Supplementary Material

Aptamer Fluorescence Anisotropy Assays for Detection of Aflatoxin B1 and Adenosine Triphosphate Using Antibody to Amplify Signal Change

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Table S1 List of oligonucleotide sequences

Name	Sequences (5' to 3')
Apt_{AFB} -5'FAM	FAM-TGC ACG TGT TGT CTC TCT GTG TCT CGT GC
Apt_{AFB}-3'FAM	TGC ACG TGT TGT CTC TCT GTG TCT CGT GC-FAM
$C14_{AFB}$	AGA CAA CAC GTG CA-Digoxin
Apt_{ATP}-5'FAM	FAM-CCT GGG GGA GTA TTG CGG AGG AAG G
$C11_{ATP}$	ACT CCC CCA GG - Digoxin

Table S2 Comparison of fluorescence polarization/anisotropy designs for detection of AFB1 and ATP with respect to affinity ligand, maximum signal change, and detection limit.

Targets	Methods	Affinity ligand	Maximum signal change	LOD	References
AFB1	Enzymes amplified FP sensing	aptamer	~0.275 （P）	0.24 pM	[25]
AFB1	FA assay using antibody as signal amplifier	aptamer	0.138 (r); 0.173 (P)	25 pM	Our assay
AFB1	FA assay using streptavidin for signal enhancement	aptamer	0.146 (r); 0.188 (P)	60 pM	[20]
AFB1	Direct FA assay for AFB1	aptamer	0.115 (r)	2 nM	[24]
AFB1	Competitive immune-FP assay using FAM-labeled AFB1	antibody	No mention	3.2 nM	[26]
AFB1	Competitive immune-FP assay using FAM-labeled AFB1	antibody	0.050 (P)	42 nM	[27]
ATP	Using quantum dots and Au nanoparticles	aptamer	~0.320 (P)	1.8 pM	[19]
ATP	Using silica nanoparticles for signal amplification	aptamer	~0.320 (P)	20 pM	[16]
ATP	Using DNA-protein hybrid nanowire assembly	aptamer	0.037 (r)	0.1 μM	[17]
ATP	Using GO for signal amplification	aptamer	~0.316 (P)	0.12 μM	[30]
ATP	FA assay using streptavidin for signal enhancement	aptamer	0.081 (r); 0.104 (P)	0.5 μM	[20]
ATP	Competitive assay	aptamer	0.174 (r); 0.230 (P)	0.5 μM	[31]
ATP	FA assay using antibody as signal amplifier	aptamer	0.060 (r); 0.080 (P)	1 μM	Our assay
ATP	Direct assay using dye-labeled aptamer	aptamer	0.047 (r) or 0.052 (r)	1 μM	[32]
ATP	Using GO for signal amplification	aptamer	~0.100 (P)	2 μM	[33]
ATP	Using antibody as mass enhancer and QDs	aptamer	~0.100 (P)	3.7 μM	[19]

r is corresponding to FA, and P is corresponding to FP.

Table S3 Comparison of EC_{50}, Cross reactivity (CR), detection limit and detection range of AFB1, AFB2, AFM1, AFM2, AFG1 and AFG2 using this method.

Target	EC_{50}/nM	CR	LOD / nM	Detection range / nM
AFB1	1.3	100%	0.025	0.025-100
AFB2	1.6	81%	0.025	0.025-100
AFM1	4.2	32%	0.39	0.39-100
AFM2	4.1	32%	0.39	0.39-100
AFG1	16	8%	1.6	1.56-800
AFG2	22	6%	1.6	1.56-800

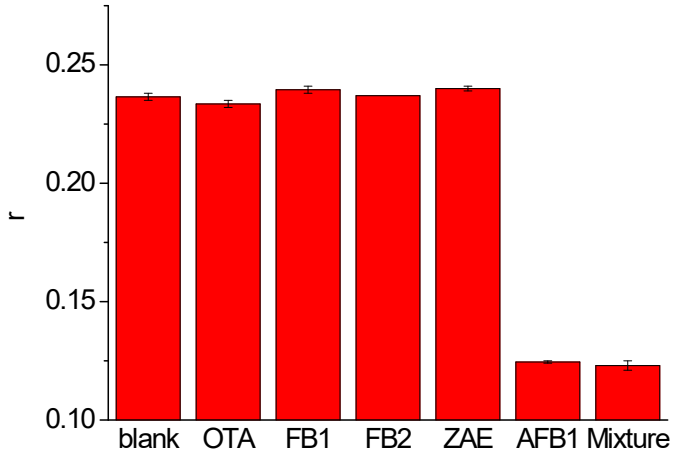

Fig. S1. Selectivity test of the aptamer FA sensor to AFB1 with other mycotoxins. Blank was the solution containing 1 nM Apt_{AFB}-5'FAM and 20 nM antibody-conjugated $C14_{AFB}$. AFB1 at 10 nM and the other mycotoxins at 200 nM were tested. Mixture sample contained 200 nM other mycotoxins and 10 nM AFB1.

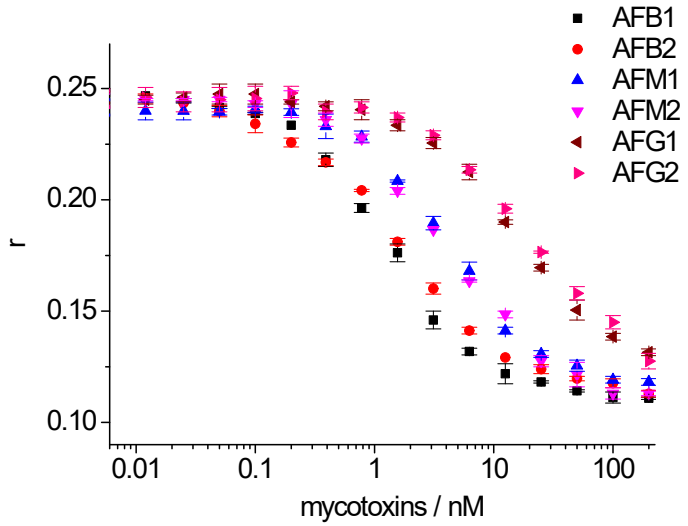

Fig. S2. FA responses corresponding to various concentrations of mycotoxins (such as AFB1, AFB2, AFM1, AFM2, AFG1, AFG2) in binding buffer using 1 nM Apt_{AFB}-5'FAM and 20 nM antibody-conjugated $C14_{AFB}$.

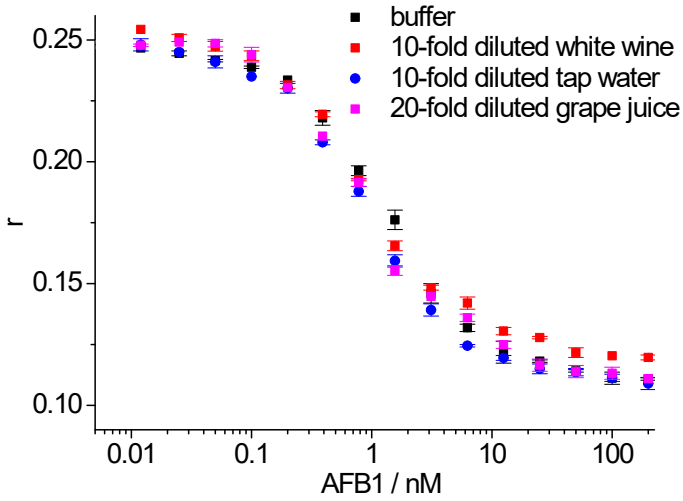

Fig. S3. FA values corresponding to various concentrations of AFB1 in binding buffer, 10-fold diluted white wine, 10-fold diluted tap water and 20-fold diluted grape juice samples. The concentration of Apt_{AFB}-5'FAM and antibody-conjugated $C14_{AFB}$ at 3' end were 1 nM and 20 nM, respectively.

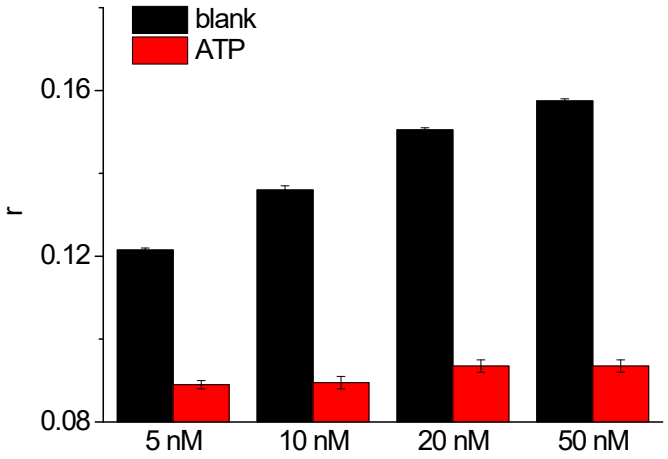

Fig. S4. Effects of concentrations of antibody-conjugated $C11_{ATP}$ on the FA values corresponding to the blank sample and the sample containing ATP (500 μM ATP). The concentration of Apt_{ATP}-5'FAM was 5 nM.

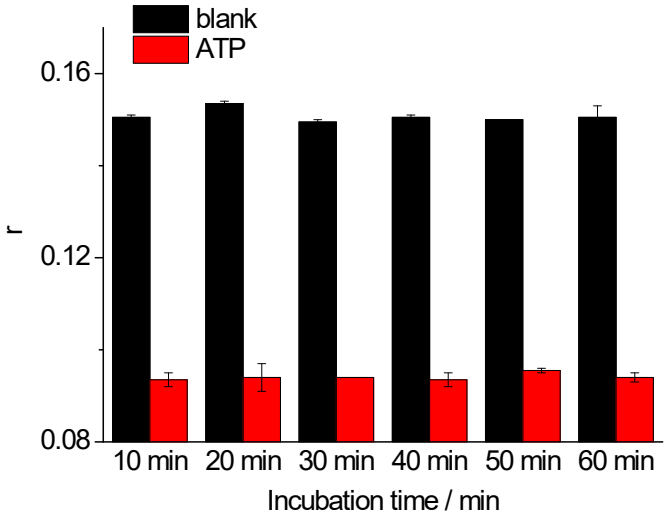

Fig. S5. Effects of incubation time on the FA values corresponding to the blank sample and the sample containing ATP (500 μM ATP). The concentration of Apt_{ATP}-5'FAM and antibody-conjugated $C11_{ATP}$ were 5 nM and 20 nM, respectively.

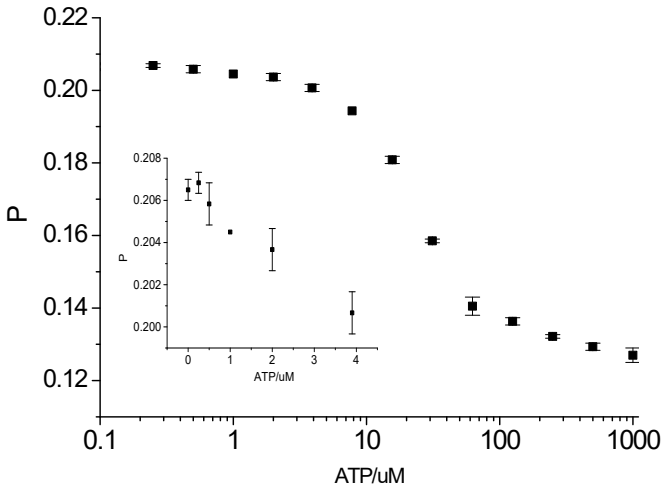

Fig. S6. Quantitative detection of ATP. FP responses versus the concentrations of ATP. The inset shows the FP signals corresponding to low concentrations of ATP.

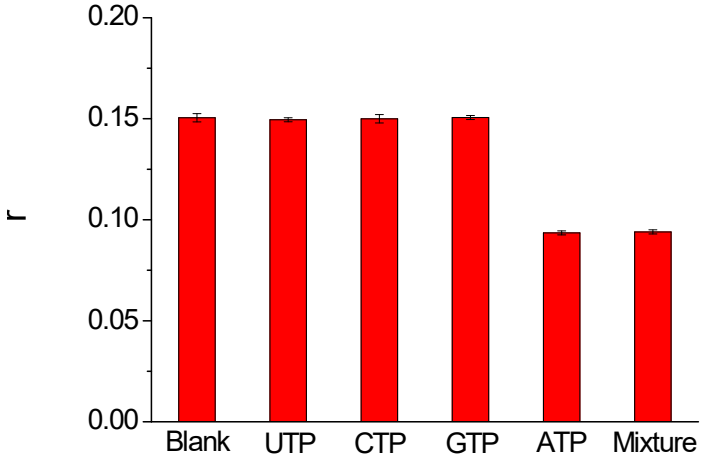

Fig. S7. Selectivity of the aptamer FA assay for ATP. Blank was the solution containing 5 nM Apt_{ATP}-5'FAM and 20 nM antibody-conjugated $C11_{ATP}$. ATP at 500 μM and the other small molecules at 500 μM were tested. Mixture sample contained 500 μM other small molecules and 500 μM ATP.

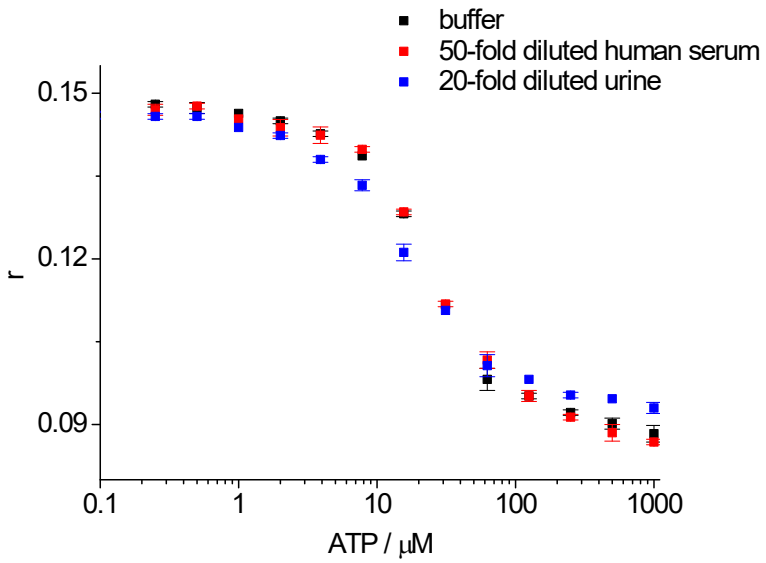

Fig. S8. FA values corresponding to various concentrations of ATP in binding buffer, 50-fold diluted human serum and 20-fold diluted urine samples. The concentration of Apt_{ATP}-5'FAM and antibody-conjugated $C11_{ATP}$ were 5 nM and 20 nM, respectively.