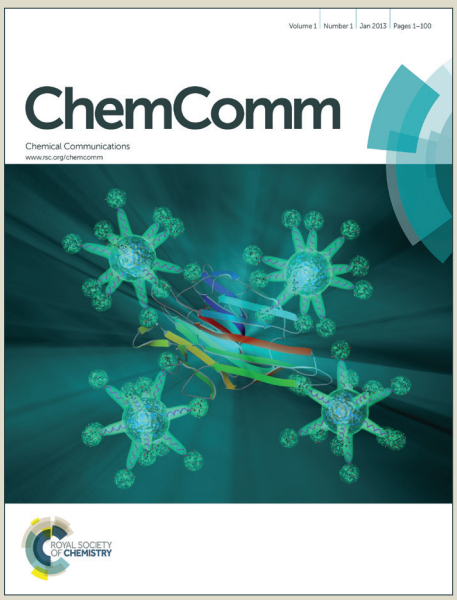
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

COMMUNICATION

A novel colorimetric sensor for Hg^{2+} based on hybridization chain reaction and silver nanowire amplification

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Shurong Tang,[a] Ping Tong,[b] Meili Wang,[b] Jinghua Chen,[a] Guangwen Li*[a] and Lan Zhang*[b]

Through the silver ion catalysis to form colored $KMnO_4$, and combined with the DNA hybridization chain reaction and silver nanowire for signal amplification, a highly sensitive and selective colorimetric sensor has been developed for the detection of Hg^{2+}.

Heavy metal pollution is known to pose a potential threat to human health. The mercury ion (Hg^{2+}) is considered to be one of the most dangerous metal ions. It can cause developmental delay and lead to the brain, nervous system, kidney, and endocrine system damage.[1] So far, some methods based on organic fluorophores[2] or chromophore[3], semiconductor nanocrystalline[4], cyclic voltammetry[5], polymeric materials[6] and protein[7] have been established for the detection of Hg^{2+}. The colorimetric sensors based on the chromophore[8] for Hg^{2+} sensing have attracted particular attention due to their simple, fast, easy readout and potential for high throughput analysis. However, there remain some disadvantages of these sensors, such as limited sensitivity or selectivity, dynamic instability, or incompatible with the water environment, limiting their practical application.

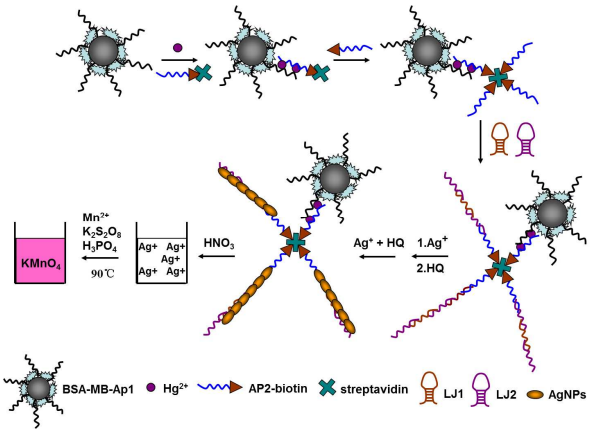
The high–specificity interaction between oligonucleotides and metal ions make them become effective tools for the analysis of specific metal species in the mixture. It has been reported that Hg^{2+} can selectively interact with two T bases and promote the T–T mismatch to form the stable T–Hg^{2+}–T base pairs.[9] This feature can be used for the construction of a highly selective sensor for Hg^{2+} in aqueous solution.

Since large amounts of metal ions are contained in single metal nanoparticles (NPs), they can be used as signal reporters to significantly amplify the detection signal. It has been calculated that there are approximately 2.9×10^5 Ag atoms in a spherical silver nanoparticle with diameter of 21 nm.[10] Some chemiluminescence (CL) systems based on Ag NPs have been developed for sensitive detection of DNA and protein[10,11]. However, in these methods, NPs need to be labeled with signal probe before testing, making the detection process more complex and time–consuming. Commonly, $KMnO_4$, which produced by the released Ag^+, was used to generate the CL signal. There are rare reports on the application of the formed $KMnO_4$ in the colorimetric sensor.

DNA–mediated metal NPs growth technique has received extensive research in the formation of conducting nanowires.[12] The high affinity between DNA and metal cations offers a new way for the formation of metal NPs along the DNA template. The formed small NPs can act as nucleation sites for further deposition of metal NPs, such as silver[13], gold[14], palladium[15] and platinum[16] NPs, etc. The detection signal is usually positively associated with the amount of metal NPs deposited on the DNA. Therefore, by the use of molecular biology technology to extend the DNA chain, the detection sensitivity will be enhanced. Hybridization chain reaction (HCR) is a non–enzyme amplification process. Long–chain DNA product can be formed after the hybridization reaction initiated by a short DNA primer. About 10 times amplification can be completed under reaction at constant temperature for 2 h[17]. These DNA–based nanomaterials can be used to construct label free and highly sensitive biosensors.[18]

Herein, base on HCR reaction and DNA mediated growth of silver nanowires (Ag NWs) for signal amplification, a novel label free colorimetric sensor has been developed for the highly sensitive detection of Hg^{2+}. The sensing principle is shown in Scheme 1. Firstly, an amino–terminated DNA (AP1) was immobilized onto the magnetic bead (MB) surface by amide bond. Then, the MB surface was blocked with BSA to prevent nonspecific adsorption. In the presence of Hg^{2+}, biotin tagged DNA (AP2–biotin)-streptavidin complex will connect to the MB surface through T–Hg^{2+}–T interaction. Then, AP2–biotin was added to combine with the rest binding site of streptavidin. With the introduction of two hairpin–structured DNA probes (LJ1 and LJ2), the HCR reaction can be initiated by AP2–biotin. First, AP2–biotin will hybridize with LJ2 to open its hairpin structure. The hairpin structure of LJ1 is then opened

[a] Department of Pharmaceutical Analysis, Faculty of Pharmacy, Fujian Medical University, Fuzhou, Fujian, 350004, China. E-mail: lgw6301@fjmu.edu.cn.
[b] Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Testing Center, Fuzhou University, Fuzhou, Fujian, 350108, China. E-mail: zlan@fzu.edu.cn.
† Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x



Scheme 1 The principle of the colorimetric sensor for Hg^{2+} based on the HCR reaction and silver nanowire amplification.

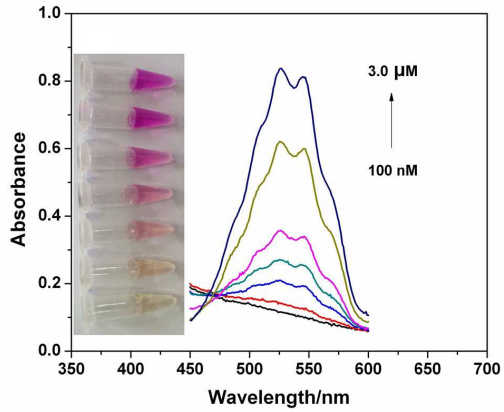


Fig.1 Absorption curves of the solution for Ag^{+} at various concentrations. Insert: The corresponding color changes of the solution.

by the opened LJ2 after hybridization. Next, the opened LJ1 will open the other hairpin–structured LJ2. Finally, a long-chain DNA can be formed by the alternating hybridization between LJ1 and LJ2. Through electrostatic interaction, Ag^{+} will bind to the negative charged long–chain DNA and then reduced to form small Ag NPs by hydroquinone (HQ). After further silver enhancement reaction, the long Ag NWs can be formed. After acid lysis, the released numerous Ag^{+} can be used as catalyst to facilitate the production of colored $KMnO_4$. Thus, highly sensitive colorimetric detection of Hg^{2+} can be achieved.

The catalytic ability of Ag^{+} for the formation of $KMnO_4$ was investigated. The mixtures of Mn^{2+}, $K_2S_2O_8$, H_3PO_4, with and without addition of Ag^{+} were heated at 90°C water bath for several minutes. Then, the obtained products were analyzed by UV–vis spectroscopy. As shown in Fig.S1, it can be seen that in the absence of Ag^{+}, the absorption signal was very small (curve a). On the contrast, the absorption signal was significantly increased after addition of 1.0 μM Ag^{+} (curve b). At the same time, the color of the solution was changed to purple. These results indicated that Ag^{+} can efficiently catalyze the formation of $KMnO_4$. By referencing the proposed model[19], the catalytic mechanism of Ag^{+} for the formation of $KMnO_4$ is elucidated as below:

$$2\,Mn^{2+} + 5\,S_2O_8^{2-} + 8\,H_2O \xrightarrow[\Delta]{Ag^{+}} 2\,MnO_4^{-} + 10\,SO_4^{2-} + 16\,H^{+} \quad (1)$$

We speculate that the reaction (1) is performed as the following two steps:

$$S_2O_8^{2-} + Ag^{+} = SO_4^{2-} + Ag^{2+} + SO_4^{\bullet -} \quad (2)$$

$$5Ag^{2+} + 2Mn^{2+} + 5SO_4^{\bullet -} + 8H_2O = 5SO_4^{2-} + 5Ag^{+} + 16H^{+} + 2MnO_4^{-} \quad (3)$$

The effect of Ag^{+} catalytic time on the absorption signal was investigated. We found that in the range of 1~12 min, the absorption signal (S) and background signal (B) were increased with the increase of catalytic time. The ratio of S/B reached the maximum value at 7 min (Fig.S2). Therefore, 7 min was used in the following experiments. To study the sensitivity of the catalytic system, a series of different concentrations of Ag^{+} were tested. The results are shown in Fig.1. With the increase concentration of Ag^{+}, the absorption value was increased and the colors of the solutions gradually became deeper. The absorption value showed a good linear relationship with the concentration of Ag^{+} in the range from 100.0 nM to 3.0 μM (Data not shown). As low as 50 nM Ag^{+} can be detected, which is adequate for the detection of Ag^{+} in drinking water (U.S.EPA define that the concentration of Ag^{+} shall not exceed 460 nM in drinking water). Thus, Ag^{+} can be used as a potential signal reporter to construct a highly sensitive colorimetric sensor for the indirect detection of various analytes.

In order to exam the feasibility of the constructed colorimetric sensor for the detection of Hg^{2+}, the absorption signals before and after addition of Hg^{2+}, with and without HCR amplification were investigated. From Fig.2, it can be observed that in the absence of Hg^{2+}, the absorption signal was relatively small (curve a). This background signal was produced by the AP1 which modified on the surface of MB. After addition of Hg^{2+}, streptavidin and AP2–biotin, the absorption signal was further increased (curve b). At the same time, the color of the solution was changed to light purple. This is due to the specific T–Hg^{2+}–T and biotin–streptavidin interaction, which resulted in formation of the MB–AP1/Hg^{2+}/AP2–biotin/avidin/biotin–AP2

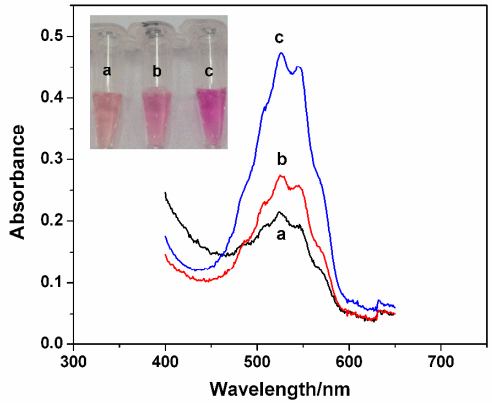


Fig.2 The absorption curves of the colorimetric sensor under different experimental conditions (Inset: the color change of corresponding solution). Before addition of Hg^{2+} (a), after addition of 1.0 nM Hg^{2+} without (b) or with (c) HCR amplification.

complex and increase of the loading amount of DNA. It is worth noting that after introduction of LJ1 and LJ2, the absorption signal was obviously increased (curve c), combining with further deepening of solution color. This is due to the fact that long–chain DNA can be formed after HCR reaction, thus remarkably increasing the loading amount of DNA. The long–chain DNA can be used as template for Ag NWs deposition, which significantly increased the absorption signal. The resulting Ag NWs were further characterized by atomic force microscopy (Fig.S3). Several micrometer–long Ag NWs were observed along the DNA skeleton. The associated height profile showed that the height of Ag NWs is ~3 nm. These experimental results presented that the constructed colorimetric sensor can be used for detection of Hg^{2+} and after HCR amplification the detection signal can be effectively amplified.

To obtained the best sensing performance, silver enhancement time (8 min) and Ag NWs dissolution time (45 min) were optimized (Fig.S4). To evaluate the sensitivity and dynamic range of the proposed colorimetric sensor, various concentrations of Hg^{2+} were tested under the optimal conditions. As shown in Fig.3A, the absorption signal increased with increasing concentration of Hg^{2+}. The more Hg^{2+} was added, the more DNA can be captured on the MB surface after HCR reaction, thus producing more Ag NWs. A good linear response of the absorption signal against the concentration of Hg^{2+} was obtained in the range from 0.05 to 3.0 nM (see Fig.3B). The limit of detection for Hg^{2+} was 45.0 pM (calculated by 3σ, where σ is the standard deviation of signal in blank solution). The detection sensitivity is satisfactorily meeting the sensitivity requirement of drinking water permitted by U.S.EPA (lower than 10 nM). Such high sensitivity was attributed to the use of HCR reaction to form long–chain DNA for the deposition of Ag NWs. After acid lysis, numerous Ag^{+} can be released from the Ag NWs, which act as signal reporter to catalyze the chromogenic reaction and significantly amplified the absorption signal.

The high selectivity of a sensor is very important for the analysis of complicated environmental and biological samples. To investigate the selectivity of the colorimetric sensor for Hg^{2+} detection, blank, Hg^{2+} (3.0 nM) and other metal ions (1.0 μM) were investigated under the same conditions. As shown in Fig.4, obvious absorption signal can be obtained only by Hg^{2+}. Importantly, the selectivity can be observed with the naked eye. Only Hg^{2+} caused significant color change of the solution to purple. These results indicated the excellent selectivity of

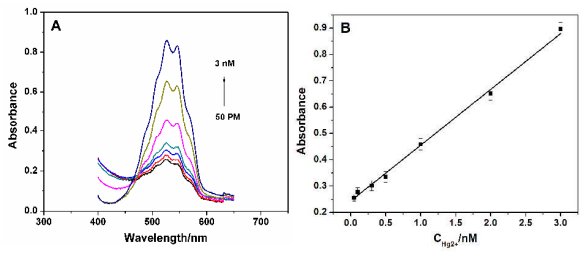


Fig.3 (A) Absorbance curves of the sensor for Hg^{2+} at various concentrations. (B) The corresponding calibration plot of absorbance values against the Hg^{2+} concentrations.

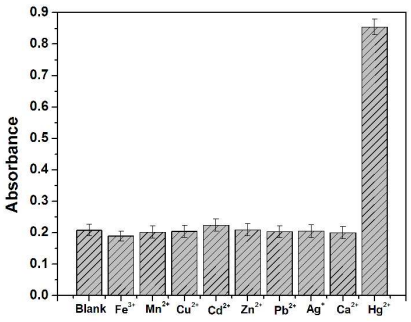


Fig.4 Selectivity investigation of the colorimetric sensing system for Hg^{2+} detection.

the colorimetric sensor for Hg^{2+}. This high selectivity was owing to the high affinity of T–Hg^{2+}–T coordination.

The practical application of the colorimetric sensor was also investigated by determination of the Hg^{2+} spiked water samples. The water samples were collected from laboratory tap water. After filtered through a 0.22 μm membrane to get rid of insolubles, standard Hg^{2+} solutions with different concentrations were added to the pretreated water sample. The spiked samples were then analyzed (each sample was parallel detected of 3 times). The results are shown in Table S1. Recovery values ranging from 98.8% to 106.0% and RSD between 4.00% to 5.95% were obtained, indicating that the proposed sensor was applicable for Hg^{2+} analysis in real samples.

In summary, a novel label–free colorimetric sensor has been developed for Hg^{2+} detection based on the HCR reaction and Ag NWs amplification. HCR is an isothermal amplification method. It has significant amplification ability without the need of protease. Through the specific T–Hg^{2+}–T interaction to capture DNA, the HCR reaction was initiated to form the long–chain dsDNA, which can be used as template for the deposition of Ag NWs in situ, avoiding the complicated label procedure. Using the specific affinity properties that one streptavidin can combine with four biotin molecule to further increase the formed amount of long–chain DNA and Ag NWs, the detection signal can be significantly amplified. Under the optimal conditions, a detection limit as low as 45.0 pM could be obtained for Hg^{2+}. This sensor also exhibits excellent selectivity for Hg^{2+} against other metal ions. This highly sensitive and selective colorimetric sensor holds great potential for the detection of environmentally toxic mercury and other target molecules.

This work was supported by the National Natural Science Foundation of China (21275029, 21375017), National Basic Research Program of China (No.2010CB732403), the National Science Foundation for Distinguished Young Scholars of Fujian Province (2013J06003), the Natural Science Foundation of Fujian Province (2015J01045), the Medical Elite Cultivation Program of Fujian, P.R.C (2014-ZQN-ZD-26).

Notes and references

1 Y. Wang, F. Yang and X. Yang, *Biosens. Bioelectron.,* 2010, **25**, 1994.
2 (a) Y. K. Yang, K. J. Yook, and J. Tae, *J. Am. Chem. Soc.,* 2005, **127**, 16760; (b) X. Guo, X. Qian and L. Jia, *J. Am. Chem. Soc.,* 2004, **126**, 2272; (c) A. Caballero, R. Martinez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tárraga, P. Molina and J. Veciana, *J. Am. Chem. Soc.,* 2005, **127**, 15666; (d) J. Wang, X. Qian and J. Cui, *J. Org. Chem.,* 2006, **71**, 4308.
3 (a) E. Coronado, J. R. Galan–Mascaros, C. Marti–Gastaldo, E. Palomares, J. R. Durrant, R. Vilar, M. Gratzel and M. K. Nazeeruddin, *J. Am. Chem. Soc.,* 2005, **127**, 12351; (b) M. K. Nazeeruddin, D. D. Censo, R. Humphry–Baker and M. Grätzel, *Adv. Funct. Mater.,* 2006, **16**, 189; (c) J. V. Ros–Lis, R. Martínez–-Máñez, K. Rurack, F. Sancenón, J. Soto and M. Spieles, *Inorg. Chem.,* 2004, **43**, 5183; (d) T. Balaji, M. Sasidharan and H. Matsunaga, *Analyst,* 2005, **130**, 1162.
4 C. Zhu, L. Li, F. Fang, J. Chen and Y. Wu, *Chem. Lett.,* 2005, **34**, 898.
5 M. A. Nolan and S. P. Kounaves, *Anal. Chem.,* 1999, **71**, 3567.
6 L. J. Fan, Y. Zhang and W. E. Jones, *Macromolecules,* 2005, **38**, 2844.
7 P. Chen and C. He, *J. Am. Chem. Soc.,* 2004, **126**,728.
8 (a) J. H. Huang, W. H. Wen, Y. Y. Sun, P. T. Chou and J. M. Fang, *J. Org. Chem.,* 2005, **70**, 5827; (b) E. Palomares, R. Vilar and J. R. Durrant, *Chem. Commun.,* 2004, **50**, 362; (c) S. Tatay, P. Gavina, E. Coronado and E. Palomares, *Org. Lett.,* 2006, **8**, 3857.
9 (a) S. Katz, *J. Am. Chem. Soc.,* 1952, **74**, 2238; (b) Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima and A. Ono, *J. Am. Chem. Soc.,* 2007, **129**, 244.
10 C. H. Liu, Z. P. Li, B. A. Du, X. R. Duan and Y. C. Wang, *Anal. Chem.,* 2006, **78**, 3738.
11 W. Chen, L. Zheng, M. Wang, Y. Chi and G. Chen, *Anal. Chem.,* 2013, **85**, 9655.
12 (a) E. Braun, Y. Eichen, U. Sivan and G. Ben-Yoseph, *Nature,* 1998, **391**, 775; (b) H. Yan, S. H. Park, G. Finkelstein, J. H. Reif and T. H. LaBean, *Science,* 2003, **301**, 1882.
13 D. Liu, S. H. Park, J. H. Reif and T. H. LaBean, *Proc. Natl. Acad. Sci. U S A,* 2004, **101**, 717.
14 A. Ongaro, F. Griffin, P. Beeeher, L. Nagle, D. Iacopino, A. Quinn, G. Redmond and D. Fitzmaurice, *Chem. Mat.,* 2005, **17**, 1959.
15 H. Liu, Y. Chen, Y. He, A. E. Ribbe and C. M. Prof, *Angew. Chem. Int. Edit.,* 2006, **45**, 1942.
16 W. E. Ford, O. Harnack, A. Yasuda and J. M. Wessels, *Adv. Mater.,* 2001, **13**, 1793.
17 (a) S. Liu, Y. Wang, J. Ming, Y. Lin, C. Cheng and F. Li, *Biosens. Bioelectron.,* 2013, **49**, 472; (b) J. Zhuang, L. Fu, M. Xu, H. Yang, G. Chen and D. Tang, *Anal. Chim. Acta,* 2013, **783**, 17; (c) Y. Chen, J. Xu, J. Su, Y. Xiang, R. Yuan and Y. Chai, *Anal. Chem.,* 2012, **84**, 7750.
18 F. Gao, J. Lei and H. Ju, *Anal. Chem.,* 2013, **85**, 11788.
19 G. P. Anipsitakis and D. D. Dionysiou, *Environ. Sci. Technol.,* 2004, **38**, 3705.