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Simultaneous G-quadruplex DNA Logic

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Simultaneous G-quadruplex DNA Logic

Antoine Bader and Scott L. Cockroft*

Abstract: A fundamental principle of digital computer operation is Boolean logic, where inputs and outputs are described by binary integer voltages. Similarly, inputs and outputs may be processed on the molecular level as exemplified by synthetic circuits that exploit the programmability of DNA base-pairing. Unlike modern computers, which execute large numbers of logic gates in parallel, most implementations of molecular logic have been limited to single computing tasks, or sensing applications. Here we report three G-quadruplex-based logic gates that operate simultaneously in a single reaction vessel. The gates respond to unique Boolean DNA inputs by undergoing topological conversion from duplex to G-quadruplex states that were resolved using a thioflavin T dye and gel electrophoresis. The modular, addressable and label-free approach could be incorporated into DNA-based sensors, or used for resolving and debugging parallel processes in DNA computing applications.

DNA is often referred to as the molecule of life due to its central role in carrying the genetic information that is translated into proteins by cells.[1] As noted by Feynman,[2] Conrad and Liberman[3] decades ago, the programmable Watson-Crick base-pairing properties of DNA make it highly suited for performing computations. Indeed, interest in the field of DNA computing blossomed following Adleman's use of DNA to solve an NP-complete Hamiltonian path problem.[4] DNA has subsequently been used to construct and operate dynamic self-assembled nanostructures,[5] logic circuits,[5e, 5f, 6] and chemical reaction networks.[7] Irrespective of their complexity, most man-made DNA-logic circuits rely on the stepwise triggering of sequentially addressable Boolean logic gates. Thus, efforts are required to achieve multi-tasking, in which multiple computational tasks are performed concurrently rather than sequentially. Moreover, novel detection methods are required for monitoring the operation of increasingly complex DNA-based logic circuits that circumvent the need for expensive fluorophore labelling.

Here we report the simultaneous operation and label-free detection of DNA-based Boolean logic gates that switch between duplex and G-quadruplex states.

Fluorescence is often the method of choice for monitoring the operation of DNA nanodevices, due to the sensitive and quantitative nature of the readout.[8] However, the increased complexity and cost of experiments involving DNA modified with fluorophores and quenchers has driven the development of label-free strategies.[9] For example, several fluorescent dyes[10] have been identified that bind non-covalently to DNA G-quadruplexes,[11] thereby allowing the sensitive, but label-free
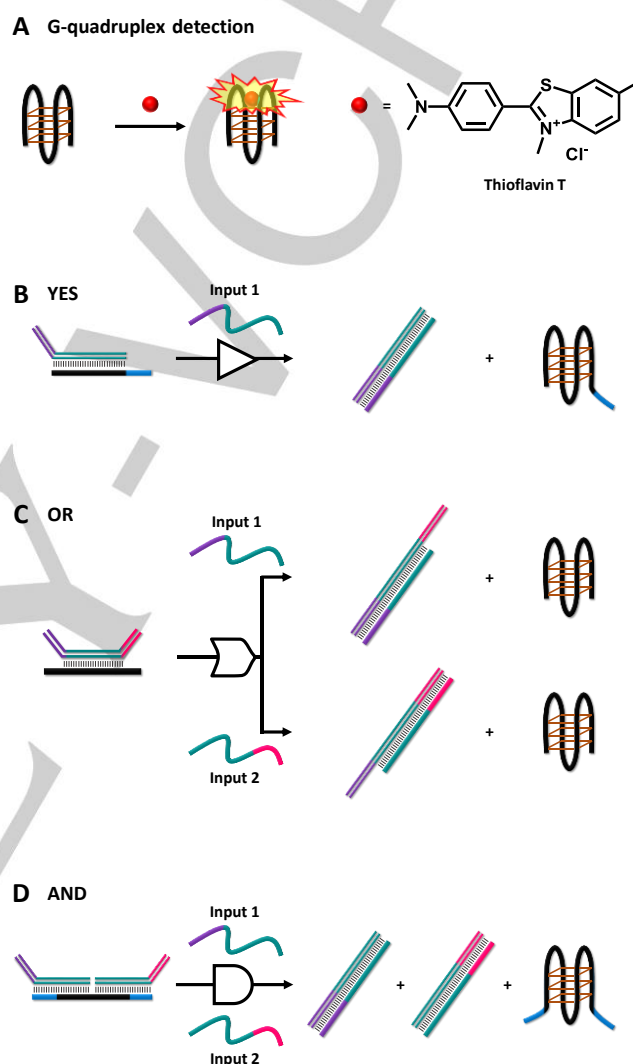
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Figure 1. (A) The fluorescence of thioflavin T is specifically enhanced upon binding to a G-quadruplex structure. (B), (C) and (D) Mechanisms for the operation of YES, OR and AND logic gates. The purple and pink domains correspond to toehold binding sites for **Inputs 1** and **2,** which induce strand-displacement reactions according to the appropriate Boolean rules to release G-rich sequences (black). The released G-rich sequences subsequently fold into G-quadruplex structures that can be detected by thioflavin T switch-on fluorescence. All sequences are provided in Table S1.

detection of such structures. Moreover, the ability of an oligonucleotide to switch between an unfolded and a G-quadruplex state[12] that binds a dye has been exploited for switch-on fluorescent detection in logical sensing applications (e.g. Figure 1A).[13] Additionally, split G-quadruplexes, where two guanine-rich strands brought in close proximity form an intermolecular quadruplex, have also been used for detecting the outcome of logical operations.[14] Thus, we reasoned that it should
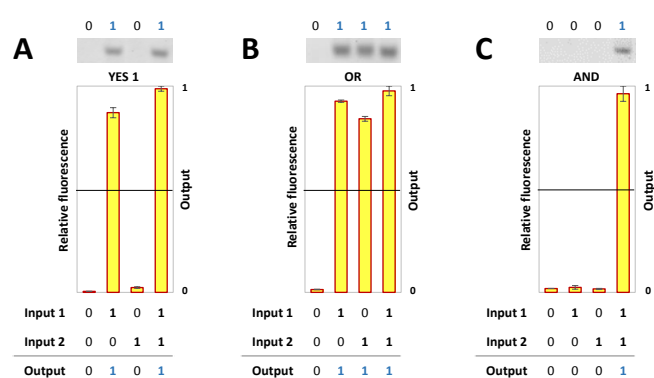
Figure 2. Experimentally observed fluorescence outputs for each of the logic gates revealed by thioflavin T gel staining. Complete gel images are provided in Figures S1 to S3.

Figure 3. (A) Circuit representation for the simultaneous operation of the YES, AND and OR G-quadruplex logic gates. (B) Individual and (C) sum total fluorescence outputs produced by each combination of inputs and the corresponding truth table. The complete gel image is provided in Figure S4.

be possible to devise a series of G-quadruplex-based Boolean logic gates that operate simultaneously in the same reaction vessel, and to detect their outputs using switch-on fluorescent detection (Figure 1).

The YES, OR and AND gates were designed such that a strand capable of folding into a G-quadruplex structure was released upon addition of the appropriate Boolean inputs (Figure 1B-D). Each G-rich output strand (black in Figure 1B-D) was initially sequestered in a duplex state by counter strands containing single-stranded overhangs (purple and pink in Figure 1B-D). Each overhang served as a toehold binding site for a complementary DNA input to invade and displace[8] a G-rich output that subsequently folds into a G-quadruplex structure. The output strands were derived from the Plas24 *Plasmodium falciparum* telomeric G-quadruplex sequence[15] which is known to switch on thioflavin T fluorescence (Figure 1A).[16] In addition, the three different G-quadruplex output strands were distinguished by having either zero, one or two single-stranded overhangs such that they could also be resolved by gel electrophoresis (blue in Figure 1).[17] Moreover, the sequences of the gates (Table S1) were addressable in parallel allowing simultaneous operation in the same reaction vessel. Furthermore, the modular design and the use of mutually compatible DNA inputs and outputs should allow integration into higher order, multi-level circuits for potential computing applications.[6b, 6c, 6f, 18] For example, G-quadruplex outputs could be used to detect the operation of connected upstream gates, while the release of single-stranded domains could serve as inputs for downstream logic gates in a larger circuit.

Initially, the YES, OR and AND gates depicted in Figure 1 were assembled from commercial oligonucleotides (see SI for details). Each Boolean combination of inputs was separately added to each gate, and subsequently analysed using gel electrophoresis to interrogate their operation. While traditional SYBR gel staining revealed all of the products and side-products of the logic operations (Figures S1 to S3, right), initial thioflavin T staining gave a much simpler fluorescent read-out by only revealing the G-quadruplex species of interest (insets above bar charts in Figure 2). These gels revealed that the YES, OR and AND gates all operated according to their respective Boolean truth tables; the YES gate was only triggered by **Input 1**, the OR
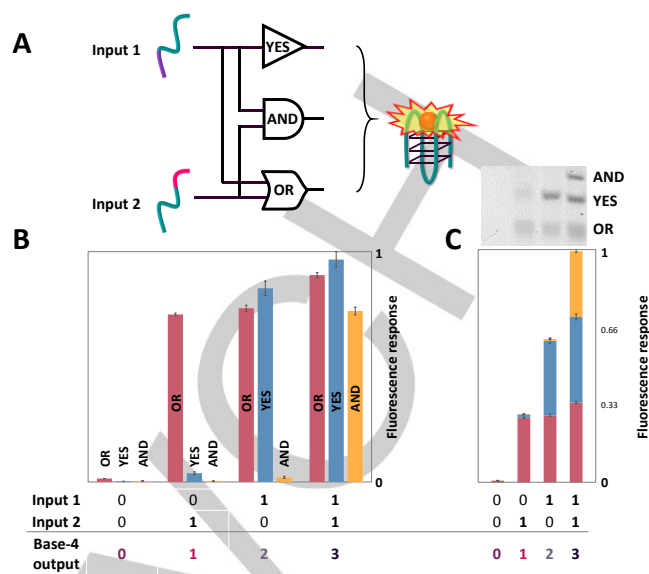
gate was triggered by **Input 1** or **2**, while the AND gate required the presence of both **Inputs 1** and **2**.

Having demonstrated correct individual operation of the YES, OR, and AND gates using G-quadruplex detection, we next sought to demonstrate simultaneous operation of all logic gates in a single reaction vessel. Individual logic gates were assembled separately and then combined in an equimolar mixture before being triggered by inputs. Correct simultaneous operation of the combined logic gates was demonstrated for all possible Boolean combinations of the inputs (Figures 3 and S4); the absence of inputs gave no signal, **Input 2** alone triggered one gate (OR), **Input 1** alone triggered two gates (YES and OR), while the simultaneous presence of **Input 1** and **2** triggered all three gates. Figure 3B shows that the fluorescent response of each of the gates is similar, thus the sum combination of these logic gate outputs presents the interesting characteristic of displaying four quantized fluorescent states depending on the inputs present (Figure 3C). Such a system extends beyond the binary (0 or 1) nature of classical Boolean logic, and like the DNA from which the gates are themselves constructed, constitutes a quaternary base-4 numeral system. Since the four possible combinations of inputs map directly onto four unique output states, a total fluorescent response of 0, 1, 2, or 3 unambiguously reveals which inputs are present to an otherwise blind external observer.[19] While the above analysis is made simple using thioflavin T-staining, the SYBR-staining of the same gel reveals that the three different G-quadruplex outputs were actually resolved against a complicated background of numerous oligonucleotide species consisting of the three logic gate assemblies, two inputs, five duplex products, in addition to multiple self-assembled side products (left vs. right in Figure S4). This underlines the power and versatility of combining G-quadruplex formation, electrophoretic separation and highly-specific and sensitive thioflavin T fluorescent

responses in resolving the operation of complicated DNA-based devices.

It is important to put the present system in the context of related DNA-based logic systems that have used G-quadruplexes as reporters. For instance, Li *et al.* demonstrated a label-free electrochemical platform that enzymatically amplified the synthesis of a G-quadruplex strand in response to a logical operation.[20] Similarly, Hu, Fei *et al.* have previously used two G-quadruplex units as logic reporters, [13h] but the heterogeneous signal response magnitudes of (0,1,1,2) contrast with the homogeneous Boolean responses (0,1,1,1) of the present system. While neither of these previous G-quadruplex systems performed multiple operations simultaneously, other DNA-based systems have made progress towards such an aim. For example, the impressive automaton described by Macdonald, Stojanovic *et al.* implemented a tic-tac-toe game using an array of deoxyribozyme-based logic gates.[5n, 21] Overall, logic gates were executed simultaneously, but activated sequentially. Since only one gate was activated per well, per play, it does not demonstrate truly simultaneous operation. However, Dong *et al.* has reported a dual-signal logic device that simultaneously computed AND and NAND operations.[22] Despite its elegance, the computational capability of the system is limited since the outputs are not fully independent, thus obtaining two positive responses from each gate was not possible. Furthermore, it would be difficult to use the non-nucleic acid outputs as inputs for downstream gates. In contrast, the present system used modular and mutually compatible DNA inputs and outputs such that other upstream or downstream DNA components could be integrated to form higher order, multi-level circuits.

In summary, we have constructed three G-quadruplex-based Boolean logic gates that crucially, can be operated and monitored simultaneously in a single reaction vessel. Key to resolving the simultaneous operation was the use of the fluorescent switch-on probe, thioflavin T, whose specificity for the G-quadruplex outputs enabled each of the gate responses to be distinguished against a complicated background of numerous oligonucleotide gates, inputs, products and side-products. The combination of gel electrophoresis and G-quadruplex-specific thioflavin T staining enabled the response of each gate in a mixed solution to either be resolved independently, or summed to give a quaternary base-4 numeral output of 0, 1, 2 or 3. In addition to resolving the operation of complicated ensembles of interacting nucleotides, the four-state quantized outputs also demonstrated the robustness of strand-displacement driven logic gates when operated simultaneously in a shared solution. Finally, the use of an extrinsic fluorescent switch-on probe side-steps the need for expensive and time-consuming covalent labelling of oligonucleotides with fluorophores or quenchers. Such a versatile, specific, and cost-efficient strategy could be employed for monitoring and debugging dynamic multi-tasking DNA-based devices, or exploited in biosensing and theranostic applications where high levels of background noise might otherwise be encountered.

Experimental Section

Operation of the logic gates

Logic gates were assembled using commercial oligonucleotides as described in the SI. One equivalent of each gate (50 µM stock) and two equivalents of the appropriate inputs (100 µM stock) were incubated in 1× TBE / 12.5 mM Mg^{2+} buffer for 1.5 h at room temperature. The resulting solution was then used for further analysis by non-denaturing polyacrylamide gel electrophoresis.

Logic gate readout

Samples were loaded on polyacrylamide gels of desired concentration supplemented with 12.5 mM $MgCl_2$. Sucrose was used as loading agent and was added to a final concentration of 15%. Gels were run at 300 V in 1× TBE / 12.5 mM Mg^{2+} buffer for the durations indicated in Figures S1 to S4 at room temperature and cooled down with an in-house fan system. The gels were then incubated for 15 min in a 0.5 µM thioflavin T solution (Sigma-Aldrich, Gillingham, UK). Gels were imaged on a Typhoon 9400 (Amersham Biosciences, Little Chalfont, UK) using the Blue1 laser (457 nm). The gels were then briefly washed with water and further stained with SYBR Gold (Life Technologies, Eugene, OR, USA) for 15 minutes before being finally imaged using the Blue2 laser (488 nm). Thioflavin T fluorescence quantification was performed using ImageQuant (Molecular Dynamics).

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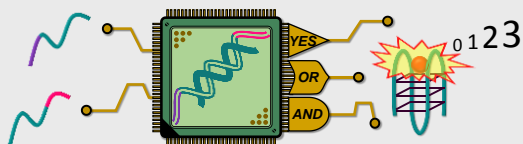
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All in one: Three DNA-based Boolean logic gates are simultaneously computed in a single reaction vessel. G-quadruplex outputs were specifically detected against a complicated background of oligonucleotide species giving a quantized four-state (0, 1, 2, 3) output, which contrasts with the binary (0, 1) nature of Boolean logic.