Detection of mercury(II) based on Hg^{2+}–DNA complexes inducing the aggregation of gold nanoparticles†

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A DNA–Au NP probe for sensing Hg^{2+} using the formation of DNA–Hg^{2+} complexes through thymidine (T)–Hg^{2+}–T coordination to control the negative charge density of the DNA strands—thereby varying their structures—adsorbed onto Au NPs.

Monitoring the levels of mercury in aquatic ecosystems is an important issue because of its severe effects on human health and the environment.[1,2] Several optical techniques have been developed for the detection of Hg^{2+}.[3–5] For example, Hg^{2+}-induced color or fluorescence changes of thiol-functionalized gold nanoparticles (Au NPs) have been demonstrated for the selective detection of Hg^{2+} in the presence of 1.0 mM pyridinedicarboxylic acid.[4a–c] The major advantages of using Au NPs for the detection of Hg^{2+} are the lack of labeling and the high sensitivity arising from the extremely high extinction coefficients (10^8–10^{10} M^{-1} cm^{-1}) of their surface plasmon resonance (SPR) absorptions and/or efficient quenching effects.[6]

Hg^{2+}-induced aggregation of DNA-functionalized Au NPs through T–Hg^{2+}–T coordination was realized.[5f] Relative to simple alkylthiol-functionalized Au NPs, DNA-functionalized Au NPs are highly selective toward Hg^{2+} ions. Nevertheless, two different sequences of thiol-functionalized DNA molecules are required for the preparation of functionalized Au NPs for the detection of Hg^{2+}. The sensitivity of the detection system is also highly dependent on the temperature.

In this paper we present a simple and rapid colorimetric assay—employing poly-T_n ss-DNA and 13-nm-diameter Au NPs in the presence of salt—for the detection of Hg^{2+} ions based on Hg^{2+}–DNA complexes inducing the aggregation of Au NPs. Random-coil DNA molecules adsorb onto Au NP surfaces through electrostatic attraction.[7] In the presence of salt, poly-T_n ss-DNA on the surface of Au NPs remains in a random-coil structure as a result of electrostatic repulsion between DNA molecules. Owing to the high negative charge density of DNA on each Au NP surface, monodisperse Au NPs exist in the salt solution (Fig. 1a). Upon formation of Hg^{2+}–DNA complexes the conformation of the poly-T_n ss-DNA changes to folded structures (Fig. 1b). As a result of the decreased zeta potential on each Au NP and the reduced degree of electrostatic repulsion between Au NPs, aggregation of the Au NPs occurs and, hence, the color of the solution changes from red to purple in a process that is detectable by the naked eye.

We first treated 2.0 nM Au NPs with 60 nM poly-T_{33} in 5 mM phosphate (pH 7.4) for 10 min in the absence and presence of 5.0 μM $HgCl_2$. Next, we added NaCl (final concentration: 50 mM) to each of the two solutions. In the absence of Hg^{2+}, the color of solution remained rose-red after adding NaCl; in the presence of Hg^{2+}, however, the color of the solution changed to purple as a result of the aggregation of Au NPs. The zeta potentials of Au NPs, DNA–Au NPs, and Hg^{2+}–DNA–Au NPs were measured to be −27.3, −39.3, and −28.0 mV, respectively. The changes in absorption (Fig. 2) and TEM images (Fig. S1†) support the Hg^{2+}-induced aggregation of Au NPs. To rule out the possibility that aggregation of the Au NPs occurred during the drying process, we conducted dark-field scattering measurements; the images in Fig. S1† further support our hypothesis that the aggregation of Au NPs occurred in the presence of DNA and Hg^{2+}. Note that strong scattering occurred once the Au NPs had aggregated.[8] CD spectra (Fig. S2†) support the formation of the folded structures from T_{33} in the presence of Hg^{2+}.[5c–e] The

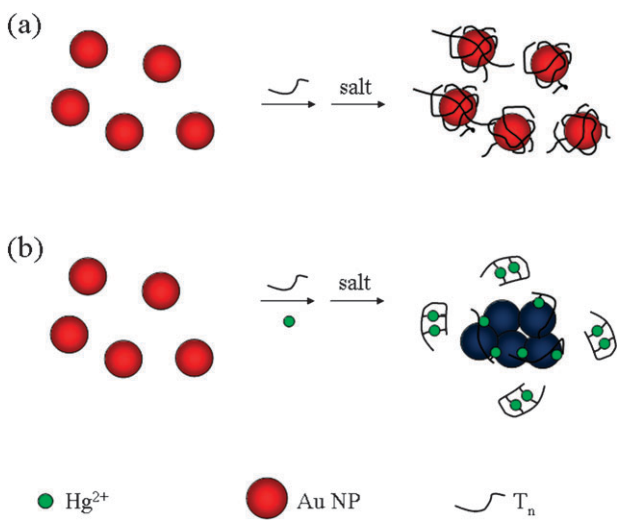

Fig. 1 Schematic representation of Hg^{2+} nanosensors.

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bulky structures of these folded Hg^{2+}–DNA complexes also minimize the number of DNA molecules that can be adsorbed onto each Au NP. As a result, the Au NPs were less well protected by DNA units, resulting in their aggregation in the presence of 50 mM NaCl and 5.0 μM Hg^{2+}. To further support our reasoning, we conducted control experiments using poly-A_{33} and random-sequence DNA (5′-TTTTTTA CCTGGGGGAGTATTGCGGAGGAAGGT-3′) under similar conditions. As expected, the addition of Hg^{2+} did not induce any significant changes in the SPR absorptions of these Au NP solutions.

Having observed that Hg^{2+} induced the formation of folded DNA structures, we suspected that the sensitivity of our analytical system would be dependent on the length of the DNA strand. Thus, we used three different ss-DNA samples—T_7, T_{33}, and T_{80}—to test our hypothesis. The extinction coefficients of the solutions at 650 and 520 nm are related to the quantities of dispersed and aggregated Au NPs, respectively. Thus, we used the ratio of the values of extinction, $Ex_{650/520}$, to express the molar ratio of aggregated and dispersed Au NPs. First, we investigated the effect of the length of the DNA strand on the stability of the Au NPs in the presence of NaCl at various concentrations (0–100 mM). We estimated that *ca.* 30 DNA strands having 30 bases were present on each 13 nm Au NP surface, which suggested that the saturation concentration of T_{33} was 60 nM when using 2 nM Au NPs.[7] To provide the same base concentration as that provided by 60 nM T_{33}, we utilized 283 and 25 nM solutions of T_7 and T_{80}, respectively. In the absence of Hg^{2+}, Fig. 3a indicates that the longer DNA strands had a better ability to stabilize the Au NPs; *i.e.*, at the same NaCl concentration, the stability of the DNA-bound Au NPs (T_n-Au NPs) increased in the order T_{80}-Au NPs > T_{33}-Au NPs > T_7-Au NPs. We suspect that when longer DNA strands adsorb onto the surfaces of Au NPs, the radius of each Au NP increases, minimizing the degree of electrostatic attraction between Au NPs. Upon increasing the NaCl concentration, salt screening reduced the surface charge density and size of the T_n-Au NPs, leading to decreased stability of the Au NPs. Fig. 3b indicates that the T_7-, T_{33}-, and T_{80}-Au NPs aggregated at NaCl concentrations greater than 25, 50, and 65 mM, respectively, in the absence of Hg^{2+}.

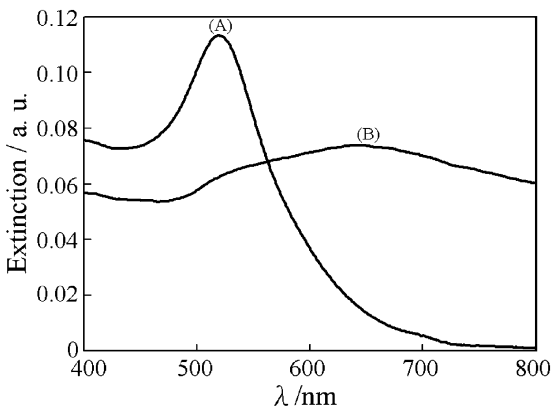

Fig. 2 UV–Vis extinction spectra of 5 mM phosphate solutions (pH 7.4) containing Au NPs (2 nM), T_{33} (60 nM), and NaCl (50 mM) in the (A) absence and (B) presence of 5.0 μM Hg^{2+}.

The degree of Hg^{2+}-induced aggregation of Au NPs in the presence of T_7 was small, mainly because of the difficulty encountered by T_7 in forming folded structures. The inset also reveals that the solution of Au NPs was more sensitive toward Hg^{2+} in the presence of T_{33} than in the presence of T_{80}, mainly because of the greater capability of T_{80} to stabilize the Au NPs. The plots suggest that the optimum conditions for sensing Hg^{2+} were the use of a 5 mM sodium phosphate solution (pH 7.4) containing 60 nM T_{33}, 2 nM Au NPs, and 50 mM NaCl.

Under the optimum conditions, we investigated the specificity of our analytical approach toward Hg^{2+} against other metal ions (each 5.0 μM). The inset to Fig. 4a indicates that only Hg^{2+} induced aggregation of the Au NPs in the presence of T_{33}. As indicated in Fig. 4a, our system responded selectively toward Hg^{2+} ions—by a factor of 50-fold or more relative to the other metal ions. The tolerance concentrations of Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} for the sensing of Hg^{2+} using our approach were at least 20 times higher than the Hg^{2+} concentration (Fig. S3†). As indicated in Fig. 4b, the

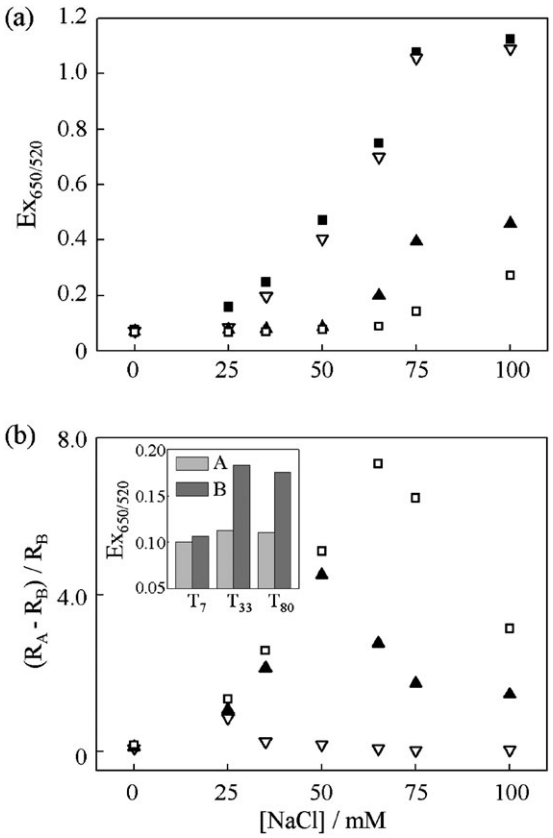

Fig. 3 (a) Plot of the values of $Ex_{650/520}$ of the Au NP solutions (2 nM) as a function of the concentration of NaCl: (■) no DNA, (▽) 283 nM T_7, (▲) 60 nM T_{33}, and (□) 25 nM T_{80}. (b) Plot of the values of $(R_A - R_B)/R_B$ of Au NP solutions (2 nM) in the absence and presence of T_n as a function of the concentration of NaCl. The descriptors R_A and R_B represent the values of $Ex_{650/520}$ of the Au NP solutions in the absence and presence of T_n, respectively. Inset: Plot of $Ex_{650/520}$ in the (A) absence and (B) presence of 1.0 μM Hg^{2+}. The concentrations of NaCl in the solutions of T_7-, T_{33}-, and T_{80}-Au NPs were 25, 50, and 65 mM, respectively. Other conditions were the same as those described in Fig. 2.

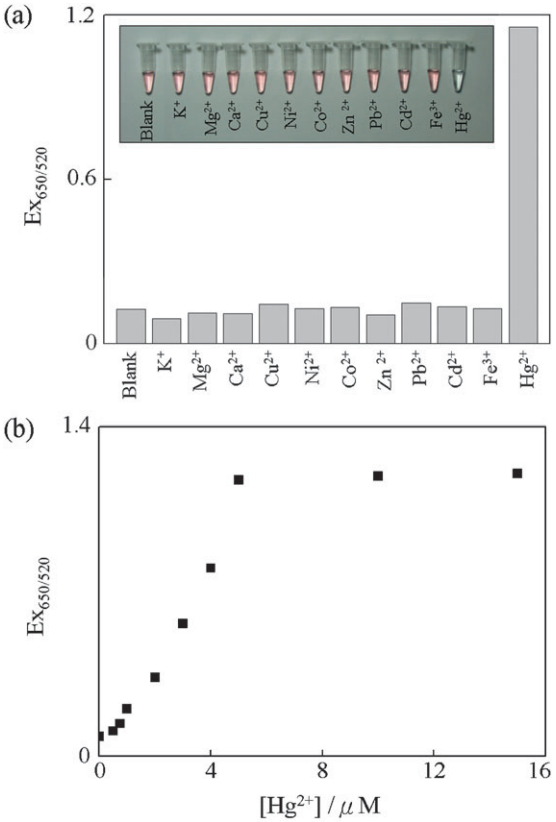

Fig. 4 (a) Values of $Ex_{650/520}$ and a photographic image (inset) of solutions containing Au NPs (2 nM), T_{33} (60 nM), and various metal ions (each 5.0 μM). (b) Plot of the value of $Ex_{650/520}$ as a function of the concentration of Hg^{2+}. Other conditions were the same as those described in Fig. 2.

$Ex_{650/520}$ ratios of solutions containing T_{33}, Au NPs, and NaCl increased upon increasing the concentration of Hg^{2+} ions. A linear correlation (R^2 = 0.96) existed between the value of $Ex_{650/520}$ and the concentration of Hg^{2+} ions over the range 0.5–5.0 μM. The limit of detection (LOD) for Hg^{2+} at a signal-to-noise ratio of 3 was estimated to be 250 nM.

To confirm that the amount of T_{33} adsorbed onto the Au NPs decreased upon increasing the Hg^{2+} concentration, we added OliGreen to the solution. DNA–OliGreen complexes fluoresce *ca.* 1000-fold more intensely that does free OliGreen, which is weakly fluorescent. We mixed T_{33} (30 nM) and Hg^{2+} (0–25 μM) together and then added them to each Au NP solution (1.0 nM). Although the DNA molecules on the surfaces of the Au NPs also interacted with OliGreen, we observed a negligible degree of fluorescence because of fluorescence resonance energy transfer between these DNA–OliGreen complexes and the Au NPs, which are efficient fluorescence quenchers. In other words, only the DNA–OliGreen complexes in the bulk solution fluoresced. The fluorescence intensity at 525 nm of the solutions upon excitation at 480 nm increased upon increasing the concentration of Hg^{2+} (Fig. S4†), supporting our hypothesis that greater amounts of DNA–OliGreen complexes existed in the bulk solution at higher concentrations of Hg^{2+} ions. To confirm this result, we detected the amount of DNA released after centrifugation. From the concentration of DNA in the supernatant, we estimated that *ca.* 25.5 and 94.8% of the adsorbed DNA strands were released into the bulk solutions in the presence of 1.0 and 10 μM Hg^{2+}, respectively (Fig. S4†).

In conclusion, we have formulated a simple and cost-effective assay—using T_{33} and Au NPs—for the sensitive and selective detection of Hg^{2+} ions. Although we have used this system to demonstrate the detection of Hg^{2+} ions only, this approach could be applied to the determination of other metal ions, DNA, or proteins through the use of other DNA molecules, DNA aptamers, and various other Au NPs.

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Notes and references

1 L. M. Campbell, D. G. Dixon and R. E. Hecky, *J. Toxicol. Environ. Health B*, 2003, **6**, 325–356.
2 M. Wang, W. Feng, J. Shi, F. Zhang, B. Wang, M. Zhu, B. Li, Y. Zhao and Z. Chai, *Talanta*, 2007, **71**, 2034–2039.
3 (*a*) E. M. Nolan and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 14270–14271; (*b*) Y.-K. Yang, K.-J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760–16761; (*c*) S. Yoon, A. E. Albers, A. P. Wong and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16030–16031; (*d*) S.-Y. Moon, N. J. Youn, S. M. Park and S.-K. Chang, *J. Org. Chem.*, 2005, **70**, 2394–2397; (*e*) E. M. Nolan, M. E. Racine and S. J. Lippard, *Inorg. Chem.*, 2006, **45**, 2742–2749.
4 (*a*) C.-C. Huang and H.-T. Chang, *Chem. Commun.*, 2007, 1215–1217; (*b*) C.-C. Huang and H.-T. Chang, *Anal. Chem.*, 2006, **78**, 8332–8338; (*c*) C.-C. Huang, Z. Yang, K.-H. Lee and H.-T. Chang, *Angew. Chem., Int. Ed.*, 2007, **46**, 6824–6828; (*d*) A. Ono and H. Togashi, *Angew. Chem., Int. Ed.*, 2004, **43**, 4300–4302; (*e*) I.-B. Kim and U. H. F. Bunz, *J. Am. Chem. Soc.*, 2006, **128**, 2818–2819; (*f*) J. M. Thomas, R. Ting and D. M. Perrin, *Org. Biomol. Chem.*, 2004, **2**, 307–312.
5 (*a*) S. Katz, *J. Am. Chem. Soc.*, 1952, **74**, 2238–2245; (*b*) T. Yamane and N. Davidson, *J. Am. Chem. Soc.*, 1961, **83**, 2599–2607; (*c*) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami and A. Ono, *J. Am. Chem. Soc.*, 2006, **128**, 2172–2173; (*d*) Y. Miyake and A. Ono, *Tetrahedron Lett.*, 2005, **46**, 2441–2443; (*e*) Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima and A. Ono, *J. Am. Chem. Soc.*, 2007, **129**, 244–245; (*f*) J.-S. Lee, M. S. Han and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2007, **46**, 4093–4096; (*g*) X. Liu, Y. Tang, L. Wang, J. Zhang, S. Song, C. Fan and S. Wang, *Adv. Mater.*, 2007, **19**, 1471–1474.
6 (*a*) A. Moores and F. Goettmann, *New J. Chem.*, 2006, **30**, 1121–1132; (*b*) Y. Kim, R. C. Johnson and J. T. Hupp, *Nano Lett.*, 2001, **1**, 165–167; (*c*) C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607–609.
7 (*a*) H. Li and L. J. Rothberg, *J. Am. Chem. Soc.*, 2004, **126**, 10958–10961; (*b*) H. Li and L. Rothberg, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 14036–14039.
8 K.-H. Lee, K.-M. Huang, W.-L. Tseng, T.-C. Chiu, Y.-W. Lin and H.-T. Chang, *Langmuir*, 2007, **23**, 1435–1442.

