Electronic Supplementary Information

"On the influence of device handle in single-molecule experiments"

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We report for the reader convenience the analytical details of the model proposed in the paper. To this end we refer to the equations in the main paper using the same notation, whereas we denote with ESI-(.) the equation (.) of this Electronic Supplementary Information. Moreover according with the main paper notation we denote with the subscripts m, d, and t to denote variables referred to the molecule, the device and the total (molecule plus device) system, respectively.

1 Hamiltonian of the system and basic definitions

The system is composed of n mass points with mass m connected by bistable springs with modulus k_m and a loading device with mass M represented as an $n+1$ spring with modulus k_d. The Hamiltonian function can be written as the sum of kinetic and elastic energy

$$H = E_K + V_t = \sum_{i=1}^{n} \frac{1}{2m} p_i^2 + \frac{1}{2M} p_{n+1}^2 + \sum_{i=1}^{n} \frac{1}{2} k_m l (\varepsilon_i - \varepsilon_u \chi_i)^2 + \frac{1}{2} k_d \alpha L \varepsilon_d^2, \tag{ESI-1}$$

where ε_u is the reference strain of the unfolded configuration, $l = L/n$ is the reference length of each element, $\alpha L \varepsilon_d$ is the elongation of the device and p_i are the momentum of the i-th oscillator. Here χ_i is an internal variable that can assume values 0 or 1 if the i-th element is *folded* or *unfolded*, respectively. We consider different boundary conditions acting on the device: assigned displacement d (*hard device*) or assigned force F (*soft device*). In the framework of equilibrium Statistical Mechanics, these two cases are described by the Helmholtz and Gibbs ensembles, respectively. The ideal hard and soft device, with assigned displacements and force, respectively, acting directly on the molecule are obtained as limit systems. Finally we consider the mechanical limit when entropic effects can be neglected and the thermodynamic limit when the number n of elements diverges.

The total displacement can be expressed as

$$d = \sum_{i=1}^{n} l \varepsilon_i + \alpha L \varepsilon_d = L(\varepsilon_m + \alpha \varepsilon_d) \tag{ESI-2}$$

where ε_m is the average strain of the molecule

$$\varepsilon_m = \frac{1}{n} \sum_{i=1}^{n} \varepsilon_i. \tag{ESI-3}$$

The relation between ε_m and the total strain ε_t is the following:

$$\varepsilon_t = \frac{d}{L(1+\alpha)} \Rightarrow (1+\alpha)\, \varepsilon_t = \varepsilon_m + \alpha\, \varepsilon_d. \tag{ESI-4}$$

By using (ESI-4) we can express the device strain as

$$\alpha\, \varepsilon_d = (1+\alpha)\, \varepsilon_t - \varepsilon_m.$$

The equilibrium condition requires a constant force F:

$$k_m(\varepsilon_i - \chi_i\varepsilon_u) = k_d\,\varepsilon_d = F.$$

By averaging with respect to I we then get the relation between the chain and the device strain

$$k_d\,\varepsilon_d = k_m(\varepsilon_m - \varepsilon_u\bar{\chi})$$

where $\bar{\chi} = \frac{\sum_{i=1}^n \chi_i}{n}$ is the fraction of unfolded domains. After introducing the non-dimensional parameter

$$\gamma = \frac{k_d}{k_d + \alpha\, k_p} \in [0, 1],$$

measuring the relative device vs total stiffness, by using (ESI-4) we get

$$\varepsilon_m = (1+\alpha)\gamma\,\varepsilon_t + (1-\gamma)\bar{\chi}\,\varepsilon_u. \tag{ESI-5}$$

2 Helmholtz Ensemble

Consider first the case of hard device, when the total displacement d is fixed. In this case we have to consider the partition function in the Helmholtz ensemble defined as

$$Z_{\mathscr{H}} = \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^{2(n+1)}} e^{-\beta H} \delta\Big(\sum_{i=1}^n l\varepsilon_i + \alpha L\varepsilon_d - d\Big) \prod_i^n dp_i\, dp_d \prod_i^n l\, d\varepsilon_i\, (\alpha L)\, d\varepsilon_d,$$

where $\beta = 1/k_B T$, k_B being the Boltzmann constant, T the absolute temperature and $\boldsymbol{\chi} = \{\chi_1, \ldots, \chi_n\} \in \{0,1\}^n$ is the vector denoting the phase (folded of unfolded) configuration. For the sake of simplicity here and in what follows we drop the domain of the vector spin variable $\boldsymbol{\chi}$. We used the Dirac function to enforce the displacement constraint (ESI-2). We can separate the contributions to $Z_{\mathscr{H}}$ of the kinetic energy and of the potential energy, so that we can split up the integral over the momenta and over the strains, respectively:

$$Z_{\mathscr{H}} = \alpha L\, l^n \int_{\mathbb{R}^{(n+1)}} e^{-\beta E_k} \prod_{i=1}^n dp_i\, dp_d \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^{(n+1)}} e^{-\beta V_t} \delta\Big(\sum_{i=1}^n l\varepsilon_i + \alpha L\varepsilon_d - d\Big) \prod_{i=1}^n d\varepsilon_i\, d\varepsilon_d.$$

We solve the Gaussian integral over the momenta to obtain

$$Z_{\mathscr{H}} = A\alpha L l^n \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^{(n+1)}} e^{-\beta\left(\sum_i \frac{1}{2}k_m l(\varepsilon_i - \varepsilon_u\chi_i)^2 + \frac{1}{2}\frac{k_d}{\alpha L}(\alpha L\varepsilon_d)^2\right)} \delta\Big(\sum_i l\varepsilon_i + \alpha L\varepsilon_d - d\Big) \prod_i d\varepsilon_i\, d\varepsilon_d,$$

where

$$A = (2\pi)^{(n+1)/2} \left(\frac{m}{\beta}\right)^{n/2} \left(\frac{M}{\beta}\right)^{1/2}. \tag{ESI-6}$$

We can also integrate out the free variable ε_d to obtain

$$Z_{\mathscr{H}} = A\, l^n \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^n} e^{-\beta\left(\sum_i \frac{1}{2}k_m l(\varepsilon_i - \varepsilon_u\chi_i)^2 + \frac{1}{2}\frac{k_d}{\alpha L}\left(\sum_i l\varepsilon_i - d\right)^2\right)} \prod_i d\varepsilon_i.$$

By using (ESI-4) we get

$$Z_{\mathscr{H}} = A\, l^n \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^n} e^{-\frac{\beta l k_m}{2}\left(\sum_i (\varepsilon_i - \varepsilon_u\chi_i)^2 + \eta n\left(\frac{1}{n}\sum_i \varepsilon_i - \varepsilon_t(1+\alpha)\right)^2\right)} \prod_i d\varepsilon_i, \tag{ESI-7}$$

where

$$\eta = \frac{k_d}{\alpha k_m} = \frac{\gamma}{1-\gamma} \quad \text{with} \quad \eta \in [0, +\infty[\,. \tag{ESI-8}$$

In order to solve the Gaussian integrals, we rearrange the exponent in the partition function as follows:

$$
-\frac{\beta l k_m}{2}\left(\left(1+\frac{\eta}{n}\right)\sum_{i=1}^{n}\varepsilon_i^2+\frac{\eta}{n}\sum_{i,j=1,i\neq j}^{n}\varepsilon_i\varepsilon_j-2\sum_{i=1}^{n}(\varepsilon_u\chi_i+\eta\varepsilon_t(1+\alpha))\varepsilon_i+\eta n\varepsilon_t^2(1+\alpha)^2+\varepsilon_u^2\sum_{i=1}^{n}\chi_i^2\right)=
$$
$$
-\frac{1}{2}\boldsymbol{A}\boldsymbol{\varepsilon}\cdot\boldsymbol{\varepsilon}+\boldsymbol{b}\cdot\boldsymbol{\varepsilon}+C, \tag{ESI-9}
$$

where we have introduced

$$
\boldsymbol{A}=\beta k_m l\begin{pmatrix}1+\frac{\eta}{n} & \frac{\eta}{n} & \cdots & \frac{\eta}{n}\\ \frac{\eta}{n} & \ddots & & \vdots\\ \vdots & & \ddots & \vdots\\ \frac{\eta}{n} & \cdots & \cdots & 1+\frac{\eta}{n}\end{pmatrix}, \tag{ESI-10}
$$
$$
\boldsymbol{b}=\{\beta k_m l\big(\varepsilon_u\chi_1+\eta\,\varepsilon_t(1+\alpha)\big),\ldots,\beta k_m l\big(\varepsilon_u\chi_n+\eta\,\varepsilon_t(1+\alpha)\big)\}^T, \tag{ESI-11}
$$
$$
\boldsymbol{\varepsilon}=\{\varepsilon_1,\ldots,\varepsilon_n\}^T, \tag{ESI-12}
$$

and

$$
C=-\frac{\beta k_m l}{2}\left(n\,\eta\varepsilon_t^2(1+\alpha)^2+\varepsilon_u^2\sum_{i=1}^{n}\chi_i^2\right)
$$

is a constant energy term. The Gaussian integration of quadratic functions can be solved explicitly (see *e.g.* [3] giving

$$
\int_{\mathbb{R}^n} e^{-\frac{1}{2}\boldsymbol{A}\boldsymbol{\varepsilon}\cdot\boldsymbol{\varepsilon}+\boldsymbol{b}\cdot\boldsymbol{\varepsilon}+C}d\boldsymbol{\varepsilon}=\sqrt{\frac{(2\pi)^n}{\det\boldsymbol{A}}}\,e^{\frac{1}{2}\boldsymbol{A}^{-1}\boldsymbol{b}\cdot\boldsymbol{b}+C}.
$$

Thus, we obtain

$$
Z_{\mathscr{H}}=K_{\mathscr{H}}\sum_{\boldsymbol{\chi}}e^{\frac{\beta l k_m}{2}\left(\sum_i\left(\varepsilon_u\chi_i+\eta\varepsilon_t(1+\alpha)\right)-\frac{\gamma}{n}\left(\sum_i\left(\varepsilon_u\chi_i+\eta\varepsilon_t(1+\alpha)\right)\right)^2-\varepsilon_u^2\sum_i\chi_i^2-\eta n\varepsilon_t^2(1+\alpha)^2\right)}
$$

with

$$
K_{\mathscr{H}}=A\,l^n\left(\frac{2\pi}{\beta k_m l}\right)^{n/2}(1-\gamma)^{1/2}.
$$

We observe that, due to the absence of non-local energy terms, all solutions with the same unfolded fraction $\bar{\chi}$ are characterized by the same energy. As a result the partition function describing the chain and the apparatus as a whole is

$$
Z_{\mathscr{H}}=K_{\mathscr{H}}\sum_{p=0}^{n}\binom{n}{p}e^{-\frac{\beta k_m l n\gamma}{2}\left(\frac{p}{n}\varepsilon_u-\varepsilon_t(1+\alpha)\right)^2}. \tag{ESI-13}
$$

Notice that the binomial coefficient gives the number of iso-energetic configurations for fixed value of p.

We then deduce that the Helmholtz free energy is given by

$$
\mathcal{F}=-\frac{1}{\beta}\ln Z_{\mathscr{H}}
$$

and the expectation value of the force can be obtained as

$$
\langle F\rangle=\frac{1}{L(1+\alpha)}\frac{\partial\mathcal{F}}{\partial\varepsilon_t}=-\frac{1}{\beta L(1+\alpha)}\frac{1}{Z_{\mathscr{H}}}\frac{\partial Z_{\mathscr{H}}}{\partial\varepsilon_t}. \tag{ESI-14}
$$

Observe that the force-strain relation can be written in the same form of Eq.(11) of the main paper

$$
\langle F\rangle=k_m\gamma(\varepsilon_t(1+\alpha)-\varepsilon_u\langle\bar{\chi}\rangle) \tag{ESI-15}
$$

after introducing the (temperature dependent) expectation value of the unfolded fraction

$$\langle\bar{\chi}\rangle = \langle\bar{\chi}\rangle_{\mathscr{H}}(\beta,\varepsilon_t) = \frac{\displaystyle\sum_{p=0}^{n}\binom{n}{p}\frac{p}{n}\,e^{-\frac{\beta k_m l n\gamma}{2}\left(\frac{p}{n}\varepsilon_u-\varepsilon_t(1+\alpha)\right)^2}}{\displaystyle\sum_{p=0}^{n}\binom{n}{p}\,e^{-\frac{\beta k_m l n\gamma}{2}\left(\frac{p}{n}\varepsilon_u-\varepsilon_t(1+\alpha)\right)^2}}. \tag{ESI-16}$$

In order to evaluate the expectation value of the molecule strain, it is convenient to start from the expression (ESI-7). We have

$$\langle\varepsilon_m\rangle = \frac{A}{Z_{\mathscr{H}}}\sum_{\boldsymbol{\chi}}\int_{\mathbb{R}^n}\left(\frac{1}{n}\sum_{i=1}^{n}\varepsilon_i\right)e^{-\frac{\beta l k_m}{2}\left(\sum_i(\varepsilon_i-\varepsilon_u\chi_i)^2+\eta n\left(\frac{1}{n}\sum_i\varepsilon_i-\varepsilon_t(1+\alpha)\right)^2\right)}\prod_{i=1}^{n} l\,d\varepsilon_i,$$

where A is given by (ESI-6). It is straightforward to show that

$$\frac{1}{L(1+\alpha)}\frac{1}{Z_{\mathscr{H}}}\frac{\partial Z_{\mathscr{H}}}{\partial\varepsilon_t} = -\beta\,k_m\frac{\gamma}{1-\gamma}(\varepsilon_t(1+\alpha)-\langle\varepsilon_m\rangle), \tag{ESI-17}$$

and, thus,

$$\langle\varepsilon_m\rangle = (1+\alpha)\varepsilon_t - \frac{1-\gamma}{k_m\gamma}\left(-\frac{1}{\beta L(1+\alpha)}\frac{1}{Z_{\mathscr{H}}}\frac{\partial Z_{\mathscr{H}}}{\partial\varepsilon_t}\right) = \varepsilon_u\langle\bar{\chi}\rangle + \gamma\big(\varepsilon_t(1+\alpha)-\varepsilon_u\langle\bar{\chi}\rangle\big). \tag{ESI-18}$$

with the same form of the mechanical limit in Eq.(12). Finally, we have

$$\langle F\rangle = k_m\left(\langle\varepsilon_m\rangle - \varepsilon_u\langle\bar{\chi}\rangle\right), \tag{ESI-19}$$

again respecting the results in Eq.(14) of the mechanical limit, with the variation due to the expectation value of $\bar{\chi}$ in (ESI-16).

3 Gibbs Ensemble

Consider now the case of assigned force (soft device). The partition function for the Gibbs canonical ensemble is

$$Z_{\mathscr{G}} = \sum_{\boldsymbol{\chi}}\int_{\mathbb{R}^{2(n+1)}} e^{-\beta\left(H-F\left(\sum_{i=1}^{n} l\varepsilon_i+\alpha L\varepsilon_d\right)\right)}\prod_i dp_i\,dp_d\prod_i l\,d\varepsilon_i\,(\alpha L)d\varepsilon_d,$$

where the Hamiltonian is defined in (ESI-1). We obtain

$$\begin{aligned}
Z_{\mathscr{G}} &= A(\alpha L\,l^n)\sum_{\boldsymbol{\chi}}\int_{\mathbb{R}^{(n+1)}} e^{-\beta\left(\frac{1}{2}\sum_{i=1}^{n}\left(k_m l(\varepsilon_i-\varepsilon_u\chi_i)^2-Fl\varepsilon_i\right)+\frac{1}{2}\frac{k_d}{\alpha L}(\alpha L\varepsilon_d)^2-F\alpha L\varepsilon_d\right)}\prod_{i=1}^{n}d\varepsilon_i\,d\varepsilon_d\\
&= A(\alpha L\,l^n)\sum_{\boldsymbol{\chi}}\int_{\mathbb{R}^{(n+1)}} e^{-\frac{\beta l k_m}{2}\left(\sum_i\left((\varepsilon_i-\varepsilon_u\chi_i)^2-\frac{2F}{k_m}\varepsilon_i\right)+\frac{1-\gamma}{\gamma}n(\alpha\varepsilon_d)^2-\frac{2F}{k_m}n\alpha\varepsilon_d\right)}\prod_i d\varepsilon_i\,d\varepsilon_d\\
&= A(\alpha L\,l^n)\,I_m\,I_d,
\end{aligned}$$

where A has the same value (ESI-6) obtained in the case of assigned displacement, we used Eq. (ESI-8), I_m and I_d correspond to the integration with respect to the ε_i and ε_d, respectively. We easily obtain

$$I_d = C_{\mathscr{G}}\,e^{\frac{\beta l n}{2k_m\gamma}(1-\gamma)\,F^2} \tag{ESI-20}$$

where we have defined the constant

$$C_{\mathscr{G}} = \frac{1}{\alpha}\left(\frac{2\pi(1-\gamma)}{\beta k_m l\gamma n}\right)^{1/2}.$$

On the other hand, the integral I_m can be rewritten as

$$I_m = \sum_{\boldsymbol{\chi}} \prod_{i=1}^{n} \int_{\mathbb{R}} e^{-\frac{\beta l k_m}{2}\left((\varepsilon_i-\varepsilon_u\chi_i)^2-\frac{2F}{k_m}\varepsilon_i\right)} d\varepsilon_i = \left(\frac{2\pi}{\beta k_m l}\right)^{n/2} \sum_{\boldsymbol{\chi}} \prod_{i=1}^{n} e^{\frac{\beta l}{2k_m}\left(F^2+F\,2k_m\varepsilon_u\chi_i\right)}.$$

Also in this case, we may observe that, due to the absence of non local energy terms, the energy of the solutions with the same unfolded fraction is invariant with respect to the permutation of the elements. Thus, we obtain the analytic expression

$$\begin{aligned} I_m &= \left(\frac{2\pi}{\beta k_m l}\right)^{n/2} \sum_{p=0}^{n} \binom{n}{p} \left(e^{\frac{\beta l}{2k_m}F^2}\right)^{n-p} \left(e^{\frac{\beta l}{2k_m}(F^2+F\,2k_m\varepsilon_u)}\right)^{p} \\ &= \left(\frac{2\pi}{\beta k_m l}\right)^{n/2} \sum_{p=0}^{n} \binom{n}{p} e^{\frac{\beta l n}{2k_m}\left(F^2+F\,2k_m\varepsilon_u\frac{p}{n}\right)} = \left(\frac{2\pi}{\beta k_m l}\right)^{n/2} e^{\frac{\beta l n}{2k_m}F^2}\left(1+e^{\beta l\varepsilon_u F}\right)^n. \quad \text{(ESI-21)} \end{aligned}$$

Finally, we find the partition function in the Gibbs ensemble:

$$Z_{\mathscr{G}} = K_{\mathscr{G}}\, e^{\frac{\beta l n}{2k_m\gamma}F^2}\left(1+e^{\beta l\varepsilon_u F}\right)^n, \quad \text{(ESI-22)}$$

where

$$K_{\mathscr{G}} = A\left(\alpha L\, l^n\right)\left(\frac{2\pi}{\beta k_m l}\right)^{n/2} C_{\mathscr{G}}.$$

Based on this result we can deduce the constitutive force-strain relation in the case of assigned force. By using the definition of average strain (ESI-3), we get

$$\langle\varepsilon_m\rangle = \frac{A\left(\alpha L\, l^n\right) I_d}{Z_{\mathscr{G}}} \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^n} \left(\frac{1}{n}\sum_{i=1}^{n}\varepsilon_i\right) e^{-\frac{\beta l k_m}{2}\sum_i\left((\varepsilon_i-\varepsilon_u\chi_i)^2-\frac{2F}{k_m}\varepsilon_i\right)} \prod_{i=1}^{n} d\varepsilon_i. \quad \text{(ESI-23)}$$

This can be rewritten as

$$\langle\varepsilon_m\rangle = \frac{A\left(\alpha L\, l^n\right) I_d}{Z_{\mathscr{G}}} \sum_{\boldsymbol{\chi}} \frac{1}{n} \sum_{i=1}^{n} \left(\prod_{j=1}^{i-1}\int_{\mathbb{R}^{i-1}} e^{-\tilde{\beta}h(\varepsilon_j,\chi_j)}d\varepsilon_j \int_{\mathbb{R}} \varepsilon_i\, e^{-\tilde{\beta}h(\varepsilon_i,\chi_i)}d\varepsilon_i \prod_{k=i+1}^{n}\int_{\mathbb{R}^{n-i-1}} e^{-\tilde{\beta}h(\varepsilon_k,\chi_k)}d\varepsilon_k\right)$$

where $\tilde{\beta} = \beta l k_m/2$ and

$$h(\varepsilon,\chi) = \left((\varepsilon-\varepsilon_u\chi)^2 - \frac{2F}{k_m}\varepsilon\right).$$

Thus, we have a product of simple Gaussian integrals (the integral over ε_i requires an integration by parts). The solution can be written as

$$\begin{aligned} \langle\varepsilon_m\rangle &= \left(\frac{2\pi}{k_m\beta l}\right)^{n/2} \frac{A\left(\alpha L\, l^n\right) I_d}{Z_{\mathscr{G}}} \sum_{\boldsymbol{\chi}} \frac{1}{n}\sum_{i=1}^{n}\left(\frac{1}{k_m}\left(F+k_m\varepsilon_u\chi_i\right)e^{\frac{\beta l}{2k_m}(F^2+2Fk_m\varepsilon_u\chi_i)} \times \right. \\ &\quad \left. \times \prod_{j=1}^{i-1} e^{\frac{\beta l}{2k_m}(F^2+2Fk_m\varepsilon_u\chi_j)} \times \prod_{k=i+1}^{n} e^{\frac{\beta l}{2k_m}(F^2+2Fk_m\varepsilon_u\chi_k)}\right) \\ &= \left(\frac{2\pi}{k_m\beta l}\right)^{n/2} \frac{A\left(\alpha L\, l^n\right) I_d}{Z_{\mathscr{G}}} \sum_{\boldsymbol{\chi}} \frac{1}{n}\sum_{i=1}^{n}\left(\frac{1}{k_m}\left(F+k_m\varepsilon_u\chi_i\right)\prod_{j=1}^{n} e^{\frac{\beta l}{2k_m}(F^2+2Fk_m\varepsilon_u\chi_j)}\right) \text{(ESI-24)} \end{aligned}$$

By simplifying (ESI-24) we get

$$\begin{aligned} \langle\varepsilon_m\rangle &= \left(\frac{2\pi}{k_m\beta l}\right)^{n/2} \frac{A\left(\alpha L\, l^n\right) I_d}{Z_{\mathscr{G}}} \sum_{\boldsymbol{\chi}} \frac{1}{n}\sum_{i=1}^{n}\left(\frac{1}{k_m}\left(F+k_m\varepsilon_u\chi_i\right)\prod_{j=1}^{n} e^{\frac{\beta l}{2k_m}(F^2+2Fk_m\varepsilon_u\chi_j)}\right) \\ &= \left(\frac{2\pi}{k_m\beta l}\right)^{n/2} \frac{A\left(\alpha L\, l^n\right) I_d}{Z_{\mathscr{G}}} \sum_{\boldsymbol{\chi}} \left(\left(\frac{F}{k_m}+\varepsilon_u\frac{1}{n}\sum_{i=1}^{n}\chi_i\right)\prod_{j=1}^{n} e^{\frac{\beta l}{2k_m}(F^2+2Fk_m\varepsilon_u\chi_j)}\right) \\ &= \left(\frac{2\pi}{k_m\beta l}\right)^{n/2} \frac{A\left(\alpha L\, l^n\right) I_d}{Z_{\mathscr{G}}} \left(\sum_{p=0}^{n}\binom{n}{p}\left(\frac{F}{k_m}+\varepsilon_u\frac{p}{n}\right) e^{\frac{\beta l n}{2k_m}\left(F^2+F\,2k_m\varepsilon_u\frac{p}{n}\right)}\right), \quad \text{(ESI-25)} \end{aligned}$$

where in the last equality we followed the same procedure used in (ESI-21). Finally, using the expression (ESI-22) of the partition function and the integrals I_m, I_d we obtain

$$\langle \varepsilon_m \rangle = \frac{F}{k_m} + \varepsilon_u \langle \bar{\chi} \rangle \tag{ESI-26}$$

that again has the same form of the molecular response Eq.(14) in the purely mechanical approximation, but in this case we consider the expectation value of the unfolded fraction $\langle \bar{\chi} \rangle$ in the Gibbs ensemble

$$\langle \bar{\chi} \rangle = \langle \bar{\chi} \rangle_{\mathscr{G}}(\beta, F) = \frac{\displaystyle\sum_{p=0}^{n} \binom{n}{p} \frac{p}{n} e^{\frac{\beta l n}{2k_m} \left(F^2 + F\, 2k_m \varepsilon_u \frac{p}{n}\right)}}{\displaystyle\sum_{p=0}^{n} \binom{n}{p} e^{\frac{\beta l n}{2k_m} \left(F^2 + F\, 2k_m \varepsilon_u \frac{p}{n}\right)}} = \frac{e^{F l \beta \varepsilon_u}}{1 + e^{F l \beta \varepsilon_u}}. \tag{ESI-27}$$

By definition, the Gibbs free energy is

$$\mathcal{G} = -\frac{1}{\beta} \ln Z_{\mathscr{G}}$$

and the expectation value of the total strain of the system, which is the variable conjugated to the force, can be obtained as

$$\langle \varepsilon_t \rangle = \frac{1}{\beta L (1+\alpha)} \frac{1}{Z_{\mathscr{G}}} \frac{\partial}{\partial F} Z_{\mathscr{G}}. \tag{ESI-28}$$

This leads to

$$\langle \varepsilon_t \rangle = \frac{1}{(1+\alpha)} \left(\frac{F}{k_m \gamma} + \varepsilon_u \langle \bar{\chi} \rangle \right), \tag{ESI-29}$$

where we have used (ESI-27), that has the same form of Eq.(11).

From (ESI-26) and (ESI-29) we can obtain the relation between $\langle \varepsilon_t \rangle$ and $\langle \varepsilon_m \rangle$

$$\langle \varepsilon_m \rangle = \varepsilon_u \langle \bar{\chi} \rangle + \gamma \big((1+\alpha) \langle \varepsilon_t \rangle - \langle \bar{\chi} \rangle \varepsilon_u \big). \tag{ESI-30}$$

consistent with Eq.(11) of the mechanical limit.

4 From Helmholtz to Gibbs ensembles: Laplace Transform

As well known [2], the partition functions in the Gibbs and Helmholtz ensembles are connected by a Laplace transform with the force F and the total displacement d as conjugate variables. From (ESI-13) we have

$$\begin{aligned}
\int_{\mathbb{R}} Z_{\mathscr{H}}\, e^{\beta F d} dd &= (1+\alpha) L \int_{\mathbb{R}} Z_{\mathscr{H}}\, e^{\beta \left(F \varepsilon_t L (1+\alpha)\right)} d\varepsilon_t \\
&= K_{\mathscr{H}} (1+\alpha) L \sum_{p=0}^{n} \binom{n}{p} \int_{\mathbb{R}} e^{-\beta l n \left(\frac{k_m \gamma}{2} \left(\frac{p}{n} \varepsilon_u - \varepsilon_t (1+\alpha) \right)^2 - F \varepsilon_t (1+\alpha) \right)} d\varepsilon_t \\
&= K_{\mathscr{H}} L \left(\frac{2\pi}{k_m l n \beta \gamma} \right)^{1/2} \sum_{p=0}^{n} \binom{n}{p} e^{\frac{\beta l n}{2 k_m \gamma} \left(F^2 + F\, 2 k_m \gamma \varepsilon_u \frac{p}{n} \right)} \\
&= K_{\mathscr{G}}\, e^{\frac{\beta l n}{2 k_m \gamma} F^2} \left(1 + e^{\beta l \varepsilon_u F} \right)^n = Z_{\mathscr{G}},
\end{aligned} \tag{ESI-31}$$

which is exactly the result obtained in (ESI-22). The other quantities $\langle \varepsilon_t \rangle$ and $\langle \varepsilon_m \rangle$ in the Gibbs ensemble can be obtained accordingly.

5 Thermodynamic limit

In this section we show how to evaluate the expression of the phase fraction expression (ESI-16) in the thermodynamic limit by using the saddle point method [3]. According to previous discussion the dependence of the response from temperature, device stiffness and discreteness parameter n is measured by the expectation value of the unfolded fraction, being the other expectation values of mechanical variable related by the same equations Eq.(11), Eq.(12), Eq.(14).

Since in the Gibbs ensemble, the formula (ESI-27) does not depend on n the thermodynamical limit behavior coincides with the one of systems with finite discreteness. We then need to study only the limit the Helmholtz ensemble $\langle\bar{\chi}\rangle$ in (ESI-16). To this end, we start considering the function f defined as

$$f(\varepsilon_t) = \sum_{p=0}^{n} \binom{n}{p} e^{-\frac{\beta k_m l n \gamma}{2}\left(\frac{p}{n}\varepsilon_u - \varepsilon_t(1+\alpha)\right)^2}. \tag{ESI-32}$$

Using the Stirling approximation, $n! \sim \left(\frac{n}{e}\right)^n \sqrt{2\pi n}$ for $n \gg 1$, (ESI-32) can be written as

$$f(\varepsilon_t) \simeq \frac{1}{\sqrt{2\pi n}} \sum_{p=0}^{n} \sqrt{\frac{1}{p/n(1-p/n)}}\, e^{n\ln n - p\ln p - (n-p)\ln(n-p) - \frac{\beta k_m l n\gamma}{2}\left(\frac{p}{n}\varepsilon_u - (1+\alpha)\varepsilon_t\right)^2},$$

where we considered both n and p large. To deduce the themodynamic limit, let us introduce the variable $x = p/n$. In the limit of large n we obtain

$$f(\varepsilon_t) \simeq \sqrt{\frac{n}{2\pi}} \int_0^1 \sqrt{\frac{1}{x(1-x)}}\, e^{-n\left(S(x) + \frac{\beta k_m l\gamma}{2}(x\,\varepsilon_u - (1+\alpha)\varepsilon_t)^2\right)} \mathrm{d}x$$

where we defined the (entropy) function

$$S(x) = x\,\mathrm{ln}x + (1-x)\mathrm{ln}\,(1-x).$$

Finally, for large n we can apply the saddle point approximation. We search for the minimum of the function

$$S(x) + \frac{\beta k_m l\gamma}{2}\left(x\,\varepsilon_u - \varepsilon_t(1+\alpha)\right)^2$$

which can be found solving the equation

$$\ln\left(\frac{x}{1-x}\right) + \varepsilon_u \beta k_m l\gamma\left(x\,\varepsilon_u - (1+\alpha)\varepsilon_t\right) = 0. \tag{ESI-33}$$

It is easy to see that there exists only one solution χ_c in the interval $]0,1[$. Thus, we can solve the integral with the saddle point method by considering the expansion around χ_c up to the second order as it follows:

$$f(\varepsilon_t) \simeq \sqrt{\frac{n}{2\pi}} \int_0^1 \sqrt{\frac{1}{\chi_c(1-\chi_c)}}\, e^{-n\left(S(\chi_c) - \frac{\beta k_m l n\gamma}{2}(\chi_c\,\varepsilon_u - (1+\alpha)\varepsilon_t)^2 - \frac{1}{2}\left(S''(\chi_c) + \beta l k_m\gamma\varepsilon_u^2\right)(x-\chi_c)^2\right)} \mathrm{d}x.$$

By substituting the variable $y = \sqrt{n}(x-\chi_c)$ we get

$$f(\varepsilon_t) \simeq \sqrt{\frac{1}{2\pi\chi_c(1-\chi_c)}}\, e^{-n\left(S(\chi_c) - \frac{\beta k_m l n\gamma}{2}(\chi_c\,\varepsilon_u - \varepsilon_t(1+\alpha))^2\right)} \int_{-\sqrt{n}\,\chi_c}^{\sqrt{n}\,\chi_c} e^{-\frac{1}{2}\left(S''(\chi_c) + \beta l k_m\gamma\varepsilon_u^2\right)y^2} \mathrm{d}y.$$

In the limit $n \to \infty$, we obtain

$$f(\varepsilon_t) \sim \frac{e^{-n\left(S(\chi_c) + \frac{\beta k_m l\gamma}{2}(\chi_c\varepsilon_u - \varepsilon_t(1+\alpha))^2\right)}}{\sqrt{1 + \beta k_m l\gamma\varepsilon_u\chi_c(1-\chi_c)}}.$$

Similarly, we can show that

$$g(\varepsilon_t) = \sum_{p=0}^{n} \binom{n}{p} \frac{p}{n}\, e^{-\frac{\beta k_m l n\gamma}{2}\left(\frac{p}{n}\varepsilon_u - \varepsilon_t(1+\alpha)\right)^2} \sim \frac{\chi_c\, e^{-n\left(S(\chi_c) + \frac{\beta k_m l\gamma}{2}(\chi_c\varepsilon_u - \varepsilon_t(1+\alpha))^2\right)}}{\sqrt{1 + \beta k_m l\gamma\varepsilon_u\chi_c(1-\chi_c)}}.$$

Finally, we get

$$\langle\bar{\chi}\rangle = \frac{g(\varepsilon_t)}{f(\varepsilon_t)} \sim \chi_c(\varepsilon_t). \tag{ESI-34}$$

6 Ideal Cases

In this section we consider the *ideal* cases, tipically considered in the literature, when the device effect is neglected and the displacement (*ideal hard device*) or the force (*ideal soft device*) are directly applied to the unfolding molecule. In this case $\varepsilon_m \equiv \varepsilon_t$ and the Hamiltonian is

$$H^{id} = \sum_{i=1}^{n} \frac{1}{2m} p_i^2 + \frac{1}{2} k_m l \sum_{i=1}^{n} (\varepsilon_i - \varepsilon_u \chi_i)^2. \tag{ESI-35}$$

6.1 Ideal Helmholtz ensemble

Using (ESI-35), the partition function in the Helmholtz ensemble for the ideal case is

$$Z_{\mathscr{H}}^{id} = \sum_{\boldsymbol{\chi}} l^n \int_{\mathbb{R}^{2n}} e^{-\beta H^{id}} \delta \left(l \sum_{i=1}^{n} \varepsilon_i - d \right) \prod_{i=1}^{n} dp_i \prod_{i=1}^{n} d\varepsilon_i. \tag{ESI-36}$$

The integrals over the momenta result in the constant

$$A_{\mathscr{H}}^{id} = l^n (2\pi)^{\frac{n}{2}} \left(\frac{m}{\beta} \right)^{\frac{n}{2}}.$$

The constraint on the total displacement is imposed by the Dirac delta as it follows:

$$\begin{aligned} Z_{\mathscr{H}}^{id} &= A_{\mathscr{H}}^{id} \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^n} e^{-\beta\left(\frac{k_m l}{2} \sum_{i=1}^{n-1} (\varepsilon_i - \varepsilon_u \chi_i)^2 + \frac{k_m l}{2} (\varepsilon_n - \varepsilon_u \chi_n)^2\right)} \delta \left(l \sum_{i=1}^{n-1} \varepsilon_i + l\varepsilon_n - d \right) \prod_{i=1}^{n} d\varepsilon_i \\ &= A_{\mathscr{H}}^{id} \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^{n-1}} e^{-\frac{\beta k_m l}{2}\left(\sum_{i=1}^{n-1} \left(\varepsilon_i - \varepsilon_u \chi_i\right)^2 + \left(\sum_{i=1}^{n-1} \varepsilon_i - \varepsilon_u \chi_n - n\, \varepsilon_m\right)^2\right)} \prod_{i=1}^{n-1} d\varepsilon_i \\ &= A_{\mathscr{H}}^{id} \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^{n-1}} e^{-\frac{1}{2} \boldsymbol{A}\boldsymbol{\varepsilon} \cdot \boldsymbol{\varepsilon} + \boldsymbol{b} \cdot \boldsymbol{\varepsilon} + C} \prod_{i=1}^{n-1} d\varepsilon_i\,, \end{aligned} \tag{ESI-37}$$

where we have introduced

$$\boldsymbol{A} = \beta k_m l \begin{pmatrix} 2 & 1 & \dots & 1 \\ 1 & 2 & & \vdots \\ \vdots & & \ddots & \vdots \\ 1 & \dots & \dots & 2 \end{pmatrix}, \tag{ESI-38}$$

$$\boldsymbol{b} = \{\beta k_m l\, (\varepsilon_u \chi_1 + \varepsilon_u \chi_n + n\, \varepsilon_m)\,, ..., \beta k_m l\, (\varepsilon_u \chi_{n-1} + \varepsilon_u \chi_n + n\, \varepsilon_m)\}^T, \tag{ESI-39}$$

$$\boldsymbol{\varepsilon} = \{\varepsilon_1, ..., \varepsilon_{n-1}\}^T, \tag{ESI-40}$$

and

$$C = \varepsilon_u^2 \sum_{i=1}^{n-1} \chi_i^2 + \varepsilon_u^2 \chi_n^2 + n^2 \varepsilon_m^2 + 2\varepsilon_u \chi_n \varepsilon_m\, n.$$

The Gaussian integration can be solved as before in the general case with the presence of the device [3]. We obtain

$$Z_{\mathscr{H}}^{id} = K_{\mathscr{H}}^{id} \sum_{\boldsymbol{\chi}} e^{\frac{\beta k_m l}{2} \left(\sum_{i=1}^{n-1} (\varepsilon_u \chi_i + \varepsilon_u \chi_n + n\, \varepsilon_m)^2 - \frac{1}{n} \left(\sum_{i=1}^{n-1} (\varepsilon_u \chi_i + \varepsilon_u \chi_n + n\, \varepsilon_m) \right)^2 - \varepsilon_u^2 \sum_{i=1}^{n-1} \chi_i^2 - \varepsilon_u^2 \chi_n^2 - n^2 \varepsilon_m^2 - 2\varepsilon_u \chi_n \varepsilon_m n \right)}$$

with

$$K_{\mathscr{H}}^{id} = A_{\mathscr{H}}^{id} \sqrt{\frac{(2\pi)^{n-1}}{(\beta k_m l)^{n-1}\, n}}.$$

Finally, we obtain the partition function for the ideal case in the Helmholtz ensemble:

$$Z^{id}_{\mathscr{H}} = K_{\mathscr{H}} \sum_{p=0}^{n} \binom{n}{p} e^{-\frac{\beta k_m l n}{2}\left(\frac{p}{n}\varepsilon_u - \varepsilon_t\right)^2}.$$

Using a procedure analogous to the general case, we deduce the formula for the expectation value of the unfolded fraction in the ideal case:

$$\langle \bar{\chi}^{id} \rangle = \langle \bar{\chi}^{id} \rangle_{\mathscr{H}}(\beta, \varepsilon_t) = \frac{\sum_{p=0}^{n} \binom{n}{p} \frac{p}{n} e^{-\frac{\beta k_m l n}{2}\left(\frac{p}{n}\varepsilon_u - \varepsilon_t\right)^2}}{\sum_{p=0}^{n} \binom{n}{p} e^{-\frac{\beta k_m l n}{2}\left(\frac{p}{n}\varepsilon_u - \varepsilon_t\right)^2}}. \tag{ESI-41}$$

6.2 Ideal Gibbs Ensemble

If we apply a fixed force at the end of the chain of n bistable elements without considering the measuring device we obtain the case of *ideal soft device.* By using (ESI-35) we can write the partition function in the Gibbs ensemble as

$$Z^{id}_{\mathscr{G}} = \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^{2n}} e^{-\beta\left(H^{id} - Fl\sum_{i=1}^{n}\varepsilon_i\right)} \prod_{i=1}^{n} dp_i \prod_{i=1}^{n} l\, d\varepsilon_i.$$

As in the Helmholtz ensemble the integral over the momenta gives the constant

$$A^{id}_{\mathscr{G}} = A^{id}_{\mathscr{H}} = l^n (2\pi)^{\frac{n}{2}} \left(\frac{m}{\beta}\right)^{\frac{n}{2}}.$$

The integrals over the strains can be rewritten as

$$Z^{id}_{\mathscr{G}} = A^{id}_{\mathscr{G}} \sum_{\boldsymbol{\chi}} \int_{\mathbb{R}^{n}} e^{-\frac{\beta k_m l}{2}\sum_{i=1}^{n}\left((\varepsilon_i - \varepsilon_u\chi_i)^2 - \frac{2F}{k_m}\varepsilon_i\right)} \prod_{i=1}^{n} d\varepsilon_i = A^{id}_{\mathscr{G}} \sum_{\boldsymbol{\chi}} \prod_{i=1}^{n} \int_{\mathbb{R}} e^{-\frac{\beta k_m l}{2}\left((\varepsilon_i - \varepsilon_u\chi_i)^2 - \frac{2F}{k_m}\varepsilon_i\right)} d\varepsilon_i.$$

The solution can be obtained exactly as in Sect. 3. We have

$$Z^{id}_{\mathscr{G}} = K^{id}_{\mathscr{G}} \sum_{p=0}^{n} \binom{n}{p} e^{\frac{\beta l n}{2k_m}\left(F^2 + 2k_m\varepsilon_u\frac{p}{n}F\right)} = K^{id}_{\mathscr{G}}\, e^{\frac{\beta l n}{2k_m\gamma}F^2}\left(1 + e^{l\beta\varepsilon_u F}\right)^n. \tag{ESI-42}$$

From (ESI-42) we can obtain, as in the previous cases, the expectation value of the strain of the molecule and the expectation value of the unfolded fraction in the ideal case

$$\langle \varepsilon_m \rangle = \frac{F}{k_m} + \varepsilon_u \langle \bar{\chi}^{id} \rangle,$$

$$\langle \bar{\chi}^{id} \rangle = \langle \bar{\chi}^{id} \rangle_{\mathscr{G}}(\beta, F) = \frac{e^{\beta l \varepsilon_u F}}{1 + e^{\beta l \varepsilon_u F}}.$$

7 Numerical test of the energy approximation

In this section we numerically verify the approximation used in the paper, i.e. the extension of the energy (parabolic) function beyond the spinodal point. We focus on the case of the Gibbs ensemble whereas the results for the Helmholtz ensemble can be found in [1]. In Fig. 1(a) we compare the force strain curves obtained via Eq.(34)-(35) of the main paper and via the integration of the partition function without approximation depending on the temperature. In particular, we have considered two different temperatures, $T = 300$K and $T = 3000$K. In both cases the curves obtained analytically (continuous line) and numerically (dashed line) are perfectly coincident. We deduce that the approximation used is very robust. As a second check, we test the approximation for different values of the spring constant of the measurement apparatus, i.e. we choose different values of γ keeping k_m fixed. The results are shown in Fig. 1(b). Also in this case, by superposing the numerical and analytical results we observe that they are perfectly superimposed.

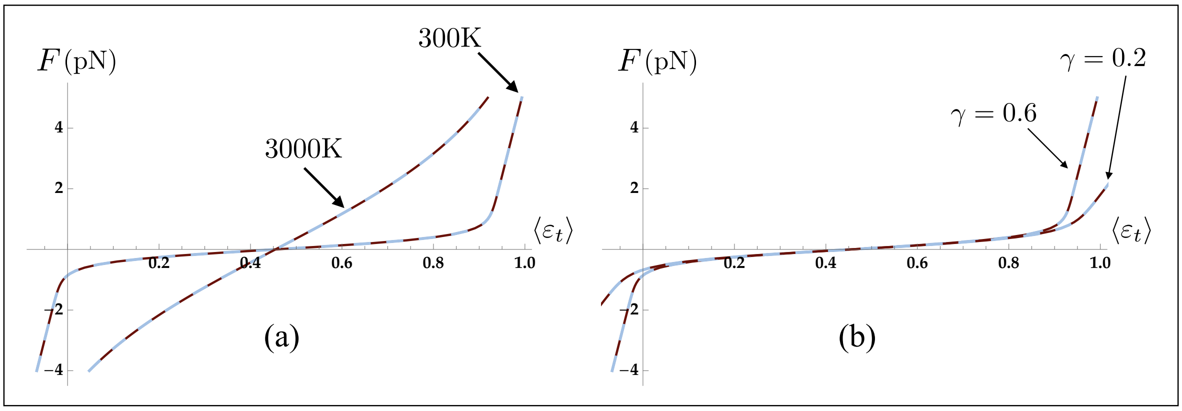

Figure 1: Comparison of the force-strain curves obtained using the analytical formulas (34)-(35) and the numerical integration of the partition function without the approximation beyond the spinodal point described in the text. (a) We have considered two different temperatures i.e. $T = 300\,\mathrm{K}$, $T = 3000\,\mathrm{K}$ with $\gamma = 0.6$. (b) We have considered two different spring constant of the measuring apparatus keeping k_m fixed i.e. $\gamma = 0.6$, $\gamma = 0.2$ with $T = 300\,\mathrm{K}$. In both cases we have fixed $N = 1$ with $l = 20\,\mathrm{nm}, \varepsilon_u = 1, k_m = 90\,\mathrm{pN}$, $\alpha = 0.1$.

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