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Supporting Information for:

A Self-Assembled Peroxidase from 5′-GMP and Heme

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Experimental Section

Materials

Guanosine 5′-monophosphate disodium salt **1**, 2′-deoxyguanosine-5′-monophosphate, disodium salt **2**, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 30% hydrogen peroxide stock solution, solid NaOH, and KCl were purchased from Sigma-Aldrich and used without further purification. Hemin (porcine, 97%) was purchased from Alfa Aesar and used as is. D_2O was purchased from Cambridge Isotope Laboratories.

UV-Vis experiments

An Agilent CARY 100 spectrophotometer was used for UV-Vis absorption measurements. Hemin and ABTS stock solutions were prepared fresh daily for kinetic experiments. Stock solutions of ABTS were made by weighing 11 mg of ABTS and dissolving in 2 mL of deionized water to give a solution of 10.0 mM ABTS. Hemin stock solutions were prepared by weighing 26 mg of hemin powder in a glass vial and adding 20 mL of 20 mM NaOH;, this solution was diluted 100-fold with DI water to give 20 μM hemin in 200 μM NaOH. To prepare samples, solid guanosine monophosphate was weighed and transferred into a glass vial. 1.4 mL of DI water was added, following addition of 0.1 mL of the 20 μM hemin solution and 0.1 mL of the 10 mM ABTS solutions. The vial was sonicated to ensure all of the guanosine has dissolved, and the sample was left to stand at room temperature for at least 1 hour and transferred into a 1 cm path length quartz cuvette. Cuvettes were placed inside the temperature control sample holder for at least 10 minutes before measurements to allow for temperature equilibration. ABTS oxidation reactions were initiated by the addition of 0.4 mL H_2O_2 solutions at the appropriate concentration, and the absorbance at 415 nm was recorded over time immediately after the addition of H_2O_2.

^{1}H NMR experiments

^{1}H NMR spectra were taken on either a Bruker AV-III (600 MHz) or a Bruker Ascend (800 MHz) NMR instrument. All ^{1}H NMR samples were prepared in D_2O. To prepare a sample, solid **1** or **2** was weighed and transferred into a glass vial. D_2O was added to the vial to give a solution of at least 0.4 mL. The solution was sonicated to completely dissolve the sample before transferring to a glass NMR tube. All spectra were recorded with a minimum of 64 scans. For variable temperature experiments the NMR sample was allowed to equilibrate for at least 10 minutes at the desired temperature before spectrum acquisition.

Circular dichroism measurements

Circular dichroism (CD) spectra were collected using a Jasco J-810 spectropolarimeter. CD spectra show the average of 5 replicate scans, with line smoothing applied. For preparation of the $(\mathbf{1}_4)_n$•Hm samples shown in Figure S3, hemin solutions were prepared as described above for UV-Vis experiments. Solid **1** was weighed and transferred into a glass vial, DI water was added and the solution was sonicated to dissolve the material. Hemin from the stock solution was added to give a final concentration of 30 μM hemin. For preparation of c-Myc22 DNA sample (in Figure S5), the stock solution of folded G-quadruplex DNA (see below for details) was diluted to 30 μM and the sample was stored in a 4 °C refrigerator until use. For sample measurements, solutions were transferred to a 1 cm path length quartz cuvette For variable temperature

experiments, the cuvette containing the sample solution was allowed to stand in the temperature control sample holder at the desired temperature for at least 15 minutes.

Procedure for c-Myc22 DNA experiments

The DNA sequence 5′-dTGAGGGTGGGGAGGGTGGGGAA-3′ (c-Myc22) was purchased from ThermoFisher as a desalted purification grade oligonucleotide. DNA stock solution was made by dissolving the solid DNA in a 0.1 M sodium phosphate buffer, pH 8.0 with 0.01 M KCl added, to give a solution containing 2.0 mM of DNA. To induce G-quadruplex folding of this DNA sequence, a procedure was adapted from that reported in Cheng et. al.[1] The solution of DNA in the potassium containing buffer was heated to 90 °C in a water bath, then left to cool and stored in a 4 °C refrigerator overnight.

Kinetic measurements and analysis

Samples were prepared as described above in the UV-Vis experiments procedure. The oxidation of ABTS was initiated by addition of H_2O_2. Thus, 0.4 mL of H_2O_2 at the appropriate concentration was pipetted into the cuvette containing 1.6 mL of a solution of hemin, ABTS, and either Na_2(5′-GMP) **1** or c-Myc22 G-quadruplex, to bring all components to the appropriate concentration in the 2 mL final volume. Proper mixing of the solution was ensured by pipetting the mixture in and out of the cuvette twice, then immediately starting data collection. Each condition was measured in triplicate. The slope of the ABTS absorbance at 415 nm over time was taken over the first 30 seconds of reaction to calculate the initial rate. The initial rate was calculated separately for each of the three replicates at a given concentration of H_2O_2. The H_2O_2 concentrations tested for both the peroxidase mimics $(\mathbf{1}_4)_n$•Hm and c-Myc22•Hm were: 0.25, 0.50, 1.00, 1.50, 2.00, and 3.00 mM H_2O_2. The initial rate data as a function of H_2O_2 concentration was fit to the Michaelis-Menten equation using a nonlinear regression, as detailed in Figure S7 below.

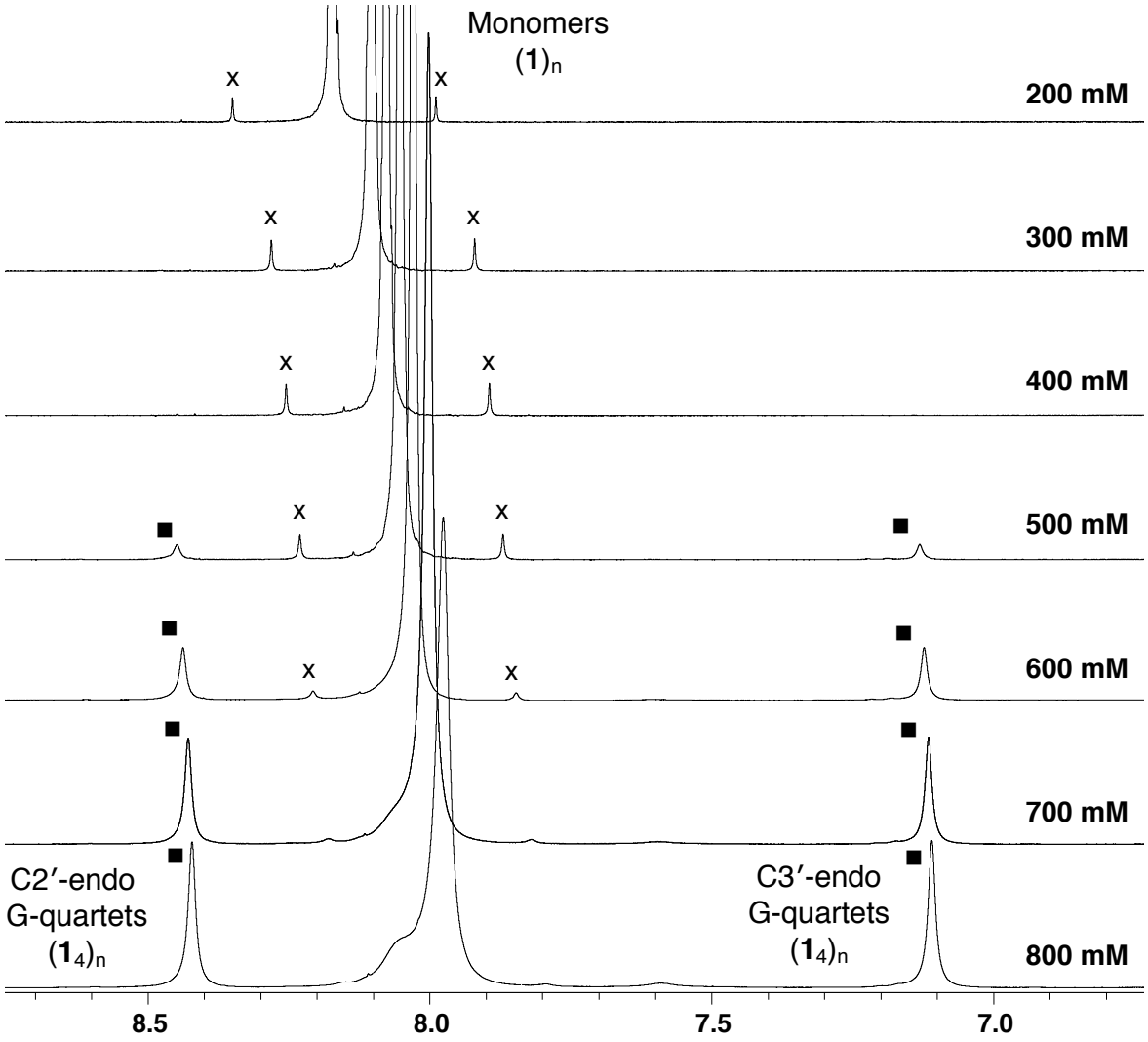


Figure S1. ^{1}H NMR (600 MHz) of Na_2(5′-GMP) **1** as a function of concentration at 25 °C. Signals corresponding to the G-quadruplex $(\mathbf{1}_4)_n$ appear and grow at δ 7.1 ppm and 8.4 ppm with increasing concentration of **1**. Peaks for monomers $(\mathbf{1})_n$, dimer $(\mathbf{1}_2)_n$ and G-quadruplex $(\mathbf{1}_4)_n$ are labeled according to published assignments.[2] Satellite signals due to ^{1}J(CH) coupling are marked with an X.

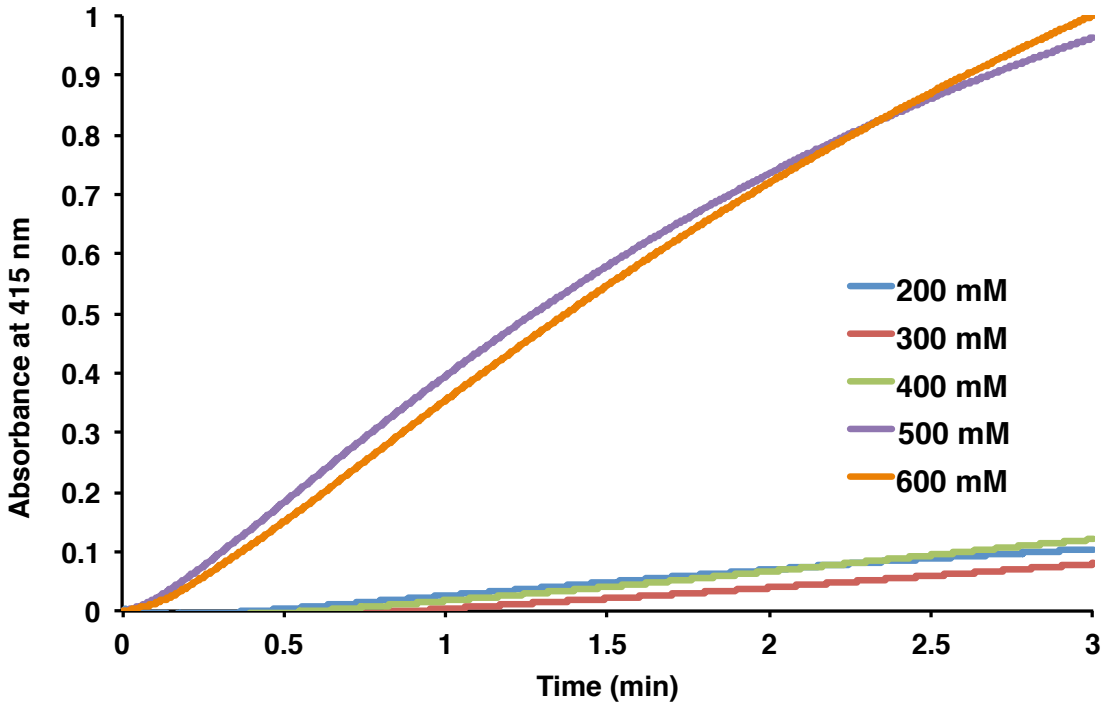


Figure S2. Absorbance of $ABTS^{\bullet+}$ (λ= 415 nm) as a function of time. In a series of experiments we followed oxidation of ABTS as a function of Na_2(5′-GMP) **1** concentration at 25 °C. Samples contain **1**, ranging from 200 mM – 600 mM in concentration, at pH 8 with hemin (1 μM), ABTS (500 μM), and H_2O_2 (250 μM). A significant increase in reaction rate is observed between the samples containing 400 mM and 500 mM of **1**. This is due to the formation of G-quadruplex $(\mathbf{1}_4)_n$ at 500 mM of **1** (see NMR data in Fig. S1). The initial rate constants calculated from these kinetic traces are plotted in Figure 2 of the paper. Each trace is an average of 3 runs.

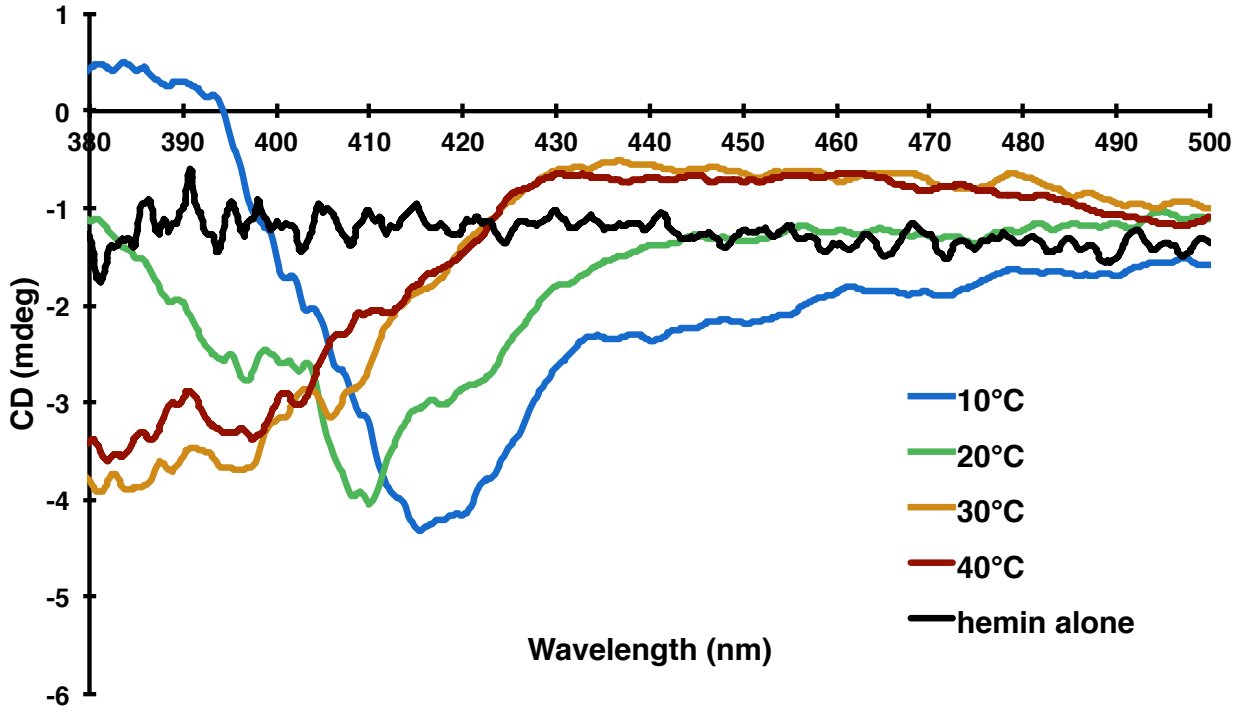


Figure S3. Induced CD of hemin when bound in the G-quadruplex-hemin complex, $(\mathbf{1}_4)_n$•Hm $(\mathbf{1}_4)_n$. All samples contain 30 μM hemin and 400 mM Na_2(5′-GMP) **1**. The "hemin alone" control was measured at 20 °C. At conditions where G-quadruplex self-assembly is favored (10 °C and 20 °C), hemin's Soret band shows an induced negative CD peak.

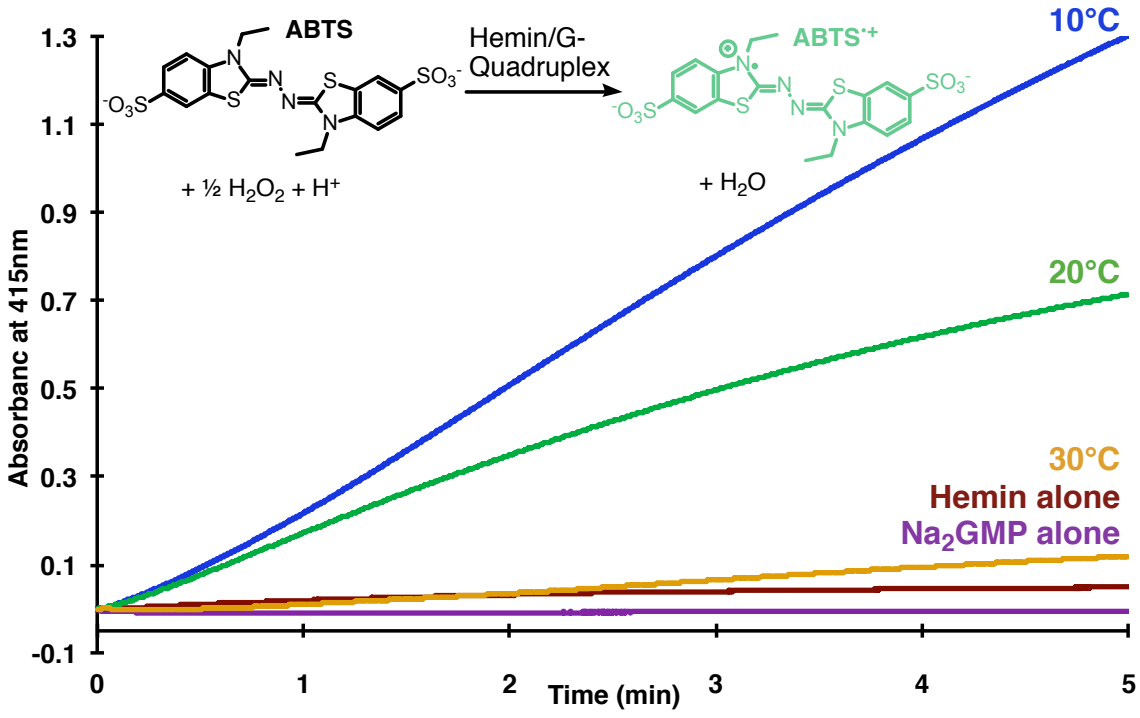


Figure S4. Absorbance of $ABTS^{\bullet+}$ (λ= 415 nm) as a function of time. In a series of experiments we followed oxidation of ABTS at different temperatures. Samples contain Na_2(5′-GMP) **1** (400 mM) at pH 8, with hemin (1 μM), ABTS (500 μM), and H_2O_2 (250 μM) Controls showed no significant activity with either hemin or **1** alone at 10 °C. Each trace is an average of 3 runs.

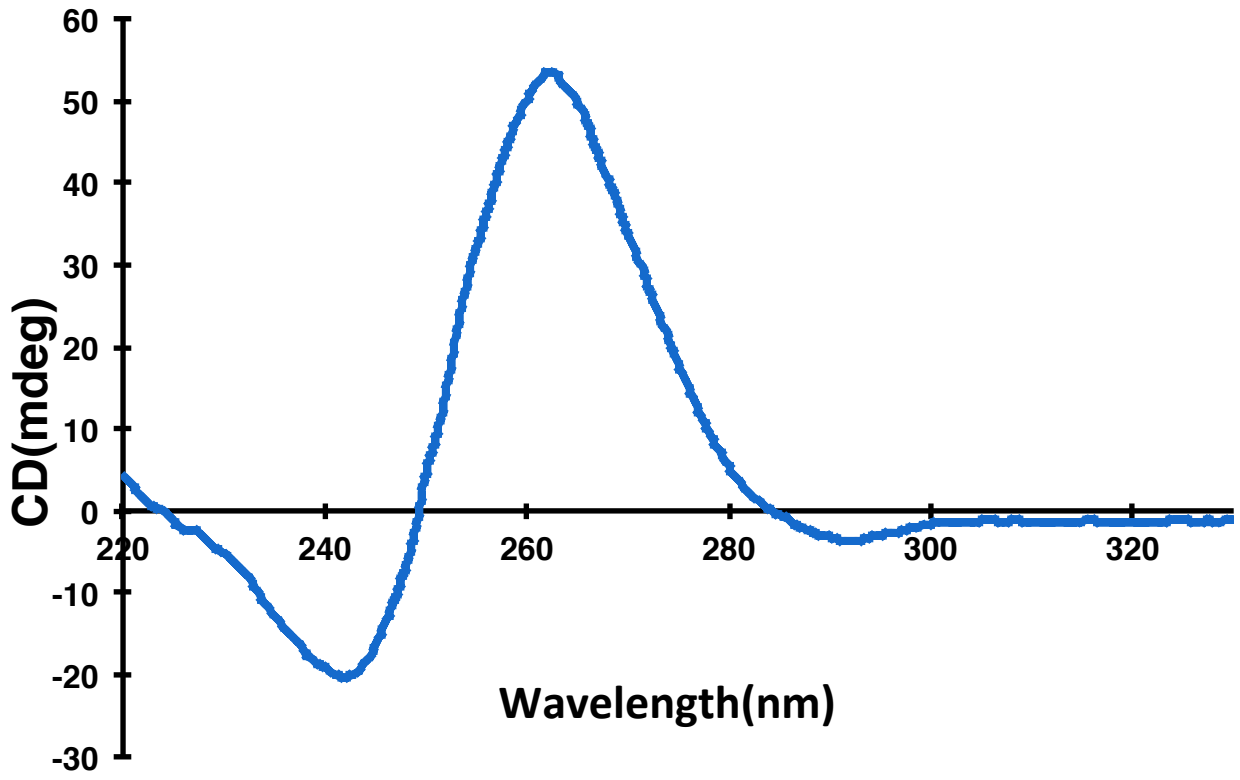


Figure S5. CD spectrum of the intramolecular G-quadruplex formed from the c-Myc22 DNA sequence 5′-d($TG_4AG_3TG_4AG_3TG_4A_2$) in 0.1 M sodium phosphate (pH 8.0) with 0.01 M KCl added. The sample contained 5 μM of DNA at 20 °C. This CD spectrum matched the spectrum previously reported for the intramolecular G-quadruplex formed by c-Myc22.[1]

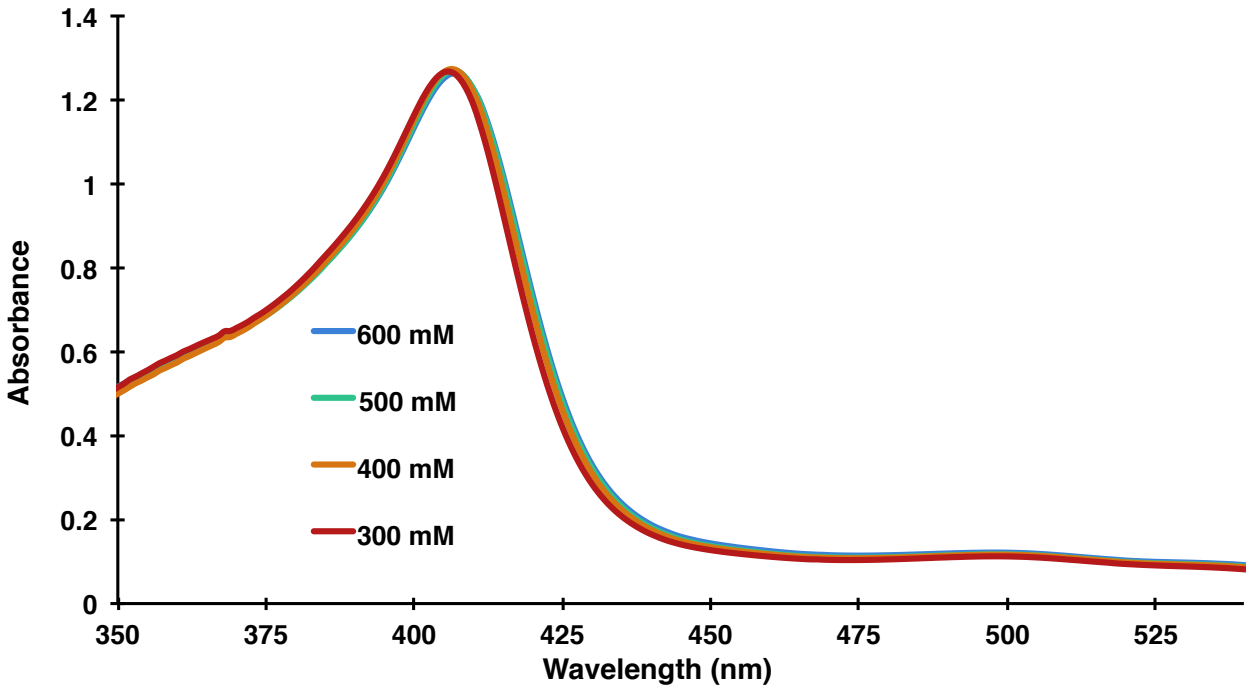


Figure S6. UV-Vis spectra showing the Soret band of hemin (20 μM) at 10 °C as a function of concentration of Na_2(5′-GMP) **1**. Hemin's Soret band does not change between 300-600 mM of **1**, indicating that under these conditions there is a sufficient concentration of G-quadruplexes $(\mathbf{1}_4)_n$ to fully bind 20 μM of hemin in forming the catalyst $(\mathbf{1}_4)_n$•Hm. Thus, we are confident that the 1 μM hemin used to determine Michelis-Menten constants (Fig. S7 and Table S1) is fully bound to the G-quadruplex and that the concentration of $(\mathbf{1}_4)_n$•Hm is also 1 μM.

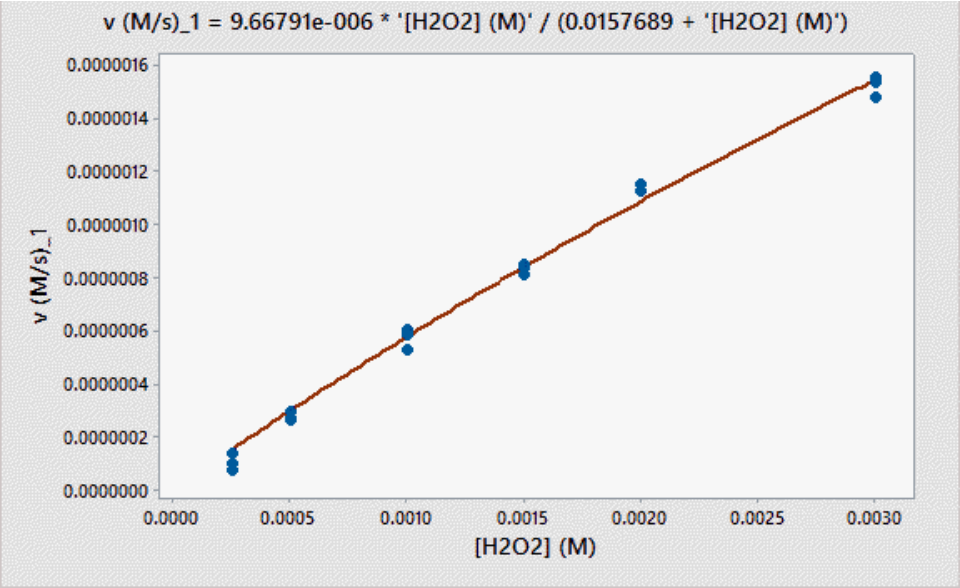
Figure S7. Kinetic analysis for the oxidation of ABTS by H_2O_2 as catalyzed by A) the supramolecular catalyst $(\mathbf{1}_4)_n$•Hm and B) the c-Myc22 G-quadruplex-hemin complex made from 5′-d($TGAG_3TG_4AG_3TG_4A_2$). Minitab® Statistical Software was used to perform a non-linear regression on the initial reaction rate as a function of H_2O_2 concentration (0.25-3.0 mM). The initial reaction rate was calculated from the slope of the reaction trace, production of $ABTS^{\bullet+}$ (λ= 415 nm), during the first 30 seconds. This data was fit to the Michaelis–Menten equation:

$$v = \frac{k_{cat}[E][H_2O_2]}{K_M + [H_2O_2]}$$

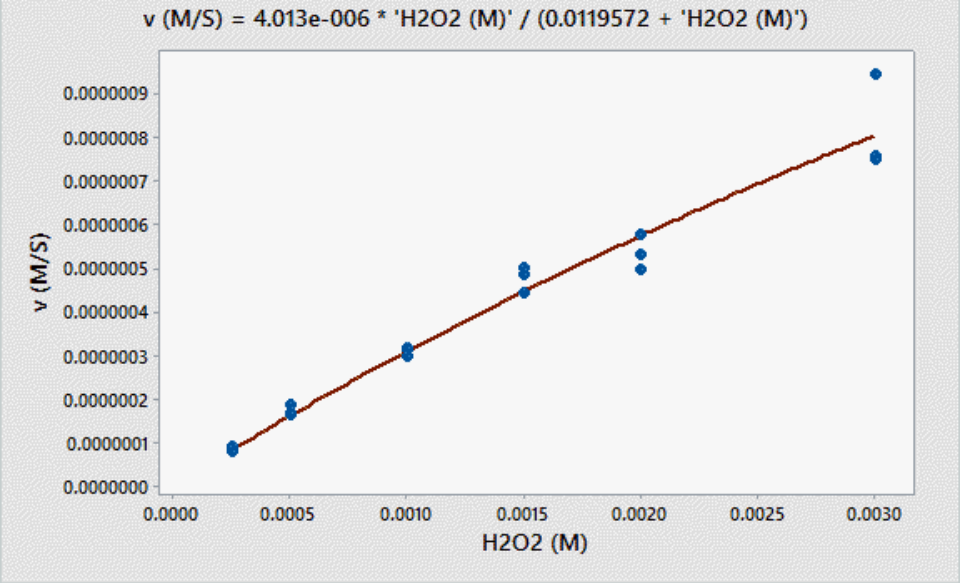
where [E] is the concentration of the peroxidase mimic $(\mathbf{1}_4)_n$•Hm, or c-Myc22•Hm, both of which have a concentration of 1 μM, since the 1 μM of hemin used in these experiments is fully complexed in both catalysts. $[H_2O_2]$ is the concentration of hydrogen peroxide used, v is the measured initial reaction rate, and k_{cat} and K_M are the Michaelis-Menten parameters.

The data fitted to the Michaelis-Menten equation, as well as the solved equation parameters are displayed below for A) 400 mM Na_2(5′-GMP) **1** and B) 30 μM c-Myc22 DNA. Kinetics were measured at 10 °C using 1 μM of hemin and 500 μM ABTS for both systems. Graph C) shows a comparison of the kinetics for the two catalysts $(\mathbf{1}_4)_n$•Hm and c-Myc22•Hm .

A)



B)


v (M/S) = 4.013e-006 * 'H2O2 (M)' / (0.0119572 + 'H2O2 (M)')
v (M/S)
0.0000009
0.0000008
0.0000007
0.0000006
0.0000005
0.0000004
0.0000003
0.0000002
0.0000001
0.0000000
0.0000
0.0005
0.0010
0.0015
0.0020
0.0025
0.0030
H2O2 (M)

C)

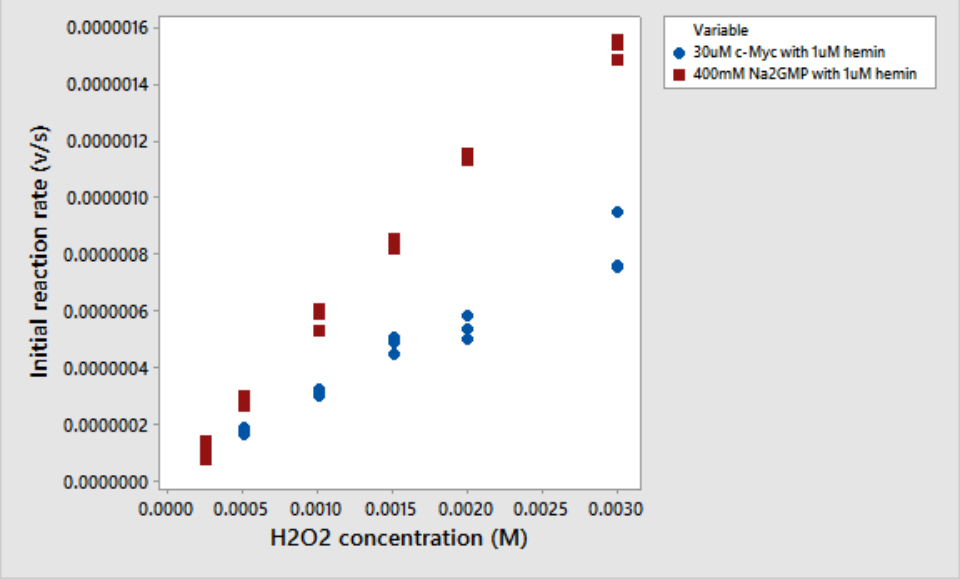

Initial reaction rate (v/s)
0.0000016
0.0000014
0.0000012
0.0000010
0.0000008
0.0000006
0.0000004
0.0000002
0.0000000
0.0000
0.0005
0.0010
0.0015
0.0020
0.0025
0.0030
H2O2 concentration (M)
Variable
30uM c-Myc with 1uM hemin
400mM Na2GMP with 1uM hemin

Table S1. Michaelis-Menten parameters for G-quadruplex/hemin peroxidase mimics with respect to H_2O_2. Measured at 10 °C, with 1 μM hemin and 500 μM ABTS

G-quadruplex type	k_{cat} (s^{-1})	K_M (mM)
$(\mathbf{1}_4)_n$•Hm (400 mM) Self-assembled	9.7 ± 2.6	16 ± 5
c-Myc22 DNA (30 μM) Intramolecular	4.0 ± 1.8 [a]	12 ± 3

[a] Our measured k_{cat} value for c-Myc22 DNA compares well with that measured by Cheng et al., namely 4.18 ± 0.22 s^{-1} at room temperature.[1]

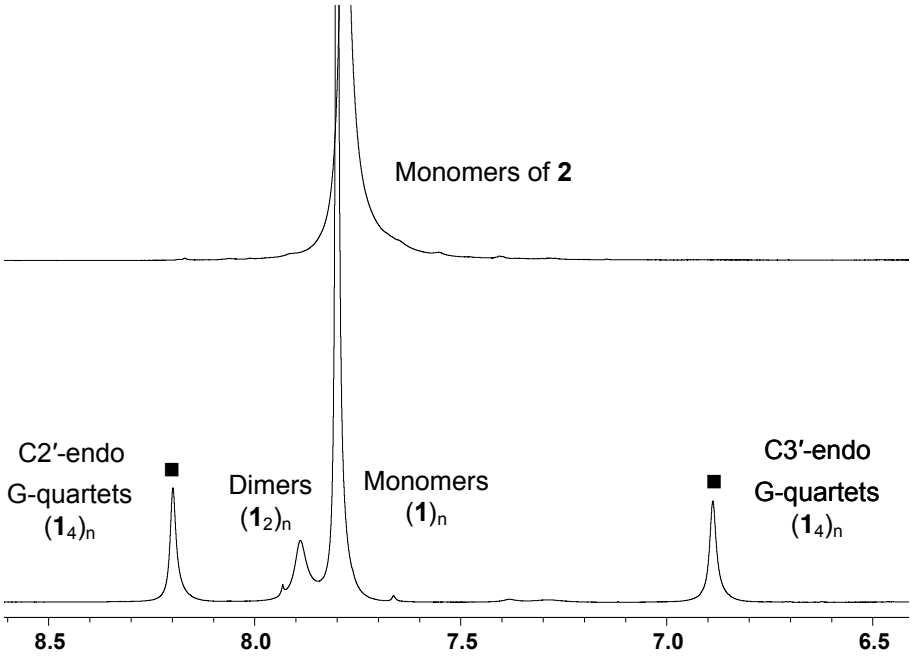


Figure S8. H8 region of the ^{1}H NMR spectra (800 MHz) for Na_2(2′-deoxyguanosine-5′-monophosphate) **2** compared with that of Na_2(5′-guanosine monophosphate) **1**. Top spectrum contains 400 mM of **2** at 10 °C, while the bottom spectrum contains 400 mm of **1** at 10 °C. Unlike 5′-GMP **1**, the sodium salt of the 2′-deoxy analog **2** does not self-assemble to give G-quadruplexes under these conditions.[4] In fact, we did not observe any NMR signals for G-quadruplexes even when the concentration of **2** was increased to 800 mM.

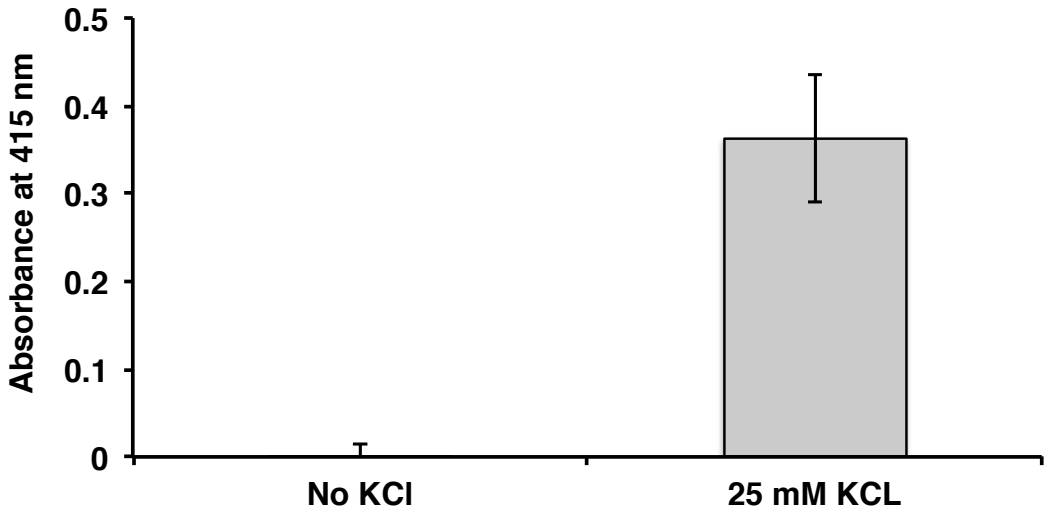


Figure S9. Absorbance of $ABTS^{\bullet+}$ (λ= 415 nm) measured 3 minutes after the addition of H_2O_2 (250 μM) to samples containing Na_2(2′deoxy-5′-GMP) **2** (400 mM), hemin (1 μM), ABTS (500 μM) with and without added KCl (25 mM). The addition of K^+ drives G-quadruplex formation by **2** (see published NMR data for self-assembly of **2** in presence of added K^+)[3] and enables peroxidase activity in the presence of hemin.

References

1. Cheng, X.; Liu, X.; Bing, T.; Cao, Z.; Shangguan, D. *Biochemistry* **2009**, *48*, 7817.

2. Wu, G.; Kwan, I. C. M. *J. Am. Chem. Soc.* **2009**, *131*, 3180.

3. Mudroňová, K.; Římal, V.; Mojzeš, P. *Vibr. Spec.* **2016**, *82*, 60.