Supplementary Information

High-throughput single molecule nanofluidic studies on *B. subtilis* Rok protein interaction with DNA

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Figure S1. A) Example of extension vs standard deviation scatter plot of $4\mu M \lambda$ -DNA incubated with 165 nM Rok, with clustering according to DNA molecule type. B) A 3D scatter plot of the same sample with mean intensity of the molecules plotted along the third axis.



Figure S2. Glass slide deposition images of 4 μ M λ -DNA incubated with: (A) 660nM, 990 nM and 1,32 μ M Rok, (B) 660nM, 990 nM and 1,32 μ M Δ Rok, and (C) 660nM, 990 nM and 1,32 μ M sRok. The scale bar in the images represents a length of 10 μ m.



Figure S3. Representation of differences in the means of binary logarithms of the relative DNA extensions of monomeric clusters from ANOVA statistical analysis.



Figure S4. Further examples of monomeric DNA molecules in a "pinched" conformation, alongside their intensity profiles, for $4\mu M \lambda$ -DNA and 3.3 μM T7-DNA incubated with 330 nM Rok, as well as for $4\mu M \lambda$ -DNA incubated with 330 nM Δ Rok.



Figure S5. Examples of circular and concatemeric DNA molecules in "pinched" or overlapping conformations, alongside their intensity profiles, for 4 μ M λ -DNA incubated with 330 nM or 660 nM Rok or with 330 nM or 660 nM Δ Rok.



Figure S6. Comparison of the consensus intensity patterns of the netropsin-bound and Rokvariant bound molecules stretched to the same length and with intensity normalized. The dark portion of the molecule (i.e. the pixels where the normalized intensity falls below the full-width at half maximum) corresponds to the AT-rich regions. The percentage of the dark portion with respect to the whole molecule for each case is as follows: Netropsin-bound: 55.95%, Rokbound: 62.8%, Δ Rok-bound: 58.33%, and sRok-bound: 56.5%.

Netropsin	0.00E+00	1.39E-02	1.00E-02	1.12E-02
ΔRok	1.39E-02	0.00E+00	7.13E-03	3.28E-03
Rok	1.00E-02	7.13E-03	0.00E+00	1.55E-02
sRok	1.12E-02	3.28E-03	1.55E-02	0.00E+00

Table S1. P-values of the Pearson cross-correlation analysis between the consensus intensity patterns of netropsin-bound DNA and Rok variant bound DNA. P<0.05 indicates that the correlation is statistically significant.