Investigating substrates Amplifu Red and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) in the colorimetric detection of DNAzyme activity localized to DNA condensates

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Supporting Information

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Introduction

Screening DNAzymes for peroxidase activity

A panel of G4 quadruplex sequences for DNAzyme exhibiting peroxidase activity was selected from the literature (Table S1). The sequences for the G4s were ordered from Integrated DNA Technologies and the DNA quantified using a NanoDrop Spectrophotometer (Table S2). Initial experiments carried out by our collaborators Olivia Zou (Caltech, Rothemund lab) and Heather Romero (UCLA, Franco lab) screened this panel of peroxidase-mimicking DNAzymes (G4 quadruplexes with hemin bound) for peroxidase activity (Figure S1). Two different methods to form the folded G4 quadruplexes were tested: annealing and snap-cooling (Figure S2), adapting procedures from reference [13].

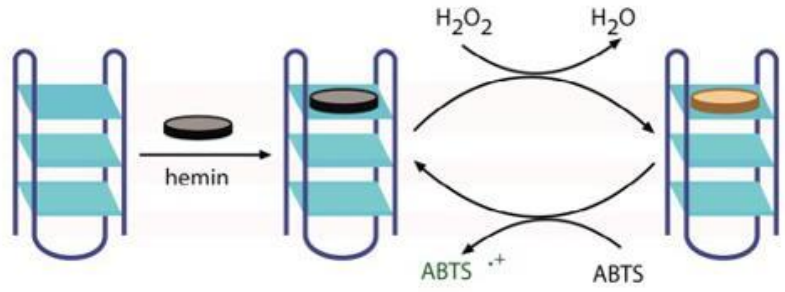


Figure S1. Overview of DNAzyme-mediated peroxidation. The DNA strand encoding the G4 quadruplex is mixed with a buffer containing KCl, heated to 95 °C for ten min and then snap-cooled by placing it in an ice water bath for 15-30 min. Hemin is incubated with the folded G4 quadruplex for 30 min at room temperature to form the active DNAzyme catalyst. Adding substrates ABTS (or Amplifu Red) and H_2O_2, results in a color change upon oxidation of ABTS. Final reaction mixture contains 100mM KCl, 10mM Tris-HCl (pH 7.5), 0.05% (v/v) Triton-X 100, 1% DMSO, 0.5μM G4-hemin catalyst, 600μM ABTS (or Amplifu Red) and 600μM H_2O_2, conditions modified from ref [13].

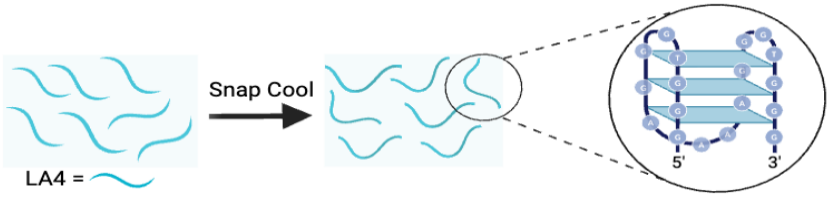


Figure S2. Formation of a G4 quadruplex LA4 using the snap cool method. The DNA strand for LA4 is first mixed with either NaCl or KCl and MilliQ water and heated to 95°C for 10 min. Snap cooling in an ice bath for 30 minutes results in the G4 quadruplex structure.

The goals of this experiment were to observe a visible blue-green color change of the DNAzyme solution as peroxidation occurs and then measure the absorbance of the reaction at 420 nm. Two different methods were used to form the G4-hemin catalyst/DNAzyme: 1) Anneal G4 strands from 95°C to 20°C, at a rate of 0.5°C/min; afterwards, incubate with hemin for 30 min at room temperature; 2) Heat G4 strands to 95°C for 10 min, then snap cool for 30 min by placing tubes

in ice water; afterwards, incubate with hemin for 30 min at room temperature and then add redox substrates ABTS/Amplifu and H_2O_2. As expected [13,14], LA4 turned blue quickly and had the darkest color change, showing that LA4 is the most 'activated' and produces the most robust color change upon peroxidation. FC3 and AS1411 also turned dark blue-green after 5 min peroxidation; 5xG3T produced a lighter blue-green in both methods tested [Figure S3].

Experimental conditions

Final reaction mixture contained:

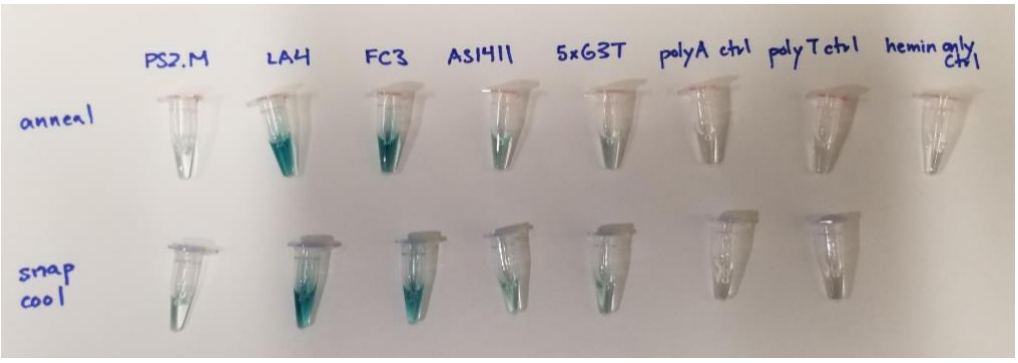
10mM Tris-HCl (pH 7.37)
100mM KCl
0.05% (v/v) Triton X-100
1% (v/v) DMSO
0.5 µM G4-hemin catalyst
600 µM ABTS
600 µM H_2O_2

These conditions were modified from ref [13], Chen *et al. ACS Catal*. (2018).

J. Chen, Y. Zhang, M. Cheng, Y. Guo, J. Šponer, D. Monchaud, J.L. Mergny, H. Ju, & J. Zhou, "How Proximal Nucleobases Regulate the Catalytic Activity of G-Quadruplex/Hemin DNAzymes." *ACS Catalysis*, vol. 8, no. 12, pp. 11352-11361, 2018.

Table S1. G4 DNA strands screened for peroxidase activity

Name	Reference	Sequence
PS2.M	1998-Travisco	GTGGGTAGGGCGGGTTGG
LA4	2018-Chen	GGGTGGGAAAAGGGTGGG
FC3	2018-Chen	GGGTGGGTGGGTGGGCCC
5xG3T	2018-Chen	GGGTGGGTGGGTGGGTGGGT
AS1411	2019-Adeoye	GGTGGTGGTGGTTGTGGTGGTGGTGG

Comparison of DNAzyme formation methods (5 min after initialization of reaction)	

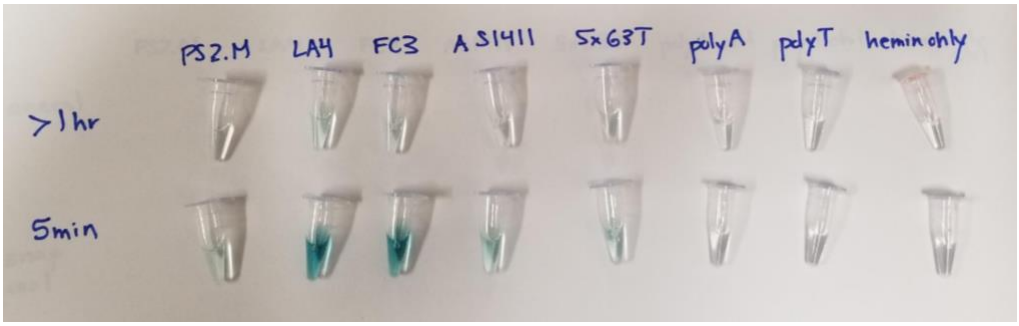
Comparison of tubes at different times after initialization of reaction	

Figure S3. Screening a panel of peroxidase-mimicking DNAzymes (G4 quadruplexes with hemin bound) using 2 different DNAzyme formation methods: 1) heating G4 strands to 95°C for 10 min, then snap cooling by placing tubes in ice water for 30 min; afterwards, incubating with hemin for 30 min at room temperature, or 2) annealing G4 strands from 95°C (ten min) to 20°C, at a rate of 0.5°C/min; afterwards, incubating with hemin for 30 min at room temperature. Both of these methods formed active DNAzyme catalysts that were used in peroxidation reactions, which were imaged at 5 min and 1 hour post reaction using an iPhone camera.

Table S2. DNA strands and concentrations determined using a NanoDrop spectrophotometer

DNA Strand ID	Concentration (µM)
nstar15_strand1	1021.5
nstar15_strand2	1046.8
nstar15_strand3	983.4
nstar15_strand4	1023.0
nstar15_strand1-5FAM	50.0
nstar15_strand4-50LA4	1938.8
nstar20_strand1	1076.7
nstar20_strand2	1014.0
nstar20_strand3	995.4
nstar20_strand4	983.2
nstar20_strand1-5TXRD	50.0
nstar20_strand4-50LA4	1905.0
nstar25_strand1	1081.0
nstar25_strand2	970.7
nstar25_strand3	1012.5
nstar25_strand4	1009.8
nstar25_strand1-5YakYel	50.0
nstar25_strand4-50LA4	190.99

Table S2a. DNAzyme sequences and concentrations

DNAzyme	Concentration	Sequence
LA4	166 µM	5’- GGG TGG GAA AAG GGT GGG -3’
polyT18	241 µM	5’ - TTT TTT TTT TTT TTT TTT -3’

Table S3. DNA strands used in forming fluorescent DNA condensates +/- G4 quadruplexes.

NS15 Strands	**NS15 Sequences**
nstar15_strand1C	CTA GTC TAC AGT GCC AAC TGG GCA GAA TTC CCA GCT AGC
nstar15_strand1C-5FAM	/56-FAM/CTA GTC TAC AGT GCC AAC TGG GCA GAA TTC CCA GCT AGC
nstar15_strand2	GGG AAT TCT GCC CAG AAC GTC ACC AGA AGC ACA GCT AGC
nstar15_strand3	GTG CTT CTG GTG ACG AAG ACG GAA TCT CCG TCA GCT AGC
nstar15_strand4	GAC GGA GAT TCC GTC AAG GCA CTG TAG ACT AGA GCT AGC
nstar15_strand4G-LA4	GGG TGG GAA AAG GGT GGG GAC GGA GAT TCC GTC AAG GCA CTG TAG ACT AGA GCT AGC
nstar15_strand4G-5xG3T	GGG TGG GTG GGT GGG TGG GTG ACG GAG ATT CCG TCA AGG CAC TGT AGA CTA GAG CTA GC

NS20 Strands	**NS20 Sequences**
nstar20_strand1	CTA CTA TGG CGG GTG ATA AAA ACG GGA AGA GCA TGC CCA TCC ACG ATC G
nstar20_strand1-5FAM	/56-FAM/CTA CTA TGG CGG GTG ATA AAA ACG GGA AGA GCA TGC CCA TCC ACG ATC G
nstar20_strand1-5TEXRD	/5TexRd-XN/CTA CTA TGG CGG GTG ATA AAA ACG GGA AGA GCA TGC CCA TCC ACG ATC G
nstar20_strand2	GGA TGG GCA TGC TCT TCC CGA ACT CAA CTG CCT GGT GAT ACG ACG ATC G
nstar20_strand3	CGT ATC ACC AGG CAG TTG AGA ACA TGC GAG GGT CCA ATA CCG ACG ATC G
nstar20_strand4	CGG TAT TGG ACC CTC GCA TGA ATT TAT CAC CCG CCA TAG TAG ACG ATC G
nstar20_strand4-LA4	GGG TGG GAA AAG GGT GGG CGG TAT TGG ACC CTC GCA TGA ATT TAT CAC CCG CCA TAG TAG ACG ATC G
nstar20_strand4-5xG3T	GGG TGG GTG GGT GGG TGG GTC GGT ATT GGA CCC TCG CAT GAA TTT ATC ACC CGC CAT AGT AGA CGA TCG

NS25 Strands	NS25 Sequences
nstar25_strand1	CGC TAC AAT ACA GTT ACA AGA ATG CAA CGC TTG ATG TAT GCA CGT ATG TTG CAC ACG TG
nstar25_strand1-5FAM	/56-FAM/CGC TAC AAT ACA GTT ACA AGA ATG CAA CGC TTG ATG TAT GCA CGT ATG TTG CAC ACG TG
nstar25_strand1-5Yakima	/5YakYel/CGC TAC AAT ACA GTT ACA AGA ATG CAA CGC TTG ATG TAT GCA CGT ATG TTG CAC ACG TG
nstar25_strand2	GCA ACA TAC GTG CAT ACA TCA AGC GAA CAT ATC TCA TAT TCG TGC CAC TAT GAC ACG TG
nstar25_strand3	CAT AGT GGC ACG AAT ATG AGA TAT GAA CAG TAG GGC AGC AAA GAC TAC GGT GAC ACG TG
nstar25_strand4	CAC CGT AGT CTT TGC TGC CCT ACT GAA GCA TTC TTG TAA CTG TAT TGT AGC GAC ACG TG
nstar25_strand4-LA4	GGG TGG GAA AAG GGT GGG CAC CGT AGT CTT TGC TGC CCT ACT GAA GCA TTC TTG TAA CTG TAT TGT AGC GAC ACG TG
nstar25_strand4-5xG3T	GGG TGG GTG GGT GGG TGG GTC ACC GTA GTC TTT GCT GCC CTA CTG AAG CAT TCT TGT AAC TGT ATT GTA GCG ACA CGT G

Table S4. DNA condensates (NS15) formed in KCl (Nanostar-15 with 5% FAM on Arm 1)

DNA strand / reagent	Initial Concentration [µM]	Final Concentration [µM]	Volume Added [µL]
nstar15_strand1	102.15	19.00	9.30
nstar15_strand2	104.68	20.00	9.08
nstar15_strand3	98.34	20.00	10.17
nstar15_strand4	102.30	20.00	9.78
nstar15_strand1-5FAM*	50	1.00	1.00
KCl mM**	2000	350.00	8.75
trisHCl mM	200	20.00	5.00
Nanopure Water***	–	–	–
TOTAL			**50.00**

**This represents the 5% of strand 1s added that have an attached FAM fluorophore.*

***NaCl had an initial concentration of 5000μM, so the volume added would simply be adjusted to maintain the final concentrations specified in the table of all other species in solution.*
****Nanopure water was not added due to the way the concentration calculations were completed in consideration of the final volume being set to 50.00μL.*

Table S5. DNA condensates (NS15-50LA4) formed in KCl (Nanostar-15 with 5% FAM on Arm 1 and 50% LA4 on Arm 4)

DNA strand / Reagent	Initial Concentration [μM]	Final Concentration [μM]	Volume Added [μL]
nstar15_strand1	102.15	19.00	9.30
nstar15_strand2	104.68	20.00	9.08
nstar15_strand3	98.34	20.00	10.17
nstar15_strand4	102.30	**10.00**	4.89
nstar15_strand1-5FAM*	50	1.00	1.00
nstar15_strand4-50LA4**	193.88	**10.00**	2.58
KCl mM***	2000	350.00	8.75
trisHCl mM	200	20.00	5.00
Nanopure Water******	–	–	–
TOTAL			**50.00**

**This represents the 5% of strand 1s added that have an attached FAM fluorophore.*
***This represents the 50% of Strand 4's added that had an attached G4 quadruplex sequence (unfolded).*
****NaCl had an initial concentration of 5000μM, so the volume added would simply be adjusted to maintain the final concentrations specified in the table of all other species in solution.*
*****Nanopure water was not added due to the way the concentration calculations were completed in consideration of the final volume being set to 50.00μL.*

Table S6. NS20 DNA condensates formed in KCl (Nanostar-20 with 5% Texas Red, Arm 1)

DNA strand / Reagent	Initial Concentration [μM]	Final Concentration [μM]	Volume Added [μL]
nstar20_strand1	107.67	19.00	8.82
nstar20_strand2	101.40	20.00	9.37
nstar20_strand3	99.54	20.00	10.05
nstar20_strand4	98.32	20.00	10.17
nstar20_strand1-5TXRD*	50	1.00	1.00

KCl mM**	2000	350.00	8.75
trisHCl mM	200	20.00	5.00
Nanopure Water***	–	–	–
TOTAL			**50.00**

**This represents the 5% of strand 1s added that have an attached Texas Red fluorophore.*
***NaCl had an initial concentration of 5000μM, so the volume added would simply be adjusted to maintain the final concentrations specified in the table of all other species in solution.*
****Nanopure water was not added due to the way the concentration calculations were completed in consideration of the final volume being set to 50.00μL.*

Table S7. DNA condensates (NS20) formed in KCl (Nanostar-20 with 5% Texas Red on Arm 1 and 50% LA4 on Arm 4)

DNA strand / Reagent	Initial Concentration [μM]	Final Concentration [μM]	Volume Added [μL]
nstar20_strand1	107.67	19.00	8.82
nstar20_strand2	101.40	20.00	9.37
nstar20_strand3	99.54	20.00	10.05
nstar20_strand4	98.32	**10.00**	5.09
nstar20_strand1-5TXRD*	50.00	1.00	1.00
nstar20_strand4-50LA4**	190.50	**10.00**	2.62
KCl mM***	2000.00	350.00	8.75
trisHCl mM	200.00	20.00	5.00
Nanopure Water******	–	–	–
TOTAL			**50.00**

**This represents the 5% of strand 1s added that have an attached Texas Red fluorophore.*
***This represents the 50% of strand 4s added that have an attached G4 quadruplex sequence (unfolded).*
****NaCl had an initial concentration of 5000μM, so the volume added would simply be adjusted to maintain the final concentrations specified in the table of all other species in solution.*
*****Nanopure water was not added due to the way the concentration calculations were completed in consideration of the final volume being set to 50.00μL.*

Table S8. NS25 DNA condensates formed in KCl (Nanostar-25, 5% Yakima Yellow on Arm 1)

DNA strand / Reagent	Initial Concentration [µM]	Final Concentration [µM]	Volume Added [µL]
nstar25_strand1	108.10	19.00	8.79
nstar25_strand2	97.07	20.00	9.79
nstar25_strand3	101.25	20.00	9.88
nstar25_strand4	100.98	20.00	9.90
nstar25_strand1-5YakYel*	50.00	1.00	1.00
KCl mM**	2000.00	350.00	8.75
TrisHCl mM	200.00	20.00	5.00
Nanopure Water***	–	–	–
TOTAL			**50.00**

**This represents the 5% of strand 1s added that have an attached Yakima Yellow fluorophore.*
***NaCl had an initial concentration of 5000µM, so the volume added would simply be adjusted to maintain the final concentrations specified in the table of all other species in solution.*
****Nanopure water was not added due to the way the concentration calculations were completed in consideration of the final volume being set to 50.00µL.*

Table S9. NS25 DNA condensates formed in KCl (Nanostar-25 with 5% Yakima Yellow on Arm 1 and 50% LA4 on Arm 4)

DNA strand / Reagent	Initial Concentration [µM]	Final Concentration [µM]	Volume Added [µL]
nstar25_strand1	108.10	19.00	8.79
nstar25_strand2	97.07	20.00	9.79
nstar25_strand3	101.25	20.00	9.88
nstar25_strand4	100.98	**10.00**	4.95
nstar25_strand1-5YakYel*	50.00	1.00	1.00
nstar25_strand4-50LA4**	190.99	**10.00**	2.62
KCl mM**	2000	350.00	8.75
trisHCl mM	200	20.00	5.00
Nanopure Water***	–	–	–
TOTAL			**50.00**

**This represents the 5% of strand 1s added that have an attached Yakima Yellow fluorophore.*
***This represents the 50% of strand 4s added that have an attached G4 quadruplex sequence (unfolded).*
****NaCl had an initial concentration of 5000μM, so the volume added would simply be adjusted to maintain the final concentrations specified in the table of all other species in solution.*
*****Nanopure water was not added due to the way the concentration calculations were completed in consideration of the final volume being set to 50.00μL.*

Table S10. Buffer reagents to form 10mL of 2X rxn buffer.

Reagent	Concentration	Volume
Tris-HCl	1M	200μL
KCl	2M	1mL
DMSO		200μL
Triton X-100		10μL
dH_2O	—	8.59mL

Table S11. Buffer reagents to perform a 1:2 dilution with the 2X rxn buffer and make 10mL of 1X rxn buffer

Reagent	Concentration	Volume
rxn buffer	2X	5mL
dH_2O	—	5mL

Table S12. Buffer reagents to prepare 2mL of 6mM H_2O_2 in 1X rxn buffer

Reagent	Concentration	Volume
rxn buffer	2X	1mL
H_2O_2	30% (w/w) (=9.79M)	1.23μL
dH_2O	—	998.8μL

Table S13. Buffer reagents to prepare 2mL of 6mM ABTS in 1X rxn buffer

Reagent	Concentration	Volume
rxn buffer	2X	1mL
ABTS*	18.226mM	658μL
dH_2O	—	342μL

**ABTS stock solution of 18.226mM was prepared by dissolving five 10mg ABTS tablets in 5mL dH_2O.*

Table S14. Buffer reagents to prepare 2mL of 50µM hemin in 1X rxn buffer

Reagent	Concentration	Volume
Tris-HCl	1M	20µL
KCl	2M	100µL
Hemin in DMSO*	5mM	20µL
Triton X-100		1µL
dH_2O	—	1.859mL

*Hemin stock solution of 5mM was made by dissolving 6.52mg of hemin in 2mL of DMSO.

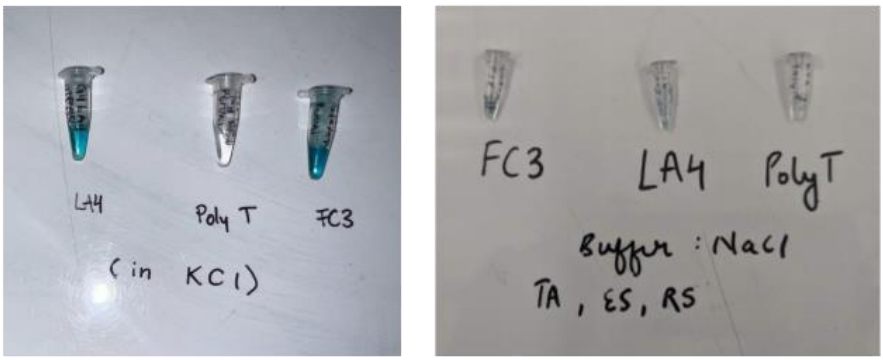
Table S15. Formation of G4-Hemin catalyst in DNA condensates in 1X buffer

Reagent	Concentration	Volume
Nanostar condensate solution	10µM	15µL
Hemin	50µM	3µL
Reaction buffer	1X	12µL
Total volume	–	30µL

Table S16. Peroxidation reagents for condensates (using ABTS)

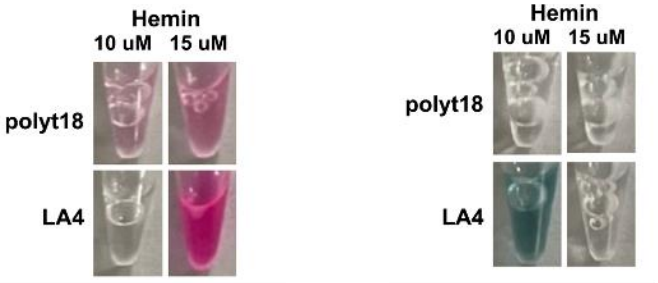
Reagent	Concentration	Volume
ABTS*	6mM	10µL
G4-hemin catalyst	5µM	10µL
H_2O_2**	6mM	10µL
Reaction buffer	1X	70µL
Total volume	–	10µL

**This table was still valid if Amplifu was used for the peroxidation reaction, as the concentration of the stock was prepared to be the same as ABTS (6mM).*
***This was added as the last step to initiate the peroxidation reaction.*



Peroxidation reaction catalyzed by Poly T (negative control), LA4 and FC3 in either KCl buffer (left) or NaCl buffer (right) i

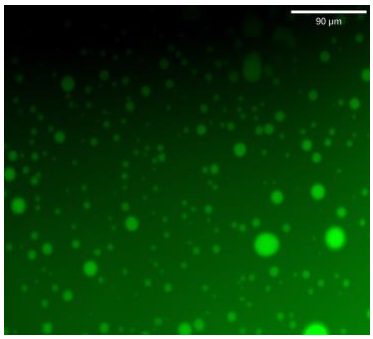
Figure S4. Buffer compatibility tests between DNAzyme-catalyzed peroxidation and DNA condensate formation. Peroxidation was carried out using DNAzymes LA4, FC3 and polyT18 (negative control) in either KCl buffer (left) or NaCl buffer (right).



Amplifu Red ABTS

Figure S5. Colorimetric detection of DNAzyme activity (LA4) using Amplifu Red or ABTS. Either 10 µM or 15 µM hemin was added to the snap-cooled LA4 to yield a DNAzyme that was used in colorimetric peroxidation using either Amplifu Red (left) or ABTS (right) showing a difference in sensitivity and detection of activity between two reagents.

To explore the relationship between substrate identity and DNAzyme activity, parallel oxidation experiments were conducted with ABTS and Amplifu Red. For ABTS-containing samples, 10µM hemin showed better DNAzyme performance compared to 15 µM, which generated a fainter color change. This could be due to a threshold limiting hemin binding to G4s. The uniform blue-green color suggested effective G4-catalyzed ABTS oxidation. Although the precise duration of the color change is unknown, all tubes were clear when visualized at 64 hours due to the light-sensitive nature of ABTS (Figure S5).



NS15, 5% FAM, 5% LA4, 10x magnifiction

Figure S6. NS15 condensates form in KCl buffer, which enhances DNAzyme activity. NS15 condensates with 5% FAM were formed in KCl buffer and with 5% LA4 attached. This is a fluorescence micrograph under FAM fluorescence at 10x magnification.

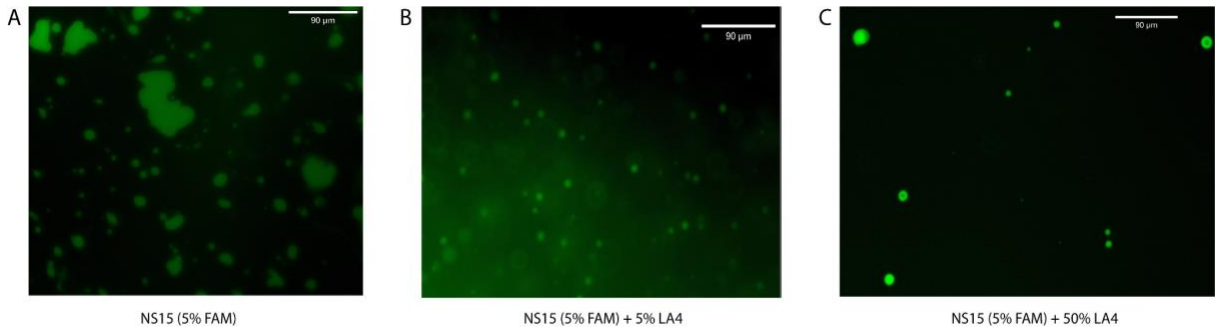


Figure S7. NS15 condensates form in KCl buffer with 5 and 50% LA4 attached. NS15 condensates with 5% FAM were formed in KCl buffer and with 5% LA4 attached. This is a fluorescence micrograph under FAM fluorescence at 10x magnification.

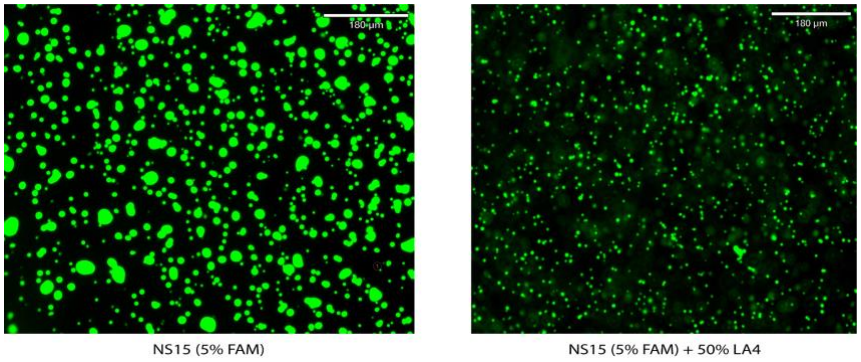


Figure S8. Formation of microscale NS15 DNA condensate with 50% G4 quadruplex LA4 in KCl buffer. DNA strands to form NS15, a 4-armed nanostar with 15 base pairs per arm, with 5% of nanostars containing FAM and 5 or 50% of strand 4 extending arm 4 with the sequence for the G4 quadruplex LA4 were added to TE buffer with KCl and water; the solution was mixed thoroughly, then heated to 95 °C for 10 min, and then slowly cooled at a rate of 1 Deg. C per min. 2.5 µL of this solution was visualized via fluorescence microscopy, 10x magnification.

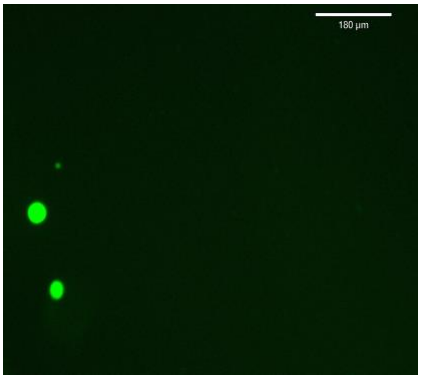


Figure S9. NS15 condensates with 50% LA4 attached, post peroxidation.
NS15 condensates with 5% FAM were formed in KCl buffer and with 50% LA4 attached and used to catalyze peroxidation; this image was taken ten minutes post peroxidation.

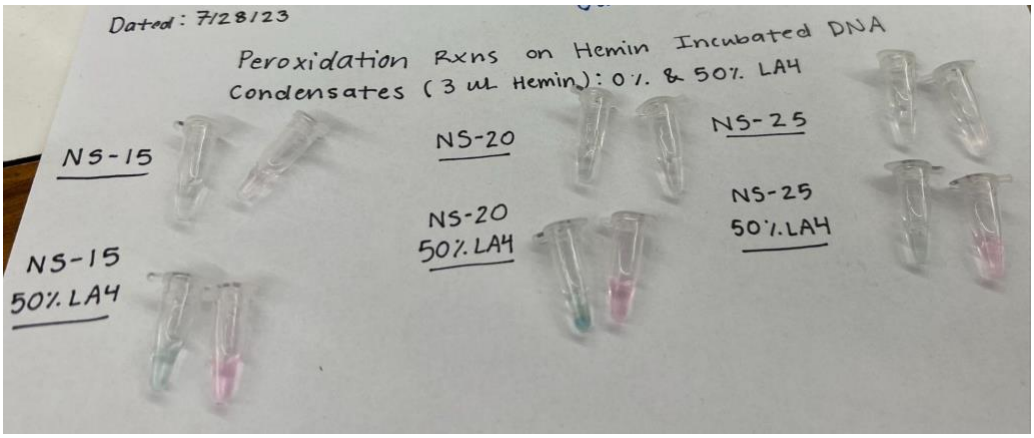


Figure S10. Peroxidation on microscale DNA condensates with G4 quadruplex LA4.
Peroxidation on microscale DNA condensates with 0 and 50% G4 quadruplex LA4. NS15 +/- 50% LA4 (left), NS20 +/-50% LA4 (middle), NS25 +/-50% LA4 (right), condensates were formed by annealing and to the resultant condensates, hemin was added and incubated for 30 min at room temperature. Either ABTS or Amplifu Red and then H_2O_2 substrates were added and the colorimetric peroxidation observed. This image was taken with an iPhone camera in lab lighting ten minutes after the substrates were added to NS25/50% LA4.

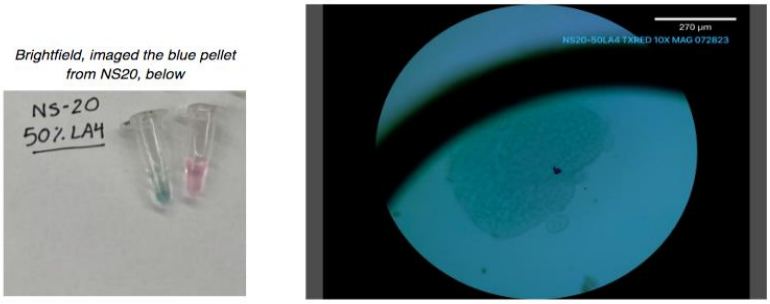


Figure S11. Peroxidation on microscale DNA condensate NS20/Texas Red with 50% G4 quadruplex LA4. Tubes showing color change in NS20 condensate solution with ABTS or Amplifu post peroxidation (left); brightfield image taken of blue pellet in the ABTS tube, 10x.

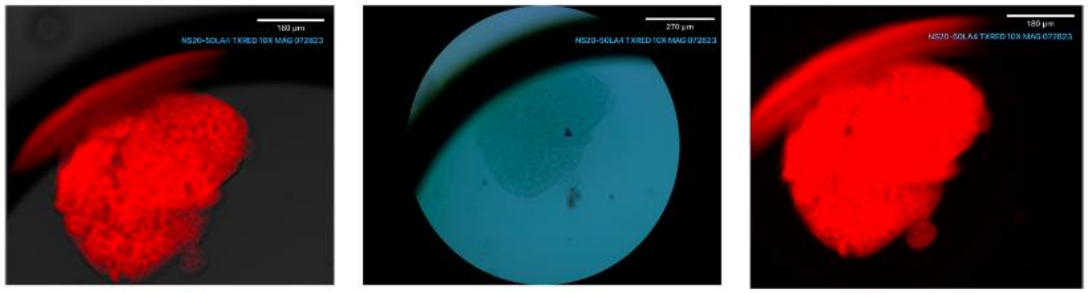


Figure S12. Micrographs, post-peroxidation on DNA condensates with 50% G4 quadruplex LA4. NS20 + 50% LA4 blue pellet pipetted onto microscope slide and visualized: overlay of brightfueld and fluorescence shows correlations between blue 'blobs' and fluorescent 'blobs'.

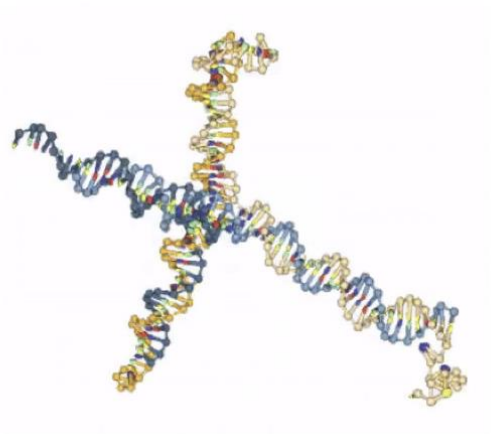


Figure S13. oxDNA simulation of a DNA nanostar NS20 with G4 tetraplex LA4 sequence extended from arm 4. Structured and rendered through oxDNA by students using simulation code. Model of NS-20 sequence of 4 armed structure including DNAzyme and fluorescent dye.

P Sulc; F.Romano; T.E. Ouldridge; L.L Rovigatti; J.P.K. Doye; A.A. Louis. Sequence-dependent thermodynamics of a coarse-grained DNA model. J. Chem. Phys. 137, 135101 (2012).
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