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Supporting information

Kinetically Grafting G-quadruplex onto DNA Nanostructure as Structure and Function Encoding via DNA Machine

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Experimental

Materials. PAGE-purified oligonucleotides were purchased from TaKaRa Biotechnology Co., Ltd (Dalian, China) and tris(hydroxymethyl)aminomethane (Tris) was obtained from Sangon Biotechnology Co., Ltd (Shanghai, China). N-methyl mesoporphyrin IX (NMM) was purchased from Frontier Scientific, Inc. (Logan, Utah, USA). Potassium chloride, magnesium chloride hexahydrate, ethylenediamine tetraacetic acid disodium, and boric acid were purchased from Beijing Chemical Works (Beijing, China). 3-Aminopropyltriethoxysilane (APTES) was purchased from Sigma-Aldrich (USA). Acrylamide, *N*, *N*′-Methylenebisacrylamide and ammonium persulfate were obtained from Beijing Dingguochangsheng Biotechnology Co, Ltd (Beijing, China). Tetramethylethylenediamine was purchased from AMRESCO Inc. (USA). Agarose was obtained from Gene Co., Ltd. (Hong kong, China). Trans2K Plus DNA marker and 20 bp DNA Ladder Marker were purchased from TransGen Biotech Co., Ltd. (Beijing, China) and TaKaRa Biotechnology Co., Ltd (Dalian, China) respectively. Gel-Dye was purchased from Biotium, Inc. (USA). The oligonucleotides were quantified using a Cary 50 Scan UV-visible spectrophotometer (Varian, USA), lyophilized and kept at –20 ^{o}C, and dissolved in Tris/Mg/K buffer (Tris-HCl 10 mM, $MgCl_2$ 4 mM, KCl 15 mM and pH 8.0) as stock solutions. All oligonucleotides except initiator strands were heated at 95 ^{o}C for 5 min and cooled to 25 ^{o}C in one hour before use. Stock solutions NMM (5 mM) was prepared in DMSO, stored in the dark at –20 ^{o}C, and diluted to the required concentration with Tris/Mg/K buffer. Double distilled water was used throughout.

Fluorescence kinetics. Both DNA machines A and B in this study employed NMM (detailed structure shown in Fig. S3), to monitor the G-quadruplex formation and machine function. The order of sample addition was the same as aforementioned general procedures: 600 μl Tris/Mg/K buffer solutions containing 1 μM NMM, 200 nM *H1* and *H2* (or *H3* and *H4*) respectively and initiators strand *SI* of various concentrations, were prepared and tested on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France) at 25 ^{o}C. Apparatus parameters: slits = 5/5 nm, excitation at 399 nm and emission at 607.5 nm.

In addition, for DNA machine A, we used a fluorescence quenching measurement to verify that DNA initiator (*SI*) can be displaced from *SI_H1* by *H2* and go to the next catalytic circuit, accompanied by formation of duplex (*H1_H2*). FAM (6-carboxyfluorescein) was tagged at 3’ end of initiator *SI*. Samples in Fig. S5 were prepared as follows: 60 μl of annealed *H1* (2 μM) and 60 μl of annealed *H2* (2 μM) were mixed firstly. Then the mixture was adjusted to 540 μl with Tris/Mg/K buffer. Finally, 60 μl of FAM-*SI*

(500 nM) was injected into solution rapidly, and fluorescent signal at 516 nm was collected immediately and at intervals. The final reaction volume was 600 µl. Apparatus parameters: slits = 2/2.5 nm, excitation at 492 nm and emission at 516 nm.

Native polyacrylamide gel electrophoresis. In Fig. 1A, annealed sample was prepared by heating the mixture solution containing *H1* (200 nM) and *H2* (200 nM) at 95 °C for 10 min, and cooling it for one hour to 25 °C, and samples were prepared by mixing 10 µl of *H1* (2 µM), 10 µl of *H2* (2 µM), and 10 µl of initiator strand *SI* (100 nM, 500 nM and 2 µM) in succession and adjusting the solution up to 100 µl with Tris/Mg/K buffer. In addition, mixture (*H1* and *H2*) and each fuel strand (*H1* or *H2*) were subject to electrophoresis with concentration of 200 nM for each. All samples were kept at 25 °C. For the reaction in Fig. 2, 40 µl of samples containing 5 µM *H3*, 5 µM *H4* and initiator strand *SI* (500 nM, 1 µM, 5 µM and 10 µM) if any, were incubated at 25 °C. Polyacrylamide gels (12 % w/v for Fig. 1A and 8 % w/v for Fig. 2) were prepared with 1×TBE buffer (Tris 89 mM, boric acid 89 mM, EDTA 2 mM and pH 8.3). 10 µl of each samples and 1.5 µl of DNA markers were mixed with 1.5 µl of Gel-Dye Super Buffer Mix and 1 µl of loading buffer, and loaded into the gels. The gels were run at 150 V for 30 min and photographed under UV light using a fluorescence imaging system (Vilber Lourmat, Marne laVallee, France).

CD measurements. Samples in Fig. S4B-C were prepared by mixing 250 µl of each fuel strand (4 µM), 375 µl pure Tris/Mg/K buffer, and 125 µl of initiator strand *SI* (4 µM), and incubated at 25 °C. Circular dichroism (CD) spectra from 210 to 350 nm were collected using a JASCO J-820 spectropolarimeter (Tokyo, Japan), the data pitch was 0.1 nm, scan speed was 200 nm/min, response time was 0.5 s, and bandwidth was 1 nm.

AFM experiments. After 5 µM *H3*, 5 µM *H4* and 1 µM initiator strand *SI* reacted for 2 days, the solution was diluted fifty times with Tris/Mg/K buffer. Freshly cleaved micas were immersed in 1 % APTES solution for 5 min, then rinsed with double distilled water and dried in a desiccator. 100 µl of diluted samples were allowed to absorb on the pretreated micas for 5 min, the surfaces were rinsed and dried. The AFM images of prepared samples were obtained on a MultiMode® 8 Scanning Probe Microscope (Veeco Instruments Inc., USA) under tapping mode at room temperature and analyzed with NanoScope® Version 8.1 software. The height measurements were conducted by analyzing the cross-section of DNA chains and the lengths of DNA polymer chains were measured by drawing short lines along the DNA contour and summating the lengths. Data points (376 for the height analysis and 209 for the length analysis) were processed into the histograms of frequency distributions (Fig. 3B and 3C), by frequency count in OriginPro 7.5.

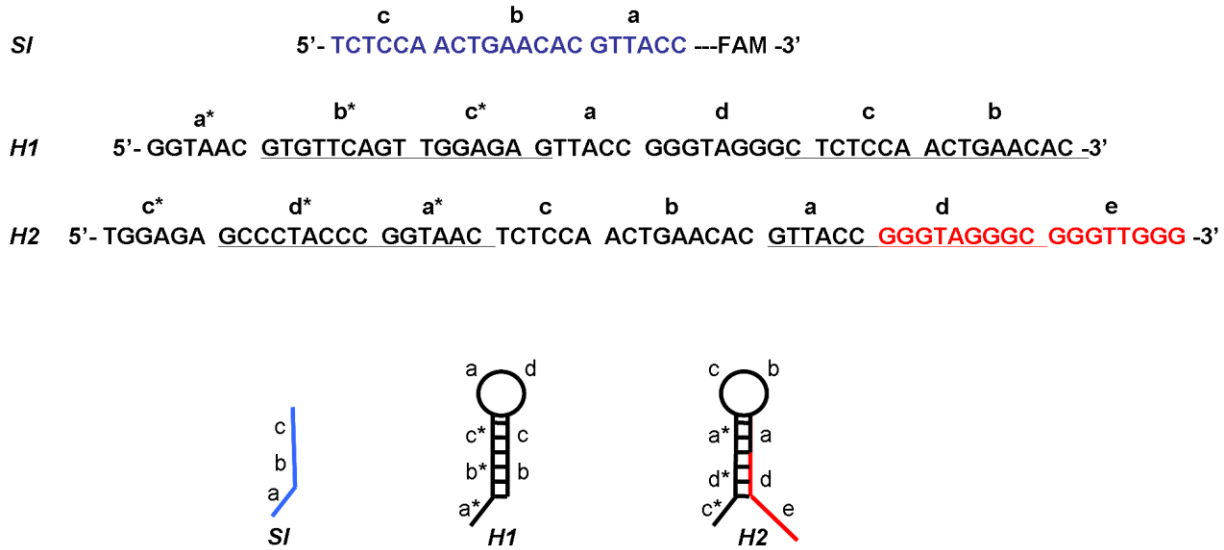

Fig. S1: DNA sequences in program A， each oligomer was divided into different domains.

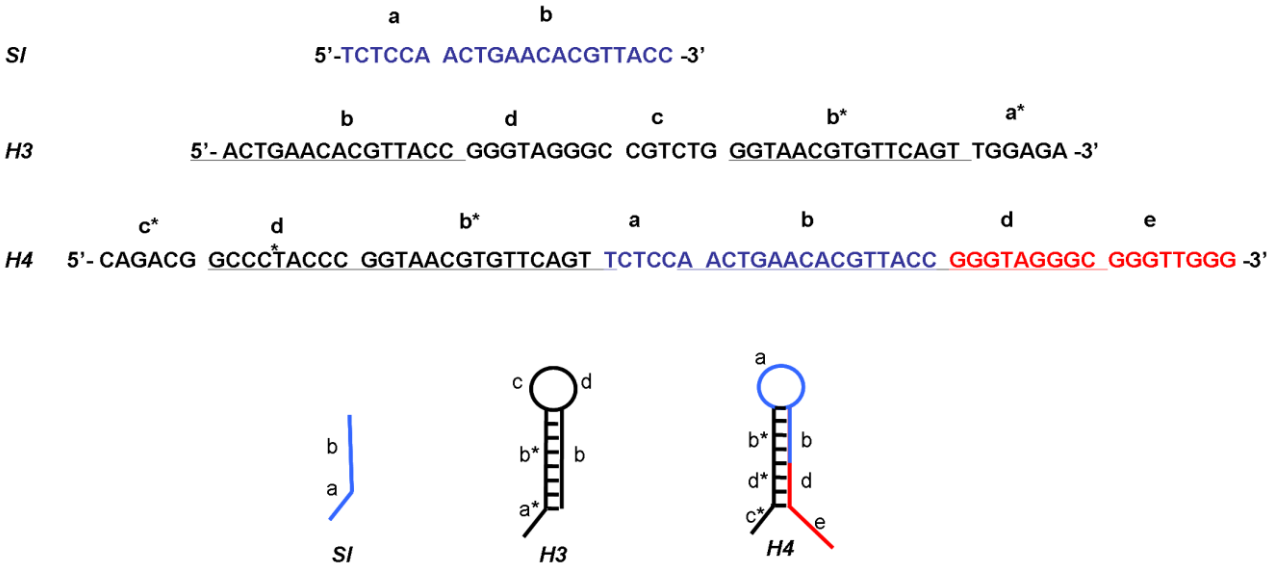

Fig. S2: DNA sequences used in program B, each oligomer was divided into different domains.

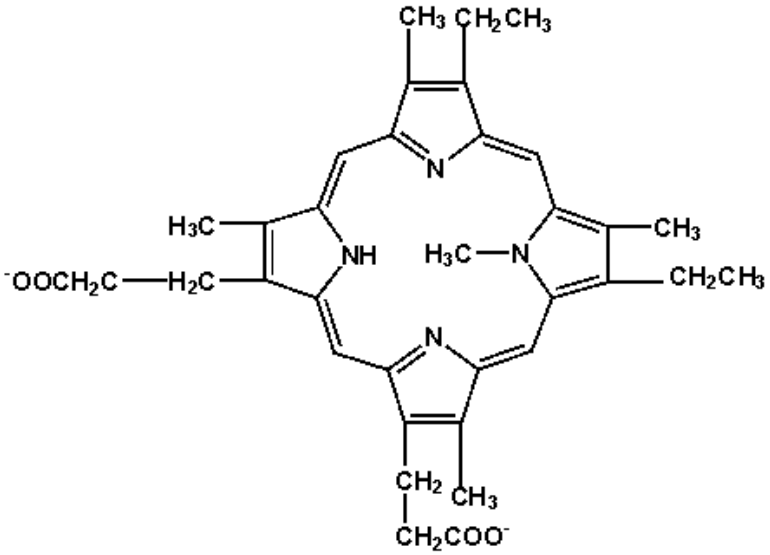

Fig. S3: Detailed structure of N-methyl mesoporphyrin IX (NMM)， which was utilized as fluorescent probe to indicate the G-quadruplex formation in this study.

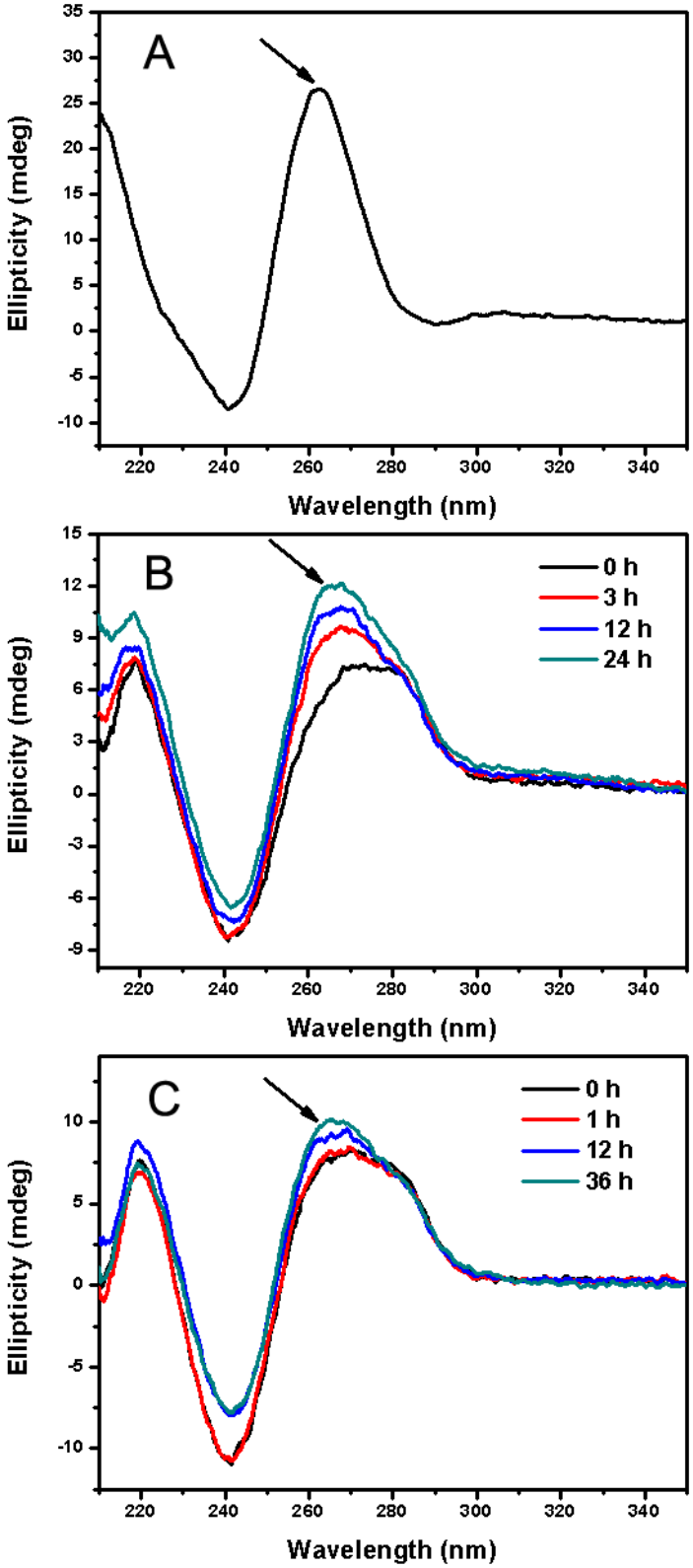

Fig. S4: (A) CD spectrum of the G-quadruplex sequence (PW17: 5'-GGGTAGGGCGGGTTGGG-3'). Experimental condition: 5 μM of PW17 in Tris/Mg/K buffer solution. (B) CD spectra of products via DNA machine A reaction after 0, 3, 12, and 24 h, demonstrating G-quadruplex formation. The concentrations of fuel strands (*H1* and *H2*) and initiator *SI* were 1 μM, 1 μM and 0.5 μM, respectively. (C) CD spectra for DNA machine B reaction after 0, 1, 12, and 36 h, demonstrating G-quadruplex formation. The concentrations of fuel strands (*H3* and *H4*) and initiator *SI* were 1 μM, 1 μM and 0.5 μM respectively.

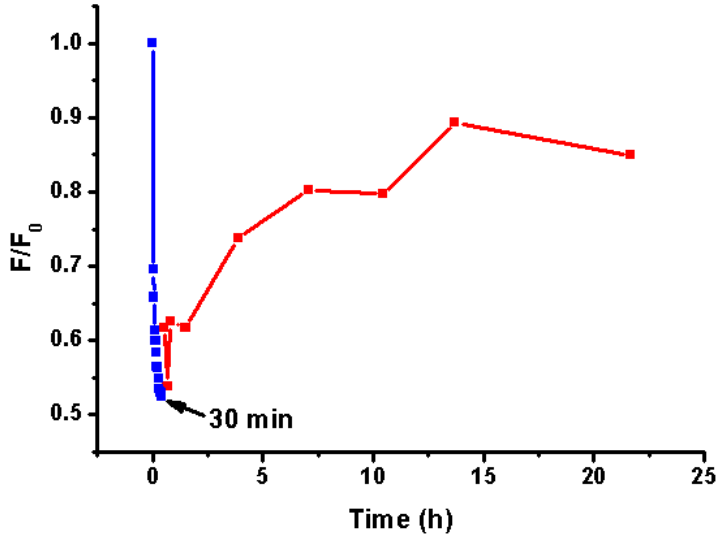

Fig. S5: Fluorescence experiment for demonstrating function of DNA machine A. FAM fluorescence was collected at emission wavelength of 516 nm. Upon mixture of *SI*, *H1* and *H2*, the fluorescence intensity of FAM (6-carboxyfluorescein) tagged at 3' end of initiator *SI* decreased dramatically because it was quenched by the guanosine at 5' end of *H1*, suggesting fast hybridization between *SI* with *H1*. After reaction for 30 minutes, its fluorescence started to increase slowly, indicating that DNA initiator *SI* started to be displaced from the hybrid of *SI_H1* by *H2* and available for next catalytic circuit.

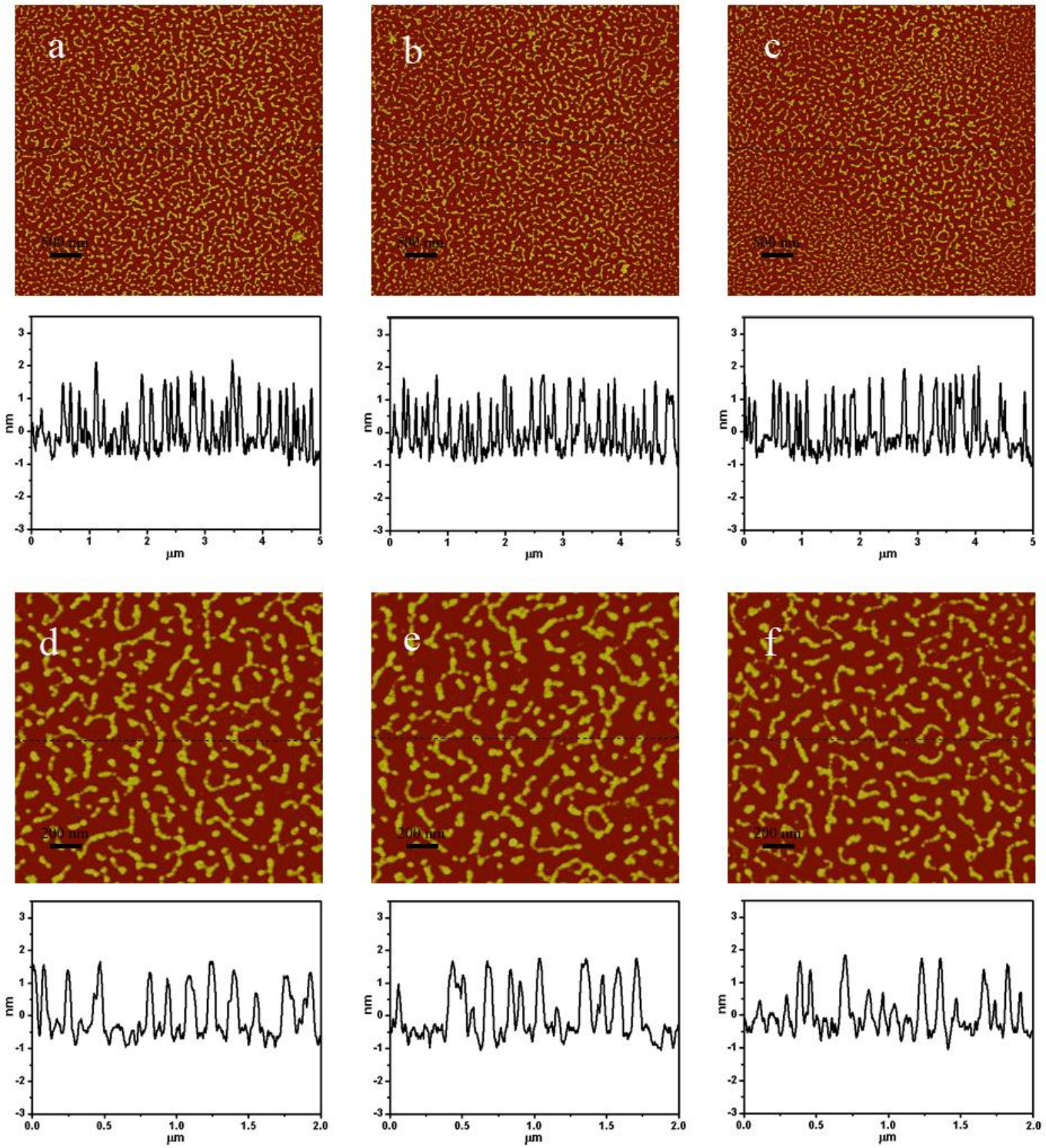

Fig. S6: Typical AFM images and their corresponding cross-section of DNA polymer chains produced via DNA machine B after 2 day's reaction. Scale bars are 500 nm (a-c) and 200 nm (d-f).

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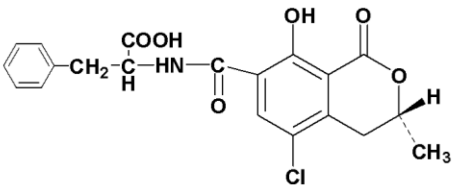
OTA inhibition on DNA machine A: Program A can also be modulated by small molecules, such as ochratoxin A (OTA), a well-known and food-contaminating mycotoxin.[1] The aptamer specific for ochratoxin A[2] was used to catalyse duplex formation of *H5_H6* as shown in Fig. S7-9. When OTA was added into the system, its aptamer folded into antiparallel G-quadruplex.[3] It has been demonstrated that significant secondary structure in initiator strand will slow down the reaction kinetics,[4] so that the function of DNA machine was inhibited at increasing the concentration of ochratoxin A. It has to be noted that the sequences of *H5* and H6 were designed in terms of OTA aptamer and sequence modularity used for *H1* and *H2* in our system. Both agarose gel electrophoresis (Fig. S8) and fluorescence measurement (Fig. S9) can verify that the small molecule can modulate the velocity of catalytic reaction. Therefore, label-free biosensors for small molecules or other targets can be constructed by means of this approach.



Ochratoxin A

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                          c        b          a
Ochratoxin A aptamer   5'- GATCGG  GTGTGGGTG  GCGTAA  AGGGAGCATCGGACA -3'

       a*      b*         c*      a       d          c       b
H5  5'- TTACGC  CACCCACAC  CCGATC  GCGTAA  GGGTAGGGC  GATCGG  GTGTGGGTG -3'

       c*      d*         a*      c       b          a       d          e
H6  5'- CCGATC  GCCCTACCC  TTACGC  GATCGG  GTGTGGGTG  GCGTAA  GGGTAGGGC  GGGTTGGG -3'
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Fig. S7: Structure of Ochratoxin A (OTA) and DNA sequences redesigned for Ochratoxin A inhibition on machine A. In the redesigned program A, OTA aptamer acted as initiator strand S*I*.

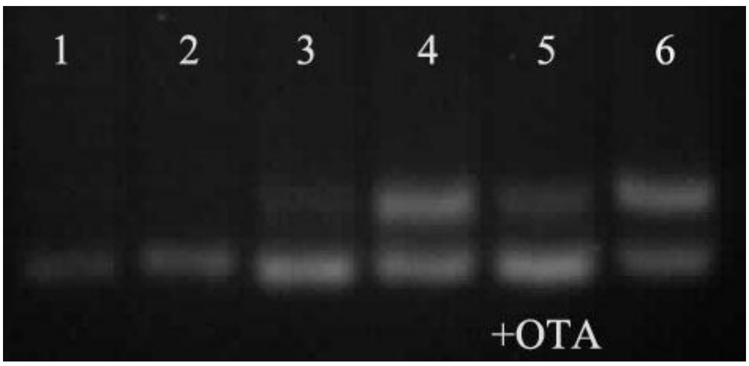

Fig. S8: Agarose gel electrophoresis demonstrating OTA inhibition on the reaction dynamic of machine A. Lane 1 and 2 represent fuel strand *H5* and *H6* respectively. Lane 3: mixture of fuel strands without OTA aptamer. After 12 hours, conversion of fuel strands to reaction products with OTA aptamer (200 nM, lanes 4), minimal conversion in the presence of OTA (10 µM, lane 5). Lane 6: annealed sample of

mixture *H5* and *H6*. The concentration of each fuel strand was 200 nM. Sample with OTA was prepared as follows: 20 μl of OTA aptamer (2 μM) and 20 μl of OTA (100 μM) were mixed, adjusted up to 140 μl with Tris/Mg/K buffer, and incubated at 25 °C for one hour firstly. Then, 20 μl of *H5* (2 μM) and 20 μl of *H6* (2 μM) were added. Other experimental details were the same as those depicted in the experimental section. All samples were kept at 25 °C.

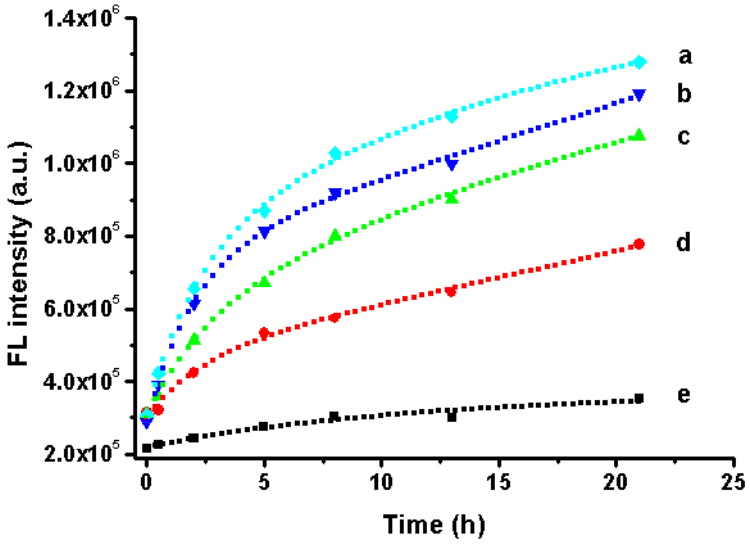

Fig. S9: Fluorescence intensity of NMM as function of time in the presence of various concentrations of OTA. In the system of redesigned DNA machine A, the concentrations of OTA from a to d were 0, 0.2, 1 and 10 μM respectively. Dotted line e represents fluorescence intensity of solution with fuel strands (*H5* and *H6*) in the absence of OTA aptamer and OTA. The reaction concentrations of *H5*, *H6* and OTA aptamer were both 200 nM. Detailed procedures were depicted as follows: 100 μl of OTA (1 μM) or 100 μl of OTA (5 μM) or 50 μl of OTA (100 μM), and 50 μl of OTA aptamer (2 μM) were mixed, adjusted up to 450 μl with Tris/Mg/K buffer, and incubated at 25 °C for one hour. Then 50 μl of NMM (10 μM) was added. After 30 min, 50 μl of annealed *H5* (2 μM) and 50 μl of annealed *H6* (2 μM) were introduced rapidly, and fluorescent signal at 607.5 nm was collected immediately and at intervals at 25 °C. The final reaction volume was 500 μl. Apparatus parameters: slits = 5/5 nm, excitation at 399 nm and emission at 607.5 nm.

References

1. K. J. Van Der Merwe, P. S. Steyn, L. Fourie, D. B. Scott and J. J. Theron, *Nature*, 1965, **205**, 1112.
2. J. A. Cruz-Aguado and G. Penner, *J. Agric. Food. Chem.*, 2008, **56**, 10456.
3. C. Yang, Y. Wang, J.-L. Marty and X. Yang, *Biosens. Bioelectron.*, 2011, **26**, 2724.
4. D. Y. Zhang and E. Winfree, *Nucleic Acids Res.*, 2010, **38**, 4182.