Supplementary Material

In vitro studies of DNA condensation by bridging protein in a crowding environment

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FCS curve fitting procedure and parameters

The external detectors are from PicoQuant, and both were controlled by computer via the producer-developed softwares, Leica Application Suite X for microscope control and imaging, and SymphoTime for FCS data acquisition and processing. The minimal lag time was set to 0.003 ms. For FCS, a minimum of 6 recordings with a duration of 90 seconds were executed, with $\kappa = z0/w0 = 4.0$ for DNA. This protocol, combined with the use of weighted arithmetic means for data analysis gave a lower error, without much subjective influence on the results. The effective focal volume was set to the default 1 fl during curve fitting, as normalized data were sufficient. The curve-fitting algorithm tends to give a high triplet-state relaxation time, often above 40 ns, and had to be adjusted manually to a more realistic number, closer to 10 ns.

The number of species for all DNA samples was assumed to be two, where the slower one of higher concentration was the DNA-dye-protein complex, and the resolution of the autocorrelation function was maximized.

The process of calculating weighted means using bootstrapping is shortly described, as follows: a recording is taken of the sample for 10 minutes; this is chopped into 6 intervals of approximately 90 seconds (in symphotime). For each of these, an autocorrelation function is constructed at maximum resolution and a lag time of 0.003 ms (symphotime). A curve fit is done with the assumption of two species and the triplet-state relaxation time manually set to 10 ns (symphotime). The resulting curve fit gives us the diffusion time of the two species where the slower one is our DNA. A "bootstrap" analysis is done on the curve fit (symphotime), and this gives us a standard deviation for the diffusion time of our species for that single 90 second interval. Then weighted arithmetic mean is calculated for all the six diffusion times by using the standard deviations from the bootstrap analysis.

Dye exclusion studies of DNA-PEG

Dye exclusion studies for plasmid DNA and PEG were performed using protocol as described previously [1].

In short, steady state fluorescence spectra were recorded using the Tecan Infinite 200-PRO multifunctional plate reader. The fluorophore used for DNA was Gelstar nucleic acid stain (Lonza), which has an emission maximum (λ_{em}) at 527 nm and an excitation maximum (λ_{ex}) at 493 nm in the presence of DNA. In order to optimize the quality of the measurement, the 10,000× concentrated stock solution of Gelstar was diluted to 10× as final working concentration.

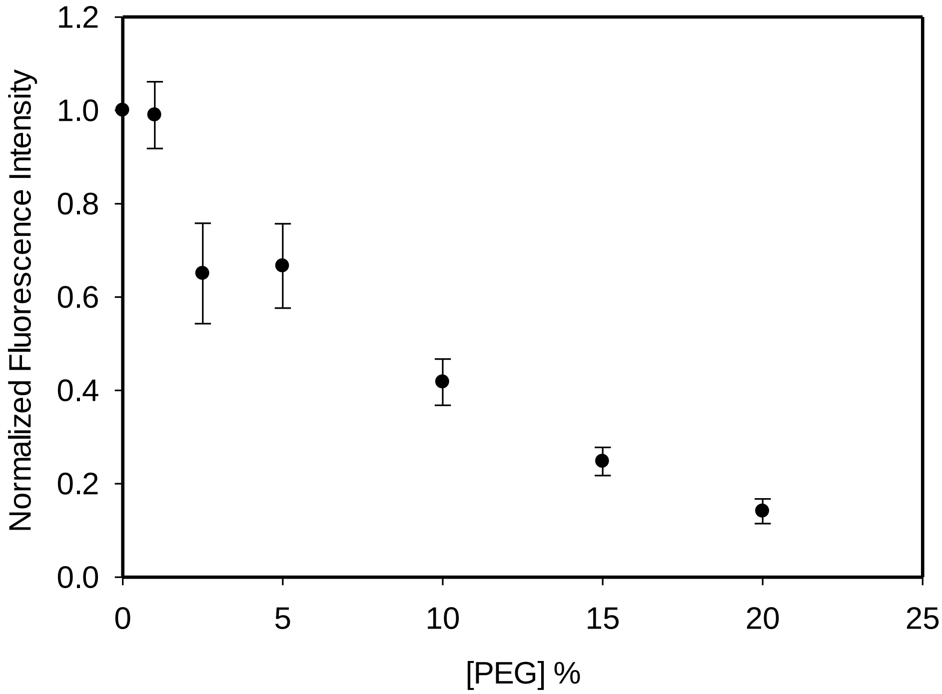

Figure S1. Fluorescence intensity of DNA–Gelstar complexes normalized to the fluorescence intensity of DNA-Gelstar complexes in the absence of PEG, I/I_0, shown as a function of PEG concentration. The final concentration of DNA was 2 µg/mL.

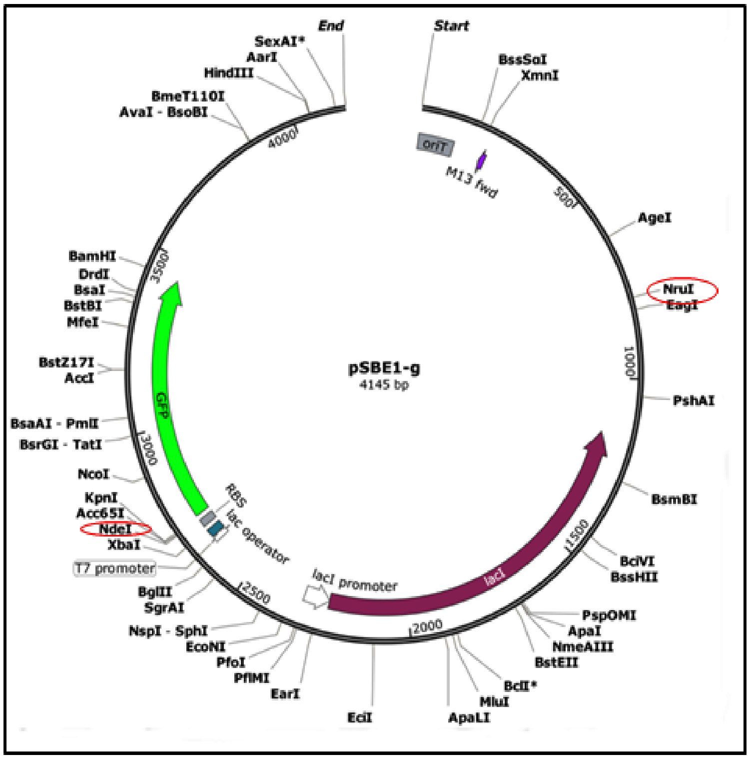

Figure S2. The map of the amplicon (4145bp) carrying a T7 promoter. The recognition sites of the restriction enzymes used in this work are highlighted. Map is regenerated by Snap Gene Viewer software

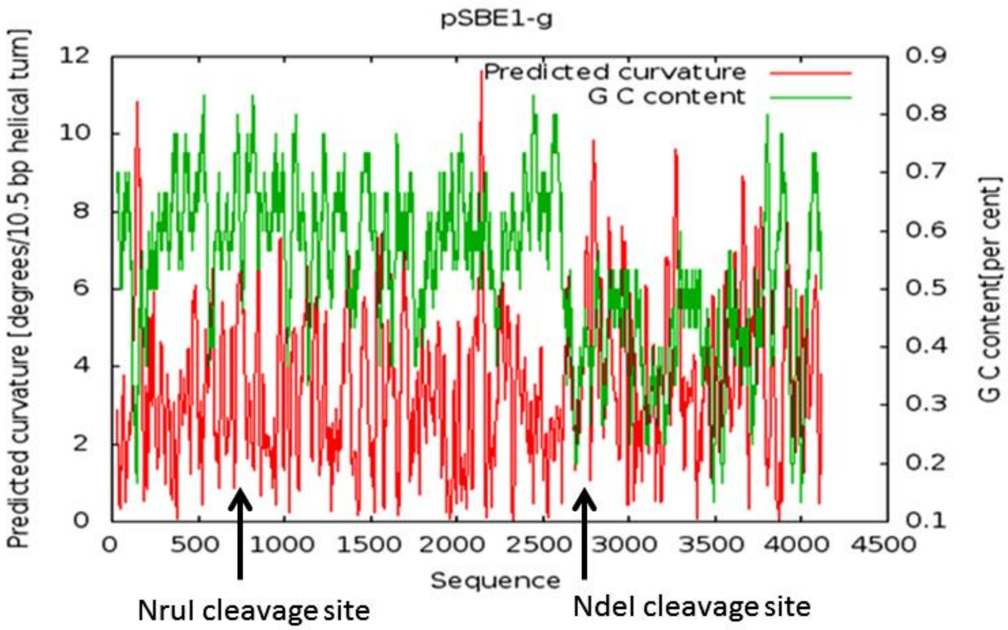

Figure S3. DNA curvature prediction: Predicted DNA curvature (red) and GC content (green) of pSBE1-g. Generated using Bend.it software.

In vitro transcription and translation assay

Production of green fluorescent protein (GFP) in the presence and absence of H-NS and PEG was followed using an *in vitro* translation assay, TnT® Quick Coupled Transcription/Translation Systems (Promega). For this assay, samples containing fixed concentrations of plasmid DNA (2 μg/mL) were premixed with varied concentrations of H-NS and PEG, as described above. The equilibrated DNA–H-NS–PEG mixtures were transferred to vials containing the translational/transcription master mix followed by the addition of methionine, required for *in vitro* translation, according to the manufacturer's protocol. Reactions were incubated for 1 h at 37 °C and GFP production was estimated using a fluorescence plate reader M200 Pro Tecan Spectrophotometer.

As mentioned in the introduction, H-NS is known to play a vital role in regulating a wide variety of genes across the genome of bacteria. In order to learn more about the gene regulatory role of H-NS in cellular conditions, we performed *in vitro* transcription/translation assays of DNA–H-NS complexes in both presence and absence of crowding agents. The results are compiled in the form of a heat map in Table S1.

Table S1. Heat map showing the fluorescence intensities of GFP, I_{525}, normalized to fluorescence intensity of GFP produced in the absence of H-NS and PEG, $I_{525,0}$.

	$I_{525}/I_{525,0}$				
[H-NS] /μM	0% PEG	0.5% PEG	1% PEG	2% PEG	4% PEG
0.00	1.00 ± 0.04	0.83 ± 0.15	0.66 ± 0.15	0.21 ± 0.06	0.11 ± 0.11
0.05	1.02 ± 0.13	0.85 ± 0.17	0.68 ± 0.15	0.21 ± 0.06	0.00 ± 0.09
0.10	1.02 ± 0.13	0.64 ± 0.09	0.68 ± 0.13	0.30 ± 0.09	0.02 ± 0.06
0.25	0.77 ± 0.13	0.77 ± 0.09	0.72 ± 0.19	0.38 ± 0.09	0.09 ± 0.09
0.50	0.36 ± 0.11	0.49 ± 0.04	0.66 ± 0.53	0.17 ± 0.17	0.06 ± 0.09
0.75	0.32 ± 0.09	0.15 ± 0.21	0.26 ± 0.04	0.17 ± 0.06	0.04 ± 0.02
1.00	0.13 ± 0.09	0.26 ± 0.06	0.17 ± 0.11	0.09 ± 0.11	0.15 ± 0.11

Excitation and emission wavelengths were 485 nm and 525 nm respectively. The indicated values are the mean of three independent sample sets and errors indicate the standard deviation from the mean.

The second column in Table 2 refers to $\frac{I_{525}}{I_{525,0}}$ of GFP expression in the presence of H-NS only. It is clear that increasing the protein concentration results in a decrease in GFP expression, in good agreement with previous reports [2]. On the other hand, increasing PEG concentration in the absence of H-NS (first row) also leads to a clear decrease in GFP production, as described previously [3, 4].

Our focus is the combined role of H-NS and PEG on gene regulation. For this we tested series of samples with DNA–H-NS complexes with varying concentrations of PEG, also compiled in Table S1. The three independent sample sets performed for these experiments showed a relatively large variability, as reflected on the large errors of some of the points in Table S1. The synergism of H-NS and PEG on gene regulation is therefore not obvious from the data, except for the samples prepared with 0.1 µM of H-NS and 0.5 % of PEG, where there is a clear decrease in the GFP expression when compared to the sets prepared in the presence of only H-NS or PEG.

DNase digestion of DNA-H-NS-PEG in the absence of Mg^{2+}

In order to check the binding affinity of H-NS to DNA in the absence of Mg^{2+}, DNase digestion assay was performed without $MgCl_2$. DNA−H-NS–PEG complexes were prepared as mentioned in materials and methods sections, except that binding buffer has no $MgCl_2$. Once formed complexes were treated with DNase enzyme and incubated for at least 20 minutes at 37 °C.

Fig S4 shows DNase digestion assay for DNA−H-NS–PEG complexes prepared without $MgCl_2$ in the binding buffer. We observe a partial protection of DNA from DNase in the presence of H-NS (see lane 4-7). Lane 9 shows the DNase digestion of DNA in the presence of 5% PEG. Addition of H-NS leads to near complete protection of the DNA, showing that the synergism of PEG in H-NS binding to DNA, and consequent protection towards DNase activity, is not affected by the presence of Mg^{2+}, although the binding mechanism of H-NS to DNA is different (see discussion in the paper). Fig S4 shows DNase digestion assay for DNA−H-NS–PEG complexes prepared without $MgCl_2$ in the binding buffer. We observe a partial protection of DNA from DNase in the presence of H-NS (lanes 4 to 7). Lane 9 shows the DNase digestion of DNA in the presence of 5% PEG. Addition of H-NS (lanes 10 to 14) leads to near complete protection of the DNA, showing that the synergism of PEG in H-NS binding to DNA, and consequent protection towards DNase activity, is not affected by the presence of Mg^{2+}, although the binding mechanism of H-NS to DNA is different (see discussion in the paper).

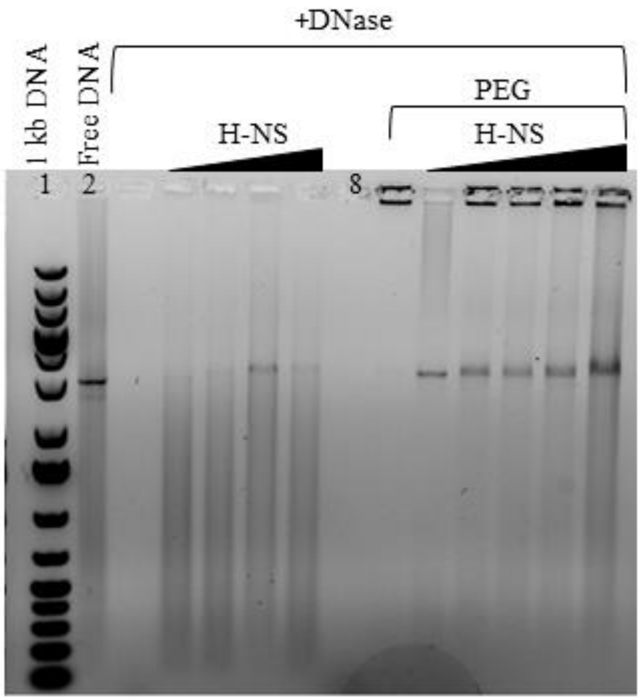

Figure S4. **DNase I protection assay in the absence of $MgCl_2$:** To examine binding activity of H-NS to linear plasmid DNA in the absence of Mg^{2+}, DNase I digestion reactions were carried out at increasing concentrations of H-NS (0.5, 1, 5, 10, 25 µM) in the absence (lanes 3 to 7) and presence of 5 % PEG (lanes 9 to 14). Lane 2 and 3 correspond to controls of free DNA with and without enzyme addition, respectively, and the last lane, 9, shows the digestion of DNA in the presence of 5 % PEG only. A final concentration of 5 µg/mL of linear plasmid DNA was used for all reactions. Lane 8 was left unloaded.

References

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