Smart Nanocrystals

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# Coating Nanocrystals with Amphiphilic Thermosensitive Copolymers**

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Nanomaterials that are sensitive to external stimuli are of emerging interest due to their great potential in many biomedical and technical applications. Tremendous development and investigation has been devoted to designing nanocrystals (NCs) with the surface coating layer sensitive to variations of temperature,[1–3] pH value,[4–7] and specific analytes.[8] Among these, thermosensitive NCs are particularly useful in sensors,[4] magnetic separation,[9] and drug-delivery systems for temperature-programmed release of payloads.[10] Poly(*N*-isopropylacrylamide) (PNIPAAm) or its copolymers are thermosensitive polymers that exhibit a lower critical solution temperature (LCST) about which the polymer undergoes a reversible phase transition. When the temperature is below the LCST, PNIPAAm is hydrophilic. The polymer chains become hydrophobic when the temperature is above the LCST.[11–13] Such a distinct thermosensitive property makes it appropriate for the temperature-sensitive coating of NCs.

To this end, several strategies have been developed for producing thermosensitive NCs. One of the prevailing methods is through direct graft of PNIPAAm brushes from the surface of NCs.[14–18] However, this approach involves multistep reactions and tedious separation procedures. Many other strategies to attach thermosensitive polymers to NCs, such as ligand exchange,[19] electrostatic interaction,[4] and soft-template in situ decomposition,[9] have been described recently. However, each of these methods is only applicable to a limited selection of particles. Therefore, it is desired to develop a more general approach to produce thermosensitive particles.

To meet this challenge, we designed an amphiphilic thermosensitive copolymer that can encapsulate a variety of NCs stabilized by lipophilic ligands irrespective of the chemical composition of NCs. These NCs are initially dispersed in nonpolar or weakly polar organic solvents and can be thereafter transferred to an aqueous solution when coated with the copolymer. The resulting NCs have very high stability in solutions over a broad range of pH values (3–13). In addition, these NCs exhibit a sharp and reversible phase transition at the LCST.

Poly(maleic anhydride-*alt*-1-octadecene) (PMAO) was employed as a backbone to prepare the amphiphilic thermosensitive copolymer. PMAO and its analogues were selected as phase-transfer reagents for hydrophobic-ligand-coated NCs because of the many features they offer.[20–23] Firstly, PMAO is inexpensive and can be obtained commercially on a large scale. Secondly, it has large number of hydrophobic alkyl chains, which can strongly associate with the hydrocarbon ligands on the NCs through hydrophobic van der Waals interactions. Thirdly, each repeating unit of PMAO has a maleic anhydride group that can be readily hydrolyzed to carboxylic groups, which not only render the NCs water dispersible, but also serve as an anchor for further conjugation to biomolecules or ligands. Finally, PMAO also provides possibilities to graft other functional molecules to the main polymeric chain owing to the highly reactive anhydride groups.

Following this strategy, we grafted amine-terminated PNIPAAm to the maleic anhydride groups to form the thermosensitive copolymer PMAO-*graft*-PNIPAAm. The reaction route is shown in Scheme 1. In short, the telechelic polymer PNIPAAm-$NH_2$ (**2**) was obtained through free radical polymerization in the presence of cysteamine hydrochloride as a chain-transfer agent. When deprotonated by triethylamine (TEA), amino groups on the end of the PNIPAAm chains can form amides with a fraction of maleic anhydride groups on PMAO (**3**). The obtained PMAO-*graft*-PNIPAAm (**4**) is poorly soluble in deionized water because of the hydrophobic nature of PMAO, despite the high water solubility of the PNIPAAm side chains.

To demonstrate the function of PMAO-*graft*-PNIPAAm, we first used $Fe_3O_4$ NCs coated with oleic acid as an example. $Fe_3O_4$ NCs were prepared by a high-temperature decomposition method.[24] Direct encapsulation of NCs in amphiphilic PMAO-*graft*-PNIPAAm whose hydrophobic ends intercalate with the organic coating on NCs is illustrated in Scheme 2. The PNIPAAm side chains give the NCs high water solubility. Briefly, an aliquot of NCs suspended in chloroform was mixed with an appropriate amount of PMAO-*graft*-PNIPAAm. To the resulting solution was added an equal volume of sodium borate buffer (pH 11), and the organic solvent was slowly evaporated. The NCs remain stable in the aqueous solution.

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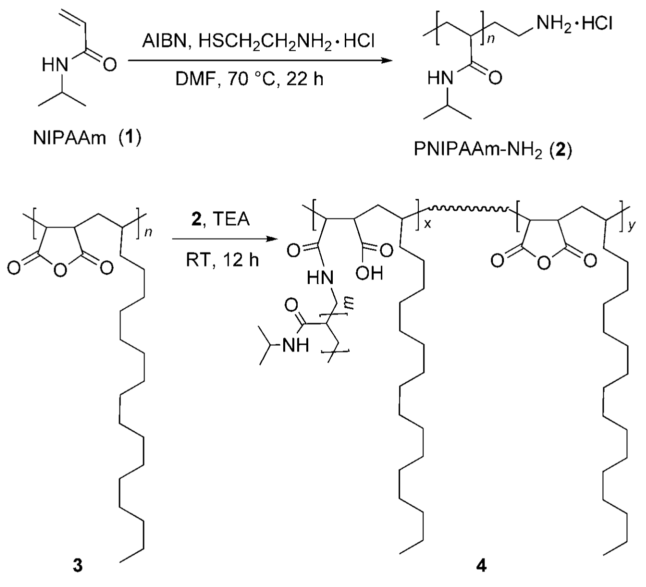


*Scheme 1.* Synthesis of PMAO-*graft*-PNIPAAm copolymer.

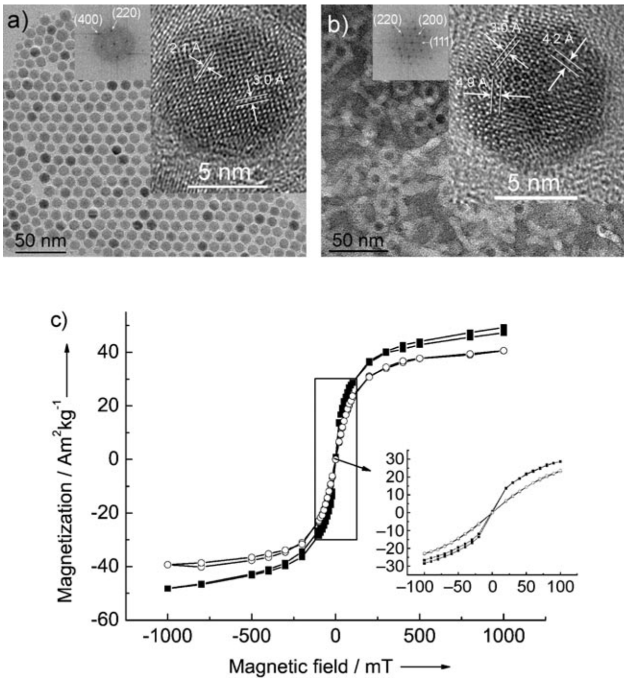
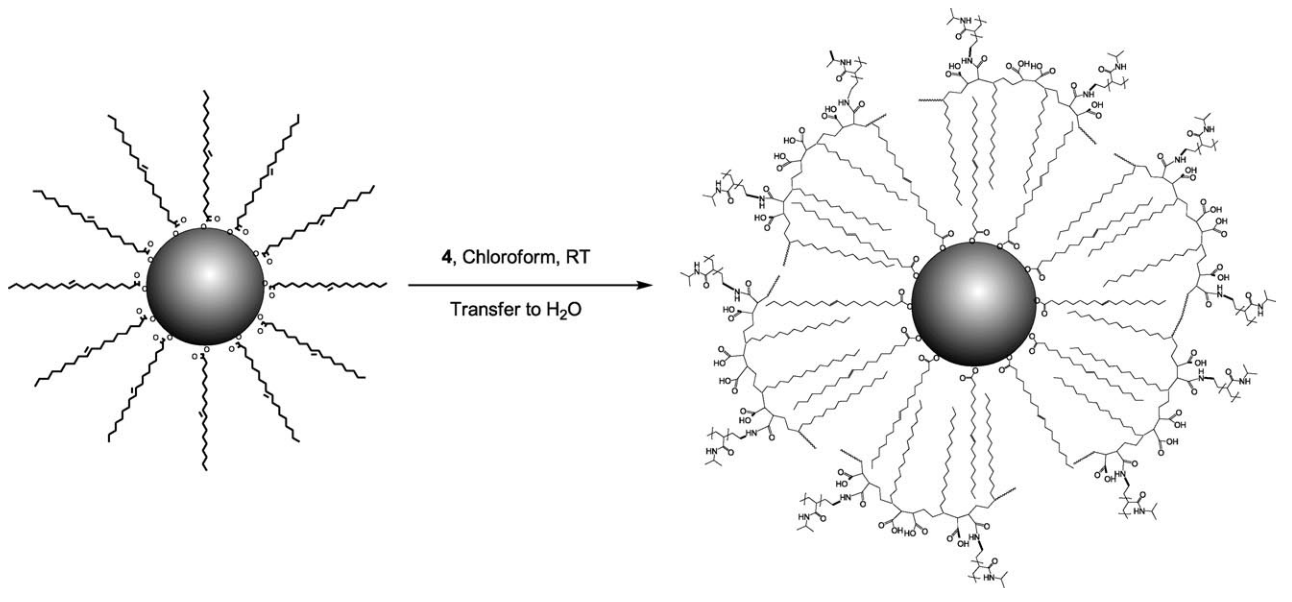


*Figure 1.* TEM and HRTEM images, as well as FFT patterns of HRTEM of $Fe_3O_4$ NCs coated with a) oleic acid and b) PMAO-*graft*-PNIPAAm copolymer, negatively stained with ammonium molybdate (2 wt%); c) room-temperature magnetization curve of as-synthesized $Fe_3O_4$ NCs (■) and $Fe_3O_4$ NCs coated with PMAO-*graft*-PNIPAAm (○).

Nearly all NCs were transferred to water phase. The unbound PMAO-*graft*-PNIPAAm polymer was removed by ultrafiltration three times during which the pH of the NC solution was tuned to 7 by buffer exchange. The obtained NCs remain stable even in the presence of high salt concentration (1M NaCl). pH-dependant ζ-potential measurements reveal that when the pH value is above 11, the negatively charged NCs are stabilized by electrostatic repulsion, whereas steric hindrance plays a role to form a stable colloid at low pH (Figure S1 in the Supporting Information).

Transmission electron microscopy (TEM) images are shown in Figure 1. The diameter of $Fe_3O_4$ NCs in chloroform is 9.9 ± 0.2 nm (Figure 1a). After coating with PMAO-*graft*-PNIPAAm, the NCs are no longer soluble in chloroform but are highly soluble in water. As expected, no size or morphology alteration can be observed for $Fe_3O_4$ NCs. The hairy PNIPAAm coating is not visible unless the TEM specimen is stained (see Figure S2 in the Supporting Information). When the specimen is negatively stained, the polymeric coating can be clearly distinguished. Full coverage of polymer was achieved (Figure 1b and Figure S3 in the Supporting Information). The aqueous solutions of NCs are very stable, and no coalescence of particles can be seen in



*Scheme 2.* $Fe_3O_4$ NCs coated with oleic acid are encapsulated in amphiphilic PMAO-*graft*-PNIPAAm copolymers through hydrophobic interactions.

TEM images because of the steric hindrance of the polymeric coating. High-resolution TEM (HRTEM) images and the corresponding fast Fourier transformed (FFT) patterns show that the $Fe_3O_4$ NCs are single crystalline and structurally uniform both before and after coated with PMAO-*graft*-PNIPAAm. The interplanar distances were measured and indexed to cubic magnetite (insets of Figure 1 a,b).[25]

The magnetic properties of $Fe_3O_4$ NCs were examined with a vibrating sample magnetometer (VSM) at room temperature. Figure 1 c shows the magnetization of $Fe_3O_4$ NCs versus applied field between −1 T and 1 T. Both coated and uncoated $Fe_3O_4$ NCs are superparamagnetic at room temperature showing no remanence or coercivity (inset of Figure 1 c). The saturated magnetization ($M_s$) of $Fe_3O_4$ NCs after coating and transfer to the aqueous phase dropped from 46.16 $Am^2 kg^{-1}$ to 41.05 $Am^2 kg^{-1}$, and the initial susceptibility decreased as well. The diameter of the magnetic cores before coating PMAO-*graft*-PNIPAAm can be derived by fitting the curve to the Langevin equation as 9.9 nm, which is in a good agreement with that measured by TEM. Since magnetite has a molar magnetic moment of 4.1 $M_B$ while that of maghemite is only 2.3 $M_B$,[26] one possible explanation for the decline of $M_s$ is partial oxidation of magnetite ($Fe_3O_4$) to maghemite ($\gamma$-$Fe_2O_3$) on the surface of NCs when transferred to aqueous solutions.[27] However, HRTEM studies cannot distinguish the difference between the magnetite and the maghemite because they share very close inverse spinel crystal structures.

The LCST of the PNIPAAm aqueous solution was determined using differential scanning calorimetry (DSC). A significant endothermic peak at 31.6 °C indicates the volume phase transition of PNIPAAm (Figure S4 in the see the Supporting Information). Since PMAO-*graft*-PNIPAAm is poorly soluble in water, we could not measure the LCST of the aqueous solution of the copolymer directly. We thus measured an aqueous solution (20 μL) of $Fe_3O_4$ NCs coated with PMAO-*graft*-PNIPAAm and observed an endothermic peak at 28.7 °C during the heating section of a heating–cooling cycle (Figure 2). An exothermic peak on the cooling section appears at 26.5 °C. Such hysteresis in phase transitions can be due to kinetic effects. Nevertheless, the good reversibility of the phase transition is demonstrated by five continuous heating–cooling cycles (Figure 2). The LCST of NCs encapsulated with PMAO-*graft*-PNIPAAm is lower (2.9 °C) than that of the pure PNIPAAm. This difference could be attributed to the reduction of conformational freedom of PNIPAAm chains as a consequence of grafting to PMAO backbone and further immobilization on the NCs. Frey and co-workers found a drop of 3–9 °C in the LCST after electrostatic binding of hyperbranched thermosensitive polymer on the surface of Au nanoparticles.[4] Li and co-workers reported a decrease of 5 °C in the LCST when the linear PNIPAAm covalently bound to Au nanoparticles through Au−S bonds.[18]

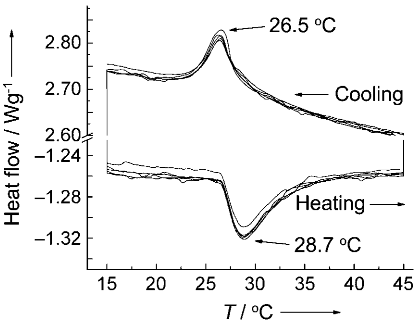


***Figure 2.*** Transition temperatures determined by five heating–cooling cycles on a sample of $Fe_3O_4$ NCs coated with PMAO-*graft*-PNIPAAm in an aqueous solution. LCST is determined on the heating ramp as 28.7 °C.

To further show the thermosensitivity of the PMAO-*graft*-PNIPAAm polymer coated $Fe_3O_4$ NCs, we measured the hydrodynamic particle size versus temperature. As shown in Figure 3 a, at 20 °C the particle size is 26.7 nm, and at 31 °C the particle size is reduced to 22.9 nm due to the collapse of the PNIPAAm chains. Although the LCST determined by DSC is 28.7 °C, the lag of response in hydrodynamic size might be due to the slow equilibration of the temperature in the measuring cell. As the temperature was increased, a sharp change in the particle size was observed and the final hydrodynamic size was 827 nm at 45 °C, indicating that large aggregates were formed owing to aroused hydrophobicity of the coating layer. This behavior is potentially useful for magnetic separation using superparamagnetic nanoparticles whose size is usually below 20 nm. For such small magnetic nanoparticles, the interaction with a magnetic field is very weak. For example, PMAO-*graft*-PNIPAAm coated $Fe_3O_4$ NCs in aqueous solution can remain stable for hours when subjected to a magnetic field of 300 mT (Figure 4 b). It is obviously not suitable for separating applications. To date, mainly large magnetic

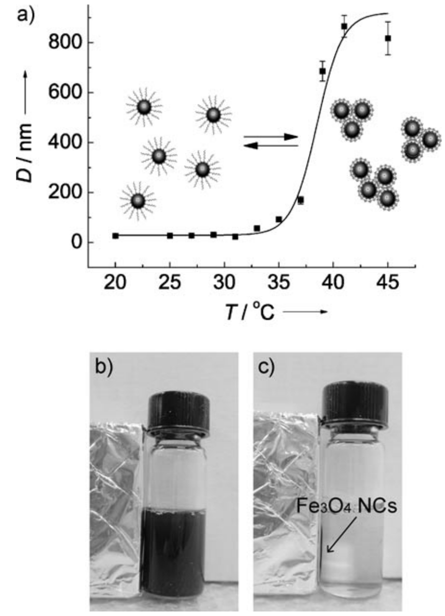


***Figure 3.*** a) Hydrodynamic diameter of $Fe_3O_4$ NCs coated with PMAO-*graft*-PNIPAAm versus temperature. The overlaid schematic illustration shows the dispersion state of particles at low and high temperature, respectively; b,c) photographs of $Fe_3O_4$ NCs coated with PMAO-*graft*-PNIPAAm suspension under the influence of a magnetic field (300 mT) at b) 20 °C and c) 40 °C.

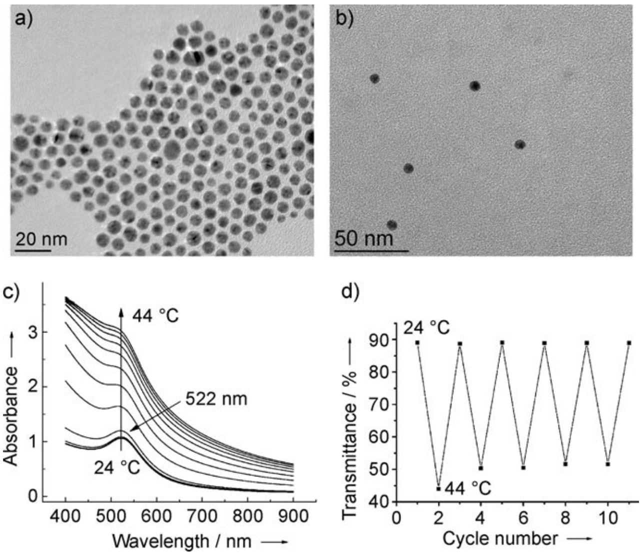


*Figure 4.* TEM images of Au NCs coated with a) oleylamine and b) PMAO-*graft*-PNIPAAm copolymer; c) UV/Vis absorption spectra of Au NCs coated with PMAO-*graft*-PNIPAAm at between 24 and 44 °C; d) changes of transmittance at $\lambda = 720$ nm during heating and cooling cycles between 24 and 44 °C.

particles or beads have been employed for magnetic separation because of the demand of rapid response and fast migration under a magnetic field. However, the use of large particles has a drawback of compromising the total surface area, leading to a limited efficiency of conjugation or adsorption of materials that need to be separated. Thermosensitive $Fe_3O_4$ NCs can remain small, but respond to an external magnetic field rapidly when the temperature is above the LCST owing to the formation of large aggregates (Figure 3c). This transition is reversible, so that when the temperature is decreased, the NCs regain water solubility.

The encapsulation of NCs using PMAO-*graft*-PNIPAAm is general for NCs coated with a layer of lipophilic ligand. We demonstrated its feasibility with Au NCs. Au NCs were prepared by high-temperature polyol reduction of gold acetate in the presence of oleylamine. The obtained Au NCs were soluble in chloroform (Figure 4a). Surface modification of the NCs with PMAO-*graft*-PNIPAAm was carried out by following the same procedure as that for $Fe_3O_4$ NCs. TEM images confirm the particles still maintain their original size and shape (Figure 4b). UV/Vis spectra were recorded for Au NCs coated with PMAO-*graft*-PNIPAAm between 24 °C and 44 °C (Figure 4c). The peak of surface plasmon band occurs at 522 nm. No red shift was observed as a result of the temperature change. Because the lower band absorbance increases as the temperature is elevated, it is not possible to observe blue shift of plasmon band when the temperature is above 30 °C. This finding is in contradiction to previous reports.[2,4] For example, a red shift of 10 nm was observed for Au nanoparticles encapsulated in PNIPAAm microgels when the temperature is increased.[2] The red shift resulted from the increase in the local refractive index induced by the collapse of the PNIPAAm shell. In our case, PNIPAAm chains on the surface of Au NCs are arranged in a loosely compact hairy form, which is unlike a dense microgel. Therefore, the conformational change of PNIPAAm chains is not significant enough to alter the local refractive index in the proximity of the NCs. Figure 4d shows that the aggregation state of these Au NCs is completely reversible over multiple cycles, revealing the robust nature of the smart PMAO-*graft*-PNIPAAm coatings.

In conclusion, we have developed a novel strategy to prepare thermosensitive $Fe_3O_4$ and Au NCs by encapsulating the NCs with the amphiphilic thermosensitive copolymer PMAO-*graft*-PNIPAAm, leading to a sharp transition upon change of temperature. This method is general for all high-quality hydrophobic nanocrystals produced by thermolysis. The resulting NCs coated with PMAO-*graft*-PNIPAAm remain stable over an extended period with no obvious agglomeration. Many important applications of the thermosensitive NCs can be envisaged in the near future, such as temperature-controlled magnetic separation, temperature-programmed MRI contrast agents, and remote-controllable drug carriers.

## Experimental Section

The telechelic PNIPAAm-$NH_2$ was synthesized by free radical polymerization. All reagents were used as received unless otherwise stated. The initiator 2,2′-azobis(2-methylpropionitrile) (AIBN) was recrystallized twice from hot methanol. In a typical experiment, NIPAAm (24 mmol), 2-aminoethanethiol·HCl (0.24 mmol), and AIBN (0.024 mmol) were dissolved in anhydrous dimethylformamide (DMF, 10 mL). The solution was degassed by means of freeze–pump–thaw cycles. The reactor was filled with dry $N_2$ and sealed. Polymerization was carried out at 75 °C for 22 h. The product was precipitated in excess diethyl ether and washed with acetone three times. The crude product was dissolved in deionized water and subjected to dialysis (molecular weight cutoff = 25 kDa) against copious amount of deionized water for 3 days with frequent refreshing of the water. PNIPAAm-$NH_2$ powder was thereafter collected after lyophilization. PMAO (MW = 30–50 kDa, 0.12 g, 0.8 mol$\,L^{-1}$ as monomer concentration) was dissolved in anhydrous chloroform (1 mL). In another container, PNIPAAm-$NH_2$·HCl (0.16 g) and triethylamine (1.6 mg) were dissolved in anhydrous chloroform (2 mL). The latter was added to PMAO solution dropwise with agitation. The reaction was allowed to complete at room temperature for 12 h. The PMAO-*graft*-PNIPAAm was collected under reduced pressure, and the waxy solid was dissolved in chloroform (2 mL) to give a solution with a monomer concentration of 0.4 mol$\,L^{-1}$. The PMAO-*graft*-PNIPAAm solution (0.5 mL) was added to $Fe_3O_4$ NCs in chloroform (1.5 mL, 1.2 μmol$\,L^{-1}$) and stirred for 30 min. Upon completion, borate buffer (pH 11, 2 mL) was added to the mixture and subjected to vigorous agitation for 1 hour. The water-soluble $Fe_3O_4$ NCs were obtained after evaporation of chloroform. Buffer exchange was performed by ultrafiltration (Amicon Ultra-4) to obtain a stable suspension at pH 7. This procedure was also applied to Au NCs.

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