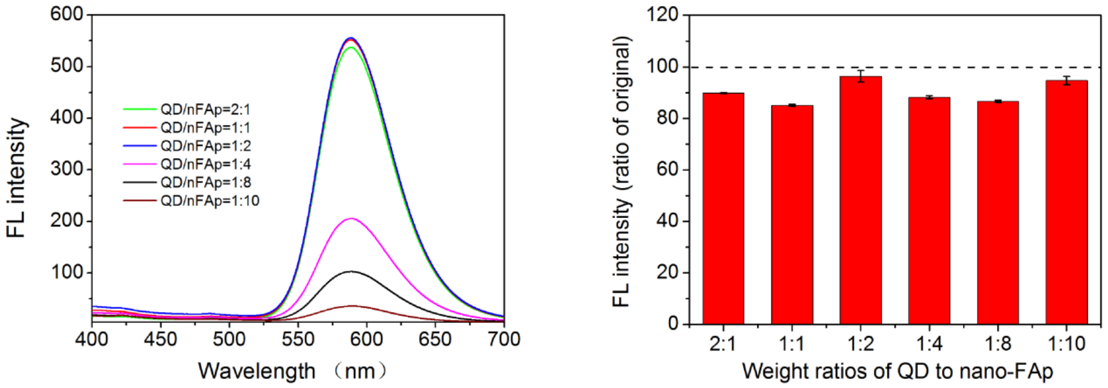

Figure S2. FL emission spectra (Left) and FL intensity change after 60 min UV irradiation (Right) of the nano-FAp-QD conjugate with different QD/nFAp weight ratios. Exciting wavelength : 365 nm.

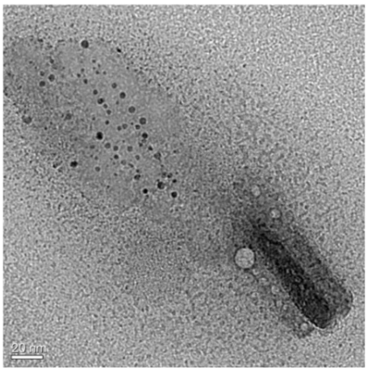

Figure S3. TEM imaging of the nano-FAp-QD nanoparticles where the crystal structure of the nano-FAp is destroyed by high-energy electron beam but the conjugated QDs was left.

References

1. R. S. Zeng, M. Rutherford, R. G. Xie, B. S. Zou and X. G. Peng, *Chem. Mat.*, 2010, 22, 2107-2113.
2. N. Pradhan and X. G. Peng, *J. Am. Chem. Soc.*, 2007, 129, 3339-3347.
3. W. J. Zhang, Y. Li, H. Zhang, X. G. Zhou and X. H. Zhong, *Inorg. Chem.*, 2011, 50, 10432-10438.
4. D. Zhu, X. X. Jiang, C. Zhao, X. L. Sun, J. R. Zhang and J. J. Zhu, *Chemical Communications*, 2010, 46, 5226-5228.
5. A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst*, 1983, 108, 1067-1071.
6. J. F. Hui, G. L. Xiang, X. X. Xu, J. Zhuang and X. Wang, *Inorg. Chem.*, 2009, 48, 5614-5616.
7. J. F. Hui and X. Wang, *Chem.-Eur. J.*, 2011, 17, 6926-6930.
8. X. Wang, J. Zhuang, Q. Peng and Y. D. Li, *ature*, 2005, 437, 121-124.