Supplementary Material and Figures

Mobile SSB on DNA stimulates RecA filament formation

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SM1. SSB interaction with the partial duplex DNA

We probed if the FRET fluctuations for ssDNA that is longer than the binding site size were due to destabilization of the duplex DNA at the ss-dsDNA junction used. SSB is known to destabilize short duplexes when single stranded overhangs are substantially shorter than the binding site size in the (SSB)$_{65}$ binding mode\(^1\). For this control experiment, two partial duplex (pd)DNA (one with 65mer long 3’ dT tail and the other with 70mer) were used where the donor and acceptor fluorophores were placed next to each other at the ss-dsDNA junction such that high FRET is observed for DNA only (Supplementary Figure 9a and b). Any significant local breathing (or melting) of the duplex DNA at the junction that lasts longer than 30 ms (time resolution of the acquired data) would be reported as a drop in FRET. Supplementary Figure 9c and d show that upon binding of SSB (marked by increase in total fluorescence intensity; data not shown), no reduction in FRET values are observed. This supports our conclusion that when bound to partial duplex DNA with overhangs similar to or larger than the binding size of SSB in the (SSB)$_{65}$ mode, SSB is incapable of disrupting the adjacent duplex region.

SM2. RecA polymerization on pre-nucleated DNA in the absence of SSB

We determined the kinetics of RecA filament formation on a ssDNA primed with a nucleation cluster (RecA-ATP$_\gamma$S) at the junction of the pdDNA in the absence of SSB. To achieve this, once the RecA-ATP$_\gamma$S filament was removed by SSB from the ssDNA tail, 10 µM (dT)$_{80}$ was added to the solution. SSB tetramers transferred from the surface immobilized DNA to the freely floating (dT)$_{80}$ molecules that were subsequently washed
with buffer flow. However, RecA-ATPγS complexes remain on the duplex DNA and serve as a nucleation cluster, and when RecA and ATP are introduced, RecA-ATP filament forms readily as the representative time trace in Supplementary Figure 10a shows. We determined the rates of polymerization initiation and filament extension on (dT)_{30+65+1} using preformed RecA-ATPγS nucleation site but in the absence of SSB by averaging such time traces after RecA-ATP flow (Supplementary Figure 10b and c). As shown in Supplementary Figure 10b (Cy5.5 FRET decay), the rate of polymerization initiation (\(k_1 = 0.2 \pm 0.02 \text{ s}^{-1}\)) is consistent with the rate measured in presence of SSB. The rate of Cy5 FRET reduction (\(k_3 = 0.25 \pm 0.03 \text{ s}^{-1}\)) is comparable to rate of polymerization initiation measured by Cy5.5 FRET (Supplementary Figure 10c). The slightly higher rate for Cy5 FRET change may be due to multiple nucleation events on the free ssDNA. Both Cy5 and Cy5.5 FRET changes demonstrate that filament extension is fast compared to other events in current assay conditions.

**SM3. Rate of hairpin disruption by SSB**

(dT)_{65+hp+3} DNA by itself in the absence of SSB displays high FRET due to close position of the dye pair. When the sample is incubated with 100 pM SSB for 5mins and the residual SSB in solution is washed away, we observe appearance of lower FRET populations (Fig. 5a and b in main text). Upon closer look at the single molecule FRET traces, three major kinds of changes are observed. As shown in Fig. 5c, 35-45% of the time traces show three-state fluctuations. We assigned these FRET states to hypothetical states of hairpin unzipping, for example, \(E_{\text{app}} \sim 0.95\) corresponding to S1 (intact hairpin), \(E_{\text{app}} \sim 0.7\) corresponding to S2 (partially disrupted hairpin) and \(E_{\text{app}} \sim 0.5\) to S3 (possibly the completely unzipped hairpin) (Supplementary Figure 11a and c). The rest of the single molecule traces show two state fluctuations between 0.95 and 0.7 (~30%) or no FRET changes (~25%). In the absence of free SSB protein in solution, we can rule out the possibility that these FRET fluctuations arise from transient binding and dissociation of SSB molecules. Hence, we attribute this observation to the melting of secondary structures achieved by the aid of a SSB tetramer bound in the near vicinity of the secondary structure. We also estimated the rates of transition between the states S1, S2
and S3 using hidden Markov model analysis\textsuperscript{d} and found rates of unzipping to be \(\sim 1.2-1.5\) s\(^{-1}\) for (dT\textsubscript{65+hp+3}) (15 molecules with 1332 transitions for each rate constant) and \(\sim 1.1-1.4\) s\(^{-1}\) for (dT\textsubscript{6+hp+65}) (16 molecules with 992 transitions) as shown in Supplementary Figure 11c. Rate of recovering the secondary structure (\(\sim 3.1\) s\(^{-1}\)) is 2-3 fold faster than the rates of unzipping, possibly due to the binding preference of SSB towards the dT sequences flanking the hairpin.

**SM4. RecA filament formation on hairpin DNA**

*RecA filament formation on single dT\textsubscript{6+hp+65} DNA-SSB complexes*

The mechanism for how RecA filament growth on the hairpin DNA can be aided by SSB can be deduced from the single molecule time trajectories (Supplementary Figure 12a). Hairpin DNA with SSB mostly displays high FRET at 200 ms time resolution where SSB-induced fluctuations to lower FRET states are mostly averaged out. Addition of 1 \(\mu\)M RecA and 1 mM ATP to the sample initiates nucleoprotein filament growth from the pre-nucleated junction end such that the FRET value drops monotonically to low FRET (\(E_{\text{app}} \sim 0.05\)). Initial drop in FRET is probably related to unzipping of the hairpin by the SSB tetramer. As RecA starts to take over the hairpin DNA, the extension of the DNA due to filament formation results in establishment of lowest FRET state (\(\sim 0.1\)). We can unambiguously distinguish this low FRET state from photobleaching of acceptor via direct excitation with red (633 nm) laser (Supplementary Figure 12a). The average rate of RecA filament growth is estimated by fitting the average FRET value of such traces (from 51 molecules) undergoing transition to low FRET state to an exponential decay function (Supplementary Figure 12b). The apparent rate of hairpin disruption and RecA growth on the ssDNA is (0.085 s\(^{-1}\)) and is likely to be limited mostly by the rate of RecA polymerization initiation from a preformed RecA-ATP\(\gamma\)S nucleation cluster (\(\sim 0.2\) s\(^{-1}\)).

*RecA filament formation on single dT\textsubscript{6+hp+65} DNA in the absence of SSB*

To compare the dynamics of RecA filament growth on hairpin DNA, we devised an assay where RecA filament formation is induced in the absence of SSB. As explained in the Supplementary Figure 12c (i-iii), DNA-SSB-RecA complex is generated using RecA in presence of ATP\(\gamma\)S as a cofactor followed by RecA removal from the ssDNA segment
with SSB. To displace the SSB on the ssDNA tail, the sample was incubated with 20 µM (dT)₈₀ ssDNA. This results in transferring of the SSB protein from the immobilized DNA to the free ssDNA in solution that is then flushed out with buffer. The FRET population in the absence of SSB returns to the narrow high FRET distribution comparable to the one observed with DNA only (Supplementary Figure 12c(iv)). Addition of 1 µM RecA with 1 mM ATP, however does not induce a clear transition to the low FRET state even after 15 minutes (Supplementary Figure 12c(v-vii)) in sharp contrast to the data obtained in the presence of SSB. The average rate of RecA growth on the hairpin DNA, estimated from the exponential fit of fraction of low FRET population with time is 40 fold slower (0.002 s⁻¹) compared to that aided by SSB.

*RecA filament formation on dT₆₅⁺hp⁺₃ DNA in the presence of SSB*

With the hairpin placed near the 3’ end of the ssDNA, we employed the same assay to check for the stimulation of RecA filament growth by SSB (Supplementary Figure 12d). Hairpin DNA displays high FRET and RecA-ATPγS filament results in generation of low FRET state (E ~0.1) (Supplementary Figure 12d (i) and (ii)). Subsequent incubation with SSB results in displacement of the RecA-ATPγS filament from the ssDNA segment and transient excursion of the SSB to the hairpin DNA generates a broad distribution of FRET values (Supplementary Figure 12d(iii)). RecA-ATP introduced into the sample however, does not stimulate rapid growth of the filament on the hairpin region (Supplementary Figure 12d(iv)) and the average rate of RecA growth over the hairpin region, estimated from the exponential fit of fraction of low FRET population with time is very low (0.003s⁻¹). It is worth mentioning that though SSB has fallen off the T₆₅⁺hp⁺₃ DNA (compare Supplementary Figure 12d (iv) to (i) and (iii)) and possibly replaced by RecA on 5’end of the hairpin, RecA filament could not grow on the hairpin region.
Supplementary Methods

All DNA constructs except for (dT)$_{69+12m}$ share the common architecture of a partial duplex DNA with a 3’ tail. The constructs were generated by annealing 5’-Cy5-GCCTCGCTGCGCTGCGCA-Biotin-3’ (BS5), 5’-Cy5.5-GCCTCGCTGCGCTGCGCA-Biotin-3’ (BS5.5) or 5’-GCCTCGCTGCGCTGCGCA-Biotin-3’ (BS) oligonucleotides to one of the long tail strands listed in Table 1. (dT)$_{69+12m}$ (annealed to 5’-Biotin-TGGCGACGGCAGCGAGG-Cy5-3’ (BSrev5)) carries a 5’ ssDNA tail and the 12m sequence is complementary to the ‘cos’ site (5’-AGGTGCGCGCC-3’) of the λ phage DNA. The oligodeoxynucleotides were synthesized with conjugated dyes using standard β-cyanoethyl phosphoramide chemistry using an ABI model 391 automated DNA synthesizer (Applied Biosystems, Foster City, CA) or purchased from IDTDNA with amino-dT modifications and conjugated to NHS-ester forms of dyes (Invitrogen). Biotin was incorporated using BiotinTEG CPG (all modification reagents were from Glen Research, Sterling, VA).

Annealing was carried out by mixing 1.5 fold excess of the non biotinylated strand to the indicated biotinylated strand (BS, BS5 or BS5.5) at 10 – 20 µM final concentration (5 min incubation at 85 °C followed by gradual cooling at room temperature over 2 - 3 hours). All the DNA constructs exhibit low apparent FRET (<0.2) in the absence of protein. Binding of SSB tetramer results in higher FRET values due to wrapping of the DNA around the protein.

For RecA-SSB assays, nucleation cluster on the partial duplex was generated by first incubating 1 µM RecA in presence of 1 mM ATPγS for 5 mins and then replacing the free RecA and ATPγS with 10nM SSB and incubating for 15 mins. RecA filament elongation was then followed in real time or measurement acquired after 2 mins from addition of 1 µM RecA and 1mM ATP that also ensure free SSB removal from the buffer.

Table 1: DNA Oligonucleotides used for Single Molecule FRET Experiments*

| DNA partial duplex | Sequence of tail strand (5’ to 3’) | FRET Experiment (Annealing) |
|--------------------|------------------------------------|-----------------------------|

*Table 1: DNA Oligonucleotides used for Single Molecule FRET Experiments*
| Strand | (dT)$_{60+4}$ | TGGCGACGGCAGCGAGGC-(T)$_{59}$-T-(T)$_{4}$ |
|--------|---------------|--------------------------------------------------|
| (dT)$_{66+1}$ | TGGCGACGGCAGCGAGGC-(T)$_{65}$-T-(T)$_{1}$ |
| (dT)$_{69+4}$ | TGGCGACGGCAGCGAGGC-(T)$_{68}$-T-(T)$_{4}$ |
| (dT)$_{69+8}$ | TGGCGACGGCAGCGAGGC-(T)$_{68}$-T-(T)$_{8}$ |
| (dT)$_{69+12}$ | TGGCGACGGCAGCGAGGC-(T)$_{68}$-T-(T)$_{12}$ |
| (dT)$_{69+18}$ | TGGCGACGGCAGCGAGGC-(T)$_{68}$-T-(T)$_{18}$ |
| (dT)$_{69+12m}$ | GGGCCGCGACCTT-T-(T)$_{68}$-TGGTACGTACATCGTCC |
| (dT)$_{Cy3+65}$ | TGGCGACGGCAGCGAGGC-Cy3-(T)$_{65}$ |
| (dT)$_{Cy3+70}$ | TGGCGACGGCAGCGAGGC-Cy3-(T)$_{70}$ |
| (dT)$_{65+hp+3}$ | TGGCGACGGCAGCGAGGC-(T)$_{65}$-T-TGGTACGTACATCGTCC-Cy5-T |
| (dT)$_{6+hp+65}$ | TGGCGACGGCAGCGAGGC-(T)$_{6}$-TGTGACTGAGACAGTCAC-
| | T-(T)$_{65}$-T |
| (dT)$_{130}$ | TGGCGACGGCAGCGAGGC-(T)$_{130}$-Cy5-T |
| (dT)$_{65+65}$ | TGGCGACGGCAGCGAGGC-(T)$_{65}$-Cy3-(T)$_{65}$-Cy5-T |
| (dT)$_{30+65}$ | TGGCGACGGCAGCGAGGC-(T)$_{30}$-Cy3-(T)$_{65}$-Cy5-T |

(2-color FRET) SSB migration (BS5) 
(2-color FRET) SSB migration blockade (BSrev5) 
(2-color FRET) Control for helix destabilization by SSB (BS5) 
(2-color FRET) Helix destabilization by SSB (BS) 
(2-color FRET) Helix destabilization by SSB (BS) 
(3-color FRET) SSB migration (BS5.5) 
(3-color FRET) RecA polymerization on SSB bound ssDNA (BS5.5)
Dye either conjugated with Amino C6 linker on underlined dT nucleotide (Cy3) or attached directly to the DNA backbone using phosphoramidite chemistry (as indicated in the sequence).

Overproducing vector pSF1, containing wt SSB gene was used for single mutation of SSB Ala to Cys at position 122 in C-terminus. Plasmid for both wtSSB and mutant SSB was transformed into E.coli K12ΔH1Δtrp host strain and protein was overproduced and purified as described with addition of double strand DNA cellulose column to remove a minor exonuclease contaminant. SSB(A122C) was labeled using Alexa Fluor 555 maleimide (Invitrogen) at 4°C. Protein was diluted to ~ 0.8mg/ml and dialyzed in reaction buffer P (50mM KH$_2$PO$_4$/K$_2$HPO$_4$, pH 7.0, 10% glycerol, 0.5M NaCl). The reaction was performed under 15 fold molar excess of TCEP by incubating protein solution (~0.4-0.5 ml) with equimolar Alexa Fluor 555 maleimide for ~ 30 mins on ice to achieve 25-30% labeling per tetramer. The reaction was stopped adding BME and the protein was separated from the free dye using site exclusion Bio-Gel P 6-DG column (MW=1000-6000, Bio-rad). Collected protein was concentrated by dialysis in storage buffer (20mM Tris, pH 8.1, 50% glycerol, 0.5 M NaCl, 1mM EDTA, 1mM BME) and stored under -80°C. The concentration of the protein was determined using $\varepsilon_{280} = 2.83\times10^5$ M$^{-1}$cm$^{-1}$ (monomer) after correction for the dye contribution at 280 nm. The extent of labeling was determined comparing with the concentration of the dye calculated using $\varepsilon_{555} = 1.5\times10^5$ M$^{-1}$cm$^{-1}$. The ability of labeled protein to bind ssDNA was checked performing bulk titrations of the protein with dT$_{70}$ by monitoring intrinsic Trp fluorescence quenching upon binding as described. The experiments demonstrate identical binding behavior of labeled SSB comparing to wild type SSB (data not shown).

Prism-type total internal reflection (TIR) microscope (IX 70, Olympus) was used to image surface immobilized DNA (via biotin-Neutravidin (Pierce)) on PEG (Nektar Therapeutics) coated quartz surface. Donor (Cy3) was excited with a 532 nm wavelength Nd:YAG laser (CrystaLaser) and direct excitation of acceptors was achieved with a 633 nm HeNe laser (Coherent) when required. Fluorescence collected using water immersion objective (60x, 1.2 numerical aperture, Olympus) passes through 545 nm long pass filter (Chroma) for laser scatter rejection. Cy3, Cy5 and Cy5.5 fluorescence was split.
using dichroic mirrors with cut-offs at 640 nm and 685 nm (Chroma) and imaged side by side on an EMCCD camera (iXon DV 887-BI, Andor) for three color FRET (only 640 nm dichroic used for two color FRET between Cy3 and Cy5). DNA with only the donor only or DNA with a donor and only one acceptor were used to estimate the leakages in each of the detection channels. Data was acquired (8-100 msec integration time) and analyzed with custom routines written in Visual C++ (Microsoft) and IDL (Research Systems), respectively.

After correcting for the leakage and background in all channels, apparent FRET efficiency was calculated as $E_{app,i} = I_{A,i}/(\Sigma I_{A,i} + I_{DA})$ where $I_A$ and $I_{DA}$ represent acceptor and donor (in the presence of acceptor) intensities, respectively and $i$ stands for Cy5 and Cy5.5. Single-molecule FRET histograms were generated by averaging for ten frames. Traces (or events) were post synchronized using MATLAB to estimate rates from a set of two or more experiments.

Auto-correlation function was calculated using the expression in equation 1,

$$G(\tau) = \int E_{app}(t)E_{app}(t-\tau)dt \quad (1)$$

where $E_{app}$ represents apparent FRET. $G(\tau)$ from >35 molecules was averaged to generate the representative $G(\tau)$ for a particular DNA (or temperature condition) and this was fit to single or bi-exponential functions.

Cross-correlation between $E_{app,5}$ and $E_{app,5.5}$ was determined by equation 2,

$$XC(\tau) = \int E_{app,5}(t)E_{app,5.5}(t-\tau)dt \quad (2)$$

from individual traces after adjacent averaging 3 time points (time resolution = 50 ms). Average XC($\tau$) is calculated from 48 traces and fit to a bi-exponential function with smaller the time constant is attributed to detection noise.

HMM analysis for single molecule FRET traces was performed using an in-house 32 node Unix cluster. The FRET traces were analyzed with 10 initial FRET states such that no prior biases were involved. The transition density plots were built by a custom
routine were single transitions from one state to the other were plotted to develop a frequency histogram of transition from one state to another.
Supplementary Figures:

Supplementary Figure 1: FRET distribution and dynamics as a function of the DNA length. (a) DNA constructs used for single molecule experiments to bind single SSB proteins. Partial duplex (pd)DNA with biotin modifications (for surface immobilization) carry two fluorophores, Cy3 and Cy5, placed 69-70 nt apart and an extension of N nt (N= 0-18). Cy3 is conjugated to the DNA using an amino-C6-dT attachment to minimize perturbation to backbone of the DNA but introduction of dyes into the backbone of the ssDNA also displayed qualitatively similar features. (b) FRET histograms displaying the distribution of the average FRET values for DNA-SSB complexes as a function of length of the DNA extensions. When the extensions are small, e.g. (dT)_{69+1}, SSB-DNA complexes exhibit mostly high FRET achieved by the close positioning of the two ends of the DNA in the (SSB)_{65} binding mode. As the length of the DNA increases, SSB
spends progressively smaller fraction of time in the segment of the DNA close to the junction with an associated decrease in average FRET. (c) Representative FRET time trajectories from single DNA-SSB complexes display characteristics unique to the length of extension DNA (each colored trace shows an individual DNA-SSB complex while corresponding color dashed horizontal line represents E=0.5). While SSB binding to different segments of ssDNA results in lower average FRET values for longer DNA, single SSB-DNA complexes still display transient high FRET values comparable to the FRET value obtained when SSB resides close to the junction). We attribute these events to dynamic repositioning of the SSB protein along the stretch of the DNA.
Supplementary Figure 2: Salt dependence of SSB migration. (a)-(c) FRET histograms generated from DNA-SSB complexes displaying FRET fluctuations show negligible dependence on salt (NaCl) concentration on poly(dT) DNA sequence. (d) When a (dT)$_{69}$ is flanked on one side with a mixed sequence, we observe a significant salt dependence in the average FRET values. Higher average FRET would signify that the SSB prefers to reside on the (dT)$_{69}$ segment with increasing salt. This is likely to be a manifestation of higher binding affinity of SSB to (dT) sequences in high salt compared to other bases$^{11}$. (e) Autocorrelation analysis of FRET fluctuations of SSB-(dT)$_{69+8}$ also exhibits no evidence for any salt dependence and hence apparent rates estimated from autocorrelation analysis reported in the main text is determined by averaging the rates at different salt conditions (inset).
Supplementary Figure 3: Hidden Markov Model (HMM) based analysis and Transition Density Plot (TDP) for SSB diffusion. (a) (dT)$_{69+12}$ DNA when bound to SSB displays low FRET (0.4-0.5) with less frequent transitions to high FRET state of 0.8 corresponding to state where SSB resides close to ss-ds junction (see Supplementary Figure 1c). HMM analysis of such FRET fluctuations yields a TDP where the frequency of the transitions is not distributed as expected from a Brownian walk with uniform stepping rates all across the different states (see top and right panels for the percentage of the transitions between each state pair). For example, 55% more transitions are identified between the lower FRET states than the higher FRET states compared to only 10% for (dT)$_{69+8}$ (data not shown). We attribute this to the presence of degenerate states exhibiting indistinguishable low FRET values. For the case of (dT)$_{69+18}$, we can no longer distinguish individual peaks in the TDP which is probably due to the breakdown of the one-to-one correspondence between the wrapped states and FRET values. (b) The TDP for short extensions ((dT)$_{69+4}$, (dT)$_{66+1}$ and (dT)$_{60+4}$ corresponding to 0, 2, 8 nts beyond the binding site size of SSB tetramer) displays monotonically decreasing number of FRET states. We assign 5, 3 and 2 states based on TDP plots to (dT)$_{69+4}$, (dT)$_{66+1}$ and (dT)$_{60+4}$ respectively.
Supplementary Figure 4: Rates of transition among the six FRET states identified with HMM analysis for (dT)_{69+8}. The rates are narrowly distributed for a data set (symbols of same color) and do not deviate from experiment to experiment (symbols with different colors) and range from 2 – 5 s\(^{-1}\) at 13 °C. Therefore, the stepping rate for each DNA length reported in the Figure 2c is averaged over transitions between all states.
Supplementary Figure 5: (dT)_{130} with two acceptors at the two ends of the DNA do not display fast fluctuations between high FRET states when bound to one SSB-Alexa555 and another unlabelled SSB in (SSB)_{65} binding mode. The representative behavior exhibited by such complexes is exemplified by the FRET trace shown. While still fluctuating alternatively between high FRET for each acceptor, there is a significant increase in the residence times (order of 3-10 sec) of the labeled SSB in each of these states suggesting restriction of SSB migration. These fluctuations could represent conformational changes in the SSB-DNA complex and need further investigation.
Supplementary Figure 6: Distribution of the time interval between drop of $E_{app,5.5}$ to zero and rise of $E_{app,5}$ to steady high value (from traces as shown in Fig. 4f). The distribution peaks at ~1 sec followed by a slow decay. The red curve, a fit to the distribution assuming two major rate limiting steps (first corresponding to RecA polymerization itself and second RecA polymerization under hindrance by SSB interaction) gives two rates $k_1 = 0.6$ s$^{-1}$ and $k_2 = 2.7$ s$^{-1}$. Assuming the slow phase to represent RecA polymerization in the presence of SSB, we attribute the rate of 0.6 s$^{-1}$ as the average rate of extension of RecA filament by 10 monomers on SSB bound ssDNA. The fast rate (27 s$^{-1}$ per monomer) compares well with estimates of RecA elongation under similar conditions in absence of SSB)\textsuperscript{12}. 

$$y = A \left[e^{k_1 t} - e^{k_2 t}\right]$$

$k_1 = 0.6$ s$^{-1}$

$k_2 = 2.7$ s$^{-1}$
Supplementary Figure S7: Rates of RecA filament formation on (dT)$_{65+65+1}$ in presence and absence of SSB on the DNA (from 20-45 molecules). (a) Rate of RecA filament initiation in presence of SSB as Cy5.5 FRET decay (b) Rate of RecA filament extension till ~65 nts from the junction observed as the increase in the Cy5 FRET due to displacement of the bound SSB molecule to the 3’ end of the DNA (c) Rate of SSB removal by RecA filament indicated by drop in Cy5 FRET (d) Rate of RecA filament initiation as drop in Cy5.5 FRET (from 0.2 to 0) (b) Rate of RecA filament extension beyond 65 nts from the junction observed as drop in Cy5 FRET.
Supplementary Figure 8: Schematic of rolling by tetrameric SSB (a) DNA wrapping and unwrapping based on DNA-SSB complex crystal structure. (b) Simplified model for SSB rolling (DNA as in black line and SSB as circle). One end of the DNA (e.g. 3' in the figure) could partially unwrap from the SSB (four subunits capable of binding DNA shown in different colors) while the other end of the DNA binds to such newly freed DNA binding site. SSB protein can undergo random walk on the DNA using this process. This mechanism is facilitated by the 'closed' wrapping of the DNA around the protein.
Supplementary Figure 9: Test for duplex destabilization by SSB. No change in FRET efficiency was observed upon SSB binding to ssDNA tail. FRET histograms for (a) (dT)$_{\mathrm{Cy3+65}}$ DNA (b) (dT)$_{\mathrm{Cy3+65}}$ + 3 nM SSB (c) (dT)$_{\mathrm{Cy3+70}}$ (d) (dT)$_{\mathrm{Cy3+70}}$ + 3 nM SSB at room temperature in imaging buffer (500mM NaCl).
Supplementary Figure 10: RecA filament growth from a pre-formed nucleation cluster in the absence of SSB. (a) Time trace showing the transition from low FRET ($E_{app,5} \approx 0.1$ and $E_{app,5.5} \approx 0.2$) state to zero FRET as RecA-ATP nucleoprotein filament extends from a RecA-ATPγS-dsDNA nucleation cluster. Flow was initiated at $t = 10$ secs and direct excitation of Cy5 and Cy5.5 by 633 nm wavelength ($t = 32$s) confirmed the presence of non-bleached dyes. Time traces from 57 molecules averaged after initiation of RecA-ATP flow. (b) Rate of RecA filament initiation estimated as drop in Cy5.5 FRET (from 0.2 to 0) (c) Rate of RecA filament extension beyond 30 nts from the junction observed as decay in the energy transfer between Cy3 and Cy5 on the 3’ end of the DNA.
Supplementary Figure 11: Kinetics of hairpin disruption by SSB. Transition density plot (TDP) for (a) (dT)$_{65}$hp+3 and (b) (dT)$_{6}$hp+65 can be broadly classified to a three state system for unzipping of hairpin from high FRET (S1) state to intermediate FRET (S2) and low FRET (S3) states. (c) Rates of unzipping for both hairpins constructs and both transitions (i.e. S1→S2 and S2→S3) are comparable and range from 1.1 – 1.5 s$^{-1}$. Rates of rezipping of the hairpin DNA ranges from 1.2 - 3.1 s$^{-1}$. 
Supplementary Figure 12: RecA filament formation on DNA hairpin (a) Single hairpin DNA-SSB complex pre-nucleated with RecA-ATPγS displays primarily high FRET (integration time=200 ms) due to close placement of the dyes in the hairpin. Upon introduction of 1 M RecA and 1mM ATP (at t = 10 s), FRET efficiency drops gradually to low FRET (E~ 0.05). Direct excitation of the acceptor with red laser (633 nm) rules out photobleaching as the cause of low FRET (t= 73-82 s) (b) Average change in FRET (from 51 molecules) fit to single exponential yields a time constant of 11.7 ± 0.3 sec. (c) RecA filament growth on hairpin DNA in the absence of SSB. c(i) DNA displays high FRET due to intact hairpin. c(ii) Formation of RecA-ATPγS filament over the majority of hairpin DNA in 5 mins results in of low FRET population. c(iii) SSB however removes RecA-ATPγS filament from the DNA hairpin and results in restoring the primarily high FRET state. c(iv) SSB is transferred to free ssDNA in solution upon addition of 20 µM 88 mer ssDNA as FRET distribution equivalent to DNA only case is recovered. c(v-vii) Addition of RecA-ATP solution cause a slow transition to the low FRET state with time. (d) When the hairpin is placed at the 3’ end of the ssDNA tail such that SSB is bound to the 5’ end of the hairpin (d(i)), RecA-ATPγS filament growth takes place efficiently (see d(ii); after 5mins) and SSB removes the RecA-ATPγS and restoring the high FRET (d(iii)). d(iv) However, addition of RecA-ATP to this nucleoprotein complex fails to induce rapid filament formation.
Supplementary References

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