Electrochemiluminescence signal amplification combining with conformation-switched hairpin DNA probe for determining methylation level and position in the Hsp53 tumor suppressor gene

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Experimental details

1. Materials and reagents.

The DNA capture probe and the Hsp53 tumor suppressor genes with different methylation statuses were purchased from Shanghai Sangon Biological Engineering Technology and Services Co.Ltd. (Shanghai, China). HAuCl₄·4H₂O was purchased from Shanghai Civi Chemical Technology Co. Ltd. (Shanghai, China). Bovine serum albumin (BSA), tri(2-carboxyethyl) phosphine hydrochloride (TCEP), (EDC) N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. 1-methylimidazol, 3-mercaptopropionic acid (MPA) and 1-Methylimidazole were purchased from J&K Scientific Ltd (Beijing, China). Na₂S and Cd(NO₃)₂.4H₂O were provided by Aladdin.

Phosphate buffer solutions (PBS, 0.1 M, pH 8.5) containing $K_2S_2O_8$ (0.05 M) were prepared by mixing stock solutions of 0.1 M K_2 HPO₄ and KH₂PO₄. Tris-HCl buffer (0.1 M) containing 0.1 M NaCl (pH 7.4) was employed for hybridization and preparation of DNA stock solutions.

All other chemicals were of analytical reagent grade. Aqueous solutions were prepared with doubly distilled water at ambient temperature.

2. Apparatus.

Transmission electron microscopy was performed with a Hitachi H-7650 microscope with an accelerating voltage of 120 kV. The UV-vis absorption spectra were obtained on a Cary 5000 UV-vis-NIR spectrophotometer (Varian, USA). The electrochemical and ECL emission measurements were conducted on an MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an, China) at room temperature. The scan rate of the cathodic potential was 0.1 V s⁻¹, and the voltage of the PMT was set at -500 V in the process of detection. All experiments were carried out with a conventional three-electrode system. The working electrode

was glassy carbon electrode (GCE), Pt wire and the saturated calomel electrode (SCE) served as the counter and reference electrodes, respectively.

3. Synthesis of Au NPs.

Au NPs were prepared through the reduction of HAuCl₄ by sodium borohydride (NaBH₄) according to the reported methods.¹ Briefly, ice cold 0.1 M NaBH₄ (0.6 mL) was added to 20 mL of aqueous solution containing 2.5×10^4 M HAuCl₄ under stirring. The solution immediately turned to a wine-red color, indicating the formation of Au NPs, and was kept stirring in an ice bath for 10 min. Then, the solution reacted at room temperature for another 3 h to resolve the surplus NaBH₄. The TEM image of Figure S1 shows that the average size of synthesized Au NPs is about 5 nm. The prepared Au NPs were stored at 4 °C for further use.



Figure S1. TEM image of Au NPs.

4. Synthesis of CdS NCs.

Five nanometer CdS NCs were synthesized as in the literature with light modification.² Briefly, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) was dissolved in 30 mL of distilled water and heated to 70 °C under stirring. Then, the mixture was injected into a freshly prepared solution of Na₂S (0.5960 g) in 30 mL of ultrapure water. Instantly, orange-yellow solution was obtained. The solution was held at 70 °C for 3 h with continuous refluxing. The final reaction precipitates were centrifugated and washed thoroughly with absolute ethanol three times and ultrapure water two times. Then, the obtained precipitate was redispersed into water for centrifugation to collect the upper

yellow solution of CdS NCs. The average size of synthesized CdS NCs is about 5 nm, as indicated by the UV-Vis spectrum and the TEM image of CdS NCs (Figure S2). The final solution was rather stable for 2 months when stored in a refrigerator at 4 °C.



Figure S2. UV-vis absorption spectrum (a) and TEM image (b) of CdS NCs.

5. Preparation of Au NPs/ DNA conjugates

In order to reduce disulfide bond, 10 μ M probe DNA hairpin (S1) solution was activated with 10 mM TCEP for 1 h. Then 500 μ L Au NPs solution which contains 0.1 M NaCl was added to the solution and stored at 4 °C for 3 hours to gain the Au-S bonding. After that, 2% BSA was used to block the nonspecific active binding sites of the Au NPs.

6. Preparation of ECL biosensor

The fabrication procedure of the ECL biosensor is illustrated in scheme 1. The GCE(3 mm in diameter) was pretreated by polishing its surface with sand papers of 800#, 1500# and 5000# before further modification. While it polished to be mirror smoothness, thoroughly rinsed with water. Then sonicating the GCE in ethanol and distilled water in turn. 10 µL of CdS NCs solution were dropped onto the surface of pretreated GCE and evaporated in air at room temperature. And the CdS NCs film was achieved.

In order to connect the probe DNA with the surface of CdS film, the CdS NCs modified GCE was dipped in 3 mM MPA(contains 0.1M NaCl and 0.1M tris-HCl) for 5 h at 4 °C. After thoroughly rinsed with 0.1 M tris-HCl buffer (pH 7.4), the electrode was immersed in 1.0 mL 0.1 M 1-methylimidazol aqueous solution containing 20 mg EDC and 10 mg NHS for 2 h at 4 °C to activate the carboxylic acid group. Subsequently, the amino-containing DNA could immobilized onto the surface of CdS film via immersing the electrode in the prepared Au NPs/DNA hairpin conjugates solution for 14 h at 4 °C. After each step, the electrode surface was rinsed with 0.1 M tris-HCl buffer to remove surplus absorbed species. At last, the AuNPs/DNA/CdS NCs/GCE was obtained.

7. Analytical procedure

The Hsp53 tumor suppressor genes with different methylation status were treated with bisulfite according to the literature method.³ After bisulfite processing, all the unmethylated cytosine residues were converted to uracil, and the methylated ones remained unchanged.

The hybridizations between the immobilized DNA probes and target DNA were performed by immersing the modified electrode in 0.1 M NaCl and 0.1 M tris-HCl buffer (pH 7.4) containing various concentrations of pretreated Hsp53 genes for 1.5 h at 37 °C. Before and after the incubation, the ECL responses of the electrode were both recorded in 0.1 M PBS (pH 8.5) containing 0.05 M $K_2S_2O_8$ as a coreactant.

References

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