SUPPLEMENTARY DATA

Enhancement of DNAzymatic activity using iterative *in-silico* maturation.

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EXPERIMENTAL SECTION

Chemicals and reagents

Oligodeoxynucleotides (ODNs) were synthesized and then purified using high-performance liquid chromatography (HPLC) (Eurofins Genomics (Tokyo, Japan)). Hemin (ferriprotoporphyrin IX chloride) was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Ready-to-Use

3,3',5,5'-tetramethylbenzidine (TMB) substrate solution containing H_2O_2, potassium chloride (KCl), and tris(hydroxymethyl)aminomethane (Tris) were obtained from Fujifilm Wako Pure Chemical Industries (Osaka, Japan).

Folding of G4/hemin complexes

All ODNs were dissolved in TE buffer (10 mM Tris-HCl (pH7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA)) to a final concentration of 50 μM, and then stored at 4 ˚C. Hemin stock solution (400 mM) was prepared in dimethyl sulfoxide and then stored in the dark at 4 ˚C. The ODNs were then diluted with Tris-HCl buffer (10 mM, pH 7.4) containing KCl (50 mM), and then heated to 95 ˚C for 5 min and gradually cooled to 25 ˚C for 30 min to fold into the G4 structures. The final G4/hemin complex was formed by mixing ODN and hemin to a final concentration of 5 μM and 20 μM, respectively.

Polyacrylamide gel electrophoresis (PAGE)

G4 structures were evaluated utilizing PAGE. Folded G4 or G4/hemin were dissolved in 5X Novex hi-density Tris-Borate-EDTA (TBE) sample buffer (Thermo Fisher Scientific, Waltham, MA, USA) and loaded on 25 % TBE polyacrylamide gel (Tefco, Tokyo, Japan), and then electrophoresed for 140 min at 180 V at 4 ˚C in TBE buffer. A Tracklt 10 bp DNA ladder (Thermo Fisher Scientific) was used for a molecular weight marker. Fluorescent staining was performed with SYBR® Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific) and viewed using a Safe Imager Blue-Light Transilluminator (Thermo Fisher Scientific).

Circular dichroism (CD)

CD spectra were used to determine the topology of G4/hemin complexes. CD spectra were obtained using a J-725 CD Spectrophotometer (JASCO, Tokyo, Japan) in a microcell with 10 mm optical path length. Scanning was performed form 230 nm to 320 nm at 25 ˚C with a step size of 0.2 nm, at scan rate of 50 nm min^{-1}, a response of 2.0 sec, and a bandwidth of 1.0 nm. Each scan was repeated 20 times and the values were averaged, and the spectrum of the buffer was subtracted. The structure was determined based on the peaks and troughs of the spectra. Parallel G4 structures have a minimum peak absorbance at *c.a.* 240 nm and a maximum peak absorbance at *c.a.* 260 nm. Antiparallel have a minimum peak absorbance at *c.a.* 260 nm and maximum peak absorbance at *c.a.* 280 nm. A mixture of the two or a hybrid structure have a maximum peak absorbance *c.a.* 260 nm and 280 nm.

Peroxidase (POD)-mimicking activity assay

POD-mimicking activity was measured using a microplate reader to select the ODN sequence with highest activity. To measure the POD-mimicking activity, 5 μL of the folded G4/hemin complex solution was added to a 96-well plate. TMB substrate solution (400 μM, 195 μL) was then added to each well. The plate was then placed in a microplate reader (MTP-880Lab; Corona Electric, Ibaraki, Japan) and the absorbance measured at 630 nm every 30 s for 10 min. The results were then analyzed using OriginPro 9 software (OriginLab, Northampton, MA, USA) with a linear fit function to determine the slope of each curve. The molar extinction efficient (Ɛ) of TMB is 39,000 $M^{-1}cm^{-1}$.

The catalytic constant for the reaction with TMB was determined by adding various amounts of TMB (from 0 to 1.6 mM) to the reaction solution. The kinetic parameters (V_{max} and K_m) were obtained using the Michaelis equation of Prism software (GraphPad, San Diego, CA, USA). The k_{cat} values were obtained from the equation $k_{cat} = V_{max} / [ODN]$.

Exemplar *in-silico* maturation

The *in-silico* maturation was performed in Microsoft Excel version 16.42. An example is below: on the parent arrays and array operations in the eighth round of array production. For the parent sequence for creating 10 sequences to be used in the eighth round and three consecutive G motifs, divided into 5', 3' end and the first to third loop is as follows.

Name	Sequence (5’ to 3')									Length (base)	Ranking
	5’ end	G_3	1st Loop	G_3	2nd Loop	G_3	3rd Loop	G_3	3’ end		
7-02	(N)	G_3	AAA	G_3	CAC	G_3	C	G_3	(N)	19	**1**
7-01	(N)	G_3	AAA	G_3	ACG	G_3	A	G_3	(N)	19	**2**
7-08	(N)	G_3	AAA	G_3	ACG	G_3	A	G_3	G	20	**3**
7-04	(N)	G_3	TAA	G_3	ACG	G_3	C	G_3	C	20	**4**
6-07	(N)	G_3	TAA	G_3	ACG	G_3	A	G_3	(N)	19	**5**

Array Operation:

1. The triple G motif is fixed and the 3' end and the 1st to 3rd loops are randomly shuffled.
2. As a result of the reproducibility evaluation of the 5th-7th round, the top 5 are used for shuffling.

3. Subsequently, for one aptamer, a mutation of 1 base is inserted in the loop part, or the 5' end and 3' end.
4. If the mutagenic portion is a loop portion A, T, G, C, N (missing), in the case of a 5 ' end A, T, G, either C, when mutated into G any of the other three bases, when mutated into 3 ' end N it is replaced with one of the four bases.
5. Producing 10 next-generation sequences.
6. Any sequences that overlap with the sequences that have appeared so far are eliminated in this step.

3' end and 1st to 3rd loop shuffling:

3' variations of the end and the first to third loops are as follows. Each corresponds to an integer from 1 to 4.

1st Loop		2nd Loop		3rd Loop		3’ end	
1	AAA	1	CAC	1	C	1	(N)
2	TAA	2	ACG	2	T	2	G
				3	A	3	C

Shuffling: Random numbers generate integers corresponding to the number of variations.

	1st Loop	2nd Loop	3rd Loop	3’ end
pre mut_8-01	2	1	2	1
pre mut_8-02	2	1	1	3

pre mut_8-03	1	1	2	2
pre mut_8-04	1	1	2	3
pre mut_8-05	2	2	1	2
pre mut_8-06	1	2	1	1
pre mut_8-07	2	1	1	2
pre mut_8-08	1	2	3	2
pre mut_8-09	1	1	3	1
pre mut_8-10	1	2	3	1

Replacing the above result with an array results in the following:

	5’ end	1st Loop	2nd Loop	3rd Loop	3’ end
pre mut_8-01	(N)	TAA	CAC	T	(N)
pre mut_8-02	(N)	TAA	CAC	C	C
pre mut_8-03	(N)	AAA	CAC	T	G
pre mut_8-04	(N)	AAA	CAC	T	C
pre mut_8-05	(N)	TAA	ACG	C	G
pre mut_8-06	(N)	AAA	ACG	C	(N)
pre mut_8-07	(N)	TAA	CAC	C	G
pre mut_8-08	(N)	AAA	ACG	A	G
pre mut_8-09	(N)	AAA	CAC	A	(N)
pre mut_8-10	(N)	AAA	ACG	A	(N)

Single point Mutation:

Introducing a mutation of one base for each sequence.

	The area where the mutation is introduced (1→5' end, 2→ 1st, 3rd→ 2nd, 4th→ 3rd, 5→3' end)	Where to introduce mutations (5' X-th from the side)	Bases to be introduced (1→a, 2→t, 3→g, 4→c, 5→N		
pre mut_8-01	1	1	1	→	a
pre mut_8-02	2	3	4	→	c
pre mut_8-03	2	2	4	→	c
pre mut_8-04	5	1	3	→	g
pre mut_8-05	1	1	2	→	t
pre mut_8-06	1	1	4	→	c

pre mut_8-07	4	1	2	→	t
pre mut_8-08	5	1	2	→	t
pre mut_8-09	3	2	2	→	t
pre mut_8-10	4	1	2	→	t

If the mutagenic portion is a loop portion A, T, G, C, N (missing), in the case of 5 'end A, T, G, either C, when mutated into G any of the other three bases, when mutated into 3 'end N it is replaced with one of the four bases.

Any of the 1-3 bases when the mutagenetic point is the first or second loop, when the 5 'end or the third loop or 3' end is introduced into the first base. When the above mutation introduction is reflected in the sequence of the loop region, it becomes as follows.

	5’ end	1st Loop	2nd Loop	3rd Loop	3’ end
pre mut_8-01	a	TAA	CAC	T	(N)
pre mut_8-02	(N)	TAc	CAC	C	C
pre mut_8-03	(N)	AcA	CAC	T	G
pre mut_8-04	(N)	AAA	CAC	T	g
pre mut_8-05	t	TAA	ACG	C	G
pre mut_8-06	c	AAA	ACG	C	(N)
pre mut_8-07	(N)	TAA	CAC	t	G
pre mut_8-08	(N)	AAA	ACG	A	t
pre mut_8-09	(N)	AAA	CtC	A	(N)
pre mut_8-10	(N)	AAA	ACG	t	(N)

The 8th round of the array:

Shows the sequence obtained from the above results.

Name	Sequence (5’ to 3')	Length (base)

8-01	aGGGTAAGGGCACGGGTGGG	20
8-02	GGGTAcGGGCACGGGCGGGC	20
8-03	GGGAcAGGGCACGGGTGGGG	20
8-04	GGGAAAGGGCACGGGTGGGg	20
8-05	tGGGTAAGGGACGGGGCGGGG	21
8-06	cGGGAAAGGGACGGGGCGGG	20
8-07	GGGTAAGGGCACGGGtGGGG	20
8-08	GGGAAAGGGACGGGGAGGGt	20
8-09	GGGAAAGGGCtCGGGAGGG	19
8-10	GGGAAAGGGACGGGGtGGG	19

* Red letters indicate the mutation introduction points.

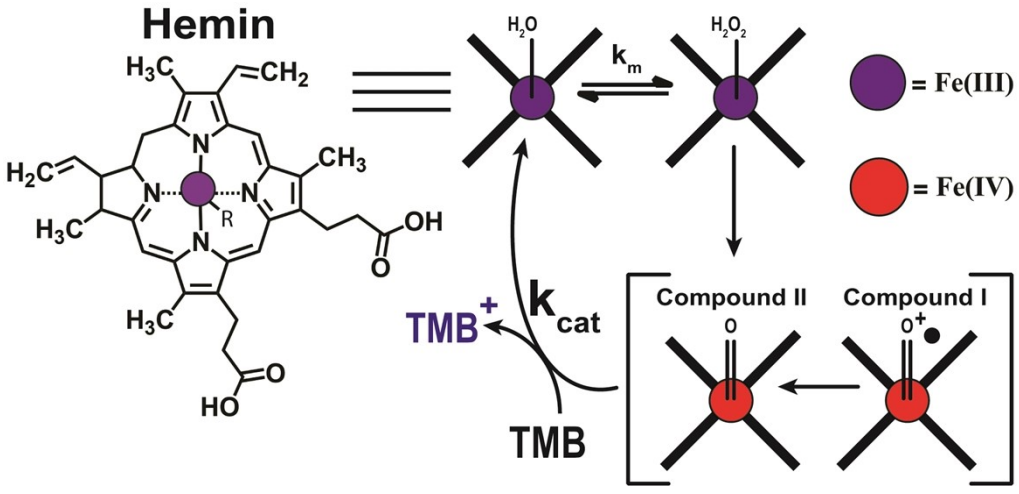

Figure S1. Structure and general reaction pathway for the iron containing protoporphyrin, hemin. The R group is replaced by a water (H_2O) molecule that is in turn replaced by a hydrogen peroxide (H_2O_2) molecule. The peroxide is then broken down into a cation π radical oxygen (Compound I) or oxygen (Compound II) resulting in the oxidation the Fe(III) into Fe(IV). The resulting Compound I can oxidize a substrate to become Compound II while Compound II is recycled back into Fe(III). G4-based biosensors rely on the formation of a G4/hemin complex which in turn exhibits peroxidase (POD)-mimicking activity.[1,2] In the presence of hydrogen peroxide (H_2O_2), the G4/hemin complex reacts with substrates such as 3,3',5,5'-tetramethylbenzidine (TMB, $Abs_{\lambda max}$ = 605 nm),[3] 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, $Abs_{\lambda max}$ = 420 nm)[4] or luminol (3-Aminophthalhydrazide),[5] to produce a colorimetric readout.

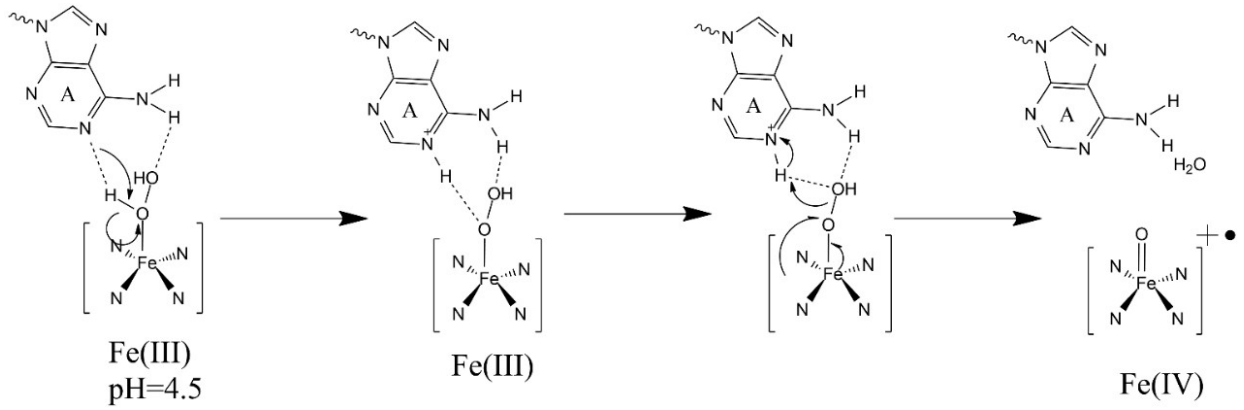

Figure S2. Proposed schematic for how an adenine nucleotide plays a role in the heterolytic cleavage of hydrogen peroxide during peroxidase-mimicking of the G4/hemin aptamer.

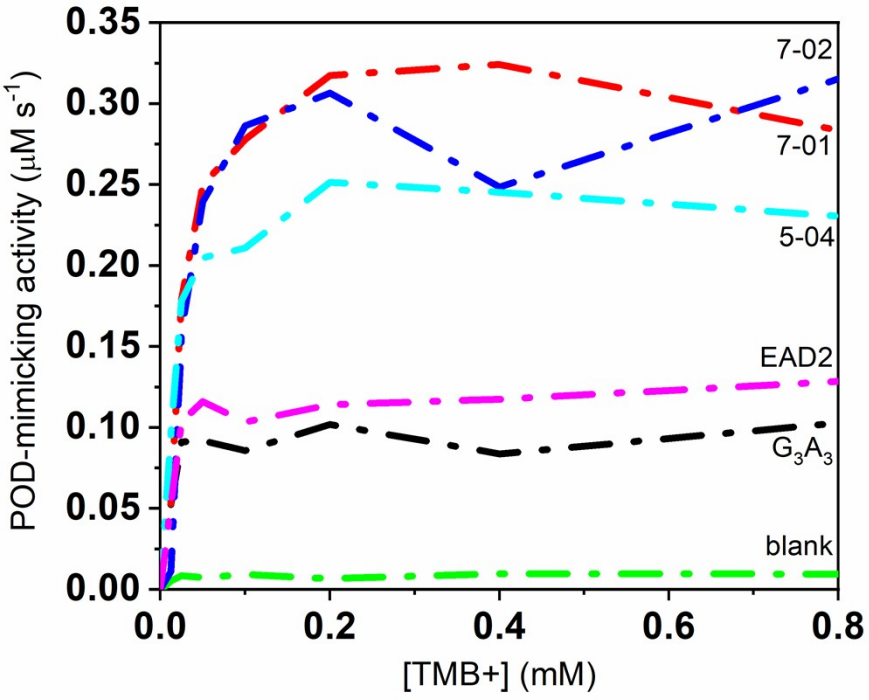

Figure S3. Michaelis-Menten curves for hemin base line (green), EAD2 (pink), G_3A_3 (black), 7-01 (red), 7-02 (blue) and 5-04 (turquois). Each line is an average of three curves and the red dotted line is the curve fit performed using OriginPro 9 applying software non-linear line fit function. Errors are ploT_2ed but not observable as they are <1%.

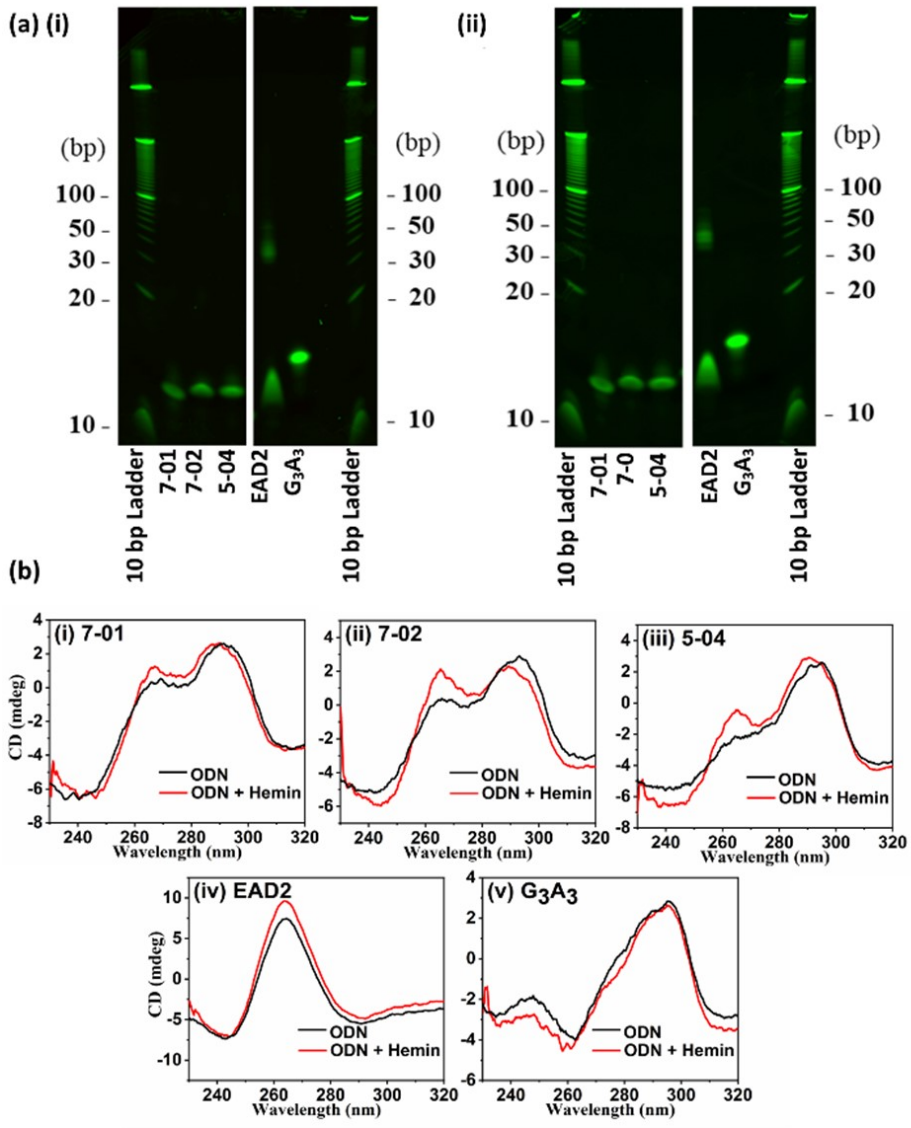

Figure S4: (a) PAGE analysis of G4s and G4/hemin complexes. Samples were run on a 10-20 %(v/v) graduated polyacrylamide gel, stained with SYBR® gold. G4 aptamers (i) without hemin and (ii) with hemin added. A 10-base pair (bp) ladder was used as a reference and (b) CD spectra for G4 ODN sequences: (i) 7-01, (ii) 7-02, (iii) 5-04, (iv) EAD2 and (v) G_3A_3. Scanning was performed from 220 nm to 320 nm at 25 ˚C with a step size of 0.2 nm, at a scan rate of 50 nm min^{-1}, a response of 2.0 s and a bandwidth of 1.0 nm. Each scan was repeated 20 times and the values averaged. The solid black lines indicate the folded G4 and the solid red lines indicate the folded G4/hemin complex.

Structural Characterization

The top 3 sequences (7-01, 7-02 and 5-04), EAD2 and G_3A_3 G4 structures were characterized using native polyacrylamide gel electrophoresis (PAGE) and circular dichroism (CD) spectroscopy as shown in Figure S5.

For the PAGE (Figure S5(a)(i and ii)), the top 3 sequences (19 nt), EAD2 (18 nt) and showed intramolecular (single stranded) G4, as the bands occur at ~21 nt, 22 nt and 30 nt, respectively. EAD2 also showed the presence of a band at ~80 nt, consistent with an intermolecular four strand G4 (80 nt / 18 nt ≈ 4.4 strands). This discrepancy in strand size and measured size is indicative of monomeric G-quadruplexes, which have larger cross-sectional areas as compared to linear duplexed DNA, decreasing distance travelled through the gel.[6] CD spectroscopy data were used to show the topology of the G4 structure (Figure S5(b)(i-v)). Parallel G4 structures have a minimum peak absorbance at *c.a.* 240 nm and a maximum peak absorbance at *c.a.* 260 nm. Antiparallel have a minimum peak absorbance at *c.a.* 260 nm and maximum peak absorbance at *c.a.* 280 nm.[7] A mixture of the two, or a hybrid structure, have a maximum peak absorbance *c.a.* 260 nm and 280 nm.[7] For the 7-01, 7-02, 5-04 G4 structures without hemin (Figure S5(b)(i-iii) black), the topology has two maxima *c.a.* 260 nm and 290 nm, indicative of hybrid structures (Figure S5(c)). The hybrid topology is a result of the truncated L_3, containing a single nucleotide (Table S3).

Upon addition of hemin there was a reduction in the peak at 260 nm, particularly for sequence 7-02, indicative of the formation of more parallel structures (Figure S5(b)(i-iii), red). From this data, it appears that the topological structures that had the largest increase in POD-mimicking activity were hybrid structures. The hybrid structures were also shown to have higher POD-mimicking

activities when compared to EAD2 (intermolecular parallel) and G_3A_3 (intermolecular antiparallel). Interestingly, after comparison of these results with the data found in Table 1, it appears that the type of G4 structure formed is not linked to enhanced POD-mimicking activity, instead it is far more reliant on the ODN sequence of the G4 itself.

Table S1. Peroxidase (POD)-mimicking activity calculated for the G4/hemin complex with the ranking of each sequence based on POD. Each sequence is listed with the mutated residues in red. The top four sequences (highest POD) are underlined. All Errors below 0.001 μM s^{-1} are not shown.

Rank	Name	ODN Sequence (5' to 3')	T_1	G_m	L_1	G_m	L_2	G_m	L_3	G_m	T_1	POD activity, mM s^{-1}
1	1-08	$\underline{CTG_3AG_3TG_3TG_3A}$	CT	G3	A	G3	T	G3	T	G3	A	0.045
2	EAD2	$\underline{CTG_3AG_3AG_3AG_3A}$	CT	G3	A	G3	A	G3	A	G3	A	0.031
3	1-02	$\underline{G_3AG_3AG_3AG_3A}$	(N)	G3	A	G3	A	G3	A	G3	A	0.028
4	1-07	$\underline{G_3AG_3TG_3TG_3A}$	(N)	G3	A	G3	T	G3	T	G3	A	0.023
5	1-09	$CTG_4AG_4AG_3AG_3A$	CT	G4	A	G4	A	G4	A	G3	A	0.021
6	1-03	$G_3GGAG_3AG_3AG_3$	(N)	G3	A	G3	A	G3	A	G3	(N)	0.022
7	1-01	$CTG_4AG_4AG_4AG_4A$	CT	G4	A	G4	A	G4	A	G4	A	0.015
8	1-10	$CTG_4AG_3AG_4AG_3A$	CT	G4	A	G3	A	G4	A	G3	A	0.013 ± 0.009
9	1-04	$G_3TG_3AG_3AG_3$	(N)	G3	T	G3	A	G3	A	G3	(N)	0.012
10	1-05	$G_3AG_3TG_3AG_3$	(N)	G3	A	G3	T	G3	A	G3	(N)	0.011
11	1-06	$G_3AG_3TG_3TG_3$	(N)	G3	A	G3	T	G3	T	G3	(N)	0.007

Table S2. Peroxidase (POD)-mimicking activity calculated for all G4/hemin complex generations that were evaluated. Each sequence is ranked based on POD-mimicking activity compared to all generations.

Rank	Name	ODN Sequence (5' to 3')	T_1	G_m	L_1	G_m	L_2	G_m	L_3	G_m	T_1	POD activity, mM s^{-1}
Parent and diversity sequences												
46	**EAD2**	$CTG_3AG_3AG_3AG_3A$	CT	G_3	A	G_3	A	G_3	A	G_3	A	0.031
28	**G_3A_3**	$G_3A_3G_3A_3G_3A_3G_3$	(N)	G_3	A_3	G_3	A_3	G_3	A_3	G_3	(N)	0.055
1st round												
68	**1-01**	$CTG_4AG_4AG_4AG_4A$	CT	G_4	A	G_4	A	G_4	A	G_4	A	0.015
48	**1-02**	$G_3AG_3AG_3AG_3A$	(N)	G_3	A	G_3	A	G_3	A	G_3	A	0.028
56	**1-03**	$G_3AG_3AG_3AG_3$	(N)	G_3	A	G_3	A	G_3	A	G_3	(N)	0.022
72	**1-04**	$G_3TG_3AG_3AG_3$	(N)	G_3	T	G_3	A	G_3	A	G_3	(N)	0.012
75	**1-05**	$G_3AG_3TG_3AG_3$	(N)	G_3	A	G_3	T	G_3	A	G_3	(N)	0.011
79	**1-06**	$G_3AG_3TG_3TG_3$	(N)	G_3	A	G_3	T	G_3	T	G_3	(N)	0.007
54	**1-07**	$G_3AG_3TG_3TG_3A$	(N)	G_3	A	G_3	T	G_3	T	G_3	A	0.023
33	**1-08**	$CTG_3AG_3TG_3TG_3A$	CT	G_3	A	G_3	T	G_3	T	G_3	(N)	0.045
55	**1-09**	$CTG_4AG_4AG_3AG_3A$	CT	G_4	A	G_4	GA	G_3	A	G_3	A	0.022
71	**1-10**	$CTG_4AG_3AG_4AG_3A$	CT	G_4	A	G_3	A	G_4	A	G_3	A	0.013
2nd round												
77	**2-01**	$CTG_3A_3G_3A_3G_3A_3G_3$	CT	G_3	A_3	G_3	A_3	G_3	A_3	G_3	(N)	0.010
73	**2-03**	$CTG_3A_3G_3A_3G_3A_3G_3A$	CT	G_3	A_3	G_3	A_3	G_3	A_3	G_3	A	0.012
67	**2-04**	$CTG_3A_3G_3TA_2G_3A_3G_3A$	CT	G_3	A_3	G_3	TA_2	G_3	A_3	G_3	A	0.017
58	**2-05**	$CG_3A_2G_3A_2G_3A_2G_3$	C	G_3	A_2	G_3	A_2	G_3	A_2	G_3	(N)	0.021
76	**2-06**	$CTG_3A_2G_3A_2G_3A_2G_3$	CT	G_3	A_2	G_3	A_2	G_3	A_2	G_3	(N)	0.010
70	**2-07**	$CTG_3A_2G_3A_2G_3A_2G_3A$	CT	G_3	A_2	G_3	A_2	G_3	A_2	G_3	A	0.014
66	**2-08**	$CTG_3A_3G_3TG_3A_3G_3A$	CT	G_3	A_3	G_3	T	G_3	A_3	G_3	A	0.017
43	**2-09**	$CTG_3A_3G_3TA_2G_3TG_3A$	CT	G_3	A_3	G_3	TA	G_3	T	G_3	A	0.033
52	**2-10**	$CTG_3A_3G_3TG_3TG_3A$	CT	G_3	A_3	G_3	T	G_3	T	G_3	A	0.024
3rd round												
47	**3-01**	$CTG_3TG_3TG_3TG_3$	CT	G_3	T	G_3	T	G_3	T	G_3	(N)	0.029
19	**3-02**	$G_3A_3G_3TCAG_3TG_3$	(N)	G_3	A_3	G_3	TCA	G_3	T	G_3	(N)	0.079
62	**3-03**	$G_3A_3G_3AG_3A_3G_3$	(N)	G_3	A_3	G_3	A	G_3	A_3	G_3	(N)	0.019
61	**3-04**	$G_3AG_3TG_3TA_2G_3A$	(N)	G_3	A	G_3	T	G_3	TA_2	G_3	A	0.019

80	**3-05**	$G_3AG_3TG_3CG_3$	(N)	G_3	A	G_3	T	G_3	C	G_3	(N)	0.006
69	**3-06**	$CTG_3AG_3A_2G_3AG_3$	CT	G_3	A	G_3	A_2	G_3	A	G_3	(N)	0.015
53	**3-07**	$T_2G_3A_3G_3AG_3AG_3A$	T_2	G_3	A_3	G_3	A	G_3	A	G_3	A	0.024
65	**3-08**	$C_2G_3A_3G_3TG_3A_3G_3$	C_2	G_3	A_3	G_3	T	G_3	A_3	G_3	(N)	0.018
22	**3-09**	$CTG_3A_3G_3A_2CG_3TG_3A$	CT	G_3	A_3	G_3	A_2C	G_3	T	G_3	A	0.076
27	**3-10**	$CTG_3TA_2G_3TA_2G_3A_3G_3$	CT	G_3	TA_2	G_3	TA_2	G_3	A_3	G_3	(N)	0.060
4th round												
78	**4-01**	$CTG_3A_3G_3TCAG_3TG_3A$	CT	G_3	A_3	G_3	TCA	G_3	T	G_3	A	0.009
5	**4-02**	$G_3A_3G_3A_2CG_3TG_3$	(N)	G_3	A_3	G_3	A_2C	G_3	T	G_3	(N)	0.139
74	**4-03**	$G_3A_3G_3TA_2G_3A_3G_3T$	(N)	G_3	A_3	G_3	TA_2	G_3	A_3	G_3	T	0.011
60	**4-04**	$G_3TA_2G_3TA_2G_3TG_3$	(N)	G_3	TA_2	G_3	TA_2	G_3	T	G_3	(N)	0.020
13	**4-05**	$G_3TA_2G_3A_2CG_3AG_3$	(N)	G_3	TA_2	G_3	A_2C	G_3	A	G_3	(N)	0.100
31	**4-06**	$TG_3A_3G_3TA_2G_3A_3G_3A$	T	G_3	A_3	G_3	TA_2	G_3	A_3	G_3	A	0.049
38	**4-07**	$AG_3TA_2G_3TA_2G_3A_3G_3$	A	G_3	TA_2	G_3	TA_2	G_3	A_3	G_3	(N)	0.040
49	**4-08**	$CTG_3AgAG_3TA_2G_3A_3G_3$	CT	G_3	AGA	G_3	TA_2	G_3	A_3	G_3	(N)	0.025
29	**4-09**	$T_2G_3TA_2G_3TCAG_3A_3G_3A$	T_2	G_3	TA_2	G_3	TCA	G_3	A_3	G_3	A	0.051
37	**4-10**	$CTG_3TA_2G_3TgAG_3A_3G_3$	CT	G_3	TA_2	G_3	TGA	G_3	A_3	G_3	(N)	0.040
5th round												
32	**5-01**	$G_3A_3G_3TCAG_3AG_3$	(N)	G_3	A_3	G_3	TCA	G_3	A	G_3	(N)	0.047
39	**5-02**	$AG_3TA_2G_3TCAG_3TG_3$	(N)	G_3	TA_2	G_3	TCA	G_3	T	G_3	(N)	0.038
42	**5-03**	$G_3A_2G_3A_2CG_3AG_3$	(N)	G_3	A_2	G_3	A_2C	G_3	A	G_3	(N)	0.034
3	**5-04**	$G_3A_3G_3A_2CG_3CG_3$	(N)	G_3	A_3	G_3	A_2C	G_3	C	G_3	(N)	0.151
14	**5-05**	$G_3A_3G_3A_2TG_3AG_3$	(N)	G_3	A_3	G_3	A_2T	G_3	A	G_3	(N)	0.099
15	**5-06**	$G_3A_3G_3A_2CG_3AG_3C$	(N)	G_3	ATA	G_3	A_2C	G_3	A	G_3	(N)	0.098
10	**5-07**	$G_3ACAG_3A_2CG_3TG_3$	(N)	G_3	ACA	G_3	A_2C	G_3	T	G_3	(N)	0.111
44	**5-09**	$G_3A_2G_3TCAG_3AG_3$	(N)	G_3	A_2	G_3	TCA	G_3	A	G_3	(N)	0.033
41	**5-10**	$G_3TA_2G_3TA_2G_3AG_3$	(N)	G_3	TA_2	G_3	TA_2	G_3	A	G_3	(N)	0.035
6th round												
17	**6-01**	$G_3ATAG_3A_2TG_3CG_3$	(N)	G_3	ATA	G_3	A_2T	G_3	C	G_3	(N)	0.088
63	**6-02**	$G_3ACAG_3ACG_3CG_3$	(N)	G_3	ACA	G_3	AC	G_3	C	G_3	(N)	0.019
20	**6-03**	$G_3ATAG_3A_2TG_3TG_3$	(N)	G_3	ATA	G_3	A_2T	G_3	T	G_3	(N)	0.079
12	**6-04**	$G_3A_3G_3A_2CG_3AG_3$	(N)	G_3	A_3	G_3	A_2C	G_3	A	G_3	(N)	0.105
34	**6-05**	$G_3AgAG_3A_2TG_3CG_3$	(N)	G_3	AGA	G_3	A_2T	G_3	C	G_3	(N)	0.043
25	**6-06**	$G_3ACTG_3A_2TG_3TG_3$	(N)	G_3	ACT	G_3	A_2T	G_3	T	G_3	(N)	0.066
6	**6-07**	$G_3TA_2G_3CACG_3AG_3$	(N)	G_3	TA_2	G_3	CAC	G_3	A	G_3	(N)	0.136
9	**6-08**	$G_3TA_2G_3A_2CG_3CG_3$	(N)	G_3	TA_2	G_3	A_2C	G_3	C	G_3	(N)	0.125
51	**6-09**	$G_3A_2G_3A_2CG_3CG_3$	(N)	G_3	A_2	G_3	A_2C	G_3	C	G_3	(N)	0.025

35	**6-10**	$G_3ATAG_3A_3G_3CG_3$	(N)	G_3	ATA	G_3	A_3	G_3	C	G_3	(N)	0.042
7th round												
2	**7-01**	$G_3TA_2G_3CACG_3AG_3$	(N)	G_3	TA_2	G_3	CAC	G_3	T	G_3	(N)	0.151
1	**7-02**	$G_3A_3G_3CACG_3CG_3$	(N)	G_3	A_3	G_3	CAC	G_3	C	G_3	(N)	0.171
26	**7-03**	$AG_3A_3G_3A_2CG_3CG_3$	A	G_3	A_3	G_3	A_2C	G_3	C	G_3	(N)	0.060
7	**7-04**	$G_3TA_2G_3CACG_3CG_3C$	(N)	G_3	TA_2	G_3	CAC	G_3	C	G_3	C	0.133
21	**7-05**	$CG_3A_3G_3A_2CG_3AG_3$	C	G_3	A_3	G_3	A_2C	G_3	A	G_3	(N)	0.078
8	**7-06**	$G_3A_2TG_3CACG_3AG_3$	(N)	G_3	A_2T	G_3	CAC	G_3	A	G_3	(N)	0.125
24	**7-07**	$G_3TA_2G_3ACG_3CG_3$	(N)	G_3	TA_2	G_3	AC	G_3	C	G_3	(N)	0.069
4	**7-08**	$G_3A_3G_3CACG_3AG_3$	(N)	G_3	A_3	G_3	CAC	G_3	A	G_3	(N)	0.144
11	**7-09**	$G_3A_3G_3CACG_3AG_4$	(N)	G_3	A_3	G_3	CAC	G_3	A	G_3	G	0.110
50	**7-10**	$G_3TA_2G_3C_2G_3CG_3$	(N)	G_3	TA_2	G_3	C_2	G_3	C	G_3	(N)	0.025
8th round												
30	**8-01**	$AG_3TA_2G_3CACG_3TG_3$	A	G_3	TA_2	G_3	CAC	G_3	T	G_3	(N)	0.050
16	**8-02**	$G_3TACG_3CACG_3CG_3C$	(N)	G_3	TAC	G_3	CAC	G_3	C	G_3	C	0.090
36	**8-03**	$G_3ACAG_3CACG_3TG_4$	(N)	G_3	ACA	G_3	CAC	G_3	T	G_3	G	0.041
23	**8-04**	$G_3A_3G_3CACG_3TG_4$	(N)	G_3	A_3	G_3	CAC	G_3	T	G_3	G	0.075
64	**8-05**	$TG_3TA_2G_3ACG_4CG_4$	T	G_3	TA_2	G_3	ACG	G_3	C	G_3	G	0.019
57	**8-06**	$CG_3A_3G_3ACG_4CG_3$	C	G_3	A_3	G_3	ACG	G_3	C	G_3	(N)	0.022
18	**8-07**	$G_3TA_2G_3CACG_3TG_4$	(N)	G_3	TA_2	G_3	CAC	G_3	T	G_3	G	0.087
45	**8-08**	$G_3A_3G_3ACG_4AG_3T$	(N)	G_3	A_3	G_3	ACG	G_3	A	G_3	T	0.031
59	**8-09**	$G_3A_3G_3CTCG_3AG_3$	(N)	G_3	A_3	G_3	CTC	G_3	A	G_3	(N)	0.021
40	**8-10**	$G_3A_3G_3ACG_4TG_3$	(N)	G_3	A_3	G_3	ACG	G_3	T	G_3	(N)	0.038

Table S3. Peroxidase (POD)-mimicking activity calculated for the G4/hemin complex with the ranking of each sequence based on POD. All Errors below 0.001 μM s^{-1} not shown.

Rank	Name	ODN Sequence (5' to 3')	T_1	G_m	L_1	G_m	L_2	G_m	L_3	G_m	T_1	POD activity, μM s^{-1}
1	7-02	$G_3A_3G_3CACG_3CG_3$	(N)	G_3	A_3	G_3	CAC	G_3	C	G_3	(N)	0.171
2	7-01	$G_3TA_2G_3CACG_3AG_3$	(N)	G_3	TA_2	G_3	CAC	G_3	A	G_3	(N)	0.151
3	5-04	$G_3A_3G_3A_2CG_3CG_3$	(N)	G_3	A_3	G_3	A_2C	G_3	C	G_3	(N)	0.151
4	7-08	$G_3A_3G_3CACG_3AG_3$	(N)	G_3	A_3	G_3	CAC	G_3	A	G_3	(N)	0.144
5	4-02	$G_3A_3G_3A_2CG_3TG_3$	(N)	G_3	A_3	G_3	A_2C	G_3	T	G_3	(N)	0.139

6	6-07	$G_3TA_2G_3CACG_3AG_3$	(N)	G_3	TA_2	G_3	CAC	G_3	A	G_3	(N)	0.136
7	7-04	$G_3TA_2G_3CACG_3CG_3C$	(N)	G_3	TA_2	G_3	CAC	G_3	C	G_3	C	0.133
8	7-06	$G_3A_2TG_3CACG_3AG_3$	(N)	G_3	A_2T	G_3	CAC	G_3	A	G_3	(N)	0.125
9	6-08	$G_3TA_2G_3A_2CG_3CG_3$	(N)	G_3	TA_2	G_3	A_2C	G_3	C	G_3	(N)	0.125
10	5-07	$G_3ACAG_3A_2CG_3TG_3$	(N)	G_3	ACA	G_3	A_2C	G_3	T	G_3	(N)	0.111
11	7-09	$G_3A_2G_3CACG_3AG_4$	(N)	G_3	A_3	G_3	CAC	G_3	A	G_3	G	0.110
12	6-04	$G_3A_2G_3A_2CG_3AG_3$	(N)	G_3	A_3	G_3	A_2C	G_3	A	G_3	(N)	0.105
13	4-05	$G_3TA_2G_3A_2CG_3AG_3$	(N)	G_3	TA_2	G_3	A_2C	G_3	A	G_3	(N)	0.100
14	5-05	$G_3A_3G_3A_2TG_3AG_3$	(N)	G_3	A_3	G_3	A_2T	G_3	A	G_3	(N)	0.099
15	5-06	$G_3A_3G_3A_2CG_3AG_3C$	(N)	G_3	ATA	G_3	A_2C	G_3	A	G_3	(N)	0.098
16	8-02	$G_3TACG_3CACG_3CG_3C$	(N)	G_3	TAC	G_3	CAC	G_3	C	G_3	C	0.090
17	6-01	$G_3ATAG_3A_2TG_3CG_3$	(N)	G_3	ATA	G_3	A_2T	G_3	C	G_3	(N)	0.088
18	8-07	$G_3TA_2G_3CACG_3TG_4$	(N)	G_3	TA_2	G_3	CAC	G_3	T	G_3	G	0.087
19	3-02	$G_3A_3G_3TCAG_3TG_3$	(N)	G_3	A_3	G_3	TCA	G_3	T	G_3	(N)	0.079
20	6-03	$G_3ATAG_3A_2TG_3TG_3$	(N)	G_3	ATA	G_3	A_2T	G_3	T	G_3	(N)	0.079
21	7-05	$CG_3A_3G_3A_2CG_3AG_3$	C	G_3	A_3	G_3	A_2C	G_3	A	G_3	(N)	0.078
22	3-09	$CTG_3A_3G_3A_2CG_3TG_3A$	CT	G_3	A_3	G_3	A_2C	G_3	T	G_3	A	0.076
23	8-04	$G_3A_3G_3CACG_3TG_4$	(N)	G_3	A_3	G_3	CAC	G_3	T	G_3	G	0.075
24	7-07	$G_3TA_2G_3ACG_3CG_3$	(N)	G_3	TA_2	G_3	AC	G_3	C	G_3	(N)	0.069
25	6-06	$G_3ACTG_3A_2TG_3TG_3$	(N)	G_3	ACT	G_3	A_2T	G_3	T	G_3	(N)	0.066
26	7-03	$AG_3A_3G_3A_2CG_3CG_3$	A	G_3	A_3	G_3	A_2C	G_3	C	G_3	(N)	0.060
27	3-10	$CTG_3TA_2G_3TA_2G_3A_3G_3$	CT	G_3	TA_2	G_3	TA_2	G_3	A_3	G_3	(N)	0.060
28	G_3A_3	$G_3A_3G_3A_3G_3A_3G_3$	(N)	G_3	A_3	G_3	A_3	G_3	A_3	G_3	(N)	0.055
46	EAD2	$CTG_3AG_3AG_3AG_3A$	CT	G_3	A	G_3	A	G_3	A	G_3	A	0.031

Table S4. Effect of K^+ ions on G4/hemin POD-mimicking activity [ODN] =0.125 μM, [hemin] = 0.5 μM.

	POD-mimicking activity, μM s^{-1} (x 10^{-3})	
G4 ODN	Without K^+ ions	With K^+ ions
7-02	1.46 ± 0.04	167.89 ± 0.10
7-01	23.26 ± 0.05	159.17 ± 0.05
5-04	3.64 ± 0.09	167.89 ± 0.05
EAD2	9.46 ± 0.09	35.61 ± 0.09
G_3A_3	13.07 ± 0.80	55.23 ± 1.45
Hemin blank	2.17 ± 0.04	2.17 ± 0.05

Potassium ions play an important role in the formation of G4 complexes and in turn the formation of G4/hemin complexes. In order to determine the K^+ ion dependence on the GA generated G4/hemin complexes, each of the top 3 GA G4 ODN sequences, G_3A_3 and EAD2 were tested for POD-mimicking activity without, and with, 50 mM K^+ ions (Table S3). Table S2 shows that all G4 complexes with no added K^+ ions have decreased POD-mimicking activity compared to with added K^+ ions (50 mM). For the sequences, 7-02 and 5-04, the POD-mimicking activity is 2 orders of magnitude higher with the added K^+ ions, with an increase from 1.46 ± 0.04 x 10^{-3} μM s^{-1} to 167.89 ± 0.10 x 10^{-3} μM s^{-1} and 23.26 ± 0.05 x 10^{-3} μM s^{-1} to 159.17 ± 0.05 x 10^{-3} μM s^{-1}, respectively. The sequence, 7-01, had the highest POD-mimicking activity of all sequences without K^+ ions (23.26 ± 0.05 x 10^{-3} μM s^{-1}). This is comparable to EAD2 (35.61 x 10^{-3} μM s^{-1}) with K^+ ions indicating that a small percentage of the G4 ODN is successfully forming a G4/hemin complex. It was concluded that the G4 ODN sequences obtained from the GA are still dependent

on the presence of a cation and would be suitable for purposes such as biosensing as the chance of false positives is still considerably low.

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