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ORIGINAL ARTICLE

DNA-based advanced logic circuits for nonarithmetic information processing

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DNA has been used as a building block to construct a series of complex logic circuits to perform nonarithmetic functions, including a multiplexer, demultiplexer, encoder and decoder. This is the first time that DNA has been used to cover this broad range of logic circuits, demonstrating the versatility and power of DNA computation to process information on the molecular level. The above logic circuits all share the same DNA platform, indicating the great prospects for DNA computation. The enzyme-free system developed here provides a novel prototype for the design of DNA-based, high-level molecular logic circuits. Considering its biocompatibility, DNA computation could find potential applications in biological and biomedical fields in the not-too-distant future.

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INTRODUCTION

DNA computation belongs to the category of molecular computing, which is a fascinating research area that studies processing information on the molecular level.[1–4] DNA is the most powerful medium to process information *in vivo*, remaining one of the most mysterious parts of life. Acting as an outstanding engineering material, DNA is endowed with amazing advantages by virtue of its easy synthesis, structural simplicity, high flexibility, sequence design space sufficiency, molecular behavior predictability and so on.[5–7] Although various fundamental DNA-based logic gates have been reported,[8] the central question in their further development is how to construct complex logic circuits for a desired purpose. The highly specific hybridization of DNA directs the implementation of reconfigurable and superposed logic functions in unimolecular systems, favoring construction of logic circuits with higher complexity for information processing.[9,10] Despite the novelty and great prospects of DNA computation, it is still a great challenge to construct complex systems with superior processing capability.

Here, for the first time, we use DNA to construct a series of advanced logic circuits to execute nonarithmetic functions, including a multiplexer (MUX), demultiplexer (DEMUX), encoder (EC) and decoder (DC). The developed complex logic circuits presented here all share the same DNA-based platform and a constant threshold value, making DNA computation a closer step. Another distinct advantage of DNA computation here lies in its massive parallelism of computation and elimination of the use of enzymes.[11,12] In addition to futuristic speculations to construct molecular computers, the biocompatibility here provides additional value in terms of potential applications in biomedical sensing, genetic engineering, intelligent diagnosis and on-demand drug delivery,[2,13–19] in comparison with previously related studies based on complex organic molecules or expensive metal complexes.[20–24]

EXPERIMENTAL PROCEDURE

Materials

All chemicals used were of analytical grade and were used without further purification. The synthesized oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and Takara Biotechnology Co., Ltd. (Dalian, China). The water used throughout all experiments was purified through a Millipore system. The stock solution of *N*-methyl mesoporphyrin IX (NMM, 1 mM) was prepared in dimethyl sulfoxide and stored in darkness at − 20 °C. The stock DNA solution was prepared with 25 mM Tris-HCl buffer (pH 7.0). DNA concentration was estimated by measuring the absorbance at 260 nm. The desired concentration of NMM or DNA was achieved by diluting the corresponding stock solution with 10 mM Tris-HCl buffer containing 5 mM $MgCl_2$ and 15 mM KCl (pH: 8.0), respectively. Each DNA concentration was 200 nM, and NMM was fixed at 400 nM. The final volume of each sample for fluorescence measurement was 500 μl.

Instruments

A Varian Cary 500 Scan UV/Vis Spectrophotometer (Palo Alto, CA, USA) was used to quantify the oligonucleotides. Fluorescence intensities were recorded on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon, Longjumeau, France).

DNA sequence design

Base mismatch has been applied in the input sequence design to increase the asymmetry and decrease the secondary structure as well as undesired crosstalk. On application of base mismatch, it is feasible to clearly control the

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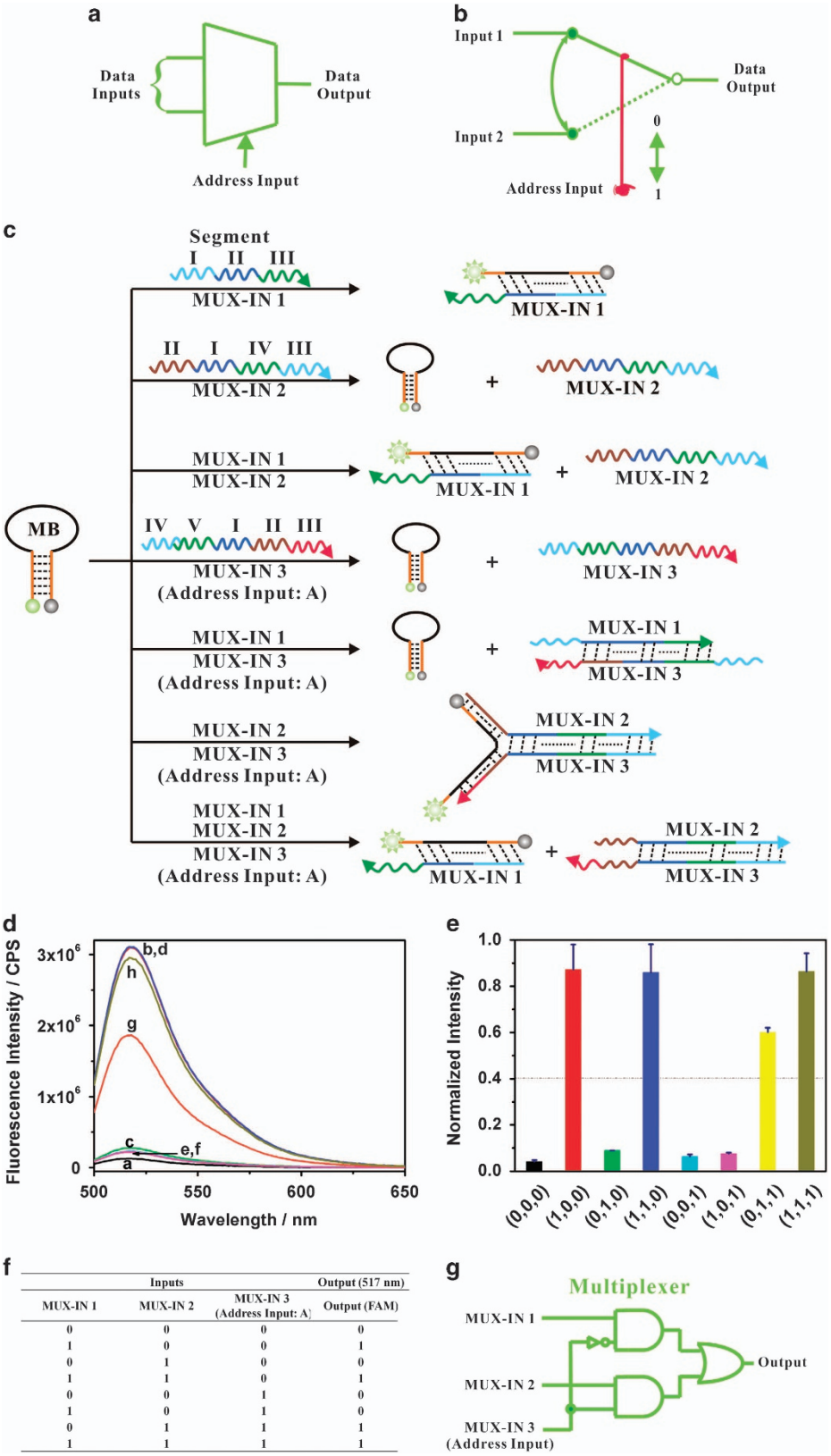


Inputs			Output (517 nm)
MUX-IN 1	MUX-IN 2	MUX-IN 3 (Address Input: A)	Output (FAM)
0	0	0	0
1	0	0	1
0	1	0	0
1	1	0	1
0	0	1	0
1	0	1	0
0	1	1	1
1	1	1	1

Figure 1 (**a**) Schematics of the 2:1 multiplexer and (**b**) its equivalent switching device. (**c**) Schematic illustration of molecular scale implementation of a 2:1 multiplexer based on DNA hybridization. (**d**) Fluorescence emission spectra of FAM under different conditions: (a) MB; (b) MB+MUX-IN 1; (c) MB+MUX-IN 2; (d) MB+MUX-IN 1+MUX-IN 2; (e) MB+MUX-IN 3 (Address input: A); (f) MB+MUX-IN 1+MUX-IN 3 (Address input: A); (g) MB+MUX-IN 2+MUX-IN 3 (address input: A); (h) MB+MUX-IN 1+MUX-IN 2+MUX-IN 3 (address input: A). (**e**) The normalized fluorescence intensity of FAM outputs. (**f**) Truth table for the 2:1 multiplexer operation. (**g**) Symbol of combinational logic network corresponding to the 2:1 multiplexer.

hybridization energy in the limited sequence design, where the concern of high symmetry exists, and drive the desired hybridization forward according to the difference in hybridization energy between duplexes. The hybridization energy can be simply described as the Gibbs free energy in directing the reaction to the formation of much more stable products. The change of free energies as well as reaction tendency were estimated with the use of the NUPACK program,[25] where coaxial stacking is not taken into consideration because of its complexity.

Spectrofluorimetry studies

To monitor 6-carboxyfluorescein (FAM) fluorescence, excitation was set at 485 nm, and the emission was monitored at 517 nm. Slit widths for the excitation and emission in recording FAM spectra were set at 3 and 5 nm, respectively. For NMM fluorescence, excitation was set at 399 nm, and the emission was monitored at 608 nm. Slit widths for both the excitation and emission in NMM fluorescence recording were set at 5 nm. To record hexachlorofluorescein (HEX) fluorescence, excitation was set at 535 nm, and the emission was monitored at 555 nm. Slit widths for both the excitation and emission to monitor HEX fluorescence were set at 3 nm. The pyrene-based excimer fluorescence was obtained by excitation at 340 nm and emission was monitored at 488 nm. Slit widths for both the excitation and emission in the excimer fluorescence scans were set at 5 nm. The error bar represents the s.d. of three independent experiments.

Native polyacrylamide gel electrophoresis

Polyacrylamide gel (12%) was prepared with 1× Tris-borate-EDTA buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). Each sample was prepared with 1× Tris-borate-EDTA buffer containing 12 mM Mg^{2+}, and the concentration of each DNA strand was 2 μM. Each sample was subjected to heating at 95 °C for 5 min and then annealed slowly from 95 °C to ~25 °C. A total of 20 μl of each sample was mixed with 2 μl of Gel-Dye Super Buffer Mix before loading into the gel. The gel was run under a constant voltage of 100 V over a period of ~2.5 h. After that, the gel was immersed in 0.5 μg ml^{-1} ethidium bromide solution for ~1 h and then washed with water twice. Photographs were taken under UV light using a fluorescence imaging system (Vilber Lourmat, Marne laVallee, France).

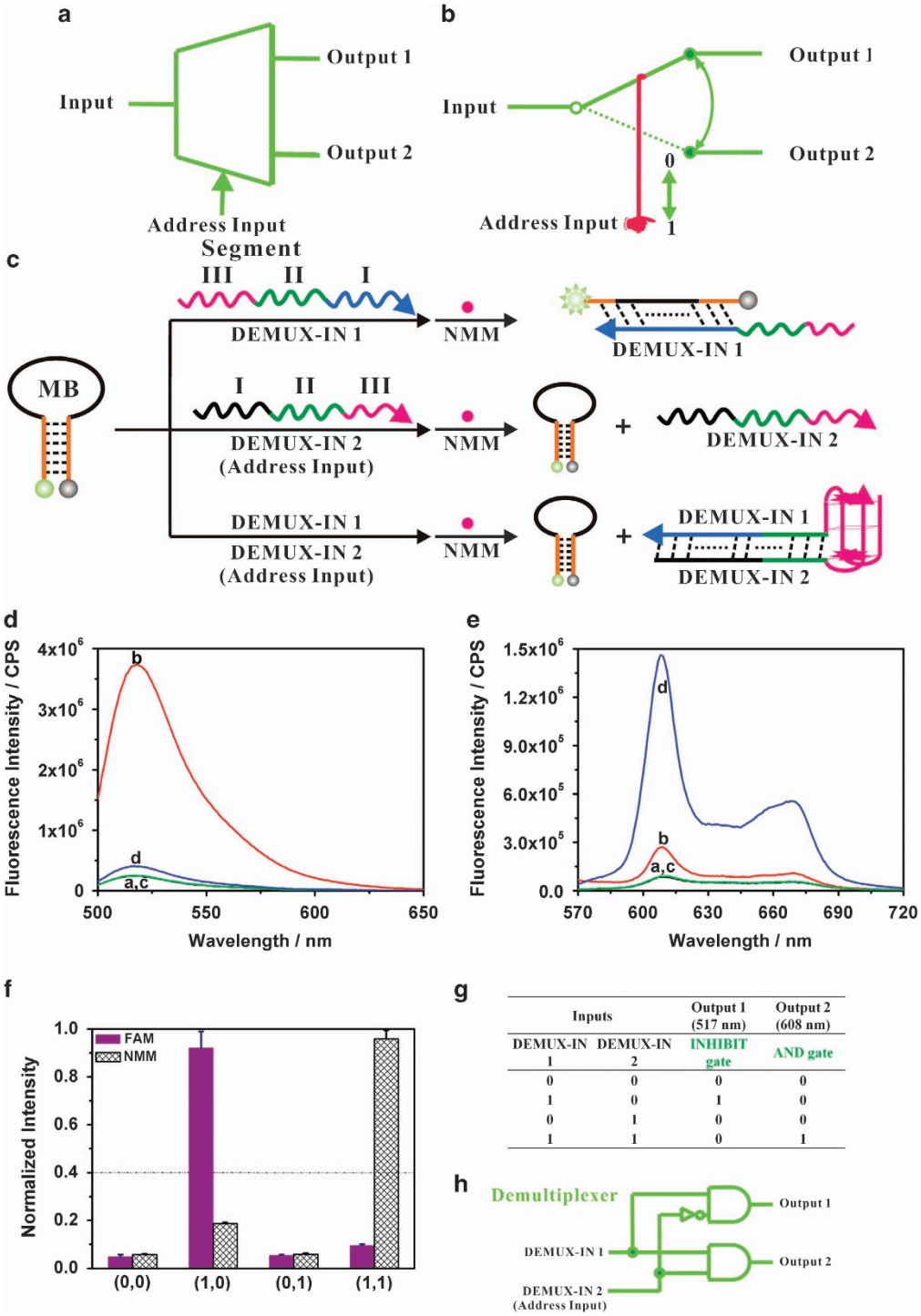
RESULTS AND DISCUSSION

As an important communication device, a digital MUX can select one binary input and then forward it into a single output channel, acting as a mechanical rotary switch.[26] A 2:1 MUX circuit involves two data inputs, one address input and one output (Figure 1a). The selection depends on the binary state of the address input, which switches between the two input channels and forwards the selection to the output channel. As shown in its equivalent switching circuit (Figure 1b), Input 1 is directed to the Output channel regardless of its binary state when the address input appears as 0. If the address input is 1, the directed input to output is taken from Input 2, also regardless of its logic value.

Here, a 2:1 MUX is designed and performed based on DNA hybridization (Figure 1c). A molecular beacon (MB) labeled with FAM and Dabcyl at each terminus is designed as the original platform, which produces weak or strong FAM fluorescence depending on its closed or open state,[27,28] serving as the signal output. FAM fluorescence is set as the only readout signal. The designed three DNA strands are defined as inputs: MUX-IN 1, MUX-IN 2 and MUX-IN 3 (Address input: A) (note that all associated DNA sequences throughout the manuscript are available in the supporting information (SI)). To mediate the complicated interactions among the three inputs and the MB platform, a two-base mismatch strategy was used to level the hybridization energy and decrease the secondary structure of DNA input, directing the desired hybridization in complex systems. In brief, two-base mismatch was embedded in the hybridization segment, facilitating subsequent interaction upon additional inputs through complementary hybridization. The strategy, sequence design, discussion on fluorescence change on different input combinations (Figure 1d) and polyacrylamide gel electrophoresis (PAGE) analysis (Supplementary Figure S4) are available in the SI. According to the fluorescence responses of FAM, a 2:1 MUX is achieved as proof-of-principle. Figure 1e shows the corresponding normalized intensity. The presence and absence of each input is defined as input '1' and '0,' respectively. The output is defined as '1' or '0' when the fluorescence signal is above or under the threshold value 0.4, respectively. The definitions are available for all of the following logic operations. A truth table (Figure 1f) obtained according to the defined threshold value fully satisfies the requirements in a 2:1 MUX operation. In the case of the address input 0 (without addition of MUX-IN 3, A = 0), the output reports the state of data input MUX-IN 1. If the address input is 1 (MUX-IN 3 added, A = 1), the output mirrors the state of data input MUX-IN 2. Its corresponding logic circuit (Figure 1g) demonstrates that one output signal is generated through the operation of two AND logic gates in parallel, and the address input has the opposite effect on them by being inverted in one of the AND gates.

In principle, a MUX encodes multiple data streams into a single data line for transmission. Conversely, a DEMUX is able to detangle data streams from a compressed single signal (Figure 2a). Figure 2b shows the equivalent switching circuit of a 1:2 DEMUX, which requires one data input signal and an additional address input to select either output channel to process the input. To date, only several studies associated with a molecular DEMUX have been reported,[11,21,22,29–31] most of which are still based on comprehensive synthetic organic molecules. It is still a great challenge to fabricate a biocompatible DEMUX considering its fundamental and practical significance not only in DNA computing but also in the biomedical field.

Complementary to the above 2:1 MUX, herein, a DNA-based 1:2 DEMUX fabrication was subsequently pursued based on the same MB platform and newly designed inputs. As a 1:2 DEMUX requires two output signals, NMM was selected as another signal reporter, presenting enhanced fluorescence responses on formation of a G-4/NMM complex (G-4: G-quadruplex).[32] FAM and NMM fluorescence were used as Output 1 and Output 2 signals, respectively. The operation principle is illustrated in Figure 2c. DEMUX-IN 1 is defined as the single input, and DEMUX-IN 2 functions as the address input (A) to forward the single input into either channel Output 1 or Output 2. The FAM and NMM fluorescence results, on using different input combinations, are shown in Figures 2d and e, respectively. The sequence design, discussion on fluorescence change on different input combinations and polyacrylamide gel electrophoresis analysis (Supplementary Figure S5) are available in the SI. After normalizing the fluorescence intensity of FAM and NMM (Figure 2f), the correspondingly obtained truth table (Figure 2g) indicates that the single input line is reported to the related output, depending on the binary state of the address input (A). When the address input DEMUX-IN 2 is absent (A = 0), the binary state of the data input is transmitted to Output 1. Otherwise, the binary data input is transmitted to Output 2 (A = 1). The corresponding logic circuit (Figure 2h) is composed of INH and AND logic gates, which are operated in parallel based on the universal MB platform and the same set of inputs, simultaneously producing two different output signals. The selection of the output channel is determined by the logic value of the address input, being inverted at one of the gates.



Inputs		Output 1 (517 nm)	Output 2 (608 nm)
DEMUX-IN 1	DEMUX-IN 2	INHIBIT gate	AND gate
0	0	0	0
1	0	1	0
0	1	0	0
1	1	0	1

Figure 2 (**a**) Schematics of a 1:2 demultiplexer and (**b**) its equivalent switching device. (**c**) Operation scheme describing molecular scale implementation of a 1:2 demultiplexer based on DNA hybridization. (**d**) and (**e**) are the fluorescence emission spectra of FAM and NMM, respectively, under different conditions: (a) MB; (b) MB+DEMUX-IN 1; (c) MB+DEMUX-IN 2 (address input: A); (d) MB+DEMUX-IN 1+DEMUX-IN 2 (address input: A). (**f**) The normalized fluorescence intensity of FAM and NMM outputs. (**g**) Truth table for the 1:2 demultiplexer operation. (**h**) Symbol of combinational logic network corresponding to the 1:2 demultiplexer.

After successful construction of a MUX and DEMUX, fabrication of an EC and DC were further pursued. An EC is a digital device that compresses information for efficient transmission or storage by converting data into a code.[23,24] In principle, a single-bit 4-to-2 EC converts 4 input bits into 2 output bits (Figure 3a). Until now, only three systems have been reported to build a 4-to-2 EC on the molecular level, two of which were performed with comprehensive synthetic organic molecules or expensive metal complexes.[23,24,33]

Here, we demonstrate the construction of a molecular 4-to-2 EC via DNA hybridization-induced optical signals. The operation of the

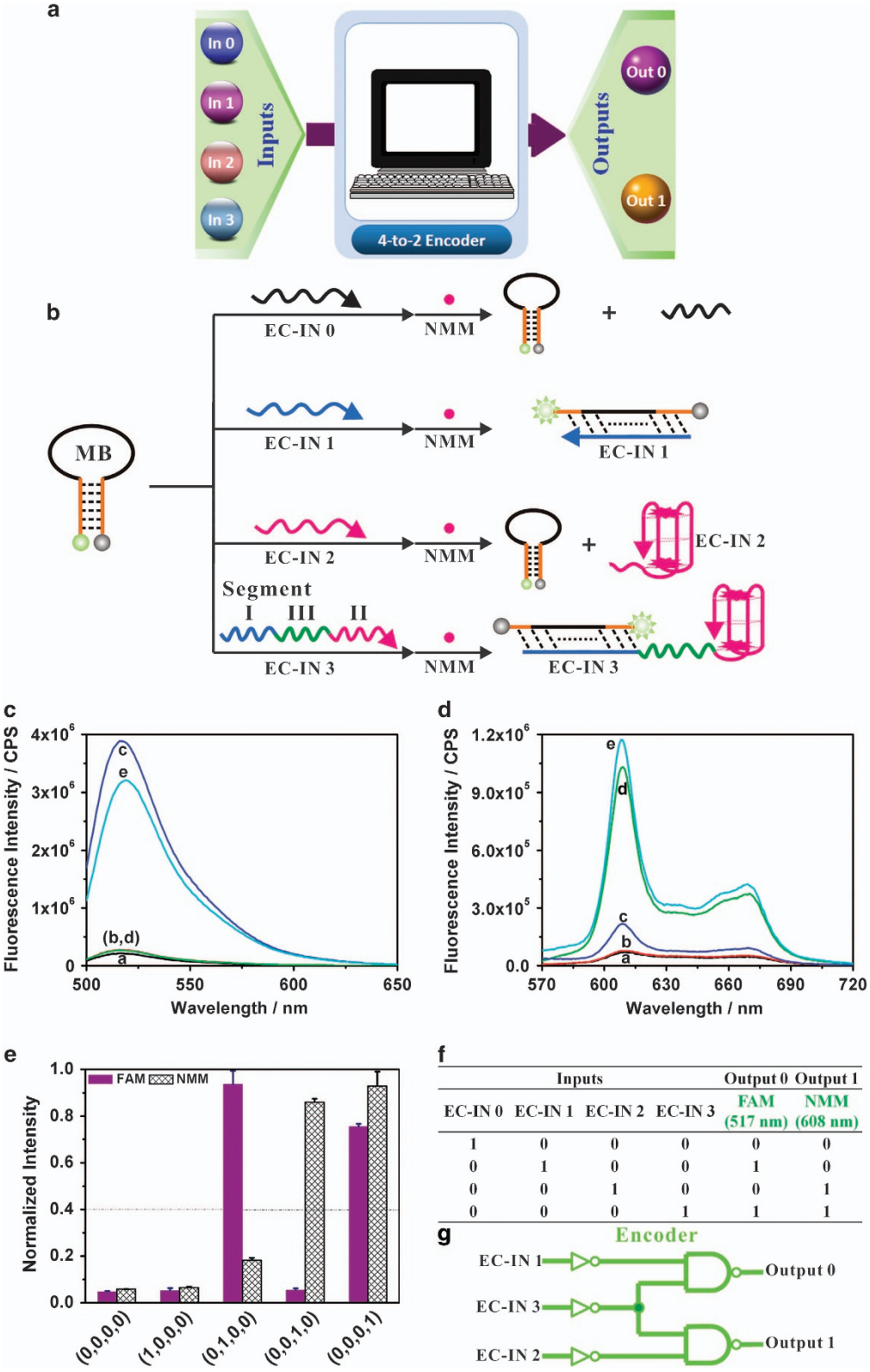
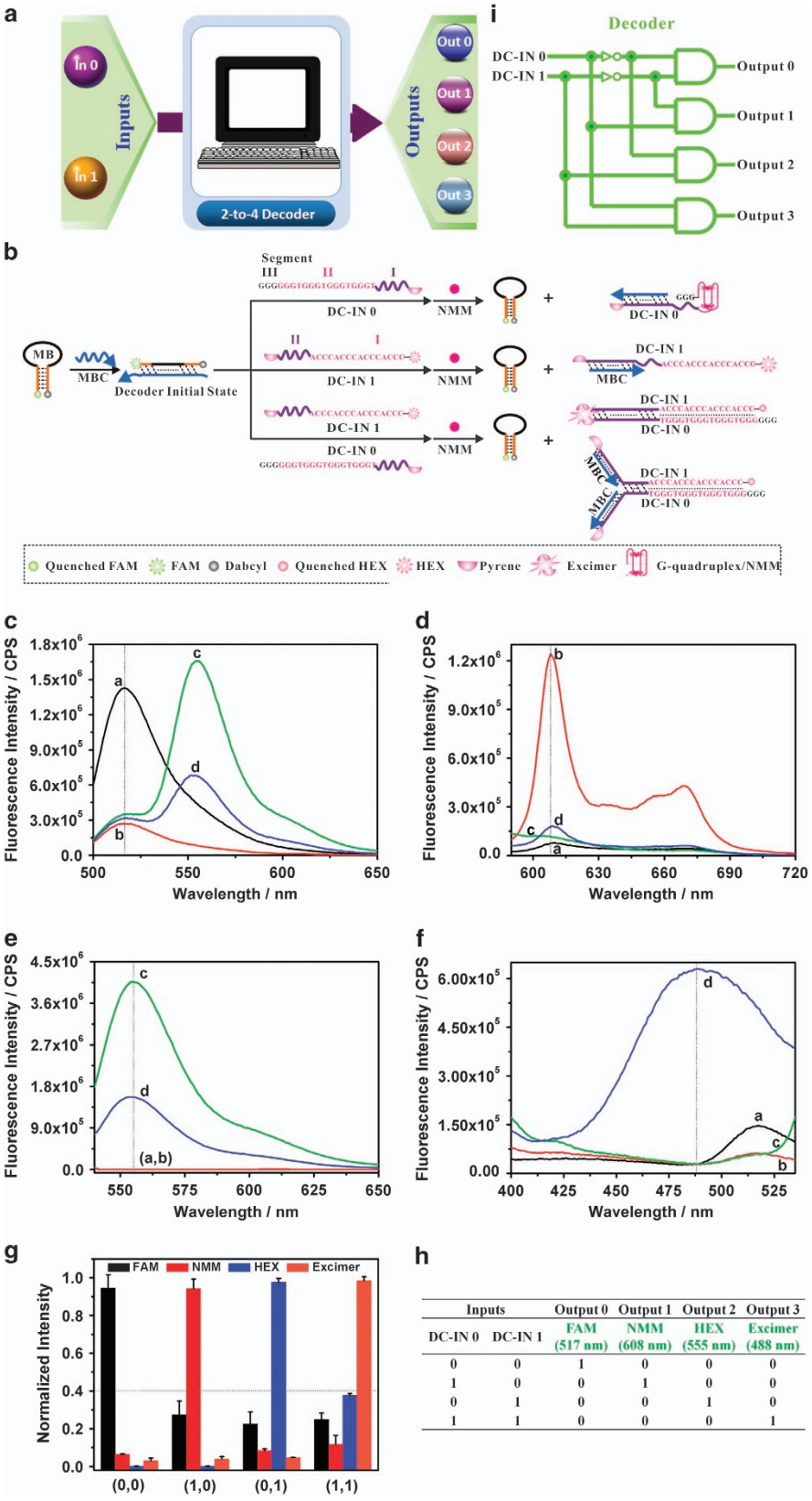


Figure 3 (**a**) Schematic representation of a 4-to-2 encoder. (**b**) Schematic illustration of molecular scale implementation of a 4-to-2 encoder based on DNA hybridization. (**c**) and (**d**) are the fluorescence emission spectra of FAM and NMM, respectively, under different conditions: (a) MB; (b) MB+EC-IN 0; (c) MB +EC-IN 1; (d) MB+EC-IN 2; (e) MB+EC-IN 3. (**e**) The normalized fluorescence intensity of FAM and NMM outputs. (**f**) Truth table for the 4-to-2 encoder. (**g**) Symbol presentation of the electronic equivalent circuitry corresponding to the 4-to-2 encoder.



Inputs		Output 0	Output 1	Output 2	Output 3
DC-IN 0	DC-IN 1	FAM (517 nm)	NMM (608 nm)	HEX (555 nm)	Excimer (488 nm)
0	0	1	0	0	0
1	0	0	1	0	0
0	1	0	0	1	0
1	1	0	0	0	1

Figure 4 (**a**) Schematic representation of a 2-to-4 decoder. (**b**) Schematic illustration of molecular scale implementation of a 2-to-4 decoder based on DNA hybridization. Fluorescence emission spectra of (**c**) FAM, (**d**) NMM, (**e**) HEX and (**f**) Excimer under different conditions: (a) MB-MBC; (b) MB-MBC+DC-IN 0; (c) MB-MBC+DC-IN 1; (d) MB-MBC+DC-IN 0+DC-IN 1. (**g**) The normalized fluorescence intensity of FAM, NMM, HEX and Excimer outputs. (**h**) Truth table for the 2-to-4 decoder operation. (**i**) Symbol presentation of the electronic equivalent circuitry corresponding to the 2-to-4 decoder.

developed 4-to-2 EC is still based on the same MB platform in combination with four new inputs (EC-IN 0, EC-IN 1, EC-IN 2, EC-IN 3), as shown in Figure 3b. The DNA hybridization-induced fluorescent FAM and NMM signals are defined as Output 0 and Output 1, respectively. The FAM and NMM fluorescence results on different input combinations are shown in Figures 3c and d, respectively. The discussion on fluorescence change on different input combinations and polyacrylamide gel electrophoresis analysis (Supplementary Figure S6) are available in the SI. After normalizing the fluorescence intensity (Figure 3e), we can obtain a truth table according to the pre-defined threshold value (Figure 3f), fully satisfying the requirements for a 4-to-2 EC operation. The logic circuit corresponding to a 4-to-2 EC is shown in Figure 3g, where input EC-IN 0 is a hidden input and equal to that without any input. The experimental results confirm the successful fabrication of a 4-to-2 EC as proof-of-principle.

Encoding and decoding are utilized in data communication, networking and storage, and both are particularly applicable to wireless communication systems. Complementary to a 4-to-2 EC, a 2-to-4 DC performs the opposite process, converting the compressed data back into the original format.[23,24] To date, only two 2-to-4 DCs have been reported based on either comprehensive synthetic organic molecules or expensive metal complexes.[23,24] Boosted by the importance of this logic device, the construction of a biomolecular system to mimic a 2-to-4 DC is highly desirable because it has potential applications in sensing and labeling as well as information manipulation.

Here, for the first time, a DNA-based molecular 2-to-4 DC was further developed based on the same MB platform, converting the two new-designed inputs (DC-IN 0 and DC-IN 1) to four readable outputs (Figure 4a). The characteristic of this two-input DC system lies in that each input combination is endowed with a unique strong fluorescent signal while the others keep mute. Considering the four resultant signals, it is a critical issue to select dye molecules that attach to DNA strands and can generate different readout signals in the emission-only mode under different conditions. To eliminate spectra overlap and secondary excitation, four different fluorescent groups are selected, including FAM, NMM, HEX and a pyrene-based excimer[34] with excitation/emission at 485/517 nm, 399/608 nm, 535/555 nm and 340/488 nm, respectively. The fluorescence of FAM, NMM, HEX and the excimer is defined as Output 0, Output 1, Output 2 and Output 3, respectively. For this purpose, a pyrene is labeled at the 5′ terminal of DC-IN 0. In addition, a HEX and a pyrene are labeled at the 5′ and 3′ terminals of DC-IN 1, respectively. To achieve the unique property of the 2-to-4 DC output signals, the fluorescence-quenching ability of G-base[35] is introduced into the fabrication of the 2-to-4 DC. The scheme of the present 2-to-4 DC operation and associated signal generation is shown in Figure 4b.

To set up the initial state in the implementation of the DC function, an assistant DNA strand (MBC) is used to hybridize with the MB, accompanied by strong FAM fluorescence (Supplementary Figure S7). The two-base mismatch strategy as mentioned above was also utilized in the sequence design to level the hybridization energy and direct the reaction tendency, as indicated in the detailed hybridization between the MB and MBC (Supplementary Figure S8). The FAM, NMM, Hex and excimer fluorescence results, on different input combinations, are shown in Figures 4c–f in turn. The discussion on fluorescence changes on different input combinations, and polyacrylamide gel electrophoresis analysis (Supplementary Figure S10) are available in the SI. To conveniently analyze the results, the fluorescence intensities of the four dye molecules in different output channels are normalized (Figure 4g). The obtained truth table fulfills the requirement for a 2-to-4 DC operation (Figure 4h). Thus, a DNA-based molecular 2-to-4 DC system was successfully constructed as proof-of-principle. As shown in its logic circuit (Figure 4i), a 2-to-4 DC integrates one NOR gate, two complementary INHIBIT gates and one AND gate,[36] which all share the same inputs and initial MB platform. The developed DC presents two distinct advantages. Firstly, only fluorescence is utilized as the single signal generation mode, bringing convenience to the operation. In previous studies, the developed DC usually involved several types of optical signals as readouts, such as light absorption, emission or transmission,[21,23,24] making the operation complicated and limiting its further development. Secondly, the utilization of a DNA biomaterial here increases the biocompatibility for potential applications in the biomedical field because each state of a 2-to-4 DC corresponds to a combination, determining whether both, either or neither of the analytes considered as inputs exists in an organism or other complex system.[23]

CONCLUSION

In summary, for the first time, a series of DNA-based comprehensive logic circuits have been achieved with the same MB platform to perform nonarithmetic information processing, including MUX, DEMUX, EC and DC functions. The constructed advanced logic circuits involve multiple inputs and outputs. In general, MUXs and ECs are used to compress data for transmission, whereas DEMUXs and DCs have the opposite functions, separating compressed data to their original form. Multiple outputs can be generated in parallel, stimulated by the same set of inputs. It is a great challenge to control the desired hybridization among various DNA strands, where high symmetry in sequence design exists. To overcome this obstacle, the suggested strategy of base mismatch has been harnessed to embed mismatched bases in appropriate positions.

The system described here has distinct advantages. (1) All of the logic circuits share the same MB platform as well as the same threshold setpoint, demonstrating the power and great perspective of DNA computation. (2) All signal outputs are set in one mode, fluorescence emission, enabling easy remote reading with low cost. This system also provides the possibility for logic implementations on several molecules or even a single molecule owing to the high sensitivity of fluorescence. (3) The developed enzyme-free system here provides a novel prototype for the design of DNA-based, high-level molecular logic circuits, which may greatly promote the development of DNA computation owing to its diverse advantages, including high flexibility, reconfigurability, massive parallelism and scalability. However, there are still some shortcomings in its further development for much more complex computation, including how to assemble these basic molecular devices, how to simplify the complex switches of output channels in fluorescence excitation, how to further increase the signal difference as required, and how to reset them in a simple way. If combined within a microfluidic chip, for example, the DNA-based platform can implement an enormous number of computations simultaneously, greatly enhancing the processing ability and providing a possible way to achieve increased computational complexity. Possibly, quantum dots might be used as signal reporters to simplify signal recording owing to their tunable emissions. Toe-hold mediated DNA strand displacement might favor sequence design to increase the difference between signal and background noise. By immobilizing an MB on the surface of magnetic beads, it is feasible to recover the MB platform, and a reset can be realized for the next logic performance. Despite these existing shortcomings, DNA computation

still could find potential applications in biological and biomedical fields in the not-too-distant future owing to its biocompatibility.[12,15,37]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the NPG Asia Materials website (http://www.nature.com/am)