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PII: S0003-2670(16)30651-1

DOI: 10.1016/j.aca.2016.05.021

Reference: ACA 234587

To appear in: *Analytica Chimica Acta*

Received Date: 6 March 2016

Revised Date: 11 May 2016

Accepted Date: 12 May 2016

Please cite this article as: Y. Tang, F. Long, C. Gu, C. Wang, S. Han, M. He, Reusable split-aptamer-based biosensor for rapid detection of cocaine in serum by using an all-fiber evanescent wave optical biosensing platform, *Analytica Chimica Acta* (2016), doi: 10.1016/j.aca.2016.05.021.

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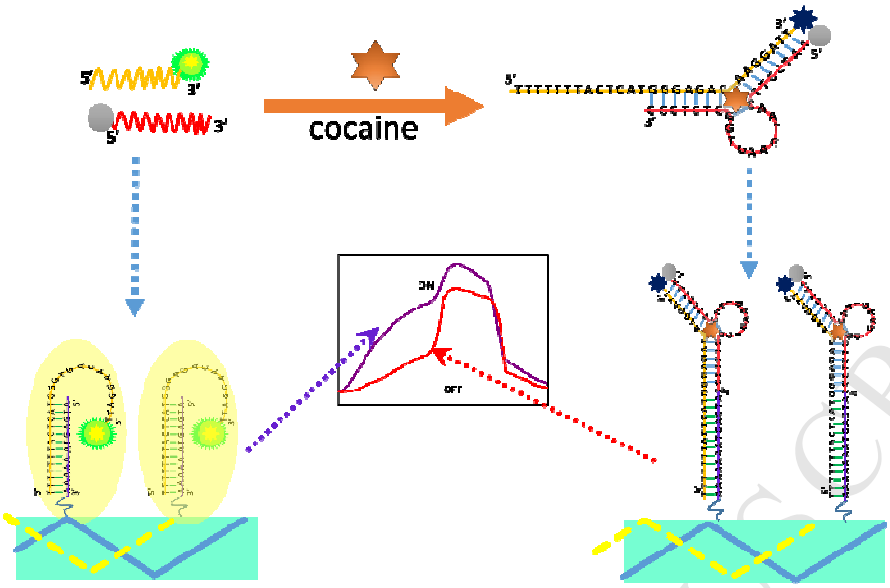

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Reusable split-aptamer-based biosensor for rapid detection of cocaine in serum by using an all-fiber evanescent wave optical biosensing platform

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Abstract: A rapid, facile, and sensitive assay of cocaine in biological fluids is important to prevent illegal abuse of drugs. A two-step structure-switching aptasensor has been developed for cocaine detection based on evanescent wave optical biosensing platform. In the proposed biosensing platform, two tailored aptamer probes were used to construct the molecular structure switching. In the existence of cocaine, two fragments of cocaine aptamer formed a three-way junction quickly, and the fluorophore group of one fragment was effectively quenched by the quencher group of the other one. The tail of the three-way junction hybridized with the cDNA sequences immobilized on the optical fiber biosensor. Fluorescence was excited by evanescent wave, and the fluorescence signal was proportional to cocaine concentration. Cocaine was detected in 450 s (300 s for incubation and 150 s for detection and regeneration) with a limit of detection (LOD) of 165.2 nM. The proposed aptasensor was evaluated in human serum samples, and it exhibited good recovery, precision, and accuracy without complicated sample pretreatments.

Keywords: Cocaine; Aptasensor; Evanescent wave; Structure switching; Fluorescence quenching

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1. Introduction

As one of the most dangerous and illegally abused drugs, cocaine has received a considerable concern because of its instantaneous and overwhelming effects on the central nervous system [1]. Traditional methods for quantitative detection of cocaine require the use of special or expensive equipment such as gas chromatography/mass spectrometry [2,3], and high-performance liquid chromatography (HPLC) [4,5]. These time-consuming methods cannot be operated in situ and on time and few of them can realize monitoring in human internals fluids due to the matrix (suppression) effect of biological samples. Therefore, developing rapid and convenient detection methods for cocaine in human fluids is imperative. Given the high selectivity, chemical stability, and relatively commercial availability of aptamers, they have been widely applied in the design of novel assay methods for recognizing various targets, such as small molecules, proteins, and cells. Moreover, aptamers are easier to produce or modify than antibodies [6]. At present, aptamers are extensively used along with different types of biosensors to detect a variety of targets [7]. Numerous aptamer-based cocaine biosensors have been developed using different signal transductions, including electrochemistry [6,8], fluorescence [9], chemiluminescence [10], colorimetry [11], and surface plasmon resonance [12]. Some aptamers undergo conformational changes after binding with their targets, and certain aptamer sensors have been constructed easier on the basis of the structure-switching property of the aptamer [13,14]. The cocaine aptamer is the structure-switching one because it turns into a hairpin at the aptamer-target binding event [15]. These cocaine biosensors were

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designed through the mechanism of conformational changes of the target-induced aptamer [16,17,18,19]. The cocaine aptamer is further constructed using the self-assembly of aptamer subunits or fragments in the presence of the target into supramolecular structures, which can introduce additional possibilities for aptasensor design [12,20]. In these approaches, the traditional single-stranded cocaine aptamer is engineered and split into two or three subunit aptamers. These subunit aptamers can bind with target molecules only in the existence of all the fragments [8,21]. These fragments can be further modified with different functional groups such as fluorophores, quenchers, or any other signal group to produce different aptasensors with specific characteristics [22,23]. Among these methods, the conversion of fluorescence change induced by the structure switching of aptamers in a biosensor system is a popular and time-saving means for rapid cocaine detection [24].

Given the new modification methods, a lower limit of detection (LOD) of cocaine than the traditional aptasensor can be obtained [25]. However, rapid quantification of cocaine remains difficult to realize because most of the aptasensors require time-consuming pretreatment or incubation [26]. Furthermore, few cocaine aptasensors are reusable, which hinders them suitable for wide application in biological fluids.

Silica optical fiber, a frequently used sensing substrate, can be easily modified with functional materials through covalent attachment and be simply regenerated [27]. Optical fiber biosensors demonstrate several advantages over other biosensors, including small size, flexible geometry, easy modification capability, easy

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operationality, and noise immunity. Therefore, optical fiber biosensors are ideal for clinical diagnosis, environmental monitoring, and process control [28].

In the present study, an aptasensor for cocaine detection is proposed on the basis of the structure switching of cocaine aptamer fragments by using the evanescent wave optical biosensing platform. In this work, a cocaine aptamer was split into two fragments (i.e., fluorophore probe and quencher probe). For the fluorophore probe, we extended 13 more bases for the hybridization with the oligonucleotide probe immobilized on the optical fiber surface. A novel "sandwich" structure strategy based on the target-induced combination of the fluorophore probe and quencher probe was then developed. Compared with traditional biosensors, the proposed sensor indicates higher reactivity and requires a shorter detection time.

2. Experiment section

2.1 Regent and material

Amikacin, ibuprofen, sulfadimethoxine (SDM), and kanamycin sulfate were obtained from Beijing Songyuan Co., Ltd. (Beijing, China). Cocaine hydrochloride, glutaraldehyde (GA), and 3-aminopropyltriethoxysilane (APTS) were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) and sodium dodecyl sulfate (SDS) were purchased from Sinapharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade if not specified and were used as received without further purification. All HPLC-grade oligonucleotides (DNA) were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The fluorophores-labeled aptamer sequence segment was 5′

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-TTTTTTTACTCATGGGAGACAAGGATT-Cy3-3′ (Fluorescence aptamer subunit probe, FP), the quencher-labeled sequence segment was 5′-BHQ-AATCCTTCAATGAAGTGGGTCTCCC -3′ (Quencher aptamer subunit probe, QP), and the aminated DNA sequence was 5′-NH_2-AAAAAAATGAGTA-3′ (Hybridization probe, HP). The stock solutions of each DNA with 100 μM were prepared by dissolving the above DNA in PBS buffer and stored at -20 °C.

2.2 Evanescent wave optical biosensing platform

The evanescent wave optical biosensing platform applied for the cocaine detection was depicted in Test S1 and Fig. S1. Briefly, a 535 nm pulse diode laser entered the multi-mode fiber (diameter = 600 μm). Subsequently, the evanescent generated and excited the fluorophores of oligonucleotides by DNA hybridization. Finally, the filtered fluorescence signal was automatic recorded.

2.3 Modification of optical fiber

The combination of tapered fiber optic probes was prepared as previously described [29]. Several procedures were performed to immobilize HP on the fiber surface (Fig. S2). Initially, the fiber was immersed in piranha solution (H_2SO_4/H_2O_2 3:1, v/v) at 100 °C for 1 h to remove possible contaminants and introduce hydroxyl group onto the fiber surface (Step 1). The hydroxyl-modified fiber was then washed with deionized water and dried in N_2. Afterward, the fiber was immersed thoroughly into 2% (v/v) APTS/toluene solution for 1 h and dried in an oven at 200 °C for 0.5 h after being washed with dry toluene and ethanol (Step 2). The amino groups on the fiber were allowed to react with 4% (v/v) GA for 1 h (Step 3). After these preparations,

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the fiber was immersed in a 250 μL solution composed of 250 nM DNA HP at 4 ℃ overnight to immobilize HP on the fiber surface (Step 4).

2.4 Analytical procedure

The mixture of equal volumes of cocaine aptamer segments with 1 μM FP and 1 μM QP in the reaction buffer was heated in a dry bath at 90 °C for 5 min, slowly cooled to room temperature, and then stored at 4 °C before use.

Different concentrations of cocaine were allowed to mix with 10 nM FP and QP oligomer in 600 μL of reaction buffer for 300 s to generate the FP-cocaine-QP conjugation. The mixture was pumped into the flow pool leaving the probes to bind with the immobilized HP. Meanwhile, the fluorescent signals were recorded until the signal intensity remained stable. The sensor surface was regenerated using the regeneration buffer and washing buffer, sequentially. For the practical detection of cocaine in biological fluids, different concentrations of cocaine in 10% serum was obtained by dilution with reaction buffer.

The HPLC tests of cocaine were carried out by an Agilent 1260 HPLC/DAD system (High Performance Liquid Chromatography/Diode Array Detector, California, USA) coupled with a C18 column (250 × 4.6 mm, 5 μm). Full details of the HPLC analysis are included in Test S2.

3. Results and discussion

3.1 Sensing mechanism

On the basis of the structure-switching property of the cocaine aptamer, we developed a novel biosensor monitoring process for the interaction between cocaine

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and aptamer splits. Scheme 1 illustrates the principle of cocaine detection. The sensor comprised two parts: the synthesis of the FP-cocaine-QP conjugation and the hybridization of the conjugation with HP on the fiber. The oligonucleotides used were Cy3-labeled FP, BHQ-labeled QP, and HP labeled to the optical fiber. FP can be divided into two segment regions. The first region is a subunit of cocaine aptamer, which can combine with QP in the existence of cocaine. The second region, which consists of 12 nucleotides, can hybridize with the complementary nucleotides of HP on the fiber.

When cocaine was added into the mixture of FP and QP (Scheme 1a), the conformational structures of FP and QP switched, and the three-way junction formed, thereby quenching the Cy3 groups on FP by BHQ groups on QP. Scheme 2b illustrates the hybridization of FP and FP-cocaine-QP with HP on the fiber. FP would hybridize with HP and induce the fluorescence activated by evanescent waves on the fiber surface in the absence of cocaine. However, when cocaine was added, the two aptamer fragments formed a three-way junction. Therefore, the distance between the fluorophore and quencher would become closer, which would further decrease the fluorescent signal. As the concentrations of cocaine increased, the fluorescent signal gradually decreased. Cocaine-induced structure switching is inspired by the recent research of Li et al., who discovered that an aptamer underwent a structure-switching process upon binding to target molecules [30]. In contrast to their design, the structure switching of our design occurs in liquid phase instead of liquid–solid interphase, which can shorten the incubation time [26]. After the recognition of FP and

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FP-cocaine-QP conjunction, SDS (pH 1.9) was introduced to denature the hybridized DNA, thereby releasing the FP-cocaine-QP complex from the fiber surface. The regenerated fiber surface was reused for the subsequent experiment.

Scheme 1. Schematic of cocaine sensing based on structure switching and fluorescence "turn off" mode on evanescent wave optical biosensing platform. (a) Structure switching of cocaine combining the two aptamer fragments; (b) Hybridization and regeneration of the aptamer with optical fiber.

3.2 Characterization of fiber modification

Fiber modification can significantly influence the detection results. Prior to cocaine detection, the surface property of the fiber should be characterized. Therefore, nonspecific adsorption and hybridization efficiency of the sensors were tested by introducing 10 nM FP into the flow cell with fiber probe at different fabrication steps (Fig. 1).

Fig. 1. (a) Surface character of fiber modification. (b) Fluorescence signals of 10 nM FP with fiber at different fabrication steps (Steps 1-4 represent the fiber at different modification steps).

A different functional group was introduced onto the fiber surface at various modification steps (Fig. 1a). For a bare optical fiber, a parallel line relative to the horizontal coordinate with few signal increases is observed after introducing FP (Fig. 1b, curve step 1). After assembling APTES on the fiber, fluorescence signals slightly increased, which may be ascribed to the nonspecific adsorption between the APTES-functionalized fiber surface and FP (Fig. 1b, curve step 2). When this amino-modified optical fiber further joined with GA, the fluorescence signals became stable (Fig. 1b, curve step 3). Finally, after being further immobilized with HP, a

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hybridization curve with continuous signal increases was obtained (Fig. 1b, curve step 4), indicating that HP was successfully modified onto the fiber surface and FP was hybridized with HP. As shown in Fig. 1b, a high signal-to-noise ratio was obtained for this biosensor, indicating the possibility of probing fluorescence changes induced by the aptamer structure switching during the sensing process.

3.3 Dose-response curve of cocaine

To adjust the performance of the design, a fluorescent spectrophotometer was used to test the signal decline after FP was mixed with QP and cocaine. After being mixed with QP, the fluorescence of FP was partly quenched because of the random distribution of the DNA oligonucleotides (Fig. S3). When cocaine was introduced in the system, the newly formed FP-cocaine-QP complex effectively quenched the fluorescence. A maximum fluorescent quenching was obtained when the concentration of cocaine was 200 μM. A series of cocaine solutions with concentrations from 100 nM to 200 μM were tested to demonstrate the applicability of the proposed fluorescent “turn off” mode assay. Fig. 2a illustrates the relative evanescent fluorescent signals. The evanescent fluorescent signals gradually decreased with the increasing cocaine concentrations within the range of 100 nM to 200 μM. The kinetics of DNA hybridization at various cocaine concentrations can also be monitored real time by using this proposed aptasensor. In this study, the fluorescent signals of different cocaine levels reached equilibrium simultaneously. These results revealed that the FP-cocaine-QP complex can still hybridize with HP, ensuring the abundant oligonucleotides that can hybridize with FP on the fiber

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remained unchanged. This design guarantees the same reaction time of different cocaine concentrations.

Fig. 2b exhibits the calibration curve for cocaine detection. The peak signal decreased logistically with the increasing cocaine concentrations at the range between 100 nM and 200 μM. The error bars corresponded to the standard deviations of data points in duplicate experiments, with the coefficient of variance of all the data points within 3%. The linear equation of cocaine assay is fitted to a 4-parameter logistic equation as follows:

$$y = N_2 + \frac{(N_1 - N_2)}{1 + (x/x_0)^p}$$

Where x is the analyte concentration; N_1 and N_2 are the upper and lower asymptote (background signal) to the simulation curve; x_0 is the analyte concentration at inflection; and p is the slope at the inflection point and the parameters attained. The LOD was 165.2 nM (S/N=3) with a correlation coefficient of 0.998 and linear concentration range was between 200 nM and 200 μM which covered three orders of magnitude. The LOD we obtained is comparable to those reported fluorescence detection of cocaine using FRET methods labelled with fluorescence group which has the LOD of 200 nM [31], and label-free fluorescence aptamer-based sensor based on isothermal circular strand-displacement amplification and graphene oxide absorption which has the LOD of 190 nM [32].

Fig. 2. (a) Typical fluorescent responses of the biosensor with a series of concentrations of cocaine: 0–200 μM; (b) Response curve obtained with different concentrations of cocaine and modification standard equation.

A time-saving method for cocaine screening is of great importance because the

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half-life period of cocaine in human body is about 1 h. Appropriately, the total reaction and regeneration time can be limited within 150 s with our aptasensor. The liquid homogeneous reaction achieves a higher reaction rate than that of the liquid–solid phase. For the aptasensor, the specific reaction between the aptamer and the target is usually the most time-consuming process and the rate-limiting reaction. However, in our work, this problem was successfully avoided by arranging the binding process in the liquid phase instead of on a liquid–solid surface. The binding dynamics using the two fluorophores-labeled aptamer sequence segments with 100 μM cocaine is shown in Fig. S4. We find that the reaction of cocaine with aptamers can get equilibrium in less than 60 s with the design of the two aptamer segments. Meanwhile, DNA–DNA hybridization on the solid surface can reach equilibrium in a few minutes when there are less than 100 base pairs [33]. The detection duration of the proposed method is compared with that of other optical approaches for cocaine detection (Table 1). The total detection time of our biosensor is 450 s, and is shorter than most of the detection methods such as the ones we mentioned in Table 1.

Table1. Available optical-based cocaine aptasensors

Method/technique	Detection time	Reference
Strand displacement amplification based aptameric sensor, Fluorescence	2 h	[9]
Strand-displacement polymerization and FRET	1000 s	[18]
Isothermal circular strand-displacement amplification	1 h	[32]
Rolling circle amplification	＞1 h	[34]
electrochemical detection and rolling circle amplification	＞2 h	[26]
All-fiber evanescent wave optical biosensing platform	450 s	This study

3.4 Selectivity, stability and regeneration property of the aptasensor

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Selectivity/specificity is one of the key parameters in the applicability of any developed aptasensor [35,36]. The selectivity of biosensor for cocaine detection is also investigated in this study. Several compounds that are environmentally relevant, including kanamycin, amikacin, SDM, and ibuprofen, were selected for this study. A control test was conducted by adding buffer 1 only instead of cocaine or other compounds. Fig. 3a displays the evanescent fluorescent signals generated by introducing 100 μM and 10 mM kanamycin, amikacin, SDM, and ibuprofen. No signal decrease is observed at the concentration of 100 μM of different compounds. The aptasensor continued to demonstrate high selectivity even at extremely high target concentrations (10 mM), indicating that no obvious cross-reaction exists for cocaine detection. The results revealed that the aptasensor exhibited high specificity and environmental adaption in cocaine monitoring.

Fig. 3. (a) Relative signal decrease of different concentration of kanamycin, amikacin, SDM, and ibuprofen against 100 μM cocaine; (b) Representative response curves for hybridization of the biosensor in multiple cycles in the absence of cocaine; the curves are hybridization signals after 1, 10, 20, and 40 regeneration cycles.

A good regeneration performance is of critical importance for the application and popularization of a robust and convenient aptasensor [37]. To evaluate the stability of the aptasensor in this work, repeatability studies were performed using 10 nM FP to hybridize with FP on the fiber surface in Buffer 1. Fig. 3b demonstrates the signals generated from the first, tenth, twentieth, and fortieth assay. The hybridization trends and the peak signals remained stable during the assay. The results indicate that the assay is highly reproducible with an excellent long-term stability. The fiber surface is

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completely regenerated by introducing 0.5% SDS (pH 1.9) for a few seconds. After regeneration, the evanescent signal of the sensor can be recovered without damaging the property of the functionalized fiber. The results illustrated that the proposed method is not the one-off, but it can be conducted repeatedly in open application tests.

3.5 Application of cocaine aptasensor in serum

Finally, the practical application of the cocaine aptasensor was applied in biological fluids testing. The detection of cocaine directly in biological fluid such as human serum is of great significance for the screening and conformation of cocaine early abuse [38]. Herein, human serum was preferred for the practical applicability of the aptasensor (Fig. 4a). A logistic relationship between the concentration of cocaine and the signal with a high correlation efficiency of 0.999 was obtained (Fig. 4b). All the signal decrease percentages at each concentration of cocaine in human serum were slightly smaller than those in the buffer. The LOD calculated from the curve was 1.01 μM (S/N=3). The slopes of the two calibration curves measured in the buffer and human serum demonstrated an acceptable coherence. Owing to the complication of serum, the LOD concentration of cocaine in the nature fluids is often higher than experimental conditions [31]. Although the LOD concentration of cocaine detection in serum was higher than that in the experimental buffer, the results clearly demonstrated the potential of our biosensor in testing real samples. The results obtained by the aptasensor are also compared with those measured by HPLC in Table S1. The results demonstrate satisfactory agreements between methods, which indicates the adequacy of the aptasensor for accurate cocaine detection. Compared with traditional methods

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such as HPLC and GC-MS, this method can directly detect cocaine in serum by diluting 10 times using reaction buffer without any complex sample pretreatment.

Fig. 4. (a) Different concentrations of cocaine in 10% human serum measured in the optical biosensor, 0–200 μM; (b) Calibration curve for cocaine detection in 10% human serum.

4. Conclusions

In summary, a novel two-step fluorescent aptasensor for sensitive, specific, and rapid detection of cocaine by using evanescent wave optical biosensing platform was developed. In addition, efficient hybridization and dissociation performance of the aptamer and the oligonucleotide on the fiber were processed. The robustness of the conjugate surface and the good surface regeneration procedures also allowed at least 40 assay cycles within an analysis time of approximately 450 s (300 s for incubation and 150 s for detection and regeneration) for each detection assay cycle without any significant loss of performance. It provides a high specificity of cocaine detection against other nonspecific compounds at high concentrations. The aptasensor was successfully applied for cocaine detection in human serum indicating its significant potential for rapid detection of cocaine.

Acknowledgements

This work is supported by the Chinese High-tech R&D (863) Program (Grant No. 2014AA06A506), the National Natural Science Foundation of China (Grant No. 21377065) and the Great National Instrument R&D Special Program (Grant No. 2012YQ030111).

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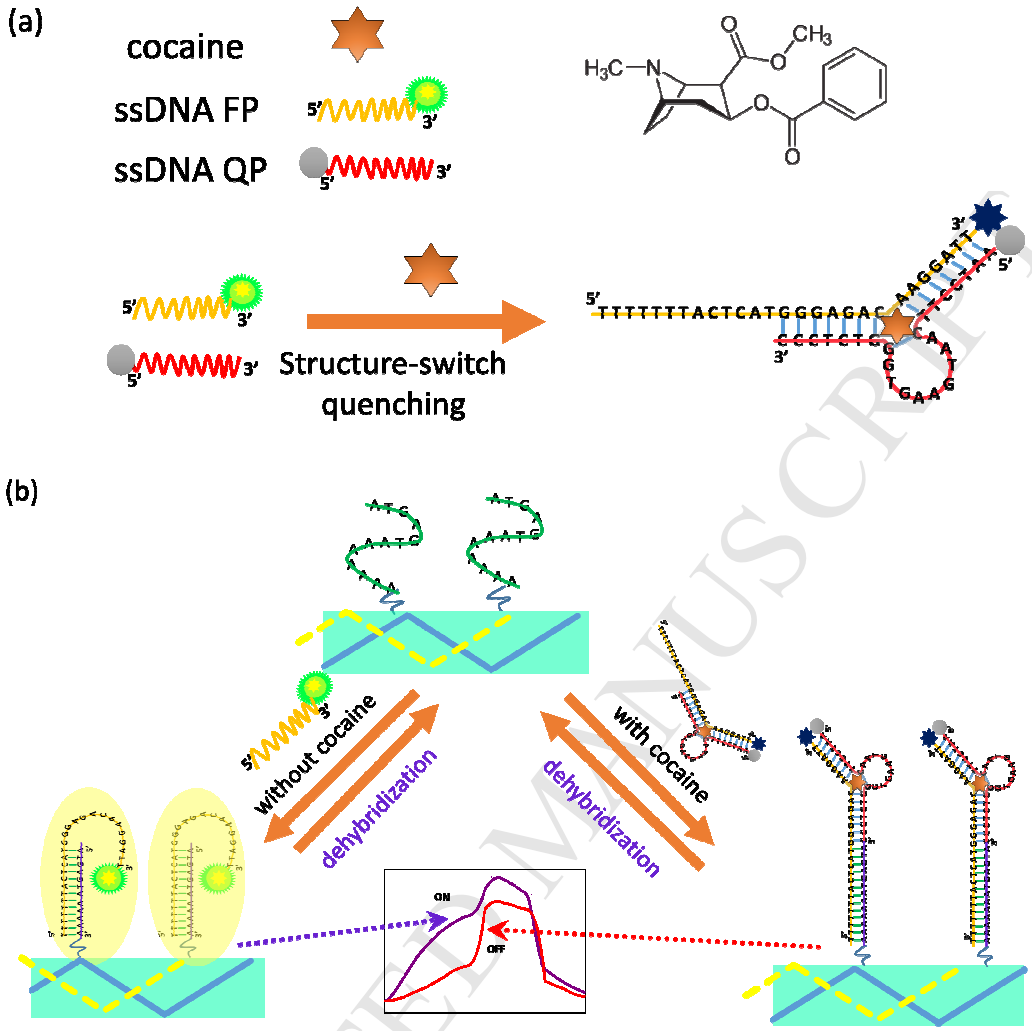

Scheme 1. Schematic of cocaine sensing based on structure switching and fluorescence "turn off" mode on evanescent wave optical biosensing platform. (a) Structure switching of cocaine combining the two aptamer fragments; (b) Hybridization and regeneration of the aptamer with optical fiber.

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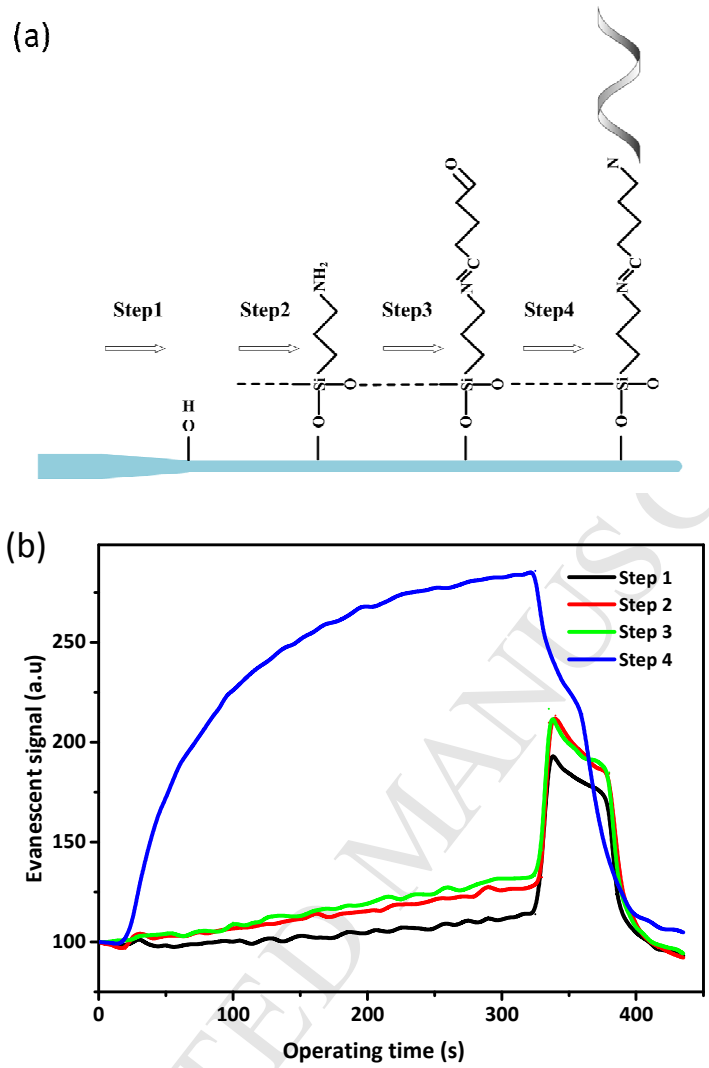

Fig. 1. (a) Surface character of fiber modification. (b) Fluorescence signals of 10 nM FP with fiber at different fabrication steps (Steps 1-4 represent the fiber at different modification steps).

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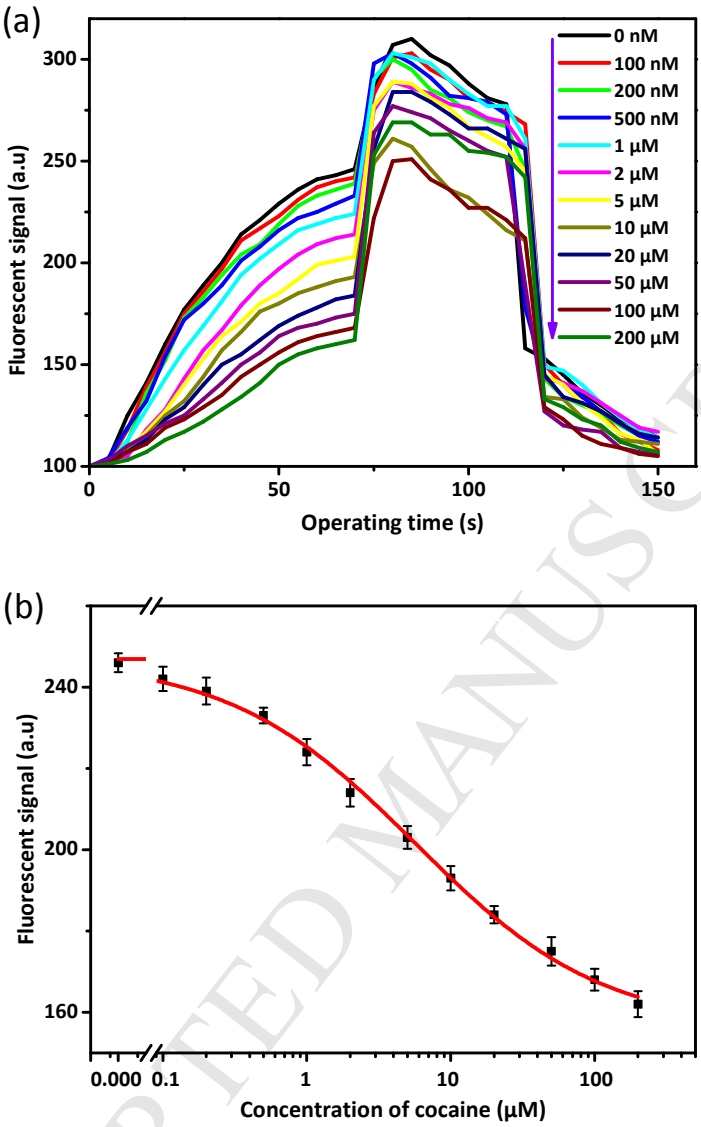

Fig. 2. (a) Typical fluorescent responses of the biosensor with a series of concentrations of cocaine: 0–200 μM; (b) Response curve obtained with different concentrations of cocaine and modification standard equation.

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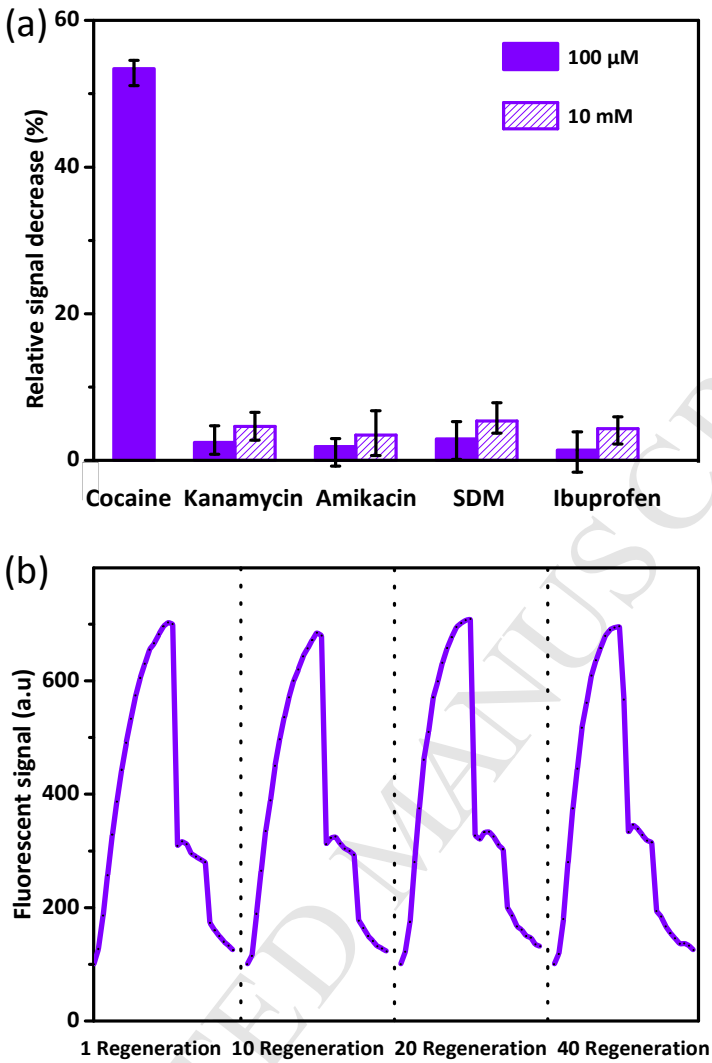

Fig. 3. (a) Relative signal decrease of different concentration of kanamycin, amikacin, SDM, and ibuprofen against 100 μM cocaine; (b) Representative response curves for hybridization of the biosensor in multiple cycles in the absence of cocaine; the curves are hybridization signals after 1, 10, 20, and 40 regeneration cycles.

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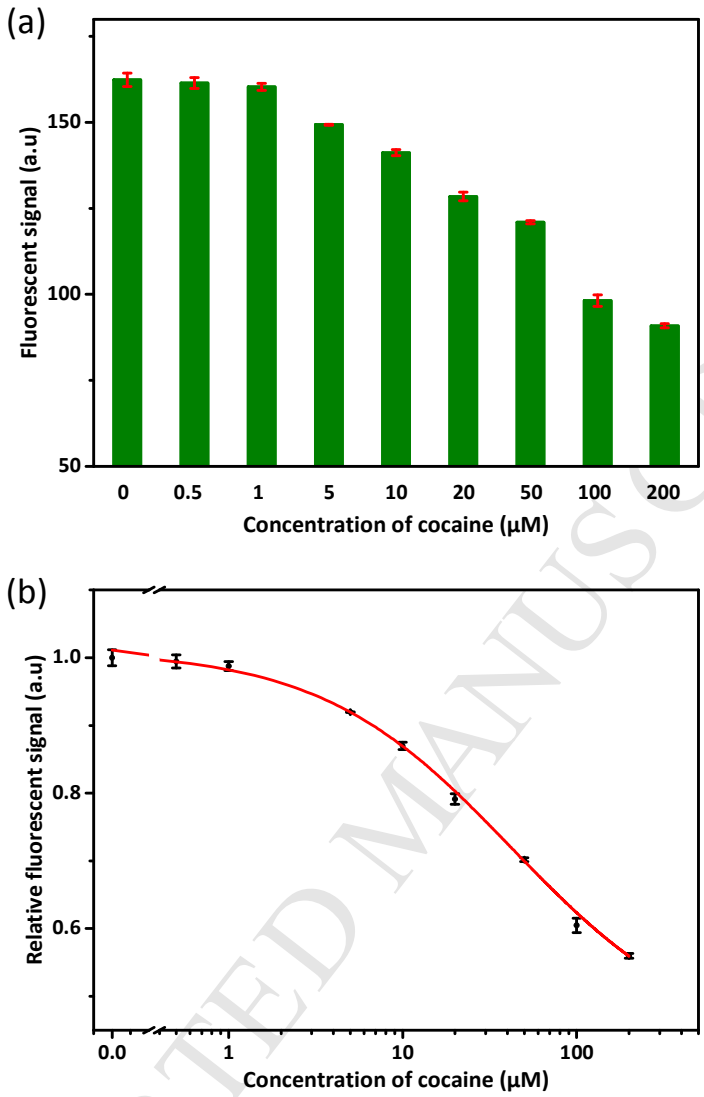

Fig. 4. (a) Different concentrations of cocaine in 10% human serum measured in the optical biosensor, 0–200 μM; (b) Calibration curve for cocaine detection in 10% human serum.

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Highlights:

- Split-aptamer was applied on fiber optical biosensing platform for the first time.
- The detection time was reduced to 450 s by the two stage reaction.
- The split-aptasensor can realize the in situ cocaine detection in serum.
- The aptasensor can be regenerated in 75 s for 40 times.

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