

Chemical Input Multiplicity Facilitates Arithmetical Processing

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Boolean functions at the molecular scale have been possible for some time through a variety of approaches¹ that harness the abilities of molecules to process information. In particular, molecular logic gates² such as AND,^{2a} XOR,^{2b} and INHIBIT^{2c} that produce light signals in response to a variety of chemical inputs have been studied extensively. The vitality of this approach was recently highlighted when basic arithmetic functions involving two, one-digit binary numbers (bits), such as addition³ or subtraction,⁴ were performed by molecules. A suggestion for more complex addition function by multiphoton spectroscopy has also been reported.⁵ The realization that several logic functions can be integrated within a single molecule has led to the design of molecular systems whose logic functions can be reconfigured either by generating multiple light signals⁶ or by altering their chemical inputs.⁷ It is likely that by combining these approaches, molecular logic gates with superior processing capabilities will emerge.

We propose a strategy based on input signal multiplicity, in which each chemical input can be recognized simultaneously at several domains within the same molecule, forming ideally distinct chemical states, each with its characteristic signaling properties. This approach resembles somewhat the way that our olfactory system works, where a specific odorant molecule, consisting of different functional groups, selectively target several receptors simultaneously.⁸ At the molecular level, a single molecular platform integrating multiple recognition sites for each input is expected to respond in parallel to a variety of reagents and thereby provide a range of Boolean functions.

We describe a first step toward this goal by demonstrating a reconfigurable molecular logic system, exhibiting parallelism in both its chemical inputs and light outputs, which by simple alternation of the input signals can perform distinct algebraic operations between two bits, solely in the fluorescence mode. The molecular arithmetic system is simple to operate, as it requires a single type of processing molecule, excited at the same wavelength of light, and uses a single measuring setup for straightforward "reading" of the arithmetic results.

Three logic gates, AND, Exclusive OR (XOR) and INHIBIT, operating in parallel, can perform simple arithmetic operations involving two bits. A half-adder^{3,5} can carry out elementary addition utilizing the XOR gate to generate the sum digit (*S*) and the AND gate to produce the carry (*C*). A half-subtractor⁴ is generated through XOR and INHIBIT logic devices, which produce the difference (*D*) and the borrow (*B*), bits, respectively. The truth table for these operations is shown in Figure 1a.

Molecule **1** (Figure 1b) possesses similar numerical processing abilities. Our design is based on a modified bacterial iron carrier⁹ (siderophore) to which two fluorophores, pyrene (donor) and protonable fluorescein (acceptor), whose emission spectra do not overlap, are attached at its termini. Upon excitation of the pyrene (344 nm) the molecule can switch between three distinct fluorescent states with emission of blue, green, or off luminescence (Figure 1c). In basic pH the fluorescein is present mainly in its mono or

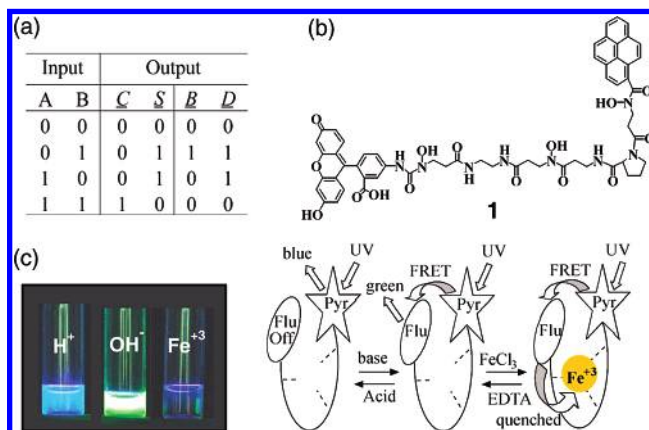


Figure 1. Truth table of half-adder and half-subtractor logic circuits and the distinct fluorescent states of molecule **1**, endowed with similar processing abilities. (a) Outputs are carry (*C*), sum (*S*), borrow (*B*), and difference (*D*). (b) Molecule **1** is composed of a siderophoric receptor, pyrene (Pyr), and protonable fluorescein (Flu). (c) Left: photograph of 0.1 mM ligand **1** in ethanol, excited with UV light, upon addition of 0.1 N aqueous sodium acetate, 0.1 N acetic acid and 1 equiv of iron(III) ions. Right: Schematic presentation describing the three fluorescent states shown in the photograph.

dianion fluorescent form,¹⁰ and therefore fluorescence resonance energy transfer (FRET) takes place between the donor and acceptor, leading to an intense green emission of the fluorescein. Turning the solution acidic causes protonation of the fluorescein to its inactive form, and the green fluorescein emission is replaced by a brilliant blue pyrene luminescence. Addition of iron(III) results in efficient fluorescent quenching^{9,11} by the bound metal ion.

However, the quenching efficiency of the fluorescein emission was found to be dependent on its protonation state. While the emission of the monoanionic fluorescein in ethanol^{10b} was fully quenched, at higher pH, when it was further deprotonated, an emission was detected even from the iron-bound fluorescein. Accordingly, the complex, **1-Fe(III)**, can be viewed as a molecular scaffold with four exchangeable domains, COH, COO⁻, CONO⁻, and Fe(III), that can be targeted either singly or in combinations with mono- or multifunctional chemical inputs to yield Boolean patterns of various fluorescence outputs (Figure 2).

The performance of the iron complex as a half-subtractor, acting simultaneously as XOR and INHIBIT logic gates, can be demonstrated by monitoring the blue and the green emissions obtained with acid and base as input signals (Figure 2A). In ethanol solution, the complex emission is inherently quenched. However, when acid (HCl) is added, the pH of the system decreases, leading to simultaneous protonation of the fluorescein and the hydroxamate units, followed by the release of the bound metal ion, as expected from siderophore-iron(III) complexes in low pH.¹² Since the fluorescence of the pyrene is not quenched and the fluorescein is in its inactive form, emission is observed solely at 390 nm. Alternatively, addition of base (sodium acetate) to the ferric complex results in a green emission at 525 nm, due to ionization of the

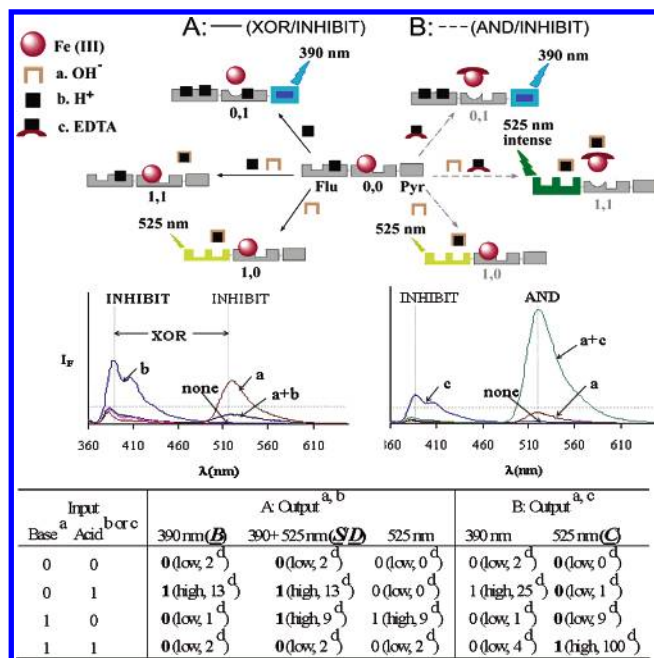


Figure 2. (Top) Schematic presentation describing the combinatorial effects of the chemical inputs on the iron complex and the resulting fluorescence emissions for (A) XOR/INHIBIT and (B) AND/INHIBIT logic patterns, together operating as a half-adder and a half-subtractor, solely in the fluorescence mode. (Bottom) Truth table for 1.5 μ M 1-Fe(III) in EtOH (1.5 mL) excited at 344 nm. Inputs: 20 μ L aqueous solution of (a) 2 M AcONa, (b) 1 M HCl, (c) saturated EDTA. Outputs: (d) fluorescence outputs, intensity in arbitrary units. (C) carry, (B) borrow, (S/D) sum/difference

fluorescein. However, when both acid and base are added, a buffer solution is formed; the iron remains bound to the receptor, and no significant change in output signal is observed. A characteristic INHIBIT logic gate is achieved when the output is read at 525 or 390 nm. A XOR gate is obtained when the outputs are read simultaneously at 390 and 525 nm. Observing multiple wavelengths simultaneously has been recognized as a powerful tool for achieving multiple logic expressions.^{6a} Consequently, by an appropriate choice of the wavelengths of detection, the molecule can be used to generate simultaneously difference (D) and borrow (B) bits of 0–0, 1–0, 0–1, and 1–1.

Molecular systems mimicking the configuration of silicon-based half-adders, with distinct AND and XOR logic gates that respond to the same chemical inputs and their outputs detected separately, have been demonstrated.³ An alternative, molecular approach, which does not necessitate the use of different processing units, is to use a molecule that can function in different modes, i.e., either as AND or as XOR logic gate.

Under identical starting conditions the same system can reconfigure its logic scheme to operate simultaneously as AND and INHIBIT logic gates (Figure 2B), simply by replacing the acid (HCl) with ethylenediamine tetraacetic acid (EDTA), which is a weaker acid and also a competitive chelator for ferric ions.⁹ With no inputs, no significant emission is detected. When EDTA is added, it protonates the fluorescein and at the same time extracts the iron-(III) ions, resulting in exclusive emission from the pyrene at 390 nm. Addition of a base (sodium acetate) causes ionization of the fluorescein, but without removal of the iron, so that a faint green emission is observed below the threshold barrier. Only when both inputs are present, namely, the solution is basic and the iron is

removed, an intense green emission of the fluorescein at 525 nm is detected. Consequently, by monitoring the emission signals at 525 nm, an AND logic gate is obtained, while detection at 390 nm results in an INHIBIT logic gate. The truth table in Figure 2 summarizes the conditions by which the operation of this AND gate in parallel to the previously described XOR logic gate offer an input-configurable half-adder which in turn can produce sum (S) and carry (C) bits of 0+0, 1+0, 0+1, and 1+1.

Molecular logic systems operating via combinatorial recognition of input molecules provide a unique signaling pattern for each reagent and therefore could handle considerable amounts of chemically encoded information.⁸ The concept described in this report is of a general nature and can be applied to extend the processing abilities of any molecular logic gate hosting a substrate to which a competitor is known by readjusting its signaling moieties to respond to the inhibitor or to some of its physicochemical properties.

Acknowledgment. We thank Mrs. Rachel Lazar, Mrs. Sara Rubinraut, and Dr. Brenda Mester for their skillful assistance. Support from the European Union (HPRN-CT-2000-00029) and the Helen and Martin Kimmel Center for Molecular Design are greatly acknowledged.

Supporting Information Available: The synthesis of 1, additional spectral data, and a representation of half-adder and half subtractor electronic logic circuits. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA0453329