Electronic Supporting Information

A label-free G-quadruplex-based switch-on fluorescence assay for the selective detection of ATP

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Materials and Methods

All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China), and the sequences of the single-stranded oligonucleotides were as follows:
ATP aptamer: 5′-AACCTGGGGGAGTATTGCGGAGGAAGGT-3′
ATP aptamer complementary strand: 5′-ACCTTCCTCCGCAATACTCC CCCAGGTT-3′
ATP aptamer shorter complementary strand: 5′- ACCTTCCTCCGCAA -3′

Crystal violet, ATP, UTP, GTP, AMP, ADP, and CTP were purchased from Sigma (St. Louis, MO). The Tris buffer used in the experiment consisted of 20 mM Tris-HCl (pH 7.0). All reagents were used without further purification. Milli-Q purified water was used to prepare all solutions.

Physical Measurement

Emission spectra and time course analyses were recorded on a PTI-QM4 fluorescence spectrophotometer. Circular dichroism (CD) spectra were recorded on a JASCO-815 spectrometer.

Emission Measurement

A mixture of anti-ATP-binding aptamer (25 μL, 100 μM) and its complementary DNA (25 μL, 100 μM) was mixed in hybridization buffer (50 μL, 20 mM Tris-HCl, pH 7.0). The mixture was annealed at 90 °C for 10 min, and was slowly cooled down from 90 °C to 25 °C. This stock solution at 25 μM duplex DNA was stored at –20 °C for further use. In the emission measurement, 100 μL of the aptamer duplex stock solution was diluted with 400 μL of Tris-HCl buffer (20 mM, pH 7.0) to obtain a 5 μM aptamer duplex solution in a cuvette. Various concentrations of ATP (final concentration ranging from 0 to 10 mM) were added to each cuvette, followed by the addition of CV at a final concentration of 12 μM. The mixture was allowed to equilibrate at 25 °C for 10 min. Fluorescence emission spectra were recorded on a PTI QM-4 spectrofluorometer at 25 °C. The fluorescence emission intensity at 600–750 nm was monitored after excitation of the sample at 580 nm.

Total cell extract preparation

HeLa cells were trypsinized and resuspended in TE buffer (10 mM Tris-HCl 7.4, 1 mM EDTA). After incubation on ice for 10 min, the lysate was centrifuged and the supernatant was collected. The cell extract was then spiked with ATP (final concentration 1–7.5 mM), and the fluorescence spectra were recorded after the addition of CV (12 μM) and DNA duplex (5 μM) and equilibration at 25 °C for 10 min.

Fig. S1 Circular dichorism (CD) spectra of the DNA duplex (1.5 μM) in the absence and presence of ATP (500 μM).

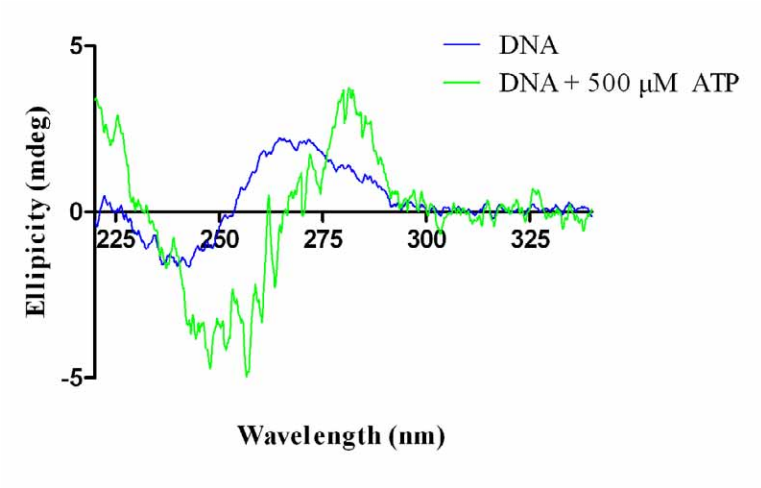


Fig. S2 Emission spectral traces of CV (12 μM) and DNA duplex (5 μM) upon addition of ATP (5 μM) in Tris-HCl buffer (20 mM, pH 7.0), showing a signal-to-noise ratio greater than 3.

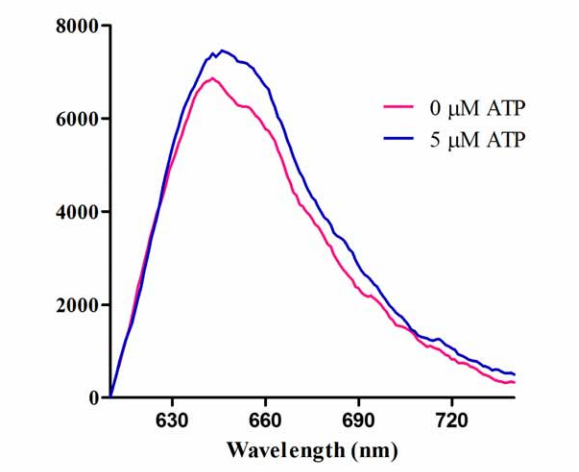


Fig. S3 Fluorescence spectra of the CV/duplex system in response to various concentrations of ATP: 0, 500, 1000, 2500, 5000, 7500 and 10000 μM. Experimental conditions: 12 μM CV, 5 μM DNA duplex, Tris-HCl buffer (20 mM, pH 7.0) containing 150 mM Na^+, excitation wavelength = 580 nm.

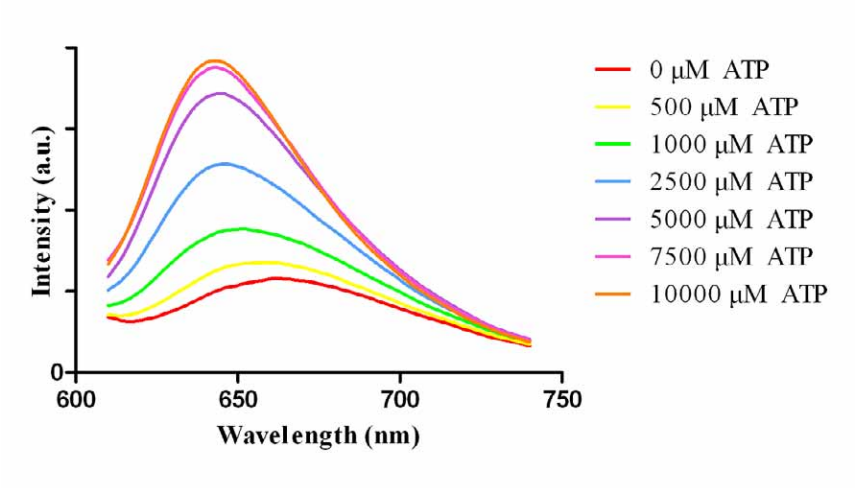


Fig. S4 Fluorescence responses of various concentrations of CV (6, 12 and 24 μM) with DNA duplex (5 μM) as a function of ATP concentration (1, 5, and 10 mM).

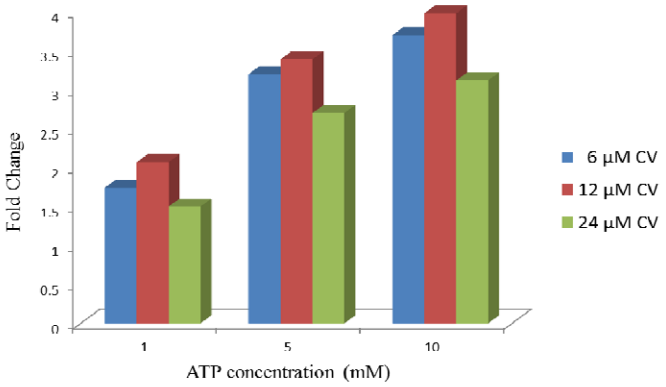


Fig. S5 Time course of fluorescence signal of CV (12 μM) and DNA duplex (5 μM) upon addition of ATP (0.5–7.5 mM) at 25 °C.

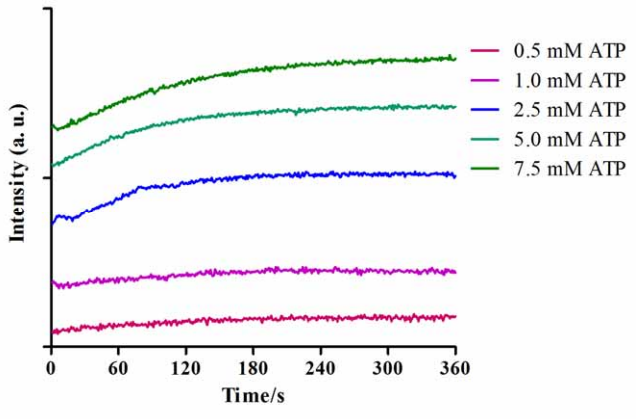


Fig. S6 Fluorescence spectra of the CV/partly hybridized duplex system in response to various concentrations of ATP: 0, 5, 250, 2500, 5000, 7500 and 10000 μM. Experimental conditions: 12 μM CV, 5 μM partly hybridized DNA duplex, 20 mM Tris-HCl buffer (pH 7.0), excitation wavelength = 580 nm.

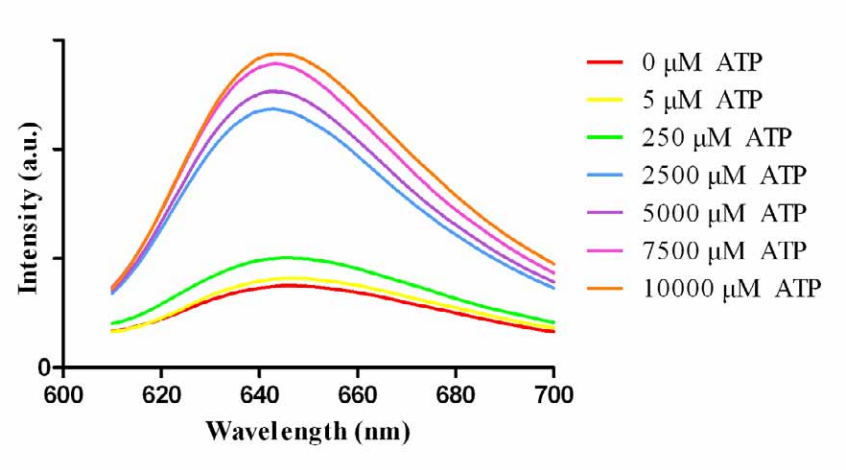


Fig. S7 Time course of fluorescence signal of CV (12 μM) and partly hybridized DNA duplex (5 μM) upon addition of ATP (2.5–10 mM) at 25 °C.

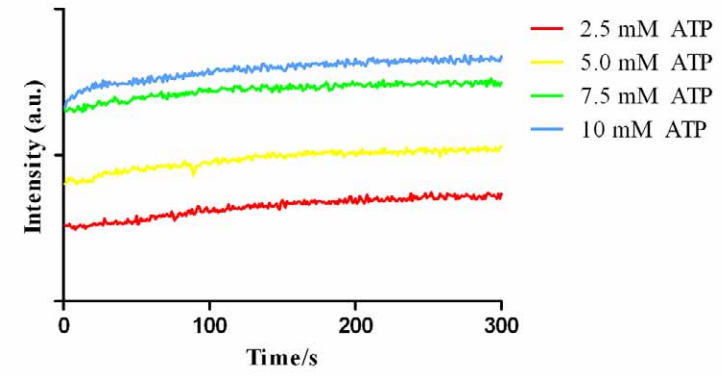


Fig. S8 Fluorescence spectra of CV (12 μM) and DNA duplex (5 μM) in whole cell extracts spiked with ATP (1–7.5 mM).

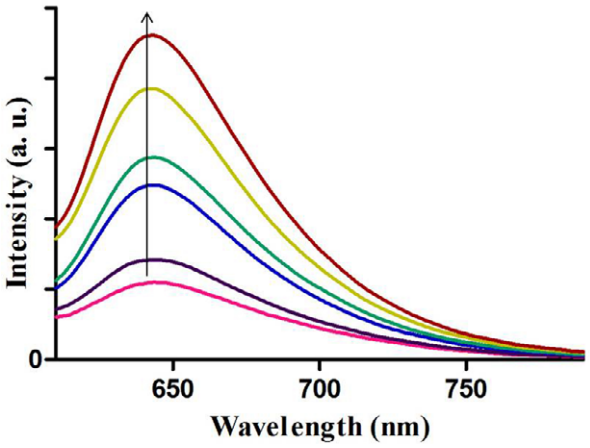
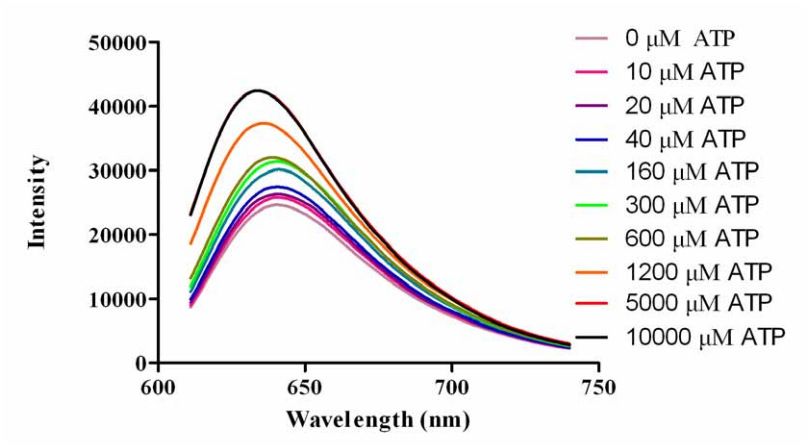


Fig. S9 Fluorescence spectra of the CV (12 μM) and ssDNA (5 μM) system in response to various concentrations of ATP (0–10 mM).



Scheme S1. Schematic representation of single strand aptamer-based method for ATP detection using G-quadruplex probe crystal violet (CV). ATP promotes the formation of aptamer–target G-quadruplex which could be subsequently detected by CV.