Photonic boolean logic gates based on DNA aptamers†

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We designed a pair of DNA-based logic gates that sense single-stranded DNAs and aptamer ligands to produce fluorescence outputs according to Boolean logic functions AND and OR.

Molecules that enable computation at molecular level should find many applications in medicine, nanotechnology and biotechnology. Molecular logic gates are synthetic molecules that produce physical or chemical output in response to physical or chemical inputs in accordance with the Boolean logic functions. For example, output of an AND logic gate is ON if and only if all inputs are ON, and an OR logic gate yields ON if at least one input is ON. To date, a number of molecular logic gates have been synthesized based on organic molecules,[1] DNA,[2] RNA,[3] peptides[4] and proteins.[5]

Molecular logic gates based on DNA and RNA have many potential advantages. The straightforward hybridization rules enable convenient interface with other molecular computation devices based on DNA and RNA. Additionally, both DNA and RNA are known to possess catalytic[6] and molecular recognition abilities[7] which can be used to provide amplification and sensor functions. In particular, the versatile molecular recognition potentials of *in vitro*-selected DNA and RNA aptamers present an opportunity to design molecular logic gates that exploit aptamer ligands as inputs.[3,8,9] While numerous aptamer-based sensors have been developed,[10] DNA and RNA logic gates that exploit multiple aptamers are rare. In this communication, we report a pair of DNA logic gates that yield fluorescence outputs in response to two aptamer ligands, adenosine and thrombin. We also demonstrate that two distinct single-stranded DNAs (ssDNAs) can be used as chemical inputs for the logic gates.

The molecular recognition strategy of the DNA logic gates are based on the structure-switching signaling aptamers described by Li and colleagues.[11] The AND logic gate was constructed by fusing the adenosine[12] and thrombin[13] DNA aptamers with an 11-nt linker with a fluorescein modification at T32 (**AFT**) (Fig. 1). Two shorter DNAs that are partially complementary to the aptamers were modified at their 5′ or 3′ end with a fluorescence quencher (**QDNA1**, **QDNA2**). In the absense of the ligands, the ternary complex **AFT**–**QDNA1**–**QDNA2** exhibits attenuated fluorescence due to the proximity of the two qencher moieties to the fluorophore. Binding of either adenosine or thrombin to the respective aptamer releases a quencher-modified DNA, but the remaining quencher strand keeps the fluorescence at low level. Addition of both ligands displaces both **QDNA1** and **QDNA2**, resulting in enhanced fluorescence (Fig. 2A, left).

An analogous response was also observed when two ssDNAs complementary to the aptamer sequences (**ADNA** and **TDNA**) were used as inputs (Fig. 2A, right). The relative fluorescence enhancement of the ON state was greater when ssDNAs were used as inputs rather than the aptamer ligands, probably due to the difference in the local environment surrounding the fluorophore.

The OR logic gate was designed by a partial modification of the AND gate. The fluorophore was removed from **AFT** to produce **AT**. The 5′ quencher of **QDNA1** was replaced with fluorescein to construct **FDNA**. As shown in Fig. 1A, **AT**–**FDNA**–**QDNA2** complex was designed to dissociate the fluorophore and the quencher when either ligand is present. The response of the logic gate to adenosine and thrombin supports the design strategy (Fig. 2B, left). A similar logic gate response was observed when **ADNA** and **TDNA** were used as inputs (Fig. 2B, right).

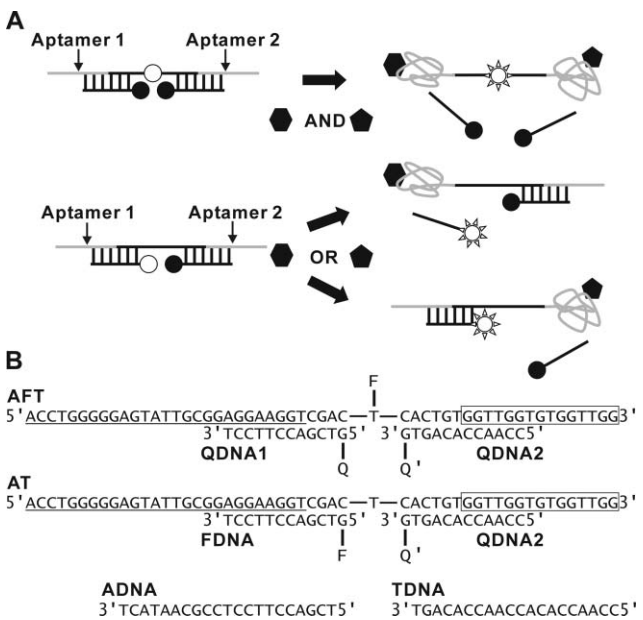

Fig. 1 DNA logic gate design strategy and sequences used. (A) Schematic representation of the aptameric AND and OR logic gates. Open circles depict the fluorophore and filled circles represent quncher moieties. (B) DNA sequences used in this study. The adenosine aptamer is underlined and the thrombin aptamer is boxed. F denotes fluorescein modification at C5 of the thymine (**AFT**) or 5′ terminus (**FDNA**) *via* a six-carbon spacer, Q represents Iowa Black FQ (IDT) quencher attachment at 5′ (**QDNA1**), and Q′ indicates a 3′ Dabcyl quencher modification (**QDNA2**). All oligodeoxynucleotides were purchased from IDT. See Supplementary Information for structural details†.

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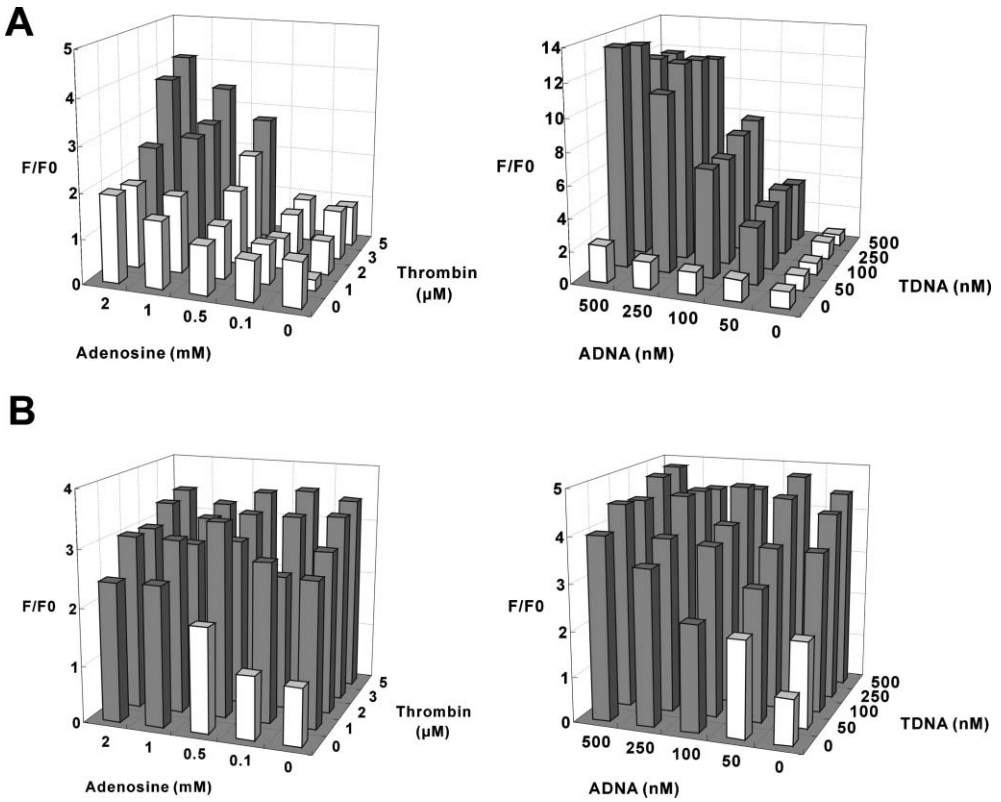

Fig. 2 Logic gate characteristics for aptamer ligands (adenosine and thrombin) and ssDNA (**ADNA** and **TDNA**) as inputs. Final composition for AND logic gate (A): **AFT** (20 nM), **QDNA1** (60 nM) and **QDNA2** (60 nM) in TKM buffer (20 mM Tris-HCl, 5 mM KCl, 0.9 mM $MgCl_2$, pH 8.3) with appropriate input molecules; OR logic gate (B): **AT** (60 nM), **FDNA** (20 nM), **QDNA2** (80 nM) in TKM buffer with appropriate input molecules. The measured fluorescence intensities (F) are normalized to the values obtained in the absence of input molecules (F_0). An universal threshold of $F/F_0 = 2.3$ was chosen to define ON ($F/F_0 > 2.3$, dark bars) and OFF ($F/F_0 < 2.3$, light bars) states of the logic gates. Detailed experimental procedures are noted in the Supplementary Information†.

The first molecular logic gate that exploited aptamers to sense its input molecules was developed by Jose *et al.* who coupled RNA aptamers for flavin mononucleotide (FMN) and theophylline to the activity of a self-cleaving hammerhead ribozyme which essentially functioned as an irreversible AND gate.[3] However, coupling of multiple aptamer–ligand interactions with ribozyme activity is a complex task involving both combinatorial and rational optimizations.

The modular aptameric sensors described by Stojanovic and Kolpashchikov may also be classified as AND gates based on aptamers in which the binding of one ligand to an aptamer is contingent upon binding of another ligand to the fused aptameric module.[8] However, optical response of the sensors depends on the malachite green aptamer, which limits their application as molecular logic gates.

Structure-switching signaling aptamers exploit the equilibrium shift of hybridization between an aptamer and its partially-complementary oligonucleotide in the presence or absence of the ligand. The strategy does not rely on specifc aptamer structures, is reversible, and the kinetic response can be optimized to suit experimental conditions. The logic gates presented here retain the advantages of structure-switching signaling aptamers and enable Boolean logic computation with a fluorescence output. Very recently, Liu and Lu reported AND and OR logic gates that yield colorimetric output based on gold nanoparticle assembly controlled by the signaling aptamers.[9] Combined with our logic gates presented here, these systems show a new direction for exploiting the versatile molecular recognition capabilities of nucleic acids for complex molecular computation.

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