

# Rapid and Highly Efficient Preparation of Water-Soluble Luminescent Quantum Dots via Encapsulation by Thermo- and Redox-Responsive Hydrogels

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A water solubilization method for hydrophobic CdSe-ZnS quantum dots (QDs) is exhibited in this paper. The thermo-sensitive and photo-cross-linkable poly(*N*-isopropylacrylamide) (PNiPAm) copolymer is used for extraction of the hydrophobic QDs in dichloromethane and transferring them to water. This was accomplished via a solvent-induced phase separation process based on the amphiphilic nature of PNiPAm and their interactions with the different solvents. In addition, the functional ferrocene-containing PNiPAm is also used for the water solubilization of the hydrophobic QDs and the hydrogel/QDs particles demonstrate the chemical redox-controlled switch on–off property.

## Introduction

The unique optical properties of semiconductor quantum dots (QDs) make them promising to be a new generation of fluorescent markers in cellular labeling, deep-tissue imaging, and fluoro-immunoassay, and as the donors for fluorescence resonance energy transfer (FRET).<sup>1</sup> Compared with the conventional organic dyes, QDs have a relatively high quantum yield, broad absorption, and narrow emission spectra. The color emissions can be fine-tuned by controlling the particle sizes and/or composition.<sup>2</sup> These advantages enable QDs to be excited simultaneously by a single wavelength and the multicolors of their respective emissions monitored in parallel.<sup>3</sup> Moreover, while an organic fluorophore is generally photochemically destroyed quickly by irradiation, QDs are highly resistant to photobleaching and degradation. Some QDs can be prepared in aqueous media directly; however, they generally have a narrow available color emission range and poor size distribution. High-quality QDs are best formed at high temperatures in organic solvents combined with pyrolysis of organo-metallic precursors, which have high crystallinity, narrow distribution of particle size, and a wide range of fluorescence emission, spanning from the ultraviolet to the near-infrared.<sup>4</sup> However, these luminescent QDs are not water-soluble due to their hydro-

phobic surface, which greatly limits their applications in biology. Therefore, much effort has been made toward transferring hydrophobic QDs to aqueous solutions.<sup>5</sup> The major routes include ligand exchange using hydrophilic small molecules,<sup>6</sup> forming a micelle-like encapsulation by amphiphilic polymer/ phospholipids,<sup>1b,7</sup> or by silica coatings.<sup>8</sup> In most cases, the ligand exchange alters the chemical and physical states of the QD surface as well as decreases the quantum efficiency. Silica coating enables QDs to be more biocompatible, but this procedure needs to be carried out in dilute solution, which is not suitable for large-scale preparations. Using amphiphilic polymer surpasses the above-mentioned strategies by forming a steady additional layer around the QDs based on the interaction of the hydrophobic chains. The advantage is no direct interaction between the polymer and the surface atoms of the QDs. However, the synthesis of amphiphilic block or alternating copolymers is complicated, and the range of commercially available ones is relatively limited.

Poly(*N*-isopropylacrylamide) (PNiPAm) is a well-known thermo-sensitive polymer, which exhibits a sharp phase transition in aqueous solution at a lower critical solution temperature (LCST) around 32 °C. This smart polymer has been extensively studied for its use in separation, drug release, and controlled cell adhesion.<sup>9</sup> The phase transition

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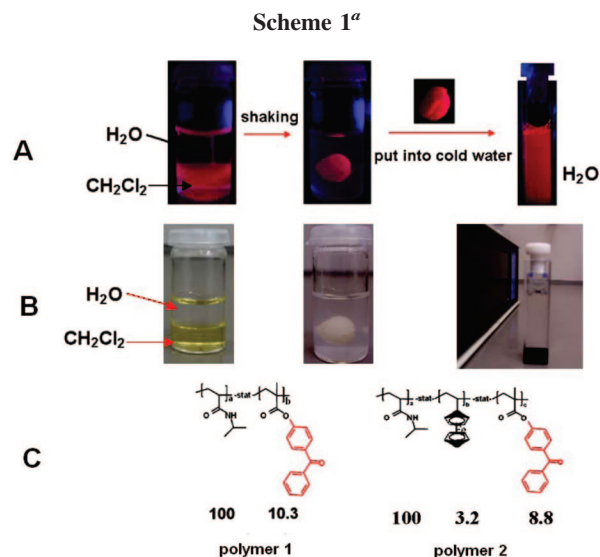
depends on the balance between hydrogen-bonding and hydrophobic effects in aqueous solutions of PNIPAM. The amphiphilic property of each PNIPAM unit suggests it to be a possible candidate for transferring hydrophobic substances into water. Surprisingly, this is rarely exploited based on the previous study.<sup>10</sup> Instead, directly embedding semiconductor nanocrystal into PNIPAM microgels was described as a way to generate QD-labeled spheres with thermo-sensitive property.<sup>11</sup> In this paper, we describe a facile and highly efficient approach for converting hydrophobic QDs into water-soluble objects by taking advantage of the “co-nonsolvent” transition of PNIPAM copolymers. This method can be easily manipulated and allows for the introduction of some desired functional groups for special applications by synthetic design in advance. To demonstrate the feasibility and flexibility of this method, 4-methacryloyloxy benzophenone (MaBP) and vinylferrocene (VF) were incorporated into PNIPAM copolymers as photo-cross-linkable and redox active groups, respectively.

## Experimental Section

**(A) Synthesis of Oleic Acid/Oleylamine-Capped Oil-Soluble CdSe-ZnS QDs.** The QDs with different emission wavelengths were prepared according to a modified literature approach.<sup>12</sup> See the details in the Supporting Information.

**(B) Synthesis of the Copolymer.** Polymer **1** was synthesized by free radical copolymerization of *N*-isopropylacrylamide (NiPAm) and 4-methacryloyloxy benzophenone (MABP), initiated by AIBN.<sup>13</sup> First, 2 g of NiPAm (17.68 mmol), 23.5 mg of MABP (0.26 mmol), and 14 mg of AIBN were dissolved in 6 mL of dioxane. After removing oxygen by two freeze–pump–thaw cycles, the reaction was allowed to proceed at 75 °C under argon and continuous stirring. After 3 h, the reaction was terminated by exposure to air. The resulting polymer was precipitated, washed by diethyl ether twice, and then dried in vacuum at 60 °C. The yield of the product is ~60%. The synthesis of polymer **2** is similar to that of polymer **1** except 76.8 mg (0.36 mmol) of vinylferrocene was added, and the yield is ~77%. Polymer **1** has an average molecular weight  $M_n$  of ~146K and a polydispersity ( $M_w/M_n$ ) of 2.5 determined by GPC measurement. The ratio of NiPAm/MaBP is 100:10.2 by the <sup>1</sup>H NMR analysis. In polymer **2**,  $M_n$  and polydispersity are 46K and 2.4, respectively. The ratio of the components of NiPAm/VF/MaBP is 100:3.2:8.8.

**(C) Transfer the Hydrophobic CdSe-ZnS QDs to Water by Encapsulation of PNIPAM Copolymer.** In a typical converting process, 0.3 mL of octadecylamine-coated nanocrystals (~2 mg/mL) and 30 mg of PNIPAM copolymer were dissolved in 2 mL of dichloromethane. After sonication of 5 min, the same volume of water was carefully added on the top of dichloromethane solution to give a sharp interface of two solvents. With hand shaking, the polymer separated out with the QDs instantly and aggregated into a big sphere at the interface of the solvents. By putting polymer/QD aggregates into fresh water and storing at 5 °C, the polymer-encapsulated QDs gradually dissolved in water within several hours,



<sup>a</sup> (A) Fluorescence photographs (under  $\lambda = 365$  nm UV light irradiation) and (B) the corresponding digital optical photographs showing the water-solubilization process of the hydrophobic QDs. QDs with light emission at  $\lambda = 626$  nm and polymer **1** were selected as an example. (C) The chemical structure of the synthesized PNIPAM copolymer with photo-crosslinkable benzophenone and redox-active ferrocene groups.

and the solution turned to optical clear. The photo-cross-linking was performed in PNIPAM/QDs aqueous solution under Stratagene UV Stratalinker 2400 operating at 75 W with a peak wavelength of 365 nm.

**(D) Characterizations.** The average molecular weight ( $M_w$ ) and the molecular weight distributions (polydispersity  $M_w/M_n$ ) of the polymers were determined by gel permeation chromatography (GPC) system with UV and RI detectors (DMF, 60 °C, and 1.0 mL/min flow rate). The <sup>1</sup>H NMR spectra were recorded on a Bruker Spectrospin 250 (250 MHz) spectrometer in methanol-*d*<sub>4</sub> solution. TEM images were collected by with a FEI Tecnai F20 operating at 200 KV. Dynamic light scattering (DLS) measurements were detected on Malvern Zetasizer 3000HS and using a He/Ne laser at 632.8 nm at scattering angles of 90. The photo-cross-linking was run on a Stratagene UV Stratalinker 2400 operating at 75 W with a peak wavelength of 365 nm.

UV–vis absorption and PL emission spectra were obtained on a diode array UV–vis spectrometer (Lambda 900, PERKIN ELMER) and a FL3095SL (J&M TIDAS 9.5, Germany), respectively. The PL efficiencies were determined by comparing the integrated emission of both Rhodamine 6G and the QDs samples, and the wavelength corresponding to the same optical density was used as the excitation wavelength for recording their emission spectra.<sup>14</sup>

## Results and Discussion

The converting process is illustrated in Scheme 1. First, oleic acid (OA)/oleylamine (OAM)-coated CdSe-ZnS and PNIPAM copolymer were dissolved in dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) and mixed homogeneously by sonication. The same volume of water was added on top of the CH<sub>2</sub>Cl<sub>2</sub> solution resulting in a sharp interface. After vigorous shaking and mixing, the polymer aggregated into a visible big sphere instantly and floated at the interface between the CH<sub>2</sub>Cl<sub>2</sub> and

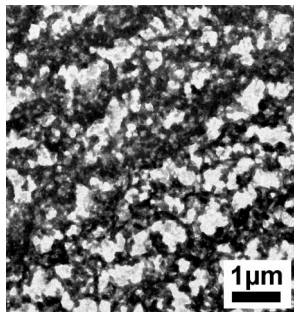
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**Figure 1.** TEM micrograph of water-soluble CdSe-ZnS QDs (QD571) encapsulated by polymer **1**.

the water layer separating spontaneously. Photographs of the fluorescence emission showed the strong luminescence of the polymer sphere and a nonluminescent organic phase, which indicated that the OA/OAm-coated QDs were extracted into the polymer sphere with a surprisingly high efficiency. The water-soluble QDs can be simply obtained by putting the polymer/QDs aggregates into water and keeping it cold (5 °C) to accelerate their dissolution. The resulting aqueous QDs solution was then irradiated in a quartz cell with  $\lambda = 365$  nm UV light for photo-cross-linking. Benzophenone was extensively employed as a cross-linking unit by reacting with C–H groups even in the presence of water as a solvent.<sup>15</sup> The kinetics of the photo-cross-linking process was characterized by UV–vis absorption spectra (Figure S1, Supporting Information). The absorption at  $\lambda = 258$  nm originating from the  $\pi$ – $\pi^*$  transition of the benzophenone moieties disappeared gradually within 45 min, which implied that the benzophenone is depleted and the polymer network was formed.

Transmission electron microscopic (TEM) pictures (Figure 1) show the typical morphology of water-soluble QDs ( $\lambda = 571$  nm light emission) converted by polymer **1** after photo-cross-linking. It can be clearly seen that the clusters of closely spaced QDs were encapsulated in PNiPAm and aggregated together with the sample spreading and drying on the carbon-coated grid, which reasons the water dispersion of hydrophobic QDs. Importantly, the photoluminescence (PL) of the QDs was maintained after organizing into bigger assemblies inside PNiPAm.<sup>16</sup> As shown in the PL images (left panel in Figure 2), the bright yellow and red luminescence originating from water-soluble QDs can be seen under a  $\lambda = 365$  nm hand-held UV light source, indicating that the QDs are distributed homogeneously in water after encapsulation and photo-cross-linkage. The PL microscope image (the right panel in Figure 2) also shows the distinguishable emission color of the mixture of water-soluble QDs after drying them on a glass slide. The fluorescence quantum yields (QYs) of the pristine QDs were 28% and 44% in  $\text{CH}_2\text{Cl}_2$  for the QD571 and QD626, respectively. After converting to water by polymer **1** and photo-cross-linking, the QYs of the polymer coated QDs were calculated to be 20% and 31%

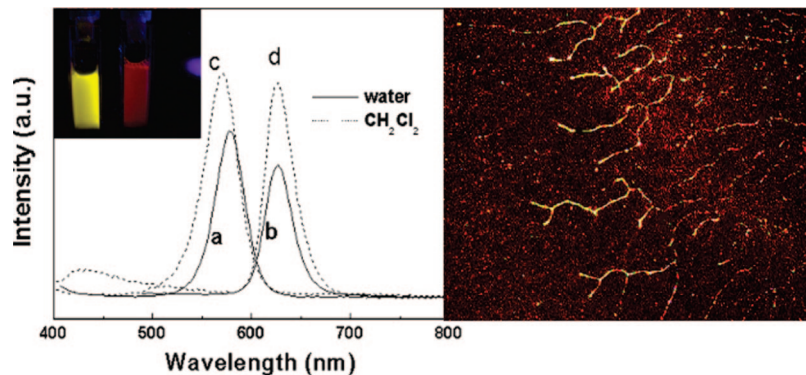
corresponding to yellow and red QDs, and it is clear that the QYs are well maintained. As compared to polymer **1**, the QY of polymer **2**-encapsulated 517 nm-emitting QDs decreases to 17%. The PL spectra show some minor differences for the QDs before and after being transferred to water. The emission bandwidths of water-soluble QDs become even narrower. In addition, a red-shift from  $\lambda = 571$  to  $\lambda = 578$  nm of the emission peak of yellow QDs ( $\sim 5$  nm in diameter) was observed after PNiPAm encapsulation. This is presumably due to energy transfer to larger QDs between neighboring QD particles in a sample with an inhomogeneous size distribution.<sup>17</sup> The temperature-dependent PL spectra (Figure S2, Supporting Information) demonstrate that the color emission further grows to longer wavelengths with the temperature rising, which could be caused by a closer packing of the QDs with the shrinking of PNiPAm copolymer.

The temperature dependence of the diameter change of PNiPAm-QDs particles was measured by dynamic light scattering (DLS) in deionized water, as shown in Figure 3A,B. As expected, a significant decrease of the particle size was observed when the temperature of the media is increased because of the shrinking of PNiPAm. Moreover, it is significant to know from DLS that there is no massive and indispersible agglomeration happening between the hydrogel/QDs particles during the photo-cross-linking because of the relatively narrow particle size distribution. It is assumed that benzophenone is a hydrophobic cross-linker, which prefers to attach to the OA/OAm surfactants on the surface of QDs, hydrophobic isopropyl groups, and the backbone of polymer, rather than be exposed in the water environment. The hydrophobic nature of benzophenone reduces the probability of cross-linking between particles. However, compared with the sizes of the initial QDs (Figure S3, Supporting Information, 5 nm for yellow-emitting and 10 nm for red-emitting QDs), it can be speculated that a large number of QDs was wrapped into a single hydrogel particle during the converting process because the hydrodynamic diameters of hydrogel/QD particles increase to 157.5 and 127.8 nm at 25 °C for yellow-emitting and for red-emitting QDs, respectively. These results are also in agreement with the TEM observation and give the reason for the temperature-dependent photoluminescence property of hydrogel/QDs in water. The average particle sizes of the PNiPAm/QDs composite decrease with the increasing of the irradiation time during the photo-cross-linking process. This should be due to the fact of improving of the cross-linking degree that makes the polymer chains bond together and constrained from free expanding (Figure S4, Supporting Information). Besides, with a ferrocene-containing PNiPAm copolymer, the hydrophobic QDs were also converted to water successfully. It has been established that the hydrophilicity of oxidized ferrocenium ions is

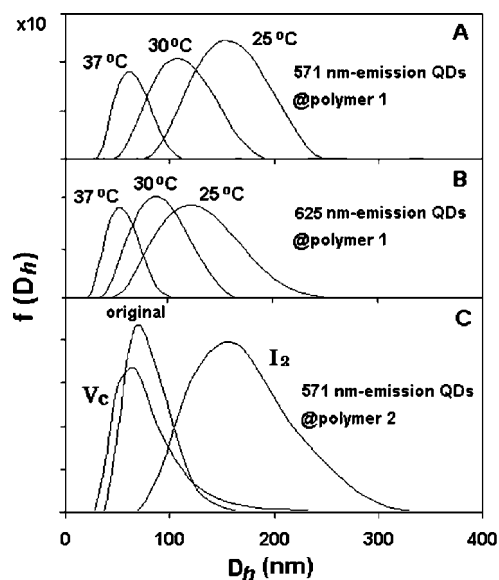
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**Figure 2.** Left panel: PL spectra (excitation wavelength is  $\lambda = 365$  nm) of the OA/OAm-coated CdSe-ZnS in  $\text{CH}_2\text{Cl}_2$  (dotted lines) and polymer encapsulated particles in water (solid lines). Inset: Digital photos showing the fluorescent water-dispersed QDs after encapsulation and photo-cross-linking. Right panel: PL microscope image ( $6 \times 5$  mm) of water-soluble QDs consists of a yellow- and red-color mixture spreading on a glass slide.

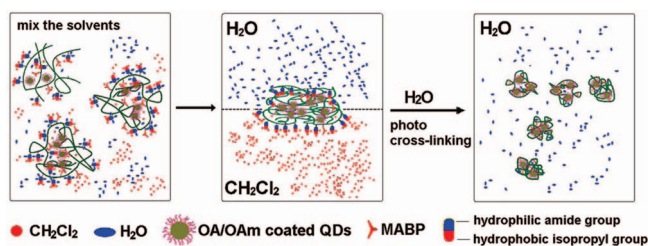


**Figure 3.** Temperature-dependent size distributions of polymer 1-QD571 (A) and polymer 1-QD626 (B). The variation of particles size according to adding  $10 \mu\text{L}$  of  $0.03$  wt %  $\text{I}_2$  and then  $10 \mu\text{L}$  of  $1$  mg/mL Vc aqueous solution to  $3$  mL of polymer 2 encapsulated QD571 water dispersion at  $37$  °C (C).

tremendously increased compared with the reduced form.<sup>18</sup> This property is utilized for altering the phase transition temperature of ferrocene-containing PNiPAM to obtain redox- or electrochemically controlled swelling/shrinking behavior.<sup>19</sup> According to the DLS result (Figure 3C), the particle size increases rapidly from  $75.2$  to  $158.1$  nm upon adding  $\text{I}_2$  (oxidative reagent for ferrocene) and reverts back to  $69.8$  nm after adding L-ascorbic acid (which is a reducing agent for ferrocenium) stepwise to the diluted aqueous dispersion of polymer 2-QD571 at  $37$  °C. This indicates that the luminescent hydrogel particles can be switched on or off according to external redox stimuli.

The possible process for the solvent-induced phase separation of PNiPAM and encapsulation of hydrophobic QDs is

### Scheme 2. Schematic Representation of Encapsulation and Transferring the Hydrophobic QDs from $\text{CH}_2\text{Cl}_2$ to $\text{H}_2\text{O}$ Using Amphiphilic Poly(NiPAM-co-MaBP) Copolymer via a Solvent-Induced Route



illustrated in Scheme 2. Its mechanism is similar with the situation of co-nonsolvency transition, in which the addition of methanol to PNiPAM water solution leads to the precipitation of the polymer.<sup>9a,20</sup> The difference is that dichloromethane and water is a completely immiscible system here. The flocculation of PNiPAM at the solvent interface should be attributed to the amphiphilic property of isopropylacrylamide groups, in which the amide group is hydrophilic and the isopropyl unit is intrinsically hydrophobic. Therefore, with mixing the  $\text{H}_2\text{O}$  and  $\text{CH}_2\text{Cl}_2$ , the water molecules might bind specifically to polar amide groups while the  $\text{CH}_2\text{Cl}_2$  prefers to attach to the nonpolar isopropylacrylamide or the backbone of the polymer, which enables the amphiphilic PNiPAM units to be attracted/repulsed by the hydrophobic/hydrophilic solvents simultaneously. These interactions result in the polymer chains collapsing and flocculating at the interface with the separation of the two solvents. At the same time, the hydrophobic OA/OAm-coated QDs prefer to be enwrapped by the polymer aggregates to avoid contacting with water and minimize the interfacial free energy. After transferring the polymer/QDs aggregates into water, the amphiphilic PNiPAM redissolves and forms the core-shell structure to draw the hydrophobic QDs into water.

### Conclusion

In summary, this report demonstrates an easy approach for water solubilization of hydrophobic CdSe-ZnS QDs by PNiPAM encapsulation via a solvent-induced phase separation method.

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Photo-cross-linkable benzophenone and redox-response ferrocene groups were integrated into the polymer, resulting in a multifunctional polymer encapsulation for CdSe-ZnS in one step. So far, the resulting aqueous dispersions are stable for months with no PL quenching or precipitation upon storage in a refrigerator. The approach is also promising to be applicable to other types of PNIPAm/hydrophobic material systems, especially for the purpose of encapsulation of hydrophobic drugs and their controlled release.

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**Supporting Information Available:** Experimental details, UV–vis spectra, PL spectra, TEM images, and hydrodynamic diameters as a function of irradiation time (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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