Hysteresis in DNA compaction by Dps is described by an Ising model

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In all organisms, DNA molecules are tightly compacted into a dynamic 3D nucleoprotein complex. In bacteria, this compaction is governed by the family of nucleoid-associated proteins (NAPs). Under conditions of stress and starvation, an NAP called Dps (DNA-binding protein from starved cells) becomes highly up-regulated and can massively reorganize the bacterial chromosome. Although static structures of Dps–DNA complexes have been documented, little is known about the dynamics of their assembly. Here, we use fluorescence microscopy and magnetic-tweezer measurements to resolve the process of DNA compaction by Dps. Real-time in vitro studies demonstrated a highly cooperative process of Dps binding characterized by an abrupt collapse of the DNA extension, even under applied tension. Surprisingly, we also discovered a reproducible hysteresis in the process of compaction and decompaction of the Dps–DNA complex. This hysteresis is extremely stable over hour-long timescales despite the rapid binding and dissociation rates of Dps. A modified Ising model is successfully applied to fit these kinetic features. We find that long-lived hysteresis arises naturally as a consequence of protein cooperativity in large complexes and provides a useful mechanism for cells to adopt unique epigenetic states.

Purified DNA behaves as an entropic spring with a radius of gyration that scales as a function of the contour length (1). In contrast, DNA in vivo is highly organized and condensed. In bacteria, this condensation is caused by nucleoid-associated proteins (NAPs) that collectively shape the chromosome (2, 3). NAPs are capable of binding genomic DNA and in doing so alter its shape, control the transcriptional expression of genes, and remodel the structure of the nucleoid in response to external stimuli (2, 3).

DNA-binding protein from starved cells (Dps) is an NAP structurally related to ferritins and associated with the response to stress. Dps is highly expressed in stationary phase (4–7) and is also involved in the cellular response to oxidative (4, 8–10), UV (8, 11), thermal (8), and pH shocks (8). In addition, Dps has been implicated in biofilm formation and tolerance to bacteriophage attacks (12). Dps monomers have a molecular mass of 19 kDa and assemble into a dodecameric shell (Fig. S1A) (13).

The resulting complex binds to both supercoiled and linear DNA to form a dense biocrystal structure (4, 7, 9, 14).

Although the crystal structure of the Dps dodecamer has been solved (13), no atomic-scale structure of Dps–DNA assemblies currently exists and little is known about complex formation. The affinity of Dps for DNA is very sensitive to buffer conditions. Like many DNA-binding proteins, Dps binds DNA more weakly in the presence of higher salt concentrations. Less typically, divalent cations such as Mg2+ can substantially weaken the affinity of Dps for DNA (9, 15). It has been proposed that fluctuations in divalent cation concentrations act as a trigger for biocrystal assembly in vivo (9, 16). Dps dodecamers have an overall negative charge that electrostatically repels the DNA backbone, whereas positively charged lysine residues located in the disordered N termini play an important role in DNA binding (10, 13, 15). Interestingly, in images of biocrystals, Dps dodecamers are also tightly packed, implying existence of extensive Dps–Dps interactions (14).

The transition into a compact Dps–DNA state seems to be cooperative (10), but the mechanism behind this transition is unclear. In bulk gel shift assays, Dps forms a massive complex with DNA and shows few intermediate-sized complexes (15). Similarly, little evidence of structural intermediates has been reported in atomic-force microscopy and EM studies performed in vitro. Here we follow Dps-mediated DNA compaction (and subsequent decompaction) at the single-molecule level in real time. These experiments provide a detailed view of Dps binding transitions. Our measurements indicate that Dps–DNA complexes shift rapidly between two stable states, compact/bound and extended/unbound, with distinct hysteresis that can be fit using an Ising model.

**Results**

DNA Compaction by Dps Occurs Abruptly. We developed a fluorescence assay to directly visualize the process of Dps–DNA complex formation at the single-molecule level without applied tension. Linear DNA molecules were attached to the surface of a flow cell and were labeled with YOYO-1 (Fig. S1B, green stars). To induce DNA compaction, a reaction buffer with 0.2 mM Dps labeled with Cy5 (Fig. S1B, red stars) was added (Materials and Methods).

We followed the binding of Dps proteins onto DNA molecules over tens of minutes using fluorescence microscopy (Movie S1). We deliberately chose low Dps concentrations, as determined by a bulk gel shift assay (Fig. S2), to slow down the arrival of Dps and resolve the process of DNA compaction in real time.

**Significance**

Cooperativity has been a fundamental concept in our understanding of biological systems for over a century. Here, we describe the observation of cooperative binding that exhibits long-lived hysteresis and cannot be described by a standard Hill model. Inspired by the Ising model of ferromagnetism, we describe this hysteresis as a consequence of cooperative binding in the limit of large complexes. We provide a method to relate the amount of hysteresis to the strength of the neighboring interactions between bound proteins and DNA. This novel kinetic feature of macromolecular complexes allows cells to create a binary response to small changes in external conditions and causes complexes to retain a memory of past conditions over long timescales.
A sequence of frames for one such DNA molecule is shown in Fig. 1A. Initially, the DNA molecule moved freely around the attachment point (0–720 s). The binding of Dps to the DNA (frames 900–1,620 s) resulted in a rapid colocalization of these two molecules into a smaller, immobile Dps–DNA complex. To further analyze the transition of the DNA into an immobile state, we measured the fluctuations of the DNA between frames and the maximum fluorescence intensity of the Dps in individual complexes (Fig. 1B). We attribute the sharp increase in Dps brightness and abrupt decrease in DNA fluctuations to the binding of labeled Dps and compaction of the DNA. For each DNA molecule, a variable delay of 200 ± 230 s (mean ± SD) was observed before the collapse (Fig. S3A). To visualize the compaction at high time resolution, we aligned the traces at the time point of collapse (Fig. S3B). An averaged trace for all observed molecules after alignment shows that the majority of Dps molecules bound in a 6-s window, with DNA compaction occurring nearly simultaneously (Fig. 1C). This sharp transition suggests a highly cooperative binding mechanism and a tight coupling between Dps binding and the compaction of DNA.

DNA Reorganization by Dps Is History-Dependent. Once Dps has assembled on DNA, we were interested in observing the dissociation of the Dps–DNA complex as well. We therefore analyzed five consecutive records of DNA molecules in the absence and presence of Dps under different ionic conditions (Fig. 1D). We first measured the average fluctuations of DNA molecules in the absence of Dps. Addition of 0.75 μM Dps in the reaction buffer resulted in a compaction of the DNA molecules (i.e., decreased fluctuations) and an abrupt increase of the peak Dps fluorescence. Upon flushing out Dps with five volumes of reaction buffer, we observed that the Dps intensity decreased but the Dps–DNA complexes remained a static structure. Addition of 3 mM MgCl₂ to the buffer weakened the affinity of Dps for DNA. This caused a sharp drop in Dps fluorescence intensity and a marked increase in DNA fluctuations, indicating the release of Dps from the DNA. Subsequent removal of the MgCl₂ by flushing in five additional volumes of the reaction buffer demonstrated that the DNA remained flexible. Dps could also be released by raising the pH to 8.1 (Fig. S3C).

In these experiments, preformed Dps–DNA complexes remained stable even after we lowered the Dps concentration to below 0.075 μM by flushing the flow cell with buffer (this estimation was made based on the reduction in the fluorescent background). In contrast, initially bare DNA did not collapse even after the addition of up to 0.1 μM Dps (Movie S2). These experiments establish that DNA compaction by Dps is history-dependent rather than being a simple function of the current Dps concentration. To probe this hysteresis in more detail, we decided to use tension to perturb Dps–DNA assemblies.

Tension Modulates the Affinity of Dps for DNA. We developed a magnetic tweezer assay (17–19) that allowed us to modulate the force applied to individual DNA molecules in the presence of Dps (Fig. S1C). By applying a slowly decreasing force (from 15 to 0.01 pN over >40 min) followed by a slowly increasing force (from 0.01 to 15 pN over >40 min) we probed for hysteresis between the assembly and disassembly of Dps–DNA complexes.

First, we consider the force-extension curves when the force is gradually decreased (solid lines, Fig. 2A). For DNA molecules without Dps in the solution, the measured extension at a given force can be approximated by the worm-like chain (WLC) model (20). In contrast, for DNA molecules in the presence of 8 μM Dps, a sharp compaction of the DNA is observed. Three example DNA traces show an abrupt collapse that occurs at a critical force $F_1 \approx 1.5$ pN. This result demonstrates that the Dps molecules can perform work on the magnetic bead to compact DNA.

Next, we consider the force-extension curves when the force is gradually increased (dashed lines, Fig. 2A). Without Dps present, the DNA extension again follows the WLC model, as expected. However, in the presence of Dps the DNA extension follows a new pattern. The DNA molecules remain highly compacted until they reach a second critical force $F_2 \approx 0$ pN. At this force, the Dps–DNA complex breaks, and the DNA returns to the extension predicted by the WLC model. We define $F_1$ and $F_2$ as the forces that correspond to a DNA extension of half the contour length ($\sim 5 \mu$m). The variation of $F_1$ and $F_2$ between molecules ranged from 10% to 25% in different conditions. We attribute this variation to experimental uncertainty, because the actual force applied to beads across the field of view can vary by as much as 24% (21).

To illustrate this behavior further, we replot the decreasing force-extension data from Fig. 2A near the critical force $F_1$ as a function of time (Fig. S4A). We observe that around $F_1$ the DNA extension decreased monotonically until it reached a fully compact state. We also replot the increasing force-extension data near the second critical force $F_2$ (Fig. S4B). The DNA extension exhibits a nearly monotonic increase in extension until it is fully extended, indicating that DNA compaction is reversible. This result demonstrates that outside of the critical force region the DNA extension converges to a single equilibrium fairly rapidly, with hundreds of Dps dodecamers binding or releasing over the course of 200–300 s. We also note that on short timescales (~1 s) the DNA extension can exhibit small (<50 nm) reversible fluctuations.

Plotting the average of multiple force-extension curves ($N=11$), we observe that the DNA extension is roughly homogeneous among the different molecules with a reproducible hysteresis (Fig. 2B). In principle, the observed hysteresis could be a...
and the narrowing of the width of the hysteresis loop (Fig. 2F) and the total width of the hysteresis loop (Fig. 3B). Increasing the concentration of monovalent salts from 50 to 150 mM destabilized the Dps–DNA complex, as demonstrated by the progressive reduction of $F_2$ and the narrowing of the width of the hysteresis loop (Fig. 3B). The addition of magnesium (2 mM) to the buffer caused a similar destabilization of the Dps–DNA complex (Fig. 3C).

We also tested whether crowding can affect DNA compaction. Interestingly, the addition of PEG 8K caused a sharp increase in the stability of the Dps–DNA complex (Fig. 3D). Finally, we observed that increasing the pH weakened the Dps–DNA complex (Fig. S5). Overall, our results revealed that Dps-induced compaction and decompaction of DNA is strongly influenced by tension applied to the DNA, ionic strength, magnesium, macromolecular crowding, and pH. These results are consistent with the trends observed in a recent single-molecule study of Dps–DNA interactions (24).

Discussion

Reversible DNA compaction by Dps is characterized by hysteresis. Here, we performed real-time in vitro measurements to study the biophysical properties of Dps–DNA complex formation at the single-molecule level. We find that a rate-limiting nucleation event stimulates the rapid incorporation of multiple Dps dodecamers on DNA, resulting in extensive compaction (Fig. 1A–C). Moreover, the degree of DNA compaction by Dps is influenced by past concentrations of Dps (Fig. 1D), that is, the system exhibits hysteresis. By changing the tension applied to Dps–DNA complexes, we show that hysteresis is also observed in force-extension curves and can be characterized by the critical forces $F_1$ and $F_2$, which define the onset of DNA compaction and decompaction, respectively (Fig. 2B). This hysteresis is nearly independent of the pulling rates (Fig. 2C). Instead, we find that within the range of forces between $F_1$ and $F_2$ the DNA is trapped in one of the two stable local equilibria, compact or extended (Fig. 2D). Moreover, the measured hysteresis in the DNA extension is strongly affected by salinity, magnesium, crowding, and pH (Fig. 3 and Fig. S5). These data, combined with bulk gel shift assays (Fig. S2), demonstrate that Dps binds DNA cooperatively.

We consider several possible models to explain the history-dependent mechanism of complex formation between a long, flexible polymer and a large number of self-interacting proteins. Cooperative binding is frequently modeled with the Hill equation (25). The resulting binding curve shows a characteristic sigmoidal shape that transitions sharply from low to high occupancy compared with a noncooperative binding curve. The Hill equation has been used to characterize Dps binding previously (10), and it reasonably fits our own bulk experimental data (Fig. S2). However, the Hill model assumes that the system can equilibrate to find the global minimum in free energy, which precludes hysteresis. Other standard models of cooperativity, such as the Koshland–Némethy–Filmer (KNF) (26), Monod–Wyman–Changeux (MWC) (27), and conformational spread (CS) (28) models, can potentially be used to model hysteresis.

Dps-Induced Compaction of DNA Is Influenced by Salt, Magnesium, pH, and Crowding Conditions. When the applied force is lowered to $F_1$, Dps must do work on the bead to compact the DNA. Later, when the force is raised to $F_2$, the bead must do work on the Dps to break the complex. The amount of work done in each case is a function of the strength of the Dps–DNA and Dps–Dps interactions. To explore how these interactions can be altered, we measured the compaction of DNA molecules exposed to Dps under a variety of buffer conditions. We performed force-extension cycles with the same pulling rate as shown in Fig. 2B, paying particular attention to shifts in the average of $F_1$ and $F_2$ and the total width of the hysteresis loop, defined as the difference between $F_1$ and $F_2$.

We recorded the compaction and decompaction of DNA at several Dps concentrations (2, 4, and 8 μM). Surprisingly, varying the Dps concentration over this range yielded relatively minor changes in the mean values of $F_1$ and $F_2$, and in the width of the hysteresis loop (Fig. 3A). Increasing the concentration of monovalent salts from 50 to 150 mM destabilized the Dps–DNA complex, as demonstrated by the progressive reduction of $F_2$ and the narrowing of the width of the hysteresis loop (Fig. 3B). The addition of magnesium (2 mM) to the buffer caused a similar destabilization of the Dps–DNA complex (Fig. 3C).

We also tested whether crowding can affect DNA compaction.
although almost all of the current literature focuses on their predictions at equilibrium. To better understand how a system can be trapped in nonequilibrium states, we turned to the Ising model of ferromagnetism.

A modified Ising model of cooperativity predicts hysteresis. The Ising model was first developed to describe interactions between magnetic dipoles placed in an external magnetic field, giving rise to ferromagnetism (29). A strong magnetic field can lock the dipoles predominantly in a single orientation, creating a stable magnetization that persists when the magnetic field is reduced. This is analogous to our observations that Dps can lock DNA in a stable complex that persists when the concentration of Dps is reduced or the tension is increased.

To apply the Ising model to Dps–DNA interactions, we assume that a DNA strand contains a fixed number of Dps binding sites, each of which can exist in an empty or occupied state. Further, we assume that Dps binding and DNA compaction at the binding site are tightly coupled, so that the number of occupied binding sites is proportional to the DNA extension. Our model then depends on only two free parameters, which are defined in limiting cases. First, if the DNA is fully extended the affinity of Dps for DNA is characterized by a dissociation constant $K_0$. Second, we assume that additional Dps–Dps and Dps–DNA interactions stabilize the complex in compact states (Fig. 56A). If the DNA is fully compacted, the sum of all of the energetic interactions between one dodecamer and its neighbors defines the cooperativity parameter $I$. For convenience we write $I$ as a dimensionless multiple of $k_BT$. Finally, for intermediate conformations some binding sites are empty, so the number of interactions between bound dodecamers will be lower than in the fully compacted conformation. Because the DNA can fold in many possible conformations, we cannot predict the exact number of interactions stabilizing a specific bound dodecamer. Instead, we use a mean-field approximation (30) to estimate that this number scales with the mean probability of Dps occupying the other binding sites. Whereas the Ising model and the mean-field approximation have been applied to study cooperative binding in other systems (31), our approach focuses specifically on how these assumptions can give rise to hysteresis.

To model the effects of force, we set the size of each binding site to 60 bp of DNA based on titration measurements of Dps dodecamers (15). Compaction of these 60 bp by Dps decreases the DNA extension by a force-dependent distance $\delta F$, which we estimate with the WLC model (20). Taken together, these parameters give rise to a transcendental equation for the probability $P$ of a given DNA binding site being occupied by a Dps dodecamer (Eq. S8):

$$P(A | F) = \frac{1}{1 + \frac{K_0^A \delta F D}{P} \left( 1 + \frac{k_B T}{P} ight)}$$

Here, $[A]$ is the concentration of Dps, $F$ is the applied force, $D = \delta F/k_BT$ is the normalized change in extension, and $K_0 = k_0 e^{-\epsilon/2}$ is the Dps concentration associated with 50% occupancy of the binding sites at zero force. The dimensionless parameter $I$ is analogous to the Hill coefficient and serves a measure of cooperativity, and $K_0$ describes how tightly Dps binds bare DNA.

To understand why our model gives rise to hysteresis we examine a concentration where the binding sites are equally likely to be occupied or empty ($[A] = K_{off} e^{\epsilon/2}$). When no cooperativity exists ($I = 0$), the occupancies of the binding sites are independent of each other, much like individual coin tosses. Therefore, if we plot the global free energy as a function of the number of bound dodecamers, it scales with the logarithm of the binding distribution, creating an entropic minimum at $P = 0.5$ (Fig. 4A and Eq. S17). When $I > 0$, a quadratic term is added to the global free energy proportional to $(1/2)P^2(1 - P)$, penalizing states near $P = 0.5$. At the critical value of $I = 4$ the solution at $P = 0.5$ switches from a stable equilibrium to an unstable equilibrium. For values of $I > 4$ a global energetic barrier arises between the majority bound/unbound states, and the Dps–DNA complex behaves collectively as a two-state system.

Before this transition to a two-state system, the Dps–DNA complex reaches equilibrium at a rate of roughly $k_{on} = k_{off} + k_{on}$. $k_{on}$ and $k_{off}$ are the individual binding and dissociation rates of Dps dodecamers (represented by the small saw-tooth peaks in Fig. 4A). After the transition, the rate will begin to scale as $k_{eq} = e^{-\frac{\epsilon}{k_BT}}(k_{on} + k_{off})$, where $H$ is the height of the global energetic barrier in units of $k_BT$. Because $H$ scales with both the number of binding sites $N$ and with cooperativity $I$, for large Dps–DNA complexes (e.g., kilobases of DNA) even small changes in the cooperativity dramatically increase the barrier height. For the $300k_BT$ barrier shown in Fig. 4A, $k_{eq}$ will be approximately $10^{30}$ times slower than $k_{on}$ and $k_{off}$.

When the barrier at $P = 0.5$ is very high, we are unlikely to observe transitions over the barrier so the positions of the local equilibria become more important than the global equilibrium. In a noncooperative binding curve ($I = 0$), only one local equilibrium exists and $P$ smoothly increases as a function of Dps concentration (Fig. 4B, gray). In a cooperative binding curve where the system can globally equilibrate, we expect $P$ to increase sharply as a function of concentration (Fig. 4B, green).

However, our Ising model predicts that at $I = 4$ (Eqs. S10 and S18), there is a region where three solutions for $P$ exist for a given Dps concentration (Fig. 4B, red). In this region, the high and low solutions represent stable local equilibria of the system. The intermediate solution is an unstable equilibrium that corresponds to the energetic barrier between the stable solutions. Therefore, our model predicts that Dps–DNA complexes can exist in either a highly compact or extended conformation depending on the path used to bring the concentration into the critical region.

**Force-Extension Experiments Measure Cooperativity and Hysteresis.** Next, we consider the effects of a changing tension applied to the Dps–DNA complex. The relationship between DNA extension and applied force is plotted for no cooperativity, a modified Hill model, and the Ising model in Fig. 4C (gray, green, and red, respectively) (Eqs. S6–S9). Similar to the case without tension, our model predicts the existence of two highly stable local equilibria and one unstable equilibrium within a critical range of forces between $F_1$ and $F_2$. The path used to bring the DNA into the critical force range determines which equilibrium is adopted. Above $F_2$, the DNA has an extended conformation. As the force is decreased below $F_2$, the DNA remains trapped at the extended local equilibrium (Fig. 2D). However, below $F_1$ no extended equilibrium exists. Therefore, when the force is then dropped below $F_1$, the Dps–DNA complex rapidly transitions to a compact conformation. Similarly, a compact DNA molecule suddenly transitions to an extended conformation only when the force is increased above $F_2$. In terms of the global free energy calculations, critical forces $F_1$ and $F_2$ correspond to the forces where the energetic barrier between the stable equilibria vanishes (Fig. S6B and Eq. S19).

The critical forces $F_1$ and $F_2$ can be used to determine the model parameters $I$ and $K_0$, provided we specify the size of the binding site $\delta F$. We estimate $F_1$ and $F_2$ by identifying where the DNA reaches 50% of its full extension (~3.5 μm) in the decreasing and increasing force-extension curves. At each critical force, a Dps dodecamer that binds DNA performs a fixed amount of work $W = F \cdot \delta F$. We define $W_{off}$ as the difference in work performed by Dps at the two critical forces $F_1$ and $F_2$ and show that it is a function of the parameter $I$ (Eq. S11):

$$W_{off} = (F_2 - F_1) \cdot \delta F = I \left( \frac{1}{1 - \frac{1}{2}} \right) - \frac{2}{1 - \frac{1}{2}}$$

We can also define $W_{on}$, the average of the work performed by Dps at the two critical forces $F_1$ and $F_2$, and show that it is given by Eq. S12:
We define the average work per Dps molecule relative to the random state in the case of: no cooperativity (gray), Hill cooperativity (with Hill coefficients $K_D$ and $K_D'$), and Ising (red) (Eqs. S5–S8). The difference $\Delta E$ is plotted as a function of $K_D$ for all buffer conditions tested in Table S1. We find that the neighboring interactions are weakened dramatically by salt, magnesium, and increasing pH. This result emphasizes that electrostatics play an important role in the binding of Dps dodecamers to each other. Conversely, the addition of crowding agents strengthens the neighboring interactions. Given that macromolecular crowding favors complexes with a smaller exposed surface area (32) this finding supports the idea that Dps dodecamers form a compact geometry on the DNA. The affinity of Dps for extended DNA, as measured by $\ln(K_D)$, is affected by buffer conditions in a similar manner to the neighboring interactions. Therefore, the overall stability of the complex, as measured by $W_{ave}$, correlates with the amount of hysteresis, as measured by $W_{diff}$ (Fig. S7).

Hysteresis in Other Models of Cooperativity. To examine whether hysteresis is unique to an Ising mechanism, we compare our model to other models of cooperativity. The KNF model (26), like our model, assumes a tight coupling between the occupancy of the DNA binding site and its conformation. Unlike our model, the KNF model assumes cooperative interactions are mediated through the conformation of neighboring DNA binding sites rather than through Dps–Dps contacts. However, given that occupancy and conformation are tightly coupled, these two interpretations produce equivalent energetic predictions. Therefore, our model can be viewed as a modification of the KNF model tailored to the flexible geometry of the Dps–DNA system.

The MWC model makes a very different physical assumption, requiring a concerted switch between a completely extended and a completely compacted DNA molecule (27). This concerted switching is not a physically realistic model of DNA dynamics, which should be uncorrelated over distances larger than the persistence length. Nevertheless, like our model the MWC model would give rise to a large global energetic barrier between two local binding equilibria and, therefore, to hysteresis (Fig. S84 and Eq. S21).

The CS model (28) is also derived from the Ising model, but it contains as many as five free parameters (33), compared with the two used in our model. With these additional parameters, the CS model can approximate the KNF model, the MWC model, or our own model when applied to a fixed lattice. The CS model assumes that the binding substrate exists in an explicit geometry, such as a 1D ring or a 2D lattice (33). Traditionally the CS model avoids a mean-field approximation, so for 2D and 3D lattices the global free energy and binding probability must be computed numerically rather than by deriving explicit equations (e.g., Eq. 1). In our system, there is no fixed lattice because the DNA can fold in many potential conformations. Therefore, an exact comparison of our model to the CS model is not possible. Instead we consider a fixed 3D lattice (27 binding sites) and demonstrate that both models predict hysteresis that arises at nearly the same amount of cooperativity (Fig. S8B). The transition of the CS model into a hysteresis regime has been noted previously (28), but has only been explored in the 1D limit (34), where the mean-field approximation breaks down.

We conclude that cooperativity can lead to hysteresis for a variety of mechanistic assumptions. However, we find our modified Ising model is particularly well-suited for modeling Dps–DNA complexes: it directly accounts for Dps–Dps interactions that are implied by the crystal structure (13), it provides an explicit prediction of the free energies and equilibria using a minimal set of free parameters, and it allows for partially collapsed states. Further, the mean-field approximation allows our model to be applied easily to a range of systems that might also be fit with a KNF or CS model while remaining agnostic about the exact geometry of the lattice. This makes our model particularly attractive when the lattice structure is undefined or too complex to precisely calculate the intermediate states.

Implications of Cooperative Hysteresis. The hysteresis described here for Dps–DNA complexes could be advantageous to bacterial survival for several reasons. First, hysteresis might ensure

$$\frac{W_{ave}}{k_B T} = \frac{I}{2} + \ln \left( \frac{[D]}{K_D} \right) = \ln \left( \frac{[D]}{K_D} \right).$$

Next, we compare this model to our empirical findings. When we change the concentration of Dps, $W_{diff}$ remains roughly constant, as predicted by Eq. 2. However, if we change the ionic strength of the buffer, we observe large changes in $W_{ave}$, indicating that $I$ is influenced by electrostatic interactions between neighboring Dps molecules (Fig. 4D). We also find that $W_{ave}$ can be roughly fit to a logarithmic function of Dps concentration as predicted by Eq. 3 and exhibits a strong dependence on electrostatic interactions (Fig. 4E). The magnitude of the change in $W_{ave}$ relative to $W_{diff}$ requires that both $I$ and $K_D$ are dependent on salt concentration. Low salt leads to tighter binding on bare DNA and exhibits a strong dependence on electrostatic interactions. Low salt leads to tighter binding on bare DNA and exhibits a strong dependence on electrostatic interactions (Fig. 4F, thin lines) to the predictions of our model (Fig. 4F, thick lines). Within the critical region between $F_1$ and $F_2$, we observe that the experimental curves track one of the two stable solutions. Outside this critical region, we find the experimental curves converge to the single stable solution.

A quantitative summary of the interaction parameters $I$ and $K_D$ (as derived from $W_{diff}$ and $W_{ave}$) for all buffer conditions tested is presented in Table S1. We find that the neighboring interactions are weakened dramatically by salt, magnesium, and increasing pH. This result emphasizes that electrostatics play an important role in the binding of Dps dodecamers to each other. Conversely, the addition of crowding agents strengthens the neighboring interactions. Given that macromolecular crowding favors complexes with a smaller exposed surface area (32) this finding supports the idea that Dps dodecamers form a compact geometry on the DNA. The affinity of Dps for extended DNA, as measured by $\ln(K_D)$, is affected by buffer conditions in a similar manner to the neighboring interactions. Therefore, the overall stability of the complex, as measured by $W_{ave}$, correlates with the amount of hysteresis, as measured by $W_{diff}$ (Fig. S7).

**Fig. 4.** An Ising model describes hysteresis. (A) Plots of $\Delta G_{global}$ (Eq. S17) demonstrate that high cooperativity creates two local equilibria: $I = 0$ (gray), $I = 4$ (blue), $I = 8$ (red), and $I = 12$ (black). (B) The probability binding DNA as a function of Dps concentration in the case of: no cooperativity (gray), Hill cooperativity ($K_D = 8$, green), and Ising cooperativity ($I = 8$, red) (Eqs. S3–S5). (C) Force-extension predictions for DNA with different binding models: no binding (black), noncooperative (gray), Hill (green), and Ising (red) (Eqs. S6–S8). (D) Work difference as a function of Dps concentration for different NaCl concentrations (mean, SD): 50 mM (dark gray circles), 100 mM (dark purple triangles), and 150 mM (light purple squares). These values were fit to Eq. 2 (solid lines). (E) Average work as a function of Dps concentration for different NaCl concentrations (mean, SD): 50 mM (dark gray circles), 100 mM (dark purple triangles), and 150 mM (light purple squares). These values were fit to Eq. 3 (solid lines). (F) Ising model force-extension curves (bold solid lines) are superimposed on the experimental data (thin solid and dashed lines) for different NaCl concentrations: 50 mM (dark gray), 100 mM (dark purple), and 150 mM (light purple) at 8 μM Dps.
that complex formation becomes binary, because our model predicts a range of intermediate binding probabilities associated only with the unstable binding equilibrium (Fig. 4). This property makes it possible for the bacteria to maintain a subcritical concentration of Dps without substantial DNA compaction. Small alterations in the pH, crowding, salinity, or magnesium concentration in the cell could then greatly increase the overall affinity of Dps for DNA, quickly inducing compaction and protecting the chromosome. Cooperative hysteresis also allows cells to maintain a memory of past conditions; therefore, cells may tailor their response to current stress conditions. For example, a previous report suggests that Dps mediates a phase transition allowing starved cells to guard against additional stresses more effectively (9). Further, a population of cells could engage in bet-hedging strategies through hysteresis, allowing otherwise identical cells to become locked into different states. The variety of responses of these cells to new stress conditions would be more robust than adopting a single response.

Given that hysteresis arises naturally from several models of cooperativity in the limit of large complexes, this behavior is likely to be observed in other systems as well. For example, other proteins that condense DNA could exhibit similar dynamics, providing a new mechanism for epigenetic regulation. Alternately, replication of some eukaryotic viruses requires many copies of self-interacting proteins to assemble into large viral factories (35). Cooperative hysteresis could provide a useful gating mechanism to prevent premature assembly of these replication factories. More generally, any large protein complex could exhibit hysteresis in assembly, especially when a 2D or 3D lattice can be formed.

Materials and Methods
Plasmid encoding the dps gene pLysS pET17b dps 2–1 was modified to insert a cysteine at position 79 (179C), then expressed and purified as described for wild-type Dps (SI Materials and Methods). Cs5 Maleimide (GE Healthcare) was incubated at room temperature for 45 min with Dps monomers at a molar ratio of 1:15 Dps monomer to dye in a buffer of 50 mM Hepes-KOH, 400 mM NaCl, 10% (vol/vol) glycerol, and 5 M GdmCl, pH 7.3. Labeled Dps was subsequently diluted 5 times with unlabeled Dps. Then, the sample was dialyzed against 50 mM Hepes-KOH and 100 mM NaCl, pH 7.3, resulting in a labeling efficiency of ~10% (~1 dye per Dps dodecamer). Labeling of Dps did not affect the binding affinity of Dps for DNA (Fig. 5). Taking into account the labeling efficiency of Dps, the concentrations used, and the penetration depth of the evanescent wave, we calculated the fluorescence intensity per Dps dodecamer in the flow cell. Based on this calculation we estimated that 4 ± 1.6 (mean ± SD) Dps dodecamers are bound per 1 kbp of DNA.

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