Supplemental Material

for

The need for speed: run-on oligomer filament formation provides maximum speed with maximum sequestration of activity

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Table S1. Western data to quantitate expression levels

SgrAI Enzyme	Protein Level (%)[2]
WT	100%
T4D	38%
S6D	70%
I7E	57%
R24E	40%
N25E	30%
P27W	82%
P27G	74%
Q34D	80%
I51E	75%
S56E	68%
S56Q	29%
A57E	62%
A57Q	108%
I59E	59%
M62E	33%
R84E	33%
R127A	100%
R131A	38%
R134A	63%

Table S2. Calculation of Local Concentration of Sites on Phage DNA

Parameter	Number	Units
Estimated molecules SgrAI per cell	100	molecules
Molecules phage per cell (at the minimum)	1	molecules
size of cell in microns	1	microns
volume of a spherical cell	$5x10^{-13}$	cm^3
	$5x10^{-16}$	L
Concentration of SgrAI in the cell[a]	**$3x10^{-7}$**	**M**
	300	**nM**
Calculation of the concentration of DNA and SgrAI recognition sites in the cell		
Concentration of phage DNA in cell	**$3x10^{-9}$**	**M**
	3	**nM**
Calculation of the local concentration of sites on the same DNA molecule[b]		
size of phage DNA	50,000	bp
Distance between sites on phage (if 5 sites per phage)[c]	8,333	bp
Distance in Ångstrom	28,333	Å
Radius of gyration	2,173	Å
Volume occupied by 2 sites in phage using radius=Rg	$4x10^{10}$	$Å^3$
	$4x10^{-14}$	cm^3
	$4x10^{-17}$	L
Local concentration of 2 SgrAI/site on phage using Rg	$8x10^{-8}$	M
	80	**nM**

[a]**Calculation of [SgrAI] in the cell:** first, the size of the cell is used to calculate volume, and with 100 molecules of SgrAI estimated, a concentration can be calculated as C=100/(Avogadro's number*volume of the cell), and is calculated to be 300 nM. With 1 copy of DNA per cell, a similar calculation gives 3 nM.

[b]**To calculate the local concentration of sites on the same phage DNA to each other**: with 3.4 Å/bp, the 8,333 bp distance between sites gives 28,333 Å between sites on linear DNA if fully extended (and in B form). The radius of gyration was calculated according to the equation $Rg=((2*P*N)/6)^{1/2}$ in Å, P is persistence length (500 Å)[1], N is the linear distance (in Å) between sites. From this radius, a volume can be calculated ($V=4/3\pi r^3$), and the concentration calculated as 2 molecules (the two DNA bound SgrAI) in a volume (calculated as $4x10^{-17}$ L), which is ~80 nM.

[c]The number of primary sites will vary with each phage genome, for example, λ phage contains 6 primary sites, though 3 are predicted based on the statistics in a 50,000 kb genome.

Table S3. Equilibria used in simulations of the ROO filament and Binary mechanisms

Reaction Step	Forward Rate Constant	Reverse Rate Constant
Equations for Run-on Oligomer Filament Mechanism		
Binding of SgrAI binding to its recognition site in DNA (site) to create the SgrAI/DNA complex R		
SgrAI + site ⇌ R	k_1	k_{-1}
Self-association of a SgrAI/DNA complexes (i.e. R)		
R + R ⇌ RR	k_2	k_{-2}
R + R ⇌ RR	k_2	k_{-2}
RR + R ⇌ RRR	k_2	k_{-2}
R + RR ⇌ RRR	k_2	k_{-2}
DNA cleavage within the ROO filament (X denotes SgrAI bound to cleaved DNA)		
RR ⇌ XR	k_3	k_{-3}
RR ⇌ RX	k_3	k_{-3}
XR ⇌ XX	k_3	k_{-3}
RX ⇌ XX	k_3	k_{-3}
RRR ⇌ XRR	k_3	k_{-3}
RRR ⇌ RXR	k_3	k_{-3}
RRR ⇌ RRX	k_3	k_{-3}
XRR ⇌ XXR	k_3	k_{-3}
XRR ⇌ XRX	k_3	k_{-3}
XXR ⇌ XXX	k_3	k_{-3}
XRX ⇌ XXX	k_3	k_{-3}
RXR ⇌ XXR	k_3	k_{-3}
RXR ⇌ RXX	k_3	k_{-3}
XXR ⇌ XXX	k_3	k_{-3}
RXX ⇌ XXX	k_3	k_{-3}
RRX ⇌ XRX	k_3	k_{-3}
RRX ⇌ RXX	k_3	k_{-3}
XRX ⇌ XXX	k_3	k_{-3}
RXX ⇌ XXX	k_3	k_{-3}
Dissociation of run-on oligomer filaments that contain some SgrAI/DNA complexes with cleaved DNA (i.e. X) and some with uncleaved (i.e. R)		
XR ⇌ R + X	k_{-2}	k_2
RX ⇌ R + X	k_{-2}	k_2
XX ⇌ X + X	k_{-2}	k_2
XRR ⇌ X + RR	k_{-2}	k_2
XRR ⇌ XR + R	k_{-2}	k_2
RXR ⇌ R + XR	k_{-2}	k_2
RXR ⇌ RX + R	k_{-2}	k_2
RRX ⇌ R + RX	k_{-2}	k_2
RRX ⇌ RR + X	k_{-2}	k_2
XXR ⇌ X + XR	k_{-2}	k_2
XXR ⇌ XX + R	k_{-2}	k_2

XRX ⇌ X + RX	k_{-2}	k_2
XRX ⇌ XR + X	k_{-2}	k_2
RXX ⇌ R + XX	k_{-2}	k_2
RXX ⇌ RX + X	k_{-2}	k_2
XXX ⇌ X + XX	k_{-2}	k_2
XXX ⇌ XX + X	k_{-2}	k_2
Dissociation of SgrAI/DNA complexes which contain cleaved DNA (i.e. X) to SgrAI and cleaved DNA		
X ⇌ SgrAI + cleaved DNA	k_4	k_{-4}
Equations for Binary Mechanism		
Binding of Binary ***enzyme E to its recognition site in DNA (site) to create the E/DNA complex R***		
E + site ⇌ R	k_1	k_{-1}
Self-association of a E/DNA complexes (i.e. R)		
R + R ⇌ RR	k_2	k_{-2}
DNA cleavage within the Binary complex (X denotes enzyme E bound to cleaved DNA)		
RR ⇌ XR	k_3	k_{-3}
RR ⇌ RX	k_3	k_{-3}
XR ⇌ XX	k_3	k_{-3}
RX ⇌ XX	k_3	k_{-3}
Dissociation of Binary ***complexes that contain some E/DNA complexes with cleaved DNA (i.e. X) and some with uncleaved DNA (i.e. R)***		
XR ⇌ X + R	k_{-2}	k_2
RX ⇌ R + X	k_{-2}	k_2
XX ⇌ X + X	k_{-2}	k_2
Dissociation of E/DNA complexes which contain cleaved DNA (i.e. X) to enzyme E and cleaved DNA		
X ⇌ E + cleaved DNA	k_4	k_{-4}

Table S4. Rate constants using in simulations

Reaction	Forward Rate Constant	Reverse Rate Constant
DNA recognition site (site) binding by **SgrAI** (ROO mechanism) or enzyme **E** (Binary mechanism)	$k_1 = 10^9\ M^{-1}\ s^{-1}$	$k_{-1} = 0.06\ s^{-1}$
Association and self-association of enzyme-substrate (**R**) and enzyme-product complexes (**X**)	$k_2 = 1.3 \times 10^5\ M^{-1}\ s^{-1}$	$k_{-2} = 0.03\ s^{-1}$
DNA cleavage by **SgrAI** (ROO mechanism) or **E** (Binary mechanism)	$k_3 = 0.8\ s^{-1}$	$k_3 = 0$ (considered irreversible)
Product release (release of cleaved DNA from **SgrAI** or **E**)	$k_4 = 0.4\ s^{-1}$	$k_{-4} = 3 \times 10^7\ M^{-1}\ s^{-1}$ (**Fig. 3A-B, D**) $k_{-4} = 3 \times 10^6\ M^{-1}\ s^{-1}$ (**Fig. 3C**)

Table S5. Equations for simulating reactions with secondary site DNA

Reaction Step	Forward Rate Constant	Reverse Rate Constant
SgrAI binding to primary site DNA or secondary site DNA into SgrAI/DNA complexes A and R, respectively		
SgrAI+PRIMARY ⇌ P	k_1	k_{-1}
SgrAI+SECONDARY ⇌ S	k_2	k_{-2}
Self-association of a SgrAI/PRIMARY complex (i.e. P) with another SgrAI/PRIMARY complex (i.e. P)		
P + P ⇌ PP	k_3	k_{-3}
P + P ⇌ PP	k_3	k_{-3}
SP + P ⇌ SPP	k_3	k_{-3}
P + PS ⇌ PPS	k_3	k_{-3}
PP + P ⇌ PPP	k_3	k_{-3}
P + PP ⇌ PPP	k_3	k_{-3}
PPP + P ⇌ PPPP	k_3	k_{-3}
P + PPP ⇌ PPPP	k_3	k_{-3}
P + PPS ⇌ PPPS	k_3	k_{-3}
P + PSP ⇌ PPSP	k_3	k_{-3}
PSP + P ⇌ PSPP	k_3	k_{-3}
SPP + P ⇌ SPPP	k_3	k_{-3}
SP+PP ⇌ SPPP	k_3	k_{-3}
SP+PS ⇌ SPPS	k_3	k_{-3}
Association of a SgrAI/PRIMARY complex (i.e. P) with a SgrAI/SECONDARY complex (i.e. S)		
P + S ⇌ PS	k_4	k_{-4}
S + P ⇌ SP	k_4	k_{-4}
PS + P ⇌ PSP	k_4	k_{-4}
P + SP ⇌ PSP	k_4	k_{-4}
PP + S ⇌ PPS	k_4	k_{-4}
S + PP ⇌ SPP	k_4	k_{-4}
S + PS ⇌ SPS	k_4	k_{-4}
SP + S ⇌ SPS	k_4	k_{-4}
S + PPP ⇌ SPPP	k_4	k_{-4}
S + PPS ⇌ SPPS	k_4	k_{-4}
SPP + S ⇌ SPPS	k_4	k_{-4}
S + PSP ⇌ SPSP	k_4	k_{-4}
SPS + P ⇌ SPSP	k_4	k_{-4}
P + SPS ⇌ PSPS	k_4	k_{-4}
PSP + S ⇌PSPS	k_4	k_{-4}
PPP + S ⇌ PPPS	k_4	k_{-4}
PPS + P ⇌ PPSP	k_4	k_{-4}
P + SPP ⇌ PSPP	k_4	k_{-4}
PP+SP ⇌ PPSP	k_4	k_{-4}
PS+PP ⇌ PSPP	k_4	k_{-4}

PS+PS ⇌ PSPS	k_4	k_{-4}
SP+SP ⇌ SPSP	k_4	k_{-4}
PP+PS ⇌ PPPS	k_4	k_{-4}
Cleavage of secondary site DNA within the ROO filament (S becomes X)		
PS ⇌ PX	k_5	k_{-5}
SP ⇌ XP	k_5	k_{-5}
PSP ⇌ PXP	k_5	k_{-5}
PPS ⇌ PPX	k_5	k_{-5}
SPP ⇌ XPP	k_5	k_{-5}
SPS ⇌ XPX	k_5	k_{-5}
PPPS ⇌ PPPX	k_5	k_{-5}
PPSP ⇌ PPXP	k_5	k_{-5}
PSPP ⇌ PXPP	k_5	k_{-5}
SPPP ⇌ XPPP	k_5	k_{-5}
PSPS ⇌ PSPX	k_5	k_{-5}
PSPS ⇌ PXPS	k_5	k_{-5}
PXPS ⇌ PXPX	k_5	k_{-5}
PSPX ⇌ PXPX	k_5	k_{-5}
SPPS ⇌ XPPX	k_5	k_{-5}
SPSP ⇌ XPXP	k_5	k_{-5}
Dissociation of a SgrAI/PRIMARY complex (i.e. P) with another SgrAI/PRIMARY complex (i.e. P)		
XPP ⇌ XP + P	k_{-3}	k_3
PPX ⇌ P + PX	k_{-3}	k_3
PPP ⇌ PP + P	k_{-3}	k_3
PPP ⇌ P + PP	k_{-3}	k_3
PPPX ⇌ P + PPX	k_{-3}	k_3
PPXP ⇌ P + PXP	k_{-3}	k_3
PXPP ⇌ PXP + P	k_{-3}	k_3
XPPP ⇌ XPP + P	k_{-3}	k_3
PPPP ⇌ PP+PP	k_{-3}	k_3
PPPX ⇌ PP+PX	k_{-3}	k_3
XPPP ⇌ XP+PP	k_{-3}	k_3
XPPX ⇌ XP+PX	k_{-3}	k_3
Dissociation of SgrAI/SECONDARY complex with cleaved secondary site DNA (i.e. X) from a SgrAI/PRIMARY complex (i.e. P)		
PX ⇌ P + X	k_{-4}	k_4
SP ⇌ X + P	k_{-4}	k_4
PXP ⇌ PX + P	k_{-4}	k_4
PXP ⇌ P + XP	k_{-4}	k_4
PPX ⇌ PP + X	k_{-4}	k_4
XPP ⇌ X + PP	k_{-4}	k_4
XPS ⇌ X + PS	k_{-4}	k_4
XPS ⇌ XP + S	k_{-4}	k_4

SPX ⇌ S + PX	k_{-4}	k_4
SPX ⇌ SP + X	k_{-4}	k_4
XPX ⇌ X + PX	k_{-4}	k_4
XPX ⇌ XP + X	k_{-4}	k_4
PPPX⇌ PPP + X	k_{-4}	k_4
PPXP ⇌ PPX + P	k_{-4}	k_4
PXPP ⇌ P + XPP	k_{-4}	k_4
XPPP ⇌ X + PPP	k_{-4}	k_4
PXPX ⇌ P + XPX	k_{-4}	k_4
PXPX ⇌ PXP + X	k_{-4}	k_4
XPPX ⇌ X + PPX	k_{-4}	k_4
XPPX ⇌ XPP + X	k_{-4}	k_4
XPXP ⇌ X + PXP	k_{-4}	k_4
XPXP ⇌ XPX + P	k_{-4}	k_4
PSPX ⇌ P + SPX	k_{-4}	k_4
PSPX ⇌ PSP + X	k_{-4}	k_4
PXPS ⇌ P + XPS	k_{-4}	k_4
PXPS ⇌ PXP + S	k_{-4}	k_4
SPPX ⇌ S + PPX	k_{-4}	k_4
SPPX ⇌ SPP + X	k_{-4}	k_4
XPPS ⇌ X + PPS	k_{-4}	k_4
XPPS ⇌ XPP + S	k_{-4}	k_4
SPXP ⇌ S + PXP	k_{-4}	k_4
SPXP ⇌ SPX + P	k_{-4}	k_4
XPSP ⇌ X + PSP	k_{-4}	k_4
XPSP ⇌ XPS + P	k_{-4}	k_4
PPXP ⇌ PP + XP	k_{-4}	k_4
PXPP ⇌ PX + PP	k_{-4}	k_4
PXPX ⇌ PX + PX	k_{-4}	k_4
XPXP ⇌ XP + XP	k_{-4}	k_4
Dissociation of cleaved SECONDARY site DNA from SgrAI		
X ⇌ SgrAI + SECONDARY^{cleaved}	k_6	k_{-6}
Binary Mechanism		
Binding of PRIMARY and SECONDARY site DNA to enzyme (E) to give complexes P and S, respectively		
E + PRIMARY ⇌ P	k_1	k_{-1}
E + SECONDARY ⇌ S	k_2	k_{-2}
Association of two enzyme-substrate complexes-enzyme bound to primary (P) may self-associate, or associate with enzyme bound to secondary (S) but enzyme bound to secondary does not self-associate		
P + P ⇌ PP	k_3	k_{-3}
P + S ⇌ PS	k_4	k_{-4}
Conversion of substrate to product (S complexes only)		
PS ⇌ PX	k_5	k_{-5}
Dissociation of Binary complex with cleaved SECONDARY site DNA		

PX ⇌ P + X	k_{-4}	k_4
Dissociation of cleaved SECONDARY site DNA from enzyme (E)		
X ⇌ E + SECONDARY^{cleaved}	k_6	k_{-6}

Table S6. Rate Constants used in simulating secondary site cleavage (equations of Table S5)

Reaction	Forward Rate Constant	Reverse Rate Constant
Binding of **SgrAI** (ROO filament mechanism) or enzyme **E** (Binary mechanism) to **PRIMARY** site DNA	$k_1 = 10^8$ $M^{-1}s^{-1}$	$k_{-1} = 0.006$ s^{-1}
Binding of **SgrAI** (ROO mechanism) or enzyme **E** (Binary mechanism) to **SECONDARY** site DNA	$k_2 = 10^8$ $M^{-1}s^{-1}$	$k_{-2} = 0.06$ s^{-1}
Association of two **SgrAI** or two enzyme **E** complexes containing **PRIMARY** site DNA (**P**)	ROO: $k_3 = 1.3x10^5$ $M^{-1}s^{-1}$ Binary: $k_3 = 1.3x10^5$ $M^{-1}s^{-1}$ (**Fig. 5A**) $k_3 = 6.0x10^5$ $M^{-1}s^{-1}$ (**Fig. 5B**)	$k_{-3} = 0.03$ s^{-1}
Association of enzyme-DNA complexes where one contains **PRIMARY** site DNA (**P**) and the other contains **SECONDARY** site DNA, cleaved or uncleaved (**S** or **X**, respectively)	ROO: $k_4 = 6.5x10^4$ $M^{-1}s^{-1}$ Binary: $k_4 = 6.5x10^4$ $M^{-1}s^{-1}$ (**Fig. 5A**) $k_4 = 3.0$ $x10^5$ $M^{-1}s^{-1}$ (**Fig. 5B**)	$k_{-4} = 0.03$ s^{-1}
Cleavage of **SECONDARY** site DNA within complex	$k_5 = 0.8$ s^{-1}	$k_{-5} = 0$ (set to 0 to be irreversible)
Dissociation of cleaved **SECONDARY** site DNA from **SgrAI** (ROO) or enzyme **E** (Binary)	$k_6 = 0.4$ s^{-1}	$k_{-6} = 0$ (set to 0 to be irreversible)

Table S7. Initial Concentrations used in the simulation of secondary site cleavage (see Tables S5-S6)

Species	Initial Concentration (nM)
SgrAI or **E**	4000 (excess to ensure complete DNA binding)
PRIMARY	3 (dotted lines) or 1000 (solid lines)
SECONDARY	3 (dotted lines) or 1000 (solid lines)

[1] Rippe, K., von Hippel, P. H., and Langowski, J. (1995) Action at a distance: DNA-looping and initiation of transcription, *Trends in biochemical sciences 20*, 500-506.