Hopping of a processivity factor on DNA revealed by single-molecule assays of diffusion

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Supporting Information

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SI Materials and Methods

Protein Labeling. UL42ΔC340 was labeled with Cy3B Mono Maleimide (Amersham Biosciences) according to the manufacturer's instructions, except that TCEP was not removed from the buffer before conjugation. MBP-UL42ΔC340 protein was labeled with fluorescein-5-maleimide (Invitrogen) according to the manufacturer's instructions. To separate labeled protein from free dye, the product of the reaction was purified on a Superdex200 HR column (GE Healthcare). The final dye/protein ratio was estimated by measuring the absorbance of the protein and dye at 280 and 558 nm, respectively, and performing calculations according to the manufacturer's instructions. Approximately 70% of the protein was labeled with Cy3B and 62% with fluorescein.

DNA Preparation for Single-Molecule Assays. λ DNA with biotin on one end was prepared by ligating a modified oligo 1 (5′-/5Phos/AGGTCGCCGCCC/3Bio/-3′; Integrated DNA Technologies) to the 12-base overhang on λ DNA (New England Biolabs) using T4 DNA ligase (New England Biolabs). λ DNA with two biotin ends was prepared by ligating modified oligo 2 (5′-/5Phos/GGGCGGCGACCT/3Bio/-3′) to the other 12-base overhang on λ DNA that already had one end modified with oligo 1. λ DNA that had one end-labeled with biotin and the other end blunt was prepared by ligating oligo 1 to one end and oligo 2 without biotin to the other end.

Single-Molecule Imaging. Single-molecule assays of protein motion were performed and analyzed as described by Blainey *et al.* (13). Briefly, to prepare the experimental flow chamber, glass coverslips (24 × 50 mm) were cleaned by sonication in KOH and ethanol. Cleaned glass coverslips were silanized with 3-aminopropyltriethoxysilane (Sigma-Aldrich) and incubated with a mixture of mPEG-SPA-5000 and Biotin-PEG-CO_2NHS-3400 polyethylene glycol (at a ratio of 100:1; Nektar Therapeutics) to produce a polyethylene glycol monolayer that minimized nonspecific interactions of the proteins and DNA with the surface. Subsequently, the functionalized surface was incubated with 0.2 mg/ml streptavidin (Sigma-Aldrich). The flow chamber was assembled with a coverslip and a cleaned glass slide (75 × 25 mm) separated by an adhesive spacer (0.12 mm thick, Grace Biolabs) with a precut section of 2 × 40 mm forming the channel. Inlet and outlet tubing was glued in holes on the slide and the chamber was connected to a withdrawing syringe pump (PhD2000, Harvard Apparatus). The streptavidin-coated flow-cell surfaces were blocked by incubation with blocking buffer [20 mM Tris, 2 mM EDTA, 50 mM NaCl, 0.2 mg/ml BSA, 0.005% Tween 20 (pH 7.5)] for 20 min. Biotin-modified DNA constructs were introduced into the flow cell at a rate of 0.1 ml/min at a concentration of 2–5 pM and allowed to bind for 5 min. These conditions resulted in an average density of ≈25 surface-tethered DNA molecules per field of view (≈50 × 50 μm^2).

Single-molecule fluorescence imaging of the motion of UL42 along DNA was performed as follows: Fluorescently labeled UL42ΔC340 was dissolved at a concentration of 5–50 pM in imaging buffer, containing 20 mM Tris (pH 7.5), 0.1 mM EDTA, 0.4 mM DTT, 40 μg/ml BSA, 4% glycerol, and varying amounts of NaCl, and was drawn into the channel by a syringe pump at a flow rate of 0.1 ml/min, creating shear flow near the surface of the coverslip. The flow cell was placed on the stage of an inverted microscope (Olympus IX71) and fluorescence was excited using the 520-nm line of an Ar/Kr laser (Coherent I-70 Spectrum). A high-N.A. microscope objective (Olympus; 60×, N.A. = 1.45) was used to illuminate the sample with total-internal reflection. The illuminated area had a diameter of 50 μm at the focal plane. The fluorescence was collected by the same objective and imaged by an EM-CCD camera (Andor iXon). Image sequences of 100–1000 frames were recorded at 0.33–14 frames/s for a total recording time of 3.3–300 s. Single-molecule tracking was performed using MetaMorph software (Molecular Devices) by subjecting each image to a 2D Gaussian filter, determining the centroid of each fluorescent object, and after the change of position of the objects by 2D cross-correlation calculation (34). Thus, an array of x and y coordinates, $(x_i, y_i)_{i=1 \text{ to } N}$, described the trajectory of each molecule, where N was the total number of points. N depended on the time interval over which each UL42ΔC340-Cy3B molecule remained attached to the DNA molecule. The mean square displacement was calculated as

$$\mathrm{MSD}(\Delta t_n) = \frac{1}{N-n} \sum_{i=1}^{N-n} [(x_{i+n} - x_i)^2 + (y_{i+n} - y_i)^2], \quad [1]$$

where $\Delta t_n = n \times \delta$, and δ is the time interval between successive frames (35). The diffusion coefficient of the UL42 molecule, D, was estimated from the initial slope of the MSD vs. Δt curve, i.e., by fitting a straight line to the MSD calculated at Δt_1, Δt_2, Δt_3, and Δt_4.

To measure UL42 dissociation from DNA ends, λ DNA was attached to the slides and incubated first with MBP-UL42ΔC340-fluorescein and then with anti-fluorescein conjugated quantum dots QD565 (Molecular Devices). The concentrations of fluorescein-MBP-UL42 used in the assay ranged from 0.001–100 pM. The concentrations of anti-fluorescein quantum dots was 5 nM. After a flushing step using a low flow rate, the flow was increased to 100 μl/min as the image recording was started. To analyze the decay of fluorescence intensity at the ends, a small rectangle was drawn around the end of each stretched DNA in the field of view, and the integrated intensity within each rectangle was normalized against its initial value. The normalized integrated intensities were then summed, and the decay of fluorescence intensity over time was fit using a single exponential.

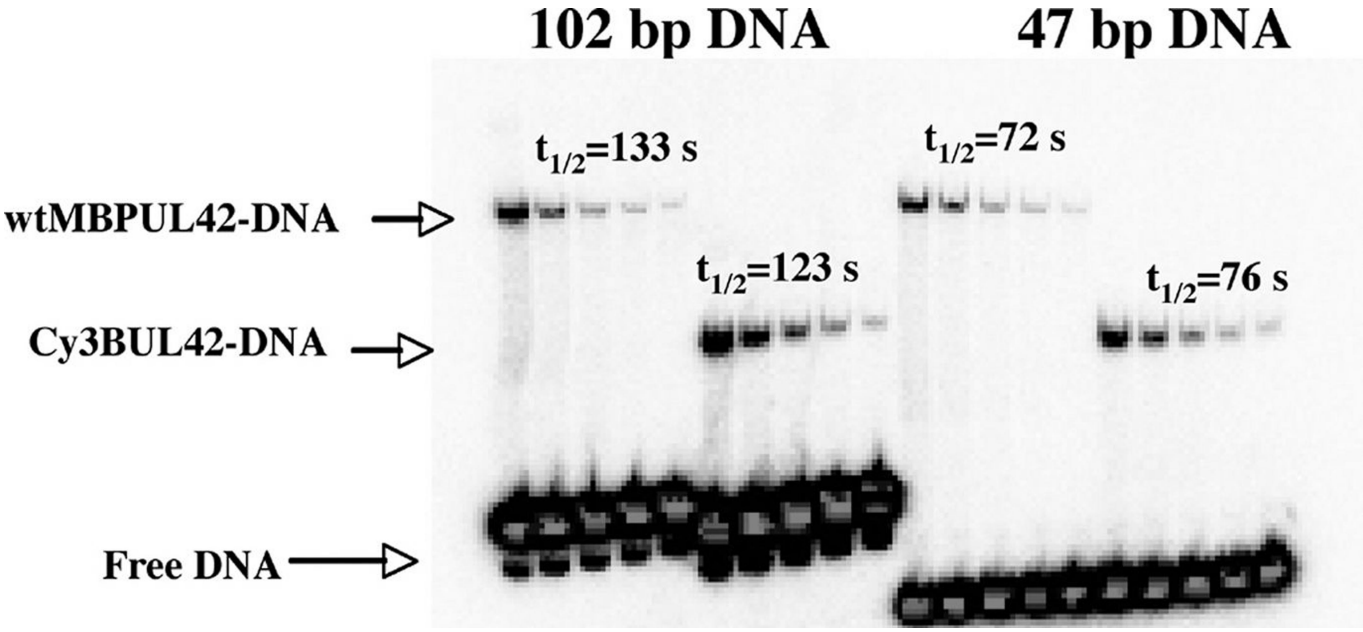


Fig. S1. Cy3B labeled UL42 behaves the same as unlabeled UL42 protein in EMSAs. Radiolabeled 102-bp or 47-bp DNA was incubated with either wild-type MBP-UL42 or Cy3B labeled UL42. Formation of the UL42-DNA complex was quenched by the addition of cold competitor DNA and at various times after quench reaction aliquots were loaded onto a running native 4% polyacrylamide gel to separate bound DNA from free probe. Calculated half lives ($t_{1/2}$) of Cy3B-labeled UL42-DNA complexes are shown.

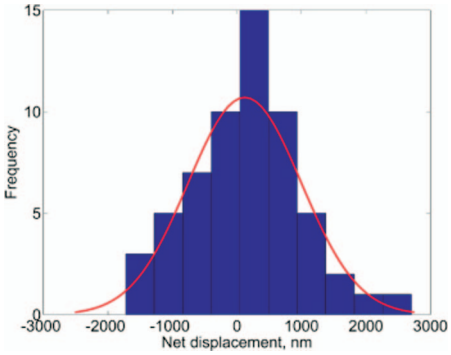
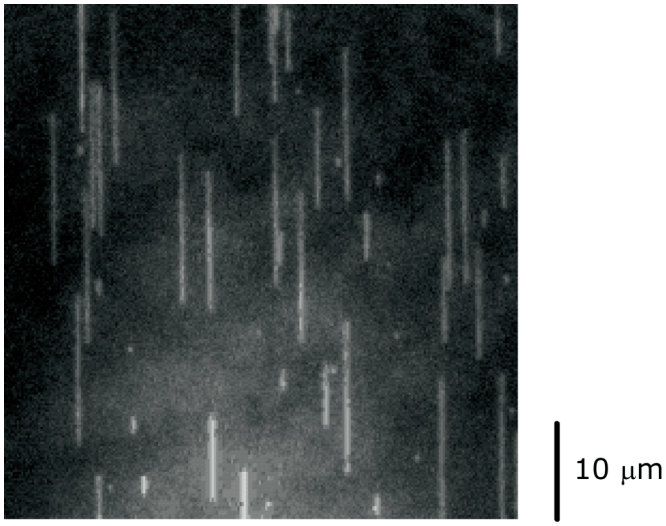
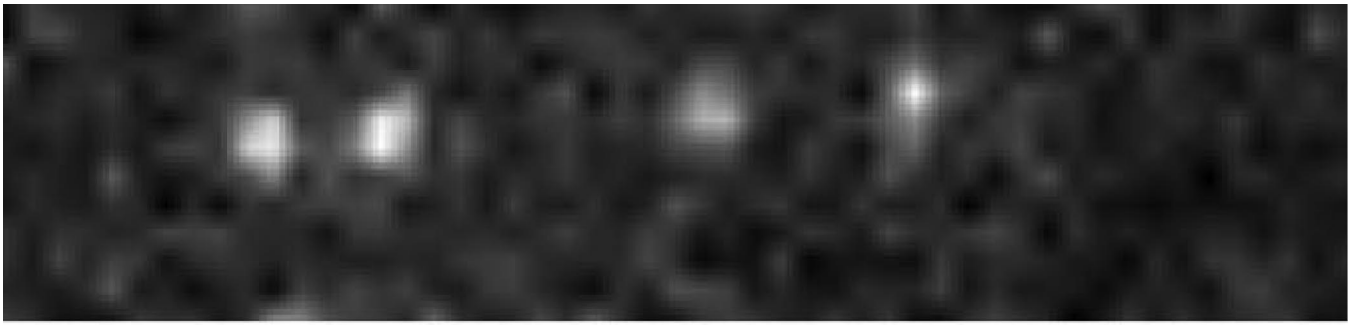


Fig. S2. Histogram showing net displacement of UL42 molecules on doubly tethered lambda DNA in the absence of buffer flow.



Movie S1. Lambda DNA molecules stained with SYTOX orange dye and stretched with buffer flow.

Movie S1

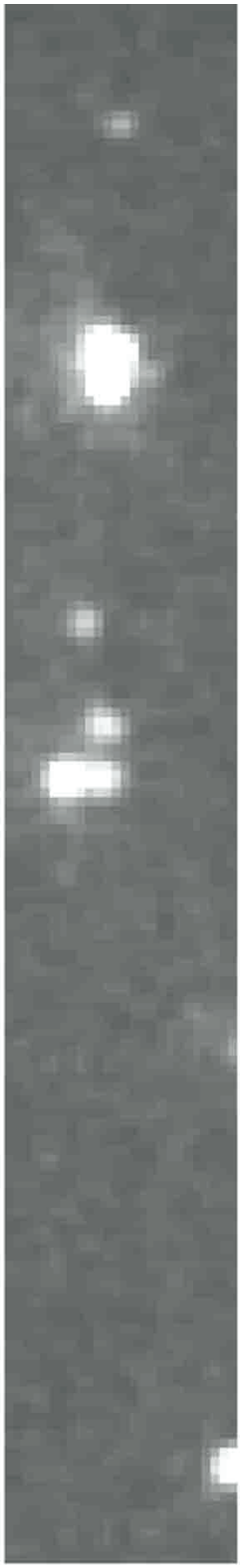


Movie S2. Diffusion of single molecules of UL42 on lambda DNA. Shown is the movement of four Cy3B-labeled UL42 molecules on DNA stretched by buffer flow. (Scale bar: 5 μM.)

Movie S2

Movie S3. Movement of Cy3B labeled UL42 molecules on stretched and doubly tethered lambda DNA in the absence of buffer flow.

Movie S3



Movie S4. Single molecules of UL42 fused to MBP and coupled to fluorescent quantum dots diffusing on doubly tethered lambda DNA in the absence of buffer flow.

Movie S4

Movie S5. Single molecules of UL42 fused to MBP and coupled to fluorescent quantum dots diffusing on doubly tethered lambda DNA in the absence of buffer flow at the beginning of the movie and with buffer flow after the start of the movie.

Movie S5

Table S1. Parameters calculated from EMSAs and single molecule assays

	Data from EMSA experiments			Data from single-molecule experiments	
[NaCl]	$k_{off(internal)}$, s^{-1}	Mean binding lifetime, s	D, bp^2/s	Mean binding lifetime, s	D, bp^2/s
10 mM	2.3×10^{-3}	430	Not detected	120	4.4×10^4
25 mM	4.7×10^{-3}	220	Not detected	Not tested	Not tested
50 mM	6.5×10^{-3}	160	12	65	5.9×10^4
100 mM	Not tested	Not tested	Not tested	22	19×10^4