Supporting Information

A novel electrochemiluminescence emitter of europium hydroxide nanorods and its application in bioanalysis

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Materials.

Labeled DNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of these three oligonucleotides employed are given below:

The 5'-amino-modified aptamer- DNA for thromin probe (aptamer):

5'-NH_2-$(CH_2)_6$-GTAGCCGTGG*TAGGGCAGGTTGGGGTGACT* -3'

The 5'- amino -modified part complementary DNA (ssDNA):

5'-NH_2-$(CH_2)_6$-GTGTGT*AGTCACCCCAACCTGCCC* -3'

The 5'- amino -modified dilute DNA (bbcDNA):

5'-NH_2-(CH2)6-GCGCGAACCGTATA -3'

Thrombin, bovine serum albumin (BSA), hemoglobin (Hb), lysozyme (Lyso), 3-aminopropyl-triethoxysilane (APS), 1,4-diisothiocyanatobenzene (98%; PDITC), tri(2-carboxyethyl) phosphine hydrochloride (TCEP), and 3-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Tris□HCl buffer (0.1 M) containing 0.1 M NaCl and 5 mM $MgCl_2$ (pH 7.4; after abbreviated as Tris buffer) was employed for hybridization and preparation of DNA stock solutions. 0.1 M phosphate buffer solution (PBS, pH 7.4) containing 0.05 M $K_2S_2O_8$ was used for ECL detection. All other reagents were of analytical grade and used as received. Millipore ultrapure water (resistivity g18.2 M Ω cm) was used throughout the experiment.

Apparatus.

The ECL emission measurements were conducted on a model MPI-E electrochemiluminescence analyzer (Xi' An Remax Electronic Science and Technology Co. Ltd., Xi' An, China) at room

temperature, All ECL detection were recorded in 0.1 M PBS (pH 7.4) containing 0.05 M $K_2S_2O_8$ from 0V to -2.20V with -600 V PMT (photo-multiplier tube). Scan rate of 100 mV s^{-1}. All experiments were carried out with a conventional three-electrode system. The working electrode was a 3 mm diameter GCE; Pt wire and SCE electrode served as the counter and reference electrodes, respectively. Transmission electron microscopy (SEM) was performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage. Transmission electron microscopy (TEM) measurements have been performed on JEOL JEM-2010 (200 kV). High resolution TEM (HRTEM) measurements have been performed on FEI Tecnai F20.

Preparation of $Eu(OH)_3$ nanorods

The EHNs were synthesized as in the literature with some modification.[1] EHNs were synthesized by using hydrothermal method through the interaction of aqueous europium(III) nitrate solution and Na_2S at atmospheric pressure in an open reflux system. $Eu(NO_3)_3 \bullet 6H_2O$ (0.4666g) was dissolved in 60 mL of ultrapure water and heated to 120℃ under stirring, then, the mixture was injected into a freshly prepared solution of Na_2S (0.6005 g) in 60 mL of ultrapure water. The solution was held at 120℃ for 3 h with continuous refluxing. The final reaction precipitates were centrifugated and washed thoroughly with absolute ethanol three times and ultrapure water three times. Then, the obtained precipitate was redistributed into water for centrifugation to collect the solution of EHNs.

Preparation of EHNs/GCE Film.

The GCE was pretreated before modification by polishing its surface with successively finer grade sand papers and then polished to a mirror smoothness with aqueous slurries of alumina powders (average particle diameters: 0.3 and 0.05 μm Al_2O_3) on a polishing silk. The GCE was thoroughly rinsed with water and then sonicated in ethanol and ultrapure water in turn. The EHNs film was achieved by dropping 10 μL of EHNs solution onto the pretreated surface of GCE and evaporated in air at room temperature. The EHNs modified GCE was stored in PBS buffer (pH=7.4) for characterization and further modification.

Fabrication of CdTe/ MNPs.

CdTe/MNPs nanocomposites were synthesized using the method reported previously.[2] Briefly, 0.069g of $CdCl_2 \bullet 2.5H_2O$ was dissolved in 25 mL of water, and 55 μL of MPA was added followed by deaeration with N_2 for 30 min. Next, oxygen-free NaHTe solution, which was freshly prepared from 0.016 g tellurium powder and 0.3 g $NaBH_4$ in 25 mL of water at 60℃, was injected into the above solution under vigorous stirring. Herein the molar ratio of Cd^{2+}/MPA/HTe^- was fixed at

1:2:0.41. The solution was then refluxed at 100℃ for 3h. The reaction mixture was purified by precipitation in absolute ethanol. Finally, the desired CdTe NCs were obtained. For the activation of carboxylic acid group on the surface of CdTe NCs, 1.0 mg CdTe NCs was dispersed in 1.0 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 25 mg EDC and 12 mg NHS and activated for 1.5 h at room temperature. The activated CdTe NCs were separated by centrifugation and washed with water and 0.01M PBS buffer (pH 7.4) alternatively for several times followed by redispersion in 1.0 mL 0.01 M PBS buffer (pH 7.4). The activated CdTe NCs were obtained

The prepared CdTe NCs were covalently bound to the synthesized amino-modified Fe_3O_4 nanoparticles by using EDC as a crosslinker in phosphate buffer solution (pH 7.4). 1.0 mg. mL^{-1} of CdTe NCs, 20 mg mL^{-1} EDC and 10 mg mL^{-1} NHS were added to a beaker one by one. The solution was reacted for 1.5 h at room temperature. Then 10 μL of 5.0 mg mL^{-1} amino-modified Fe_3O_4 was added dropwise in the solution. The solution was incubated for 2 h at room temperature to form the nanocomposites. The CdTe/Fe_3O_4 nanocomposites were then separated from the solution by using magnet force and washed several times with ethanol. The obtained CdTe/MNPs conjugates were denoted CdTe/MNPs.

Preparation of ssDNA/CdTe/MNPs/ composites

The NH_2-modified ssDNA was connected with the carboxyl groups on the surface of the CdTe/MNPs by covalently binding. Firstly, 1.0 mg CdTe/MNPs were suspended in 300 μL 0.1M Tris-HCl buffer (pH 7.4) to form CdTe/MNPs dispersion, 300 μL 1 μM mixture of ssDNA and bbcDNA (molar ratio=1:4) was added, followed by incubation for 12 h at 4 ◦C. Finally, the ssDNA/CdTe/MNPs conjugates were centrifuged and washed with the Tris-HCl buffer for three times to remove the excess ssDNA, and redispersed in 300 μL Tris-HCL buffer and kept at 4 ◦C. The obtained ssDNA/CdTe/MNPs conjugates were denoted ssDNA/CdTe/MNPs.

Fabrication of the ECL Aptasensor.

The EHN_S/GCE was dipped in 2% APS aqueous solution for 45 min. Then, the GCE was rinsed with Tris-HCl buffer and immersed into 1 mM PDITC N,N-dimethylformamide (DMF) solution for 2 h. The modified GCE was taken out and incubated in Tris-buffer containing 0.85 μ M aptamer 1 for about 16 h to produce an aptamer/PDITC/APS/EHNs attached GCE. Finally, 2 wt % BSA solution was used to block the nonspecific binding sites of the EHNs at 4 □C for 1 h. The electrode surface was rinsed with PBS buffer after each step to remove nonspecifically adsorbed species. Then, ssDNA/CdTe/MNPs conjugates were assembled to the surface of aptamer/PDITC/APS/EHNs/GCE via immersing the aptamer/PDITC/APS/EHNs/GCE into 60 μ L of ssDNA/CdTe/MNPs conjugates for the formation of ds-DNA structures between aptamer and ssDNA. The hybridization reaction was carried out for 1 h at 37℃ with mechanical shaking.

Subsequently, the electrode was washed thoroughly with Thris -HCl to remove unhybridized ssDNA/CdTe/MNPs conjugates.

Analytical Procedure.

Sample solutions containing various concentrations of thrombin were prepared in Tris- buffer. In a typical test, the assembled aptasensor was incubated in 100 μL of sample solution for 40 min at 37℃, followed by thoroughly washing with the same buffer to remove unbound thrombin and replaced ssDNA/CdTe/MNPs conjugates. Before and after the incubation, the ECL responses of the electrode were both recorded in 0.1 M PBS (pH7.4) containing 0.05 M $K_2S_2O_8$ as a coreactant. The linear scan potential was applied with a scan rate of 100 mV/s, and the voltage of the PMT was set at 600 V. ECL signals related to the thrombin concentrations could be measured.

Real samples analysis

The human serum from healthy volunteers was diluted 10-fold in PBS (pH 7.4). Three concentrations of TB (5.0, 10.0, and 100 pM) were detected in the 10-fold diluted human sera. Human serum samples were obtained from the affiliated hospital of Yantai University

Table S1 Recovery of TB assay at different concentrations spiked into human serum samples[a]

Sample	Spiked (fM)	Found (fM)	Recovery (%)	RSD (%)
1	5	4.8	96.0	4.2
2	10	10.6	106.0	6.2
3	100	105.6	105.6	5.7

a: Each sample was repeated for three times and averaged to obtain the recovery and RSD values

References

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2. C.Y. Tian, J. J. Xu, H. Y. Chen, *Chem. Commun.*, 2012, **48**, 8234–8236.