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A Photoelectrochemical Immunosensor for Benzo[a]pyrene Detection Amplified by Bifunctional Gold Nanoparticles

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Supplementary Information

Experiment section

Chemicals and Reagents. Titanium foil (99.8% purity, 0.127mm thick) was purchased from Aldrich (Milwaukee, WI). Ascorbic acid, H_2O_2 (30%), sodium fluoride, sodium hydrogen sulfate, sodium dihydrogen phosphate and disodium hydrogen phosphate at analytical grade were purchased from commercial sources and used as received. Benzo(a)pyrene (BaP), 1-aminopyrene, naphthalene, acenaphthene, anthracene, and horseradish peroxidase (HRP) were purchased from Sigama Aldrich. Chitosan, glutaraldehyde, tween 20, bovine serum albumin (BSA) were purchased from Amresco (U.S.). PAH mouse monoclonal antibody was purchased from Santa Cruz Biotechnology, Inc. Twice distilled water was used throughout the experiment.

BSA-PAH Synthesis. BSA-PAH was synthesized by use of the diazotizating reaction between 1-aminopyrene and BSA. Briefly, 21.72 mg 1-aminopyrene was dissolved in 2 mL pH 2.0, ice-cold HCl solution. 7.59 mg $NaNO_2$ (pH 2.0, ice-cold HCl solution) was added slowly to the above solution to start the diazotizating reaction at 4 °C for 1 h. Then, 5 mL PBS buffer (pH 7.5, 0.1 M) containing 68 mg BSA was added drop by drop to the reaction solution. Mixture pH was adjusted to 11 by 1.0 M NaOH and incubated overnight at 4 °C. BSA-PAH was obtained after dialysis the reactant in 0.9 % physiological saline for 24 h.

BGNPs Synthesis and Characterization. Au nanoparticles (Au NPs) were synthesized by the reduction of tetrachloroauric acid in trisodium citrate solution according to the previously reported method by Adriano et al.[1] Briefly, 100 mL of 0.01% $HAuCl_4$ solution was refluxed upon which 1.5 mL of 1% trisodium citrate solution was added to the solution. After the solution turned red, it was refluxed for another 30 minutes then allowed to cool. A quantity of 5 mL of the resultant solution was transfered to a small bottle, and the pH was adjusted to 9.0 by 0.1 M K_2CO_3. Surface modification was carried out by adding a mixture of 200 μL HRP (10 mg/mL) and 20 μL BSA-PAH to the above solution then stirred for 12 h at room temperature. After that, 0.8 mL 5% BSA was added and stirred for 8 h. To remove excess chemicals the solution was centrifuged at 10000 rpm for 0.5 h at 4°C. The clear supernatant was carefully removed, and the BGNPs obtained by re-suspending the precipitate in 2 mL of PBS buffer. Ultraviolet-visible (UV-vis) spectra were recorded on a CARY 300 UV-vis spectrophotometer (Varian, USA). Fourier transform infrared (FTIR) spectra were collected on Nicolet 5700 FTIR spectrometer (Thermoelectron, USA).

Immunosensor Construction. TiO_2 NTs were prepared by anodizing titanium foils at a constant potential of 15 V in an electrolyte containing 0.1 M NaF and 0.5 M $NaHSO_4$ for 3 h in a two-electrode configuration with a platinum cathode.[2] Before anodization, the titanium foil was pretreated by sonicating in 3% HF solution for several minutes, then washed in water. After anodization the TiO_2 NTs film was immediately washed with water and then dried in air. The anodized substrate was annealed at 450°C in oxygen for 3 h to convert the amorphous phase to crystalline anatase. A field-emission scanning electron microscope (FE-SEM) (JSM 6700F; JEOL, Tokyo, Japan) was used to characterize the topology of the substrate surface. The TiO_2 NTs/Ti substrate (0.5 cm × 2.5 cm) was coated by 20 μL chitosan solution dissolved in 1% acetic acid and dried at 50 °C for 4 h. After washing with 0.1 M NaOH and water, the substrate was dipped in 5% glutaraldehyde solution for 30 min, and then rinsed with water to remove the physically adsorbed glutaraldehyde. 10 μL of 0.04 mg/mL PAHs antibody was dropped onto the glutaraldehyde-activated substrate and incubated at 4 °C overnight. The as-prepared antibody-modified photoelectrochemical immunosensor was then rinsed with 3% (w/v) BSA for 1 h to block unspecific sites and washed with PBS washing buffer (pH 7.4, 0.01 M PBS, 0.05% tween 20). The Antibody-modified electrode is referenced as Ab /TiO_2 NTs.

Photoelectrochemical Measurements. Photocurrent was recorded on a CHI660C Workstation (CH Instruments, Inc. USA) in a standard three-electrode system with a Pt counter electrode and a saturated Calomel electrode (SCE) reference electrode irradiated under a 300 W Xe lamp. The incident light intensity through a UV cut-filter was 100 mW cm^{-2} measured by a radiometer (OPHIR, Newport, USA). As for BaP detection, 30 μL BGNPs solution mixed with different concentrations of BaP was applied on the sensor. After incubating at 39 °C for 3 h, the photocurrent was collected in a 0.1 M PBS (pH 7.0) solution containing 7.5 mM H_2O_2 and 0.1 M ascorbic acid. Ascorbic acid was used as oxidative quencher. The background was obtained with 30 μL PBS instead of the BGNPs solution. The sensor response to BGNPs in the absence of BaP was detected as the control (I_c). BaP was quantified based on the photocurrent changes with respect to I_c. The sensor responses to naphthalene, acenaphthene, and anthracene were also measured to help estimate the sensor selectivity.

Characterization section

A FE-SEM image of the top surface of the as-prepared TiO_2 NT arrays is shown in Fig. S1. The well-aligned TiO_2 NTs are vertically oriented from the Ti foil substrate providing a good sensor platform with excellent optical properties.

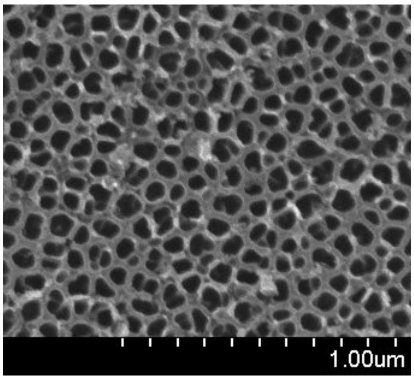

Fig. S1. SEM image of the annealed TiO_2 NTs

Fig. S2A and S2B show, respectively, UV-vis and FTIR spectra of Au NPs and BGNPs. The Au NPs exhibit an UV-vis absorption peak at 534 nm (curve a) while the BGNPs have a peak at 541 nm (curve b). As anticipated, the modification results in a red shift of 7 nm, which is consistent with the difference between Au NPs before and after modifications.[3-5] Compared with the FTIR spectra of the Au NPs, which exhibit hardly any peaks (curve c), the BGNPs have peaks at 3280 nm (ν (NH_2)), 1652 nm (ν_{as} (C=O), ν_{phenyl} (C=C)), 1531 nm (ν_{phenyl} (C=C)) and 3030 nm assigned to the benzene rings (curve d), confirming the successful coating of Au NPs with HRP and BSA-PAH.

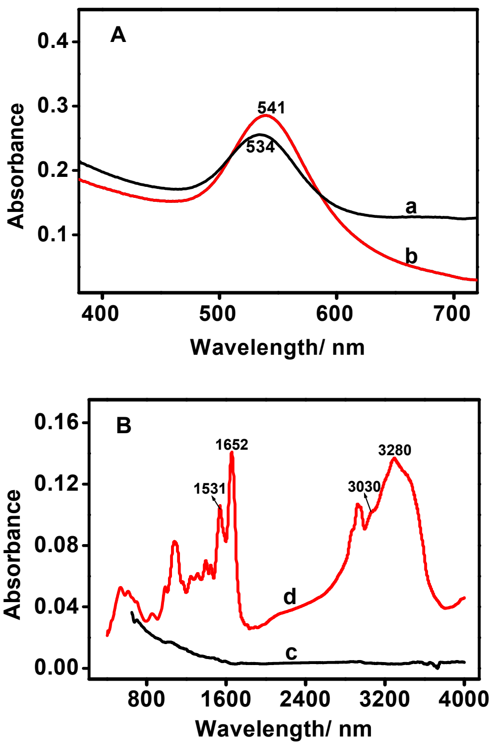

Fig. S2. UV-vis (A) and FTIR (B) spectra of Au NPs (a, c) and BGNPs (b, d).

Optimization of experimental conditions

The effect of the H_2O_2 concentration, immunoreaction temperature and incubation time on the photocurrent responses was examined to obtain the optimum conditions. Fig. S3 shows the H_2O_2 concentration-dependent photocurrent amplitude, with the greatest value observed at 7.5 mM H_2O_2. The possible reason is that too little H_2O_2 would not cause visible amplification of the photocurrent, while too much H_2O_2 might directly react with ascorbic acid. Considering the immunoreaction, the region of 25-55°C was chosen to investigate the effect of reaction temperature, with 39°C found the optimal condition for performance (Fig. S4A). Fig. S4B exhibits the effect of incubation time, with 3 h giving the greatest response.

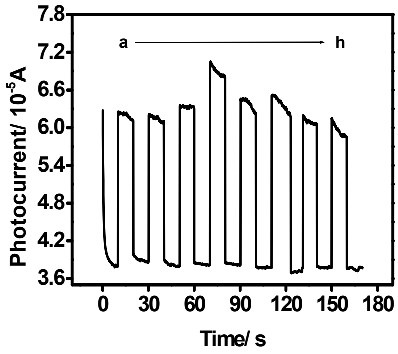

Fig. S3. Dependence of the photocurrent intensity of the Ab/TiO_2 NTs working electrode on the concentration of H_2O_2 in 0.1 M PBS (pH 7.4) containing BGNPs and 0.1 M ascorbic acid. From a to h the concentration of H_2O_2 (mM) is: 0, 1.25, 3.75, 7.5, 10, 12.5, 15, 20.

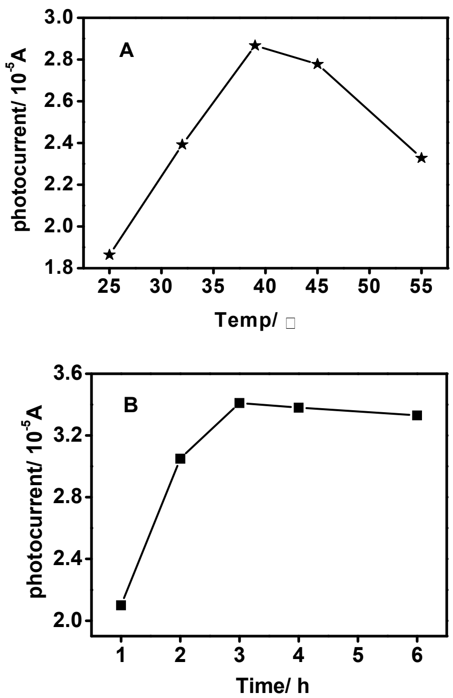

Fig. S4. The effect of (A) temperature and (B) incubation time on the photocurrent intensity of Ab/TiO_2 NTs electrode in 0.1 M PBS (pH 7.4) containing BGNP, 0.1 M ascorbic acid, and 7.5 mM H_2O_2.

The recovery rates

The recovery rates were investigated by analyzing water samples spiked with BaP in the concentrations of 10.5 pM, 31.5 pM and 105 pM. Recovery rate is defined as the ratio of the added amount detected to the actually added amount, i.e R=(m_a-m_b)/m, where m_a/m_b the detected value after/before addition of the target, and m the added amount of target. Table S1 lists the recovery rates.

Table S1. Real sample detection and Recoveries

sample	Added (pM)	Detected (pM)	Recovery (%)	RSD (%)
River water	0	14.2		
	10.5	27.1	123	6.12
	31.5	44.7	96.8	3.89
	105	108.9	90.2	5.91
Tap water	0	Nd[a]		
	10.5	11.1	106	5.15
	31.5	32.5	103	2.66
	105	101.3	96.5	4.73

[a] Nd: not detected

Notes and references

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