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Supporting Information

Sandwich-type electrochemical immunosensor for carcinoembryonic antigen detection based

on the cooperation of gold-vertical graphene electrode and gold@silica-methylene blue

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1. Experimental section

1.1. Materials and chemicals

CEA, CEA antibodies (Monoclonal antibody to CEA) (specificity: Ab₁(Ab202), human CEA; Ab₂ (Ab205), reacts with a protein determinant belonging to the "Gold" epitope group 5(3); Ab206, Ig G₁, human CEA), CA 199, CA 125, CA 153, and alpha-fetoprotein (AFP) were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). CEACAM5 rabbit polyclonal antibody (polyclonal-Ab) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA, 97%) and human serum specimen were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄•3H₂O) was purchased from Alfa Aesar (USA). Curcumin and vanillin were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Methylene blue (MB) was obtained from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). Citric acid was obtained from Tianjin Kewei Co., Ltd. (Tianjin, China). Rosehip oil was purchased from Weifang Naao Cosmetics Co., Ltd. (Weifang, China). SiO₂ nanoparticles were purchased from Siping Gaosida Nano Material Equipment Co., Ltd. (Siping, China). Potassium ferricyanide (K₃[Fe(CN)₆]), potassium ferrocyanide (K₄[Fe(CN)₆]), NaH₂PO₄, Na₂HPO₄, KCl, and glycine were purchased from Tianjin Guangfu FINE Chemical Research Institute (China). Sodium hydroxide (NaOH) was purchased from Tianjin Jiangtian Unified Technology Co., Ltd (China). Tris(hydroxymethyl)aminomethane (Tris-HCl) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (China). Ultrapure water (18.25 MΩcm, 24 °C) was used in all the experimental processes.

1.2. Materials characterization

Scanning electron microscope (SEM) images were obtained using ZEISS MERLIN compact field-

emission environmental SEM (Germany). Transmission electron microscope (TEM) images and energy-dispersive spectrometer (EDS) element mapping were obtained from FEI Talos F-200 TEM (USA) with Oxford INCA EDS analysis (UK). X-ray photoelectron spectra (XPS) were captured on a Thermo Scientific ESCALAB 250Xi X-ray photoelectron spectrometer (USA). X-ray powder diffraction (XRD) patterns were acquired with a Rigaku D/Max 2500/PC X-ray diffractometer (Japan). Fourier-transform infrared (FTIR) spectra were recorded by Bruker Tensor 27 (Germany). Raman spectra were obtained from LabRAM HR Evolution HORIBA Raman spectroscope with a laser wavelength of 532 nm (Japan). UV-Vis spectrophotometric measurements were conducted on a Hitachi U-3900H spectrophotometer (Japan). Zeta-potential was measured by Malvern ZetaSizer Nano ZS 90 electrophoretic light scattering system (UK).

1.3. Electrochemical measurements

All electrochemical measurements were performed on CHI660E electrochemical workstation using a three-electrode system, which consisted of an Au-VG/Ta electrode (use area: 1 cm²) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum sheet as the counter electrode. Differential pulse voltammetry (DPV) was performed using a pulse amplitude of 0.05 V, pulse width of 0.05 s, pulse period of 0.15 s, and potential range of -1 to 1 V. Electrochemical impedance spectroscopy (EIS) was performed in 0.1 M KCl containing 5 mM [Fe(CN)₆]³⁻ and 5 mM [Fe(CN)₆]⁴⁻ probe ions at the open circuit potential, with an amplitude of 5 mV and frequency range of 10^{-2} to 10^{5} Hz. All electrochemical experiments were repeated 5–9 times at room temperature. Phosphate buffered saline (PBS; pH 4-9) was prepared by compounding the solution of 0.1 M NaH₂PO₄, 0.1 M Na₂HPO₄, 0.1 M H₃PO₄, and 0.1 M NaOH.

2. The calculation of detection limit (LOD):

The LODs were calculated based on the DPV current intensity of three times as large as the noise (S/N=3). The detail calculation is shown as follows:

A standard curve between the logarithm of CEA concentration (x, pg/mL) and the DPV current of the characteristic peak (y, mA) is established. The regression equation is $y = a + b \log x$, where "a" and "b" are the intercept and slope of the equation, respectively. The LOD is calculated when y

 $= y_{blank} + 3\sigma_{, SO} LOD = 10^{\frac{y_{blank} + 3\sigma - a}{b}}, \text{ where "}y_{blank}$ " is the average current of 10 blank measurements and " σ " is the standard deviation of 10 blank measurements.

3. Characterization



Fig. S1 Potential window of Ta electrode.



Fig. S2 Multi-potential step curve.



Fig. S3 Cross-sectional SEM image of VG layer on Ta substrate.



Fig. S4 (a) TEM image of Au-VG nanosheets and corresponding EDS elemental mapping of (b) C,

(c) Au, and (d) O components.



Fig. S5 (a) Survey scans XPS spectrum as well as (b) O 1s high-resolution XPS spectrum of Au-VG electrode.



Fig. S6 SEM images of SiO₂, Au@SiO₂, and Au@SiO₂-MB powders.



Fig. S7 TEM image of Au@SiO₂-MB nanocomposite.

Zeta Potential Distribution



Fig. S8 Zeta potential distribution of SiO₂ NPs.



Fig. S9 Zeta potential distribution of Au@SiO₂ NPs.



Fig. S10 (a) XRD pattern of Au@SiO₂ carrier. (b) FTIR spectra of SiO₂, Au@SiO₂, Au@SiO₂-MB, and Ab₁-SiO₂. (c) UV-vis spectra of Au@SiO₂, Au@SiO₂-MB, Ab₁, Ab₂, and Ab₁(or Ab₂)-Au@SiO₂-MB labels.



Fig. S11 CV at (a) Gr, (b) Au-Gr, (c) Ab₁-Au-Gr, (d) BSA- Ab₁-Au-Gr, (e) CEA-BSA- Ab₁-Au-Gr,

(f) Label@Ab₁- CEA-BSA- Ab₁-Au-Gr electrodes in 0.1 M KCl containing 5 mM [Fe(CN)₆]^{3-/4-}.



Fig. S12 UV-Vis absorption spectra in a mixed suspension of 50–1000 μ M MB and 0.5 mg mL⁻¹

Au@SiO₂ carrier.



Fig. S13 DPV curves of sensors in 0.1 M PBS solution (pH 7.4). (a) Sensor with label@Ab₂. (b) Sensor with label@Ab₁. Au@SiO₂ carriers in labels are soaked in different concentrations of MB solution. CEA: 30 ng mL⁻¹.



Fig. S14 (a, b) DPV curves of Au-VG electrode in 0.1 M PBS solution with different pH values containing 50 μ M MB (A) and 600 μ M MB (B). (c) DPV curves of label@Ab₂-CEA-Ab₁-Au-VG electrode in 0.1 M PBS solution with different pH values. CEA: 30 ng mL⁻¹; carrier soaked in 600 μ M MB. (d) Variations of peak potential vs. pH value.



Fig. S15 DPV curves of sensors in 0.1 M PBS (pH 7.4). (a) Ab₁-Au-VG electrode with different Ab₁ concentrations. (b) Label@Ab₁ with different Ab₁ concentrations. Unspecified parameters: incubation times are 2 h for Ab₁-Au-VG, 30 min for CEA, 10 min for BSA, and 20 min for label, 30 ng mL⁻¹ CEA solution.



Fig. S16 DPV curves of label@Ab₂-CEA-Ab₁-Au-VG electrodes: (a) with different CEA incubation times; (b) regeneration method of sensor. Unspecified parameters: 1500 ng mL⁻¹ Ab₁ solution, 1 mg mL⁻¹ label dispersion with 1000 ng mL⁻¹ Ab, incubation times are 2 h for Ab₁, 30 min for CEA, 20 min for label, and 10 min for BSA, 30 ng mL⁻¹ CEA solution, and 0.1 M PBS (pH 7.4).



Fig. S17 DPV curves of sensor based on label@Ab₂-CEA-Ab₁-Au-VG electrodes incubated in different concentrations of CEA solution. MB soaking concentration: (a) 600 μ M, (b) 50 μ M.



Fig. S18 DPV responses of different sensors for 30 ng mL⁻¹ CEA: (a) Series of new sensors. (b) Effects of various interfering substances, such as 9.9 U mL⁻¹ CA 125, 9.96 U mL⁻¹ CA153, 10 U mL⁻¹ CA199, 20 ng mL⁻¹ AFP, 1 μ M curcumin, 1 vol% rosehip oil, 1 μ M coumarin, and 1 μ M vanillin. (c) Obtained from the sensor after storage for various time periods. (d) DPV responses of sensor for serum and mixed solutions with 5 vol% serum and various concentrations of CEA.

CEA in	Added	Measured after addition					Average	RS	Recover
5vol%	CEA	(pg/mL)					value	D	у
serum	(pg/mL							(%,	(%, n=5)
(pg/mL)							n=5)	
)									
0.075	1	1.062	1.089	1.071	1.078	1.072	1.074	0.93	99.9
(serum:	10	9.224	10.268	9.586	10.235	10.687	10.000	5.86	99.3
1.5)	100	98.148	100.127	97.918	104.842	96.918	99.591	3.17	99.5
	1000	989.542	997.822	987.243	976.728	985.112	987.289	0.77	98.7
	6000	5987.413	5857.361	5946.785	5928.728	5918.391	5927.736	0.80	98.8
	10000	10014.27	10008.82	10009.13	10007.71	10011.23	100010.23	0.03	100.1
		8	4	9	7	8	9		

 Table S1 Determination of CEA in human serum sample.