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Supplemental Information

Role of Salt Valency in the Switch of H-NS Proteins between DNA-Bridging and DNA-Stiffening Modes

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- Supporting Material –

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MODEL AND SIMULATIONS

Temperature T is assumed to be 298 K throughout the study. The model for the DNA molecule consists of a circular chain of $n = 2880$ beads with radius $a = 1.0$ nm separated at equilibrium by a distance $l_0 = 2.5$ nm and enclosed in a sphere with radius $R_0 = 120$ nm. Two beads represent 15 DNA base pairs. The contour length of the DNA molecule and the cell volume correspond approximately to 1/200th of the values for *E. coli* cells, so that the nucleic acid concentration of the model is close to the physiological one. The potential energy of the DNA chain consists of 4 terms, namely, the stretching energy V_s, the bending energy V_b, the electrostatic repulsion V_e, and a confinement term V_wall

$$E_\mathrm{DNA} = V_\mathrm{s} + V_\mathrm{b} + V_\mathrm{e} + V_\mathrm{wall} \ . \tag{S1}$$

The stretching and bending contributions write

$$\begin{aligned} V_\mathrm{s} &= \frac{h}{2}\sum_{k=1}^{n}(l_k - l_0)^2 \\ V_\mathrm{b} &= \frac{g}{2}\sum_{k=1}^{n}\theta_k^2, \end{aligned} \tag{S2}$$

where $\mathbf{r}_k$ denotes the position of DNA bead k, $l_k = \left\|\mathbf{r}_k - \mathbf{r}_{k+1}\right\|$ the distance between two successive beads, and $\theta_k = \arccos((\mathbf{r}_k - \mathbf{r}_{k+1})(\mathbf{r}_{k+1} - \mathbf{r}_{k+2}) / (\left\|\mathbf{r}_k - \mathbf{r}_{k+1}\right\| \left\|\mathbf{r}_{k+1} - \mathbf{r}_{k+2}\right\|))$ the angle formed by three successive beads. The stretching energy V_s is a computational device without biological meaning, which is aimed at avoiding a rigid rod description. The stretching force constant h is set to $h = 100\ k_\mathrm{B}T / l_0^2$, which insures that the variations of the distance between

successive beads remain small enough (1). In contrast, the bending rigidity constant is obtained from the known persistence length of the DNA, $\xi = 50$ nm, according to $g = \xi\, k_{\mathrm{B}}T / l_0 = 20\, k_{\mathrm{B}}T$.

Electrostatic repulsion between DNA beads that are not close neighbours along the chain is written as a sum of repulsive Debye-Hückel terms with hard core

$$V_{\mathrm{e}} = \left(\frac{e_{\mathrm{DNA}}}{Z}\right)^2 \sum_{k=1}^{n-4} \sum_{K=k+4}^{n} H(\|\mathbf{r}_k - \mathbf{r}_K\|) \ , \tag{S3}$$

where

$$H(r) = \frac{1}{4\pi\varepsilon r} \exp\left(-\frac{r-2a}{r_D}\right) \ . \tag{S4}$$

Interactions between close neighbours ($1 \le |k-K| \le 3$) are not included in Eq. (S3) because it is considered that they are already accounted for in the stretching and bending terms. $\varepsilon = 80\, \varepsilon_0$ denotes the dielectric constant of the buffer. The value of the Debye length r_D is set to 3.07 nm, which corresponds to a concentration of monovalent salt of 0.01 M. e_{DNA} is the electric charge, which is placed at the centre of each DNA bead when considering that the buffer contains only monovalent cations ($Z = 1$). The numerical value $e_{\mathrm{DNA}} = -3.525\, \bar{e}$, where $\bar{e}$ is the absolute charge of the electron, is the product of l_0 and the net linear charge density derived from Manning's counterion condensation theory ($-\bar{e} / \ell_B \approx -1.41\ \bar{e}/\mathrm{nm}$, see the main text). The charge placed at the center of each bead reduces to e_{DNA} / Z when considering that the buffer contains cations of effective valency $Z > 1$.

Finally, the confinement term V_{wall} is taken as a sum of repulsive terms

$$V_{\mathrm{wall}} = 10\, k_{\mathrm{B}}T \sum_{k=1}^{n} f(\|\mathbf{r}_k\|) \ , \tag{S5}$$

where *f* is the function defined according to

if $r \le R_0$: $f(r) = 0$

if $r > R_0$: $f(r) = \left(\frac{r}{R_0}\right)^6 - 1$. (S6)

H-NS dimers are modeled as chains of 4 beads with radius a separated at equilibrium by a distance $L_0 = 4.0$ nm. For each protein chain *j*, charges $e_{j1} = e_{j4} = 3\bar{e}$ are placed at the centre of terminal beads $m = 1$ and $m = 4$, and charges $e_{j2} = e_{j3} = -3\bar{e}$ at the centre of central beads $m = 2$ and $m = 3$. The values of these effective charges were obtained from a

naive counting of the number of positively and negatively charged residues in published crystallographic structures (2). It is considered that the density of charges along the naked protein chain is small enough for counterion condensation not to take place in the range $1 \le Z \le 2$. Moreover, the terminal beads of each protein chain can bind either to the beads of the DNA chain or to the central beads of other protein chains, so that the model accounts for both H-NS oligomerization and binding of H-NS to the DNA chain. In most simulations, $P = 200$ protein chains were introduced in the confining sphere together with the DNA chain, which corresponds to a protein concentration approximately twice the concentration of H-NS dimers during the cell growth phase and six times the concentration during the stationary phase (3).

The potential energy of the protein chains consists of 4 terms

$$E_{\mathrm{P}} = V_s^{(\mathrm{P})} + V_b^{(\mathrm{P})} + V_e^{(\mathrm{P/P})} + V_{\mathrm{wall}}^{(\mathrm{P})} , \tag{S7}$$

where the stretching, bending, and confining energies are very similar to their DNA counterparts

$$\begin{aligned} V_s^{(\mathrm{P})} &= \frac{h}{2} \sum_{j=1}^{P} \sum_{m=1}^{3} (L_{jm} - L_0)^2 \\ V_b^{(\mathrm{P})} &= \frac{G}{2} \sum_{j=1}^{P} \sum_{m=1}^{m=2} \Theta_{jm}^2 \\ V_{\mathrm{wall}}^{(\mathrm{P})} &= 10\, k_{\mathrm{B}} T \sum_{j=1}^{P} \sum_{m=1}^{4} f(\|\mathbf{R}_{jm}\|) , \end{aligned} \tag{S8}$$

with $\mathbf{R}_{jm}$ the position of bead *m* of protein chain *j*, L_{jm} the distance between beads *m* and *m*+1 of protein chain *j*, and Θ_{jm} the angle formed by beads *m*, *m*+1, and *m*+2 of protein chain *j*. The value of the bending constant is assumed to be as low as $G = 2\, k_{\mathrm{B}} T$, in order to account for the flexible linker that connects the C-terminal and N-terminal domains of H-NS.

The interaction energy between protein chains, $V_e^{(\mathrm{P/P})}$, is taken as the sum of (attractive or repulsive) Debye-Hückel terms with hard core and (repulsive) excluded volume terms, with the latter ones contributing only if the corresponding Debye-Hückel term is attractive

$$\begin{aligned} V_e^{(\mathrm{P/P})} = & \sum_{j=1}^{P} e_{j1} e_{j4} H(\|\mathbf{R}_{j1} - \mathbf{R}_{j4}\|) + \sum_{j=1}^{P-1} \sum_{m=1}^{4} \sum_{J=j+1}^{P} \sum_{M=1}^{4} e_{jm} e_{JM} H(\|\mathbf{R}_{jm} - \mathbf{R}_{JM}\|) \\ & + \chi \sum_{j=1}^{P} \sum_{m=1,4} \sum_{\substack{J=1 \\ J \neq j}}^{P} \sum_{M=2,3} F(\|\mathbf{R}_{jm} - \mathbf{R}_{JM}\|) , \end{aligned} \tag{S9}$$

where *F* is the function defined according to

if $r \le r_0$: $F(r) = \frac{r_0 - 2a}{r - 2a}(\frac{r_0 - 2a}{r - 2a} - 2) + 1$ (S10)

if $r > r_0$: $F(r) = 0$,

and r_0 denotes the threshold distance below which the excluded volume term, taken as the repulsive part of a 2^h order Lennard-Jones-like function with hard core, creates a repulsion force between oppositely charged beads. The first term in the right-hand side of Eq. (S9) insures that the two terminal beads of the same chain do not overlap. The numerical values of the two parameters of the excluded volume potential, $\chi = 1\, k_B T$ and $r_0 = 3.5$ nm, were adjusted manually in order that the enthalpy change upon forming a complex between two protein chains is comparable to the experimentally determined value for H-NS. As shown in Fig. S1, this enthalpy change is equal to $-12.0\, k_B T$ for two protein chains at equilibrium approaching one another perpendicularly, which is close to the experimentally determined value for H-NS ($\approx -10.2\, k_B T$ (4)).

Finally, the potential energy describing the interactions between the DNA chain and the protein chains, $E_{\mathrm{DNA/P}}$, is similarly taken as the sum of (attractive or repulsive) Debye-Hückel terms with hard core and (repulsive) excluded volume terms, with the latter ones contributing only if the corresponding Debye-Hückel term is attractive

$$E_{\mathrm{DNA/P}} = \frac{e_{\mathrm{DNA}}}{Z} \sum_{k=1}^{n} \sum_{j=1}^{P} \sum_{m=1}^{4} e_{jm} H(\left\| \mathbf{r}_k - \mathbf{R}_{jm} \right\|) + \chi \sum_{k=1}^{n} \sum_{j=1}^{P} \sum_{m=1,4} F(\left\| \mathbf{r}_k - \mathbf{R}_{jm} \right\|) . \quad \text{(S11)}$$

It is emphasized that the attraction term between DNA beads and terminal protein beads scales as $1/Z$ in Eq. (S11), while the attraction term between terminal beads of a protein chain and central beads of another protein chain does not depend on *Z* in Eq. (S9). This is a key point of the model, see the main text. The potential energy felt by protein chains at equilibrium approaching perpendicularly the linear DNA chain at equilibrium is shown in Fig. S2 for an effective valency of the cations $Z = 1.37$. For this particular value of *Z*, the enthalpy change upon binding of a protein chain to the DNA chain is $-12.0\, k_B T$, which is equal to the enthalpy change upon binding of a protein chain to another protein chain, and comparable to experimentally determined values ($\approx -11.0\, k_B T$ (5)). In contrast, as illustrated in Fig. 1 of the main text, binding of protein chains to the DNA chain is favored for $Z < 1.37$, while binding to other protein chains is favored for $Z > 1.37$. It may also be noted in this figure that the evolution of the enthalpy change upon binding of H-NS to DNA deviates slightly from an

$1/Z$ law, which results from the fact that the excluded volume term is assumed to be independent of Z in Eq. (S11).

The total potential energy of the system, E_{pot}, is the sum of the energies of DNA and protein chains and DNA/protein interactions

$$E_{\text{pot}} = E_{\text{DNA}} + E_{\text{P}} + E_{\text{DNA/P}} \ . \tag{S12}$$

The dynamics of the model was investigated by integrating numerically the Langevin equations of motion with kinetic energy terms neglected. Practically, the updated position vector for each bead (whether DNA or protein), $\mathbf{r}_j^{(n+1)}$, is computed from the current position vector, $\mathbf{r}_j^{(n)}$, according to

$$\mathbf{r}_j^{(n+1)} = \mathbf{r}_j^{(n)} + \frac{D_t \, \Delta t}{k_B T} \mathbf{F}_j^{(n)} + \sqrt{2 \, D_t \, \Delta t} \; \xi^{(n)} \ , \tag{S13}$$

where the translational diffusion coefficient D_t is equal to $(k_B T)/(6\pi\eta a)$ and $\eta = 0.00089$ Pa s is the viscosity of the buffer at $T = 298$ K. $\mathbf{F}_j^{(n)}$ is the vector of inter-particle forces arising from the potential energy E_{pot}, $\xi^{(n)}$ a vector of random numbers extracted at each step n from a Gaussian distribution of mean 0 and variance 1, and Δt the integration time step, which is set to 1.0 ps for $P = 200$ protein chains and 0.5 ps for $P = 1000$ chains. After each integration step, the position of the center of the confining sphere was slightly adjusted so as to coincide with the center of mass of the DNA molecule.

SUPPORTING REFERENCES

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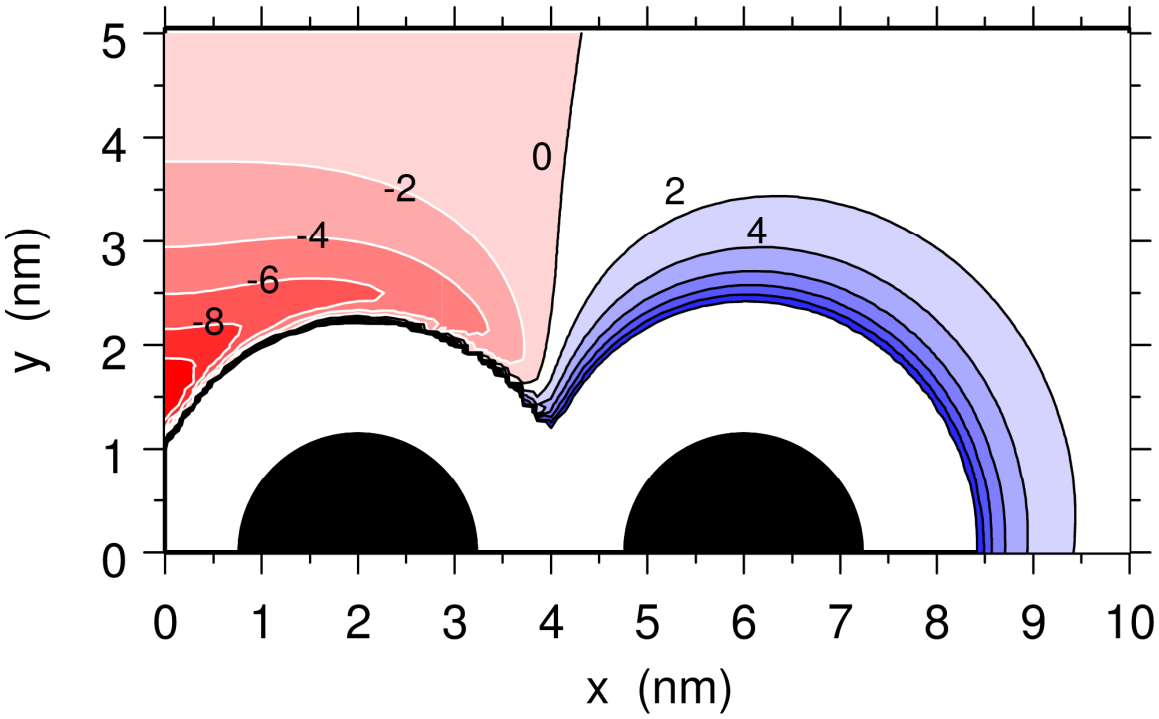

Figure S1 : Potential energy felt by a protein chain aligned along the *y* axis when approaching another protein chain elongated along the *x* axis and centered on (0,0). Both chains are at equilibrium with respect to their stretching and bending degrees of freedom. The black disks represent two beads of the protein chain elongated along the *x* axis. In this geometry, the potential is symmetric with respect to the *y* axis, in addition to having rotational symmetry along the *x* axis. (*x*,*y*) denote the coordinates of the center of the terminal bead of the vertical chain that lies closest to the horizontal chain. The minimum of the potential energy surface ($-12.0\,k_{\mathrm{B}}T$) is located on the *y* axis. Contour lines are separated by $2\,k_{\mathrm{B}}T$.

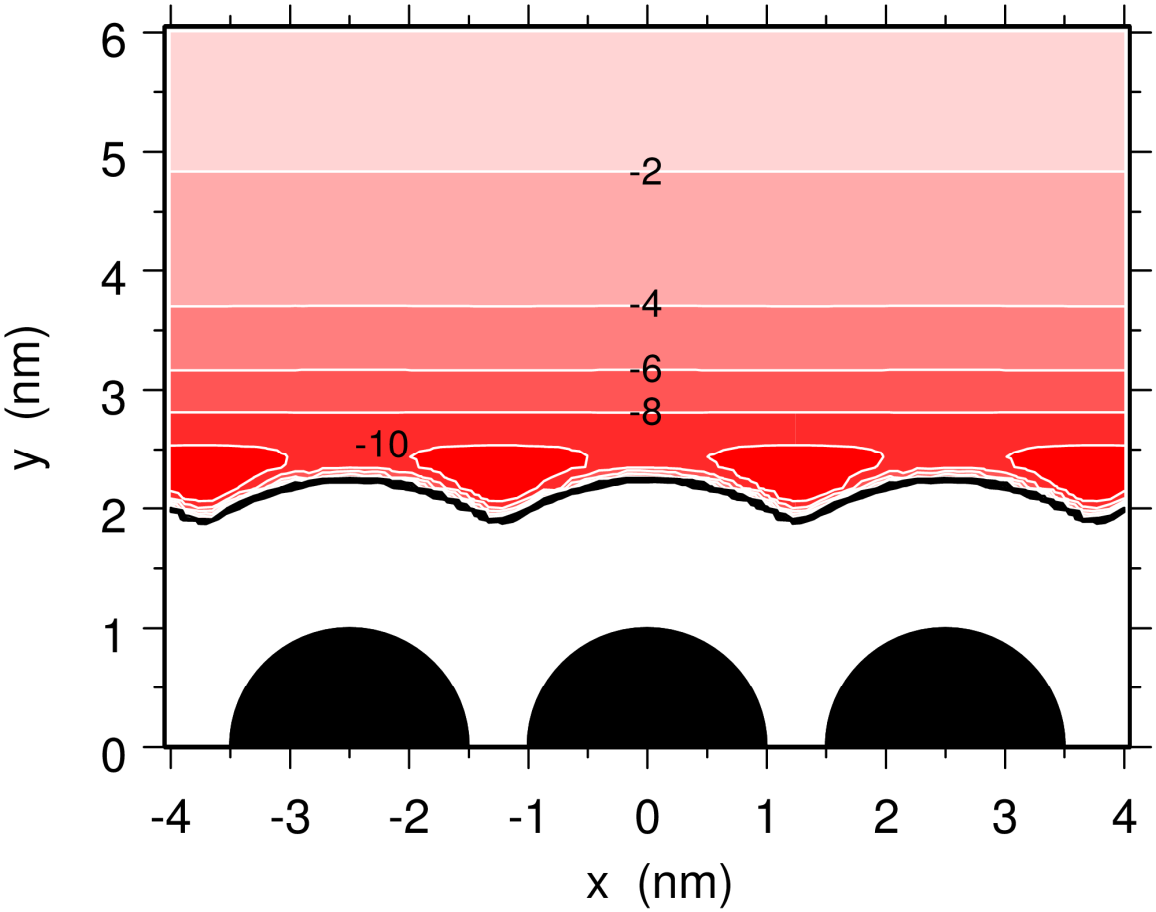

Figure S2 : Potential energy felt by a protein chain aligned along the *y* axis when approaching an infinite DNA chain elongated along the *x* axis, for an effective valency *Z*=1.37. Both chains are at equilibrium with respect to their stretching and bending degrees of freedom. The black disks represent DNA beads. In this geometry the potential has rotational symmetry along the *x* axis. (*x*,*y*) denote the coordinates of the center of the terminal bead of the vertical protein chain that lies closest to the horizontal DNA chain. The minima of the potential energy surface ($-12.0\,k_{\mathrm{B}}T$) are located at equal distances from two successive DNA beads. Contour lines are separated by $2\,k_{\mathrm{B}}T$.

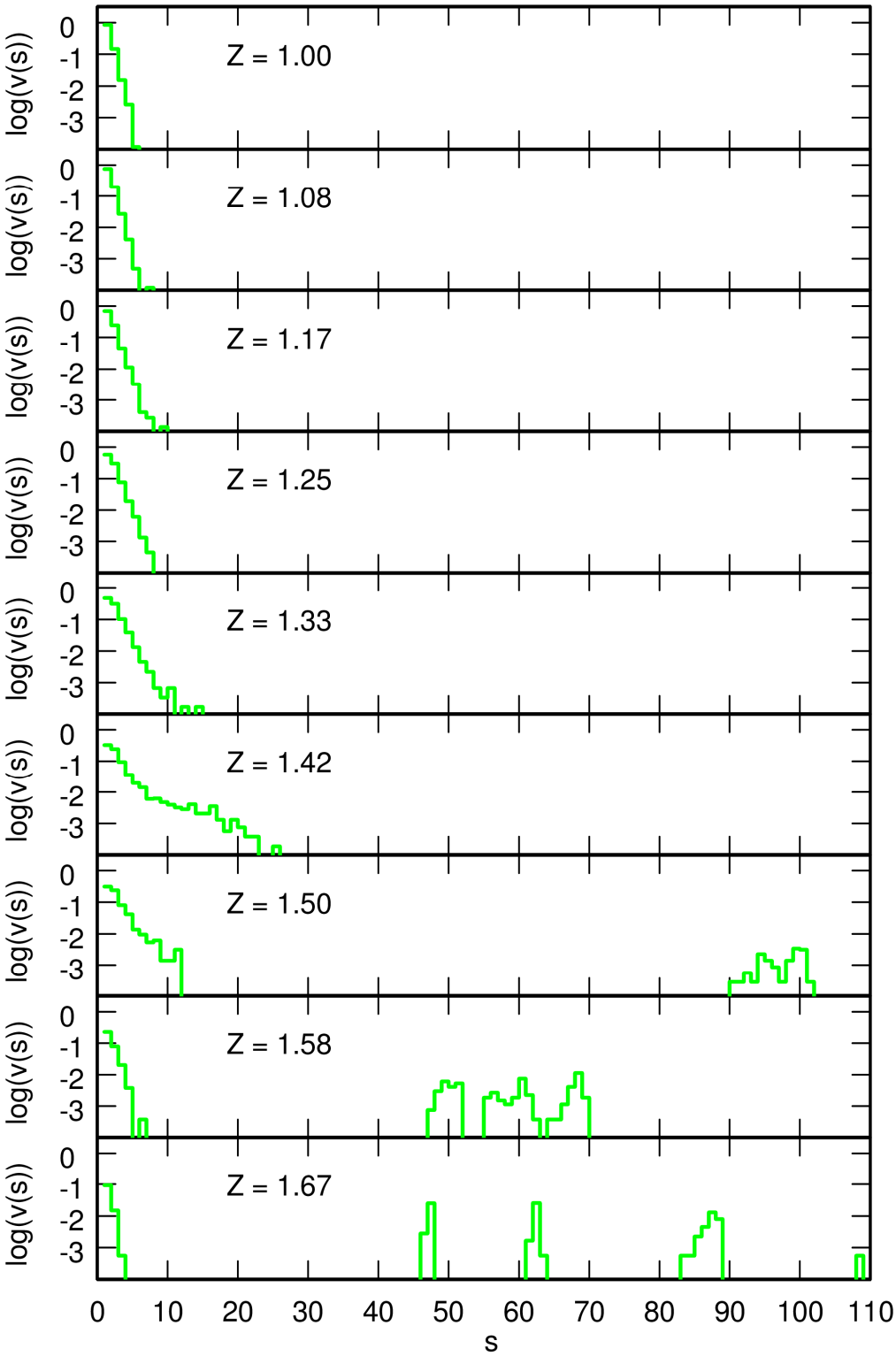

Figure S3 : Decimal logarithm of $v(s)$, the probability distribution for a protein cluster to contain s protein chains, for values of Z increasing from 1.00 to 1.67. Each plot was obtained from a single equilibrated simulation with 200 protein chains by averaging over a time interval of 0.1 ms.

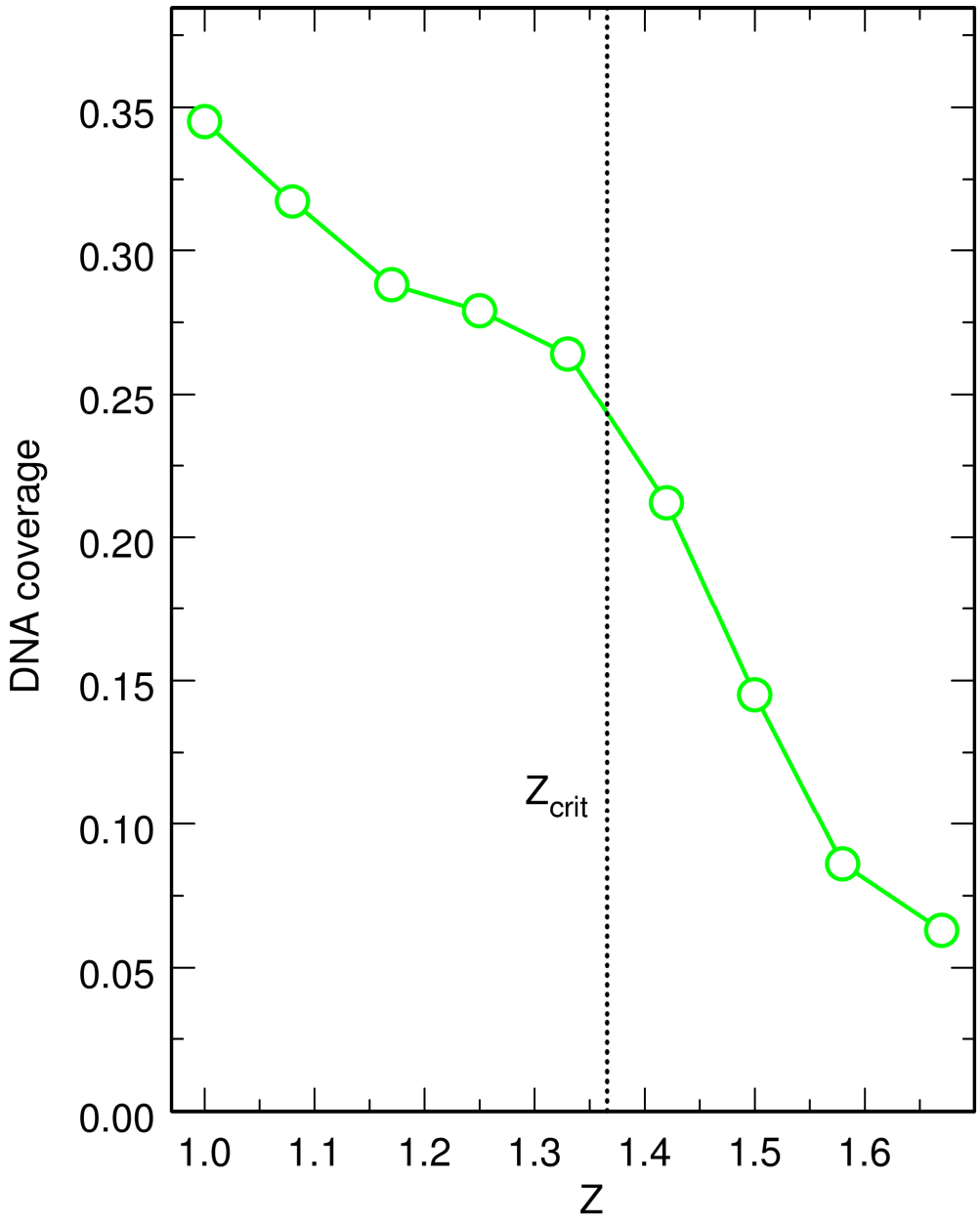

Figure S4 : Plot, as a function of Z, of the fraction of the DNA chain covered by protein chains, for 200 protein chains. A bead *k* of the DNA chain is considered to be "covered" by a protein chain if it is bound to a protein chain or surrounded, in the range $[k-5, k+5]$, by two beads that are bound to protein chains belonging to the same cluster.