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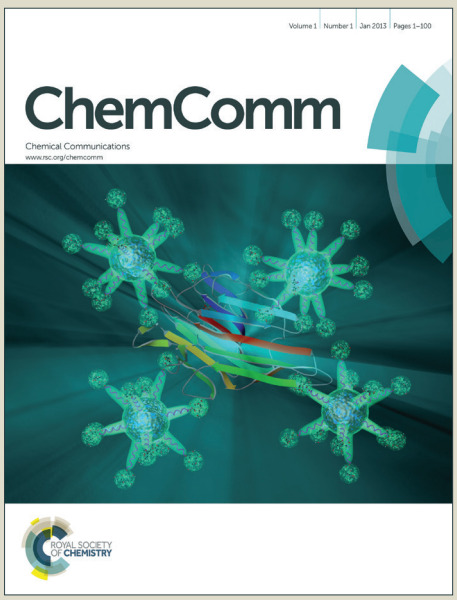

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ARTICLE TYPE

Scaling Up Electrochemical Signal with Catalytic Hairpin Assembly Coupling Nanocatalyst Label for DNA Detection

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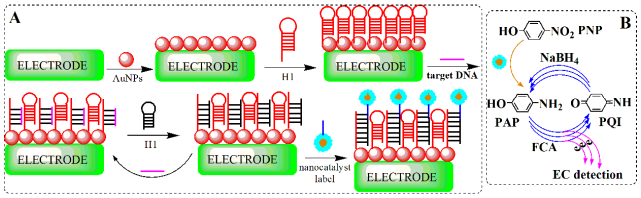
A strategy for the electrochemical detection of DNA based on catalytic hairpin assembly combined with nanocatalyst label-based redox cycling reaction signal amplification. A superior detection limit of 0.3 aM toward DNA can be achieved.

The ability to detect DNA sensitively with simple and inexpensive methods is essential in clinical diagnosis, gene expression analysis, and biomedical studies.[1] The amplified detection of DNA has spurred substantial research efforts. The amplification approaches include polymerase chain reaction (PCR),[2] rolling circle amplification (RCA),[3] loop-mediated isothermal amplification (LAMP),[4] cascade enzymatic signal amplification (CESA),[5] exponential amplification reaction (EXPAR),[6] nanoparticles labelling,[7] conjugated-polymer-based methods,[8] et al.. PCR, RCA, LAMP and CESA have been employed to enrich the target DNA or the target relative molecule. Although nanoparticles labelling and conjugated-polymer-based methods can amplify the detection signal, generally the sensitivity is low. Recently, nicking endonucleases signal amplification technology is employed to amplify the signals of DNA/RNA detections.[9] However, nicking endonucleases are sequence-specific and thus are limited in the number of target DNA sequences against which they can be used.[9]

In recent years, an alternative nucleic acid amplification technique, catalytic hairpin assembly (CHA),[10] has been adapted to the detection of DNA instead. This amplification method is achievable at constant temperature simply by mixing target DNA, hairpin species and DNA probe. CHA based on DNA machines attracted researchers' attention to design the sensitive analysis of targets for their autonomous circular amplification and simple operation. Unlike conventional nucleic-acid amplification reactions such as PCR, this reaction does not require exogenous primer, which often causes primer dimerization or non-specific amplification.[10a] CHA was also a simple isothermal enzymatic-free process. Because of its chemical simplicity, this scheme is expected to allow the development of enzyme-free DNA circuits substantially more simple and effective than previous enzyme-dependent examples.[11]

Inspired by these strategies, we envisioned that CHA technology should be reasonably conceivable for DNA determination with high sensitivity. As a proof of this idea, Bimetalic nanocatalysts, core/shell Au@Pt nanospheres (Au@PtNPs), were synthesized and functionalized with DNA acting as the nanocatalyst labels firstly (Scheme 1). The hairpin H1 was immobilized onto AuNPs modified gold electrode. In the absence of target DNA, H1 self-hybridized into a stem–loop structure with a 15-nt-long stem. Whereas, in presence of target DNA, the stem–loop structure of H1 opened due to binding to the target DNA and forming the double strand product with 21 bases hybridization. This resulted in the opening of hairpin H2 and forming the partially complementary dsDNA with 39 bases hybridization. And the target DNA would be displaced and released. The released target DNA could open another H1. Through such a recycle the target DNA could be reused. And more hairpin DNA was opened and more H2 hybridized with H1. When the nanocatalyst label which consisted of Au@PtNPs and probe DNA was added the sticky ends of the formed double-strand DNA would hybridize with probe DNA. The carried Au@PtNPs can catalyze reduction of p-nitrophenol (PNP) to produce p-aminophenol (PAP) in the prescence of $NaBH_4$. After that, the generated PAP was electrooxidized to p-quinone imine (PQI) by ferrocene in the solution, and then the produced PQI was reduced back to PAP by $NaBH_4$. This leads to the occurrence of a redox cycling between PAP and PQI with the aid of self-produced PAP reactant and the current response is thus amplified. As a result, the electrochemical (EC) response was produced and amplified through this process. A enzyme-free method was fabricated with high sensitivity.



Scheme 1. Schematic illustration of signal amplified strategy based on catalytic hairpin assembly and nanocatalyst label for DNA detection. (A)

† Electronic Supplementary Information (ESI) available: Experimental details. See DOI: 10.1039/b000000x/

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Catalytic hairpin assembly and nanocatalyst label; (B) Redox cycling of PAP to PQI induced by Au@PtNPs catalyze PNP.

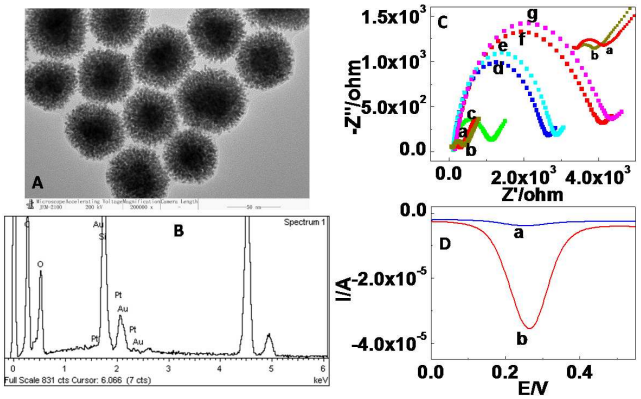

Fig. 1. The TEM images of Au@PtNPs (A) and EDS spectra of the nanoparticles (B). And electrochemical impedance spectroscopy of different electrodes (C): (a) GE; (b) AuNPs/GE; (c) H1/AuNPs/GE; (d) H1/AuNPs/GE blocked with MCH; (e) after target DNA added into (d); (f) after H2 added into (e); (g) after nanocatalyst label added into (e). EC response without (a) and with (b) target DNA1 (2.3×10^{-13} M) (D).

By coupling CHA and nanocatalyst label-based redox cycling reaction for signal amplification, our system provides a electrochemical detection limit of 0.3 aM, which is 3 or 4 orders of magnitude more sensitive than that of a AuNPs label method or sandwich assay format. Because of its chemical simplicity, this scheme is expected to allow the development of enzyme-free DNA sensor substantially more robust than previous enzyme-dependent examples.[11a, 12]

Fig. 1 (A) show the transmission electron microscopy (TEM) of Au@PtNPs. And the formation of the Au@PtNPs was also investigated using the Energy Dispersive Spectrometer (EDS) (Fig. 1 (B)). The average diameter of the formed gold nanoparticle was about 25 ± 4 nm. The TEM and EDS spectra of the nanoparticles indicated the formation of Au@PtNPs. The assembly of nucleic acids on electrodes and the hybridization of target DNA and hairpin DNA, and the capture of nanocatalyst label were also characterized by electrochemical impedance spectroscopy (EIS). The electron transfer resistance (Ret), which was derived from the semicircle domains of impedance spectra (Fig. 1 (C)), can be estimated to be 77 Ω, 19.6 Ω 568Ω, 948Ω, 2387Ω, 3205Ωand 5301 Ω on (a) GE; (b) AuNPs/GE; (c) H1/AuNPs/GE; (d) H1/AuNPs/GE blocked with MCH; (e) after target DNA added into (d); (f) after H2 added into (e); (g) after nanocatalyst label added into (e), respectively. It have been reported that the link DNA3 can not open the hairpin DNA2 without of target DNA 1.[13] In order to test this, only link DNA3 and EC probe were added. There is no obvious peak current (Fig. 1(D)). It suggested that the existence of target DNA 1 is necessary for link DNA3 to open the target DNA 1. So the target DNA1 can be detected through the DNA recycling and EC probe labeling strategy.

The synthetic nanomaterials with enzyme-like activities have garnered increasing interest due to their favourable reactivity, ease of fabrication and low-cost storage, superior to organic molecules. And platinum flowers (PtNFs), platinum–cerium oxide (CeO_2–Pt) and thionine-modified cerium oxide (Thi–CeO_2) hybrid nanostructures et al. [14] have been reported for catalytic recycling. And the EC redox cycling behavior of PNP in the presence of PtNPs was investigated in detail by

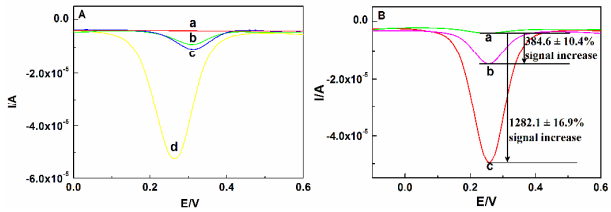

Fig. 2. (A) DPV responses of biosensor after reaction with target DNA with concentration of 9.0×10^{-14} M using Au@PtNPs nanocatalyst labels (a) in pH 7.4 phosphate buffer solution, (b) in pH 7.4 phosphate buffer solution containing 5 mM FCA, (c) in pH 7.4 phosphate buffer solution containing 5 mM FCA + 5 mM PNP, (d) in pH 7.4 phosphate buffer solution + 5 mM FCA + 5 mM PNP + 5 mM $NaBH_4$. (B) DPV of biosensor after reaction with target DNA with concentration of 2.0×10^{-14} M using various nanocatalyst labels: (a) without PtNPs or Au@PtNPs ; (b) PtNPs; (c) Au@PtNPs.

previous articles.[14a] And this mechanism was proved to be useful in amplifying the current signal response for the detection of different biomolecules. In this method, the Au@PtNPs was used as the nanocatalyst label. We firstly verified that if Au@PtNPs can also execute the reduction of PNP to PAP, and further to trigger the oxidation of PAP to PQI on the electrode in the presence of ferrocenecarboxylic acid (FCA). To test this issue, the newly developed assay was used for the detection of 9.0×10^{-14} mol/L target DNA, and the characteristics were investigated in the different supporting electrolytes. As seen from Fig. 2A, no redox peak was observed in pH 7.4 phosphate buffer solution (curve a). When FCA was added, a couple of redox peaks were obtained (curve b), which was mainly derived from the added FCA. And the peak currents were not significantly changed after PNP was added (curve c). The result suggested that the PNP could not be catalytically reduced by the Au@PtNPs and FCA in the absence of $NaBH_4$. When $NaBH_4$ was added, the anodic peak current was largely increased (curve d). The increase in the peak current originated from the catalytic reduction of PNP by Au@PtNPs with the aid of $NaBH_4$ and the redox cycling between PAP and PQI.

Moreover, PtNPs and Au@PtNPs were used as the nanocatalyst label respectively for comparison. The developed bioassay was applied for the detection of 2.0×10^{-14} mol/L target DNA by using PtNPs or Au@PtNPs as trace tags. As shown in Fig. 2B, a relatively low background signal was achieved in pH 7.5 phosphate buffer solution absence of PtNPs or Au@PtNPs (curve a). When PtNPs was used as trace tags, favorably, a obvious catalytic current was acquired (curve b). Relative to the background signal, PtNPs resulted in a 384.6 ± 10.4% signal increase in the anodic current. Interestingly, the peak current was largely increased when Au@PtNPs was used as trace tags (curve c). Au@PtNPs resulted in a 1282.1 ± 16.9% signal increase in the anodic current. During this process, the PAP reactant could be produced in situ by using the Au-Pt nanocatalysts. This phenomenon is coincident with the reported results.[15] It is confirmed that the Au@PtNPs effectively catalyze PNP to PAP and the current can increase greatly through the redox cycling. Coupling the CHA the detection signal can be amplified and the sensitivity can be gained.

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Under the optimized test condition (See as ESI, Fig. S1), the peak current increased with the increasing of the

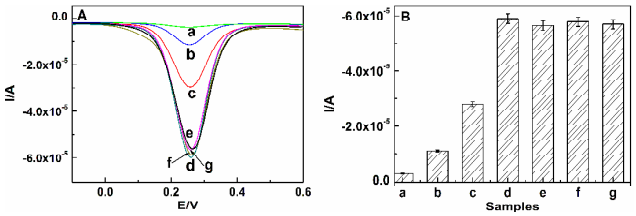

Fig 3. EC responses of different DNA: (a) Three mismatched target DNA sequence. (b) Two mismatched target DNA sequence; (c) One mismatched target DNA sequence; (d) Perfect-matched target DNA sequence. EC responses of artificial complex: (e) Target DNA without random sequences DNA, (f) Target DNA with random DNA, (g) Target DNA spiked with serum. The concentration of DNA was 1.3×10^{-8} M for (a), (b), (c) and (d); 4.0×10^{-9} M for (e), (g) and 4.0×10^{-9} M target DNA and 4.0×10^{-8} M random sequences DNA for (f).

concentration of target DNA. The results showed that the peak current linear increased with the concentration of target DNA in the range from 1.0×10^{-18} to 1.0×10^{-7} M (Fig. S2) and the detection limit was 3.0×10^{-19} M. The regression equation I_p =5.010 log C + 95.38 with a regression coefficient of 0.9990 (C, M; I_p, μA). This is more than 10^6-fold lower, respectively, than the previously reported detection limits of most sensitive assay for target DNA[2b, 8e, 16] and equal to the most sensitive assay (Table S1).[2c, 17]

The control experiment was carried out in catalytic hairpin assembly with FAC labeled AuNPs (CHA with AuNPs label method) or sandwich hybridization with nanocatalyst label (sandwich with nanocatalyst label method). The experimental results suggested that CHA with AuNPs label method gave the linearity range from 1.0×10^{-14} M to 1.0×10^{-10} M with LOD of 3.0×10^{-15} M. And sandwich with nanocatalyst label method gave the linearity range from 2.0×10^{-15} M to 1.0×10^{-11} M with LOD of 7.0×10^{-16} M. The detection limit of the catalytic hairpin assembly and nanocatalyst label amplification was 10 000 or 2 300 times higher than that obtained in the CHA with AuNPs label method or sandwich with nanocatalyst label method. The developed method is more sensitive than the most current approaches and enzyme-based methods (ESI) Thus the high sensitivity of this method was mainly attributed to the amplification of CHA and redox cycling process, which increased the repeat use of target DNA and the number of electrons on the surface of AuNPs modified electrode. The generality of the developed method was also tested by detecting other one target DNA (ESI) .

We evaluated the intra-assay precision of the method by analyzing the same concentration target DNA 7 times with multiple replicates and the inter-assay precision by analyzing the same concentration target DNA on 7 consecutive days. The intra- and inter-assay precision are 6.0%, 5.4%, 4.5%, 4.4%; 5.5%, 5.4%, 5.0%, 4.8% for seven parallel measurements of 1.0×10^{-17} M, 1.0×10^{-15} M, 1.0×10^{-13} M, 1.0×10^{-11} M target DNA respectively. The results indicated that the electrochemical bioassay could be used repeatedly, and further verified the possibility of batch preparation.

The specificity of the electrochemical bioassay was monitored by challenging the system with mismatch base DNA (Fig. 3). The gene-sensor exhibits the different response signals. As is shown in Fig. 3 (B), the response signals gradually decreased with the increasing of the mismatch bases. The response for artificial complex samples was investigated (Table S2). The results indicated that the selectivity of the developed gene-sensor is sufficient for DNA detection.

In summary, a new strategy for the electrochemical detection of DNA based on CHA combined with nanocatalyst label-based redox cycling reaction signal amplification was demonstrated. Combined with CHA and nanocatalyst label-based redox cycling reaction signal amplification a superior detection limit of 0.3 aM was achieved.

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