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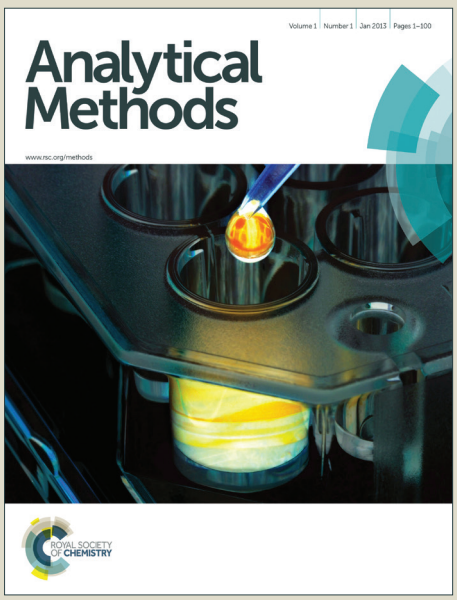

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COMMUNICATION

Fluorescent G-Quadruplex-NMM DNA Probe for Detection of Sliver Nanoparticles in Aqueous Media

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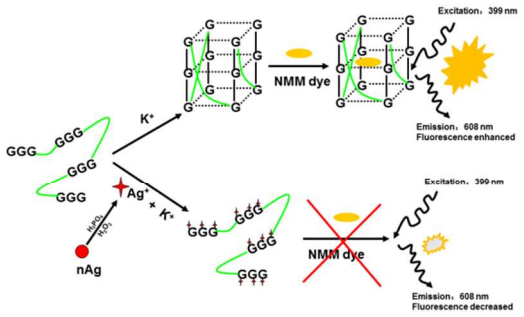
This communication reports the development of a new homogeneous assay based on G-quadruplexes (G-DNA) and N-methylmesoporphyrin IX (NMM) for its sensitive and selective detection of silver nanoparticles.

Due to the intrinsic antimicrobial activity, sliver nanoparticles (AgNPs) and its various products have been wildly applied in our daily life, such as medicine and consumer products.[1,2] It is almost certain that AgNPs can be inevitably released into the environment and enter the biological system through various entry points during the processes of synthesis, manufacturing and AgNPs products applications.[3] Many studies demonstrated that AgNPs could cause potentially negative impacts on environment and human health.[4,5] For instance, along with its good antimicrobial activity it is also reported to be toxic to aquatic environment.[6,7] In mammals, AgNPs was found to reach the nervous and circulatory system and influence the function of human organisms.[8,9] More importantly, nanoparticles, such as AgNPs, could be bioaccumulated and biomagnified through the food chain, showing a potential concern for human beings.[10,11]

Traditional methods, such as atomic absorption and inductively coupled plasma mass spectrometry (ICP-MS) which require sophisticated equipments operated by highly skilled professionals, have been used to measure trace levels of AgNPs.[12,13] Meanwhile, numerous simpler detection tools such as organic fluorogenic probes or semiconductor quantum dots were developed for the simple and rapid detection of silver ions/AgNPs in various situations.[14,15] Although these probes or sensors provide a highly sensitive and selective detection Ag^+/AgNPs, they have the limitation for practical use due to the complex processes or low water solubility etc.[16] Therefore, the effective methods with simple manipulation and high sensitivity are urgently required for the detection of AgNPs in the environment.[17]

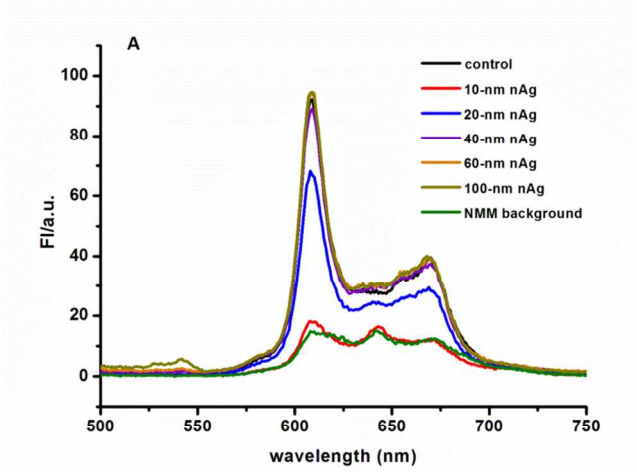
G-quadruplexes (G-DNA) is a unique higher-order structure in which G-rich nucleic acid sequences form stacked arrays of G-quartets connected by Hoogsteen-type base pairing.[18] In recent decades, G-DNA has been developed into various probes for sensitive analyses.[19-22] In the present study, we develop a simple, rapid and label-free assay for highly selective detection of AgNPs using G-DNA and a small molecule dye N-methylmesoporphyrin IX (NMM) (Scheme 1). In the process, NMM was able to form a supramolecular structure with G-DNA and significantly enhanced its fluorescence.[23,24] Fluorescence spectroscopy was then utilized to indicate AgNPs or Ag^+-induced changes of G-DNA conformation and fluorescence of NMM interaction with G-DNA.



Scheme 1 Schematic of utilizing the fluorescent G-DNA-NMM probe for detection of AgNPs based on oxidation reaction by H_2O_2 under acid condition.

Our results showed that NMM at 1 μM exhibited weak fluorescence in a solution of 10 mM Tris-Ac, 2 mM EDTA, 1 mM KAc, 0.05% Triton X-100. On the addition of 0.4 μM G-DNA, the fluorescence of NMM was strongly enhanced and showed a positive peak near 608 nm, indicating that NMM bound with G-DNA. When incubated with 10- and 20-nm AgNPs, a remarkable decrease of the fluorescence was observed. However, this phenomenon was not found with 2-, 40-, 60-, and 100-nm AgNPs (Fig. 1A and B), suggesting that 10- and 20-nm AgNPs could directly disturb the interaction between NMM and G-DNA, and the 10-nm AgNPs have the strongest inhibition effect. It was reported that G-DNA could bind with hemin to form complexes which exhibited peroxidase-like catalytic activity, and it catalyzed the oxidation of 2,2'-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) with H_2O_2.[25,26] We also discovered that the peroxidase activity of G-DNA could be strongly inhibited by small size AgNPs (Fig. S1). A calibration curve derived from 10-nm AgNPs standard solutions was shown in the inset of Fig. 1B. This curve exhibited a linearity in the range of 1-10 μM, indicating that this probe can be used to directly detect AgNPs

with size about ~10-nm.



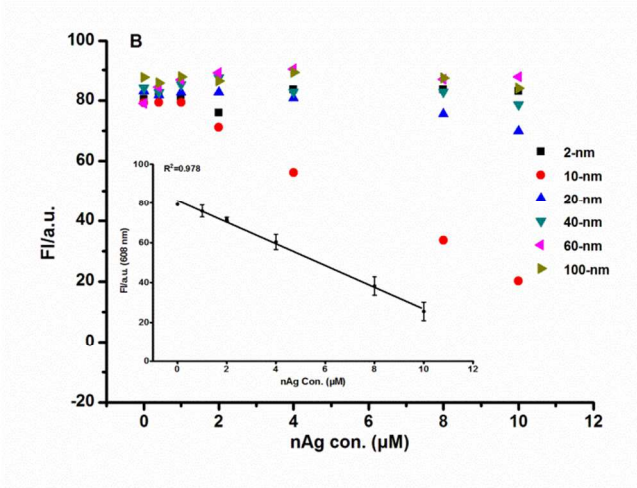
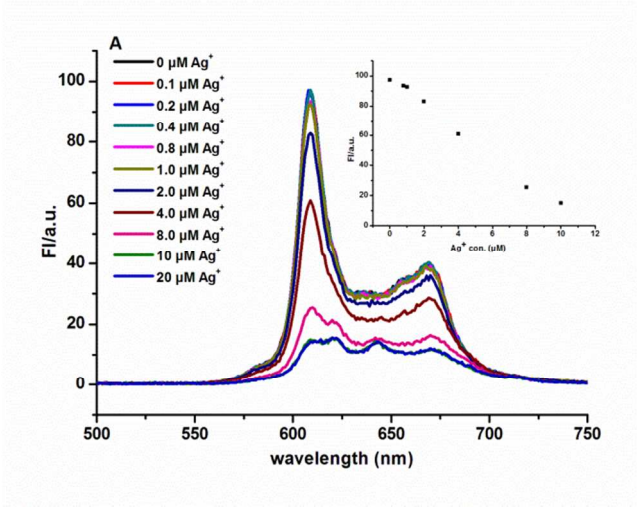


Fig. 1 (A) Fluorescence spectra of G-DNA-NMM (0.4 μM) in the presence of different sizes of AgNPs (10 μM). (B) The fluorescence intensity (608 nm) plotted against the concentrations of different size AgNPs (0.4-10 μM). The inset: the fluorescence intensity (608 nm) plotted against the concentrations of 10 nm AgNPs (1-10 μM) with error bars.

Although G-DNA-NMM probe in the present study has an excellent response to 10-nm AgNPs, it showed little effect to smaller or larger size AgNPs. To overcome this problem and for the detection of all state AgNPs in environment, AgNPs were firstly oxidized to Ag^+ ions with H_2O_2 under acidic condition.[14,16] Fig. S2 revealed that the reaction for the oxidation of AgNPs to Ag^+ reached completion in 30 min. Ag^+ is capable of selectively chelating guanine bases at the binding sites, which disturbs the formation of G-quadruplexes.[27] Based on this feature, a highly sensitive and selective Ag^+-detection method based on G-quadruplex-hemin DNAzymes was developed with a detection limit to nanomolar.[28] As the peroxidase-like catalytic activity of DNAzymes can be destroyed by H_2O_2 used to transform AgNPs to Ag^+ ions (Fig. S3), the next step for the present study is how to make G-DNA probe surviving the oxidation conditions during equilibrating AgNPs to Ag^+ by H_2O_2. We found that the fluorescence of G-DNA-NMM complexes was not changed after addition of 1 mM H_2O_2, and even for the H_2O_2 concentration reaching 10 mM (Fig. S4), indicating the G-DNA-NMM probe might be explored for detection of AgNPs under the oxidation conditions. To test our hypothesis, the feasibility of this method for Ag^+ quantitation was first investigated and the fluorescent signal of the probe solution as a function of Ag^+ concentration was examined. The analysis was done in Tris-Ac buffer (10 mM Tris, 2 mM EDTA, 1 mM KAc, 0.05% Triton-X 100, pH 8.0) with 0.4 μM G-DNA and 1 μM NMM. Fig. 2A showed that with increasing concentration of Ag^+, the fluorescence at 550-700 nm continuously decreased. The inset of Fig. 2A indicated the dependence of fluorescence at 608 nm on Ag^+ concentration, with a correlation coefficient (R^2=0.996) in the range of 0.8-10 μM, demonstrating the usefulness of probe for the quantification of Ag^+ under oxidation conditions.

To confirm the specificity of probe toward Ag^+, other control metal ions were used instead of Ag^+ under identical conditions. The fluorescence intensity at 608 nm of G-NDA-NMM did not show any obvious change on the addition of 10 μM other metal ions (Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Mn^{2+}, Cr^{5+},Cd^{2+}, and Co^{2+}) other than Ag^+ (Fig. 2B). When Ag^+ and other metal ions were both added to the probe solution, a similar result was observed, indicating that the presence of other metal ions did not interfere with the detection of Ag^+. These results demonstrated the high specificity of this probe for detection of Ag^+.



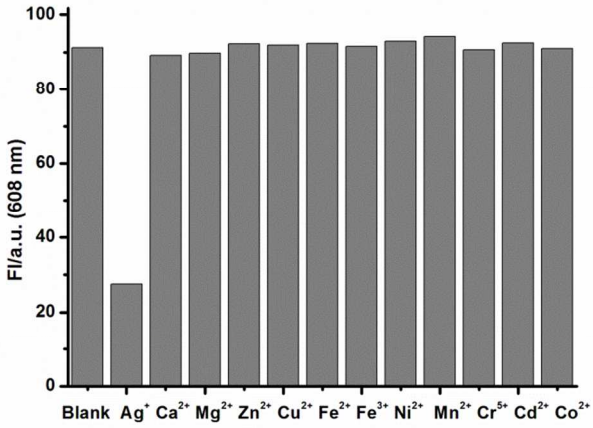


Fig. 2 (A) Fluorescence spectra of G-DNA-NMM (0.4 μM) in the presence of different Ag^+ concentrations (from top to bottom: 0, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 4.0, 8.0, 10, 20 μM) (excitation at 399 nm). The inset: the fluorescence intensity (608 nm) plot depending on the concentration of Ag^+ (0.8-10 μM). (B) Relative fluorescence intensity at 608 nm of G-DNA-NMM on the addition of other metal ions (10 μM).

For the detection of AgNPs using this detection system, AgNPs were firstly oxidized to Ag^+ with H_2O_2 under acidic condition. As shown in Fig. 3A, after the same amount of different size AgNPs was equilibrated to Ag^+ ions, they could inhibit the fluorescent intensity of G-DNA-NMM complex to the same level. Thus, different concentrations of AgNPs (20 nm) were added to a solution of 1.0 mM H_2O_2 and 1.0 μM H_3PO_4, and the resulting mixture was directly detected by our detection system. Fig. 3B showed the fluorescence of G-DNA-NMM complexes decreased linearly depending on the concentrations of AgNPs, and the correlation coefficient for the determination of AgNPs in the ranges 0.4 to 8 μM was 0.999 with a detection limit of 0.4 μM (inset in Fig. 3B). And our probe had a good specificity in the presence of other nanoparticles like ZnO, TiO_2 and Fe_2O_3 (Fig. S5).

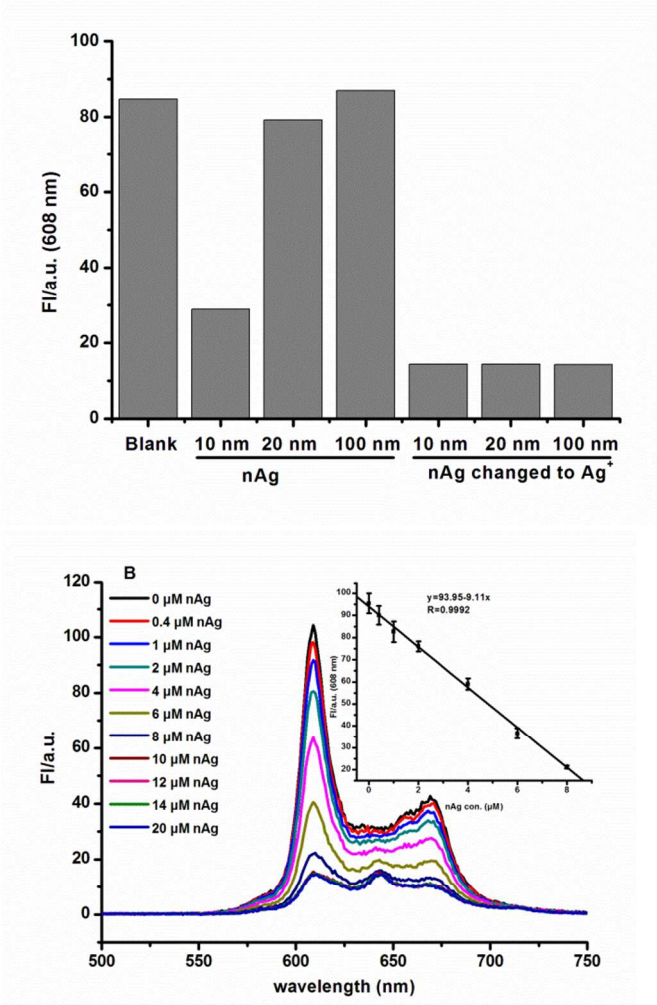


Fig. 3 (A) Fluorescence intensity at 608 nm of G-DNA-NMM (0.4 μM) in the presence of different sizes of AgNPs (10 μM), which has been treated with 1.0 mM H_2O_2 and 1.0 μM H_3PO_4. (B) Fluorescence spectra of G-DNA-NMM (0.4 μM) after 1 h upon addition of different concentrations AgNPs (20 nm) (from top to bottom: 0, 0.4, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 12, 14, 20 μM), which has been treated with 1.0 mM H_2O_2 and 1.0 μM H_3PO_4. The inset: the fluorescence intensity (608 nm) plotted against the concentrations of AgNPs (0.4-8 μM) with error bars.

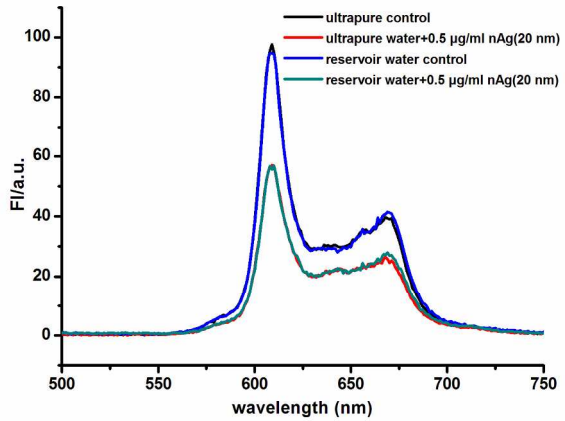


Fig. 4 Fluorescence spectra of G-DNA-NMM (0.4 μM) on the addition of ultrapure water or reservoir water, which has been spiked with AgNPs (~4.6 μM) and treated with 1.0 mM H_2O_2 and 1.0 μM H_3PO_4.

The AgNPs detection protocol was applied for the quantification for AgNPs in environment water sample. The same amount of AgNPs was added to ultrapure water or environmental water sample which was collected from Dongpu Reservoir (Hefei, China). The samples were treated with aqueous H_2O_2 and H_3PO_4 followed by addition of G-DNA and NMM. After formation G-DNA-NMM complexes, the mixture was subjected to the fluorescence measurement. There was no difference between the two samples from the fluorescence inhibition (Fig. 4). On the basis of a calibration curve, this detection system gave the same values within experimental errors compared with ICP-MS. This demonstrated that the G-DNA-NMM probe provided a candidate for environmental water sample AgNPs detection.

Conclusions

In summary, we have introduced a label-free fluorescent method for highly specific detection of aqueous Ag^+/AgNPs using the probe of G-NDA and NMM. Our probe not only has a direct response to AgNPs about ~10 nm and Ag^+ ions, but also can be survived under oxidation condition where larger AgNPs are equilibrated to Ag^+ by H_2O_2. This is a simple, cost-effective, rapid and high specific method compared with traditional methods. The sensitivity of this probe toward Ag^+/AgNPs should be further improved by optimizing the G-DNA sequence, concentration, or combining with electrochemical methods. Furthermore, we suggest that this probe provides a candidate method for quantification of AgNPs in water environment.

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Notes and references

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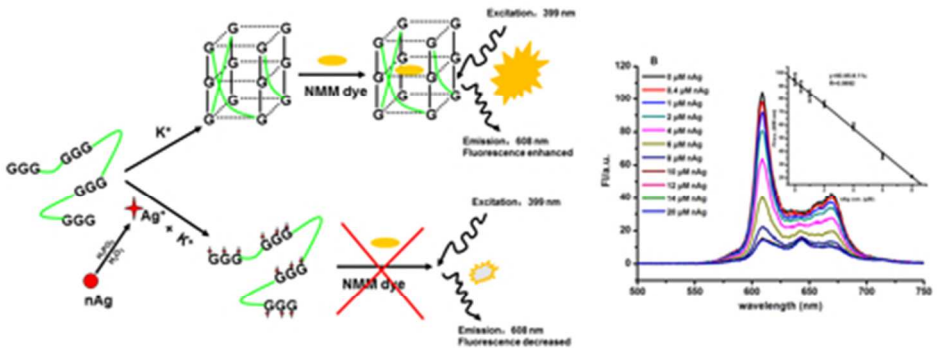
† Electronic Supplementary Information (ESI) available: Experimental details and Fig. S1-S4. See DOI: 10.1039/c000000x/

1 L. Sintubin, W. Verstraete and N. Boon, *Biotechnol Bioeng*, 2012, **109**, 2422-2436.
2 K. Chaloupka, Y. Malam and A. M. Seifalian, *Trends Biotechnol*, 2010, **28**, 580-588.
3 M. A. Maurer-Jones, I. L. Gunsolus, C. J. Murphy and C. L. Haynes, *Anal Chem*, 2013, **85**, 3036-3049.
4 S. W. P. Wijnhoven, W. J. G. M. Peijnenburg, C. A. Herberts, W. I. Hagens, A. G. Oomen, E. H. W. Heugens, B. Roszek, J. Bisschops, I. Gosens, D. Van De Meent, S. Dekkers, W. H. De Jong, M. van Zijverden, A. J. A. M. Sips and R. E. Geertsma, *Nanotoxicology*, 2009, **3**, 109-138.
5 F. M. Christensen, H. J. Johnston, V. Stone, R. J. Aitken, S. Hankin, S. Peters and K. Aschberger, *Nanotoxicology*, 2010, **4**, 284-295.
6 M. Matzke, K. Jurkschat and T. Backhaus, *Ecotoxicology*, 2014, **23**, 818-829.
7 N. Seltenrich, *Environ Health Perspect*, 2013, **121**, A220-225.
8 N. Hadrup, K. Loeschner, A. Mortensen, A. K. Sharma, K. Qvortrup, E. H. Larsen and H. R. Lam, *Neurotoxicology*, 2012, **33**, 416-423.
9 M. van der Zande, R. J. Vandebriel, E. Van Doren, E. Kramer, Z. Herrera Rivera, C. S. Serrano-Rojero, E. R. Gremmer, J. Mast, R. J. Peters, P. C. Hollman, P. J. Hendriksen, H. J. Marvin, A. A. Peijnenburg and H. Bouwmeester, *ACS Nano*, 2012, **6**, 7427-7442.
10 J. D. Judy, J. M. Unrine and P. M. Bertsch, *Environ Sci Technol*, 2011, **45**, 776-781.
11 R. Werlin, J. H. Priester, R. E. Mielke, S. Kramer, S. Jackson, P. K. Stoimenov, G. D. Stucky, G. N. Cherr, E. Orias and P. A. Holden, *Nat Nanotechnol*, 2011, **6**, 65-71.
12 J. M. Zook, S. E. Long, D. Cleveland, C. L. Geronimo and R. I. MacCuspie, *Anal Bioanal Chem*, 2011, **401**, 1993-2002.
13 S. J. Yu, J. B. Chao, J. Sun, Y. G. Yin, J. F. Liu and G. B. Jiang, *Environ Sci Technol*, 2013, **47**, 3268-3274.
14 A. Chatterjee, M. Santra, N. Won, S. Kim, J. K. Kim, S. B. Kim and K. H. Ahn, *J Am Chem Soc*, 2009, **131**, 2040-2041.
15 W. Sun, J. Yao, T. Yao and S. Shi, *Analyst*, 2013, **138**, 421-424.
16 Y. H. Lin and W. L. Tseng, *Chem Commun (Camb)*, 2009, 6619-6621.
17 F. Gottschalk, T. Sonderer, R. W. Scholz and B. Nowack, *Environ Sci Technol*, 2009, **43**, 9216-9222.
18 A. T. Phan, V. Kuryavyi and D. J. Patel, *Curr Opin Struct Biol*, 2006, **16**, 288-298.
19 C. Teller, S. Shimron and I. Willner, *Anal Chem*, 2009, **81**, 9114-9119.
20 J. Elbaz, B. Shlyahovsky and I. Willner, *Chem Commun (Camb)*, 2008, 1569-1571.
21 T. Li, S. Dong and E. Wang, *Anal Chem*, 2009, **81**, 2144-2149.
22 T. Li, E. Wang and S. Dong, *Anal Chem*, 2010, **82**, 1515-1520.
23 Y. Li, C. R. Geyer and D. Sen, *Biochemistry*, 1996, **35**, 6911-6922.
24 H. Arthanari, S. Basu, T. L. Kawano and P. H. Bolton, *Nucleic Acids Res*, 1998, **26**, 3724-3728.
25 P. Travascio, Y. F. Li and D. Sen, *Chem Biol*, 1998, **5**, 505-517.
26 P. Travascio, A. J. Bennet, D. Y. Wang and D. Sen, *Chem Biol*, 1999, **6**, 779-787.
27 R. M. Izatt, J. J. Christensen and J. H. Rytting, *Chem Rev*, 1971, **71**, 439-481.
28 X. H. Zhou, D. M. Kong and H. X. Shen, *Anal Chem*, 2010, **82**, 789-793.



G-DNA-NMM fluorescent prboe for detection of silver nanoparticles
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