Effect of elastic colored noise in the hopping dynamics of single molecules in stretching experiments

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The influence of colored noise induced by elastic fluctuations in single-molecule stretching experiments is theoretically and numerically studied. Unlike in the thermal white noise case currently considered in the literature, elastically induced hopping dynamics between folded and unfolded states is manifested through critical oscillations showing smaller end-to-end distance fluctuations ($\delta x \sim 1.25$ nm) within the free energy wells corresponding to both states. Our results are derived by analyzing the elastic coupling between the Handle-Molecule-Handle system and the laser optical tweezers (LOT) array. It is shown that an Ornstein-Uhlenbeck process related to this elastic coupling may trigger the hopping transitions via a colored noise with an intensity proportional to the elastic constant of the LOT array. Evolution equations of the variables of the system were derived by using the irreversible thermodynamics of small systems recently proposed. Theoretical expressions for the corresponding stationary probability densities are provided and the viability of inferring the shape of the free energy from direct measurements is discussed.

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I. INTRODUCTION

The study of individual macromolecules like DNA or RNA is determinant in the development of biotechnology as well as in the understanding of the biological functioning of living beings. In particular, RNA's folding dynamics presents valuable information about the gene expression mechanism in these macromolecules. That is why a suitable model of RNA's folding dynamics emerges as an aid to a better understanding of this particular biological mechanism [1].

Commonly, experiments using laser optical tweezers (LOT) are implemented in order to produce an external force, which induces conformational changes on the structure of RNA or DNA molecules [2–7]. The system considered in these works is made up by a RNA hairpin joined at its ends through two appropriate polymeric arms to two polystyrene beads. These polymers, which act as handles, are characterized by their contour and persistence lengths L and l_p, respectively. The system is immersed in a thermal bath with constant temperature T, which may be able to produce fluctuations of the position of the bead trapped by the LOT and the Handle-RNA-Handle system. The main reason for introducing this particular array is to facilitate the manipulation of the tiny RNA hairpin with LOT. The system discussed here is called "Handle-RNA-Handle." Roughly, the LOT manipulation of the system is performed by fixing one end of the Handle-RNA-Handle to a substrate. The other end is attached to a bead that may be manipulated with the LOT by applying a constant tension or by regulating the length of the system. In this way, when the force versus extension curve of the system is near the unstable region, the hairpin shows episodes of hopping between the folded and unfolded states, spontaneously. Many details related to the LOT system and the experimental protocols are well described in Refs. [8,9].

In this scenario, it has been considered that bead fluctuations are negligible and therefore that the two main variables describing the dynamic of the system are the extension x of the RNA hairpin serving as a reaction coordinate, and the tension τ implemented by the LOT [10]. In addition, it is assumed that the fluctuations of the RNA extension or end-to-end distance are due to thermal noise, which is considered as a white noise. As a consequence, the corresponding evolution equation describing the dynamics of this system is directly assimilable to a Langevin equation in the overdamped regime [10,11].

However, in this article we study the effect of thermal fluctuations on the polystyrene beads on the dynamics of the Handle-RNA-Handle system and how, due to the coupling with the hairpin via the handles, these fluctuations affect the hopping dynamics of the macromolecule. Colored noise in the fluctuations of an extended DNA molecule were reported by optical trapping in Ref. [2]. It was recently shown that, for several proteins, the dependence of folding and unfolding rates on solvent viscosity does not obey Kramers' theory and thus, indicating the necessity to use colored noise descriptions [3]. These descriptions may be implemented through non-Markovian Langevin equations. This approach is very rich and was recently used to explain important issues of the transport of cargoes by protein motors in living cells [12,13]. Other examples of studies showing the importance of non-Markovian Langevin equations in the realm of biophysics were devoted to analyze the mean square displacement of chromosomal segments in cell nucleus [14–16] and of the relative position between donor and acceptor in proteins [17]. The results obtained allowed one to better quantify important aspects of the biological and physicochemical function of these entities.

These considerations suggest that the fluctuations of the position of the polystyrene beads may perturb the tension exerted by LOT over the system and, in this way, influence its dynamics in a different way as it has been considered in the literature (see Fig. 1). The analysis we present in this work shows that it is indeed a colored noise the agent giving rise to extension fluctuations. Our approach to the problem comprises the derivation of the fundamental stochastic kinetic equation of a Langevin type of the Handle-RNA-Handle by means of the

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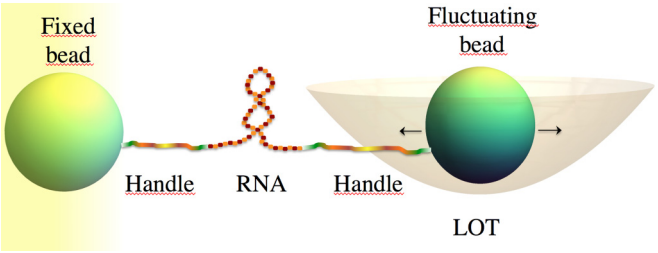

FIG. 1. (Color online) Configuration of the system Handle-RNA-Handle. Each handle is attached to a bead. Maintaining one bead fixed to a substrate while the other bead is manipulated by LOT, creating fluctuations of this bead within a harmonic potential.

Onsager's irreversible thermodynamics [11]. This derivation together with the results obtained show the generality and utility of our approach, in particular when considering important effects as intrinsic handle and polystyrene bead fluctuations. These kinds of fluctuations cannot be obtained by following the traditional approach based on master equations [18–20].

The article is organized as follows. First we discuss a situation in which the source of noise is attributable to the dynamic of the bead, resulting in a model described by a Langevin equation with additive colored noise. After this, we introduce changes in the dynamics of the bead, having as a consequence, that the model which represents the dynamics of the system Handle-RNA-Handle plus polystyrene beads is now a Langevin equation with multiplicative colored noise. In both cases we present simulations of their respective Langevin equation's solution. We also calculate the corresponding entropies produced during the process and their stationary probability density functions. Finally we present our conclusions.

II. SINGLE MOLECULE IRREVERSIBLE THERMODYNAMICS

As a starting point, we will consider that the system is the *single* RNA molecule of average length $\langle x\rangle$ subject to a stretching force τ which can be controlled as an external parameter. The system is in contact with a heat bath at temperature T, pressure P, and volume V and hence, we may consider the Gibbs free energy G as the adequate thermodynamic potential to describe the system. The definition of the Gibbs free energy is $G = U + PV - TS$ with U the internal energy and S the entropy. The differential change of the Gibbs free energy G is

$$dG = dU + PdV + VdP - SdT - TdS. \tag{1}$$

The first law of thermodynamics is

$$dU = dQ - PdV + \tau d\langle x\rangle, \tag{2}$$

where we have taken into account the work done by the stretching force $dW_{\text{stretching}} = \tau d\langle x\rangle$. We may write the reversible exchange of heat between the system and the heat bath in terms of the reversible exchange of entropy in the form $dQ = Td_eS$. Furthermore, in the general case we have to consider that the total entropy change of the system is given by

$$dS = d_eS + d_iS, \tag{3}$$

where d_iS is the entropy produced during the transformation due to the existence of irreversibilities that may be internal to the molecule and/or due to its interaction with the heat bath (friction). Substitution of Eq. (2) in the first term and of Eq. (3) in the last term at the right-hand side of (1), yields the final result,

$$dG = \tau d\langle x\rangle + VdP - SdT - Td_iS. \tag{4}$$

For process at constant pressure, temperature, and extension, this equation and the second law of thermodynamics imply that the Gibbs free energy must satisfy the relation $dG = -Td_iS \leqslant 0$. For processes in which the extension is not conserved, the second law of thermodynamics imposes the condition,

$$Td_iS = \tau d\langle x\rangle - dG \geqslant 0. \tag{5}$$

Here, it is convenient to recall that for equilibrated transformations ($d_iS = 0$) the internal and external tensional forces are equal in magnitude, $\tau_{\text{int}} = \tau$, and therefore the internal work done by the molecule, $dG = dW_{\text{rev}}$, is equal to the external work thus satisfying the relation: $dG = dW_{\text{rev}} = \tau d\langle x\rangle$. For nonequilibrated transformations, internal and external forces are not equal ($\tau_{\text{int}} \neq \tau$) and therefore we have $dG = dW_{\text{irr}} \neq \tau d\langle x\rangle$. From this discussion it follows that the general definition of the internal tension is

$$\tau_{\text{int}} \equiv \frac{\partial G}{\partial\langle x\rangle}. \tag{6}$$

For irreversible processes, the entropy production per unit time can be computed by taking the variation in time of Eq. (5) from which it follows,

$$T\frac{d_iS}{dt} = \left[-\frac{\partial G}{\partial\langle x\rangle} + \tau\right]\frac{d\langle x\rangle}{dt}. \tag{7}$$

Since the second law imposes that the entropy production must be non-negative, $d_iS/dt > 0$, we may assume, without loss of generality, the linear relationship,

$$\frac{d\langle x\rangle}{dt} = \frac{1}{\gamma}\left[-\frac{\partial G}{\partial\langle x\rangle} + \tau\right], \tag{8}$$

which is the overdamped dynamic equation for the average extension of the molecule. Here, $\gamma > 0$ is an Onsager coefficient which plays the role of an effective friction coefficient whose dimensions must be force over velocity. This coefficient may be in general a function of the state variables, T, P, τ, and $\langle x\rangle$. The dependence on temperature and pressure is not important for the purposes of this work. However, the dependence on τ and $\langle x\rangle$ can be of importance. A general criterion to determine this dependence is by imposing mathematical conditions that make the equation physically consistent. In the present case, due to the fact that the free energy profiles are obtained from simulations [10], one may assume, in first approximation a linear response regime in which the Onsager coefficient is considered constant. It is convenient to mention that the coefficient γ is associated with the extension of the H-RNA-H system and, hence, it should be taken as an effective friction coefficient for longitudinal motions along the system's axis and not like the friction coefficient associated with translational motions of the whole molecule. This point will be further discussed in Sec. III A.

Equation (8) is the starting point in the calculation of the evolution of the single molecule when the external tension takes a value in the unstable region of the force-extension curve. However, since in such a region the system is strongly sensitive to noise because it is intrinsically unstable, then the evolution Eq. (8) should be replaced by its corresponding stochastic counterpart. This way to proceed is very general and allows one to consider the effect of white or colored noises.

The simplest stochastic model that can be formulated using Eq. (8) as a starting point is based on Onsager's approach to nonequilibrium fluctuations and their relation with irreversible thermodynamics. Given the regression law Eq. (8), the corresponding stochastic formulation can be established by simply adding the random force $\xi(t)$ as an additive contribution, that is,

$$\frac{dx}{dt} = \frac{1}{\gamma}\left[-\frac{\partial G}{\partial x} + \tau\right] + \xi(t), \tag{9}$$

where the brackets were removed since now the dynamics is stochastic and $\xi(t)$ is a white noise which satisfies the following statistical properties in the overdamped case,

$$\langle \xi(t)\rangle = 0 \quad \text{and} \quad \langle \xi(t)\xi(t')\rangle = \frac{2k_BT}{\gamma}\delta(t-t'). \tag{10}$$

Here k_B is the Boltzmann constant and the second relation in Eq. (10) is the fluctuation dissipation theorem [21,22]. Notice that we have considered the noise correlation coming from the complete nonoverdamped Langevin equation. The results obtained for individual realizations of the dynamics of the Handle-RNA-Handle system in the unstable region of the force-extension curve were in agreement with previous computer simulations [10] and discussed in Ref. [11].

III. INFLUENCE OF POLYSTYRENE BEAD FLUCTUATIONS ON SYSTEM'S DYNAMICS

In this work we analyze the critical oscillations of the Handle-RNA-Handle system by assuming that the fluctuations in the position of the polystyrene beads induce in turn fluctuations of the tension exerted on the Handle-RNA-Handle. For this situation, it can be said that the coupling with the bead dynamics introduces a fluctuating force that is correlated for different times of evolution, that is, the fluctuating force can be treated as a colored noise source. Therefore, the evolution equation of the system should satisfy the equation,

$$\frac{dx}{dt} = \frac{1}{\gamma}\left[-\frac{\partial G}{\partial x} + \tau\right] + \xi_c(t), \tag{11}$$

where $\xi_c(t)$ is a random force with finite correlations (colored noise) which satisfies the following statistical properties:

$$\langle \xi_c(t)\rangle = 0 \quad \text{and} \quad \langle \xi_c(t)\xi_c(t')\rangle = \Gamma(t-t'). \tag{12}$$

Here, the fluctuation dissipation theorem obeyed by the random force $\xi_c(t)$ has a finite time correlation function $\Gamma(t-t')$. In the next we will analyze two possible physical origins of this term and give an explicit expression for $\Gamma(t-t')$. A particular important case is, however, when the noise source $\xi_c(t)$ is a Ornstein-Uhlenbeck process for which the fluctuation-dissipation relation can be written in the form,

$$\langle \xi_c(t)\xi_c(t')\rangle = \frac{D}{t_c}e^{-|t-t'|/t_c}, \tag{13}$$

where t_c and D are the characteristic correlation time and the diffusion coefficient of the process, respectively.

A. Additive colored noise

In Fig. 1 we present a diagram in which we show the features of the configuration of the Handle-RNA-Handle system. Each handle is attached to a polystyrene bead; one bead is fixed to a substrate and the other one is free to be manipulated by LOT. It is clear that the position of this second bead is influenced by the LOT, the thermal noise originated in the heat bath, and the reaction force of the Handle-RNA-Handle system. The second factor induces fluctuations of the position of the bead whereas the forces due to LOT and the Handle-RNA-Handle system restrict the motion of the bead. In first approximation, this restriction can be modeled by means of an effective harmonic potential with effective elastic constant $\kappa_b = k_{lot} + k_m$, where k_{lot} and k_m are the elastic constants of LOT and the Handle-RNA-Handle system, respectively (see Ref. [10]). Reference values for both elastic constants are reported in Ref. [10] and are $k_{lot} = 0.1$ pNnm^{-1} and $k_m = 0.01$ pNnm^{-1}. Other possible values of these constants are considered in Refs. [8,9]. Considering the force that the Handle-RNA-Handle system exerts on the bead as an effective linear force is an approximation having analytical advantages, in the sense that Fokker-Planck equations for the distribution function of the system's extension can be explicitly deduced in both the additive and multiplicative cases.

Thus, if we denote the position of the bead by $z_b(t)$, in the overdamped approximation its dynamics can be modeled by the Ornstein-Uhlenbeck process [10],

$$\frac{dz_b}{dt} = -\frac{\kappa_b}{\tilde{\gamma}}z_b + \tilde{\xi}(t), \tag{14}$$

where $\tilde{\gamma} = 6\pi\eta a$ is the Stokes frictional coefficient of the polystyrene bead with η the dynamic viscosity of the medium and a the radius of the bead. In addition $\tilde{\xi}(t)$ is a white noise with zero average and obeying the fluctuation-dissipation theorem,

$$\langle \tilde{\xi}(t)\tilde{\xi}(t')\rangle = \frac{2k_BT}{\tilde{\gamma}}\delta(t-t'). \tag{15}$$

Therefore, the time correlation of $z_b(t)$ obeys the formula,

$$\langle z_b(t)z_b(t')\rangle = \frac{k_BT}{\kappa_b}e^{-\frac{\kappa_b}{\tilde{\gamma}}|t-t'|}. \tag{16}$$

Hence, assuming that the fluctuations in the position of the bead induce additive fluctuations in the force applied on the Handle-RNA-Handle system, we may write

$$\tau = \tau_{lot} + \delta\tau, \tag{17}$$

where τ_{lot} is the constant external tension exerted by LOT on the Handle-RNA-Handle system and $\delta\tau = \kappa_b z_b(t)$ is the fluctuating tension term arising from the fluctuations in the

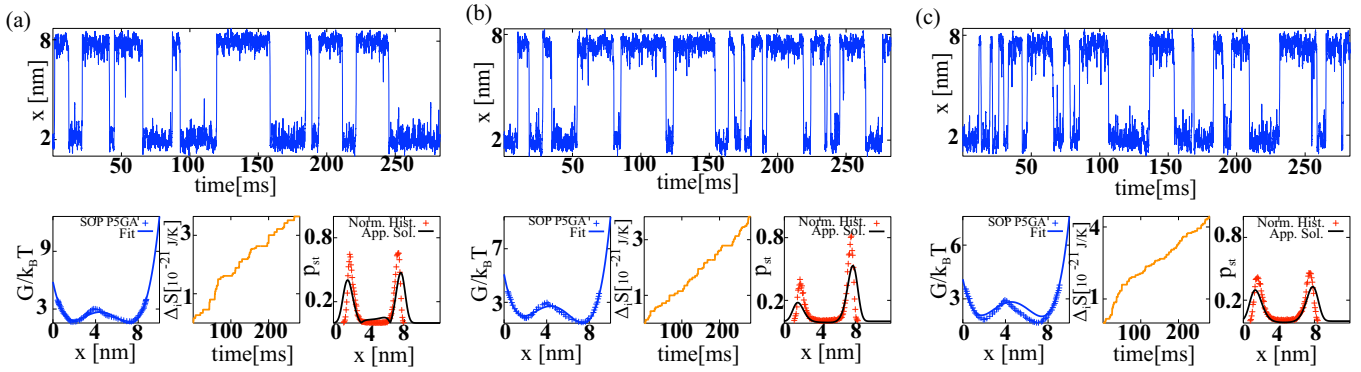

FIG. 2. (Color online) Simulation outputs for the elastically induced transitions in the additive case for the systems. (a) Rigid, $l_p/L = 7$; (b) semiflexible, $l_p/L = 2.8$; (c) flexible, $l_p/L = 0.12$. In all cases the effective spring constant was taken as $k_b = k_{lot} + k_m = 0.11$ pN/nm and $\tilde{\gamma}/\gamma = 0.55$. Also shown is the fit (solid line) of the free energy (symbols) reported from simulations in Ref. [10] (left), the staircase shape of the entropy produced by the system when performing the elastically induced transitions between folded and unfolded states (middle), and the comparison between the theoretical prediction (21) for the probability density (black line) and its empirical (red crosses) counterpart (right). The comparison is much better for higher values of k_b than for small values due to the form of Eq. (21); see the discussion in the text.

position of the bead. Substitution of Eq. (17) into (8) yields

$$\frac{dx}{dt} = \frac{1}{\gamma}[\tau_{lot} - \tau_{\rm int}] + \frac{\kappa_b}{\gamma} z_b(t). \tag{18}$$

Here, $\tau_{\rm int}(x) = \partial G/\partial x$, takes into account the internal tension of the system Handle-RNA-Handle.

It is interesting to stress here that, within the range of the unstable region of the free energy (corresponding to the energy barrier in Fig. 2, for instance), the order of magnitude of the terms at the right-hand side of Eq. (18) are $\Delta\tau = \tau_{lot} - \tau_{\rm int} \sim 1 - 5$ pN for the deterministic term whereas for the noise we have $\kappa_b z_b \sim 0.7$ pN, meaning that the effect of bead fluctuations cannot be neglected. In addition, the left-hand side dictates that fluctuations have a characteristic time of the order of microseconds. These rough estimates indicate that the transition time between folded and unfolded states should be about two orders of magnitude larger, that is, milliseconds. These expectations are confirmed by the simulations shown in Figs. 2 and 3.

Hence, by comparing Eq. (18) with (11) we can identify $\xi_c(t) = \frac{\kappa_b}{\gamma} z_b(t)$ and, therefore, from Eqs. (13) and (16) we can infer the characteristic time t_c and the diffusion coefficient D of the process:

$$t_c = \tilde{\gamma}/\kappa_b \quad \text{and} \quad D = \frac{k_B T}{\gamma}\frac{\tilde{\gamma}}{\gamma}. \tag{19}$$

Given that $\tilde{\gamma} = 6\pi 10^{-6}$ pN/nm and $\kappa_b = 0.11$ pN/nm we estimate $t_c = 1.7 \times 10^{-4} s$. This value is two orders of magnitude smaller than the average residence time $t_r \simeq 10$ ms inferred from our simulations (see Figs. 2 and 3). Hence, our analysis agrees with previous theoretical analysis reported in Refs. [23,24].

Using these results it is possible to deduce an approximate expression for the Fokker-Planck equation for the probability density $p(x,t)$ corresponding to the process determined by Eq. (18). Following Ref. [21], a decoupling approximation scheme can be applied in the Stratonovich interpretation in order to obtain

$$\frac{\partial p}{\partial t} = \frac{\partial}{\partial x}\left\{-\frac{\Delta\tau}{\gamma}\, p + \frac{k_B T}{\gamma}\frac{\tilde{\gamma}}{\gamma}\frac{\partial}{\partial x}\left[\left(1 + \frac{\tilde{\gamma}}{\gamma}\frac{d}{dx}\frac{\Delta\tau}{\kappa_b}\right)p\right]\right\}. \tag{20}$$

In writing this equation we assumed a constant κ_b and used the definition $\Delta\tau = \tau_{lot} - \tau_{\rm int}$. The equilibrium stationary solution of Eq. (20) is given by the approximate expression,

$$p_{st}(x) \simeq \left[1 - \frac{1}{\kappa_b}\left(\frac{\tilde{\gamma}}{\gamma}\frac{d\Delta\tau}{dx} + \frac{(\Delta\tau)^2}{2k_B T}\right)\right]\frac{e^{-\frac{\tilde{G}}{k_B T}\frac{\gamma}{\tilde{\gamma}}}}{Z}, \tag{21}$$

where Z is the partition function and we have introduced the effective Gibbs free energy $\tilde{G}$,

$$\tilde{G} \equiv G - \tau_{lot} x. \tag{22}$$

It is convenient to point out that another approximation for calculating the stationary probability solution of the system's extension is by deriving the Fokker-Planck equation for the two-dimensional stochastic process defined by the vector $(x(t), z_b(t))$ and distributed by a bivariate probability density $f(x, z_b, t)$. The resulting stationary distribution $p_{st}(x)$ leads to quantitative results that do not differ appreciably from those obtained by Eq. (21); see Ref. [21].

In Eq. (21), the thermal energy felt by the system RNA is scaled with the factor $\tilde{\gamma}/\gamma$ that compares the characteristic dissipation coefficients of the bead ($\tilde{\gamma}$) and of the Handle-RNA-Handle system (γ) when subject to extensional deformations. The value of the effective friction coefficient γ can be estimated by following the approximation of Refs. [25,26], which is based on the Rousse model for polymers [27]. The basic physical idea is to consider the polymer as a sequence of monomers having spherical shape. Locally, each monomer vibrates and moves surrounded by the solvent. All the dissipation comes from this interaction since binding interactions are due to conservative (elastic) forces. The interaction between the monomer and the solvent is thus dissipative and proportional to the friction of each monomer with the fluid. Hence, the total friction coefficient of the polymer can be approximated in terms of the total number of monomers.

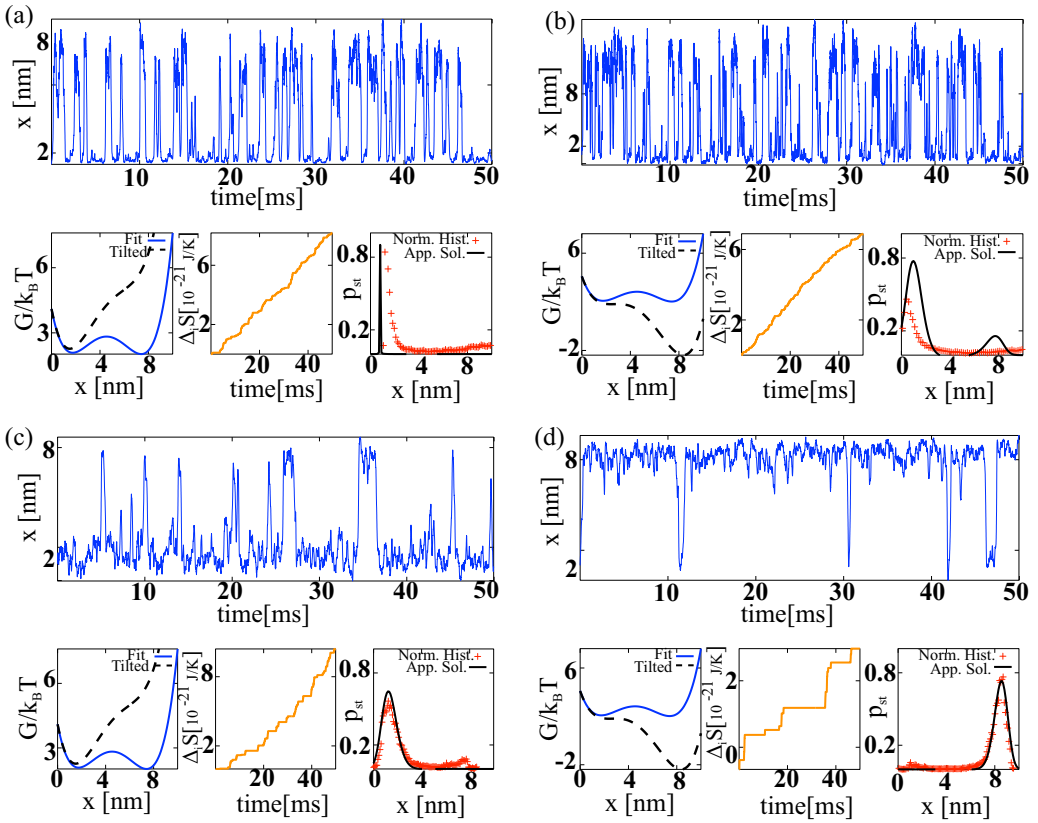

FIG. 3. (Color online) Simulation results for the extension x of the flexible system with $l_p/L = 0.12$ in tilted free energy profiles. The original profile corresponds to the same as Fig. 2(c). In (a) and (b) we present the results of the multiplicative noise case for which $c = 1.27$ and $c = -1.27$, respectively. In (c) and (d) the same tilts were considered in the case of additive noise. For reference, simulation results without tilting are those of Fig. 2(c). Noticeable is the fact that the average residence time in each state is reduced in the case of multiplicative noise and the fact that the tilt of the potential does not change considerably the hopping dynamics for the same initial condition. On the contrary, the hopping dynamics in the additive case varies considerably even for the same initial condition by increasing considerably the average residence times. Agreement between the theoretical and empirical distributions is very good in the additive case. The multiplicative case shows that the approximate Fokker-Planck approach fails to reproduce the corresponding distribution due to the fact that the parameter c is too large.

This approximation models well the friction of the polymer under quasilongitudinal deformations and predicts that the friction coefficient associated with deformations of an RNA molecule of about 3000–7000 base pairs is approximately of the order $\gamma \sim 6 - 14 \times 10^{-9}$ Kg s^{-1} or larger [25,26]. For friction coefficients of the bead about $\tilde{\gamma} \sim 2 \times 10^{-9}$ Kg s^{-1}, one may expect the ratio $\tilde{\gamma}/\gamma \sim 0.33 - 0.14$. These values are indicative that, in general, $\tilde{\gamma}/\gamma < 1$. Hence, energy transduction by elastic fluctuations of the system suggests that the effect of the effective friction coefficient γ of the RNA molecule (or the Handle-RNA-Handle system) should be to reduce the actual value of the thermal energy along the molecule. This result is interesting because it may imply that, if the elastic colored noise controls the hopping dynamics between the folded and unfolded states, then the intrinsic elastic noise dominates because of the presence of finite time correlations along the Handle-RNA-Handle system. We will show and discuss in the next section that this may be the case since simulations with a color noise source lead to a behavior of the system extension $x(t)$ that better resembles the experimental observations, essentially characterized by low fluctuations of $x(t)$ in the unfolded and folded states. On the contrary, the high thermal energy associated with direct thermal fluctuations make much more noisy the behavior of $x(t)$, as it will be shown in agreement with previous results in the literature [10].

The theoretical probability density (21) can be compared with the normalized histogram arising from simulations. Comparisons are shown in Fig. 2, showing in general a good agreement. We have to recall here that, for not too large time intervals, the time averaged histogram (the empirical probability distribution function) cannot be identical to the ensemble average given by the stationary solution of the Fokker-Planck equation.

B. Linear multiplicative colored noise

A more general approach to the coupling between the bead and Handle-RNA-Handle system dynamics should consider the feedback effect of the Handle-RNA-Handle system on the corresponding bead energy potential. This feedback effect can be modeled in first approximation in terms of multiplicative noise effects on the Handle-RNA-Handle system, for which the tilting and the shape of the corresponding free-energy profile will fluctuate. In this case, the force applied to the

Handle-RNA-Handle system can be expressed in the form,

$$\tau = \tau_{lot} + (1 - c\,x)\kappa_b z_b, \tag{23}$$

where c is the constant coupling the colored noise term with the linear term of the internal tension, corresponding to the feedback effect. The second term at the right-hand side of Eq. (23) is the already analyzed additive term, inducing a fluctuating tilting of the free energy. The third term in Eq. (23) is associated with shape deformations of the free energy landscape.

Introducing Eq. (23) in Eq. (18) we obtain the following stochastic equation:

$$\gamma \frac{dx}{dt} = \Delta\tau + g(x)\kappa_b z_b(t), \tag{24}$$

where we have introduced $g(x) = (1 - cx)$. Using one more time the decoupling approximation in the Stratonovich interpretation, we obtain the following approximate Fokker-Planck equation [21]:

$$\frac{\partial p}{\partial t} = -\frac{\partial}{\partial x}\left(\frac{\Delta\tau}{\gamma}\,p\right) + \frac{k_B T}{\gamma}\frac{\tilde{\gamma}}{\gamma}\frac{\partial}{\partial x}\left\{g\frac{\partial}{\partial x}\left[g\left(1 + \frac{\tilde{\gamma}}{\gamma}\frac{b}{\kappa_b}\right)p\right]\right\}, \tag{25}$$

where $b(x) = g\frac{d}{dx}(\Delta\tau/g)$ was defined for notation convenience. For weak feedbacks, $cx \ll 1$ and if $\Delta\tau'/\kappa_b < 1$, then the stationary solution of Eq. (25) can be written in a similar form as Eq. (21):

$$p_{st}(x) \simeq \frac{1}{Z}\frac{1}{g\left(1 + \frac{\tilde{\gamma}}{\gamma}\frac{b}{\kappa_b}\right)}\exp\left[-\frac{\tilde{G}}{k_B T}\frac{\gamma}{\tilde{\gamma}}\right], \tag{26}$$

where Z is the corresponding partition function. Comparison of the theoretical result with the empirical probability distribution is similar to that of the additive colored noise case, although it is more sensitive to κ_b.

The solutions (21) and (26) have a form similar to the stationary solution of the system when it is perturbed by additive noise. That is, the solution can be expressed in terms of an exponential of the reduced effective free energy multiplied by a correction term associated with the nonlinear elastic term. However, it is remarkable that in both the additive and multiplicative colored noise cases, the thermal energy is scaled by a factor proportional to the ratio: $\tilde{\gamma}/\gamma$. Thus, beside the fact that bistable distributions can be expected for this two-state model system, our results indicate that the inference of the free energy landscapes and the associated thermodynamic properties that can be obtained from them may strongly depend on the peculiarities of the experimental array.

IV. RESULTS FROM SIMULATIONS

We are considering the folding-unfolding dynamics of the system that arises when the Handle-RNA-Handle system is in the unstable region of the force-extension curve. In this region, the free energy has to be a bistable function of the extension whose two equilibrium configurations correspond to the folded and unfolded states. Hence, in first approximation the effective free energy can be modeled in the form of a fourth-order polynomial,

$$\tilde{G} = a_4 x^4 - a_3 x^3 + a_2 x^2 + (\tau_{\rm int} - \tau_{lot})x + a_0, \tag{27}$$

where the coefficients $a_i, i = 1, \ldots, 3$ and $\Delta\tau = \tau_{lot} - \tau_{\rm int}$ can be determined by fitting experimental data of the free energy profile for a given external tension τ_{lot} applied on the system. This polynomial approximation does not reproduce certain characteristics of the barrier of the free energy. However, it is a good first approximation for the purposes of this work.

Assuming a fourth-order polynomial for the free energy profile implies that the internal tension $\tau_{\rm int}$ in our evolution Eq. (18) is a third-order polynomial. Taking this last into account, the evolution Eq. (18) takes the final form,

$$\gamma \frac{dx}{dt} = -b_3 x^3 + b_2 x^2 - b_1 x + (\tau_{\rm int} - \tau_{lot}) + \kappa_b z_b(t), \tag{28}$$

where now the coefficients b_i whose coefficients are related to those of the free energy a_i by the relation $b_i = (i + 1)a_{i+1}$ for $i = 0,1,2,3$.

Equations (14) and (28) were solved numerically by using simultaneously the Euler-Maruyama approximation [28]. In order to illustrate the results from simulations, we have taken three representative cases reported in Ref. [10] for the conformation of the Handle-RNA-Handle system. These correspond to rigid, semiflexible, and flexible handles.

The criterion for determining the rigidity of the system comes from the effective elastic constant k_m in Eq. (14). Large values of the elastic constant ($k_m/k_0 \gg 1$) imply a rigid system, whereas small values ($k_m/k_0 \ll 1$) imply a flexible polymer. Values around the unity ($k_m/k_0 \simeq 1$) imply a semiflexible system where k_0 is a characteristic elastic constant of the system.

This criterion can be expressed by means of the aspect ratio l_p/L, between the persistence length l_p and the total length L of the polymer by the fact that both the elastic constant and the persistent length are proportional to the Young's modulus E: $k_m = E/L$ and $l_p = \epsilon_b/k_B T$, where $\epsilon_b = E\,I$ is the bending energy of the polymer and I is the moment of inertia. Thus, in first approximation we have $k_m = k_0 l_p/L$, where the characteristic constant is given by $k_0 = k_B T/I$. In view of the previous discussion, in the next we will consider the flexible system having $l_p = 0.6$ nm and $L = 5$ nm with $l_p/L = 0.12$; the near semiflexible system having $l_p = 70$ nm and $L = 25$ nm with $l_p/L = 2.8$; and the rigid system having $l_p = 70$ nm and $L = 10$ nm with $l_p/L = 7$.

In addition, we have to mention that the previous criterion may also be applied by considering that the first-order term in Eq. (28) represents the intrinsic elastic response of the Handle-RNA-Handle system, and thus that b_1 plays the role of an intrinsic elastic coefficient. For the cases shown in Fig. 2 (from rigid to flexible) $b_1 = 4.58$ pNnm^{-1} for Fig. 2(a); $b_1 = 3.72$ pNnm^{-1} for Fig. 2(b); and $b_1 = 2.64$ pNnm^{-1} for Fig. 2(c). The decreasing value from rigid to flexible is consistent with the criterion explained in the previous paragraphs.

A. Simulation with additive colored noise

Three realizations of the elastically induced hopping process for Handle-RNA-Handle systems having three different

rigidities are shown in Fig. 2. Each subfigure also shows the fit of the free energy profile from simulations of Ref. [10], the cumulative entropy produced as a function of time,

$$\Delta_i S(t) = \int \frac{d_i S}{dt} dt, \tag{29}$$

obtained from Eq. (7), and the comparison between the empirical probability density $p_{\rm emp}(x)$ (red crosses), and the corresponding theoretical prediction by Eq. (21) (black lines).

Our aim was to reproduce sequences of hopping events similar to those observed within intervals about 250 ms. An interesting feature emerges from the hopping dynamics shown in Fig. 2. For the case when the effective elastic constant is $\kappa_b = 0.11$, the dependence of the extension on time shows very clear transitions during the 250 ms, and internal basin fluctuations of the extension of about $\delta x \sim \pm 1.25$ nm. The magnitude of the internal basin fluctuations are considerably smaller than the ones corresponding to thermal white noise for the same number of transitions in the simulated time interval.

In addition, we observed the expected behavior when the intermediate free energy barrier between both configurations is large enough, that is, the same white noise amplitude acting on the bead leads to a much slower hopping dynamics between folded and unfolded states or even to a suppression of the hopping events. In this second case, the intensity of the white noise acting on the bead should be increased in order to reproduce transitions within the same time range. The consequence of this is that the fluctuations of the extension within each free energy well are increased. Similar effects arise when increasing or decreasing the value of the effective elastic constant k_b by increasing or decreasing the elastic constant k_{lot} of LOT. The stochastic hopping behavior is also shown in the inset representing the entropy produced as a function of time. When the energy barrier is larger than the thermal energy, then the entropy produced has an irregular staircase shape; see Fig. 2(a). For more noisy behaviors of the simulation output this staircase behavior is loosed; see Figs. 2(b) and 2(c). In all cases, however, the entropy produced per unit time was positive, reflecting the irreversible character of the folding-unfolding process [11]. Finally, we also stress that, in general, the comparison between the theoretical density and the empirical probabilities for each realization is better for the case of flexible and rigid systems than for semiflexible ones suggesting that the use of these configurations could be better for determining the thermodynamic parameters of the system.

B. Simulation with multiplicative colored noise

Simulations with multiplicative elastic noise lead to similar qualitative results as those already discussed for the additive elastic noise case. However, transitions are more frequent and intrabasin fluctuations are larger and more irregular. Hence, here we show results focused on the effect of the control parameter c appearing in Eq. (23). This parameter may be changed to regulate the tilt of the potential as it is shown in Fig. 3. Figures 3(a) and 3(b) correspond to the multiplicative noise case whereas Figs. 3(c) and 3(d) correspond to the additive case. The results shown in Fig. 3 should be compared with those of Fig. 2(c) where there is no appreciable tilting of the free energy.

The general expected result is that, depending on tilting of the free energy landscape, the folding dynamics has a preference for a particular configuration state, and the transitions between folded and unfolded states decease significantly in the same time interval. The corresponding probability densities should present a peak preferentially about the position of the global minimum of the free energy. From simulations we observe that this behavior is better reproduced in the additive noise case than in the multiplicative case. In the case of multiplicative noise the simulations are much more sensitive to the tilting of the free energy since the multiplicative character of the noise depends on the parameter c. This makes correlations stronger and therefore induces a drastic reduction of the residence time in the global minimum of the free energy. For such large tilts as used in Fig. 3, the comparison between the empirical probability and the theoretical prediction fails completely. This is in agreement with the hypothesis behind the derivation of the corresponding Fokker-Planck equation, requiring that the residence time should be much larger than the correlation time. These results strongly suggest that multiplicative nonlinear noise is not playing an important role in determining the folding-unfolding transitions reported in experiments.

In simulations with the multiplicative model we considered the case of the flexible system having $l_p/L = 0.12$ as in Fig. 2(c), with a tilting constant $c = 1.27\ \mathrm{pN/nm^2}$ in Fig. 3(a) and $c = -1.27\ \mathrm{pN/nm^2}$ in Fig. 3(b). The features of the single realizations are well reproduced in the additive case by the theoretical prediction for the stationary probability density approximation.

C. Free energy landscape and probability histograms

In view of the results of previous subsections, we will concentrate our discussion in the case of additive noise. A comparison between simulations (blue circles) and the corresponding approximate stationary theoretical distributions obtained from the ensemble description given by the Fokker-Planck equation (20) (lines) is shown in Fig. 4 for the Handle-RNA-Handle systems considered in Fig. 2; see the caption for details.

The first point to emphasize is shown in Fig. 4(a). One can observe a large difference between the distribution function associated with the pure white noise case (magenta dotted line) and the elastic noise (orange dash-dotted and red solid lines). The white noise case needs larger intensities to reproduce the hopping events between folded and unfolded states and therefore produces wider probability distributions than those associated with the elastic case. As a consequence of this fact, the maxima of the probability distributions are, in general, significantly smaller than those of the colored case. In addition, the distance between maxima of the distribution is smaller than in the elastic colored noise case. These differences are more pronounced for rigid and semiflexible Handle-RNA-Handle systems. The quantitative comparison between simulation histograms and theoretical distributions of the elastically induced transitions is good in all cases with the first-order approximated solution represented by the red solid line.

The results above discussed imply that the free energy profile of the Handle-RNA-Handle system is proportional to

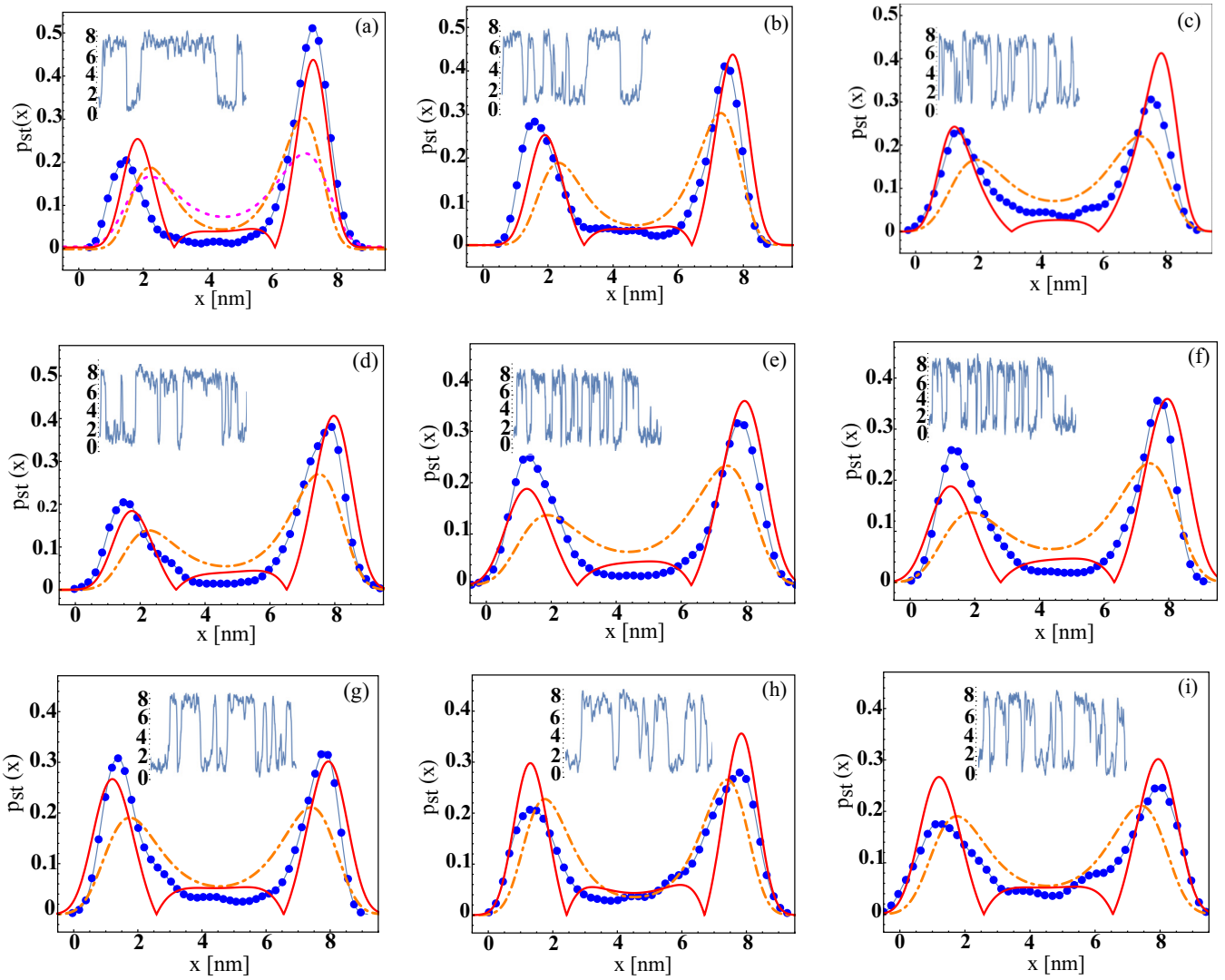

FIG. 4. (Color online) Comparison of the theoretical prediction of the stationary distribution function (lines) with the empirical single realization histogram (blue circles). (a)–(c) Correspond to a rigid Handle-RNA-Handle system with $\kappa_b = 0.11$ pNnm^{-2}, $l_p = 70$ nm, $L = 10$ nm, $l_p/L = 7$, and $\tilde{\gamma}/\gamma = 0.6, 0.5$, and 0.6, respectively. (d)–(f) Correspond to a semiflexible system with $\kappa_b = 0.11$ pNnm^{-2}, $l_p = 70$ nm, $L = 25$ nm, $l_p/L = 2.8$, and $\tilde{\gamma}/\gamma = 0.8, 1.0$, and 1.0, respectively. We increased the spring constant to $\kappa_b = 0.18$ pNnm^{-2} in the case of (e) and (f). (g)–(i) Correspond to a flexible system with $\kappa_b = 0.11$ pNnm^{-2}, $l_p = 0.6$ nm, $L = 5$ nm, $l_p/L = 0.12$, and $\tilde{\gamma}/\gamma = 0.7, 0.4$, and 0.6, respectively. We increased the spring constant to $\kappa_b = 0.18$ pNnm^{-2} in case (i). The magenta dotted line shown only in case (a) represents the theoretical distribution associated with the pure white noise case. The orange dot-dashed line is the zero order solution from Eq. (21), that is, the exponential term only and preserves the position of the maxima of the white noise case. However, the scaling of the free energy makes the maxima of the function more marked. The red solid line is the absolute value of the first-order solution containing the correction proportional to the derivative of the tension.

the logarithm of the empirical histogram only in the case when additive white noise drives directly the folding-unfolding process [11].

However, what follows from Eqs. (21) and (26) is that if the dynamics of the system is driven by elastic colored noise, additive or multiplicative, then the previous inference is incorrect, because the logarithm of the empirical histogram yields a nonlinear and nonhomogeneous second-order differential equation for the effective Gibbs free energy $\tilde{G}$, that is,

$$-k_B T \ln[p_{\rm emp}(x)] \simeq \tilde{G}(x)\frac{\gamma}{\tilde{\gamma}} - \ln\left|1 - \frac{\tilde{\gamma}}{\gamma}\frac{d}{dx}\frac{\Delta\tau}{\kappa_b}\right|, \qquad (30)$$

for Eq. (21) (we neglected the second-order contribution) and

$$-k_B T \ln[p_{\rm emp}(x)] \simeq \tilde{G}(x)\frac{\gamma}{\tilde{\gamma}} + \ln\left|g\left(1 + \frac{\tilde{\gamma}}{\gamma}\frac{b}{\kappa_b}\right)\right|, \qquad (31)$$

for Eq. (26). In these expressions one has to recall that $\Delta\tau(x) = \partial\tilde{G}/\partial x$ and $\Delta\tau' = \Delta\tau/dx$; see Eq. (22). These expressions show that the effect of additive and multiplicative noises, or their combination, may predict considerably different values of the free energy barriers and profiles ΔG associated with the folding-unfolding process, just as we have remarked in the previous section.

The other comparisons between the empirical, single-realization histogram and the theoretical prediction (ensemble)

of Eq. (21) shown in Figs. 4(b)–4(i) were done to study the effect of increasing the value of the spring constant of LOT and the value of the ratio $(\tilde{\gamma}/\gamma)$ between the friction constants of Eqs. (18) and (24), γ, with respect to that in Eq. (14), $\tilde{\gamma}$, for which the correlations between histograms and theoretical predictions are good. In three cases (see caption of Fig. 3 for details) we analyzed the effect of changing the value of the spring constant by increasing it moderately to $\kappa = 0.18$ pNnm^{-2}. In all cases, we have monitored the behavior of the bead position $z_b(t)$, predicted by Eq. (14) in such a way that it has to be consistent with the fluctuation dissipation theorem Eq. (15).

D. Interplay between colored and white additive noises

Finally, we have also performed simulations in which the evolution equation for the extension of the macromolecule is perturbed by both elastic and thermal fluctuations,

$$\gamma \frac{dx}{dt} = -b_3 x^3 + b_2 x^2 - b_1 x + (\tau_{in} - \tau_{lot}) + \kappa_b z_b + \xi. \tag{32}$$

Figure 5 shows the effect of adding direct thermal fluctuations on the hopping dynamics of the extension $x(t)$ of a semiflexible system. It is observed that Gaussian white noise should be very large (unphysical) in order to modify the number of transitions between folded and unfolded states. For acceptable values of the thermal white noise intensity the intrawell fluctuations become much more noisy. In practical terms, this effect may not be evident from experimental measurements, since the characteristic acquisition time should be much larger than the characteristic time of thermal fluctuations. This is why the top panel of Fig. 5 seems to better reproduce the shape of $x(t)$.

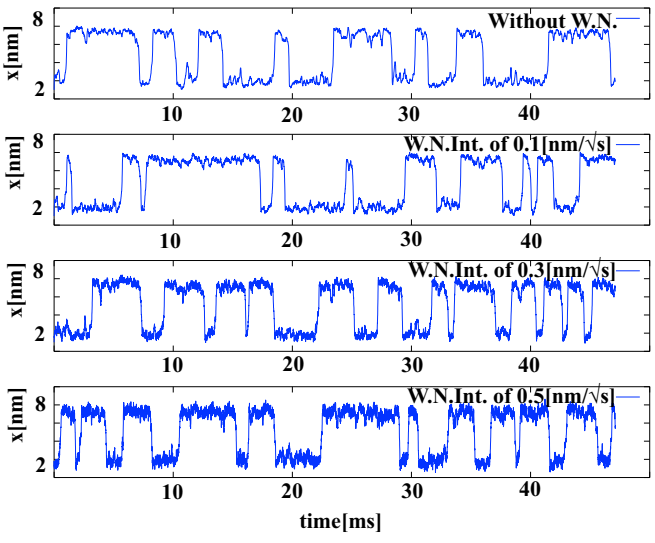

FIG. 5. (Color online) Numerical analysis of the effect of additive white noise on the elastically activated hopping dynamics of the Handle-RNA-Handle system. Hopping events between folded and unfolded states are dominated by the colored noise contribution. The Gaussian white noise contribution makes the output dynamics more noisy but the distribution of hopping events is not affected by this fact. The intensity of the white noise is indicated in each inset by the notation W. N. Int of 0.1, 0.3, and 0.5.

V. SUMMARY AND CONCLUSIONS

In this work, we have performed a detailed theoretical and numerical analysis of the possible influence of colored noise fluctuations of elastic origin on single-molecule stretching experiments. The stochastic hopping dynamics of the extension of the Handle-RNA-Handle system considered, coupled to the fluctuating dynamics of the dielectric bead of the LOT array was studied by using the irreversible thermodynamics of small systems. General nonlinear evolution equations for the variables of the system, RNA extension $x(t)$ and bead position $z_b(t)$ were deduced after calculating the entropy production associated with this hopping dynamics and using linear relationships between thermodynamic forces and fluxes.

The coupling between the bead and the Handle-RNA-Handle system implies that an elastic contribution to the fluctuating force entering into the evolution equation for the molecule extension is present. This contribution has intensities about 10% of the forces involved in the dynamics and modifies substantially the fluctuating dynamics of the extension in both the folded and unfolded configurations, when compared with the white noise case. The main feature is that critical oscillations between the folded and unfolded states are better defined because the magnitude of the extension fluctuations in each well is about $\delta x \sim 1.2$ nm. The intensity of the elastic fluctuations are proportional to the elastic constant of the LOT array and therefore follow an Ornstein-Uhlenbeck process. The colored nature of the resulting noise in both the additive and multiplicative cases studied here allowed us to find theoretical expressions for the corresponding stationary probability densities. The differences with respect to the white noise case were discussed in terms of the variances of the corresponding distributions.

The differences between the colored and white noises we have obtained suggest that the direct inference of the free energy landscape from the logarithm of the empirical probability density is valid only in the case of additive white noise. Clearly, this is the case because Gaussian white noise is an alternative way to represent equilibrium fluctuations. However, our theoretical and simulation results indicate that the critical oscillations observed in experiments do not necessarily correspond to an equilibrium fluctuation process. In fact, the two-peaked nature of the probability density function, much more marked for the elastic noise case, suggest the opposite.

In the colored noise case, the width of the folded and unfolded basins are narrower than that corresponding to the case of white noise, indicating that the experimental and simulation results may be extremely sensitive to entropic forces associated with chain conformations. On the other hand, the values of the maxima of the bimodal probability of the folded and unfolded states in the colored noise case differ considerably from those of the white noise case, thus suggesting that the energetic contribution to the dynamics is responsible for larger binding energies than those predicted by the white noise model (even differences about 40% for the maximum corresponding to the unfolded state in the left panel). The good agreement of the theoretical prediction and the empirical histogram, and their quantitative differences with respect to the thermal case suggest in its turn that the inference

of the free energy profile should be further analyzed under the scope of the present results, in particular in terms of the nonlinear differential Eqs. (30) and (31).

Finally, we have to stress that our simulations considering the influence of both elastic and thermal noises indicate that although the direct influence of thermal noise on the state of the molecules does not affect considerably the transition times of the hopping dynamics, it controls the width of the resulting empirical probability distribution via the internal basin fluctuations. A more accurate description of single molecule stretching experiments should be given in terms of the interplay of these two forces. Therefore, the results obtained in this work encourage theoretical work in order to find the corresponding Fokker-Planck equation and its stationary solution for the case of a system simultaneously perturbed by elastic and thermal noises.

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