Supporting Information

Label-free fluorescent assay of ATP based on aptamer-assisted light-up of Hoechst dyes

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Experimental Section

Reagents and materials. The oligonucleotides used in this study were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China) with the following sequences: Apt: 5'-ACCTGGGGGAGTATTGCGGAGGAAGGT-3'. 10×$NaNO_3$-MOPS buffer (500 mM $NaNO_3$ and 200 mM 3-(4-morpholinyl)-1-propanesulfonic acid, pH 7.0) was prepared using metal-free reagents in distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA) with an electrical resistance of 18.2 MΩ. All chemicals used in this work were of analytical grade and obtained from commercial sources and directly used without additional purification. The artificial urine was prepared according to the reported literature.[1]

Instrumentation. Fluorescence was measured in a fluorescence microplate reader (Bio-Tek Instrument, Winooski, USA) using a black 384 well microplate (Fluotrac 200, Greiner, Germany). Photographs were taken with a digital camera.

Assays for ATP using the Apt-Hoechst solution. The Apt-Hoechst solution (Apt, and Hoechst 33342 were used) was prepared in 50 mM $NaNO_3$ and 20 mM 3-(4-morpholinyl)-1-propanesulfonic acid buffer (pH 7.0), and the mixture was incubated for 10 min at room temperature. For the fluorescent "on" detection of ATP, an aliquot of the tested ATP or control samples or Mill-Q water (as blank sample) was added to the Apt-Hoechst solution. The final concentration of Apt and Hoechst 33342 was 0.1 μM and 0.01 mg/mL, respectively. The mixture was vortexed to mix all the reagents and then incubated for 10 min at room temperature and after that an aliquot of 0.1 mL mixture was placed in the black 384 well microplate to measure the luminescence intensity (excited at 360 nm).

Data analysis. The GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA) was employed to perform the data processing. Each sample was repeated in duplicate, and data were averaged.

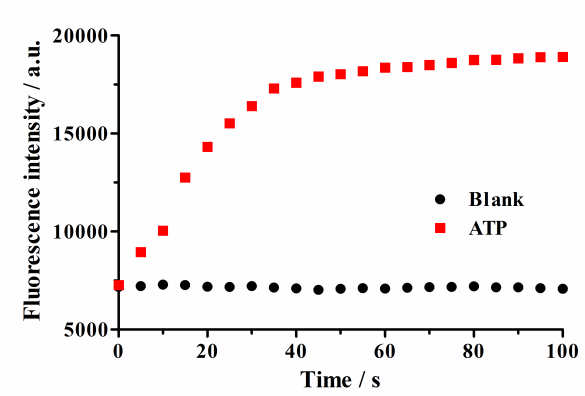

Figure S1. Kinetics investigation of fluorescence response of the Apt-Hoechst solution to ATP. 0.1 μM Apt, 0.01mg/mL Hoechst 33342 and 2 mM ATP were used.

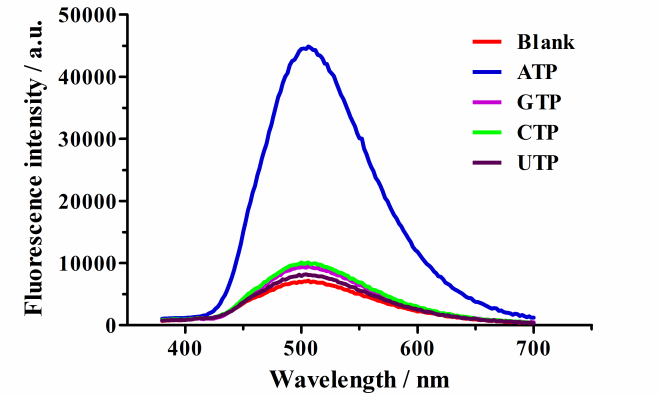

Figure S2. Fluorescent response of Apt-Hoechst solution upon the addition of 5 mM ATP, 10 mM GTP, CTP and UTP excited at 360 nm, respectively.

Reference:

1. T. Brooks and C. W. Keevil, *Lett Appl Microbiol*, 1997, 24, 203-206.