RESEARCH ARTICLE

Figures and figure supplements

A new insight into RecA filament regulation by RecX from the analysis of conformation-specific interactions

Aleksandr Alekseev *et al*

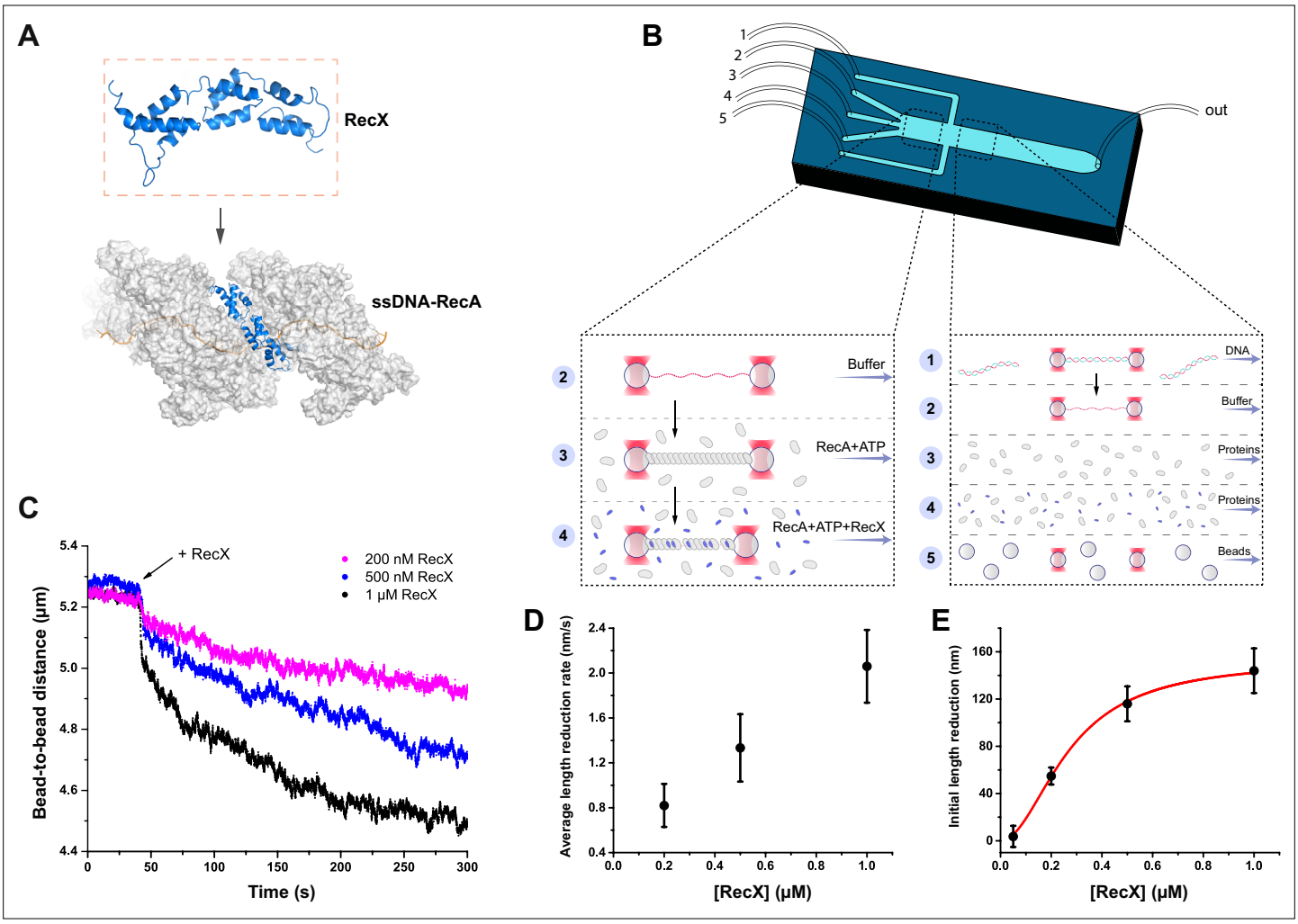

Figure 1. The study of the RecX effect on the RecA-ssDNA filaments. (**A**) A schematic of RecX binding along the groove of the active RecA-ssDNA. Atomic structure model for RecA::RecX::ssDNA is adopted from ***Shvetsov et al., 2014***. (**B**) A schematic of a five-channel microfluidic flow cell (Lumicks). Dash line highlights two working regions. The three-channel region was used to study the effect of RecX on the RecA-ssDNA filament. In the five-channel region, the beads trapping, DNA tether formation, and generation of ssDNA by force-induced melting were performed. (**C**) The change in the length of RecA-ssDNA filament upon transition from the channel containing 1 μM RecA and 1 mM ATP to the channel containing 1 μM RecA, 1 mM ATP, and various concentrations of RecX. During incubation, a constant tension of 3 pN was applied to the tether. (**D**) The impact of RecX concentration on the average rate of reduction in the RecA-ssDNA filament length over 250 s after initial steep decrease. (**E**) The dependence of the RecX induced initial sharp decrease in RecA-ssDNA filament on the RecX concentration. Solid curve - fit of experimental data with Hill equation with a Hill coefficient of 2.0±0.3. Each data point in (**D**) and (**E**) is a mean value of at least three measurements, bars represent SD.

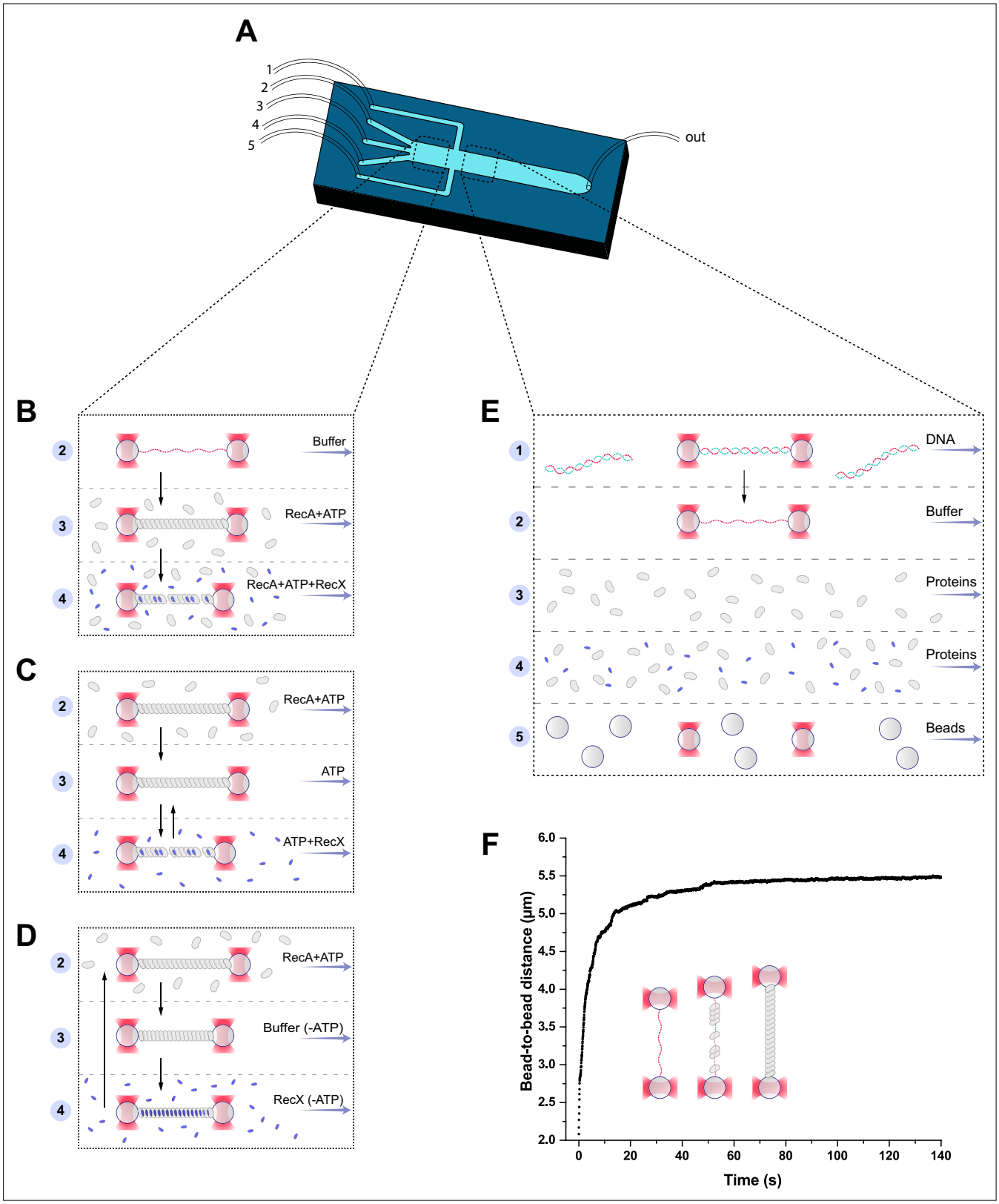

Figure 1—figure supplement 1. Single-molecule assay. Single-molecule manipulations were performed within a five-channel microfluidic flow chip (**A**). Two working regions are highlighted with a dash line. The three-channel region (left) was used to study the effect of RecX on the RecA-ssDNA filament (**B–D**). In the five-channel region, the beads trapping, DNA tether formation, and generation of ssDNA by force-induced melting were performed (**E**). The RecA-ssDNA filaments were assembled by applying a stretching force of 12 pN to the ssDNA molecule in the channel containing 1 μM RecA and 1 mM ATP. Binding of RecA to ssDNA was followed by an increase in the end-to-end distance (**F**).

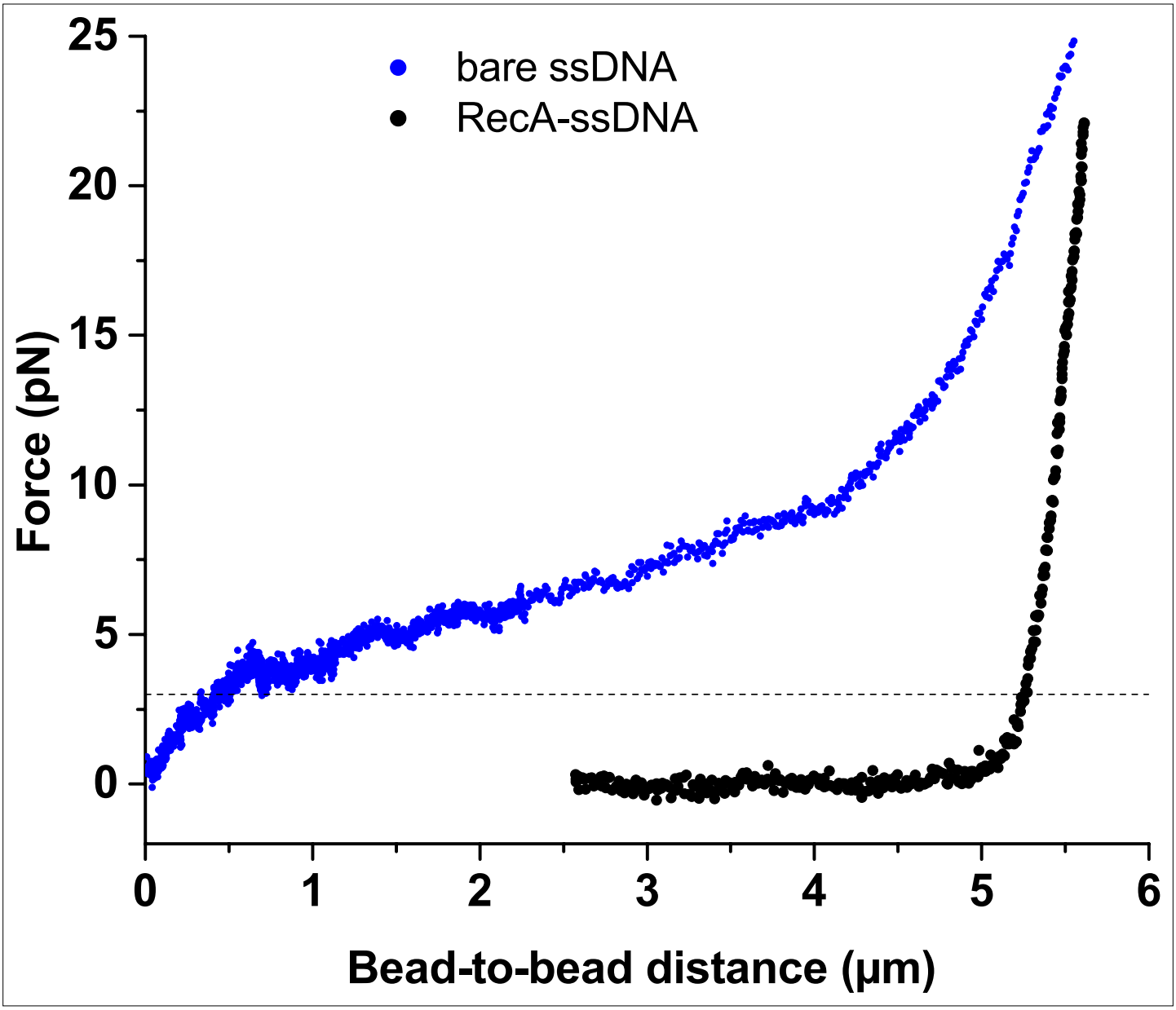

Figure 1—figure supplement 2. The comparison of force-extension behavior of bare ssDNA (blue) and the ATP-bound RecA-ssDNA filament (black). Adopted from ***Alekseev et al., 2020b***. The dash line indicates 3 pN tension.

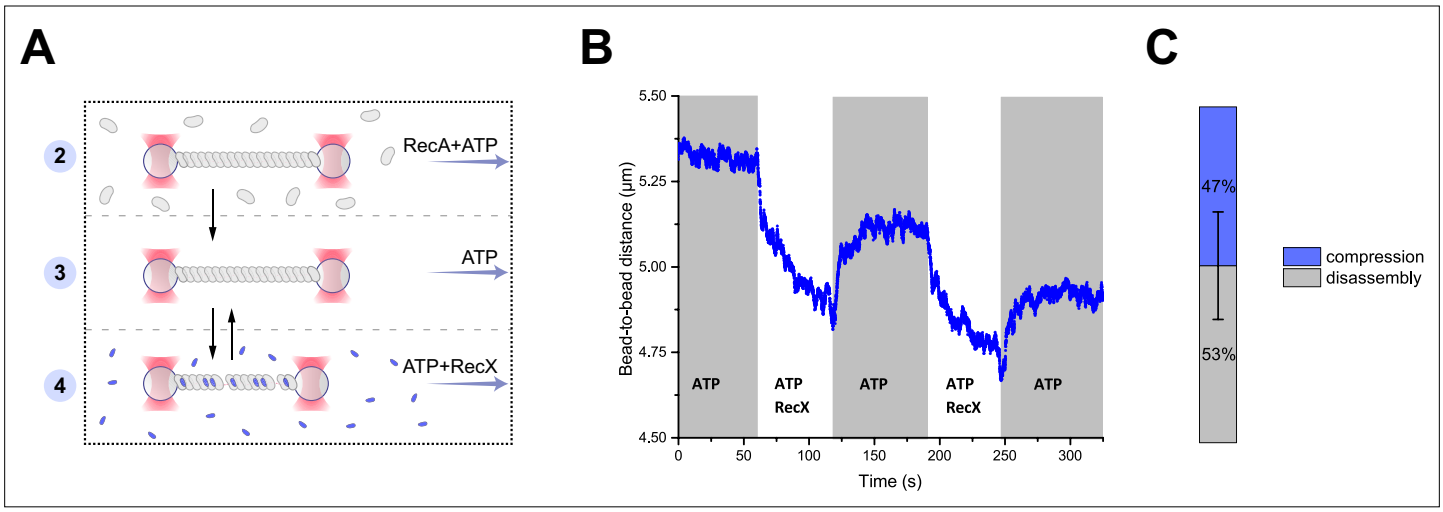

Figure 2. RecX-induced reversible changes in the RecA-ssDNA filament structure. (**A**) A schematic of the experiment revealing that RecX is able to induce reversible structural changes in the RecA-ssDNA filaments. (**B**) RecX induces reversible changes in the RecA-ssDNA filament structure in the presence of ATP. (**C**) A comparison of the reversible (compression) and the irreversible (disassembly) reduction in RecA-ssDNA filament length. Stacked histogram represents multiple measurements for six different molecules. Bars represent SD.

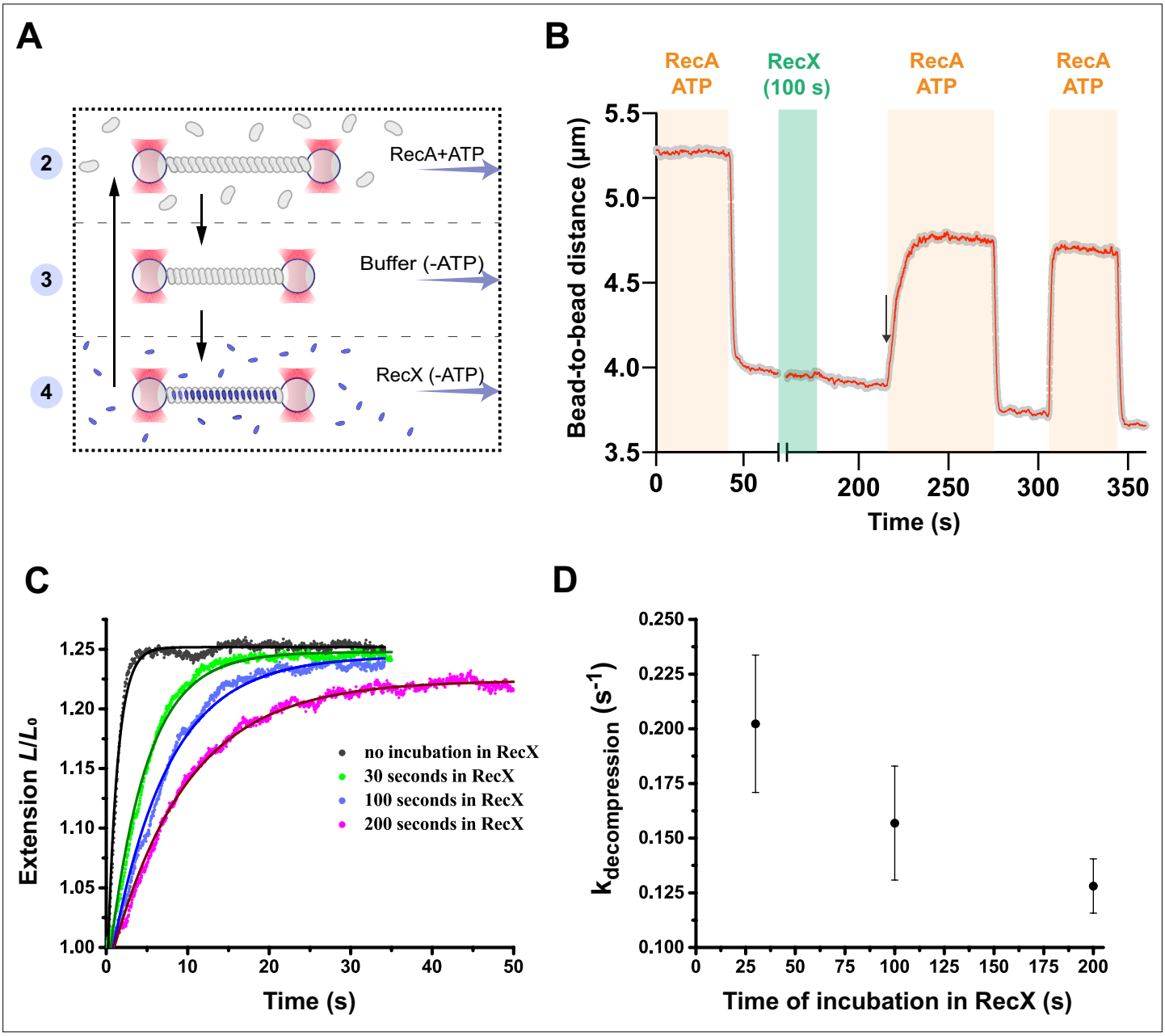

Figure 3. RecX affects the conformational transition of RecA-ssDNA filament from the inactive state to the active state. (**A**) A schematic of the experiment revealing that RecX binds inactive RecA-ssDNA filaments. (**B**) The change of the RecA-ssDNA filament length upon conformational transitions between *apo* and ATP-bound states. Incubation of *apo* RecA-ssDNA filament with 500 nM RecX (green area) leads to a slowdown of the subsequent decompression of the RecA-ssDNA filament (black arrow points the beginning of the slowed down decompression). A constant tension of 3 pN was applied to the tether during incubation and transitions. (**C**) Relative extension of the RecA-ssDNA filament in the course of decompression after incubation of inactive RecA-ssDNA filament with 500 nM RecX for 30, 100, and 200 s. (**D**) Corresponding rate constants of the decompression obtained by exponential fitting (solid line in (**C**)) of the elongation profiles. Each point is a mean of at least six measurements. Bars represent SD.

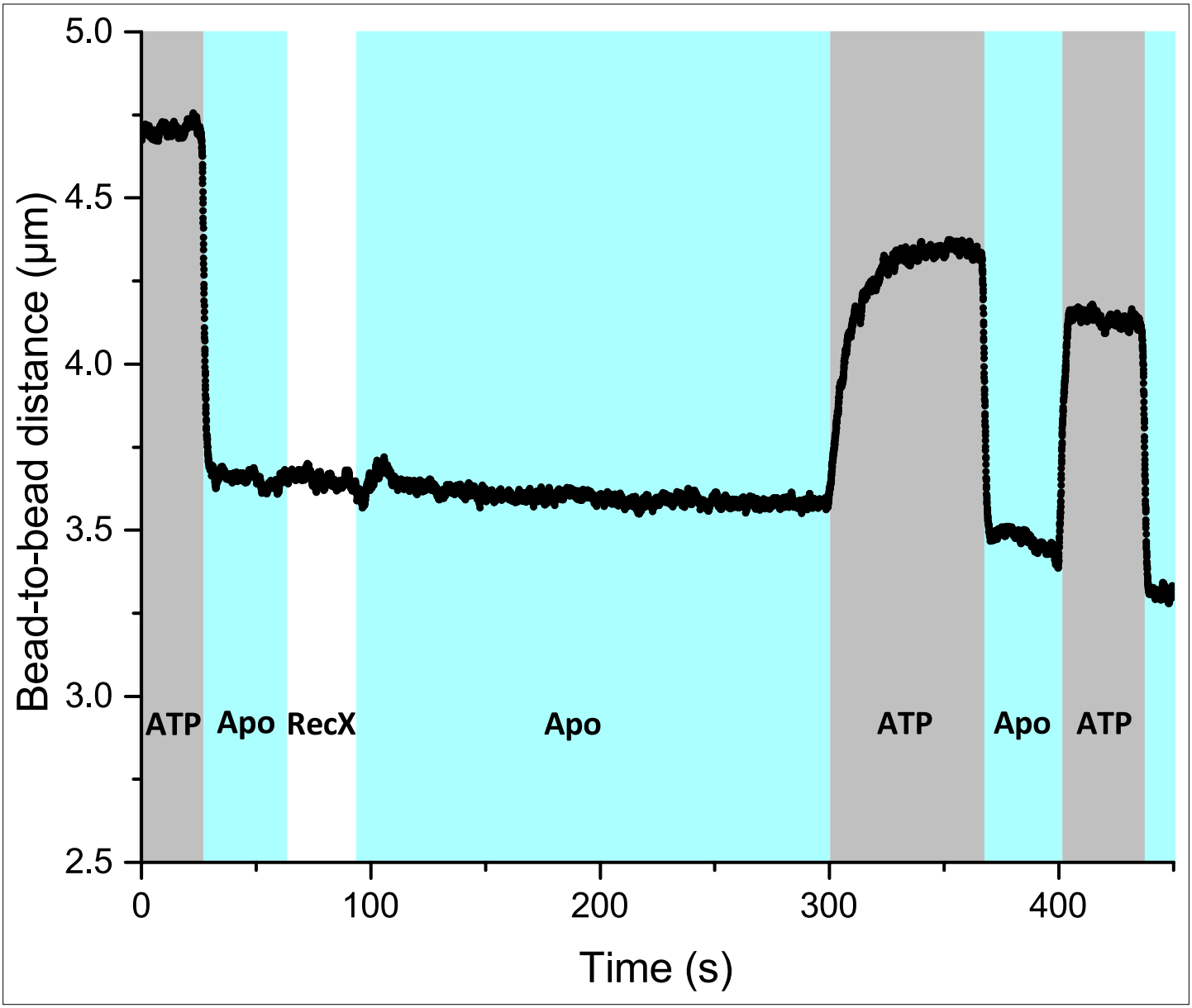

Figure 3—figure supplement 1. The effect of the slowed down decompression retains when RecA-ssDNA filament is incubated in the RecX-free buffer after short incubation with RecX. ATP-containing channel was also supplemented with free RecA.

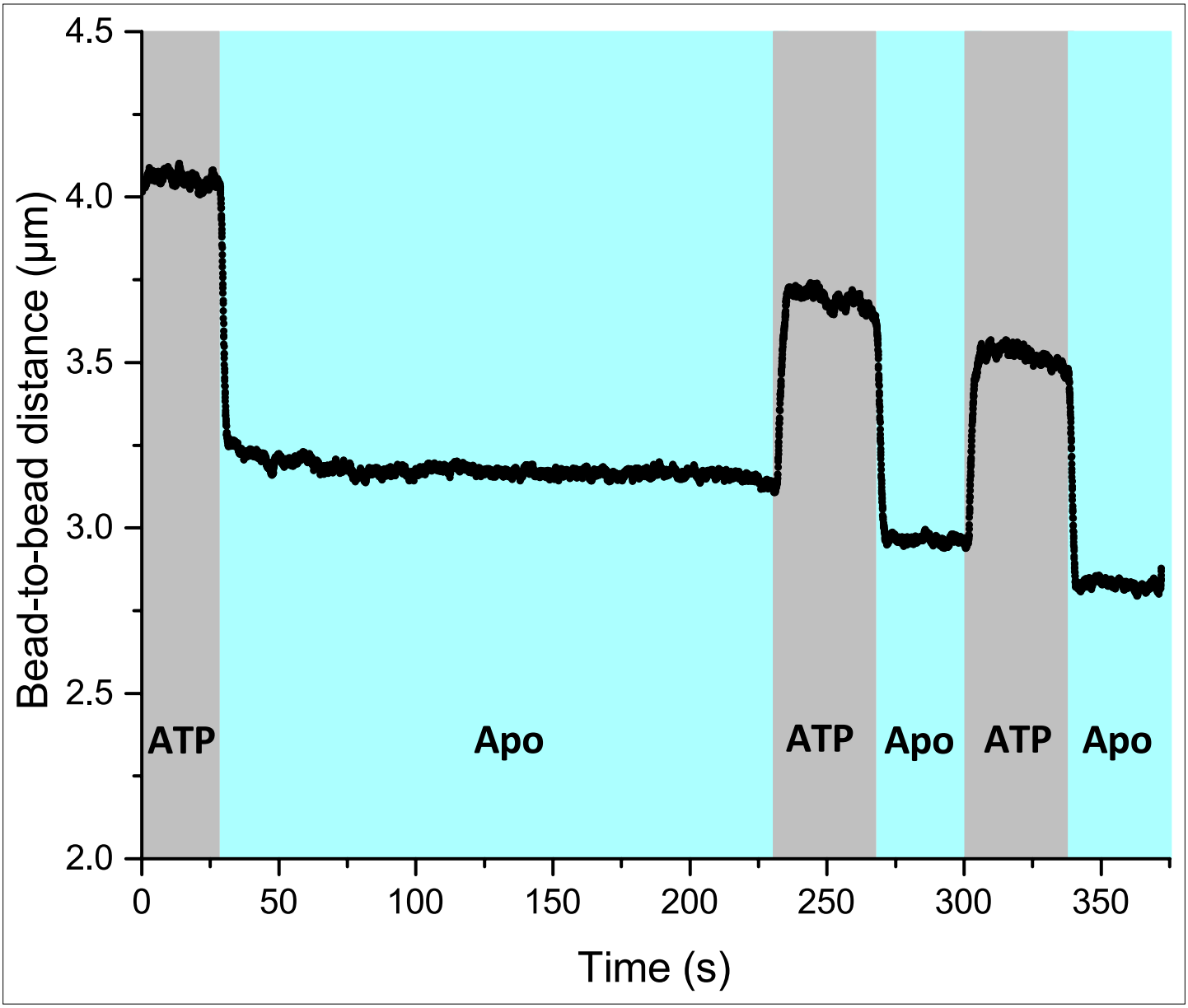

Figure 3—figure supplement 2. The effect of the slowed down decompression is independent of incubation time of the RecA-ssDNA filament in the *apo* channel in the absence of RecX. ATP-containing channel was also supplemented with free RecA.

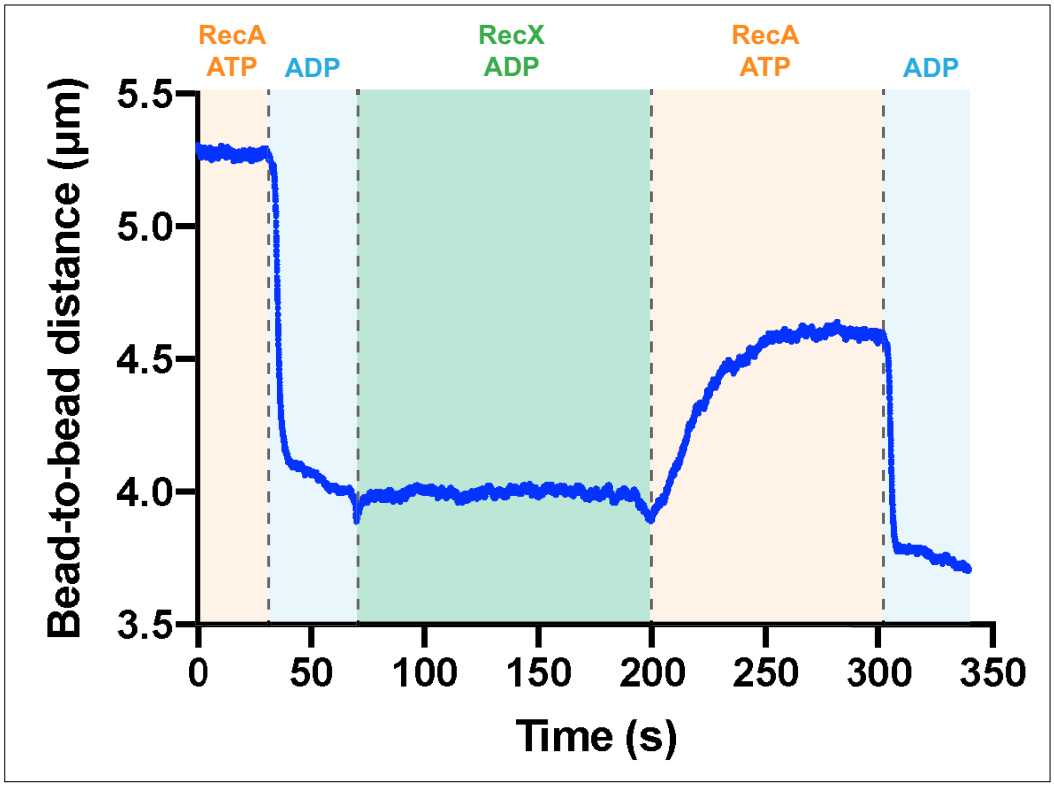

Figure 3—figure supplement 3. Incubation of ADP-bound form of the RecA-ssDNA filament with RecX results in the slowdown of the following decompression.

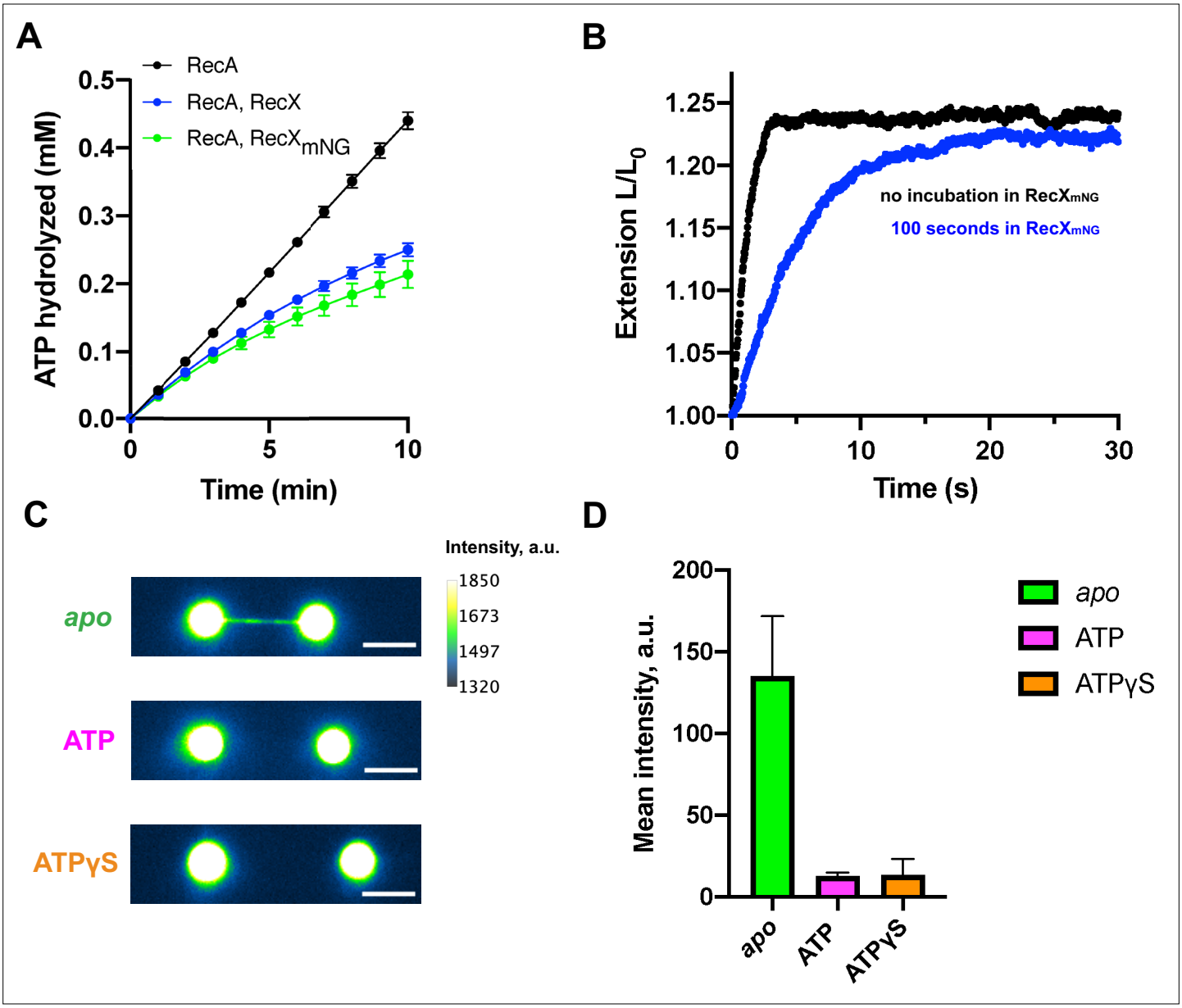

Figure 4. Fluorescent visualization reveals that RecX dissociates from the ATP-bound state of the RecA-ssDNA. (**A**) Inhibition of RecA ATPase activity by wild-type RecX (blue) and fluorescent mNeonGreen-RecX ($RecX_{mNG}$) (green). ATP hydrolysis by RecA in the absence of RecX is shown in black. Each data point represents the average of three independent experiments (error bars – SD). (**B**) Relative extension of the RecA-ssDNA filament in the course of *apo*-ATP transition without incubation in $RecX_{mNG}$ (black curve) and after incubation of *apo* RecA-ssDNA filament with 500 nM $RecX_{mNG}$ for 100 s (blue curve). (**C**) Fluorescent images of: RecA-ssDNA filament in *apo* (top) and ATP-bound state (middle) after incubation with 1 μM $RecX_{mNG}$ for 30 s; RecA-ssDNA filament assembled in the presence of ATPgS (bottom) after incubation with 1 μM $RecX_{mNG}$ for 30 s. Scale bar is 5 μm. (**D**) Comparison of the average intensity of the tether after incubation with $RecX_{mNG}$ for *apo* (N=6 molecules), ATP-bound RecA-ssDNA filament (N=3 molecules), and the filament assembled in the presence of ATPgS (N=6 molecules) (consistently with (**B**)). Data are representative of three independent experiments, and values are expressed in mean ± SD.

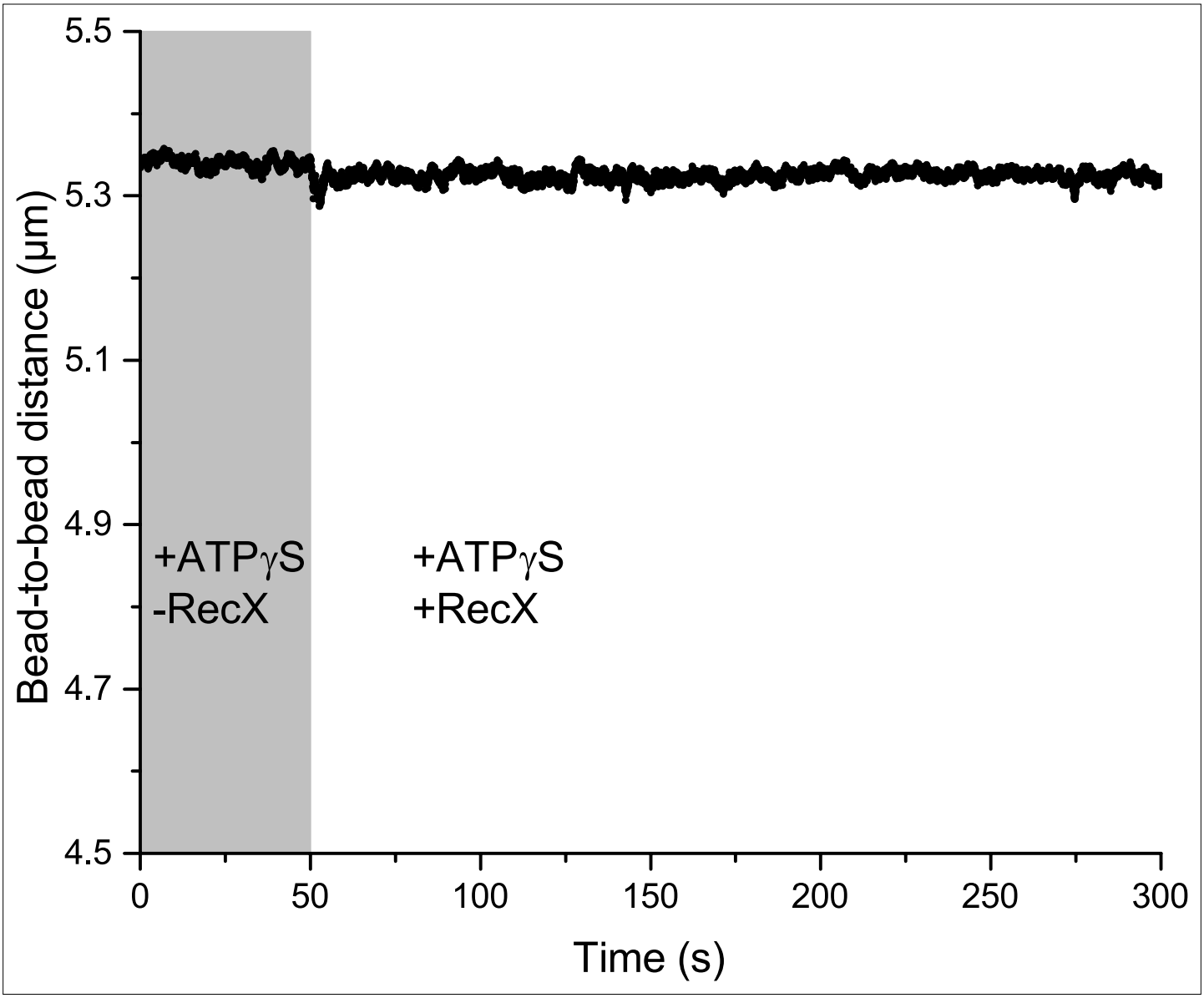

Figure 4—figure supplement 1. The effect of 1 μM RecX on the dynamics of RecA-ssDNA filament formed in the presence of 0.5 mM ATPγS.

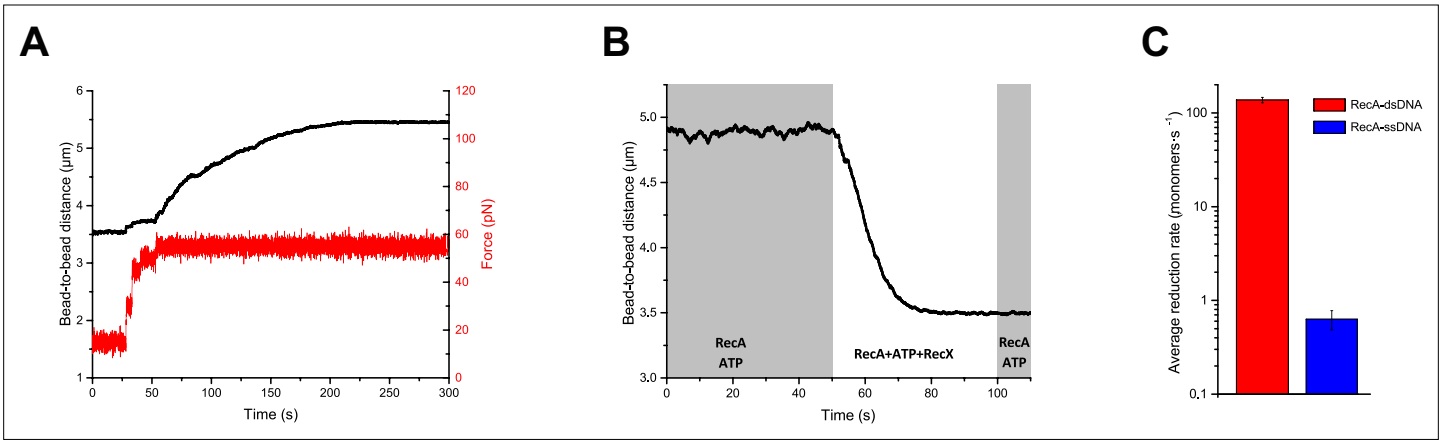

Figure 5. RecX effectively promotes disassembly of RecA-dsDNA filaments. (**A**) The assembly of the RecA-dsDNA filament. (**B**) The disassembly of the RecA-dsDNA filament in the presence of 200 nM RecX. (**C**) The comparison of the average length reduction of RecA-dsDNA (N=4) and RecA-ssDNA (N=4) filament induced by 200 nM RecX. The data for RecA-ssDNA is consistent with ***Figure 1D***. Data are representative of at least three independent experiments, and values are expressed in mean ± SD.

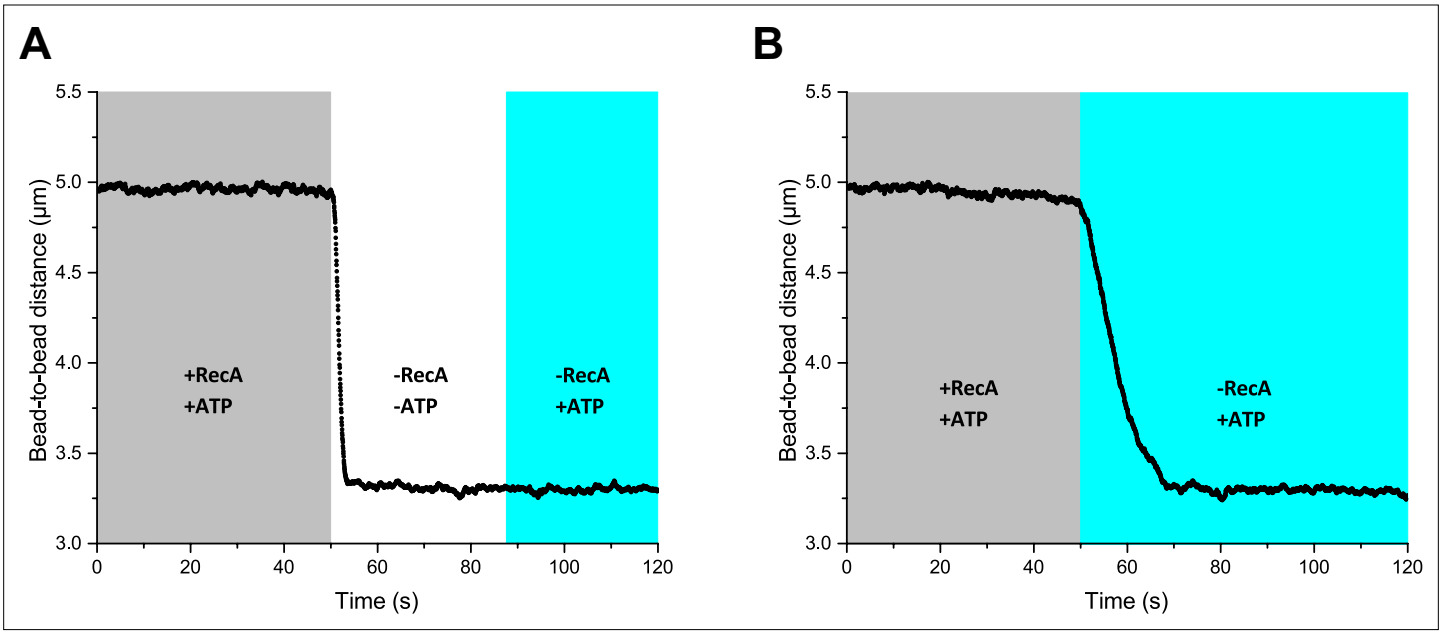

Figure 5—figure supplement 1. RecA-dsDNA filament is stable only in the presence of both free RecA and ATP. (**A**) ATP elimination leads to rapid RecA-dsDNA filament disassembly. (**B**) The elimination of free RecA promotes RecA-dsDNA filament disassembly.

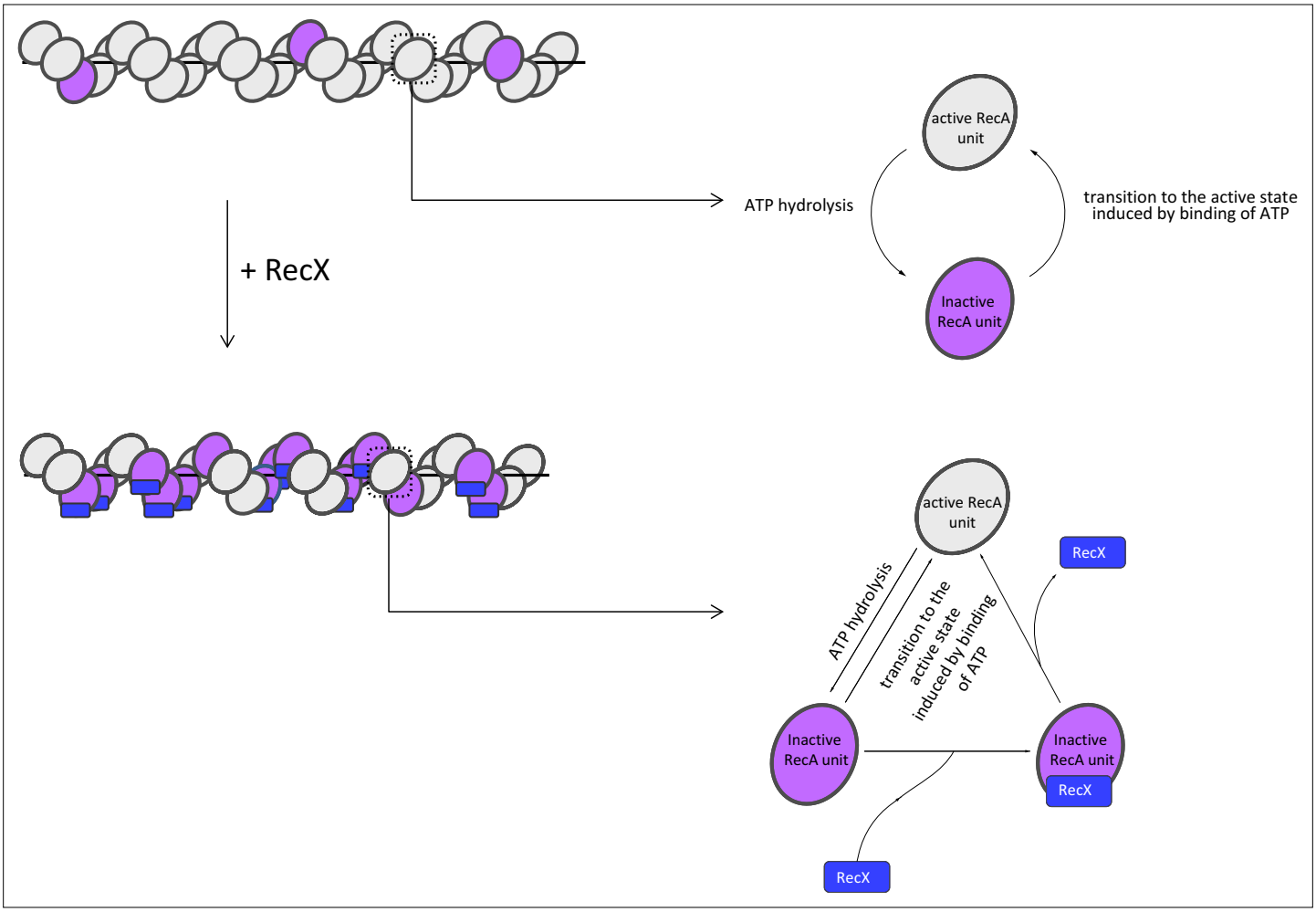

Figure 6. Model of RecX interaction with RecA-ssDNA filaments under conditions of continuous ATP hydrolysis. In the presence of ATP, RecX binds inactive patches within RecA-ssDNA filaments and hampers the transition to the active state (see text for details).