Published in final edited form as:

New J Chem. 2014 April 1; 38(4): 1546–1550. doi:10.1039/C3NJ01019H.

Highly Sensitive Simultaneous Detection of Mercury and Copper Ions by Ultrasmall Fluorescent DNA-Ag Nanoclusters

Shengliang Li^{a,b,*}, Weipeng Cao^{a,*}, Anil Kumar^a, Shubin Jin^a, Yuanyuan Zhao^a, Chunqiu Zhang^a, Guozhang Zou^a, Paul C. Wang^c, Feng Li^b, and Xing-Jie Liang^a

^aLaboratory of Nanomedicine and Nanosafety, Division of Nanomedicine and Nanobiology, National Center for Nanoscience and Technology of China, and CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Chinese Academy of Sciences, Beijing, 100190, China

^bDepartment of Neurobiology and Anatomy, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China

^cLaboratory of Molecular Imaging, Department of Radiology, Howard University, Washington DC 20060, USA

Abstract

Fluorescent metal nanoclusters (NCs) have given rise to a new class of fluorescent nanomaterials for the detection of heavy metals. Here, we design a simple, rapid and highly sensitive sensing nanosystem for the detection of Hg²⁺ and Cu²⁺ based on fluorescence quenching of ultrasmall DNA-Ag NCs. The fluorescence intensity of DNA-Ag NCs was selectively quenched by Hg²⁺ and Cu²⁺, and the limit of detection (LOD) was found to be 5 nM and 10 nM, respectively. The technique was renewable employment by EDTA addition and successfully applied to detection of Hg²⁺ and Cu²⁺ in domestic water samples. The quantum yield (QY) of DNA-Ag NCs was significantly higher to ~30% compared to traditional water-soluble fluorescent metal NCs. The DNA-Ag NC detection system make it potentially suitable for detecting Hg²⁺ and Cu²⁺ and monitoring water quality in a wide range of samples regulated under the Environmental Protection Agency.

Introduction

Recently, metal nanoclusters (NCs), made up from a few to a hundred atoms within a core structure, are emerging as novel luminescent nanomaterials and have received extensive attention for their potential uses in real-time applications.^{1–3} With sizes comparable to the Fermi wavelength of electrons, NCs exhibit unique photoluminescence properties including strong photoluminescence, high emission rates, large Stokes shifts, excellent photostability

© The Royal Society of Chemistry [year]

Correspondence to: Feng Li; Xing-Jie Liang.

*These authors contributed equally to this work

[†]Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

and two-photon absorption cross sections.^{4–7} More importantly, the emission wavelength of water-soluble metal NCs can be easily tuned from blue to near-infrared by varying the type of ligand or controlling the size.^{8–10} Owing to their ultras-small size and attractive photoluminescence properties, metal NCs are employed as a new class of biocompatible fluorophores for applications in biological imaging and bioassays.^{11, 12}

Heavy and transition metal ions are essential for the physical activities of biological and environmental processes.^{13, 14} However, excess amounts of these metal ions are toxic and can induce a series of health and environmental problems. Mercury is one of the most toxic heavy metal ions and poses significant toxicity even after minute exposure. Mercury damages biological systems by disrupting biological processes and can result in a variety of serious diseases.^{15–17} Copper, the third most abundant transition metal in the human body, plays vital roles in many fundamental biological processes. However, Cu²⁺ can cause serious damage to the liver and kidney at high concentrations.^{18–20} Thus, methods for detecting the presence of these ions are urgently needed. Although a number of effective approaches for Hg²⁺ and Cu²⁺ detection have been developed, existing methods need complex equipment or sophisticated operations.^{21–22} Simple and sensitive methods for detecting metal ions are still required.

In this work, we report the design of a sensing system to detect Hg²⁺. This system is based on the interaction between DNA-Ag NCs and Hg²⁺, which causes fluorescence quenching. We also found that the DNA-Ag NCs responded not only to Hg²⁺, but also to Cu²⁺. Hg²⁺ caused a more obvious fluorescence change than Cu²⁺. Further studies were carried out to investigate the detection of Hg²⁺ and Cu²⁺ in water samples. The highly sensitive and selective fluorescence quenching by Hg²⁺ and Cu²⁺ demonstrates that DNA-Ag NCs have great potential as a nano-sensing system for monitoring water quality in a wide range of samples.

Materials and methods

Materials and reagents

All oligonucleotides were synthesized by Beijing Sunbiotech Co. Ltd (Beijing, China). The oligonucleotides were purified by desalting. Gold (III) chloride trihydrate (HAuCl₄·3H₂O), silver nitrate (AgNO₃) and sodium borohydride (NaBH₄) were purchased from Sigma-Aldrich (St Louis, USA). Ultrapure water (18.2 MΩ cm⁻¹) produced by a Milli-Q system (Millipore Co., USA) was used in all the experimental work. All chemicals used were of analytical grade.

Synthesis of oligonucleotide-Ag NCs

Oligonucleotide-AgNCs were prepared as described elsewhere with slight modifications.^{2, 23} Briefly, DNA (oligonucleotide sequence: 5'-ACC CGA ACC TGG GCT ACC ACC CTT AAT CCC C-3') was dissolved in deionized water to prepare a stock solution. 6 μl DNA (250 μM) and 9 μl AgNO₃ (1 mM) were sequentially added to sodium phosphate buffer (PBS, 20 mM, pH 6.6), and the mixture was incubated at room temperature, in the dark, for 20 minutes. Freshly prepared NaBH₄ solution (90 μM) was then

added to the reaction mixture with vigorous shaking for 30 seconds. The reaction was kept in the dark at room temperature for 4 hours before use.

Characterization of DNA-Ag NCs

UV-Vis absorption spectra were measured with a Lambda 950 UV/vis/NIR spectrophotometer (Perkin-Elmer, USA). The fluorescence spectra were obtained in a microcell with 1 cm path length using a Perkin-Elmer LS55 luminescence spectrometer. The fluorescence lifetime of DNA-Ag NCs was measured with an Edinburgh FL 900 single-photon counting fluorescence lifetime instrument using a laser lamp (Edinburgh Instruments, Scotland). Transmission electron microscopy (TEM) imaging was performed with an FEI Tecnai F20 U-TWIN electron microscope (FEI Company, Philips, Netherlands). The size and zeta-potential distribution of DNA-Ag NCs was measured by a Zetasizer Nano ZS (Malvern, UK) at 25°C. X-ray photoelectron spectroscopy (XPS) was carried out on an X-ray photoelectron spectrometer (Perkin-Elmer, PHI-5300).

Determination of the fluorescence spectra of DNA-Ag NCs in the presence of metal ions

To explore the interaction of DNA-Ag NCs with different metal ions, 100 μL of the as-synthesized DNA-Ag NCs were mixed with different metal ions at 1 mM. The fluorescence spectra of DNA-Ag NCs in the presence of metal ions were recorded using a luminescence spectrometer after the samples were incubated for 5 minutes.

Detection of metal ions

The fluorescence activity of the DNA-Ag NCs was monitored over a range of Hg^{2+} and Cu^{2+} concentrations to evaluate the lowest concentration at which metal ions could be detected. For detection of mercury ions, concentrations of Hg^{2+} varying from 0 to 2 μM were added to a solution of DNA-Ag NCs (100 μL) to obtain the limit of detection and metal ion response range of the DNA-Ag NC probes. After 5 minutes incubation, the changes in fluorescence behavior of the Hg^{2+} -treated solutions were observed using a UV lamp and a luminescence spectrometer. The corresponding UV-Vis absorption spectra were also recorded. The same procedure was repeated for the detection of Cu^{2+} .

Analysis of real water samples

To analyze the activity of the DNA-Ag NCs, we used the system to detect metal ions in real water samples from different sources. Spring and laboratory tap water samples were collected for analysis. For the detection procedure, we prepared the samples by spiking them with standard solutions of Hg^{2+} and Cu^{2+} (the final concentration of each ion was 1 μM). The protocol for detection was similar to that described above. In order to confirm the practicality of the DNA-Ag NC system, the concentrations of Hg^{2+} and Cu^{2+} in the real water samples were detected by ICP-MS.

Results and discussion

Synthesis and characterization

The unique optical properties of fluorescent nanoclusters have attracted attention for use in bioassays and biological imaging.^{1,3} Here we develop a highly sensitive and selective fluorescence sensor for detecting the metal ions Cu^{2+} and Hg^{2+} based on the fluorescence quenching of DNA-Ag NCs. An oligonucleotide (a polymer consisting of 31 nucleotides) was employed as a stabilizer, and NaBH_4 was used as a reducing agent in a one-step synthesis of DNA-Ag NCs.

As shown in Fig. 1a (insert), the light brown solution of DNA-Ag NCs emitted an intense red fluorescence under UV light (365 nm), and fluorescence spectrophotometer analysis showed that the emission spectrum of DNA-Ag NCs had a single broad transition maximizing at 615 nm with a large Stokes shift (>80 nm) while excited at 535 nm. The quantum yield (QY) of DNA-Ag NCs was measured to be $\sim 30\%$, using Rhodamine B (QY=0.95 in ethanol) as the reference. In the past, various approaches have been taken to synthesize water-soluble fluorescent metal NCs, but the QY was extremely low, in the range of 10^{-10} to 10^{-2} .^{24, 25} These low yields were insufficient for biomedical applications.

TEM images of DNA-Ag NCs indicate that the fluorescent nanoparticles are well dispersed and the average diameter is 3.1 nm (Fig. 1b). Energy-dispersive-X-ray spectroscopy (EDX) experiments confirmed the presence of elemental Ag in DNA-Ag NC samples (Figure S1). To confirm these results, dynamic light scattering (DLS) was used to show that the hydrodynamic diameter and zeta-potential of these particles is 6.5 nm and -29 mV, respectively (Fig. 1c and 1d). The negative potential of DNA-Ag NCs implies that they possess good colloidal stability in physiological conditions.

Fluorescence lifetime measurements shed light on the underlying mechanism of fluorescence enhancement of DNA-Ag NCs. To confirm the fluorescent properties of DNA-Ag NCs, their fluorescence decay were measured. As showed in Fig. 2a, the DNA-Ag NCs possess a characteristic multi-exponential fluorescence decay that fitted well with two exponential functions, with lifetimes (fractional weights) of 5.42 ns (84.14%) and 2.6 ns (15.86%). In order to verify this result, a known fluorescein isothiocyanate (FITC) was chosen as a control for fluorescence decay measurements. The results showed that the fluorescence lifetime of FITC was 3.7 ns. In order to further confirm the mechanism of DNA-Ag NC fluorescence, X-ray photoelectron spectroscopy (XPS) measurements were carried out. As shown in Fig. 2c, the XPS spectra indicated that the DNA acted as a ligand on the surface of Ag NCs. The valence states of silver in the DNA-Ag NCs were also analyzed. The Ag 3d XPS spectrum showed that the binding energies (BE) of Ag 3d_{5/2} and Ag 3d_{3/2} were 367.98 eV and 373.98 eV, respectively (Fig. 2d). The BEs of Ag 3d_{5/2} and Ag 3d_{3/2} were close to that of Ag(0), which confirmed the formation of DNA-Ag NCs. The high luminescence of the as-synthesized DNA-Ag NCs is particularly favorable for use in biosensors.

Detection of Hg^{2+} and Cu^{2+} and mechanism

To evaluate the interaction of DNA-Ag NCs with Hg^{2+} , DNA-Ag NCs were mixed with different concentrations of Hg^{2+} ions. Fig. 3a displays the fluorescence emission spectra of DNA-Ag NCs as an indicator of Hg^{2+} concentration from 0 to 1 μM . The fluorescence intensity of the DNA-Ag NCs gradually decreased as the Hg^{2+} concentration increased, and the emission was quenched to less than 5% in the presence of 1 μM Hg^{2+} . These results indicated that the fluorescence quenching effect can be developed as a DNA-Ag NC-based sensing system for detection of Hg^{2+} . There is a good linear relationship between fluorescence quenching and the logarithm of the Hg^{2+} concentration at low Hg^{2+} concentrations (Fig. 3b). When the sensitivity of the DNA-Ag NC sensing system was examined, it was found that the limit of detection (LOD) of Hg^{2+} was 5 nM. This sensitivity meets with the Hg^{2+} detection requirement for drinking water by the U.S. Environmental Protection Agency (EPA, 10 nM) and the European Union (5 nM).

We next evaluated whether the DNA-Ag NCs can selectively detect different metal ions. We tested abundant cellular cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+} and Al^{3+}), essential cellular transition metal ions (Fe^{2+} , Fe^{3+} , Cu^{2+} , Mn^{2+} , Co^{2+} , and Ni^{2+}) and environmentally relevant heavy metal ions (Hg^{2+} , Pb^{2+} and Cd^{2+}) at a concentration of 1 μM (Fig. 3d). Only Hg^{2+} and Cu^{2+} caused pronounced and immediate fluorescence quenching, and Hg^{2+} caused stronger quenching than Cu^{2+} . Because Cu^{2+} could interfere with the detection of Hg^{2+} if Hg^{2+} and Cu^{2+} exist in the same analyte, we also studied the fluorescence responses of the DNA-Ag NCs to different concentrations of Cu^{2+} . As shown in Fig. 4, the DNA-Ag NC sensing system showed a relationship between fluorescence intensity and Cu^{2+} concentration. The LOD of Cu^{2+} was estimated to be 10 nM which met the detection requirement of the U.S. EPA (20 μM).²⁶ These results imply that simultaneous detection of Hg^{2+} and Cu^{2+} can easily be achieved by monitoring the fluorescence change of the DNA-Ag NCs with a UV lamp.

In order to investigate the underlying mechanism by which Hg^{2+} and Cu^{2+} quenched the fluorescence of DNA-Ag NCs, fluorescence lifetime measurements were carried out. In the presence of Hg^{2+} and Cu^{2+} , there was no distinct change in the fluorescence decay of the DNA-Ag NCs and the average fluorescence lifetime was 5.4 ns for Hg^{2+} and 5.2 ns for Cu^{2+} (Fig. S2). XPS measurements demonstrated that Hg^{2+} and Cu^{2+} had no effect on the valence states of silver in the DNA-Ag NCs (Fig. 5b). Previous studies suggested that the fluorescence of DNA-Ag NCs could also be quenched through Hg^{2+} -mediated interparticle aggregation.²⁷ In our study, we used TEM imaging to show that the DNA-Ag NCs formed aggregates in the presence of Hg^{2+} but not Cu^{2+} (Fig. 5a). Hg^{2+} binds to thymine (T) residues in single-stranded DNA, and the interaction of Hg^{2+} with the oligonucleotides on the surface of the nanoparticles probably causes the aggregation of the DNA-Ag-NCs.^{28–31} However, our results rule out the possibility that Hg^{2+} -induced aggregation of DNA-Ag NCs is responsible for the fluorescence quenching.

We speculate that Cu^{2+} could quench the fluorescence of DNA-Ag NCs due to the specific and strong metallophilic interaction between Cu and Ag atoms in our DNA-Ag NCs. Next, we investigated the effect of the chelating agent ethylene diamine tetracetic acid (EDTA) on the ability of DNA-Ag NC to sense Hg^{2+} and Cu^{2+} . As shown in Fig. S3, adding EDTA to the sensor system “turned on” the fluorescence of the DNA-Ag NC/ Hg^{2+} and DNA-Ag

NC/Cu²⁺ complexes, and the fluorescence intensity of Hg²⁺ and Cu²⁺ increased to 80% and 62% respectively in the presence of 10 μ M EDTA (data not shown). This indicates that EDTA forms stronger complexes with Hg²⁺ than with Cu²⁺. These results suggested that the DNA-Ag NC sensor system can be renewed by using EDTA.

In order to evaluate the potential application of DNA-Ag NCs in detecting ions in real water samples, we tested the sensitivity and specificity of the fluorescence response of Hg²⁺ and Cu²⁺ in tap water and spring water. As shown in Fig. S4, the real water samples did not induce fluorescence quenching of the DNA-Ag NC solution. Thus, the concentrations of Hg²⁺ and Cu²⁺ in tap water and spring water were lower than the detection limit of the DNA-Ag NCs. ICP-MS analysis was used to determine the Hg²⁺ and Cu²⁺ concentrations in the real water samples, and the data confirmed the results from the DNA-Ag NC system. However, the fluorescence of DNA-Ag NCs was immediately quenched when Hg²⁺ and Cu²⁺ were added to the real water. Taken together, our data suggest that the label-free DNA-Ag NC assay system is a potential tool for Hg²⁺ and Cu²⁺ sensing in environmental samples.

Conclusions

In summary, DNA-Ag NCs were synthesized through reduction by NaBH₄ at room temperature, which markedly increased the QY of fluorescence emission. These DNA-Ag NCs are of uniform ultrasmall size and possess good photophysical properties. In the present work, we developed a simple, rapid (<5 min) and low-cost sensing system for Hg²⁺ and Cu²⁺ detection using DNA-Ag NCs. This system has unprecedented sensitivity with a limit of detection (LOD) of 5 nM and 10 nM for Hg²⁺ and Cu²⁺, respectively. The fluorescence response of DNA-Ag NCs is remarkably specific for Hg²⁺ and Cu²⁺ in the presence of other metal ions, and thus meets the requirements of environmental and industrial monitoring applications. This novel sensing system is simple and can be easily carried out by simple mixing and incubation of the sample at room temperature. It has also been successfully applied to practical detection of Hg²⁺ and Cu²⁺ in real water samples. Because of its simplicity, rapidity and sensitivity, the DNA-Ag NC sensing system demonstrated here shows potential for monitoring water quality in developing regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Chinese Natural Science Foundation project (No.81171455), a National Distinguished Young Scholars grant (31225009) from the National Natural Science Foundation of China, the National Key Basic Research Program of China (2009CB930200), the Chinese Academy of Sciences (CAS) "Hundred Talents Program" (07165111ZX), the CAS Knowledge Innovation Program and the State High-Tech Development Plan (2012AA020804). This work was also supported in part by NIH/NIMHD 8 G12 MD007597, and USAMRMC W81XWH-10-1-0767 grants.

Notes and references

1. Qian H, Zhu M, Wu Z, Jin R. Acc. Chem. Res. 2012; 45:1470. [PubMed: 22720781]

2. Li J, Zhong X, Cheng F, Zhang J-R, Jiang L-P, Zhu J-J. *Anal. Chem.* 2012; 84:4140. [PubMed: 22482827]
3. Wu X, He X, Wang K, Xie C, Zhou B, Qing Z. *Nanoscale.* 2010; 2:2244. [PubMed: 20835443]
4. Zhu M, Lanni E, Garg N, Bier ME, Jin R. *J. Am. Chem. Soc.* 2008; 130:1138. [PubMed: 18183983]
5. Jin R. *Nanoscale.* 2010; 2:343. [PubMed: 20644816]
6. Maretta L, Billone PS, Liu Y, Scaiano JC. *J. Am. Chem. Soc.* 2009; 131:13972. [PubMed: 19788331]
7. Xie J, Zheng Y, Ying JY. *J. Am. Chem. Soc.* 2009; 131:888. [PubMed: 19123810]
8. Richards CI, Choi S, Hsiang J-C, Antoku Y, Vosch T, Bongiorno A, Tzeng Y-L, Dickson RM. *J. Am. Chem. Soc.* 2008; 130:5038. [PubMed: 18345630]
9. Le Guével X, Spies C, Daum N, Jung G, Schneider M. *Nano Res.* 2012; 5:379.
10. Sharma J, Rocha RC, Phipps ML, Yeh HC, Balatsky KA, Vu DM, Shreve AP, Werner JH, Martinez JS. *Nanoscale.* 2012; 4:4107. [PubMed: 22648534]
11. Shang L, Dong S, Nienhaus GU. *Nano Today.* 2011; 6:401.
12. Zhu M, Lanni E, Garg N, Bier ME, Jin R. *J. Am. Chem. Soc.* 2008; 130:1138. [PubMed: 18183983]
13. Trautwein, AX., editor. *Bioinorganic Chemistry.* Wiley-VCH: Weinheim; 1997.
14. Aragay G, Pons J, Merkoci A. *Chem. Rev.* 2011; 111:3433. [PubMed: 21395328]
15. Clarkson TW, Magos L. *CRC Critical Reviews in Toxicology.* 2006; 36:609. [PubMed: 16973445]
16. Knecht M, Sethi M. *Analytical and Bioanalytical Chemistry.* 2009; 394:33. [PubMed: 19189085]
17. Clarkson TW, Magos L, Myers GJN. *Engl. J. Med.* 2003; 349:1731.
18. Que EL, Domaille DW, Chang CJ. *Chem. Rev.* 2008; 108:1517–1549. [PubMed: 18426241] Liu M, Zhao H, Chen S, Yu H, Zhang Y, Quan X. *Chem. Commun.* 2011; 47:7749.
19. Lin Y-W, Huang C-C, Chang H-T. *Analyst.* 2011; 136:863. [PubMed: 21157604]
20. Guo Y, Wang Z, Shao H, Jiang X. *Analyst.* 2012; 137:301. [PubMed: 22048157]
21. Wang H, Wang Y, Jin J, Yang R. *Anal. Chem.* 2008; 80:9021. [PubMed: 19551976]
22. Wang J, Liu B. *Chem. Commun.* 2008:4759. Pramanik M, Bhaumik A. *Chem Eur J.* 2013; 19:8507. [PubMed: 23650095]
23. Gwinn EG, O'Neill P, Guerrero AJ, Bouwmeester D, Fyngenson DK. *Adv. Mater.* 2008; 20:279.
24. Link S, Beeby A, FitzGerald S, El-Sayed MA, Schaaff TG, Whetten RL. *J Phys Chem B.* 2002; 106:3410.
25. Huang T, Murray RW. *J Phys Chem B.* 2001; 105:12498.
26. Adhikari B, Banerjee A. *Chem. Mater.* 2010; 22:4364.
27. Huang CC, Yang Z, Lee KH, Chang HT. *Angewandte Chemie.* 2007; 119:6948.
28. Chiang C-K, Huang C-C, Liu C-W, Chang H-T. *Anal. Chem.* 2008; 80:3716. [PubMed: 18363331]
29. Du J, Liu M, Lou X, Zhao T, Wang Z, Xue Y, Zhao J, Xu Y. *Anal. Chem.* 2012; 84:8060. [PubMed: 22957843]
30. Xu H, Zhu X, Ye H, Yu L, Liu X, Chen G. *Chem. Commun.* 2011; 47:12158.
31. Zhu Z, Xu L, Zhou X, Qin J, Yang C. *Chem. Commun.* 2011; 47:8010.

Fig. 1. Characterization of the properties of DNA-Ag NCs. (a) Fluorescence spectrum of DNA-Ag NCs. Inset: pictures of the Ag NCs solution under normal room lighting (left) and UV irradiation (right). (b) Typical TEM image of DNA-Ag NCs. (c) The hydrodynamic diameter of DNA-Ag NCs. (d) Zeta potential distribution of the DNA-Ag NCs.

Fig. 2.

(a) The fluorescence decay curves of DNA-Ag NCs and multi-exponential fits. (b) Fluorescence lifetime parameters of DNA-Ag NCs and FITC. (c) XPS spectrum of DNA-Ag NCs and (d) the expanded spectrum of Ag_{3d}.

Fig. 3.

(a) Fluorescence emission spectrum of DNA-Ag NCs in the presence of Hg^{2+} concentrations increasing from 0 to 2 μM (top to bottom, excitation at 535 nm). The insert shows an image of the Hg^{2+} -treated DNA-Ag NCs under UV irradiation (0 μM Hg^{2+} , left, to 2 μM , right). (b) Relative fluorescence intensity of DNA-Ag NCs (F/F_0 , where F and F_0 are the fluorescence intensities at 615 nm in the presence and absence of Hg^{2+} , respectively) versus the logarithm of the Hg^{2+} concentration up to 100 nM. (c) Fluorescence response of DNA-Ag NCs in the presence of different metal ions (all at 1 μM). (d) Histogram analysis of (c).

Fig. 4. Sensitivity and fluorescence response of DNA-Ag NCs toward Cu^{2+} ions. Emission spectra of DNA-Ag NCs in the presence of different concentrations of Cu^{2+} increasing from 0 to 2 μM (top to bottom, excitation at 535 nm). The insert shows an image of the Cu^{2+} -treated DNA-Ag NCs under UV irradiation (0 μM Cu^{2+} , left, to 2 μM , right).

Fig. 5.
TEM imaging (a) and XPS spectrum analysis (b) of DNA-Ag NCs in the absence and presence of Hg²⁺ (1 μ M) or Cu²⁺ (1 μ M).