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A SERS DNAzyme biosensor for lead ion detection†

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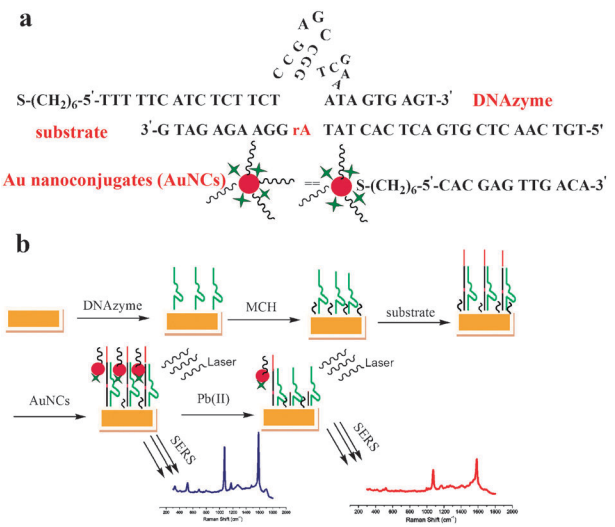
SERS biosensor for sensitive and selective detection of lead ions (Pb^{2+}) based on DNAzyme was developed by taking advantage of the specific catalytic reaction of DNAzyme upon binding to Pb^{2+} ions. Detection was accomplished by SERS nanoprobe labeled with DNA and Raman reporters for signal amplification.

Surface enhanced Raman Scattering (SERS) is a physical phenomena based on the magnitude of the electromagnetic field around a roughened gold or silver metal surface,[1] such as gold or silver nanoparticles.[2] Since its discovery, SERS has been developed into a powerful tool for the non-destructive and ultrasensitive detection of proteins, DNA and small molecules,[3–5] making it a viable alternative to fluorescence sensors. Detection of lead ions (Pb^{2+}), a common environmental contaminant, is very important to human health and environmental monitoring. A number of analytical methods have been developed including the classical atomic absorption/emission spectroscopy,[6] electrochemical[7] and fluorescent techniques.[8] SERS has been used to detect Pb^{2+} ions with a 4-(2-pyridylazo)resorcinol (PAR) coating modified with a disulfide, which provided a strong anchor to a roughened silver substrate.[9] The limit of detection using this method was ~522 ppb. However, the selectivity is a big issue for this method. Recently, artificially designed nucleic acid sequences (aptamers) have been developed to either recognize target analytes or catalyze specific chemical reactions (catalytic nucleic acid containing ribozymes, deoxyribozymes, and aptazymes).[10] Based on the binding specificity of these artificially designed nucleic acids to its target, various types of sensors could be designed for the detection of proteins, small molecules as well metal ions using fluorescent,[11] electrochemical[12] and colorimetric approaches.[13] Our and other group's published works have shown that SERS is a powerful tool for the detection of protein (thrombin) and small molecules (cocaine and adenosine) based on synthesized oligonucleotides.[5,14] Here we report the detection of Pb^{2+} ions by SERS based on the artificially designed nucleic acid sequences. We will show that a DNAzyme concept with SERS sensitivity will provide a new means to detect metal ions (Pb^{2+} ions) with high sensitivity and selectivity.

Gold nanoparticles (AuNPs), owing to its unique optical properties, have been widely utilized in colorimetric and electrochemical biosensors.[12,15] Due to the electromagnetic field produced around the gold surface, AuNPs of size greater than 20 nm have been shown to provide strong Raman enhancement of the reporters therein.[16] Because of its stability and mono-dispersity, AuNPs could be easily conjugated with Raman reporters and DNA sequences to detect ultra-low concentration of contaminants.

Therefore, in this work we take advantage of the unique optical property of AuNPs, high sensitivity of SERS and the specfic catalytic reaction of DNAzyme upon binding to Pb^{2+} ions, to propose a novel SERS DNAzyme biosensor for specific and sensitive detection of Pb^{2+} ions.

Scheme 1 details the fabrication of SERS DNAzyme biosensor for Pb^{2+} ion detection. DNAzyme (termed 17 E according to the report, which is capable of cleaving a single RNA linkage within a DNA substrate), the cleavage substrate (17DS, a DNA/RNA chimera in which γ A represents the ribonucleotide adenosine)[17] was chosen in this study. Details of the sequence are provided in Scheme 1a. It should be noted that the sequence of the cleavage substrate was extended on the 5′ end to hybrid with the short DNA sequence conjugated



Scheme 1 Schematic of the fabrication of SERS biosensor for the detection of Pb^{2+} ions based on the DNAzyme.

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onto the AuNPs. AuNPs with the average size of 45 nm in diameter (Fig. S1A, ESI†) were selected as the enhancing substrate because of its strong Raman enhancement and good stability to enable functionalization with biomolecules and Raman reporters.[18] The conjugation of AuNPs with DNA and Raman reporters was accomplished according to the method reported by our pervious work[18] (details in ESI†). Scheme 1b provides a detailed account of the SERS DNAzyme biosensor proposed for Pb^{2+} ion detection. The DNAzyme with thiol group were first immobilized onto a gold-coated surface through the strong Au–S bond. The surface was then blocked by a small molecule 6-mercaptohexane. The cleavage substrate (17DS) was then hybridized to the DNAzyme on the gold surface. Due to the extension of the cleavage substrate at the 5′ end, it can be used to further hybridize specifically to the AuNPs conjugates. Upon binding of Pb^{2+} ions to the substrate a proportional amount of AuNPs conjugates will be cleaved from the gold surface to result in a decreased Raman signal, herein, to realize a sensitive and specific SERS DNAzyme biosensor for the detection of Pb^{2+} ions. As a first step, the conjugation of the extended oligonucleotide sequence and the Raman reporters to AuNPs were demonstrated by the UV-Vis absorption spectra as shown in Fig. S1B (ESI†). AuNPs show a typical surface Plasmon resonance (SPR) peak at 526 nm (curve a), after conjugation with the capture DNA and the Raman reporters, the SPR of AuNPs band was red-shifted to 530 nm (curve b), indicating the successful conjugation of DNA and Raman reporters to AuNPs for SERS detection. After hybridization of the AuNPs-DNA nanoconjugates to the cleavage substrate on gold surface, AuNPs were bound thereon, which were characterized by scanning electron microscopy (SEM) as shown in Fig. 1A. The uniform distribution of AuNPs on the gold surface indicates the interaction of AuNPs-DNA nanoconjugates with the cleavage substrate through the highly specific interaction to complete the sensor construction. Upon exposure of the DNAzyme SERS biosensor to Pb^{2+} ions, the cleavage substrate cleaves the AuNPs conjugates from the gold surface thereby releasing the AuNPs. The decrease in the number of AuNPs apparent from the magnified SEM images (inset in Fig. 1) and the related SERS intensity of the Raman reporter could be used as an effective indicator of the concentration of Pb^{2+} ions.

SERS spectra of Raman reporters (mercaptobenzoic acid-MBA) with different concentrations of Pb^{2+} ions after binding to the gold surface are shown in Fig. 2A. An obvious decrease in the intensity of characteristic peaks (1078 and 1587 cm^{-1}, assigned to the ν_{8a} and ν_{12} aromatic ring vibrations, respectively)[19] of MBA attached to AuNPs could be noted corresponding to an increase in the concentration of Pb^{2+} ions. As the concentration of Pb^{2+} ions increased, the numbers of AuNPs nanoconjugates on the gold surface decreased proportionately, weakening the Raman signal on the gold surface. To clearly demonstrate the detection for Pb^{2+} ions with this design, a semi-quantative approach was used and an appropriate calibration curve was developed. First Raman spectra of silica at 520 cm^{-1} (Si_{520}) were used for system calibration.[20] Spectra of control substrate without Pb^{2+} ions was used as a reference for normalization. Ten replicate SERS spectra of MBA were obtained at each concentration (from 20 nM to 1 μM) and the averaged intensity of the characteristic peaks at 1078 cm^{-1} and 1584 cm^{-1} were used to calibrate the SERS intensity with reference of the same peak got from the control ($R = \Delta I/I_0$, I_0, without Pb^{2+}). Based on this concept, the calibration curve between the SERS signal intensity at 1584 cm^{-1} and the concentrations of Pb^{2+} ions were obtained as shown in the Fig. 2B, illustrating that the detection range for Pb^{2+} using the SERS DNAzyme biosensor is from 20 nM to 1 μM and the lowest concentration for Pb^{2+} ions detection is 20 nM, which is comparable to the reported DNAzyme biosensor for Pb^{2+} ions detection using electrochemical and

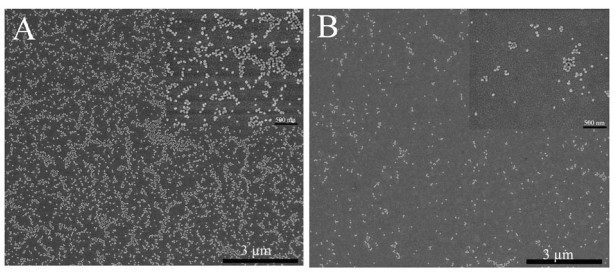

Fig. 1 SEM images of AuNPs on gold surface before (A) and after (B) reaction with Pb^{2+} ions at 500 nM concentration. Inset shows magnified SEM images.

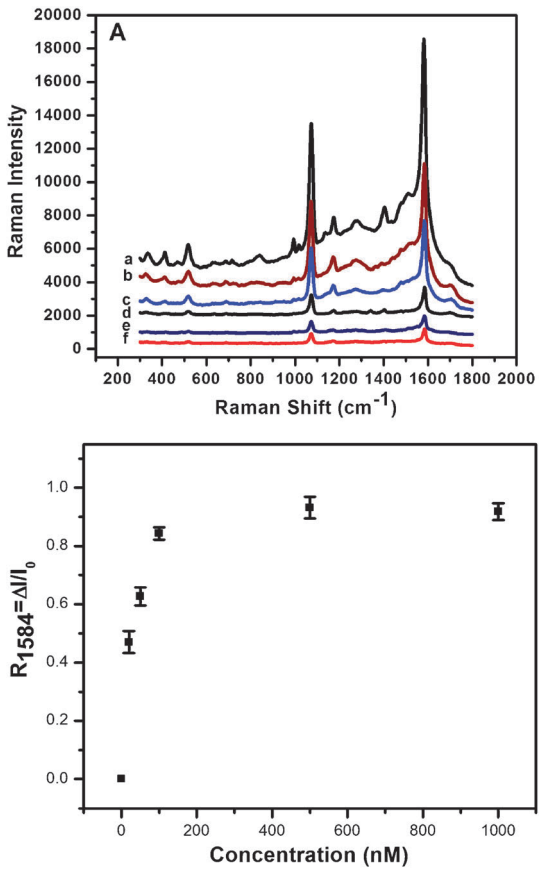

Fig. 2 A. SERS spectra of MBA with different concentration of Pb^{2+} ions (a. 0 nM; b. 20 nM; c. 50 nM; d. 100 nM; e. 500 nM; f. 1 μM). B. Calibration curve of SERS intensity at 1584 cm^{-1} as a function of Pb^{2+} ion concentration. (All of the averaged intensity was normalized with respect to the control samples, *i.e.* surface without Pb^{2+}).

flourescent methods in the literature reported.[21] The SERS intensity was saturated when the Pb^{2+} ions concentration was above 1 μM. The reproducibility of the SERS signal for Pb^{2+} ion detection was also demonstrated as shown in Fig. S2 (ESI†). The large area SEM image of the gold surface shows a uniform surface and consistent Raman signal from the different points randomly selected for Raman measurement shows good reproducibility of the fabricated SERS biosensor for Pb^{2+} ion detection. One another point should be noted that saturate absorption of DNAzyme on the gold surface is necessary and important for sensitive Pb^{2+} detection (ESI†).

The selectivity of the SERS biosensor was determined in the presence of various other divalent metal ions (Zn^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+} and Mg^{2+}) at a high concentration of 2 μM as shown in Fig. 3A and B according to the same quantitative method used above and typical SERS spectra for the Zn^{2+} detection were shown in the Fig. S2D (ESI†). These metal ions were tested because they showed relatively high catalytic activity toward the cleavage by 17 E DNAzyme.[22] It can be clearly seen that only in the presence of Pb^{2+} ions, the SERS signal decreased significantly, while in the presence of other metal ions, only a minor or no decrease in signal intensity was observed, indicating the high selectivity of the designed SERS DNAzyme biosensor for Pb^{2+} ion detection.

In summary, we have developed a unique and simple SERS biosensor for the detection of Pb^{2+} ions based on the DNAzyme concept. Due to the high sensitivity of SERS, as low as 20 nM Pb^{2+} ions can be detected. Due to the high binding specificity of the DNAzyme to Pb^{2+} ions and the portability of Raman spectroscopy, the SERS DNAzyme biosensor scheme could be developed into a highly selective portable devise. More importantly, the developed SERS biosensor concept could be extended to detect multiple metal ions using different Raman reporters, based on the DNAzyme catalytic reaction with SERS amplification.

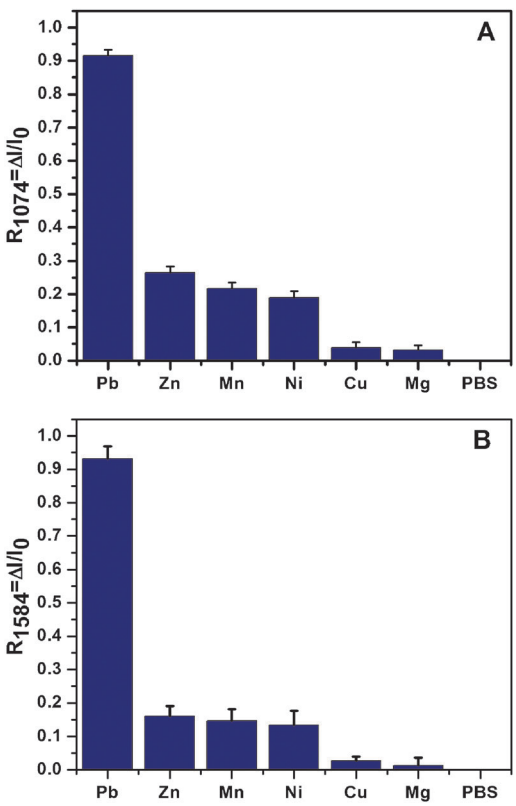

Fig. 3 Selectivity of SERS DNAzyme biosensor for Pb^{2+} detection. The intensity ratio of ν_{8a} (A) and ν_{12} (B) of MBA for control (PBS) and various divalent metal ions (500 nM for Pb^{2+} ions and 2 μM for other metal ions, Zn^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}).

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Notes and references

1 M. Fleischmann, P. J. Hendra and A. J. Mcquillan, *Chem. Phys. Lett.*, 1974, **26**, 163; D. L. Jeanmaire and R. P. Van Duyne, *J. Electroanal. Chem.*, 1977, **1**, 84; Y. C. Liu and C. C. Wang, *J. Phys. Chem. B*, 2005, **109**, 5779.

2 J. A. Creighton, C. G. Blatchford and M. G. Albrecht, *J. Chem. Soc., Faraday Trans. 2*, 1979, **75**, 790; K. Faulds, R. E. Littleford, D. Graham, G. Dent and W. E. Smith, *Anal. Chem.*, 2004, **76**, 592.

3 J. Ni, R. J. Lipert, G. B. Dawson and M. D. Porter, *Anal. Chem.*, 1999, **71**, 4903; Y. L. Wang, K. Lee and J. Irudayaraj, *Chem. Commun.*, 2010, **46**, 613.

4 L. Fabris, M. Dante, G. Braun, S. J. Lee, N. O. Reich, M. Moskovits, T. Q. Nguyen and G. C. Bazan, *J. Am. Chem. Soc.*, 2007, **129**, 6086; G. Braun, S. J. Lee, M. Dante, T. Q. Nguyen, M. Moskovits and N. Reich, *J. Am. Chem. Soc.*, 2007, **129**, 6378.

5 J. Chen, J. Jiang, X. Gao, G. Liu, G. Shen and R. Yu, *Chem.–Eur. J.*, 2008, **14**, 8374; J. Chen, X. Liu, K. Feng, Y. Liang, J. Jiang, G. Shen and R. Yu, *Biosens. Bioelectron.*, 2008, **24**, 66.

6 X. P. Yan and Z. M. Ni, *J. Anal. At. Spectrom.*, 1991, **6**, 483; M. Temprano, M. R. F. Delacampa and A. Sanzmedel, *J. Anal. At. Spectrom.*, 1993, **8**, 821.

7 C. Babyak and R. R. Smart, *Electroanalysis*, 2004, **16**, 175.

8 C. L. Davis, B. W. Smith and J. D. Winefordner, *Microchem. J.*, 1995, **52**, 383.

9 L. G. Crane, D. X. Wang, L. M. Sears, B. Heyns and K. Carron, *Anal. Chem.*, 1995, **67**, 360.

10 A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818; C. Tuerk and L. Gold, *Science*, 1990, **249**, 505; M. P. Robertson and G. F. Joyce, *Nature*, 1990, **344**, 467.

11 B. L. Li, H. Wei and S. J. Dong, *Chem. Commun.*, 2007, 73; J. Wang, Y. Jiang, C. Zhou and X. Fang, *Anal. Chem.*, 2005, **77**, 3542; C. C. Huang and H. T. Chang, *Chem. Commun.*, 2008, 1461.

12 L. Shen, Z. Chen, Y. Li, S. He, S. Xie, X. Xu, Z. Liang, X. Meng, Q. Li, Z. Zhu, M. Li, X. C. Le and Y. Shao, *Anal. Chem.*, 2008, **80**, 6323; Y. Du, B. L. Li, H. Wei, Y. Wang and E. Wang, *Anal. Chem.*, 2008, **80**, 5110.

13 H. Wei, B. L. Li, J. Li, S. J. Dong and E. K. Wang, *Nanotechnology*, 2008, **19**, 095501; D. Li, A. Wieckowska and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 3927.

14 Y. Wang, K. Lee and J. Irudayaraj, *Chem. Commun.*, 2010, **46**, 613–615; Y. Wang, H. Wei, B. Li, W. Ren, S. Guo, S. Dong and E. Wang, *Chem. Commun.*, 2007, 5220–5222.

15 C. Huang, Y. Huang, Z. Cao, W. Tan and H. Chang, *Anal. Chem.*, 2005, **77**, 5735; J. Lee, P. Ulmann, M. Han and C. Mirkin, *Nano Lett.*, 2008, **8**, 529.

16 P. N. Njoki, I. S. Lim, D. Mott, H.-Y. Park, B. Khan, S. Mishra, R. Sujakumar, J. Luo and C.-J. Zhong, *J. Phys. Chem. C*, 2007, **111**, 14664.

17 J. Li and Y. Lu, *J. Am. Chem. Soc.*, 2000, **122**, 10466; S. W. Santoroand and G. F. Joyce, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 4262.

18 L. Sun, C. Yu and J. Irudayaraj, *Anal. Chem.*, 2007, **79**, 3981; L. Sun, C. Yu and J. Irudayaraj, *Anal. Chem.*, 2008, **80**, 3342.

19 S. Xu, X. Ji, W. Xu, X. Li, L. Wang, Y. Bai, B. Zhao and Y. Ozaki, *Analyst*, 2004, **129**, 63; C. J. Orendorff, A. G. Tapan, K. Sau and C. J. Murphy, *Anal. Chem.*, 2005, **77**, 3261.

20 K. Kim and J. K. Yoon, *J. Phys. Chem. B*, 2005, **109**, 20731; K. Kim and H. S. Lee, *J. Phys. Chem. B*, 2005, **109**, 18929.

21 M. Moshe, J. Elbaz and I. Willner, *Nano Lett.*, 2009, **9**, 1196.

22 J. W. Liu and Y. Lu, *Anal. Chem.*, 2003, **75**, 6666; A. K. Brown, J. Li, C. M. B. Pavot and Y. Lu, *Biochemistry*, 2003, **42**, 7152.

