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LETTER TO THE EDITOR

Bubble dynamics in DNA**Andreas Hanke¹ and Ralf Metzler²**¹ Institut für Theoretische Physik, Universität Stuttgart, Pfaffenwaldring 57,
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Online at stacks.iop.org/JPhysA/36/L473**Abstract**

The formation of local denaturation zones (bubbles) in double-stranded DNA is an important example of conformational changes of biological macromolecules. We study the dynamics of bubble formation in terms of a Fokker–Planck equation for the probability density to find a bubble of size n base pairs at time t , on the basis of the free energy in the Poland–Scheraga model. Characteristic bubble closing and opening times can be determined from the corresponding first passage time problem, and are sensitive to the specific parameters entering the model. A multistate unzipping model with constant rates recently applied to DNA breathing dynamics (Altan-Bonnet *et al* 2003 *Phys. Rev. Lett.* **90** 138101) emerges as a limiting case.

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1. Introduction

Under physiological conditions, the equilibrium structure of a DNA molecule is the double-stranded Watson–Crick helix. At the same time, in essentially all physiological processes involving DNA, for docking to the DNA, DNA binding proteins require access to the ‘inside’ of the double helix, and therefore the unzipping (denaturation) of a specific region of base pairs [1, 2]. Examples include the replication of DNA via DNA helicase and polymerase, and transcription to single-stranded DNA via RNA polymerase. Thus, double-stranded DNA has to open up locally to expose the otherwise satisfied bonds between complementary bases.

There are several mechanisms how such unzipping of double-stranded DNA (dsDNA) can be accomplished. Under physiological conditions, local unzipping occurs spontaneously due to fluctuations, the *breathing* of dsDNA, which opens up bubbles of a few tens of base pairs [3]. These breathing fluctuations may be supported by accessory proteins which bind to transient single-stranded regions, thereby lowering the DNA base pair stability [2]. Single-molecule force spectroscopy opens the possibility of inducing denaturation regions of controllable size,

by *pulling* the DNA with optical tweezers [4]. In this way, the destabilizing activity of the ssDNA-binding T4 gene 32 protein has been probed, and a kinetic barrier for the single-strand binders identified [5]. Denaturation bubbles can also be induced by *under-winding* the DNA double helix [6]. A recent study of the dynamics of these twist-induced bubbles in a random DNA sequence shows that small bubbles (less than several tens of base pairs) are delocalized along the DNA, whereas larger bubbles become localized in AT-rich regions [7]. Finally, upon *heating*, dsDNA exhibits denaturation bubbles of increasing size and number, and eventually the two strands separate altogether in a process called denaturation transition or melting [8, 9]. Depending on the specific sequence of the DNA molecule and the solvent conditions, the temperature T_m at which one-half of the DNA has denatured typically ranges between 50 °C and 100 °C. Due to the thermal lability of typical natural proteins, thermal melting of DNA is less suited for the study of protein–DNA interactions than force-induced melting [4]. On the other hand, the controlled melting of DNA by heating is an important step of the PCR method for amplifying DNA samples [10], with numerous applications in biotechnology [11].

The study of the bubble dynamics in the above processes is of interest in view of a better understanding of the interaction with single-stranded DNA binding proteins. This interaction involves an interplay between different time scales, e.g., the relaxation time of the bubbles and the time needed for the proteins to rearrange sterically in order to bind [12]. Dynamic probes such as single-molecule force spectroscopy [4] and molecular beacon assays [13] may therefore shed light on the underlying biochemistry of such processes.

In a recent experiment by Altan-Bonnet *et al* [14], the dynamics of a single bubble in dsDNA was measured by fluorescence correlation spectroscopy. It was found that in the breathing domain of the DNA construct (a row of 18 AT base pairs sealed by more stable GC base pairs) fluctuation bubbles of size 2 to 10 base pairs are formed below the melting temperature T_m of the AT breathing domain. The relaxation dynamics follows a multistate relaxation kinetics involving a distribution of bubble sizes and successive opening and closing of base pairs. The characteristic relaxation time scales were estimated from the experiment to within the range of 20 to 100 μ s. Also in [14], a simple master equation of stepwise zipping–unzipping with constant rate coefficients was proposed to successfully describe the data for the autocorrelation function, showing that indeed the bubble dynamics is a multistate process. The latter was confirmed in a recent UV light absorption study of the denaturation of DNA oligomers [15].

In this work, we establish a general framework to study the bubble dynamics of dsDNA by means of a Fokker–Planck equation, based on the bubble free energy function. The latter allows one to include microscopic interactions in a straightforward fashion, such that our approach may serve as a testing ground for different models, as we show below. In particular, it turns out that the phenomenological rate equation approach, corresponding to a diffusion with constant drift in the space of bubble size n used by Altan-Bonnet *et al* [14] to fit their experimental data corresponds to a limiting case of our Fokker–Planck equation. However, the inclusion of additional microscopic interactions in such a rate equation approach is not straightforward [16]. In what follows, we first establish the bubble free energy within the Poland–Scheraga (PS) model of DNA denaturation [8, 9], and then derive the Fokker–Planck equation to describe the bubble dynamics both below and at the melting temperature of dsDNA.

2. Bubble free energy

In the PS model, energetic bonds in the double-stranded, helical regions of the DNA compete with the entropy gain from the far more flexible, single-stranded loops [8, 9]. The stability of the double helix originates mainly from stacking interactions between adjacent base pairs,

apart from the Watson–Crick hydrogen bonds between bases. In addition, the positioning of bases for pairing out of a loop state gives rise to an entropic contribution. The Gibbs free energy $G_{ij} = H_{ij} - TS_{ij}$ for the dissociation of two paired and stacked base pairs i and j has been measured, and is available in terms of the enthalpic and entropic contributions H_{ij} and S_{ij} [17]. In the following we consider a homopolymer for simplicity, as suitable for the AT breathing domain in [14]. For an AT-homopolymer ($i = j = \text{AT}$), the Gibbs free energy per base pair in units of $k_B T$ yields $\gamma \equiv \beta G_{ii}/2 = 0.6$ at 37 °C for standard salt conditions (0.0745M Na⁺). Similarly, for a GC-homopolymer one finds the higher value of $\gamma = 1.46$ at 37 °C. The condition $\gamma = 0$ defines the melting temperature T_m [17, 18], thus $T_m(\text{AT}) = 66.8$ °C and $T_m(\text{GC}) = 102.5$ °C (we assume that G_{ij} is linear in T , cf [19]). Above T_m , γ becomes negative. For given $\gamma = \gamma(T)$, the statistical weight for the dissociation of n base pairs is obtained as

$$W(n) = \exp(-n\gamma). \quad (1)$$

Additional contributions arise upon formation of a loop within dsDNA. Firstly, an initial energy barrier has to be overcome, which we denote as γ_1 in units of $k_B T$. From fitting melting curves to long DNA, $\gamma_1 \approx 10$ was obtained, so that the statistical weight for the initiation of a loop (cooperativity parameter), $\sigma_0 = \exp(-\gamma_1)$, is of order 10^{-5} [9, 17]. As the energy to extend an existing loop by one base pair is smaller than $k_B T$, DNA melts as large cooperative domains. Below the melting temperature T_m , the bubbles become smaller, and long range interactions beyond nearest neighbours become more important. In this case, the probability of bubble formation is larger, and γ_1 ranges between 3 and 5, thus $\sigma_0 \lesssim 0.05$ [7] (cf section 5 in [9]). According to [18], the smallness of σ_0 inhibits the recombination of complementary DNA strands with mutations, making recognition more selective. Secondly, once a loop of n base pairs has formed, there is a weight $f(n)$ of mainly entropic origin, to be detailed below. The additional weight of a loop of n open base pairs is thus

$$\Omega(n) = \sigma_0 f(n). \quad (2)$$

For large bubbles one usually assumes the form $f(n) = (n+1)^{-c}$ [9, 17]. Here, the value of the exponent $c = 1.76$ corresponds to a self-avoiding, flexible ring [8, 9, 20], which is classically used in denaturation modelling within the PS approach. Recently, the PS model has been considered in view of the order of the denaturation phase transition [21–25]. Zocchi *et al* [15] find by finite size scaling analysis of measured melting curves of DNA oligomers that the transition is of second order. In [21], the value $c = 2.12$ was suggested, compare the discussion in [7, 18, 24]. For smaller bubbles (in the range of 1 to a few tens of base pairs), the appropriate form of $f(n)$ is more involved. In particular, $f(n)$ will depend on the finite persistence length of ssDNA (about eight bases), on the specific base sequence, and possibly on interactions between dissolved but close-by base pairs (cf section 2.1.3 in [9]). Therefore, the knowledge of $f(n)$ provides information on these microscopic interactions.

For simplicity, we here adopt the simple form $f(n) = (n+1)^{-c}$ for all $n > 0$, and consider the loop weight

$$\Omega(n) = \sigma_0 (n+1)^{-c}. \quad (3)$$

We show that at the melting temperature the results for the relaxation times for the bubbles are different for the available values $c = 1.76$ and $c = 2.12$ quoted above. This shows that the specific form of $f(n)$ indeed modifies experimentally accessible features of the bubble dynamics [14].

In what follows, we focus on a single bubble in dsDNA, neglecting its interaction with other bubbles. Since due to $\sigma_0 \ll 1$ the mean distance between bubbles ($\sim 1/\sigma_0$ [8]) is large, this approximation is justified as long as the bubbles are not too large [7]. It also corresponds

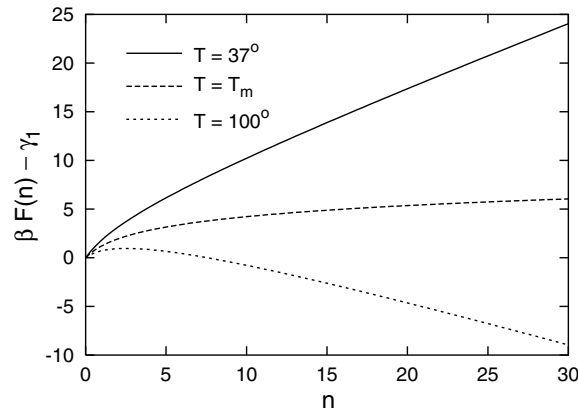


Figure 1. Variable part of the bubble free energy (4), $\beta\mathcal{F}(n) - \gamma_1 = n\gamma(T) + c \ln(n+1)$, as a function of the bubble size n for $T = 37^\circ\text{C}$ ($\gamma \approx 0.6$), $T_m = 66^\circ\text{C}$ ($\gamma = 0$) and $T = 100^\circ\text{C}$ ($\gamma \approx -0.5$). In the latter case, a small barrier precedes the negative drift towards bubble opening as dominated by the $\gamma < 0$ contribution.

to the situation studied in the recent experiment by Altan-Bonnet *et al* [14]. According to the above, the total free energy $\mathcal{F}(n)$ of a single bubble with $n > 0$ open base pairs follows in the form

$$\beta\mathcal{F}(n) = -\ln[W(n)\Omega(n)] = n\gamma(T) + \gamma_1 + c \ln(n+1) \quad (4)$$

where the dependence on the temperature T enters only via $\gamma = \gamma(T)$. We show the free energy (4) in figure 1 for $c = 1.76$ and for the parameters of an AT-homopolymer, for physiological temperature $T = 37^\circ\text{C}$, at the melting temperature $T_m = 66^\circ\text{C}$, and at $T = 100^\circ\text{C}$, compare the discussion below.

3. Bubble dynamics

In the generally accepted multistate unzipping model, the double strand opens by successively breaking Watson–Crick bonds, like opening a zipper [26, 27]. As γ becomes small on increasing the temperature, thermal fluctuations become relevant and cause a random walk-like propagation of the zipper locations at both ends of the bubble–helix joints. The fluctuations of the bubble size can therefore be described in the continuum limit through a Fokker–Planck equation for the probability density function (PDF) $P(n, t)$ to find at time t a bubble consisting of n denatured base pairs, following a similar reasoning as pursued in the modelling of the dynamics of biopolymer translocation through a narrow membrane pore [28]. To establish this Fokker–Planck equation, we combine the continuity equation (compare [28])

$$\frac{\partial P(n, t)}{\partial t} + \frac{\partial j(n, t)}{\partial n} = 0 \quad (5)$$

with the expression for the corresponding flux,

$$j(n, t) = -D \left(\frac{\partial P(n, t)}{\partial n} + \frac{P(n, t)}{k_B T} \frac{\partial \mathcal{F}}{\partial n} \right) \quad (6)$$

where it is assumed that the potential exerting the drift is given by the bubble free energy (4). In equation (6), we incorporated an Einstein relation of the form $D = k_B T \mu$, where the mobility μ has dimensions $[\mu] = \text{s g}^{-1} \text{cm}^{-2}$, and therefore $[D] = \text{s}^{-1}$ represents an inverse

time scale. By combination of equations (4), (5) and (6), we retrieve the Fokker–Planck equation for $P(n, t)$:³

$$\frac{\partial P(n, t)}{\partial t} = D \left(\frac{\partial}{\partial n} \left\{ \gamma + \frac{c}{n+1} \right\} + \frac{\partial^2}{\partial n^2} \right) P(n, t). \quad (7)$$

Thus, we arrived at a reduced 1D description of the bubble dynamics in a homopolymer, with the bubble size n as the effective ‘reaction’ coordinate. For a heteropolymer, the position of the bubble within the double helix, i.e., the index m of the first open base pair, also becomes relevant. In this case, the bubble free energy \mathcal{F} and thus the PDF depend on both m and n , and on the specific base sequence; the corresponding generalization of equation (7) is straightforward. In a random sequence, additional phenomena may occur, such as localization of larger bubbles [7]. Finally, to establish the Fokker–Planck equation (7), we assume that changes of the bubble size n occur slower than other degrees of freedom of the PS free energy within the bubble region (e.g., Rouse–Zimm modes). This assumption seems reasonable due to the long bubble dynamics’ relaxation time scales of 20 to 100 μs [14], and the good approximation of bubble independence [7].

By rescaling time according to $t \rightarrow Dt$, the Fokker–Planck equation (7) can be made dimensionless, a representation we are going to use in the numerical evaluation below. The formulation in terms of a Fokker–Planck equation makes it possible to derive the characteristic times for bubble closing and opening in terms of a first passage time problem. That is, for bubble closing, the associated mean closing time follows as the mean first passage time to reach bubble size $n = 0$ after starting from the initial bubble size n_0 . We now determine these characteristic times for the three regimes defined by γ with respect to temperature T .

- (i) $T < T_m$. In this regime, the drift consists of two contributions, the constant drift $D\gamma$ and the loop closure component $Dc/(n+1)$, which decreases with n . For large n , we can therefore approximate the drift by the constant term $D\gamma$, and in this limit the Fokker–Planck equation (7) is equivalent to the continuum limit of the master equation used in [14] to describe the experimental bubble data. In particular, we can identify our two independent parameters D and γ with the rate constants k_+ and k_- to open and close a base pair introduced in [14], respectively: $D \equiv (k_+ + k_-)/2$ and $\gamma \equiv 2(k_- - k_+)/ (k_+ + k_-)$. In this approximation, the correlation functions used successfully to fit the experimental results in [14] can be derived from the Fokker–Planck equation (7). Moreover, we can deduce the mean first passage time PDF for a bubble of initial size n_0 to close in the exact analytical form (compare [29])

$$f(0, t) = \frac{n_0}{\sqrt{4\pi Dt^3}} \exp \left\{ -\frac{(n_0 - D\gamma t)^2}{4Dt} \right\} \quad (8)$$

which decays exponentially for large n . In particular, from (8) the characteristic (mean) first passage time for bubble closing,

$$\tau = n_0/(D\gamma) \quad (9)$$

follows, which is linear in the initial bubble size n_0 . In figure 2, we compare this analytical result for the value $\gamma(37^\circ\text{C})$ with the characteristic closing times using the full drift term from equation (7), obtained from numerical integration. It can be seen that the qualitative behaviour for both cases with and without the $Dc/(n+1)$ term is very similar, but that in the presence of the loop closure component the characteristic times are reduced.

³ Note that the operator $\frac{\partial}{\partial n}$ acts also on $P(n, t)$.

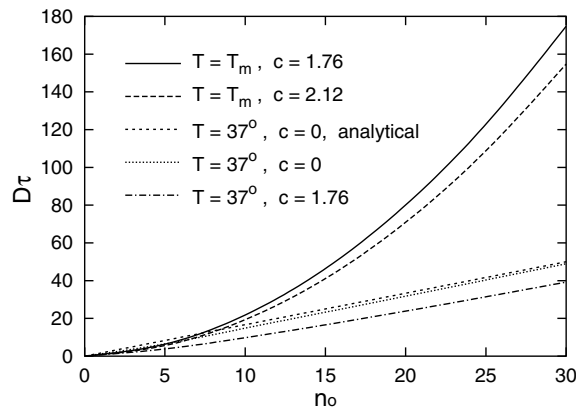


Figure 2. Characteristic bubble closing times τ as a function of initial bubble size n_0 for an AT-homopolymer, obtained from the Fokker–Planck equation (7) by numerical integration. At $T = 37^\circ\text{C}$, the result for $c = 1.76$ is compared to the approximation $c = 0$ which leads to somewhat larger closing times. The analytical solution for $c = 0$ compares well with the numerical result, the slight discrepancy being due to the reflecting boundary condition applied in the numerics, in comparison to the natural boundary condition at $n \rightarrow \infty$ used to derive equation (8). At the melting temperature $T_m = 66^\circ\text{C}$, the closing times for the values $c = 1.76$ and $c = 2.12$ can be distinguished.

- (ii) $T = T_m$. At the melting temperature, the drift in equation (7) is solely given by the loop closure term $Dc/(n+1)$. In figure 2, we plot the characteristic bubble closing time obtained numerically. In comparison to the above case $T < T_m$, the faster than linear increase as a function of initial bubble size n_0 is distinct. Keeping in mind that $Dc/(n+1)$ becomes very small for increasing n , this behaviour can be qualitatively understood from the approximation in terms of a drift-free diffusion in a box of size n_0 , in which the initial condition $P(n, 0) = \delta(n - n_0)$ is located at a reflecting boundary, and at $n = 0$ an absorbing boundary is placed. This problem can be solved analytically, with the result $\tau = n_0^2/(2D)$ for the characteristic escape time⁴ in which the quadratic dependence on n_0 contrasts the linear behaviour in the result (9). Thus, at the melting temperature T_m , the tendency for a bubble to close becomes increasingly weaker for larger bubble sizes, and therefore much larger bubbles can be formed, in contrast to the case $T < T_m$. A further comparison to the value $c = 2.12$ mentioned above demonstrates that a clear quantitative difference in the associated closing times exists, see figure 2. However, the qualitative behaviour remains unchanged. In principle, the study of the bubble dynamics can therefore be used to discern different models for the loop closure factor.
- (iii) $T > T_m$. Above the melting temperature, the drift is governed by the interplay between the loop closure component $Dc/(n+1)$ tending to close the bubble, and the bubble free energy $D\gamma(T > T_m) < 0$, which causes a bias towards bubble opening. In figure 1, we show for the AT-homopolymer case how the overall drift potential after a small initial activation barrier becomes negative, and the dynamics is essentially governed by the γ -contribution. As a consequence, from the result (8), we find that the associated mean first passage time diverges, i.e., the bubble on average increases in size until the entire DNA is denatured, as expected from the PS model.

By symmetry, similar results hold for the bubble opening process. However, the existence of the bubble initiation energy γ_1 involves an additional Arrhenius factor, which is not included

⁴ This result can, for instance, be obtained through the method of images, compare [30].

in the Fokker–Planck equation (7), and which reduces the opening probability, causing an increase of the associated opening time, cf [14].

4. Conclusions

From the bubble free energy of a single, independent bubble in the Poland–Scheraga theory of DNA melting, we derived a Fokker–Planck equation for the PDF to find a bubble created by denaturation of n base pairs at time t . This formulation allows for the calculation of the characteristic times scales for bubble closing and opening in terms of first passage time problems. Three different regimes were distinguished: (i) below the melting temperature, the characteristic bubble closing time increases linearly in bubble size, and the drift can be approximated by the constant value $D\gamma$. In this approximation, the Fokker–Planck equation matches the continuum version of the master equation employed previously in an experimental study of DNA breathing [14]. (ii) At the melting temperature, an approximately quadratic growth of the bubble closing time as a function of bubble size is observed, which can be explained by noting that the $1/(n+1)$ -dependence of the loop closure drift component can be neglected for larger n , leading to pure diffusion. In this approximation, the exact analytical results indeed lead to the quadratic dependence observed in the numerical results. (iii) Above the melting temperature, the characteristic closing time in our model diverges, consistent with the fact that on average the DNA follows the trend towards the thermodynamically favourable state of complete denaturation.

The expression for the Gibbs free energy used in our approach involves a purely entropic contribution for a single-stranded bubble. It was suggested in [14] that also in denaturation bubbles a residual stacking energy ε_s is present. In this case, the value of γ would have to be corrected by this ε_s .

From the Fokker–Planck equation (7), in which the microscopic interactions enter via the free energy (4), one can derive measurable quantities such as moments and dynamic correlation functions [16]. In principle, the Fokker–Planck equation involves one free parameter, the time scale $1/D$, while the values for the other parameters are known. However, by fitting sufficiently accurate experimental data for DNA bubble dynamics at different temperatures, the values for additional parameters may be extracted with the help of our general Fokker–Planck framework probing suitable free energy functions.

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