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DNA Nanostructure-Decorated Surfaces for Enhanced Aptamer-Target Binding and Electrochemical Cocaine Sensors

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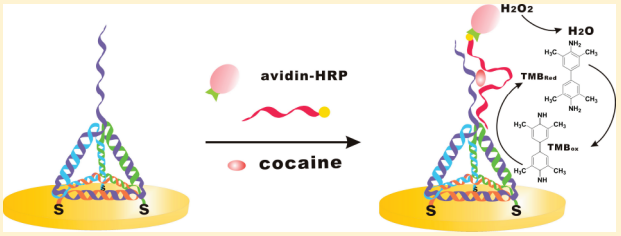
Supporting Information

ABSTRACT: The sensitivity of aptamer-based electrochemical sensors is often limited by restricted target accessibility and surface-induced perturbation of the aptamer structure, which arise from imperfect packing of probes on the heterogeneous and locally crowded surface. In this study, we have developed an ultrasensitive and highly selective electrochemical aptamer-based cocaine sensor (EACS), based on a DNA nanotechnology-based sensing platform. We have found that the electrode surface decorated with an aptamer probe-pendant tetrahedral DNA nanostructure greatly facilitates cocaine-induced fusion of the split anticocaine aptamer. This novel design leads to a sensitive cocaine sensor with a remarkably low detection limit of 33 nM. It is also important that the tetrahedra-decorated surface is protein-resistant, which not only suits the enzyme-based signal amplification scheme employed in this work, but ensures high selectivity of this sensor when deployed in sera or other adulterated samples.



Cocaine is a stimulant substance first isolated from the leaves of the coca plant, which has been well-recognized, because of the addictive properties of cocaine. Consequently, cocaine-related biomedical and psychosocial problems remain a major public concern,[1,2] and its illegal use represents part of the main constituent of police apprehensions.[3] It is thus highly desirable to develop simple and sensitive cocaine detection methods for law enforcement and clinical diagnostics. Nevertheless, conventional chromatography-based[4−8] or spectroscopy-based[9−14] methodologies are usually expensive, cumbersome, and time-consuming. More recently, electrochemical cocaine sensors have proven of high utility in this regard, mostly because of the readily available low-cost and small-sized electrochemical detectors.[15−23] In this study, we report a DNA nanotechnology-based platform for the development of a novel electrochemical aptamer-based cocaine sensor (EACS). In this design, we employ an engineered split aptamer for cocaine that ensures high-selectivity recognition of cocaine, with one fragment of the split aptamer anchored on rigid and spatially isolated DNA nanostructures that are immobilized on gold electrode surfaces. We have demonstrated that this architecture greatly facilitates aptamer-cocaine binding at the surface, leading to a cocaine sensor with low detection limit.

Aptamers are in-vitro-selected, single-strand functional nucleic acid receptors with antibody-like high ligand-binding affinity and specificity.[24,25] Because of their chemical robustness and synthesis convenience, aptamers have been actively explored to realize various molecular sensing and therapeutic applications,[26−34] including cocaine assays.[10,35−40] While there have been several successful examples for electrochemical cocaine sensors with the anticocaine aptamer, it remains challenging to realize a low detection limit for cocaine detection, partially because of the surface-induced perturbation of the aptamer structure and the reduced accessibility of the target molecule to probes at a heterogeneous and locally crowded surface.[41−44]

In order to circumvent these problems, we devise a novel nanostructured electrode surface decorated with an aptamer-pendant DNA tetrahedral structure. With the rapid emergence of DNA nanotechnology, it has been able to "bottom-up" construct exquisite DNA nanostructures with excellent controllability and high precision arising from unmatched self-recognition properties of DNA molecules.[45−55] Our previous study has demonstrated that a DNA tetrahedron nanostructure modified with sulfur at three vertices can be rapidly and firmly adsorbed at gold surfaces. This homogeneous self-assembled monolayer (SAM) of DNA nanostructure has proven to be well-ordered and highly stable.[54−58] More significantly, the presence of bulky tetrahedral structure not only avoids interprobe entanglement via spatially segregating pendant probes, but also reduces surface effects and places the probes in a solution-phase-like environment.[56]

EXPERIMENTAL SECTION

Materials. All oligonucleotides were synthesized and purified by TaKaRa, Inc. (Dalian, PRC), and the sequences are shown in

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Table 1. Synthetic Oligonucleotide Probes

name	sequence (5′−3′)
Tetra-aca-1	GGGAGTCAAGAACTTTTTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Tetra-B	SH-C_6TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
Tetra-C	SH-C_6-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
Tetra-D	SH-C_6-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
Biotin-aca-2	GTTCTTCAATGAAGTGGGACGACA-BIOTIN′
SH-aca-1	GGGAGTCAAGAAC-C_6−SH

Table 1. The TMB substrate (where TMB = 3,3′,5,5′-tetramethylbenzidine) was purchased from Neogen in the format of a ready-to-use reagent (K-blue low activity substrate, H_2O_2 included). Horseradish peroxidase-conjugated avidin (avidin-HRP) was from Roche Diagnostics (Mannheim, Germany). Ethylenediaminetetraacetic acid (EDTA), mercaptohexanol (MCH), and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Sigma. Fetal calf serum (Sigma−Aldrich) was used as-received. Sucrose and soda were purchased from a local retail supermarket and used as received. Enzyme diluent was 0.1 M PBS buffer with 0.5% casein (pH 7.2). All other chemicals were of analytical grade, and all chemicals were used without further purification. All solutions were prepared with Milli-Q water (resistivity = 18 MΩ cm) from a Millipore system.

Self-Assembly of the Anticocaine Aptamer-Appended Tetrahedron Probes at Gold Electrode Surfaces. Gold electrodes were cleaned following the reported protocol.[59] Anticocaine-aptamer-appended tetrahedron probes (Tetra-aca-1) were formed as reported in the literature.[54] Four strands—tetra-aca-1, B, C, and D—were dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), yielding a final concentration of 50 μM. A quantity of 2 μL of each strand was combined with 5 μL of TCEP (30 mM) and 37 μL of TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0), and the resulting mixture was heated to 95 °C for 2 min, then cooled to 4 °C over 30 s, using a Peltier Model PTC-200 thermal cycler (MJ Research, Inc., SA). Then, 3 μL of tetra-aca-1 probes were added to the cleaned gold electrode and allowed to react overnight at room temperature. Thiolated aca-1 (SH-aca-1) probes, immobilized to Au electrodes without using tetrahedra, were used for comparison: the SH-aca-1 immobilization buffer, 10 mM Tris, 1 mM EDTA, 3 mM TCEP, and 1 M NaCl (pH 7.4). A quantity of 3 μL of 2 μM SH-aca-1 probes were added to the surface of the gold electrodes and then allowed to sit overnight at room temperature. The SH-aca-1 modified electrode was further treated with 2 mM MCH for 2 h to obtain a well-aligned DNA monolayer. The electrode was rinsed with 0.01 M phosphate buffered saline (PBS) and dried lightly with N_2 before hybridization.

Surface Plasmon Resonance (SPR) Measurements. All surface plasmon resonance (SPR) experiments were performed at 25 °C on a Biacore 3000 instrument (Biacore, Inc.). To prepare the biosensor surface with TSP, 1 μM TSP in running buffer was automatically injected onto untreated gold surface sensor chips (Sensor Chip Au, GE Healthcare, Inc.) to the saturated density in different flow cell. DNA was diluted in running buffer.

Cocaine Detection with the Tetrahedron-Based EACS. Cocaine detection was carried out in the sandwich format. The target cocaine was first mixed with the biotinylated reporter aptamer fragment (biotin-aca-2, 100 nM) in 10 mM phosphate buffer (PB) and 1 M NaCl (pH 7.4) and heated to 75 °C for 5 min. The mixture was cooled to room temperature, and then a 3-μL droplet was pipetted at the tetrahedra-based electrode surface. After 30 min of incubation at room temperature, the sensor was rinsed with 0.01 M PBS and 0.8 M NaCl buffer and then incubated with 3 μL of avidin-HRP (0.5 U/mL) for 15 min at room temperature. The sensor was then extensively rinsed with 0.01 M PBS and subjected to electrochemical measurements. Detection of the cocaine analogues, benzoyl ecgonine (BE) and methylecgoine (ME), was performed using the same method.

Cocaine samples adulterated with soda or sucrose were prepared in a 1:1 ratio (m/m) of adulterant to cocaine, and these sampels were allowed to equilibrate in buffered solution for at least 5 min prior to testing. The cocaine solutions in 10% serum were prepared via the addition of 5 μL of a 1 mM cocaine solution and 5 μL of calf serum to 40 μL of a 10 mM PB buffer/1 M NaCl (pH 7.4) mixture and in 50% serum via the addition of 5 μL of a 1 mM cocaine solution and 25 μL of calf serum to 20 μL of a 20 mM PB buffer/2 M NaCl (pH 7.4) mixture.

Electrochemical Measurements. Electrochemical measurements were performed with a Model CHI 630 electrochemical workstation (CH Instruments, Inc., Austin, TX) and a conventional three-electrode configuration was employed throughout the experiment, which involved a gold working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (3 M KCl) reference electrode. Cyclic voltammetry (CV) was carried out at a scan rate of 50 mV/s. Amperometric detection was fixed at 150 mV, and the electroreduction current was measured at 100 s after the HRP redox reaction reached steady state.

RESULTS AND DISCUSSION

There have been various ways to realize DNA- and DNA aptamer-based electrochemical sensors,[60−63] with the "sandwich-type" strategy as the mostly popularly employed one.[60,61] Since only one aptamer exists for the small-molecule cocaine that does not support the sandwich strategy, we thus engineer the single-stranded anticocaine aptamer (aca) by splitting it into two fragments: aca-1 and aca-2. One fragment was appended to the rigid DNA tetrahedron on electrode surfaces as the capture element (aca-1). The other one (aca-2) was modified with a biotin tag to introduce signals. In the presence of cocaine, the two halves of the aptamer were expected to be reorganized to form a sandwich structure.[17] Notably, although aca-1 and aca-2 has 6-base complementary sequences, they do not form stable duplexes at room temperature (data not shown). The biotin tag can bind specifically to horseradish peroxidase-conjugated avidin (avidin-HRP), which catalyze the reduction of hydrogen peroxide to generate quantitative electrochemical current signals in the presence of the TMB (3,3′,5,5′-tetramethylbenzidine) cosubstrate (shown in Scheme 1).

Figure 1A displays cyclic voltammograms (CVs) of the HRP-based electrocatalytic process in this tetrahedra-based EACS.

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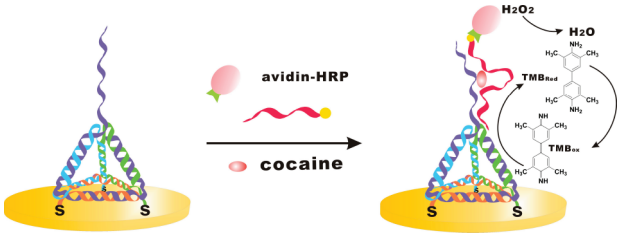
Scheme 1. Scheme for EACS, an Apatamer-Based Electrochemical Cocaine Sensor Using Tetrahedron-Decorated Gold Electrodes[a]



[a] The gold surface is anchored with aca-1 (purple)-appended tetrahedra. The presence of cocaine fuses aca-1 and biotinylated aca-2 (red). This binding process is transduced to electrochemical signals via the specific binding of avidin-HRP conjugates to the biotin tag of aca-2. HRP catalyzes electro-reduction of hydrogen peroxide in the presence of an electroactive cosubstrate, TMB, thus generating quantitative amperometric signals.

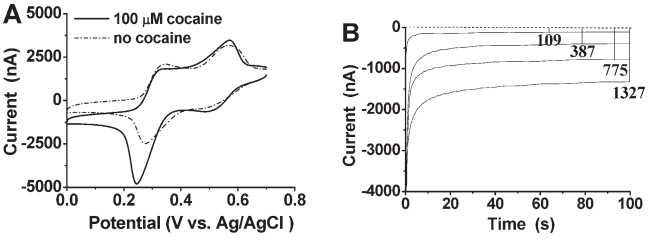


Figure 1. (A) Cyclic voltammograms (CVs) for tetrahedra-based electrochemical aptamer-based cocaine sensor (EACS) in the (– · –) absence and (—) presence of 100 μM of cocaine. Scan rate: 50 mV/s. (B) Amperometric curves (current (I) versus time (t)) for EACS tested in solution with a series of cocaine concentrations. From top to bottom: 0, 1, 100, and 1000 μM. Amperometric detection was fixed at 150 mV. A K-blue solution from Neogen with TMB and H_2O_2 was employed for the assays.

When cocaine was absent, we observed two pairs of well-defined redox peaks that were assigned to the two-electron reduction and oxidation reactions of TMB, suggesting that the presence of the tetrahedron nanostructure did not significantly interfere with the electron communication between TMB and the underlying gold electrode.[60] This indicates that, although the monolayer is relatively thick (~6 nm, as estimated from the duplex length), it is still amenable to electrochemical transduction with enzymatic amplification, because of the fact that the tetrahedra are hollow structures. This unique property makes it particularly useful for the development of high-sensitivity aptamer-based cocaine sensors, because it reduces the surface effect (increased layer thickness) without sacrificing electrochemical reactivity.

In the presence of cocaine, we found that the reduction peak located at ~270 mV apparently increased, leading to a pair of asymmetric redox peaks that was characteristic of the occurrence of electrocatalysis. This implied that the two halves of the aptamer formed the sandwich structure, leading to the binding of avidin–HRP to the biotin tag of aca-2 and, subsequently, the localization of the HRP enzyme to the electrode surface. Here, TMB served as an electron shuttle that could diffuse into and out of the redox site of the HRP, which coupled the catalytic reduction of H_2O_2 to the electrode surface and resulted in the observed electrocatalytic peaks.[62]

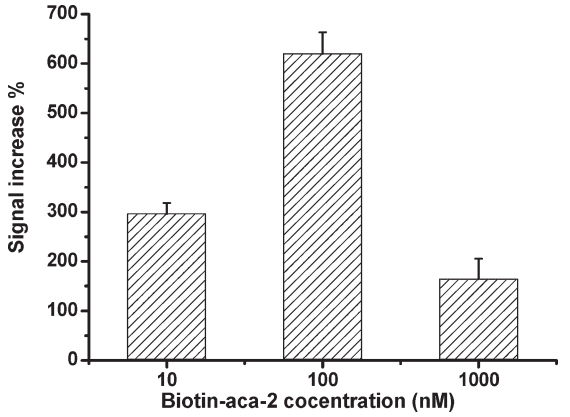


Figure 2. Optimization of the concentration of biotin-aca-2 for tetrahedra-based EACS (for 100 μM of cocaine). The signal increase was calculated as follows: signal increase (%) = $[(I_{cocaine}/I_{blank}) - 1)] \times 100$.

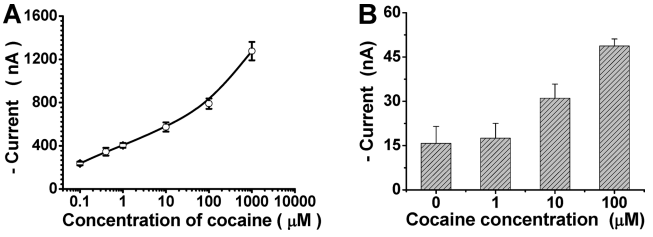


Figure 3. (A) Logarithmic plot of amperometric current versus cocaine concentration for tetrahedra-based EACS. (B) Concentration profile for the detection of cocaine, using single-stranded thiolated aca-1. Error bars represent standard deviations for measurements taken from at least three independent experiments.

Amperometry offers a direct way to characterize the HRP-catalyzed electrochemical process. A decay curve for the I–t relationship was observed instantly after the onset of the potential, which reached a plateau (steady-state current) within ~100 s (see Figure 1B). We found that the amperometric current for 1 mM of cocaine (~1200 nA) was ~10 times larger than that in the absence of cocaine (~110 nA). Importantly, the electrochemical signal of EACS was dependent on the concentration of aca-2. Apparently, an insufficient supply of the signaling part (aca-2) reduces the signal, while excessive addition of the aca-2 increases the background. In our experiments, we found that a concentration of 100 nM led to the highest electrochemical signal of EACS for cocaine detection (shown in Figure 2).

We then challenged the EACS with a series of concentrations of cocaine, covering a range of 4 orders of magnitude (100 nM to 1 mM). The amperometric signal increased monotonically (and nearly linearly) with the logarithm concentration of cocaine (see Figure 3A). Significantly, the signal for 100 nM of cocaine (~236 nA) could be easily distinguished from the background (~110 nA). The detection limit was calculated to be 33 nM (>3 standard deviations (SDs)), which exceeds that of a variety of previously reported electrochemical cocaine sensors with aptamers,[15−18,64] as well as aptamer-based colorimetric[10,38] or fluorescent assays (see comparison in Table 2).[9,11] More significantly, our sensitive EACS satisfactorily meets the U.S. government guideline cutoff level established by the Substance Abuse & Mental Health Services Administration (SAMHSA) (300 ng/mL or 1 μM for the initial test, and 150 ng/mL or 0.5 μM for the confirming test).

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Table 2. Comparison of Sensors for Cocaine Detection

detection methods	strategy	detection limit	sensor mode
electrochemistry			
this work	engineered aptamer−pendant DNA tetrahedral structure	33 nM	turn-on
ref 15	microfluidic electrochemical aptamer-based sensor	10 μM	turn-on
ref 16	cocaine-Pt-NPs−aptamer subunits	10 μM	turn-on
ref 17	Methylene Blue (MB)-tagged engineered aptamer	1 μM	turn-on
ref 18	Methylene Blue (MB)-tagged aptamer	10 μM	turn-on
colorimetric			
ref 10	engineered aptamer and AuNPs	2 μM	turn-off
ref 38	cyanine dye−aptamer combination	2 μM	turn-off
fluorescence			
ref 9	aptamer-based FRET	10 μM	turn-off
ref 11	autonomous aptamer-based machine	5 μM	turn-on

In order to further substantiate the advantage of using nanostructured gold surfaces with DNA tetrahedra, we designed a control system without using tetrahedral nanostructure. Thiolated aca-1 was directly anchored to Au electrodes via the Au−S chemistry, and the detection performance of the two systems was compared. Similar to that in the sensor with DNA tetrahedra, aca-1 captured cocaine that brought biotinylated aca-2 to the Au surface, and then avidin-HRP was employed to generate electrocatalytic signals. As shown in Figure 3B, the signal for 100 μM of cocaine was ~50 nA, with a signal-to-background ratio only of ~3. When the cocaine concentration was <10 μM, the signal was indistinguishable from the background. This limit of detection was higher than that of the tetrahedron-based EACS, by ~3 orders of magnitude.

This marked difference clearly suggests that the use of surfaces decorated with DNA nanostructures greatly enhanced the accessibility of cocaine to the surface-bound aptamer and possibly the affinity of cocaine to the split aptamer. While the target-induced fusion of two split aptamers at surfaces have not been studied extensively, previous theoretical and experimental studies on DNA surface hybridization provides a hint toward explaining this increased recognition at the interface.[42−44,65,66] DNA hybridization often is largely suppressed at surfaces immobilized with single-stranded probes, compared to solution-phase hybridization, because of the presence of significant electrostatic and packing constraints at crowded surfaces.[44,66] Such suppressed surface recognition could be mitigated at surfaces with low-to-medium probe densities. Nevertheless, while biomolecular recognition is favored at low-density surfaces, the surface preparation usually becomes less reproducible than that of high-density surfaces. Interestingly, the introduction of rigid DNA tetrahedra effectively reduces the surface density without compromising the reproducibility and ordering of the surface. The surface density of the tetrahedra-based surface was calculated to be 4.8×10^{12} probe/cm^2 (8.0 pmol/cm^2), using surface plasmon resonance (SPR) measurements (also see Figure S1 in the Supporting Information), which was converted to an interprobe space of 4.6 nm.[56] Based on the previously reported map of surface hybridization regimes, this density falls outside of the suppress-hybridization regime, thus ensuring relatively high accessibility of cocaine and aca-2.

A control experiment was carried out to confirm that the observed current change was only specific to the binding of cocaine with the split aptamer. As shown in Figure 4A, when the two cocaine analogues (1 mM each), benzoyl ecgonine (BE) and methylecgoine (ME), were employed, they produced signals only slightly larger than the background (~160 nA vs ~110 nA), which was markedly lower than that for 1 mM of cocaine (~1200 nA). This study confirms that (i) the split aptamer retains the selectively of the original aptamer and (ii) EACS possesses excellent selectivity for cocaine.

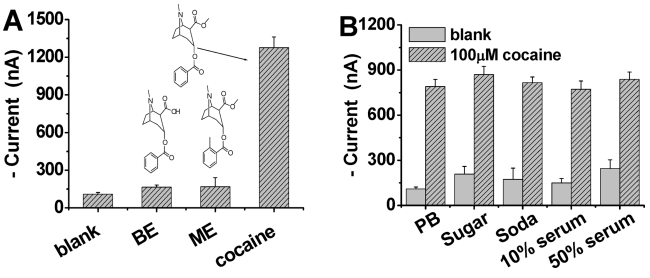

Figure 4. (A) Selectivity tests of tetrahedra-based EACS, using cocaine and two analogues (benzoyl ecgonine (BE) and methylecgoine (ME)) (1 mM each). (B) Selectivity tests of tetrahedra-based EACS in a range of media. From left to right: 100 μM of cocaine in phosphate (PB) buffer, 100 μM of cocaine (1:1 m/m) "masked" with sugar and baking soda, 100 μM of cocaine adulterated in 10% and 50% serum. Error bars represent standard deviations for measurements taken from at least three independent experiments.

It is critically important to evaluate the real applicability of EACS by challenging this sensor in various media. Serum is a complicated biological fluid containing a large number of proteins and other interference materials. Given that the tetrahedral-based surface is resistant to protein adsorption,[56] we tested EACS for 100 μM of cocaine in the presence of 10% serum. Significantly, we found that the signal was almost the same as that in a pure phosphate buffer, with only a slight increase in the background. In fact, EACS worked even in the presence of 50% serum, although the signal-to-background ratio was decreased. Similarly, we found that the EACS could be effectively used in tainted samples ("masked" (1:1 m/m) with baking soda or sugar), with minimal perturbation of the performance (shown in Figure 4B).

CONCLUSION

We have developed an ultrasensitive and highly selective electrochemical aptamer sensor for the detection of cocaine in

adulterated samples. The introduction of the tetrahedral nanostructure greatly facilitates aptamer-cocaine binding at the surface, which is directly translated into marked increase in signal-to-background ratio (e.g., ~800% increase in signal for 100 μM of cocaine detection versus an ~200% increase for the sensor without using tetrahedra). It is also important that the tetrahedron-decorated surface is protein-resistant, which not only suits the enzyme-based amplification system employed in this work, but ensures high selectivity of the sensor in sera or other adulterated samples.

■ ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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