Force-extension behavior of DNA in the presence of DNA-bending nucleoid associated proteins

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Interactions between nucleoid associated proteins (NAPs) and DNA affect DNA polymer conformation, leading to phenomena such as concentration dependent force-extension behavior. These effects, in turn, also impact the local binding behavior of the protein, such as high forces causing proteins to unbind, or proteins binding favorably to locally bent DNA. We develop a coarse-grained NAP-DNA simulation model that incorporates both force- and concentration-dependent behaviors, in order to study the interplay between NAP binding and DNA conformation. This model system includes multi-state protein binding and unbinding, motivated by prior work, but is now dependent on the local structure of the DNA, which is related to external forces acting on the DNA strand. We observe the expected qualitative binding behavior, where more proteins are bound at lower forces than at higher forces. Our model also includes NAP-induced DNA bending, which affects DNA elasticity. We see semi-quantitative matching of our simulated force-extension behavior to the reported experimental data. By using a coarse-grained simulation, we are also able to look at non-equilibrium behaviors, such as dynamic extension of a DNA strand. We stretch a DNA strand at different rates and at different NAP concentrations to observe how the time scales of the system (such as pulling time and unbinding time) work in concert. When these time scales are similar, we observe measurable rate-dependent changes in the system, which include the number of proteins bound and the force required to extend the DNA molecule. This suggests that the relative time scales of different dynamic processes play an important role in the behavior of NAP-DNA systems. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5016177>

I. INTRODUCTION

The genomic material in a prokaryotic cell is contained within the cellular structure known as the nucleoid, composed of DNA and nucleoid associated proteins (NAPs). NAPs act as architectural proteins by helping to organize the overall nucleoid structure.^{1–7} These proteins bind non-specifically to the DNA and can alter the local structure. For example, the NAP HU (histone-like protein) can form weakly bent hairpin turns or stiffen straight DNA, while NAPs IHF (integration host factor) and FIS (factor for inversion stimulation) can form stiff local bends.^{5,8–10} These small changes in the local structure have effects at longer length scales, which play a role in nucleoid organization.^{6,11,12} In addition to the direct architectural role these proteins play, they also indirectly play a role in several biological functions, such as DNA replication, transcription, and gene expression.^{2,3,12–17}

Many of these biological processes can exert forces on the DNA, such as the forces stemming from DNA and RNA polymerases.^{18–20} These pN scale forces can affect how proteins interact with the DNA, especially those that bind weakly such as NAPs. Single molecule studies can give us information about how forces affect the behavior of protein-DNA interactions. Studies done with NAPs such as FIS,

IHF, and HU have shown a range of force and concentration dependent extension behaviors.^{8,10,21–27} When low concentrations of these proteins are bound to the DNA, the extension at a given force decreases, indicating that these proteins are compacting the DNA.^{8,10,21–27} However, at high concentrations of proteins, reduced compaction or even a stiffening effect can be observed.^{8,10,21–27} The underlying mechanisms by which these behaviors occur are difficult to determine by single molecule experiments alone.

Simulations can give us insights into how the local interactions between the proteins and DNA are affecting the conformational behavior that is observed in these single molecule experiments. Previous studies have provided evidence that the NAP FIS has multiple binding states, where a protein can be partially bound or fully bound to a DNA strand.²⁸ Other NAPs such as HU, H-NS (histone-like nucleoid structuring protein), and IHF may also exhibit multiple binding states due to their dimeric nature.^{2,7,29} These multiple bound states are thought to play a role in these different extension behaviors. Simulations allow us to directly track the states (and number) of bound proteins and how they correlate with the overall behavior of the DNA molecule.

Simulations have investigated protein-DNA interactions on a coarse-grained scale. For example, Takada *et al.* have investigated the relationship between protein sliding and DNA bending using the NAP protein HU by using a three beads per nucleotide model for DNA (3SPN.2C)³⁰ and an

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atomistic interaction-based coarse-grained model (AICG) for proteins.³¹ They are able to look at the local dynamic interactions between the protein and the DNA. This type of coarse-grained model can be used to study other protein-DNA interactions, such as the transcription factor protein p53,^{32–34} zinc finger proteins,³⁵ and nucleosomes,^{36–38} but this level of coarse-graining cannot be extended past systems on the order of 1 μm .³⁰ There are also highly coarse-grained protein-DNA models, where there are multiple base pairs per DNA bead, and a protein is modeled with just a few particles.^{39–43} Many of these large-scale models are capable of reaching lengths on the order of nucleoid ($\sim\text{mm}$), but the interactions between the DNA and protein are often phenomenologically motivated.⁴¹ We want to create a model that is capable of capturing the equilibrium and dynamic behavior of NAP/DNA interactions that is built on physically motivated local interactions.

This work investigates the force-extension behavior of DNA as a function of concentration of FIS, whose binding behavior has been well characterized.^{22,44–50} By controlling the dependence of FIS's binding affinity on the local shape of the DNA, we can demonstrate changes in the number of proteins bound as a function of force. The different local deformations that are based on FIS's bound state are the main drivers of the different force-extension behaviors. Our model allows us to look at the behavior of FIS/DNA interactions as the DNA strand is being pulled out of equilibrium.

II. MATERIALS AND METHODS

We aim to create a simulation model that allows us to not only study NAP/DNA interactions at equilibrium but also investigate their dynamics. In order to achieve this, we model single molecule systems of interest with a coarse-grained Brownian Dynamics (BD) simulation. A strand of dsDNA is represented by a chain of $N = 100$ beads of radius $a = 4$ nm, resulting in a simulated strand that corresponds to approximately 2350 base pairs, as shown in Fig. 1(a). The size of the bead is chosen to correspond to the approximate size of a binding site of a FIS protein.^{21,45,48} One end of the DNA strand is tethered, and the other end of the DNA strand is constricted to movement only along the x axis. Distance, energy, and time in the system are non-dimensionalized by the radius of a bead a ($\tilde{r}_{ij} = r_{ij}/a$), $k_B T$ ($\tilde{U} = U/k_B T$), and the diffusion time of

a single particle $\tau_D = 6\pi\eta a^3/(k_B T)$ ($\tilde{t} = t/\tau_D$), respectively. Normalized (and dimensionless) values are denoted by a tilde.

The DNA strand can interact with a concentration field of implicit proteins. Each DNA bead can be in one of the four bound states: no protein bound (unbound, or U), a protein fully bound (F), a protein partially bound (leaving one site per DNA bead open, or P), or a saturated bead (S) with two proteins partially bound to it. A representative schematic can be seen in Fig. 1(b). This simplified four state model is motivated by previous simulations^{28,51,52} and experiments.^{21,22,53} Movement between two states, A and B, is controlled by binding and unbinding energy barriers, $\Delta\tilde{E}_{a,b}$. We use arbitrary energy barriers that result in simulated binding kinetics that are much faster than experimentally measured values in order to make our simulations computationally tractable.²¹ An example set of energy barriers for movement between the states P and F is shown in Fig. 1(c).

Force dependent binding and unbinding behavior has been experimentally observed.^{23,24} We incorporate this into our model by accounting for the energy penalty and the force required to bend the DNA into a new equilibrium position. Bare DNA (with no bound proteins) has an equilibrium angle of $\theta_{0,U} = 0.0$. Motivated by the DNA bending induced by the fully bound NAP FIS, we choose $\theta_{0,F} = \pi/3$.^{9,48,49,54,55} $\theta_{0,S} = \pi/6$ and $\theta_{0,P} = 0.0$ are parameterized to qualitatively match the force versus DNA extension curves seen experimentally.^{10,21}

The energy barrier for binding events (moving from U to P, from P to F, and from P to S) has the following form:

$$\Delta\tilde{E}_{a,b} = \Delta\tilde{E}_B^* + \delta_{b,F}\tilde{\sigma} + \frac{\tilde{\kappa}_B}{2}(\theta - \theta_{0,b})^2, \quad (1)$$

where $\Delta\tilde{E}_B^* = 3.0$ is the constant binding energy and $\delta_{a,F}\tilde{\sigma} = 2.0$ is the entropic loss associated with moving from the singly bound state to the fully bound state. The last term is included in order to cause binding events to be more likely when the DNA strand is in a preferential conformation ($\theta_{0,b}$). This is not a direct effect of the applied force since the force applied to the DNA cannot affect a protein until it is bound. The force instead biases DNA into states that are far from ideal binding configurations, leading to an indirect effect on binding likelihood.

The force-dependent energy barrier for unbinding events (movement from P to U, from F to P, and from S to P) has the

FIG. 1. (a) A snapshot of the tethered DNA strand from a simulation with the bending angle θ shown in the inset. (b) Schematic of the four different coarse-grained DNA states. The small gold beads represent DNA binding sites, and the large yellow circle represents a single simulated DNA bead. Blue dimers represent protein dimers. (c) An example energy landscape of the binding/unbinding model of the partially bound and fully bound states. Each ΔE is a function of θ , as described in Eqs. (1) and (2).

following form:

$$\Delta\tilde{E}_{b,a} = \Delta\tilde{E}_{UB,a}^* + \lambda[\tilde{\kappa}_B(\theta - \theta_{0,b})], \quad (2)$$

where $\Delta\tilde{E}_{UB,a}^*$ are the constant unbinding energies associated with changing the bound state. These values are set to $\Delta\tilde{E}_{UB,F}^* = 10.0$ and $\Delta\tilde{E}_{UB,P}^* = \Delta\tilde{E}_{UB,S}^* = 12.0$. The last term is associated with the change in energy landscape due to the bending force, which is related to the force applied to the DNA strand. $\lambda = 0.7$ is the strength of this force dependence and is chosen to see a measurable change in the binding behavior in our simulations.

When the DNA strand is being pulled by a low force, there are more local bends in the strand (higher average θ). This leads to a decrease in the binding energy barriers [last term in Eq. (1)] and an increase in the unbinding energy barriers [last term in Eq. (2)], making it easier for a protein to move from an unbound state to a bound state. Alternatively, at high force, there are fewer local bends in the DNA strand (lower average θ), which corresponds to an increase in binding energies and a decrease in unbinding energies. Proteins therefore have an easier time moving from a bound state to an unbound state at high force.

A Monte Carlo type update of each DNA bead's bound state, denoted by Ω_i , occurs every $\tilde{\tau}_{MC} = 0.05$. This update time step is chosen to match the average constant binding time, τ_B , to the diffusion time, τ_D , of a single bead, given that a protein is in a position to bind, which is dependent on concentration. This update also shows the statistical results of a Bell Model type reaction.⁵⁶ A random number ζ is generated, and the binding update occurs as follows:

$$\Omega_i(t) = \begin{cases} \begin{cases} \text{P if } \zeta < \gamma e^{-\Delta E_{U,P}} \\ \text{U if } \zeta > \gamma e^{-\Delta E_{U,P}} \end{cases} & \text{if } \Omega_i(\tilde{t} - \tilde{\tau}_0) = \text{U} \\ \begin{cases} \text{F if } \zeta < e^{-\Delta E_{P,F}} \\ \text{P if } \zeta > e^{-\Delta E_{P,F}} \end{cases} & \text{if } \Omega_i(\tilde{t} - \tilde{\tau}_0) = \text{P} \\ \begin{cases} \text{P if } \zeta < e^{-\Delta E_{F,P}} \\ \text{F if } \zeta > e^{-\Delta E_{F,P}} \end{cases} & \text{if } \Omega_i(\tilde{t} - \tilde{\tau}_0) = \text{F} \\ \begin{cases} \text{U if } \zeta < e^{-\Delta E_{P,U}} \\ \text{P if } \zeta > e^{-\Delta E_{P,U}} \end{cases} & \text{if } \Omega_i(\tilde{t} - \tilde{\tau}_0) = \text{P} \\ \begin{cases} \text{S if } \zeta < \gamma e^{-\Delta E_{P,S}} \\ \text{P if } \zeta > \gamma e^{-\Delta E_{P,S}} \end{cases} & \text{if } \Omega_i(\tilde{t} - \tilde{\tau}_0) = \text{P} \\ \begin{cases} \text{P if } \zeta < e^{-\Delta E_{S,P}} \\ \text{S if } \zeta > e^{-\Delta E_{S,P}} \end{cases} & \text{if } \Omega_i(\tilde{t} - \tilde{\tau}_0) = \text{S} \end{cases}. \quad (3)$$

The γ coefficient corresponds to the concentration dependence of binding. We impose a chemical potential on our system, $\mu = \mu_o + \ln(c)$, where c is the concentration of the protein in μM and γ takes the form $\gamma = e^{-\mu} = e^{-\mu_o}c$. This impacts the probability that the DNA bead will move from the unbound state to the P (or S) state. The values of γ were found by matching to simulations done with explicit, freely diffusing proteins, which indicated that $\mu_o = 1.7 \mu\text{M}$.

The movement of each DNA bead i is governed by the Langevin equation,

$$\frac{d\mathbf{r}_i}{dt} = - \sum_j^{N_{\text{total}}} \mu_{ij} \cdot \nabla_j U(t) + \xi_i, \quad (4)$$

where $\mathbf{r}_i$ is the position of bead i , $\mu_{ij} = \delta_{ij}\delta/(6\pi\eta a)$ is the freely draining Stokes mobility matrix, η is the solvent viscosity, δ is the identity matrix, δ_{ij} is the Kronecker delta, and ξ_i is a random velocity that satisfies the fluctuation dissipation theorem, $\langle \xi_i \xi_j \rangle = 2k_B T \mu_{ij} \delta_{ij}$.

U is the sum of the potential energies of the system: stretching (U_S), Lennard-Jones interaction (U_{LJ}), and bending (U_B). DNA beads are connected by Hookean springs, which gives the equation

$$\tilde{U}_S = \frac{\tilde{\kappa}_S}{2} \sum_{i,i+1} (\tilde{b} - \tilde{b}_0)^2, \quad (5)$$

where $\tilde{b}$ is the distance between adjacent beads i and j and $\tilde{b}_0 = 2.0$ is the equilibrium distance between two beads. $\tilde{\kappa}_S$ is set to an arbitrary value of 200.0 to ensure that deviations from $\tilde{b}_0$ are small.

The interaction potential between beads is of the shifted Lennard-Jones (LJ) form,

$$\begin{cases} \tilde{U}_{LJ} = \tilde{\epsilon} \sum_{ij} \left[\left(\frac{\tilde{b}_0}{\tilde{r}_{ij}} \right)^{12} - 2 \left(\frac{\tilde{b}_0}{\tilde{r}_{ij}} \right)^6 + 1 \right] & \tilde{r}_{ij} \leq r_c \\ 0 & \tilde{r}_{ij} > r_c \end{cases}, \quad (6)$$

where $\tilde{\epsilon} = 0.41$ controls the magnitude of the potential and $\tilde{b}_0 = 2.0$ is where the potential is at its minimum. A cutoff of $\tilde{r}_c = 5.0$ is chosen due to the negligible forces at distances greater than 5.0a. Repulsive beads placed at the midpoint of the bond vectors also interact with this potential at a cutoff of $\tilde{r}_c = 2.0$ (to include only the repulsive portion of this potential), which is needed to prevent the DNA strands from crossing.

The bending potential is included to maintain the appropriate stiffness of DNA,

$$\tilde{U}_B = \frac{\tilde{\kappa}_B}{2} \sum_{i=1}^{m-1} (\theta_i - \theta_0(\Omega_i))^2, \quad (7)$$

where $\tilde{\kappa}_B = 6.25$ is chosen to correspond to a DNA persistence length of 50 nm and θ_i is the angle between the bonds of beads $i-1$ and i , and i and $i+1$. $\theta_0(\Omega_i)$ is the equilibrium angle of the DNA bead in its bound state Ω .

A summarized list of simulation parameters can be found in Table I, grouped into four rows. Row 1 contains parameters defining the simulated DNA strand, corresponding to a DNA strand of 800 nm length. Row 2 contains parameters defining the equilibrium angle of DNA in a given bound state used

TABLE I. Summary of parameters used in the simulation. Tildes denote normalized values.

Simulation parameters	
$N = 100$	$a = 4 \text{ nm}$
$\theta_{0,U} = 0$	$\theta_{0,F} = \pi/3$
$\theta_{0,P} = 0$	$\theta_{0,S} = \pi/6$
$\Delta\tilde{E}_B^* = 3.0$	$\Delta\tilde{E}_{UB,P}^* = 12.0$
$\Delta\tilde{E}_{UB,F}^* = 10.0$	$\Delta\tilde{E}_{UB,S}^* = 12.0$
$\lambda = 0.7$	$\tilde{\tau}_{MC} = 0.05$
$\tilde{b}_0 = 2.0$	$\tilde{\epsilon} = 0.41$
$\tilde{\kappa}_S = 200.0$	$\tilde{\kappa}_B = 6.25$

in Eqs. (1), (2), and (7). Row 3 contains the energy barrier values used in Eqs. (1) and (2) and other binding parameters, all of which are chosen for computational convenience. Row 4 contains parameters used in the potential energies of the system [Eqs. (5)–(7)].

III. RESULTS AND DISCUSSION

A. Binding equilibrium

To verify that our 4-state model captures the appropriate binding behavior, we compare the expected binding equilibrium results from statistical mechanics to those obtained from our simulations. These simulations can also show us the effect of force on the number of NAPs bound to the DNA. Similar measurements can be calculated from experimental force versus extension curves.²³ Simulations allow us to measure the number of DNA beads in a bound state directly, and they also allow us to measure the number of DNA sites in a specific state. At low forces, there is a large change in energy between the partially and fully bound states, $\Delta\tilde{E}_2 = \Delta\tilde{E}_{P,F} - \Delta\tilde{E}_{F,P}$ (and similarly between the partially bound and saturated states, $\Delta\tilde{E}_3 = \Delta\tilde{E}_{P,S} - \Delta\tilde{E}_{S,P}$). This is due to the higher values of the unbinding energies ($\Delta\tilde{E}_{F,P}$ and $\Delta\tilde{E}_{S,P}$) and lower values of the binding energies ($\Delta\tilde{E}_{P,F}$ and $\Delta\tilde{E}_{P,S}$). Force thus drives the system away from both the fully bound and saturated states toward the partially bound state.

We observe the result of these force-dependent transitions in the binding behavior of our model NAP. The changes in $\Delta\tilde{E}_2$ and $\Delta\tilde{E}_3$, caused by the force exerted on the DNA molecule, change the number of proteins bound at equilibrium, as shown by the data points for forces of 0.0 pN and 10.0 pN in Fig. 2. At high forces, there are fewer total proteins bound because there is a lower change in energy between states. If we look at the differences between the different bound states (P, F, and S), we see that at high forces, there is an increase in the number of DNA sites in the partially bound state compared to the low

force value. This can be explained by the increase in energy required to move from the partially bound to fully bound state ($\Delta\tilde{E}_{P,F}$), leading to more proteins staying in the partially bound state. The differences in the fully bound state behavior at low and high forces are again described by the changes in $\Delta\tilde{E}_{P,F}$ and $\Delta\tilde{E}_{F,P}$. At high forces, there is an overall lower $\Delta\tilde{E}$ between unbound and fully bound states, leading to fewer proteins fully bound to the DNA strand. The saturated state is the state most dependent on the concentration of proteins in the system, and we start to see the force effects at high concentrations. We see fewer DNA sites in the saturated states at lower force due to the high number of DNA sites in the fully bound state. At higher forces, there are fewer DNA beads in the fully bound state, which allows the DNA to become over-saturated with partially bound proteins (beads are in the S state).

We compare the values from our simulation to the value predicted by statistical mechanics,

$$\langle n_p \rangle = k_B T \frac{\partial \ln \Xi}{\partial \mu}, \quad (8)$$

where n_p is the number of bound proteins and Ξ is the grand partition function of our system,

$$\Xi = \left[e^{(\Delta\tilde{E}_1 + \tilde{\mu})} + e^{(\Delta\tilde{E}_1 + \Delta\tilde{E}_3 + 2\tilde{\mu})} + e^{(\Delta\tilde{E}_1 + \Delta\tilde{E}_2 + \tilde{\mu})} + 1 \right]^N. \quad (9)$$

Substituting this expression into Eq. (8), we get

$$\langle n_p \rangle = N \frac{e^{\Delta\tilde{E}_1 + \tilde{\mu}} + 2e^{(\Delta\tilde{E}_1 + \Delta\tilde{E}_3 + 2\tilde{\mu})} + e^{\Delta\tilde{E}_1 + \Delta\tilde{E}_2 + \tilde{\mu}}}{e^{\Delta\tilde{E}_1 + \tilde{\mu}} + e^{(\Delta\tilde{E}_1 + \Delta\tilde{E}_3 + 2\tilde{\mu})} + e^{\Delta\tilde{E}_1 + \Delta\tilde{E}_2 + \tilde{\mu}} + 1} \quad (10)$$

for the average total number of NAPs bound to the DNA. We can also modify Eq. (10) to calculate the number of DNA beads in each state by doing the derivative of Ξ with respect to an arbitrary μ_a , which will give us the number of DNA beads in state a . The predicted values for $\langle n_a \rangle$ are plotted as lines in Fig. 2 and demonstrate quantitative matching with simulations (points).

FIG. 2. The number of DNA beads in each bound state at a force of 10.0 pN (darker lines and square data points) and 0.0 pN (lighter lines and circle data points). The data points correspond to simulation data, and lines are calculated from Eq. (8).

B. Force-extension at equilibrium

Elasticity behavior of a strand of DNA, such as extension (z) as a function of force (f), can be dependent on the number of proteins bound to it (which depends on the concentration of proteins, c).^{8,10,21,23–27} Figure 3(a) shows the simulated force versus extension behavior obtained by exerting a constant force on the DNA strand and measuring the extension of the chain at equilibrium. From $c = 0 \mu\text{M}$ to $c = 10.0 \mu\text{M}$, extension decreases at a given force as more proteins are added. This is due to the increase in the number of DNA sites that are in a fully bound state and the local bending that occurs at each of these sites. At $c = 10.0 \mu\text{M}$, the DNA strand is nearly “saturated” with fully bound sites [see Fig. 3(b)], leading to a high number of sites with the highest degree of local bending. When the concentration of proteins increases past $c = 10.0 \mu\text{M}$, more proteins bind to the DNA, which start to displace some of the fully bound states with saturated states, and above $c = 1000 \mu\text{M}$, the DNA is “saturated” with two proteins bound per DNA site (S) [see Fig. 3(b)]. The DNA sites in this state have a higher degree of bending than the

FIG. 3. (a) Force versus extension behavior as a function of NAP concentration. The extension of a DNA molecule decreases as the concentration is increased to $10.0 \mu\text{M}$ but begins to re-extend as protein concentration is increased to $1000 \mu\text{M}$ as the DNA strand becomes saturated with proteins. Data points are from simulations, and the lines are provided to guide the eye. (b) Simulation snapshots of the DNA at different protein concentrations. Orange beads represent unbound DNA beads, gray beads are partially bound DNA beads, dark blue beads are fully bound DNA, and cyan beads are saturated DNA beads. (c) Theoretical concentration dependent force-extension behavior from Eq. (16).

bare DNA, but less than the DNA saturated with fully bound sites.

We adapt a theory developed in the literature to describe the force-extension behavior of DNA that can undergo local bending when proteins are bound to it.^{57–60} The model is based on a semi-flexible polymer composed of discrete segments, each of which can adopt a bent conformation at equilibrium when a protein is bound. We expand this model to account for the multiple binding modes in our model. The energy of a strand is

$$\begin{aligned} \tilde{E} = & \sum_{i=1}^N \left[-bfu_i \cdot \hat{x} + \frac{\kappa_B}{2} (\text{acos}(u_i \cdot u_{i+1})) \delta_{iU} \right. \\ & + \left\{ \frac{\kappa_B}{2} (\text{acos}(u_i \cdot u_{i+1}) - \theta_S) - E_1 - \mu \right\} \delta_{iP} \\ & + \left\{ \frac{\kappa_B}{2} (\text{acos}(u_i \cdot u_{i+1}) - \theta_S) - E_1 - E_3 - 2\mu \right\} \delta_{iS} \\ & \left. + \left\{ \frac{\kappa_B}{2} (\text{acos}(u_i \cdot u_{i+1}) - \theta_F) - E_1 - E_2 - \mu \right\} \delta_{iF} \right], \quad (11) \end{aligned}$$

where b is the length of a segment, f is the force, $\hat{x}$ is a unit vector in the direction of the force, u_i are the normalized bond vectors, θ_a are the equilibrium bending angles for the binding state and μ is the chemical potential.

To get the force extension behavior, we numerically solve the partition function using a transfer matrix formalism. We use a discretized set of $t = 1000$ possible bond vectors (u_i) that are uniformly distributed along the surface of a unit sphere. We consider a $4t$ -component vector ψ_i , with each component representing the partition function where the i th monomer has a given unit vector u_i and state Ω_i . This vector ψ_i can be related to the vector ψ_{i+1} describing a chain with $i + 1$ monomers via a $4t \times 4t$ transfer matrix, $\mathbf{T} = (u_{i+1}, \Omega_{i+1} | u_i, \Omega_i)$. This matrix is composed of the Boltzmann factors that incorporate the $i + 1$ -th monomer contributions into the i -monomer partition function, with this new monomer u_{i+1} in the unbound ($\Omega = U$), partially bound ($\Omega = P$), saturated ($\Omega = S$), or fully bound ($\Omega = F$) states, given that the i th monomer had a segment u_i in a state Ω ,

$$\mathbf{T}(u_{i+1}, U | u_i, \Omega_i) = \exp \left[- \left(\frac{\tilde{\kappa}_B}{2} \right) (\text{acos}(u_i \cdot u_{i+1})) + \tilde{b}\tilde{f}u_{i+1} \cdot \hat{x} \right], \quad (12)$$

$$\mathbf{T}(u_{i+1}, P | u_i, \Omega_i)$$

$$= \exp \left[- \left(\frac{\tilde{\kappa}_B}{2} \right) (\text{acos}(u_i \cdot u_{i+1} - \theta_S) + \tilde{E}_1 + \tilde{\mu}) + \tilde{b}\tilde{f}u_{i+1} \cdot \hat{x} \right], \quad (13)$$

$$\begin{aligned} \mathbf{T}(u_{i+1}, S | u_i, \Omega_i) = & \exp \left[- \left(\frac{\tilde{\kappa}_B}{2} \right) (\text{acos}(u_i \cdot u_{i+1} - \theta_S) \right. \\ & \left. + \tilde{E}_1 + \tilde{E}_3 + 2\tilde{\mu}) + \tilde{b}\tilde{f}u_{i+1} \cdot \hat{x} \right], \quad (14) \end{aligned}$$

$$\begin{aligned} \mathbf{T}(u_{i+1}, F | u_i, \Omega_i) = & \exp \left[- \left(\frac{\tilde{\kappa}_B}{2} \right) (\text{acos}(u_i \cdot u_{i+1} - \theta_F) \right. \\ & \left. + \tilde{E}_1 + \tilde{E}_2 + \tilde{\mu}) + \tilde{b}\tilde{f}u_{i+1} \cdot \hat{x} \right]. \quad (15) \end{aligned}$$

The partition function is calculated by operating on a single-monomer vector ψ_0 with $\mathbf{T}$ N -times to obtain ψ_N . The components of this final ψ_N are summed to obtain the partition function $\mathcal{Z} = \mathbf{1} \cdot \psi_N$ ($\mathbf{1}$ is a vector of 1s) for a strand of N segments. The extension of the strand (z) is calculated by taking the derivative of $(\ln \mathcal{Z})$ with respect to the force,

$$z = \frac{\partial \ln \mathcal{Z}}{Nb\partial \tilde{f}}. \quad (16)$$

Figure 3(c) shows the theoretical force-extension behavior as a function of chemical potential, μ . The theoretically predicted behavior is qualitatively similar to what is seen in our simulations and in previous experiments.^{10,21} The quantitative differences between the theoretical prediction and the simulation data are due to simulation parameters, such as excluded volume interactions, that cannot be included in the theoretical model.

C. Rapid force-extension

Our model allows us to look past this system at equilibrium, and we can anticipate the interplay of time scales as the system is driven out of equilibrium. We investigated the behavior of a DNA strand with stretching, bending, and LJ potentials as a function of pulling rate, v , and concentration, c , where each variable has associated time scales. The simulations are initiated in a fully extended state ($z = 200a$) and are slowly compressed to $z = 50a$ to allow protein binding to equilibrate. The strand is then pulled at both ends at a rate $v/2$

back to its fully extended state. 40 trajectories are averaged for each v , which range from 1.0×10^{-4} to $1.0 \times 10^{-1} a/\tau_D$ (or 0.15 to 151 $\mu\text{m/s}$), and each c , ranging from 0 to 1000 μM , to obtain the force and number of bound proteins as a function of extension. The system allows us to examine time scales associated with the pulling rate and FIS-DNA interactions: the time it takes for a protein to go from fully bound to partially bound, $\tilde{\tau}_{\text{UB}}$, and the time it takes for the DNA strand to move one bead length, or $2a$, $\tilde{\tau}_P$. The average time it takes for a protein to partially unbind (F to P) can be calculated by

$$\tilde{\tau}_{\text{UB}} = \tilde{\tau}_{\text{MC}} e^{\Delta \tilde{E}_{F,P}}, \quad (17)$$

where $\Delta \tilde{E}_{F,P}$ is calculated from Eq. (2), which is dependent on force. At low extensions ($\tilde{z} = 50$) where the DNA strand can adopt many conformations, the average $\Delta \tilde{E}_{F,P} \approx 9.1$, leading to an average $\tilde{\tau}_{\text{UB}} = 405$. At high extensions ($\tilde{z} = 200$), where the pulling force and bending force are strongly competing, $\Delta \tilde{E}_{F,P} \approx 7.7$, leading to an average $\tilde{\tau}_{\text{UB}} = 107$. The characteristic pulling time, $\tilde{\tau}_P = 1/v$, ranges from 10 000 for $v = 1 \times 10^{-4}$ to 20 for $v = 1.0 \times 10^{-1}$. The relationship between these two time scales can be described by the dimensionless number $\Gamma = \tilde{\tau}_P / \tilde{\tau}_{\text{UB}}$.

We can measure the force as a function of extension and v , and an example force-extension curve at $c = 0.0 \mu\text{M}$ can be seen in Fig. 4(a). At low extensions, the forces measured for the slower pulling rates ($v < 5.0 \times 10^{-3}$) are nearly identical. At higher extensions, shown in the inset of Fig. 4(a),

we see an increase in measured force, which matches for all $v < 5.0 \times 10^{-3}$. For $v > 5.0 \times 10^{-3}$, we see a measurably different behavior. Instead of a constant force at low extensions, we see different linear increases in the measured force. At high extensions and high v , we see a similar sharp increase in force just as we did for low v . This is due to hydrodynamic drag effects, which are negligible at low v . The work dissipated because the drag can be calculated by the following integral:

$$W_D = \int_{z_i}^{z_f} dz (f_v(z) - f_{eq}(z)), \quad (18)$$

where f_v is the measured force at the pulling rate v at an extension z and f_{eq} is the measured force at extension z at equilibrium. This value increases as we increase v since there are higher drag forces at higher pulling rates.

We also compared the force-extension curves of different concentrations at a constant pulling rate, which can be seen in Fig. 4(b). We see qualitatively similar non-monotonic behavior with constant v at high extensions as we did with constant f , as shown previously in Fig. 3(a). As the concentration is increased, we observe a higher measured force at a given extension as we increase the concentration from 0 μM to 10.0 μM . We then see the expected decrease in force as concentration increases further to 1000 μM . The inset of Fig. 4(b) shows the behavior at $v = 1.0 \times 10^{-1}$ and shows the same qualitative concentration-dependent trends as the low pulling rate but also exhibits hydrodynamic drag effects.

FIG. 4. (a) Force versus extension behavior of the bare DNA ($c = 0 \mu\text{M}$) as a function of pulling rate v . The lines are a moving average of 50 data points, where each data point is an average of the force at a given extension of 40 trajectories. The inset shows the force-extension behavior at a high extension ($z \geq 160a$). (b) Force versus extension behavior as a function of NAP concentration at $v = 1.0 \times 10^{-4}$, which we take to be the equilibrium force-extension behavior. The inset shows the force-extension behavior as a function of NAP concentration at $v = 1.0 \times 10^{-1}$. (c) The force versus extension data at ($c = 5.0 \mu\text{M}$). We see an increase in the measured force as we increase v that is not solely due to drag, especially above $z = 140a$. The inset shows the force measured at high extension, where the differences in measured force at different rates are most pronounced. [(d) and (e)] The number of DNA beads in the fully bound state as a function of extension and v at $c = 0.05 \mu\text{M}$ (d), $c = 0.5 \mu\text{M}$ (e), and $c = 10.0 \mu\text{M}$ (f). The inset of (d) is a magnification of the number of fully bound states at $c = 0.05 \mu\text{M}$.

Figure 4(c) shows the force-extension behavior when $c = 5.0 \mu\text{M}$, which corresponds to the concentration where the most DNA sites are in the fully bound state. We see many qualitative similarities to the DNA only system, such as the constant force at low v and low z , hydrodynamic drag at high v and low z , and a sharp increase in the measured force at high extensions for all v . The effects of proteins on the system are most evident at high extensions, which are shown in the inset of Fig. 4(c). The forces measured at extensions above $180a$ increase with increasing v , which is not observed for all v in the bare DNA system. We see this effect when most of the DNA beads are in the fully bound state at low force with low numbers of the saturated bound state, which occurs between $c = 0.01 \mu\text{M}$ and $c = 10.0 \mu\text{M}$. These fully bound proteins cause the greatest local bends in the backbone (via the equilibrium bending angle θ_0), which leads to a higher force required to extend the DNA molecule. This force also changes the binding and unbinding energies of these proteins, and these proteins “pop off” more easily as the force is increased. We see a very similar behavior when $v < 2.5 \times 10^{-3}$, which corresponds to $\Gamma < 1$. When $\Gamma \geq 1$, we see marked changes in the force-extension behavior.

In addition to measuring the force as a function of extension, we can track the number of DNA sites in a given state as a function of extension and v . The most significant changes occur with the fully bound state, which corresponds to the differences seen in the equilibrium binding behavior, as shown in Figs. 4(d)–4(f). At low concentrations ($c \leq 0.05 \mu\text{M}$), there are slight decreases in the number of beads in the fully bound state as a function of extension [Fig. 4(d)], which are most pronounced for the low pulling rates. For all concentrations, the number of proteins bound can re-equilibrate at slow pulling rates due to the extremely high Γ , where the time scales associated with binding and unbinding are far faster than the time scales associated with pulling. At a faster pulling rate v , the number of proteins bound in a given state cannot re-equilibrate as the strand is extended, leading to a less of a decrease in the number of proteins fully bound. The total number of DNA beads in any bound state (P, F, or S) changes at low v , but at high v proteins do not have time to become fully unbound from the DNA strand, leading to near constant values of total number of DNA beads in any bound state.

This v dependent unbinding behavior as a function of extension is most pronounced at concentrations, where there is a large difference in the number of fully bound DNA beads at low extension ($\tilde{z} = 50$) and high extension ($\tilde{z} = 200$), which we call ΔC . The maximum value of ΔC occurs at $c = 0.5 \mu\text{M}$ [Fig. 4(e)]. The DNA strand starts with ≈ 65 beads in the fully bound state and decreases to ≈ 14 beads when allowed to re-equilibrate during slow pulling, leading to $\Delta C = 51$. As we increase v , we see a decrease in ΔC , and at the fastest pulling rate $v = 1.0 \times 10^{-1}$, $\Delta C = 17$. The difference in these ΔC s (denoted by $\Delta\Delta C$) is the difference in the number of fully bound proteins between the slow and fast pulling rates, leading to $\Delta\Delta C = 34$ for $c = 0.5 \mu\text{M}$.

We can compare these ΔC s and $\Delta\Delta C$ to those at $c = 10.0 \mu\text{M}$, where we see the largest number of fully bound DNA beads, as shown in Fig. 4(f). At equilibrium, $\Delta C = 42$,

and at $v = 1.0 \times 10^{-1}$, $\Delta C = 23$, leading to $\Delta\Delta C = 19$. Even though there are more DNA beads in the fully bound state at $c = 10.0 \mu\text{M}$, there is a bigger $\Delta\Delta C$ between the slow and fast pulling rates at $c = 0.5 \mu\text{M}$.

This effect caused by the change in the number of fully bound proteins becomes less important as the system increases in concentration beyond $c = 10.0 \mu\text{M}$, and we begin to see more DNA beads in the saturated state at both low and high forces. When a fully bound protein undergoes an unbinding event, it opens up a site on the DNA bead, which can be filled by another partially bound protein at high enough concentrations. The time it takes for that empty site to fill with another protein decreases as we increase the concentration. In this high concentration regime, we move from a highly bent state ($\theta_{0,F} = \pi/3$) to a less highly bent state ($\theta_{0,S} = \pi/6$), which does not decrease the force required to extend the DNA as much as movement between a highly bent state to a no-bent state.

We look at the value $\Delta C' = \Delta C - \Delta C_S/2$, where ΔC_S is the change in the number of DNA sites in the saturated states. The factor of 0.5 is included because the saturated state bends the DNA less than the fully bound state. When $c < 5.0 \mu\text{M}$, $\Delta C \approx \Delta C'$ because there are very few DNA sites in the saturated state. At higher concentrations, these two values differ due to the increase in the number of saturated states (and consequently a decrease in the number of fully bound states). At $500 \mu\text{M}$ and at equilibrium, $\Delta C' = 11$, while the change in the number of fully bound states is $\Delta C = 20$. If we look at the overall change in the number of DNA bent sites between equilibrium and $v = 1.0 \times 10^{-1}$, we observe that $\Delta\Delta C' = 4$ at $500 \mu\text{M}$, while $\Delta\Delta C = 10$.

We can compare the cooperative effects of pulling rate and concentration by looking at the rate- and concentration-normalized dissipative work, $\check{W}_D$, where the drag force is negligible. We can use Eq. (18) at a given concentration to calculate the work dissipated due to the effect of rate on the binding/unbinding behavior of the proteins. These normalized work values ($\check{W}_D$) are shown in Fig. 5(a). We observe a region (the red area of the plot) where there is a large amount of work dissipated due to pulling rate effects on protein binding and unbinding behavior. This corresponds to the area where $\Delta\Delta C$ is high; this is caused by the greater change in force required to extend the DNA strand at higher v . This stems from two attributes of this region: (1) DNA beads tend to be in the fully bound state in equilibrium but leave the strand at a high force (a high $\Delta C'$) and (2) the DNA is being pulled sufficiently quickly that the proteins do not have time to unbind. We demonstrate the first attribute by plotting $\Delta C'$ in Fig. 5(b), which demonstrates a maximum at a concentration corresponding to the high-dissipation region seen in Fig. 5(a). This concentration regime is the range where we see the greatest change in the number of DNA beads in a bent state ($\Delta C'$), which is the underlying cause of protein unbinding, regardless of the pulling rate. The second attribute reflects the dependence on pulling rate, and we thus see that large values of $\check{W}_D$ occur where $\Gamma \leq 1$ [shown by the black line in Fig. 5(a)]. Here, τ_P becomes less than τ_{UB} , and therefore, proteins do not have time to re-equilibrate as the strand is pulled.

FIG. 5. (a) A heat map of the concentration normalized dissipative work. Data points (at a given c and v) are calculated using Eq. (18), where f_{eqm} is the extension-dependent force at c and $\tilde{f}_v$ is the drag-normalized extension-dependent force at v . The black line is where $\Gamma \approx 1$. (b) A representative plot of $\Delta C'$ at equilibrium.

IV. CONCLUSION

NAP-DNA interactions affect DNA conformations in ways that have been measured experimentally with single-molecule studies. This work uses coarse-grained models to demonstrate how local interactions, specifically the bending induced by the protein FIS and corresponding force-dependent binding and unbinding energies, impact DNA equilibrium and dynamic behavior in a system with external forces. The local bending equilibrium angle is a function of the bound state of DNA, and we can thus change the force-extension behavior as a function of protein concentration in our model. This reflects previous experimental studies that postulate that FIS can partially bind to DNA,^{28,51–53} and this partial binding behavior leads to a non-monotonic force-extension behavior.^{21,23,25} We are able to show how the interplay of time scales of the system can impact dynamic behavior.

This model made several simplifying assumptions about the FIS-DNA system; for example, the bending and twisting rigidity of the DNA is not changed when NAPs become bound. This may be important if we want to capture effects such as DNA stiffening behavior as seen with the NAP HU,²⁴ or more extreme bending that can cause a change in twisting rigidity such as the hairpins caused by IHF.⁵ Our simulation method can be easily manipulated to account for these types of effects. We can see different types of concentration dependent force-extension behaviors by changing (for example) the local equilibrium angle's stiffness κ_B or the extent that force affects the unbinding energy barrier λ . In order to take into account

effects such as the dependence of binding behavior on salt, we can change the binding and unbinding energies or add in other particle-DNA interactions.^{24,53}

While the focus of this work was to demonstrate fundamental equilibrium and dynamic competitions in DNA-NAP systems by mimicking experimental single molecule studies, our coarse-grained BD model can be extended past the single molecule scale. This will allow us to investigate behaviors that occur at the nucleoid scale using a model that is built up from local interactions.

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