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Biothiols as Chelators for Preparation of N-(aminobutyl)-N-(ethylisoluminol)/Cu^{2+} Complexes Bifunctionalized Gold Nanoparticles and Sensitive Sensing of Pyrophosphate Ion

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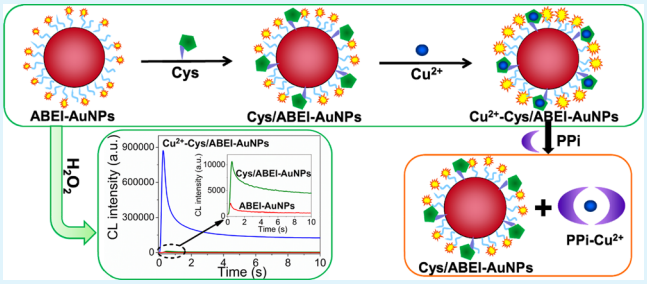
Supporting Information

ABSTRACT: In this work, chemiluminescence (CL) reagent and catalyst metal ion complexes bifunctionalized gold nanoparticles (BF-AuNPs) with high CL efficiency were synthesized via an improved synthesis strategy. Biothiols, such as cysteine (Cys), cysteinyl-glycine (Cys-Gly), homocysteine (Hcy), and glutathione (GSH), instead of 2-[bis[2-[carboxymethyl-[2-oxo-2-(2-sulfanylethylamino)ethyl]amino]-ethyl]amino]acetic acid (DTDTPA), were used as new chelators. N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was used as a model of CL reagents and Cu^{2+} as a model of metal ion. In this strategy, biothiols were first grafted on the surface of ABEI-AuNPs by Au−S bond. Then, Cu^{2+} was captured onto the surface of ABEI-AuNPs by the coordination reaction to form BF-AuNPs. The CL intensity of Cu^{2+}-Cys/ABEI-AuNPs was 1 order of magnitude higher than that of DTDTPA/Cu^{2+}-ABEI-AuNPs synthesized by the previous work. Moreover, strong CL emission of Cu^{2+}-Cys/ABEI-AuNPs was also observed in neutral pH conditions. In addition, the present BF-AuNPs synthesis method exhibited advantages over the previous method in CL efficiency, simplicity, and synthetic rate. Finally, by virtue of Cu^{2+}-Cys/ABEI-AuNPs as a platform, a simple CL chemosensor for the sensitive and selective detection of pyrophosphate ion (PPi) was established based on the competitive coordination interactions of Cu^{2+} between Cys and PPi. The method exhibited a wide detection range from 10 nM to 100 μM, with a low detection limit of 3.6 nM. The chemosensor was successfully applied to the detection of PPi in human plasma samples. It is of great application potential in clinical analysis. This work reveals that BF-AuNPs could be used as ideal nanointerface for the development of novel analytical methods.



KEYWORDS: *chemiluminescence, gold nanoparticles, catalyst, biothiols, pyrophosphate ion*

INTRODUCTION

Gold nanomaterials have attracted considerable interest due to their unique physical properties, excellent stability, good biocompatibility, and ease of functionalization.[1] Novel functional gold nanomaterials with special properties can be synthesized, benefiting from the rational design of the surface chemistry of gold nanoparticles (AuNPs) with various functional molecules.[2−5] Recently, a new concept regarding chemiluminescence (CL) reagent and catalyst metal ion complexes bifunctionalized gold nanoparticles (BF-AuNPs) has been proposed by our group.[6] N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was used as a model of CL reagents. 2-[bis[2-[carboxymethyl-[2-oxo-2-(2-sulfanylethylamino)thyl]-amino]ethyl]amino]acetic acid (DTDTPA) was used as chelator. DTDTPA/metal ion complexes were grafted onto the surface of ABEI functionalized AuNPs (ABEI-AuNPs) by Au−S bond to form BF-AuNPs. However, previous work is at the proof-of-concept stage and functional AuNPs with high CL efficiency and stability are far from fully developed. Particularly, the chelator DTDTPA was synthesized with multistep reactions and purifications, which was tedious and time-consuming. Furthermore, the analytical application of such BF-AuNPs has not been explored.

Biothiols are natural and environmental-friendly thiol compounds occurring in biological systems.[7] Cysteine (Cys), cysteinyl-glycine (Cys-Gly), homocysteine (Hcy), and glutathione (GSH) are common biothiols that bear a thiol group, an amino group, and a carboxyl group simultaneously. The thiol group could interact with AuNPs through the formation of Au−S bond.[8] Meanwhile, the amino and carboxyl groups could act as chelation group to chelate metal ions.[9] They are supposed to be ideal chelators for the synthesis of BF-AuNPs. Therefore, in this work, the synthesis of BF-AuNPs was explored by using biothiols (Cys, Cys-Gly, Hcy, GSH) as new chelators via an improved synthesis strategy. ABEI was used as a model of CL reagents and Cu^{2+} as a model of metal ion. In

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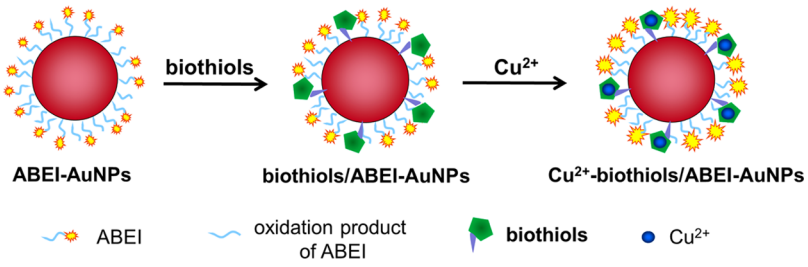
Scheme 1. Schematic Illustration for Synthesis of Cu^{2+}-Biothiol/ABEI-AuNPs



the present strategy, biothiols were first grafted on the surface of ABEI-AuNPs by Au−S bond to form a layer of coordination. Then, Cu^{2+} was captured onto the surface of ABEI-AuNPs by the coordination reaction to form BF-AuNPs. Taking Cu^{2+}-Cys/ABEI-AuNPs as a model of BF-AuNPs, the assembly process, CL performance, and CL mechanism of BF-AuNPs were studied. A comparison of the improved synthesis method with the previous method was made. The effects of pH and chelators on the CL efficiency of BF-AuNPs were also studied. Finally, the analytical application of BF-AuNPs was explored.

Pyrophosphate ion ($P_2O_7^{4-}$, PPi) is an important biological anion that plays a key role in a wide range of critically biological processes including energy transduction and several major metabolic processes.[10−12] For example, PPi concentrations can provide critical information on DNA replication and hence can be used in cancer diagnosis by monitoring telomerase elongation process.[13] The detection of PPi can also help identifying diseases such as chondrocalcinosis or calcium pyrophosphate dehydrates deposition (CPPD) disease.[14,15] Hence, great effort has been made to develop chemosensors for the detection of PPi in the past decade.[16−20] Even though PPi detection methods based on fluorescence changes have been developed, a majority of the studies are limited to organic or mixed aqueous media due to the strong hydration of anions and low solubility of organic fluorescent probes.[21−24] Several CL methods have also been reported for the detection of PPi. However, most of them determine PPi via indirectly analyzing the amount of Pi or ATP released in enzymatic cascade reactions involving PPi, which are multistep coupled enzymatic assays, complex, and time-consuming.[25,26] The development of simple, sensitive, and highly specific chemosensors for the detection of PPi in aqueous medium is of important significance.

By virtue of Cu^{2+}-Cys/ABEI-AuNPs as a platform, a simple CL chemosensor for the sensitive and selective detection of PPi in 100% aqueous medium was established. In the presence of PPi, a decrease in CL intensity of Cu^{2+}-Cys/ABEI-AuNPs was observed due to the higher coordination reactivity between Cu^{2+} and PPi than that between Cu^{2+} and Cys, resulting in catalyst Cu^{2+} being removed from the surface of Cu^{2+}-Cys/ABEI-AuNPs. The PPi concentration is determined directly based on changes in CL intensity. To the best of our knowledge, this is the first example of enzyme-free CL chemosensor focusing on PPi detection through competitive coordination chemistry in 100% aqueous medium.

EXPERIMENTAL SECTION

Chemicals and Materials. $HAuCl_4$ stock solution (0.2% $HAuCl_4$, w/w) was prepared by dissolving 1.0 g of $HAuCl_4{\cdot}4H_2O$ (Shanghai Reagent, China) in 412 mL of purified water and stored at 4 °C. A 4.0 mM stock solution of ABEI (TCI, Japan) was prepared by dissolving ABEI in 0.1 M NaOH solution and was kept at 4 °C. Cys, Cys-Gly, Hcy, GSH, cysteine (Cyss), homocystine (Hcyss), glutathione disulfide (GSSG), arginine (Arg), threonine (Thr), glycine (Gly), lysine (Lys), proline (Pro), phenylalanine (Phe), and valine (Val) were purchased from Shanghai Sangon Biotechnology Co. Let. PPi was purchased from Sinopharm Chemical Reagent Col, Ltd. All other reagents were of analytical grade. Ultrapure water was prepared by a Milli-Q system (Millipore, France) and used throughout. White 96-well plates were obtained from Pierce (U.S.A.).

Synthesis of BF-AuNPs. ABEI-AuNPs were prepared by the reduction of $HAuCl_4$ with ABEI in aqueous solution at room temperature as described previously.[27] Briefly, a 7 mL portion of 6 mM $HAuCl_4$ stock solution was mixed with 45 mL ultrapure water. While stirring vigorously, 5 mL of 4 mM ABEI stock solution was added rapidly, the solution was maintained at room temperature for 2 h. Then, another 6 mL portion of $HAuCl_4$ stock solution was added and the reaction was kept on for another 2 h to form ABEI-AuNPs. 100 μL 6 mM biothiols (Cys, Cys-Gly, Hcy, or GSH) aqueous solution was added to 1 mL of ABEI-AuNPs colloid and incubated at 4 °C for 5 h. Then, the mixture was further added with 100 μL 6 mM Cu^{2+} solution and kept on for 5 min at room temperature. Finally, the resulting solution was centrifuged at a speed of 12 500 rpm for 15 min (Universal 320, Hettich, Germany). After the supernatant was removed, the soft precipitant was dispersed in 1 mL water or other medium for further use.

Synthesis of DTDTPA/Cu^{2+}-ABEI-AuNPs. DTDTPA/Cu^{2+}-ABEI-AuNPs was synthesized as described previously.[6] Briefly, DTDTPA/Cu^{2+} complexes were prepared by mixing 100 μL 6 mM of DTDTPA aqueous solution with 100 μL 6 mM of Cu^{2+} solution and keeping it for 5 min at room temperature. Then, the DTDTPA/Cu^{2+} complex aqueous solution was added to 1 mL ABEI-AuNPs. After stirring overnight, the resulting solution was centrifuged at a speed of 12 500 rpm for 15 min. After the supernatant was removed, the soft precipitant was dispersed in 1 mL water for further use.

CL Measurements. CL measurements were performed with a microplate luminometer (Centro LB 960, Berthold, Germany) equipped with two injectors. For a typical CL measurement, 100 μL functionalized AuNPs colloid was added into a microwell of 96-well plates. When 100 μL of 0.1 M H_2O_2 in 0.1 M NaOH or other buffer was injected into the microwell, CL emission was detected. The measurement time was optimized as 10 s with a time interval of 0.1 s.

The CL spectrum of BF-AuNPs was also measured on a fluorescence spectrophotometer (Shimadzu, RF 5301 PC, Japan) operated in luminescence mode.

Detection of PPi. First, Cu^{2+}-Cys/ABEI-AuNPs were synthesized by using Cys with the concentration of 1 mM and Cu^{2+} with the concentration of 0.5 mM. 1 mL Cu^{2+}-Cys/ABEI-AuNPs colloid was centrifuged and the precipitant was dispersed with 1 mL water for the further use. For the CL detection of PPi, 50 μL of PPi with different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100 μM) was mixed with 50 μL of the as-prepared Cu^{2+}-Cys/ABEI-AuNPs aqueous dispersion within a microwell of 96-well plates and stirred at room temperature for 10 min. Then, the CL kinetic curves were recorded by injecting 100 μL of 0.1 M H_2O_2 in 0.1 M NaOH (pH 13.0) into the microwell. The morphologies of Cu^{2+}-Cys/ABEI-AuNPs before and after the addition of PPi were characterized by transmission electron microscopy (TEM, JEOL Ltd., JEOL-2010, Japan). To investigate the selectivity of the chemosensor, different kinds of anion species were

measured instead of PPi. The practical applicability of the chemosensor was studied by measuring PPi in human plasma samples. Deproteinization of the plasma samples by ultrafiltration (3000 Da cutoff) were carried out prior to the measurements.

RESULTS AND DISCUSSION

Synthesis of BF-AuNPs. An improved strategy for the synthesis of BF-AuNPs is shown in Scheme 1. Biothiols (Cys, Cys-Gly, Hcy, GSH) were chosen as chelators because of their easy availability, environmental-friendly, outstanding chelating ability with Cu^{2+},[9] as well as ease of assembly with AuNPs via the Au−S bond.[8] To start with, ABEI-AuNPs were prepared as described previously.[27] Then biothiols were grafted on the surface of ABEI-AuNPs by Au−S bond to form a layer of coordination. Then, catalyst metal ion Cu^{2+} was further grafted onto the surface of ABEI-AuNPs by the coordination reaction between biothiols and Cu^{2+} to form BF-AuNPs.

Taking Cu^{2+}-Cys/ABEI-AuNPs as a model of BF-AuNPs, the assembly process of BF-AuNPs was studied by monitoring the CL responses at different synthetic stages, as shown in Figure 1.

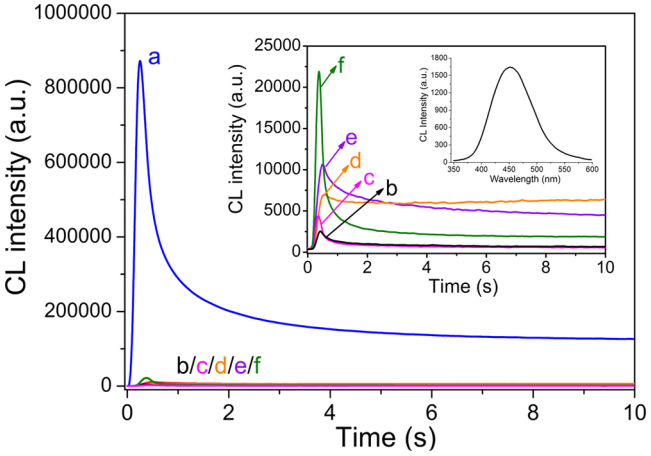


Figure 1. CL kinetic curves for the reaction of (a) Cu^{2+}-Cys/ABEI-AuNPs, (b) ABEI-AuNPs, (c) mixture of ABEI-AuNPs with Cu^{2+}, (d) mixture of ABEI-AuNPs with Cys, (e) Cys/ABEI-AuNPs, (f) Cu^{2+}/ABEI-AuNPs, with H_2O_2. Inset: magnification of curves b−f, CL spectrum of Cu^{2+}-Cys/ABEI-AuNPs-H_2O_2 system. Reaction conditions: 100 μL 0.1 M H_2O_2 in 0.1 M NaOH (pH 13.0) was injected into 100 μL AuNPs dispersion in a microwell.

Cu^{2+}-Cys/ABEI-AuNPs exhibited excellent CL activity (Figure 1a), while ABEI-AuNPs exhibited a rather weak CL activity (Figure 1b). The CL intensity of Cu^{2+}-Cys/ABEI-AuNPs was more than 2 orders of magnitude higher than ABEI-AuNPs (354-fold). The CL intensities of ABEI-AuNPs mixed with Cys and Cu^{2+}, respectively, without incubation, were measured. The CL intensities of the mixture of ABEI-AuNPs with Cu^{2+} (Figure 1c), and Cys (Figure 1d) were enhanced by 2-fold and 3-fold, respectively, compared with ABEI-AuNPs. The CL intensities of ABEI-AuNPs incubated with Cys and Cu^{2+}, respectively, were also measured. Cys could be grafted on the surface of ABEI-AuNPs by Au−S bond with incubation to form Cys/ABEI-AuNPs. It has been reported that ABEI and its oxidized product N-(aminobutyl)-N-(ethyl phthalate) coexisted on surface of ABEI-AuNPs.[27] The structural formula of ABEI and N-(aminobutyl)-N-(ethyl phthalate) are shown in Supporting Information Figure S-1. The O atoms in the carboxyl groups of N-(aminobutyl)-N-(ethyl phthalate) molecule could chelate Cu^{2+}. Thus, some Cu^{2+} may be captured on the surface of ABEI-AuNPs directly with incubation to form Cu^{2+}/ABEI-AuNPs. The CL intensities of Cys/ABEI-AuNPs (Figure 1e) and Cu^{2+}/ABEI-AuNPs (Figure 1f) were enhanced by 5-fold and 8-fold, respectively, compared with ABEI-AuNPs. The CL intensity of Cys/ABEI-AuNPs was still 2-fold higher than that of the mixture of ABEI-AuNPs with Cys, and the CL intensity of Cu^{2+}/ABEI-AuNPs was still 5-fold higher than that of the mixture of ABEI-AuNPs with Cu^{2+}, indicating that catalyst Cu^{2+}, and Cys not immobilized on the surface of ABEI-AuNPs could catalyze the CL reaction to some extent, but their catalytic ability was much weaker than Cu^{2+}, and Cys immobilized on the surface of ABEI-AuNPs. Moreover, the CL intensities of Cu^{2+}/ABEI-AuNPs and Cys/ABEI-AuNPs were much weaker than that of Cu^{2+}-Cys/ABEI-AuNPs. The CL intensity of Cu^{2+}-Cys/ABEI-AuNPs was 43-fold, and 83-fold higher than that of Cu^{2+}/ABEI-AuNPs, and Cys/ABEI-AuNPs, respectively. These results demonstrated that catalyst grafted on the surface of AuNPs have unique catalytic activity on the CL reaction, superior to that in the liquid phase, and BF-AuNPs have been successfully assembled as expected.

The CL spectrum for the reaction of Cu^{2+}-Cys/ABEI-AuNPs with H_2O_2 exhibited a peak centered at around 450 nm as shown in Figure 1, insert, which was consistent with that of the CL reaction of ABEI with H_2O_2. The results revealed that the CL emission resulted from the CL reaction of ABEI on the surface of the AuNPs with H_2O_2.

The excellent CL efficiency of Cu^{2+}-Cys/ABEI-AuNPs might be due to the synergistic catalysis effect of Cys, Cu^{2+}, and AuNPs on the ABEI-H_2O_2 CL system. According to our earlier study about BF-AuNPs,[6] the CL catalysis and enhancement of Cu^{2+}-Cys/ABEI-AuNPs was suggested as follows: Cys, Cu^{2+}, and AuNPs facilitate the formation of $-CO_4^{\bullet 2-}$, $O_2^{\bullet -}$, and $HO^{\bullet}$, which could react with ABEI to facilitate the formation of ABEI radicals. Meanwhile, AuNPs as nanosized platform stimulate electron transfer and promote radical-involved CL reaction, resulting in strong light emission. In addition, a heterogeneous catalysis process was involved in the Cu^{2+}-Cys/ABEI-AuNPs-H_2O_2 CL system. The rate of heterogeneous catalysis increases when the available catalytic active sites are present on the surfaces of particles of smaller size for a given amount of catalyst material.[28,29] Both of Cu^{2+}, and Cys were highly concentrated on the surface of ABEI-AuNPs, the rate of heterogeneous catalysis would increase greatly and further enhance the CL intensity.

Comparative Study of Two BF-AuNPs Synthesis Methods. A comparison of the present BF-AuNPs synthesis method with the previous method was carried out. Cys/Cu^{2+}-ABEI-AuNPs was synthesized according to the previous method by using Cys instead of DTDTPA, and Cu^{2+}-DTDTPA/ABEI-AuNPs was synthesized according to the present method by using DTDTPA instead of Cys. A comparison of CL intensity among Cu^{2+}-Cys/ABEI-AuNPs, Cys/Cu^{2+}-ABEI-AuNPs, Cu^{2+}-DTDTPA/ABEI-AuNPs, and DTDTPA/Cu^{2+}-ABEI-AuNPs was carried out, as shown in Figure 2. The CL intensities of Cu^{2+}-Cys/ABEI-AuNPs (Figure 2a), Cys/Cu^{2+}-ABEI-AuNPs (Figure 2b), and Cu^{2+}-DTDTPA/ABEI-AuNPs (Figure 2c) were 1 order of magnitude, 3-fold, and 2-fold higher than that of DTDTPA/Cu^{2+}-ABEI-AuNPs synthesized by the previous method (Figure 2d), respectively. These results demonstrated that the CL intensity of BF-AuNPs could be enhanced both by using Cys as a new chelator and by using the improved synthesis strategy. On the other hand, in the present strategy, chelators were grafted on the surface of

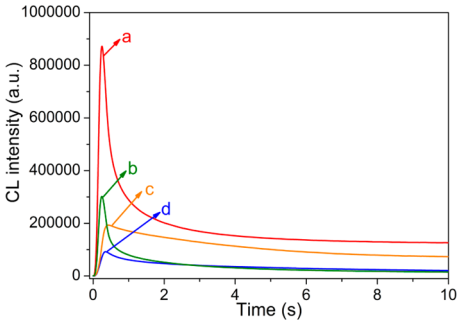


Figure 2. CL kinetic curves for reactions of (a) Cu^{2+}-Cys/ABEI-AuNPs, (b) Cys/Cu^{2+}-ABEI-AuNPs, (c) Cu^{2+}-DTDTPA/ABEI-AuNPs, (d) DTDTPA/Cu^{2+}-ABEI-AuNPs, with H_2O_2. Reaction conditions: 100 μL 0.1 M H_2O_2 in 0.1 M NaOH (pH 13.0) was injected into 100 μL BF-AuNPs dispersion in a microwell.

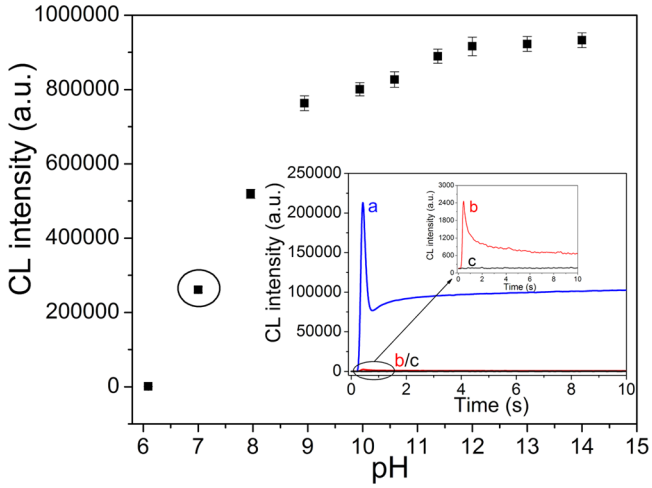


Figure 3. Effect of pH of H_2O_2 on the CL intensity of Cu^{2+}-Cys/ABEI-AuNPs. Reaction conditions: 100 μL 0.1 M H_2O_2 in Britton-Robinson buffer (pH 6.1−11.2), and NaOH solution (pH 12.0−14.0) was injected into 100 μL Cu^{2+}-Cys/ABEI-AuNPs water dispersion in a microwell. Insert: CL kinetic curves for the reactions of (a) Cu^{2+}-Cys/ABEI-AuNPs in neutral pH, (b) ABEI-AuNPs in optimal alkaline pH, (c) 0.1 mM ABEI solution in neutral pH, with H_2O_2.

ABEI-AuNPs to form a layer of coordination prior to the chelation reaction. It was supposed that more Cu^{2+} could be captured due to the concentrated coordination and highly energetic metal−ligand coordinative interactions.[30] Besides, Cys is a kind of readily available commercial reagent. However, DTDTPA was synthesized with multistep of reactions and purifications. Therefore, the synthesis of Cu^{2+}-Cys/ABEI-AuNPs is much simpler than that of DTDTPA/Cu^{2+}-ABEI-AuNPs. In addition, the present synthesis strategy also takes much less time than the previous method. Thus, the improved BF-AuNPs synthesis method exhibits advantages over the previous method in CL efficiency, simplicity and synthetic rate.

Effect of pH. pH has important influence on CL reactions. The effect of pH of dispersion solution on the CL intensity of Cu^{2+}-Cys/ABEI-AuNPs was studied, as shown in Supporting Information Figure S-2. The centrifuged Cu^{2+}-Cys/ABEI-AuNPs was dispersed in water, carbonate buffer, and NaOH solution with different pH values. The results showed that no significant difference in the CL intensity was observed for Cu^{2+}-Cys/ABEI-AuNPs dispersed in water, in carbonate buffer, and in NaOH solution when the pH was lower than 13.0. This may be due to that pH of total CL reaction system was dominated by pH of H_2O_2 (in 0.1 M NaOH, pH 13.0). The color of Cu^{2+}-Cys/ABEI-AuNPs turned blue and the CL intensity decreased when Cu^{2+}-Cys/ABEI-AuNPs were dispersed in 0.1 M NaOH (pH 13.0) and 1 M NaOH (pH 14.0). This may be due to that Cu^{2+}-Cys/ABEI-AuNPs aggregated in high ionic strength medium.

The effect of pH of H_2O_2 on the CL intensity of Cu^{2+}-Cys/ABEI-AuNPs dispersed in water was also examined, as shown in Figure 3. 0.1 M H_2O_2 with different pH values were obtained by diluting H_2O_2 with Britton-Robinson buffer (pH 6.1−11.2), and NaOH solution (pH 12.0−14.0). The CL intensity increased rapidly with the increase of pH from 6.1 to 9.0, continued to increase slowly with the increase of pH from 9.0 to 12.0, and trended to level off when the pH was higher than 12.0. High pH could promote the dissociation of H_2O_2 to form more $HO^{\bullet}$ radicals, which dissociate into superoxide radical $O_2^{\bullet-}$ at high pH, leading to an increase in CL intensity.[31] In particular, a relatively strong CL emission was observed at pH of 7.0, which was even 2 orders of magnitude stronger than the CL emission of ABEI-AuNPs in optimal alkaline pH. No CL emission was observed with ABEI solution (0.1 mM) at pH of 7.0. CL emission of luminol type compounds in neutral pH condition has rarely been reported. Strong CL emission of Cu^{2+}-Cys/ABEI-AuNPs might be due to the synergistic catalysis effect of Cys, Cu^{2+}, and AuNPs on the ABEI-H_2O_2 CL system.

Effect of Chelators. BF-AuNPs were synthesized by using different biothiol chelators such as Cys, Cys-Gly, Hcy, and GSH. The effect of chelators on CL efficiency of BF-AuNPs was investigated. The CL intensities of the mixture of ABEI-AuNPs with Cys, Cys-Gly, Hcy, and GSH without incubation, ABEI-AuNPs incubated with Cys, Cys-Gly, Hcy, and GSH, and BF-AuNPs synthesized by using Cys, Cys-Gly, Hcy, and GSH as chelators, respectively, were measured. Cyss, Hcyss, and GSSG, which do not bear thiol group, are the corresponding disulfides of Cys, Hcy, and GSH. As a control experiment, the CL intensities of the mixture of ABEI-AuNPs with Cyss, Hcyss, and GSSG without incubation, ABEI-AuNPs incubated with Cyss, Hcyss, and GSSG, and BF-AuNPs assembled by using Cyss, Hcyss, and GSSG as chelators, respectively, were also measured. The CL kinetic curves of the mixture of ABEI-AuNPs with Cys, Hcy, GSH, Cyss, Hcyss, and GSSG, respectively, are shown in Figure 4A. The results showed that the CL intensities of the mixture of ABEI-AuNPs with Cys, Cys-Gly, Hcy, GSH, and GSSG could be enhanced by 3-fold, 2-fold, 7-fold, 19-fold, and 15-fold, respectively, compared with ABEI-AuNPs. Small inhibition effects were observed with Cyss and Hcyss. The CL intensity of ABEI-AuNPs was 3-fold and 2-fold higher than that of the mixture of ABEI-AuNPs with Cyss and Hcyss, respectively. The CL kinetic curves of ABEI-AuNPs incubated with Cys, Cys-Gly, Hcy, GSH, Cyss, Hcyss, and GSSG, respectively, are shown in Figure 4B. Centrifugation and dispersion procedures were carried out after incubation to remove free molecules before the CL measurements. The CL intensities of ABEI-AuNPs incubated with Cys, Cys-Gly, Hcy, and GSH, respectively, was enhanced by 5-fold, 3-fold, 5-fold, and 9-fold, respectively, compared with ABEI-AuNPs. In contrast, the CL intensities of ABEI-AuNPs incubated with Cyss, Hcyss, and GSSG, respectively, were similar to that of ABEI-AuNPs. Cys, Cys-Gly, Hcy, and GSH could be grafted on the surface of ABEI-AuNPs by Au−S bond. ABEI and N-(aminobutyl)-N-(ethyl phthalate) molecules were grafted on

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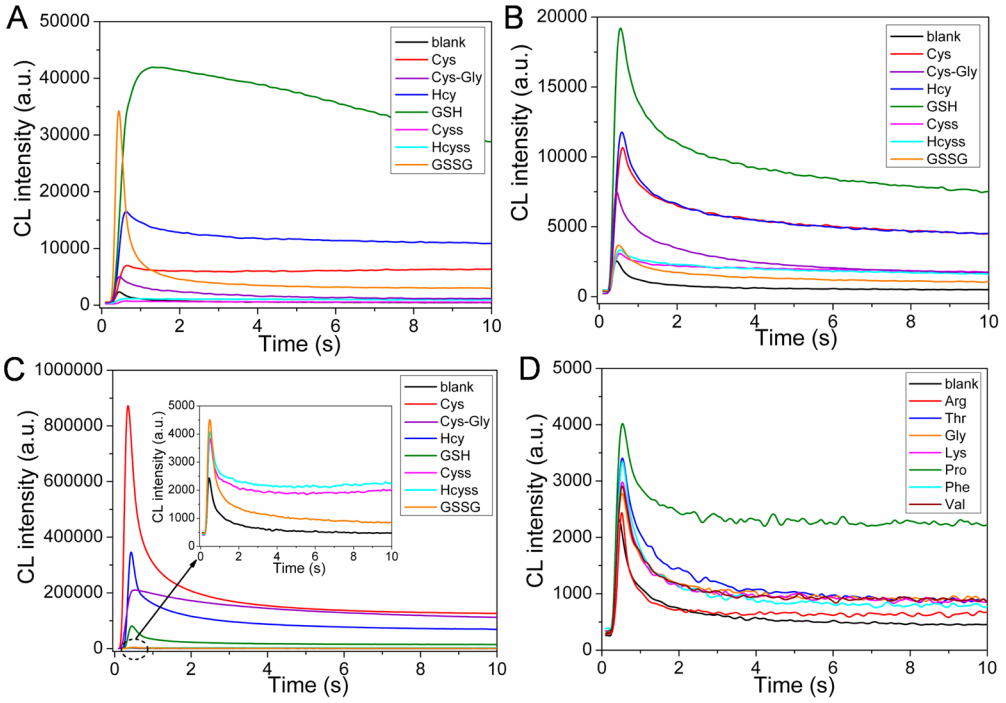


Figure 4. CL kinetic curves for reaction of (A) mixture of ABEI-AuNPs with Cys, Cys-Gly, Hcy, GSH, Cyss, Hcyss, and GSSG, respectively, (B) ABEI-AuNPs incubated with Cys, Cys-Gly, Hcy, GSH, Cyss, Hcyss, and GSSG, respectively, (C) BF-AuNPs synthesized by using Cys, Cys-Gly, Hcy, GSH, Cyss, Hcyss, and GSSG, respectively, (D) BF-AuNPs synthesized by using Arg, Thr, Gly, Lys, Pro, Phe, and Val, respectively, with H_2O_2. All of the blank is the CL kinetic curve for reaction of ABEI-AuNPs with H_2O_2. Reaction conditions: 100 μL 0.1 M H_2O_2 in 0.1 M NaOH (pH 13.0) was injected into 100 μL AuNPs dispersion in a microwell.

the surface of AuNPs through the weak covalent interaction between gold and N atoms in their amino groups.[27] Cyss, Hcyss, and GSSG bearing an amino group may also be grafted on the surface of ABEI-AuNPs by Au−N covalent interaction. However, the results indicated that chelators bearing thiol group could compete the AuNPs binding site with ABEI and N-(aminobutyl)-N-(ethyl phthalate) molecules through the formation of stronger Au−S bond, while the disulfides bearing amino group could not connect to the surface of ABEI-AuNPs owing to the steric hindrance effect. The CL kinetic curves of BF-AuNPs by using Cys, Cys-Gly, Hcy, GSH, Cyss, Hcyss, GSSG, respectively, as chelators are shown in Figure 4C. The CL intensities of BF-AuNPs synthesized by using Cys, Cys-Gly, Hcy, and GSH as chelators were enhanced by 354-fold, 94-fold, 146-fold, and 34-fold, respectively, compared with ABEI-AuNPs. In contrast, the CL intensities of BF-AuNPs assembled by using Cyss, Hcyss, and GSSG as chelators were similar to that of ABEI-AuNPs. Furthermore, the CL kinetic curves of BF-AuNPs assembled with several amino acids (Arg, Thr, Gly, Lys, Pro, Phe, Val), which do not bear thiol group, were also measured (Figure 4D). The CL intensities of BF-AuNPs assembled by using amino acids above as chelators were also similar to that of ABEI-AuNPs. The results demonstrated that BF-AuNPs could not be successfully synthesized by using either disulfides or amino acids which do not bear thiol group. Thus, chelators bearing thiol group and chelation group simultaneously were critical factors for the successful synthesis of BF-AuNPs. From Figure 4, we can see that the CL intensities of BF-AuNPs synthesized by using Cys, Hcy, and GSH, as chelators follow the order: Cu^{2+}-Cys/ABEI-AuNPs > Cu^{2+}-Hcy/ABEI-AuNPs > Cu^{2+}-Cys-Gly/ABEI-AuNPs > Cu^{2+}-GSH/ABEI-AuNPs. In contrast, when only these chelators were coated on the surface of ABEI-AuNPs without Cu^{2+}, the order of the CL intensity was reverse: Cys-Gly/ABEI-AuNPs < Cys/ABEI-AuNPs < Hcy/ABEI-AuNPs < GSH/ABEI-AuNPs. The reason for a decrease in CL efficiency of BF-AuNPs may be due to the increased steric hindrance effect relate to more complex molecular structures from Cys to Hcy to Cys-Gly to GSH on the surface of ABEI-AuNPs, leading to binding of less chelators and Cu^{2+}. Cu^{2+}-Cys/ABEI-AuNPs was used in the following experiments on account of its excellent CL performance.

Effect of Metal Ions. It has been reported that various metal ions such as Cu^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, Cr^{3+}, and Ni^{2+} could catalyze the CL reactions of luminol and its analogues with H_2O_2.[32] In this work, Cu^{2+} was used as a model of metal ion to synthesize BF-AuNPs. The effect of other metal ions on the CL efficiency of BF-AuNPs was also investigated. A series of BF-AuNPs were synthesized by using several catalyst metal ions, including Cu^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, Cr^{3+}, and Ni^{2+}. As shown in Figure 5, BF-GNPs synthesized by using Cu^{2+}, Co^{2+}, and Hg^{2+} exhibited excellent CL activity, showing over 2 orders of magnitude higher CL intensity than ABEI-AuNPs. The CL intensity follows the order: Cu^{2+} > Co^{2+} > Hg^{2+} > Pb^{2+} > Cr^{3+} > Ni^{2+}. The CL intensity order with Cys as chelator was different from our previous study with DTDTPA as chelator, which may be due to a difference in chelator property. In this work, BF-AuNPs synthesized with Cu^{2+} exhibited much stronger CL intensity than the others. This may be due to that more catalyst metal ion could be captured onto the surface of Cys/ABEI-AuNPs by using Cu^{2+} as catalyst metal ion, because the stability constant of the complex formed by Cu^{2+} and Cys is higher than that of the other metal ions.[33]

Detection of PPi. PPi is also an effective chelator. It has been reported that the stability constant (K) of the complex formed by Cu^{2+} and Cys is log $K_{Cu\text{-}Cys}$ = 7.88,[33] and the stability

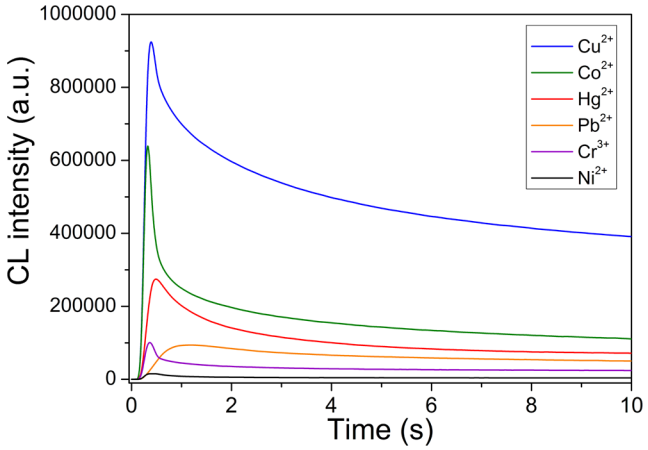
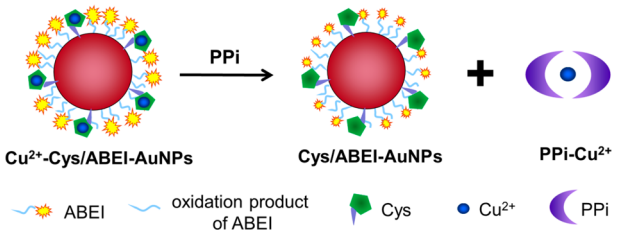


Figure 5. CL kinetic curves for reaction of various BF-AuNPs with H_2O_2. Reaction conditions: 100 μL 0.1 M H_2O_2 in 0.1 M NaOH (pH 13.0) was injected into 100 μL BF-AuNPs dispersion in a microwell.

constant for the complex formed by Cu^{2+} and PPi is log $K_{Cu\text{-}PPi}$ = 12.45.[34] Therefore, Cu^{2+} may be detached from the surface of Cu^{2+}-Cys/ABEI-AuNPs in the presence of PPi due to stronger binding between Cu^{2+} and PPi than between Cu^{2+} and Cys. Indeed, a decrease in CL intensity of Cu^{2+}-Cys/ABEI-AuNPs was observed in the presence of PPi, indicating that Cu^{2+} was removed from the surface of Cu^{2+}-Cys/ABEI-AuNPs. Accordingly, by virtue of Cu^{2+}-Cys/ABEI-AuNPs as a platform, a simple CL chemosensor for the sensitive and selective detection of PPi was developed based on the competitive coordination reactions of Cu^{2+} between PPi and Cys, as illustrated in Scheme 2.

Scheme 2. Schematic Illustration of Proposed CL Chemosensor for the Detection of PPi by Using Cu^{2+}-Cys/ABEI-AuNPs as Sensing Platform



The morphologies of Cu^{2+}-Cys/ABEI-AuNPs before and after the addition of PPi were characterized by TEM (Figure 6). Since monomolecular layer modification normally is undetectable by TEM, the results reflect the size of the gold core. It can be seen that Cu^{2+}-Cys/ABEI-AuNPs in absence and presence of PPi are all monodispersed sphericity with a diameter around 20 nm, demonstrating the good stability of Cu^{2+}-Cys/ABEI-AuNPs as platform for the construction of the PPi chemosensor.

In order to get better analytical performance, the concentrations of Cu^{2+} and Cys used in the fabrication of the chemosensor were optimized. It was found that the color of Cu^{2+}-Cys/ABEI-AuNPs turned purple with the increase of the concentrations of Cu^{2+} and Cys, as well as the increased concentration ratios of Cu^{2+} to Cys, which might result from the Cu^{2+}-triggered aggregation of AuNPs. Best analytical performance with the widest detection range and lowest detection limit for PPi sensing was obtained by using Cu^{2+}-Cys/ABEI-AuNPs synthesized with 1 mM Cys and 0.5 mM Cu^{2+}, and by using H_2O_2 in 0.1 M NaOH (pH 13.0).

The calibration curve for the determination of PPi is shown in Figure 7. The results indicated that the CL intensity decreased with the increasing of PPi concentration and exhibited a linear response toward the logarithm of the concentration of PPi from 0.01 μM to 100 μM. The regression equation was $\Delta I = 11818 - 2446 \times \log C$ (unit of C is μM) with a correlation coefficient of 0.997. ΔI was the relative CL intensity calculated by $I_0 - I$, where I_0 and I were the CL intensity of Cu^{2+}-Cys/ABEI-AuNPs in the absence and presence of PPi. The detection limit (LOD) at a signal-to-noise ratio of 3 ($S/N = 3$) for PPi was 3.6 nM, which was lower than most of the previously reported PPi assays (Table S-1 in Supporting Information). The RSD of seven replicate detections of 10 μM PPi within a day (intraday precision, n = 7) was 3.6%, and the RSD in different days (interday precision, n = 7) was 7.5%, indicating good repeatability of the proposed chemosensor. The results indicate that the proposed chemosensor was suitable for PPi detection.

To investigate the selectivity of the chemosensor, various species, including ATP, ADP, AMP, and other anions (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, HCO_3^-, CO_3^{2-}, NO_3^-, SO_4^{2-}, Cl^-) that possibly interfere with the PPi sensing, were tested instead of PPi (Figure 8). It can be seen that the highest ΔI was observed only for the detection of PPi, confirming that the chemosensor

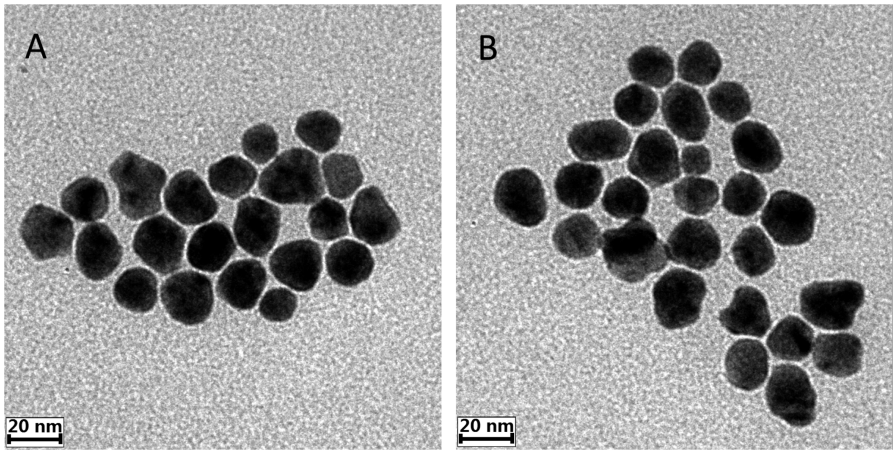


Figure 6. TEM images of Cu^{2+}-Cys/ABEI-AuNPs in the absence (a) and presence (b) of 10 μM PPi.

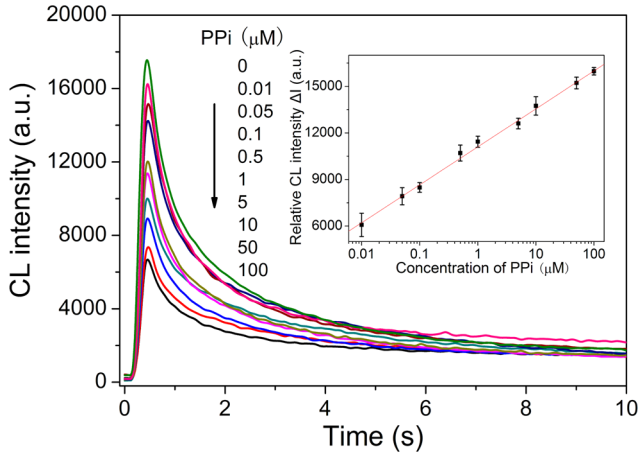


Figure 7. CL responses of proposed chemosensor with various concentrations of PPi. Inset: calibration curve for PPi. The relative CL intensity ΔI is calculated by $I_0 - I$, where I_0 and I are the CL intensity of Cu^{2+}-Cys/ABEI-AuNPs in the absence and presence of PPi. Reaction conditions: 100 μL 0.1 M H_2O_2 in 0.1 M NaOH (pH 13.0) was injected into 50 μL Cu^{2+}-Cys/ABEI-AuNPs water dispersion mixed with 50 μL PPi of various concentrations.

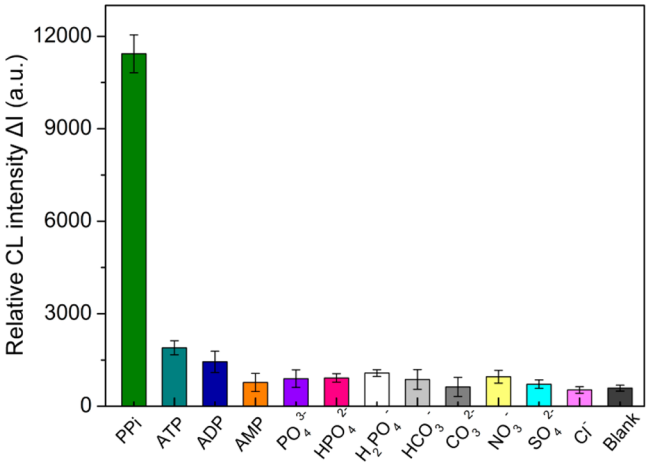


Figure 8. CL intensity changes of the proposed chemosensor to PPi and those of different interfering anions (1 μM). Reaction conditions: 100 μL 0.1 M H_2O_2 in 0.1 M NaOH (pH 13.0) was injected into 50 μL Cu^{2+}-Cys/ABEI-AuNPs water dispersion mixed with 50 μL various anions.

can distinguish PPi from other investigated species, including phosphate anions. Histidine may remove Cu^{2+} attached to the surface of Cu^{2+}-Cys/ABEI-AuNPs.[36] Thus, the interference of histidine for determining PPi was also studied. The tolerable limit was taken as a relative error $\leq \pm 10\%$. The tolerable concentration ratio with respect to 10 μM PPi was higher than 200 for histidine. When the concentration of histidine was 500-fold higher than PPi, histidine will interfere with the determination of PPi.

The potential applicability of the chemosensor for the detection of PPi in practical samples was also investigated by determining PPi in human plasma samples. Deproteinization of the plasma samples with ultrafiltration were carried out to eliminate the possible effect of macromolecular proteins on the CL responses. PPi in human plasma samples was determined, as shown in Table 1. The concentration of PPi in tested normal human plasma samples was in the range 1.4–4.7 μM, which is

Table 1. Determination of PPi Concentration in Human Plasma Samples

plasma samples	PPi detected (mean ± σ, n = 3) (μM)	PPi added (μM)	total PPi detected (mean ± σ, n = 3) (μM)	recovery (%)
1	2.3 ± 0.1	10	12.4 ± 0.3	101
2	4.7 ± 0.2	10	14.5 ± 0.4	98
3	1.4 ± 0.06	10	11.9 ± 0.3	105

in good agreement with the data obtained in the literature (1.19–5.65 μM of PPi in plasma).[35] The results show that the chemosensor is reliable. The recovery of PPi ranged from 98 to 105%, demonstrating that the CL chemosensor is applicable to monitor PPi in the practical human plasma samples.

CONCLUSION

In conclusion, BF-AuNPs have been successfully synthesized through an improved synthesis strategy by taking ABEI as a model of CL reagents, Cu^{2+} as a model of metal ion and biothiols (Cys, Cys-Gly, Hcy, GSH) as new chelators. The results showed that chelators bearing thiol group and chelation group simultaneously were critical factors for the successful synthesis of BF-AuNPs, and BF-AuNPs synthesized by using Cys as chelators exhibited much higher CL intensity than Hcy and GSH. The CL intensity of Cu^{2+}-Cys/ABEI-AuNPs was also 1 order of magnitude higher than that of DTDTPA/Cu^{2+}-ABEI-AuNPs synthesized by the previous method. Moreover, it was observed that CL emission of Cu^{2+}-Cys/ABEI-AuNPs in neutral pH condition was even 2 orders of magnitude stronger than that of ABEI-AuNPs in optimal alkaline pH. This CL emission in neutral pH conditions is well compatible with biological systems and may find future applications in biological systems. In addition, the present BF-AuNPs synthesis method exhibited advantages over the previous method in CL efficiency, simplicity, and synthetic rate. Finally, by virtue of Cu^{2+}-Cys/ABEI-AuNPs as platform, a simple CL chemosensor for the sensitive and selective detection of PPi was established based on the competitive coordination interactions of Cu^{2+} between Cys and PPi. The presented chemosensor exhibited a wide dynamic range from 10 nM to 0.1 mM, with a low detection limit of 3.6 nM. The chemosensor was also successfully applied for the detection of PPi in human plasma samples. This work reveals that BF-AuNPs could be used as ideal nanointerface for the development of novel analytical methods. BF-AuNPs may find more applications in bioassays, sensors, and biological imaging.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text, including structural formulas of ABEI and N-(aminobutyl)-N-(ethyl phthalate), effect of pH of dispersion solution on CL intensity of Cu^{2+}-Cys/ABEI-AuNPs, and a comparison of different chemosensors for the detection of PPi. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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