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Kinetically grafting G-quadruplexes onto DNA nanostructures for structure and function encoding *via* a DNA machine†

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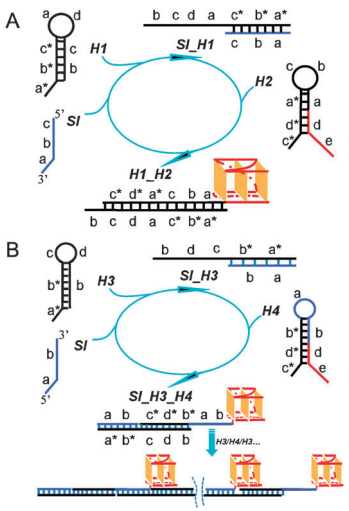
Kinetically grafting G-quadruplexes onto one-dimensional DNA nanostructures with precise positioning was realized in this study. The programs hold great promise for label-free and enzyme-free detection of various targets as a result of signal amplification from G-quadruplexes, and building DNA nanostructures as scaffolds due to the molecular recognition capacity of G-quadruplex aptamers.

Controlled positioning of functional motifs onto the DNA scaffold has been in the forefront of DNA nanotechnology,[1] which in recent years has attracted widespread interest in various fields, such as nanoelectronics,[2] biosensors,[3] drug delivery[4] and single molecule analysis.[5] However, kinetic incorporation of biorelevant motifs into these DNA scaffolds has not been paid much attention until the recent discovery of several DNA machines, such as assembly chain reaction,[6] hybridization chain reaction[7] and cascaded assembly and disassembly reaction,[8] which have provided biomimetic approaches in programmed assembly of one-dimensional DNA nanostructures and two-dimensional dendritic nanostructures.[9]

The G-quadruplex structure as a biorelevant motif exists at the telomere and protein-sensitive region which is critical for gene activity[10] and has been utilized as building block for construction of DNA nanostructures.[11] One key feature of these nanostructures is that G-quadruplexes act as connectors, contributing to the integrity of DNA nanostructures. One disadvantage is that the trunks of DNA nanostructures composed of metastable G-quadruplex prevent further manipulation and also may decrease the binding affinity of G-quadruplexes toward protein targets. Furthermore, due to interference between duplex and G-quadruplex, it was difficult to construct large nanostructures.[12] Therefore, grafting G-quadruplex structure onto large DNA nanostructures with G-quadruplexes as branches and duplex chains as trunks was pursued *via* the DNA machine as mentioned above.[6,7,8b]

In this study, we designed two DNA machines (A and B) to kinetically graft G-quadruplex onto the end of duplex DNA (program A) and a periodic array of G-quadruplexes onto one-dimensional DNA nanostructure (program B), respectively, which have not been realized in previous studies. In order to make the programs tunable, we subdivide the strands into domains (Fig. S1 and S2†). Both pathways include one initiator strand and two fuel hairpin-strands.

Briefly, for program A in Scheme 1, single-stranded DNA (*SI*) containing domain a nucleates at the accessible toehold a* of hairpin *H1*, which initiates the branch migration and exposes new toehold c, leading to duplex intermediate (*SI_H1*) formation. Opened hairpin *H1* with the new exposed toehold c can function as a new initiator, leading to nucleation between domain c of hairpin *H1* and domain c* of hairpin *H2*. As a result, domain c, b, a, and d of hairpin *H2* are made fully accessible *via* branch migration. The accessible domain c, b and a



Scheme 1 (A) Catalytic circuit for autonomous formation of duplex ended with G-quadruplex. (B) Construction of one-dimensional DNA nanostructure grafted with a periodic array of G-quadruplexes *via* hybridization chain reaction.

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† Electronic supplementary information (ESI) available: Experimental materials and procedures, detailed sequences of fuel strands (*H1*, *H2*, *H3* and *H4*) and initiator strand (*SI*) in the programs, the structure of NMM, CD spectra of PW17 and products of DNA machine A, a fluorescence quenching experiment, AFM images and OTA inhibition experiment. See DOI: 10.1039/c1cc13973h

of hairpin *H2* start to displace single stranded initiator *SI* from the complex *SI_H1*, coincidently with the self-assembly and formation of G-quadruplex composed of domain d and e of hairpin *H2*. The released *SI* will start the new cycle.

For program B in Scheme 1, the single-stranded DNA (*SI*) hybridizes with *H3* at the nucleation site a*, leading to the displacement reaction and exposure of domain c, d and b. The new exposed domains can start further nucleation and branch migration, which make the domain a, b, d and e of *H4* accessible. The exposed domain a, b of *H4* have the same DNA sequence as *SI*, which can start a subsequent reaction cycle; the domain d and e of *H4* will self assemble into a G-quadruplex structure. By this method, we can dynamically graft a periodic array of G-quadruplexes onto the DNA polymer.

A series of experiments were carried out to validate the program A. Duplex formation was corroborated by conducting native polyacrylamide gel electrophoresis (native PAGE, Fig. 1A). With increasing the concentration of DNA initiator (*SI*), the bands corresponding to the duplex *H1_H2* became distinguishable from lane 3 to lane 6, and had the same shift distance as that lane corresponding to the annealed duplex *H1_H2* (lane 7), confirming exquisite function of DNA machine A.

In addition, we selected one porphyrin derivative, *N*-methyl mesoporphyrin IX (NMM) (Fig. S3†) which can selectively bind to G-quadruplex instead of double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) with the result of its fluorescence enhancement,[13] as a fluorescent probe to indicate the formation of G-quadruplex. Fig. 1B shows the time course of fluorescence enhancement of NMM at various concentrations of DNA initiator with a fixed concentration of *H1* and *H2*. With increasing the concentration of initiator *SI*, the fluorescence intensity of the solution at a higher concentration of *SI* increased faster, indicating that a higher amount of G-quadruplex was continuously formed. In addition, circular dichroism (CD) spectroscopy was used to confirm the formation of the G-quadruplex composed of domain d and e. The enhanced ellipticity signal near 265 nm was observed with reaction time, which directly reflected parallel-G-quadruplex generation (PW17, Fig. S4A and S4B†) during the function of DNA machine A.[14] We also constructed a fluorescence quenching measurement which verified the catalytic circuit of the program A (Fig. S5†).

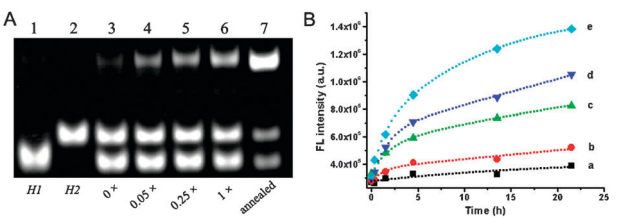

Fig. 1 (A) Native PAGE demonstrating catalytic circuit of DNA machine A after 7 h. Conversion of fuel strands to reaction products using substoichiometric initiator *SI* (lanes 3, 4, 5 and 6). Lane 1, 2 and 7 represent fuel strand *H1*, *H2* and annealed mixture *H1* and *H2*, respectively. The concentration of each fuel strand was 200 nM. (B) Fluorescence intensity of fluorescent probe (NMM) as function of time in the presence of various concentrations of initiator strand *SI*. The concentrations of *SI* from a to e were 0, 10, 25, 50 and 100 nM respectively. The reaction concentrations of fuel strands were both 200 nM. All data were obtained at an emission wavelength of 607.5 nm.

Another program B was designed based on the expectation that G-quadruplexes were grafted at well-defined place along the DNA polymer chain. As revealed in Fig. 2A and B, gel shift assays disclosed, that in the presence of initiator strand *SI* (lane 3–6), DNA polymer chain mainly composed of *H3* and *H4* was continuously prolonged within 2 days of the test. From analysis of the continuous bands after 48 h, the DNA polymer chain attained a length corresponding to over 1000 base pairings (bp) (lane 3 and 4), and the average length of products decreased obviously with the initiator strand (*SI*) concentration increasing, while unobservable bands in lane 2 suggested little leakage for the DNA machine B.

The products after 2 days of reaction were also characterized by atomic force microscopy (AFM). One-dimensional DNA products were observed clearly in Fig. 3A and Fig. S6.† The height and length of the visualized products were analyzed statistically and the results were shown in Fig. 3B and C. The peak heights of about 1.5 nm and 2.0 nm in Fig. 3C represent single G-quadruplex structure and single G-quadruplex–duplex hybrid structure, respectively (the height of single duplex DNA is around 0.5 nm).[12,15] In Fig. 3C, the DNA polymer chains with lengths of over 167 nm are consistent with those in gel electrophoresis assays that exhibit bands above 500 bp. The one-dimensional products with lengths of around 334 nm can also be observed, which correspond to the bands of 1000 bp (Fig. 2B, lane 4), according to the nm-to-bp value of 0.334.[16] These data indeed illustrate that G-quadruplex being grafted onto one-dimensional DNA nanostructures can be produced *via* DNA machine B.

As revealed in Fig. 4, time courses of fluorescence enhancement of NMM at various concentrations of initiator strand (*SI*) suggested that the incorporation of G-quadruplex was not interrupted during 48 h of the test. We noted that the incorporation of G-quadruplexes onto DNA polymer chains was much slower than the process corresponding to machine A, which was probably due to the steric hindrance of the G-quadruplex. To further directly confirm G-quadruplex formation *via* machine B, CD spectra were also collected (Fig. S4C†). The ellipticity value at 265 nm increased obviously

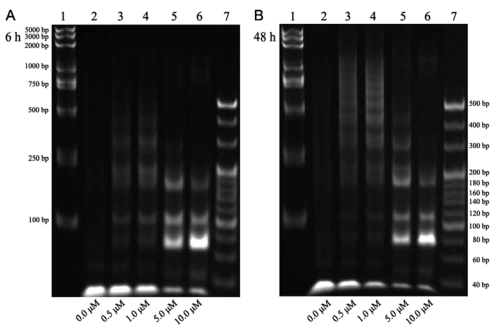

Fig. 2 Native PAGE confirming the formation of DNA polymer chain *via* DNA machine B after 6 h (A) and 48 h (B). Conversion of fuel strands to the final products using different concentrations of initiator *SI* (lanes 2–6). Both the concentrations of *H3* and *H4* were 5 μM. Lane 1 and 7 represent DNA markers.

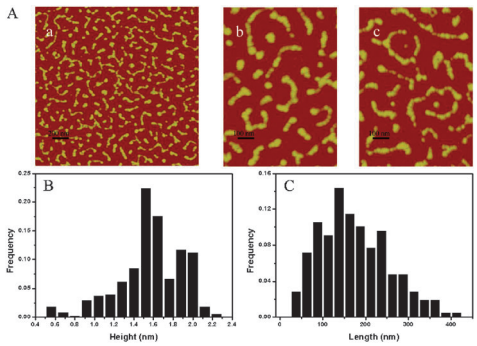

Fig. 3 (A) AFM images of the one-dimensional products *via* DNA machine B after 2 days reaction. Scale bars are 200 nm (a) and 100 nm (b and c). (B and C) Frequency distributions of height (B) and length (C) of DNA polymer chains. The concentrations of *SI*, *H3* and *H4* were 1 μM, 5 μM and 5 μM.

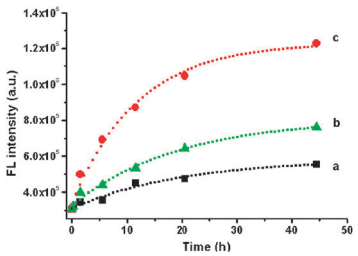

Fig. 4 Fluorescence intensity of NMM in DNA machine B as function of time with various concentrations of initiator strand *SI*. The concentrations of *SI* from a to c were 0, 25 and 50 nM. The concentration of each fuel strand was 200 nM.

during the time course of reaction, demonstrating G-quadruplex formation with the prolongation of DNA polymer chains.

In our two systems, the exquisite design of *SI*, *H1*, *H2*, *H3*, and *H4* is essential and significant to realize our concept. In our systems, fuel strand sequences were designed based on the initiator strand *SI* that was random single-stranded DNA. There have been two concerns which have to been taken into account: leakage and reaction rate. From aforementioned PAGE results for the two programs, little leakage existed, which benefited from stable hairpin stems and no complementarity between hairpin loops of fuel strands.[17] However, stable hairpin stems impeded the strand displacement and decreased the reaction rate. It has been demonstrated that the toeholds can greatly enhance the rate of strand displacement,[17,18] therefore toeholds of six nucleotides attached on all fuel hairpin-strands were utilized to accelerate the reaction rate of the two DNA machines.

Based on the modularity of the sequences in our design, program A can be fashioned into enzyme-free DNA circuits, which continuously produce G-quadruplexes leading to signal amplification (G-quadruplexes bind with hemin to form peroxidase or bind with dye molecules to enhance its fluorescence intensity), therefore, interesting label-free biosensors can be constructed (Fig. S7–S9†).[19] Program B provides an enzyme-free alternative of rolling circle amplification (RCA) to produce one-dimensional DNA polymers.[20] As a constant-temperature cascading approach, it can prevent the error formation during DNA assembles, compared to other assembling approaches, *e.g.* tile-based assembly.[21] As such, program B is a very promising method for delivering functional motifs (such as G-quadruplex aptamers), onto DNA nanostructures with nanoscale spatially-resolved patterning.

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