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

Jiangtao Ren,ab Jiahai Wang,a Lei Han,ab Erkang Wang*ad and Jin Wang*a†c

Received 4th July 2011, Accepted 29th July 2011
DOI: 10.1039/c1cc13973h

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 delivery4 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 activity10 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.5,7,8

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.

†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, as a fluorescent probe to distinguish 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.

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, 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. We also constructed a fluorescence quenching measurement which verified the catalytic circuit of the program A (Fig. S5†).

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). 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. 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. 1](image1.png)

**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.

![Fig. 2](image2.png)

**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.
and SI our systems, fuel strand sequences were designed based on the
tarity between hairpin loops of fuel strands.17 However, stable
which benefited from stable hairpin stems and no complemen-

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.

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,
account: leakage and reaction rate. From aforementioned

Fig. 4 Fluorescence intensity of NMM in DNA machine B as func-
tion 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.

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.

This work was supported by National Natural Science
Foundation of China (No. 20905056 and 2011CB911000),
the 973 Project (2009CB930100 and 2010CB933600).

Notes and references

1 (a) F. C. Simmel and W. U. Dittmer, Small, 2005, 1, 284;
(b) U. Feldkamp and C. M. Niemeyer, Angew. Chem., Int. Ed.,
2006, 45, 1856; (c) Y. Weizmann, A. B. Braunschweig, O. I. Wilner,
105, 5289.
2 (a) E. Braun, Y. Eichen, U. Sivan and G. Ben-Yoseph, Nature,
1998, 391, 775; (b) K. Keren, M. Krueger, R. Gilad, G. Ben-
3 (c) C. Lin, Y. Liu and H. Yan, Nano Lett., 2007, 7, 507; (b) F. Xia,
R. J. Whi, X. Zuo, A. Patterson, X. Xiao, D. Kang, X. Gong,
K. W. Plaxx and A. J. Heeger, J. Am. Chem. Soc., 2010,
132, 14346; (c) Z. Zhang, D. Zeng, H. Ma, G. Feng, J. Hu,
L. He, C. Li and C. Fan, Small, 2010, 6, 1854.
4 B. P. Timko, T. Dvir and D. S. Kohane, Adv. Mater., 2010,
22, 4925.
5 (a) F. Kukolka, B. K. Müller, S. Paternoster, A. Arndt,
C. M. Niemeyer, C. Bräuchle and D. C. Lamb, Small, 2006,
2, 1083; (b) S. Rinker, Y. Ke, Y. Liu, R. Chhabra and H. Yan,
2009, 131, 2422.
2004, 101, 15275; (b) J. Huang, W. Yu, W. Chen, Z. Zhu, X. Yang,
C. I. Yang, K. Wang and W. Tan, Angew. Chem., Int. Ed., 2010,
50, 401.
8 (a) D. Y. Zhang, A. J. Turberfield, B. Yurke and E. Winfree,
Science, 2007, 318, 1121; (b) P. Yin, H. M. T. Choi, C. R. Calvert
9 S. Venkataraman, R. M. Dirks, P. W. K. Rothemund, E. Winfree
11 (a) T. C. Marsh, J. Vesenka and E. Henderson, Nucleic Acids Res.,
1995, 23, 696; (b) R. P. Fahliman and D. Sen, J. Am. Chem. Soc.,
1999, 121, 11079; (c) M. Biyani and K. Nishigaki, Gene, 2005,
364, 130; (d) D. P. N. Gonzales, T. L. Schmidt, M. B. Koeppe
and A. Heckel, Small, 2010, 6, 1347.
12 K. Dutta, T. Fujimoto, M. Inoue, D. Miyoshi and N. Sugimoto,
1998, 26, 3724.
15 (a) L. T. Costa, M. Kerkmann, G. Hartmann, S. Endres,
P. M. Bisch, W. M. Heckl and S. Thalhammer, Biochem. Biophys.
Res. Commun., 2004, 313, 1065; (b) K. J. Neaves, J. L. Huppert,
R. M. Henderson and J. M. Edwardson, Nucleic Acids Res., 2009,
37, 6269.
27, 63.
17 S. J. Green, D. Lubrich and A. J. Turberfield, Biophys. J., 2006,
91, 2966.
18 B. Yurke and A. Mills, Genetic Programming and Evolvable Machines, 2003, 4, 111.
20 W. Zhao, M. M. Ali, M. A. Brook and Y. Li, Angew. Chem., Int.
Ed., 2008, 47, 6330.
321, 1795.