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Target triggered self-assembly of Au nanoparticles for amplified detection of *Bacillus thuringiensis* transgenic sequence using SERS

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ABSTRACT

The research methods for DNA detection have been widely extended since the application of nanotechnology, but it remains a challenge to detect specific DNA sequences or low abundance genes in the biological samples with accuracy and sensitivity. Here we developed a SERS biosensing platform by target DNA (tDNA) triggered self-assembly of Au nanoparticles (Au NPs) probes on DNA nanowires for signal amplification in DNA analysis. Based on the hybridization chain reactions (HCR) and surface enhanced Raman scattering (SERS) technology, the SERS intensity reveals a good linearity with tDNA ranging from 50 pM to 500 pM under optimal conditions. The specific detection of tDNA sequence was realized with a detection limit of 50 pM (S/N=3). To demonstrate the specificity and universality of the strategy, the single-base mismatches in DNA and the *Bacillus thuringiensis* (Bt) transgenic sequence were successively applied in the SERS assay. The results showed that the sensitivity and accuracy of the SERS-based assay were comparable with real-time PCR. Besides, the method would provide precise and ultra-sensitive detection of tDNA but also informative supplement to the SERS biosensing platform.

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1. Introduction

As a specific DNA sequence, *Bacillus thuringiensis* (Bt) transgene has aroused attention worldwide since its introduction into crops for insect control, which brings benefits to the farmers but also raises people's great concern in food security and bio-security (Hutchison et al., 2010; Quist and Chapela, 2001). Therefore, it is imperative to develop certain accurate and rapid methods for testing transgenes in food and feed. Recently, many important technological advances have been made in the development of multiple sensors for monitoring the DNA interactions and recognition events (Morisset et al., 2008; Hiroshi et al., 2005). Wherein, the detection methods are mostly based on DNA hybridization pattern recognition, which possess high sensitivity and specificity but usually require amplification by polymerase chain reaction (PCR). Despite its high sensitivity and stability, the PCR method typically suffers from the disadvantages such as high cost, high complexity and time consuming (Zhu et al., 2008; Qiu et al., 2011). Moreover, it still remains a challenge to detect specific DNA sequences or low abundance genes in the biological samples with accuracy and sensitivity (Wang et al., 2011). Hence, it is of great importance to establish a time-saving, reliable and sensitive detection method for detecting specific DNA sequence.

To develop newly amplified detection methods for the analysis of DNA, various sensing strategies have been employed in bioassay field based on electrochemical and optical technology (Wang et al., 2014; Hsu and Huang, 2004). These methods often adopt an enzymatic reaction (Wan et al., 2013) or rolling circle amplification (RCA) (Bi et al., 2010) for signal amplification in ultra-sensitive detection of DNA. However, these approaches are confronted with the difficulties of harsh reaction conditions and long amplification period (Zhang et al., 2012). At present, new strategies for the detection of trace DNA have aroused broad interest (Tang et al., 2012). One of the impressive arts is designed upon the recognition of target DNA (tDNA) triggering hybridization chain reaction (HCR) to construct self-assembly DNA nanowires (Dirks and Pierce, 2004). Based on the self-assembly amplification system, the biosensing platform enables sensitive DNA analyses without the assistance of enzymes and can be implemented at room-temperature. The method for constructing DNA biosensors via HCR makes it an attractive protein-free, room-temperature alternative to PCR and RCA for signal amplification in DNA analysis (Huang et al., 2011; Ren et al., 2011).

Because of its high sensitivity and abundant structural information content for molecules, surface enhanced Raman scattering (SERS) technology has become a well-established analytical tool

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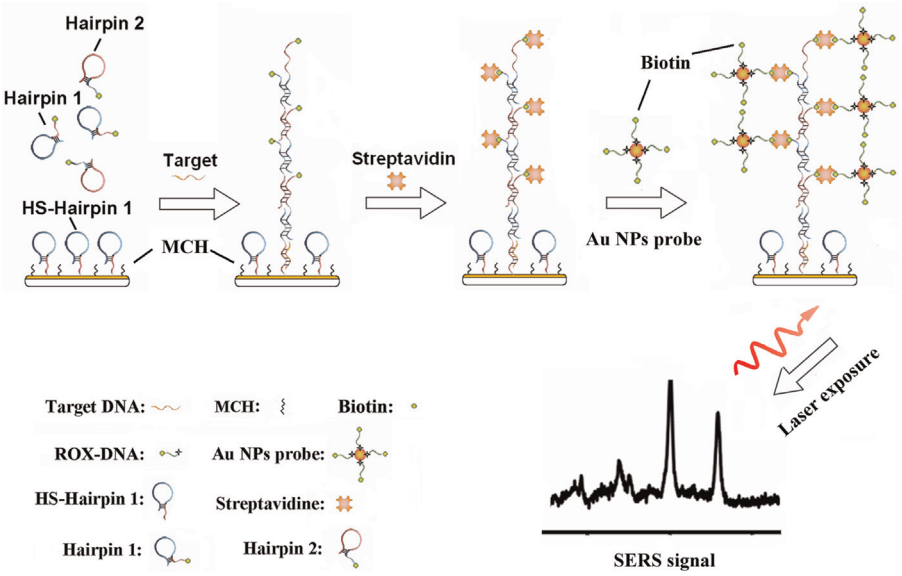

Fig. 1. Schematic illustration of the SERS biosensing platform.

for chemical and biological sensing by combining with Ag or Au nanoparticles (Au NPs) (Gao et al., 2013; Lim et al., 2010). Herein, on the basis of HCR, we present a universal biosensing platform for DNA sequences assay via SERS technology coupled with Au NPs probes. Fig. 1 illustrates the target triggered self-assembly of Au NPs on the biosensing platform for the ultra-sensitive detection of tDNA amplified by HCR. First, HS-hairpin 1 (HS-H1) was fixed on the substrate followed by a blocking procedure of 6-mercapto-1-hexanol (MCH). Second, tDNA was injected and hybridized with HS-H1, which was unfolded and triggered HCR by alternative cross-hybridization of biotinylated hairpin 1 (bio-H1) and hairpin 2 (bio-H2) forming a long DNA nanowire. Third, the Au NPs probes grew along the DNA nanowires after the addition of streptavidin (SA). Finally, under laser exposure, the sensing platform could provide SERS signals originated from Raman active molecules (X-rhodamine: ROX) on the surface of Au NPs. The results demonstrated that the proposed strategy possesses many advantages such as simple procedures, low experimental requirements and high sensitivity (50 pM).

2. Material and methods

2.1. Chemicals and materials

Biotin, streptavidin, and 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich; 6-mercapto-1-hexanol (MCH) was from J&K Chemical Ltd.; $HAuCl_4$, sodium citrate and other relevant reagents were from Sinopharm Chemical Reagent Co. Ltd.; all chemicals and solvents were of analytical grade and used as received without further purification.

HS-H1, H1, H2, bio-H1, bio-H2, tDNA, mismatch DNA (mis-DNA) and Bt transgenic gene (tDNA-Bt) were synthesized by AuGCT biotechnology Co. Ltd. (Beijing, China). ROX–DNA and dHS-H1 were synthesized by TaKaRa Biotechnology Co. Ltd. (Dalian, China). The oligonucleotide sequences are listed in Table S1. Detailed sequence information of Bt is available in GenBank database of the National Center for Biotechnology Information (NCBI).

Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used throughout the experiment.

2.2. Instrumentation

All Raman spectra were performed using in Via Raman spectrometer (Renishaw, UK) equipped with a co-focusing microscope (Leica, German). The samples were excited under a He–Ne laser (633 nm) with laser power of approximately 10 mW. The band of a silico wafer at 520 cm^{-1} was used to calibrate the spectrometer. Unless otherwise mentioned, the SERS spectra were acquired with 10 s exposure and one time accumulation. Spectral manipulation such as baseline adjustment and smoothing was performed using the WiRE 2.0 software package (Renishaw).

The UV–vis absorption spectra were obtained using a Nicolet Evolution 300 UV–vis spectrometer (Thermo Nicolet, America). Transmission electron microscopy (TEM) images were acquired by a JEM-2010 transmission electron microscope (JEOL, Japan). Hydrodynamic diameters were measured with a Zetasizer Nano ZS90 DLS system (Malvern, England). Electrochemical impedance spectroscopy (EIS) was performed by an electrochemical workstation (CHI660D Instruments, Shanghai Chenhua Instrument Corp., Shanghai, China).

2.3. Preparation and modification of Au NPs

Au NPs were synthesized according to the procedures described in the literature (Frens, 1973). The obtained solution was centrifugated at 6000 rpm to remove excess ions and the precipitate was resuspended in water. Au NPs probes were prepared according to our previous method elaborated in Supplementary information.

2.4. SERS substrate and electrode modification, DNA detection

The whole fabrication process of SERS sensing platform is illustrated in Fig. 1. The preparation of DNA nanowires is specified in Supplementary information. To amplify the SERS signal, 0.1 mM SA was subsequently added onto the obtained SERS sensing substrate and incubated at room temperature for 5 min. SA was anchored to the bio-H1 and bio-H2 through biotin–SA specific interaction. Then, after thoroughly rinsing with 10 mM PBS (pH = 7.0), Au NPs probes were added to the substrate and remained for 10 min to combine with the SA assembled on the DNA nanowires. Finally the completed SERS sensing substrate was washed carefully with water to get rid of the nonspecifically

bounded Au NPs. The processing steps of gold electrode were similar to the gold-plated mica sheets, and the detailed procedures are listed in Supplementary information.

The modified SERS sensing substrate obtained during stepwise modification was characterized using Raman spectroscopy, and the gold electrode was characterized using EIS in 0.1 M KCl solution containing 5.0 mM $[Fe(CN)_6]^{3-}$ with scanning frequency ranging from 10 kHz to 50 MHz at 5 mV.

3. Results and discussion

3.1. Characteristics of Au NPs and Au NPs probes

As depicted in Fig. S1, absorption spectra of Au NPs probes showed a 2 nm red-shift to the absorption of original Au NPs at 523 nm, which was consistent with the local plasmon resonance of Au NPs with an average diameter of 20 nm. The red-shift was primarily attributed to the modification of ROX–DNA onto the gold surface.

The hydrodynamic diameters increased when the ROX–DNA was conjugated with Au NPs, demonstrating that the average particle size in water was enlarged by surface coverage of ROX–DNA. TEM image showed that the functionalized Au NPs were around 20 nm in diameter (Fig. S2C). No obvious aggregation was found when the ROX–DNA conjugated with Au NPs, which was in good agreement with UV–vis spectra and hydrodynamic diameter measurements (Fig. S2A and B), and again confirmed the good dispersity and stability of the prepared Au NPs. These facts indicated that Au NPs SERS probes could be obtained by the aforementioned procedures.

3.2. Characteristics of SERS sensing platform

As depicted in Fig. S3B, the SERS spectra of the sensing substrate without tDNA were flat between 500 cm^{-1} and 2000 cm^{-1}, indicating the cleanliness of SERS sensing substrate and the stability of hairpin DNA at room temperature. However, the characteristic SERS band of ROX appeared as the tDNA existed in the sensing substrate (Fig. S3A). Wherein, the strongest characteristic Raman peak appears at 1504 cm^{-1}, which was selected as the quantitative peak of tDNA in the following section.

To verify the detection process of tDNA, the performance of the SERS sensing substrate was also evaluated by EIS. There are differences in the electron-transfer resistance (R_{et}) upon the presence and absence of tDNA (Fig. S4). In comparison, the R_{et} of tDNA-present Au electrode (curve a) is larger than that of tDNA-absent electrode (curve b), mainly due to the electrostatic repulsion between negative charges of DNA nanowires and electroactive probe $[Fe(CN)_6]^{3-/4-}$ (Jiang et al., 2011). When tDNA was hybridized with dHS-H1, DNA nanowires were generated through the HCR between H1 and H2, and the negative charge at the electrode surface was essentially increased. As a result, the R_{et} (curve a) increased obviously than that of HS-H1 modified

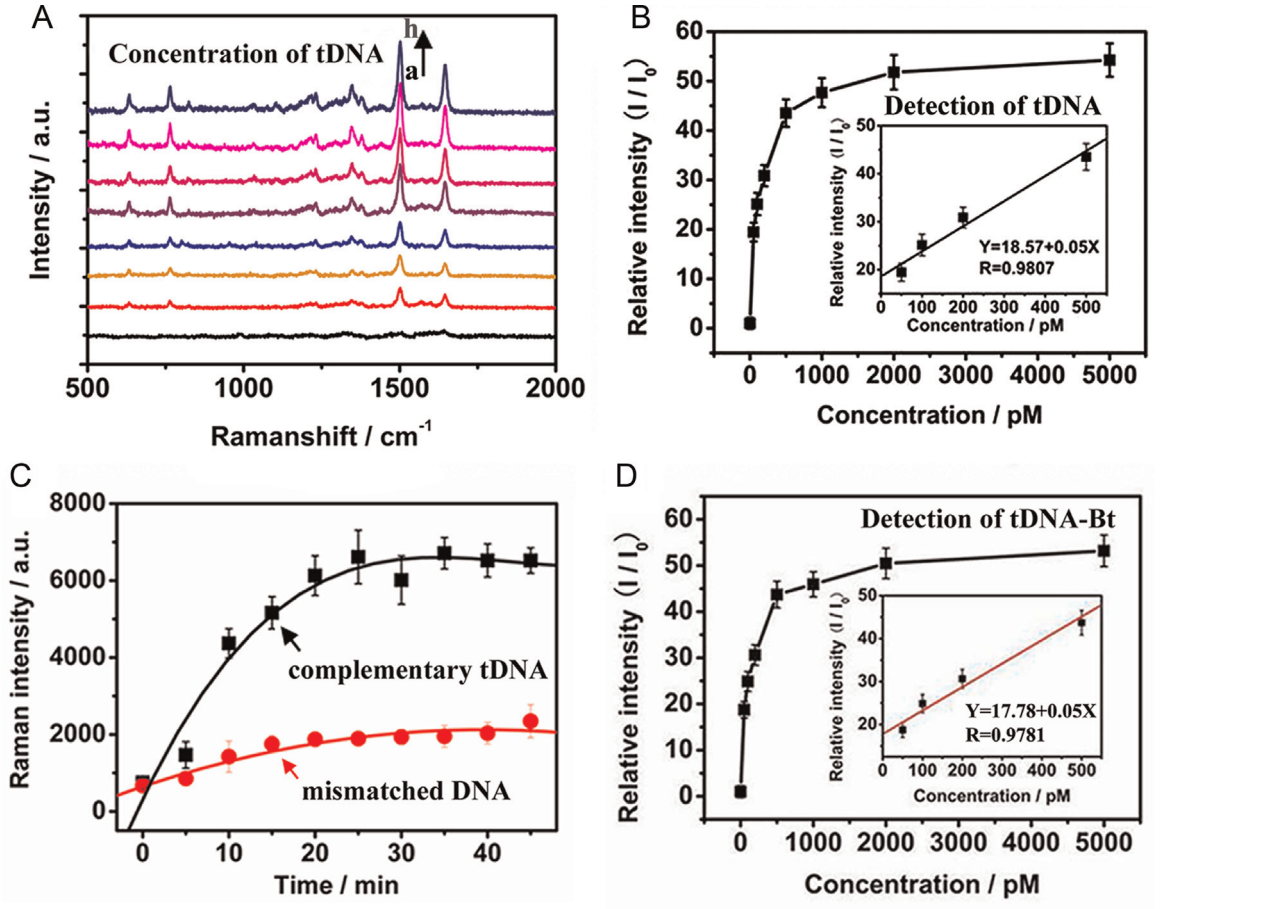

Fig. 2. (A) Representative SERS spectra of the sensing platform for 0, 50, 100, 200, 500, 1000, 2000, and 5000 pM tDNA (from a to h). (B) Raman intensity response to 0, 50, 100, 200, 500, 1000, 2000, and 5000 pM tDNA (inset: linear relationship between SERS intensity and tDNA concentration). (C) Time-dependent SERS intensity upon the analysis of 0.5 nM tDNA (square) and 0.5 nM mis-DNA (circle) using the SERS sensing platform. (D) Raman intensity response to 0, 50, 100, 200, 500, 1000, 2000 and 5000 pM tDNA-Bt (inset: linear relationship between SERS intensity and tDNA-Bt concentration). All the error bars were calculated based on the standard deviation of eight measurements.

electrode (curve b). The excellent EIS performance could also be applied to the detection of tDNA.

The results of SEM further verified the HCR between H1 and H2 triggered by tDNA, and demonstrated the self-assembly of Au NPs SERS probes induced by the strong affinity of biotin–SA system. As shown in Fig. S5, the nanowires consisting of Au NPs twisted crossover on the SERS substrate with the length of no less than 1000 nm, which was in accordance with the published paper (Shimron et al., 2012). It also can be recognized that the nanowires were composed of arranged nanoparticles with the size of 20 nm, which was in agreement with the prepared Au NPs.

3.3. Optimization of SERS sensing platform

When HS-H1 was chosen as the fixed hairpin to perform SERS and EIS tests, the SERS intensity and impedance both decreased as HCR time increased. The results revealed that the nanowires were not strong enough to anchor on the substrate with a single thiol. Thus, dHS-H1 with dual thiols was chosen to serve as the anchor of SERS sensor in the experiment. Fig. S6 displays the results of time-dependent SERS intensity upon different diluted tDNAs (the dilution folds are 2, 4, 8, 16, 32, and 64). Under the same concentration of tDNA, the SERS intensity increases as time prolonged in the premier 20 min. However, the SERS intensity nearly remained the same after 20 min, which implied that dHS-H1 has stronger anchor than HS-H1, and no more nanowires were scraped from the substrate surface due to the overmuch chain growth. Meanwhile, it also manifested that HCR is faster than enzymatic reaction and RCA, and can achieve the ideal amplification in 20 min.

3.4. HCR signal amplification for SERS detection of tDNA

Fig. 2A shows the results of SERS intensity responded to the different concentrations of tDNA. It could be seen that the SERS intensity increased with the increasing concentration of tDNA. To further investigate the sensitivity of the SERS sensing platform, we took the relative intensity at 1504 cm^{-1} as a variable and discussed its relationship with the concentration of tDNA (Fig. 2B). The results revealed that the intensity increased with the increase of tDNA concentration and showed a linear response to the tDNA in the range of 50–500 pM. The regression equation was $Y=18.57+0.05X$ (X: M) with $R=0.9807$, and the detection limit was 50 pM (S/N=3). This level of sensitivity was comparable to that of non-PCR based method in previously published reports for DNA determination (Table 1). Besides, compared with the enzyme reaction and RCA method, this work required fewer steps and did not need any enzyme in the process of signal amplification (Wan et al., 2013; Bi et al., 2010).

3.5. Detection of the single-base mismatches in DNA

The specificity of the SERS sensing platform was investigated at the same concentration of completely complementary tDNA and single-base mismatched DNA. As shown in Fig. 2C, the intensity of the complementary sequences was nearly 6 times stronger than single-base mismatched DNA sequences after 20 min. The results demonstrate that the target sequences can be effectively recognized by the proposed amplification method with high specificity.

3.6. Selectivity of Bt transgenic gene

To further test the performance of the SERS sening platform in the practical samples and expand its scope of application, a new haipin DNA (H3) was introduced to create a biosensing platform for the detection of Bt transgenic gene fragment (tDNA-Bt). The additional H3 contained complementary sequences that could hybridize with tDNA-Bt. When the tDNA-Bt was injected and hybridized with H3, the hairpin structure of dHS-H1 was unfolded, and then triggered HCR between bio-H1 and bio-H2 resulting in DNA nanowires. Au NPs probes then grew along the nanowires by the strong affinity of the biotin–SA system. The Au NPs probes packed closely and formed hot spots between the particles, which could produce a strong SERS signal in the laser irradiation. Fig. 2D reveals the SERS intensity response to different concentrations of tDNA-Bt. The response concentration to tDNA-Bt was covered from 50 pM to 5000 pM and showed a linearity in the range of 50–500 pM. The regression equation was $Y=17.78+0.05X$ (X: M) with $R=0.9781$, and the detection limit was 50 pM (S/N=3). The results demonstrated that the additional H3 had no obvious effects on the detection of tDNA and the proposed method could provide a universal sensing platform. Moreover, compared with our previously reported work (Jiang et al., 2011; Chen et al., 2012), this method can be applied to genomic DNA detection and operated at room temperature without any enzyme assistance.

4. Conclusions

This work obtained ultra-sensitive signal of tDNA based on the SERS sensing platform by introducing HCR into SERS detection. With further development, the method was constructed as a universal SERS sensing system and successfully applied to the ultra-trace detection of Bt transgene. The results revealed that the SERS sensing platform has high sensitivity and specificity in the detection of tDNA. Besides, compared with the enzyme reaction and RCA method, it required fewer operation steps, non-enzyme assistance and can be implemented at room-temperature in the process of signal amplification. Still, the present DNA sensing method also needs cumbersome label procedure, and further researches on the label-free DNA detection by SERS and HCR are underway. Overall, this report provides a new strategy for SERS signal amplification in the ultra-trace detection of DNA, and

Table 1
Comparison of the proposed method with the reported techniques for DNA determination.

Detection technique	Liner range (M)	Detection limit (M)	R	Reference
QCM	$0–5.0\times10^{-7}$	2.5×10^{-8}	0.985	Scarano et al. (2009)
FRET	1.0×10^{-10}– 5.0×10^{-8}	7.7×10^{-11}	0.9926	Xu et al. (2011)
SOI	6.25×10^{-7}– 12.50×10^{-6}	4.39×10^{-8}	0.993	Zhang et al. (2013)
CL	7.5×10^{-10}– 1.0×10^{-8}	7.5×10^{-10}	0.9826	Wang et al. (2014)
EIS	–	1.0×10^{-10}	–	Gong et al. (2009)
SES	5.0×10^{-11}–5.0×10^{-10}	5.0×10^{-11}	0.9807	This work

QCM: quartz crystal microbalances; FRET: fluorescence resonance energy transfer; SOI: silicon-on-insulator ware; EIS: electrochemical impedance spectroscopy; CL: chemiluminescence.

could be extended to the risk assessment of food safety such as genetically modified food.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.06.046.

References

Bi, S., Li, L., Zhang, S., 2010. Anal. Chem. 2, 9447–9454.
Chen, K., Han, H., Luo, Z., Wang, Y., Wang, X., 2012. Biosens. Bioelectron. 34, 118–124.
Dirks, R.M., Pierce, N.A., 2004. Proc. Natl. Acad. Sci. U. S. A. 101, 15275–15278.
Frens, G., 1973. Nature 241, 20–22.
Gao, F., Lei, J., Ju, H., 2013. Anal. Chem. 85, 11788–11793.
Gong, H., Zhong, T., Gao, L., Li, X., Bi, L., Kraatz, H.B., 2009. Anal. Chem. 81, 8639–8643.
Hiroshi, A., Takahiro, W., Kaoru, W., Shinsuke, N., Shuji, Y., Kozue, S., Ryoko, C., Frank, S., Akihiro, H., Tamio, M., 2005. Anal. Chem. 77, 7421–7428.
Hsu, H.Y., Huang, Y.Y., 2004. Biosens. Bioelectron. 20, 123–126.
Huang, J., Wu, Y.R., Chen, Y., Zhu, Z., Yang, X.H., Yang, C.J., Wang, K.M., Tan, W.H., 2011. Angew. Chem. Int. Ed. 50, 401–404.
Hutchison, W.D., Burkness, E.C., Mitchell, P.D., Moon, R.D., Leslie, T.W., Fleischer, S.J., et al., 2010. Science 330, 222–225.
Jiang, X., Chen, K., Han, H., 2011. Biosens. Bioelectron. 28, 464–468.
Lim, D.K., Jeon, K.S., Kim, H.M., Nam, J.M., Suh, Y.D., 2010. Nat. Mater. 9, 60–67.
Morisset, D., Dobnik, D., Hamels, S., Zel, J., Gruden, K., 2008. Nucleic Acids Res. 36, e118.
Qiu, B., Zheng, Z.Z., Lu, Y.J., Lin, Z.Y., Wong, K.Y., Chen, G.N., 2011. Chem. Commun. 47, 1437–1439.
Quist, D., Chapela, I.H., 2001. Nature 414, 541–543.
Ren, J.T., Wang, J.H., Han, L., Wang, E.K., Wang, J., 2011. Chem. Commun. 47, 10563–10565.
Scarano, S., Spiriti, M.M., Tigli, G., Bogani, P., Buiatti, M., Mascini, M., Minunni, M., 2009. Anal. Chem. 81, 9571–9577.
Shimron, S., Wang, F., Orbach, R., Willner, I., 2012. Anal. Chem. 84, 1042–1048.
Tang, W., Wang, D., Xu, Y., Li, N., Liu, F., 2012. Chem. Commun. 48, 6678–6680.
Wan, Y., Xu, H., Su, Y., Zhu, X., Song, S., Fan, C., 2013. Biosens. Bioelectron. 41, 526–531.
Wang, C., Xiao, R., Dong, P., Wu, X., Rong, Z., Xin, L., Tang, J., Wang, S., 2014. Biosens. Bioelectron. 57, 36–40.
Wang, F., Elbaz, J., Orbach, R., Magen, N., Willner, I., 2011. J. Am. Chem. Soc. 133, 17149–17151.
Xu, L., Zhu, Y., Ma, W., Kuang, H., Liu, L., Wang, L., Xu, C., 2011. J. Phys. Chem. C 115, 16315–16321.
Zhang, B., Liu, B., Tang, D., Niessner, R., Chen, G., Knopp, D., 2012. Anal. Chem. 84, 5392–5399.
Zhang, H., Jia, Z., Lv, X., Zhou, J., Chen, L., Liu, R., Ma, J., 2013. Biosens. Bioelectron. 44, 89–94.
Zhu, D.B., Tang, Y.B., Xing, D., Chen, W.R., 2008. Anal. Chem. 80, 3566–3571.