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**Highlight Review**

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**Abstract**

Poly(ethylene glycol)-b-poly(2-(N,N-dimethylamino)ethyl methacrylate) block copolymers possessing a reactive group at the PEG chain end (R-PEG-b-PAMA) was synthesized by two different polymerization techniques. Using R-PEG-b-PAMA block copolymer thus obtained, gold nanoparticles (GNPs) were prepared. The PEG-b-PAMA worked as not only reducing agent of aurate ions but also coordination on the obtained nanoparticle surface. The obtained GNP had controlled sizes and narrow size distributions. It is also showed extremely high dispersion stability due to the PEG tethering chains on the surface. The obtained GNP had controlled sizes and narrow size distributions. It is also showed extremely high dispersion stability due to the PEG tethering chains on the surface. The same strategy could be applicable to other nanoparticles such as semiconductor quantum dot and inorganic porous nanoparticles. Using PEGylated GNPs thus prepared, several applications such as molecular recognitions, siRNA release and enzyme immobilizations were carried out. The functionalities of these materials are described in this review.

**Introduction**

Recently, nanometer-scaled metal particles have attracted growing interest as a center of nanomaterial science and technologies.\(^1\) Especially, they are widely applied to biorelated fields such as bioseparations,\(^2\) diagnostics,\(^3\) bioimaging,\(^4\) and therapy.\(^5\) GNPs with a size range of several to hundreds of nanometers show a bright-pinkish color due to plasmon resonance and have been widely utilized for preparation of stained glasses for several hundred years. A simple and facile means of anchoring different ligand molecules onto particle surfaces allows their utilization as colloidal sensors.\(^6\) Particularly, color changes induced by association of nanometer-sized gold particles provide a basis of a simple, yet highly selective, method of anchoring different ligand molecules and receptor molecules in the milieu. Mirkin and co-workers have shown that gold colloidal particles modified with oligonucleotides form large assemblies through the hybridization with complementary oligonucleotide strands, providing a new method for colorimetric detection of targeted DNA sequences.\(^7\)

The growing interest in colloidal GNPs in the bioanalytical field lends a progressive impetus to the development of their novel preparation methods.\(^8\) Most of these methods are based on the reduction of tetrachloroauric acid (HAuCl\(_4\)) in aqueous medium. GNPs thus prepared are dispersed in solution by ionic repulsion of adsorbed ions such as AuCl\(_{4}^-\) on their surface.\(^9\) Under physiological salt concentration, however, the ionically stabilized GNPs tend to aggregate because of charge shielding. To improve their dispersibility in solution with appreciably high ionic strength, various types of low molecular weight stabilizers as well as water-soluble polymers such as starches are often added in the solution.\(^10\) An alternative way to stabilize the dispersion is to form a brush layer of hydrophilic polymer strands on the surface of the GNPs. Indeed, a brush layer of PEG was successfully prepared on GNPs using end-thiolated PEG.\(^11\) Functionalization of the α-end of ω-thiolated PEG was further accomplished to install specific ligands for molecular sensing.\(^12\) α-Lactosyl-ω-thiolate-PEG-anchored GNPs were demonstrated to undergo sensitive and quantitative aggregation responding to a lactose-specific lectin, RCA120.\(^13\)

Though thiol chemistry has been widely used for the modification on gold surfaces, the functionality of thiolate-modified GNPs in vivo is limited to only a few days because of the limited oxidative stability of thiolate species,\(^13\) as well as exchange reactions with thiolated compounds inside the body. Thiolate-modified surfaces are also damaged by exposure to light, high temperature and oxygen.\(^14\) The stability of PEGylated GNPs via a thiol–gold bond, therefore, is not sufficiently long-lasting, especially in vivo.

An alternative method was proposed using water-soluble polymers possessing coordination ability with metals such as poly(2-vinylpyridine) and poly(ethylenimine) as a stabilizer for metal colloids.\(^15\) For example, Antonietti and co-workers reported the preparation of PEG-stabilized gold colloids through the mixing of AuCl\(_4^-\) with poly(ethyleneimine)-poly(ethylene glycol) graft copolymer (PEI/PEG).\(^16\) The amino group in the PEI segment of the PEI/PEG graft copolymer was considered to have the ability to reduce auric cations to form a gold colloid in the AuCl\(_4^-\) incorporated PEI/PEG micelles. The stability of graft copolymer on metal surface however, is not always high enough.\(^17\)

In order to improve the long-term stability of PEGylated GNPs under physiological conditions, we have used PEG-b-polyamine block copolymers. Polyspecification of amino groups in the side chain of the block copolymer plays an important role for the stabilization of the GNPs. This review describes synthesis of PEG/polyamine block copolymers and their application for GNP preparation. Other nanoparticles such as semiconductor quantum dots (QDs), upconversion nanophosphor and inorganic porous nanoparticles are also prepared using R-PEG-b-PAMA block copolymers. Applications of the obtained GNPs are discussed.
PEG-b-PAMA possesses functional groups at both chain ends prepared by two different polymerization reactions described in Supporting Information. Using these polymerization techniques, R-PEG-b-PAMA with different PAMA chain length \((DP = 3 - 43)\) was prepared \((M_w (PEG) = 5000)\). The block copolymers used in this study is shown in Scheme 1.

**Synthesis of End-reactive PEG-b-PAMA Block Copolymers**

Wuelfing et al. and our group have reported the preparation of highly dispersion-stable GNP by PEGylation with thiol-ended PEG. Although the S–Au linkage is strong enough \((197 \text{ kJ/mol})\) for the construction of a dense PEG brush on the colloid surface, it can be easily cleaved through the oxidation of the thiol group. This strategy is not suitable when PEGylated GNP are used in physiological conditions, because biofluid is known to contain numerous oxidation species such as active oxygens. Our idea was to explore alternative methods of synthesizing PEGylated GNP, which can eventually be utilized in vivo. Since the N–Au linkage is not as strong \((25 \text{ kJ/mol})\) as the S–Au linkage, we employed PEG possessing an oligoamine segment at one end. The effect of the polyvalency interaction of the N–Au linkage on the PEGylation chemistry was investigated.

An unprecedented finding is that the block copolymer even facilitated auto-reduction of auric cations to obtain GNP without using any additional reducing reagent. The tetra-chloroauric acid solution changed from colorless to bright red due to the formation of colloidal GNP when acetal-PEG-b-PAMA was added to the solution in an appropriate ratio. The tertiary amino groups in the PAMA segment play a crucial role in the reduction of auric cations as well as the anchoring of PEG on the surface of the GNP.

The insert of Figure 1 (right) shows typical surface plasmon absorption of GNP obtained by acetal-PEG-b-PAMA block copolymers under a molar ratio of amino group in block copolymer versus aurate cation \((\text{N/Au}^{3+}) = 8\). The insert of Figure 1 (left) is an image of transmission electron microscopy (TEM) of the obtained GNP. From the TEM image, the average size and the distribution index \((D_w / D_s)\) of the obtained GNP, were 11.8 nm and 1.01, respectively. Figure 1 shows size and its distribution index as a function of \(N/Au^{3+}\) molar ratio. With increasing the ratio from 4 to 16, the average particle size decreased from 16 to 6 nm. Note that these acetal-PEG-b-PAMA-stabilized GNP have a substantially narrower distribution than those prepared by conventional methods, including that using the PEI/PEG graft copolymer. This is obviously an advantage of using block copolymers as stabilizers because they can form a multimolecular micelle structure with a definite association number and well-defined core–shell architecture: an aurate-complex core segregated and surrounded by a hydrophilic shell layer.

The obtained GNP were purified by centrifugation at 45000 \(\times g\), for 30 min at 20 °C. The precipitated particles were resuspended in water or in various buffer solutions with a wide range of pH. The centrifugations were repeated several times to remove free block copolymers. No color change was observed, while retaining the transparency of the solution. This behavior clearly indicates the high dispersion stability of the acetal-PEG-b-PAMA-anchored GNP.

Commercially available GNP, prepared by citrate, were dispersed in an aqueous solution through the ionic repulsion of the surface-adsorbed ions. Thus, the citrate-reduced GNP have an appreciably negative \(\zeta\)-potential \((-20 \text{ mV})\) at neutral pH, yet they undergo aggregation in the acidic region \((<pH 5)\) due to the neutralization of the surface negative charge. On the other hand, the \(\zeta\)-potential of the GNP prepared by the acetal-PEG-b-PAMA was close to zero regardless of the environmental pH. This result indicates that the PAMA segment in the block copolymer coordinates with the gold surface, allowing the PEG segment to be tethered from the surface into the aqueous exterior to shield the surface charge. The clear phase separation of the PEG layer and the anchored PAMA segment should be an important factor in shielding the cationic charge of the PAMA segment.

In order to obtain information on the interaction between PEG-b-PAMA to the gold surface, modification of citrate-reduced GNP by PEG-b-PAMA was carried out under various conditions.

The protonation degree of the amino groups in the block copolymer depended on the environmental pH. The effect of the state of the amino groups on surface modification was investigated using PEG-b-PAMA \((43)\) and varying the environmental pH. In order to confirm the dispersion stability, dynamic light scattering (DLS) measurement was carried out after the PEG-b-PAMA modification. Figure 2a shows plots of the particle size of the PEG-b-PAMA modified GNP solutions for 18 h at room temperature after preparation under different pH conditions in 10 mM phosphate buffer (closed plots). The particles prepared under acidic conditions increased in size after 18 h at room temperature, indicating the coagulation of the particles in the solution. On the contrary, the particle size did not increase much under alkaline conditions in the same phosphate buffer \((pH <8.5)\). However, in phosphate buffer solution at pH 8.5, a slight but definite increase in particle size was observed. In order to further investigate the preparation conditions in the higher pH region, the buffer of the preparation experiment was changed.
they are not cleaved even in neutral pH. The pKb conditions (pH 10.5). This probably means that, once the neutral PEG–GNPs were prepared from deprotonated PEG–PAMA, the particles. It is rather surprising that the purified PEG–GNPs which was maintained for 14 d without any change in size of the particles. It is rather surprising that the purified PEG–GNPs thus prepared, the free excess polymers in the solutions was removed by centrifugation (2.0 × 10^2 g, 4 times), and the solvent was substituted with pH 7.4 phosphate buffer saline. Plots were obtained from the data in ref 19.

to carbonate buffer (open plot). Even in carbonate buffer at pH < 10, a slight increase in particle size was observed. At pH > 10, on the contrary, an almost constant size (ca. 37 nm) with narrow size distribution was obtained even after 18 h. The size of the modified GNP prepared at pH 10, 37 nm, is reasonable for a PEG brush composed of PEG with a molecular weight of 4.2 kDa coating the commercial citrate-reduced GNPs (18.4 nm), assuming a single GNP for each particle. It has been reported previously that the pKb value of the amino groups of PEG-b-PAMA is 7.0, which means that all the amino groups in the PAMA segment of the block copolymer are completely deprotonated at pH 10. This means that the GPNPs are stabilized by the neutral PEG-b-PAMA, probably via coordination of amino nitrogen atoms on the GNP surface, but not via electrostatic interaction between the protonated PAMA and the negative GNP surface, caused by the absorbed citric acid. As stated above, although the N–Au bond is not strong enough, unlike the S–Au bond, the polyvalency coordination of the tertiary amino groups of PEG-b-PAMA might work effectively on the GNP surface.

Dispersion stability of the modified GPNPs under physiological conditions was examined as a function of PAMA chain length in the block copolymers. Figure 2b shows change in sizes of the particles after 14 d incubation in the presence of 4.5 mg/mL BSA in PBS (pH 7.4) at 4 °C as a function of numbers of amino groups in the PAMA segment of the block copolymer. When block copolymers with longer PAMA chain were used as stabilizer, the size increased, indicating coagulation took place during incubation. When the GPNPs were modified with PEG-b-PAMA having short AMA units less than 10, on the contrary, the PEG–GNPNPs showed excellent dispersion stability, which was maintained for 14 d without any change in size of the particles. It is rather surprising that the purified PEG–GNPNPs (DP = 3 and 6) were stable at neutral pH even though they were prepared from deprotonated PEG-b-PAMA under alkaline conditions (pH 10.5). This probably means that, once the neutral amino groups in PEG-b-PAMA coordinate on the GNP surface, they are not cleaved even in neutral pH. The pKb value of the amino groups, which coordinate on the gold surface might change. When the GPNPs were modified with PEG-b-PAMA having long polyamine segments, a part of the amino groups in the single polymer chain could not coordinate on the particle surface even at high pH, due to the conformational restriction of the PAMA segments. The protonation of the free amino groups in this case may decrease the dispersion stability under physiological conditions.

On the basis of these results, it is confirmed that PEG-b-polyamine possessing several amino groups shows excellent characteristics for both preparation and stabilization of GPNPs under physiological conditions.

*Preparation of PEGylated Quantum Dots and Other Nanoparticles*

The optical properties of quantum dots (QDs) have been intensively studied for the past several decades. Since the use of ligand-conjugated QDs as fluorescent biolabeling reagents were reported in 1998 by the groups of Alivisatos et al. and Chan and Nie, many approaches to QD applications have been done in the bioanalytical field such as DNA sequencing, tissue immuno diagnostics and single molecular imaging. Several advantages of QDs as biolabeling agents are i) tunable fluorescent wavelength by size, ii) sharp and symmetrical fluorescent peak, iii) strong and long life emission, and iv) wide excitation wavelength. Though the QDs are expected to be an important nanomaterials in the bioanalytical field, their dispersion stability in liquid significantly decreases with their decreasing size, especially in the range of nanometers due to an increased surface area. In addition, decrease in fluorescent characteristics is another serious issue in aqueous media, which may be due to surface erosion of the QDs.

We recently found that PEG-b-polyamine can be utilized for both preparation and stabilization of CdS QD. Figure 3 shows the results of the coprecipitation of CdCl2 with Na2S in the absence and presence of several water soluble polymers. When CdCl2 and Na2S were mixed in water, CdS crystalline was formed. However, the size of the crystalline increased too large to disperse under aqueous solution without any additive. Even in the absence of commercially available PEG, the phenomenon was the same as in the absence of any polymer. In the presence of the PAMA homopolymer with the molecular weight of 5000,
no precipitation was observed at low salt concentrations. The solution was transparent with a pale yellow color. With increasing salt concentration, however, CdS particles immediately precipitate, indicating the low dispersion stability of the QD for the PAMA homopolymer. In addition, almost no emission spectrum was observed by excitation at 400 nm. When CdS QD was prepared in the presence of the PEG-b-PAMA block copolymer, on the contrary, a strong emission spectrum was observed at 540 nm using the same excitation wavelength. The CdS QD thus prepared was fairly stable. Actually, no precipitation was observed in 0.3 M NaCl solution at least for several days. It is known that the photoluminescent (PL) emission of CdS QD is affected by the surface charge state. Several approaches to PL activation have been reported, which are based on the confinement of charge carriers within the QD cores. For example, coordination of electron-donating compounds such as an amine and phosphine oxide significantly improve the fluorescent performance. In the case of the QD stabilization by the PAMA homopolymer, the amino groups in the side chain of PAMA were used not only for coordination on the CdS surface but also for solubilization in aqueous phase. Thus, PAMA homopolymer is softly bound to the surface of the CdS QD. For the PEG-b-PAMA block copolymer stabilization, on the contrary, the PAMA segments are strongly coordinated to the surface of the CdS QD, which is probably due to the immiscibility of the block copolymer segments with each other, viz., PAMA segments are segregated from PEG segments, settling on the QD surface to minimize interfacial free energy, like PEG-b-PAMA immobilized gold nanoparticles. Biotin-installed PEGylated CdS QD also showed specific biorecognition, which was monitored by fluorescent energy transfer method.

Preparation of PEGylated nanoparticles by PEG-b-PAMA can be applied to other materials such as inorganic porous nanoparticles, upconversion nanophosphor, fullerene, and so on (Scheme 2).

♦ Application of PEGylated Nanoparticles

We have already published specific biorecognition of lectin proteins by sugar–PEG–SH modified GNPs as stated above. In the same manner, biotin-PEG-b-PAMA could be utilized for stable bionano-gold particles with high biorecognition ability. Due to the limitation of space in this review, data on the selective molecular recognition by ligand-installed PEGylated GNPs are skipped.

Small interfering RNAs (siRNAs) have attracted much attention as a new class of nucleic acid medicines, because they can be used to target mRNA for sequence-specific gene silencing via an RNA interference (RNAi) process in the cytoplasm. Nevertheless, the therapeutic value of siRNAs under in vivo conditions is still controversial due to their low stability against enzymatic degradation, low permeability across the cell membrane, and preferential liver and renal clearance. A major key to the success of the cytoplasmic delivery of siRNAs is the development of effective carrier systems, which achieve the modulated disposition in the body as well as a smooth release of siRNA in response to intracellular chemical stimuli such as pH, ions, and glutathione.

PEG-b-PAMA-modified GNP can be applicable for thiol-ended siRNA carrier system. We have previously found that thiol-ended single-stranded oligonucleotide can be immobilized on the PEG-b-PAMA-immobilized gold sensor surface, which improved hybridization ability, suppressing mismatch hybridization. In the same manner, when the PEG-b-PAMA-modified GNP was mixed with thiol-ended siRNA, PEG/siRNA co-immobilized GNP was constructed, which is promising as a very high-performance RNA interference carrier. As stated above, the PEGylated GNPs formed from PEG-b-PAMA showed excellent stability under physiological conditions even in the presence of high concentrations of thiol compounds, due to the polyvalent coordination between the gold surface and the tertiary amino groups of the PAMA segment. In the same manner, biotin–Au interaction often causes an exchange reaction between R–SH/Au and thiol-containing compounds such as dithiothreitol (DTT) and glutathione, leading to the efficient dissociation of R–SH from the gold surface. It suggests that the smart PEGylated GNPs composed of PEG-b-PAMA and SH–siRNA facilitate the specific glutathione-mediated release of siRNA in the cytoplasm, because the concentrations of glutathione, which is an abundant thiol-containing compound in most cells, are in a millimolar range (1–10 mM) in cytoplasm, whereas those in the blood are in the micromolar range (2 μM), indicating that R–SH/GNPs might be stable in the blood stream but show the glutathione-mediated release of R–SH in the cytoplasm.

To evaluate the RNAi activity (gene inhibition effect) of PEGylated GNP containing SH–siRNA, we carried out a dual luciferase reporter assay in HuH-7 cells (human hepatoma cells) in the presence of 10% fetal bovine serum, as shown in Figure 4a. Almost no RNAi activity was observed for the free siRNA even at a siRNA concentration of 100 nM. The lack of RNAi activity
here indeed occurred through a sequence-specific RNAi effect. That the inhibition of firefly luciferase expression observed renilla luciferase expression were not observed at all, suggesting some) (60% inhibition). Cytotoxicity and the inhibition of the RNAi activity of PEGylated GNPs containing SH–siRNA is remarkably similar to that of OligofectAMINE (cationic liposome) and the same.

Figure 4. Left: RNAi activities against firefly luciferase gene expression in cultured HuH-7 cells. Normalized ratios between firefly luciferase activity (firefly luc.) and the renilla luciferase activity (renilla luc.) are shown as the vertical of the figure. The plotted data are averages of triplicate experiments ± SD. Data points marked with asterisks are statically significant compared with mock (buffer-treated cells: mock) (P* < 0.05).

Right: Stability of enzyme activity against thermal treatments. To a 0.5 mL of substrate (0.5 mM) in phosphate buffer (pH 7.0, I = 25 mM), 1 mL of the enzyme-immobilized colloid solution (6 mg/L-lipase in phosphate buffer, pH 7.0, I = 25 mM) was added. Thermal treatment means that once the enzyme–colloid complex solution was heated at 58°C for 10 min, followed by cooling to 25°C to measure the enzyme activity. This heating treatment was repeated several times and the enzymatic activity was monitored as a function of the heating times. Right figure is reproduced from ref 32 by courtesy of publishers, American Chemical Society, USA.

for the free siRNA may be ascribed to the low tolerance against enzymatic attack. Both the PEGylated GNPs containing siRNA (without the thiol group) and the SH–siRNA revealed RNAi activity and, in particular, PEGylated GNPs containing SH–siRNA achieved a far more effective RNAi activity (65% inhibition) than the PEGylated GNPs containing siRNA (25% inhibition). In addition, the RNAi activity of the PEGylated GNPs containing SH–siRNA at a siRNA concentration of 50 nM (47% inhibition) was found to be higher than that of the PEGylated GNPs containing siRNA even at siRNA concentration of 100 nM (25% inhibition), strongly suggesting that the pronounced RNAi activity of the PEGylated GNPs containing SH–siRNA is not due to the difference in the amount of siRNA molecules immobilized onto the PEGylated GNPs. Thus, the PEGylated GNPs containing SH–siRNA engage in thiol–Au interaction, which exchanges SH–siRNA with glutathione in the cytoplasm; in other words, the efficient release of siRNA from the PEGylated GNPs occurred synchronously with the increase in glutathione concentration, leading to enhancement of RNAi activity. On the other hand, the PEGylated GNPs containing siRNA, engaging only in electrostatic interaction, which is a weak form of interaction under extremely diluted conditions, led to the dissociation of the siRNA in the medium. Note that the RNAi activity of PEGylated GNPs containing SH–siRNA is remarkably similar to that of OligofectAMINE (cationic liposome) (60% inhibition). Cytotoxicity and the inhibition of the renilla luciferase expression were not observed at all, suggesting that the inhibition of firefly luciferase expression observed here indeed occurred through a sequence-specific RNAi effect.

Complexiation of GNP with oligonucleotide thus worked as a stable carrier. One of the other features of PEGylated GNPs is an immobilization of protein such as enzymes (Scheme 3). Lipase was first employed for co-immobilization on GNP surface along with PEG-b-PAMA block copolymers. Since the lipase (Candida rugosa (Sigma) EC 3.1.1.3) possesses an isoelectronic point of ca. 4.5, it is negatively charged under a physiological condition. Thus, the adsorption of the lipase on the gold colloid surface was not by electrostatic interaction because of the negatively charged gold colloid prepared by the citrate reduction. PEG modifications were carried out after the lipase modification on the gold colloid using acetal-PEG-b-PAMA block copolymers. The acetal group can be utilized for the installation of a second function to the colloid complex such as a ligand molecule for specific molecular recognition for produg therapy. Using 4-nitrophenyl propionate (NPP) as a substrate, enzymatic reactivity of the colloids was then investigated. The enzymatic reaction was monitored by the change in absorption of a liberated 4-nitrophenol by the hydrolysis reaction of NPP as a function of time. In order to obtain the information on the thermal stability of the lipase on the gold colloid complex, the enzymatic reactivity was monitored after thermal treatments, viz., once the enzyme–colloid complex solution was heated at 58°C for 10 min, followed by cooling to 25°C to measure the enzyme activity. This heating treatment was repeated several times and the enzymatic activity was monitored as a function of the heating times. It is interesting to note that the initial enzymatic activity of the lipase on the gold surface as well as the PEG-modified gold surface showed almost the same reactivities (0.054 and 0.053 mM/s, respectively) as compared to the native enzyme (0.054 mM/s). The adsorption of lipase on the gold surface did not reduce its activity. When free lipase was heated once to 58°C for 10 min, the enzyme lost more than half of its activity. After the second heating of the free enzyme solution, less than one third of the activity remained. As Sastry et al. pointed out, the activity of the enzyme on the gold colloid surface was more stable than that of the free enzyme though the mechanism was not confirmed in detail. In the case of lipase–gold colloid complex in this study, ca. 60% of the enzymatic activity was retained after the 5-fold thermal treatments as shown in Figure 4b (closed square). It is rather surprising that the lipase–gold colloid possessing PEG tethered chains on the surface retained more than a 95% enzymatic activity even after the 5-fold thermal treatments. The gold colloid possessing both the enzyme and PEG tethered chain surface showed an extremely high stability having the same enzymatic activity as that of the free enzyme. The PEG condensed layer between the immobilized enzyme on the gold colloid may prevent the denaturation of the enzyme at high temperature.

The same phenomena was observed by alternative enzymes such as glucose dehydrogenase (GDH), which is a novel redox-type enzyme, drawing attention due to its oxygen-insensitivity and high catalytic efficiency.

**Conclusion**

We have found that PEG-b-polyamine works as both reducing and stabilizing agent, when mixed with AuCl₃⁻ in aqueous media. The obtained PEG-b-polyamine-immobilized GNP is extremely dispersion stable under physiological conditions. The coordination of nitrogen to the gold surface plays a crucial role for the stable immobilization but not via electrostatic interaction. Three nitrogen atoms at the end of the PEG chain are enough for polyvalent coordination on the gold nanoparticles. The same
strategy could be employed for the preparation of PEGylated nanoparticles such as quantum dots, porous clay, and upconversion nanoparticles. PEG-polyamine-immobilized nanoparticles can be utilized for versatile applications such as calorigraphic bioanalysis, bioimaging, siRNA carrier, and stabilization of enzymes.

References and Notes
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