Anomalous dynamics of DNA hairpin folding

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By means of computer simulations of a coarse-grained DNA model we show that the DNA hairpin closing dynamics is anomalous, i.e. the characteristic time $\tau$ scales non-linearly with $N$, the hairpin length: $\tau \sim N^\alpha$ with $\alpha > 1$. This is in sharp contrast with the prediction of the zipper model for which $\tau \sim N$. We show that the anomalous dynamics originates from an increase in the friction during closing due to the tension built in the closing strands. From a simple polymer model we get $\alpha = 1 + \nu = 1.59$ with $\nu$ the Flory exponent, a result which is in agreement with the simulations. We discuss transition path times data where such effects should be detected.

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The folding dynamics of DNA (or RNA) hairpins, which are single stranded molecules forming a stem-loop structure, has been a topic of broad interest within the biophysics community for a long time [1–7]. Hairpin folding is a prototype example of secondary structure formation [8] and shares common features with the more complex case of protein folding [9]. In both cases the folding process is described by a one-dimensional reaction coordinate performing a diffusive motion across a free energy potential barrier (see e.g. [10]). Recent advances in experimental single molecule techniques allow to monitor the folding of hairpins [7] and of proteins [11] with an unprecedented time resolution. These and future experiments are expected to elucidate many aspects of the folding dynamics [12], the reason being that the actual conformational changes occur on timescales which can be typically a few orders of magnitude smaller than the total folding time [11].

The aim of this letter is to investigate the folding dynamics of DNA hairpins, focusing in particular on the rapid closing which follows the formation of a stable nucleus of a few base pairs. The latter process is generally much slower as initially the hairpin undergoes a large number of failed nucleation attempts. We show here that the closing time $\tau$ scales with the hairpin length $N$ as $\tau \sim N^\alpha$ with $\alpha > 1$. This conclusion is based on extensive simulations of coarse-grained model of DNA and on scaling arguments for polymer dynamics. Our results are at odds with the zipper model [13] which assumes that the hairpin closes like a zipper following a biased random walk dynamics, which implies $\alpha = 1$. The results give insights on the forces involved in the folding process and in particular in the role of frictional forces. In addition, as argued at the end of this letter, recent experiments on transition path times [7] appear to be better described by a non-linear dependence of closing time vs. $N$, supporting the results reported here.

Despite neglecting the fine atomistic details, coarse-grained models are expected to provide an accurate description of the structure and dynamics of DNA [14–17]. Our simulations were performed using the three sites per nucleotide (3SPN) model [14]. Here a nucleotide is mapped to three “mesoscopic” beads representing sugar, phosphate and base as shown in the snapshot of Fig. 1. The force fields contain interaction terms for bonds, angles and dihedral angles with equilibrium values reproducing the B-DNA structure. In addition there are base-pairing, stacking and electrostatic interactions [14, 18]. We performed Langevin dynamics simulations using the BBK integrator [19] and with force fields parametrized as in Ref. [20]. Simulations were performed at different

![FIG. 1. Snapshot of a DNA hairpin at the end of the folding process as simulated by the 3SPN model. The 3 mesoscopic beads are sugar (S), phosphate (P) and one of the four different bases (A,T,C and G).](image1)

![FIG. 2. Plot of $n(t)$, the number of bound native base pairs, as a function of time for five different molecular dynamics runs of folding of an hairpin with stem of length 40 at $T = 10^\circ C$. In the simulations only native base pairs interactions are taken into account. The folding is characterized by a long timescale for the formation of a nucleus of a few base pairs ($t_a$), followed by a rapid closing ($t_b$).](image2)
temperatures $T = 10^\circ C$ and $T = 30^\circ C$ and for hairpins of different lengths with sequences selected as follows. A single master sequence with a random alternation of AT and CG base pairs was generated. The hairpin sequences were taken from the master sequence starting from its origin so that two hairpins of different lengths $N_1 > N_2$ share the same $N_2$ pairs of nucleotides.

As the focus of this paper is the zippering dynamics which follows the formation of a few native contacts, we consider base-pairing interactions only between native base pairs, as in the original 3SPN model [14]. Figure 2 shows a plot of $n(t)$ vs. $t$, the number of native contacts as a function of time. Two timescales are visible in the plot: the formation of a stable nucleus ($t_a$) is followed by a rapid zippering ($t_b \ll t_a$). The analysis of the simulations reveals that the nucleation predominantly occurs at nucleotides close to the middle of the strand. Hence, in order to speed up the simulations, we used an initial state of a “clamped” configuration as that shown in Fig. 3(a): a high binding energy was assigned to four pairs of nucleotides close to the middle of the DNA strand. This energy was chosen sufficiently high so that the base pairs never unbind during the simulation runs. A single stranded segment of four adenine nucleotides joins these two clamped regions together on one side, forming the loop of the hairpin. During an initial relaxation stage the attractive part of the base pairing interactions between all the bases in the two strands were turned off, except for the four clamped base pairs. At a given time ($t = 0$) the attractive energies on the two strands are turned on and the zippering starts (see Fig. 3(b)). Note that the repulsive part of the base pairs interaction is however always on.

Figure 4 shows a plot of the number of formed base pairs vs. time in a log-log scale for a simulation temperature of $T = 30^\circ C$ and for hairpins of length $N = 10$ to $N = 48$. Here $N$ indicates the maximal number of base pairs which can be bound during the simulation, excluding the initially clamped pairs. Hence counting the eight bases which are clamped and four in the loop, a given $N$ corresponds to a sequence of a single strand with $2N + 12$ nucleotides. In Fig. 4 we plot the linear law $n(t) \sim t$ expected in the zipper model; clearly the dynamics is slower than predicted from the zipper model. The data instead follow a power-law scaling which is consistent with $n(t) \sim t^{1/(1+\nu)}$, where $\nu = 0.59$ is the Flory exponent [21]. This behavior matches the theory discussed below. We estimate the characteristic zippering time by requiring that the number of formed base pairs is a fraction of the total $N$, i.e. $n(\tau) = \lambda N$, where we took different values for $\lambda$ in the range $0.4 \leq \lambda \leq 0.8$. The inset of Fig. 4 shows a plot of $\tau$ (circles) obtained by setting $\lambda = 0.7$ vs. the hairpin length $N$. The data follow a power-law behavior $\tau \sim N^\alpha$ with $\alpha = 1.59(2)$ (circles). The simulations were repeated at $T = 30^\circ C$ (squares) with a similar result. Taking into account the results from both temperatures, and the variations arising from the different possible choices of $\lambda$, we arrive at the aforementioned final result of $\alpha = 1.60(3)$, which is consistent with $\tau \sim N^{1+\nu}$. Figure 5 shows a plot of $R_{ee}(t)/R_{ee}(0)$ and $n(t)/N$ vs. $t$. The end-end distance $R_{ee}(t)$ starts from its maximal value and drops to a small constant...
value when the hairpin closes, while \( n(t) \) increases as the zipper ing proceeds. By comparing the two quantities at equal times (dashed vertical line) one sees that \( R_{ee} \) still largely retains its initial value while roughly a quarter of the base pairs have already formed. This indicates that only a part of the single strands are set into motion when the hairpin starts forming, while the far ends of the two strands are still in their equilibrium configuration. Such conformation is known in polymer physics as a stem-flower shape (see Fig. 5 b)) and it is the cause of the anomalous dynamics, as discussed below. The inset of Fig. 5 shows a plot of the initial value of the \( R_{ee} \) as a function of \( N \), showing that in the 3SPN model the asymptotic regime \( \sim N^\nu \) is reached at around \( N = 20 \) (note that \( N \) is the length of a single strand, hence \( R_{ee} \), the distance between the end points refers to a separation of \( 2N \) nucleotides).

The stem-flower dynamics has been discussed in the context of the absorption of polymers to a flat surface [22]. The number of bound base pairs \( n(t) \) is expected to follow the equation

\[
\gamma(n) \dot{n} = f
\]

where \( f \) is the constant force due to base pairing (averaging over differences between AT and CG base pairs), while the friction \( \gamma \) is assumed to be \( n \)-dependent, since it arises from the stretched stems whose length varies in time (as the flower remains static, it does not contribute to the friction). We thus expect \( \gamma \) to scale as the number of bases in the stem: \( \gamma \sim N_s \) [22]. We can work out the \( n \)-dependence of the friction coefficient by noticing that the distance between the static flowers (AB in Fig. 3) scales as the end-end separation of a single strand of \( 2n + 2N_s \) nucleotides in equilibrium. During zipper ing, this distance is bridged by the two stems which are stretched back-to-back yielding a separation \( \sim 2N_s \). As such [22, 23]:

\[
\gamma(n) \sim N_s \sim (n + N_s)^\nu
\]

where we have assumed that the conformation of a strand with \( n + N_s \) nucleotides is described by a self-avoiding walk statistics. Furthermore for \( n \) sufficiently large we approximate \( \gamma(n) \sim n^\nu \) [25]. Hence Eq. (1) becomes \( n^\nu \dot{n} = f \) which has solution (with \( n(0) = 0 \):

\[
n(t) \sim t^{1/(1+\nu)}
\]

and the total zipper ing time obtained from \( n(\tau) \sim N \) is

\[
\tau \sim N^{1+\nu}.
\]

The theory discussed here is valid in the asymptotic limit of long DNA strands such that their equilibrium properties are described by the self-avoiding walks statistics \( R_{ee} \sim N^\nu \). This behavior is seen in the 3SPN model simulations reported in the inset Fig. 5. Although the hairpin simulated are rather short the data of Fig. 3 show good convergence to the expected asymptotic behavior. We note that hydrodynamics interactions do not modify the predicted exponent in the stem-flower regime, as the friction originates from the stretched parts of the single strands. Hence the scaling \( \tau \sim N^{1+\nu} \) is expected to be relevant for experiments.

Anomalous dynamics in polymers has been studied a lot in the past decade ([22, 24, 26–31]). Besides the already mentioned case of polymer absorption to a planar substrate [22] the exponent \( 1+\nu \) also governs the dynamics of driven translocation through a small pore (see e.g. 27, 28). The formation of a stem-flower shape in DNA hairpin dynamics is also supported by polymer physics arguments. Let us consider a single polymer pulled by a constant force \( f \) applied to one of its end monomers [33]. A stem-flower conformation arises if the force is large enough such that [27, 33]

\[
\Sigma = \frac{fa}{k_B T} \gtrsim 1
\]

where \( a \) is the monomer-monomer distance. In DNA hairpins \( f \) is the force due to base pairing (see Fig. 3), which can be estimated from the hybridization free energy per nucleotide: \( \Delta G \approx fa \). For \( \Delta G \) we use the experimentally determined values from the nearest-neighbor model from Ref. [33]. Distinguishing between weak (AT) and strong (CG) base pairings we obtain estimates \( 1 \lesssim \Sigma \lesssim 3.6 \) at \( T = 37^\circ \text{C} \) and \( 2 \lesssim \Sigma \lesssim 5 \) at \( T = 10^\circ \text{C} \). This suggests that the base-pairing in real DNA hairpin is sufficiently strong to produce a stem-flower conformation.
The TPT recently measured in nucleic acids of different types of biomolecular folding. There has been quite some recent interest in the experimental determination of the transition path times (TPT), which are the short timescales in which the folding process actually takes place \[ \tau \sim N^{1.6} \] . In analogy to what is shown in Fig. 2, the TPT are much shorter than the total folding time and their measurement is very challenging. The fat lines in the inset of Fig. 4 and those of the 3SPN model of Fig. 6 are shown in Fig. 4. We note that there is a difference in absolute timescales of the simulations from the 3SPN model of the inset of Fig. 4 and those of the experiments. This is because the coarse-grained model contains some simplifications: for instance it does not include explicit solvent effects \[ 14 \], which usually slow down the dynamics. However the exponent characterizing the dynamical laws is expected to be universal, despite the difference in absolute times. A weighted fit, which weights the error bars in each point, of the data of Fig. 6 yields \( \tau \sim N^{1.6} \). The limited data favor a superlinear scaling compared to a linear scaling as expected from the zipper model. We note that only very recently TPT have been measured, therefore a limited amount of data is available. In addition, the TPT are obtained indirectly as via energy landscape theory \[ 7 \], using some assumptions on the underlying dynamics. It would be interesting to extend the TPT measurements to test the anomalous dynamics scenario, which, as shown in this work, is supported by theory and simulations.

Our results show that the simple diffusive motion predicted by the zipper model cannot explain the simulations. However, the data are compatible with a diffusive dynamics with a \( n \)-dependent diffusion coefficient, obtained from the fluctuation-dissipation relation \( D(n) = k_B T / \gamma (n) \). Diffusion coefficients which depend on the reaction coordinate have been recently discussed in the protein folding literature \[ 10 \]. In the context of the stem-flower folding in DNA hairpin dynamics the coordinate-dependence arises naturally from the increasing friction of the closing strands, which should lead to a decrease in \( D(n) \). We expect that this should also happen in the folding of other biomolecule domains; for instance in the formation of an alpha helix the two ends of the unfolded polypeptide are pulled towards the helical domain, producing frictional forces similar to those described here. Therefore, this discussion could be useful to rationalize the observed diffusion coefficients in other types of biomolecular folding.
We note that $0 \leq n \leq N$, while $0 \leq N_s \leq N^\nu$, therefore for large $n$ (and $N$) the approximation is justified. To estimate the range of $n$ for which this relation is valid we rewrite the right hand side of Eq. (2) using the appropriate prefactors as

$$2aN_s = aN_p \left( \frac{2n + 2N_s}{N_p} \right)^\nu$$

(6)

Here $2aN_s$ is the distance between the two points AB in Fig. 3, $a$ is the distance between two nucleotides and $aN_p$ is the persistence length of single stranded DNA. We solve Eq. (6) numerically to get $N_s$ as a function of $n$, from which we obtain $\gamma(n) \propto N_s(n)$. The single stranded DNA persistence length is estimated in the range $2 \leq N_p \leq 4$ [14]. Taking $N_p = 2$, in the range $20 < n < 50$ the friction is approximated by a power-law $\gamma(n) \approx n^\delta$ with $\delta \approx 0.54$, while for $N_p = 4$ we get $\delta \approx 0.52$. These values are not far from the asymptotical value $\nu = 0.59$, which suggests that the range of hairpin simulated the assumption $\gamma(n) \sim n^\nu$ is a good approximation. We note that for $N_p = 2$ we get for $n = 35$ the value $N_s \approx 10$, hence the approximation $\gamma(n) \sim n^\nu$ does not require $n \gg N_s$ to hold strongly.

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