

SCIENTIFIC REPORTS



OPEN

A novel and highly sensitive nanocatalytic surface plasmon resonance-scattering analytical platform for detection of trace Pb ions

Received: 11 February 2016

Accepted: 21 March 2016

Published: 13 April 2016

Lingling Ye*, Guiqing Wen*, Huixiang Ouyang, Qingye Liu, Aihui Liang & Zhiliang Jiang

Gold nanoparticles (AuNP) have catalysis on the reaction of $\text{HAuCl}_4\text{-H}_2\text{O}_2$. The produced AuNP have strong resonance Rayleigh scattering (RRS) effect and surface-enhanced resonance Raman scattering (SERS) effect when Victoria blue B (VBB) and rhodamine S (RhS) were used as probes. The increased RRS/SERS intensity respond linearly with the concentration of gold nanoparticles (AuNP_B) which synthesized by NaBH_4 over 0.038–76 ng/mL, 19–285 ng/mL, 3.8–456 ng/mL respectively. Four kinds of tested nanoparticles have catalysis on the $\text{HAuCl}_4\text{-H}_2\text{O}_2$ particles reaction. Thus, a novel nanocatalysis surface plasmon resonance-scattering (SPR-S) analytical platform was developed for AuNP. The DNAzyme strand hybridized with the substrate strand to form double-stranded DNA (dsDNA) which couldn't protect AuNP_C to aggregate to AuNP_C aggregations, having strong RRS effect. Upon addition of Pb^{2+} , dsDNA could be cracked by Pb^{2+} to produce single-stranded DNA (ssDNA) that adsorbed on the AuNP_C surface to form $\text{AuNP}_C\text{-ssDNA}$ conjugates. The conjugates have strong catalysis on $\text{HAuCl}_4\text{-H}_2\text{O}_2$ reaction. With increased Pb^{2+} concentration, the concentration of $\text{AuNP}_C\text{-ssDNA}$ increased and lead to the catalytic activity stronger. The increased RRS intensity responds linearly with Pb^{2+} concentration over 16.7–666.7 nmol/L. The SERS intensity responded linearly with the concentration of Pb^{2+} over 50–500 nmol/L.

Nanoparticles are of advantages of unique optical, electrical and chemical properties, and have been used for protein and nucleic acid analysis, biosensors, biochips and nanocatalytic analysis^{1–8}. Noble metal nanoparticles have high electron density, good biocompatibility, good catalysis and good stability, easy preparation, so it has attracted people's attentions. Haruta found that nanogold was a good catalyst which load on the transition metal oxides⁹, not only has high catalytic activity for CO oxidation at low temperature, but also have the advantages of good water resistance, stability and the enhanced effect of humidity¹⁰. It has broken the traditional ideas that nanogold has no catalytic activity. In the analysis of trace contaminants, nanocatalysis provides opportunities to establish a high sensitive and selective analysis method to amplify analytical signal, and improve selectivity that combine with immunoreaction and nucleic acid aptamer reaction^{11–16}. Xu *et al.*¹⁵ have reported a new light scattering method for determination of nucleic acid using immunonanogold catalytic amplification, with a detection limit of 10 fmol/L. Our group developed two new technologies including immunonanocatalysis and aptamer-modified nanoparticle catalysis, that have been used for detecting 7.2 pg/mL urine albumin and 0.09 ng/mL IgG^{14,17,18}. These demonstrate that exploring new highly sensitive nanocatalysis analytical reaction is very significant. Among the nanoparticles, nanogold in solution has best stability and strong catalysis. H_2O_2 not only has no effect for subsequent SPR research but also is colorless, accessible and with non-toxic product. As far as we know, there are no reports about $\text{H}_2\text{O}_2\text{-HAuCl}_4\text{-nanogold}$ catalytic analytical reaction and used for the SPR-S analysis platform.

Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection of Ministry Education, Guangxi Normal University, Guilin 541004, China. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to A.L. (email: gqwen@mailbox.gxnu.edu.cn) or Z.J. (email: zljjiang@mailbox.gxnu.edu.cn)

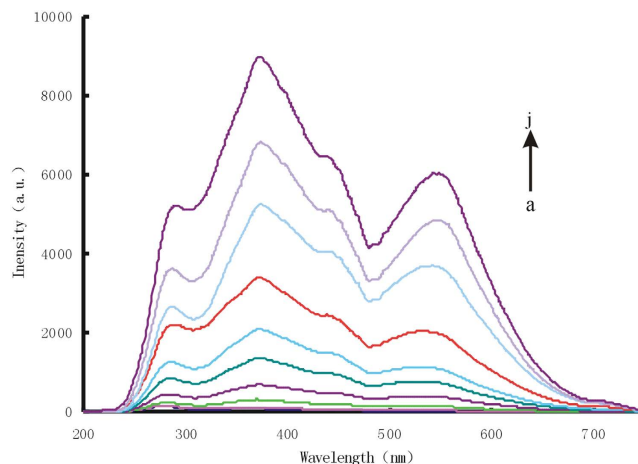


Figure 1. RRS spectrum of AuNP_c-HAuCl₄-H₂O₂ system. (a) 4.48 μmol/L HAuCl₄+0.67 mmol/L HCl+3.33 mmol/L H₂O₂ (b) a+1.9 ng/mL AuNP_c; (c) a+3.8 ng/mL AuNP_c; (d) a+7.6 ng/mL AuNP_c; (e) a+19 ng/mL AuNP_c; (f) a+34.2 ng/mL AuNP_c; (g) a+47 ng/mL AuNP_c; (h) a+85.5 ng/mL AuNP_c; (i) a+133 ng/mL AuNP_c; (j) a+161.5 ng/mL AuNP_c.

The SPR-S techniques included the RRS and SERS, which the former is elastic and the later is inelastic scattering that both were based on the nanoparticle scattering. RRS is simple, sensitive spectral analysis method and has been used for protein, nucleic acid and metal ions analysis^{19–23}. Lead is a harmful heavy metal, which has been listed as key detection project in food, drugs, environmental pollutants and supervision inspection. Based on the reaction of Pb²⁺ reacting with excessive I⁻ to form [PbI₄]²⁻, and further associated with rhodamine 6 G (Rh6G) to produce particles with a strong RRS peak, Luo *et al.*²⁴ developed a RRS method for detection of Pb²⁺ as low as 0.04 μg/mL. Luo *et al.*²⁵ reported a RRS method for detection Pb²⁺ as low as 1.0 nmol/L, based on the binding of Pb(II) with thrombin and aptamer. Pb⁴⁺ was reduced to PbH₄ gas by NaBH₄ and the gas trapped by Au³⁺ to form nanogold that exhibited a RRS effect at 286 nm. This principle was used to detect Pb²⁺ as low as 7.0 × 10⁻⁸ mol/L²⁶. Based on the dsDNA cracked by Pb(II) to release a short single-stranded DNA that conjugated gold nanoparticles (AuNPs) to form a stable AuNPs-ssDNA complex, and its nanocatalysis of HAuCl₄-vitamin C particle reaction, a sensitive RRS method was developed for detection of Pb(II)²⁷. However, there are no reports about the HAuCl₄-H₂O₂ nanogold catalysis SPR-RS analytical platform being utilized to detect trace Pb²⁺, combining with the DNA enzymes cracked reaction. SERS is a sensitive and selective molecular spectrometry, based on the molecular probes adsorbed on rough surface of nanoparticles^{28–33}. Although there are many SERS detection techniques, a few SERS quantitative methods have been reported, with good accuracy, simplicity and practicality. Liu *et al.*³⁴ proposed a SERS biosensor to detect lead ion, combining the DNAzyme cracking and nanocatalytic reaction. Zhang *et al.*³⁵ used the prepared tree-shape nanogold-DNA as a signal amplifier to fabricate a SERS biosensor for detection of 100 pmol/L Pb²⁺. A label-free rhodamine 6G SERS probe was reported for detection of trace Pb(II) in Au_{core}-Ag_{shell} nanosol substrate, based on the Pb(II) cracking the DNAzyme³⁶. However, there are no reports about aptamer combining with the nanocatalysis of H₂O₂-HAuCl₄ in SERS quantitative analysis of Pb²⁺. In this paper, we have considered the new nanocatalytic reaction of AuNP-HAuCl₄-H₂O₂, and two new SPR methods were developed for detection of Pb(II), combining the analysis platform with the DNAzyme cracking.

Results

RRS spectra. The RRS signals of small particle size gold, silver, platinum and palladium nanosol are very weak. Different concentrations of AgNO₃ were added to preparation of AuNR₁, AuNR₂ and AuNR₃, with the diameter of 32 nm, 37 nm and 43 nm respectively that RRS values gradually reduced (Fig. S1). With the increase of AuNR concentration, the RRS peak linear increased at 370 nm (Fig. S1D). Nanoparticles can catalytic hydrogen peroxide reduction of HAuCl₄ under the condition of 0.67 mmol/L HCl, and with the increase of nanogold solution concentration, the RRS intensity of system linear increase at 370 nm (Fig. 1, Fig. S2). The catalytic activity of AuNP_B was better than that of AuNP_C because the particle size of AuNP_B was smaller than AuNP_C, which lead to the surface energy more larger and the surface of AuNP_B nanoparticles can absorb more HAuCl₄. Different particle size of AuNR was added as catalyst, with the AuNR concentration increased, the RRS peak linear increased at 370 nm (Figs S3–S5). When AgNPs, PdNPs, PtNPs nanosol solution was used as catalyst, with the increase of nanoparticles concentration, the RRS peak linear increased at 370 nm (Fig. S6). It can be used to quantitative of HAuCl₄ and H₂O₂ through this catalytic system, with the increase of HAuCl₄ concentration, the RRS peak of system linear increased and color from colorless gradually became red (Fig. S7), with the increase of H₂O₂ concentration, the RRS peak of system linear increased (Fig. S8). When AuNP_c-ssDNA solution was used as catalyst, AuNP_c modified by aptamer catalytic activity is stronger than AuNP_c solution, with the increase of AuNP_c-ssDNA concentration, the RRS peak linear increased at 370 nm (Fig. S9).

DNAzyme catalytic strand hybridize with substrate strands to form double-stranded DNA (dsDNA). In pH 8.0 Tris-HCl buffer solution and 6.7 mmol/L NaCl, AuNP_c were aggregated to the AuNP_c aggregations which exhibited a strong RRS peak at 370 nm. Upon addition of Pb²⁺, the substrate chain of dsDNA could be cracked

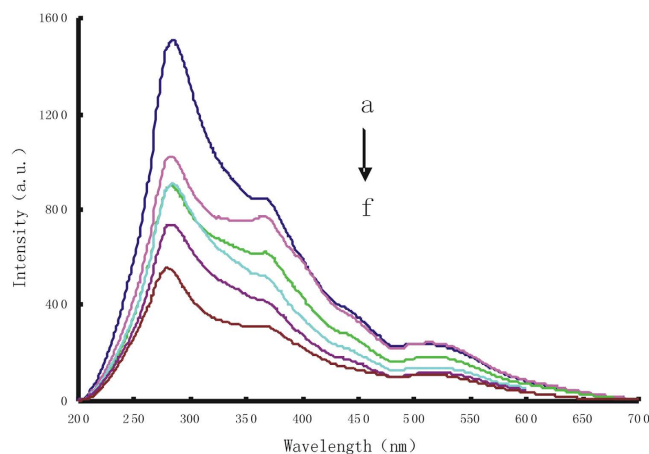


Figure 2. RRS spectrum of Pb^{2+} -aptamer-AuNP_c system. (a) 4 mM pH 8.0 Tris-HCl-50 μL hybrid solution-9.55 $\mu\text{g}/\text{mL}$ AuNP_c-10 mmol/L NaCl; (b) a+0.125 $\mu\text{mol}/\text{L}$ Pb^{2+} ; (c) a+0.175 $\mu\text{mol}/\text{L}$ Pb^{2+} ; (d) a+0.25 $\mu\text{mol}/\text{L}$ Pb^{2+} ; (e) a+0.375 $\mu\text{mol}/\text{L}$ Pb^{2+} ; (f) a+0.425 $\mu\text{mol}/\text{L}$ Pb^{2+} .

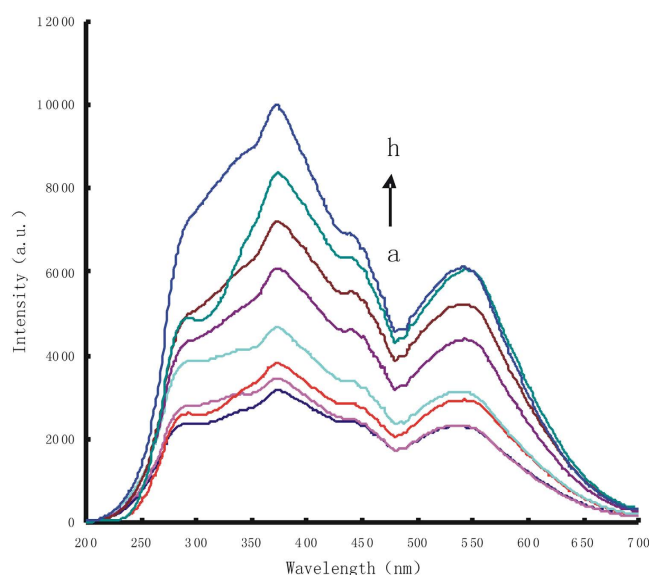


Figure 3. RRS spectrum of Pb^{2+} reaction solution-HAuCl₄-H₂O₂ nanocatalytic system. (a) 4.48 $\mu\text{mol}/\text{L}$ HAuCl₄+0.67 mmol/L HCl+3.33 mmol/L H₂O₂; (b) a+16.7 nmol/L Pb^{2+} reaction solution; (c) a+33.3 nmol/L Pb^{2+} reaction solution; (d) a+83.3 nmol/L Pb^{2+} reaction solution; (e) a+166.7 nmol/L Pb^{2+} reaction solution; (f) a+333.3 nmol/L Pb^{2+} reaction solution; (g) a+500 nmol/L Pb^{2+} reaction solution; (h) a+666.7 nmol/L Pb^{2+} reaction solution.

catalytically by Pb^{2+} to produce a short single-stranded DNA (ssDNA) that adsorbed on the AuNP_c surface to form stable AuNP_c-ssDNA conjugate to prevent aggregation by NaCl. With the increase of Pb^{2+} concentration, the RRS peak linearly decreased at 370 nm (Fig. 2). The AuNP_c-ssDNA probe of the apt-AuNP_c- Pb^{2+} system reaction solution has strong catalytic effect on the slow reaction between H₂O₂ and HAuCl₄, the products gold nanoparticles had a stronger RRS peak at 370 nm, with the increase of Pb^{2+} concentration, the RRS peak linearly increased at 370 nm (Fig. 3).

SERS spectra. Au³⁺ was reduced to Au and growing around the surface of nano-gold under the action of reducing agent H₂O₂, and the irregular shape, big particle size of nanoparticles was obtained. Upon addition of Rh_s, it was adopted on the surface of gold nanoparticles which exhibited SERS peaks at 618 cm⁻¹, 732 cm⁻¹, 1199 cm⁻¹, 1277 cm⁻¹, 1356 cm⁻¹, 1507 cm⁻¹, 1527 cm⁻¹ and 1645 cm⁻¹. Among them, the SERS peak at 1645 cm⁻¹ is the biggest, and the SERS peak intensity linearly increased with the concentration of AuNP_B increasing (Fig. S10). Upon addition of VBB, VBB molecular probes exhibited SERS peaks at 795 cm⁻¹, 1167 cm⁻¹, 1200 cm⁻¹, 1364 cm⁻¹, 1394 cm⁻¹ and 1612 cm⁻¹. Among them, the SERS peak at 1612 cm⁻¹ is the biggest, and the SERS peak intensity linearly increased with the concentration of AuNP_B increasing (Fig. 4). Upon addition of Tibetan red T, Tibetan red T molecular probes exhibited SERS peaks at 349 cm⁻¹, 612 cm⁻¹, 1240 cm⁻¹,

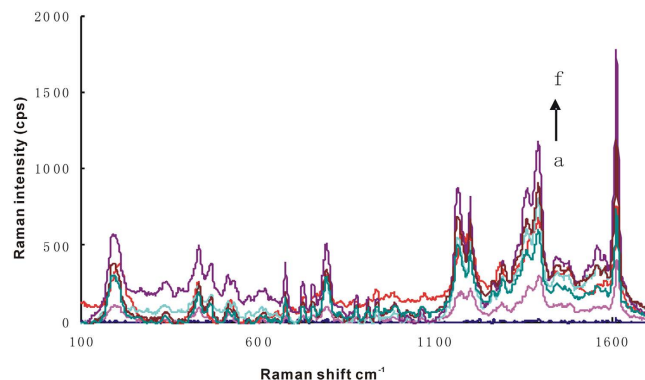


Figure 4. SERS spectrum of AuNP_B-HAuCl₄-H₂O₂-VBB system. (a) 4.48 $\mu\text{mol/L}$ HAuCl₄+0.67 mmol/L HCl+3.33 mmol/L H₂O₂+1.3 $\mu\text{mol/L}$ VBB (b) a+19 ng/mL AuNP_B; (c) a+38 ng/mL AuNP_B; (d) a+95 ng/mL AuNP_B; (e) a+190 ng/mL AuNP_B; (f) a+285 ng/mL AuNP_B.

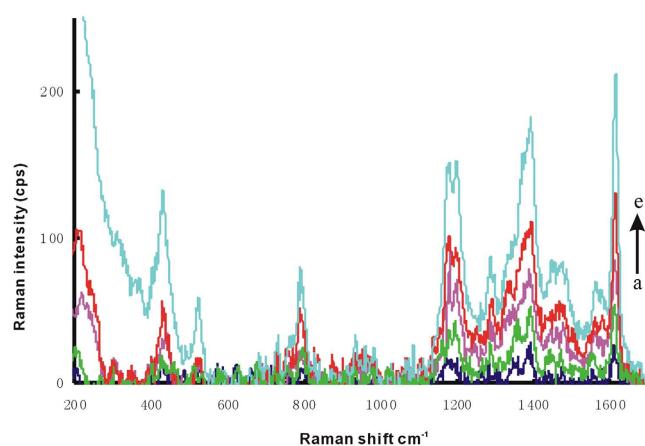


Figure 5. SERS spectrum of Pb²⁺ reaction solution-HAuCl₄-H₂O₂-VBB nanocatalytic system. (a) 4.48 $\mu\text{mol/L}$ HAuCl₄+0.67 mmol/L HCl+3.33 mmol/L H₂O₂+1.3 $\mu\text{mol/L}$ VBB; (b) a+50 nmol/L Pb²⁺ reaction solution; (c) a+167 nmol/L Pb²⁺ reaction solution; (d) a+250 nmol/L Pb²⁺ reaction solution; (e) a+500 nmol/L Pb²⁺ reaction solution.

1372 cm^{-1} , 1551 cm^{-1} and 1639 cm^{-1} . Among them, the SERS peak at 1372 cm^{-1} is the biggest, and the SERS peak intensity linearly increased with the concentration of AuNP_B increasing (Fig. S11), and we can know that the SERS signal strength of Tibetan red T is weaker than that of RhS and VBB. When Rh6G was used as SERS probe, the SERS signal is very weak. When PdNPs solution was used as catalyst and VBB was used as SERS probe, with the increase of PdNPs concentration, the SERS peak linearly increased at 1612 cm^{-1} (Fig. S12). For the apt-nanogold-Pb²⁺ catalytic system, VBB and RhS was used as SERS probe respectively, the SERS peak intensity linearly increased with the concentration of AuNP_C increasing (Fig. 5, Fig. S13).

Scanning electron microscopy (SEM). According to the procedure to get the aptamer reaction solution, a 1.0 mL the solution was taken into a 1.5 mL centrifuge tube, and centrifuged in 15000 r/min for 20 min before abandoned the supernatant. A 1.0 mL water was added into the centrifuge tube and dispersed by ultrasonic 30 min, and centrifuged again. The operation was repeated, and the dispersed sample solution was dropped onto a silicon wafers and dried naturally, then the scanning electron microscope (SEM) was recorded. The size of gold nanoparticles and silver nanoparticles are uniform and small (Fig. S14a,b). Different concentration of AgNO₃ was added to preparation of AuNR₁, AuNR₂, AuNR₃, the diameter of them was 32 nm, 37 nm, 43 nm respectively (Fig. S14c-e). For AuNP_B-HAuCl₄-H₂O₂ system, in the absence of AuNP_B, the reaction of H₂O₂ and HAuCl₄ is slow under the condition of 0.67 mmol/L HCl medium and 60 °C, and the products of gold nanoparticles is less (Fig. 6a). Upon addition of the AuNP_B, the reaction was accelerated by nano-catalyst of small gold nanoparticles (AuNP_B), and it would reacted to form a large number of gold nanoparticles which was irregular shape, big particle size. With the increase of AuNP_B concentration, the amount of gold nanoparticles increased which had high SERS and RRS signals (Fig. 6b,c). When AuNP_C-ssDNA was used as catalyst, the products gold nanoparticles were gathered into small clusters (Fig. 6d). Compared with the same concentration of nanogold, the catalytic activity of nanogold modified by aptamer was better due to its size was smaller. For the apt-AuNP_C-Pb²⁺ system, with the increase of Pb²⁺ concentration, the amount of reaction product gold nanoparticles increased (Fig. 6e,f).

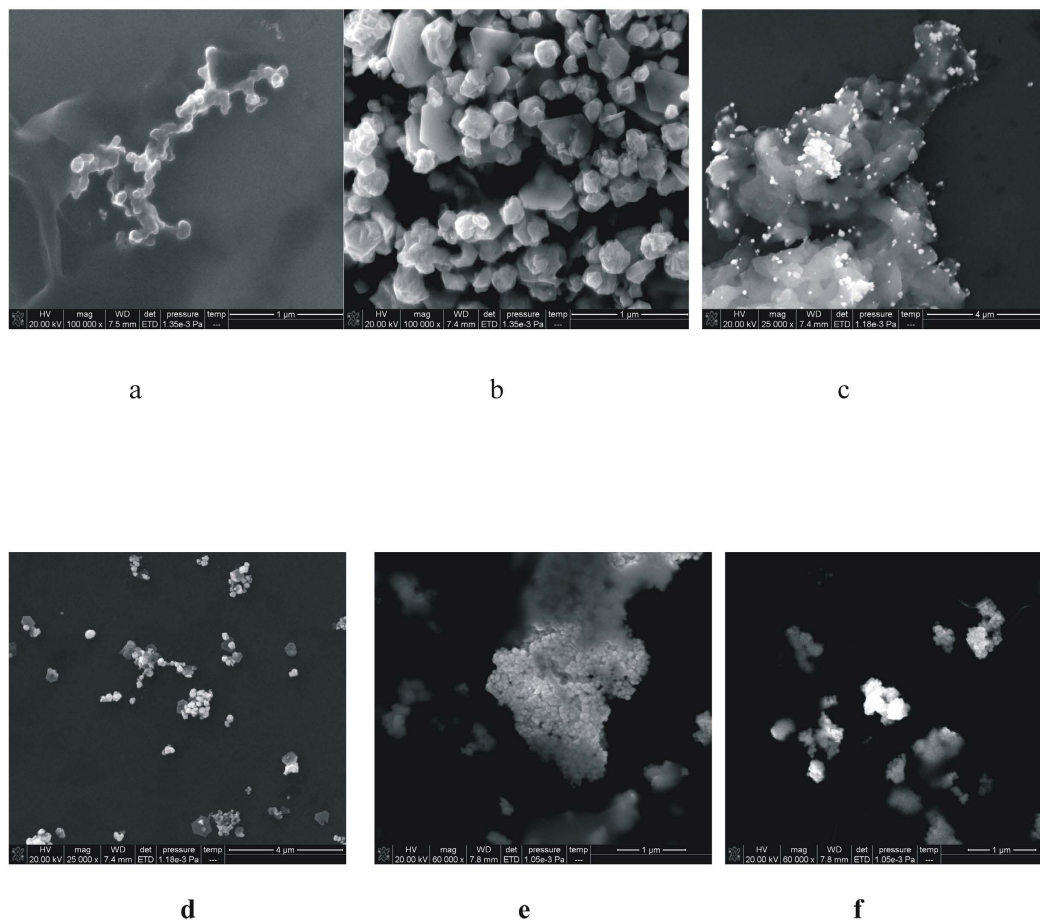


Figure 6. SEM of the nanoparticles. (a) 0.67 mmol/L HCl+4.48 μ mol/L HAuCl₄+3.33 mmol/L H₂O₂; (b) 0.67 mmol/L HCl + 4.48 μ mol/L HAuCl₄+3.33 mmol/L H₂O₂+19 ng/mL AuNP_B; (c) 0.67 mmol/L HCl +4.48 μ mol/L HAuCl₄+3.33 mmol/L H₂O₂+57 ng/mL AuNP_B; (d) 0.67 mmol/L HCl+0.152 μ g/ml AuNP_B+3.33 mmol/L H₂O₂+19 ng/mL Apt- AuNP_B; (e) 26.7 μ M pH 8.0Tris-HCl-0.33 μ L hybrid solution-63.7 ng/mL AuNP_C- 0.067 mmol/L NaCl- 0.67 mmol/L HCl +4.48 μ mol/L HAuCl₄+3.33 mmol/L H₂O₂-16.7 nmmol/L Pb²⁺; (f) 26.7 μ M pH 8.0Tris-HCl-0.33 μ L hybrid solution -63.7 ng/mL AuNP_C-0.067 mmol/L NaCl-0.67 mmol/L HCl+4.48 μ mol/L HAuCl₄+ 3.33 mmol/L H₂O₂- 333 nmmol/L Pb²⁺.

Research of gold nanoparticle-HAuCl₄-H₂O₂ reaction. The effect of HCl concentration was examined. It was found that the influence of hydrochloric acid concentration has a great influence on the formation of gold nanoparticles. The results showed that the ΔI value reached its maximum when the concentration was 0.5 mmol/L (Fig. S15). But under this condition, the color of the blank was pink and the RRS value was 3506 which indicated that the blank had formed a large number of gold nanoparticles. Thus, the 0.67 mmol/L was chosen for use which RRS value was 506 and colorless. The effect of HAuCl₄ and H₂O₂ concentration was studied. The results showed that the ΔI value reached its maximum when the concentration was 4.48 μ mol/L and 3.33 mmol/L H₂O₂ respectively (Figs S16 and S17). Thus a 4.48 μ mol/L of HAuCl₄ and 3.33 mmol/L H₂O₂ solutions were chosen for use. The effect of the incubation temperature was considered, when the temperature was greater than 60 °C, with the increase of temperature, the RRS value and color of blank increased gradually. When the incubation temperature was 60 °C, the blank RRS value was 745 and the color was colorless, meanwhile the catalytic reaction was very slow below 60 °C. Therefore the best temperature was 60 °C (Fig. S18). The effect of incubation time on the catalytic reaction was considered, a fixed reaction time of 15 min was chosen for use, giving a good compromise between high sensitivity, short analytical time and low blank (Fig. S19). After quenching the catalytic reaction, cooling with ice water to quench the reaction, the scattering intensity was constant within 90 min (Fig. S20). The effect of Raman probe RhS, VBB and Tibetan red T concentration were examined, and the results showed that the ΔI value reached its maximum when their concentration were 7 μ mol/L, 13.2 μ mol/L, 6.7 μ mol/L respectively (Figs S21–S23).

The gold nanoparticle reaction of HAuCl₄-H₂O₂ was slow in diluted HCl solution at 60 °C and was accelerated by nano-catalyst of small gold nanoparticles (AuNP_B, AuNP_C, AgNPs, PdNPs and PtNPs). Under the optimal conditions, the RRS intensity for different AuNP concentrations (C) was recorded and the working curves were drawn according the relationship between C and their corresponding ΔI values. We have investigated the influence of different kinds of AuNP on the working curve (Fig. 7, Fig. S24). Table 1 showed that the AuNP_B system

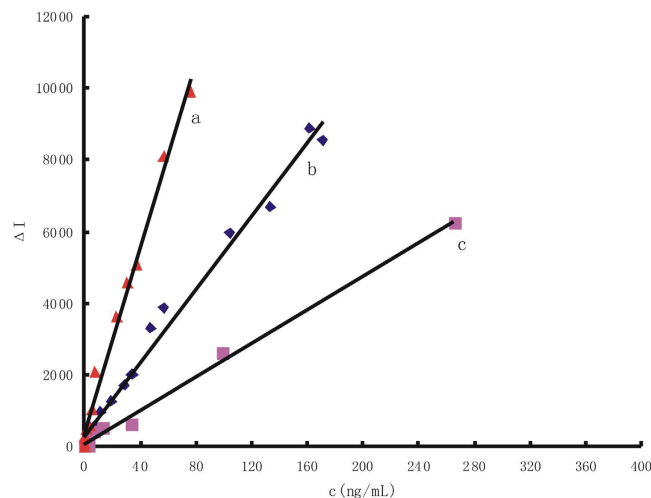


Figure 7. Relationship between nanogold concentration of H_2O_2 - HAuCl_4 catalytic system with I_{RRS} . (a) $4.48 \mu\text{mol/L HAuCl}_4$ - 0.67 mmol/L HCl - $3.33 \text{ mmol/L H}_2\text{O}_2$ - AuNP_B ; (b) $4.48 \mu\text{mol/L HAuCl}_4$ - 0.67 mmol/L HCl - $3.33 \text{ mmol/L H}_2\text{O}_2$ - AuNP_C ; (c) $4.48 \mu\text{mol/L HAuCl}_4$ - 0.67 mmol/L HCl - $3.33 \text{ mmol/L H}_2\text{O}_2$ - AuNPs .

system	Detection method		The regression equation	Linear range (ng/mL NP)	The correlation coefficient
H_2O_2 - HAuCl_4 - AuNP_B	RRS		$\Delta I = 131 C + 300$	0.038–76	0.9951
	Different probe	RhS	$\Delta I = 5.9 C + 86$	3.8–456	0.9898
		VBB	$\Delta I = 2.9 C - 73$	19–285	0.9966
	SERS	Tibetan red T	$\Delta I = 1.5 C - 9.1$	4–190	0.9923
	UV		$\Delta A = 2.6 \times 10^{-3} C + 0.0406$	9.5–180	0.9825
H_2O_2 - HAuCl_4 - AuNP_C	RRS		$\Delta I = 51 C + 267$	0.38–171	0.9941
	UV		$\Delta A = 2 \times 10^{-3} C - 0.0407$	38–228	0.9808
H_2O_2 - HAuCl_4 - AuNR_1	RRS		$\Delta I = 0.51 C + 54$	32.5–975	0.9840
H_2O_2 - HAuCl_4 - AuNR_2	RRS		$\Delta I = 0.37 C + 21$	32.5–2600	0.9901
H_2O_2 - HAuCl_4 - AuNR_3	RRS		$\Delta I = 0.24 C + 73$	32.5–1950	0.9784
H_2O_2 - HAuCl_4 - AgNPs	RRS		$\Delta I = 23 C + 73$	3.3–265	0.9971
	UV		$\Delta A = 0.001 C + 0.0203$	13–265	0.9701
H_2O_2 - AuCl_4 - PdNPs	RRS		$\Delta I = 0.12 C + 2.6$	200–9920	0.9939
	SERS		$\Delta I = 14.71 C + 2.06$	500–5950	0.9913
	UV		$\Delta A = 2 \times 10^{-5} C + 0.011$	298–1587	0.9939
H_2O_2 - HAuCl_4 - PtNPs	RRS		$\Delta I = 0.05 C + 21$	200–600	0.9729
H_2O_2 - HAuCl_4 - AuNP_C - Apt	RRS		$\Delta I = 70.7 C + 257$	0.95–76	0.9936

Table 1. Different nanoparticles catalytic systems analysis feature.

was the best, with the most wide linear range and lowest detection limit. We have investigated the influence of different size of AuNR on the working curve (Fig. S25), Table 1 showed that with the increase of AuNR particle size, the catalytic activity was weaker. As for AuNP_B - HAuCl_4 - H_2O_2 system, under the optimal conditions, RhS, VBB, and Tibetan red T was added as SERS probe respectively, the increased SERS intensity responded linearly with the concentration of AuNP_B over 3.8–456, 19–285, 4–190 ng/mL respectively, with a linear regression equation of $\Delta I_{1645 \text{ cm}^{-1}} = 2.28 C + 72.77$, $\Delta I_{1612 \text{ cm}^{-1}} = 5.94 C + 86$, $\Delta I_{1372 \text{ cm}^{-1}} = 1.47 C - 9.1$ respectively (Fig. 8). Results showed that when VBB used as SERS probe was the most sensitive. When PdNPs was used as catalyst and VBB was used as SERS probe, the increased SERS intensity at 1612 cm^{-1} responded linearly with the concentration of PdNPs over 500–5950 ng/mL, with a linear regression equation of $\Delta I_{1612 \text{ cm}^{-1}} = 14.71 C + 2.06$ (Fig. S26). We have investigated the influence of different AuNP on the working curve, and the results (Table 1) showed that the AuNP_B system was the best, with the lowest detection limit.

The gold nanoparticle reaction of HAuCl_4 - H_2O_2 was slow in diluted HCl solution at 60°C . Upon addition of the nanoparticles, HAuCl_4 would be adsorbed on the surface of nanoparticles catalyst. The surface energy was higher when the nanoparticles particle size was very small, and it can absorb a large number of HAuCl_4 in the surface of the nanoparticles. When Au^{3+} was reduced to Au and growing around the nano-gold surface under the action of reducing agent H_2O_2 , irregular shape, big particle size of nanoparticles were obtained. The products had

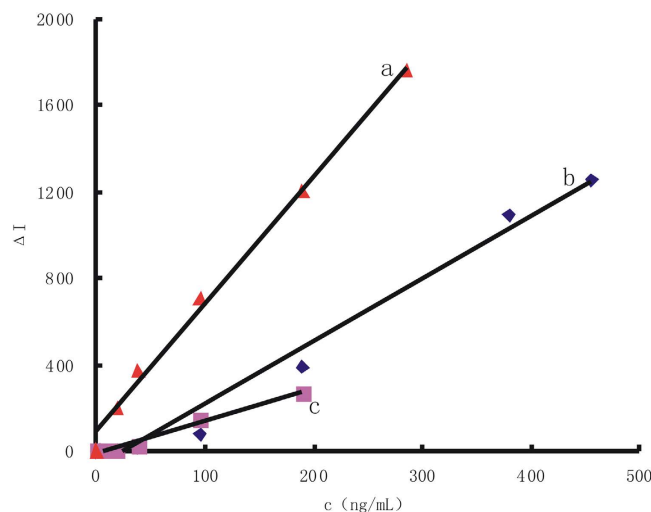


Figure 8. Relationship between NG concentration of H_2O_2 - HAuCl_4 catalytic system with I_{SERS} . (a) 4.48 $\mu\text{mol/L}$ HAuCl_4 -0.67 mmol/L HCl -3.33 mmol/L H_2O_2 - AuNP_B -1.3 $\mu\text{mol/L}$ VBB; (b) 4.48 $\mu\text{mol/L}$ HAuCl_4 -0.67 mmol/L HCl -3.33 mmol/L H_2O_2 - AuNP_B -6.97 $\mu\text{mol/L}$ RhS; (c) 4.48 $\mu\text{mol/L}$ HAuCl_4 -0.67 mmol/L HCl -3.33 mmol/L H_2O_2 - AuNP_B -6.7 mmol/L Tibetan red T.

a strong RRS signal because of the particle size was very large. When VBB, RhS and Tibetan red T were added as SERS probe respectively, the products had strong SERS signal because of the shape of gold nanoparticles was random. From Table 1, we can know that the AuNP_B RRS system was the best.

Optimization of aptamer detection of Pb^{2+} system analysis conditions. The effect of Tris-HCl concentration and pH were examined, the results showed that the ΔI value reached its maximum when the concentration was 4 mmol/L and pH was 8.0 (Figs S27 and 28). The effect of AuNP_c and NaCl concentration were examined, the results showed that the ΔI value reached its maximum when the concentration were 9.55 $\mu\text{g/mL}$ and 10 mmol/L respectively (Figs S29 and 30), thus, 9.55 $\mu\text{g/mL}$ of AuNP_c and 10 mmol/L of NaCl solution were chosen for use.

Effect of foreign substances. According to the procedure, the effect of foreign substances on the determination of 0.167 $\mu\text{mol/L}$ Pb^{2+} was tested, with a relative error within $\pm 10\%$. Results (Table S1) showed that common ions did not interfere with the determination, which indicated that this method had good selectivity.

Working Curve. Under the optimal conditions, the RRS intensity for different Pb^{2+} concentrations (C) were recorded and the working curves were drawn according the relationship between C and their corresponding ΔI values. With the increase of Pb^{2+} concentration, the RRS peak increased at 370 nm and the decreased RRS intensity responded linearly with the concentration of Pb^{2+} over 125–425 nmol/L with a linear regression equation of $\Delta I_{370\text{nm}} = 1.26C - 20.56$, coefficient R^2 of 0.9836. For the apt-nanogold- Pb^{2+} catalytic system, The increased RRS intensity at 370 nm responded linearly with the concentration of Pb^{2+} over 16.7–666.7 nmol/L, the linear regression equation is $\Delta I_{370\text{nm}} = 9.85C + 470$, coefficient R^2 of 0.9856 (Fig. S31). VBB and RhS were added as SERS probe, the SERS intensity $I_{1612\text{cm}^{-1}}$ and $I_{1645\text{cm}^{-1}}$ responded linearly with the concentration of Pb^{2+} over 17–250, 17–167 nmol/L respectively (Fig. S32).

Sample analysis. Three natural water samples were filtered to obtain water sample solutions, and were analyzed according to the procedures. Results (Table S2) showed that two of them had been detected out of Pb^{2+} . A known amount of Pb^{2+} was added into the water sample to obtain the recovery. The relative standard deviation was in the range of 4.4–5.5%, and the recovery was in the range of 98.0–102%.

Discussion

Analytical principle. Nanocatalytic reaction is an important way to amplify the signal of analysis method, so explore a new method to use nanocatalytic reaction is great significance. It was found that, the gold nanoparticle reaction of HAuCl_4 - H_2O_2 is slow in diluted HCl solution at 60 °C, upon addition of nanoparticles such as AuNP_B , AuNP_c , AuNR , AgNPs , PdNPs and PtNPs , HAuCl_4 would be adsorbed on the surface of nanoparticles catalyst. The specific surface area is larger because of the nanoparticles particle size is very small, therefore it can absorb a large number of HAuCl_4 in the surface of the nanoparticles, owing to the fact that the small nanoparticles have a high surface energy. When Au^{3+} was reduced to Au and growing around the nano-gold surface under the action of reducing agent H_2O_2 , it can obtain irregular shape, big particle size of nanoparticles (Fig. 9), which have highly SERS signals and RRS signals. Thus the nanogold catalytic reaction can be used to build SPR-S analysis platform.

The DNzyme catalytic strand hybridized with substrate strands to form double-stranded DNA (dsDNA) which couldn't protect AuNP_c in pH 8.0 Tris-HCl buffer solution containing 6.7 mmol/L NaCl, and were aggregated to AuNP_c aggregations with a strong RRS peak at 370 nm. Upon addition of Pb^{2+} , the substrate chain of

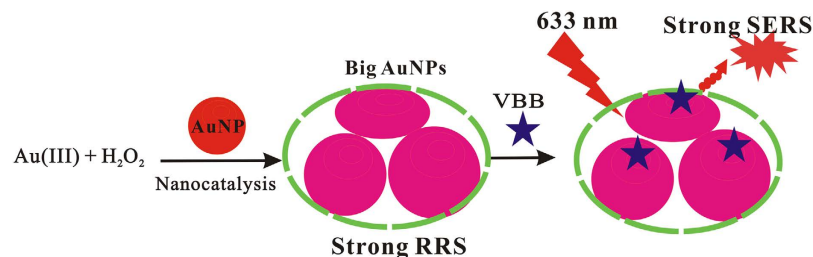


Figure 9. Small AuNPs catalyze the H_2O_2 reduction of HAuCl_4 to big AuNPs with SERS and RRS effects.

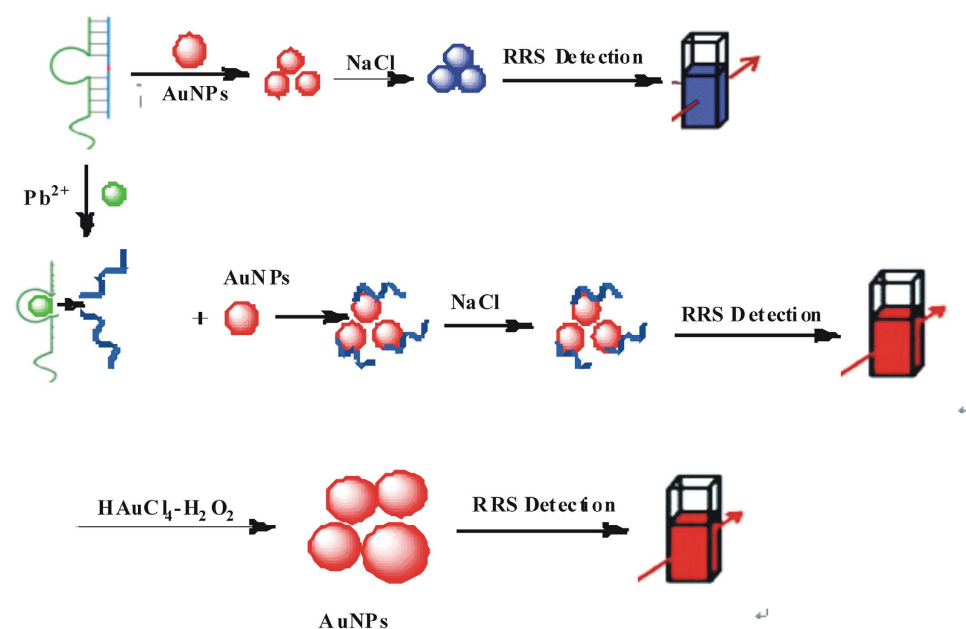


Figure 10. Principle of aptamer nanogold catalytic detection of Pb^{2+} with RRS effects.

dsDNA could be cracked catalytically by Pb^{2+} to produce a short single-stranded DNA (ssDNA) that adsorbed on the AuNPc surface to form stable AuNPc-ssDNA conjugate to prevent aggregation by NaCl. Combining the nanocatalytic SPR-RRS analytical platform and the DNazyme cracking reaction, the AuNPc-ssDNA conjugates have strong catalytic activity to $\text{HAuCl}_4\text{-H}_2\text{O}_2$ particles reaction, and its product gold nanoparticles had a stronger RRS peak at 370 nm. With the increase of Pb^{2+} concentration, the concentration of AuNPc-ssDNA probe increase and lead to the catalytic activity stronger (Fig. 10). Based on this, the new sensitive RRS and SERS quantitative analysis methods were developed for detection of Pb^{2+} .

Conclusion

In 0.67 mmol/L HCl medium at 60°C , HAuCl_4 adsorbed on the surface of nanoparticles catalyst, Au^{3+} was reduce to Au and growing around the nano-gold surface, the products have highly SERS signals and RRS signal, thus the AuNP- $\text{HAuCl}_4\text{-H}_2\text{O}_2$ nanogold catalytic reaction RSS and SERS analysis platform were built. The AuNPc-ssDNA probe of the apt-AuNPc- Pb^{2+} system reaction solution has strong catalytic effect on the slow reaction between H_2O_2 and HAuCl_4 . Combing the nanocatalysis and the DNazyme reaction, a new nanocatalysis analysis platform was developed for the detection of Pb^{2+} by the RRS and SERS, with advantages of high sensitivity, good selectivity, simplicity and rapidity. Compared with the reported methods, the methods are easier to operate and more sensitive. Further more, it is a rapid RRS and SERS quantitative method for Pb^{2+} (Table S3).

Methods

Apparatus. A model of DXR smart Raman spectrometer (Thermo companies in the United States) with a laser wavelength of 633 nm and power of 2.5 mW, a model of the F-7000 Hitachi Fluorescence spectrometer (Hitachi Company, Japan), a model of the TU-1901 double-beam UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China), a model of FEI 200 FEG field emission scanning electron microscope (Dutch philips), and a model of C-MAG HS7 incubation magnetic stirrer (Germany IKA company) were used.

Reagents. A 1.0 $\mu\text{mol/L}$ DNazyme catalytic strand with sequence of 5'-(T)10 CAT CTC TTC TCC GAG CCG GTC GAA ATA GTG AGT-3', 1.0% HAuCl_4 , 1.0% sodium citrate, 10 mmol/L sodium borohydride, 0.2 mol/L cetyltrimethyl ammonium bromide (CTAB), 4.0 mmol/L AgNO_3 , 77.8 mmol/L vitamin C (VC),

0.01 mol/L HCl, 0.3% H₂O₂ (0.1 mol/L), 50 mmol/L pH 7.4 Tris-HCl, 50 mmol/L pH 8.0 Tris-HCl, 5×10^{-5} mol/L PdCl₂ and 1.45×10^{-2} mol/L PdCl₂, 2.9×10^{-2} mol/L HPtCl₆ and 5.23×10^{-5} mol/L RhS solution were prepared. A pH 7.0 Na₂HPO₄-citric acid buffer solution was prepared as follows, a 16.5 mL 0.2 mol/L Na₂HPO₄ and 3.5 mL 0.1 mol/L citric acid solution were mixed together to obtain a concentrations 0.16 mol/L Na₂HPO₄. A 1.0×10^{-3} mol/L VBB solution was prepared as follows, 0.0250 g VBB was dissolved in 5.0 mL ethanol, and diluted to 50 mL with water. The nanosols and ssDNA-AuNP were prepared as in the SI³⁷.

Procedure of HAuCl₄- nanoparticles -H₂O₂ system. A 80 μL 0.1% HAuCl₄ (84 μmol/L), 100 μL 0.01 mol/L HCl, a certain amount of nanoparticles including AuNP_B, AuNP_C, AuNR, AgNPs, PdNPs and PtNPs, and 50 μL 0.3% (0.1 mol/L) H₂O₂ were added into a 5 mL marked test tube and mixed well, and diluted to 1.5 mL. The mixture was heated at 60 °C for 15 min, cooling with ice water to quench the reaction. A part of the solution was transferred into a 1 cm quartz cell. The RRS spectra were recorded by synchronous scanning excited wavelength λ_{ex} and emission wavelength λ_{em} ($\lambda_{ex} - \lambda_{em} = \Delta\lambda = 0$), a PMT voltage of 400 v, both excited and emission slit width of 5 nm, emission filter of 1%T attenuator on fluorescence spectrophotometer. The RRS intensity at 370 nm ($I_{370\text{nm}}$) and the blank value ($I_{370\text{nm}})_0$ without nanoparticles were recorded. The value of $\Delta I_{370\text{nm}} = I_{370\text{nm}} - (I_{370\text{nm}})_0$ was calculated. 200 μL 1.0×10^{-5} mol/L VBB, 20 μL 5.23×10^{-5} mol/L RhS or 100 μL 1×10^{-4} mol/L tibetan red T was added in the mixture respectively, The SERS intensity corresponding at 1612 cm⁻¹, 1645 cm⁻¹, 1370 cm⁻¹ and the blank value I_0 without nanoparticles were recorded. The value of $\Delta I = I - I_0$ was obtained.

Procedure of apt-AuNP_C-Pb²⁺ system. A 500 μL 2 μmol/L Substrate strand, 500 μL 1 μmol/L DNAzyme catalytic strand, 1 mL 50 mM pH 7.4 Tris-Hcl buffer solution and 50 μL 1 mol/L NaCl were mixed well, incubated at 65 °C water bath for 10 min, then gradually cooled to room temperature over 2 h, and hybrid solution was obtained. In a 5 mL marked test tube 120 μL 50 mM pH 8.0 Tris-Hcl buffer solution, 50 μL hybrid solution, a certain amount of Pb²⁺ was added respectively, mixed well and diluted to 1.5 mL. Then the tube was placed at 37 °C water bath for reaction 60 min before cooling with ice water to quench the reaction. After that 250 μL AuNP_C and 30 μL 0.5 mol/L NaCl were added in the mixture and mixed well to obtain Pb²⁺ aptamer reaction solution, then a part of the solution was transferred into a 1 cm quartz cell. The RRS spectra were recorded by synchronous scanning excited wavelength λ_{ex} and emission wavelength λ_{em} ($\lambda_{ex} - \lambda_{em} = \Delta\lambda = 0$), a PMT voltage of 450 v, both excited and emission slit width of 5 nm, The RRS intensity at 370 nm ($I_{370\text{nm}}$) and the blank value ($I_{370\text{nm}})_0$ without Pb²⁺ were recorded. The value of $\Delta I_{370\text{nm}} = (I_{370\text{nm}})_0 - I_{370\text{nm}}$ was calculated.

Procedure of apt-nanogold-Pb²⁺ catalytic system. A 80 μL 0.1% HAuCl₄ (84 μmol/L), 100 μL 0.01 mol/L HCl, 100 μL Pb²⁺ aptamer reaction solution and 50 μL 0.3% (0.1 mol/L) H₂O₂ were added into a 5 mL marked test tube and mixed well, and diluted to 1.5 mL. The mixture was heated at 60 °C for 15 min, cooling with ice water to quench the reaction. A part of the solution was transferred into a 1 cm quartz cell. The RRS spectra were recorded by synchronous scanning excited wavelength λ_{ex} and emission wavelength λ_{em} ($\lambda_{ex} - \lambda_{em} = \Delta\lambda = 0$), a PMT voltage of 400 v, both excited and emission slit width of 5 nm, emission filter of 1%T attenuator on fluorescence spectrophotometer. The RRS intensity at 370 nm ($I_{370\text{nm}}$) and the blank value ($I_{370\text{nm}})_0$ without Pb²⁺ were recorded. The value of $\Delta I_{370\text{nm}} = I_{370\text{nm}} - (I_{370\text{nm}})_0$ was calculated. 200 μL 1.0×10^{-5} mol/L VBB 20 μL or 5.23×10^{-5} mol/L RhS was added in the mixture, The SERS intensity at 1612 cm⁻¹ and the blank value I_0 without Pb²⁺ were recorded. The value of $\Delta I = I - I_0$ was obtained.

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Acknowledgements

This work supported by the National Natural Science Foundation of China (No. 21267004, 21367005, 21307017, 21465006, 21477025, 21567001, 21567005), the Research Funds of Key Laboratory of Ecology of Rare and Endangered Species and Environmental Conservation of Education Ministry, the Research Funds of Guangxi Key Laboratory of Environmental Pollution Control Theory and Technology, and the Natural Science Foundation of Guangxi (No. 2013GXNSFFA019003, 2014GXNSFAA118059).

Author Contributions

G.Q., L.L. and A.H. performed the experiment and prepared Figs 1–6 and Fig. 8. L.L., G.Q., H.X., Z.L. and A.H. wrote the main manuscript text and prepared Fig. 7. Z.L., A.H., Q.Y. and G.Q. contributed to the discussion and measurement analysis. All authors contributed to the preparation of the manuscript and reviewed the manuscript.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Ye, L. *et al.* A novel and highly sensitive nanocatalytic surface plasmon resonance-scattering analytical platform for detection of trace Pb ions. *Sci. Rep.* **6**, 24150; doi: 10.1038/srep24150 (2016).



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