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Spotlighting of Cocaine by an Autonomous Aptamer-Based Machine

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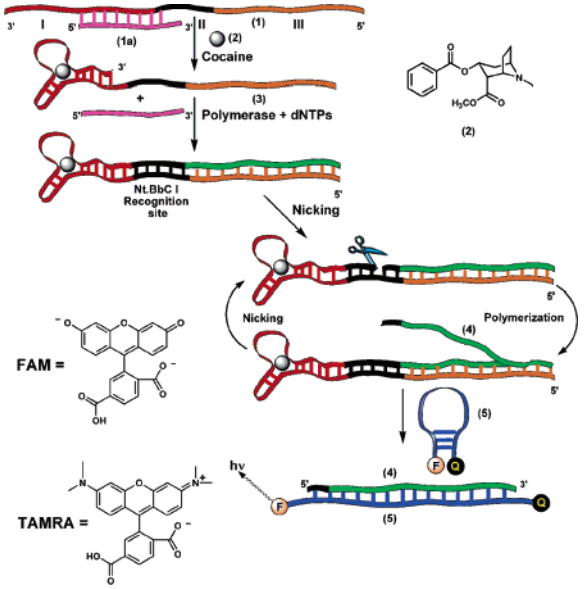
Aptamers are nucleic acids with specific recognition properties toward low-molecular-weight substrates or macromolecules such as proteins. The aptamers are selected from a rich library of 10^{16} nucleic acids using the SELEX procedure, amplified by PCR, and sequenced to yield a defined recognition unit.[1] Recent research efforts integrate aptamers as recognition matrices in optical[2] or electrochemical[3] sensors (aptasensors). Albeit substantial progress was accomplished, a major disadvantage of aptamers is their relatively low association constants to their substrates that lead to low detection limits. Thus, the development of amplification paths for aptamer-based sensing is essential. The coupling of catalytic nanoparticle labels to aptasensors was used for the amplified electrochemical,[4] chemiluminescence,[5] or microgravimetric[6] detection of the respective analytes. Similarly, semiconductor nanoparticles such as CdS or PbS were used as tracers for the amplified electrochemical detection of aptamer−analyte complexes.[7] Recently, DNA-based machines were employed to amplify the analysis of DNAs. In these systems, mechanical transformations occurring on a nucleic acid "track", as a result of hybridization with the appropriate DNA, yielded a nucleic acid "product" that provided a readout signal for the operation of the machine and the primary hybridization with the analyte DNA.[8] The machinelike operations were, for example, replication or scission, while the nucleic acid product could be a DNAzyme that catalyzes the generation of chemiluminescence.[9] In the present study, we introduce the paradigm of an autonomous aptamer-based machine that amplifies the recognition event between the aptamer and its substrate through the operation of the "machine". The operation of the machine and the readout of the analysis of the substrate are accomplished by a fluorescence signal.

Scheme 1 outlines the principles for analyzing cocaine by the aptamer-based machines. The nucleic acid **1** provides the skeleton that self-assembles to the functional "machine" in the presence of cocaine, **2**. The nucleic acid **1** consists of three regions. Region I includes the aptamer region for cocaine. Region II is the "heart" of the machine, and upon the formation of a double strand of this region a nicking site for Nt.BbvC I is formed. Region III consists of a sequence that is complementary to the nucleic acid sequence that is designed to act as the "product" that leads to the transduction of the cocaine-activated machine. The nucleic acid **1** is blocked with the nucleic acid **1a** to prevent uncontrolled folding of **1** to an active "machine" configuration. The double-strand **1/1a** is interacted with polymerase, dNTPs mixture, and the nicking enzyme Nt.BbvC I. This assembly exists in an inactive mute configuration. Note that the blocker **1a** is terminated at the 3′-end with a domain noncomplementary base to prevent undesired replication of the **1**/**1a** assembly.

Addition of cocaine **2** to the system separates the blocked double-stranded assembly and folds the aptamer into its stable aptamer−cocaine complex, **3**, that includes a 7-base duplex structure that

Scheme 1. The Amplified Analysis of Cocaine by an Autonomous Aptamer-Based Machine



can initiate replication. In the presence of the dNTPs mixture as fuel (the reagents that enable the operation of the machine), the polymerase-induced replication of the single-stranded domain activates the autonomous operation (spontaneous activity that cannot be stopped) of the machine. Replication of the single strand yields the duplex that includes the nicking site for Nt.BbvC I. Scission of the replicated strand results in a new replication site for polymerase and the concomitant displacement of the nicked strand **4**. The system includes as reporter unit the hairpin nucleic acid **5**, that is substituted at the ends of the stem with the dyes, FAM and TAMRA. In the closed configuration of the hairpin, **5**, the photoexcitation of FAM at $\lambda = 480$ nm results in the fluorescence resonance energy transfer (FRET) to TAMRA and the fluorescence of TAMRA at 580 nm, with only a residual minute emission of FAM at 520 nm. The displaced nucleic acid generated by the machine, **4**, is, however, complementary to the single stranded loop of **5**. This leads to the hybridization of **4** with **5**, resulting in the hairpin opening. The spatial separation of the dyes prohibits the FRET process between the donor−acceptor pair of dyes, resulting in the emission from the FAM component.

Figure 1 shows the changes in the fluorescence of FAM at time-intervals of operation of the machine, upon analyzing cocaine at a concentration corresponding to 0.4 mM. As the time interval for operating the machine is prolonged, the fluorescence of FAM is intensified. The time-dependent operation of the "machine" reveals, however, an induction time and an "S"-shape kinetics. This is because a sufficiently high concentration of the product, **4**, must be accumulated to stimulate the opening of **5** at a reasonable rate.

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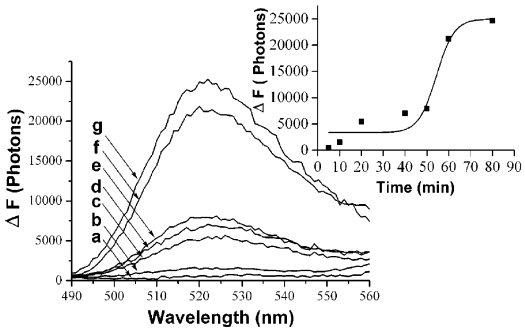

Figure 1. Time-dependent fluorescence spectra, measured at 10 min time-intervals a−g, observed upon operating the aptamer-based machine in the presence of cocaine, 0.4 mM. The analysis is performed in the presence of polymerase 10 units, Nt.BbvC I, 20 units, dNTPs, 0.1 mM, and **1**, 5×10^{-8} M. To the reaction product the hairpin **5**, 6.7×10^{-8} M, was added, and the fluorescence was recorded after a time-interval of 8 min. All systems consisted of a Tris buffer, 25 mM, pH = 8.2 solution that included 100 mM NaCl, 50 mM KAc, 10 mM $MgAc_2$, and 1 mM DTT. (The background fluorescence was substracted from each of the recorded spectra.) The inset shows the time-dependent fluorescence changes at $\lambda = 520$ nm upon operating the aptamer-machine, $\lambda_{ex} = 480$ nm.

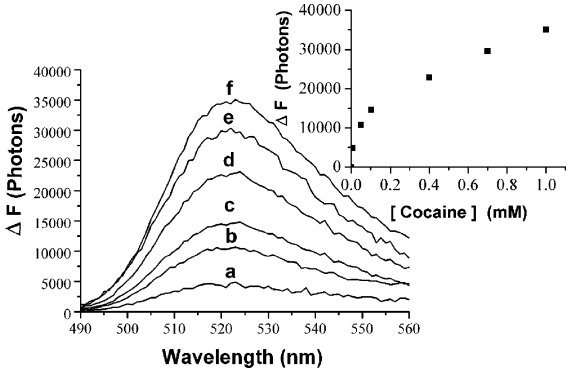

Figure 2. Fluorescence spectra observed upon analyzing different concentrations of cocaine in the presence of **1** at 5×10^{-8} M: (a) 0.005, (b) 0.05, (c) 0.1, (d) 0.4, (e) 0.7, (f) 1 mM. Experimental conditions for operating the system are given in Figure 1. The inset shows the derived calibration curve.

(The time-dependent opening of the beacon **5** is described as Supporting Information S1.)

Figure 2 shows the changes in the fluorescence intensities of the system upon analyzing different concentrations of cocaine by the aptamer-based machine **1**/**1a**. The machine was operated for 60 min, followed by 10 min of beacon opening. Figure 2 (inset) shows the respective calibration curve. Using a time-interval of 60 min for operating the machine, the detection limit for analyzing cocaine is 5×10^{-6} M, a value that is slightly better than the detection limits for analyzing cocaine by an electrochemical aptasensor[3c] or the optical detection of cocaine by aptamer−nanoparticle conjugates.[10] Also, the method is comparable in its sensitivity to enzyme-linked immunoassays for cocaine.[11]

Control experiments revealed that the **1**/**1a** system generated a small background fluorescence of FAM upon operating the machine in the absence of cocaine. This background fluorescence is attributed to minute quantities of unhybridized **1** that folds to the active

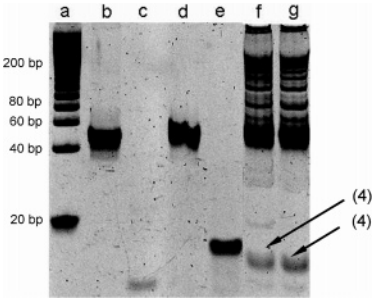

Figure 3. Nondenaturating PAGE analysis of the "product", **4**, generating upon the operating of the aptamer machine for 1 h and analyzing cocaine at concentrations: 0.4 mM (lane f) and 1 mM (lane g). Lanes correspond to (a) reference ladder, (b) the template **1**, (c) the blocker **1a** only, (d) **1** hybridized with **1a**, and (e) the "product" only.

aptamer structure that activates the machine even in the absence of cocaine. Similarly, treatment of the machine **1**/**1a** with the foreign substrate adenosine leads to the residual fluorescence observed in the absence of cocaine, indicating that the machine **1**/**1a** is selectively activated by the cocaine substrate.

Further support that confirms the activation of the aptamer machine by cocaine was obtained by complementary gel-electrophoresis experiments, Figure 3. The runs f and g correspond to the system where the machine is activated for 1 h using 0.4 mM and 1 mM of cocaine, respectively; product **4** is generated in the absence of the probing beacon **5**. As the concentration of cocaine increases, the band of **4** is intensified, consistent with the enhanced formation of the product.

Thus, the present study demonstrated the novel amplified detection of a small molecule by an aptamer-based machine.

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Supporting Information Available: The sequences **1**, **1a**, **4**, and **5**; the time-dependent opening of **5** by **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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