Supporting information for:

Investigation and Improvement of Catalytic Activity of G-quadruplex/Hemin DNAzyme Using Designed Terminal G-tetrads with Deoxyadenosine Caps

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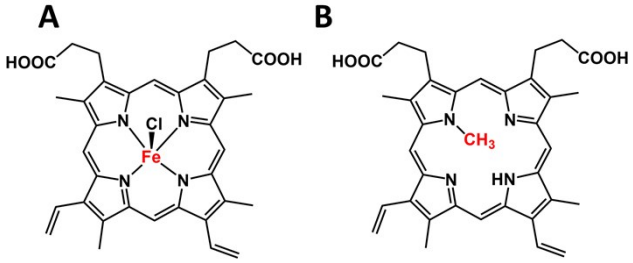

Scheme S1. The chemical structures of **A)** hemin, and **B)** N-methyl mesoporphyrin IX **(**NMM).

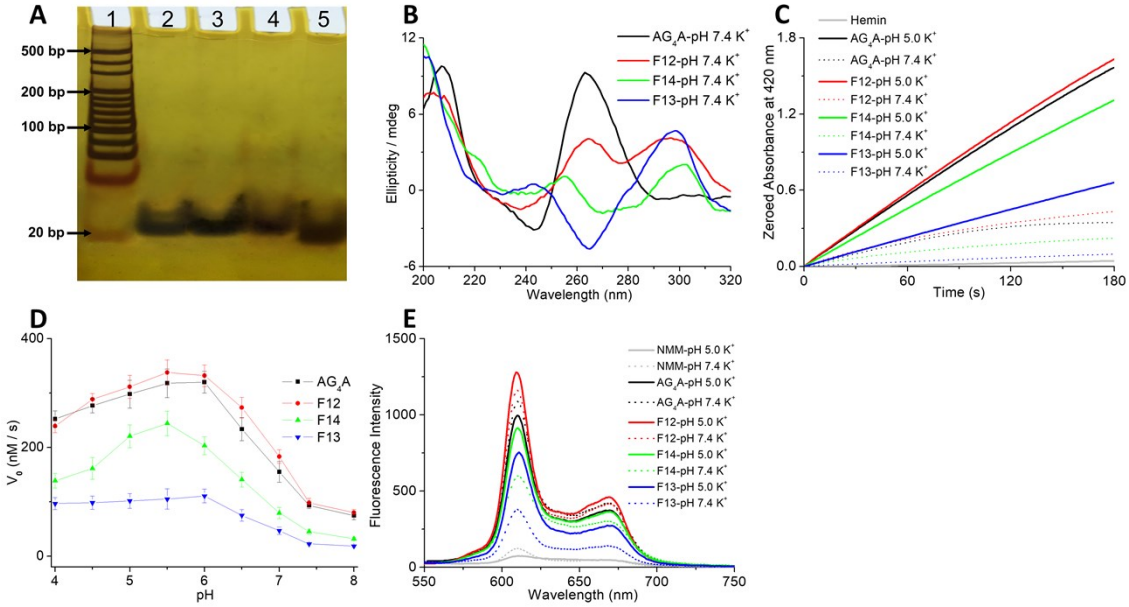

Figure S1. A) Native PAGE using 12% polyacrylamide gel of various DNA samples (10 μM each) in KCl buffer, pH 5 (lane 1: a DNA marker of 20 - 500 bp, lane 2: AG_4A, lane 3: F12, lane 4: F14, and lane 5: F13). **B)** CD spectra of four DNA strands, AG_4A, F12, F14 and F13 at the concentration of 4 μM in 20 mM MES buffer with 10 mM KCl at pH 7.4. **C)** Plots of absorbance at 420 nm versus reaction time for DNAzymes of AG_4A, F12, F14 and F13 (1.2 μM of each oligomer and 0.6 μM hemin) in 20 mM MES buffer with 10 mM KCl at pH 5 or 7.4. **D**) Plots of V_0 values of AG_4A, F12, F14 and F13 versus pH values. **E**) Fluorescence spectra of 1.8 μM NMM after titration with the four DNA strands (2.4 μM of each oligomer) with 10 mM KCl in 20 mM MES buffer at pH 5 or 7.4.

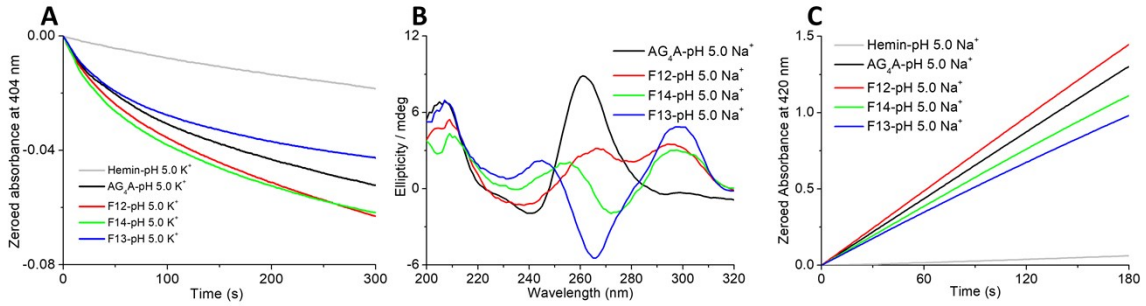

Figure S2. A) Absorbance dynamics at 404 nm of the four DNAzymes during the exposure to 0.5 mM H_2O_2. **B**) CD spectra of four DNA strands, AG_4A, F12, F14 and F13 at the concentration of 4 μM in 20 mM MES buffer supplemented with 10 mM NaCl (pH 5). **C**) Plots of absorbance at 420 nm versus reaction time for DNAzymes of AG_4A, F12, F14 and F13 (1.2 μM of each oligomer and 0.6 μM hemin) in 20 mM MES buffer supplemented with 10 mM NaCl (pH 5).

Table S1. K_d values of hemin binding to the various G4s at pH 5 and absorbance changes at 404 nm during absorption titration processes.

G4 scaffold	K_d [μM]	ΔA_{max} at 404 nm	G4 scaffold	K_d [μM]	ΔA_{max} at 404 nm
$[AG_4A]_4$	7.2 ± 2.1	0.061 ± 0.012	$[AG55]_4$	2.5 ± 0.6	0.179 ± 0.019
$[F12]_4$	6.6 ± 0.7	0.066 ± 0.006	$[A33G55]_4$	2.0 ± 0.6	0.115 ± 0.014
$[F14]_4$	9.8 ± 1.6	0.063 ± 0.013	$[A55G33]_4$	0.7 ± 0.3	0.023 ± 0.005
$[F13]_4$	21.4 ± 8.6	0.024 ± 0.004	$[AG33]_4$	ND	0
$[AGS55]_4$	2.3 ± 0.4	0.197 ± 0.015			

* Each data obtained is the average of two measurements. Here, "ND" means "not determined".

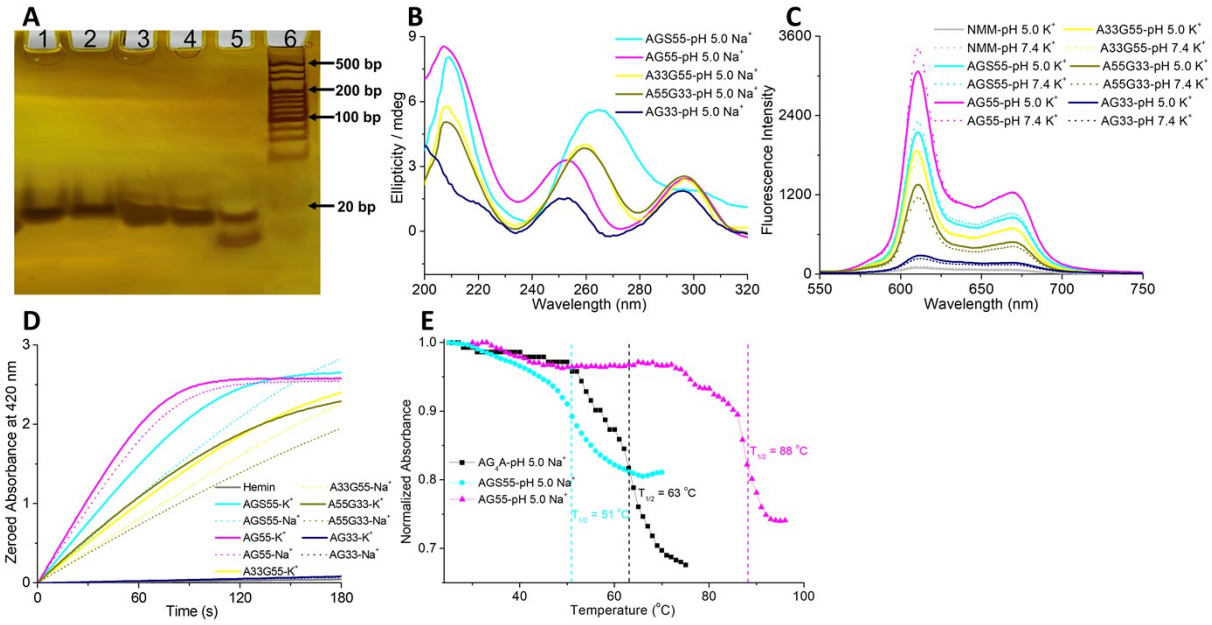

Figure S3. A) Native PAGE using 12% polyacrylamide gel of various DNA samples in KCl, pH 5 (lane 6: DNA marker (20-500 bp), lane 1: AGS55, lane 2: AG55, lane 3: A33G55, lane 4: A55G33, and lane 5: AG33). **B**) CD spectra of five DNA strands, AGS55, AG55, A33G55, A55G33 and AG33 at the concentration of 4 µM in 20 mM MES buffer supplemented with 10 mM KCl (pH 5). **C**) Fluorescence spectra of 1.8 µM NMM after titration with various DNA strands: AGS55, AG55, A33G55, A55G33, and AG33 (2.4 µM of each oligomer) with 10 mM KCl in 20 mM MES buffer at pH 5 or 7.4. **D**) Plots of absorbance at 420 nm versus reaction time for DNAzymes of AGS55, AG55, A33G55, A55G33 and AG33 (1.2 µM of each oligomer and 0.6 µM hemin) in 20 mM MES buffer supplemented with 10 mM KCl or NaCl (pH 5). **E**) UV melting curves of three strands, AG_4A, AGS55 and AG55 (8 µM of each DNA strand) in 20 mM MES buffer (pH 5) supplemented with 20 mM NaCl.

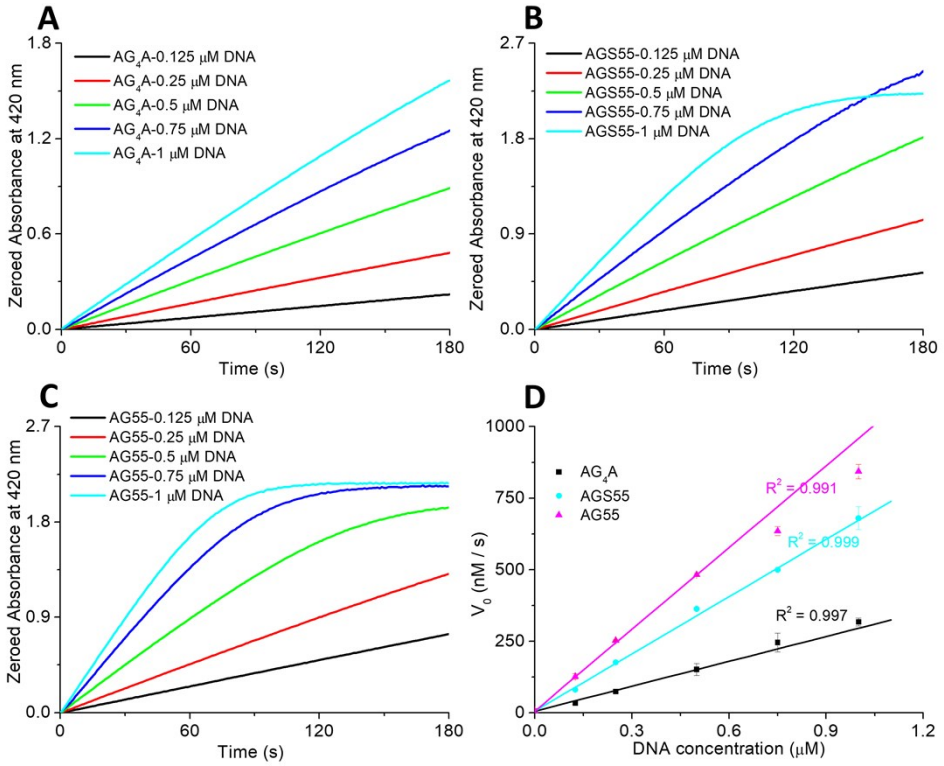

Figure S4. A) - **C**) Plots of absorbance at 420 nm versus reaction time for DNAzymes of AG_4A (A), AGS55 (B) and AG55 (C) in 20 mM MES buffer supplemented with 10 mM KCl or NaCl (pH 5.5). The final concentration of hemin was controlled at 0.5 μM and each oligomer was ranging from 0.125 to 1 μM. **D**) Changes of V_0 values at various DNA concentrations at pH 5.5.

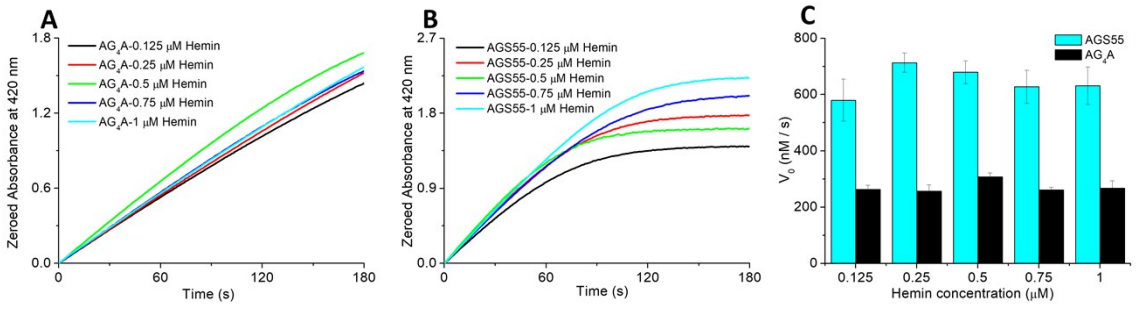

Figure S5. A) - **B**) Plots of absorbance at 420 nm versus reaction time for DNAzymes of AG_4A (A) and AGS55 (B) in 20 mM MES buffer supplemented with 10 mM KCl (pH 5). **C**) V_0 values of the DNAzymes of AGS55 and AG_4A in KCl solution (pH 5.5) versus changes of hemin concentrations. The concentration of each oligomer was controlled at 1 μM.

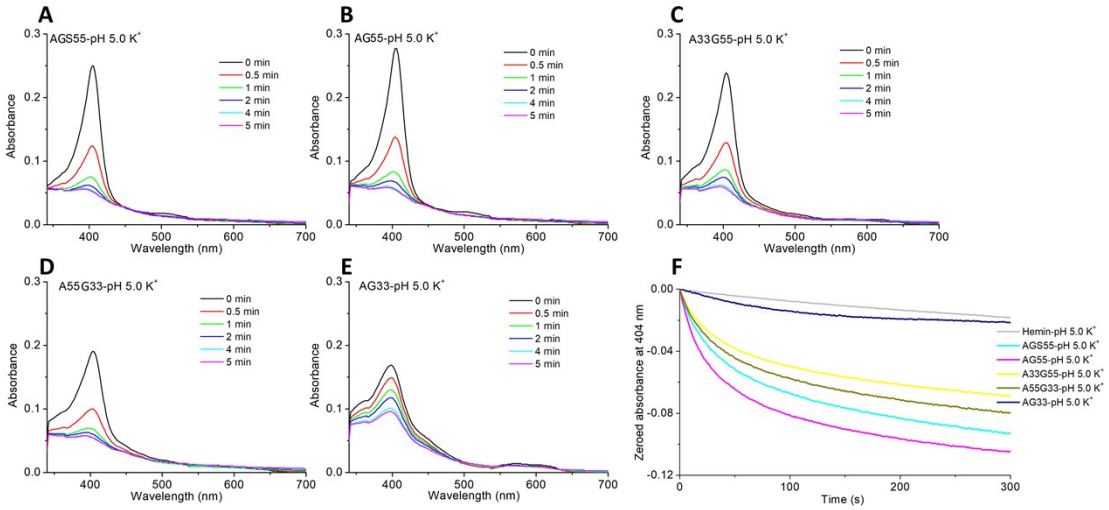

Figure S6. Inactivation dynamics of G4/hemin DNAzymes exposed to different concentrations of H_2O_2. **A**) - **E**) Absorption spectra of AGS55-, AG55-, A33G55-, A55G33- and AG33-based DNAzymes exposed to 3.5 mM H_2O_2 at different times. **F**) Absorbance dynamics at 404 nm of the five DNAzymes during the exposure to 0.5 mM H_2O_2. All DNAzymes were formed in the presence of 5 μM DNA strand (each) and 4 μM hemin in 20 mM MES buffer with 10 mM KCl at pH 5.

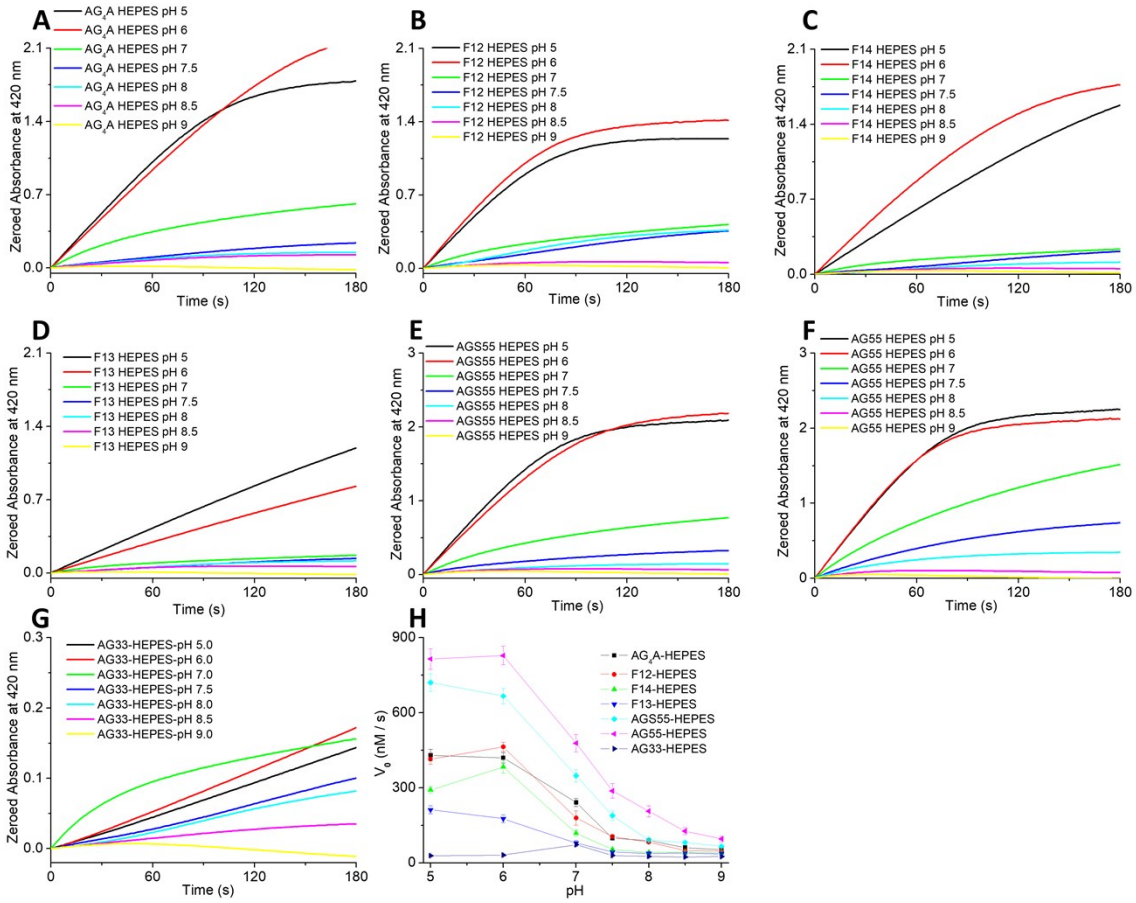

Figure S7. A) - **G**) Plots of absorbance at 420 nm versus reaction time for DNAzymes of AG_4A (A), F12 (B), F14 (C), F13 (D), AGS55 (E) AG55 (F) and AG33 (G) in 10 mM HEPES buffer supplemented with 10 mM KCl at various pH values. **H**) Changes of V_0 values of AG_4A, F12, F14, F13 AGS55, AG55 and AG33 at various pH values. Each sample contained 0.6 μM hemin, 1.2 μM DNA strand and 10 mM KCl, and the reaction was initiated by the addition of 1.2 mM ABTS and 1.2 mM H_2O_2.

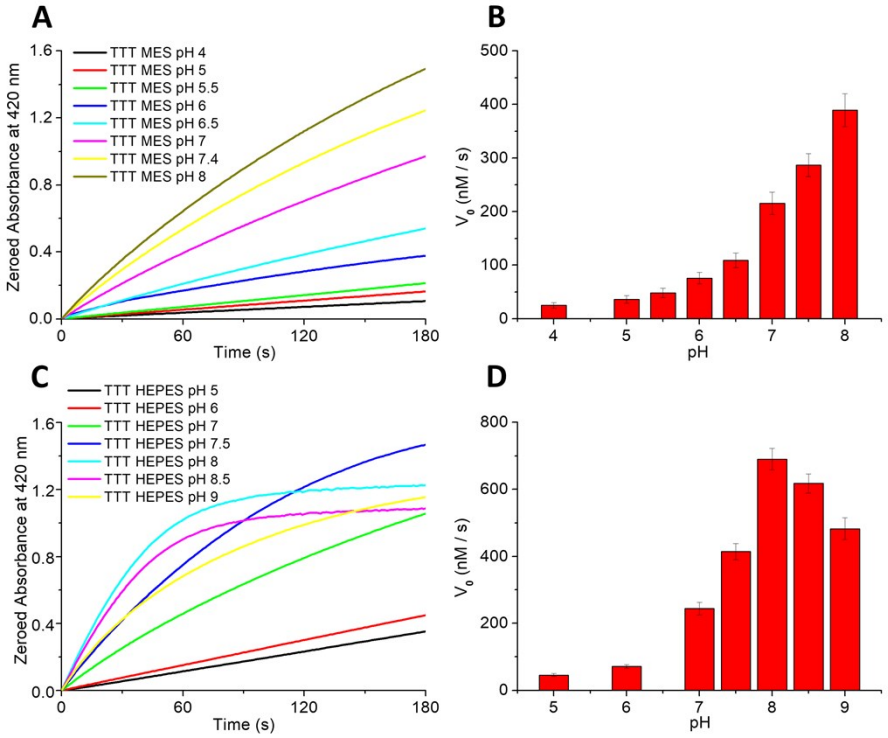

Figure S8. A) - **B**) DNAzymes of TTT in MES buffer: plots of absorbance at 420 nm versus reaction time (A) and V_0 values of TTT versus changes of pH values (B); **C**) - **D**) DNAzymes of TTT in HEPES buffer: plots of absorbance at 420 nm versus reaction time (C) and V_0 values of TTT versus changes of solution pH (D). Each sample contained 1.2 μM hemin, 1.2 μM DNA strand and 10 mM KCl, and the reaction was initiated by the addition of 1.2 mM ABTS and 1.2 mM H_2O_2.

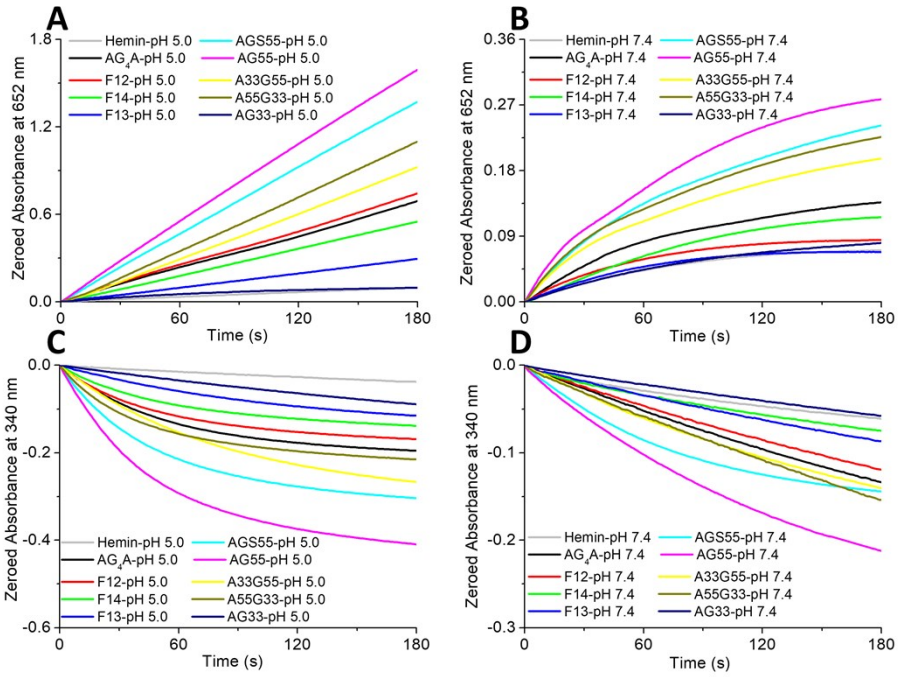

Figure S9. Plots of absorbance at 652 nm (**A**, **B**) and 340 nm (**C**, **D**) versus reaction time for DNAzymes of AG_4A, F12, F14, F13, A33G55, A55G33, AGS55, AG55 and AG33 in 20 mM MES buffer at pH 5 (A, C) or pH 7.4 (B, D). Each sample contained 0.6 μM hemin, 1.2 μM DNA strand and 10 mM KCl, and the reaction was initiated by the addition of each substrate (0.6 mM TMB or 120 μM NADH) and 1.2 mM H_2O_2.

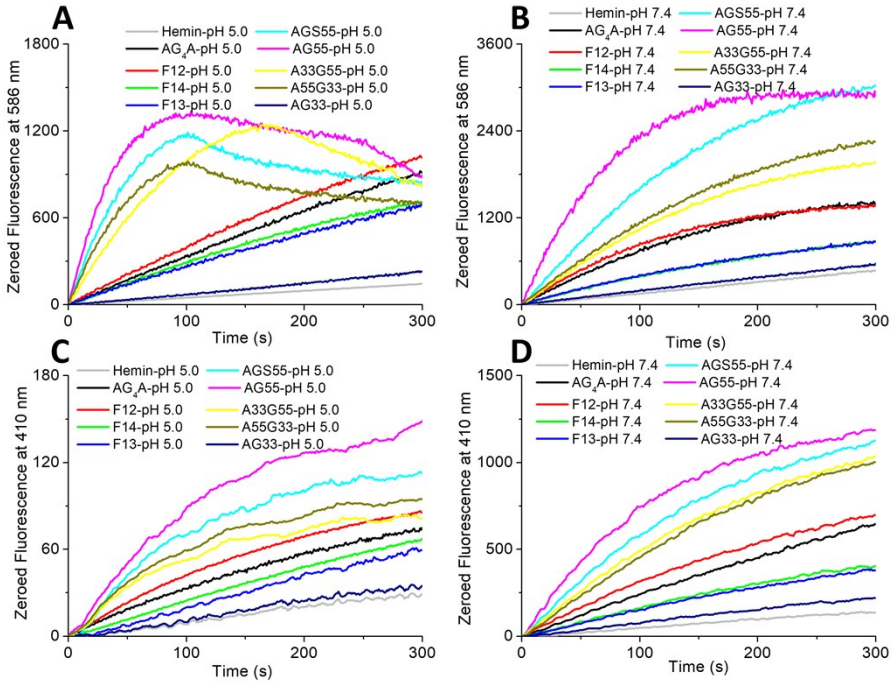

Figure S10. Plots of fluorescence at 586 nm (**A**, **B**) and 410 nm (**C**, **D**) versus reaction time for DNAzymes of AG_4A, F12, F14, F13, A33G55, A55G33, AGS55, AG55 and AG33 in 20 mM MES buffer at pH 5 (A, C) or pH 7.4 (B, D). Each sample contained 0.6 μM hemin, 1.2 μM DNA strand and 10 mM KCl, and the reaction was initiated by the addition of each substrate (15 μM AR or 240 μM Tyramine-HCl) and 0.8 mM H_2O_2.