Electronic Supplementary Information:

Catalytic signal amplification of gold nanoparticles combining with conformation-switched hairpin DNA probe for hepatitis C virus quantification

Wen Li, Ping Wu*, Hui Zhang and Chenxin Cai*

Jiangsu Key Laboratory of New Power Batteries, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210097, P.R. China.

* Corresponding author, E-mail: wuping@njnu.edu.cn, cxcai@njnu.edu.cn

Fax: 86 25 85891767; Tel: 86 25 85891780.

1. Experimental details

1.1 Chemicals

All chemicals and solvents were of reagent grade or better. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), tris(hydroxymethyl)aminomethane (Tris), *N*-hydroxysuccinimide (NHS, 98%), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide (EDC), tri(2-carboxyethyl)phosphine hydrochloride (TCEP), and *p*-aminobenzoic acid (ABA) were purchased from Sigma-Aldrich and used as received. All solutions were prepared with doubly distilled water.

The synthetic thiol-capped single-stranded 31-base hairpin DNA probe (S1) and 17-base modified HCV-1b cDNA containing one mismatch base, two mismatch bases, and three mismatch bases, respectively, were purchased from *BioSune* Biotechnology Co. (Shanghai, China). The probe DNA was designed to hybridize to the highly conserved 5' noncoding region of the HCV genome. The samples of target cDNA were obtained from the highly conserved region from HCV-1a, HCV-1b, HCV-1, and HCV-6a, respectively, by nested reverse transcription PCR (RT-PCR) and processed by the complete Amplicor Hepatitis C Virus test procedure. The concentration was determined by a quantitative assay (Cobas Amplicor HCV Monitor 2.0, Roche). The samples were kept in freezer. The oligonucleotide sequences used in this work are summarized in Table S1.

1.2 Preparation of AuNPs

AuNPs were prepared using the procedures reported previously.[1] Briefly, all glassware used in the preparation was thoroughly cleaned in *aqua regia* (3 parts concentrated HCl, 1 part concentrated HNO_3), rinsed in distilled H_2O, and oven-dried prior to use. ***Caution: aqua regia is extremely dangerous and should be handled with extreme caution. Gloves and eye protection are required for handling***. In a 1 L round-bottom flask equipped with a condenser, 500 mL of 1 mM $HAuCl_4$ was brought to a rolling boil with vigorous stirring. Rapid addition of 50 mL of 38.8 mM sodium citrate to the vortex of the solution resulted in a color change from pale yellow to burgundy. Boiling was continued for 10 min; the

heating mantle was then removed, and stirring was continued for an additional 15 min. After the solution reached room temperature, it was filtered through a 0.8 μm Nylon membrane filter. Transmission electron microscopic (TEM) indicated a particle size of 15-20 nm (Fig. S1).

1.3 Immobilization of probe DNA and conjugation of AuNPs

A glassy carbon electrode (GC, 3-mm in diameter, CH Instruments) was used as substrate for assembly of probe DNA. Prior to use, the electrode was polished sequentially with metallographic abrasive paper (No. 6) and slurries of 0.3 and 0.05 μm alumina to create a mirror finish, and then sonicated with absolute ethanol and double distilled water for about 1 min, respectively. It was rinsed thoroughly with double distilled water and dried under ambient temperature.

For assembly of DNA probe, a monolayer of the bifunctional molecule of *p*-aminobenzoic acid (ABA) was first formed on the GC electrode surface by cyclic scanning the electrode in a solution of 10 mM phosphate buffer (PBS, pH 7.4) containing 1 mM ABA (6 cycles, at the scan rate of 10 mV/s, step (a), Fig. 1). ABA exhibits a single irreversible oxidation peak at ca. 0.8 V (versus SCE) at the GC electrode in PBS (Fig. S2). This anodic peak is ascribed to one-electron oxidation of the amino group turning into its corresponding cation radical,[2] which forms carbon-nitrogen linkage at the GC electrode surface resulting the assembly of ABA. The peak current decreases quickly with the successive scanning (Fig. S2), indicating the grafting of ABA on electrode surface.

The probe DNA (S1) was assembled on the electrode surface by forming the amide between the group of –COOH at ABA and $–NH_2$ moiety at 3'-terminus of S1 via the EDC-NHS coupling (step (b), Fig. 1). First, the –COOH was activated by immersing the ABA-assembled GC electrode into EDC-NHS mixture (5 mM EDC, 10 mM NHS in 10 mM Tris-HCl buffer, pH 7.4) for 30 min. Then, the electrode was transferred into the solution of S1 (10 μM, 10 mM Tris-HCl buffer, pH 7.4) for 3 h. The $–NH_2$ moiety at S1 would react with the activated –COOH group, resulting to covalently link S1 at the GC electrode surface (step (c), Fig. 1).

The AuNPs was conjugated on the electrode via formation of Au–S bond by incubating the S1-assembled GC electrode in the AuNPs solution for 1 h (step (d), Fig. 1). Prior to conjugated AuNPs, the probe DNA was incubated for 1 h in 2 μM TCEP solution to reduce disulfide bonds. The electrode was then thoroughly rinsed with Tris-HCl buffer and water in turn, and stored in the buffer. The cyclic voltammetric result indicates the characteristic feature of the redox reaction of Au with the oxidation peak at ca. +1.1 V (versus SCE) corresponding to the formation of Au oxide, and a reduction peak at ca. +0.9 V corresponding to the reduction of the Au oxide (Fig. S3),[3,4] verifying the conjugation of AuNPs on the electrode.

1.4 Hybridization and electrocatalysis

Hybridization was conducted at 37 °C by immersing the AuNPs-conjugated S1 in Tris-HCl buffer containing the target 17-base cDNA from HCV-1b, HCV-1a, HCV-1, HCV-6a, or synthetic mismatched DNA for 60 min (step (e), Fig. 1). After hybridization, the electrode was thoroughly rinsed with water.

The electrocatalytic reduction of O_2 by the conjugated AuNPs was conducted in air-saturated PBS (10 mM, pH 7.4). The electrocatalytic currents were recorded before and after the hybridization of AuNPs-conjugated S1 with target DNA (including those DNAs containing mismatched bases), and the value of Δi was calculated at the different concentration of target DNA.

1.5 Apparatus

Transmission electron microscopy (TEM) image was obtained with a JEOL-2010 transmission electron microscope operating at an accelerating voltage of 120 kV. The cyclic voltammetric (CV) experiments were performed with an Autolab PGSTAT302N electrochemical station (Metrohm). A two-compartment three-electrode cell with a sample volume of 5 mL was employed. A coiled Pt wire and a saturated calomel electrode (SCE) were used as the counter and the reference electrode, respectively.

2. Evaluation on the assay repeatability and precision of the method

Different aspects regarding the assay repeatability and precision of the method were evaluated. The RSD (relative standard deviation) for assay of 1.5 nM 17-base cDNA from HCV-1b is ~2.0% obtained with five different and freshly fabricated detection systems, revealing an acceptable repeatability of the method. The assay precision is estimated with the slopes of calibration plots obtained from five independent assay systems. The RSD of these slopes is ~2.2%. These results demonstrate the great potential for practical application of the proposed model.

References

1 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735.

2 J. Liu, L. Cheng, B. Liu and S. Dong, *Langmuir*, 2000, **16**, 7471.

3 Y. Hu, H. Zhang, P. Wu, H. Zhang, B Zhou and C. X. Cai, *Phys. Chem. Chem. Phys.*, 2011, **13**, 4083.

4 Y. Hu, J. Jin, P. Wu, H. Zhang and C. X. Cai, *Electrochim. Acta*, 2010, **56**, 491.

Table S1. The sequences of oligonucleotide used in this work

description	sequence (5' to 3')
31-base hairpin DNA probe (S1)	5'-SH-$(CH_2)_6$-TCG CTG AGA TCG GGA TCC CCA AAA TCA GCG A-$(CH_2)_6$-NH_2-3'
17-base HCV-1b cDNA (S2, perfect matched target DNA)	5'-GGG GAT CCC GAT CTC AG-3'
17-base modified HCV-1b cDNA (one-base mismatch, 1MM)	5'-G*T*G GAT CCC GAT CTC AG-3'
17-base modified HCV-1b cDNA (two-base mismatch, 2MM)	5'-G*T*G GAT CC*T* GAT CTC AG-3'
17-base modified HCV-1b cDNA (three-base mismatch, 3MM)	5'-G*T*G GAT CC*T* GAT CTC *T*G-3'
17-base modified HCV-1b cDNA (one-base mismatch. The mismatched base locates at the 5'-terminus, HM)	5'-G*T*G GAT CCC GAT CTC AG-3'
17-base modified HCV-1b cDNA (one-base mismatch. The mismatched base locates at the middle, MM)	5'-GGG GAT CC*T* GAT CTC AG-3'
17-base modified HCV-1b cDNA (one-base mismatch. The mismatched base locates at the 3'-terminus, TM)	5'-GGG GAT CCC GAT CTC *T*G-3'
17-base HCV-1a cDNA	5'-CCG GGC GCC AAG CAG GA-3'
17-base HCV-1 cDNA	5'-GGT CTC CTT ACA CCA GG-3'
17-base HCV-6a cDNA	5'-ACG GTG TTG GGC ATT GG-3'

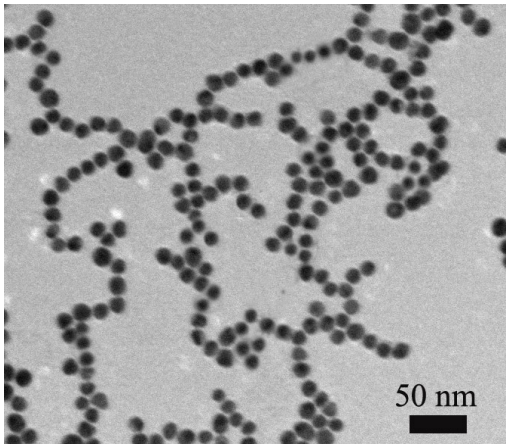

Fig. S1 Typical TEM image of the prepared AuNPs.

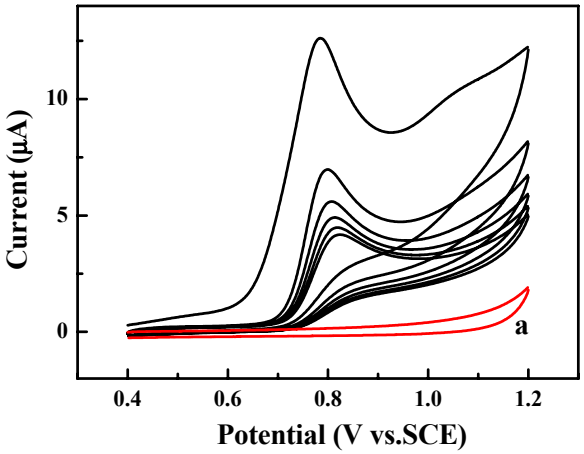

Fig. S2 Cyclic voltammetric responses of 1 mM ABA in PBS (10 mM, pH 7.4) at the GC electrode. Curve (a) is the cyclic voltammetric response of the electrode PBS in the absence of ABA. The scan rate is 10 mV/s.

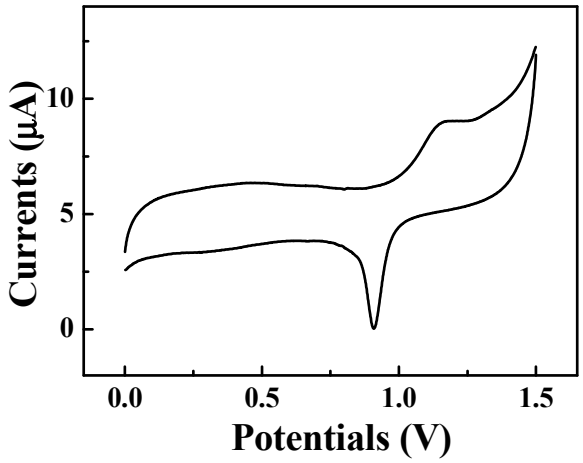

Fig. S3 Cyclic voltammograms of AuNPs-conjugated S1 in 0.1 M H_2SO_4 solution at a 10 mV/s.

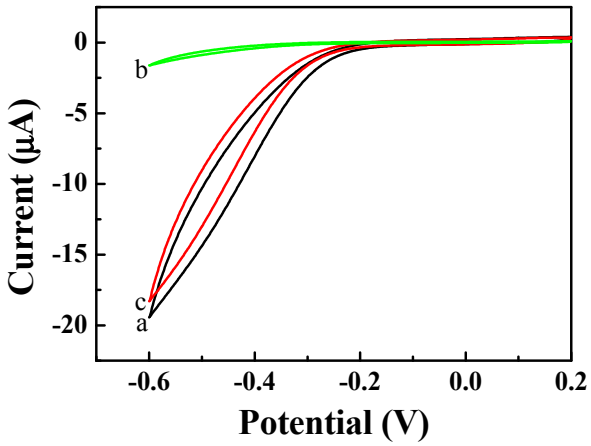

Fig. S4 Voltammetric responses of the AuNPs-conjugated S1 in air-saturated PBS (10 mM, pH 7.4) before (a) and after (b) hybridized with S2 (10 nM) for 60 min. Curve (c) depicts the voltammetric response of the duplex DNA of AuNPs-S1/S2 in air-saturated PBS after the duplex DNA was washed with a solution of dimethyl sulfoxide for 30 s. The dimethyl sulfoxide denatures double-stranded DNA and recovers the hairpin structure of probe DNA. The scan rate is a 10 mV/s.

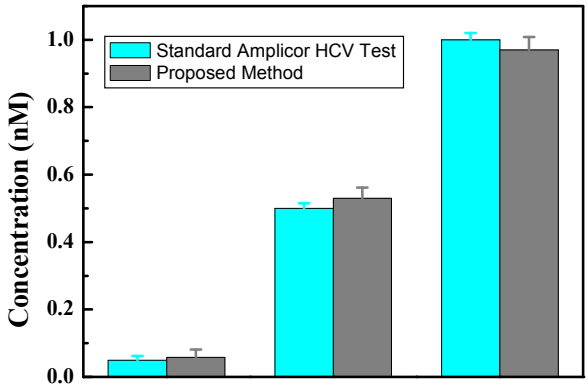

Fig. S5 Comparison of the results obtained by the standard Amplicor HCV test (commercial method) and the developed method. The data presented here are the average values of three measurements.