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Ensemble inequivalence in single-molecule experiments

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In bulk systems the calculation of the main thermodynamic quantities leads to the same expectation values in the thermodynamic limit, regardless of the choice of the statistical ensemble. Single linear molecules can be still regarded as statistical systems, where the thermodynamic limit is represented by infinitely long chains. The question of equivalence between different ensembles is not at all obvious and has been addressed in the literature, with sometimes contradicting conclusions. We address this problem by studying the scaling properties of the ensemble difference for two different chain models as a function of the degree of polymerization. By characterizing the scaling behavior of the difference between the isotensional (Gibbs) and isometric (Helmholtz) ensembles in the transition from the low-stretching to the high-stretching regime, we show that ensemble equivalence cannot be reached for macroscopic chains in the low force regime, and we characterize the transition from the inequivalence to the equivalence regime.

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I. INTRODUCTION

Among the technical developments that influenced the research in molecular biophysics during the last two decades, the possibility of manipulating single molecules by means of different techniques has certainly played a pivotal role and has been proved to be an invaluable tool to gain insight into the structural properties and function of macromolecules involved in many biological processes [1]. The techniques devised to manipulate single molecules are collectively known as single-molecule experiments (SMEs) [1–3] and include, among others, approaches based on atomic force microscopy (AFM) [4–8], optical tweezers [9–12], fluorescence detection of Förster resonance energy transfer [13], elongational flow [14–16], and magnetic tweezers [17–19].

It is widely known that equilibrium thermodynamics can be recovered from a statistical mechanics description in the limit of infinite system size (the thermodynamic limit) and that, except when in proximity of a phase transition, all statistical ensembles provide the same average values for the observables of interest. In other words, in the thermodynamic limit different ensembles become equivalent.

In case of SMEs, on the contrary, the outcome of a measure explicitly depends on the control parameters, that is, on the choice of which quantities are kept constant and which ones are allowed to vary. For this reason, the efforts in providing satisfying theoretical descriptions of small, out of equilibrium, systems have been intensified during the last decade. In particular, the validation of the hypothesis made by Flory [20], that ensemble equivalence for a SME on a linear polymer should be obtained in the limit of infinite chain length, has been often subject of investigations [1,21,22]. Since one of the basic means of extracting information regarding a linear polymer in a SME is to analyze the molecular force-extension curve (FEC) [1], it is natural to introduce two different conjugate ensembles, namely, the Helmholtz or isometric ensemble and the Gibbs or isotensional ensemble. In the isometric ensemble the position of the chain ends is employed as a control parameter, fixing also the end-to-end distance, while in the isotensional case the control parameter is represented by the force applied on one loose end. The conditions under which the equivalence between these two ensembles can be obtained have been investigated in many works, both from a theoretical point of view [21,23–40] and by means of computer simulations [41–50]. Some of the authors concluded that ensembles are equivalent in the infinitely long chain limit for a Gaussian chain [30,47], as well as for a generic chain [21] and toy lattice chain models [21,35,36]. Some other works states that ensembles are not equivalent in the thermodynamic limit for a single chain [27,28,44,45].

The present paper addresses the problem of ensemble equivalence of Gaussian chains from the point of view of computer simulations, employing coarse-grained Langevin dynamics simulations. In Sec. II, we discuss the basic theoretical background and the appropriate way of quantifying the ensemble equivalence. After we describe the employed simulation techniques in Sec. III, we present the analysis of the force-extension curves in the Helmholtz and Gibbs ensembles for Gaussian chains with zero and nonzero average bond vectors in Sec. IV. There we also discuss the analysis of the scaling behavior of the ensemble difference with respect to the chain length, confirming the prediction of Neumann [22] on the ensemble inequivalence in the limit of vanishing applied forces, before we conclude in Sec. V.

II. CONJUGATE ENSEMBLES AND THEIR EQUIVALENCE IN A SME

In statistical mechanics the connection with thermodynamics is realized by defining the thermodynamic potentials

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from the partition functions in the limit that every extensive control parameter is going to infinity. As a general result, given a statistical ensemble and a control parameter, it is possible to construct a conjugate ensemble using as a conjugate control parameter the derivative of the ensemble's thermodynamic potential. It is generally assumed that in the thermodynamic limit, two conjugate ensembles should be equivalent, namely, they should provide the same expectation value for the thermodynamic quantities [51]. Let us take as an example the case of the canonical ensemble, whose partition function will be denoted by $Q(N,V,T)$ and whose thermodynamic potential is the Helmholtz energy A, defined as

$$-\beta A = \lim_{(N,V)\to\infty} \ln Q(N,V,T).$$

Here $\beta=1/k_BT$, where k_B is Boltzmann's constant. The main thermodynamic quantities can be then derived by computing the derivatives of the thermodynamic potentials with respect to their parameters. Given any generic ensemble, characterized by its thermodynamic potential $P(Y_1,Y_2,\ldots)$, it is possible to generate a conjugate ensemble by choosing any quantity, Y_i, and applying a Legendre transform which involves the pair of conjugate variables Y_i and $\partial P/\partial Y_i$ [52–54]. In the example of the canonical ensemble, by choosing the volume V and the pressure $p=-\partial A/\partial V$ as a conjugate pair, the isothermal-isobaric ensemble (NpT) is generated.

The Legendre transform applied to the Helmholtz energy yields the thermodynamic potential of the isothermal-isobaric ensemble, namely, the Gibbs energy $G(N,p,T)=A+pV$. The conjugate partition function, $\mathcal{Z}(N,p,T)$, can be written in a natural way as a weighted sum of the canonical partition functions,

$$\mathcal{Z}(N,p,T) = \int dVQ(N,V,T)\exp(-\beta pV). \tag{1}$$

Consequently, the ensemble average $\langle\mathcal{A}\rangle$ of any observable $\mathcal{A}(V)$ can be expressed in the conjugate ensemble as

$$\langle\mathcal{A}\rangle = \mathcal{Z}^{-1}\int dVQ(N,V,T)\exp(-\beta pV)\mathcal{A}(V). \tag{2}$$

The ensemble equivalence problem can then be stated as follows: can the *thermodynamic potential* of the conjugated ensemble be obtained from the thermodynamic limit of the conjugate partition function? If this is true, the thermodynamic quantities computed in either ensembles will lead to the same expectation values. Conversely, if at a given thermodynamic point a generic function of state assumes different values, depending on the ensemble in which it is computed, then the ensembles are inequivalent.

As pointed out first by Flory [20], single polymers can be regarded as statistical ensembles, and their thermodynamical limit can be realized by infinitely long chains. Among the interesting control parameters that can be employed to characterize the statistical ensemble of a molecule is the molecular end-to-end (displacement) vector $\mathbf{X}$. Following the convention of Neumann [22] we will call isometric the statistical ensemble generated by keeping this vector constant. Obviously, $\mathbf{X}$ *plays no privileged role with respect to other observables*, and other choices for the control parameter (like the end-to-end distance) are possible. Here we will restrict our analysis to the end-to-end vector $\mathbf{X}$ (as a control parameter) because we consider it to be relevant for experiments. For example, the end-to-end distance $|\mathbf{X}|$ would be the control parameter in an experiment for which the molecular ends are kept at constant distance but where the end-to-end vector is free to rotate. This ensemble, although perfectly licit, would obviously be difficult to be realized experimentally.

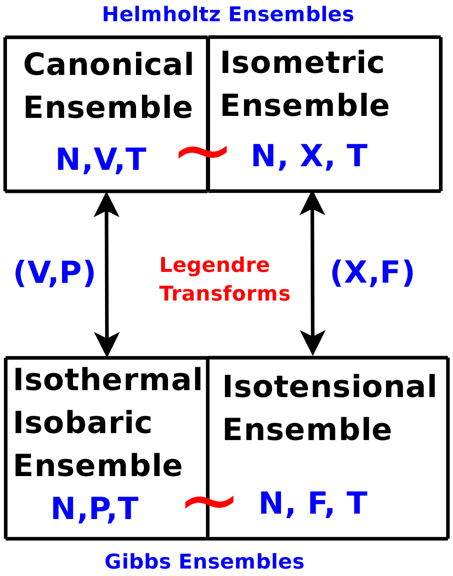

FIG. 1. (Color online) The sketch of analogies between conjugate chain ensembles and standard statistical mechanical ensembles.

Keeping the other control parameters (temperature, number of particles, etc.) fixed, the work done on the system by displacing the chain end by an amount $d\mathbf{X}$ is $dU=\mathbf{F}\cdot d\mathbf{X}$. As a consequence, the force $\mathbf{F}$ acting on one end is equal to the derivative with respect to $\mathbf{X}$ of the thermodynamic potential. Therefore, $\mathbf{F}$ is the variable conjugate to $\mathbf{X}$. Note that since a conjugate pair $(Y_i,\ \partial P/\partial Y_i)$ is uniquely determined by the ensemble and a control parameter, in the isometric ensemble defined above the force $\mathbf{F}$ is the only possible conjugate variable to $\mathbf{X}$. This leads, via a Laplace transform analogous to Eq. (1), to the definition of the ensemble conjugate to the isometric one, namely, the isotensional one. In the isotensional ensemble a constant force is applied on one molecular ending. A schematic representation of the formal analogy between the isometric, isotensional, canonical, and isothermal-isobaric ensembles is presented in Fig. 1.

In experiments in which single molecules are directly manipulated, as is the case for AFM or optical tweezers, the manipulating device is basically acting on the terminal part of the molecule, and depending on the strength of the interaction of the tool with the molecule, the former can be employed either to hold firmly one molecular end or to measure the force which is acting on it while the other end is attached to some rigid support [21]. Both the isometric and the isotensional ensembles can then be realized by changing the force constant of the cantilever in the case of AFM or spring constants in the case of optical tweezer experiments [55], where the use of high force constants will lead to a sampling of the isometric ensemble. It is therefore of particular interest, both from a fundamental and from a practical point of view, to understand if these two ensembles are equivalent. This knowledge can tell, e.g., whether measurements performed in the two ensembles are comparable, or, which kind of theoretical model has to be employed in order to fit experimental data, which is a key step to extract information from SMEs [56,57].

The term "ensemble equivalence" indicates, as it is stated in textbooks on statistical mechanics [51,54,58], that in the thermodynamic limit the partition functions of two ensembles become indistinguishable. As a result, if two ensembles are equivalent, the expectation values of *any* observable measured in the two ensembles have to coincide.

Ensemble equivalence requires that a calculation of *any observable* in the thermodynamic limit should yield the *same expectation value* for both ensembles [59], and this is a sound definition of equivalence from the practical point of view. Surely it is possible to find some specific observable that has the same expectation values in the two ensembles, but this is by no means a proof of ensemble equivalence, unless one checks this for every observable. In this sense the negative proof is an easier task: if one manages to find an observable which behaves differently in the two ensembles, their inequivalence is proven. We will show that the expectation values for the end-to-end distance $X=|\mathbf{X}|$ sampled in the isometric and isotensional ensembles converge only by choosing a thermodynamic point characterized by high values of $\langle \mathbf{F} \rangle$, while they do not converge in the weak force regime. This will prove that in the part of the phase diagram characterized by weak applied forces the two ensembles are not equivalent.

To summarize, the basic steps taken to define the isotensional and isometric ensembles and to check their inequivalence are the following

(1) The end-to-end vector $\mathbf{X}$ is chosen as a control parameter. We denote the ensemble generated by keeping this parameter fixed as *isometric*.

(2) The variable conjugate to $\mathbf{X}$ is $\mathbf{F}$ (since the work done on the system is $dU=\mathbf{F}\cdot d\mathbf{X}$). We denote the ensemble generated by keeping $\mathbf{F}$ fixed as *isotensional*. It is realized by applying a constant force on one molecular end.

(3) The value of the applied force (in the isotensional ensemble) or of the average force $\langle \mathbf{F} \rangle$ (in the isometric ensemble) identifies a thermodynamic point of the system.

(4) The extension $X=|\mathbf{X}|$ is chosen as the observable to be compared in the two ensembles. This observable assumes constant values in the isometric ensemble and fluctuates in the isotensional one.

(5) The relative difference between the average values of $|\mathbf{X}|$ is sampled in the two ensembles at different thermodynamic points, that is, different values of $\langle \mathbf{F} \rangle$, and its scaling is investigated in the limit of long chains.

In a system which does not exhibit any directional preference, due to symmetry reasons the average force $\langle \mathbf{F} \rangle$ points along $\mathbf{X}$. For that, a thermodynamic point can be uniquely identified by the modulus of the average force $|\langle \mathbf{F} \rangle|$ or, equivalently, by the average projected force defined as $\langle F \rangle \equiv \langle \mathbf{F}\cdot\mathbf{X}/X \rangle$ since it holds that $\langle F \rangle = |\langle \mathbf{F} \rangle|$. Therefore, checking the convergence of $|\mathbf{X}|$ at different thermodynamic points is equivalent to checking the convergence of the force-extension curves $\langle F \rangle(X)$ and $F(\langle X \rangle)$.

In case of ensemble equivalence, these two graphs should, in the limit of infinitely long chains, become indistinguishable. Note, however, that some care has to be taken, about the precise meaning of this statement, as will be discussed in Sec. III.

III. ENSEMBLE EQUIVALENCE FOR GAUSSIAN CHAINS

In general terms, if the probability density distribution for the end-to-end vector at equilibrium in the free case is denoted by $\mathcal{P}(\mathbf{X})$, then the partition functions for the isometric and isotensional cases can be written, respectively, as

$$Z_{\mathbf{X}} = \mathcal{P}(\mathbf{X}), \tag{3}$$

$$Z_{\mathbf{F}} = \int d\mathbf{X}\mathcal{P}(\mathbf{X})\exp(-\beta\mathbf{F}\cdot\mathbf{X}), \tag{4}$$

where the formal analogy with the canonical and its conjugate isothermal-isobaric ensemble is evident. Often, as is the case with computer simulations and different theoretical approaches, one needs to refer to a specific molecular model, which will explicitly determine the form of the probability density $\mathcal{P}(\mathbf{X})$. In case of long linear chains, however, the probability density is very well approximated by a Gaussian distribution [60],

$$\mathcal{P}(\mathbf{X}) = b^3\pi^{-3/2}\exp(-b^2X^2), \tag{5}$$

where the characteristic length b^{-1} is proportional to the root-mean-square end-to-end distance $\sqrt{\langle|\mathbf{X}|^2\rangle_0}=\sqrt{3/2}/b$ and to the mean end-to-end distance $\langle X \rangle_0 = 2/(b\sqrt{\pi})$ (the notation $\langle \ldots \rangle_0$ denotes an ensemble average in the free case, i.e., with no applied force). This distribution can be recovered as a limiting case for a wide class of different models representing polymers with discrete units. This is the case, for example, for a freely jointed chain of N elements of length a, whose end-to-end distribution is well approximated by the Gaussian distribution when $Na \gg X$. In this case, $b^2 = 3/(2Na^2)$ and the total contour length L is $L=Na$. Moreover, the Gaussian chain model can be easily simulated by means of series pointlike particles connected by harmonic springs.

Given this explicit form for the partition function, it is possible to make some analytical prediction on the model. As Neumann [22] noted in a critical analysis on the interpretation of stretching experiments, from the partition functions of the Gaussian chain it is possible to derive Hookean-type relations between the force and end-to-end vectors. This is easily seen by computing the derivative of the free energy with respect to the end-to-end vector in the isometric case

$$\langle \mathbf{F} \rangle = \beta^{-1}\frac{\partial}{\partial\mathbf{X}}\ln Z_{\mathbf{X}} = 2b^2\mathbf{X}/\beta \tag{6}$$

and computing the average end-to-end vector $\langle \mathbf{X} \rangle$ in the isotensional case

$$\mathbf{F} = 2b^2\langle \mathbf{X}\rangle/\beta. \tag{7}$$

This analogy has usually led to the erroneous interpretation of an equivalence between the isometric and isotensional ensembles, which moreover seems to hold for every chain length and not just in the thermodynamic limit of infinite chain length.

Disregarding the fact that this equivalence is not true for other observables has been often a source of misinterpretations, for example, when theoretical force-extension curves that predict zero extension at zero applied force are employed (see, e.g., [14,61–63]) either to fit experimental data or to build theoretical models. In these cases *ad hoc* corrections or hypotheses have been admittedly introduced. This could have been avoided by employing the right ensemble and, consequently, nonvanishing extensions at zero applied force.

In fact, it is clear that these relations cannot hold for other observables such as, e.g., the end-to-end distance X (remember that in order to attain ensemble equivalence every observable should converge to the same limiting expectation value). This can be inferred from the well known fact that in the free case the average end-to-end distance $\langle X\rangle_0$ does not vanish, whereas Eq. (6) yields zero force at zero distance. Therefore, by choosing observables other than $\mathbf{X}$, the convergence of their expectation values is not a trivial matter anymore. A direct evaluation [22] of $\langle X\rangle$ in the isotensional ensemble, in fact, leads to

$$\langle X\rangle = \frac{\langle X\rangle_0}{2}\left[e^{-\nu^2/4} + (\nu + 2/\nu)\int_0^{\nu/2} e^{-t^2} dt \right], \tag{8}$$

where $\nu = \beta F/b$. This relation can be usefully approximated, for small external forces, as $\langle X\rangle = \langle X\rangle_0[1+\frac{1}{12}\nu^2+\mathcal{O}(\nu^4)]$, demonstrating a nonzero extension in the weak force limit, in fact, for all chain lengths. Therefore, the F vs X force-extension curves measured in the two different ensembles cannot coincide. The question of ensemble equivalence has then to be investigated more carefully by examining the scaling behavior (i.e., the finite-size effects) of an observable that quantifies the difference between the expectation values of X when measured in different ensembles.

In recent investigations the fundamental difference in meaning between Eqs. (6) and (7) was not appreciated resulting in a general consensus that in the thermodynamic limit, equivalence between the two ensembles is obtained. In particular, employing techniques such as renormalization group theory [47], maximum entropy approach [30], and standard analytical approaches [21,35], the authors conceived the idea that the isotensional and the isometric ensembles are actually equivalent in the usual thermodynamic limit. Recently, however, Neumann [22] pointed out a particular feature of the statistical mechanics of the single chain, namely, that ensemble equivalence cannot be obtained for small values of the external force. In other words, there is theoretical evidence that by choosing an appropriately small external force, the ensemble difference for any (finite but arbitrarily large) chain length can be maintained constant. To our knowledge, this subtle point in the investigation of the equivalence has never been tested by means of computer simulation, and a part of this paper is devoted to clarify it, in particular, by analyzing the scaling behavior of the ensemble difference as a function of the applied force regime.

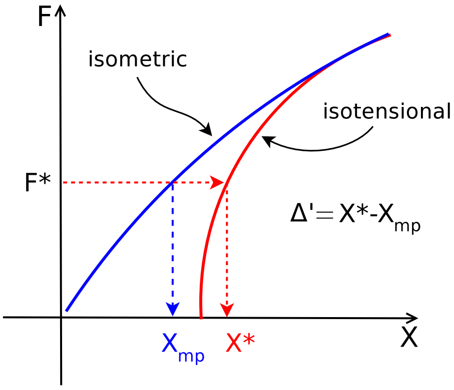

FIG. 2. (Color online) Definition of ensemble difference Δ' between isotensional and isometric ensembles for a given value F^* of the force applied in the isotensional ensemble.

Let us turn now to the problem of evaluating ensemble difference and its scaling. In order to make any statements about equivalence in the thermodynamic limit, there is a need for an observable which quantifies how much two ensembles differ. Given a point (F^*,X^*) on the graph of the isotensional force-extension curve $F(\langle X\rangle)$, the measure of the difference between two ensembles is then defined as

$$\Delta = \frac{X^* - X_{mp}}{X^*}, \tag{9}$$

where X_{mp} is the value of the extension in the isometric ensemble that solves the equation $\langle F\rangle(X^*)=F^*$. (The notation stems from the fact that this extension actually coincides with the maximum in the probability distribution function of the isotensional ensemble [21].) This definition (which of course is not restricted to Gaussian chains) plays a crucial role since the difference between the two ensembles *should not depend on a reparametrization* of the control parameter. So, while $\Delta'(X)=X^*-X_{mp}$ could in principle seem to be a reasonable alternative, it is easily seen that using the same functional form $\Delta'(\xi)=\xi^*-\xi_{mp}$ for the relative extension $\xi = X/N$ would lead to completely different results. This does not happen using relative measure (9), which behaves correctly under reparametrization.

A schematic view of the identification of X^* and X_{mp} from the graphs of the isotensional and isometric force-extension curves is given in Fig. 2.

The correct definition of the ensemble difference shows (at least for the Gaussian chain) that in the moderate or strong stretching regime the ensemble difference indeed goes to zero when the number of monomers tends to infinity, i.e., that ensemble equivalence is attained. In particular, if the chain end-to-end distance is supposed to scale linearly with the number of monomers in the overstretched regime, the ensemble difference should scale [21] like $\Delta \sim (N-1)^{-1}$. However, the behavior of the ensemble difference at low-stretching regimes is markedly different, inasmuch as in this regime both X^* and X_{mp} scale as $\sqrt{N-1}$, and therefore the ensemble difference does not scale with system size and remains constant. For any given chain length (i.e., for a macroscopic though not strictly infinite system) it is then possible to find a small enough force for which the ensemble difference does not vanish and, moreover, does not decrease appreciably when increasing the chain length. The ensemble difference in the free case Δ_0, as a limiting case of zero forces, can be written as

$$\Delta_0 = 1 - \frac{X_{mp,0}}{X_0^*}.$$

Since for Gaussian chains $X_{mp,0}=0$, the ensemble difference takes the limiting value of $\Delta_0=1$, which for long enough chains is a model-independent result, as long as the Gaussian approximation is valid. Summarizing, the analytical argument of Neumann demonstrates that, in principle, if one chooses a thermodynamical point characterized by a small enough force, then the expectation values for X, measured in the two ensembles will not converge, therefore exhibiting ensemble inequivalence in the vanishing force regime. However, the presence of two simultaneous limiting procedures (i.e., of vanishing forces and of diverging chain lengths) introduces some difficulties in its interpretation and poses the serious question whether the set of states which exhibits inequivalence is of practical importance or, instead, is limited to such a tiny range of forces which would make it vanishing for the sake of every operational purpose. Besides checking the validity of the analytical approach, the computational analysis described in Secs. IV and V will also try to address this problem by investigating the transition from the nonequivalence to the equivalence regimes.

IV. SIMULATION DETAILS

The investigations on the ensemble difference have been carried out by simulating the Langevin dynamics of two different chain models by sampling the isometric and the isotensional ensembles for different chain lengths using the ESPRESSO simulation package [64]. Each chain consists of a given number of monomers, which are interacting only with their respective first neighbors via an harmonic potential. Therefore no excluded volume interactions are present and in the free case the chains perform a pure random walk. The role of Langevin equation,

$$\frac{d^2\mathbf{x}_i}{dt^2} = \mathbf{F}_i - \gamma\frac{d\mathbf{x}_i}{dt} + \mathbf{W}_i(t), \tag{10}$$

is basically that of providing a thermostat for the chain, where the position of the monomer i is $\mathbf{x}_i$ and $\mathbf{F}_i$ represents the conservative force acting on the monomer. As usual, the thermostat is acting via a friction coefficient γ, and a random force $\mathbf{W}_i(t)$ with zero mean and square deviation $\langle \mathbf{W}_i(t)\cdot\mathbf{W}_j(t')\rangle = 6k_BT\gamma\delta_{ij}\delta(t-t')$, in order to satisfy the fluctuation-dissipation theorem.

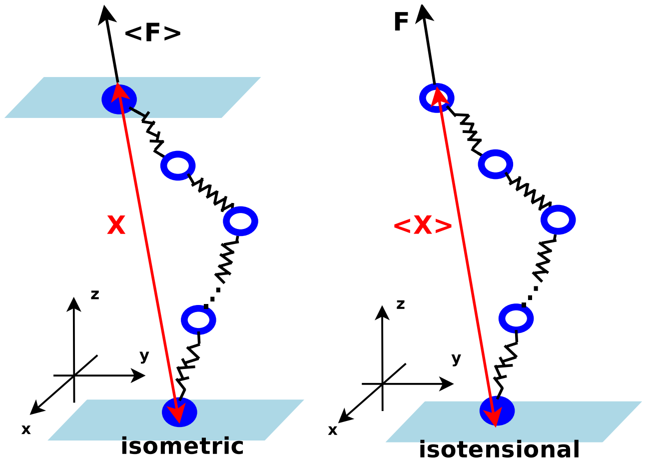

FIG. 3. (Color online) Sketch of the chains in the isometric and isotensional ensembles.

By measuring energies in units of k_BT, distances in arbitrary units d, and considering unitary masses it is possible to express the energy of a chain consisting of N monomeric units, depicted in Fig. 3, as

$$U = \frac{1}{2}k\sum_{i=1}^{N}(|\mathbf{x}_i - \mathbf{x}_{i-1}| - r_{eq})^2, \tag{11}$$

where $k=20/d^2$ is the spring constant and r_{eq} is the equilibrium distance between a pair of connected monomers.

We focused our attention on two specific cases, namely, that of $r_{eq}=d$ and that of zero equilibrium distance. In particular the latter case models precisely a Gaussian probability distribution for the end-to-end distance in the free case. For every simulation, the friction coefficient was set to $\gamma=0.5/\tau$ and the integration time step was set to 0.01τ, where $\tau=d$ is the characteristic time.

The sampling of the isometric ensemble was realized by fixing the spatial position of both terminal monomers, x_1 and x_N. In the isotensional ensemble, the end monomer of the chain was fixed, while in the other monomers one was free to move and a given force, constant in modulus and direction, was applied on it.

The simulation procedures employed for the isometric ensemble consisted of constraining the positions of the first and last monomers at the desired distance X, then performing a relaxation run, up to at least ten times the autocorrelation time of the observable of interest, F and eventually computing the average $\langle F\rangle = \langle \mathbf{F}\cdot\mathbf{X}/X\rangle$, where $\mathbf{X}=\mathbf{x}_N-\mathbf{x}_1$. In the isotensional ensemble case, the chains started from a straight conformation where the position of the first monomer was constrained, and a constant force $\mathbf{F}$ was applied to the last monomer. Then, after relaxing the system, the end-to-end distance X was sampled. By varying over suitable ranges the end-to-end distance in the isometric ensemble and the magnitude of the applied force in the isotensional ensemble, we sampled the force-extension curves $\langle F\rangle(X)$ and $F(\langle X\rangle)$ for a number of different chain lengths ranging from 6 to 500 monomeric units. Every point in the force-extension

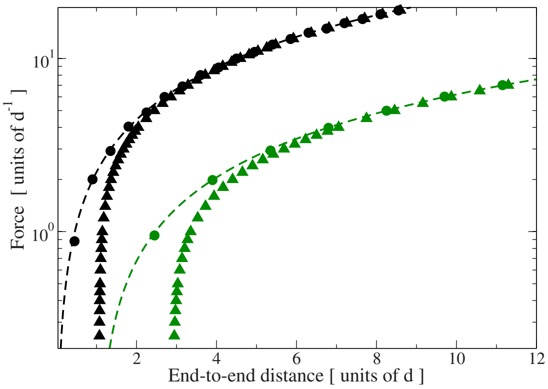

FIG. 4. (Color online) The force-extension curve for the $r_{eq}=0$ case in the isometric (circles) and isotensional (triangles) ensembles. From left to right, $N=10$ (black) and $N=30$ (green). The dashed lines show the result of linear interpolation. Data have been offset along the x axis for the sake of clarity.

curves was generated from the average taken during a 10^8 step long run.

V. RESULTS AND DISCUSSIONS

In Figs. 4 and 5 we present the measured force-extension curves for the $r_{eq}=0$ and $r_{eq}=d$ cases, respectively. From the qualitative point of view, every force-extension curve displays the same pattern, namely, a difference $\Delta'(X)$ which decreases with increasing applied (or measured) force. This behavior is somewhat expected and can be qualitatively explained in the following way. In the isotensional ensemble, when the external force is vanishing, $\langle X\rangle$ is expected to have a nonzero average value $\langle X\rangle_0$, the exact value of which depends on r_{eq}. In the isometric case, on the other hand, although the projected force $\langle F\rangle$ is not strictly defined at zero end-to-end distance, simple symmetry arguments show that the force acting on each of the terminal beads has, on average, to be zero. Therefore, a vanishing value of $\langle F\rangle$ is expected in this limit. While this arguments account for the differences in the weak stretching regime, in the high-stretching regime the energetic contributions to the free energy are expected to dominate over the entropic ones, and therefore a narrowing of the distance between the force-extension curves is expected. In Fig. 5 the result of a linear fit on the whole spanned x range is also included, showing the perfect Hooke behavior of chain in the isometric ensemble. In contrast to the $r_{eq}=0$ case, the response for the model with a nonzero equilibrium distance does not display a linear behavior over the whole end-to-end distance range. This feature appears because in this case two characteristic distances enter the description of the model, namely, the root-mean-square displacement of a bead around the equilibrium position and the equilibrium distance itself. Indeed, since the spring constant k is relatively high with respect to the thermal energy, at low applied forces (or, equivalently, at short end-to-end distances) the chain behaves much like a freely jointed chain, while when the applied force increases and the springs are significantly stretched with respect to r_{eq}, a different effective bond length becomes relevant.

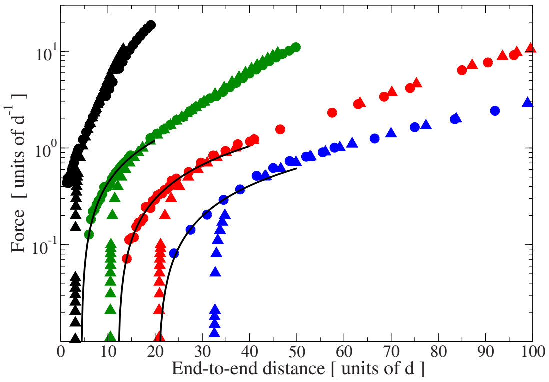

FIG. 5. (Color online) The force-extension curve for the $r_{eq}=d$ case in the isometric (circles) and isotensional (triangles) ensembles. From left to right, $N=10$ (black), $N=30$ (green), $N=60$ (red), and $N=90$ (blue). The solid lines represent the result of linear interpolation in the low-stretching regime. Data have been offset along the x axis for the sake of clarity.

The definition of a weak and a high-stretching region is of course somewhat arbitrary, although by dimensional analysis the obvious threshold is set by $F^c=k_BT/a$, where a now identifies the model-dependent effective bond length. This can be defined [60] according to the scaling behavior of a linear chain in the free case, $a^2\equiv\langle \mathbf{X}^2\rangle_0/(N-1)$. Since in the weak stretching regime the force is expected to be a small perturbation with respect to the thermal fluctuations, the question naturally arises whether and to which extent do the scaling arguments hold in the isometric and isotensional ensembles that are valid for the free case only. In order to check this, the force-extension curves have to be rescaled. The end-to-end distance behavior is obviously $X\sim\sqrt{N-1}$, while from Eq. (7), and remembering that in a Gaussian chain $b\sim 1/\sqrt{N-1}$, one can obtain the scaling behavior for the force $F\sim 1/\sqrt{N-1}$. By plotting the rescaled end-to-end distance and forces, namely,

$$\tilde{X}=X/\sqrt{N-1},$$

$$\tilde{F}=F\sqrt{N-1},$$

for every different chain length, one can see (Fig. 6) that in the low-stretching regime both the isotensional and isometric force-extension curves collapse onto the same universal curve. The force-extension curves in the two ensembles fall onto two different universal curves only up to a certain point, after which they start to depart from the universal behavior of free chains and, at the same time, the difference between the isometric and isotensional cases (for a given chain length) diminishes dramatically. In fact, as long as the scaling laws of free chains are fulfilled, they retain the characteristic differences in the force-extension curves. On the other hand, when the chains start being moderately or strongly stretched, the scaling law changes and the ensemble difference becomes less pronounced. The universal behavior of the chains in the low-stretching regime already points out that the ensemble difference Δ should become constant in

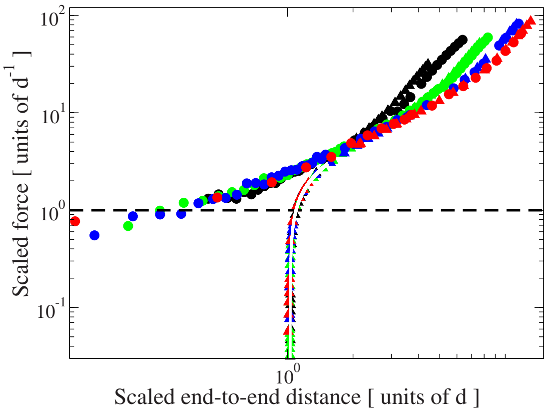

FIG. 6. (Color online) Universality of the force-extension curves in the low-stretching regimes for the $r_{eq}=d$ case, reporting the rescaled force $\widetilde{F}$ as a function of the rescaled end-to-end distance $\widetilde{X}$ for the isometric (circles) and isotensional (triangles) ensembles. The white solid line on top of the isotensional sampled curve in the low-stretching regime is a fit to Eq. (8), while the horizontal dashed line represents an estimate for the low-stretching regime from dimensional analysis.

this regime since both $\Delta'(X)$ and X^* scale like $\sqrt{N-1}$. The only requirement for the loss of ensemble equivalence is that the scaled force $\widetilde{F}$ is roughly less than 1.0. This implies that, for example, in order to keep a constant ensemble difference, the applied forces should decrease as $1/\sqrt{N-1}$ with increasing chain length.

Although the scaling arguments are helpful in showing the ensemble inequivalence in the low-stretching regime, it is instructive to explicitly look at the dependence of the ensemble difference on the force for different chain lengths and, also, to look for the scaling behavior of Δ in different force regimes. In Fig. 7, the ensemble difference is shown, as a function of the applied force, for different chain lengths for the $r_{eq}=0$ case. As it has been already noticed from the qualitative analysis of the force-extension curves, the stronger the applied force, the smaller the ensemble difference is. It can also be observed that the decay of the ensemble difference becomes faster when the chain length is increased. This shows that, compared to the high stretching regime, weaker forces are required in the moderate one to reach ensemble equivalence. On the other hand, the behavior of the ensemble difference in the weak stretching regime suggests that all the curves tend to converge to the limiting value of 1 no matter what the system size is.

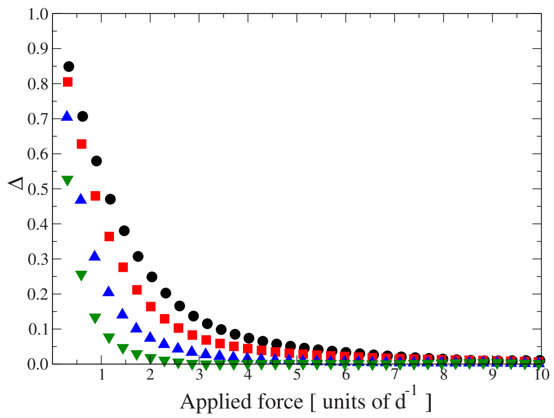

FIG. 7. (Color online) Dependence of the ensemble difference Δ as a function of the force applied in the isotensional ensemble for different chain lengths, $N=15$ (circles), $N=25$ (squares), $N=60$ (upper triangles), and $N=170$ (lower triangles) in the $r_{eq}=0$ case.

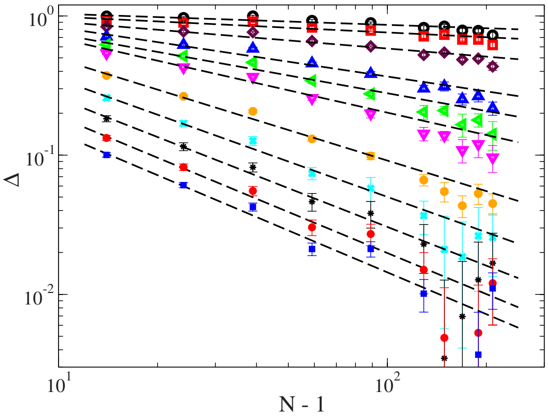

FIG. 8. (Color online) Logarithmic plot of the ensemble difference Δ as a function of the number of beads, $N-1$, for the $r_{eq}=0$ case. Different symbols correspond to different applied forces in the isotensional ensemble, going from the low-stretching regime (smaller slope) to the high-stretching regime (larger slope). Dashed lines correspond to the best fit to a power law. The applied forces ranged from 0.035 to 3.25 d^{-1}.

If one focuses instead of the force dependence on the dependence of the number of monomers on the ensemble difference, it is possible to investigate the scaling properties of the ensemble difference in different force regimes. In this way we perform a finite-size study of the convergence of the isometric and isotensional ensembles. In Figs. 8 and 9 the scaling of Δ with respect to the number of units in the chains is shown for the $r_{eq}=0$ and $r_{eq}=d$ cases, respectively.

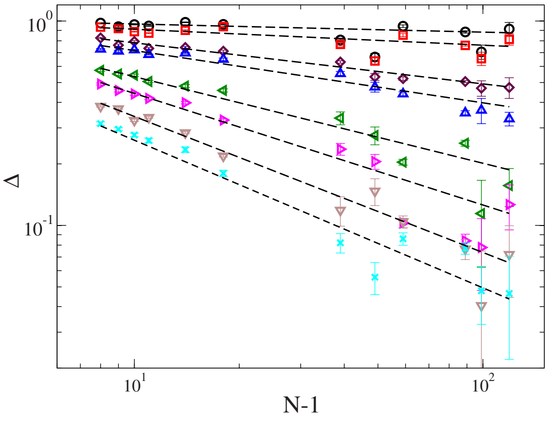

FIG. 9. (Color online) Logarithmic plot of the ensemble difference Δ as a function of the number of beads, $N-1$, for the $r_{eq}=d$ case. Different symbols correspond to different applied forces in the isotensional ensemble, going from the low-stretching regime (smaller slope) to the high-stretching regime (larger slope). Dashed lines correspond to the best fit to a power law. The applied forces ranged from 0.035 to 0.7 d^{-1}.

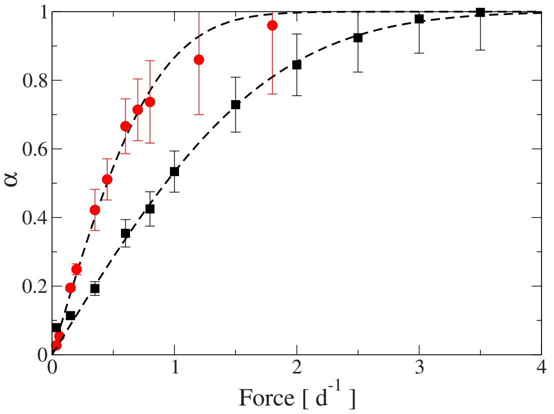

FIG. 10. (Color online) Dependence of the fitted scaling exponent α on the applied force for the $r_{eq}=0$ (squares) and $r_{eq}=d$ (circle) cases. The dashed lines are the result of a best fit to the error function.

For both models we observe the same trend, and for each value of the force the ensemble difference actually follows a power law $\Delta(N)=A(N-1)^{-\alpha}$ (the result of a best fit being also shown in the plots), where the actual value of the exponent strongly depends on the value of the force itself. For diminishing values of the applied force, the curves in the logarithmic plot show a decreasing slope, displaying the clear tendency to become a constant (namely, one) in the limit of vanishing forces. This result not only confirms the inequivalence of the isometric and isotensional ensembles in the vanishing force limit but also shows the development of the scaling behavior in the intermediate force regime. The limiting behavior in the high-stretching regime can be derived by evaluating the ensemble difference using Eqs. (8) and (6), and looking for the asymptotic behavior at large values of ν, leading to $\Delta \sim (N-1)^{-1}$ in the limit of large ν. The two limiting behaviors of the ensemble difference are obviously characterized by the ratio of the typical energies with the thermal one, $\beta F/b$, which is vanishing and greater than 1 in the free and overstretched limits, respectively. The way the overstretched region is approached, in terms of scaling exponents, will therefore depend on the typical length $1/b$ associated with the model. In Fig. 10 the value of the scaling exponent of Δ as a function of the applied force is shown for the two models. Indeed, the high-stretching exponent $\alpha=1$ is reached much faster in the $r_{eq}=1$ case. A phenomenological fitting function is also reported, which describes surprisingly well the switch between the two regimes is $\alpha(F)=\mathrm{erf}(cF)$, where $c\simeq 0.5$ and 1.0 for $r_{eq}=0$ and $r_{eq}=d$, respectively (notice that the effective bond lengths a for the two cases are roughly 0.35 and 1.03). The important information that is conveyed from this analysis, as anticipated in Sec. III, is that the transition from the inequivalence to the equivalence regime actually encompasses a surprisingly broad range of forces, being the scaling exponent still sensibly different from one at reduced forces of about 1 and 2 for the $r_{eq}=1$ and $r_{eq}=0$ cases, respectively. The scenario of a scaling exponent which would reach unity for values of reduced force much smaller than 1 has therefore been ruled out. This result thus demonstrates that the ensemble inequivalence has actually an important impact from the operational point of view on measurements in single-molecule experiments.

VI. CONCLUSIONS

Computer simulations have been employed to study the behavior of linear model polymers in two different ensembles, namely, the isotensional and isometric ones, which are representative for a class of single-molecule experiments. In particular, we have addressed the question of the equivalence of these two ensembles in the thermodynamic limit of growing chain lengths. Finite size effects of order $(N-1)^{-1}$ for the ensemble difference have been demonstrated as a limiting case for a Gaussian chain experiencing high tensions and verified for bead-spring models with zero and nonzero equilibrium distance. In this case ensemble equivalence is reached in the infinite chain length limit. By switching to the low-stretching regime, a dramatic change in the scaling behavior appears. Here we find that the force-extension curves exhibit a universal scaling behavior that is typical for free Gaussian chains in equilibrium. This, in turn, leads to the fact that ensemble equivalence can indeed never be obtained in the vanishing force limit, as has been pointed out by Neumann [22]. Our computer simulations confirm and enhance (by showing the relevance of the inequivalence to equivalence regime transition) the analysis by Neumann [22] that care has to be taken when considering the thermodynamics of single molecules, which presents many subtle differences with respect to bulk systems despite the formal analogies between the two.

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