SUPPLEMENTARY DATA

Double-strand DNA end binding and sliding of the toroidal CRISPR-associated protein Csn2

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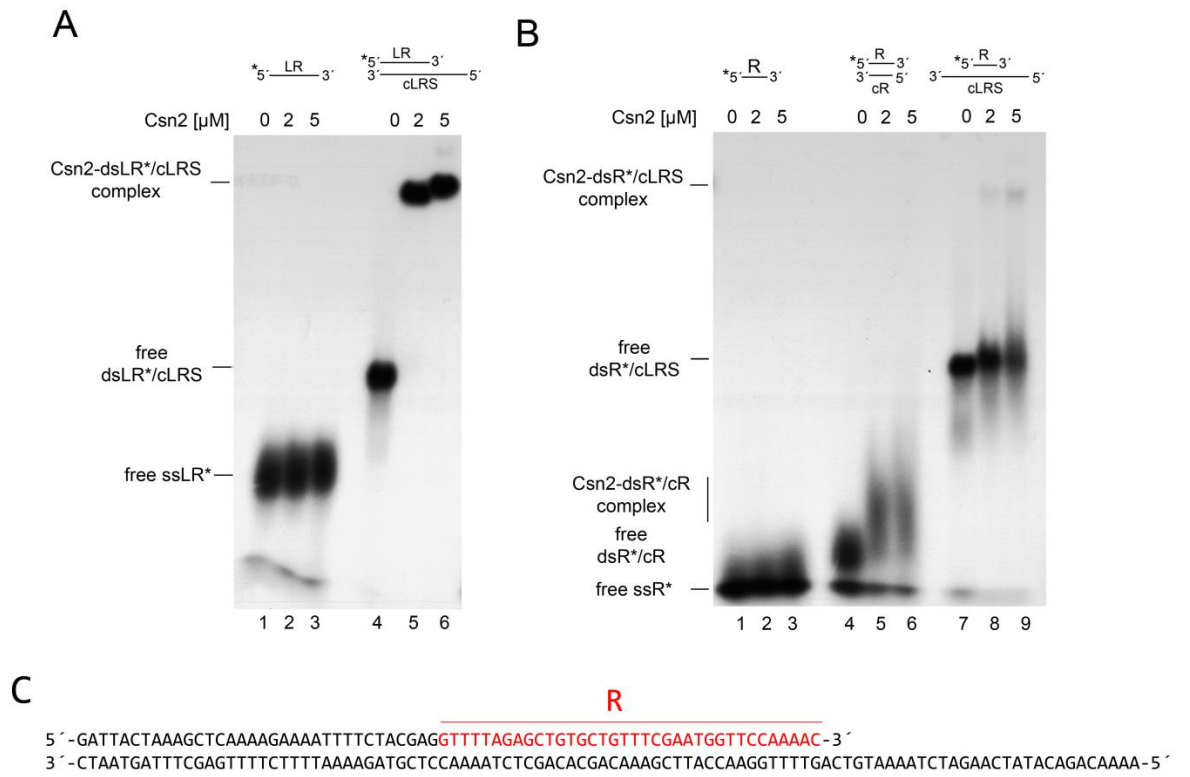

Figure S1: Csn2 binds to dsDNA with single-stranded DNA tails

Electrophoretic mobility shift assays of Csn2 with ssDNA or dsDNA are shown. (A) 5´-labeled ssLR oligonucleotide (70 nt in length) was incubated with 0 (lane 1), 2 µM (lane 2) or 5 µM (lane 3) Csn2. No complexes are formed with ssLR DNA. The ssLR oligonucleotide was hybridized with 100 nt complementary oligonucleotide cLRS to generate dsDNA with 5´-overhang. The dsLR/cLRS DNA was incubated with 0 (lane 4), 2 µM (lane 5) or 5 µM (lane 6) Csn2. Unbound DNA and Csn2-DNA complexes are indicated on the left. Asterisks indicate the labeled DNA strand. (B) Similar analyses as shown in (A) with either ssR* oligonucleotide (36 nt), dsR*/cR DNA or dsR*/cLRS (3´ and 5´ overhangs) are presented. (C) The upper strand corresponds to the sequence of the ssLR oligonucleotide. The sequence of the ssR oligonucleotide is colored in red. The lower strand corresponds to the sequence of the cLRS oligonucleotide.

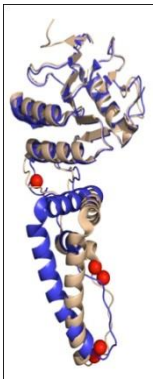

Figure S2: Superposition of the two Csn2 monomers (PDB ID: 3QHQ) (30) Superposition of two Csn2 protomers is shown. The lack of Ca^{2+} at the linker domain of one protomer leads to conformational change of the α-helices in the leg domain (30).

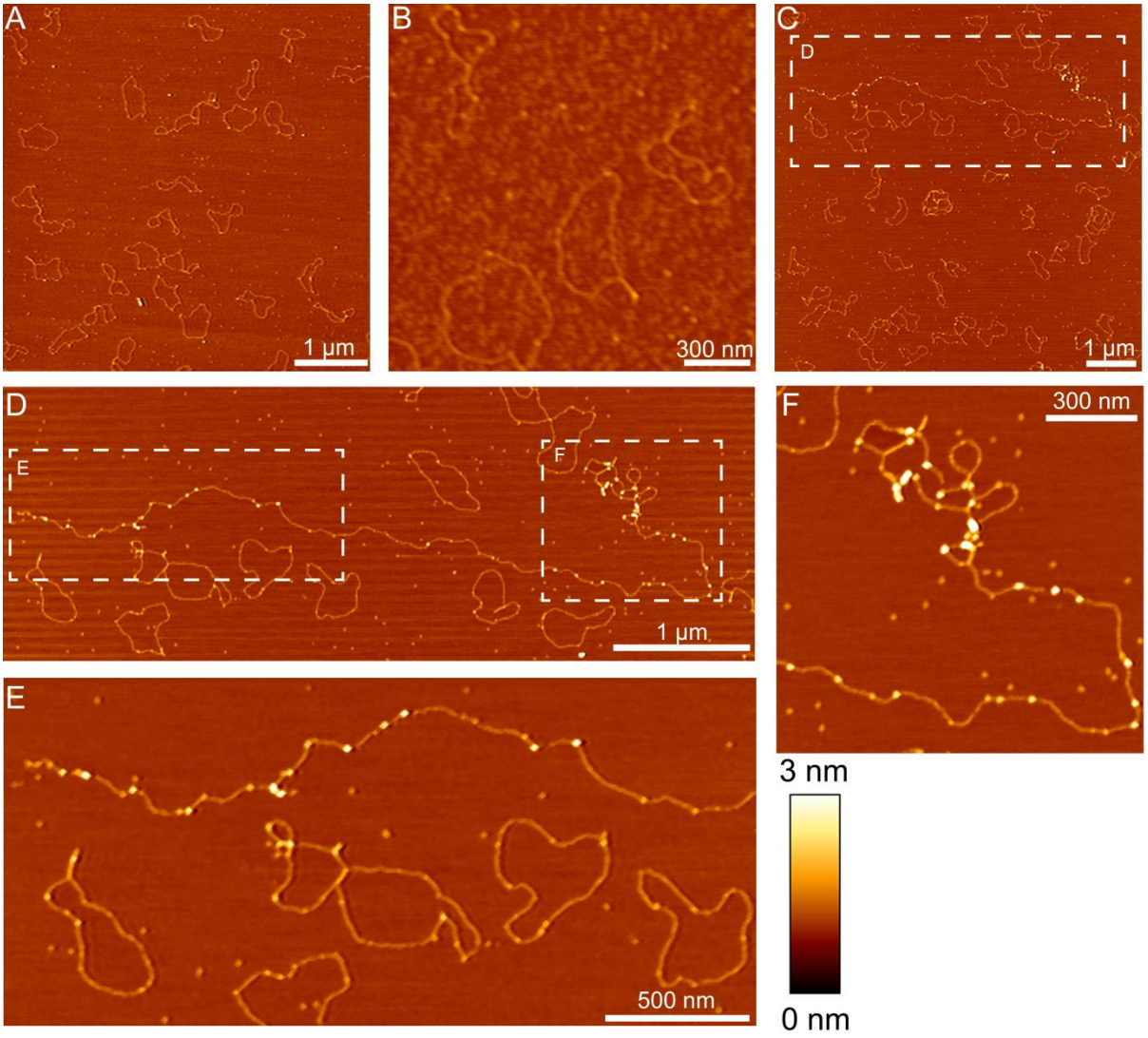

Figure S3: Csn2 does not bind to closed circular DNA.

AFM images of circular plasmid DNA incubated with Csn2 are presented. 2.6 nM of 5125 bp relaxed plasmid DNA was incubated with 176 nM Csn2. No accumulation of Csn2 on the closed circular plasmid was observed (A-E). Several Csn2 proteins are associated with a long linear DNA fragment (roughly more than 8 µm in length, corresponding to 24 kbp), likely a co-purified chromosomal DNA fragment (C-F). The DNA end segments of this linear DNA were occupied by Csn2 (D-F). The relative color scale range is 0 to 3 nm in all images.

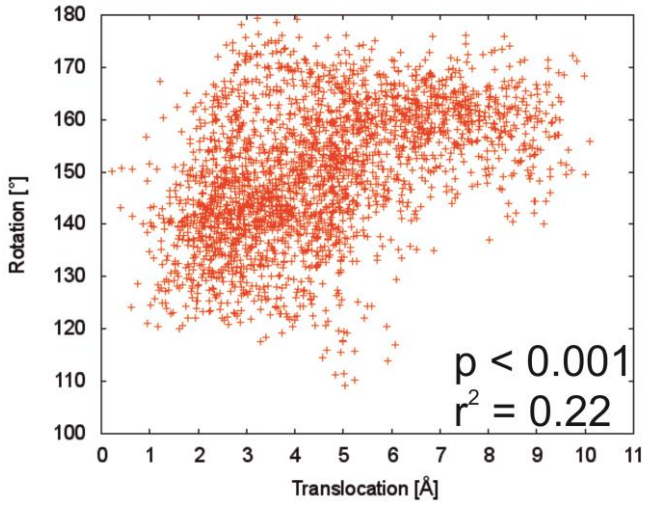

Figure S4: Coupled motions of translocation and rotation of Csn2 when moving along the DNA over the last 50 ns of the second MD simulation.
To determine the rotational motion, all conformations of the trajectories were aligned with respect to the phosphorous atoms of the DNA. Note that, while the magnitude of the slope of the correlation line (~9°/Å) is similar to the one determined for the first simulation (Figure 6B), its sign is opposite. This is because the translocation is symmetric with respect to the DNA center, and Csn2 moves in opposite directions in both simulations.

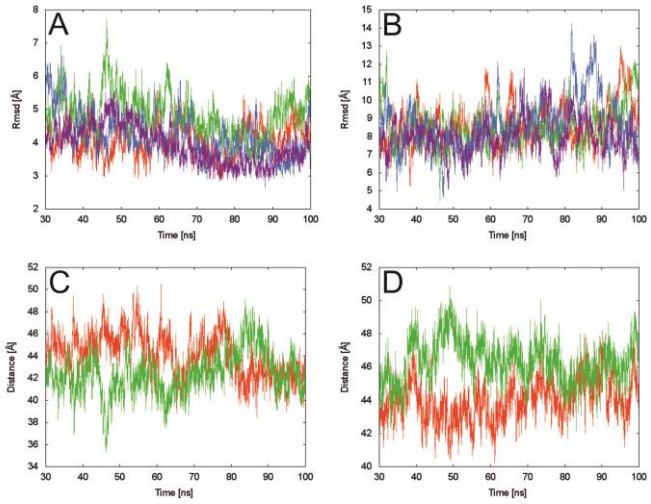

Figure S5: Root mean-square deviations and pore movements during molecular dynamics simulations of Csn2-DNA complexes

(A) Rmsd of Cα atoms of Csn2 with respect to the crystal structure after aligning the protein with respect to the Cα atoms. Red and green lines: two independent simulations of Csn2-DNA complexes with Ca^{2+} ions; magenta and blue lines: two independent simulations of Csn2-DNA complexes without Ca^{2+} ions. (B) Rmsd of phosphorous atoms of DNA with respect to an ideal B-DNA after aligning the DNA with respect to the phosphorous atoms. See panel A for the color code. (C) Distance between the average coordinates of the Cα atoms of the two 3_{10} helices located in the head domains of Csn2 on one side of the tetramer. See panel A for the color code. (D) Distance between the average coordinates of the Cα atoms of the two pairs of H5 helices; each pair of helices is located in one of the legs of the tetrameric structure. See panel A for the color code.