

Electronic Supplementary Information (ESI) for Chemical Communications

**Picomolar level profiling of the methylation status of p53
tumor suppressor gene by label-free electrochemical
biosensor**

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1. Experimental details

1.1. Chemicals and reagents

The thiolated peptide nucleic acid (PNA) capture probe with a sequence of 5'-TC ATC CAA ATA CTC CAC ACG CAA ATT TCC TTC CAC TCG-3' was purchased from Panagene (Korea). The p53 tumor suppressor genes with different methylation statuses were obtained from Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. (Shanghai, China). Their base sequences are as follows: 5'-CGA GTG GAA GGA AAT TTG CGT GTG GAG TAT TTG GAT GA-3'; 5'-mCGA GTG GAA GGA AAT TTG CGT GTG GAG TAT TTG GAT GA-3'; 5'-CGA GTG GAA GGA AAT TTG mCGT GTG GAG TAT TTG GAT GA-3'; 5'-mCGA GTG GAA GGA AAT TTG mCGT GTG GAG TAT TTG GAT GA-3'. H₂PO₄ and choline (Ch) were purchased from Shanghai Chemical Co. Ltd. (Shanghai, China). They were used as received without further purification. Phosphate buffer solutions (PBS, 0.1 M) of different pH were prepared by mixing stock solutions of 0.1 M KH₂PO₄ and Na₂HPO₄, and adjusted by 0.1 M H₃PO₄ or NaOH (Beijing Chemical Reagent Company, Beijing, China).

All other chemicals not mentioned here were of analytical reagent grade. Aqueous solutions were prepared with doubly distilled water at ambient temperature. High purity nitrogen was used for deaeration of the prepared aqueous solutions.

1.2. Apparatus and measurements

Electrochemical experiments including cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were carried out on CHI 760C electrochemical workstation (Chenhua, Shanghai, China). A conventional three-electrode electrochemical system was used for all electrochemical experiments, which consisted of a working electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode. A glassy carbon disk electrode (GCE) was used as the basal working electrode. The electrochemical solutions were thoroughly deoxygenated by N₂ for 15 min before sampling and N₂ atmosphere was maintained throughout the experiments.

Field emission scanning electron microscope (FE-SEM) image was obtained on JSM-6700F field emission scanning electron microanalyzer (JEOL, Japan).

1.3. Electrode preparation and modification

Prior to use, GCE was carefully polished with 1.0, 0.3 and 0.05 μm alumina powder sequentially and then washed ultrasonically in doubly distilled water and ethanol for 10 min, respectively. The Ch monolayer modified GCE (Ch/GCE) was prepared by immersing the GCE in 0.1 M pH 7.0 PBS containing 2.0 mM Ch and scanning between -1.70 and 1.80 V for 6 cycles under the scan rate of 25 mV s⁻¹. The obtained Ch/GCE was rinsed with distilled water and sonicated for 10 min to remove the physically adsorbed materials. The construction of Au nanoparticles on the Ch/GCE was carried out by CV in 0.1 M pH 7.0 PBS containing 0.25 mg mL⁻¹ HAuCl₄ with a potential scanning from 0.20 to -1.0 V at 50 mV s⁻¹ for 12 cycles. The

resulting electrode was denoted as nano-Au/Ch/GCE.

The immobilization of PNA capture probe on the nano-Au/Ch/GCE was prepared by immersing the nano-Au/Ch/GCE in 0.1 M pH 7.0 PBS containing 20 μ M thiolated PNA probe for 4 h at room temperature, and then rinsed successively with PBS and doubly distilled water. The obtained electrode was described as PNA/nano-Au/Ch/GCE.

The hybridizations between the immobilized PNA probes and p53 tumor suppressor genes with different methylation statuses were performed by immersing the PNA/nano-Au/Ch/GCE in 0.1 M pH 7.0 PBS containing 1.0 μ M pretreated p53 tumor suppressor genes for 1 h at 52 °C. After that, the resulting electrodes were washed successively with PBS and distilled water, and then dried with nitrogen steam.

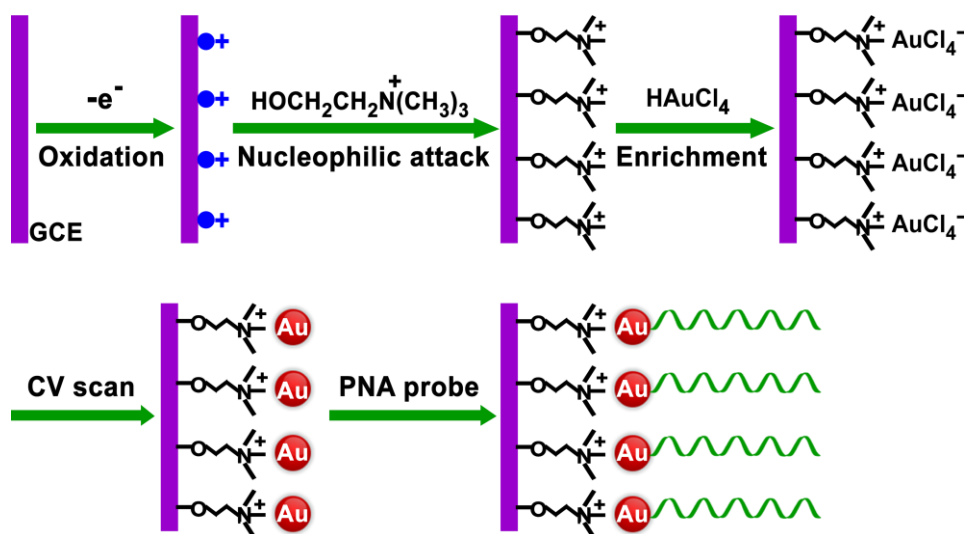
1.4. Bisulfite pretreatment of p53 tumor suppressor genes

The p53 tumor suppressor genes were treated with bisulfite according to the literature method with minor modifications.^{1,2} In brief, the p53 tumor suppressor genes were firstly denatured in 0.3 M NaOH for 30 min at 37 °C. Then, the bisulfite reaction was carried out in 3.2 M sodium bisulfite containing 0.5 mM hydroquinone for 16 h at 55 °C. The bisulfite-modified p53 tumor suppressor genes were desalted with the Wizard DNA Clean-up System (A7280, Promega, Madison, WI, USA) and desulfurated in 0.3 M NaOH for 15 min at 37 °C, neutralized by ammonium acetate, alcohol precipitated, dried, and then dissolved in 0.1 M pH 7.0 PBS. After bisulfite processing, all unmethylated cytosine residues were converted to uracil, whereas the

methylated ones remained unchanged.

2. Supplementary scheme

The construction mechanism of the proposed biosensor was shown in Scheme S1. The bare GCE was oxidized to generate cationic radicals on the surface. The Ch was then covalently linked to the GCE surface through the nucleophilic attack reaction between cation radical and the hydroxyl of Ch. As a consequence, a stable and positively charged Ch monolayer was obtained, which made the AuCl_4^- in HAuCl_4 solution easily approach to the surface of Ch/GCE through static gravitation. The Au nanoparticles were then synthesized by the use of in situ electrodeposition method. The obtained Au nanoparticles were assembled to form a flowerlike structure, which could provide a suitable supporting substrate and favorable local microenvironment for the immobilization of PNA capture probe.



Scheme S1 The construction mechanism of the proposed biosensor.

3. Reproducibility of the method

For evaluating the precision of this method, a series of repetitive voltammetric measurements were carried out with the same biosensor. The relative standard deviation (R.S.D.) for ten successive determinations of 1.0 μM methylated p53 tumor suppressor gene was 3.7%, demonstrating an excellent detecting reproducibility. Moreover, the fabrication reproducibility was also estimated by detecting 1.0 μM methylated p53 tumor suppressor gene in duplicate with ten biosensors prepared in the same manner independently. The R.S.D. was 4.9%. Therefore, the reproducibility of the proposed method was good enough for clinical detection.

4. Interference investigation

The investigation of possible interference for the detection of 1.0 μM methylated p53 tumor suppressor gene was performed by adding various foreign species into the proposed biosensing system. It was found that common metal ions and acid radical ions in physiological fluids exhibited almost no interference on the determination in a 500-fold concentration, such as Ca^{2+} , K^+ , Na^+ , Zn^{2+} , Fe^{3+} , Mg^{2+} , Ba^{2+} , CO_3^{2-} , Cl^- , Ac^- , NO_3^- and PO_4^{3-} . The influences of other biological molecules which may exist in actual samples were also examined, such as protein, glucose and neurotransmitter. The results revealed that the current variation of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ indicator was less than 5% of the original current signal after the addition of the following compounds: serum

albumin (100), hemoglobin (50), myoglobin (50), thrombin (50) and glucose (100), where the data in the brackets were the concentration ratios. Although norepinephrine, dopamine and serotonin could produce oxidation currents at 0.15, 0.22, 0.38 V, respectively, their peak potentials were far from that of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ indicator (-0.21 V), indicating that they did not interfere with the determination in a 20-fold concentration. With regard to the interference behavior of detergent, anionic surfactant of sodium dodecyl benzene sulfonate (SDBS) and cationic surfactant of cetyltrimethyl ammonium bromide (CTAB) were studied. The results demonstrated that no significant interference was observed for SDBS in a 50-fold concentration (signal change < 5%), while CTAB exhibited serious interference in a 20-fold concentration (signal change > 10%). The interference of CTAB was attributed to the fact that cationic surfactant could be adsorbed onto the phosphate backbone of sensing interface through electrostatic interaction, which occupied the binding site for the loading of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ indicator. Accordingly, the use of cationic surfactant should be avoided in the sample preparation step. Based on the above results, we may conclude that the proposed biosensing system is promising for analysis of real samples without separation procedure.

References

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