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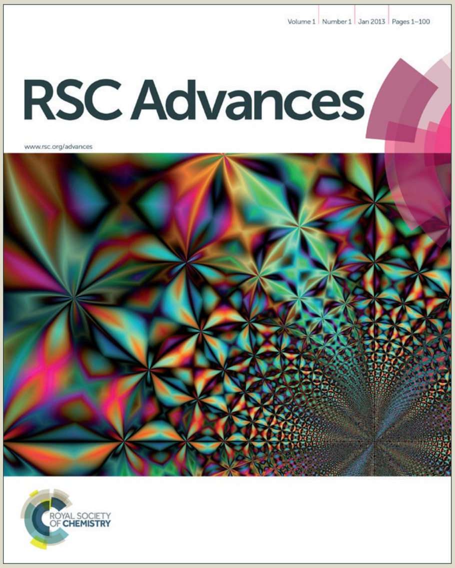

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Highly Water-Dispersed Superparamagnetic Magnetite Colloidal Nanocrystal Clusters From Multifunctional Polymeric Nanoreactors: Synthesis and Properties

Junjing Bai,[§] *Xiaobing Wang,*[§] *Peng Fu,* Zhe Cui,* Qingxiang Zhao,* Xinchang Pang,* and Minying Liu**

School of Materials Science and Engineering, Zhengzhou University, Zhengzhou 450001, China

*To whom correspondence should be addressed. [§]These authors contributed equally to this work.

Email: lmy@zzu.edu.cn, xinchang.pang@mse.gatech.edu, cuizhezzu@126.com, fupeng@zzu.edu.cn and zhaoqingxiang1@126.com

Abstract:

An unconventional but robust strategy to fabricate uniform hybrid inorganic-organic core-shell superparamagnetic magnetite (Fe_3O_4) colloidal nanoclusters in situ was introduced based on water-soluble multi-arm star-shaped brush-like block copolymers as multifunctional polymeric nanoreactors, composed of poly(ethylene oxide)(PEO) as main chain, poly(acrylic acid) (PAA) as functional graft chains, and the second PEO block as shell (i.e., multi-arm star-shaped brush-like block copolymer $[(PEO\text{-}g\text{-}PAA)\text{-}b\text{-}PEO]_{18}$) with different molecular weights and grafting density. After $FeCl_3$ and $FeCl_2$, as precursors of Fe_3O_4, were loaded into graft chain PAA template domain of multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ polymeric nanoreactors followed by the in situ reaction into Fe_3O_4 nanoclusters. The dimensions of clusters can be tuned precisely by changing the chain length of PEO backbones of PAA grafting region. In addition, the density of subunits can be also tailored by adjusting grafting density of PAA side chains, determined by the molar ratio of ethoxyethyl glycidyl ether (EEGE) to EO during the anionic copolymerization. The Fe_3O_4 colloidal nanocrystal clusters with superparamagnetic behavior at room temperature are highly water-dispersed because of surface-tethered PEO polymer shell as hydrophilic ligands.

Keyword: Multifunctional polymeric nanoreactors; anionic ring-opening copolymerization; atom transfer radical polymerization; superparamagnetic magnetite; hybrid inorganic-organic core-shell colloidal nanocrystal clusters

Introduction

Owing to their unique optical, electrical, catalytic and magnetic properties, the fabrication of high-quality colloidal inorganic nanocrystals with tunable dimensions and shape is of key importance for applications in optics, catalysis, electronics, and magnetic resonance imaging for medical diagnosis, to name but a few.[1-3] As one of the most important manipulations for the applications, the assembling of inorganic nanocrystals into the secondary nanostructures is highly desired in order to combine the properties of individual nanocrystals and their secondary structures due to interactions of the subunits.[4-6] Fabrication approaches of the secondary nanostructures, nanocrystal clusters, have become a focus of synthetic efforts in recent years by self-assembly or solution growth in situ.[7-10] Among various strategies for the secondary nanostructures of nanocrystals, template-assisted method is an important process to the synthesis of nanocrystal clusters. For example, amphiphilic block copolymers, polystyrene-*block*-poly(4-vinylpyridine) (PS-*b*-P4VP), have been utilized as soft templates to fabricate a series of nanoclusters with highly ordered secondary patterns (e.g., Au, Ag and Pt, etc.).[11, 12] However, these macromolecular micelles as templates by conventional linear amphiphilic block copolymers were formed by equilibrium multi-molecular aggregates.[13-15] The shapes and characteristics of these multi-molecular micelles with equilibrium structures can change with varying ambient conditions, such as concentration, solvent, temperature, pH and so on.[16] Besides block copolymers, dendritic polymers as soft templates have been shown to be useful for fabricating different nanoclusters.[17] However, these approaches often require certain stringent experimental conditions, are difficult to tune dimensions of secondary structures and density of subunits.

Magnetite (Fe_3O_4) as a common magnetic iron oxide with a cubic inverse spinel structure has been broadly applied as ferrofluid, colloidal liquids made of nanoscale ferromagnetic, or ferrimagnetic, particles suspended in a carrier fluid (usually an organic solvent or water).[18, 19] Especially, colloidal Fe_3O_4 nanocrystals with superparamagnetic behavior and water dispersibility have various medical applications, for example, magnetic resonance imaging for medical diagnosis, AC magnetic field-assisted cancer therapy, drug delivery, and bioseperation.[18, 20] In addition, narrow size distribution of nanocystals is also important so that the nanostructures have uniform physical and chemical properties. Owing to interactions between primary nanocrystals, assembling these small Fe_3O_4 nanocrystals into big clusters with controllable size and shape is still desirous for future applications. In recent years, copolymer brushes have received considerable attentions due to their unique chemical and physical properties as well as their applications in biomaterials, nanotechnology, supramolecular science and so on.[21, 22] Even though various well-defined copolymers with complicated structures have been synthesized by different controlled/living polymerization techniques (e.g., anionic polymerization, ATRP, etc.), [23, 24] limited work was reported about the assembling of copolymer brushes with two functional hydrophilic segments into star-shaped architecture.[25]

Here we report an unconventional but robust method for the in situ fabrication of a series of water-dispersed Fe_3O_4 colloidal secondary nanostructures, nanocrystal clusters, composed of small primary Fe_3O_4 nanocrystals as subunits, with precisely tunable dimensions and subunit density by exploiting rationally designed, novel water-soluble star-shaped brush-like block copolymers as multifunctional polymeric nanoreactors, composed of PEO as main chain, PAA as functional graft chains and the second PEO block as hydrophilic ligands (i.e., multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$) with different molecular weights and grafting density. A series of water-soluble multi-arm star-shaped graft block copolymer based on α-cyclodextrin as core, star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$, were firstly prepared by a combination of anionic copolymerization and atom transfer radical polymerization (ATRP).[25] Subsequently, $FeCl_3$ and $FeCl_2$, as precursors of Fe_3O_4, were encapsulated into graft chain PAA template domain of star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ polymeric nanoreactors by coordination interaction between precursors and carboxyl groups of PAA domain, following by the in situ reaction into Fe_3O_4 nanocrystal clusters. The dimensions of clusters can be adjusted precisely by changing the length of PEO backbones in grafting domain. In addition, the density of subunits can be also tailored by adjusting grafting density of PAA graft side chains, determined by the feed molar ratio of EEGE to EO during the anionic copolymerization. The Fe_3O_4 colloidal nanocrystal clusters with superparamagnetic behavior at room temperature are highly water-dispersed because of surface-tethered hydrophilic PEO polymer shell.

Experimental Section

Materials.

α-cyclodextrin (α-CD, ≥99%), ethylene oxide (EO, ≥99.5%),*N,N,N′,N″,N″*-pentamethyldiethylene triamine (PMDETA, 99%), benzenecarbonyl chloride (≥99.0%), 1-methyl-2-pyrrolidinone (NMP, anhydrous, 99.5%), and trifluoroacetic acid (TFA, 99%) were purchased from Sigma-Aldrich without further purification. CuBr (98%, Sigma-Aldrich) was purified by stirring in acetic acid, subsequentially filtered, washed with ethanol and diethyl ether successively, and dried in vacuum. *N,N*-dimethylformamide (DMF, Fisher Scientific, 99.9%), *tert*-butyl acrylate (*t*BA, Sigma-Aldrich 98%) and methyl ethyl ketone (99.9%, Fisher Scientific) were dried by CaH_2 and distilled under reduced pressure prior to use. Tetrahydrofuran (THF, 99%, BDH) was dried by refluxing over sodium, and then distilled from sodium naphthalenide solution. THF solution of diphenylmethyl sodium (DPMNa) (c=0.51 M) was synthesized according to the literature.[26] Ethoxyethyl glycidyl ether (EEGE) was synthesized by protecting the hydroxyl group of glycidol (Gly) with ethyl vinyl ether based on previously reported method.[26] Iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$, ≥99%), iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, ≥98%) and ammonium hydroxide solution ($NH_3.H_2O$, ACS reagent, 28.0-30.0% NH_3 basis) were purchased from Sigma-Aldrich and used as starting materials without further purification. All other reagents were purified by common purification methods.

Characterizations.

Molecular weights of copolymers were measured by gel permeation chromatography (GPC, Agilent1100) equipped with a G1310A pump, a G1362A refractive detector and a G1314A variable wavelength detector. THF was used as as the mobile phase at the flow rate of 1.0 mL/min at 35°C. One LP 5 μm gel column and two 5 μm gel LP mixed bed column were calibrated with 10 polystyrene (PS) standard samples with molecular weights ranging from 200 to 3×10^6 g/mol. ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectra were obtained using a Bruker 400 MHz spectrometer with the solvent resonances with tetramethylsilane (TMS) as the internal standard. $CDCl_3$ and methanol-d_4 (CD_3OD) were used as the solvent. FTIR spectra were obtained on a Magna-550 Fourier transform infrared spectrometer. Morphology of star-shaped brush-like (PEO-*g*-PAA)-*b*-PEO architectures and resulting colloidal Fe_3O_4

nanocrystal clusters were characterized by transmission electron microscope (JEOL 1200EX TEM; operated at 80 kV). TEM samples of template copolymers were prepared by applying a drop of star-shaped brush-like block copolymer (PEO-*g*-PAA)-*b*-PEO DMF solution (~5 μL at c=1 mg/mL) onto a carbon coated copper TEM grid (300 mesh) and allowing DMF to evaporate in a vacuum oven at room temperature. Then the copolymer samples were stained with uranyl acetate. A droplet of freshly prepared saturated uranyl acetate aqueous solution (~20 μL) was deposited onto dried TEM samples.[27, 28] The excess solution was removed using a filter paper after 5 min. TEM images were analyzed using standard TEM image analysis software (Image Pro). Morphologies of the structures of multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ were measured by atomic force microscopy (AFM, Bruker Dimension 3000) in the tapping mode. AFM characterization samples were prepared by spin-coating the DMF solution (c=1 mg/mL) on a silicon substrate at 2000 rpm for 1 min. The crystalline structures of samples were measured by X-ray diffraction (XRD; SCINTAG XDS-2000, Cu Kα radiation). The energy dispersive spectroscopy (EDS) microanalysis of samples was conducted by field emission scanning electron microscopy (FE-SEM; FEI Quanta 250). A superconducting quantum interference device (SQUID) magnetometer (Quantum Design MPMS-5S) was used to determine the magnetic properties of Fe_3O_4 nanocrystal clusters at room temperature.

Synthesis of Water-Soluble 18-Arm Star-Shaped Brush-Like Block Copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$.

Firstly, multi-arm star-shaped brush-like block copolymer [(PEO-*g*-P*t*BA)-*b*-PEO]$_{18}$ was synthesized by ATRP technique. ATRP of *tert*-butyl acrylate (*t*BA) was carried out by using multi-arm star-shaped block copolymer [poly(EO-*co*-BiBGE)-*b*-PEO]$_{18}$ as a star-shaped macroinitiator possessing side initiation sites on each arm according to our earlier work (**Supporting Information**).[25] In a typical process, an dry ampoule charged with CuBr (0.0707 g), PMDETA (0.1707 g), star-shaped macroinitiator (0.1 g), *t*BA (23.4 mL), and 23 mL methyl ethyl ketone as solvent was degassed by three freeze-pump-thaw cycles in liquid nitrogen, then sealed and placed in an oil bath at 60 ^{o}C. The polymerization process was terminated by dipping the ampoule into ice/water bath at desired reaction time. The crude solution was then diluted with acetone and passed through a neutral alumina column to remove the catalyst, and precipitated in the mixture of methanol/water (v/v = 1/1). The final product multi-arm star-shaped brush-like block copolymer [(PEO-*g*-P*t*BA)-*b*-PEO]$_{18}$ was purified by dissolution/precipitation twice with acetone and methanol/water, and dried at 50 ^{o}C in vacuum oven for 12h.

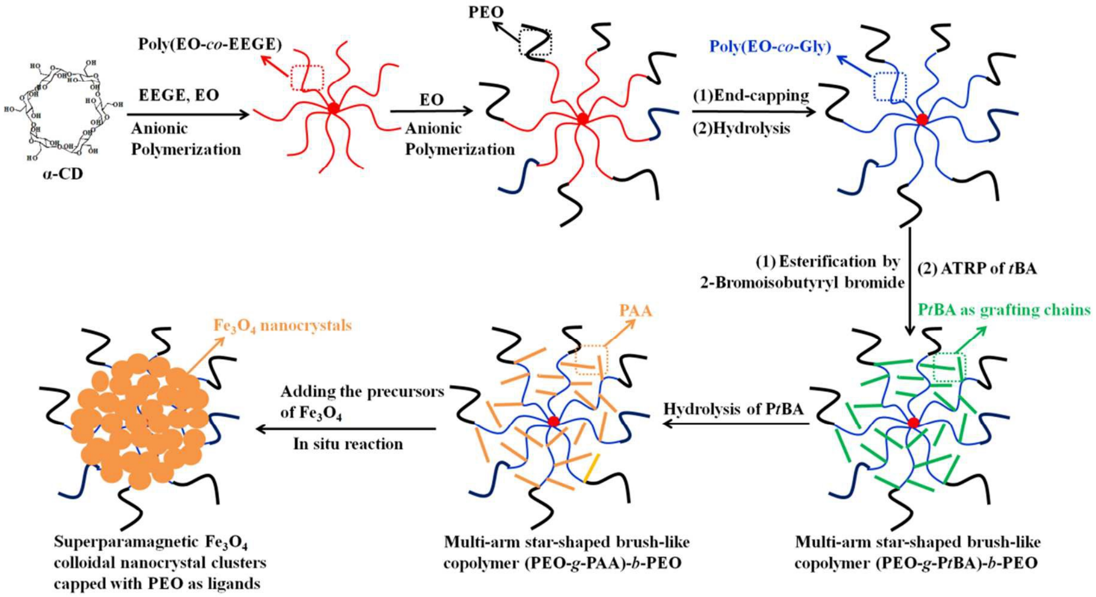
After multi-arm star-shaped brush-like block copolymer [(PEO-*g*-P*t*BA)-*b*-PEO]$_{18}$ was obtained, water-soluble star-shaped brush-Like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ was prepared by hydrolysis of the *tert*-butyl ester groups of P*t*BA graft chains. In a typical hydrolysis process, purified multi-arm star-shaped brush-like block copolymer [(PEO-*g*-P*t*BA)-*b*-PEO]$_{18}$ (1.0 g) was dissolved in 50 mL CH_2Cl_2, and then 10 mL trifluoroacetic acid was introduced. The reaction system was stirred at room temperature for 24 h. Notably, the resulting water-soluble star-shaped brush-like copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ was gradually precipitated from CH_2Cl_2 during the hydrolysis process. The final product was washed with CH_2Cl_2, and then dried under vacuum at 50 ^{o}C for 10h.

Fabrication of Highly Water-Dispersed Hybrid Inorganic-Organic Core-Shell Superparamagnetic Fe_3O_4 Colloidal Nanocrystal Clusters Capped with Hydrophilic PEO as Shell.

The fabrication of superparamagnetic Fe_3O_4 colloidal nanocrystal clusters capped with hydrophilic PEO chains as shell, using water-soluble multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ as templates, was described as follow: 10 mg star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ template was dissolved in a 10 mL DMF at room temperature, followed by the addition of an appropriate amount of precursors ($FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, and $NH_3.H_2O$ as precursors system) that were selectively loaded into the inner grafting PAA polymer chain template domain. The fabrication reaction was carried out at 100^{o}C for 1h under argon. The final products were purified by the magnet, sequentially washed by ethanol three times, and then dispersed in DI water.

Results and Discussion

As illustrated in **Scheme 1**, according to our earlier work,[25] a series of novel water-soluble multi-arm star-shaped brush-like block copolymers with narrow molecular distribution of each segment based on α-cyclodextrin as core were firstly rationally designed and synthesized by a combination of anionic copolymerization and atom transfer radical polymerization (ATRP), composed of poly(ethylene oxide)(PEO) as main chain, poly(acrylic acid) (PAA) as functional graft chains, and the second PEO block as shell (i.e., multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$) (**see the Supporting Information**).[25] Four samples were synthesized as **Table 1**. Subsequently, these water-soluble multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ were utilized as multifunctional polymeric nanoreactors to in situ deposit a series of water-dispersed Fe_3O_4 colloidal secondary nanostructures, nanocrystal clusters, composed of small primary Fe_3O_4 nanocrystals as subunits, with precisely tunable dimensions and subunit density.



Scheme 1. Schematic illustration of the fabrication of superparamagnetic Fe_3O_4 colloidal nanocrystal clusters capped with PEO as ligands using water-soluble multi-arm star-shaped brush-like block copolymer $[(PEO\text{-}g\text{-}PAA)\text{-}b\text{-}PEO]_{18}$ as multifunctional polymeric nanoreactors.

Table 1. Summary of multi-arm star-shaped brush-like copolymers of $[(PEO\text{-}g\text{-}PtBA)\text{-}b\text{-}PEO]_{18}$ and their corresponding water-soluble multi-arm star-shaped brush-like block copolymers of $[(PEO\text{-}g\text{-}PAA)\text{-}b\text{-}PEO]_{18}$

Sample	$M_{n,\ GPC}$ [a] (kg/mol)	PDI[b]	$M_{n,PtBA}$ [c] (kg/mol)	$M_{n,PAA}$ [d] (kg/mol)
A-1b	218.5	1.12	4.2	2.4
A-2b	321.3	1.15	4.9	2.8
B-1b	179.5	1.14	4.6	2.6
B-2b	268.9	1.10	5.1	2.9

[a] Number-average molecular weights of multi-arm star-shaped brush-like copolymers of $[(PEO\text{-}g\text{-}PtBA)\text{-}b\text{-}PEO]_{18}$ determined by GPC. [b] The polydispersity (PDI) of multi-arm star-shaped brush-like copolymers of $[(PEO\text{-}g\text{-}PtBA)\text{-}b\text{-}PEO]_{18}$ determined by GPC. [c] Mn of each P*t*BA graft chain calculated from ^{1}H-NMR data. [d] Number average molecular weight, Mn of each PAA block calculated from the molecular weight difference between P*t*BA block (before hydrolysis) and PAA block (after hydrolysis).

Owing to their ability to direct synthesis of functional inorganic nanomaterials with well-defined structures, multimolecular micelles by aggregation of linear amphiphilic block copolymers were used as an attractive preparation strategy to fabricate functional colloidal nanocrystals by combination with sol-gel processes.[13] Multimolecular micelles based on equilibrium aggregates of linear amphiphilic block copolymers are formed when the concentration is above the critical micelle concentration, and their morphology and structures in a given system depend heavily on temperature and solvent properties.[13, 29] With the changing of concentration, solvent properties, temperature, pH values, etc., the shapes and structures of multimolecular micelles may vary.[15] Comparing with multimolecular template, the multifunctional polymeric nanoreactors from multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ with uniform and structurally stable spherical structures are static rather than dynamic.[16, 30]

Specific interactions between PAA and PEO segments in aqueous solutions resulting in the formation of interpolymer complexes have been extensively studied during more than four decades.[31, 32] In aqueous solutions, the pH values of solutions governs the behavior of PAA/PEO mixtures. When the pH value of aqueous solution was below a certain critical pH of complexation between PAA and PEO segments (usually in acidic conditions), polymers can form interpolymer associate. Above the critical pH of complexation, copolymers consisting of PAA and PEO segments do not form association product or polycomplex. In the polymeric template system of multi-arm star-shaped brush-like block copolymers of [(PEO-*g*-PAA)-*b*-PEO]$_{18}$, in order to exploit them as polymeric nanoreactors, the interpolymer associate from complexation between PAA and PEO segments should be avoided. In our system, pure DMF was chosen as polar aprotic solvent, a good solvent for both PAA and PEO segments. After the water-soluble multi-arm star-shaped brush-like block copolymer (PEO-*g*-PAA)-*b*-PEO was completely dissolved in polar solvent DMF, polymeric template system were formed. AFM characterizations were performed to measure the characteristics of polymeric template structures. Clearly, the water-soluble multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ formed spherical structures with an average diameter of 42±4.6 nm (**Figure S12**). In order to further investigate the architectures of star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ structures, TEM characterization was carried out. The dark spherical structures in the TEM micrographs corresponded to the hydrophilic PAA brush core as uranyl acetate preferentially stained the PAA segments of polymeric architectures (**Figure 1**). The average diameter of cores was 33±3.6 nm, and the size was smaller than that of AFM results about 9nm owing to the second PEO as shell.

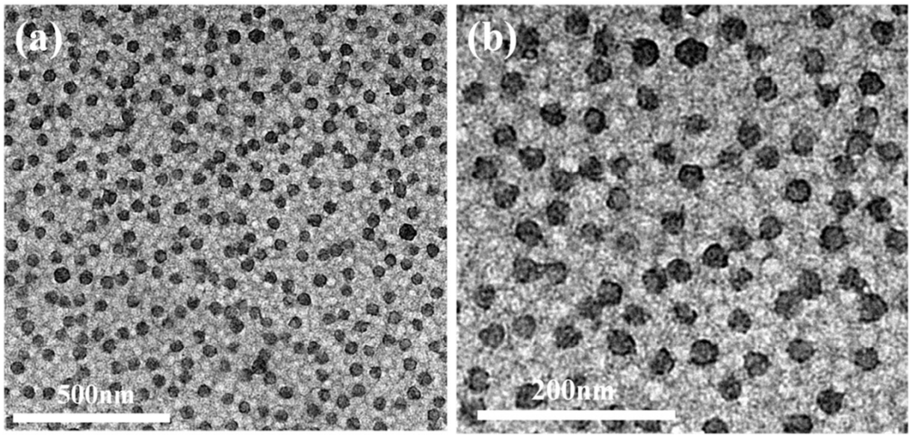


Figure 1. TEM images of polymeric nanoreactors from the water-soluble multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ (Sample A-1b as precursor in **Table 1**) with different scale bars. The samples were treated with uranyl acetate before imaging to selectively stain the hydrophilic core PAA segments.

Subsequently, the water-soluble multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ was utilized as multifunctional polymeric nanoreactors to structure-direct the precursors of Fe_3O_4 into superparamagnetic magnetite colloidal nanocrystal clusters. Highly water-dispersed Fe_3O_4 colloidal nanocrystal clusters were fabricated in situ by exploiting the water-soluble multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ as templates. $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, and $NH_3.H_2O$ were used as precursors system (Fe^{3+}:Fe^{2+}=3:2; the molar ratios of Fe_3O_4 precursors to AA units in PAA domain=2:1), DMF as polar solvent. Precursors were loaded into PAA region of templates owing to the strong coordination of carboxyl groups with metal ions (e.g., iron cations).[3, 33, 34] Introduction of $NH_3.H_2O$ into reaction system increased the alkalinity of the reaction solution, and is propitious to hydrolysis of $FeCl_2$ and $FeCl_3$ for the formation of Fe_3O_4 colloidal nanocrystal clusters. Finally, Fe_3O_4 nanoclusters are formed through dehydration of the resulting $Fe(OH)_3$ and $Fe(OH)_2$, within which spherical Fe_3O_4 colloidal nanocrystal clusters were intimately and permanently capped with hydrophilic PEO chains on the surface. The morphology of three-dimensional Fe_3O_4 nanocrystal clusters with an average diameter of 30±3.2 nm was characterized by TEM, as demonstrated in the representative TEM images at different magnification in **Figure 2**. According to the close observation of these images, these uniform spherical colloids comprise small primary Fe_3O_4 nanocrystals. The subunits of clusters can be investigated more clearly, and crystal lattices were clearly shown in **Figure 2(d)**, high resolution TEM (HR-TEM) image. Clearly, the spherical clusters consist of small primary nanocrystals as subunits with the average diameter of ~3.6nm. X-ray diffraction (XRD) measurements also confirm the subunit crystal structure of Fe_3O_4 colloidal nanocrystal clusters, and the diffraction pattern of cubic magnetite was shown in **Figure S13**. In addition, energy dispersive spectroscopy (EDS) microanalysis was also used to confirm the composition of iron oxide as magnetite by combining the XRD characterization, as shown in **Figure S14**.

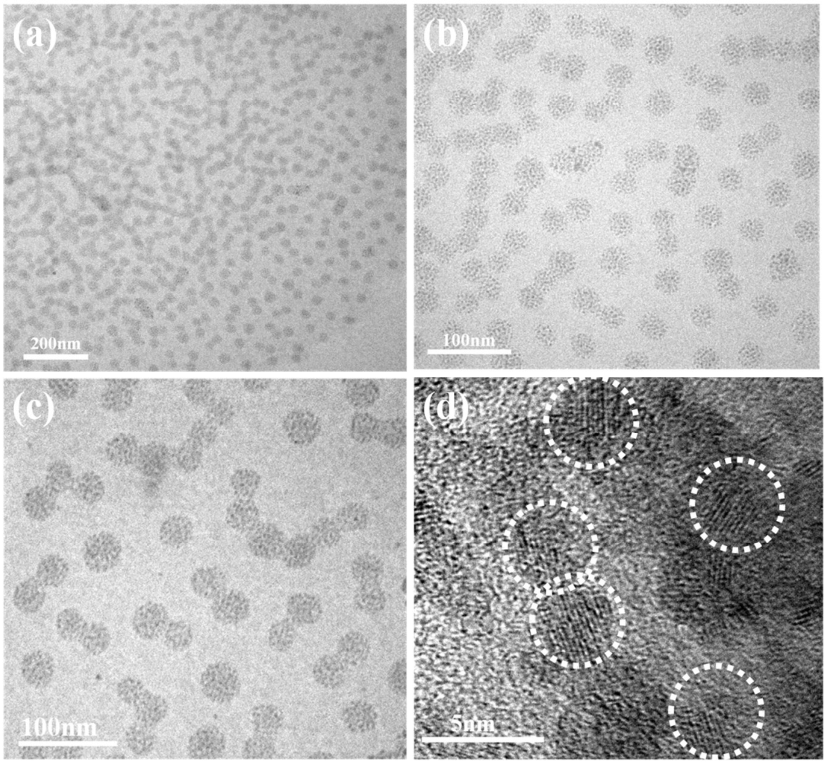


Figure 2. Representative TEM and HR-TEM images of Fe_3O_4 colloidal nanocrystal clusters with different scale bars, the water-soluble multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ (Sample A-1b as precursor in **Table 1**) used as multifunctional polymeric nanoreactor. (a, b, c) Representative TEM images. (d) Typical HR-TEM image.

The dimension of Fe_3O_4 colloidal nanocrystal clusters can be precisely tuned by adjusting the molecular weight of PEO backbones in PAA grafting chains region of multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$. When multi-arm star-shaped copolymer [poly(EO-*co*-EEGE)]$_{18}$ was synthesized, the molecular weight, in another word, the length of the grafting backbone of the final template copolymers can be tailored by changing the monomer conversion and the concentration of initiators during the anionic copolymerization. With the length of grafting backbones increasing, the diameter of the spherical domain of PAA grafting chains may also increase, so the size of the resulting Fe_3O_4 colloidal nanocrystal clusters based on copolymer templates became larger. As shown in **Figure 3**, with the increased molecular weight of each arm poly(EO-*co*-EEGE) in multi-arm star-shaped copolymer $[poly(EO\text{-}co\text{-}EEGE)]_{18}$ from 17.2kg/mol to 34.9kg/mol (Sample A-1 and Sample A-2 in **Table S1**, respectively), the average diameter of Fe_3O_4 colloidal nanocrystal clusters increased from 30±3.2 nm to 42±4.5 nm. Clearly, the density of Fe_3O_4 primary nanocrystals in spherical clusters can be also changed by tuning the grafting density of PAA side chains (i.e., the number of EEGE units in multi-arm star-shaped copolymers of $[poly(EO\text{-}co\text{-}EEGE)]_{18}$ while keeping other parameters fixed (**Figure 4**). Owing to the change of the number of EEGE units in multi-arm star-shaped copolymers of $[poly(EO\text{-}co\text{-}EEGE)]_{18}$, the average number of grafting PAA chains on each arm of final template copolymer can be accordingly adjusted. Sequentially, the PAA region as template phase with lower density of PAA side chains can load smaller precursors even if the molar ratio of precursors to AA unit was fixed. As demonstrated in **Figure 4**, according to the approximate calculation based on the volume of spherical clusters and the average number of subunits in one cluster, the density of Fe_3O_4 primary nanocrystals can be adjusted from about 29 nanocrystals per 1×10^3 nm^3 to about 18 nanocrystals per 1×10^3 nm^3 while the number of EEGE units in each arm of multi-arm star-shaped copolymers of $[poly(EO\text{-}co\text{-}EEGE)]_{18}$ was changed from 41 to 20 by tuning the feed molar ratio of two monomers at the stage of preparing multi-arm star-shaped copolymers of $[poly(EO\text{-}co\text{-}EEGE)]_{18}$.

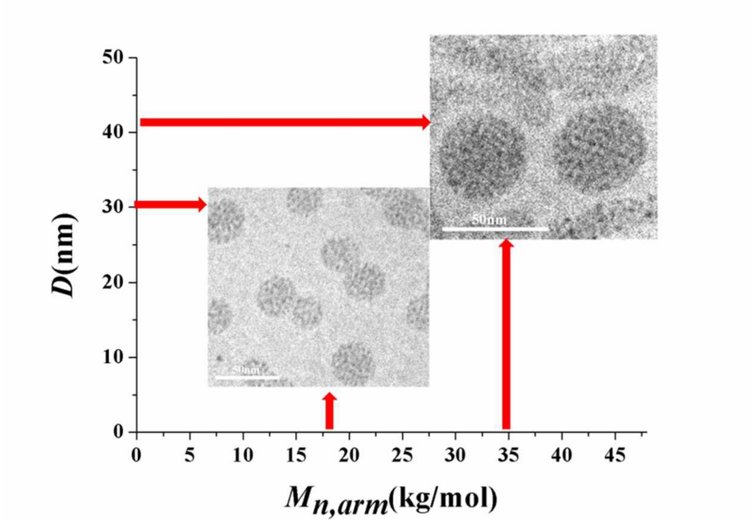


Figure 3. Dependence of the diameter of Fe_3O_4 colloidal nanocrystal clusters (i.e., 30±3.2 nm and 42±4.5 nm) on molecular weights of each arm poly(EO-*co*-EEGE) in multi-arm star-shaped copolymer $[poly(EO\text{-}co\text{-}EEGE)]_{18}$ (Sample A-1 and Sample A-2 in **Table S1**, respectively).

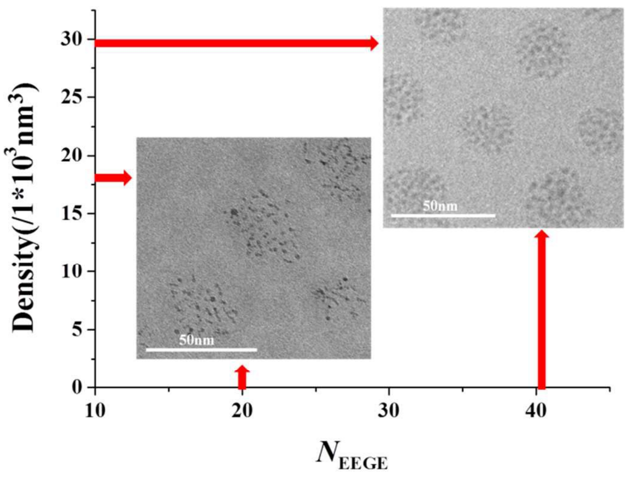


Figure 4. Dependence of the density of Fe_3O_4 primary nanocrystals on the grafting density of PAA side chains (i.e., the number of EEGE units in each arm of multi-arm

star-shaped copolymers of [poly(EO-*co*-EEGE)]$_{18}$, Sample A-1 and Sample B-1 in **Table S1**, respectively).

Fe_3O_4 colloidal nanocrystal clusters with the unique architecture (i.e., the aggregates of small primary Fe_3O_4 nanocrystals) show superparamagnetic behavior at room temperature. The hysteresis loops of ~30nm Fe_3O_4 colloidal nanocrystal clusters composed of primary nanocrystals prepared by using different molar ratios of Fe_3O_4 precursors to AA units in PAA domain were measured at 300 K, as shown in **Figure 5(a)**. Both of two clusters (i.e., the aggregates of small primary Fe_3O_4 nanocrystals, not individual primary magnetite nanoparticles) show no remanence or coercivity at 300 K, that is, superparamagnetic behavior.[35] In order to quantitatively evaluate the magnetic response of Fe_3O_4 colloidal nanocrystal clusters to the applied magnetic field, the mass magnetization was characterized at 300 K. The saturation magnetization of Fe_3O_4 colloidal nanocrystal clusters capped with hydrophilic PEO on the surface was determined to be 21.9 and 15.5 emu/g for Fe_3O_4 colloidal nanocrystal clusters (*D*: ~30nm) consisted of Fe_3O_4 primary nanocrystals when the different molar ratios of Fe_3O_4 precursors to AA units in PAA domain were used. Even if the sizes of two Fe_3O_4 colloidal nanocrystal clusters are same (*D*: ~30nm), their saturation magnetization values are different. The increasing saturation magnetization should be due to the size of the subunit increasing .[35] This size increase of primary nanocrystals may be the result of higher concentration of precursors, and caused more precursors loading into PAA compartment for the formation of larger nanocrystals.

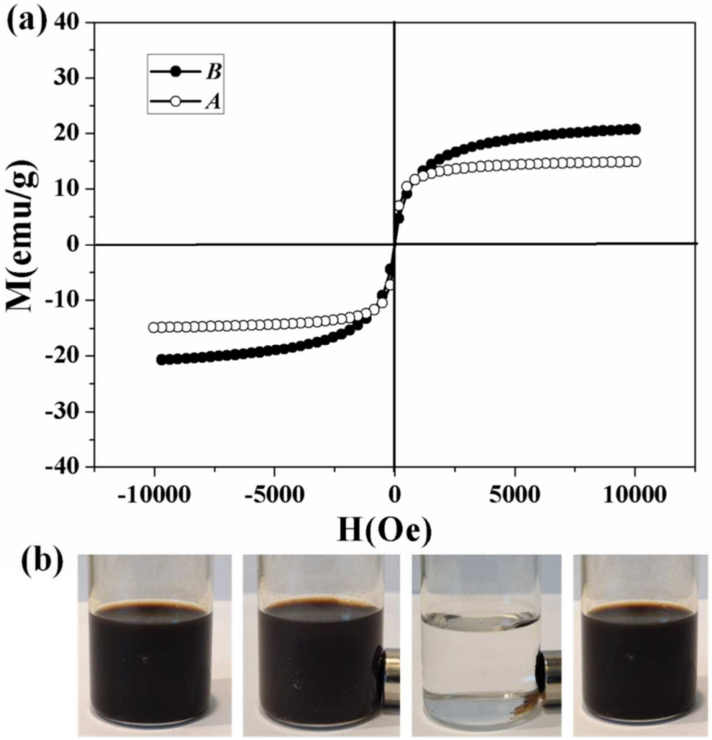


Figure 5. Superparamagnetic behavior of Fe_3O_4 colloidal nanocrystal clusters (*D*: ~30nm) by using multi-arm star-shaped brush-like block copolymer $[(PEO\text{-}g\text{-}PAA)\text{-}b\text{-}PEO]_{18}$ as multifunctional polymeric nanoreactor (Sample A-1b as precursor in **Table 1**). (a) Mass magnetization *M* as a function of applied external field *H* at 300K when the different molar ratios of Fe_3O_4 precursors to PAA grafting chain repeating units were used (*A*=2:1; *B*=6:1). (b) Digital images of an aqueous Fe_3O_4 colloidal nanocrystal clusters shown in **Figure 2**. Without magnetic field, with magnetic field for 0 and 10min, and after the magnetic field was removed (from left to right).

Owing to surface-tethered hydrophilic PEO polymer chains as shell, the resulting hybrid inorganic-organic core-shell Fe_3O_4 colloidal nanocrystal clusters by multifunctional polymeric nanoreactor are highly water-dispersed. The external magnetic responses were observed by dispersing Fe_3O_4 colloidal nanocrystal clusters into DI water. As shown in **Figure 5(b)**, Fe_3O_4 colloidal nanocrystal clusters deposited on the wall of the vial under the influence of external magnetic field within several minutes. When external magnet was removed, the aggregated Fe_3O_4 colloidal nanocrystal clusters can be back into the original aqueous solution. Even if six cycles of magnetic sequestration/redispersion were made, comparing with TEM images in **Figure 2**, TEM measurements show that the morphology of Fe_3O_4 colloidal nanocrystal clusters almost remained unchanged (**Figure S15**).

Conclusion

In summary, we have developed an unconventional but robust strategy to in situ create water-dispersed hybrid inorgniac-organic core-shell Fe_3O_4 colloidal nanocrystal clusters composed of small primary nanocrystals as subunits, with precisely tunable dimensions and subunit density by exploiting rationally designed, novel water-soluble star-shaped brush-like block copolymers as multifunctional polymeric nanoreactors, consisted of PEO as backbone, PAA as functional graft chains, the second PEO block as hydrophilic ligands (i.e., multi-arm star-shaped brush-like block copolymer $[(\text{PEO-}g\text{-PAA})\text{-}b\text{-PEO})]_{18}$ with different molecular weights and grafting density. The dimensions of clusters can be tuned precisely by adjusting the length of PEO backbones of grafting domain. In addition, the density of subunits can be also tailored by adjusting grafting density of PAA graft side chains, determined by the molar ratio of EEGE to EO during the anionic copolymerization. The Fe_3O_4 colloidal nanocrystal clusters with superparamagnetic behavior at room temperature are highly water-dispersed because of surface-tethered PEO polymer chains as hydrophilic shell. Hence, multifunctional polymeric nanoreactor strategy may stand out as an emerging methodology to fabricate a myriad of other functional inorganic colloidal nanocrystal clusters with novel and tailorable physical properties, for a range of intriguing nanoelectric, sensor, magnetic devices, catalytic application and other areas.

Acknowledgements

We gratefully acknowledge funding support from National High Technology Research and Development Program of China (863 Program, No. 2011AA02A204-03) , 1000 Young Talent (to Xinchang Pang), Postdoctoral Research Sponsorship in Henan Province (2014004, to Zhe Cui) and Outstanding Young Teacher Development Fund of Zhengzhou University (1421320045, to Peng Fu).

Supporting Information

Synthesis procedures and summary of characterization data for the final multi-arm star-shaped brush-like block copolymer [(PEO-*g*-PAA)-*b*-PEO]$_{18}$ as template and intermediates: GPC, ^{1}H-NMR and FTIR; XRD and EDS of Fe_3O_4 colloidal secondary nanostructures.

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TOC Graphic

An unconventional but robust strategy to fabricate uniform superparamagnetic Fe_3O_4 colloidal nanocrystal clusters in situ was introduced based on a novel water-soluble multi-arm star-shaped brush-like block copolymers as multifunctional polymeric nanoreactor, composed of PEO as main chain, PAA as functional graft chains, and the second PEO block as shell with well-defined architectures. The dimensions of clusters can be tuned precisely by changing the chain length of PEO backbones of grafting polymeric region. In addition, the density of subunits can be also tailored by adjusting grafting density of PAA side chains, determined by the molar ratio of EEGE to EO during the anionic copolymerization. The Fe_3O_4 colloidal nanocrystal clusters with superparamagnetic behavior at room temperature are highly water-dispersed because of surface-tethered hydrophilic PEO chains as shell.