Supplemental Information:

A novel aptasensor for the ultra-sensitive detection of adenosine triphosphate via aptamer/quantum dot based resonance energy transfer

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Materials and Methods:

1. Materials

1.1 Chemicals and Reagents:

The ATP aptamer was synthesized and its 3’-end was labeled with Cy3 (5’-ACCTGGGGGAGTATTGCAGGAAGGT-Cy3, Shanghai Shenggong, China). The complementary sequence of the ATP aptamer was modified with amino group at its 5’ end through a \((\text{CH}_2)_{12}\) linker (3’-TGGACCCCCTCATAACGCCTCCTCCA-(\text{CH}_2)_{12}-\text{NH}_2, Shanghai Shenggong, China). The EDC (1-ethyl-3-[3-dimethylaminopropyl carbodiimide hydrochloride]) and NHS (N-hydroxysulfosuccinimide) were purchased from Thermo Scientific. The ATP, ADP, CTP, GTP and TTP were purchased from Shanghai Shenggong, China. PBS buffer (137 mM NaCl, 2.7 mM KCl, 2 mM KH_2PO_4, 10 mM Na_2HPO_4, pH 7.4). MES buffer (0.1M MES,0.5M NaCl, pH6.0).

2. Equipments:

UV Visible Spectrophotometer (Thermo-Fisher, Biomate 3S); Spectrometer (Edinburgh FLS920); Circular dichroism spectropolarimeter (Tokyo JASCO J-720); Centrifuge (Thermo microCL 21R); Micro-Shaker (Allsheng MSC-100).

2. Experimental Methods:

2.1 The synthesis of the QDs:

The QDs were synthesized according to the literature \cite{1}. In brief, green-emission
oleylamine-capped CdSe QDs were obtained as following: First, 0.2 mM B$_2$Se$_3$ and 0.6 mM CdCl$_2$ was separately loaded into 3mL oleylamine in sealed vials and dissolved by heating at 110ºC for 20 minutes. After the solutions were cooled down to room temperature, they were mixed together and heated at 75ºC for 1 hour in a convectional oven. Then, 0.1 mM P$_5$S$_5$, 0.5g mercaptopropyl acid (MPA) and 0.3 mL butylamine were heated at 110ºC for 20 minutes in 10mL 1-methyl-2-pyrrolidinone (NMP) in a sealed vial to dissolve the sulfide. In a separate vial, 0.5mM ZnCl$_2$, 0.5g MPA and 0.3mL butylamine were mixed with 10mL NMP and heated to dissolve in the same way. After cooling down to room temperature, 0.01g oleylamine-capped CdSe core particles were dissolved into the P$_5$S$_5$ solution, and the whole solution was mixed with ZnCl$_2$ solution. The mixture solution was heated at 70ºC for 1 hour in a conventional oven, yielding the MPA-capped CdSe/ZnS core/shell products. The CdSe/ZnS core/shell QDs were purified by solvent extraction 5 times using hexane, and re-dispersed in PBS buffer (137 mM NaCl, 2.7 mM KCl, 2 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$, pH 7.4).

2.2 The construction and the purification of the ATP aptamer based aptasensor:

The complementary sequence of the ATP aptamer was immobilized on the QDs as follows: the reaction mixture containing QDs, the complementary sequence of the ATP aptamer, EDC and sulfo-NHS with a ratio of 1:10:300:300 in PBS buffer (137 mM NaCl, 2.7 mM KCl, 2 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$, pH 7.4) was prepared and incubated for 2hrs at room temperature with gentle shaking (300rpm). Then, the mixture was purified using an ultra-filtration membrane by six rounds of centrifugation with 200µL PBS buffer. The conjugated QDs were collected and dissolved in PBS buffer. Afterwards, the modification of the QDs surface was further confirmed by 1% agarose
electrophoresis. To further remove the exceeding free nucleic acids, the surface modified QDs were subject to dialysis in PBS buffer at 4°C for 24hrs. The dialyzed ODs were collected, and further hybridized to the Cy3 modified ATP aptamer for 1hrs at room temperature with gentle shaking (300rpm) in PBS buffer. Afterwards, the aptasensor was purified by using an ultrafiltration membrane by six rounds of centrifugation with 200μL PBS buffer. To further remove the free ATP aptamer, the exceeding free Cy3 modified ATP aptamer was removed by dialyzing in PBS buffer for another 24hrs at 4°C. Finally, the ATP aptasensor was collected and stored in 4°C for further usage.

2.3 The ATP affinity measurement of the Cy3 modified ATP aptamer

The ATP affinity measurement was performed according to the literature [2]. In brief, 1mL ATP-agarose (Sigma) was pre-equilibrated with approximately 25mL PBS buffer (137 mM NaCl, 2.7 mM KCl, 2 mM KH₂PO₄, 10 mM Na₂HPO₄, pH 7.4) at 4°C for 20min with rotation. Then, different concentration of ATP aptamer was loaded onto the ATP-agarose and incubated at 4°C for 3hrs. Afterwards, the ATP-agarose was washed by three rounds of centrifugation with 5 mL of PBS buffer for 1 min at 4°C. The Cy3 fluorescence intensity of the supernatant was measured, and the according aptamer concentration was calculated. The remaining ATP aptamer was specifically eluted with 3mL of elution buffer (137 mM NaCl, 3mM ATP, 2.7 mM KCl, 2 mM KH₂PO₄, 10 mM Na₂HPO₄, pH 7.4). The Cy3 fluorescence intensity of the elution was measured, and the according aptamer concentration was calculated based on the fluorescence Intensity. The bound ATP aptamer concentration and the input concentration were plotted, and the affinity was calculated based on the plotting. Here, the ATP affinity of the Cy3 modified ATP aptamer is 8μM, which is similar to the original reported affinity of this aptamer [2].
2.4 CD measurement of the constructed aptasensor

The CD spectra of the constructed duplex aptasensor and none-hybridized nucleic acid-QD complex in PBS buffer (137 mM NaCl, 2.7 mM KCl, 2 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$, pH 7.4) were collected by Jasco J-720 CD spectrometer. The CD spectra were examined from 220 nm to 300 nm (Scanning speed 50 nm/min, band width 1 nm). The background of the buffer was subtracted from the CD spectra data. The CD spectrum of the none-hybridized nucleic acid-QD complex consists of a positive band at 260 nm and a negative band at 240 nm, which is consistent with the reported CD feature of ss-DNA; the CD spectrum of the constructed duplex aptasensor consists of a positive band a 284 nm and a negative band at 252 nm, which is well fitted with the classic CD spectrum of the ds-DNA.

References


Supplementary Figure Legends:

FigS 1. (A) The Cy3 spectrum of the constructed duplex aptasensor ($\lambda_{ex}$=510nm, the width of the slits for both excitation and emission was 4nm); (B) The CD spectrum of the none-hybridized nucleic acid-QD complex (black line, a), and the CD spectrum of the constructed duplex aptasensor (red line, b); (C) the absorption spectrum (black line) and
the emission spectrum (blue line) of the constructed duplex aptasensor.

Fig S 2. The emission spectra of the constructed duplex aptasensor in the presence of 1000nM ATP (red line) and in the absence of ATP (black line) from 500nm to 580nm ($\lambda_{ex} = 480$nm, the width of the slits for both excitation and emission was 4nm).

Fig S 3. (A) The emission spectra of the duplex aptasensor from 500nm to 545nm (the first emission peak) in the presence of different concentration of ATP; (a-g), the responses of the aptasensor to different concentration of ATP, 0nM, 0.1nM, 1nM, 10nM, 100nM, 1µM and 10µM respectively; (B) the corresponding fluorescence intensity change (525nm) in the presence of different concentration of ATP, and the linear fit of the change to the concentration of ATP. $\Delta F_{525}$ represents the fluorescence intensity at 525nm in the presence of ATP subtract the fluorescence intensity at 525 in the absence of ATP.

Fig S 4. The stability of the duplex aptasensor when it was stored in 4°C over days in the absence of ATP. The ratio of 525/560 of the duplex emission spectrum was calculated as an indicator of the stability.

Fig S 5. (A) The spectra of the constructed ATP aptasensor from 500nm to 580nm ($\lambda_{ex} = 480$nm, the width of the slits for both excitation and emission was 4nm) in the presence of different concentration of ATP. (a-f), the responses of the aptasensor to different concentration of ATP, 0nM, 0.1nM, 0.2nM, 0.3nM, 0.4nM and 0.5nM respectively. (B) The calculated responses of the aptasensor and the linear fit of the responses to the concentration of ATP. $R_0$ represents the ratio of 525/560 in the absence of ATP, $R$ represents the ratio of 525/560 in the presence of ATP.
FigS 1. (A) The Cy3 spectrum of the constructed duplex aptasensor ($\lambda_{\text{ex}}=510\text{nm}$, the width of the slits for both excitation and emission was 4nm); (B) The CD spectrum of the none-hybridized nucleic acid-QD complex (black line, a), and the CD spectrum of the constructed duplex aptasensor (red line, b); (C) the absorption spectrum (black line) and the emission spectrum (blue line) of the constructed duplex aptasensor.
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Figure S 5

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