DNA stabilized silver nanoclusters for ratiometric and visual detection of Hg^{2+} and its immobilization in hydrogels

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Abstract

DNA oligmers are particularly interesting templates for making silver nanoclusters (AgNCs) as different emission colors can be obtained by varying the DNA sequence. Many AgNCs have been used as Hg^{2+} sensors since Hg^{2+} induces fluorescence quenching. From an analytical chemistry standpoint, however, these 'light off' sensors are undesirable. In this work, taking advantage that some AgNCs are not as effectively quenched by Hg^{2+}, we design a sensor with AgNCs containing two emission peaks. The red peak is strongly quenched by Hg^{2+} while the green peak shows concomitant increase, producing an orange-to-green visual fluorescence transformation. Using this AgNC, we demonstrate ratiometric detection with a detection limit of 4 nM Hg^{2+}. This sensor is further immobilized in a hydrogel matrix and this gel is also capable of detecting Hg^{2+} with a visual response.

Keywords: mercury, silver nanoclusters, fluorescence, ratiometric, biosensors

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1. Introduction

Mercury is a highly toxic metal and long term exposure to even low concentrations of mercury causes severe damages to various organs (Nolan and Lippard 2008). The US Environmental Protection Agency (EPA) has defined the maximal contamination level for mercury to be just 10 nM. Recent work on fluorescent metal nanoclusters (NCs) has made it a new platform for developing mercury sensors (Diez and Ras 2011; Han and Wang 2012; Jin 2010; Latorre and Somoza 2012; Zheng et al. 2007). Au or AgNCs are usually prepared by reducing their metal salts in the presence of a polymeric ligand such as DNA or protein (Kennedy et al. 2012; Petty et al. 2004; Richards et al. 2008; Xie et al. 2009). It has been reported that the fluorescence of many Au and Ag NCs is strongly quenched by Hg^{2+} (Guo and Irudayaraj 2011; Guo et al. 2009; Huang et al. 2007; Lan et al. 2011; Lan et al. 2010; Xie et al. 2010). As such, most of the current Hg^{2+} sensors involving metal NCs are 'light-off' sensors (Guo and Irudayaraj 2011; Guo et al. 2009; Huang et al. 2007; Lan et al. 2011; Lan et al. 2010; Xie et al. 2010), which are undesirable from an analytical chemistry standpoint due to the limited room for signal change, large background variation and susceptibility to false signals. To solve this problem, Dong and co-workers designed a bulged DNA duplex with several thymine bases flanking a cytosine bulge, where Hg^{2+} can stabilize a cytosine loop and produce fluorescent AgNCs (Deng et al. 2011; Ono and Togashi 2004). In the absence of Hg^{2+}, little fluorescence was observed. In another report, Chang and co-workers discovered that with a particular DNA sequence, Cu^{2+} could enhance the fluorescence of AgNC when both metal ions were present during the reduction reaction (Lan et al. 2010). Although these are 'light-up' sensor designs, the synthesis of AgNC is an integral step of their detection processes.

A large number of AgNCs with a broad spectrum of emission colors have been prepared using various cytosine-rich DNA sequences (Driehorst et al. 2011; Gwinn et al. 2008; Lan et al. 2011; O'Neill et al. 2009; Richards et al. 2008; Ritchie et al. 2007; Yeh et al. 2010). From the literature, it appears that the majority of work on Hg^{2+} sensing was performed using red emitting AgNCs. An

interesting question is whether all the AgNCs have the same sensitivity to Hg^{2+} or not. We postulate that if Hg^{2+} sensitive and insensitive AgNCs of different emission colors can be produced, it might be possible to achieve emission color change and ratiometric detection. Herein, we communicate such AgNCs for visual and ratiometric Hg^{2+} detection. We further immobilize thet AgNCs in hydrogels, where visual detection is also achieved.

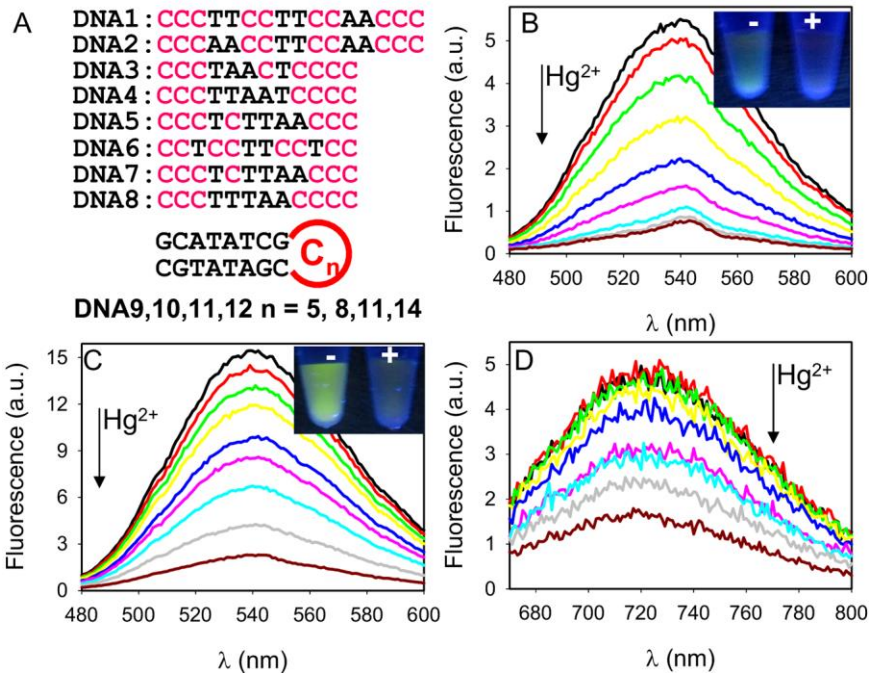

Figure 1. (A) DNA sequences used in this work. Fluorescence spectra of AgNCs synthesized using DNA1 (B), DNA2 (C) and DNA3 (D) titrated with increasing Hg^{2+} concentrations (Hg^{2+} = 0, 10, 20, 30, 50, 70, 100, 150 and 200 nM). Insets: photographs of sample fluorescence in the absence (left) or presence (right) of 4 μM Hg^{2+}.

2. Experimental

2.1. Chemicals. All of the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA) and purified by standard desalting. $AgNO_3$, $NaBH_4$, $Hg(ClO_4)_2$, $MgCl_2$, $CaCl_2$, $SrCl_2$,

$BaCl_2$, $FeCl_3$, $CoCl_2$, $NiSO_4$, $ZnCl_2$, $CuSO_4$, $Pb(OAc)_2$, $Cd(NO_3)_2$ and 2,6-pyridinedicarboxylic acid were from Sigma-Aldrich. Acrylamide and bisacrylamide (29:1) stock solution (40%), ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) were from VWR. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and its sodium salt were purchased from Mandel Scientific (Guelph, ON). Milli-Q water was used for making buffers and dilutions.

2.2. AgNC preparation. For DNA1-8, 50 μM DNA and 300 μM $AgNO_3$ were dissolved in 10 mM HEPES buffer (pH 7.6). To 200 μL of this solution, 30 μL of 2 mM $NaBH_4$ was added and rapidly mixed. For DNA9-13, 15 μM DNA and 120 μM $AgNO_3$ were mixed and 12 μL of 2 mM $NaBH_4$ was added. AgNCs were assayed after 4-8 hr reaction in dark.

2.3. Fluorescence spectroscopy. 30 μL of the AgNCs were dissolved in 570 μL 10 mM HEPES buffer (pH 7.6). The excitation peak was determined first and then the emission peaks were scanned. All fluorescence spectra were collected using a Varian Eclipse fluorometer. A few microliters of 5 or 20 μM Hg^{2+} solutions were added to achieve the designated Hg^{2+} concentration.

2.4. UV-vis spectroscopy. 80 μL of as synthesized AgNCs were transferred into a quartz micro-cuvette and the spectra were collected using an Agilent 8453 UV-vis spectrophotometer. Hg^{2+} was then added using 100 μM stock solutions and the spectra were measured again.

2.5. Hydrogel preparation. A hydrogel was prepared by mixing 40% acrylamide:bisacrylamide (29:1), $NaNO_3$ (2 M), HEPES (pH 7.6, 0.5 M), acrydite-modified DNA (0.5 mM), and water. This mixture contained a final gel percentage of 4% and 100 mM $NaNO_3$, 50 mM HEPES, and 10 μM DNA. To initiate polymerization, a fresh initiator solution was prepared by dissolving 50 mg APS in 500 μL water and 25 μL TEMED. The volume of the initiator was kept to be 5% of the final mixture. A 96-well plate was used for gel preparation. To each well 75 μL of the gel solution was added. The gels were polymerized for 1 hr at room temperature and then soaked in water in dark three times (each soaking for 3 hr) to remove free monomers, initiator, and unincorporated DNA. The gels were then

soaked in 10 μM $AgNO_3$ solution and were further soaked in water to remove unbound Ag^+. Then 0.2 mM $NaBH_4$ was added to induce AgNC formation. After 2 hr, the samples were then soaked twice in water to further purify the gels.

2.6. Imaging. 80 μL of the AgNCs were treated with Hg^{2+} (3 μM) and compared with the same sample without adding Hg^{2+}. The fluorescence signal was observed in a dark room with the excitation of 254 nm light from a handheld UV lamp. The samples were documented using a digital camera (Canon PowerShot SD1200 IS).

3. Results and discussion

We first employed eight DNA sequences previously reported in the literature to prepare AgNCs (DNA1-8, Figure 1A) (Lan et al. 2011; Richards et al. 2008). These DNAs have several cytosine bases on the ends of the strands. Three samples with relatively strong fluorescence (Figure S1, Supporting Information) were further examined for Hg^{2+} detection. DNA1 and DNA2 produced green/yellow fluorescence that was effectively quenched by Hg^{2+} (Figure 1B, C). AgNCs templated by DNA3 showed near-IR fluorescence with a peak at 720 nm. Although invisible to the human eye, spectroscopic measurements confirmed its sensitivity to Hg^{2+} (Figure 1D).

In addition to DNAs rich in terminal cytosines, we also tested DNAs with cytosines on the loop of a DNA hairpin (DNA9-12, Figure 1A), where the loop size was varied from 5 to 14 cytosines. Some of the small hairpins are known to be less sensitive to Hg^{2+} (Morishita et al. 2013). In the current work, we further test their visual fluorescence responses. We obtained red/orange fluorescent AgNCs for all the hairpin samples. AgNCs templated by DNA9 (5 cytosines) showed very little visual fluorescence change (inset of Figure 2A). AgNCs synthesized with DNA10 (8 cytosines) was quenched by ~40% (Figure 2B). The brightest fluorescence was obtained with DNA11 containing eleven cytosines, whose red fluorescence was completely quenched by Hg^{2+} (Figure 2C). Further increase to a 14-cytosine loop

(DNA12) also resulted in a Hg^{2+} sensitive sample but with a lower quantum yield (Figure 2D). Therefore, DNA11 appears to be the best candidate amongst the tested DNAs for visual detection.

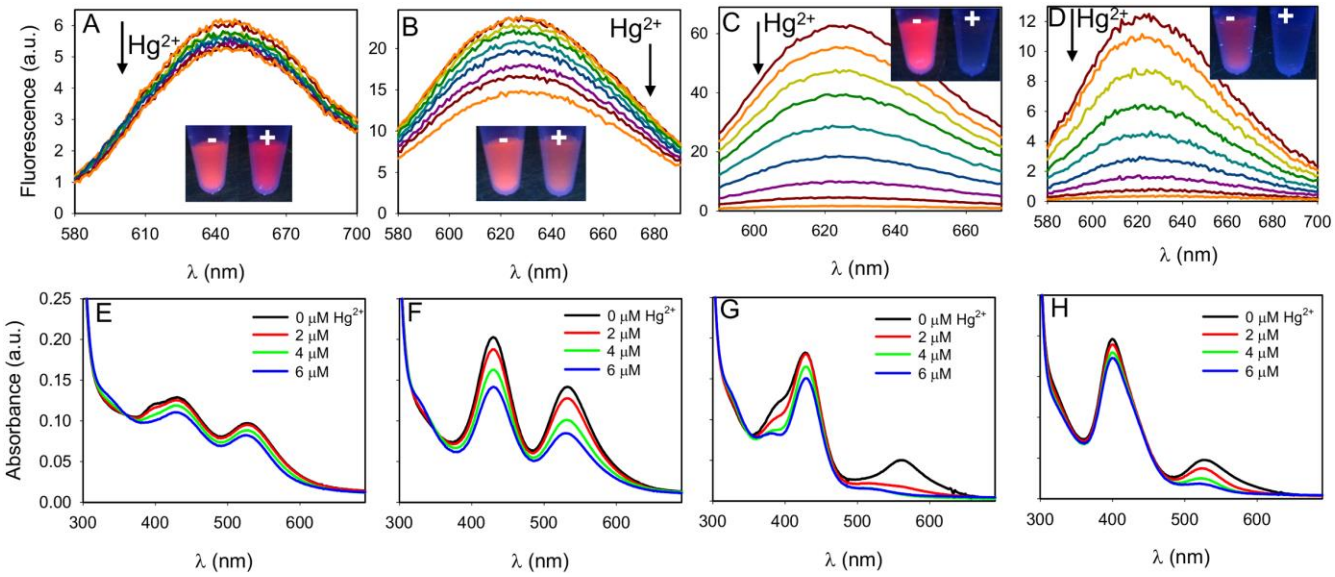

Figure 2. Fluorescence quenching induced by up to 200 nM Hg^{2+} using AgNCs templated by DNA9 (A), DNA10 (B), DNA11 (C) and DNA12 (D). The excitation wavelength was 550. Insets: photographs of sample fluorescence in the absence (left) or presence (right) of 4 μM Hg^{2+}. UV-vis spectra as a function of Hg^{2+} concentration of AgNCs templated by DNA9 (D), DNA10 (E) and DNA11 (F).

All the four hairpin DNAs produced red emission peaks. Their sensitivity to Hg^{2+} was compared, where DNA9 (i.e. smallest loop) was the least sensitive and DNA11 and 12 (i.e. largest loops) were the most sensitive. To further understand this observation, we measured the UV-vis absorption of the AgNCs in the presence of Hg^{2+}. For each AgNC sample, there were two absorption peaks, one at 430 nm and the other at 540 nm (Figure 2E-H) (Lan et al. 2011; Morishita et al. 2013). The 540 nm peak corresponded to the red fluorescence excitation maximum and thus should be responsible for the red fluorescence emission. AgNCs templated by DNA9 did not show much spectral

change after the addition of Hg^{2+} (Figure 2E). About 40% drop was observed with DNA10 (Figure 2F), while the absorption dropped to the background level with DNA11 and DNA12 (Figure 2G, H). Therefore, the change of the absorption peak at 540 nm shares the same trend as the fluorescence change. Our data support the conversion of fluorescent AgNCs to other species that disrupted the electronic absorption of the original fluorescent AgNCs. This observation is consistent with previous fluorescence lifetime studies, where fluorescence quenching was attributed to the ground state instead of excited state events (Guo et al. 2009). It appears that a smaller cytosine loop (e.g. DNA9, 5 cytosines) allows tighter binding of the red emitter, hindering the reaction with Hg^{2+} and thus retaining fluorescence.

Although a visual fluorescence change was achieved with DNA11 and DNA12, further optimization of DNA sequence is still needed since these are still 'light-off' sensors. To this end, we employed DNA13 shown in Figure 3A. A hairpin structure is likely to form in the presence of Ag^{+}, which can stabilize the C-Ag^{+}-C base pair (Ono et al. 2008). The individual loop cytosines might also bind Ag^{+}. Interestingly, AgNCs templated by this DNA showed orange fluorescence in the absence of Hg^{2+}. Addition of Hg^{2+} produced green fluorescence (inset of Figure 3B). To quantitatively measure Hg^{2+} sensitivity, a careful titration experiment was performed. We found two emission peaks at 620 nm and 520 nm, respectively. The 620 nm red peak was completely quenched by Hg^{2+} while the 520 nm green peak increased by ~30% (Figure 3B), explaining the orange-to-green change. The fact that two peaks are present allows for ratiometric detection (Figure 3C). We obtained a detection limit of 4 nM Hg^{2+} based on signal being three times higher than that of background variation (inset). This is comparable with many other NC-based Hg^{2+} sensors (Guo and Irudayaraj 2011; Guo et al. 2009; Lan et al. 2011; Lan et al. 2010; Xie et al. 2010). Selectivity was tested against several common metal ions. The ratio remained low in the absence of Hg^{2+} and Hg^{2+} was still capable of inducing quenching (Figure 3D). Cu^{2+} was the only interfering ion that also induced significant fluorescence quenching by

itself. This is a commonly observed for AgNCs and Cu^{2+} can be masked by adding 2,6-pyridinedicarboxylic acid (see Figure S2) (Guo and Irudayaraj 2011; Li et al. 2008).

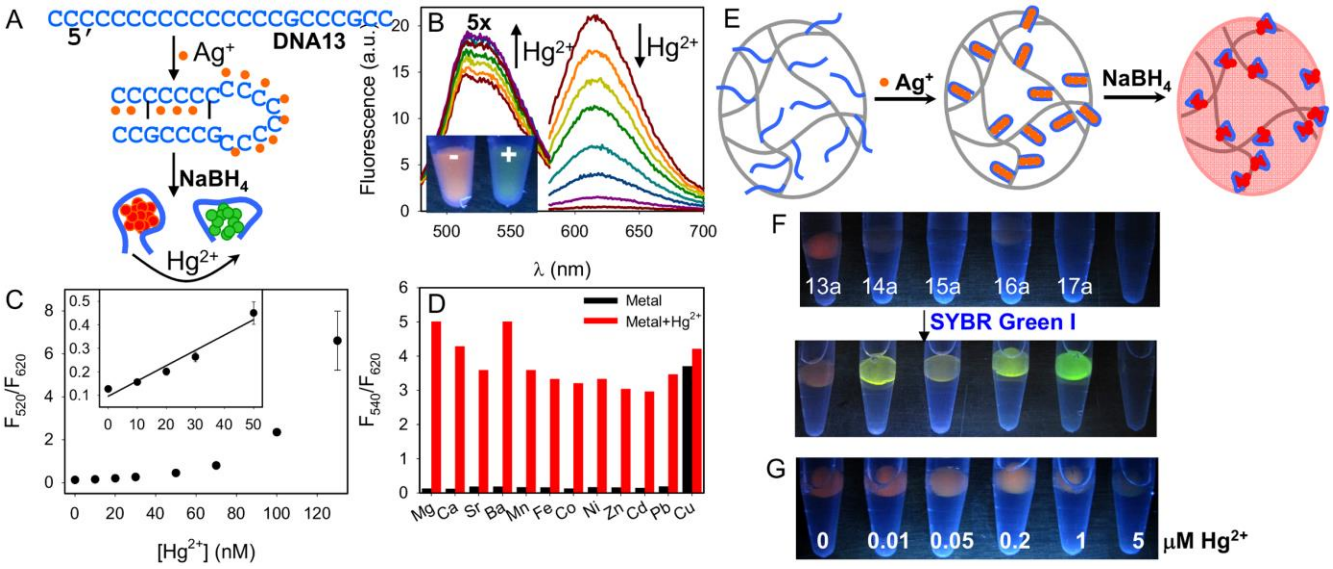

Figure 3. (A) DNA13 templated AgNC synthesis producing both red and green emitters. Adding Hg^{2+} can further induce the conversion from red to green and the quenching of red emitter. (B) Fluorescence spectra of AgNCs templated by DNA13 excited at 436 nm (emission peak = 520 nm) or excited at 557 nm (emission peak = 620 nm). Both red and green emissions are shown. Inset: a photograph of sample fluorescence in the absence (left) or presence (right) of 3 μM Hg^{2+}. (C) Fluorescence intensity ratio as a function of Hg^{2+} concentration. Inset: response at low Hg^{2+} concentrations. (D) Sensor response to competing metal ions (Mg^{2+} and Ca^{2+} = 1 mM; the rest = 1 μM) and to 100 nM Hg^{2+} in the presence of competing metal ions. Note that the Cu^{2+} interference can be masked (see Figure S2). (E) Schematics of immobilizing AgNCs in hydrogels. (F) Fluorescence of hydrogel immobilized with different DNA sequences and responses after adding SYBR Green I. See Supporting Information for DNA sequences. (G) Photograph of immobilized sensor in the presence of various concentrations of Hg^{2+}.

DNA hairpin templated dual emitters have been studied by a number of techniques including electrophoresis, mass spectrometry and fluorescence spectroscopy (Driehorst et al. 2011; Morishita et al. 2013; O'Neill et al. 2009; Ritchie et al. 2007). For example, the red and green emitters templated by another hairpin DNA are assigned to be Ag_{13} and Ag_{11}, respectively. Our AgNCs templated by DNA13 showed very similar spectroscopic properties and thus may be from similar species. Hg^{2+} has a strong effect on the red emitter to quench its fluorescence. At the same time, at least a fraction of the red emitter is converted to the green emitter. The green emitter may have a lower quantum yield, which could explain why the green peaked increased only slightly. An alternative explanation for this color change is that each DNA can template two AgNCs, emitting green and red fluorescence, respectively. These two form a fluorescence resonance energy transfer (FRET) pair and the red emitter acts as an energy acceptor to quench the green emitter. We collected the full range emission spectrum by exciting the green peak (Figure S3). However, no red peak was produced by this excitation, arguing against the energy transfer possibility.

Finally, we aim to test whether this AgNC-based sensor can be immobilized in a hydrogel matrix, since immobilized sensors can be more easily manipulated and for device incorporation. To this end, an acrydite-modified DNA13 (named DNA13a) was co-polymerized with acrylamide. After soaking the gel in $AgNO_3$, $NaBH_4$ was added as the reducing agent (see Figure 3E for this process). The resulting gel emitted similar red fluorescence under UV excitation (Figure 3F) and addition of Hg^{2+} caused green fluorescence. The detection limit was ~50 nM Hg^{2+} based on visual inspection. To ensure that the AgNCs were templated by the C-rich DNA instead of the gel matrix, we compared the effect of different acrydite-modified DNA sequences. As shown in Figure 3G, the brightest fluorescence was observed with the C-rich DNA. Very weak fluorescence was observed with T-rich or other sequences while no fluorescence was observed in the absence of DNA. All the DNAs have been

successfully conjugated to the hydrogels since adding SYBR Green produced bright fluorescent for all the samples. Therefore, it is the immobilized C-rich DNA13a that templated the formation of AgNCs.

4. Conclusions

In summary, we have reported the first metal-NC-based fluorescence Hg^{2+} sensors with a visual color change and allowing ratiometric detection. By varying the DNA loop size, AgNC sensitivity to Hg^{2+} can be precisely tuned and larger hairpin gives higher sensitivity to Hg^{2+}. Hg^{2+} can also increase the emission intensity of certain fluorescence peaks, making ratiometric detection possible. This sensor has been immobilized in a monolithic hydrogel matrix.

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Supplementary Information available*: materials and methods, kinetics, effect of salt and pH, masking agent for* Cu^{2+}*. See DOI...*

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