Randomly arrayed G-rich DNA sequence for label-free and real-time assay of enzyme activity

Zhuoliang Liu, Wang Li, Zhou Nie*, Feifei Peng, Yan Huang, and Shouzhuo Yao

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China. Fax: +86-731-88821848; Tel: +86-731-88821626; E-mail: niezhou.hnu@gmail.com

EXPERIMENTAL SECTION

Materials and instruments.

Terminal deoxynucleotidyl transferase (TdT) was purchased from Fermentas Inc. (Vilnius, Lithuania). 2,2'-amino-di(2-ethyl-benzothiazoline sulphonic acid-6) ammonium salt ($ABTS^{2-}$), deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP), the oligonucleotide, SYBR Green II and 2-(4-morpholino) ethanesulfonic acid (MES) were obtained from Sangon (Shanghai, China). Hemin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Human blood serum samples were obtained from Hunan Provincial People's Hospital, China. All other chemicals were of analytical reagent grade and used without further purification. Ultrapure water with an electric resistance of 18.3 MΩ was obtained from a Millipore filtration system and used throughout.

UV-vis absorption spectra were obtained on a Beckman DU-800 spectrophotometer. The circular dichroism (CD) spectra of DNA were collected by a JASCO J-815 spectropolarimeter (Tokyo, Japan). Fluorescence spectra were measured on a QuantaMaster™ fluorescence spectrophotometer, PTI (Canada).

The DNA sequence was:

DNA-primer 5′-AAT ACA ACC TCT CA-3′

Enzymatic generation of G-rich random sequence by TdT.

The typical polymerization experiment was performed in 10 μL of TdT buffer (1×, 0.2 M potassium cacodylate, 0.025 M Tris, 0.01% (v/v) Triton X-100, 1 mM $CoCl_2$, pH 7.2) containing 1 μM DNA-primer, 1 mM dNTP (e.g. 40 % dATP and 60 % dGTP), and 4 U of TdT at 37 °C for 2 h, and terminated by heating the solution at 75 °C for 10 min. To evaluate the effect of the dNTP composition on the activity of randomly synthesized DNAzyme, the polymerization experiments were conducted under the same condition as the typical process except using different compositions of dNTP including various combinations of dGTP (percentage ranging from 50 % to 100 %), dATP (percentage ranging from 0 % to 50 %) and dTTP (percentage ranging from 0 % to 50 %). To detect different concentration of TdT enzyme, the polymerization experiments were conducted under the same condition as the above-mentioned process except using different concentrations of TdT ranging from 0.2 U to 8 U.

Assessment of TdT activity by colorimetric detection.

After the polymerization reaction, 1 μL of 10 μM hemin (prepared in DMSO), 32 μL of 2.5×2-(4-morpholino) ethanesulfonic acid (MES) buffer (final concentration was 100 mM MES-Tris, 40 mM KCl, and 0.05% Triton X-100, pH 5.5), and 21 μL of H_2O were added to the sample. Half an hour later, 8 μL of 20 mM 2,2'-amino-di(2-ethyl-benzothiazoline sulphonic acid-6) ammonium salt ($ABTS^{2-}$) and 8 μL of 20 mM H_2O_2 were added to initiate the bio-catalytic oxidation of $ABTS^{2-}$. UV-vis absorption spectra were obtained on a Beckman DU-800 spectrophotometer at 415 nm in 4 min at 27 ± 2 °C.

Assessment of TdT activity by fluorescence detection.

After the polymerization reaction, 2 μL of 100 μM ThT, 50 μL of 2× Tris-HCl buffer (final concentration was 50 mM Tris-HCL, 50 mM KCl, pH 7.2), and 38 μL of H_2O were added to the sample. Twenty minutes later, the emission spectra were monitored from 445 to 600 nm with excitation at 425 nm.

Real-time fluorescent detection of TdT activity.

The real-time fluorescent detection of TdT activity was conducted in 100 μL of TdT buffer (1×, 0.2 M potassium cacodylate, 0.025 M Tris, 0.01% (v/v) Triton X-100, 1 mM $CoCl_2$, pH 7.2) containing 1 μM DNA-primer, 1 mM dNTP (e.g. 10% dTTP, 40 % dATP and 50 % dGTP), and 20 μM ThT at 37 °C. Different concentration of TdT (0 – 20 U) was added to start the TdT polymerization reaction and the reaction process was monitored by the fluorescence intensity at 485 nm on the fluorescence spectrometer.

TdT activity assay by denaturing 8% PAGE.

For the denaturing 8% PAGE analysis of the TdT polymerization product, TdT polymerization process was conducted as the same as aforementioned. After TdT reaction, the sample was loaded into a denaturing 8% PAGE and the electrophoresis was carried in 1× tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, 7 M urea, pH 8.0) at 85 V for 3 h. The gel was stained by SYBR Green II for 20 min and scanned by a ChemiDoc™ MP System (Bio-Rad).

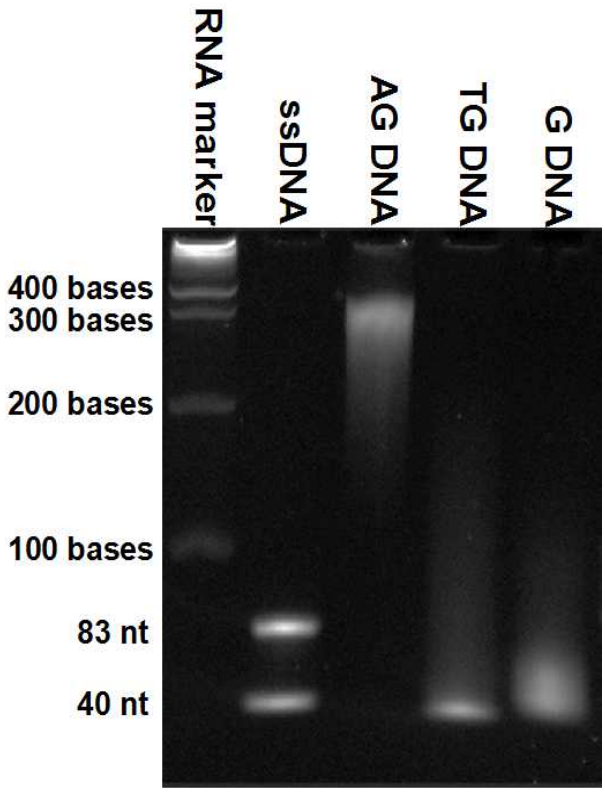

Fig. S1 Denaturing 8% PAGE analysis of the DNA synthesized by TdT. lane 1: RNA marker (RL 1000); lane 2: 40 nt and 83 nt single-stranded DNA; lane 3: AG DNA; lane 4: TG DNA; lane 5: G DNA.

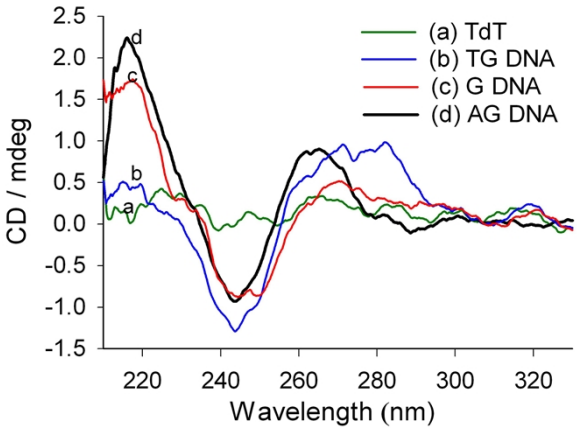

Fig. S2 CD spectra for characterizing the DNA conformation of the TdT-synthesized DNAzyme: (a) TdT only; (b) TG DNA; (c) G DNA; (d) AG DNA. The final concentration of primer DNA and TdT was 0.375 μM and 40 U, respectively.

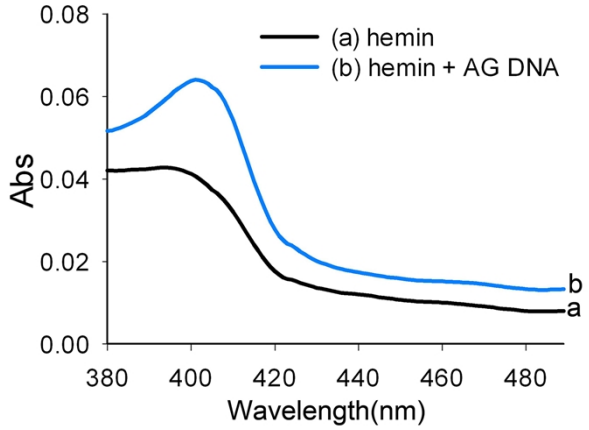

Fig. S3 The assessment of the soret bands of hemin and the complex of TdT-generated G-quadruplex with hemin in MES buffer: (a) 500 nM hemin; (b) 500 nM hemin incubated with AG DNA.

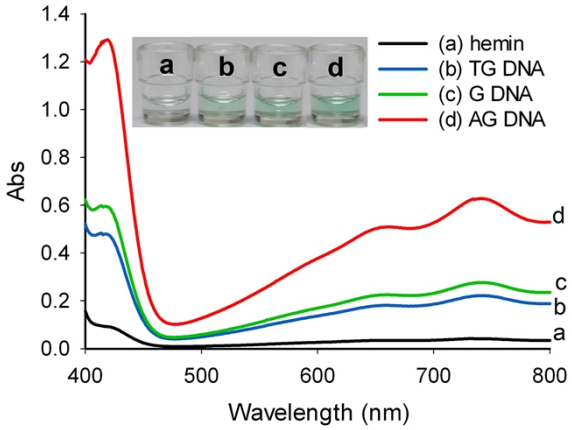

Fig. S4 UV-vis absorption spectra of DNAzyme samples generated by TdT with different dNTP substrate content: (a) hemin only; (b) TG DNA (40% dATP and 60% dGTP) ; (c) G DNA (dGTP only); (d) AG DNA (40% dATP and 60% dGTP). The inset picture shows the visualization analysis of (a) hemin, (b) TG DNA, (c) G DNA, and (d) AG DNA in the $ABTS^{2-}$-H_2O_2 system.

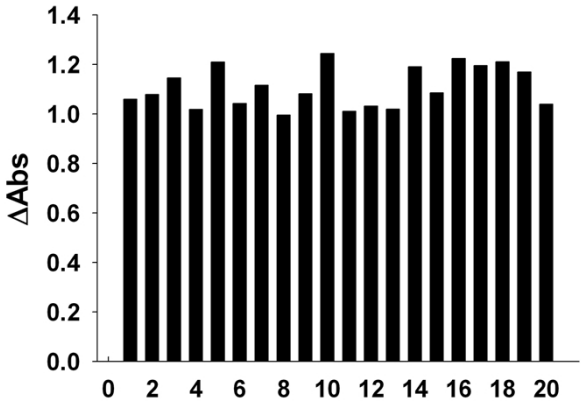

Fig. S5 The repeatability of the catalytic capability of TdT-generated DNAzyme. Each TdT reaction mixture contained 1 μM primer, 4 U TdT, and 1 mM dNTPs (40% dATP and 60% dGTP) and the polymerization reaction took 2 h. The catalytic activity was evaluated by the absorbance change at 415 nm in 4 min obtained in the peroxidation mixture containing 0.125 μM TdT polymerization product, 0.125 μM hemin, 2 mM H_2O_2 and 2 mM $ABTS^{2-}$. The x-coordinate of the figure represents the number of samples.

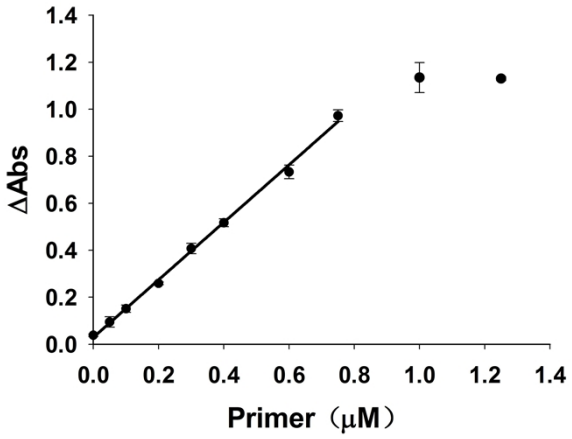

Fig. S6 Effect of the DNA primer concentration on the catalytic capability of TdT-generated DNAzyme. The TdT polymerization was performed with 4 U TdT and 1 mM dNTP (40% dATP and 60% dGTP), and lasted for 2 h.

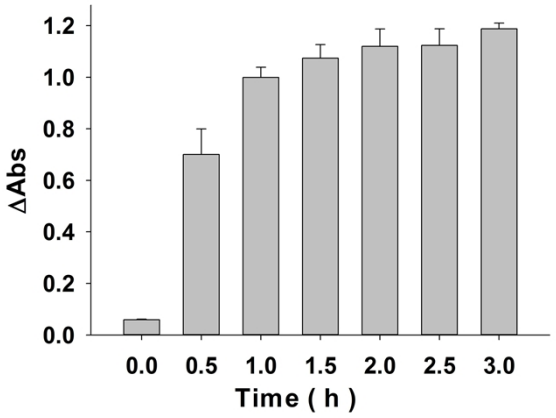

Fig. S7 Effect of TdT reaction time on the catalytic capability of TdT-generated DNAzyme. The TdT polymerization was performed with 1 μM DNA primer, 4 U TdT, and 1 mM dNTP (40% dATP and 60% dGTP).

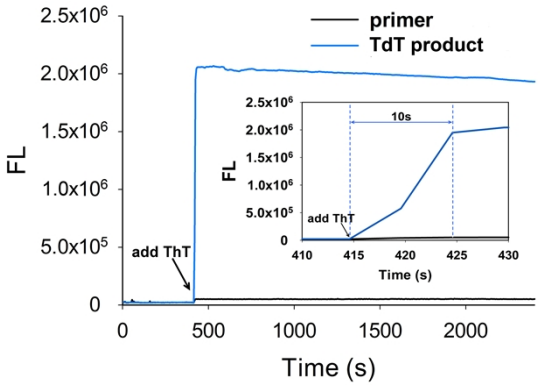

Fig. S8 Kinetic study of fluorescence change after addition of ThT to the TdT-generated G-rich sequence. The TdT-generated G-rich sequence was prepared in the reaction mixture containing 10 μM DNA primer, 40 U TdT, and 10 mM dNTP (10% dTTP, 40% dATP and 50% dGTP). The final concentration of ThT was 20 μM. The total volume of the sample was 1 mL.

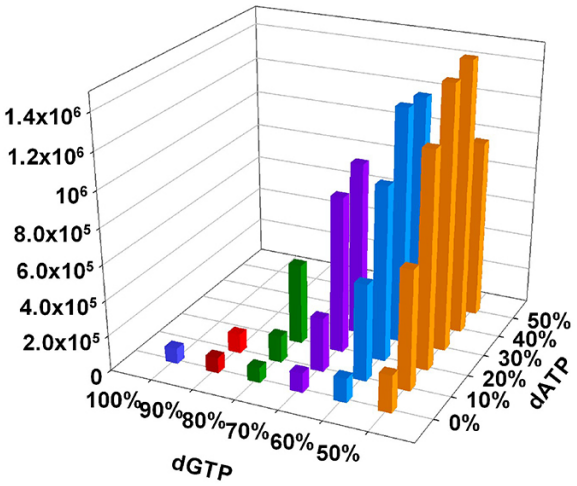

Fig. S9 The ThT fluorescence enhancement via binding with TdT polymerization products as a function of different compositions of three-component substrate pool (dGTP + dATP + dTTP). In all experiments, the TdT reaction mixtures contain DNA primer (1 μM) and TdT (4 U).

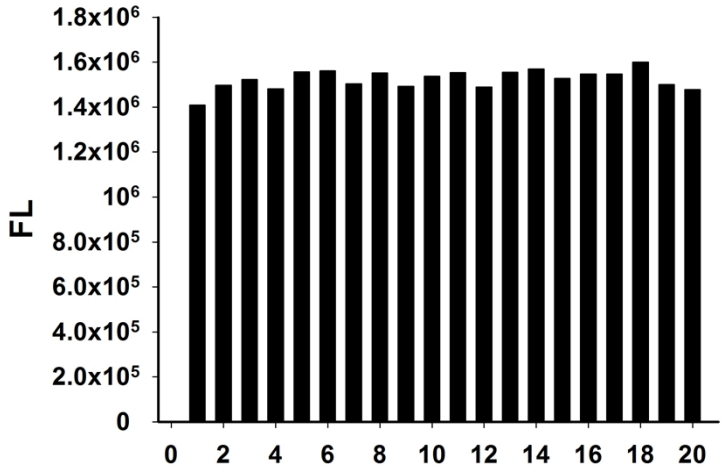

Fig. S10 The repeatability of the fluorescence intensity of TdT-geneated G-quadruplexes. Each TdT reaction mixture contained 1 μM primer, 4 U TdT, and 1 mM dNTPs (50% dGTP, 40% dATP and 10% dTTP) and the polymerization reaction took 2 h. The fluorescence intensity was measured at 485 nm. The x-coordinate of the figure represents the number of samples.

Table S1 Measurement results of TdT in 10% human blood serum.

Sample number	Added (U)	Found (U)[a]	Recovery (%)[a]	RSD(%)[a]
1	1	0.96	96	1.4
2	2	1.97	99	0.7
3	3	2.93	98	1.2
4	4	3.93	98	1.6

[a] the average value of three parallel determinations.