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General Avenue to Multifunctional Aqueous Nanocrystals Stabilized by Hyperbranched Polyglycerol

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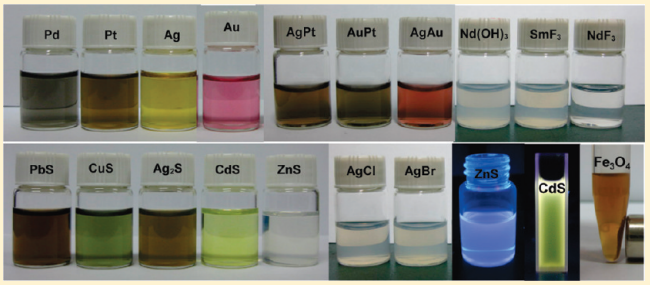
Supporting Information

ABSTRACT: A general strategy to synthesize multifunctional aqueous nanocrystals is presented and demonstrated in this paper. Using multihydroxy hyperbranched polyglycerol (HPG) with high molecular weight (>20 KDa) as a stabilizer, a variety of nanocrystals (e.g., monometallic, alloy of noble metal, semiconductor, magnetic, rare-earth, and silver halide nanocrystals) are readily and rapidly synthesized at room temperature in water or *N*,*N*-dimethylformamide (DMF) with high yield (>90%). The resulting HPG-stabilized nanocrystals show uniform and small sizes, good solubility in water and polar organic solvents (e.g., DMF, methanol, ethylene glycol (EG), and ethanol), favorable biocompatibility, excellent stability, and multihydroxyl groups. The surface hydroxyl groups of nanocrystals can be further tailored with various functional molecules (e.g., amino acids). Our methodology paves the way for fast, facile, and large-scale fabrication of multifunctional aqueous nanocrystals, opening up enormous opportunities to use the nanocrystals for many technological applications.



KEYWORDS: nanomaterials (nanoparticles, nanotubes, etc.), semiconductors, hybrid inorganic/organic materials

INTRODUCTION

Nanocrystals are of great interest in both fundamental research and technological applications, because of their unique size-dependent electrical, optical, magnetic, and chemical properties, compared with their bulk counterparts.[1] Nanocrystals can now be produced with good control over size and shape in organic medium.[2] However, the obtained nanocrystals cannot be directly dissolved in water, which greatly limits their applications, especially in the biological field, which is one of the most widely and potentially applied fields for nanocrystals. Besides, many of the nanocrystals synthesized in organic solvents have no or rare functional groups, precluding them from directly chemical modification for tailoring structures and properties.[3] Therefore, exploring a facile and general approach to direct synthesis of water-soluble nanocrystals with multiple functional groups is quite exigent.

Until now, water-soluble nanocrystals can be directly prepared using either functional small molecules (e.g., trisodium citrate,[4] thiol molecules,[5] and 2-pyrrolidone,[6] etc.) or polymers as stabilizers. Compared to small molecules, polymeric stabilizers are more robust, not only because they can play the role of multivalent ligand or nanoreactor to control the size and size distribution of nanocrystals, but also because they can provide sufficient hindrance to prevent the aggregation of the as-prepared nanocrystals from individual particles.[7] Accordingly, various topologies of functional polymers including linear polymers, hyperbranched polymers (HPs), and dendrimers have been used as stabilizers to synthesize nanocrystals (see Part II and Figure S1 in the Supporting Information for details).

Generally, linear homopolymer stabilizers are easily or even commercially available, but the resulting nanocrystals usually show bigger size and broad size distribution, lower stability, and less-reactive surface functional groups, compared with dendrimers and HPs. Specifically, for block polymer (BP) stabilizers, even though they can facilitate the formation of nanocrystals with more uniform size and higher stability than linear homopolymers, many of the generated nanocrystals are not water-soluble;[8] otherwise, the resulting water-soluble nanocrystals are prepared under a higher temperature (>250 °C), using organic solvents as the reaction medium.[9] By comparison, dendrimers such as poly(amido amine) (PAMAM) could be used to prepare multiple water-soluble noble metal and semiconductor nanocrystals with uniform and small sizes, as well as abundant surface functional groups.[10−12] However, tedious repeat steps are needed to synthesize and purify the PAMAM dendrimers, especially for the high-generation ones, and, meanwhile, further modification such as PEGylated reaction is required to decrease the toxicity of the PAMAM dendrimers.[13] Notably, HPs possess comparable unique characters to dendrimers such as high solubility, low viscosity, abundant functional groups, and three-dimensional topology;

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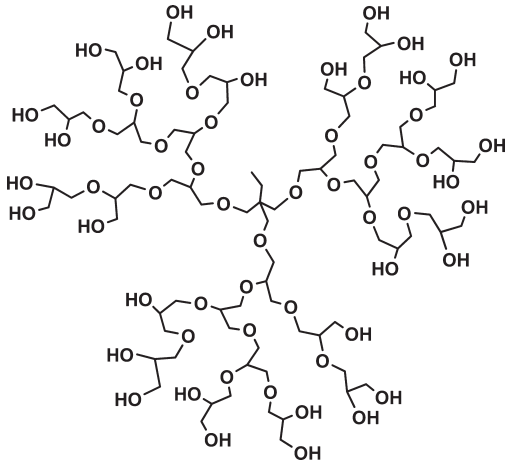
Scheme 1. Chemical Structure of Hyperbranched Polyglycerol (HPG)



moreover, they can be readily prepared by one-step polymerization.[14] So HPs are recognized to be superior over dendrimers in the real applications, especially with the need of large scale. With a structure and components similar to those of PAMAM dendrimers, the HPs, such as hyperbranched polyethylenimine (HPEI)[15] and hyperbranched poly(amido amine) HPAMAM,[16] can also be utilized to form a small portion of water-soluble nanocrystals (e.g., noble metals, semiconductors, and magnetic nanocrystals), whereas most of the HPs stabilizers used, such as hyperbranched polyurethane (HPU),[17] amphiphilic hyperbranched polyglycerol (AHPG),[18] and hyperbranched poly(amido ester) (HPAE),[19] can only form organosoluble nanocrystals. Consequently, a general approach to direct, cost-effective preparation of water-soluble nanocrystals possessing uniform and small size, good biocompatibility, and abundant surface functional groups is still a challenge.

To resolve the aforementioned problem, here, we carefully choose hyperbranched polyglycerol (HPG) with high molecular weight as a stabilizer to explore a facile and general approach to aqueous nanocrystals (see Scheme 1). To our delight, with the stabilization of HPG, a variety of inorganic nanocrystals with unique attributes and excellent properties can indeed be readily produced. The synthesis methodology shows remarkably combined advantages. First, the methodology is general and various water-soluble nanocrystals ,including monometallic and alloys of noble metal, magnetic, semiconductor, rare-earth, silver halides, and other nanocrystals could be readily and directly synthesized at room temperature. Second, the resulting HPG-stabilized nanocrystals show good biocompatibility, as verified by in vitro cell evaluations. Third, compared to dendrimers, HPG can be controllably synthesized via a one-pot process, favoring the large-scale production of polymer-stabilized nanocrystals; compared with linear polymers, the HPG-stabilized nanocrystals are much more stable, even at high temperature for a long time, and simultaneously, they possess a high density of reactive surface hydroxyl groups. Fourth, HPG-containing nanocrystals show excellent solubility in both water and polar organic solvents such as *N*,*N*-dimethylformamide (DMF), methanol, and ethylene glycol (EG), which is very convenient for biological applications and further modifications. We believe that this work will open up enormous opportunities for the synthesis and application of multifunctional aqueous nanocrystals.

EXPERIMENTAL SECTION

Synthesis of HPG-Stabilized Monometallic or Bimetallic Noble-Metal Nanocrystals in Aqueous Solution. HPG-stabilized monometallic or bimetallic noble-metal nanocrystals (e.g., Ag, Au, Pt, Pd, Ag−Au alloy, Ag−Pt alloy, Au−Pt alloy) could be synthesized by blending one or two types of noble-metal salts with HPG in aqueous solution or DMF or methanol or EG, respectively, followed by a single reduction step using $NaBH_4$ as a reducing agent. Typically, for the synthesis of HPG-stabilized Ag nanocrystals (HPG-Ag), aqueous solutions of HPG (0.1 mM) with different molecular weights (M_n = 2.2, 6.4, 22, 55, 86, or 128 KDa) was prepared first. To this solution, 50, 115, or 300 equiv of a freshly prepared 30 mM $AgNO_3$ aqueous solution was added to yield the HPG-$(Ag^+)_n$ (n = 50, 115, or 300) solution, respectively. The solution then was subjected to stirring for 15 min at room temperature before the addition of a 3-fold molar excess of $NaBH_4$ from a freshly prepared 0.5 M aqueous solution. The product was precipitated in acetone and then redispersed in water (yield: 90%−94%). For comparison, polymers other than HPG, such as PVA, PAA, and PEG, were used as stabilizers by keeping other parameters constant during the synthetic process. Note that the concentrations of HPG and other polymer stabilizers are all based on their molecular weights.

Synthesis of HPG-Stabilized Magnetic Fe_3O_4 Nanocrystals in Aqueous Solution. HPG-stabilized magnetic Fe_3O_4 (HPG-Fe_3O_4) was prepared as follows. First, a 1-mL aqueous solution of 0.1 mM HPG (M_n = 86 KDa), a 1-mL aqueous solution of $FeCl_3$ (30 mM), and a 0.5-mL aqueous solution of $FeCl_2$ (30 mM) were mixed in a flask. The mixture was then stirred for 15 min at 55 °C before the addition of 0.5 mL of NaOH (0.45 M) or $NH_3 \cdot H_2O$ (0.45 M). The product was precipitated in acetone and then redispersed in water (yield: 90%−93%).

Synthesis of HPG-Stabilized Semiconductor and Other Nanocrystals in Aqueous Solution. HPG-stabilized semiconductor and other nanocrystals, such as Ag_2S, PbS, CuS, AgCl, AgBr, $PbCrO_4$, CaF_2, SmF_3, NdF_3, $Sm(OH)_3$, and $Nd(OH)_3$, were prepared through a facile coprecipitation method in the presence of HPG in aqueous solution, DMF, methanol, or EG. Typically, for the synthesis of HPG-stabilized Ag_2S (HPG-Ag_2S) nanocrystals, a 1.5-mL aqueous solution of 0.1 mM HPG (M_n = 86 KDa) and a 1-mL aqueous solution of $AgNO_3$ (30 mM) were mixed in a flask. Then, 0.5 mL aqueous solution of $Na_2S \cdot 9H_2O$ (30 mM) was added and the mixture was stirred for 15 min. The product was precipitated in acetone and then redispersed in water (yield: 90%−92%).

Synthesis of HPG-Stabilized ZnS and CdS Nanocrystals in Polar Organic Solvents. Note that HPG-stabilized ZnS and CdS nanocrystals cannot be directly synthesized in aqueous solution. For HPG-stabilized ZnS and CdS nanocrystals, DMF and EG are suitable solvents. Typically, for the synthesis of HPG-stabilized CdS (HPG-CdS) nanocrystals, a 3-mL DMF solution of 0.1 mM HPG (M_n = 86 KDa) and a 0.1-mL aqueous solution of $CdCl_2$ (100 mM) were mixed in a flask. Then, a 0.1-mL aqueous solution of $Na_2S \cdot 9H_2O$ (100 mM) was added and the mixture was stirred for 15 min. The product was precipitated in acetone and then redispersed in water (yield: 90%−92%).

Modification of the HPG-Au nanocrystals. Because of the use of HPG as a stabilizer, there are many hydroxyl groups on the surface of the HPG-stabilized nanocrystals, and these hydroxyl groups can be further utilized to conjugate other functional molecules. Here, we have chosen amino acid, *N*-(*tert*-butoxycarbonyl) glycine (Boc-Gly-OH), as a model molecule to functionalize the HPG-Au nanocrystals. Typically, 53 mg (0.3 mmol) of Boc-Gly-OH was mixed with 5 mL of a DMF solution containing dicyclohexylcarbodiimide (DCC, 80 mg, 0.39 mmol) and 4-(dimethylamino) pyridine (DMAP, 45 mg, 0.37 mmol). The HPG-Au (40 mg) was then added to the mixture at room temperature under magnetic stirring for 12 h. After removing the insoluble dicyclohexylurea (DCU) by filtration and centrifugation, the resulting sample (designated

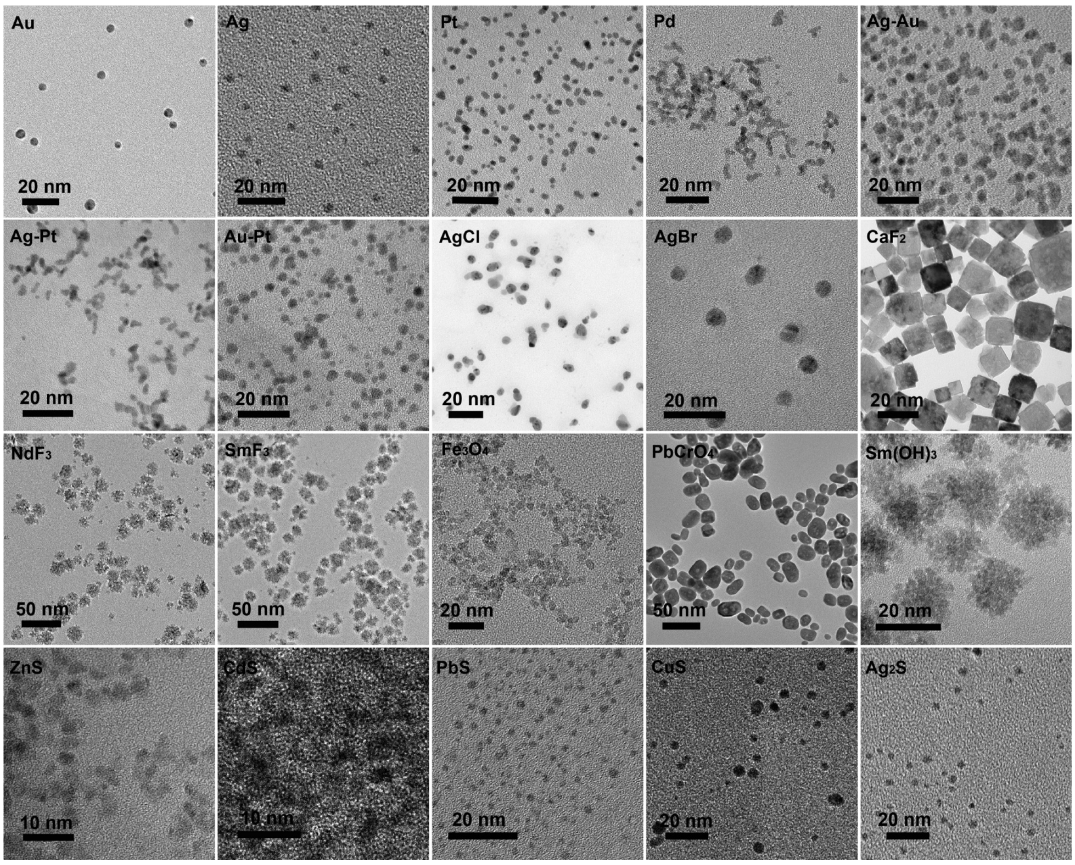


Figure 1. TEM images of nanocrystals, using HPG-86KDa as a stabilizer: Au, Ag, Pt, Pd, Ag−Au alloy, Ag−Pt alloy, Au−Pt alloy, AgCl, AgBr, CaF_2, NdF_3, SmF_3, Fe_3O_4, $PbCrO_4$, $Sm(OH)_3$, ZnS, CdS, PbS, CuS, and Ag_2S.

as HPG-Au-Boc, in the centrifugal liquid) was precipitated by acetone. The HPG-Au-Boc nanocrystals can well dissolve in nonpolar solvents such as chloroform and dichloromethane but cannot dissolve in the polar solvents such as water and methanol anymore. To deprotect the *tert*-butoxycarbonyl (Boc) groups, typically, 30 mg of HPG-Au-Boc nanocrystals, 0.3 mL of trifluoroacetic acid (TFA), and 5 mL of dichloromethane were mixed in a flask and stirred for 2 h at room temperature. The deprotected HPG-Au-Boc nanocrystals (designated as HPG-Au-Gly) were precipitated at the bottom of the flask. The as-obtained HPG-Au-Gly nanocrystals with numerous terminal amino groups can well dissolve in polar solvents, such as water and methanol, again.

RESULTS AND DISCUSSION

Selection of Polymer Stabilizer. Among the numerous reported HPs, HPG is of particular interest, because of its features of water solubility, biocompatibility, and abundant terminal hydroxyls. Moreover, it can be scalably synthesized via one-pot ring-opening polymerization of glycidol with excellent control over molecular weight and polydispersity.[20−23] Recent reports have even shown that the molecular weight of HPG can be readily tuned by anionic emulsion polymerization in a wide range from several KDa to hundreds of KDa,[21] comparable to the generation tunability of dendrimers, paving the way to investigate the effect of molecular weight on the formation of nanocrystals. Previously, only the generation effect of dendrimers was revealed possibly,[10,11] while it is beyond the imagination to address the molecular weight effect of HPs, because of the inaccessibility for controlled synthesis of HPs in a wide range of molar mass. Therefore, we choose HPG as the stabilizer of nanocrystals to meet the challenge of integrated attributes of multifunctional groups, biocompatibility, and water solubility for nanocrystals, as well as generality and simplicity of synthesis protocol.

Note again that there are few published reports on the use of modified amphiphilic HPG comprising of hydrophilic HPG core and hydrophobic shell as a stabilizer to synthesize organosoluble nanocrystals (e.g., Ag, Au, Pd, CdS, and CdSe)[18] in the nonpolar organic solvents such as chloroform and toluene. Besides, the HPG core used in the previous studies of amphiphilic HPG usually had low molecular weight (<20 KDa), whereas, in this article, we find that the unmodified HPG with low molecular weight (<20 KDa) cannot well stabilize the aqueous nanocrystals. Meanwhile, the synthesis of amphiphilic HPs requires additional purification and separation steps, and the characterization of shell arms such as their density, distribution, and linking sites is extremely difficult. To our surprise, until now, no reports on the direct use of unmodified HPG as a stabilizer for the synthesis of nanocrystals can be found. This huge omission is likely caused by two main facts. One is that HPG consists of ether bonds and hydroxyl groups that are normally believed to have very poor interactions with metal ions, compared to charged units such as amino and carboxylic groups, as well as the well-known thioether bonds, which may lead to the instability of nanocrystals. The other is that HPG with a molecular weight lower than 20 KDa generally shows bad performance in the preparation of nanocrystals, as shown below, while the early developed classic approach to the synthesis of HPG by bulk/solution anionic polymerization of glycidol only results in low-molecular-weight products (~6 KDa)[23] and the

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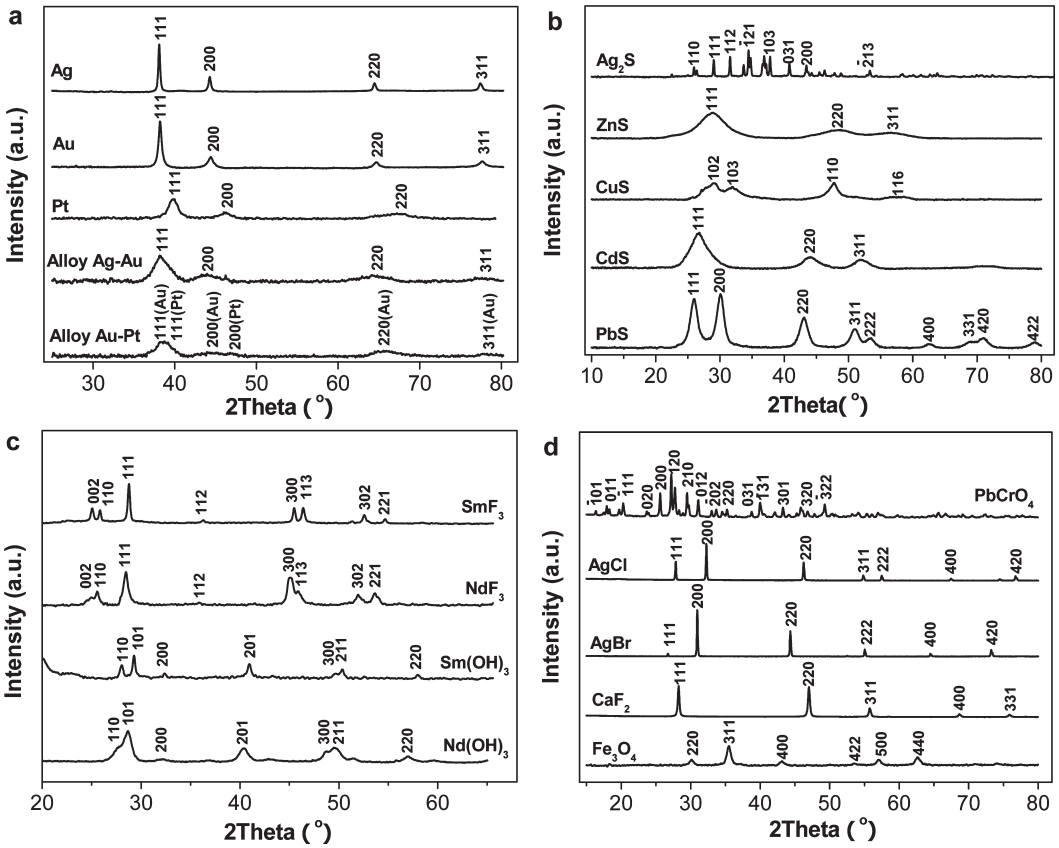

Figure 2. XRD patterns of nanocrystals using HPG-86KDa as a stabilizer: (a) noble-metal nanocrystals (Ag, Au, Pt, Ag−Au alloy, and Au−Pt alloy); (b) semiconductor and sulfide nanocrystals (Ag_2S, ZnS, CuS, CdS, and PbS); (c) rare-earth nanocrystals (SmF_3, NdF_3, $Sm(OH)_3$, and $Nd(OH)_3$); and (d) halide, magnetic, and other nanocrystals ($PbCrO_4$, AgCl, AgBr, CaF_2, and Fe_3O_4).

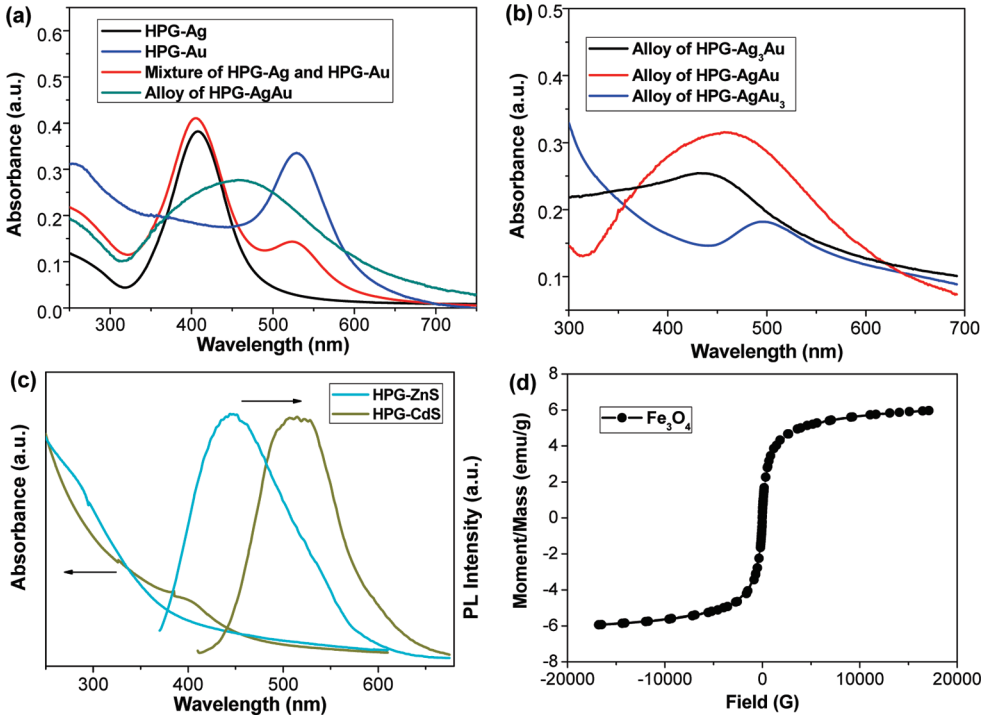

Figure 3. (a) Absorption spectra of HPG-Ag, HPG-Au, alloy of HPG-AgAu, and mixture of HPG-Ag and HPG-Au. (b) Absorption spectra of HPG-AgAu, HPG-$AgAu_3$, and HPG-Ag_3Au. (c) Absorption and photoluminescence spectra of HPG-CdS and HPG-ZnS. (d) Magnetization curves at 300 K for HPG-Fe_3O_4. The stabilizer is HPG-86KDa.

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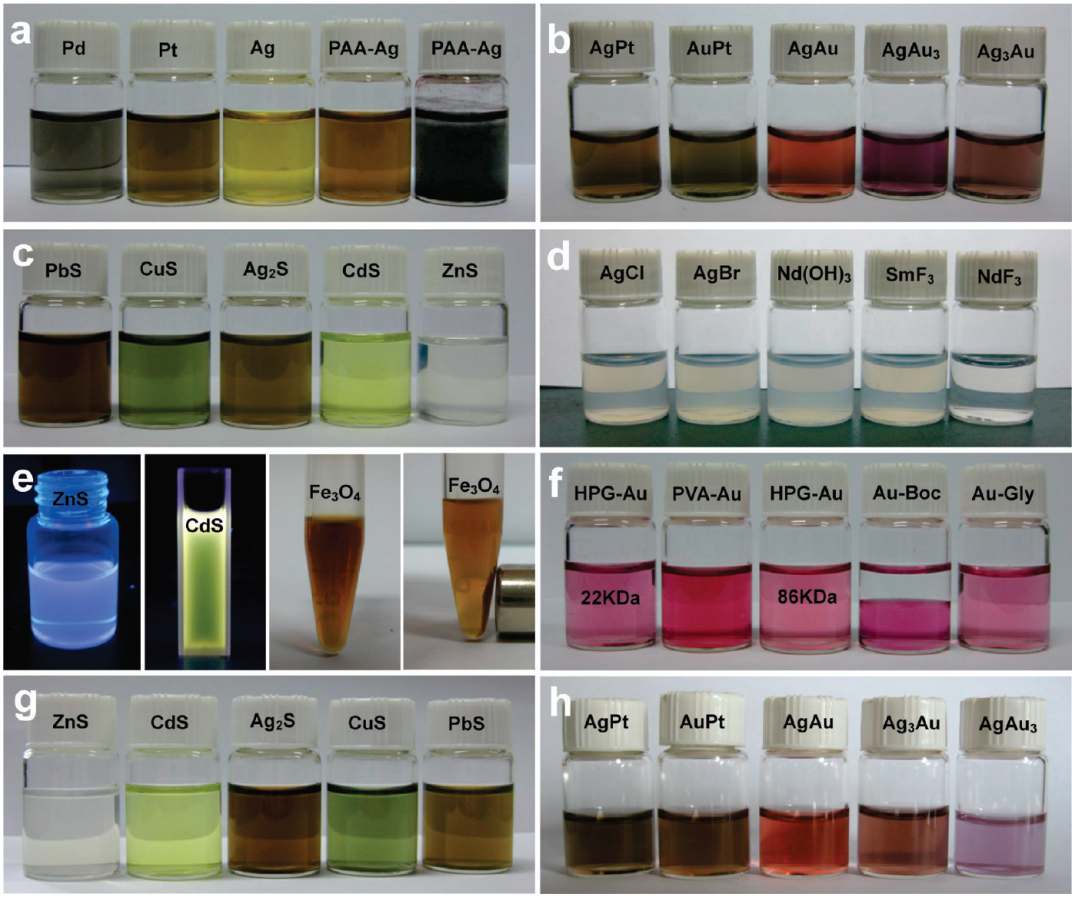

Figure 4. Photographs of the aqueous solutions (panels a−f) and DMF solutions (panels g and h) of nanocrystals: (a) noble-metal nanocrystals using HPG-86KDa as a stabilizer and Ag nanoparticles using PAA as a stabilizer newly prepared and after only one week; (b) bimetallic noble-metal nanocrystals using HPG-86 KDa as a stabilizer; (c) semiconductor nanocrystals using HPG-86KDa as a stabilizer; (d) silver halide and rare-earth nanocrystals using HPG-86KDa as a stabilizer; (e) ZnS and CdS under UV light (365 nm), and Fe_3O_4 before and after placement of a magnet, using HPG-86KDa as a stabilizer; (f) Au nanoparticles, using PVA (PVA-Au) and HPG (HPG-Au) with different molecular weights (M_n = 22 KDa or 86 KDa) as stabilizers, and HPG-Au nanoparticles modified by amino acid Boc-Gly-OH before (Au-Boc) and after (Au-Gly) deprotection; (g) DMF solutions of semiconductor nanocrystals using HPG-86KDa as a stabilizer; and (h) DMF solutions of bimetallic noble-metal nanocrystals using HPG-86KDa as a stabilizer.

solution for high-molecular-weight HPG was reported recently via emulsion anionic polymerization.[21] Thus, the synthesis method and the resulting nanocrystals presented in this paper are totally different from the previous reports.

Synthesis and Characterization of HPG-Based Nanocrystals. We choose noble-metal nanocrystals as a representative to demonstrate the stabilization function of HPG. In a typical synthesis, a 2-mL aqueous solution of HPG (0.1 mM, M_n = 86 KDa) and 50 equiv of a freshly prepared 30 mM aqueous solution of noble-metal salts (e.g., $AgNO_3$, $Pd(NO_3)_2$, $HAuCl_4$, or K_2PtCl_4) were added to a flask. For the synthesis of bimetallic noble-metal alloy, the mixed aqueous solution of two types of noble-metal salts was added. The mixture was then allowed to stir for 15 min at room temperature. After the addition of a 3-fold molar excess of $NaBH_4$ (for the synthesis of other nanocrystals, the difference lies in the addition of corresponding pair salts instead of the reducing agent), the solution changed color immediately (for example, yellow to red for Au), indicating the successful reduction. After a period of one to tens of minutes, the water-soluble noble-metal nanoparticles can be collected by precipitation in acetone and then can be redissolved in water, DMF, EG, ethanol, and methanol.

Figure 1 shows the transmission electron microscopy (TEM) images of noble-metal nanoparticles. For Au, Ag, Pt, Ag−Au alloy, and Au−Pt alloy nanocrystals, generally they exhibit uniform and small sizes without obvious aggregation (also see Part II and Figures S2−S8 in the Supporting Information). For the Pd and Ag−Pt alloy nanocrystals, aggregated nanoclusters can be observed.

Powder X-ray diffraction (XRD) patterns (Figure 2a) confirmed the successful synthesis of face-centered cubic Ag, Au, Pt, and Pd nanocrystals. Energy-dispersive spectroscopy (EDS) analysis showed that the Ag−Au alloy and Au−Pt alloy nanoparticles were composed of Ag and Au and Au and Pt, respectively (see Figure S26 in the Supporting Information). Because the Ag, Au, and Pt have similar lattice parameters, the XRD pattern of Ag−Au and Au−Pt alloy shows a homogeneous phase and becomes broader than that of single Ag or Au.

The successful formation of Ag−Au alloy was also demonstrated by UV absorption spectra (Figure 3a). The mixture of Ag and Au shows two absorption bands, at 407 and 524 nm, corresponding to the surface plasmon resonances of Ag and Au nanoparticles, respectively, whereas the Ag−Au alloy shows only a broad band at 465 nm, confirming the alloy structure.[24] In addition, as the feed molar ratio of Ag to Au varies from 3:1 to

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Table 1. Effects of Molecular Weight of HPG and the Molar Ratio of [Au^{3+}]:[HPG] on the Size of Au Nanoparticles

$M_{n,HPG}$ (KDa)	[Au^{3+}]:[HPG] molar ratio	average size (nm)
2.2	20	bulk[a]
6.4	20	28
6.4	50	46
22	50	10
22	115	18
55	50	6
55	115	10
86	50	5
86	115	7.5
86	200	9
86	300	13
128	50	5
128	115	6
128	300	10

[a] Au particles precipitated immediately after the addition of the reducing agent $NaBH_4$.

Table 2. Effects of Polymer Stabilizer and the Molar Ratio of [Ag^+]:[Polymer] on the Size of Ag Particles

polymer stabilizer	[Ag^+]:[polymer] molar ratio	average size (nm)
HPG (M_n = 22 KDa)	50	2.5
HPG (M_n = 22 KDa)	115	3.0
HPG (M_n = 22 KDa)	300	6.6
PVA (M_n = 89 KDa)	50	11
PVA (M_n = 89 KDa)	115	17
PEG (M_n = 20 KDa)	10	bulk[a]
PEG (M_n = 20 KDa)	50	bulk[a]
PAA (M_n = 1.8 KDa)	15	3.5[b]
PAA (M_n = 1.8 KDa)	50	8.7[b]

[a] Ag particles precipitated immediately after the addition of the reducing agent $NaBH_4$. [b] Ag nanoparticles precipitated from the aqueous dispersions after one week.

1:3, the broad absorption band increases from 436 nm to 495 nm, indicating that the optical properties of the Ag−Au alloy can be tuned in a wide range of wavelength by simply adjusting the feed ratio (see Figure 3b).

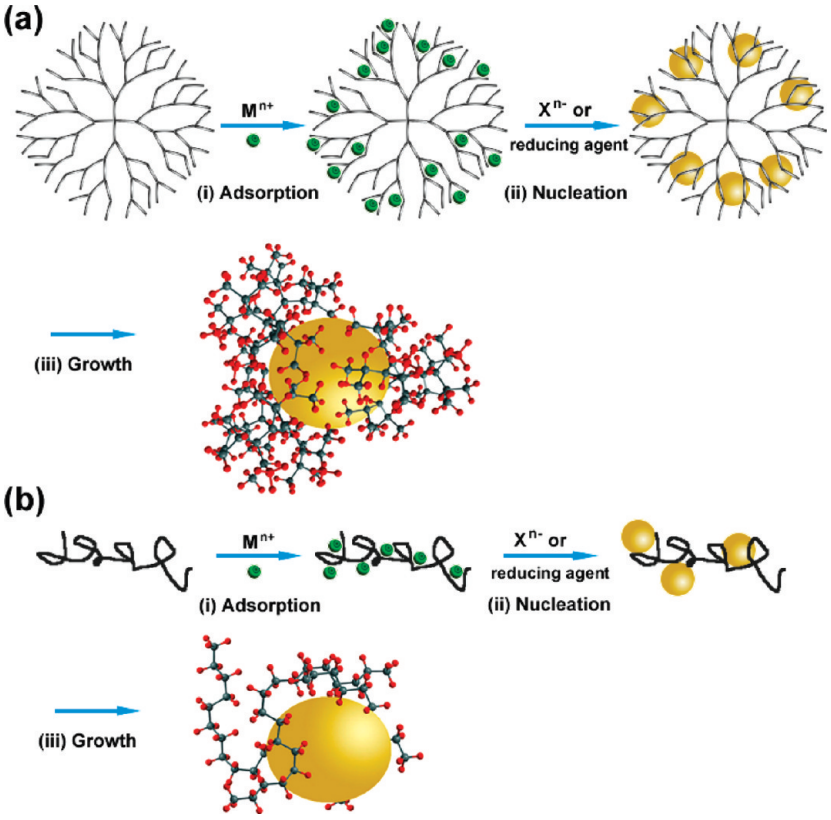
The HPG-stabilizing strategy can also be used to rapidly and facilely synthesize other nanocrystals including silver halides (AgCl and AgBr), magnetic (Fe_3O_4), semiconductor (ZnS, CdS, PbS, CuS, and Ag_2S), rare-earth (NdF_3, SmF_3, $Nd(OH)_3$, and $Sm(OH)_3$), and other (CaF_2 and $PbCrO_4$) nanocrystals in aqueous solution or other polar solvents, such as DMF and EG at room temperature (55 °C for Fe_3O_4), as confirmed by TEM (Figure 1; also Figures S9−S22 in the Supporting Information), EDS (Figure S26 in the Supporting Information), and XRD (Figure 2) characterizations. These results affirm the generality, reproducibility, and simpleness of our methodology.

Significantly, all of the resulting nanocrystals show an amphibious solubility that can be well-dissolved or well-dispersed in both water and organic solvents such as DMF and methanol (Figure 4), which is very convenient for the further modification and applications (for example, biological fields, catalysis, and fabrication of electronic devices) of the nanocrystals. Particularly, the magnetic Fe_3O_4 nanoparticles show superparamagnetic property because of their small size, which is promising for the applications in biomedical fields such as cellular labeling, cell separation, magnetic resonance imaging (MRI), and drug delivery (Figure 3d).[25] The semiconductor nanocrystals show very uniform and small size (below 5 nm) and good dispersion (see Figure 1 and Figures S18−S22 in the Supporting Information). The semiconductor ZnS and CdS nanocrystals show strong fluorescence under UV light, which is attractive for the biolabeling and optical devices applications (Figure 3c).[26] The rare-earth nanocrystals of both fluorides and hydrates exhibit an interesting cactus structure with a mean diameter in the range of 17−23 nm (Figure 1 and Figures S13−S16 in the Supporting Information). Silver halide nanoparticles <15 nm in size showed promising applications in high-speed photographic materials, X-ray films, and catalysis, but they are extremely hard to synthesize in water, because of their strong aggregation tendency.[27] Interestingly, with the templating of unmodified HPG, AgCl and AgBr nanocrystals with mean diameters of 10.1 and 9.9 nm were smoothly produced using our method (see Figure 1 and Figures S9 and S10 in the Supporting Information). Other nanocrystals, such as CaF_2 and $PbCrO_4$, with mean diameters of <30 nm, were also directly synthesized in the aqueous phase using unmodified HPG as a stabilizer (see Figure 1 and Figures S12 and S17 in the Supporting Information).

Comparison Studies. We find that the size of the nanocrystals can be well controlled by using HPGs with different molecular weights and different molar ratios of metal ions to HPG. In general, the higher molecular weight of HPG and lower [metal ions]:[HPG] ratio could lead to smaller sizes of nanocrystals (see Table 1 and Figure S24 in the Supporting Information). For example, the mean diameters of Au nanocrystals using HPG with M_n = 6.4 and 86 KDa at a ratio of [Au^{3+}]:[HPG] = 50 are 46 and 5 nm, respectively. These results may be due to the HPG with high molecular weight possessing more terminal hydroxyl groups, and thereby the interaction between the HPG and metal ion is stronger, resulting in the formation of smaller-sized nanocrystals. The strong interaction between the HPG and metal ion was demonstrated by the UV−vis spectra of HPG-Ag^+ and HPG-Au^{3+} (see Figure S23 in the Supporting Information). Compared to an aqueous solution of HPG and Ag^+ (or Au^{3+}), the new peak at 294 nm (309 nm for HPG-Au^{3+}) derived from the strong interaction between the HPG and Ag^+ (or Au^{3+}) can be clear observed. In addition, we think that several macromolecules of HPG, rather than only one, stabilized the nanocrystals. The bigger HPG shall have a better protection (or separation) effect against the contacting and fusion of different particles, resulting in a smaller size of the final nanocrystals. Nevertheless, the detailed mechanism is still an open question that awaits further deep study in the future.

To study the characteristics of HPG as a stabilizer well, other stabilizers of linear polymers such as PEG, PVA, and PAA were also tried in an effort to produce nanocrystals (see Table 2 and Figure S25 in the Supporting Information). None of the nanocrystals can be obtained using PEG as a stabilizer, even though the [metal

Scheme 2. Cartoon Illustration for Synthesis of Aqueous Inorganic Nanocrystals Using (a) Dendritic Polymer and (b) Linear Polymer as Stabilizers[a]



[a] The polymer stabilizers could play three important roles in the nanocrystal synthesis: (i) scaffold for the adsorption of metal ions, (ii) cradle for the nucleation, and (iii) container/network for the growth of crystals.

ions]:[PEG] molar ratio is as low as 10:1. Aqueous nanocrystals such as Ag, Au, and Fe_3O_4 can be prepared using PVA as a stabilizer, but they show much bigger sizes and wider polydispersity, compared to the cases of HPG. For PAA, aqueous Ag nanocrystals with small (below 10 nm) and uniform size can also be synthesized. However, the as-prepared PAA-Ag nanoparticles are easy to aggregate and would precipitate from their aqueous dispersions after being allowed to stand for one week (Figure 4a, vial 5). In contrast, the HPG-Ag shows extraordinarily good stability in water and no precipitation was observed in its aqueous solution after three months. We think these results are mainly due to the following two reasons: (i) the dendritic macromolecules possess much more efficiently functional groups than linear macromolecules, and, therefore, they show better performance for the controlled growth and stabilization of the nanocrystals; (ii) compared to the free-to-move linear macromolecule chains, the three-dimensional (3D) globular dendritic macromolecules cannot move freely or vibrate, so the formed nanocrystals are very stable. Scheme 2 shows the supposed formation processes/mechanisms of nanocrystals with dendritic and linear polymers as stabilizers. Besides, the reaction medium can affect the polarity and electronegativity of the stabilizers, and thereby affect the formation of nanocrystals.

Stability, Cytotoxicity, and Post-Modification Studies. Compared to linear polymer stabilizers, HPG-stabilized nanocrystals are very stable, even at high temperature for a long time. For instance, Au nanocrystals stabilized by poly(*n*-butyl acrylate) would precipitate after 3.5 h when bathed at 80 °C,[28] while the aqueous solution of HPG-Au is stable even after 12 h at 80 °C and is stable at room temperature for at least three months, as demonstrated by UV/vis spectra (Figure 5a). The absorption bands of HPG-Au before and after heating treatment are observed almost at the same value, indicating that no obvious aggregation occurred. Such stability derives from the multivalent dendritic structure of HPG, as previously discussed. In addition, most of other HPG-stabilized nanocrystals (e.g., Ag, Pt, Pd, Ag−Au alloy, Ag−Pt alloy, Au−Pt alloy, CdS, PbS, Ag_2S, CuS, and so on) are very stable in aqueous solution, and no obvious precipitate can be observed even after being stored for three months. For the aqueous solutions of rare-earth nanocluster compounds such as $Sm(OH)_3$, $Nd(OH)_3$, SmF_3, and NdF_3, precipitates can be observed after two days.

As an excellent biocompatible polymer, HPG is often used to modify inorganic nanoparticles to endow them with good biocompatibility.[29] To determine whether our HPG-based nanocrystals exhibit toxicity or not, we chose some representative nanocrystals (including HPG-Au, HPG-Fe_3O_4, and HPG-PbS) to perform in vitro cell evaluations with COS-7 and SPCA-1 cells. To our delight, the results show that none of the nanocrystals exhibit obvious toxicity, even if their concentrations are as high as 0.5 mg/mL (see Figure 5b; also see Figures S27 and S28 in the Supporting Information). This good biocompatibility benefits from the HPG coverage on the surface of nanocrystals; meanwhile, the neutral hydroxyls of HPG are superior in biological applications over charge groups such as amino and carboxyl groups associated with conventional stabilizers such as PAMAM, PEI, and PAA.

The merit of the hydroxyl groups of the HPG-based nanocrystals lies in that they can be easily converted to the required

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functional groups such as amino or carboxyl ones. Herein, to demonstrate the high reactivity of the surface hydroxyl groups, we chose a representative amino acid, *N*-(*tert*-butoxycarbonyl) glycine (Boc-Gly-OH), to functionalize HPG-Au nanocrystals, as shown in Scheme 3. The entire process was monitored using various techniques. The presence of HPG on the Au surface was confirmed by the ^{1}H nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared (FTIR) spectroscopy, and thermogravimetric analysis (TGA) measurements (see Figure 6). Specifically, TGA indicates that HPG-Au nanocrystals contained ca. 30 wt % HPG between 100 °C and 500 °C, corresponding to a hydroxyl density of 4.1 mmol/g, which provides a good platform for further conjugation of desired molecules such as drugs and

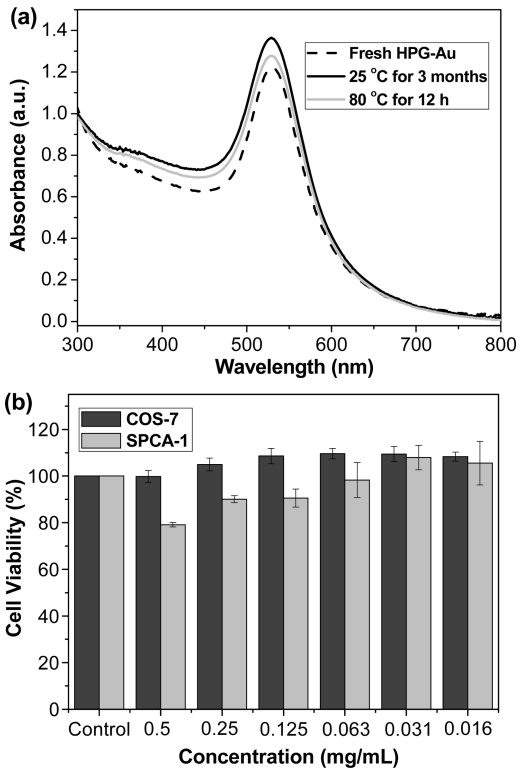


Figure 5. (a) Absorption spectra of fresh HPG-Au and HPG-Au stored for 3 months at room temperature or at 80 °C for 12 h in aqueous solution (using HPG-86KDa as a stabilizer). (b) Relative cell viability of HPG-Au nanocrystals obtained from COS-7 and SPCA-1 cell lines using standard MTT colorimetric assays.

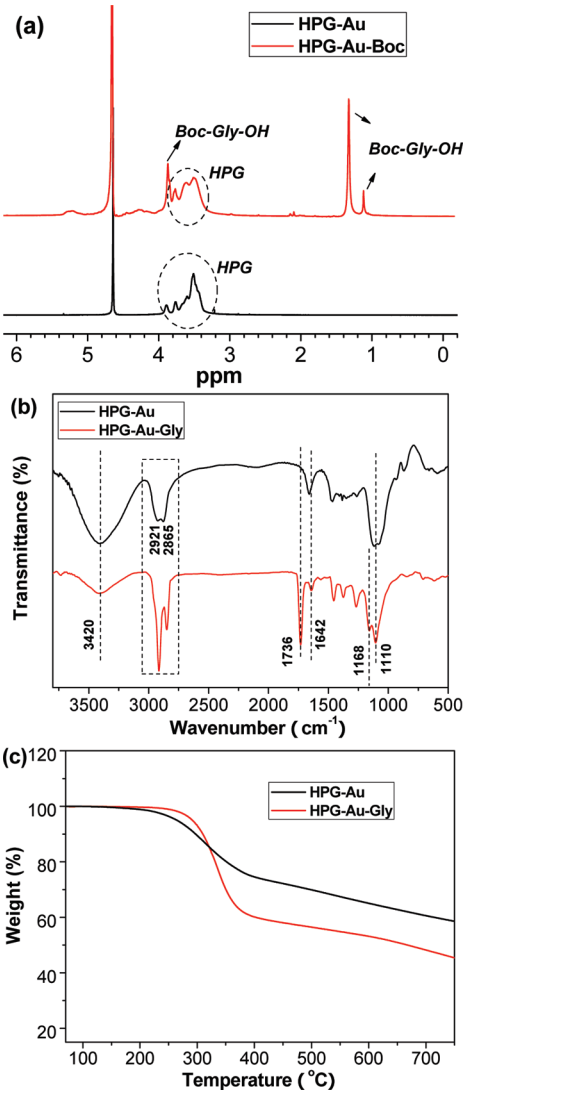
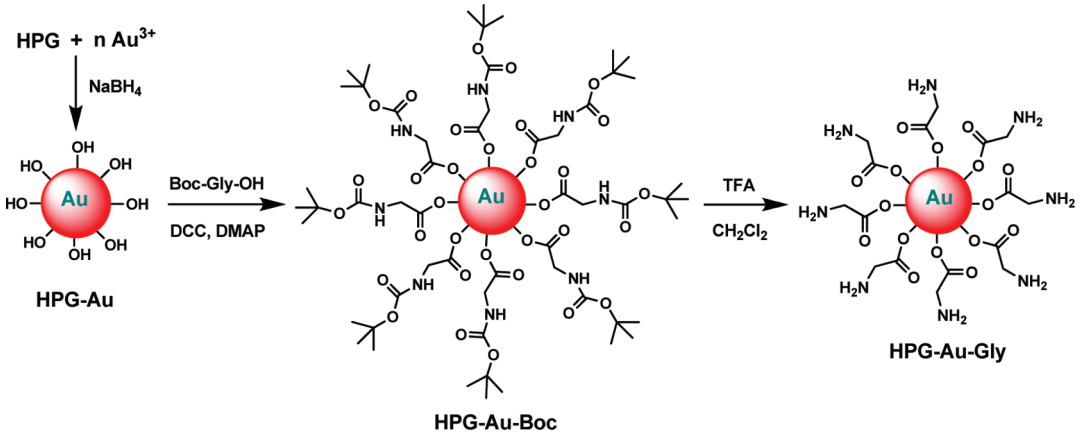


Figure 6. (a) ^{1}H NMR spectra of HPG-Au (D_2O) and HPG-Au-Boc ($CDCl_3$). (b) FTIR spectra of HPG-Au and HPG-Au-Gly. (c) TGA curves of HPG-Au and HPG-Au-Gly.

Scheme 3. Schematic Illustration for the Synthesis Protocol of HPG-Au, HPG-Au-Boc, and HPG-Au-Gly



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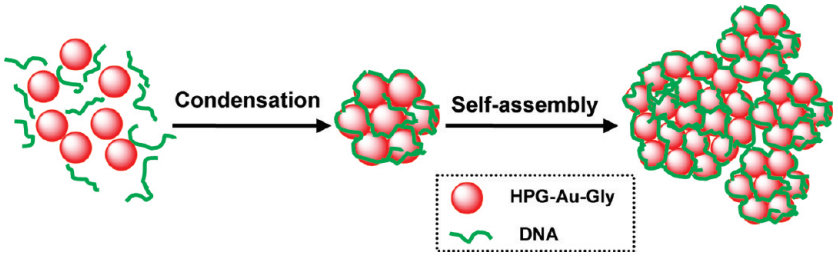
Scheme 4. Schematic Illustration of the Condensation of DNA with HPG-Au-Gly



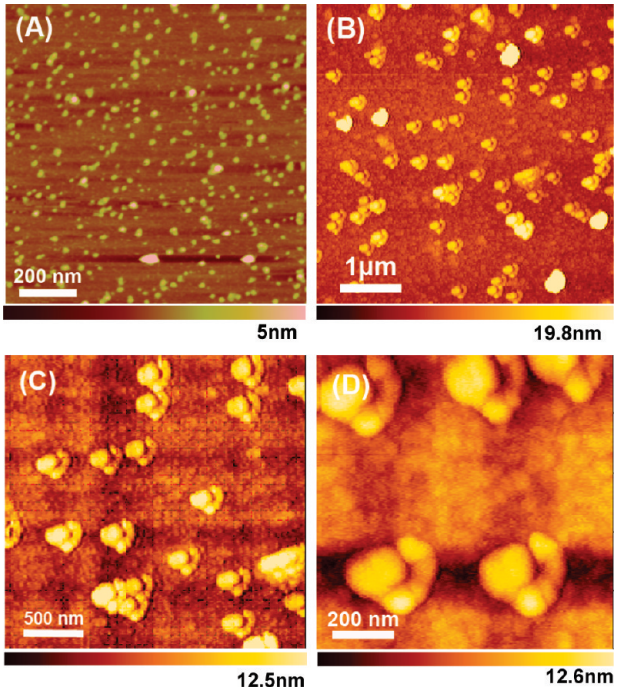


Figure 7. AFM images of HPG-Au (A) before and (B−D) after the condensation of DNA.

biomolecules (see Figure 6c). After the reaction with Boc-Gly-OH, new character peaks at 1.0−1.4 and 3.8−3.9 ppm associated with the Boc-Gly-OH appeared, indicating the successful modification of the HPG-Au (see Figure 6a). Note that the HPG-Au cannot dissolve in nonpolar organic solvents, while the resulting HPG-Au-Boc particles show good solubility in nonpolar organic solvents such as chloroform and dichloromethane but poor dispersibility in polar solvents such as water and methanol, further confirming the successful modification (see Figure 4f, vials 4 and 5). Deprotecting the Boc groups of the HPG-Au-Boc nanocrystals in the mixture of dichloromethane and trifluoroacetic acid, the Au nanocrystals gradually precipitated and could dissolve in polar solvents again, suggesting that HPG-Au-Gly nanocrystals with numerous surface amino groups were obtained. Compared with HPG-Au, the obvious new peaks at 1168, 1642, and 1736 cm^{-1} are assigned to the C−N, N−H, and C═O stretching, respectively, confirming the presence of amino groups (see Figure 6b). Meanwhile, the weight loss of HPG-Au-Gly increased to 43 wt %, corresponding to an amino groups density of 3.3 mmol/g (see Figure 6c).

Furthermore, the binding of HPG-Au-Gly and DNA was carried out, as depicted in Scheme 4. The binding of positively charged inorganic particles or organic molecules with DNA has been attracting increased interest, because it can condense the extended DNA double helices into compact and orderly particles and, therefore, has potential applications in nonviral gene therapies and self-assembly of DNA.[30] In this respect, Au nanocrystal is one of the most widely used nanocrystals in gene therapy.[31] Herein, because of the numerous amino groups on the surface of HPG-Au-Gly, interesting Au-DNA assemblies were formed, indicating the versatility and promising applications of the HPG-stabilized nanocrystals (see Figure 7).

CONCLUSIONS

We have shown that the HPG-stabilizing approach can be used to prepare a wide variety of inorganic nanocrystals with excellent properties such as good stability and biocompatibility, multiple functionality, and excellent solubility in water and polar organic solvents. It is the first time that a stabilizer can be used to synthesize so many types of multifunctional aqueous nanocrystals, even compared to the widely used stabilizer of PAMAM dendrimers. We have also systematically investigated the influence factors of the aqueous nanocrystals using HPG with different molecular weights as well as different types of linear polymers as stabilizers. It is found that the polymer component and topology are two key aspects for the formation of stable nanocrystals. We believe that the work presented here would open avenues to the synthesis, assembly, functionalization, and application of aqueous nanocrystals.

ASSOCIATED CONTENT

Supporting Information. Materials and characterization, the synthesis of HPG, cytotoxicity evaluation, the binding of HPG-Au-Gly with DNA, literatures about using polymer as a stabilizer for the synthesis of nanocrystals, TEM images and EDS of the HPG-based nanocrystals, UV/vis spectra of the HPG-Au and HPG-Ag, and TEM images of Ag and Au particles with different $[Ag^{+}]$:[polymer stabilizer] and $[Au^{3+}]$:[HPG], respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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