

Supporting information

Preparation of A Novel Sandwich-Type Electrochemical Immunosensor for AFP Detection Based on ATRP and Click Chemistry Technique

Xiaoqing Zhao^a, Na Wang^a, Hou Chen^{a*}, Liangjiu Bai^{a*}, Hui Xu^a, Wenxiang Wang^a,
Huawei Yang^a, Donglei Wei^a, Lixia Yang^a, Zhenping Cheng^b

^aKey Laboratory of High Performance and Functional Polymer in the Universities of Shandong Province, Collaborative Innovation Center of Shandong Province for High Performance Fibers and Their Composites; School of Chemistry and Materials Science, Ludong University, Yantai 264025, China. E-mail: chenhou@ldu.edu.cn (H. Chen), bailiangjiu@ldu.edu.cn (L. J. Bai).

^bJiangsu Key Laboratory of Advanced Functional Polymer Design and Application, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China.

Experimental Section

2.1 Reagents

2-Hydroxyethyl methacrylate (HEMA, $\geq 99\%$, J&K SCIENTIFIC LTD) was passed through a column of Al_2O_3 to remove the inhibitor and stored at $4\text{ }^\circ\text{C}$. Acrylonitrile (AN, $\geq 98\%$, Tianjin FuChen Chemical Reagents, China) was distilled and stored at $4\text{ }^\circ\text{C}$. Ethyl 2-bromoisobutyrate (EBiB, $\geq 98\%$, Aladdin Chemistry Co., Ltd), copper bromide (CuBr_2 , $\geq 98.5\%$, Tianjin DaMao Chemical Reagents Co., Ltd, China). 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA, $\geq 98\%$) was purchased from Beijing HWRK Chem. Anthraquinone-2-carboxylic acid ($\geq 99.5\%$) was obtained from Zhengzhou Alfachem. Tetrahydrofuran (THF, $\geq 99.5\%$) and ascorbic acid (V_C , $\geq 99.7\%$) were brought from Tianjin Bodi Chemical Company. Bovine serum albumin (BSA) and 2-bromoisobutyryl bromide were purchased from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and *N*-hydroxysuccinimide (EDC and NHS, $\geq 99.5\%$, Shanghai Yuanye Biological Technology Company, China), sodium azide (NaN_3 , $\geq 98\%$, ChengDu Micxy Chemical CO., Ltd, China), ammonium chloride (NH_4Cl , $\geq 99.5\%$, Sinopharm Chemical Reagent Co., Ltd, China), diethylenetriamine (DETA, $\geq 99\%$, Sinopharm Chemical Reagent Co., Ltd, China) and 2-bromoisobutyryl bromide ($\geq 98\%$, Aladdin Chemistry Co., Ltd) were all used as received. EDC/NHS, AFP, primary antibodies (Ab1) and secondary antibodies (Ab2) were all dissolved in phosphate buffered saline (10 mM, pH = 7.4). The above solutions were all prepared with ultra-pure water (18 M Ω cm). Real human serum was obtained from healthy human, which was a donation from Yuhuangding Hospital affiliated to Qingdao University. Blood samples were thoroughly centrifuged for 10 min, and the serum was stored in $4\text{ }^\circ\text{C}$ for further use. Finally, the obtained human serum was further diluted for 100 times with 10 mM PBS.

2.2 Characterization

The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) of the synthesized PHEMA and PAN were analyzed by Gel Permeation Chromatograph (GPC, Waters 1515) equipped with a refractive-index detector (Waters 2414) using HR column (7.8×300 mm). The Raman spectra of GO, GO@Br and GO@PAN were measured on Jobin Yvon Horiba spectrometer (France, Model T64000) at an excitation wavelength of $\lambda_{\max} = 514$ nm. Argon-Krypton mixed ion gas laser (MODEL 2018 RM, Make Spectra Physics, USA) was used as an excitation source. X-ray diffraction (XRD) patterns were collected using a Rigaku D/max 2500 VPC X-ray diffractometer. The FT-IR spectra measurements were performed on a Nicolet is 50 (Thermo Fisher Nicolet, United States). Fourier transform infrared spectrometer equipped with Thermo Nicolet corporation OMINIC 32 software. Energy dispersive spectrometer (EDS) was measured on Bruker XFlash 6160 under a nitrogen atmosphere. The CHI 660C electrochemical workstation (CH Instruments, Shanghai) with traditional three-electrode system was used to carried out the square wave voltammetry (SWV).

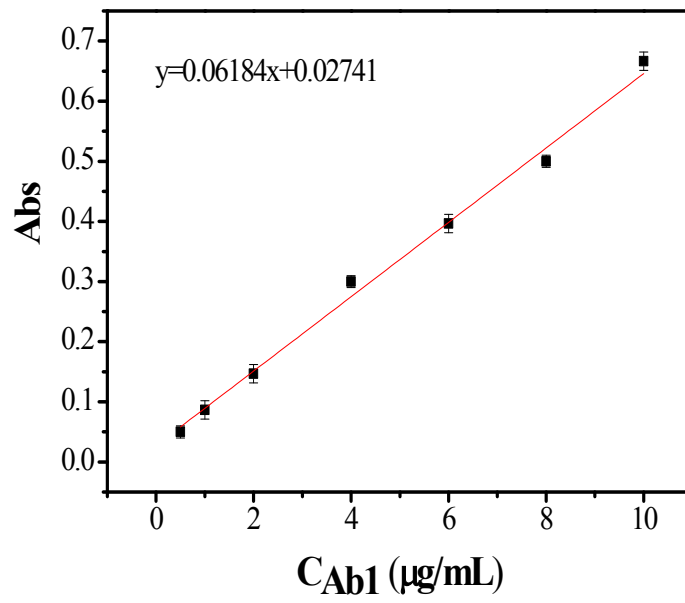


Figure S1. The visible calibration curve of standard anti-AFP.

SEM has been used to characterize the formation of sandwich-type electrochemical immunosensor. The assembly process is roughly divided into four parts, the morphology of GCE have been characterized as observed in Fig. S2. The rGO/AuNPs was coated o the surface by electrochemical deposition. From the Fig. S2a, some gold nanoparticles and graphene oxide were successfully modified to the GCE. After the antibody Ab1 was added to the surface, the GCE was covered with a layer of large protein molecules in Fig. S2b. The antigen was then added and combined with the Ab1, so the particle size of the molecules was increased as shown in Fig. S2c. Finally, Fig. S2d clearly showed that the nanoprobe was successfully loaded on the surface of GCE. The whole sandwich-type electrochemical immunosensor was proved to successfully assemble.

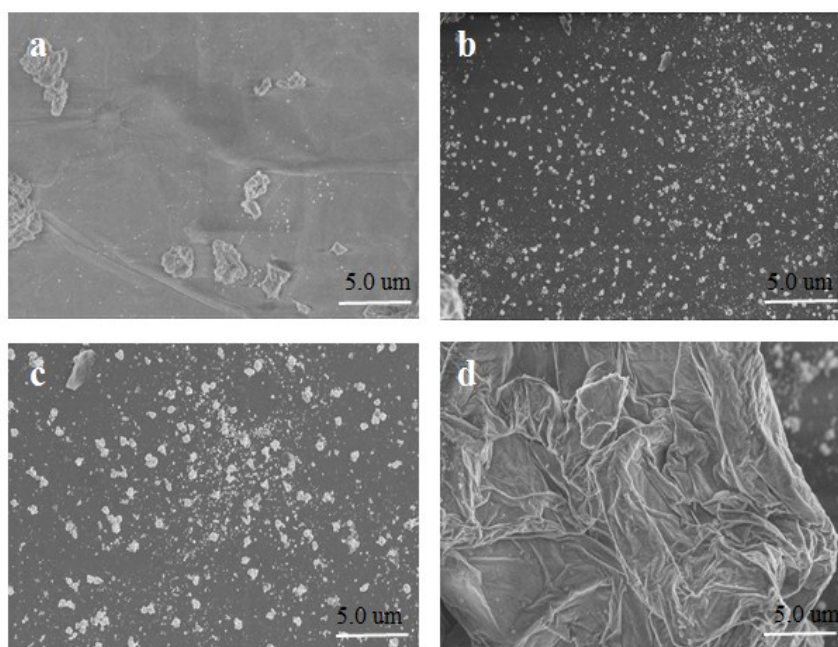


Figure S2. The SEM images of (a) rGO/AuNPs/GCE, (b) Ab1/rGO/AuNPs/GCE, (c) antigen/Ab1/rGO/AuNPs/GCE, (d) nanoprobe/antigen/Ab1/rGO/AuNPs/GCE.

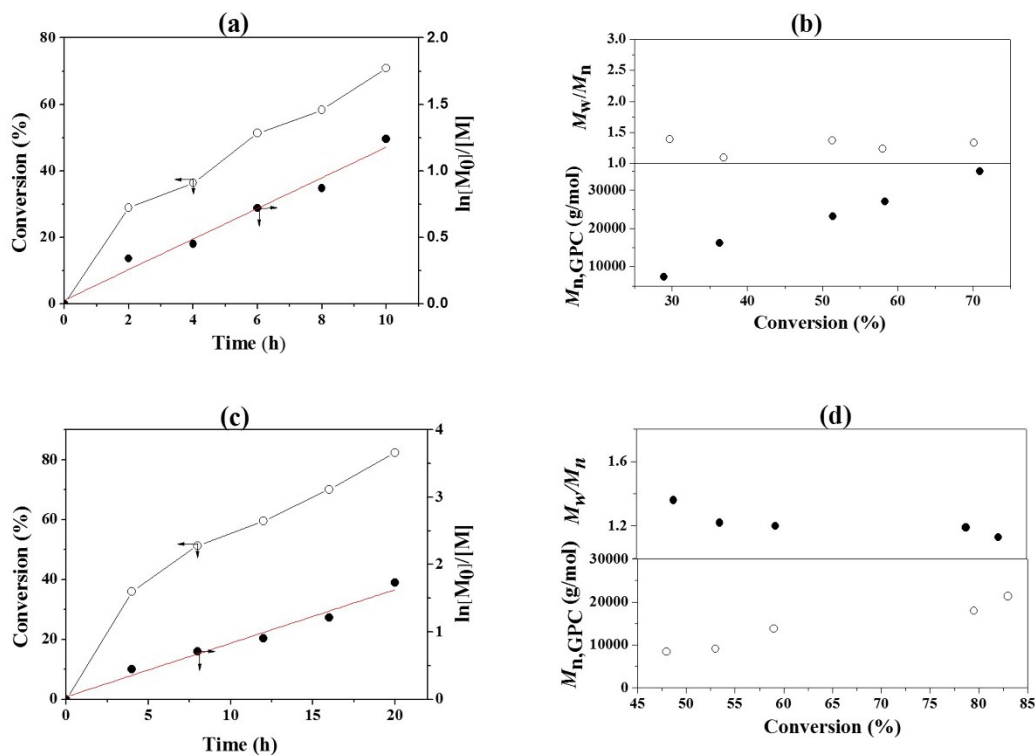


Figure S3. (a) Pseudo-first-order kinetics investigation of PAN initiated by EBiB in presence of GO@Br; (b) Number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) vs monomer conversion for the SI-ATRP of PAN; (c) Pseudo-first-order kinetics investigation of PHEMA. (d) Number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) vs monomer conversion for the ATRP of PHEMA.

Table S1. Contributions of individual chemical moieties in the high-resolution C1s spectra of GO and GO composites.

Samples	XPS (atom%)			
	C	O	Br	N
GO	62.25	28.05	0	0
PAN-g-GO	57.53	2.40	1.24	29.73
P(VT-co-HEMA)-g-GO	48.95	23.54	0.94	20.93