Theory of force-extension curve for modular proteins and DNA hairpins

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We study a model describing the force-extension curves of modular proteins, nucleic acids, and other biomolecules made out of several single units or monomers. At a mesoscopic level of description, the configuration of the system is given by the elongations of each of the units. The system free energy includes a double-well potential for each unit and an elastic nearest neighbor interaction between them. Minimizing the free energy yields the system equilibrium properties whereas its dynamics is given by (overdamped) Langevin equations for the elongations, in which friction and noise amplitude are related by the fluctuation-dissipation theorem. Our results, both for the equilibrium and the dynamical situations, include analytical and numerical descriptions of the system force-extension curves under force or length control, and agree very well with actual experiments in biomolecules. Our conclusions also apply to other physical systems comprising a number of metastable units, such as storage systems or semiconductor superlattices.

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I. INTRODUCTION

Nowadays technological advances allow manipulation of single molecules with sufficient precision to study many mechanical, kinetic and thermodynamic properties thereof. Recent reviews of techniques used and results obtained in single-molecule experiments (SMEs) can be found in Refs. [1–3]. A typical outcome of a SME is a force-extension curve (FEC) characterizing the molecule elasticity and providing information about its processes of folding and unfolding [4][7]. The force-extension curves are different depending on whether the total length or the force are controlled. When the total length of the protein is used as a control parameter (length-control), the stretching transition is accompanied by a drop in the measured force and a sawtooth pattern is the typical force-extension curve [5][7][11]. When the force is the control parameter (force-control), unfolding of several or all single protein domains may occur at a constant value of the force [13]. Other questions are related to the rate at which the control parameter (length or force) sweeps the force-extension curve: depending on the loading rate, stochastic jumps between folded and unfolded protein states can be observed [1][8][10][12][13].

The analysis of the force vs. extension curves provides valuable information about the polyprotein, the DNA or the RNA hairpin. Let us consider atomic force microscope (AFM) stretching of a modular protein comprising a number of identical folds (modules or units) [5]. The typical value of the force \( F_c \) at which the unfolding takes place is related to the mechanical stability of the units: a larger value of the force is the signature of higher stability. Nevertheless, it should be stressed that the unraveling of a domain is a stochastic event and occurs for forces within a certain range. A second feature of the sawtooth FEC is the spacing between consecutive force peaks. This spacing is directly related to the difference of length between the folded and unfolded configurations of one unit. This is the reason that the peaks of the FEC of artificially engineered modular proteins are regularly spaced. A typical example is I27\(_8\), composed of eight copies of immunoglobulin domain 27 from human cardiac titin. The spacing between peaks for this protein is 28.4 ± 0.3 nm at an unfolding force of 204 ± 26 pN [5][7]. This length increment is found by fitting several peaks of the FEC with the worm-like chain (WLC) model of polymer elasticity [14][15]. More recently, force-controlled AFM experiments with an I27 single-module protein have been reported [16][17]. These experiments provide data free from the module to module variations that even an artificially engineered polyprotein has. Berkovich et al. have interpreted their results using a simple Langevin equation model that includes an effective potential with two minima for a range of the applied force [16].

The thermodynamics of pulling experiments is well established under both force and length control. For controlled force, the relevant thermodynamic potential is a Gibbs-like free energy, whereas for controlled length it is a Helmholtz-like free energy [10][18][19]. Interestingly, the sawtooth structure of the FEC of biomolecules is already present at equilibrium, as shown very recently in a simple model with a Landau-like free energy [20]. However, the control parameter in real experiments with biomolecules (force or length) changes usually with time at a finite rate [1][8][7][10][12][13][21]. Knowledge of these dynamical situations is not as complete as in the equilibrium case. Under force control, we can write a Langevin equation (or the associated Fokker-Planck equation) in which noise amplitude and effective friction are linked by a fluctuation-dissipation relation, as done in Refs. [16][22]. On the other hand, under length control, the situation is more complex: the force is no longer...
a given function of time but an unknown that must be calculated by imposing the length constraint. This has lead to the proposal of simple dynamical algorithms such as the quasi-equilibrium algorithm of Ref. [10]. While being successful in reproducing experimentally observed behavior, these algorithms do not correspond to the integration of well-defined evolution equations.

In some cycling experiments, the biomolecule is first pulled until it completely unfolds and Afterwards is pushed back with the same rate [1, 8, 10, 12, 13, 21]. The unfolding typically occurs at a force \( F_u \) that is larger than the refolding force \( F_r \). Therefore, some hysteresis is present and, moreover, the unfolding (refolding) force typically increase (decrease) with the pulling rate. A reversible curve in which \( F_u = F_r \) is only observed for a small enough rate. Some authors have claimed that this is a signature of irreversible non-equilibrium behavior and thus used these experiments to test non-equilibrium fluctuation theorems [12, 23, 24]. On the other hand, for a simple model for which only the force-controlled situation could be analyzed [25], it has been found that the observed behavior in biomolecules can be understood as the system sweeping a certain part of the metastable equilibrium region of the FEC that surrounds \( F_c \). In this way, the system is exploring metastable minimum of the system free energy landscape. One of the main goals of this work is to determine if this physical picture also holds for length-controlled experiments.

In this paper, we add two important ingredients of real biomolecule pulling experiments to a simple model with independent domains and Landau-like free energy whose equilibrium analysis is given in Ref. [20]. We add: (i) dynamical effects and (ii) interacting units. Dynamical effects are introduced by means of Langevin or Fokker-Planck equations, both under force and, most interestingly, length control. Therefrom, we can carry out a systematic investigation of the dynamical FEC, when the control parameter (force or length) is varied at a finite rate. The simplest way to introduce interaction between modules is via a harmonic potential trying to drive of them to global equilibrium. In this way, the creation of bubbles, that is, regions of unfolded modules inside regions of folded ones, has a free energy cost. This is expected to be most relevant for systems in which the unfolding/refolding of units is mainly sequential, as in the unzipping of DNA hairpins [1]. Interestingly, the complex and force-sensitive behavior of polyproteins observed in force-clamp experiments has been recently explained by sequential unfolding [20].

The main ingredients of our model are bistability of protein modules and, in the length-controlled case, a global constraint that introduces a long-range interaction among modules. These features are quite general in physics, as they appear in many different fields. For instance, many particle storage systems such as the storage of lithium in multi-particle electrodes of rechargeable lithium-ion batteries [27, 28], air storage in interconnected systems of rubber balloons [29], or voltage biased weakly coupled semiconductor superlattices [30–34]. Throughout the paper, the analogies and differences that arise in these different physical situations will be discussed.

The rest of the paper is as follows. The model we use is described in Section [I], in which we write down both the Langevin and the Fokker-Planck equations in Secs. [I A] and [I B] respectively. In Section [II] we investigate an ideal modular protein comprising many identical, non-interacting, units. In Sec. [II A] we show that the equilibrium FEC corresponding to our Landau-like double-well free energy has multiple branches. Statistical mechanics considerations determine the stability of the equilibrium branches for: (a) force-control in Sec. [II B] and (b) length-control in Sec. [II C]. We also consider dynamical situations when the control parameter (either force or length) varies at a finite rate. Section [IV] deals with a real chain, in which the nearest neighbor modules interact via an extra harmonic term. First, we study the equilibrium situation in Sec. [IVA], in which we show that the size of the branches is reduced, as compared to the ideal case. Sections [IV B], [IV C], and [IV D] analyze the changes that the dynamics brings to the equilibrium picture by considering deterministic dynamics, quenched disorder and finite temperature dynamics (thermal noise), respectively. Final remarks are made in Section [V] and the appendices deal with some technical aspects not covered in the main text.

II. MODEL

To be specific, let us consider AFM stretching of modular proteins: They are stretched between the tip of the microscope cantilever and a flat, gold-covered substance (platform), whose position is externally controlled. The forces acting on the molecule bend the cantilever which, in turn, determines the applied force with pN precision. See Fig. 1 of Ref. [7] for an idealized situation. In force-controlled experiments with a single module protein, the free energy of an extending protein comprises at least two distinct components, an entropic term that accounts for chain elasticity and an enthalpic component that includes the short-range interactions arising between the neighboring amino acids as the protein contracts [16, 17]. In a certain force range, these two components cause the single-module free energy to have two minima [16]: The enthalpic (entropic) minimum corresponds to the folded (unfolded) state of the domain.

The \( j \)-th module extends from \( x_j \) to \( x_{j+1} \), so that its extension is \( \eta_j = x_{j+1} - x_j \), \( j = 1, \ldots, N \). The configuration \( \eta = \{ \eta_j \} \) defines the polyprotein state at a mesoscopic level of description. When isolated, the free energy of the \( j \)-th unit is \( u(\eta_j; Y, \delta_j) \), a double-well potential whose minima correspond to the folded and unfolded states discussed above. \( Y \) is the set of relevant intensive parameters, like the temperature \( T \) and the pressure \( p \) of the fluid (thermal bath) surrounding our system. The
If all the linkers are identical, \( k_j \) accounts for the slight differences from unit to unit: \( \delta_j = 0, \forall j \), if all units are identical and thus no quenched disorder is present in the system.

As part of the tertiary structure of the polyprotein, modules are weakly interconnected by linkers in a structure-dependent way \cite{35}. It seems reasonable that this weak interaction acts on the unfolding/refolding time scale and tries to bring the extensions of the modules to a common value, corresponding to global mechanical equilibrium. For the sake of simplicity, we model the linkers as harmonic springs. Thus the system free energy \( A \) for a given configuration of module extensions \( \eta \) is

\[
A(\eta; Y) = \sum_{j=1}^{N} a(\eta_j; Y, \delta_j) + \sum_{j=1}^{N+1} \frac{k_j(Y)}{2} (\eta_j - \eta_{j-1})^2.
\]

(1)

If all the linkers are identical, \( k_j = k \) for all \( j = 2, \ldots, N \), and the elastic constants may depend on the intensive parameters. The coordinates for the platform and the tip of the cantilever are \( \eta_0 \) and \( \eta_{N+1} \), respectively. Therefore, the elastic terms proportional to \( k_1 \) (\( k_{N+1} \)) account for the interaction of the first (last) module of the chain with the platform (the tip of the cantilever). The length \( L \) of a polyprotein in a configuration \( \eta \) is

\[
L(\eta) = \sum_{j=1}^{N} \eta_j.
\]

(2)

Let us consider that external forces \( \pm F \) are applied to the ends of the modular protein. We have to add a term

\[
- F \sum_{j=1}^{N} \eta_j = F \xi_1 - F \xi_{N+1}
\]

to the free energy \( A(\eta) \). In this way, we obtain a Gibbs free energy \( G(\eta; Y, F) = A(\eta, Y) - FL(\eta) \),

\[
G(\eta; Y, F) = \sum_{j=1}^{N} g(\eta_j; Y, F, \delta_j) + \sum_{j=1}^{N+1} \frac{k_j(Y)}{2} (\eta_j - \eta_{j-1})^2,
\]

(3a)

\[
g(\eta_j; Y, F, \delta_j) = a(\eta_j; Y, \delta_j) - F\eta_j.
\]

(3b)

The experiments are carried out at either (i) force-control \( F = F(t) \) (ii) length-control \( L(\eta) = L(t) \), conditions. In case (ii), \( F \) is the unknown force required to have the prescribed length \( L(t) \), to be calculated by imposing the constraint.

### A. Langevin dynamics

The extensions \( \eta_j \) obey coupled Langevin equations with the appropriate thermodynamic potential. The friction coefficient and the amplitude of the white noise are related by a fluctuation-dissipation theorem. The source for both the friction and the stochastic force is the fluid the modules are immersed in, which is assumed to remain in equilibrium at temperature \( T \). We assume that the modules’ inertia can be neglected and thus their evolution equations are overdamped,

\[
\gamma_j \dot{\eta}_j = F - \frac{\partial}{\partial \eta_j} A(\eta; Y) + \sqrt{2T \gamma_j} \xi_j(t),
\]

(4a)

\[
\langle \xi_j(t) \rangle = 0, \quad \langle \xi_j(t) \xi_j(t') \rangle = \delta_{jj} \delta(t - t'), \quad j = 1, \ldots, N.
\]

(4b)

Here \( \gamma_j \) is the friction coefficient for the \( j \)-th module, and we measure the temperature in units of energy (\( k_B = 1 \)). In force-controlled experiments, \( F = F(t) \) is a known function, whereas in length-controlled ones we have \( L(\eta) = L(t) \), and \( F(t) \) is determined by imposing this constraint, which yields

\[
F = \frac{\gamma}{N} \left[ \frac{dL}{dt} + \sum_{j=1}^{N} \frac{1}{\gamma_j} \frac{\partial A(\eta; Y)}{\partial \eta_j} - \sum_{j=1}^{N} \frac{2T}{\gamma_j} \xi_j \right].
\]

(5a)

\[
\gamma^{-1} = \frac{1}{N} \sum_{j=1}^{N} \gamma_j^{-1}.
\]

(5b)

The parameter \( \gamma \) is an average friction coefficient. In the case of identical units, \( \gamma_j = \gamma, \forall j \). We split \( F \) in two terms, a “macroscopic term” \( F_{FP} \) and a “fluctuating term” \( \Delta F \), as follows:

\[
F = F_{FP} + \Delta F,
\]

(6a)

\[
F_{FP} = \frac{\gamma}{N} \left[ \frac{dL}{dt} + \sum_{j=1}^{N} \frac{1}{\gamma_j} \frac{\partial A(\eta; Y)}{\partial \eta_j} \right],
\]

(6b)

\[
\Delta F = - \frac{\gamma}{N} \sum_{j=1}^{N} \sqrt{\frac{2T}{\gamma_j}} \xi_j.
\]

(6c)

We prove in Sec. III B that \( F_{FP} \) is the force appearing in the flux term of the Fokker-Plack equation. Note that (i) for any \( N \), \( \langle \Delta F \rangle = 0 \) and then \( \langle F \rangle = \langle F_{FP} \rangle \), (ii) for large \( N \), \( \Delta F \) becomes very small since it tends to its zero average value. In force–extension experiments, the length is usually uniformly increased/decreased with time \( t \), \( dL/dt = \mu \) with a constant \( \mu \).

The boundary conditions for this chain are provided by giving relations for the coordinates of the cantilever tip and the platform, \( \eta_0 \) and \( \eta_{N+1} \). The simplest situation arises by assuming that the interaction between the protein and the platform/tip of the cantilever does not contribute to the free energy of the system, that is

\[
\eta_0 = \eta_1, \quad \eta_{N+1} = \eta_N.
\]

(7)

This boundary condition holds if the contribution due to the interactions with the platform/tip is negligible compared to the sum of the on-site terms and the elastic interaction between the modules of the protein.

It is convenient to render our equations dimensionless. We set the length unit \([\eta]\) equal to the difference between
the extensions of the two free energy minima of a single unit for a certain applied force. It is natural to adopt the critical force, at which the two minima are equally deep, as the unit of force, \([F] = F_c\). The parameters \([\eta] \) and \([F]\) depend on the specific choice of the double-well potential \(a(\eta; Y, 0)\). The free energy unit is then \([F][\eta]\). We select the time scale as \([\tau] = \gamma[\eta]/[F]\), where \(\gamma\) is the typical friction coefficient experienced by the units. The typical value of \(\gamma\) can be obtained from the value of the diffusion coefficient \(D = T/\gamma\) of a single module protein being stretched [17]. In principle, we introduce a new notation for the dimensionless variables, \(F^* = F/[F]\), etc. but, in order not to clutter our formulas, we drop the asterisks in the remainder of the paper.

**B. Fokker-Planck equation and equilibrium distributions**

In force controlled experiments, \(F(t)\) is a given function of time, and the set of Langevin equations (1a) is equivalent to the following Fokker-Planck equation for the probability density \(\mathcal{P}(\eta, t)\) of finding the system with extension values \(\eta = \{\eta_1, \ldots, \eta_N\}\) at time \(t\),

\[
\frac{\partial \mathcal{P}}{\partial t} = \sum_{j=1}^{N} \frac{1}{\gamma_j} \frac{\partial}{\partial \eta_j} \left[ G \frac{\partial \mathcal{P}}{\partial \eta_j} \right] + T \sum_{j=1}^{N} \frac{1}{\gamma_j} \frac{\partial^2 \mathcal{P}}{\partial \eta^2_j}. 
\]

(8)

where \(G = A - FL\), as given by Eq. (3a). If the force \(F\) is kept constant, Eq. (8) has a stationary solution, which is the statistical mechanics prescription,

\[
\mathcal{P}^\text{eq}(\eta) \propto e^{-G(\eta; Y, F)/T}.
\]

(9)

Therefore, the equilibrium values of the module extensions \(\eta^\text{eq}\) are the functions of \(F\) that maximize \(\mathcal{P}\) or, equivalently, minimize \(G\), that is, they verify

\[
\left. \frac{\partial G}{\partial \eta_j} \right|_{Y,F} = 0 \Rightarrow \eta_j = \eta_j^\text{eq}(Y, F), \quad j = 1, \ldots, N.
\]

(10)

If there is only one minimum, this is the equilibrium configuration. If there is more than one, the absolute minimum is the thermodynamically stable configuration, while the other minima correspond to metastable states in the thermodynamic sense. For each equilibrium configuration, either stable or metastable, the equilibrium value of the free energy \(G\) is

\[
G^\text{eq}(Y, F) = G(\eta^\text{eq}(Y, F); Y, F).
\]

(11)

Taking into account Eq. (10), we have

\[
\left. \frac{\partial G^\text{eq}}{\partial F} \right|_{Y} = - \sum_{j=1}^{N} \eta_j^\text{eq}(Y, F) = -L^\text{eq}(Y, F), \quad \left. \frac{\partial G}{\partial \eta_j} \right|_{Y} = 0, \quad j = 1, \ldots, N.
\]

(12)

which gives the equilibrium FEC under force control.

Let us consider now the length control. The correct Fokker-Planck equation follows from the first two moments of the extensions \(\eta\), taking into account that not all the extensions \(\eta\) are independent and that the force \(F\) is given by eq. (5a).

\[
\frac{\partial \mathcal{P}}{\partial t} = \sum_{j=1}^{N} \frac{1}{\gamma_j} \frac{\partial}{\partial \eta_j} \left[ (\frac{\partial A}{\partial \eta_j} - F_{FP}) \mathcal{P} \right] + T \sum_{j=1}^{N} \frac{1}{\gamma_j} \sum_{k=1}^{N} (\delta_{jk} - \frac{\gamma_j}{N \gamma_k}) \frac{\partial^2}{\partial \eta_j \partial \eta_k} \mathcal{P}. \quad (13)
\]

Here \(F_{FP}\) is given by Eq. (6a). If the length is kept constant, \(dL/dt = 0\), Eq. (13) has a stationary solution,

\[
\mathcal{P}^\text{eq}(\eta) \propto \delta(L(\eta) - L) e^{-A(\eta; Y)/T},
\]

(14)

as can be easily verified by inserting (14) into (13). This means that \(A\) is the relevant potential for the statistical mechanics description at equilibrium, as was expected. To obtain the equilibrium values for the extensions, we look for the minima of \(A\) with the constraint given by the delta function in (14), \(L(\eta) = L\). We have to introduce a Lagrange multiplier \(F\) and look for the minima of \(A - FL\), that is, the same minimization as in the force-controlled case. However, the Lagrange multiplier is an unknown that must be calculated at the end of the process by imposing the constraint, \(F = F(L)\). This Lagrange multiplier is, from a physical point of view, the force that must be applied to the system in order to have the desired length. The equilibrium extensions \(\eta^\text{eq}_j(L)\) are thus given by the solutions of

\[
\left. \frac{\partial A}{\partial \eta_j} \right|_{Y} = F; \quad j = 1, \ldots, N; \quad - \sum_{j=1}^{N} \eta_j^\text{eq}(Y, F) = L. \quad (15)
\]

The last equation gives the FEC, \(L = L(Y, F)\) or \(F = F(Y, L)\), from which we obtain \(\eta^\text{eq} = \eta^\text{eq}(Y, L)\). The thermodynamic potential \(A^\text{eq}\) is the Legendre transform of \(G^\text{eq}\) with respect to \(F\). In fact, the equilibrium value of \(A\), \(A^\text{eq}(Y, L) = A(\eta^\text{eq}(Y, L); Y)\), verifies that

\[
\left. \frac{\partial A^\text{eq}(Y, L)}{\partial L} \right|_{Y} = F. \quad (16)
\]

The proper variables for \(A^\text{eq}\) are the set of intensive parameters \(Y\) (temperature \(T\), pressure \(p\), ...) of the fluid in which the polyprotein is immersed) and the extensive length \(L\) [36], while the proper variables for \(G^\text{eq}\) are all intensive, \(Y\) and \(F\). In this sense, \(A^\text{eq}\) plays the role of Helmhotz free energy, while \(G^\text{eq}\) is the analogous of Gibbs free energy. It should be stressed that (i) however, different notations are found in the literature for these two thermodynamic potentials; (ii) as in the case of magnetic systems [37], there is a difference of sign with respect to the usual free energy terms with the pressure \(p\) and the volume \(V\).
III. THE IDEAL CHAIN

In this Section, we analyze the case of an ideal chain, in which the identical units do not interact either among themselves or with the cantilever/platform, $k_j = 0$ and $\delta_j = 0$ for all $j \neq 0$. We analyze the equilibrium situation and thus solve the minimization problems for the force-controlled and length-controlled cases of the previous section. We also investigate the dynamical situation arising in processes in which the force or length varies in time at a finite rate, and compare these dynamical FECs to the equilibrium ones.

A. Double-well potential. Equilibrium branches.

In order to keep the notation simple, we omit the dependence on the intensive parameters $Y$ of the free energy parameters. We consider the polynomial form, à la Landau, for the free energy [20]

$$A(\eta) = \sum_{j=1}^{N} a(\eta_j), \quad a(\eta) = F_c \eta - a \eta^2 + \beta \eta^4. \quad (17)$$

The parameters $F_c$, $\alpha$ and $\beta$ are all positive functions of the intensive parameters $Y$. The possible equilibrium extensions $\eta^{eq}$ are the minima of $a(\eta) - F \eta$, $a'(\eta^{eq}) = F$, or, equivalently,

$$- 2a \eta^{eq} + 4 \beta \left( \eta^{eq} \right)^3 = \varphi, \quad \varphi \equiv F - F_c. \quad (19)$$

We have introduced the notation $\eta^{eq}$ because Eq. (19) has three solutions in the metastability region, given by $|\varphi| = |F - F_c| < \varphi_0 = (2a/3)^{3/2} \beta^{1/2}$. We set the indexes by choosing $\eta^{eq} < \eta^{eq+} < \eta^{eq-}$. They depend on the force $F$ through $\varphi$ (and on the intensive variables $Y$ through $\{\alpha, \beta, F_c\}$). The extensions $\eta^{eq}(\varphi)$ and $\eta^{eq+}(\varphi)$ are locally stable because they correspond to minima of $a_j - F \eta_j$, while $\eta^{eq-}(\varphi)$ corresponds to a maximum and is therefore unstable. The curvatures at the folded and unfolded states are $\chi^{(i)}(\varphi) = a''(\eta^{eq}(\varphi)) = 12 \beta [\eta^{eq}(\varphi)]^2 - 2a$, $i = 1, 3$. Both curvatures (i) are positive in the metastability region and (ii) vanish at their limits of stability, $\chi^{(1)}(\varphi^{eq})$ at $\varphi = \varphi_0$ ($\varphi = -\varphi_0$).

The situation is similar to that analyzed by Landau [28] for a second order phase transition under an external field, with $\eta$ and $\varphi = F - F_c$ playing the role of the order parameter and the external field, respectively. At the critical force $\varphi = 0$, the stable equilibrium values of the extensions are

$$\eta^{eq} = - \eta^{eq-} = \left( \frac{\alpha}{2 \beta} \right)^{1/2}. \quad (20)$$

They are equiprobable, since $a - F \eta$ is an even function of $\eta$ for $F = F_c$, and $a^{eq} - F_c \eta^{eq} = a^{eq-} - F_c \eta^{eq-}$, where we have introduced the notation $a^{eq} = a(\eta^{eq})$, $a^{eq+} = a(\eta^{eq+})$. For $F \neq F_c$, the “field” $\varphi$ favors the state with $\text{sgn}(\varphi) = \text{sgn}(\eta)$. Therefore, in the metastability region $|\varphi| < \varphi_0$, we have the following picture: For $F < F_c$, the thermodynamically stable state is the folded one $\eta^{eq} < 0$ and the unfolded one $\eta^{eq} > 0$ is metastable. For $F > F_c$, the situation is simply reversed. On the other hand, the folded $\eta^{eq}$ (unfolded $\eta^{eq}$) state also exists for forces below (above) the metastability region $\varphi < - \varphi_0$ ($\varphi > \varphi_0$). In their respective regions of existence, both locally stable extensions $\eta^{eq}$ and $\eta^{eq-}$ are increasing functions of $\varphi$ (or $F$), since Eq. (18) implies that $\chi^{(i)}(\varphi)d\eta^{eq}/d\varphi = 1$. At zero force, one module can be folded or unfolded if $\varphi_0 > F_c$, while we have only the folded state if $\varphi_0 < F_c$.

Either module can be either folded or unfolded in the metastability region, and thus a FEC with $N + 1$ branches shows up, as seen in Fig. 1. The $J$-th branch of the $F - L$ curve corresponds to $J$ unfolded modules and $N - J$ folded ones, $J = 0, \ldots, N$. Since there is no coupling among the units, the equilibrium value of $A$ over the $J$-th branch is

$$A^{eq}_J = (N - J)a^{eq} + J a^{eq-}. \quad (21a)$$

The corresponding length is

$$L_J = (N - J)a^{eq} + J a^{eq-}. \quad (21b)$$

Both $A^{eq}_J$ and $L_J$ are functions of $F$ and the intensive parameters $Y$ through the equilibrium extensions. Eq. (21b) is the FEC, both for the force and length controlled cases. It is interesting to note that similar multistable equilibrium curves appear in quite different physical systems: from storage systems [27,29] to semiconductor superlattices [30,31]. For instance, see Fig. 3 of Ref. [27] and Fig. 6 of Ref. [28] for the chemical potential vs. charge curve in storage systems, and Fig. 8.13 of Ref. [33] for the current-voltage curve of a superlattice.

As discussed in the previous section, we have chosen $|F| = F_c = 1$ and $|\eta| = \eta^{eq} = \eta^{eq-} = 1$ as units of force and length. Using Eq. (20), $\beta = 2a$ and $\eta^{eq} = - \eta^{eq-} = 1/2$. Moreover, the folded state $\eta^{eq}$ is the most stable one at zero force. This means that the unstable state $\eta^{eq-}$ is closer to the metastable state $\eta^{eq}$ for the simple Landau potential we are using [39]. For the sake of concreteness, we take $\eta^{eq-} - \eta^{eq} = 0.9(\eta^{eq-} - \eta^{eq})$ at zero force, which leads to $\alpha = 273/2/1672 \approx 2.697787$ and $\varphi_0 = 91/2/836 \approx 1.083878$. It should be stressed that all the normalized plots in this section are independent of this particular choice of parameters. A more conventional definition of protein length could be to select at zero force (a) zero extension for the folded modules (b) the difference between the unfolded and folded configurations as the length unit. This ‘physical’ definition would give a nondimensional extension

$$u = \frac{\eta - \eta^{eq}(F = 0)}{\eta^{eq}(F = 0) - \eta^{eq-}(F = 0)}, \quad (22a)$$
It is worth recalling $\eta^{(3)} - \eta^{(1)} = 1$ in nondimensional units. The free energy (23) produces
$$\frac{d}{dF} (G_N^{eq} - G_0^{eq}) = -N \left( \eta^{(3)}(F) - \eta^{(1)}(F) \right) < 0, \quad \forall F,$$
consistently with Eq. (12). Then the basin of attraction of the completely folded branch is the largest one for $F < F_c$, whereas the completely unfolded branch has the largest basin of attraction for $F > F_c$. All the intermediate metastable branches with $J \neq 0, N$ are not “seen” by the system in a quasi-static process that takes infinite time to occur, see the top panel of Fig. 2.

B. Force control

In force-controlled experiments, the Gibbs free energy is the relevant thermodynamic potential because it appears in the equilibrium distribution (9). As discussed in Sec. III B, the stable state corresponds to the absolute minimum of $G$. All the units in our ideal chain are independent under force control. Therefore, by increasing quasi-statically the force, the equilibrium FEC (21b) is swept. Over the $J$-th branch with $J$ unfolded modules,

$$G_{j}^{eq} = (N - J)g^{(1)} + Jg^{(3)}, \quad g^{(i)} = a^{(i)} - F\eta^{(i)}.$$  \hspace{1cm} (23)

For $F < F_c = 1$ ($F > F_c$), the absolute minimum of $G$ corresponds to the folded (unfolded) state $\eta^{(1)}$ ($\eta^{(3)}$) and the system moves over the force–extension branch in which none (all) of the units are unfolded, $J = 0$ ($J = N$).

Unfolding is a first-order phase transition between these states that occurs at the critical force $F_c = 1$ defined by continuity of forces and of the Gibbs free energies, $G_N^{eq}\big|_{F_c} = G_0^{eq}\big|_{F_c}$. At $F_c = 1$, all the units unfold simultaneously. The length, that is a function of $F$ given by Eq. (12), has a discrete jump

$$\Delta L_c = L_N(F_c) - L_0(F_c) = N \left( \eta^{(3)}_c - \eta^{(1)}_c \right).$$  \hspace{1cm} (24)

For a real, non-quasi-static process, the simple equilibrium picture above is not realized. Depending of the rate of variation of the force and the strength of the ther-
In length-controlled experiments, the length constraint introduces a long-range interaction between the protein modules. The equilibrium probability of any configuration $\eta$ is now given by Eq. (14). Then the equilibrium configuration $\eta_{eq}$ is found by minimizing $A$ with the constraint (4), and the difference between values of $A^{eq}$ at adjacent branches in the $F - L$ diagram governs the stability thereof. The length $\ell_j$ at which there is a change in the relative stability of two consecutive branches, with $J - 1$ and $J$ unfolded units, is determined by the equality of their respective free energies $A^{eq}$. The corresponding forces $f_j = F_{j-1}(\ell_j)$ and $f^+_j = F_j(\ell_j)$ over the branches with $J - 1$ and $J$ unfolded units obey the system of two equations

$$A^{eq}_{j-1}f_j = A^{eq}_j|f^+_j, \quad L^{eq}_{j-1}|f_j = L^{eq}_j|f^+_j. \quad (26)$$

The force rips at $L = \ell_j$ are $N$ first-order equilibrium phase transitions because (i) the thermodynamic potential $A^{eq}$ is continuous at the transition, (ii) $F = \langle \partial A^{eq}/\partial L \rangle_T$ has a finite jump, from $f_j^-$ to $f_j^+$ at the $J$-th transition. In the top left panel of Fig. 3 we explicitly show $f_j^-$ and $f_j^+$. We have the following picture: As observed in Fig. 1, the branches $J - 1$ and $J$ coexist on a certain range of lengths. Inside this range, Eq. (16) implies

$$\left(\frac{\partial}{\partial L} [A^{eq}_j - A^{eq}_{j-1}] \right) = F_j(L) - F_{j-1}(L) < 0, \quad (27)$$

where we have used (15). At equal length values $L$, the force is larger on the branch with a smaller number of folded units, $F_j(L) < F_{j-1}(L), \forall J$. Therefore, $A^{eq}_{J-1} < A^{eq}_J$, and then the branch $J - 1$ is the stable one and $J$ is metastable for $L < \ell_j$. The situation reverses for $L > \ell_j$, and there are not more stability changes between these branches because $A^{eq}_j - A^{eq}_{j-1}$ decreases monotonically as a function of $L$, as given by (27). Each intermediate branch $(J = 1, \ldots, N - 1)$ is thus stable between $\ell_j$ and $\ell_{j+1}$, that is, between $f^*_j$ and $f^*_j$ (see top left panel of Fig. 3). A sawtooth pattern arises in the $F - L$ curve, with $N$ transitions between the $N + 1$ branches at lengths $\ell_1, \ldots, \ell_N$.
and (ii) $f^\pm_J$ increase with the number of unfolded units $J$ for moderate values of $N$. The equilibrium case is illustrated by Fig. 4. Interestingly, the increase with $J$ of the rips forces has been observed in modular proteins $^3$ $^7$ ($N \sim 10$), whereas the rips forces are basically independent of $J$ for nucleic acids experiments (larger $N$) $^1$ $^13$ $^21$.

IV. CHAINS WITH ELASTIC INTERACTIONS BETWEEN IDENTICAL MODULES

In this Section, we investigate the effect of the harmonic potential in Eq. (1) (proportional to $(\eta_j - \eta_{j-1})^2$), on the FECs. This term tends to minimize the number of “domain walls” separating regions with folded units from regions with unfolded units, as the domain walls give a positive contribution to the free energy that is proportional to their number. This elastic interaction is expected to be more relevant in experiments in which the unfolding/refolding of units is basically sequential, as in the case of unzipping/rezipping of DNA/RNA hairpins. The harmonic potential does not completely prevent the formation of “bubbles”, regions of unfolded units inside a domain of folded ones, but adds a free energy cost thereto. The same elastic interaction is responsible for the so-called depinning transition of wave fronts $^33$ $^43$ $^45$. The latter has been recently related to the experimentally observed stepwise unfolding of modular proteins under force-clamp conditions $^26$.

A. Equilibrium states

First, we consider the case in which there is no disorder, all $k_j = k$ and $\delta_j = 0$. The equilibrium extensions $\eta^{eq}$ solve the minimization problem in Eqs. (10) or (15),
that is,

\[ a'(\eta_j^{eq}) - F + k(2\eta_j^{eq} - \eta_{j+1}^{eq} - \eta_{j-1}^{eq}) = 0, \quad j = 1, \ldots, N. \] (28)

Alternatively, the extensions \( \eta_j^{eq} \) can be regarded as the stationary solutions of the evolution equations (2a) with zero noise. Again, in the length-controlled case, \( F \) is a Lagrange multiplier, calculated by imposing the constraint \( L = L(\eta) \). The equilibrium extensions may be found by solving numerically (25), but they can also be built analytically by means of a perturbative expansion in powers of \( k \), as we now show.

1. Pinned wave fronts for \( k \ll 1 \)

Substituting the expansion

\[ \eta_j^{eq} = \sum_{n=0}^{\infty} \eta_{j,n}^{eq} k^n, \quad j = 1, \ldots, N, \] (29)

into Eq. (28), we obtain

\[ a'(\eta_{j,0}^{eq}) = F, \] (30a)

\[ \chi_j \eta_{j,1}^{eq} = \eta_{j+1,0}^{eq} + \eta_{j-1,0}^{eq} - 2\eta_{j,0}^{eq}, \] (30b)

\[ \chi_j \eta_{j,2}^{eq} = \eta_{j+1,1}^{eq} + \eta_{j-1,1}^{eq} - 2\eta_{j,1}^{eq} - \frac{1}{2} \zeta_j (\eta_{j,1}^{eq})^2, \] (30c)

where

\[ \chi_j = a''(\eta_{j,0}^{eq}), \quad \zeta_j = a'''(\eta_{j,0}^{eq}). \] (31)

For \( k = 0 \) we recover the results of the previous section, Eq. (30a) is the same as Eq. (18). The number of “unfolded” units \( J \) having extensions \( \eta^{(3)} \) determines the equilibrium values of Helmholtz free energy \( A \), length \( L \) and Gibbs free energy \( G \) of the considered configuration, as given by eqs. (21a), (21b), and (23), respectively. There are \( N!/(J!(N-J)!) \) configurations yielding the same values of \( L, A \), and \( G \) for \( k = 0 \), a degeneracy that is partially broken at order \( k \) by the elastic interaction. If three consecutive units \((j-1, j, j+1)\) are in the same potential well (either folded or unfolded) for \( k = 0 \), then \( \eta_{j+1}^{eq} = 0 \) and the stationary extension of the \( j \)-th unit does not vary. Therefore, only the modules at the domain walls separating domains where \( \eta_j = \eta_j^{(1)} \) from others where \( \eta_j = \eta_j^{(3)} \) change their extension. At the domain walls,

\[ \eta_j^{eq} = \begin{cases} \eta_j^{(1)} + k \frac{\eta_j^{(3)} - \eta_j^{(1)}}{\chi_j^{(3)} - \chi_j^{(1)}} + O(k^2) & \eta_j^{eq} = \eta_j^{(1)} \\ \eta_j^{(3)} - k \frac{\eta_j^{(3)} - \eta_j^{(1)}}{\chi_j^{(3)} - \chi_j^{(1)}} + O(k^2) & \eta_j^{eq} = \eta_j^{(3)} \end{cases} \] (32)

The length of the folded (unfolded) unit is slightly increased (decreased), as observed in Fig. 5 for \( k = 1.615 \). Therewith, the second-order corrections in \( k \) are already very small. Thus, in the remainder of this section, we neglect \( O(k^2) \) terms, that is, we write all the expressions up to the linear corrections in \( k \). The equilibrium length and free energy for \( J \) unfolded units and \( M \) domain walls are,

\[ L_{J,M} = (N-J)\eta_j^{(1)} + J\eta_j^{(3)} \]
\[ + kM (\chi^{(3)} - \chi^{(1)}) (\eta_j^{(3)} - \eta_j^{(1)}) \chi_j^{(1)} \chi^{(3)}, \] (33a)

\[ G_{J,M} = (N-J)\eta_j^{(1)} + J\eta_j^{(3)} + kM \frac{(\eta_j^{(3)} - \eta_j^{(1)})^2}{2}. \] (33b)

Thus, each domain wall contributes \( k(\eta_j^{(3)} - \eta_j^{(1)}) (\chi_j^{(1)} - \chi_j^{(3)})^{-1} \) to the length and \( k(\eta_j^{(3)} - \eta_j^{(1)})^2/2 \) to the free energy. An equivalent Ising model may be introduced to describe these equilibrium configurations, see Appendix \( A \) The configurations with the fewest number of domain walls minimize the free energy \( G \). For the boundary conditions (7), the minimal configurations have a single domain wall for a given value of the number of unfolded units \( J \). The extension \( \eta_j^{eq} \) increases with \( j \) from \( \eta_j^{(1)} \) to \( \eta_j^{(3)} \), slowly across the sites inside either the folded and unfolded domains, and suddenly at the domain wall, see Fig. 4.

2. Stability analysis

The pinned wave front solutions in Fig. 5 are stable in a certain range of forces, as proven in the literature [33, 43–45]. Here, we investigate the stability for small \( k \), by looking at the second variation of the relevant thermodynamic potential. We have

\[ \delta^2 G = \frac{\delta^2 A}{2} = \sum_{j=1}^{N} a''(\eta_j^{eq}) (\delta \eta_j)^2 + \frac{k}{2} \sum_{j=1}^{N+1} (\delta \eta_j - \delta \eta_{j-1})^2, \] (34)
where $\delta\eta_j = \eta_j - \eta_j^{eq}$. Note that the second variations of $A$ and $G$ are identical because the term proportional to $F$ does not contribute to $\delta^2 G$. It must be stressed that the non-diagonal terms of the symmetric matrix corresponding to this quadratic form are of order $k$, namely $\partial^2 A/\partial \eta_j \partial \eta_{j+1} = \partial^2 G/\partial \eta_j \partial \eta_{j+1} = -k$, and they have not to be taken into account in our stability analysis.

Let us consider a domain of folded (unfolded) units, whose lengths are $\eta^{(1)}(\eta^{(3)})$ for the ideal chain with $k = 0$. Inside a domain of either folded or unfolded units, there is an additional positive contribution $2k$ to the diagonal terms $\partial^2 A/\partial \eta^2_j$, so that stability is reinforced. Instability may arise at the domain walls, where

$$\frac{\partial^2 A}{\partial \eta^2_j} = \chi^{(i)} + k \left[ 2 - \frac{\zeta^{(i)}(\eta^{(3)} - \eta^{(1)})}{\chi^{(i)}} \right], \quad i = 1, 3. \quad (35)$$

Consistently with the notation introduced in Eq. (31), $\zeta^{(i)} = a''m(\eta^{(i)}) = 24 \beta \eta^{(i)}$, $i = 1, 3$. Then $\zeta^{(1)} < 0 < \zeta^{(3)}$ because $\eta^{(1)} < 0 < \eta^{(3)}$. The first and last branch of the FEC correspond to all-folded and to all-unfolded modules, respectively. Their configurations do not involve domain walls and therefore $\partial^2 A/\partial \eta^2_j = \chi^{(i)}$ for them, as in (35) with $k = 0$. These branches are stable until $\chi^{(i)} = 0$ at the extrema of $a'(\eta^{eq})$. In contrast to this, the other FEC branches have configurations with one domain wall and the linear corrections in Eq. (35) cause $\partial^2 A/\partial \eta^2_j$ to vanish for intermediate elongations between the extrema of $a'(\eta^{eq})$. As the limit of stability of the FEC branches is given by the condition $\partial^2 A/\partial \eta^2_j = 0$, this reduces their size. This reduction in the branch size with $k$ is clearly observed in Fig. 6. We further illustrate this result in Fig. 7 where we plot the second derivatives of the on-site potential at the domain wall, both for $k = 0$ and with the linear correction in $k$ (only for the folded unit at the domain wall, the curves for the unfolded unit are just the symmetrical ones with respect to $F_c = 1$).

![Figure 5](image1.png)

**FIG. 5.** Stable stationary wave front with increasing profile from $u^{(1)}$ to $u^{(3)}$ (corresponding to $\eta^{(1)}$ and $\eta^{(3)}$, respectively) pinned at a particular point $j = J$ of an infinitely long chain, for $k = 1.615$. The specular reflection of this pinned wave with respect to the center of the chain $j = N/2$ gives a pinned wave with decreasing profile from $\eta^{(3)}$ to $\eta^{(1)}$.

![Figure 6](image2.png)

**FIG. 6.** FECs for a system with $N = 8$ modules. (Top) Stable stationary branches for $k = 0.055$, quite similar to those for $k = 0$ (see Fig. 1). (Bottom) Stable stationary branches, each corresponding to a wave front pinned at a different site $j = J, J = 1, \ldots, 8$, for $k = 0.55$. The completely folded and unfolded branches are basically unchanged, but the size of the intermediate branches is considerably reduced. Here $L$ refers to the physical length (22b) that vanishes at $F = 0$.

### B. Deterministic dynamics

As the interacting chain is more complex than the ideal one, we start by neglecting thermal noise. This corresponds to the so-called deterministic (or macroscopic) approximation of the Langevin equation [46]. Alternatively, this can be presented as solving the dynamical equations [4] at $T = 0$. In a later Section, we will consider the changes introduced by a finite value of the temperature. Borrowing the usual terminology in classical mechanics, we refer to slow processes at $T = 0$ as *adiabatic*, as they can no longer be regarded quasi-static because ergodicity is broken.

In Fig. 8 we plot two such processes. In the first one (top panel) we increase the length adiabatically in a stepwise manner, at each value of the length the system relaxes for a time $\Delta t$, after which the length is increased in $\Delta L$. We have chosen $\Delta L = 0.2$ and $\Delta t = 300$, for $k = 0.5$. As compared to the equilibrium branches...
in Fig. 6 we observe that the $J$-th branch is swept as long it is locally stable, that is, until we reach the maximum value of the force $F_{J,\text{max}}$ at which $\delta^2 A$ in (34) is no longer positive definite. Then the completely unfolded branch $J = 0$ is swept to a higher force than all the intermediate branches: Its size is not reduced with respect to the $k = 0$ case and $F_{0,\text{max}} > F_{J,\text{max}}$, $J = 1, \ldots, N - 1$, as discussed in Sec. IV A 2. In the force-controlled case (bottom panel), we first increase the force adiabatically from $F = 0$. The system moves over the branch of folded units, $J = 0$, until it reaches the maximum thereof, $F_{0,\text{max}}$, at which the length jumps by $\Delta L = N[\eta_j^{(3)}(F_{0,\text{max}}) - \eta_j^{(0)}(F_{\text{max}})]$ to the completely unfolded branch where $\eta_j = \eta_j^{(3)}(F_{\text{max}})$ for all $j$. If the force is now adiabatically decreased, the system moves over the branch of unfolded units, $J = N$, until the force reaches its minimum possible value and the system jumps back to the completely folded branch. Thus, for both length-controlled and force-controlled conditions, the largest possible hysteresis cycles appear, similar to the ones obtained in storage systems, see Fig. 5 of Ref. [27] or Fig. 7 of Ref. [28].

C. Influence of quenched disorder

The units comprising a chain are not identical and therefore their on-site double-well potentials $a(\eta_j)$, their friction coefficients $\gamma_j$ and the spring constant between modules $k_j$ may depend on $j$. These considerations are much more important for DNA or RNA hairpins than for modular proteins, whose units have been artificially engineered to be as similar to each other as possible. In the equivalent experiment to find the current-voltage curves of superconductor superlattices, quenched disorder arises from fluctuations of the doping density at different wells [30, 31]. Including the natural variation in the free energy parameters amounts to adding quenched noises to them. To be concrete, we consider a potential whose strength depends on a random number $\delta_j$:

$$a'(\eta_j; \delta_j) = (1 + \delta_j)a'(\eta_j, \delta_j = 0).$$

which are i.i.d. random variables uniformly distributed on an interval $[-\beta, \beta]$ ($\beta < 1$).

Quenched disorder modifies both the stability of the FEC and the dynamics of the chain. When we depict the solutions corresponding to a wavefront pinned at particular locations as in Fig. 6, the presence of disorder moves the solution branches up and down and affects the dynamical behavior of the system. We show a hysteresis cycle under length-controlled conditions in the top panel of Figure 9. We have used a large disorder ($\beta = 0.5$) which produces large variations in the length and height of the high-energy states, as shown by the shaded areas. Our results for the high-energy states are consistent with Ref. [27] as well as with a recent numerical study which suggests that the degree of disorder has an important role in the observed behavior [32].
FIG. 9. FEC for a DNA hairpin as in Figure 5 but with $N = 40$ and disorder as in Eq. (36) (strength of the potential with $\beta = 0.5$ and $k = 1$). (Top) Hysteresis under length controlled conditions. The upper (lower) curve corresponds to adiabatically increasing (decreasing) length, with a rate $|dL/dt| = 1.2 \times 10^{-3}$ or smaller. (Bottom) Hysteresis under force-controlled conditions. Similarly, the upper (lower) curve corresponds to adiabatically increasing (decreasing) force, with a rate $|dF/dt| = 3 \times 10^{-3}$ or smaller. In the plots, $L$ is the physical length introduced in Eq. (22b).

of the branches. Under force-controlled conditions, up and down sweeping the FEC, we obtain the much wider hysteresis cycles of the bottom panel of Fig. 9. Since the disorder changes the length and size of the force-extension branches, additional steps are seen in the hysteresis cycles, as compared to the case of identical units.

D. Influence of thermal noise

In the last Section, we considered the effect of quenched disorder, but we still had zero temperature. Thermal noise allows random jumps between stable branches, provided the system has sufficient waiting time to escape the corresponding basins of attraction. As the control parameter (force or length) changes more slowly, the behavior of the system approaches the corresponding equilibrium statistical mechanics curve.

Let us first consider length-controlled simulations. For an ideal biomolecule with identical modules, at $T = 0$ adiabatic sweeping the FEC produces hysteresis cycles similar to the ones shown in Fig. 3 for a very low temperature. For finite temperature, (i) the size of the hysteresis cycles depends on the sweeping rate and becomes smaller as the rate decreases; (ii) there appear random jumps between stable branches that correspond to the same extension. Both effects have been observed in experiments with DNA hairpins, for which noise is much more important than in the case of modular proteins [8, 10, 12, 13]. Also, some branches are not swept and the distinction between different branches is blurred, as shown in the top panel of Fig. 10. For a similar situation in semiconductor superlattices, see Fig. 2 in Ref. [31], which shows a current-voltage curve for a sample comprising 40 periods of 9nm wide GaAs wells and 4nm wide AlAs barriers. It is also interesting to note that there is always some “intrinsic” hysteresis in the last (first) rip of the FEC, even for the lowest rate for which a perfect reversible behavior was obtained in the ideal case. This behavior has been observed experimentally in the unzipping/rezipping of DNA, see Fig. 1C and Fig. S4 of Ref. [21], and also in superlattices, see Fig. 1 of Ref. [30] and Fig. 1 of Ref. [34]. As explained in Section IV A 2, the FEC branch size is reduced in the non-ideal case ($k \neq 0$) except for
the first and last branches whose configurations do not possess a domain wall. Then the non-zero interaction between neighboring modules makes the metastable regions in the first (completely folded) and the last (completely unfolded) branches wider than the rest.

In the force-controlled simulations, the effect of a finite temperature is shown in the bottom panel of Fig. 10. We observe a behavior similar to that in Fig. 2 for the ideal chain, and also to the one found in other models [25, 40]. The physical picture is completely consistent with the experimental findings in nucleic acids [13].

V. FINAL REMARKS

We have proposed a biomolecule model that includes an on-site quartic double-well potential and an elastic harmonic interaction among its modules in the free energy thereof. Despite its simplicity, it captures the main features of FECs in real biomolecules while allowing us to identify the main physical mechanisms and to keep a mathematically rigorous approach. This can be done in equilibrium but also for the dynamics, for which we have written the relevant Langevin (or Fokker-Planck equations). It should be stressed that the Fokker-Planck equation for the length-controlled case is not trivial, since the force $F$ appearing in the Langevin equation is an unknown that must be calculated by imposing the length constraint. The relevant thermodynamic potential, Gibbs-like (Helmholtz-like) for the force-controlled (length-controlled) case, has been shown to be the stationary solution of the Fokker-Planck equation.

Equilibrium FECs show multistability in a certain range of forces: There are multiple FEC branches corresponding to different number of folded/unfolded units. Under force-controlled conditions, there is an equilibrium phase transition between the all-modules-folded to the all-modules-unfolded, the lengths across the jump being determined by continuity of force and Gibbs free energy. Under length-controlled conditions, there appears a saw-tooth FEC consisting of a number of branches with force jumps between them in which the number of unfolded modules differs by one. The forces across the jump are determined by continuity of length and Helmholtz free energy.

Dynamical FECs are obtained when the control parameter (either the force $F$ or the length $L$) is changed at a finite rate: Some hysteresis is present and the unfolding (refolding) forces increase (decrease) with the rate, as observed in experiments [7, 8, 10, 12, 13, 23, 47]. Assume that the process is slow over the time scale characterized by the friction but fast as compared to the Arrhenius time scale for surpassing the energy barrier separating the folded and unfolded state. Then the size of the hysteresis cycle is maximum and it approaches the deterministic case. Only when the variation of the control parameter is slow over this Arrhenius time scale, the process can be considered quasi-static and the equilibrium FECs are recovered. Our results show that, in these elasticity experiments, biomolecules display what may be called a “metastable equilibrium behavior”. They follow stationary FEC branches that can be obtained out of the equilibrium solution of the Fokker-Planck equation, and dynamic out-of-equilibrium excursions do not depart too much from them. The hysteresis cycles, completely similar to those observed in real experiments, stem from equilibrium multistability: At the highest loading rates, the system is not able to reach the absolute minimum but sweeps a certain part of the metastable region (the narrower the smaller the rate is) of the equilibrium free energy landscape. There are techniques to obtain single molecule free energy differences from time-dependent driving about hysteresis cycles [8, 10, 12, 23]. In addition, the complete single molecule free energy landscape can be obtained using model-dependent algorithms [24]. Although there is some evidence of glass-like behavior in force-clamp experiments with proteins [45], hysteresis in these unfolding/refolding experiments seems to be quite different from the more complex out-of-equilibrium hysteresis of glassy systems in cooling/heating cycles. When cooled down to low temperatures, glassy materials depart from the equilibrium curve and end up in a far from equilibrium state; when reheated, they return to equilibrium approaching a normal curve, which typically overshoots the equilibrium one [19, 53].

We have also discussed in detail the role of the interaction between neighboring units of the chain. The main effect of this interaction is the reduction of the width of the of the metastability region. When the elastic interaction is absent, all the configurations with the same number $J$ of unfolded units have the same free energy. This “entropic term” is reduced when the elastic interaction is taken into account, since the free energy also depends on the number of domain walls separating regions of unfolded and folded units and the configuration with only one domain wall (pinned wave front) is favored. From a physical point of view, this decrease is responsible for the reduction of force fluctuations, which are at the root of the width of the metastability region. Thus, we expect that the same behavior will be present for more realistic interaction potential between modules. In real biomolecules for which their on-site potentials and number of modules are similar, a smaller size of the rips may be linked to a stronger interaction between the neighboring units. The relevance of the interaction between units is also clearly shown by the fact that the metastability regions in the first and last branches are wider than those of the intermediate ones. This leads to the existence of some “intrinsic” hysteresis in the first/last force rips of the FEC under length-controlled conditions, even for very low pulling rates, close to the quasi-static limit. Interestingly, this effect has been reported in experiments with DNA molecules, see for instance the FECs in Fig. 1C (reziping) and Fig. 5 (unzipping) of Ref. 21. In the unzipping (reziping) experiment, the physical reason is the “extra” free energy cost $k(\eta^{(3)} - \eta^{(1)})^2/2$ for creat-
ing (removing) the domain wall separating the folded and unfolded regions of the molecule. As explained in Section IV A 2, this produces a reduction in branch size for all FEC branches except for the first and last ones whose configurations lack domain walls. This causes the metastable regions in the first (completely folded) and the last (completely unfolded) branches to be wider than the rest. Thus, the existence or not of this intrinsic hysteresis may be used to discriminate the importance of the coupling between units. For instance, compare Fig. 1 of 34 (or of 30) to Fig. 2 of 31 for the current-voltage curve obtained in the analogous experimental situation in semiconductor superlattices.

Many of the main characteristic behaviors observed here: multistability (multiple branches for a certain region of parameters like those in Fig. 1), the associated sawtooth FECs for length-controlled experiments, hysteresis effects when the control parameters are changed at a finite rate, etc. also occur in quite different physical situations, such as particle storage systems 27 29 and weakly coupled semiconductor superlattices 30 34. This analogy stems from the following common feature: all these systems comprise a number of similar bistable units whose individual states may be determined by a long-range interaction introduced by a global constraint (total charge 27 29, fixed voltage bias 30 34). Of course, fine-detail differences appear in the observed behavior in each physical situation, depending on the relevance of non-ideal effects, such as interactions among modules, quenched disorder, or the thermal noise considered here. For instance, the maximum size hysteresis cycles, basically identical to the deterministic case, have been observed in Refs. 27 28 for storage systems. This seems to indicate a lesser relevance of fluctuations in the latter.

The static and dynamic behaviors of biomolecules described here do not differ qualitatively from hysteretic behavior in static current–voltage curves of voltage biased semiconductor superlattices. The latter systems are definitively out-of-equilibrium: electrons are continuously injected and extracted from contacts, and their behaviors include time-periodic and chaotic oscillations besides hysteretic behavior 32. Nonlinear charge transport in superlattices cannot be described with the free-energy scaffolding available for biomolecules. Instead, discrete drift–diffusion models based on sequential tunneling between neighboring quantum wells are used 32 33. Nevertheless, the present paper shows that the methodology developed for these discrete systems can be adapted to describe FECs of biomolecules. As experiments with semiconductor superlattices are much more controllable than those with biomolecules, it would be interesting to see what the interpretation of measurements given in Refs. 8 10 12 23 24 produces in the superlattice case.

According to the above discussion, our main conclusions are quite general. They are applicable not only to biomolecules but to any physical system composed of repeated similar bistable units. Of course, we need naming appropriately variables for each relevant physical situation. For instance, force-extension curves must be replaced by chemical potential-charge ones in storage systems 27 28 or by current-voltage curves in semiconductor superlattices 32 33. Depending on the system, some of the necessary experiments are not yet available. For instance, there are no precise current-controlled experiments on semiconductor superlattices. Thus our investigations open new interesting perspectives for experimental research in these fields.

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Appendix A: Equivalent Ising model for the free energy minima

We can write down the length and Gibbs free energy in an Ising-like manner. Let us assign a spin-down variable to the folded units, so that \( \sigma_j = -1 \) if \( \eta_{j,0}^{eq} = \eta^{(1)} \), and an spin-up \( \sigma_j = +1 \) to the unfolded ones, with \( \eta_{j,0}^{eq} = \eta^{(3)} \). The number of unfolded units and domain walls are

\[
J = \sum_{j=1}^{N} \frac{1 + \sigma_j}{2}, \quad M = \sum_{j=1}^{N-1} \frac{1 - \sigma_j \sigma_{j+1}}{2}. \quad (A1)
\]

Except for an additive constant, the free energy becomes

\[
G^{eq}(\sigma) = -H \sum_{j=1}^{N} \sigma_j - \Xi \sum_{j=1}^{N-1} \sigma_j \sigma_{j+1} + O(k^2), \quad (A2)
\]

an Ising system with an external field \( H \) and ferromagnetic nearest neighbor coupling \( \Xi \) given by

\[
H = \frac{g^{(3)} - g^{(1)}}{2}, \quad \Xi = \frac{[\eta^{(3)} - \eta^{(1)}]^2}{4} > 0. \quad (A3)
\]

Interestingly, a similar expression for the free energy was proposed in Ref. 54. The sign of \( H \) determines which minimum of the Gibbs free energy \( g(\eta) \) is deepest, \( \eta^{(1)} \) or \( \eta^{(3)} \); at the critical force \( F_c = 1 \), that is, \( H = 0 \), they are equally deep. The ferromagnetic coupling \( \Xi \propto k \) favors the configurations with domains of parallel spins and thus a minimal number of domain walls for a given number of unfolded units \( J \). Then \( M = 0 \), when all the units are either folded or unfolded, or \( M = 1 \), when there are both folded and unfolded units, produce the minimum free energy.
Given (A1), the length of the system at equilibrium is

$$L^a(\sigma) = \frac{N}{2} \left[ (n^{(1)} + n^{(3)}) + (N - 1) \Delta \right] + \frac{n^{(3)} - n^{(1)}}{2} \sum_{j=1}^{N} \sigma_j - \Delta \sum_{j=1}^{N-1} \sigma_j \sigma_{j+1}. \quad (A4)$$

where

$$\Delta = \frac{k \left[ \chi^{(3)} - \chi^{(1)} \right]}{2 \chi^{(1)} \chi^{(3)}}. \quad (A5)$$

The parameter $\Delta$ can be positive or negative. For the simple quartic potential we are considering, $\Delta = 0$ at the critical force $F_c = 1$, $\Delta > 0$ for $F < F_c$, and $\Delta < 0$ for $F > F_c$.

We have not considered here the quadratic corrections, proportional to $k^2$, which only affect sites at the domain walls and their nearest neighbors. In this equivalent Ising description, they (i) change the first order coupling constants $\Xi$ and $\Delta$, and (ii) introduce a second-nearest-neighbor interaction. Similarily, by taking into account higher order corrections, up to order $k^n$, we get an Ising model with longer-ranged interactions up to the $n$th-nearest-neighbors.

**Appendix B: Lyapunov function for the deterministic dynamics in the length-controlled case**

Unlike the Gibbs free energy $G$ in the force-controlled case, the Helmholtz free energy $A$, as given by Eq. (4), is no longer a Lyapunov function of the zero-noise dynamics under length-controlled conditions with a known length dependence $L(t)$. However,

$$\dot{A}(\eta) = A(\eta) + \sum_{j=1}^{N} \left[ \frac{k}{2} (\eta_{j+1} - \eta_j)^2 - \eta_j \left( \sum_{k=1}^{N} a'_{(\eta_k)} + \frac{dL}{dt} \right) - \frac{a_{(\eta_j)} - a_{(\eta_j')}}{N} \right]. \quad (B1)$$

is a Lyapunov function in this case. In fact, the governing nondimensional equations can be written as

$$\frac{d\eta_j}{dt} = -\frac{\partial}{\partial \eta_j} \dot{A}(\eta), \quad (B2)$$

after eliminating $F$ by means of Eq. (5a). Then

$$\frac{d}{dt} \dot{A}(\eta) = -\sum_{j=1}^{N} \left[ \frac{\partial}{\partial \eta_j} \dot{A}(\eta) \right]^2 \leq 0.$$

Also,

$$\dot{A}(\eta) \geq N \min_u \left[ a(u) - Fu - \frac{a(u) - ua'(u)}{N} \right]$$

for

$$F_m < F = \frac{1}{N} \sum_{j=1}^{N} a'_{(\eta_j)} + \frac{1}{N} \frac{dL}{dt} < F_M.$$
In biomolecules at zero force, the folded state is much more localized than the unfolded state but this feature cannot be reproduced with the simple Landau-like potential we are using. Due to its simplicity, the barrier separating the two minima is always closer to the metastable state. In order to make the “width” of the folded state smaller than that of the unfolded state, more realistic potentials like the one in Refs. [16, 17] must be used.

This time interval is much longer than the time step used to integrate the Langevin equations, but much shorter than the total time for pulling (or pushing) the molecule. As a result, the plotted force is close to $F_{exp}$, Eq. (6b), because the time average of $\Delta F$ is very small.