Electronic Supplementary Information (ESI) for Chemical Communications

Amplified logic gate driven by in situ synthesis of silver nanoclusters for identification of biomarkers

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Experimental Section

Chemicals: Sodium borohydride ($NaBH_4$), tris(hydroxymethyl) aminomethane (Tris), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), silver nitrate ($AgNO_3$), and 6-mercapto-1-hexanol (MCH) were supplied by Aladdin Chemical Co., Ltd. (China). The detailed oligonucleotide sequences listed in Table S1 were bought from Takara Biotechnology Co., Ltd. (Dalian, China).

Table S1 DNA sequences used in the proposed system.

Name	Sequence (from 5' to 3')
Methylated target DNA	mCGAGTGGTGATAAGmCGATGAAGTAGGATGTGATGA
Unmethylated DNA	CGAGTGGTGATAAGCGATGAAGTAGGATGTGATGA
Head methylated DNA	mCGAGTGGTGATAAGCGATGAAGTAGGATGTGATGA
Intermediate methylated DNA	CGAGTGGTGATAAGmCGATGAAGTAGGATGTGATGA
MicroRNA (miRNA) 122	UGGAGUGUGACAAUGGUGUUUG
Assistant probe (AP)	TGGAGTGTGACAATGGTGTTTG
AP used for miRNA 122 detection	CGAGTGGTGATAAGCGATGAAGTAGGATGTGATGA
Displacement probe (DP)	CAAACACCATTGTTTTCTACTTCATCGCTTATCACCACTCG
Immobilization probe (IP) 1	GTGATAAGCGATGAAGTAGTTTTTT-$(CH_2)_6$-SH
IP2	SH-$(CH_2)_6$-TTTTTTCAATGGTGTTTG
Linker probe (LP)	TCATCACATCTCACACTCCA
Signal probe (SP)	CAAACACCATTGCCCCCCCCCCC

Sensing interface fabrication: Prior to use, the gold electrodes (AuEs, 2 mm) were first immersed in the piranha solution (mixture of 98% H_2SO_4 and 30% H_2O_2 at the volume ratio of 3:1) for 0.5 h, and then polished sequentially using 0.3 and 0.05 μm alumina slurries to acquire the mirror-like AuEs surfaces, followed by the ultrasonic wash in ethanol and ultrapure water. Subsequently, the cyclic voltammetric treatment was performed in 500 mM H_2SO_4 solution from −0.3 to 1.55 V until achieving the stable voltammetric peak. Ultimately, the well-pretreated AuEs were washed using water and dried under a nitrogen stream for subsequent reaction.

The V-shaped probe (VSP) was prepared based on mixing the IP1 (500 nM), IP2 (500 nM), and DP (600 nM) for annealed hybridization in 100 mM Tris buffer (10 mM $MgCl_2$, 5 mM KCl, 0.1 M NaCl, pH 7.4) through heating at 90 °C for 10 min and cooling to 25 °C within 1 h. Then, the VSP (500 nM) was incubated with TCEP (10 mM) for 60 min to open the disulfide bond. Subsequently, 10 μL VSP (500 nM) was incubated with AuE for 12 h. Finally, the sensing electrode was washed using Tris buffer and then reacted with 1 mM MCH for 120 min to acquire the MCH/VSP/AuE.

Operation of the sensing system: The methylated DNA targets were first denatured at 37 °C for 0.5 h in NaOH solution (0.3 M), followed by reacting with $NaHSO_3$ solution (3.2 M) containing 0.5 mM hydroquinone for 15 h at 55 °C. The bisulfite-modified targets were recovered using the Wizard DNA cleanup kit (Promega), desulfonated in 0.3 M NaOH for 20 min at 37 °C, and precipitated by ethanol. The bisulfite treatment would convert the unmethylated C residues to U, and the methylated C residues could keep invariant. The MCH/VSP/AuE sensing interface was incubated with LP (0.5 μM) and AP (0.5 μM) under different inputs of methylated target DNA or miRNA 122 at room temperature for 90 min in Tris buffer. Then SP (0.5 μM) was dropped on the sensing interface for hybridization for 1 hour, followed by incubating $AgNO_3$ (0.1 mM) in 20 mM citrate buffer (pH 7.0) for 0.5 h. After rinsing with buffer, the interface was finally reacted with $NaBH_4$ (500 μM) for 5 min in dark for reducing $AgNO_3$ to silver nanoclusters (AgNCs).

Live subject statement: The liver injury patient and healthy volunteer serum samples were collected from the affiliated hospital of Xuzhou Medical University (Jiangsu, China), and all of the human subjects signed an informed consent form prior to the assay. The detection was carried out in agreement with the World Health Organization (WHO) guidelines on blood drawing (WHO Publication ISBN-13: 978-92-4-159922-1, 2010) and was approved by the Ethics Committee of the affiliated hospital of Xuzhou Medical University.

Extraction of miRNA 122 from human serum samples: The miRNeasy RNA isolation kits were applied to extract miRNA 122 from serum samples using Qiagen's instructions.

Briefly, serum samples (200 μL) were firstly injected into Qiazol solution (1.0 mL), and stirred at 25 °C for 5 min to dissociate nucleoprotein complexes. Then, chloroform (200 μL) was added to the mixed solution and vortexed for 0.5 min. The purification of miRNA in the upper phase was conducted when centrifugated at 12 000 × g for 15 min at 4 °C, followed by precipitation using the recommended assay protocol.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) experiments: The extracted RNA was first converted to complementary DNA (cDNA) using the Revert Aid Premium Reverse Transcriptase Reverse Transcription Kit (Thermo) following the manufacturer's instructions. The concentration of total RNA was then detected using SMA4000 spectrophotometer (Merinton Instrument, Inc.). The reaction conditions were listed as follows: 10 min at 20 °C, 0.5 h at 50 °C for reverse transcription, and 85 °C for 5 min to stop the reaction. Subsequently, the obtained cDNA solution was tested by qRT-PCR assay, which was conducted on a Step One Plus real-time PCR instrument using the Fast qPCR Master Mix kit. The reaction system was incubated at 95 °C for 3 min, followed by 45 cycles of 95 °C for 5 s and 60 °C for 0.5 min.

Apparatuses and measurements: Linear sweep voltammetry (LSV), cyclic voltammetry (CV), and differential pulse voltammetry (DPV) were conducted on a CHI 660E electrochemical workstation (Shanghai Chenhua Instrument, China), and the electrochemical system consisted of an Ag/AgCl reference electrode, a modified AuE working electrode, and a platinum wire counter electrode. The native polyacrylamide gel electrophoresis (PAGE) experiment was conducted on the DYCZ-24DN electrophoresis instrument (Beijing LIUYI Biotechnology Co., LTD.). The nucleic acid samples were first mixed with loading buffer (6×) with a volume ratio of 5:1, and then loaded into 16% PAGE, followed by running in Tris-boric acid-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.0) for 120 min at 100 V. PAGE was finally stained with ethidium bromide about 30 min and taken a picture using the Bio-Rad imaging system (Hercules,

CA, U.S.A.). Transmission electron microscopy (TEM) image was tested on a FEI Tecnai G2 Spirit Twin microscope operated at 120 kV.

Supplementary Results and Discussion

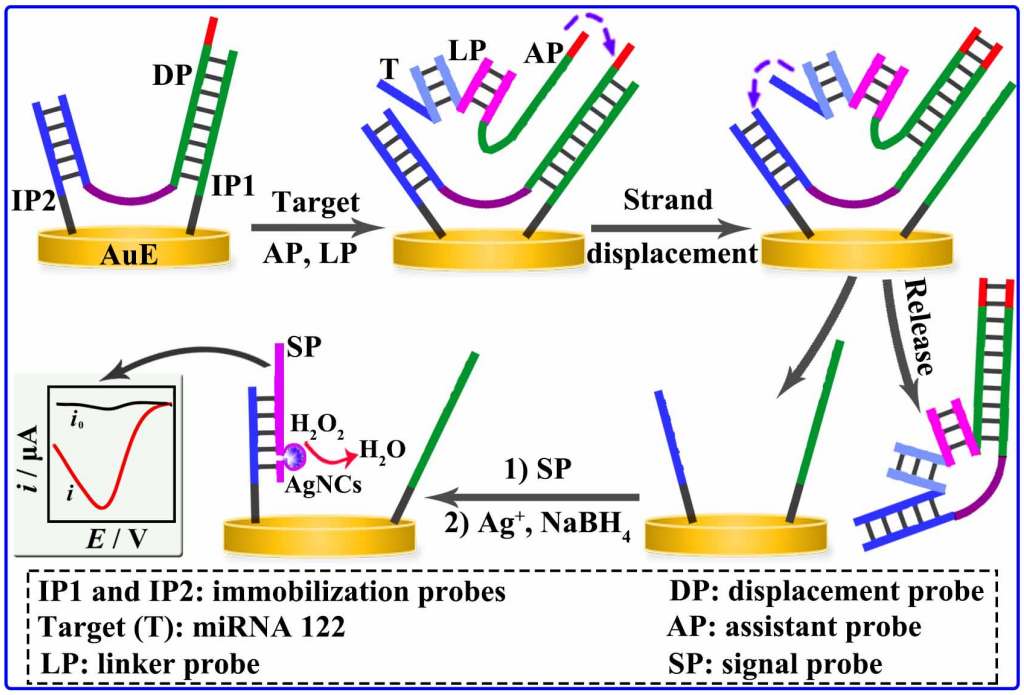

Fig. S1 Schematic diagram of the convenient and amplified sensing platform for detection of miRNA 122. AP sequence was substituted with 5'-CGAGTG GTGATA AGCGAT GAAGTA GGATGT GATGA-3', while other strands remained unchanged.

The native PAGE experiment was first performed to demonstrate the preparation of VSP. As shown in Fig. S2A, IP1, IP2, and DP exhibited a distinct single band in lanes 1, 2, and 3, respectively. When IP1, IP2 and DP were mixed for hybridization, a new band with lower mobility can be seen in lane 4, suggesting that the VSP structure was prepared. We further used the CV test to record the fabrication process for demonstrating the modification of the sensor. It can be seen from Fig. S2B, bare AuE displayed excellent redox peak currents (curve a) in 1 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl. After assembling the VSP structure on the electrode, the redox current of $[Fe(CN)_6]^{3-/4-}$ significantly decreased (curve b), because the

electrostatic repulsion between the $[Fe(CN)_6]^{3-/4-}$ and DNA probes inhibited the electron transfer. When MCH has subsequently blocked on the electrode surface, some physically adsorbed DNA probes would be removed from the sensing interface, resulting in the increase of the redox currents (curve c). The addition of methylated target DNA (T), AP and LP caused a further increased current response owing to the disassembly of VSP (curve d). When the SP (curve e) and AgNCs (curve f) were progressively modified on AuE, redox peak currents gradually decreased. The results suggested that the sensing interface was successfully constructed.

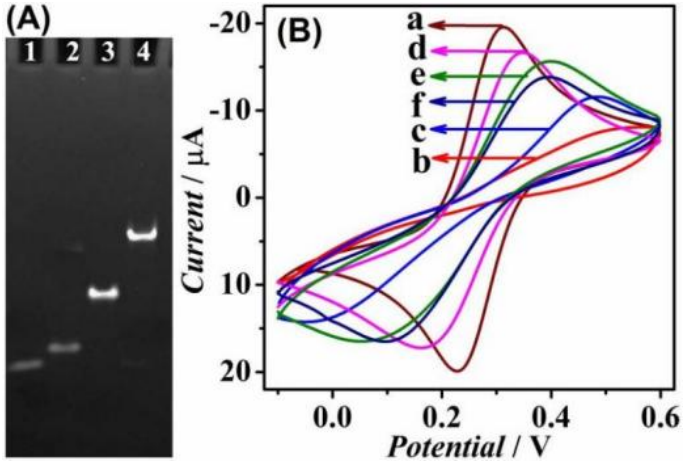

Fig. S2 (A) PAGE characterization: Lane 1, IP1 (0.5 μM); Lane 2, IP2 (0.5 μM); Lane 3, DP (0.5 μM); Lane 4, IP1 (0.5 μM) + IP2 (0.5 μM) + DP (0.5 μM); (B) CV responses of different interfaces in $[Fe(CN)_6]^{3-/4-}$ solution: (a) bare AuE, (b) VSP/AuE, (c) MCH/VSP/AuE, (d) (T+AP+LP)/MCH/VSP/AuE, (e) SP/(T+AP+LP)/MCH/VSP/AuE, (f) AgNCs/SP/(T+AP+LP)/MCH/VSP/AuE.

The synthesis and electrocatalytic ability of AgCNs were further characterized. As displayed in Fig. S3A, MCH/VSP/AuE did not exhibit an obvious LSV current peak (curve a). When MCH/VSP/AuE was incubated with SP in the presence of methylated DNA (T), AP, and LP for synthesizing AgCNs, a sharp current peak at 0.1 V could be observed. According to the inset in Fig. S3A, we can see that spherical AgCNs were uniformly distributed with a mean diameter of 5 nm. The

experiment results thus verified that AgCNs were in situ synthesized on the sensing interface. The catalytic ability of AgCNs to H_2O_2 redox reaction was investigated by conducting CV test in N_2 saturated phosphate buffer (10 mM, pH 7.4) containing 1 μM H_2O_2. According to Fig. S3B, MCH/VSP/AuE exhibited no reduction or oxidation current (curve a) from −0.8 to 0 V, suggesting that the MCH/VSP/AuE displayed no catalytic ability to H_2O_2 redox reaction. While AgNCs/SP/(T+AP+LP)/MCH/VSP/AuE would result in a significant reduction current to 1 μM H_2O_2 at around −0.5 V (curve b), manifesting the electrocatalytic capability of AgNCs to H_2O_2 reduction.

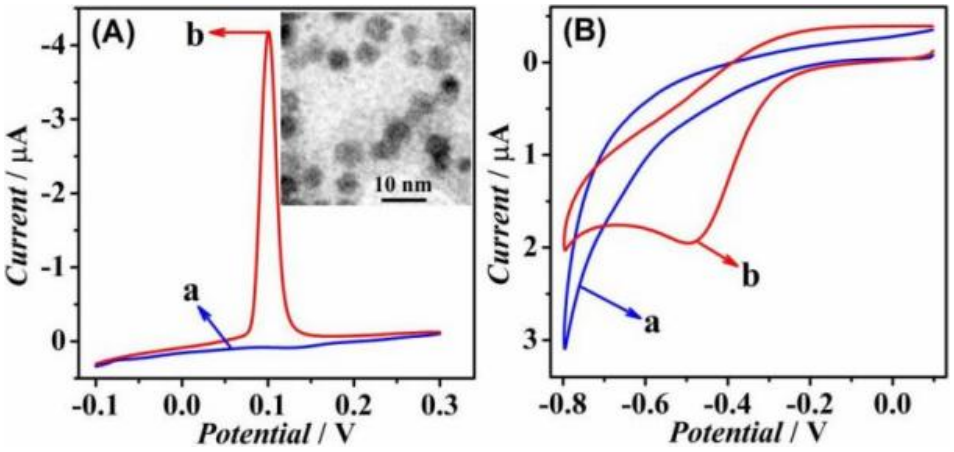

Fig. S3 (A) LSV responses of (a) MCH/VSP/AuE and (b) AgNCs/SP/(T+AP+LP)/MCH/VSP/AuE. The inset was TEM image of AgNCs. (B) CV responses of (a) MCH/VSP/AuE and (b) AgNCs/SP/(T+AP+LP)/MCH/VSP/AuE in 10 mM phosphate buffer (pH 7.4) containing 1 μM H_2O_2 with the scan rate of 50 mV s^{-1}.

The detection ability of the proposed sensor for detecting two biomarkers individually was studied by DPV measurements. As shown in Fig. S4A, when the sensing electrode was incubated with LP and AP, no obvious current would be obtained (curve a). The addition of SP in the absence of target caused an insignificant change in DPV current (curve b). And the negligible current response was achieved by the incubation of methylated DNA without SP (curve c). While the remarkable current could be obtained after the sensing electrode was reacted with

SP and methylated DNA (curve d), suggesting the feasibility of the sensor for methylated DNA detection. When AP sequence was substituted with 5'-CGAGTG GTGATA AGCGAT GAAGTA GGATGT GATGA-3', the sensing system was applied to detect miRNA 122 (Schematic illustration was shown in Fig. S1). According to the experiment results shown in Fig. S4B, three control experiments exhibited little current responses when the MCH/VSP/AuE was reacted with LP and AP (curve a) in the presence of SP (curve b) or miRNA 122 (curve c). Importantly, the sensing interface would obtain the obvious current after reacting with LP, AP, and SP in the presence of miRNA 122 (curve d). Therefore, the above results demonstrated the sensor can be applied to detect the two biomarkers individually.

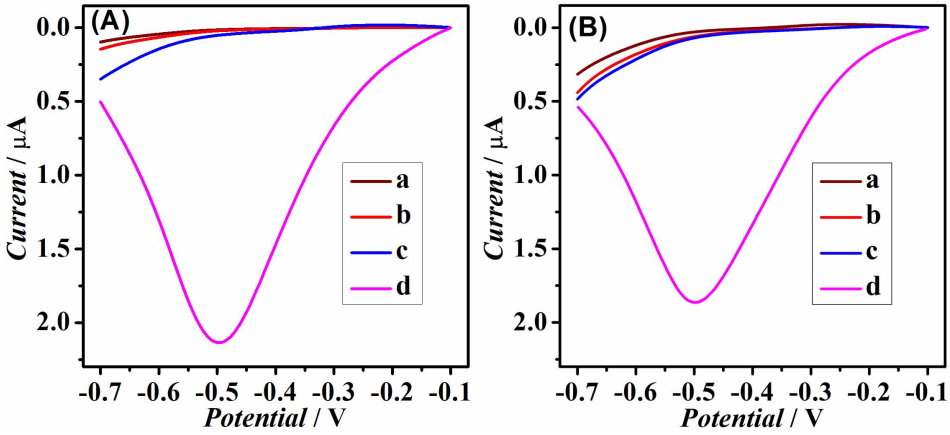

Fig. S4 (A) DPV responses of MCH/VSP/AuE to (a) LP (0.5 μM) and AP (0.5 μM), (b) the mixture of (a) with SP (0.5 μM), (c) the mixture of (a) with methylated target DNA (100 pM), (d) the mixture of (a) with SP (0.5 μM) and methylated target DNA (100 pM) in 10 mM phosphate solution (pH 7.4) including 1 μM H_2O_2. (B) DPV responses of MCH/VSP/AuE to (a) LP (0.5 μM) and AP (0.5 μM), (b) the mixture of (a) with SP (0.5 μM), (c) the mixture of (a) with miRNA 122 (100 pM), (d) the mixture of (a) with SP (0.5 μM) and miRNA 122 (100 pM) in 10 mM phosphate solution (pH 7.4) including 1 μM H_2O_2.

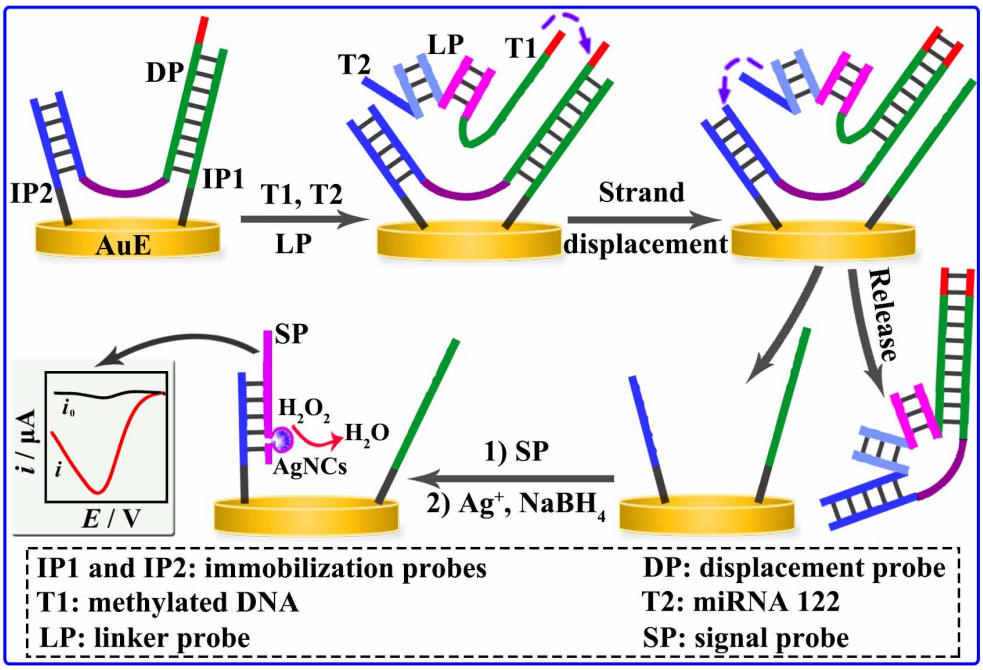

Fig. S5 Schematic illustration of AND logic gate. The logic sensing system was operated without the AP strand.