A salt-out strategy for purification of amphiphilic polymers-coated quantum dots

Zhi-Liang Chen [a,b]*, *Yi Lin* [b], *Chun-Nan Zhu* [c], *Zhi-Ling Zhang* [b], *and Dai-Wen Pang* [b]

[a] School of Pharmacy, Shaoyang University, Shaoyang, 422000, P. R China.

[b] College of Chemistry and Molecular Sciences, State Key Laboratory of Virology, The Institute for Advanced Studies, and Wuhan Institute of Biotechnology, Wuhan University, Wuhan 430072, P. R. China.

[c] Hubei Key Laboratory of Medical Information Analysis and Tumor Diagnosis & Treatment, College of Biomedical Engineering, South-Central University for Nationalities, Wuhan 430074.

Corresponding author: Dr. Zhi-Liang Chen

E-mail: chengzhiliang0411@163.com.

Phone: +86-739-5308282

Experimental Section

Materials

1-Octadecene (ODE, 90%), zinc acetate ($Zn(AC)_2 \cdot 2H_2O$, 99%), cadmium oxide (CdO, 99.99%), 1-octanethiol (98.5%), oleic acid (90%), oleylamine (90%), 1-ethyl-3-(3-dimethylamino-propyl)- carbodiimide (EDC), polyacrylic acid (MW 1800), and selenium powder (Se, 99.99%) were purchased from Aldrich. Amine-PEG-carboxyl (NH_2-PEG-COOH, MW 2000) was purchased from Laysan Bio. Anti-rabbit second antibody and anti-mouse second antibody were purchased from Bioss. Other chemicals including sodium chloride (NaCl), potassium chlorid (KCl), Magnesium chloride (Mg_2Cl) N, N-dimethylformamide (DMF), chloroform, toluene, and octylamine were purchased from China National Pharmaceutical Group Corporation. Ultrapure water (18 MΩ. cm^{-1}) was prepared by a Millipore Milli-Q system.

Synthesis of CdSe/ ZnSe QDs

The CdSe/ZnSe QDs was synthesized according to previous reports[1−3]. Briefly, approximately 60 mg of CdO, 2 mL of oleylamine, 25 mL of ODE, 2 mL of oleic acid, and 30 mg of $Zn(AC)_2 \cdot 2H_2O$ were added to a 250 mL flask. The resulting mixture was heated to 120 °C under vacuum for 30 min to remove water and oxygen. After purging 5 times with argon, the mixture was further heated to 320 °C. Approximately 35 mg of Se powder dispersed in 5 mL of TOP solution was swiftly injected into the flask. The resulting mixture was then cooled and maintained at 290 °C. Subsequently, 0.5 mL of octanethiol dispersed in 10 mL of ODE was added into the flask at a flow rate of 1.5 mL/min. Finally, by removing heating mantle, the resulting CdSe/ZnSe QDs were cooled to room temperature and were precipitated with ethanol. Different-colored CdSe/ ZnSe QDs were prepared by varying the amount of CdO and octanethiol.

Synthesis of OPA

The OPA was synthesized according to our previous literature[4]. Briefly, 500 mg of polyacrylic acid mixed with 792 mg of EDC were dispersed in 20 mL of DMF. Approximately 341 μL of octylamine was added dropwise into the flask and stirred for 12 h under argon. Subsequently, DMF was removed under vacuum, and hydrochloric acid (1 M) was added to obtain

a sticky white precipitate. After centrifugation and washed with ultrapure water, the sticky white precipitate was dissolved in ethanol and dried by rotary evaporation. The as-prepared OPA was stored a desiccator for QDs water-solubilization.

Preparation and characterization of OPA-QDs and OPA micelles

Hydrophobic QDs (1×10^{-9} mol) and 3 mg of OPA were dispersed in 20 mL of chloroform. Subsequently, a membrane layer contained OPA-QDs was obtained by removing chloroform with a rotary evaporation and was dissolved in 4 mL of borate saline buffer (pH 9, 50 mmol/L) to obtain OPA-QDs solution which contains of the self-assembly OPA micelles. Similarly, OPA of the same amount was dispersed in chloroform, and a membrane was also obtained by removing chloroform and dissolved in 4 mL of borate saline buffer (pH 9, 50 mmol/L) to obtain OPA micelles. Then, OPA-QDs solution were separated by size exclusion chromatograph (Shimadzu Corporation) to obtain OPA-QDs alone and OPA micelles for the dynamic light scattering characterization. An UV-Vis detector (SPD-20A, Shimadzu Corporation) at 210 nm was utilized to monitor the purification process. A Shodex KW-804 size exclusion column (Showa Denko scientific instruments (Shanghai) Co., Ltd.) was used to separate OPA micelles from OPA-QDs solution. An automatic fraction collector was utilized to collect the OPA-QDs and OPA micelles. Borate saline buffer (pH 7.2, 20 mmol/L) containing NaCl (0.2 mol/L) was utilized as the mobile phase with a flow rate of 0.5 mL/min. Typical injection volume was 100 μL, and all the purification was performed at room temperature.

Transmission electron microscope (TEM) images were obtained on a JEM2010FEF (UHR) microscope with an acceleration voltage of 200 kV. Approximately 0.5 μL of sample (1×10^{-11} mol) mixed with 2 μL of phosphotungstic acid (2%) were dropped onto copper grids and dried at room temperature for TEM characterization.

Agarose gel electrophoresis was carried out on a DYY-6D gel electrophoresis apparatus (Beijing LiuYi instrument factory) with 1% (w/v) agarose gel, the gel picture was obtained by alphaimager HP system (Alpha Innotech). Zeta potential measurements were performed on a Malvern Nano ZS-ZEN 3600 instrument (Malvern Instruments, UK).

Salting-out of OPA-QDs solution

Approximately 1 mL of NaCl (2 mol/L) were added into 50 μL of OPA-QDs (3 μmol/L) solution, OPA-QDs solution change turbid aqueous phases. After 2 hours, the NaCl-treated OPA-

QDs solution could be again recovered to transparent. The resulting samples were filtered by a centrifugal filter device with the MWCO of 50 kDa to remove NaCl, and the purified OPA-QDs was dispersed in borate saline buffer (pH 7.4, 20 mmol/L) and stored in room temperature for further characterization. The large-scale purification of OPA-QDs are as follows, approximately 100 mL of NaCl (2 mol/L) were added into 5 mL of OPA-QDs (3 μmol/L) solution at room temperature, filtered by a centrifugal filter device to remove NaCl, the resulting OPA-QDs were dispersed in borate saline buffer (pH 7.4, 20 mmol/L).

Preparation of OPA-QDs-PEG and QDs-IgG nanoprobes

The preparation of OPA-QDs-PEG and QDs-IgG nanoprobes was carried out according to our previous literature.[4] Briefly, 10 mg of EDC and 10 mg of NH_2-PEG-COOH (MW 2000) dispersed in borate saline buffer (pH 7.4, 20 mmol/L) were mixed with 2 mL of purified OPA-QDs (3×10^{-9} mol). After stirred at 160 rpm for 1.5 h, the resulting OPA-QDs-PEG were purified by gel filtration on NAP-10 column (GE Healthcare) to remove the excess of NH_2-PEG-COOH. QDs-IgG nanoprobes were prepared by conjugating IgG to the surface of OPA-QDs-PEG. Briefly, 400 μg of IgG and 20 μg of EDC dispersed in 1.5 mL borate saline buffer containing 0.8 nmol OPA-QDs-PEG (pH 7.4, 20 mmol/L), After stirred at 160 rpm for 1.5 h, the resulting QDs-IgG nanoprobes were filtered by a centrifugal filter device with the MWCO of 50 kDa and purified by a column with the medium of Superdex 200 prep grade (GE Corporation).

QDs-Based Double Immunofluorescence Labeling

The QDs-Based Immunofluorescence labelling procedure was carried out according to the instructions by Wuhan Jiayuan Quantum Dots Co., Ltd., Wuhan, China. The paraffin-embedded tonsil tissues were provided as a gift from Hubei Cancer Hospital. Briefly, tonsil tissues were dewaxed with xylene and dehydrated with 100%, 95%, 80%, and 70% alcohol, respectively. Human complement receptor type2 (*CR2*) and nuclear protein *Ki-67* (*Ki-67*) retrieve were performed in boiling EDTA (pH 9.0, 10 mM) solution for 3 min. Subsequently, tonsil tissues were incubated with rabbit anti-human *CR-2* monoclonal antibody and mouse anti-human *Ki-67* monoclonal antibody at 4 °C overnight, and washed three times with TBS-T (0.1 M Tris-base, 0.5% Tween, 0.8% NaCl, pH 7.6) to remove the excess primary antibodies. Then the as-prepared two kinds of QDs-IgG nanoprobes were simultaneously added to incubate with *CR2* and *Ki-67* on tonsil tissues at 37 °C for 1 h, and washed three times with TBS-T to remove the excess QDs-IgG

nanoprobes. The fluorescence signal of QDs was collected using the Caliper multispectral microscopy imaging system (Caliper Life Sciences, Hopkinton, MA, USA).

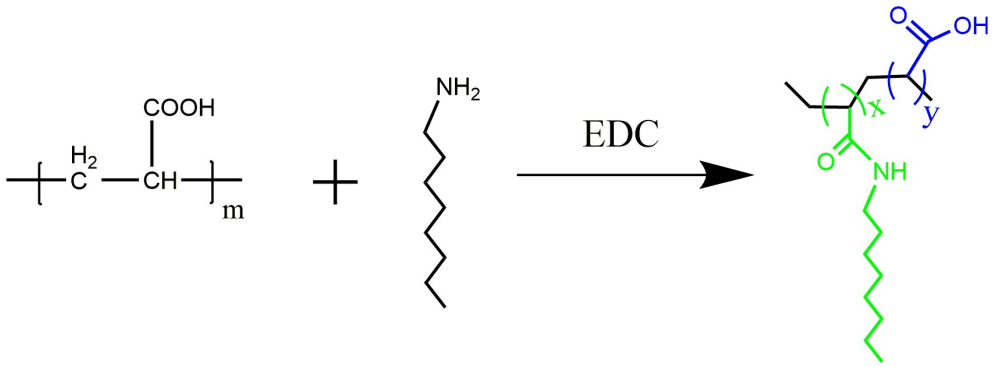

Fig. S1 Schematic diagrams of the synthesis of OPA

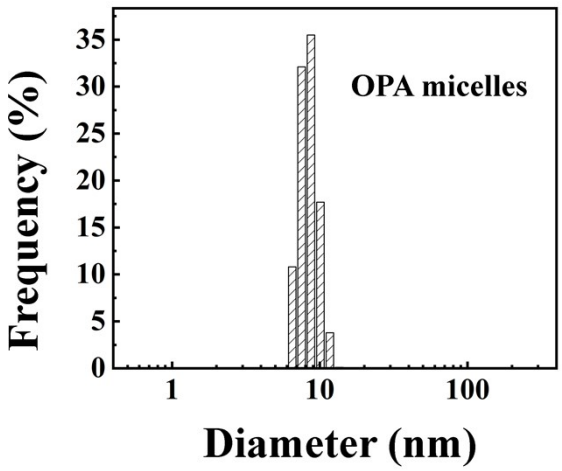

Fig. S2 Hydrodynamic diameter distributions of OPA micelles

Fig. S3 Hydrodynamic diameter distributions of OPA-QDs

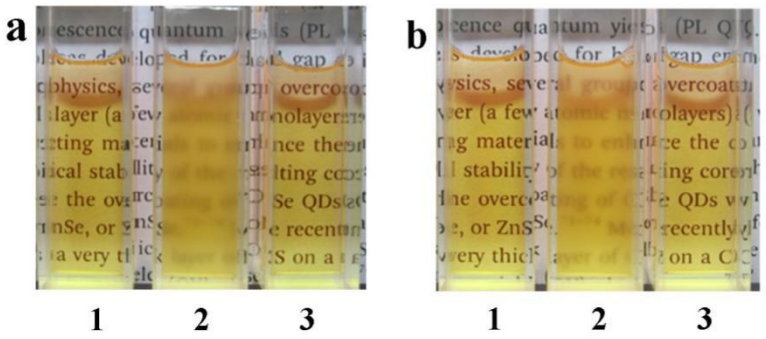

Fig. S4 (a) Picture of OPA-QDs before and after adding NaCl (1: no NaCl; 2: adding NaCl; 3: 2 hours after adding NaCl). (b) Picture of OPA-QDs before and after adding KCl (1: no KCl; 2: adding KCl; 3: 2 hours after adding NaCl)

Fig. S5 (a) Picture of OPA-QDs solution after adding $MgCl_2$

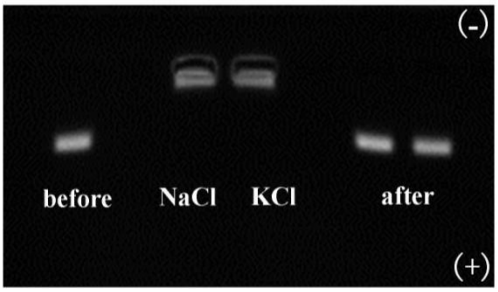

Fig. S6 Agarose gel electrophoresis image of OPA-QDs before and after salt-out with NaCl or KCl.

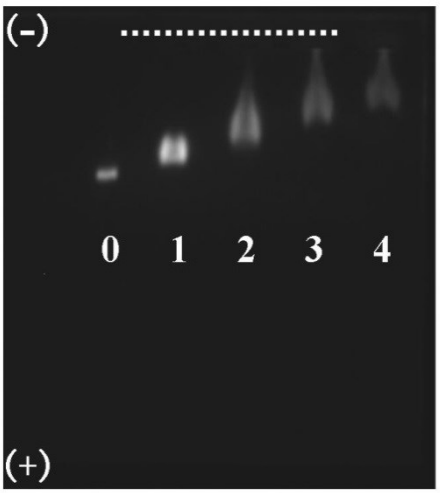

Fig. S7 Agarose gel electrophoresis image of OPA-QDs before and after salt-out with NaCl (0, 1, 2, 3, 4 M). The dashed line indicates the location of the loading wells.

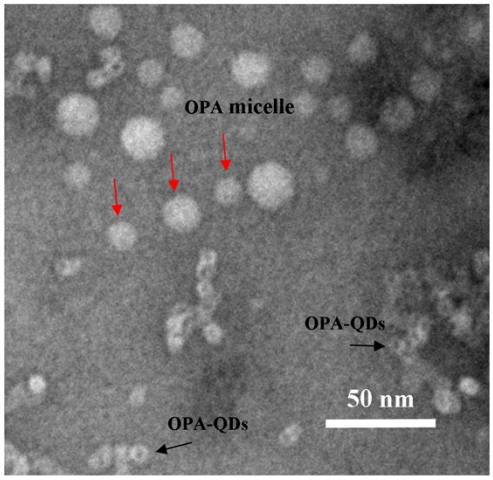

Fig S8 TEM of original OPA-QDs solution

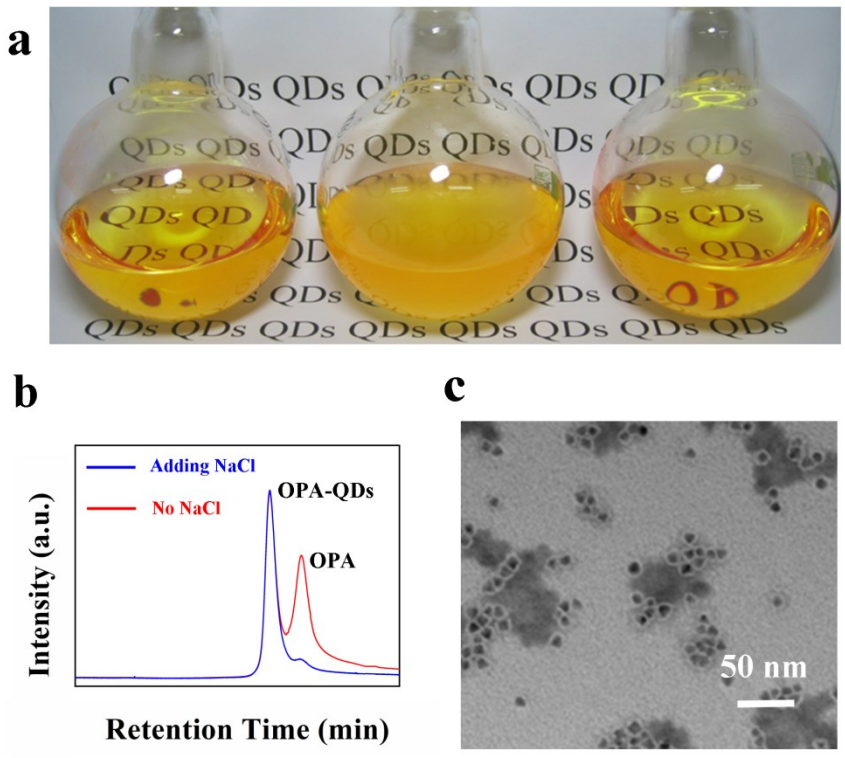

Fig. S9 (a) Large-scalable purification picture of OPA-QDs before and after adding NaCl, (b) chromatograms of OPA-QDs before and after adding NaCl, (c) TEM of purified OPA-QDs with PTA (1%).

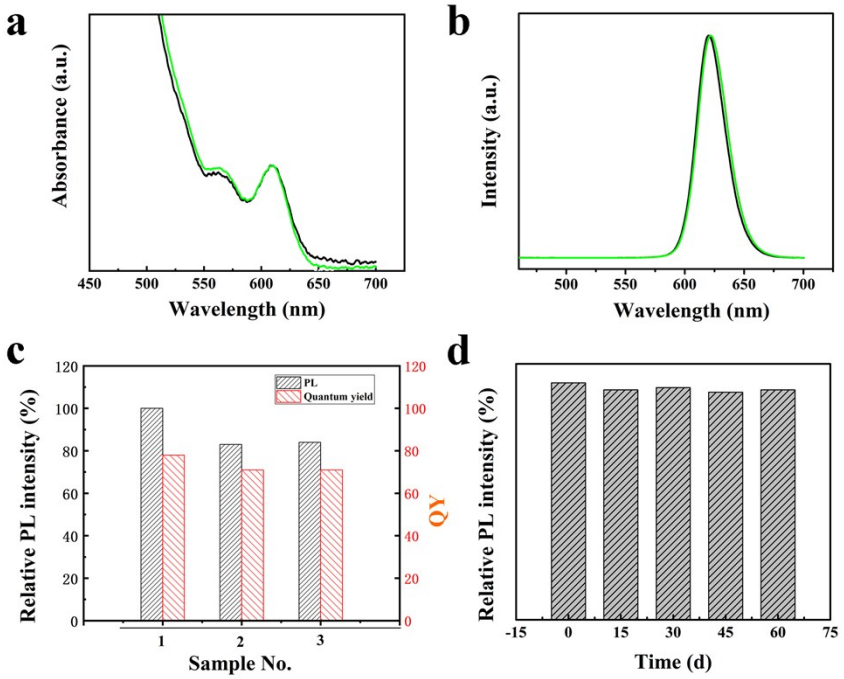

Fig. S10 (a) Absorption and (b) PL emission spectral of OPA-QDs before (black line) and after (green line) the addtion of NaCl, (c) PL intensity and quantum yield of OPA-QDs before and after adding NaCl (1: original OPA-QDs, 2:adding NaCl, 3: removing NaCl), (d) PL stability of purified OPA-QDs as function of time. The quantum dots were measured by a commercialized system (XPQY-EQE-350-1100, Guangzhou Xi Pu Optoelectronics Technology Co., Ltd.), the excitation was 450 nm.

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