-Supporting Information-

A Novel DNA Aptasensor for the Detection Adenosine in Cancer Cells by Electrochemiluminescence of Nitrogen Doped TiO_2 Nanotubes

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Experimental section

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 adenosine probe (aptamer):

5'-NH_2-$(CH_2)_6$-ACCTGGGGGAGTATTGCGGAGGAAGGT

The 5'-NH_2-modified part complementary DNA (ssDNA):

5'-NH_2-$(CH_2)_6$-CTTCCAAGACGCAATACTCCCCCAGGT

The 5'-NH_2-modified dilute DNA (bbcDNA):

5'-NH_2-$(CH_2)_6$-ACTAGGGTGTACCTGGCAT

Titanium foils (99.8% pure, 0.127mm thick) were purchased from Aldrich (Milwaukee, WI). Adenosine triphosphate (ATP), cytosine triphosphate (CTP), guanosine triphosphate (GTP) and uridine triphosphate (UTP) were obtained from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China), and their stock solutions (1.0 mM) were prepared by doubly distilled water. The resulting solution was further consecutively diluted with doubly distilled water in order to obtain the proper solution used for ECL detection. 3-Mercaptopropionic acid (MPA), oxalic acid (OA), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were from Aldrich. Fe_3O_4 magnetic nanoparicles (MNPs, ~ 100 nm, 5 mg. mL^{-1}) modified by amino were obtained from BaseLine Chromtech Research Centre. 0.1 M phosphate buffer solution (PBS, pH 7.4) containing 0.05 M $K_2S_2O_8$ was used for ECL detection. 0.1 M PBS buffer (pH 7.4) containing 0.1 M NaCl was used for the preparation of DNA solutions. Other reagents of analytical grade were obtained from Beijing Chemical Company (China) and were used as received. Millipore ultrapure water (resistivity of 18.2 MΩ cm) was used throughout the experiment.

Apparatus.

The electrochemical measurements were performed on CHI 660A electrochemical workstation (Shanghai CHI Instruments Co., China). The ECL signals were conducted on a model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science and Technology Co. Ltd., Xi'An, China). ECL spectrum was obtained by a

series of optical filters (from 420 nm to 660 nm, spaced 20 nm, Omega Optical, Inc, US.). All these experiments were carried out with a conventional three-electrode system. A TiO_2-N NTs electrode, a Pt wire and a saturated calomel electrode (SCE) served as the working, counter and reference electrodes, respectively. The UV-vis absorption spectra were obtained on a Shimadzu UV-3600 UV-vis NIR photospectrometer (Shimadzu Co.). TiO_2-N NTs were characterized by S-3400N scanning electron microscopy (SEM, HITACHI Co.) equipped with EX-250 Energy-dispersive X-ray spectroscopy (EDX, HORIBA Co.).

Fabrication of TiO_2-N NTs electrode.

The TiO_2-N NTs were synthesized according to the previous works,[1] similarly to that described by Peng et al.[3] Prior to anodization, a titanium ribbon (4cm×0.5cm) was ultrasonically cleaned in a dilute HF solution then water for 5 min each. The cleaned titanium ribbon with the area of 0.5 cm × 0.5 cm was immersed in an electrolyte containing 1.0 M NaF and 0.5% M $(NH_4)_2SO_4$ and anodized at 20 V with a platinum cathode (performed on the DC power supply) at room temperature for 3 hour. The un-anodized top part of the titanium ribbon was used as the electrical contact. After anodic oxidation, it was immersed into 0.1M $NH_3 \cdot H_2O$ aqueous solution for 24 h and the resulting amorphous TiO_2 nanotubes were annealed at 450ºC for 2 h with heating and cooling rates of 10ºC/min. Then the samples were sonicated with ultrapure water, and dried in a N_2 stream. Figure S1 shows the stable ECL emission of TiO_2-N NTs electrode under continuous cyclic scan of 20 cycles.

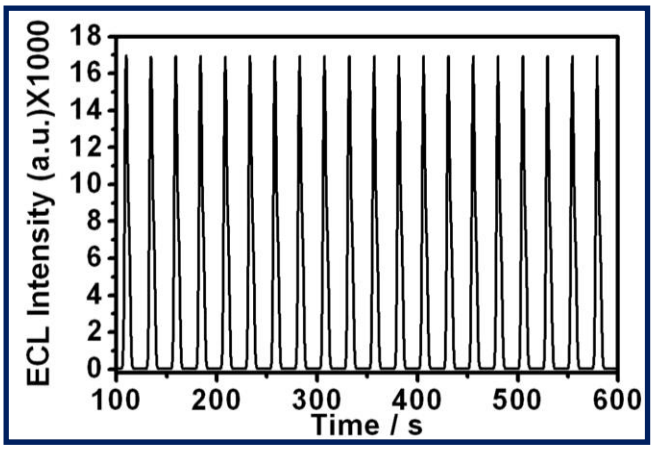

Figure S1 ECL emission from TiO_2 –N NTs electrode under continuous 20 cycles of cyclic scan in 0.05M $K_2S_2O_8$ and 0.1 M PBS (pH 7.4) with scan rate of 100 mV/s.

Fabrication of CdTe/ MNPs.

CdTe/MNPs nanocomposites were synthesized using the method reported previously.[2] Briefly, 0.069g of $CdCl_2 \cdot 2.5H_2O$ 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.

Figure S2 A is the TEM of the CdTe/MNPs NCs. The MNPs were coated with a relatively higher density of NPs with a diameter of about 5-10 nm. As show in Figure S2B, the absorption spectra demonstrated that the CdTe/MNPs NCs aqueous suspension possessed a broad absorption in the wavelength range of 400–800 nm with no excitonic emission peak, indicating that the obtained CdTe/MNPs composite was a black-body-like material. And it could be an excellent ECL quencher by efficiently absorbing ECL emission from TiO_2 –N NTs electrodes

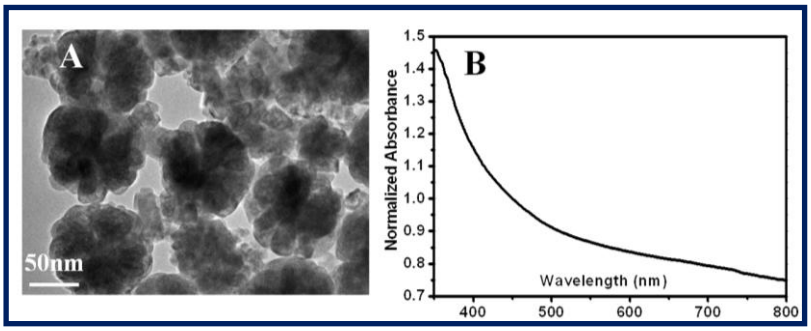

Figure S2 A) The TEM and B) The normalized UV-Vis absorption of CdTe/MNPs

Preparation of ssDNA/CdTe/MNPs/ composites

As shown in Scheme S1, the ssDNA and bbcDNA was immobilized to the surface of the magnetic bead through the linkage of an amidization reaction between carboxyl group on the surface of CdTe of magnetic beads and amino group on the terminal of the ssDNA and bbcDNA. Firstly, 1.0 mg CdTe/MNPs were suspended in 300 μL 0.1M PBS 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 PBS buffer for three times to remove the excess ssDNA, and redispersed in 300 μL PBS buffer and kept at 4 °C. The obtained ssDNA/CdTe/MNPs conjugates were denoted ssDNA/CdTe/MNPs.

Calculation of the surface coverages of ssDNA and bbcDNA on CdTe/MNPs used in this study

Preparation of UV-vis Calibration Curve of DNA: Standard ssDNA solutions were prepared from the solution of 1.0μM ssDNA with PBS buffer. The UV-vis absorbance calibration curve of ssDNA is shown in Fig. S3, the regression equation could be expressed as Y = 0.02049X – 0.00092 (X is the concentration of DNA, 10^{-7} M; Y is the absorbance of UV-vis, n = 16, R = 0.9986).

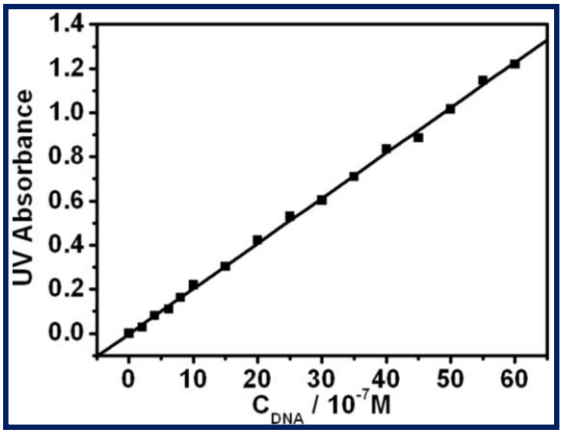

Figure S3 UV absorbance calibration curve of DNA solution.

Determination of the surface coverages of ssDNA and bbcDNA on CdTe/ MNPs: 2 mL of 10 × 10^{-7} M ssDNA and 40× 10^{-7} bbcDNA (molar ratio=1:4) was added into the activated CdTe/ MNPs（preparated by 33 μl, 5 mg.L^{-1} MNPs）and incubated 12 h at 4° C. Then the supernatant was taken for UV-vis absorbance detection. The number of ssDNA immobilized on the CdTe/ MNPs can be quantitatively calculated from the absorbance difference at 260 nm between the DNA solution before and after immobilization, which is calculated as below.

UV absorbance of the supernatant before immobilization: 1.0236

Concentration of total DNA (ssDNA and bbcDNA) before immobilization: 49.87× 10^{-7} M

UV absorbance of the supernatant after immobilization**:** 0.3704

Concentration of DNA after immobilization: 18.12 × 10^{-7} M

Moles of total DNA immobilized on CdTe/MNPs: (49.87 × 10^{-7} － 18.12× 10^{-7}) × 2 × 10^{-3} =6.35× 10^{-9}

Calculation of moles of MNP in a given preparation

(Assume that the process of fabrication of CdTe/ MNPs and ssDNA/CdTe/MNPs/ composites, the amount of the MNPs was conservative)

MNP diameter = 0.1 × 10^{-4} cm

MNP Volume = $4/3\pi r^3$ =5.233× 10^{-16} cm^3

Mass MNP = $\rho_{MNPs} \times V_{MNPs}$ =1.18 × 5.233 × 10^{-16} =6.175× 10^{-16}(g /MNPs)

The amount of CdTe/MNPs in 33 μL , 5 mg.mL^{-1}CdTe/MNPs for the preparing

0.033×0.005/6.175× 10^{-16}/6.02 × 10^{23}=4.439× 10^{-13} moles

Table S1 Quantitive Determination of the surface coverages of ssDNAs and bbcDNAs on MNPs

CdTe/MNPs (mol)	Total DNA (mol)	ssDNA (mol)	bbcDNA (mol)	Ratio of CdTe/MNPs/ ssDNA/bbcDNA
4.439×10^{-13}	6.35×10^{-9}	1.270×10^{-9}	5.08×10^{-9}	1/2.861×10^3/1.144×10^4

Preparation of ECL aptasensor

As shown in Scheme 1B, the obtained TiO_2–N NTs electrode was immersed in 1.0 mL of 3.0 mM OA solution for about 5 h at 4 ºC. After rinsed thoroughly with water and PBS buffer, the terminal carboxylic acid groups of the OA/TiO_2–N NTs were activated by immersion in 1.0 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS for 1 h at room temperature. Then the electrode was rinsed with 0.1 M PBS buffer (pH 7.4) to wash off the excess EDC and NHS. Subsequently, the resulting electrode was soaked in 150 μL 1 μM aptamer solution at 4 °C for at least 12 h. Finally, 100 μL 2 wt% BSA was dropped on the electrode at

4°C for 2 h to block non-specific binding sites of OA/TiO_2–N NTs electrode. The resulted aptamer-OA/TiO_2-N NTs were denoted aptamer/TiO_2-N NTs. For the hybridization with ssDNA/CdTe/MNPs/, 150 μl ssDNA/CdTe/MNPs/ dispersion was dropped on the aptamer/TiO_2-N NTs electrode. The hybridization reaction was held at 37℃ for 2 h. The obtained CdTe/MNPs/dsDNA/ TiO_2-N NTs were thoroughly rinsed with water and PBS buffer to wash off the excess ssDNA/CdTe/MNPs and denoted the aptasensor. The aptasensor was stored in PBS buffer at 4 ℃.

ECL detection of ATP

The as-prepared aptasensor was incubated with 150 μL PBS buffer (pH 7.4) containing different concentrations of ATP for 1 h at 37 ℃for the binding of ATP to aptamer DNA, followed by thoroughly washing with PBS (pH 7.4) to remove unbound ATP. The aptasensor before and after the formation of aptamer-ATP complex was in contact with 0.1 M PBS (pH 7.4) containing 0.05 M $K_2S_2O_8$ and scanned from 0 to -1.25 V. ECL signals related to the ATP concentrations could be measured. The preparation of ssDNA/CdTe/MNPs composites along with the principle of aptamer-based assay for ATP using CdTe/MNPs as ECL quencher is shown in scheme 1.

For the preparation of aptamer-TiO_2-N NTs electrode, we studied the effect of density of aptamer on ATP detection. Increasing the aptamer concentration in the preparation process of aptamer-TiO_2-N NTs electrode from 1nM to 12 μM for 1mM ATP detection, the changes of the ratio of ECL recovery signal to the quenching signal (RS/QS %) is shown in Figure S4. It reveals that when the aptamer concentration below 5 μM, the recovery efficiency close to 100%. When the concentration was over 5 μM, the recovery efficiency reduced sharply. This may be due to the aptamer too density on the surface of the electrode, and form mutliple bondings for each CdTe/MNPs, then ATP could not compete to release the quencher strand. Thus, 1μM aptamer was chosen in the preparation of ECL aptasensor.

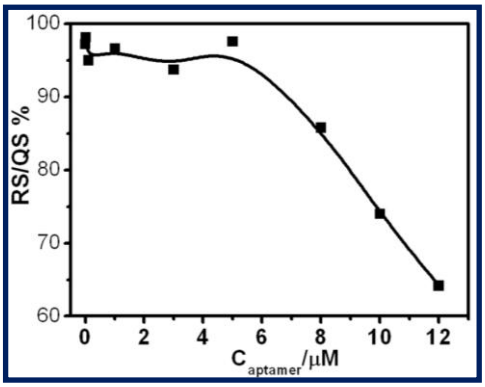

Figure S4 The ratio of ECL quenching signal and the recovery signal (RS/QS %) with different aptamer concentrations (μM) for 1mM ATP.

Preparation of adenosine Extracts from Cancer Cells.

The K562 leukemia cells were kindly provided by the Gulou Hospital, Nanjing,China. The K562 leukemia cells were cultured in RPMI 1640 medium (GIBCO) supplemented with fetal bovine serum (10%) (FBS, GIBCO), penicillin (60 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37℃ in a humidified atmosphere containing CO_2 (5%). After 48 h, the cells were collected and separated from the medium by centrifugation at 1500 rpm for 5 min and then washed twice with sterile PBS (pH 7.4). The sediment was resuspended in PBS to obtain a homogeneous cell

suspension of 1.5 × 10^6 cells (1.0 mL) at a certain concentration. Finally, the cells were disrupted by sonication for 30 min at 0 °C. To remove the homogenate of cell debris, the lysate was centrifuged at 16 000 rpm for 20 min at 4 °C.

References

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2. (a) X. Lan, X. Cao, W. Qian, W. Gao, C. Zhao and Y. Guo, J. *Solid State Chem.*, 2007, **180,** 2340-2345. (b) X. L. Wang, L. Wei, G. H. Tao and M. Q. Huang, *Chin. Chem. Lett.*, 2011, **22**, 233-236.