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ARTICLE TYPE

Toehold-mediated DNA logic gates based on host-guest DNA-GNPs

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A simple, toehold-mediated two-way input DNA machine has been developed. Utilizing symmetric and asymmetric protector sequences, INH, XOR logic gates and a half-subtractor are designed based on this two-way structure.

Toehold-mediated DNA strand displacement reactions are utilized as a powerful and multitalented nanoscale tool for engineering dynamic DNA nanodevices.[1-6] Utilizing these reactions, a series of dynamic DNA devices, such as nanomachines,[7] logic gates,[8-10] and catalytic amplifiers,[11] have been wisely constructed. In general, the displacement of a single-stranded DNA on a double-stranded complex could be triggered by an invasive single-stranded DNA.[4, 12] In addition, DNA-functionalized gold nanoparticles (DNA-GNPs) which boast both nucleic acids' extraordinary capability of programming and the distinguished assembly ability of GNPs,[13, 14] have opened an exciting window to the fabrication of multifunctional nanomaterials.[15] Recently, the engineering of the control of dynamic DNA-GNPs devices by programmed sequestration and activation of the toehold, such as blocking the toehold and activating it by subsequent exposure, has drawn great attention.[7, 16] In these systems, oligonucleotide chains act as catalysts or inputs that fuel the DNA-based molecular machine through a series of toehold strand displacement reactions.

Despite the success in the above fields, the strategies have been mainly limited to the construction of complexes on each GNPs probes and have increased the difficulty in probe preparation to develop regulative methods toward the control of strand displacement.[16, 17] It is still a challenge to create new schemes of toehold sequestration and activation based on host-guest model for additional levels of controlling DNA strand displacement reactions. As toehold displacement could occur at either 3' or 5' end of a DNA duplex, by controlling the length of toehold region, a two-way input model on one DNA duplex could potentially be developed.

In this work, we develop a novel two-way toehold activation strategy based on the tunable toehold protector on host GNPs, which can finely control the DNA strand displacement results by regulating the sequences. The study of the ability of toehold protector at different positions and lengths in controlling sequence release and toehold activation can help the design of DNA nanodevices. Furthermore, due to the spectral overlap, using fluorescent and colorimetric methods as the readout tool might limit the development of multichannel nanodevices.[12, 17, 18] We firstly introduce the surface enhanced Raman scattering (SERS) combined with UV-vis spectroscopy to DNA logic gates as the readout tool which features multichannel detection ability.[19] This strategy provides additional design flexibility for dynamic DNA devices.

To achieve this two-way strategy, a special DNA machine was designed. Fig. 1 shows the principle of the design and DNA sequences used for the DNA molecular machine. The design is based on DNA duplex with dual toehold region, which can be tuned to asymmetric or symmetric structure. Asymmetric structure refers to the unequal numbers of unpaired bases at two ends while symmetric structure means equal numbers. For optical measurement, the DNA duplex were immobilized onto host GNPs. When the duplex conformation is maintained, the strands on guest GNPs cannot hybridize with the complementary strands of the duplex due to the blockage by the protector sequence. When appropriate DNA input is introduced and hybridized with the duplex, the protector oligonucleotide can be displaced and released from the complex, producing a subsequent duplex with increased number of unpaired bases. Host GNPs are then cross-linked with guest GNPs through hybridization, as the length of toehold region is sufficiently long to activate second toehold displacement. Besides, H1 GNPs and H2 GNPs were labelled by Raman dye malachite green (MG) and cresol violet (CV) respectively for SERS detection. When toehold protector blocks the hybridization with guests, UV-vis absorbance of dispersed GNPs at 530 nm is high and SERS intensity of SERS dyes is low, which is defined as low output ("0"), otherwise the high outputs are designated "1".

The symmetric two-way structure (Fig. 1a) is a DNA complex containing a 12 nt protector N3 and 28 nt host H with toehold regions at each end with 5'-end-linked pegylated thiol for GNPs immobilization. Inputs I_A and I_B are 14 nt DNA strands. Based on the sequence assignment, both I_A and I_B can displace the protector from two terminals respectively to expose a hidden toehold region and lead to the assembly of host and guest GNPs. The guest GNPs probes are modified with 18 nt single strand G which is complementary to the centre region of H.

It is reasonable to believe that the protector length have an important effect on the behaviour of the protector-oligomer displacement through the input oligomer. In a symmetric structure, toeholds for G formed by the host-oligomer and the protector oligomer N3 are 3 nt and for inputs are 8 nt at each sides. Unexpectedly, more than 3 nt length toehold for G at both

sides (protector N1 or N2) can lead to a low level aggregation (Fig. S1). This behaviour is different from that observed in

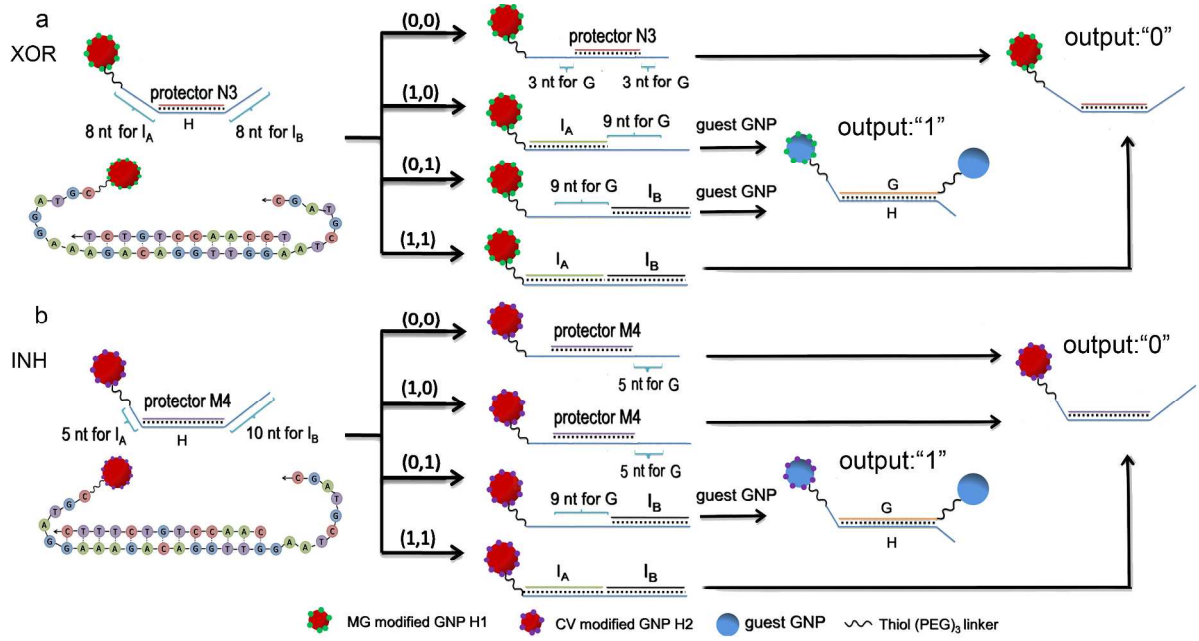


Fig. 1 Graphical representation of the symmetric (a) and asymmetric (b) two-way DNA machine and the mechanism of DNA-GNPs assembly.

one-way pure DNA toehold systems, where at least six-base toehold were needed to give a observable extent of the toehold-mediated stand displacement reaction.[20] The reason for this difference might arise from the synergy of two toehold domains. The experimental results indicated that only when the protector was 12 nt long would the sealing effect be suitable for the design. Hence, a 12 nt protector N3 covering the centre of the host oligomer was used in the following observations of symmetric structure (Fig. 1a). 8 nt at both sides would be left unprotected upon formation of the complex of the host-oligomer and the input-oligomer. This might guarantee a higher reaction rate when the protector-oligomer is displaced by the input-oligomer. Either I_A or I_B is capable of activating the aggregation of host GNPs and guest GNPs while simultaneously introducing the two inputs would block the H, thus preventing the aggregation. Furthermore, the study showed that the aggregation extent of DNA-GNPs mediated by I_A was relatively smaller compared with that by I_B (Fig. S1) which could be attributed to the steric hindrance and surface charge on GNPs. The aggregation of DNA-GNPs was detected via UV-vis absorption spectroscopy at 530 nm.

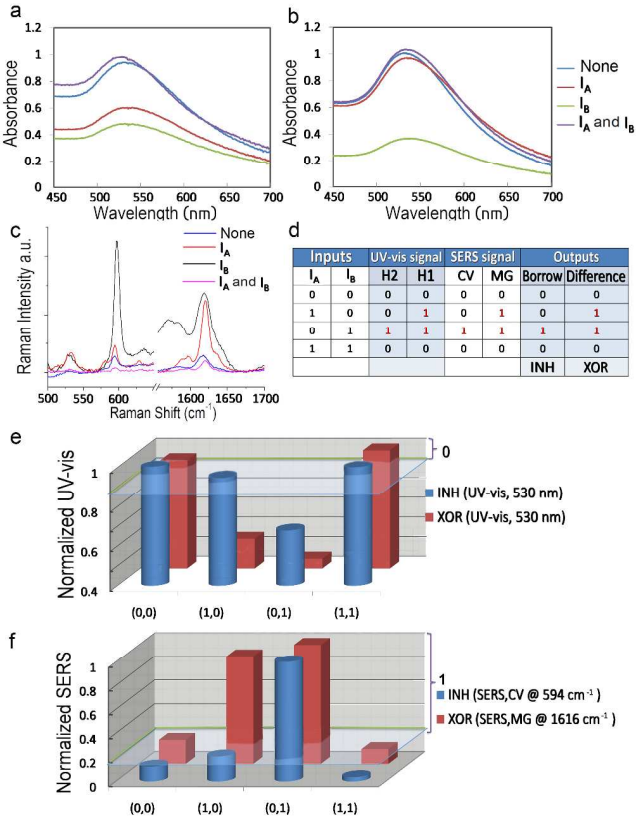
When the sequence of protector was adjusted to form an asymmetric structure, for example M4, the toehold length at 5' end was 0 and 3' end was 5 for G (Fig. 1b). The tuning protector sequence design gives this strategy flexibility to make some changes to achieve a new model of the machine by blocking I_A. Meanwhile, The toehold length for I_A was 5 nt, which was not enough for invasive hybridization. The function of the invasion of I_B was similar to that of the symmetric structure. Particularly, I_A was also able to attach to H strand when the invasion of I_B exposes a longer toehold region. The aggregation of guest GNPs and host GNPs were not generated when there was no input because the one-way displacement reaction needs more than 5 bases toehold.[4] If the I_B is the sole input in the solution, the guest GNP can be hybridized with host GNP which generates "hot spot" sites that enhances the Raman signal of dye.[21, 22]

In practical applications, both high extent of reaction and high stability of DNA-GNPs are desired. Thus, experiments were carried out to ensure that these two characteristics were present at acceptable levels using the host-guest DNA-machine-driven assembly strategy. After careful optimization, a suitable NaCl concentration of 200 mM was selected for the strategy (Fig S3.). Interestingly, the DNA-GNPs showed high stability and an evident aggregation extent comparable to that of the lower salinity. This indeed proved that salt concentration is an effective parameter to control the extent of reaction, which would be particularly important in the future application of the DNA-GNPs-machine-driven assembly scheme.

As indicated above, these DNA-GNPs may find applications as probes in molecular computing. The value of the tunable assembly scheme was demonstrated by building XOR and INH logic gates, which is currently being studied in pure DNA systems.[5] XOR (exclusive OR) represents the inequality function, i.e., the output is true if the inputs are not alike, otherwise the output is false.[23, 24] INH (inhibit) is a two-input AND gate with one input carrying a NOT gate.[9] Symmetric structure was used to build the XOR gate. Inputs oligonucleotides and guest DNA-GNPs were successively added into the solution containing H1 DNA-GNPs, triggering aggregation of the DNA-GNPs and resulting in the construction of the XOR gate. The results showed that DNA-GNPs aggregation could be triggered as long as either of the inputs, I_A or I_B, was present in the solution containing the two types of DNA-GNPs, otherwise it's not activated (Fig. 2a). Asymmetric DNA machine was used to construct the INH gate, a complex in which H hybridized with off centre protector M4 was prepared. The DNA-fuelled INH gate could be triggered only when I_B was present in the solution (Fig. 2b).

Furthermore, a half-subtractor logic circuit was built. A half-subtractor logic circuit uses two binary digits for subtraction and

produces a borrow-bit and a difference-bit as outputs using XOR and INH logic gates, respectively (Fig. 2d).[25] Two optical approaches, UV-vis and SERS, were employed to read out the outputs of the circuit. To acquire the outputs, two measurements are needed for UV-vis while only one measurement is needed for SERS. Fig. 2a and Fig. 2b are the UV-vis results of XOR and INH respectively. To form a half-subtractor, XOR and INH were combined, the result of which is shown in Fig. 2e. The absorption intensity of the results above the threshold of 0.9 were defined as "0". The spectrum of mixed solution that contain both two gates can be easily acquired in one measurement due to the different spectral fingerprints of Raman dyes (Fig. 2c). As the state when the relative peak intensity was under the threshold of 0.2 was designated as "0", the difference between "0" and "1" is quite obvious as shown in Fig. 2f. All the results were perfectly identical with the truth table of half-subtractor (Fig. 2d), which proved the success of our design.



Inputs		UV-vis signal		SERS signal		Outputs	
I_A	I_B	H2	H1	CV	MG	Borrow	Difference
0	0	0	0	0	0	0	0
1	0	0	1	0	1	0	1
0	1	1	1	1	1	1	1
1	1	0	0	0	0	0	0
						INH	XOR

Fig.2 (a) and (b) are the UV-vis outputs of XOR and INH gate respectively. (c) is the SERS outputs of mixture of two gates which agree with the truth table of half-subtractor (d). The histogram (e) shows the normalized UV-vis intensity at 530 nm of both XOR and INH gate. The histogram (f) is the outputs of half-subtractor (mixture of XOR and INH) based on the characteristic SERS peaks of CV (594 cm^{-1}) and MG (1616 cm^{-1}) respectively.

In summary, we have developed a toehold-mediated two-way input DNA machine whose capabilities in driving the assembly of host and guest DNA−GNPs have been demonstrated as well. This nanomachine provides an applicable and flexible platform that allows the development of the two-way DNA strand displacement-based inorganic nanoparticles assembly. The applicability of this strategy has been successfully exemplified in the construction of XOR and INH logic gates, and the construction of the half-subtractor logic circuit assembled with the two gates. This strategy can easily be extended to the building of other DNA-GNPs based computing systems such as ANDNOT and NOR gate. Worth of mentioning, for the first time, SERS was used as a readout tool in DNA logic gates. Due to its capability of reading out the results of multichannel outputs at one measurement, SERS has shown great potential in the reading of large scale integrated circuit. The effort to produce more attractive toehold-mediated DNA-GNPs based computing systems is underway.

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Notes and references

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