Exploring toehold-mediated strand displacement as a strategy to program DNAzyme-catalyzed peroxidation localized to DNA condensates

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Supporting Information

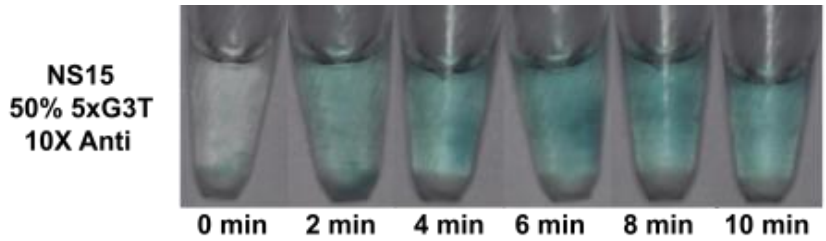

Fig. S1. DNAzyme-catalyzed peroxidation reaction in a single layer system on the microscale with 10X concentration of displacer strand and hemin added pre-annealing. After forming DNA nanostar condensates on the microscale by adding hemin and heating to 95°C for ten minutes, then cooling slowly to room temperature at a rate of 1°C/min in a thermocycler, peroxidation was carried out by adding ABTS and H_2O_2 substrates directly into the solution. As in the same experiment conducted in Figure 4 with 2X and 5X concentrations of displacer and hemin added pre-annealing, the reaction mixture turned just as blue as the positive control, suggesting that toehold-mediated strand displacement was not successful in this experiment.

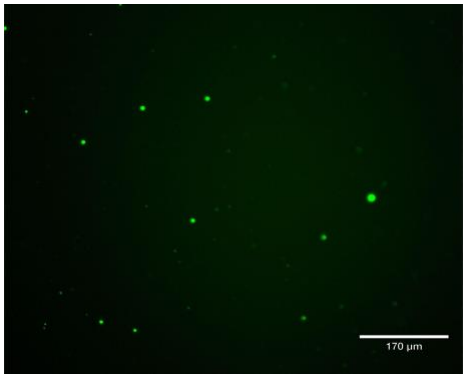

Fig. S2. Micrograph of microscale NS15/50% 5xG3T-T1 DNA condensates with 10X displacer strand concentration (Fig. S1) visualized under the FITC channel of an Echo Revolve fluorescence microscope after addition of dilute SYBR gold dye.

Table S1. Summary of DNA strands used in this study

Name	Concentration	Sequence
polyT18	120 µM	5'- TTT TTT TTT TTT TTT TTT -3'
5xG3T	189 µM	5'- GGG TGG GTG GGT GGG TGG GT -3'
5xG3T-T1-displacer	162 µM	5'- ACC CAC CCA CCC ACC CAC CCA CCT CTC A -3'
nstar15_strand1	973 µM	5'- CTA GTC TAC AGT GCC AAC TGG GCA GAA TTC CCA GCT AGC -3'
nstar15_strand2	934 µM	5'- GGG AAT TCT GCC CAG AAC GTC ACC AGA AGC ACA GCT AGC -3'
nstar15_strand3	901 µM	5'- GTG CTT CTG GTG ACG AAG ACG GAA TCT CCG TCA GCT AGC -3'
nstar15_strand4	925 µM	5'- GAC GGA GAT TCC GTC AAG GCA CTG TAG ACT AGA GCT AGC -3'
nstar15_strand4-5xG3T	181 µM	5'- GGG TGG GTG GGT GGG TGG GTG ACG GAG ATT CCG TCA AGG CAC TGT AGA CTA GAG CTA GC -3'
nstar15_strand4-5xG3T-T1	171 µM	5'- TGA GAG GTG GGT GGG TGG GTG GGT GGG TGA CGG AGA TTC CGT CAA GGC ACT GTA GAC TAG AGC TAG C -3'

Table S2. Forming NS15/50% 5xG3T Reaction Mixture (Positive Control)

Reagent	Initial Concentration (µM)	Final Concentration (µM)	Volume Added (µL)
nstar15_strand1	97.3	19.00	9.76
nstar15_strand2	93.4	20.00	10.71
nstar15_strand3	90.1	20.00	11.10
nstar15_strand4	92.5	10.00	5.41
nstar15_strand4-5xG3T	181	10.00	2.76
KCl (mM)	2000	350.00	8.75
trisHCl (mM)	200	20.00	5.00
Total Volume	—	—	**50.00**

**Initial concentrations for strands 1-4 (excluding strands with 5xG3T or T1) are based on concentrations after a tenfold dilution from the concentrations listed in Table S1.*
***Enough milliQ water was added to the positive control tubes to balance the volume of displacer strands that would be added to the experimental tubes*

Table S3. Forming NS15/50% 5xG3T-T1 2X Displacer Reaction Mixture

Reagent	Initial Concentration (µM)	Final Concentration (µM)	Volume Added (µL)
nstar15_strand1	97.3	19.00	9.76
nstar15_strand2	93.4	20.00	10.71
nstar15_strand3	90.1	20.00	11.10
nstar15_strand4	92.5	10.00	5.41
nstar15_strand4-5xG3T-T1	171	10.00	2.93
5xG3T-T1-displacer*	162	20.00	6.18
KCl (mM)	2000	350.00	8.75
trisHCl (mM)	200	20.00	5.00
Hemin in 1X Buffer**	—	—	6.00
Total Volume	—	—	**50.00**

**The displacer strand was added before annealing or after annealing (2 minutes after peroxidation had begun) depending on the experimental tube.*
***The hemin buffer solution was added before annealing or after annealing depending on the experimental tube.*

Table S4. Forming NS15/50% 5xG3T-T1 5X Displacer Reaction Mixture

Reagent	Initial Concentration (µM)	Final Concentration (µM)	Volume Added (µL)
nstar15_strand1	97.3	19.00	9.76
nstar15_strand2	93.4	20.00	10.71
nstar15_strand3	90.1	20.00	11.10
nstar15_strand4	92.5	10.00	5.41
nstar15_strand4-5xG3T-T1	171	10.00	2.93
5xG3T-T1-displacer*	162	50.00	15.46
KCl (mM)	2000	350.00	8.75
trisHCl (mM)	200	20.00	5.00
Hemin in 1X Buffer**	—	—	6.00
Total Volume	—	—	**50.00**

**The displacer strand was added before annealing or after annealing (2 minutes after peroxidation had begun) depending on the experimental tube.*
***The hemin buffer solution was added before annealing or after annealing depending on the experimental tube.*

Table S5. Forming NS15/50% 5xG3T-T1 10X Displacer Reaction Mixture

Reagent	Initial Concentration (µM)	Final Concentration (µM)	Volume Added (µL)
nstar15_strand1	97.3	19.00	9.76
nstar15_strand2	93.4	20.00	10.71
nstar15_strand3	90.1	20.00	11.10
nstar15_strand4	92.5	10.00	5.41
nstar15_strand4-5xG3T-T1	171	10.00	2.93
5xG3T-T1-displacer*	162	100.00	30.91
KCl (mM)	2000	350.00	8.75
trisHCl (mM)	200	20.00	5.00
Hemin in 1X Buffer**	—	—	6.00
Total Volume	—	—	**50.00**

**The displacer strand was added before annealing with the 10X displacer experimental tube.*
***The hemin buffer solution was added before annealing with the 10X displacer experimental tube.*