Nanodendritic Gold/Graphene-Based Superwettable Biosensor for Tri-Mode miRNA Sensing

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Supporting Video

SI Video 1. The graphene stability on nanodendritic gold substrate by rinsing with water and ethanol.

Materials and instruments

The oligonucleotides sequences were synthesized from Sangon Biotech (Shanghai, China). The sequences were as follows:

Probe DNA: 5'-(CH2)6-ROX- T CAC GCG AGC CGA ACG AAC AAA-Ferrocene-3'

The miRNA-375 was purchased from GenePharma

miRNA-375: 5'-UUU GUU CGU UCG GCU CGC GUG A-3'

Indium tin oxide (ITO) (resistivity of ca. 10-20 Ω/cm) glass was obtained from Asahi Glass (Japan). Ethanol (>99.8%, GR), sulfuric acid (H_2SO_4, 98%, AR), acetone (>99.5%, AR), and Rhodamine 6G were purchased from Sigma-Aldrich.

$K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and Sodium chloride were purchased from Sinopharm Chemical Reagent Co. Ltd, China. The hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Alfa Aesar and the dodecylmercaptan was purchased from J&K Chemical. All chemicals were analytical-grade reagents and were used without any further purification and prepared by dilution using ultrapure water (Milli-Q, 18.2 MN·cm).

The morphologies of dendritic gold/graphene nanostructures were characterized by a scanning electron microscope (SEM, JSM-6700F, Japan). The electrochemical detection process and deposition of nanodendritic gold were carried out by a homemade device connected with a CHI-660D electrochemical workstation (CHI instruments, shanghai, China. Contact angle images were characterized by an OCA20 system (Data-Physics, Germany). The Micro Raman Spectroscopy system (InVia-Reflex, Renishaw, England) connected to a CCD detector was used to R6G and ROX SERS detection. Before the detection, the equipment was calibrated with standard Raman signal from Si at 520 cm^{-1}.

The electrochemical performance of nanodendritic gold/graphene-based biochip

For nanodendritic gold/graphene-based biochip, we recorded the cyclic voltammetry curves with the increase of scanning rate between -0.1 and 0.4 V by two electrode system. The anodic and cathodic peak currents are nearly symmetric and the peak potential increased the linearly with scan rates (from 10 to 200 mV) with a correlation coefficient of 0.997(**SI Figure 6a**), indicating that the redox process of nanodendritic gold/graphene microwell is a surface controlled electrochemical process.

The tri-mode detection

The 10 μL droplet with 10 μM probe DNA modified Rhodamine X (ROX) as the

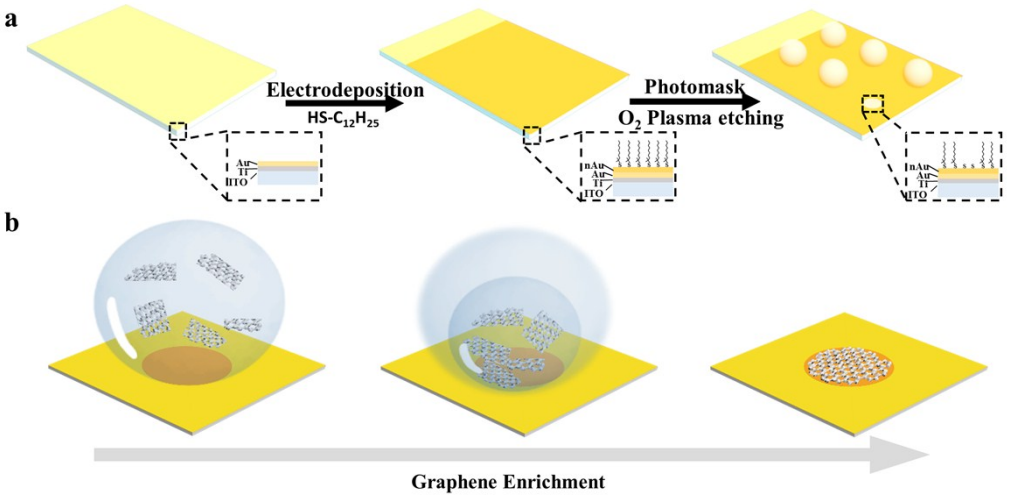
fluorescent and SERS signal molecule and ferrocene as electrochemical signal molecule was dropped in superhydrophilic microwell and incubated 2 h at room temperature for the graphene adsorption adequately. Cleaning the microwell with ultrapure water to remove unfixed probe DNA for weakening background signal. Then the desorption process was carried out in 10 μL 0.01 M phosphate buffered saline (pH = 7.4) containing miRNA-375 concentrations of 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10} and 10^{-9} M at 37 ℃ incubator for 2 h to release double-stranded nucleic acid in supernatant. The supernatant was diluted to 100 μL and transferred to the fluorescent dish for recording with the 578 nm excitation wavelength. The microwell containing the remaining probe DNA was interrogated by a 633 nm excitation laser source for SERS detection. Subsequently, the electrochemical sensing was performed by differential pulse voltammetry (voltage varies from −0.05 to +0.04 V) in the PBS buffer containing the 0.1 M NaCl. With the double-stranded nucleic acid desorption process, the biochip increases the fluorescent signal in supernatant and weakens the electrochemical and SERS signal in substrate gradually.

The specificity of nanodendritic gold/graphene-based biochip

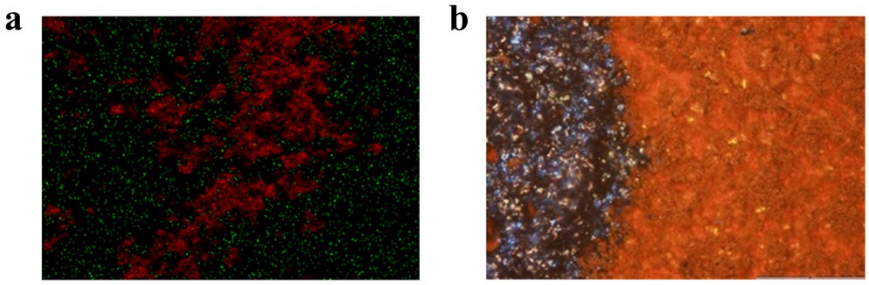
To evaluate the specificity of the nanodendritic gold/graphene-based biochip, the miRNA-141 and miRNA-21 were conducted as the control molecular for tri-mode sensing in superhydrophilic microwells as shown in SI Figure 8. For fluorescence sensing, the supernatant containing the interference miRNAs did not exhibit the obviously fluorescence intensity as low as the background and the prominent fluorescence intensity was obtained in the presence of the miRNA-375. For SERS sensing, only the miRNA-375 gave a weakened Raman response and the control groups maintained the same obvious signal as the blank group. For electrochemical sensing, the substrate had significantly smaller electrochemical signal when adding the miRNA-

375 and regardless of adding miRNA-21 and miRNA-141. Thus the results indicated high specificity of such nanodendritic gold/graphene-based biochip.

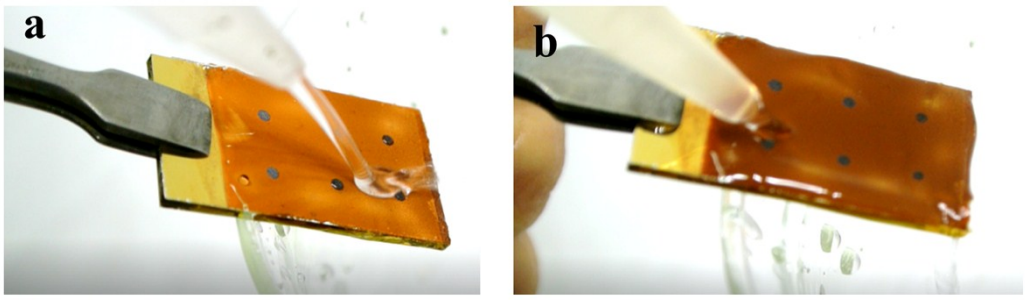
Supporting Figure



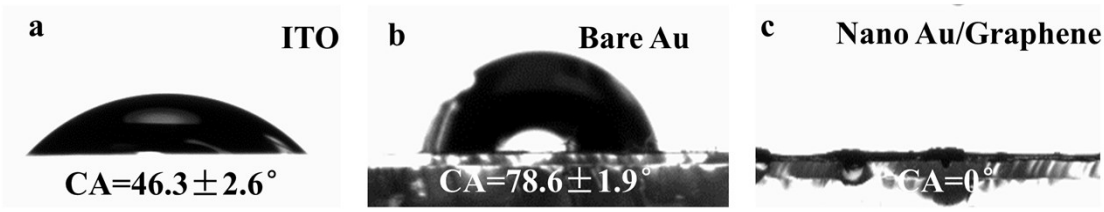
SI Fig. 1 Fabrication processes of the superwettable nanodendritic gold/graphene-based tri-mode biosensor. a) Fabrication of superwettable nanodendritic gold microchip following by sputtering the layer of Ti and Au on an ITO glass, electrodeposition, superhydrophobic modification, and O_2 plasma etching. b) Assembly processes of graphene in the superhydrophilic microwell.



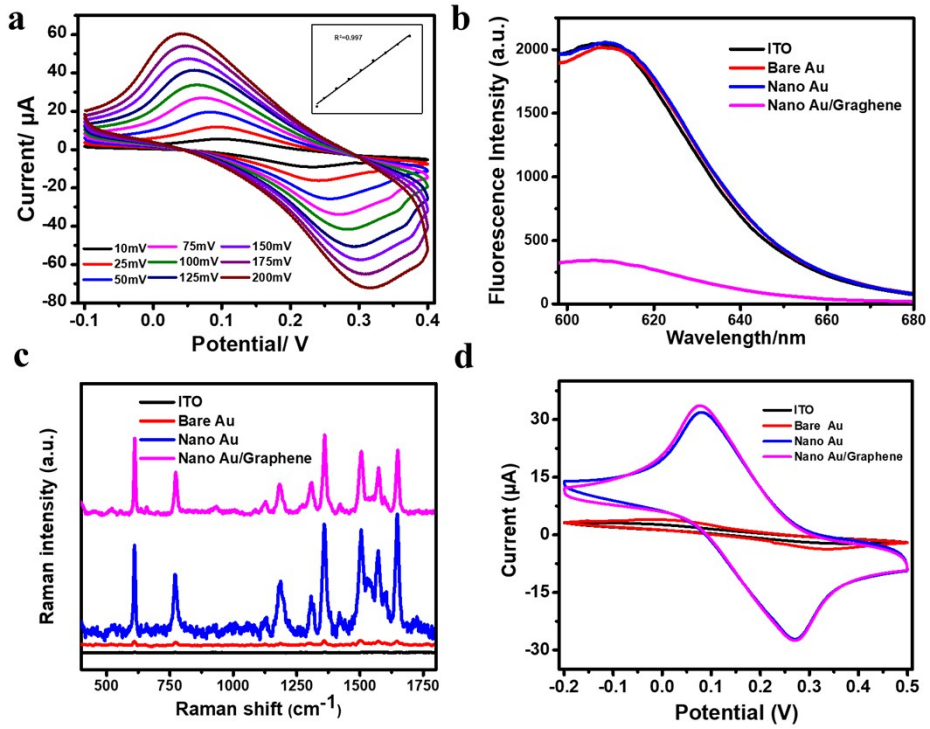
SI Fig 2. a) EDX elemental map of nanodendritic gold/graphene substrate. d) Optical microscope image of demarcation of nanodendritic gold/graphene and gold nanodendrites.



SI Fig 3. Characterization of the graphene stability on nanodendritic gold substrate by rinsing with water (a) and ethanol (b).

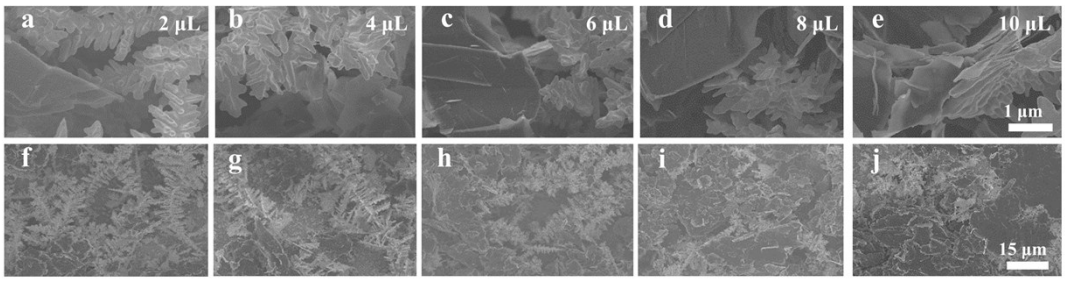


SI Fig 4. Water contact angles of bare ITO (a), bare Au (b) and Nano Au/Graphene (c) substrates.

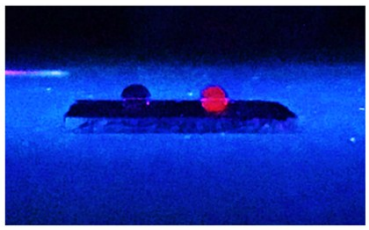


SI Fig 5. a) Cyclic voltammetry curves of nanodendritic gold/grephene electrode at increasing scan rates. Inset: The linear relationship between the peak currents and the

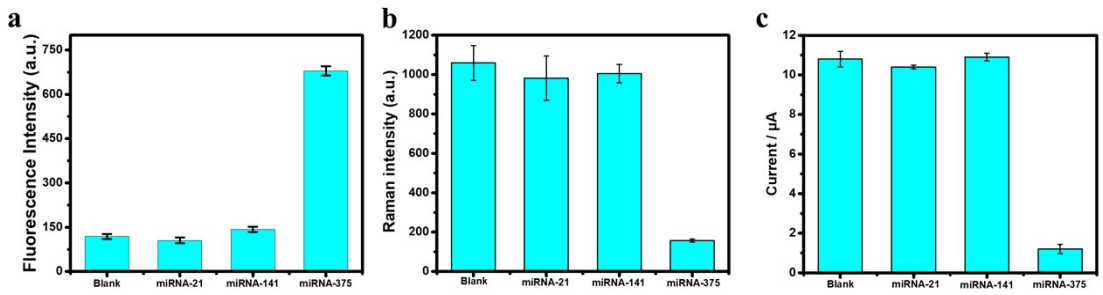
scan rates. b) Supernatant fluorescence spectra, c) SERS spectra and d) Cyclic voltammetry curves (scan rate: 100 mV/s) comparison of ITO, bare Au, nano Au and nanodendritic gold/grephene substrates.



SI Fig 6. High (a, b, c, d, e) and low (f, g, h, i, j) magnified SEM images of 2, 4, 6, 8 and 10 μL 0.5 mg/mL graphene in superhydrophilic microwell.



SI Figure 7. Visual fluorescence comparison photograph of adding droplets without miRNA-375(left) and with miRNA-375(right) in microwells.



SI Figure 8. The specificity of the superwettable microchip. a) fluorescence intensity, b) SERS intensity at 1644 cm−1 and c) electrochemical signal in the presence of buffer, miRNA-21, miRNA-141 and miRNA-375.