Supporting Information

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SI Materials and Methods

Strains, Plasmids, Media, and Growth Conditions. Plasmids were maintained in TOP10 *Escherichia coli* cells from Invitrogen [F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G]. All bacterial cells were grown in LB medium or on LB plates. *sopA-gfp-his*$_6$ was synthesized with the linker sequence 5′GGTAGT, which inserted the two-residue linker GS between SopA and GFP-His_6 of the fusion protein. The genes *sopA-his*$_6$ (pX7), *sopB-his*$_6$ (pX8), or *sopA-gfp-his*$_6$ (pX2) were expressed under the control of a bacteriophage T7 promoter in the vector pET15b (Novagen). The pBR322::*sopC* plasmid substrate (4,738 bp) used for total internal reflection fluorescence microscopy (TIRFM) studies was created by PCR amplification of a *sopC* fragment containing a 5′ BamHI site and a 3′ SphI site. The fragment and vector (pBR322, 4,361 bp) were digested with BamHI and SphI and ligated. pBluescript::*parS* plasmid construction and plasmid labeling with Alexa Fluor 647 has been described elsewhere (1). The average plasmid:dye ratio was calculated to be 1:4.

Proteins. The plasmid pX2, pX7, or pX8 was introduced into BL21 (AI) cells (Invitrogen) for protein expression. Overnight cultures (4 × 10 mL) were used to inoculate 4 L of LB broth supplemented with 100 μg/mL carbinecillin, which then was incubated with aeration at 20 °C to an OD_{600} of ∼0.6. Protein expression was induced with 1 mM isopropylthio-β-galactoside and 0.2% arabinose, and the cells were grown at 16 °C overnight. The cells were harvested by centrifugation in a Beckman JLA8.1 rotor at 4,500 rpm for 1 h. The cell pellet was frozen with liquid nitrogen and stored at −80 °C. The cell pellet was resuspended up to 150 mL with lysis buffer [50 mM Hepes·KOH (pH 7.6), 200 mM KCl, 10% glycerol, 5 mM imidazole, 2 mM β-mercaptoethanol (BME)] containing Complete Protease Inhibitor Mixture Tablets (Roche), and lysed with a Microfluidizer (Microfluidics Corp.). The lysate was cleared by ultracentrifugation using a Beckman 45Ti rotor at 35,000 rpm for 40 min and filtration through a 0.45-μm filter and was loaded onto a 5-mL HisTRAP HP cassette (GE Healthcare). The column was washed with high-KCl buffer [50 mM Hepes·KOH (pH 7.6), 1 M KCl, 10% glycerol, 20 mM imidazole, 2 mM BME] and low-KCl buffer (same composition as the high-KCl buffer but with 200 mM KCl). The protein then was eluted with a 20 mM–1 M imidazole gradient. Peak fractions were pooled, and imidazole was removed using a 26/10 salt-exchange column (GE Healthcare) before ion exchange chromatography.

SopA-His_6 and SopA-GFP-His_6 were loaded onto a 1-mL MonoQ column (GE Healthcare) equilibrated in Q-buffer [50 mM Hepes·KOH (pH 7.6), 200 mM KCl, 10% glycerol, 0.1 mM EDTA, 2 mM DTT] and eluted with a 0.2–1 M KCl gradient. SopB-His_6 was loaded onto a 1-mL MonoS column equilibrated in S-buffer [50 mM MES (pH 6), 80 mM KCl, 10% glycerol, 0.1 mM EDTA, 2 mM DTT] and eluted with a 0.08–1 M KCl gradient. For all proteins, the peak fractions were pooled, concentrated, and passed over a Superdex 200 gel-filtration column (GE Healthcare) equilibrated with Q-buffer, except that the salt concentration was 150 mM KCl for SopB-His_6 and 600 mM KCl for both SopA proteins. For all proteins, the peak fractions corresponding to dimer (as determined by gel-filtration molecular-weight standards) were stored at −80 °C. P1 ParA-GFP (2) and ParB (3) were expressed and purified as described previously.

SopB Labeling. DTT was removed from the SopB-His_6 sample to be labeled using a 26/10 salt exchange column equilibrated in Q-buffer (at 150 mM KCl) without DTT. Alexa Fluor 647 C_2 maleimide (250 μM) (Invitrogen) was incubated with 50 μM SopB-His_6 in a final reaction volume of ∼2 mL for 30 min at 23 °C. The labeling reaction was quenched with 10 mM DTT, and free label was removed using a 26/10 salt exchange column equilibrated in Q-buffer (at 150 mM KCl) including 2 mM DTT. The labeled protein was concentrated using an Amicon Ultra-4 10K molecular weight cut-off device. The average labeling efficiency was determined with a NanoDrop 2000 spectrophotometer by comparing absorbencies at 280 nm and 647 nm. The average SopB monomer:dye ratio was calculated to be 1:1.

Flowcell. The flowcells were assembled on fused silica slides in which one or two inlet holes and an outlet hole had been drilled (Esco Products). The slides were washed overnight with NOCHROMIX glass cleaning reagent (Sigma), rinsed thoroughly with deionized water, and air dried. A 4-mm-wide by 3-cm-long flow-channel was cut on a piece of 25-μm-thick acrylic transfer tape (3M) which was placed between a cover glass and the fused silica slide. Nanoport N-333–01 inlets (Upchurch) were attached to the fused silica slides above the holes using Optical Adhesive 61 (Norland Products). The flowcell was baked at 100 °C for 45 min. A two-inlet flowcell was used for buffer-switch experiments (Fig. S2).

DNA Carpet. Flowcell surfaces were carpeted with sonicated salmon sperm DNA as described previously (1). The DNA-carpeted flowcell was washed with 500 μL Sop buffer containing 1 mg/mL α-casein and was incubated for 30 min at 23 °C. The flowcell then was washed with 500 μL Sop buffer containing 0.1 mg/mL α-casein. Failure to pacify the surfaces led to nonspecific sticking and protein denaturation. For SopA, inadequate blocking led to the formation of nondynamic, filament bundles of dead protein, which also were observed near air bubbles. The formation of SopA aggregates on glass slides not treated with blocking agents has been reported previously (4).

TIRFM Setup. The prism-type TIRFM setup was essentially as described previously (5). The typical camera settings were digitizer, 3 MHz (14-bit gray scale); preamplifier gain, 5.2; vertical shift speed, 2 MHz; vertical clock range: normal, electron-multiplying gain 40, EM CCD temperature set at −98 °C, baseline clamp ON, exposure time 100 ms, frame rate 0.5 Hz. The baseline of ∼100 camera units was subtracted from the data. The setup for single-particle acquisition was similar except that the electron-multiplying gain was 200, vertical shift speed was 1.11 MHz, and frame rate was 10 Hz.

The excitation for SopA-GFP and SopB-Alexa647 was provided by 488-nm diode-pumped solid-state (sapphire, Coherent) and 633-nm HeNe (Research Electro-Optics) lasers, respectively. Laser powers were calibrated, and the intensity data for different laser power levels were normalized according to the settings. Total internal reflection fluorescence illumination had a Gaussian shape in the field of view with measured horizontal and vertical half maximum widths of ∼65 μm × 172 μm at 488 nm. Intensity data for the DNA carpet-bound populations of SopA-GFP and SopB-Alexa647 were taken from the middle of the illumination profile. The typical laser power of 488-nm and 633-nm illumination was 15 μW and 500 μW, respectively. For single-particle tracking, the 488-nm and 633-nm laser power was 6.5 mW and 6 mW, respectively. Metamorph 7 software (Molecular Devices) was used for camera control, image acquisition, and analysis. The display brightness and contrast were set individually for each

picture or movie for the best representation of the features of interest. ImageJ software (National Institutes of Health) was used for final conversion of Metamorph movies (.stk) into the QuickTime file format (.mov). Movie accelerations are indicated in the figure legends. Adobe Illustrator was used for movie conversion into figures.

Single Particle Tracking Analysis. Movies were cropped to a 200 pixel × 200 pixel square centered where illumination intensity was highest and uniform. Background was subtracted using a sliding window length of 40 pixels with the Mosaic Plugin for ImageJ (6), and a fast Fourier transformation was performed with a bandpass that filtered large structures down to 10 pixels and small structures up to 3 pixels. The Octane plugin for ImageJ (7) was used for particle tracking with the following options: subpixel fitting algorithm, polyfit Gaussian weight; kernel size, 2 pixels; maximum displacement, 10 pixels; blinking, 3 pixels; and residue threshold, 100. After Octane analysis, only the particles that had trajectory lengths greater than 20 frames (2 s) were analyzed further. Particle movement then was inspected by eye to remove erroneous trajectories. The data were exported to Wolfram Mathematica 8 for graphing and statistical analysis (analysis program written by V. Ivanov).

1. Hwang LC, et al. (2013) ParA-mediated plasmid partition driven by protein pattern self-organization. *EMBO J*, 10.1038/emboj.2013.34.
2. Vecchiarelli AG, et al. (2010) ATP control of dynamic P1 ParA-DNA interactions: A key role for the nucleoid in plasmid partition. *Mol Microbiol* 78(1):78–91.
3. Bouet JY, Funnell BE (1999) P1 ParA interacts with the P1 partition complex at *parS* and an ATP-ADP switch controls ParA activities. *EMBO J* 18(5):1415–1424.
4. Lim GE, Derman AI, Pogliano J (2005) Bacterial DNA segregation by dynamic SopA polymers. *Proc Natl Acad Sci USA* 102(49):17658–17663.
5. Ivanov V, Mizuuchi K (2010) Multiple modes of interconverting dynamic pattern formation by bacterial cell division proteins. *Proc Natl Acad Sci USA* 107(18):8071–8078.
6. Sbalzarini IF, Koumoutsakos P (2005) Feature point tracking and trajectory analysis for video imaging in cell biology. *J Struct Biol* 151(2):182–195.
7. Niu L, Yu J (2008) Investigating intracellular dynamics of FtsZ cytoskeleton with photoactivation single-molecule tracking. *Biophys J* 95(4):2009–2016.

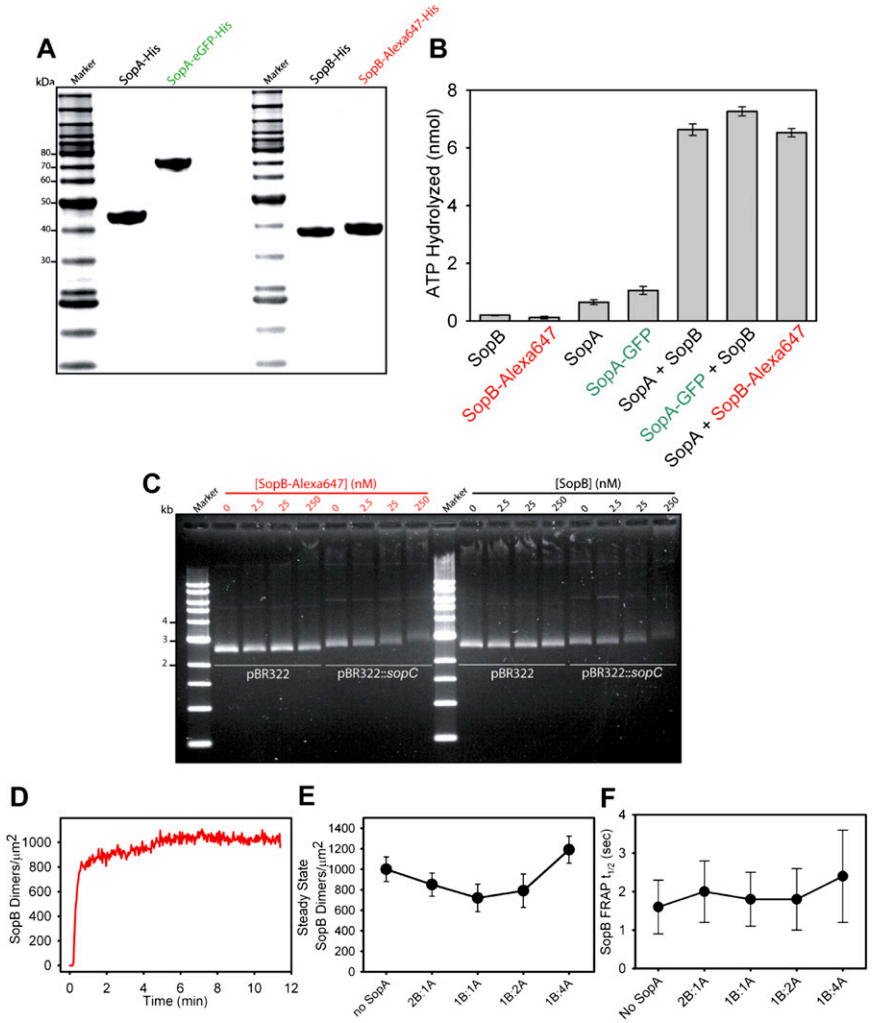


Fig. S1. Purification and in vitro characterization of unlabeled and fluorescent-labeled Sop proteins. (*A*) Coomassie stained SDS/PAGE. (*B*) C-terminal fluorescent fusions of SopA have been shown to be as efficient as native SopA in stabilizing Δ*sopA* mini-F plasmid in vivo (1, 2). To confirm that the proteins were functional in vitro, SopA ATPase activity, which is stimulated by DNA and SopB, was measured. The indicated proteins were incubated with 1 mM [γ-^{32}P] ATP and 0.1 mg/mL sonicated salmon sperm DNA for 1 h at 30 °C. The hydrolysis product then was separated and measured using TLC. SopB and SopB-Alexa647 showed no significant contaminating ATPase activity. SopA and SopA-GFP both displayed weak ATPase activity that was stimulated by SopB or SopB-Alexa647. (*C*) Site-specific binding of SopB to *sopC* was tested to determine whether Alexa647 labeling of SopB perturbed this activity. The supercoiled plasmid substrate, pBR322 or pBR322::*sopC* (1 nM), was incubated with SopB or SopB-Alexa647 at 30 °C for 30 min. The 20-μL sample then was run on a 0.8% agarose gel. Binding of SopB-Alexa647 to plasmid DNA was dependent upon the presence of *sopC*, and the binding affinity was similar to that of unlabeled SopB. (*D*) SopB-Alexa647 interaction with the DNA carpet. SopB (0.5 μM) mixed 9:1 with SopB-Alexa647 was infused onto the DNA carpet up to steady-state binding. (*E*) SopB was infused as in *D*, except that SopA-GFP also was present at the ratios indicated. Steady-state binding of SopB to the DNA carpet was not influenced by SopA. For *D* and *E*, the *y* axis is the SopB-Alexa647 intensity converted to dimers per square micrometer (*SI Materials and Methods*). Conversion included the contribution of unlabeled SopB molecules. (*F*) FRAP analysis of SopB on the DNA carpet showed that the presence of SopA had no effect on SopB recovery time. The *y* axis is the time required for 50% recovery. Results and SD (indicated by error bars) are from at least three independent experiments.

1. Vecchiarelli AG, et al. (2010) ATP control of dynamic P1 ParA-DNA interactions: A key role for the nucleoid in plasmid partition. *Mol Microbiol* 78(1):78–91.
2. Hatano T, Yamaichi Y, Niki H (2007) Oscillating focus of SopA associated with filamentous structure guides partitioning of F plasmid. *Mol Microbiol* 64(5):1198–1213.

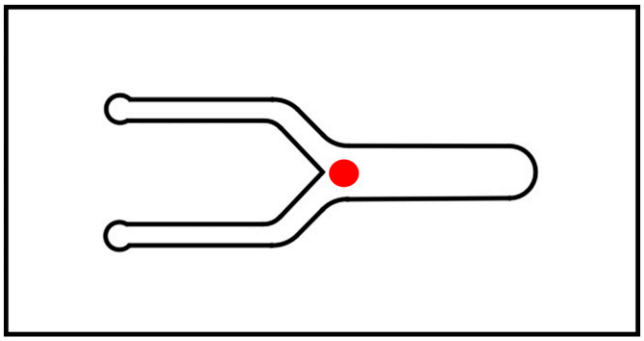


Fig. S2. Schematic of the two-inlet flow cell. The two-inlet flowcell used for buffer switch experiments had a Y-shaped flow channel. Observations were made at a position close to the point of flow convergence (red circle) to eliminate the influences of protein rebinding.

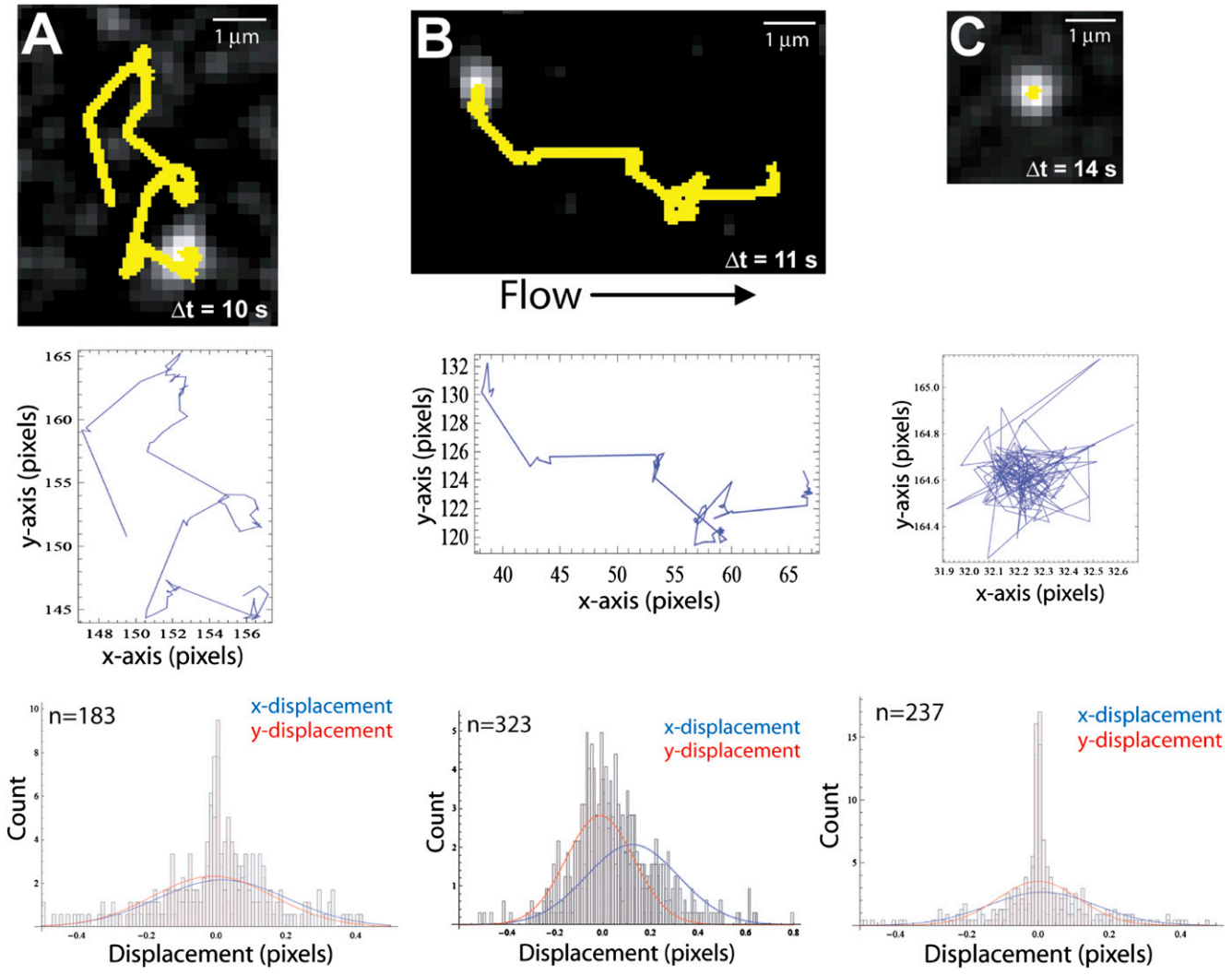


Fig. S3. Single-molecule tracking statistics of SopA-GFP. (*A*) SopA-GFP particle diffusion at low SopA density (~1% of saturation) on the DNA carpet in the absence of flow. (*B*) As in *A* but in the presence of 10 μL/min flow. (*C*) As in *A* but at full SopA saturation density on the DNA carpet. In *A*–*C* all trajectories (yellow lines) start when SopA-GFP binds to the DNA carpet and end at the point of release or photobleaching. The *x*- and *y*-axis displacements of individual particles with residence times greater than 20 frames (2 s) were plotted (*Middle Row* shows examples). The *x*- and *y*-axis displacements per 0.1 s of all tracked particles were plotted on a histogram and fit to a Gaussian distribution (*Bottom Row*). With flow, *x*-displacement was biased in the direction of flow. At high SopA density, a significant fraction of particles exhibited restricted mobility. Refer to Movie S2.

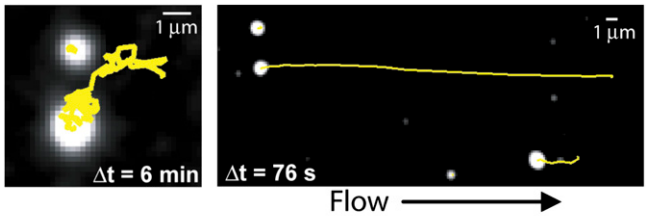
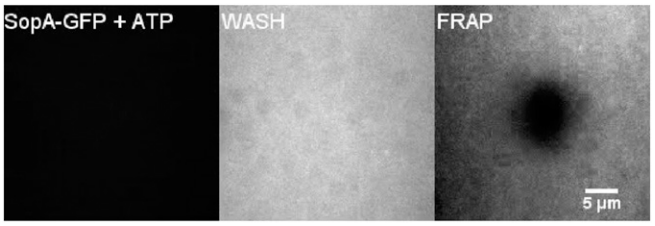
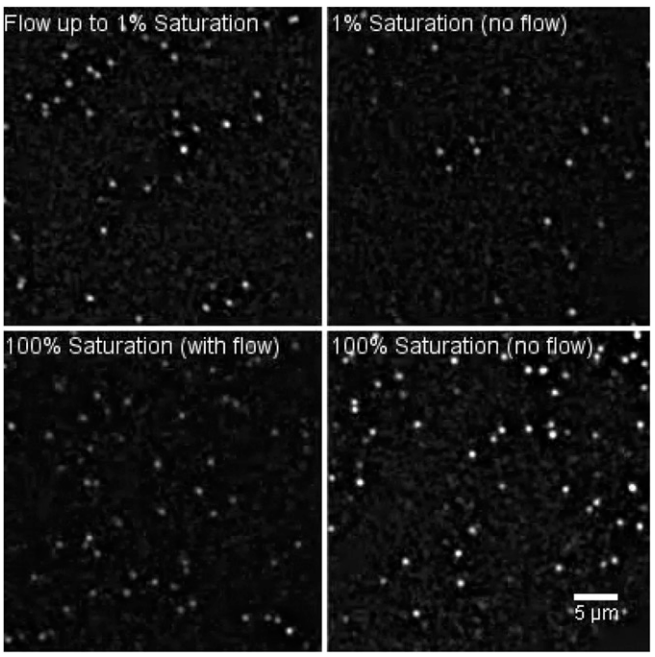


Fig. S4. Examples of fluorescent plasmid clusters diffusing laterally on the DNA carpet. SopA-GFP (0.5 μM), SopB (1 μM), and *sopC*-647 (0.1 nM) were pre-incubated together with 1mM ATP for 15 min at 23 °C and infused into the flowcell at 20 μL/min for 2 min before flow stoppage. (*Left*) Plasmid clusters were tracked (yellow line) to the point of release from the DNA carpet. 2D movement was random in the absence of flow. (*Right*) When flow was initiated many plasmids rolled in the direction of flow while maintaining contact with the DNA carpet. Only *sopC*-647 fluorescence is displayed.



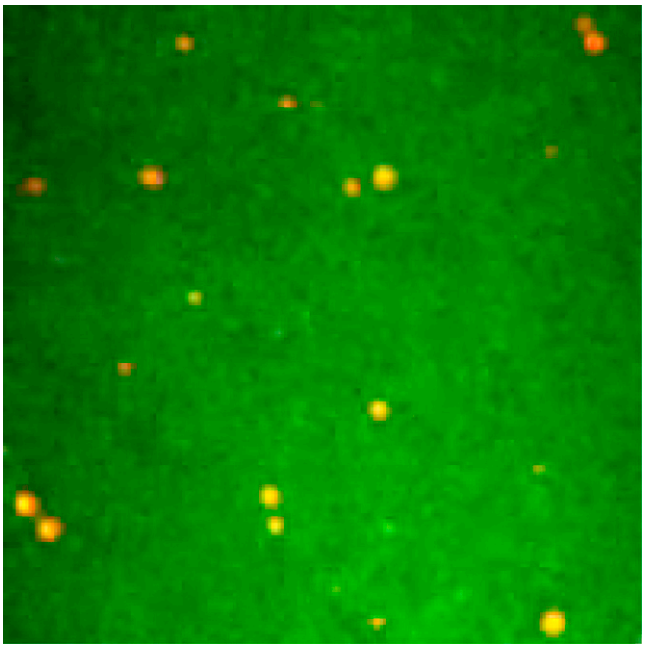
Movie S1. Binding, release, and exchange of SopA-GFP on a DNA carpet. SopA-GFP (0.5 μM) was mixed with 1 mM ATP during infusion at 20 μL/min onto the DNA carpet (*Left*). Once SopA-GFP binding reached saturation, the flow was switched to wash buffer, and dissociation was acquired (*Center*). FRAP analysis of SopA-GFP exchange on the DNA carpet (*Right*). Videos are 30 times faster than real time. Display areas are 33 × 33 μm.

Movie S1



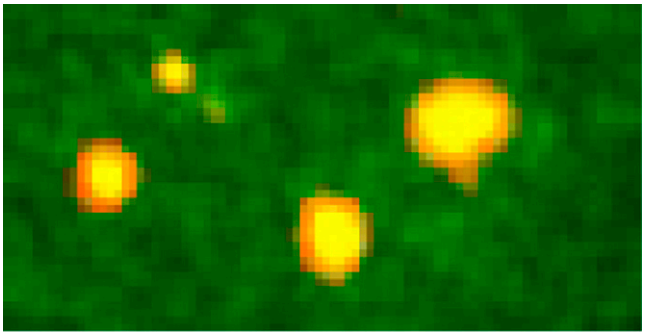
Movie S2. Single-molecule tracking of SopA-GFP. SopA (500 nM) and SopA-GFP (50 pM) were mixed with 1 mM ATP and infused onto the DNA carpet at 10 μL/min up to ~1% of the SopA saturation density (*Upper Left*). Flow then was stopped (*Upper Right*). Flow was reinitiated until SopA binding to the DNA carpet reached saturation (*Lower Left*), and flow was stopped once again (*Lower Right*). Videos are 1.5 times faster than real time. Display areas are 35 × 35 μm. Buffer flow was from left to right when used.

Movie S2



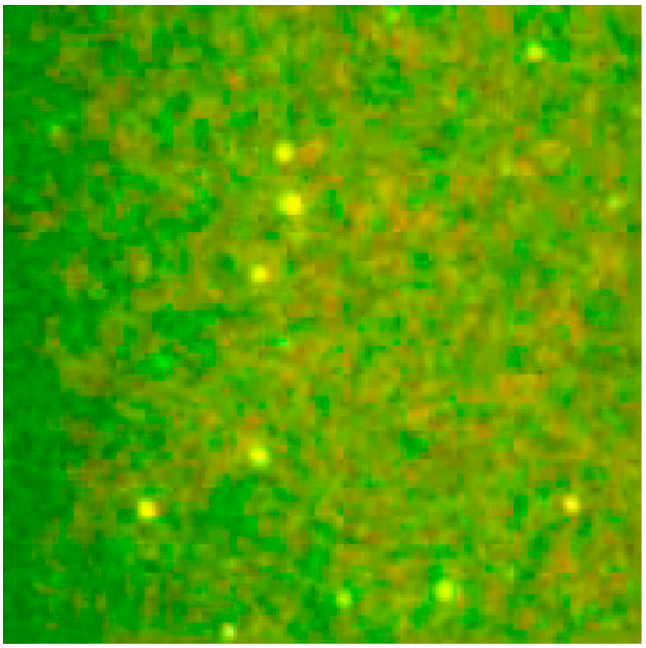
Movie S3. Formation of the partition complex cluster on the DNA carpet. SopA-GFP (0.5 μM, green), SopB (1 μM), and *sopC*-647 (0.1 nM, red) were preincubated together with 1mM ATP for 15 min at 23 °C and were infused onto the DNA carpet at 20 μL/min for 2 min before flow stoppage. The video rate is 60 times faster than real time, and the display area was cropped to 33 × 33 μm.

Movie S3



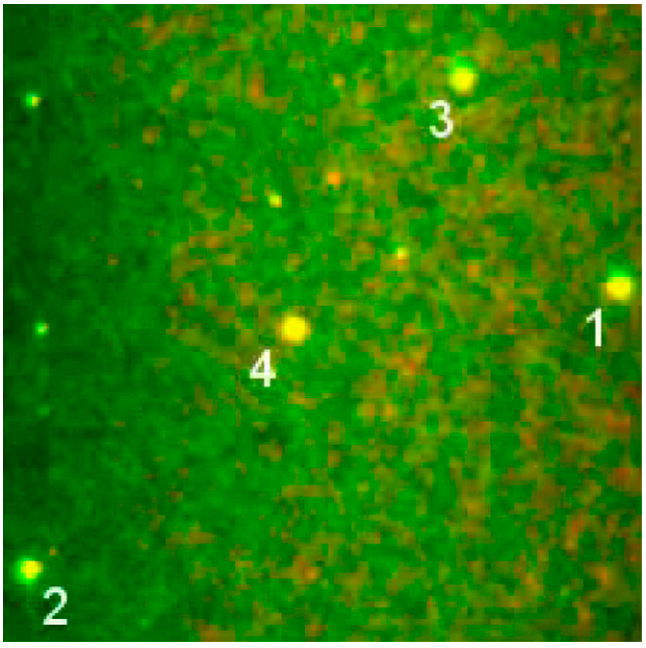
Movie S4. SopA-GFP (green) and *sopC*-647 (red) dynamics on the DNA carpet after preincubation. Conditions are as in Movie S3, except the display area was cropped to 8 μm × 16 μm.

Movie S4



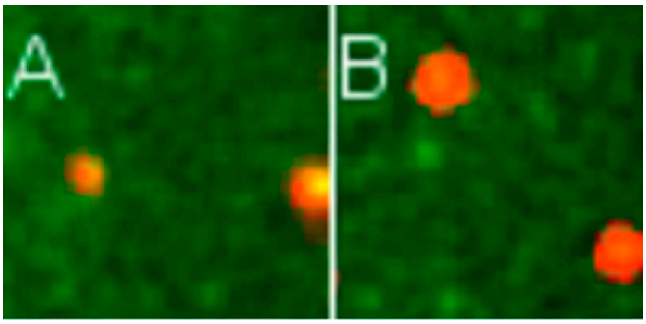
Movie S5. SopA-GFP (green) and SopB-647 (red) dynamics on plasmid clusters. SopA-GFP (0.5 μM), SopB (1 μM mixed 9:1 with SopB-Alexa647), and pBR322::*sopC* (0.1 nM) were preincubated with 1 mM ATP for 1 h at 23 °C and were infused into the flowcell at 20 μL/min for 2 min before flow stoppage. The video rate is 60 times faster than real time, and the display area is 40 × 40 μm.

Movie S5



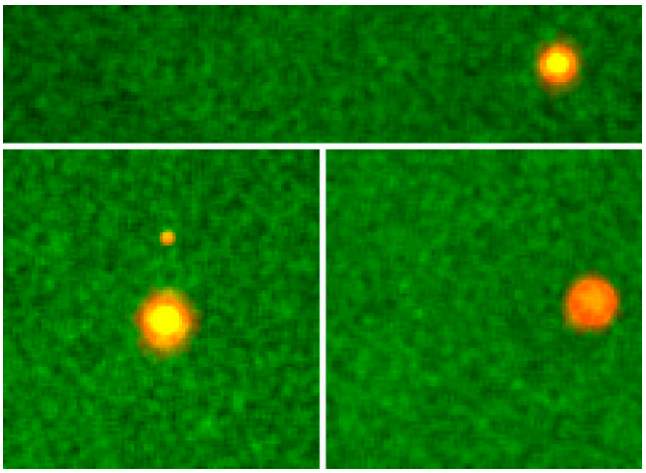
Movie S6. SopA-GFP (green) and SopB-647 (red) dynamics on plasmid clusters. Conditions are as in Movie S5, except plasmid foci are numbered in the order in which they detached from the carpet. The video rate is 60 times faster than real time, and the display area is 40 × 40 μm.

Movie S6



Movie S7. Examples of F plasmid clusters traveling across the DNA carpet. (*A*) Conditions are as in Movie S3, except the imaged areas were cropped to plasmid clusters that diffused laterally on the carpet before their first release. The video rate is 60 times faster than real time. (*B*) Conditions are as in *A*, except the video was acquired 90 min after flow stoppage, and the plasmid clusters were revisiting the carpet after their initial release. The video is 1.5 times faster than real time. Display areas are 10 × 10 μm.

Movie S7



Movie S8. Examples of P1 plasmid clusters revisiting and traveling across the DNA carpet. ParA-GFP (0.5 μM), ParB (0.5 μM), and pBluescript::*parS*-Alexa647 (0.5 nM) were preincubated with 1 mM ATP for 1.5 h at 23 °C and were infused into the flowcell at 5 μL/min for 3 min. Movies were acquired after flow was stopped. Plasmid cluster movement was biased from right to left because of residual flow in flowcell. The video rate is 10 times faster than real time. Display areas are 200 × 45 μm (*Upper*) and 100 × 100 μm (*Lower*).

Movie S8