Superwettable Electrochemical Biosensor toward Detection of Cancer Biomarkers

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Materials and instruments

The oligonucleotides sequences were purchased from TaKaBa Biotechnology (Dalian) Co. China, as follows:

PSA aptamer: 5'-SH-$(T)_{10}$-ATTAAAGCTCGCCATCAAATAGCTGC-3'

Target miRNA-141: 5'-UAA CAC UGU CUG GUA AAG AUG G-3'

Detection probe for miRNA-141: 5′-/Fc-$(CH_2)_6$ **TTA GC**C CAT CTT TAC CAG ACA GTG TTA **GCTAA**- $(CH_2)_6$ –SH-3'

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

Detection probe for miRNA-375: 5′-/Fc-$(CH_2)_6$ **TTA GC**T CAC GCG AGC CGA ACG AAC AAA **GCTAA**- $(CH_2)_6$ –SH-3'

Saline sodium citrate (SSC), $NaClO_4$, hydroxymethylferrocene ($C_{11}H_{12}FeO$) and hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$) were obtained from Alfa Aesar. Photomask with pore sizes of 500, 1000, 1500, and 2000 μm were custom made from Beijing Zhongjingkeyi Technology Co., Ltd, China. Acetone (>99.5%, AR), dodecanethiol, ethanol (≥ 99.8%, GR), ammonia solution ($NH_3 \cdot H_2O$, 25%, AR), Sulfuric acid (H_2SO_4, 98%, AR), Phosphate buffered saline (pH = 7.4), $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (potassium ferricyanide/ferrocyanide), and potassium chloride (KCl) were purchased from Sinopharm Chemical Reagent Co. Ltd, China. Indium tin oxide (ITO) glasses (resistivity of ca. 10-20 Ω/cm) were obtained from Asahi Glass (Japan). All chemicals were analytical-grade reagents and were used without any further purification and prepared by dilution using ultrapure water (Milli-Q, 18.2 MΩ•cm).

All experiments were carried out at room temperature (25 °C). Electrochemical deposition of gold nanostructure and electrochemical detection procedures were carried out in a homemade cell connected with a CHI-660D electrochemical workstation (CHI instruments, shanghai, China). Scanning electron microscope (SEM, JSM-6700F, Japan) was used to characterize the morphologies of dendritic gold nanostructures after electrochemical deposition. Water contact angles (CA) of gold nanostructure and dodecanethiol-modified gold nanostructure were measured on an OCA20 system (Data-Physics, Germany) at ambient temperature. Average CA values were obtained by measuring at five different positions on the same sample. O_2 plasma treatment of dodecanethiol-modified gold nanostructure was carried out with a DT-03 plasma processor (Suzhou OPS Plasma Technology Co., Ltd., China).

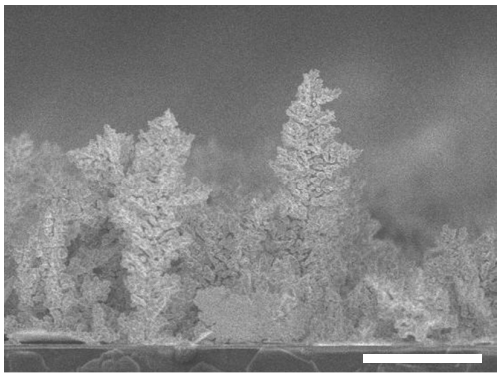
Electrochemical performance comparison of nanodendritic Au and bare Au electrode. Bare Au electrode were obtained by vapor deposition, and nanodendritic Au were obtained as previous section. The comparison of electrochemical performance of nanodendritic Au and bare Au electrode was carried out in a 140 mM NaCl solution with 0.25 M $C_{11}H_{12}FeO$. The electrical signal (DPV) of nanodendritic Au is about 10.5 times compared with that of bare Au electrode as shown in **SI Fig. 2**. Such result reveals the great sensitivity of the superwettable electrochemical biosensor.

Electrochemical detection miRNA in serum. A 5μL water droplet with 10 μM miRNA-141 detection probe was dropped into the microwell and incubated at room temperature for 1h, and incubated at room temperature for probe DNA self-assembly

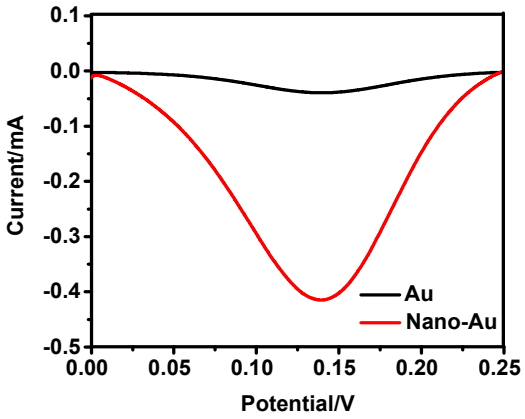
and automatically ringing on nanodendritic gold surface. Prior to measurement, the microchip was immersed in ultrapure water to remove unfixed DNA. Then a 5μL goat serum (containing various proteins, 1:10 diluted with 0.1 M PBS) contains 140 mM NaCl, 100 mM $NaClO_4$, and selective markers (blank, 100 nM miRNA-375, 100 pM PSA and 100 nM miRNA-141) were dropped in microwells for 30 min to ensure the sufficient hybridization of DNA probe. Such result reveals that the miRNA-141 detection probe can only specifically combine with miRNA-141 in the real biological sample.

Electrochemical detection PSA in serum. 5 μL PSA aptamer (20 μM) was dropped into the microwell and was incubated at room temperature for 1h. Then a 5μL goat serum (containing various proteins, 1:10 diluted with 0.1 M PBS) contains 1 mM $C_{11}H_{12}FeO$, 1 mM $MgCl_2$, 140 mM NaCl, and different selective markers (100 nM miRNA-141, 100 nM miRNA-375 and 100 pM PSA) was dropped into microwell for 30 min to ensure the full combination. Such result reveals that the immobilizing aptamer can specifically combine with PSA in the real biological sample.

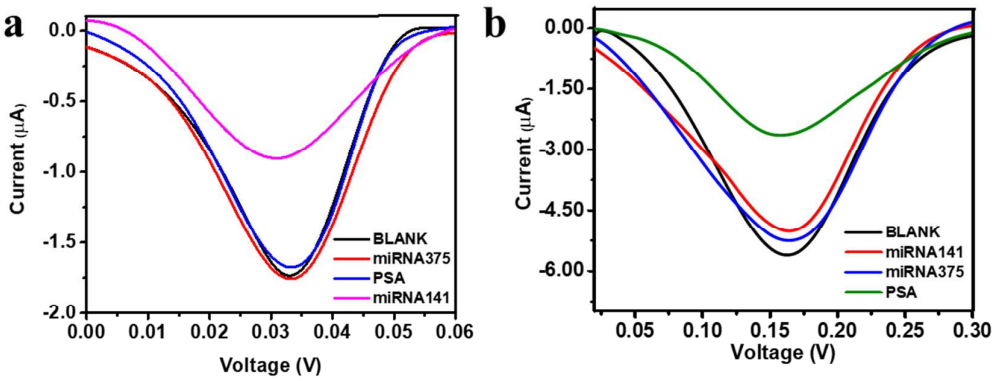
Supporting Figure



SI Fig. 1 Side view of the gold nanodendritic structure. Scale bar: 5 μm.



SI Fig. 2 Electrochemical performance comparison of nanodendritic Au and bare Au electrode. Differential pulse voltammetry (DPV) of nanodendritic Au and bare Au electrode in 140 mM NaCl solution with 0.25 M $C_{11}H_{12}FeO$. The electrical signal of nanodendritic Au is about 10.5 times compared with that of bare Au electrode. (Votage: −1.8 V; Time: 1800 s; Sensor surface area: 1 cm x 1 cm)



SI Fig. 3 The selectivity of the superwettable microchip in serum. a) Selectivity of 100 nM miRNA-141 vs blank solution, 100 nM miRNA-375 and 100 pM PSA. b) Selectivity of 100 pM PSA vs blank solution, 100 nM miRNA-375 and 100 nM miRNA-141.