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Synthesis of cationic quantum dots *via* a two-step ligand exchange process†

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A new class of quaternary ammonium derivatives has been used to synthesize cationic CdSe/ZnS quantum dots with exceptional stability in water as well as in biological media.

Semiconductor quantum dots (QDs) are important class of nanomaterials due to their inherent physical properties such as size/composition-tunable fluorescence emission, high fluorescence quantum yield, extended fluorescence lifetime and photostability.[1] QDs can provide better sensitivity and stability for biological application than that of traditional organic dyes and protein-based fluorophores.[2] The unique optical properties of QDs make them appealing for bio-molecular interaction studies[3] and biomedical applications including sensing,[4] labelling[5] and intracellular imaging.[6]

The essential prerequisite for biological applications of QDs is solubility and stability in water and biofluids. Common synthetic routes for QDs, however, generate particles capped with hydrophobic surface ligands such as trioctylphosphine oxide (TOPO) and trioctylphosphine (TOP).[7] Several strategies have been developed to solubilize QDs in aqueous medium, including encapsulation and ligand exchange.[8] Hydrophilic ligands, featuring negative, positive and neutral termini have also been used to displace the hydrophobic ligands on the QDs and provide solubility.[9]

Positively charged QDs are of particular interest for biological applications, providing a complementary surface binding for negatively charged proteins[10] and nuclei acids[11] *via* electrostatic interactions. This supramolecular design enables applications including intracellular delivery and sensing.[12] Additionally, positively charged QDs have higher stability at low pH[13] and possess higher cellular permeability than uncharged and anionic analogs.[14] Several groups have generated cationic QDs by using the amine-containing ligands or polymers, using either thiol capping or hydrophobic encapsulation.[15] However, amine-functionalized QDs have a limited useful pH range due to the protonation/deprotonation of amine groups as QDs tend to aggregate and precipitate upon deprotonation at higher pH.[16] This pH dependence complicates both synthesis and applications of these cationic particles.

Despite the potential advantages of permanently cationic QDs, to our knowledge there have been no examples of particle of this sort reported to date. To provide QDs with a permanent positive charge featuring extended pH and biofluid stability profiles we have explored protocols for the synthesis of quaternary ammonium functionalized QDs. The surface properties, *i.e.* hydrophobicity and hydrophilicity, of these cationic QDs are tunable through the choice of terminal head group.

The cationic QDs were synthesized *via* a two-step ligand exchange reaction featuring conversion of hydrophobic QDs to amphiphilic QDs followed by creation of cationic QDs from the amphiphilic QD intermediate. The subsequent change in ligand polarity is the key factor in providing solubility of the QDs. This process is highly efficient, providing an essentially quantitative yield of the cationic particle from the hydrophobic precursor. These particles feature high purity, pH stability, and dispersibility in biofluids including serum, making them excellent candidates for biological applications.

Our ligand design features dihydrolipoic acid (DHLA) as the bidentate anchor on the surface[17] and a tetra(ethylene glycol) (TEG) spacer to minimize non-specific protein and cell interactions.[3a] Positive charge and enhanced water solubility are then imparted by the quaternary ammonium terminus, whose physicochemical properties can be tuned through head group choice (Scheme 1a). The general synthetic scheme for synthesizing dithiol cationic ligands is given in Scheme 1c, with details available in the ESI.†

Our initial efforts to produce cationic QDs focused on direct functionalization of TOPO/TOP-capped QDs (TOPO/TOP-QDs). This process, however, provided a very low yield of QD ($< 10\%$). To address this problem, we developed a two-step ligand exchange process. As shown in Scheme 1b, the first step involves the phase transfer conversion of hydrophobic TOPO/TOP-capped QDs to amphiphilic HS-C5-TEG capped QDs using MeOH as solvent. The particles are then purified, resuspended in MeOH and the corresponding dithiol ligand added to provide hydrophilic DHLA–TEG–$N^+(CH_3)_2$–R capped QDs. These particles are quite clean; mass

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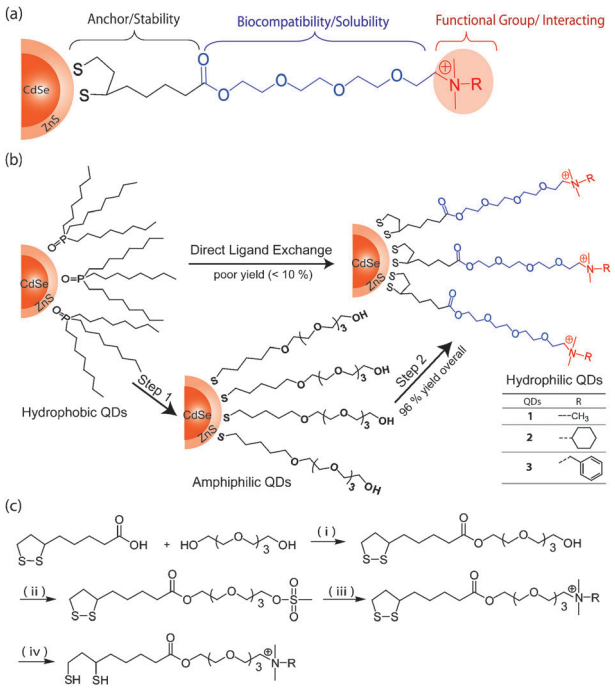

Scheme 1 (a) Design of dithiol cationic ligand. (b) Ligand structure for functionalization of QDs and synthetic route for a two-step ligand exchange reaction: Step 1: conversion of hydrophobic QDs to amphiphilic QDs into MeOH; Step 2: conversion of amphiphilic QDs to hydrophilic QDs into water. (c) Synthetic route of the cationic ligands. *Reagents and conditions*: (i) EDC, HOBt, DIPEA, DCM, rt, 24 h; (ii) MsCl, NEt_3, DCM, 0 °C to rt, 24 h; (iii) $N(CH_3)_2$–R/EtOH, 35 °C, 48 h; (iv) $NaBH_4$, $EtOH/H_2O$, rt, 2 h.

spectrometric analysis revealed complete removal of both the initial capping ligand (TOPO/TOP) and the intermediate amphiphilic ligands (HS-C5-TEG) (Fig. S1, ESI†).

Further characterization of the particles was obtained by transmission electron microscopy (TEM). As expected, the micrographs showed primarily single dots, indicating that the

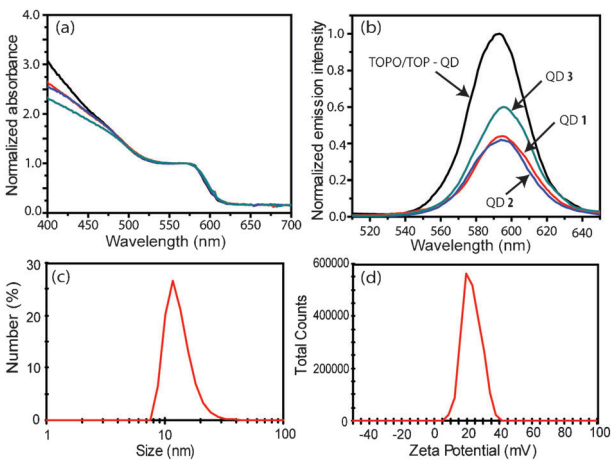

Fig. 1 (a) Absorption and (b) emission spectra of cationic QDs after a two-step ligand exchange reaction: TOPO/TOP-QDs in toluene (black), QD **1** in water (red), QD **2** in water (blue) and QD **3** in water (green). (c) Hydrodynamic diameter and (d) surface zeta potential of QD **1**.

Table 1 Physical properties of cationic QD **1–3**

	Absorption peak/nm	Emission peak/nm	Hydrodynamic diameter/nm	Zeta potential/mV
TOPO/TOP-QD	578	593	—	—
QD **1**	578	595	11.7 ± 3.9	19.7 ± 5.7
QD **2**	578	594	11.7 ± 2.7	19.4 ± 6.3
QD **3**	578	595	14.6 ± 2.9	24.4 ± 7.8

ligand exchange reaction did not cause QD aggregation, with the size of the CdSe/ZnS core–shell was about 5 nm diameter, same as the precursor TOPO/TOP-QDs (Fig. S2, ESI†). Fig. 1a and b presents the absorption and emission spectra of QDs before and after the ligand exchange process. The absorption and emission maxima of the hydrophobic and hydrophilic QDs were unchanged (Table 1), indicating the optical properties of cationic QDs exhibited no major changes after transferring into water. However, the relative emission intensity indicates that the cationic QDs in aqueous media had photoluminescence quantum yield (QY) 40–60% of those of the precursor particles in organic solvent, an observation for QDs after their phase transfer into water.[18] The hydrodynamic diameter and the surface charge of the QD **1** were measured using Malvern Zetasizer Nano ZS (Fig. 1c and d). The hydrodynamic diameter of cationic QDs **1–3** ranged from 11 to 15 nm due to the different sized head group, and all QDs showed a net positive surface charge (20–25 mV) with a narrow charge distribution (Table 1). The narrow size distribution further confirms that the cationic QDs were uniformly dispersed in aqueous media.

Colloidal stability in physiological media is a significant challenge for biological applications of QDs. The stability of the cationic QDs were monitored by photoluminescence intensity (λ_{max} at 590 nm) for 8 h. Fig. 2a–c show that cationic QDs are exceptionally stable in Tris buffer, media, and serum. As shown in Fig. 2d the photoluminescence intensity and hence stability of QD **1** was maintained for 6 h in phosphate buffers ranging from pH 4 to pH 11. In addition, the QDs were stable in the saturated NaCl solution after 8 h incubation (Fig. S3, ESI†).

The stability of the QDs enables their application in cell imaging. QD **1** was incubated with HeLa cell for 3 h (Fig. 3).

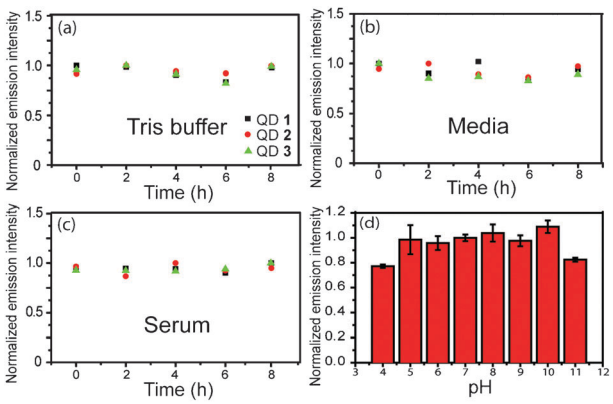

Fig. 2 Stability of cationic QDs in (a) Tris buffer, (b) media and (c) serum with different QDs: QD **1** (black), QD **2** (red) and QD **3** (green). (d) QD **1** in different pH phosphate buffers after incubation for 6 h.

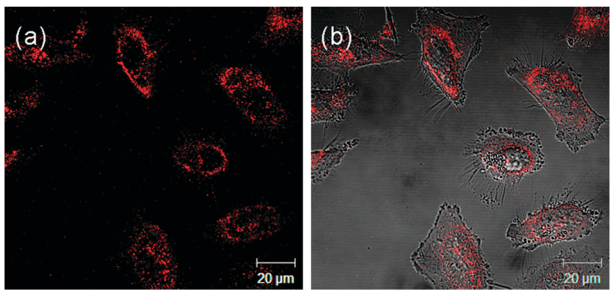

Fig. 3 Imaging of photoluminescent QD **1** (5 nM) internalized in HeLa cells after 3 h incubation. (a) Fluorescence image of QD **1** with 488 nm excitation. (b) Fluorescence image merged with differential interference contrast (DIC) image.

Live cell confocal fluorescence imaging shows effective entry of the particle to the cell enabled by electrostatic interaction between positively charged QDs and negatively charged cell membrane. As expected from prior cell uptake studies,[19] the intracellular fluorescence from QD **1** is punctate, indicating vesicular entrapment of the QDs. The readily tunable surface properties of these ligands, however, might enable the creation of particles capable of escape into the cytosol.

In summary we have demonstrated a two-step method for the creation of permanently cationic QDs. This method prevents uncontrolled aggregation during ligand exchange process, resulting in high yields of cationic QD. The resultant cationic QDs exhibit uniform dispersibility in aqueous solution and high stability under biological conditions. The stability and high cellular permeability of these cationic QDs make them potentially useful imaging agents. Furthermore, the tunability of the particle surface enables their application in delivery and sensing applications.[20]

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