Supporting Information

The noncovalent dimerization of a G-quadruplex/hemin DNAzyme improves its biocatalytic properties

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G-quadruplex is short as GQ in both main text and supporting material.

Contents

Materials and methods

DNA samples and reagents

Ultra-PAGE or HPLC purified DNA oligonucleotides were purchased from Sangon Biotech (Shanghai, China) without further purification. All the DNA sequences used for the formation of DNAzymes are listed in **Table S1**. Distilled and deionized H_2O (18.2 MΩ, Milli-Q A10) was used for all the experiments. The strand concentration of each DNA sample in H_2O was determined by measuring the UV absorbance at λ=260 nm, using the corresponding molar extinction coefficient value. *For clarity, the concentrations of all oligonucleotides were given as the concentration of GQ, which contains three stacked G-quartets in this work.* Unless otherwise stated, *DNA samples were prepared in 100 μM GQ concentration with 100 mM KCl and heated at 95 ℃ for 3 min, cooled slowly to room temperature, and then diluted to corresponding GQ concentration before use*. Oligonucleotide stock solutions were incubated under 4 ℃ for at least one week before use in order to achieve the dynamic equilibrium between folded GQ and random coil strands.[1] Prior to perform enzyme assay or characteristic experiments, GQ was diluted to corresponding concentration and incubated at room temperature for at least one hour. All experiments in this work were repeated at least three times.

N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) (99.5%, Aladdin) was dissolved in H_2O to 50 mM. Hemin (97%, Sigma) was dissolved in DMSO, diluted to 1 mM and stored in the dark at 4℃. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) (98%, Sigma), NADH disodium salt (92%, Sangon) and dopamine hydrochloride (99%, Alfa) were dissolved in H_2O to 50 mM. L-tyrosine (99%, Alfa), D-tyrosine (99%, Alfa) and 3, 4-dihydroxyl-L-phenylalanine (L-dopa) (99%, Adamas) were dissolved in 1 M HCl to 50 mM. Luminol (97%, Sigma) was dissolved in 0.1 M NaOH to 100 mM. 3, 3', 5, 5'-tetramethylbenzidine (TMB) (99%, Aladdin) was dissolved in DMSO to 5 mM. Thioanisole (95%, Alfa) and methyl phenyl sulfoxide (98%, TCI) were dissolved in CH_3CN to 0.5 M. Unless otherwise stated, experiments were conducted in 40 mM pH 7.5 HEPES buffer containing 100 mM KCl (99%, Alfa). For analysis of pH effects, the experiments were conducted in 40 mM HEPES buffer (pH from 3.0 to 10.0) containing 100 mM KCl. For analysis of ion effects, the experiments were conducted in 40 mM HEPES (pH 7.5) containing 100 mM NaCl (99%, Macklin) or 100 mM NH_4Cl (99.5%, Kermel) or different concentrations of KCl ranging from 0 mM to 300 mM.

Circular dichroism (CD) spectroscopy

Circular dichroism (CD) experiments were carried out on a dual beam DSM 1000 CD spectrophotometer (Olis, Bogart, GA) equipped with a Peltier temperature controller, using a quartz cell of 1.0-cm path length and 3.0 mL volume. The scanning rate was automatically selected by the Olis software as a function of the signal intensity to optimize data collection. CD spectra were recorded both in the absence and presence of hemin. The final concentration of GQ was 10 μM in 40 mM pH 7.5 HEPES buffer containing 100 mM KCl and 0.05 % (v/v) triton X-100. The final concentration of hemin was 10 μM. Spectra were recorded from λ=220 to λ=320 nm at 20℃. For CD melting experiments, temperatures were increased from 20 ℃ to 80 ℃, with 5℃ as an increment and spectra were recorded after the incubation of 10 min at the corresponding temperature. All CD spectra were averaged from five scans. A background spectrum of the buffer was subtracted for CD spectra.

Non-denaturing polyacrylamide gel electrophoresis (PAGE)

Unless otherwise stated, 50 μM GQs were incubated in 1×Tris-Borate-EDTA (TBE) buffer in the absence or presence of 100 μM hemin at 25℃ for 30 minutes (For samples containing hemin, 0.05% (v/v) triton X-100 was added). Samples were run on a 20% native polyacrylamide gel (7 cm × 10 cm), made up in 1×TBE buffer containing 100 mM KCl (1×TBE buffer containing 20 mM KCl used for the running buffer) for 3 or 4 hours at 100 V. Gels were stained with Stains-all (Sigma, 95%) and then destained under the sunlight. Gel pictures were taken by the camera of smart-phone and given in this manuscript without any further processing.

1D ^{1}H NMR

^{1}H NMR experiments were performed on a 700 MHz Bruker Avance III HD spectrometer equipped with a cryogenic QCI probe at 20 ℃. The final concentration of GQ was 100 μM in 40 mM KPi buffer (pH 7.5) in the absence or presence of hemin, and the corresponding total potassium concentration was complemented by adding extra KCl. For potassium concentration dependent NMR experiments, samples were 100 μM GQ in H_2O containing corresponding KCl. Only in NMR experiments, hemin was dissolved in 50 mM KOH, no triton X-100 was added and the final concentration of hemin was 100 μM. For all experiments, 10 % (v/v) D_2O was added and the WATERGATE pulse program was used to recording ^{1}H spectra while suppressing the water signal.

Size exclusion chromatography (SEC) - HPLC

SEC-HPLC analysis[2] was performed on an Agilent 1100 Series instrument with the eluent of 40 mM pH 7.5 HEPES buffer containing 20 mM KCl at the velocity of 0.15 ml/min, using a AdvanceBio SEC column (4.6×150 mm; 2.7 μm high-porosity silica particles, 300 Å pore size; Part number: PL1580-3301). Absorbance was detected at λ=260 for samples without hemin or λ=404 nm for samples with hemin. Unless otherwise stated, samples were 10 μM GQ in 40 mM pH 7.5 HEPES buffer containing 100 mM KCl in the absence or presence of 2 μM hemin. For samples containing hemin, 0.05% (v/v) triton X-100 was added. It should be noted that the column efficiency will decrease resulting from the adsorption of hemin to column. Completely removing hemin from column should be confirmed before the next injection.

DNAzyme activity assay

The annealed DNA samples (100 μM) were diluted by HEPES buffer containing 0.05% (v/v) triton X-100, and incubated with hemin for 30 minutes at 25 ℃. After the formation of GQ/hemin complexes, substrate was added, and then H_2O_2 was added to start the reaction. For ABTS, TMB, L-tyrosine, D-tyrosine, L-Dopa and dopamine as substrates, the product formations were tracked by monitoring the corresponding absorbances using a Shimadzu 2450 spectrophotometer equipped with a Peltier temperature control accessory. The samples were measured in a sealed quartz cell of 1.0-cm path length and 3.0-mL volume. The total volume of reaction system is 2.0 mL. For NADH as the

substrate, consumption of NADH was monitored by the same way. For luminol as the substrate, the chemiluminescence (CL) emission within 3 minutes was recorded by fluorescence spectrophotometer (Edinburgh, Inc.). For thioanisole as the substrate, the product was monitored by a chiral HPLC, which has been described in our previous work.[3]

Activity of the DNAzyme was evaluated by the initial rate (V_0), which was obtained from the slope of the initial linear portion (the first 10 seconds) of the plot of absorbance versus reaction time, using molar extinction coefficients ε of 36000 M^{-1} cm^{-1} for $ABTS^{\cdot+}$ at λ=414 nm, 39000 M^{-1} $cm^{-1}$for $TMB^{\cdot+}$ at λ=652 nm and 59000 M^{-1} cm^{-1} for diimmine at λ=450 nm, 6220 M^{-1} cm^{-1} for NADH at λ=340 nm. V_0 was calculated according to equation 1:

$$V_0 = \frac{|dC|}{dt} = \frac{|dA|}{dt \times b \times \varepsilon} \qquad (1)$$

Where C is the real-time concentration of substrate or product; t is the reaction time; A is the absorbance of corresponding substrate or product; b is the light path length, which is 1.0 cm; ε is the molar extinction coefficient. For luminol, A denotes the integrated chemical luminescence. All the measurements were repeated at least three times, and the background activity of hemin alone was subtracted.

Kinetic of DNAzyme degradation

DNAzyme degradation experiments were carried out on a Shimadzu 2450 spectrophotometer equipped with a Peltier temperature control accessory. 0.25 μM GQs were incubated with 0.5 μM hemin in 40 mM HEPES buffer (pH 7.5) containing 100 mM KCl and 0.05% (v/v) triton X-100 at 25 ℃ for 30 minutes. After addition of 0.5 mM H_2O_2, the absorbance at λ=404 nm was monitored for 30 minutes and the initial apparent degradation velocity V_d was obtained from the initial linear portion (the first 2 minutes) in the plot of absorbance versus reaction time, by using equation 2：

$$V_d = \frac{|dC_{complex}|}{dt} = \frac{|dA|}{dt \times b \times \varepsilon_{complex}} \qquad (2)$$

Where $C_{complex}$ is the real-time concentration of GQ/hemin complex; t is the reaction time of degradation; A is the absorbance of GQ/hemin complex at λ=404 nm; b is the light path length, which is 1.0 cm; ε is the molar extinction coefficient of GQ/hemin complex at 404 nm, which is obtained from **Figure S11** and summarized in **Table S3**.

UV/Vis absorption spectroscopy

All the experiments were carried out in a Shimadzu 2450 equipped with a Peltier temperature control accessory and spectra were collected from λ=300 nm to λ=700 nm in 40 mM HEPES containing KCl and 0.05% (v/v) triton X-100. GQs were incubated with hemin at 25 ℃ for 30 minutes before experiments.

To measure the characteristic absorption of DNAzymes, 5 μM GQ and 2 μM hemin were used to keep the hemin been bound by DNA. For pH titration experiments, GQ was 5 μM and hemin was 5 μM. For Job plot experiments, the total concentrations of GQ and hemin were kept 10 μM, with the concentrations of GQ varying from 0 to 10 μM. For measurement of dissociation constant (K_d), a spectrophotometric titration of 2 μM hemin with increasing concentrations of DNA in 0 to 13 μM range was performed. The titration was stop until no change of absorbance at λ=404 nm. The saturation curves for the binding of hemin with GQs were determined by plotting fraction of bound hemin (α, determined by equation 3) as a function of GQ concentration and fitting with a one-site binding model to get the K_d value for every DNA sequence:

$$\alpha = \frac{A_x - A_0}{A_\infty - A_0} \quad (3)$$

where A_x is the absorbance at λ=404 nm of hemin incubated with varied concentration of DNA, A_∞ and A_0 are the absorbances at λ=404 nm in the presence of saturating DNA and in the absence of DNA, respectively. Data was fitted by Hill equation by Origin software, and K_d of hemin with GQ could be got from the fitting result.

Tables

Table S1 DNA sequences used in this work.

3' variant		5' variant	
Name	Sequence(5'→3')	Name	Sequence(5'→3')
$TTAG_3TT$	TTAGGGTT	$TT(G_3T)_4$	TTGGGTGGGTGGGTGGGT
$TTAG_3T$	TTAGGGT	$T(G_3T)_4$	TGGGTGGGTGGGTGGGT
$TTAG_3p$	TTAGGGp [b]	$(G_3T)_4$	GGGTGGGTGGGTGGGT
$TTAG_3$	TTAGGG [a]		
$TTAG_3AA$	TTAGGGGAA	$AA(G_3T)_4$	AAGGGTGGGTGGGTGGGT
$TTAG_3A$	TTAGGGA	$A(G_3T)_4$	AGGGTGGGTGGGTGGGT
$TTAG_4TT$	TTAGGGGTT		
$TTAG_4T$	TTAGGGGT		
$TTAG_4$	TTAGGGG [a]		
$TTAG_5TT$	TTAGGGGGTT		
$TTAG_5T$	TTAGGGGGT		
$TTAG_5$	TTAGGGGG [a]		
TTG_3	TTGGG		
AAG_3	AAGGG		
TAG_3	TAGGG		
ATG_3	ATGGG		
TTAGCG	TTAGCG		
TTACCG	TTACCG		

[a] The truncated sequences of $TTAG_n$ (n=3-5) adopt a homodimer of two GQ units through π-π stacking of the external G-quartet at their 3' interface.[4-6] Others mainly adopt a monomer because of the steric effect introduced by terminal one or two thymine (T) nucleotides, d(T) or d(TT).[4-6]

[b] Phosphorylation at 3' end. It predominantly folds into a monomer tetramolecular GQ according to the finding that negative charge-charge repulsion between terminal phosphate groups inhibits the dimerization of GQs to some extent.[7]

Table S2 Percent of dimeric GQ formed by various sequences obtained by SEC-HPLC experiments.[a]

Complex ([KCl]/ mM)	[GQ] / μM	Dimer (%)	Monomer (%)
$TTAG_3$	1	59±3	41±3
	2.5	61±3	39±3
	5	60±3	40±3
	10	61±1	39±1
	25	60±2	40±2
	50	60±2	40±2
$TTAG_3$ (0) [b]	10	1±0.02	99±0.02
$TTAG_3$ (10) [b]		7±0.5	93±0.5
$TTAG_3$ (25) [b]		37±0.6	63±0.6
$TTAG_3$ (50) [b]		47±0.1	53±0.1
$TTAG_3$ (100) [b]		61±1	39±1
$TTAG_3$ (300) [b]		73±0.4	27±0.4
$TTAG_3TT$	10	3±0.1	97±0.1
$TTAG_3TT$+hemin		3±0.01	97±0.01
$TTAG_3T$		37±2	63±2
$TTAG_3T$+hemin		37±2	63±2
$TTAG_3p$		31±0.2	69±0.2
$TTAG_3p$+hemin		31±0.8	69±0.8
$TTAG_3$+hemin		61±1	39±1
$TT(G_3T)_4$		0	100
$TT(G_3T)_4$+hemin		4±0.2	96±0.2
$T(G_3T)_4$		7±1	93±1
$T(G_3T)_4$+hemin		9±0.4	91±0.4
$(G_3T)_4$		100	0
$(G_3T)_4$+hemin		100	0

[a] Unless otherwise stated, experiments were carried out in presence of 100 mM potassium.

[b] 5'-$TTAG_3$-3' was annealed in different concentrations of KCl and measurements were carried in corresponding concentrations of KCl. Number in the parentheses are the potassium concentrations: 0, 10, 25, 50, 100, 300 mM.

Table S3 Kinetic measurement results of DNAzymes.[a]

Complex	V_{max} / nM s^{-1}	k_{cat} / s^{-1}	K_m / mM	k_{cat}/K_m / (s^{-1} mM^{-1})
$TTAG_3TT$	49	0.10	0.34	0.29
$TTAG_3T$	158	0.32	0.75	0.43
$TTAG_3p$	190	0.38	0.66	0.58
$TTAG_3$	860	1.72	1.1	1.56
$TTAG_3$*	4095	8.19	3.81	2.15
$TTAG_4TT$	55	0.11	0.45	0.24
$TTAG_4T$	49	0.098	0.16	0.61
$TTAG_4$	482	0.96	0.78	1.23
$TTAG_5TT$	26	0.053	0.18	0.29
$TTAG_5T$	39	0.078	0.17	0.46
$TTAG_5$	255	0.51	0.41	1.24

[a] Data were obtained from **Figure S8**.

Table S4 Catalytic activities of mutated and covalent multimeric DNAzymes.[a]

Complex	V_0 / (nM^{-1} s^{-1})
TAG_3	246
AAG_3	228
TTG_3	229
ATG_3	210
$TTAG_3$	263
TTAGCG	8
TTACCG	10
46AG[b]	11
70AG[b]	12
HT50[c]	9

[a] Data were obtained from **Figure S10**.

[b] 46AG and 70 AG were covalent multimeric DNA sequences.[8]

[c] HT50 was used as migration markers for dimer GQs in the PAGE analysis.[9]

Table S5 Interaction (apparent dissociation constant K_d, acid-alkaline transition pK_a) and UV-visible absorption parameters (Soret, E and D band) between GQ and hemin.

Complex ([KCl]/ mM)	K_d / μM [a]	pK_a [b]	Soret band [c]		E band [c]	D band [c]		Spin/ Coord.
			λ (nm)	ε_M (M^{-1} cm^{-1})	λ (nm)	nm	ε_M (M^{-1} cm^{-1})	
hemin	---	---	---	---	---	---	---	---
$TTAG_3TT$	n.d. [e]	6.92 ± 0.05	394 404	1.2×10^5 1.1×10^5	---	---	---	---
$TTAG_3T$	5.96 ± 0.20	7.67 ± 0.04	403	1.3×10^5	---	---	---	---
$TTAG_3p$	3.91 ± 0.20	8.35 ± 0.09	404	1.4×10^5	502	630	3.4×10^4	HS/6C
$TTAG_3(10)$ [d]	n.d. [e]	n.d. [e]	398	1.1×10^5	---	---	---	---
$TTAG_3(25)$ [d]	5.68 ± 1.08	8.23 ± 0.07	403	1.3×10^5	---	---	---	---
$TTAG_3(50)$ [d]	4.16 ± 0.26	8.03 ± 0.21	404	1.5×10^5	502	628	3.3×10^4	HS/6C
$TTAG_3(100)$ [d]	2.84 ± 0.20	8.72 ± 0.20	404	1.7×10^5	501	630	3.4×10^4	HS/6C
$TTAG_3(300)$ [d]	0.96 ± 0.06	8.46 ± 0.17	405	2.0×10^5	502	625	3.2×10^4	HS/6C
$TTAG_4TT$	---	---	404	1.0×10^5	---	---	---	---
$TTAG_4T$	---	---	404	1.4×10^5	---	---	---	---
$TTAG_4$	---	---	404	1.6×10^5	---	---	---	---
$TTAG_5TT$	---	---	404	1.0×10^5	---	---	---	---
$TTAG_5T$	---	---	404	1.5×10^5	---	---	---	---
$TTAG_5$	---	---	404	1.5×10^5	---	---	---	---
$TT(G_3T)_4$	0.82 ± 0.06	8.37 ± 0.08	404	1.8×10^5	500	632	3.6×10^4	HS/6C
$T(G_3T)_4$	0.96 ± 0.07	8.34 ± 0.05	404	1.9×10^5	500	632	3.6×10^4	HS/6C
$(G_3T)_4$	0.95 ± 0.10	8.10 ± 0.06	404	1.8×10^5	500	632	3.6×10^4	HS/6C
PS2.M	0.027 ± 0.002 [f]	8.70 ± 0.03 [g]	404 [h]	1.9×10^5 [h]	500 [h]	625 [h]	1.9×10^4 [h]	HS/6C [h]
Met Mb [i]	---	8.93 ± 0.03 [g]	404 [h]	1.9×10^5 [h]	502 [h]	630 [h]	0.4×10^4 [h]	HS/6C [h]
HRP [j]	---	10.9 ± 0.4 [g]	402 [h]	1.1×10^5 [h]	497 [h]	645 [h]	0.3×10^4 [h]	HS/5C [h]

[a] Unless otherwise stated, different concentrations of GQ were titrated into hemin (2 μM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100 (**Figure S12**).

[b] Absorbance from λ=300 to λ=700 nm were recorded within GQ (5 μM) and hemin (5 μM) in 40 mM HEPES buffer (pH 3.0 to 10) with 100 mM KCl and 0.05% (v/v) triton X-100 (**Figure S13**).

[c] Measurement were performed with GQ (5 μM) and hemin (2 μM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100 (**Figure S11**).

[d] 5'-$TTAG_3$-3' was annealed in different concentrations of KCl and measurements were carried in corresponding concentrations of KCl. Number in the parentheses are the potassium concentrations: 10, 25, 50, 100, 300 mM.

[e] It cannot be determined because of the weak interaction.

[f] Quoted value is from reference.[10]

[g] Quoted values are from reference.[11]

[h] Quoted values are from reference.[12]

[i] Met Mb is short for metmyoglobin.

[j] HRP is short for horseradish peroxidase.

Figures

Figure S1 1D ^{1}H NMR spectra of GQs with varied potassium concentration.

Six peaks assigned to the imino protons of guanines in the chemical shift range of 12-10 ppm, which are indicative of the formation of Hoogsteen hydrogen bonding of G-quartet, were observed in the spectra of 5'-TTAG$_3$-3' in 100 mM potassium. Three of them belong to monomeric GQ and the remaining three belong to dimer.[4-5] Only monomer formed by 5'-TTAG$_3$T-3' or 5'-TTAG$_3$TT-3' was detected by NMR spectra, which is consistent to previous NMR studies.[4-5,13-14]

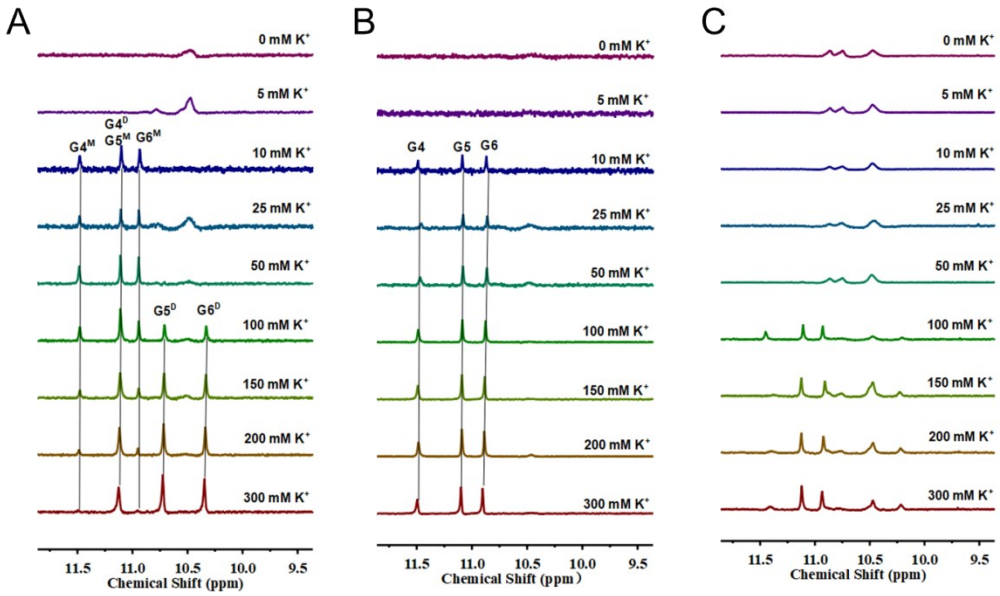


Figure S1 ^{1}H NMR spectra of (**A**) 5'-TTAG$_3$-3', (**B**) 5'-TTAG$_3$T-3' and (**C**) 5'-TTAG$_3$TT-3' (100 μM) in different K^+ concentrations (0, 5, 10, 25, 50, 100, 150, 200, 300 mM) in 10% (v/v) D_2O/H_2O was added ,at 20 ℃. The imino protons were assigned according to the previously published articles.[4,13]

Figure S2 CD spectra of DNA with and without hemin.

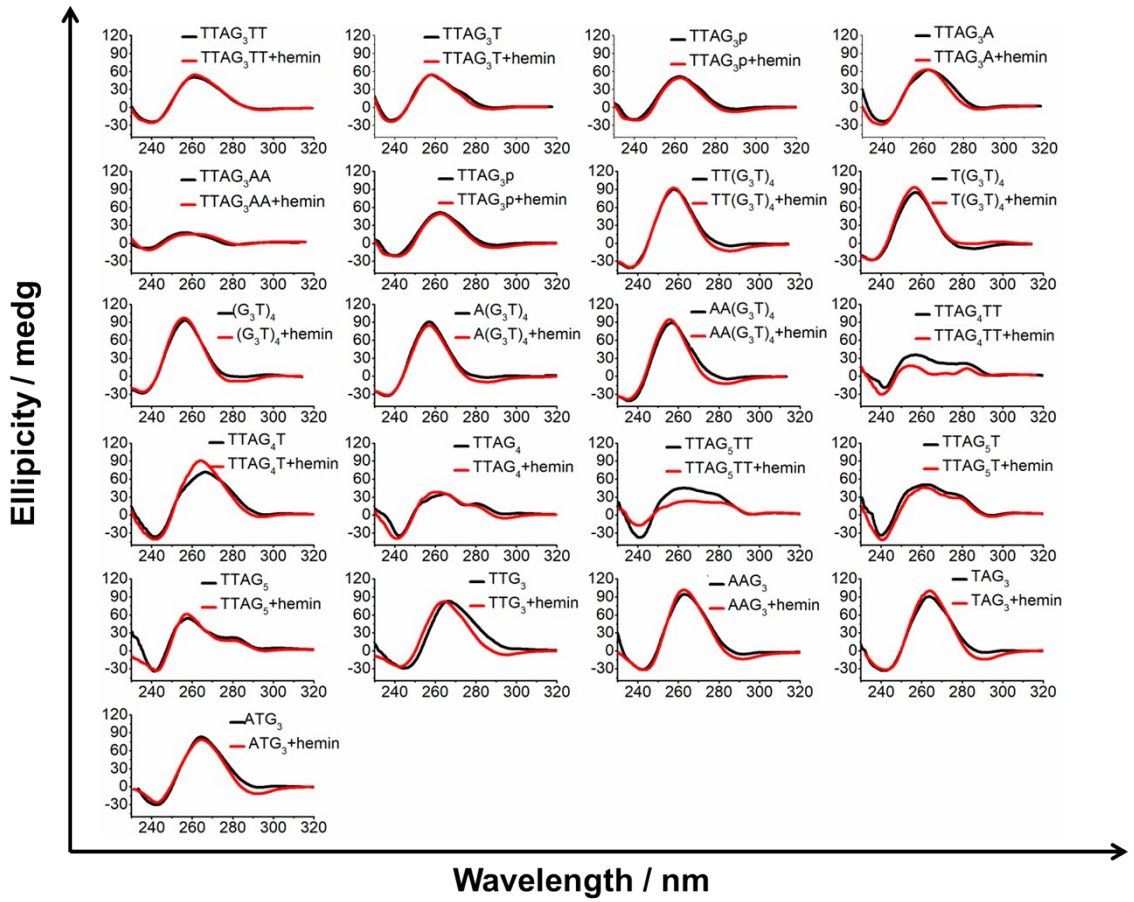


Figure S2 CD spectra from λ=230 nm to λ=320 nm of all the DNA sequences used in the experiment in absence and presence of hemin (10 μM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl, and 0.05% (v/v) triton X-100,at 20 ℃. Sequences were listed in **Table S1**.

Figure S3 Non-denaturing PAGE of DNA samples.

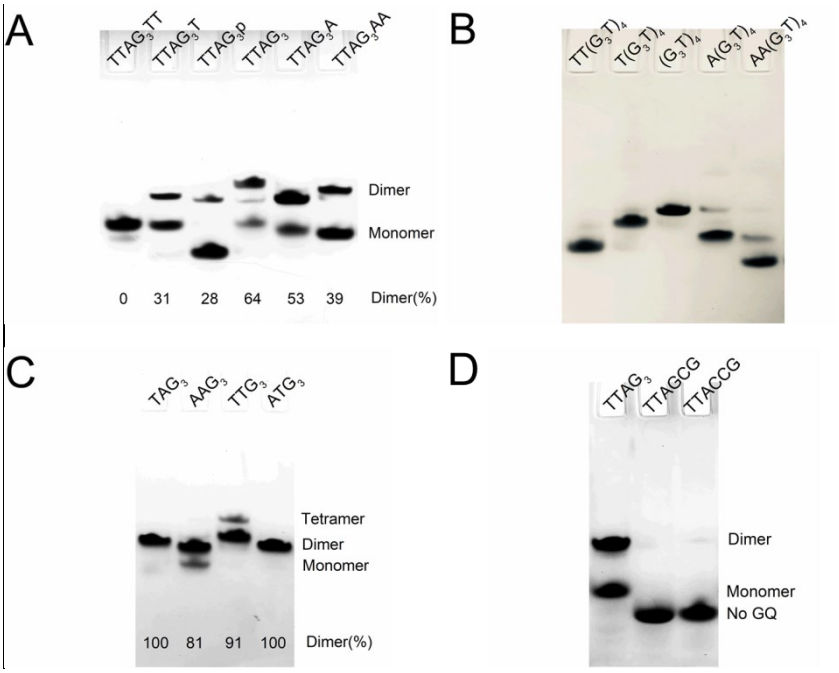


Figure S3 PAGE analysis of the dimerization of (**A**) 5'-TTAG$_3$TT-3', 5'-TTAG$_3$T-3', 5'-TTAG$_3$p-3', 5'-TTAG$_3$-3', 5'-TTAG$_3$A-3', 5'-TTAG$_3$AA-3'; (**B**) 5'-TT(G$_3$T)$_4$-3', 5'-T(G$_3$T)$_4$-3', 5'-(G$_3$T)$_4$-3', 5'-A(G$_3$T)$_4$-3', 5'-AA(G$_3$T)$_4$-3'; (**C**) 5'-TAG$_3$-3', 5'-AAG$_3$-3', 5'-TTG$_3$-3', 5'-ATG$_3$-3'; (**D**) 5'-TTAG$_3$-3', 5'-TTAGCG-3', 5'-TTACCG-3' in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl. Loading samples containing 50 μM GQ and 1×TBE buffer were run on a 20% native polyacrylamide gel made up in 1× TBE buffer with 100 mM KCl (1× TBE buffer with 20 mM KCl used for the running buffer). Samples were run for 3 hours at 100 V, except for **S3B** for 4 hours at 100 V. Gels were stained with Stains-all (Sigma, 95%) and then destained under the sunlight. Percent of dimer (%) is given. Sequences are listed in **Table S1**.

Figure S4 CD melting experiment of 5'-TTAG$_3$-3'/hemin DNAzyme.

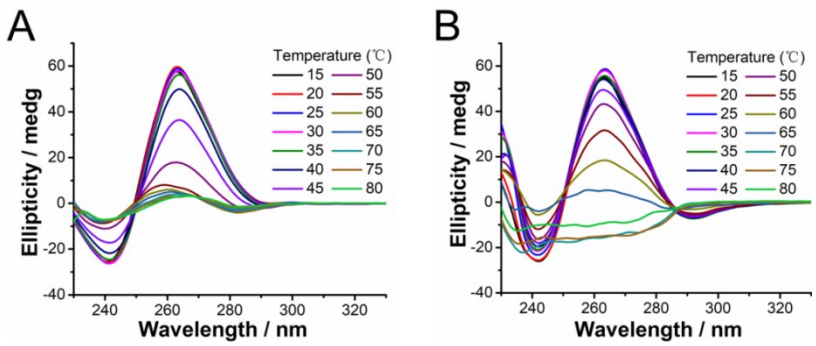


Figure S4 CD melting experiment of 5'-TTAG$_3$-3'/hemin DNAzyme. CD spectra from λ=230 nm to λ=330 nm of 5'-TTAG$_3$-3' (10 μM) in absence (**A**) and presence of hemin (5 μM, **B**) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% triton X-100, from 15 to 80 ^{o}C.

Figure S5 5'-TTAG$_3$-3' in Li^+, Na^+, NH_4^+ and Mg^{2+}.

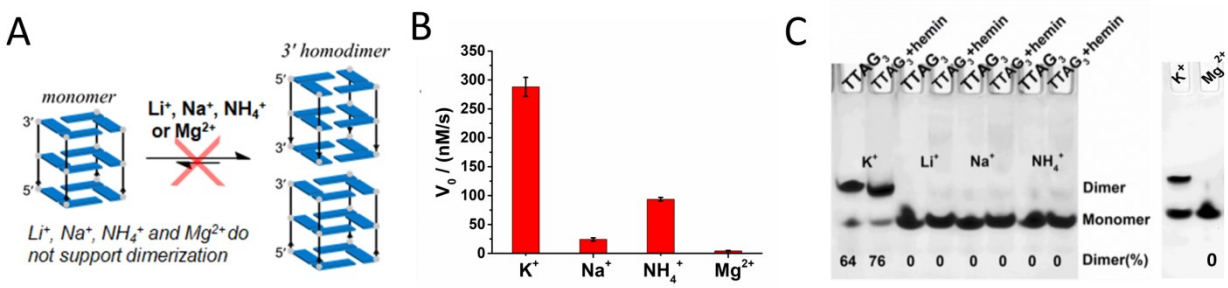


Figure S5 (**A**) Schematic representation of the Li^+, Na^+, NH_4^+ and Mg^{2+} do not support GQ dimerization of 5'-TTAG$_3$-3'. (**B**) Catalytic activities of 5'-TTAG$_3$T-3' in different cations. The reactions were performed with GQ (0.25 μM) and hemin (0.5 μM) in presence of ABTS (0.5 mM) and H_2O_2 (0.5 mM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl, or LiCl, or NaCl, or NH_4Cl or 10 mM $MgCl_2$ and 0.05% (v/v) triton X-100, at 25 °C. (**C**) PAGE analysis of 5'-TTAG$_3$-3' (50 μM), with and without hemin (100 μM), in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl, LiCl, NaCl and NH_4Cl (left). PAGE analysis of 5'-TTAG$_3$-3' (50 μM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 10 mM $MgCl_2$ (right).

Figure S6 Influence of the pH and temperature on the DNAzyme experiments.

The buffer pH influences the catalytic activities of DNAzymes through controlling the exchange rate of OH^- or H^+ with H_2O_2 to coordinate the iron center of hemin.[15] Therefore, catalytic activities are dependent on the pH of solution and the optimal pH is near to 7.5 for both 3' and 5' variant DNAzymes (panels **A** and **B**). Similarly, the temperature of the experiments can influence the catalytic rate of the DNAzyme. Here, increasing the temperature (from 20 to 45°C) results in a decrease of the catalytic activity (**C**).

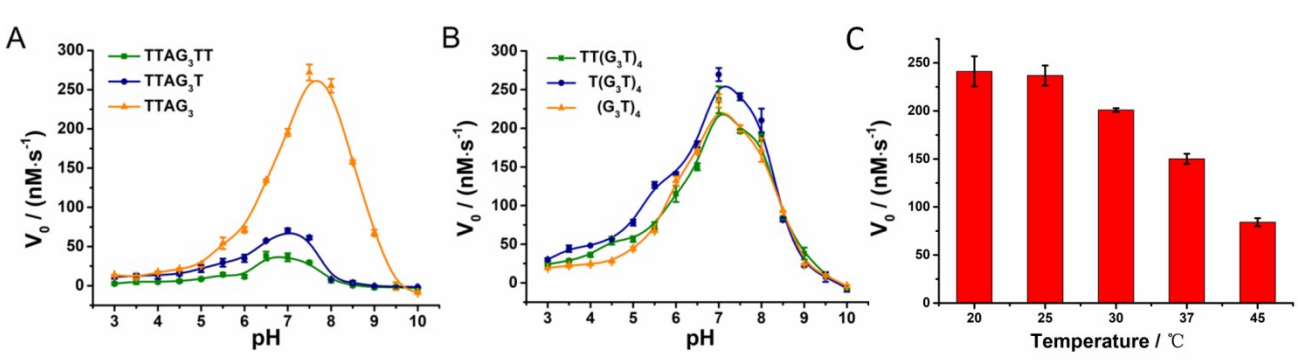


Figure S6 Catalytic activities of non-covalent dimer GQ-DNAzymes formed from 3'-homodimers (**A**, 5'-$TTAG_3TT$-3', 5'-$TTAG_3T$-3' and 5'-$TTAG_3$-3') and 5'-homodimers (**B**, 5'-$TT(G_3T)_4$-3', 5'-$T(G_3T)_4$-3' and 5'-$(G_3T)_4$-3'). The reactions were performed with GQ (0.25 μM) and hemin (0.5 μM) in presence of ABTS (0.5 mM) and H_2O_2 (0.5 mM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100, at 25 °C. (**C**) Catalytic activity of 5'-$TTAG_3TT$-3' at 20, 25, 30, 37 and 45 °C. The reactions were performed with GQ (1 μM) and hemin (0.1 μM) in presence of ABTS (2 mM) and H_2O_2 (1 mM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100

Figure S7 DNA concentration dependent experiment of 5'-TTAG$_3$-3' /hemin DNAzyme.

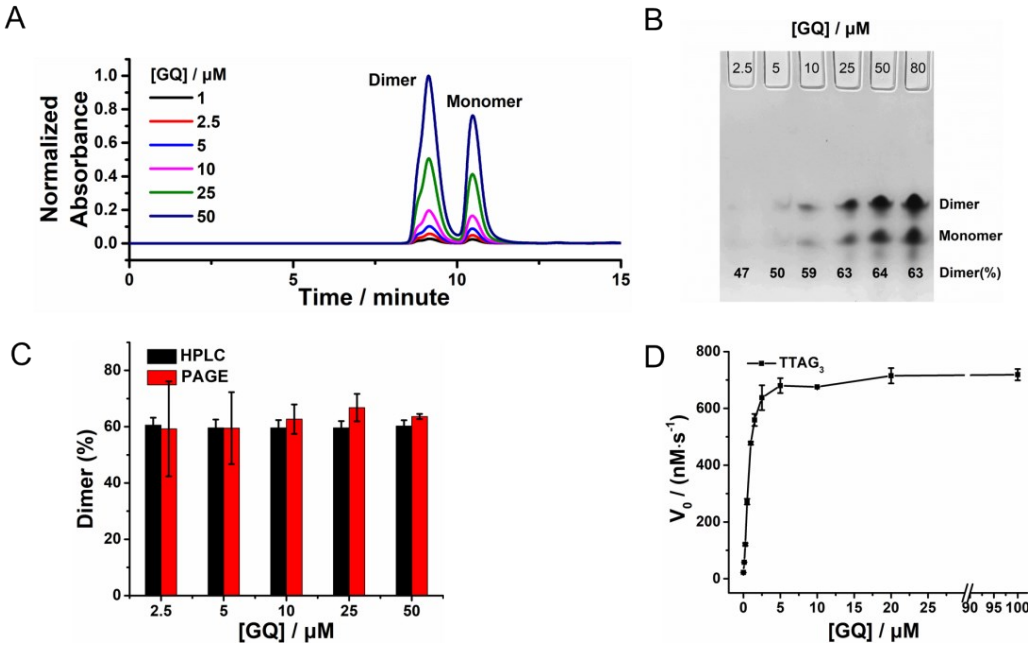


Figure S7. SEC-HPLC (**A**) and PAGE analysis (**B**) of 5'-TTAG$_3$-3' in different GQ concentrations (1 to 100 μM) in 40 mM HEPES buffer (pH 7.5) with100 mM KCl. (**C**) Comparison of the percentage of the dimeric form of 5'-TTAG$_3$-3' obtained by both SEC-HPLC and PAGE in different GQ concentrations. (**D**) Catalytic activities of 5'-TTAG$_3$-3' as a function of GQ concentration. The reactions were performed with GQ (from 0 to 100 μM) and hemin (0.5 μM) in presence of ABTS (0.5 mM) and H_2O_2 (0.5 mM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100, at 25 ^oC.

Figure S8 Kinetic measurements of DNAzymes.

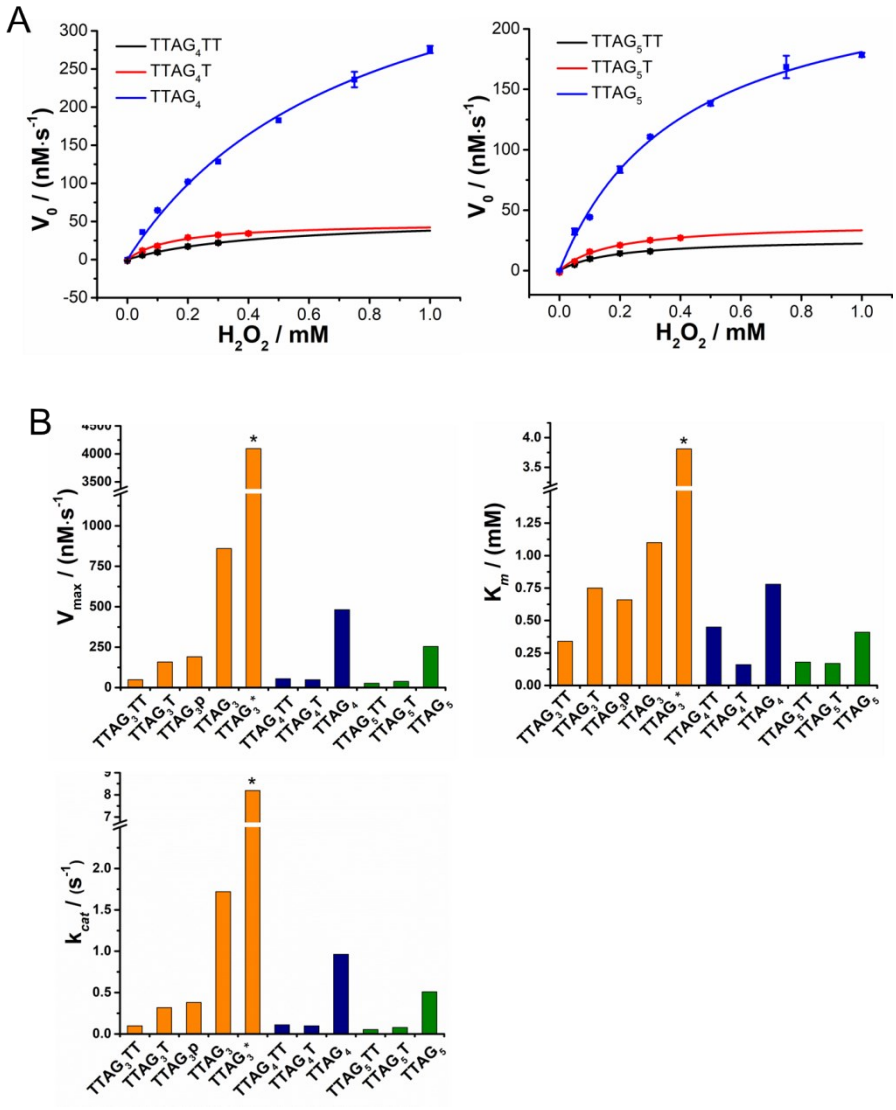
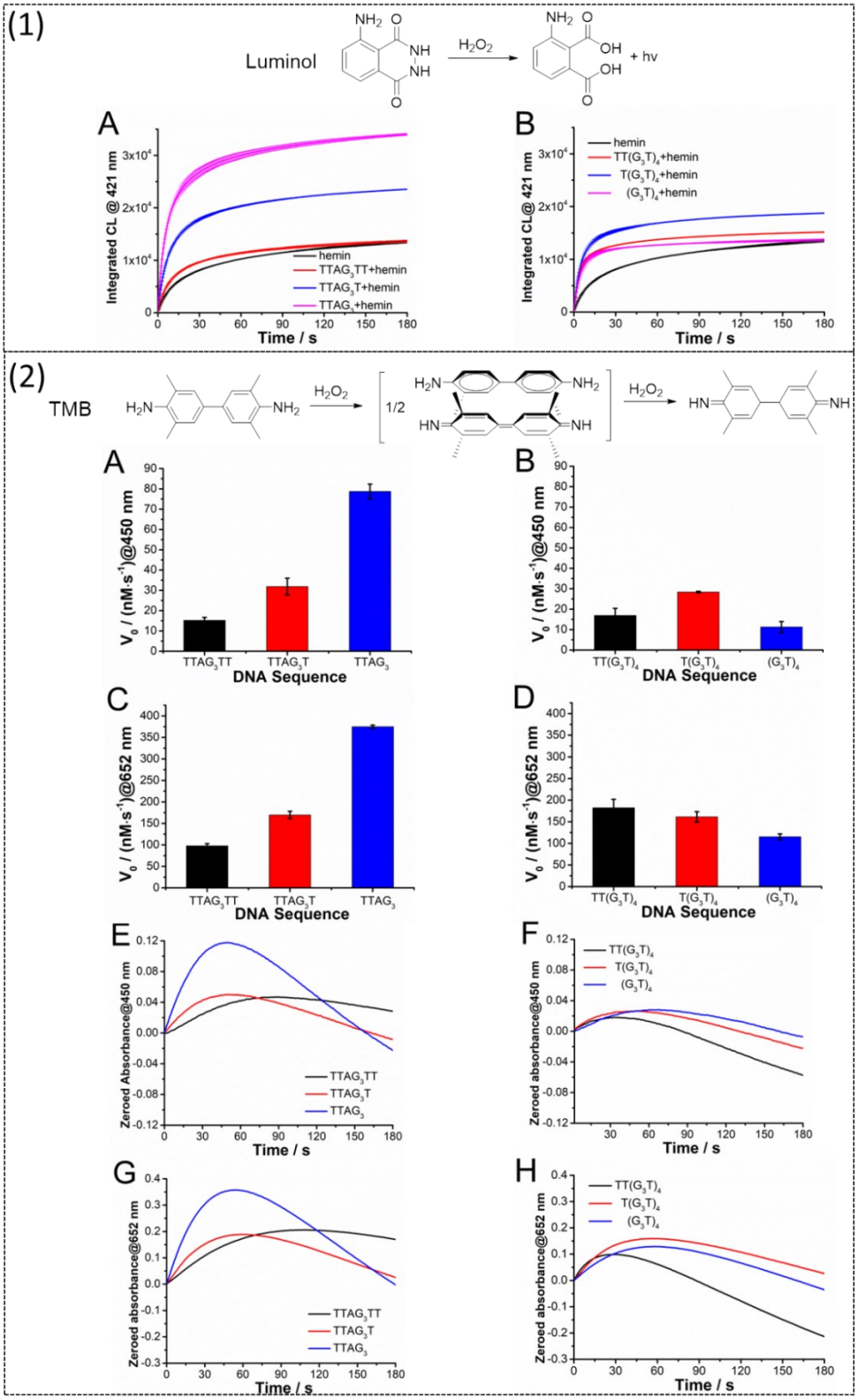
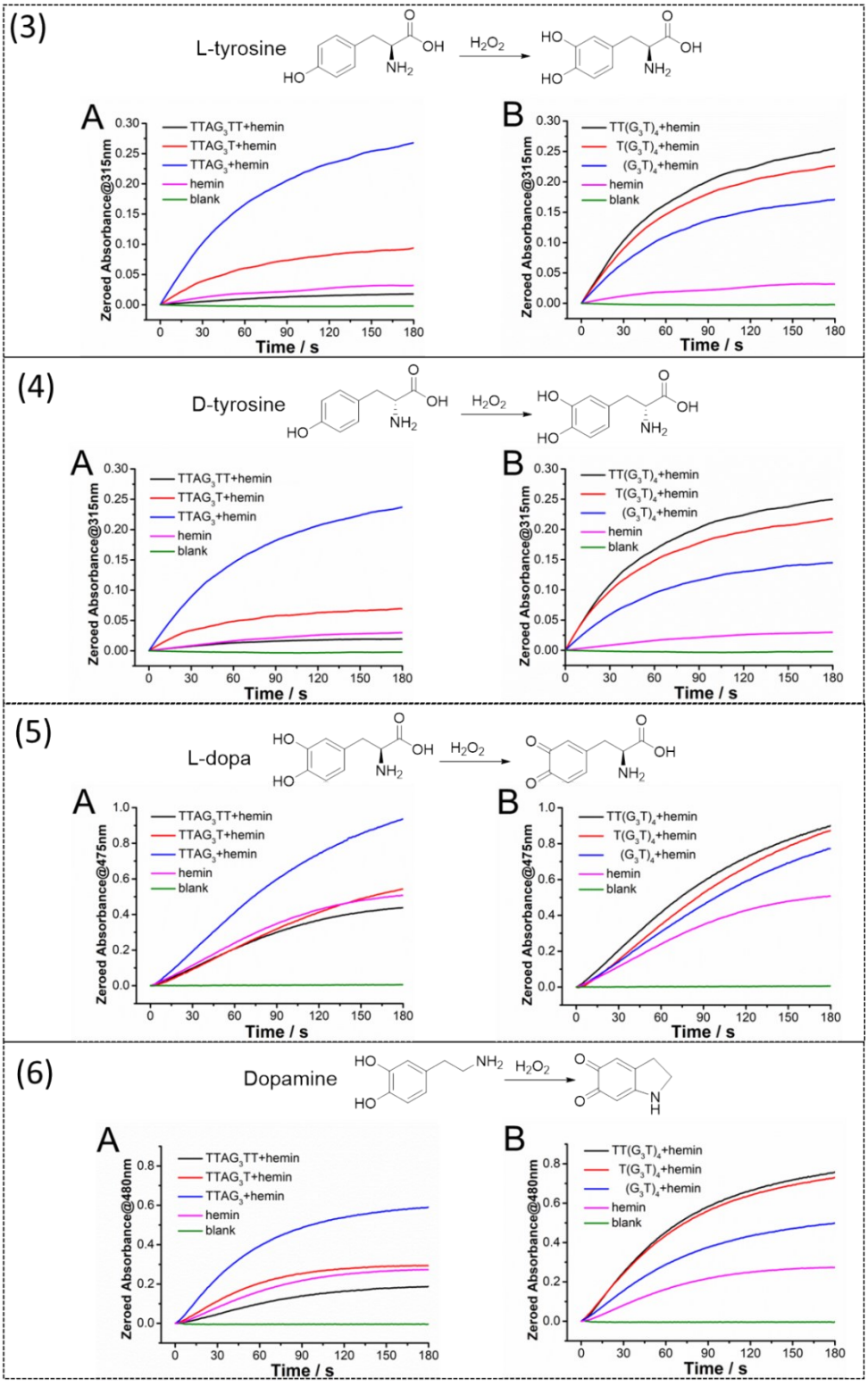


Figure S8 Michaelis-Menten analysis (**A**) of non-covalent dimeric GQ-DNAzyme from 5'-TTAG$_4$TT-3', 5'-TTAG$_4$T-3', 5'-TTAG$_4$-3', 5'-TTAG$_5$TT-3', 5'-TTAG$_5$T-3' and 5'-TTAG$_5$-3' and V_{max}, K_m, k_{cat} (**B**) of 3'-homodimeric DNAzymes formed from GQ (0.25 μM) and hemin (0.5 μM) in presence ABTS (0.5 mM) and increasing H_2O_2 concentrations (0 to 1.0 mM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v), at 25 ^{o}C.

Figures S9 Substrate scope.



Figures S9 Substrate scope (*Continued_01*).



Figures S9 Substrate scope (*Continued_02*).

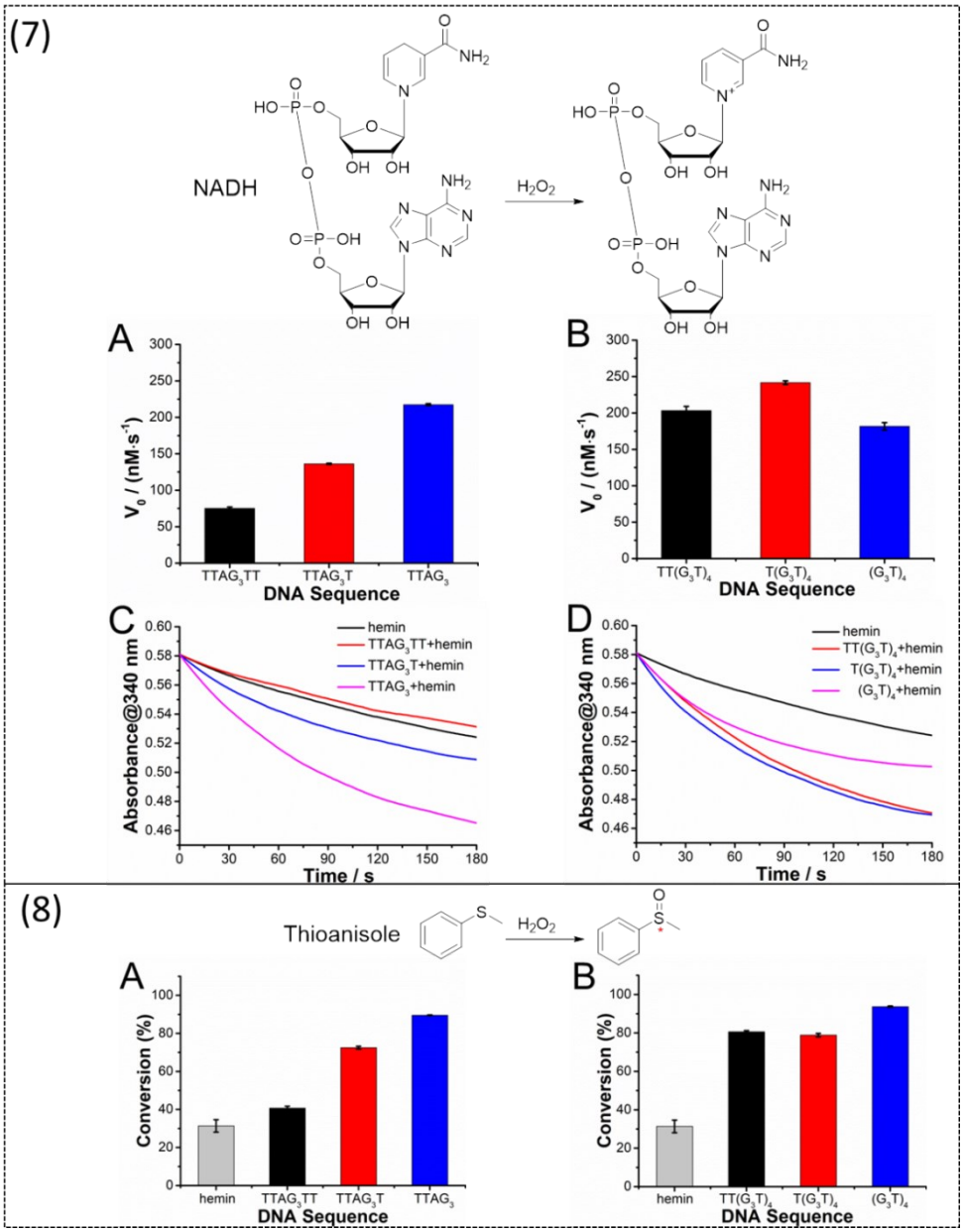


Figure S9 Substrate scope. Catalytic activity measurements using different substrates. All the reactions were performed in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100. For lines marked as hemin, reaction conditions were the same except no DNA was added. For lines marked as hemin, reaction conditions were the same except no DNA was added. For lines marked as blank, reaction conditions were the same except no DNA or hemin was added. Experimental details are given in the section of 'Materials and method'.

(**1**) **Luminol**[16]: Plots of integrated chemiluminescence at λ=421 nm versus reaction time. Reactions were performed with GQ (1 μM), hemin (2 μM), luminol (2.5 mM) and H_2O_2 (100 mM).

(**2**) **TMB**[17]: Initial velocity (V_0) of different DNAzymes at λ = 450 nm (**A** & **B**) and 652 nm (**C** & **D**). Plots of absorbance versus reaction time at λ = 450 nm (**E** & **F)** and 652 nm (**G** & **H**). Reaction were performed with GQ (0.25 μM), hemin (0.5 μM), TMB (0.25 mM) and H_2O_2 (5 mM).

(**3**) **L-tyrosine**[18]: Plots of absorbance at λ=315 nm versus reaction time. Reaction were performed with GQ (1 μM), hemin (2 μM), L-tyrosine (1 mM) and H_2O_2 (10 mM).
(**4**) **D-tyrosine**: Plots of absorbance at λ=315 nm versus reaction time. Reaction were performed with GQ (1 μM), hemin (2 μM), D-tyrosine (1 mM) and H_2O_2 (10 mM).
(**5**) **L-dopa**[19]: Plots of absorbance at λ=475 nm versus reaction time. Reaction were performed with GQ (0.5 μM), hemin (1 μM), L-dopa (0.5 mM) and H_2O_2 (5 mM).
(**6**) **Dopamine**[20]: Plots of absorbance at λ=480 nm versus reaction time. Reaction were performed with GQ (0.5 μM), hemin (1 μM), dopamine (0.5 mM) and H_2O_2 (5 mM).
(**7**) **NADH**[21]: (**A** & **B**) Initial reaction velocity (V_0) and (**C** & **D**) plots of absorbance at λ=340 nm versus reaction time. Reaction were performed with GQ (0.5 μM), hemin (1 μM), NADH (0.1 mM) and H_2O_2 (0.5 mM).
(**8**) **Thioanisole**[3]: Conversion of substrate thioanisole to product methyl phenyl sulfoxide. Reaction were performed with GQ (2 μM), hemin (4 μM), thioanisole (1 mM) and H_2O_2 (1.5 mM).Substrate and product content were detected by HPLC and conversions were calculated from the correction cofactor referring to the previous result in our group[3]. HPLC traces 1-4 are in the last part of Supporting Information.

Figure S10 Catalytic activities of DNAzymes mutated at 5' terminal, DNAzymes mutated at G tract of 5'-TTAG$_3$-3' and covalent multimeric DNAzymes,

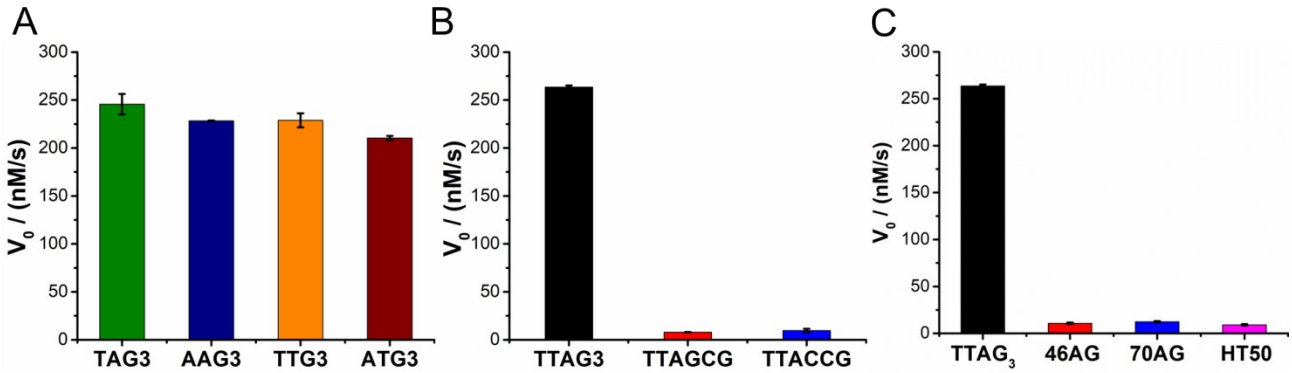


Figure S10 Catalytic activities 3'-homodimers mutated at their 5'-ends (**A**, 5'-TAG$_3$-3', 5'-AAG$_3$-3', 5'-TTG$_3$-3' and 5'-ATG$_3$-3' and mutated at G-tract (**B**, 5'-TTAG$_3$-3', 5'-TTAGCG-3' and 5'-TTACCG-3') and of covalent multimeric DNAzymes (**C**, 46AG[8], 70AG[8] and HT50[9]). The reactions were performed with GQ (0.25 μM) (for 46AG and HT50, DNA sequence concentration was 0.125 μM; for 70AG, DNA sequence concentration was 0.083 μM) and hemin (0.5 μM) in presence of ABTS (0.5 mM) and H_2O_2 (0.5 mM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100, at 25 ^oC.

Figure S11 Absorption characteristic of DNAzymes.

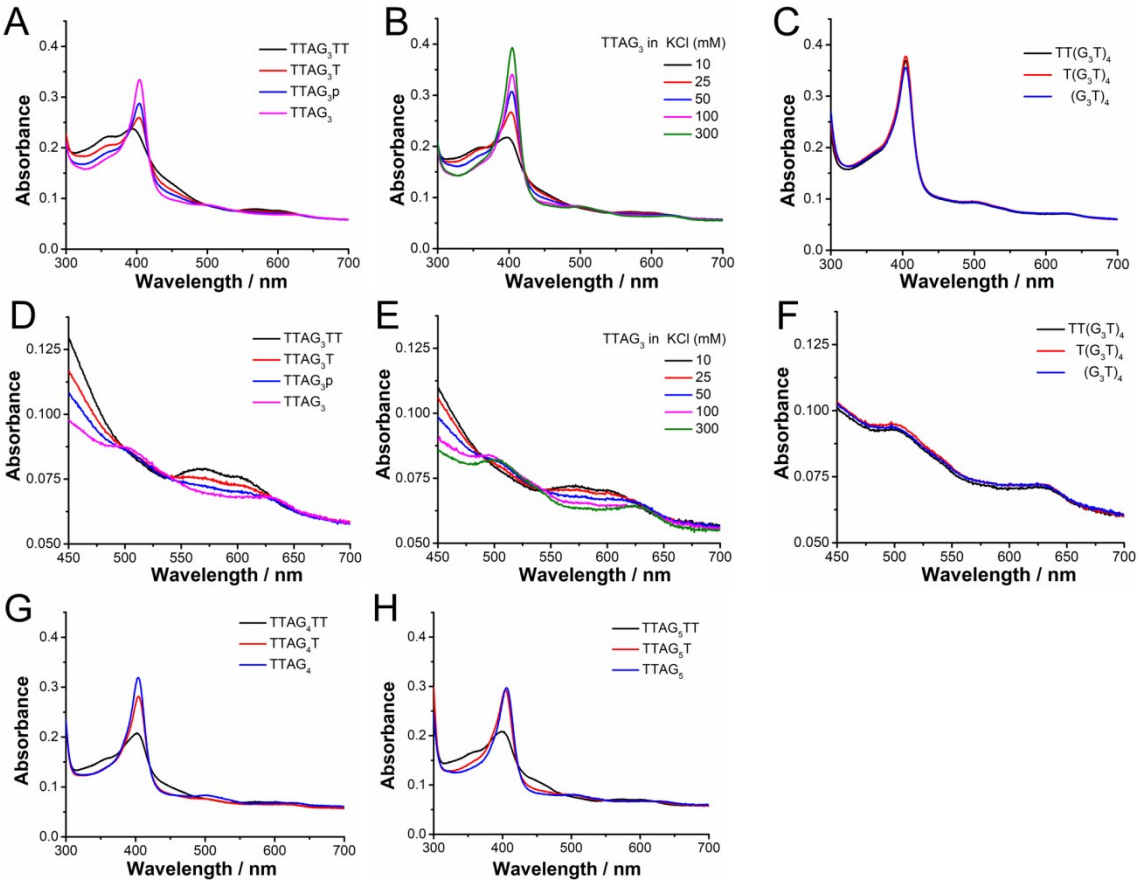


Figure S11 UV absorbance from λ=300 nm to λ=700 nm of 3'-homodimers (**A** & **D**, 5'-TTAG$_3$TT-3', 5'-TTAG$_3$T-3', 5'-TTAG$_3$p-3' and 5'-TTAG$_3$-3' with 100 mM KCl; **B** & **E**, 5'-TTAG$_3$-3' with 10, 25, 50, 100, 300 mM KCl; **G**, 5'-TTAG$_4$TT-3', 5'-TTAG$_4$T-3' and 5'-TTAG$_4$-3' with 100 mM KCl and **H**, 5'-TTAG$_5$TT-3', 5'-TTAG$_5$T-3' and 5'-TTAG$_5$-3' with 100 mM KCl) and 5'-homodimers (**C** & **F**, 5'-TT(G$_3$T)$_4$-3', 5'-T(G$_3$T)$_4$-3' and 5'-(G$_3$T)$_4$-3' with 100 mM KCl). Measurements were performed with GQ (5 μM) and hemin (2 μM) in 40 mM HEPES buffer (pH 7.5) with 0.05% (v/v) triton X-100, at 25 ^{o}C.

Figure S12 K_d measurements.

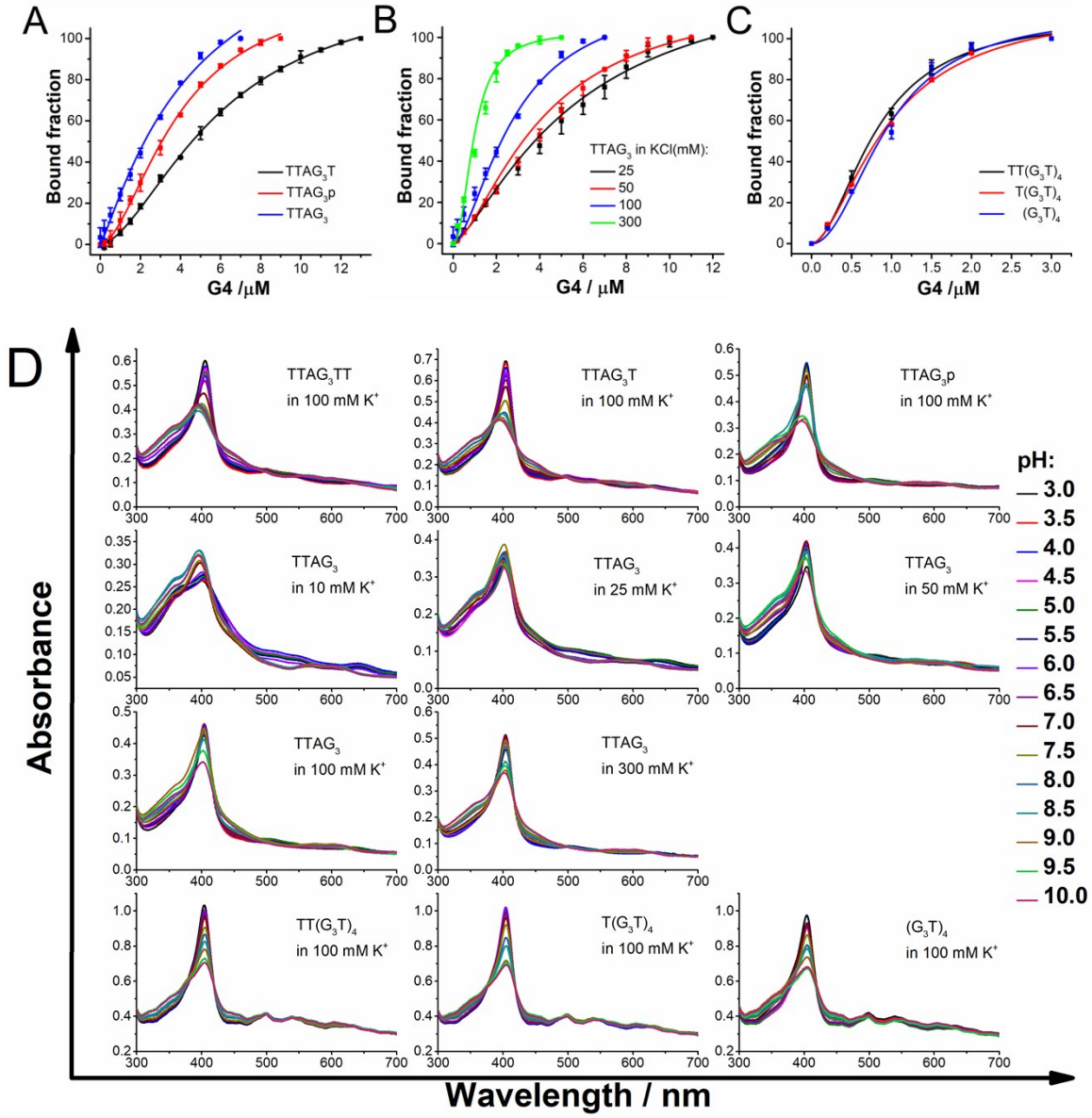


Figure S12 (**A-C**) Plots of bound fraction of hemin to GQs versus GQ concentration and (**D**) absorbance from λ=300 nm to λ=700 nm. Measurement were performed with hemin (2 μM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl (For 5'-$TTAG_3$-3' in 10, 25, 50, 300 mM K^+, with 10, 25, 50, 300 mM KCl, respectively) and 0.05% (v/v) triton X-100, at 25 °C.

Figure S13 pH titration experiments of DNAzymes.

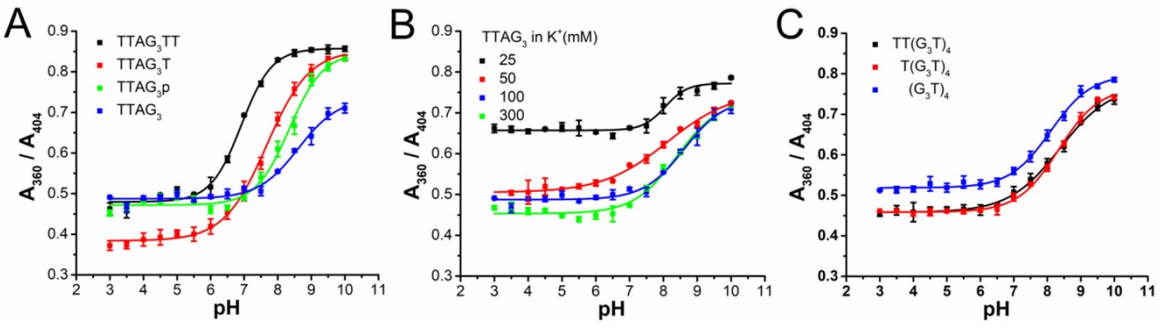


Figure S13 (**A-C**) Ratio of absorbance at λ=360 nm to λ=404 nm versus pH. Data were fitted by DoseResp equation in the origin, and pK_a of different DNAzymes could be got from the fitting result; (**D**) Absorbance from λ=300 nm to λ=700 nm. Measurement were performed in GQ (5 μM) and hemin (5 μM) in 40 mM HEPES buffer (pH from 3.0 to 10.0) with 100 mM KCl (For 5'-$TTAG_3$-3' in 10, 25, 50, 300 mM K^+, with 10, 25, 50, 300 mM KCl, respectively) and 0.05% (v/v) triton X-100, at 25 °C.

Figure S14 Oxidative degradation of DNAzymes.

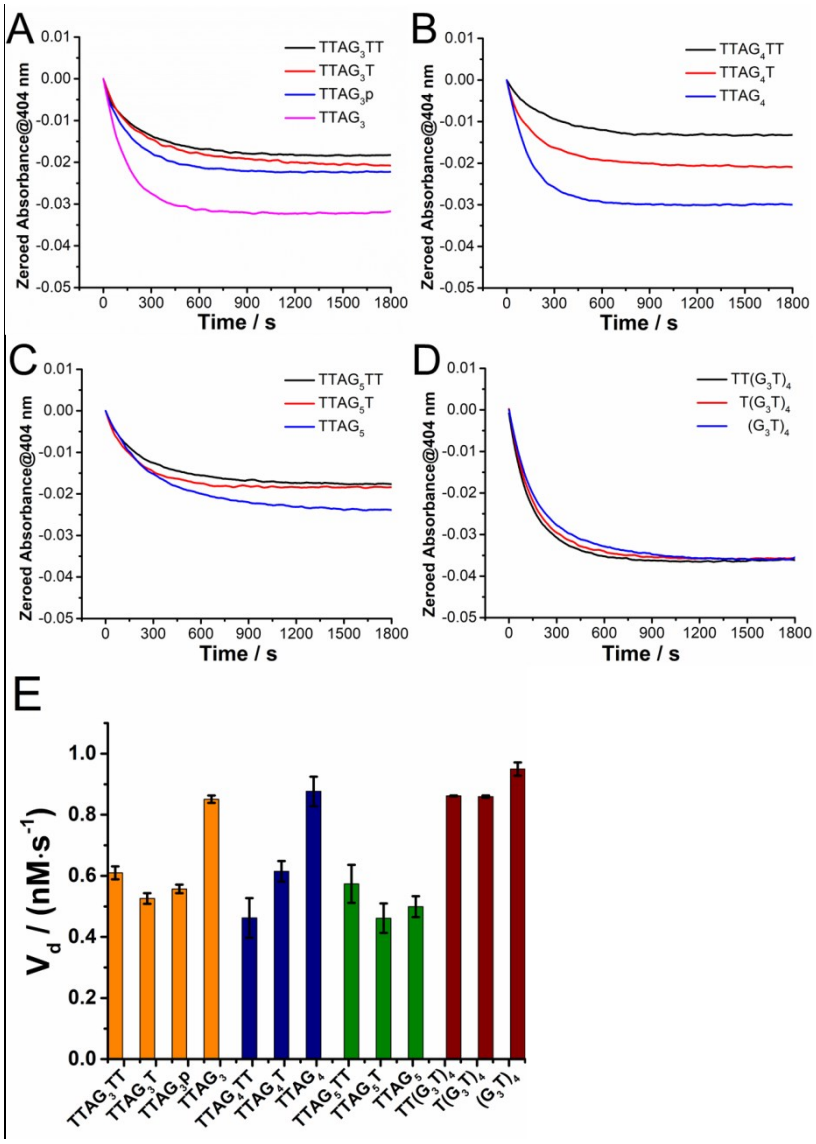
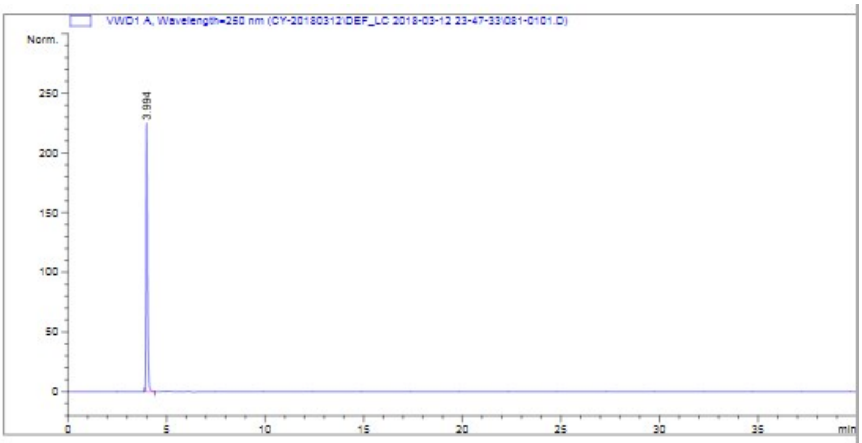


Figure S14 Absorbance at λ=404 nm of 3'-homodimers (**A**, 5'-TTAG$_3$TT-3', 5'-TTAG$_3$T-3', 5'-TTAG$_3$p-3' and 5'-TTAG$_3$-3'; **B**, 5'-TTAG$_4$TT-3', 5'-TTAG$_4$T-3' and 5'-TTAG$_4$-3'; **C**, 5'-TTAG$_5$TT-3', 5'-TTAG$_5$T-3' and 5'-TTAG$_5$-3') and 3'-homodimers (**D**, 5'-TT(G$_3$T)$_4$-3', 5'-T(G$_3$T)$_4$-3' and 5'-(G$_3$T)$_4$-3') and (**E**) initial apparent degradation velocity (V_d) of different DNAzymes. Reaction were performed with GQ (0.25 μM) and hemin (0.5 μM) in presence of H_2O_2 (0.5 mM) in 40 mM HEPES buffer (pH 7.5) with 100 mM KCl and 0.05% (v/v) triton X-100.

Chiral HPLC traces for sulfoxidation

HPLC Trace 1 Thioanisole

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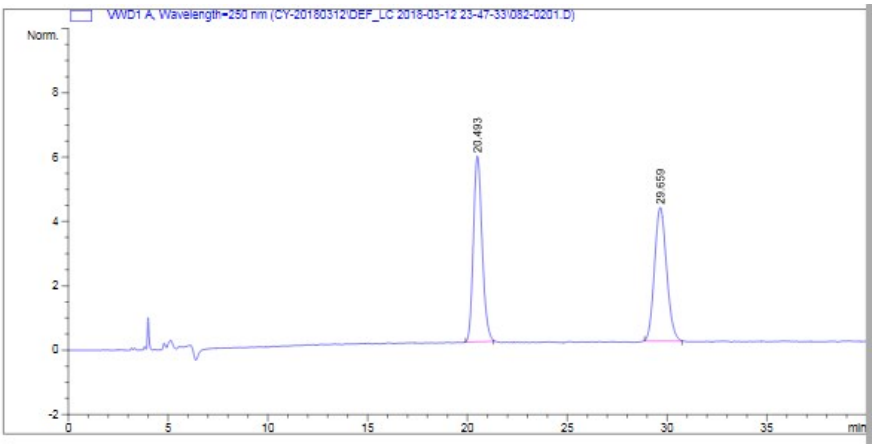
================================================================================
                            Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   3.994 BB    0.0799 1175.75146  224.89717 100.0000
```

HPLC Trace 2 Racemic sulfoxides

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================================================================================
                            Area Percent Report
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Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  20.493 BB    0.4733  176.06847    5.78043  50.0955
   2  29.659 BB    0.6366  175.39706    4.15517  49.9045
```
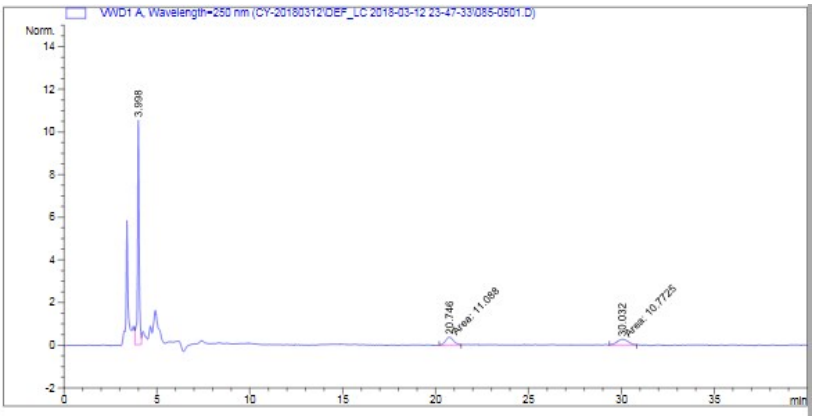

HPLC Trace 3 Thioanisole catalyzed by hemin alone

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Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]     %
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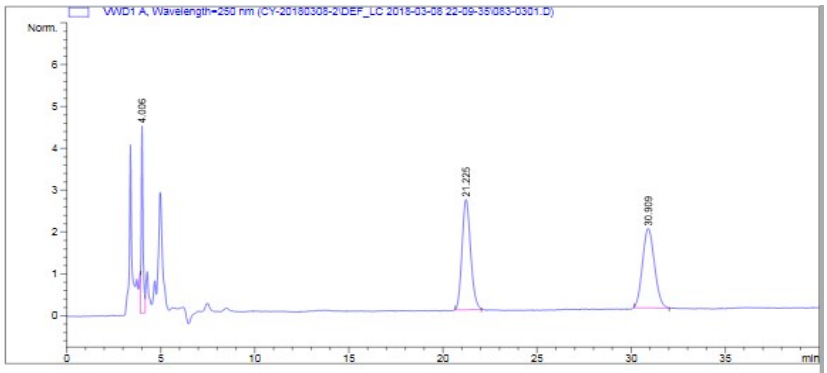
   1   3.998 VV    0.0887   64.28892   10.52368  74.6249
   2  20.746 MM    0.5061   11.08801 3.65148e-1  12.8707
   3  30.032 MM    0.6711   10.77247 2.67527e-1  12.5044
```

HPLC Trace 4 Thioanisole catalyzed by 5'-TTAG$_3$-3'/hemin DNAzyme

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=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]     %
----|-------|----|-------|----------|----------|--------|
   1   4.006 VV    0.0916   28.51055    4.48434  14.5345
   2  21.225 BB    0.4750   83.50619    2.63200  42.5710
   3  30.909 BB    0.6113   84.14053    1.89461  42.8944
```

References:

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