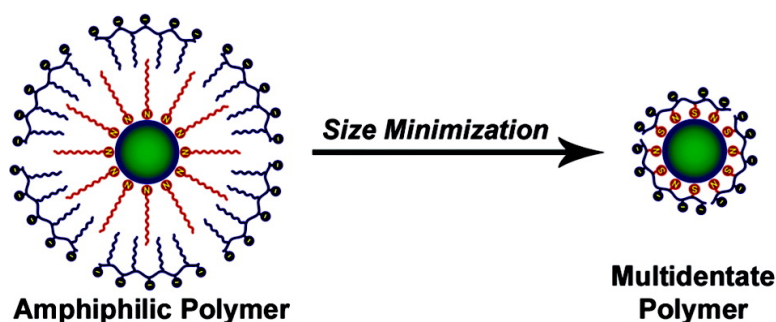


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Minimizing the Hydrodynamic Size of Quantum Dots with Multifunctional Multidentate Polymer Ligands

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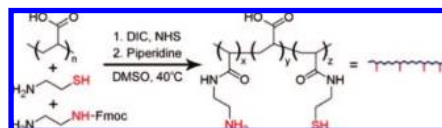
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Semiconductor quantum dots (QDs) are light-emitting nanocrystals with unique optical and electronic properties that are not available from organic dyes or fluorescent proteins.¹ In the near term, one of the most promising applications of these particles is for use as fluorescent probes for molecular, cellular, and in vivo imaging.² However, a major problem is the large size of conventional QD probes, which adversely affects their molecular binding and in vivo biodistribution. This bulkiness is not an intrinsic problem of QD nanocrystals, but arises mainly from organic surface coatings used for encapsulation and stabilization. In fact, small 4–7 nm QDs have been shown to have hydrodynamic sizes (diameters) of 20–40 nm when coated with amphiphilic polymers.³ Small hydrophilic and cross-linked ligands such as thioglycerol and dihydrolipoic acid have been used to reduce the coating layer thickness,^{4–7} but the resulting dots often suffer from low colloidal stability, photobleaching, or low quantum yield. Consequently, these size-reduced QDs have found only limited utility in live cell and in vivo applications.²

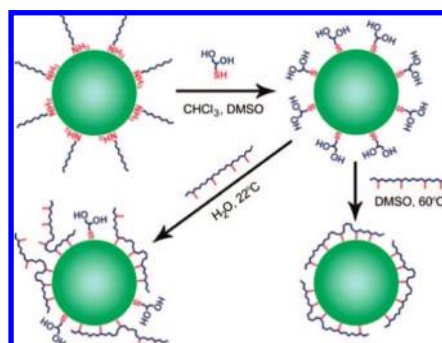
Here we report a new class of multifunctional multidentate polymer ligands not only for minimizing the hydrodynamic size of QDs but also for overcoming the colloidal stability and photobleaching/signal brightness problems encountered in previous research. A major finding is that a mixed composition of thiol (-SH) and amine (-NH₂) groups grafted to a linear polymer chain can lead to a highly compact QD with long-term colloidal stability, strong resistance to photobleaching, and high fluorescence quantum yield. In contrast to the standing brushlike conformations of PEGylated dihydrolipoic acid ligands and monovalent thiols, we believe that these multidentate polymer ligands can wrap around the QD in a closed “loops-and-trains” conformation.⁸ This structure is highly stable from a thermodynamic perspective and is thus responsible for the excellent colloidal and optical properties observed. As a result, we have prepared a new generation of bright and stable CdTe QDs with small hydrodynamic diameters between 5.6 and 9.7 nm, with fluorescence emission tunable from the visible (515 nm) to the near-infrared (720 nm). In addition to CdTe nanocrystals, we find that this new class of multidentate polymers is applicable to a broad range of core nanocrystals as well as core/shell nanostructures including CdS, ZnSe, CdSe/ZnS, and CdTe/CdS, more versatile and compact than amphiphilic phytochelatin peptides.⁹

As depicted in Scheme 1, the multidentate polymer was synthesized by covalently modifying about 35% of the carboxylic acids of poly(acrylic acid) (PAA, MW ≈ 1800) with cysteamine and *N*-Fmoc-ethylenediamine using diisopropylcarbodiimide (DIC) and *N*-hydroxysuccinimide (NHS). After deprotection of the amine with piperidine and purification, each polymer molecule contained approximately 3.5 active thiols and 3.0 active amines, as determined via Ellman's reagent and fluorescamine assays (see Supporting Information). For coating QDs, this balanced composition of amines and thiols was found to provide superior monodispersity, photostability, and fluorescence quantum yield compared to either amines or thiols alone. These multifunctional, multidentate polymers are soluble only in strongly polar solvents such as water, DMSO, and DMF. Because the CdTe

Scheme 1



Scheme 2



QDs were prepared in a high temperature organic solvent using hydrophobic ligands (see Supporting Information), it is necessary to first exchange the native ligands with thioglycerol (Scheme 2). These polar monovalent ligands are then replaced with the multidentate ligand. A surprising finding is that stable, compactly coated QDs are produced only after heating (60–70 °C) for 1–2 h in DMSO under inert conditions. It is energetically favorable for the linear multidentate polymer to wrap around the QD in a closed configuration, but this highly ordered structure is kinetically slow to form at room temperature (see Scheme 2), so elevated temperatures are needed to speed up ligand exchange and loop closure.

Figure 1 compares the optical properties and hydrodynamic sizes of CdTe QDs (2.5 nm) coated with a traditional amphiphilic polymer (octylamine-modified poly(acrylic acid)) or the mixed thiol/amine multidentate ligand. Although the amphiphilic polymer and the multidentate ligand are prepared from the same molecular-weight poly(acrylic acid) backbone, the QDs coated with the multidentate ligand are considerably smaller in size and also much brighter in fluorescence. Dynamic light scattering measurements show that the multidentate polymer coating is only 1.5–2 nm in thickness. This compact shell matches the geometric predictions of a polymer conformation with a high degree of adsorption on the QD surface, enabled by its high affinity and low molecular weight. In comparison, the coating thicknesses are on the order of 4–7 nm for amphiphilic polymers and even some monovalent molecular ligands.^{3b} It is also worth noting that the CdTe QD is not protected with an electronically insulating inorganic shell (e.g., ZnS or CdS) and its fluorescence is retained with the multidentate polymer, but nearly completely quenched by the amphiphilic polymer.

As shown in Figure 2, the fluorescence quantum yield, monodispersity and photostability of these polymer-coated QDs are strongly

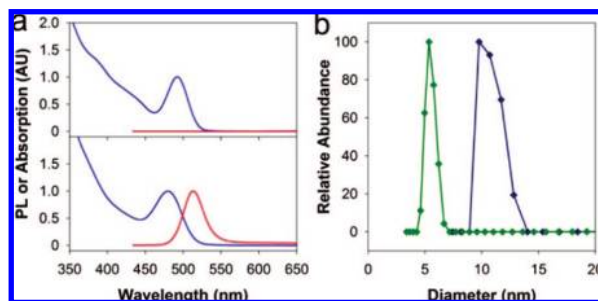


Figure 1. Comparison of optical and hydrodynamic properties of CdTe QDs (2.5 nm) solubilized in water with an amphiphilic polymer (octylamine-modified poly(acrylic acid)) or a multidentate polymer ligand. (a) Absorption (blue curves) and fluorescence emission (red curves) spectra of CdTe QDs with amphiphilic polymer (upper) or multidentate polymer (lower) coatings. (b) Dynamic light scattering size data of QDs with amphiphilic polymer (blue curve) and multidentate polymer (green curve) coatings. PL = photoluminescence, AU = arbitrary units. All samples were dissolved in phosphate buffered saline.

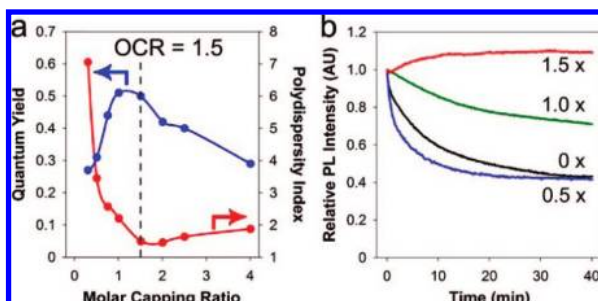


Figure 2. Effects of polymer capping ratios on QD properties: (a) QD (2.5 nm) fluorescence quantum yield (blue curve) and polydispersity index (red curve) as a function of molar capping ratio, and (b) photostability data at various capping ratios (MCR = 1.5, 1.0, or 0.5) and in the absence of polymer (MCR = 0). See text for details.

dependent on the molar capping ratio (MCR), which is calculated by dividing the sum of basic groups (amine and thiol) on the polymer by the sum of cadmium and tellurium atoms on the QD surface (see Supporting Information). When the MCR values are below 1.0, the amount of polymer is not sufficient to completely coat 2.5-nm CdTe QDs, resulting in polydisperse nanocrystals (as measured by the polydispersity index or PDI in gel filtration chromatograms). When the MCR values are above 2.0, the excess polymer leads to better size monodispersity and colloidal stability, but a reduced fluorescence quantum yield. Between these two limits is the optimal capping ratio (OCR) of approximately 1.5 (Figure 2A), yielding small, monodisperse nanocrystals (PDI < 1.5) with bright fluorescence (~50% quantum yield) and exceptional photostability (Figure 2B). The OCR is QD size-dependent, and its value changes to 1.0 for 3.0 nm cores and to 0.5 for 4.0 nm cores. The multidentate polymer-coated QDs are stable at room temperature for over 6 months, with no significant changes in gel filtration chromatograms. The quantum yield is also entirely retained under these conditions when stored in the dark. Furthermore, these dots can undergo dialysis for more than 1 week without deleterious effects, in contrast to QDs coated with monovalent ligands that generally aggregate within 2–3 h.

Figure 3 shows a size comparison of multidentate polymer-coated QDs (four emission colors) with globular protein standards, as measured by gel filtration chromatography. The results demonstrate that the coated green-emitting QDs (515 nm) have a hydrodynamic size slightly larger than fluorescent proteins (MW = 27–30 kDa), while the yellow-emitting QDs (562 nm) dots are slightly smaller than serum albumin (MW = 66 kDa). Even the near-infrared emitting dots (720 nm) are similar to antibodies (MW = 150 kDa) in hydrodynamic size.

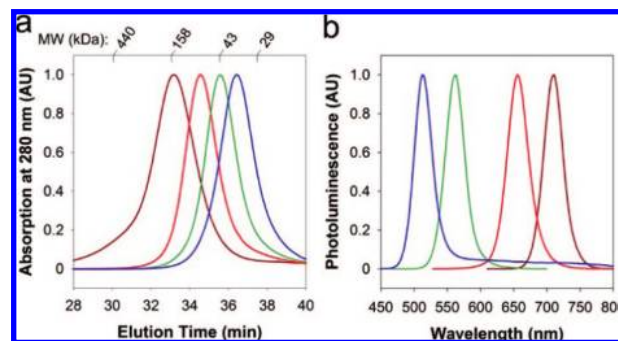


Figure 3. (a) Gel filtration chromatograms of multidentate polymer coated CdTe QDs showing direct size comparison with protein standards (29, 43, 158, 440 kDa). (b) Fluorescence emission spectra from the corresponding QDs. The QD hydrodynamic sizes are 5.6 (2.5 core, blue), 6.6 (3.1 core, green), 7.8 (4.0 core, red), and 9.7 nm (6.0 nm core, brown).

In summary, we have reported a new strategy to minimize the hydrodynamic size of QDs by using multifunctional, multidentate polymer ligands. A novel finding is that a balanced composition of thiol and amine groups yields a highly compact coating for QDs, with a hydrodynamic thickness of only 1.5–2 nm. This has led to a new generation of highly bright and stable QDs with hydrodynamic diameters similar to proteins (5.6–9.7 nm) with tunable fluorescence emission from the visible to the near-infrared. These size-minimized QDs open new possibilities in multicolor molecular and cellular imaging at the level of single molecules and single nanoparticles.

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Supporting Information Available: Detailed methods and calculations, as well as spectroscopic and chromatographic data with various coatings. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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