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Combining Ligand Design with Photoligation to Provide Compact, Colloidally Stable, and Easy to Conjugate Quantum Dots

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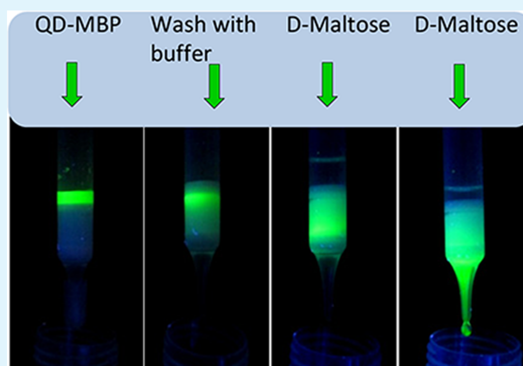
Supporting Information

ABSTRACT: We describe the design and synthesis of two compact multicoordinating (lipoic acid-appended) zwitterion ligands for the capping of luminescent quantum dots, QDs. This design is combined with a novel and easy to implement photoligation strategy to promote the in situ ligand exchange and transfer of the QDs to buffer media. This method involves the irradiation of the native hydrophobic nanocrystals in the presence of the ligands, which promotes in situ cap exchange and phase transfer of the QDs, eliminating the need for a chemical reduction of the dithiolane groups. Applied to the present LA-zwitterion ligands, this route has provided QDs with high photoluminescence yields and excellent colloidal stability over a broad range of conditions, including acidic and basic pH, in the presence of growth media and excess salt conditions. The small lateral extension of the capping layer allowed easy conjugation of the QDs to globular proteins expressing a terminal polyhistidine tag, where binding is promoted by metal-affinity interactions between the accessible Zn-rich surface and imidazoles in the terminal tag of the proteins. The ability to carry out conjugation in acidic as well as basic conditions opens up the possibility to use such self-assembled QD-protein conjugates in various biological applications.



KEYWORDS: *quantum dots, fluorescence, surface-functionalization, biocompatibility, colloidal stability*

INTRODUCTION

Even though several synthetic routes have been reported for preparing a wide range of semiconductor nanocrystals, including growth in reverse micelles and growth via arrested precipitation and in solid substrates, the highest quality nanocrystals with narrow size distribution and high fluorescence quantum yields are primarily prepared by reacting organometallic precursors at high temperature in coordinating solutions.[1−5] Hence, QDs with a broad combination of core materials with high quantum yields, large extinction coefficients, along with tunable and in most cases narrow emission profiles have been reported in the past decade.[4] The same synthetic route has been used to grow a thin overcoating layer of wider band gap semiconducting materials over the native core, which increases the quantum yield and enhances the long-term photostability. Among the materials prepared thus far, nanocrystals that are overcoated with a layer of ZnS (or ZnSeS), such as CdSe-ZnS (or CdSe-ZnSeS), exhibit the highest quality optical and spectroscopic properties, including minimal red shift of the emission compared to the native cores and high quantum yields.[2,6−8] As prepared QDs are capped with a hydrophobic layer that is primarily composed of a mixture of alkylphophines (trioctylphosphine/trioctylphosphine oxide, TOP/TOPO) and alkylamines.[1,2,9,10] This hydrophobic coating promotes the QD solubility only in nonpolar organic solvents. Those properties combined have generated a tremendous interest to develop these nanocrystals as fluorescent platforms in biology. A key requirement for a successful integration in biological system, however, involves developing effective and easy to implement strategies to promote solubility in buffer media and biocompatibility.

Several approaches have been put forward to achieve this goal. These range from coating the TOP/TOPO-capped nanocrystals within a silica coating and encapsulation within amphiphilic polymer shells or lipid micelles, to exchanging the native cap with bifunctional hydrophilic ligands.[11−16] Surface ligand exchange is simple to implement and can provide high quality water-soluble QDs that are compact and functional, to allow further manipulation and coupling to target biomolecules.[11] Nanocrystals with narrow emission (fwhm = 30−40 nm, hydrodynamic radius not exceeding 10 nm, a photoluminescence (PL) quantum yield exceeding 20%, and a great long-term colloidal stability are desired aspects for these materials. However, this route can be ineffective when implemented with simple monothiol-alkyl molecules, since the resulting dispersions have limited long-term and pH stability.[11,17] The use of one or two dihydrolipoic

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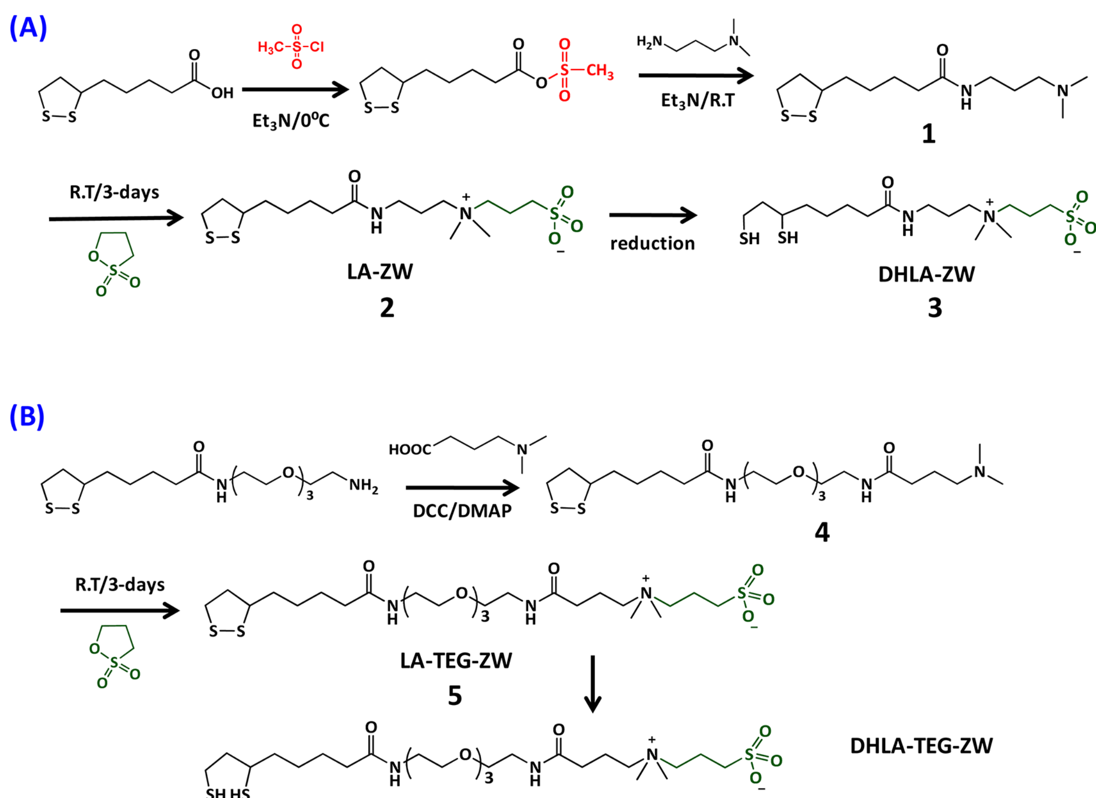

Figure 1. Schematic representation of the chemical structures and synthetic strategies of the LA-based zwitterion ligands and their reduced forms for: (A) LA-ZW, (B) LA-TEG-ZW.

acid (DHLA) anchoring groups combined with the insertion of a short polyethylene glycol (PEG) within the ligand structure produces greatly enhanced stability over a broad pH range, to added electrolytes and reducing agents, while providing reactivity.[17−20] More recently, a new set of multithiol-appended ligands presenting zwitterion (sulfobetaine) groups have been designed and shown to provide hydrophilic dispersions with great colloidal stability.[21−23]

To perform cap exchange on CdSe and ZnS-overcoated QDs, reduction of the 1,2-dithiolane using a large excess of $NaBH_4$ is required to produce dihydrolipoic acid (DHLA)-appended ligands; the oxidized ligands do not cap these QDs.[16,20,21,24,25] Though effective, chemical reduction of the dithiolane ring using $NaBH_4$ is tedious and introduces additional steps to the phase transfer. Moreover, the DHLA-based ligands need to be stored under an inert atmosphere. In order to address these problems, we have recently introduced a new strategy to transfer QDs to polar and buffer media using lipoic acid-based ligands.[26] The ligand exchange and phase transfer are promoted photochemically, because the photoreduction of a lipoic acid (the stable oxidized but non chelating form) in the presence of UV irradiation at 350 nm is coupled to ligation of DHLA onto QDs. This approach eliminates the need for chemical reduction of the dithiolane using $NaBH_4$, and is compatible with sensitive functional groups.

In this report, we combine the design of two types of multicoordinating, zwitterion ligands with the photoligation strategy to prepare a set of compact and reactive hydrophilic QDs. The ligands are made of lipoic acid anchoring group, on which we chemically appended either a zwitterion group directly (LA-ZW), or a tetraethylene glycol-zwitterion group (LA-TEG-ZW). The resulting QDs are compact and fully compatible with the ubiquitous metal-histidine conjugation to proteins expressed with a terminal polyhistidine tag.

We will provide the detailed synthesis of the ligands, and describe how to implement the photoligation strategy. We will also outline the most important parameters that control the phase transfer and provide experimental results related to the characterization of the ligands and resulting QDs. Metal-histidine conjugation of maltose binding protein onto the QDs will be tested using simple (and visual) assay, involving binding of the conjugate to amylose on a gel column followed by release with soluble maltose.

■ EXPERIMENTAL SECTION

Materials. All chemical reactions were performed under dry nitrogen that passed through an O_2 scrubbing tower, unless otherwise stated. Air-sensitive solids were handled in a glovebox (MBraun Labmaster 130) or using Standard Schlenk techniques. Lipoic acid, LA (or DL-thioctic acid, TA), D-(+)-maltose monohydrate, triethylamine, methanesulfonyl chloride, organic solvents (MeOH, $CHCl_3$, etc.), PBS buffer, and salts (such as NaCl, Na_2SO_4, Na_2CO_3) were purchased from Sigma Chemicals (St. Louis, MO). N,N-Dimethyl-1,3-propanediamine, 4-(dimethylamino)butyric acid hydrochloride, and 1,3-propanesultone were purchased from Alfa-Aesar (Ward Hill, MA). Purification of the compounds was performed using silica gel chromatography (60 Å, 230−400 mesh, from Bodman Industries, Aston, PA). Deuterated solvents used for NMR experiments were purchased from Cambridge Isotope Laboratories (Andover, MA). All the chemicals and solvents were used as received, unless otherwise specified. Disposable plastic columns and amylose resin were, respectively, purchased from Fisher thermoscientific (Rockford, IL) and New England Biolabs Inc. (Ipswich, MA).

Instrumentation. ^{1}H NMR spectra of all compounds were recorded on a Bruker SpectroSpin 600 MHz spectrometer, while mass characterization of the ligands was carried out using a JEOL AccuTOF JMS-T100LC ESI mass spectrometer (ESI-MS). Fourier transform

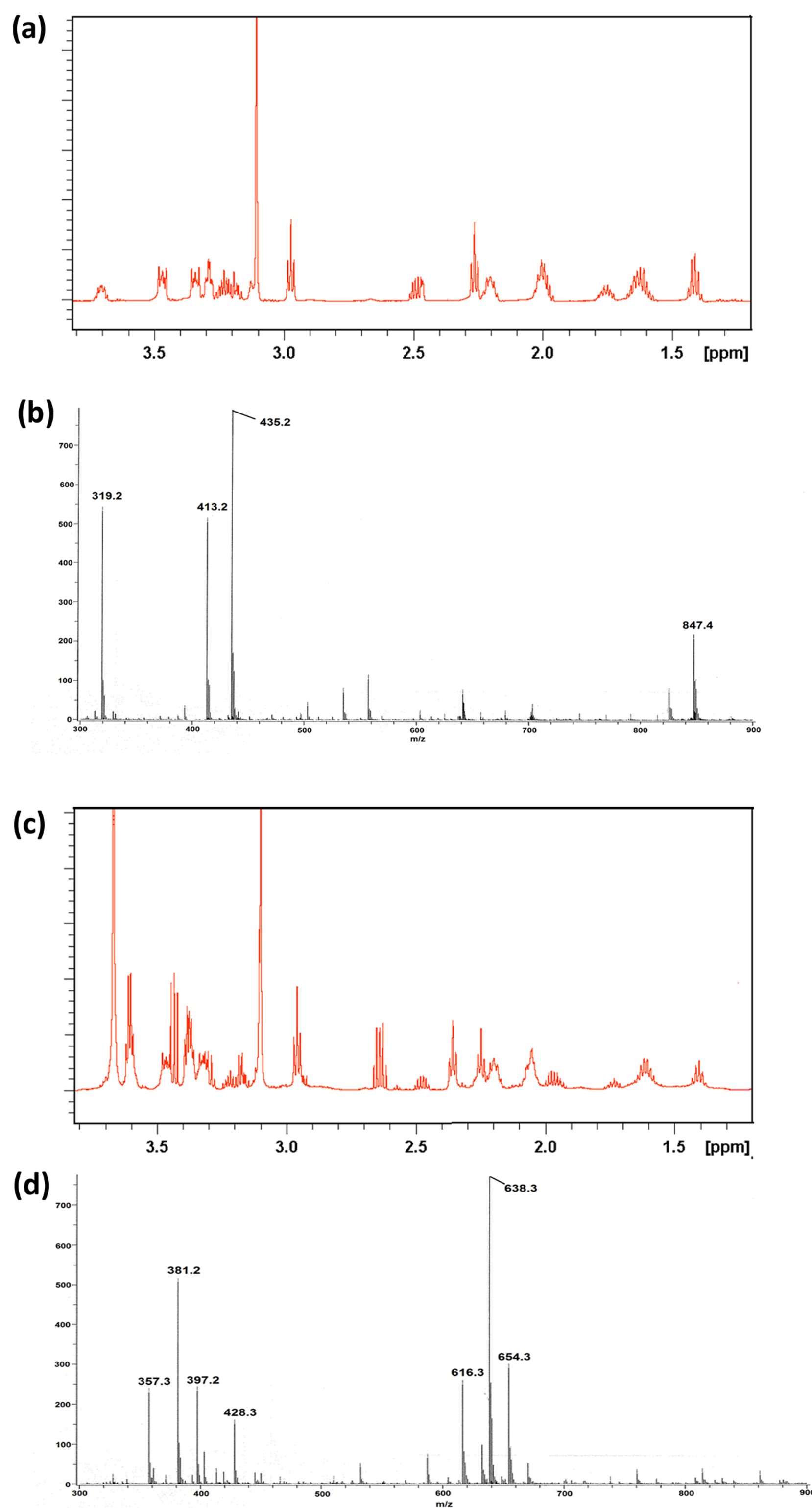

Figure 2. (a) ^{1}H NMR spectra of LA-ZW (**2**); (b) mass spectra of LA-ZW (**2**); (c) ^{1}H NMR spectra of LA-TEG-ZW (**5**); (d) mass spectra of LA-TEG-ZW (**5**). The NMR spectra are collected using D_2O.

infrared (FT-IR) spectra were measured using a Perkin-Elmer FT-IR spectrometer. The photoirradiation experiments on the QD-plus-ligand were performed using a photoreactor (Luzchem UV lamp, Model LZC-4 V) containing fourteen lamps, loaded on top (6 lamps) and the two sides (4 lamps each). UV–vis absorption spectra were collected using a Shimadzu spectrophotometer (UV 2450 model). The fluorescence spectra were collected on a Fluorolog-3 spectrometer (Jobin Yvon Inc., Edison, NJ) equipped with TBX PMT detector and an air cooled CCD camera.

Ligand Synthesis. The polyethylene glycol-appended lipoic acid ligands used in this study, namely LA-PEG$_{750}$-OCH$_3$ and LA-TEG-NH$_2$ were prepared, characterized, and purified using the procedures we described in previous reports.[17,18,27] The LA-appended with zwitterion (LA-N,N-dimethyl-propanediamine-sulfobetaine) group was synthe-

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sized following the rationale described in reference,[23] with a few modifications. We have also designed and prepared a new lipoic acid-appended zwitterion ligand made of a tetra-ethylene glycol bridge (LA-TEG-ZW) starting from LA-TEG-NH_2. All these ligands were used for the phase transfer of TOP/TOPO-capped QDs. Chemical reduction of the dithiolane group to provide DHLA-zwitterion ligands was also carried out using sodium borohydride. These ligands (DHLA-ZW and DHLA-TEG-ZW) were applied for the phase transfer of QDs using the conventional route, and the resulting dispersions provided reference samples for comparison to those prepared via the UV-mediated photoligation strategy using the oxidized LA-based ligands. Figure 1 shows the chemical structures of the various ligands along with a schematic description of the synthetic steps involved. Below, we discuss the synthesis of the zwitterion ligands used in this study.

1. Synthesis of LA-Zwitterion. A. Compound 1 (LA-N,N-Dimethyl-1,3-propanediamine). Lipoic acid (3 g, ~15 mmol), triethylamine (1.47 g, ~15 mmol) and 30 mL CH_2Cl_2 were placed in three-necked bottom flask. The mixture was cooled to ~0 °C under ice-cold conditions while being continuously flushed with N_2, and was then left stirring for 30 min. Methanesulfonyl chloride (1.67 g, ~15 mmol) was added dropwise and the mixture was slowly warmed up to room temperature, and left stirring for 5 h. A mixture of N,N-dimethyl-1,3-propanediamine (1.24 g, ~12 mmol) and triethylamine (0.61 g, ~6 mmol) in 20 mL CH_2Cl_2 was slowly added, followed by overnight stirring at room temperature and under N_2 atmosphere; a slight excess of LA was used. The reaction mixture was washed with water (30 mL, two times), then with saturated Na_2CO_3 solution (two times). The organic layer was dried over Na_2SO_4 and filtered and the solvent was evaporated, yielding compound **1** as yellow oil. The reaction yield was ~70%. The ^{1}H NMR spectrum for compound **1** is shown in the Supporting Information (Figure S1).

^{1}H NMR (600 MHz, DMSO-d_6) of compound **1**. δ (ppm) 6.69 (s, 1H), 3.54−3.59 (m, 1H), 3.32−3.34 (m, 2 H), 3.09−3.20 (m, 2H), 2.43−2.48 (m, 1 H), 2.37 (t, 2H, J = 6 Hz), 2.23 (s, 6H), 2.15 (t, 2H, J = 6 Hz), 1.88−1.93 (m, 1H), 1.67−1.74 (m, 1H), 1.52−1.61 (m, 5H), 1.41−1.52 (m, 2H). ESI-MS (EI$^+$) calculated for $C_{16}H_{32}N_2O_4S_3$ $(M+H)^+$ 413.2; found 413.2.

B. Compound 2 (LA-Zwitterion). Compound **1** (4 g, ~14 mmol) was dissolved in 60 mL of $CHCl_3$ and purged with N_2 for 30 min. 1,3-Propanesultone (1.8 g, ~15 mmol) was added at room temperature and the reaction mixture was left stirring for 3 days. A slight turbidity progressively built up as the reaction progressed, due to limited solubility of LA-zwitterion in chloroform. Once the reaction was complete, the solvent was evaporated and the product was further dried under vacuum, yielding a solid paste that is no longer dispersible in $CHCl_3$. The crude product was rinsed with $CHCl_3$ three times (to remove any impurities) followed by drying, to yield compound **2** as foamy solid. The reaction yield was ~90%. The corresponding ^{1}H NMR and mass spectra are shown in Figure 2.

^{1}H NMR (600 MHz, D_2O) of compound **2**. δ (ppm) 3.68−3.72 (m, 1H), 3.45−3.48 (m, 2H), 3.32−3.36 (m, 2H), 3.28−3.30 (m, 2H), 3.16−3.25 (m, 2H), 3.11 (s, 6H), 2.97 (t, 2H, J = 6 Hz), 2.46−2.50 (m, 1H), 2.26 (t, 2H, J = 9 Hz), 2.18−2.22 (m, 2H), 1.96−2.03 (m, 2H), 1.73−1.79 (m, 1H), 1.58−1.67 (m, 4H), 1.39−1.44 (m, 2H).

2. Synthesis of LA-TEG-Zwitterion. A. Compound 4 (LA-TEG-4-(Dimethylamino) Butyric Acid). Lipoic acid appended with amine-terminated tetraethylene glycol (TEG-NH_2) was prepared according to the procedure described in our previous reports.[17,27] In a round-bottom flask, 4-(dimethylamino)butyric acid hydrochloride (0.33 g, ~2.0 mmol) was mixed with DCC (0.41 g, ~2.0 mmol), DMAP (0.05 g, 0.4 mmol), and triethylamine (0.29 g, ~3.0 mmol) in 30 mL of $CHCl_3$ under ice cold condition. Then LA-TEG-NH_2 (0.5 g, 1.3 mmol) dissolved in 20 mL $CHCl_3$ was added to the flask dropwise under N_2 atmosphere. The reaction mixture was gradually warmed to room temperature and left stirring for ~9 h. The mixture was then filtered through Celite, followed by washing with water two times (30 mL each time), and saturated Na_2CO_3 aqueous solution three times (30 mL each time). The $CHCl_3$ layer was collected, dried with Na_2SO_4 and the solvent was evaporated to yield compound **4** as yellow oil; the reaction yield was ~60%. The ^{1}H NMR spectrum for compound **4** is shown in the Supporting Information (Figure S1).

^{1}H NMR (600 MHz, $CDCl_3$) of compound **4**. δ (ppm) 6.84 (s, 1H), 6.33 (s, 1H), 3.42−3.64 (m, 17H), 3.08−3.18 (m, 2H), 2.42−2.47 (m, 1H), 2.32 (t, 2H, J = 9 Hz), 2.24 (t, 2H, J = 9 Hz), 2.22 (s, 6H), 2.18 (t, 2H, J = 9 Hz), 1.86−1.92 (m, 1H), 1.77−1.82 (m, 2H), 1.61−1.72 (m, 4H), 1.4−1.49 (m, 2H).

B. Compound 5 (LA-TEG-Zwitterion). Compound **4** (0.3 g, ~0.6 mmol) was mixed with 1,3-propanesultone (0.06 mL, 0.7 mmol) in 30 mL $CHCl_3$ and continuously stirred for 3 days at room temperature under N_2 atmosphere. Once the reaction was complete, the solvent was evaporated. The crude product was purified by rinsing with ethyl acetate 3 times followed by drying to yield compound **5** as yellow oil; the reaction yield was ~90%. The ^{1}H NMR and mass spectra for compound **5** are shown in Figure 2.

^{1}H NMR (600 MHz,D_2O) of compound **5**. δ (ppm) 3.59−3.68 (m, 15H), 3.45−3.48 (m, 2H), 3.36−3.39 (m, 4H), 3.31−3.36 (m, 2H), 3.16−3.25 (m, 2H), 3.1 (s, 6H), 2.96 (t, 2H, J = 6 Hz), 2.62−2.66 (m, 2H), 2.47−2.5 (m, 1H), 2.36 (t, 2H, J = 6 Hz), 2.25 (t, 2H, J = 6 Hz), 2.19−2.22 (m, 2H), 2.05−2.09 (m, 2H), 1.96−1.99 (m, 1H), 1.71−1.77 (m, 1H), 1.57−1.66 (m, 3H), 1.38−1.43 (m, 2H). ESI-MS (EI$^+$) calculated for $C_{25}H_{49}N_3O_8S_3$ $(M+H)^+$ 616.3; found 616.3.

Quantum Dots. We used different color CdSe-ZnS QDs with emission varying from 520 nm (blueish-green) to 624 nm (red). All QD samples were synthesized using high-temperature reduction of organometallic precursors in a coordinating solvent mixtures made of alkylphosphines, alkyphosphine-carboxyl, and alkylamines, as described in previous reports.[9,10]

Transfer of QDs via UV-Irradiation of Two-Phase. Cap exchange of the TOP/TOPO-capped QDs with LA-ZW or LA-TEG-ZW ligands is carried out using a two-phase reaction starting from TOP/TOPO-QDs dispersed in hexane and ligands dissolved in methanol. This route is essentially imposed by the stringent solubility requirement of the zwitterion ligands, because LA-ZW and LA-TEG-ZW ligands are soluble only in methanol or water.[21] Nonetheless, a single phase reaction described in our recent report can be applied with LA-PEG ligands as these can be easily dissolved in several polar solvents including methanol, ethanol, propanol and butanol.[26] Below, we briefly describe the protocol applied to the phase transfer using LA-ZW; a similar protocol can be applied to LA-TEG-ZW. QDs capped with hydrophobic TOP/TOPO (200 μL of ~7 μM stock solution in a hexane/toluene mixture) was first precipitated as a paste using ethanol, and then redispersed in 550 μL of hexane. In a separate vial, 35 mg of LA-ZW ligand was solubilized in 500 μL of methanol with a slight heating and stirring for 2 h to ensure a homogeneous solution; catalytic amount of tetra methyl ammonium hydroxide, (TMAH, 10 mM) was added. Heating for longer time may be necessary to provide a homogeneous solution of ligands in methanol. These two solutions were mixed in a scintillation vial, a magnetic stirring bar was inserted, the vial was sealed, and the atmosphere was switched to nitrogen. The vial was placed inside the UV reactor, and irradiated for 30−40 min. A colored precipitate made of the QDs is deposited on the vial walls, leaving the methanol and hexane layers clear (devoid of QD materials). The newly capped QDs are not dispersible in either solvent. The solvents containing free TOP/TOPO and excess zwitterion ligands were removed using a glass pipet. Further washing with methanol (2 times) followed by centrifugation allowed removal of the excess free ligands. Finally, the QDs were dried under vacuum and redispersed in 500 μL of DI water resulting in a homogeneous dispersion (Figure 3). Our attempts to carry out the transfer using a one phase reaction where the TOP/TOPO-QDs are first precipitated (as a paste) then mixed with the ligand plus methanol (or a mixture of methanol and butanol), produced inefficient or partial ligand exchange, as the final dispersion was always slightly turbid.

Expression and Purification of 8-Residue-Histidine-Tagged Maltose Binding Protein, MBP-His$_8$. The sequence coding for MBP spanning K1 to T366 was modified within the pMal-c2 vector (New England Biolabs, Beverly, MA) to introduce a 8x Histidine-tag. More precisely, the polylinker region was modified to contain extra NSSSHHHHHHHHSSGLVPRGSS residues at the C-terminus of MBP. The protein was expressed in BL21 cells (Novagen, Rockland,

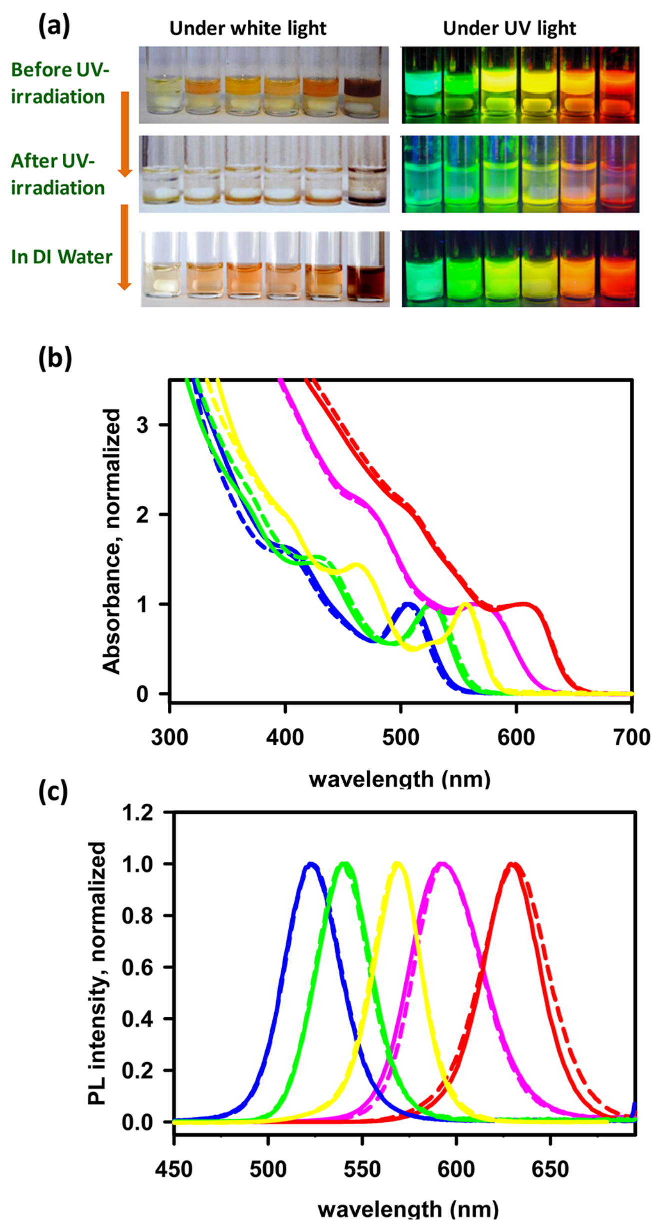

Figure 3. (a) White light and fluorescence images of five different size QDs before (in organic solvent) and after photoligation and transfer to water media. (b) UV−vis absorption and (c) PL Spectra of the various QDs (λ_{em} = 520, 540, 568, 585, and 624 nm); dotted lines represent hydrophobic QDs in organic solvents, and solid lines represent hydrophilic QDs phase transferred using LA-ZW. The absorption and PL spectra were normalized with respect to the band edge peak and the emission maximum, respectively.

MA). Luria−Bertani broth was inoculated 1:100 with saturated culture and grown to an OD_{600} of 0.6, then treated with 0.4 mM isopropyl α-D-1-thiogalactopyranoside (Fisher Scientific, Fair Lawn, NJ). The culture was harvested for 3 h after induction and lysed in binding buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 10 mM imidazole) using a microfluidizer. Clarified lysate was initially purified on a His-Select Nickel Affinity Gel (Sigma, St. Louis, MO), then proteins were eluted using a binding buffer containing 250 mM imidazole. Fractions were diafiltrated into 20 mM Tris, pH 7.4, 2 mM EDTA buffer using YM-10 Centricon units (Millipore, Billerica, MA) and purified to homogeneity on an anion exchange HPLC (Mono Q, GE Healthcare, Piscataway, NJ) using a linear gradient from 0 to 1 M NaCl, 20 mM Tris, 2 mM EDTA buffer pH 7.4 at the Analytical Core Facility (Florida State University, Tallahassee, FL). The purified proteins were diafiltrated into PBS buffer (11.9 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4) using Centricon units and stored at 4 °C.

Self-Assembly of QD-MBP-His_8 Conjugates. In an eppendorf tube, 8.2 μL of MBP-$(His)_8$ (537 μM stock solution) was mixed with PBS buffer to a total volume of 100 μL. In a separate tube, 50 μL of a stock solution of DHLA-ZW-QDs or DHLA-TEG-ZW-QDs (7.3 μM) was diluted in PBS buffer to a total volume of 200 μL. The MBP solution was added to the tube containing the QDs and the mixture was incubated at ∼4 °C for 30−45 min to allow for self-assembly. The molar ratio of QD-to-MBP-His was maintained at 1:12 in all cases. Affinity chromatography was performed to test the QD-MBP conjugate formation. This assay is based on the specific binding of MBP (fusion protein) to amylose (e.g., gel column loaded with amylose). Briefly, 1.5−2 mL of amylose stock gel was loaded onto a 10 mL of capacity column and washed with 10 mL of buffer three times. The QD and MPB-His mixture (above) was then loaded onto the column and washed with PBS buffer pH 7.3 (4 times, 5 mL each). A colored band visible under hand hold UV light (365 nm) formed on the top of the column and was unaffected by multiple buffer washes, indicating binding to the amylose gel (see below). Adding 5 mL of a 20 mM solution of D-(+)-maltose to the column progressively displaced the luminescent band (QD-conjugates), which was collected and further characterized.

RESULTS AND DISCUSSION

Design and Synthesis of Zwitterion Ligands. The design of zwitterion ligands is motivated by the desire to reduce the overall hydrodynamic diameter (HD) of the QDs, which is a stringent requirements for certain applications such as energy transfer-based sensing, in vivo imaging, and protein tracking.[19,28−30] The LA-ZW ligands were prepared using relatively simple synthetic rationales. N,N-Dimethyl-1,3-propanediamine moiety was first coupled onto the lipoic acid, followed by reaction with 1,3-propanesultone to produce LA-zwitterion ligand. The purification steps required are relatively simple and reaction yields are high. For LA-TEG-ZW, LA-TEG-NH_2 was first coupled to 4-(dimethylamino)butyric acid in the presence of DCC and DMAP, followed by reaction with 1,3-propane sultone; in both cases, the second reaction step involves the ring-opening of the sultone to provide the final zwitterion-terminated ligands. We should stress that the easy purification of these ligands is a rather important feature, as zwitterion-appended molecules tend to require stringent solubility conditions, often only in water.[21,23,31]

Cap Exchange and Characterization of the Hydrophilic QDs Following Phase Transfer. When applied to QDs, cap exchange with lipoic acid presenting ligands has necessitated the reduction of the disulfide groups, because the oxidized LA form does not coordinate onto the nanocrystal surfaces.[16,18,19] Reduction of the lipoic acid to DHLA has thus far been achieved chemically using large excesses of $NaBH_4$. Driven by a desire to simplify the phase transfer, reduce the number of steps required, and eventually improve the ligand exchange efficiency, we explored the use of optical means to promote the transfer. More precisely, we found that reduction of the dithiolane ring combined with rapid ligand exchange can be achieved under UV irradiation (at 350 nm) of TOP/TOPO-QDs in the presence of LA-based ligands.

Prior work by Sander and co-workers showed that partial reduction of pure lipoic acid in water can be realized with UV-excitation/irradiation.[32] We have also found that UV irradiation at 350 nm of the LA-based compounds (LA-PEG and LA-ZW) resulted in a near complete disappearance of the absorption peak at 340 nm characteristic of the lipoic group (see the Supporting Information, Figure S2).[26] This transformation is attributed to an optically induced transformation of the dithiolane rings through

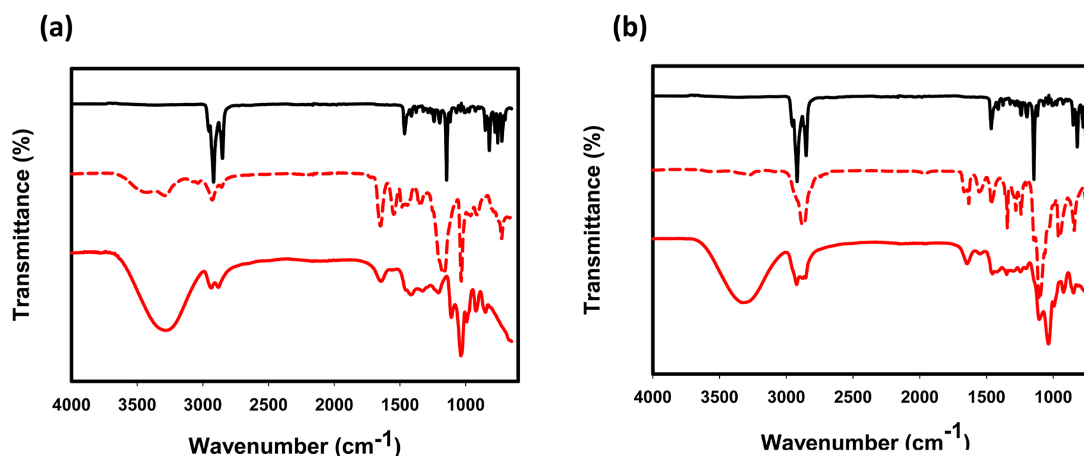

Figure 4. (a) FTIR spectra of TOP/TOPO-capped QDs in toluene (black, solid line), LA-ZW ligand (red, broken line), and QDs photoligated with LA-ZW dispersed in water (red, solid line). (b) FTIR spectra of TOP/TOPO-capped QDs in toluene (black, solid line), LA-PEG-OCH_3 ligand (red, broken line), and QDs photoligated with LA-PEG-OCH_3 in water (red, solid line). The latter is provided as a reference.

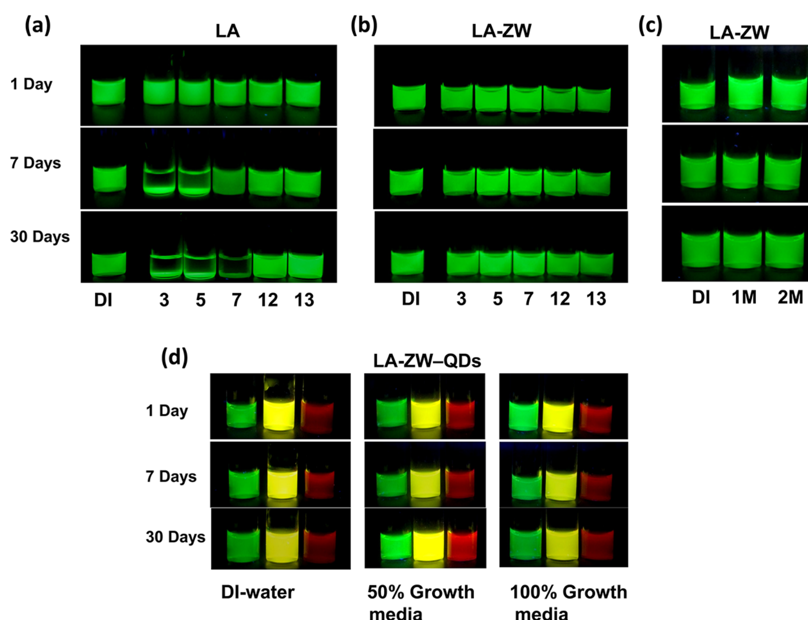

Figure 5. Stability tests. PL images of QD dispersions (λ_{em} = 540 nm) in phosphate buffer at different pHs, which were photoligated using: (a) lipoic acid and (b) LA-ZW. (c) PL images of QDs dispersions photoligated using LA-ZW in the presence of excess electrolyte (1 and 2 M NaCl). (d) PL images of different size QDs (λ_{em} = 540, 574, and 624 nm) photoligated using LA-ZW dispersed in RPMI growth media (50% and 100%) collected after different storage times. The QDs dispersed in DI water also shown as control.

radical formation. QDs are also known to engage (either as or photoexcited) in charge transfer interactions with proximal redox/metal complexes.[33−35] Thus, we believe that in situ reduction of the dithiolane groups while mixed with the photoexcited TOP/TOPO-capped QDs promotes rapid ligand exchange. This approach also eliminates the need to prepare and store chemically reduced ligands, and thus removes a few experimental steps. By removing the need for chemical reduction, the new photoligation strategy adds another potential benefit when using LA-ZW ligand. Borohydride reduction of LA-ZW is tedious, due to the limited solubility of both oxidized and reduced forms of the ligand; modified ZW ligands are highly hydrophilic by nature.

The photoligation strategy was primarily applied to heterogeneous two immiscible phase mixtures, one nonpolar phase containing the native TOP/TOPO-QDs, whereas the other polar phase contains the LA-based ligands. Such procedure was successfully applied to a wide array of QDs with photoemission ranging from green-blue to red, and in the presence of either LA-ZW or LA-TEG-ZW, where a complete precipitation of the QDs following UV irradiation resulted for all samples (see Figure 3). Simple removal of the solvents, followed by gentle drying, produced nanocrystals that readily dispersed in buffer media as shown in Figure 3. We consistently observed ∼40−50% loss in the PL intensity upon transfer to water compared to the native TOP/TOPO-QDs in toluene (e.g., TOP/TOPO-QDs with a QY of 60% produced water-dispersion with ∼30−35%). The absorption and emission spectra of the different color QDs measured in organic solvent (prior to ligand exchange) and for the same QDs after transfer to DI water using both LA-ZW ligands are nearly identical, as shown in Figure 3. The absorption and emission spectra are identical to those measured for dispersions of QDs phase transferred in the presence of LA-TEG-ZW (see Supporting Information, Figure S3). This

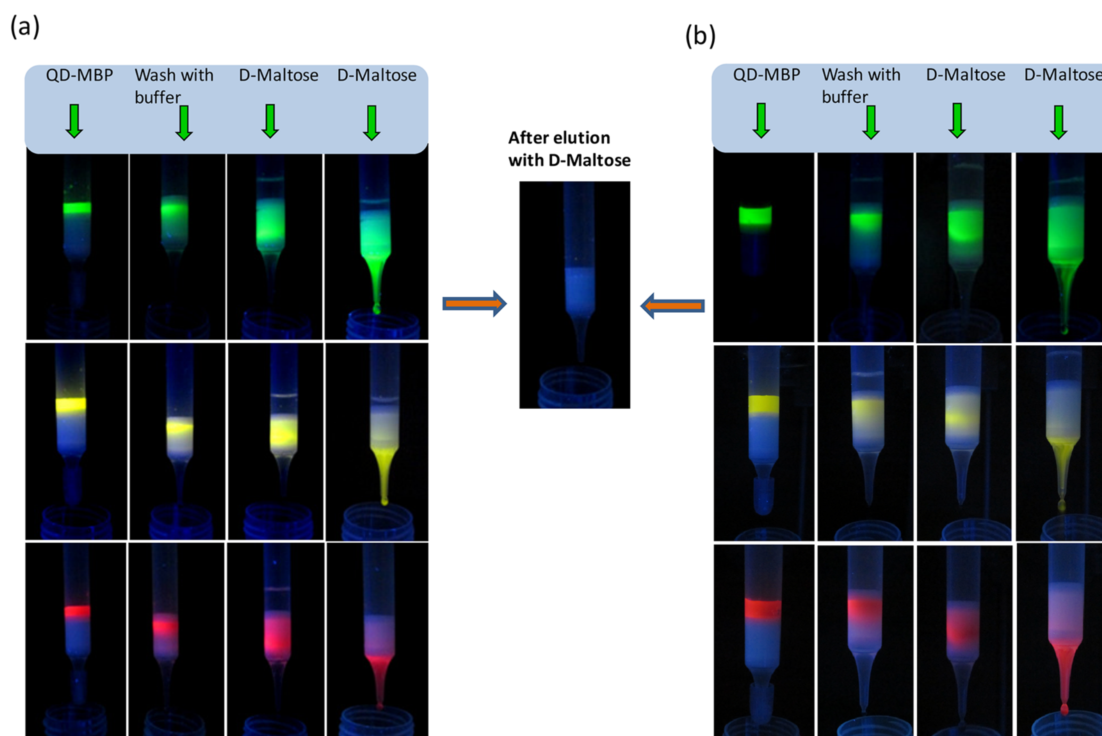

Figure 6. Binding assay of QD−MBP assemblies onto amylose column and release by soluble maltose: (a) conjugates were prepared using QDs photoligated using LA-ZW; shown are green-, yellow-, red-emitting (λ_{em} = 540, 574, and 624 nm) nanocrystals. (b) Conjugates of the same QDs prepared using QDs photoligated using LA-TEG-ZW.

indicates that the new photopromoted ligation does not alter any of the photophysical characteristics of the nanocrystals. The new strategy also provides nanocrystals with better photophysical properties than those prepared using ligand exchange with DHLA-ZW ($NaBH_4$-reduced). Indeed, QD dispersions in water prepared using the conventional route, using chemically reduced ligands, exhibited slightly weaker PL than those transferred via photoligation; we attribute this to the difficulties associated with the chemical reduction and purification of ZW-appended lipoic acid ligands. We also found that hydrophilic DHLA-ZW-QDs prepared using the photoligation strategy consistently showed higher PL quantum yields than those phase transferred in the presence LA-PEG-OCH_3 (see the Supporting Information, Figure S4).

The effectiveness of the photoligation process with LA-ZW was further verified by FTIR spectroscopy. Figure 4a shows that the distinct bands characteristic of TOP/TOPO measured from the native QDs have disappeared following the photomediated cap exchanged with LA-ZW ligand, while new bands at 1650 cm^{-1} (C═O stretch) and 1550 cm^{-1} (N−H bending) appeared. The spectrum collected for the DHLA-ZW-QDs was similar to the one collected for the free ligand, further confirming the effectiveness of the photomediated cap exchange combined with the newly synthesized LA-zwitterion ligands. Similar FTIR results were measured for nanocrystals photoligated with LA-PEG-OCH_3, where new bands at 1650 cm^{-1} (C═O stretch) and 1550 cm^{-1} (N−H bending) were measured (Figure 4b).[26]

Colloidal Stability of the Nanocrystals. We tested the long-term colloidal stability of QDs capped with LA-ZW ligands at several pHs, and in the presence of large excess electrolytes (NaCl) and cell growth media. We used phosphate buffers (prepared using different combinations of NaH_2PO_4, Na_2HPO_4, Na_3PO_4, and H_3PO_4) plus 0.137 mM NaCl. Aliquots of stock solutions of DHLA-ZW-QDs were added to the desired buffer to achieve a final nanocrystal concentration of 0.5 μM.

Stability tests of the QDs photoligated with LA-ZW and LA-TEG-ZW were compared to those of dispersions of LA-QDs (also prepared via photoligation). Figure 5 shows fluorescence images of green-emitting CdSe-ZnS QDs capped with DHLA-ZW dispersed in buffers over the pH range 3−13 and in the presence of added 1 and 2 M NaCl; additional stability tests are provided in the Supporting Information (Figure S5). Data indicate that QDs photoligated with LA-ZW ligand are stable across the pH range 4−13 for at least 2 months of storage at 4 °C (the duration of the test). Similarly, QDs dispersion stayed homogeneous in the presence of 1 M NaCl and 2 M NaCl. The observed long-term stability can be attributed to the nature of the zwitterion where the combination of a quaternary amine and the sulfonate group provide charge balance, maintaining well dispersed nanocrystals over a broad pH range. Similar data were collected for QDs photoligated with LA-TEG-ZW ligands (not shown). Conversely, dispersions of nanocrystals photoligated with LA showed limited stability, as precipitation started to build up after 1 week of storage at acidic pH, with full scale aggregation occurred at pH $\leq$ 7 after 1 month. Stability in basic media (pH $\geq$7) is promoted by the formation of carboxylate groups, which provide steric stabilization to the nanocrystals.[16] These data are consistent with stability tests of DHLA-QDs prepared using chemically reduced ligand.[16,17] We further monitored the stability of DHLA-zwitterion capped QDs in cell culture media. Figure 5 shows that QDs photoligated with LA-zwitterion stayed stable in both 50% and 100% growth media for at least one month; additional stability tests are provided in the Supporting Information (Figure S5).

Protein Conjugation. Self-assembly of QD bioconjugates driven by metal-histidine interaction between the nanocrystals

and His-tagged proteins and peptides has been explored by several groups over the past decade.[19,28,36−41] This effort is driven by the fact that proteins are routinely expressed with polyhistidine tag so that they can be purified using easy to implement Ni-NTA affinity chromatography. Direct coordination of His-tagged proteins onto ZnS-overcoated QDs was used by a few groups including ours, but such approach requires that the tag accesses the metal ions on the dot surface.[36,42] To achieve this, we have used DHLA-capped QDs, and that has limited our effort to working in basic buffer conditions, because these nanocrystals tend to progressively aggregate at pH <7. Conversely, using PEGylated-ligands or polymer encapsulation shields the metal-rich surface and increases the size of QDs, rendering it ineffective for direct coordination of imidazoles onto the QD surface.[43] There have been several attempts to address this by, for example, inserting a flexible linker between the protein and the histidine tag to allow conjugation onto DHLA-PEG-QDs.[44] Alternatively, Ni-NTA chelating groups have been attached at the end of DHLA-PEG ligands (on DHLA-PEG-QDs), or on polymer-encapsulated nanoparticles, to make them reactive with the His-tag of the proteins.[37,40,45−48] Direct access to the Zn-rich surface of the nanoparticles is simpler, more effective, and it removes the need for additional steps for both QD preparation and protein expression, but requires QDs capped with small ligands (e.g., DHLA).[19,36,49,50]

The present zwitterion-modified ligand design, namely LA-zwitterion and LA-TEG-zwitterion, addresses some of these issues. Such ligands provide dispersions that are colloidally stable over a broad range of conditions, and nanocrystals with a hydrophilic layer thin enough to allow direct access of the polyhistidine to the Zn-rich QD surface. Indeed, dynamic light scattering measurements showed that QDs photoligated with these LA-based zwitterion ligands are small, with hydrodynamic size comparable to the one measured for the native TOP/TOPO-capped QDs and slightly smaller than that reported for DHLA-capped QDs (see the Supporting Information, Figure S6, and ref 42). We also found that such QDs easily and tightly self-assemble with MBP-His_8, and the resulting conjugates maintain the protein biological activity. Affinity chromatography relying on the competition between the binding of MBP to amylose gel and MBP to its substrate maltose in solution proves that QDs photoligated with LA-ZW- and LA-TEG-ZW have easily self-assembled with MBP-His_8. Following incubation of the QDs with MBP-His_8 protein, the resulting QD-MBP-His_8 conjugates tightly bound onto an amylose column as shown in Figure 6 by the fluorescent band visible under UV excitation in the columns. The QD-MBP conjugates stayed bound to the gel even after several washes with buffers (Figure 6). Binding of the conjugates to the gel was strong and stable, as indicated by the complete lack of fluorescence in the eluted buffer. Here the protein allowed specific interaction with the amylose gel while the QDs provided a visual tool for locating the conjugates. Upon addition of 20 mM D-(+)-maltose solution, fluorescent band immediately started eluting and was completely displaced from the amylose column. Figure 6 shows that similar binding to the column and release by soluble maltose was observed for several color QDs (green, yellow or red) and with both zwitterion ligands (DHLA-ZW and DHLA-TEG-ZW). This clearly confirms the compactness of the QDs capped with these new ligands. It may also reflect the benefit of using slightly longer polyhistidine tag compared to earlier protein expression where 5 and 6 histidine tags were used. We believe that these nanocrystals will be extremely useful for conjugate assemblies with a variety of proteins and peptides modified with polyhistidine tags. The ability to manipulate the various components over a broad of pHs and salt conditions is particularly promising for using these conjugates in in vitro and in vivo experiments.

■ CONCLUSION

We combined the synthesis of two compact zwitterion-modified lipoic acid ligands with a simple to implement photoligation strategy to form hydrophilic CdSe-ZnS QDs that are highly luminescent, colloidally stable and biocompatible. Being small, these zwitterion ligands reduce the hydrophilic capping shell and thus the overall size of the nanocrystals, while expanding the colloidal stability over a broad pH range, compared to DHLA for example. As such QDs phase-transferred using this combined strategy are highly luminescent, stay well dispersed in acidic and basic buffers, and are compatible with conjugation to His-tagged proteins. This design also removes the need for further modification of the capping shell with Ni-NTA groups, as required when nanocrystals encapsulated within block copolymers or large-scale ligands are used. We anticipate that nanocrystals prepared using this strategy will find great use in application such as sensor design based on energy transfer interactions, in vivo imaging, and other cell based assays.

■ ASSOCIATED CONTENT

Supporting Information

Details on the phase transfer, ligand structure, ^{1}H NMR spectra, colloidal stability of the QD dispersions, UV−vis Abs data, FT-IR data, and dynamic light scattering data. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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