Supplementary Information

Ultrasensitive detection of ATP based on ATP regeneration amplification and its application in cell homogenate and human serum

Yingshu Guo[a*], Xiaofeng Sun[b], Guangxu Yang [b], Jia liu [b]

[a] School of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, China;

[b] Key Laboratory of Biochemical Analysis, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

* To whom correspondence should be addressed. Tel. +86 -539-8766867.

Email: yingshug@126.com

Instrumentation

The electrochemical measurements was carried out on a CHI660 electrochemical working station (Shanghai CH Instruments Co., China) using a three-electrode system. The detection was carried out in a electrochemical cell containing a glassy carbon working electrode (3 mm-diameter), an Ag/AgCl reference electrode, and a platinum wire counter electrode. The quartz crystal microbalance (QCM) measurements were conducted using a Q-sense E1 (Vastra Frolunda, Sweden). TEM imaging was performed with a JEM-2000EX/ASID2 (HITACHI, Japan) instrument.

Chemicals and Materials

Oligonucleotide sequences (Table S1)were synthesized by Shanghai Sangon Biotech Co. Ltd. (Shanghai, China). 2-Mecaptoethanol (MCE), Cadmium chloride ($CdCl_2$), sodium sulfide nonahydrate ($Na_2S{\cdot}9H_2O$) and mercaptoacetic acid ($HSCH_2COOH$, MCA, 99%) were obtained from Fluka. Adenosine triphosphate (ATP, AB0020), cytidine triphosphate (CTP, CD0134T), guanosine triphosphate (GTP, GD0250T) were all purchased from Shanghai Sangon Biotech Co. Ltd. (Shanghai, China). Polystyrene microspheres (-COOH, 1.0- 2.0 μm) was obtained from Baseline Chromtech Research Centre (Tianjin, China). E. coli DNA ligase was purchased from TaKaRa Biotechnology Co. Ltd. (Dalian, China). Phi 29 DNA polymerase and ATP sulfurylas were obtained from New England Biolabs. adenosine 5'-phosphosulfate (APS) and apyarse was purchased from Sigma Co. Ltd.

Table S1. DNA Sequences Used in this Study

Name	**Sequence (5'-3')**
DNA1	5'-TCACACAAAATGATGATGAAAGTGTGAGGACCTGGGGGAGTATTGCGGAGGAAGGTCC-3'

DNA2	5’-NH_2-TTTTTTGGACCTTCCTCCGC-3’
DNA3	5’-CATCATCAT-3’
DNA4	5’-SH-TTTTTT-GCGGAGGAA-3’
DNA5	5’-SH-TTTTTTTTT
DNA6	5’-ACAAAATGATGATGTTT-SH-3’
DNA7	5’-CCACCCCCTCATAACTTT-SH-3’

Synthesis of CdS NPs

CdS NPs were synthesized as in the previously reported method with slight modification.[S1] 3.66g $CdCl_2$ was dissolved in 200 mL of ultrapure water, followed by an injection of 60 mmol of MCA under continuous stirring. The pH was adjusted to 7.0 with NaOH. After stirring for 30 min, 1.56 g Na_2S in 100 mL of ultrapure water was slowly injected at room temperature. The reaction proceeded for 10 h at room temperature, with N_2 protection and continuous stirring. The final reaction precipitates were centrifugated and washed with ethanol and ultrapure water to ensure the removal of most excess MCA molecules and other impurities such as Cl^- and Na^+, and then redispersed into deionized water.

Preparation of Bio-bar-code DNA-Functionalized CdS NPs Probe

According to literature[S2] with slight modifications, bio-bar-code DNA -functionalized CdS NPs probe (bbc-CdS NP probe) were prepared as follows. The 20 μL of 1.0×10^{-4} M DNA 5 and 1.0×10^{-5} M DNA4 solution and 100 μL of CdS NPs solution were mixed together. After standing for 24 h at room temperature, the mixed solution was brought to 0.15 M NaCl and the particles were aged for an additional 10 h. The NaCl concentration was then raised to 0.2 M, and the mixture was allowed to stand for a further 40 h before centrifugalization. Finally, the bbc-CdS NP probes were redispersed into 200 μL of PBS buffer (10 mM, pH = 7.4) and stored at 4 °C for

future usage.

Fabrication of the DNA-Functionalized Polystyrene Microspheres Interface

DNA-functionalized polystyrene microspheres were fabricated as follows. 50 μL carboxylate-modified polystyrene microspheres were washed twice with 100 μL of 0.1M MES buffer (pH 4.5), followed by the addition of a freshly prepared solution of 1.0×10^{-3} M EDC. The mixture was incubated at room temperature for 30 min. Then, 50 μL of 1.0×10^{-6} M DNA 2 was added, and the mixture was incubated at ambient temperature for 1 h. The DNA 2- microspheres were washed four times with 100μL of 0.1 M KCl, 4 mM $MgCl_2$, and 0.05% Tween 20 in 30 mM Tris-HCl (pH 7.8) and diluted to 10 μL, stored in 4°C. And then 50 μL of 1.0×10^{-5} M DNA 1 was added, and the mixture was incubated at ambient temperature for 1 h followed washing four times to form DNA 1/2- microspheres .The DNA-functionalized microspheres were finally resuspended in 50 μL of 0.1 M KCl, 4 mM $MgCl_2$, and 0.05% Tween 20 in 30 mM Tris-HCl (pH 7.8) solution and diluted to 10 μL, stored in 4°C.

Cell Culture

Ramos cells were cultured at 37 °C in a flask with a RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/ mL streptomycin in an incubator (5% CO_2). The density of cells was calculated using a hemocytometer. After the concentration of cells reached 1.0×10^6 cells/mL, the cells were collected using centrifugation at 3000 rpm for 5 min.

Preparation of ATP Extracts from Cells

The preparation of ATP extracts from cells was conducted according to a reported method.[S3]Briefly, 2.0×10^6 cells were washed four times with PBS buffer, then disrupted by sonication for 25 min in ice bath. The lysate was centrifuged at 4 °C to remove the cell debris

homogenate. Finally, the cell lysate was filtrated to deproteinate.

Electrode Cleaning and Pretreatment

The gold electrode with 4 mm diameter was polished with 1.0 μm, 0.3 μm and 0.05 μm α-Al_2O_3 and then washed ultrasonically with pure water, followed by electrochemically cleaning in 0.5 M H_2SO_4 by potential scanning between -0.2 to 1.5 V until a reproducible cyclic voltammogram was obtained. Then it was rinsed thoroughly with pure water and finally dried in nitrogen stream at room temperature.

ATP-Triggered Regeneration Cycle Amplification

10 μL of ATP was mixed with 50 μL of DNA 1/2-functionalized polystyrene microspheres and incubated at 37 °C for 30 min. Ligation was carried out at 37 °C water bath for 1 h with 30 μL of solution containing 10.0 mM $(NH_4)_2SO_4$, 4.0 mM $MgCl_2$, 1.0 mM EDTA and 0.1 mM NAD, 30.0 mM Tris-HCl (pH 7.8) and 8.0 U *E. coli DNA* ligase. Then, the complex was incubated with 30 μL of the reaction mixture containing 1.0×10^{-6} M DNA 3, 40.0 U Phi 29 DNA polymerase, 1.0 mM dNTP, 10.0 mM $(NH_4)_2SO_4$, 15.0 mM KCl, 2.0 mM $MgSO_4$, 0.1% Tween-20 and 20.0 mM Tris-HCl (pH 7.8). The RCA reaction was conducted for 1 h in a 37 °C water bath. 0.1 U apyrase was added at room temperature for 30 minutes and heat-inactivated at 95°C for 3 minutes. The excess dNTP were degrades by apyrase. After the reaction was cooled to room temperature, the complex was incubated with 200 μL of the reaction mixture containing 0.1 M Tris-HCl (pH7.6), 2 mM EDTA, 10 mM $MgCl_2$, 0.1% BSA, 0.4 mg/mL PVP, 1mM DTT, 15 μM APS and 0.04 U ATP sulfurylase. Subsequently, the bbc-CdS NP probes were added and allowed to hybridize with the products for about 1 h prior to the electrochemical analysis.

Electrochemical Analysis

After bbc-CdS NP probes were captured by polystyrene microspheres, the polystyrene microspheres was rinsed with buffer and immersed into HNO_3 (200 μL, 1.0 M) for 30 min to dissolve the residual CdS NPs. The volume of the solution was adjusted to 2 mL with acetate buffer (0.2 M, pH 5.0) containing $HgCl_2$ (300 μM). Then, the concentrations of ATP were determined with electrochemical methods.

Preparation of DNA 7-Functionalized CdS NPs

The 20 μL of 1.0×10^{-4} M DNA 7 and 100 μL of CdS NPs solution were mixed together. After standing for 24 h at room temperature, the mixed solution was brought to 0.15 M NaCl and the particles were aged for an additional 10 h. The NaCl concentration was then raised to 0.2 M, and the mixture was allowed to stand for a further 40 h before centrifugalization. Finally, the DNA 7-CdS NPs were redispersed into 200 μL of PBS buffer (10 mM, pH = 7.4) and stored at 4 °C for future usage.

Characterization of the CdS NPs and bbc-CdS NPs

UV−vis spectroscopy was used to confirm the binding function between DNA and CdS NPs. In Figure S1, CdS NPs show an absorption peak at 550 nm. After DNA was attached to CdS NPs, an obvious absorption peak occurs at 260 nm as shown in Figure S1, which is a characteristic of the DNA strand, indicating the successful binding between DNA and CdS NPs. In addition, the TEM image of CdS NPs (Figure S2) indicated that CdS NPs used were nearly uniform and monodisperse.

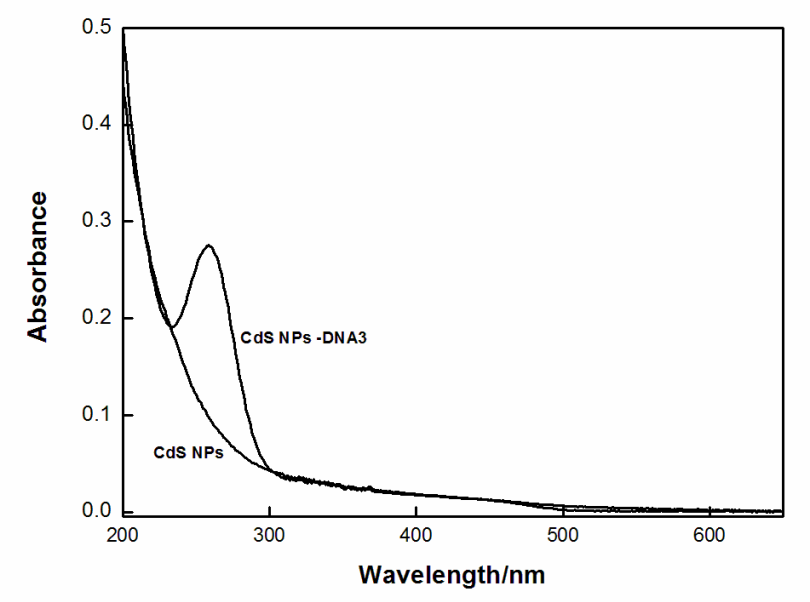

Figure S1. UV−vis absorption spectrum of CdS NPs and CdS NPs –DNA3.

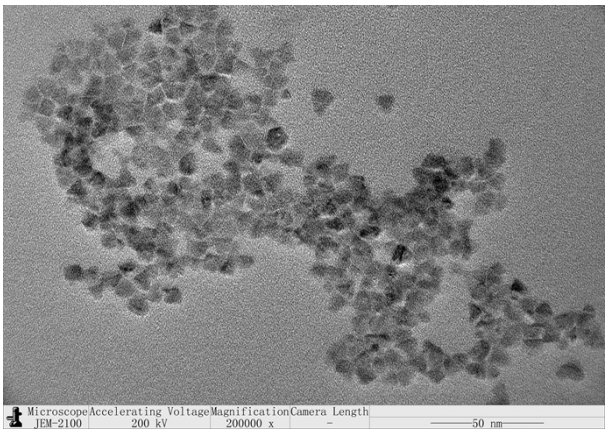

Figure S2 TEM image of CdS NPs

Quartz Crystal Microbalance Characterization of the RCA Products

A QCM method for characterization of RCA products is described below (Figure S3). 10 μL of sample (0 or 1.0×10^{-8} M ATP) was mixed with 50 μL of DNA 1/2-functionalized polystyrene microspheres and incubated at 37 °C for 30 min. Ligation was carried out at 37 °C water bath for 1 h with 30 μL of solution containing 10.0 mM $(NH_4)_2SO_4$, 4.0 mM $MgCl_2$, 1.0 mM EDTA and 0.1 mM NAD, 30.0 mM Tris-HCl (pH 7.8) and 8.0 U *E. coli DNA* ligase. Then, the complex was incubated with 30 μL of the reaction mixture containing 1.0×10^{-6} M DNA 3, 40.0 U Phi 29 DNA

polymerase, 1.0 mM dNTP, 10.0 mM $(NH_4)_2SO_4$, 15.0 mM KCl, 2.0 mM $MgSO_4$, 0.1% Tween-20 and 20.0 mM Tris-HCl (pH 7.8). The RCA reaction was conducted for 1 h in a 37 °C water bath and obtained the RCA products. DNA 6 as a capture probe was initially immobilized on the gold chip surface by a Au-S affinity binding. Subsequently, the mixture of RCA products and DNA 7- CdS NPs were injected and hybridized with DNA 6. Finally, the substrate was washed with buffer and the response signal was recorded by QCM (Figure 1).

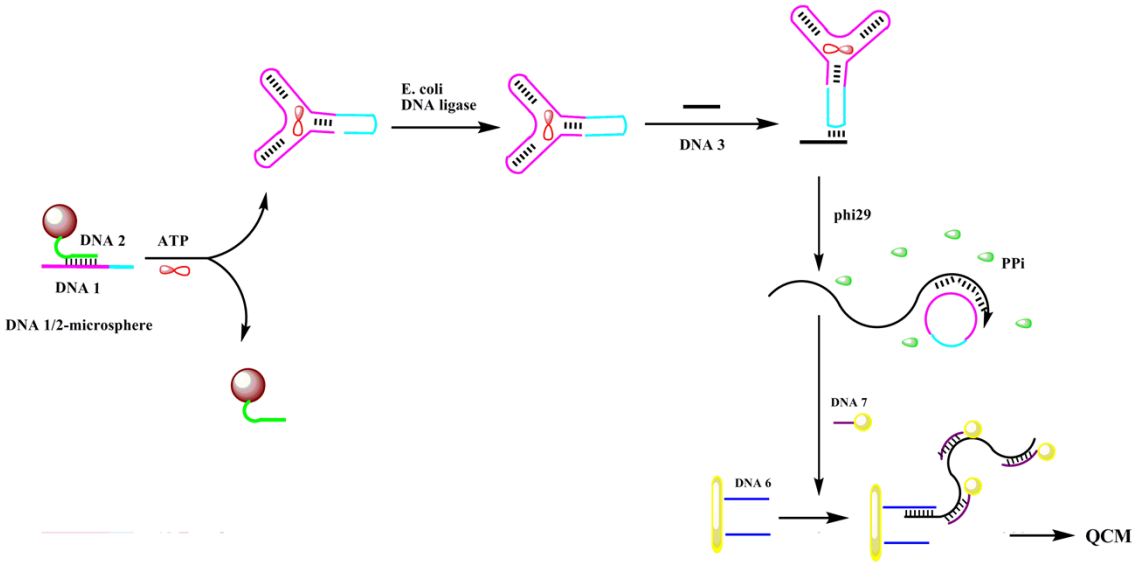

Figure S3 A QCM method for characterization of RCA products

Non-amplification Strategy for ATP Detection

As compared to amplification strategy for ATP detection (Scheme 2), the non-amplification strategy for ATP detection was no conversion based on APS and ATP sulfurylase (Figure S4) and PPi molecule can not be converted to ATP. This process is as follows. 10 μL of ATP was mixed with 50 μL of DNA 1/2-functionalized polystyrene microspheres and incubated at 37 °C for 30 min. Ligation was carried out at 37 °C water bath for 1 h with 30 μL of solution containing 10.0 mM (NH4)2SO4, 4.0 mM MgCl2, 1.0 mM EDTA and 0.1 mM NAD, 30.0 mM Tris-HCl (pH 7.8)

and 8.0 U E. coli DNA ligase. Then, the complex was incubated with 30 μL of the reaction mixture containing 1.0 × 10-6 M DNA 3, 40.0 U Phi 29 DNA polymerase, 1.0 mM dNTP, 10.0 mM $(NH_4)_2SO_4$, 15.0 mM KCl, 2.0 mM $MgSO_4$, 0.1% Tween-20 and 20.0 mM Tris-HCl (pH 7.8). The RCA reaction was conducted for 1 h in a 37 °C water bath. 0.1 U apyrase was added at room temperature for 30 minutes and heat-inactivated at 95°C for 3 minutes. The excess dNTP were degrades by apyrase. After the reaction was cooled to room temperature, the complex was incubated with 200 μL of the reaction mixture containing 0.1 M Tris-HCl (pH7.6), 2 mM EDTA, 10 mM MgCl2, 0.1% BSA, 0.4 mg/mL PVP, 1mM DTT. Subsequently, the bbc-CdS NP probes were added and allowed to hybridize with the products for about 1 h prior to the electrochemical analysis.

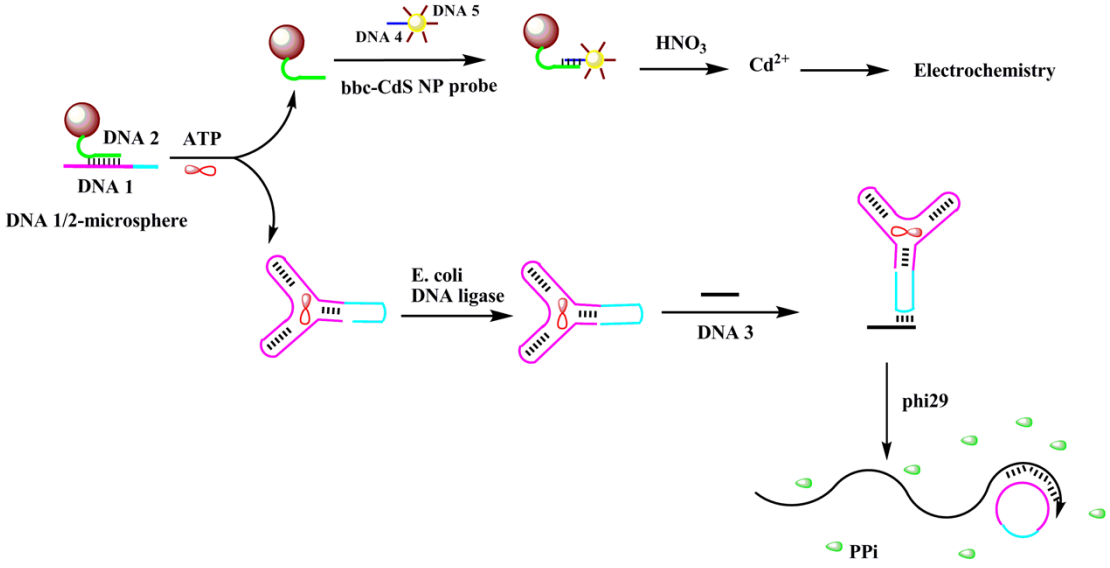

Figure S4 Figure representation of the non-amplification strategy for ATP detection

The detection results were shown in Figure S5. As the increasing of ATP concentrations from 2.0×10^{-8} to 1.0×10^{-6} M, the peak current values increased. The regression equation was Y = 0.968 X + 0.0499 (Y is the relative current value $\Delta I=I-I_0$, μA, where I and I_0 are the current value in the presence and absence of ATP inputs, respectively. X is the concentration of ATP, μM), and the

correlation coefficient was 0.995. The limit of detection was 2.0×10^{-8} M ATP.

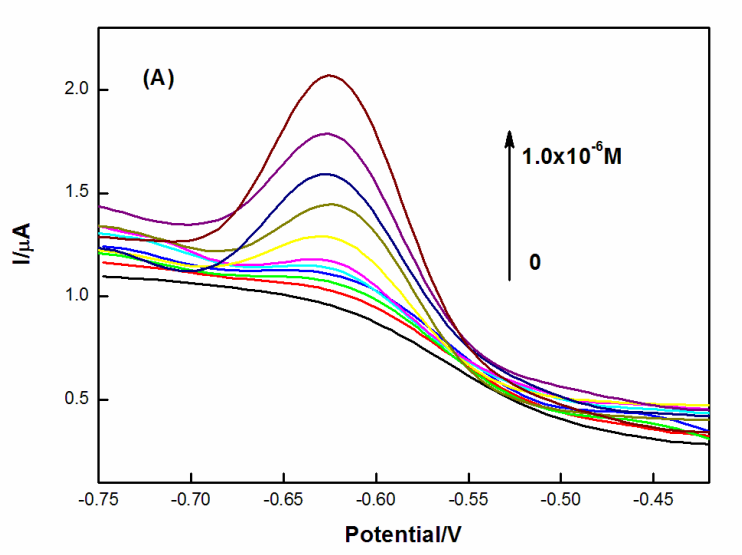

Figure S5 (A) DPV response to ATP at different concentrations; (B) the polt of peak current vs ATP concentration from 0 to 1.0×10^{-6} M.

References

[S1] J. O. Winter, N. Gomez, S. Gatzert, C. E. Schmidt and B. A. Korgel, *Colloids Surf. A*, 2005, **254**, 147.

[S2] G. P. Mitchell, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1999, **121**, 8122.

[S3] J. Li, H. Fu, L Wu, A Zheng, G Chen and H. Yang, *Anal. Chem.*, 2012, **84**, 5309.