PHYSICAL REVIEW E **72**, 030901(R) (2005)

Coupled dynamics of DNA breathing and of proteins that selectively bind to single-stranded DNA

Tobias Ambjörnsson* and Ralf Metzler†
NORDITA, Blegdamsvej 17, DK-2100 Copenhagen Ø, Denmark

(Received 25 November 2004; revised manuscript received 16 March 2005; published 27 September 2005)

We study the size fluctuations of a local denaturation zone in a DNA molecule in the presence of proteins that selectively bind to single-stranded DNA, based on a (2+1)-dimensional master equation. By tuning the physical parameters we can drive the system from undisturbed bubble fluctuations to full, binding-protein-induced denaturation. We determine the effective free-energy landscape of the DNA bubble and explore its relaxation modes.

DOI: 10.1103/PhysRevE.72.030901 PACS number(s): 87.15.−v, 82.37.−j, 87.14.Gg

Under physiological conditions the Watson-Crick double helix is the thermodynamically stable configuration of a DNA molecule. This stability is effected by the specific Watson-Crick H bonding, whose key-lock principle guarantees a high level of fidelity during replication and transcription; and by the stronger base stacking between neighboring base pairs causing hydrophobic interactions between the planar aromatic bases [1,2].

The initial breaking of the stacking interactions in an unperturbed DNA molecule is associated with an activation barrier $\sigma_0 \simeq 10^{-5,\ldots,-3}$ [4–6]. Once this barrier is overcome, the free energy of breaking an additional base pair is of the order of 1 to $2k_BT$ [3,4], effecting local, single-stranded DNA denaturation zones of a few tens of broken base pairs [7]. By thermal activation, these DNA bubbles fluctuate in size by a zipper motion at the two forks where the bubble meets the intact double strand [8]. By fluorescence correlation methods this DNA breathing can be explored on the single molecule level, revealing multistate (un)zipping kinetics with a typical single (un)zipping step time scale around 50 μs and a typical bubble lifetime of the order of 1 ms [9].

A long-standing puzzle had been why the presence of selectively single-stranded DNA binding proteins (SSBs) does not lead to full DNA denaturation, as SSB binding is thermodynamically favorable [10]. Detailed single-molecule optical tweezers studies, by overstretching of the DNA molecule to bring the effective temperature close to the melting temperature T_m in the presence of bacteriophage T4 gene 32 SSBs, showed quantitatively [11] that there exists a kinetic block for SSB binding [12]: Below T_m the bubble lifetime is shorter than the typical SSB binding time, counteracting helix destabilization.

In this paper, we develop a dynamical model to quantify the coupled dynamics between a fluctuating DNA bubble and SSBs that attempt to bind to it, in terms of the system parameters (temperature, external force, SSB binding rate and strength, SSB size). We demonstrate that the presence of SSBs leads to enhanced bubble lifetime. Effectively, the bubble free energy is lowered, and even SSB-induced denaturation can occur.

The system we have in mind (Fig. 1) resembles the DNA construct from Ref. [9], in which a homopolymer bubble region is clamped at both ends. The one-bubble approximation used here is generally valid below T_m due to $\sigma_0 \ll 1$. Figure 1 also illustrates that the typical binding size λ of an SSB ($\simeq$10 bases), is of the same order as the bubble size. It is therefore necessary to consider the statistical weight from SSBs explicitly, instead of defining an effective chemical potential [14].

To quantify the system, we define the probability distribution $P(m,n,t)$ to find a bubble of size m, with n bound SSBs at time t. Its time evolution is governed by the (2+1)-dimensional master equation

$$\begin{aligned}\partial P(m,n,t)/\partial t &= \mathbf{t}^+(m-1,n)P(m-1,n,t) + \mathbf{t}^-(m+1,n)P(m+1,n,t) \\ &\quad - [\mathbf{t}^+(m,n) + \mathbf{t}^-(m,n)]P(m,n,t) \\ &\quad + \mathbf{r}^+(m,n-1)P(m,n-1,t) + \mathbf{r}^-(m,n+1)P(m,n+1,t) \\ &\quad - [\mathbf{r}^+(m,n) + \mathbf{r}^-(m,n)]P(m,n,t), \qquad (1)\end{aligned}$$

owing to the discrete nature of the problem. In Eq. (1), the transfer rates $\mathbf{t}^\pm$ describe changes in the bubbles size m, and $\mathbf{r}^\pm$ changes in the number n of bound SSBs. The boundary conditions for the $\mathbf{r}^\pm$ are reflecting at $n=0$ and $n_{\max}=2[m/\lambda]$, the maximum number of SSBs that can bind to a bubble of m denatured base pairs. Similarly, we impose a reflecting boundary condition at $m=0$ and $m=M$, the maximum bubble size. Moreover, we have to consider that a bubble cannot zip close if its size is a multiple of the SSB size, $m=k\lambda$ ($k \in \mathbb{N}$) and the number of bound SSBs is $n_{\max}$ or

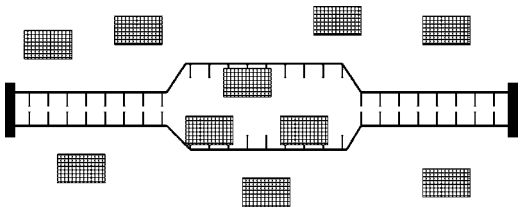

FIG. 1. Clamped DNA bubble in a region of size M, immersed in a bath of SSBs. SSBs do not bind across zippers.

*Electronic address: ambjorn@nordita.dk
†Electronic address: metz@nordita.dk

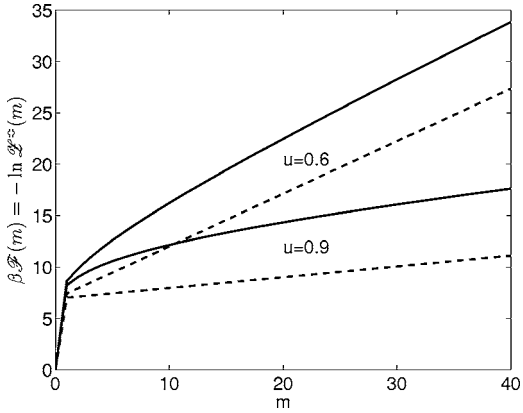

FIG. 2. Bubble free energy in absence of SSBs as function of bubble size m (solid line: $c=1.76$; dashed line: $c=0$). We chose $\sigma_0=10^{-3}$.

$n_{\max}-1$, i.e., at least one of the arches of the bubble is fully occupied. To define the transfer rates based on the statistical weight $\mathcal{Z}(m,n)$ to find a state (m,n), we assume detailed balance [15,16]:

$$\mathbf{t}^+(m-1,n)\mathcal{Z}(m-1,n)=\mathbf{t}^-(m,n)\mathcal{Z}(m,n), \tag{2}$$

$$\mathbf{r}^+(m,n-1)\mathcal{Z}(m,n-1)=\mathbf{r}^-(m,n)\mathcal{Z}(m,n). \tag{3}$$

The statistical weight $\mathcal{Z}(m,n)=\mathcal{Z}^{\circ}(m)\mathcal{Z}^{\mathrm{SSB}}(m,n)$ has two contributions. The bubble part according to the Poland-Scheraga model for DNA melting is [17,18]

$$\mathcal{Z}^{\circ}(m)=\sigma_0 u^m(1+m)^{-c}, \quad m\geqslant 1, \tag{4}$$

with the bubble initiation factor σ_0, the weight $u=e^{-\beta E}$ for breaking a base pair, and the loop closure factor $(1+m)^{-c}$ for creating a polymer loop of size m, with the offset by 1 due to persistence length corrections [4,19]. We choose typical values, $\sigma_0=10^{-3}$ and $c=1.76$ [18,20]. Eq. (4) is completed by $\mathcal{Z}^{\circ}(0)=1$. In comparison to the continuum description of DNA breathing in absence of SSBs by a Fokker-Planck equation involving the gradient of the free energy [21], we note that the discrete approach (1) explicitly includes the constant activation barrier σ_0 (cf. Fig. 2).

The contribution from the SSBs has the form [14,15]

$$\mathcal{Z}^{\mathrm{SSB}}(m,n)=\kappa^n\Omega(m,n), \tag{5}$$

with the binding strength $\kappa=c_0K^{\mathrm{eq}}$, involving the SSB concentration c_0 and equilibrium binding constant $K^{\mathrm{eq}}=v_0\exp(\beta|E_{\mathrm{SSB}}|)$, where v_0 is the typical SSB volume and E_{SSB} its binding energy. The weight $\Omega(m,n)$ counts all possible ways of putting n SSBs onto the two arches of the bubble, including potential gaps between SSBs due to the fact that their size λ is larger than a base. Explicitly,

$$\Omega(m,n)=\sum_{n'=0}^{n}\omega(m,n')\omega(m,n-n'), \tag{6}$$

with $n,(n-n')\leqslant n^{\max}/2$, and the combinatorial term

$$\omega(m,n)=\binom{m-(\lambda-1)n}{n}. \tag{7}$$

By detailed balance and taking the rate $\mathbf{t}^+$ for breaking a base pair to be proportional to the activation factor u (including loop closure effects), and assuming that the rate $\mathbf{r}^-$ for SSB unbinding is proportional to the number n of bound SSBs, we arrive at the expression for the transfer coefficients, i.e., the rates for the bubble size increase or decrease

$$\mathbf{t}^-(m,n)=k\Omega(m-1,n)/\Omega(m,n), \tag{8a}$$

$$\mathbf{t}^+(m,n)=ku([1+m]/[2+m])^c, \quad m\geqslant 1, \tag{8b}$$

with the bubble initiation rate $\mathbf{t}^+(0,0)=2^{-c}k\sigma_0u$, and

$$\mathbf{r}^-(m,n)=n\gamma k, \tag{9a}$$

$$\mathbf{r}^+(m,n)=\gamma k\kappa(n+1)\Omega(m,n+1)/\Omega(m,n), \tag{9b}$$

for the SSB number transfer rates [22]. Here, we introduced the dimensionless ratio $\gamma\equiv q/k$ of the SSB unbinding rate q and the base pair zipping rate k.

To solve the master equation (1), we introduce an eigenmode expansion of the form

$$P(m,n,t)=\sum_p c_pQ_p(m,n)\exp(-t/\tau_p), \tag{10}$$

in which the coefficients c_p of a given eigenmode p are determined via the initial conditions. The corresponding eigenvalue equation for the bubble size-SSB number eigenfuction Q_p determines the mode relaxation times τ_p, and can be solved numerically, or, in some limits, analytically [15]. From the bubble-size autocorrelation function

$$A(t)=\langle m(t)m(0)\rangle=\sum_{p\neq 0}A_pe^{-t/\tau_p}, \tag{11}$$

a typical quantity determined in experiment, we obtain the relaxation time spectrum $\{\tau_p\}$ with the corresponding amplitudes $A_p=[\Sigma_{m,n}mQ_p(m,n)]^2$. The slowest mode $\tau_{\mathrm{relax}}=\tau_1$ determines the characteristic equilibration time [23]. These measures can be used to quantify the bubble-SSB system for different cases:

(i) In absence of SSBs, Eq. (1) reduces to a $(1+1)$-dimensional master equation. If we neglect the loop closure factor, we can obtain an analytic result using orthogonal polynomials, from which we infer the inequalities for the eigenvalues τ_p [15]

$$k^{-1}(1+u^{1/2})^{-2}\leqslant\tau_M<\cdots<\tau_1\leqslant k^{-1}(1-u^{1/2})^{-2}, \tag{12}$$

The equal signs hold in the limit $M\to\infty$. The characteristic relaxation time τ_{relax} tends to very large values (and diverges for $M\to\infty$) in the limit $u\to 1$, i.e., on approaching T_m. In Fig. 3, we plot the corresponding relaxation time spectrum for three temperatures. Well below T_m, we see the multistate relaxation observed experimentally [9]. For temperatures closer to T_m, the slowest eigenmode becomes increasingly dominant ($\approx$ two-state behavior). To estimate the rate constant k for zipping we find from Fig. 3 that at

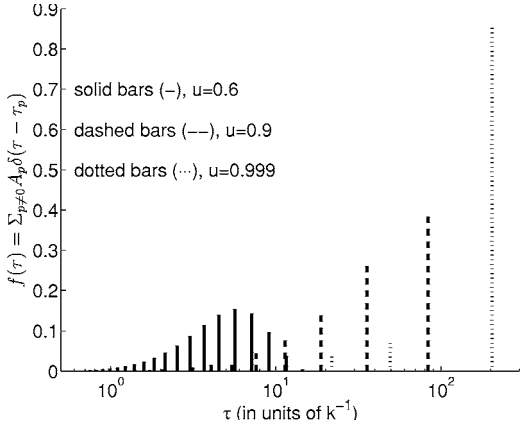

FIG. 3. Relaxation time spectrum for $\sigma_0=10^{-3}$ and $M=40$. For $u=0.999$, the longest relaxation time dominates.

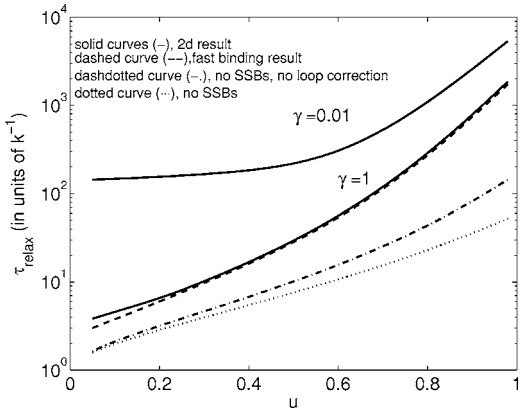

FIG. 5. Longest relaxation time as function of u, for $\kappa=0.5$, $\sigma_0=10^{-3}$, $M=20$, $\lambda=5$.

$u=0.6\tau_{\rm relax}\simeq 20/k$; comparing to the experimental result $\tau_{\rm relax}\simeq 1$ ms, we consistently extract $k\simeq 50$ μs.

(ii) The fast binding limit corresponds to $\gamma\gg 1$, i.e., SSB (un)binding being much faster than the bubble zipping. By adiabatic elimination [16], the master equation (1) reduces to an equation in the bubble size m as in case (i) but where the bubble free-energy landscape $\beta\mathcal{F}=-\ln\mathcal{Z}_{\rm ad}$, with $\mathcal{Z}_{\rm ad}=\Sigma_n\mathcal{Z}(m,n)$, is dressed by the ongoing SSB (un)binding. This effective free energy is lowered in comparison to case (i), as demonstrated in Fig. 4 for two cases: (a) the effective free energy is reduced, but the overall slope still positive; (b) the SSB dynamics causes a negative slope of the effective free energy, ultimately leading to complete denaturation of unclamped DNA.

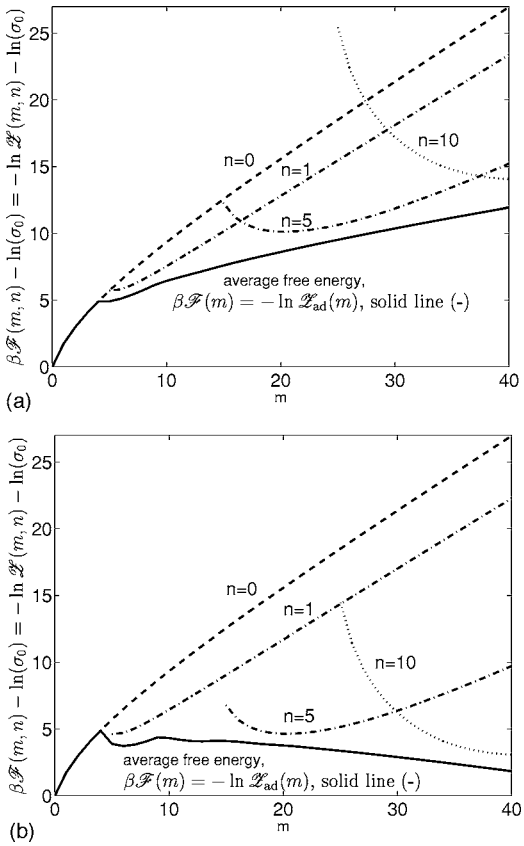

FIG. 4. Effective free energy in the limit $\gamma\gg 1$ (—), and free energy for various fixed n ($u=0.6$, $M=40$, $c=1.76$, $\lambda=5$). Top: $\kappa=0.5$; bottom: stronger binding, $\kappa=1.5$.

We note a characteristic feature of the effective energy in Fig. 4, namely, the finite size effects due to the SSB size λ: the bubble has to open up to a minimum size $m=\lambda$, before it is able to accommodate the first SSB, etc. This also effects a nucleation barrier for SSB-assisted DNA denaturation even in the case of negative slope of the dressed free energy for larger m. Numerically, we determine the critical binding strength $\kappa_{\rm crit}\approx 1$ for the parameters of Fig. 4, at which the effective $\beta\mathcal{F}(m)$ is almost flat. Fast binding is, e.g., realized for gp32p mutants at elevated effective temperature [11].

(iii) General case. If SSB (un)binding is not sufficiently fast but occurs within the typical bubble relaxation time in absence of SSBs, the full master equation (1) needs to be solved. By tuning the relative SSB unbinding rate γ and SSB binding strength κ, we can move from the situation with practically no binding to the fast binding case. In Fig. 5, we illustrate this behavior via the characteristic relaxation time $\tau_{\rm relax}$.

Experimentally, u and κ are changed by variation of temperature and concentration of SSBs in solution, respectively.

To (un)zip close (open) a base pair, the two single strands making up the bubble have to be pulled closer towards (pushed away from) the zipper fork. This additional effect may be included using similar arguments as in Ref. [24]. The adjustment of pulling (pushing) propagates along the contour of the chain until a bend (inflexion) is reached, a distance that scales as the gyration radius, i.e., $\simeq m^{\nu}$. Having in mind Rouse-type dynamics, this would slow down the (un)zipping rates by a factor $m^{-\nu}$ [15]. The relevance of this effect for the rather small bubble sizes well below T_m is not obvious, and has to be based on more accurate experimental or numerical data.

At temperatures well below T_m, our description will be valid for unclamped DNA homopolymers due to $\sigma_0\ll 1$. Explicit boundary and heteropolymer structure effects can be included by introducing the bubble position as an additional variable in the master equation (1) [25]. Our description will also hold under moderate chain tension, e.g., in combination

with optical tweezers setups. The pulling force f then gives rise to blobs of size $\xi=k_BT/f$ in which the DNA is undisturbed [26]. Conversely, in the case of strong pulling twist is progressively taken out of the DNA molecule, corresponding to a torque $\mathcal{T}$ and the twist energy $\theta_0\mathcal{T}$, where $\theta_0=2\pi/10.35$ denotes the twist angle per base. The statistical weight is modified according to $u\to u\exp(\beta\theta_0\mathcal{T})$ [13].

Our (2+1)-dimensional master equation approach provides a quantitative framework for the coupled dynamics of the size fluctuations of DNA denaturation bubbles and the binding of SSBs. It explains the different regimes, from free bubble breathing to SSB-induced denaturation, that correspond to experimentally accessible in vitro as well as *in vivo* situations. In particular, we expect that this scheme is useful to the design of future experiments, and to estimate in vivo conditions based on the SSB binding strength κ, the base stacking factor u, and the ratio γ of SSB unbinding and base pair zipping rates.

Given the rather general formulation in terms of a master equation for the probability distribution $P(m,n,t)$, our approach may be useful to various other systems where particles bind to a substrate whose binding surface fluctuates in time (wetting, oxidation, or binding of biomolecules to membranes); in particular, in the presence of finite size effects. In addition, the coupled DNA bubble-SSB binding dynamics studied here is a generic example of how a stochastic process (partially) can rectify another one.

We are happy to thank Richard Karpel, Oleg Krichevsky, and Mark Williams for helpful discussions.

[1] A. Kornberg, *DNA Synthesis* (W. H. Freeman, San Francisco, 1974).
[2] S. G. Delcourt and R. D. Blake, J. Biol. Chem. **266**, 15160 (1991).
[3] J. SantaLucia, Jr., Proc. Natl. Acad. Sci. U.S.A. **95**, 1460 (1998).
[4] R. D. Blake *et al.*, Bioinformatics **15**, 370 (1999).
[5] R. M. Wartell and A. S. Benight, Phys. Rep. **126**, 67 (1985).
[6] R. Blossey and E. Carlon, Phys. Rev. E **68**, 061911 (2003).
[7] M. Guéron, M. Kochoyan, and J.-L. Leroy, Nature (London) **328**, 89 (1987).
[8] C. Kittel, Am. J. Phys. **37**, 917 (1969).
[9] G. Altan-Bonnet, A. Libchaber, and O. Krichevsky, Phys. Rev. Lett. **90**, 138101 (2003).
[10] D. E. Jensen and P. H. von Hippel, J. Biol. Chem. **251**, 7198 (1976).
[11] K. Pant, R. L. Karpel, and M. C. Williams, J. Mol. Biol. **327**, 571 (2003).
[12] R. L. Karpel, IUBMB Life **53**, 161 (2002).
[13] T. Hwa *et al.*, Proc. Natl. Acad. Sci. U.S.A. **100**, 4411 (2003).
[14] T. Ambjörnsson and R. Metzler, Physical Biology **1**, 77 (2004).
[15] T. Ambjörnsson and R. Metzler J. Phys.: Condens. Matter **17**, S1841 (2005).
[16] H. Risken, *The Fokker-Planck Equation* (Springer-Verlag, Berlin, 1989).
[17] D. Poland and H. Scheraga, *Theory of Helix-Coil Transitions in Biopolymers* (Academic, New York, 1970).
[18] C. Richard and A. J. Guttmann, J. Stat. Phys. **115**, 925 (2004).
[19] M. Fixman and J. J. Freire, Biopolymers **16**, 2693 (1977).
[20] The generic behavior of the model does not change substantially as long as $\sigma_0\ll 1$.
[21] A. Hanke and R. Metzler, J. Phys. A **36**, L473 (2003).
[22] Different choices are possible as detailed balance does not fully determine the transfer rates.
[23] In certain cases, it may be of advantage to use the characteristic relaxation time and not the longest.
[24] E. A. Di Marzio, C. M. Guttman, and J. D. Hoffman, Faraday Discuss. Chem. Soc. **68**, 210 (1979). It is stated here that the effect should pertain to both fast and slow pulling.
[25] T. Ambjörnsson and R. Metzler (unpublished).
[26] P.-G. de Gennes, *Scaling Concepts in Polymer Physics* (Cornell University Press, Ithaca, 1979).