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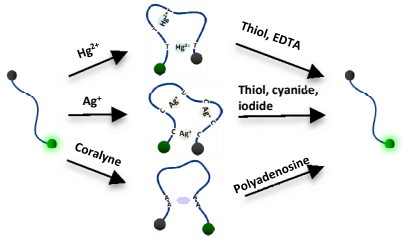
A single, resettable, and sensitive molecular beacon has been developed to operate two-input, three-input, and set-reset logic gates



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ARTICLE TYPE

Design of two and three input molecular logic gates using non-Watson-Crick base pairing-based molecular beacons

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This study presents a single, resettable, and sensitive molecular beacon (MB) used to operate molecular-scale logic gates. The MB consists of a random DNA sequence, a fluorophore at the 5′-end, and a quencher at the 3′-end. The presence of Hg^{2+}, Ag^{+}, and coralyne promoted the formation of stable T–Hg^{2+}–T, C–Ag^{+}–C, and A_2–coralyne–A_2 coordination in the MB probe, respectively, thereby driving its conformational change. The metal ion or small molecule-mediated coordination of mismatched DNA brought the fluorophore and quencher into close proximity, resulting in collisional quenching of fluorescence between two organic dyes. Because thiol can bind Hg^{2+} and remove it from the T–Hg^{2+}–T-based MB, adding thiol to a solution of the T–Hg^{2+}–T-based MB allowed the fluorophore and quencher to be widely separated. A similar phenomenon was observed when replacing Hg^{2+} with Ag^{+}. Because Ag^{+} strongly binds to iodide, cyanide, and cysteine, they were capable of removing Ag^{+} from the C–Ag^{+}–C-based MB, restoring the fluorescence of the MB. Moreover, the florescence of the A_2–coralyne–A_2-based MB could be switched on by adding polyadenosine. Using these analytes as inputs and the MB as a signal transducer, we successfully developed a series of two-input, three-input, and set-reset logic gates at the molecular level.

Introduction

Developing molecular beacon (MB) techniques for highly sensitive DNA detection is of great interest to many researchers, because MB techniques are simple, rapid, specific, and sensitive. An MB is a single-strand oligonucleotide that consists of a stem-loop structure, a reported fluorophore, and a non-fluorescent quencher or another fluorophore at its 5′- and 3′-ends.[1] Watson-Crick hydrogen bonding forms the stem by joining the 5–7 nucleotides at the 5′-end, and joining the 5–7 complementing nucleotides at the 3′-end. The resulting stem reduces the fluorescence of the fluorophore through fluorescence resonance energy transfer (FRET), collisional quenching, and contact quenching between the two organic dyes.[2] When the central loop hybridizes with the complementary target DNA or RNA, the MB can change from a stem-loop to an open-chain form. This conformational change subsequently restores the fluorescence of the reported fluorophore. Recently, non-Watson-Crick base-pairing-based MBs have become alternative emerging sensors for detecting a wide variety of analytes, because the presence of an analyte forces the stem to close or open. Alternative MBs based on metal ions or small molecule-mediated base pairs in the stem, including thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) coordination,[3-5] cytosine–Ag^{+}–cytosine (C–Ag^{+}–C) binding,[6] adenosine$_2$–coralyne–adenosine$_2$ (A_2–coralyne–A_2) interaction,[7] and K^{+}–G-quadruplex complexes, have been developed.[8] Without requiring a precise temperature control, these types of MBs are capable of discriminating perfectly matched DNA from single-base mismatched DNA, at room temperature.[3-4, 6] The stem of an MB containing a T-rich sequence was used for fluorescent sensing of Hg^{2+} because Hg^{2+}-induces coordination of T–Hg^{2+}–T in the stem, resulting in energy transfer from the fluorophore to the quencher.[9] Because of the strong binding of thiol-containing molecules to Hg^{2+}, a T–Hg^{2+}–T-based MB was capable of detecting glutathione (GSH) and homocysteine through the thiol-induced removal of Hg^{2+} from the T–Hg^{2+}–T coordination.[10-11] When the stem containing a T-rich sequence was replaced with that containing a C-rich sequence, the selective detection of Ag^{+} was accomplished using a C–Ag^{+}–C-based MB.[12] An MB probe consisting of an adenosine analog-binding aptamer in the loop and a C-rich sequence in the stem was devised to recognize adenosine triphosphate.[13] Using the unique features of coralyne that interact with the A_2–A_2 mismatch,[14] an MB containing a stem of a pair of 12-mers served as a light-up probe for detecting heparin in plasma.[15]

Much effort has been dedicated to constructing molecular-scale logic chemical computing, which generates various Boolean logic systems based on host-guest supramolecular systems, enzymatic biochemical networks, and biopolymer-ligand interactions.[16-17] DNA molecules can be used as active components for logic gate functions,[18] and are more attractive compared with other molecules because they provide structural simplicity, strand-specific hybridization, and the ability to interact with specific molecules (e.g, metal ions, small molecules, and proteins). For example, a simple and universal MB probe was used to construct a complete set of two-input logic gates, using

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† Electronic Supplementary Information (ESI) available: Figure S1-S6 and Table S1-3. See DOI: 10.1039/b000000x/

single-stranded DNA as inputs and the change of fluorescence intensity as the output.[19] A series of DNA logic gates was devised based on the formation of T–Hg^{2+}–T and C–Ag^{+}–C mismatched based pairs, the specific interaction between aptamer and small molecules, and the ion-driven conformational change of the DNA G-quadruplex.[20-28] However, most DNA logic gates are unable to perform resettable and multiple logic operations, which are required for the future development of DNA circuits.

In this study, an MB containing 58 bases (5'-GTC TCT GTG TGC GCC AGA GAA CAC TGG GGC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3') of random DNA was modified using a reporter of carboxyfluorescein (FAM) at the 5′-end, and a quencher of 4-([4-(dimethylamino)phenyl]azo)-benzoic acid (DABCYL) at the 3′-end. **Fig. 1A** shows that The presence of Hg^{2+}, Ag^{+}, and coralyne induced a conformational change of the MB through the coordination of T–Hg^{2+}–T, C–Ag^{+}–C, and A_2–coralyne–A_2, resulting in collisional fluorescence quenching between FAM and DABCYL.[29] Because adding the input resulted in the removal of Hg^{2+}, Ag^{+}, or coralyne from the MB, the designed MB generated a set of two-input (OR, AND, INHIBIT, NOR, NAND, and REVERSE IMPLICATION) and three-input (OR, NOR, and AND) molecular logic gates. Moreover, this MB was capable of operating reversibly to perform the set-reset function.

EXPERIMENTAL

Chemicals and reagents

$Hg(ClO_4)_2$, $AgNO_3$, coralyne, hydrogen peroxide (30% v/v), cysteine, GSH, S-(5′-adenosyl)-L-homocysteine (SAH), S-(5′-adenosyl)-L-homocysteine hydrolase (SAHH; rabbit erythrocytes, 50,000units/L; MW 240,000), KI, NaCN, ethylenediaminetetraacetic acid (EDTA), NaH_2PO_4, and Na_2HPO_4 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Silver nanoparticles (AgNPs) with diameter of 20 nm were purchased from were purchased from Ted Pella Inc. (Redding, California). All DNA samples were synthesized from Neogene Biomedicals Corporation (Taipei, Taiwan). Water used in all of the experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA).

Quantification of Hg^{2+}, Ag^{+} and coralyne.

The MB probe (20 nM, 450 μL) was separately incubated with Hg^{2+} (50 μL, 0.1–100 μM), Ag^{+} (50 μL, 0.2–40 μM), and coralyne (50 μL, 0.06–40 μM) in 5 mM phosphate buffer (pH 7.4) at ambient temperature for 5 min. The fluorescence spectra of the resulting solutions were recorded by operating the fluorescence spectrophotometer at an excitation wavelength of 480 nm. Additionally, Hg^{2+}, Ag^{+}, and coralyne-induced change in the conformation of MB were observed using a JASCO model J-810 circular dichroism (CD) spectrapolarimeter (JASCO, Corporation, Tokyo, Japan).

MB-based logic operations in solution.

20 nM MB probes were separately incubated with 5 μM Hg^{2+} and 1 μM Ag^{+} for 5 min at ambient temperature, generating an output of 0 in the absence of input 1 (i_1) and input 2 (i_2). The "AND and OR" logic gates were performed in a solution containing a T–Hg^{2+}–T-based MB, while the INHIBIT gate was operated in a solution containing a C–Ag^{+}–C-based MB. (a) AND gate: the MB probe was mixed with 10 μM SAH, 40 units/L SAHH, or both. Homocysteine was generated from the reaction of 10 μM SAHH and 40 units/L SAH at 37 °C for 30 min. (b) OR gate: the MB probe was initiated by adding 10 μM cysteine, 100 μM EDTA, or both. (c) INHIBIT gate: the MB probe was incubated with 10 μM cysteine, 100 mM H_2O_2, or both. 10 μM cysteine reacted with 100 mM H_2O_2 for 5 min at ambient temperature to produce cystine. For the construction of NAND, NOR and REVERSE IMPLICATION gates,[30] the fluorescence intensity at 520 nm of 20 nM MB was defined to produce an output of 1. (d) NAND gate: the MB probe was mixed with 630 pM AgNPs, 10 mM H_2O_2, or both 10 mM H_2O_2 reacted with 630 pM AgNPs for 5 min at ambient temperature to produce Ag^{+}. (e) NOR gate: the fluorescence of the MB probe was quenched by adding 5 μM Hg^{2+}, 1 μM Ag^{+}, or both. (f) IMPLICATION gate: the MB probe was incubated with 5 μM Hg^{2+}, 10 μM GSH, or both.

The three-input NOR gate was constructed by introducing 5 μM Hg^{2+}, 1 μM Ag^{+}, and/or, 1 μM coralyne as inputs to quench the fluorescence of the MB probe (20 nM). For three-input OR gate, a C–Ag^{+}–C-based MB (20 nM MB and 1 μM Ag^{+}) was activated by adding 10 μM cyanide, 10 μM iodide, and/or 10 μM cysteine. For three-input AND gate, 20 nM MB was incubated with a mixture of 5 μM Hg^{2+}, 1 μM Ag^{+}, and, 0.3 μM coralyne for 5 min. The as-prepared MB was activated by adding a mixture of 10 μM EDTA, 10 μM iodide, and 1 μM polyadenosine.

A set of two and three input logic gates, as described above, was incubated in 5 mM phosphate buffer (pH 7.4) for 5 min at ambient temperature. Their fluorescence spectra were recorded by operating the fluorescence spectrophotometer (F-7000, Hitachi, Tokyo, Japan) at an excitation wavelength of 480 nm.

Results & discussion

We initially compared the fluorescence spectra of the designed MB in the absence or presence of Hg^{2+}, Ag^{+}, and coralyne. When excited at 480 nm, the MB fluoresced strongly in 5 mM of a phosphate buffer at pH 7.4 (Curve a in **Fig. 1B**). This result indicates that FAM and DABCYL were separated far from each other. Note that quenching of FAM fluorescence is only effective at a short distance between two organic dyes.[29] Adding 5 μM Hg^{2+}, 1 μM Ag^{+}, or 0.2 μM coralyne to a solution of the MB resulted in approximately 65% quenching of FAM fluorescence, indicating that these three compounds triggered a change in the conformation of the MB, and caused FAM to be close to DABCYL (Curves b-d in **Fig. 1B**). The thermal stability of the MB was next measured in the absence or presence of Hg^{2+}, Ag^{+}, and coralyne. The melting point was defined as the temperature at which the fluorescence of FAM reached 50% of its initial value. The fluorescence intensity at 520 nm of the MB alone remained almost constant as a result of no hydrogen bonds between DNA bases (**Fig. S1**, ESI). Once the same concentrations of Hg^{2+}, Ag^{+}, and coralyne were present in a solution of the MB, the melting point of the folded MB was determined to be 50, 71, and 49 °C, respectively. Apparently, Hg^{2+}, Ag^{+}, and coralyne were able to stabilize the MB with T:T, C:C, and A_2:A_2-mismatched base pairs, respectively. Because the MB contained more C than A and T bases, the melting point of the T–Hg^{2+}–T-based MB was higher than that of the C–Ag^{+}–C- and A_2–coralyne–A_2-based MBs.

CD spectroscopy was used to monitor changes in the conformation of the MB during the binding events. Compared to the CD spectrum of the MB, adding Hg^{2+} or Ag^{+} to a solution of the MB caused a remarkable change in ellipticity at the region of 250–300 nm (**Fig. S2**, ESI). In contrast to the CD spectrum of the MB, the presence of coralyne generated a new band between 320 and 350 nm, reflecting that coralyne triggers a dramatic change in the conformaion of the MB (**Fig. S2D,** ESI). Likewise, Xing et al. demonstrated that the adding coralyne to a solution of

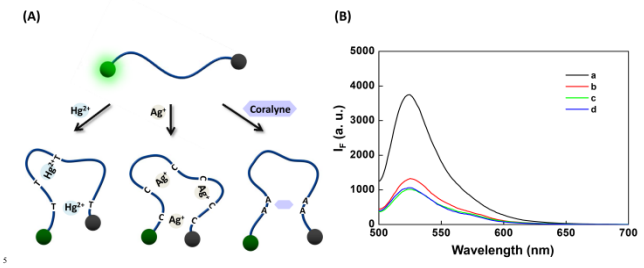

Fig. 1 (A) Schematic illustration of fluorescence detection of Hg^{2+}, Ag^{+}, and coralyne using the designed MB. (B) Fluorescence spectra of a solution of 20 nM MB in the (a) absence and (b-d) presence of (b) 5 μM Hg^{2+}, (c) 1 μM Ag^{+}, and (d) 200 nM coralyne. The MB probe was incubated with analyte in 5 mM phosphate buffer (pH 7.4) for 5 min at ambient temperature.

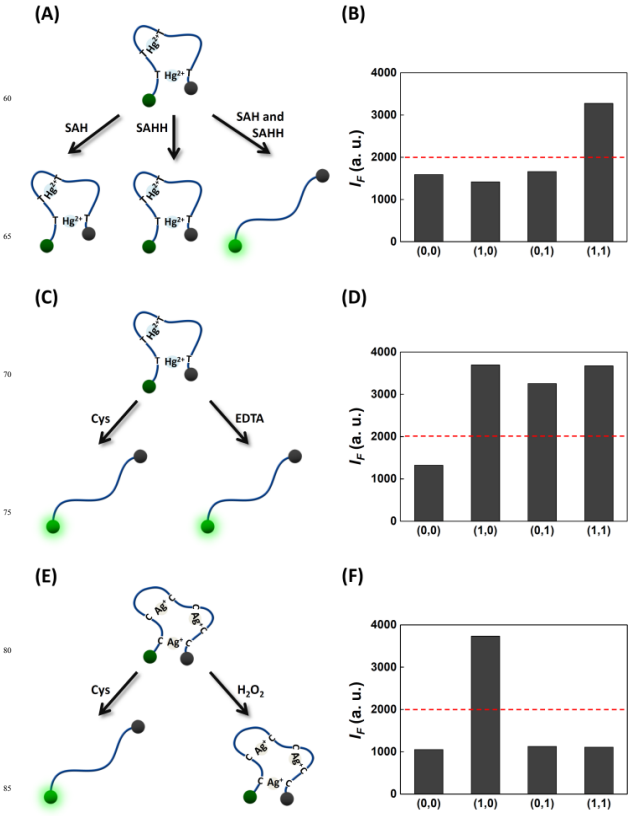

Fig. 2 Schematic illustration of the designed MB for the operation of two input (A) AND, (C) OR, and (E) INHIBIT gate. Fluorescent intensity at 520 nm of (B, D) a complex of 20 nM MB and 5 μM Hg^{2+} and (F) a complex of 20 nM and 1 μM Ag^{+} obtained without and with the addition of (B) 10 μM SAH, 40 units/L SAHH, or both, (D) 10 μM cysteine, 100 μM EDTA, or both, and (F) 10 μM cysteine, 100 mM H_2O_2, or both. (B, D, F) The MB probe was incubated with input in 5 mM phosphate buffer (pH 7.4) for 5 min at ambient temperature.

polyadenosine led to the appearance of a new band between 320 and 350 nm.[31] These results signifies that Hg^{2+}, Ag^{+}, and coralyne are able to bridge T:T, C:C, and A_2:A_2 bases separately to drive the conformational change of the MB. The sensitivity of the MB toward Hg^{2+}, Ag^{+}, and coralyne was tested to determine the threshold values. **Fig. S3** (ESI) shows that the fluorescence intensity at 520 nm of FAM gradually decreased as the concentrations of Hg^{2+}, Ag^{+}, and coralyne increased in the range of 0.01–5, 0.02–1, and 0.006–1 μM, respectively. The limits of detection at a signal-to-noise ratio of 3 for detecting Hg^{2+}, Ag^{+}, and coralyne were estimated to be 3, 6, and 2 nM, respectively, which are comparable to those of most existent DNA-based sensors for detecting Hg^{2+}, Ag^{+}, and coralyne (**Table S1-S3**, ESI). The fluorescence quenching of the MB reached a saturation level after adding 5 μM Hg^{2+}, 1 μM Ag^{+}, or 1 μM coralyne, at which the fluorescence intensity at 520 nm of the MB was lower than 2000. Thus, fluorescence intensities at 520 nm of the MB probe higher and lower than 2000 were defined as an output of 1 and 0, respectively. In other words, when 5 μM Hg^{2+}, 1 μM Ag^{+}, or 0.2 μM coralyne was present in a solution of the MB, the output was 0.

To demonstrate that the MB probe can be used as a universal component for developing molecular logic gates, the AND logic gate was first designed using SAH and SAHH as inputs, and the fluorescence change of the T–Hg^{2+}–T-based MB was set as the output (**Fig. 2A**). In the absence of SAH and SAHH (i_1 = 0, i_2 = 0), Hg^{2+}-induced fluorescence quenching of the MB probe produced an output value of 0. The fluorescence of the T–Hg^{2+}–T-based MB remained almost unchanged when SAH (i_1 = 1, i_2 = 0) or SAHH (i_1 = 0, i_2 = 1) was added, indicating that the T–Hg^{2+}–T-based MB remained in the OFF state (output 0). When SAHH catalyzed the hydrolysis of SAH,[32] the generated homocysteine (i_1 = 1, i_2 = 1) removed Hg^{2+} from the T–Hg^{2+}–T-based MB through the formation of S-Hg-S bonds. As a result, the fluorescence of the MB was restored, thereby switching the output to the ON state (output 1). This result provided clear evidence that the T–Hg^{2+}–T-based MB was adapted to monitor the output of enzyme-catalyzed reactions. **Fig. 2B** shows that the fluorescence intensity at 520 nm of the T–Hg^{2+}–T-based MB displayed different combinations of input signals corresponding to the "true table" of the AND logic gate. We suggest that this system be used to monitor other enzyme reactions, such as the GSH reductase-catalyzed cleavage of GSH disulfide,[33] homocysteine thiolactonase-induced hydrolysis of homocysteine thiolactone,[34] and acetylcholinesterase-triggered hydrolysis of acetylthiocholine.[35] Because of the strong interaction between Ag^{+} and thiol groups, the AND logic gate can be operated by replacing a T–Hg^{2+}–T-based MB with a C–Ag^{+}–C-based MB. Furthermore, *a* T–Hg^{2+}–T-based MB can be used to design the OR gate when cysteine and EDTA are used as inputs (**Fig. 2C**). With no input (i_1 = 0, i_2 = 0), the fluorescence of the T–Hg^{2+}–T-based MB was identified as an output of 0. Because both cysteine and EDTA have a strong binding affinity for Hg^{2+}, the presence of either (i_1 = 1, i_2 = 0; i_1 = 0, i_2 = 1) or both (i_1 = 1, i_2 = 1) as inputs switched on the fluorescence of the T–Hg^{2+}–T-based MB, and generated an output value of 1. EDTA is commonly used in medicine, food technology, and the chemical industry, whereas cysteine in plasma and urine is implicated in several clinical disorders. This OR logic gate is a potential sensor for detecting cysteine or EDTA. **Fig. 2D** shows the fluorescence change at 520 nm of the T–Hg^{2+}–T-based MB at different input states. The OR logic gate can be constructed using a T–Hg^{2+}–T-based MB instead of a C–Ag^{+}–C-based MB, and using cysteine and CN^{-} as inputs.

Subsequently, we designed the INHIBIT gate, in which cysteine and H_2O_2 act as inputs, and the fluorescence change of a C–Ag^{+}–C-based MB is the output (**Fig. 2E**). In the absence of inputs (i_1 = 0, i_2 = 0), the C–Ag^{+}–C-based MB fluoresced weakly, generating an output value of 0. Because of the coordination

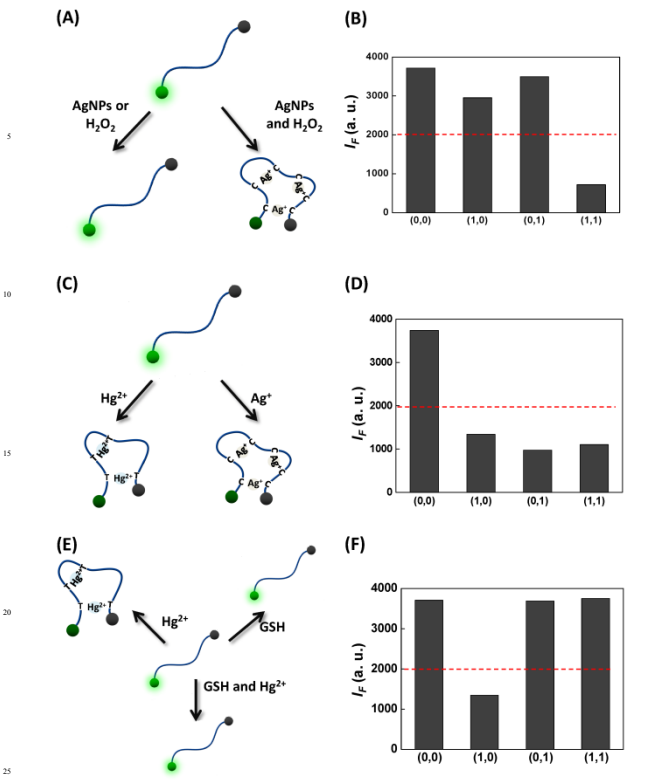

Fig. 3. Schematic illustration of the designed MB for the operation of two input (A) NAND, (C) NOR, and (E) REVERSE IMPLICATION gate. Fluorescent intensity at 520 nm of 20 nM MB obtained (B) without and with the addition of 630 pM AgNPs, 10 mM H_2O_2, or both, (D) without and with the addition of 5 μM Hg^{2+}, 1 μM Ag^+, or both, and (F) without and with the addition of 5 μM Hg^{2+}, 10 μM GSH, or both. (B, D, F) The MB probe was incubated with analyte in 5 mM phosphate buffer (pH 7.4) for 5 min at ambient temperature.

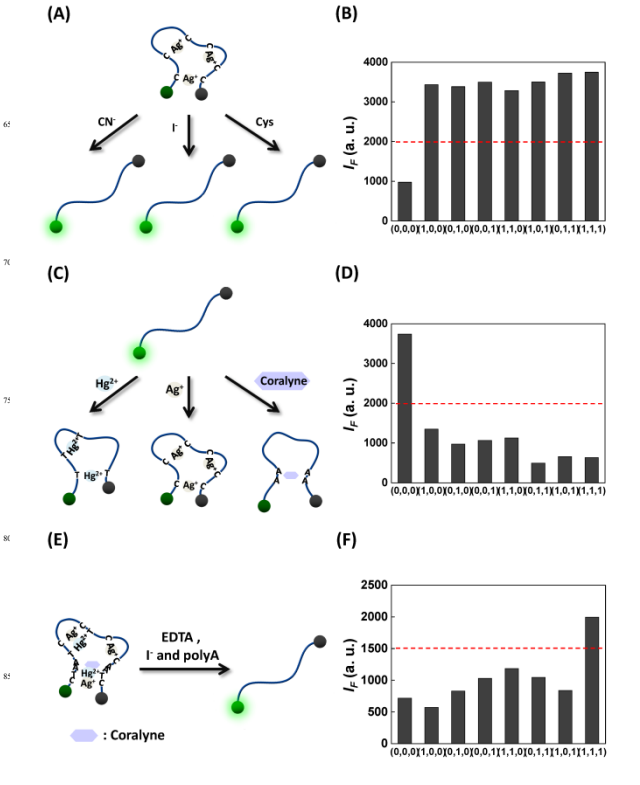

Fig. 4. Schematic illustration of the designed MB for the operation of three input (A) OR, (C) NOR, and (E) AND gate. Fluorescent intensity at 520 nm of (B) 20 nM MB, (D) a complex of 20 nM MB and 1 μM Ag^+, and (F) a complex of 20 nM MB, 5 μM Hg^{2+}, 1 μM Ag^+, and 0.3 μM coralyne obtained without and with the addition of (B) 10 μM cyanide, 10 μM iodide, and/or 10 μM cysteine, (D) 5 μM Hg^{2+}, 1 μM Ag^+, and/or 0.2 μM coralyne, and (F) 10 μM EDTA, 10 μM iodide, and/or 1 μM polyadenosine (A_{58}). (B, D, F) The MB probe was incubated with input in 5 mM phosphate buffer (pH 7.4) for 5 min at ambient temperature.

between cysteine and Ag^+, adding cysteine (i_1 = 1, i_2 = 0) to a solution of the MB probe caused an increase in fluorescence intensity at 520 nm, resulting in an output value of 0. By contrast, using H_2O_2 (i_1 = 0, i_2 = 1) as an input did not change the fluorescence of the C–Ag^+–C-based MB. When a mixture of H_2O_2 and cysteine was added (i_1 = 1, i_2 = 1), the fluorescence of the C–Ag^+–C-based MB remained almost constant. This occurred because H_2O_2 can oxidize cysteine to cysteine disulfide (cystine),[36] which is incapable of interacting with Ag^+. Hence, the logic gate produced a true output only when cysteine was present in a solution of the C–Ag^+–C-based MB, which is consistent with the truth table of the INHIBIT gate (**Fig. 2F**). Because specific oxidase catalyzes the oxidation of its substrate to generate H_2O_2, we suggest that this INHIBIT gate be applied for sensing numerous oxidases and their substrates (e.g., glucose oxidase and glucose).

To demonstrate the flexibility of the MB probe, we created other types of two-input logic gates, including NAND, NOR, and REVERSE IMPLICATION,[30] because the fluorescence intensity at 520 nm of the MB probe was defined as an output of 1 in the absence of any inputs (i_1 = 0, i_2 = 0). The NAND, NOR, and REVERSE IMPLICATION gates were generated by inverting the AND, OR, and INHIBIT gates, respectively. The NAND gate was constructed using AgNPs and H_2O_2 as inputs (**Fig. 3A**). The fluorescence intensity at 520 nm of the MB probe did not change after adding either AgNPs (i_1 = 1, i_2 = 0) or H_2O_2 (i_1 = 0, i_2 = 1), as shown in **Fig. 3B.** When the MB probe was subjected to the mixture (i_1 = 1, i_2 = 1) of AgNPs and H_2O_2, the produced Ag^+ disrupted the conformation of the MB probe through the coordination of C–Ag^+–C, and quenched their fluorescence. Because H_2O_2 oxidizes AgNPs to Ag^+,[37] it is evident this system can mimic the operation of the NAND logic gate. Additionally, this logic gate could be applied for quantifying toxic AgNPs in consumer products such as hand sanitizer gel and fabric softener. We mimicked the operation of the NOR gate by using Hg^{2+} (i_1 = 1, i_2 = 0) and Ag^+ (i_1 = 0, i_2 = 1) as inputs (**Fig. 3C**). As expected, the existence of either or both Hg^{2+} and Ag^+ (i_1 = 1, i_2 = 1) switched off the fluorescence of the MB probe and generated an output value of 0 (**Fig. 3D**). The REVERSE IMPLICATION gate was created using Hg^{2+} (i_1 = 1, i_2 = 0) and GSH (i_1 = 0, i_2 = 1) as inputs (**Fig. 3E**). Because GSH strongly binds to Hg^{2+} (i_1 = 1, i_2 = 1), adding only Hg^{2+} to a solution of the MB led to fluorescence quenching and yielded an output value of 0 (**Fig. 3F**). These results indicate that the designed MB probe could be used as a universal component to operate various logic gates at the molecular level. Compared with other DNA-based logic gate,

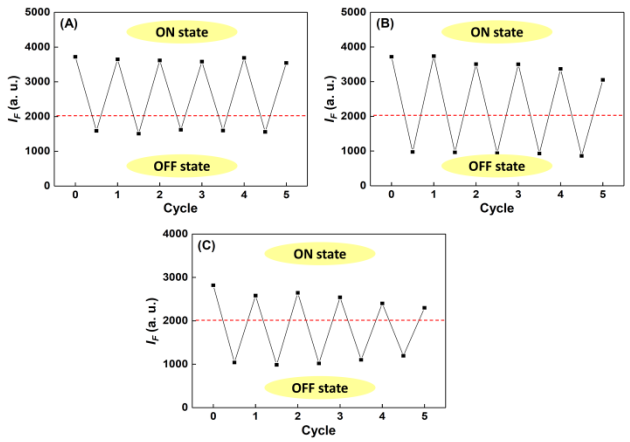

Fig. 5 Reversible switching of the MB probe (20 nM) between the ON and OFF states through the alternating addition of (A) Hg^{2+} and GSH, (B) Ag^{+} and cysteine, and (C) coralyne and polyadenosine (A_{58}). The MB probe was incubated with input in 5 mM phosphate buffer (pH 7.4) for 5 min at ambient temperature. Fluorescence intensities at 520 nm of the MB probe greater and less than 2000 are defined as ON and OFF states, respectively.

this MB probe was able to operate several types of logic gates without changing the DNA sequence.[21] **Table 1** shows the truth tables of AND, OR, INHIBIT, NAND, NOR, and REVERSE IMPLICATION Boolean logic gates.

The major challenge in logic systems is increasing their complexity by assembling multicomponents or individual logic gates. Therefore, we developed three-input logic gates, including OR, NOR, and AND gates. The three-input OR gate was manipulated using **cyanide,** iodide, and cysteine as inputs, whereas the fluorescence change of the C–Ag^{+}–C-based MB was the output (**Fig. 4A**). Because Ag^{+} can form complexes with cyanide, iodide, and cysteine, each input and combined inputs were induced to restore the fluorescence of the C–Ag^{+}–C-based MB. This result was consistent with a three-input OR gate behavior (**Fig. 4B**). Moreover, adding Hg^{2+}, Ag^{+}, and/or coralyne promoted a conformational change of the MB probe through the formation of mismatched T–Hg^{2+}–T, C–Ag^{+}–C, or A_2–coralyne–A_2 base pairs, respectively, thereby trigging fluorescence quenching (**Fig. 4C**). This result allowed the construction of a true table for building a three-input NOR gate (**Fig. 4D**). The MB probe consisting of Hg^{2+}, Ag^{+}, and coralyne was used to construct the three-input AND gate (**Fig. 4E**); fluorescence intensities higher and lower than 1500 were set as outputs of 1 and 0, respectively. By controlling the concentration of Hg^{2+} (5 μM), Ag^{+} (1 μM), and coralyne (0.3 μM), the fluorescence intensity of the MB probe was adjusted to be below a predetermined threshold value, producing an output value of 0. Any one or two of the three inputs was unable to exceed a threshold value. The combination of EDTA, iodide, and polyadenosine simultaneously removed Hg^{2+}, Ag^{+}, and coralyne from the MB probe, resulting in a change in the fluorescence intensity beyond a threshold value. This result confirmed that this system is able to operate a three-input AND gate (**Fig. 4F**).

The reusability of the MB probe was examined by adding GSH, cysteine, and polyadenosine to remove Hg^{2+}, Ag^{+}, and coralyne from the MB. As shown in **Fig. 5A,** the alternate addition of GSH and Hg^{2+} allowed the fluorescence intensity of the MB to be switched between the ON and OFF state. The repeated switching behavior was also observed in the cyclic treatment of the MB probe with cysteine and Ag^{+} or polyadenosine and coralyne (**Fig. 5B and 5C**). In the ON state, the fluorescence intensity of the MB probe decreased gradually with an increasing number of cycles. This is because the concentration of the MB decreased gradually with the addition of compounds in every cycle. These results demonstrate that the MB probe is able to perform the set-reset logic function.

Conclusions

We established an easy method for constructing a set of two-input (AND, OR, INHIBIT, NAND, NOR, and REVERSE IMPLICATION) and three-input (OR, NOR, and AND) logic gates based on the Hg^{2+}/thiol-, Hg^{2+}/EDTA-, Ag^{+}/thiol-, Ag^{+}/cyanide-, Ag^{+}/iodide-, and coralyne/polyadenosine-mediated fluorescence change of the MB probe. The designed MB provides several distinct advantages over conventional DNA-based logic gates. First, it allows the sensitive detection of Hg^{2+}, Ag^{+}, and coralyne through the coordination of T–Hg^{2+}–T, C–Ag^{+}–C, and A_2–Hg^{2+}–A_2. The selectivity of the MB probe could be further improved by adding suitable masking agents. For example, this probe could become selective for coralyne by adding iodide to mask the interferences from Hg^{2+} and Ag^{+}. Second, the MB probe can operate a set of two-input and three-input logic gates without altering the DNA sequence, indicating that the designed MB is a convenient, universal, and low-cost alternative for constructing logic gates. Third, the MB probe can be used for determining enzyme activity and inhibition, and quantifying its substrate. Finally, the MB can mimic the set-reset logic function through the alternate addition of GSH/Hg^{2+}, cysteine/Ag^{+}, or polyadenosine/coralyne. We expect that the designed MB can operate sophisticated networks of logic circuits by simply using DNA, thiol, metal ions, anions, and coralyne as inputs, and attaching two different fluorescent labels on the MB.

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