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# A novel electrochemical aptasensor based on H-shape structure of aptamer-complimentary strands conjugate for ultrasensitive detection of cocaine

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ABSTRACT

Cocaine is one of the most commonly abused stimulant which could affect the central nervous system. In this work, an electrochemical aptasensor was designed for sensitive and selective detection of cocaine, based on complimentary strands of aptamer (CSs), H-shape structure of Aptamer (Apt)-CSs conjugate and gold electrode. This aptasensor inherits properties of gold such as high electrochemical conductivity and large surface area, as well as high selectivity and sensitivity of aptamer toward its target and property of H-shape structure of Apt-CSs conjugate to act as a gate for the access of redox probe to the surface of electrode. In the absence of cocaine, the gate is closed, so that the electrochemical signal is weak. In the presence of cocaine, aptamer binds to target, leaves the surface of electrode and the gate is opened, leading to a strong electrochemical signal. The fabricated electrochemical aptasensor exhibited good selectivity toward cocaine with a limit of detection (LOD) as low as 0.228 nM. Furthermore, the designed aptasensor was successfully used to detect cocaine in serum with a LOD as low as 0.273 nM.



## 1. Introduction

Cocaine acts as a powerful stimulant on the central nervous system. Abuse of cocaine could cause the serious side effects on human, including organ damage, cardiac arrest, anxiety and spread of human immunodeficiency [1–3]. Cocaine addiction is a common worldwide problem [4]. The sensitive detection of cocaine is essential for clinical diagnosis and law enforcement [5,6].

Radioimmunoassay, high performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS) and enzyme-linked immunosorbent assay (ELISA) are the common analytical approaches for cocaine detection. Most of these analytical techniques are expensive, time-consuming and need extensive sample preparation [3,4,7,8].

Aptamers are short single-stranded oligonucleotides, selected by an in vitro process called SELEX (systematic evolution of ligands by exponential enrichment) [9,10]. Aptamers bind to their pre-selected targets, ranging from small substances to cells with high specificity and affinity [11,12]. Relative to antibodies, aptamers exhibit unique properties such as low cost, ease of synthesis and modifications, excellent thermal stability and lack of immunogenicity and toxicity [10,13–15]. Because of these properties, aptamer-based sensors (aptasensors) have attracted substantial attention.

Gold has been commonly used for molecular sensing, owing to its good biocompatibility, large surface area and unique electronic and optical properties [16–18]. Gold usually applies to modify the surface of electrodes, owing to its high affinity of binding to thiol-labeled molecules and high electro-transfer ability [11].

Among the different sensing approaches, electrochemical aptasensors possess unique advantages, such as simplicity, rapid response, high sensitivity and low cost [19–21]. Compared to optical aptasensors, electrochemical-based apasensors need less quantity of target for detection and do not require fluorescent labeling [6].

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In this work, an electrochemical aptasensor was developed for the first time for detection of cocaine, based on complimentary strands of aptamer, H-shape structure of aptamer-complimentary strands conjugate and gold electrode. In this study, a 44-mer ssDNA aptamer, which binds to cocaine with high affinity [22], was applied as targeting agent.

## 2. Materials and methods

### 2.1. Materials

The cocaine aptamer (Apt) was adopted from [22], 5′-CCATAGGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCC-3′, and its complimentary strands (CSs), 5′-AGGTATCGTG-Thiol-3′ (CS1) and 5′-TGGAGACGTG-Thiol-3′ (CS2), were purchased from Microsynth (Switzerland). Plasma from rat, chloramphenicol, cocaine, propranolol, morphine, diazepam, 6-mercaptohexanol (MCH), Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), Potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$) and potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) were obtained from Sigma (USA).

### 2.2. Apparatus

Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) measurements were performed using a μstat 400 portable Biopotentiostat/Galvanostat (DropSens, Spain). Screen-printed gold electrodes (SPGEs) were purchased from DropSens (Spain). The data were analyzed using DropView8400 software.

### 2.3. Preparation of Apt-CSs-modified electrode

CS1 (1 μM final concentration) and CS2 (1 μM final concentration) were pretreated with 10 mM TCEP in immobilization buffer (1 mM EDTA, 100 mM NaCl, 10 mM Tris–HCl, pH 7.4) for 1 h at room temperature. 9 μl of the above solution was added on the surface of electrode and incubated for 12 h at room temperature under 100% humidity. Then, 9 μl Apt (1 μM final concentration) in hybridization buffer (1 mM EDTA, 10 mM Tris–HCl, pH 7.4) was added on the surface of electrode. After incubation for 1 h at room temperature, the surface of SPGE was treated with 1 mM MCH solution (10 μl) for 1 h to block the left sites of electrode. Then, the surface of SPGE was rinsed thoroughly with the hybridization buffer.

### 2.4. Function study of the fabricated electrochemical aptasensor

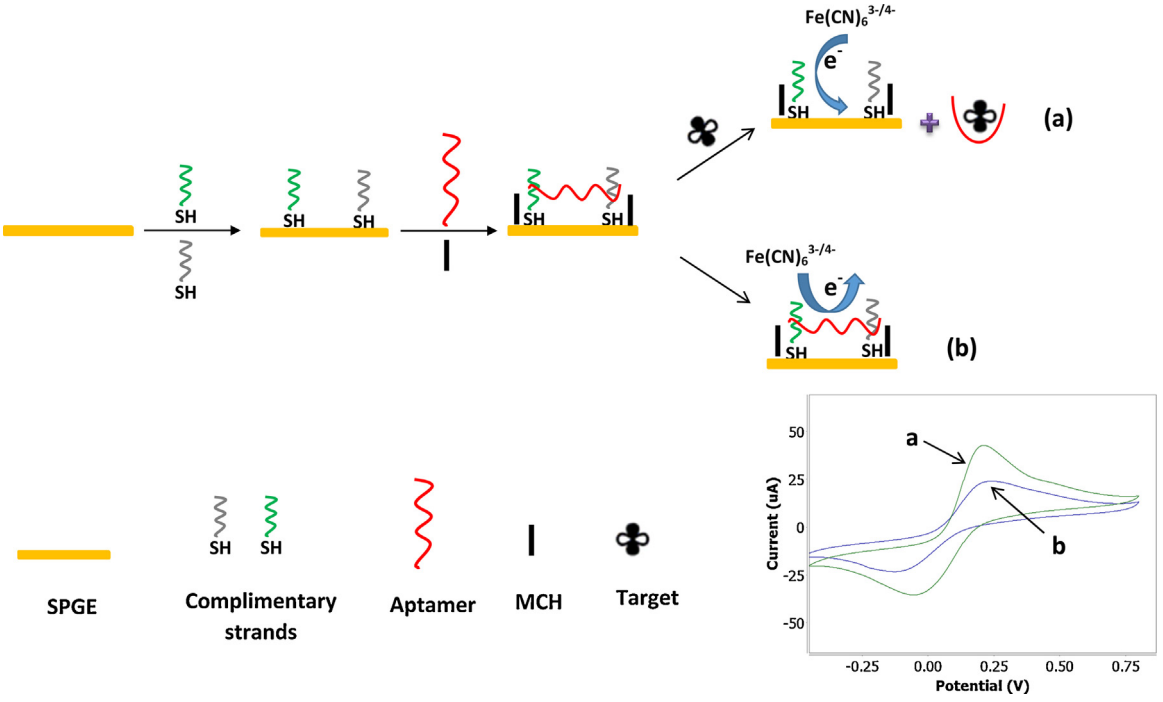
The interaction of the designed aptasensor and cocaine was assessed by electrochemical measurement. The Apt-CSs-modified electrode was incubated with 9 μl droplet of cocaine (15 nM) in phosphate buffer saline (10 mM PBS, pH 7.4) for 30 min at room temperature. The electrode was rinsed thoroughly with phosphate buffer saline (PBS, pH 7.4) and the electrochemical signals were measured using CV. CV measurements were performed in 2 mM $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ (redox probe) solution containing 0.1 M KCl, scanning from −0.5 V to 0.8 V at a scan rate of 50 mV/s.

### 2.5. Cocaine detection based on electrochemical measurements

Apt-CSs-modified electrodes were incubated with 9 μl droplet of different concentrations of cocaine (0–60 nM) for 30 min at room temperature. Next, the surface of electrodes were rinsed with PBS. The electrochemical signals were recorded using DPV. DPV measurements were carried out by scanning the potential from 0 V to 0.3 V with the pulse time of 25 ms and pulse potential of 30 mV.

### 2.6. Selectivity

The selectivity of the designed electrochemical aptasensor was analyzed in the presence of 15 nM cocaine, chloramphenicol, morphine, propranolol and diazepam (the concentration of each substance was 15 nM).




**Scheme 1.** Schematic illustration of cocaine detection based on an electrochemical aptasensor. In the absence of cocaine, the H-shape structure of Apt-CSs conjugate is intact and redox probe does not have access to the surface of electrode, leading to a weak electrochemical signal (b). In the presence of cocaine, aptamer binds to cocaine, leaves the CSs and redox probe have access to the surface of electrode, leading to enhancement of the electrochemical signal (a).