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A Molecular NAND Gate Based on Watson–Crick Base Pairing

H. Tarik Baytekin and Engin U. Akkaya*

Middle East Technical University, Department of Chemistry, Ankara TR-06531, Turkey

akkayaeu@metu.edu.tr

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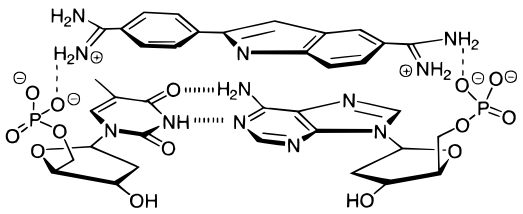
ABSTRACT



A DNA-binding dye, 4′,6-diamidino-2-phenylindole (DAPI) signals AT base pairing with a shift in the fluorescence emission spectrum. The signaling follows W–C base-pairing rules, and both dAMP and dTMP are required for the largest spectral shift. Thus, the dye with its two phosphate receptor sites functions as a molecular NAND gate accepting nucleotides as inputs. Moreover, when the observation wavelength is changed from 470 to 411.5 nm, the gate functions in TRANSFER logic.

The design and construction of supramolecular systems that respond to chemical and/or photonic inputs by generating output signals that are in accordance with logic gate behavior has attracted considerable attention.[1−5] Most successful systems designed so far function via photoinduced electron transfer (PET) mechanisms. Molecular analogues of AND,[1] OR,[2] NOR,[3] INHIBIT,[3] XOR,[4] YES, and NOT[5] gates have already been proposed. The first example of molecular arithmetic was also reported[6] recently. While PET effects may generate significant signal differences between the off (0) and on (1) states, more complex PET and non-PET signaling possibilities need to be studied especially for a wider variety of gate functions and gate implementation. The leap from molecular analogues of gates to true molecular information processing is gigantic, but it is recognized that the potential is equally impressive.

Among the 16 logic functions with two variables, some are equal to a constant and some are not associative or commutative, so only 8 of them, AND, OR, NOR, XOR, NAND, COMPLEMENT, TRANSFER, and EQUIVALENCE, are used as standard gates in digital design. The NAND gate is particularly important; it is considered to be a universal gate, since every other gate function can be generated by successive implementation of NAND gates.[7]

In our own work directed toward molecular information processing, we would like to exploit base-pairing interactions in nucleic acids that are ultimately responsible for the storage and transmittal of genetic information. This unique capability of nucleic acids has attracted attention in the context of molecular computing.[8] The Hamiltonian path problem[9] is

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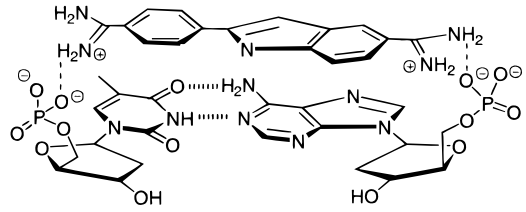
readily solvable by massively parallel DNA computing approaches taking advantage of well-established rules of base pairing at the nucleic acid level. However, conventional serial and faster parallel processing of information may require construction of molecular logic gates. Thus, we wanted to study signaling opportunities at the single base level. The hydrogen bonding interactions, although weak in water, can be considerable[10] in aprotic solvents.

For base-pairing interactions to trigger a fluorescence response, the fluorophore/receptor molecule should have functional groups that can be affected by the ternary complex formation either in the ground or excited state (Scheme 1).

Scheme 1. Putative Ternary Complex That Leads to Fluorescence Intensity Changes



DNA-binding molecule 4′,6-diamidino-2-phenylindole (DAPI) looked like a promising candidate. It has a number of different binding modes, including intercalation, and displays a significant bias for AT-rich regions of DNA.[11] Amidino functions are likely to interact with the phosphodiester chain, and the phenyl−indole base is a very good match for a purine−pyrimidine base pair. Fluorescence change on binding to DNA could be due to both microenvironment change in a DNA base stack[12] and changes in the protonation state of the amidino functions. In fact, Czarnik[13] showed that on binding to polyanionic species, such changes in protonation states of polyamines linked to fluorophores could result significant alterations in the fluorescence emission intensity of the fluorophore, mostly by the changes in the efficiency of PET process.

The base inputs are best introduced as deoxynucleotides, i.e., 5′-monophosphates. In this way, once the base pairing is established according to Watson−Crick rules, further complex stabilizing interactions between the amidino and phosphate groups would be generated, and the DAPI fluorescence would be altered. We found that a solvent system comprising 99% DMSO and 1% aqueous buffer solution (Tris, pH 7.5) gave the best results. A 0.75 μM solution of DAPI in this solvent system gave a characteristic emission spectrum with an emission maximum of 475 nm when excited at 345 nm. When an input in the form of 0.4 mM dTMP was added, no significant change in either the emission intensity or the emission maximum was observed. When the same concentration of dAMP was added, a small blue shift in the emission maximum was observed, but the signal intensity change was insignificant. When both inputs were added at the same concentration, however, a larger spectral shift, in addition to a drop in the emission intensity was observed. When the emission intensity was followed near the isoemissive point of DAPI and DAPI + dAMP emissions (455 nm), a larger than 2-fold drop in intensity was observed when both inputs were present with no changes in any other conditions (Figure 1). Thus, the signal changes

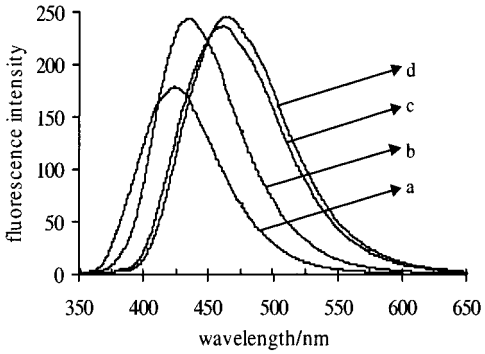


Figure 1. Fluorescence spectra of DAPI in the absence or presence of deoxynucleotides. DAPI concentration was 0.75 μM, and nucleotides were added to a final concentration of 0.4 mM. The solvent was 99% DMSO/1% buffer (50 mM Tris, pH 7.5) Excitation was at 345 nm: (a) dAMP + dTMP + DAPI, (b) dAMP + DAPI, (c) dTMP + DAPI, and (d) DAPI alone.

at that wavelength are in accordance with a NAND logic gate behavior (Table 1). The presence of a hypsochromic shift brings a further advantage, since the observation wavelength can be modified at will; by changing it from 455 nm to 411.5, the supramolecular assembly is in effect transformed to a TRANSFER gate. The truth table for this type of gate, with the actual emission intensity data is shown in Table 2.

Table 1. DAPI Fluorescence Response in NAND Logic[a,b]

input 1	input 2	output (normalized)
0	0	high (1.00)
0.4 mM dAMP	0	high (0.91)
0	0.4 mM dTMP	high (0.98)
0.4 mM dAMP	0.4 mM dTMP	low (0.48)

[a] DAPI (0.75 μM) emission in 99% DMSO and 1% buffer. The excitation wavelength was 345 nm, and the emission data at 455 nm were collected. NAND logic requires that the signal is 0 only when both inputs are 1, and 0 in all other cases. [b] Relative emission intensities were normalized to the emission intensity of DAPI alone at 0.75 μM concentration.

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Table 2. DAPI Fluorescence Response in TRANSFER Logic[a,b]

input 1	input 2	output (normalized)
0	0	low (0.28)
0.4 mM dAMP	0	high (1.00)
0	0.4 mM dTMP	low (0.32)
0.4 mM dTMP	0.4 mM dAMP	high (1.00)

[a] DAPI (0.75 mM) emission in 99% DMSO and 1% buffer. The excitation wavelength was 345 nm, and the emission data at 411.5 nm were collected. TRANSFER logic requires that the signal is 0 when both inputs are 0, and when only one of the inputs is 1. When the other input is 1 or when both are 1, the output is 1. [b] Relative emission intensities at 411.5 nm were normalized to the emission intensity of DAPI + dAMP + dTMP (0.75 μM, 0.4 mM, and 0.4 mM, respectively).

To further establish the specificity of the fluorescence signal, we studied the fluorescence response of the other base pair combinations, both matching and nonmatching (Table 3). No other response comparable to the dAMP−dTMP pair was observed, including dGMP−dCMP. Apparently, DAPI shows some selectivity for the AT pair which is not surprising, considering that in both longer DNA sequences and oligomers, it is selective for AT-rich regions.

Table 3. Fluorescence Emission at 455 nm in the Presence of Indicated Pairs of Deoxynucleotides Normalized to DAPI Emission[a]

added deoxynucleotide	normalized emission at 455 nm
dAMP + dTMP	0.48
dAMP + dGMP	0.93
dAMP + dCMP	0.99
dTMP + dGMP	1.09
dCMP + dGMP	0.98
dCMP + dTMP	1.04
DAPI alone	1.00

We believe selective deprotonation (a decrease in the pK_a) of the amidino functions is at least in part responsible for the small blue shift and decrease in the emission intensity seen in the AT−DAPI complex. The detailed study of ternary complex formation is highly complicated, since in principle four different ternary complexes and four binary complexes are possible. To complicate the matters further, solubility of the nucleotides in the mixed solvent system is limited; a concentration of 0.4 mM represents the maximum solubility for the nucleotides dTMP, dCMP, and dGMP. Nevertheless, a stable ternary complex is clearly implicated on the basis of an earlier report[14] of a similar complex involving an equal number of phosphate−guanidinium interactions.

Thus, we show that base-pairing interactions can be exploited in the construction of logic gates with appropriate receptor molecules. Different fluorophores with different geometrical requirements for signal enhancement or quenching may lead to other gate structures and functions. In the system described here, we acknowledge that the change in the signal is low, but this DAPI-based system is the first report of a gate design in which hydrogen bonding interactions have been utilized. With these inputs that make use of more spatially defined molecular interactions, new examples of higher level gate implemenation seem to be more likely. Our work to that end is in progress.

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