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Design of enzyme-interfaced DNA logic operations (AND, OR and INHIBIT) with an assaying application for single-base mismatch†

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We devised AND, OR and INHIBIT logic gates. They were based on the enzyme-induced DNA base flipping mechanism, which caused significant local conformation changes in DNA. This can be monitored *via* photonic signals as outputs. We also provided a prototype of a built-in biosensor capable of distinguishing single-base mismatches in a DNA duplex.

The Boolean circuit revolutionised the way human beings maneuver logic questions. Modern man-made automatic machines heavily rely on this paradigm, as evidenced by an array of digital data storage devices, computational operations, information processing and digital components. Boolean circuits can be defined in terms of logic gates, which use simplified inputs of 'true' (1, high value) or 'false' (0, low value).[1] These inputs are correspondingly converted to Boolean outputs. Over recent decades, bio-molecular-level computations have been performed with macromolecules in solutions,[2] on functionalised surfaces[3] and even within living organisms,[4] which highlight themselves as alternatives to conventional computations. These mimics have extended the reach of computation and promise to be beneficial. Logic gates have been re-constructed in a number of systems such as DNA,[5] DNA-conjugates,[6] peptides,[7] small[8] or supramolecular molecules,[9] and enzymatic,[10] mammalian[11] and bacterial networks.[12] Amongst those, the DNA-based systems have been exhaustively explored. Despite the ubiquity of DNA–enzyme interactions found in all kingdoms of life, they are as such rarities for producing logic gates.[13]

Here we reported the progress toward the goal to create OR, AND and INHIBIT logic gates using the EcoP15I–DNA interaction. EcoP15I, a member of type III restriction–modification (R–M) enzyme of *Escherichia coli*, is heterotrimeric and composed of enzyme Res_1Mod_2 (Res: restriction subunit; Mod: modification subunit). It has a 5′-CAGCAG-3′ recognition site and possesses both restriction and modification functions subject to conditions. When acting as a methyltransferase, it methylates the second adenine of 5′-CAGCAG-3′ in its double-stranded DNA (dsDNA) substrate.[14] We have previously shown that EcoP15I uses a base flipping mechanism to accomplish this biochemical modification.[15] Base flipping was first identified in the crystal structure of HhaI methyltransferase in complex with its substrate DNA (Fig. 1A).[16] It is a striking example of how enzymes can manipulate DNA in which the DNA base subject to modification is rotated outside from the axis of the DNA double helix to reach a flipped-out state towards the enzyme active site. As such, the highly stacked base conformation is significantly abolished. In aqueous solution, researchers used 2-aminopurine (2AP) to substitute for the based-flipped nucleotide in order to probe this phenomenon. 2AP (Fig. 1B) is an isomer to naturally occurring adenine (6-aminopurine, Fig. 1C), which is intrinsically fluorescent and able to give a high quantum yield in solution, as much as 0.68. It has an emission maximum at ~370 nm. More importantly, its excitation maximum is at ~303 nm, lies to the red absorption of natural DNA bases and proteins, which allows it to be excited selectively in DNA or DNA–protein complexes.[17] 2AP is an environment-sensitive fluorophore and highly quenched when placed within DNA. When 2AP nucleobase is incorporated in dsDNA, a most highly stacked structure can be maintained,

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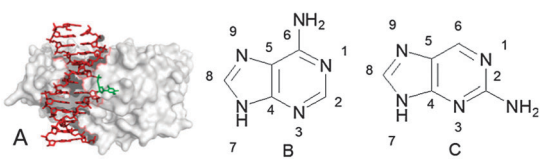

Fig. 1 (A) Is the crystal structure that illustrates base flipping. HhaI methyltransferase is in a complex with its substrate dsDNA. The chemical structures of adenine (B) and 2AP (C), respectively.

which is favourable for both intra- and inter-strand charge transfer. Therefore, its fluorescence intensity is most repressed. In the case when 2AP is flipped outside of the dsDNA to reach an exhelical state, it experiences significantly alleviated quenching and thereby displays up to several hundred-fold increases in emission. Commonly, with respect to the fluorescence intensity in the same DNA sequence contexts, base-flipped 2AP > 2AP in corresponding ssDNA > 2AP in corresponding dsDNA. This can be well recorded by both steady-state and time-resolved fluorometers.[18]

In these binary arithmetic operations, the fluorescence changes of 2AP upon enzyme binding generated readable photonic signals as outputs. The outputs of the gate were measured at 370 nm fluorescence upon excitation at 315 nm. For all three logic gates of interest, the two-digit inputs were listed in the bracket; for instance (0,1), '0' meant there was no DNA sequence as input 1, whereas '1' indicated DNA of interest was added into the system as input 2. For the outputs, '0' was defined as low fluorescence, whereas '1' stood for high fluorescence. All DNA sequences used in this study are listed in Table S1 (ESI†).

Experiments were performed by testing the AND gate (Fig. 2). When there was no input DNA, defined as (0,0), there was no detectable fluorescence output, which resulted in output '0'. For (1,0), the ANDin 1 (AND gate input 1) was a 2AP-labeled ssDNA, which missed its complementary strand. This input was unable to trigger base flipping even in the presence of EcoP15I, thus showing a mild fluorescence as registered as '0' output. For input (0,1), the ANDin 2 was an unmodified ssDNA, which was devoid of its complementary strand ANDin 1 to form a target DNA duplex. This gave a '0' output. For (1,1), the cognate dsDNA with 2AP modification was recognised by EcoP15I, which subsequently incurred base flipping. The flipped 2AP base displayed a markedly enhanced fluorescence as output '1'.

In contrast to the AND gate, an OR gate (Fig. 3) is activated in the presence of either of the two inputs but not both. For input (1,0), the ORin 1 (OR gate input 1) was the cognate DNA duplex possessing the recognition site of EcoP15I. In this duplex, the second adenine within 5′-CAGCAG-3′ site was substituted with 2AP. The emission at 370 nm was highly enhanced upon enzyme binding on the account of base flipping, and it produced output '1'.

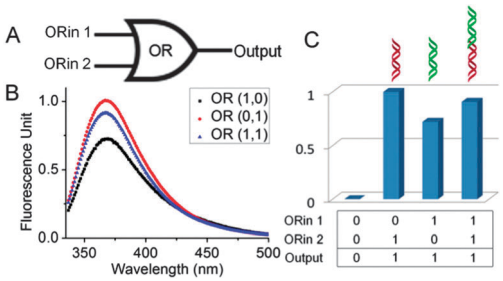

Fig. 3 The fluorescence readouts of OR logic gate. (A) The electrical symbol for AND gate. (B) The fluorescence spectra of the logic gate. (C) The bar graph representation and truth table of the outputs of the OR logic gate. The red and green duplexes above the bar chart indicate the cognate 2AP-labeled oligonucleotides subject to base flipping.

The input (0,1) adopted another cognate DNA duplex ORin 2, and likewise OR (1,0), Ecop15I–DNA binary complex had a remarkably raised fluorescence. The input (1,1) was a mixture of ORin 1 and ORin 2 (1 : 1 equiv.); the measured fluorescence increased accordingly, which brought about output '1'.

We then tried to establish an INHIBIT gate (Fig. 4) simply, for INHIBIT (1,0), a DNA hairpin termed as INHin 1 (INHIBIT gate input 1) with cognate sequence was adopted. EcoP15I was able to bind to it and flipped the 2AP base, thereby a '1' output was obtained. INHIBIT (0,1) gave a '0' output since it used a non-labeled INHin 2, which was the complementary strand to INHin 1 apart from four mismatches introduced in the 5′-CAGCAG-3′ recognition site. The (1,1) combination was mixed with both inputs (1 : 1 equiv.), the favourable complementarity led to unfolding of the stem–loop structure, and a putative duplex structure was eventually obtained. Because the perfectly matched recognition site has been removed, the base flipping could not take place. Therefore, the highly quenched 2AP had a considerably decreased fluorescence. The base flipping was only triggered in the presence of EcoP15I and dsDNA substrates with an accurate recognition site. It is notable that a minimum eight-fold dynamic range in fluorescence intensities was observed in order to finely distinguish the 'high' and 'low' Boolean outputs for both the AND and INHIBIT gates.

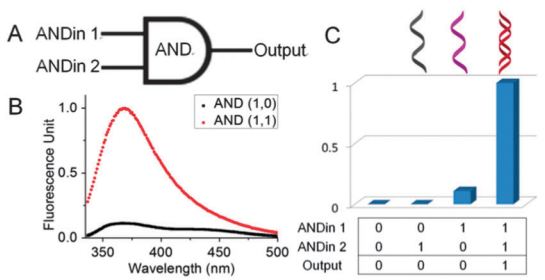

Fig. 2 The fluorescence readouts of an AND logic gate. (A) The electrical symbol for AND gate. (B) The fluorescence spectra of the AND logic gate. (C) The bar graph representation and truth table of the outputs of the AND logic. The oligonucleotides above each column represent the DNA input for each logic gate. The black and purple ones indicate the unmodified and 2AP-modified DNA, respectively, while the red duplex indicates the cognate 2AP-labeled dsDNA subject to base flipping.

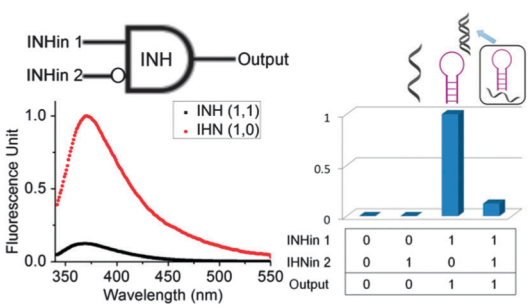

Fig. 4 The fluorescence readouts of INHIBIT gate. (A) The electrical symbol for INHIBIT gate. (B) The fluorescence spectra of the INHIBIT gate. (C) The bar graph representation and truth table of the outputs of the INHIBIT gate. The DNA hairpin above the bar chart indicates the cognate 2AP-labeled oligonucleotides subject to base flipping. The black ssDNA indicates the unmodified DNA and it has some complementarities to the DNA hairpin.

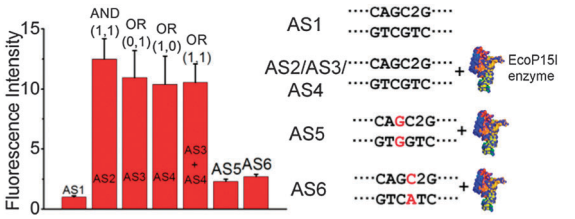

Fig. 5 The application of AND and OR logic gates in a complex biological system. All experiments were performed in triplicate and the error bars are given.

Single-base mismatch detection is a key process for monitoring genetic mutations, which is crucial for early diagnosis, clinical prognostics and disease prevention.[21] Next, we tested the applicability of these proposed logic gates for practical uses in such detection. The OR and AND logic gates were operated in samples (50 mM Tris-HCl, 20 mM NaCl, 20 mM $MgCl_2$, pH 7.8, containing BSA and four types of non-relevant oligonucleotides) to mimic a complex biological system (Fig. 5). AS1 denoted a base-paired duplex in the absence of EcoP15I. AS2 used the same sequence as AS1 but in the presence of EcoP15I. In the AND (1,1) logic gate, as shown in Fig. 5, the two fully complementary ssDNA served as two inputs, and they were able to form a duplex containing the recognition site, whose fluorescence enhanced as expected (output '1') on account of base flipping. The fluorescence for the other two duplexes (AS5 and AS6) with even a single-base mismatch difference compared with AS1 gave a '0' output for the reason that the strict sequence specificity of EcoP15I did not allow strong binding of DNA duplex and base flipping. For the OR logic gates, the fluorescence was high when using AS3 and/or AS4 spiked in samples as inputs (the 3rd to 5th columns in Fig. 5). The DNA sequences used are listed in Table S1 (ESI†), and the fluorescence emission spectra for Fig. 5 are displayed in Fig. S1 (ESI†).

The advantages afforded by enzyme–DNA interactions are their sequence or structure-dependent properties.[19] Benefitting from these types of interactions, we constructed an approach to build 2-input logic operations, including AND, OR and INHIBIT. This choice of logic gates was selected as it represented one example of a complete set of logic gates, indicating that they can be theoretically assembled into multi-level circuits.[20] Our results validated the application of enzyme–DNA interactions as platforms for the fabrication of addressable elementary arithmetic operations. The EcoP15I could be replaced by other nucleic acid-manipulating enzymes, which greatly expands the output possibilities. Moreover, enzyme-interfaced oligonucleotides have barely been incorporated into logic gate design. This may show a few advantages, including reducing background signals, ruling out possibilities of false positives and extending the tool kits for DNA computation.[22] Last, this simple system could be utilised for sensing nucleic acid molecules at a single base mismatch resolution, avoiding some demanding instruments (*e.g.*, MALDI-TOF mass spectrometry, Raman spectroscopy and cyclic voltammetry) and chemical labeling.[23]

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