SUPPLEMENTAL METHODS

Overview – Purification of individual subunits and reconstitution of the γ complex has been described previously as referenced below. These procedures were followed with minor modifications, and unless otherwise noted all steps were carried out at 4 °C. Typical yields are 60 – 75 mg of pure protein per liter of bacterial culture, and procedures are based on starting with 2 L of bacterial culture. For convenience, these procedures are described in together here.

Buffers – Buffer A contains 20 mM Tris•HCl pH 7.5, 2 mM DTT, 0.5 mM EDTA, and 10 % glycerol. Buffer B contains 30 mM HEPES pH 7.2, 0.5 mM EDTA, 2 mM DTT, and 10 % glycerol.

Cell lysis – Cell pellets were thawed on ice and resuspended in Buffer A or Buffer A supplemented with 0.1M NaCl (4 mL/g of cells). Cells were lysed by two passes through a French press at about 17,000 psi. The cell lysate was clarified by centrifugation at 20,000 x g for 30 min. The proteins are present in the supernatant and cell debris was discarded.

Purification of the β-clamp (*1*) – Ammonium sulfate (0.194 g/mL, 35% saturation) was added to the clarified cell lysate. The precipitate was removed by centrifugation at 31,000 x g for 30 min and discarded. Additional ammonium sulfate (0.218 g/mL, 70% saturation) was added to the supernatant, and the precipitate was recovered by centrifugation at 9800 x g for 20 min. The pellet was resuspended in Buffer A and dialyzed against Buffer A. The dialyzed protein was loaded onto two 5-mL HiTrap Q columns (GE Healthcare), joined in tandem, washed with 3 column volumes of Buffer A, and eluted with a linear gradient of 0 – 500 mM NaCl. Fractions containing a peak eluting at about 0.225 M NaCl were pooled and dialyzed against 10 mM sodium acetate pH 7.5 and 0.5 mM EDTA. Dialyzed protein was loaded onto two 5-mL HiTrap Heparin columns equilibrated in 10 mM sodium acetate pH 7.5 and 0.5 mM

EDTA. The material that was not retained on the column was collected and adjusted to pH 6.0 with acetic acid before loading onto two 5-mL HiTrap Heparin columns equilibrated in 10 mM sodium acetate pH 6.0 and 0.5 mM EDTA. The column was washed with 3 column volumes of the equilibration buffer and eluted with a linear gradient of 0 – 500 mM NaCl over 10 column volumes. Fractions containing β, eluting at about 240 mM NaCl were pooled, dialyzed against Buffer A that did not contain DTT, aliquoted and stored at -80 °C.

Purification of the γ subunit (*2*) – Ammonium sulfate (0.226 g/mL) was added to the clarified cell lysate (Buffer A) to precipitate the γ subunit. The precipitate was isolated by centrifugation at 31,000 x g for 30 min, and the pellet was washed with a solution of ammonium sulfate at 35% saturation (0.20 g/mL) followed by a solution of ammonium sulfate at 30% saturation (0.16 g/mL). The washed pellet was resuspended in Buffer A supplemented with 20 mM NaCl and dialyzed against the same buffer. The dialyzed sample was loaded onto two 5-mL HiTrap Heparin columns (GE Healthcare) in tandem and washed with 3 column volumes of Buffer A supplemented with 20 mM NaCl. A linear gradient from 20 to 500 mM NaCl was applied to the column and the γ subunit eluted at about 65 mM NaCl. Fractions containing γ were pooled, dialyzed against Buffer A supplemented with 50 mM NaCl, and loaded onto a 5-mL HiTrap Q column (GE Healthcare). The γ subunit was eluted at about 180 mM NaCl in a linear gradient of 50 to 500 mM NaCl. Fractions containing γ were pooled, dialyzed against Buffer A supplemented with 50 mM NaCl, and loaded onto an ATP agarose column (N6-linked, Sigma/Aldrich). The column was washed with 5 column volumes of Buffer A supplemented with 50 mM NaCl and the γ subunit was eluted with Buffer A supplemented with 1 M NaCl. The purified protein was dialyzed against Buffer A supplemented with 100 mM NaCl and stored at -80 °C.

Purification of the δ subunit (*3*) – The clarified cell lysate was diluted with Buffer A supplemented with 0.1M NaCl to double the volume and loaded onto a 70-mL Q sepharose FF column equilibrated in the same buffer. The column was washed with 3 column volumes of Buffer A supplemented with 0.1 M NaCl and eluted with a linear gradient of 0.1 to 0.5 M NaCl. Fractions containing δ were pooled and diluted with Buffer A to reduce the concentration of NaCl to approximately 0.1 M prior to loading the sample on two 5-mL HiTrap Heparin columns in tandem equilibrated with Buffer A plus 0.1 M NaCl. The column was washed with 6 column volumes of Buffer A plus 0.1 M NaCl and δ was eluted with a linear gradient of 0.1 to 0.5 M NaCl. Fractions containing δ were pooled and dialyzed against Buffer A adjusted to pH 8.0 plus 0.1 M NaCl. Dialyzed protein was loaded onto a 5-mL HiTrap Q column equilibrated with the same buffer used in dialysis. The column was washed with 5 column volumes of start buffer and eluted with a linear gradient of 0.1 to 0.5 M NaCl. Fractions containing δ were pooled and dialyzed against Buffer A plus 0.3 M NaCl because δ precipitates at NaCl concentrations below 0.1 M. Protein was stored at -80 °C.

Purification of the δ' subunit (*3*) – The clarified cell lysate was diluted to 80 mL with Buffer A and 16.8 g of ammonium sulfate was added with stirring over 1.5 hours to reach 37.5% saturation. The precipitate containing δ' was recovered by centrifugation at 31,000 x g for 30 min, resuspended in approximately 20 mL of Buffer B, and dialyzed against the same buffer. The dialyzed protein was loaded onto two 5-mL HiTrap heparin columns joined in tandem and equilibrated with Buffer B. The column was washed with 3 column volumes of Buffer B and eluted with a linear gradient of 0 to 0.3 M NaCl. Fractions containing δ' were pooled and dialyzed against Buffer A before loading onto two 5-mL HiTrap Q columns joined in tandem and equilibrate with the same buffer. The HiTrap Q columns were washed with 3 column

volumes of Buffer A and eluted with a linear gradient of 0 – 0.3 M NaCl. Fractions containing δ' were pooled, dialyzed against Buffer A supplemented with 0.1 M NaCl, and stored at -80 °C.

Purification of a χψ complex (*4*) - The clarified cell lysate was diluted to 80 mL with Buffer A before adding 20.64 g of ammonium sulfate with stirring over about 1.5 hours to 45% saturation. The pellet containing χψ was recovered by centrifugation at 31,000 x g for 30 min, resuspended in about 17 mL of Buffer A, and dialyzed against Buffer A supplemented with 20 mM NaCl. Dialyzed protein was loaded onto two 5-mL HiTrap Q columns joined in tandem and equilibrated with Buffer A plus 20 mM NaCl. The HiTrap Q columns were washed with 3 column volumes of Buffer A plus 20 mM NaCl and eluted with a linear gradient of 20 – 200 mM NaCl. Fractions containing χψ were pooled and dialyzed against Buffer A adjusted to pH 7.0, and loaded onto a 5-mL HiTrap SP columne equilibrated in Buffer A at pH 7.0 and supplemented with 20 mM NaCl. The column was washed with 5 column volumes of the starting buffer, and eluted with a linear gradient of 20 – 200 mM NaCl. Fractions containing cy were pooled, dialyzed against Buffer A supplemented with 0.1 M NaCl, and stored at -80 °C.

Reconstitution of γ complex (*5*) – Aliquots of 50 nmol each of δ of δ'were mixed and incubated at 15 °C for 15 min before adding 150 nmol of γ and 50 nmol of χψ. The five-subunit mixture was incubated for 30 min more at 15 °C. The salt concentration was reduced to 0.1 mM NaCl by addition of Buffer A supplemented with 0.05 M NaCl. The γ complex was purified from individual subunits by loading the mixture on two 1-mL HiTrap Q columns joined in tandem and equilibrated with Buffer A plus 100 mM NaCl. The column was washed with 5 column volumes of Buffer A plus 100 mM NaCl and eluted with a linear gradient of 100 – 500 mM NaCl. Fractions containing the γ complex ($\gamma_3\delta\delta'\chi\psi$) were pooled, dialyzed against Buffer A supplemented with 50 mM NaCl, and stored at -80 °C.

Simulation of reaction time courses in Fig. 7C - Time courses for DNA binding, ATP hydrolysis, and clamp release were simulated (Fig. 7C) based on the simple model shown in the schematic diagram to Fig. 7. The original model simulates a single turnover as measured experimentally in clamp release assays by inclusion of excess unlabeled β trap. Steps were added to the model to simulate the steady-state phases of DNA binding and ATP hydrolysis to match these experimental assay conditions. Four steps, shown in brackets in Supplemental Fig. 1, were added to the model in Fig. 7 (also shown in Supp. Fig. 1) to allow the clamp loader to "recycle" and generate the steady-state phase. These steps included: 1) ADP dissociation, 2) ATP binding, 3) an ATP-induced conformational change, and 4) β binding. For ATP hydrolysis experiments, a step was included in which inorganic phosphate (P_i) binds MDCC-PBP to increase the fluorescence of MDCC. Published rate constants for P_i binding and dissociating of 136 $\mu M^{-1}s^{-1}$ and 13.6 s^{-1}, respectively, were used (*6*).

SUPPLEMENTAL REFERENCES

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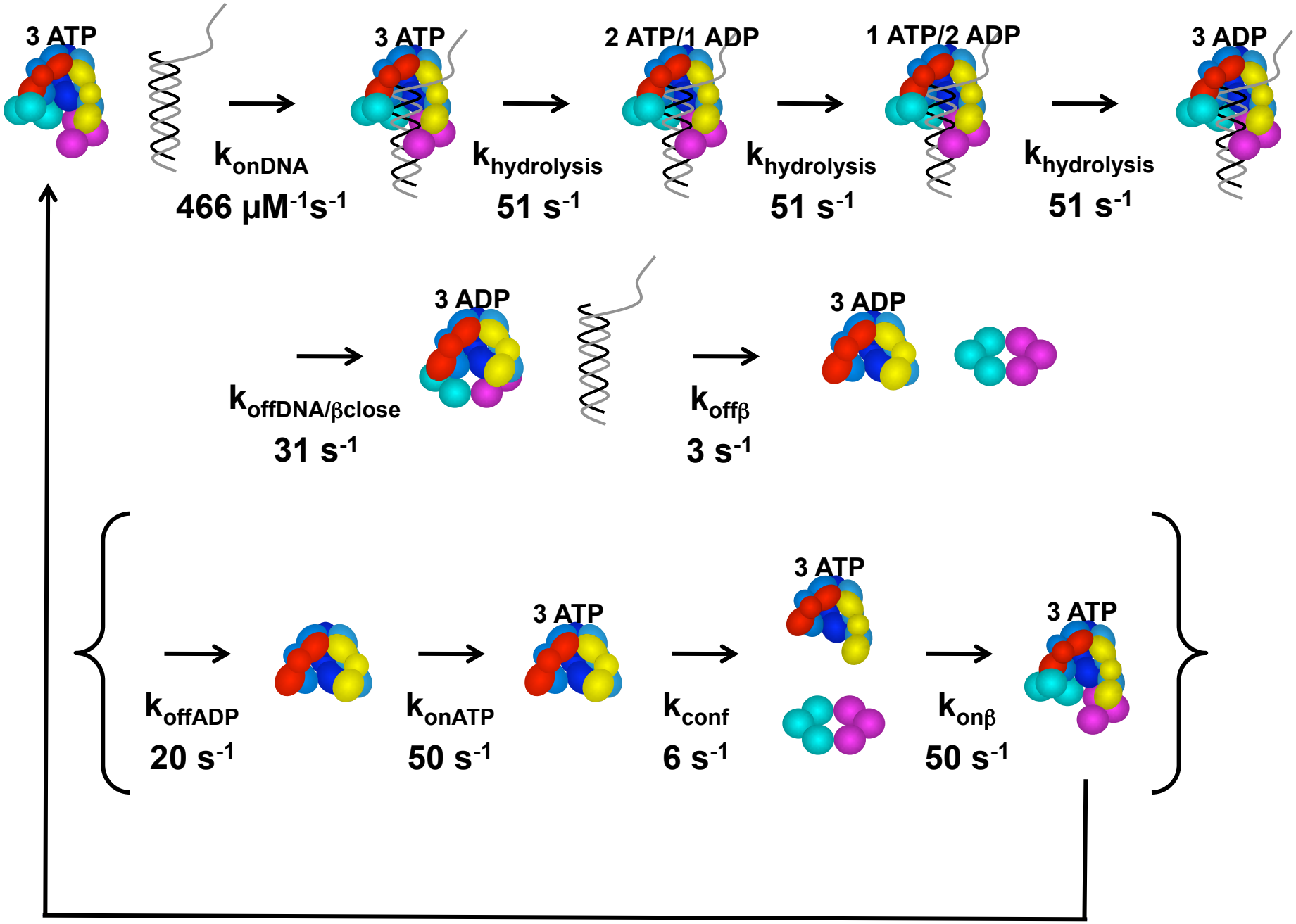
Supp. Fig. 1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k_{onDNA}
466 μM^{-1}s^{-1}
3 ATP
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2 ATP/1 ADP
$k_{hydrolysis}$
51 s^{-1}
1 ATP/2 ADP
$k_{hydrolysis}$
51 s^{-1}
3 ADP
$k_{offDNA/\beta close}$
31 s^{-1}
3 ADP
$k_{off\beta}$
3 s^{-1}
3 ADP
k_{offADP}
20 s^{-1}
k_{onATP}
50 s^{-1}
3 ATP
k_{conf}
6 s^{-1}
3 ATP
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50 s^{-1}
3 ATP