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Article

Bifunctional Multidentate Ligand Modified Highly Stable Water-Soluble Quantum Dots

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We have designed and synthesized a multidentate polymer ligand used for water-solubilization of luminescent quantum dots (QDs). The synthesis of the multidentate ligand (PAA-*g*-MEA) was based on several thiol groups grafted to a linear polymer chain through a simple carboxy-amine coupling reaction between poly(acryl acid) (PAA) and mercaptoethylamine (MEA). Water-soluble QDs capped with these PAA-*g*-MEA ligands were prepared via ligand exchange from the original hydrophobic ones. The resulting PAA-*g*-MEA capped water-soluble QDs with relatively small hydrodynamic diameters possess higher photoluminescence quantum yields than the initial hydrophobic QDs, extraordinary stability over extended periods of time and over a broad pH range (3−14), salt concentrations (up to saturated NaCl solution), and thermal treatment at 100 °C.

Introduction

Semiconductor quantum dots (QDs) have generated an increasing interest because of their narrow and size-tunable emission spectra, broad absorption profiles, high quantum yields, and good chemical stability.[1−4] One of the most promising applications of these luminescent QDs is served as an alternative fluorophore for molecular, cellular, and in vivo imaging.[5−10] They have the potential to overcome many of the limitations encountered by conventional organic fluorophores and genetically engineered fluorescent proteins in a variety of biological applications.[11−14] For biomedical applications, it is imperative to preserve the water dispersible and highly luminescent properties of the QDs. However, high-quality luminescent QDs are usually synthesized in organic media, and the resulting QDs capped with the native hydrophobic ligands (such as trioctylphosphine oxide, alkyl amines etc.) are not soluble in aqueous solutions.[15−19] To overcome this hurdle, many strategies have been developed to render these initially hydrophobic QDs water-soluble and biocompatible, which include silica coating,[20−23] encapsulation

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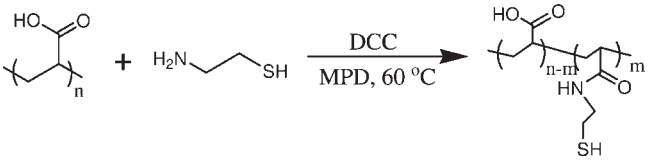
with amphiphilic polymers (adopted by most commercial water-soluble QDs),[24−28] and ligand replacement with hydrophilic ligands.[29−37] Silica shell and amphiphilic polymer encapsulation provide QDs with good aqueous solubility and preserve high photoluminescence quantum yields (PL QYs), but the approach to modify or synthesize the polymer is complex and gives relatively low conversion yield and large hydrodynamic diameters (HDs) on the level of 30−40 nm.[24−28] The excessive size of amphiphilic polymer-coated QDs, which are often much larger than the cellular receptors being labeled, presents a barrier to the widespread implementation of QDs for biomedical imaging.[38,39] Ligand exchange with bifunctional ligands, such as mercaptopropionic acid (MPA), is one of the most widely used phase transfer techniques for QDs, owing to its simplicity, speed, and providing QDs with small size.[29−37] Notwithstanding, the known major limitation of this route is the instability and drastic decrease in the QYs of the resulting water-soluble QDs. Thus, the ligand exchange procedure to obtain water-soluble QDs with high quantum yield and high stability is still a great challenge.

It has been well-established that, besides the nature of the inorganic core, the stability of nanocrystals is also heavily dependent on the nature and structure of the surface ligands and the interface between the inorganic core and the organic ligands.[40,41] The dissociation of organic ligands from the inorganic core has a direct effect on the stability issue related to nanocrystal/ligand complexes. To prevent the dissociation of organic ligands from the inorganic core and accordingly improve the stability of the nanocrystals, the most direct and effective method is based on the use of capping molecules possessing strong bonding capability with the surface atoms of the inorganic core. Since thiol groups have the strongest binding affinity for metal atoms on the surface of QDs, the thiolated aliphatic carboxylic acids, such as MPA, are most often chosen to serve as the bifunctional ligand to replace the native hydrophobic ligands and thus make the QDs water-soluble, in which the thiol end binds onto the QDs surface while the carboxyl moiety confers water solubility.[29−37] To achieve higher stability of the resulting QDs, bidentate ligands such as dihydrolipoic acid (DHLA) or its derivatives were used extensively for ligand exchange to render QDs water-soluble.[33−37] It has been proven that the QDs covered by monothiol ligands are prone to aggregate over time because of the lability of the thiol−QD bond, and the substitution of mono- by dithiol ligands improves longer-term stability. The enhanced stability is attributed to the bidentate chelating effect afforded by the dithiol groups, which have stronger bonding affinity for the QDs as compared to monothiol-terminated ligands. We can expect that multidentate thiolated ligands could render the QDs more stable than either monothiol or dithiol ligands do. It has been verified that capping by trithiol species, or multithiolated ligands such as artificial peptides with multiple cysteine residues, renders the functionalized particles more stable toward ligand exchange than the mono- and dithiol ligands do.[42−45] In a recent report,[43] QDs were first ligand-exchanged with mercaptoundecanoic acid, and then cross-linked by either lysine or diaminopimelic acid through the carboxy-amine coupling by the dicyclohexylcarbodiimide method. However, these cross-linked QDs have an overall HD of 22−30 nm, even larger than their polymer- or silica-coated counterparts.

Scheme 1. Synthesis of the Bifunctional Multidentate Ligand PAA-*g*-MEA



On the basis of the above expectation, we have designed and synthesized a bifunctional multidentate ligand, namely, mercaptoethylamine (MEA) grafted poly(acrylic acid) (PAA) (noted as PAA-*g*-MEA hereafter), through the simple carboxy-amine coupling reaction between PAA and MEA in an aprotonic solvent in the presence of dicyclohexylcarbodiimide (DCC) (Scheme 1). As the strategy adopted for most bifunctional ligands, our current designed ligand uses the multithiol unit for strong anchoring onto the QD surface while the carboxyl groups on the PAA chain confer hydrophilicity and biocompatibility. The designed capping ligand PAA-*g*-MEA has several advantageous features: (i) facile synthesis with high yield; (ii) multidentate thiolated ligand possesses a stronger bonding affinity for the surface atoms of QDs than the conventional mono- or bidentated ligands and accordingly provides higher stability of the resulting QDs; (iii) the polymerized PAA hydrophobic aliphatic chain backbone can ensure a dense and compact coverage on the surface of the QDs, which forms an effective barrier against solvent molecules from coming into contact with the QD surface, and accordingly resulted in high chemical and colloidal stability of the QDs. Water-soluble QDs capped

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with PAA-*g*-MEA ligand (noted as PAA-*g*-MEA-QDs, hereafter) were prepared from the initial oil-soluble QDs via ligand exchange with the native hydrophobic ligands. The resulting water-soluble PAA-*g*-MEA-QDs with relatively small HDs possess higher PL QY than the original hydrophobic QDs, extraordinary stability over extended periods of time and over a broad pH range (3−14), salt concentrations (up to nearly saturated NaCl solution), and thermal treatment at 100 °C. This reported bifunctional multidentate polymer ligand not only minimizes the hydrodynamic size of QDs but also overcomes the colloidal stability and luminescence brightness problems encountered in previous research. We expect that these water-dispersible QDs with such superior performances would be promising fluorophores for ultrasensitive, multicolor, and multiplexing assay applications.

Experimental Section

Chemicals. Poly(acrylic acid) (PAA, 63 wt % solution in water; approximately M.W. 2000, Acros Organics). 2-Mercaptoethylamine hydrochloride (MEA·HCl, 98+%), 3-mercaptopropionic acid (MPA, 99%), 1-methyl-2-pyrrolidione (MPD, anhydrous, 99.5%), tetramethylammonium hydroxide pentahydrate (TMAH, 98%), dicyclohexylcarbodiimide (DCC, 99%) were purchased from Alfa Aesar. The commercial source of MPD was further purified and dried by vacuum distillation over anhydrous sodium sulfate. Dry PAA in powder form was obtained by freeze-drying of the concentrated PAA solution. Neutralized MEA was obtained by freeze-drying the neutralized MEA solution. Deionized water was used throughout.

PAA-*g*-MEA Ligand Synthesis. MEA grafted PAA molecules (PAA-*g*-MEA) were synthesized according to the literature method with minor modifications.[46] In a typical reaction for a nominal 15% grafting percentage of PAA (that is, 15% of the carboxylic acid groups are nominally modified with MEA portion), dried PAA powder (4.5 g, 62.5 mmol based on −COOH group) was dissolved in 150 mL of MPD at 60 °C for 24 h. Then, the solutions of dried MEA (1.1 g, 9.4 mmol) dissolved in 15.0 mL of MPD and 2.2 g (10.5 mmol) of DCC dissolved in 10.0 mL of MPD were introduced into the PAA solution under vigorous stirring. After a reaction for 72 h at 60 °C to give a bifunctional grafted PAA polymer with 15% of the carboxylic acid functional groups modified with MEA portion, the system was cooled to room temperature, centrifuged, and the precipitation was discarded, followed by addition of 100 mL of 40 wt % NaOH solution to precipitate the polymer. The precipitate was washed three times with 50 mL of hot MPD (60 °C) and then with 60 mL of methanol at room temperature. After filtration, the solid product was dissolved in 10 mL of water at 30 °C, and precipitated in 100 mL of methanol (two times). The product was exposed to the air for 30 min, and then dissolved into 10 mL of water. The final dry product (4.7 g with yield of 86% based on MEA) was obtained by freeze-drying. Each polymer molecule contained approximately 3.6 thiol groups as determined by NMR measurement (typical NMR spectrum is available in Figure S2 of the Supporting Information). The MEA substitution level was determined from the peak areas' ratio between CH_2 groups from PAA backbone (broad peak, 1.35 ppm) and CH_2 groups connected to SH group from the MEA portion (triplet peak, 2.32 ppm).

Water Solubilization of Oil Soluble QDs with PAA-*g*-MEA or MPA Ligand. The initial oil-soluble CdSe/CdS/ZnS QDs were prepared according to literature method (detailed procedure and relative characterization are available in the Supporting Information).[47] The MPA capped water-soluble QDs (noted as MPA-QDs hereafter) were prepared strictly according to the modified procedure developed by Lee.[48] The detailed procedure for the preparation of PAA-*g*-MEA-QDs was described as follows. Typically, 120 mg of TMAH was mixed well with PAA-*g*-MEA (50 mg) in a mixture solution of ethanol (0.4 mL) and $CHCl_3$ (1.6 mL), and the resulting pH of the solution was about 10. Then the PAA-*g*-MEA solution was added dropwise into the purified QDs solution in chloroform (5.0 mL of 1 mg mL^{-1} solution) with vigorous stirring for 20 min. Subsequently, deionized water was added into the solution. The QDs were found to be successfully transferred from the chloroform phase in the bottom to the water phase on the top. The underlying organic phase was discarded, and the aqueous phase containing the QDs was collected. The excess amount of free PAA-*g*-MEA ligands was removed by centrifugation. The supernatant was discarded and the pellet was then redissolved in water, and this centrifugation-decantation cycle was repeated twice to get the purified QDs aqueous solutions.

Characterization. The pH value of a solution was measured by a PHS-3C pH meter. UV−vis and PL spectra were obtained on a Shimadzu UV-2450 spectrophotometer and Cary Eclipse (Varian) fluorescence spectrophotometer, respectively. The room temperature PL quantum yield (PL QY) was estimated following the literature procedure by comparing the integrated emission of the QD samples in water with that of a fluorescent dye (rhodamine 6G in ethanol, QY = 95%),[49] with identical optical density. Excitation wavelengths were set at the first absorption peak of the QD samples. ^{1}H NMR spectra were recorded on a Varian Inova-400 NMR spectrometer operating at 400 MHz at room temperature. Samples were dissolved in D_2O at about 2 wt %. FT-IR spectra were collected using a Nicolet 730 FT-IR spectrometer. Transmission electron microscopy (TEM) images were taken on a JEOL JEM-1400 at an acceleration voltage of 100 kV. TEM samples were prepared by depositing a drop of dilute dispersion of nanoparticles on a copper grid coated with carbon film. Dynamic light scattering (DLS) analysis in aqueous solution was conducted with a Zeta Sizer nano series laser light scattering system (Malvern Instrument Corporation).

Results and Discussion

Synthesis of Bifunctional Ligand and Water Solubilization of QDs. Scheme 1 shows the chemical structures and synthetic procedure for the bifunctional multidentate ligand PAA-*g*-MEA used in the ligand exchange for water solubilization of QDs. The designed ligands are the MEA grafted PAA molecules, synthesized following the standard carbodiimide chemistry.[46] MEA grafting onto linear PAA polymer with average formula weight of 2000 was conducted using dicyclohexylcarbodiimide (DCC) in an aprotonic solvent MPD. Reaction of a fraction of the carboxylate groups of a PAA molecule with MEA molecules leads to the formation of the multithiol groups that are used for intercalation with the surface of QDs. The rest of the free carboxylate groups provide water solubility and biocompatibility of the capped QDs. By optimizing the balance between the thiol and the carboxylate groups in the ligand, the resulting ligand can get the optimal performance to preserve the high luminescence

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brightness and good stability of the modified QDs in aqueous media. If the nominal thiol grafting percentage is too low (less than 6%), the binding affinity of the ligand for the surface atoms of QDs becomes too weak to transfer the hydrophobic QDs into aqueous phase. While if the nominal thiol grafting percentage is too high (greater than 25%), aggregation of QDs occurs when transferred into aqueous media. This could be due to undesired interparticle cross-linking between neighboring QDs through multithiol groups in the ligand molecules. We have found that a 15% nominal grafting percentage (that is, 15% of the carboxylic acid groups in the linear PAA polymer are modified with MEA side chains, about 3.6 thiol groups in a single ligand molecule) is nearly optimal for solubilization of the hydrophobic QDs in water. Notably, this designed ligand was synthesized via a simple one-step reaction in high yields.

The CdSe/CdS/ZnS QDs used were prepared from the 3.5 nm CdSe cores and consecutively coated with 3 monolayers of CdS shell and 2 monolayers of ZnS shell. The overall diameter of the initial oil-soluble QDs is 7.0 nm with emission wavelength of 605 nm and QYs in the range of 50−60%. Water-soluble QDs capped with the designed bifunctional ligands were obtained via ligand exchange from the native hydrophobic QDs following a literature method.[48] The obtained PAA-*g*-MEA ligands show superior performance for the phase transfer of the hydrophobic QDs into aqueous solutions. The phase transfer process was rapid (less than 0.5 h) and occurred with nearly 100% efficiency, which was determined by observing almost no luminescence emission and no absorption corresponding to QDs in the optical spectra of the organic phase after the phase transfer. The extra free ligands in the solution were removed by repeated redissolution and precipitation with the addition of acetone for three cycles. FT-IR spectra of the original oleylamine-capped QDs and the as-received water-soluble PAA-*g*-MEA-QDs (Supporting Information, Figure S3) clearly show displacement of oleylamine shell by the PAA-*g*-MEA after phase transfer. In the spectrum of PAA-*g*-MEA-QDs, the absence of the absorption peaks for oleylamine (ν_{C-H} of CH_2 chain at 2923 and 2852 cm^{-1}; and δ_{N-H} at 1379 cm^{-1}); while the characteristic absorption peaks from PAA-*g*-MEA (υ_{COO-} at 1570 and 1407 cm^{-1}, $\upsilon_{C=O}$ of −CONH- at 1659 cm^{-1}) are obvious; therefore, the displacement mechanism also holds true for our PAA-*g*-MEA-QDs water-solubilization.

To test the stability of water-soluble PAA-*g*-MEA-QDs, the common bifunctional ligand MPA was also used to transfer the same batch of hydrophobic QDs into aqueous media using the modified procedure.[48] It should be noted that before the invention by Lee,[48] phase transfer by MPA usually led to significant loss of QYs and resulted in poor stability of the QDs in water.[50−53] While with the use of this modified procedure the QY of the resulting water-soluble MPA-QDs is comparable to those

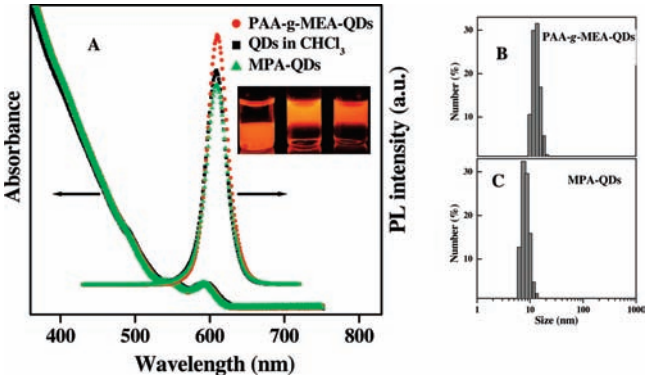

Figure 1. (**A**) UV−vis and PL (λ_{ex} = 360 nm) spectra of initial hydrophobic CdSe/CdS/ZnS QDs in chloroform (black curve), the corresponding PAA-*g*-MEA-QDs (red) and MPA-QDs (green) in aqueous media. Note: PL spectra were measured under the same conditions. Inset: Luminescence images of initial oil-soluble QDs in chloroform (left), PAA-*g*-MEA-QDs (middle), and MPA-QDs (right) in water under UV light irradiation. Representative dynamic light-scattering histograms of PAA-*g*-MEA-QDs (**B**) and of MPA-QDs (**C**) with inorganic core diameter of 7.0 nm in aqueous solutions.

of native QDs in organic phase, they exhibit high colloidal stability for several months in neutral and weak basic buffers. As the monothiol ligand MPA can get one of the best results for water-soluble QDs via the modified phase transfer procedure, we only compared the performance of the water-soluble QDs functionalized by our designed ligand PAA-*g*-MEA with that by the MPA through the modified procedure. After phase transferring into water, the absorption profiles of both PAA-*g*-MEA-QDs and MPA-QDs exhibited a negligible change in comparison with the original hydrophobic QDs. The PL emission peak of the PAA-*g*-MEA-QDs shifted slightly to longer wavelength (∼1 nm) compared with those of the initial oil-soluble QDs in $CHCl_3$ and the MPA-QDs in water (Figure 1A). This negligible peak shift indicates that the surface ligands have no effects on the electronic properties of the inorganic QD cores and no aggregation or surface degradation of the QDs occurred upon phase transfer. It is highlighted that the water-soluble PAA-*g*-MEA-QDs showed ∼16% higher PL QY than the initial hydrophobic QDs dispersed in organic phase, while the MPA-QDs showed almost the same QY as the original oil-soluble QDs, which was in accordance with the previous report.[48] The higher QY of PAA-*g*-MEA-QDs can be seen from the luminescence images of the QDs samples under the irradiation of a UV lamp (Inset of Figure 1A), where the PAA-*g*-MEA-QDs showed brighter luminescence in comparison with both the original oil-soluble one and the MPA-coated one. It should be highlighted that loss of luminescence brightness of QDs following phase transfer into aqueous solutions was commonly observed in previous reports.[29−37,50−53] The exceptional superior optical performance observed here may be due to both the multishell structured inorganic core and the extraordinary bonding affinity of the designed ligand for the surface atom of the QDs. For the CdSe/CdS/ZnS core/shell/shell QDs, because of the multishell overcoating with high bandgap materials (CdS and ZnS), the electronic confinement of both photogenerated holes and electrons are located almost completely inside the core materials

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CdSe, which should efficiently suppress the nonradiative recombination of the charge carrier as reported previously.[47,54] From the aspect of the ligand, it is energetically favorable for the linear multidentate polymer to wrap around the QD in a closed conformation.[43b,55,56] In contrast to the standing brushlike conformation of monovalent thiols, we believe that the suggested closed conformation is highly stable from thermodynamic and steric perspectives and is thus responsible for the excellent optical properties and the extraordinary colloidal and chemical stability as discussed later.

Dynamic light scattering (DLS) measurements indicated that the average hydrodynamic diameter (HD) of PAA-*g*-MEA-coated QDs was 12.9 nm, and the HD of the corresponding MPA-QDs was 7.8 nm (Figures 1B, C). This indicates that the hydrodynamic thickness of the capping layer PAA-*g*-MEA is about 3.0 nm based on the 7.0 nm size of the inorganic core. This compact shell matches the geometric prediction of a polymer conformation with a high degree of adsorption on the QDs surface, enabled by its high affinity and low molecular weight. The HD of these PAA-*g*-MEA-QDs is significantly smaller than that of QDs coated with amphiphilic polymeric shells (e.g., commercial QDs), which have HDs on the order of 14−60 nm.[57] The reduced size of these QDs relative to commonly used commercial QDs makes them attractive for cell labeling applications. In addition, the monomodal size distribution shown by the DLS histogram (Figure 1B) indicates that these QDs form well-dispersed aggregate-free solutions. TEM measurements showed that the PAA-*g*-MEA-QDs were individually well isolated and no aggregation occurred. In addition, the particles have nearly the identical size (7.0 nm in average) and shape compared to their initial hydrophobic counterparts (Figure 2). The overall dimension of PAA-*g*-MEA-QDs measured by TEM (7.0 nm) is remarkably smaller than their hydrodynamic sizes (12.9 nm). This is expected because of the inability of electron microscopy to resolve surface-associated solvent molecules and also because of the shell compaction that occurs during the drying process. These above features suggest that this new ligand befits the surface stabilization of QDs for their high fluorescence quantum yield, well monodispersibility, and relatively small HD.

To explore their suitability in intracellular imaging, we have examined the cellular uptake of PAA-*g*-MEA-coated QDs in cell culture medium. The Chang liver cells were incubated in solution containing PAA-*g*-MEA-coated QDs (1.0×10^{-6} M). After 30 min of incubation, the fluorescence images were obtained. Supporting Information, Figure S4 shows that a significant number of QDs were internalized and accumulated in cells by through endocytosis or macropinocytosis (nonspecific uptake, not mediated by receptors). This demonstrated that the PAA-*g*-MEA-coated QDs were stable in cells and suitable for in vitro cell labeling, cell tracking, and other bioimaging applications.

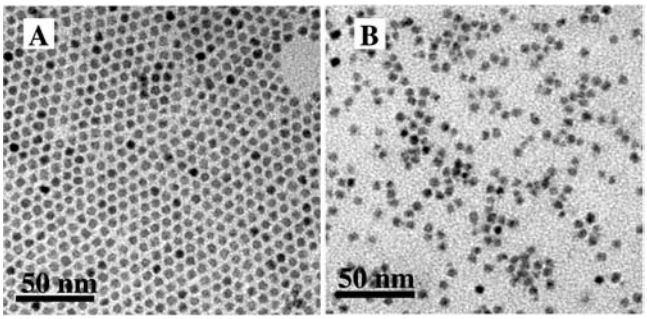

Figure 2. TEM images of QDs capped with the native hydrophobic ligand shell from the synthesis in organic solvent (**A**) and with PAA-*g*-MEA (**B**) after ligand exchange.

pH Sensitivity. For the pH sensitivity study, 100 μL of concentrated purified QDs aqueous solution was added and mixed well in a 5.0 mL buffer solution with different pH values (different pH values were obtained with the addition of HCl or NaOH solution to the 50 mM phosphate buffer with initial pH of 7.0). The obtained QD solutions with various pH values were sealed and stored in the dark, and their PL spectra was monitored over time. By this procedure the concentrations of QDs in different pH solutions could be taken as identical and kept constant over time. Since the PL spectra of all the samples were measured under the same instrument conditions, the PL intensities of the corresponding QDs can represent their corresponding QYs and can be used to compare their pH sensitivity. As expected, the obtained PAA-*g*-MEA-QDs can remain high PL QYs and colloidal stability, aggregation-free over extended periods of time (months) in a broad pH range (3−14) (Figure 3A). The instant PL spectra of PAA-*g*-MEA-QDs dispersed in solutions with pH values from 1 to 14 exhibited similar spectral profiles though with different PL intensities (Inset of Figure 3A). At pH 3−14, PL peak intensities in all samples did not show significant change although some fluctuation was observed within 5% relative intensity variation; while under strong acidic condition of pH = 2 or 1, the PL intensity decreased to 41 or 35% of the value in neutral condition. After 10 days, the PL QYs of PAA-*g*-MEA-QDs samples at pH 3−14 were not affected by the media pH; however, the QDs in the pH 1−2 range lost their photobrightness furthermore (decreased to about 10% of the value at neutral condition). Figure 3C shows representative luminescence photographs of PAA-*g*-MEA-QDs samples at pH 1−14 stored for 20 days. Except for the samples at pH 1 or 2, all the samples show very high photoluminescence brightness. The PAA-*g*-MEA-QDs in pH 3−14 buffers were stable for more than 1 month, where their PL spectral profiles kept unchanged and PL intensities remained practically stable. The ideal working pH range of the PAA-*g*-MEA-capped QDs is therefore between pH 3.0 and 14.

In comparison, MPA-QDs were also well-dispersed and retained their PL spectral profiles and kept their high photobrightness in weak acidic to weak basic media (Figure 3B), but showed progressive aggregation and eventual precipitation under strong acidic or strong basic

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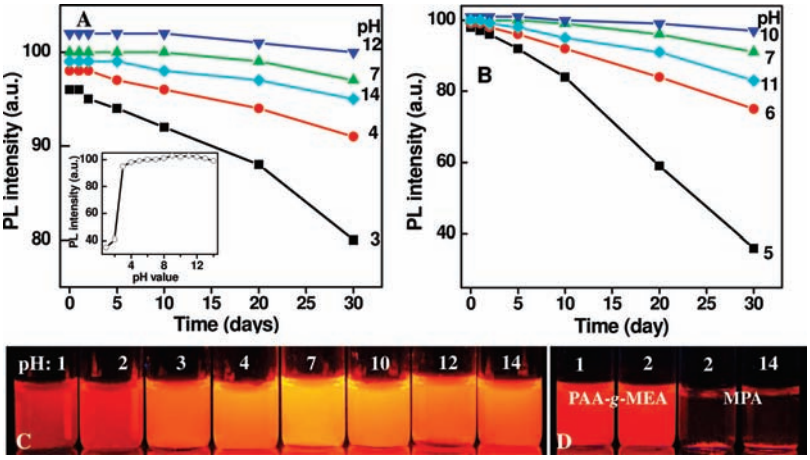

Figure 3. PL stability of PAA-*g*-MEA-QDs (**A**) and MPA-QDs (**B**) over 1 month at different pH values. Inset in (**A**): the instant relative PL intensities of PAA-*g*-MEA-QDs at different pH values. (**C**) Luminescence images of the PAA-*g*-MEA-QDs samples at different pH values stored for 20 days. (D) Luminescence photographs of PAA-*g*-MEA-QDs samples at pH 1 or 2 and MPA-QDs samples at pH 2 or 14 stored for 24 h.

conditions. Figure 3D shows that the MPA-QDs at pH 2 or 14 precipitated completely after storing for 24 h, while the PAA-*g*-MEA-QDs at pH 1 or 2 could still retain homogeneously dispersible and keep certain amount luminescence brightness. Unlike the case of PAA-*g*-MEA-QDs, which are photostable in the pH range of 3−14, the MPA-QDs keep photostable only in the pH range of 5−12 and show PL quenching accompanied with particle aggregation beyond this range. These results indicate that the PAA-*g*-MEA-QDs are more tolerant to different pH conditions than the MPA-QDs.

Deprotonation of carboxyl groups is known to be a crucial factor to solubilize carboylated ligand coated QDs in aqueous solutions, and the colloidal stability of those QDs is commonly observed in neutral to weak basic solutions. The water-dispersibility of these PAA-*g*-MEA-QDs also depends on the deprotonation of carboxyl groups in the PAA backbone, but they show quite superior colloidal and luminescent stability in acidic condition in comparison with MPA-QDs. This may be partly due to a localized buffering of multicarboxyl groups, thus increasing the local pH. Another reason can be ascribed to the closed conformation adopted by the multidentate polymer ligands wrapping around the surface of QDs.[43b,55,56] Such closed conformation creates a hydrophobic shell around the nanocrystal surface, which can provide additional protection for the hydrophilic nanocrystals as this shell can prevent hydrophilic reagents from reacting with the nanocrystal surface. In previous reports, extremely high stability over pH and salt concentration was also observed for QDs coated with ligands possessing a similar hydrophobic shell as the PAA-*g*-MEA ligand.[58,59] The colloidal and luminescent stability of PAA-*g*-MEA-QDs in acidic media suggests that they may be good candidates as intracellular imaging probes for QD applications because most intracellular organelles such as endosomes and lysosomes are acidic (pH 4−6).

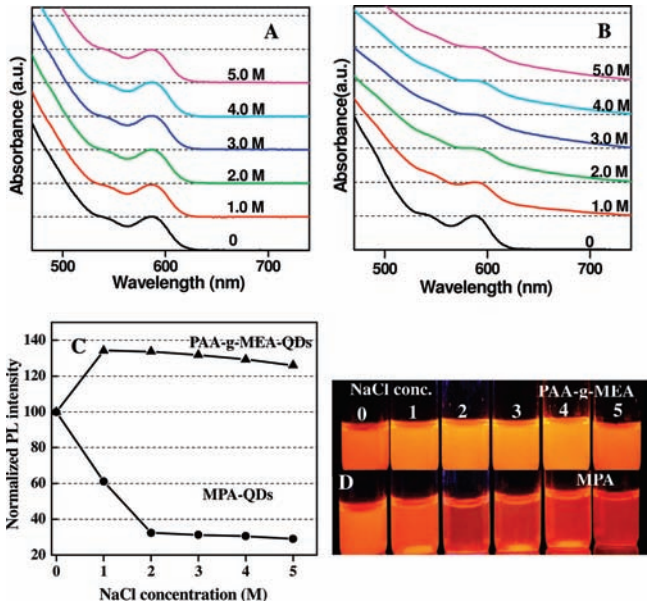

Figure 4. UV−vis spectra of PAA-*g*-MEA-QDs (**A**) and MPA-QDs (**B**) at various NaCl concentrations. (C) Normalized PL intensities of PAA-*g*-MEA-QDs (▲) and MPA-QDs (●) under various NaCl concentrations. (D) Photographs of PAA-*g*-MEA-QDs (top) and MPA-QDs (bottom) under various concentrations of NaCl solutions.

Salt Solution Stability. The use of QDs in any sensing scheme requires that they exhibit long-term stability in solutions that span a wide range of electrolyte concentrations. The aggregation of QDs at high NaCl concentration may define the limitations of some biological applications in high ionic strength media, such as intracellular and in vivo studies, where the ionic concentration is known to be high. To test the colloidal stability of the PAA-*g*-MEA-QDs, a series of NaCl solutions with concentration ranging from 0 to 5.0 M (nearly saturated concentration) were prepared. A 100 μL concentrated purified QDs aqueous solution was added to a 5.0 mL NaCl solution with a specific concentration and mixed well for the measurement of optical spectra. Figure 4 presents the instant absorption spectra of the PAA-*g*-MEA-QDs and MPA-QDs dispersed in an aqueous solution containing different NaCl concentrations.

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For the absorption spectra of PAA-*g*-MEA-QDs, up to the saturated NaCl solution condition, the baselines were all horizontal and no absorption tail at the long wavelength side was observed. This indicates that scattered light from the colloidal dispersions did not exist, and thus no aggregation occurred for the QDs dispersions throughout the whole NaCl concentration range. Furthermore, at the nearly saturated NaCl solution media (5.0 M NaCl), the PAA-*g*-MEA-QDs could still keep homogeneously dispersed after being stored for more than 1 month. The PL spectra of PAA-*g*-MEA-QDs dispersed in different concentrations NaCl solutions kept a similar profile but with different intensities (Figure 4C). It is highlighted that the PL QYs have a 23−35% increase when the QDs dispersed in solutions containing 1.0−5.0 M NaCl compared with those dispersed in pure water, and the highest PL QYs are observed in the NaCl concentration of 1−2.0 M. In contrast, for the MPA-QDs, the instant absorption spectra had a heavy deviation from the baseline and show a tail in the long wavelength side when the NaCl concentration in the QDs solution increased (Figure 4B). Furthermore, because of the particle precipitation in NaCl solutions, the measured absorbance at the first exitonic absorption peak was very low even though the same concentration of QDs existed in each measured sample. The absorption tail at the long wavelength side indicates that scattered light from the colloidal dispersions exists, and aggregation occurs for the QDs dispersions. When the NaCl concentration was up to 2.0 M, the dispersed MPA-QDs precipitated instantly, which can be observed by naked eyes. Figure 4D shows the luminescence photographs of PAA-*g*-MEA-QDs and MPA-QDs dispersed in solutions containing different concentrations of NaCl. In contrast to the PAA-*g*-MEA-QDs, whose PL QYs have an improvement with the increase of NaCl concentration, the PL QYs of MPA-QDs diminish significantly with the increase of NaCl concentration in the dispersed solution. Unlike the case of PAA-*g*-MEA-QDs, the PL peak position and shape are independent of the NaCl concentration in the media, while in the case of MPA-QDs, the PL peak position shows a significant redshift and the peak width gets broadeer and asymmetric when the NaCl concentration increases in the dispersed solution because of the aggregation of the QDs. It is noteworthy that the extraordinary stability of PAA-*g*-MEA-stabilized QDs in high concentration salt solutions can be comparable to that of QDs functionalized by Tween derivatives,[58] and even higher than that of gold nanocrystals used in commercial biomedical diagnosis.[60] This property is of special interest to expand their applications to biology and biomedicine.

Thermal Stability. Temperature is another important parameter for biological applications (e.g., cell-incubation studies, polymerase chain reaction (PCR), DNA sensors). The purified PAA-*g*-MEA-QDs and MPA-QDs were loaded in a closed container and heated from room temperature to 100 °C in a period of 10 min and kept at this temperature for a certain period. Aliquots were taken, and their corresponding optical spectra measured over time. Timing started when the temperature reached 100 °C. Compared to monothiol ligands, the designed ligand molecule is a multidentate chelating one and should have stronger affinity for the surface atom of QDs; thus, an increased thermal stability of the resulting QDs is expected. Experimental results show that the PAA-*g*-MEA-QDs exhibit excellent stability in boiling water. As shown in Figure 5 when the samples were heated to 100 °C from room temperature, the corresponding PL intensities of PAA-*g*-MEA-QDs and MPA-QDs dropped about 20% and 35%, respectively. With extended heating time at 100 °C, the PL intensity decrease was much sharper in MPA-QDs in comparison with that of PAA-*g*-MEA-QDs. For MPA-QDs heated at 100 °C, on one hand, the PL intensity decreased sharply (in less than 1 h, the PL intensity decrease about 65%); on the other hand, the particles aggregated and precipitated gradually in a period of 1 h heating. As the PL intensity is difficult to be quantitatively measured when aggregation occurs, PL measurement ended then. For PAA-*g*-MEA-QDs, the PL intensity decreased much more gently, and the PL intensity decreased only 30, 57, and 66% with the heating time of 1, 2, and 3 h, respectively. Furthermore, the particles were still homogenously dispersible in solution with the heating time up to 3 h. This difference may come from the stronger capping capability of PAA-*g*-MEA to the surface of QDs compared with MPA. These findings suggest that PAA-*g*-MEA is superior to MPA for the surface stabilization of QDs.

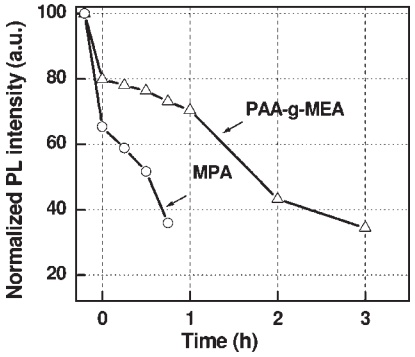

Figure 5. Temporal evolution of PL peak intensities of PAA-*g*-MEA-QDs and MPA-QDs in the process of heating at 100 °C. The first measurement point corresponds to samples at room temperature.

Conclusions

In summary, we have reported a facile and effective approach for preparing high-quality hydrophilic QDs through the ligand exchange reaction based on the use of the designed multidentate ligand PAA-*g*-MEA. These multidentate polymer ligands had a relatively small hydrodynamic thickness of 3.0 nm, and the capped water-soluble QDs possessed extraordinary stability over extended periods of time and over a broad pH range (3−14), salt concentrations (up to saturated NaCl solution), and thermal treatment at 100 °C. The relative PL QYs of the water-soluble PAA-*g*-MEA-QDs were even higher than those of the original hydrophilic QDs in organic solvents. These superior performances of the resulting water-soluble QDs could be attributed to both the multishelled core/shell/shell structure of the inorganic nanocrystals and the multidentate thiol groups,

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polymerized PAA hydrophobic aliphatic chain backbone, together with multiple negative charges derived from the carboxylate groups in the capping ligand. This reported bifunctional multidentate polymer ligand not only minimizes the hydrodynamic size of QDs but also overcomes the colloidal stability and luminescence brightness problems encountered in previous research. On the other hand, the multiple negative charges of the ligand also cause the capped QDs to have high non-specific adsorption, rendering them unsuitable for single-particle imaging where low background is essential.[61] One possible route to mitigate this shortcoming can use ligand mixtures containing the uncharged poly(ethylene glycol) (PEG) molecules. We expect that these water-dispersible QDs with such superior performance would be promising fluorophores for ultrasensitive, multicolor, and multiplexing assay applications.

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Supporting Information Available: Details of procedure for the synthesis of the initial oil-soluble CdSe/CdS/ZnS QDs. This material is available free of charge via the Internet at http://pubs.acs.org.

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