

Correlation of photobleaching, oxidation and metal induced fluorescence quenching of DNA-templated silver nanoclusters†

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Few-atom noble metal nanoclusters have attracted a lot of interest due to their potential applications in biosensor development, imaging and catalysis. DNA-templated silver nanoclusters (AgNCs) are of particular interest as different emission colors can be obtained by changing the DNA sequence. A popular analytical application is fluorescence quenching by Hg^{2+} , where d^{10} – d^{10} metallophilic interaction has often been proposed for associating Hg^{2+} with nanoclusters. However, it cannot explain the lack of response to other d^{10} ions such as Zn^{2+} and Cd^{2+} . In our effort to elucidate the quenching mechanism, we studied a total of eight AgNCs prepared by different hairpin DNA sequences; they showed different sensitivity to Hg^{2+} , and DNA with a larger cytosine loop size produced more sensitive AgNCs. In all the cases, samples strongly quenched by Hg^{2+} were also more easily photobleached. Light of shorter wavelengths bleached AgNCs more potently, and photobleached samples can be recovered by NaBH_4 . Strong fluorescence quenching was also observed with high redox potential metal ions such as Ag^+ , Au^{3+} , Cu^{2+} and Hg^{2+} , but not with low redox potential ions. Such metal induced quenching cannot be recovered by NaBH_4 . Electronic absorption and mass spectrometry studies offered further insights into the oxidation reaction. Our results correlate many important experimental observations and will fuel the further growth of this field.

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Introduction

Few-atom noble metal nanoclusters (NCs) have emerged as a class of promising materials for biosensor development, imaging and catalysis.^{1–6} Unlike metal nanoparticles, NCs possess well-defined structures and discrete electronic energy bands that allow radiative relaxation to produce fluorescence with a high quantum yield.⁷ Compared to semiconductor quantum dots,⁸ gold and silver NCs have fewer toxicity concerns. As a bridge between traditional organometallic chemistry and nanoparticle science, NCs are important for fundamental studies. Most NCs are prepared by reducing metal salts. To prevent a rapid growth into nanoparticles, such reduction reactions are often carried out in the presence of a polymeric ligand (e.g. proteins, DNA, or synthetic polymers).^{2,7,9–17} Cytosine-rich DNAs bind to Ag^+ strongly,^{9,18} and many DNA-stabilized AgNCs with various emission colors have been prepared by changing the DNA sequence.^{19–23} On the

practical side, by rational incorporation of DNA aptamers, the fluorescence signaling of AgNCs has been successfully coupled with the molecular recognition property of DNA to design biosensors and smart imaging probes.^{24–36}

An interesting and useful discovery is that the fluorescence of many NCs is strongly quenched by Hg^{2+} , allowing its detection at low nM and even sub-nM concentrations.^{20,37–42} Despite its importance in analytical chemistry, our fundamental understanding of NCs is still quite limited. For example, Hg^{2+} -induced quenching has often been attributed to the d^{10} – d^{10} metallophilic interactions with Au^+ or Ag^+ that may be present in NCs.^{37,43} However, Zn^{2+} and Cd^{2+} do not induce NC fluorescence quenching, even though they also possess the d^{10} electronic structure. In addition, we noticed that most AgNCs used for Hg^{2+} detection emit red fluorescence, but it is unclear whether different NCs have the same sensitivity to Hg^{2+} or not. Furthermore, some AgNCs are susceptible to oxidation. Dickson and co-workers showed that oxidized NCs have a completely different fluorescence property.⁴⁴ Several groups have also reported the conversion of AgNCs with different emission colors through an oxidation reaction.^{44–46} In some cases, oxygen was identified as a requirement to produce fluorescent AgNCs; in an oxygen-free condition, only non-fluorescent species were produced.⁴⁷ Finally, many AgNCs have relatively low photostability.^{44,48,49} In this work, we take advantage of the fact that AgNCs of different emission colors can be produced using

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different DNA sequences, and we prepared a total of eight AgNCs. We show that AgNCs have different sensitivity to Hg^{2+} and different photostability. Instead of the d^{10} – d^{10} metal-ligand interaction, we propose that redox reaction is the main force driving metal ions to interact with AgNCs. This work unifies oxidation, photobleaching and Hg^{2+} sensitivity to the redox property of AgNCs.

Materials and methods

Chemicals

All of the DNA samples were purchased from Integrated DNA Technologies (IDT, Coralville, IA) and purified by standard desalting. AgNO_3 , NaBH_4 , $\text{Hg}(\text{ClO}_4)_2$, HAuCl_4 , CuSO_4 , MnCl_2 , CoCl_2 , NiSO_4 , ZnCl_2 , $\text{Pb}(\text{OAc})_2$ and $\text{Cd}(\text{NO}_3)_2$ were from Sigma-Aldrich. 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.

AgNC preparation

For fluorescence measurements, each sample contained 15 μM DNA and 120 μM AgNO_3 dissolved in 25 mM HEPES buffer (pH 7.6) with 270 μM NaBH_4 as the reducing agent. NaBH_4 was freshly prepared right before its addition. After the addition of NaBH_4 , the sample was rapidly mixed and stored in the dark for overnight reaction. Alternatively, if 120 μM NaBH_4 was used, the incubation was ~ 2 h.

Fluorescence spectroscopy

AgNCs from the above preparation (30 μL) were dissolved in 570 μL of 10 mM HEPES buffer (pH 7.6). The excitation peak in the visible region was determined first and then the emission peaks were scanned at the excitation maximum. All fluorescence spectra were collected using a Varian Eclipse fluorometer at room temperature.

Light and Hg^{2+} sensitivity

For light sensitivity, the fluorescence spectra were collected before and after 5, 10, 15, 20, 25 and 30 min exposure to a fluorescent tube light. For Hg^{2+} sensitivity, fluorescence spectra of the samples were collected at 0, 10, 20, 30, 50, 70, 100, 150 and 200 nM Hg^{2+} . A few microliters of 5 or 20 μM Hg^{2+} solutions were added to achieve the designated Hg^{2+} concentration. To test the sensitivity of AgNCs to other metal ions, solutions of the metal salts were prepared and fluorescence spectra were collected at 0, 200 and 2000 nM metal ion concentrations. All the reactions were carried out in 10 mM HEPES buffer (pH 7.6). The slopes of Hg^{2+} from 0 to 100 nM and light exposure from 0 to 5 min were used for calculating sensitivity.

Fluorescence recovery using NaBH_4

After an initial scan, the samples were exposed to 200 nM Hg^{2+} or 30 min continuous fluorescent tube light. The emission spectrum was collected again, and then 1 μL of 2 mM NaBH_4

was added to the samples. The samples were moved to the dark for one hour to allow for fluorescence recovery. They were then scanned once more to check for fluorescence recovery. This procedure of Hg^{2+} /light exposure and the addition of NaBH_4 was repeated two more times. A fluorescence spectrum was collected after each step, yielding a total of 7 measurements for each sample.

Light wavelength sensitivity

Fluorescence spectra were obtained after 0, 2, 5, 10 and 15 min light exposure from the lamp in our fluorometer. The wavelength of exposure light was fixed by the fluorometer (slit width = 10 nm). The wavelengths tested were 225, 250, 275, 300, 400, 436, and 557 nm (DNA1), and 225, 250, 275, 300, 350, and 466 nm (DNA4).

Mass spectrometry

Samples containing 150 μM DNA1, 1.2 mM AgNO_3 and 1.35 mM NaBH_4 were dissolved in water without additional buffer. After vigorous mixing and overnight reaction in the dark, mercury was added, yielding an Hg^{2+} concentration of 40 μM . A small amount of NaBH_4 was added to the sample without light or Hg^{2+} exposure right before the measurement to eliminate oxidized AgNCs. The samples were centrifuged and the supernatant was removed and mixed with water–acetonitrile (1 : 1) containing 0.5% NH_4OH to facilitate ionization. Mass spectra were obtained using a Micromass Q-TOF Ultima Global mass spectrometer using electrospray ionization (ESI).

UV-vis spectroscopy

The electronic absorption of AgNCs was monitored using a UV-vis spectrophotometer (Agilent 8453A). The samples for UV-vis were prepared in the same way as that in the mass spectrometry studies. The final DNA concentration in the cuvette was 30 μM and the Hg^{2+} concentration was 20 μM . The samples were dissolved in water without additional salt or buffer.

Results and discussion

Correlation between light and Hg^{2+} exposure

Using a cytosine-rich DNA that can fold into a hairpin in the presence of Ag^+ (DNA1, see Fig. 1B for the DNA sequence), we prepared a dual emitting AgNC sample with both red and green fluorescence peaks. Such hairpin-stabilized dual emitters have also been reported by using other DNA sequences.⁴⁵ As shown in Fig. 2A, the red peak was almost completely quenched by 200 nM Hg^{2+} , whereas the green peak increased slightly in the same process. Since these two AgNCs were tested in the same tube, we can conclude that the red emitter is much more sensitive to Hg^{2+} than the green emitter. Some of the red emitters might even be converted to the green emitter by Hg^{2+} . This is the first report that AgNCs have different sensitivity to Hg^{2+} . In this case, the green and red peaks were respectively excited at 436 nm and 557 nm to achieve a high quantum yield for their emissions at 520 and 620 nm. To monitor both emission peaks at the same time, excitation at 230 nm was also employed.

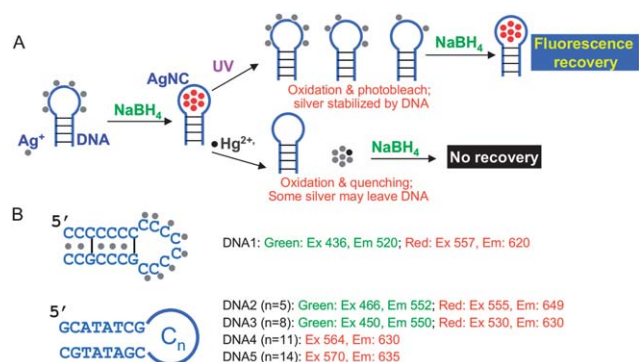


Fig. 1 (A) Schematics of DNA templated synthesis of a fluorescent AgNC and its reaction with UV light and metal ions of high oxidation power. The gray dots are Ag^+ ions and the red dots are silver in fluorescent AgNCs. Note that the sensitivity to UV and to Hg^{2+} is correlated through oxidative reactions but through different reaction mechanisms. UV-induced quenching can be recovered by adding NaBH_4 , but Hg^{2+} -induced AgNC quenching cannot be recovered since the AgNC may dissociate from the DNA. (B) DNA sequences used in this study and the excitation and emission wavelength of AgNCs produced by these DNAs. Some DNAs produce dual emitters.

Although 260 nm is likely to provide a more efficient excitation *via* DNA base absorption,⁵⁰ we refrained from choosing 260 nm to avoid a double frequency artifact that masks the 520 nm emission peak. As shown in Fig. 2B (black spectrum), both peaks were excited simultaneously with a comparable initial emission intensity. As more fluorescence scanning was performed, the green peak increased while the red peak dropped even in the absence of Hg^{2+} . After 9 scans with each scan taking 3 min, the red peak dropped $\sim 80\%$. The presence of an isosbestic point at 560 nm is a strong indication of the conversion between the two emitters. Since UV light exposure produced similar results to those achieved with the addition of Hg^{2+} (*e.g.* increased green and decreased red), we want to test whether there is such a correlation for other AgNCs. It needs to be pointed out that light induced production of green fluorescence appears to be more efficient than that induced by Hg^{2+} . Therefore, the exact chemical process might not be the same for these two stimuli but they may share the same origin.

To test whether there is a general correlation between light and Hg^{2+} sensitivity, we employed four additional DNA hairpins

(DNA2-5, see Fig. 1B) with various sized poly-cytosine loops. DNA2 and 3 also produced dual emitters with both green and red emissions, while only red fluorescence was observed with DNA4 and 5. Since three of the DNAs produced dual emitters, a total of eight emitters were measured. For each emitter, the excitation maximum in the visible region was used to collect the fluorescence spectrum, since UV excitation might change its fluorescence properties during the measurement. The light sensitivity was obtained by exposing the samples to a fluorescent tube lamp at 5 min intervals. The fluorescence spectra of a light sensitive sample templated by DNA4 are presented in Fig. 3A. Within 30 min, the fluorescence was quenched by $\sim 80\%$. For the Hg^{2+} sensitivity test, the sample was challenged with up to 200 nM Hg^{2+} and its fluorescence was quenched by $\sim 90\%$ (Fig. 3B). The same procedure was repeated for all the other AgNCs.

It is particularly interesting to compare DNA2-5 since they produced a similar red emission color but were stabilized by DNA hairpins of different sizes. The AgNCs templated by DNA2 (*e.g.* 5-cytosine loop) were quenched only $\sim 20\%$ by Hg^{2+} and were also the least sensitive to light exposure (Fig. 3C and D, black dots). For DNA4 and 5 with 11 and 14 cytosines, respectively, the majority of the fluorescence was quenched by light and Hg^{2+} . Therefore, as the hairpin loop size was increased, both light and Hg^{2+} sensitivity of the red emitters increased. The sensitivities of the green emitters from DNA2 and DNA3 as well as the two emitters from DNA1 are presented in Fig. 3E and F. The green emitter from DNA1 was increased by both light and Hg^{2+} , while the red emitter was almost completely quenched. If the sample is stored in dark, the decrease of fluorescence is $\sim 1\%$ every 30 min (Fig. S1, ESI†).

Since the initial quenching induced by Hg^{2+} appeared to be linear for most samples, we used the initial slope (*e.g.* Hg^{2+} concentration from 0 to 100 nM) to quantify Hg^{2+} sensitivity. For light sensitivity, the initial linear part was also calculated. The relationship between Hg^{2+} and light sensitivity is plotted in Fig. 4. A linear relationship was observed for all tested AgNCs with an R^2 value of 0.971. The green emitter templated by DNA1 was the only one that showed a significant increase in fluorescence and thus positive sensitivity for both light and Hg^{2+} exposure. This linear correlation strongly suggests that Hg^{2+}

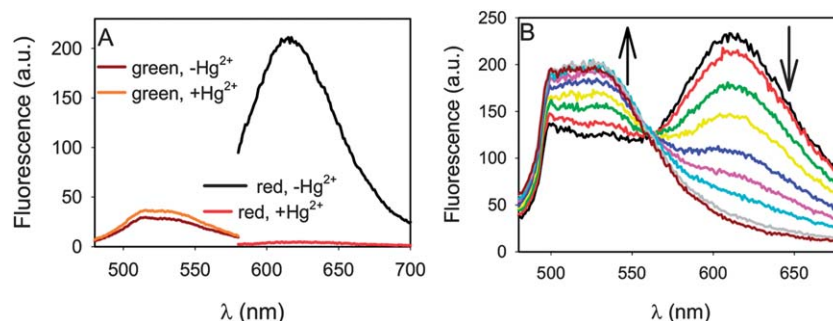


Fig. 2 (A) Fluorescence spectra of AgNCs templated by DNA1 before and after adding 200 nM Hg^{2+} . There are two emitters in this sample, and the excitation wavelengths are shown in Fig. 1B. The green emitter is enhanced slightly by Hg^{2+} and the red emitter is quenched. (B) Fluorescence spectra of AgNCs templated by DNA1 excited at 230 nm. A total of 9 scans were measured, each taking ~ 3 min. The initial spectrum is in black. The 620 nm emission peak intensity is decreased while the 520 nm peak is increased as the number of scans is increased. No Hg^{2+} was added to this sample.

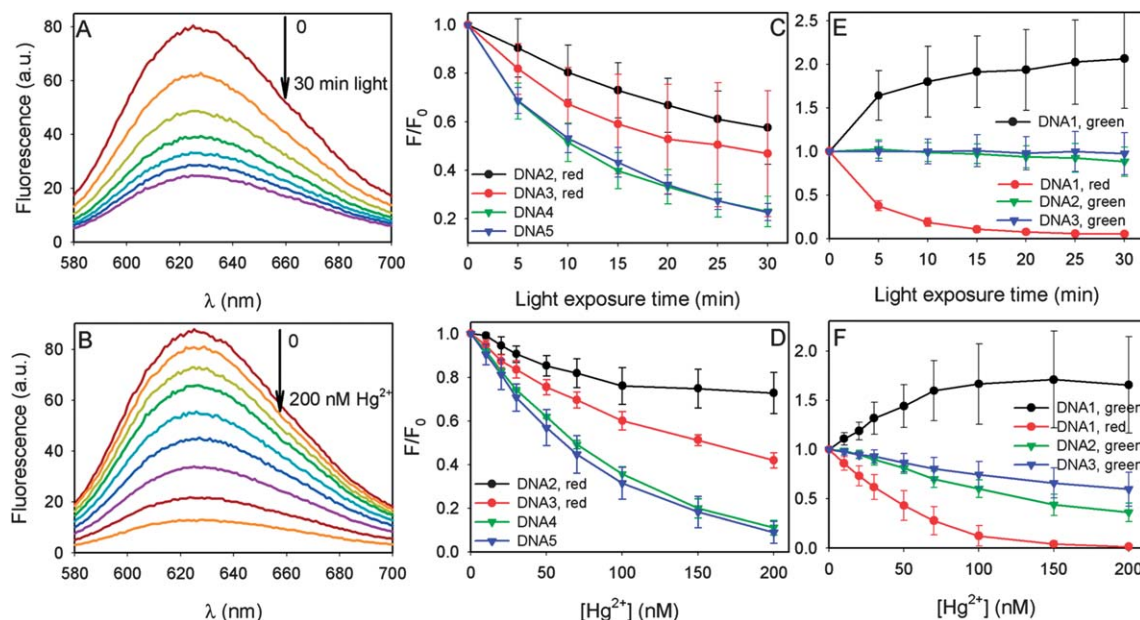


Fig. 3 Fluorescence spectra of AgNCs templated by DNA4 with light (A) or Hg^{2+} exposure (B). Fluorescence sensitivity to Hg^{2+} (D and F) and light (C and E) of various AgNCs.

binding and light sensitivity may share the same origin (note that the reactions themselves are likely to be different). This study also indicates that the DNA sequence plays a crucial role in affecting the interaction between AgNCs and Hg^{2+} or light. It is likely that a smaller hairpin allows tighter binding of AgNCs, blocking their interaction with Hg^{2+} .

Sensitivity to the wavelength of light

Since the fluorescent tube light contains a broad range of wavelengths, to systematically test the wavelength dependency of photobleaching, we exposed AgNCs templated by DNA1 and 4 to selected wavelengths from the fluorometer lamp. DNA1 was chosen to represent dual emitters, and it showed an interesting red-to-green conversion. DNA4 was chosen because it produced a highly fluorescent red emitter (quantum yield = $\sim 45\%$ using Rhodamine-B as standard). For the emitter by DNA4, the strongest bleaching occurred with the shortest 225 nm exposure (Fig. 5A). No bleaching was observed with wavelengths longer

than 300 nm. Even with its direct excitation wavelength of 566 nm exposure, little photobleaching occurred. Similar observations were also made for AgNCs templated by DNA1, which produced dual emitters (Fig. 5B). The green emitter intensity increased and the red was quenched upon UV light exposure. The change of intensity was again more drastic with shorter wavelength exposure. Exposure to its direct excitation wavelengths at 436 and 557 nm also failed to induce emission change (data not shown). Therefore, the energy of incoming light is critical for the photobleaching of AgNCs. DNA-templated AgNCs can be excited either *via* DNA base absorption and subsequent energy transfer or directly *via* visible light.⁵⁰ Since 225 nm exposure showed a stronger bleaching effect than 250 nm exposure, photobleaching should not occur *via* DNA base absorption (*e.g.* DNA bases absorb at ~ 260 nm). To minimize photobleaching, it is thus better to excite AgNCs in the visible region for fluorescence spectral measurements. Short wavelength UV light generates reactive oxygen species with strong oxidation power.⁵¹ Therefore, oxidation might be the ultimate reason for the photobleaching of AgNCs.

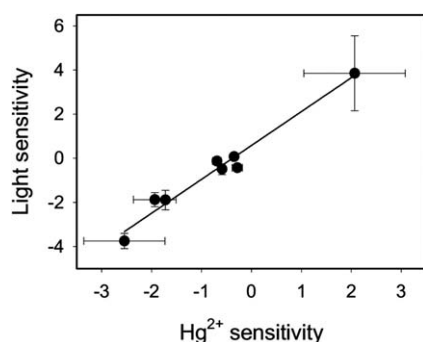


Fig. 4 Correlation between Hg^{2+} sensitivity and light sensitivity of the AgNCs prepared in this study.

Photobleaching and oxidation

Excited fluorophores have higher energy and more easily react with molecules such as oxygen to generate non-fluorescent species. Such light-induced fluorescence decrease is called photobleaching, which is usually an irreversible process for organic fluorophores. We observed that UV light can achieve the conversion between the red and green emitters templated by DNA1 (Fig. 2). In addition, shorter wavelengths are more effective for the bleaching reaction. Therefore, we postulate that the effect of light on AgNCs is to induce oxidation. We first tested this hypothesis using AgNCs templated by DNA4. After measuring its initial fluorescence intensity, the sample was exposed to light for

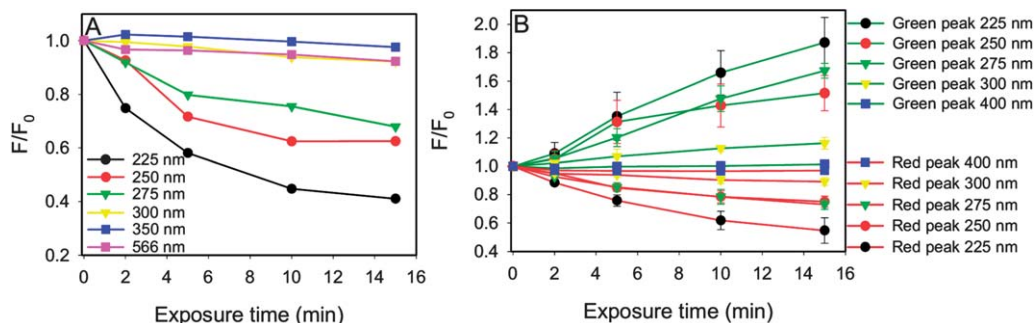


Fig. 5 Relative fluorescence change of AgNCs templated by DNA4 (A) and DNA1 (B) after exposure to light at designated wavelengths over time. DNA1 produced two emitters, where the green emitter is enhanced by light exposure.

30 min, which quenched its fluorescence (Fig. 6A). The addition of a reducing agent (NaBH_4) resulted in fluorescence exceeding the original value, suggesting that the initial sample was already partially oxidized. This process can be repeatedly performed, suggesting that photobleaching is a reversible oxidation reaction. DNA1 produced dual emitters and we also studied this sample (Fig. 6B). There is an anti-correlation between the red and green emitters. The green fluorescence increased upon light exposure but decreased upon NaBH_4 treatment, suggesting that it is the oxidized species while the red emitter is the reduced species. This bleaching and recovery operation can also be carried out for many cycles. Using these two examples, we reason that light-induced bleaching is through oxidation of AgNCs. The oxidation product can be non-fluorescent (e.g. DNA4) or a different fluorescent cluster in the case of DNA1.

Since a strong correlation between light and Hg^{2+} sensitivity has been shown in Fig. 4, a related question is whether the Hg^{2+} treated samples can also be recovered by adding NaBH_4 . Ying and co-workers showed partial fluorescence recovery of BSA

stabilized AuNCs quenched by Hg^{2+} .³⁷ However, only one regeneration step was demonstrated in that study. For our AgNCs templated by DNA1, red fluorescence recovery and the anti-correlation between red and green were observed (Fig. 6D). In addition, multiple regeneration cycles were also achieved. Compared to the light exposure/recovery in Fig. 6B, however, the fluorescence intensities decayed significantly as the number of recovery cycles increased for the Hg^{2+} treated sample (e.g. $\sim 50\%$ loss for each regeneration). Therefore, while the effect of Hg^{2+} is similar to that of light as an oxidation agent, Hg^{2+} also induced irreversible reactions. This irreversible component was even more pronounced for DNA4 (Fig. 6C), where fluorescence decreased upon the first addition of Hg^{2+} and a slight recovery was achieved by the first addition of NaBH_4 . However, no subsequent recovery was observed by adding more Hg^{2+} and NaBH_4 . We attribute this initial recovery to the reduction of oxidized species (previously oxidized by air) by NaBH_4 , while Hg^{2+} induced quenching cannot be recovered by NaBH_4 . Once these regenerated emitters were also quenched by Hg^{2+} in its

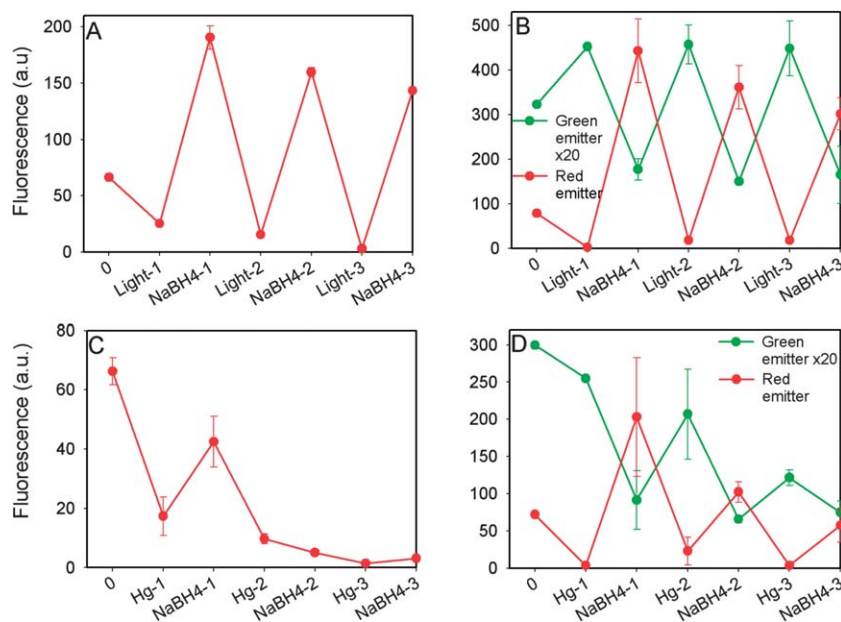


Fig. 6 Fluorescence recovery of AgNCs by adding NaBH_4 for samples templated by DNA4 after exposure to light (A) or to 200 nM Hg^{2+} (C) and for samples templated by DNA1 after exposure to light (B) or to Hg^{2+} (D). The light source is ambient fluorescent tube light.

second addition, no more regeneration could take place. Bringing these results together, Hg^{2+} acts as an irreversible oxidizing agent while light induced oxidation is reversible. DNA1 stabilizes two emitters; the green emitter is less affected by Hg^{2+} and thus acts as a reservoir for the red emitter during the NaBH_4 reaction. The addition of Hg^{2+} converts some of the red emitters back to green, and the rest of the red emitters are irreversibly quenched. The percentage of the red emitters that undergo Hg^{2+} induced irreversible quenching or reversible conversion to green is comparable, leading to only $\sim 50\%$ recovery for the red peak each time (Fig. 6D). This is not the case for DNA4 since it has only one emitter. Hg^{2+} eliminates this emitter and it cannot be reversed by adding NaBH_4 . To ensure that fluorescence quenching upon light exposure is not related to DNA damage, we performed a control experiment by exposing DNA to light first followed by AgNC synthesis. In that case, we observed the same AgNC fluorescence intensity regardless of whether DNA was exposed to light (for 30 min) or not (see Fig. S2, ESI†).

To further understand these reactions, the AgNCs were analyzed by UV-vis spectroscopy. AgNCs templated by DNA4 show two absorption peaks at ~ 440 nm and 560 nm (Fig. 7A, black curve). Note that the 560 nm peak matches the excitation wavelength of this AgNC. The addition of Hg^{2+} eliminated the light absorption at 560 nm (red curve), which is reflected by the color change (inset) and is the direct reason for fluorescence quenching. This indicates that the 560 nm (absorption) emitter is converted to other species that does not show absorption features in the visible region. Exposure of the same sample to light also eliminated the 560 nm peak, but the 420 nm peak increased in the same process (green curve), indicating the conversion of the red emitter to non-fluorescent but still absorbing clusters. The 560 nm peak recovered after treating the sample with NaBH_4 (Fig. S3, ESI†), confirming reversibility. For AgNCs templated by DNA1, two absorption peaks were again observed. In this case, both peaks produce fluorescence emissions. The addition of Hg^{2+} reduced the 500 nm peak more than the 400 nm peak, which is similar to that for DNA4. Light exposure only reduced the 500 nm peak while the 400 nm appeared to increase just slightly.

For both DNA samples, light exposure seems to convert the red emitters to other species that can still be detected by light

absorption. Hg^{2+} exposure, on the other hand, seems to eliminate the red emitters without generating new species that can absorb in the visible region. This may explain the difference between light and Hg^{2+} exposure in terms of fluorescence recovery by adding NaBH_4 .

To further understand the reaction mechanism, we followed the Hg^{2+} and light reaction using mass spectrometry. Fig. 8A shows the mass distribution of the AgNCs templated by DNA1. It has a peak of free DNA1, the complex of the DNA1-Ag₁₀ cluster as a major peak and also DNA1-Ag₁₃. After Hg^{2+} addition, the color of the sample changed from brown to yellow but the speciation in the mass spectrum changed only slightly (Fig. 8C). The relative abundance of the Ag₁₀ peak decreased by $\sim 50\%$. Interestingly, the Ag₁₃ peak disappeared while a new peak of Ag₁₂ appeared. Since Hg^{2+} completely quenches the fluorescence of the sample, it is unlikely that Ag₁₀ is the fluorescent species. We assigned Ag₁₃ to be the red emitter, which agrees with the literature report.^{44–46} Nevertheless, the amount of Ag associated with DNA was significantly reduced after reacting with Hg^{2+} . The light-exposed sample showed a completely different pattern (Fig. 8B). Complexes formed by DNA1 with Ag₁ to Ag₇ were identified. The relative abundance of the free DNA is decreased. This result also explains that light induced oxidation is reversible since the silver species are still associated with DNA and the addition of NaBH_4 re-forms AgNCs. In the presence of Hg^{2+} , the overall association between Ag and DNA is weakened and even disrupted, leading to an irreversible reaction.

The commonly proposed reaction mechanism for Hg^{2+} induced AgNC quenching can be generalized in eqn (1) of Fig. 8D, where Hg^{2+} is believed to associate with AgNCs. It may also displace some of the silver in the cluster. However, this mechanism is not supported by our mass spectrometry data. We propose in eqn (2) that Hg^{2+} causes the AgNC to leave the DNA scaffold, or at least the binding is weakened so that dissociation occurs under mass spectrometry conditions. It needs to be pointed out that we do not have evidence that shows whether or how Hg^{2+} is associated with silver after the reaction. The mechanism of a light induced reaction is proposed in eqn (3), where the released silver species are still associated with DNA. To further test our mechanism, EDTA was added to the sample containing Hg^{2+} and no fluorescence recovery was observed

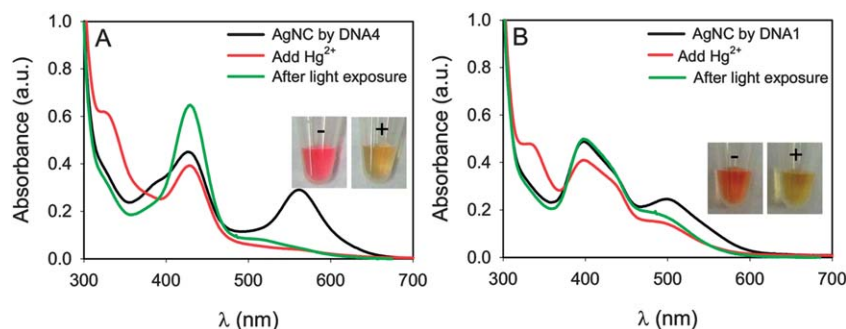


Fig. 7 UV-vis spectra of AgNCs templated by DNA4 (A) and DNA1 (B) upon Hg^{2+} and light exposure. Insets: photographs of AgNCs in the absence and presence of Hg^{2+} . The Hg^{2+} concentration is 40 μM in the inset.

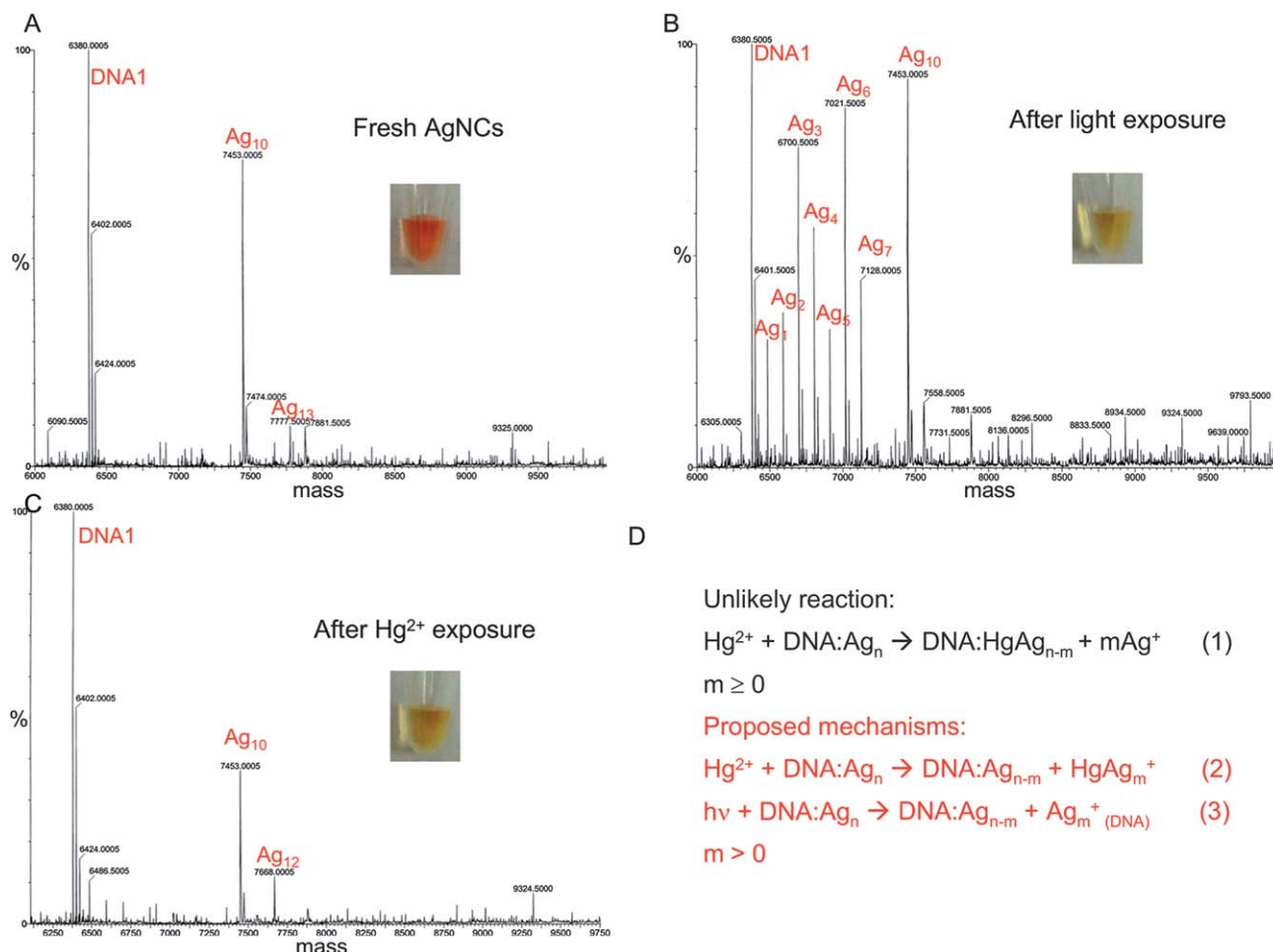


Fig. 8 ESI mass spectrometry characterization of AgNCs templated by DNA1 (A) and its reaction with light (B) or Hg²⁺ (C). (D) Previously implied reaction mechanism (1); proposed reaction mechanisms with Hg²⁺ (2) and light (3) in this work.

(Fig. S4A, ESI[†]). If EDTA was added before Hg²⁺ addition, then Hg²⁺ cannot induce fluorescence quenching (Fig. S4B[†]), suggesting the high affinity between Hg²⁺ and EDTA. This experiment also argues against the metallophilic mechanism since Hg²⁺ might be removed by EDTA in that case.

We reason that the sensitivity to light and Hg²⁺ correlates with each other because they are both related to the ability of this AgNC to be oxidized. However, the mechanism of each reaction is different. We call the light induced reaction to be an oxidation reaction since the fluorescent product can be recovered by adding a reducing agent. It needs to be noted that oxidation does not mean the insertion of oxygen into the AgNC. In fact, such oxygen adducts were not detected. It is more likely to be a number of splitting reactions involved in reducing the size of the AgNCs, where Ag⁺ ions are produced and electrons are lost (likely to oxygen) in this process.

Sensitivity to other metal ions

To provide more evidence to link the Hg²⁺ reaction to the redox property of AgNCs, we examined a number of metal ions with different redox potentials. Similar tests have been previously performed to demonstrate selectivity for Hg²⁺, where highly

oxidative metals such as gold and silver were often omitted. We added 200 nM and 2 μM of metal ions (Fig. 9) to DNA4 templated AgNCs. Cu²⁺, Ag⁺, and Au³⁺ also showed significant quenching of the fluorescence spectra (Fig. 9A–C). Other lower oxidation potential ions showed little effect (Fig. 9D–I). This further supports the redox mechanism of the Hg²⁺ reaction.

While it may appear odd to use Hg²⁺ to oxidize Ag because of its anti-galvanic nature, there are a few examples which show that noble metal NCs can be oxidized by metal ions (*e.g.* Cu²⁺ and Pb²⁺) whose oxidation power is even weaker than Hg²⁺. Murray and co-workers reported the oxidation of a Au₂₅ NC by Ag⁺, Cu²⁺ and Pb²⁺ using CH₂Cl₂ as a solvent.⁵² In that case, it was considered that the redox potential of a nanoparticle or cluster might differ from the bulk metal and that the solvent may also influence the potentials. This Au₂₅ NC has a number of charged states. To rule out that its anionic form may have a different reducing ability, Wu studied the neutral Au₂₅ and confirmed that it reduces Cu²⁺ and Ag⁺ but not Pb²⁺, Fe²⁺ or Ni²⁺.⁵³ These experiments were again performed in organic solvents. For many Hg²⁺ sensors based on AgNCs, Cu²⁺ is a common interfering ion. All these literature reports are in line with our hypothesis to link Hg²⁺ with redox reactions. Another method to confirm oxidation is to use X-ray photoelectron

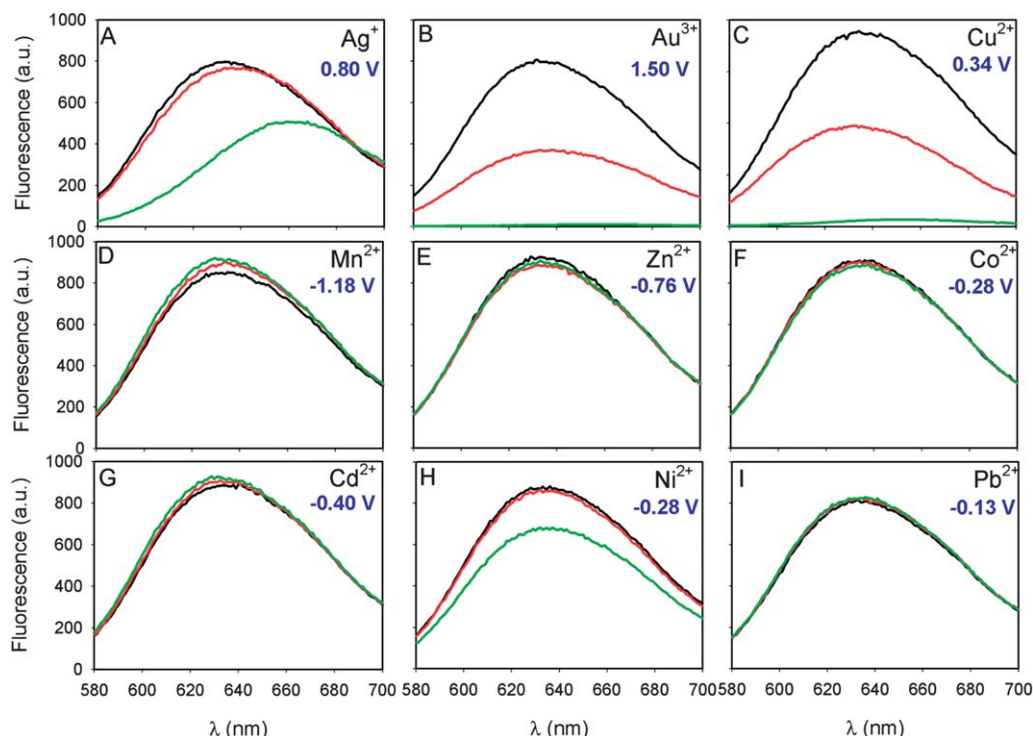


Fig. 9 Fluorescence spectra of AgNCs templated by DNA4 (black spectra) and after the addition of 200 nM (red) and 2 μ M (green) of various metal ions. The redox potentials for the half reaction of turning metal ions to elemental metal are also shown.

spectroscopy (XPS) to measure the oxidation state of silver. However, this requires sample drying. Due to the instability of our sample, significant loss of fluorescence was observed even after drying in vacuum (Fig. S5, ESI[†]), preventing us from performing meaningful XPS experiments.

Attributing all metal induced Au or AgNC quenching to redox reactions is likely to be over-simplified. Other fluorescence quenching mechanisms may also come into play. Shang and Dong noticed a significant quenching of AgNCs stabilized by poly(methacrylic acid) and they attributed it to the binding of Cu^{2+} to the polymer.⁴¹ This was supported by the lack of electronic absorption spectrum change upon Cu^{2+} addition and the inhibited quenching by adding the free monomer. This quenching was reversed by adding a Cu^{2+} chelating agent. The energy transfer from AgNC to the nearby Cu^{2+} was also proposed to be a possibility. Chang and co-workers synthesized AuNCs capped by a carboxyl ligand and demonstrated fluorescence quenching in the presence of Pb^{2+} , Hg^{2+} or Cd^{2+} .⁵⁴ It is likely that quenching was achieved by AuNC aggregation since these metals can be chelated by the surface ligand. Energy transfer to quenchers was another mechanism to achieve quenching.⁵⁵ Wang and co-workers showed the lack of fluorescence lifetime change by Hg^{2+} induced AgNC quenching, suggesting disruption of the ground state of AgNCs by Hg^{2+} , which is consistent with our UV-vis and mass spectrometry measurement.³⁹ Ying and co-workers first proposed the direct interaction between Hg^{2+} and Au^+ in a protein-stabilized AuNC (*i.e.* d^{10} - d^{10} metal-philic interaction).³⁷ The latter two systems are more directly related to our current study, where we propose that the force driving Hg^{2+} to interact with AgNCs is from redox reactions.

Conclusions

In summary, based on the previous work that fluorescent AgNCs with different emission properties can be produced using different DNA sequences, we further demonstrated that these AgNCs also have different sensitivity to Hg^{2+} and light exposure. In this regard, DNA serves as a tool that allow us to synthetically access various AgNCs. Some AgNCs showed a sharp decrease in fluorescence after exposure to light and Hg^{2+} , while other sequences can effectively protect the associated clusters or even enhance fluorescence. A linear relationship between the sensitivity to light exposure and to Hg^{2+} was identified, suggesting a common origin for AgNCs to respond to these two stimuli. The energy of the light is critical for its photobleaching activity, and shorter wavelengths produce more effective quenching. Photobleaching can be recovered by adding NaBH_4 , suggesting that the nature of photobleaching is an oxidation reaction. UV-vis spectroscopic studies indicate that the fluorescent AgNCs are converted to other species upon light exposure, while mass spectrometry data indicate that this conversion involves a reduction of the number of silver atoms. On the other hand, Hg^{2+} weakens the binding between AgNCs and DNA, and Hg^{2+} induced quenching cannot be recovered by adding NaBH_4 . The correlation between light and Hg^{2+} sensitivity suggests the redox nature of Hg^{2+} reacting with AgNCs, which is further confirmed by measuring AgNC response to other metal ions with different redox potentials. The overall mechanism is summarized in Fig. 1A. In general, AgNCs that can be effectively quenched by Hg^{2+} are also more easily bleached by light, and the nature of photobleaching is an oxidation reaction. This

work unifies many important experimental observations and is important for a better understanding and further development of these NCs for various applications.

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