Communication

Magnetic Fe₃O₄-Based Sandwich-Type Biosensor Using Modified Gold Nanoparticles as Colorimetric Probes for the Detection of Dopamine

Zhiyong Wang *, Yanyan Bai, Wenchao Wei, Ning Xia * and Yuhui Du

College of Chemistry and Chemical Engineering, Anyang Normal University, Anyang 455000, Henan, China; E-Mails: ybhxx1@163.com (Y.B.); wwchxx@sina.com (W.W.); yhduhxx@163.com (Y.D.)

* Authors to whom correspondence should be addressed; E-Mails: zywanghxx@163.com (Z.W.); xianing82414@csu.edu.cn (N.X.); Tel.: +86-732-290-0040 (N.X.).

Abstract: In this work, we designed a visual biosensor for dopamine (DA) detection using magnetic Fe₃O₄ particles and dithiobis(sulfosuccinimidylpropionate)-modified gold nanoparticles (DTSSP-AuNPs) as the recognition elements. Specifically, DA molecules were assembled onto the surface of DTSSP-AuNPs via the amine coupling reaction between the amino group of DA and activated carboxyl group of DTSSP. Accordingly, DA-anchored DTSSP-AuNPs were captured by Fe₃O₄ through the interaction of catechol and iron. In a magnetic field, the formed Fe₃O₄-DA-DTSSP-AuNPs conjugates were easily removed from the solution, leading to fading of the AuNPs suspension and decrease of the UV/Vis signal. As a result, a detection limit of 10 nM for DA was achieved. The theoretical simplicity and high selectivity demonstrated that the sandwich-type strategy based on Fe₃O₄ and AuNPs would lead to many colorimetric detection applications in clinical study by rationally designing the surface chemistry of AuNPs and Fe₃O₄.

Keywords: gold nanoparticles; magnetic particle; biosensor; dopamine; colorimetric assay

1. Introduction

Gold nanoparticles (AuNPs) based colorimetric assays have been widely applied in a variety of research fields due to the high extinction coefficient (3–5 orders of magnitude higher than those of organic dye molecules [1]) and the unique size-dependent optical property of AuNPs [2–7]. Such

methods are very promising in that they require very simple sample handling procedures and minimum instrumental investment and can be conducted in the field with portable devices. The rational design of the surface chemistry of AuNPs can promote specific interactions between the receptors and analytes and, as a result, renders the measurements highly selective [8]. Magnetic particles (MPs) are also one of the most popular materials that have been widely exploited in medical diagnostics, controlled drug release and separation technologies [9,10]. Even before the term "nanotechnology" was popular, iron oxide (Fe_3O_4) nanoparticles were used to magnetically isolate and purify proteins, DNA, viruses and even whole mammalian cells [11]. Recently, bifunctional Au-Fe_3O_4 nanoparticles, recognized as a major advancement in nanobiotechnology generating, from the two components, excellent surface chemistry, special optical properties, and superparamagnetic properties, have been synthesized by various strategies [12–18], and many applications in a wide variety of fields have been found, such as DNA hybridization assay and protein detection [19–23].

Dopamine (DA) is a monoamine neurotransmitter distributed in the central neural system brain tissues and body fluids of mammals. It plays pivotal roles in the function of the central nervous, renal, hormonal and cardiovascular system. Detection of DA is important in diagnoses, monitoring, prevention and treatment of some certain diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, epilepsy, pheochromocytoma and neuroblastoma [24]. Herein, we reported a sandwich-type strategy for DA detection using magnetic Fe_3O_4 particles and dithiobis(sulfosuccinimidylpropionate) (DTSSP)-modified AuNPs (DTSSP-AuNPs). This method is based on the following two facts: (1) DA can form stable, robust anchor onto the surface of iron oxide by the interaction of catechol and iron, facilitating the conjugation of biomolecules onto the Fe_3O_4 surface via the amine coupling reaction between DA and biomolecules [25–30]; and (2) the amine-reactive succinimidyl residues on the surface of AuNPs can react selectively with the primary amine group of DA [4,31]. As a result, decrease in the UV/Vis absorbance of DTSSP-AuNPs suspension was observed in the external magnetic field when DA and Fe_3O_4 were successively added to the DTSSP-AuNPs suspension. The method is sensitive and selective to DA detection without the requirement of expensive and complicated instruments. To the best of our knowledge, this is the first magnetic Fe_3O_4-based biosensor with AuNPs as colorimetric probes.

2. Experimental Section

2.1. Reagents and Materials

Dithiobis(sulfosuccinimidylpropionate) (DTSSP) was purchased from Chemsky International Co., Ltd. (Shanghai, China), dihydroxyphenylacetic acid (DOPAC), glutamic acid, tyrosine and trisodium citrate were obtained from Sigma-Aldrich (Shanghai, China). Norepinephrine and dopamine hydrochloride were obtained from Aladdin reagent Inc. (Shanghai, China). All of other reagents were analytical-grade reagents and were used without further purification. DA and norepinephrine were prepared with HCl solution (pH 2) and diluted to the desired concentration with phosphate buffer solution (PBS, 2 mM, pH 7.2) containing 0.1 mM Na_2SO_3. The other small-molecule organic chemicals were dissolved in deionized water. Unless otherwise noted, the reactions were conducted at room temperature.

2.2. Synthesis of DTSSP-AuNPs

The citrate-stabilized AuNPs were prepared using a trisodium citrate reduction method. The DTSSP-modified AuNPs were prepared by ligand-exchange reaction between DTSSP and citrate-stabilized AuNPs, as in previous reports [4,31,32]. Briefly, trisodium citrate (5 mL, 38.8 mM) was rapidly added to a boiling solution of $HAuCl_4$ (50 mL, 1 mM), and the solution was boiled continually for an additional 30 min to yield a wine-red solution. Ligand-exchange reaction was performed at room temperature by mixing 10 mL of the diluted AuNPs suspension with 10 μL of DTSSP solution under stirring for 30 min. After filtering the solution through a 0.45-μm membrane filter to remove the precipitate, the filtrate was used for DA assay. The particle concentration of the AuNPs solution was calculated by using a molar absorptivity of 2.7×10^8 M^{-1} cm^{-1} at 520 nm [33]. The optimal molar ratio of DTSSP to AuNPs was presented in the Result section. The morphology of DTSSP-AuNPs was characterized by an FEI Tecnai G2 T20 transmission electron microscope (TEM).

2.3. Synthesis of Magnetic Fe_3O_4 Particles

Magnetic Fe_3O_4 particles were prepared via the co-precipitation [34]. Briefly, $FeCl_3 \cdot 6H_2O$ (5.40 g) and $FeSO_4 \cdot 7H_2O$ (2.78 g) were dissolved under a N_2 atmosphere in 100 mL of deoxygenated water, which had been bubbled for 15 min with nitrogen gas. After the Fe^{3+}/Fe^{2+} mixed solution was stirred in a water bath at 80 ℃ for 30 min, aqueous ammonia (28%) was dropped into the solution at 80 ℃ with stirring until pH 9 was reached. The mixture was then stirred continuously for 30 min in the water bath at 80 ℃. With the completion of the reaction, an external magnet was used to separate the Fe_3O_4 from the sample solution. The isolated Fe_3O_4 MPs were washed repeatedly with hot water to remove untreated impurities and then dried in an oven at 90 ℃ for 30 min.

2.4. Detection of DA

Three hundred and thirty microliters of dispersions of the DTSSP-AuNPs were first diluted with 660 μL PBS. Then, DA (10 μL) was added to the diluted solution to react with DTSSP-AuNPs for 30 min. This step is followed by the addition of 1 mg of magnetic Fe_3O_4 particles. Color change was observed with the naked eyes and the absorption spectra were recorded with a Cary 50 UV/Vis spectrometer. The photographs were taken with a Sony Cyber-shot digital camera. Reaction and detection were conducted at room temperature.

3. Results and Discussion

3.1. Mechanism of DA Detection

In view of the magnetic nature of Fe_3O_4, Fe_3O_4-based biosensors have been developed to detect DNA and protein with carbon nanotubes and the quantum dot as the recognition elements [34,35]. The principle of our strategy with colorimetric AuNPs as the recognition elements is shown in Figure 1. The Fe_3O_4 MPs and DTSSP-AuNPs can form a sandwich structure of Fe_3O_4-DA-DTSSP-AuNPs. DA molecules were first assembled onto the surface of DTSSP-AuNPs via the standard amine coupling reaction between the amino group of DA and activated carboxyl group of DTSSP. Accordingly,

DA-anchored DTSSP-AuNPs can be separated by Fe_3O_4 MPs in the external magnetic field through the interaction of catechol and iron [25–30], leading to fading in the color of the DTSSP-AuNPs suspension and a decrease in the UV/Vis absorbance. The method based on this concept should be very sensitive because Fe_3O_4 MPs have excellent separation ability and AuNPs have high extinction coefficient.

Figure 1. Schematic illustration of the strategy for dopamine (DA) detection using dithiobis(sulfosuccinimidylpropionate)-modified gold nanoparticles (DTSSP-AuNPs) and Fe_3O_4 magnetic particles (MPs) (**A**) and the interaction between DA, Fe_3O_4 and DTSSP-AuNPs (**B**).
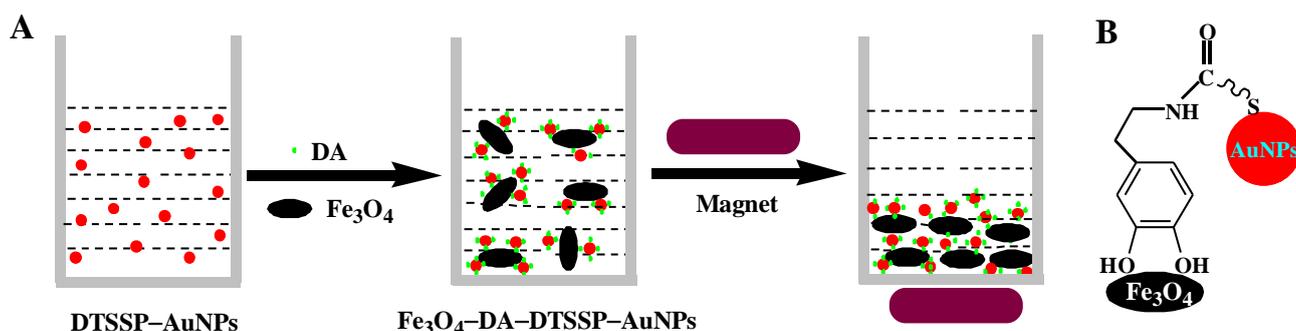
3.2. Colorimetric Assay for DA

The synthesized DTSSP-AuNPs were first characterized by UV/Vis spectroscopy and TEM. The average size of the DTSSP-AuNPs was determined to be 12.8 nm from the TEM image (Figure 2A). The red DTSSP-AuNPs suspension exhibited an absorption peak at 520 nm (A_{520}), which was ascribed to its surface plasmon resonance (vial 1 and black curve in Figure 2B). No obvious change was observed upon the addition of Fe_3O_4 (vial 2 and black curve), indicating that AuNPs did not absorb onto the surface of Fe_3O_4 MPs. However, when DA and Fe_3O_4 were added successively to the solution of DTSSP-AuNPs in the external magnetic field, the red color disappeared (vial 3). Note that DA did not cause the aggregation and color change of DTSSP-AuNPs in the absence of Fe_3O_4. Meanwhile, the DA-induced conjugate of DTSSP-AuNPs and Fe_3O_4 was also monitored by UV/Vis spectroscopy. As shown in the blue curve, the original absorbance of AuNPs at 520 nm almost dropped to the background level with the addition of DA and Fe_3O_4.

We also optimized the molar ratio of DTSSP to AuNPs by measuring the absorbance change of DTSSP-AuNPs in the presence of DA and Fe_3O_4. ΔA_{520} ($\Delta A_{520} = A_{520}' - A_{520}^0$, where A_{520}' represents the absorbance of AuNPs with modification of different amounts of DTSSP in the presence of DA and Fe_3O_4, and A_{520}^0 represents that in the absence of DA) was used here to evaluate the performances of the sensor. As shown in Figure 3A, ΔA_{520} reaches maximum at the DTSSP concentration of around 2.8 μM. This is understandable since free DTSSP in the solution could compete with DTSSP-AuNPs to react with DA, leading to the decrease in the amount of DA molecules anchored onto AuNPs surface. From the result, DTSSP at the concentration of 2.8 μM could be completely absorbed by 3.6 nM AuNPs. Thus, the average number of DTSSP molecules per gold nanoparticle was calculated to be around 778. Moreover, solution pH affects not only the stability of AuNPs and DTSSP, but also the reactivity of DA to DTSSP. The effect of pH on the ΔA_{520} was also examined over a range from

5.5 to 8.0. As shown in Figure 3B, ΔA_{520} reaches a maximum at pH around 7.2. Thus, we chose pH 7.2 PBS solution as the reaction medium. The signal decreases remarkably at low and high pH, probably due to the poor reactivity of amine to DTSSP and the ionization of DTSSP molecules [36].

Figure 2. (**A**) Transmission electron microscope (TEM) of DTSSP-AuNPs and (**B**) UV/Vis absorption spectra of DTSSP-AuNPs with and without addition of DA (4 μM) and Fe_3O_4 MPs. The inset in panel B shows the visual color change.
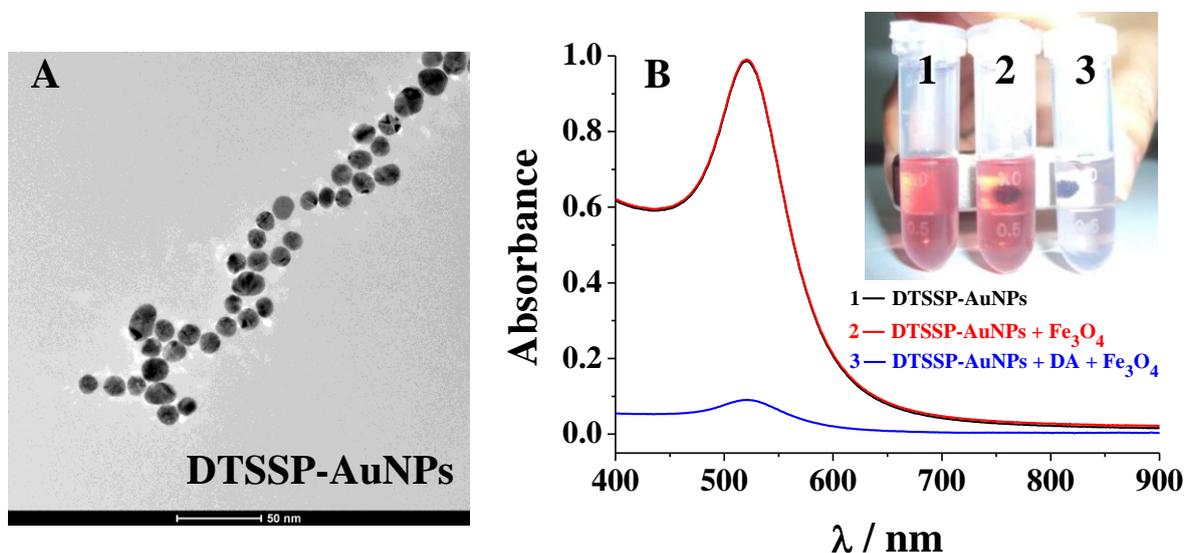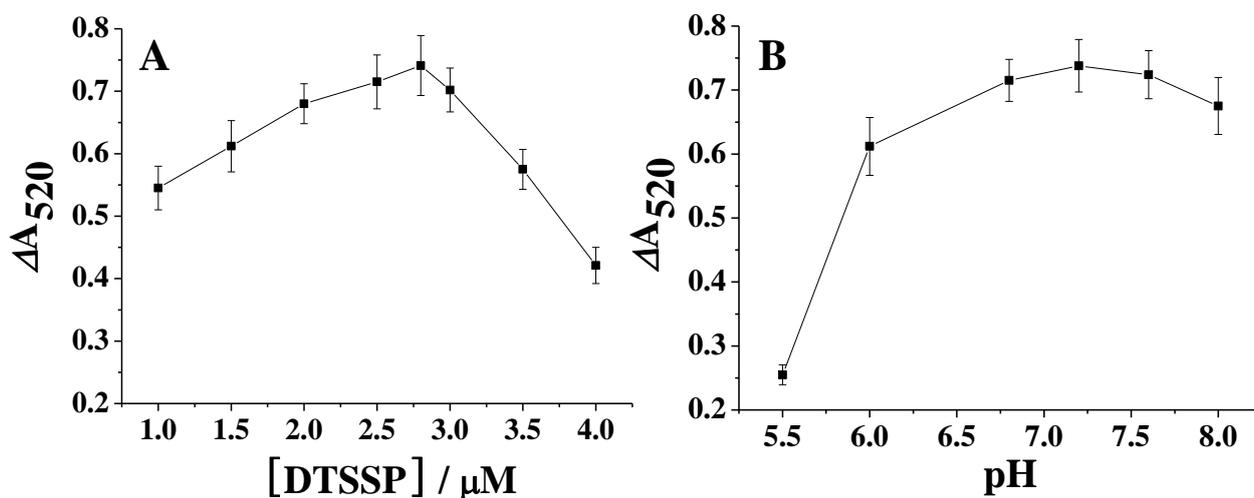

Figure 3. (**A**) Signal intensity variation of AuNPs functionalized by different amounts of DTSSP toward the addition of DA (2 μM) and Fe_3O_4 MPs; (**B**) Effect of pH on the ΔA_{520}.


3.3. Sensitivity

To demonstrate the performance of the sensor for naked eye detection of DA by the mechanism mentioned above, different amounts of DA were added to the DTSSP-AuNPs solution. The results were presented in Figure 4A. Upon the addition of increasing concentrations of DA, the red color of DTSSP-AuNPs faded gradually. These results were also confirmed by the UV/Vis spectroscopy. As shown in Figure 4B, with the addition of increasing concentrations of DA to the solution of

DTSSP-AuNPs, obvious decrease in the absorption peak at 520 nm was observed. A linear relationship was found between A_{520} and DA concentration over the range of 0.02–0.80 μM, which can be expressed using $A_{520} = 0.87 - 0.53$ [DA] (μM) ($R^2 = 0.99$). The detection limit for DA was estimated to be 10 nM.

Figure 4. (**A**) Color change with increasing DA concentrations; (**B**) Absorbance response for different concentrations of DA; (**C**) The corresponding plot of A_{520} *vs.* DA concentration from 0, 0.02, 0.04, 0.08, 0.16, 0.24, 0.40, 0.80, 2.00, 3.00, 4.00 to 5.00 μM.
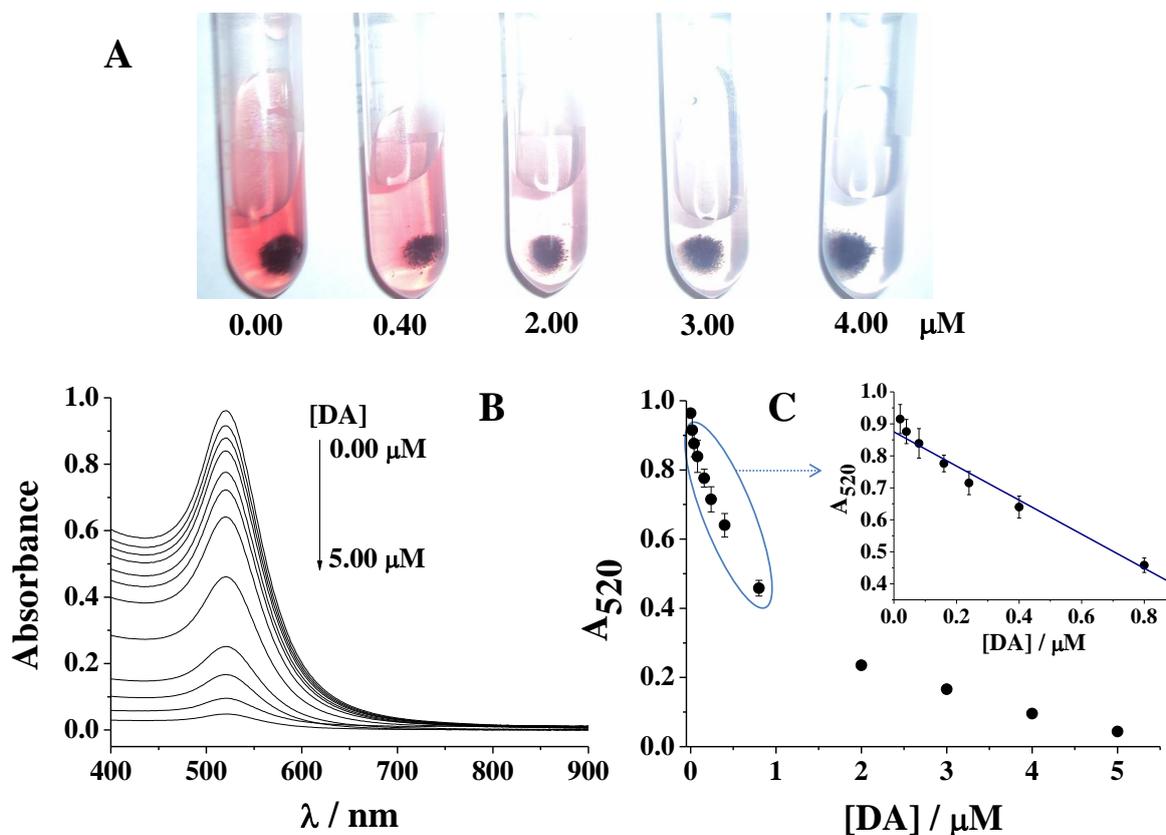

3.4. Selectivity

The selectivity of the present approach was evaluated by testing other members of the catechol family and amino acids that may coexist in the body fluids of mammals, such as DOPAC and norepinephrine, glutamic acid and tyrosine. Interestingly, we found that no obvious intensity changes were observed when all of the above four compounds were added to the dispersion of AuNPs (Figure 5). For glutamic acid and tyrosine, this result is understandable because they have no (or poor) affinity to Fe_3O_4 although they can absorb onto the surface of DTSSP-AuNPs through the formation of amino bond. For DOPAC, a reasonable explanation is that it has no amine group and cannot be assembled onto the DTSSP-AuNPs surface via the amine coupling reaction, although it can be captured by Fe_3O_4. However, although NE can also be captured by Fe_3O_4 and has primary amine group, its addition induced a slight change in the intensity. We presume that this lack of obvious change is probably due to the differential reactivity of the amines with DTSSP on the surface of AuNPs [4,24].

4. Conclusions/Outlook

In this study, we report for the first time, a sandwich-type biosensor using magnetic Fe$_3$O$_4$ and colorimetric AuNPs. In the work, DTSSP-AuNPs were connected with Fe$_3$O$_4$ MPs by DA and formed a sandwich structure of Fe$_3$O$_4$-DA-DTSSP-AuNPs. The method was developed to detect DA by observing the color change of DTSSP-AuNPs suspension and by measuring the UV/Vis signal. A detection limit of 10 nM for DA was achieved. The theoretical simplicity and high selectivity reported herein demonstrated that the detection strategy will likely find many applications in clinical study by rationally designing the surface chemistry of AuNPs and Fe$_3$O$_4$.

Figure 5. Selectivity of the sensing protocol. DOPAC: dihydroxyphenylacetic acid; GA: glutamic acid; NE: norepinephrine; Tyr: tyrosine. The concentrations of DA and interferences are 2 and 20 μM, respectively.
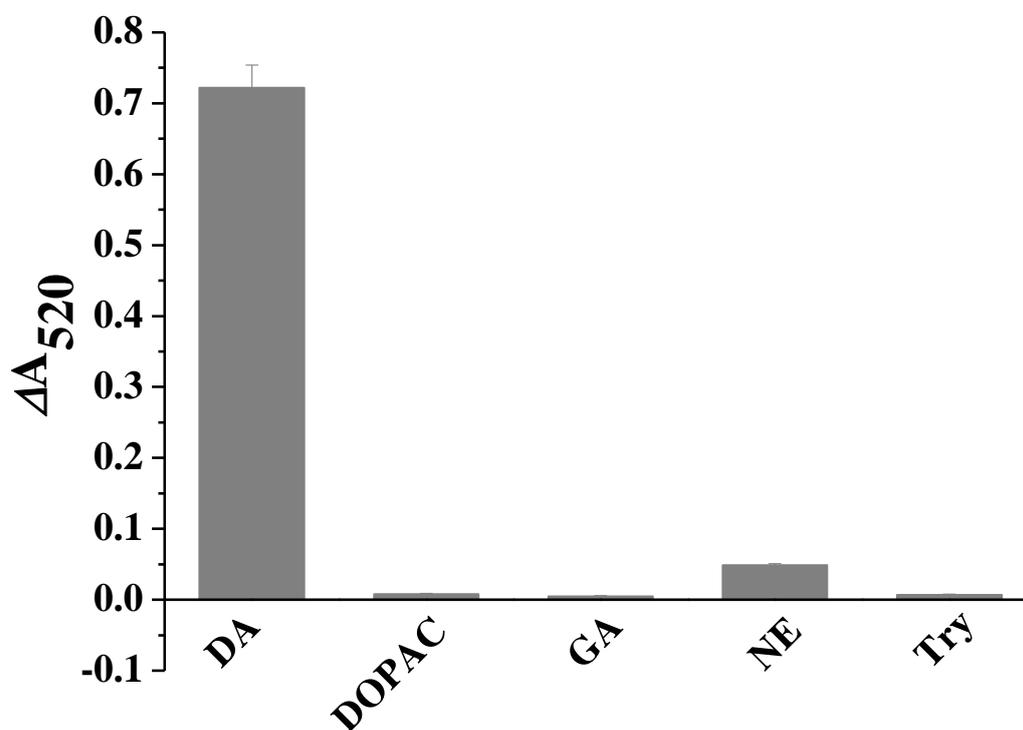
Acknowledgments

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Yguerabide, J.; Yguerabide, E.E. Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications—I. Theory. *Anal. Biochem.* **1998**, *262*, 137–156.

2. Cao, R.; Li, B.; Zhang, Y.; Zhang, Z. A simple and sensitive method for visual detection of heparin using positively-charged gold nanoparticles as colorimetric probes. *Chem. Commun.* **2011**, *47*, 12301–12303.

3. Huang, C.-C.; Huang, Y.-F.; Cao, Z.; Tan, W.; Chang, H.-T. Aptamer-modified gold nanoparticles for colorimetric determination of platelet-derived growth factors and their receptors. *Anal. Chem.* **2005**, *77*, 5735–5741.

4. Kong, B.; Zhu, A.; Luo, Y.; Tian, Y.; Yu, Y.; Shi, G. Sensitive and selective colorimetric visualization of cerebral dopamine based on double molecular recognition. *Angew. Chem. Int. Ed.* **2011**, *50*, 1837–1840.

5. Liu, C.-W.; Hsieh, Y.-T.; Huang, C.-C.; Lin, Z.-H.; Chang, H.-T. Detection of mercury(II) based on Hg(2+)-DNA complexes inducing the aggregation of gold nanoparticles. *Chem. Commun.* **2008**, 2242–2244.

6. Nam, J.-M.; Thaxton, C.S.; Mirkin, C.A. Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science* **2003**, *301*, 1884–1886.

7. Wei, H.; Li, B.; Li, J.; Wang, E.; Dong, S. Simple and sensitive aptamer-based colorimetric sensing of protein using unmodified gold nanoparticle probes. *Chem. Commun.* **2007**, 3735–3737.

8. Wilson, R. The use of gold nanoparticles in diagnostics and detection. *Chem. Soc. Rev.* **2008**, *37*, 2028–2045.

9. Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Elst, L.V.; Muller, R.N. Magnetic iron oxide nanoparticles: Synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chem. Rev.* **2008**, *108*, 2064–2110.

10. Aw, M.S.; Addai-Mensah, J.; Losic, D. Magnetic-responsive delivery of drug carriers using titania nanotube arrays. *J. Mater. Chem.* **2012**, *22*, 6561–6563.

11. Perez, J.M. Iron oxide nanoparticles: Hidden talent. *Nat. Nanotechnol.* **2007**, *2*, 535–536.

12. Stoeva, S.I.; Huo, F.; Lee, J.-S.; Mirkin, C.A. Three-layer composite magnetic nanoparticle probes for DNA. *J. Am. Chem. Soc.* **2005**, *127*, 15362–15363.

13. Bao, J.; Chen, W.; Liu, T.; Zhu, Y.; Jin, P.; Wang, L.; Liu, J.; Wei, Y.; Li, Y. Bifunctional $Au\text{-}Fe_3O_4$ nanoparticles for protein separation. *ACS Nano* **2007**, *1*, 293–298.

14. Lyon, J.L.; Fleming, D.A.; Stone, M.B.; Schiffer, P.; Williams, M.E. Synthesis of Fe oxide core/Au shell nanoparticles by iterative hydroxylamine seeding. *Nano Lett.* **2004**, *4*, 719–723.

15. Yu, H.; Chen, M.; Rice, P.M.; Wang, S.X.; White, R.L.; Sun, S. Dumbbell-like bifunctional $Au\text{-}Fe_3O_4$ nanoparticles. *Nano Lett.* **2005**, *5*, 379–382.

16. Xu, C.; Xie, J.; Ho, D.; Wang, C.; Kohler, N.; Walsh, E.G.; Morgan, J.R.; Chin, Y.E.; Sun, S. $Au\text{-}Fe_3O_4$ dumbbell nanoparticles as dual-functional probes. *Angew. Chem. Int. Ed.* **2008**, *47*, 173–176.

17. Xu, Z.; Hou, Y.; Sun, S. Magnetic core/shell Fe_3O_4/Au and $Fe_3O_4/Au/Ag$ nanoparticles with tunable plasmonic properties. *J. Am. Chem. Soc.* **2007**, *129*, 8698–8699.

18. Zhao, X.; Cai, Y.; Wang, T.; Shi, Y.; Jiang, G. Preparation of alkanethiolate-functionalized core/shell Fe_3O_4@Au nanoparticles and its interaction with several typical target molecules. *Anal. Chem.* **2008**, *80*, 9091–9096.

19. Kouassi, G.K.; Wang, P.; Sreevatan, S.; Irudayaraj, J. Aptamer-mediated magnetic and gold-coated magnetic nanoparticles as detection assay for prion protein assessment. *Biotechnol. Prog.* **2007**, *23*, 1239–1244.

20. Kouassi, G.K.; Irudayaraj, J. Magnetic and gold-coated magnetic nanoparticles as a DNA sensor. *Anal. Chem.* **2006**, *78*, 3234–3241.

21. Zhang, H.; Meyerhoff, M.E. Gold coated magnetic particles for solid phase immunoassays: Enhancing immobilized antibody binding efficiency and analytical performance. *Anal. Chem.* **2006**, *78*, 609–616.

22. Gan, N.; Jin, H.J.; Li, T.H.; Zheng, L. Fe_3O_4/Au magnetic nanoparticle amplification strategies for ultrasensitive electrochemical immunoassay of alfa-fetoprotein. *Int. J. Nanomed.* **2011**, *6*, 3259–3269.

23. Zhou, H.K.; Gan, N.; Li, T.H.; Cao, Y.T.; Zeng, S.L.; Zheng, L.; Guo, Z.Y. The sandwich-type electrochemiluminescence immunosensor for alpha-fetoprotein based on enrichment by Fe_3O_4-Au magnetic nano probes and signal amplification by CdS-Au composite nanoparticles labeled anti-AFP. *Anal. Chim. Acta* **2012**, *746*, 107–113.

24. Liu, L.; Du, J.; Li, S.; Yuan, B.; Han, H.; Jing, M.; Xia, N. Amplified voltammetric detection of dopamine using ferrocene-capped gold nanoparticle/streptavidin conjugates. *Biosens. Bioelectron.* **2013**, *41*, 730–735.

25. Xie, J.; Chen, K.; Lee, H.-Y.; Xu, C.; Hsu, A.R.; Peng, S.; Chen, X.; Sun, S. Ultrasmall c(RGDyK)-coated Fe_3O_4 nanoparticles and their specific targeting to integrin $\alpha_v\beta_3$-rich tumor cells. *J. Am. Chem. Soc.* **2008**, *130*, 7542–7543.

26. Gu, H.W.; Yang, Z.M.; Gao, J.H.; Chang, C.K.; Xu, B. Heterodimers of nanoparticles: Formation at a liquid-liquid interface and particle-specific surface modification by functional molecules. *J. Am. Chem. Soc.* **2005**, *127*, 34–35.

27. Li, H.; Wei, Q.; He, J.; Li, T.; Zhao, Y.; Cai, Y.; Du, B.; Qian, Z.; Yang, M. Electrochemical immunosensors for cancer biomarker with signal amplification based on ferrocene functionalized iron oxide nanoparticles. *Biosens. Bioelectron.* **2011**, *26*, 3590–3595.

28. Shultz, M.D.; Reveles, J.U.; Khanna, S.N.; Carpenter, E.E. Reactive nature of dopamine as a surface functionalization agent in iron oxide nanoparticles. *J. Am. Chem. Soc.* **2007**, *129*, 2482–2487.

29. Wang, B.D.; Xu, C.J.; Xie, J.; Yang, Z.Y.; Sun, S.H. pH controlled release of chromone from chromone-Fe_3O_4 nanoparticles. *J. Am. Chem. Soc.* **2008**, *130*, 14436–14437.

30. Xu, C.; Xu, K.; Gu, H.; Zheng, R.; Liu, H.; Zhang, X.; Guo, Z.; Xu, B. Dopamine as a robust anchor to immobilize functional molecules on the iron oxide shell of magnetic nanoparticles. *J. Am. Chem. Soc.* **2004**, *126*, 9938–9939.

31. Durocher, S.; Rezaee, A.; Hamm, C.; Rangan, C.; Mittler, S.; Mutus, B. Disulfide-linked, gold nanoparticle based reagent for detecting small molecular weight thiols. *J. Am. Chem. Soc.* **2009**, *131*, 2475–2477.

32. Lee, M.H.; Yang, Z.; Lim, C.W.; Lee, Y.H.; Dongbang, S.; Kang, C.; Kim, J.S. Disulfide-cleavage-triggered chemosensors and their biological applications. *Chem. Rev.* **2013**, *113*, 5071–5109.

33. Xia, N.; Deng, D.; Zhang, L.; Yuan, B.; Jing, M.; Du, J.; Liu, L. Sandwich-type electrochemical biosensor for glycoproteins detection based on dual-amplification of boronic acid-gold nanoparticles and dopamine-gold nanoparticles. *Biosens. Bioelectron.* **2013**, *43*, 155–159.

34. Hu, P.; Huang, C.Z.; Li, Y.F.; Ling, J.; Liu, Y.L.; Fei, L.R.; Xie, J.P. Magnetic particle-based sandwich sensor with DNA-modified carbon nanotubes as recognition elements for detection of DNA hybridization. *Anal. Chem.* **2008**, *80*, 1819–1823.

35. Xiao, S.J.; Hu, P.P.; Wu, X.D.; Zou, Y.L.; Chen, L.Q.; Peng, L.; Ling, J.; Zhen, S.J.; Zhan, L.; Li, Y.F.; *et al*. Sensitive discrimination and detection of prion disease-associated isoform with a dual-aptamer strategy by developing a sandwich structure of magnetic microparticles and quantum dots. *Anal. Chem.* **2010**, *82*, 9736–9742.

36. Liu, L.; Deng, D.; Xing, Y.; Li, S.; Yuan, B.; Chen, J.; Xia, N. Activity analysis of the carbodiimide-mediated amine coupling reaction on self-assembled monolayers by cyclic voltammetry. *Electrochim. Acta* **2013**, *89*, 616–622.