Electronic Supplementary Material (ESI) for Chemical Communications

Simultaneous electrochemical immunoassay of three liver cancer biomarkers using distinguishable redox probes as signal tags and gold nanoparticles coated carbon nanotubes as signal enhancer

Ya Li[a,1], Zhaoyang Zhong[b,1], Ruo Yuan[a,1],Yaqin Chai[a], Zhongju Song[a], Ying Zhuo[a], Huilan Su[a], Siming Liu[a], Dong Wang[b,*]

[a]Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

[b]Cancer Center, Daping Hospital and Research Institute of Surgery, Third Military Medical University, Chongqing 400042, PR China

[*] Corresponding author. Tel.: +86 23 68252277; fax: +86 23 68253172.

E-mail address: yuanruo@swu.edu.cn (R. Yuan), dongwang64@hotmail.com (D. Wang).

[1] These authors contributed equally to this work.

(Supplementary Material)

EXPERIMENTAL SECTION

Reagents and Materials. Monoclonal primary antibody alpha-fetoprotein variants (AFP-L3) and abnormal prothrombin (APT) (capture anti-AFP-L3,capture anti-APT) and tracer secondary anti-AFP-L3 and anti-APT (signal anti-AFP-L3, signal anti-APT), AFP-L3 and APT standard solutions were purchased from Leibao Fu Co. (Shanghai, China). Monoclonal primary antibody α-fetoprotein (capture anti-AFP) and tracer secondary anti-AFP (signal anti-AFP), and AFP standard solutions were obtained from Biocell Co. (ZhengZhou, China). Gold chloride tetrahydrate, sodium citrate, ferrocene (Fc-COOH), 2,2′-bipyridine-4,4′-dicarboxylic acid ($Co(bpy)_3^{3+}$), and thionine (Thi) were obtained from Sigma Chemical Co. (St. Louis., MO., USA). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDC) and N-hydroxy succinimide (NHS) were purchased from Shanghai Medpep Co. Ltd (Shanghai, China). Cobaltous chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) were purchased from Chemical Reagent Co. (Chongqing, China). Tris-HCl was purchased from Xiasi Biotechnology Co. Ltd (Beijing, China). Serum specimens provided by Daping Hospital of Third Military Medical University (Chongqing, China) were stored at 4°C in a freezer. All chemicals used were of analytical grade and were used as received without further purification. Double distilled water was used throughout all experiments. Phosphate-buffered solution (PBS, 0.1 M) with pH 7.4 were prepared with stock standard solution of Na_2HPO_4 and NaH_2PO_4. The supporting electrolyte was 0.1 M KCl.

The AFP, AFP-L3 and APT were stored at 4 ˚C, and the standard solutions were diluted with PBS to prepare a series of analyte solutions. Gold colloidal nanoparticles with mean size of 16 nm (the graph not shown) were prepared by reducing gold chloride tetrahydrate with sodium citrate at 100 ˚C for half an hour.

Apparatus. All electrochemical measurements were performed with a CHI 852C electrochemistry workstation (Shanghai CH Instruments Co., China). A three-electrode system contained a modified glassy carbon electrode (GCE, ϕ = 4 mm) as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. Transmission electron microscope (TEM, TECNAI 10, Philips Fei Co., Hillsboro, OR) was performed to characterize the morphology and size of AuNps attached CNTs.

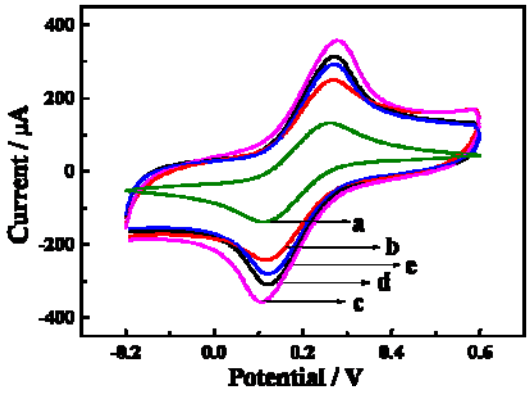
Electrochemical characterization of the electrode surface: Cyclic voltammetry (CV) was used to investigate the electrochemical characteristics of $[Fe(CN)_6]^{3-/4-}$ after each assemble step. CVs of $[Fe(CN)_6]^{3-/4-}$ on the different modified electrodes were shown in FigureS 1. The redox probe $[Fe(CN)_6]^{3-/4-}$ exhibited a reversible CV on the bare GCE (a). The peak current increased greatly after the pretreated GCE was modified with GR, owing to the increase of the electroactive surface area by the GR. The introduction of the GR also provided the conducting bridges for the electron-transfer of $[Fe(CN)_6]^{3-/4-}$ (b). After DpAu monolayer was electrodeposited on the GR-modified electrode, the peak current further increased (c), indicating that the promotion of electron transfer by DpAu. The current peak obviously decreased after PA was immobilized onto the DpAu/GR/GCE (d), which was attributed to the

fact that PA hindered the transmission of electrons to the electrode surface. When antibodies were immobilized successfully on the modified electrode, the peak current decreased (e).

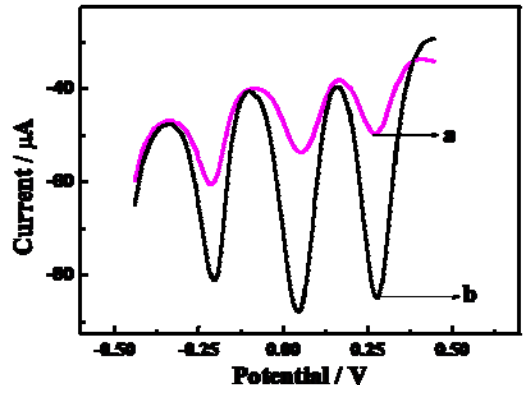
The influence of gold nanoparticles coated carbon nanotubes on the sensitivity of the assay: In order to make a comparison of different signal amplification strategies, we used "redox probe labeled antibody" (FigureS 2a) and "redox probe labeled antibody on AuNPs attached CNTs" (FigureS 2b) as the secondary antibody for sandwich-type immunoassay under the same experimental conditions, respectively. As shown in FigureS 2, the obvious increase of peak currents were observed for the SMIAs with the addition of AuNPs attached CNTs, then AuNPs attached CNTs was selected for preparating the bioconjugate. Due to the high affinity of AuNPs/CNTs nanomaterial, relatively high content of Ab_2 were immobilized onto the electrode, and the concentration of electro-active material was increased correspondingly. The increase of the responses was due to the high binding ability of the bioconjugate at the high amount of antibody in the sandwich-type immunoreactions.

Analytical performance: The method was challenged by detecting 10 human serum samples from normal individuals. The results were summarized in TableS 1. (Note: The analytes with higher original concentrations in the samples were appropriately diluted). The relative deviations of 10 human serum samples were found to vary from -7.14% to 8.45% for AFP, -7.89% to 8.50% for AFP-L3 and -7.64% to 7.65% for APT, respectively. Comparison between the experimental results obtained by the proposed immunoassay and the enzyme-linked immunosorbent assay (ELISA) was also

performed via a least-squares regression method. Good correlations were achieved after comparison with the conventional assay for AFP, AFP-L3 and APT in 10 human serum samples. Assays on serum from normal individuals clearly indicated that this method was suitable for the analysis of real samples in clinical diagnosis.



FigureS 1. CVs of different modified electrodes in pH 7.4 PBS containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) as redox probe: (a) bare GCE, (b) GR/GCE, (c) DpAu/GR/GCE, (d) PA/DpAu/GR/GCE, (e) Ab/PA/DpAu/GR/GCE.



FigureS 2. DPV responses of the the different signal tags when detecting a mixture solution of 5 ng/mL AFP, 2.7 ng mL^{-1} AFP-L3 and 3.6 ng mL^{-1}APT: (a) antibody@redox probe, (b) AuNPs/CNTs-antibody@redox probe.

sample no.	multiplexed immunoassay[a]/ng mL^{-1}			ELISA[a]/ng mL^{-1}			*relative deviation/%*		
	AFP	AFP-L3	APT	AFP	AFP-L3	APT	AFP	AFP-L3	APT
1	2.34	219.40	35.47	2.52	202.22	33.56	-7.14	8.50	5.69
2	63.24	299.24	60.98	58.92	286.77	57.14	7.33	4.35	6.72
3	28.57	106.26	5.98	27.54	100.06	5.70	3.74	5.20	4.91
4	163.54	71.98	7.84	153.78	77.16	7.42	6.35	-6.71	5.66
5	2.31	168.28	22.64	2.46	161.71	24.13	-6.10	4.06	-6.17
6	10.76	217.85	38.82	10.14	226.88	41.28	6.11	-3.98	-5.96
7	6.42	79.18	11.68	6.66	85.96	10.85	-3.60	-7.89	7.65
8	36.27	61.86	7.25	34.35	59.54	7.85	5.59	4.06	-7.64
9	31.57	632.54	114.97	29.11	589.75	109.85	8.45	7.26	4.66
10	153.52	396.48	82.93	146.66	373.09	77.71	4.68	6.27	6.72

[a] The values shown here are the average values from three measurements.

TableS 1. Experimental results comparison of two methods obtained in serum samples.