Electronic Supplementary Information

Double signal amplification through functionalized nanoporous Au-Ag alloy microwire and Au nanoparticles: development of an electrochemical •OH sensor based on self-assembled layer of 6-(ferrocenyl) hexanethiol

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Electronic Supplementary Information (ESI)

Experimental Section

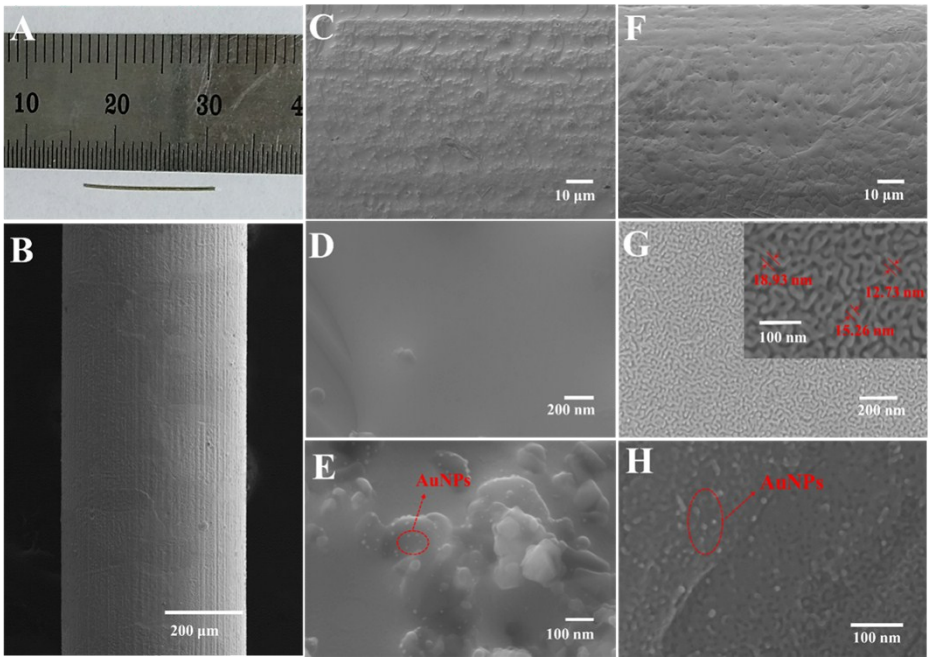
Chemicals and apparatus: Au-Ag alloy microrod (AMW) (40:60 wt %) with a diameter of 400 μm was purchased from Suzhou Coldstones Tech. Co. Ltd. (Suzhou, China). Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), 1,6-hexanedithiol (HDT), 2,2-azobis(2-methylpropionamidine) dihydrochloride (AAPH), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and 1-octadecanethiol (ODT) were supplied by Adamas Reagent, Ltd. (Shanghai, China). 6-(Ferrocenyl)hexanethiol (6-FcHT), phorbol 12-myristate 13-acetate (PMA), ascorbic acid and dimethyl sulphoxide (DMSO) were bought from Sigma-Aldrich Co. LLC. (Shanghai, China). EDTA disodium salt, sodium hypochlorite solution (11% active chlorine basis), 30% hydrogen peroxide and trisodium citrate were obtained from Sinopharm Chemical Reagent Co. Ltd. All other regents were of analytical grade and used without further purification. The solutions were prepared with double distilled water. Colloidal gold solution was prepared by the reduction of $HAuCl_4 \cdot 3H_2O$ in the presence of trisodium citrate and stored at 277 K. Hydroxyl radical (•OH) was generated by Fenton reaction (Fe(II)- EDTA: H_2O_2 = 1:1) and has the same concentration of H_2O_2. Alkyl peroxyl radical (ROO•) was generated by thermolysis of AAPH in aqueous at 37 ˚C for 30 min. Hypochlorite anion (ClO^-) was generated from the diluted commercial NaClO solution. Singlet oxygen ($^1O_2$) was generated from the reaction of NaClO solution with H_2O_2 in ethanol solution. Superoxide anion (O_2^-) was prepared by dissolving KO_2 into DMSO. PMA was dissolved in DMSO and diluted in different concentrations with complete growth medium.

All electrochemical measurements were carried out on a CHI 760E electrochemical workstation (Shanghai Chenhua, China) with a conventional three-electrode system. A prepared electrode (15 mm in length, 0.4 mm in diameter) was employed as working electrode, Ag/AgCl electrode as reference and platinum wire as counter electrode. The influences of free radical on prepared electrode were recorded within 10 min reaction time. All cyclic voltammogram (CV) and square wave voltammogram (SWV) texts were conducted in 0.1 M $HClO_4$. Absorption spectrum of colloidal gold nanoparticles (AuNPs) was obtained at room temperature with a UV–vis spectrometer (UV-2600, Shimadzu Co.). Size and size-distribution of AuNPs were measured by using a Zetasizer NanoS90 (Malvern Instruments Ltd., UK). Scanning electron microscopy (SEM, Hitachi S-4800, Tokyo, Japan) and field-emission transmission electron microscopy (TEM, FEI TecnaiG2 F20, USA) were applied for morphology and composition characterization of the prepared electrode. X-ray photoelectron spectroscopy (XPS) measurements were used to track the modification processes of FcHT with an Al Kα X-ray source (Escalab 250, Thermo Fisher Scientific, USA).

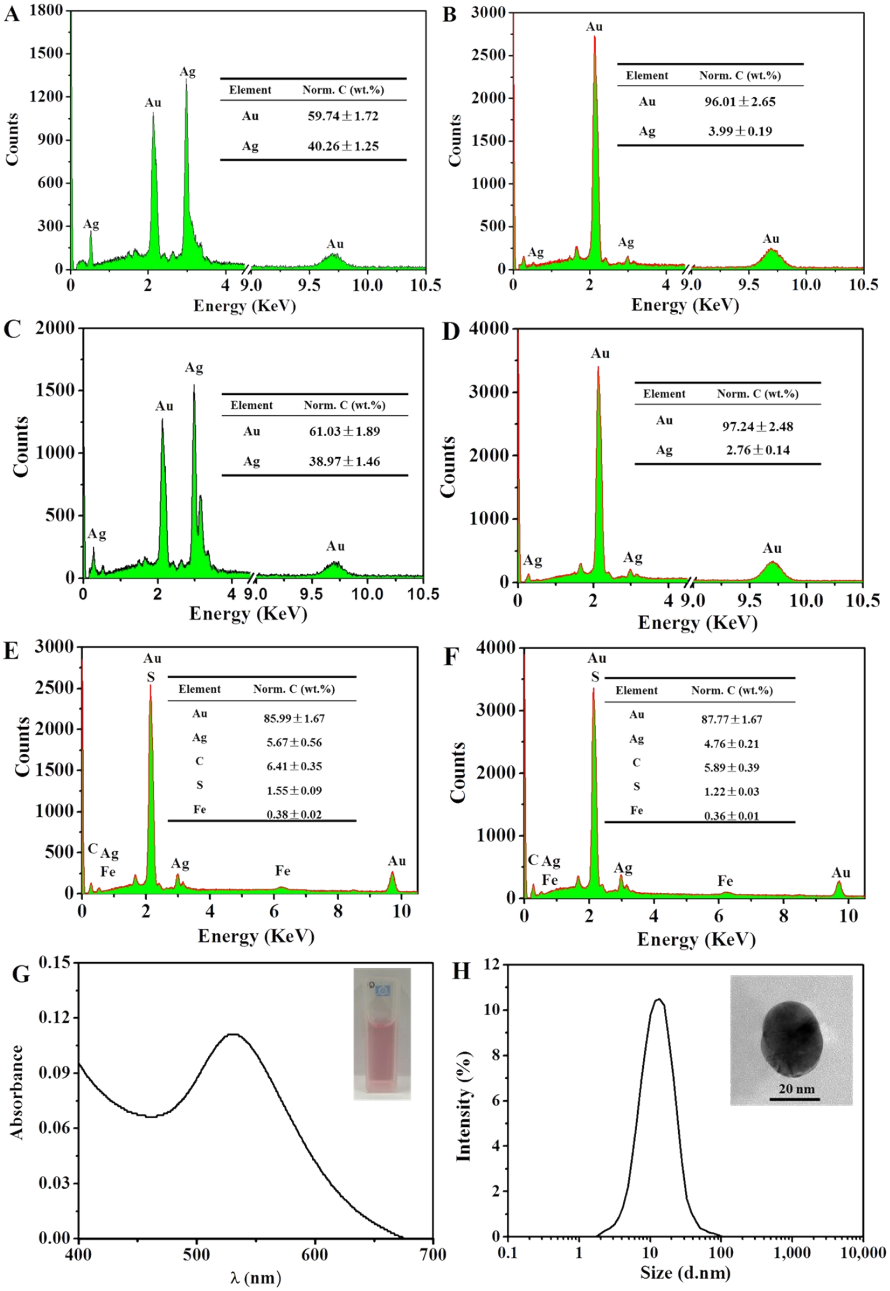
Electrode fabrication: 3D nanoporous Au-Ag alloy microwire (NPAMW) was prepared by corroding AMW in nitric acid (65vol%) for 10 min and then annealed at 900 °C for 9 h in a muffle furnace. After sequential ultrasonic treatment in acetone, ethanol and deionized water, NPAMW was dried with N_2 flow and immersed into an acetonitrile solution containing 0.5% HDT at 301 K for 3 h. Afterwards, AuNPs were assembled on the surface of HDT-decorated NPAMW by Au-S bond at 277 K for 24 h (AuNPs/NPAMW). Finally, AuNPs/NPAMW was immersed in an acetonitrile solution including 1 mM 6-FcHT and 1 mM ODT at 277 K for 18 h (6-FcHT/AuNPs/NPAMW). Before electrochemical tests the electrode was rinsed with acetonitrile and deionized water repeatedly and dried with nitrogen gas. The stepwise preparation process was illustrated in Scheme 1. For comparison, 6-FcHT/AMW and 6-FcHT/NPAMW were also prepared by the same process in the absence of corrosion and GNPs, respectively.

Cell culture and detection of •OH in HepG2 cells: HepG2 cells were cultured in complete growth medium including basic Eagle's Minimum Essential Medium (EMEM) and the following components: 10% (vol/vol) fetal bovine serum, 100 U/mL penicillin and 100 mg/L streptomycin. HepG2 cells (2×10^5 cell/mL) were seeded in a 96-well plate and incubated in a humidified incubator at 37 °C, under 5% CO_2 for 24 h. The growth medium was removed and discarded. Then HepG2 cells were treated with 500 ng mL^{-1} PMA for 4 h to stimulate cell generation and release of •OH. Finally, 6-FcHT/GNP/NPAMW was placed in the 96-well plate for 10 min to allow continuous attack from •OH. For comparison, the other groups of cells untreated with PMA and treated with 2.5 mM ascorbic acid (a recognized radical scavenger) for 1 h before stimulation with PMA were also investigated to ensure that the signals of electrode arise exclusively from the generated •OH.

Characterization of AMW, NPAMW and AuNPs/NPAMW

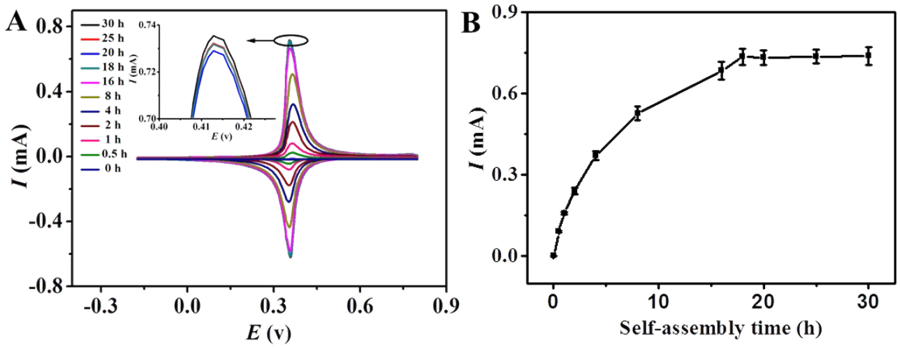


E.S.I. Figure 1. A typical optical image of AMW (A); SEM images of AMW before self-assembly of AuNPs under different magnifications (B-D) and after self-assembly of AuNPs (E); SEM images of NPAMW before self-assembly of AuNPs under different magnifications (F-G) and after self-assembly of AuNPs (H).



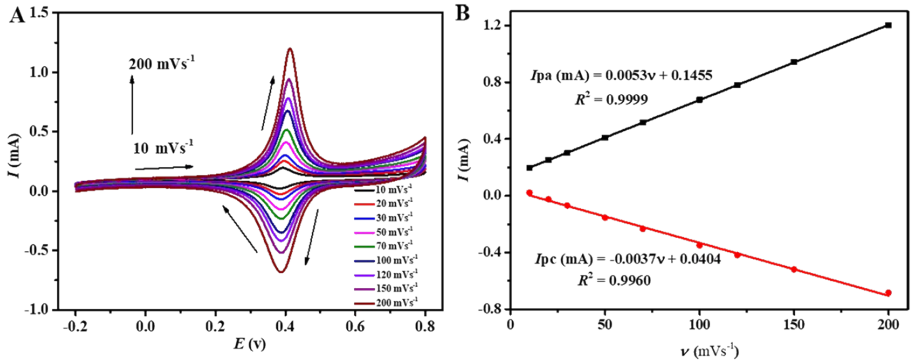
E.S.I. Figure 2. EDS spectra of AWM (A), NPAMW (B), AuNPs/AMW (C), AuNPs/NPAMW (D), FcHT/NPAMW(E) and FcHT/AuNPs/NPAMW(F); UV-Vis absorbance spectrum of AuNPs (G); Diameter distribution map of AuNPs (H). The inserts of (A), (B), (C), (D), (E) and (F) are the table of element contents; the insert of (G) is the optical photo of colloidal gold; the insert of (H) is the typical TEM image of AuNP.

Effect of self-assembly time of FcHT on FcHT/AuNPs/NPAMW



E.S.I. Figure 3. (A) Cyclic voltammograms and (B) plots of electrochemical response versus self-assembly time from 0 to 30 h by using FcHT/AuNPs/NPAMW in 0.1 M $HClO_4$. The scan rate is 100mV s^{-1} and the inset of (A) is partially amplified cyclic voltammograms.

Effect of scan rates on FcHT/AuNPs/NPAMW



E.S.I. Figure 4. Cyclic voltammograms of FcHT/AuNPs/NPAMW at the scan rate from 10 to 200 mV s^{-1}. The electrolyte contained 0.1 M $HClO_4$ (A); Relationship between peak currents and scan rate (ν) at FcHT/AuNPs/NPAMW.

E.S.I. Table 1. Comparison of the major characteristics of the reported electrochemical sensors used for •OH detection

Methods	Dynamic range (nM)	LOD (nM)	Sensitivity (nA nM^{-1})	References
PANI[a]/Si_3N_4/Si/GE[b]	200 ~ 800	200	-	[1]
FcHT[c]-ODT[d]/AuNPs[e]/APTMS[f]/ITO[g]	5 ~ 45	0.37	340	[2]
ODT/GF[h]/ITO	1.8 ~ 43.8	1.8	-	[3]
DNA/MCH[i]/GE	0.125 ~ 625	0.08	-	[4]
MBs[j]/DNA/AgNPs/GCE[k]	50 ~ 4000	10	1.48	[5]
FcHT/NPAMW[l]	0.5 ~ 80	0.26	86240	This work
FcHT/AuNPs/NPAMW	0.002 ~ 80	0.00064	174800	This work

[a] PANI: Peroxidase (HRP)-immobilized polyaniline, [b] GE: Gold electrode, [c] FcHT: 6-(Ferrocenyl)hexanethiol, [d] ODT: 1-octadecanethiol, [e] NPs: Nanoparticles, [g] ITO: Indium tin oxide, [h] GF: Gold flower, [i] MCH: 6-mercaptohexanol, [j] MBs: Magnetic beads, [k] GCE: Glassy carbon electrode, [l] NPAMW: 3D nanoporous Au–Ag alloy microwire

5. References

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