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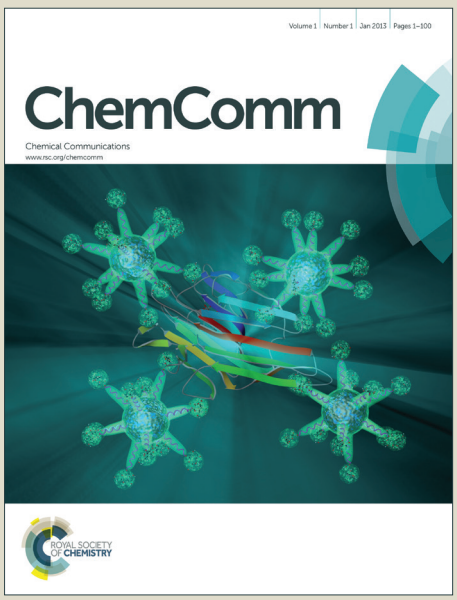

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Construction of DNA logic gates utilizing an H^+/Ag^+ induced i-motif structure

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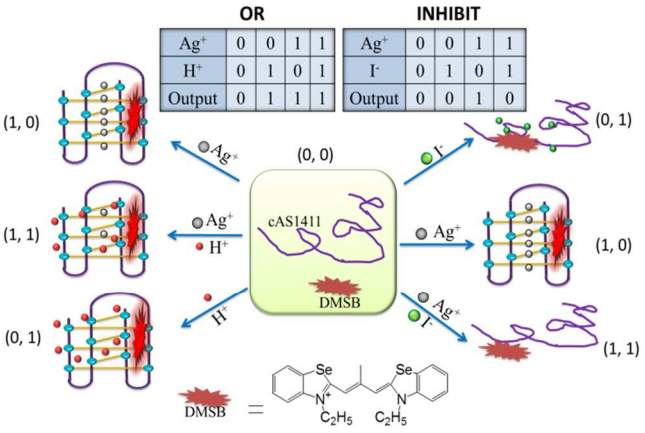
A simple technology to construct diverse DNA logic gates (OR and INHIBIT) has been designed utilizing an H^+ or/and Ag^+ induced i-motif structure. The logic gates are easily controlled and also show a real time response towards inputs. The research provides a new insight for design DNA logic gates by using i-motif DNA structure.

Molecular logic gates, especially the design of chemical or biological computers, have opened a way for an alternative approach to silicon-based computing[1]. DNA, as a biological molecule, has the unique features such as data-storage and information processing capacity, highly specific binding properties, and particular hybridization ability. Thus design and construction of DNA logic gates have attracted considerable attention. Numerous DNA-based logic gates based on the base pairing or reactivity toward enzymes of duplex DNA have been designed.[2,3] However, the researchers generally are confused with the complicated handling procedures and complex designs, which also limit the application of these DNA logic gates to a certain extent.

Recently, there is increasing interest in utilizing four-stranded DNA structures (G-quadruplex[4] and i-motif[5]) to construct logic gates owing to their great advantages in the high specific binding properties, polymorphic versatility, and self-assembly into catalytic nucleic acids (DNAzyme).[6-8] The four-stranded DNA logic systems generally require the coexistence of G-quadruplex- and i-motif-forming oligonucleotides,[6,8] and thus need elaborate design and careful operation in order to meet the conditions for regulating the both structures. There are also the logic gates constructed by solely using the G-quadruplex-forming oligonucleotides, which are mainly based on the catalytic property relevant to G-quadruplex conformation.[7] Tough they are simply designed and easily controlled, their applications are still limited. There is a pressing expectation that the i-motif structure can also be solely used to constructed logic gates and extend or open a new application area.

I-motif is a type of four-stranded nucleic acid secondary structure comprised of two parallel duplexes hydrogen bonded together by intercalated C^+-C base pairs.[5] Formation of i-motif is promoted by slightly acid pH, which is needed to protonate the N3 in cytosine.[9] Recently, it is found that i-motif can also form at neutral pH with Ag^+ present owing to the strong interaction of Ag^+ with cytosine to form a C-Ag^+-C complex.[10] This property provides a possible way to construct DNA logic gates in response to H^+, Ag^+, and I^-. Inspired by this idea, we have developed a simple platform technology to construct diverse logic gates (OR and INHIBIT) based on the response of i-motif DNA to H^+, Ag^+, and I^- inputs. Meanwhile, we also designed a fluorescence probe named DMSB (2, 2'-diethyl-9-methylselenacarbocyanine bromide) to recognize i-motif formation, the fluorescence signal of which was used as the outputs (Scheme 1).



Scheme 1 Schematic representation of the structural interconversion of cAS1411 induced by adding Ag^+, H^+, and I^-, and the fluorescence variance of DMSB with the structural switch of cAS1411. The sheets show the two logic gates being comprised of cAS1411-DMSB: the OR logic gate system using both Ag^+ and H^+ as inputs; the INHIBIT logic gate system using both Ag^+ and I^- as inputs.

The complementary sequence of the aptamer AS1411 (cAS1411) was chosen as the motif-forming oligonucleotides because its motif structure caused more obvious variance of the probe's fluorescence than that other oligonucleotides did (Fig. S1, ESI†). The response of cAS1411 oligonucleotides to H^+ and Ag^+ was determined by measuring the circular dichroism (CD) spectra of cAS1411 under different amounts of H^+ and Ag^+, respectively. The CD spectral

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feature of i-motif structure is that it has a positive peak around 286 nm.[10] In the absence of Ag^+ and H^+, the CD spectrum of cAS1411 showed a negative peak around 242 nm, and a positive peak around 275 nm (Fig. S2, ESI†), indicative of unfolded DNA structure.[10] Titration of H^+ and Ag^+ caused the positive peak of cAS1411 to red-shift to 284 nm, corresponding to the formation of i-motif (Fig. S2, ESI†). The response of cAS1411 toward to H^+ and Ag^+ enables logic gates to be constructed with H^+ and Ag^+ as inputs.

With H^+ or Ag^+ being input, the cAS1411 oligonucleotides formed i-motif structure. To output the signals relevant to i-motif formation, an external probe that can recognize i-motif structure is required. It is well known that numerous probes have been developed to recognize G-quadruplex. These probes generally have a large planar aromatic structure, which facilitates them to stack onto the face of G-quartet.[11] Compared with G-quadruplex, i-motif has no large planar structure, and thus the probes with a large planar aromatic structure are unsuitable. Recently, we designed the cyanine dye DMSB, which has a narrow molecular plane, to specifically insert into the grooves of G-quadruplex DNA structure.[12] The grooves of i-motif are similar to that of G-quadruplex, and thus we think that DMSB can also insert into the grooves of i-motif to realize recognition of i-motif formation. Just as speculated, DMSB showed an obvious enhancement of fluorescence when interacting with i-motif (Fig. S3, ESI†). This phenomenon did not appear when DMSB interacted with single-stranded DNA (Fig. S3, ESI†). This result indicates the property of DMSB to recognize i-motif formation. Furthermore, DMSB exhibited a dramatic enhancement of fluorescence intensity with increasing the concentrations of either H^+ or Ag^+ in the presence of cAS1411 (Fig. 1), further validating the performance of DMSB recognizing H^+/Ag^+-induced formation of i-motif structure.

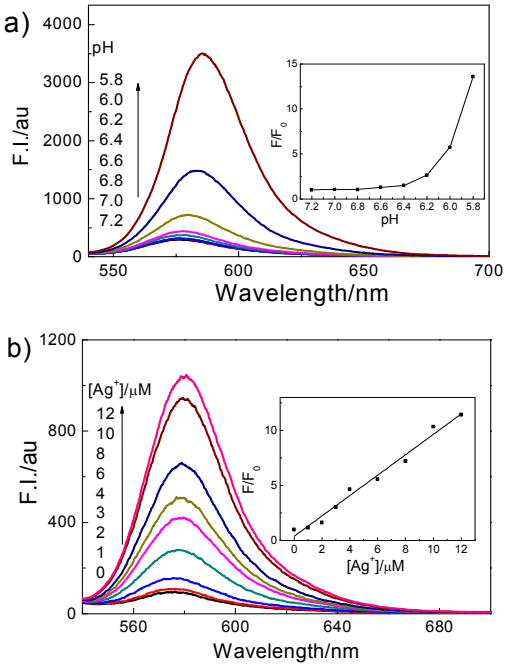

Fig. 1 The fluorescence spectra of 5 μM DMSB with a) variant pH and b) increasing concentrations of Ag^+ in the presence of 2 μM cAS1411 DNA in PB solution. The insets show the plot of the fluorescence intensity ratio F/F_0 at 585 nm versus pH and [Ag^+], respectively. F represents the fluorescence intensity of DMSB with H^+ or/and Ag^+ present and F_0 represents the fluorescence intensity of DMSB without H^+ and Ag^+.

DMSB being selected as the probe of i-motif structure, we proceeded to construct the OR gate by using both Ag^+ and H^+ as inputs. For input, we defined the presence of H^+ or Ag^+ as 1 and their absence as 0. The fluorescence intensity ratio (F/F_0) at a wavelength of 585 nm was defined as the output (1 or 0). The single-stranded structure of cAS1411 in the absence of H^+ and Ag^+ was confirmed based on the CD spectra (Fig. 2a). Titration of H^+ and Ag^+ caused obvious red-shift of the positive peak (Fig. 2a), meaning i-motif was induced. Meanwhile, over 5-fold enhancement of the DMSB fluorescence intensity was observed with H^+ (Fig. S4) or/and Ag^+ being added in (Fig. 2b), owing to the interaction of DMSB with i-motif. This result is in accordance with the feature of an OR logic gate.

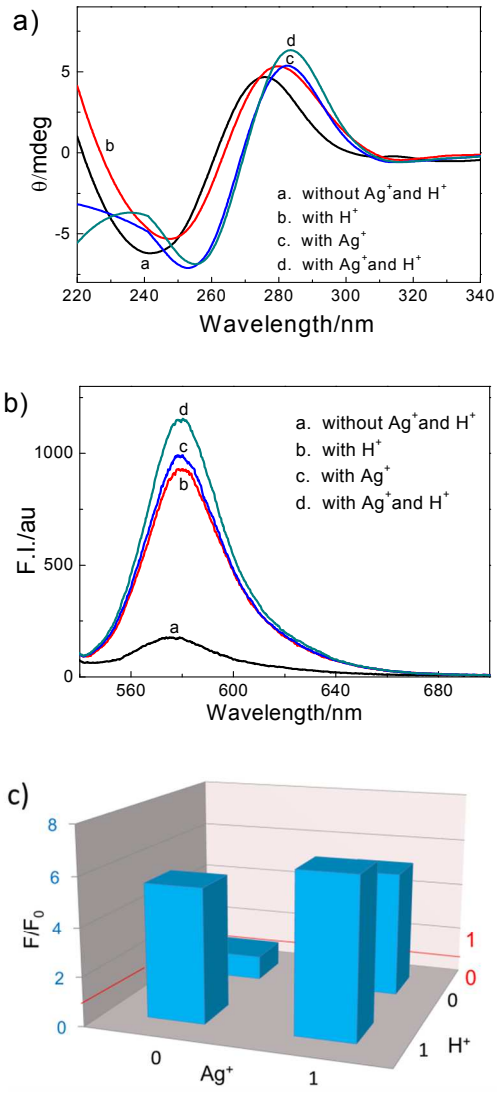

Fig. 2 a) The CD spectra of 2 μM cAS1411 and b) the fluorescence spectra of 5 μM DMSB with 2 μM cAS1411 in PB buffer solution under the conditions without Ag^+ and H^+ (pH 7.4), with H^+ (pH 5.7), with Ag^+ (pH 7.4), and with Ag^+ and H^+ (pH 5.7), respectively. c) Fluorescence intensity ratio F/F_0 of the DMSB-cAS1411 at λ_{em} = 585nm in the presence of different inputs. F represents the fluorescence intensity of DMSB with H^+ or/and Ag^+ present and F_0 represents the fluorescence intensity of DMSB without H^+ and Ag^+.

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The INHIBIT logic gate was further constructed by using both Ag^+ and I^- as inputs. In this system, the presence of Ag^+ or I^- was defined as 1, and the fluorescence intensity ratio (F/F_0) at a wavelength of 585 nm was still defined as the output (1 or 0). With no input, no change was observed in the CD spectra of cAS1411 (Fig. 3a) and the fluorescence spectra of DMSB (Fig. 3b), and the output was 0 (Fig. 3c). With iodide input alone, or with the two inputs together, no red-shift in the CD spectra was observed, but the DMSB fluorescence was obviously weakened, and the output also was 0. With Ag^+ input alone, the CD peaks of cAS1411 red-shifted and DMSB exhibited an over 5-fold fluorescence enhancement, giving an output signal of 1. This result validates the INHIBIT logic gate. It is known that Ye et al. also constructed an INHIBIT logic gate based on thymine (T) and Hg^{2+} forming T- Hg^{2+}-T complex.[13] Compared with the Ye's logic gate, our proposed gate has the important advantage of using unmodified oligonucleotides and simple operation.

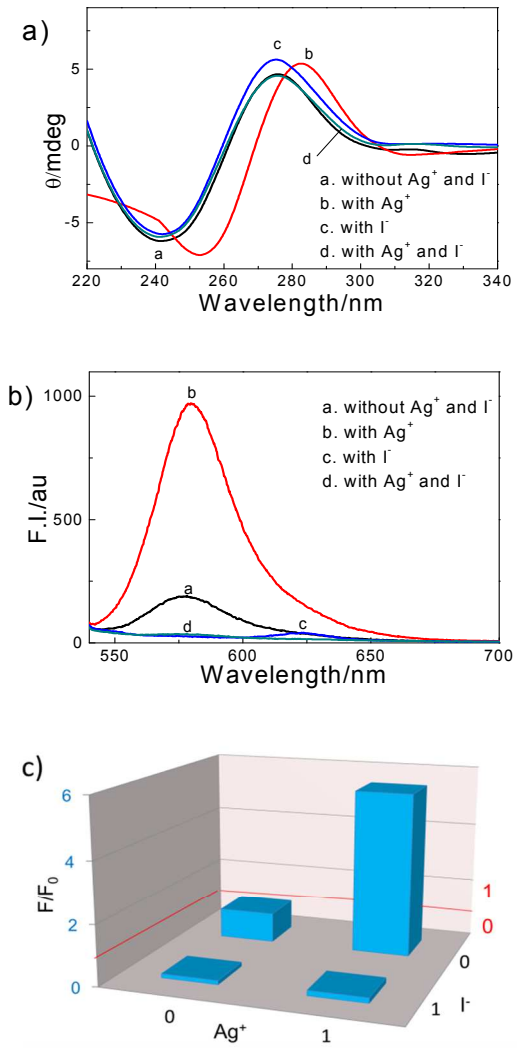

Fig. 3 a) The CD spectra of 2 μM cAS1411 and b) the fluorescence spectra of 5 μM DMSB with 2 μM cAS1411 in PBNa buffer solution (pH 7.4) under the conditions without Ag^+ and I^-, with Ag^+, with I^-, and with Ag^+ and I^-, respectively. c) Fluorescence intensity ratio F/F_0 of the DMSB-cAS1411 at λ_{em} = 585nm in the presence of different inputs. F represents the fluorescence intensity of DMSB with Ag^+ or/and I^- present and F_0 represents the fluorescence intensity of DMSB without Ag^+ and I^-.

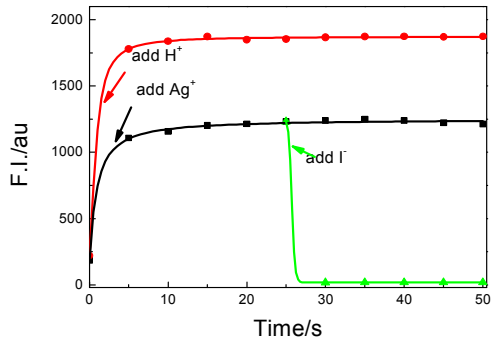

Fig. 4. The fluorescence intensity at 585 nm of DMSB with time after addition of Ag^+, H^+, and I^- in the presence of cAS1411.

The response of the both logic gates towards inputs is in real time. The time-dependent fluorescence measurement was done once H^+, Ag^+, and I^- was injected in, respectively. DMSB exhibited a sharp enhancement of the fluorescence intensity at 585 nm once H^+ and Ag^+ was added in but a dramatic decrease once I^- was titrated in (Fig. 4). The fluorescence intensity reached the maximum or minimum value within 10 seconds according to the time appeared in the inflection points, indicating the logic gates has an excellent performance in quick response.

In summary, we have designed a simple platform technology to construct diverse logic gates (OR and INHIBIT) utilizing an H^+ or/and Ag^+ induced i-motif for the first time. A novel label-free fluorescent probe has been designed to recognize formation of i-motif structure, which was further used to output signals in the logic gate systems. Unlike the previous four-stranded DNA logic gates which required coexistence of G-quadruplex- and i-motif-forming oligonucleotides, our proposed logic gates just need the motif-forming oligonucleotides. Thus the logic gates are easily controlled, cost-effective, and simply operated. The research will provide a new insight for design DNA logic gates by using i-motif DNA structure.

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