Supplementary Materials

Stochastically multimerized ParB orchestrates DNA assembly as unveiled by single-molecule analysis

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Supplementary Figures S1 – S19
Supplementary Table S1
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Supplementary Figure S1. Construction of the 8x parS DNA templates.

A. The 21.5-kbp DNA template containing 8x parS sites consists of three DNA segments, L1, L2, and a parS segment. The 1x parS DNA template was generated similarly except that the parS segment was prepared by annealing two partially complementary oligonucleotides (Supplementary Table S1). B. The 18.6-kbp DNA template containing two copies of 8x parS sites a ligation product of two parS-containing segments. See the Materials and Methods section for the detailed procedures for the construction of these two DNA templates.
The 8x *parS*-containing DNA sequence: 5’-...TGTTACACGTGAAACACGCAC
TCAGCTTGACATGGCGCTAAATATCGGCCTTTACACGTGAAACACGCAC
ATACACCCGGCATCGCGTCTCTTGTTACACGTGAAACAGCCATCCAGATTGTACTC
ACCGATGGACACAACGTGTTACACGTGAAACACGCACCGGATCCAGATTGTACTC
ACCGATGGACACAACGTGTTACACGTGAAACACGCACCGGATCCAGATTGTACTC
ACCGATGGACACAACGTGTTACACGTGAAACACGCACCGGATCCAGATTGTACTC
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ACCGATGGACACAACGTGTTACACGTGAAACACGCACCGGATCCAGATTGTACTC
ACCGATGGACACAACGTGTTACACGTGAAACACGCACCGGATCCAGATTGTACTC

The *parS* motifs are colored red.
Supplementary Figure S2. Characterization of the purified ParB-eGFP protein.

A. SDS-PAGE analysis of the purified ParB-eGFP (68 kDa) protein. B. Chromatographic analysis of the ParB-eGFP protein. Size exclusion chromatography of ParB-eGFP eluted from the Superdex 200 Increase 10/300 GL column (red arrow). The black arrows 1 - 3 indicate marker proteins with molecular weights of 440 kDa, 158 kDa, and 75 kDa, respectively. These data suggest that the ParB-eGFP protein preferentially exists in a dimeric form (136 kDa). C. CTPase activity of ParB proteins in the presence of parS-containing or nonspecific DNA measured by malachite green detection of inorganic phosphate. Error bars represent SEM. D. Electrophoretic mobility shift assay (EMSA) to examine and compare the nsDNA binding activities of WT ParB and ParB-eGFP.
The CTPase activity of ParB proteins was measured by Malachite Green colorimetric detection. The sequences of the 1x parS DNA and the 58-bp nsDNA used in this experiment are listed in Supplementary Table S1. The sample containing 0.5 μM DNA, 4 μM protein, and 2 mM CTP was mixed and incubated at room temperature for 30 min. The diluted sample was mixed with the working reagent from the Malachite Green Phosphate Assay Kit (Sigma, MAK307) and transferred to a 96-well plate for a 30-min incubation. The absorbance of the samples at a wavelength of 620 nm was measured using the SpectraMax i3x (Molecular Devices). All experiments were repeated three times. This set of data indicates CTPase activity of ParB-eGFP similar to that of WT ParB. In contrast, two ParB mutants lack the CTPase activity as expected.

A Cy5-labeled 53-bp nsDNA was used in the EMSA. WT ParB and ParB-eGFP at indicated concentrations were incubated with 0.5 nM nsDNA for 10 min, and the reaction was then quenched by the addition of 10 μl of 50% glycerol. Samples were resolved on 8% TBE PAGE gel at 150 V at 4 °C for 45 min. Gels were exposed to a phosphor screen and subsequently scanned by Typhoon FLA 9500 (GE Healthcare) for imaging. The purified ParB-eGFP protein exhibited nsDNA binding activity similar to that of WT ParB.
Supplementary Figure S3. DNA condensation detected under 250 nM ParB-eGFP and 0.1 pN.

Representative DNA length vs. time of a λ DNA shows ParB at 250 nM condensing DNA under 0.1 pN.
Supplementary Figure S4. DNA dissociation of nonmultimeric ParB proteins in the absence and presence of CTP.

A. A representative kymograph of a λ DNA molecule under 1 pN. After a 1-min incubation in the 20 nM ParB-eGFP channel, the DNA tether was transported to the buffer channel containing no CTP. B. A representative kymograph of a λ DNA molecule under 1 pN. After a 1-min incubation in the 20 nM ParB-eGFP channel, the DNA tether was transported to the buffer channel containing 10 mM CTP. Scale bar, 5 μm. C. Three-dimensional plot of the corresponding fluorescence intensity of ParB-eGFP along the DNA in A at indicated time points. D. Three-dimensional plot of the corresponding fluorescence intensity of ParB-eGFP along the DNA in B at indicated time points. E. Time-evolution of the average overall fluorescence intensity of ParB-eGFP along the DNA in the absence (n = 15) and presence (n = 19) of CTP.
Supplementary Figure S5. ParB multimer assembled on DNA under high salt concentration.

Representative kymograph and DNA length vs. time of a DNA tether show the formation of the ParB multimer in the buffer channel containing 100 mM NaCl. The final fluorescence intensity profile of the DNA molecule is shown alongside the kymograph. The scale bar represents 5 μm.
Supplementary Figure S6. Multimers formed by ParB-AF555 and ParB-Cy5 and the stable DNA binding of the ParB-eGFP multimer exhibited by a protein exchange assay.

A. Schematic representation of ParB showing a conserved architecture consisting of an N-terminal domain (NTD), a central DNA-binding domain (DBD), and a C-terminal domain (CTD) (1). The cysteine was inserted adjacent to the N-terminus of ParB for fluorescent labeling. B-C. A kymograph of a DNA molecule under 1 pN showing the multimers formed by ParB-AF555 (B) and ParB-Cy5 (C) in the buffer channel. Scale bars represent 5 μm and 10 s, respectively. D. Representative kymographs of a DNA molecule showing DNA binding of ParB molecules in the ParB-eGFP (blue) channel and the ParB-Cy5 (red) channel. A merged kymograph is shown below. Scale bars represent 5 μm and 10 s, respectively. E. The overall fluorescence intensity of multimeric and nonmultimeric ParB as a function of time. The nonmultimeric ParB-eGFP proteins were quickly replaced by the ParB-Cy5 proteins, whereas the multimeric ParB-eGFP remained stable binding to DNA.
Supplementary Figure S7. Quantification of the ParB-eGFP protein in multimer.

A. Confocal image of a dCas9-eGFP/sgRNA complex bound to λ-DNA molecule with a 10% excitation intensity (11.9 μW) and the fluorescence intensity profile of the DNA. The green arrow highlights the DNA-bound dCas9-eGFP/sgRNA complex. B. An individual dCas9-eGFP molecule from (A). The intensity profiles in the x and y directions (black dots) were fitted to a Gaussian function (solid blue line). C. A Gaussian fitting of the fluorescence intensity of individual eGFP, n = 31. D. Confocal image of a ParB multimer bound to λ-DNA molecule with a 10% excitation intensity and the fluorescence Intensity profile of the DNA. The red arrow highlights the DNA-bound ParB multimer. E. An individual ParB multimer from (D). The intensity profiles in the x and y directions (black dots) were fitted to a Gaussian function (solid blue line).

To calculate the number of ParB-eGFP molecules in each multimer, we first quantified the fluorescence intensity of a single eGFP protein as previously described (2). Using the bead as a reference, the intensity distribution along the DNA was aligned to confirm the location of the dCas9-eGFP/sgRNA probe on
the λ DNA. The total fluorescence intensity and the background intensity under the given imaging conditions were calculated by integrating the number of photons within an ROI of 20 x 20 pixels around the probe. The fluorescence intensity of a single eGFP was calculated by subtracting the background intensity. Gaussian fitting was performed on the experimental data to calculate the average intensity of a single eGFP. Next, the fluorescence intensity of a ParB multimer was measured and calculated under the same experimental conditions. The ParB-eGFP protein in each multimer was quantified by dividing the total intensity of the ParB multimer by the intensity of a single eGFP.
Supplementary Figure S8. Representative kymograph of diffusive ParB multimers in the presence of 10 mM CTP.

A representative kymograph of a DNA molecule under 1 pN shows the diffusion of three ParB multimers in the presence of 10 mM CTP. Scale bars represent 5 μm and 10 s, respectively. The final fluorescence profile of ParB proteins along the DNA is shown alongside the kymograph.
Supplementary Figure S9. The diffusion of ParB-Cy5 and ParB-AF555 multimers along DNA.

Representative kymographs of DNA molecules in the buffer channel containing 10 mM CTP after a 1-min incubation in the channel containing 50 nM ParB-Cy5 or ParB-AF555. Scale bars represent 5 μm and 10 s, respectively. Consistent with the findings with ParB-eGFP, CTP also supports a long-term diffusion of ParB-Cy5 (red) and ParB-AF555 (cyan) multimers along DNA.
Supplementary Figure S10. Characterization of the diffusion of the ParB multimer.

A. A representative trajectory of a diffusive ParB multimer in the presence of 10 mM CTP. A first-order derivative of the trajectory was calculated and plotted below. The reversion of the sliding direction of the ParB multimer is indicated by red arrows. The sliding distance and rate are calculated between two reversion events. B. Mean square displacement (MSD) of diffusive ParB multimers in the presence of 0, 2, and 10 mM CTP. The MSDs in kbp² as a function of time were linearly fitted (red). Regression evaluation indexes ($R^2$) are shown. The dotted red line indicates imperfect fitting.
Supplementary Figure S11. The diffusion of the multimers formed by two CTP binding and/or hydrolysis-deficient ParB mutants.

A. Schematic representation of the NTD, DBD, and CTD domains of ParB. The locations of the mutant genes of ParB are indicated by the black arrows. B. Representative kymographs of diffusive multimers formed by the two mutants (R80A and N112S) under 2 mM CTP. C. The diffusion rates of the ParB-eGFP, R80A, and N112S multimers under 2 mM CTP. n = 31, 15, 31 respectively. D. The processivities of the ParB-eGFP, R80A, and N112S multimers under 2 mM CTP. n = 32, 14, 31 respectively. E. The diffusion coefficients of the ParB-eGFP, R80A, and N112S multimers under 2 mM CTP. n = 32, 17, 37 respectively.
Supplementary Figure S12. ParB Binding to an 8x *parS*-containing DNA template and a non-*parS* DNA template.

**A.** ParB multimer formation on the 26.8-kbp DNA template. The 26.8-kbp DNA template with the 8x *parS* motifs located in the middle of DNA was constructed similarly. A representative kymograph of the DNA molecule in the buffer channel after a 1-min incubation in the 20 nM ParB channel indicates the binding of a ParB multimer to the *parS* motifs. Scale bars represent 3 μm and 10 s, respectively.

**B.** ParB multimer formation on the 21.5-kbp DNA template. The 21.5-kbp DNA template containing no *parS* was constructed similarly to the one containing *parS* except that the *parS* segment was replaced by a 1-kbp nsDNA segment. Scale bars represent 1 μm and 10 s, respectively. The examined DNA molecules did not show preferential binding of ParB multimers to the 1-kbp DNA, supporting that the detected fluorescence signals around the *parS* region result from the specific association of ParB multimers with the *parS* sites.
Supplementary Figure S13. ParB multimer escaping from parS sites.

A. Schematic of the 8x parS DNA template showing the position of the parS sites (highlighted in red) in DNA (not to scale). Representative kymograph and derived real-time trajectory of a ParB multimer showing it escaped from the 8x parS sites in the presence of 10 mM CTP. The yellow star indicates the positions of the 8x parS sites. Scale bars represent 2 μm and 10 s, respectively. B. Schematic of the 1x parS DNA template showing the position of the parS site (highlighted in red) in DNA (not to scale). Representative kymograph and derived real-time trajectory of a ParB multimer showing it escaped from the 1x parS sites in the presence of 10 mM CTP. The yellow star indicates the position of the 1x parS site. Scale bars represent 2 μm and 10 s, respectively.
Supplementary Figure S14. The ParB multimer collision on nsDNA.

A. Representative kymographs and derived real-time trajectories of ParB multimers show that a diffusive multimer remains independent after a collision with another diffusive or immobile multimer on nsDNA. Scale bars represent 5 μm and 10 s, respectively. The yellow and blue arrows indicate the occurrence of collision events. B. Representative kymographs and derived real-time trajectories of ParB multimers showing that two multimers fuse after a collision on nsDNA. Scale bars represent 5 μm and 10 s, respectively. The yellow and blue arrows indicate the occurrence of collision events. 36% of the multimers fused after a collision.
Supplementary Figure S15. Confocal images of multimeric and nonmultimeric ParB-eGFP and captured DNA.

A. Representative images present a ParB-eGFP multimer capturing two DNA molecules. B. Representative confocal images show that nonmultimeric ParB-eGFP on λ DNA is incapable of capturing free DNA. Schematics of the stretched λ DNA and free DNA used are shown. Scale bars, 5 μm.
Supplementary Figure S16. DNA bridging and transportation mediated by the ParB-Cy5 multimer.

A. Representative images of DNA (green) bridging mediated by the ParB-Cy5 multimer (red) Merged images and zoom-in views of the selected regions (yellow) are shown on the right. B. Representative images of merged confocal images showing the movements of the complex (indicated by gray arrows) formed by the ParB-Cy5 multimer and parS-containing DNA. The dotted reference line is shown. Scale bar, 2.5 μm.
Supplementary Figure S17. CTP promotes DNA bridging and transportation of the ParB-eGFP multimer.

A. Representative kymographs of a DNA-bound ParB-eGFP multimer (green) bridging and transporting parS-oligo-Cy5 (red) in the absence of CTP. B. Representative kymographs of a DNA-bound ParB-eGFP multimer (green) bridging and transporting ns-oligo-Cy5. Scale bars represent 5 μm and 10 s, respectively. C. The percentage of DNA-bound ParB-eGFP multimer that bridges parS-oligo or ns-oligo in the absence and presence of CTP. n = 72, 77, 34, and 72 from left to right. D. The diffusion coefficients of the ParB-eGFP multimer-parS oligo complex in the absence and presence of CTP. n = 17 and 17, respectively.

Based on these findings, we conclude that CTP promotes DNA bridging and transportation mediated by the ParB-eGFP multimer.
Supplementary Figure S18. DNA-bound ParB-eGFP multimers transport bridged 18.6-kbp DNA.

Confocal images of the DNA-bound ParB-eGFP multimer (green) bridging and transporting parS-containing (A) and nonspecific (B) DNA (red). The merged images are shown in yellow on the right. The ParB-DNA complexes are indicated by gray arrows. Scale bar, 2 μm.
Supplementary Figure S19. Colocalization of the ParB-eGFP multimer with the parS-oligo-Cy5 during diffusion.

Representative kymographs of a DNA molecule showing the colocalization of the ParB-eGFP multimers (green) with parS-oligo-Cy5 (red) and their simultaneous diffusion in the presence of 2 mM CTP. Scale bar, 3 μm. The fluorescence intensity profiles of numbered ParB-eGFP multimers (green) and parS-oligo-Cy5 (red) at indicated times are shown on the right.
**Supplementary Table S1. Oligonucleotides and primers used for the construction of the DNA templates.**

<table>
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<tr>
<th>Segment</th>
<th>Oligonucleotide or Primer</th>
<th>Sequence</th>
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<tr>
<td>L1</td>
<td>Forward (L1)</td>
<td>5'- biotin-TGATAAGCAGAATGGCATCGTTCC - 3'</td>
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<tr>
<td></td>
<td>Reverse (L1)</td>
<td>5'- CCAAAATCGTGGAGGCATCCACCC AAAATTCAG - 3'</td>
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<td></td>
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<td></td>
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The parS motifs are colored red.
Supplementary References
