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Abstract

Detection of very low amount of illicit drugs such as cocaine in clinical fluids like serum continues to be important for many areas in the fight against drug trafficking. Herein, we constructed a label-free nanopore biosensor for cocaine rapid and highly sensitive detection in human serum and saliva samples based on target-induced strand release strategy. In this bioassay, an aptamer for cocaine was pre-hybridized with a short complementary DNA. Owing to cocaine specific binding with aptamer, the short DNA strand was displaced from aptamer and translocation of this output DNA through α-hemolysin nanopore generated distinct spike-like current blockages. When plotted in double-logarithmic scale, a linear relationship between target cocaine concentration and output DNA event frequency was obtained in a wide concentration range from 50 nM to 100 μM of cocaine, with the limit of detection down to 50 nM. In addition, this aptamer-based sensor method was successfully applied for cocaine detection in complex biological fluids like human saliva and serum samples with great selectivity. Simple preparation, low cost, rapid, label-free and real sample detection are the motivating factors for practical application of the proposed biosensor.

Keywords

Nanopore, α-hemolysin, Cocaine detection, Target-induced strand release, Aptamer, Label-free, Complex biological fluid, Small molecule

Cocaine, derived from the leaves of the *Erythroxylum coca* plants, is a strong central nervous system addictive stimulant drug. Abuse of cocaine can cause cardiovascular problems, loss of appetite, anxiety, paranoia, insomnia, organ damage, hyper excitability and violent behavior.[1,2] More than two million people in the U.S. only are addicted to cocaine.[3] Therefore, rapid and sensitive detection of cocaine is substantially important for clinical diagnostics and law enforcement. Traditional methods proposed for detection of cocaine are commercial enzyme-linked immunosorbent assays (ELISA),[4] capillary electrophoresis,[5] chromatography,[6,7] and spectroscopy-based techniques.[8,9] The use of these assays is often limited because of lengthy procedures, high cost and sophisticated instrumentation.

Nucleic acid has been emerged as a powerful tool in construction of biosensors in recent years. A variety of nucleic acid (DNA or RNA) biosensors have been designed.[10-12] Among them, aptamers are single-stranded nucleic acids that selectively bind to low molecular weight organic or inorganic substrate or to relatively large macromolecules.[13-17] Numerous aptameric sensors based on electrochemical,[18-20] fluorescence,[21,22] and colorimetric,[23-25] assays have been developed for detection of cocaine. Electrochemical biosensors, though sensitive but are time consuming, need chemical modification and labelling of substrates. Fluorescent assays also have drawbacks like high background, false positives and difficulty in labelling of some probes. Colorimetric techniques seem attractive but still share the problems like preparation of nanoparticles, complicated samples, low sensitivity and high cost. Serum is one of the most complex media containing variety of proteins and other interference. Saliva has become a widely accepted alternative matrix for drugs of abuse detection due to its easy availability, non-invasiveness, and most importantly drugs are found in parent form in saliva. Cocaine detection in unprocessed biological fluids like diluted human serum samples with most of the reported biosensors faces high matrix effect which hinders their practical usage.[21] Thus, it is highly desirable to construct a sensitive and label-

free biosensor for cocaine detection which also works well in complex biological fluids to fight against the illegal use of cocaine.

Recently, nanopore techniques have remarkable applications in biosensing.[26] α-hemolysin (α-HL) is released by bacterium *Staphylococcus aureus* and is the first identified member of the pore forming β-barrel toxin family. The α-HL heptamer consists of a vestibule of about 2.6 nm diameter and a constriction with an internal diameter of about 1.4 nm.[27] When a charged molecule passes through protein nanopore under an applied potential, it induces a transient change in the ionic current and current variation events recorded electrically. The analyte can be quantified by the frequency of occurrence of current events while characteristic current signatures reveal analyte identity. This nanopore sensor has notable advantages such as simple, label-free, highly sensitive and high signal-to-noise ratio. These properties make it a powerful tool in the detection of various analytes including small molecules,[28-31] metal ions,[32] DNA,[33] RNA,[34] peptides and proteins.[35] Various aptamers' conformations and their ligand binding properties at the single-molecule level have been discriminated using nanopore.[36-42] In addition, α-hemolysin protein nanopore has also been used to investigate biomolecule binding with its target,[43] and is under examination for rapid and low-cost next-generation DNA sequencing technology.[44,45]

Selective detection of small molecules at single-molecule level is difficult as they pass through α-HL nanopore because their blockage signal cannot be observed for much smaller size than the protein pore. Different strategies are utilized to circumvent this problem. Previously reported works of nanopore have utilized either engineered version of α-hemolysin or protein pore occupied with cyclodextrin adapters for detection of small molecules, which generally lack selectivity and need alteration in the protein channel. Takeuchi[46] and Wu[47] have reported the use of α-HL nanopore for the detection of illicit drug cocaine based on aptamer. However, to the best of our knowledge, until now, there is no

report of employing nanopore sensing technology for the quantitative determination of cocaine in clinical samples like human serum and saliva with high sensitivity.

Here, we combined DNA probes with the α-HL for highly selective cocaine detection. The small molecule target detection was converted to oligonucleotide detection by target-induced strand release strategy. Cocaine binding aptamer was hybridized with a short DNA probe. The presence of target cocaine caused the aptamer-probe duplex to unwind and released the DNA probe which produced spike-like signature current events with α-HL nanopore. The application of strand displacement strategy provides a highly sensitive nanopore sensor for detection of cocaine at the nanomolar concentration level. The use of label-free oligonucleotide probes is simple and cost effective. In addition, the proposed method has been successfully applied for cocaine detection in human saliva and serum samples without performing multiple separation or pretreatment steps.

Experimental section

Materials Lipid 1,2-Diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Wild type α-HL for the formation of nanopore was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received without further treatment. Tris·HCl, KCl, theophylline, dopamine and cocaine hydrochloride were purchased from Sigma-Aldrich. Adenosine tri-phosphate (ATP) and adenosine di-phosphate (ADP) were obtained from Dingguo Biological Products Co. (Beijing, China). All the other chemicals utilized were of analytical grade. All solutions for analytical studies were prepared in ultrapure water. The DNA samples were custom synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd (Shanghai, China) and purified by denaturing polyacrylamide gel electrophoresis (PAGE). DNA sequences utilized for target cocaine detection in this nanopore analysis are as follows:

Probe **A**: 5'- GGGAGACAAGGAAAATCCTTCAATGAAGTGGGTCTCCCTAG -3';

Probe **B**: 5'- A_{25} CTAGGGAGAC -3'.

The underlined bases represent complementary bases of the oligonucleotide probes, while probe **A** is the cocaine aptamer.

Target-induced Strand Release Reaction DNA stock solutions (100 μM) were prepared in ultrapure water. DNA probe **AB** was prepared by mixing DNA probe **B** (20 μL, 100 μM) with cocaine binding aptamer probe **A** (40 μL, 100 μM) in 40 μL hybridization buffer (0.15 M NaCl and 25 mM Tris-HCl, pH 7.2), heating at 95 °C for 5 min and then slowly cooled to room temperature. Cocaine hydrochloride was dissolved in PBS buffer and stored at 4 °C. The concentration of cocaine stock solution was 20 mM. The DNA probe **AB** was incubated with various concentrations of cocaine from 100 μM to 50 nM for 30 min at room temperature in 1000 μL test buffer before adding into the *cis* chamber. The test buffer consisted of 1 M KCl, 10 mM PBS and 10 mM EDTA at pH 7.2. The final concentration of probe **AB** was 100 nM.

Preparation of Cocaine Samples in Biological Fluids Human saliva was collected by a volunteer and Serum was obtained from Lablead Biotech. Co., Ltd. (Beijing, China). Human serum or saliva samples were centrifuged at 12000 rpm for 15 min and supernatants were collected. The cocaine solutions in biological fluids were prepared as follow, probe **AB** 100 nM, saliva or serum supernatants 10 μL and target cocaine with different concentrations in 1 mL *cis* test buffer incubated at room temperature for 30 min.

Single-Channel Recording A vertical chamber setup (Warner Instruments, Hamden, CT, USA) was used for electrical current recordings. The setup consisted of two chambers, which were separated by a 150 μm aperture on the *cis* chamber cup. Both chambers contained 1 mL test buffer (1 M KCl, 10 mM PBS, 10 mM EDTA, pH 7.2). For the generation of lipid bilayer, each side of cuvette aperture was pretreated by 0.5 mg mL^{-1} of DPhPC in hexane, and then,

30 mg mL^{-1} of DPhPC in decane was applied to the aperture for the formation of the bilayer. DNA samples were added to the *cis* compartment, which was connected to ground. The *trans* compartment was connected to the head-stage of the amplifier. The α-HL solution was injected adjacent to the aperture in the *cis* chamber. Once insertion of single protein nanopore was confirmed, transmembrane potentials of +120, +130, +140, +150 mV were applied from *cis* side by Ag/AgCl electrodes.

Single-channel currents were recorded with a HEKA EPC10 patch-clamp amplifier (Lambrecht/Pfalz, Germany). The signal was filtered with a low-pass Bessel filter set to 5 kHz at a sampling rate of 20 kHz, by using a LIH 1600 A/D converter (HEKA Elektronik).

Data Analysis Data analysis was performed by using homemade MATLAB (R2011b, MathWorks) programs and OriginLab 8.0 software (OriginLab Corporation). $\mathbf{\Delta I}/I_0$ is the normalized current blockage, where $\mathbf{\Delta I} = I_0 - I_{block}$, I_0 is the open-pore ionic current, I_{block} is the current value of a block signal and $\mathbf{\Delta I}$ is the amplitude of the current blockage. The mean dwell time for DNA probe **B** was obtained from the dwell time histogram by fitting the distributions to single exponential function by the Levenberg-Marquardt procedure. The mean value of $\mathbf{\Delta I}/I_0$ was acquired by fitting the data to Gaussian functions. Data of at least 5 min was recorded for the calculation of frequency. All the data were based on mean ± standard deviation (s.d.) of three separate experiments.

Results and Discussion

Label-free nanopore assay based on target-induced strand release strategy The principle of nanopore assay based on target-induced strand release strategy for cocaine detection is depicted in scheme 1. The narrow constriction of α-HL nanopore (~1.4 nm diameter) only allows output probe **B** (~1 nm) translocation, which generates spike-like short current blockade events. Frequency of these output events is used to quantify the target cocaine. In

contrast owning to the double-stranded nature and complex secondary structure of aptamer, the diameters of probe **AB** and target bound probe **A** are larger than the constriction of α-HL nanopore, which make them difficult to translocate through nanopore, leading to extended interaction time within protein nanopore under the applied electric field. The duplex probe **AB** only produced single-level blockages in ionic current and nearly no multi-level blocks for duplex DNA unzipping were observed. It indicates the trapped probes **AB** in the pore vestibule**,** with the size larger than narrow constriction of α-HL nanopore, were preferentially released back to *cis* solution instead of being unzipped and translocated through the pore. This result is in agreement with the previous report studying the duplex DNA interactions with α-HL nanopore.[48] As a result, only output probe **B** could threads into the pore constriction and pass thoroughly, and its short dwell time is a signature parameter for the differentiation of output probe **B** signals from the other probes.

The differentiation of output probe **B** from probe **AB** and target bound probe **A** is displayed in Figure 1. The addition of probe **AB** on *cis* side generated events with longer duration (~13 ms in Figure 1Ac) in recording traces with mean dwell time of 4.27 ± 0.70 ms and mean current blockade $\Delta I/I_0$ of 0.88 ± 0.03 (Figure 1A). Probe **A** (cocaine binding aptamer) in the presence of 100 μM of target cocaine also produced prolonged events (~10 ms in Figure 1Bc) in current traces with mean duration of 7.24 ± 0.40 ms and $\Delta I/I_0$ of 0.86 ± 0.03 (Figure 1B). Relatively, single-stranded output probe **B**, generated events with short average dwell time of 0.24 ± 0.20 ms and a little smaller value of $\Delta I/I_0$ to 0.82 ± 0.01 (Figure 1C), so distinctions between the output probe **B** and other probes were readily attained based on the translocation time. Target cocaine was tested in the same experimental conditions without any DNA probe, which did not generate any signal, showing no involvement to oligonucleotide probes detection (Figure S1A).

Detection of Cocaine With double-stranded DNA probe **AB**, a sensitive nanopore sensor for the detection of cocaine was developed based on binding-induced strand release approach. From the results presented above, signals with $\Delta I/I_0$ larger than 0.70 and duration shorter than 1 ms caused by probe **B** translocations were defined as output events, separated from translocation events of probe **AB** and target bound probe **A** strands in duration (>1 ms). Figure 2A displays the results of control experiment that was only probe **AB** without target cocaine. Translocation of probe **AB** through nanopore generated output signals at a small frequency of 8.8 ± 1.2 min^{-1} (n = 3), which were recognized as background signal. Background was induced by the target-independent release of output probe **B**. In control experiment, most of the signals were located in dwell time region larger than 1 ms on the dwell-time histogram (Figure 2Ab) and $\Delta I/I_0$ was 0.88 ± 0.03. But probe **AB** pre-incubated with 100 μM of target cocaine, translocation through the nanopore resulted in large output probe signals in recording traces at the frequency of 47.1 ± 2.0 min^{-1}, which can also be observed in dwell time histogram with a characteristic dwell time of less than 1 ms. The mean current blockage ($\Delta I/I_0$) dropped to 0.84 ± 0.02 of the open pore current (Figure 2B). These results suggest that specific binding of target cocaine was the triggering process for the successful binding-induced strand release.

Linear Range and Detection Limit Optimized conditions were selected in order to improve the sensitivity of the nanopore bioassay. Translocation of biomolecule through nanopore is greatly affected by applied transmembrane potential.[49] Thus, we carried out the cocaine detection experiments with probe **AB** at different applied potentials. With the increase in potential, the frequency of output events increased considerably (Figure 2C). Voltage of +150 mV was selected for further experiments due to high frequency of output events at this value. The ratio for probe **A** to probe **B** for making probe **AB** was also optimized. As shown in Figure 2D, [A]/[B] ratio of 2/1 was selected due to low nonspecific background and good

sensitivity. The reaction incubation time was 30 min at room temperature. The concentration of probe **AB** was set to 100 nM, due to the low nonspecific background at this concentration as compared to the higher probe **AB** concentrations (data not included in the manuscript).

These optimized experimental conditions were used to quantify cocaine from 50 nM to 100 μM. As shown in Fig 3A, the frequency of output event increased with an increase in the concentration of target cocaine. A linear relationship was observed between output event frequency and target cocaine concentration ranging from 50 nM to 100 μM in a double logarithmic coordinate. The linear regression equation can be described as a function, $f = 23.69\ c^{0.15}$ with the correlation coefficient of $R^2 = 0.996$, where f represents output DNA signals frequency and c is the concentration of cocaine (Figure 3B). In relationships of the form $y = ax^k$ polynomials, appear as straight lines in a log-log graph, with the power and constant term corresponding to slope and intercept of the line. Detection limit of cocaine was down to 50 nM (S/N=3) in about 15 min of nanopore experiment, with pre-incubated mixture of 100 nM probe **AB** and target cocaine. The response time of the designed nanopore biosensor was comparable to the detection time of cocaine in a GC-based system, but surpassed the need for cumbersome equipment and highly trained technicians. The limit of detection was much better than variety of traditional aptamer-based cocaine detection methods.[50-52] Also our nanopore based biosensor met the federal workplace cutoff value for cocaine (300 ng/mL or 1 μM for the initial test, and 150 ng/mL or 0.5 μM for the confirming test).[53] It should be noted that, the output event frequency possesses non-linear relationship with target cocaine concentration rather than linear. It is because that we have detected a wide range of cocaine concentrations, about four orders of magnitude only by using 100 nM of probe **AB**.

Selectivity of biosensor Selectivity is an important performance factor for practical application of a sensor. Thus several nonspecific small molecules, such as ATP, ADP,

dopamine and theophylline, were investigated by this assay using the same experimental conditions as described above. As control experiment, 100 μM of each small molecule was tested by nanopore without DNA probe **AB** and the results are shown in Figure S1, B-E. Then these compounds were tested with 100 nM probe **AB** and results show that only cocaine was able to trigger target-induced strand release reaction and thus generated large output events while other compounds were unable to bind with cocaine binding aptamer. Figure 4A shows the current signals for the nonspecific molecules incubated with probe **AB**. In comparison to cocaine, the other molecules generated only few output events at frequency of ~9 min^{-1}, nearly similar with the blank control (Figure 4B). Thus, the method is highly selective for its target cocaine as compared to other molecules.

Detection of cocaine in biological fluids It is critically important to evaluate the real applicability of this nanopore based biosensor by challenging this sensor in various media (serum and saliva). Given that this nanopore sensor is resistant to interference materials, we tested the platform for cocaine detection in the presence of human serum and saliva samples. At first human serum and saliva samples without DNA probes were tested which produced only noise-like blocks in current traces (Figure 5Aa and 5Ba). The signal to noise ratio of the nanopore current was not disturbed by complex biological fluid species. Blank control (only contained probe **AB** in human serum or saliva samples without target cocaine) translocation through nanopore generated only few output events. After the addition of target cocaine in the mixture of serum or saliva and probe **AB**, frequency of output events increased tremendously as presented in Figure 5 (Expanded view of characteristic events for cocaine detection in human serum is shown in Figure S2). In order to evaluate accuracy, the proposed biosensor was used for determining the recoveries by spiking different amounts of cocaine into human serum and saliva samples. As given in Table 1, even though the concentrations of

target cocaine of the two samples differed by four orders of magnitude, the average recovery ratios of samples were 96 % and 103 %.

The designed biosensor selectively detected cocaine at very low concentration of 50 nM in human saliva or serum samples. Most importantly, we found that the signal was almost the same as that in buffer suggesting that there was minimum matrix effect (shown in Figure 5C). These results reveal that the proposed aptamer-based sensor platform has a great potential for practical application. In our method, we combined the selectivity of binding-induced strand release strategy and sensitivity of α-hemolysin nanopore to develop a label-free assay for the detection of cocaine with reduced sample consumption. For comparison with previous studies, we have summarized the detection limit, type of DNA used, detection time and the real sample application of other reported methods in Table S1 (see SI). Some of these methods require modification of DNA, which is more expensive than the unmodified DNA used in our method. Only some of them could perform in real samples, such as diluted human serum and saliva samples. The comparison table therefore demonstrates the fact that the sensitivity of our assay is comparable or better than previous reported techniques and our assay can perform in different kinds of biological samples, which has not been established for some of the previous methods.

Conclusion

In summary, we have constructed a biosensor for cocaine quantification by α-hemolysin nanopore combined with binding-induced strand release strategy. The designed biosensor showed the advantages of good selectivity and excellent sensitivity with a detection limit down to 50 nM. The proposed biosensor provides simple, rapid, label-free and amplication-free technique, and has been successfully applied to cocaine detection in real and complex biological fluids like human saliva and serum samples. This bioassay can have extension for

development of variety of simple biosensors for small molecules capable of working in complex media.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website http://pubs.acs.or

Additional figures, including representative current traces for small molecules used in this work, representative current traces with expanded view and comparison table for analytical performance of various different techniques for detection of cocaine (PDF)

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Table 1. Results of the recovery test of cocaine in real samples

Sample	Added (µM)	Found (µM)	Recovery (%) n = 3
Human saliva	0.050	0.049 (± 0.001)	98
	100	98.900 (± 0.840)	99
Human serum	0.050	0.048 (± 0.002)	96
	100	103.100 (± 2.190)	103

Figure Legends

Scheme 1. Schematic diagram of aptamer-based nanopore sensor for cocaine detection by binding-induced strand displacement strategy. Cocaine binding aptamer (probe **A**) is hybridized with short complementary DNA strand (probe **B**). In the prescence of the target (red circle), the binding of target with aptamer induces the conformational change of aptamer to release output probe **B** which produces a distinguished short spike-like signal with α-hemolysin nanopore. Frequency of these output events is used to quantify the target cocaine.

Figure 1. Discrimination of output probe **B** with other probes. (A-C) (a) Schematic illustration of probe **AB**, target bound probe **A** and probe **B**, interacting with or translocating through α-HL nanopore (from up to down). (A-C) (b) Representative single-channel current traces for the experiments of probe **AB**, target bound probe **A** and probe **B**. (A-C) (c) expanded view of the events indicated in the traces by the red triangles. (A-C) (d) Histograms of dwell times for probe **AB**, target bound probe **A** and probe **B**. Histograms for probe **AB** and target bound probe **A** were fit to Gaussian distributions and the histogram for probe **B** was fit to an exponential function. (A-C) (e) Histograms of normalized current blockade $\mathbf{\Delta I}/I_0$ for probe **AB**, target bound probe **A** and probe **B**. Each histogram was fit to a Gaussian distribution. The final concentration of all DNA probes were 100 nM. Test for target bound aptamer was performed by incubating 100 nM of probe **A** with 100 μM target cocaine before adding to *cis* chamber. All the experiments were performed in solution containing 1 M KCl

and 10 mM EDTA, 10 mM PBS (pH = 7.4), with transmembrane potential of +150 mV. Each experiment was repeated three times.

Figure 2. Nanopore based detection of target cocaine. (a) Representative single-channel current traces of the events induced by probe **AB** without (A) and with (B) 100 μM target cocaine. Red triangles mark the output events in current traces. (b,c) Histograms of dwell time and normalized current blockade $\Delta I/I_0$ for probe **AB** without (A) and with (B) 100 μM target cocaine. Blue boxes represent the output event regions on the dwell time histograms. Each ionic-current histogram was fit to Gaussian distributions. (C) Dependence of output event frequency on the applied potential. For all the groups, nanopore tests were performed with 100 nM probe **AB,** pre-incubated with 50 μM of target cocaine. All the experiments were performed in solution containing 1 M KCl and 10 mM EDTA, 10 mM PBS (pH = 7.4), with transmembrane potential held at +120, +130, +140 and +150 mV. Each experiment was repeated three times. (D) Dependence of output event frequency on the ratio of probe **A** to **B**. The tests were conducted with pre-incubated solution of 100 μM target cocaine and probe **AB** with various ratios. There was no target cocaine in every blank group. Different ratios were achieved by changing the concentration of probe **B** with a constant concentration of probe **A**. All the experiments were performed in solution containing 1 M KCl and 10 mM EDTA, 10 mM PBS (pH = 7.4), with transmembrane potential of +150 mV. Each experiment was repeated three times.

Figure 3. Dependence of output event frequency on concentration of target cocaine. (A) Representative single-channel current traces of 100 nM probe **AB** incubated with various concentrations of target cocaine added to the *cis* chamber. The concentrations of target cocaine were ranging from 50 nM to 100 μM, respectively (from up to down). Red triangles

represent the output events in current traces. (B) Variance of the output event frequency in response to different concentration of target cocaine. The tested cocaine concentrations are 50 nM, 100 nM, 200 nM, 500 nM, 1 μM, 10 μM and 100 μM. The curve shows linear response from 50 nM to 100 μM on a log-log coordinate. All the experiments were performed in solution containing 1 M KCl and 10 mM EDTA, 10 mM PBS (pH = 7.4), with transmembrane potential of +150 mV. Each experiment was repeated three times.

Figure 4. (A) Representative single-channel current traces of DNA probe **AB** in presence of cocaine (a) or nonspecific small molecules, ATP (b), ADP (c), dopamine (d) and theophylline (e). In the blank (f), all experimental conditions were the same as (a), except that there was no cocaine. The concentrations of probe **AB** and all the small molecules are 100 nM, 100 μM, respectively. Red triangles mark the output events in current traces. (B) Comparison of the output event frequency of probe **AB** with target cocaine and other nonspecific small molecules. All the experiments were performed in solution containing 1 M KCl and 10 mM EDTA, 10 mM PBS (pH = 7.4), with transmembrane potential of +150 mV. Each experiment was repeated three times.

Figure 5. (A) Representative current traces for human serum sample (a) and detection of cocaine in human serum samples without (b) or with (c) 100 μM of target cocaine. (B) Representative current traces for human saliva sample (a) and detection of cocaine in human saliva samples without (b) or with (c) 100 μM target cocaine. The DNA probe **AB** in (a-c) is

in concentration of 0 nM, 100 nM and 100 nM, respectively. Red triangles mark the output events in current traces. (C) Comparison of cocaine detection capability in a range of media. From left to right: 100 μM of cocaine in test buffer, 100 μM of cocaine adulterated in 10 μL human serum and 10 ul human saliva samples respectively. All the experiments were performed in solution containing 1 M KCl and 10 mM EDTA, 10 mM PBS (pH = 7.4), with transmembrane potential of +150 mV. Each experiment was repeated three times.

Scheme 1

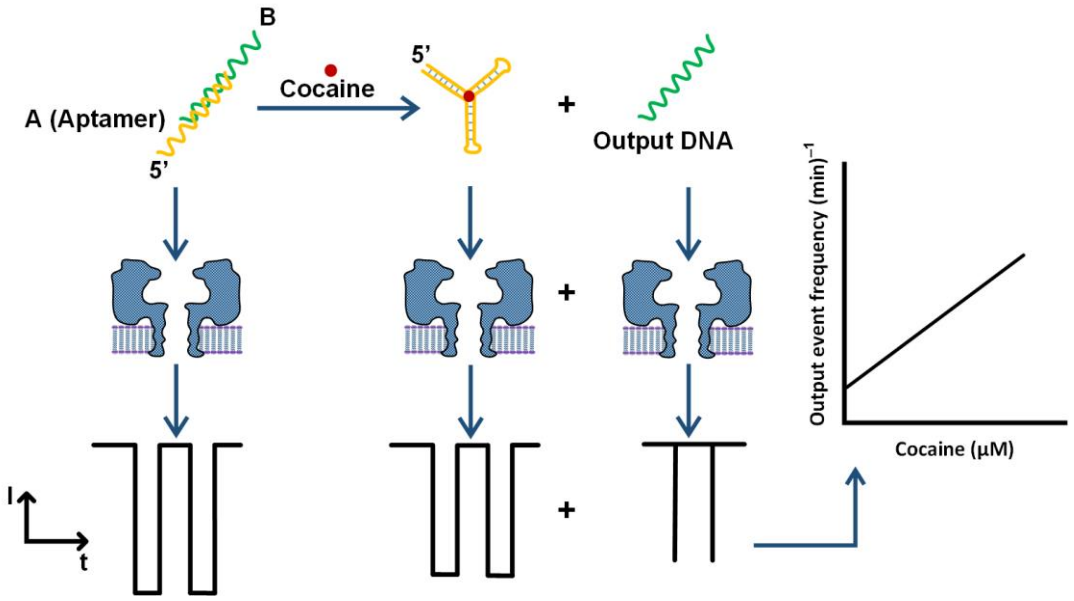

B
A (Aptamer)
5'
Cocaine
5'
+
Output DNA
+
+
I
t
Output event frequency (min)$^{-1}$
Cocaine (μM)

Figure 1

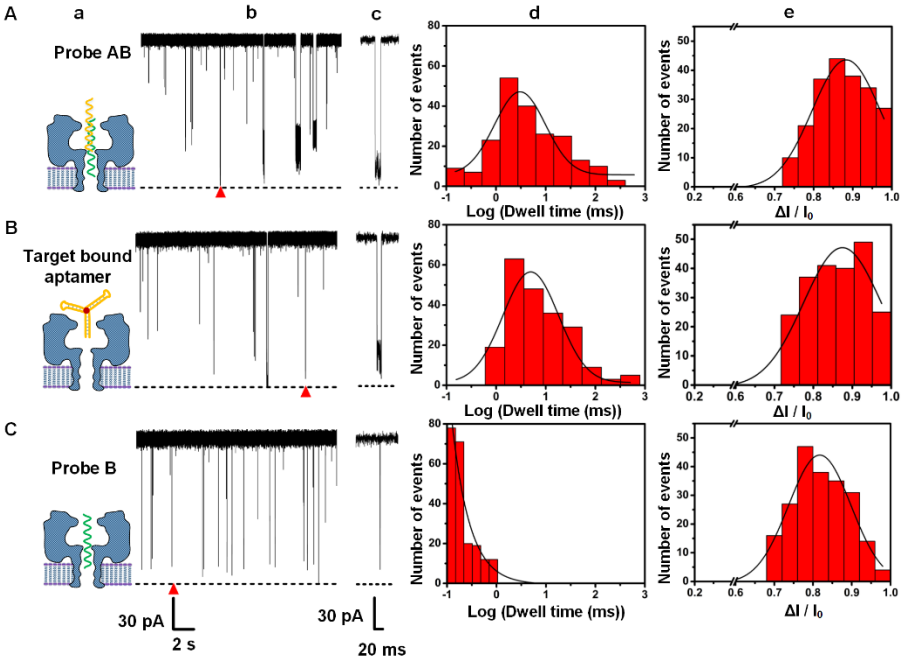

A
a
b
c
d
e
Probe AB
B
Target bound aptamer
C
Probe B
30 pA
2 s
30 pA
20 ms
Number of events
Log (Dwell time (ms))
ΔI / I0

Figure 2

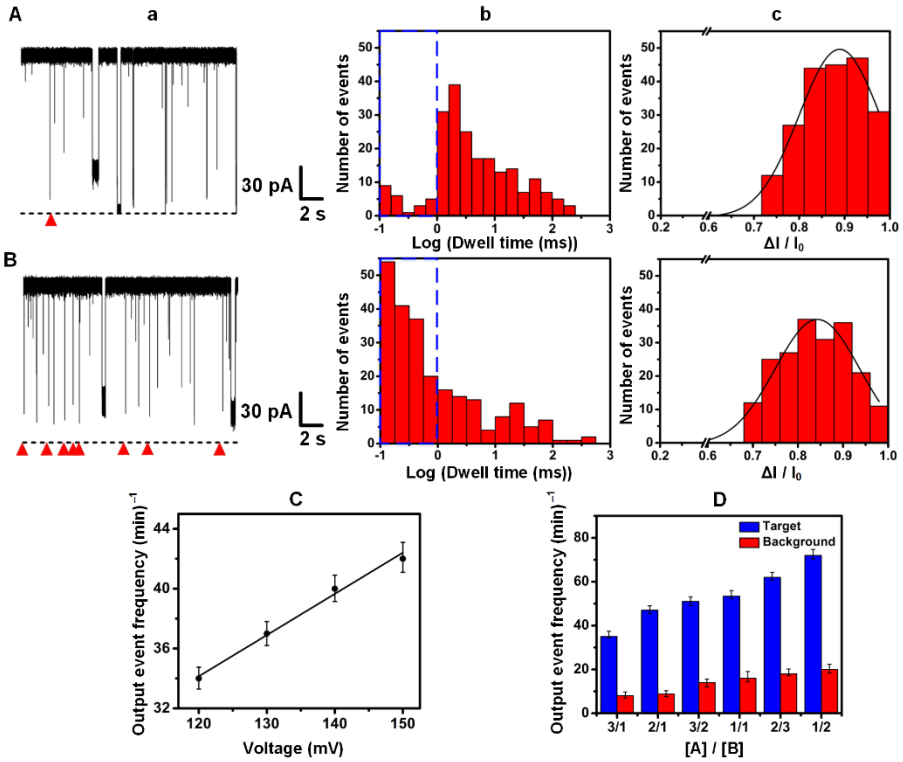

A
a
30 pA
2 s
b
Number of events
Log (Dwell time (ms))
c
Number of events
ΔI / I0
B
30 pA
2 s
Number of events
Log (Dwell time (ms))
Number of events
ΔI / I0
C
Output event frequency (min)−1
Voltage (mV)
D
Target
Background
Output event frequency (min)−1
3/1
2/1
3/2
1/1
2/3
1/2
[A] / [B]

Figure 3

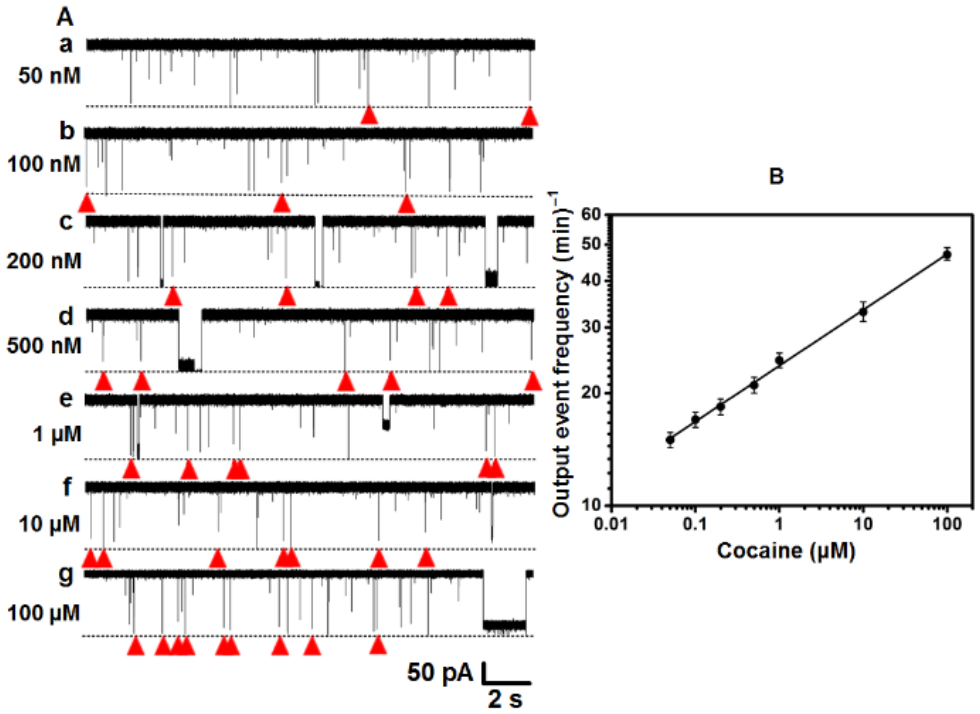

A
a
50 nM
b
100 nM
c
200 nM
d
500 nM
e
1 μM
f
10 μM
g
100 μM
50 pA
2 s
B
Output event frequency (min)$^{-1}$
60
50
40
30
20
10
0.01
0.1
1
10
100
Cocaine (μM)

Figure 4

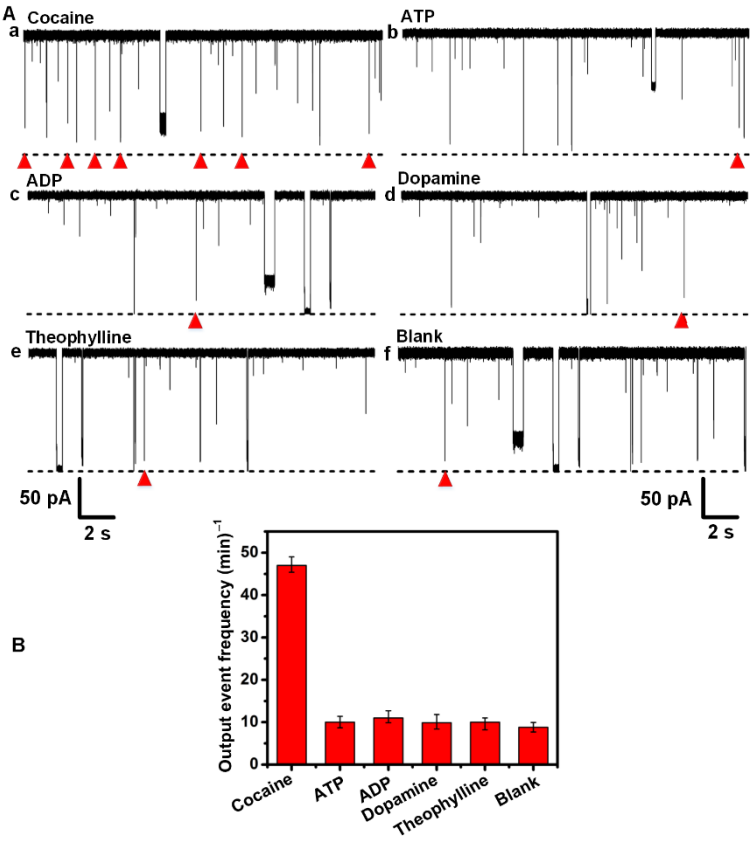
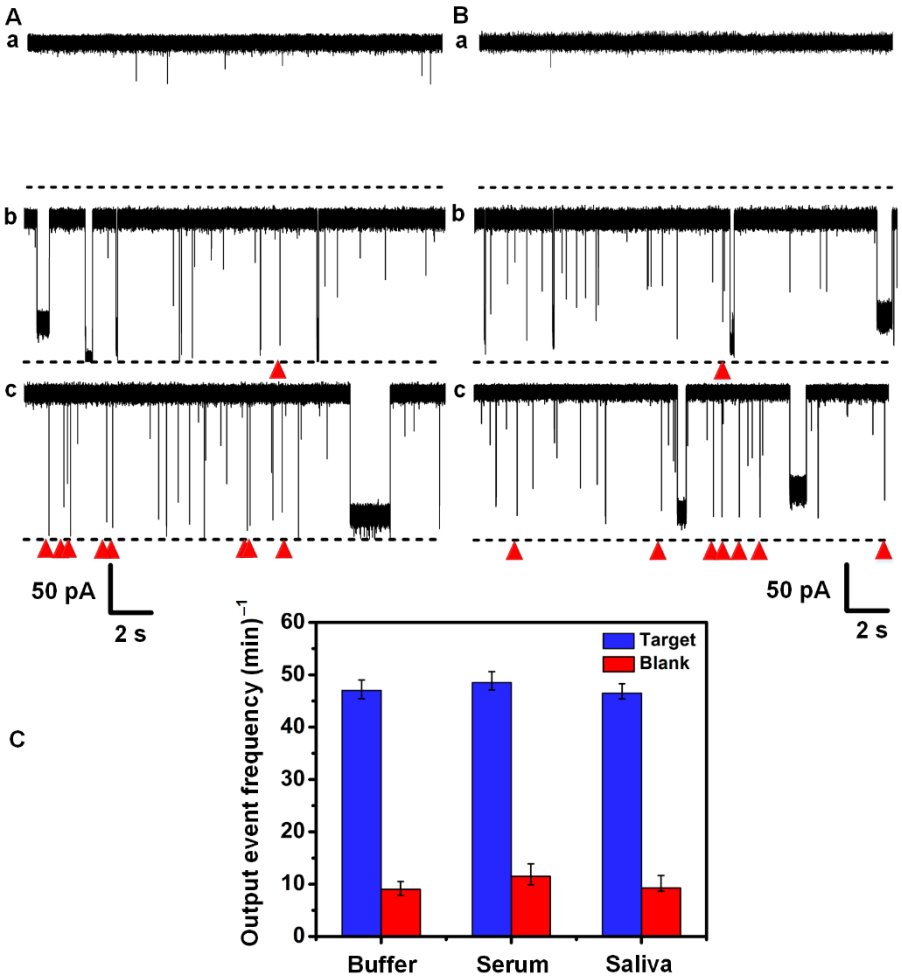
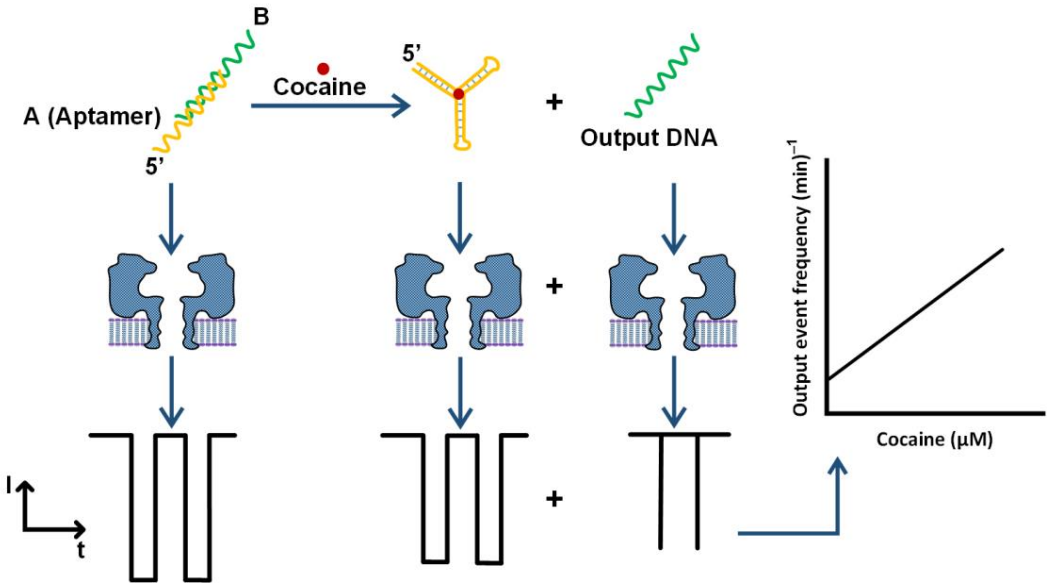

A
a
Cocaine
b
ATP
c
ADP
d
Dopamine
e
Theophylline
f
Blank
50 pA
2 s
50 pA
2 s
B
Output event frequency (min)$^{-1}$
0
10
20
30
40
50
Cocaine
ATP
ADP
Dopamine
Theophylline
Blank

Figure 5


A
a
B
a
b
b
c
c
50 pA
2 s
50 pA
2 s
C
Output event frequency (min)$^{-1}$
60
50
40
30
20
10
0
Target
Blank
Buffer
Serum
Saliva

For TOC Only


B
A (Aptamer)
5'
Cocaine
5'
+
Output DNA
+
+
I
t
Output event frequency (min)$^{-1}$
Cocaine (μM)