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# Cooperative Conformational Transitions Keep RecA Filament Active During ATPase Cycle

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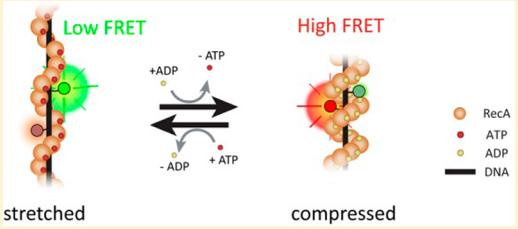
Supporting Information

**ABSTRACT:** The active, stretched conformation of the RecA filament bound to single-stranded DNA is required for homologous recombination. During this process, the RecA filament mediates the homology search and base pair exchange with a complementary sequence. Subsequently, the RecA filament dissociates from DNA upon reaction completion. ATP binding and hydrolysis is critical throughout these processes. Little is known about the timescale, order of conversion between different cofactor bound forms during ATP hydrolysis, and the associated changes in filament conformation. We used single-molecule fluorescence techniques to investigate how ATP hydrolysis is coupled with filament dynamics. For the first time, we observed real-time cooperative structural changes within the RecA filament. This cooperativity between neighboring monomers provides a time window for nucleotide cofactor exchange, which keeps the filament in the active conformation amidst continuous cycles of ATP hydrolysis.




## INTRODUCTION

The RecA protein is involved in recombinational DNA repair in bacteria.[1,2] RecA forms a filament on single-stranded (ss) DNA with a right-handed helical structure.[3] The filament is in a stretched conformation in the ATP bound state (pitch length = 94 Å) and changes to a compressed conformation in the inactive ADP bound state (75 Å).[4,5] The stretched conformation is called the active state and is capable of mediating a strand exchange reaction between ssDNA and a homologous double-stranded (ds) DNA. Given its indispensable role in the maintenance of genomic integrity, RecA and its homologues are found in virtually all species.

The RecA filament needs to be maintained in an active, stretched state during strand-exchange reaction. Upon reaction completion, the filament rapidly disassembles from the final product. ATP binding and hydrolysis are critical for both processes.[6−12] Each ATP molecule is sandwiched by two adjacent RecA monomers within a filament and serves to maintain the filament in the stretched conformation. This network of interactions is lost upon ATP hydrolysis, which may potentially trigger conformational change to the inactive, compressed state.[5,13−16] An intriguing feature of the RecA filament is that only monomers at the filament ends are licensed to dissociate, although all monomers within the filament are continuously engaged in ATP hydrolysis.[10,17,18] Given that the interactions between neighboring RecA monomers are in a constant state of flux, it remains unclear how RecA monomers in the middle of a filament remain bound to DNA (without dissociating) while simultaneously maintaining the stretched conformation which is critical for catalytic activity.

Early biochemical and electron microscopy (EM) studies suggested that the structure of monomers within a filament is highly cooperative, and the stretched and compressed conformations are not directly interconvertible.[6,14,16,19] It was proposed that the conversion between the two conformations requires dissociation of RecA monomers from DNA and rebinding. More recently, direct visualization of RecA filaments assembled on long stretches of DNA in the presence of different cofactors presented evidence for the possible conversion between active and inactive conformations in the absence of any RecA dissociation.[20−23] However, these experiments were confined to measuring the end-to-end distance of the filament consisting of thousands of monomers with heterogeneous cofactor occupancies and were unable to monitor the structural state of individual monomers within the filament. Also, unlike the case of Rad51 where cooperativity between adjacent monomers was proposed to form the repeated dimeric structure of the filament,[24] no such pattern was found in the crystal structure of RecA.[5] Hence, the origin of

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cooperativity between RecA monomers within the filament remains unclear.

Here, we utilized the high spatial and temporal resolution of single-molecule fluorescence resonance energy transfer (smFRET)[25,26] to elucidate how the conformational change of a RecA filament is related to its nucleotide cofactor bound states (Figure 1 of the Supporting Information). We observed that changes between the stretched and compressed forms occur in a cooperative manner such that several adjacent RecA monomers simultaneously change their conformations. We precisely defined the rates of conversions of the RecA filament between different cofactor bound states and showed how cooperativity between RecA monomers keeps the RecA filament in the active, stretched state. We propose a model that this cooperativity provides a time window for nucleotide cofactor exchange which allows for maintaining an active RecA filament without leading to dissociation.

## RESULTS

To understand how RecA monomers in the middle of a filament constantly consume ATP molecules without dissociation,[27] we probed the conformational dynamics of RecA in the middle of the filament. We designed a ssDNA with a donor (Cy3) and an acceptor (Cy5) internally labeled with a 9-nt separation (Figure 1a) to measure structural changes of DNA induced during ATP hydrolysis by RecA monomers in the middle of the filament. As each RecA monomer occupies three bases within a filament, FRET efficiency between the dyes would reflect structural status of three RecA monomers that are interacting with the bases between the dyes. We positioned the pair of fluorophores more than 21 nt away from the DNA ends to avoid FRET changes which might arise from monomer dissociation or binding at the filament ends. In addition, we maintained RecA in solution at all times to avoid complete disassembly of the filament. The FRET efficiency ($E$) distribution of stretched RecA filaments formed with ATP is peaked at E of ~0.4 (Figure 1b, gray boxes). With ADP, the peak shifted to a higher value, $E \sim 0.7$ (Figure 1b, shaded boxes) is clearly distinct from that of bare DNA ($E \sim 0.8$, Figure 1b, empty boxes). Our observation is consistent with recent single molecule studies of RecA and Rad51 in which DNA length changes upon conversion between the stretched and the compressed filaments were observed.[20−23] In the presence of both ATP and ADP, we observed a bimodal distribution at $E \sim 0.4$ and $E \sim 0.7$, and their relative ratio changed with the ADP/ATP ratio (Figure 2 of the Supporting Information). In all cases, we did not observe the bare DNA population at $E \sim 0.8$, consistent with the idea that the filament does not entirely disassemble during cycles of ATP hydrolysis. However, transiently populated states may not be detected from steady-state distributions.

Therefore, we attempted to observe the compression and stretching processes in real time. Initially, a saturating concentration of ATP was present, resulting in a FRET efficiency of approximately 0.4. After a solution carrying ADP was injected at time zero, the average FRET efficiency changed to 0.7 (Figure 1c and Figure 3a of the Supporting Information). More than 70% of single-molecule traces exhibited a single-step transition from $E \sim 0.4$ to $E \sim 0.7$ within our time resolution of 100 ms, indicating that the conformational change of the three monomers bound between the dye pair is cooperative (see below for details). No intermediate states, including the bare ssDNA state at $E \sim 0.8$, were identifiable within our spatial and

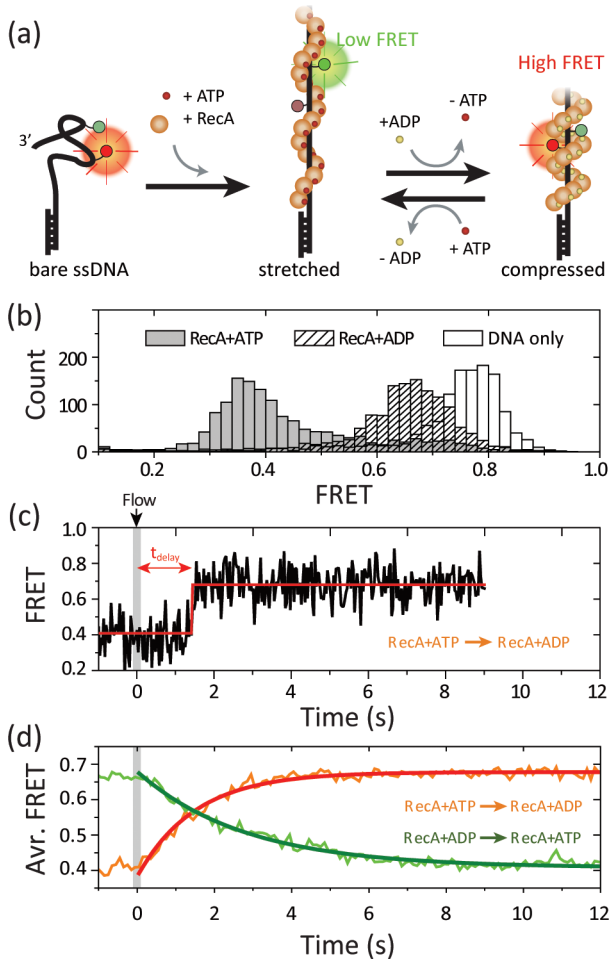


**Figure 1.** Real-time measurements of the filament compression and stretch processes. (a) A model of the RecA filament stretch and compression processes. (b) Single-molecule FRET histograms in the presence of 1 $\mu$M RecA with 1 mM ATP (gray boxes) or 1 mM ADP (shaded boxes). Bare DNA in the absence of RecA is shown as empty boxes. (c) A representative time trace of the RecA filament compression process with its idealized trace estimated by hidden Markov method. At time zero, a buffer carrying RecA and ADP flowed into the channel in which a RecA filament was previously formed with ATP. (d) Averaged FRET time trajectory from the selected single-molecule traces of the compression (orange line, 51 molecules) and stretch (green line, 43 molecules) processes with single exponential fits (red and dark green lines).

time resolution. We interpreted the time delay between the buffer exchange and the abrupt FRET increase as the time required for ATP hydrolysis and subsequent conformational change within the filament. We selected 39 single RecA filaments and obtained an averaged FRET time course (Figure 1d, orange). The averaged trace was well-fitted by a single exponential function with a rate of $k_{compression} = 1.0 \pm 0.3\ s^{-1}$. Because we have exchanged the buffer from the saturating ATP to non-ATP condition, this rate is an upper limit of the compression process of the RecA filament.

The reverse process of filament compression, induced by replacing ADP with ATP, also occurred mostly as a single-step process from $E \sim 0.7$ to $E \sim 0.4$ with a time delay characterized by $k_{stretch} = 0.5 \pm 0.2\ s^{-1}$ (Figure 1d, green and Figure 3b of the Supporting Information), suggesting that the stretching process (conformational change from compressed to stretched state) is also cooperative. The stretching rate of cofactor-free RecA


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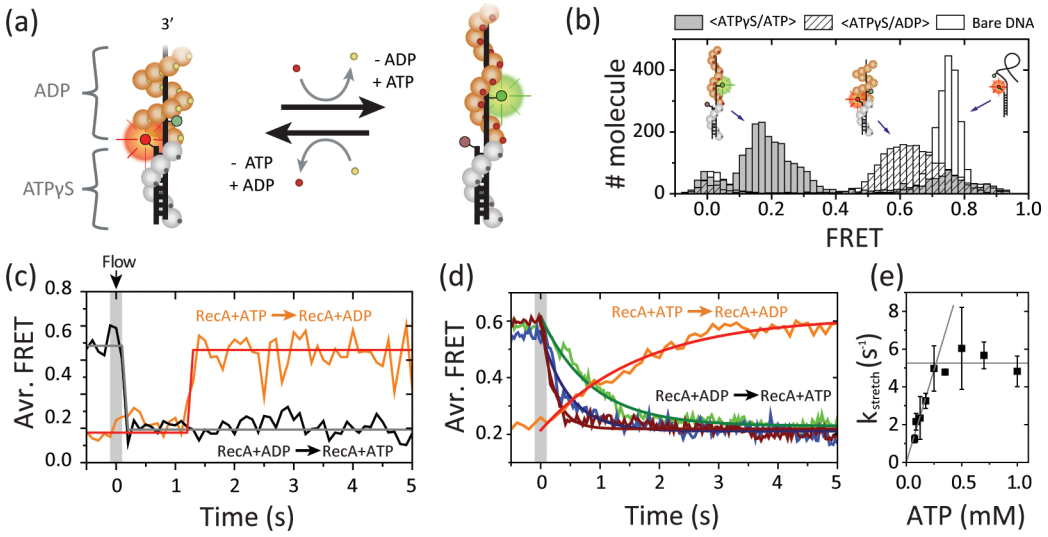


**Figure 2.** Measurement of the filament compression and stretch at the junction of a stretched and compressed filament. (a) A model of a hybrid RecA filament connected (on ssDNA) to a permanently stretched RecA filament (on dsDNA with ATPγS). (b) Single-molecule FRET histograms of the hybrid filaments in the presence of 1 μM RecA with 1 mM ATP (gray boxes) or 1 mM ADP (shaded boxes). Bare DNA observed prior to any filament formation is shown as empty boxes. (c) Representative single-molecule FRET time traces of compression (orange line) and expansion (black line) processes with their idealized traces found by a hidden Markov method. (d) Averaged FRET time trajectories from the selected single-molecule traces undergoing compression (orange) upon exchange of the buffer from 1 mM ATP to 1 mM ADP in the presence of RecA. Average FRET time trajectories for stretch processes upon exchange of the buffer from 1 mM ADP to 0.075 mM (green, 32 molecules), 0.125 mM (blue, 18 molecules), and 1 mM (brown, 44 molecules) ATP. Solid lines are single exponential fits to the data. (e) The stretching rates obtained from the flow of the buffer carrying different ATP concentrations show upper bound at 5 $s^{-1}$ due to the limit of our buffer-exchange speed. The error bars are standard errors from three data sets. Gray lines are guides to the eye.

filaments was 2-fold higher, possibly because a new ATP does not need to wait for the release of a prior ADP molecule that already occupies the ATP binding pocket ($k_{stretch}$ = 1.0 ± 0.2 $s^{-1}$ and Figure 3c of the Supporting Information).

The single step compression and stretching of the filaments may be mediated by cooperative interactions between adjacent monomers. To test the cooperativity hypothesis further, we prepared a RecA filament in which a portion of the filament is maintained in a stretched form and an adjacent portion can be stretched or compressed, depending on the solution condition (Figure 2a and Figure 4a of the Supporting Information). This experimental configuration, based on our previous study,[18] would test whether the stretched portion of the filament can influence the conformation change of the neighboring monomers. A RecA filament assembled in the presence of ATPγS can be extended to the duplex DNA portion of the immobilized DNA substrate (Figure 4a of the Supporting Information). Subsequent removal of free RecA in solution results in RecA dissociation from the single-stranded portion but not from the duplex region. The duplex region with bound RecA/ATPγS filament is more extended compared to the canonical B-form dsDNA, and further changes in the solution conditions do not affect the stretched state.[18] Thus, the RecA/ATPγS filament on dsDNA can now act as a nucleation site for the binding of additional RecA monomers to the adjoining ssDNA.

We then grew the preformed RecA/ATPγS filament on dsDNA to the ssDNA portion by adding RecA and ATP to form a hybrid filament, wherein the ssDNA portion of RecA is ATP-bound while the dsDNA portion is bound by ATPγS (Figure 2a). To monitor the monomers near the junction, we positioned an acceptor at the junction and a donor on the ss-tail with 13 nt separation (Figure 2a). The filaments formed with ATP appeared at $E \sim 0.2$ (Figure 2b, gray boxes). The population at $E \sim 0$ is due to donor-only molecules where the acceptor is missing or inactive. We then flowed 1 mM ADP and observed filament compression to $E \sim 0.6$ (Figure 2b, shaded boxes). The transition was observed mostly as single step (Figure 2c, orange and Figure 4b of the Supporting Information) at a rate of $k_{compression}$ = 0.5 ± 0.2 $s^{-1}$ (Figure 2d, orange). This rate is 2-fold slower than that observed without the stretched neighbors (Figure 1d, orange), showing that the stretched conformation of the preformed filament inhibits the transition to the compressed filament. When we replaced ADP by 1 mM ATP, filament stretching occurred also in a single step (Figure 2c black and Figure 4c of the Supporting Information) at a rate of 5.3 ± 0.6 $s^{-1}$ (Figure 2d, brown), which is more than 10-fold faster than the rate measured in the absence of a stretched RecA filament in the vicinity ($k_{stretch}$ = 0.5 ± 0.2 $s^{-1}$; Figure 1d, green). ATP concentration dependence data showed that the stretching rate increases with increasing ATP concentration and reaches a saturation at around 5 $s^{-1}$, which is likely limited by our solution exchange rate (Figure 2e, and Figure 5 of the Supporting Information). This set of experiments shows that the filament stretching is greatly enhanced when neighboring monomers exist in a stretched conformation.

## DISCUSSION

We made two important observations in the compression and stretching processes. (1) The compression process exhibits a single-step transition (Figure 1c and Figure 3a of the Supporting Information), implying that the three RecA monomers between the dye pair changed their conformation simultaneously. (2) The stretching process is greatly enhanced if a part of a filament is in a stretched conformation (Figure 2d), showing intercommunication between monomers. These findings provide the most direct evidence for the cooperative


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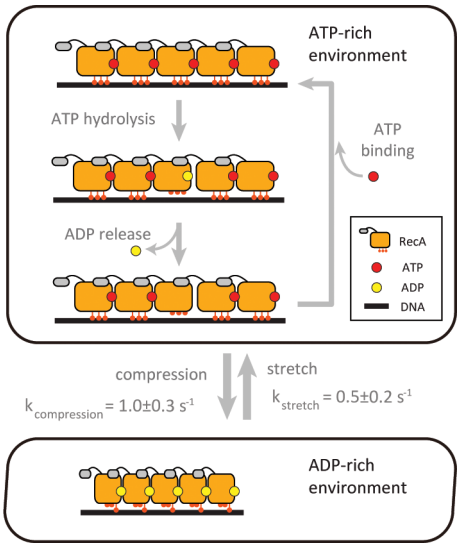


**Figure 3.** Models of the dynamics of RecA filaments. A model of the ATP hydrolysis cycle and conformational change of a RecA filament.

structural transition proposed by previous studies.[14,16,19,24] In addition, our real-time measurement provides the timescale of the cooperative conformational changes.

We summarize our findings in Figure 3. In the middle of a filament, the ATP hydrolysis cycle begins with the binding of an ATP molecule to a RecA monomer. The monomer then hydrolyzes the ATP, resulting in the breakage of the interactions with the DNA and with one of the neighboring monomer. However, the internal RecA monomer is constrained by its two neighboring monomers and therefore does not dissociate after ATP hydrolysis. Instead, the filament maintains its stretched structure due to the cooperative nature of the conformational change, which forces the internal monomer to reload a new ATP molecule quickly instead of being compressed after the ADP is released. Therefore, in an ATP-rich environment, even though a monomer in the middle of a filament continuously hydrolyzes ATP molecules, the structure of the filament is maintained in its active stretched state.

Our single-molecule time traces showed two FRET states of the stretched and compressed states without any clear sign of an intermediate corresponding to a bare ssDNA portion (Figure 1c and Figure 3, panels a and b, of the Supporting Information), indicating that complete disassembly did not occur during filament compression. Consistently, we did not detect such disassembly species at any ADP/ATP ratio within our experimental scheme (Figure 2 of the Supporting Information). While these observations are contradictory to the conclusion drawn by early biochemical and electron microscopic studies that the conversion between the two conformations requires dissociation and rebinding of RecA,[6,14,19] there is growing evidence of direct structural interconversion reported by recent single molecule studies,[20−23] in which repeated stretching and compression processes were observed even after removal of free RecA from the solution. Given the limitations in the spatial and temporal resolution of our single molecule measurements, one possibility is the presence of an intermediate state that is rapid and transient or one that is accompanied only by a subtle structural change. Hence, if disassembly during the transition suggested by the early biochemical and EM studies does occur, it could only involve a brief loss of the contact between RecA and DNA but not a complete dissociation of RecA from its substrate DNA.

We have observed that at least three neighboring RecA monomers change their conformation simultaneously (Figure 1c and Figure 3a of the Supporting Information). An ensuing question is how ATP hydrolysis triggers the cooperative conformational change. Do several adjacent monomers hydrolyze ATP simultaneously? Because RecA dissociates mainly in a monomeric form,[18] it is unlikely that a group of adjacent monomers hydrolyze their ATP molecules simultaneously (distant, nonadjacent monomers that are periodically located may still hydrolyze ATP molecules at the same time as proposed by Cox et al.).[28] One possible explanation is that individual RecA monomers do not change their conformation at the moment of ATP hydrolysis but wait until their neighbors also hydrolyze ATP. When we provide the ADP/ATP mixture above the ratio of ∼2 (equivalently, ADP contents >65%), we observed complete compression of RecA filaments and no stretched filament even though there are free ATP molecules still available (Figure 2 of the Supporting Information). Therefore, compression may occur even before all of the monomers within a filament hydrolyze ATP. Thus, there may exist a threshold for the compression process in terms of the percentage of monomers that hydrolyzed ATP within a filament. This model also provides an explanation of the greatly enhanced stretching process in the presence of neighboring monomers of stretched form (Figure 2d) because such an enhancement would not occur if stretching requires that all the RecA monomers exchange their nucleotide cofactor. Notably, the delayed conformational change in compression process (Figure 1c), owing to its cooperative nature, provides a time window for the monomers to exchange nucleotide cofactors after hydrolysis while the filament maintains its stretched conformation in ATP-rich environment.

Conformational change of RecA filament may occur when ATP/ADP ratio in cytoplasm changes with the cellular condition. For example, ATP/ADP ratio increases during SOS response[29] and this may reinforce the active, stretched form of the RecA filaments during the SOS response in which the RecA-mediated DNA repair mechanism is activated.

## MATERIALS AND METHODS

**Reaction Condition.** RecA was purchased from New England Biolabs and used without further purification. The reaction was performed in a solution consisting of 10 mM $Mg(OAc)_2$, 100 mM NaOAc, and 10 mM TrisOAc at pH 7.5 with 1 mM ATP or ADP (Sigma) unless otherwise specified. Two milligrams per milliliters 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Sigma), 1 mg/mL glucose oxidase (Sigma), 0.8% (w/v) dextrose (Sigma), and 0.04 mg/mL catalase (Sigma) were included in the reaction buffer to stabilize the fluorescence dyes (Cy3 and Cy5, GE Healthcare). All measurements were carried out at room temperature.

**DNA.** Single-stranded DNA molecules were purchased from IDTDNA. During its synthesis, a DNA with the sequence 5′-GCC TCG CTG CCG TCG CCA-biotin-3′ was labeled with biotin. If necessary, Cy5 was used to label the 5′ phosphate of the DNA molecule. For the internal double labeling, Cy3 and Cy5 dyes were randomly labeled at the amine-modified thymine bases on the DNA, which has the sequence 5′-TGG CGA CGG CAG CGA GGC-$(dT)_{30}$-T*-$(dT)_8$-T*-$(dT)_{22}$-3′, where T* denotes the amine-modified dT. For the ATPγS/ATP hybrid filament structure, we used 5′-TGG CGA CGG CAG CGA GGC-$(dT)_{13}$-T*-$(dT)_{46}$-3′. The two ssDNAs were hybridized to obtain a partial duplex DNA with a 3′ single-strand tail and immobilized via a streptavidin−biotin linker on a


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polyethylene glycol-coated quartz surface to avoid the nonspecific adsorption of RecA.

**Single-Molecule FRET Assay.** The experimental details on the smFRET assay are described in our previous work.[18] The fluorescently labeled DNA was immobilized on the quartz slide surface and illuminated by a 532 nm laser (CrystaLaser) through total internal reflection to reduce the background noise (Figure 1 of the Supporting Information). The fluorescence signals from the donor and acceptor were collected with an objective lens (Olympus, NA 1.2 water immersion) and separated spectrally with a dichroic mirror before being imaged on an electron multiplying charge-coupled device (EMCCD) with 100 ms exposure. The fluorescence intensity time traces were extracted from the recorded movie, and the FRET efficiency was calculated using home-built software. Given that our primary goal is to determine relative changes in FRET values which are indicative of transitions between different monomer/cofactor occupancy states, we did not determine changes in gamma factor as a function of time or due to changes in local protein environment[30] but applied a single gamma value ($\gamma = 1$) for all of the FRET pairs. The most plausible FRET time trajectories were estimated using the hidden Markov method (HMM), with which the optimal number of discrete states, the FRET value of each state, and the transition rates between the states were determined out of the noisy time trace.[31] FRET histograms were built from thousands of single molecules for which the FRET values were determined by averaging the first 10 data points of the traces.

**Flow Experiment.** In the flow experiments, we first incubated the immobilized DNA with 1 $\mu$M RecA and 1 mM of ATP for 1 min. We then flowed 1 $\mu$M RecA with the indicated nucleotide cofactor while observing the FRET efficiency in real time. We prepared compressed filaments, ADP or nucleotides free, by first forming stretched filaments in the presence of ATP and then replacing ATP by ADP or no nucleotide solution via solution exchange. For the measurements of filament stretching and compression at the junction of the stretched and compressed filaments, we first incubated the DNA with RecA and ATP$\gamma$S to form a stable filament in the double-stranded region. The removal of RecA and ATP$\gamma$S and the subsequent thorough washing with RecA-free buffer resulted in filament dissociation from the single-stranded region while keeping the filament that was bound to the double-strand region intact.[18] Because the preformed RecA filament in the double-stranded region provides a stable binding platform, the introduction of RecA even at a very low concentration (~20 nM) restored the RecA filament on the single-strand region. We then flowed 1 $\mu$M RecA solution with 1 mM ADP to observe the change in the FRET. We used a higher time resolution of 30 ms to observe the rapid change in FRET accompanying the stretching process at the junction between the stretched and compressed filaments.

## ■ ASSOCIATED CONTENT

### Supporting Information

Schematics of experimental setup, representative single molecule time traces, and other control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

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