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Label-Free, Dual-Analyte Electrochemical Biosensors: A New Class of Molecular-Electronic Logic Gates

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Electronic logic gates, transistor-based binary switches whose input conditions (0 or 1) determine their output state (0 or 1), form the basis of conventional computer microprocessors. By analogy, molecular logic gates may enable the development of molecular-scale computers and "autonomously regulated" chemical systems, ideas that have attracted significant recent interest.[1,2] Toward this goal, a range of diverse DNA logic gates have been designed and constructed in recent years, with effort primarily focused on the fabrication of the basic logic functions AND, OR, and NOT.[3−8]

Most of the chemical logic gates reported to date employ small molecules or macromolecules as their inputs and fluorescent or colorometric signals as their outputs.[3−8] Willner and co-workers have, for example, developed optically reported "AND", "OR", and "SET-RESET" logic gate operations employing ion-driven conformational changes in a DNA G-quadruplex as inputs and fluorescence intensity as outputs.[11] Wang and co-workers have likewise constructed an optical-output "INHIBIT" logic gate utilizing K^+ or Pb^{2+}-switched DNA structures.[12] A potential limitation, however, of these important proof-of-principle examples is that interfacing their optical outputs with nonmolecular-based technologies may prove cumbersome. In response we report here the fabrication of reagentless, molecular logic gates that instead produce electronic (electrochemical) signals as their outputs.

As the basis of our logic gates we have emplaced two previously described electrochemical sensor architectures[9,10] on a single electrode. These approaches, which share a common read-out modality, are each comprised of a specific DNA probe oligonucleotide modified on one terminus with a redox reporter (here methylene blue) and attached to an electrode at the other (Figure 1a). The first of these two devices, a stem-loop "E-DNA" sensor, targets a 17-base DNA sequence from the *Salmonella typhimurium* (*gyr*B gene) sequence, turning "off" (i.e., the faradaic current observed via alternating current voltammetry (ACV) is reduced) when hybridization of the target to the stem-loop forces the redox reporter away from the electrode.[9] The second device, a cocaine-responsive E-AB sensor,[10] responds to its target by bringing the redox reporter into proximity to the electrode, thereby increasing the observed current upon ACV interrogation.[10] Both devices are also responsive to the denaturant urea, which unfolds their DNA probes and thus serves as a third input factor. Together, this two-device, three-analyte system supports both the commonly employed, two-input XOR and three-input logic gating.

As a first test we have designed a two-analyte XOR logic device that defines the concentration of cocaine and the concentration of a cDNA target (cDNA) as inputs and the change in faradaic current from the attached redox reporter as output (see Figure S1 in Supporting Information for details of the ACV protocols employed). For input, the presence of cocaine at >250 μM and cDNA at >50 nM define the "on" or "1" states, and lower (to 0 M) concentrations of these molecules define the "off" or "0" states. For output, we define the signal changes (changes in the reduction peak of the methylene blue) of greater than ±5% and less than ±5% as "1" and "0", respectively. Thus, a logic operation can be realized by controlling the concentration of cocaine and cDNA producing a truth table and schematic representation of the logic gates presented in Figure 1. From the truth table, we see that a HIGH output (1) results if one, and only one, of the inputs to the gate is HIGH (1); if both inputs are LOW (0) or both are HIGH (1), a LOW output (0) results. Thus, this label-free, dual-analyte device serves as an XOR gate.

Starting from the XOR gate we have also fabricated a three-input logic gate by employing urea, which, by unfolding our structured DNA probes alters their ability to transfer electrons, as the third input. For input, the presence of urea concentrations >1 M defines the "on" or "1" state, and lower (to 0 M) concentrations define the "off" or "0" state. As before we define signal changes greater than ±5% and less than ±5% as "1" and "0," respectively. Based on the above definitions, a three-input logic operation is thus realized by controlling the concentrations of cocaine, cDNA, and urea. The truth table and schematic representation of this logic gate are presented in Figure 2.

Because all of the components in E-AB and E-DNA sensors are strongly adsorbed onto the sensor surface these reagentless devices are readily reusable (see Figure S2a in the Supporting Information). Thus the logic gates we have built from these devices can be employed for the continuous monitoring of time-evolving processes (Figure 3). Likewise, the folding-based signal transduction mechanism and electrochemical readouts of these sensors are relatively impervious to nonspecific interferants,[10] and thus these logic gates perform well even in relatively complex sample matrices (see Figure S2b in Supporting Information; see also refs 9 and 10).

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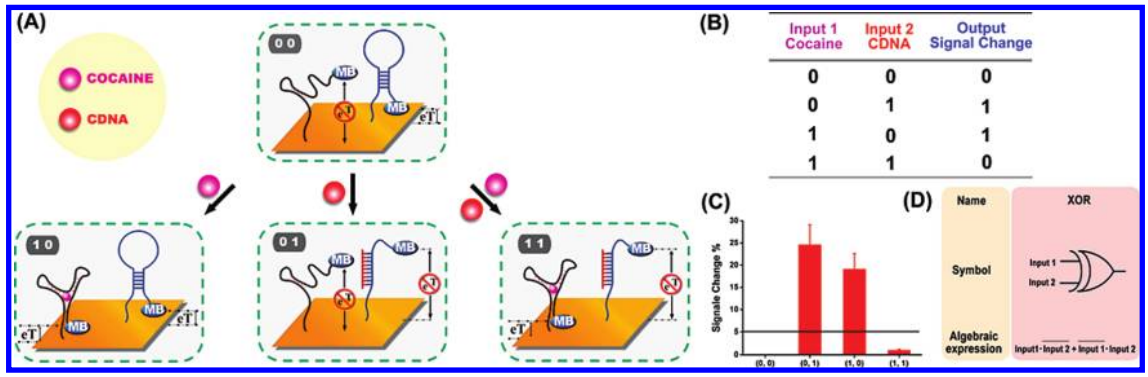

Figure 1. (A) A schematic presentation of an "XOR" gate built from two label-free electrochemical sensors, and the activation of this logic gate via changing concentrations of their respective targets as inputs. To fabricate this two-input logic device we have employed a previously described E-DNA sensor[9] composed of a stem-loop oligonucleotide and a previously described E-AB sensor,[10] composed of a cocaine-binding DNA aptamer, both of which are modified with reporting methylene blue moieties and immobilized on the surface of a single gold electrode via self-assembled monolayer chemistry. When interrogated via alternating current voltammetry (ACV) these respond to their respective targets (a complementary cDNA and cocaine, respectively) via a decrease and an increase faradaic current respectively. Together these sensors comprise a logic gate for which (B, C) four possible input combinations induce different electrochemical output currents when probed via ACV. (B) The truth table for this two-input logic gate is shown. The values in parentheses in the output column indicate the experimental signal change. (D) The name, symbol, and algebraic expression for this logic gate.

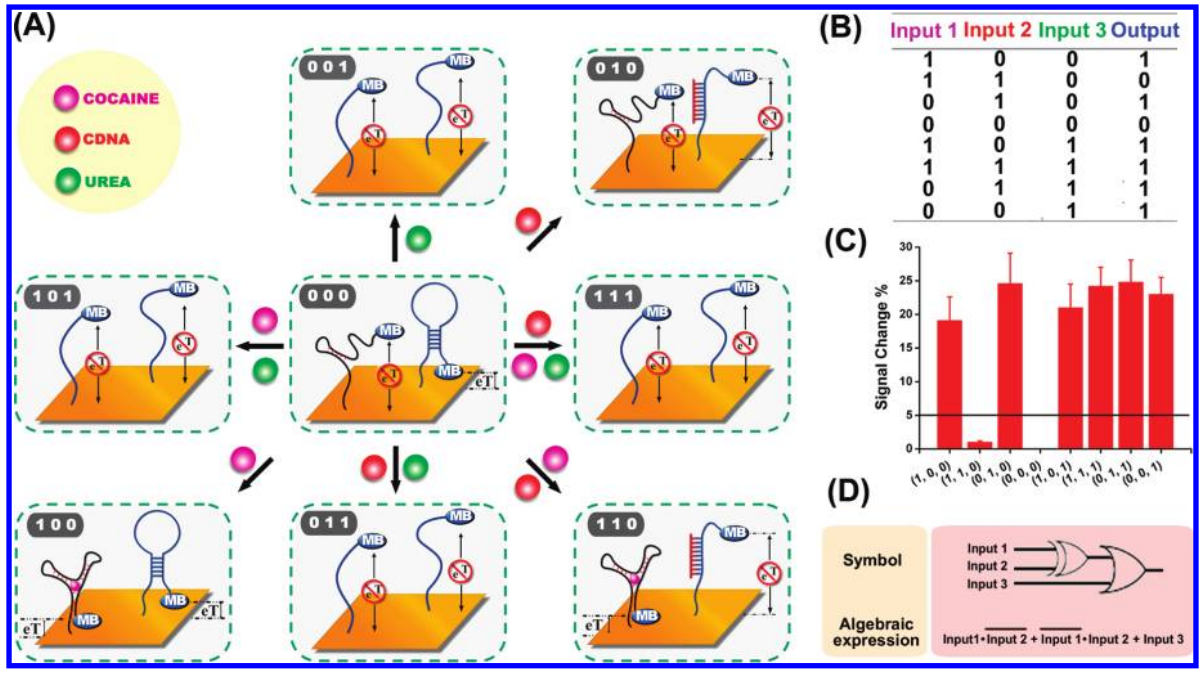

Figure 2. The two components of our gate, the cocaine E-AB sensor and the cDNA responsive E-DNA sensor, are also sensitive to urea, which unfolds their DNA probes producing a decrease in faradaic current upon ACV interrogation. This provides a means of producing a three input logic gate in which urea serves as the third input. (A) Shown is a schematic representation of a three-input logic gate and the activation of the gate using varying concentrations of cocaine, cDNA, and urea as inputs. (B) Truth table for the two-input logic gate. (C) The eight input combinations induce different electrochemical signal changes. The values in parentheses in the output column indicate the experimental signal change. (D) The symbolic and algebraic expression describing the action of this logic gate.

Given their ability to continuously monitor chemical status, this new class of molecular-electronic logic gates may be useful for monitoring, for example, industrial processes. To illustrate this, we have employed the three-input logic gate to monitor a simulated process based on the following working assumptions: First, we define the cocaine and the cDNA as two components of the process to be monitored. Second, only when the ratio of cocaine to cDNA is 5000:1 does the process achieve peak efficiency. Third, excess urea "poisons" the process, leading to reduced efficiency. When the ratio of the first two components is 5000:1, the signal change is below 5% (state 1, 3, 5 in Figure 3A), corresponding to the "0" (translated by the three-input logic gate) in state 1, 3, 5 (Figure 3B), which corresponds to conditions in which the process proceeds at high efficiency. When the ratio of the two components moves away from optimal, say to 0:1, the signal change is greater than 5%, corresponding to the "1" state (translated by the our three-input logic gate), which signals that the process is now in a low efficiency regime. Likewise, if the "poison" (urea) is present, the signal change once more climbs above 5% even if the ratio of cocaine to cDNA is at its optimal 5000:1 value (Figure 3), signaling that the process is again operating at suboptimal conditions.

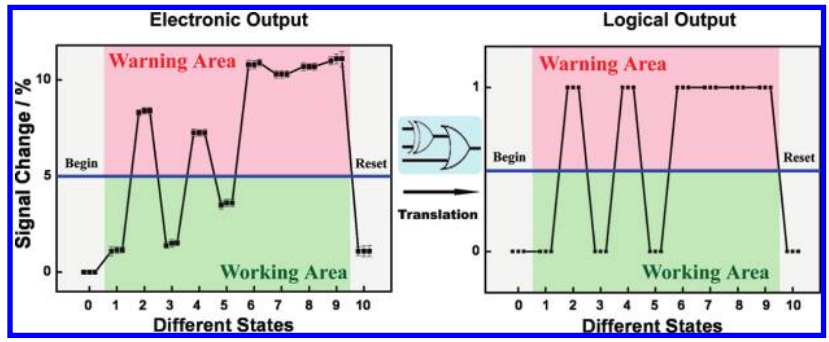

Figure 3. The three-input logic gates translate molecular (concentration) signals into electrical signals (Left) and thence into logic signals (Right). (Left) The ([cocaine]/μM, [cDNA]/nM, [urea]/M) for different states are (state 0: (0, 0, 0)), (state 1: (250, 50, 0)), (state 2: (0, 50, 0)), (state 3: (250, 50, 0)), (state 4: (500, 0, 0)), (state 5: (500, 100, 0)), (state 6: (0, 0, 1)), (state 7: (750, 0, 1)), (state 8: (0, 150, 1)), (state 9: (750, 150, 1)), and (state 10: (0, 0, 0)), respectively. (Right) Correspondingly, the input is (state 0: (0, 0, 0)), (state 1: (1, 1, 0)), (state 2: (0, 1, 0)), (state 3: (1, 1, 0)), (state 4: (1, 0, 0)), (state 5: (1, 1, 0)), (state 6: (0, 0, 1)), (state 7: (1, 0, 1)), (state 8: (0, 1, 1)), (state 9: (1, 1, 1)), and (state 10: (0, 0, 0)), respectively. When the logical signal is "0", the "process" runs efficiently. When the logical signal is "1", tune the ratio of cocaine to cDNA to 5000: 1 recovers the "0" setting of the logic gate. If it does not, this is a signal that excessively high levels of urea are present.

We have demonstrated a label-free, dual-analyte device that functions as a chemical XOR gate and a three-input logic gate but that, in contrast to prior efforts in this arena, produces an electronic rather than an optical output. Moreover, these logic gates are based on reagentless, reusable sensing elements and are thus suitable for continuous monitoring. Moving forward, we envision that more complex operations can be performed by attaching several different exogenous redox labels on an individual electrode, inducing more complex outputs that would, in turn, enable the monitoring of more involved processes.

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Supporting Information Available: Experimental details and supporting figures and discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information

Label-free, Dual-analyte Electrochemical Biosensors:

A New Class of Molecular-Electronic Logic Gates

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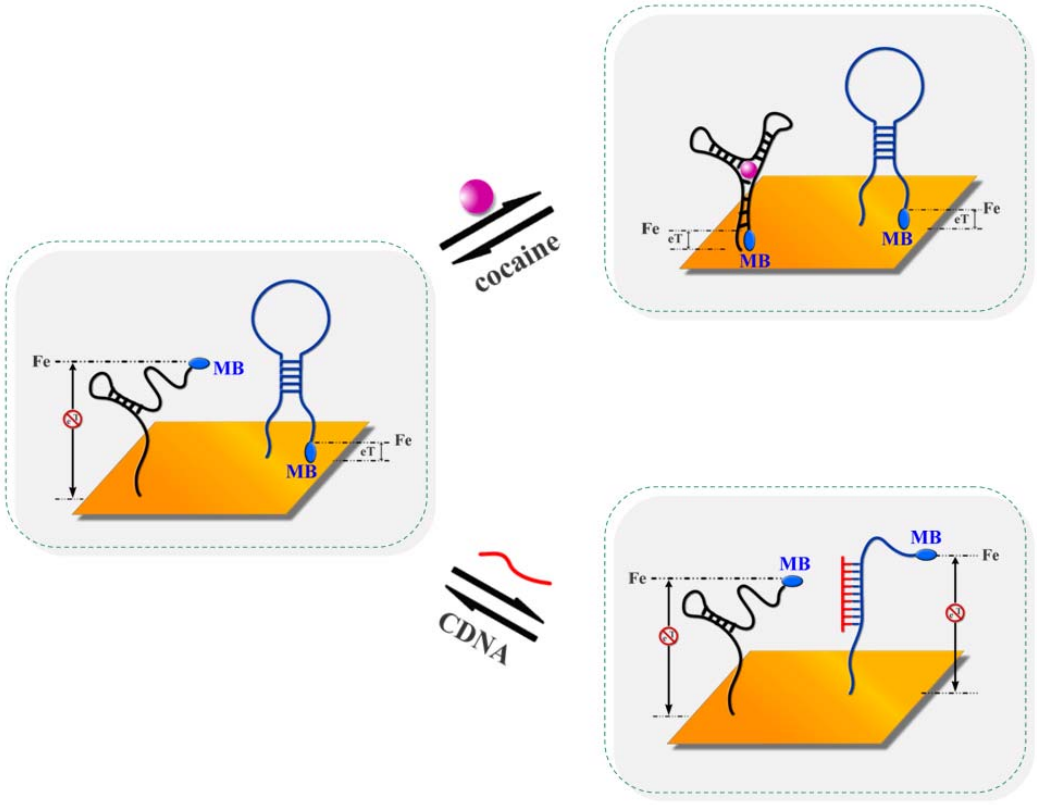
1. How the Label free, Dual-analyte Bio-Sensor Device works.

The logic gate is composed of two previously described electrochemical sensors fabricated on a single interrogating electrode. The first is an electrochemical aptamer-based (E-AB) sensor. In the presence of its target, cocaine, the aptamer in this sensor is thought to fold into a cocaine-binding three-way junction, altering electron transfer from an attached methylene blue moiety and increasing the faradaic reduction peak observed upon alternating current (ACV) interrogation (Scheme S1). The second is an electrochemical E-DNA sensor, which is composed of a methylene-blue-modified DNA stem-loop that responds to the presence of its target analyte via a reduction in faradaic current upon ACV interrogation.



Scheme S1. Dual-analyte devices system contains a stem–loop oligonucleotide and a cocaine-sensitive aptamer possessing terminal thiol and a methylene blue (MB) group are immobilized at a gold electrode through self-assembly. In the absence of target, specific

sequence, the stem–loop structure holds the redox reporter into close proximity with the electrode surface, thus ensuring rapid electron transfer and efficient redox of the MB. On hybridization with the target sequence, a large change in redox currents is observed, presumably because the MB is separated from the electrode surface. In contrast, when there is no cocaine, the aptamer is thought to remain partially unfolded, with only one of its three double-stranded stems intact. In the presence of cocaine the aptamer is thought to fold into the cocaine-binding three-way junction, altering electron transfer and increasing the observed electrochemical signal.

When cocaine concentration changed from 20 μM to 1000 μM, the observed electrochemical signal is increased from 4.3 % to 19.1 % relative to the no-cocaine no-cDNA background current (Figure S1A, B). While the concentration of specific sequence varied from 10 nM to 200 nM, the signal gain is increased from 2.5 % to 24.6 % relative to the same background (Figure S1C, D).

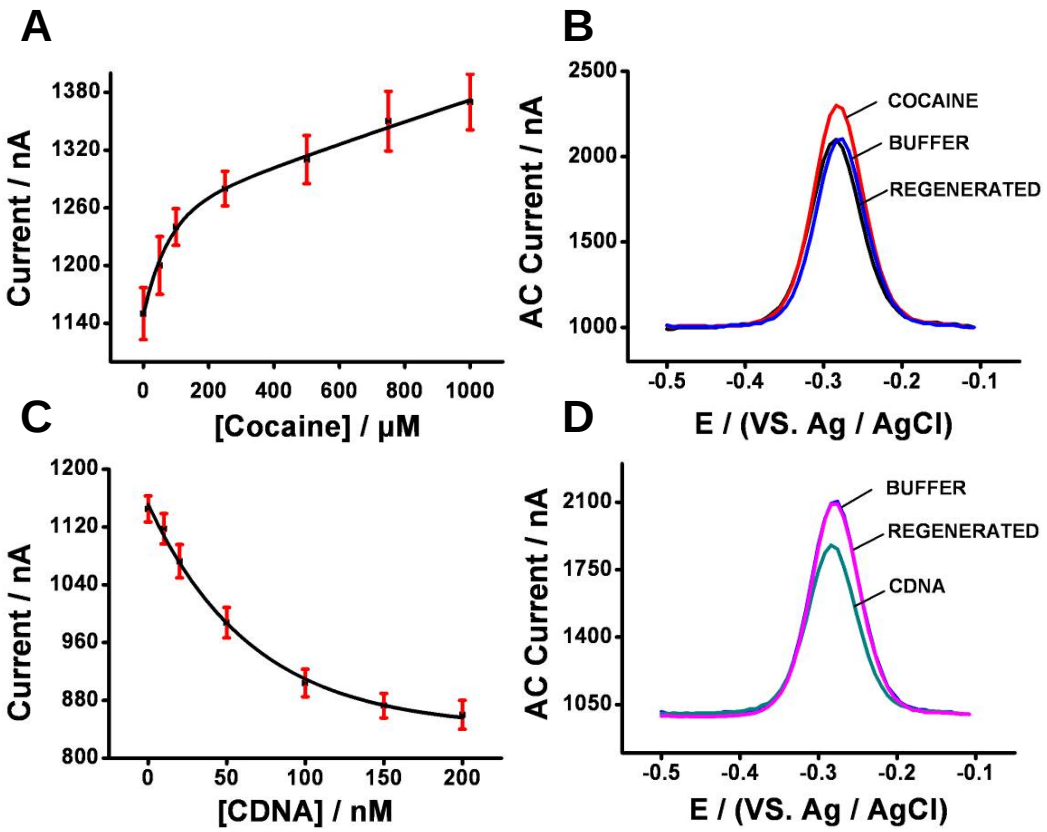


Figure S1. Dose-response curve for the E-AB (A) and E-DNA (B) sensor in Tris buffer. AC voltammograms of the E-AB (C) and E-DNA (D) sensor recorded in Tris buffer. Upon addition of 1000 uM cocaine, a signal increase is observed (red). With the appearance of 200 nM specific sequence, a signal decrease is observed (green). The sensors were then regenerated by immersing the electrode in room temperature deionized water (black and pink).

2. The regeneration and detection in Adulterated Samples.

Because signal generation in the sensor is based on a hybridization-linked change in the flexibility of the probe and not on simple adsorption to the sensor surface, E-DNA is largely impervious to false positives arising from the adsorption of interferants to the sensor surface; both sensors perform well in highly complex samples, such as blood serum and soil extracts[1-3] Consistent with this, the dual-analyte sensors is performs well even when challenged directly in "pure baking soda" (From Arm & Hammer, Princeton, New Jersey (Figure S2). Sensor regeneration is critical in order to ascertain that a signal is arising due to specific interactions with the target and is not simply due to some other nonspecific modification of the probe or sensor head. The dual-analyte sensor is stable enough to allow for ready regeneration: room temperature deionized water is sufficient to recover about 96% (Figure S2). Moreover, because both sensors are stable and rapidly equilibrating, logic gates built from them can be used to continuously monitor time-evolving processes without requiring to regeneration between evaluations (see text Figure 3).

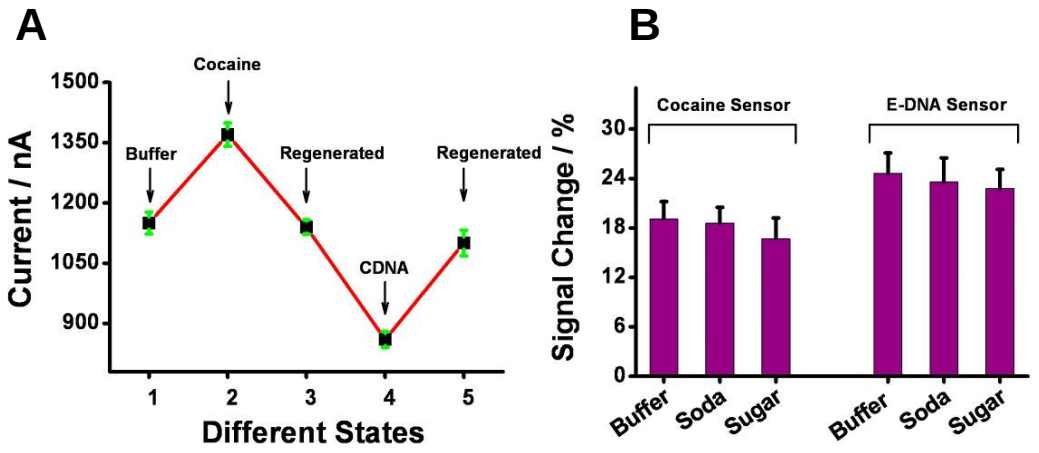


Figure S2. (A) The dual-analyte sensor regeneration can be simply achieved via immersing in room temperature deionized water. The signal regeneration is about 96%. (B) The dual-analyte device performs well in complex samples, such as "Pure Baking Soda" (From Arm & Hammer, Princeton, New Jersey) "Pure Granulated White Cane Sugar" (From C&H Sugar Company, Inc., Crockett, California).

3. Experimental Section:

Material: The two DNA probe sequences employed were commercially synthesized with their required methylene blue and thiol modifications (Biosearch Technologies, Novato, CA) and used as received. The cocaine aptamer is the oligonucleotide sequence developed by Stojanovic *et al.*.[4, 5] This sequence, GGGAGACAAGGAAAATCCTTCAATGAAGTGGGTCG, was modified with a six-carbon disulfide group at the 5' terminus and a C7-linked methylene blue group at the 3' terminus. E-DNA sensor and target's sequences we have employed are based on the *Salmonella typhimurium* (gyrB gene) sequence. The sequences of the redox-tagged probe oligonucleotides and target oligonucleotides are as follows: stem-loop, 5′-GACACTGGATCGGCGTTTTATTGTGTC-MB-3′; target, 5'-AATAAAACGCCGATCCA-3'. All sensors sequence were purified by HPLC and PAGE, and verified by mass spectrometry by the vendor. Target DNA concentrations were confirmed via UV absorbance measurements at 260 nm and used

as received. The above DNA probes were diluted in Tris buffer (0.1 M Tris pH 7.4 with 140 mM NaCl, 20 mM $MgCl_2$, and 20 mM KCl) to different concentration. This buffer was employed in all sensor experiments in this paper. 6-Mercaptohexanol (Sigma-Aldrich, St. Louis, MO), tris(2-carboxyethyl) phosphine hydrochloride (Molecular Probes, Carlsbad, CA), and guanidine hydrochloride (Pierce, Rockford, IL) were used as received.

Fabrication and Characterization of E-DNA Sensors: The E-DNA and E-AB sensors were fabricated as previously described.[1] In short, polycrystalline gold disk electrodes (2 mm diameter, CH Instruments, Austin, TX) were prepared by polishing with 1 μm diamond and 0.5 μm alumina (Buehler, Lake Bluff, IL), sonicating in water, and electrochemically cleaning (a series of oxidation and reduction cycles in 0.5 M H_2SO_4, 0.01 M KCl/0.1 M H_2SO_4, and 0.05 M H_2SO_4) before modification with probe DNA by incubating the clean electrode in 0.5 μM cocaine aptamer and 0.5 μM DNA probe / 2 μM TCEP (tris(2-carboxyethyl) phosphine hydrochloride) in Tris buffer pH 7.4 for 45 min. The surface was then rinsed with water and subsequently passivated with 5 mM 6-mercaptohexanol in Tris buffer, for 1 h. Prior to use, electrodes were rinsed with deionized water. For a more thorough review of the sensor fabrication please see Reference 1.

Electrochemical analysis was performed using alternating current voltammetry (ACV) at 10 Hz frequency, 25 mV amplitude, over a potential range of -0.1 to -0. 5V, using a CHI 630B potentiostat (CH Instruments, Austin, TX) in a standard cell with a platinum counter electrode and Ag/AgCl (3 M NaCl) reference electrode. All reported values represent the mean and standard error of the mean of three measurements conducted using three independently fabricated electrodes. We have employed the three-input logic gate to monitor a simulated process. We sequential detect the alternating current voltammetry for different states: ([cocaine] / μM, [cDNA] / nM, [urea] / M) for different states are (state 0: (0, 0, 0)), (state 1: (250, 50, 0)), (state 2: (0, 50, 0)), (state 3: (250, 50, 0)), (state 4: (500, 0, 0)), (state 5: (500, 100, 0)), (state 6: (0, 0, 1)), (state 7: (750, 0, 1)), (state 8: (0, 150, 1)), (state 9: (750, 150, 1)) and (state 10: (0, 0, 0)), respectively.

Preparation of Adulterated Cocaine / DNA Samples: A stock solution of 0.1 M cocaine hydrochloride (Sigma-Aldrich, St. Louis, MO) in Tris buffer, pH 7.4, was prepared and used within a few days to avoid hydrolysis of the cocaine, which readily occurs at basic conditions. Substances commonly used to cut (dilute) or mask cocaine were purchased at a local retail supermarket. Baking Soda (Arm & Hammer, Princeton, NJ) and Granulated White Cane Sugar (C&H Sugar Company, Inc., Crockett, CA), were used as received. Cocaine samples adulterated with each of the above substances were prepared in a 1:1 ratio (m/m) of adulterant to cocaine, and allowed to equilibrate in buffered solution for at least 30 minutes before testing.

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