Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Polyamines stimulate RecA-mediated recombination by condensing duplex DNA and stabilizing intermediates

Naciye Esma Tirtom[1], Yang Hsu[2], Hung-Wen Li[1]*

1 Department of Chemistry, National Taiwan University, Taiwan
2 Department of Chemistry, National Taiwan Normal University, Taiwan

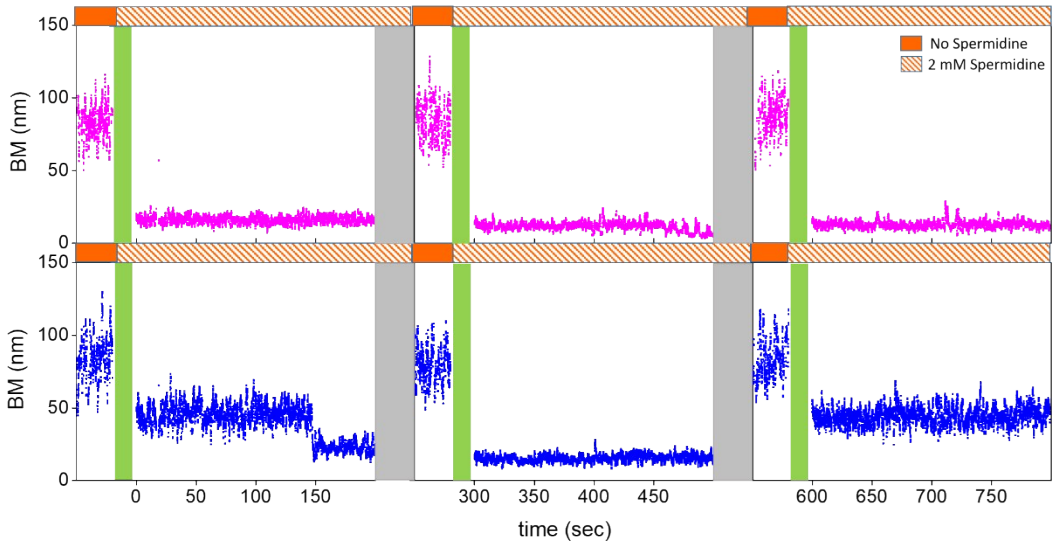
Primer (Sequence) #	Sequence	Label	Direction	Substrate
1	CTTCCTAATGCAGGAGTC		Forward	127 nt - bio - ssDNA
2	TTTTTAGACAGTCATAAGTGCGG	biotin	Reverse	
3	ACTACGATACGGGAGGGC		Forward	231 nt - bio - ssDNA
4	CACCACGATGCCTGCAC	biotin	Reverse	
5	CGTCACCCTGGATGCTGTAG	digoxigenin	Forward	1162 bp - dsDNA
6	GGCTTCCATTCAGGTCG		Reverse	
7	GGCTTCCATTCAGGTCG	biotin	Reverse	1162 bp - bio - dsDNA
8	ACTACGATACGGGAGGGC	digoxigenin	Forward	231 nt - dig - ssDNA
9	CACCACGATGCCTGCAC		Reverse	
10	GCGCTGGCTGGTGGTCTAGA		Forward	150 nt - ssDNA
11	CCCAGTGCTGCAATGATACC		Reverse	
12	CCAAAGACACCACAGACCACACACAAGAATCGAG AGGGACACCAACGTCGTGACTGGGAAAACCCT	biotin		66 nt Non-homologous ssDNA

Table S1. The primer list for preparing the fully duplex 1162 bp dsDNA and 231/150 bp hybrid DNA, as well as invading 127 nt and 231 ssDNAs.

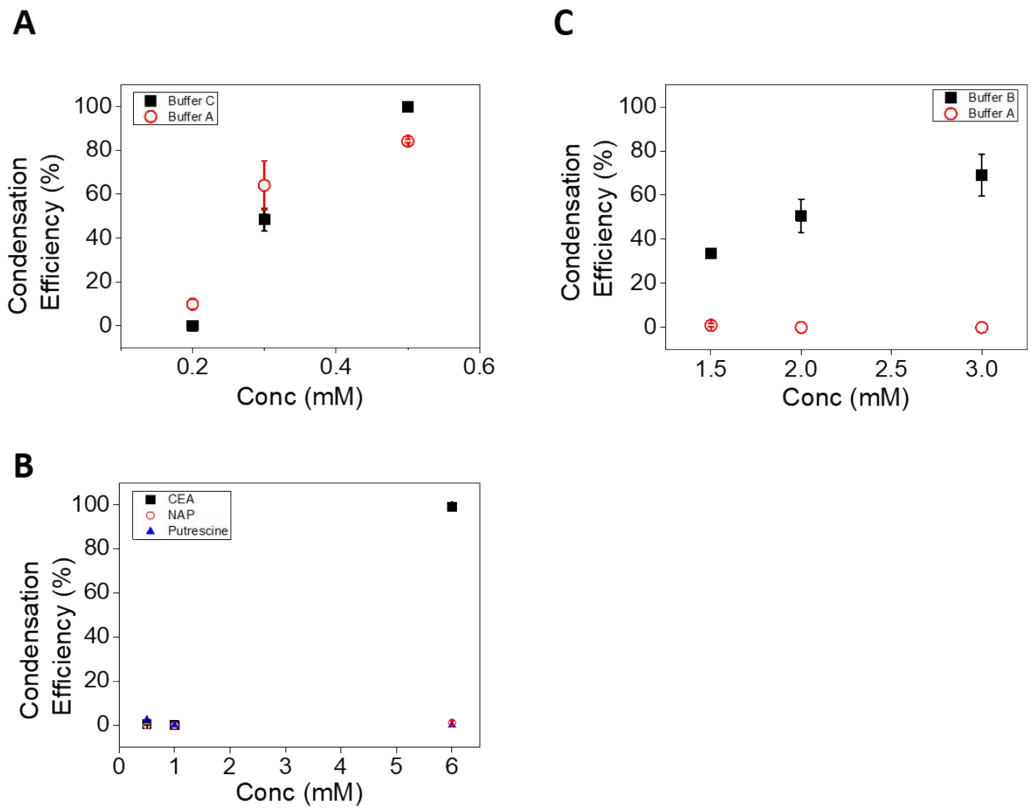
Invading ssDNA	[Spermine] (μM)	RecA	Tether Count	
Homologous	-	-	0.1 ± 0.07	0.004
Non-homologous	-	-	0.13 ± 0.11	0.006
Homologous	-	+	0.59 ± 0.08	0.03
Non-homologous	-	+	0.63 ± 0.11	0.03
Homologous	300	+	19.9 ± 0.84	0.8
Non-homologous	300	+	7.54 ± 0.77	0.3
Homologous	500	-	36.4 ± 2.35	1.5
Non-homologous	500	-	24.1 ± 4.67*	1*
Homologous	500	+	61.3 ± 4.37	2.5
Non-homologous	500	+	19.0 ± 2.44	0.8

* Reference point

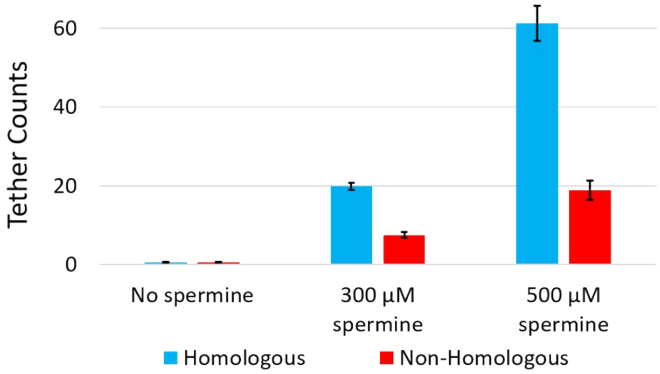
Table S2. Stable tether count values for TPM duplex capture assay with 1162 bp DNA substrate, without RecA or with 2 μM RecA. Non-homologous, 500 μM spermine condition is taken as a reference. Data is an average from 4 independent experiments, and errors are standard error of the mean. Spermine concentration dependency of tether counts in invading assay is plotted in Supplementary Fig. 3.



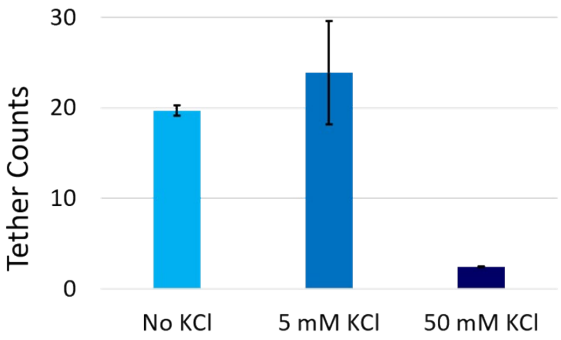
Supplementary Figure 1. Exemplary time-courses for stepwise condensation of DNA substrates. Brownian motion changes in 3 cycles of addition-and-removal of 2 mM spermidine can be seen here. In above molecule, condensation took place rather fast in all 3 cycles, while below molecule showed stepwise condensation in 2 of the cycles (first and last), and rather fast condensation in the middle cycle.



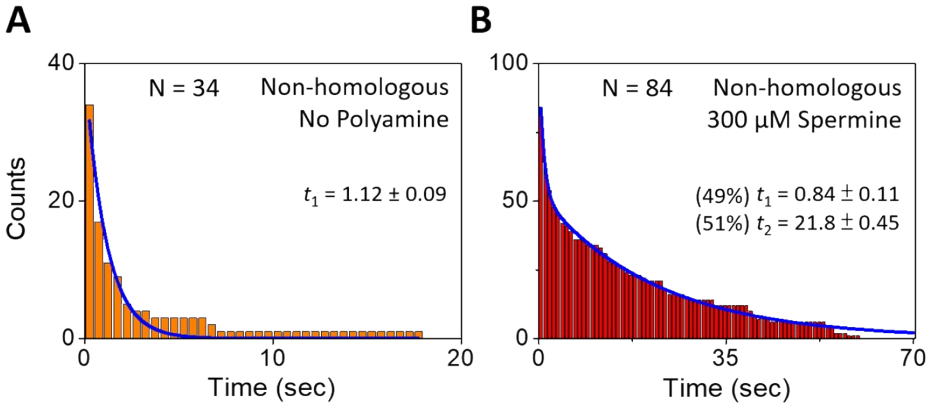
Supplementary Figure 2. A. Condensation efficiency of spermine in different reaction buffers were compared, the data for buffer A are reported in Figure 2. Efficiency values in both buffers are close to each other, in proximity of single standard error of the mean, suggesting that buffers of similar ionic strength will result in similar condensation efficiencies. **B.** Condensation efficiencies of spermidine in Buffer A and B. Spermidine's condensation efficiency greatly depends on the reaction buffer's buffering capacity and also salt. **C.** Natural polyamine, putrescine and synthetic polyamines, Carboxyethyl-γ-aminobutyric acid (CEA) and N-(2-Aminoethyl)-1,3-propanediamine (NAP) were shown to be less effective compared to spermine and spermidine. At 6 mM, only CEA showed significant condensation activity.



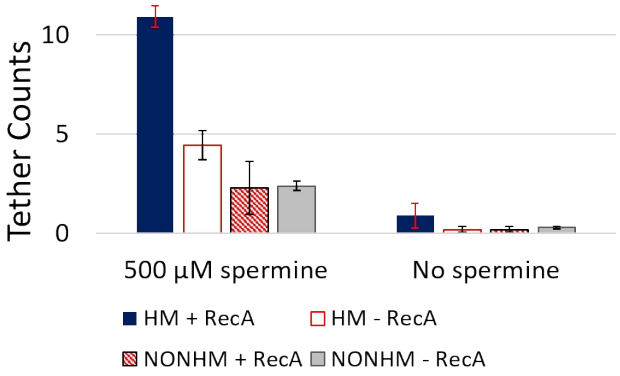
Supplementary Figure 3. Tether counts depend on polyamine concentration. As the spermine concentration is increased to 300 µM (~50% condensation efficiency), both tether counts increased significantly, compared to no spermine condition. Further increase in spermine concentration to 500 µM (89.1 % condensation efficiency) emphasized this effect more. Data is an average from 4 independent experiments, and errors are standard error of the mean.



Supplementary Figure 4. Salt abolishes the polyamine's stimulation on duplex capture assay for 1162 bp DNA substrate. 5 mM KCl did not alter the polyamine's effect. 50 mM KCl, however, nearly abolished the stimulation. Homologous ssDNA, 300 µM spermine and 2 µM RecA is used. Data is the average from 5 different fields-of-view, and error bars refer to the standard deviation.



Supplementary Figure 5. Dwell time histograms of transient tethers from the duplex capture reactions without spermine or with 300 µM spermine, in the presence of non-homologous ssDNA. A table of the dwell times of these histograms is provided in Table 2. In the absence of spermine, data is well fitted with single-exponential, giving 1 sec-scale of short dwell time. In the presence of 300 µM spermine, data is fitted to bi-exponential. The short dwell time coincides with the 1 sec-scale dwell time of the no polyamine condition, representing the unproductive collision events. The longer dwell time, represents the polyamine-stimulated three-strand intermediate formation. Data is obtained from 4 independent experiments. For non-homologous and no polyamine case (A), N = 34; non-homologous and 300 µM spermine (B), N = 84



Supplementary Figure 6. Polyamines stimulate RecA duplex capture reaction also for 231/150 bp hybrid DNA substrate. Here, dark blue solid square refers to the homologous ssDNA with RecA, red open square to the homologous ssDNA without RecA, red-patterned square to the non-homologous ssDNA without RecA, and gray-shaded square to the non-homologous ssDNA with RecA. Data are from 2-3 independent experiments.