Supporting Information

A Simple Strategy for Fabrication of Au-modified Single Nanopore and its Application for miRNA Sensing

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EXPERIMENTAL SECTION

Chemicals and Materials

All DNA, and miRNA were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of DNA and miRNA were shown in Table S1. KCl, KH$_2$PO$_4$, K$_2$HPO$_4$, Triton X 100 (TX-100), glucose (Glu), ethylene glycol (EG) and ethanol (EtOH) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and gold (III) chloride Hydrate, sodium borohydride are obtained from Sigma Aldrich. Human serum was from Beijing BioDee Biotechnology, Co., Ltd. (Beijing, China). All other chemicals are of analytical grade, and all chemicals are used without further purification. All solutions are prepared with ultrapure water (resistivity=18.5 MΩ·cm). The glass capillaries (o.d. = 1.0 mm, i.d. = 0.7 mm) was obtained from Sutter Instrument Company.

Graft of Probe DNA on Nanopore and Hybridization

The graft of probe DNA was achieved by immersing the Au-modified nanopores into a 5'- thiol modified probe DNA (0.2 μM) in 10 mM phosphate buffer (pH = 7.4) containing 100 mM KCl for 5 h via the formation of Au–S bonds. After washing with buffer, the probe DNA modified nanopore was then immersed into the mismatched target DNA (0.4 μM) for 8 h and double strand DNA could be formed, which led to the shrinking of the diameter of nanopore.

Hybridization of miRNA

The double-stranded DNA on the inner surface of the nanopore was exposed to the miRNA solution for various times. After hybridization, the channel was washed thoroughly with PBS buffer solution. After washing, the I–V curves were measured.
using 100 mM KCl prepared in PBS (pH = 7.4) as an electrolyte for the examination of successful hybridization.

**Preparation of the glass nanopores**

Quartz capillaries with an outer diameter of 1.0 mm and an inner diameter of 0.7 mm (Sutter Instrument Company) were used for all experiments.\(^1\)\(^-\)\(^3\) Prior to pulling the capillaries, the glass pipettes were thoroughly cleaned by sonicating in acetone and ethanol for a duration of 5 min in each step. This removed any contamination resulting from the production process or dirt acquired during transport, storage, and handling. Residual ethanol from the cleaning process was removed with gaseous nitrogen. The glass capillary was mounted on a P-2000 laser microelectrode puller, with the two moving bars stabilized using a homemade aluminum clamp and passed the following heating procedures: heating = 550, filament = 3, velocity = 99, delay = 20, Pull = 50, the heating program is applied for 40 seconds, and then the cooling period is 20 seconds. The above heating and cooling process are repeated 3-4 times; Drawing procedure: The clamp was removed from the puller and the following drawing procedure was performed to pull the microporous preform into two ultrasharp nanotips: heating = 660, Filaments = 1, Velocity = 60, Delay = 165, Pull = 225. It should be noted that there is some variation between P2000 pullers due to local temperature and humidity therefore these pulling parameters only serve as an example.

**Preparation of Ultra-thin Gold Modified Single Glass Nanopore**

The modification of inner surface of glass nanopore by ultra-thin gold film was approached by a UV irradiation method (see Figure S1, Supporting information). A
solution of Au (III) chloride was prepared by dissolving chloroauric acid in distilled water. Triton X-100 (TX-100, named as poly(oxyethylene)iso-octylphenyl ether) solution was prepared by dissolving appropriate volume in water. The decoration of gold onto the inner surface of the bare nanopore with a diameter of 25 nm was done by a photochemical approach. First, HAuCl₄ solution and TX-100 solution were mixed together, the final concentration of Au (III) ion was $1.2 \times 10^{-3}$ M and the concentration of TX-100 was $8.1 \times 10^{-4}$ M. The prepared solution was then backfilled into the glass nanopore. After that, the glass nanopore filled with the mixed solution was placed at a distance of 3 cm from a light source by a hand-held ultraviolet analyzer (365 nm, 8 W, Model ZF-7, Shanghai Jihui Scientific Analyze Instrument Co., Ltd., Shanghai, China) and was irradiated for a while. Finally, the nanopore was rinsed with water and ethanol, dried (room temperature) and annealed (100 °C) for 2 h.

**Characterizations**

The electrochemical characterization of the nanopores and its derivative products were performed by using two-electrode cells in a 100 mM KCl solution as electrolytes. The symmetric Ag/AgCl electrodes were used as working and counter electrode. CHI 660C electrochemical analyzer (CH Instruments, Chenhua Co., Shanghai, China) was used to measure the electrochemical responses. In all cases, the nanopore electrode was filled with the same electrolyte as the bulk solution.

UV−vis spectra were acquired on a Thermo Fisher Scientific Evolution 3000 UV−vis spectrophotometer (Perkin-Elmer). TX-100 solution was used as reference solution. TEM images were obtained with a transmission electron microscope...
(HITACHI HT-7700, Japan). Samples for TEM were prepared by placing cut the tip of the glass nanopore on a carbon-coated Cu grid (Beijing Zhongjingkeyi technology Co., Ltd). Fluorescent microscope images were observed through a fluorescence microscopy (Olympus CX31 microscope). The gold nanoparticles images were measured using a Scanning electron microscope (Hitachi S-4800) operating. Samples for SEM were prepared by placing a drop of solution containing nanoparticles on a gold particulate film formed on a planar glass slide.

**MiRNA-15 Assay.**

The performance of the biosensor was analysed by the standard addition method, in order to determine the miRNA-15 concentration in 10% diluted commercial normal human serum. MiRNA-15 concentrations in samples were set to different levels, equal to 50 fM, 100 fM and 300 fM. Always lying within the linear range of the device. All the assays were conducted in triplicate.

**Mechanism of Gold Film Formation.**

The mechanism of formation of gold particles can be formulated as follows. The Au(III) from AuCl- 4 is reduced by the hydroxymethyl radical generated by the photolysis of TX-100 [R-O-CH₂CH₂OH where R=(CH₃)₃-CCH₂C(CH₃)₅C₆H₄(OCH₂CH₂)~9-] into Au (II). The next step is the fast disproportionation to Au(I) and Au (III). Then, accumulated aurous ions Au(I) are reduced by the hydroxymethyl radical to Au (0), and the primary hydroxyl function of TX-100 is oxidized to the carboxylate group. The atoms are formed with a homogeneous distribution throughout the solution. The atoms, then, tend to dimerize when encountered in pairs or associated with excess ions. But
the surfactant molecules inhibit this association through the capping/template effect and thus act as a particle stabilizer. Other small reductants, such as ethylene glycol, ethanol and glucose,\textsuperscript{5-8} can also be decomposed and hydroxy radical will be generated, which will act as the same function of hydroxymethyl radical generated by the photolysis of TX-100. However, such small reductants can’t be acted as particle stabilizer and the large aggregates can be found from the TEM images (see Figure S7, ESI)

References

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<th>Oligo Name</th>
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<tr>
<td>Probe-DNA</td>
<td>SH-CACAAACCATTATGTGCTGCTA</td>
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<td>Probe-DNA2</td>
<td>SH-TCAACATCAGTCTGATAAGCTA</td>
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<tr>
<td>Probe-DNA3</td>
<td>SH-CCCCTATCAGATTAGCATTAA</td>
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Table S2. Recovery of miRNA-15 Added in 10% Diluted Normal Human Serum.

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<th>Added amount (fM)</th>
<th>Found amount (fM)</th>
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<th>Recovery (%)</th>
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<td>305.68, 289.15, 295.49</td>
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**Figure S1.** Schematic representation of the photochemical preparation of ultrathin gold-decorated single glass nanopore. This scheme is not drawn to scale.
Figure S2. Current–voltage curves of 5 representative nanopipettes using 100 mM KCl (pH = 7.4).
Figure S3. Current-Voltage (I-V) responses of bare nanopore (black curve), gold-modified nanopore (red curve) and probe DNA modified Au-decorated nanopore (green curve). Supporting electrolyte was 100 mM KCl (pH =7.4).
Figure S4. Fluorescence microscope images of the gold coated (A) and unmodified (Au-free) (B) single conical glass nanopores.
Figure S5. (A) TEM image of an ultrathin Au-decorated glass nanopore blocked with solution-formed big AuNPs and lumps after continuous UV irradiation for 80 min. Scale bar, 300 nm. (B-D) SEM images of the gold particle film formed on the planar glass slide were irradiated under UV for 20 min (scale bar, 1 μm), 40 min (scale bar, 1 μm), 60 min (scale bar, 2 μm) and 80 min (scale bar, 2 μm), respectively.

We also performed a control photochemical experiment using a flat glass slide instead of a tapered glass nanopipette. We can use SEM images to show that the 40-minute UV irradiation time will produce the highest quality gold film. Fig. S5 B-E is an SEM image of a gold particle film formed on a flat glass slide irradiated with UV for 20, 40, 60 and 80 minutes, respectively. As shown in Fig.
S5B, we found that Au was not fully formed within the 20-minute irradiation time. The 40-minute UV exposure time is ideal for high quality ultra-thin gold nanofilms (Fig. S5C, ESI). When the irradiation time was extended to more than 60 minutes, some of the gold nanoparticles in the solution grew and precipitated, and the Au nanoparticles were largely agglomerated at the irradiation time of 80 minutes (Fig. S5E, ESI).

As mentioned previously,⁹ the formation of Au film on nanopore surface is complicated, and the quality of film should be affected by gold salt concentration, irradiation intensity and time, and the types and concentration of reducing agents used. In our case, the good quality of Au film is formed at the irradiation time of 40 min, which may be due to the synergistic effect of factors listed above.
Figure S6. UV-vis absorptions of the reaction mixture (supernatant, diluted by 75x)
with UV irradiation, using Glu, EtOH and EG as reductant agent, respectively, recorded with different time period.

**Figure S7.** SEM images of the gold particle film formed on the planar glass slide were irradiated under UV for 40 min using Glu (scale bar, 5 μm), EtOH (scale bar, 10 μm) and EG (scale bar, 5 μm) as reductant agent (from top to bottom), respectively.
Figure S8. I-V curves of nanopore sensor to DNA sequences. (black line) addition of Probe DNA sequence, (red line), and (green line) addition of 0.4 μM DNA1, and DNA2, respectively. Supporting electrolyte was 100 mM KCl.
Figure S9. (A) I-V curves of probe-DNA2 modified Au nanopore (black line), DNA3 modified probe DNA/Au nanopore (red line), and treated with miRNA-21 (green line, 300 fM), respectively. (B) I-V curves of probe-DNA3 modified Au nanopore (blue line), DNA4 modified probe DNA/Au nanopore (yellow line) and treated with miRNA-155 (gray line, 300 fM), respectively.

Probe DNA2 and probe DNA3 are the aptamers of miRNA-21 and miRNA-155, whose sequences are perfectly matched each other. However, probe DNA2 and probe
DNA3 are not completely complementary to DNA3 and DNA4, respectively. After hybridization of probe DNA2 (or probe DNA3)-DNA3 (or DNA4), the ion current drops sharply from the ON state (Fig. S9, black curve or blue curve) to the OFF state (Fig. S9 red curve or yellow curve) measured at -1 V, which indicates that the passage of ions through the nanopore is completely blocked. miRNA-21 or miRNA-155 is fully complementary to the probe-DNA2 or probe-DNA3 immobilized on the gold surface, and the ion current is re-opened to the passage of nanopore after treatment with miRNA-21 or miRNA-155 (Fig. S9, green curve or gray curve).
Figure S10. (A) The relationship between the rectification ratio and the reaction time (ranging from 1 h to 16 h), Error bars represent the standard deviation of three different glass nanopores independent experiments. (B) The relationship between the rectification ratio and the temperature (ranging from 25 °C to 47 °C), Error bars represent the standard deviation of three different glass nanopores independent experiments.
Figure S11. Detect specific miRNA sequences. I-V curves of (black line) ds-DNA2 sequence, (orange line), (green line), (red line) and (blue line) addition of miRNA-21, miRNA-25, miRNA-221 and miRNA-155, respectively.