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Polyethyleneimine-Functionalized Platinum Nanoparticles with High Electrochemiluminescence Activity and Their Applications to Amplified Analysis of Biomolecules

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Abstract: Polyethyleneimine-functionalized platinum nanoparticles (PtNPs) with excellent electrochemiluminescence (ECL) properties were synthesized and applied to the amplified analysis of biomolecules. These particles were prepared at room temperature, with hyperbranched polyethyleneimine (HBPEI) as the stabilizer. The UV/Vis absorption spectra and transmission electron microscopy images clearly confirmed the formation of monodisperse PtNPs. Such particles proved to possess high stability against salt-induced aggregation, enabling them to be employed even under high-salt conditions. Owing to the existence of many tertiary amine groups, these particles exhibited excellent ECL behavior in the presence of tris(2,2′-bipyridyl)ruthenium(II). An HBPEI-coated particle possessed an ECL activity that was at least 60 times higher than that of a tripropylamine molecule. Furthermore, these particles could be immobilized on the 3-aminopropyltriethoxysilane-treated quartz substrates to amplify the binding sites for carboxyl groups. Through this approach, PtNPs were applied to the amplified analysis of the hemin/G-quadruplex DNAzyme by using the luminol/H_2O_2 chemiluminescence method.

Keywords: DNA · electrochemiluminescence · enzyme catalysis · platinum nanoparticles · polymers

Introduction

In the past decades, nanoparticles have exhibited their versatility in the fields of physics, chemistry, biology, medicine, and material science owing to their novel optical, electronic, thermal, or catalytic properties.[1,2] Another significant advantage of nanoparticles is that the particle properties are easily tailored by surface coating or modification with biomolecules, inorganic and organic materials (e.g., polyelectrolytes)[3] to prepare various functional materials with desired properties for different purposes.[4–8] Polyelectrolytes are charged polymers that are capable of combining both steric and electrostatic stabilization,[1,9] and they have served as ideal protectants to prepare various nanoparticles with desired properties.[3,10–13] To date, these polyelectrolyte-protected particles have been widely employed in various fields such as catalytic reactions,[6,8] gene delivery mediation,[7] and DNA interaction,[14] indicating their great potential in applications to chemical and biological analysis.

Since Bard et al. opened the door to exploring the electrochemiluminescence (ECL) properties of nanoparticles in 2002,[15] the preparation and application of various nanomaterials with ECL activity have attracted considerable attention.[16–26] The encapsulation of ECL reagents (e.g., tris(2,2′-bipyridyl)ruthenium(II) ($[Ru(bpy)_3]^{2+}$) and luminol) in silica nanomaterials pioneers a new approach to preparing nano- and/or microparticles with high ECL activity.[17–23] These functional particles have been employed to develop some solid-state ECL detectors,[17–20] and construct ECL sensors for biological/chemical analysis.[21–23] In the presence of a peroxide such as H_2O_2 and $[S_2O_8]^{2-}$, some semiconductor nanomaterials (e.g., CdS and CdSe) exhibit ECL activity as well.[24–26] Under these conditions, this kind of luminescent materials is also used to fabricate ECL sensors with various applications. It is well known that molecules with tertiary amine groups (e.g., tripropylamine) can serve as effective coreactants to catalyze the ECL emission of $[Ru(bpy)_3]^{2+}$.[27] Accordingly, we hypothesize that those particles coated with

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plenty of tertiary amine groups should possess high ECL activity in the presence of $[Ru(bpy)_3]^{2+}$. However, to the best of our knowledge, the investigation of the ECL properties of polyamine-coated nanoparticles has not been reported so far.

Recently, the preparation of catalytic DNAs (also called DNAzymes) for use as new biocatalysts in the biochemical fields has attracted increasing interest.[28,29] Lu et al. have selected a metal ion-dependent DNAzyme that is capable of cleaving a single RNA linkage in vitro.[30] This specific deoxyribozyme can serve as a novel sensing element for the detection of metal ions, especially Pb^{2+},[31] enabling the development of colorimetric biosensors for Pb^{II},[32,33] or adenosine[34] based on the DNAzyme-directed assembly of gold nanoparticles (AuNPs). An interesting kind of G-quartet-based DNAzyme formed by hemin and guanine-rich DNA aptamers possesses peroxidase-like activities,[35] catalyzing the H_2O_2-mediated oxidation of 2,2-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid[36,37] or luminol.[38,39] Thus, these DNAzymes can serve as catalytic beacons for DNA analysis[37] and protein detection[40]. By introducing a monolayer of the hemin/G-quartet DNAzyme onto gold substrates, Willner et al. have developed a few chemiluminescence (CL) aptasensors for the detection of DNA and telomerase activity.[38,39] In their work, AuNPs were employed to amplify the immobilization of thiolated aptamers on the substrate surface; thus the detection sensitivity for DNA analysis was improved by over 10-fold.[39] Because of its two carboxyl groups, hemin was also immobilized on the amino group-functionalized substrate by peptide bond formation to construct another kind of DNAzyme-based aptasensor.[41] Presumably, the nanoparticles coated with plenty of primary amine groups can also be employed to amplify the immobilization of hemin, thus improving the capability of aptamer-based CL sensors for bioanalysis (e.g., DNA detection). With this idea in mind, here we explore platinum nanoparticles (PtNPs) functionalized with hyperbranched polyethyleneimine (HBPEI) for potential bioapplications.

HBPEI is a hyperbranched polyamine. This specific polyelectrolyte is chosen as the protectant to prepare PtNPs because it has a unique molecular structure where numerous tertiary and primary amine groups coexist, contributing significantly to the versatility of nanoparticles. In this work, the HBPEI-coated PtNPs were investigated by cyclic voltammetry in the presence of $[Ru(bpy)_3]^{2+}$ to explore their ECL properties. Furthermore, these functional particles were used for amplifying the immobilization of hemin/G-quartet DNAzyme on the amino group treated quartz substrates to reveal their potential in bioapplications.

Results and Discussion

HBPEI-protected PtNPs were prepared by reducing chloroplatinic acid (H_2PtCl_6) with sodium borohydride ($NaBH_4$) at room temperature in the presence of HBPEI (see the Experimental Section). The formation of PtNPs was monitored by UV/Vis absorption spectra (Figure 1). The main absorption center of H_2PtCl_6 was observed at 259 nm, with a weak absorption at 212 nm (Figure 1, curve a). After the addition of HBPEI, the color of the solution changed to pale yellow.

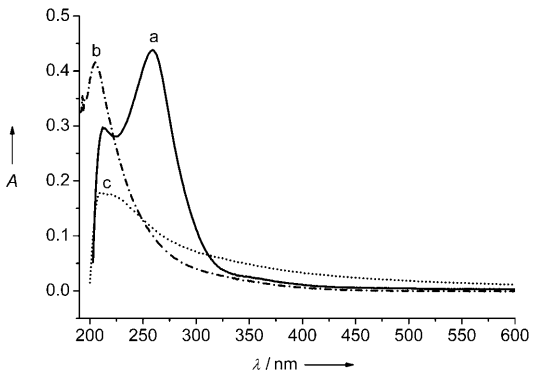


Figure 1. UV/Vis absorption spectra of a) 37 μM H_2PtCl_6; b) after the addition of 0.566 mg mL^{-1} HBPEI; c) after the addition of 1 mg $NaBH_4$ in a 45 min reaction period.

The UV/Vis spectra showed that the main absorption center of $[PtCl_6]^{2-}$ disappeared whereas another strong peak was observed at 205 nm (Figure 1, curve b). These phenomena indicated there was a complexation between HBPEI and the Pt atom, which might contribute to the stability of HBPEI-protected PtNPs.[42] The maximal absorption of colloid platinum is generally observed at about 215 nm.[43] The addition of $NaBH_4$ resulted in a change of the solution color from pale yellow to dark brown, and a peak was observed at 212 nm in the absorption spectrum (Figure 1, curve c), indicating the formation of HBPEI-protected nanoparticles. However, reducing H_2PtCl_6 with $NaBH_4$ without the protectant HBPEI led to a black precipitate. This indicates that the HBPEI coated onto the particle surface prevents the PtNPs from aggregating in aqueous solutions.

Transmission electron microscopy (TEM) clearly illustrated the high monodispersity of the as-prepared PtNPs (Figure 2A). The particle sizes varied from 1.8 to 3.2 nm, with an average size of 2.4 nm. Furthermore, HBPEI-protected PtNPs exhibited high stability against salt-induced aggregation. It is well known that gold particles are subject to aggregation in the presence of a high concentration of salt solution (e.g., 0.2 M NaCl), resulting in a color change from red to blue.[44] However, we found that the HBPEI-stabi-

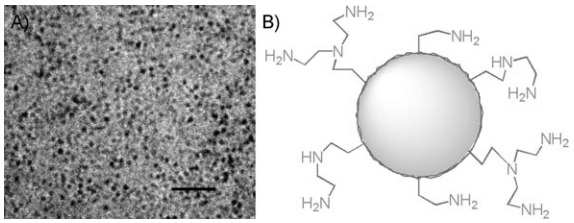


Figure 2. TEM image (A) and the proposed surface structure (B) of HBPEI-coated PtNPs. Scale bar: 20 nm.

lized PtNPs remained monodisperse after the addition of 0.5 M NaCl into the as-prepared colloid (data not shown). By adding a large volume of ethanol to the colloid in the presence of citrate, PtNPs were deposited out and separated from the excess stabilizer. In this process, no aggregation of particles was observed throughout. The high stability against salt-induced aggregation enabled the HBPEI-protected PtNPs to be used for bioanalysis even under high-salt conditions, for example, the $[Ru(bpy)_3]^{2+}$-based ECL reaction and biomolecule analysis.

Similarly, other nanoparticles such as Au and CdTe can also be synthesized with HBPEI as the protectant (see the Supporting Information), but these particles are subject to aggregation in high-salt conditions. So, only PtNPs are adopted here, owing to their high stability. More importantly, these particles had specific surface structures owing to the coating of HBPEI (Figure 2B). Many tertiary and primary amine groups coexisted on the surfaces of the PtNPs, giving them new properties and potential in chemical/biological applications.

It is well known that tertiary amines possess high ECL activity in the presence of $[Ru(bpy)_3]^{2+}$.[27] Accordingly, we predicted that the HBPEI protectant should exhibit a high ECL activity owing to numerous tertiary amine groups existing in its structure, which was confirmed by cyclic voltammetry on a platinum disk electrode in the $[Ru(bpy)_3]^{2+}$ solution. Figure 3A shows that this polyamine possesses a remarkable ECL activity. Under the detection conditions, the intensity of 5 μM HBPEI was approximate to that of 30 μM TPA. That is, the ECL activity of this polyamine was about six times higher than that of TPA. It was observed that the ECL intensity of HBPEI was directly dependent on the pH of the detection solution (Figure 3B). The maximum of the ECL signal of HBPEI was observed at pH 8.0, revealing the typical ECL characteristics of tertiary amines.

Under the above conditions, the HBPEI-coated PtNPs exhibited high ECL activity as well, as shown in Figure 4. When investigated by cyclic voltammetry in 5 mM $[Ru(bpy)_3]^{2+}$ dissolved in pH 8.0 phosphate buffer (Figure 4, curve a), the addition of 10 nM particles (the corresponding concentration of Pt atoms is 3.7 μM) gave rise to an obvious increase in the ECL signal (Figure 4, curve b). As the concentration of PtNPs increased, the ECL signal increased remarkably (Figure 4, curve c). It was observed that the ECL intensity of 6 μM TPA was lower than that of 100 nM particles (Figure 4, curve d), that is, the average ECL activity of a particle was at least 60 times higher than that of a TPA molecule, indicating the excellent ECL behaviors of HBPEI-functionalized PtNPs. It might be attributed mainly to two factors: 1) The protectant HBPEI possesses the intrinsic ECL properties (shown in Figure 3). The coating of numerous HBPEI on the surface of PtNPs gives them high ECL activity. 2) The Pt particles might adsorb onto the Pt working electrode and contribute to the ECL efficiency owing to increasing the electrode area, but this contribution is negligible as compared with that from the coating of HBPEI (see the Supporting Information).

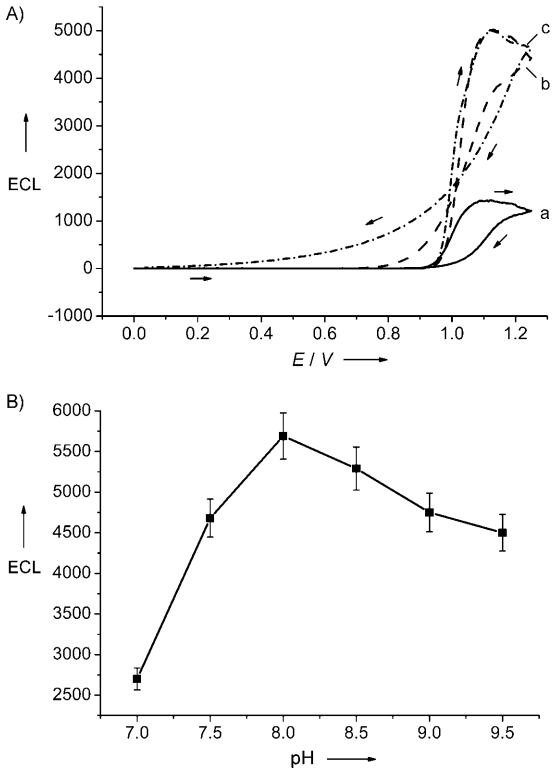


Figure 3. Investigation of the ECL properties of HBPEI by using cyclic voltammetry in the potential range of 0–1.25 V (vs Ag/AgCl) on a platinum disk electrode (ø = 500 μm) in 5 mM $[Ru(bpy)_3]^{2+}$ dissolved in 50 mM phosphate buffer of pH 8.5. A) Voltammograms of a) 5 mM $[Ru(bpy)_3]^{2+}$, b) after the addition of 5 μM HBPEI, c) after the addition of 30 μM TPA. The arrows represent the scan direction (the scan rate is 0.05 V s^{-1}). B) Dependence of the ECL intensity of 5 μM HBPEI on pH of the detection conditions. The voltage of the photomultiplier tube is set at 850 V.

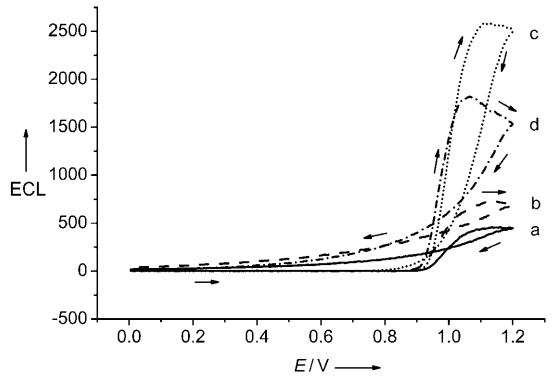


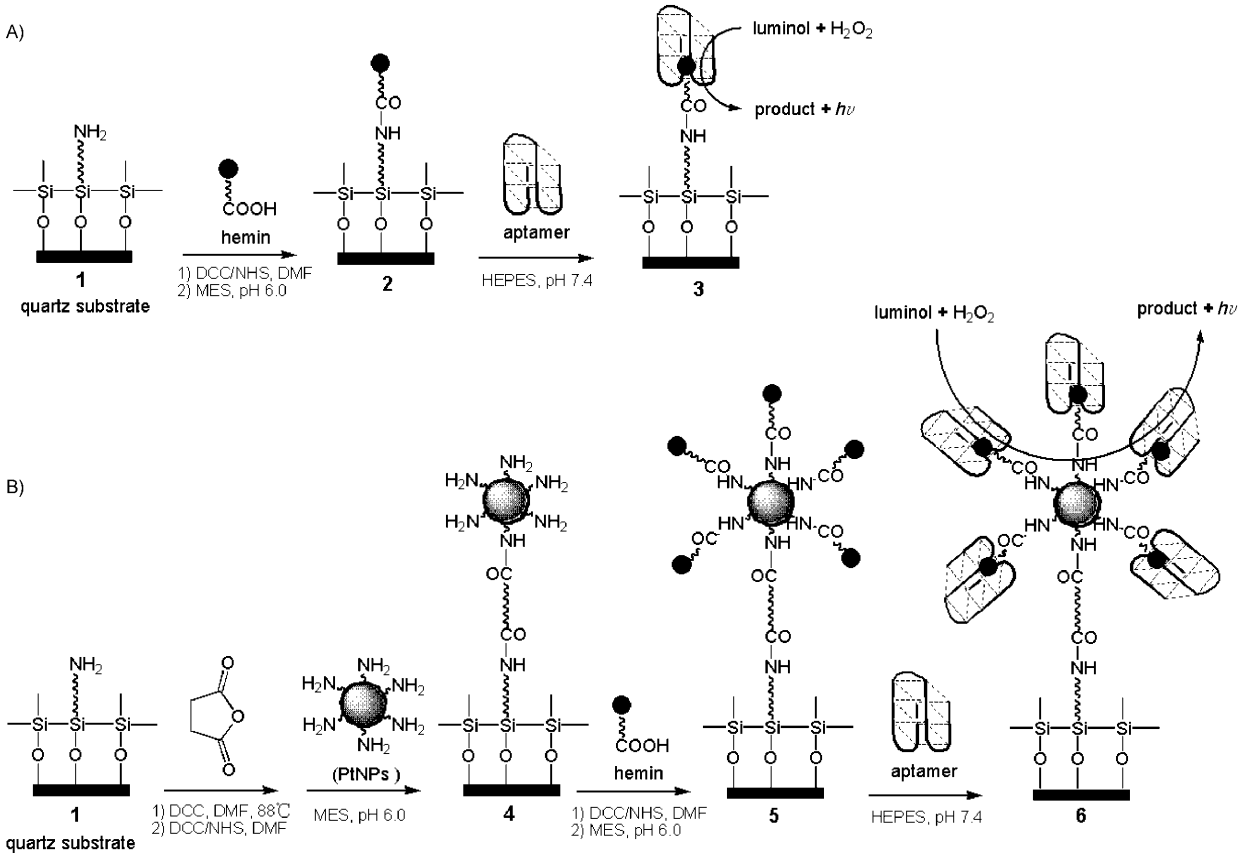
Figure 4. Comparison between the ECL activity of HBPEI-protected PtNPs and TPA by using cyclic voltammetry in the presence of 5 mM $[Ru(bpy)_3]^{2+}$ (pH 8.0). a) 0 nM particles; b) 10 nM particles; c) 100 nM particles; d) 6 μM TPA. The arrows represent the scan direction. Other experimental conditions are equal to those in Figure 3.

It should be noted that, although numerous polymer-protected nanoparticles have been synthesized, there is very little research directed at exploring the ECL properties of this kind of particles. In comparison with those ECL nanomaterials reported previously,[15,16] the HBPEI-coated PtNPs revealed relatively high ECL activity, mainly owing to the

existence of many tertiary amine groups on their surfaces. The excellent ECL properties and high stability against salt-induced aggregation enabled such particles to serve as ECL labels for bioanalysis. For example, this kind of ECL nanoprobes was employed to label target proteins or peptides, so that the targets could be sensitively detected by using the $[Ru(bpy)_3]^{2+}$-based ECL method.

Besides tertiary amine groups, there are plenty of primary amine groups on the surfaces of HBPEI-functionalized PtNPs (Figure 2B). Therefore, these functional particles can be employed to amplify the binding sites for carboxyl groups, and thus improve the sensitivity for analyzing biomolecules. Here we applied the HBPEI-coated PtNPs to amplify the immobilization of hemin on quartz substrates, thus introducing more hemin/G-quartet DNAzyme onto the substrate surface (Scheme 1). First, the substrates were treated with 3-aminopropyltriethoxysilane (APTES) to construct a monolayer of amine groups on the surface. Hemin, an anion porphyrin with carboxyl groups, was then immobilized on the substrates by peptide bond formation. Finally, the hemin-modified substrates were incubated with an 18-mer DNA aptamer, d(GTGGGTAGGGCGGGTTGG), to form a supramolecular complex with peroxidase-like activity, which can catalyze the oxidation of luminol by H_2O_2 to generate strong CL emission.[38,39] Scheme 1A depicts the direct immobilization of hemin and the formation of hemin/G-quadruplex DNAzyme on the substrates. In this case, the immobilization capacity of hemin and DNAzyme was mainly dependent on the density of amine group sites on the substrate surface. However, after modification with the HBPEI-functionalized PtNPs by the linkage of succinic anhydride, the binding sites for hemin on the substrates were amplified to great extent. Through this approach, more hemin and DNAzyme were introduced onto the substrates (Scheme 1B).

Because both hemin and DNAzyme can catalyze the H_2O_2-mediated oxidation of luminol, the catalyst-modified substrates were characterized by using the luminol/H_2O_2 CL method (Figure 5), revealing that such particles were capable of effectively amplifying the luminescent readout. When the hemin-modified substrates (**2** and **5**) were investigated, the use of HBPEI-protected PtNPs amplified the CL signal by about 5-fold (Figure 5A). After incubation with the DNA aptamer, hemin interacted with the G-quadruplex structure to form the G-quadruplex-based DNAzyme with relatively high catalytic activity (substrates **3** and **6**), reflected by an approximately 3-fold increase in the CL signal (Figure 5B). In comparison with the direct immobilization



Scheme 1. Procedure of the use of HBPEI-protected PtNPs for the amplified analysis of DNAzyme. A) Direct immobilization of hemin/G-quadruplex DNAzyme on the quartz substrate. **1**: APTES-treated substrate; **2**: hemin-modified substrate; **3**; DNAzyme-modified substrate. B) Amplified immobilization of DNAzyme on the quartz substrate by using the HBPEI-coated PtNPs. **1**: APTES-treated substrate; **4**: PtNPs-amplified substrate; **5**: hemin-modified substrate; **6**: DNAzyme-modified substrate.

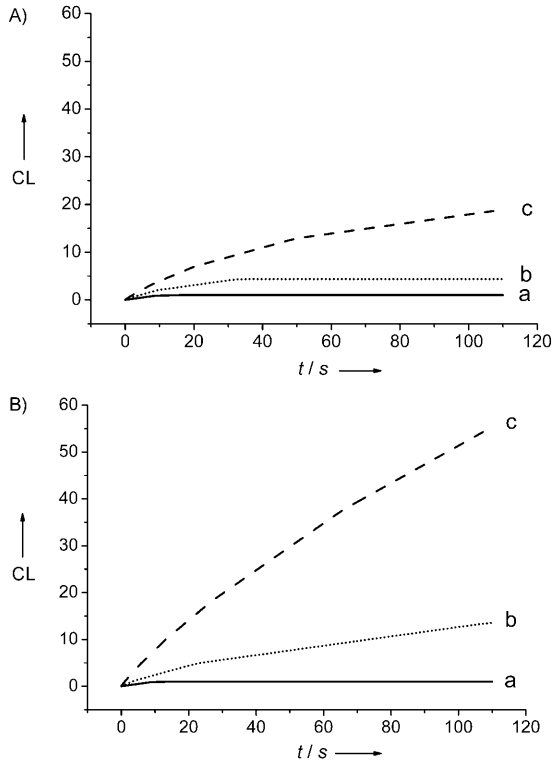


Figure 5. Chemiluminescence characterization of quartz substrates in different cases by using the luminol/H_2O_2 method. A) Integrated CL signal of analyzing the hemin-modified substrates: a) before modification (substrate **1**), b) after direct modification with hemin (substrate **2**), c) after PtNP-amplified modification with hemin (substrate **5**). B) Integrated CL signal of analyzing the DNAzyme-modified substrates: a) before modification (substrate **1**), b) after direct modification with DNAzyme (substrate **3**), c) after PtNP-amplified modification with DNAzyme (substrate **6**). Experimental conditions: [luminol] = 0.5 mM, [H_2O_2] = 30 mM in 25 mM HEPES (pH 8.0) containing 20 mM KCl and 200 mM NaCl. The voltage of the photomultiplier tube is set at 300 V.

approach, the use of PtNPs increased the immobilization capacity of DNAzyme, thus resulting in an approximately 4.5-fold improvement in the catalytic activity. The results indicated such particles, in fact, served as effective signal amplifiers.

With a supramolecular G-quadruplex-based DNAzyme as the catalytic label, Willner et al. have presented a novel approach for sensing DNA.[41] In their work, hemin was immobilized on gold substrates, and then bound by two guanine-rich single-strand DNAs to form a hemin/G-quadruplex complex that catalyzed the oxidation of luminol by H_2O_2. This DNAzyme has two free nucleic acid parts. A single-strand DNA with a complementary sequence of the two parts is capable of decomposing this G-quadruplex-based DNAzyme, giving rise to a decrease in the CL signal. By using this approach, the complementary DNA was detected at 0.6 μM. Similarly, we have employed this supramolecular DNAzyme to develop a colorimetric aptasensor for the sensitive detection of various single-strand DNAs with different sequences.[45] Accordingly, we presumed that the HBPEI-functionalized PtNPs could be employed to construct a CL aptasensor for the amplified detection of DNAs by using the suparmolecular DNAzyme as the catalytic label (see the Supporting Information). Presumably, the sensitivity for analyzing single-strand DNAs would be improved at least 4-fold using HBPEI-functionalized PtNPs as the signal amplifiers.

According to a previous report,[46] bare Pt particles could catalyze the H_2O_2-mediated oxidation of luminol. However, the HBPEI-coated PtNPs were here found to possess poor catalytic behavior in the luminol/H_2O_2 system. In contrast, the G-quadruplex-based DNAzyme exhibited peroxidase-like activity, which was at least 15-fold higher than that of PtNPs under the same conditions (see the Supporting Information). Furthermore, from Figure 5 it could be concluded that there were about five DNAzyme molecules on the surface of one Pt particle, that is, the CL signal yielded by DNAzyme was about 75-fold higher than that by HBPEI-coated PtNPs. Therefore, such particles had little influence on the CL readout when they served as signal amplifiers for the DNAzyme-based analysis of biomolecules.

Conclusions

In this work, HBPEI-functionalized PtNPs with high ECL activity have been prepared and applied to the amplified analysis of the hemin/G-quadruplex DNAzyme. These nanoparticles were synthesized at room temperature, with HBPEI as the stabilizer. The UV/Vis absorption spectra and TEM images clearly confirmed the formation of monodisperse PtNPs. Such particles proved to possess high stability against salt-induced aggregation, enabling them to be used for biological/chemical analysis even under high-salt conditions. More importantly, the polyamine coating on the surface of PtNPs gave them some new properties and potential in bioapplications. Owing to the existence of many tertiary amine groups, these particles exhibited excellent ECL behavior in the presence of $[Ru(bpy)_3]^{2+}$. A functional particle possessed an ECL activity that was at least 60 times higher than that of a TPA molecule, enabling its use as an ECL label in bioanalysis. Furthermore, the functional particles could be immobilized on APTES-treated quartz substrates to amplify the binding sites for carboxyl groups. Through this approach, the PtNPs were applied to the amplified analysis of the hemin/G-quadruplex DNAzyme by using the luminol/H_2O_2 CL method. The CL signal of analyzing DNAzyme was thus improved ca. 4.5-fold. It was reasonably predicted that the HBPEI-coated PtNPs are also applicable to the amplified analysis of other biomolecules (e.g., DNAs and proteins). Our results suggest that such functional particles possess great potential in bioapplications.

Experimental Section

Materials: Hyperbranched polyethyleneimine (HBPEI), sodium borohydride ($NaBH_4$), 3-aminopropyltriethoxysilane (APTES), tripropylamine (TPA), tris(2,2′-bipyridyl)ruthenium(II) ($[Ru(bpy)_3]^{2+}$), N,N′-dicyclohex-

ylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), succinic anhydride, N,N′-dimethylformamide (DMF), and luminol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Hemin, DNA aptamer (5' GTG GGT AGG GCG GGT TGG 3′), 2-(N-morpholino)ethanesulfonic acid monohydrate (MES), and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were obtained from Sangon Biotechnology Co., Ltd (Shanghai, China). Chloroplatinic acid (H_2PtCl_6) and 30% H_2O_2 were purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). All reagents were used as received without further purification. The stock solution of hemin (5 mM) was prepared in DMSO and stored in the dark at −20 °C. The 5 mM working solution of $[Ru(bpy)_3]^{2+}$ was freshly prepared by diluting the 10 mM stock solution with 0.1 M phosphate just before use. 18 MΩ water purified by a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

Instrumentation: A CARY 500 Scan UV/Vis-NIR spectrophotometer (Varian, USA) was used to record the absorption spectra of the colloids at room temperature. A JEOL 2010 transmission electron microscope was used to obtain the TEM images. The accelerating voltage was set at 200 kV. A Model MCDR-A Chemiluminescence Analyzer Systems (Xi′An Remax Analytical Instrument Co. Ltd., Xi′An, China) was used to record the luminescence signal. A Model CHI800 voltammetric analyzer (CH Instruments, Austin, TX) was used for cyclic voltammetry measurement in the ECL experiments.

Preparation of HBPEI-protected PtNPs: All glassware used in the synthesis was immersed in aqua regia overnight, and rinsed thoroughly with water. H_2PtCl_6 (200 μL, 19.3 mM) and HBPEI (39 μL, 0.15 g mL^{-1}) were added to water (10 mL) in a round-bottom flask. Freshly prepared $NaBH_4$ solution (100 μL, 0.01 g mL^{-1}) was added to this under vigorous stirring, resulting in a color change from pale yellow to dark brown within about 10 min. The solution was kept stirring for another 35 min at room temperature, and then stored at 4 °C. The formation of PtNPs was monitored by UV/Vis absorption spectroscopy. The TEM images showed a particle size varying from 1.8 to 3.2 nm, with an average size of 2.4 nm. The as-prepared colloid remained stable at 4 °C for several months. NaCl (0.5 M, final concentration) was added to this colloid, and no aggregation was observed. Before being used for further investigation and application, an equal volume of citrate solution (38.8 mM) was added to the as-prepared colloid followed by a large volume of anhydrous ethanol. The suspension was then centrifuged at 10000 r min^{-1} for 10 min. The deposit was collected and redissolved in water to prepare the PtNP solution. The separation experiments were repeated twice to remove the excess protectant from the colloid.

ECL properties of the PtNPs: The purified colloid was added into a solution of 5 mM $[Ru(bpy)_3]^{2+}$ dissolved in 50 mM phosphate buffer (pH 8.0). The ECL behavior of the PtNPs was investigated by cyclic voltammetry on a Pt disk electrode (500 μm diameter) in the potential range of 0 to 1.3 V (vs Ag/AgCl). The ECL signal changing with the potential in the first cycle was recorded by an MCDR-A system. The voltage of the photomultiplier tube was set at 850 V.

Amplified immobilization of hemin on substrates: Before use, all quartz substrates (10×5×2 mm) were immersed overnight in piranha solution freshly prepared by mixing 98% H_2SO_4 with 30% H_2O_2 at a volume ratio of 3:1 (***Warning**: piranha solution violently reacts with organic compounds and should be handled with extreme caution!*). The substrates were rinsed thoroughly with water and ethanol, and then immersed overnight in anhydrous ethanol (400 μL) containing 10% (v/v) APTES. After rinsing with ethanol to remove the excess APTES, the substrates were heated in an oven at 120 °C for 30 min to solidify the cross-linkage of siloxane. The APTES-treated substrate was immersed in dry DMF solution (400 μL) containing 0.013 g succinic anhydride, heated at 88 °C for 5 h, and then cooled to room temperature. To this solution were added NHS (0.032 g) and DCC (0.027 g), and the mixture was shaken overnight under anhydrous conditions at room temperature to construct a monolayer of NHS active ester on the substrate surface. Then, the substrate was immersed in a solution of PtNPs prepared by mixing the purified colloid (200 μL) with an equal volume of MES buffer (0.1 M MES, 0.5 M NaCl, pH 6.0), and was shaken at room temperature for 2 h. Finally, the substrate was incubated with the NHS active ester of hemin (5 μM) in the MES buffer for 2 h to immobilize hemin onto the PtNP surfaces by peptide bond formation. The hemin–NHS active ester was freshly prepared by shaking the hemin overnight with DCC/NHS in anhydrous DMF at room temperature.

Construction of the monolayer of hemin/G-quadruplex DNAzyme: Before use, 5 OD (optical density) of the hemin-binding aptamer was dissolved in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4) and heated at 88 °C for 10 min to dissociate any intermolecular interaction, then allowed to cool to room temperature gradually. An equal volume of hybridization buffer (50 mM HEPES, pH 7.4, 40 mM KCl, 400 mM NaCl, 0.1% (w/v, g mL^{-1}) Triton X-100, 2% (v/v) DMSO) was added to this DNA solution and the mixture was kept at room temperature for 40 min, allowing the aptamer to fold into the G-quartet structure. The hemin-modified substrate was immersed in the hybridized aptamer solution consisting of 2 μM aptamer, 25 mM HEPES (pH 7.4) 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100, and 1% DMSO, and was then kept at room temperature for 2 h to form the hemin/G-quartet complex, that is, a monolayer of catalytic DNAzyme was constructed on the substrate.

Chemiluminescence Analysis of DNAzyme-Modified Substrates: The DNAzyme-modified substrates were characterized by using the luminol/H_2O_2 CL method. Typically, the substrates were placed into a quartz cuvette containing 0.5 mM luminol and 30 mM H_2O_2 dissolved in the detection buffer (25 mM HEPES, pH 8.0, 20 mM KCl, 200 mM NaCl). The integrated CL signal was recorded by an MCDR-A system. The voltage of the photomultiplier tube was set at 300 V.

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[1] X. Sun, S. Dong, E. Wang, *Polymer* **2004**, *45*, 2181–2184.
[2] Y. C. Cao, R. Jin, C. A. Mirkin, *Science* **2002**, *297*, 1536–1540.
[3] K. S. Mayya, B. Schoeler, F. Caruso, *Adv. Funct. Mater.* **2003**, *13*, 183–188.
[4] A. Rogach, A. Susha, F. Caruso, G. Sukhorukov, A. Kornowski, S. Kershaw, H. Mohwald, A. Eychmuller, H. Weller, *Adv. Mater.* **2000**, *12*, 333–337.
[5] W. S. Choi, H. Y. Koo, J.-H. Park, D.-Y. Kim, *J. Am. Chem. Soc.* **2005**, *127*, 16136–16142.
[6] S.-L. Xu, H. Cui, *Luminescence* **2007**, *22*, 77–87.
[7] S. Zaitsev, R. Cartier, O. Vyborov, G. Sukhorukov, B.-R. Paulke, A. Haberland, Y. Parfyonova, V. Tkachuk, M. Bottger, *Pharm. Res.* **2004**, *21*, 1656–1661.
[8] A. B. R. Mayer, J. E. Mark, *J. Polym. Sci. Part A: Polym. Chem.* **1997**, *35*, 3151–3160.
[9] T. L. Pugh, W. Heller, *J. Polym. Sci.* **1960**, *47*, 219–227.
[10] P.-L. Kuo, W.-F. Chen, H.-Y. Huang, I.-C. Chang, S. A. Dai, *J. Phys. Chem. B* **2006**, *110*, 3071–3077.
[11] D. Radziuk, A. Skirtach, G. Sukhorukov, D. Shchukin, H. Mohwald, *Macromol. Rapid Commun.* **2007**, *28*, 848–855.
[12] M. V. Vasylyev, G. Maayan, Y. Hovav, A. Haimov, R. Neumann, *Org. Lett.* **2006**, *8*, 5445–5448.
[13] W. P. Cheng, A. I. Gray, L. Tetley, T. L. B. Hang, A. G. Schatzlein, I. F. Uchegbu, *Biomacromolecules* **2006**, *7*, 1509–1520.
[14] B. Steitz, H. Hofmann, S. W. Kamau, P. O. Hassa, M. O. Hottiger, B. von Rechenberg, M. Hofmann-Amtenbrink, A. Petri-Fink, *J. Magn. Magn. Mater.* **2007**, *311*, 300–305.
[15] Z. Ding, B. M. Quinn, S. K. Haram, L. E. Pell, B. A. Korgel, A. J. Bard, *Science* **2002**, *296*, 1293–1297.
[16] Y. Bae, N. Myung, A. J. Bard, *Nano Lett.* **2004**, *4*, 1153–1161.
[17] L. Zhang, S. Dong, *Anal. Chem.* **2006**, *78*, 5119–5123.
[18] L. Qian, X. Yang, *Adv. Funct. Mater.* **2007**, *17*, 1353–1358.
[19] L. Zhang, S. Dong, *Electrochem. Commun.* **2006**, *8*, 1687–1691.

[20] L. Zhang, B. Liu, S. Dong, *J. Phys. Chem. B* **2007**, *111*, 10448–10452.
[21] X. Wang, J. Zhou, W. Yun, S. Xiao, Z. Chang, P. He, Y. Fang, *Anal. Chim. Acta* **2007**, *598*, 242–248.
[22] Z. Chang, J. Zhou, K. Zhao, N. Zhu, P. He, Y. Fang, *Electrochim. Acta* **2006**, *52*, 575–580.
[23] L. Zhang, X. Zheng, *Anal. Chim. Acta* **2006**, *570*, 207–213.
[24] G. Jie, B. Liu, H. Pan, J. Zhu, H. Chen, *Anal. Chem.* **2007**, *79*, 5574–5581.
[25] H. Jiang, H. Ju, *Anal. Chem.* **2007**, *79*, 6690–6696.
[26] S.-N. Ding, J.-J. Xu, H.-Y. Chen, *Chem. Commun.* **2006**, 3631–3633.
[27] M. M. Richter, *Chem. Rev.* **2004**, *104*, 3003–3036.
[28] R. R. Breaker, *Nat. Biotechnol.* **1997**, *15*, 427–431.
[29] G. M. Emilsson, R. R. Breaker, *Cell. Mol. Life Sci.* **2002**, *59*, 596–607.
[30] J. Li, W. Zheng, A. H. Kwon, Y. Lu, *Nucleic Acids Res.* **2000**, *28*, 481–488.
[31] J. Li, Y. Lu, *J. Am. Chem. Soc.* **2000**, *122*, 10466–10467.
[32] J. Liu, Y. Lu, *J. Am. Chem. Soc.* **2003**, *125*, 6642–6643.
[33] J. Liu, Y. Lu, *J. Am. Chem. Soc.* **2005**, *127*, 12677–12683.
[34] J. Liu, Y. Lu, *Anal. Chem.* **2004**, *76*, 1627–1632.
[35] P. Travascio, P. K. Witting, A. G. Mauk, D. Sen, *J. Am. Chem. Soc.* **2001**, *123*, 1337–1348.
[36] P. Travascio, Y. Li, D. Sen, *Chem. Biol.* **1998**, *5*, 505–517.
[37] Y. Xiao, V. Pavlov, T. Niazov, A. Dishon, M. Kotler, I. Willner, *J. Am. Chem. Soc.* **2004**, *126*, 7430–7431.
[38] V. Pavlov, Y. Xiao, R. Gill, A. Dishon, M. Kotler, I. Willner, *Anal. Chem.* **2004**, *76*, 2152–2156.
[39] T. Niazov, V. Pavlov, Y. Xiao, R. Gill, I. Willner, *Nano Lett.* **2004**, *4*, 1683–1687.
[40] D. Li, B. Shlyahovsky, J. Elbaz, I. Willner, *J. Am. Chem. Soc.* **2007**, *129*, 5804–5805.
[41] Y. Xiao, V. Pavlov, R. Gill, T. Bourenko, I. Willner, *ChemBioChem* **2004**, *5*, 374–379.
[42] Z. Q. Tian, S. P. Jiang, Z. Liu, L. Li, *Electrochem. Commun.* **2007**, *9*, 1613–1618.
[43] A. Henglein, B. G. Ershov, M. Malow, *J. Phys. Chem.* **1995**, *99*, 14129–14136.
[44] H. Li, L. J. Rothberg, *J. Am. Chem. Soc.* **2004**, *126*, 10958–10961.
[45] T. Li, S. Dong, E. Wang, *Chem. Commun.* **2007**, 4209–4211.
[46] R. Gill, R. Polsky, I. Willner, *Small* **2006**, *2*, 1037–1041.

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