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Supplemental Data

Real-Time Observation of RecA Filament Dynamics with Single Monomer Resolution

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Supplemental Experimental Procedures

DNA Preparation

ssDNA molecules were purchased from IDT DNA or were provided as a generous gift from Dr. T. Lohman. Labeling of dye and biotin at 5′ or 3′ end of ssDNA was done during DNA synthesis. In case of internally-labeled ssDNA, Cy3 (NHS-ester from GE Healthcare) was attached to the base of a dT through a C6 amino linker in order to keep the DNA backbone intact. Annealing of dsDNA was performed by heating a sample that contained two complementary ssDNA molecules at micromolar concentrations to 90 °C and slowly cooling for 2 hours at the room temperature. The sequence of biotinylated ssDNA forming the dsDNA is: 5′-GCC TCG CTG CCG TCG CCA-biotin-3′ and that of ssDNA with 3′ tail is 5′- TGG CGA CGG CAG CGA GGC -(tail)- 3′. The sequences of $(dT)_{5+54}$, $(dT)_{10+49}$ and $(dT)_{13+46}$ are 5’ TGG CGA CGG CAG CGA GGC-T5-T*-T53-3’, 5’ TGG CGA CGG CAG CGA GGC-T10-T*-T48-3′ and 5′ TGG CGA CGG CAG CGA GGC-T13-T*-T46-3’ where T* stands for an amine-modified dT with Cy3 labeled.

Protein-Repelling Surface

All the experiments were carried out on quartz slides, which were coated by polyethylene glycol (PEG) in order to minimize any possible interaction between a protein and a surface. Quartz slides and coverslips were cleaned as follows. The slides were sonicated in a glass staining dish for 20 min in 10 % alconox, 5 min in water, 15 min in acetone and 20 min in 1 M KOH. The coverslips were sonicated in another glass staining dish for 20 min in 1 M KOH. Then, the slides and the coverslips were burned by a propane torch for one minute and a few seconds, respectively. Finally, both were placed back in the previous glass staining dishes and stored in methanol until the next step.

The slides and coverslips were next amino-modified as follows. In a flask which was cleaned with methanol in 5 minute sonication, 100 ml of methanol (Fisher, ACS grade), 5 ml of acetic acid (Fisher, ACS grade) and 1 ml of aminosilane (A0700, UCT) were mixed. The methanol in the glass staining dishes was replaced by the mixture. They were incubated for 20 minutes at room temperature and sonicated for 1 minute during the incubation. Then, the solution was replaced by methanol and the slides and the coverslips were stored under the condition until next step.

The amine-modified surface of the slides and the coverslips was polymer-coated by amine-reactive PEG as follows. The slides and the coverslips were rinsed by water (MilliQ, 18.5 MΩ) and placed well-leveled. 70 μl of the following solution was dropped on each slide and the coverslips were laid over them; the solution was made by dissolving 0.2 mg of biotin-PEG (BIO-NHS-5000, MW 5000, Nektar Therapeutics) and 16mg of mPEG (M-SPA-5000, MW 5000,

Nektar Therapeutics) in 64 µl of freshly made 0.1 M sodium bicarbonate, pH 8.5 and by centrifuging it for a minute to remove bubbles. Then the overlapped slides and coverslips were incubated in dark and humid for 2–3 hr. Finally, they were disassembled, rinsed with water (MilliQ) and stored in dark and dry at –20°C until use.

Fluorescence and Absorption Spectra

The spectra of 50 nM partial dsDNA molecules with $(dT)_{21}$ and $(dT)_{13}$ tail labeled with Cy3 and/or Cy5; and 10 nM 77mer ssDNA (5′ TGG CGA CGG CAG CGA GGC-T13-T*-T45 3', T* is amine-modified dT with Cy3 labeled) were measured by Cary Eclipse (Varian) in the identical chemical condition as single-molecule measurements after 10 min incubation at the room temperature. The fluorescence signal was recorded by exciting with 540 nm (640 nm in case of directly exciting Cy5) light and by collecting signal from 550 to 750 nm (650 to 750 in case of directly exciting Cy5). The recorded fluorescence signal was corrected for the wavelength-dependent sensitivity of the detector. The absorption spectrum was measured by Cary Bio 100 (Varian) under the identical condition.

ATPase Rate Measurement

50 nM of labeled 77mer ssDNA molecules (5' TGG CGA CGG CAG CGA GGC-T13-T*-T45 3′, T* is amine-modified dT with Cy3 labeled) and unlabeled DNA (5′ TGG CGA CGG CAG CGA GGC -T59 3′) were, respectively, mixed with 1.3 µM RecA and 2 mM ATP in the identical chemical condition as single-molecule measurements at room temperature. ATPase activity was measured by EnzChek kit (Invitrogen) based on the increase in absorption at 360 nm, which was detected by Cary Bio 100 (Varian). The rates were determined by fitting the change of absorption in the first minute. The measurement was repeated three times for each sample.

DNase I Footprinting

600 nM of a partial dsDNA molecules with $(dT)_{13}$ tail was incubated with RecA and 1 mM cofactor (ATPγS or ATP) for a given time, in a buffer that contains 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1% v/v 2-mercaptoethanol, and 10 mM Tris-HCl (pH 7.6). Next, 10 µl of the solution was cleaved by 0.05 U/µL of DNase I (New England Biolabs) for 20 seconds, which was stopped by adding SDS, EDTA and tRNA (0.3%, 70 mM and 1 µg/ml in final). The solution was ethanol-precipitated, then, the DNA-pellet was dissolved in 10µl formamide. It was run in 12% denaturing PAGE for 2 hr (20W) and imaged in a fluorescence imager (FLA-3000, Fujifilm) with 633 nm light exciting Alexa647 that is attached to the dsDNA.

Interaction between a Fluorophore and a RecA Filament

Effects of a RecA Filament on Fluorescence

A fluorescence spectrum of a partial dsDNA with $(dT)_{13}$ ssDNA tail that was labeled with Cy3 and Cy5 (Figure S2A) was measured in ensemble (Supplemental Experimental Procedures). While DNA alone shows high FRET (red in Figure S2Ba), introduction of RecA with ATPγS leads to 60% decrease in FRET (green in Figure S2Ba) due to the filament formation.

To study an effect of a RecA filament on Cy3 (donor), the measurement was repeated with Cy3-only sample (Figure S2Bb). Cy3 attached at the end of ssDNA tail became 70% brighter when the RecA filament formed with ATPγS, which has been also observed in single-molecule measurements (Figures S3C-D). The increase in fluorescence signal is likely due to conformational constraint imposed by a protein nearby which decreases the non-radiative decay

rate and consequently increasing the fluorescence quantum yield of Cy3. While the donor quantum yield change does affect the absolute FRET efficiency, it does not affect the apparent FRET efficiency we and other typically use for single molecule FRET analysis as shown below.

The donor quantum yield ϕ_D is given by $\phi_D = \frac{k_r}{k_r + k_{nr}}$, where k_r is the radiative decay rate and k_{nr} is the non-radiative decay rate. The apparent FRET efficiency E is given by $E \equiv \frac{I_A}{I_A + I_D} = \frac{\eta_A I_A^0}{\eta_A I_A^0 + \eta_D I_D^0}$, where I_D and I_A are the measured intensities of donor and acceptor molecules, I_D^0 and I_A^0 are the true intensities of donor and acceptor emission, η_D and η_A are the detection efficiencies of donor and acceptor signals determined by instrumentation. Since $I_D^0 \propto \frac{k_r}{k_{ET} + k_r + k_{nr}}$ and $I_A^0 \propto \phi_A \frac{k_{ET}}{k_{ET} + k_r + k_{nr}}$ where k_{ET} is the energy transfer rate and ϕ_A is the acceptor quantum yield, we obtain $\frac{I_D^0}{I_A^0} = \frac{k_r}{\phi_A k_{ET}}$. Finally, inserting this into the formula for E, we obtain $E = \frac{1}{1 + (\eta_D / \eta_A)(k_r / \phi_A k_{ET})}$ which is independent of changes in the non-radiative decay rate of the donor, k_{nr}.

In addition, the RecA filament formation did not cause any change in the fluorescence emission spectrum of Cy3. The same measurement was carried out with a ssDNA internally-labeled with Cy3 (that was used in Figure 2A), with which we again observed the increase in fluorescence signal (50%) but no change in the emission spectrum (Figure S2Eb).

A similar measurement was carried out by directly exciting Cy5 (acceptor) that is positioned at the junction of ss-dsDNA (Figure S2Bc). We observed a decrease of the signal (30%), smaller than that in FRET (60%), when RecA formed a filament with ATPγS. One possible reason for the fluorescence decrease is photoblinking of Cy5 which may be enhanced by the filament formation on the ssDNA as well as dsDNA. The photoblinking frequently showed up in single-molecule traces in the same condition (Figures S2C). We can easily distinguish such photoblinking ($E = 0$) from a normal active state of Cy5 ($E > 0$) in single-molecule traces based on a FRET value. Regardless of the source of Cy5 signal reduction, our data analysis on FRET trajectories do not rely on the absolute values of FRET but rather on its relative changes. Therefore, the kinetic rates measured 1) from all-or-none events of a nucleation cluster (Figure 1), (2) from stepwise change in FRET (Figures 2–4) and (3) from gradual change in FRET (Figure 5) would not be influenced by the decrease in Cy5 signal.

As shown in single-molecule traces taken with $(dT)_{13}$ and $(dT)_{21}$ tail (Figures S2C and S2D), the low-FRET state which results from a RecA filament formed with ATPγS is distinguished from photoblinking or photobleaching of Cy5 by the difference in FRET ($E > 0$ versis $E = 0$). We further checked the activity of Cy5 by exciting Cy5 directly using a 633 nm laser (Figure S2D).

When absorption spectra were measured, there was only slight change observed (Figures S2Bd, Ec), indicating insignificant influence of a RecA filament on the absorption properties.

An Effect of a Dye on a RecA Filament

We have measured ATPase activity with 77mer ssDNA (Supplemental Experimental Procedures) ATP hydrolysis rates were 0.14 ± 0.00 and 0.14 ± 0.01 (sec^{-1} $RecA^{-1}$) with labeled and unlabeled ssDNA, respectively, indicating that the dye (Cy3) positioned in the middle of DNA does not affect ATPase activity of a RecA filament. The minimal influence of organic dyes on a RecA filament were reported previously (Bazemore et al., 1997; Gourves et al., 2001; Xiao and Singleton, 2002). We could also carry out RecA-mediated homologous strand exchange with a ssDNA that was internally-labeled in a single-molecule assay (unpublished observation).

Filament Formation on a Short ssDNA

The Shorter the ssDNA, the Higher the FRET Efficiency

When ssDNA tail (10–17 nt) of a partial dsDNA (Figure S3A) is free from a RecA filament, FRET efficiency is high ($E > 0.7$) and slightly depends on the length of the ssDNA (Figure S3B, gray). When a RecA filament forms with ATPγS, FRET efficiency drops sharply (Figure S3B, black). The new FRET values strongly depend on the length of the ssDNA—the longer the ssDNA, the lower the FRET efficiency, reflecting the difference in the contour lengths of the ssDNA.

RecA Filament Formation with ATP

Figure S3C shows traces taken with $(dT)_{21}$ tail in the same condition as Figure 1D. A fraction of filament assembly/disassembly events are slow enough to display intermediate FRET values during transition (Figures S3Cc and S3Cd).

The change in FRET is well-correlated with the change in total fluorescence intensity as has been discussed above (Figure S2Bb). When a two-dimensional density plot is made for E vs. "fluorescence intensity," the anticorrelation between FRET and intensity is clearly visualized. This anti-correlated property was carefully considered in analyzing traces, especially if FRET is too low ($0 < E << 1$) to distinguish from an inactivated state of acceptor molecules ($E = 0$).

RecA Filament Formation with Different Types of NTP Factors

$(dT)_{21}$ ssDNA tail free from a RecA filament shows high FRET ($E \sim 0.55$) (Figures S4Ba and 1Ca), while it shows low FRET ($E \sim 0.1$) with the filament (Figures S4Bb and 1Ca). When dATP is used as a NTP factor, a stable filament forms (Figures S4Bc; arrows) in contrast to the brief filament formation with ATP (Figures S4Ba) (Menetski and Kowalczykowski, 1989). The single molecule trace also shows more stable low-FRET states (Figure S4Ca). A similar property was observed with $(dT)_{17}$ tail (Figure S4Cb) while the filament formation was rarely observed with ATP (Figure 1Eb).

In the absence of NTP factors, RecA still forms a filament but in a collapsed form with a lower pitch than usual (Bell, 2005; Story et al., 1992). The FRET efficiency ($E \sim 0.3$) is higher than that with ATPγS (E ~ 0.05) possibly reflecting the lower pitch (Figure S4Bd). The broad distribution in a FRET histogram is due to two-state fluctuation between $E \sim 0.3$ (filament) and ~0.55 (no filament) (Figure S4Cc).

Disassembly of <ssDNA|ATPγS>

After <ssDNA|ATPγS> forms around a short oligonucleotide by incubating with RecA and ATPγS, if we remove RecA and ATPγS from solution, the filament disassembles (Figure 3Ac). We found that, if ATPγS is kept in solution, single-molecule traces recorded during this

disassembly visualize individual states (M_i) of $(dT)_{13}$ since monomers dissociate slowly one by one (Figure S8A). Among the molecules that exhibit a monotonic and stepwise change in FRET, we could count up to ~5 states as shown in an example time trace (Figure S8Ba), consistent with expectation from the ratio between RecA and nucleotides. Molecules with 4 states are also observed with a similar frequency (Figure S8Bb). The same experiment was carried out with $(dT)_{10}$ tail that showed four different states in maximum (Figure S8Ca), while there were a similar number of molecules that show three states only (Figure S8Cb). Not all the molecules show a monotonic and/or stepwise change in FRET, therefore, it should be noted that this assay is only for counting the maximum number of FRET states but not for understanding the disassembly process of <ssDNA|ATPγS>.

Filament Formation around a dsDNA Mediated by ssDNA Tail

Filament Formation on dsDNA Is Not Local to the ss-dsDNA Junction

In order to test whether <dsDNA|ATPγS> forms all over the dsDNA or only a part of it (e.g. only near the junction of ss-dsDNA), we relocated Cy5 at the junction (Figure 3B) into the middle of the dsDNA as shown in Figure S9A. (The sequence of the ssDNA that is labeled with biotin and Cy5 is 5′-GCCTCGC T* GCCGTCGCCA-biotin-3′, where T* stands for an amine-modified dT with Cy5 labeled.) While $E \sim 0.6$ without a RecA filament, E becomes as low as ~0.4 when RecA and ATPγS are added (Figure S9Ab). Since the change in FRET reflects a filament formation over a portion of dsDNA that is far from the junction and since <dsDNA|ATPγS> cannot form with a blunt end of the dsDNA (Figure S9C), <dsDNA|ATPγS> is not likely to form only near the junction, but rather to form over the entire length of 18 bp duplex DNA.

<dsDNA|ATPγS> Protects dsDNA from Digestion by an Endonuclease

DNase I footprinting was carried out to check the presence of <dsDNA|ATPγS> using an independent, non-FRET based method. A dsDNA with 3' $(dT)_{13}$ tail was labeled with a dye at the junction (Figure S9B). The DNA was incubated with RecA and ATPγS, which was followed by cleavage by DNase I (Supplemental Experimental Procedures). When the DNA was incubated with RecA and ATPγS for a different amount of time (lanes 1–5 in Figure S9Bb) before cleavage reaction, a different degree of cleavage activity was observed—the longer incubation, the less cleavage. Similarly, when the DNA was incubated with RecA and ATPγS for 10 minutes but with different RecA-concentrations, the less cleavage reaction was observed with the larger amount of RecA (lanes 8–11). Such protection of dsDNA by RecA was not observed when ATP was used (lane 7). Therefore, this footprinting assay further supports the formation of a filament around dsDNA with ATPγS mediated by 3' ssDNA tail, which has not been reported previously.

<dsDNA|ATPγS> Cannot Form without ssDNA Tail

When the same DNA as that in Figure 3B but without ssDNA tail (Figure S9C) was incubated with RecA and ATPγS, we could not observe any filament formation around dsDNA for up to 2 hours (Figures S9Cc). This rules out the possibility that <dsDNA|ATPγS> which formed in a 3′ tailed DNA was due to the dyes acting as nucleation sites, as the blunt dsDNA used for the control here also contained the dyes in the same location but did not show evidence of filament formation.

Formation and Disassembly of <dsDNA|ATPγS> at High Temperature (35°C)

The same experiment as Figure 3 was carried out at 35°C instead at the room temperature (Figure S10). The formation of <ssDNA|ATPγS> was observed when RecA and ATPγS were added in the same way as at the room temperature, resulting in a transition from high to low FRET (Figures S10Aa and S10Ab). When RecA and ATPγS were removed from solution, the disassembly of <ssDNA|ATPγS> was observed as at the room temperature but at a faster rate (<1min) (Figures S10Ac and S10Ad). Finally, when RecA and ATP were added after 10 minute-incubation in the previous condition (no RecA and ATPγS in solution at 35°C), only ~50% of the molecules in the high-FRET state shifted into the low-FRET state (Figures S10Ae and S10Af). Therefore, <dsDNA|ATPγS> is less stable at 35°C than at the room temperature. Note that <dsDNA|ATPγS> is still more stable than <ssDNA|ATPγS> at 35°C for a short 3′ tail (13 nt).

Similarly, the formation of <dsDNA|ATPγS> was directly observed at 35°C (Figure S10Ba). A half of the molecules lost the filament 10 minutes after RecA and ATPγS were removed from solution at 35°C (Figure S10Bb) consistent with the result above (Figure S10Af).

Formation of <dsDNA|ATPγS> Mediated by 5' ssDNA Tail

The same experiment as shown in Figure 3 was carried out with a dsDNA with 5′ $(dT)_{20}$ ssDNA tail, instead of 3′ tail (Figure S11). When RecA and ATPγS were added, a filament formed (Figures S11Aa and S11b) in less than half a minute. When RecA and ATPγS were removed from solution, the disassembly of <ssDNA|ATPγS> was observed in a similar time scale as that with 3′ tail (~5 min) (Figures S11Ac, d). Finally, when RecA was added with ATP, most of the population in the high-FRET state was shifted into low FRET (Figures S11Ae and S11Af). Such transition was not observed in the absence of <dsDNA|ATPγS> (Figure S11B).

However, a small population were in high and intermediate FRET (Figure S11f, arrows), the origin of which is revealed when single-molecule traces are analyzed. As shown in Figure S11Ag, *E* fluctuates between high and low FRET dynamically. This large fluctuation is in clear contrast with a trace recorded with 3′ $(dT)_{19}$ tail (Figure S11C), where *E* always stays low. This difference between 3′ and 5′ tails, observed in FRET histograms and single-molecule traces, is anticipated since <ssDNA|ATP> on 5′ tail corresponds to the unstable 5′-disassembly end of a filament (K_D ~ 100 nM), while <ssDNA|ATP> on 3′ tail corresponds to the stable 3′-extending end (K_D ~ 8 nM). Conversely, it implies that the polarity of <dsDNA|ATPγS>, which appears to act as a nucleation cluster, is not random but rather follows that of <ssDNA|ATPγS>.

Formation of <dsDNA|ATPγS> was directly observed by attaching the dye pair at two ends of the dsDNA (Figure S11Da) and <dsDNA|ATPγS> remained stable in the absence of RecA and ATPγS for ~2 hr until it started to disassemble after ~3 hr (Figure S11Db).

Extension Speed of a RecA Filament

RecA Extends a Filament Swiftly, Regardless of the Presence of SSB

As shown in Figure 5Cd, RecA forms a filament displacing SSB with a rate of 0.55 (sec^{-1}). We carried out a similar experiment as Figure 5B but removed DNA-bound SSB before adding RecA and ATP in order to find out how much SSB influences the formation/extension of a RecA filament. SSB was removed efficiently by adding 100 nM of 80-mer ssDNA (Figure S12Ae), which restored the FRET histogram into that of DNA-only (*E* ~ 0.1) (Figure S12Ab). When RecA and ATP were added, FRET became even lower (Figure S12Af). The transitions are shown in Figure S12B. After RecA was added at t = 0, FRET dropped from *E* ~ 0.1 to 0 through

two different ways; 1) FRET becomes high briefly, right before *E* becomes 0 (Figure S12Ba), or 2) it changes monotonically (Figure S12Bb). The origin of the brief high-FRET state is not known at this point. The time taken for the transition was measured from 55 molecules, which gave the transition rate of ~0.8 (sec^{-1}) (Figure S12Bc), which is close to the value obtained in the presence of SSB, 0.55 (sec^{-1}) (Figure 5Cb). Note that the former rate is over-estimated since it reflects not only the filament extension but also the filament formation de novo, which makes the two values even closer. It suggests that SSB is removed by the extending RecA filament at the rate of regular filament extension, implying that the binding of SSB must be weak compared to the force exerted during extension of a RecA filament.

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Figure S1. Single-Molecule Spectroscopy for FRET

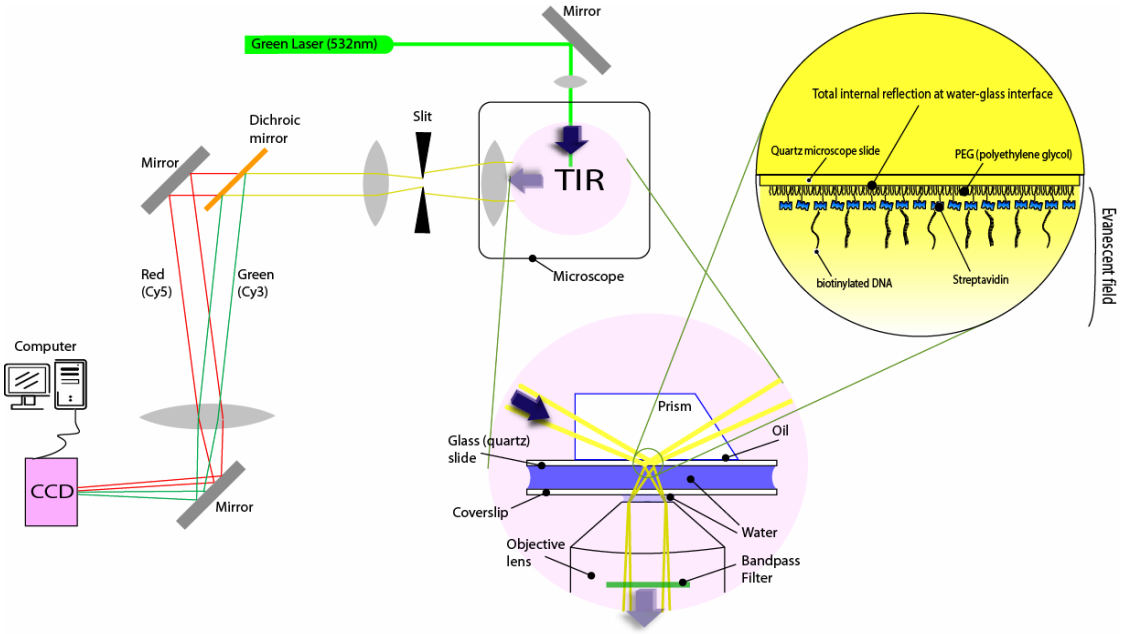
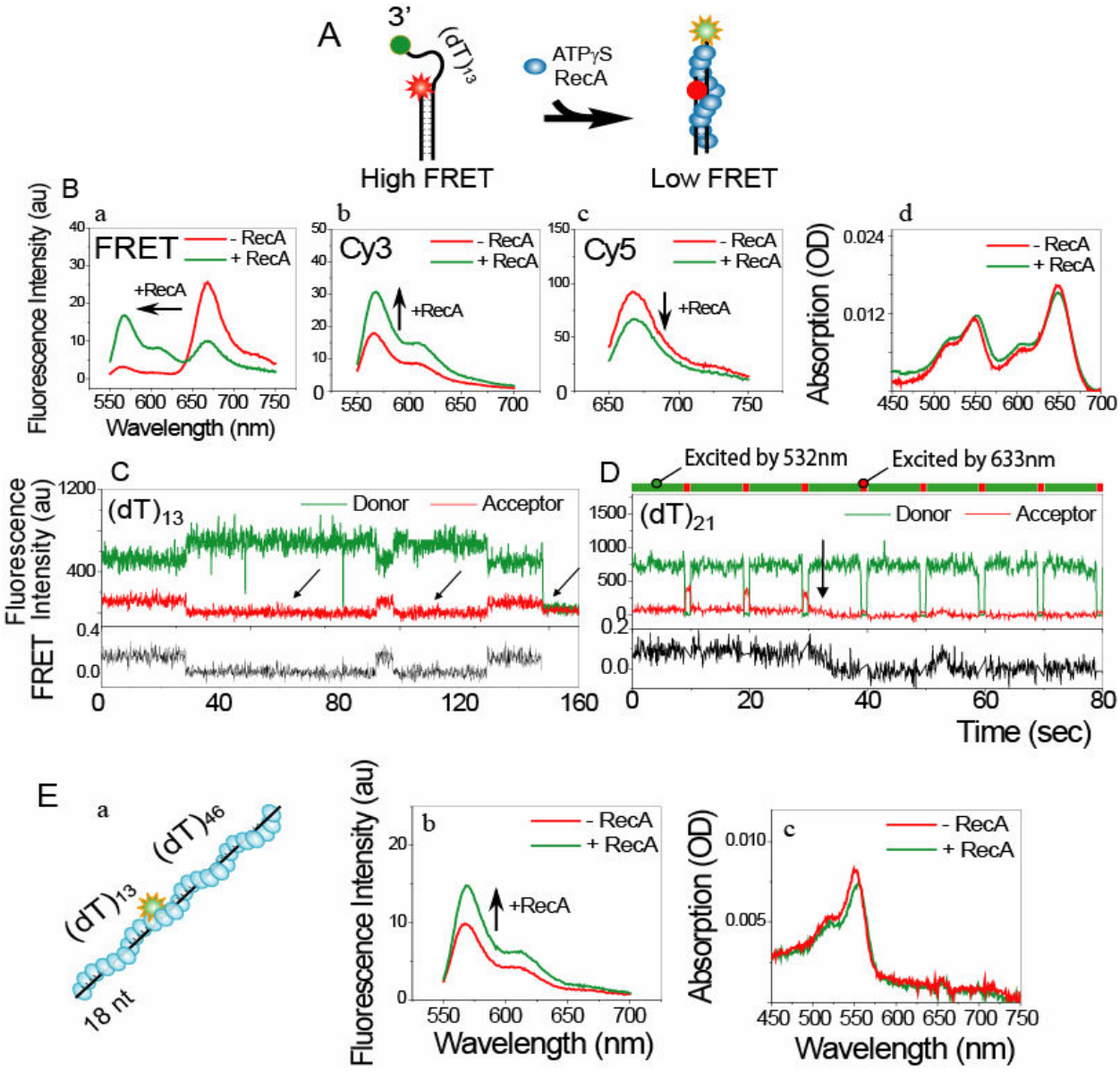


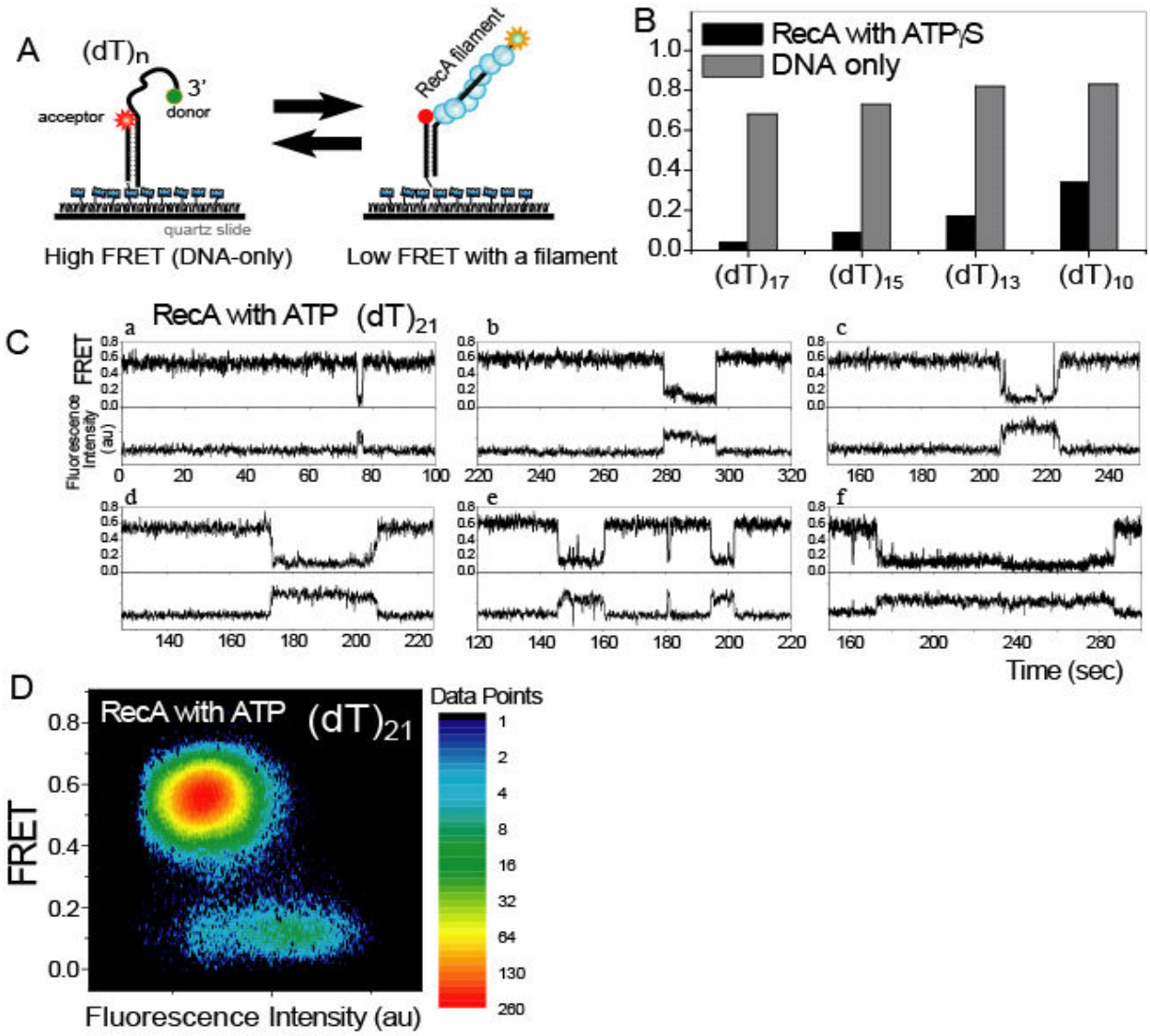
Figure S2. Fluorescence and Absorption spectra of Cy3 and Cy5 That Are Conjugated to DNA



(A) Formation of a filament on a dsDNA with 3′ ssDNA tail
(B) (a) While $(dT)_{13}$ ssDNA tail alone shows high FRET (red), the filament formation with RecA and ATPγS results in 60% decrease in FRET (green, after 10 min incubation) (b) The same DNA but with Cy3 only. Fluorescence of Cy3 becomes enhanced when a filament forms with ATPγS, by 70% (after 10 min incubation). (c) Fluorescence signal of Cy5 decreases when the filament forms with ATPγS, by 30% (after 10 min incubation) (d) Absorption spectra of Cy3 and Cy5 molecules without and with a RecA filament formed, with ATPγS (after 10 min incubation)
(C) Time traces (100 ms time resolution) with RecA and ATPγS shows photoblinking of Cy5 at t ~30–90 and ~95–130 s before photobleaching of Cy3 at t ~ 150 s (arrows).

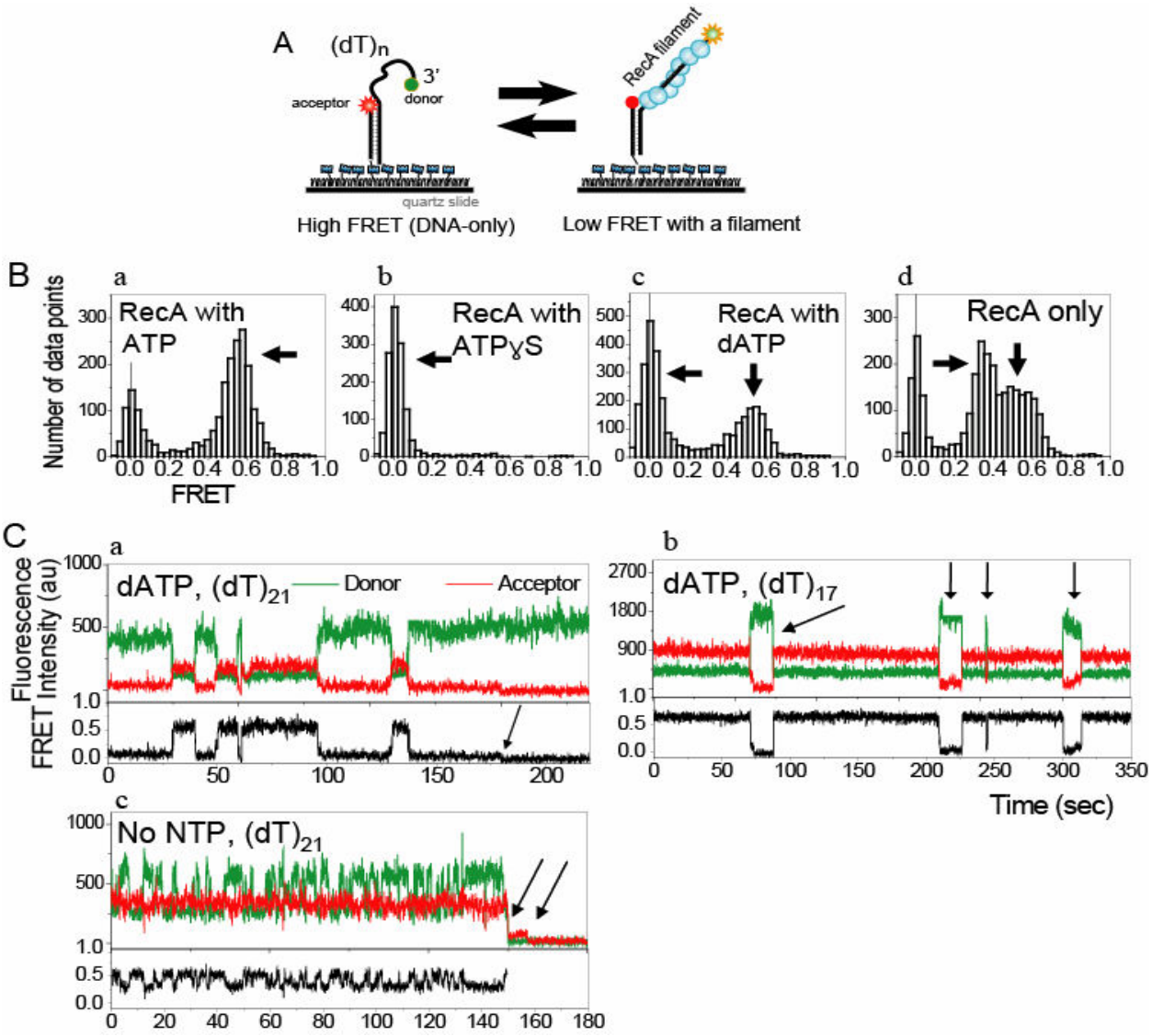
(D) Time traces (100 ms time resolution) taken by alternative excitation of green and red laser light. A dsDNA with $(dT)_{21}$ tail was excited by 532 nm light for 9 seconds and by 633 nm for 1 second, alternatively. Until photobleaching of Cy5 at t ~ 32 (sec) (arrow), not only there is Cy5 signal by FRET (*E*~0.1) but also there is Cy5 signal when directly excited by 633 nm light. After the photobleaching of Cy5, there is neither FRET (E~0) nor Cy5 signal by direct excitation.
(E) (a) Formation of a filament on a ssDNA with Cy3-labeled in the middle that was used in Figure 2A (b) Fluorescence of Cy3 becomes enhanced when a filament forms with ATPγS, by 50% (after 10 min incubation) (c) Absorption spectra of Cy3 molecule without and with a RecA filament formed, with ATPγS (after 10 min incubation).

Figures S3. Filament Formation on Short ssDNA Tail with ATP and ATPγS



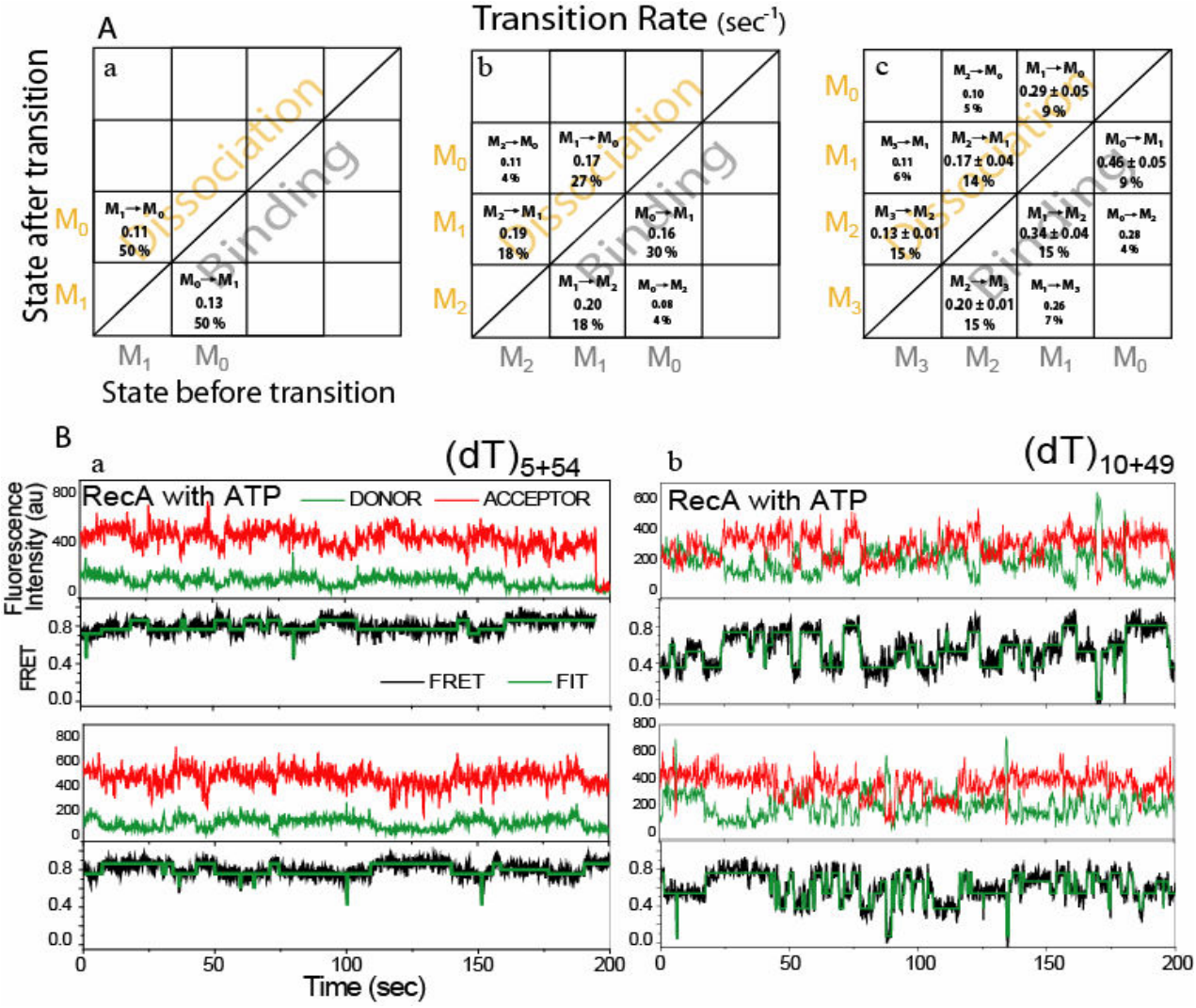
(A) Formation of a filament on a dsDNA with 3′ tail.
(B) ssDNA-length dependent FRET efficiency without (gray) and with (black) RecA and ATPγS
(C) FRET and total-intensity trajectories (100 ms time resolution) from $(dT)_{21}$ tail with RecA and ATP in solution. *Top:* FRET stays high ($E \sim 0.55$) most of the time but frequently becomes low ($E \sim 0.1$). *Bottom:* Accompanying the shift to $E \sim 0.1$ is an overall increase in the total fluorescence signal.
(D) 2-dimensional density plot of E vs. 'fluorescence intensity' from 114 molecules

Figure S4. Filament Formation on Short ssDNA Tail with Different Ypes of NTP Factors



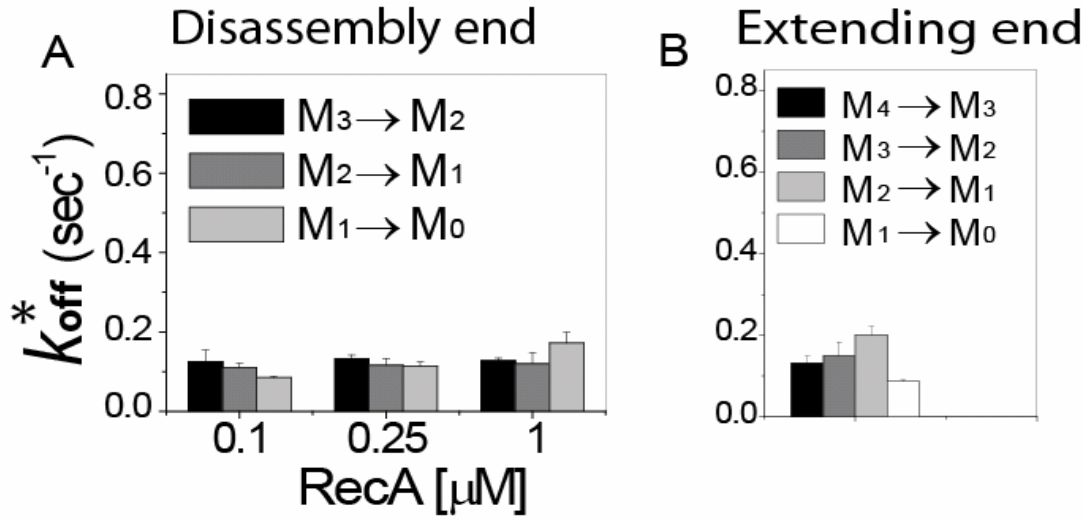
(A) Formation of a filament on a dsDNA with 3′ tail.
(B) FRET histograms with $(dT)_{21}$ tail in the presence of RecA (a) with ATP (after 1 min incubation), (b) with ATPγS (after 1 min incubation), (c) with dATP (after 1 min incubation), and (d) without any NTP (after 1 min incubation).
(C) Time traces with a 100 msec time resolution (a) $(dT)_{21}$ tail with RecA and dATP in solution. Several low-FRET states are observed until photobleaching of Cy5 (arrow). (b) $(dT)_{17}$ tail with RecA and dATP. Formation of a nucleation cluster is observed several times (arrows). (c) $(dT)_{21}$ tail with RecA only. Frequent two-state transitions between $E \sim 0.3$ and ~ 0.55 are observed until Cy3 and Cy5 photobleach, respectively (arrows).

Figure S5. The Dynamics at the 5′-Disassembly End (I)



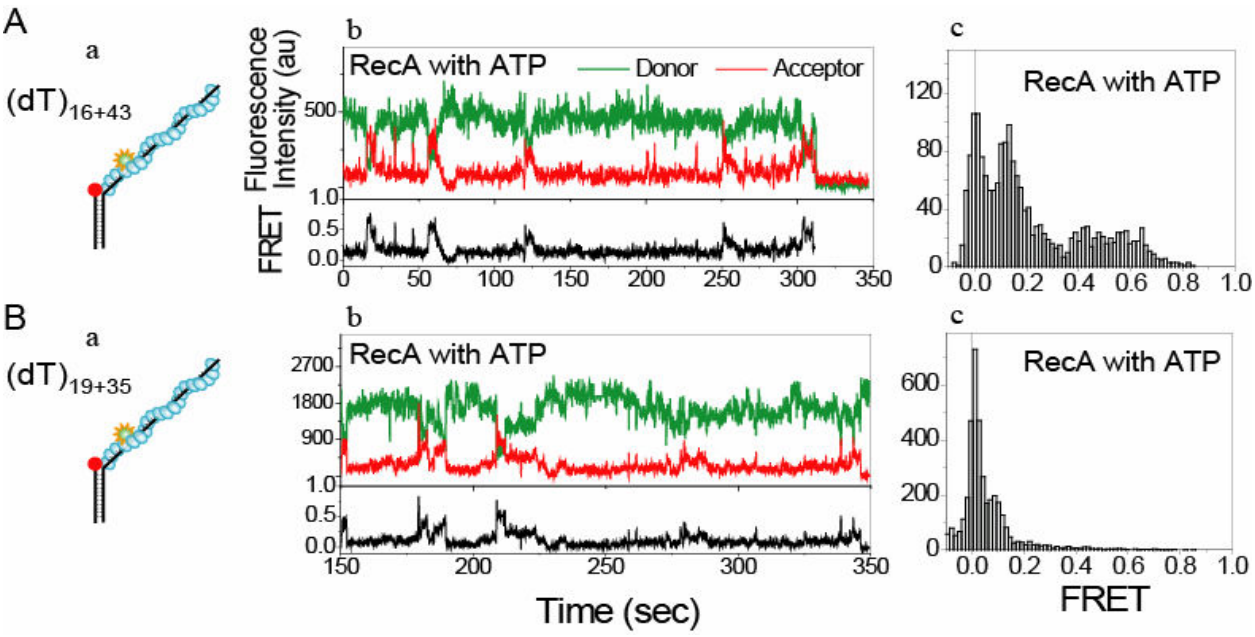
(A) For better comparison in kinetic rates, Figures 2Db, 2Fb, and 2Fd are re-arranged here. The rates and frequencies of transitions observed with (a) $(dT)_{5+54}$, (b) $(dT)_{10+49}$ and (c) $(dT)_{13+46}$ tails. (B) Time traces with (a) $(dT)_{5+54}$ and (b) $(dT)_{10+49}$ tails with RecA and ATP (200 ms time resolution). The fit by HMM is in green, overlaid on each FRET trajectory in black.

Figure S6. Corrected Dissociation Rates



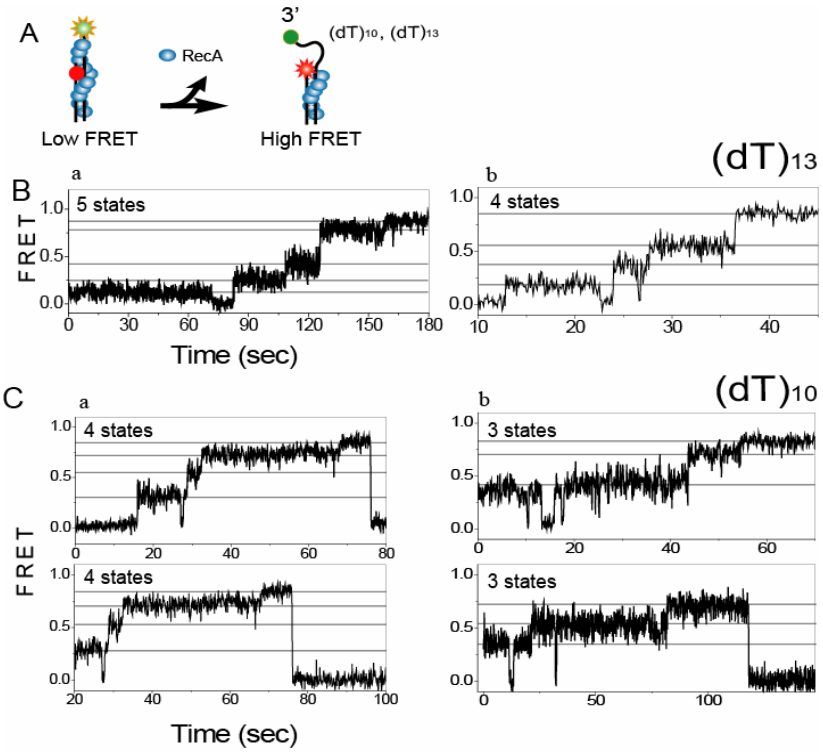
Corrected dissociation rates based on the data in (A) Figure 2Eb and (B) Figure 4Cc. The errors are standard deviations of (A) three and (B) two data sets each.

Figure S7. The Dynamics at the 5′-Disassembly End (II)



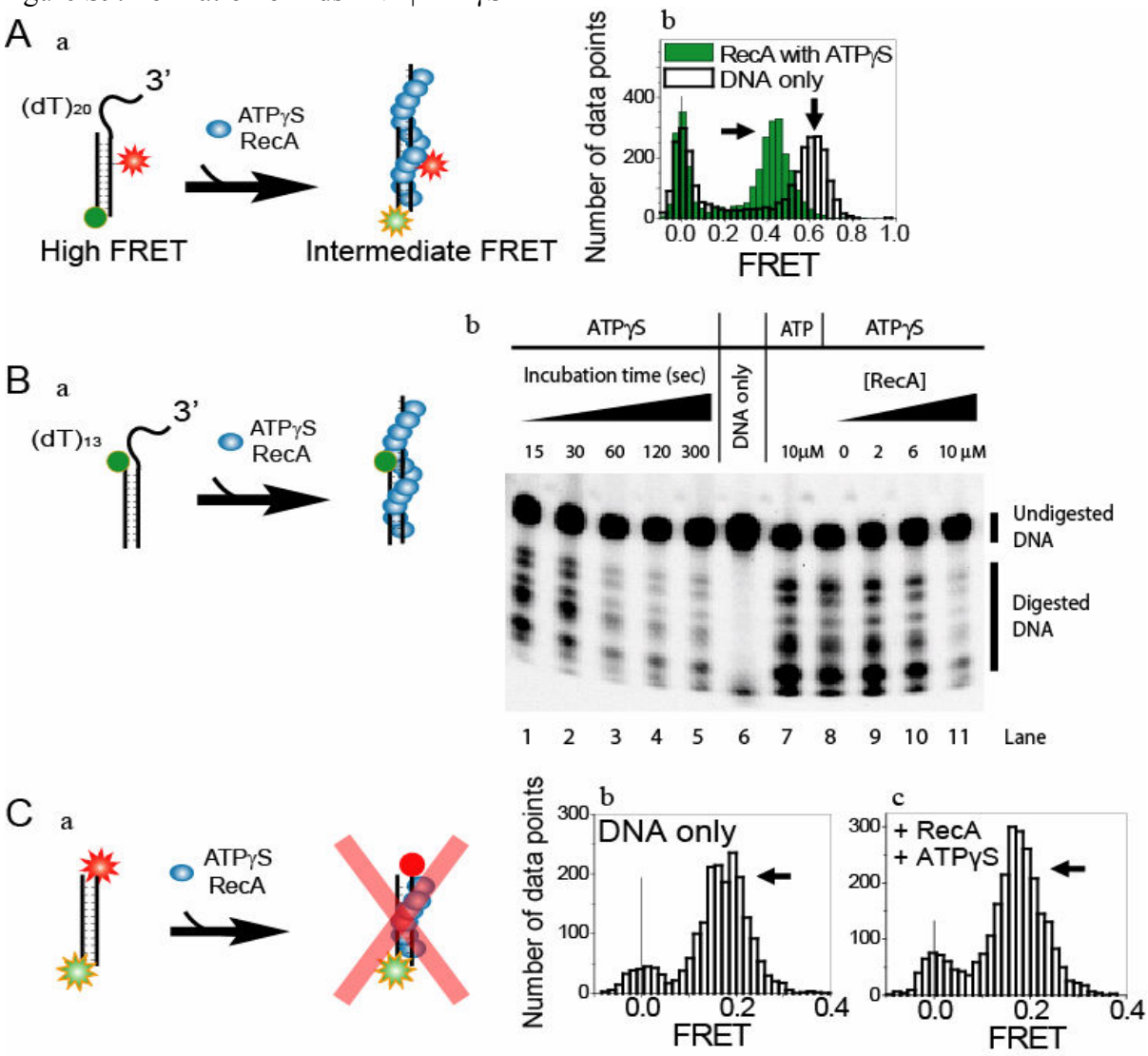
(A) (a) A schematic of a dsDNA with $(dT)_{16+43}$ tail. (b) Time traces with RecA and ATP in solution (200 ms time resolution). They stay in low FRET, most of the time, with infrequent visit to high FRET. (c) A FRET histogram (after 1 min incubation).
(B) (a) A dsDNA with $(dT)_{19+35}$ tail (b) Time traces with RecA and ATP in solution (100 ms time resolution). They stay in low FRET, most of the time, with infrequent visit to high FRET. (c) A FRET histogram display a majority of the population in low FRET (after 1 min incubation).

Figure S8. Disassembly of <ssDNA|ATPγS>



(A) Disassembly of <ssDNA|ATPγS> from $(dT)_{13}$ and $(dT)_{10}$ ssDNA tails.
(B) After 30 min incubation with RecA and ATPγS, upon removal of RecA from solution but with ATPγS kept in solution, monotonic stepwise increase in FRET is observed with $(dT)_{13}$. Time traces show (a) five and (b) four different FRET states during disassembly.
(C) The same assay as (B) but with $(dT)_{10}$ after 60 min incubation with RecA and ATPγS. Time traces show (a) four and (b) three different states during disassembly.

Figure S9. Formation of <dsDNA|ATPγS>

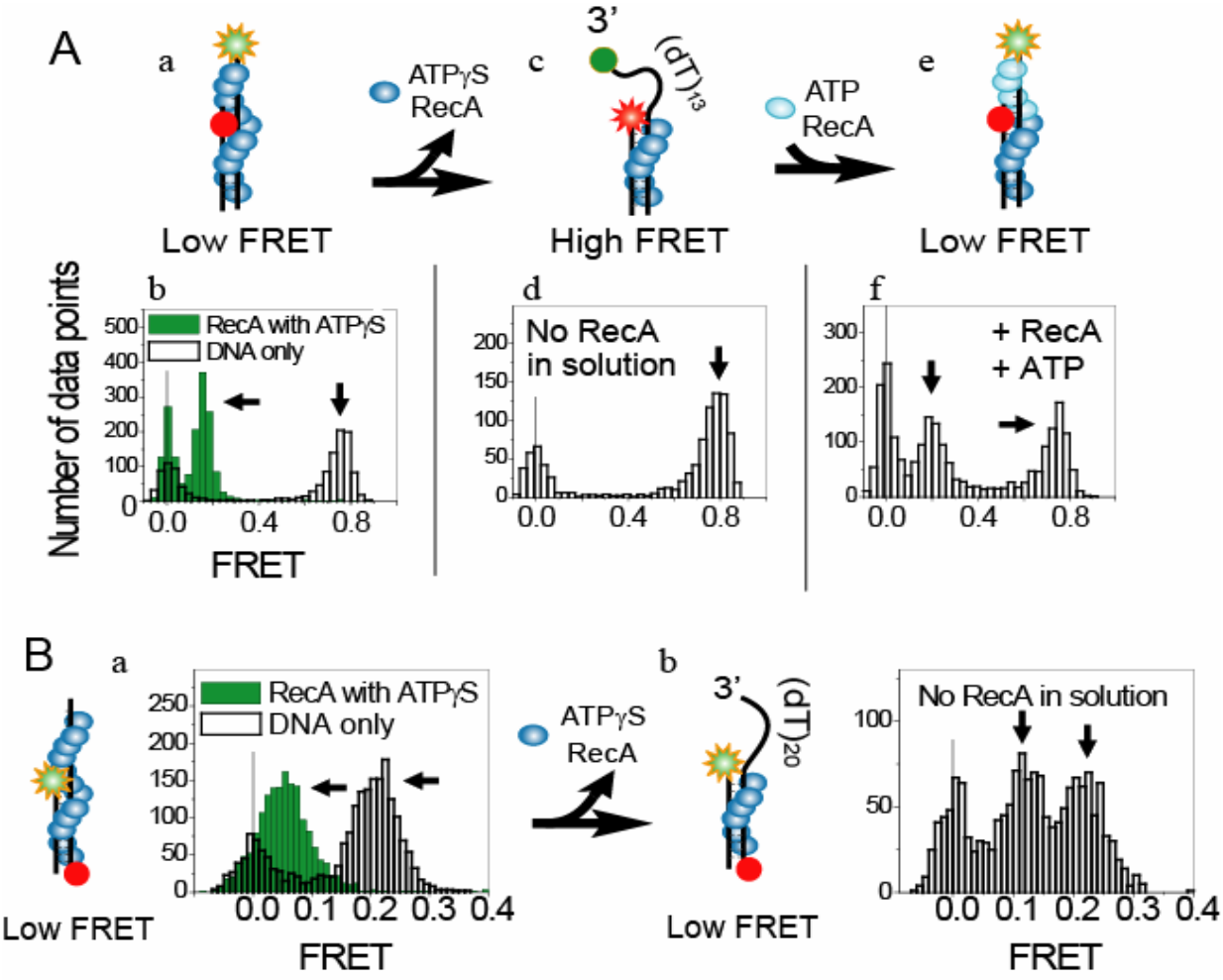


(A) (a) Filament formation on a dsDNA with 3′ $(dT)_{20}$ ssDNA tail. Cy5 is labeled in the middle of a dsDNA instead of at the junction of ss-dsDNA. (b) Change in a FRET histogram when RecA is added with ATPγS indicates a filament formation on the dsDNA (after 15 min incubation).

(B) Protection of dsDNA by <dsDNA|ATPγS> from an endonuclease (a) dsDNA with 3' $(dT)_{13}$ tail was labeled with Alexa647 at the junction. It is incubated with RecA and ATPγS before cleavage by DNase I. (b) The 600nM DNA molecules were incubated with 10 μM RecA and 1 mM ATPγS for 15, 30, 60, 120, and 300 seconds before cleavage (lanes 1-5). The DNA (600 nM) was incubated with 10 μM RecA and 1 mM ATP (lane 7); and with 0, 2, 6, and 10 μM RecA and 1 mM ATPγS, for 10 minutes before cleavage (lanes 8-11). Lane 6 is for a DNA without any RecA and DNase I.

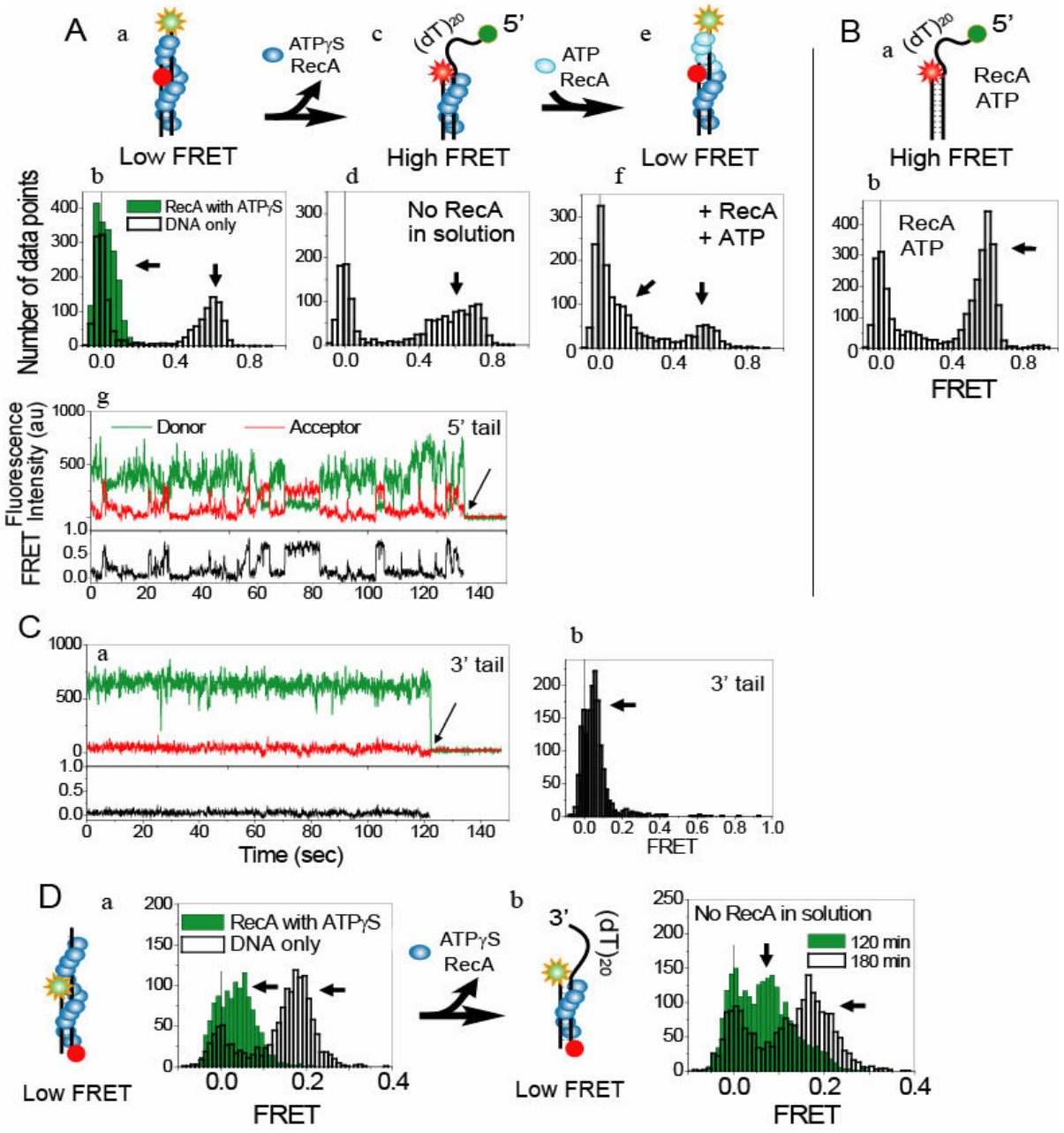
(C) (a) No filament formation on a dsDNA with blunt ends, which is labeled with Cy3 and Cy5. FRET histograms (b) without RecA and (c) with RecA and ATPγS in solution (after 2 hr incubation).

Figure S10. Formation and Disassembly of <dsDNA|ATPγS> at 35°C



(A) The same experiment as Figure 3A but at high temperature (35°C). (a and b) Filament formation on a 3′-$(dT)_{13}$ tail of a partial dsDNA results in low FRET (green, $E \sim 0.2$, after 30 min incubation), while DNA-only shows high FRET (white, E~0.75). (c and d) <ssDNA|ATPγS> disassembles after RecA and ATPγS are removed from solution thus FRET is restored to the DNA-only value (Ab, white) (after 10 min incubation). (e and f) After 10 min incubation in the condition of (Ac), RecA and ATP are added and a stable <ssDNA|ATP> forms on ~50% of the molecules, assisted by <dsDNA|ATPγS> (after <1 min incubation).
(B) The same experiment as Figure 3B but at high temperature (35°C). (a) Filament formation on a dsDNA with $(dT)_{20}$ tail results in E ~0.05 (green, after 30 min incubation) compared to $E \sim 0.2$ of DNA-only (white). (b) About a half of <dsDNA|ATPγS> disassembles during the first 10 min after removal of RecA and ATPγS from solution (after 10 min incubation).

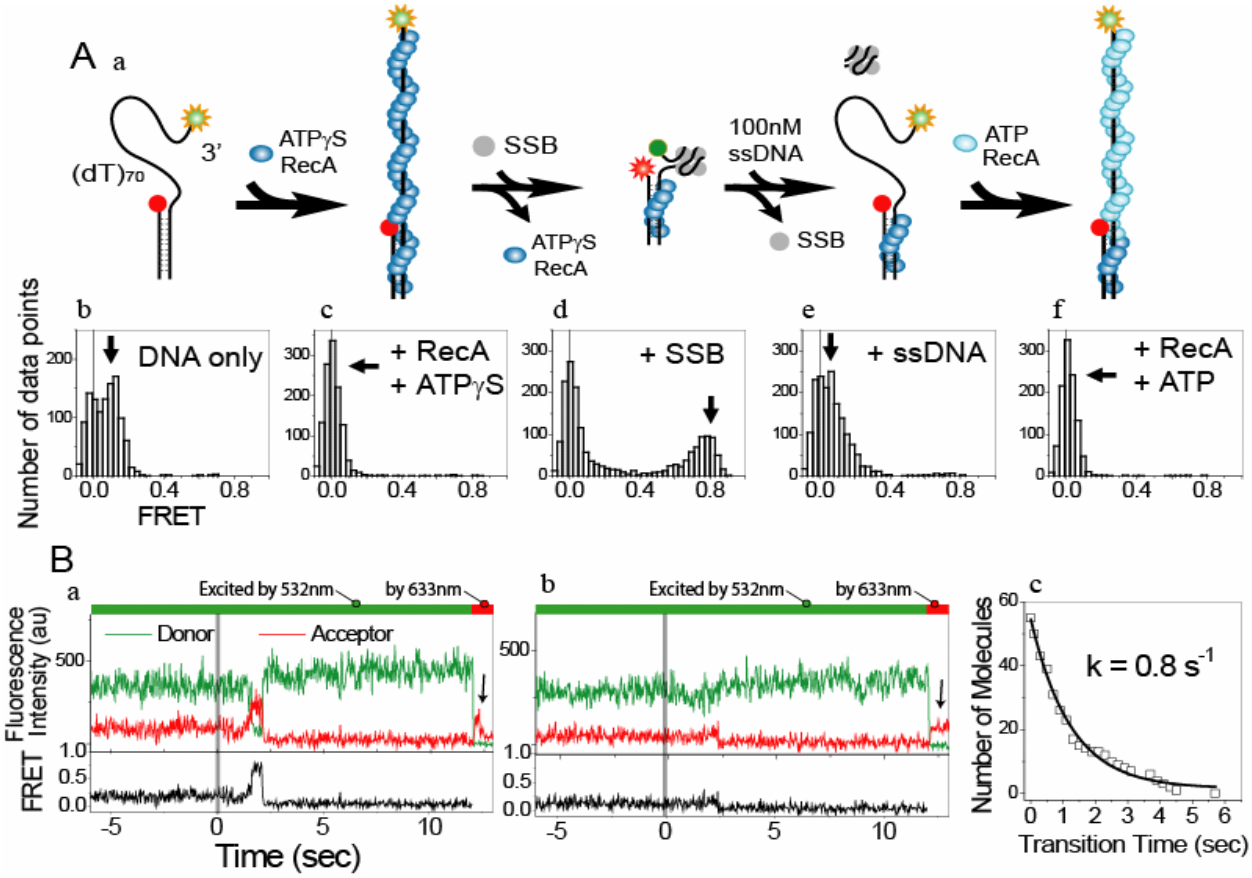
Figure S11. Formation and Disassembly of <dsDNA|ATPγS> Mediated by 5′-Tail ssDNA



(A) (a and b) Filament formation on 5′-$(dT)_{20}$ ssDNA tail of a partial dsDNA results in low FRET (E ~ 0.05, green, after 30 min incubation), while FRET is high with DNA only (E ~ 0.6, white). (c and d) <ssDNA|ATPγS> disassembles in 5 minutes after RecA and ATPγS are removed from solution (after 30 min incubation). (e and f) After 30 min incubation in the condition of (Ac), when RecA and ATP are added, <ssDNA|ATP> forms assisted by <dsDNA|ATPγS> (after <1 min incubation). (g) Time trace (100 ms time resolution) in the condition of (Af). Cy3 photobleached at t ~30 sec (arrow).

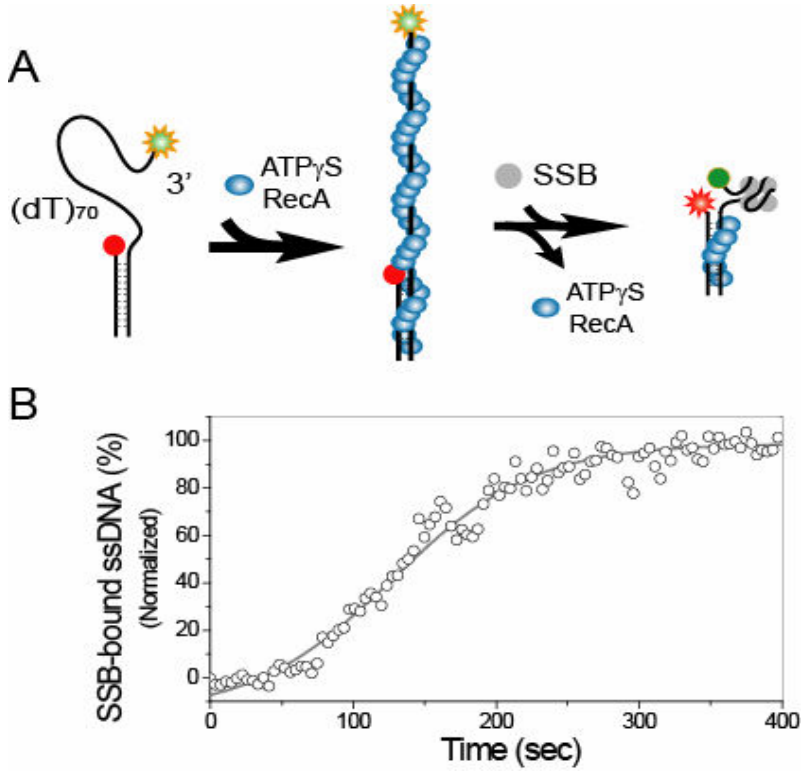
(B) A FRET histogram when RecA and ATP are added, in the absence of <dsDNA|ATPγS> (after 1 min incubation). Longer incubation does not change the shape of the distribution.
(C) (a) Time trace (100 ms time resolution) in the same condition and configuration as (Ag) but with 3′-$(dT)_{19}$ tail. Cy3 photobleached at t ~ 120 s (arrow). (b) A FRET histogram with 3′ $(dT)_{19}$ tail. In contrast to (Af), no intermediate or high FRET states are observed.
(D) (a) Filament formation on the dsDNA with $(dT)_{20}$ tail results in E ~ 0.05 (green, after 30 min incubation) compared to E ~ 0.2 of DNA only (white). (b) It takes more than 2 hr for <dsDNA|ATPγS> to disassemble after RecA and ATPγS are removed from solution.

Figure S12. Formation of <ssDNA|ATP> in the Presence of <dsDNA|ATPγS>



(A) (a) Extension of a RecA filament from <dsDNA|ATPγS> into <ssDNA|ATP> in the absence of DNA bound SSB (b–d) The same procedures as Figures 5Bb–5Bd (e) When 100 nM $(dC)_{80}$ is introduced, SSB is transferred to $(dC)_{80}$ within a minute, leaving ssDNA tail naked but keeping <dsDNA|ATPγS> intact (after 2 min incubation). (f) When RecA and ATP are added in solution, a filament extends rapidly assisted by <dsDNA|ATPγS> (after <1 min incubation).
(B) (a and b) Time traces recorded during the transition from (Ae) to (Af). RecA and ATP are added at t = 0. To distinguish the low FRET ($E \sim 0$) from photobleaching of Cy5, the activity of acceptor was checked by directly exciting acceptor at t > 12 s (arrows). (c) The transition rate is 0.8 (sec^{-1}), determined from 55 molecules.

Figure S13. <ssDNA|ATPγS> Is Removed by SSB



(A) The same procedures as Figures 5Bb–d. <ssDNA|ATPγS> is displaced by SSB when RecA and ATPγS are removed from solution and 10 nM SSB is added. (B) The change in the population of SSB bound ssDNA is calculated from the change in FRET value. Each data point is based on the normalized average FRET value from more than 100 molecules (recording for 2 seconds). Different areas were imaged for individual data points. t = 0 when SSB is introduced and RecA is removed from solution.