Dynamics of the interaction of RecG protein with stalled replication forks

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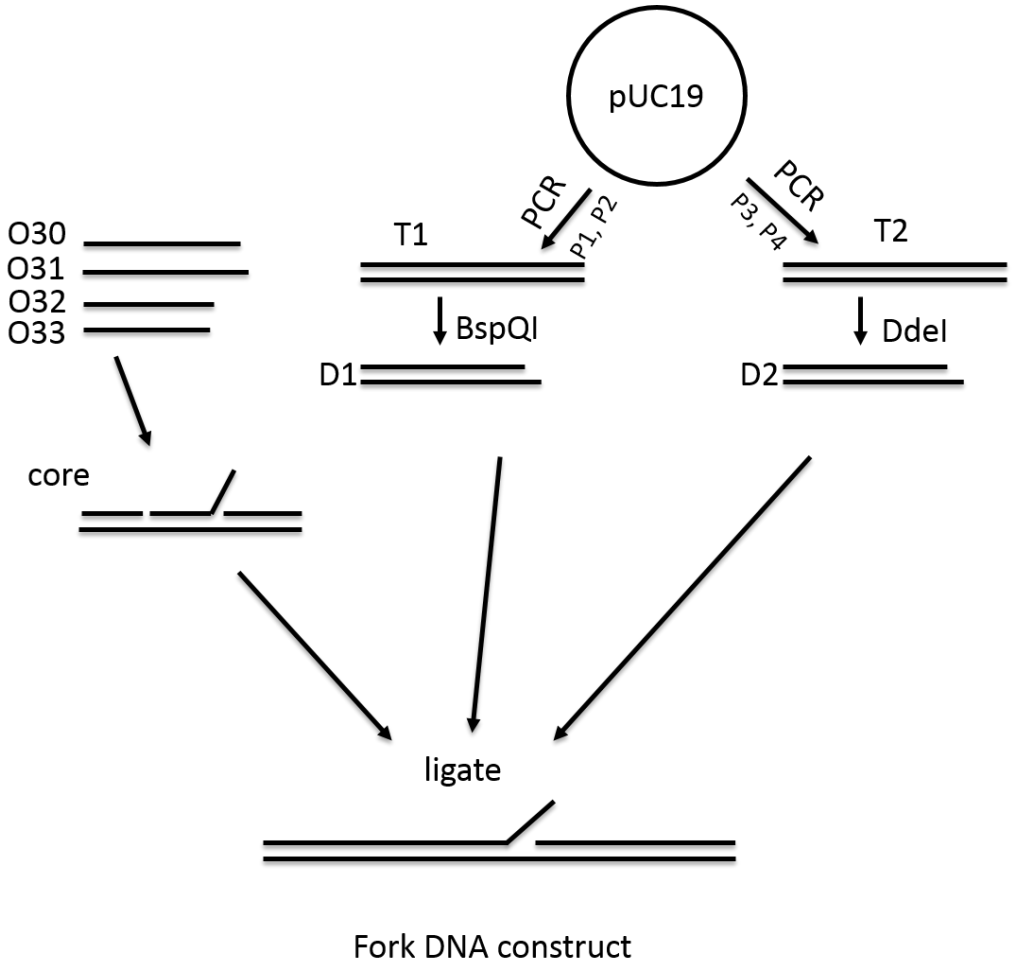
Keywords: DNA replication; replication fork regression; DNA helicases; RecG protein; SSB protein, AFM; time-lapse AFM; nanoscale dynamics; computational modeling.



Scheme S1. The assembly of the fork DNA constructs.

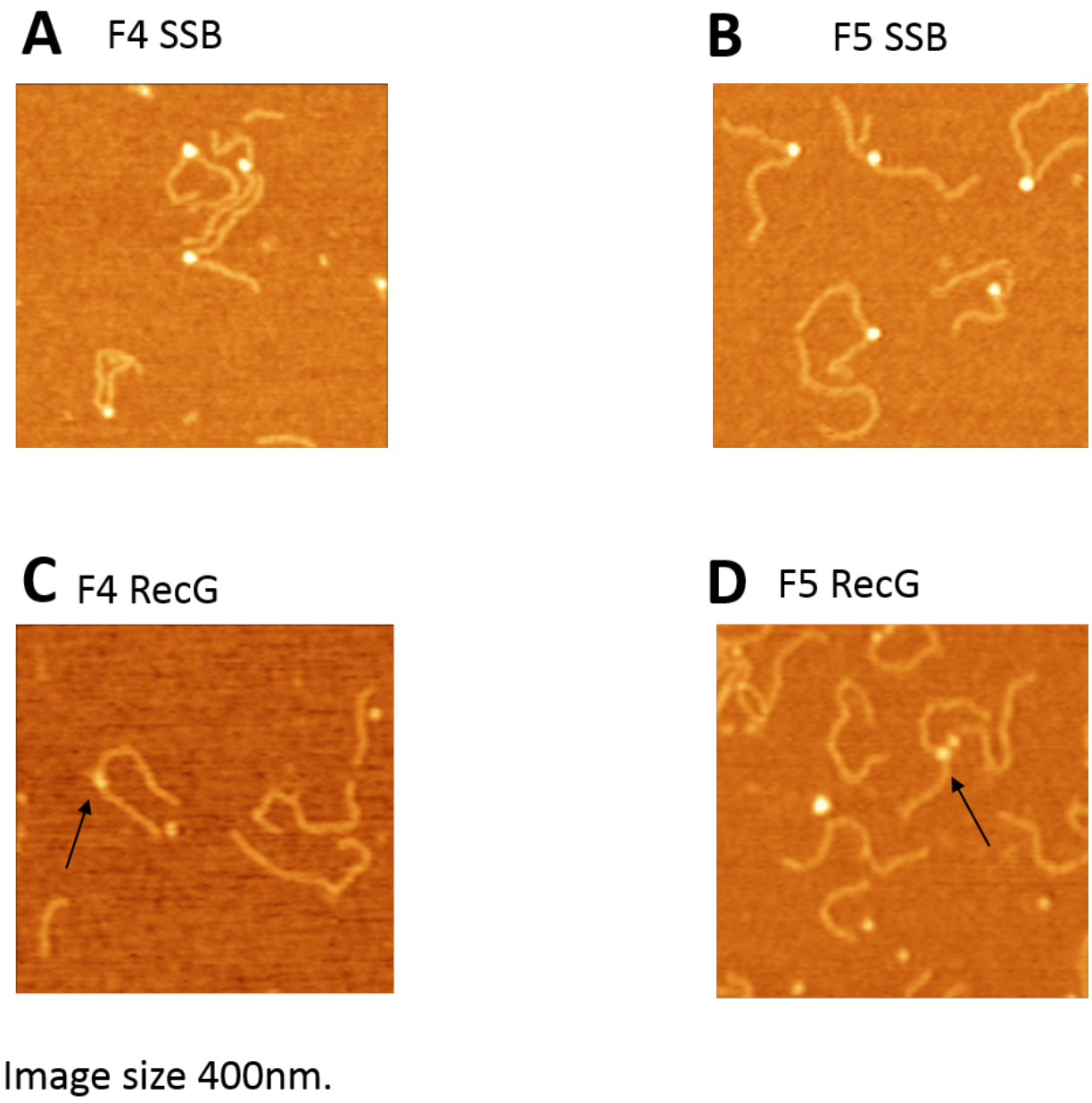


Figure S1. The AFM images of complexes of DNA substrates and proteins: F4 with SSB (A), F5 with SSB (B), F4 with RecG (C), F5 with RecG (D).

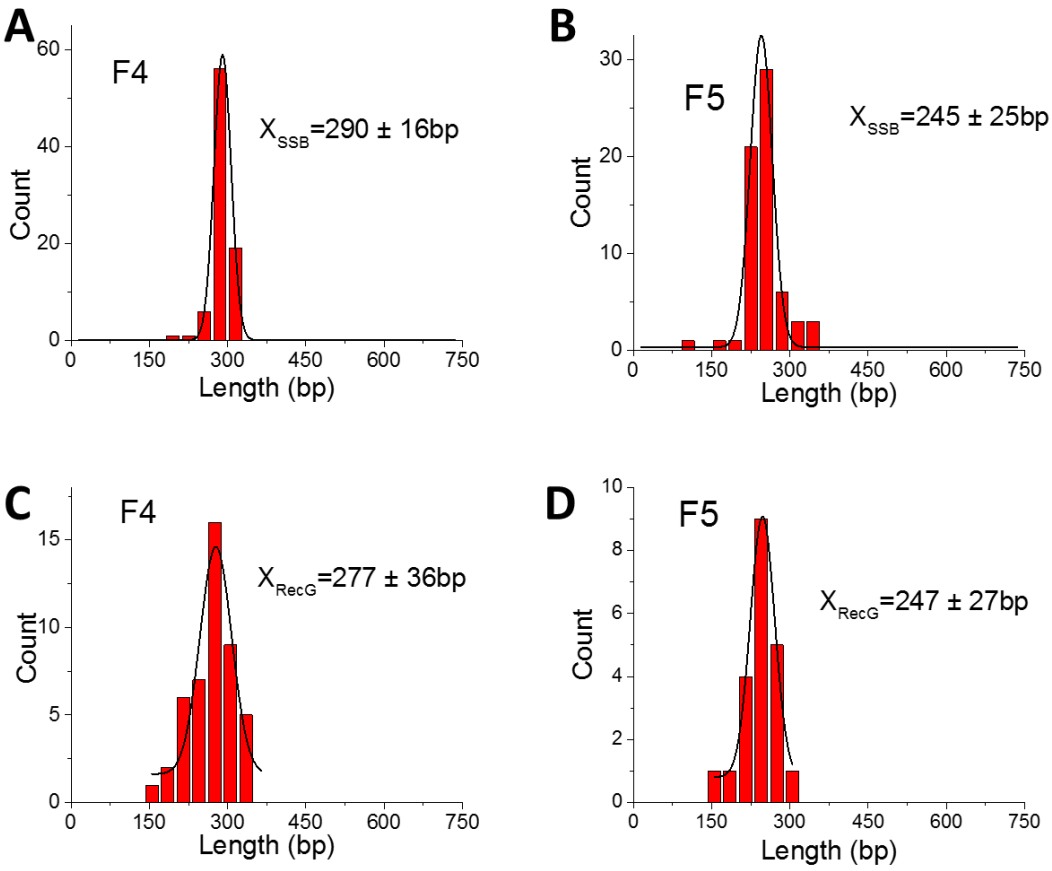


Figure S2. The histograms of distributions of proteins location on different fork substrates. The location of SSB are shown in panels (A) and (B) for F4 and F5 substrates, respectively. Similarly, the RecG location on F4 and F5 are shown in (C) and (D), respectively.

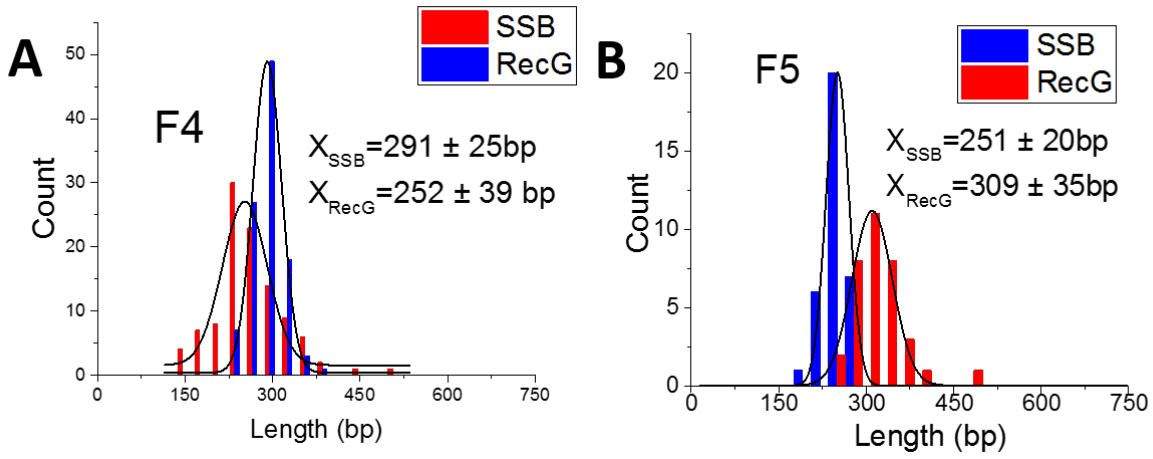


Figure S3. The histograms for positions of RecG (red) and SSB (blue) for constructs F4 (A) and F5 (B). Histograms have been fitted with Gaussian functions and mean values with standard deviations are shown.

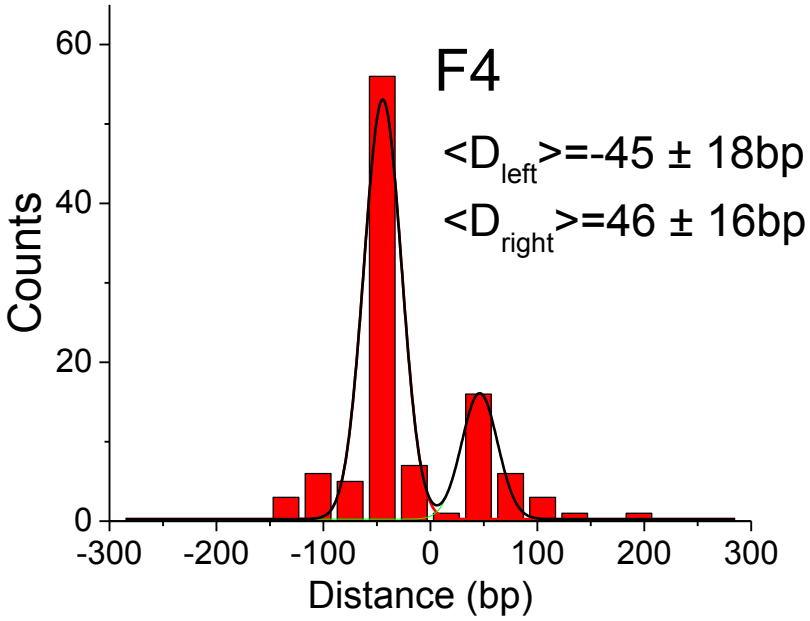


Figure S4. Distribution of distances between RecG and SSB on F4 DNA substrate. The negative values represent RecG translocation to the parental strand, while positive values show the translocation to the daughter strand. The histogram has been fitted with Gaussian functions and mean values with standard deviations are shown.

RecG remains bound to DNA during translocation

In order to check if RecG dissociates from DNA during translocation, we measured the distance between RecG and DNA using cross-sectional profiles of the frames in movie S3 as shown in Fig. S5A. The cross-section (green line) was made over the protein and the protein position determined. The DNA height appears as a shoulder on this cross-section (cross-section profile 1 in A). DNA position was determined based on another cross-section (yellow line) at the edge of the protein to eliminate the overlap between the height for protein and DNA (cross-section profile 2). These measurements were done over the entire movie set and the dependence of the distance between the DNA and protein is shown in graph (Fig. S5B). The data shows that while the distance between RecG and DNA fluctuates, there is no gap between them.

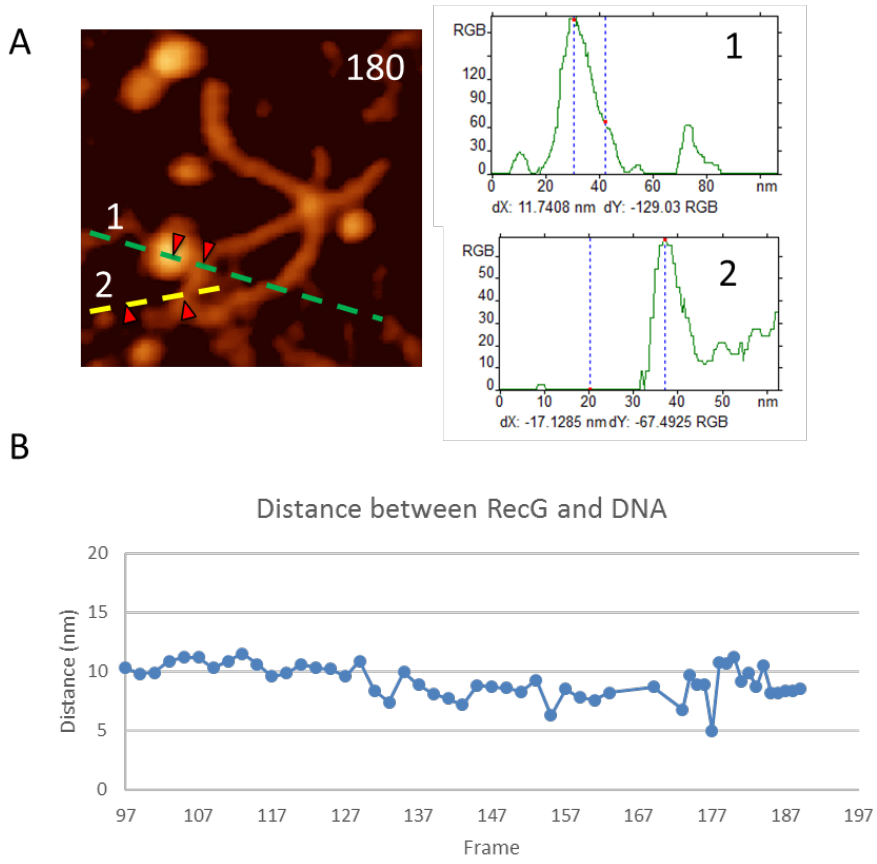


Figure S5. The cross-sectional distance between RecG and DNA on the different frames in Fig. 4. (A) Typical image (left) and the cross-sections (right) of RecG and DNA. The distance from the center of protein (right column, the arrow on the tall peak) to the center of DNA (the short peak) was measured (green dashed line). The yellow cross-section shows the height of DNA which is similar to the DNA in the green cross-section. (B) The distance between the center of RecG and DNA from Fig. 4 (movie S3), measured using cross-sectional profiles.

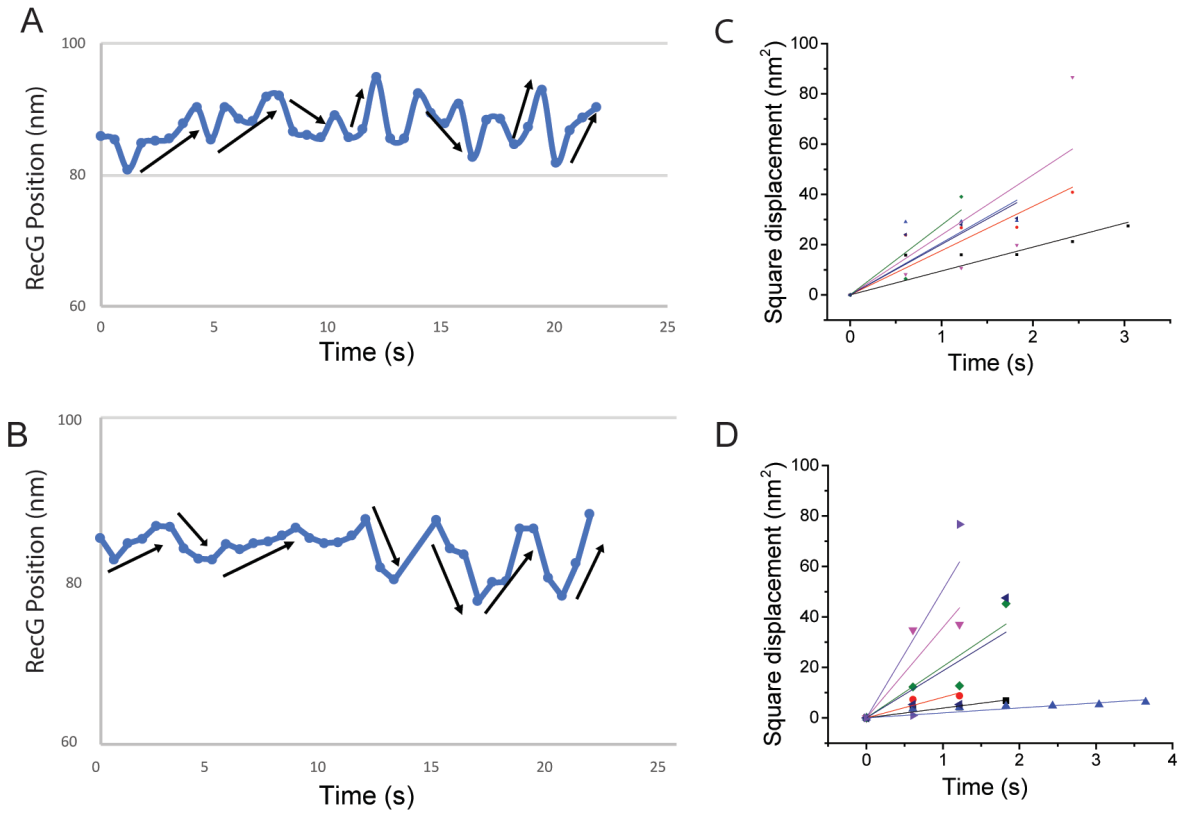


Figure S6. Figure S6. Analysis of one-dimensional diffusion of RecG for data shown in Figs. 3 and 5. (A, B) Time-dependent position of RecG measured from short end of DNA for movies S1 and S3 without and with SSB dissociation respectively. Arrows indicate RecG movement along selected segments. (C, D) Time-dependent squared displacements of RecG for trajectories in A-B, respectively. Solid lines are the linear fits of the data for each selected segment.

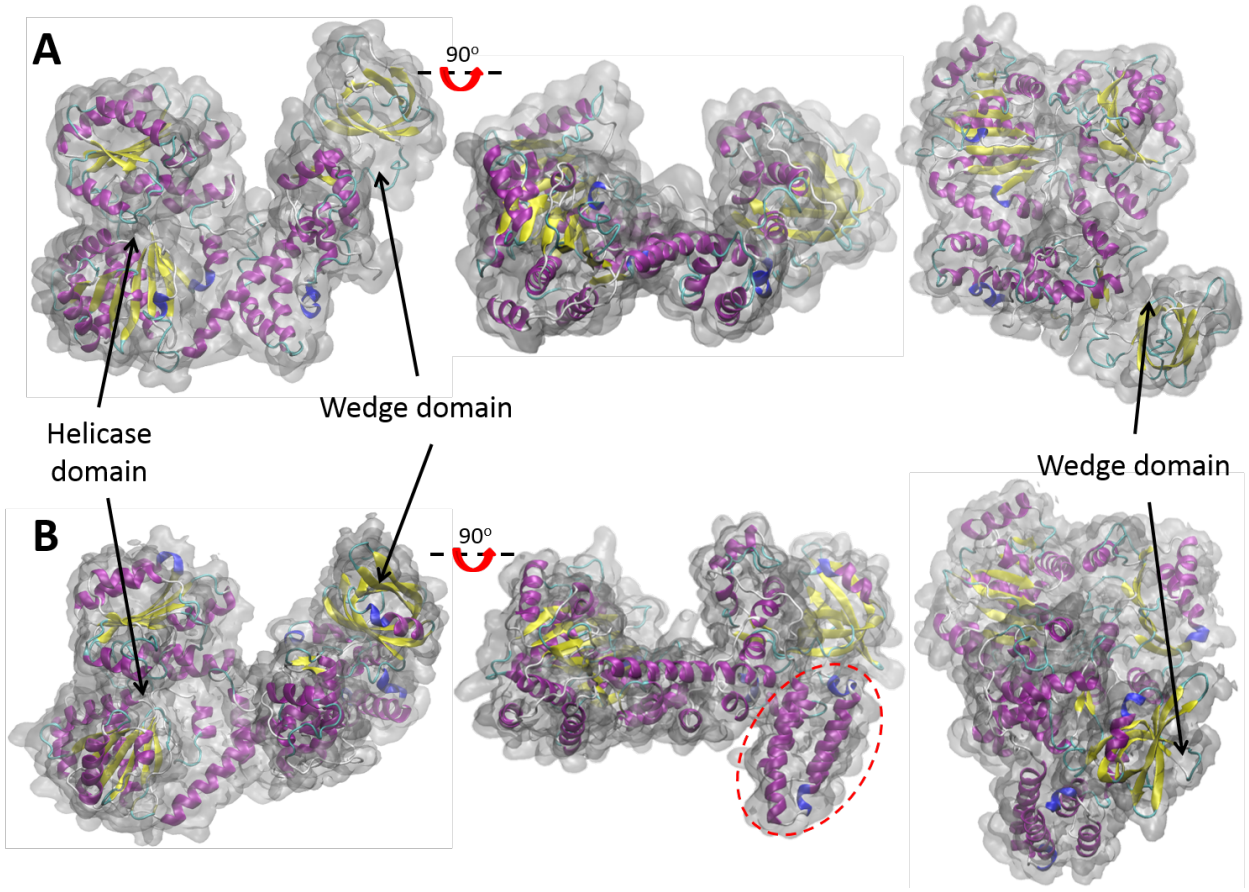


Figure S7. RecG 3D structures. (A) *E. coli* RecG 3D structure, obtained from I-TASSER threading and refined using 20 ns all-atom MD simulation. (B) *T. maritima* RecG 3D crystal structure from RecG-DNA complex (PDB ID: 1GM5); the N-terminal helix bundle is highlighted with a dashed ellipse. Proteins are shown from three different projections, top (left), side (center), and front (right) and key features are highlighted with arrows.

Figure S8. The secondary structure map of *E. coli* RecG (A) and *T. maritima* RecG (from PDB: 1GM5) (B) determined using DSSP.

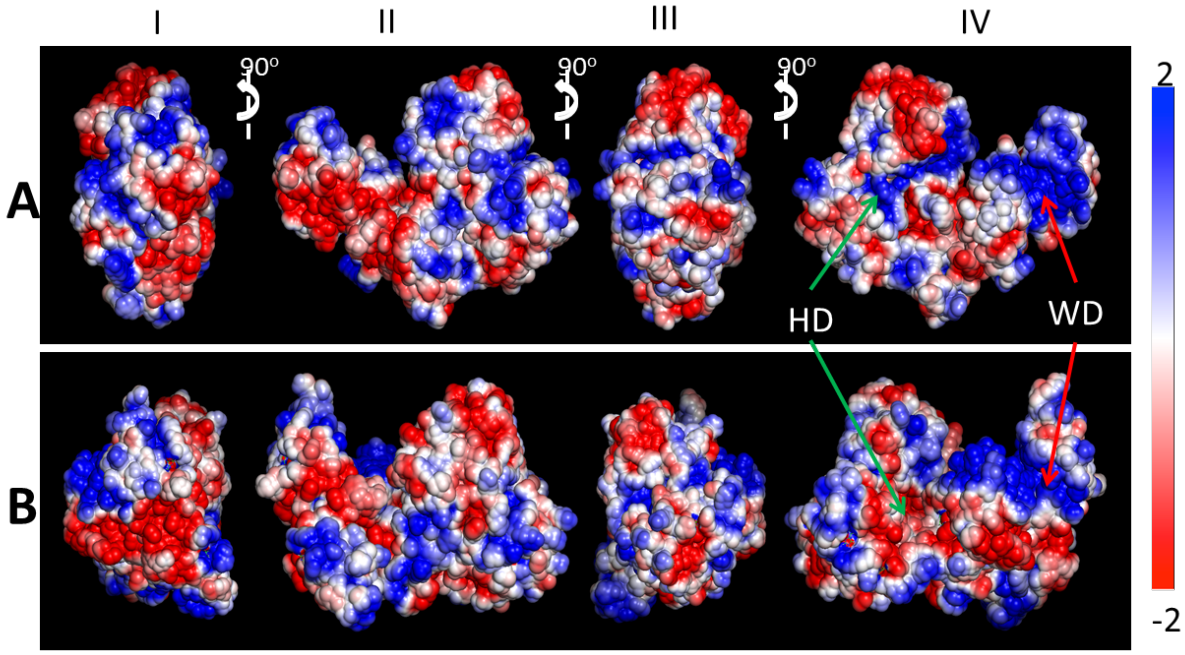


Figure S9. Projection of electrostatic potential of the RecG protein onto the solvent accessible surface of *E. coli* RecG with closed helicase domain (A) and *T. mairitima* RecG from PDB: 1GM5 (B). Wedge domain (WD) and helicase domain (HD) have been highlighted with arrows. Each frame is rotated 90 degrees counter clockwise around the Z-axis, starting from column I; each column shows the same projection of the different proteins. Color scale is in units of k_B*T/e_C, showing negatively charged regions in red and positively charged in blue.

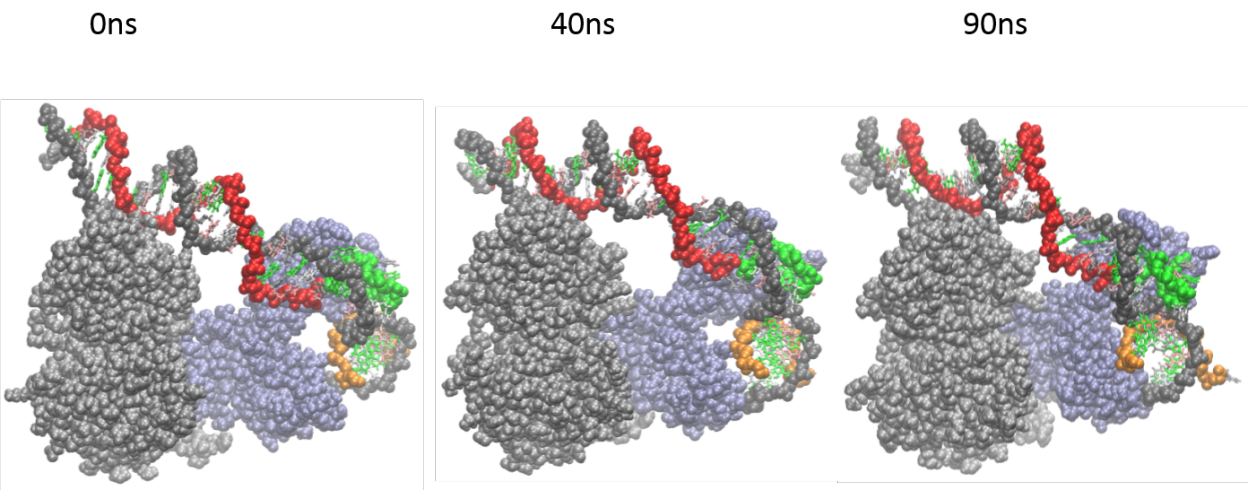


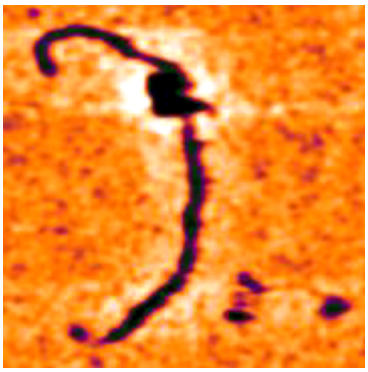
Figure S10. The interaction for *E. coli* RecG with replication fork substrate with a ssDNA nick. The RecG-fork complex from Fig. 6B, after 150 ns, is used as the initial structure; a nick in the parental leading strand introduced a new 9 nt ssDNA (green). RecG is presented as van der Waal spheres with wedge domain colored in blue and helicase domain in grey. DNA strands are shown in different colors indicating each chain.

Table S1. Statistics of yields for RecG-DNA complexes obtained in three independent experiments.

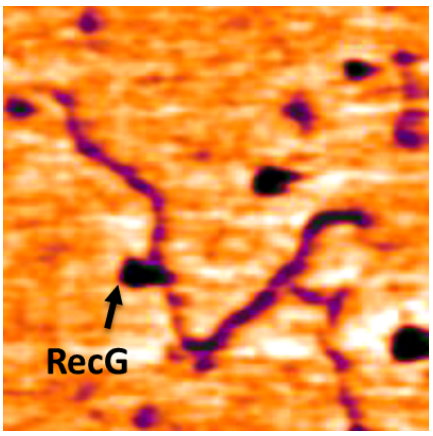
	F4		F5	
	yield	number of complexes	yield	number of complexes
Experiment 1	10.2	26	5.6	20
Experiment 2	8.6	21	4.5	19
Experiment 3	13.2	20	7.5	15

Table S2. The calculated diffusion coefficients for the events in Fig. S6 (column A and B corresponded to Fig. S6 A and C).

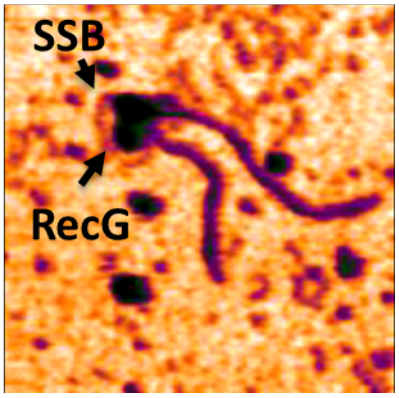
Events number	A (nm^2/s)	B (nm^2/s)
1	9.5	3.9
2	17.6	8.1
3	20.6	2
4	23.9	35.8
5	27.8	20.3
6	20.1	18.6
7		50.8
Mean value	19.91±5.66	19.92±11.64



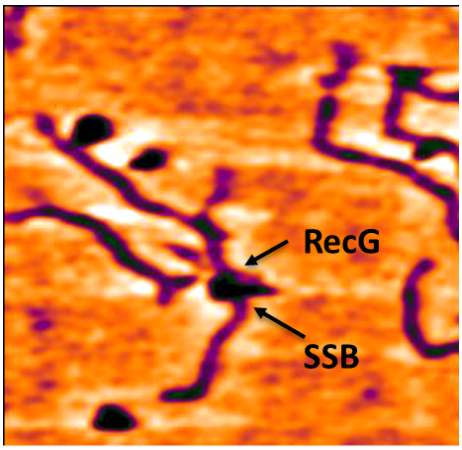
Movie S1. Time-lapse AFM of SSB and RecG interacting with replication fork substrate F4. The size of each frame in the movie is 180nm x180nm.



Movie S2. Time-lapse AFM of RecG interacting with replication fork substrate F4. The size of each frame in the movie is 190nm x190nm.



Movie S3. Time-lapse AFM of SSB and RecG interacting with replication fork substrate F4. The size of each frame in the movie is 180nm x180nm.



Movie S4. Time-lapse AFM of SSB and RecG interacting with replication fork substrate F5. The size of each frame in the movie is 230nm x 230nm.