Imparting Nanoparticle Function with Size-Controlled Amphiphilic Polymers

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Semiconductor and metallic nanoparticles (NPs) have attracted enormous attention due to their unique optical and electronic properties.[1] These materials have been used to advance basic scientific studies in such diverse fields as molecular electronics and medicine. As work in these areas progress, the need to create more complex nanoparticle functional systems will be paramount to realize the true potential of NPs. In this vein, we have sought to develop facile chemical methods to functionalize NP materials in aqueous environments to create interesting material systems for studying biological phenomena.[2]

Several methods for solubilizing hydrophobic NPs within aqueous solutions have been developed over the past decade.[3] While these accomplishments are very significant, NPs must be further derivatized to impart functionality such as biological compatibility or chemical sensitization. Consequently, a facile method of water solubilization may produce a stable, aqueous NP suspension that is unfortunately difficult or impossible to subsequently functionalize; considerable effort has been spent to develop methods to circumvent this fact.[4] Herein we report our method of micellular encapsulation of a variety of NP materials within highly uniform amphiphilic polymers that contain a single organic moiety which acts as a functionalizable "chemical handle." Our process begins by synthesizing hydrophilic polymers via a controlled polymerization process followed by partial hydrophobic modification of the polymer. Next, we couple the NP and amphiphilic polymer which is then easily dispersed in water. We demonstrate here that we may use a built-in chemical handle incorporated into the polymer to synthesize several types of multifunctional NPs. A diagram of one such coupled construct is shown in Figure 1; all procedures are explained in detail in the Supporting Information.

Generally, methods that have been developed to impart aqueous solubility to NPs involve organic cap exchange or micelle encapsulation.[3c] In our experience we have found that cap exchanged aqueous fluorescent NP suspensions (such as CdSe/ZnS) have reduced brightness and may precipitate after a few weeks (or sometimes days) in solution. However, NP encapsulation into micelles appears to be robust for a variety of systems and is stable in solution on a time scale of years. Micellular emissive NP materials are not as quenched compared to cap exchanged systems. Our specific interest in micelle encapsulated NPs began with the first reports of forming aqueous NP suspensions using amphiphilic modified polyacrylic acid (PAA).[5] We first sought to study this material system by examining any possible NP/polymer size relationship; however a highly uniform PAA source is required as commercially available polymers are very polydisperse. To this end we synthesized PAA via Reversible Addition−Fragmentation Chain Transfer (RAFT), which utilizes blocking agents that reversibly bind to the end of growing polymer chains during polymerization resulting in enhanced size contol.[6] After 40% octylamine modification and hydrolysis of the RAFT agent located at the "head" of the polymer, we found that these materials can solubilize such diverse
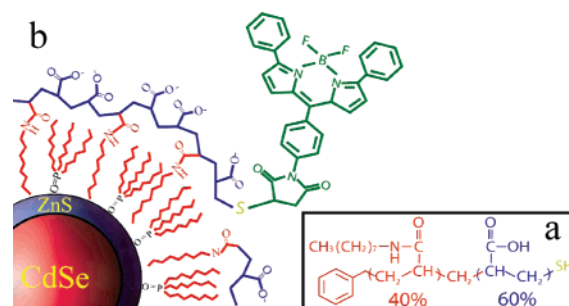

Figure 1. (a) RAFT synthesized polyacrylic acid is 40% modified with octylamine to make a amphiphilic polymer. Note the polymer functionalities are randomly placed. (b) Subsequent encapsulation of a hydrophobic CdSe/ZnS NPs imparts solubilization of the NP in water and a free thiol to conjugate to maleimide functional dye, here BODIPY 577/618 as shown in green.
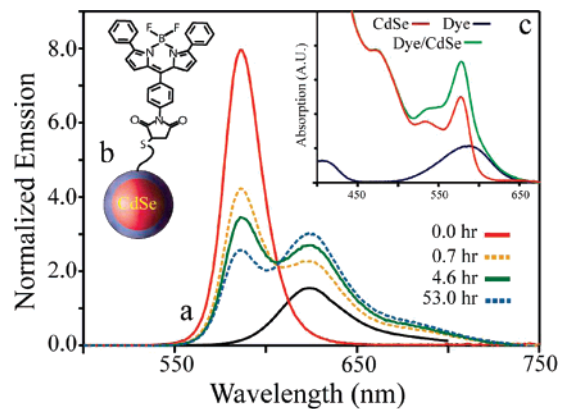

Figure 2. (a) Emission of RAFT polymer water solubilized CdSe/ZnS NCs following the addition of BODIPY 577/618 maleimide over time. The emission from the aqueous dye/polymer complex alone is shown in black. (b) Schematic of the coupled NP/dye system. (c) Absorption spectra of the bare NP, the NP/dye couple, and the dye alone.

NPs as CdSe/ZnS, CdS/ZnS, Fe_2O_3, and ZnSe/ZnMnSe/ZnS NPs[7] into water.

During this time we began to explore if the polymer thiol "head" group, formed from the hydrolysis of the RAFT agent, was in fact a functionalizable moiety, as we first assumed the thiol would be bound to the surface of the NP and thus unreactive. We first water solubilized 580 nm emitting CdSe/ZnS NPs using RAFT amphiphilic polymers. The emissive NPs were then exposed to thiol-reactive BODIPY 577/618 maleimide in a pH 6 phosphate buffered solution overnight while monitoring the emission; the excitation wavelength was set at 475 nm where the BODIPY dye has very little absorption. As shown in Figure 2 the increase of the emission of the dye and concomitant suppression of the NC emission over time must be the result of efficient Förser Resonant Energy Transfer (FRET) from the NP to the dye,[8] which indicates that the NC and dye are coupling together under these mild conditions. However, we cannot rule out that the hydrophobic nature of the BODIPY
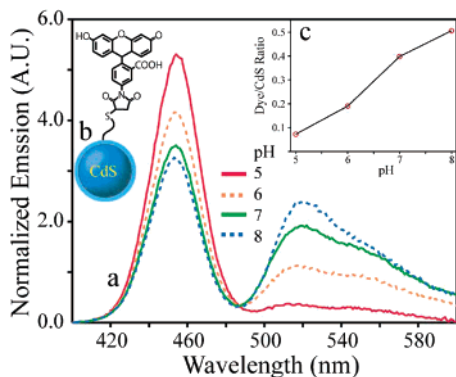
Figure 3. (a) Emission of a fluorescein/CdS/ZnS NP results in a ratiometric fluorescence sensor for pH. An isosbestic point is observed at ∼490 nm. (b) Cartoon of the coupled construct. (c) Ratio of the fluorescein (520 nm) to CdS/ZnS (455 nm) emission as a function of pH.

Figure 4. (a) Absorption spectra of water solubilized Fe_2O_3 NP blank and fluorescein conjugates as a function of pH (blank = pH 8). (b) NP/dye conjugate absorption spectra after blank subtraction displays fluorescein-like character as a function of pH. (c) Photograph of a blank Fe_2O_3 NP sample and fluorescein conjugates as a function of pH under room light conditions and (d) under UV illumination. (e) Emission spectra of the Fe_2O_3 NP blank and fluorescein conjugates as a function of pH (blank = pH 8).

contributes to the NP/dye interaction beyond thiol−maleimide chemical coupling; we thus performed all further studies using a more polar functional dye.

To accurately characterize the coupling efficiency we next turned to a more polar fluorescein maleimide dye which we conjugated to RAFT amphiphilic polymer coated CdS/ZnS NPs in pH 6 water. We also sought to examine whether our coupling strategy could create a ratiometric NP/dye pH sensor similar to a previous report.[9] First, CdS/ZnS NPs[10] were water solubilized with RAFT amphiphilic PAA and were exposed to fluorescein maleimide overnight. After excess dye was removed, we found that the conjugation was highly efficient with $30 \pm 10\%$ of the fluorescein maleimide reacting with the NP materials. Next, we examined the optical properties as a function of solution pH as shown in Figure 3. The increase in the dye absoprtion with increasing pH quenches the CdS/ZnS emission with concomitant fluorescein brightening as discussed previously.[9] A clear isosbestic point is observed which is indicative of chemical (pH) sensing via modulation of FRET energy transfer.[9]

We next solubilized magnetic Fe_2O_3 NPs[11] into pH 6 water using the RAFT amphiphilic polymers and conjugated the materials to pH sensitive fluorescein maleimide dye. After the mixture stirred for 1 day, we removed the excess dye and measured the NP/dye conjugate's absorption and emission spectra as a function of pH. As shown in Figure 4, the absorption spectra of the coupled constructs reveal the existence of fluorescein-like absorptions upon subtraction of the blank oxide NPs. These materials also demonstrate green fluorescein emission upon excitation of the dye. After correcting for the NP absorption we found that the dye is quenched by ∼45% relative to unbound fluorescein. We also confirmed that the fluorescein is sensitive to the local environment as the dye brightens considerably in basic solution. Consequently, the use of RAFT synthesized amphiphilic polymers represents a novel way to create a variety of functional nanoscopic material systems such as magnetic fluorescent nanoparticle chemical sensors.

In conclusion, we have shown that amphiphilic RAFT synthesized PAA is capable of encapsulating a variety of NP materials for solubilization within an aqueous environment. The thiol functionality of the polymers is available to react with maleimide functionalized vectors, here organic dyes, although several biological agents such as biotin and streptavidin are commercially available with thiol-reactive functionality. These reactions proceed with high yield and are performed under very mild aqueous environments, producing thioether bonds that are stable under physiological conditions. Overall, our synthetic strategy appears to be very robust
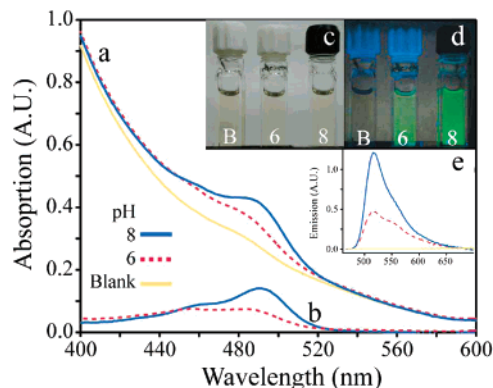
and removes the necessity for secondary coupling agents that may cause NP precipitation.[4a]

Supporting Information Available: All chemicals, procedures, processing methods as well as characterization, including kinetic data, GPC traces, quantum yields, FRET efficiencies, and MALDI-TOF spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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