Supporting Information

A cost-effective fluorescence biosensor for cocaine based on "mix-and-detect" strategy

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1. Experimental Section

Table S1. Sequences of cocaine aptamer used in this work

Name	Sequence (5'-3')
MNS-4.1	GGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCGATA
coc.ap2	GGGAGACAAGGA**CAG**TCCTTCAATGAAGTGGGTC**TCCC**
coc.ap3	GGGAGACAAGGAAAATCCTTCAATGAAGTGGGTC**TCCC**
coc.ap4	GGGAGACAAGGA**CAG**TCCTTCAATGAAGTGGGTCGACA
coc.P1	**AGC**GGGAGACAAGAACGAA
coc.P2	TTCGTTCTTCAATGAAGTGGGTCGACA**GCT**
38-GC	GGGAGACAAGGAAAATCCTTCAA**C**GAAGTGGGTC**TCCC**
38GC-2	GGGAGACAAGGAAAATCCTTCAA**C**GAAGTGGGTCGACA
38-GC-3	GGGAGACAAGGAAAATCCTTCAA**C**GA**C**GTGGGTC**TCCC**
coc.ap2-GC	GGGAGACAAGGA**CAG**TCCTTCAACGAAGTGGGTC**TCCC**
coc.ap4-GC	GGGAGACAAGGA**CAG**TCCTTCAA**C**GAAGTGGGTCGACA
coc.ap2-GC-M1	GGGAGACAAGGACAGTCCT**CT**AACGAAGTGGGTCTCCC
coc.ap2-GC-M2	GGGAGACAAGGACAGTCCT**AC**AACGAAGTGGGTCTCCC

The bases of variants different from aptamer MNS-4.1 is marked in red.

2. Supplementary results

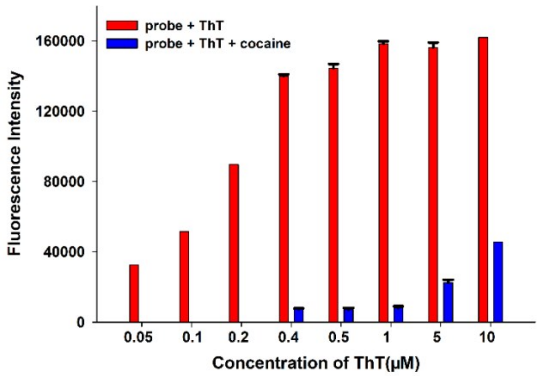

Fig. S1 Effect of different concentrations of ThT on fluorescence intensity in the presence or absence of cocaine. The concentration of the probe and cocaine were 200 nm and 500 μM, respectively. Error bars represent the SDs of three measurements.

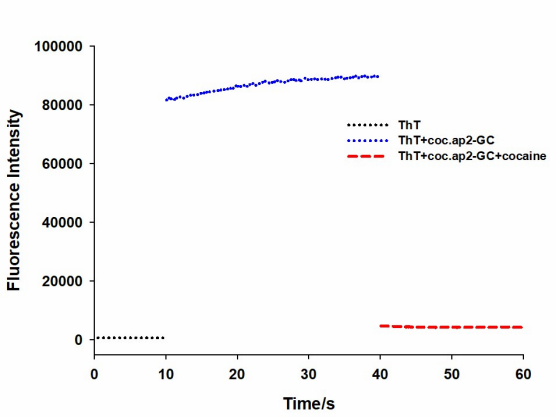

Fig. S2 Dynamic fluorescence response of the ThT for the progress of the ThT has been bound to the coc.ap2-GC and ThT replacement by cocaine within one minute. The concentration of the coc.ap2-GC, ThT and the cocaine were 200 nM, 1 μM, and 500 μM, respectively.

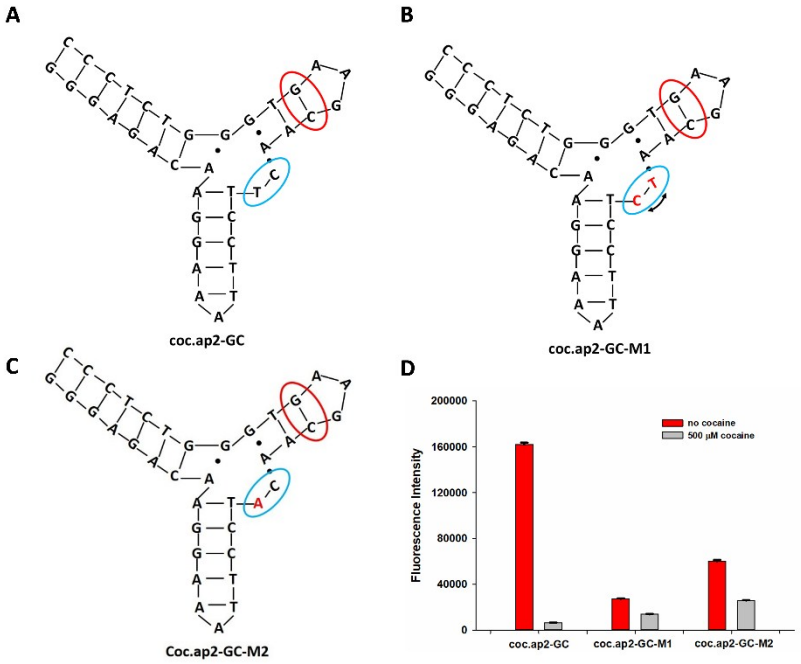

Fig. S3 Aptamer variants: (A) coc.ap2-GC; (B) coc.ap2-GC-M1; (C) coc.ap2-GC-M2. (D) Fluorescence analysis of the interaction between ThT with different aptamer variants in the present or absent of the cocaine. The concentration of the variants, ThT and the cocaine were 200 nM, 1 μM, and 500 μM, respectively. Error bars represent the SDs of three measurements.

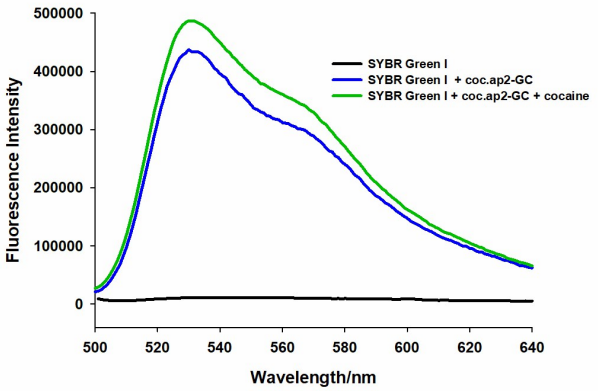

Fig. S4 The fluorescence spectra of the SYBR Green I/cocap2-GC aptasensor. Experimental conditions: [cocap2-GC]=200 nM, [SYBR Green I]=0.006 × and [cocaine]=500 μM, with excitation at 490 nm and emission range from 500 to 640 nm.

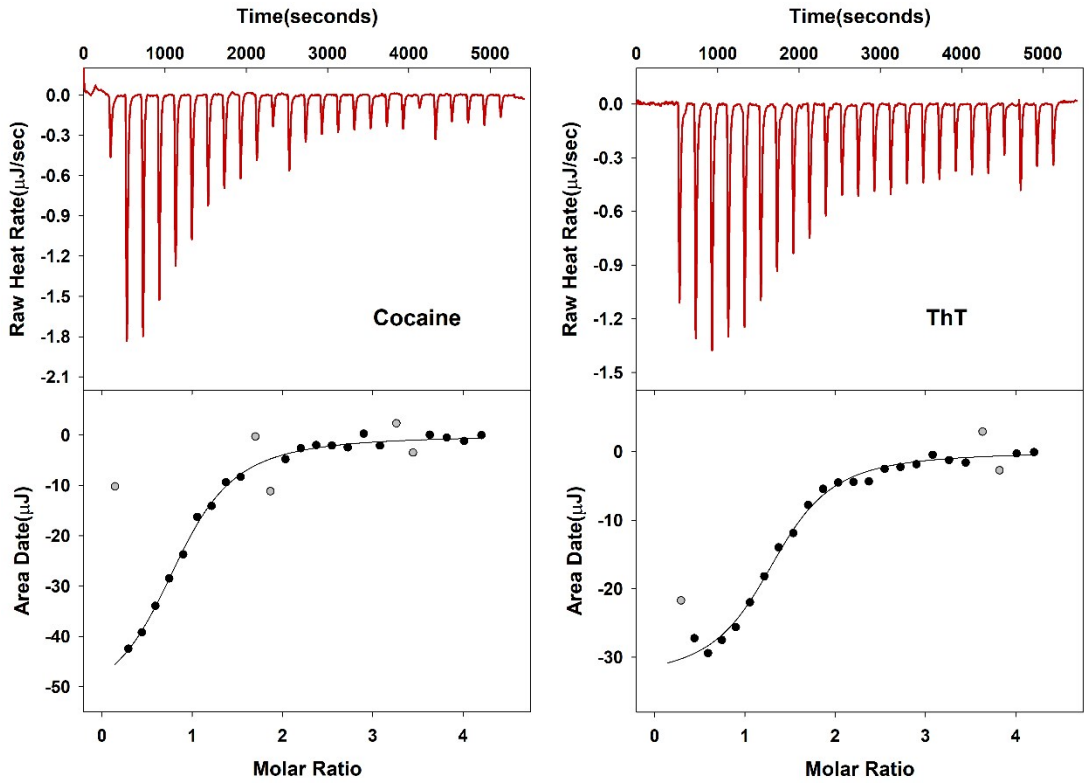

Fig.S5. (A) ITC data demonstrated that cocap2-GC binds both cocaine and ThT. The top is the heat generated from each injection of cocaine or ThT into the cocap2-GC aptamer solution. The bottom is the integrated heat plot after correcting for the heat of dilution. The concentration of cocap2-GC, cocaine, and ThT were 20 μM, 500 μM, and 500 μM, respectively.

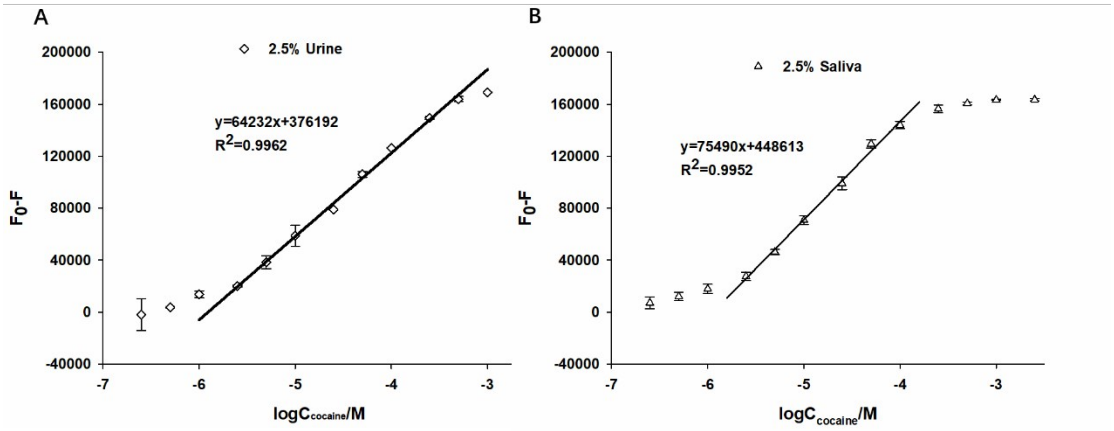

Fig.S6. Calibration curves for ThT/coc.ap2-GC-competitive sensor cocaine detection in body fluids samples. A) in 2.5 urine B) in 2.5% saliva. The concentration of the coc.ap2-GC and ThT were 200 nM, 1 μM, respectively. Error bars represent the SDs of three measurements.

Table S2 Comparison of Analytical Performance of Various Different Methods for the Determination of Cocaine

Method	Limit of detection	Response time
Cy7 displacement[1], Colorimetric	2 μM	12 hours
SYBR-gold binding[2], Fluorescence	5 μM	3 hours
Strand-displacement amplification[3], Fluorescence	1 nM	2 hours
Enzyme-assisted target recycling[4], Fluorescence	0.2 μM	2 hours
DNAzyme-based amplification[5], Colorimetric	1 μM	15 min
Exonuclease inhibition[6], Fluorescence	0.1 μM	25 min
Microcantilever[7], Interferometric	5 μM	25 min
Aptamer conformational change[8], Electrochemistry	10 μM	Seconds
ATMND-38-GC complex[9], Fluorescence	0.2 μM	Seconds
ThT-coc.ap2-GC complex, Fluorescence (this work)	0.25 μM	Seconds

Table S3 Results of the recovery test of cocaine in real sample

Sample	Added (μM)	Assayed (μM)	Recovery (%) n=3	RSD (%) n=3
Human serum (2.5%)	5.00	4.62	92.4	0.57
	10.00	9.96	99.6	0.64
	50.00	51.75	103.5	2.18
Human urine (2.5%)	5.00	5.19	103.8	0.15
	10.00	10.60	106.0	2.66
	50.00	50.55	101.1	2.60

The healthy human serum and urine samples were obtained from Dongguan Tungwah Hospital. All values were obtained as the average of three repetitive determination plus standard deviation.

2. References

1. M. N. Stojanovic and D. W. Landry, *J. Am. Chem. Soc.*, 2002, **124**, 9678-9679.
2. D. Zheng, R. Zou and X. Lou, *Anal. Chem.*, 2012, **84**, 3554-3560.
3. H. Jing-Lin, W. Zai-Sheng, Z. Hui, W. Hong-Qi, J. Jian-Hui, S. Guo-Li and Y. Ru-Qin, *Anal. Chem.*, 2010, **82**, 1358-1364.
4. C. H. Lu, J. Li, M. H. Lin, Y. W. Wang, H. H. Yang, X. Chen and G. N. Chen, *Angew. Chem. Int. Ed.*, 2010, **49**, 8454-8457.
5. Y. Luo, H. Yu, O. Alkhamis, Y. Liu, X. Lou, B. Yu and Y. Xiao, *Anal. Chem.*, 2019, **91**, 7199-7207.
6. J. Canoura, Z. W. Wang, H. X. Yu, O. Alkhamis, F. F. Fu and Y. Xiao, *J. Am. Chem. Soc.*, 2018, **140**, 9961-9971.
7. K. Kang, A. Sachan, M. Nilsen-Hamilton and P. Shrotriya, *Langmuir*, 2011, **27**, 14696-14702.
8. B. R. Baker, R. Y. Lai, M. C. S. Wood, E. H. Doctor, A. J. Heeger and K. W. Plaxco, *J. Am. Chem. Soc.*, 2006, **128**, 3138-3139.
9. D. Roncancio, H. Yu, X. Xu, S. Wu, R. Liu, J. Debord, X. Lou and Y. Xiao, *Anal. Chem.*, 2014, **86**, 11100-11106.