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Electrochemical, Photoelectrochemical, and Surface Plasmon Resonance Detection of Cocaine Using Supramolecular Aptamer Complexes and Metallic or Semiconductor Nanoparticles

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Metallic or semiconductor nanoparticles (NPs) are used as labels for the electrochemical, photoelectrochemical, or surface plasmon resonance (SPR) detection of cocaine using a common aptasensor configuration. The aptasensors are based on the use of two anticocaine aptamer subunits, where one subunit is assembled on a Au support, acting as an electrode or a SPR-active surface, and the second aptamer subunit is labeled with Pt-NPs, CdS-NPs, or Au-NPs. In the different aptasensor configurations, the addition of cocaine results in the formation of supramolecular complexes between the NPs-labeled aptamer subunits and cocaine on the metallic surface, allowing the quantitative analysis of cocaine. The supramolecular Pt-NPs aptamer subunits-cocaine complex allows the detection of cocaine by the electrocatalyzed reduction of H_2O_2. The photocurrents generated by the CdS-NPs-labeled aptamer subunits-cocaine complex, in the presence of triethanol amine as a hole scavenger, allows the photoelectrochemical detection of cocaine. The supramolecular Au-NPs aptamer subunits-cocaine complex generated on the Au support allows the SPR detection of cocaine through the reflectance changes stimulated by the electronic coupling between the localized plasmon of the Au-NPs and the surface plasmon wave. All aptasensor configurations enable the analysis of cocaine with a detection limit in the range of 10^{-6} to 10^{-5} M. The major advantage of the sensing platform is the lack of background interfering signals.

Metallic[1−4] or semiconductor[5,6] nanoparticles (NPs) find growing interest as electrical, photoelectrochemical, or optical labels for biosensing events. The solubilization of metallic or semiconductor NPs acting as labels for biorecognition events and the electrochemical detection of the solubilized ions were used to amplify different biosensing events.[7−11] Also, metallic NPs such as Pt-NPs were used as electrocatalysts for the electrochemical detection of DNA or proteins.[12,13] Photoexcitation of semiconductor NPs and the generation of the electron-hole pair provides the basis for the photoelectrochemical effect and the generation of photocurrents. Indeed, semiconductor NPs were used as labels for sensing biocatalytic processes[14−16] or DNA sensing[17] through the generation of photocurrents.

The aggregation of Au-NPs as a result of biorecognition events, and the accompanying red-to-blue color change as a result of interparticle coupling of plasmons, was widely used for optical biosensing.[18] Also, the biocatalytic growth of Au-NPs and the resulting absorbance changes were used to follow biocatalytic reactions and the substrates of the respective enzymes.[19] Similarly, the coupling between the localized plasmon of Au-NPs and the surface plasmon wave associated with thin gold films was used for the amplified surface plasmon resonance (SPR) detection of biorecognition processes. For example, the formation of antigen-antibody complexes[20] or DNA hybridization[21] was amplified by labeling the recognition complexes linked to Au surfaces with Au-

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NPs, and the effects of the size of the NPs on the coupling efficiency was reported.[22−24] Aptamers are nucleic acids with specific binding properties toward low-molecular-weight substrates or biopolymers, and these are elicited by the Systematic Evolution of Ligands by Exponential Enrichment, SELEX, process.[25,26] The specific binding features of aptamers were, recently, implemented to develop electrochemical or optical biosensors (aptasensors).[27−31] Different electrochemical aptasensors were developed including the use of redox labels,[32] enzyme labels that yield electroactive substrates,[33] and label-free detection of low-molecular-weight substrates on field-effect-transistors.[34] Also, Au-NPs functionalized with aptamer subunits were used for the amplified detection of aptamer-substrate complexes using ion-sensitive field-effect transistor devices or impedimetric means.[35] Different optical aptasensors were reported using semiconductor quantum dots or organic dyes as fluorescent labels,[36,37] and the aggregation of Au-NPs upon formation of the aptamer-substrate complexes enabled the development of colorimetric aptasensors.[38] Also, the coupling of DNAzymes as labels that produce colored products, as a result of the biocatalytic reaction, were conjugated to the aptamer-substrate complexes, and this allowed the amplification of the sensing events.[39,40] Recently, the construction of aptasensors based on the self-assembly of aptamer subunits, in the presence of the analyte-substrate, into supramolecular structures, was introduced as a general platform for aptasensors. According to this method, the aptamer is divided into two subunits that do not interact with one another in the absence of the analyte. In the presence of the respective substrate, however, a tricomponent supramolecular aptamer complex is generated. By the appropriate labeling of the aptamer subunits with fluorophores,[41] semiconductor quantum dots/dyes,[42] or pyrene units,[42] the formation of the supramolecular aptamer-substrate complexes were optically followed by fluorescence resonance energy transfer (FRET) or by the pyrene excimer emission. Also, the labeling of one of the aptamer subunits with a redox label allowed the electrochemical readout of the formation of the supramolecular aptamer subunits-analyte complex on electrode surfaces.[43] Similarly, by the labeling of the aptamer subunits with enzymes or cofactor/enzyme units, the formation of the supramolecular aptamer-substrate complex was amplified by enzyme cascade or by the cofactor/enzyme coupled biocatalytic transformation.[44] Here, we wish to report on the functionalization of metallic or semiconductor NPs with a subunit of the anticocaine aptamer and on the application of the modified NPs as labels for the electrochemical, photoelectrochemical, or surface plasmon resonance detection of the cocaine-aptamer complexes on surfaces.

EXPERIMENTAL SECTION

Materials and Reagents. Ultrapure water from NANOpure Diamond (Barnstead Int., Dubuque, IA) was used throughout the experiments. Bis(sulfosuccinimidyl) suberate (BS^3) was purchased from Pierce Biotechnologies. All other chemicals were purchased from Sigma-Aldrich and used as received without further purification.

The sequences of the oligonucleotides used in this study are

(**1**) 5′-GGGAGTCAAGAACGAAAAAAA$(CH_2)_3$SH-3′

(**2**) 5′-HS$(CH_2)_6$AAAAAATTCGTTCTTCAATGAAGTGGGACGACA-3′

(**3**) 5′-$NH_2(CH_2)_6$TTCGTTCTTCAATGAAGTGGGACGACA-3′

(**4**) 5′-GGGAGTCAAGAACGAA$(CH_2)_3$SH-3′

(**5**) 5′-HS$(CH_2)_6$TTCGTTCTTCAATGAAGTGGGACGACA-3′

All of the chemically purchased thiol-functionalized aptamer subunits were activatd by reacting the oligonucleotides in 0.1 M phosphate buffer (pH = 7.4) containing 0.1 M dithiothreitol for at least 2 h. Subsequently, aliquots of the deprotected DNA solution were purified using a microspin (G-25) column.

Preparation and Functionalization of NPs with the Aptamer Subunits. *Preparation of Pt Nanoparticles (Pt-NPs).* Platinum NPs were prepared by heating 100 mL of a 1 mM K_2PtCl_6 solution to reflux, while adding 10 mL of a 38.8 mM aqueous sodium citrate solution. After 20 min of boiling, an additional 10 mL of 38.8 mM an aqueous sodium citrate solution was added, resulting in an instant change of color from clear to dark brown. The solution was heated for an additional 30 min, after which, the solution was allowed to cool down to room temperature and, then, stirred for an additional time interval of 48 h. The resulting solution was filtered through a 0.45 μm cellulose acetate filter (Schleicher and Schuell, Keene, NH), and rinsed once through a 30 000 MW cutoff Microcon tube (Millipore Inc., Billerica, MA) with water. The resulting NPs were dispersed in a 10 mM phosphate buffer (100 mM NaCl, pH = 7.4). The size of the NPs was determined by a transmission electron microscope (TEM) to be ca. 4 nm (diameter).

Preparation of DNA-Modified Pt-NPs. The DNA-aptamer-modified Pt-NPs were prepared by mixing 450 μL of filtered and washed

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Pt-NPs solution with 50 μL of 10 mM phosphate buffer (pH = 7.4) containing 1×10^{-5} M of the thiol-functionalized ss-DNA (**1**) for 18 h. Then, NaCl was slowly added to the mixture to yield a 0.1 M NaCl solution that was stirred for 2 h. Subsequently, the solution was centrifuged for 30 min at 14 000 rpm using a 30 000 MW cutoff Microcon tube (Millipore Inc., Billerica, MA). The aptamer subunit-modified Pt-NPs were resuspended in 500 μL of 10 mM phosphate buffer (100 mM NaCl, pH = 7.4).

Preparation of CdS Nanoparticles. A dioctyl sulfosuccinate sodium salt (AOT)/*n*-heptane water-in-oil microemulsion was prepared by the solubilization of 2.0 mL of water in 100 mL of *n*-heptane in the presence of 7.0 g of AOT as surfactant. The mixture was stirred until a clear phase was generated. The resulting mixture was separated into 60 and 40 mL of reverse-micelle subvolumes. Aqueous solutions of $Cd(ClO_4)_2$ (240 μL, 1.35 M) and Na_2S (160 μL, 1.33 M) were added to the 60 and 40 mL subvolumes, respectively, and the two microemulsion volumes were combined and stirred for 1 h to yield the CdS-NPs. For the preparation of cystamine dihydrochloride-capped CdS-NPs, a mixture consisting of an aqueous solution of 2-mercaptoethane sulfonic acid sodium salt (200 μL, 0.32 M) and cystamine dihydrochloride (200 μL, 0.32 M) was added to the resulting CdS-NPs micellar solution, and the mixture was stirred for 14 h under argon. Pyridine, 20 mL, was, then, added to the system, and the precipitated NPs were centrifuged and washed with *n*-heptane, petrol ether, butanol, ethanol, methanol, and diethyl ether (twice with each of the solvents).

Preparation of DNA-Modified CdS-NPs (CdS-NPs). A stock solution of CdS-NPs was prepared by dissolving 2 mg of the cystamine modified-CdS in 500 μL of 10 mM HEPES buffer solution (pH = 7.5). To 70 μL of the stock solution, diluted by 1.5 fold with 10 mM HEPES buffer (pH = 7.5), was added an excess of the BS[3] linker, and the mixture was shaken for 30 min. The resulting NPs were precipitated by centrifugation, followed by decantation, and were subsequently reacted with 100 μL of 10 mM HEPES buffer solution (pH = 7.5) that included 1×10^{-5} M of the nucleic acid (**3**) for 2 h. Finally, the resulting NPs were purified by precipitation by centrifugation followed by decantation of the solvent. The resulting (**3**)-modified CdS-NPs were, then, suspended in a 10 mM phosphate buffer (100 mM NaCl, pH = 7.4).

Preparation and Functionalization of Au-NPs. The 13 nm Au-NPs were prepared using a standard citrate method.[45] The Au-NPs were functionalized by derivatizing aqueous Au colloid with deprotected thiol-oligonucleotides (**5**) (final concentration of 10 μM for oligonucleotides and 10 nM for AuNPs, respectively). The resulting Au-NPs and oligonucleotide mixture were incubated at room temperature and gently shaken for 24 h. The modified Au-NPs solution was, then, incubated for an additional time interval of 24 h at room temperature while gradually adding a $NaNO_3$ solution to yield a final 100 mM solution. (Gradual addition of the nitrate solution is essential to avoid precipitation of the NPs.) The resulting DNA-Au-NPs were purified by three consecutive precipitation/resuspension steps by centrifugation (14 000 rpm for 35 min) and resuspension of the Au-NPs in a 10 mM phosphate buffer (pH 7.0).

Preparation of Aptamer-Modified Gold Electrodes. *Preparation of Thiolated Aptamer-Modified Gold Electrodes for Electrochemical and Photoelectrochemical Experiments.* Gold slides (Au-coated glass microarray slides were purchased from Nalge Nunc International, Rochester, NY) were cut to the size of 22×22 mm and 9×25 mm for electrochemical and photoelectrochemical detection, respectively. The slides were placed in boiling ethanol solution for 2 min, followed by their sonication for 5 min in an ethanol solution at room temperature. The electrodes were, then, rinsed with water and treated with a piranha solution (70% sulfuric acid, 30% H_2O_2), followed by rinsing them with water and finally drying under argon. *Caution: Piranha solution reacts violently with many organic materials and should be handled with great care.*

The functionalization of the Au electrodes was achieved by placing a 400 μL (for the 22×22 mm electrode) or 200 μL (for the 9×25 mm electrode) drop of a 1×10^{-5} M solution of the thiolated cocaine aptamer subunit ((**2**) and (**4**) for electrochemical and photoelectrochemical experiments, respectively). The solutions were allowed to interact with the electrodes for 18 h, rinsed, dried, and placed in a solution of 1 mM mercaptohexanol in 10 mM phosphate buffer (100 mM NaCl, pH = 7.4) for 1 h. This procedure yielded the DNA/mercaptohexanol mixed-monolayer sensing surface. The resulting electrodes were rinsed with a phosphate buffer solution and, then, dried under argon.

Preparation of Thiol-Aptamer-Modified SPR Gold Electrodes. Au-coated semitransparent glass slides (0.5 mm thickness, Mivitec GmbH, Analytical μ-Systems, Germany) were used for the SPR measurements. Prior to modification, the Au electrode was cleaned in hot ethanol for 5 min, followed by a gentle rinse with water and subsequently dried under nitrogen. The clean Au SPR slides were reacted with 1×10^{-4} M of (**4**) for 24 h in a 10 mM phosphate buffer solution (100 mM $NaNO_3$, pH = 7.4) at room temperature. The slides were, then, rinsed with the same buffer solution and, then, dried under argon.

Experimental Protocol. *Electrochemical and Photoelectrochemical Protocol.* The analysis of cocaine by these systems was accomplished by drop casting 200 μL (CdS) or 400 μL (Pt) of a 2-fold dilution of the aptamer-functionalized NPs solution, which contained the appropriate cocaine concentration, allowing it to interact with the respective aptamer subunits-functionalized electrode for 30 min.

SPR Experimental Protocol. The detection of cocaine was performed by first exposing the aptamer subunit-modified electrode to 300 μL of phosphate buffer until the stabilization of the SPR signal was reached. Subsequently, a solution of the aptamer-subunit-functionalized-Au-NPs and the respective (variable) concentrations of cocaine were added to the cell. Sensograms were recorded by the primary interaction of the SPR gold surface with a solution of 200 μL of modified-Au-NPs, followed by the addition of cocaine required to reach the appropriate concentration.

It should be noted that a two-compartment SPR cell was used in the experiment. The Au surfaces in the two compartments were functionalized with the probe aptamer subunit (**4**) and the (**5**)-subunit-functionalized Au-NPs were added at the same concentra-

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tion into the two compartments. Cocaine at variable concentrations was added to the analyzing cell while adjusting the reference cell with an identical volume of the buffer solution. The resulting sensogram corresponded to the reflectance changes as compared to the reference compartment. Thus, any reflectance changes originating from nonspecific binding of the (**5**)-functionalized NPs are excluded.

It should also be noted that washing of the two-compartment cell with a phosphate buffer solution, pH = 7.4, 2 mL, did not alter the SPR curve, indicating that the cocaine-aptamer subunit complex is stable upon these rinsing conditions.

Experimental Setup. All electrochemical experiments were carried out using an Autolab electrochemical system (ECO Chemie, The Netherlands) driven by GPES software. Cyclic voltammograms and linear sweep voltammograms were recorded by introducing the modified gold slides into 3 mL of 10 mM phosphate buffer solution (100 mM NaCl, pH = 7.4) containing and 10 mM H_2O_2 and recording the voltammetric response using a saturated calomel electrode as a reference and a carbon counter electrode.

Photoelectrochemical experiments were performed using a home-built photoelectrochemical system that included a 300W Xe lamp (Oriel, model 6258), a monochromator (Oriel, model 74000, 2 nm resolution), and a chopper (Oriel, model 76994). The electrical output from the cell was sampled by a lock-in amplifier (Stanford Research model SR 830 DSP). The shutter chopping frequency was controlled by a Stanford Research pulse/delay generator, model DE535. The photogenerated currents were measured between the modified Au working electrode and the carbon counter electrode. The measurements were performed in a 10 mM phosphate buffer solution (100 mM NaCl, pH = 7.4), containing 20 mM triethanolamine.

A surface plasmon resonance (SPR) Kretschmann type spectrometer Nano SPR 321 (NanoSPR devices, USA) with a LED light source, $\lambda = 650$ nm, and with a prism refraction index of $n = 1.61$ was used in this work. The in situ measurements were conducted using a home-built fluid cell (0.2 cm^2 area solution exposed to Au surface).

UV−vis spectroscopy measurements were carried out using a Shimdzu UV-2401PC spectrophotometer.

TEM images were recorded on a Tecnai F20 G2 (FEI Co.) using an accelerating voltage of 200 kV. Samples were prepared by placing a 5 μL drop of the Pt-NPs solution on a 3 mm copper TEM grid and allowing the droplet to evaporate to dryness.

RESULTS AND DISCUSSION

Scheme 1 path A outlines the electrochemical detection of cocaine using the aptamer subunits approach. The Pt-NPs, 4.0 nm in diameter, were functionalized with the nucleic acid (**1**). The loading of (**1**) on Pt-NPs was determined spectroscopically to be ca. 20 per particle. The thiolated nucleic acid (**2**) was assembled on a Au electrode. The surface coverage of (**2**) assembled on the electrode was determined by Tarlov's method[46] to be ca. 8.3×10^{-13} mol/cm^2. In the presence of cocaine, the supramolecular complex consisting of cocaine and the two aptamer subunits is formed on the electrode, resulting in the labeling of the complex with the catalytic Pt-NPs. This enabled, then, the electrocatalytic reduction of H_2O_2, a process that amplifies the formation of the cocaine-aptamer complex. Figure 1A, shows the time-dependent electrocatalytic cathodic currents upon interaction of the (**2**)-modified Au-electrode in the presence of the (**1**)-functionalized Pt-NPs and cocaine, 1×10^{-3} M. As the time of interaction of the electrode with the components in solution is prolonged, the electrocatalytic cathodic currents are intensified, and they level off to a saturation value after ca. 30 min, Figure 1A, inset. The time-dependent increase of the cathodic currents corresponds to the dynamics of the self-assembly of the supramolecular complex on the electrode surface. The saturated cathodic current corresponds to the equilibrium reaction generated at this specific concentration of the cocaine analyte. Control experiments reveal that in the absence of cocaine only a residual electrocatalytic current is observed, Figure 1A, curve (a), implying that the supramolecular cocaine-aptamer fragments is formed on the electrode surface only in the presence of the analyte. Accordingly, we monitored the electrocatalytic cathodic currents generated by the Pt-NPs-labeled supramolecular complexes formed in the presence of variable concentrations of cocaine, Figure 1B. In these experiments, the electrodes were treated with different concentrations of cocaine for a fixed time interval corresponding to 30 min, to yield the equilibrated complex on the electrode surface. As the concentration of cocaine increases, the electrocatalytic cathodic currents are intensified, consistent with the formation of a higher content of the labeled cocaine-aptamer complex on the electrode surface. The derived calibration curve is depicted in Figure 1B, inset. The detection limit for analyzing cocaine corresponds to 1×10^{-5} M. Assuming a Langmuir-type binding process to the surface, a dissociation constant of $(3.5 \pm 0.4) \times 10^{-5}$ M was derived. For the effect of ascorbic acid on the electrochemical sensing of cocaine, see the Supporting Information.

It should be noted that a redox-labeled intact anticocaine aptamer was previously reported for the electrochemical detection of cocaine.[32] The detection limit of our system is comparable with the sensitivity reported for the intact aptamer system. The major advantage of our approach that involves the self-assembly of the aptamer subunit rests, however, on the fact that our system has no background signal. The aptamer subunit-functionalized Pt-NPs bind to the electrode surface only in the presence of cocaine, and thus, the electrocatalytic currents are observed only in the presence of the analyte. A further advantage of our system involves the electrochemical detection of the analyte at a potential of ca. −0.6 V vs SCE, where the electrochemical oxidation of interfering components is eliminated. For the future applications of the sensor system to analyze cocaine in biological samples, we examined the effect of added albumin (50 mg/mL), on the performance of the electrode. We did not find any decrease in the voltammetric response of the electrode under these conditions.

One further aspect in the characterization of the cocaine-Pt-NPs-aptamer subunit system is related to the possibility to regenerate the sensing surface. We find that rinsing the electrode modified with the cocaine-Pt-NPs-aptamer subunits with distilled water or the thermal treatment (50 °C for 20 min) in distilled water did not separate the complexes in the electrode surface. This apparent stability of the cocaine-aptamer subunits is presumably due to the multisite ligation of the (**1**)-functionalized Pt-NPs with

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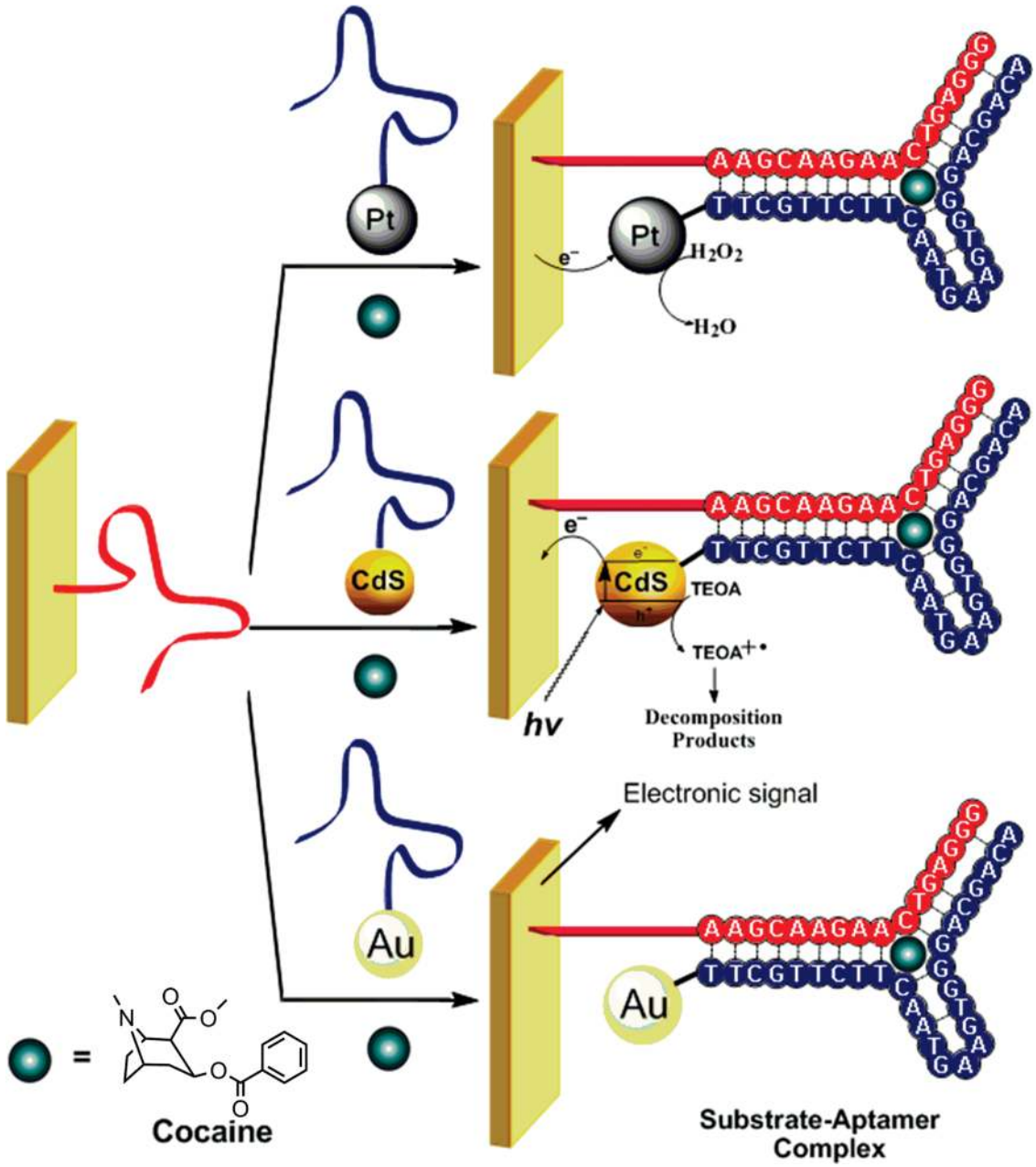
Scheme 1. Electrochemical (Path A), Photoelectrochemical (Path B), and SPR (Path C) Analysis of Cocaine through the Self-Assembly of Supramolecular Complexes of Pt-NPs-, CdS-NPs-, or Au-NPs-Functionalized Aptamer Subunits and Au-Surfaces Functionalized with the Second Aptamer Subunit in the Presence of Cocaine



the surface. We find, however, that the treatment of the electrode modified with the cocaine-Pt-NPs-aptamer subunit complex with urea 6 M (25 min, room temperature) completely regenerated the sensing interface, and the reusability of the electrode was demonstrated, Figure 1C.

The use of semiconductor nanoparticles as labels for the photoelectrochemical readout of biorecognition events or biocatalytic transformations attracts substantial recent research efforts.[47,48] For example, DNA hybridization,[49] biocatalytic transformations, and inhibition of enzymes,[3,50] were followed by the photocurrents generated by biomolecule-semiconductor NPs hybrid systems linked to electrodes. In the present study, we used CdS-NPs as photoelectrochemical labels for the readout of the formation of the supramolecular complex between the anticocaine aptamer subunits and cocaine, Scheme 1 path B. The CdS-NPs (3 nm) were modified with one of the aptamer subunits (**3**). The loading of (**3**) on the CdS-NPs was estimated spectroscopically to be ca. 16 units per particle. The second thiolated aptamer subunit (**4**) was immobilized on a Au electrode. In the presence of cocaine, the supramolecular complex between the aptamer subunits and cocaine is formed on the surface. Photoexcitation of the CdS-NPs yield the electron hole pair in the NPs. The ejection of the conduction-band electrons into the electrode and the concomitant scavenging of the valence-band holes by the sacrificial electron

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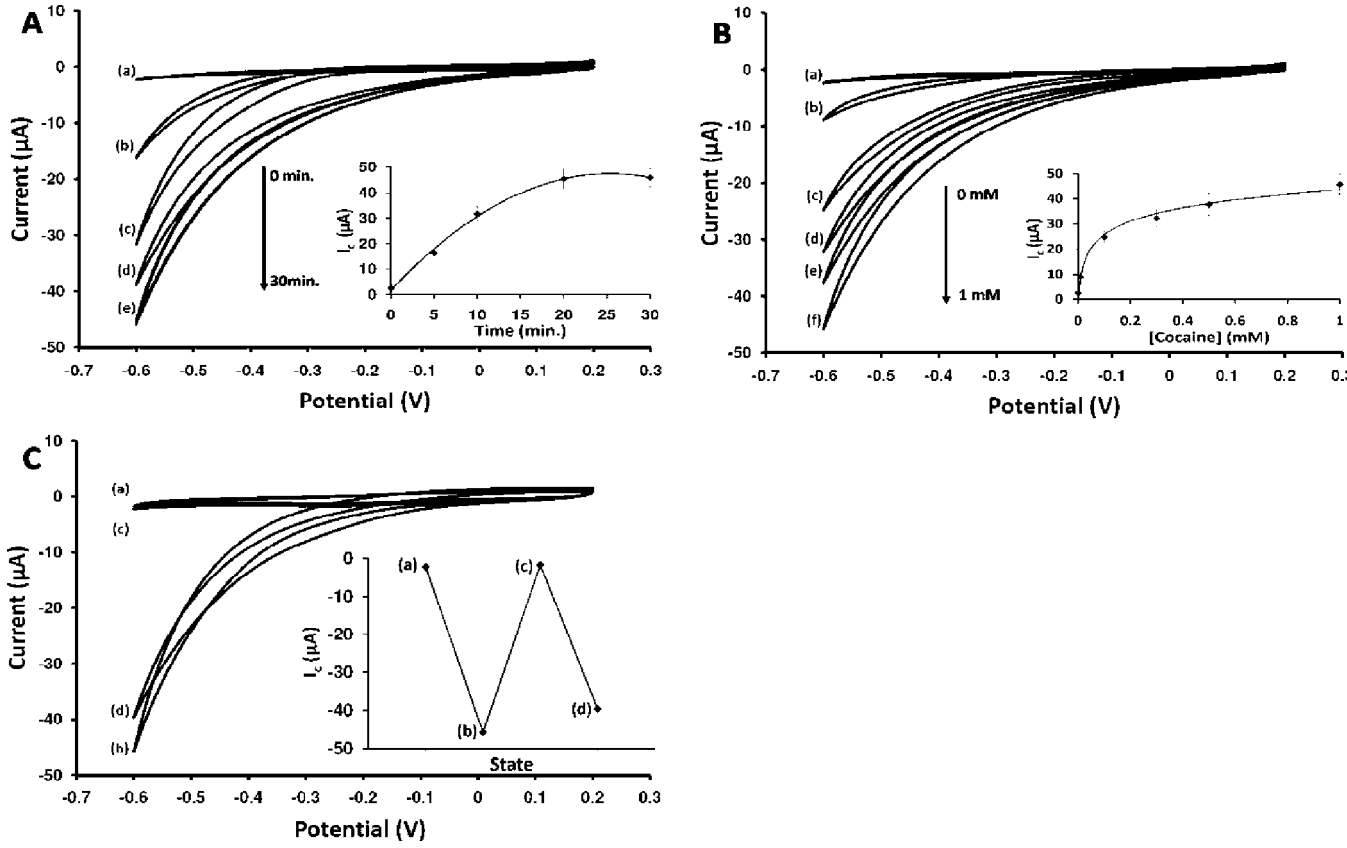

Figure 1. (A) Cyclic voltammograms corresponding to the (**2**)-modified Au electrode in the presence of (**1**)-functionalized Pt-NPs (a) in the absence of cocaine. (The voltametric response is not altered with time.) (b−e) in the presence of cocaine, 1×10^{-3} M at time intervals corresponding to 5, 10, 20, and 30 min of hybridization, respectively. Error bars were based on $N = 5$ experiments. Inset: Electrocatalytic cathodic currents at −0.6 V vs SCE at different time intervals corresponding to the self-organization of the Pt-NPs-labeled aptamer subunits-cocaine complexes on the electrode. Error bars were based on $N = 5$ experiments. (B) Cyclic voltammograms corresponding to the analysis of different concentrations of cocaine: (a) 0 M, (b) 1×10^{-5} M, (c) 1×10^{-4} M, (d) 3×10^{-4} M, (e) 5×10^{-4} M, and (f) 1×10^{-3} M by the Pt-NPs-labeled complexes on the electrode. Inset: The derived calibration curve corresponding to the analysis of cocaine. All experiments were performed in 10 mM phosphate buffer (pH = 7.4), in the presence of 10 mM H_2O_2; scan rate = 0.01 V/sec. Error bars were based on $N = 5$ experiments. (C) Cyclic voltammograms corresponding to the (**2**)-modified Au electrode (a) in the absence of cocaine, (b) after interaction with the (**1**)-functionalized Pt-NPs in the presence of 1×10^{-3} M cocaine, (c) after treatment of the electrode with 6 M urea for 25 min, and (d) after a second interaction with the (**1**)-functionalized Pt-NPs in the presence of 1×10^{-3} M cocaine. Inset: The cyclic response of the system represented by electrocatalytic cathodic currents at −0.6 V vs SCE of the (**2**)-modified Au electrode (a) in the absence of cocaine, (b) after interaction with the (**1**)-functionalized Pt-NPs in the presence of 1×10^{-3} M cocaine, (c) after treatment of the electrode with 6 M urea for 25 min, and (d) after a second interaction with the (**1**)-functionalized Pt-NPs in the presence of 1×10^{-3} M cocaine. Error bars were based on $N = 5$ experiments.

donor, triethanolamine, solubilized in the electrolyte solution, results in the formation of a photocurrent. The intensities of the resulting photocurrents are controlled by the amount of supramolecular cocaine-aptamer complexes associated with the electrode. Figure 2A shows the time-dependent photocurrent spectra observed upon interacting the (**4**)-modified electrode with the (**3**)-functionalized CdS-NPs, in the presence of cocaine, 1×10^{-3} M. As the time of interaction is prolonged, the photocurrents are intensified, and the photocurrent levels-off to a saturation value after ca. 20 min, Figure 2A, inset. Time time interval that corresponds to the equilibration of the supramolecular complex on the electrode surface. The photocurrent action spectra overlap the absorption spectrum of the CdS-NPs, indicating that the photocurrents originate from the excitation of the semiconductor NPs. Exclusion of triethanolamine from the system prohibited the formation of any photocurrent, implying that the scavenging of the valence-band holes by the electron donor is essential to generate the steady-state photocurrents. Further, control experiments revealed that no photocurrent was generated by the system when cocaine was excluded from the system. These control experiments imply that the photocurrent is formed only upon the formation of the CdS-labeled supramolecular complex consisting of the cocaine-aptamer subunits on the electrode surface. Thus, the resulting photocurrent may be used as a readout signal for the analysis of cocaine. Figure 2B shows the photocurrent action spectra generated by the equilibrated supramolecular cocaine-aptamer complexes formed on the electrode in the presence of different concentrations of cocaine. The photocurrents are intensified as the concentration of cocaine is elevated, consistent with the higher coverage of the electrode with the photoelectrochemically active supramolecular complex. The method enabled the analysis of cocaine with a detection limit that corresponded to 1×10^{-6} M. Assuming a Langmuir-type binding of the cocaine and the subunits to the surface, the derived dissociation constant was calculated to be $(2.5 \pm 0.4) \times 10^{-6}$ M. This value agrees with previously reported value.[31,41]

A further method for the amplified detection of cocaine, by means of the aptamer subunits-cocaine supramolecular structure

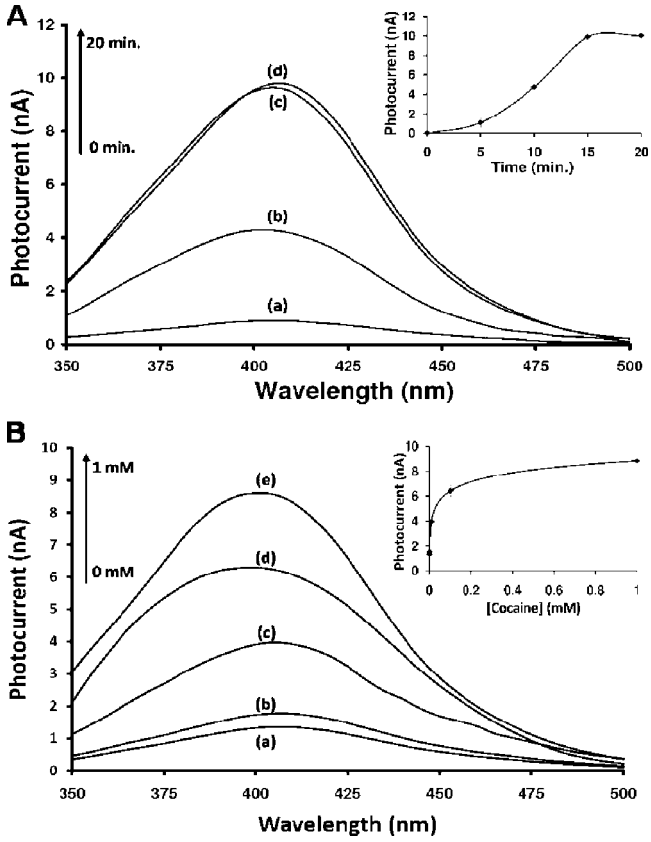

Figure 2. (A) Time-dependent photocurrent action spectra corresponding to the (**4**)-functionalized electrode treated with the (**3**)-modified CdS-NPs in the presence of cocaine, 1×10^{-3} M. Time intervals corresponding to (a) 5, (b) 10, (c) 15, and (d) 20 min. Inset: Time-dependent photocurrent (at $\lambda = 415$ nm) generated by the supramolecular complexes generated on the electrode between the CdS-labeled with the aptamer subunit, the modified electrode, and cocaine, 1×10^{-3} M. Error bars were based on $N = 5$ experiments. (B) Photocurrent action spectra corresponding to the (**4**)-functionalized electrode treated with the (**3**)-modified CdS-NPs in the presence of variable concentrations of cocaine: (a) in the absence of cocaine, (b) 1×10^{-6} M, (c) 1×10^{-5} M, (d) 1×10^{-4} M, and (e) 1×10^{-3} M. Inset: The derived calibration curve with a detection limit of 1×10^{-6} M. All experiments were performed in a 10 mM phosphate buffer solution (pH =7.4) in the presence of 20 mM triethanolamine. Error bars were based on $N = 5$ experiments.

included the use of Au-NPs as amplifying labels for the surface plasmon resonance readout of the sensing process. The electronic coupling between the localized plasmon of metallic NPs (e.g., Au-NPs) and the surface plasmon wave associated with metallic films results in a pronounced shift in the surface plasmon resonance spectrum. Thus, the labeling of the recognition elements, linked to a metal surface, with Au-NPs provides a means to amplify the SPR detection of the sensing process. Indeed, Au-NPs-labeled nucleic acids or antibodies were used to develop SPR-based DNA sensors[51] or immunosensors.[52] Similarly, functionalized Au-NPs were applied to follow biocatalytic transformations.[53] While the application of aptamers as recognition elements for sensing seems natural, the reports on SPR-based aptasensors are quite limited.[54–56] The fact is that the sensing of analytes by SPR spectroscopy requires a measurable dielectric constant change upon formation of the aptamer-substrate complex, thus limiting the method to analyze the formation of aptamer-protein complexes and sensing difficulties to detect low-molecular-weight substrates. The labeling of the recognition complexes with Au-NPs could, then, amplify the sensing process by the coupling of the localized NPs plasmons with the suface plasmon wave, thus enabling the SPR deection of the complexes between low-molecular-weight substrates and their aptamers. In one recent report[57] a competitive aptasensor for adenosine monophosphate was reported using nucleic acid-functionalized Au-NPs. The complexity of the analytical steps and high background signal of this method, together with the fact that the detection of the analyte is accompanied by the decrease of the SPR signal (rather than a "positive" increase in the readout signal), are serious drawbacks of this aptasensor configuration.

We have applied Au-NPs as labels for the sensing of cocaine by means of the assembly of the labeled aptamer-subunits-cocaine supramolecular complexes on Au surfaces and the readout of the formation of the structures by surface plasmon resonance spectroscopy, SPR, Scheme 1 path C. The nucleic acid, (**4**), being one subunit of the anticocaine aptamer, was assembled on the Au electrode. The Au-NPs (13 nm) were functionalized with the second anticocaine subunit, (**5**). The loading of (**5**) on the Au-NPs was determined spectroscopically, and it corresponded to ca. 3 units per particle. In the presence of the (**5**)-functionalized Au-NPs and cocaine, the Au-NPs-labeled supramolecular complex composed of cocaine and the aptamer subunits is self-organized on the Au surface. The surface plasmon resonance shift resulting upon the electronic coupling between the localized plasmon on the NPs and the surface plasmon wave provides, then, the readout signal for detection of cocaine. Figure 3A shows the SPR curve of the (**4**)-modified surface in the presence of the (**5**)-functionalized Au-NPs in the absence of added cocaine (curve a) and in the presence of added cocaine, 1×10^{-3} M (curve b). Clearly, a shift in the SPR curve is observed upon addition of cocaine. Control experiments reveal that the SPR curve of the (**4**)-modified surface in the absence and presence of the (**5**)-functionalized Au-NPs (in the absence of cocaine) are overlapping, suggesting that the (**5**)-modified Au-NPs do not interact with the surface. Also, the SPR curve of the (**4**)-functionalized surface is unaffected by the addition of cocaine. These control experiments suggest that the supramolecular complex formed between the aptamer subunits and cocaine on the Au surface lead to the changes in the SPR spectrum of the surface. Furthermore, treatment of the (**4**)-functionalized surface with the aptamer subunit (**5**) that lacks the Au-NPs labels, in the presence of cocaine, 1×10^{-3} M, does not lead to any significant change in the SPR spectrum. This latter control experiment indicates that the formation of the unlabeled aptamer subunits-cocaine complexes on the surface does not alter the interfacial properties of the surface to a measurable

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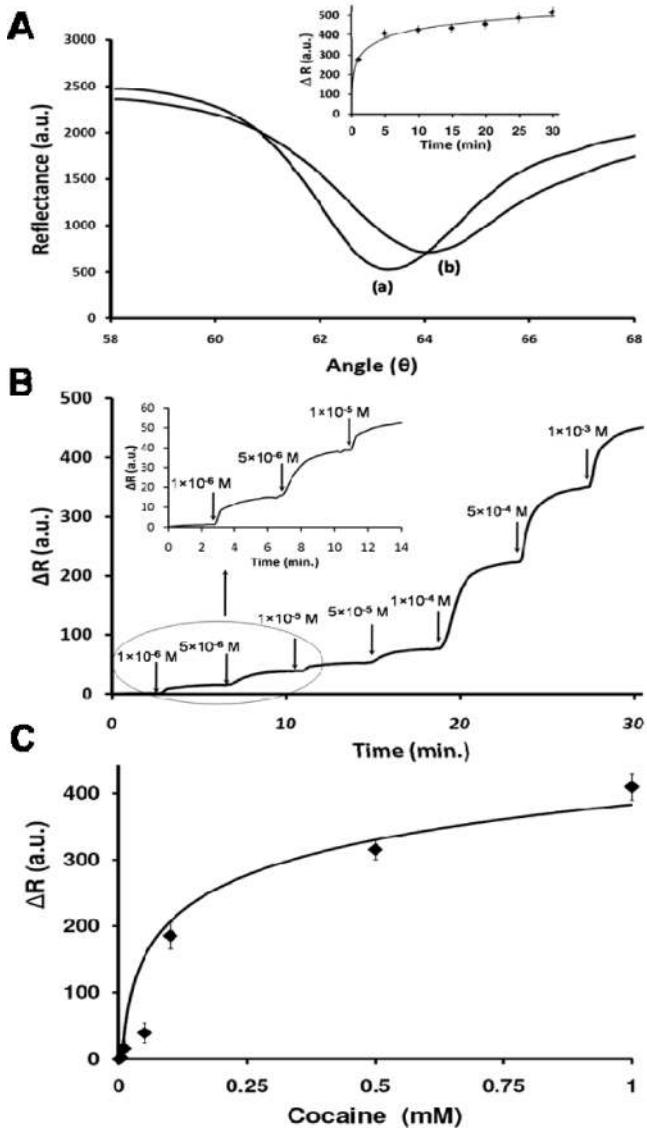

Figure 3. (A) SPR curves corresponding to the (**5**)-modified Au-surfaces treated with the (**5**)-modified Au-NPs (a) in the absence of cocaine and (b) in the presence of 1×10^{-3} M of cocaine. Time-dependent reflectance changes (ΔR) upon the formation of the supramolecular complex consisting of the aptamer subunits and cocaine, 1×10^{-3} M, on the Au surface. (B) The sensogram corresponding to the (**4**) modified gold electrode, in the presence of the (**5**)-modified Au-NPs, and variable concentration of cocaine. The reflectance changes were followed, at $\theta = 61.3°$. Arrows indicate the time of addition of the analyte. Inset: Enlarged time-dependent reflectance changes at low concentrations of cocaine. (C) Calibration curve corresponding to the reflectance changes at different concentrations of cocaine. Error bars were based on $N = 5$ experiments.

degree by the SPR spectrum. Thus, the labeling of the complexes with Au-NPs is essential to follow the formation of the aptamer-cocaine complexes on the surfaces. Figure 3A, inset, shows the time-dependent reflectance changes at a constant angle of 61.3°, upon interacting the (**4**)-modified Au surface with cocaine, 1×10^{-3} M, and the (**5**)-labeled Au-NPs. The reflectance changes level off to a saturation value after ca. 30 min, which correspond to the time interval for equilibrating the aptamer fragment-cocaine complexes on the surface. Figure 3B depicts the sensogram corresponding to reflectance changes of the (**4**)-modified surface upon analyzing different concentrations of cocaine. Figure 3C presents the derived calibration curve. The system allowed the analysis of cocaine with a detection limit that corresponds to 1×10^{-6} M. From the calibration curve and assuming a Langmuir-type association of cocaine to the aptamer subunits, the derived dissociation constant of the complex corresponded to $(8.9 \pm 0.4) \times 10^{-6}$ M.

The three different configurations to analyze cocaine, which were discussed in the present study, rely on the same principle of self-organization of aptamer subunits-cocaine supramolecular complexes by the application of different nanoparticles as labels, resulting in the readout signal of the cocaine sensing events. The Pt-NPs enabled the electrocatalytic detection of cocaine, the CdS-NPs allowed the photoelectrochemical readout of the analysis of cocaine, and the Au-NPs enabled the surface plasmon resonance detection of cocaine through the reflectance changes occurring upon coupling of the localized Au-NPs plasmon with the surface plasmon wave. All three configurations reveal a common advantage over the available aptasensors, which is reflected by the lack of any background signal in the absence of cocaine. The lack of the background signal originates from the use of aptamer subunits as the structural components to assemble the aptamer subunits-cocaine complex. While the entire aptamer sequence includes complementary domains that enable the folding of the aptamer structure even in the absence of the substrate, the base-paired domains of the aptamer subunits are too weak to organize the aptamer complex, in the absence of cocaine.

The response times of the different sensing configurations is controlled by the time interval required to allow the self-assembly of the aptamer subunits and cocaine on the respective surfaces. We find that the response times for the different systems is ca. 30 min, implying that the nature of the NPs label does not significantly affect the self-assembly process. Finally, the detection limits of all three configurations are very similar 1×10^{-6} to 1×10^{-5} M. The sensitivities of the sensors are controlled by the dissociation constant of the aptamer-cocaine complex (ca. 1×10^{-6} M). It should be noted that for any future applications of these aptamer-based sensors in complex composite mixtures, it will be essential to maintain delicate and precise ionic-strength conditions to stabilize the substrate-aptamer structures.

In conclusion, the present study has demonstrated the analysis of cocaine by means of the self-assembly of supramolecular complexes composed of aptamer subunits and cocaine on transducer surfaces. We have shown that the electrocatalytic, photoelectrochemical, and optical properties of metallic or semiconductor NPs allow their versatile use as labels for the sensing events. The different aptasensor configurations may be extended to analyze other low-molecular-weight substrates or proteins.

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