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Intelligent Medical Diagnostics via Molecular Logic

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Molecular logic gates and molecular computational systems have used a variety of recognition mechanisms, including proteins,[1−5] biochemical pathways in living cells,[6−15] and DNA.[16−31] Molecular logic and computation may also be applied to medical diagnostics. For example, if abnormal results were detected during a medical examination, they could be interpreted using Boolean logic, resulting in intelligent diagnostics. In this communication, we report the integration of microarray sensor technology with logic capability for screening combinations of proteins and DNA in a biological sample. In this system, the reporter and receptor molecules perform simple logic operations by coupling multiple molecular recognition inputs to a fluorescence signal output.

Previously developed molecular AND gates from the de Silva group allowed the creation of a "lab on a molecule" prototype that responded to inputs of the electrolytes Na^+, H^+, and Zn^{2+} in water, leading to an enhanced fluorescence signal as the output.[28] In addition, the NOTIF logic function was previously demonstrated in designed synthetic peptide networks that mimic some basic logic functions of more complex biological networks.[32] Here we demonstrate both AND and INHIBIT (NOTIF) logic gates that respond to the presence of both protein and DNA in a sample. Such a system potentially could be used for performing smart diagnostics. For instance, increased airway obstruction in patients with chronic obstructive pulmonary disease (COPD) or bronchial asthma is frequently associated with bacterial respiratory infections, especially *Haemophilus influenzae*, and is accompanied by the secretion of proinflammatory cytokines, such as IL-8 protein.[32−37] It is possible to describe the resulting logic network of bacterial DNA and IL-8 protein using a Boolean operator truth table (Figure 1A).

A.

Input 1 (Target DNA)	Input 2 (Protein)	Outlet AND gate	Outlet INH gate	Outlet OR gate
1	1	1	0	1
1	0	0	0	0
0	1	0	1	1

B.

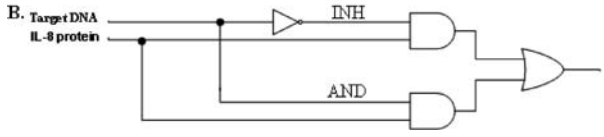

Figure 1. (A) Truth table for logic gates. (B) AND and INHIBIT logic gates.

Our system is designed to determine whether both a protein and a nucleic acid sequence or only protein is present in a sample. Schematic illustrations of the protein−DNA and protein-only sensors are shown in Figure 2A,B, respectively. The fiber-optic microarray was prepared by loading monoclonal antibody (mAb)-functionalized microspheres into microwells created by selectively etching the distal end of optical-fiber bundles.[38] Every microsphere on the array was encoded with a unique optical barcode consisting of fluorescent dye incorporated into each microsphere. The identity of each microsphere in the random array was then determined using image-processing techniques to allow positional registration of the entire array [Figure 3A(1),B(1)]. mAb-functionalized microspheres were used as receptors to capture protein on the surface and then to bind a secondary antibody labeled with DNA (the capture probe) via a biotin−avidin bridge (for details, see the Supporting Information).

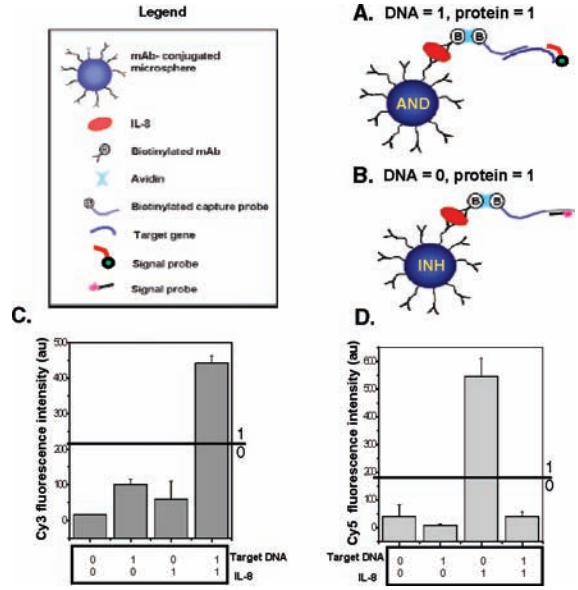

Figure 2. Schematic illustrations of (A) protein−DNA and (B) protein-only detection. Output-averaged fluorescence intensities from (C) Cy3 and (D) Cy5 in the absence (0) or presence (1) of different inputs [for protein, (1) = 10 nM; for target DNA, (1) = 50 nM].

To use this platform for both protein-only detection and protein−DNA detection, two fluorescent probes were designed for the detection step. When both DNA and protein are present in the sample, the Cy3-labeled signal probes hybridize to the complementary target DNA sequence (Figure 2A) while the remaining secondary antibodies free of target DNA hybridize to Cy5 signal probes (Figure 2B). No Cy5 fluorescence is obtained when the capture probe is saturated with a high concentration of target DNA (50 nM) [Figure 2D(1,1)]. The detection of the specific DNA sequence in the designed system is possible only if the protein is also present. Therefore, a Cy3 signal indicates the detection of both target DNA and protein. Conversely, if target DNA is not present in the sample solution, only the Cy5-labeled signal probes hybridize to the secondary antibody labeled with the complementary sequence (Figure 2B).

This biochemical system can be described as a simple functional model of AND and INHIBIT (INH) logic gates (Figure 1B). In our system, the absence of input protein has the power to disable the entire system regardless of the presence of the other input−DNA.

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The INH function can be interpreted as a particular integration of AND and NOT logic functions, where the output signal is inhibited by one of the active inputs. Therefore, the INH logic gate presented here queries protein presence or absence in a sample. If only protein is present in the sample [input (0,1)], the gate switches on and Cy5 fluorescence emission is produced (output 1) [Figure 3A(2)]. If protein is not present in the sample [inputs (0,0) and (1,0)], then the fluorescence output is "inhibited"; consequently, neither Cy5 nor Cy3 fluorescence is produced (output 0). AND logic is represented by the situation where the output of the gate occurs only when both inputs are present. The AND logic gate presented here queries two biological species (protein and DNA) in a sample to determine in a single test whether they are both present. Experiments were performed by testing the AND gate sensor with all four possible input combinations of protein and DNA (Figure 2C). In the presence of both protein and DNA inputs in a sample (1,1), the AND gate switches on and Cy3 fluorescence emission is produced (output 1) [Figure 3B(2)]. No Cy5 fluorescence is obtained for (1,1) because the capture probe is saturated with a high concentration of target DNA (50 nM) [Figure 2D(1,1)]. If one (0,1 or 1,0) or neither (0,0) of the analytes is present in the sample, no Cy3 fluorescence is produced (output 0).

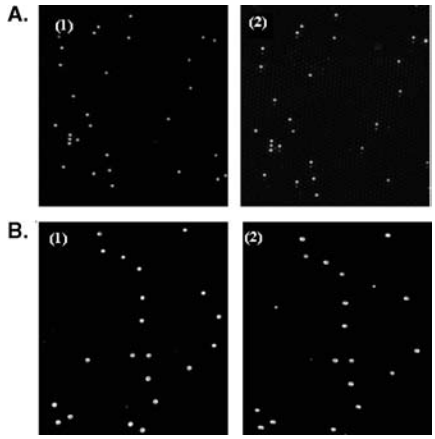

Figure 3. Fluorescence micrographs of a small section of a fiber-optic microarray [A(1), B(1)]. Positional registration of the entire europium-dye-encoded microsphere array: [A(2)] Cy5 microarray signal image of IL8 protein (10 nM) and target DNA (0 nM); [B(2)] Cy3 microarray signal image of IL8 protein (10 nM) and target DNA (50 nM). The correspondence between the microspheres encoded with the Eu dye and the microspheres showing fluorescence in the signal images should be noted.

In summary, we have demonstrated the use of a single platform amenable to both protein-only and protein−DNA detection using molecular logic gates. The pattern of protein and DNA inputs to fluorescence outputs executed according to the truth table for AND and INHIBIT gates demonstrates the feasibility of performing medical diagnostics using a logic gate design. One possible application of this technique would be direct screening of various medical conditions that are dependent on combinations of diagnostic markers.

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

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