Supplementary Information

The nucleoid-associated protein Dan organizes chromosomal DNA through rigid nucleoprotein filament formation in *E. coli* during anoxia

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Supplementary Methods

FE curves WLC model fitting

The Marko-Siggia worm-like chain (WLC) model can be used to model DNA stretching by an applied force (1). Below is the Marko-Siggia WLC model.

$$\frac{fA}{K_BT} = \frac{z}{L} + \frac{1}{4\left(1-{}^{z}/_{L}\right)^2} - \frac{1}{4} \quad (1)$$

z is the measured DNA extension, f is the applied stretching force, L is the DNA contour length and A is the DNA persistence length. The WLC model can be written at the high-force limit where $fA \geq 1$ (i.e. $f > 0.08$ pN for A of 50 nm, naked DNA).

$$\frac{z}{L} = 1 - \sqrt{\frac{k_BT}{4fA}} \quad (2)$$

The above equation 2 can then be used to fit our single-DNA force-extension (FE) curves where the experimental DNA stretching force is from 0.1-10 pN and above. The DNA extension, z will be the dependent variable while force, f will be the independent variable, which will yield two fitted parameters; DNA contour length L and persistence length, A.

Occupancy fraction calculation

DNA stiffening protein increases the DNA apparent persistence length and the measured DNA persistence length at different protein concentration can be used to calculate the occupancy fraction. Assuming the saturated persistence length of the DNA-protein complex is known. The DNA-protein WLC model can be written as a normalized composite function of a naked DNA segment and a DNA-protein segment.

$$\frac{z_{measured}}{L} = \alpha \frac{z_{DNA-protein}}{L} + (1-\alpha)\frac{z_{DNA}}{L} \qquad (3)$$

Where α is the occupancy fraction of the protein on DNA. Equation 3 can be re-arranged to yield the following equation where the occupancy fraction is expressed in terms of the measured persistence length, $A_{measured}$, DNA-protein persistence length, $A_{DNA\text{-}protein}$, and DNA persistence length, A_{DNA}.

$$\boldsymbol{\alpha} = \frac{\sqrt{\frac{1}{A_{measured}}} - \sqrt{\frac{1}{A_{DNA}}}}{\sqrt{\frac{1}{A_{DNA-protein}}} - \sqrt{\frac{1}{A_{DNA}}}} \qquad (4)$$

The DNA persistence length can be calculated from the WLC model fitting of a fresh DNA FE curve without any protein addition while the DNA-protein persistence length value can be calculated from the WLC model fitting of a saturated DNA-protein FE curve.

Supplementary Figures

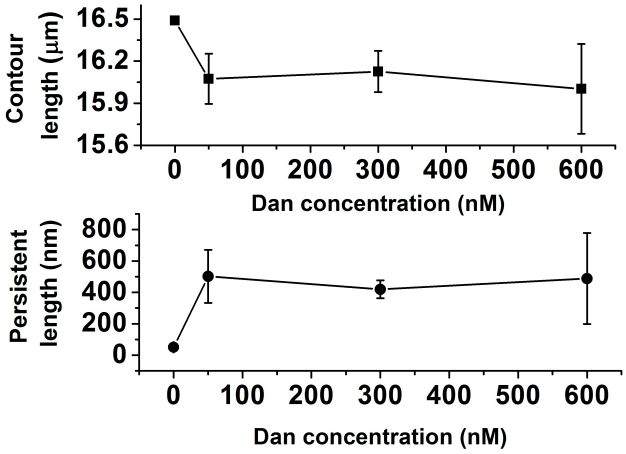

Figure S1. WLC model fitting values from 3 independent single-DNA stretching experiments at different Dan concentration. The apparent DNA contour length is slightly reduced from 16,490 nm to 16,002 ± 320 nm at 600 nM Dan concentration. The DNA contour length is more or less stabilized at 50 nM Dan concentration onwards. In contrast, the apparent DNA persistence length is significantly increased from the naked DNA value of ~ 50 nm to 488 ± 290 nm, which is more than 8-fold increase in value.

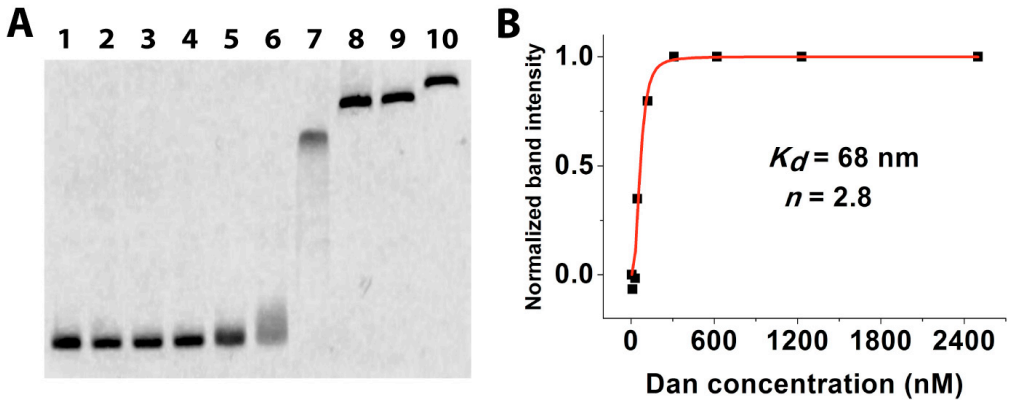

Figure S2. Electrophoresis mobility shift assay (EMSA) of Dan-DNA complex. EMSA of linear 576 bp DNA (40 ng, 50% CG rich random sequence) incubated with 0-2500 nM of Dan protein. Lane 1:10 – 0,6.2,12,31,46,120,310,620,1230,2500 nM Dan concentrations. It can be seen that at 310 nM Dan concentration (lane 7), there is a large shift in the DNA band position, indicating strong Dan binding. Beyond 310 nM, the DNA band shift is more or less stabilized, suggesting saturated DNA-binding. (B) Normalized DNA occupancy fraction against Dan concentration plot with Hill equation curve fitting (red line) from the EMSA result yielded the fitted dissociation constant, K_d and Hill's coefficient, n values of 66.8 ± 6.4 nM and 2.8 ± 0.5 respectively. The positive > 1 Hill's coefficient of 2.8 suggests Dan binds DNA in a positive cooperative manner.

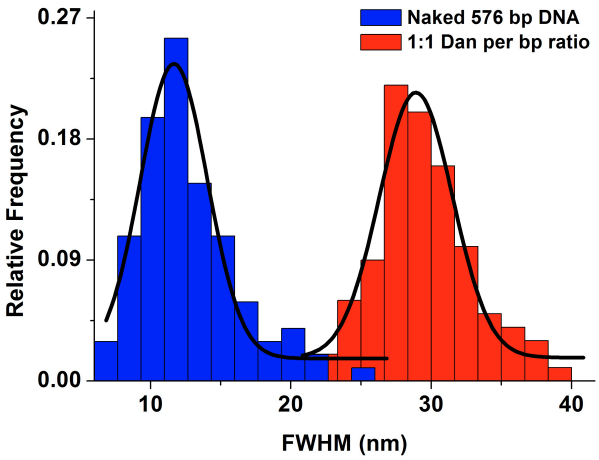

Figure S3. Dan protein filaments thickness measurements. Fully-coated Dan nucleoprotein filaments were obtained by incubating the DNA at 1:1 Dan/bp ratio. AFM width analysis estimated the width/thickness (Full width half max, FWHM) of the naked DNA to be 11.67 ± 2.39 nm (blue histogram) while that of the fully-coated 576 bp Dan nucleoprotein filaments to be 28.91 ± 2.61 nm (red histogram). To estimate the thickness of the Dan protein filament, we subtract the Dan nucleoprotein width value with the naked DNA width value. This yielded an estimated Dan protein filament thickness/width value of 17.24 nm.

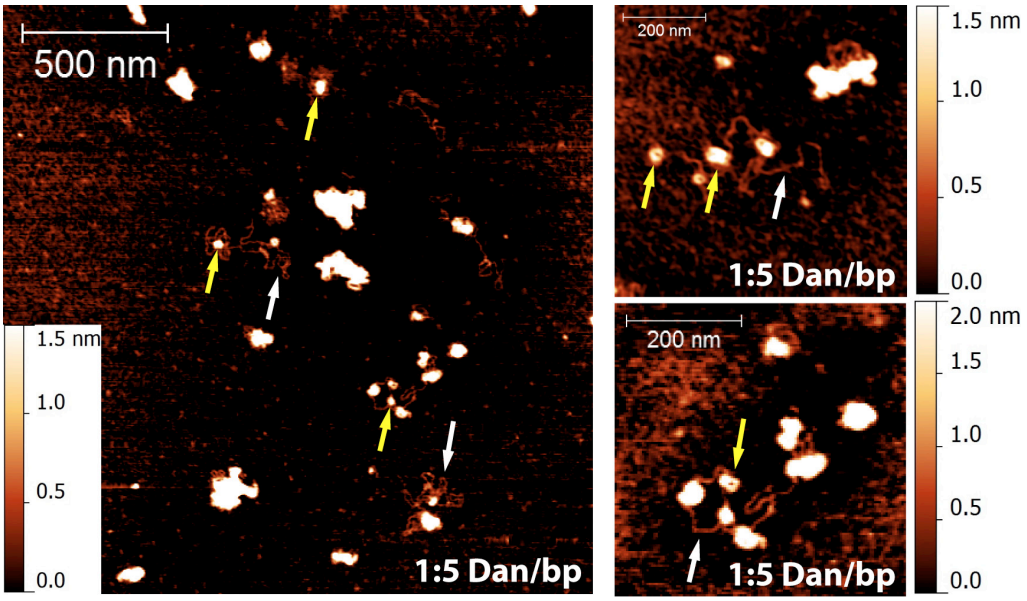

Figure S4. Unsaturated Dan DNA-binding leads to DNA compaction. At 1:5 Dan/bp ratio, linearized φX174 DNA are compacted. Dan DNA-binding at this protein/DNA bp ratio is unsaturated, as can be seen by the naked DNA segments (white arrows). In addition, Dan DNA compaction is also highly localized (yellow arrows).

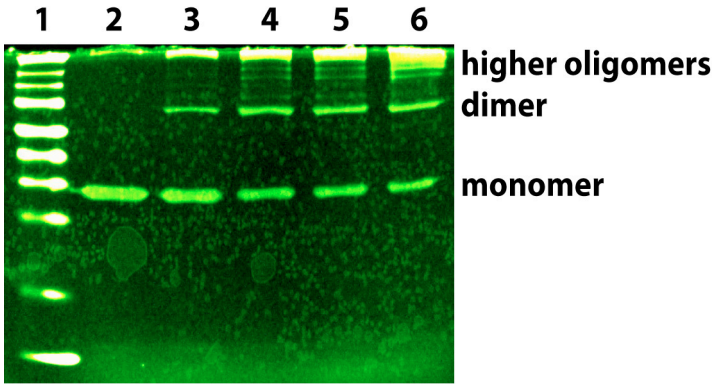

Figure S5. Dan oligomerization properties. Protein cross-linking assay using glutaraldehyde shows Dan can form higher order oligomers; dimers and above. Lane 1 is the protein ladder. Lane 2 is the negative control of 6 μM Dan in the absence of glutaraldehyde. Lane 3-6 is 6 μM Dan incubated with glutaraldehyde (lane 3 = 0.005%, lane 4 = 0.01%, lane 5 = 0.02% & lane 6 = 0.04% glutaraldehyde) for 10 minutes to allow protein-protein cross-linking to take place.

Supplementary References

1. Marko, J.F. and Siggia, E.D. (1995) Stretching DNA. *Macromolecules*, **28**, 8759-8770.