Surface PEGylation and Ligand Exchange Chemistry of FePt Nanoparticles for Biological Applications

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FePt magnetic nanoparticles (MNPs) were functionalized with a mixed monolayer of poly(ethylene glycol)-terminated thiol and dopamine ligands. The resulting nanoparticles were soluble and stable in aqueous media, including water, ionic solutions, and cell culture medium. The surface thiol ligands are readily exchanged with other thiols bearing chain-end functionalities. MNPs featuring either a cationic or an anionic surface were synthesized by ligand exchange chemistry to afford ligand peripheries capable of binding biomolecules. Surface binding of cationic MNPs to DNA and anionic MNPs to chymotrypsin was enabled by incorporation of a charged functionality on the nanoparticle surface. This approach represents a general strategy to synthesize functionalized FePt nanoparticles that form stable solutions in water and facilitates the use of these magnetic FePt nanoparticles in biological applications.

Introduction

The biological application of nanoparticles is of growing importance in biotechnology.[1] Magnetic nanoparticles (MNPs) have applications[2] in magnetic separation,[3] sensing,[4] hyperthermia,[5] and magnetic resonance imaging (MRI) as contrast agents.[6] Although scalable preparative routes to high-quality magnetic nanoparticles are well-established,[7] surface modification chemistries are far less developed, which limits their utility in biological applications.

The useful magnetic properties of FePt nanoparticles have inspired synthetic advances that afford monodisperse particles of interest for ultra-high-density data storage applications. Proper functionalization of FePt nanoparticles would be of great benefit to bionanotechnology. Recently, Xu et. al. reported the use of nitrilotriacetic acid-functionalized FePt nanoparticles as a general agent to separate polyhistidine-labeled proteins.[3a] Farle and co-workers reported the use of tetramethylammonium hydroxide to stabilize FePt nanoparticles in aqueous solutions through the formation of an ionic double layer around the nanoparticles.[8] However, the aqueous stability of these nanoparticles, including that in biological media, has not been established. Furthermore, a general route to decorate FePt nanoparticles with diverse functionalities has yet to be developed.

Here we report mixed-monolayer-functionalized FePt nanoparticles with enhanced stability and improved versatility (Figure 1). As poly(ethylene glycol) (PEG) and PEGylated materials are well-known for their biocompatibility,[9] PEGylation of colloidal nanoparticle surfaces (i.e., Au,[10] CdSe,[11] CdSe/ZnS,[12] and iron oxide[13]) has been shown to reduce cytotoxicity and nonspecific protein binding. We report here the first example of surface PEGylation of FePt nanoparticles. By using a mixture of PEGylated thiol and dopamine ligands, FePt nanoparticles were functionalized and found to be soluble and stable in a variety of aqueous media.

We found that the thiol ligands on FePt nanoparticles are exchangeable, comparable to the "place exchange reaction" on colloidal gold nanoparticle surfaces.[14] Since the develop-

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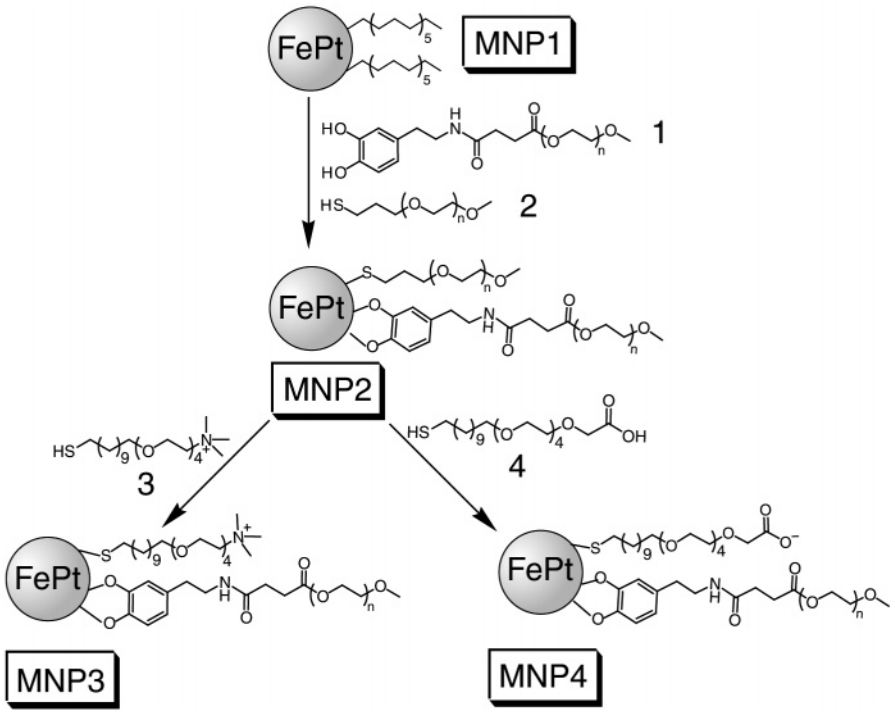

Figure 1. Thiol and dopamine ligands **1**−**4** used for FePt nanoparticle functionalization and the resulting nanoparticles **MNP1**−**3**. The average molecular weight of mPEG used in this work was 550 (i.e., $n \approx 11$).

ment of the place exchange reaction on colloidal gold nanoparticles, a large number of different functional ligands, from simple functional chemical moieties to complicated biomacromolecules, have been incorporated into surface monolayers of gold nanoparticles. This has facilitated numerous applications of these hybrid inorganic−organic materials in biotechnology.[15] Given the chemical similarity between Au and Pt, we anticipated similar ligand binding behavior of thiol to Au/Pt. To our knowledge, this is the first report of a place exchange reaction on FePt nanoparticles and represents a general strategy to synthesize FePt nanoparticles with a biocompatible and tunable ligand periphery.

Results and Discussion

Surface PEGylation. FePt nanoparticles were synthesized according to Sun and co-workers.[16] Attempts to functionalize these FePt nanoparticles with thiol-terminated PEG **2** did not result in water-soluble nanoparticles.[17] However, the use of ammonium-terminated (**3**) and carboxylate-terminated (**4**) PEG-thiols did provide water-soluble FePt nanoparticles. This observation can be attributed to a low coverage of the FePt nanoparticle surface by the neutral PEG-thiol ligand **2**. Although the degree of FePt surface functionalization of **3** and **4** should be similar to that of **2**, the presence of charge at the ligand chain end confers water solubility on the nanoparticles through interparticle repulsion.[18] The stability of these water-soluble nanoparticles in buffers such as phosphate-buffered saline (PBS), however, was not satisfactory for biological applications. Aggregation and precipitation were observed in less than 1 h when **3**-modified nanoparticles were kept in PBS.

On the basis of the above observations, co-incorporation of an iron-binding ligand into the PEG-containing monolayers was expected to improve the water solubility and stability of the FePt nanoparticles. Due to their Fe-chelating capability,[19] 1,3-diols and 1,2-enediols have been used as Fe-binding ligands for γ-Fe_2O_3 nanoparticles.[20] Since the surface Fe atoms of FePt nanoparticles have been shown to be oxidized,[8,21] we envisioned dopamine, a 1,2-enediol, could bind strongly to the FePt nanoparticle surface. Thus, a thiol−dopamine mixed-monolayer system was used to functionalize FePt nanoparticles, where thiols cap surface Pt atoms and dopamine ligands cap the Fe sites.

The PEGylation of hydrophobic FePt nanoparticle **MNP1** was accomplished by incubating **MNP1** with a mixture of ligands **1** and **2** (see the Experimental Section for details). The resulting FePt nanoparticles, **MNP2**, were found to be stable in aqueous solutions. Moreover, **MNP2** was found to be amphiphilic. **MNP2** was soluble in both methylene chloride and water (Figure 2), owing to the properties of PEG. The improved water solubility of **MNP2** relative to that found with only thiol **2** coverage demonstrates the role of dopamine as a key coligand. We further tested the stability of these nanoparticles in biologically relevant media, such

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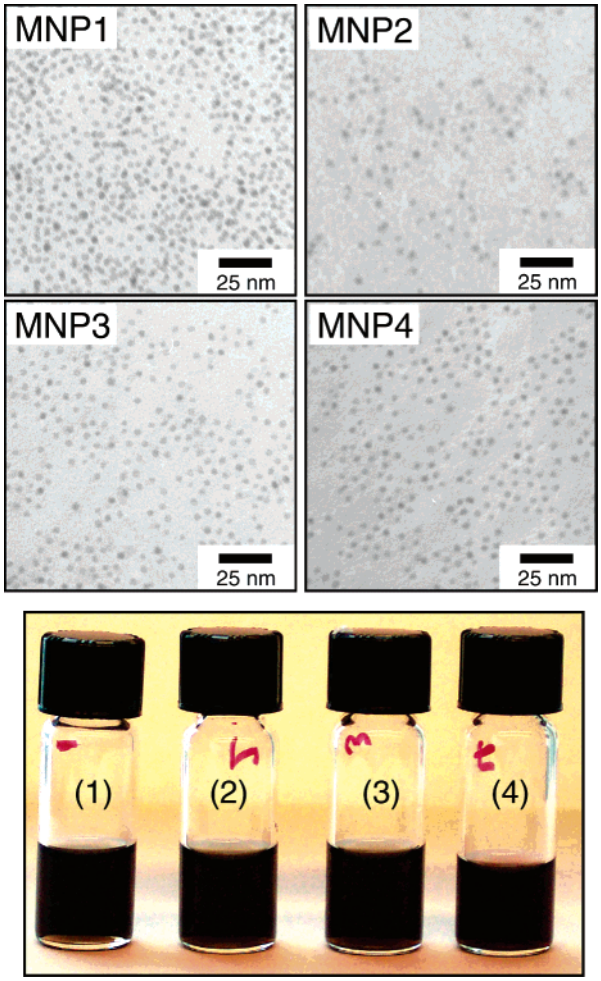

Figure 2. (Top) TEM images of FePt nanoparticles **MNP1**−**MNP4**. (Bottom) Photograph of stable dispersions of **MNP2** in the following solvents after 24 h incubation: (1) methylene chloride; (2) water; (3) PBS; (4) cell culture medium.

as PBS and cell culture medium (minimum Eagle's essential medium supplemented with 10% horse serum). As shown in Figure 2, there was no detectable precipitation of nanoparticles after incubation in the respective medium for 24 h.

Place Exchange Reactions. The methoxypoly(ethylene glycol) (mPEG)-covered nanoparticle **MNP2** was used as the starting material for place exchange reaction with ammonium- and carboxylic acid-terminated PEG-thiols **3** and **4**. The resulting nanoparticles **MNP3** and **MNP4** were found less soluble in DCM than **MNP2** as expected. **MNP3** was readily solubilized in water, and **MNP4** was solubilized in aqueous solution upon addition of base, such as NaOH, suggesting the incorporation of carboxylate ligands on the FePt nanoparticle surface.[22]

The success of this ligand functionalization chemistry was confirmed by X-ray photoelectron spectroscopy (XPS), performed to characterize the surface composition of nanoparticles **MNP2**−**4**. In the C(1s) region of the spectrum (Figure 3) the peak at 286 eV is characteristic of carbon adjacent to oxygen, while the signal at 284 eV indicates carbon−carbon bonding. For all the nanoparticle samples, a peak at 286 eV was observed, providing further evidence of

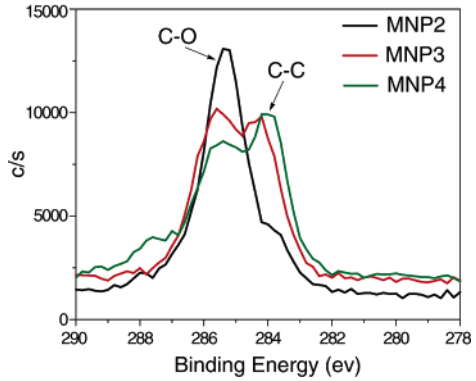

Figure 3. XPS spectrum of the C(1s) region of the magnetic nanoparticles **MNP2**−**4**.

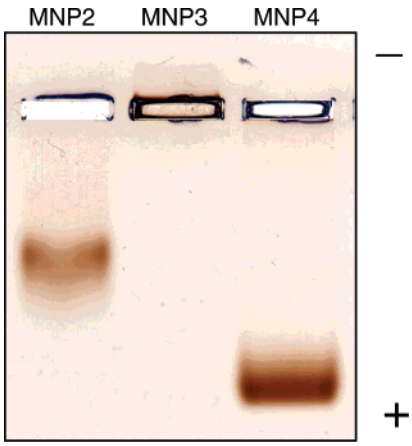

Figure 4. Gel electrophoresis of magnetic nanoparticles **MNP2**−**4**.

the deposition of PEG ligands on nanoparticles. Prior to the exchange reaction, the **MNP2** spectra revealed a peak around 286 eV with a small shoulder around 284 eV. This was in agreement with the chemical structure of the ligands in the surface monolayer, as PEG moieties dominated the monolayer composition. Upon exchange with ligand **2** or **3**, the PEG content decreased from about 11 repeat units per thiol ligand to 4, while the alkyl content increased from 3 methylene groups to 11. This was directly reflected in the spectrum as the relative intensity of the C−O peak (286 eV) decreased while that of the C−C peak (284 eV) increased. In addition, a peak at 402 eV was recorded in the spectrum of **MNP3**, characteristic of the N(1s) binding energy in a quaternary ammonium group. This peak was not present prior to the thiol exchange reaction. It is important to note that the ligand exchange reactions did not change the size of the nanoparticles nor lead to nanoparticle aggregation, as judged by the transmission electron microscopy (TEM) images (Figure 2; see the Supporting Information for histograms).

Gel electrophoresis was performed on samples of **MNP2**, **MNP3**, and **MNP4** to evaluate the impact of their surface monolayer on their mobility in the electric field (Figure 4). Both **MNP2** and **MNP4** were observed to migrate toward the positive electrode of the gel. The mobility of **MNP2** may be attributed to the MNP itself, rather than the neutral ligand.[23] **MNP4** showed higher mobility than **MNP2**, indicating an increased negative charge character of these

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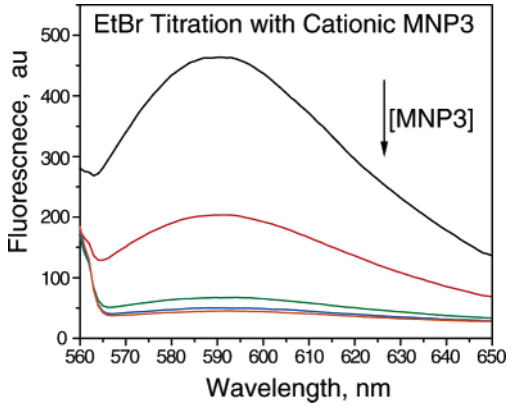

Figure 5. Ethidium bromide titration of DNA with cationic **MNP3** (1 mg/mL).

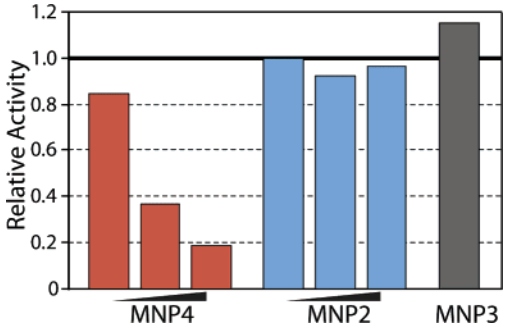

Figure 6. Relative enzymatic activity of ChT with **MNP2**−**4** (1 mg/mL) normalized to ChT alone. Aliquots of 1, 5, and 10 μL of **MNP4** and **MNP2** solutions were added to ChT solutions prior to the addition of the substrate, while 5 μL of **MNP3** was used as a control.

nanoparticles, due to the incorporation of carboxylate into the surface monolayer. The tight bands observed in the gel suggest excellent colloidal stability and narrow size dispersity of the nanoparticles following surface functionalization. The cationic **MNP3** nanoparticles were found to collect in the loading well on the wall toward the negative electrode in the gel, which was in sharp contrast to **MNP2**. The surface charge nature of **MNP2**−**4** was further characterized by ζ potential measurements, and values of −8.2, 10.6, and −23.5 mV were recorded for **MNP2**, **MNP3**, and **MNP4**, respectively, correlating closely with the electrophoresis data.

FePt MNP−**Biomolecule Interactions**. Surface-functionalized nanoparticles have recently been recognized as potent scaffolds for binding with biomolecules. We have previously demonstrated that electrostatically driven interactions between nanoparticles (Au and CdSe cores) and biomolecules (protein and DNA) can be modulated by tailoring the nanoparticle surface monolayer.[11,24,26] To probe the interaction between the cationic **MNP3** and the negatively charged DNA,[24] ethidium bromide titration experiments were conducted. Ethidium bromide, a DNA intercalation agent, exhibits intense fluorescence only when intercalated between DNA base pairs.[25] Nanoparticle binding of the DNA quenches ethidium bromide emission, likely due to the exclusion of ethidium bromide from the DNA base pairs. As shown in Figure 5, decreased ethidium bromide emission was observed upon addition of **MNP3** to a solution of DNA and ethidium bromide. In a control experiment, when anionic **MNP4** was titrated into DNA solution under the same conditions, fluorescence quenching was not observed. These results confirmed the importance of the nanoparticle monolayer and its ability to regulate interactions with DNA. To probe the interactions of the functionalized FePt nanoparticles with proteins, activity assays of chymotrypsin (ChT) with **MNP2**−**4** were performed. On the basis of our previously studies,[11,26] we expected the negatively charged **MNP4** to inhibit the enzymatic activity of ChT, while positively charged **MNP3** would not. As shown in Figure 6, a dose-dependent inhibition of ChT was observed for **MNP4**, due to the surface binding of ChT onto **MNP4** through charge complementarity. As expected, the cationic **MNP3** did not show any inhibitory effect on ChT, indicating unfavorable electrostatic interactions between ChT and **MNP3**. Interestingly, although shown to be negatively charged by electrophoresis and ζ potential measurements, **MNP2** did not disturb the enzymatic activity of ChT at concentrations identical to those of **MNP4**. This observation suggests that the negative charge of **MNP2** is located near the surface of the FePt core rather than at the periphery of the surface monolayer. In this case, the neutral PEG monolayer precludes electrostatic interactions between **MNP2** and ChT.

In conclusion, we have developed a general strategy to PEGylate and further functionalize magnetic FePt nanoparticles with charged groups. By covering FePt nanoparticles with PEGylated thiol and dopamine ligands, water-soluble FePt nanoparticles with great stability have been synthesized. Both thiol and dopamine moieties were determined to be essential to impart water solubility and stability to the nanoparticles. More significantly, we have shown that the surface thiol ligands were exchangeable. In our preliminary investigation, we have exchanged neutral thiol ligands with both positively and negatively charged ligands. The properties of the resulting nanoparticles were found to be highly consistent with the chemical properties of the ligands introduced. The ease of the exchange reaction greatly enhanced the ability to functionalize FePt nanoparticles for biological applications. We have successfully demonstrated surface binding of biomolecules, both DNA and protein, to tailored magnetic FePt nanoparticles. Besides the capability of using these charged nanoparticles in controlling nanoparticle−biomolecule interactions, they should also find applications in constructing nanoparticle−polymer composite materials through electrostatic interactions.[27]

Experimental Section

Instrumentation. TEM samples were analyzed using a JEOL 200CX electron microscope with an acceleration voltage of 200 keV. XPS spectra were recorded on a Physical Electronics Quantum 2000 XPS spectrophotometer using a monochromatic A1 Kα source. The ζ potential was measured on a MALVERN Zetasizer

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Nano ZS by using 1× PBS (pH 7.8) as solvent. The buffer was filtered through a 0.22 μM filter prior to the measurement.

Synthesis of Ligands. See the Supporting Information for details.

Synthesis of MNP2. FePt nanoparticles were synthesized according to the method developed by Sun et al. The FePt nanoparticles, after the purification steps in the literature were followed, were stored in a hexane solution in the presence of excess hexylamine and 1-hexanoic acid. Before the exchange reaction, the nanoparticles were precipitated in EtOH and centrifuged. The precipitate was dissolved in methylene chloride and once again precipitated by adding EtOH. The resulting FePt nanoparticles (∼10 mg) were added to ligands **1** (15 mg) and **2** (20 mg) in ∼4 mL of DCM. The reaction mixture was stirred at 30−40 °C for 2 days. DCM was then removed, and the residue was dissolved in a small volume of DCM to which was added diethyl ether. Nanoparticles precipitated out from solution and were collected by centrifugation. This purification was repeated once more, and the resulting **MNP2** was kept in a DCM solution. After removal of DCM, the nanoparticles were readily solubilized in aqueous solution.

Synthesis of MNP3 and MNP4. A solution of **MNP2** (∼1 mg/mL) in DCM was used for exchange reaction. To synthesize **MNP3**, ligand **3** (10 mg) was added to a 3 mL solution of **MNP2**. The mixture was then stirred at 30−40 °C. After about 4 h, the solution became slightly turbid, indicating the decreased solubility of the exchanged nanoparticles in DCM. After 4 days, solvent was removed and ∼1 mL of DCM was added to the residue. The nanoparticles were found to be partially soluble and were precipitated by the addition of 1 volume of diethyl ether. The nanoparticles were separated by centrifugation and were washed one more time. After being dried under a nitrogen flow, the resulting **MNP3** was kept in a Milli-Q water solution. **MNP4** was synthesized by following the same procedure as that for **MNP3**. It was not directly soluble in DCM or water, but was solubilized in aqueous solution upon the addition of dilute NaOH solution.

EtBr Titration of DNA with MNP3 and MNP4. A solution of 1 μL of DNA 37-mer (50 μM) and 1 μL of EtBr (1 mM) in 498 μL of TE buffer (pH 7.41) was prepared. MNP solution (1 mg/mL) was added as 1 μL aliquots into the DNA solution. Emission spectra of EtBr upon mixing the MNP and DNA solution were recorded on a Shimadzu RF-5301 PC fluorimeter, $\lambda_{ex} = 545$ nm). The excitation and emission slit widths were set at 10 and 15 nm, respectively.

ChT Activity Assays. ChT activity was followed by the catalytic hydrolysis of substrate glutaryl-Phe-pNA (GPNA; Aldrich) at 405 nm for 30 min using a microplate reader (EL808IU, Bio-Tek Instruments, Winooski, VT).[28] All the experiments were preformed in 5 mM sodium phosphate buffer at pH 7.4. The final concentrations of ChT and GPNA were 3.2 μM and 1 mM, respectively. MNP solutions of ∼1 mg/mL were prepared in water and were added to the ChT solution before the addition of the substrate.

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Supporting Information Available: Syntheses of the ligands and histograms of MNPs (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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