

Towards understanding of poly-guanine activated fluorescent silver nanoclusters

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Abstract

It has been recently reported that the fluorescence of some DNA templated silver nanoclusters (AgNCs) can be significantly enhanced upon hybridizing with a partially complementary DNA containing a G-rich overhang near the AgNCs. This discovery has found a number of analytical applications but many fundamental questions remain to be answered. In this work, the photostability of these activated AgNCs is reported. After adding the G-rich DNA activator, the fluorescence intensity peaks in ~1 h and then starts to decay, where the decaying rate is much faster with light exposure. The lost fluorescence is recovered by adding NaBH₄, suggesting that the bleaching is an oxidative process. Once activated, the G-rich activator can be removed while the AgNCs still maintain most of their fluorescence intensity. UV-vis spectroscopy suggests that new AgNC species are generated upon hybridization with the activator. The base sequence and length of the template DNA have also been varied, leading to different emission colors and color change after hybridization. G-rich aptamers can also serve as activators. Our results indicate that activation of the fluorescence by G-rich DNA could be a convenient method for biosensor development since the unstable NaBH₄ is not required for the activation step.

Keywords: DNA, fluorescence, silver, biosensor, nanoclusters

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(Some figures may appear in color only in the online journal)

1. Introduction

In recent years, DNA-templated fluorescent silver nanoclusters (AgNCs) have attracted extensive interest in biosensor development since they are highly fluorescent, cost-effective to produce, and less toxic compared to semiconductor quantum dots. In addition, different DNA sequences produce various emission colors [1-6], allowing multiplexed detection. The emission color is likely to relate to the arrangement of silver species and their interaction with the surrounding nucleotides [6]. Their fluorescence property is also sensitive to heavy metal ions, change of pH, light, and nearby nucleotide composition [7-15]. Since DNA is an intrinsic component of such AgNCs, it is quite convenient to harness the molecular recognition property of DNA. Beyond sensing complementary DNA (cDNA), aptamers can recognize a broad range of analytes. A number of aptamer and AgNC based sensors have already been reported [16-20].

A few strategies have been developed to design biosensors using AgNCs. 1) Most AgNCs are sensitive to high redox potential metal ions such as Hg^{2+} and Cu^{2+} , which strongly quench the fluorescence of both Au and AgNCs [21-25]. A few mechanisms have been proposed to explain fluorescence quenching and we recently showed that oxidation might be an important one [26]. 2) The production of AgNCs is usually facilitated by a loop of cytosine-rich DNA. Generation of such loops in the presence of target analytes has been utilized for sensing [27-31]. However, Ag^+ reduction by an unstable chemical (NaBH_4) is a required step in the detection process, which is a challenge for field applications. 3) AgNCs have been used as a simple fluorophore label that can be quenched by external dark quenchers [32-35]. 4) Werner, Martinez and co-workers reported that a G-rich DNA could enhance the fluorescence of a DNA-templated AgNC by over 500-fold (see Figure 1B) [36]. A few biosensors have been designed by manipulating the distance between the G-rich DNA and AgNCs [36-40].

A problem limiting practical applications of AgNCs is photostability; most DNA templated AgNCs are easily bleached upon light exposure [26, 41]. We reason that G-rich DNA activated AgNCs

might provide a solution for the photostability problem. G-rich DNA is highly stable and easy to handle, making it possible to activate the fluorescence on-demand without using the unstable NaBH_4 . In this work, we systematically test the stability of such AgNCs. In addition, we study a number of different templating and activating DNA sequences to have a more complete understanding on this system.

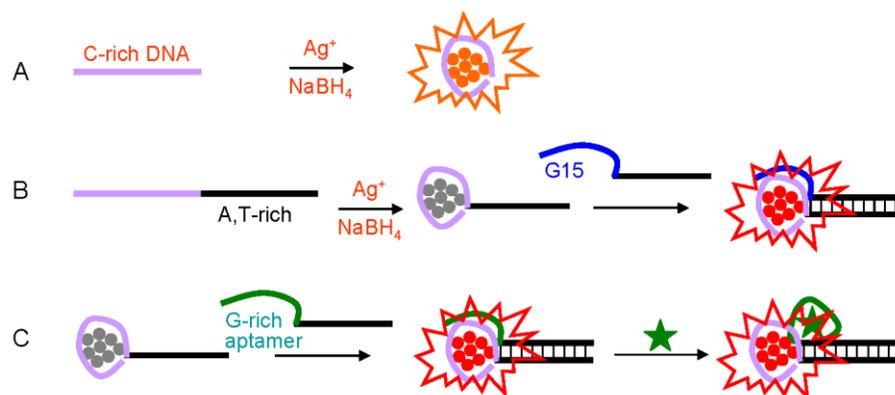


Figure 1. Schemes of DNA templated AgNC synthesis and fluorescence generation. (A) Producing AgNCs with a C-rich DNA template and NaBH_4 as the reducing agent. (B) The extended DNA produces non-fluorescent AgNCs, which become fluorescent upon hybridizing with a DNA containing a G-rich overhang. (C) Some G-rich aptamers can also activate the fluorescence, but aptamer binding to its target has little influence on fluorescence intensity.

2. Materials and Methods

2.1. Chemicals. All of the DNA samples were purchased from Integrated DNA Technologies (IDT, Coralville, IA) and purified by standard desalting. The DNA sequences are shown in Table 1. AgNO_3 and NaBH_4 were from Sigma-Aldrich. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), its sodium salt, NaH_2PO_4 , NaNO_3 , and adenosine and other ribonucleosides were purchased from Mandel Scientific (Guelph, ON). Milli-Q water was used for making buffers and dilutions.

2.2. Preparation of AgNCs. DNA/Ag NCs preparation procedure was based on the literature [36]. Concentrations of starting solutions were 100 μM for DNA, 1 mM for AgNO_3 , 1 mM for NaBH_4 , 500 mM for sodium phosphate buffer (pH 6.6). AgNCs were formed by firstly preparing the mixture solution containing final concentrations of 15 μM DNA, 90 μM AgNO_3 , 90 μM NaBH_4 and 20 mM for sodium phosphate buffer, where freshly prepared NaBH_4 was added at the last step. The NaBH_4 solution was prepared by dissolving the powder in water and the required volume was added to the DNA/ Ag^+ mixture within 30 sec followed by vigorous shaking for 5 sec. The reaction was kept in dark at room temperature for 24 h before hybridization. In case of DNA7a, NaBH_4 concentration was 120 μM and the incubation time after adding NaBH_4 was 2 h.

2.3. Hybridization with G-rich sequences. After adding NaBH_4 for 24 h, DNA_G15 (11.5 μL of 100 μM solution) was added to the samples (50 μL of reaction solution). The ratio DNA_G15/DNA1-7 was approximately 1.5:1. The final concentrations of DNA1 (or other template DNA) and DNA_G15 were 12.2 μM and 18.7 μM , respectively. This was followed by 1 h incubation in dark at room temperature before fluorescence spectra were measured.

2.4. Fluorescence measurements. Fluorescence was measured using a Varian Cary Eclipse Fluorescence Spectrophotometer. The samples (50 μL of DNA-Xa or DNA-X samples and 61.5 μL of DNA-X + DNA_G15 samples, X = 1-7) were diluted with water so that the final volume was 600 μL and final concentration of ssDNA (DNA-X and DNA-Xa) or dsDNA (DNA-X + DNA_G15) was 1.25 μM . Also, excitation wavelength was dependent on each sample and respective excitation wavelengths are in the legend describing the spectra plots. Just before spectra measurement a photograph of each sample exposed to a handheld UV lamp was taken using a Canon SD1200 IS camera.

2.5. Photobleaching. The prepared DNA1-AgNC/DNA_G15 samples were either stored in dark for 72 h or exposed to the light for 1 h. In both cases, this was followed by adding an additional portion of NaBH_4 solution (9 μl of 1 mM NaBH_4 to 122.5 μL of the bleached sample) and incubating at room temperature in dark for 3 h before spectral measurements.

2.6. UV-vis spectroscopy. The procedure of DNA1/AgNCs preparation is the same as described above. UV-vis spectra were measured using an Agilent 8453A spectrometer. Firstly, spectrum for 100 μ L of DNA-X/AgNCs ($X = 1-7$) sample was measured and then 22.5 μ l of 100 μ M DNA_G15 or DNA_A/T was added and spectra measured after different times.

2.7. Aptamer binding. The procedure for AgNC synthesis was the same but a different DNA sequence was used. The aptamer activated AgNCs were diluted with buffer so that the final concentrations of DNA1 and Mg^{2+} ions were 1.25 μ M and 2 mM, respectively. Then, adenosine, thymidine or cytidine was added and the maximum intensity at 630 nm with 570 nm excitation was measured. The experiment was repeated three times.

Table 1. DNA sequences used in this work.

Names	Sequences (from 5' to 3')
DNA1a	CCCTTAATCCCC
DNA1	CCCTTAATCCCCTATAATAAATTTTAAATATTATTTATTAAT
DNA2a	CCCTCCCTCCCC
DNA2	CCCTCCCTCCCCTATAATAAATTTTAAATATTATTTATTAAT
DNA3a	CCCCCCCCCCCC
DNA3	CCCCCCCCCCCCCTATAATAAATTTTAAATATTATTTATTAAT
DNA4a	CCCTAACTCCCC
DNA4	CCCTAACTCCCCTATAATAAATTTTAAATATTATTTATTAAT
DNA5a	CCCAACCTTCCAACCC
DNA5	CCCAACCTTCCAACCCTATAATAAATTTTAAATATTATTTATTAAT
DNA6a	CCCTTCCTTCCAACCC
DNA6	CCCTTCCTTCCAACCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
DNA7a	CCCCCCCCCCCCCGCCGCC
DNA7	CCCCCCCCCCCCCGCCGCCCTATAATAAATTTTAAATATTATTTATTAAT
DNA_G15	ATTAATAAATAATATTTAAAATTTATTATAGGGTGGGGTGGGGTGGGG
DNA_A/T	ATTAATAAATAATATTTAAAATTTATTATA
Ade_apt	ATTAATAAATAATATTTAAAATTTATTATAACCTGGGGGAGTATTGCG GAGGAAGGT
cAde_apt	ACCTTCCTCCGCAATACTCCCCAGGTTATAATAAATTTTAAATATTATTT ATTAAT
Pb ²⁺ _apt	ATTAATAAATAATATTTAAAATTTATTATAGGGTTAGGGTTAGGGTTAGGG

3. Results and Discussion

3.1. Stability of fluorescence signal. For sensing applications, it is important to have stable fluorescence signal. We recently reported that UV light bleaches DNA-templated AgNCs, which was attributed to oxidation [26]. Since hybridization with G-rich DNA appears to be a novel way of signal generation and the presence of nearby guanines might result in different properties, we herein studied their stability. AgNCs were prepared using DNA1 (see Table 1 for DNA sequences) as the template, which is the published sequence and has been used by most researchers for this purpose [36]. As expected, no fluorescence was observed for this sample (Figure 2A, zero time point). After adding the G-rich DNA (DNA_G15), red fluorescence rapidly increased, reaching ~80% of its maximal intensity in the first 15 min. The fluorescence then started to decrease after 1 h even when the sample was stored in dark, turning into faint yellow after 24 h. The fluorescence emission spectra of these samples were measured and a peak at 640 nm was observed at each time point (Figure 2B). This peak decayed almost linearly from 1 to 6 h (Figure 2C, red dots). A second emission peak at 550 nm was also observed. At 1 h, the intensity of the 640 nm peak was more than 20-fold stronger than that of the 550 nm peak, producing red fluorescence. At 24 h, their intensities were comparable due to the growth of the 550 nm peak (Figure 2D) and the drop of the 640 nm one (Figure 2B), which explained the yellow fluorescence (Figure 2A). This conversion of red to green emission has been observed by a few groups for different DNA sequences [26, 42-44], and this is attributed to oxidation of the AgNCs. The reduced AgNCs emit red light, and the oxidized AgNCs emit green. Therefore, such G-rich DNA activated AgNCs also suffer from decay of fluorescence signal, potentially because of oxidation, similar to most AgNCs that do not require this activation step. Having a G-rich DNA nearby the AgNC does not directly increase photostability.

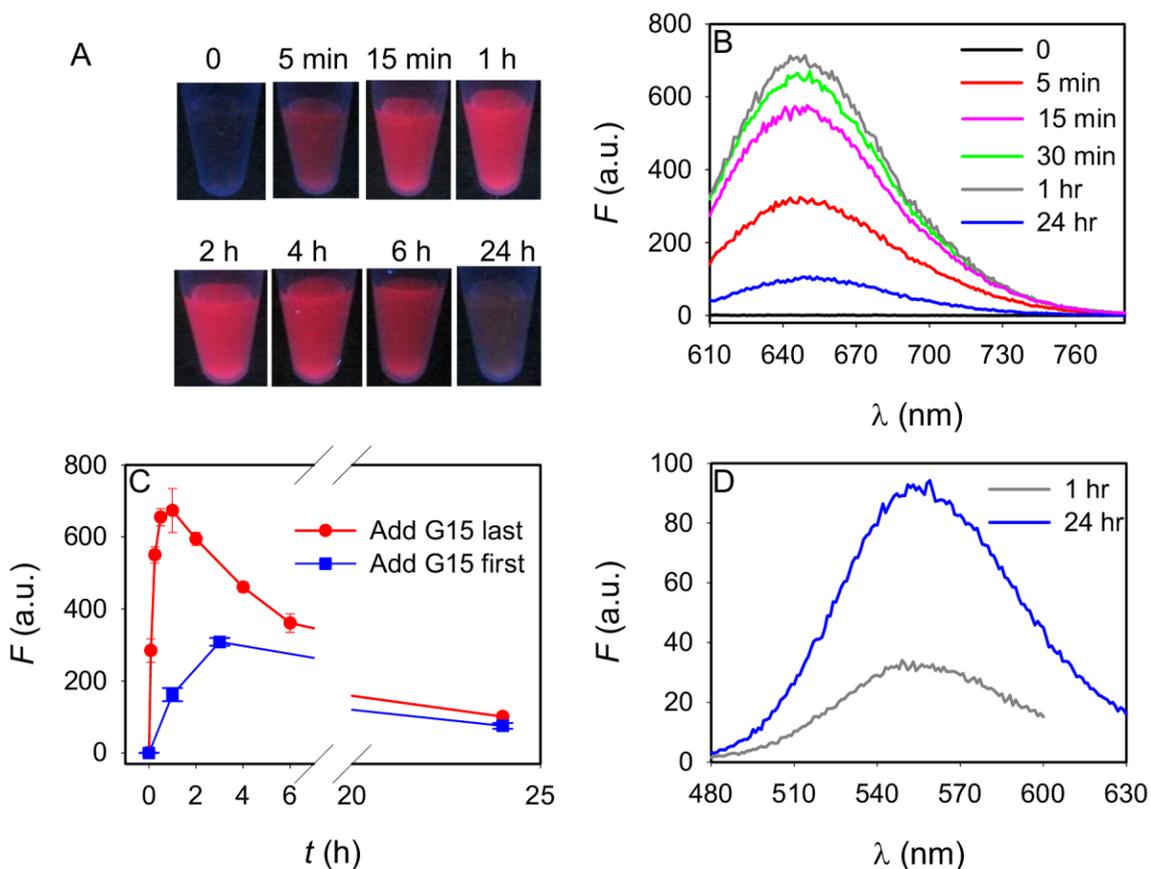


Figure 2. (A) Photographs of DNA1-templated AgNCs at various time points after adding DNA_G15. The scheme of fluorescence activation is shown in Figure 1B. The final mixture contained 15 μ M DNA, 90 μ M AgNO₃, 90 μ M NaBH₄ and 20 mM phosphate buffer (pH 6.6). NaBH₄ was freshly prepared. (B) Fluorescence spectra of the samples in (A) by exciting at 596 nm. (C) Intensity of the 640 nm emission peak as a function of time for samples in (B) (red dots). In addition, DNA_G15 was hybridized to DNA1 before adding NaBH₄ and the fluorescence intensity was recorded after adding NaBH₄ as a function of time (blue dots). (D) Fluorescence spectra of the 1 h and 24 h samples in (A) by exciting the green emitter at 458 nm.

To test whether the time-dependent fluorescence decay in Figure 2B is related to oxidation of AgNCs, a reducing agent (NaBH_4) was added to an aged sample. Indeed, fluorescence recovery was achieved and the emission peak remained at the same position (Figure 3A). During this aging process, the above samples were stored in dark to minimize photobleaching. Next we tested whether light exposure could accelerate fluorescence bleaching. A highly fluorescent sample after hybridization with DNA_G15 was exposed to a fluorescent tube light for 1 h, dropping the fluorescence to the background level (Figure 3B, red spectrum). Note that it takes more than 24 h to drop fluorescence to a similar value when the sample is stored in dark. Addition of NaBH_4 to this bleached sample also recovered the fluorescence. However, the sample only reached $\sim 80\%$ of the initial intensity after recovery, which is attributed to the loss of some silver species by forming large non-fluorescent silver nanoparticles (AgNPs). There is always a competition between forming AgNCs and AgNPs. Nevertheless, similar to the normal DNA-templated AgNCs [26], samples activated by DNA_G15 can also be bleached by UV light and recovered with NaBH_4 . Photobleaching needs to be considered for biosensing or imaging applications.

In the previous samples, DNA_G15 was added at the final step to activate fluorescence. Next, we hybridized DNA_G15 with DNA1 first, followed by adding AgNO_3 and NaBH_4 . Similarly, red fluorescence was observed (Figure 3C), although the intensity was not as strong as the sample with DNA_G15 added at the last step (e.g. compare the blue and red dots in Figure 2C). The weaker fluorescence was attributed to the presence of more DNA during AgNC synthesis and thus some Ag^+ might be associated with the activator. In addition, the template and activator may partially hybridize (one is C-rich and one is G-rich), weakening Ag^+ binding. Therefore, it is better to form AgNCs with the template DNA alone first. For such samples, the fluorescence still decayed over time (Figure 2C, blue line).

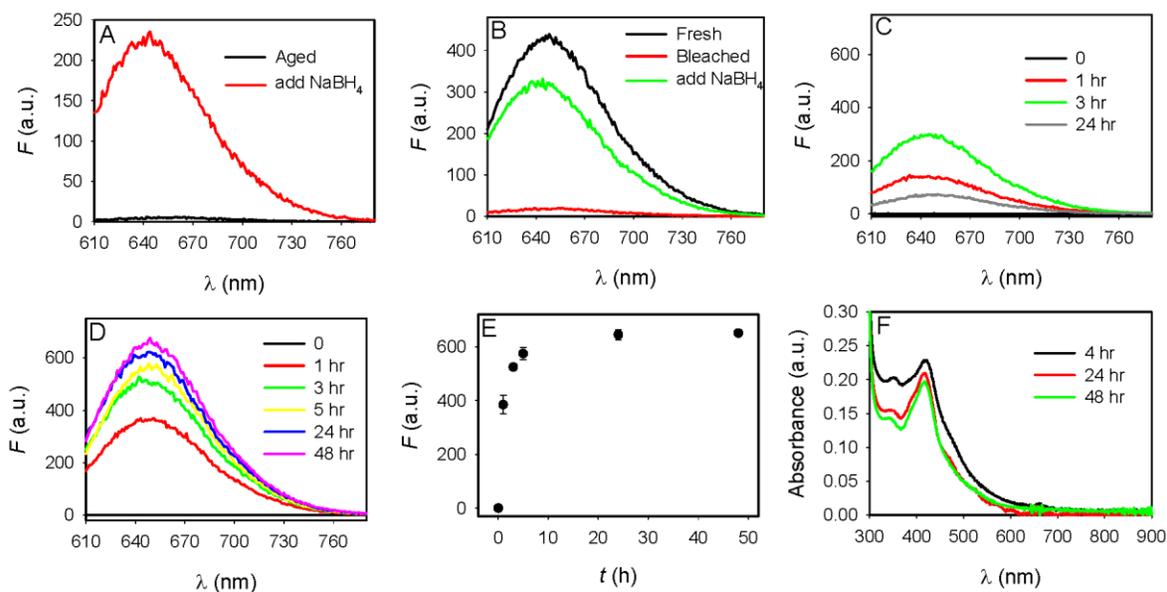


Figure 3. (A) Fluorescence spectra of DNA1-templated AgNCs after hybridizing with DNA_G15 and stored in dark for 3 days (black spectrum) and 1.5 h after adding NaBH_4 to this aged sample (red spectrum). (B) The freshly prepared AgNC spectrum (black), fluorescence after exposure to a fluorescent tube light for 1 h (red) and recovered for 3 h after adding NaBH_4 (green). (C) AgNC fluorescence spectra of samples prepared by hybridizing DNA_G15 with DNA1 first before adding NaBH_4 . Spectra were collected at different time after adding NaBH_4 . (D) Fluorescence spectra and (E) peak intensity quantification for AgNCs aged for different time after adding NaBH_4 . (F) UV-vis spectra of DNA1 templated AgNCs at different time points after adding NaBH_4 (DNA_G15 was not added).

Such time-dependent fluorescence decay poses a challenge for analytical applications. Even though fluorescence can be recovered by adding NaBH_4 , NaBH_4 is an unstable chemical and needs to be freshly prepared, making it undesirable for field applications. This problem might be partially solved by this DNA_G15 activation reaction. To test this, we studied whether DNA1-templated AgNCs are stable before hybridizing with DNA_G15. We waited for various time after adding NaBH_4 , but before

DNA_G15 was added. As shown in Figure 3D, E, the final fluorescence intensity was very stable as long as the previous incubation step (addition of NaBH₄) was more than 5 h. No decay was observed even after 48 h. Therefore, the AgNCs are in a stable (but non-fluorescent) state before adding DNA_G15. Once activated by DNA_G15, however, the time window for operation is only a few hours. UV-vis spectroscopy results also confirmed the stability of such AgNCs over two days (Figure 3F). This observation might partially solve the fluorescence stability problem of AgNCs, allowing assembly of sensors without using unstable NaBH₄. Although the time window of experiment is only ~2 h, using a DNA for activation is more convenient than using NaBH₄.

3.2. Changing the template DNA sequence. It is well-established that there is a correlation between DNA sequence and the resulting fluorescence property of AgNCs [1-6], although the underlying chemical principle remains unclear. For the current system, there are two DNA components: a template DNA for making AgNCs and an activator DNA. Different emission colors can be obtained by simply changing the overhang sequence in the activator DNA, where poly-G DNA gives red emission and poly-T DNA gives green emission [36, 45]. However, the effect of the template DNA has not been systematically explored. To study this, we changed the sequence and length of the C-rich DNA templates and studied six more sequences (DNA2-7). The experiment was conducted by comparing the fluorescence of the C-rich DNA template alone without extension (DNA1a-7a), the C-rich DNA with the A/T-rich DNA extension (DNA1-7), and after hybridizing with DNA_G15. Note that DNA1a-7a have been reported previously to template AgNCs with different emission properties [1, 46]. For each sequence, the emission spectrum was measured at the excitation maximum in the visible region (Figure 4). Both the fluorescence spectra and the photographs under UV excitation are presented.

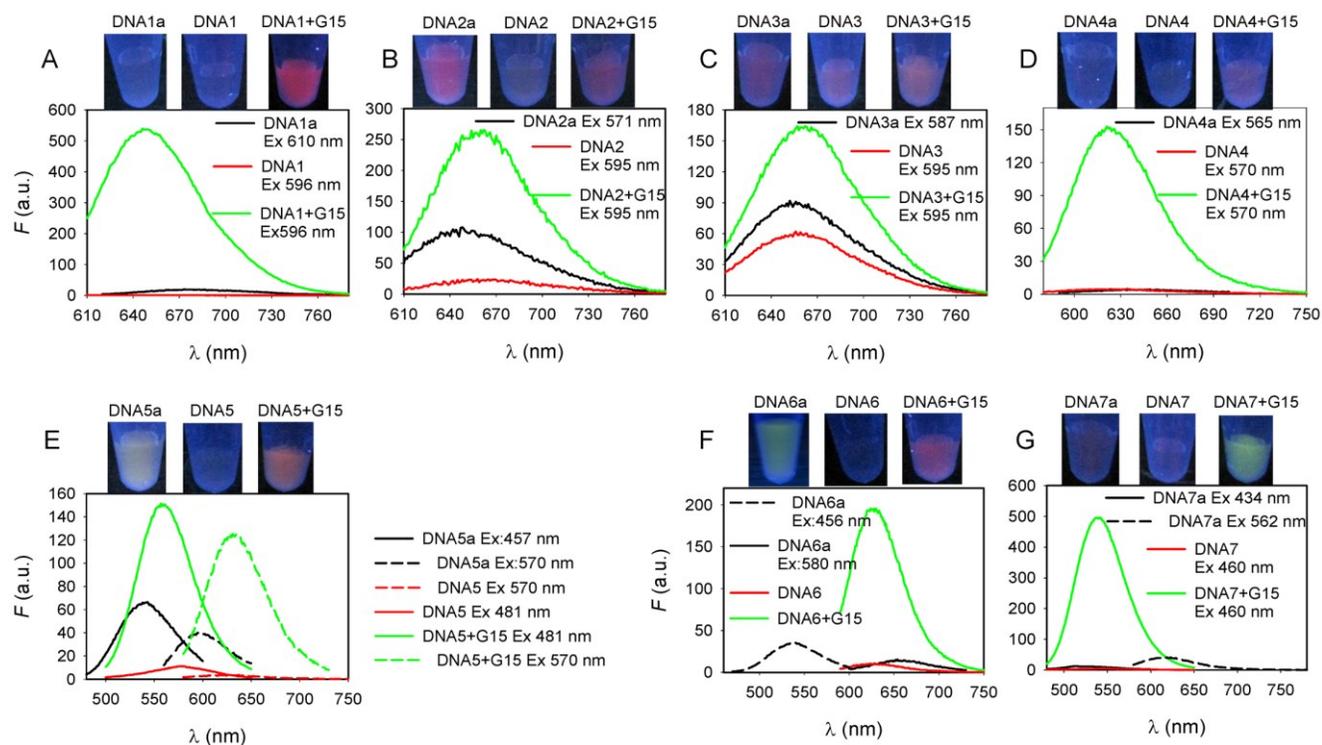


Figure 4A-G. Fluorescence spectra and photographs of the DNA1a-7a, DNA1-7 and after hybridizing DNA_G15 with DNA1-7. For (E-G), some samples showed dual emission peaks, which are denoted by spectra of the same color but with either continuous or dashed lines. The conditions are the same as that described in Figure 2.

A few interesting observations were observed. 1) By comparing the first two tubes in each panel, the AgNC fluorescence is often reduced when the DNA contains the A/T tail. Therefore, the A/T tail might have fluorescence quenching property or have affected the formation of AgNCs. 2) By comparing the last two tubes, a large increase in fluorescence intensity is often observed, and sometimes even a change in emission color, suggesting this poly-G activated fluorescence works for many DNA sequences. 3) DNA1-4 contained 12-mer C-rich DNA and they all produced red emitters. DNA1 has thus far been the best candidate for signaling since it has high fluorescence enhancement and also strong final fluorescence. DNA4 also has large enhancement but the final fluorescence is ~3-fold lower

than that from DNA1. DNA2 and DNA3 are poor choices since the background fluorescence before adding DNA_G15 is quite high. On the other hand, the three longer C-rich DNA (DNA5-7) all produced emission color changes. This is due to the presence of dual emitters either before or after hybridizing with DNA_G15 (or both), which can be observed from the different emission spectra. In Figure 4E-G, the spectra in the same color are from AgNCs templated by the same DNA but with different excitation wavelengths.

We previously studied DNA7, which produces both green and red emitters [26, 46]. The switching from red to green emission normally takes a day in dark and is related to an oxidation reaction. When DNA_G15 was used, this conversion occurred within 1 h in dark. This suggests that DNA_G15 has facilitated oxidation of the red emitter and accelerated the conversion to green emission.

3.3. UV-vis spectroscopy. To further understand G-rich DNA activated fluorescence, UV-vis spectroscopy was used to follow the samples after adding DNA_G15. In Figure 5A, DNA1-templated AgNCs initially absorbed at ~420 nm. While this is close to the surface plasmon band of AgNPs, the surface plasmon peak is usually much sharper. Therefore, at least a fraction of it should be from AgNCs. A new peak grew at 575 nm after adding DNA_G15, accompanying a decrease in the 420 nm peak. Such an absorption peak shift indicates the formation of new AgNC species and it occurred at a time scale comparable to its fluorescence signal generation (Figure 5B). Therefore, DNA_G15 DNA is likely to cause re-organization of AgNCs in such a way that the new AgNCs have stronger fluorescence. In other word, the activation step is not a simple providing of a G-rich environment but is related to the formation of new emitters. As a control experiment, if we use only the A/T rich DNA without the G-rich part (DNA_A/T in Table 1), no such shift was observed (Figure 5C). In addition, this UV-vis spectral shifting is general and occurs with all the tested DNAs. Another example of such is presented in Figure 5D for DNA7.

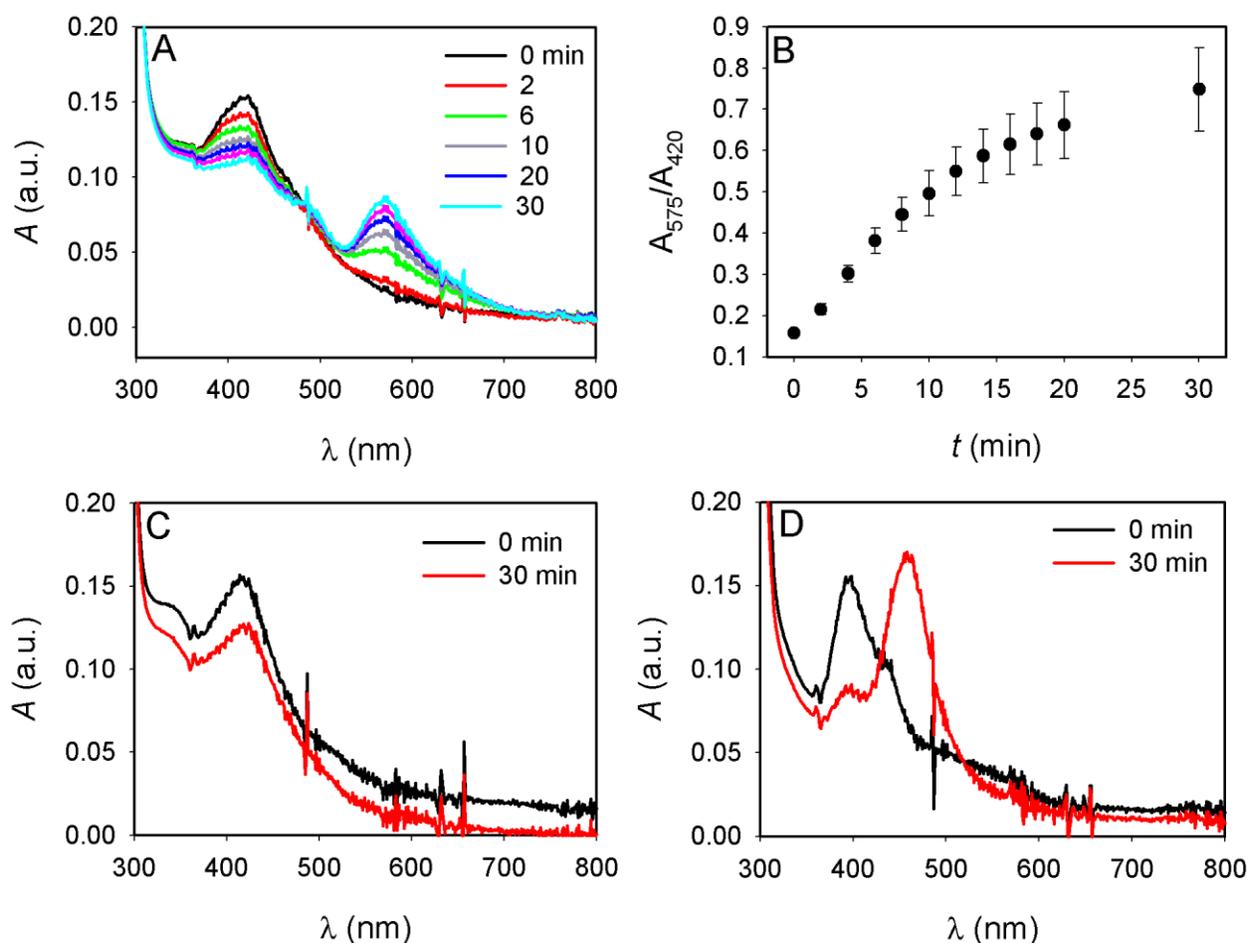


Figure 5. (A) Time-dependent UV-vis spectra of DNA1 templated AgNCs after adding DNA_G15. (B) Kinetic of the change of the peak ratio for the spectra in (A). (C) Spectra collected after adding DNA_A/T lacking the G-rich sequence. (D) Spectra collected using DNA7 and after adding DNA_G15. The samples were measured directly after synthesis without dilution.

3.4. Removal of the activator. An interesting and unresolved question is whether fluorescence is again quenched if the G-rich activator DNA is removed after the activation step. To investigate this, we employed structure switching aptamers (Figure 1C). Many aptamers are rich in guanine and may also serve as an activator. Indeed, both the Pb^{2+} aptamer (Figure 6A) and the adenosine aptamer (Figure 6B) produced strong fluorescence enhancement. The enhancement with the adenosine aptamer was

compared to that with DNA_G15 (Figure 6C), while the Pb^{2+} aptamer only achieved ~ 50 -fold enhancement. It is interesting to note that the number of guanine bases in DNA_G15 (15 guanines), adenosine aptamer (13 guanines) and Pb^{2+} aptamer (12 guanines) is quite similar. However, the enhancement with the Pb^{2+} aptamer was significantly lower, indicating that the distribution of the guanine bases in the DNA is also important. Aptamers that are not rich in guanine do not enhance the fluorescence (Figure S1).

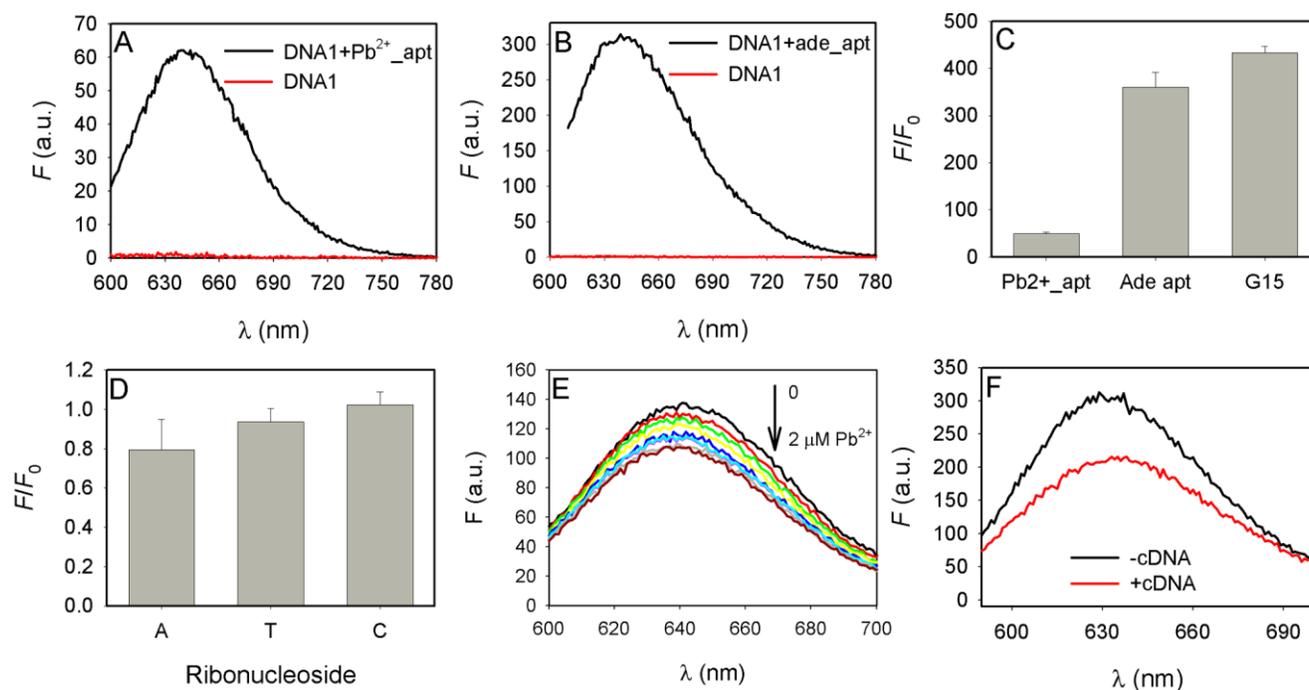


Figure 6. Fluorescence spectra of AgNCs templated by DNA1 and after hybridizing with DNA containing a Pb^{2+} aptamer overhang (A) or an adenosine aptamer overhang (B). (C) Fluorescence enhancement after hybridizing DNA1 with various G-rich DNA. (D) Decrease of AgNC fluorescence upon addition of 1 mM of adenosine, thymidine or cytidine. The buffer also contained 2 mM Mg^{2+} . (E) Titrating Pb^{2+} to the Pb^{2+} aptamer sample. (F) Change of fluorescence after adding the cDNA of the adenosine aptamer to remove it from the AgNC. The cDNA concentration (2.5 μM) was twice of that for the adenosine aptamer to ensure complete removal.

It is well-established that the aptamer folds when adenosine is added as shown in Figure 1C [47, 48]. However, with 1 mM adenosine, the fluorescence quenching was just ~20% (Figure 6D), although control experiment with cytidine and thymidine showed slightly less fluorescence quenching. This suggests that the influence of aptamer binding to its target on fluorescence is quite moderate. Similarly, we observed very little fluorescence change when Pb^{2+} was added to the Pb^{2+} aptamer sample (Figure 6E), where Pb^{2+} is known to fold the DNA into a G-quadruplex. To further confirm it, we added the cDNA of the adenosine aptamer (cAde_apt) to completely remove it from the AgNC and the fluorescence drop was still only ~30% (Figure 6F). Therefore, once activated, removal of the G-rich DNA has minimal effect on the fluorescence. In other words, the activated AgNCs do not need the G-rich DNA environment to maintain its fluorescence. Since guanine is easily oxidized, the G-rich overhang may serve as a reducing agent to reduce the nearby AgNCs [36]. After the reaction, the G-rich DNA is no longer needed.

4. Conclusions. In summary, we have performed a comprehensive study on G-rich DNA activated AgNCs in terms of fluorescence stability, fluorescence generation kinetics, effect of DNA sequence, photobleaching and recovery of fluorescence. Using G-rich DNA to activate AgNC fluorescence might partially solve the photostability problem of AgNCs since fluorescence activation can be achieved using the stable G-rich DNA alone without the need of unstable NaBH_4 ; NaBH_4 is needed only in the initial synthesis step. We demonstrated that the emission color can be controlled by tuning the C-rich DNA template, which complements to the previous report using different overhang sequences to achieve the same goal [45]. In addition, UV-vis spectroscopy suggested a change in the structure of AgNCs after adding the G-rich DNA. Many aptamer sequences are rich in guanine; we showed that these aptamers can also be used for fluorescence activation. After the activation step, the G-rich DNA is no longer needed for maintaining the fluorescence. It is quite likely that the AgNCs we produced are

a mixture of various species. Ideally, single species are more valuable for mechanistic studies. This might be achieved by purifying with HPLC, but that requires that the DNA/AgNC complex to be stable and does not dissociate when free Ag⁺ is removed. Follow up work to improve stability of AgNCs is a future direction to be investigated.

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References:

- [1] Richards C I, Choi S, Hsiang J-C, Antoku Y, Vosch T, Bongiorno A, Tzeng Y-L and Dickson R M 2008 Oligonucleotide-stabilized Ag nanocluster fluorophores *J. Am. Chem. Soc.* **130** 5038
- [2] Lan G Y, Chen W Y and Chang H T 2011 Control of synthesis and optical properties of DNA templated silver nanoclusters by varying DNA length and sequence *RSC Adv.* **1** 802
- [3] Gwinn E G, O'Neill P, Guerrero A J, Bouwmeester D and Fygenson D K 2008 Sequence-dependent fluorescence of DNA-hosted silver nanoclusters *Adv. Mater.* **20** 279
- [4] Liu G, Feng D-Q, Mu X, Zheng W, Chen T, Qi L and Li D 2013 DNA-functionalized silver nanoclusters as a chemopalette: Tunable fluorescence for turn-on detection of cysteine *J. Mater. Chem. B* **1** 2128
- [5] Sharma J, Yeh H-C, Yoo H, Werner J H and Martinez J S 2010 A complementary palette of fluorescent silver nanoclusters *Chem. Comm.* **46** 3280
- [6] Schultz D, Gardner K, Oemrawsingh S S R, Markešević N, Olsson K, Debord M, Bouwmeester D and Gwinn E 2013 Evidence for rod-shaped DNA-stabilized silver nanocluster emitters *Adv. Mater.* **25** 2797

- [7] Zheng J, Nicovich P R and Dickson R M 2007 Highly fluorescent noble-metal quantum dots *Annu. Rev. Phys. Chem.* **58** 409
- [8] Parker J F, Fields-Zinna C A and Murray R W 2010 The story of a monodisperse gold nanoparticle: Au₂₅L₁₈ *Acc. Chem. Res.* **43** 1289
- [9] Jin R C 2010 Quantum sized, thiolate-protected gold nanoclusters *Nanoscale* **2** 343
- [10] Shang L, Dong S J and Nienhaus G U 2011 Ultra-small fluorescent metal nanoclusters: Synthesis and biological applications *Nano Today* **6** 401
- [11] Han B Y and Wang E K 2012 DNA-templated fluorescent silver nanoclusters *Anal. Bioanal. Chem.* **402** 129
- [12] Latorre A and Somoza Á 2012 DNA-mediated silver nanoclusters: Synthesis, properties and applications *ChemBioChem* **13** 951
- [13] Diez I and Ras R H A 2011 Fluorescent silver nanoclusters *Nanoscale* **3** 1963
- [14] Obliosca J M, Liu C and Yeh H-C 2013 Fluorescent silver nanoclusters as DNA probes *Nanoscale* **5** 8443
- [15] Latorre A, Lorca R and Somoza A 2013 Fluorescent DNA stabilized silver nanoclusters as biosensors *J. Chem.* **2013** 631421
- [16] Sharma J, Yeh H-C, Yoo H, Werner J H and Martinez J S 2011 Silver nanocluster aptamers: In situ generation of intrinsically fluorescent recognition ligands for protein detection *Chem. Comm.* **47** 2294
- [17] Liu J-J, Song X-R, Wang Y-W, Zheng A-X, Chen G-N and Yang H-H 2012 Label-free and fluorescence turn-on aptasensor for protein detection via target-induced silver nanoclusters formation *Anal. Chim. Acta* **749** 70
- [18] Sun Z, Wang Y, Wei Y, Liu R, Zhu H, Cui Y, Zhao Y and Gao X 2011 Ag cluster-aptamer hybrid: Specifically marking the nucleus of live cells *Chem. Comm.* **47** 11960

- [19] Yin J J, He X X, Wang K M, Qing Z H, Wu X, Shi H and Yang X H 2012 One-step engineering of silver nanoclusters-aptamer assemblies as luminescent labels to target tumor cells *Nanoscale* **4** 110
- [20] Zhou Z, Du Y and Dong S 2011 DNA-Ag nanoclusters as fluorescence probe for turn-on aptamer sensor of small molecules *Biosens. Bioelectron.* **28** 33
- [21] Xie J, Zheng Y and Ying J Y 2010 Highly selective and ultrasensitive detection of Hg^{2+} based on fluorescence quenching of Au nanoclusters by Hg^{2+} - Au^+ interactions *Chem. Comm.* **46** 961
- [22] Wei H, Wang Z D, Yang L M, Tian S L, Hou C J and Lu Y 2010 Lysozyme-stabilized gold fluorescent cluster: Synthesis and application as Hg^{2+} sensor *Analyst* **135** 1406
- [23] Lin Y H and Tseng W L 2010 Ultrasensitive sensing of Hg^{2+} and CH_3Hg^+ based on the fluorescence quenching of lysozyme type vi-stabilized gold nanoclusters *Anal. Chem.* **82** 9194
- [24] Su Y-T, Lan G-Y, Chen W-Y and Chang H-T 2010 Detection of copper ions through recovery of the fluorescence of DNA-templated copper/silver nanoclusters in the presence of mercaptopropionic acid *Anal. Chem.* **82** 8566
- [25] Zhang M and Ye B-C 2011 Label-free fluorescent detection of copper(II) using DNA-templated highly luminescent silver nanoclusters *Analyst* **136** 5139
- [26] Morishita K, MacLean J L, Liu B, Jiang H and Liu J 2013 Correlation of photobleaching, oxidation and metal induced fluorescence quenching of DNA-templated silver nanoclusters *Nanoscale* **5** 2840
- [27] Guo W, Yuan J, Dong Q and Wang E 2009 Highly sequence-dependent formation of fluorescent silver nanoclusters in hybridized DNA duplexes for single nucleotide mutation identification *J. Am. Chem. Soc.* **132** 932
- [28] Li T, Zhang L B, Ai J, Dong S J and Wang E K 2011 Ion-tuned DNA/Ag fluorescent nanoclusters as versatile logic device *ACS Nano* **5** 6334

- [29] Deng L, Zhou Z X, Li J, Li T and Dong S J 2011 Fluorescent silver nanoclusters in hybridized DNA duplexes for the turn-on detection of Hg²⁺ ions *Chem. Comm.* **47** 11065
- [30] Ono A and Togashi H 2004 Molecular sensors: Highly selective oligonucleotide-based sensor for mercury(II) in aqueous solutions *Angew. Chem., Int. Ed.* **43** 4300
- [31] Guo W W, Yuan J P and Wang E K 2011 Strand exchange reaction modulated fluorescence "Off-on" Switching of hybridized DNA duplex stabilized silver nanoclusters *Chem. Comm.* **47** 10930
- [32] Zhang L, Zhu J, Guo S, Li T, Li J and Wang E 2013 Photoinduced electron transfer of DNA/Ag nanoclusters modulated by g-quadruplex/hemin complex for the construction of versatile biosensors *J. Am. Chem. Soc.* **135** 2403
- [33] Dong H, Jin S, Ju H, Hao K, Xu L-P, Lu H and Zhang X 2012 Trace and label-free microRNA detection using oligonucleotide encapsulated silver nanoclusters as probes *Anal. Chem.* **84** 8670
- [34] Liu X, Wang F, Aizen R, Yehezkeli O and Willner I 2013 Graphene oxide/nucleic-acid-stabilized silver nanoclusters: Functional hybrid materials for optical aptamer sensing and multiplexed analysis of pathogenic DNAs *J. Am. Chem. Soc.* **135** 11832
- [35] Tao Y, Lin Y, Huang Z, Ren J and Qu X 2012 DNA-templated silver nanoclusters-graphene oxide nanohybrid materials: A platform for label-free and sensitive fluorescence turn-on detection of multiple nucleic acid targets *Analyst* **137** 2588
- [36] Yeh H C, Sharma J, Han J J, Martinez J S and Werner J H 2010 A DNA-silver nanocluster probe that fluoresces upon hybridization *Nano Lett.* **10** 3106
- [37] Yeh H-C, Sharma J, Shih I-M, Vu D M, Martinez J S and Werner J H 2012 A fluorescence light-up Ag nanocluster probe that discriminates single-nucleotide variants by emission color *J. Am. Chem. Soc.* **134** 11550
- [38] Li J, Zhong X, Zhang H, Le X C and Zhu J-J 2012 Binding-induced fluorescence turn-on assay using aptamer-functionalized silver nanocluster DNA probes *Anal. Chem.* **84** 5170

- [39] Zhang M, Guo S-M, Li Y-R, Zuo P and Ye B-C 2012 A label-free fluorescent molecular beacon based on DNA-templated silver nanoclusters for detection of adenosine and adenosine deaminase *Chem. Comm.* **48** 5488
- [40] Zhang L, Zhu J, Zhou Z, Guo S, Li J, Dong S and Wang E 2013 A new approach to light up DNA/Ag nanocluster-based beacons for bioanalysis *Chem. Sci.* **4** 4004
- [41] Sharma J, Rocha R C, Phipps M L, Yeh H-C, Balatsky K A, Vu D M, Shreve A P, Werner J H and Martinez J S 2012 A DNA-templated fluorescent silver nanocluster with enhanced stability *Nanoscale* **4** 4107
- [42] O'Neill P R, Velazquez L R, Dunn D G, Gwinn E G and Fygenson D K 2009 Hairpins with poly-C loops stabilize four types of fluorescent Ag(n):DNA *J. Phys. Chem. C* **113** 4229
- [43] Ritchie C M, Johnsen K R, Kiser J R, Antoku Y, Dickson R M and Petty J T 2007 Ag nanocluster formation using a cytosine oligonucleotide template *J. Phys. Chem. C* **111** 175
- [44] Driehorst T, O'Neill P, Goodwin P M, Pennathur S and Fygenson D K 2011 Distinct conformations of DNA-stabilized fluorescent silver nanoclusters revealed by electrophoretic mobility and diffusivity measurements *Langmuir* **27** 8923
- [45] Hsin-Chih Y, Sharma J, Han J J, Martinez J S and Werner J H 2011 Nanocluster beacon - a new molecular probe for homogeneous detection of nucleic acid targets. In: *Nano/Micro Engineered and Molecular Systems (NEMS), 2011 IEEE International Conference on*, pp 267
- [46] MacLean J L, Morishita K and Liu J 2013 DNA stabilized silver nanoclusters for ratiometric and visual detection of Hg²⁺ and its immobilization in hydrogels *Biosens. Bioelectron.* **48** 82
- [47] Nutiu R and Li Y 2003 Structure-switching signaling aptamers *J. Am. Chem. Soc.* **125** 4771
- [48] Liu J and Lu Y 2006 Fast colorimetric sensing of adenosine and cocaine based on a general sensor design involving aptamers and nanoparticles *Angew. Chem., Int. Ed.* **45** 90