



Figures and figure supplements

Synergy between RecBCD subunits is essential for efficient DNA unwinding

Rani Zananiri et al

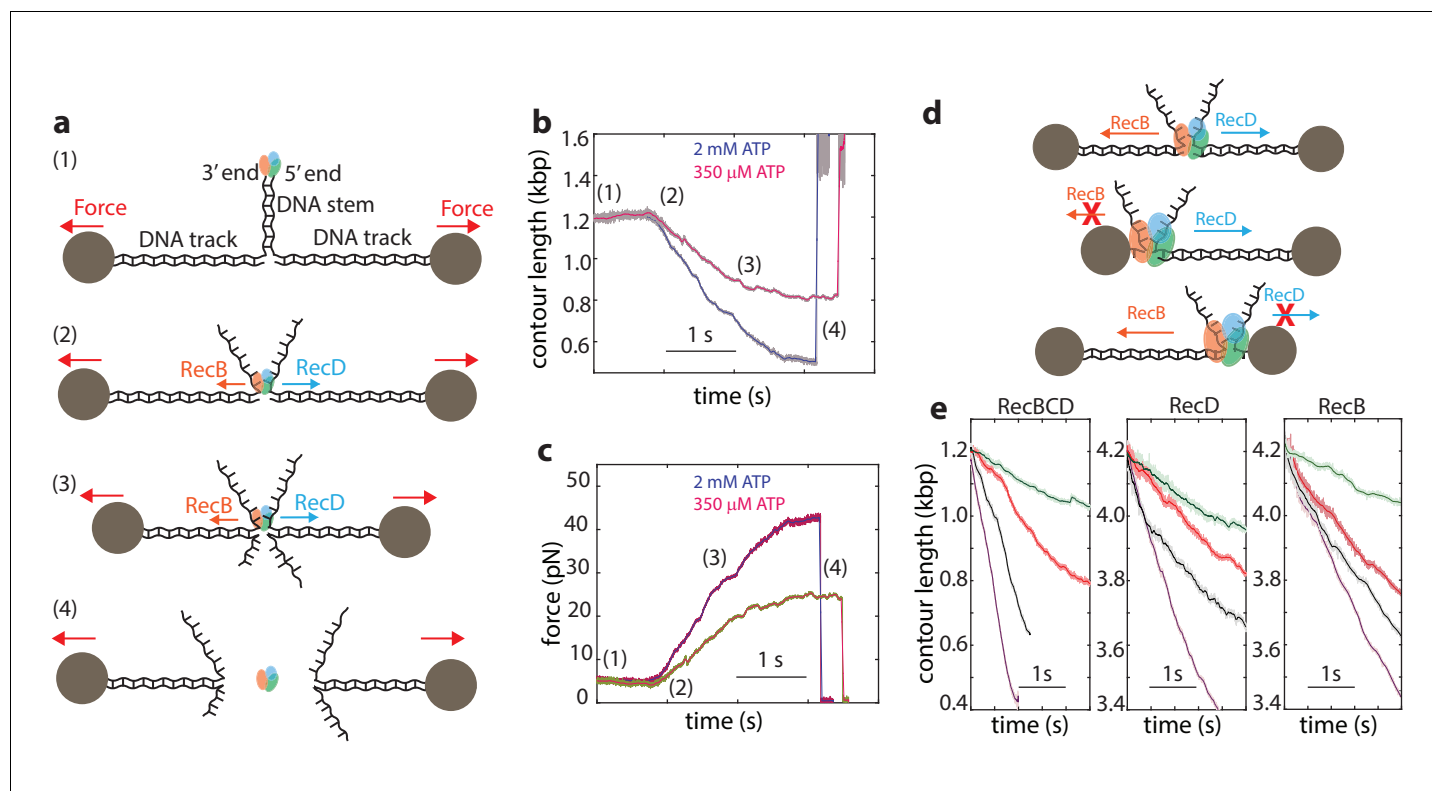


Figure 1. Monitoring RecBCD and its individual subunits 'in complex'. (a) Schematic representation of the experimental optical tweezers setup. From top to bottom: RecBCD binds to, and translocates on a DNA stem connected to optically trapped beads through DNA 'tracks'. Upon reaching the fork, the helicase subunits translocate in different directions due to their opposing polarities, shortening the tether length and increasing the tension on it. The force increases up to a point where RecBCD dissociates from the construct. (b) Two representative contour-time traces, for [ATP] = 2 mM (blue) and [ATP] = 350 μM (purple). Raw data is shown in grey, filtered data ($f_c = 250$ Hz) is shown in blue and purple. (c) The corresponding force-time trace. Raw data is shown in grey; filtered data is shown in blue. (d) Symmetric molecular construct (top, 600 bp and 600 bp) to probe RecBCD and asymmetric constructs (middle, 35 nt and 4200 bp, and bottom, 4200 bp and 35 nt) to probe RecB and RecD, respectively. (e) Representative traces probing both translocases (left), RecD (middle) and RecB (right), at different ATP concentrations (purple, 2 mM; gray, 1 mM; red, 100 μM; green, 20 μM). Raw data is shown in light colors, filtered data ($f_c = 250$ Hz) is shown in dark colors.

DOI: <https://doi.org/10.7554/eLife.40836.002>

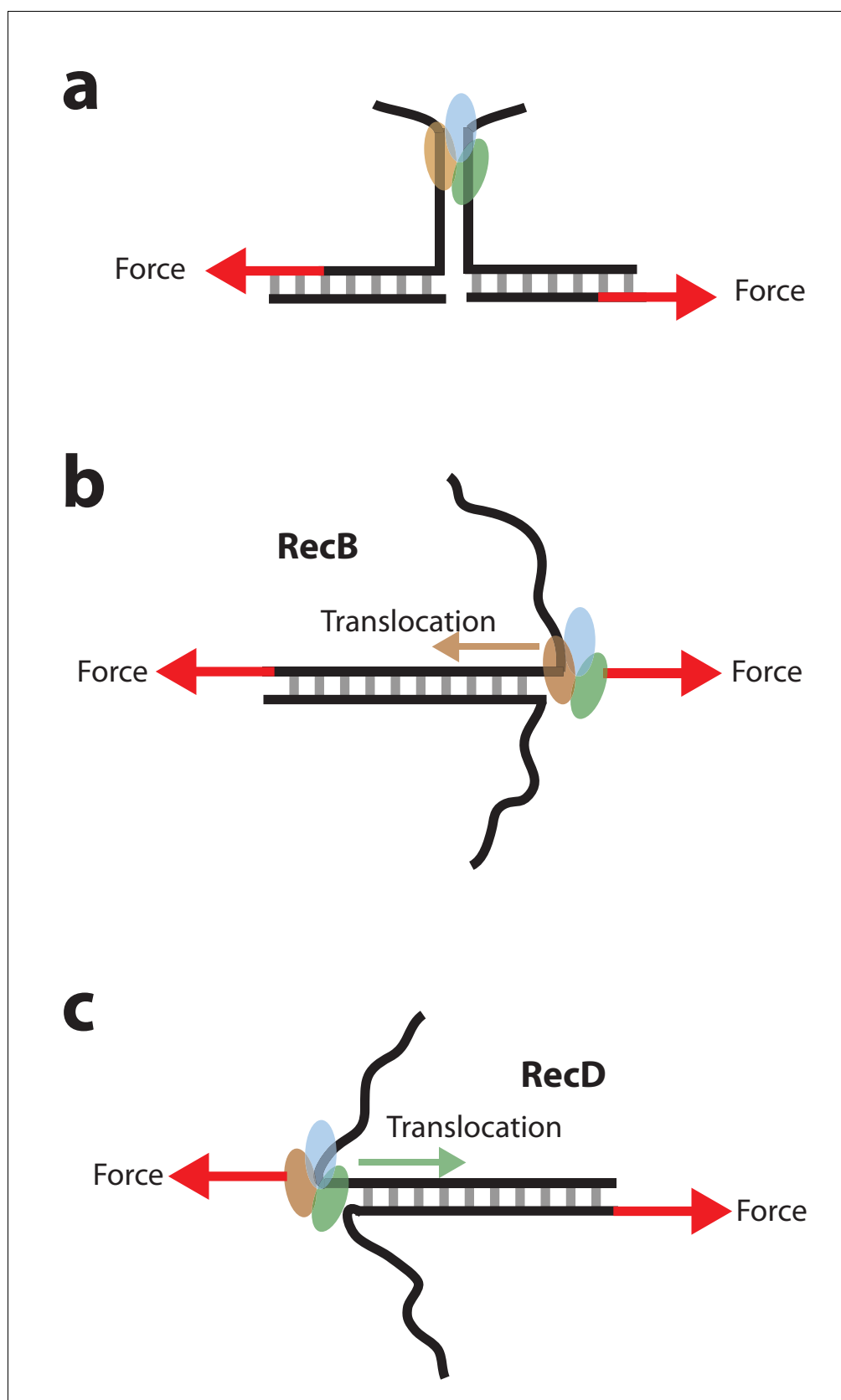


Figure 1—figure supplement 1. Schematic description of the force effect. (a) During unwinding of the 'stem', the force acts on the DNA fork, not on RecBCD, and is below the force required to 'unzip' the DNA. (b–c) As RecBCD finishes unwinding the stem and each subunit engages its track, an

Figure 1—figure supplement 1 continued on next page

Figure 1—figure supplement 1 continued

effective force acts pulling the DNA on one side and each subunit on the other, in the direction opposite to its translocation. This opposing force functions as an inhibitor of the translocation step.

DOI: <https://doi.org/10.7554/eLife.40836.003>

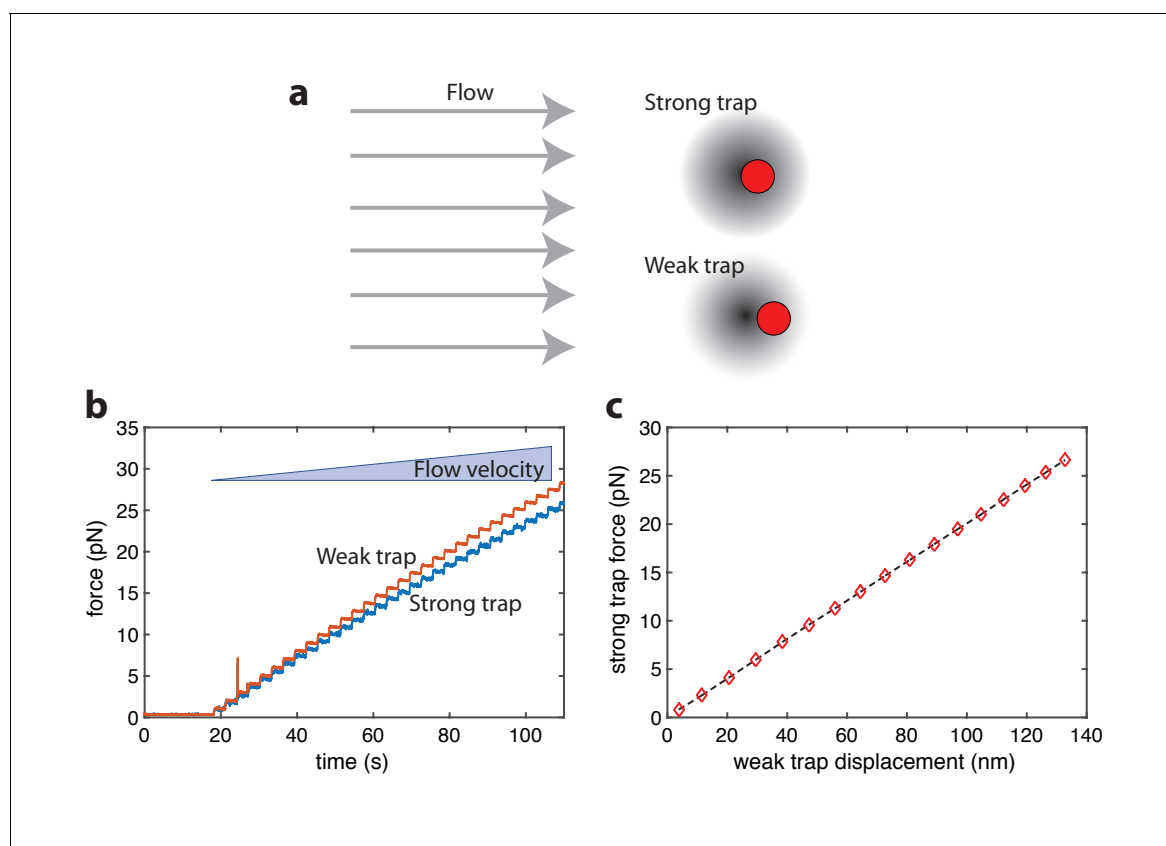


Figure 1—figure supplement 2. Linearity of the optical traps at large forces. (a) To verify that all force measurements are in the traps' linear regime, two similar beads are simultaneously trapped in a weak ($k \sim 0.2$ pN/nm) and strong ($k \sim 0.75$ pN/nm) trap, respectively. A flowing buffer creates a Stoke's flow on the trapped beads, and thus a displacement that depends on the traps' stiffness. Since the displacement of the bead in the stronger trap is small (<35 nm) the force measurement is assumed to be in the linear regime, and enables to check the linearity of the weak trap, where the bead's displacement is larger. (b) Calculated forces, based on the beads' displacement and the traps' calibrated stiffness. The difference in slope stems from the variability ($\sim 10\%$) in the size of the beads. (c) The force, measured using the strongly trap bead, remains linear (within 1%) with the displacement of the weakly trapped bead up to the measured displacement of ~ 140 nm.

DOI: <https://doi.org/10.7554/eLife.40836.004>

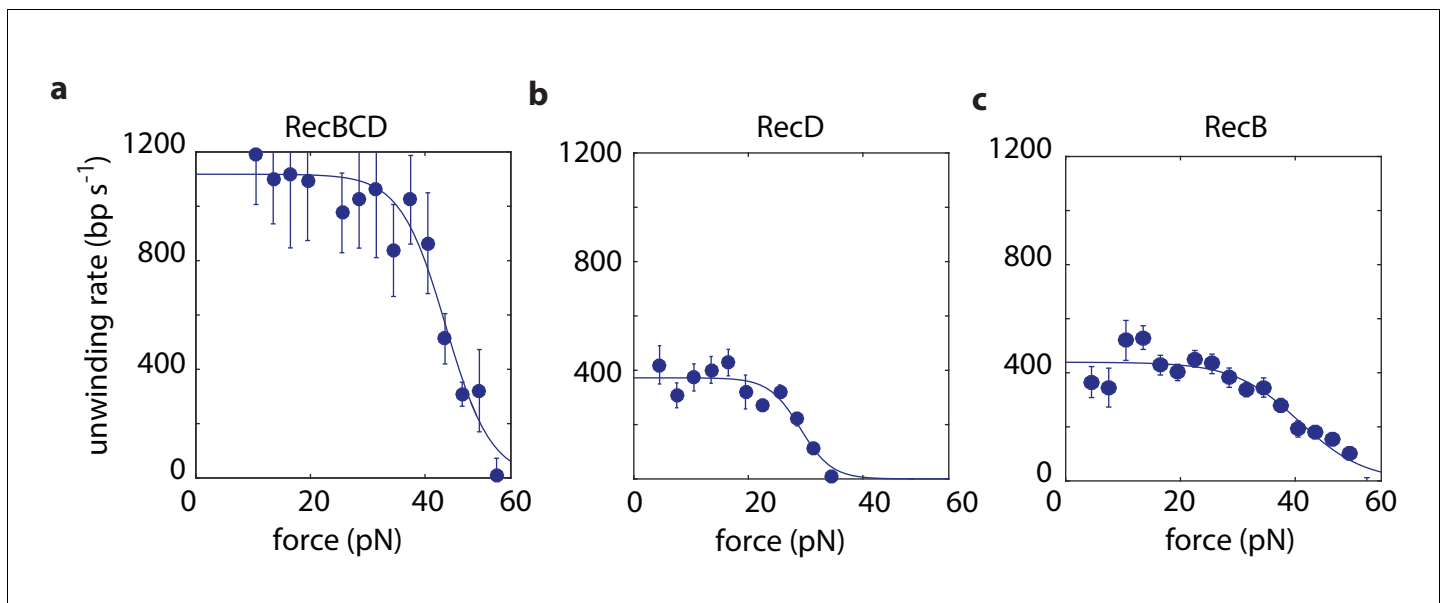


Figure 2. Force-velocity curves for RecBCD and its subunits in complex at 2 mM ATP. Force velocity curves at [ATP] = 2 mM for (a) RecBCD, (b) RecD and (c) RecB. Data shown as mean \pm s.e.m.; the number of experiments is listed in **Supplementary file 4**. Lines through the data are best fits to **Equation 1**.

DOI: <https://doi.org/10.7554/eLife.40836.005>

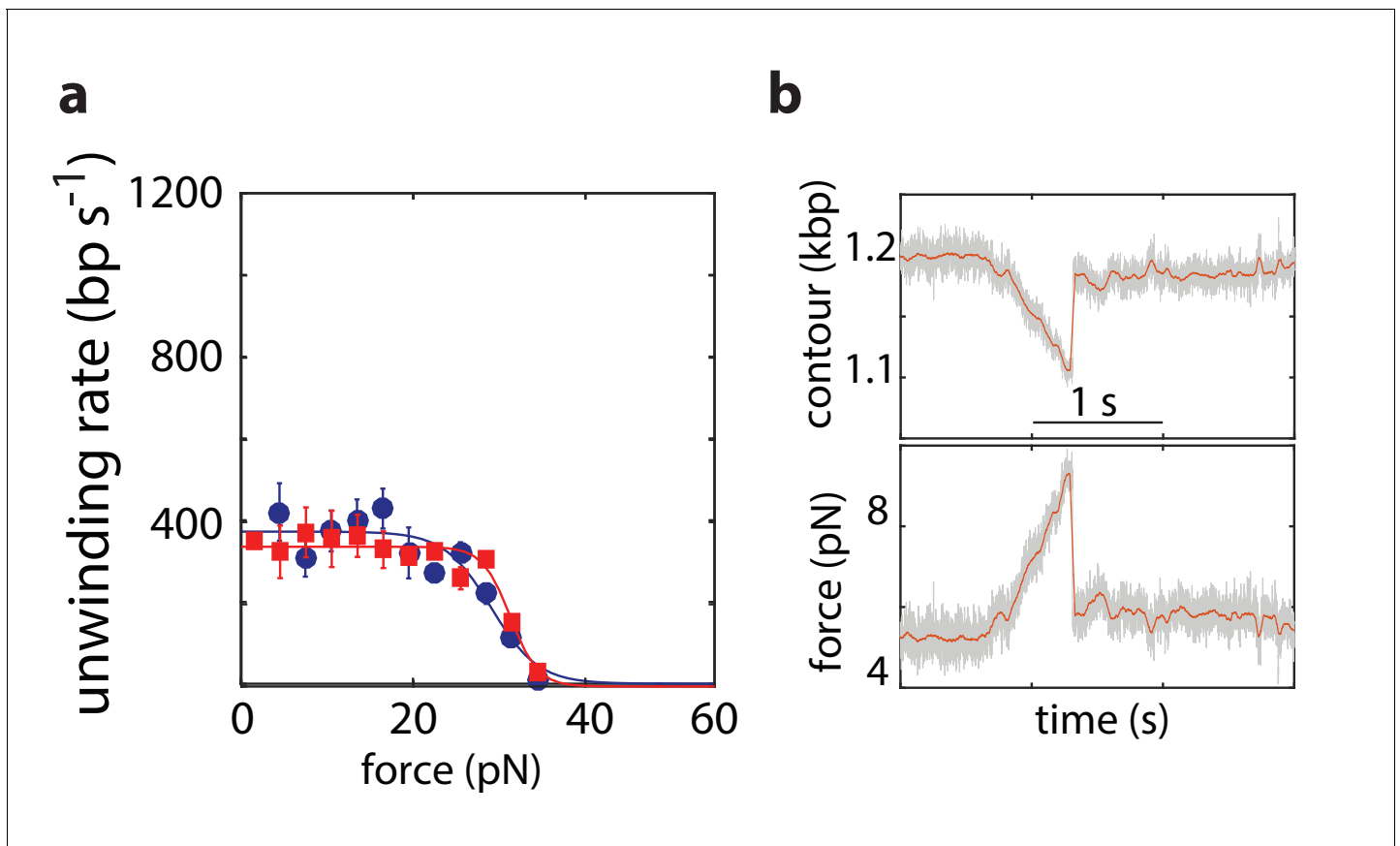


Figure 2—figure supplement 1. The secondary translocase of RecB does not affect the force-velocity measurements of RecD. (a) Force-velocity curves on the asymmetric construct probing RecD, for RecBCD (blue, data similar to **Figure 2d**) and RecB^{K29Q}CD (red), at [ATP] = 2 mM. Data shown as mean \pm s.e.m., lines through the data are best fits to **Equation 1**. The number of experiments is listed in **Supplementary file 4**. (b) Representative trace for RecBC activity on the symmetric construct. RecBC's secondary translocase can sustain forces up to 8.3 ± 1.2 pN (mean \pm s.e.m.). Raw data is shown in grey, filtered data ($f_c = 250$ Hz) is shown in red.

DOI: <https://doi.org/10.7554/eLife.40836.006>

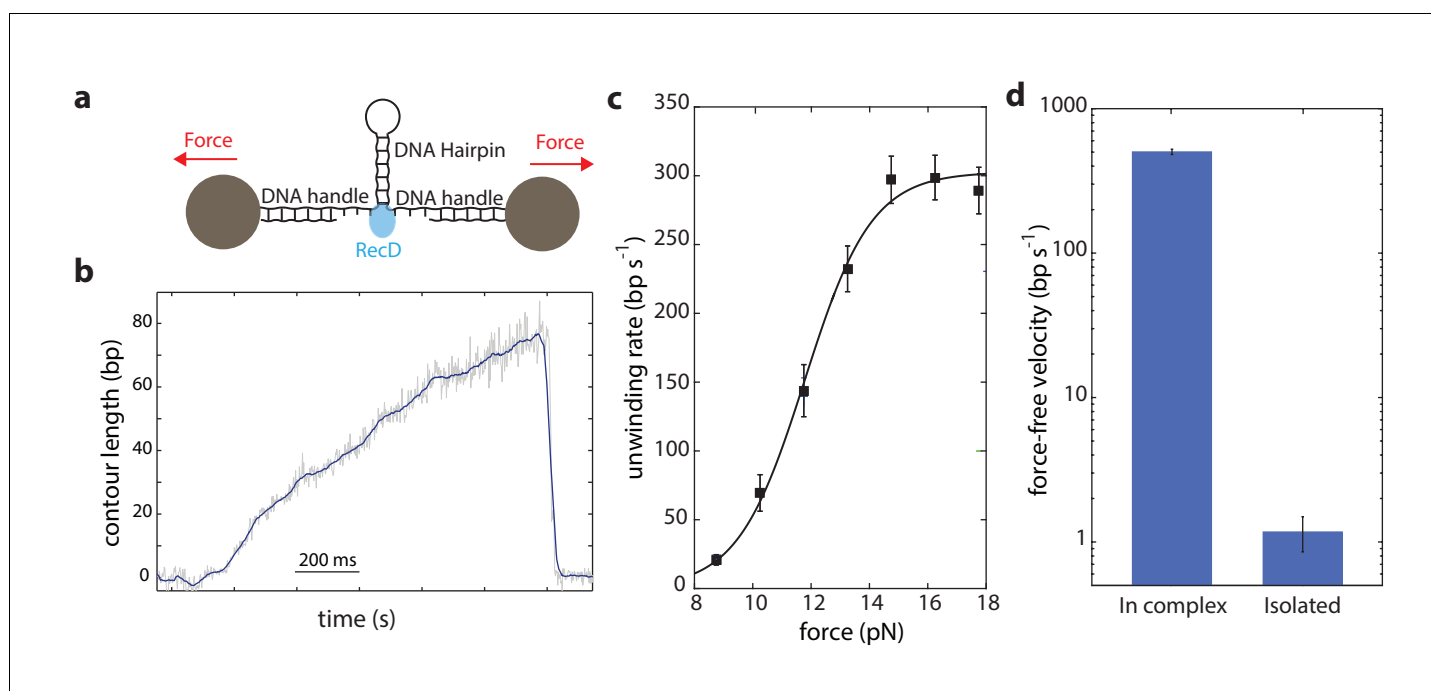


Figure 3. Unwinding of a hairpin under tension by the isolated RecD. (a) Schematic representation of RecD unwinding in optical tweezers. (b) Representative unwinding trace. Raw data is shown in grey, filtered data ($f_c = 250$ Hz) is shown in blue. [ATP] = 2 mM. (c) Velocity-force curve for the isolated RecD at 2 mM ATP follows a sigmoidal-like behavior saturating at high forces. Data shown as mean \pm s.e.m., number of traces in **Supplementary file 4**. The line through the data is a best fit to Equation S29, **Supplementary file 3**. (d) Force-free velocities for RecD, obtained from (c) and **Figure 2b** by extrapolating to $F = 0$, show ~ 2 – 3 orders of magnitude difference between the enzyme in-complex and the isolated one (Extrapolated result \pm s.e.).

DOI: <https://doi.org/10.7554/eLife.40836.007>

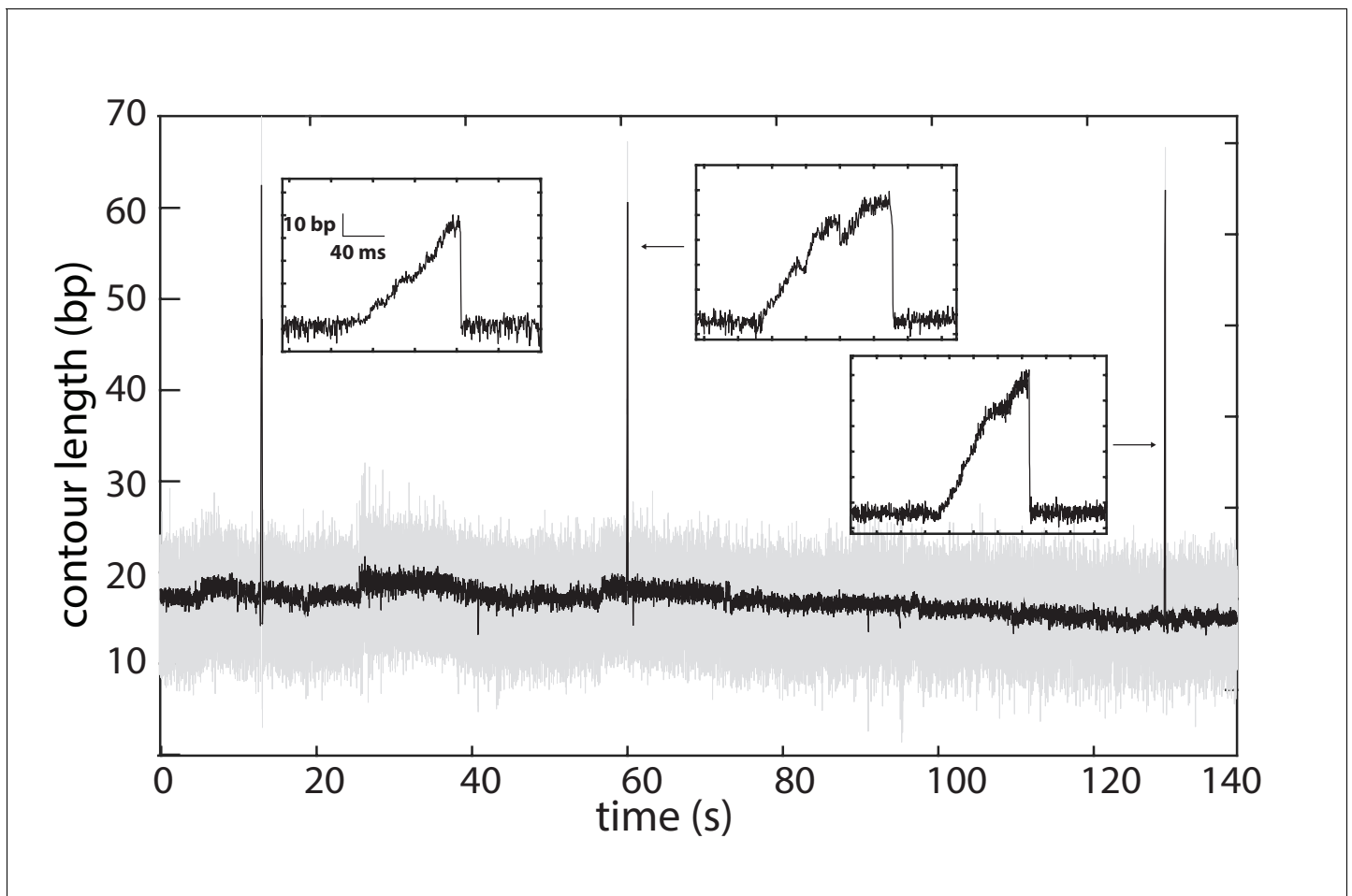


Figure 3—figure supplement 1. Multiple unwinding events by RecD occur on the same DNA molecular construct. Contour-time trace for long timescales shows peaks of RecD activity. Zoomed-in windows indicate individual activity events of RecD followed by dissociation. [ATP] = 2 mM. Raw data is shown in grey, filtered data ($f_c = 250$ Hz) is shown in black.

DOI: <https://doi.org/10.7554/eLife.40836.008>

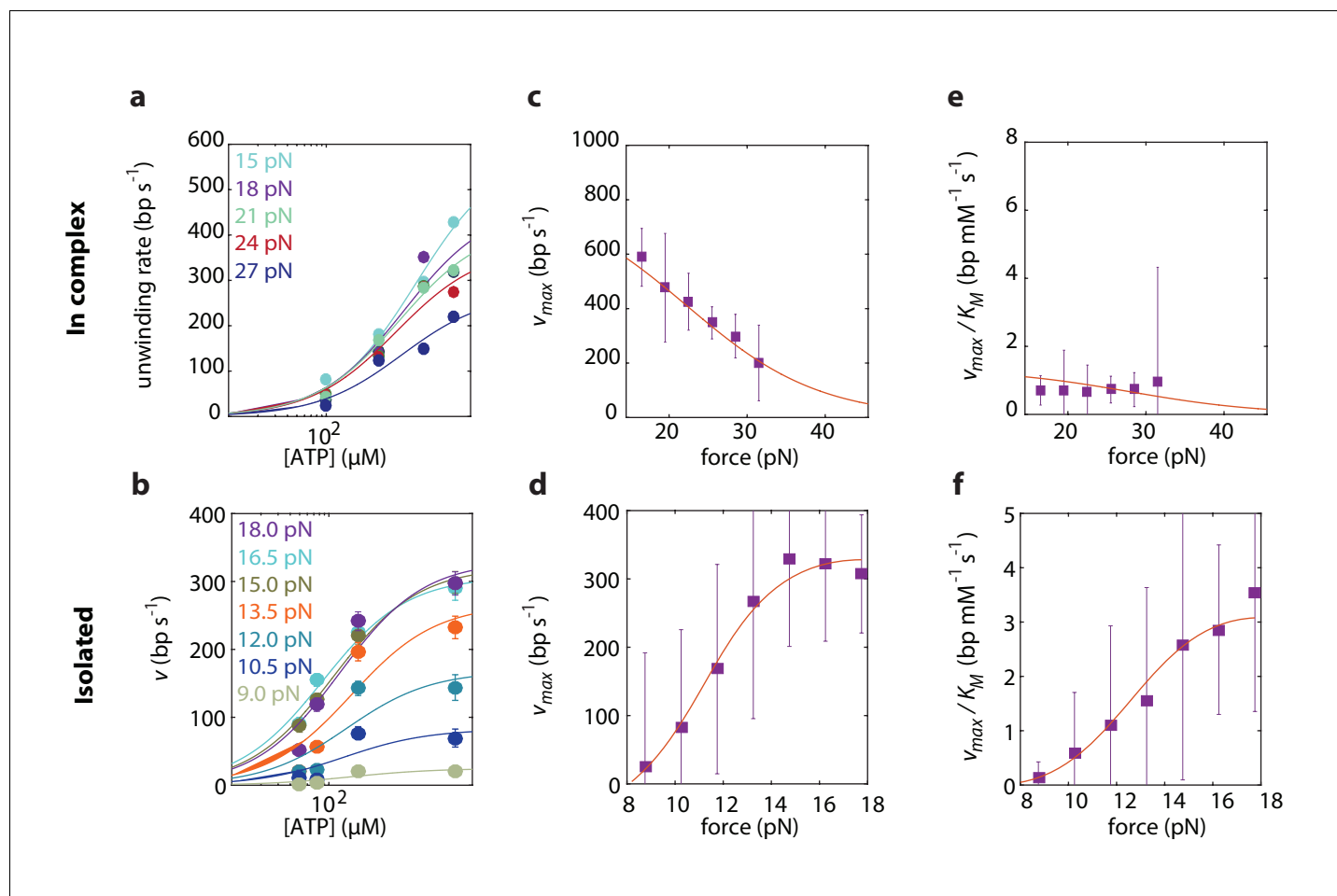


Figure 4. Force dependent Michaelis-Menten curves for RecD in complex (a) and isolated (b). Data shown as mean ± s.e.m. Lines through the data are the result of a global fit to a kinetic model of a Brownian ratchet before ATP binding (Equations S7 and S8 in **Supplementary file 2** and Equations S19 and S20 in **Supplementary file 3**). Different colors indicate different forces. (c–d). Force-dependent v_{max} for RecD in complex (c) and isolated RecD (d). Discrete data points indicate v_{max} as a result of fitting Michaelis-Menten curves to the data in a and b for each force separately (shown as fit result ± s.e.). (e–f) Force dependent v_{max}/K_M for RecD in complex (e) and isolated (f). Discrete data points indicate v_{max}/K_M as a result of fitting Michaelis-Menten curves to the data in a and b for each force separately (shown as fit result ± s.e.). Lines through the data indicate the results of the global fitting.

DOI: <https://doi.org/10.7554/eLife.40836.009>

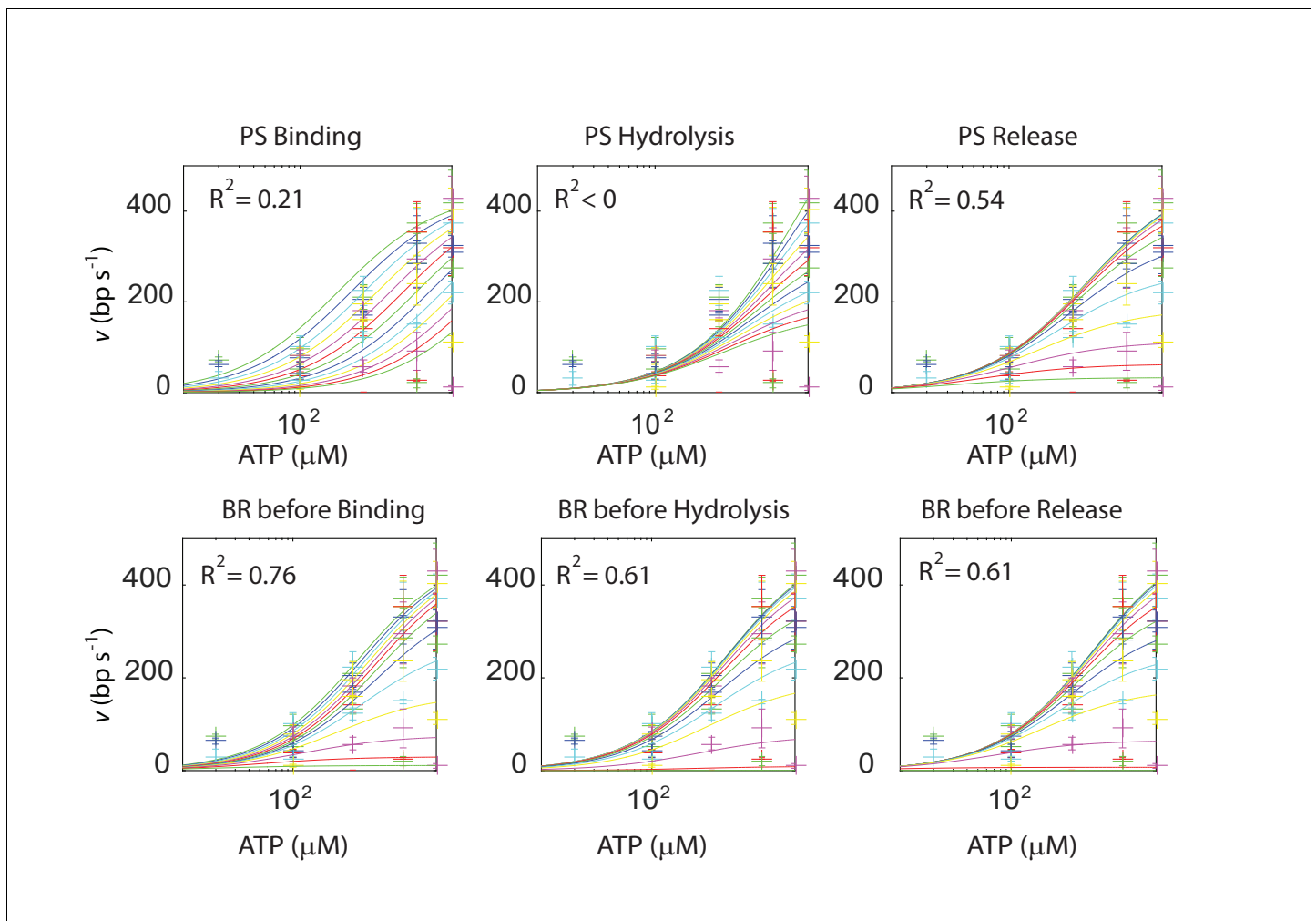


Figure 4—figure supplement 1. Global fits for different translocation mechanisms of in-complex RecD under force. Different colors indicate different forces (Materials and methods). Data shown as mean ± s.e.m. Lines show the results of fitting the data with the kinetic models described by Equations S1-S12, **Supplementary file 2**.

DOI: <https://doi.org/10.7554/eLife.40836.010>

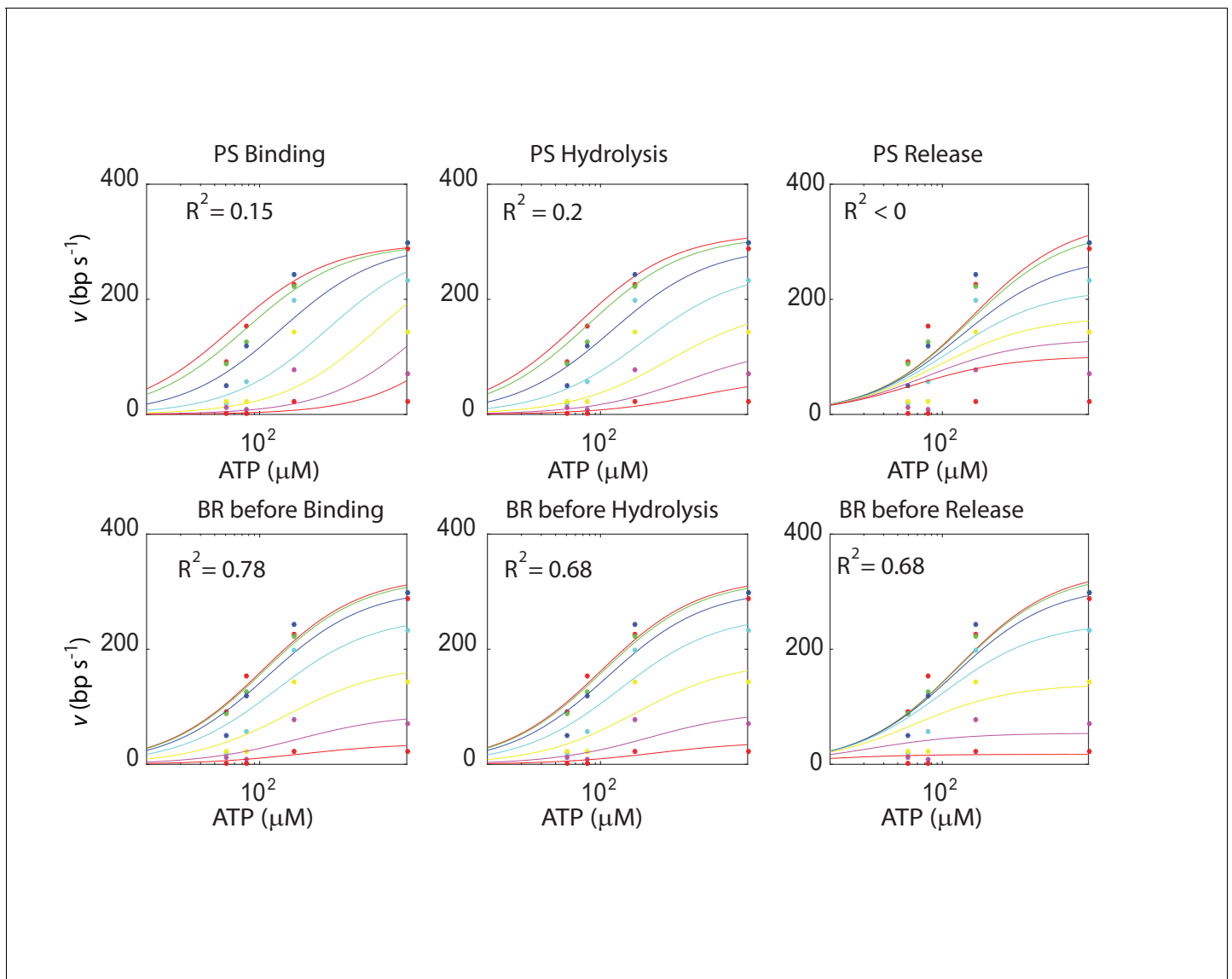


Figure 4—figure supplement 2. Global fits for different translocation mechanisms of isolated RecD under force. Different colors indicate different forces (Materials and methods). Data shown as mean \pm s.e.m. Lines show the results of fitting the data with the kinetic models described by Equations S13-S24, **Supplementary file 3**.

DOI: <https://doi.org/10.7554/eLife.40836.011>

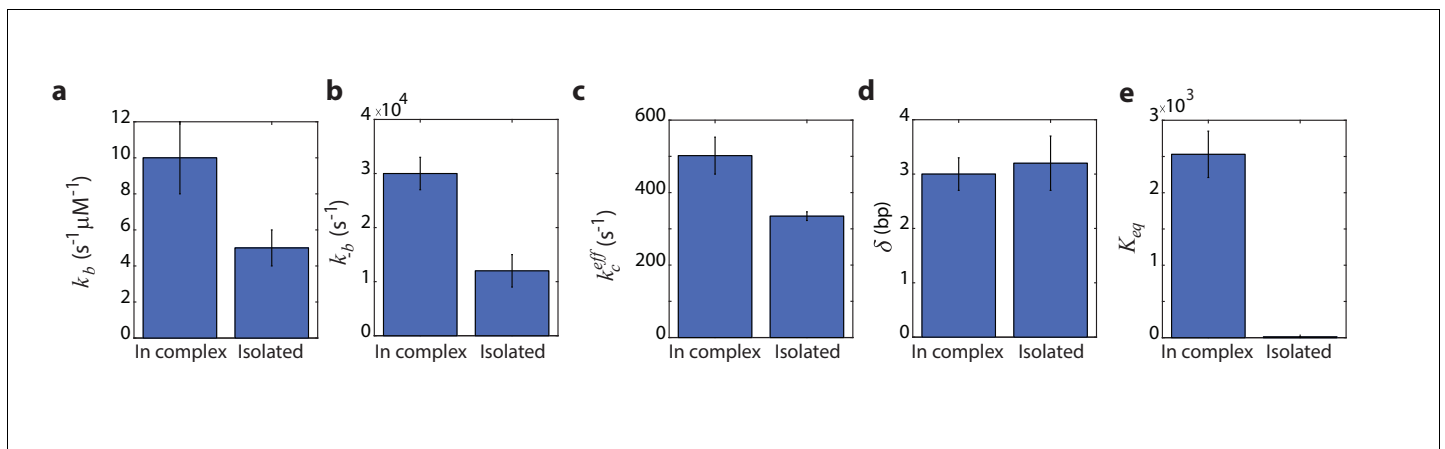


Figure 5. The global fitting parameters reveal a shift towards the post translocation state of RecD when in complex. (a) Forward ATP binding rate, k_b . (b) Backward binding rate, k_{-b} . (c) effective catalytic rate, k_c^{eff} . (d) Step size, δ . (f) Equilibrium constant of translocation, shifted by the complex toward the post-translocation state.

DOI: <https://doi.org/10.7554/eLife.40836.012>