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Communicating Chemical Congregation: A Molecular AND Logic Gate with Three Chemical Inputs as a "Lab-on-a-Molecule" Prototype

David C. Magri,* Gareth J. Brown, Gareth D. McClean, and A. Prasanna de Silva*

School of Chemistry and Chemical Engineering, Queen's University, Belfast BT9 5AG, Northern Ireland

Received December 6, 2005; E-mail: d.magri@qub.ac.uk; a.desilva@qub.ac.uk

Several major phenomena in different fields require the presence of more than two independent variables. The concept of volume depends on the third spatial dimension.[1] Signal amplification in electronic devices, such as in transistors, requires a third terminal.[2] The old adage "two's company, three's a crowd"[3] reminds us that congregations start with three entities. We now present the first AND logic gate[4−6] driven by three chemical inputs as a direct way of detecting congregations of chemical species. A fluorescent signal is switched "on" only when three distinct species exceed pre-set concentration thresholds. Such a device could have application for inexpensive disease screening. Rather than testing for three disease parameters by three separate tests, followed by manual consideration of the data by a practitioner, a medical condition could be directly diagnosed by a single rapid test.[7] For instance, renal failure is indicated by elevated levels of creatinine, urea, and potassium.[8] We illustrate this idea by constructing AND gate **1**, which responds to the electrolyte inputs Na^+, H^+, and Zn^{2+} in water with an enhanced fluorescence signal. **1** is a "lab-on-a-molecule" prototype since a clinically relevant result would emerge from on-board information processing of several sensory channels simultaneously in targeted cases. Fluorescent molecules driven by single inputs are already useful in probing the environment of biological systems, including individual living cells,[9] and demonstrate the capabilities of molecular devices.[10]

The logic gate **1** is constructed according to the design principles of modular photoinduced electron transfer (PET) systems.[11] More specifically, **1** possesses a "receptor$_1$−spacer$_1$−fluorophore−spacer$_2$−receptor$_2$−spacer$_3$−receptor$_3$" format (Figure 1) by incorporating known components. The receptors (red) incorporated in the design are a benzo-15-crown-5 ether as receptor$_1$ for Na^+, a tertiary amine as receptor$_2$ for H^+, and a phenyliminodiacetate as receptor$_3$ for Zn^{2+}. The latter, due to Gunnlaugsson,[12] is one of a range of receptors arising from modern Zn^{2+} sensor research.[13] An anthracene moiety is used as the fluorophore (blue). The three spacer units between adjacent receptors and the fluorophore are methylene spacers (green) to minimize the distance between the receptors and the fluorophore, thus facilitating conditions for efficient PET, notably if there are folded conformers.

The logic characteristics of AND gate **1** in water were determined by observing the fluorescence spectra under eight possible input conditions (Figure 2). In the presence of significantly high levels of the three chemical inputs, the emission intensity is significantly high. Otherwise, in all other cases, the fluorescence output is low because of PET occurring from either the benzocrown ether in the absence of Na^+, the tertiary amine in the absence of H^+, or the anilinic moiety in the absence of Zn^{2+} to the excited anthracene fluorophore. A fluorescent system with three PET processes is known, but has different input-output characteristics.[14]

Table 1 gives the three-input AND logic truth table for **1**. The high levels of Na^+, H^+, and Zn^{2+} concentrations were chosen to achieve essentially complete binding to their respective receptors.

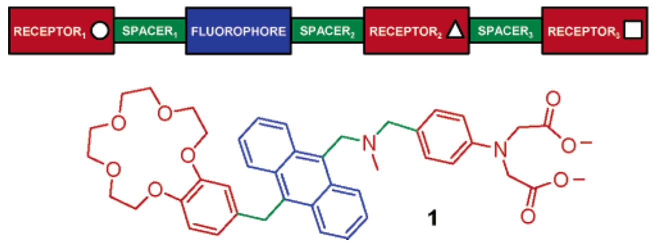

Figure 1. The design principle (top) and the molecular structure of the three-input AND logic gate **1** (bottom).

Conversely, low levels of analytes are either zero, or in the case of H^+, chosen to leave the tertiary amine unprotonated. The nature of Zn^{2+} restricts the lowest possible H^+ concentration that can be usefully maintained in those cases containing the metal ion to pH 8.0. The fluorescence signal continues to decrease at pH values greater than 8.0, but the precipitation of $Zn(OH)_2$ is a significant interference. Thus, the fluorescence quantum yield (ϕ_F) of **1** in the presence of Na^+ and Zn^{2+} and absence of H^+ is estimated at pH 9.5 by extrapolation of the corresponding pH titration results at pH < 8.0 according to the equation[15] $\log [(\phi_{Fmax} - \phi_F)/(\phi_F - \phi_{Fmin})] = pH - pK_a$. The pK_a value is found to be 7.7.[16] A corresponding equation was employed to determine the other two cation binding constants by measuring the ϕ_F value as a function of a chosen cation concentration while holding the other two cations in excess. Log β_{Na^+} and log $\beta_{Zn^{2+}}$ were determined to be −0.3[17] and 4.0[18] in water at pH 6. These ion binding constants represent the thresholds that each ionic concentration must exceed before the input can be considered to be high. Hence, the AND gate **1** switches on only when all three ion concentrations have exceeded their respective thresholds. The thresholds could be adjusted by choosing different receptors or by substitutional tuning of the current set.[19] Alternatively, aqueous organic solvent mixtures can be employed (see Table

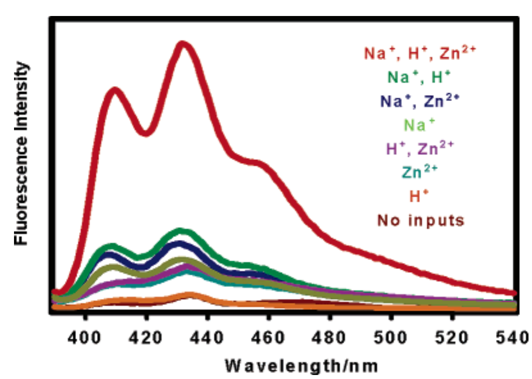

Figure 2. Fluorescence spectra of 10^{-5} M **1** in water excited at 379 nm under the eight experimental conditions (color coded) required to demonstrate three-input AND logic. Only when Na^+, H^+, and Zn^{2+} are all present in high enough concentrations is a significant fluorescence enhancement observed (spectrum in red). See Table 1 for detailed data.

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Table 1. Truth Table for the Three-Input AND Logic Gate **1** in Water

input$_1$ (Na^+)[a]	input$_2$ (H^+)[b]	input$_3$ (Zn^{2+})[c]	output emission (ϕ_F)[d]
0 (low)	0 (low)	0 (low)	0 (low, 0.001)
0 (low)	1 (high)	0 (low)	0 (low, 0.001)
0 (low)	0 (low)	1 (high)	0 (low, 0.002)[e]
0 (low)	1 (high)	1 (high)	0 (low, 0.003)
1 (high)	0 (low)	0 (low)	0 (low, 0.006)
1 (high)	1 (high)	0 (low)	0 (low, 0.007)
1 (high)	0 (low)	1 (high)	0 (low, 0.006)[f]
1 (high)	1 (high)	1 (high)	1 (high, 0.020)

[a] High input level 5 M sodium methanesulfonate. Low input level maintained with no added sodium salt. [b] High and low input levels correspond to $10^{-6.0}$ and $10^{-9.5}$ M protons adjusted with methanesulfonic acid and tetramethylammonium hydroxide. [c] High input level 9.1 mM zinc sulfate with 10 mM IDA corresponding to pZn = 3.1 at pH 6.0 and 4.8 at pH 8.0. Each of these pZn values is sufficient to saturate the zinc receptor in **1** at the corresponding pH. The low input level maintained by scavenging any trace heavy metals with 1 mM EDTA. [d] Output is considered high when $\phi_F > 0.010$. ϕ_F obtained by comparison with 9,10-bis[di(hydroxyethyl)aminoethyl]anthracene ($\phi_F = 0.66$)[15] in 1:4 MeOH:H_2O (v/v) at pH = 3.5. ϕ_F uncertainty is ±10%. [e] Based on conditions at pH 8. [f] Extrapolated to pH 9.5 by curve fitting data between pH 5.5 and 8.0 for ϕ_F and ϕ_{Fmin} to log $[(\phi_{Fmax} - \phi_F)/(\phi_F - \phi_{Fmin})] = pH - pK_a$.[15]

S1 in Supporting Information). Thus, **1** processes data concerning three ion concentrations and produces a composite result in the form of an easily detected fluorescence signal.

Since **1** is a prototype, the receptor modules were chosen more for synthetic convenience rather than their ion binding selectivities. Nevertheless, the large fluorescence switching of **1** (cf. first and eighth rows of truth Table 1) indicates it is robust toward the presence of other ions at similar concentrations, notably K^+ versus Na^+ and Ca^{2+} versus Zn^{2+}.[4b,12a] Device operation should not be impaired. In addition, the absence of one input cannot be compensated for by the presence of a large excess of another. Control experiments in the presence of 0.1 M Zn^{2+} confirm that there is no significant fluorescence enhancement with a quantum yield of only 0.004 even at pH 6.

Boolean logic processing of concentration information by a molecular device could be particularly useful for fast screening of medical conditions. For three species with concentration inputs *A*, *B*, and *C*, AND logic gate **1** delivers a fluorescence signal for the Boolean algebraic expression $A{\cdot}B{\cdot}C$. A three-input INHIBIT logic gate[20] delivers an expression $\bar{A}{\cdot}B{\cdot}C$ corresponding to high concentrations of two species (*B* and *C*) and a low concentration of the other (*A*). When the present result is viewed alongside this latter example, direct screening for various medical conditions opens up in general since different medical conditions depend on various logical combinations of individual indicators.[8]

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Supporting Information Available: Synthesis, characterization, and the truth table S1 for three-input AND logic gate **1** in 1:1 methanol/water. This material is available free of charge via the Internet at http://pubs.acs.org.

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