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Digital Analysis of Protein Properties by an Ensemble of DNA Quadruplexes

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There is a growing interest in the development of pattern-based detection arrays, inspired by the mammalian olfactory system, which might afford efficient protein identification within a single apparatus.[1] Recently, we have demonstrated a step toward this goal with a homogeneous sensing ensemble, based on DNA quadruplexes, capable of detection within a microliter scale solution of various proteins.[2] Beyond the need to identify proteins, an important diagnostic goal would involve a device that can also analyze the changing properties of a particular protein. Various cancers, for example, are associated with changes in concentration levels of a hormone or a growth factor, rather than with the appearance of a distinct protein. Similarly, diseases such as Parkinson's and Huntington's require the detection of a conformational change that occurs in a single protein species. Following these events in real time would be even more challenging, as they are dynamic and normally accompanied by additional cellular analytes.

A conceptually different approach for analyzing changes in conformation, concentration, and combination of analytes[3] has been developed in the area of molecular logic gates.[4] Unlike combinatorial sensory arrays[1,2] that transduce chemical signals into composite signatures, molecular logic systems[5] can provide a clear-cut binary output[6] corresponding to a particular state of interest. Therefore, we envisioned that applying Boolean logic to a library of combinations of associated fluorophores might enable it to analyze a range of medicinally relevant samples. Here we report a sensing ensemble that can not only generate protein fingerprints[2] but also provide a direct analysis of their properties.

A mixture of DNA quadruplexes, assembled from G-rich oligonucleotides modified with three fluorophores (pyrene (P), fluorescein (F) and tamra (T)), whose emission spectra are distinct but overlapping, produces unique emission patterns from differential contacts (see Supporting Information) with different proteins (Figure 1A).[2] In this paper we show that homogeneous, pattern generating systems can lend themselves to the application of logic gate strategies such that, by using only three wavelengths, the status of protein concentration levels and analyte combinations reflecting conformational changes can be monitored.

The simplest logic gates involve a single input (0 or 1) and a single output that comes in four combinations: YES (0, 1), NOT (1, 0), PASS 1 (1, 1), and PASS 0 (0, 0). Prior to this work, de Silva et al. had elegantly shown the use of these and more complex logic gates in the covalent tagging and identification of microscopic objects (e.g., polymer beads).[7] In our system the input represents a given protein and the outputs were chosen as the intensity of the fluorescence emission at two wavelengths, 525 and 590 nm. We can set the threshold of emission intensity above and below the outputs which are defined as 0 and 1, respectively. Importantly, these signal changes come from noncovalent interactions of the analytes with the ensemble and so can be used to encode changes in protein concentration levels.

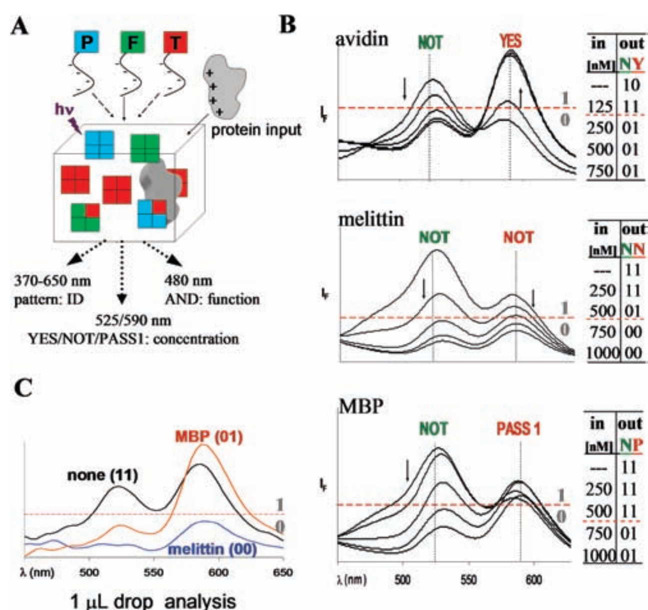

Figure 1. (A) A scheme summarizing the properties of a quadruplex sensing ensemble generated from G-rich oligonucleotides, modified with pyrene (P), fluorescein (F), or tamra (T). Emission patterns at 370−650 nm provide protein fingerprints,[2] while logic gates at discrete wavelengths encode their concentration or follow their function. (B) Parallel gates at 525/590 nm encode high protein levels: NOT-YES code for [avidin] $\geq$ 250 nM; NOT-NOT encode [melittin] $\geq$ 750 nM and NOT-PASS 1 code for [MBP] $\geq$ 750 nM. Excitation: 344 nm. (C) Microliter drop analysis.

Multiple sclerosis (MS) is a test case, as it is associated with increased levels of myelin basic protein (MBP) in the cerebrospinal fluid. The quadruplex ensemble acts as a configurable logic system[4a] that responds to increasing concentrations of MBP, as well as melittin and avidin proteins (as controls), in three distinct ways (Figure 1B). Analyzing these changes according to Boolean logic provides a direct analysis of protein levels in respect to a given threshold. In the case of avidin, a 250 nM concentration of protein activates both NOT and YES logic gates at 525 and 590 nm, respectively. In contrast, NOT NOT gates or NOT PASS 1 gates are triggered by 750 nM concentrations of melittin or MBP. Consequently, simple binary codes for levels of each protein are generated, such that outputs 01, 00, and 01 encode high levels of avidin, melittin, and MBP, respectively. Any other code would reflect a lower concentration or no protein at all. The potential of wireless, configurable logic gates to provide real time diagnosis of small protein samples is demonstrated in Figure 1C, showing digital codes produced by a single microliter drop loaded with only 8 ng of melittin or 48 ng of MBP.

A molecular AND logic gate involves several inputs and a single output and thus is a more advanced analytical device that can potentially signal a combination of analytes.[3] A protein sensor of

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this kind could be particularly useful in following dynamic events, such as protein conformational changes induced by metal ions. Calmodulin (CaM) and calcium (Ca^{2+}) were chosen as a protein−metal ion model system, as CaM is an acidic protein (pI = 4.09) that, by itself, should not induce the signaling of the ensemble.[2] Upon calcium binding, however, CaM undergoes a significant conformational change, in which a large buried hydrophobic patch opens up, enabling it to bind other proteins and regulate their function (Figure 2A). Pyrene has a high affinity for hydrophobic domains with consequent enhancement of its fluorescence emission.[8] We therefore anticipated that the CaM−(Ca^{2+}) state might be detected by the ensemble.

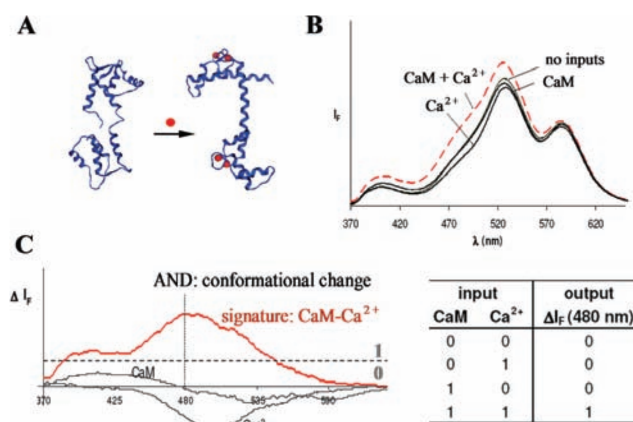

Figure 2. (A) Change in conformation of CaM upon addition of Ca^{2+} (red circle). (B) CaM−Ca^{2+} systems as followed in the fluorescence mode. (C) Changes in emission at 370−650 nm correspond to CaM(Ca^{2+}) signature, while detection at 480 nm provides an AND gate that responds to both Ca^{2+} (1 mM) and CaM (20 μM), indicating a conformational change. Right: truth table.

We first established that the conformational change does occur in the presence of the G-quartet mixtures (see Supporting Information). We then tested the response of the ensemble under the same conditions (Figure 2B). Acidic CaM was, as expected, relatively invisible to the ensemble. Ca^{2+} alone induced a small decrease in fluorescence intensity, which may result from an interaction with the negatively charged DNA scaffold. However, upon addition of Ca^{2+} to the ensemble−CaM solution, interaction with the protein took place, resulting in an increased intensity at the pyrene emission region (390 nm, excimer: 500 nm) and a new pattern corresponding to the CaM(Ca^{2+}) complex.

Extension of the CaM surface area and the exposure of a large hydrophobic domain might be responsible for the ensemble−CaM(Ca^{2+}) interaction, although calcium ions may also play a role. Hence, an AND gate (Figure 2C) that signals a change in protein conformation within a high metal ion environment could be a useful tool for following its function. Other molecular logic gates that process various inputs with a high affinity and selectivity have been reported.[5] Combinatorial sensing, however, enables Boolean logic to be applied to understanding protein function, whose selective targeting can normally be achieved only with antibodies. In this example, monitoring at 370−650 nm provides a unique signature[1,2] for the CaM(Ca^{2+}) protein. However, an AND gate at 480 nm is enough to monitor CaM and Ca^{2+} interactions, as they rise and fall in response to external stimuli (Figure 2C).

A drawback of the current system is the relatively small changes in the signals caused by different proteins. This limitation could be overcome, for example, by modifying the ODNs with fluorescence quenchers,[1c] to provide better on−off ratios, or with specific protein recognition groups,[1b] to enable selective analysis within complex biochemical mixtures.

To the best of our knowledge this is the first diagnostic system that combines two different disciplines in supramolecular analytical chemistry.[9] While principles of the olfactory neural system enable the identification various proteins,[2] the logic of an electronic circuit is applied to analyze their properties. In a more general perspective, this work demonstrates ways by which molecular Boolean logic can provide combinatorial recognition systems with the ability to process information, such that a straightforward analysis of diverse biochemical samples can, in principle, occur within a single molecular digital device.

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Supporting Information Available: Complete fluorescence spectra and logic gate analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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