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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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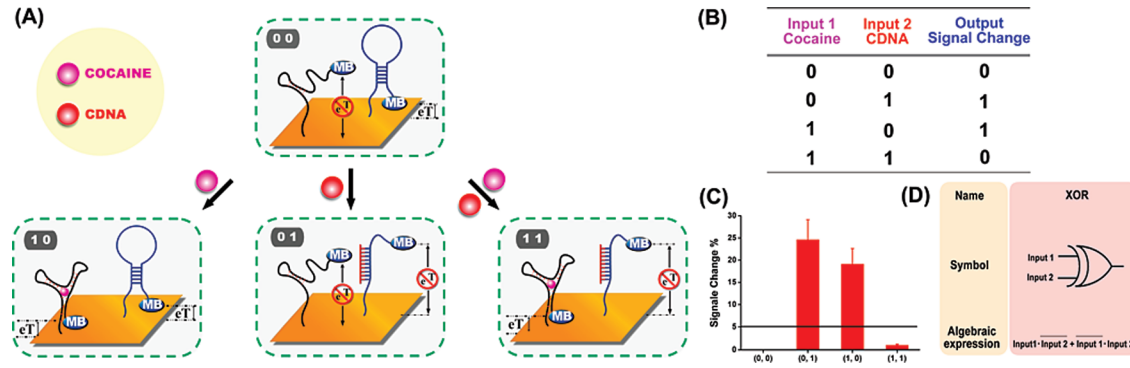

Figure 1. (A) A schematic presentation of an "XOR" gate built from two label-free electrochemical sensors, and the activation of this logic gate via changing concentrations of their respective targets as inputs. To fabricate this two-input logic device we have employed a previously described E-DNA sensor[9] 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.

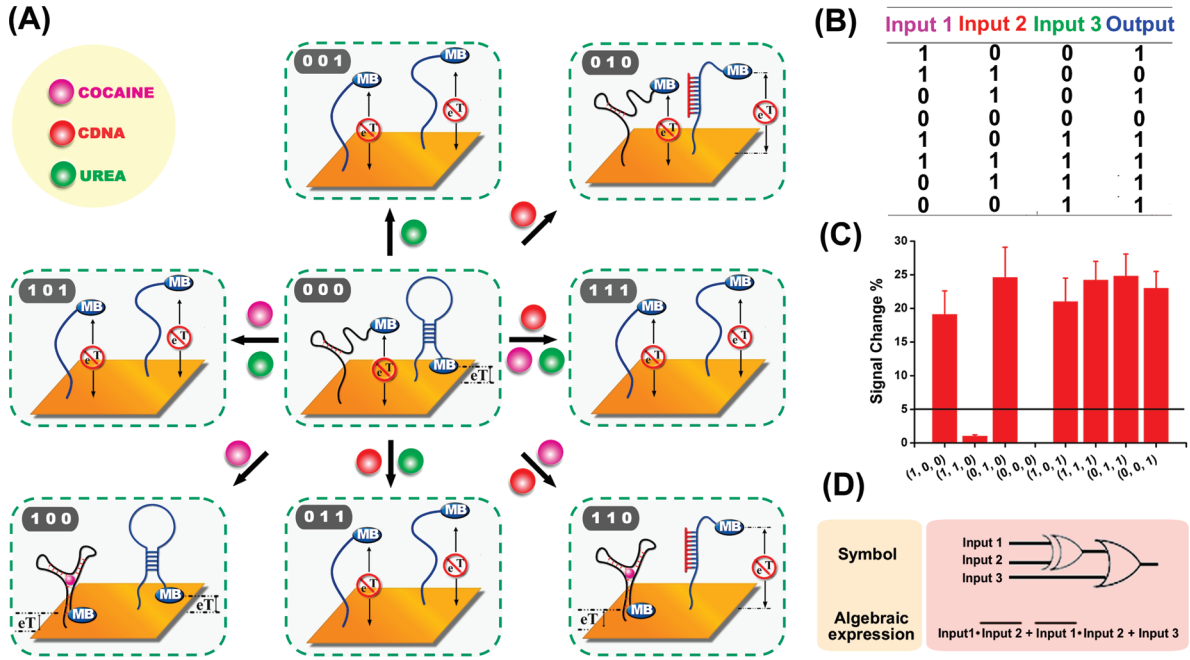

Figure 2. The two components of our gate, the cocaine E-AB sensor and the cDNA responsive E-DNA sensor, are also sensitive to urea, which unfolds their DNA probes producing a decrease in faradaic current upon ACV interrogation. This provides a means of producing a three input logic gate in which urea 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.

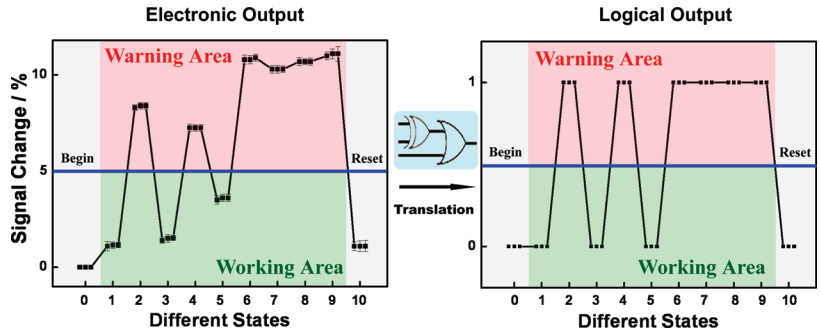

Figure 3. The three-input logic gates translate molecular (concentration) signals into electrical signals (Left) and thence into logic signals (Right). (Left) The ([cocaine]/μM, [cDNA]/nM, [urea]/M) for different states are (state 0: (0, 0, 0)), (state 1: (250, 50, 0)), (state 2: (0, 50, 0)), (state 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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