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# PAPER

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# Spin variable approach for the statistical mechanics of folding and unfolding chains

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The force-extension response of chains composed of bistable (or multistable) units strongly depends on the applied boundary conditions. As a matter of fact, isotensional conditions (soft devices) lead to a plateau-like response, whereas isometric conditions (hard devices) lead to a sawtooth-like pattern. We develop an equilibrium statistical mechanics methodology, based on the introduction of a set of discrete or spin variables, which is able to describe the thermal and mechanical properties of a folding and unfolding chain under arbitrary external conditions. In particular, we will work within the Gibbs and Helmholtz ensembles, which correspond to soft and hard devices, respectively. We introduce a one-dimensional system composed of multistable units and a bistable freely jointed chain. For both systems we obtain explicit expressions for the force-extension relation and we study the spinoidal behavior induced by the isometric conditions.

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# 1 Introduction

In recent years, the folding and unfolding of single macromolecules have been largely investigated through atomic-force microscopes (AFMs), laser optical tweezers (LOTs) and magnetic tweezers (MTs), which are able to apply external forces directly to chemical entities.<sup>1-5</sup> In particular, single-molecule micromanipulation has been largely employed for biopolymers such as proteins,<sup>6-10</sup> RNA,<sup>11,12</sup> and DNA.<sup>13-18</sup> The direct quantification of the elasticity of individual molecules (force spectroscopy) has for the first time allowed the investigation of the thermodynamics and the statistical mechanics of small systems.<sup>19,20</sup> In addition, this approach permitted detecting the length of domains in proteins,<sup>21</sup> and identifying an individual block in a copolymer chain,<sup>22</sup> with an efficiency similar to that of nuclear magnetic resonance or X-ray crystallography. The mechanical manipulation of molecular chains has also been achieved by micro-electro mechanical systems (MEMSs), such as silicon nanotweezers (SNTs),<sup>23</sup> recently used to evaluate DNA damage under ionizing radiation.24,25

Force spectroscopy methods offer the possibility of directly measuring the force–extension response of macromolecules and therefore have stimulated the development of theoretical approaches to predict and compare their thermo-mechanical properties.<sup>26–28</sup> Classical theories include the freely-jointed chain (FJC) model<sup>26,29</sup> (describing, *e.g.*, single-stranded DNA and RNA) or the worm-like chain (WLC) model<sup>14,28,30</sup> (well representing

the double-stranded DNA behavior), and several enriched generalizations.<sup>31-35</sup>

Mechanically induced unfolding of monomers or molecular elements of a chain (overstretching regime) is a fundamental phenomenon encountered across the scales from polypeptides to cytoskeletal actin networks and nucleic acids. From the chemical point of view, it is based on the conformational transformation of a molecular structure exhibiting two (or more) metastable configurations. On the other hand, from the mechanical point of view, it can be represented by an interaction potential composed of two (or more) potential wells, corresponding to the stable conformational states. As said above, the unfolding process can be experimentally examined by measuring the force–extension relation using force spectroscopy techniques (see Fig. 1 for the AFM scheme). Devices employed have different equivalent



**Fig. 1** Representation of the unfolding process of a macromolecular chain realized through AFM. The sequence of folded and unfolded domains is tethered onto a given substrate on one side, and stretched by the AFM cantilever on the other side. A laser-detector system is able to measure the cantilever displacement.

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stiffnesses, being in the 10<sup>-4</sup>-1 pN nm<sup>-1</sup> range for optical or magnetic tweezers, and in the  $10-10^2$  pN nm<sup>-1</sup> range for the AFM.<sup>4</sup> Therefore, depending on the equivalent stiffness of the device, the stretching experiment corresponds to a situation placed in between the Gibbs and Helmholtz ensembles of the statistical mechanics. In particular, experiments performed at constant applied forces (extremely soft devices) correspond to the Gibbs statistical ensemble (see Fig. 2a), and experiments performed at prescribed displacements (extremely hard devices) are a realization of the Helmholtz statistical ensemble (see Fig. 2b).<sup>36-38</sup> Typical experimental force-extension curves are also shown in Fig. 2. The Gibbs (plateau-like) response can be interpreted by supposing that the conformational change occurs simultaneously for all the domains at a given threshold force (cooperative process).<sup>15,16,39-41</sup> On the other hand, the Helmholtz (sawtoothlike) response shows that the domains unfold progressively in reaction to the increasing extension (non-cooperative process).6,8,42-44

It is important to remark on the meaning of the word 'cooperativity' in this context. Here we introduce this word to underline the synchronization of the unfolding process. Usually in biology, the word 'cooperativity' is introduced when the transition



**Fig. 2** Scheme of the statistical Gibbs (a) and Helmholtz (b) ensembles, corresponding to the isotensional and isometric conditions applied to a chain of *N* domains (with  $\ell$  being the extension of a folded domain). The plots correspond to typical experimental results measuring the normalized force *versus* the normalized extension under the two distinct conditions. While in the Gibbs ensemble (a) we apply the force *f* and we measure the position  $\langle z \rangle$ , in the Helmholtz ensemble (b) we impose the position *z* and we measure the force  $\langle f \rangle$ .

of one element affects the transition of the others. In this context, we have no interactions among the elements and we simply observe if the unfolding processes take place at the same time or not. We maintain the use of the term 'cooperativity' to be coherent with the existing literature on this subject.

Several theoretical models have been proposed to better understand the force-induced transformations under the two different conditions mentioned above. For example, the plateaulike response observed for double-stranded DNA has been studied through thermodynamic approaches,45,46 and using molecular dynamics simulations,47 leading to interpretation of the overstretching transition as a melting transition. However, another statistical analysis supported the conformational transformation from double-stranded DNA to a new form named stretched-DNA, or S-DNA.48 Recent results show that the stress induced unfolding of double-stranded DNA leads to melting or S-DNA depending on the relative contents of Adenine-Thymine (AT) and Guanine-Cytosine (GC) pairs.<sup>40</sup> In addition, a statistical mechanics study on the force-extension behavior of folding polymers has been applied to different nucleic acid configurations.49 The plateau-like response has also been observed for long polysaccharides, such as dextran,<sup>36,42</sup> and interpreted by means of a continuous two-state model.<sup>50</sup>

Similarly, some theories have been developed to explain the sawtooth-like response observed in other experiments. A model has been proposed for macromolecules unfolded in AFM, and validated for titin and RNA hairpins.<sup>51</sup> An equilibrium statistical mechanics theory has been developed by introducing a Landau-like free energy, eventually predicting a series of firstorder phase transitions in correspondence to the unfolding processes.<sup>52</sup> Another approach is based on the minimization of the total energy of a two-state system and it is in good agreement with the sawtooth pattern observed in titin experiments.<sup>53</sup> Besides, the mechanical unfolding of proteins has been studied by introducing interactions among individual domains described by the Ising model.<sup>54</sup> Furthermore, force–extension curves with marked picks have been theoretically predicted in force spectroscopy of polymer desorption.<sup>55</sup> Finally, an exhaustive understanding of chain behaviors driven by hard or soft devices has been achieved by analysing discrete systems with folding/unfolding units,56,57 Fermi-Pasta-Ulam chains of bistable elements,<sup>58</sup> and structures undergoing discrete phase transformations.<sup>59</sup>

It is important to remark that the differences between the force–extension curves measured under Gibbs or Helmholtz conditions can be noticed only for a moderately small number N of domains of the chain under consideration (small systems). Indeed, for a large number of domains, *i.e.* in the thermodynamic limits (ideally with  $N \rightarrow \infty$ ), the different Gibbs and Helmholtz statistical ensembles are equivalent and they are described by the same constitutive force–extension response.<sup>31,60–62</sup>

As discussed above, several models exist to describe the response of folding/unfolding chains within either the Gibbs or the Helmholtz ensemble. We propose here a universal statistical mechanics methodology for the analysis of the equilibrium behaviour of chains composed of bistable (or multistable) elements and subjected to arbitrary boundary conditions. In simple cases our model can be analytically solved for both the Gibbs and Helmholtz ensembles, eventually giving closed form expressions useful to better understand the physics underlying the bistability (or multistability) in complex systems (at thermodynamic equilibrium).

The idea is as follows. The exact mathematical analysis of a double-basin energy potential is rather complicated from the statistical mechanics point of view because of the critical calculation of the partition function. This point is even more striking when dealing with the Helmholtz ensemble because of the particular constraint imposed by the prescribed chain extension. Therefore, to describe the system more easily, we define additional internal variables (taking part of the phase space of the system and considered as standard variables of the statistical mechanics), which are discrete and behave like spin variables. These discrete quantities (defined for any element of the chain) assume a finite number of values and allow us to identify the basin explored by each element. Consequently, each basin of the potential energy can now be described by a simple spring-like (quadratic) potential, thus facilitating the mathematical analysis of the problem. From the historical point of view, the first biomechanical analysis based on a spin variable has been performed to model skeletal muscles.63,64 This formalism has been recently readdressed to study a broad range of allosteric systems.<sup>65,66</sup> It is important to remark that, as mentioned above, the proposed approach can be adopted only for systems at thermodynamic equilibrium. Indeed, if we are interested in the out-of-equilibrium dynamics, the simplified representation provided by the sequence of basins and spin variables is not sufficient since the relaxation times of the system depend on the energy barriers between the potential wells. This is consistent, e.g., with the Kramers rate formula, originally formulated to deal with chemical reaction rates.<sup>67</sup> Coherently, the rate dependent response of systems with a nonconvex energy landscape has recently been investigated by properly considering the energy barriers among the potential wells.<sup>68,69</sup> To conclude, the introduced approach allows strongly simplifying the analysis of systems with bistable (or multistable) units, eventually giving analytical solutions describing the behavior of the same chain under different boundary conditions or, equivalently, different statistical ensembles.

While we did not discuss in the present paper the quantitative comparison of our statistical mechanics models with data from experiments described in the literature, we illustrate the crucial points of our results, relevant to better interpreting a given stretching experiment going from the entropic regime up to the unfolding process.

The structure of the paper is as follows. In Section 2 we propose the analysis of a simple one-dimensional system composed of multistable units. We develop the statistical mechanics analysis under both Gibbs and Helmholtz conditions. In Section 3 we introduce the bistable freely jointed chain, which is the generalization of the classical FJC model with bistable elements. For both systems we obtain explicit expressions for the force–extension relation under isotensional and isometric conditions and we study the spinoidal behavior (negative slope in the force–extension curve) induced by the isometric conditions. The cooperativeness of the folding/ unfolding process is finally quantitatively measured through the average value of the spin variables.

# 2 One-dimensional system

The aim of the present section is to propose a toy-model which can be analytically solved for both the Gibbs (isotensional) ensemble and the Helmholtz (isometric) ensemble, eventually giving closed form expressions useful to better understand the physics of the phenomena underlying the bistability in complex systems.

We consider a one-dimensional system composed of N elements with mechanical bistability. It means that each element of the chain can be described by a symmetric potential energy function U(x) exhibiting four minima (equilibrium points) at  $x = \pm \ell$  and  $x = \pm \chi \ell$ , where  $\chi$  represents the elongation ratio between the unfolded and folded configurations (see Fig. 3). Moreover, the potential energy assumes the minimum value U = 0 for  $x = \pm \ell$  and the minimum value  $U = \Delta E$  for  $x = \pm \gamma \ell$ . In order to perform a simplified analysis of the system, instead of considering the complex potential function represented in Fig. 3 (blue dashed lines), we introduce an additional discrete variable y, which behaves as a spin. It takes part of the phase space of the system and, therefore, is a standard variable of the statistical mechanics. The variable y assumes the values in the set  $\mathcal{S} = \{\pm 1, \pm \chi\}$  and is able to identify what basin the system explores. So, the multimodal energy function is substituted with the simpler expression

$$U(x,y) = v(y) + \frac{1}{2}k_0(y)(x - y\ell)^2,$$
 (1)

where we consider  $v(\pm 1) = 0$ ,  $v(\pm \chi) = \Delta E$ ,  $k_0(\pm 1) = k$  and  $k_0(\pm \chi) = h$ . The potential energy in eqn (1), by varying the value of the spin variable in S, generates the four parabolic wells represented in Fig. 3 (red solid lines). They are able to fully describe the behavior of the system represented by the multimodal energy profile when we assume to work at thermodynamic equilibrium. Similar assumptions have been introduced and analysed in the recent literature.<sup>58,69</sup>

We add some comments on the energy profile proposed in Fig. 3. We introduced four potential wells (two folded and two



**Fig. 3** Multistable symmetric potential energy of a single domain (blue dashed line) and its approximation by means of four parabolic profiles (red solid lines).

#### Paper

unfolded) for the following reasons. Since we study a onedimensional system, the consideration of positive (x > 0) and negative (x < 0) orientations of the elements allows modeling entropic, enthalpic, unfolding and over-stretching regimes. Indeed, if we consider the system with the first end-terminal tethered at x = 0 and the second end-terminal free to fluctuate (without externally applied forces), we have that the average value of the position of the second end-terminal is zero because of the random orientation of the domains (almost domains folded, 50% towards x > 0 and 50% towards x < 0). Incidentally, it means that the force-extension curve passes through the origin of the axes. With a weak applied force (positive or negative), we are in the entropic regime and we will have a slight increase of the number of domains oriented in the direction of the force. Under these conditions, the mechanical reaction of the system is governed by the stochastic distribution of the differently oriented domains (entropy) and not by the actual spring-like behavior of the elements. Then, with a larger force we act on the real elasticity of the domains (enthalpic regime) up to the unfolding process and the over-stretching regime, described below for both isotensional and isometric conditions.

In other words, the entropic regime corresponds to the transition between the two branches around x = 0 (with the same zero energy levels). Then, an enthalpic regime follows and results in a second transition corresponding to the element unfolding (with different energy levels 0 and  $\Delta E$ ). Finally, another enthalpic behavior corresponds to the over-stretching regime (also enthalpic). We note that the well identified by  $y = -\chi$  remains unexplored for positive forces and the well identified by  $y = \chi$  remains unexplored for negative forces. We also remark that we have given a different interpretation of the transition at x = 0 (named entropic since the energy jump is zero) and the other transition (named unfolding since the energy jump is  $\Delta E \neq 0$ ), although they are represented by the same physical process (but with different energy jumps).

In conclusion, the four well energy profile is appropriate in order to correctly represent the entropic behavior of the system for a weak applied force. This aspect is naturally introduced in two- or three-dimensional models by the higher dimensionality, which automatically induces a larger number of degrees of freedom (see Section 3 for details).

#### 2.1 The Gibbs ensemble

The total potential energy of the system under the Gibbs condition (isotensional ensemble) is given by

$$U_{\text{tot}}^{\text{G}}(\vec{x}, \vec{y}; f) = \sum_{i=1}^{N} U(x_i - x_{i-1}, y_i) - fx_N, \qquad (2)$$

where *f* is the force applied to the last element,  $\vec{x} = (x_1, x_2, ..., x_N)$  (continuous variables) and  $\vec{y} = (y_1, y_2, ..., y_N)$  (discrete variables). For this system, we can define the partition function  $Z_G$ , as follows:

$$Z_{\rm G}(f) = \sum_{y_1 \in \mathscr{S}} \cdots \sum_{y_N \in \mathscr{S}} \int_{\Re} \cdots \int_{\Re} e^{-\frac{U_{\rm tot}}{k_{\rm B}T}} \mathrm{d}x_1 \dots \mathrm{d}x_N, \qquad (3)$$

where the variable  $\vec{x}$  is integrated, whereas  $\vec{y}$  is summed. We have not considered the kinetic energy entering the total Hamiltonian of the system since it merely produces a non-influential multiplicative constant in the partition function. Indeed, since we use orthogonal coordinates, the kinetic energy depends only on the linear momentum variables and the potential energy only on  $\vec{x}$  and  $\vec{y}$ . Therefore, the two contributions are fully uncoupled and the integral over the momentum variables simply generates, as mentioned above, a multiplicative constant in  $Z_G(f)$ . We can now substitute eqn (2) in eqn (3). To evaluate the integral we apply the change of variables  $x_1 - x_0 = \xi_1, x_2 - x_1 = \xi_2, \dots, x_N - x_{N-1} = \xi_N$ , from which we get  $x_n = \sum_{j=1}^N \xi_j$  (with  $x_0 = 0$ ). The change of variables within the multiple integral must be applied by considering that  $d\vec{r} = Id^{\vec{k}}$ 

multiple integral must be applied by considering that  $d\vec{x} = Jd\vec{\xi}$ , where the quantity *J* is the Jacobian of the transformation, *i.e.*  $J = |\det[\partial \vec{x}/\partial \vec{\xi}]|$ , where  $[\partial \vec{x}/\partial \vec{\xi}]_{ij}$  is the matrix of the partial derivatives  $\partial x_i/\partial \xi_j$ . It is not difficult to prove that J = 1 for our change of variables and, therefore, we get  $d\vec{\xi} = d\vec{x}$ . Hence, we easily obtain

$$Z_{\mathbf{G}}(f) = \sum_{\vec{y} \in \mathscr{S}^{N}} \int_{\Re^{N}} \exp\left\{\sum_{j=1}^{N} \left[-\frac{U(\xi_{j}; y_{j})}{k_{\mathbf{B}}T} + \frac{f\xi_{j}}{k_{\mathbf{B}}T}\right]\right\} d\vec{\xi}$$
$$= \left\{\sum_{y \in \mathscr{S}} \int_{-\infty}^{+\infty} \exp\left[-\frac{U(\xi; y)}{k_{\mathbf{B}}T} + \frac{f\xi}{k_{\mathbf{B}}T}\right] d\xi\right\}^{N} \qquad (4)$$
$$= \left\{\sum_{y \in \mathscr{S}} \exp\left[-\frac{v(y)}{k_{\mathbf{B}}T}\right] \mathscr{I}(f)\right\}^{N},$$

where the integral  $\mathscr{I}(f)$  is defined as

$$\mathscr{I}(f) = \int_{-\infty}^{+\infty} \exp\left[-\frac{k_0(y)}{2k_{\rm B}T}(\xi - y\ell)^2 + \frac{f\xi}{k_{\rm B}T}\right] \mathrm{d}\xi,\qquad(5)$$

and it can be calculated in the closed form by means of the wellknown expression

$$\int_{-\infty}^{+\infty} e^{-\alpha x^2} e^{\beta x} dx = \sqrt{\frac{\pi}{\alpha}} \frac{\beta^2}{e^{4\alpha}} (\alpha > 0).$$
 (6)

We eventually obtain the result

$$\mathscr{I}(f) = \sqrt{\frac{2\pi k_{\rm B}T}{k_0(y)}} \exp\left[\frac{y\ell f}{k_{\rm B}T} + \frac{f^2}{2k_{\rm B}Tk_0(y)}\right].$$
 (7)

Coming back to the partition function, we have

$$Z_{\rm G}(f) = (2\pi k_{\rm B}T)^{\frac{N}{2}} \Biggl\{ \sum_{y \in \mathscr{S}} \sqrt{\frac{1}{k_0(y)}} \exp\left[\frac{1}{k_{\rm B}T} \left(\frac{f^2}{2k_0(y)} + y\ell f - v(y)\right)\right] \Biggr\}^{N}$$
(8)

$$= (8\pi k_{\mathrm{B}}T)^{\frac{N}{2}} \left\{ \sum_{s \in \{1,\chi\}} \sqrt{\frac{1}{k_{0}(s)}} \exp\left[\frac{1}{k_{\mathrm{B}}T} \left(\frac{f^{2}}{2k_{0}(s)} - \nu(s)\right)\right] \cosh\frac{s\ell f}{k_{\mathrm{B}}T} \right\}^{N}$$
(9)

The last sum can be evaluated by obtaining

$$Z_{\rm G}(f) = (8\pi k_{\rm B}T)^{\frac{N}{2}} \mathscr{D}^N, \qquad (10)$$

where

$$\mathscr{D} = \sqrt{\frac{1}{k}} \exp\left(\frac{f^2}{2k_{\rm B}Tk}\right) \cosh\frac{\ell f}{k_{\rm B}T} + \phi \sqrt{\frac{1}{h}} \exp\left(\frac{f^2}{2k_{\rm B}Th}\right) \cosh\frac{\chi\ell f}{k_{\rm B}T},$$
(11)

and  $\phi = \exp\left(-\frac{\Delta E}{k_{\rm B}T}\right)$ . It is important to remark that within the

Gibbs ensemble the elements of the chain do not interact and this point leads to a partition function which is in the form of a power with exponent N. A similar result can be found in eqn (34) of ref. 58, describing the statistical behavior of a Fermi-Pasta-Ulam chain of bistable elements. Also in this case a soft device leads to the independence of the bistable units.

The extension of the chain can be directly calculated through the expression  $x_N = -\partial U_{tot}/\partial f$  and its average value is therefore  $\langle x_N \rangle = \langle -\partial U_{tot}/\partial f \rangle$ . It can be simply evaluated by means of the partition function, as  $\langle x_N \rangle = k_B T \partial / \partial f (\log Z_G)$ . The calculation eventually gives

$$\frac{\langle x_N \rangle}{N\ell} = \left\{ \sqrt{\frac{1}{k}} \exp\left(\frac{f^2}{2k_{\rm B}Tk}\right) \left[\frac{f}{k\ell} \cosh\frac{\ell f}{k_{\rm B}T} + \sinh\frac{\ell f}{k_{\rm B}T}\right] + \chi \phi \sqrt{\frac{1}{h}} \exp\left(\frac{f^2}{2k_{\rm B}Th}\right)$$

$$\times \left[\frac{f}{\chi\ell h} \cosh\frac{\chi\ell f}{k_{\rm B}T} + \sinh\frac{\chi\ell f}{k_{\rm B}T}\right] \right\} / \mathcal{D}.$$
(12)

In the simpler case with k = h, we get

$$\frac{\langle x_N \rangle}{N\ell} = \frac{f}{k\ell} + \frac{\sinh\frac{\ell f}{k_{\rm B}T} + \chi\phi\sinh\frac{\chi\ell f}{k_{\rm B}T}}{\cosh\frac{\ell f}{k_{\rm B}T} + \phi\cosh\frac{\chi\ell f}{k_{\rm B}T}}.$$
(13)

We can also calculate the average value of the spin variable  $\langle y \rangle = \langle y_i \rangle \forall i$ , which is independent of the element considered in the chain and is given by

$$\langle y \rangle = \langle y_i \rangle = \frac{\mathcal{N}}{\mathscr{D}} \forall i,$$
 (14)

where

$$\mathcal{N} = \sqrt{\frac{1}{k}} \exp\left(\frac{f^2}{2k_{\rm B}Tk}\right) \sinh\frac{\ell f}{k_{\rm B}T} + \chi \phi \sqrt{\frac{1}{h}} \exp\left(\frac{f^2}{2k_{\rm B}Th}\right) \sinh\frac{\chi \ell f}{k_{\rm B}T},$$
(15)

and  $\mathcal{D}$  is given in eqn (11). In the simpler case with k = h, we get

$$\langle y \rangle = \frac{\sinh \frac{\ell f}{k_{\rm B}T} + \chi \phi \sinh \frac{\chi \ell f}{k_{\rm B}T}}{\cosh \frac{\ell f}{k_{\rm B}T} + \phi \cosh \frac{\chi \ell f}{k_{\rm B}T}}.$$
 (16)



**Fig. 4** Responses of the one-dimensional system with multistable elements under Gibbs conditions (*h* = *k*): average spin variable (blue line) and normalized extension (red line) *versus* dimensionless force. We adopted the parameters  $\Delta E = 30k_{\rm B}T = 12.4 \times 10^{-20}$  J,  $\chi = 8$ ,  $\ell = 0.5$  nm and  $h = k = 2.5k_{\rm B}T/\ell^2 = 0.0414$  N m<sup>-1</sup> (at T = 300 K). The results are independent of *N* within the Gibbs ensemble.

By combining eqn (13) with eqn (16), we immediately obtain  $\frac{\langle x_N \rangle}{N\ell} = \frac{f}{k\ell} + \langle y \rangle$  or, equivalently,

$$f = k \left( \frac{\langle x_N \rangle}{N} - \ell \langle y \rangle \right). \tag{17}$$

This constitutive equation represents a spring-like behavior with an equilibrium length directly modulated by the average value of the spin variables.

An example of the force-extension response and spin variable behavior is presented in Fig. 4 for h = k. In the curve of  $\frac{f\ell}{k_{\rm B}T}$  versus  $\frac{\langle x_N \rangle}{N\ell}$  we observe a force plateau corresponding to  $f^* = \pm \frac{\Delta E}{(\chi - 1)\ell}$  or, equivalently,  $\frac{f^*\ell}{k_BT} = \pm \frac{\Delta E}{(\chi - 1)k_BT}$ . Similarly, in the curve of  $\frac{f\ell}{k_{\rm P}T}$  versus  $\langle y \rangle$  we can identify the regions  $\langle y \rangle = \pm 1$  and  $\langle y \rangle = \pm \chi$  with a transition corresponding to the same force  $f^*$ . The interpretation of this behavior is based on a cooperative process inducing the transition of all elements of the chain at the same value of force  $f^*$ . The cooperative transition is indeed manifest in both the force-extension curve and the spin variable behavior. Of course, the slope of the plateau observed depends on the temperature. Indeed, for higher values of the temperature the response is smoother and the elements undergo transitions in a more uncorrelated fashion. Anyway, the value of the threshold force  $f^*$  can be explained as follows. Due to the symmetry of the response, we limit the following discussion to the region with f > 0 and  $\langle x_N \rangle > 0$ . We suppose that the system has two potential energies  $U_1(x) = \frac{1}{2}k(x-\ell)^2 - fx$  and  $U_2(x) = \Delta E + \frac{1}{2}k(x-\chi\ell)^2 - fx$ , corresponding to the potential wells of the system identified

by y = 1 and  $y = \chi$ . The related equilibrium positions are defined by  $\partial U_i/\partial x = 0$  and can be found as  $x_1 = \ell + f/k$  and  $x_2 = \chi \ell + f/k$ . Hence, the unfolded configuration is more stable than the folded one if and only if  $U_2(x_2) < U_1(x_1)$ , that is to say,  $f < \frac{\Delta E}{(\chi - 1)\ell}$ , as observed in Fig. 4. Notably, the value of the plateau force inducing the conformation transition does not depend on the spring constant, nor on the temperature. Such a result is readily interpreted in the framework of the Bell expression, originally derived in the context of the adhesion of cells.<sup>70</sup>

#### 2.2 Validation of the model and limits of applicability

In order to validate the introduction of the discrete or spin variables identifying the energy wells of the system, we analyse using different methods a real multistable system described by the following potential energy (we begin with the case where h = k):

$$U(x) = \begin{cases} \frac{1}{2}k(x-\ell)^2 & \text{if } 0 < x < x_1, \\ \mathscr{A} - \frac{1}{2}k_0(x-\chi_0\ell)^2 & \text{if } x_1 < x < x_2, \\ \Delta E + \frac{1}{2}k(x-\chi\ell)^2 & \text{if } x > x_2, \end{cases}$$
(18)

defined such that U(-x) = U(x) (see Fig. 3). As before, we consider a four-well energy profile in a one-dimensional system to properly introduce the entropic regime. Here  $k_0 > 0$ ,  $1 < \chi_0 < \chi$  and  $\mathscr{A}$  represents the energy barrier between the wells. The conditions of continuity and derivability of *U* at points  $x_1$  and  $x_2$  give the relations

$$\chi_0 = \frac{\sqrt{\mathscr{A} - \Delta E} + \chi \sqrt{\mathscr{A}}}{\sqrt{\mathscr{A} - \Delta E} + \sqrt{\mathscr{A}}},\tag{19}$$

$$\frac{1}{k_0} = \frac{\ell^2 (\chi - 1)^2}{2 \left(\sqrt{\mathscr{A} - \Delta E} + \sqrt{\mathscr{A}}\right)^2} - \frac{1}{k},$$
(20)

$$x_1 = \frac{k + k_0 \chi_0}{k + k_0} \ell,$$
 (21)

$$x_2 = \frac{k\chi + k_0\chi_0}{k + k_0}\ell.$$
 (22)

Therefore, once  $\Delta E$ , k,  $\chi$  and  $\mathscr{A}$  are fixed, we can easily find  $x_1$ ,  $x_2$ ,  $k_0$  and  $\chi_0$ . The equilibrium thermodynamics of this system can be studied exactly (without the introduction of the spin variables) with two fully independent techniques: (i) Monte Carlo (Metropolis) simulations and (ii) exact calculation of the partition function. As a check of the procedure, we verified that the two approaches always give exactly the same results. Then, both methods have been applied and compared with the solution of our spin model given in eqn (13) and in Fig. 4. The results of such comparison can be found in Fig. 5. In particular, it is important to remark that the perfect agreement has been observed for any value of the energy barrier  $\mathscr{A}$  larger than or equal to the jump energy  $\Delta E$ , provided that  $\Delta E \gg k_BT$ . As a



**Fig. 5** Comparison of exact results (Monte Carlo or analytical) for the potential energy in eqn (18) (green continuous line) and the spin variable expression given in eqn (13) (red dashed line). We adopted the same parameters of Fig. 4 (in particular, we assumed h = k). The perfect agreement has been found for any value of  $\mathscr{A} \ge \Delta E$  provided that  $\Delta E \gg k_{\rm B}T$ .

matter of fact, the adopted approximation based on the spin variables is valid only if the temperature is low enough to clearly identify the folded and unfolded states of the elements of the chain. Therefore, for the present case with h = k the only condition to fulfil for the validity of our model is  $\mathscr{A} \ge \Delta E \gg k_{\rm B}T$ . Indeed, very high values of temperature may generate some deviations, but these values of temperature are not interesting for typical applications to biophysics.

In order to further investigate the limits of applicability of our model based on discrete or spin variables, we also analyse the case with h > k. An example can be seen in Fig. 6, where we



**Fig. 6** Responses of the one-dimensional system with multistable elements under Gibbs conditions (h > k): average spin variable (blue line) and normalized extension (red line) versus dimensionless force. We adopted the parameters  $\Delta E = 15k_{\rm B}T = 6.2 \times 10^{-20}$  J,  $\chi = 8$ ,  $\ell = 0.5$  nm,  $k = 1.25k_{\rm B}T/\ell^2 = 0.0207$  N m<sup>-1</sup> and  $h = 7.5k_{\rm B}T/\ell^2 = 0.124$  N m<sup>-1</sup> (at T = 300 K). The shaded areas represent the regions where the model gives incorrect results. The response is independent of *N* within the Gibbs ensemble.

reported the force–extension response given by eqn (12) and the spin variable behavior given by eqn (14) for a system with h > k. In the force–extension curve, we observe two force plateaux: the first one, which corresponds to the correct conformational transition of the elements of the chain, and the second one, for large values of the applied force, which is non-physical. It means that the model, in this case, cannot be applied with forces exceeding a given threshold. The shaded areas in Fig. 6 represent the regions where the model gives incorrect results. Also, the spin variable shows the first transition from  $\langle y \rangle = 1$  to  $\langle y \rangle = \chi$  (which represents the correct unfolding) and the second opposite transition from  $\langle y \rangle = \chi$  to  $\langle y \rangle = 1$  (which is non-physical).

This point is confirmed by an independent analysis based on the following potential energy (we consider now  $h \neq k$ ):

$$U(x) = \begin{cases} \frac{1}{2}k(x-\ell)^2 & \text{if } 0 < x < x_1, \\ \mathscr{A} - \frac{1}{2}k_0(x-\chi_0\ell)^2 & \text{if } x_1 < x < x_2, \\ \Delta E + \frac{1}{2}h(x-\chi\ell)^2 & \text{if } x > x_2, \end{cases}$$
(23)

defined such that U(-x) = U(x) (see Fig. 3). As before,  $k_0 > 0, 1 < \chi_0 < \chi$  and  $\mathscr{A}$  represents the energy barrier. The conditions of continuity and derivability of U at points  $x_1$  and  $x_2$  give

$$(\mathscr{A} - \Delta E)(\chi_0 - 1)^2 - \mathscr{A}(\chi - \chi_0)^2 = \frac{2\mathscr{A}(\mathscr{A} - \Delta E)(h - k)}{hk\ell^2}, \quad (24)$$

which is a second degree equation for  $\chi_0$ , and

1 1

$$k_0 = \frac{2\mathscr{A}k}{k\ell^2(\chi_0 - 1)^2 - 2\mathscr{A}},$$
 (25)

$$x_1 = \frac{k + k_0 \chi_0}{k + k_0} \ell,$$
 (26)

$$x_2 = \frac{h\chi + k_0\chi_0}{h + k_0}\ell.$$
 (27)

Therefore, once  $\Delta E$ , k, h,  $\chi$  and  $\mathscr{A}$  are fixed, we can easily find  $x_1$ ,  $x_2$ ,  $k_0$  and  $\chi_0$ . As before, the behavior of this system can be studied exactly (Monte Carlo simulations or exact calculation of the partition function). This approach has been applied to our system and compared with the spin model described by eqn (12) and is presented in Fig. 6. The comparison can be found in Fig. 7, where we observe that the spin model gives incorrect results for values of the force exceeding a given threshold (shaded areas).

This problem can be explained as follows. In Fig. 8 we can observe the energy potential of one element of the chain (blue dashed line) given in eqn (23) and the profiles of the energy wells corresponding to the folded and unfolded configurations, respectively (we adopted the same parameters of Fig. 6 and 7 and an energy barrier  $\mathscr{A} = 17k_{\rm B}T$ ). The intersection point A corresponds to the folding/unfolding transition (the first correct transition already observed in Fig. 6 and 7). On the other hand, point B corresponds to another potential transition



**Fig. 7** Comparison of exact results (Monte Carlo or analytical) for the potential energy in eqn (23) (green continuous line) and the spin variable expression given in eqn (12) (red dashed line). We adopted the same parameters of Fig. 6 (in particular, we assumed  $h \neq k$ ). The green continuous line is nearly independent of  $\mathscr{A}$  provided that  $\mathscr{A} \geq \Delta E \gg k_{\rm B}T$ . The shaded areas represent the regions where the spin model (red dashed line) gives incorrect results.

to the folded configuration, which is non-physical because the parabolic branch  $\frac{1}{2}k(x-\ell)^2$  does not exist on the right of point A in a real multistable system. Therefore, in this example, the results of the spin model are limited to a specific range of the applied force.

To conclude this discussion, it is useful to better identify the threshold value of the force, limiting the validity of the model. Due to the symmetry of the response, we limit the following analysis to the region with f > 0 and  $\langle x_N \rangle > 0$ . We suppose that the system has two independent potential energies  $U_1(x) = \frac{1}{2}k(x-\ell)^2 - fx$  and  $U_2(x) = \Delta E + \frac{1}{2}h(x-\chi\ell)^2 - fx$ . The related equilibrium positions are defined by  $\partial U_i/\partial x = 0$  and can be found as  $x_1 = \ell + f/k$  and  $x_2 = \chi\ell + f/h$ . Hence, the second system is favoured with respect to the first one if and only if  $U_2(x_2) < U_1(x_1)$ ; that is to say,

$$\frac{1}{2}f^2\left(\frac{1}{k}-\frac{1}{h}\right)-f\ell(\chi-1)+\Delta E<0.$$
(28)

If h > k and  $\Theta = (\chi - 1)^2 \ell^2 - 2\Delta E(1/k - 1/h) > 0$  (positive discriminant), the unfolded configuration is preferred in the interval

$$\frac{2\Delta E}{(\chi - 1)\ell + \sqrt{\Theta}} < f < \frac{2\Delta E}{(\chi - 1)\ell - \sqrt{\Theta}},$$
(29)

corresponding to the positions between A and B in Fig. 8. While the first endpoint of the interval corresponds to the force of the real folding/unfolding transition, the second endpoint corresponds to the force of the non-physical transition introduced by the spin method, and it can be considered as the threshold force defining the region of validity of the proposed model.



**Fig. 8** Plots of the energy potential given in eqn (23) (blue dashed line) with the same parameters of Fig. 6 and 7 and with a barrier  $\mathscr{A}_{1} = 17k_{\text{B}}T$ . We also plotted the energy profiles corresponding to the wells  $\frac{1}{2}k(x-\ell)^{2}$  and  $\Delta E + \frac{1}{2}h(x-\chi\ell)^{2}$ . The intersection points A and B between them are highlighted.

This kind of limitation on the range of admissible force values must be carefully taken into account when applying this technique to multistable chains, especially when we have different elastic responses of the energy wells composing the system. However, we finally observe that the proposed spin method offers a very high accuracy when we remain within the interval of validity of the approximation. Of course, this limitation on the force range must be always coupled with the condition  $\mathscr{A} \geq \Delta E \gg k_{\rm B}T$ , as previously discussed.

#### 2.3 The Helmholtz ensemble

We consider now the chain of multistable elements with both end-terminals tethered at the points  $x_0 = 0$  and  $x_N = x$ , respectively. It means that the total potential energy of the system can be written as

$$U_{\text{tot}}^{\text{H}}(\vec{x}, \vec{y}; x_N) = \sum_{i=1}^{N} U(x_i - x_{i-1}, y_i), \qquad (30)$$

where  $x_N = x$  is the fixed extremity of the chain,  $\vec{x} = (x_1, x_2, ..., x_{N-1})$  (continuous variables) and  $\vec{y} = (y_1, y_2, ..., y_N)$  (discrete variables). In eqn (30) the potential energy U(x,y) of a single element is given in eqn (1). The partition function of this system can be written as

$$Z_{\mathrm{H}}(x_{N}) = \sum_{y_{1} \in \mathscr{S}} \cdots \sum_{y_{N} \in \mathscr{S}} \int_{\Re} \cdots \int_{\Re} \mathrm{e}^{-\frac{U_{\mathrm{tot}}^{\mathrm{H}}}{k_{\mathrm{B}}T}} \mathrm{d}x_{1} \dots \mathrm{d}x_{N-1}.$$
(31)

Since  $x_N = x$  is fixed the direct evaluation of the integral in eqn (31) is rather difficult. However, by comparing eqn (3) and (31) we deduce that the two partition functions  $Z_G$  and  $Z_H$  are related through a bilateral Laplace transform, as follows:

$$Z_{\rm G}(f) = \int_{-\infty}^{+\infty} Z_{\rm H}(x) \exp\left(\frac{fx}{k_{\rm B}T}\right) \mathrm{d}x.$$
 (32)

Moreover, if we let  $f = -i\omega k_{\rm B}T$ , we simply obtain

$$Z_{\rm G}(-i\omega k_{\rm B}T) = \int_{-\infty}^{+\infty} Z_{\rm H}(x) \exp(-i\omega x) dx, \qquad (33)$$

which means that the Fourier transform of  $Z_{\rm H}$  gives the analytical continuation of  $Z_{\rm G}$  on the imaginary axis. Thus, we can invert the Fourier transform, by getting

$$Z_{\rm H}(x) = \int_{-\infty}^{+\infty} Z_{\rm G}(-i\omega k_{\rm B}T) \exp(i\omega x) \mathrm{d}\omega.$$
(34)

It means that the behavior of the system in the Helmholtz ensemble can be studied through eqn (34), which considers the partition function of the Gibbs ensemble as the starting point. Anyway, from eqn (10) and (11), we have

$$Z_{\rm G}(-i\omega k_{\rm B}T) = c \left\{ \sqrt{\frac{1}{k}} \exp\left(-\frac{k_{\rm B}T\omega^2}{2k}\right) \cos(\ell\omega) + \sqrt{\frac{1}{h}} \exp\left(-\frac{k_{\rm B}T\omega^2}{2h}\right) \phi \cos(\chi\ell\omega) \right\}^{N}$$

$$= c \sum_{p=0}^{N} \binom{N}{p} \sqrt{\frac{1}{h^p}} \sqrt{\frac{1}{k^{N-p}}} \phi^{p}$$

$$\times \exp\left[-\frac{1}{2} k_{\rm B}T\left(\frac{p}{h} + \frac{N-p}{k}\right) \omega^2\right]$$

$$\times \cos^{p}(\chi\ell\omega) \cos^{N-p}(\ell\omega),$$
(35)

where c is an unimportant multiplicative constant. By using the Newton development

$$\cos^n x = \frac{1}{2^n} \mathrm{e}^{-inx} \sum_{t=0}^n \binom{n}{t} \mathrm{e}^{2itx},\tag{36}$$

we obtain from eqn (34) and (35)

$$Z_{\rm H}(x) = c \sum_{p=0}^{N} \sum_{q=0}^{p} \sum_{s=0}^{N-p} {N \choose p} {p \choose q} {N-p \choose s} \times \sqrt{\frac{1}{h^p}} \sqrt{\frac{1}{k^{N-p}}} \phi^p \times \int_{-\infty}^{+\infty} \exp\left[-\frac{1}{2} k_{\rm B} T \left(\frac{p}{h} + \frac{N-p}{k}\right) \omega^2\right] \times \exp[i\omega(2\chi\ell q - \chi\ell p + 2\ell s - \ell N + \ell p + x)] d\omega.$$
(37)

The integral in eqn (37) can be done with the help of the standard expression

$$\int_{-\infty}^{+\infty} e^{-\alpha x^2} e^{i\beta x} dx = \sqrt{\frac{\pi}{\alpha}} e^{-\frac{\beta^2}{4\alpha}} (\alpha > 0), \qquad (38)$$

eventually obtaining

$$Z_{\rm H}(x) = c \sum_{p=0}^{N} \sum_{q=0}^{p} \sum_{s=0}^{N-p} {N \choose p} {p \choose q} {N-p \choose s}$$

$$\times \frac{1}{\sqrt{\frac{p}{h} + \frac{N-p}{k}}} \sqrt{\frac{1}{h^p}} \sqrt{\frac{1}{k^{N-p}}} \phi^p e^{-\phi^2},$$
(39)

where

$$\varphi = \frac{2\chi\ell q - \chi\ell p + 2\ell s - \ell N + \ell p + x}{\sqrt{2k_{\rm B}T\left(\frac{p}{h} + \frac{N-p}{k}\right)}}.$$
(40)

It is interesting to remark that the partition function obtained cannot be written as a power with exponent *N*. It means that within the Helmholtz ensemble there is an effective interaction among the elements. The origin of this interaction is not explicitly defined in the potential energy of the system (as, *e.g.*, in the Ising model), but comes from the specific boundary conditions characterising the Helmholtz ensemble. Our result can be compared with eqn (20) of ref. 58, obtained for a Fermi–Pasta–Ulam chain with bistable elements. Also this work confirms that hard devices lead to an interaction among elements (see also Fig. 13 of ref. 58 for the comparison of the mechanical response with soft or hard devices).

Now we can evaluate the average value of the overall force  $\langle f \rangle = -k_{\rm B}T\partial/\partial x \log Z_{\rm H}$  applied to the system and the average value of the spin variables  $\langle y \rangle = \left\langle \frac{1}{N} \sum_{i=1}^{N} y_i \right\rangle$  describing the transitions, as follows:



**Fig. 9** Responses of the one-dimensional system with multistable elements under Helmholtz conditions for N = 5: average spin variable (blue line) and dimensionless force (red line) *versus* normalized extension. We adopted the parameters  $\Delta E = 30k_{\rm B}T = 12.4 \times 10^{-20}$  J,  $\chi = 8$ ,  $\ell = 0.5$  nm and  $h = k = 2.5k_{\rm B}T/\ell^2 = 0.0414$  N m<sup>-1</sup> (at T = 300 K), as in Fig. 4. For comparison, we also plotted the Gibbs force–extension response (green dashed line).

force–extension relation and by the sequence of steps characterizing the average value of the spin variable. To sum up, while in the Gibbs ensemble all the domains unfold collectively (cooperative process), leading to a plateau in the force–extension curve, in the Helmholtz ensemble the domains unfold individually (non-cooperative process), leading to the sawtoothlike force–extension curve.

$$\langle f \rangle = \frac{\sum_{p=0}^{N} \sum_{q=0}^{p} \sum_{s=0}^{N-p} \binom{N}{p} \binom{p}{q} \binom{N-p}{s} \frac{\sqrt{2k_{\rm B}T}}{p} \sqrt{\frac{1}{h^{p}}} \sqrt{\frac{1}{k^{N-p}}} \phi^{p} \varphi e^{-\varphi^{2}}}{\sum_{p=0}^{N} \sum_{q=0}^{p} \sum_{s=0}^{N-p} \binom{N}{p} \binom{p}{q} \binom{N-p}{s} \frac{1}{\sqrt{\frac{p}{h} + \frac{N-p}{k}}} \sqrt{\frac{1}{h^{p}}} \sqrt{\frac{1}{k^{N-p}}} \phi^{p} e^{-\varphi^{2}}},$$

$$\langle y \rangle = \frac{\frac{1}{N} \sum_{p=0}^{p} \sum_{s=0}^{N-p} \binom{N}{p} \binom{p}{q} \binom{N-p}{s} \frac{1}{\sqrt{\frac{p}{h} + \frac{N-p}{k}}} \sqrt{\frac{1}{h^{p}}} \sqrt{\frac{1}{k^{N-p}}} \phi^{p} (\chi p - 2\chi q + N - p - 2s) e^{-\varphi^{2}}}{\frac{N-p}{p} \sum_{q=0}^{N} \sum_{s=0}^{p} \sum_{s=0}^{N-p} \binom{N}{p} \binom{p}{q} \binom{N-p}{s} \frac{1}{\sqrt{\frac{p}{h} + \frac{N-p}{k}}} \sqrt{\frac{1}{h^{p}}} \sqrt{\frac{1}{k^{N-p}}} \phi^{p} e^{-\varphi^{2}}}$$

$$(42)$$

An application of eqn (41) and (42) can be found in Fig. 9 where the average spin variable and the dimensionless force are represented *versus* the normalized extension for h = k and N = 5(with the same parameters used in Fig. 4). For comparison, in the same figure one can also find the force–extension response in the Gibbs ensemble. These results show a non-cooperative behavior characterized by the progressive unfolding of the domains with the increasing total length of the system. This is confirmed by the marked picks visible in the Helmholtz In Fig. 10 we can also find the force–extension curve in the Helmholtz ensemble for different values of N = 4, 6, 8, 10 and 12. We note that for an increasing number N of elements, the Helmholtz response converges to the Gibbs one, by progressively reducing the pick-to-pick distance in the sawtooth pattern. This is in perfect agreement with recent results concerning the ensembles' equivalence in the thermodynamic limit.<sup>61,62</sup>

In Fig. 11 one can find the behavior of the system within the Helmholtz ensemble with increasing values of temperature.



**Fig. 10** Force-extension responses of the one-dimensional system with multistable elements under Helmholtz (H) conditions for N = 4, 6, 8, 10, and 12. We also reported the Gibbs (G) response to show the equivalence of the ensembles in the thermodynamic limit. We adopted the parameters  $\Delta E = 30k_{\rm B}T = 12.4 \times 10^{-20}$  J,  $\chi = 8$ ,  $\ell = 0.5$  nm and  $h = k = 2.5k_{\rm B}T/\ell^2 = 0.0414$  N m<sup>-1</sup> (at T = 300 K), as in Fig. 4 and 9.

We observe that the picks in the force-extension curves (panel a) are smeared out by increasing the temperature, as expected since the fluctuations are able to attenuate the non-cooperative response. This point can be better observed in terms of effective elastic stiffness, defined as  $k_{\text{eff}} = \partial \langle f \rangle / \partial x$  (panel b of Fig. 11). For sufficiently low temperatures, we have force-extension curves which are nonmonotone and we observe a negative stiffness in the so-called spinoidal regions, where the slope of the forceextension curves is negative.<sup>68,69</sup> However, it is interesting to note that a critical temperature  $T^{c}$  can be defined by the condition that the stiffness is always positive for supercritical temperatures (in the example of Fig. 11, we have  $T^{c} \simeq 2070$  K). Hence, the real non-cooperative response can be observed only at subcritical temperatures. The critical temperature can be viewed as the Curie temperature controlling the ferromagnetic/ paramagnetic transition in magnetism. This analogy has been recently discussed in the context of the Huxley-Simmons model.<sup>65,66</sup> The decrease of non-cooperativeness can be easily

represented in terms of the average value of the spin variable (panel c of Fig. 11). Indeed, for increasing temperatures we gradually lose the staircase shape of the  $\langle y \rangle(x)$  curve, observing a quite linear growth of  $\langle y \rangle$  *versus x* for supercritical temperatures.

To characterize this spinoidal behavior, we can consider panel b of Fig. 11 and we can identify, for a given temperature, the regions of the x-axis with negative stiffness. This analysis results in a sequence of intervals  $(x_i^-(T), x_i^+(T))$  with i = 1, ..., N, which are represented in Fig. 12 (panel a) versus the temperature of the system. The blue curves represent the endpoints of such intervals versus the temperature. We observe that each interval, corresponding to a given pick of the force-extension response, becomes degenerate and converges to a single point  $x_i^{-}(T_i^{c}) = x_i^{+}(T_i^{c})$  at the specific critical temperature  $T_i^{c}$  of the pick under consideration (red circles in Fig. 12, panel a). Hence, the global critical temperature of the system is the highest value among all specific critical temperatures of the picks. For each interval of the x-axis with spinoidal behavior, we can determine the corresponding interval on the  $\langle f \rangle$ -axis. The intervals of force  $(\langle f \rangle_i^+(T), \langle f \rangle_i^-(T))$  (i = 1, ..., N) with spinoidal behavior are indeed presented in Fig. 12 (panel b) as a function of temperature. Once again, we can identify the specific critical temperature  $T_i^c$  of each pick of the sawtooth response by observing the degeneration of the intervals to a single point  $\langle f \rangle_i^+(T_i^c) = \langle f \rangle_i^-(T_i^c)$  (blue circles) in Fig. 12, panel a). Finally, the spinoidal working regions characterising the critical behavior of the chain can be directly represented on the  $(\langle f \rangle, x)$  plane, as shown in Fig. 12 (panel c). Here, the N = 5 solid curves represent the parametric plots of  $(\langle f \rangle_i^+(T), \mathbf{x}_i^-(T))$  and  $(\langle f \rangle_i^-(T), \mathbf{x}_i^+(T))$  (i = 1, ..., N) for a temperature range from T = 300 K (blue) to  $T = T^{c}$  (red). These curves correspond to the loci of the maxima and minima of the forceextension curve with varying temperature. On the same plot, the dashed (yellow) line represents the force-extension response for T = 300 K.

#### 3 Bistable freely jointed chain

In this section we elaborate a generalization of the classical freely jointed chain model in order to introduce the bistable



**Fig. 11** Effect of the temperature on the spinoidal behavior in the chain under Helmholtz conditions with N = 5. The average force  $\langle f \rangle \langle x \rangle$  (panel a), the effective stiffness  $k_{\text{eff}}(x)$  (panel b), and the average spin variable  $\langle y \rangle \langle x \rangle$  (panel c) are plotted versus x > 0 for different values of temperature (T = 600, 900, 1200, 1500, 1800 and 2100 K). We adopted the parameters  $\Delta E = 12.4 \times 10^{-20}$  J,  $\chi = 8$ ,  $\ell = 0.5$  nm and h = k = 0.0414 N m<sup>-1</sup>.



**Fig. 12** Critical behavior of the chain with multistable elements. The spinoidal intervals  $(x_i^-(T), x_i^+(T))$  on the *x*-axis (panel a), the spinoidal intervals  $(\langle f_i^+(T), \zeta_i^-(T) \rangle)$  on the  $\langle f_i^- \alpha x_i^+(T) \rangle$  on the  $\langle f_i^- \alpha x_i^-(T) \rangle$  on the  $\langle f_i^- \alpha x_i^$ 

behavior in the elements of the system. We develop the model in the three-dimensional space and we suppose that the potential energy of a single element of the chain is described by a bistable behavior (see Fig. 13, dashed blue line). The two potential wells can be represented by

$$U(\vec{r},s) = v(s) + \frac{1}{2}k(s)[||\vec{r}|| - \ell(s)]^2,$$
(43)

where  $\vec{r}$  is the vector joining the initial point with the final point of the element (see Fig. 13, red lines). The parameter  $s \in \{0,1\}$ represents a spin variable identifying two potential wells explored by the vector  $\vec{r}$ . The quantities v(s), k(s) and  $\ell(s)$  stand for the basal energy, the elastic stiffness and the equilibrium length of the potential wells, respectively. We remark that, in the classical freely jointed chain, the elastic stiffness diverges to infinity or, equivalently, the length of each element is kept constant. Consequently, the mechanical behavior is fully governed by entropic forces, with the elastic contribution being simply absent. Here, for mathematical convenience, it is better to proceed from eqn (43), with finite elastic constants,



**Fig. 13** Potential energy of a single element of the bistable freely jointed chain (dashed blue curve). The potential wells are approximated through two parabolic profiles identified by s = 0 and 1.

and to analyse the limiting cases in a following phase. We will study this system within both the Gibbs and Helmholtz ensembles.

#### 3.1 The Gibbs ensemble

We take into consideration a chain of N elements described by eqn (43) with a force applied to the last one. Hence, we can write the total potential energy of the system as

$$U_{\text{tot}}(\vec{q}, \vec{s}; \vec{f}) = \sum_{i=1}^{N} U(\vec{r}_i - \vec{r}_{i-1}, s_i) - \vec{f} \cdot \vec{r}_N,$$
(44)

where  $\vec{q} = (\vec{r}_1, ..., \vec{r}_N)$  is the generalized coordinate vector containing all positions  $\vec{r}_1, ..., \vec{r}_N, \vec{s} = (s_1, ..., s_N)$  is the vector of all spin variables, and  $\vec{f}$  is the force applied to the last element of the chain. The partition function can be therefore calculated by summing the discrete (spin-like) variables and integrating the continuous (coordinates) ones, as follows:

$$Z_{\rm G}(\vec{f}) = \sum_{s_{\rm I} \in \{0,1\}} \cdots \sum_{s_{\rm N} \in \{0,1\}} \int_{\Re^{3N}} e^{-\frac{U_{\rm tot}(\vec{q},\vec{s};f)}{k_{\rm B}T}} \mathrm{d}\vec{q}.$$
 (45)

The integral over the vector  $\vec{q}$  can be elaborated by means of the change of variables  $\vec{\xi}_1 = \vec{r}_1 - \vec{r}_0$ ,  $\vec{\xi}_2 = \vec{r}_2 - \vec{r}_1, \dots, \vec{\xi}_N = \vec{r}_N - \vec{r}_{N-1}$ , leading to  $\sum_{k=1}^N \vec{\xi}_k = \vec{r}_N - \vec{r}_0$  and  $d\vec{q} = d\vec{\xi}_1 \dots d\vec{\xi}_N$ . By fixing  $\vec{r}_0$  at the origins of the axes, we obtain

$$Z_{\rm G} = \left\{ \sum_{s \in \{0,1\}} \int_{\Re^3} \exp\left[ -\frac{U(\vec{\xi},s)}{k_{\rm B}T} + \frac{\vec{f} \cdot \vec{\xi}}{k_{\rm B}T} \right] \mathrm{d}\vec{\xi} \right\}^N, \tag{46}$$

which means that the partition function is multiplicative with respect to the elements of the chain. With the system being spherically symmetric, we can choose an arbitrary direction for the applied force. Hence, to further simplify eqn (46), we set  $\vec{f} = (0,0,f)$  and we change the variables according to  $\vec{\xi} = (\xi \cos \varphi \sin \vartheta, \xi \sin \varphi \sin \vartheta, \xi \cos \vartheta)$ . Since  $d\vec{\xi} = \xi^2 \sin \vartheta d\xi d\varphi d\vartheta$ ,

 $\|\vec{\xi}\| = \xi$  and  $\vec{f} \cdot \vec{\xi} = f\xi \cos \vartheta$ , we get the following simpler form of the partition function

 $Z_{G}$ 

$$= c \left\{ \sum_{s \in \{0,1\}} e^{-\frac{\nu(s)}{k_{\mathrm{B}}T}} \int_0^\infty \exp\left[-\frac{k(s)}{2k_{\mathrm{B}}T} [\xi - \ell(s)]^2\right] \times \frac{\sinh\left(\frac{f\xi}{k_{\mathrm{B}}T}\right)}{\frac{f\xi}{k_{\mathrm{B}}T}} \xi^2 \mathrm{d}\xi \right\}^N,$$
(47)

where c stands for a noninfluential multiplicative constant. As already observed for the one-dimensional model, within the Gibbs ensemble the elements of the chain do not interact and this point leads to a partition function which is in the form of a power with exponent N.

Now, we can explicitly specify the properties of the two potential wells, namely v(0) = 0,  $\ell(0) = \ell$ , k(0) = K and  $v(1) = \Delta E$ ,  $\ell(1) = \chi \ell$ , k(1) = K, where  $\chi$  is the ratio between the unfolded and folded equilibrium lengths. Then, we get

$$Z_{\rm G} = c \left\{ \int_0^\infty e^{-\frac{K}{2k_{\rm B}T}[\xi-\ell]^2} \frac{\sinh\left(\frac{f\xi}{k_{\rm B}T}\right)}{\frac{f\xi}{k_{\rm B}T}} \xi^2 d\xi + \phi \int_0^\infty e^{-\frac{K}{2k_{\rm B}T}[\xi-\chi\ell]^2} \frac{\sinh\left(\frac{f\zeta}{k_{\rm B}T}\right)}{\frac{f\xi}{k_{\rm B}T}} \xi^2 d\xi \right\}^N,$$

$$(48)$$

where  $\phi = \exp\left(-\frac{\Delta E}{k_{\rm B}T}\right)$ . This form of the partition function can be used to perform the limit for *K* approaching infinity, useful to properly define the bistable freely jointed chain. To this aim, we can use the Dirac delta function property  $\sqrt{\frac{\alpha}{\pi}}e^{-\alpha(x-x_0)^2} \xrightarrow[\alpha \to \infty]{} \delta(x-x_0)$ , eventually yielding

$$Z_{\rm G} = c \left\{ \frac{\sinh y}{y} + \chi \phi \frac{\sinh \chi y}{y} \right\}^N, \tag{49}$$

where we introduced the dimensionless force  $y = \frac{\ell f}{k_{\rm B}T}$ . When we remove the bistability from the system, the second term in eqn (49) vanishes, and we obtain

$$Z_{\rm G} = c \left\{ \frac{\sinh y}{y} \right\}^N,\tag{50}$$

which is the classical partition function of the freely jointed chain model.<sup>26,29,31</sup> The force–extension response for the bistable freely jointed chain can be found through the standard relation  $\langle r \rangle = k_{\rm B} T \frac{\partial \log Z_{\rm G}}{\partial f}$ , producing the important result

$$\langle r \rangle = N\ell \frac{\mathscr{L}(y) + \chi^2 \phi \mathscr{L}(\chi y) \frac{\sinh \chi y}{\sinh y}}{1 + \chi \phi \frac{\sinh \chi y}{\sinh y}},$$
(51)

where  $\mathscr{L}(y) = \coth y - \frac{1}{y}$  is the Langevin function. If we define the average value of the spin variables as  $\langle s \rangle = \frac{1}{N} \left\langle \sum_{i=1}^{N} s_i \right\rangle$ , it is not difficult to prove that  $\langle s \rangle = -k_{\rm B}T \frac{\partial \log Z_{\rm G}}{N \partial \Delta E}$ . We can therefore obtain the second important achievement

$$\langle s \rangle = \frac{\chi \phi \frac{\sinh \chi y}{\sinh y}}{1 + \chi \phi \frac{\sinh \chi y}{\sinh y}}.$$
(52)

By combining eqn (51) and (52), we can find the relationship

$$\langle r \rangle = N[(1 - \langle s \rangle)\ell \mathscr{L}(y) + \langle s \rangle \chi\ell \mathscr{L}(\chi y)], \qquad (53)$$

affirming that the average extension of the bistable system is given by a linear combination of the responses of an FJC model with length  $\ell$  and an FJC model with length  $\chi\ell$ , with the coefficients being controlled by the average value of the spin variable. In other words, when  $\langle s \rangle$  varies from 0 to 1 the element unfolds progressively with an effective equilibrium length increasing from  $\ell$  to  $\chi\ell$ .

An application of eqn (51) and (52) is shown in Fig. 14, where the average normalized extension and the normalized spin variable are represented *versus* the dimensionless force. In the force–extension curve, we observe a cooperative behavior characterized by the collective unfolding of all domains at the threshold value of the dimensionless force  $\frac{f^*\ell}{k_BT} = \frac{\Delta E}{(\chi - 1)k_BT}$ , as predicted in Section 2.1. This behavior is confirmed in the spin variable curve, where a clear transition from 0 to 1 is exhibited for the same threshold value of the force. Since we have developed the theory in the limit of  $K \to \infty$ , in the bistable freely jointed chain model we cannot observe a second transition for a larger force, as in the case shown in Fig. 6 of



**Fig. 14** Average normalized extension (red line) and normalized spin variable (blue line) *versus* the dimensionless force for the bistable freely jointed chain under Gibbs conditions. We adopted the parameters  $\Delta E = 30k_{\rm B}T$  and  $\chi = 3$  (at T = 300 K). The curves are independent of N within the Gibbs ensemble.

Section 2.2. Therefore, the results are correct for any value of the applied force.

#### 3.2 The Helmholtz ensemble

We consider now the chain of bistable elements with both endterminals tethered at the points  $\vec{r}_0 = \vec{0}$  and  $\vec{r}_N = \vec{r}$ . It means that the total potential energy of the system can be written as

$$U_{\text{tot}}^{\text{H}}(\vec{q}, \vec{s}; \vec{r}_{N}) = \sum_{i=1}^{N} U(\vec{r}_{i} - \vec{r}_{i-1}, s_{i}),$$
(54)

where  $\vec{r}_N = \vec{r}$  is the fixed extremity of the chain,  $\vec{q} = (\vec{r}_1, \dots, \vec{r}_{N-1})$  is the generalized coordinate vector containing all positions  $\vec{r}_1, \dots, \vec{r}_{N-1}$ , and  $\vec{s} = (s_1, \dots, s_N)$  is the vector of all spin variables. In eqn (54) the potential energy  $U(\vec{r}, s)$  of a single element is given in eqn (43). The partition function of this system can be written as

$$Z_{\rm H}(\vec{r}_N) = \sum_{s_1 \in \{0,1\}} \cdots \sum_{s_N \in \{0,1\}} \int_{\Re^{3(N-1)}} e^{-\frac{U_{\rm tot}^{\rm H}(\vec{q},\vec{s};\vec{r}_N)}{k_{\rm B}T}} d\vec{q}.$$
 (55)

By comparing eqn (45) and (55), we deduce that the two partition functions  $Z_{\rm G}$  and  $Z_{\rm H}$  are related through a three-dimensional bilateral Laplace transform, as follows:

$$Z_{\rm G}(\vec{f}) = \int_{\Re^3} Z_{\rm H}(\vec{r}) \exp\left(\frac{\vec{r} \cdot \vec{f}}{k_{\rm B}T}\right) d\vec{r}.$$
 (56)

Moreover, by considering the spherical symmetry of the problem we easily obtain the relationship

$$Z_{\rm H}(r) = c \int_{-\infty}^{+\infty} Z_{\rm G}(i\eta) \frac{\eta}{r} \sin \frac{\eta r}{k_{\rm B} T} \mathrm{d}\eta, \qquad (57)$$

and by substituting eqn (49) we get the important integral expression

$$Z_{\rm H}(r) = c \int_{-\infty}^{+\infty} \left\{ \frac{\sin y}{y} + \chi \phi \frac{\sin \chi y}{y} \right\}^N \frac{y}{r} \sin \frac{ry}{\ell} \mathrm{d}y.$$
(58)

Interestingly enough, when we remove the bistable behavior of the elements, we find the partition function

$$Z_{\rm H}(r) = c \int_{-\infty}^{+\infty} \left\{ \frac{\sin y}{y} \right\}^N \frac{y}{r} \sin \frac{ry}{\ell} dy, \tag{59}$$

which has been largely studied by Rayleigh,<sup>71</sup> Polya,<sup>72</sup> Treloar,<sup>73</sup> and Wang and Guth,<sup>74</sup> to analyze the behavior of chains and chain networks. Here, we elaborate eqn (58) in order to obtain a closed form expression useful to better explain the chain behavior within the Helmholtz ensemble. The function to integrate in eqn (58) is regular on the real axis and analytical on a strip  $|\Im my| < M$  for an arbitrary  $M \in \Re$ . Then, instead of integrating on the whole real axis we can use the path  $\Gamma$  shown in Fig. 15. Therefore, we can write

$$Z_{\rm H}(r) = -ic \int_{\Gamma} \left\{ \frac{\sin y}{y} + \chi \phi \frac{\sin \chi y}{y} \right\}^N \frac{y}{r} {\rm e}^{\frac{ry}{\ell}} {\rm d}y.$$
(60)

By developing the power in the previous expression and by using the expansion

$$\sin^{n} x = \frac{1}{(2i)^{n}} e^{inx} \sum_{t=0}^{n} \binom{n}{t} (-1)^{t} e^{-2itx},$$
 (61)



Fig. 15 Definition of the contour  $\Gamma$  on the complex plane.

we obtain

$$Z_{\rm H}(r) = \frac{c}{2^N i^{N+1} r} \sum_{k=0}^N \sum_{p=0}^{N-k} \sum_{q=0}^k \binom{N}{k} \binom{N-k}{p} \times \binom{k}{q} (-1)^{p+q} (\chi \phi)^k \int_{\Gamma} \frac{{\rm e}^{-iAy}}{y^{N-1}} {\rm d}y,$$
(62)

where we defined

2

$$\Lambda = k - N + 2p - \chi k + 2\chi q - \frac{r}{\ell}.$$
 (63)

We prove in Appendix A that

$$\int_{\Gamma} \frac{e^{iay}}{y^m} dy = \begin{cases} 0 & \text{if } a > 0\\ -2\pi i^m \frac{a^{m-1}}{(m-1)!} & \text{if } a \le 0 \end{cases},$$
(64)

and then we find from eqn (62) the result

. .

$$Z_{\rm H}(r) = \frac{\pi c}{2^{(N-1)}(N-2)!r} \sum_{k=0}^{N} \sum_{p=0}^{N-k} \sum_{q=0}^{k} \binom{N}{k} \binom{N-k}{p} \times \binom{k}{q} (-1)^{p+q} (\chi \phi)^{k} (-\Lambda)^{N-2} \mathbf{1}(\Lambda),$$
(65)

written in terms of the Heaviside step function 1(x), defined as 1(x) = 1 if  $x \ge 0$ , and 1(x) = 0 if x < 0. Similarly to the



**Fig. 16** Average dimensionless force (red line) and average spin variable (blue line) *versus* normalized extension for the bistable freely jointed chain under Helmholtz conditions. We adopted the parameters  $\Delta E = 30 k_B T$ ,  $\chi = 3$  and N = 20 (at T = 300 K). We also plotted for comparison the force–extension Gibbs response (dashed green line).



**Fig. 17** Force-extension responses of the bistable freely jointed chain with under Helmholtz (H) conditions for N = 10, 15, and 20. We also reported the Gibbs (G) response to show the equivalence of the ensembles in the thermodynamic limit. We adopted the parameters  $\Delta E = 30k_{\rm B}T$  and  $\chi = 3$ .

one-dimensional case, this partition function cannot be written as a power with exponent *N*. It means that within the Helmholtz ensemble there is an effective interaction among the elements induced by the boundary conditions.

The knowledge of the partition function allows us to obtain the force–extension response through the expression

$$\langle f \rangle = -k_{\rm B}T \frac{\partial \log Z_{\rm H}}{\partial r},$$
 (66)

and the average value of the spin variable, as follows:

$$\langle s \rangle = \left\langle \frac{1}{N} \sum_{i=1}^{N} s_i \right\rangle = -\frac{1}{N} k_{\rm B} T \frac{\partial \log Z_{\rm H}}{\partial \Delta E}.$$
 (67)

Of course, both  $\langle f \rangle$  and  $\langle s \rangle$  could be written in the closed form by performing the derivatives indicated. However, for the sake of brevity, we omit this development and we show an example of application of eqn (66) and (67) in Fig. 16. In the force– extension response, we can note the typical sawtooth curve corresponding to a non-cooperative process. It means that the domains unfold individually, one by one, as also confirmed by the average value of the spin variable, which exhibits a series of steps corresponding to each unfolding process.

In Fig. 17 we can also find the force–extension curve in the Helmholtz ensemble for different values of N = 10, 15 and 20. We note that for an increasing number N of elements, the Helmholtz response converges to the Gibbs one, by progressively reducing the pick-to-pick distance in the sawtooth pattern. Once again, this confirms the equivalence of the ensembles in the thermodynamic limit, as recently demonstrated for a large class of non-confined polymer chains.<sup>61,62</sup>

The temperature behavior of the bistable freely jointed chain and its spinoidal character can be studied as described in Section 2.3 for the simpler one-dimensional model. The corresponding results are shown in Fig. 18. Also in this case, the picks of the sawtooth curve are less pronounced for high temperatures. Therefore, the spinoidal intervals in the force-extension response, with negative elastic stiffness, degenerate to single points for specific critical temperatures (see Fig. 18). In particular, we plotted the sequence of intervals  $(x_i^{-}(T), x_i^{+}(T))$ , with i = 1, ..., N, as a function of temperature in the 300 K < T <2500 K interval. We observe that each interval degenerates to a single point  $x_i^-(T_i^c) = x_i^+(T_i^c)$  at the specific critical temperature  $T_i^c$  of the pick under consideration. Of course,  $T^c = \max_i \{T_i^c\}$ . For each interval of the x-axis with spinoidal behavior, we can determine the corresponding interval on the  $\langle f \rangle$ -axis. Therefore, we also show the parametric plot of  $(\langle f \rangle_i^+(T), x_i^-(T))$ and  $(\langle f \rangle_i^-(T), x_i^+(T))$  (i = 1, ..., N) for a temperature range from



**Fig. 18** Temperature behavior and spinoidal character of the bistable freely jointed chain. The spinoidal intervals ( $x_i^-(T)$ ,  $x_i^+(T)$ ) on the x-axis for 300 K < T < 2500 K (panel a), and the parametric plots of ( $\langle f \rangle_i^+(T), x_i^-(T)$ ) and ( $\langle f \rangle_i^-(T), x_i^+(T)$ ) for T in the range from 300 K to  $T^c$  (panel b) are shown. In the latter panel the colour gradation of the parametric plots corresponds to the temperature (blue for T = 300 K and red for  $T = T^c$ ). In the same panel, the dashed (yellow) line represents the force–extension response for T = 300 K. We adopted the parameters  $\Delta E = 12.4 \times 10^{-20}$  J,  $\ell = 0.5$  nm and  $\chi = 3$ .

T = 300 K (blue) to  $T = T^{c}$  (red). These curves correspond to the loci of the maxima and minima of the force–extension curve with varying temperature.

# 4 Conclusions

In this paper we introduced an equilibrium statistical mechanics methodology able to describe the thermal and elastic behavior of chains composed of bistable (or multistable) elements. Each element of the chain is associated with a discrete quantity (spin variable), whose values identify the (two or more) basins of the energy landscape. This approach permits considering simple quadratic forms for the energy wells, thus simplifying the calculus of the pertinent partition functions. Therefore, this technique can be applied to both isotensional and isometric conditions, which are the limiting cases employed in real force spectroscopy experiments. Besides, they correspond to the Gibbs and Helmholtz ensembles of the statistical mechanics. The closed form expressions for the corresponding partition functions are useful to directly evaluate the force-extension curves in good qualitative agreement with the plateau-like response and the sawtooth pattern observed in real measurements, under isotensional and isometric conditions, respectively. Moreover, the partition functions are necessary to evaluate the average values of the spin variables, which correspond in our system to the occupancy states of the potential energy basins. As a consequence, the variations of these average values with the applied force (Gibbs ensemble) or with the prescribed extension (Helmholtz ensemble) allow us to give a quantitative interpretation of the cooperative or non-cooperative response of the folding-unfolding process. As a matter of fact, a single transition in the average spin variable reveals a cooperative process characterized by a simultaneous folding/unfolding of the bistable units. On the other hand, a stepwise behavior reveals a non-cooperative process, where the units fold/unfold sequentially under the external action. Therefore, the average values of the spin variables represent a quantitative measure of the cooperativeness, which is a crucial point for the physical interpretation of the folding/unfolding processes. It is important to remark that some limitations can be considered to the range of admissible forces for the spin model, especially when we deal with a multi-basin energy profile described by different elastic responses. On the other hand, the approximation introduced by the use of the spin variables leads to a very high accuracy when we remain within the limits of applicability of the model.

Concerning the partition function within the Gibbs ensemble, we remark that its calculation can be directly performed in consequence of the introduction of the spin variables, which simplifies the mathematical form of the multi-basin energy landscape. Conversely, within the Helmholtz ensemble the partition function calculation is more involved because of the condition imposed prescribing the extension of the chain. To overcome this issue, we take advantage of the Laplace transform relationship existing between the Gibbs and Helmholtz

partition functions. This property is usually employed to demonstrate the equivalence of the different statistical ensembles in the thermodynamic limit. Indeed, it is not difficult to prove that for non-confined polymer chains the Laplace transformation of the partition functions turns into the Legendre transformation of the corresponding thermodynamic potentials for a large number of elements of the chain. Moreover, the Legendre transformation between the thermodynamic potentials leads to the same constitutive equation within different statistical ensembles, that is to say, to the equivalence of the ensembles.61,62 Differently, in our approach we exploit the Laplace transformation between the partition functions as an explicit method to make the calculation of the Helmholtz partition function easier. Instead of integrating the coordinates pertaining to all the chain elements, we can reduce the integration to only one variable (force or extension), describing the Laplace transform itself.

The proposed approach opens the possibility of studying more general situations, as summarized below. As discussed in the Introduction, the real force spectroscopy experiments are placed in between the Gibbs and Helmholtz statistical ensembles, depending on the device stiffness.<sup>37</sup> While in this work we dealt only with the limiting cases corresponding to the two pure statistical ensembles (soft or hard devices), it is possible to generalize the theory by considering a finite elasticity for the adopted device, thus defining a continuous class of statistical ensembles. Another important generalization concerns the type of interaction between contiguous chain elements. We considered here freely jointed chains with bistability. It is important to improve the theory by introducing semi-flexible chains, characterized by a specific persistence length. This point could lead to the definition of the bistable worm-like chain, which is the more appropriate model to describe the DNA over-stretching and the unfolding of several proteins.<sup>1</sup> From the statistical mechanics point of view, it means that one should study the Ising model with bistable or multistable magnetization. A further perspective concerns the dynamics of such folding/unfolding systems. While the dynamics of the occupancy state of the basins of the energy landscape have recently been studied,68 it would be interesting to study the complete dynamics of the system, including the continuous coordinates and the discrete spin variables. This point will encourage the study of stochastic processes composed of a mixing of continuous and discrete variables. In particular, we will envisage the development of Langevin and/or Fokker-Planck equations with mixed variables.

Finally, it is interesting not only to observe that the statistical mechanics of folding/unfolding processes for understanding the behavior of bistable chains but also to model other biological phenomena such as cell adhesion, flip-flopping of macromolecular hairpins and other allosteric transitions where the bistability is driven by biochemical actions.<sup>66</sup> Moreover, a sawtooth pattern appears in the force–extension response of several non-biological systems undergoing discrete phase transformations: ferromagnetic alloys, nano-indented substrates and plastic materials.<sup>59,69</sup>

# Conflicts of interest

There are no conflicts to declare.

## Appendix

#### A An integral evaluation

We calculate here the integral  $\int_{\Gamma} \frac{e^{iay}}{y^m} dy$  over the contour  $\Gamma$  shown in Fig. 15. To begin with, we suppose  $a \in \Re$  and a > 0. In this case, we observe that on the imaginary axis we have  $e^{iay} = e^{-a\Im my}$ , which is a decreasing to zero function for  $\Im my > 0$ . So, we consider the contour in Fig. 19 and we write

$$\oint_{\Gamma_R \cup C_R} \frac{e^{iay}}{y^m} dy = 0,$$
(68)

since the function is holomorphic within  $\Gamma_R \cup C_R$ . We also have

$$\int_{\Gamma_R} \frac{e^{iay}}{y^m} dy + \int_{C_R} \frac{e^{iay}}{y^m} dy = 0.$$
 (69)

Since  $\lim_{R \to \infty} \int_{C_R} \frac{e^{iay}}{y^m} dy = 0$  for the Jordan lemma and  $\Gamma_R \to \Gamma$ when  $R \to \infty$ , we obtain the first result

$$\int_{\Gamma} \frac{\mathrm{e}^{iay}}{y^m} \mathrm{d}y = 0 \text{ if } a > 0.$$
(70)

We consider now the case with a < 0. On the imaginary axis we have  $e^{iay} = e^{-a\Im my}$ , which is a decreasing to zero function for  $\Im my < 0$ . Therefore, we introduce the contour shown in Fig. 20. The function is not holomorphic within  $\Gamma_R \cup C_R'$  since it presents a pole of order *m* for y = 0. Hence, we have

$$\oint_{\Gamma_R \cup C_R'} \frac{e^{iay}}{y^m} dy = -2\pi i \operatorname{Res}\left\{\frac{e^{iay}}{y^m}, 0\right\},\tag{71}$$

or

$$\int_{\Gamma_R} \frac{e^{iay}}{y^m} dy + \int_{C_R} \frac{e^{iay}}{y^m} dy = -2\pi i \operatorname{Res}\left\{\frac{e^{iay}}{y^m}, 0\right\}.$$
 (72)

Since a < 0, for the Jordan lemma we have  $\lim_{R \to \infty} \int_{C_R} \frac{e^{iay}}{y^m} dy = 0$ .



Fig. 19 Contour in the complex plane used to solve the integral for a > 0.



Fig. 20 Contour in the complex plane used to solve the integral for a < 0.

Moreover, the residue can be calculated as follows:

$$\operatorname{Res}\left\{\frac{e^{iay}}{y^{m}},0\right\} = \frac{1}{(m-1)!} \lim_{y \to 0} \frac{d^{m-1}}{dy^{m-1}} \left(y^{m} \frac{e^{iay}}{y^{m}}\right)$$
  
$$= \frac{(ia)^{m-1}}{(m-1)!}.$$
(73)

Summing up, we easily obtain the second result

$$\int_{\Gamma} \frac{e^{iay}}{y^m} dy = -2\pi i^m \frac{a^{m-1}}{(m-1)!} \text{ if } a \le 0.$$
 (74)

To conclude, eqn (70) and (74) prove eqn (64) of the main text.

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