Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2013

Electronic Supplementary Information (ESI)

High Sensitive Fluorescent Detection of Small Molecules, Ions, and Proteins Using a Universal Label-Free Aptasensor

*Zhenzhen Lv, Jinchuan Liu, Yu Zhou, Zheng Guan, Shuming Yang, Cheng Li, and Ailiang Chen**

Institute of Quality Standards & Testing Technology for Agro-Products, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

*Author to whom correspondence should be addressed: ailiang.chen@gmail.com

1. Materials.

All the oligonucleotide sequences used in this study were synthesized and purified through HPLC by Sangon Biotechnology Co., Ltd. (Shanghai, China), and sequence information was listed in Table S1. The fluorescent dye PicoGreen (200× concentrated) was purchased from Invitrogen (CA). Thrombin from human plasma (Cat #T6884) and bovine plasma (Cat #T4648) was purchased from Sigma (St. Louis, MO). Fetal Bovine Serum (Cat #SH30070.03) was purchased from Hyclone (Logan, UT). Adenosine, guanosine cytidine and uridine were purchased from Amresco (Solon, OH). All other reagents were of analytical grade and bought from AccuStandard (Beijing, China).

2. Fluorescent Detection of adenosine.

For adenosine detection by our method, 25 μL of adenosine aptamer solution (0.5 μM) was mixed with 50 μL of 1× adenosine binding buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5) containing different concentrations of adenosine in microplate wells. Samples were incubated at room temperature for 20 min. Then, 25 μL of 0.5 μM complementary strand of adenosine aptamer and 10 μL 10× PicoGreen was added to the sample wells. After incubation for 3 min, the fluorescence intensities were scanned with LS-55 Fluorescence Spectrometer (Perkin e Elmer, Norwalk, CT) with an excitation of 480 nm and emission of 523 nm or recorded using a multifunction microplate reader (Tecan Infinite 200, Tecan Austria GmbH, Austria) with an excitation of 480 nm and emission of 520 nm.

3. Fluorescent Detection of K^+.

For K^+ detection by our method, the assay procedure was same as that used for the detection of adenosine, except that the adenosine aptamer and complementary strand were replaced by K^+'s and some adjustment for reagent concentration. In brief, 25 μL of aptamer solution (5 μM) was mixed with 50 μL of 1× potassium binding buffer (50 mM Tris-HCl, 2 mM $MgCl_2$, pH 8.3) containing different concentrations of K^+ in microplate wells. Samples were incubated at room temperature for 20 min. Then, 25 μL of 5 μM complementary strand of K^+ aptamer and 20 μL 10× PicoGreen was added to the sample wells. After incubation for 5 min, the fluorescence intensities were recorded as above.

4. Fluorescent Detection of thrombin.

All assay procedure was the same as those used for the detection of adenosine and K^+. In brief, 50 μL of thrombin was incubated with 25 μL of 0.5 μM thrombin aptamer in thrombin binding buffer (100 mM Tris-HCl, 140 mM NaCl, 20 mM $MgCl_2$, 20 mM KCl) for 1h in black microplate. Then, 25 μL of 0.5 μM complementary strand of thrombin aptamer and 10 μL 10× PicoGreen was added to the sample wells. After incubation for 3 min, the fluorescence intensities were recorded as above.

5. Application

This journal is © The Royal Society of Chemistry 2013

In order to test the practical applicability and the accuracy of this sensor, serum and urine were chosen as real-world samples. For adenosine detection in serum, Fetal Bovine Serum was diluted 20-fold with adenosine buffer and spiked with different concentrations of adenosine. For K^+ detection in urine, urine samples were harvested from three members of our laboratory and were filtered through 0.22 μM membranes. Then the urine samples were extracted three times by methylene dichloride (volume ratio: sample/methylene dichloride = 3:1) and diluted 50-fold with potassium binding buffer. The detection process of adenosine in human serum and K^+ in urine were the same as in the clean buffer by the label-free aptasensor. For K^+ measurements using Atomic Absorption Spectrometry (Z-2300 Atomic Absorption Spectrometry, Hitachi, Japan), after microwave digestions of urine samples, the concentration of K^+ were detected.

This journal is © The Royal Society of Chemistry 2013

Table S1. Aptamer and complementary sequences used in this study.

Description	Sequence(5'-3')
Adenosine aptamer	ACCTGGGGGAGTATTGCGGAGGAAGGT
Complementary strand	ACCTTCCTCCGCAATACTCCCCCAGGT
Potassium aptamer	TGAGGGTGGGGAGGGTGGGGAA
Complementary strand	TTCCCCACCCTCCCCACCCTCA
Thrombin aptamer	GGTTGGTGTGGTTGG
Complementary strand	CCAACCACACCAACC
Sulforhodamine B aptamer	CCGGCCAAGGGTGGGAGGGAGGGGGCCGG
Complementary strand	CCGGCCCCCTCCCTCCCACCCTTGGCCGG
Sulfadimethoxine aptamer	GAGGGCAACGAGTGTTTATAGA
Complementary strand	TCTATAAACACTCGTTGCCCTC

Table S2. Potassium detection in urine samples

Sample	Atomic Absorption Spectrometry (mM)	Aptasensor (mM)	Ratio
Urine 1	25.65±0.26	23.82±1.12	92.86±4.30%
Urine 2	18.89±0.18	16.74±1.58	88.61±8.77%
Urine 3	11.23±0.24	9.01± 1.26	80.23±5.25%

This journal is © The Royal Society of Chemistry 2013

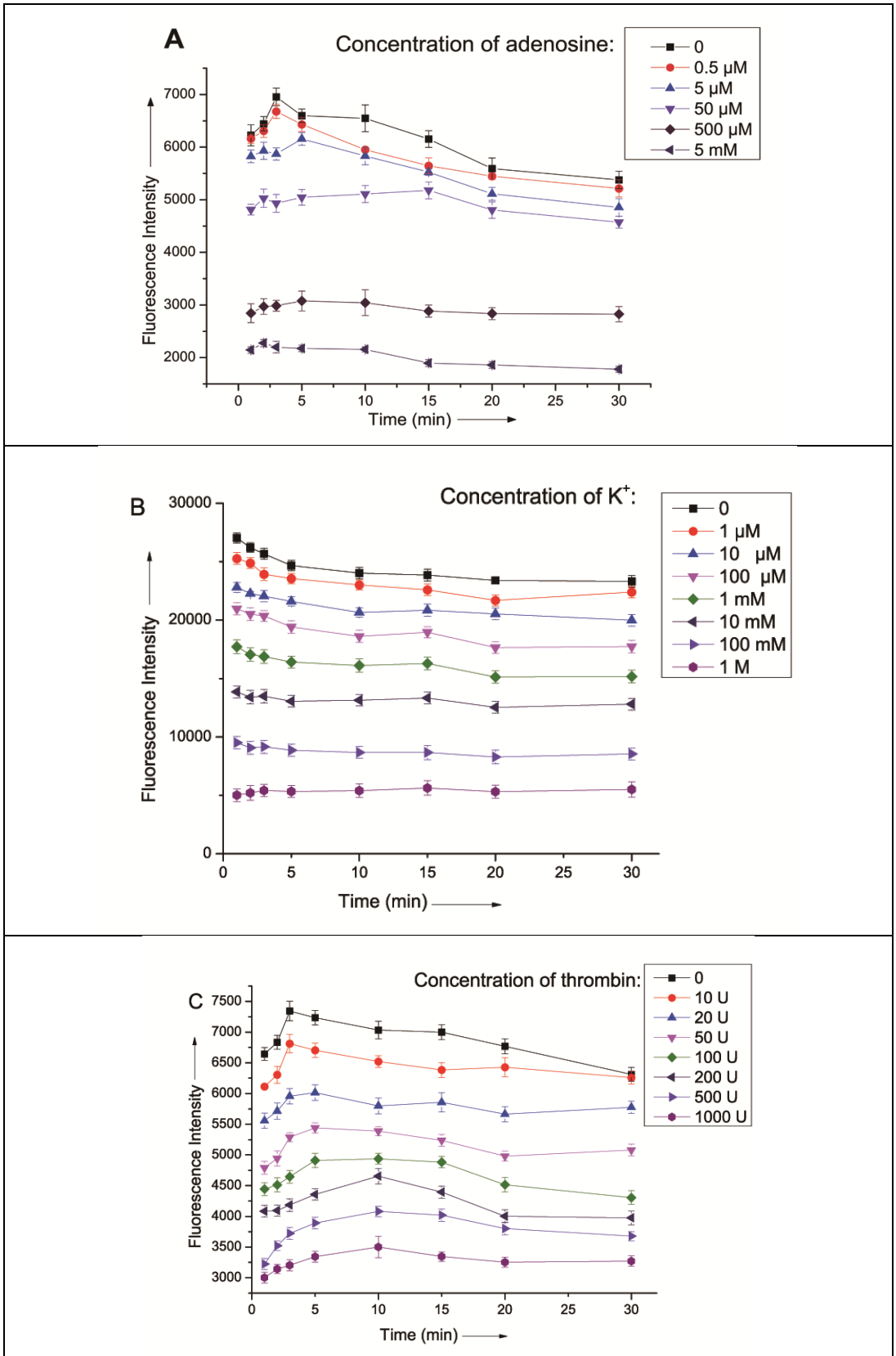

Figure S1. Optimization of the incubation time after aptamer complementary strand and PicoGreen were added into the different concentration of target and aptamer mixtrure. (A) Adenosine, (B) K^+, (C) Thrombin

This journal is © The Royal Society of Chemistry 2013

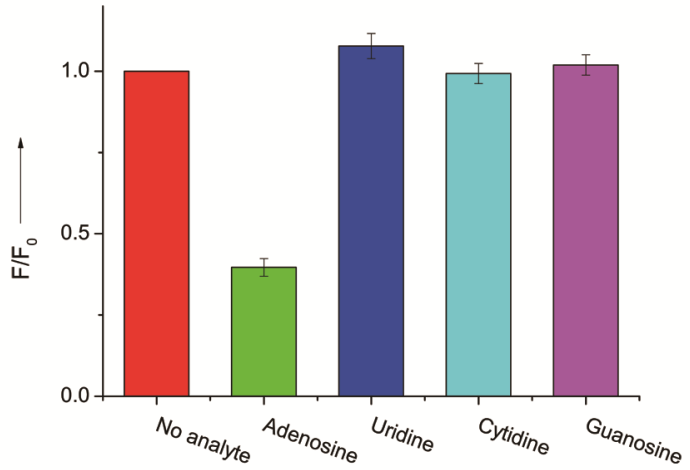

Figure S2. Selectivity evaluation of the aptasensor for adenosine against uridine, cytidine and guanosine in same series contrations.

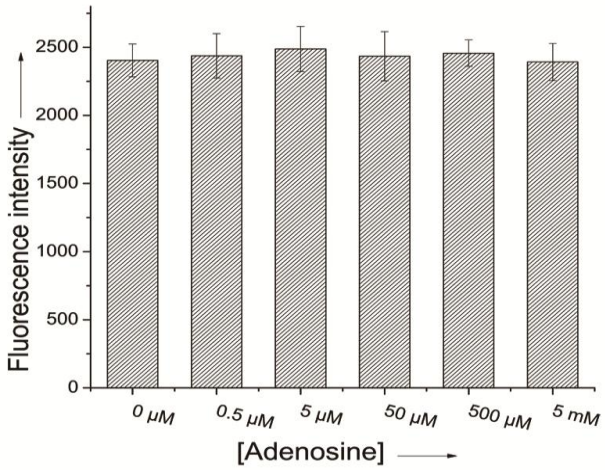

Figure S3. Selectivity evaluation of the aptasensor for adenosine using sulforhodamine B aptamer and its complementary strand.

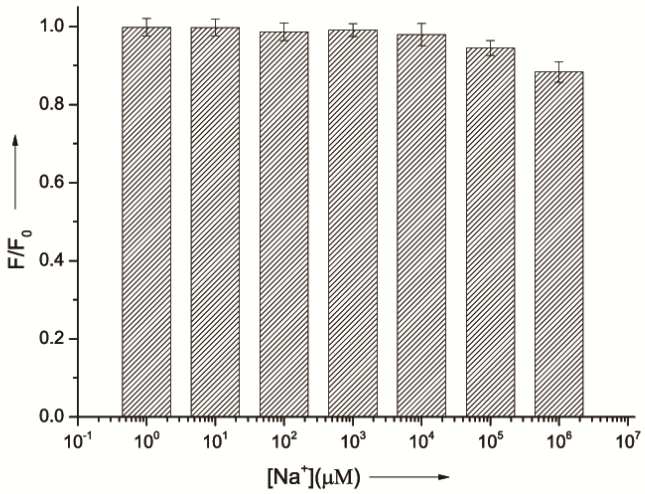

Figure S4. Selectivity evaluation of the aptasensor for K^+ using a series concentration of Na^+.

This journal is © The Royal Society of Chemistry 2013

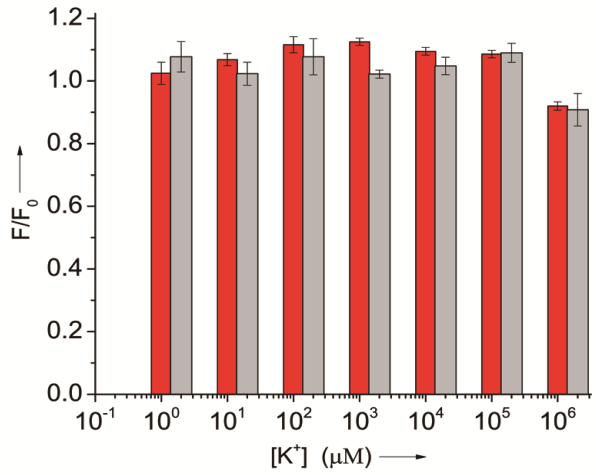

Figure S5. Selectivity evaluation of the aptasensor for K^+ using the control dsDNA supplied by the PicoGreen kit (red) and sulfadimethoxine aptamer and its complementary strand (gray).

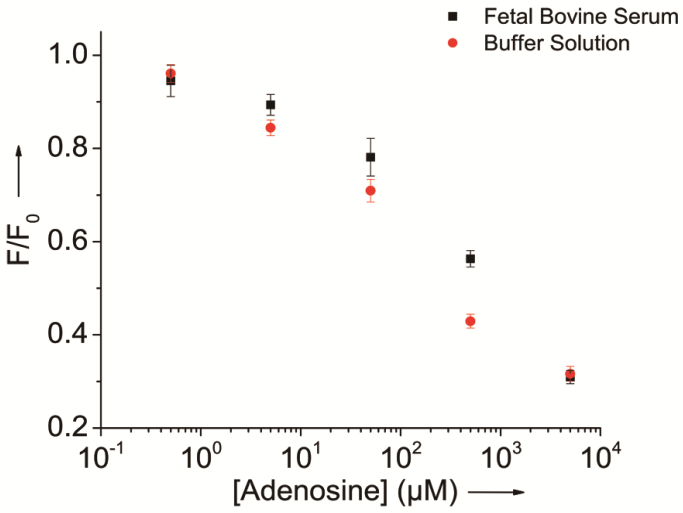

Figure S6. The linear fit of the fluorescence intensity in 20-fold diluted Fetal Bovine Serum (black) versus buffer solution (red) on a logarithmic scalefrom 0.5 μM to 5 mM.