SUPPORTING INFORMATION

Amplified Optical Aptasensors through the Endonuclease-Stimulated Regeneration of the Analyte

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1. Non-amplified aptamer system for ATP detection

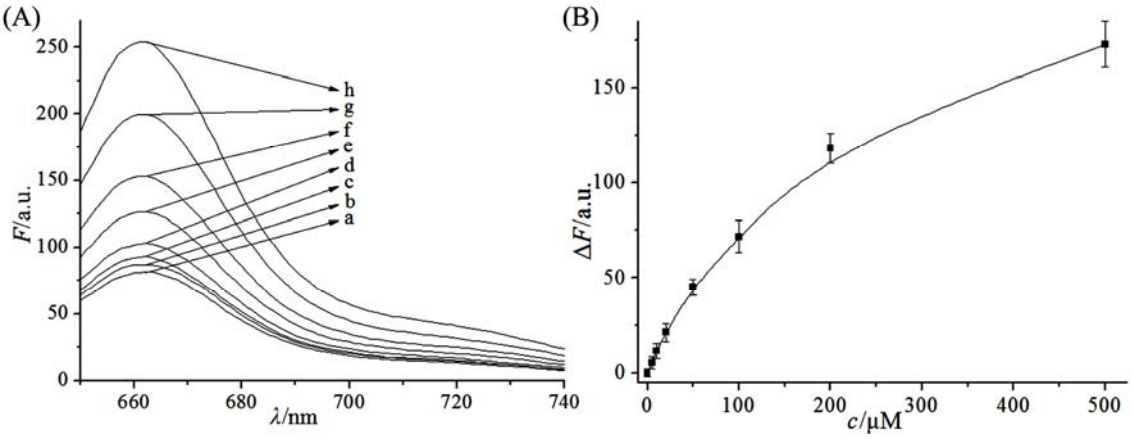

Fig. S1 (A) Fluorescence spectra corresponding to the analysis of different concentrations of ATP according to Figure 1A. Spectra recorded after a fixed time interval of 2 h. Concentrations of ATP correspond to (a) 0 μM; (b) 5 μM; (c) 10 μM; (d) 20 μM; (e) 50 μM; (f) 100 μM; (g) 200 μM; (h) 500 μM. (B) Calibration curve corresponding to the resulting fluorescence intensities at λ = 662 nm upon analyzing different concentrations of ATP.

2. Polyacrylamide gel electrophoresis

To support the endonuclease-mediated cleavage of the aptamer subunits/ATP complex we performed complementary gel electrophoresis experiments. Nonetheless, to follow the different fragmentation products we modified the blocker unit (**3**) and one of the aptamer subunits (**2**) by tethering to their 5'- and 3'-ends non-relevant nucleic acid chains that are aimed to increase the molecular weight of the respective oligonucleotides, as shown in **Fig. S2 (A)**. The blocker unit (**3a**) and the subunit (**2a**) were used as modified components

2a: 5'-**TTT**TAGGCCTGGGGGAGTATCAGCTGTA**TT**-3'

3a: 5'-**TTTTTTTTTTTTTTTTTTTTTTTTTTT**CTTCCTCCGCTCAG-3'

After the endonuclease-mediated cleavage of the aptamer subunits (**1**)(**2a**)-ATP complex, the fragmented nucleic acid **X** being a part of (**2a**) and the oligonucleotide composed of sequence **Y**, that hybridizes with the blocker (**3a**), are formed.

X= CCTGGGGGAGTATCAG

Y= CTGAGCGGAGGAAGG

Fig. S2(B) shows the denatured gel electrophoresis of the resulting system. The fragmented components are clearly observed only in the system that includes the endonucleases.

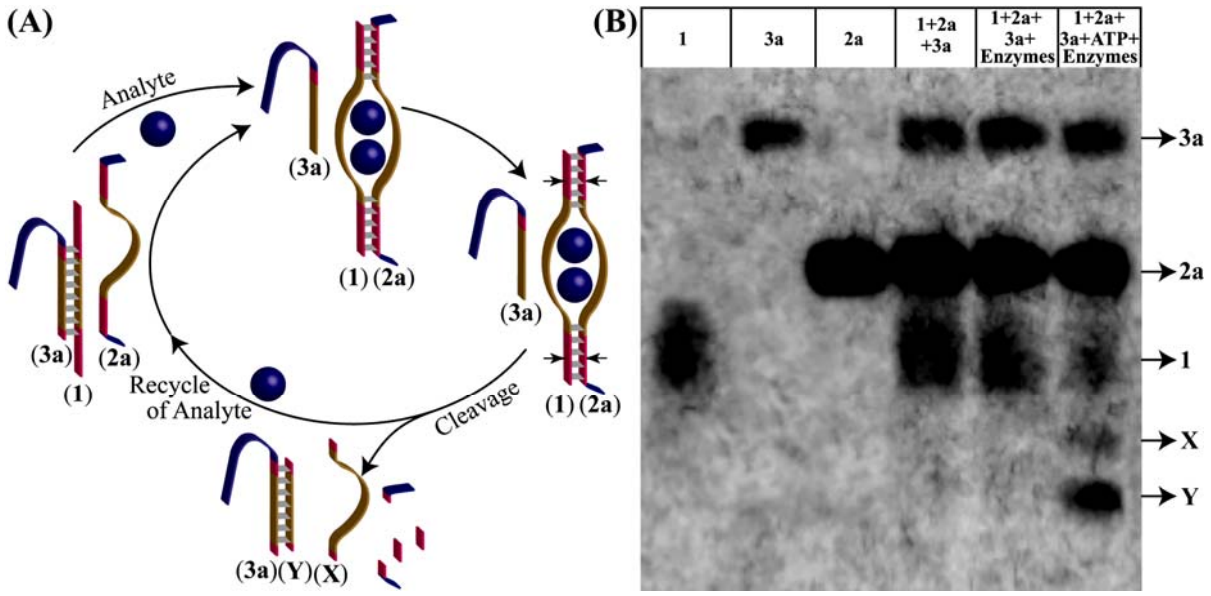

Fig. S2 (A) Schematic amplified detection of ATP. (B) The denatured gel electrophoresis.

3. Non-amplified aptamer system for VP detection

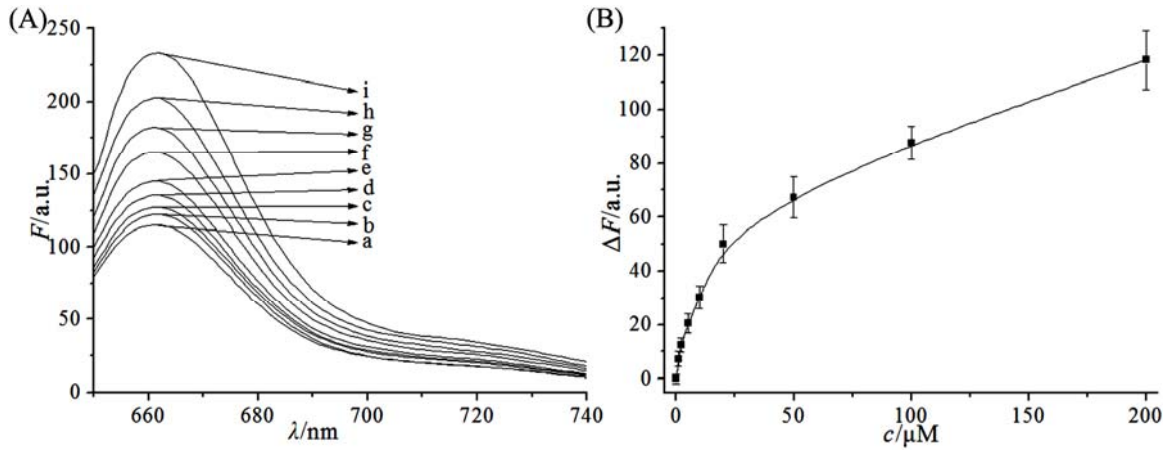

Fig. S3 (A) Fluorescence spectra corresponding to the analysis of different concentrations of VP using the non-amplified aptamer subunits system. Spectra recorded after a fixed time interval of 2 h. Concentrations of VP correspond to: (a) 0 μM; (b) 1 μM; (c) 2 μM; (d) 5 μM; (e) 10 μM; (f) 20 μM; (g) 50 μM; (h) 100 μM; (i) 200 μM. (B) Calibration curve corresponding to the resulting fluorescence intensities at λ = 662 nm upon analyzing different concentrations of VP.

4. Non-amplified aptamer system for cocaine detection

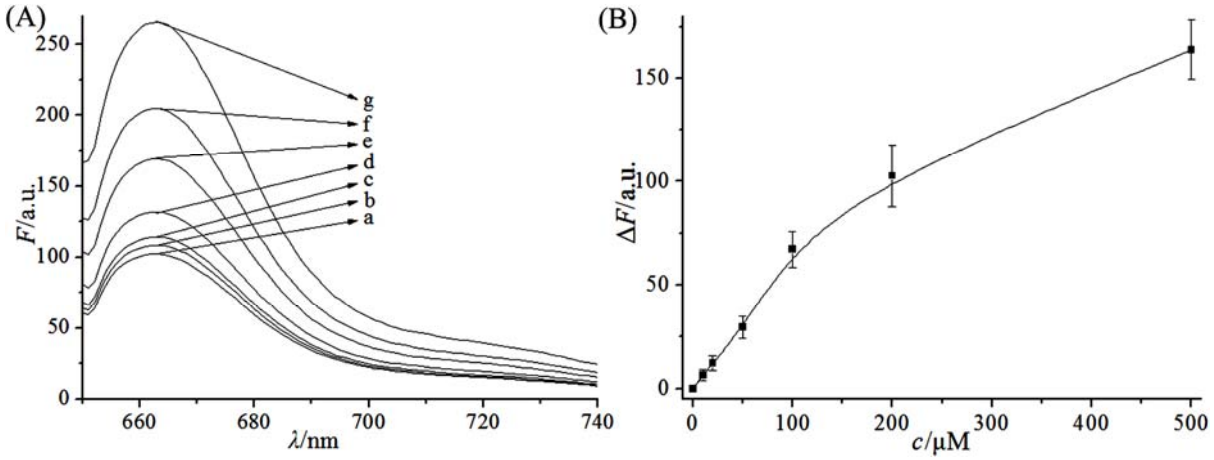

Fig. S4 (A) Fluorescence spectra corresponding to the analysis of different concentrations of cocaine using the non-amplified aptamer subunits system. Spectra were recorded after a fixed time-interval that corresponded to 2 h. Concentrations of cocaine correspond to: (a) 0 μM; (b) 10 μM; (c) 20 μM; (d) 50 μM; (e) 100 μM; (f) 200 μM; (g) 500 μM. (B) Calibration curve corresponding to the resulting fluorescence intensities at λ = 662 nm upon analyzing different concentrations of cocaine.