Electronic Supplementary Information

Split Aptamer Mediated Endonuclease Amplification for Small-Molecule Detection

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Experimental section

Martials and reagents. 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. Adenosine, thymine, cytidine, uridine and inosine were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Morphine hydrochloride (MHC), caffeine, theophylline, benzoylecgonine and cocaine were obtained from Beijing Institute for Drug Control (Beijing, China) and used without further purification. The human serum samples were obtained from the local hospital. The Endonuclease IV and 10 × NEBuffer 3 (1000 mM NaCl, 500 mM Tris-HCl (pH 7.9), 100 mM $MgCl_2$ and 10 mM DTT) were purchased from New England Biolabs Ltd (Beijing, China). Deionized and sterilized water (resistance >18.2 MΩ) was used throughout the experiments.

Fluorescence analysis for the three-way junction structure mediated endonuclease amplification. Detection probe with AP site one-base, two-base, three-base and four-base away from junction sites were incubated with different DNA probes (DNA probe **1** and Endo IV; DNA probe **2** and Endo IV; DNA probe **1**, DNA probe **2** and Endo IV). Detection probe 1 μM, DNA probe **1** 100 nM, DNA probe **2** 100 nM, Endo IV 1 U, 1 × NEBuffer 3 were used for all the experiments. After 2.5 h incubation time at 37 ºC, fluorescence spectrum of each sample was recorded at room temperature in a 100 μL quartz cuvette on a Fluorolog-Tau-3 spectrofluorometer (Jobin Yvon Inc., NJ) with the slit set to be 2.5 nm for both the excitation and the emission using 494 nm for excitation. For the Endo IV mediated cleavage reaction

study, a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with a CFX96 was used to record real-time fluorescence intensity at 518 nm. Real-time fluorescence intensity was monitored in 30 s intervals using the FAM/SYBR Green channel. The cleavage reactions were conducted at different concentrations of DNA substrate with detection probe at 1 μM and Endo IV at 1 U in 1 × NEBuffer 3. The initial reaction velocity was determined from the slope of the progress curve from the first 10 min data points after Endo IV addition.

Gel electrophoresis analysis of SAMEA assay. The assay was performed with different DNA probes in the presence or in the absence of adenosine. Each sample was incubated at for 37 ºC 2.5 h. The resultant mixture was collected and analyzed using gel electrophoresis in 7% (w/w) agarose stained by 0.5 μg/mL goldview and 0.5 μg/mL ethidium bromide. Electrophoresis was performed at a constant voltage of 101 V for 120 min with a load of 10 μL of sample in each lane. The gel was visualized using a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company, China).

Fluorescence analysis of SAMEA strategy. For adenosine assay, adenosine AFP1, adenosine AFP2, detection probe were incubated in 1 × NEBuffer 3 at 37 ºC for 20 min. Then, Endo IV was added into the reactions system and incubating at 37 ºC for 2.5 h before allowing fluorescence detection. The final concentration in the 30 μL reaction system were 100 nM adenosine AFP1, 100 nM adenosine AFP2, 1μM detection probe and 1 U Endo IV. Concentrations of adenosine were ranged from 0 to 5 mM. For cocaine detection, cocaine AFP1 and cocaine AFP2 were used instead of

adenosine AFP1, adenosine AFP2. Concentrations of cocaine were ranged from 0 to 5 mM. The fluorescence spectra were measured at room temperature in a 100 μL quartz cuvette on a Fluorolog-Tau-3 spectrofluorometer (Jobin Yvon Inc., NJ) with the slit set to be 2.5 nm for both the excitation and the emission. Time-dependent fluorescence responses of the SAMEA assay were performed with a time interval of 30 s in a 384-well black microplate on a Tecan Infinite M-1000 microplate reader. The excitation wavelength was 494 nm, and the emission wavelength was 518 nm with both excitation and emission bandwidths of 10 nm.

Results section

Design of three-way junction structure mediated endonuclease amplification.

To investigate the possibility of Endo IV-mediated amplification for split aptamer, we design a three-way junction structure using two DNA probes, 1 and 2, with a detection probe (Table S1). As shown in Fig. S1, these two DNA probes can hybridize with each other to form a DNA duplex with two tail sequences extended at one side. The detection probe is designed as a dually-labeled probe with an apurinic or apyrimidinic (AP) site flanked by two sequences complementary to the two tail sequences of DNA probes, 1 and 2, respectively. This probe delivers a low fluorescent background in its intact state because of efficient förster resonance energy transfer (FRET) between these two labels, the fluorophore FITC and the quencher TAMRA. Detection probe only has a short sequence such that the melting temperature for the duplex between probe and its perfectly matched sequence is ~50 ^{o}C, as calculated by DNA folding (http://www.bioin- fo.rpi.edu/applications/).[1] This design implies that probe 3 can be annealed on two tail sequences of the DNA duplex between probes **1** and **2** and generate a three-way junction structure. On the other hand, provided that probe is cleaved by Endo IV, its fragments are too short to stably hybridize with the tail sequences. That is, provided that probe is cleaved by Endo IV in the three-way DNA junction, the tail sequences in the duplex of probes **1** and **2** are released and allowed to hybridize with another detection probe, mediating a cycling of the cleavage of the detection probe. This cycling is able to activate an intense fluorescence signal because of the separation of the fluorophore FITC from the

quencher TAMRA and thus creates substantial signal amplification for detecting the duplex of probes **1** and **2**. Fig. S2 depicts typical fluorescence spectral responses of Endo IV-mediated amplification from this three-way DNA junction. One observed that incubation of the detection probes separately with probe **1** or **2** mostly gave very weak fluorescence signals, but the detection probe with the AP site four bases away from the junction displayed a slightly large fluorescence response. This slight fluorescence activation was ascribed to cleavage of the detection probe by one of the tail sequence. In contrast, incubation of the detection probes with the duplex of probes **1** and **2** resulted in fluorescence activation to a degree dependent upon the distance from the AP site to the junction. In the cases when the AP site was one- or two-base away from the junction, the fluorescence responses were relatively low, presumably because the steric hindrance of the junction prevented Endo IV from approach and cleaving the AP site. For detection probes with the AP site three- or four-base away from the junction, the fluorescence activation signal became very intense, indicating efficient cleavage of the detection probe. This maximized signal-to-background ratio was obtained for the detection probe with the AP site three-base away from the junction, which was attributed to the minimized steric hindrance and the optimized selectivity for the three-way junction mediated cleavage of the detection probe. A further analysis of Endo IV mediated cleavage reactions revealed that the Michaelis constant for Endo IV mediated cleavage of this three-way junction was ~1.61 μM, which was merely slightly larger than the Michaelis constant (~0.71 μM) for Endo IV mediated cleavage of the linear DNA duplex as shown in Fig. S3. These data implied

a finding that the three-way junction was an active substrate for Endo IV, provided the AP site was three-base away from the junction. It is noteworthy that because the three-way junction is a very common design in nucleic acid chemistry,[2] this finding is expected to hold great potential for the development of new sensitive strategies for nucleic acid based analysis.

Table S1. Sequences of DNA probes[a]

Name	Sequence (5′-3′)
DNA probe 1-1	ACCTTCCTCCGCA CGAAC *TAGATTGTAT*
DNA probe 2-1	*GTTGAGGTAG* GTTCGTGCGGAGGAAGGT
DNA probe 1-2	ACCTTCCTCCGCA CGAAC *GTAGATTGTAT*
DNA probe 2-2	*GTTGAGGTA* GTTCGTGCGGAGGAAGGT
DNA probe 1-3	ACCTTCCTCCGCA CGAAC *AGTAGATTGTAT*
DNA probe 2-3	*GTTGAGGT* GTTCGTGCGGAGGAAGGT
DNA probe 1-4	ACCTTCCTCCGCA CGAAC *TAGTAGATTGTAT*
DNA probe 2-4	*GTTGAGG* GTTCGTGCGGAGGAAGGT
Cocaine AFP1	**GGGAGTCAAGAAC***AGTAGATTGTAT*
Cocaine AFP2	*GTTGAGGT***GTTCTTCAATGAAGTGGGACGACA**
Adenosine AFP1	**ACCTGGGGGAGTAT**CGAAC*AGTAGATTGTAT*
Adenosine AFP2	*GTTGAGGT*GTTCG**TGCGGAGGAAGGT**
Detection probe	ATACAAT(FITC)CXACT(TAMRA)ACCTCAAC

[a]Boldface type indicates split aptamer sequences of cocaine and adenosine. Italic type in AFP 1 and AFP 2 shows tail sequence. The stabilizer sequences are highlighted in red. X represents abasic sites. DNA probes 1-1 and 2-1, 1-2 and 2-2, 1-3 and 2-3, 1-4 and 2-4 are paired separately with the detection probe to form three–way junction structures. The AP site in the detection probe is at one-base, two-base, three-base, or four-base away from the junction site with DNA probes 1-1 and 2-1, 1-2 and 2-2, 1-3 and 2-3, 1-4 and 2-4 accordingly.

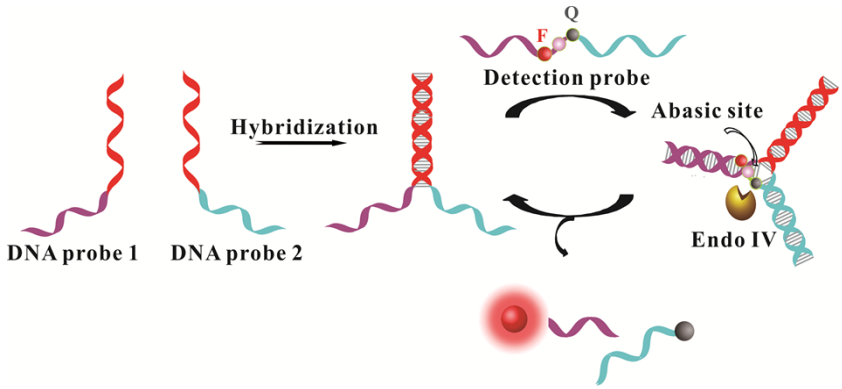

Fig. S1. Design of three-way junction structure mediated endonuclease amplification.

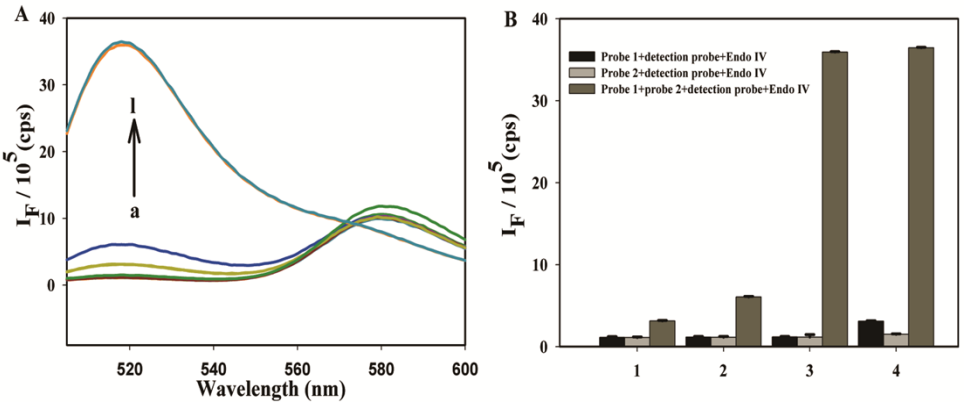

Fig. S2. (A) Fluorescence spectral responses for Endo IV-mediated amplification under different conditions. Detection probe with AP site one-base, two-base, three-base and four-base away from junction sites incubated with DNA probe 1 and Endo IV (b, d, f, i); DNA probe 2 and Endo IV (a, c, e, g); DNA probe 1, DNA probe 2 and Endo IV (h, j, k, l). (B) The corresponding fluorescence peak intensities at 518 nm of detection probe with AP site one-base (1); two-base (2); three-base (3); and four-base (4) away from junction sites incubated with different DNA probes and Endo IV.

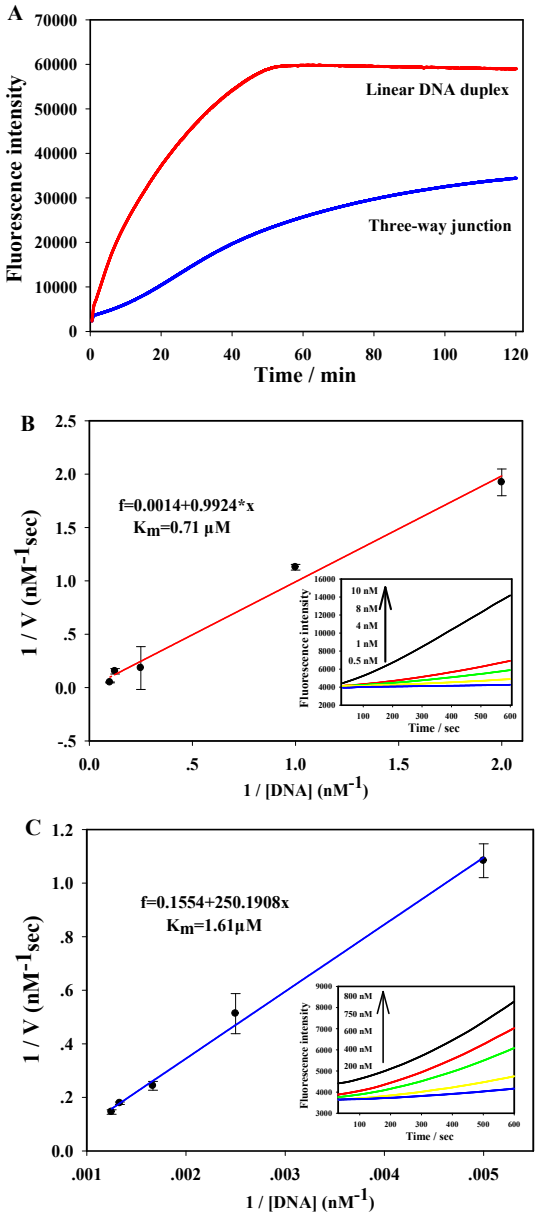

Fig. S3. Fluorescence activation rates of linear DNA duplex and three-way DNA junction structure. Fluorescence-based progress curves of the Endo IV-catalyzed reaction as a function of time (A); Double reciprocal (Lineweaver-Burk) plot of the initial degradation velocity as a function of concentrations of linear DNA duplex (B) and three-way DNA junction structure (C). Error bars are standard deviation of three repetitive experiments.

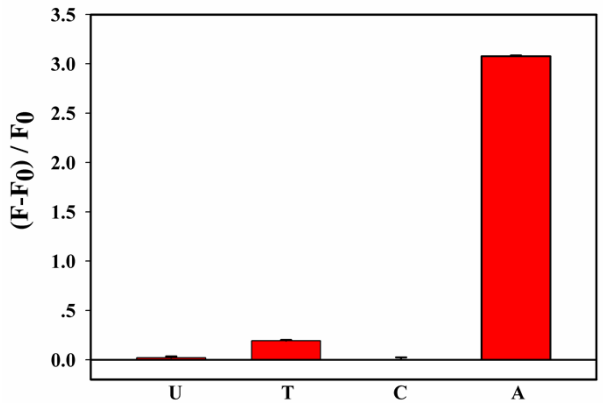

Fig. S4. Selectivity for adenosine assay using the SAMEA strategy. The concentration of each analyte was 5 mM. F and F_0 correspond to fluorescence intensities obtained in the presence or absence of analytes, respectively. Error bars are standard deviation of three repetitive experiments.

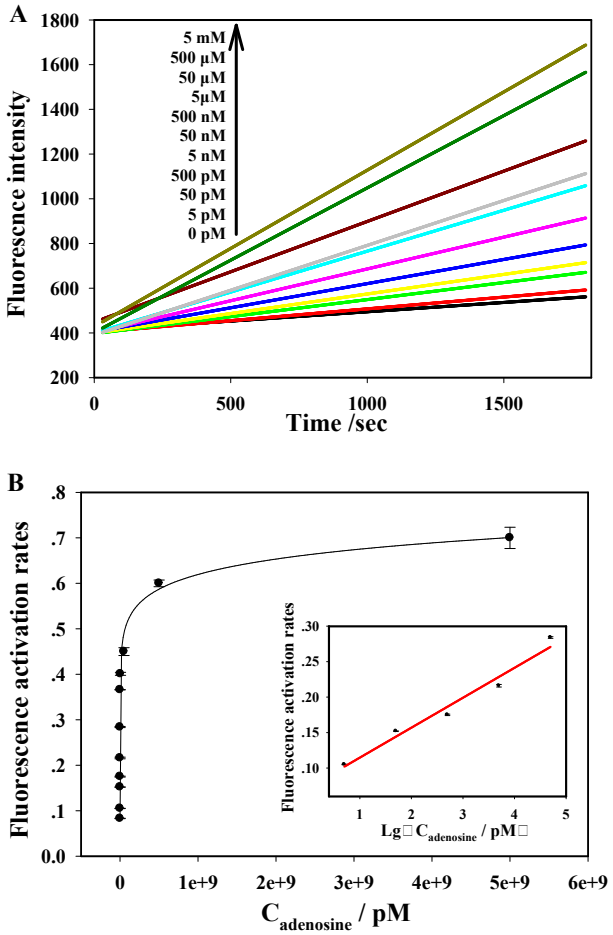

Fig. S5. (A) Time-dependent fluorescence responses of SAMEA assay for various concentration of adenosine in 30 min. (B) Fluorescence activation rates versus adenosine concentrations. Inset: linear relationship between the fluorescence activation rates and the logarithm of adenosine concentration. Error bars are standard deviation of three repetitive experiments.

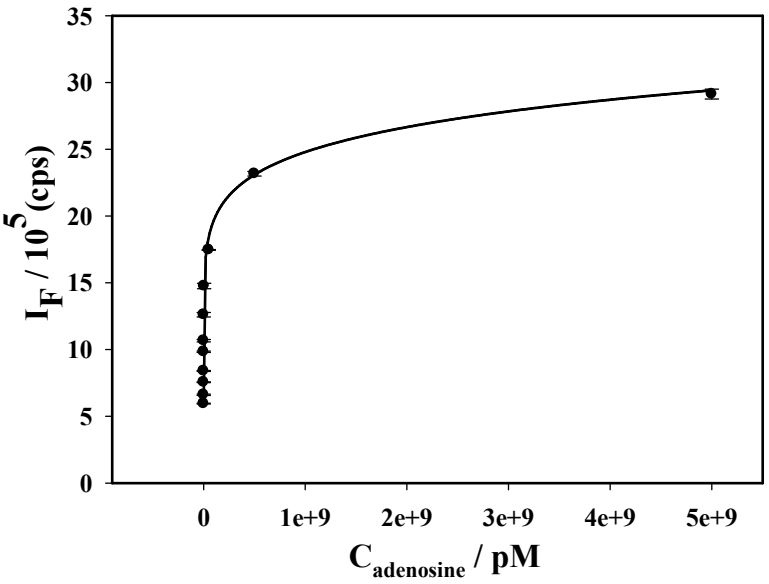

Fig. S6. The calibration curve for adenosine detection in diluted serum. Error bars were estimated from three replicate measurements.

Table S2. Recovery experiments of adenosine in 10% human sera samples

Samples	Added adenosine (nM)	Detected adenosine[a] (nM)	Recovery (%)
1	0	0.0252 ± 0.001	
2	0.005	0.0301 ± 0.001	98.0
3	0.05	0.073 ± 0.004	95.6
4	0.5	0.477 ± 0.017	90.4
5	5	5.443 ± 0. 3	108.4
6	50	50.602 ± 2	101.2

[a]Average of three determinations ± standard deviation

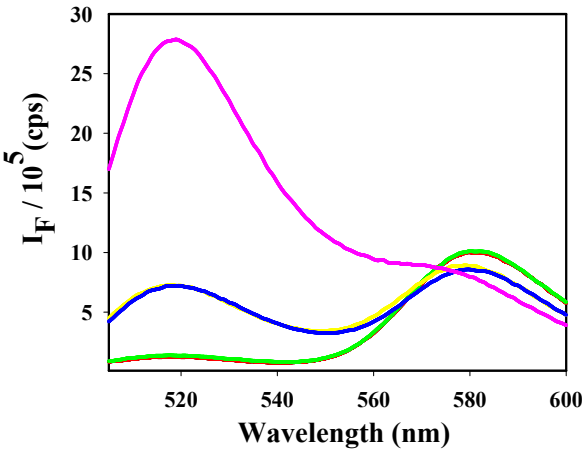

Fig. S7. Typical fluorescence spectral responses for cocaine detection. Cocaine AFP1 + detection probe + Endo IV (red), cocaine AFP2 + detection probe + Endo IV (green), cocaine AFP1 + cocaine AFP2 + detection probe + Endo IV (cyan), benzoylecgonine + cocaine AFP1 + cocaine AFP2 + detection probe + Endo IV (blue), cocaine + cocaine AFP1 + cocaine AFP2 + detection probe + Endo IV (pink). Reactions were performed at 37 ºC for 2.5 h and detection probe 1 μM, cocaine AFP1 100 nM, cocaine AFP2 100 nM, cocaine 5 mM, benzoylecgonine 5 mM, Endo IV 1U were used for all experiments.

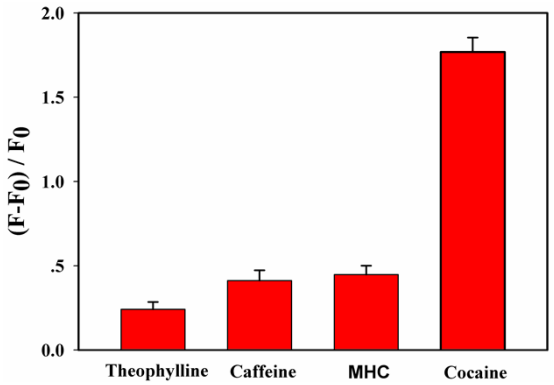

Fig. S8. Selectivity for cocaine assay using the SAMEA strategy. The concentration of each analyte was 1 mM. F and F_0 correspond to fluorescence intensities obtained in the presence or absence of analytes, respectively. Error bars are standard deviation of three repetitive experiments.

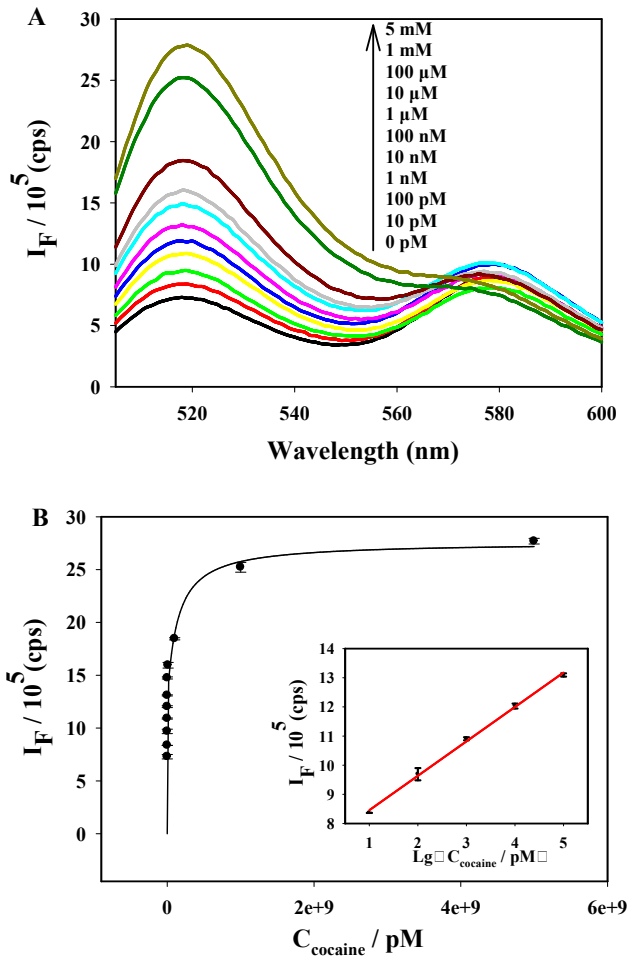

Fig. S9. (A) Fluorescence spectra of SAMEA assay in response to cocaine of various concentrations. (B) The corresponding fluorescence response at 518 nm of the SAMEA assay. Inset: linear relationship between fluorescence peak intensity and the logarithm of cocaine concentration. Error bars are standard deviation of three repetitive experiments.

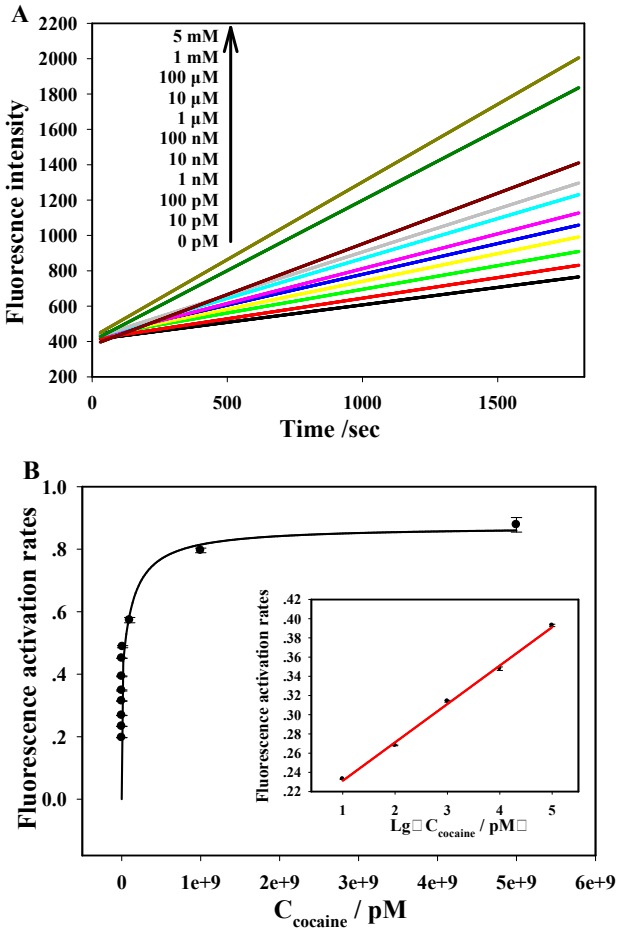

Fig. S10. (A) Time-dependent fluorescence responses of SAMEA assay for various concentration of cocaine in 30 min. (B) Fluorescence activation rates versus adenosine concentrations. Inset: linear relationship between the fluorescence activation rates and the logarithm of cocaine concentration. Error bars are standard deviation of three repetitive experiments.

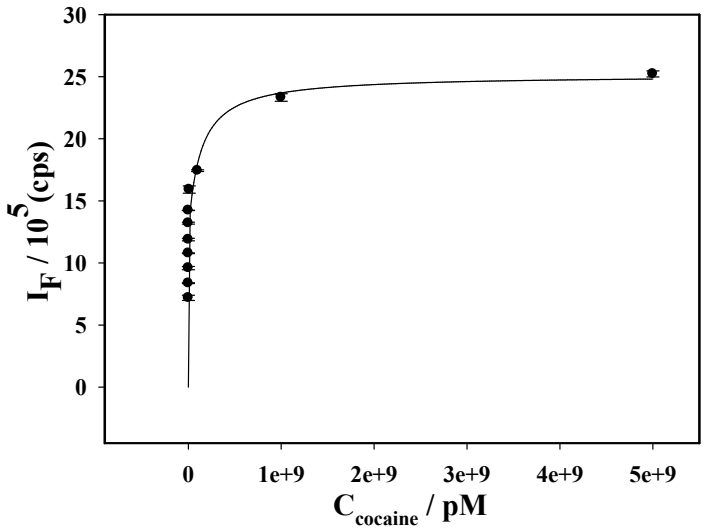

Fig. S11. The calibration curve for cocaine detection in diluted serum. Error bars were estimated from three replicate measurements.

Table S3. Recovery experiments of cocaine in 10% human sera samples

Samples	Added cocaine (nM)	Detected cocaine [a] (nM)	Recovery (%)
1	0	0.000003 ± 0.0000002	
2	0.01	0.00945 ± 0.0002	94.5
3	0.1	0.0915 ± 0.005	91.5
4	1	0.936 ± 0.07	93.6
5	10	10.51 ± 0.38	105.1
6	100	105.7 ± 6	105.7

Average of three determinations ± standard deviation

References:

1. M. Zuker, *Nucleic Acids Res.,* 2003, **31**, 3406–3415.

2. F. Li, Y. W. Lin and X. C. Le, *Anal. Chem.,* 2013, **85**, 10835 −10841.